HANDBOOK OF FOOD SCIENCE, TECHNOLOGY, AND ENGINEERING

Volume 1

Edited by

Y. H. HUI

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CRC Taylor & Francis
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HANDBOOK OF FOOD SCIENCE, TECHNOLOGY, AND ENGINEERING

Volume 1

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Taylor & Francis
Taylor & Francis Group
Boca Raton London New York

A CRC title, part of the Taylor & Francis imprint, a member of the
Taylor & Francis Group, the academic division of T&F Informa plc.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-9847-9 (Set)
International Standard Book Number-10: 1-57444-551-0 (Vol 1)
International Standard Book Number-10: 0-8493-9848-7 (Vol 2)
International Standard Book Number-10: 1-57444-552-9 (Vol 3)
International Standard Book Number-10: 0-8493-9849-5 (Vol 4)
International Standard Book Number-13: 978-0-8493-9847-6 (Set)
International Standard Book Number-13: 978-1-57444-551-0 (Vol 1)
International Standard Book Number-13: 978-0-8493-9848-3 (Vol 2)
International Standard Book Number-13: 978-1-57444-552-7 (Vol 3)
International Standard Book Number-13: 978-0-8493-9849-0 (Vol 4)
Library of Congress Card Number 2005050551

Library of Congress Cataloging-in-Publication Data

Handbook of food science, technology, and engineering / edited by Y.H. Hui.
p. cm. -- (Food science and technology ; 148)
Includes bibliographical references and index.
ISBN 1-57444-551-0 (v. 1 : alk. paper) -- ISBN 0-8493-9848-7 (v. 2 : alk. paper) -- ISBN 1-57444-552-9 (v. 3 : alk. paper) -- ISBN 0-8493-9849-5 (v. 4 : alk. paper)
1. Food industry and trade--Handbooks, manuals, etc. 2. Food--Analysis--Handbooks, manuals, etc. 3. Food--Composition--Handbooks, manuals, etc. I. Hui, Y. H. (Yiu H.) II. Food science and technology (Taylor & Francis) ; 148.

TP370.4.H38 2005
664--dc22 2005050551

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Preface for Volumes 1 and 2

In the last 30 years, progress in food science, food technology, and food engineering has advanced exponentially. As usual, information dissemination for this progress is expressed in many media, both printed and electronic. Books are available for almost every specialty area within these three disciplines, numbering in the hundreds.

Collective works on the disciplines are also available, though in smaller number. Examples are encyclopedias (food science, food engineering, food packaging) and handbooks (nutrition, food processing, food technology). Because handbooks on these topics are limited, this four-volume treatise is released by Taylor & Francis to fill this gap. The title of these four volumes is *Handbook of Food Science, Technology, and Engineering* with individual volume title as follows:

- Volume 1: Food Science: Properties and Products
- Volume 2: Food Science: Ingredients, Health, and Safety
- Volume 3: Food Engineering and Food Processing
- Volume 4: Food Technology and Food Processing

This preface introduces Volumes 1 and 2. Each volume contains about 1,000 printed pages of scientific and technical information. Volume 1 contains 55 chapters and Volume 2 contains 46 chapters. Volume 1 presents the following categories of topics, with the number of chapters indicated:

- Food components and their properties, 14
- Food categories, 26
- Food analysis, 9
- Food microbiology, 6

Volume 2 presents the following categories of topics, with the number of chapters indicated:

- Food attributes, 7
- Food fermentation, 8
- Food and workers safety, food security, 12
- Functional food ingredients, 15
- Nutrition and health, 4

A brief discussion of the coverage for each volume is described below.

In Volume 1, the first group of topics covers the components and properties of food such as carbohydrate, protein, fat, vitamins, water, and pigments. The second group of topics covers the different categories of food products including, but not limited to, beverages, bakery, cereals, legumes, vegetables, fruits, milk, meat, poultry, fats, oils, seafood, and wine. The third group of topics describes the analysis of food such as basic principles and various techniques (chemical method, spectroscopy, chromatography, mass spectrometry, and other analytical methodology). The last group of topics covers food microbiology such as basic considerations, spoilage, land and marine animals, and analytical methodology.

In Volume 2, the first group of topics covers the attributes of food such as sensory science, data base concepts, flavor, texture, and color. The second group of topics covers food fermentation including basic principles, quality, flavor, meat, milk, cultured products, cheese, yeasts, and pickles. The third group of topics covers food from the perspective of safety, workers health, and security, especially in the United States, such as food standards, food protection methods, filth, pathogens, migratory chemicals, food plant sanitation, retail food sanitation, establishment safety, animal feeds and drugs, and bio-terrorism. The fourth group of topics covers major functional food ingredients including, but not limited to, antioxidants, colors, aroma, flavor, spice, enzyme, emulsifiers, phytates, sorbates, artificial sweeteners, eggs, gums. The last group of topics covers special topics in nutrition and health such as food allergy, Chinese edible botanicals, dietary supplements, and health related advertisement in the United States.

When studying the information in this two-volume text, please note two important considerations:

1. Although major topics in the discipline are included, there is no claim that the coverage is comprehensive.
2. Although the scientific information is applicable worldwide, a small number of topics with legal implications are especially pertinent in the United States.

These two volumes are the result of the combined effort of more than 150 professionals from industry, government, and academia. They are from more than 15 countries with diverse expertise and background in the discipline of food science. These experts were led by an international editorial team of 13 members from 8 countries. All these individuals, authors and editors, are responsible for assembling 2,000 printed pages of scientific topics of immense complexity. In sum, the end product is unique, both in depth and breadth, and will serve as an essential reference on food science for professionals in government, industry, and academia.

The editorial team thanks all the contributors for sharing their experience in their fields of expertise. They are the people who make this book possible. We hope you enjoy and benefit from the fruits of their labor.

We know how hard it is to develop the content of a book. However, we believe that the production of a professional book of this nature is even more difficult. We thank the editorial and production teams at Taylor & Francis for their time, effort, advice, and expertise. You are the best judge of the quality of this book.

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Dr. Susan E. Duncan is a professor in the Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA. She earned her Ph.D. in food technology and science, The University of Tennessee, Knoxville. She is the director of the Macromolecular Interfaces with Life Sciences Program, a multidisciplinary graduate program integrating polymer chemistry and life sciences. Dr. Duncan is a sensory specialist with a focus on quality issues of dairy, lipids, nutraceutical, and water/beverage products, emphasizing interactions with packaging materials. She has authored 50 peer-reviewed research publications and 7 book chapters. She is a member of the Institute of Food Technologists and American Dairy Science Association.

Dr. Eunice C. Y. Li-Chan is a professor of food science at the University of British Columbia, Faculty of Agricultural Sciences, Food Nutrition & Health Program. Her significant research contributions include pioneering studies that launched the use of Raman spectroscopy and fluorescent hydrophobic probes as tools to study food protein systems, research that established the potential and protocols for using egg yolk antibodies in lieu of mammalian polyclonal antibodies in immunochemistry and immunoaffinity techniques, and the isolation and characterization of value-added proteins and peptides as functional food ingredients. Her publication record includes authorship or coauthorship in over 75 original articles in peer-reviewed scientific journals, more than 25 chapters in books, and a book entitled *Hydrophobic Interactions in Food Systems* (1988, CRC Press).

Dr. Isabel Guerrero Legarreta is a profesor of food science, Department of Biotechnology, Universidad Autónoma Metropolitana, Iztapalapa, México. She received a B.Eng. degree (1972) in chemical engineering from the Universidad Nacional Autónoma de Mexico, Mexico City, an M.Sc. degree (1975) in food science from the University of Reading, England, and a Ph.D. (1983) in food science from the University of Guelph, Canada. Her research and teaching work has been focused on meat and fish preservation and utilization in subtropical areas. She has also studied the obtainment of products from marine resources, stressing the utilization of marine underutilized material and its by-products. Her professional contributions include over 100 papers, book chapters, and a patent on industrial carotenoid pigment separation from shrimp wastes.

Dr. C. Y. Ma obtained his Ph.D. in food chemistry from the University of British Columbia, Canada. After working as a research scientist in Agriculture and Agri-Food Canada for 16 years, he is now a professor of food science at the University of Hong Kong. His current research activities include the study of structure-function relationships of food proteins and bioactive peptides. The molecular structure and conformation of selected proteins with potential uses as food ingredients and peptides possessing biological/pharmaceutical activities are studied by various physical and chemical techniques. Professor Ma also studies the potential uses of under-utilized protein sources from cereal and legume seeds, and the improvements of functional properties of these proteins by various chemical and physical methods.

Dr. Charles Manley received his Ph.D. from the University of Massachusetts–Amherst for research in the area of food and flavor chemistry. He received a B.S. degree in chemistry at University of Massachusetts–Dartmouth. He has worked as a research chemist for the Givaudan Company, and in various research and management positions within a number of Unilever Companies, including manager of Beverage Development and Technology for Thomas J. Lipton, director of Flavor Operations for the National Starch and Chemical Company, and as vice president, International Business Development for Quest International. Currently he serves as vice president of science and technology for Takasago International Corporation (U.S.A.). Takasago is one of the leading Global Flavor and Fragrance Companies with sales volumes in the top five. His major corporate responsibilities have been in managing commercialization of scientific research efforts and departments at both Unilever and Takasago. He has made major professional contributions, including over 150 publications, patents, and presentations in the field of flavor ingredient safety, food processing and science, and natural product chemistry. He has served as the president of the Institute of Food Technologists (IFT) and the Flavor and Extract Manufacturers' Association (FEMA).

Professor Tom McMeekin holds a personal Chair of Microbiology at the University of Tasmania and is co-director of the Australian Food Safety Centre of Excellence. He is a Fellow of the Australian Academy of Technological Sciences and Engineering, Scientific Fellow of Food Standards Australia New Zealand, and Chair of the Food Safety Information Council. Professor McMeekin has contributed to more than 200 publications, including the monograph "Predictive Microbiology: Theory and Application," and has made greater than 30 invited international conference and workshop presentations. He is an executive board member of the International Committee of Food Microbiology and Hygiene and an editor of the *International Journal of Food Microbiology*. Awards include the JR Vickery Medal (International Institute of Refrigeration, 1987), the Annual Award of Merit (Australian Institute of Food Science and Technology, 1998), and International Leadership Award (International Association of Food Professionals, 2002).

Dr. Wai-Kit Nip is a food technologist emeritus from the Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu. Dr. Nip received his B.S. degree (Food Technology, 1962) from National Chung-Hsing University, Taiwan, and an M.S. degree (Food Technology, 1965) and Ph.D. (1969) from Texas A&M University, College Station, Texas, U.S.A. He has taught classes in food processing, food safety, and experimental foods. Research activities include handling and processing of tropical fruits and vegetables, and aquatic foods. He has published numerous refereed articles, proceeding papers, and book chapters, and coedited several books in the food science and techology area. He is also the senior contributor of a patent. He has served at various capacities in local and national scientific organizations.

Dr. Leo M. L. Nollet is a professor of biotechnology at Hogeschool Ghent, Ghent, Belgium. The author and coauthor of numerous articles, abstracts, and presentations, Dr. Nollet is the editor of the *Handbook of Water Analysis*, *Food Analysis by HPLC*, and *Handbook of Food Analysis (3 volumes)* (all titles Marcel Dekker). His research interests include food analysis techniques, HPLC, and environmental analysis techniques. He received an M.S. degree (1973) and a Ph.D. (1978) in biology from the Katholieke Universiteit, Leuven, Blegium.

Dr. Mohammad Shafiur Rahman is an associate professor at the Sultan Qaboos University, Sultanate of Oman. He is the author or coauthor of over 150 technical articles and the author of the internationally acclaimed and award-winning *Food Properties Handbook* published by CRC Press, Boca Raton, FL. He is editor of the *Handbook of Food Preservation* published by Marcel Dekker, New York, which was translated into Spanish by Acribia, Spain in 2003. He is one of the editors for the *Handbook of Food and Bioprocess Modeling Techniques*, which will be published by Taylor & Francis. Dr. Rahman has initiated the *International Journal of Food Properties* (Marcel Dekker) and has been serving as the founding editor for more than 6 years. He is one of the section editors for the Sultan Qaboos University *Journal of Agricultural Sciences* (1999). In 1998 he was invited to serve as a food science adviser for the International Foundation for Science (IFS) in Sweden. He received B.Sc.Eng. (chemical) (1983) and M.Sc.Eng. (chemical) (1984) degrees from Bangladesh University of Engineering and Technology, Dhaka, an M.Sc. degree (1985) from Leeds University, England, and a Ph.D. (1992) in food engineering from the University of New South Wales, Sydney, Australia. Dr. Rahman has received numerous awards and fellowships in recognition of research/teaching achievements, including the HortResearch Chairman's Award, the Bilateral Research Activities Program (BRAP) Award, CAMS Outstanding Researcher Award 2003, and the British Council Fellowship.

Dr. Fidel Toldrá holds a B.Sc. degree in chemistry (1980), M.Sc. degree in food technology (1981), and a Ph.D. in chemistry (1984). Currently, he is research professor and head of the Laboratory of Meat Science, Department of Food Science,

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Dr. Youling L. Xiong is professor of food chemistry at the Department of Animal and Food Sciences, University of Kentucky. He obtained a Ph.D. from Washington State University (1989) and received postdoctoral training at Cornell University. Professor Xiong also holds joint appointments with the Graduate Center for Nutritional Sciences and the Center for Membrane Sciences at the university. Dr. Xiong's research focuses primarily on food protein chemistry and biochemistry, functionality, and applications, with an emphasis on muscle food processing. His fundamental work in food protein oxidation and the study of enzymic modification of soy, whey, wheat, and potato proteins to obtain physicochemically and biologically functional peptides has earned him several prestigious national awards. Dr. Xiong has published more than 130 research papers, contributed to 18 book chapters, and coedited two food science books. He also teaches undergraduate and graduate food chemistry, food protein, and meat science courses.

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Part A

Components

1 Carbohydrate Chemistry

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CONTENTS

I. INTRODUCTION TO CARBOHYDRATES

Carbohydrates, which in their basic form exhibit the general chemical formula $C_n(H_2O)_n$, are a class of organic compounds that were historically designated "hydrates of carbon" due to their observed elemental composition. As the most abundant class of organic compounds on Earth, carbohydrates are the primary constituents of plants and exoskeletons of crustaceans and insects. Therefore, carbohydrates are virtually an unavoidable element of daily life, as they are encountered in food (glucose, sucrose, starch, etc.), wood, paper, and cotton (cellulose). Carbohydrates

themselves can be sub-grouped according to the number of sugar building blocks comprising their respective structures from monomers (monosaccharides) right through to polymers (polysaccharides). In addition, the diversity of carbohydrates occurring within nature arises from the number of carbon atoms comprising sugar monomer units (monosaccharides of 3 to 9 carbon atoms), the varied chemical structure of monosaccharides (including substituent groups), and the nature of linkages joining monosaccharide units.

II. MONOSACCHARIDES

Monosaccharides, which represent the most basic carbohydrate elements, are polyhydroxy aldehydes and ketones commonly referred to as aldoses and ketoses, respectively. In addition, the number of carbon atoms present in the molecule also aids classification of monosaccharides. For sugars comprised of 3, 4, 5, 6, and 7 carbon atoms, the analogous aldose sugars are referred to as *trioses*, *tetroses*, *pentoses*, *hexoses*, and *heptoses*, respectively, while the same ketoses are correspondingly and officially named *triuloses*, *tertruloses*, *pentuloses*, *hexuloses*, and *heptuloses*, respectively. They may also be unofficially grouped with names such as ketopentose and ketohexose. The simplest aldose and ketose monosaccharides are the two entantiomers of glyceraldehyde (D and L) (Figure 1.1) and 1,3-dihydroxyacetone (Figure 1.2), respectively. Aldoses exhibit one additional chiral center compared to ketoses for the same number of carbon atoms. With the addition of an extra carbon atom to a growing monosaccharide chain, the number of possible stereoisomers increases. For the total number of chiral or asymmetric centers (n) possessed by a

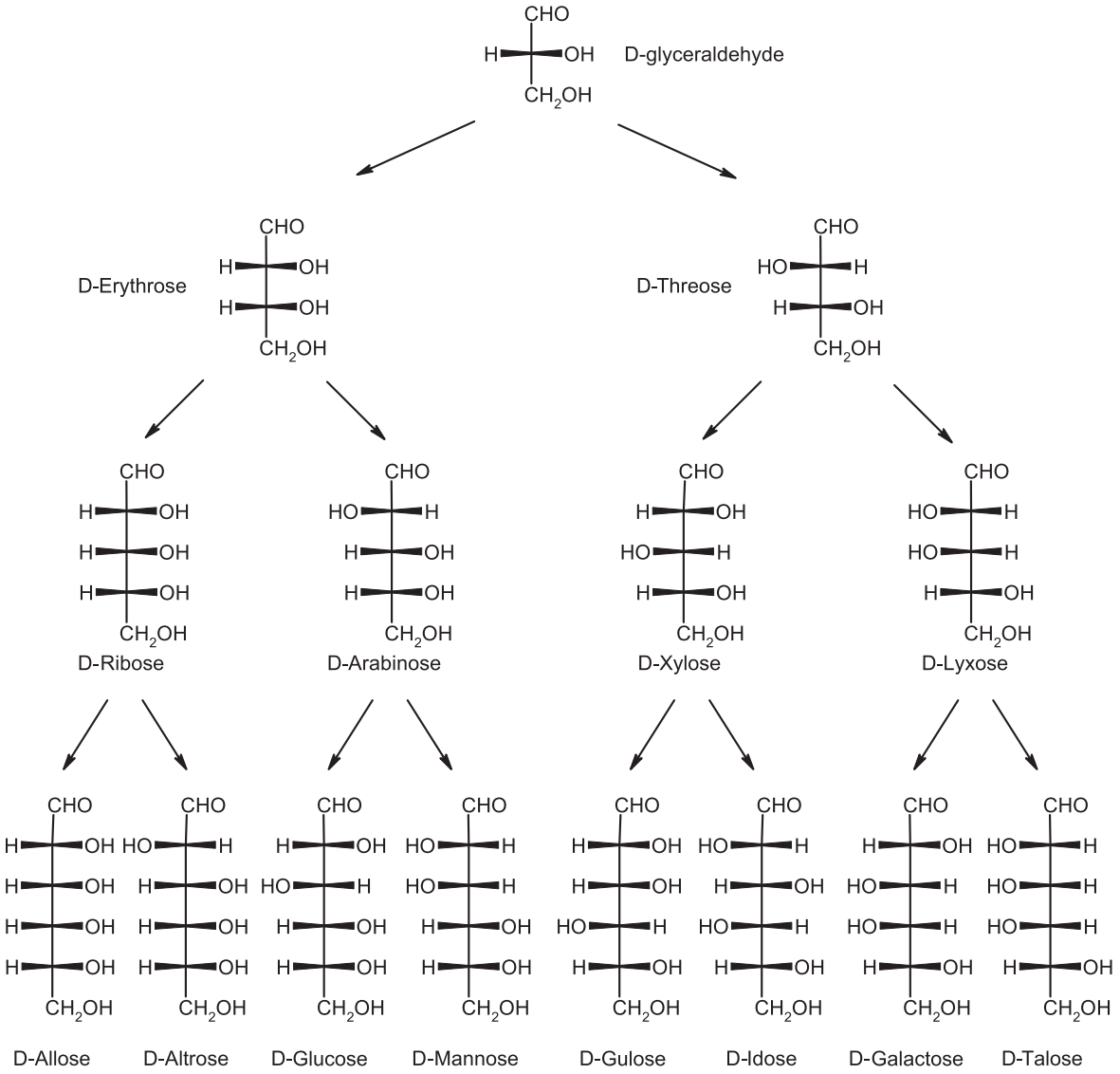


FIGURE 1.1 Acyclic form of the D-aldose series.

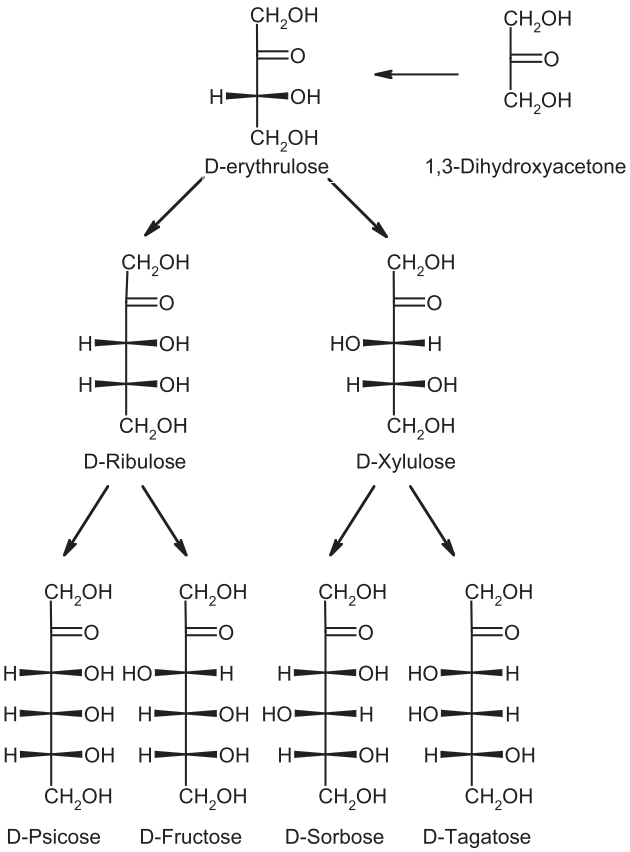


FIGURE 1.2 Acyclic form of the D-ketose series.

monosaccharide, there are 2^n possible arrangements. The reference monosaccharide is considered to be D-glyceraldehyde, which provides a template for generation of acyclic carbon skeletons (from 3 to 6 carbon atoms) as outlined in Figure 1.1 (Fischer projection format). For D-sugars, the hydroxyl group of the highest numbered asymmetric carbon atom (the one furthest from the carbonyl group) is situated on the right-hand side of the Fischer projection, while for L-sugars, the same hydroxyl group is positioned on the left. Thus, the analogous L-aldose series (for brevity not shown) is represented by the exact mirror image structures presented for the D-aldose series. Most sugars found in nature are of the D-configuration, though some common exceptions include L-arabinose, L-rhamnose, L-fucose, L-guluronic acid and L-iduronic acid. Monosaccharide units that differ only in the configuration about a single chiral carbon atom are referred to as epimers (diastereomers). For example, D-glucose and D-galactose are C-4 epimers. Similar to the pattern previously presented for the aldoses, the ketose acyclic series begins with 1,3-dihydroxyacetone; however, the chiral template series starts at D-erythrulose (Figure 1.2) [1,2].

The carbonyl group of aldoses and ketoses is reactive and readily forms an intramolecular cyclic hemiacetal. Therefore, most monosaccharides (except glyceraldehydes, 1,3-dihydroxyacetone and tetrulose) form energetically stable 5- (furan) and 6- (pyran) membered ring structures. Through cyclization, an additional chiral center is formed (compared to the acyclic form) at C-1 (aldoses) or C-2 (ketoses), which is designated the anomeric carbon atom. At the new chiral center, there are two possible anomeric configurations, α and β, which denote the hydroxyl group below and above the ring plane, respectively (true for D-sugars, while the opposite designation is true for L-sugars). The cyclic hemiacetal formation for both pyranose and furanose ring structures (Haworth projections) is illustrated in Figure 1.3 for D-glucose. The actual conformation of the glucopyranosyl structure exists predominantly in the form of a chair-shaped ring (not all ring atoms within the same plane) with the bulky hydroxyl groups in an equatorial arrangement to minimize steric (1,3-*syn*-diaxial) interactions and lessen bond angle strain. For example, β-D-glucopyranose is shown in the $^4C_1$ conformation (Figure 1.3). The superscript and subscript numbers of the conformational notation denote the numbers of the carbon atoms above and below the plane of the ring, respectively [1,2].

Aldoses and ketoses (both hemiacetals) can readily react with alcohols to produce acetals called glycosides. The

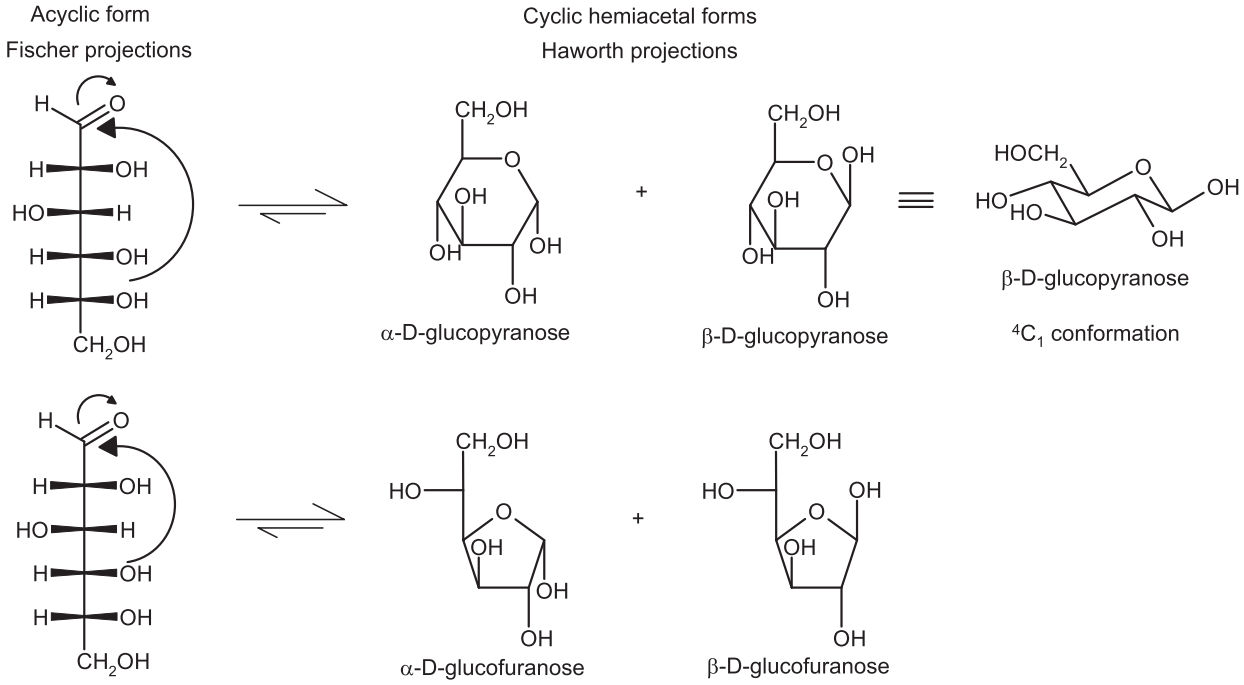


FIGURE 1.3 Cyclic hemiacetal formation of D-glucose and ring conformation.

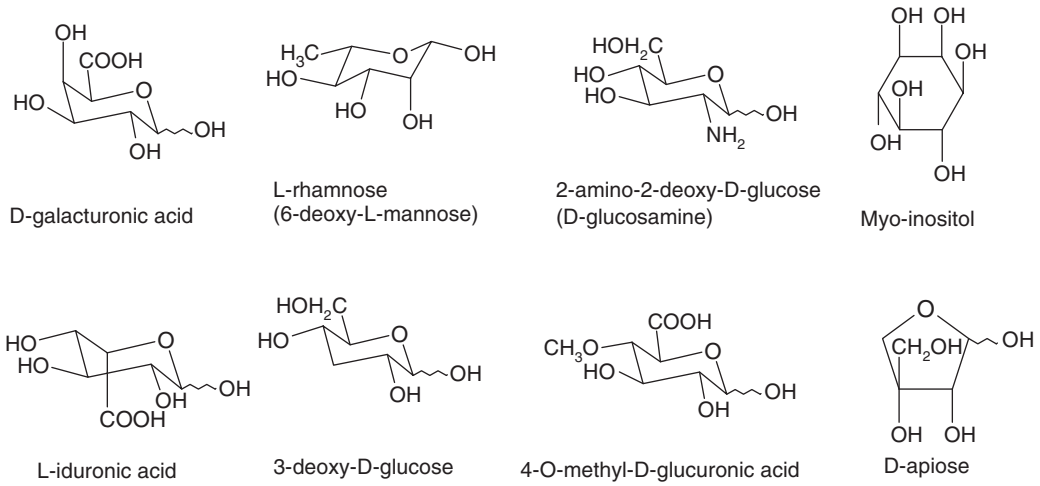


FIGURE 1.4 Structures of other common monosaccharides and inositol.

suffix *-ide* indicates an acetal linkage. For example, D-xylose reacting with methanol produces a mixure of methyl α-D-xylopyranoside and methyl β-D-xylopyranoside [1]. The alcohol (methanol in the above example) portion of the glycoside is called the *aglycon*. In nature, the aglycon (alcohol) is most often another monosaccharide unit, and the covalent bond joining two monosaccharide units is termed a glycosidic bond. This concept can be used to describe two (disaccharide) or more monosaccharide units attached through glycosidic linkages, including extensive polymeric chains (e.g., polysaccharides) comprised of many monosaccharide units.

In addition to the stereoisomeric configurations of sugars, the chemical diversity of monosaccharides can include chemical functionalities such as: carboxyl groups at the primary hydroxyl group position (uronic acids), amino groups in place of hydroxyl groups (amino sugars), hydroxyl groups replaced with hydrogen atoms (deoxy sugars), double bonds (unsaturated derivatives), branch chain sugars, ether substituents, and ester substituents. Examples of these diverse structures are shown in Figure 1.4. A uronic acid is an aldose in which the primary alcohol group (e.g., C-6) has been converted to a carboxylic acid (e.g., α-D-galacturonic acid). A deoxy monosaccharide involves the replacement of

a hydroxyl group with a substituent such as a hydrogen atom (e.g., 6-deoxy-L-mannopyranose, commonly known as L-rhamnose; 2-deoxy-D-*erythro*-pentose, also known as 2-deoxy-D-ribose; 3-deoxyl-D-*ribo*-hexose, also known as 3-deoxy-D-glucose). An amino sugar is a monosaccharide, in which a hydroxyl group is replaced by an amino group (e.g., 2-amino-2-deoxy-β-D-glucopyranose). A branch chain sugar is C-substituted at a non-terminal carbon (e.g., 3-C-hydroxymethyl-D-*erythro*-tetrose, also known as D-apiose). Ether and ester carbohydrate derivatives will be discussed later.

Polyhydroxycyclohexanes, also known as cyclitols or inositols, are discussed here due to their similarities to pyranoses. Nine stereoisomers are possible, and the most widespread in nature is *myo*-inositol (Figure 1.4). Methyl ether derivatives of inositols are also common.

III. REACTIONS OF CARBOHYDRATES

A. HYDROLYSIS

Glycosides, including disaccharides and polymeric chains (oligosaccharides and polysaccharides), undergo hydrolysis in aqueous acids to yield free sugars. The process somewhat randomly cleaves glycosidic bonds to reduce large carbohydrate chains into smaller fragments, which can in turn be further depolymerized to monosaccharide units. Hydrolysis is initiated in glycosides by protonation of the *exocyclic* oxygen atom followed by breakdown of the conjugate acid (cleavage of the bond between the anomeric carbon atom and the *glycosidic* oxygen atom) resulting in the formation of a cyclic carbocation, which is attacked by water to yield the hemiacetal product (Figure 1.5). Glycosidic bonds can also be cleaved by enzymes, which are very specific to the type of sugar residue (e.g., D-galactosyl vs. D-glucosyl), anomeric configuration (α or β), and the glycosidic linkage site (e.g., 1→3). Both acid- and enzyme-catalyzed hydrolysis are commonly employed in the manufacture of maltodextrins and corn syrups, as well as in the commercial production schemes of polysaccharides.

B. OXIDATION/REDUCTION

Aldoses can be readily oxidized to aldonic acids. Because during the oxidation there is a concurrent reduction of the oxidizing agent, aldoses are called reducing sugars (Figure 1.6). Aldonic acids can readily cyclize to form a stable lactone under neutral or acidic conditions. This oxidation reaction has been successfully exploited either chemically (Fehling solution, $Cu(OH)_2$; bromine solution; Tollens reagent) or enzymatically (glucose oxidase) to quantitatively determine sugars [1,2]. In contrast, ketoses must first be isomerized to an aldose (under alkaline conditions), which can then undergo oxidation.

Reduction of an aldose or ketose results in the formation of an alditol or sugar alcohol (denoted by the *-itol* suffix). Commercial-scale operations typically use high-pressure hydrogenation in conjunction with nickel catalyst for such reductions. Sorbitol (D-glucitol) is a commonly occurring alditol in fruits, and is 50% as sweet as sucrose. Sugar alcohols, such as D-glucitol, D-mannitol, and D-xylitol, are frequently used as alternative sweeteners (noncariogenic) in chewing gum and confectionary applications.

C. THERMAL REACTIONS

Heating of reducing sugars results in a complex series of reactions called caramelization. The process is a cascade of

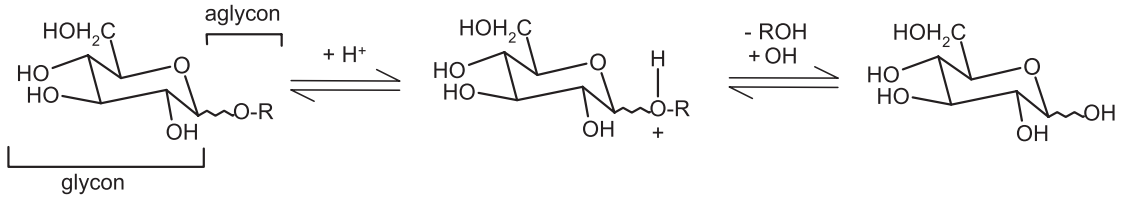


FIGURE 1.5 Abbreviated mechanism of acid-catalyzed hydrolysis of a glycoside.

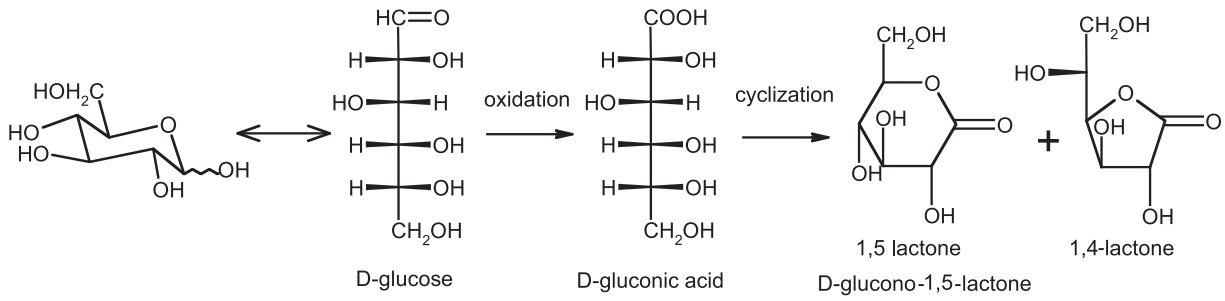


FIGURE 1.6 Oxidation of an aldose to an aldonic acid with subsequent formation of D-gluconolactone.

dehydration reactions that form semi-volatile anhydrides (e.g., 1,6-anhydro-β-D-glucopyranose (levo-glucosan)) and unsaturated compounds (e.g., 5-hydroxy-methyl-furaldehyde (HMF) and furaldehyde) as shown in Figure 1.7 [1]. Catalysts such as salts and acids are added to promote the reaction.

Reducing sugars in the presence of amines (such as proteins and amino acids) undergo a thermal reaction called the Amadori rearrangement. In the case of D-glucose, reaction with an amine ($R\text{-}NH_2$) will form a derivative of 1-amino-1-deoxy-D-fructose and D-glucosylamine (Figure 1.8a). If the reaction continues under acidic conditions, it will undergo dehydration reactions to form HMF. Above pH 5, reactive Amadori intermediates yield complex polymerized dark-colored products via the poorly understood non-enzymatic browning or Maillard reaction, which contributes both color and flavor components to a wide range of food systems (e.g., bread crust, chocolate, caramels, etc.) Recently, acrylamide has been detected in a myriad of high-temperature processed foods (French fries, bread, breakfast cereal, popcorn, etc.), and seems to be primarily derived by the reaction between D-glucose and asparagine. The reaction likely proceeds via the glucosyl-asparagine derivative, and then undergoes decarboxylative deamination to form acrylamide (Figure 1.8b) [4]. To date, it is not known whether the low levels (ppb) detected in food pose any significant health risk to humans.

D. Ester/Ether Formation

Hydroxyl groups of sugars can form esters with organic and inorganic acids. Reaction of hydroxyl groups with acyl chlorides or acid anhydrides in the presence of a catalyst (base) produces esters. Industrially, starches are esterified (acetates, phosphates, succinates, adipates, etc.) to improve their food-use properties. Acetates, sulfates, and phosphates are commonly found as native constituents of carbohydrates. For example, acetyl groups are present in certain polysaccharides such as the plant hemicelluloses (xylan and glucomannan), certain pectins, and xanthan, while sugar phosphates are common intermediates in the biosynthesis of monosaccharides and polysaccharides. The polysaccharide carrageenan contains sulfate half-ester substituents. In addition to esters from sugar hydroxyl groups, esterified uronic acid units are found in polysaccharides. The best example is pectin, in which some of its D-galacturonic acid units exist in the methyl ester form.

The hydroxyl groups of carbohydrates can also form ethers. In nature, ether groups are not common, though some D-glucuronic acid units, particularly in hemicelluloses, such as glucurononxylan, are methylated at the O-4 position (4-O-methyl-D-glucuronic acid). Industrially, starches and celluloses are methylated (cellulose), hydroxypropylated (starch, cellulose), and carboxymethylated (cellulose) to improve the properties of these polysaccharides for a variety of food applications.

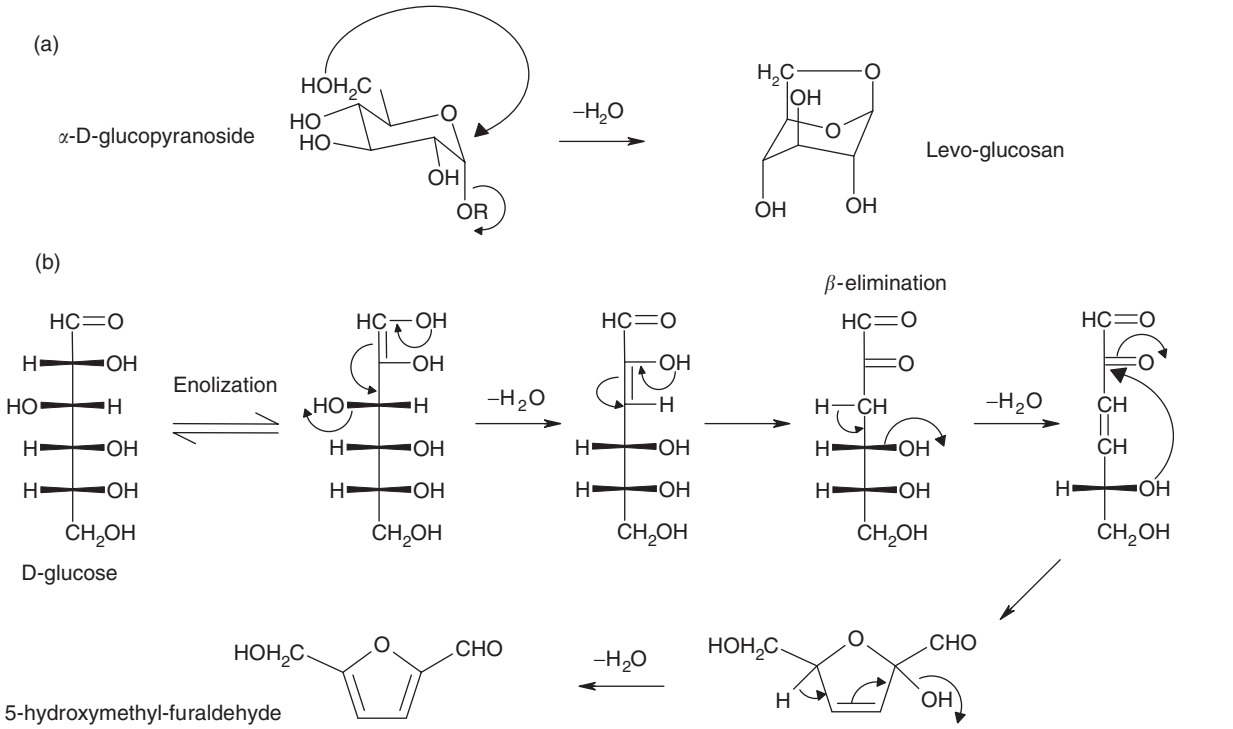


FIGURE 1.7 Reaction mechanism for the formation of (a) levo-glucosan and (b) HMF.

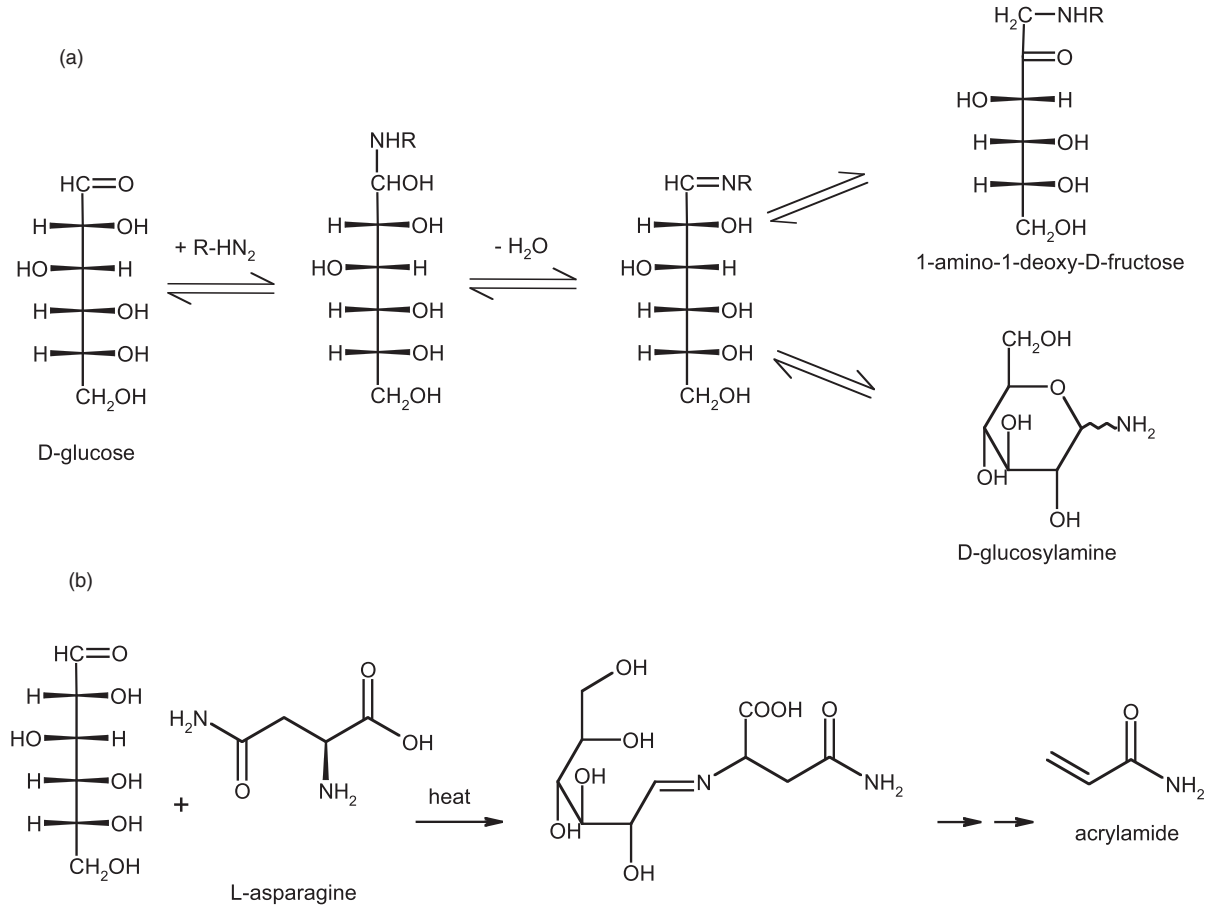


FIGURE 1.8 (a) Amadori reaction scheme and (b) formation of acrylamide.

IV. OLIGOSACCHARIDES

Oligosaccharides are comprised of 2 to 20 glycosidically-linked monosaccharide units [3]. In nature, enzymes called glycosyltransferases catalyze the biosynthesis of both oligosaccharides and larger polymeric carbohydrates (e.g., polysaccharides). These very specific enzymes link specific monosaccharide units together according to a defined anomeric configuration and linkage position (e.g., C-3) on the aglycon sugar. Commercially, oligosaccharides also can be generated through enzyme- or acid-catalyzed hydrolysis of polysaccharides. The following section will briefly discuss common disaccharides, trisaccharides, and fructo-oligosaccharides.

A. Disaccharides

Disaccharides are composed of two monosaccharide units joined by a glycosidic bond. Disaccharides can either be reducing (e.g., maltose and lactose, Figure 1.9) or non-reducing (e.g., sucrose, Figure 1.10), depending on whether one or both anomeric carbon atoms are involved in the disaccharide glycosidic bond. Maltose (Figure 1.9), a disaccharide formed by enzymatic hydrolysis of starch, is produced commercially from the malting of barley, and is the primary fermentable sugar used in the production of beer [3]. The structure of maltose (α-D-glucopyranosyl(1→4)-D-glucopyranose) can be written in shorthand notation as α-Glc*p*(1→4)Glc*p*. The shorthand abbreviation for a monosaccharide unit is based on its first three letters, except for glucose, which is designated as Glc. The position of the linkage is designated as (1→4) from carbon atom 1 of the glycosyl unit to carbon atom 4 of the agylcon unit. The sugar ring size is denoted as *p* for pyranose or *f* for furanose, while the anomeric configuration is designated as either α or β. In the case of D or L configuration, it is only necessary to stipulate L-sugars (D-sugars are assumed unless noted otherwise). This shorthand notation can be used to define both oligosaccharide and more complex polymeric (polysaccharide) carbohydrate structures.

Lactose (β-Gal*p*(1→4)Glc*p*; Figure 1.9) is found in milk at concentrations between 4 and 9%, and is the primary carbohydrate source for developing mammals. For energy utilization, it is necessary that lactose be hydrolyzed by the enzyme lactase (β-galactosidase) to D-galactose and D-glucose in the small intestine to facilitate absorption into the bloodstream. In some individuals, lactose is not (or is only partially) hydrolyzed (lactase deficiency), which

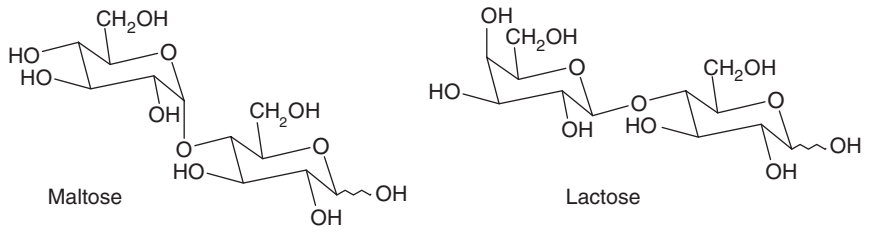


FIGURE 1.9 Structures of maltose and lactose.

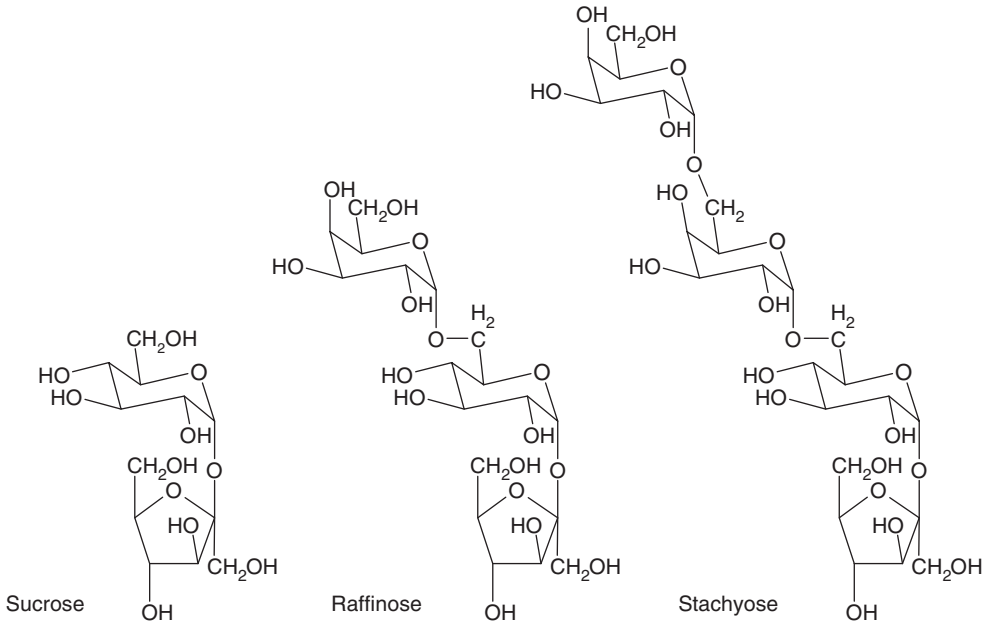


FIGURE 1.10 Structures of sucrose, raffinose, and stachyose.

condition is clinically termed lactose intolerance, and results in the bacterial, anaerobic fermentation of lactose in the large intestine to lactic acid and gaseous products [3].

Sucrose (αGlc*p*(1↔2)βFru*f*; Figure 1.10) is composed of an α-D-glucopyranosyl unit linked (reducing end to reducing end) to a β-D-fructofuranosyl unit, and therefore is non-reducing, because it has no free carbonyl (aldehyde) group. Sucrose (table sugar) is one of the most common low-molecular-weight carbohydrates in the human diet. It is found in plants (e.g., sugar beets, sugarcane, and fruit), where it represents an easily transportable energy and carbon source and an intermediate in starch and cellulose biosynthesis. Another attribute of sucrose is its solubility in water to form highly concentrated solutions, which result in the lowering of the freezing point of water (anti-freeze) and resistance against dehydration in plants and fruits [3]. As a food ingredient, sucrose is utilized due to its water-solubility, desirable sweet taste, effects on colligative properties (e.g., boiling and freezing point regulation), preservative function (osmotic effect), and texturizing effects.

In certain plants, some sucrose molecules are α-galactosylated to form the non-reducing trisaccharide, raffinose (αGal*p*(1→6)αGlc*p*(1↔2)βFru*f*), the tetrasaccharide, stachyose (αGal*p*(1→6)αGal*p*(1→6)αGlc*p*(1↔2)βFru*f*) as shown in Figure 1.10, and the pentasaccharide, verbascose. These oligosaccharides are found especially in beans, onions, and sugarcane. They are non-digestible and are responsible for causing the flatulence (due to microbial fermentation in the colon) associated with the eating of beans and onions [3].

B. Fructooligosaccharides

Fructans, which are polymers (polysaccharides) consisting of β-D-fructofuranosyl units, are found in higher plants, and are composed of two types, inulins and levans (Figure 1.11). Inulins consist of (2→1)-linked β-D-fructofuranosyl units and are found in Jerusalem artichoke, chicory, and dahlia tubers, while levans, consisting of (2→6)-linked β-D-fructofuranosyl units, are found in grasses. Both types of fructans are terminated at the reducing end with a sucrose unit [2]. Fructo-oligosaccharides, which are smaller versions of fructans are used in prebiotic food applications, and are believed to serve as a

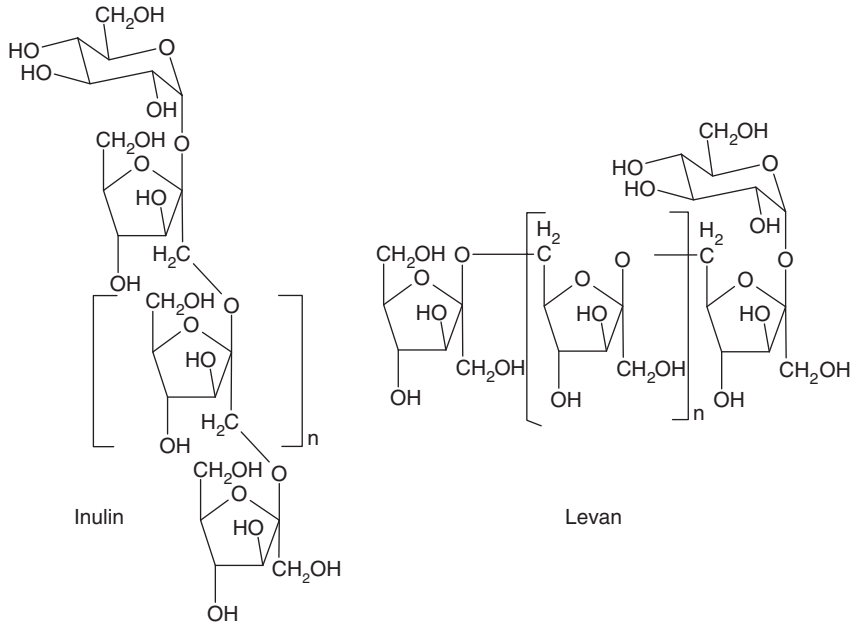


FIGURE 1.11 Structures of inulin and levan oligosaccharides.

TABLE 1.1
Categorization of Select Polysaccharides according to Origin[1]

Origin/Source	Polysaccharide Examples
Higher plants	
Cell wall associated	Cellulose, hemicellulose, pectin
Energy stores (seeds, roots, tubers)	Starch, guar gum, locust bean gum
Exudates	Gum arabic, gum karaya
Marine plants (seaweed extracts)	Carrageenan, alginate, agar
Microorganisms (bacterial fermentation)	Xanthan, gellan
Chemical derviatives (of varied native origin)	Hydroxypropylstarch, starch acetate, starch phosphate, carboxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose
Synthetic	Polydextrose

[1]Adapted from Ref. [3].

preferred substrate to promote colonization of beneficial gut microflora (e.g., bifidobacteria).

V. POLYSACCHARIDES

By definition, polysaccharides (glycans) are long-chain, carbohydrate polymers comprised of, at minimum, 20 glycosidically linked monosaccharide (monomer) units [3]. The number of individual monosaccharide units that comprise a particular polysaccharide is referred to as the *degree of polymerization* (*DP*). Most indigenous polysaccharides possess DPs far in excess of the stated minimum (200–3000 DP is typical), though extremes are observed in nature at both ends of the DP spectrum [3]. While polysaccharides are present in a wide range of plant and animal biological systems, most glycans of commercial significance occur in higher plants (though a few are produced by bacteria). Collectively, polysaccharides from varied origins offer a multitude of structural and functional diversity consistent with their respective intended roles (e.g., structure, energy storage, hydration, etc.) within biological systems. Of the various carbohydrate classes, polysaccharides are by far the most abundant in nature [3], and, as a class of compounds, represent the greatest single component of biomass on the planet. Their relative abundance combined with their diverse structural and functional characteristics make them a superb source of biopolymers for utilization in a wide range of food applications.

A. Classification of Polysaccharides

Though commonly classified by source (Table 1.1), polysaccharides may also be categorized according to the number of different monosaccharide types contained

TABLE 1.2
Categorization of Select Polysaccharides according to Multiple Classification Schemes Related to Structure and Behavior[1]

Origin/Source	Polysaccharide Examples
By Shape	
Linear	Cellulose, starch (amylose[2]), pectin,[3] alginate, agar, carrageenan, gellan, cellulose derivatives (carboxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose)
Branched	Guar gum, locust bean gum, xanthan
Branch-on-branch	Starch (amylopectin), gum arabic
By Number of Types of Monomeric Units	
Homoglycan	Cellulose, starch (amylose, amylopectin)
(Di)Heteroglycan	Guar gum, locust bean gum, alginate, agar, carrageenan, pectin[3]
(Tri)Heteroglycan	Xanthan, gellan
(Tetra)Heteroglycan	Gum arabic
By Charge	
Neutral	Cellulose, starch (amylose, amylopectin[4]), agar,[5] guar gum, locust bean gum, methylcellulose, hydroxypropylmethylcellulose, hydroxypropylstarch, starch acetate
Anionic	Xanthan, gellan, alginate, carageenan, pectin, gum arabic, gum karaya, carboxymethylcellulose, starch phosphate
By Rheological Properties	
Gelling	Starch and starch derivatives, alginate, agar, carrageenan (κ- and ι-types), pectin, gellan, hydroxypropylmethylcellulose, methylcellulose
Non-gelling	Celulose, xanthan,[6] locust bean gum,[7] guar gum, carrageenan (λ-type), gum arabic,[8] carboxymethylcellulose, polydextrose

[1] Adapted from Ref. [3].

[2] Depending on botanical source, amylose can contain some minor short branches toward the molecular reducing end [3].

[3] Categorization does not account for native pectin hairy regions (regions of extensive branching composed of multiple monosaccharide units), most of which are lost during processing to commercial grade pectin [26].

[4] Some starch amylopectin molecules (i.e., potato) may possess small amounts of native starch monophosphate [3].

[5] Agar does possess small amounts of sulfate [30], but is considered to be largely neutral.

[6] Though xanthan solutions do not gel, xanthan does form synergistic gels with locust bean gum, agar, and κ-carrageenan [3].

[7] Though primarily a thickener, locust bean gum exhibits synergistic gelling behavior with xanthan, agar, and κ-carrageenan [3].

[8] Forms gels at very high concentrations [3].

within their molecular structure (e.g., homoglycan: one type vs. heteroglycan: more than one type), molecular shape (e.g., branched vs. linear), electrostatic charge (e.g., neutral vs. anionic) and properties (e.g., gelling vs. non-gelling) (Table 1.2).

In addition, polysaccharides differ from proteins and nucleic acids in that they are both *polydisperse* and *polymolecular* [3]. With regard to polydispersity, a particular polysaccharide type (e.g., pectin) is not defined by a specific number of monomeric units or a defined molecular weight, but rather possesses a range of DPs and molecular weights. Further, the majority of polysaccharides are not chemically homogeneous (cellulose and bacterial polysaccharides are exceptions); they are polymolecular in the sense that individual molecules within a polysaccharide type (e.g., pectin) may differ from one another with respect to fine structure (monosaccharide sequence, proportion of monosaccharide constituents, linkage type, branching frequency). Thus, it is important to keep in mind that the described structure of a polysaccharide type often is not absolute; rather it is an idealized, statistical representation for a population of macromolecules. For every polysaccharide, the reported molecular weight is also an average value.

B. Structural Regimes of Polysaccharides

Nevertheless, structural aspects of polysaccharides may be defined on several different organizational levels (analogous to protein primary, secondary, tertiary, and quaternary structural regimes) [5]. Polysaccharide *primary structure* refers to the sequence of monosaccharide units and the configuration of accompanying glycosidic

linkages. However, it is the glycan primary structure that ultimately dictates the nature and extent of intramolecular and intermolecular associations within a polysaccharide system that lead to development of three-dimensional molecular order (secondary, tertiary, and quaternary structures). Of the two defining elements of primary structure, linkage type generally exerts a greater influence on molecular conformation than monosaccharide type [6]. While there is free rotation about glycosidic bonds, the extent of rotation is limited to a narrow range of thermodynamically favored conformations that coincide with potential energy minima (as a function of hydrogen bonding, van der Waals, polar, and torsional interactions) [5]. These preferred conformations define the proximity of adjacent glycosyl units one to another, and dictate the polysaccharide long-range, three-dimensional shape. This principle is illustrated by the classic comparison of cellulose, amylose, and dextran polysaccharides, which are all linear chains of polyglucose, differing only in the nature of their glycosidic linkages (Figures 1.12a–c) [3].

The equatorial-equatorial β(1→4) glycosidic linkage of cellulose, which facilitates a strong hydrogen bonding interaction between the ring oxygen atom and the C-3 hydroxyl group of adjacent glycosyl units, gives rise to a flat, ribbon-like molecular conformation. On the contrary, the axial-equatorial α(1→4) linkage of amylose leads to a more open, coiled, helical structure, based on favorable hydrogen bonding between the C-2 and C-3 hydroxyl groups of neighboring glucosyl units. Finally, the α(1→6) glycosidic linkage inherent to dextran introduces an additional bond (C-5–C-6), about which free rotation can occur. This additional bond also increases the distance between adjacent glycosyl units such that hydrogen bonding cannot occur. The resulting consequence is that dextran molecules do not generally possess an ordered three-dimensional conformation, but instead adopt the structure of a random coil (possess no defined shape).

The ability to form ordered secondary structure is favored by a high degree of chain uniformity (regularity of monosaccharide sequence and glycosidic linkage) [3], while a random coil results from the lack thereof. In summary, the *ribbon*, *helix*, and *random coil* conformations described for cellulose, amylose, and dextran, respectively, effectively demonstrate the range of *secondary structure* typical of polysaccharide systems.

An example of polysaccharide *tertiary structure* is observed with starch amylose molecules, which can associate to form sections of ordered, double-helical arrangements [5]. Triple-helical tertiary structures have also been reported to exist for various polysaccharides [7,8]. Most polysaccharide tertiary structures are typically stabilized through intermolecular hydrogen bonds.

Temperature and physical state also influence the tendency for a polysaccharide to adopt an ordered secondary

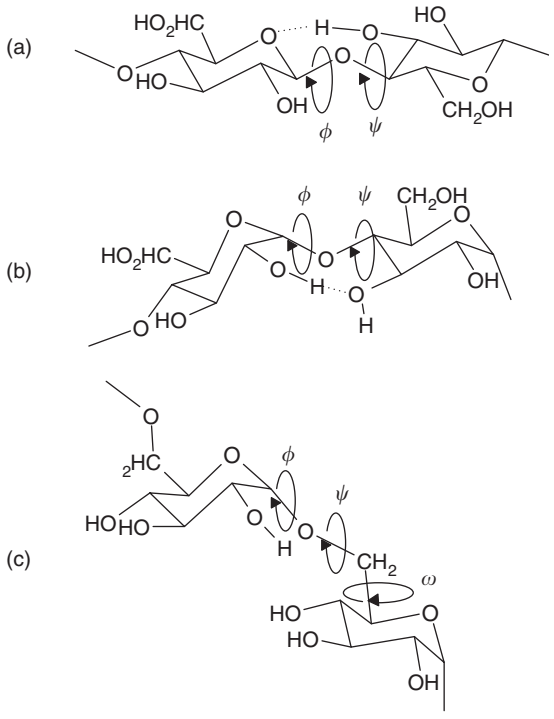


FIGURE 1.12 Rotation about glycosidic bonds (ϕ and ψ) exhibited by polyglucose chains of (a) cellulose, (b) amylose, and (c) dextran (also exhibits free rotation about C5–C6 bond, ω) that provide the basis for long-range, three-dimensional conformational structure (ribbon, helix, and random coil, respectively). Dotted lines between adjacent glucosyl units depict stabilizing hydrogen bonds.

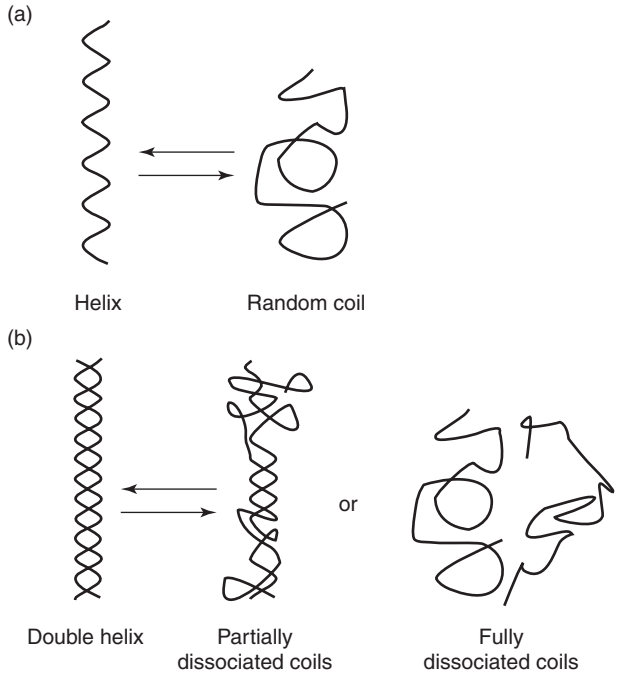


FIGURE 1.13 Depiction of the conformational changes associated with the thermoreversible order to disorder transition for (a) single- and (b) double-helical structures.

or tertiary structure. A polysaccharide in an ordered conformation typically undergoes a reversible order (helix) to disorder (random coil) transition with an increase in temperature sufficient to disrupt hydrogen bonds that stabilize the ordered conformation (Figure 1.13a) [9]. Under these circumstances, double-stranded tertiary structures generally unfold (Figure 1.13b). Upon cooling below the transition temperature, polysaccharide molecules are again able to regain their respective ordered secondary and/or tertiary arrangements. For polysaccharides capable of forming ordered secondary structures, the crystalline state generally favors the existence of the ordered conformation, while the solution state (in water) often results in adoption of a random coil [5]. In the solution state, competing hydrogen bonds between solute (polysaccharide) and solvent (water) molecules tend to minimize the stabilizing effects of intramolecular (solute-solute) hydrogen bonds that would otherwise stabilize an ordered polysaccharide secondary structure. Nevertheless, the solution state does not necessarily impede the formation of double-helical tertiary structures, though solvent conditions necessary for development of such structures may vary with polysaccharide type.

The ability to form some degree of ordered secondary or tertiary structure is generally a prerequisite (but not a guarantee) for polysaccharides to participate in advanced quaternary supramolecular structures. *Quaternary structure* develops through alignment and aggregation of secondary- and/or tertiary-ordered polysaccharide molecules [5], and is typically stabilized by non-covalent interactions (electrostatic, non-polar, hydrogen bond associations) under requisite solvent conditions. Such quaternary order is responsible for the intermolecular associations that lead to development of both gel (junction zone) and other crystalline structures, which are important to processed foods and native plant cell wall systems. However, in discussing any level of polysaccharide three-dimensional structure, it is important to note that polysaccharide molecules in solution are in a constant state of dynamic flux, and likely exist in a wide range of physical forms (helix, double helix, random coil, etc.) at any point in time (even though a statistically favored conformation may be dominant) [3,5]. Nevertheless, the three-dimensional structures discussed here provide a basis for many of the observed properties of polysaccharide systems. A more detailed description of molecular features impacting polysaccharide conformation and physical properties is presented next.

C. Impact of Polysaccharide Molecular Features on Physical Properties

While polysaccharides possess ring oxygen and hydroxyl groups capable of interacting with water through hydrogen bonds [3], physical properties such as solubility, viscosity, and gelling capability are additionally influenced by other molecular features inherent to a polysaccharide. Water solubility of a polysaccharide is generally enhanced by molecular features that prevent formation of

TABLE 1.3
General Description[1] of Polysaccharide Molecular Features and Conditions That Promote Water-Solubility, Viscosity Development, Gelling Behavior

Polysaccharide Feature	Water-Solubility	Viscosity Development	Gelling Behavior/Stability
Backbone linkage and/or monosaccharide repeat	Irregular	Regular (rigid structures)	Mixed (both regular and irregular segments)
Backbone shape	Branch-on-branch structure	Linear, extended structures	Linear, extended structures
Degree of branching and/or substitution	Regular, even distribution of sidechains or substituents along polymer chains	Regular, even distribution of short sidechains or substituents along polymer chains	Sporadic or irregular distribution of side chains or substituents along polymer chains
Molecular charge (if charged)	Even distribution of charge (repulsive) along polymer chains	Even distribution of charge (repulsive) along polymer chains	Uneven distribution of charge (repulsive) along polymer chains
Degree of solvation	Maximum	High	Balanced (segments of both polymer-polymer and polymer-water interactions)
Molecular size	Low	Intermediate to high	Low to intermediate

[1] It is important to note that polysaccharides do not necessarily need to possess all suggested molecular features or conditions to exhibit a particular property, though the greater number of molecular features present will increase the likelihood for a particular property to be exhibited. Exceptions do also exist.

an ordered three-dimensional structure (e.g., irregular backbone structure) or that present physical barriers to intermolecular interactions (e.g., uniform sidechains, backbone repulsive charge) (Table 1.3). An irregular polysaccharide glycosidic linkage or monosaccharide repeat tends to promote polymer flexibility, which can reduce opportunity for intermolecular association and aid solubility. The presence of regular sidechains or derivatized polysaccharide hydroxyl groups can introduce steric hindrance and molecular repulsion (if substituents are charged), which minimize polysaccharide intermolecular associations, leading to increased solubility [3].

The basis for the increased viscosity of polysaccharide solutions (relative to pure water) varies according to polysaccharide concentration. The viscosity of a polysaccharide system within the dilute regime arises from the restructuring of water at the polysaccharide-water interface, and represents the collective (additive) effect of individual polysaccharide molecules in solution [10]. At more intermediate concentrations, typical of industrial applications, intermolecular effects become more predominant. As a result of being in constant dynamic motion, a polysaccharide molecule in solution sweeps out or occupies a theoretical volume or domain of spherical shape [10]. With increasing polysaccharide concentration, the probability for individual polysaccharide molecular domains to collide or overlap becomes increasingly likely, leading to entanglements, internal friction, and increased viscosity [3,11]. The polysaccharide concentration at which interpenetration of polymer domains occurs is referred to as the overlap concentration, and coincides with a concurrent rise in the slope of the viscosity increase in response to an increasing polysaccharide concentration [11].

Aside from concentration effects, molecular characteristics of polysaccharides greatly influence solution viscosity. The greater the theoretical volume swept out by a polysaccharide molecule in motion, the greater the resulting viscosity (assuming a constant concentration). Thus, in principle, the volume swept out by a polysaccharide in solution is a function of both molecular size (DP) and shape (three-dimensional structure) [3]. While a polysaccharide of high molecular weight or DP might generally be expected to sweep out a greater volume compared to a glycan of relatively smaller size, the factor of molecular shape must also be considered. A random coil (highly flexible) structure will occupy a smaller spherical solution domain than that of a stiff, rod-like extended structure of equal molecular size (Figures 1.14a and 1.14b) [3]. Likewise, with the continued assumption of equal molecular weight, a highly branched polysaccharide is anticipated to exhibit a more compact shape and smaller volume in solution compared to that of a highly linear, extended glycan (Figures 1.14b and 1.14c) [3]. Thus, linear, high-molecular-weight polysaccharides capable of forming ordered secondary (helical) and/or tertiary (double-helical) rod-like, extended structures generally produce highly viscous solutions (at relatively low concentrations). As previously described, formation of ordered secondary or tertiary structures is generally favored by extended regions of chain uniformity (regularity of monosaccharide sequence and glycosidic linkage). Nevertheless, some degree of chain disruption (presence of sidechain, charged, or derivatized moieties

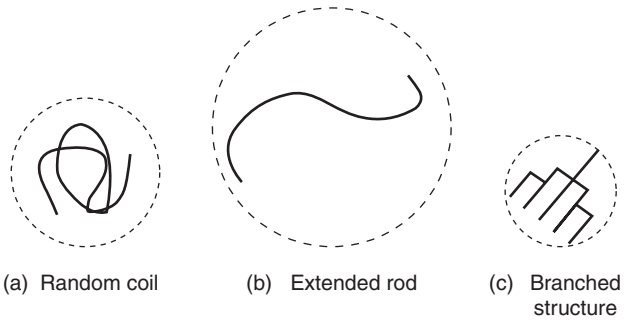


FIGURE 1.14 Comparison of theoretical solution volumes occupied or swept out by (a) a random coil, (b) a somewhat rigid rod, and (c) a branched macromolecule with the assumption of identical molecular weight.

along backbone, etc.) is often necessary to retain polysaccharide solubility (Table 1.2) [3,5]. In particular, charged groups along the polysaccharide backbone tend to keep polysaccharides in extended form by way of intramolecular repulsion, and enhance solubility and increase viscosity.

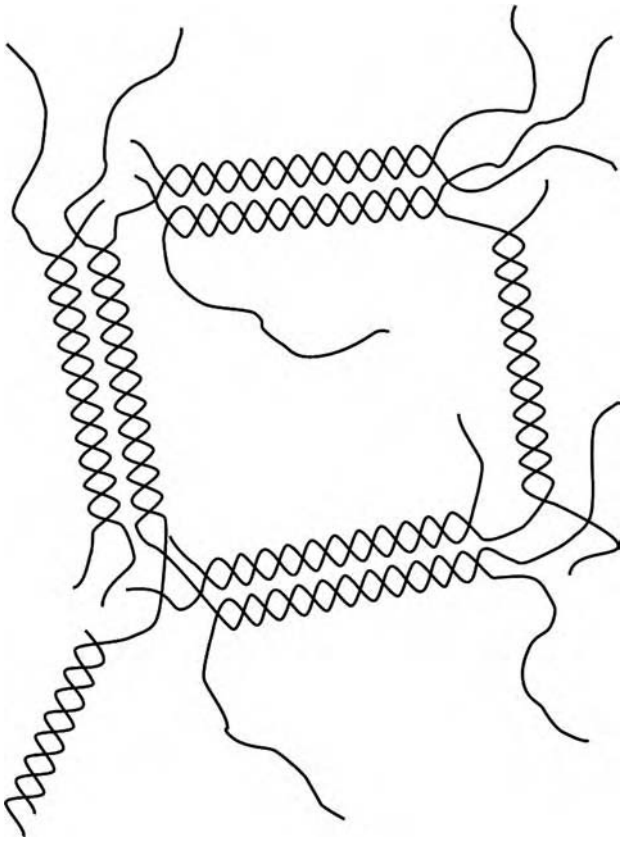


FIGURE 1.15 Schematic representation of a generalized polysaccharide gel structure consisting of segments of aggregated, ordered polysaccharide molecules (double helices) that comprise junction zones (intermolecular cross-links) stabilizing a porous, continuous three-dimensional network or suprastructure. Void spaces are occupied by entrapped solvent (water) and unordered (fully solvated) portions of polysaccharide molecules to yield a viscoelastic material.

The ability to form viscoelastic (combination of both liquid-like (viscous) and solid-like (elastic) behavior) gels represents another significant physical property inherent to many polysaccharide systems. A polysaccharide gel typically consists of some form of an open, continuous, three-dimensional network of aggregated solute macromolecules (polysaccharides) capable of entrapping significant volumes of solvent molecules (water) (Figure 1.15) [3]. The polysaccharide network is generally reinforced through limited aggregation of secondary- and/or tertiary-ordered polysaccharide molecules that form regions of supramolecular quaternary structure termed *junction zones* (intermolecular cross-links) [3,5]. Junction zones may be anchored by a range of stabilizing forces (hydrogen bonds, hydrophobic interactions, electrostatic forces, van der Waals attractions, molecular entanglement, etc.) defined by the polysaccharide structure and solvent conditions. Regions of polysaccharide molecules not involved in junction zone structure maintain strong interaction with water molecules to achieve a delicate balance between the solute-solute (junction zone structure) and solute-solvent (soluble polysaccharide) interactions that constitute a gel.

In general, the polysaccharide structural features that promote gel formation (junction zone development) are similar to those previously described to favor development of secondary- and/or tertiary-ordered structures (characteristics that encourage chain regularity). Nevertheless, to achieve gel stability, most gelling polysaccharides also possess some degree of structural perturbation or disruption that breaks up or limits the formation of the ordered arrangement at sites along the length of polysaccharide chains (Table 1.3) [5]. Such disruptions prevent excessive growth or development of junction zones that would otherwise lead to syneresis (loss of water-holding capacity) and gradual precipitation of polysaccharide molecules [3]. Specific structural features that serve this purpose include: occasional irregularity within the chain primary structure (e.g., carrageenan); occurrence of mixed blocks of monsaccharides within the primary chain (e.g., alginate); and presence of short, sporadic sidechains (e.g., locust bean gum in mixed gel systems with xanthan or carrageenan), substituent groups (e.g., hydroxypropylated starch), or charged moieties (e.g., high-methoxyl pectin). Formation of a stable gel structure also requires manipulation of solvent conditions to meet gelling requirements imposed by the specific structural features of a polysaccharide. Addition of low-molecular-weight solutes (acids, salts, sugar, etc.) or adjustment of temperature may also be used to encourage polysaccharide interaction (reduction of solvation), and regulate the balance of attractive and repulsive forces that coincide with the formation of a stable gel system.

D. Polysaccharide Stability and Reactivity

Polysaccharides are subject to a range of environments and conditions in food systems that have the potential to alter not only their conformations, but also their chemical structures and behaviors. A primary means by which molecular structure is significantly altered occurs through the cleavage of glycosidic bonds (depolymerization), which transpires by two primary means, *hydrolysis* and β-*elimination* reactions. The mechanism of chain cleavage by hydrolysis, which may be initiated by acids or enzymes, was described in an earlier section (Section II, Figure 1.5). While the rate of acid-catalyzed hydrolysis is influenced by pH (lower = faster rate), temperature, and time of exposure, it also varies with the nature of the glycosidic linkage [3]. For example, the rate of acid-catalyzed hydrolysis for uronic acid-based polysaccharides (e.g., alginate) is significantly slower than for corresponding neutral polysaccharides. For enzyme-catalyzed hydrolysis, polysaccharides such as starch can be readily hydrolyzed into maltose and branched oligosaccharides by treatment with β-amylase (*exo*-glucanase), which cleaves terminal maltosyl residues from starch polysaccharides. In contrast, α-amylase (*endo*-glucanase) cleaves α(1→4)-linked bonds at random points along the polysaccharide chain affording oligosaccharide products. Thus, for various polysaccharides, the pattern of enzymatic hydrolysis may differ according to the specific enzymes employed. Lastly, polysaccharide depolymerization by means of beta-elimination is favored under alkaline conditions, and requires oxidation at O-2, O-3, or O-6 for the reaction to proceed as depicted below (Figure 1.16). Aside from conditions encountered within food systems, it is important to note that depolymerization reactions are often intentionally employed in the production schemes of many commercial polysaccharides [3].

The reactivity of polysaccharides is also frequently manipulated to improve and extend their physical properties. The reactions described earlier in relation to monosaccharides (Section II) are also pertinent to polysaccharides, and generally involve derivatization of polysaccharide hydroxyl groups. The extent of chemical modification is most commonly described by the *degree of substitution* (*DS*). Most individual monosaccharide units within a polysaccharide structure possess an average of three hydroxyl groups available for reaction. The DS, which may exhibit a maximum value of three, depicts the average number of modified hydroxyl groups per glycosyl unit [3]. For reactions in which it is possible for a substituent group resulting from reaction with a polysaccharide hydroxyl group to further react with another reagent molecule, the degree of reaction is described in terms of *molar substitution* (*MS*), which is defined as the average number of moles of reactant per glycosyl unit [3].

VI. POLYSACCHARIDE STRUCTURES AND FUNCTIONS

Polysaccharides of commercial significance will be discussed in terms of their structural constituents that are ultimately responsible for their observed properties. The discussion of specific polysaccharides is anticipated to highlight the diversity of structures and functions common to food systems, but is not intended to represent a comprehensive list of polysaccharides present in foods either naturally or as added ingredients.

A. Starch and Its Derivatives

As the primary storage medium in higher plants, *starch* in its simplest form consists of two diverse homopolymers, *amylose* (linear structure) and *amylopectin* (branch-on-branch structure), both of which are comprised exclusively of D-glucosyl units (Figure 1.17a and 1.17b). The linear fraction, amylose, consists of (1→4)-linked α-D-glucopyranosyl units, and has a molecular weight in the range of 30,000 to greater than 10^6, depending on source [3]. While the amylopectin backbone exhibits a primary structure identical to that of amylose, it also possesses sidechains of (1→4)-linked α-D-glucosyl units (average chain length

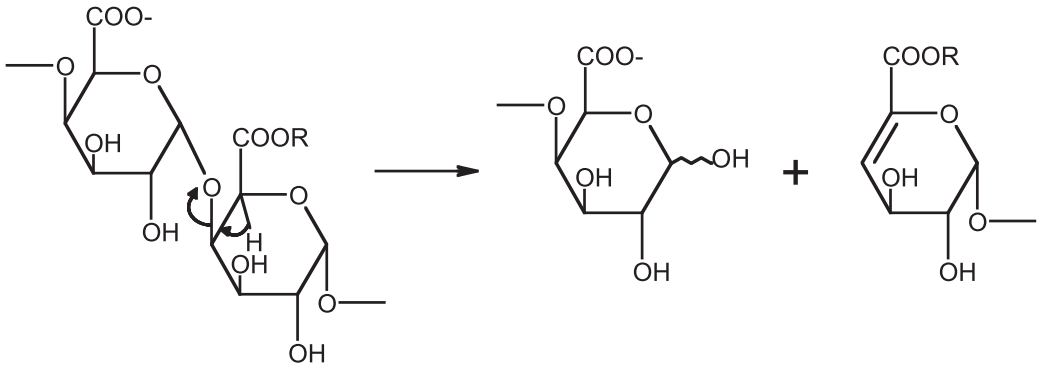


FIGURE 1.16 A possible mechanism for depolymerization of pectin, which possesses native carboxylate and carboxy methyl ester groups, via β-elimination.

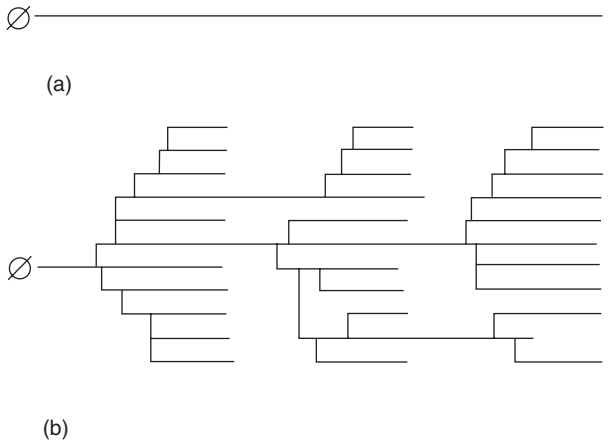


FIGURE 1.17 Idealized diagrams depicting the linear and branch-on-branch structures of starch molecules, (a) amylose and (b) amylopectin, respectively (∅ depicts the molecular reducing end).

of 20–30 units) attached to the main chain through α(1→6) linkages. The sidechains themselves give rise to further branches to yield large, yet compact, branch-on-branch structures of significant molecular weight (approaching 10^9) (Figure 1.17b) [3,12]. Starch is unique in the sense that amylose and amylopectin molecules are biosynthesized and assembled in the form of semi-crystalline aggregates, called *granules*, which vary in size (1–100 μm) and shape (spherical, elliptical, angular, lenticular, etc.) according to the botanical source. Starch granules, which are stabilized by regions of complex molecular order (double-helical association of polymer chains), are insoluble in room temperature water. Slurries of starch granules in water require heating sufficient to disrupt the native granular structure to achieve solubility and realize the functionality of starch [3].

Heating of starch granules in water brings about *gelatinization* or the irreversible loss of granular order, which is accompanied by increased granule hydration, swelling, and leaching of soluble components (primarily amylose) [3,12–14]. In the presence of shear, the fragile, swollen granules are reduced to a paste composed of granule remnants dispersed within a continuous phase of solubilized starch. As the paste is cooled, the linear amylose molecules *retrograde* (crystallize), adopting regions of double-helical structure, which through aggregation, form junction zones that comprise a continuous three-dimensional gel network (Figure 1.18) [15]. The dispersed phase of a starch gel network consists of amylopectin-rich regions and granule remnants. The branched nature of amylopectin limits its intermolecular association, and favors initial water solubility, though amylopectin chains do slowly interact (crystallize) in time [3,12]. Thus, waxy starches, which contain only amylopectin, lack the ability to form strong gel networks, but are nevertheless capable of generating highly viscous solutions over time at starch levels above the overlap concentration via the development of weak intermolecular associations.

Due to their properties and abundance, starches of varied biological origin are frequently exploited as thickeners, gelling agents, binding agents, texture modifiers, and substrates in diverse food applications. However, most food starch (≈75%) added as an ingredient is first chemically and/or physically modified [16], while yet in the granular form, to enhance the physical properties of starch polymers in accordance with the intended end-use. Several categories of starch derivatives will be discussed briefly, though in reality most commercial starch derivatives undergo multiple modifications.

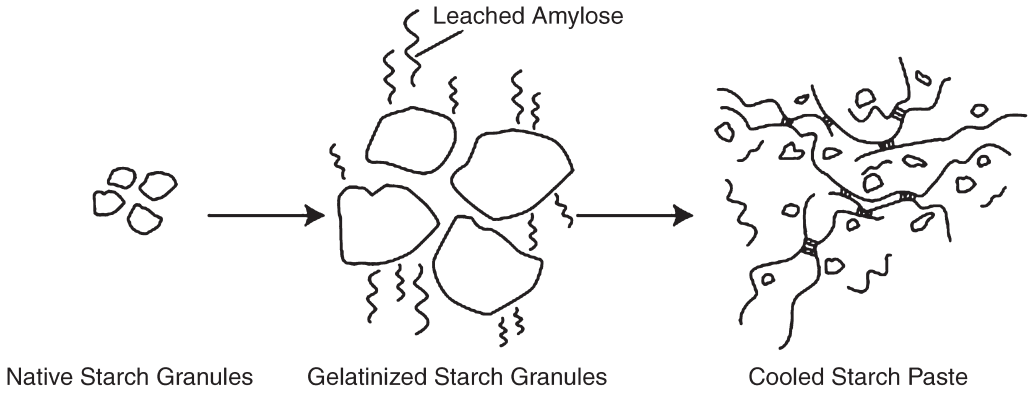


FIGURE 1.18 Schematic representation of the structural changes associated with the gelatinization and pasting of native starch granules. Gelatinization (loss of granular molecular order) is accompanied by granule swelling and leaching of soluble starch components (amylose) during aqueous heating. With the application of shear, swollen granules undergo further disintegration to yield a paste, which is composed of a continuous phase of solubilized starch and a dispersed phase of granule remnants. Upon cooling, amylose retrogradation (depicted by the cross-hatching between molecules within the paste) results in the formation of a gel network.

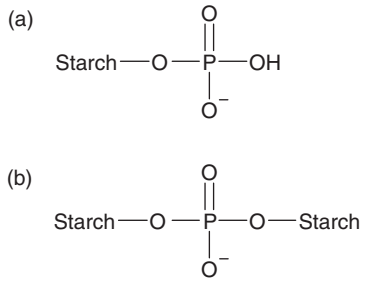


FIGURE 1.19 Chemical structures of a (a) stabilized (starch monophosphate) and (b) cross-linked (distarch phosphate) starch derivatives.

Starch *stabilization* generally involves conversion of starch hydroxyl groups to phosphate monoesters (DS $\leq$ 0.002), acetate esters (DS $\leq$ 0.09), or hydroxypropyl ethers (MS $\leq$ 0.1) [3]. Modification is employed to overcome the tendency for syneresis of native starch pastes, which occurs due to excessive junction zone growth. The periodic incorporation of bulky (hydroxypropyl) and/or charged (phosphate monoester) substituent groups onto starch molecules (particularly amylose) introduces a physical and/or electrostatic impediment to intermolecular association and formation of ordered structures (Figure 1.19a) [3,17]. By regulating junction zone growth, stabilized starches exhibit improved paste clarity and syneresis/freeze-thaw stability in comparison to their native counterparts [3,17].

Cross-linked food starches are most frequently generated through reaction with phosphorus oxychloride or sodium trimetaphosphate, and exhibit of low levels of distarch phosphate ester cross-links (one per 1000–2000 glycosyl units) between adjacent starch molecules and/or chains (Figure 1.19b) [17]. While the presence of cross-links generally reduces the swelling of granules during gelatinization, it also contributes stability and rigidity to the swollen granule structure (less breakdown with shear), leading to a higher ultimate paste viscosity (compared to the unmodified starch) [17]. Due to the reinforced granule structure, cross-linked starches display good stability to shear, acidic conditions, and extended heating, and are utilized in a broad array of food systems (retorted, extruded, frozen, baked, and dehydrated applications) [3].

Acid-modified starch results from treatment of granular starch with dilute acid to effect partial hydrolysis of starch molecules within granule amorphous (disordered) regions [17]. While retaining their granular shape, acid-modified starch granules display minimal swelling and almost complete disintegration upon heating in water. Most importantly, hot pastes of acid-modified starches exhibit very low viscosities (breakdown of swollen granules), and are easily pumped while hot, but form stiff, opaque gels upon cooling [17]. Acid treatment of starch increases the proportion of linear starch molecules (due to hydrolysis of branched starch chains), which facilitate development of tertiary- and quaternary-ordered structures that comprise a gel network. Primary applications of acid-thinned starches involve production of gelled candy products [3].

Generation of *pregelatinized* or *cold-water swelling* starches requires the partial or complete disruption of the native granule structure (molecular order) by pre-processing (heating) a starch slurry under prescribed conditions [3]. The resulting starch products exhibit either ambient temperature solubility (pregelatinized) or granule swelling (cold-water swelling) to achieve viscosity development in aqueous environments without the requirement of additional heating. Pregelatinized and cold-water swelling starches are incorporated as both thickening and gelling agents in dehydrated and/or instant food products that do not require heat preparation.

Lastly, starch is the substrate for an assortment of carbohydrate ingredients classified as starch *hydrolyzate products*, which include maltodextrins, dextrose (commercial name for glucose), corn syrups, and high fructose syrups (HFS) [18]. Generation of these products involves variable degrees of acid and/or enzyme conversion of starch to lower-molecular-weight polysaccharides, oligosaccharides, and glucose. With the exception of some maltodextrins (bulking agent), all other noted starch hydrolyzate products (sweeteners) are reduced in molecular size to the point they are no longer classified as polysaccharides.

B. Cellulosics

As the most abundant component of biomass on the planet, *cellulose* is the key structural constituent of plant primary cell walls. It consists of long, linear chains composed solely of (1→4)-linked β-D-glucopyranosyl units (Figure 1.12a) [3]. As previously described, the nature of the cellulose glycosidic linkage, its regular monosaccharide sequence, and its linear backbone causes cellulose molecules to adopt flat, rigid, ribbon-like secondary structures that readily aggregate to form crystalline, water-insoluble superstructures [3]. Thus, in the native state, while cellulose represents a good source of dietary fiber in indigenous whole foods or in isolated form (referred to as *powdered cellulose*), it generally requires further processing or derivatization to enhance functionality for broader food use. Several such cellulose derivatives will be highlighted below.

Microcrystalline cellulose (*MCC*), which is generated by acid-catalyzed hydrolysis of native crystalline cellulose fibers, can be categorized into two primary types, powdered and colloidal, based on processing scheme and function. Both are insoluble in water. For powdered MCC, hydrolysis is conducted to generate small crystalline

fragments, which are spray-dried and agglomerated to yield open, porous, aggregates of crystals of desired size (20–100 μm typical) [3]. Powdered MCC is used as a bulking agent and flow aid in food systems. On the other hand, colloidal MCC is produced by applying mechanical shear to crystalline fragments (obtained by acid hydrolysis) sufficient to further reduce crystallite size to the colloidal range (0.2 μm) [3]. A second polysaccharide (generally one with a backbone negative charge) is added to stabilize the dispersed phase (cellulose crystals) by providing a physical and or electrostatic barrier to aggregation [3,19]. Functioning as a protective colloid, the second polysaccharide interacts with cellulose crystals along uncharged segments of its backbone, while its charged regions provide electrostatic repulsion to prevent excessive association of cellulose particles. The dried dispersion, known as colloidal MCC, functions in food as an emulsion stabilizer, thickener, or fat replacer depending upon the properties of the protective colloid.

Production of *carboxymethylcellulose* (*CMC*) involves reaction of cellulose with chloroacetic acid, and converts native hydroxyl groups to carboxymethyl ethers (Figure 1.20a). For food applications, typical DS levels range from 0.4–0.95 [20]. The introduction of charged substituents along the cellulose backbone greatly enhances solubility (relative to that of native cellulose) by way of intermolecular repulsion [3]. At pH values above the carboxyl pK_a, CMC molecules occur as extended linear structures and sweep out large molecular domains to form high viscosity solutions. Commercially, CMC is available in a range of molecular weights (viscosity grades) as are most food gums. It is utilized primarily as a thickener in a wide range of food applications [20].

Methylcellulose (MC) and *hydroxypropylmethylcellulose* (*HPMC*) are additional ether derivatives that offer unique properties to food systems. Methylcellulose is achieved through reaction of cellulose with methyl chloride (MS levels 1.6–1.9) (Figure 1.20b), while production of HPMC involves additional derivatization with propylene oxide (DS levels 0.07–0.34) (Figure 1.20c) [3]. Relative to CMC, significantly higher derivatization levels are required to achieve water-solubility of methylcellulose, which is only marginally enabled by the presence of bulky (but nonpolar) substituent groups distributed along the length of cellulose chains. The marginal solubility of MC becomes further reduced at increased temperatures (due to loss of water molecules of solvation, which facilitates intermolecular association of polymer chains, through hydrophobic interactions). The result is thermoreversible gelation over the temperature range of 50–90°C [3,20]. Due to the ability to form thermal gels, MC may provide a physical barrier against moisture loss and fat uptake during high-temperature frying operations. While HPMC also exhibits thermal gelation behavior, gels are typically weaker (relative to those of MC), and increase in softness with an increasing degree of hydroxypropylation (decreases hydrophobic nature and provides a physical barrier to intermolecular associations) [20]. In addition, HPMC exhibits good surface activity as a foam stabilizer [3].

C. Galactomannans: Locust Bean and Guar Gums

Galactomannans of significance include *guar* and *locust bean* (carob) gums, which commercially are the ground crude flours of their respective seed endosperm [3]. The primary polysaccharide component of both guar and locust bean gums possesses a backbone structure comprising of (1→4)-linked β-D-mannopyranosyl units with the occurrence of solitary α-D-galactopyranosyl units attached glycosidically at C-6 of main-chain mannosyl units (Figure 1.21) [3,21].

While guar and locust bean gums have only low to moderate molecular weights (200,000 and 80,000, respectively) [21], the mannan backbone (extended ribbon-like structure) contributes molecular rigidity that facilitates a large hydrodynamic volume and development of high viscosity solutions [3]. The presence of sidechains (impede aggregation) enhances the solubility of both guar and locust bean gums relative to unsubstituted mannan, which forms insoluble, crystalline, intermolecular aggregates (akin to native cellulose) [21]. Though the two gums have similar structures, substitution with D-galactosyl units is more frequent in guar gum (about 1 of 2 backbone units substituted) and more evenly distributed over the length of the polysaccharide chains as compared to locust bean gum

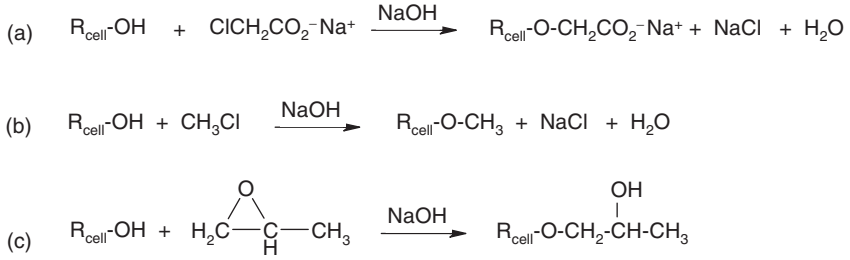


FIGURE 1.20 Reactions used for generation of (a) carboxymethyl-, (b) methyl-, and (c) hydroxypropylcellulose derivatives.

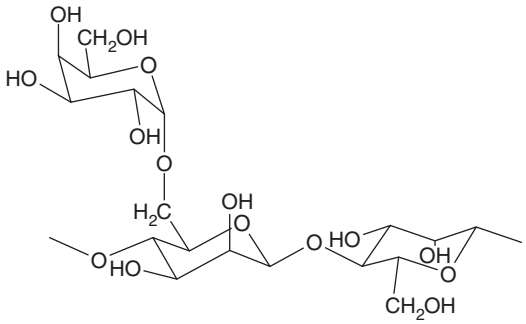


FIGURE 1.21 Generalized structural repeat of galactomannans.

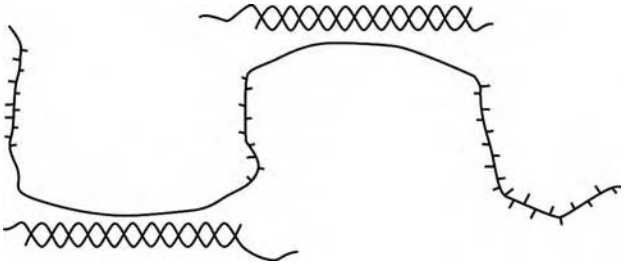


FIGURE 1.22 Schematic representation of the junction zone gel structure between locust bean gum "naked regions" and xanthan or carrageenan double-helical segments.

(about 1 of 4 backbone units substituted with irregular sidechain distribution) [3,21]. The regular substitution pattern of guar gum minimizes intermolecular associations, and explains the excellent water solubility and nongelling behavior of this polysaccharide. The "naked regions" (large polymer sections devoid of sidechains) of locust bean gum afford open segments of the main chain capable of intermolecular interaction, and account for the gel-forming capabilty with xanthan gum and κ-carrageenan (Figure 1.22) [3,21]. Thus, it is the differing patterns of sidechain substitution that primarily account for the basic differences in the properties of locust bean and guar gum.

D. Alginate

Extracted from brown seaweeds, *alginates* are complex, linear, block copolymers composed of (1→4)-linked β-D-mannopyranosyluronic acid and α-L-gulopyranosyluronic acid (occurs in $^1C_4$ chair conformation) units [3,5,22]. Three major types of primary structure generally describe the polymer backbone of alginate: 1) uninterrupted sections of D-mannuronate units (M blocks), 2) uninterrupted regions of L-guluronate units (G blocks), and 3) intermingled sequences of D-mannuronate and L-guluronate units (mixed or MG blocks) (Figure 1.23a) [23]. The occurrence of multiple, primary structural regimes within a single molecule has significant consequences on alginate three-dimensional structure and properties. Due to backbone charge, alginate molecules adopt an extended solution structure consisting of sections of M blocks (ribbon-like structure), G blocks (buckled shape), and MG blocks (irregular coil). In the presence of divalent cations, alginate forms gel structures that are described by the *egg-box* model (Figure 1.23b) [24]. In this model, junction zones are stabilized by divalent cations, which provide electrostatic cross-bridges between oriented G block regions of adjacent molecules. While the M and MG blocks do not participate in junction zone formation, they do serve to balance intermolecular associations by breaking up G block regions and limiting excessive junction zone growth. At excessively low pH values (below the pK_a of the carboxylate group), intermolecular electrostatic repulsion is lost, and precipitation can occur [3].

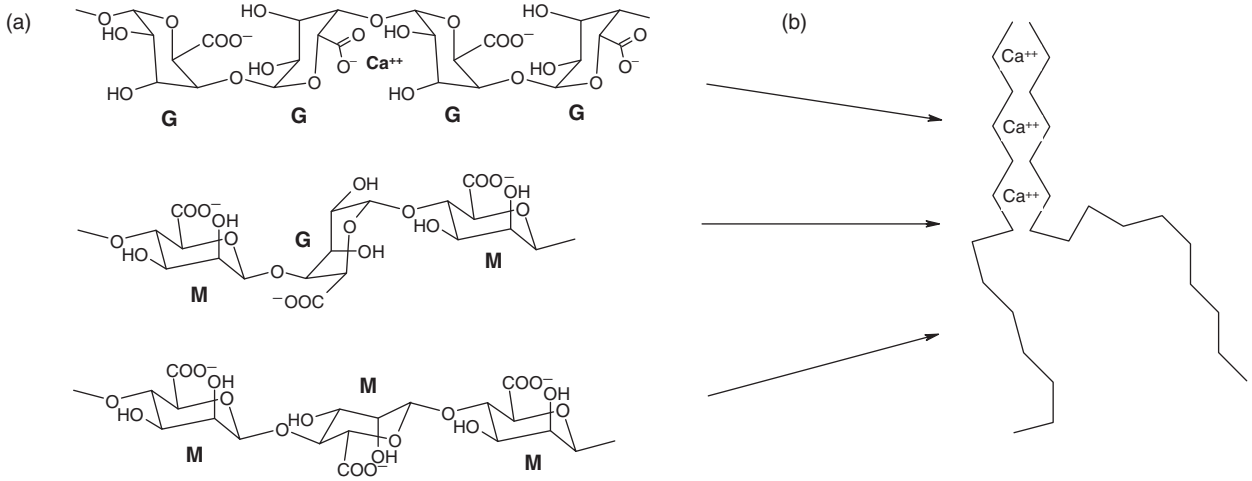


FIGURE 1.23 Depiction of alginate (a) G block, M block, and MG (mixed) block conformational structures and (b) the contribution of each respective conformation to junction zone gel structure characterized by the egg-box model.

E. Pectin

Pectin, a cell-wall associated polysaccharide of higher plants, is a predominantly linear glycan consisting of α-D-galactopyranosyluronic acid units, some of which are present in a methyl ester form [3,25,26]. The polygalacturonate chain may also be disrupted by the occasional insertion of an α-L-rhamnopyranosyl unit [3,25,26] and the presence of sporadic, highly branched segments (hairy regions) [25,26], both of which introduce backbone irregularity (though hairy regions are mostly removed during preparation of commercial pectin). Commercially, pectins are categorized according to their degree of esterification as either low-methoxyl (LM; <50% esterified) or high-methoxyl (HM; > 50% esterified), which designation also defines the optimum conditions in which they gel [3]. LM pectins form gels in the presence of divalent cations, and align to form an "egg box" junction zone structure similar to that previously depicted for alginate (LM pectin and alginate G blocks possess almost mirror image secondary structures).

For HM pectin, solvent conditions must be adjusted to reduce both polysaccharide solvation and intermolecular repulsion (due to ionized carboxylate groups) to facilitate junction zone development. In food systems, the addition of competing solute (usually sugar; 55% minimum) and acid (to achieve a pH <3.5 and reduce the amount of negative charge) provide conditions that lead to gelation [3]. Junction zones, which are stabilized by a combination of both hydrogen bonds and hydrophobic interactions (between methyl ester groups) on adjacent molecules [27], are also effectively limited in size by occasional backbone irregularity (insertion of rhamnose, presence of hairy regions) to benefit gel stability [3,25].

F. Carrageenans

Carrageenans represent a family of linear polysaccharides isolated from red seaweed species, and are generally categorized into three primary classes (κ-, ι-, and λ-types) according to chemical structure and physical properties [3]. All three classes exhibit a common, idealized, disaccharide repeat consisting of 3-O-substituted β-D-galactopyranosyl and 4-O-substituted α-D-galactopyranosyl units, but differ primarily with respect to their degree of sulfation (at C-6 and C-2), which generally follows the trend $\kappa < \iota < \lambda$ (Figure 1.24a–c) [3,28]. Both κ- and ι-types also possess significant proportions of 3,6-anhydro ring structures.

Due to the charged backbone arising from the presence of sulfate substituents, all classes of carrageenan (only sodium salt forms for κ- and ι-types) are highly soluble in water and adopt rigid, extended coil solution structures [3]. Below a particular transition temperature (40–70°C), both κ- and ι-types form regions of double-helical secondary order at sites along the polymer chain [3,5]. In the presence of K^+ and Ca^{2+} cations (κ- and ι-types, respectively), the shielding of negative charge by counterions facilitates aggregation of double-helical regions of polymer chains to form junction zones and a characteristic gel structure (Figure 1.25) [3,5]. Periodic irregularity (kinks) in the backbone structure limits the length of double-helical segments, which in turn regulate junction zone growth and promote gel stability [3,5].

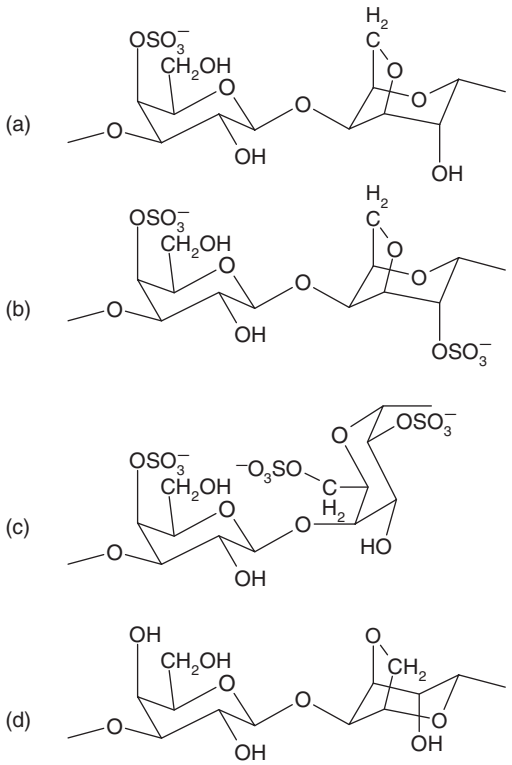


FIGURE 1.24 Idealized structural repeats for (a) κ-carrageenan, (b) ι-carrageenan, (c) λ-carrageenan, and (d) agar that provide insight into the physical properties of each respective polysaccharide.

In contrast, the high degree of negative charge and the different shape of the disaccharide repeat inherent to λ-type carrageenan molecules preclude significant intermolecular association and gel formation under any conditions common to food systems [3]. For carageenans, gel strength decreases in the order $\kappa > \iota > \lambda$ (non-gelling), which is inversely related to the degree of polysaccharide molecular charge.

G. Agar

Agar, which is also derived from specific species of red seaweed, exhibits a chemical structure similar to that of κ-carrageenan, except that the second unit of the characteristic disaccharide repeat is a 3,6-anhydro-α-L-galactopyranosyl unit (in carrageenans, D-entantiomer is present instead) (Figure 1.24d) [29]. Similar to the carrageenans, the backbone structure is interrupted by an occasional kink (presence of sulfate hemiester at C-6 of the α-L-galactosyl unit),

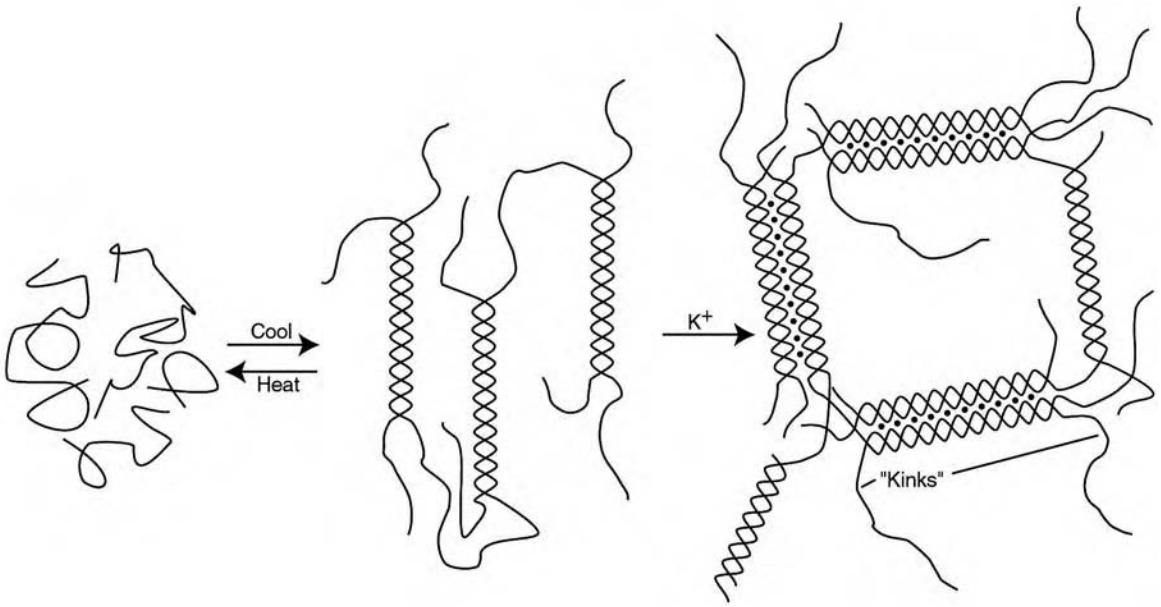


FIGURE 1.25 Representation of the gelation mechanism and junction zone structure for κ-carrageenan gels, in which potassium ions (•) shield backbone negative charges to allow intermolecular interaction of double-helical polysaccharide segments. The presence of "kinks" (occasional backbone structural irregularity) lends stability to the gel by limiting junction zone size and growth. Agar gels are believe to possess a similar gel mechanism and structure, except that potassium ions are not required to bring about gelation.

though the sulfate content of agar is nominal (1.5–2.5%) compared to that of carrageenan (>20%) [30]. While heating (85°C) is required to bring about water solubility, the lack of consistent negative charge along the length of the agar backbone results in a fairly flexible, non-extended polymer chain of relatively low viscosity (in comparison to carrageenan) [29]. However, upon cooling (40°C), agar molecules undergo a transition to an ordered double-helical tertiary structure, which leads to intermolecular aggregation of sections of ordered polymer chains and development of a quaternary gel structure [23]. Agar junction zone and gel structure are thought to mimic that of gelling carrageenans, with the exception that counterions are not required to promote gel formation in agar (lack significant negative charge that would require shielding for intermolecular association to occur) (Figure 1.25). Similar to carrageenan, occasional kinks in backbone structure disrupt the double-helical arrangement, which in turn prevents excessive growth of junction zones and aids gel stability [29].

H. Xanthan

Xanthan is the common name for the heteroglycan isolated from the bacterium *Xanthomonas campestris*. While xanthan has a backbone primary structure identical to that of cellulose, it differs from cellulose in that it possesses a trisaccharide sidechain glycosidically attached to O-3 of alternating backbone units [3,31]. The sidechain consists of two mannosyl units separated by a glucuronic acid unit (Figure 1.26). Approximately half of the terminal mannose units of the sidechain contain pyruvic acid, linked at C-4 and C-6 via a cyclic acetal structure, while the nonterminal mannosyl units contain an acetyl substituent attached at C-6.

The presence of the trisaccharide sidechain, which reduces intermolecular associations (due to electrostatic repulsion and steric hindrance), is thought to account for the excellent water solubility of xanthan relative to that of native cellulose (water-insoluble) [32]. Xanthan forms highly viscous, pseudoplastic (shear-thinning) solutions at low concentrations, which solutions are stable to viscosity change over a wide range of pH (1–12), salt concentration (up to 0.7%), and temperature (0–95°C) [3]. The relatively high-viscosity solutions are attributable to a high molecular weight ($2–10 \times 10^6$) and molecular rigidity derived from its ordered conformation, which is thought to consist of an extended double-stranded helix [33]. At a temperature of 120°C, xanthan solutions lose up to 98% of their original viscosity due to loss of molecular order and rigidity [34]. At reduced temperature, xanthan solutions regain up to 80% of their original viscosity as molecules appear to reform the ordered conformation [34]. Due to its unique solution behavior, xanthan is used as a multipurpose thickener in a wide range of food applications.

I. Gum Arabic

Gum arabic, also known as acacia gum, is the exudate material of the acacia tree common to the Sahel zone of

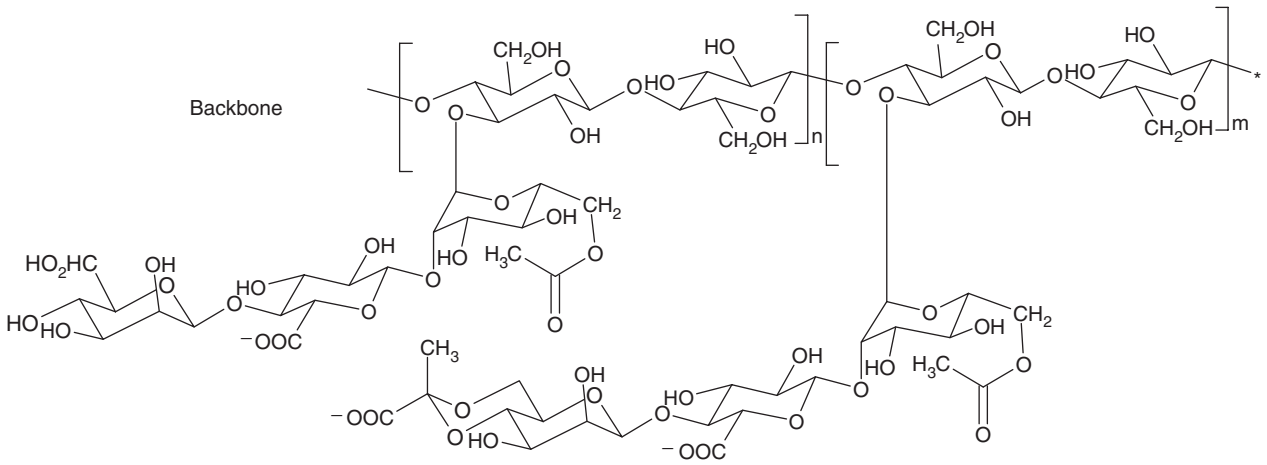
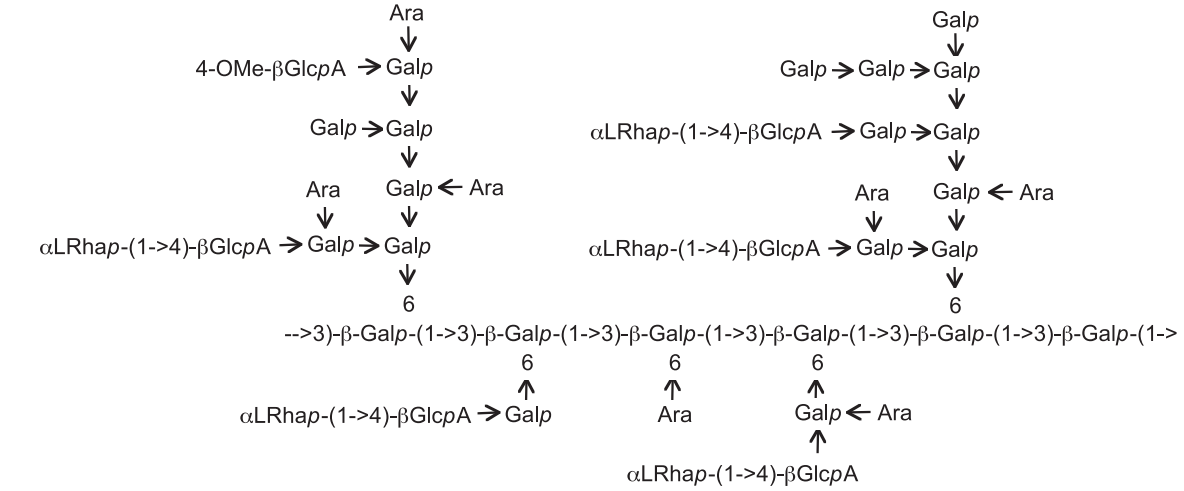


FIGURE 1.26 Chemical structural repeat of xanthan.



Galp = (1-> 3)- or (1->6)-linked β-D-Galp-residues

Ara = L-Araf , or (1->3)-linked LAraf oligosaccharide side chain, or αGal-(1->3)-LAraf

FIGURE 1.27 Representation of the branched chemical structure and composition of gum arabic.

Africa [35,36]. The gum contains 2–3% protein (gives rise to emulsification capability), which is covalently bound to the polysaccharide component [3]. Chemically, gum arabic has a (1→3)-linked backbone of β-D-galactopyranosyl units, which constitute approximately 40% of the total monosaccharide content of the gum (Figure 1.27) [3,36]. Further, the gum arabic backbone is highly substituted with sidechains (which themselves may give rise to further branching), producing a highly branched structure. It contains at least four additional types of monosaccharide units (L-arabinofuranosyl, L-rhamnopyranosyl, D-glucopyranosyluronic acid, and 4-*O*-methyl-D-glucopyranosyluronic acid units) attached to the branched backbone [3,35].

Due to its highly branched nature, gum arabic, though of substantial molecular weight (580,000), possesses a very compact three-dimensional structure, which provides the basis for its most unique physical properties, its astronomical solubility, and low viscosity (up to 50% gum solutions may be prepared) [3]. The compact nature of gum arabic molecules is best comprehended by the fact that gum solutions of up to 10% (w/v) display Newtonian flow behavior, and that it is not until 30% (w/v) solutions are achieved that steric overlap of individual molecular domains begins to occur accompanied by a more substantial rise in viscosity as a function of increasing gum concentration [36].

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2 Carbohydrates: Physical Properties

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CONTENTS

I. INTRODUCTION

Carbohydrates include monosaccharides, oligosaccharides, and polysaccharides as well as substances derived from them by various reactions such as reduction, oxidation, esterification, etc. Monosaccharides are the basic units from which all carbohydrates are built. Linking of monosaccharides via glycosidic bonds leads to the formation of oligosaccharides (2 to 20 monomers) and polysaccharides (more than 20 monomers). The term "sugars" is often used to refer to the monosaccharides and some disaccharides (e.g., sucrose). Polysaccharides are grouped into two major classes: (1) simple polysaccharides, which contain only monosaccharides and their derivatives (esters and ethers), and (2) conjugate polymers made up of a polysaccharide linked to another polymer, such as polypeptide. It is the purpose of this chapter to focus on the physical properties of simple carbohydrates and associated characterization techniques that are important to food sciences.

As one of the three major food components, carbohydrates have enormous functions and applications. They not only supply most of the energy in the diet of humans, but also have various functionalities which are used to confer desired texture in foods. In these latter applications, the physical properties of carbohydrates, such as solubility, water holding capacity, and solution rheology, play important roles. Although containing similar building blocks, mono-, oligo-, and poly-saccharides have different physical properties. An extreme example of this is the contrast between the highly soluble monomeric glucose and the completely insoluble cellulose, which is a polymer of glucose. It has long been known that the configuration and conformation of sugars are the determinants of their chemical and physical properties, and those of oligosaccharides and polysaccharides inevitably depend on the constituent monosaccharide as well as the intermonomeric linkages. Abundant evidence has shown that most of the physical properties of carbohydrates depend on the size, shape, charge, and polarity of the individual molecules. The study of structure-function relationships has been an important topic of carbohydrate research, and advances in physical techniques continue to improve our understanding and provide more insight into these relationships.

II. CONFORMATION OF CARBOHYDRATES

A. MONOSACCHARIDES

Most monosaccharides and their derivatives encountered in foods are polyhydric alcohols carrying a "reducing" keto or aldehydo unit, and they exist primarily in cyclic tetrahydropyran and tetrahydrofuran forms, with the latter occurring less frequently than the former. However, the common ketosugars are more likely than aldosugars to exist as furanoses. Seven-membered rings occur but are not common in foods. Free reducing sugars in solution may exist in different cyclic forms, which are in equilibrium via the acyclic aldehydo or keto form.

There are three potentially stable shapes for the six-membered saturated sugar rings, namely chair, boat, and skew (Figure 2.1). The chair conformation predominates in most cases because the widest separation of the electronegative oxygen atoms is usually achieved through equatorial orientations of most of the hydroxyl and CH_2OH groups. The anomeric hydroxyl unit differs in that it may adopt two orientations (α or β), which are strongly influenced by the ring oxygen. Similarly, there are two principal conformations for saturated furanoid rings, described as envelope (E) and twist (T) (Figure 2.1), each with four or three coplanar atoms, respectively. Because of the low energy barriers between the E and

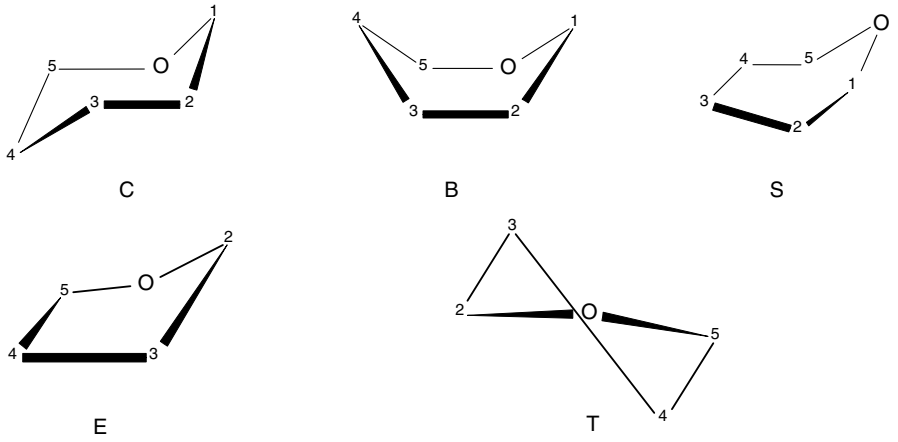

FIGURE 2.1 Examples of chair (C), boat (B), and skew (S) forms for pyranoid rings and envelope (E) and twist (T) forms for furanoid rings.

T conformers, interconversions of these occur more readily than between the pyranoid forms. The shapes of acyclic aldehydo and keto carbohydrates and their reduced forms are usually described as either a linear (zig-zag) or a sickle shape. The advent of diffraction and NMR techniques has allowed the determination of the configuration and conformation of almost all the important monosaccharides (1). In crystals, most molecules adopt a single conformation, whereas in solution there is generally more than one conformation undergoing fast interconversion.

For a more detailed treatment of monosaccharide chemistry and nomenclature, the readers are referred to standard textbooks (2).

B. Oligosaccharides

The conformation of oligosaccharides is less well documented than that of monosaccharides, although the naturally occurring common oligosaccharides are well characterized. Most data from x-ray diffraction and NMR analysis are limited to oligosaccharides having less than four monomeric units. There is considerable experimental difficulty encountered when applying these techniques to large oligosaccharides (3). However, although based on limited amount of data, some general features about the conformation of oligosaccharides can be drawn. Once incorporated into an oligosaccharide or polysaccharide chain, the monosaccharide ring is relatively rigid and the ring geometry becomes effectively fixed. Thus, the overall shape of oligosaccharides become more determined by the two torsion (dihedral) angles ϕ and ψ across the two single bonds of their connecting glycosidic linkage than by the unit geometries. Wells of minimum potential energy may be calculated, which limit the values adopted by ϕ and ψ but not rigidly so. Generally speaking, disaccharides should have a preference for staggered conformations about the two linkage bonds, unless there are geometric constraints imposed by, for example, a hydrogen bond between the two rings. The crystal structures of many oligosaccharides have been elucidated (3). Monosaccharides and certain oligosaccharides possess definite crystalline structures, and thus have well-defined melting points and solubilities.

C. Polysaccharides

Similar to polypeptides, polysaccharides also have different levels of structures, although higher level structures are less well defined. The primary structure describes the covalent sequence of monosaccharide units and the respective glycosidic linkages. The secondary structure describes the characteristic shapes of individual chains such as ribbons and helices, which arise from repetition of units adopting a particular average orientation in shape. Polysaccharide chains with well-defined secondary structure (or sufficient areas of such) may interact with each other, leading to further ordered organizations incorporating a group of molecules. This is known as the tertiary structure. Further association of these ordered entities results in large quaternary structures.

1. Ordered Structures in the Solid State

A repeated sequence of monomers or oligomers leads to an ordered and periodic conformation of polysaccharide molecules. The different linkage types, arising from the anomeric nature of glycosidic linkage and the orientation of OH units through which it is attached, impose certain general features on oligosaccharide and polysaccharide conformations because of the limitations placed on the dihedral angles. Fundamentally there are four different types of chain shapes: ribbons, hollow helices, loosely jointed, and crumpled types (4). For example, for β-(1→4) linked D-glucopyranosyl units, the two bonds from the ring to its two bridging oxygens define a zig-zag form, which promotes a tendency to adopt a flat, extended, ribbon-like conformation, in the polymer (Figure 2.2a). In contrast, when the links between the D-glucopyranosyl units are β-(1→3) or α-(1→4), they define a U-turn form (Figure 2.2b); this geometry extended over multiple units often produces a hollow helical conformation, which becomes stabilized in multiple helices. The linkage through the primary hydroxyl units, such as between (1→6) linked hexopyranose units, leads to a loosely jointed type of conformation and marked molecular flexibility in the resultant polysaccharides. This arises from the extra single bond and torsion angle (ω) between the two sugar rings that separates the rings, reducing inter-unit interactions and allowing a greater range of conformational

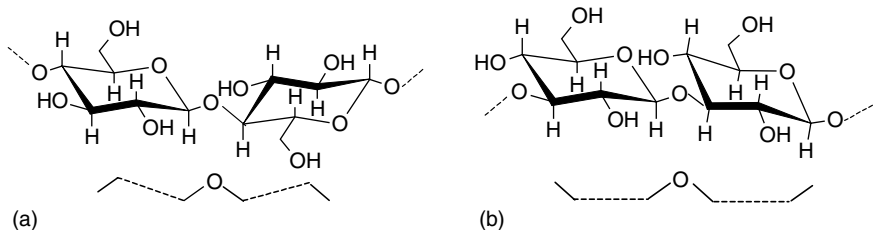

FIGURE 2.2 Examples of geometrical relationships across sugar rings. (a) Zig-zag relationship across 1,4-linked β-D-glucopyranose; (b) U-turn relationship across 1,3-linked β-D-glucopyranose.

possibilities. A further type of conformation known as "crumpled," such as in β-(1→2) linked glucopyranoxyl units, is less common in food carbohydrates.

The regular conformation of polysaccharides can always be described as a helix, which may be defined by just two parameters, the number of units per turn of the helix, and the translation of each repeating residue along the helical axis. The resultant single helix may associate to form multiple helices, which are then further packed in various ways to form higher ordered structures in the solid state. The majority of polysaccharides in their native form exist in an amorphous structure, examples being the antiparallel, extended twofold ribbon-like organized chain structure in the family of mannans and galactomannans (5). A relatively small number of polysaccharides are organized into a repeating crystalline or partly crystalline structure, examples being cellulose, starches, chitin, and some β-D-glucans. The crystalline element is usually capable of existing in different polymorphic forms. The ordered structures of polysaccharides have been extensively studied by x-ray and electron diffraction (6), and the x-ray structures of more than 50 well-defined polysaccharides are known (7).

2. Secondary and Tertiary Structures in Solutions and Gels

The extensively ordered conformation of a polysaccharide in the solid state may not be retained following hydration in solutions and gels. Polysaccharide chains tend to adopt a more or less coiled shape in solutions and fluctuate continuously between different local and overall conformations. A large group of non-gelling polysaccharides, or gelling polysaccharides in non-gelling conditions, exist in solutions with a conformation known as disordered random coils. Since polysaccharide molecules contain a large number of hydroxyl groups, they have a high tendency to associate into supramolecular aggregates through hydrogen bonding in aqueous solutions. For example, combining static and dynamic light scattering, a fringed micelle model was proposed for the aggregates formed in solutions by a number of neutral polysaccharides including tamarind xyloglucan (8) and cereal (1→3)(1→4)-β-glucans (9). The association of molecules in such a form markedly increases the stiffness of the single chains, leading to enhanced solution viscosity. More ordered structures may be developed, in solution through the so-called cooperative interactions, especially for polysaccharides in which identical repeat units result in a regularity of sequence. Conformational transitions in solution between random coils and helices have been well recognized and characterized for a number of polysaccharides such as curdlan, xanthan, and gellan (10). Under favorable conditions, these ordered structures may further associate, leading to the formation of three-dimensional gel networks.

D. Physical Techniques Used to Study Carbohydrate Conformation

A wide range of physical techniques has been used to study the structures of carbohydrates at different levels, i.e., molecular, macromolecular, and supramolecular structures (11). The use of such means as mass spectroscopy and molecular spectroscopy to elucidate the primary structure of carbohydrates will not be covered in this chapter. The purpose is to include only those physical techniques used for studying the conformation of carbohydrates in general, and for probing the higher level structures of polysaccharides. Generally there is a need to combine several physical techniques to provide complementary information about the structure of carbohydrates.

1. X-Ray Diffraction

a. Background

X-ray diffraction and other types of diffraction methods (electron and neutron) have contributed to our understanding of the molecular geometry of carbohydrates. Diffraction is essentially a scattering phenomenon. When a monochromatic x-ray beam travels through a test specimen, a small proportion of the radiation is scattered with mutual reinforcement of a large number of scattered rays, and the resultant x-ray intensity in specific directions depends on the arrangement of the scattering atoms within the sample. X-ray scattering techniques are divided into two categories: wide-angle x-ray scattering (WAXS) and small-angle x-ray scattering (SAXS). Typically, SAXS gives information on a scale of ~ a few nanometers and smaller, while WAXS gives information on a scale of 1–1000 nm. WAXS is used to measure crystal structure and related parameters, which is the topic of this section. SAXS will be discussed in the next section together with light and neutron scattering techniques.

The diffraction pattern, commonly recorded on photographic film, consists of an array of spots (reflections) of varying intensities, from which structural information for a chemical repeat may be deduced. If a large enough size of crystal can be prepared, it is usually possible to determine the crystal structure and hydrogen bonding to a high degree of accuracy. Information such as repeating unit cell dimensions, lattice type, space group symmetry and bond lengths, and valence angles can be derived from the analysis.

b. Monosaccharides and oligosaccharides

For almost all monosaccharides and many oligosaccharides with low degrees of polymerization, it is not a major problem to prepare single crystals for x-ray measurement. X-ray characterized structures are available for most of these molecules (3, 12–16). As an example, in the study of mannotriose (O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-α-D-mannopyranose) (14), the unit cell was determined as monoclinic with dimensions of

a = 0.1183 nm, b = 0.1222 nm, and c = 0.9223 nm, and β = 112.34°; the space group was $P2_1$. The crystal structure includes three water molecules, two of which are involved in hydrogen bonding such that the mannotriose molecules occur effectively as sheets of long parallel chains, with each consecutive sheet having chains lying at approximately right angle to those in a neighboring sheet.

c. Polysaccharides

Large oligosaccharides rarely and polysaccharides never form single crystals that are good enough for classical x-ray crystallography. They tend to form fibers that are amorphous, or at best only partly crystalline, starch being a typical example of the latter. X-ray study of starches mostly measures the degree of crystallinity and identifies different polymorphic forms. To obtain useful x-ray diffraction data from other more amorphous non-starch polysaccharides, oriented fibers or films are used (6). These polycrystalline fibers or films are prepared in such a way that the polysaccharide helices are preferentially oriented with their long axes nearly parallel. The x-ray diffraction intensities then provide information about the helical structures such as repeat spacing of the helix and helix screw symmetry, and if the diffraction pattern is sufficiently "crystalline," the unit cell dimensions and lattice type. However, the x-ray data alone are inadequate to solve a fiber structure, and interpretation requires supplementation with molecular modeling analysis using existing stereochemical information derived from surveys of crystal structures of related mono- or oligosaccharides (7, 17).

X-ray fiber diffraction is of great value in the determination of the conformations of polysaccharides. Studies of the (1→3)-β-D-glucan family, curdlan, schizophyllan, and scleroglucan, are good examples. Curdlan is a linear (1→3)-β-D-glucan, whereas schizophyllan and scleroglucan also contain some β-(1→6)-glucosyl branches. These (1→3)-β-D-glucans usually form triple-stranded helices (18). The structure of curdlan (in both hydrated and anhydrous forms), determined from oriented fibers, assume a right-handed, parallel, six-fold triple-helical conformation. There are interstrand O2···O2 hydrogen bonds in the hexagonal unit cell, with parameters a = b = 1.441 nm and c = 0.587 nm. The space group is $P6_3$ and there is one helix per unit cell (19). The short-branch substitutions on the main chain primary hydroxyls in schizophyllan and scleroglucan do not seem to affect the fundamental triple-helical structure (20).

2. Light, X-Ray, and Neutron Scattering

a. Background

The principles on which light, x-ray, and neutron scattering depend are basically similar and can be treated by the same fundamental sets of equations. For all three modes of scattering, angular dependence of the normalized scattering intensity provides information on the size and shape of the macromolecules. The resolving power of scattering techniques is related to the wavelength of the scattered radiation (21). The wavelengths are 0.1–0.3 nm for SAXS, 0.2–1.0 nm for small angle neutron scattering (SANS), and ~500 nm for light scattering. Conventional light scattering typically reveals only the global dimensions of a macromolecule, which may be tens to hundreds of nanometers for a typical polysaccharide. SAXS and SANS can probe molecular structures at closer ranges of about 2–25 nm (22). SANS may additionally observe the Gaussian behavior of polymer chains in their own bulk (solid), which conventional light scattering cannot. Light scattering is effective in measuring the angular dependence of intensity typically in the range 30° to 135°. SAXS can be carried out at very small angles, typically less than 1°, and is thus superior for the determination of the size and shape of macromolecules, but it is less convenient for the determination of molecular weight and second virial coefficient.

b. Application to polysaccharides

Scattering measurements can be carried out in two modes, static and dynamic. The former measures the average scattering intensity within a selected time period, whereas the latter measures the fluctuation of the intensity over time. From static measurements, the weight average molecular weight (M_w), z-average radius of gyration (R_g), and the second virial coefficient can be extracted. From dynamic measurement, the translational diffusion coefficient is obtained from which the hydrodynamic radius (R_h) can be determined. The parameter, $\rho = R_g/R_h$, may provide information on the architecture of the macromolecules and their aggregates (23). From the combination of static and dynamic scattering data, other information may be derived including the linear mass density, Kuhn segment length, and polydispersity index. To obtain as much structural information as possible, experimental data from scattering are usually processed and presented through various plots, and need to be interpreted using molecular model such as the worm-like chain model (23).

Light scattering was applied to study the solution properties of amyloses and the retrogradation of amyloses as early as the 1960s (24–26). A typical flexible chain behavior was observed for high-molecular-weight amyloses in freshly prepared aqueous solutions. With decreasing molecular weight, the tendency to aggregate increased considerably so that a stable aqueous solution could not be prepared. The many studies on amylopectin and glycogen demonstrated how scattering techniques may be used for investigating the branching behavior of polysaccharides (27). The branching nature of amylopectin and glycogen can be detected clearly by light scattering from the Zimm plot, which shows an upturn (28, 29).

Scattering techniques can be used to probe the conformational transition of polysaccharides in solution. For

example, the thermal transition evident in low ionic strength xanthan solutions was followed by light scattering (30). It was observed that the apparent hydrodynamic radius significantly decreases with increasing temperature in the vicinity of the helix-coil transition temperature. As discussed above (Section II.C.2), light scattering is also useful in investigating aggregation properties of polysaccharides.

3. Chiroptical Methods

a. Background

Optical activity is one of the most readily and often measured physical properties of carbohydrates. Carbohydrates contain several similarly substituted asymmetric carbon atoms and are therefore all optically active. The optical activity can be determined by optical rotation (OR), optical rotatory dispersion (ORD), and circular dichroism (CD). OR is measured by a polarimeter at a single wavelength, usually the sodium D line (589 nm), and expressed as specific (or molecular) rotations $[\alpha]_D$. A number of approaches, all of them empirical in nature, have been devised to interpret the relationship between the measured optical rotations and structural features of carbohydrates (31). Specific rotations are used extensively to characterize new derivatives and to recognize known ones. Instead of using a single wavelength, optical rotatory dispersion measures the optical rotation angle (φ) over a wide range of wavelengths, and circular dichroism measures the differential absorption of right- and left-circularly polarized light as a function of wavelength. Both ORD and CD spectra can exhibit marked changes in slope in the vicinity of the absorption maximum of a chromophore attached to the chiral center, known as the Cotton effect.

b. Optical rotation

In a monosaccharide molecule, several chiral carbons contribute to the overall optical rotation, but the configuration of the carbon atoms attached to the ring oxygen atom have the greatest influence on the overall rotation value. For many monosaccharides and reducing oligosaccharides, the initial optical rotation in aqueous solutions changes with time until reaching a constant value. This phenomenon is known as mutarotation, most often the outcome of interconversion between α and β ring isomers, until reaching an equilibrium.

Similar to monosaccharides, oligo- and polysaccharides have optical activity. With advances in the understanding of carbohydrate stereochemistry, it has become generally recognized that the overall optical rotation is determined more by the relative orientation of adjacent monosaccharide residues (defined by dihedral angles) than by the additive contributions from each asymmetric center. The optical activity of these is therefore beyond those arising from the simple monosaccharides, but is rather associated with the conformation of larger molecules or macromolecules. Stevens and co-workers developed a chiroptical technique to investigate disaccharide conformation (32), based upon the estimates of variation in the optical activity of a particular disaccharide as a function of its glycosidic conformation. A number of disaccharides, including sucrose, maltose and cellobiose, have been characterized using this method (33).

The optical rotation of polysaccharides at long wavelengths is usually dominated by the optical activity of the polymer backbone. Measurement of optical rotation at long wavelengths remains a standard and practical technique for polysaccharide systems despite the advent of ORD and CD instruments. For example, OR is used frequently for monitoring the progress of cooperative conformational transitions of polysaccharides (34).

c. Circular dichroism and optical rotatary dispersion

Monosaccharides of most food carbohydrates exist in cyclic forms, thus do not possess the unsaturated chromophores necessary to display a Cotton effect at long wavelengths. In the absence of unsaturated chromophores, two very short wavelength transitions associated with conformational transitions of carbohydrate backbone may be used (35–37). These can be observed by modern vacuum UV polarimetry. One such transition is centered near 175 nm, attributed to the $n \rightarrow \sigma^*$ transitions of the acetal oxygen atoms. The second is usually found around 150 nm and is closely related to the optical rotation at long wavelengths. CD and ORD experiments show that the variation in intensity of these two bands in polysaccharides is correlated to their composition and conformation (38). Thus, CD and ORD offer powerful tools to study structural and conformational transitions.

Some polysaccharides contain chromophores that absorb at substantially longer wavelengths than the polymeric backbone and thus give significant CD and ORD bands at wavelengths above ~185 nm. Examples are acyl and pyruvate ketal constituents and the carboxyl groups. In these cases, the CD spectra are close to those of the isolated monosaccharides, with little direct influence from the chain geometry. Since CD is very sensitive to the local environment of chromophores, conformational changes caused by, for example, specific site binding of uronate segments are usually accompanied by dramatic changes in CD spectra (39). This provides an alternative approach to study the gelation mechanisms of polysaccharides containing carboxyl groups such as alginate, pectin, xanthan, and gellan (40, 41).

4. Microscopy Techniques

a. Background

Direct imaging of polysaccharides using microscopy provides an important additional method for physical

characterization of polysaccharides. Two types of microscopy are especially of interest. First, electron microscopy (EM) is the traditional type, like light microscopy, but instead uses an electron beam to probe smaller structures than possible with light. Atomic force microscopy (AFM) senses forces such as electrostatic, magnetic, capillary, or van der Waals forces, as the molecular surface is approached by a probe. EM has considerable power to study supramolecular assemblies such as starch granules and mixed structures such as composite gels, whereas AFM has wide potential applications in investigating the structures of single molecules, as well as supramolecular assemblies and gel networks.

b. Electron microscopy

In EM, an electron beam produced from an electron gun is employed as an illuminating source instead of visible light. In transmission electron microscopy (TEM), when a fine electron beam hits the specimen, the electrons are transmitted after a series of interactions with the specimen, and then magnified to produce the image on a fluorescent screen or a photographic film. In scanning electron microscopy (SEM), the secondary electrons originating from ionization of the specimen atoms by the incident primary electrons are collected by an electron detector. The incident beam is scanned over a small area corresponding to the area of the micrograph. EM gives a better resolution than light microscopy because the wavelength of an electron beam is shorter than that of visible light.

A critical part of electron microscopy is adequate preparation of the specimen to minimize structural changes and to avoid artifacts. In most cases, the samples are exposed to a series of treatments prior to observation such as dehydration (or solidification), sectioning, and coating with electrical conducting materials. Thus, the image shapes obtained from the specimens may differ from their true shapes in the hydrated state.

Information can be obtained from EM on how macromolecules associate into supramolecular assemblies, and under favorable conditions, form gel networks (42). EM was used to monitor the conformational changes of polysaccharides that often initiate gelation such as coil-helix transitions (42). Direct visualizing of the structure of gel networks using EM has helped the understanding of structure-function relationships of polysaccharide gelation. In addition to these qualitative assessments of structural features, it is also possible to quantify properties like contour length, persistence length, linear mass density, and thickness of strands, using advanced image analysis systems (42, 43). Polysaccharides like xanthan and various β-D-glucans, all with a persistence length in the order of 100 nm, are ideally suited for such EM investigations. Since EM only provides a two-dimensional projection of the specimen, it is important to compare the parameters derived from EM with those obtained from other physical techniques, or from specimens prepared by different techniques.

c. Atomic force microscopy

AFM is still a relatively new form of microscopy and has only been applied to the study of biopolymers since the late 1990s. It generates images by sensing the changes in force between a probe and the sample surface as the sample is scanned. Using a variety of probing methods (44), a three-dimensional image with sub-nanometer resolution of the surface topography of tested samples can be produced (45). Thus, AFM affords an opportunity to directly image individual molecules and the helical structures of polysaccharides with minimal sample preparation (44, 46, 47). The polysaccharides are simply deposited from aqueous solution onto the surface of freshly cleaved mica, air dried, and then imaged directly under appropriate liquid (45).

For highly flexible polysaccharides such as dextrans, the AFM images show globular structures representing time-averaged pictures of the random coil structure. For more extended polysaccharides, such as xanthan and β-D-glucans, the AFM images may be quantified to yield persistence length, contour length, and its distributions (48). The dimensions observed by AFM are often larger than those derived from conventional techniques (44). This is believed to be due to the polymer-surface interactions which occur when the molecules are absorbed onto the mica surface prior to observation. AFM can be used to investigate the nature of association in junction zones, and also the overall structure of gel networks (49–51).

The use of EM and AFM has led to an improved understanding of the functional properties of polysaccharides at a molecular level. Furthermore, the ability to provide direct information about heterogeneity makes microscopy not simply complementary to other physical techniques, but also indispensable for obtaining additional detailed structural information.

5. Nuclear Magnetic Resonance

a. Background

Nuclear magnetic resonance (NMR) spectroscopy provides detailed structural information of carbohydrates, such as identification of monosaccharide composition, elucidation of α or β configurations, and establishment of linkage patterns and sequence of the sugar units in oligosaccharides and polysaccharides. Recent advances in two-dimensional NMR techniques allow the elucidation of some polysaccharides without chemical analysis (52). NMR can also be used to determine the conformation and chain stiffness/mobility of oligosaccharides and some polysaccharides in solution and to monitor coil-helix transitions and gel formation (53).

The principle of NMR spectroscopy is based on the magnetic property of the nucleus in atoms associated with

spins. The most useful nuclei in carbohydrate research are ^{1}H and ^{13}C, which by absorbing radio frequency energy in a strong magnetic field, jump to higher energy levels. Spins at the higher energy levels tend to relax to lower energy levels, and the transitions are dependent on the magnetic field strength in the local environment of the nucleus. Therefore, every nuclear spin in a molecule is influenced by the small magnetic fields of the nuclei of its nearest neighbors. Hence, the signal released by the nucleus reveals structural information of the nucleus in specific environment. The analysis of these individual signals relative to a standard, expressed by chemical shift and spin-coupling between nuclei, can yield detailed information on the structure and shape of molecules.

One-dimensional NMR experiments are limited to the portrayal of response intensity as a function of the observation frequency under the applied field. Two-dimensional NMR techniques utilize a second frequency domain, which greatly expands the information contained in the spectrum. The introduction of this second domain allows correlations to be established and hence connectivity information can be obtained. These are very useful in determining molecular structures, particularly of complex oligosaccharides and polysaccharides. For example, COSY (COrrelation SpectroscopY) and TOCSY (TOtal Correlation SpectroscopY) are used to establish connectivities around monosaccharide rings. Long-range correlation experiments, such as Nuclear Overhauser Effect (NOE), a through-space phenomenon, can be used in the study of shape and conformation. Long-range heteronuclear correlation experiments can establish interresidue connectivity, and the sequences of complex oligosaccharides and polysaccharides can therefore be determined.

b. NMR in molecular dynamics and conformational analysis

NMR relaxation data (T_1 and T_2^*) provide information on the dynamics of oligosaccharides and polysaccharides involving several different types of internal motion (53). NOESY provides information on inter-glycosidic spatial constraints, which helps define linkage conformations. Since they are able to provide conformational analysis of oligosaccharides in solution, NMR techniques are important means to obtain information on the three-dimensional structures free of crystal lattice constraints. NMR measurements of vicinal long-range homonuclear couplings ($^{3}J_{H,H}$) and long-range heteronuclear couplings ($^{3}J_{C,H}$) provide information on both intra- and inter-residue conformation(s) by measuring the parameters controlled by the dihedral angles between constituent monosaccharides of oligosaccharides and polysaccharides. In a recent application of NMR spectroscopy, long-range heteronuclear coupling constants were measured across the glycosidic linkages of a series of eight α- or β-linked disaccharides in solution (54). The $^{3}J_{C,H}$ values were determined by multiple ^{13}C site-selective excitation experiments using ^{1}H decoupling under pulsed field gradient-enhanced spectroscopy. The experimentally determined long-range three-bond heteronuclear coupling constants were converted to calculate values of the glycosidic dihedral angles of each disaccharide using a Karplus-type equation. Wide applications of NMR in solution dynamics, conformational analysis, and prediction of helical structure of oligosaccharides and polysaccharides can be found in the literature (55–57).

In summary, NMR spectroscopy is a very powerful tool not only for analyzing the primary structures of carbohydrates to provide information such as anomeric configuration, linkage sites, and sequences of monosaccharides, but also to determine the dynamics and shape of carbohydrates in solutions. The information can be further enhanced by combining with molecular modeling techniques. In this way, a deeper understanding of the dynamic properties and three-dimensional conformation of oligosaccharides and polysaccharides, and hence, the structure-property relationships, are obtained.

III. MOLECULAR WEIGHT AND MOLECULAR WEIGHT DISTRIBUTION

A. Polydispersity and Molecular Weight Averages

Monosaccharides and oligosaccharides have well-defined chemical structures, and specific molecular weights. However, polysaccharides contain molecules with different numbers of monosaccharide units (thus different molecular weights) and are said to be polydisperse. The distribution of molecular weight (MWD) varies, depending on the synthetic pathway and environments, as well as the extraction conditions to isolate the polysaccharides. The distribution may be described as mono-, bi-, or polymodal. Before we discuss how to quantitatively describe this polydispersity in molecular weight, we have to introduce the concept of molecular weight averages.

There are four statistically described molecular weight averages in common use, number average molecular weight (M_n), weight average molecular weight (M_w), z-average molecular weight (M_z), and viscosity average molecular weight (M_v). The mathematical descriptions of

* T_1 relaxation, or spin-lattice relaxation, is characterized by the longitudinal return of the net magnetization to its ground state of maximum length in the direction of the main magnetic field through energy loss to the surrounding lattice. T_2 relaxation, or spin-spin relaxation, is characterized by the exchange of energy of spins at different energy levels, and does not lose the energy to the surrounding lattice.

these averages in terms of the numbers of molecules N_i having molecular weight M_i are:

$$M_n = \frac{\sum_{i=1}^{\infty} M_i N_i}{\sum_{i=1}^{\infty} N_i} \quad (2.1)$$

$$M_w = \frac{\sum_{i=1}^{\infty} M_i^2 N_i}{\sum_{i=1}^{\infty} M_i N_i} \quad (2.2)$$

$$M_z = \frac{\sum_{i=1}^{\infty} M_i^3 N_i}{\sum_{i=1}^{\infty} M_i^2 N_i} \quad (2.3)$$

$$M_v = \left[\frac{\sum_{i=1}^{\infty} M_i^{1+\alpha} N_i}{\sum_{i=1}^{\infty} M_i N_i} \right]^{1/\alpha} \quad (2.4)$$

In Equation 2.4, α is the Mark Houwink exponent (Section V.B.2). Most of the thermodynamic properties are dependent on M_n and bulk properties such as viscosity are particularly affected by M_w. M_w and M_z emphasize the heavier molecules to a greater extent than does M_n. M_v is usually between M_w and M_n and closer to M_w; when $\alpha = 1$, $M_w = M_v$. For very stiff polysaccharides with $\alpha > 1$, M_v exceeds M_w.

A convenient measure of the range of molecular weights present in a distribution is the ratio M_w/M_n, called the polydispersity index (PI). In a random MWD produced by condensation syntheses, as with polysaccharides, PI is typically around 1.5~2.

B. Physical Methods for Molecular Weight Determination

Absolute techniques for MW determination include membrane osmometry, static light scattering and equilibrium sedimentation. These techniques require no assumptions about molecular conformation and do not require calibration employing standards of known MW. Relative techniques include gel permeation chromatography (GPC), dynamic light scattering, velocity sedimentation and viscometry, and require either knowledge/assumptions concerning macromolecular conformation or calibration using standards of known MW. Combined techniques use information from two or more methods, such as velocity sedimentation combined with dynamic light scattering, velocity sedimentation combined with intrinsic viscosity measurements, and GPC combined with on-line (or off-line) static light scattering or equilibrium sedimentation.

1. Osmometry

Polymer solutions exert osmotic pressure across a porous boundary because the chemical potentials of a pure solvent and the solvent in a polymer solution are unequal. There is a thermodynamic drive toward dilution of the polymer-containing solution with a net flow of solvent through a separating membrane, toward the side containing the polymer. When sufficient pressure is built up on the solution side of the membrane, equilibrium is restored. The osmotic pressure π depends on M_n and polymer concentration c as follows (58):

$$\pi = RT\left(\frac{c}{M_n} + A_2c^2 + A_3c^3 + \cdots\right) \quad (2.5)$$

where R is the molar universal gas constant, T is the absolute temperature, and A_2 and A_3 are the second and the third virial coefficients, respectively. In very dilute solutions, it is usually sufficient to consider only the first two terms in the equation, which can then be rearranged as:

$$\frac{\pi}{c} = \frac{RT}{M_n} + RTA_2c \quad (2.6)$$

where π/c is called the reduced osmotic pressure. According to the above equation, M_n may be determined by a plot of π/c versus c extrapolated to zero concentration. The intercept gives RT/M_n, and the slope of the plot yields A_2.

For neutral polysaccharides, osmotic pressure measurements can be made in water. However, for charged polysaccharides, salt solutions should be used to suppress the charge effects on apparent molecular weights. Usually 0.1–1 M NaCl or LiI is of sufficient ionic strength. Since osmotic pressure is dependent on the number of molecules present in solution, it is less sensitive to high MW polysaccharides. In practice, this method is only useful for polysaccharides having MW less than 500,000 g/mol (59).

2. Static Light Scattering

Static light scattering is widely used for determining the MW of macromolecules and measures M_w. For a highly dilute solution, the normalized intensity of scattered light R(q) as a function of scattering wave vector (q) and concentration (c) is given as (60):

$$\frac{Kc}{R(q)} = \frac{1}{M_wP(q)} + 2A_2c \quad (2.7)$$

where K is a contrast constant and P(q) is the particle scattering factor. For a random coil, P(q) is expressed by:

$$P(q) = 1 - \frac{q^2R_g^2}{3} + \ldots \quad (2.8)$$

$$\text{and } q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right) \quad (2.9)$$

where λ is the wavelength, θ is the scattering angle, and R_g is the radius of gyration. Equations 2.7–2.9 form the

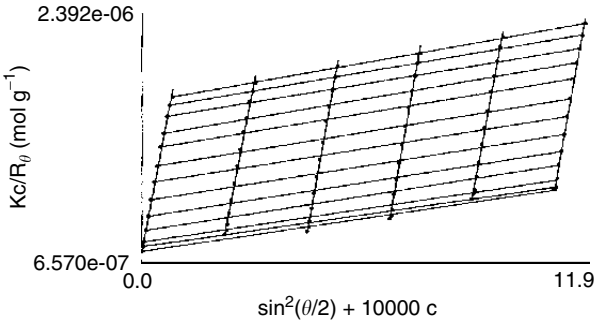

FIGURE 2.3 Zimm plot of tricarbanilate of β-D-(1→3) (1→4)-glucan measured in dioxan.

basic theory for MW determination using static light scattering. In practice, this is done by measuring the angular dependence of scattered light from a series of dilute solutions. The scattering data are then processed in the form of a Zimm plot or other associated plots (Berry and Gunniur plots). In a typical Zimm plot, Kc/R(q, c) is plotted against q^2 + kc, where k is an arbitrary constant to separate the angle-dependent curves from different concentrations. The double extrapolation to c = 0 and q = 0 (i.e., θ=0) results in two limiting curves intersecting the ordinate at the same point. This point gives $1/M_w$. The initial slope of the curve at θ = 0 is $2A_2$, and from the initial slope of the curve at c = 0, R_g is obtained. Figure 2.3 is a Zimm plot of (1→3) (1→4)-β-D-glucan tricarbanilate measured in dioxan by static light scattering.

The measurement of the MW of polysaccharides by light scattering has not been an easy task when compared to many other macromolecules. The major difficulty is the preparation of optically clear solutions that are free of dust and molecular aggregates. A detailed procedure for the preparation and clarification of polymer solutions is given by Tabor (61) and Harding *et al*. (59). The measurement of MW is especially complicated by the existence of aggregates. Extreme caution has to be taken in interpreting the data. Poor reproducibility is often an indication of the presence of aggregates. Extensive efforts have been made to eliminate aggregates by the selection of appropriate solvents (9, 62, 63) or by chemically transforming the polysaccharides to reduce H-bonding, using derivatives such as carbanilates (64).

3. Sedimentation

Sedimentation methods are of two types, sedimentation equilibrium and sedimentation velocity. The equilibrium technique employs a centrifugal field to create concentration gradients in a polymer solution contained in a special centrifuge cell. For a solute under appropriate conditions (sedimentation equilibrium), sedimentation and diffusion become comparable so that there is no net transport of the solute. Analysis of the distribution of the solute concentration along the centrifugal field at such an equilibrium provides a means to study the MWD and the average MW. For polysaccharides, such an equilibrium distribution is generally achieved in 24–48 hours depending on the nature of the solute and experimental conditions (59).

The basic equation describing the distribution of solute concentration J(r) at sedimentation equilibrium is given for an ideal system as (65):

$$\frac{d\ln J(r)}{d(r^2)} = \frac{M_w(r)(1 - \upsilon\rho)\omega^2}{2RT} \tag{2.10}$$

where r is the distance of a given point in the cell from the center of the rotor, ω is the rotor speed (rad/s), υ is the partial specific volume (ml/g), and ρ is the solution density. The solute concentration profile is recorded, usually by a Rayleigh interference optical system, and transformed into plots of log J(r) versus r^2, from which the (point) weight average molecular weight can be obtained. The whole-cell M_w can then be calculated as

$$M_w = \frac{J(b) - J(a)}{J_0(b^2 - a^2)} \frac{2RT}{\omega^2(1 - \upsilon\rho)} \tag{2.11}$$

where a and b are the distance from the center of the rotor to the cell meniscus and cell bottom, respectively, and J_0 is the initial loading concentration.

Sedimentation equilibrium can cover a very wide range of molecular weights compared to light scattering and osmotic pressure methods. However, since the procedure is inherently time consuming and the thermodynamic non-ideality of polysaccharides can complicate interpretation of the measurements, the technique is not frequently applied in polysaccharide research.

As with equilibrium sedimentation, velocity sedimentation is based on the principle that the sedimentation rate of a polymer under a centrifugal field is directly proportional to its MW and shape. Velocity sedimentation monitors the boundary movement during ultracentrifugation by an optical method, from which the sedimentation coefficient, and hence MW, can be estimated provided the conformation of the molecule is known. By the use of high angular velocities, initial sedimentation may occur before diffusion effects become important. Compared to equilibrium sedimentation, velocity sedimentation is less time consuming, but can only provide qualitative information on average MW and MWD.

4. Viscometry

Because of the simple experimental setup and ease of operation, viscometry is extensively used to determine the MW of polysaccharides. The method simply requires the measurement of the relative viscosity η_r and polymer concentration of dilute solutions. Experimentally, η_r can be measured either by a capillary viscometer, a rotational viscometer, or

a differential viscometer (66). The MW of the polysaccharides is then calculated via the Mark-Houwink relationship (Equation 2.18). The Mark-Houwink constants K and α are usually determined experimentally using a series of ideally monodisperse substances with known molecular weights. More discussion of this method will follow (Section V.B).

Caution is needed when applying this relative method to polysaccharides with chemical heterogeneity. Any factors that may change chain extension lead to changes in K and α values; examples are degree of branching (as with amylopectin and dextrans) and the distribution and/or substitution of certain monosaccharide units (as with alginates and galactomannans). The chemical composition and structure of the material under test should resemble those of the calibration substances.

5. Gel Permeation Chromatography

Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) is widely used for the determination of MW and MWD of polysaccharides. In GPC, the polymer chains are separated according to differences in hydrodynamic volume by the column packing material. Separation is achieved by partitioning the polymer chains between the mobile phase flowing through the column and the static liquid phase that is present in the interior of the packing material, which is constructed to allow access of smaller molecules and exclude larger ones. Thus, larger molecules are eluted before smaller ones.

Conversion of the retention (or elution) volume of a polymer solute on a given column to MW can be accomplished in a number of ways. Narrow MWD standards with known MW, such as pullulan and dextran, may be used to calibrate the column. As with viscometry, the difference in structure between the calibration standards and the tested sample may lead to over- or underestimating the MW. To overcome this, a universal calibration approach may be applied in which the product of intrinsic viscosity [η] and MW, being proportional to hydrodynamic volume, is used (67). For different polysaccharides, a plot of log [η] MW versus elution volume emerges to a common line, the so-called "universal calibration curve." The calibration is usually obtained using narrow MWD standards from which the MW of a test sample can be read, provided the intrinsic viscosity is known.

In the last two decades or so, methods for the determination of MWD have been facilitated by combining GPC with a laser light scattering detector (68, 69). These methods provide absolute measurement of average MW and information on MWD and molecular conformations.

6. Other Methods

There are a number of other less frequently used methods for MW determination of carbohydrates, such as mass spectrometry, end group analysis, and NMR. The readers are referred to the review by Harding (59) for a detailed discussion of alternative methods on MW determination of carbohydrates. In addition, recent development in AFM has shown that it is a potential means for MW determination of polysaccharides. The power of this approach is that it permits MW measurements of single polysaccharide molecules rather than mixtures of single molecules and aggregates. All the other methods described above determine the apparent MW of samples that often include molecular aggregates. Round *et al.* (46) found that M_n and M_w obtained from AFM is 2–3 times smaller than that for similar samples measured by conventional techniques.

IV. HYDRATION AND SOLUBILITY OF CARBOHYDRATES

A. Low-Molecular-Weight Carbohydrates

Carbohydrates contain both polar -OH groups and non-polar -CH groups. In an aqueous system, the numerous hydroxyl groups of carbohydrates may hydrogen bond strongly with water molecules. Also, the ring oxygen atom and the glycosidic bridging oxygen atom can form hydrogen bonds with water. Franks and coworkers discussed the thermodynamic data of small carbohydrates in the context of NMR and dielectric relaxation data (70). They found no solute-solute interactions in aqueous solutions even at fairly high concentrations. Both the sites of hydration and their relative conformations are important factors in the resultant hydration properties. Molecular dynamics studies have revealed that hydroxyl groups make on average between two and three hydrogen bonds with solvent (71, 72). Because of the proximity of adjacent hydroxyl groups, many water molecules were found to simultaneously hydrogen bond to two hydroxyl groups (71). The geometric requirements of these solute-solvent hydrogen bonds favor one conformation over another, leading to some solutes experiencing less favorable interactions with water, and hence being less soluble (73, 74). Nevertheless, low-molecular-weight carbohydrates, with degrees of polymerization less than 15~20, are generally very soluble in water and other polar solvents (75). The solubility decreases with increasing degree of polymerization because of increased solute-solute interactions.

Addition of polar organic solvents to solutions of carbohydrates results in the precipitation of an amorphous or crystalline form of the carbohydrates. Increasing the concentration of alcohol decreases the solubility of mono- and oligosaccharides, and they are only slightly soluble when the alcohol concentration is higher than 80% (76).

B. Polysaccharides

Polysaccharides display a wide range of solubilities conventionally described as easily soluble, intermediately

soluble, and insoluble. There is no clear boundary between the three groups but the general consensus is: easily soluble polysaccharides are readily dissolved in cold water; intermediately soluble ones are only soluble in hot water; and insoluble ones cannot be dissolved even in boiling water. Structure and molecular weight are the two primary factors that determine solubility. Polysaccharides with a highly regular conformation that can form crystalline or partial crystalline structures (Section II.C.1) are usually insoluble in water. Linear polysaccharides with high regularity in structure, such as 1→4 or 1→3 linked β-D-glucans, and 1→4 linked β-D-mannans, are examples of this group. Although (1→4)-β-D-mannan can be dissolved in 5% alkaline solution, neutralization leads to reassociation and precipitation. Cellulose is insoluble, but swells in strong alkaline solutions such as 18% sodium hydroxide (77). Only cellodextrins with DP of about 15–80 can be dissolved or dispersed in such alkaline solutions; for DP less than 15, there is solubility in neutral aqueous solutions (75). Amylose, an α-(1→4)-homoglycan, is insoluble in cold water but can be dissolved in hot water.

A decrease in uniformity/regularity of molecular structure is always accompanied by an increase in solubility. The irregularity of the molecular chains prevents the formation of a closely packed structure, allowing many polysaccharides to readily hydrate and dissolve when water is available. The mixed linkage (1→3) (1→4)-β-D-glucans from cereals differ from cellulose only by the introduction of occasional single (1→3) linkages. The insertion of these linkages introduces "kink" points into the otherwise stiff cellulosic backbone, rendering the polymer soluble in water. Branching or substitution of the polysaccharide chain also reduces the possibility of intermolecular association and usually increases solubility. Examples are easily seen by comparing the solubility of galactomannans with that of (1→4)-β-D-mannan. By introducing single α-D-galactopyranosyl constituents (1→6) linked to the mannan backbone, the resulting galactomannans are fairly soluble in water. Any structures which contain especially flexible units such as (1→6) linkages will lead to higher solubility because of a larger favorable entropy of solution. Highly branched polysaccharides are almost always very soluble in water as in the case of amylopectin which has a much better solubility compared to its linear counterpart, amylose.

C. Dissolution Kinetics

The ability of a substance to be solvated is governed by the fundamental thermodynamic equation:

$$\Delta G = \Delta H - T\Delta S \tag{2.12}$$

where ΔG, ΔH, and ΔS are the changes of Gibbs free energy, enthalpy, and entropy of mixing, respectively. T is the absolute temperature of the system. A homogeneous solution is obtained when the Gibbs free energy is negative. For an ideal system, ΔH is usually small, so dissolution is an entropically driven process.

For low-molecular-weight carbohydrates, dissolution of the molecules is promoted by a large increase in entropy on mixing. The dissolution rate is mainly controlled by the diffusion or convective transport of solute from the interfacial boundaries to the bulk solution, which in turn is determined by the difference between the solute concentration and the saturated concentration at a given temperature. The dissolution process is generally fast as long as the solution is not close to the saturation point. Increase in the hydrodynamic field, such as stirring, promotes dissolution.

For polysaccharides, the contribution of entropy changes during dissolution is limited because of conformational constraints of the polymer chains. Most linear polysaccharides only form colloidal dispersions in aqueous systems that are not in thermodynamic equilibrium. In the initial stage of dissolution, amorphous polymer starts to swell as a result of water diffusing into the particle with a simultaneous transition from a glassy state to a rubbery gel-like state. Consequently, a gel layer forms on the surface of the polymer particle. The dissolution rate may be determined by a number of factors, either individually or combined together, including the rate of water penetration into the polymer, the rate of disentanglement of the polymer from the gel layer, and the diffusion or convective transport of solute from the interfacial boundaries to the bulk solution. In the case of high MW polysaccharides, the disentanglement of molecules is often the limiting step of dissolution. Thus the dissolution rate is expected to decrease with increasing MW because disentanglement of large molecules from the gel layer takes a longer time. The dissolution rate of guar gum was shown to be inversely related to the MW of the galactomannan (78). Diffusion or transport of solutes may also be the controlling factor in combination with disentanglements, such as in the case of a low MW polymer in low hydrodynamic environment (low temperature, low agitation), or when the viscosity of the solvent phase has built up significantly (78). The initial solvent content may also affect the dissolution of certain polysaccharides, but in various ways. Theoretical work and experiments suggest that dissolution rate increases with the level of residual solvent in the solid polymer (79, 80). However, if the presence of low levels of solvent leads to an increase in structure ordering, the suggested enhanced dissolution may not occur. For example, it has been observed that purified (1→3) (1→4)-β-D-glucan is very difficult to dissolve in water when it is precipitated from an aqueous solution and air dried. Solvent exchange using isopropanol before drying greatly improves solubility and dissolution. This is presumably due to the presence of water in the polymer, resulting in increased ordering of

the polymer and poorer solubility. Other factors such as particle size and porosity of the polymer may also influence dissolution rate.

V. RHEOLOGICAL PROPERTIES OF POLYSACCHARIDES

A. Concentration Regime

Rheology is the study of flow and deformation of materials, and for any given polysaccharide, concentration is of course of primary importance. A dilute polymer solution is one in which each polymer coil and the solvent associated with it occupies a discrete hydrodynamic domain within the solution. The isolated macromolecules provide their individual contribution to the rheological properties of the system almost independently of the imposed shear rate. As the concentration of polymer increases, a stage is reached at which the individual molecular domains begin to touch one another frequently. The corresponding concentration is called the overlap concentration c*. At polymer concentration c>c*, the solution is called semi-dilute and when c>>c* the solution is concentrated.

B. Dilute Solutions

1. Steady Shear Viscosity

The ratio of applied shearing stress (τ) to rate of shear ($\dot{\gamma}$) for an ideal viscous fluid is called the coefficient of viscosity, or simply viscosity (η), which is a measure of the resistance to flow. The term "fluidity," which is the reciprocal of viscosity, is sometimes used in the food industry. The viscosity increase due to the contribution of dissolved or dispersed solutes over the solvent is described by the relative viscosity (η_r) and specific viscosity (η_{sp}):

$$\eta_r = \frac{\eta}{\eta_s} \tag{2.13}$$

$$\eta_{sp} = \frac{\eta - \eta_s}{\eta_s} = \eta_r - 1 \tag{2.14}$$

where η_s is the solvent viscosity and η the overall solution viscosity. For most polysaccharides, especially of the random coil type, dilute solutions under shear flow show essentially Newtonian behavior. That means the viscosity of the solution is a constant independent of share rate. However, non-Newtonian flow behavior is observed for dilute solutions of some rigid polysaccharides, such as xanthan and some other β-glucans (81). For these systems, the apparent viscosity falls as the shear rate increases — a phenomenon called shear thinning. The shear thinning behavior of such polysaccharide solutions is a result of progressive orientation of the stiff molecules in increasing field of shear.

2. Intrinsic Viscosity

In dilute solutions, viscosity usually increases with concentration according to the Huggins and the Kramer equations:

$$\eta_{sp} = [\eta]c + K'[\eta]^2c^2 \tag{2.15}$$

$$\ln(\eta_r) = [\eta]c + (K' - 0.5)[\eta]^2c^2 \tag{2.16}$$

where K′ is the Huggins coefficient. [η] is known as intrinsic viscosity and is the limit of reduced viscosity (η_{sp}/c) as c→0:

$$[\eta] = \lim_{c\to 0}(\eta_{sp}/c) \tag{2.17}$$

Experimentally, [η] is usually determined from the measurement of η_r or η_{sp} over a series of dilute solutions. By plotting η_{sp}/c or ln (η_r)/c versus c, [η] is obtained as the average of the two intercepts at the ordinate via graphic extrapolations of c→0.

Intrinsic viscosity is not actually a viscosity but is a characteristic property of an isolated polymeric molecule in a given solvent, and is a measure of its hydrodynamic volume. It has a unit of volume per unit weight. Mark (82) and Houwink (83) independently correlated the intrinsic viscosity with the viscosity average molecular weight M_v

$$[\eta] = KM_v^{\alpha} \tag{2.18}$$

where both K and α are constants for a given polysaccharide-solvent pair at a given temperature. The exponent α is a conformation-sensitive parameter and usually lies in the range of 0.5–0.8 for random coil polymers, and increases with increasing chain stiffness. It can be as high as 1.8 for polysaccharides with a stiff rod conformation. Low values of α (<0.5) tend to indicate significant branching or a compact structure. Values of K and α for some food polysaccharides have been documented by Harding (66).

C. Semi-Dilute and Concentrated Solutions

1. Steady Shear Viscosity

Polysaccharides of sufficiently high molecular weights and concentrations can form an entangled network in solution which impedes flow. Such a system usually shows strong shear thinning behavior. Unlike dilute solutions, the shear thinning behavior of a semi-dilute or concentrated solution is associated with the continuous interchange between entanglement and disentanglement. At high shear rates, the newly formed entanglements cannot compensate for those being disentangled, which leads to a decrease in viscosity. A typical viscosity-shear rate

flow curve consists of a low shear rate Newtonian plateau, followed by a shear thinning region where viscosity decreases as shear rate increases (Figure 2.4). In theory, there exists an upper Newtonian plateau at very high shear rates, but this is not accessible for most polysaccharide solutions using current instrumentation. As shown in Figure 2.4, the increase in viscosity with concentration is more pronounced at low shear rates. Therefore, for meaningful comparison of different systems, zero-shear rate viscosity (η_0) should be used. η_0 is obtained by measuring viscosity at a range of low shear rates and extrapolating to zero concentration, as illustrated in Figure 2.4 (84).

Shear thickening of polysaccharide solution, although rare, is observed occasionally. This phenomenon usually results from a shear-induced formation of ordered structures (10).

2. Concentration and Molecular Weight Effects

As with dilute solutions, the viscosity of semi-dilute or concentrated polysaccharide solutions also increases with increasing concentration. The relationship normally can be described by the power-law type correlation:

$$\eta_{sp} = ac^n \tag{2.19}$$

where η_{sp} is zero-shear specific viscosity. At a concentration above the overlap concentration c*, a more pronounced increase in both the zero-shear viscosity and the shear rate dependence of viscosity develops. For most random coil systems, the exponent n lies between 1~1.5 when c < c*, and 3.5~5 at c > c* (85).

Precisely, the viscosity generated by disordered polymer coils is dependent on the degree of space-occupancy by the polymer, which is determined by both concentration and molecular weight. In general, for linear polysaccharides in a given solvent, solution viscosity increases proportionally to their molecular weight and concentration. The space occupancy is characterized by the dimensionless product of concentration and intrinsic viscosity c[η], since [η] is a measure of volume occupancy of the isolated coil in the solvent. Morris *et al.* (86) found that the double-logarithmic plots of η_{sp} vs. c[η] for a number of different disordered polysaccharides and the same polysaccharides with different molecular weights are virtually identical.

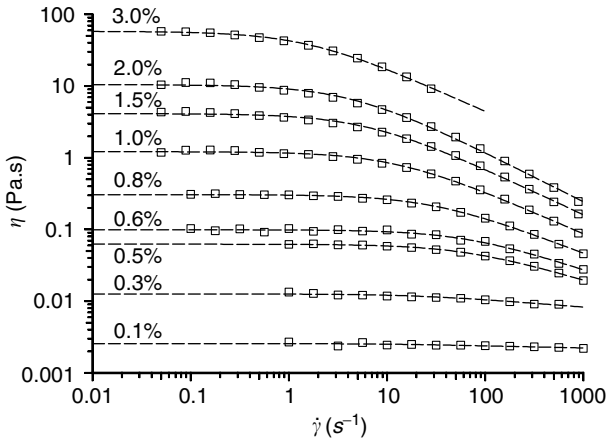

FIGURE 2.4 Shear rate ($\dot{\gamma}$) dependence of viscosity (η) for xyloglucan from *Detarium senegalense* Gmelin in aqueous solutions at different concentrations. From Wang *et al.*, 1997 (84).

3. Temperature and Ionic Strength Effects

Another important factor that may affect the viscosity values and the profile of flow curves is temperature. In the absence of temperature-induced conformational changes, increasing the temperature leads to a monotonical decrease of solution viscosity and an increase of the shear rate of onset of the non-Newtonian region. For polysaccharides with charged groups, the shear viscosity is also sensitive to ionic strength and pH. Due to an expanded chain conformation caused by like-charge repulsions, a higher viscosity is normally obtained at lower ionic strength. Electrostatic repulsions are suppressed at higher ionic strength, leading to a less extended chain conformation and hence lower viscosity.

4. Dynamic Properties

Polysaccharide solutions are viscoelastic substances, i.e., have both solid and liquid characteristics. An important experimental approach to the study of the viscoelasticity of a polymer solution is to use a dynamic oscillatory measurement. A sample is subjected to a small sinusoidal oscillating strain (γ); this generates two stress components in viscoelastic materials, an elastic component which is in phase with the applied strain and a viscous component which is 90° out of phase with the strain:

$$\sigma_0 = G'\gamma_0 \sin\omega t + G''\gamma_0 \cos\omega t \tag{2.20}$$

$$\tan\delta = \frac{G''}{G'} \tag{2.21}$$

where G′ is the elastic or storage modulus, G″ is the viscous or loss modulus, tanδ is the loss tangent, and ω is the frequency of oscillation. The loss tangent is the ratio of the energy dissipated to that stored per cycle of deformation. The frequency dependency of these viscoelastic quantities allows specific features of different classes of polysaccharides to be distinguished. Based on the relative magnitudes of G′ and G″ in a frequency sweep experiment within the linear viscoelastic strain range, three types of polysaccharide systems may be distinguished: solutions, weak gels, and gels (85). For dilute solutions of polysaccharides, G″ values are higher than G′, with $G'' \propto \omega$ and $G' \propto \omega^2$ at low frequency. When the frequency or concentration is increased, there is a crossover between G′ and G″, implying that the system passes from being a more and

more viscous liquid to being a viscoelastic solid. Also, both G′ and G″ become less frequency dependent as the frequency is increased; a "rubbery" plateau of G′ is seen at high frequencies. Gels have a very different spectrum, with G′ remaining almost constant and G″ only increasing slightly as frequency increases; and G′ values are higher than G″ at all frequencies, with tanδ around 10^{-1} for a weak gel and 10^{-2} for a true gel.

D. Polysaccharide Gels

1. Gelation Mechanism

Under certain conditions, the association of hydrated polysaccharides results in a three-dimensional polymeric network (a gel) that fills the liquid available rather than precipitation of the polysaccharide. In these resultant gels, polysaccharide molecules or portions of these are aggregated in the junction zones through interactions such as hydrogen bonding, hydrophobic association, and cation-mediated cross-linking. To induce gelation, polysaccharides usually have to be first dissolved or dispersed in a solution, in order to disrupt mostly the hydrogen bonds from the solid state. The subsequent transformation of sols to gels is achieved by treatments such as heating and cooling, addition of cations, and change of pH.

The adoption of an ordered secondary and tertiary structure such as a helix or flat ribbon is a primary mechanism for the gelation of polysaccharides. The familiar gelation of algal polysaccharides agarose and κ-carrageenan, and bacterial polysaccharide gellan, involves the formation of helices (87). These helices may further associate to form a quaternary structure (gel network) through intermolecular hydrogen bonding or incorporation of counterions in the case of some charged polymers. The gelation of some other polysaccharides is through the formation of pleated sheets, sometimes described as an egg-box structure. Familiar examples of this are gels of low-methoxyl pectin and alginate. In this structure, the polysaccharides associate into matched aggregates in a twofold ribbon-like conformation, with the metal ions cooperatively bound during the process, sitting inside the electronegative cavities like eggs in an egg box.

2. Physical Properties of Polysaccharide Gels

Polysaccharides are able to form a vast range of gel structures which can be controlled by the properties of polysaccharides themselves and by the gelling conditions. A list of gelling food polysaccharides and a comparison of their relative textural characteristics are given by Williams and Phillips (88). Some polysaccharides form thermo-reversible gels and examples exist where gelation occurs on either the cooling or heating cycle. Thermal hysteresis may exist in some of the thermo-reversible gels; the melting temperature of the gel is significantly higher than the setting temperature. Thus, gelation occurs when hot agarose solutions are cooled to below 40°C, but this gel does not melt until the temperature is raised to above ~90°C. Some polysaccharides form thermally irreversible gels, which are usually formed by cross-linking polysaccharide chains with divalent cations.

Gel formation occurs above a critical minimum concentration for each polysaccharide, and gel strength normally increases with increasing concentration. Molecular weight is also important. Intermolecular associations of polysaccharides are stable only above a minimum critical chain length necessary for the cooperative nature of the interaction, typically in the range of 15–20 residues (75). Gel strength normally increases significantly as MW increases up to a certain point, then becomes MW independent at higher values.

The gelation of anionic polysaccharides is also dependent on the type and concentration of associated cations because the association of the charged tertiary structures may be promoted by specific counterions whose radii and charges are suitable for incorporation into the structure of the junction zones.

Mixed gels from two or three polysaccharides may impart novel and improved rheological characteristics to food products. Synergy is observed for a number of binary systems including pectin-alginate, xanthan-galactomannan or glucomannan, and agarose or carrageenan–galactomannan or glucomannan. In these mixtures, synergism confers either enhanced gelling properties at a given polysaccharide concentration, or gelation under conditions in which the individual components will not gel. Although the gelation mechanisms for mixed polysaccharides are still controversial, there is evidence that some form of binding and structure compatibility has to be present between the two polysaccharides (87).

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3 Carbohydrates: Starch

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CONTENTS

Starch is the major source of calories and dietary energy in most human food systems. As the primary human metabolic substrate, starch is preferentially digested, absorbed and metabolized. Most diets worldwide have a substantial starchy component as a main or side item. For instance, potatoes are a major item in most northern European diets, rice is popular in Asian diets, maize-based foods are common in Latin America, and starchy root and tuber crops constitute a significant part of the diet in most tropical areas.

Starch occurs naturally in plants and is the storage polysaccharide of plants. It is heterogeneous, consisting of two glucose polymers: amylose and amylopectin. It is a polymer of glucose and a complex carbohydrate, which finds multiple applications in various industries such as pharmaceuticals, textiles, paper, and the food industry.

Starch performs various functions in food systems. It is used as a carrier in various products, as a texture modifier, as a thickener, and as a raw material for the production

of other valuable food ingredients and products. Physiologically, it is a source of energy. In addition, starches that are resistant to amylase digestion have properties similar to soluble fiber.

I. STARCH COMPOSITION AND STRUCTURE

Starch is composed of two basic molecular components: amylose and amylopectin. These are identical in their constituent basic units (glucose), but differ in their structural organization (linkages). These variations in the linkages in turn affect their functionality in food applications. Amylose is a straight chain molecule, while amylopectin is a branched molecule. In addition, each is hydrolyzed, digested, and absorbed differently. Amylose is hydrolyzed mainly by amylases, while amylopectin requires debranching enzymes such as pullulanase for complete hydrolysis. As a result of their structure, the nature and products of hydrolysis of amylose and amylopectin differ. The proportions of amylose and amylopectin in foods therefore influence the extent of digestibility of the starch.

The ratios of amylose to amylopectin vary among starch sources and play a considerable role in determining reactions and physicochemical properties of starches in processing and food applications (1–6). Most tuber starches contain high levels of amylopectin, imparting a "waxy" texture.

Amylose and amylopectin are the polymers which constitute the starch granule (Table 3.1).

A. AMYLOSE

Amylose is composed of D-glucose molecules, which are linked in an α-1→4 conformation. The glucose monomers therefore form a linear straight chain polymer.

Amylose is less predominant (about 20%) and typically constitutes about 20–40% in proportion (7).

Amylose contains α-1→4 glycosidic bonds and is slightly soluble in water. Amylose molecules are arranged in a helical conformation. This facilitates formation of complexes with iodine, lipids, and other polar substances (2,8,9). The iodide ions are sequestered in the central tunnel of the helix. Amylose forms a blue complex with iodine, which can be read at about 650 nm.

The starch iodine test is often used to determine amylose content of various starches and starch types (4,10). Amylose is more suitable for the starch-iodine test. The affinity of pure amylose for iodine is 19–20% compared to only about 1% for amylopectin (1,2,6). Amylose would adsorb 19–20.5 g of iodine per 100 g compared to only about 1.2 g for amylopectin. The starch iodine test is often considered to be a measure of apparent amylose content of the starch.

Amylose is the key component involved in water absorption, swelling, and gelation of starch in food processing. High amylose starches are therefore most commonly applied in food products that require quick-setting gels such as candies and confectionery. Amylose is more susceptible to gelatinization and retrogradation, and hence is most commonly involved in resistant starch formation.

TABLE 3.1
Properties of Amylose and Amylopectin (1–6,9)

Property	Amylose	Amylopectin
Structure	Linear (branched chains isolated from some starches)	Branched chains (long segments of linear chains in some starches)
Molecular weight	Up to 1 000 000	Up to 5 000 000
Glycosidic linkage	α-1→4	α-1→4 and α-1→6
Iodine complex	Blue	Purple
Iodine affinity	19–20.5%	0–1.2%
Blue value	1.2–1.6	0–0.05
Polar agents	Complexes with polar agents	Does not complex with polar agents
X-ray diffraction pattern	Crystalline	Amorphous
Phosphorus	Phosphorus-free	0.06–0.9% phosphorus (mostly in root/tuber starches)
Association with lipids	High	Low
α-Amylase hydrolysis products	Glucose, maltose, maltotriose, mainly oligosaccharides	Small amounts of reducing sugars, mainly oligosaccharides
Hydrolysis to maltose	100%	55–60 (100% with limit dextrinase and β-amylase)
Pullulanase	No effect	Debranches α-1→6 linkages
Gel stability	Firm, translucent, quick-setting gels	Clear viscous gels
Susceptibility to retrogradation	Retrogrades readily	Mostly stable
Common sources	Typically higher in cereal starches (e.g., corn starch)	Typically high in tubers and root starches (e.g., tapioca starch)

B. Amylopectin

Amylopectin consists of D-glucose units which are linked in an α-1→4 conformation as is the case with amylose, as well as D-glucose units in an α-1→6 conformation. Amylopectin is therefore highly branched as the α-1→4 linear chains are punctuated with the α-1→6 linkages. The α-1→6 constitute about 5% of the structure of amylopectin and gives rise to the branching (11). The amylopectin molecule therefore is much larger than the amylose molecule. The larger molecular size of amylopectin from amylose facilitates separation of these two polymers by size exclusion chromatography (2,7). Negligible amounts of unbranched amylopectin (A chains) in some starches have also been reported. In addition, there are long unbranched portions of the glucose polymer in some amylopectin molecules. While the straight chain of amylose is readily hydrolyzed by β-amylases, de-branching enzymes have to be used to obtain full hydrolysis of amylopectin (2,3,8).

Amylopectin has short branched chains and branch linkages, and thus cannot form the helical complex with iodine. The branched dextrin of amylopectin, however, gives a purple color with the iodine complex, identifiable at about 550 nm (3,9).

The enzymes required for amylopectin hydrolysis vary from those required for hydrolysis of amylose. Pullulanase, an enzyme which is specific for the α-1→6 glycosidic linkage, and other debranching enzymes are needed to hydrolyze amylopectin.

The properties of amylopectin in food applications differ considerably from those of amylose. Amylopectin gels are more flexible and resistant. Amylopectin also is much more resistant to retrogradation than amylose. High amylopectin starches (waxy starches) are therefore commonly used in noodle processing and in some baked products to extend shelf-life. They also are used to improve freeze-thaw stability due to their resistance to retrogradation.

C. The Starch Granule

The basic components of starch, amylose and amylopectin, are located in granules. The size, shape, and characteristics of the granules are specific to the plant source. The growth and development of the granule originates at the center of the granule, which is known as the hilum. Under magnification and polarized light, native starch granules typically appear to have a cross-like structure, similar to a maltese cross, exhibiting birefringence. The size and shape of this cross-like shape varies among botanical starch sources. For instance, starch from pinto bean has elliptical shaped lobes, while some starches have more than four lobes (12).

The ordered arrangement of amylopectin molecules intertwines to form three-dimensional double helices between adjacent branches of the same amylopectin molecule or between adjacent clusters. The double helices are stabilized by weak van der Waals and hydrogen bonds. The various arrangements of the helices result in the presence of crystalline regions on the granule (11,13,14). The nature of these regions becomes clear by their X-ray diffraction patterns. The crystalline patterns vary under X-ray diffraction patterns. These polymorphic arrangements occur in two patterns, which are classified as A or B, and an intermediate form or mixture of A and B forms, known as C type (11,14,15). Most cereal starches have A type patterns. Root and tuber starches such as potato starch contain mostly B type patterns, while legume starches have a combination of both polymorphic A and B forms, and hence are classified as C forms (11,14,15). The crystalline nature and diffraction of starch is greatly altered by processing (16).

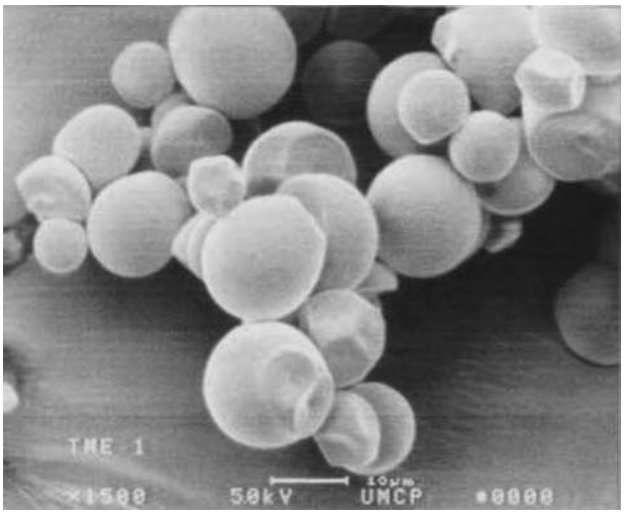

FIGURE 3.1 Tapioca starch granules.

Natural starch granules are insoluble in water, which is why starch is separated by sedimentation. This shape is disfigured and lost as starch loses its structure with modification such as heat and moisture.

Granules vary in shape and size and are characteristic of the starch sources. These shapes may be round, lenticular, or oval (11,17). Starch granule properties are used as diagnostic characteristics for identification and characterization of starches, based on structure and shape (Figure 3.1).

D. Non-Starch Components

Various non-starch components are covalently linked to amylose or amylopectin in starch. Structural and functional proteins are present which surround the starch granule. The protein friabilin, responsible for hardness of the endosperm in most cereals, is located on the granule. In addition, the enzyme responsible for starch synthesis, granule-bound starch synthase (GBSS), is located on the granule (18,19). Wheat starch characteristics are especially influenced by the presence of proteins. The proportion of protein in starches could be up to 0.5%.

Most starches contain glycolipids, complexed to amylose or amylopectin. Wheat starch, for instance, contains amylose-lipid complexes. The nature of lipids present in starches differs depending on the origin and nature of the starch. Lipids such as monoglycerides and lysophosphatidyl choline form complexes with amylose and amylopectin. Lipids may occur on the surface of the starch granule as well as in the interior of the granule. Lipids that occur within the starch granule are typically monoacyl lipids which could either be mostly free fatty acids or lysophospholipids (20). In addition, most of the lipids on the surface of the granules are monoacyl lipids. Some lipids are non-starch lipids and not associated with starch, but rather occur in the endosperm (21).

Phosphorus is a common constituent of many starches, occurring primarily as phosphate monoesters on amylose and amylopectin. Rice starch, corn starch, wheat starch, and potato starch contain various proportions of organic phosphorus or phosphate groups (22,23). Banana starches are reported to contain potassium and magnesium (24).

Non-starch components such as protein and lipids influence starch behavior in food applications. Functional properties such as water absorption, gelatinization, and starch hydrolysis are influenced by the presence of these components. The presence of lipids, for instance, affects water absorption and hence gelatinization properties. This in turn influences the formation of resistant starch and starch susceptibility to enzymatic digestion.

II. STARCH SOURCES

Starch is obtained from various plant sources. The most common sources of dietary and industrial starch are grains, such as maize and wheat, and roots such as potato and cassava (tapioca). Roots and tubers are significant sources of dietary starch (25).

A. Grain Starches

The grains primarily used as dietary and industrial starch sources include various cereal grains, mainly maize, wheat, and rice. Legumes and pulses also contribute considerably to dietary starch consumption. Corn starch, from maize (*Zea mays*), is the most commonly used source of industrial starch. Corn contains about 86% starch on a dry weight basis. As a high amylose starch, it forms heavy and easy setting gels, and therefore is commonly used for thickening. Corn starch is also used as a carrier, as an ingredient for various applications, and as raw material for other industrial products. For instance, it is hydrolyzed in various ways to obtain sweeteners and glucose. Starch from wheat (*Triticale aestivum*) and rice (*Oryza sativa*) is also a predominant ingredient in food industry applications. Other cereal grains such as sorghum (*Sorghum bicolor*) and barley (*Hordeum distichon*) are sources of starch, less commonly used than maize or wheat starch (Figure 3.2).

Grain starches tend to have high levels of amylose. Furthermore, these starches typically contain amylopectin in the crystalline regions. The amylose of these starches meanwhile may form complexes with glycolipids (26). Most cereal starch sources such as maize and wheat starches are A-type starches (15).

Various legumes contain up to 45% starch (12). Legumes commonly used as sources of starch include pinto bean, faba bean, moth bean, chickpea, and mung bean. As a result of their high amylopectin content, some legume starches such as mung bean starch have restricted swelling and increased overall stability during processing. They are therefore of high suitable quality for application in food products such as starch noodles (27).

Most legumes contain B-type starches that are generally more resistant to digestion (28,29). In addition, other legume starches such as pea starch meanwhile contain C-type starches. Legume starches have lower digestibility than other starches and hence result in a lower post-prandial glycemic and insulin response (30) (Figure 3.3).

B. Root and Tuber Starches

Among the root starches, potato (*Solanum tuberosum*) starch and tapioca (*Manihot esculenta*) or cassava starch are the most predominant industrial starch sources. Root starches have high amylopectin content and therefore have greater clarity, minimal flavor, and acceptable water absorption, and subsequently swelling capacity. Tapioca (cassava) starch is a major ingredient in dietary and industrial starch application. Also known as yucca or manioc, this root crop is the primary source of dietary energy in various tropical regions of the world. Tapioca starch has unique attributes that make it particularly desirable in food applications.

Potato is a dietary staple of most European and Scandinavian diets. Potato starch has high water-binding capacity and a bland taste, and is commonly used in the food industry in many applications for thickening and texture modification.

Other dietary and industrially important root and tuber starch sources include banana and plantain (*Musa* spp.), taro (*Colocasia esculenta*), cocoyams (*Xanthosoma* spp.), and various yams (*Dioscorea* spp.), sweet potato (*Ipomea batatas*) (31,32). Even though there are multiple sources of dietary starch in the tropics, including grains and legumes, roots and tubers constitute dietary staples in most areas as their cultivation is suited to the hot humid tropics. When freshly harvested, they are high in moisture, containing about 70–80% moisture and between 16–24% starch (32). Starchy foods are generally processed in some manner prior to utilization in food preparation. In addition, they are processed into raw material for secondary products. These therefore satisfy needs for calories, food preferences, and convenience foods (Figure 3.4).

Some of these find limited use in industrial applications such as is the case with yam starches (32). Root

starches contain amylopectin in the crystalline regions, while amylose is more common in the amorphous regions of the starch granule (26).

C. Other Sources of Starch

In addition to the major sources of dietary and industrial starch such as maize and tapioca starch, other starch-containing plants find considerable application as dietary and commercial sources of starch. These include lesser known sources of starch such as sago (*Metroxylon sagu*), arrow root (*Maranta arundinacea*), and edible canna (*Canna edulis*). Sago starch is obtained from the trunk of the plant *Metroxylon sagu*. The starch is used in various food products as it has high storage stability. Refined sago starch finds application in noodles, as well as raw material in industry for monosodium glutamate (MSG), glucose, and caramel (33). It is susceptible to enzymatic hydrolysis to glucose, which can then be fermented to produce fermentation products.

Arrowroot starch contains up to 23% amylose and is used in dietary applications as a thickener in various

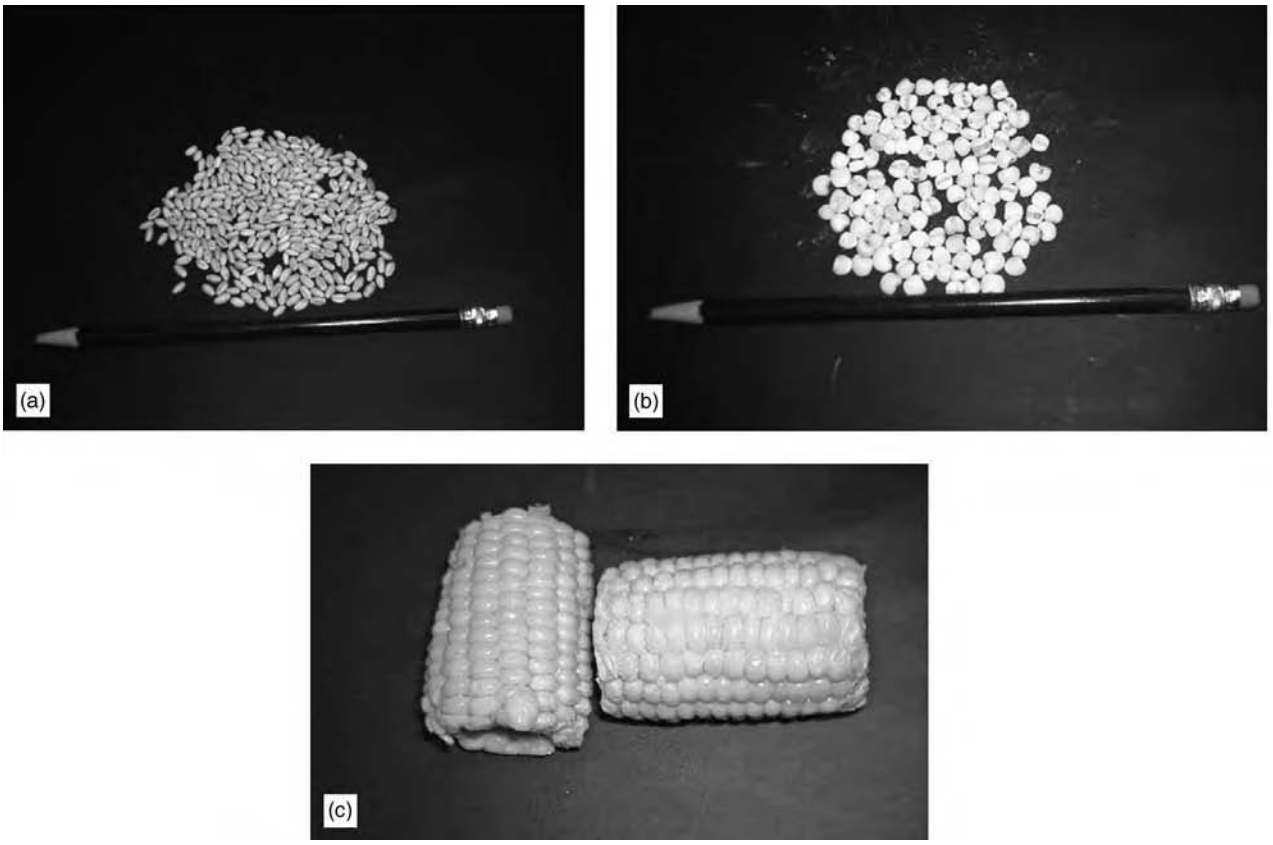

FIGURE 3.2 Some common cereal grain starch sources: (a) wheat, (b) barley, (c) maize.

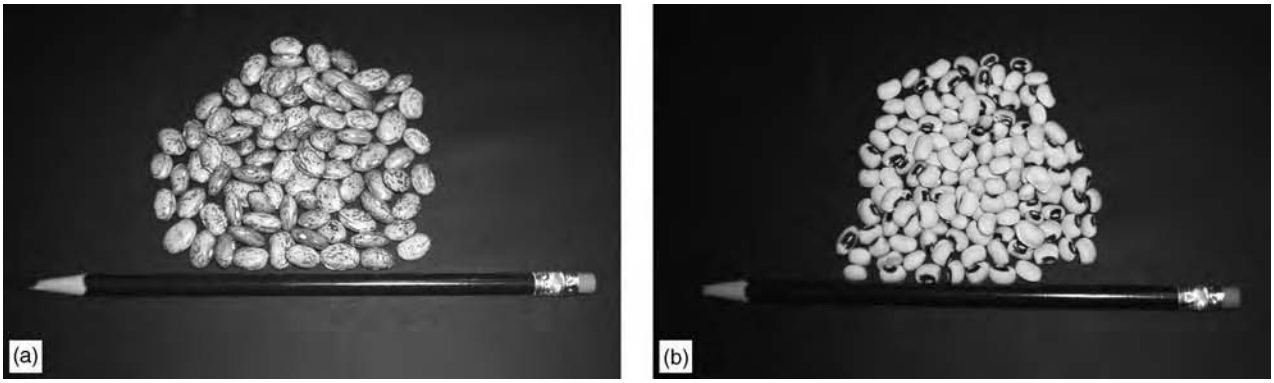

FIGURE 3.3 Some legume starch sources: (a) pinto bean, (b) black-eye pea.

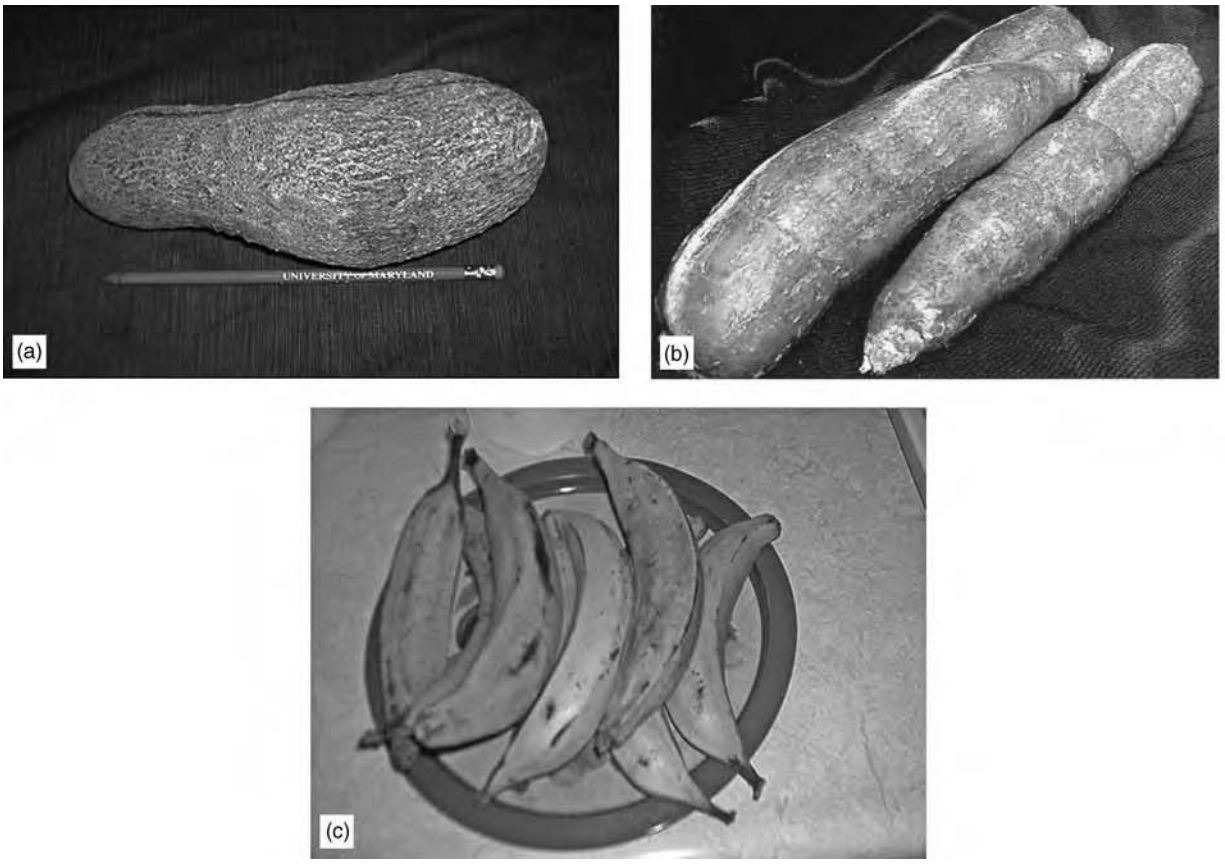

FIGURE 3.4 (a) Brazil yam, (b) cassava (tapioca) root, (c) plantain.

sauces. Another lesser known root crop commonly used in starch production for food application is edible canna, obtained from *Canna edulis* (34). The root contains up to 16% high amylose starch. The separated starch is used in production of noodles in various parts of Asia, particularly Vietnam. The properties of the canna starch are desirable for noodle production.

III. STARCH PHYSICOCHEMICAL PROPERTIES AND FUNCTIONALITY

The application of starch in food products is greatly influenced by its physicochemical properties and interactions with various components. The reaction of starch molecules in foods is essential for the multiple properties that they contribute to the quality of food products. For instance, water absorption and gel formation are extremely essential for the thickening properties of starch. In addition, hydrolysis and digestion of starch, for instance, are not feasible if starch is not gelatinized as the amylases and starch hydrolysis enzymes do not interact with intact, ungelatinized granules.

Characteristics such as gelatinization temperature, granule size, and shape are specific to the type of starch. They are diagnostic properties characteristic of native starches and can therefore be used for identification. The quality of products formulated with starch, such as carriers and thickeners, is largely affected by its functional and pasting properties (35,36).

Water-holding capacity, solubility, and paste viscosity are important parameters that influence the quality of products such as carbohydrate-based fat substitutes (37). These in turn influence gelling ability, water- and fat-binding ability, slicing ability, and hence textural quality of food products. Functionality and physicochemical properties vary among starches as they are influenced by the ratios of amylose to amylopectin. High amylopectin starches for instance are preferred for high viscosity products. In addition, the presence of phosphate esters in some starches such as potato starch may influence starch water-binding capacity by weakening the bonds between starch molecules due to ionic repulsion.

A. Starch Gelatinization

Gelatinization occurs when the ordered structure of the starch granule is disrupted and reorganized in the presence of heat and sufficient moisture. The granules are disrupted with absorption of water, losing their organized molecular structure, to facilitate swelling (29,38).

Starch gelatinization is critical in the utilization of starch in food applications. Native starch granules are insoluble in cold water and gelatinization is essential to facilitate water absorption and enhances the chemical and physical reactivity of inert starch granules in food processing (11). Granular characteristics of starches are characteristic of the plant source. The structure of the granule in turn influences the structure of the gels or pastes formed on heating.

Gelatinization results in starch swelling, and formation of a viscous paste that may be opaque or translucent depending on the nature of the starch (12). Gelatinization is followed by gelation, a process in which the swollen granules are disrupted and amylose is released into the starch-water medium. The leaching of amylose from gelatinized granules contributes to the thickening characteristics of starch and gel formation, a colloidal dispersion of starch in water. The leached amylose in the starch-water system associates to form a structural network to entrap the granules, resulting in the formation of a gel.

Viscosity of starches such as maize and tapioca starch are greatly influenced by ratios of amylose to amylopectin (10). Genetically modified high amylose starches form highly resistant and firmer gels (39). Increasing amylose content also increases early onset of gelation. Starches with low levels of amylose such as waxy maize — less than 1% amylose — do not form gels effectively. Instead, they form clear pastes that are generally resistant to syneresis (11).

The strength of the starch gel is influenced by the presence of ionic components which may interact with the negatively charged starch molecules. Water absorption and swelling of starch is limited by the presence of amylose-lipid complexes (20).

B. Starch Retrogradation

Cooling of gelatinized starch results in the re-association of the leached amylose from gelatinized granules. This is the process of retrogradation. Retrogradation is also referred to as setback, and occurs with re-crystallization of amylose. Amylose is much more susceptible to retrogradation and amylopectin is only minimally involved in starch retrogradation even though amylopectin has been shown to influence retrogradation and syneresis in corn starch gels (5). This re-association and re-crystallization of amylose causes release of the water absorbed and bound during gelatinization, leading to the phenomenon known as syneresis.

Retrogradation of starch in food products is a concern as it affects product quality. The stability of starch-containing products during cold storage in particular is greatly affected by the extent of retrogradation. Freeze-thaw cycles result in extensive retrogradation and syneresis.

Retrogradation of starch in some instances enhances quality as such starches are resistant to enzyme hydrolysis and hence more stable. Cooling retrograded starches at room temperature prior to freezing at −20°C results in the formation of resistant starch as the retrograded starch is no longer susceptible to enzyme hydrolysis (29). This procedure is used in the production of industrial resistant starch.

C. Starch Damage

Starch damage is the modification or destruction of starch granule structure to the extent that it affects physicochemical properties such as water absorption. This in turn influences functionality of damaged starch in food applications, and subsequently, the quality of the final product. Starch damage results from various processes such as milling of grains. Starch damage affects the susceptibility of starch to hydrolysis and reactions as enzymes do not properly interact with the restructured granules.

Starch damage by processing or mechanical action causes a cracked appearance to granules. Extensive starch damage causes disruptions in the molecular structure of the starch. Modification to the starch granule therefore results in increased swelling ability and is more susceptible to enzymatic hydrolysis (19). In addition, cold water solubility of starch is enhanced. This affects the applicability in baking and food applications.

D. Interactions with Acids, Sugar, and Salts

The presence of chemical components such as sugar and salts has a great effect on the characteristics of starch in food systems. The granule surface structure is affected and restructured in the presence of acid, as there is de-polymerization and hydrolysis of amylose and amylopectin (40). This results in lower viscosities of starch pastes. Solubility of starch is enhanced by acid. These effects are due to the disintegration of the component amylose and amylopectin at the low pHs typical of highly acidic solutions.

Starch competes with sugars such as glucose, fructose, and sucrose for water absorption. Gelling and swelling of a starch is therefore modified in the presence of sugars. This is because sugars contain hydrophilic hydroxyl groups identical to the glucose monomers of starch. As a result they decrease the water activity of the starch-water system. There is an overall increase in the free volume of water, reducing its effectiveness as a desirable plasticizer required to facilitate starch gelatinization (41,42). Slade and Levine (1988) report that sugar has an anti-plasticization effect on starch. The sugars bind the water, reducing its availability for starch gelatinization (43). Consequently, sugars elevate the temperature at which the gelatinization of various starches occurs.

The ionic nature of salts is responsible for their interaction with starch and the subsequent effects on starch physicochemical properties. Starch molecules possess a

weakly charged ionic structure. In the presence of cations, the granules are stabilized and protected, whereas in the presence of anions, the hydrogen bonds are ruptured. This destabilizes the granules, enhancing and facilitating gelatinization (44,45).

Various salts such as phosphates form complexes with the amylose and amylopectin, a property exploited for use in industrial in starch modification. Salts overall delay loss of birefringence and depress overall extent of gelatinization.

Sodium chloride has great influence on starch physicochemical properties. Sodium chloride increases the gelatinization temperature of various starches. At concentrations of 6–9%, sodium chloride solution inhibits starch gelatinization (46). Various procedures for starch pre-treatment are commonly used in food processing. A common procedure is alkalization, in which alkalizing agents are added to maize, wheat, or rice in the preparation of tortillas, Chinese wheat noodles or rice dumplings, respectively. Common alkalizing agents include sodium hydroxide, or sodium and potassium carbonate (47).

Addition of alkali contributes to improving starch swelling capacity. The presence of lime (calcium hydroxide) has been shown to decrease starch crystallinity in corn (48). Gelatinization temperature of corn starch is also increased by the presence of lime, attributed to cross linking of calcium with the starch, as well as due to ionic interactions with hydroxyl groups on the starches (47). It is expected that these would in turn lead to variations in gelatinized starch quality characteristics — color, gelation, and retrogradation tendency.

IV. STARCH HYDROLYSIS

Starch hydrolysis is the cleaving of the starch polymer to short chain fragments such as dextrins and maltose, or to the glucose monomers. Starch hydrolysis is essential in many aspects of the application of starch. For instance, starch is hydrolyzed by various means for the production of sweeteners. Hydrolysis products of starch are multifold and include products such as dextrins and simple sugars. Starch hydrolysis is carried out primarily by the use of enzymes or chemicals, or in combination.

A. Enzyme Hydrolysis

Enzymatic hydrolysis of starch is carried out for various purposes, but most notably for the industrial production of maltose syrup (49). The key enzymes used for starch hydrolysis are β-glucosidases, which hydrolyze the amylose and amylopectin in starch. These include α-amylase, amyloglucosidase, and pullulanase.

The extent of starch hydrolysis is quantified by various parameters. This could be the hydrolysis index (HI), or by the dextrose equivalent (DE). The hydrolysis index quantifies the proportion of starch hydrolyzed. Dextrose equivalent describes the potential for starch conversion to dextrose (glucose) and is defined as the sum of reducing sugars expressed as dextrose. This is because starch in its native form has few reducing sugar ends. The number of reducing ends is influenced by the proportion of amylopectin. Degree of polymerization is indicative of the number of glucose residues. Amylose from starches such as maize or wheat have DP of 200–1200, while amylose from potato or tapioca starch have DP of about 1000–6000.

Hydrolysis of starch to maltodextrins is achieved by use of α-amylase enzymes. These enzymes are categorized either as endoamylases, exoamylases, debranching enzymes, or transferases (26). The endoamylases, the most common being α-amylase (EC 3.2.1.1), are specific for the α-1→4 linkage in amylose and amylopectin. Their hydrolysis products from starch hydrolysis are mainly oligosaccharides and dextrins (26). Exoamylases, on the other hand, have the ability to hydrolyze both the α-1→4 and α-1→6 bonds of amylose and amylopectin. A common example is amyloglucosidase (EC 3.2.1.20). β-Amylase is an exoamylase that has the ability to hydrolyze the α-1→4 bond of amylose.

Debranching enzymes used in starch hydrolysis are targeted at hydrolyzing the α-1→6 bonds in amylopectin. These include pullulanases. Hydrolysis products of these are mainly maltose and maltotriose. The transferases have low activity with regard to starch hydrolysis but are involved in formation of new glycosidic linkages (26).

Enzymatic hydrolysis of starch is influenced by the presence of non-starch components such as lipids, particularly lipids bound to amylose. This is because the presence of these complexes renders the amylose less susceptible to hydrolysis enzymes (20). Additional enzymes such as lysophospholipase are therefore sometimes required for complete hydrolysis of starch in the production of glucose from starch.

Amylase enzymes produced by lactic acid bacteria — *Lactobacillus plantarum*, *Lactobacillus amylophilus*, and *Lactobacillus delbruecki*, in particular — are used for industrial hydrolysis of starch for conversation of starch to glucose. This is a process known as saccharification (50). Yeasts such as *Saccharomyces cerevisiae*, which produce α-amylase, are also used in bioreactors for enzymatic hydrolysis of starch and subsequent fermentation of the hydrolysis product (glucose) by the yeast strains (33). These micro-organisms produce heat-stable amylase which can survive the high bioreactor process temperatures required for gelatinization and hydrolysis of the starch. The lactobacilli produce enzymes that hydrolyze the starch to glucose, and then the bacteria ferment the starch of the industrial production of lactic acid.

Immobilized enzymes also are used in industrial hydrolysis of starch. The enzymes are extracted from an

industrial source, usually microorganisms such as *Aspergillus*, and then immobilized on inert particles such as silica (51). This ensures that the enzyme has optimum activity and access for starch hydrolysis. Co-enzymes and ionic particles such as calcium are required for starch hydrolysis.

While traditionally acids (mainly hydrochloric acid) have been used for hydrolysis of starch, there has been an increase in use of industrial enzymes for starch hydrolysis. Most of these convert starches for the production of maltodextrin, modified starches, glucose syrup, or fructose syrup. Hydrolysis of starch in foods is increased by processing. Enzymatic hydrolysis of starch in various legumes for instance is enhanced by soaking and sprouting. Gelatinization of starch is required prior to enzymatic hydrolysis.

B. Acid Hydrolysis

Acids are used to facilitate the hydrolysis of starch. The α-1$\rightarrow$4 linkages in amylose and amylopectin are susceptible to hydrolysis at the low pH typical of acids. Hydrochloric acid at low concentrations (0.36% w/v) hydrolyzes starch (52).

The use of acids in combination of alcohols has been suggested for starch hydrolysis. Formation of limit-dextrins with varying degrees of polymerization occurs in the presence of various alcohols such as methanol, ethanol, and propanol. These alcohols are possibly involved in disrupting the hydrophobic and hydrogen bonds of the starch helical structure in the granule. Increase in temperature further increases the susceptibility of starch to acid hydrolysis in alcohol (52).

C. Alkaline Hydrolysis

Alkaline hydrolysis of starch is enhanced and influenced in the presence of heat and inorganic salts. There is complete hydrolysis of starch with microwave heating in the presence of metal chlorides (53). The theoretical yield of glucose (111%) is obtained in the presence of chloride salts such as lithium chloride, barium chloride, and iron trichloride. On the other hand, acid hydrolysis of starch is greatly limited in the presence of sulfate salts. In the presence of sulfate salts — sodium sulfate, magnesium sulfate and or zinc sulfate — acid hydrolysis is actually greatly impeded (53).

D. Heat-Induced Hydrolysis

Extrusion of starch is used in combination with enzymes for effective starch hydrolysis. The starch is treated under conditions of high temperature, high pressure, shear, and moisture (54). Heat stable amylase is used for starch hydrolysis. Extrusion cooking facilitates disruption of the granule structure and the crystallinity. This renders the amylose and amylopectin susceptible to gelatinization (55). Application of extrusion in starch hydrolysis has the advantage in that the process conditions can be modified such that the extent of hydrolysis is controlled for desired end products and dextrins (55).

V. STARCH MODIFICATION

Native starches such as tapioca starch often require considerable modification to enhance quality and versatility in food applications, and for storage stability. The components of starch — amylose and amylopectin — are highly sensitive to shear, stress, acidity, and high temperatures, and are typically altered by heat-moisture conditions of processing (15). Most native starches such as tapioca starch have limited swelling power and solubility. Modification is essential to improve paste clarity, paste stability, resistance to degradation, and freeze-thaw stability. Modification of starch is important to improve the reactivity of glucose, as well as introduce reactive side chains (56). The integrity and structure of the granule is also enhanced by modification. Additional side chains interfere with potentially deleterious post-process starch properties such as retrogradation. Most starches used in food applications are modified starches.

Modification of starches is by physical and chemical procedures. Modification procedures include acetylation, hydroxypropylation, and a combination of hydroxypropylation and cross-linking (57). Hydroxypropylated starches are most commonly used in the food industry (57). Stabilization of starch is facilitated by use of acetates and hydroxypropyl esters (58). These modification procedures greatly increase freeze-thaw stabilization and increase resistance to process conditions such as heat and shear.

Cross-linking is commonly carried out with various chemical agents such as phosphorus oxychloride, sodium trimetaphosphate, and anhydrides (58). Cross-linked starches are more resistant to process conditions such as temperature and acidity as a result of the fact that the hydrogen bonds have been reinforced and act as bridges. These are useful in preventing re-crystallization of amylose and the subsequent retrogradation in processed starchy foods.

Some procedures that have been shown to be effective in modification of banana starch include cross-linking with sodium trimetaphosphate, formation of starch phosphate with sodium tripolyphosphate, and hydroxypropylation using a combination of sodium hydroxide and sodium sulphate (24). These procedures result in starches with enhanced water-binding capacity, and in most cases, increased solubility. Starch phosphates in particular have increased freeze-thaw stability.

Acid-thinned starches are obtained by reducing the concentration of concentrated starch slurry with a mineral

acid at 40–60°C, to obtain a desirable viscosity. The starch is recovered after the acid is neutralized (59). The granule structure of the starch is not destroyed in the process, but various changes to the properties of the starches occur. Starch solubility and gel strength for instance are increased, while starch viscosity is decreased (60). Rate of starch hydrolysis is increased with increasing concentration of acid (59).

Physical modification procedures that have been used include pre-gelatinization. Pre-gelatinization increases swelling power and paste clarity of banana starch (24). Extrusion cooking of starch is used to improve quality and characteristics of starch.

VI. STARCH IN FOOD APPLICATIONS

Starch is a functional ingredient in many food products. There are multiple functions of starch in food products. Most commonly, starch is used as a bulking agent, binder, carrier, in fat-replacers, as a texture-modifier, and as raw material for other starch-related products. It is a basic ingredient in products such as breads, puddings, marinades, and sauces, and also serves a considerable function in other products such as powdered spices and beverages. The applicability and utility of a starch in food products is enhanced by factors such as its composition and functionality. Starch is a substrate for lactic acid bacteria in fermentation to produce lactic acid (61).

Starch-based foods play a major role in the diet in various areas because of their bulking quality, and ability to contribute to satiety. Fermentation of cassava for instance imparts a sour taste that is sometimes highly desirable.

A. Functional Properties

Starch is used as to facilitate thickening and gel formation in various food products such as fruit preparations (62). The consistency of products such as tapioca pudding and many custards would not be attainable without the thickening and stabilizing properties of starch.

High amylose starches have high viscosity, and form thick gels. This enhances their properties as thickeners in food products. Starches with lower amylose content are better suited for use in certain types of noodles, such as Japanese noodles (23). High amylose starches are desirable for application in fried products as they have minimal fat absorption. High amylose starches are also applicable as thickeners and for use as gelling agents in foods such as jellies. High amylose starch gels set rapidly hence are desirable in production of confectionary and candies (56). Other desirable properties of high amylose starches include their flexibility, water resistance, and tensile strength (63).

Starches with high swelling ability and high viscosity are desirable for various types of Asian noodles (18). Starches high in amylopectin and low in amylose (waxy starches) such as waxy wheat are produced for use in such products. The higher levels of amylopectin further contribute to extending shelf-life, by reducing retrogradation and staling in baked products. High amylopectin starches are less susceptible to retrogradation, and hence very applicable in improving freeze-thaw stability (63).

Modified starches are highly effective as stabilizers in products such as yogurt (57). The presence of side groups such as acetyl and hydroxyl groups in modified starches, however, results in interactions with amylose and amylopectin, improving overall stabilizing ability.

Fermented, sun-dried cassava starch is commonly used in baked products in various parts of South America and Brazil. This is unique in that the fermentation facilitates expansion which is desirable in the baked products (64). Viscosity of sour starch pastes is lower than for non-fermented starch, attributable to the solubilization of amylopectin.

Starch is used to improve quality of extruded food products. Addition of cassava starch to cassava flour prior to extrusion increases water solubility, but decreases water absorption and bulk density properties (65).

Shear thinning of starch is an important characteristic with regards to stability of starch pastes during processing, particular in food products that require extensive stirring and agitation. Removal of lipids (defatting) in sorghum starch has been associated with increased shear-thinning characteristics (66). Starches that are resistant to shear thinning are generally highly desirable to ensure product stability and suitable consistency.

Products of starch hydrolysis find considerable application in food products. Maltodextrins, for instance, are commonly used in heat-stable gels (67).

B. Value-Added Food Applications

Starch is used as a basic ingredient in starch-based fat substitutes. These simulate the functional properties of fats, particularly texture modification, but with less caloric value. Various starch-based fat substitutes are commonly used in industry. Some examples of these include TrimChoice™ (Specialty Grain products, NE) made from hydrolyzed oat starch, Amalean™ (American Maize Products, IN) made from modified high-amylose corn starch, and SlenderLean™ (National Starch, NJ) made from tapioca starch (37).

Starch-based fat substitutes are especially applicable in baked products and value-added foods.

Resistant and minimally digestible starches are used in value-added food products. Most of these products are targeted at the management of diet-related diseases such as obesity and type II diabetes. An example of such a product is Extend™, a snack bar formulated with resistant starch (corn and rice starch), which has been formulated for the

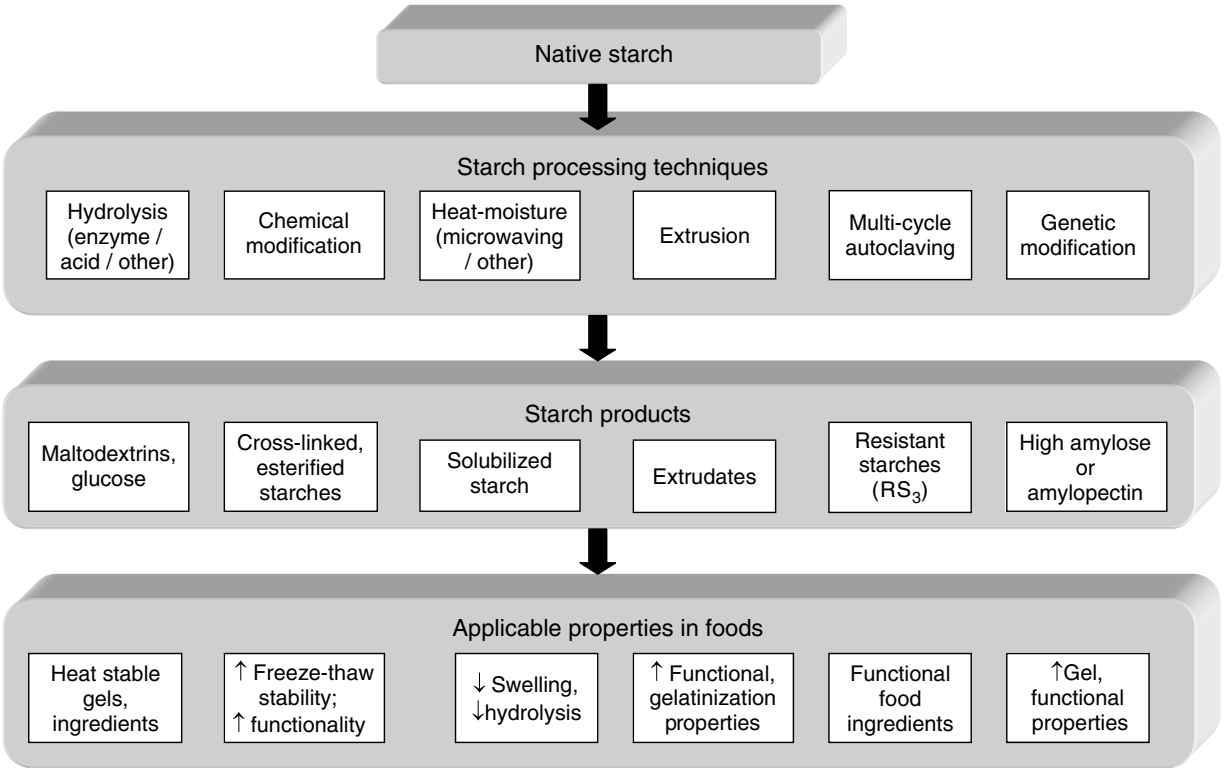

FIGURE 3.5 Outline of starch processing and applications in foods.

management of type II diabetes. The product ensures a slow and sustained digestion of the resistant starch, and minimal absorption of glucose, mitigating the problems of high post-prandial blood glucose levels (Figure 3.5).

VII. STARCH NUTRITIONAL QUALITY

Starch is the primary nutrient involved in energy intake and regulation. Typically, most adults require about 200 g carbohydrate daily to facilitate brain and muscle function. The digestibility and absorption of starch has significant nutritional and physiological implications. As the primary source of energy, starch is rapidly metabolized and absorbed. The extent of starch digestibility is influenced by the nature of the starch, food processing, and physiological status.

There is a dichotomy of starch functionality as a nutrient. On the one hand, it is a source of glucose, the primary substrate for cell metabolism. On the other hand, starch resistant to digestion (resistant starch) is minimally digestible and only minimally absorbed, and therefore is not physiologically available.

A. Starch and Glycemic Index

Starch is the primary source of metabolizable energy, and therefore its availability and digestion are important. High starch foods are rapidly digested and metabolized. Glycemic index (GI), the post-prandial blood glucose response to a particular food, has been used to differentiate the metabolic response to dietary carbohydrates (68). Glycemic index is indicative of the relationship between a food and the implications of starch digestibility, absorption, and metabolism. Foods that are high in readily digestible starch result in high levels of glucose in the blood. These are classified as high GI foods. Typically, most tropical root starchy staples such as cassava and yams have high levels of readily digestible waxy starches (high amylopectin). These are rapidly and readily absorbed, resulting in elevated levels of glucose in the blood. This is, however, modified to a large extent by other factors associated with the nature of the starch, its processing, preparation, and consumption.

Starch is hydrolyzed by salivary and pancreatic amylases to yield monosaccharides such as glucose and fructose, and maltodextrins. These are transported via the hepatic portal vein and available for metabolism. Starch digestion and metabolism has been classified into three categories: rapidly digestible starch, slowly digestible starch, and resistant starch (69). Rapidly digestible starch, which typically is completely digested, is associated with post-prandial glucose response, and hence has effect on insulin levels. Rapidly available glucose meanwhile describes glucose and sucrose obtained as hydrolysis

products of rapidly digestible starch. Rapidly digestible starch occurs most commonly in highly processed foods such as puffed wheat cereal, while slowly digestible starch occurs in foods such as legumes and pasta (69).

Starch digestion and glycemic index also have been associated with satiety. Rapidly digestible starches are quickly absorbed and metabolized, whereas slower digesting starches are only slowly absorbed and therefore improve satiety (70). These have also been shown to improve exercise endurance (71).

There are differences in the metabolic response to dietary carbohydrates. Post-prandial blood glucose and insulin responses vary depending on the nature of carbohydrates, particularly starch. Physiological conditions such as type II diabetes and obesity have been associated with starch metabolism. Other conditions such as coronary heart disease are linked to metabolism of glucose derived from dietary starch. The physiological consequence of starch consumption is influenced by the extent of its digestibility and metabolism. Digestibility of starch is determined by its availability and susceptibility to digestive enzymes. Susceptibility of starch to digestive enzymes is in turn influenced by the chemical nature of the starch and the changes that result from processing.

Starch digestibility is influenced by various factors such as processing, storage, amylose content, and presence of dietary fiber (72). The ratios of amylose to amylopectin are important in starch digestion and metabolism. Consumption of modified high amylopectin starches have been shown to result in an increase in serum free fatty acids and serum glucose levels. This is probably as a result of gluconeogenesis. Conversely, modified amylose cornstarch is highly digestible, and results in lower insulin levels. Starch that is digested and absorbed, however, has physiological effects, some of which have been linked to disease conditions.

Researchers have demonstrated that the consumption of high starch diets in human test subjects apparently leads to an overall decrease in overall energy intake, compared to a high-sucrose (simple sugar) or high fat meals (70,73). This indicates that high starch may have potential for a high satiety value, but with low caloric density, and hence the lowered energy intake.

Type II diabetes, a condition that results from inadequate production of insulin to facilitate glucose uptake, is exacerbated by the presence of glucose in the blood. Clinical manifestations of Type II diabetes include fainting and dizzy spells as a result of low brain glucose levels. Starch and glucose metabolism have been also associated with obesity and accumulation of fat, as glucose is involved in fat metabolism.

The nature of starch and the level of amylose in the starch play a considerable role in the diabetic process and insulin response. Long-term consumption of a high amylose corn starch (70% amylose) by hyper-insulinemic subjects results in a normal insulin response (74). High amylose starch in the diet reduces insulin response (75). Meanwhile legume starches such as pure pea starch have been shown to be even more effective than corn starch in reducing hyperglycemia, as has been demonstrated with purified pea starch (76).

The conversion of sugars, which are starch hydrolysis products, into fat has been implicated in diabetes and cardiovascular disease and obesity. The consumption of simple sugars and refined grain foods has been linked to higher rates of cardiovascular disease and Type II diabetes, particularly in instances of insulin resistance (68,77).

B. Resistant Starch

Resistant starch is non-digestible starch which occurs in foods in various forms.

Resistant starch is described in various ways, including as starch and starch degradation products not absorbed in the gut (78,79).

Resistant starch occurs in four categories, primarily dependent on their mode of origin. These are described as: type 1 (RS_1): physically entrapped starch in the cell matrix of whole or partially milled grains, hence is inaccessible; type 2 (RS_2): native granular starch, mostly B-type legume starches which are may be ungelatinized during processing; type 3 (RS_3): retrograded starch, particularly from food processing; and type 4 (RS_4): chemically modified starch (29,79–82).

Resistant starch levels in food products are influenced by various factors including the nature of the starch, the mode of food processing and preparation, and storage conditions (83). Physical inaccessibility such as cell wall structure and the presence of dietary fiber influences levels of resistant starch, particularly in legumes (72).

Type 3 resistant starch (RS_3), which is retrograded starch, is the most commonly occurring form of resistant starch in processed foods. Starch in cooked then cooled foods such as pasta, rice, and lentils exhibits considerably reduced susceptibility to enzymatic digestion, indicating the formation of resistant starch (72). This is attributable to the retrogradation which occurs following cooling of gelatinized starch. Amylose is more susceptible to retrogradation than amylopectin. Resistant starch formation is therefore influenced by ratios of amylose to amylopectin.

Processing of starchy foods, in addition to factors such as starch amylose: amylopectin ratios and chemical modification, influences their digestibility. This is as a consequence of the disruption of the physical and chemical structure of the starch (84,85). Retrogradation of amylose with processing is mainly thought to be responsible for this alteration in susceptibility to digestive enzymes. In some cases, however, partial damage to starch molecules which would otherwise be physically entrapped

by the cell wall and inaccessible to digestive enzymes, may improve their susceptibility to digestion (86).

Consumption of resistant starch yields physiological effects similar to soluble fiber (82). Fermentation products include short chain fatty acids such as acetate, propionate, and butyrate, which facilitate absorption of minerals, excretion of bile acids, and consequently protect against colorectal cancer.

Resistant starch-containing foods such as legumes, and retrograded starches, however, have been associated with disease prevention. Fermented corn porridge, commonly consumed among some indigenous populations, has been shown to contain considerable amounts of starch that is resistant to digestion and subsequently is protective against various colon conditions (87). Experimental evidence using high resistant starch breakfast cereals in humans shows an improved glucose tolerance (88). In rural South Africa, however, consumption of cold maize porridge, which is high in retrograded starch and has rather low starch digestibility and a low glycemic index, has been associated with low levels of diabetes mellitus (87,89).

High resistant starch foods which have low glycemic index are effective in lowering the concentrations of high-density-lipoprotein (HDL) cholesterol and in improving glucose tolerance in incidence of diabetes and insulin resistance (77,88,90).

Root and tuber starches, unlike grain starches, are high in amylopectin and do not have the same restricting nature of the cell wall. They are, therefore, generally more digestible. Processing techniques such as autoclaving to reduce starch digestibility by increasing resistant starch levels have been suggested in foods (91). Digestibility of legume starch is increased by processes such as soaking and sprouting (30).

VIII. NEW STARCH TECHNOLOGIES

The functionality and applicability of starch in so many food applications and its importance as a food ingredient have led to continuous efforts to improve and optimize properties and versatility of starch. Some techniques currently used include genetic modification to modify starch yields and quality, multi-cycle autoclaving for production of resistant starch, and new processes such as microwave hydrolysis of starch.

A. Genetic Modification

Genetic modification of starch most commonly targets the enzymes of the starch biosynthetic pathway. The activities of these enzymes dictate and determine the quantities of starch synthesized as well as specific characteristics such as ratios of amylose to amylopectin. Their activity therefore influences starch properties: its reactivity, functionality, and applicability in food processing and in food applications.

Genetic modification of cereal starch is commonly employed to modify ratios of amylose to amylopectin, and hence improve functionality and nutritional quality of starch.

The primary enzymes involved in starch synthesis include the starch synthases, starch branching enzymes and adenosine diphosphate-glucose pyrophosphorylase (ADP-glucose-phosphorylase). The starch synthases occur both as a granule-bound synthase (GBSS) or located in the soluble phase, and catalyze the formation of the α-1$\rightarrow$4 glucan chains by adding ADP-glucose to the non-reducing end of the primer. The starch branching enzyme catalyzes formation of the α-1$\rightarrow$6 branches of amylopectin molecules. The ADP-glucose pyrophosphorylase catalyzes the formation of ADP-glucose (56). Other important enzymes are starch debranching enzymes and phosphorylases.

A major contribution of genetic modification is the modification of various cereal starches to reduce amylose content and produce high amylopectin starch. These waxy starches are desirable for various characteristics. They are desirable in various noodles, for modification of amylose characteristics in extrudates, and to extend the shelf-life of baked goods (18).

Waxy starches are produced by modification of the enzyme involved in amylose synthesis, granule-bound starch synthase (GBSS). While naturally occurring mutations in various wheats have resulted in waxy wheat starch, biotechnology to modify the expression of the GBSS genes is used to produce waxy starches, including rice, maize and wheat. Modification by decreasing the levels of enzymes such as starch synthase and starch branching enzyme is employed to increase amylose levels.

Modification by decreasing levels of GBSS results in increased amylopectin levels.

Regular cereal starches (up to 27% amylose) typically form opaque pastes and firm gels. Genetic modification techniques are applied to either decrease or increase the amylose to amylopectin ratios. Low amylose cereal starches (waxy maize, waxy rice, waxy wheat) lack GBSS, and therefore contain less than 1% amylose. These therefore do not effectively form gels but instead clear pastes. Genetically modified high amylose starches form highly resistant and firmer gels (41). Increasing amylose content also increases early onset of gelation. High amylose maize starch — amylomaize — is modified to have high levels of amylose, 50–70%. The granules of amylomaize are more resistant to swelling and therefore form much firmer and more rigid gels (11,92).

Genetically modified potato starch has been shown to be suitable in processing and preparation of starch noodles, as these have greater transparency and higher flexibility (93). This may be due to higher amylose content. Modification of starch synthesis to increase overall yields of starch in food products is carried out by modifying

levels of adenosine triphosphatase (ATPase) and starch branching enzymes (56).

Genetic modification of reactive groups such as phosphates is used to change the composition of starch by decrease of the starch branching enzyme (56).

B. Resistant Starch Production by Autoclaving

Autoclaving and steam processing are used in the production of resistant starch. Resistant starch produced by this technique is retrograded starch (RS_3), as it involves gelatinization and subsequent retrogradation of starch, rendering it resistant to digestive amylases. Autoclaving has been shown to modify resistant starch content in grain sorghum (94). High amylose starches which are most susceptible to retrogradation are therefore preferred for this process.

High pressure autoclaving has been standardized for the production of resistant starch (91,95). Starch with a high volume of water is gelatinized in a high pressure autoclave with stirring until a homogenous gel is obtained. The mixture is then cooled and frozen to facilitate retrogradation.

C. Other Procedures

Other procedures employed in starch modification include microwave solubilization (96,97). Corn starch modified by microwave heating for a short period of time (32–90 seconds) at 900 W has decreased swelling ability (96). Microwave pre-solubilization of starch at 180 W for 10 minutes is employed in food analysis (97). In the presence of dilute hydrochloric acid, there is complete hydrolysis of starch in 5 minutes of microwave processing. This is attributable to the superheating produced by the presence of the ions. These procedures are proposed to substitute for the more expensive and time-consuming enzyme hydrolysis procedures commonly used.

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4 Functional Properties of Carbohydrates: Polysaccharide Gums

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CONTENTS

I. INTRODUCTION

Gums are long chain polysaccharides widely used in the food and many other industries as thickeners, stabilizers, and texture modifiers. Gums and related polysaccharides are produced in nature as storage materials, cell wall components, exudates, extracellular substances from plants or microorganisms, and in some cases from exoskeletons of shellfish such as lobsters, shrimps and crabs (e.g., chitosan). Some polysaccharides are simple in sugar composition, such as cellulose and β-D-glucans, which contain only one type of monosaccharide (e.g., β-D-glucose), while others are rather complex and may contain up to six types of monosaccharides plus one or two types of uronic acids. Common monosaccharides and uronic acids present in natural polysaccharides include D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, L-rhamnose, L-fucose, D-galacturonic acid, D-gulucuronic acid, D-mannuronic acid, and L-guluronic acid. The primary structure of a polysaccharide, i.e., monosaccharide composition, linkage patterns, and molecular weight, defines the solubility and conformation of the polymer chains in aqueous solutions, which in turn dictate the functional properties of the gums exhibited in food and other systems.

Polysaccharides can be linear or branched polymers. With the same molecular weight, linear polysaccharides generally have poorer solubility and higher viscosity than branched counterparts due to their extended conformation in solutions (if soluble or dispersible). Perfectly linear homoglycans such as cellulose are either difficult to dissolve or insoluble in aqueous medium due to excessive intra- and intermolecular interactions (mainly through hydrogen bonding), which make them less useful as hydrocolloidal gums. Irregularity introduced by substitution or branching to the linear chain increases solubility. Highly branched polysaccharides are usually very soluble but exhibit lower viscosity in solutions because of their smaller hydrodynamic volumes compared to liner molecules with the same molecular weight. The variations in monosaccharide composition, linkage patterns, molecular weight, and molecular weight distribution of gums contribute to the unique functional properties exhibited by each gum.

The goal of the present chapter is to provide information on the basic structural and functional properties and major applications of all commercial gums and some emerging gums in food and other industries. For detailed descriptions of chemical structure, molecular characterization, physicochemical properties, and applications of these gums, readers are referred to several comprehensive books and chapters (1–5).

II. FUNCTIONAL PROPERTIES OF POLYSACCHARIDE GUMS

A. Viscosity Enhancing or Thickening Properties

When polysaccharide gums are dissolved into solution, one remarkable phenomenon is the considerable increase in solution viscosity; gums restrict the movement of water molecules and in extreme cases gels are formed. The ability of polysaccharide gums to increase viscosity or to thicken the aqueous system is the most important property of such polymers. The shape and conformation of polysaccharides are determined by their primary sequence structure. Once the structure is determined, the shape and/or conformation of a polysaccharide are more or less fixed, and the molecular weight (size) and number of polysaccharide molecules in a given volume (concentration) become important in determining their functional properties. In addition, environmental factors such as solution pH, temperature, presence of certain ions, and ionic strength of the system have significant influences on the conformation of polysaccharide chains, and hence their functional properties.

Solution viscosity of a gum almost always increases with concentration, but not necessarily in a linear manner. At low concentrations, dilute gum solutions normally exhibit Newtonian flow behavior (independent of shear rate) in which polymer molecules are free to move independently without intermolecular entanglements. For most random coil polysaccharides, the relationship between zero-shear specific viscosity (η_{sp}) and concentration (c) follows $\eta_{sp} \propto c^{1.1–1.3}$. When the polymer concentration is increased to a critical point (critical concentration C*), the viscosity of the solution increases sharply due to entanglement of polymer molecules. This is called the semi-dilute region within which polysaccharide gums usually exhibit shear thinning flow behavior where viscosity decreases with increase in shear rate.

The viscosity of most gum solutions decreases with increased temperature, although some gums are more resistant to temperature changes. For example, the viscosity of xanthan gum solution is relatively unchanged over a wide range of temperatures (−4°C to 93°C) (4). There are other extremes, such as methyl cellulose, where the viscosity increases as the temperature increases, and eventually gels are formed at higher temperature. Other factors influencing viscosity include pH, ionic strength, and presence of co-solutes with effects differing in individual gums.

B. Gelling Properties

All hydrocolloids have viscosity enhancing or thickening properties, but only a few are able to gel. Gelation of polysaccharides is caused by the cross-linking (covalently

and/or non-covalently) of long polymer chains to form a continuous three-dimensional network which traps and immobilizes water and forms a firm and rigid structure resistant to flow under force. Gelation of polysaccharides usually involves three stages: 1) Polysaccharide gums have to be dissolved/dispersed at temperature above the melting point. At this stage, polymer chains exist in a coiled conformation. Upon cooling, polymer chains start to form ordered structures such as helices. 2) Formation of a gel network with further cooling. At this stage, helices begin to aggregate by forming cross-links or super-junctions, and a continuous network is eventually developed. 3) Aging stage where existing helices or aggregations are further enhanced and some new helices are formed. Contraction of gel networks may occur with the liberation of free water ("weeping" or syneresis). Most of the polysaccharide gels are thermally reversible below 100°C with defined setting and melting temperature ranges. There is a minimum concentration for each polysaccharide, below which gel cannot be obtained. Some gels exhibit thermal hysteresis where the melting temperature is significantly higher than the setting temperature, e.g., agarose gels (6). However, there are a few gelling gums which do not follow the above rules. Some gums form gels upon heating while others can form gels by changing ionic strengths and pH or introducing specific ions. A wide range of gels with different textures, such as soft, elastic, very firm, and brittle, can be prepared by selecting different types of polysaccharides and by varying gelation conditions.

C. Surface Activity and Emulsifying Properties

Although polysaccharides are hydrophilic compounds not conventionally perceived to be surface active, many polysaccharide gums are used to stabilize emulsions that already contain an emulsifier (proteins or surfactants). The universal role of gums in emulsion systems is to thicken the continuous phase, thereby inhibiting or slowing droplet flocculation and/or creaming. There are a few exceptions of gums that actually exhibit surface activity; these gums play double roles in emulsion systems: as an emulsifier and a thickener. In most cases, surface activity of these gums is attributed to the protein component associated with the polysaccharides, while in other situations, the surface activity is due to the presence of hydrophobic functional groups, such as in the cases of methyl cellulose and propylene glycol alginate. Although it is still controversial regarding what is responsible for the surface activity, fenugreek gum does exhibit excellent emulsifying and emulsion stabilizing properties even at very low protein content (e.g., $<0.5\%$) (7). Detailed applications of these gums as emulsifiers are described in the following sections.

III. CHEMISTRY, FUNCTIONAL PROPERTIES, AND APPLICATIONS OF POLYSACCHARIDE GUMS IN FOOD AND OTHER INDUSTRIES

A. Gums from Exudates

The earliest gum known to humans is from plant exudates. Many plants exude a viscous, gummy liquid when wounded and the liquid will dry to form hard, glassy, tear-drop-like balls or other shapes of masses. The exudates are hand collected, sorted/graded, and further processed to meet the application needs. Gum arabic, tragacanth, karaya, and ghatti are exuded gums that are commercially significant.

1. Gum Arabic

a. Source and structure

Gum arabic, or acacia gum, is prepared from the exudate of *Acacia* trees, mostly from *senegal* species, and sometimes mixed with *seyal* species. Natural gum is in the form of spherical balls resembling tear drops, collected by hand and processed before use. Almost all commercial gum arabic is produced from the Sahelian regions of Africa.

Gum arabic consists of a mixture of a relatively low-molecular-weight polysaccharide ($\sim 0.25 \times 10^6$ daltons, a major component) and a high-molecular-weight hydroxyproline-rich glycoprotein ($\sim 2.5 \times 10^6$ daltons, a minor component) (8). It is a heavily branched polysaccharide; the main chain consists of (1→3)-linked β-D-galactopyranosyl residues. The side chains are two to five units in length made of (1→3)-linked β-D-galactopyranosyl units, joined to the main chain by (1→6)-linkage. Both main and side chains are substituted by α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl-β-D-glucuronopyranosyl units. The monosaccharide composition of gum arabic varies with gum sources, e.g., gum from *Acacia senegal* contains about 44% galactose, 27% arabinose, 13% rhamnose, and 16% glucuronic acid of which only 1.5% are 4-O-methylated. In contrast, gum arabic from *Acacia seyal* contains 38% galactose, 46% arabinose, 4% rhamnose, and 12% total glucuronic acid (of which 5.5% are 4-O-methylated) (8). These compositional and structural differences affect their functionalities, e.g., gum arabic from *Acacia senegal* is a much better emulsifier than gum from *Acacia seyal*.

b. Functional properties and applications

Gum arabic is readily dissolved in water to give clear solutions with light colors ranging from very pale yellow to orange brown. It is a typical low viscosity gum and the solutions exhibit Newtonian flow behavior even at concentrations as high as 40%. Higher concentration solutions can be

prepared up to 55%. A major functional property of gum arabic is its ability to stabilize oil-in-water emulsions. The protein-rich high-molecular-weight species are preferentially adsorbed onto the surface of oil droplets while the carbohydrate portion inhibits flocculation and coalescence by electrostatic repulsions and steric forces (8). The major application of gum arabic is in the confectionary and beverage industries for stabilizing emulsions and flavor encapsulation. In the confectionary industry, gum arabic is used to prevent sugar crystallization and to emulsify the fatty components. Examples of such products include pastilles, caramel, and toffee. Gum arabic has also been used in chewing gums, cough drops, and candy lozenges. Good stability under acidic conditions makes gum arabic useful in beverages, e.g., it is used as an emulsifier in the production of concentrated citrus juices and cola flavor oils of soft drinks (3). Gum arabic stabilized flavor oils can be spray-dried to form microencapsulated powders that can be easily incorporated into dry food products such as soup and dessert mixes.

2. Tragacanth Gum

a. Source and structure

Tragacanth gum is dried exudates from branches and trunks of *Astragalus gummifer* Labillardiere or other species of *Astragalus* grown in West Asia (mostly in Iran, some in Turkey). After hand collection, the exudates are graded, milled, and sifted to remove impurities. Tragacanth gum is composed of a water-soluble fraction and a water-insoluble fraction. The water-soluble fraction, accounting for 30–40% of total gum, is a highly branched neutral polysaccharide consisting of L-arabinose side chains attached to D-galactosyl backbones (9, 10). The D-galactosyl residues in the core chains are mostly 1→6-linked, sometimes 1→3-linked, whereas the branching L-arabinosyl residues are mutually joined by 1→2-, 1→3-, and/or 1→5-linkages. The water-insoluble fraction, the major fraction (60–70%), is an acidic polysaccharide consisting of D-galacturonic acid, D-galactose, L-fucose, D-xylose, L-arabinose, and L-rhamnose, and is called tragacanthic acid or bassorin. It has a (1→4)-linked α-D-galacturonopyranosyl backbone chain with randomly substituted xylosyl branches linked at the 3 position of the galacturonic acid residues. Some of the xylosyl residues are attached by an α-L-fucosyl or a β-D-galactosyl residue at the 2 positions (3, 11).

b. Functional properties and applications

Tragacanth gum swells rapidly in both cold and hot water to form a viscous colloidal suspension rather than a true solution. When added to water, the soluble tragacanthin fraction dissolves to form a viscous solution while the insoluble tragacanthic acid fraction swells to a gel-like state, which is soft and adhesive. When more water is added, the gum first forms a uniform mixture; after 1 or 2 days, the suspension will separate into two layers with dissolved tragacanthin in the upper layer and insoluble bassorin in the lower layer. The viscosity of the suspension reaches a maximum after 24 hours at room temperature, and hydration can be accelerated by an increase in temperature. The suspension typically exhibits shear thinning behavior. The ability to swell in water, forming thick, viscous dispersions or pastes, makes it an important gum in the food, pharmaceutical, and other industries. It is the most viscous of the natural water-soluble gums and is an excellent emulsifying agent with good stability to heat, acidity, and aging.

Food applications of tragacanth gum include salad dressings, oil and flavor emulsions, ice creams, bakery fillings, icings, and confectionary. In the pharmaceutical and cosmetic industries, tragacanth gum is used as an emulsifier and stabilizer in medicinal emulsions, jellies, syrups, ointments, lotions, and creams. Gum tragacanth is also a good surface design thickener since it is a good medium for mixing with natural dyes and conveying controlled design onto fabric. It allows easy painting, stamping, and stenciling, and ensures a good control over color placement.

3. Gum Karaya

a. Source and structure

Gum karaya is from the exudates of *Sterculia urens*, trees of the Sterculiaceae family grown in India. It is a branched acidic polysaccharide with high molecular weight. Gum karaya contains 37% uronic acid and 8% acetyl groups. The backbone chain consists of (1→4)-linked α-D-galacturonic acid and (1→2)-linked α-L-rhamnosyl residues with side chains of (1→3)-linked β-D-glucuronic acid, or (1→2)-linked β-D-galactose on the galacturonic acid unit where one half of the rhamnose is substituted by (1→4)-linked β-D-galactose (12, 13). The quality of the gum varies significantly depending on the season of collection: summer usually gives high yields and high viscosity gum. During storage, the viscosity of gum karaya can be lost when exposed to high temperature and high humidity. The decrease in viscosity is more significant when the particle size is small. Preservatives may be added to prevent viscosity loss.

b. Functional properties and applications

Similar to gum tragacanth, gum karaya does not dissolve in water to give a clear solution but swells to many times its own weight to give a dispersion. The type of dispersion is influenced by the particle size of the product. For example, coarse granulated gum karaya produces a discontinuous, grainy dispersion whereas a fine powdered product gives a homogenous dispersion. Dispersion of gum karaya exhibits Newtonian flow behavior at low concentration ($<0.5\%$) and shear thinning behavior at semi-dilute concentrations ($0.5\% < c < 2\%$) (13). Further increase in gum concentration produces a paste resembling spreadable gels. An increase in temperature improves solubility in water, but excessive heat will cause degradation of the polysaccharides, resulting in non-recoverable loss of viscosity. At

extreme pHs and in the presence of sodium, calcium, and aluminium salts, the viscosity of gum karaya dispersion decreases.

Gum karaya is used to stabilize packaged whipped cream products, spread cheeses and other dairy products, frozen desserts and salad dressings, and as acid-resistant stabilizers in acidified products. It is also used as a water binder in bread, processed meats, and low-calorie dough-based products such as pasta (11). Other applications of karaya gum include dental adhesives, bulk laxatives, and adhesives for ostomy rings. Gum karaya is also used in the manufacture of long-fibered, lightweight papers in the paper industry and as a thickening agent in the textile industry to help print the dye onto cotton fabrics.

4. Gum Ghatti

a. Source and structure

Gum ghatti is an amorphous translucent exudate of *Anogeissus latifolia,* a tree of the Combretaceae family grown in India. It contains L-arabinose, D-galactose, D-mannose, D-xylose, and D-glucuronic acid in the ratio of 10:6:2:1:2, plus traces of a 6-deoxyhexose. The detailed structure of gum ghatti has not been clearly established. Its main chain consists of β-D-galactopyranosyl residues connected by (1→6)-linkages and D-glucopyranosyluronic acid units connected by (1→4)-linkages (9).

b. Functional properties and applications

Similar to gum karaya and tragacanth, gum ghatti does not dissolve in water to give clear solutions, but can be dispersed to form a colloidal dispersion. The dispersion exhibits non-Newtonian flow behavior and its viscosity is between those of gum arabic and gum karaya dispersions at the same concentration. Gum ghatti is an excellent emulsifier and can be used to replace gum arabic in more complex systems (13). The pH of gum ghatti dispersions is 4.8, and the viscosity increases with increase in pH, reaching a maximum at pH 8 (3). The viscosity of gum ghatti dispersions increases with time regardless of solution pH; however, addition of sodium salts, such as sodium carbonate and sodium chloride, results in decrease in viscosity. Loss in viscosity also occurs when the gum dispersions are not protected by preservatives against bacterial attack. Gum ghatti is used as an emulsifier and stabilizer in beverages and butter-containing table syrups, and as a flavor fixative for specific applications. Gum ghatti is also used to prepare powdered, stable, oil-soluble vitamins, and as a binder in making long-fibered, lightweight papers.

B. Gums from Plants

Gums of plant origin other than exudates are also important for food use. These include storage polysaccharides from seeds and tubers, mucilages from seed coats, and cell wall materials from fruits and cereals.

1. Galactomannans (Locust Bean, Tara, Guar, and Fenugreek Gums)

a. Source and structure

Galactomannans are a group of storage polysaccharides from various plant seeds. There are four major sources of seed galactomannans: guar (*Cyamopsis tetragonoloba*), locust bean (*Ceratonia siliqua*), tara (*Caesalpinia spinosa* Kuntze) and fenugreek (*Trigonella foenum-graecum* L.). Among these, only guar and locust bean gums are of considerable industrial importance and the use of tara and fenugreek is limited due to availability and price. Most of the guar crop produced worldwide is grown in India and Pakistan. The plant has also been cultivated in tropical areas such as South and Central America, Africa, Brazil, Australia, and the semi-arid regions of the southwest United States. Locust bean is produced mostly in Spain, Italy, Cyprus, and other Mediterranean countries. Fenugreek is grown in northern Africa, the Mediterranean, western Asia, and northern India, and has been recently cultivated in Canada.

The production of commercial guar, locust bean, and tara gums is similar, involving separation of endosperms from the seed hull and germ, grinding and sifting of the endosperm to a flour of fine particle size and sometimes purifying by repeated alcohol washings. The final product is a white to cream-colored powder. The amount and molecular weight of galactomannans found in the endosperm extract can vary significantly depending on the source of seed and growing conditions. Most commercial gums contain >80% galactomannan. Low-molecular-weight grades are produced from acid, alkaline, or enzyme hydrolysis of native gums. Fenugreek gum is extracted from the endosperm or ground whole seed with water or dilute alkali, and yields vary from 13.6% to 38%, depending on the variety/cultivar and extraction methods (14). Commercial fenugreek gum products, such as Fenu-pure and Fenu-life, contain over 80% galactomannas with about 5% proteins. Laboratory-prepared material involves pronase treatment of the gum samples, which produces a product of much higher purity with less than 0.6% protein contaminates (15).

Seed galactomannans consist essentially of a linear (1→4)-β-D-mannopyranose backbone with side groups of single (1→6)-linked α-D-galactopyranosyl units. The molar ratio of galactose to mannose varies with origins, but are typically in the range 1.0:1.0~1.1, 1.0:1.6–1.8, 1.0:3.0, and 1.0:3.9~4.0 for fenugreek, guar, tara, and locust bean gums, respectively. The distribution of D-galactosyl residues along the backbone chain is considered irregular, where there are longer runs of unsubstituted mannosyl units and block condensation of galactosyl units (16, 17).

b. Functional properties and applications

The solubility of galactomannan gums increases with the degree of galactose substitution. Guar and fenugreek gums are readily dissolved in cold water whereas locust bean gum is only slightly soluble in cold water but can be dissolved in

hot water. The hydration rate and solution viscosity depend on factors such as particle size, pH, temperature, etc. Guar gum solutions are reported to be stable over the pH range 4.0–10.5, and the highest hydration rate is reported at ~ pH 8.0 (18). Hydration rates are reduced in the presence of salts and other water-binding agents such as sucrose.

Like many polysaccharides found in nature, these galactomannans are polydisperse, high-molecular-weight polymers. Average molecular weight varies, typically from 1.0 to 2.5 million Daltons. The galactomannan molecules exist as an extended ribbon-like structure at solid state and adopt a flexible coil-like conformation in solution. All four types are highly efficient thickening agents. Given the same molecular weight and polymer concentration, the thickening powder decreases in the order of the increase of galactose contents, i.e., locust bean > tara > guar > fenugreek. The rheological properties of some galactomannan solutions show a considerable departure from classical random coil-like behavior (19). In particular, there is a lower coil overlap parameter $C^*[\eta] \sim 2.5$ in comparison with $C^*[\eta] \sim 4$ for most other disordered coils, and a stronger dependence of specific viscosity on concentration ($\eta_{sp} \propto c^{\sim 4.5}$ in contrast to $\eta_{sp} \propto c^{\sim 3.3}$). This is attributed to intermolecular associations at high concentrations.

Galactomannan gums are compatible with most hydrocolloids. There is a useful synergistic increase in viscosity and/or gel strength by blending galactomannan gums with certain linear polysaccharides including xanthan, κ-carrageenan, and agarose. The synergistic interactions are more pronounced with galactomannans of lower galactose contents.

Fenugreek and guar gums are non-gelling polysaccharides whereas locust bean and tara gum solutions may form weak gels upon freeze-thaw treatment, or by adding large amounts of ethylene glycol or sucrose. Gelation of galactomannans can also be induced by the addition of cross-linking agents such as borax and transition metal ions. The synergistic interactions of locust bean and tara gums with some gelling polysaccharides, such as κ-carrageenan and agarose, may enhance gelation, impart a desirable elastic character, and retard syneresis in these gels. These mixed gels have been used to form sheeted, fruit-flavored snack products and to produce hair gels.

Polysaccharides are generally considered non-surface active agents and the apparent surface activity is frequently attributed to the presence of small amounts of proteins. However, purified fenugreek gum (with less than 0.6% proteins and used in less than 1%) appears to be more efficient than guar and locust bean gums in lowering interfacial free energy. Fenugreek gum is also more concentration-efficient than gum arabic and xanthan gum in stabilizing oil/water emulsions.

Guar and locust bean gums are the most extensively used gums in the world. In the food industry they are widely used as thickening and stabilizing agents, usually in amounts of <1% of the food weight. They are used as stabilizers to improve shelf-life, to prevent creaming or settling in salad dressings, soft drinks, and fruit juices, to influence crystallization, and to improve freeze-thaw behavior of frozen products such as ice-cream and frozen desserts. They are also used as thickener or water-binding agents in pie fillings, icings, meat products, and pet foods. Addition of guar gum to baked products and pastry reduces the degree of starch retrogradation and improves texture and shelf-life. Guar gum is a potential dietary supplement in weight control and treatment of diabetes and hyperlipidemia. Guar and locust bean gums and their derivatives also have wide applications in other industries including pharmaceutical, mining, paper, textile, and construction (18).

2. Pectins

a. Source and structure

Pectin is a generic name for a group of polysaccharides extracted from cell walls of plant tissues. The main commercial pectins are produced from citrus peels and apple pomace, although other raw materials are also used including sugar beet pulp, sunflower seed head, peach pulp, and potato pulp. Pectin is mainly composed of an α-(1→4)-linked D-galacturonic acid backbone chain interrupted by α-(1→2)-linked L-rhamnose residues and the mole-percent of rhamnose varies with the source of pectin (20). The carboxyl groups of galacturonic acids are partly methylesterified and in certain pectins are partially acetylated. The degree of esterification higher than 50% is defined as high methoxyl (HM) pectin; the degree of esterification lower than 50% is defined as low methoxyl (LM) pectin. The molecules have "smooth regions" consisting of blocks of galacturonic acid residues and "hairy regions" with condensation of the side-chains. Often, arabinan, galactan, or arabinogalactan side chains are attached to the C-4 position of the rhamnose residues. Other sugars such as glucuronic acid, L-fucose, D-glucose, D-mannose, and D-xylose are sometimes found in the side-chains.

b. Functional properties and applications

In the solid state, pectin molecules exist as right-handed helices which are stabilized by intra- and intermolecular hydrogen bonding and/or intermolecular calcium ions forming an ordered and fairly stiff structure. It is generally accepted that the backbone adopts a worm-like conformation in solution. Several studies on the solution properties of pectins suggested that pectin molecules are further aggregated into either rods or segmented rods in solution and held together by non-covalent forces (21).

Pectins are highly heterogeneous with respect to their molecular weight. For isolated and purified pectins, the molecular weight is largely determined by the extraction modes and conditions. The average molecular weight from various fruit sources is typically the order of

10^4~10^5 daltons (22). Pectins are generally soluble in water and the solubility usually decreases with increasing ionic strength and molecular weight and decreasing degree of esterification. Aqueous pectin dispersions show rheological behavior similar to many other commercial polysaccharides, and the viscosity decreases with increasing temperature but increases with increasing concentration. The viscosity of aqueous pectin solutions also depends on molecular weight, degree of esterification, electrolyte concentration, type and concentration of co-solute, and pH. Addition of salts of monovalent cations, such as sodium chloride to pectin, dispersions reduces viscosity (23), whereas the addition of calcium or other polyvalent cations increases viscosity of pectin dispersions. In a calcium-free solution, viscosity decreases when pH is increased. Because of its low molecular weight, pectin is not an efficient thickener compared to other high-molecular-weight polysaccharides.

For both high methoxyl (HM) and low methoxyl (LM) pectins, gels can be prepared at concentration above 0.5~1%. Both high molecular weight and high concentration favor gel formation and enhance gel strength. Other intrinsic and extrinsic factors such as pH, amount and type of co-solutes, and the degree of esterification (DE) are also important with different effects on HM and LM pectins. HM pectins form thermally irreversible gels at sufficiently low pH (pH<~3.6) and in the presence of sugars or other co-solutes at a concentration of greater than ~50% by weight. The DE and overall distribution of hydrophilic and hydrophobic groups have major effects on gelation. Commercial HM pectin is classified as rapid-set, medium-set, and slow-set types. Sucrose and other co-solutes also affect gelation although to a lesser extent. The amount of co-solute required increases with increasing DE. LM pectins require calcium or other divalent cations for gelation, and the reactivity to calcium is governed by the proportion and distribution of carboxyl groups and DE. The relative amount of calcium affects the gelling properties of LM pectins considerably. Gelation is favored by increased soluble solids and decreased pH. LM pectin gels prepared at low pH are thermally reversible whereas those prepared at neutral pH are thermally irreversible.

The main uses of pectins are as gelling agents in various food applications including dairy, bakery, and fruit products. HM pectins have long been used in traditional jams and jellies, whereas LM pectins are used in low-calorie, low-sugar jams and jellies. Pectin gels can be used as an alternative to gelatine in fruit desserts and amidated LM pectins are used to prepare milk gels and desserts. Pectins are also used as a protein dispersion stabilizer in acidified dairy products such as yoghurt and milk-based fruit drinks and other protein drinks prepared from soya and whey. The enhanced emulsion stability is caused by interaction between pectin and protein particles (24).

3. Konjac Glucomannan

a. Source and structure

Konjac mannan, or glucomannan, is prepared from the tubers of the konjac plant (*Amorphophallus konjac*). Konjac is a perennial plant unique to Asia and is specially cultivated in Japan. In the production of konjac flour, two-year-old tubers are sliced into thin chips and subsequently dried and milled. Further purification of konjac flour is achieved by washing with ethanol aqueous solutions to remove proteins, lipids, and soluble salts. Purified konjac flour contains typically 88% carbohydrates, 1.7% ash, 0.9% lipids, and 0.5% protein (25). Konjac polysaccharide is a copolymer consisting of random blocks of β-D-glucose and β-D-mannose in the ratio of 2:3. All the monosaccharide units are joined together by 1→4 linkages with occasional substitutions of acetyl group (5–8%) attached at the 3 position of the mannose or both mannose and glucose residues (26, 27).

b. Functional properties and applications

In the solid state, konjac glucomannan has a crystalline structure. X-ray diffraction characterization revealed that it has extended two-fold helices (28). Konjac glucomannan yields a high viscosity solution, but extra efforts such as extensive stirring are needed to dissolve the gum. The solution is a typical non-Newtonian system showing shear thinning behavior. A thermo-irreversible gel is formed when konjac glucomannan solution is heated in alkali conditions. A possible gelling mechanism is that hydrolysis of the acetyl groups under alkali conditions promotes intra- and intermolecular hydrogen bonding leading to "cross-linking" of the glucomannan molecules. However, this hypothesis cannot explain why the gel is thermo-irreversible. Another interesting property of konjac glucomannan is its synergism with other hydrocolloids. Mixture of konjac glucomannan and xanthan gum form gels at a total concentration as low as 0.1%. Thermo-reversible gels can be prepared when konjac glucomannan is mixed with agarose or carrageenan solutions.

Alkali konjac gel is a popular traditional Japanese food (*Kon-nyaku*). Thermo-reversible gels formed from interactions between konjac glucomannan and other gums also have applications in products such as health jellies. Konjac flour is suitable for thickening, gelling, texturing, and water binding. It is a major ingredient for some vegetarian meat products and is also used in fat-free or low-fat meat products.

4. Soluble Soybean Polysaccharides

a. Source and structure

Water-soluble soybean polysaccharide (SSPS) is the cell wall material of the cotyledons of soybeans. Commercial SSPS is extracted with dilute acid from by-products produced from the manufacturing of soy proteins or tofu.

The extract is filtered and/or centrifuged, and dried to give a yield of ~45% (29).

SSPS is a highly branched pectic polysaccharide. The backbone consists of a galacturonan (GN) and a rhamnogalacturonan (RG). The RG structure consists of a diglycosyl repeating unit: (1→4)-α-D-galacturonic acid-(1→2)-α-L-rhamnopyranose units with β-D-galactan side-chains. The side-chains are substituted with L-fucosyl and L-arabinosyl residues, which are linked to the C-4 of the rhamnosyl residues. The degree of polymerization is estimated to be 43–47 which is longer than those of fruit pectins. SSPS is highly polydisperse in molecular weight. Three to four fractions with molecular weights ranging from 4,700 to 542,000 daltons were identified (30).

b. Functional properties and applications

Similar to gum arabic, SSPS is a low viscosity gum. It is soluble in cold water and exhibits Newtonian flow behavior at a concentration as high as 10%. When the concentration is increased to 20%, it exhibits shear thinning flow behavior. Unlike pectins, the viscosity of SSPS solution is not sensitive to salts, including NaCl, $CaCl_2$ and KCl; however, the viscosity increases with increase in sugar concentration (e.g., sucrose). The viscosity is also sensitive to pH and temperature, decreases with decrease in pH and increase in temperature, and the effect is reversible.

SSPS could be used in the food industry as a stabilizing and thickening agent. Addition of 10% SSPS has less effect than wheat bran on color and surface smoothness of Chinese steamed bread but has a stronger detrimental effect on volume and texture (29). Recent applications of SSPS include stabilizing milk proteins under acidic conditions (31).

5. Flaxseed Gum

a. Source and structure

Flaxseed gum is extracted from the seed or hull (seed coat) of the flax plant (*Linum usitatissimum*). Flaxseed gum is composed of a neutral arabinoxylan and acidic pectic polysaccharide. The neutral arabinoxylan has a (1→4)-β-D-xylosyl backbone to which arabinose side-chains are attached to the 2 and/or 3 positions. The acidic polysaccharide has a backbone of (1→2)-linked α-L-rhamnopyranosyl and (1→4)-linked D-galactopyranosyluronic acid residues, with side-chains of L-fucose and D-galactose, with the former at the non-reducing end. The ratio of L-rhamnose, L-fucose, D-galactose, and D-galacturonic acid is 2.6:1:1.4:1.7 (5, 32).

b. Functional properties and applications

Flaxseed gum exhibits Newtonian flow behavior at low concentrations and shear thinning behavior at high concentrations. Flaxseed gum is a low to medium viscosity gum and the viscosity is influenced by pH, with higher viscosity at pH 6–8, and lower viscosity at pH 2–6. Viscosity of flaxseed gum solutions decreases with increase in temperature. Flaxseed gum demonstrates surface activity and the ability to stabilize oil/water emulsions.

In food applications, flaxseed gum has been used as an egg white substitute in bakery products and ice creams. It can be used in medicinal preparations, e.g., ointments and pastes containing flaxseed gum are effective in the treatment of furunculosis, carbunculosis, impetigo, and ecthyma (33). Flaxseed gum is used as a bulk laxative, a cough emollient agent, and a stabilizer in barium sulphate suspensions for X-ray diagnostic preparations (34). Flaxseed gum solution is also used as a saliva substitute because it possesses lubricating and moisture-retaining characteristics resembling those of natural saliva (35). The stringy and fast drying properties of flaxseed gum make it suitable for hairdressing preparations and hand cream formulations. At 2.5% concentration, flaxseed gum is a good base for eye ointments. Other applications of flaxseed gum include printing, textile, and cigar papers.

6. Yellow Mustard Gum

a. Source and structure

Yellow mustard gum is a mucilage deposit in the epidermal layer of yellow mustard seed (*Sinapis alba)*. It is water-soluble and can be extracted from whole seed or seed coat (bran). Yellow mustard gum is a mixture of two polysaccharides, a linear 1,4-linked β-D-glucan with ethyl substitutes and a branched acidic rhamnogalacturonan composed of, disaccharide repeating unit: (1→2)-α-L-Rhamp-(1→4)-α-D-GalpA as backbone chains. The side-chains are composed of a terminal non-reducing end 4-O-Me-β-D-GlcpA, and/or (1→6)-β-D-Galp which is attached at the 4 position of the rhamnose residues in the backbone chain. The ratio of the 4-substituted and unsubstituted rhamnose is 2:1 (5).

b. Functional properties and applications

Yellow mustard gum solution/dispersion is a non-Newtonian system exhibiting shear thinning flow behavior and weak gel structures resembling xanthan gum. It also demonstrates surface activity and emulsifying capacity. The gum solution has a high viscosity over a wide range of pH. Similar to xanthan gum, yellow mustard gum has synergistic interactions with galactomannans leading to considerable increase in solution viscosity or formation of thermo-reversible gels (e.g., with locust bean gum).

Yellow mustard gum is an ideal stabilizer for salad dressings and fruit juice concentrates. It is also used in cosmetics, e.g., a skin care moisturizing lotion prepared using this gum gives a favorable hand feeling (36).

7. Cereal β-Glucan

a. Source and structure

Mix linked (1→3)(1→4)-β-D-glucans are cell wall polysaccharides of cereal endosperm and aleurone cells. The content of β-D-glucans in cereals follows the order of

barley (3–11%), oats (3.2–6.8%), rye (1–2%), and wheat (<1%). It is a linear, unbranched polysaccharide containing a single type of monosaccharide, β-D-glucose. β-D-Glucans from different cereals share a common structural feature: with consecutive blocks of (1→4)-linked β-D-glucose (mostly 2 or 3, and sometimes up to 14) interrupted by a single (1→3)-linked β-D-glucose. The β-(1→3)-linked cellotriosyl unit (trisaccharide) is the major building block (58–72%), followed by β-(1→3)-linked cellotetraosyl unit (tetrasaccharide, 20–34%). The ratio of cellotriose/cellotetraose is about 2.0–2.4 in oats, 3.0 in barley, and 3.5 in wheat (37).

b. Functional properties and applications

β-D-Glucan solution exhibits a wide range of rheological behavior, from viscoelastic fluid, weak gel to real gel, depending on molecular weight and concentration. Solutions of high-molecular-weight β-D-glucans are typically viscoelastic fluids of the "random coil" type. In contrast, low-molecular-weight β-glucans are able to form gels at reduced temperature or favored solvent properties. The gelling property depends on molecular weight and structural regularity (tri-/tetra ratio). The higher the tri-/tetra ratio, the more regular is the structure, and the easier in gel formation. Above the minimum molecular weight for gelation, lower molecular weight favors gel formation (5, 38).

Commercially, there are two β-D-glucan products: Oatrim and Glucagel. Oatrim is made by treating oat bran or flour with a thermo-stable α-amylase at high temperature. Its main component is dextrins, but its functionality appeared to be attributed to β-glucans and dextrins. Oatrim is used in bakery products, frozen desserts, processed meats, sauces, and beverages. Because it mimics the texture of fats, Oatrim is also used as a fat replacer in low-fat or no-fat products (39).

Glucagel is produced by partial hydrolysis of barley β-D-glucan and is a low-molecular-weight product (15,000 to 150,000 daltons) which can form gels at 2% concentration. Glucagel has gelling and fat mimetic properties and is used as a fat substitute in bakeries, dairy products, dressings, and edible films (40, 41).

Oat β-D-glucan is recognized as having important health benefits. It may lower cholesterol levels and reduces glycemic response, and is recommended as a good dietary fiber supplement. Oat β-D-glucan is also used in cosmetics as a moisturizer in lotions and hand creams.

8. Psyllium Gum

a. Source and structure

Psyllium gum is from the seed of *Plantago*, a plant comprising about 200 species of herbs or shrubs widely distributed in the temperate regions of the world. The gum is deposited in the seed coat (husk, hull), and has a long history of medicinal use.

Psyllium gum is a mixture of polysaccharides containing heteroxylans with a (1→3)- and (1→4)-linked β-D-xylopyranosyl backbone with short side-chains attached to position 2 of some of the 1,4-linked β-D-xylopyranosyl residues and to position 3 of other 1,4-linked β-D-xylopyranosyl residues (42). The side-chains consist of β-D-xylopyranosyl and α-L-arabinofuranosyl residues. 1→4-linked α-D-GalpA residues are also present in addition to small amounts of 1,2,4-linked Rha*p* and 1,3-, 1,6-, and 1,3,6-linked Gal*p*. The latter monosaccharides may arise from a small portion of pectic polysaccharides.

b. Functional properties and applications

Psyllium gum does not dissolve completely in water but swells to a mucilagenous dispersion with the general appearance of wallpaper paste (33, 43). A 2.0% psyllium gum dispersion exhibits gel-like structure, similar to that of xanthan gum which generates a "weak-gel" network by entanglement of rigid, ordered molecular structures (44). Increasing the concentration of psyllium gum from 1% to 2% gives a significant increase in gel strength. However, freshly prepared solutions/dispersions of psyllium gum (1%) show flow properties similar to those of disordered coils, with a Newtonian plateau at low shear rate. Upon aging, psyllium gum solutions form cohesive gels and show obvious syneresis. The gels continue to contract on storage over long periods (to about 30% of their original volume after three months). This contraction process can be accelerated by freezing and thawing cycles. However, psyllium gum is stable in high-salt solutions (e.g., 2.5 M NaCl) which are formed by neutralization of the alkaline extract over a prolonged storage, with no evidence of gelation or precipitation.

Psyllium gum is used with other gums in bakeries to replace wheat gluten. Adding psyllium gum and hydroxypropylmethylcellulose (HPMC) at 2% and 1%, respectively, to rice flour gives a loaf volume close to that of hard wheat control (43). The effectiveness of the psyllium-HPMC system arises from the psyllium network stabilizing gas cells formed during proving, and preventing them from collapsing during initial stages of heating in the baking oven. When temperature is further increased, HPMC starts to gel and stabilizes the gas cells.

As a dietary fiber, psyllium gum lowered plasma LDL-cholesterol levels by 6–20% in mildly hypercholesterolemic individuals, although conflicting results were reported (45, 46). Psyllium gum has been used as a demulcent in dysentery, erosion of intestines, dry coughs, burns, excoriations, and inflammations of the eyes (33, 47). It is extensively used as a bulk laxative. When ingested with the proper amount of water, it swells and increases the size of the fecal mass, and has a lubricant effect equal to that of oil but without oil's disadvantages. The water-holding capacity and gelling property of psyllium gum can be used to delay and reduce allergic reactions by holding toxins and allergens in the gel structure.

9. Pentosans/Arabinoxylans

a. Source and structure

Arabinoxylans or pentosans are the primary construction material of the cell wall in some cereals, such as wheat and rye, and are also present in oats and barley. According to solubility, cereal arabinoxylans are classified as water-soluble and water-insoluble. The water-soluble wheat arabinoxylans are present mainly in the flour (endosperm cell walls), while the water-insolubles can be found in both the bran and flour fractions. Insoluble arabinoxylans are cross-linked with proteins through phenolic ester bonds which can be rendered water-soluble by treating with alkali or specific enzymes.

Arabinoxylans consist of a backbone of (1→4)-linked β-D-xylopyranose residues to which α-L-arabinofuranose units are attached through O-3 and/or O-2,3 positions of the xylose residues. The distribution of the α-L-arabinosyl branches along the backbone chain are random with some regions heavily substituted and others unsubstituted (smooth domain) (48). The pattern and degree of substitution vary with cereal sources and tissue locations in the grain.

b. Functional properties and applications

Arabinoxylans are random coil polysaccharides in aqueous solutions, and exhibit Newtonian flow behavior at low concentrations and shear thinning behavior at higher concentrations. Because of the presence of ferulic acid, wheat flour arabinoxylans can form thermo-irreversible gels upon oxidation (48). In comparison, arabinoxylans from wheat bran can form thermo-reversible gels upon cooling (5). Wheat and rye arabinoxylans are important functional ingredients in baked products affecting water binding, dough rheology, and starch retrogradation. They also protect gas retention in dough due to viscous influence on the gluten-starch films (49).

C. Gums from Seaweeds

Seaweed or algae is an important natural source for hydrocolloidal gums. Most seaweed extracts are gelling agents. However, their chemical compositions and structures vary significantly due to the sources of raw material and processing conditions, so do their gelling mechanism.

1. Agar

a. Source and structure

Agar, or agar agar, is extracted from red seaweeds (*Rhodophycae*). It is commercially obtained from *Gelidium* spp. and *Gracilariae* spp. The Japanese are pioneers in processing agar, called "*Kanten*" in Japanese, meaning "cold days" or "cold weather," reflecting the weather conditions used to remove water from agar gels through freeze-thaw cycles which are still being used in modern agar processing plants. The alternative procedure is a syneresis method, in which the absorbed water is eliminated by applying a proper force.

Agar contains a major component, agarose, and a minor component, agaropectin. Agarose is the gelling component which has a molecular weight about 120,000 daltons. It is a linear polymer consisting of a (1→3)-β-D-galactopyranosyl and (1→4)-3,6-anhydro-α-L-galactopyranosyl residues as building units. Agaropectin is a heterogeneous mixture of polysaccharides of lower molecular weights. Their structures are similar to agarose but slightly branched and sulfated, and they may also have methyl and pyruvic acid ketal substituents (6, 50).

b. Functionality and applications

Agar is referred to as the queen of the gelling agents. The gelling property is due to the three equatorial hydrogen atoms on the 3,6 anhydro-L-galactose residues of agarose to form hydrogen bonds. The gel network contains double helices formed from left-handed threefold helices. These double helices are stabilized by the presence of water molecules bound inside the double helical cavity. Exterior hydroxyl groups allow aggregation of these helices to form suprafibers. The gelling temperature is around 38°C and the melting temperature is about 85°C, which gives very high gelling hysteresis. With this enormous gelling power and hysteresis property, agar has found broad applications in food and other industries. It is used in the baking industry because of its heat-resistant gel properties, and is widely used as a stabilizer in pie fillings, icings, toppings, and glazes. The heat-resistant gelling properties help to prevent chipping, cracking or sweating of icings, toppings, and glazes in baked goods. Agar is particularly effective in forming a stiff gel at low concentrations and it is compatible with most other gums. At around 40°C and upon setting, agar gel forms an impervious moisture barrier between icing and wrapping and it has the unique property of not sticking to the wrapping. Agar is also widely used in the confectionary industry as a gelling agent, and in preparing canned meat, fish, and poultry products.

Addition of a small portion of locust bean gum to *Gelidium* agar (1:9) may enhance gel strength and improve gel texture; in particular, it increases the elasticity of the gel (6). This is due to the synergistic interaction between locust bean gum and agar which has practical applications in the food industry where less brittle texture is desired. However, this interaction is only limited to agar from species of *Gelidium* and *Pterocladia.* Due to its excellent gelling characteristics and high resistance to metabolization by microorganisms, agar is used in biotechnology applications such as in microbiological culture media where it forms clear, stable, and firm gels.

2. Algin (Alginates)

a. Source and structure

Alginates or algin are the salts and derivatives of alginic acid, an acidic polysaccharide extracted from the brown seaweeds (Phaeophyceae). The main species of commercial significance include *Laminaria hyperborea*, *L. digitata*, *L. japonica, Macrocystis pyrifera, Ascophyllum nodosum*, *Eclonia maxima, Lessonia nigrescens, Durvillea antarctica*, and *Sargassum* spp. Natural alginate is insoluble due to its salt derivatives obtained during growth in seawater, including calcium, magnesium, and barium salts. In order to extract the material, a pre-extraction treatment is necessary to convert the insoluble cations into protons by treating milled algae tissue with dilute mineral acids. The next step is to neutralize with sodium carbonate or sodium hydroxide, and alginic acid is converted into water-soluble sodium salt. Following the removal of algal particles by sifting, floatation, centrifugation, and filtration, sodium alginate is precipitated by alcohol, calcium chloride, or mineral acids. A derivative of alginate, propylene glycol alginate (PGA), is produced from alginate by an esterification reaction with propylene oxide.

Alginate is an unbranched polymer containing β-(1→4)-linked D-mannuronic acid (**M**) and α-(1→4)-linked L-guluronic acid (**G**) residues (3, 51). It is a copolymer composed of three types of blocks: two homopolymeric blocks consisting of the same residues of M or G, respectively, and a block with strictly alternating residues (i.e., GMGMGMGM). The lengths and sequences of the three blocks vary considerably with the source of algae, place and season of collection, as well as part of the algae from which the alginate is extracted. The differences in chemical structures account for their differences in functionalities and applications.

b. Functional properties and applications

In commercial alginate products, the following factors are important in determining functionality: type of cations, M/G ratio, molecular weight and residual calcium. Sodium salt is the most common form of alginates. Potassium, ammonium, and calcium salts and free alginic acid are also available (3).

The primary function of alginates is to form thermally stable cold-setting gels in the presence of calcium ions. Alginate gels can be heat treated without melting although they may eventually degrade. Gelling properties and gel strengths of alginate gels depend on the type and mode of ion binding ($Mg^{2+} << Ca^{2+} < Sr^{2+} < Ba^{2+}$) (52). Therefore, the control of cation addition is critical for the production of homogeneous gels. High **G** alginates produce strong brittle gels with good heat stability (except if present in low-molecular-weight molecules), but water weepage (syneresis) occurs on freeze-thaw whereas high **M** alginates produce weaker but more elastic gels with good freeze-thaw stability. Two basic methods have been used to control the introduction of cross-linking ions: diffusion and internal setting. The diffusion method lets the cross-linking ion (e.g., Ca^{2+}) diffuse from an outer reservoir into an alginate solution which is used for the restructuring of foods such as artificial berries, pimiento strips, and onion rings. Internal setting differs from the former in that the Ca^{2+} ions are released in a controlled fashion from an inert calcium source within the alginate solution. The controlled release of Ca^{2+} is usually achieved by a change of pH and/or by limited solubility of calcium salt. The internal setting gels found wide applications in food, pharmaceutical, and cosmetic applications, such as cold water dessert gel, instant imitation bakery jelly, and facial masque (52).

The molecular weights of commercial alginates are between 12,000 and 190,000 daltons. Higher-molecular-weight favors gel strength and viscosity enhancement. However, it is sometimes desirable to use a higher concentration of low-molecular-weight alginates to obtain strong gels and to avoid excessively high viscosity prior to gelation.

Chemically modified alginates, e.g., propylene glycol esters of alginic acid (PGAs), are very different from alginates in functionality. They are much more tolerant to calcium ions and acidic environments such as in milk-based products and salad dressings (51). PGAs also exhibit surface activities due to esterification, and can be used as an emulsifier and foam stabilizer.

3. Carrageenans

a. Source and structure

Carrageenans are popular seaweed gums extracted from red seaweed (Rhodophycae). The main species used are *Eucheuma cottonii, E. spinosum. E. cottonii*, and *E. spinosum* which are spiny bushy plants growing along the coasts of the Philippines, Indonesia, and other islands in the Far East. Other species of commercial significance include *Chrondrus crispus* from around the coasts of the North Atlantic and *Gigartina* sp. from the cold deep coastal waters of South America. Based on the origins and processing conditions, three types of carrageenan gums are on the market, namely kappa (κ), iota (ι), and lambda (λ) carrageenans. The process of producing carrageenan gums is rather complex, involving washing and cleaning of the seaweed, alkaline extraction, followed by coarse and fine filtrations, solvent precipitations and/or freeze-thaw cycles and finally, drying, grinding, and blending.

Carrageenan gums consist of alternating (1→3)-linked-β-D-galactopyranose and (1→4)-linked-α-D-galactopyranose sugar units. The three gums differ from one another in their content of 3,6-anhydro-D-galactose and number and position of ester sulfate groups (53).

κ-Carrageenan is composed of alternating (1→3)-β-D-galactopyranose-4-sulfate and (1→4)-3,6-anhydro-α-D-galactopyranose units. It is produced by alkaline elimination of μ-carrageenan isolated mostly from the tropical seaweed *Eucheuma cottonii* (also known as *Kappaphycus alvarezii*). Typical food grade κ-carrageenans contain 25% of ester sulfate and 35% of 3,6-anhydro-D-galactose, which is close to the theoretical maximum.

ι-Carrageenan is composed of alternating (1→3)-β-D-galactopyranose-4-sulfate and (1→4)-3,6-anhydro-α-D-galactopyranose-2-sulfate units. ι-Carrageenan is produced by alkaline elimination of μ-carrageenan isolated from the Philippines seaweed *Eucheuma denticulatum* (also called *Spinosum*). The major difference between κ- and ι-carrageenan amount of 2-sulfate on the 1,4-linked 3,6-anhydro-D-galactose. As the degree of sulfation increases from 25% in κ- to about 50% in ι-carrageenans, there is a noticeable weakening of gelling properties arising from decreased potassium sensitivity.

λ-Carrageenan is mainly composed of (1→3)-β-D-galactopyranose-2-sulfate-(1→4)-α-D-galactopyranose-2,6-disulfate repeating. It is isolated mainly from *Gigartina pistillata* or *Chondrus crispus* and can be converted into θ-carrageenan (theta-carrageenan) by alkaline elimination.

b. Functional properties and applications

All carrageenan gums are highly flexible molecules which, at higher concentrations, interact with each other to form double-helical zones (3). Food-grade carrageenans have molecular weights in the range of 2–4 × 10^5 daltons. Gel formation of κ- and ι-carrageenans requires gel-inducing and gel-strengthening cations, such as K^+ and/or Ca^{2+} and it involves helix formation upon cooling from a hot solution. Carrageenans are used mainly as gelling, thickening, and suspending agents. κ-Carrageenan gels are firm, clear, and brittle with poor freeze-thaw stability. These gels may be softened (and are generally regarded to be synergistically strengthened) by mixing with locust bean gum. Ionic binding of ι-carrageenan is less specific and increasing ionic strength promotes formation of junction zones leading to soft elastic gels with good freeze-thaw stability. λ-Carrageenan is non-gelling as it lacks 3,6-anhydro groups in the 1→4-linked α-D-galactopyranosyl residues which are necessary for forming the initial double helix. λ-Carrageenan can act as a cryoprotectant, and a combination of λ-carrageenan with locust bean gum improves the freeze-thaw behavior of frozen products (54).

κ-Carrageenan stabilizes dairy products due to its interactions with casein micelles (~200 nm diameter), preventing whey separation. It is used in ice creams as a second stabilizer to prevent LBG and β-casein from phase separation (55). Carrageenan is also used as a water binder in cooked meats and as a thickener in toothpaste and puddings.

D. Gums from Microbial Fermentation

Some microorganisms produce slimy materials such as extracellular polysaccharides, structural polysaccharides, or intracellular storage polysaccharides. The extracellular polysaccharides are water-soluble and exhibit unique functional properties, and are hence an important source of hydrocolloids. Since the fermentation process can be controlled and processed in large quantity, gums from microbial production are more consistent than naturally occurring gums.

1. Xanthan Gum

a. Source and structure

Xanthan gum is an extracellular polysaccharide produced commercially by aerobic submerged fermentation from *Xanthomonas campestris* (56). The primary structure of xanthan gum is a β-(1→4)-D-glucopyranosyl backbone with tri-sugar unit side-chains consisting of a β-D-glucuronic acid residue between two D-mannopyranosyl residues, which is attached to the backbone at the 3-position of alternative β-D-glucosyl residues. In the side-chains, the terminal β-D-mannosyl residue is glycosidically linked to the 4-position of the β-D-glucuronic acid, which in turn is linked to the 2-position of the α-D-mannose. About 40% of the terminal mannose residues are 4,6-pyruvated while almost all the inner mannose is 6-acetylated (57).

b. Functional properties and applications

Xanthan is considered a non-gelling gum and is best known for its unique shear thinning flow behavior and weak gel structures. It hydrates rapidly in cold water, but proper hydration depends on particle size, solvent quality, and rate of agitation. Since the viscosity of xanthan gum solutions/dispersions is relatively temperature independent and the polymer is resistant to acids, alkali, and enzymes, it is a popular thickener and stabilizer in the food industry. The most striking property of xanthan gum is the very high low-shear viscosity coupled with its strongly shear-thinning characteristics. The relatively low viscosity at high shear rate provides the advantages of easy to mix, pour, and swallow, and the high viscosity at low shear rate gives good suspension and coating properties to colloidal suspensions at rest. This makes xanthan gum a perfect stabilizer for salad dressings, sauces, gravies, syrups, and toppings. In dairy products, such as ice creams, sour cream, and sterile whipping cream, xanthan gum provides optimal viscosity, long-term stability, improved transfer characteristics during processing, heat shock protection, and ice crystal control. In baked goods, xanthan gum contributes to smoothness, air incorporation and retention, hence improved volume, texture, and moisture retention of refrigerated dough. Blending of xanthan gum with dry cake ingredients helps uniform hydration

and batter mixing, which are important for the overall quality of finished cakes, particularly after storage (58).

Xanthan gum exhibits pronounced synergistic interactions with galactomannans and glucomannans (59, 60). These interactions result in increased viscosity, and in some cases formation of thermo-reversible, soft elastic gels. Experimentally, mixture of xanthan-locust bean gum forms thermo-reversible gels at polymer concentrations as low as 0.1%; in contrast, xanthan-guar gum mixtures only exhibit increased viscosity regardless of the concentration. It is necessary to heat the mixtures to about 90 to 95°C to achieve maximum synergism as this not only unfolds the xanthan structure but also helps to fully hydrate the locust bean gum. The extent of interaction between xanthan gum and galactomannans is reduced at low pH and high salt concentrations. The optimum ratio of interaction is 50:50 for xanthan–LBG mixture and 20:80 for xanthan–guar mixture (4).

2. Gellan Gum

a. Source and structure

Gellan gum is a bacterial exocellular polysaccharide, commercially produced by inoculating a fermentation medium with *Sphingomonas elodea* (ATCC 31461). Gellan gum is composed of a linear tetrasaccharide repeating unit: →3)-α-D-glcp- (1→4)-β–D-glcpA-(1→4)-β-D-Glcp-(1→4)-α-L-rhap-(1→ (61, 62). There are two acyl substitutions on the 3-linked glucose, a L-glyceryl at O(2) and an acetyl at O(6) positions. The average degree of substitution is one glycerate per repeat unit and one acetate every two repeats.

b. Functionality and applications

The functional property of gellan gum is dependent on the degree of acylation and presence of counter ions. High acyl (HA) gellan gum can form soft, elastic, transparent, and flexible gels by cooling the hot solutions when concentration is above 0.2%. The gels set and melt at 70–80°C with no thermal hysteresis and the gelling property is not affected by the presence of counter ions. In contrast, low acyl (LA) gellan gum forms hard, non-elastic brittle gels when binding with a wide variety of ions, such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and H^{+}; the divalent ions are more efficient than the monovalent ions in promoting gelation. The gel strength of LA gellan gum increases with increasing ion concentration until a maximum is reached. Increase of ion concentration also results in increased gel setting and melting temperatures. Sugar has a negative influence on the gelation of LA gellan gum which is often described to have a 'snap set' since gelation occurs rapidly once the setting temperature is reached. In contrast to HA gellan gum, significant thermal hysteresis is observed for LA gellan gels. However, under most conditions, LA gellan gels are not thermo-reversible below 100°C. The minimum gelling concentration of LA gellan gum can be as low as 0.05% in the presence of cations (63).

Both LA and HA gellan gums are commonly used as gelling agents and can be used to prepare dessert jellies with a variety of textures. Gellan gum is used in dairy products and sugar confectionery. LA gellan gum is used to modify traditional gelatin dessert jellies. An interesting application of gellan gum is to prepare structured liquids by applying shear stress either during or after gelation. These structured liquids are extremely efficient suspending agents (61).

3. Curdlan Gum

a. Source and structure

Curdlan gum is an extracellular polysaccharide commercially produced by microbial fermentation of a mutant strain of *Alcaligenes faecalis* var. *myxogenes*. Curdlan is a linear, homopolymer composed entirely of (1→3)-β-D-glucosidic linkages. Native curdlan has a granular structure and is water-insoluble but can be dissolved in sodium hydroxide solutions (64), cadoxen aqueous solution, and dimethyl sufoxide (DMSO).

b. Functional properties and applications

Curdlan gum can form gels by heating the suspensions or reduce the pH of an alkali solution. Heat treatment of curdlan suspensions may produce two types of gels, depending on temperature. A thermo-reversible low-set gel is obtained by heating gum suspensions at 55°C followed by cooling. A thermo-irreversible high-set gel requires heating up to 80°C or higher. The gel strength increases with heating temperature (gelation starts at 55°C) but stays constant between 60–80°C. Further increase in temperature results in steady increase of gel strength (64, 65). At the same heating temperature, gel strength increases with increased concentration. The addition of co-solute also has significant effects on gel strength, e.g., adding borate to curdlan suspension significantly increases gel strength, and adding urea markedly reduces gel strength (65).

Curdlan is a tasteless, odorless, and colorless product with wide food applications. It is used in making tofu noodles and other processed cooked foods as texture modifier, binding, and moisture improvement agents. Curdlan is used in noodle dough to reduce leaching of soluble ingredients, giving a clear broth with improved texture and mouthfeel. Addition of curdlan in processed meats modifies texture and improves water-holding capacity. Other food applications include processed rice cake, ice creams, jellies, sausages, and hams. It is also a popular low-energy ingredient in dietetic foods.

4. Dextran

a. Source and structure

Dextran is a group of α-glucans produced by exocellular bacteria Lactobacillaceae, particularly the genera *Lactobacillus, Leuconstoc*, and *Streptococcus*. Commercially important dextran is produced by *Leuconstoc mesenteroides* B-512 (F) (66). Dextran is a homo-polysaccharide composed primarily of 1→6-linked α-D-glucopyranose units (~95%) with branches at C-3 and/or C-4, occasionally at C-2. About 80% of the branches are single D-glucose. The molecular weight of dextrans can be 40 to 600 million daltons, however, the molecular weight of commercial food-grade dextrans is below 100,000 daltons.

b. Functional properties and applications

Dextran is a fine, white powder, very soluble in both cold and hot water giving clear solutions with low viscosity. A solution can be made to contain up to 50% dextran. The viscosity of dextran solution (2%) is independent of shear rate and unaffected by co-solutes, salts, or changes in the pH (3–10). Dextran has good water-holding capacity and imparts good bodying attributes to liquid systems. It also exhibits effective emulsifying and stabilizing properties.

The most important use of dextran is as a blood plasma extender with strict control of molecular weight (75,000 ± 25,000 daltons). Other pharmaceutical applications include cryo-protective agent to protect cells from freezing damage, suspending agent for X-ray opaque compositions, binder for tablets, stabilizing agent for water-insoluble vitamin preparations and a stomach-irritant preventive, a pharmaceutical taste masking agent, and a drug encapsulating agent in combination with methycellulose.

Dextrans with molecular weight below 100,000 daltons are permitted by the U.S. Food and Drug Administration as Generally Regarded as Safe (GRAS) until their deletion in 1973 due to the lack of use (67). The apparent lack of interest in dextran in foods is probably due to its high solubility and low solution viscosity (66). Bread prepared from yeast-raised dough containing 1–2% dextran was soft with a greater volume and longer shelf-life (68). The addition of dextran (up to 0.5%) to ice cream conferred excellent stability and heat-shock resistance. Dextran can be used as a stabilizer for confectionery to prevent crystallization, improve moisture retention, increase viscosity, and maintain flavor. It can also be used in soft drinks, flavor extracts, milk beverages, and icing compositions. Other food applications include fish products, meats, vegetables, and cheeses.

E. Chemically Modified Gums

Cellulose is the most abundant polysaccharide in plants. Natural cellulose is completely insoluble in aqueous media due to extensive intra- and intermolecular hydrogen bonding. However, certain treatments and chemical modification can render cellulose water-soluble and the derived products exhibit unique functional properties, making them useful as hydrocolloids in many industrial applications. About a third of the purified cellulose is used as base material for water-soluble derivatives and a wide range of products with designed properties, depending on the groups involved and degree of derivatization.

1. Microcrystalline Cellulose (MCC)

a. Source and structure

Microcrystalline cellulose (MCC) is produced by treating purified cellulose with a strong mineral acid, such as hydrogen chloride (69). Acid hydrolysis removes the amorphous regions of cellulose and gives a product consisting primarily of crystallite aggregates. Powdered MCC is produced by drying the acid hydrolysates. Dispersible MCC can be prepared by further processing the hydrolysates by wet mechanical disintegration, which breaks up the aggregates to microcrystals, followed by co-processing with a hydrophilic barrier or other hydrocolloids, such as guar gum, xanthan gum, or carboxylmethylcellulose.

b. Functional properties and applications

Colloidal MCC exhibits thixotropic flow behavior in which viscosity decreases with increase in shear stress, and once the stress is removed, viscosity will recover gradually over time (70). MCC dispersions have good thermo-stability which is useful in the preparation of heat-stable products. MCC can also be used to modify textures, resulting in a cleaner mouthfeel and good flavor release, and making a useful fat replacer in emulsion products. In addition, MCC is a good suspending agent for particles and solids.

MCC has many applications in pharmaceutical, food, and paper industries. Examples include bar mixes to add creaminess and pulpiness. In batters and breadings, MCC improves cling and reduces drying time and fat absorption during frying. MCC also adds creaminess in chocolate drinks in addition to its ability to suspend solids and add opacity to the product (69).

2. Carboxymethylcellulose (CMC)

a. Source and structure

Carboxymethylcellulose (CMC) is produced by reacting alkali cellulose with monochloroacetic acid. The substitution is mostly at 2-O and 6-O, occasionally at 3-O positions. The degree of substitution (DS) is generally 0.6–0.95 per monomeric unit (maximum DS is 3) (62, 71).

b. Functional properties and applications

CMC is readily dissolved in cold water, with maximum viscosity and best stability at pH 7–9. The rheological

properties of CMC are determined by the uniformity of substitution and degree of polymerization (DP). CMC solutions with medium to high DP are pseudoplastic, whereas solutions of low DP exhibit low viscosity and are less pseudoplastic. Medium and high viscosity gum solutions with 0.4–0.7 DS, especially those that are less uniformly substituted, are thixotropic. In contrast, evenly substituted, high DS, medium and high DP gums show no or less thixotropic behavior in solutions (71).

Gelation of CMC can be induced by adding trivalent metal ions (e.g., aluminum) or subjecting the solution to high shear. Gels ranging from soft to very firm can be prepared by selecting different grades of CMC and types of metal ions. The gel structure is also dependent on polymer concentration, DP, pH, and metal cation to carboxylate anion ratio.

Food grade CMC is widely used in the food industry as a thickener, stabilizer, and suspending agent. CMC can be used to improve the volume yield during baking by encouraging gas bubble formation; it is also used in frozen desserts and soft-serve ice creams to control ice crystal growth, and to improve mouthfeel, body, and texture. CMC is used in pet food to bind water, thicken gravy, aid extrusion, and bind fines. Because CMC is insoluble in acidic stomach fluids but soluble in alkaline intestinal fluids, it is a good coating for powders and tablets in the pharmaceutical industry. In the cosmetic industry, CMC is used as a stabilizer for hand lotions and vitamin-oil emulsions. CMC also found applications in textiles and detergents (62).

3. Methylcellulose

a. Source and structure

Methylcellulose (MC) is prepared by etherifying the available hydroxyl groups of cellulose chain by conversion of cellulose into alkali cellulose in sodium hydroxide, followed by reaction with methyl chloride (62). Many MC derivatives are available, but the most important one is hydroxypropylmethylcellulose (HPMC).

b. Functional properties and applications

MC solutions exhibit shear thinning flow behavior at 0.5% concentration and above. The pseudoplastic property is enhanced with increases in concentration and molecular weight. A unique functional property of MC and HPMC is their inverse temperature solubility and thermo-gelling property; they can form gels when the temperature reaches above a critical level due to hydrophobic interactions between high-substituted regions, which consequently stabilizes intermolecular hydrogen bonding. The gels break down upon cooling. Another unique property of MC and HPMC is their surface activity, making them useful emulsifiers for oil-in-water emulsions.

Due to the multifunctional properties, MC and HPMC are used as emulsifiers and stabilizers in French dressings and in low-oil or no-oil salad dressings (72). MC is often used in fried foods to reduce oil absorption through film formation and thermal gelation. In addition, batter adhesion and matrix food cohesion are improved by adding MC and/or HPMC. MC is used in baked goods including cakes, doughnuts, breads, cookies, fruit pie fillings, icings, and glazes. The thermal gelation property prevents boil-over of pastry fillings and aids gas retention during baking. MC retards water migration in frozen baked products during freeze-thaw cycles. MC is also used in non-dairy whipped toppings because of its surface active properties. In the pharmaceutical industry, MC is used in tablet film coating, controlled drug release and ointments. MC and its derivatives are also used as adhesives for wallpapers, controlled release agents for pesticides and fertilizers, and emulsifiers and stabilizers in shampoo and hair conditioners.

4. Hydroxypropylcellulose and Hydroxyethylcellulose

a. Source and structure

Hydroxypropylcellulose (HPC) and hydroxyethylcellulose (HEC) are prepared by reactions of alkali cellulose with propylene oxide and ethylene oxide, respectively, at elevated temperature and pressure (62, 73). The substitution patterns of HPC and HEC are random and lead to significant number of hydroxyl groups unsubstituted along the backbone chain. The molar substitution (MS) of commercial HPC is between 3–4; in comparison, commercial water-soluble grades HEC has a much wider MS value, from 1.8 to 3.8. The molecular weight of HPC and HEC ranges from 50k to 1,300k daltons.

b. Functional properties and applications

HPC is soluble in cold water, but becomes insoluble at temperature above 45°C. It has good film forming and surface active properties and can be used to stabilize toppings, especially at high ambient temperatures. HPC is soluble in ethanol, and has potential applications in alcoholic beverages. It is also used as a thickener in solvent-based adhesives, alcohol-based hair dressings, grooming aids, perfumes, inks, and paint removers. In the polymer industry, HPC is used as a secondary stabilizer in the suspension polymerization of vinyl chloride.

HEC with a DS greater than 1.6 is soluble in hot or cold water. The viscosity decreases with increase in temperature. HEC solutions exhibit Newtonian flow behavior at low shear rate and shear thinning behavior at high shear rate. It is useful as a thickener, viscosity control additive, protective colloid, binder, suspending agent, and film former in many industrial applications, including latex paints, emulsion polymerization, petroleum, paper, and pharmaceutical products. The largest use of water-soluble HEC is

for thickening latex paints because of its solubility, low foaming characteristics, high thickening efficiency, and good compatibility with universal coloring systems.

5. Chitin and Chitosan

a. Source and structure

Chitin is the second most abundant natural biopolymer on Earth and is composed of α-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucose (N-acetylglucosamine). Chitin is found in exoskeletons, peritrophic membranes, and cocoons of insects. Chitin is water-insoluble, but can be converted to water-soluble derivatives such as chitosan. Commercial chitin is prepared from shells of lobster, crab, or shrimp which are grounded and treated with 5% hydrochloric acid to remove minerals. The demineralized shell is further processed with pepsin or trypsin to remove proteins. Alkali deproteinization is also used and preferred if the final product is deacetylated chitin, i.e., chitosan. Chitosan is prepared by N-deacetylation of chitin under strong alkali conditions. The degree of acetylation of commercial chitosans is about 0.20 (74, 75).

b. Functional properties and applications

Chitosan is insoluble in organic solvents but soluble in aqueous acidic media. Following protonation of the amino groups, it forms a unique polycationic structure, while other polysaccharides usually give a neutral or anionic structure. Unlike most gelling polysaccharides, chitosan forms gels through chemical and enzymatic reactions. A thermo-irreversible gel can be prepared by treating chitosan in acetate salt solution with carbodiimide to restore acetamido groups. Gelation is probably attributed to hydrophobic interactions which are thermally favored. Chitosan gel can also be prepared by introducing large organic counter ions, such as 1-naphthol-4-sulphonic acid or 1-naphthylamine-4-sulphonic acid.

Chitosan is also chemically modified for specific applications, e.g., a stable and self-supporting gel can be obtained by enzyme treatment of tyrosine glucan, which is synthesized by reacting chitosan with 4-hydroxyphenylpyruvic acid. The enzyme tyrosinase oxidizes phenol to quinine which forms cross-links with the free amino groups. Chitosan can also be cross-linked with glutaraldehyde in lactic acid to produce a colorless, rigid, and infusible gel up to 200°C (74).

Chitin, chitosan, and their derivatives have a wide range of applications in pharmaceutics, biomaterials, foods, water treatments, biotechnology, cosmetics, textiles, and membranes. Chitosan is a good film former and is recommended for textile finishing, paper sheet formation, glass fiber coating, dye application, shrink proofing of wool, photographic application, and cement setting retardation. Chitosan is also used as a dietary fiber for weight control.

IV. FUTURE PROSPECTS AND NEW DEVELOPMENT

There have been continuous efforts in the field of hydrocolloids to reduce production costs, improve functionality, explore novel applications of existing gums, and discover new gums from natural resources. Although the use of new hydrocolloid gums in foods is limited by extremely high costs required to pass legislative approval, there are gums that have long history of use in foods which could easily get approval for food uses. Currently, we have a good understanding of the basic structures and functional properties of most gums, but problems arise on a daily basis at the production level due to inconsistency of the gum supplies and lack of knowledge on how these gums interact with other food ingredients, such as starch, proteins, oils, etc. Therefore, the following areas are recommended for future research: 1) improve manufacturing practice to produce consistent gum products; 2) further understand the structure-function relationships of hydrocolloids and their interactions with other food ingredients; 3) develop new hydrocolloids from natural sources. Most hydrocolloids, particularly non-starch polysaccharides, are conceived as dietary fibers. Some natural polysaccharides or oligosaccharides are biologically active, such as tissue repairing, anti-carcinogenic, anti-inflammatory, and immuno-modulatory. These polysaccharides are potential ingredients for functional foods or neutraceuticals in addition to their role as thickeners and stabilizers. More research should be directed to these bioactive polysaccharides.

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5 Food Protein Analysis: Determination of Proteins in the Food and Agriculture System

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CONTENTS

I. INTRODUCTION

A. Definitions and Perspectives

Protein analysis is the development of methods, instrumentation, and strategies for obtaining information about the quantity and composition of proteins within a sample; analysis also extends to the study of the kinetic and thermodynamic behavior of proteins. A food protein is any protein of interest, in food science and technology, in relation to enhancing the supply of food, which is wholesome, nutritious, affordable, and safe. Two broad areas of food protein analysis are readily recognizable: (i) quantitative analysis – determination of the total amount of protein, and (ii) qualitative analysis – fractionation, characterization, and identification of proteins. Of course, so-called qualitative methods also yield quantitative (i.e., numerical) data related to the concentrations of specific proteins in a mixture, molecular mass, isoelectric point, etc.

The major areas of application for food protein analysis are: (a) protein quantitation, (b) protein speciation – including the detection of cultivars, varieties, genetic polymorphism, and adulteration, (c) tests for physical functionality – defined as protein physicochemical properties of interest in food processing, (d) tests for nutritional quality, and (e) detection of bioactive agents including allergens, inhibitors, microbial toxins, as well as functional ingredients. Figure 5.1 summarizes some of the major techniques for food protein analysis

The aim of this chapter is to provide a brief outline of selected techniques for food protein analysis. The principles behind different techniques are well described in the general literature. Less well discussed are the many practical and scientific issues confronted by food protein analysts. In the remainder of Section I, we consider the scope for food protein analysis. Section II covers protein quantitation methods, Section III deals with protein fractionation analysis by

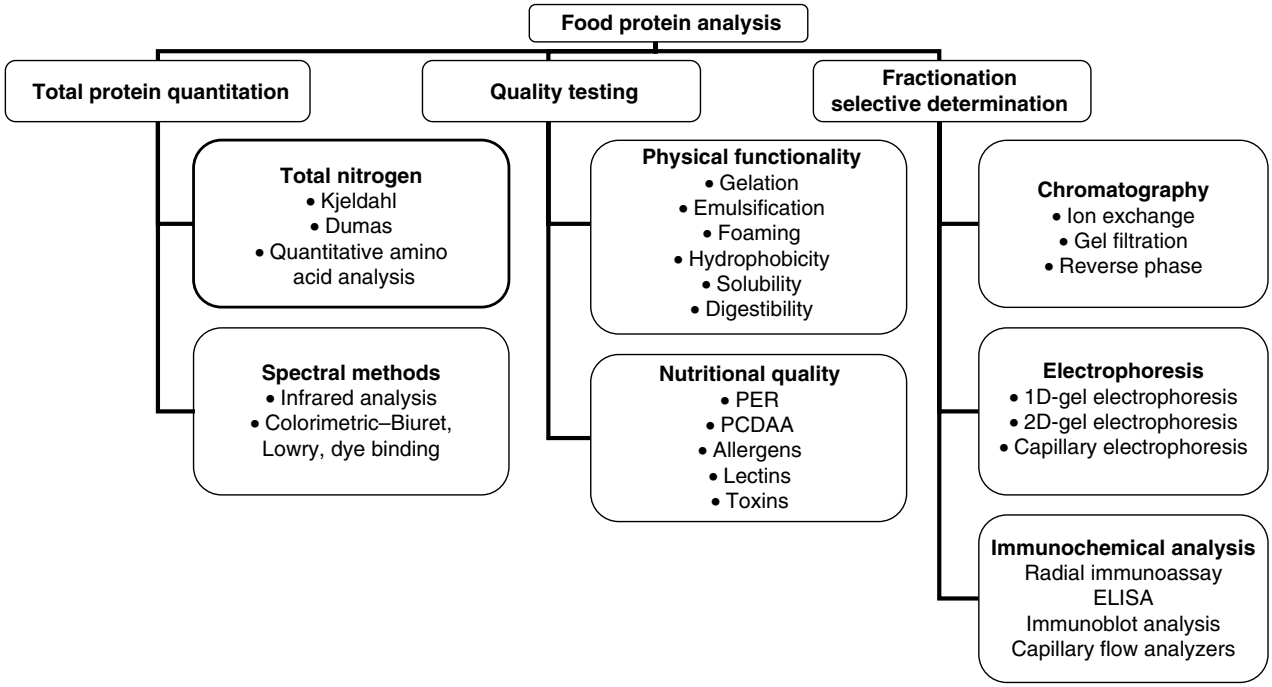

FIGURE 5.1 Some of the major areas of food protein analysis.

electrophoresis, chromatography and mass spectrometry. Section IV provides a brief overview of proteomics, which features high-throughput techniques for protein identification. Food protein analysis is a vast discipline. Only selected themes are introduced in this chapter. Some spectral methods, i.e., colorimetric (e.g., biuret, Lowry, Coomassie blue, bicinchoninic acid, etc.) or spectrophotometric (e.g., ultraviolet absorbance) methods of analysis, were reviewed recently (1) and are not covered here. The following monographs and reviews provide further reading (1–5).

B. Food Proteins

The major food protein groups are listed in Table 5.1. They are mostly industrial proteins produced from well-known food commodities, including milk, wheat, maize, soybean, peanut, canola, fish, egg, meat, some animal by-products. The manufacturing processes involve standard unit operations, e.g., particle size reduction, solubilization, membrane separation, ion exchange, and dehydration. Processing can lead to changes in protein structure, not all of which are "undesirable." Denaturation is effectively harnessed to create interesting high-protein foods (5).

A key requirement for food protein analysis is reproducibility. Consistent protein readings are necessary for fair decision-making about product quality, pricing, and compliance with food legislation. Other desirable assay characteristics include high speed, and low capital and running costs for analysis. Assay sensitivity is not a premium because food samples are readily available. On the contrary, large sample sizes lead to concerns about representative sampling. Accuracy, the closeness of a measurement to a real value, is not of paramount concern because bias (deviation from the true reading) is readily determined by calibration.

TABLE 5.1
Schema for Food Protein Analysis-1

Some Major Protein Ingredients	Specific Proteins
Animal protein ingredients	Specific animal proteins
• Blood proteins	• Blood serum albumins
• Egg protein	• β-Lactoglobulin, α-lactalbumin, casein
• Milk protein	• Ovalbumin
• Muscle – fish, poultry and meat	• Myosin
• Skin & feather – gelatin	• Collagen
• Carcass and by-products, bone meal	
Plant protein ingredients	Specific plant proteins
• Cereals – vital gluten	• Albumins, globulins
• Legume – protein isolates (soy, peas, sesame)	• Prolamins, glutelins
• Tuber – potato protein	• Residue proteins*
• Leaf protein	
Microbial proteins or single cell protein (SCP)	

* Residue proteins refer to cereal storage proteins not extracted using conventional Osborne solvents.

Table 5.2 shows the movement of protein within the food system from agricultural production through to the consumer. Protein analysis is important at all levels of the food system. Farm gate prices for many commodities, e.g., grain and milk, are partly determined by their protein

TABLE 5.2
Schema for Food Protein Analysis-2

The Food System	IFT[a] Expert Areas in Food Science
Agricultural production	Food chemistry & analysis
↓	Food safety & microbiology
Processing & packaging	Food processing & engineering
↓	Nutrition
Transportation & distribution	Other - legislation
↓	
Wholesale & retail	
↓	
Consumption	

[a] IFT refers to the Institute of Food Technologists.

content. Pricing of farm inputs (e.g., animal feeds) is also affected by levels of protein nitrogen. Recent legislation requires that feed intended for ruminants is free from animal protein. Protein ingredient manufacturers and users require functionality testing, ingredient standardization, optimization, and quality control (Figure 5.1). High protein foods (cheese, milk, meat, fish) are potential targets for adulteration and undeclared substitution – leading to a need for methods to ensure product authenticity. Detection of protein allergens and toxins assumes considerable importance at the consumer end of the food system. Table 5.2 lists also the main food science expertise areas recognized by the Institute of Food Technologists (IFT). All the sub-disciplines in food science offer some opportunity for protein analysis. The schemes in Figure 5.1 and Tables 5.1 and 5.2 provide an indication of the wide scope for food protein analysis.

C. Food Protein Analysis-General Considerations

According to accepted guidelines (6), one should consider the scope and purpose of food protein analysis – type of method (empirical, definitive, screening) and their intended use (research, quality control, surveillance, enforcement). Assay selection should follow desktop research (literature review) and discussions with people having relevant experience. One should obtain some notion of the strengths and disadvantages of different assays (bias, equipment cost, running cost, limit of detection, precision, specificity, speed, etc.). The degree of training necessary is another factor. Initial method development should be followed by optimization. As a rule, most off-the-shelf protein assays are not optimized for specific food samples with regard to interferences (additives, contaminants, flavors) or food matrix effects. Consider whether to use a commercially available ready-made kit. Sample clean-up may be necessary for complex foods, whether raw or processed. Particle size reduction and adequate mixing are important before sampling. Accuracy can be confirmed by comparing the results of one assay with that of an established method. Consider forms of sample storage and transport where protein tests are performed off-site.

II. QUANTITATION OF FOOD PROTEINS

A. Kjeldahl Method

Biological materials are transformed water, carbon dioxide, sulfur dioxide and ammonia when heated at 370–400°C in concentrated sulfuric acid. About 100–250 mg of food material requires 3–10 ml of concentrated sulfuric acid along with potassium sulfate, hydrogen peroxide, or metal oxide catalyst for digestion. There is sample charring followed by decomposition to form a clear-green liquid. Nitrogen (from urea, amino acids, peptides, proteins, nucleic acid, etc.) is converted to ammonium sulfate. Collection and quantitation of ammonia involves (a) neutralization with sodium hydroxide, (b) distillation and absorption by 4% standard boric acid, and (c) titrimetric analysis in the presence of a suitable titration indicator. Sample nitrogen, S_N (g-N per 100 g foodstuff), can be calculated from Eq. 5.1:

$$S_N = \frac{14.01 * V_{TITRANT} * N_{TITRANT} * 100}{Wt(g) * 1000} \qquad (5.1)$$

where $V_{TITRANT}$ is the volume of titrant corrected for blank readings, N is the normality of titrant (0.1M) and Wt(g) is the weight of food sample (grams) digested. Finally, values for S_N are converted to crude protein (cP) by multiplying with K_F (the nitrogen-to-protein conversion factor). For animal proteins K_F has a default value of 6.25 assuming that such proteins contain 16% N.

$$\text{cP (\%)} = S_N \text{ (g-N per 100 g foodstuff)} * K_F \text{(g-protein per g-N)} \qquad (5.2)$$

The accuracy of Kjeldahl analysis is impaired when samples contain high levels of non-protein nitrogen (NPN). For plant foods a default K_F-value of 6.25 overestimates protein content (7). Yeoh & Wee (8) determined K_F for 90 plant species from first principles. Leaf protein content was determined by amino acid analysis (next section). Then S_N was determined via the Kjeldahl method. From Eq. 5.2, we see that $K_F = cP/S_N$. Three distinct K_F parameters were identified: (i) K_A – ratio of protein to amino acid-N excluding amide-N from glutamine and asparagine residues, (ii) K'_A – ratio of protein to amino acid-N and ammonia, or (iii) Kp – Kjeldahl factor; this is protein content (from amino acid analysis) divided by S_N including contributions from NPN. As a matter of interest, the value for K_A, K'_A or Kp was 6.16 ± 0.05, 5.72 ± 0.12 or 4.43 ± 0.4 (g-protein per g-N), respectively.

Plant leaves contained 0.9–12% free amino acid and ≤30% NPN. Ezeagu et al. (9) reported K_F for 13 tropical seeds with a mean protein content of 19.83 ± 6.43% for

10-leguminoseae. They found $K_A = 7.13 \pm 0.23$, $K'_A = 5.94 \pm 0.34$, and $K_p = 4.97 \pm 1.07$. Clearly the default K_p value is not 6.25 for legumes. Multiplying the average value for S_N (4.14%) by K_p gave accurate estimates for cP (20.5%).

Mushrooms and other fungi have high levels of NPN, mostly ammonia, urea, free amino acids, and chitin. Mattila et al. (10) found that K_p was 4.7 ± 0.21 for several mushroom species. Protein levels reported for brown or white *Agaricus bisporus* (27% dry weight) were comparable to values found by Weaver et al. (11), but significantly lower than the value of 7% DW reported by others (12).

Kjeldahl analysis remains one of the most reliable methods for protein quantitation. Further research is needed to address the relation between Kjeldahl and other methods of food protein analysis (13), accuracy issues for K_p (7–10), rapid (colorimetric) methods for ammonia determination (14), and collaborative testing for novel foods (15,16).

B. Dumas Method — Combustion Nitrogen Analyzers

The sample is heated in a combustion nitrogen analyzer (CNA) oven at 950–1000°C within a high (99+%) oxygen atmosphere. Most of the gases produced by combustion (carbon dioxide, sulfur dioxide, and water vapor) are removed using adsorbents. The remaining nitrogen oxide is reduced to elemental nitrogen and measured by a thermal conductivity detector. Instrument output (S_N) is converted to cP after multiplying by K_F (Eq. 5.2). Table 5.3 shows some examples of CNA applications for food analysis. The Dumas method is approved by the CGC (Canadian Grain Commission), AOAC (Association of Official Analytical Chemist), AOCS (American Oil Chemists' Society), ASBC (American Society of Brewing Chemists), AFI (American Feed Industry), BRF-International (Brewing Research Foundation-International), IOB (Institute of Brewing), and EBC (European Brewing Convention).

TABLE 5.3
Analysis of Food Proteins Using the Dumas or Combustion Method

Sample	Reference
Animal feeds	Sweeney (17)
Brewing grains	Buckee (18), Johnson & Johansson (19)
Cereal grains	Bicsak (20), Williams, Sobering & Antoniszyn (21)
Dairy products	Wiles, Gray & Kissling (22), Simonne et al. (23)
Fruit	Huang et al. (24)
Infant food	Bellemonte, Costantini & Giammorioli (25)
Meat products	King-Brink & Sebranek (26)
Oilseeds	Duan & DeClercq (27), Berner & Brown (28)
Potatoes	Young, Mackerron & Davies (29)

Source: Adapted from Ref. 1.

Williams (21) discussed sources of error during combustion analysis. Equipment error is possible from infrequent or sporadic instrument use, poor maintenance, and instrument malfunction. Common sources of sampling error include incorrect weighing and the presence of trapped air (nitrogen). EDTA (ethylenediaminetetraacetic acid) used for calibration should be highly pure and low in moisture.

C. Quantitative Amino Acid Analysis

Amino acid concentration (C_T; mole per g) is routinely determined during the nutritional evaluation of many foodstuffs. Multiplying C_T by the formula weight for each amino acid (F_i, g per-mole) gives the weight of that amino acid (AA_m) per gram of sample (Eq. 5.3a). Protein content is the sum of all amino acids within the sample (Eq. 5.3b).

$$AA_m = C_T * F_i \tag{5.3a}$$

$$cP = \Sigma (AA_m) \tag{5.3b}$$

Results from quantitative amino acid analysis (QAA) are normally corrected for proline and tryptophan. Such corrections are necessary because acid hydrolysis of proteins destroys tryptophan, and many colorimetric reagents for detection of amino acids fail to react with proline (1). Nowadays, QAA is used mainly to assess values for K_F, which are needed for both the Kjeldahl and Dumas protein assays (7–10). Examples of QAA include the determination of proteins in peanuts (30), vegetables from Japan (31), meat, seafoods, baked goods, and fruits (32).

D. Near Infrared Analysis

Protein determination by near infrared (NI) analysis began in the 1970s (33,34). Protein, moisture, fat, starch, and fiber levels can be determined simultaneously. Other advantages of NI analysis include high sensitivity, high sample throughput, and high precision. NI analysis is non-destructive and the instrumentation is rugged and well suited for on-site operation. The AACC has approved NI analysis for cereals and grains including barley, oats, rye, triticale, wheat of all classes, and soybean (35). More recent developments have led to NI analysis for on-line or real time monitoring. Several monographs (36,37) and reviews (38–40) provide further details about NI analysis. There are very few reviews dealing solely with NI analysis of food proteins.

During near infrared reflectance (NIR) measurements, radiation reflected from the sample is sensed using a lead sulfide detector and an integrating optical sphere (41). Log (1/R) is linearly related to protein concentration. In the NI transmission (NIT) mode, a double beam of radiation passes through the sample and reference cells. Protein concentration is directly proportional to log (1/transmittance). Commercial NIR instruments include the Grain Quality Analyzer (Neotech Corporation, Silver

Spring, MD), Grain Analyzer Computer (Dickey-John, Auburn, IL), Infratec 1225 (Tectator AB, Sweden), Inframatic 8100 & 8144 (PerCon Instruments), InfraAlyzer 300 & 400 (Technicon) and the NIRSystems Model 6500 instrument (NIRSystems,[1] Silver Spring, MD). The following instruments were used in an NIT mode: MilkoScan 104, 203B, 605, etc. (Foss Electric), Multipec M (Shields Instruments, UK) and the NIRSystems Model 6500 instrument (NIRSystems, Silver Spring, MD). Recent developments in NI instrumentation have been reviewed (36,37). Collaborative trials were reported for NI analysis for milk (42), barley (43), wheat (44–46), meat (47), and kernel wheat (48).

1. Near Infrared Analysis of Grain Proteins

A recent example of grain analysis by NIR is described by Corbellini & Canevara (49) using 100 bread wheat (*T. aestivum* L.) samples from Italy. Analysis was performed for ground wheat as well as kernel wheat. Over the range of 7.8–14.7% protein ($S_N \times 5.7$) content, the correlation coefficient (r) for NIR and Kjeldahl results was > 0.98. Other protein-related quality attributes are measurable by NIR (Table 5.4). It is possible to correlate NI instrument output with grain moisture content, kernel hardness, sedimentation value, water absorption, and loaf volume. In addition to multilinear regression analysis, sophisticated multicomponent analysis, such as principal component analysis or partial least squares calibration, enable grain quality assessment by NIR (50, 51).

TABLE 5.4
Characterization of Grain Quality Using Near Infrared Analysis[a]

Sample-Type of Analysis	References
Bread making quality — water absorption and protein content	Delwiche & Weaver (52), Delwiche et al. (53), Millar & Bar L'Helgouac'h (54)
Essential amino acids	Fontaine et al. (55)
Grain grading, hard red winter vs. spring wheat — protein content	Delwiche et al. (56)
Heat damage (wheat kernels)	Wang et al. (57)
Insect infestation — insect protein, chitin & moisture	Ridgway & Chambers (58)
Kernel hardness — γ-zein content	Eyherabide et al. (59)*
Vitreous vs. non-vitreous wheat	Dowell (60), Wang et al. (61)

[a] NIR unless otherwise indicated with (*) for NIT analysis.

[1] A number of corporate takeovers have affected producers of NI instruments. Neotec Corporation (1966–1981) introduced the first commercial NI instrument in 1972 and microprocessor-controlled NI instruments in 1974. The company was bought by Pacific Scientific Corporation in 1981. In 1989, Perstorp Analytical (Sweden) purchased the NIR operations from Pacific Scientific and renamed it NIRSystems. Ownership passed to Foss-Electric (Denmark) in 1997. Foss-NIRSystems is based in Silver Spring, MD, the home of Neotech Corporation. Extract from http://www.foss.dk/c/p/default.asp?width = 1024.

2. Near Infrared Analysis of Meat and Milk Proteins

NIR results for ground lamb and beef were affected by sample homogeneity, particle size, and temperature (62). Results from NIR and Kjeldahl analysis showed only moderate correlation ($r = 0.67–0.8$). Higher correlation of $r \approx 0.99$ was reported in more recent studies for protein determination in fresh chicken (63), mutton (64), trout (65), and meat patties (66). The precision of NIR results appears to match or surpass those from Kjeldahl results. Furthermore, the NIR format leads to greater reproducibility as compared to NIT. However, the coefficient of variation for meat protein analysis (2.71–3.43%) was considered too high for NIR to be adopted for regulatory purposes (67). Sources of error for NIR and NIT measurements (21) include instrumental error, temperature variations, changes in relative humidity, and stray light. Operator error is often the result of inadequate training. NIR was applied for protein determination in milk powder and semi-solid dairy products including whey protein (68), nonfat dried milk (69), cheese (70,71), and fermented products (72,73).

III. FRACTIONATION ANALYSIS OF FOOD PROTEINS

A. Electrophoresis and Chromatography

Electrophoresis and chromatography are the foremost methods for food protein fractionation (74,75). High resolution polyacrylamide gel electrophoresis (PAGE) is available in a range of formats. Chromatography also offers a variety of separation chemistries (Table 5.5). The principles of electrophoresis and chromatography are described in References (76–78). During PAGE analysis, protein molecules migrate under the influence of an applied potential difference. The rate of migration is

TABLE 5.5
Methods for Food Protein Fractionation*

Electrophoresis	Chromatography
Gel electrophoresis	*HPLC or FPLC*
Native PAGE, acid-PAGE, urea-PAGE, SDS-PAGE, Isoelectric focusing (IEF)-PAGE, two-dimensional PAGE	Size exclusion, ion exchange, hydrophobic interaction, reverse phase, hydroxyapatite, affinity
Capillary electrophoresis	
CAE, CGE, CIEF, HICE	

* See accompanying text for explanation of abbreviations.

dependent on protein charge-to-mass ratio. Protein bands separated by PAGE are visualized by staining (for example, with Coomassie blue dye or silver stain), destaining, and scanning with a densitometer. Native-PAGE is widely applied for identifying genetic polymorphism in milk and cereal proteins. A single amino acid difference for beta-lactoglobulin A and B leads to distinct protein bands during native-PAGE. Acid (acid-PAGE) or urea (urea-PAGE) can be added to gels in order to improve protein solubility. Acid-PAGE allows the identification or differentiation of cereal cultivars based on the analysis of their storage proteins (79,80). Fractionation of milk caseins frequently involves urea-PAGE (81,82).

Addition of ampholyte to native or urea-PAGE formats yields isoelectric focusing-PAGE (IEF-PAGE), which fractionates proteins on the basis of their isoelectric point (pI). IEF-PAGE requires high voltages and consequently the gels are thinner than those used for conventional PAGE to allow increased heat dissipation. Another PAGE format of interest involves sodium dodecylsulfate (SDS). Addition of this detergent encourages protein subunit dissociation, denaturation, and transformation into negatively charged polymers. During SDS-PAGE, proteins are separated on the basis of their molecular mass. Examples of food proteins analyzed by PAGE are provided in References 74, 75, and 83.

Proteins can be transferred from polyacrylamide gels by electroblotting and fixed to synthetic (nitrocellulose, polypropylene, or polyvinylinefluoride) membranes before staining. The membrane-bound protein can also be analyzed using highly specific antibodies as probes. Immunoblotting is an important screening method for food allergens (84,85). Finally, protein zones from PAGE can be excised for analysis using techniques such as mass spectrometry (see below).

Capillary electrophoresis is a relatively recent development in food protein analysis (86,87). Proteins are separated within a small diameter capillary. Various detectors are compatible with CE including fluorescence, refractive index, UV absorbance, and mass spectrometers. A range of protein separation methods is possible, such as capillary affinity electrophoresis (CAE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), and hydrophobic interaction capillary electrophoresis (HICE). The principles of CE are described in several reviews (77,86,88,89). Applications to dairy and cereal products are discussed in References 90 and 91, respectively.

B. Mass Spectrometry of Food Proteins

The principles of mass spectrometry (MS) are well known. Only a brief outline is provided here. A food sample is bombarded with high energy electrons in an evacuated chamber. Molecular ions produced by bombardment are accelerated via an electric field, past a set of electromagnets, towards a detector. The detection time for molecular ions is proportional to their mass-to-charge ratio $(m_i/z_i)^{½}$. Techniques for generating protein ions for MS analysis were only developed in the mid-1980s. The best known of these desorption techniques are fast atom bombardment (FAB), matrix assisted laser desorption ionization (MALDI), and electrospray ionization (ESI). The theory, principles, and instrumentation for protein MS analysis have been reviewed (92). Table 5.6 summarizes each desorption method and types of information available from MS analysis of proteins. Process-induced changes in food proteins appear to be readily detectable. For example, ESI-MS analysis of beta-lactoglobulin from 109 cows showed it to be covalently modified by a 324 Da species, probably lactose (93). The mechanism of heme protein denaturation was also examined by ESI-MS (94). Proteolysis of caseins during cheese manufacture and ripening was readily followed by MALDI-MS (95). Virtually all the major food protein groups have been analyzed by MS including milk, egg, meat and cereal proteins as reviewed in References 96–98.

TABLE 5.6
Mass Spectrometric Analysis of Food Proteins

Technique	Comments
FAB	• Protein + glycerol are bombarded by Xe or Cs ions at 8–40 keV. Sample is introduced into MS port.
ESI	• Protein solution disintegrates from the tip of capillary polarized at ±3000–5000 V. Electrospray is fed to MS port.
MALDI	• Protein + large excess of crystalline matrix is irradiated by a laser. The matrix absorbs energy, vaporizes, and ionizes protein.
Application areas*	
Aggregation	Molecular mass analysis
Cheese ripening and maturation	Polymorphism
Denaturation	Post-translational modification
Glycation	Process effects
Heat effects	Proteolysis
Irradiation	Purity
Meat postmortem	Sequence determination
	Sulfur/disulfide exchange

* Compiled from References 92–98.

IV. PROTEOMICS

The term proteome was introduced by Wilkins et al. in 1995 to describe the "entire PROTEin complement expressed by a genOME, or by a cell or tissue type" (99). Proteomics is the wholesale identification of proteins comprising a proteome using large-scale, high-throughput technologies, primarily 2D gel electrophoresis and MS. In

the so-called post-genomics era, interest has extended to the subsidiary topics of transcriptomics, proteomics, and metabolomics (Figure 5.2). The transcriptome and metabolome refer to the total profile of RNA and metabolites within a cell, respectively (100). Protein functions other than metabolism – the entirety of enzymatic reactions within a cell – are also of interest. Proteomics is considered a protein-based method for gene expression analysis.

The rationale for proteomics can be seen from the central dogma of molecular biology, postulated by Francis Crick in 1958. The direction of information flow within cells is DNA → RNA → protein → function (101). Transcription of DNA leads to time-dependent expression of cell function. However, the relation between genome and cell function is complicated due to the presence of regulatory mechanism, redundancy, or amplification processes during information transfer. The study of proteomics is clearly essential because (i) proteins are the machinery that perform day-to-day functions within a cell, (ii) there is a low correlation between the number of genes and the number of proteins within a cell, (iii) differential gene expression occurs at different times and in different parts of the organism, and (iv) single genes can encode for more than one protein due to post-translational modification.

There are five essential steps for proteomics research: (i) sample preparation – cells, tissues, or organelles are homogenized to produce a protein extract. Care is needed to avoid protein modification by endogenous proteases, chemical modification or denaturation; (ii) protein separation by 2D electrophoresis. Typically, IEF (1D) and SDS-PAGE (2D) analysis is followed by protein visualization and densitometry leading to a 2D digital representation of the separated proteins. With the new generation of densitometers it is possible to perform a comparative image analysis of hundreds of protein spots resolved by 2D electrophoresis. The key is to discover differences between specific protein (spots) for the control and treatment sample; (iii) protein identification – protein spots of interest are excised from the 2D gel, digested with trypsin, then subjected to HPLC or CE analysis; (iv) peptide sequencing – the products of proteolysis are analyzed by MALDI-MS or ESI-MS as described in Section IIIB. Sophisticated MS instrumentation can now provide protein molecular mass as well as sequence information, (v) Bioinformatics – the application of computerized informatics tools to biological data. Peptide sequences are compared with the DNA- sequence database in order to identify the protein of interest. Digitized 2D gel patterns can also be compared directly with computerized library data for protein identification (99).

• DNA→	• mRNA→	• Proteins→	• Function

(A)

Genome	Transcriptoms	Proteome	Metabolome
Genomics	Transcriptomics	Proteomics	Metabolomics

(B)

FIGURE 5.2 Information flow in cells is from DNA (genome) to mRNA (transcriptome) to proteins (proteome) to a variety of protein functions (phenome, physiome, and metabolome).

The impact of proteomics on food science and technology could be considerable (100,102). It may be possible to correlate changes in protein expression or post-translational modification with specific treatments, be they developmental, environmental, nutritional, or hormonal. Potential areas for proteomics research in food related areas include the study of protein structure function relations, functional ingredients, food-borne pathogens (103), allergens (104), food adulteration, novel ingredients, starter cultures (105), muscle or meat science (106), and the identification of protein markers for grain quality (107).

V. CONCLUSION

Aspects of food protein analysis are reviewed in this chapter. Food proteins were defined as those proteins which are of interest in food science. Food protein analysis is a vast topic but still emerging as an integrated discipline. Novel techniques for protein quantitation and characterization are being developed. The food system and food science expertise areas (Tables 5.1–5.2) were suggested as schema for defining the perspective of this rapidly evolving subject. The first part of the chapter emphasized "approved" methods for protein quantitation which are used within the food industry (Figure 5.1). Further research on Kjeldahl and Dumas analysis is needed to establish national and international protocols for a wide range of foods. Further developments in NIR analysis are needed in the areas of instrumentation and pattern recognition software. On-line NIR analysis for products on conveyor belts will probably increase (108). An example of a commercially available NIR on-line instrument is the MM170 analyzer from NDC Infrared Engineering (109). The trend towards diode array NIR instrumentation will lead to more rapid acquisition of spectra, increased portability, and more affordable instrumentation. Section III dealt with selected methods for protein fractionation, and the chapter culminated with a discussion of proteomics (Section IV). Though less than 10-years old, high-throughput protein analysis within a proteomics framework is now firmly on the food science agenda.

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6 Protein: Denaturation

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CONTENTS

I. INTRODUCTION

Proteins perform various functions in processed foods. The functional properties of proteins, such as foaming, emulsifying, gelling, thickening, texturizing, dough forming, whipping, curdling, water binding, flavor binding, and fat binding properties, are important for imparting desirable sensory attributes in a variety of food products. These various functional properties of proteins emanate from two molecular attributes of proteins, namely hydrodynamic properties and physicochemical attributes of the protein's surface (1). While the hydrodynamic properties relate to the size and shape of the molecule, the properties of a protein's surface relate to its topology and to the pattern of distribution of polar and non-polar patches. Although these molecular attributes of proteins in their native state can be determined fairly precisely from their crystallographic structure, it has been difficult to predict their functionality in a complex food milieu. This is principally due to denaturation of proteins that inevitably occurs during food processing, which might alter both their hydrodynamic attributes (shape and size) and surface characteristics. In the denatured state, the intensity of intermolecular interactions between proteins, or between proteins and other constituents in the food milieu, would dramatically alter the proteins' functionality.

The extent of denaturation of proteins in a food milieu depends on the susceptibility of intra-molecular interactions that hold the compact native protein structure to temperature, pressure (shear), pH, ionic strength, types of ions, and specific and non-specific interactions with other food components such as sugars, polysaccharides, lipids, and other additives. It should be recognized, however, that from a food application standpoint, protein denaturation during processing is not always undesirable. In fact, in some cases it is highly desirable. For instance, partial denaturation of proteins at the air-water and oil-water interfaces improve their foaming and emulsifying properties (2, 3), whereas excessive thermal denaturation of soy proteins diminishes their foaming and emulsifying properties (4). In protein beverages, where high solubility and dispersibility of proteins is a necessity, even partial denaturation of protein during processing may cause flocculation and precipitation during storage and thus may adversely affect the sensory attributes of the product. Thus, to develop appropriate processing strategies, a basic understanding of the environmental and other factors that affect structural stability of proteins in food systems is imperative.

II. PROTEIN STRUCTURE

Protein structure is a highly complex architecture. A polypeptide chain containing hundreds of main chain covalent bonds can theoretically assume numerous configurations in three-dimensional space. Yet, under physiological pH, temperature, and ionic strength conditions, proteins always assume a particular native folded conformation. This native conformation represents a thermodynamic equilibrium state in which the free energy of the molecule is usually at the lowest possible level and it is achieved through optimization of various intra-molecular interactions within the polypeptide chain as well as interactions of its constituent amino acid residues with the surrounding aqueous medium.

At the molecular level, protein structure can be defined in terms of four levels. The primary structure denotes the linear sequence of amino acid residues in the polypeptide chain. Intuitively, one would expect this linear structure to possess chain flexibility similar to that of random coil polymers. However, this is not the case. The amide linkage between amino acid residues, which constitute one-third of the covalent bonds in the backbone of a polypeptide chain, possesses a partial double bond character and this substantially restricts the flexibility of the chain. Furthermore, depending on the amino acid sequence, the bulky side chains of amino acid residues impose additional restrictions on local flexibility of polypeptide chains. As a result of such sequence-imposed local restrictions on flexibility, polypeptides do not behave like other natural polymers, such as polysaccharides or nucleic acids, and synthetic polymers in solution. These restrictions on local flexibility play the critical role of guiding the protein toward attaining a particular folded native conformation. Thus, the primary structure of a protein is believed to possess the coded information for its final three-dimensional structure.

The secondary structure refers to regular conformations in polypeptide chains. This regularity of conformation occurs when the dihedral angles of each amino acid residue in a segment of the polypeptide assume the same set of values. The α-helix and β-sheet are the two regular structures found in polypeptides. Segments of polypeptide chain where consecutive amino acid residues assume different sets of dihedral angles tend to be in a disordered state and those regions are termed a periodic structures. The secondary structures in proteins arise as a result of short-range non-covalent interactions that tend to minimize local free energy of the protein chain.

The tertiary structure refers to the overall three dimensional arrangement of the folded polypeptide chain. For most proteins, the folded tertiary structure is roughly spherical in shape with irregular topography. The crevices on the surface, which are inaccessible to solvent water, are non-polar in nature. In food proteins, these crevices act as binding sites for hydrophobic ligands, such as fat and flavor molecules. The tertiary structure of a protein is the net result of optimization of various short and long-range interactions within the polypeptide chain and represents a state that has the lowest possible free energy under physiological conditions. In a monomeric protein, i.e., a protein that contains only one polypeptide chain, the surface of the protein that contacts with surrounding solvent water is predominantly polar and hydrophilic. However, in some proteins, depending on amino acid composition and sequence, some surface regions of the tertiary fold may be non-polar. In such instances, the protein molecules aggregate via hydrophobic interaction; an aggregated structure of the protein, which contains more than one polypeptide chain, is referred to as the *quaternary* structure.

A. Non-Covalent Forces

Folding of a protein from a nascent unfolded state to a folded native conformation is driven by several non-covalent interactions within the molecule. These include van der Waals forces, steric strains, hydrogen bonding, electrostatic, and hydrophobic interactions. The van der Waals interactions are short range in nature and therefore involve interactions between neighboring atoms. Although most of the main chain and side-chain covalent bonds in proteins are single bonds, their rotational freedom is hindered because of steric constraints from side-chain groups. Thus, steric strains indirectly limit the number of configurations accessible to various segments of polypeptides.

Proteins contain several groups that can form hydrogen bonds. The greatest number of hydrogen bonds in proteins occurs between the NH and CO groups of main chain amide bonds. The maximum strength of hydrogen bonds formed between N-H and C=O groups in proteins is about 4.5 Kcal mol^{-1}. The majority of hydrogen bonds in proteins occur in α-helix and β-sheet structures. The stability of these secondary structures is partly attributable to these hydrogen bonds. The vectorial orientation of hydrogen bonds in α-helices renders it to behave like a macro dipole. When two such α-helices come close and orient themselves in anti-parallel directions and form a bundle, the macro dipole-dipole interactions between these helices can stabilize such conformations (5). However, because water itself can hydrogen bond with amide groups in proteins, formation of hydrogen bonds between amide groups in proteins is not thermodynamically stable in an aqueous environment. More importantly, they do not, and cannot, act as the driving force for protein folding. Thus, their existence in α-helix and β-sheet structures might be the result of other interactions that create a non-polar environment where the hydrogen bonding and other macro dipole-dipole interactions become stable. Based on these considerations, it is fair to say that hydrogen bonds in proteins are only pseudo-stable and their stability depends on maintenance of the non-polar environment.

Electrostatic interactions in proteins at neutral pH arise mainly between the positively charged ε-amino groups of lysine, arginine, and histidine residues and the side-chain carboxyl groups of glutamate and aspartate residues. Depending on the relative numbers of these groups, a protein assumes either a net positive or a net negative charge at neutral pH. The stability of electrostatic interactions is dependent on the dielectric constant of the local environment. They are stronger in a non-polar environment than in a polar environment. In aqueous solutions, because of the high dielectric screening effect of water on charged groups, attractive and repulsive electrostatic interactions between charged groups in proteins are very insignificant. Thus, charged groups located on the surface of the protein do not greatly influence the stability of protein structure. On the other hand, if a salt bridge occurs between two buried opposite charges in the interior of a protein, where the dielectric constant is about 2–4, it can contribute very significantly to the structural stability of the protein.

Hydrophobic interactions between non-polar side-chain groups are considered to be the major driving force for protein folding in aqueous solutions. These interactions arise as a result of thermodynamically unfavorable interaction between solvent water and non-polar groups in proteins. The unfavorable free energy change occurs neither because of phobia between hydrocarbon and water nor because of attraction between hydrocarbons. In fact, the negative free energy change for dipole-induced dipole interaction between water and hydrocarbons is greater than the induced dipole-induced dipole interactions between hydrocarbons. The origin of hydrophobic interactions is rooted in the fact that the affinity between water molecules is much greater than between water and hydrocarbon; that is, the negative free energy change for water-water interaction is greater than water-hydrocarbon interaction. Because of this thermodynamically driven preferential interaction, water tends to minimize the surface area of direct contact with hydrocarbon chains and maximize its interaction with other water molecules. Water accomplishes this by forcing the hydrocarbon chains to aggregate. In proteins, this hydrophobic aggregation/association process is the main driving force for the folding of the protein chain. As the non-polar residues are removed from the aqueous environment, the non-polar regions created within the molecule allow formation of hydrogen bonds in such water-deficient regions as the interior of α-helix and β-sheet structures.

From the above discussions, it can be summarized that the folding of a protein from a nascent unfolded state to a folded native state is driven by a simple but fundamental thermodynamic requirement that a majority of non-polar groups be buried in the interior of the protein, away from contact with the aqueous phase, and that a majority of hydrophilic polar and charged groups be located on the surface of the protein in contact with the surrounding aqueous phase in such a manner that the global free energy of the molecule is at the lowest possible level (1). In partial accordance with this dictum, almost all charged and hydrophilic groups are found on the surface of the protein and most, but not all, of the non-polar groups are buried in the interior. The inability to bury all non-polar groups is essentially related to steric constraints imposed by the polypeptide chain. In most globular proteins, about 40% of the protein's surface is non-polar.

The structural stability of the folded state under a given set of solution conditions depends on two opposing forces: the sum of the energetics of hydrophobic interactions and other non-covalent interactions, such as hydrogen bonds, attractive and repulsive electrostatic interaction, and van der Waals interactions which favor folding of the polypeptide chain, and the conformational entropy of the polypeptide chain which opposes folding of the chain. Thus, the net stability of a folded protein molecule is

$$\Delta G_{U\leftrightarrow N} = (\Delta G_{H\text{-}bond} + \Delta G_{ele} + \Delta G_{H\phi} + \Delta G_{vdW}) - T\,\Delta S \quad (6.1)$$

Here $\Delta G_{H\text{-}bond}$, ΔG_{ele}, $\Delta G_{H\phi}$, and ΔG_{vdW} are free energy changes for hydrogen bonding, electrostatic, hydrophobic, and van der Waals interactions, respectively and $T\Delta S$ is the free energy change arising from the decrease in configurational entropy of the polypeptide chain as a result of folding at temperature T. For most proteins the transformation from an unfolded state (U) to the folded state (N) is spontaneous, implying that $\Delta G_{U\leftrightarrow N}$ is negative. The net stability, $\Delta G_{U\leftrightarrow N}$, is in the range of -5 to -20 kcal mol^{-1} (Table 6.1). This marginal stability suggests that the favorable free energy change emanating from numerous non-covalent interactions is greatly offset by the unfavorable free energy change arising from the loss of configurational entropy of the chain.

TABLE 6.1
Free Energy Change for Unfolding of Proteins at 25°C

Protein	$\Delta G_{N\leftrightarrow U}$ (kcal mol^{-1})	Ref.
Actin	6.6	63
Bovine serum albumin	7.2	
Carbonic anhydrase	10.5	64
α-Chymotrypsin (bovine)	12.9	65
Cytochrome c	8.3	65
Lysozyme (chicken)	13.5	65
α-Lactalbumin	5.3	66
β-Lactoglobulin	6.6	67
Ovalbumin	5.9	68
Papain	20.7	69
Pepsin	10.8	70
Ribonuclease A	6.0	65
Alkaline phosphatase	20.0 (30°C)	71
Troponin	4.7 (37°C)	72

The spontaneous transformation of a protein from an unfolded state to a folded conformation in an aqueous medium often resembles that of a hetero-polymer chain collapsing on itself from an expanded state to a compact state driven by the non-covalent interactions (6). This is schematically shown in Figure 6.1. Note that because of the partial double bond character of the amide linkage and local steric restrictions caused by bulky side-chains, protein chains do not behave as a true random coil polymer even in a fully unfolded, denatured state.

III. DENATURATION

The low $\Delta G_{U\leftrightarrow N}$ values indicate that the tertiary structure of proteins is only marginally stable. Any change in the thermodynamic environment of the protein, such as pH, ionic strength, temperature, pressure, or presence of other solutes, can readily cause a shift in the equilibrium in favor of the denatured state. Two terminologies are often used to define alterations in protein structure. Subtle changes in the tertiary structure that do not greatly alter the topographical features of the protein are usually identified as conformational adaptation. This kind of change in structure occurs when a substrate, inhibitor, or a low-molecular-weight ligand binds to a protein. Breakdown of the tertiary fold along with unfolding of the secondary structures is often termed "denaturation."

While the native structure of a protein is a well-defined entity with structural coordinates for each and every atom in the molecule obtainable from its crystallographic structure, it is not the case for the denatured state. Denaturation is a phenomenon wherein a well-defined initial state of a protein formed under physiological conditions is transformed into an ill-defined final state under non-physiological conditions by the application of a denaturing agent. It does not involve any chemical changes in the protein. In the denatured state, because of a greater degree of rotational motions of dihedral angles of the polypeptide chain, the protein can assume several configuration states differing only marginally in free energy (Figure 6.1).

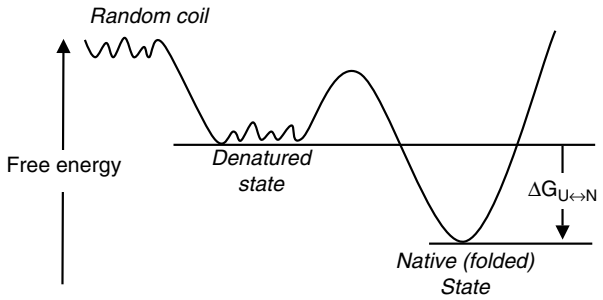

FIGURE 6.1 Schematic representation of the free energy of a protein as a function of its conformational state.

A. Measurement of Denaturation

Since conformation of a protein is not a quantifiable property, and the "denatured state" is not a precisely definable one, the thermodynamics of transformation of a protein from the native state to a denatured state is often determined by measuring changes in physical and chemical properties, such as viscosity, sedimentation coefficient, extinction coefficient, fluorescence, optical rotation, circular dichroism, enzyme activity, and reactivity of sulfhydryl groups of the protein. These intrinsic properties of proteins are conformation-dependent and they are modified significantly as the protein is progressively unfolded from the native state to a denatured state.

The majority of globular proteins so far studied exhibit a "two-state transition" denaturation model. The two-state model stipulates that a protein molecule can only exist either in the native or in the denatured state, but not in an intermediate state. The experimental evidence for this comes from the fact that when changes in a physical property y of a protein are monitored as a function of the concentration of a denaturant or temperature, the property y changes abruptly within a narrow range of denaturant concentration (or temperature), indicating that the transition from the native state to the denatured state is a highly cooperative process. That is, when certain critical intra-molecular interactions in the native state are destabilized by the denaturant, other interactions in the protein become highly unstable, and as a consequence the whole structure unravels within a small range of increment of denaturant concentration. An example of this phenomenon is shown in Figure 6.2 for urea and guanidine hydrochloride-induced denaturation of cytochrome c (7).

For a two-state transition model, the equilibrium between the native and the denatured state is given by

$$N \xleftrightarrow{K_D} D$$

$$K_D = \frac{[D]}{[N]} \tag{6.2}$$

Here K_D is the equilibrium constant. If $y_{(X)}$ is the value of the property y in the presence of a denaturant at concentration X, and $y_{N(X)}$ and $y_{D(X)}$ are the values of the native and denatured states, respectively, at denaturant concentration X, then the equilibrium constant for denaturation can be expressed as

$$K_{D(X)} = \frac{f_{D(X)}}{f_{N(X)}} = \frac{y_{(X)} - y_{N(X)}}{y_{D(X)} - y_{(X)}} \tag{6.3}$$

where $f_{D(X)}$ and $f_{N(X)}$ are the fraction of molecules in the denatured and native states, respectively, at denaturant concentration X. The free energy change $\Delta G_{D(X)}$ is determined from the equation

$$\Delta G_{D(X)} = -RT \ln K_{D(X)} \tag{6.4}$$

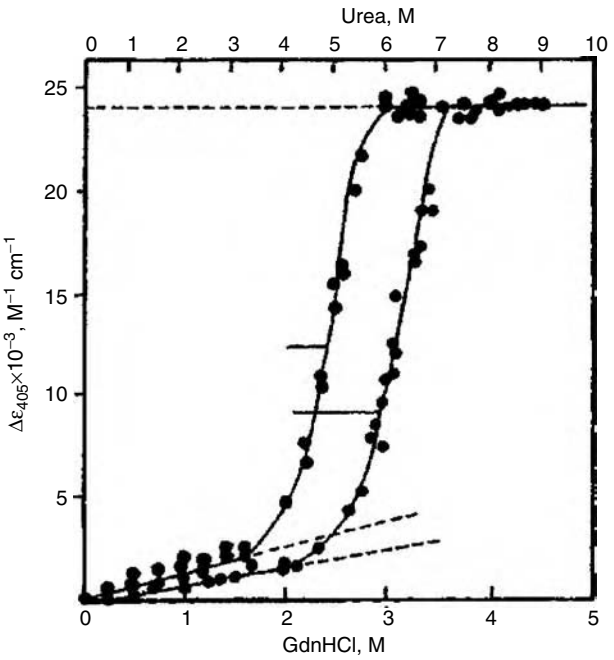

FIGURE 6.2 Guanidine hydrochloride (curve 1) and urea- (curve 2) induced unfolding of cytochrome c at pH 6 and 25°C (from Ref. 7). The ordinate represents change in molar extinction coefficient of the protein at 293 nm as a function of denaturant concentration.

where R is the gas constant and T is the temperature. The effect of the denaturant concentration on $\Delta G_{D(X)}$ generally follows the linear relation (7)

$$\Delta G_{D(X)} = \Delta G_{D(0)} - m_d [X] \qquad (6.5)$$

where $\Delta G_{D(0)}$ is the free energy change in the absence of the denaturant, obtained from the intercept, and m_d is the slope.

Although many proteins follow the two-state denaturation model, recent evidence suggests that certain proteins, such as α-lactalbumin, carbonic anhydrase B, and bovine growth hormone, show deviations from the two-state model (8–11). These proteins, under certain denaturation conditions, follow a three-state transition model

$$N \rightleftharpoons A \rightleftharpoons D \qquad (6.6)$$

where A is a stable intermediate state. The intermediate state is described as the "molten globule" state. The conformation of protein in the molten globule state is neither in the fully folded form nor in the fully unfolded form. The characteristic features of the molten globule state, which is sometimes referred to as the third thermodynamic state (11), are 1) its secondary structure content in terms of α-helix, β-sheet, and aperiodic structures, is very similar to that of the native conformation, 2) its overall shape and dimension is very close to that of the native state such that its intrinsic viscosity is almost the same as that of the native state, 3) the environment of the side-chains, as measured by tryptophan fluorescence, is very similar to that of the native state, and 4) in spite of the presence of secondary structures, the enthalpy of the molten globule is almost the same as that of the denatured state (9).

Acid- or alkali-induced transitions in proteins typically produce an unfolded state characterized as the molten globule state. This phenomenon has been very well studied in the cases of α-lactalbumin, carbonic anhydrase B, and bovine growth hormone. The stability of this state depends on the ionic strength. At low ionic strength, the molten globule, also known as the A state, becomes fully unfolded because of electrostatic repulsion. In addition to acid and alkaline induced transitions, structural states similar to that of molten globule are also produced under other conditions. For instance, complete removal of bound calcium from α-lactalbumin produces a partially unfolded state that is identical to that produced in the acid transition (12, 13). Stable but partially denatured state produced by mild heating, which has a structure different from that of the A state, is also categorized as a molten globule (14, 15). The molten globule state is also formed by reduction of intra-molecular disulfide bonds in proteins (16). Thus, it appears that the molten globule can be defined as any partially unfolded but stable and roughly globular state of a protein formed under a variety of experimental conditions, but having an enthalpy content similar to that of the fully unfolded state.

IV. MECHANISM OF DENATURATION

A. Temperature-Induced Denaturation

Many unit operations in food processing and preservation involve heating and cooling. These processes invariably cause protein denaturation. Typically, most proteins undergo thermal denaturation at elevated temperatures, but certain proteins, e.g., myoglobin, exhibit "cold denaturation" at sub-ambient temperatures as well. In such proteins, the $\Delta G_{N\leftrightarrow U}$ versus temperature profile exhibits a bell-shaped curve with an optimum temperature for maximum stability.

Proteins exhibit vast differences in their thermal stability. For instance, most enzymes are unstable even at 45°C, which is only a few degrees above the physiological temperature at which they function optimally, whereas other enzymes, such as alkaline phosphatase, are stable at higher temperatures. Non-enzyme proteins, especially food proteins such as whey proteins and legume proteins, are usually stable up to 70–80°C. In general, enzymes/proteins from thermophilic and thermotropic organisms are more heat stable than those from psychrophilic and mesophilic species.

The mechanism of temperature-induced denaturation of proteins primarily involves the effect of temperature on the stability of non-covalent interactions. In this respect, the hydrogen bonding and electrostatic interactions, which

are exothermic in nature, are destabilized, and hydrophobic interactions, which are endothermic, are stabilized as the temperature is increased. In addition to non-covalent interactions, temperature dependence of conformational entropy, $T\Delta S_{conf}$, also plays a major role in the stability of proteins. The net stability of a protein at a given temperature is then the sum total of these interactions.

In globular proteins, the majority of charged groups exist on the surface of the protein molecule, fully exposed to the high dielectric aqueous medium. Because of the dielectric screening effect of water, attractive and repulsive electrostatic interactions between charged residues are greatly reduced. For instance, the strength of electrostatic interaction between two charged residues at a distance of 5 Å in water is only about ± 0.8 kcal mol^{-1}, which is comparable to thermal kinetic energy at 25°C. In contrast, in an environment having a dielectric constant of 4, which is comparable to that of protein interior, the strength of the same ion-pair interaction would be about ± 16 kcal mol^{-1}. In addition, at physiological ionic strength, screening of charged groups in proteins by counter ions further reduces electrostatic interactions in proteins. Because of these facts, the influence of temperature on electrostatic interactions in proteins would be negligible. Similarly, hydrogen bonds are unstable in an aqueous environment and therefore their stability in proteins is dependent on hydrophobic interactions that create local low dielectric environment. This implies that so long as a non-polar environment is maintained, the hydrogen bonds in proteins would remain intact when the temperature is increased. These facts suggest that although polar interactions are affected by temperature, they generally do not play a significant role in heat-induced denaturation of proteins. Based on these considerations, the stability of the native state of a protein can be simply regarded as the net free energy difference emanating from hydrophobic interactions that tend to minimize the nonpolar surface area of the protein molecule and the positive free energy change arising from the loss of conformational entropy of the chain ($T\Delta S$). That is,

$$\Delta G_{fold} = \Delta G_{H\phi} + \Delta G_{conf} \tag{6.7}$$

The temperature dependence of the stability of the protein at constant pressure is then given by (17)

$$\frac{\partial \Delta G_{fold}}{\partial T} = \frac{\partial \Delta G_{H\phi}}{\partial T} + \frac{\partial \Delta G_{conf}}{\partial T} \tag{6.8}$$

Hydrophobic interactions are strengthened at higher temperatures; therefore, $\partial \Delta G_{H\phi}/\partial T < 0$. Conformational entropy increases upon unfolding of the protein; therefore, $\partial \Delta G_{conf}/\partial T > 0$. As the temperature is increased, the interplay between these opposing forces reaches a point at which $\partial \Delta G_{fold}/\partial T > 0$. The temperature at which this occurs signifies the denaturation temperature of the protein.

In dilute solutions under certain heating conditions, thermal denaturation of globular proteins is completely reversible. At high concentration, e.g., >1%, protein-protein interaction between unfolded protein molecules hinders refolding of the protein. Prolonged heating of protein solutions at high temperatures, viz., >90°C, can cause irreversible denaturation of proteins regardless of the protein concentration. This is mainly due to chemical changes in proteins, such as destruction of cysteine and cystine residues and deamidation of asparagine and glutamine residues at high temperatures (18, 19).

In addition to denaturation at above ambient temperatures, several proteins have been shown to undergo denaturation at cold temperatures. For instance, myoglobin exhibits maximum stability at about 30°C and is destabilized at lower and higher temperatures (20). Several food proteins also undergo reversible dissociation and denaturation at cold temperatures. For example, glycinin undergoes reversible aggregation and precipitation when stored at 2°C (21). The cold temperature-induced denaturation of proteins is mainly due to a decrease in the stability of hydrophobic interactions at low temperatures.

The fact that the hydrophobic (solvophobic) force and the conformational entropy are the two important forces manifestly controlling thermodynamic stability of proteins tentatively suggests that the stability of proteins might be in some way depend on the amino acid composition. However, studies have shown that thermal denaturation temperature of proteins is not correlated with the mean hydrophobicity of amino acid residues in proteins (22). On the other hand, correlations between certain groups of amino acid residues and thermal stability have been found. For instance, statistical analysis of 15 proteins of known amino acid composition and thermal denaturation temperatures has revealed that the denaturation temperature of these proteins increased linearly with the number percentage of the sum of Asp, Cys, Glu, Lys, Leu, Arg, Trp, and Tyr residues, whereas it decreased linearly with the number percentage of the sum of Ala, Gly, Ser, Thr, Val, and Tyr residues (23). No other combinations of the 20 different amino acid residues in proteins showed a statistically significant correlation with the denaturation temperature. It is likely that attractive interactions between positively and negatively charged groups and the hydrophobic Leu, Trp, and Tyr residues in the first group might be responsible for imparting stability, whereas the highly flexible Gly, Ala, and Ser residues in the second group may tend to increase conformational entropy of proteins and thereby contribute to instability.

Thermal stability of proteins from thermophilic and hyperthermophilic organisms, which can withstand extremely high temperatures, is attributed to their unique amino acid composition (24). These proteins contain lower levels of Asn and Gln residues than those from mesophilic organisms. The implication here is that because Asn and Gln are susceptible to deamidation at high temperatures,

higher levels of these residues in mesophilic proteins may partly contribute to instability. The Cys, Met, and Trp contents, which can be oxidized easily at high temperatures, are also very low in thermostable proteins. On the other hand, thermostable proteins have high levels of Ile and Pro (25, 26). It is believed that the high Ile content might help in better packing of the interior core of the protein (27), which reduces buried cavities or void spaces. Absence of void spaces can reduce mobility of the polypeptide chain at high temperatures and this will minimize the increase in its configurational entropy of the polypeptide chain at high temperatures. A high content of Pro, especially in the loop regions of the protein chain, is believed to provide rigidity to the structure (28, 29). However, comparison of packing volumes of hydrophobic residues in the interior core of homologous glutamate dehydrogenases from mesophilic and hyper-thermophilic organisms has shown no significant difference between them (30), implying that the Ile content and the packing volume may not be really critical for thermostability. On the other hand, examination of crystallographic structures of several proteins/enzymes from thermophilic organisms shows that these thermostable proteins contain a significantly higher number of ion-pairs in crevices on the surface and a substantially higher amount of buried water molecules engaged in hydrogen bonding bridge between segments than in their mesophilic counterparts (30, 31). In other words, it appears that polar interactions (both salt bridges and hydrogen bonding between segments) in the protein interior are responsible for thermostability of proteins from thermophilic and hyper-thermophilic organisms. As discussed earlier, it is conceivable that the presence of 3 to 4 salt bridges between oppositely charged groups in the protein interior could increase the stability of protein structure by about 64 kcal/mol. In spite of these findings, a real understanding of the molecular factors contributing to hyper-thermostability of proteins is still elusive. Apparently, a combination of salt bridges, a strong hydrophobic core, and reduced conformational flexibility seems to be involved in thermostability of proteins.

Water content of dry protein powders affects their thermal denaturation. As the water content is increased from zero to about 0.4 g/g, which incidentally corresponds to monolayer coverage for most proteins, the denaturation temperature of proteins decreases asymptotically and reaches a value that is similar to the denaturation temperature of the protein in a dilute solution (32). This is due to the plasticizing effect of water, which promotes segmental mobility in proteins.

Small-molecular-weight solutes, such as salts and sugars, affect the denaturation temperature of proteins. For instance, addition of 0.5 M NaCl to α-lactalbumin increases its denaturation temperature by about 5°C (33). Sucrose and glucose at 50 wt% level also elevates the denaturation temperature by about 5°C in α-lactalbumin (33). On the other hand, the denaturation temperature of soy glycinin is elevated by about 15°C and that of soy conglycinin by about 12°C in the presence of 0.5 M NaCl compared to the control (34). Interaction of vanillin with ovalbumin decreases both the temperature and the enthalpy of denaturation of ovalbumin (35). Addition of up to 40 wt% sucrose to whey protein isolate solution significantly increased both the denaturation temperature and the gelation temperature (36). The gels formed in the presence of sucrose were more rigid than the control. Addition of calcium at 1–10 mg/g of protein during isoelectric precipitation of soy protein significantly increased the denaturation temperature of soy proteins, especially the 11S fraction; the enthalpy of denaturation, however, was not affected (37). These observations suggest that the magnitude of impact of small-molecular-weight solutes on thermal stability of proteins is dependent on the intrinsic properties of proteins and their response to changes in the external environment.

B. Pressure-Induced Denaturation

Proteins are inherently highly flexible. This high flexibility is the underlying reason for their marginal stability under physiological conditions. This flexibility and marginal stability, which arise due to various competing and balancing forces, are necessary requirements for functioning of enzymes under physiological conditions, because a highly rigid protein cannot exhibit conformational adaptability required for binding of substrates and other ligands.

The flexibility of proteins arises because of void spaces or cavities in the interior of the protein. The void spaces in the interior are created by imperfect packing of the residues as the protein chain collapses on itself during folding. In aqueous solutions, the partial specific volume, $\bar{v}^0$, of a protein consists of

$$\bar{v}^0 = V_c + V_{cav} + \Delta V_{sol} \tag{6.9}$$

Here V_c is the sum of constitutive volumes of atoms in the protein, V_{cav} is the volume of cavities in the protein, and ΔV_{sol} is the volume change due to hydration (38). Among these three parameters, V_c is constant for a given protein molecules since atomic volumes are incompressible. Thus, V_{cav} and ΔV_{sol} are the main parameters that affect partial specific volumes of proteins.

The pressure-induced denaturation is caused by compressibility of the cavities or void spaces in the interior of a protein. Differentiation of Equation 6.9 with pressure under iso-entropic (i.e., adiabatic) conditions results in

$$(\partial \bar{v}^0/\partial \mathrm{P}) = (\partial \mathrm{V}_{cav}/\partial \mathrm{P}) + (\partial \Delta \mathrm{V}_{sol}/\partial \mathrm{P}) \tag{6.10}$$

The adiabatic compressibility is defined as

$$\bar{\beta}_s = -\frac{1}{\bar{v}^0}\frac{\partial \bar{v}^0}{\partial P} \tag{6.11}$$

Thus, Equation 6.10 can be expressed in terms of adiabatic compressibility as

$$\overline{\beta}_s = -\frac{1}{\overline{v}^0}\left[\frac{\partial V_{cav}}{\partial P} + \frac{\partial V_{sol}}{\partial P}\right] \quad (6.12)$$

When hydrostatic pressure is applied, compression of the cavities results in a reduction in volume, i.e., $\partial V_{cav}/\partial P < 0$, but disruption of the hydration shells of charged and polar surfaces causes an increase in volume, i.e., $\partial V_{sol}/\partial P > 0$ (38). Because of these two opposing effects, the net compressibility or decrease in the volume is only marginal. However, if the compressibility arising from $\partial V_{cav}/\partial P$ alone is considered, the compressibility of proteins is an order of magnitude greater than water (38).

The adiabatic compressibility, $\overline{\beta}_s$, of some proteins is shown in Table 6.2. It should be noted that fibrous proteins, such as gelatin, F-actin, myosin, and tropomyosin, have negative compressibility, indicating that the increase in volume due to disruption of hydration shells, i.e., $\partial V_{sol}/\partial P > 0$, is more dominant than the volume decrease due to elimination of cavities. It is possible that these fibrous proteins might be devoid of cavities altogether and therefore $\partial V_{cav}/\partial P$ might be almost close to zero. The positive compressibility values of globular proteins suggest that the negative volume change due to compression of cavities is much greater than the positive volume change from hydration effects. It should be noted that although the partial specific volume of β-casein (0.744 ml g^{-1}) is greater than that of α_s-casein (0.739 ml g^{-1}) and κ-casein (0.739 ml g^{-1}), its adiabatic compressibility is significantly lower than those of α_s- and κ-caseins. This might be related to higher proline content of β-casein (17%) than α_s- and κ-caseins. Because of lack of rotational freedom of the dihedral angle ϕ of the N-C_α bond of proline residues, protein segments containing proline residues often behave like a stiff rod (39). It is conceivable that the uniform distribution of 35 proline residues along the backbone chain in β-casein may render the protein to behave as a stiff rod with only limited flexibility. Among the globular proteins listed in Table 6.2, cytochrome c has the lowest adiabatic compressibility.

TABLE 6.2
Adiabatic Compressibility, $\overline{\beta}_s$, of Proteins[1]

Protein	$\overline{\beta}_s$ ($cm^2\ dyn^{-1}\ 10^{12}$)
Gelatin	−2.5
F-actin	−6.3
Myosin	−18.0
Tropomyosin	−41.0
α-Casein	7.74
α_s-Casein	5.68
β-Casein	3.80
κ-Casein	7.49
Whole casein	6.67
α-Lactalbumin	8.27
β-Lactoglobulin	8.45
Lysozyme	4.67
Myoglobin	8.98
Peroxidase	2.36
Ovalbumin	9.18
Ovomucoid	3.38
Bovine serum albumin	10.50
Cytochrome c	0.066

[1] Compiled from References 73 and 74.

Under very high hydrostatic pressure, the collapse of the cavities formed as a result of imperfect packing of amino acid residues causes unfolding of the protein. In the unfolded state, elimination of the cavities decreases the volume, and hydration of the exposed hydrophobic residues also leads to a reduction in the volume of the solvent. Thus, pressure-induced denaturation usually results in a net reduction in the volume of the system (protein+water).

The free energy change under pressure-induced denaturation of protein is related to its volume change according to the equation

$$\Delta V = d(\Delta G)/dP \quad (6.13)$$

The pressure-induced denaturation of single-chain proteins has been measured by monitoring changes in fluorescence emission and UV absorption spectra of tryptophan residues, and changes in turbidity (40–42). A critical review of the data in the literature suggests the following. In single chain proteins, pressure-induced denaturation occurs only at very high pressures. For most proteins, the midpoint of pressure-induced structural transition is in the range of 4–8 kilobars (43). In oligomeric proteins, dissociation of subunits occurs at 1–2 kilobars, followed by unfolding of the subunits at higher pressures. The volume change in proteins upon pressure-induced unfolding is typically about 0.5% of the total volume of the protein molecule, which is considerably smaller than the theoretically predicted value of about 2%. This tentatively suggests that proteins may retain some residual folded structures even at pressures as high as 10 kilobars. This may partly explain complete reversibility of pressure-induced unfolding in most proteins, including oligomeric proteins. For most single chain proteins, the free energy change at the midpoint of transition at neutral pH and ambient temperature is in the range of 10–20 kcal mol^{-1}. This value is comparable to that obtained from heat-induced or urea and guanidine hydrochloride-induced denaturation. Taken together, the smaller than expected volume change in proteins might indicate that proteins may assume a molten globule state, instead of the completely unfolded state, under high pressure since the enthalpy content of the molten globule state and the fully unfolded state are almost the same.

In addition to causing protein denaturation, high hydrostatic pressure disrupts the integrity of macromolecular assemblies, such as biomembranes, ribosomes, and

bacterial cell walls. Disruption of cell membrane is often irreversible, which results in inactivation of vegetative cells. For this reason, high-pressure treatment of food products is being examined for its effectiveness as a tool for food preservation (44–46). At sufficiently high protein concentration, pressure denaturation can cause non-thermal gelation of proteins (47, 48). Exposure of myosin to pressures up to 800 MPa for 20 min causes denaturation and polymerization of myosin; the polymerized structure contains both hydrogen bonds (as judged from differential scanning calorimetry (DSC)) and disulfide cross-links (49). Pressure-induced gels are softer in texture than the heat-induced gels. However, pressure-induced gels retain color, flavor, and vitamins and other nutrients that are destroyed to some extent in thermally processed foods and in heat-induced gels.

C. Denaturation by Small-Molecular-Weight Additives

Several small-molecular-weight solutes, such as urea, guanidine hydrochloride, detergents, sugars, and neutral salts, affect protein stability in aqueous solutions. While urea, guanidine hydrochloride, and detergents destabilize the native conformation of proteins, sugars tend to stabilize the native structure. In the case of neutral salts, while certain salts, such as sulfate and fluoride salts of sodium, termed as kosmotropes, stabilize protein structure, other salts, such as bromide, iodide, perchlorate, and thiocyanate, termed as chaotropes, destabilize protein structure.

The stabilizing or destabilizing effects of small-molecular-weight additives on proteins is believed to follow a general mechanism. This is related to their preferential interaction with the aqueous phase and the protein surface. Additives that stabilize protein structure bind very weakly to the protein surface but enhance preferential hydration of the protein surface (Figure 6.3). Such additives are generally excluded from the region surrounding the protein; that is, their concentration near the protein is lower than in the bulk solution. This concentration gradient presumably creates an osmotic pressure gradient surrounding the protein molecule, sufficient enough to elevate the thermal denaturation temperature. For instance, studies on protein stabilization by glycerol using the electrospray ionization mass spectrometry (ESI-MS) technique have shown that in glycerol solutions lysozyme assumes a slightly compressed state compared with its state in water (50). This might be due to creation of an exclusion zone around the protein surface for glycerol and development of an osmotic pressure gradient.

In the case of additives that destabilize protein structure, the opposite seems to be true. That is, those additives that decrease the stability of proteins preferentially bind to the protein surface and cause dehydration of the protein. In such cases, water molecules are excluded from the region surrounding the protein and the concentration of the additive in this water-excluded region is higher than in the bulk solvent. Favorable interaction of such additives with protein surface, particularly the non-polar surface, promotes unfolding of the protein so that the buried non-polar surfaces are further exposed for favorable interaction with the additive.

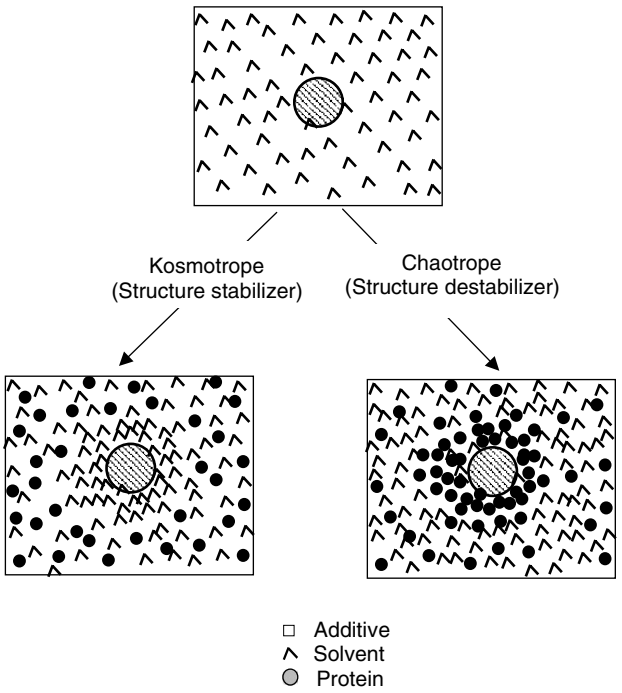

FIGURE 6.3 Schematic representation of preferential binding of solute and solvent molecules with proteins in the cases of kosmotropes (structure stabilizers) and chaotropes (structure destabilizers).

When a protein is exposed to a mixture of stabilizing and destabilizing solutes, the net effect on protein stability generally follows an additivity rule. For example, sucrose and polyols are considered to be protein structure stabilizers, whereas guanidine hydrochloride is a structure destabilizer. When sucrose is mixed with guanidine hydrochloride, the concentration of guanidine hydrochloride required for unfolding proteins increased with increase of sucrose concentration (51). It was also observed that the structure of water was altered in the presence of guanidine hydrochloride and urea. However, addition of polyhydric compounds such as sucrose countered this urea and guanidine hydrochloride-induced change in water structure. Thus, the increase in guanidine hydrochloride and urea concentrations required for unfolding proteins in the presence of sucrose and other polyhydric compounds might be due to mutually opposing effects of these compounds on water structure (51). This also underscores that changes in water structure in the presence of additives are involved in some fundamental way in the

transmission of the effects of additives on protein stability. The exact mechanism is still elusive, partly because "water structure" is not yet a well-defined concept.

Theoretical statistical thermodynamic analyses have shown that the excluded volume of additives has three distinct types of effects on protein stability (52): 1) Small-size solutes strongly denature proteins, 2) medium-size solutes stabilize proteins at low solute concentrations and destabilize them at high concentrations, and 3) large-size solutes stabilize the native state of proteins across the whole liquid region. In agreement with this theory, stabilizers tend to be large-size molecules such as sugars, polymers, polynols, nonionic, and anionic surfactants while denaturants tend to be small-size molecules such as alcohols, glycols, amides, formamides, ureas, and guanidium salts.

The mechanism of preferential binding to or exclusion from protein surface of additives in aqueous solutions is a complex one. Various molecular interactions between the additive and protein (both with the protein surface and protein interior) are involved. These interactions include electrostatic and van der Waals interactions (consisting of Debye-Keesom and London dispersion interactions) with proteins, as well as their interaction with solvent water. It has been pointed out (53, 54) rather convincingly that preferential binding or exclusion of ions from a protein surface in aqueous electrolyte solutions can be fully explained only by including, in addition to electrostatic interactions, dispersion interactions between ions and the protein.

1. Urea and Guanidine Hydrochloride

Both urea and guanidine hydrochloride denature proteins at very high concentrations. Guanidine hydrochloride is a more potent denaturant than urea. For globular proteins the midpoint of unfolding transition occurs at 4–6 M urea and at 3–4 M guanidine hydrochloride concentrations. Generally, globular proteins are completely denatured in 8 M urea and 6 M guanidine hydrochloride. The mechanism of denaturation of proteins by urea and guanidine hydrochloride is related to their solubilizing effect on both polar and non-polar amino acid residues. Solubility studies on non-polar amino acids have shown that the free energy change for transfer of non-polar groups from water to urea and guanidine hydrochloride solutions is favorable (55). This transfer free energy is proportional to the accessible surface area of the non-polar solute, which is about –7.1 cal mol^{-1} $Å^{-2}$ for 8 M urea and –8.3 cal mol^{-1} $Å^{-2}$ for 6 M guanidine hydrochloride (55). Because urea and guanidine hydrochloride interact more favorably with both polar and non-polar groups (via ion-dipole and dispersion interactions) on the protein surface than does water, they preferentially bind to proteins. This preferential interaction further leads to unfolding and solubilization of buried non-polar residues. This shift in equilibrium from native state to the unfolded state is driven by the thermodynamic requirement to increase the area of contact between protein non-polar surfaces and the denaturant.

2. Neutral Salts

Neutral salts affect protein stability via two different mechanisms. At low concentrations (<0.5 M), non-specific electrostatic interactions with charged groups on protein surface results in neutralization of electrostatic (repulsive) interactions within a protein and this often leads to stabilization of protein structure. Salts also affect the pK_a of ionizable groups in proteins, especially the acidic residues, which causes changes in thermal stability of proteins (56). At higher concentrations, neutral salts exert ion-specific effects on protein stability depending on their relative position in the Hofmeister Series (56). The effects of anions on protein stability are greater than the cations and follows the order $F^- > SO_4^{2-} > Cl^- > Br^- > I^- > ClO_4^- > SCN^- > Cl_3CCOO^-$. F^- and SO_4^{2-} actually increase the stability of protein structure (kosmotropes), whereas Br^-, I^-, ClO_4^-, SCN^-, and Cl_3CCOO^- ions destabilize protein structure (chaotropes). The stabilizing and destabilizing effects of these ions on protein structure are manifested in the form of elevation and depression of the denaturation temperature (T_d) of proteins. For instance, Figure 6.4 shows the effect of neutral salts on T_d of β-lactoglobulin (58). At high concentrations, Na_2SO_4 and NaCl significantly raise the denaturation temperature, whereas NaSCN and $NaClO_4$ lower the denaturation temperature of β-lactoglobulin.

Several theories have been proposed to explain the effects of neutral salts on protein structure and stability (57, 59). Experimental evidence indicates that the effects of salts on protein stability are related to their relative ability to bind to proteins and affect its hydration. Salts that stabilize protein structure weakly bind to the protein surface and enhance hydration of the protein, whereas salts that destabilize protein structure bind strongly to protein surface and cause dehydration of the protein (60). However, the fundamental reasons for differential binding of salts, for example monovalent salts, to proteins are not well understood. It appears that ion binding to proteins cannot be simply explained using classical electrostatic potential on the surface. It might also involve dispersion forces between the ion and the apolar interior of the protein (54). The dispersion potential, which relates to ion-dipole polarizability effects, is dependent on ionic radius and concentration and the dielectric susceptibility of the protein. On a more fundamental level, differences in interaction of salts with proteins must be related to free energy differences between interaction with solvent water and protein surface. In this case, ions might affect the thermodynamic state of water and alter its solvent properties. It has been shown that salts that stabilize protein structure also enhance hydrogen-bonded structure of water, and salts that destabilize protein structure also break down the hydrogen-bonded structure of water (59).

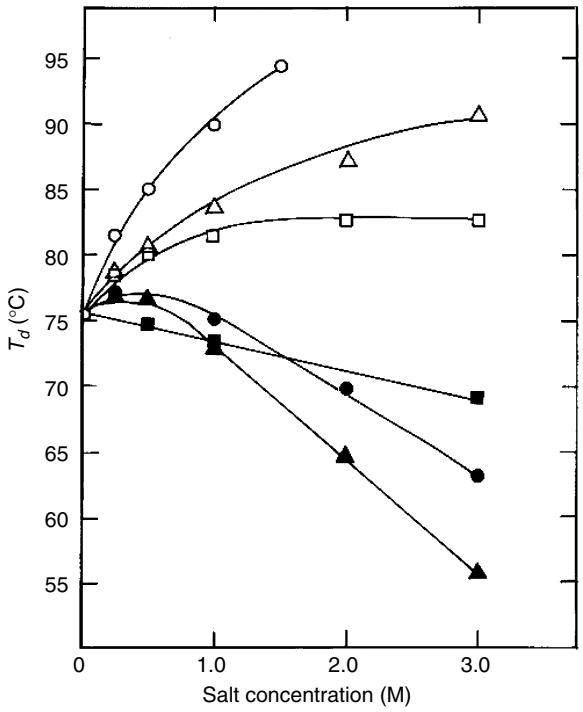

FIGURE 6.4 Effects of various sodium salts on thermal denaturation temperature of β-lactoglobulin. ○, Na_2SO_4; △, NaCl; □, NaBr; ●, $NaClO_4$; ▲, NaSCN; ■, urea (from Ref. 55).

3. Detergents

Detergents, especially anionic detergents such as sodium dodecyl sulfate (SDS), are potent denaturing agents. Unlike urea and guanidine hydrochloride, SDS can denature proteins at 3–8 mM concentration. The mechanism of denaturation involves strong binding of SDS to hydrophobic groups in the crevices of the protein molecule, which results in destabilization and solubilization of buried hydrophobic regions. Proteins bind up to about 1.4 g of SDS per gram of protein. Because of this high binding capacity, proteins in SDS solution become highly negatively charged and consequently electrostatic repulsion between segments also play a role in protein unfolding. Unlike in other denaturing environments, the denatured state of proteins in SDS solution is generally not in a random or aperiodic conformation; invariably they assume a helical rod-type shape. Because of strong binding via hydrophobic interactions, detergent-induced denaturation of proteins is mostly irreversible, whereas denaturation by urea and guanidine hydrochloride is generally reversible under appropriate conditions.

4. Organic Solvents

The mechanism of protein denaturation by organic solvents such as ethanol, propanol, and acetone is complex. This is because these water miscible organic solvents affect various molecular forces, such as electrostatic, hydrogen bonding, and hydrophobic interactions, in proteins differently. However, the effects are mediated mainly via a decrease in the dielectric constant of the solvent medium. First, when a water-miscible organic solvent is added to a protein solution, the decrease in the permittivity of the medium increases electrostatic repulsion between like charges and enhances attraction between unlike charges. The repulsive interactions tend to unfold the protein, whereas the attractive electrostatic interactions tend to stabilize the structure. The latter becomes crucial if the protein contains a salt bridge between a carboxyl and an amino group in a partially exposed crevice on the surface of the protein. Second, because apolar residues in proteins are more soluble in an organic solvent and in an aqueous-organic solvent mixture than in water, the hydrophobic interactions within the protein are weakened and consequently the buried non-polar groups tend to become exposed to the solvent, which results in unfolding of the tertiary structure of the protein. Third, although the tertiary (and quaternary) structure of the protein may unfold, the secondary structures, viz., α-helix and β-sheet, which are stabilized by hydrogen bonds, may not unfold. In the low dielectric environment of the aqueous-organic solvent mixture, strengthening of these dipole-dipole interactions may actually stabilize the secondary structure. In fact, a recent study has shown that in the entire range of aqueous-organic mixtures, that is, from pure water to pure organic solvent, the secondary structure of lysozyme and subtilsin remained essentially intact (61). It is noteworthy that several enzymes retain their activity in neat organic solvents and that is the basis for non-aqueous enzymology (61). Hexane extraction of oil from dry soybeans and other oilseeds at low temperature causes very little denaturation of soy proteins. However, when the moisture content of soybean is high (>10%), hexane extraction often leads to partial denaturation of soy proteins.

Exposure of proteins to polar organic solvents at elevated temperature usually causes extensive denaturation. In the case of food proteins, this results in poor aqueous solubility of the protein. For example, hot aqueous ethanol treatment (55% ethanol at 80°C) caused complete denaturation of pea protein isolate and a large reduction in its protein dispersibility index (62). A significant reduction in the trypsin inhibitory activity of pea protein isolate also was observed. However, treatment with higher ethanol concentration (65%) and lower treatment temperature (65°C) minimized protein insolubility and caused only a marginal reduction in trypsin inhibitory activity.

5. pH-Induced Denaturation

Proteins are either negatively or positively charged at neutral pH. Since this represents the physiological pH, the native structure of the protein represents an equilibrium structure with a global minimum free energy that has

already taken into account the preexisting repulsive and attractive electrostatic interactions. However, at pH values away from the neutral pH, changes in the state of ionization of various charged residues in proteins alter the electrostatic free energy of the protein, resulting in conformational changes. Most proteins are very stable at their isoelectric pH, where the net charge of the protein is zero and electrostatic repulsive interactions are at a minimum. However, many proteins unfold at pH values below 5 and above 10. This unfolding is not simply because of changes in the ionization state of the charged residues on the surface of the protein, but is related to ionization of residues that are partially or fully buried in the protein. Specifically, in many proteins histidine ($pK_a = 6.0$) and tyrosine residues ($pK_a = 9.6$) are buried in the un-ionized form in proteins. When the pH of the solution is decreased below 5.0, ionization of the buried histidine residues can cause unfolding. Similarly when the pH of the solution is increased above 10, ionization of buried tyrosine residues can unravel the protein structure. The pH-dependent ionization of charged residues on the surface of the protein also may contribute to protein denaturation. Protonation of carboxyl groups ($pK_a = 4.6$) at acidic pH eliminates negative charge and the protein becomes highly positively charged. On the other hand, deprotonation of lysine residues ($pK_a = 10.2$) at pH above 10.5 decreases the positive charge and the protein becomes highly negatively charged. In both situations, increased electrostatic repulsion between surface charges may also contribute to unfolding of the protein. If salt bridges between positively and negatively charged residues in partially buried surface crevices of proteins are involved in the stability of native structure of proteins, then protonation (of the carboxyl group) and/or deprotonation (of the amino group) of one of the groups of the salt bridge can significantly perturb protein structure. In many cases, the pH-induced denaturation of proteins is reversible, but prolonged exposure to extreme pH values can cause hydrolysis of certain peptide bonds, deamidation of asparagine and glutamine residues, and destruction of cysteine residues via β-elimination reaction. Such chemical alterations in proteins would result in irreversible denaturation.

V. SUMMARY

Despite the presence of numerous intra-molecular interactions, proteins exist in a metastable state. Thermodynamically, the net stability of the native structure of proteins is only about 5–20 kcal mol^{-1}. Because of this, destabilization of a few critical interactions in a protein by temperature, salts, pH, and other small-molecular-weight denaturants can cause cooperative unraveling of the native structure. In the case of food proteins, the majority of which are oligomeric proteins, the denaturation phenomenon is more complex than that in monomeric proteins. In oligomeric proteins, the denaturation process may follow a three-state model, the first being the dissociation of the oligomer into monomers and the second being unfolding of the monomers. In some proteins these two processes may occur in a de-coupled manner and in other proteins these may be coupled together. Such complex structural transitions in proteins may affect their functional properties as food protein ingredients. They also affect the nutritional properties of proteins, e.g., digestibility. Proteins that are resistant to thermal denaturation also are less digestible, e.g., soy proteins. Thus, a fundamental understanding of the structural stability of food proteins and their denaturation behavior under various environmental conditions as encountered in a food milieu is essential for understanding their structure-function relationships in food systems.

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7 Food Protein Functionality

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CONTENTS

I. INTRODUCTION

Functionality has been described as the non-nutritive roles that food constituents play in a food system. More formally, functional properties are the physical and chemical properties that affect the behavior of molecular constituents in food systems. Functionality of ingredients is important in the preparation, processing, storage, quality, and sensory attributes of foods (1). Proteins in foods are multifunctional and may be the principal structural component in many food systems, including products from meat and poultry, eggs, dairy, cereals, and legumes. Proteins contribute significantly to the sensory attributes and overall quality of products of which they are a component. Knowledge of protein functionality is critical for the development of new products and the improvement of existing ones. An example is the use of less expensive protein sources as replacements in traditional food products. Use of less expensive proteins not only allows for cost reduction, but also can increase the utilization of food materials that previously might have been considered waste products. As our society increases in complexity,

TABLE 7.1
Important Functional Properties of Proteins in Several Food Systems

Functional Property	Food System(s)
Solubility	Infant formulas, protein beverages, beer, yogurt drinks
Water-holding ability (capacity)	Tumbled hams, deli meats, frankfurters, poultry products, yogurt
Gelation	Frankfurters, custards, gelatin, comminuted meat and poultry products
Emulsification	Ice cream, liquid coffee creamers, salad dressings, milk, mayonnaise, gravies
Foaming	Angel and sponge cakes, meringues, soufflés, marshmallows, whipped cream and toppings

the demand for new food products requires us to understand protein functionality so that we can modify or control their behavior in new food systems.

Functional properties commonly associated with proteins include solubility, gelation, emulsification, foaming, and water-holding capacity. Table 7.1 lists these functional properties and gives examples of foods which commonly exhibit them. Smith (2) classified functional properties into three broad categories: 1) hydration properties such as solubility or water retention, 2) protein-protein interactions such as gelation, and 3) surface properties such as emulsification and foaming.

In many foods systems, proteins are multifunctional in that they might play a number of different functional roles in a given food. Additionally, a given food might require several different proteins so that all of the functional needs of the food are met. Excellent examples of this are highly ground or comminuted muscle foods systems such as frankfurters or bologna. Those products require proteins that exhibit good solubility, gelation, water-holding capacity, and emulsification. Because the naturally occurring proteins in muscle might not perform all of these roles well, it is necessary to add functional proteins to these formulations in the form of food protein ingredients. Protein additives available for use in formulated foods come from many sources and are in high demand by the food industry. As seen in Table 7.2, protein ingredients are extracted and purified (or partially purified) from both plant and animal sources. Besides native source, the major functional protein in each is also listed. Typically, protein ingredients are called concentrates if they contain between 50–80% protein and isolates if their concentration is greater than 90% (2).

The major factors that influence the functional properties of a protein are its biochemical nature and how it was affected by extraction and purification into a food ingredient. Perhaps of paramount importance is the primary structure, or amino acid sequence, of the protein. This sequence dictates how the protein might fold in regular patterns (secondary structure) and how it might appear in three dimensions (tertiary structure). Whether or not a protein is a single unit or contains multiple subunits (quaternary structure) is also a function of primary structure. The tertiary and quaternary structures of proteins are very important to the overall functionality of the proteins. These structures dictate the physicochemical properties of the protein including surface charge, hydrophobicity, stability to heat and chemicals, molecular flexibility, and dissociation behavior. The physicochemical properties of proteins are what determine how it functions in a food system. Thus, if we can understand the physical and chemical properties of a protein we can begin to predict how it might behave in a food. Understanding these principles also allows us to manipulate the physical and chemical characteristics of a protein during food preparation and subsequent processing. We can also use this knowledge to perhaps alter the properties of a protein ingredient to change how it functions in a food.

Figure 7.1 illustrates how the physicochemical properties of a protein might be altered. Food proteins can be altered by processing operations, environmental conditions, or interactions with other ingredients in the food system. Processing conditions that might alter functional properties include heating, drying, freezing, mixing or shearing, and pressurizing. Environmental conditions that influence protein functionality are ionic strength, pH, types of salts present, and oxidation-reduction potential. Environmental conditions tend to overlap with ingredient interactions, since many ingredients have a strong

TABLE 7.2
Major Functional Proteins: Their Sources and Food Use Examples

Major Functional Protein	Source	Use
Gluten: glutenin, gliadin	Wheat	Bakery, pasta, bread
Legumin: 11S and 7S globulins	Legumes	Soy protein isolates, texturized vegetable protein, infant formulas
Casein	Milk	Whipped toppings
Alpha lactalbumin Beta lactoglobulin	Whey	Whey protein isolates, ice cream, infant formula
Myosin	Muscle (beef, poultry)	Frankfurters, deli meats, massaged and tumbled hams, lunchmeats, surimi
Gelatin	Rendering of beef, fish (collagen)	Gelatin desserts, yogurt
Ovalbumin, ovomucin	Egg white	Angel and sponge cakes, soufflés, meringues

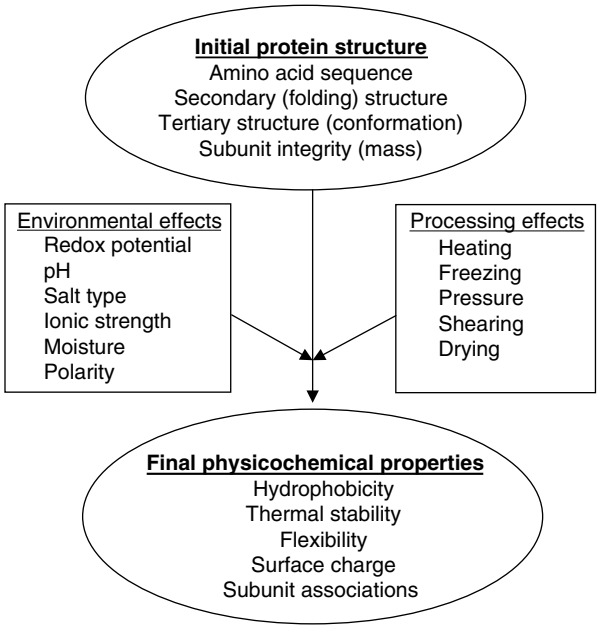

FIGURE 7.1 Effects of environment and processing on the structure and physicochemical properties of proteins.

influence on the environment in which a protein exists. An example is the presence and type of ions and salts that might be added in the form of an ingredient. The presence and types of lipids or fats have a powerful influence on the physicochemical properties of proteins. An example is the inclusion of a small amount of egg yolk in a mixture of egg white proteins that are meant to be foamed. The lipids present in the egg yolk will interact with the egg white proteins and markedly reduce their ability to foam in terms of overall volume and foam stability.

Small changes in formulation (and thus environment) or processing can greatly alter the functionality of food proteins. A classic example is the effect of salt on the functional properties of muscle proteins in processed meats such as frankfurters and lunchmeat (3). The addition of 1.5–2.5% salt solubilizes the meat proteins and allows them to bind water and form an elastic, rubbery gel. This results in the typical texture and sensory quality that we expect in a hot dog or frankfurter. If the salt is not present, the hot dog has poor water binding and gelation, which results in a brittle, tough product.

Storage of food ingredients or finished foods will also influence the functionality of food proteins. Proteins may partially unfold or denature during storage, leading to aggregation and loss of solubility. If a food system contains oxidizing lipid, proteins may react with lipid breakdown products to form unstable aggregates, brown polymers, and otherwise dramatically change in terms of function in the food. When frozen for extended periods, proteins may lose their ability to bind and hold water, thus creating excessive drip in the thawed product.

Because food systems are so complex, protein functionality has been traditionally studied in model systems where many of the interactions with ingredients or processing can be controlled or removed. These studies involve the use of very controlled conditions which are carefully defined so that reproducible results might be obtained. A number of model systems have been developed for each functional property. Functional property tests should be based on a fundamental physical property of a protein. An error that some researchers have repeated is the development of tests that are based solely on equipment available in the researchers' laboratory. In the next section we will examine some of the individual functional properties of proteins that determine their use and application in various foods.

II. FUNCTIONAL PROPERTIES OF FOOD PROTEINS

A. Protein Solubility

The solubility of a protein many times determines its use in foods. Solubility may be the most important aspect of functionality for a protein. In beverages, foams, and emulsions, a protein must be soluble to have functionality. Foaming, gelation, and water binding are also influenced by solubility. In some cases, e.g., water binding, less soluble proteins may function more efficiently. Using classical biochemical terminology, albumins are water-soluble proteins; globulins are salt soluble; glutelins are soluble at high pH values; and prolamines are most soluble in alcohols. The solubility of a protein is determined by its primary structure—the sequence of amino acids in the protein chain. If a protein has a polar surface due to the presence of polar amino acids, it will have good solubility in a polar solvent such as water. Since the pH of a solution will influence the overall charges on a protein, the use of a protein in a given food is many times dictated by the pH of the food system. Proteins with higher contents of hydrophobic amino acids, fewer charges on their surface, or those which contain many subunits tend to have limited water solubility.

As we will see, heat-treated proteins unfold and tend to form less soluble aggregates. Thus, solubility may be used as an index of denaturation of the protein due to exposure to heat, processing or storage. Solubility is markedly affected by pH, polarity of the solvent, temperature, and concentration of dissolved salts.

1. Effect of pH

Surfaces of proteins have net charges due to their amino acid content and the pH of their environment. Amino acid residues such as argenine or lysine can provide positive charges for protein surfaces, while aspartic or glutamic

acids can yield negative charges. The charges present depend on the pH of the food system. When a protein has equal numbers of positive and negative charges on its surface it has minimal solubility. This is called the pI or isoelectric point of the protein. At this point the protein has a net charge of zero. There is minimal solubility because intermolecular repulsion is at a minimum and proteins will tend to aggregate. At pH values above the pI of a protein it has a net negative charge. At pH values below the pI, it will have a net positive charge. In both cases the presence of pronounced surface charges will result in intermolecular repulsion and enhanced solubility.

2. Effect of Salt Concentration

Solubility is also affected by the type and concentration of salts in a food system. As salts increase in concentration proteins become more soluble. This is called "salting in" of a protein and is attributed to the ability of salt ions to enhance the surface charges on proteins. In foods, sodium chloride is commonly used for this purpose. Salt concentrations may be adjusted in a product to enhance the functional properties of the food system. At high salt concentrations, usually above 1 molar, protein solubility decreases. This is thought to be due to salt competing with the proteins for available water for solvation. This effect is called "salting out" and is rarely seen in foods as the concentration of salt required is so high that the food would be inedible.

3. Protein Denaturation

In their natural state, proteins tend to have highly folded structures. Many are "globular" or roughly spherical in shape. This is called their "native" structure. These native structures tend to be very sensitive to conditions which would cause them to change their shape or conformation in a food setting. If a protein unfolds or unravels due to large changes in its surface charges and conformation, it is said to be denatured. For most proteins, denaturation is an irreversible process. Proteins can be denatured by very low or high pH values, organic solvents, high salt concentrations, mechanical shear, and elevated temperatures. Single strands of denatured protein are unstable, leading them to form aggregates which can form gels, foams, or precipitates. The temperature at which proteins denature varies widely due to differences in amino acid sequence and other environmental conditions, such as pH or salt concentration. As denaturation proceeds, protein molecules change in regard to surface charges, shape, size, and hydrophobicity. Most thoroughly denatured proteins are insoluble. When foods are cooked or otherwise thermally processed, the majority of proteins in them become denatured. Denatured protein functions differently from native proteins. Since the solubility of a protein is many times dependent on native structure, denaturation can be simply measured by looking at how soluble a known protein is under certain conditions.

Since denaturation results in loss of solubility, it is undesirable in many food systems where solubility is important. An example is the preparation of protein products for use in foods that rely on protein functionality. Egg whites are pasteurized to destroy pathogenic bacteria; however, the process to do so must be sufficiently gentle so that the finished product can be used in the baking industry to make products such as angel food cake. If the whites were harshly heated, the desired proteins would denature and the ingredient would not function in the finished food. On the other hand, denaturation may cause desirable changes in some food systems, such as in the manufacture of gelatin from collagen or the gelling of egg whites during the cooking of an egg. Some ingredients, such as whey protein concentrates, are available in a variety of degrees of denaturation for application in different products (4, 5). Whey products used for foaming applications have less denaturation than those sold for use as water-binding agents.

4. Measurements of Solubility

One must always remember that any test used to measure a functional property is empirical and thus the results will vary with the test conditions selected. Many solubility tests are based on suspending and stirring a known amount of protein in a buffered solution, followed by centrifugation to remove insoluble components with subsequent protein analysis (colorimetric or Kjeldahl) of the supernatant. The results depend greatly on the protein concentration used, pH, buffer ionic strength, and centrifugal force. Over 70 methods have been published to measure the water-holding capacity and solubility of dairy proteins (6). To obtain results that can be compared to other data and laboratories, it is always best to use a standard method if available. Two commonly used standard methods are published by the American Oil Chemists Society (AOCS) (7) and the American Association of Cereal Chemists (AACC) (8).

B. Gelation

A protein gel is a three-dimensional cross-linked network of protein molecules imbedded in an aqueous solvent (9). Many processed foods are gels such as custards, yogurts, cheeses, frankfurters, and gelatin-based desserts. Most gels are very high in water content (up to 95–98%), yet still have characteristics of solid or rigid food materials. Gelation is based on the denaturation of proteins, followed by their intermolecular association to form matrices which trap water, fat, and other food ingredients. The formation of gels is influenced by heat, pH, pressure or shearing, and the presence of various solvents. The majority of edible food gels based on protein are formed due to heating.

Food gels fall into two categories: thermally reversible and thermally irreversible. Thermally irreversible gels are also called "thermoset" gels and represent the largest group of edible gels. Included in the group are gels based on the muscle protein myosin and ovalbumin, an egg protein. Thermoset gels form chemical bonds that will not break during reheating of the gel and thus remain rigid if reheated. An example would be reheating a cooked egg white. Thermoplastic gels are thermoreversible and will "melt" when reheated due to the reversible nature of the bonds which hold the strands of the gel together. The collagen breakdown product gelatin is the most common example of a thermally reversible gel. Some types of protein, such as alpha-lactalbumin from whey, will form either type of gel depending on the environmental conditions in the food system during heating (5).

1. Properties of Thermoset (Irreversible) Gels

Most thermoset gels are the result of protein unfolding and denaturation followed by aggregation of the molecules into a cross-linked network. During this process, heated proteins partially unfold and form clumps or aggregates. As the "gel-point" temperature is reached, these aggregates unfold further and rapidly cross-link to form a gel (4). This network is generally formed via noncovalent bonds such as hydrophobic interactions and hydrogen bonds. Occasionally, disulfide bonds may be involved. In order for a stable gel to set, there must be a balance between the charges on the denatured protein surfaces and the water molecules in the system. If the protein-protein attraction is too weak, the proteins will remain in solution and a viscous fluid will result. If the attractive forces are too strong, the proteins will aggregate and precipitate. The proper amount of denaturation will result in proteins that interact sufficiently to form a matrix that will also hold a large amount of water.

2. Factors Affecting Thermoset Gel Properties

As one might predict, environmental conditions such as pH and salt concentration, as well as heating rate and final temperature greatly affect the properties of a thermoset gel. Thermoset gels can be opaque and turbid or transparent and clear. Turbid gels are formed when repulsive forces between the protein molecules are low (such as pH values close to the isoelectric point). When heated, the proteins tend to form grape-like aggregate clusters which then are cross-linked together upon further heating (10). Transparent gels are more likely to form when protein molecules are highly charged and repulse each other until denaturation occurs. In transparent gels the protein molecules tend to form structures which resemble "strings of pearls" where individual molecules form chains which occasionally cross-link with other strands. Various researchers have examined the differing properties of gels formed from a single type of protein when conditions of pH, ionic strength, and heating are varied (11). Manipulation of environmental factors can produce turbid, transparent, or hybrid gels from the same protein.

3. Thermoplastic Gels (Thermoreversible)

The most extensively studied protein which forms thermoplastic gels is gelatin, a product of refined collagen. Collagen is a triple-stranded helical protein used in a variety of structural roles in many species. When heated it dissociates into single proteins strands called gelatin that assume random coil configurations. When gelatin is dissolved in hot water, it attempts to reform its original triple helix structure. If the mixture is cooled, gelatin strands will randomly reform sections of the helical structure resulting in the formation of a cross-linked matrix. The sections where intermolecular bonding occurs are called "junction zones." The bonds used to form the matrix are hydrogen bonds, which are thermally reversible. Heating a gelatin gel will cause the hydrogen bonds in a junction zone to dissociate and the matrix/gel will melt. Upon cooling, the gel will reform.

4. Measurements of Gel Properties

As with solubility, a range of functional tests for protein gels exists. Although it might seem appropriate to use a model system where only the protein and water are studied, the impact of other food system components, such as fat or ground particulate matter (such as spices), can be very great and cannot always be ignored. In general, the more simple a model system is, the less likely it will mimic what occurs in a real food, such as a frankfurter (2).

Quality control operations in the food industry have developed a number of empirical tests to relate the properties of gelled products to their acceptability by consumers. An example would be measuring the amount of force required to shear through a cross-section of a frankfurter. Samples of finished product can be gathered off the processing line and sheared as a means of ensuring proper product formulation and processing. However, these types of tests are only valuable for measuring specific products which that particular company produces.

A common test used to test gel strengths is the "torsion test" (12). A gel of appropriate size and shape is twisted in a rheometer until the gel breaks or ruptures. The force required to rupture the cross-sectional area of the gel can be calculated and compared to other properties, such as sensory evaluation results. Several gel strength tests have been widely accepted for use in this manner (13, 14).

C. Water Binding

Water binding is an important functional property for several reasons: 1) most foods contain high amounts of water

and if their chemistry changes in a manner that would cause the formation of free water or drip loss, consumers would be displeased, 2) increasing the amount of water a product can hold effectively can increase the profitability of a given product, and 3) both product yield and sensory quality are highly dependent on the proper moisture content of a finished food. Water-binding capacity is the amount of water that is bound or retained by a protein under highly defined conditions.

Water is usually be bound to the surface of a protein by hydrogen bonding, which is sometimes called dipole bonding. Hydrogen bonding results from water's interaction with the R group of amino acids which are dipoles, such as serine and threonine, or ionic, such as lysine or aspartic acid. Water bound to the surface of proteins in this manner is called "monolayer" water and is very tightly associated with the protein. Other water associated with the protein or protein matrices can be trapped in capillary structures and pores. In food systems, water can also be held in cells and other structural networks. Water that is not associated with the monolayer on the protein surface is called free water and moves unhindered throughout the food system. Depending on the food or model system, water-binding capacity tests measure a mixture of the free and bound water present.

1. Factors Influencing Water Binding

For a given food system, the protein concentration, temperature, salt type and concentration, and degree of denaturation impact on the water binding exhibited. In addition, small polar molecules, such as sugars or sugar alcohols, will enhance water binding by proteins in general. As the protein concentration increases, so does the water binding observed. Generally, water binding increases as temperature increases. In some cases the proteins will form gels, which will enhance the binding of water by the system. Many types of whey protein and soy protein isolates will form gels when heated above 80°C. Enhanced binding in gels is due to water being bound by both hydrogen bonds and by being trapped in pores.

Ions also influence water binding. Sodium chloride binds to charged groups on protein surfaces and weakens intermolecular bonds. This is a positive effect in systems which utilize muscle fibers as part of the structural elements of the food. Salt allows the muscle proteins to distance themselves from others within the muscle fiber and thus increase the number of sites for water to bind. In various muscle types, water binding may be increased 2–3 fold by the presence of salt (15).

The pH of a system markedly influences its ability to bind water. This is due to changes in the surface charges on a protein as the pH is altered. Water binding is lowest at the isoelectric point (pI) of a protein. As the pH is adjusted away from the isoelectric point the ionic charges on the protein increase dramatically and water binding is enhanced. As the pH is lowered from the isoelectric point the protein assumes a net positive charge. Conversely, as the pH is increased, a net negative charge is seen.

2. Methods of Measurement

Water-binding model systems must be carefully defined if reproducible results are expected. Most of the methods are empirical and are usually designed for a specific product or application. At least 15 different methods have been developed to measure the water binding of various muscle/meat proteins (16). In general, two main types of tests exist. One is based on sorption—the adsorptions of water by a dry powder of protein. The other is sometimes called "expressible moisture" where a product is subjected to a force and the amount of moisture expelled is measured. The force is usually pressure or centrifugation. These types of tests must be carefully designed so that the actual internal structure of the gel or food is not destroyed when the pressure is applied. This would lead to an underestimation of the water binding for the system since water that normally would have been trapped in the matrix would be expressed (17).

D. Emulsification

An emulsion is a mixture of two immiscible liquids in which one is dispersed in the other in the form of droplets (18). It is common practice to call the liquid in the droplets the dispersed, internal, or discontinuous phase. The surrounding phase is called the external or continuous phase. Emulsions in which the dispersed phase is a lipid are called "oil in water" emulsions (o/w). Water in oil emulsions contain droplets of water dispersed in a lipid continuous phase. Egg yolk and milk are examples of natural emulsions. Many manufactured foods are intentional emulsions including ice cream, salad dressings, chocolate, mayonnaise, cakes, frosting, butter, and spreads. Food emulsions are far more complex than a droplet of one phase suspended in another. Foods contain many other materials such as air, particulates or other dispersed solids, partially crystallized fat, and gels.

1. Principles of Emulsification

When a liquid is exposed to air, the surface between them is in a state of tension. This so-called "surface tension" is due to the attractive forces between molecules in the liquid that are enhanced by exposure to air. In lay terms, the molecules "bunch" together to decrease their exposure to the air surface. When two immiscible liquids, such as water and oil are in contact, the region of contact is called the interface, with the development of "interfacial tension." As the interfacial area increases, the stability of the mixture decreases. An example would be the creation of

temporary emulsion of oil in water by forming millions of small droplets of oil suspended in the water phase. One can imagine creating this mixture by blending several milliliters of oil into a cup of water in a high speed mixer. This type of mixture is called a "temporary emulsion," for although there would initially be millions of oil droplets, they would rapidly coalesce into a separate oil layer which would form on top of the aqueous phase. Coalesced droplets have less surface area exposed to the water, and thus are more stable. Vinegar and oil salad dressing is a classic example of temporary emulsions.

To stabilize emulsions it is necessary to add molecules which decrease the interfacial tension between mixtures of lipids and water. Surfactants are molecules that contain both hydrophobic (non-polar) and hydrophylic (polar) regions in their structure. When added to a system that contains both lipids and water, surfactants rapidly migrate to the interfaces between the two phases. At the interface, the surfactants orient their polar region towards the aqueous phase and their non-polar region towards the lipid phase. Since proteins contain amino acid residues that can be polar and non-polar they can be excellent surfactants in food systems. Figure 7.2 illustrates the coating of an oil or fat droplet with a protein molecule. Once the droplet or air bubble is coated, the interfacial tension between the two phases is markedly lowered and the tendency to coalesce is greatly reduced. The simplistic illustration in Figure 7.2 shows a single molecule of protein unfolding on the oil surface. In a real emulsion, there are thousands of molecules involved on the surface of a single droplet. If a sufficient reduction in interfacial tension is achieved, the emulsion can be stable for long periods of time.

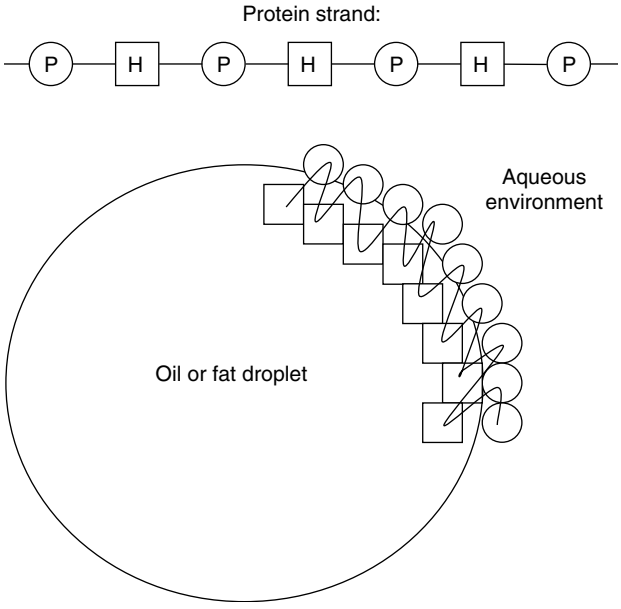

FIGURE 7.2 Formation of a protein stabilized emulsion. The protein is represented by the connected circles and squares, which are defined as follows:
Squares are hydrophobic (H) amino acid residues which orient towards the oil or fat phase.
Circles are hydrophylic (P) amino acid residues which orient towards the aqueous phase.
The continuous line represents the peptide bonds connecting the amino acids.

2. Factors Affecting Protein-Based Emulsions

Food emulsions are complex systems which are normally created by using large amounts of energy to form very small particles of one phase which become suspended in another. Higher energy inputs normally result in smaller droplets and enhanced activity of any surfactants that are present. To work well as a surfactant, a protein must be able to migrate to the interface, orient polar and non-polar side chains into the proper phases, and form a stable film around the droplet. In many cases proteins may partially unfold or denature during these activities. After unfolding and orientation towards the proper phase, proteins form multiple layers on the droplet surface due to intermolecular ionic, hydrogen, and hydrophobic bonding between unfolded protein strands. The formation of superior emulsions by proteins relates to their ability to form viscous yet flexible films around the surface of droplets (19).

Most food emulsions are oil in water. In forming o/w emulsions, the initial water solubility of the protein is very important. Therefore, factors such as protein concentration, pH, salts, ionic strength, and temperature strongly affect the emulsification ability of proteins. Other factors that influence their ability to emulsify are related to the physicochemical properties of the protein, such as surface charge, surface hydrophobicity, molecular flexibility, ease of denaturation, and dissociation behavior of subunits. Table 7.3 lists the factors which can be important to the formation and stability of protein-based emulsions.

Since proteins vary in physicochemical properties, it is not unexpected that researchers have seen varying results when studying the effects of pH, salts, and temperature on the emulsification ability of proteins. Because proteins have unique primary, secondary, and quaternary structures, environmental effects such as pH must be determined for each individual protein. In general, proteins near their isoelectric point (pI) are poor emulsifiers, presumably due to poor solubility. As the pH is adjusted away from the pI, improvement in emulsification is generally seen and is probably due to enhanced solubility. Very low or high pH may lead to poor emulsification even though the proteins might be very soluble. In this case, the proteins are so highly charged they do not interact to form films on the surface of the dispersed phase. If proteins are coated with an excess of charges they will not form cohesive, flexible films (19). Proteins with multiple subunits, such as the soybean storage protein legumin, may exhibit

TABLE 7.3
Factors Affecting Formation and Stability of Protein-Based Emulsions

Environmental factors	Temperature, pH, salt concentration, salt type, ionic strength, other ions, other food components
Protein characteristics	Solubility, ease of denaturation, surface hydrophobicity, surface charge, isoelectric point, flexibility, elasticity of protein film
Processing parameters	Amount and rate of energy input (shear), oil or fat type, oxidative state of lipid, temperature
Aqueous phase	Viscosity

improved emulsification activity at pH values where the subunits are encouraged to dissociate (20).

Several researchers have shown that surface hydrophobicity can be highly related to the ability of proteins to act as emulsifiers (21). In general, proteins that are water soluble but which have large numbers of hydrophobic groups on their surface tend to be superior emulsifiers. With increased surface hydrophobicity, a larger portion of the protein strand might be able to interact with the oil or fat droplet and form stronger protein films (22). Results have shown that proteins with high surface hydrophobicities, such as casein or bovine serum albumin (BSA), are superior emulsifiers compared to proteins with low surface hydrophobicities, such as collagen or gelatin (21).

3. Methods of Measurement

As with other model systems tests, the results of protein emulsification evaluations are often highly empirical. Comparison of results between laboratories is only possible when strict testing conditions are maintained. Although the absolute numbers obtained in these tests may vary from lab to lab, the ranking of different proteins in terms of ability to emulsify or emulsion stability are often similar. The results seen in rapid tests may not give the same results seen in a true food system. Many food companies, however, cannot wait a year for results when they are evaluating proteins for use in products under development and subject to an accelerated development time table.

Emulsions are commonly tested for two parameters: stability and capacity. Emulsification capacity is normally measured as the maximum amount of oil a protein may emulsify. A classic test of emulsification capacity is that of Wang and Kinsella (23), where the maximum amount of oil that a protein can emulsify is determined by a titration method. Oil is added to a protein solution in a blender or mixer until the emulsion fails, which is determined by a rapid decrease in viscosity. Advantages are that the procedure is quick and simple. Disadvantages are that the results are highly dependent on the equipment used to generate the emulsions and the protein:lipid ratios encountered in the test are not commonly encountered in food systems (24). Another widely used technique is the Emulsification Activity Index (EAI) developed by Pearse and Kinsella (25). In this assay, the interfacial area created in an emulsion is measured by a spectrophotometric assay. As the ability of a protein to emulsify increases, smaller and smaller emulsion droplets are formed, which can be measured by light scattering principles. Advantages of this method are that it is a rapid technique that does not rely on the use of an external force to break the emulsion. Disadvantages are: 1) emulsions must be prepared under very standardized conditions (e.g., sample container volume, homogenizer or mixer manufacturer, speed or power settings) and 2) several studies have found poor correlations between EAI and emulsion stability (24).

Emulsion stability can be measured by a variety of methods. Choice of method depends on the type of instability or emulsion breakdown observed in the actual food system where the protein may be used. Separation and clustering of fat or oil droplets on the top of an emulsion is called "creaming" and is a marked sign of emulsion breakdown. A standard test for creaming (26) involves the placement of an emulsion in a graduated cylinder which is held in an environmental chamber. The height of the boundary between the top of the creaming layer and the residual emulsion is measured as it decreases with time. Advantages of the test are that it can be set up rapidly and it can be easily used to determine how pH, ionic strength, and protein concentration impact emulsion stability. Disadvantages are that lengthy storage times under highly controlled conditions are required (24). A more rapid test is the Emulsion Volume Index where centrifugation in microhematocrit tubes is used to accelerate the forces which cause emulsion breakdown (27). Advantages are that analysis times are much shorter and other researchers have found correlations between EVI and longer term stability testing of actual products (24). If time is not essential, it is possible to use a long-term storage test to evaluate emulsion stability by measuring droplet size distribution and concentration in a hermetically sealed container (28). Although the test can duplicate actual storage conditions of products, it may take over a year to complete (24).

E. Foaming

Emulsions and foams are similar types of food systems in that they contain two distinct phases. In foams, the liquid or solid continuous phase surrounds a dispersed gaseous phase which is usually air. Many times a protein which

emulsifies well will also foam well. The first step in the formation of a foam is the migration of proteins to the interface between air bubbles and the aqueous phase. At the interface the protein will unfold and orient their nonpolar regions toward the air phase. As proteins adsorb on the bubble surface, they begin to form layers of partially denatured proteins which encapsulate the air bubble and prevent the foam from collapsing (29). Foams may be produced by mechanical agitation (whipping) or sparging, which is the injection of gas through very small orifices to produce bubbles. Most food foams are produced by whipping and include meringues, soufflés, whipped cream, non-dairy whipped toppings, angel food or sponge cakes, and ice cream. Yeast leavened breads are foams that are produced by the trapping of carbon dioxide bubbles by the gluten protein matrix.

1. Factors Affecting Foaming

As with other functional properties, solubility plays a critical role in foaming. Good foaming proteins also exhibit one or more of the following molecular properties: 1) high rates of diffusion and adsorption at the interface, 2) ability to unfold and denature at the interface, and 3) ability to form intermolecular associations with other molecules that result in the formation of cohesive films around the air bubble (1). The surface hydrophobicity of proteins correlates with its ability to form foams (21). Since it is important for a protein to unfold on the interface, the foaming ability of some proteins can be improved by a mild heat treatment or chemical modification which loosens the protein structure and allows it to unfold more rapidly. Enhanced film formation through increased intermolecular bonding is also generally seen. Excessive denaturation will decrease foam formation and stability by decreasing initial protein solubility and causing protein films to form at the interface that are inflexible and stiff. This is a result of excessive intermolecular interaction between protein strands.

In regard to foaming ability, egg whites (albumen) form some of the highest quality foams due to the properties of the constituent proteins, ovalbumin, globulins, and ovomucoid. During the foaming of egg whites, acids such as cream of tartar (potassium acid tartrate) are added after an initial whipping period. The acid lowers the pH and reduces the net charge on the protein which allows the protein strands to interact more strongly. It also facilitates the denaturation of proteins to increase the elasticity of protein films around the air bubbles. Overall, a more stable film results. Addition of the tartrate before the initial foaming causes the proteins to unfold prematurely and interact before they reach the air bubble surface, which results in decreased foam volumes and stabilities (1).

In most cases, the foaming ability of proteins in inhibited by the presence of lipids. Contamination of egg white with as little as 0.03% egg yolk completely inhibits foaming (30). It is theorized that lipid is absorbed at the air/water interface within the foam and causes the protein film to rupture. Conversely, high concentrations of saturated fat can stabilize foams. An example is whipped dairy cream in which cold coalesced fat droplets can surround protein-encapsulated air bubbles, resulting in a very stable foam.

The amount of energy used during foam formation impacts on foam stability. Energy inputs must be sufficient to create very small air bubbles and assist in the denaturation of proteins due to shear forces. Over-whipping, however, may cause the foam to dehydrate and collapse, leading to decreased foam volume and stability. Energy input must not cause the proteins to denature prior to encountering air bubbles. Denatured protein may fail to reach the air bubble surface and precipitate by interacting with other protein molecules in solution. The denatured protein may also form more brittle films on the air bubble surface.

2. Methods of Measurement

As previously mentioned, the results of functional tests many times are empirical and depend on the methods used and the laboratory in which they are performed. Several methods have been published for foaming proteins, including shaking, sparging (gas injection), and whipping (31). Most food foams are prepared by whipping. Results from a model system test that is based on whipping might be better correlated to actual foods since the method of preparation more closely resembles actual food preparation. Both foam volume and foam stability are important in the manufacture of foods, so it would be valuable to gather data in a model system on those two parameters. Wilde and Clark (29) have published foamability and stability methods that have been widely accepted. The methods use inexpensive equipment but care should be taken to use identical procedures between different laboratories. Another widely used test determines the percent overrun of a foam and involves the removal of samples at various times to determine foam density (32). Stability tests for foams commonly use a "half-life" determination where the time for half of the foam to break down or half the original liquid volume to return to the liquid state is measured.

III. APPLICATION OF FUNCTIONAL PROPERTIES

A. Intentional Modification of Protein Functional Properties

The manufacture of many modern food products relies on the use of proteins for their functionality. The proteins

may be a natural constituent of the food, such as in muscle, or the proteins may be added as an ingredient due to their ability to solubilize, bind water, gel, emulsify, or foam. As we have discussed, the functional properties of proteins are interrelated and associated with the structures of the protein molecule. Although we can obtain protein from a multitude of sources, not all proteins are highly functional due to their primary, secondary, tertiary, or quaternary structures. For example, several yeast proteins and many plant proteins have limited functionality when used in their native conformation. Solubility may be a problem for some types of proteins and require the addition of salts to enhance their functionality. An example is in the manufacture of frankfurters where 0.5–1.5% salt is commonly used to enhance the functionality of myosin in gel formation. Protein modification has the potential to enhance the use of many less functional and less expensive proteins to perhaps make foods more economical and perhaps more desirable in a nutritional sense.

Modification is the intentional alteration of the physicochemical properties of a protein by chemical, physical, or enzymatic means to improve functional properties (33). Many of the alterations simply increase solubility, since it is paramount to most functional properties. For proteins with substantial quaternary structures, such as the legume storage proteins, improvement is related to the disruption of the bonding between individual subunits. In other cases it might be the alteration of amino acids on the protein surface to make them more polar or the disruption of hydrophobic cores of proteins to decrease their molecular density.

As we have learned, a protein's ability to emulsify or foam is related to its ability to migrate to an interface and denature or unfold. Some types of modification will "loosen" up the native structure of a protein through chemical, physical (e.g., heating), or enzymatic means to allow the protein to migrate, unfold, and interact more substantially on the interface in either emulsions or foams. These types of treatments can also enhance the ability of water to bind within a protein or the ability of protein strands to interact to form gels; thus both water-binding and gelation behavior of proteins can be enhanced by modification.

1. Chemical Modification

A variety of chemical modifications have been used to enhance the functionality of proteins from plants, animals, and microorganisms. These generally involve the use of a chemical agent to modify the protein by reacting to covalently alter the amino acid residues on its surface. Acylation reactions that involve the direct addition of chemical groups through the R groups (side chains) of amino acids have the most potential to modify proteins. Researchers have examined modification of surface charge via the creation of esters with acetic, succinic, phosphoric, and a number of fatty acids. Sugars and other oligosaccharides have also been attached to the surfaces of proteins (33).

The common sites for acylation are the epsilon and alpha amino groups of proteins. Lysine is a particularly reactive amino acid, followed by tyrosine and cysteine. Alteration of surface charge and surface hydrophobicity through these modifications is the likely mechanism for functionality improvement.

For chemically altered proteins to be acceptable for human foods they must be nontoxic and digestible (34). Typically, modified proteins can be tested using protocols outlined by the Food and Drug Administration (FDA). A number of different tests can be used to determine the impact on nutritive value (33). Because lysine is an essential amino acid for many species and is also a major site for modification, decreases in nutritive value are seen in chemically modified proteins. Protein Efficiency Ratios (PER) for modified proteins typically range from 40–100% of the values for their unmodified counterparts (33). Since physical and enzymatic methods do not alter amino acid composition dramatically, they are preferred methods for modifying proteins.

2. Enzymatic Modification

Enzymatic modification of proteins includes partial hydrolysis, covalent attachment of functional groups, and the incorporation of cross-links between protein molecules (35).

Protein hydrolysis is the most widely used of these techniques. Proteolysis is easy to control, very rapid, and occurs under ambient conditions. There is very low risk for the formation of toxic residues. Proteolysis is considered by many to be the most cost-effective way to enhance protein functionality (35). Because proteolysis produces peptides that are smaller in size and which contain less secondary structure than the original proteins, their solubility is increased. Enhanced solubility is directly related to the degree of hydrolysis and related to increases in functional properties such as foaming and emulsification. Partial hydrolysis has been used to improve the functionality of a wide variety of proteins from beef, chicken, dairy, fish, yeast, corn, wheat, and peas. Proteolysis is commercially used to prepare functional protein products for use as food ingredients. Depending on the source of protein, optimal peptide size for one functionality, such as foaming, does not always equate to optimal functionality for another, such as emulsification. Proteolysis is quite interesting in that it is possible to fine tune the process to obtain protein ingredients that are optimized for a given functionality.

Enhanced functionality due to cross-linking or covalent attachment of a hydrophylic or hydrophobic residue

is also an area of intense research. Several very functional natural proteins, such as ovomucin, the superior foaming protein from egg white, have surfaces that are highly polar due the presence of glycosyl groups. Researchers have been able to produce protein products with excellent foaming abilities from soy and casein using enzymes which attach very polar groups to their surface (36). Another enzyme, peptidoglutaminase, has also been examined as a means of improving functionality (37). The enzyme increases the negative charges on a protein by converting glutamine and aparagine to their negatively charged counterparts, glutamic and aspartic acids. This technology appears to have potential for widespread use since many insoluble proteins from soy, peas, beans, dairy, and wheat have substantial numbers of glutamine and aparagine residues on their surface.

3. Physical Modification

Heat is the major way in which physical modifications are carried out with proteins. For example, the industry is replete with many soy protein products that have been modified by heating to improve foaming, emulsification, water-binding, and gelation properties (38). Heat causes proteins to partially denature through changes in secondary and primary structure. In theory, this should increase the functionality of the protein by improving its ability to unfold at interfaces and form films around air bubbles or lipid droplets. In particular, the gelling ability of several proteins, such as those from dairy whey, has been substantially improved through heat treatment (4). Many of the procedures used to manufacture heated protein ingredients for use in formulated foods are proprietary. It is known, however, that the procedures used to maximize the functionality of a protein source for one application are usually not the same for another. In other words, heating techniques used to enhance foaming or gelling are not the same as for emulsification (4).

A specific type of modification of plant proteins using heat has widespread application. Texturized Vegetable Protein (TVP) is commonly used in the processed food and food service industry to bind water and fat. Most TVP products are made from soy and are manufactured under very specific environmental conditions of temperature, pH, ionic strength, and pressure. TVP is produced using extrusion techniques where the proteins are subjected to heat, pressure, and shear forces simultaneously. The proteins are extensively denatured and insolublized; however, the resulting matrix is almost sponge-like in its ability to bind other liquids, such as excess fat and moisture, in food systems.

One interesting application of physical alteration of proteins is the manufacture of several fat substitutes through the manipulation of their physical characteristics. Using a process generically called "microparticulation," proteins from milk and/or eggs are denatured and refolded into smaller, denser particles that can act as lubricants during the chewing and swallowing of foods. These materials have the mouthfeel of lipids, but are actually a blend of proteins and water. Simplesse (registered trade name by the NutraSweet Company) is an example. Since these ingredients are proteins, they cannot be used in products that will see extremes of environment, such as heat or pH, since they would most likely denature under such conditions. Their use is probably limited to products that will only see low temperature heating processes, such as pasteurization.

IV. SUMMARY

The functional properties of proteins are defined as their physical or chemical properties that affect foods during their preparation, processing, storage, and consumption. They contribute greatly to the quality and acceptability of a wide range of natural and processed/prepared foods in the food supply. Proteins are considered by many experts to be the most multifunctional components of foods in that they can play many different roles in foods. Natural products made from meat, poultry, dairy, eggs, cereal grains, and legumes, as well as the majority of formulated foods developed and marketed through the food industry, all rely on the functionality of proteins for their acceptability and quality.

The functional properties include solubility, water-holding ability or capacity, gelation, emulsification, and foaming. Solubility is paramount for the successful use of proteins in most food systems. To function in a food the protein must be able to migrate throughout the aqueous phase to seek interfaces (foaming and emulsification), hold water, or form extensive three-dimensional networks (gelation). The solubility of a protein is determined by its amino acid sequence and is greatly influenced by environmental factors such as solvent polarity, pH, temperature, and concentration of dissolved salts.

Food gels are diverse structures primarily composed of immobilized water held within a cross-linked protein matrix. Both thermally reversible (gelatin) and thermally irreversible (frankfurters) gels are important food products. The ability of a protein to gel is primarily evaluated by measuring the texture or strength of the gel using a torsion test.

Water binding by proteins is influenced by temperature, concentration of protein, concentration of salt, degree of protein denaturation, and the presence of other compounds, such as sugars or alcohols. Two main methods, water absorption and expressible moisture, are commonly used to measure the water binding of proteins.

Emulsification and foaming are cousins in the protein functionality family. They both rely on similar properties of the protein in the food system, such as solubility and

ease of denaturation. Proteins must be able to migrate to the interface formed with the second phase of oil in emulsions and air in foams. Proteins must be able to form cohesive, multilayered, flexible films around droplets of lipid or air. The main model tests involve measuring the overall ability of a protein to emulsify or foam, as well as the stability of the emulsion or foam created.

The functional properties of proteins are often measured using model systems that can vary widely from researcher to researcher. To make the results of our research valuable, we must strive to use protocols that are widely accepted and used by our colleagues. In many cases, model systems do not mimic the conditions seen in real food systems. We tend to measure, and correctly so, the fundamental properties that are related to the desired functional property seen in the food.

The modification of proteins by chemical, physical, and enzymatic methods has increased the utilization of less conventional protein sources and decreased the costs of manufacture for a number of food products. Most methods attempt to increase the solubility of proteins. Although chemical methods are effective, there are questions regarding safety and nutritional losses. Enzymatic and physical methods have been adapted for use in the protein ingredients industry. Heating remains the most widely used modification technique and has been very successful in increasing the functionality of a wide range of proteins, particularly those from soybeans and whey. Texturization produces very useful protein ingredients which have the ability to adsorb excess moisture and fat in foods. Lastly, the ability of proteins to be compressed allows them to be used as fat substitutes in foods.

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8 Lipid Chemistry and Biochemistry

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CONTENTS

I. INTRODUCTION

Although the intake of excess fat may result in significant health problems, it is important to note that fats are a critical part of a proper diet. The human body can produce most fatty acids but certain "essential" fatty acids (e.g., linoleic and linolenic acid) are typically derived from lipid-containing foods since the body cannot produce them. In addition, fats in foods are sources of vitamins A, D, E, and K. There is considerable evidence that therapeutic components that can improve human health are endogenous to food fats. Fats also contribute unique

flavors and physical properties to the foods that consumers find desirable.

The two major classes of fats in foods are phospholipids (PL) and triacylglycerols (TAG). Phospholipids are constituents of cell membranes while TAG are fatty globules that exist primarily as coalesced droplets in biological tissues. Other types of fats include sterols, waxes, and carotenoids. Lipid is a term used to encompass all types of compounds that are soluble in organic solvents and therefore classified as fats. Olive oil contains only triacylglycerols while muscle foods contain both PL and triacylglycerols. Food emulsions can contain only TAG (e.g., margarine) or TAG and PL (e.g., mayonnaise). The egg portion of mayonnaise supplies PL, which cause the water phase and the oil phase to be continuous. In other words, yolk phospholipids act as emulsifiers. Phospholipids and TAG are each susceptible to oxidation during storage that results in the formation of off-odors and off-flavors, which effectively end shelf life. Loss of nutritional value can also occur due to oxidation of the lipid and co-oxidation of proteins and vitamins (1). Triacylglycerols and phospholipids can be modified so that desirable functional properties are made available. These functional properties include physical attributes, flavor stability, caloric value, therapeutic effects, and nutrient content. This chapter will primarily focus on 1) factors that promote or inhibit lipid oxidation processes, and 2) chemical modification of lipids that improve their functional and nutraceutical properties. Further, the chemistry involved in food processes such as oil refining, frying, and food irradiation will be addressed.

II. NOMENCLATURE

The core portion of any lipid comprises repeating units of the hydrocarbon group $(-CH_2)_n$. Saturated hydrocarbons are typically named with a numerical prefix and the termination "ane" (e.g., octane is an 8-carbon saturated hydrocarbon). The suffix "ene" indicates the presence of double bonds also expressed as "unsaturation" in the hydrocarbon. Fatty acids contain an acid group (-COOH) bound to the hydrocarbon tail (Figure 8.1). At pH values above the pKa for each fatty acid, the fatty acid will exist mainly in its conjugated base form (COO^-). The charge on the fatty acid will control its reactivity.

The shorthand for c-9 octadecenoic acid (oleic acid) is 18:1ω9. This indicates there are 18 carbons and one double bond. The ω9 indicates that the double bond is nine carbons in from the end of the hydrocarbon portion. c-11 octadecenoic acid (asclepic acid, 18:1ω7) also exists in nature but is less prevalent than oleic acid. This molecule is identical to oleic acid except that the double bond is seven carbons in from the hydrocarbon tail. The omega symbol (ω) is assigned because nutritional and health impact of different fatty acids are related to the locations of the double bonds. Omega-3, ω-3, and n-3 are often used interchangeably. Linolenic acid (18:3ω6 or 18:3 cis-6, cis-9, cis-12) indicates that there are three double bonds. The 6, 9, 12 are the positions where the double bonds begin counting the carboxylic acid group as the first carbon (Figure 8.1). The *cis* conformation is indicative that both alkyl groups adjacent to the double bond are aligned in the same direction. *Trans* fatty acids have the alkyl groups pointing in opposite directions. Most polyunsaturated fatty acids have 1,4-pentadiene structures as illustrated in Figure 8.1. Conjugated fatty acids will have one of the double bonds shifted one carbon closer to the adjoining double bond. Desaturases and elongases cause smaller and more saturated fatty acids (e.g., linoleic acid 18:2ω6) to be converted to longer and less saturated fatty acids (e.g., arachidonic acid 20:4 ω6).

Each membrane phospholipid contains two fatty acids and a single polar head group esterified to glycerol 3-phosphate (Figure 8.2). Typical head groups bound to the phosphate portion include ethanolamine, choline, and serine. These head groups are polar and orient themselves toward the aqueous phase while the fatty acids assemble away from the water phase to form a lamellar membrane bilayer (Figure 8.3). Sterols, proteins, and glycolipids imbedded in the membrane are not illustrated. Other molecular arrangements of phospholipids are possible including inverted micelles (2). Triacylglycerols contain a glycerol backbone on which three fatty acids are esterified. Locations of the fatty acids are designated as sn-1, sn-2, and sn-3. A more extensive review of nomenclature of lipids including different sphingolipids, waxes, sterols, and carotenoids is available (3).

III. LIPID OXIDATION

Lipid oxidation needs to be controlled during storage in order to prevent the formation of off-odors and off-flavors in foods. Desired color and nutritional attributes are also compromised when oxidation of lipids is unimpeded

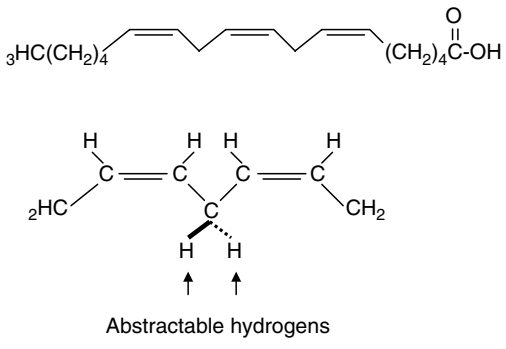


FIGURE 8.1 (Top) Structure of cis-6, cis-9, cis-12 linolenic acid (18:3ω6). (Bottom) The 1,4-pentadiene unit within most polyunsaturated fatty acids.

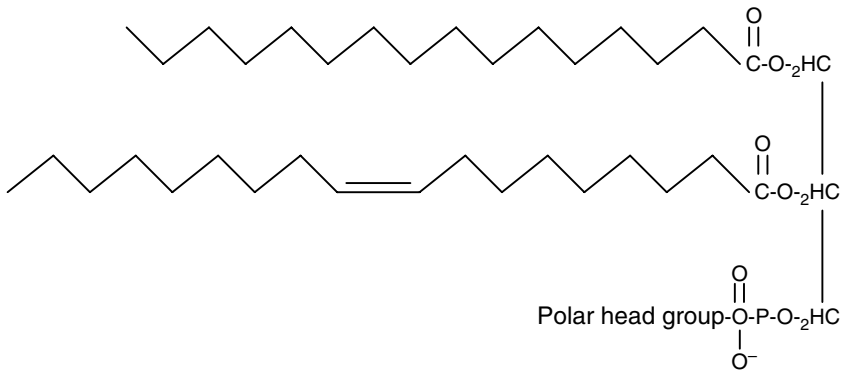


FIGURE 8.2 Basic structure of a glycerophospholipid.

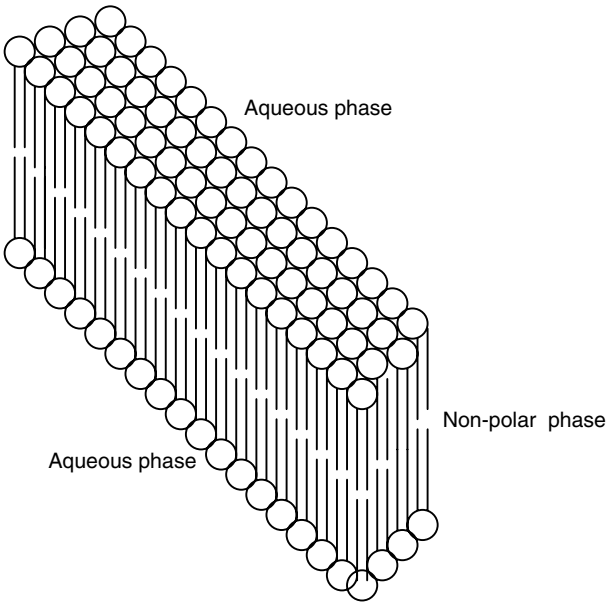


FIGURE 8.3 A simplified representation of a membrane bilayer.

during storage. The mechanism and rate of lipid oxidation will vary depending on the system under investigation. Interfacial properties, processing temperature, particle size, fatty acid unsaturation, and non-lipid constituents will differ among different systems (e.g., a bulk oil, salad dressing emulsion, raw muscle, cooked meat). Therefore the optimal strategies used to inhibit lipid oxidation processes will change from system to system. This should be kept in mind as general principles of lipid oxidation reactions are discussed. Attention to lipid oxidation has rapidly expanded in recent years due to the growing accumulation of evidence that the onset of various disease states may be related to intake of oxidized food lipids and ingesting critical amounts of antioxidants may improve human health (4, 5).

The classical description of lipid oxidation processes begins with the initiation step where a hydrogen atom is abstracted from a polyunsaturated fatty acid (Figure 8.4).

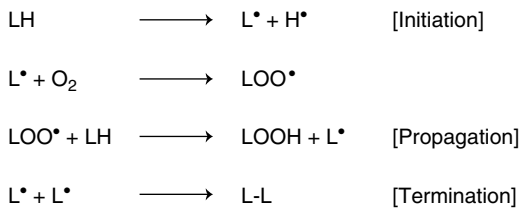


FIGURE 8.4 Initiation, propagation and termination of lipid peroxidation.

This causes the formation of a lipid alkyl radical (L•) that reacts with oxygen to form a peroxyl radical (LOO•). The peroxyl radical can then abstract a hydrogen atom from a different polyunsaturated fatty acid to form a lipid hydroperoxide (LOOH) and another alkyl radical (propagation). It should be kept in mind that small amounts of pre-formed lipid hydroperoxides exist in freshly processed oils, muscle foods and even biological tissues *in vivo* (6, 7). This may decrease the importance of controlling true 'initiators' of lipid oxidation and switch the emphasis to a better understanding of food constituents that break down lipid hydroperoxides. The breakdown of lipid hydroperoxides by metals, heme, heme proteins, and hydroperoxide lyases causes the formation of key volatiles (e.g., aldehydes and ketones) that are responsible for the undesirable odors and flavors associated with lipid oxidation.

Oleic acid (18:1) is generally more susceptible to lipid oxidation than any saturated fatty acids. This is because the lower bond energy of hydrogen atoms adjacent to a double bond allows for more easy abstraction compared to hydrogen atoms in fatty acids lacking double bonds. Most fatty acids with two or more double bonds possess the 1,4-pentadiene structure (Figure 8.1). Hydrogen atoms in the center of the 1,4-pentadiene are around 20 times more abstractable than the abstractable hydrogen of oleic acid (8). Increasing the number of double bonds increases the number of 1,4-pentadiene units and thus increases susceptibility to lipid oxidation. The relative rates of lipid oxidation of oleate (1 double bond): linoleate (2 double bonds): linolenate (3 double bonds) was reported to be 1:12:25 (9).

This was the case in pure lipid systems. In different liposome preparations, oxidative stability decreased as the degree of fatty acid unsaturation increased (10). However, the order of oxidative stability was found to increase with increasing degree of unsaturation when different fatty acids were dispersed in 1% Tween 20 and exposed to iron salts and ascorbate (11). Although atypical, this indicates that under certain conditions highly unsaturated fatty acids are more resistant to lipid oxidation than more saturated ones. In oil-in-water emulsions, fatty acid composition, the physical states of the lipids, content of tocopherols, and activity of transition metals were implicated as factors controlling rates of lipid oxidation (12).

In muscle foods the situation is even more complex. A multitude of components are present that can either accelerate or slow down lipid oxidation processes. Water-washing of muscle fibers from various animal species was used to obtain a wide range of fatty acid unsaturation (13). Washing the muscle removes aqueous antioxidants and pro-oxidants while insoluble myofibrillar proteins and membrane phospholipids remain after washing. Metmyoglobin or iron was added to the washed fibers to stimulate lipid oxidation. The general trend was that lipid oxidation occurred more rapidly in washed muscle containing elevated levels of polyunsaturated fatty acids (fish > chicken > beef). It has also been shown that the ability of fish, poultry, and beef hemoglobins to promote lipid oxidation in a washed muscle system occurred in the following order (fish > chicken > beef) (14). Therefore, the reactivity of different hemoglobins in various muscle foods should be considered as a causative factor in addition to fatty acid unsaturation and endogenous antioxidant capacity.

Compartmentation of cellular and extracellular reactants should be critical in controlling rates of lipid oxidation. Takama et al. (15) suggested that minced flesh of trout was susceptible to rancidity due to the dispersed blood pigments in the flesh caused by the mechanical destruction of the tissue. Crushing plant tissue brings formerly segregated reactants together to stimulate various reactions including oxidation of lipid (16). Critical cellular components and additional factors that control rates of lipid oxidation are discussed below. In any discussion pertaining to lipid oxidation, it is important to realize that any component that accelerates lipid oxidation under one set of conditions can be inhibitory under different conditions.

A. Metals

Low-molecular-weight metals are potent catalysts of lipid oxidation. Copper and iron are two of the more potent metal catalysts in biological systems. Only ferrous ions and oxygen are needed to produce hydrogen peroxide (H_2O_2) as seen in reactions 1 and 2:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + {}^{-\bullet}O_2 \qquad \text{[reaction 1]}$$

$$^{-\bullet}O_2 + 2H^+ \rightarrow H_2O_2 \qquad \text{[reaction 2]}$$

Ferrous iron (Fe^{2+}) can then react with H_2O_2 or preformed lipid hydroperoxides to produce hydroxyl or alkoxyl and hydroxyl radicals, respectively (reactions 3 and 4). Hydroxyl and alkoxyl radicals are capable of abstracting a hydrogen atom from a polyunsaturated fatty acid and hence initiate/propagate lipid oxidation (17).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{-\bullet}OH \qquad \text{[reaction 3]}$$

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + {}^{-\bullet}OH + LO^{-\bullet} \qquad \text{[reaction 4]}$$

Reaction 3 is termed the Fenton reaction. Hydroxyl radical can also be produced via the Haber-Weiss reaction (reaction 5). A "ferryl ion" is produced from Fenton reagents and relevant as an initiator of lipid oxidation (reaction 6); even in the absence of H_2O_2, ferryl ion can be produced from the reaction of Fe^{2+} and the "perferryl ion" complex ($Fe^{2+}O_2$) (18).

$$^{-\bullet}O_2 + H_2O_2 \rightarrow O_2 + OH^- + {}^{\bullet}OH \qquad \text{[reaction 5]}$$

$$Fe^{2+} + H_2O_2 \rightarrow \underset{\text{Ferryl ion}}{Fe^{2+}O} + H_2O \qquad \text{[reaction 6]}$$

Chelators such as ethylenediaminetetraacetic acid (EDTA) and adenosine diphospate (ADP) are widely used to enhance the ability of iron to promote lipid peroxidation (19). Ascorbate increased the ability of iron to stimulate lipid oxidation by reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) (20). Antioxidant properties of ascorbate and metal chelators are discussed later (Section V.F and V.G). Ferric iron can also be reduced enzymatically (e.g., membrane bound reductase, ADP and NADH). Although Fe^{2+} did eventually stimulate lipid oxidation in sarcoplasmic reticulum, increasing concentrations of Fe^{2+} increased the lag phase prior to lipid oxidation; this suggested that Fe^{2+} initially was an antioxidant by reducing membrane antioxidant radicals to their active form (21). Fe^{2+} could then stimulate lipid oxidation after the antioxidant capacity was exhausted. Lipolysis, cooking temperatures, ascorbate, peroxides and extended storage times have the ability to increase iron concentrations in biological systems by stimulating the release of iron from proteins including ferritin, transferrin, hemoglobin, and myoglobin (22–26).

B. Heme Proteins

Hemoglobin and myoglobin are the predominant heme proteins (HP) in muscle foods. The blood protein hemoglobin is a tetrameric protein while myoglobin from the interior of muscle cells is a monomer (single polypeptide chain). Hemoglobin tetramers dissociate into monomers and dimers upon dilution and with decreasing pH (27, 28).

Certain aquatic and land animals possess multiple hemoglobins with different chromatography characteristics (29, 30). These factors can cause erroneous determination of hemoglobin and myoglobin content in tissue extracts. Heme proteins consist of a globin chain(s) and a heme ring(s), the latter containing an iron atom. The iron is primarily in the ferrous state (HP-Fe^{2+}) *in vivo*. Met heme protein (HP-Fe^{3+}) accumulates post mortem via a proton or deoxygenated HP mechanism (reactions 7 and 8) (31). A general term for the formation of metHP from ferrousHP is heme protein autoxidation.

$$\text{oxy}(+2)\text{HP} + \text{H}^+ \rightarrow \text{met}(+3)\text{HP} + {}^{\bullet}\text{OOH} \qquad \text{[reaction 7]}$$

$$\text{deoxy}(+2)\text{HP} + \text{O}_2 \rightarrow \text{met}(+3)\text{HP} + {}^{-\bullet}\text{O}_2 \qquad \text{[reaction 8]}$$

Met heme protein formation is likely critical to the onset of lipid oxidation since metHP reacts with either H_2O_2 or lipid hydroperoxides to form the ferryl HP radical that is capable of initiating lipid oxidation (reactions 9 and 10) (32). MetHP is much more likely to unfold and release its heme group compared to ferrous HP (33, 34). Released or displaced heme can react with lipid peroxides to form various lipid radical species that have the ability to propagate lipid oxidation processes (reaction 11) (35–37). Bohr effects occur in certain fish hemoglobins (38). This decreases oxygen affinity of the heme protein at post mortem pH values and hence increases met heme protein formation (reaction 8). It is still unclear if ferrous forms of heme proteins can react with lipid hydroperoxides to stimulate lipid oxidation processes although some potential pathways have been suggested involving oxyHP and deoxyHP (39, 40).

$$\text{met}(+3)\text{HP} + \text{H}_2\text{O}_2 \rightarrow \text{HP}^{+\bullet}\text{ferryl}(+4) = \text{O} + \text{H}_2\text{O} \qquad \text{[reaction 9]}$$

$$\text{met}(+3)\text{HP} + \text{LOOH} \rightarrow \text{HP}^{+\bullet}\text{ferryl}(+4) = \text{O} + \text{LOH} \qquad \text{[reaction 10]}$$

$$\text{heme}(+3) + \text{LOOH} \rightarrow \text{Heme}(+3)\text{-O}^{\bullet} + \text{LO}^{\bullet} \qquad \text{[reaction 11]}$$

C. Peroxides

There are numerous sources of hydrogen peroxide in biological systems. Equations 1 and 2 describe an iron, oxygen, and proton-mediated mechanism of formation. NADPH-cytochrome P450 reductase produces ${}^{-\bullet}O_2$ that dismutates to H_2O_2 (19). Production of H_2O_2 in mitochondrial and peroxisomal fractions has been described (41). H_2O_2 production in erythrocytes was mainly attributed to hemoglobin autoxidation (42). H_2O_2 was produced at a rate of 14 nmol/g of fresh weight/30 min in turkey muscle at 37°C (43). High concentrations of H_2O_2 will cause release of iron from the heme ring of heme proteins (25).

Like H_2O_2, lipid hydroperoxides (LHP) react with metals or heme proteins to produce free radical species that propagate lipid oxidation. Further, the collection of volatiles that produce rancid odor result from LHP breakdown. Trace amounts of LHP are required for lipoxygenase activity, converting iron in the active site from the ferrous to ferric form (44). Reduction of lipid hydroperoxides to alcohols with compounds such as ebselen and triphenylphosphine often abolishes any lipid oxidation that was observed prior to reduction (45, 46). Tocopherol-mediated lipid peroxidation was found to require Cu^{2+} and low levels of lipid hydroperoxides (47). Fe^{2+} reacts with lipid hydroperoxides around 20 times faster than with hydrogen peroxide (48). Protein hydroperoxides may also exacerbate lipid oxidation processes (49). Non-lipid surfactant hydroperoxides increased rates of lipid oxidation in oil-in-water emulsions (50).

D. Role of Oxygen

Oxygen not only peroxidizes alkyl radicals to propagate lipid oxidation (Figure 8.4) but also is a source of activated oxygen species (Figure 8.5). Unlike ${}^{-\bullet}O_2$, ${}^{\bullet}OOH$ can cross membranes, which may increase its pro-oxidative character (51). The oxygen concentration in marine oils is fairly constant between 20°C and 60°C but rapidly decreases between 60°C and 80°C (52). The O_2 concentrations in these oils at 20°C (0.44 to 1.25 mM) exceed the O_2 concentration found in water at the same temperature (around 0.30 mM). In 80% oxygen and 20% carbon dioxide packaging, oxygen penetrated 1.7 to 11 mm into different muscle foods (beef > pork > lamb) (53). At high ratios of $[O_2]/[H_2O_2]$, the ferryl ion initiation (reaction 6) is believed to dominate while Fenton reagents (reaction 3) are more prevalent at lower ratios (18). In CCl_4-induced lipid peroxidation of hepatocytes, a distinct maximum was obtained at 7 mm Hg oxygen while iron-mediated lipid oxidation in microsomes differed in oxygen dependence depending on whether initiation or propagation phases were considered (54). Metmyoglobin formation in beef occurred most rapidly at around 11 mm Hg oxygen (55). Non-destructive oxygen sensors are available to measure the oxygen content in headspace of different packaging systems (56). Carotenoids are believed to be more effective antioxidants at low oxygen concentrations compared to higher oxygen concentrations (57).

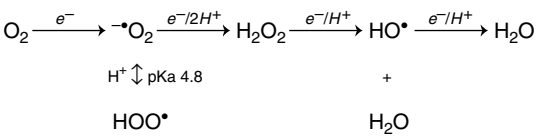


FIGURE 8.5 One-electron reductions of oxygen.

E. Lipoxygenases and Myeloperoxidases

Lipoxygenases are responsible for flavor deterioration in beans during frozen storage (58). Lipoxygenases "initiate" lipid oxidation processes by hydrogen abstraction from a polyunsaturated fatty acid. The off-flavor is due to the volatiles that are produced from breakdown of the lipoxygenase-derived lipid hydroperoxides. Fresh fish aromas are believed to result in part from lipoxygenases that enzymically peroxidize fatty acid substrates (59). These enzymes may also be responsible for formation of rancid odors by providing critical amounts of lipid hydroperoxides that can be broken down by metals or heme proteins to produce rancid odor. Some of the confusion surrounding the presence or absence of lipoxygenases in animal tissues may be due to the quasi- lipoxygenase activities of myoglobin and hemoglobin (60, 61). Esculetin has been utilized as a specific lipoxygenase inhibitor; however, esculetin is a phenolic compound that has general free radical scavenging ability and should not be considered a specific inhibitor of lipoxygenase.

Myeloperoxidases are found in white blood cell neutrophils. Myeloperoxidase catalyzes the reaction of chloride and hydrogen peroxide that produces hypochlorous acid which in turn reacts with $^{\bullet-}O_2$ and yields hydroxyl radical (62). This reaction was found to be six orders of magnitude faster than the Haber-Weiss reaction (reaction 5) and does not require iron.

F. Lipolysis

Lipolysis results in the formation of free fatty acids. Lipolysis occurs due to enzyme action or heat and moisture. Free fatty acids are responsible for both undesirable and desirable flavors (e.g., milk rancidity or positive flavors in cheese, bread, and yogurt). Cabbage phospholipase D decreased the formation of lipid oxidation products in beef homogenates and egg yolk phosphatidylcholine liposomes (63). Adding free fatty acids to fresh salmon flesh, at levels of free fatty acids that accumulated during 6 months at –10°C storage, increased taste deterioration in fresh minced salmon (64). The amount of taste deterioration from each fatty acid was 22:6n-3 > 16:1n-7 > 18:2n-6 > 20:5n-3). This suggested that hydrolysis of triacylglycerols negatively impacted sensory quality. A review on lipolysis effects in fish muscle indicated that triacylglycerol hydrolysis results in increased lipid oxidation while phospholipid hydrolysis was inhibitory (65).

G. Photoactivated Sensitizers (Singlet Oxygen)

Oxygen can exist in the triplet ($^3O_2$) or singlet state ($^1O_2$). Triplet oxygen is the normal state of oxygen while singlet oxygen is generated via photosensitization by natural pigments in food (e.g., riboflavin or chlorophyll). The two electrons in the antibonding 2p orbitals of $^3O_2$ have the same spin and are in different orbitals. This creates a small repulsive electronic state. In $^1O_2$, the two electrons are in a single antibonding orbital and have opposite spins; therefore, electrostatic repulsion will be great. $^1O_2$ is thus at a higher energy state than $^3O_2$, and $^1O_2$ is more electrophillic than $^3O_2$. This causes $^1O_2$ to react readily with moieties of high electron density such as double bonds in unsaturated fatty acids (8). This direct addition of $^1O_2$ to unsaturated fatty acids initiates lipid oxidation without the need for hydrogen abstraction as is the case with free radical-mediated initiation. Nine or more conjugated double bonds (e.g., carotenoids) are required for physical quenching of singlet oxygen (66). Other compounds such as tocopherols and amines can quench singlet oxygen by a charge transfer mechanism (67).

H. Fat Content

Release of c-9 aldehydes into headspace decreased with increasing oil content in oil-in-water emulsions (68). This suggested that the impact of certain odor compounds is decreased by elevated levels of fat via solubilization of the component into the oil phase. A study was conducted that examined the effect of added triacylglycerols on rates of hemoglobin-catalyzed oxidation of washed cod muscle lipids. No difference in rate or extent of lipid oxidation catalyzed by hemoglobin was obtained when washed cod muscle (around 0.7% phospholipids) was compared to the washed cod muscle containing up to 15% added triacylglycerols (69). This indicated that triacylglycerols did not accelerate rates of lipid oxidation during storage. Similar non-effects of added triacylglycerols were obtained in cooked lipid-extracted muscle fibers (70). Increasing fat contents did not increase oxidized oil odor in frozen stored catfish (71).

I. Effect of Cooking

Consumers are finding less time to prepare meals. The food industry is responding to this by increasing the availability of pre-cooked meats. A major problem with pre-cooked meats is the development of an objectionable warmed-over flavor via lipid oxidation (72). This warmed-over flavor occurs more rapidly during refrigerated compared to frozen storage temperatures. It has been suggested that released iron from heme proteins promotes warmed-over flavor in pre-cooked beef (23). The evidence for this was that the low-molecular-weight fraction in an aqueous extract of beef muscle stimulated lipid oxidation of washed muscle fibers much better than the high-molecular-weight fraction (73). On the other hand, in pre-cooked fish, heme proteins were believed to be the active catalysts due to higher pro-oxidative activity in the high-molecular-weight fraction of the fish muscle (74).

Polyphosphates inhibited lipid oxidation in precooked beef, which may be due to iron chelating properties of the phosphates (73). Inhibitors of warmed-over flavor were produced in meat during retorting but could not be extracted from raw beef. This suggests that the high temperature processing caused formation of products that inhibit lipid oxidation (75). Browning reactions that involve carbohydrates and amino acids were believed to impart this antioxidant effect.

Lipid oxidation is much less of a problem in precooked meats that are cured. Cured meats contain nitrite in the formulation. The primary way that nitrite is believed to exert its antioxidant effect is by conversion of nitrite to nitric oxide (NO) that binds to the iron atom in the heme ring of heme proteins. The NO-ligand may be antioxidative by preventing release of heme or iron during cooking and storage or by decreasing heme protein reactivity. Nitrite can also act as an antioxidant by chelating metals and scavenging free radicals. Nitrite may be toxic at elevated levels and therefore it is critical to control the residual nitrite content in the product.

IV. MEASURING RATES OF LIPID OXIDATION IN FOOD SYSTEMS

Lipid hydroperoxides are primary lipid oxidation products that are precursors to rancidity. Lipid hydroperoxides need to be broken down to form the low-molecular-weight volatile compounds (secondary products) that impart rancidity. It is imperative to measure primary and secondary lipid oxidation products. To accentuate this point, tocopherol enriched lipoproteins had higher levels of conjugated dienes (primary product) than lipoproteins containing little tocopherol (76). Standing alone, this errantly suggests that tocopherol was a pro-oxidant. Fortunately, these researchers also measured thiobarbituric reactive substances (TBARS) which indicated less formation of the secondary products in the tocopherol enriched samples. Apparently, tocopherol stabilized the hydroperoxides. Thus, a more complete picture is realized when measuring both primary and secondary lipid oxidation products.

Sensory analysis should be done whenever possible since human subjects can determine the point at which the product becomes undesirable which ultimately determines shelf life. Degree of rancidity or quality perception is harder to pinpoint using chemical indicators of lipid oxidation. Single time point measurements are also discouraged. Primary and secondary lipid oxidation products commonly increase, reach a maximum, and then decrease substantially. This can create a situation where one sample is perceived to be minimally oxidized but in fact had undergone extensive oxidation well before the measurement. Thus, measuring lipid oxidation products at multiple time points during storage is suggested so that a kinetic curve can be obtained which demonstrates a lag phase, exponential phase and plateau, or decrease phase.

Common lipid oxidation indicators that are measured during storage of lipid-containing foods include lipid peroxides, conjugated dienes, headspace volatiles, thiobarbituric acid reactive substances (TBARS), anisidine value, oxygen consumption, and carotene bleaching. A description of these and other methods including those used in fried products is available (8). Very good correlations between TBARS and headspace volatiles (e.g., hexanal, pentenal) were determined in cooked turkey during 4°C storage (77). TBARS are unlikely to provide useful results if the starting material has already undergone considerable oxidation. Rancidity can develop before any detectable change in fatty acid composition occurs. For example, no difference in fatty acid composition was found when fresh mackerel muscle was compared to extensively rancid mackerel muscle (78). This should not be a surprise considering that extremely small amounts of fatty acid precursors are required to produce the amount of volatiles needed for sensory impact (79).

Numerous pitfalls exist when measuring rates of lipid oxidation. Thermogravimetric methods entail weighing the sample until a rapid increase in weight occurs due to oxygen adding to the lipid. This can be done under isothermal conditions or programming from ambient to elevated temperatures. The drawback is that by the time a spike in weight occurs, detection of rancidity had previously occurred. Bulk oils are sometimes heated to 90°C to shorten the storage period needed to produce quantifiable levels of lipid oxidation. The amount of oxygen that is soluble in oil decreases substantially at elevated temperatures. This causes the mechanism of oxidation to be different from that which would occur at lower temperatures. Both the AOM and Rancimat method have been considered unreliable due to the high temperatures that are used (80). More reasonable methods to accelerate the rate of lipid oxidation in oils and emulsions are to store samples at 50°C and add metals or hemin to the system. It is interesting to note that fish held at –10°C was more susceptible to lipid oxidation than muscle stored at around 0°C. The temperature deceleration effect was apparently less substantial than the effect of freeze concentration of reactants (81). The mechanism of lipid oxidation at –20°C (commercial storage) may also be different than –10°C considering that less tissue damage should occur at the lower temperature due to faster freezing rate and smaller sized ice crystals.

V. ANTIOXIDANTS

Food antioxidants are used to inhibit lipid oxidation reactions that cause quality deterioration (e.g., flavor, color, texture, nutrient content). It is important to note that any compound that is antioxidative under one set of conditions

can become pro-oxidative under different conditions. As an example of this point, ascorbate has been found to both inhibit and accelerate lipid oxidation depending on the concentration of linoleate hydroperoxides in the system (82). The main antioxidant mechanisms are free radical scavenging, chelation of metals, removal of peroxides or reactive oxygen species, and quenching of secondary lipid oxidation products that produce rancid odors (83).

A. Free Radical Scavengers

Some typical free radicals that can initiate/propagate lipid oxidation and hence be scavenged by antioxidants include hydroxyl ($^{\bullet}OH$), alkoxyl ($LO^{\bullet}$), peroxyl radicals ($LOO^{\bullet}$), and ferryl heme protein radicals ($HP^{+\bullet}$ferryl(+4)=O) (84). $^{\bullet}OH$ is one of the strongest biological oxidants (Table 8.1) and therefore will react with nearly any molecule that it encounters. This might limit the amount of $^{\bullet}OH$ that will react with fatty acids. Peroxyl radicals are likely prevalent since the reaction of oxygen with alkyl radicals that forms after hydrogen abstraction from a fatty acid is highly favored both thermodynamically and kinetically. Alkoxyl radicals will form due to breakdown of lipid hydroperoxides by heme or reduced metal complexes. Alkoxyl radicals can undergo β-scission reactions that produces a short chain alkyl radical ($RCH_2^{\bullet}$) that reacts readily with O_2 to form peroxyl radicals (17).

There are numerous free radical scavengers (FRS) that are either endogenous to the food or incorporated during processing. The antioxidant effectiveness will depend on hydrogen bond energies (85). The donation of hydrogen from a generic phenolic antioxidant to an alkoxyl radical is depicted in Figure 8.6. The ability of a particular FRS to donate hydrogen to a free radical can be predicted from standard one-electron reduction potentials (17). Any compound that has a reduction potential lower than that of a free radical is capable of donating hydrogen to that free radical (Table 8.1). For example, catechol has a lesser reduction potentials than alkoxyl radical. Thus, catechol can donate hydrogen to the alkoxyl radical. This donating ability of catechol competes with the undesirable reaction of alkoxyl radicals with polyunsaturated fatty acids (PUFA-H) (Table 8.1) and hence inhibits lipid oxidation processes. It should be kept in mind that the reduction potential of a compound changes as a function of pH, temperature, and concentration of the compound(s) of interest.

A potential drawback is that the FRS becomes a free radical itself after donating hydrogen to the alkoxyl radical (Figure 8.6) (Table 8.1). The most efficient FRS exist as low energy radicals after scavenging. The benefit of existing as a low energy radical is that the radical is unlikely to abstract hydrogen from polyunsaturated fatty acids. Low energy radicals result from resonance delocalization (Figure 8.6). The conjugated ring structure of the phenolic allows the phenolic radical to reside at multiple sites on the molecule. As the radical migrates from site to site, a low energy radical results that possesses low reactivity. Evidence of low reactivity can be gleaned from the one-electron reduction potentials. Any radical with reduction potential less than a polyunsaturated fatty acid (e.g., catechol radical) cannot abstract hydrogen from the fatty acid; hence the antioxidant radical cannot initiate/propagate lipid oxidation processes (Table 8.1).

Efficient FRS in their radical form should also not react with oxygen. If reaction with oxygen occurs, a free radical peroxide forms (FR-OOH). The free radical peroxide cannot be regenerated by reducing equivalents as can occur when the FRS is in a resonance delocalized form ($FR^{\bullet}$). The net effect is depletion of the antioxidant upon reaction with oxygen. Further free radical peroxides can decompose to species capable of furthering oxidation. Note that ascorbate has a one-electron reduction potential that is less than tocopherol (Table 8.1) and thus ascorbate can regenerate tocopherol from tocopheroxyl radicals.

Thus, phenolic compounds are efficient FRS due to their hydrogen donating properties and resonance delocalization of the phenoxyl radical. There is a multitude of phenolic free radical scavengers available to food scientists. The synthetic phenolics butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), tertiary butyl hydroquinone (TBHQ), and propyl gallate (PG) (Figure 8.7) are commonly used in the food industry due to their low cost of production although consumers prefer natural FRS such as tocopherols and plant phenolics.

TABLE 8.1
Standard One-Electron Reduction Potentials of Components Involved in Free Radical Reactions

[Oxidized / Reduced] Couple	E° ′ (mV)
$HO^{\bullet}$, H^+ / H_2O	2310
$LO^{\bullet}$, H^+/ LOH	1600
$LOO^{\bullet}$, H^+ / LOOH	1000
PUFA$^{\bullet}$*, H*$^+$ */ PUFA-H*	600
Catechol$^{\bullet}$, H^+ / catechol	530
α-Tocopheroxyl$^{\bullet}$, H^+ / α-tocopherol	500
Ascorbate$^{\bullet -}$, H^+ / ascorbate$^-$	282

Adapted from Ref. 17.

B. Synthetic Phenolics

Propyl gallate (PG) is poorly soluble in oils and sensitive to heat degradation (e.g., frying temperatures). Substitution of the propyl group with octyl or dodecyl groups provides more heat stability and lipid solubility. Gallates have been used to stabilize meat products, baked goods, fried products, confectionaries, nuts, and milk products (86). Butylated hydroxyanisole (BHA) volatilizes upon frying,

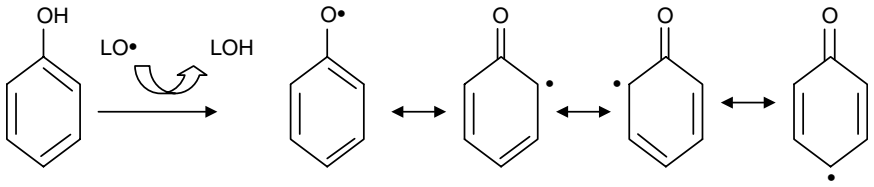


FIGURE 8.6 Free radical scavenging by a phenolic compound and resonance stabilization of the resulting phenoxyl radical. Adapted from Ref. 85.

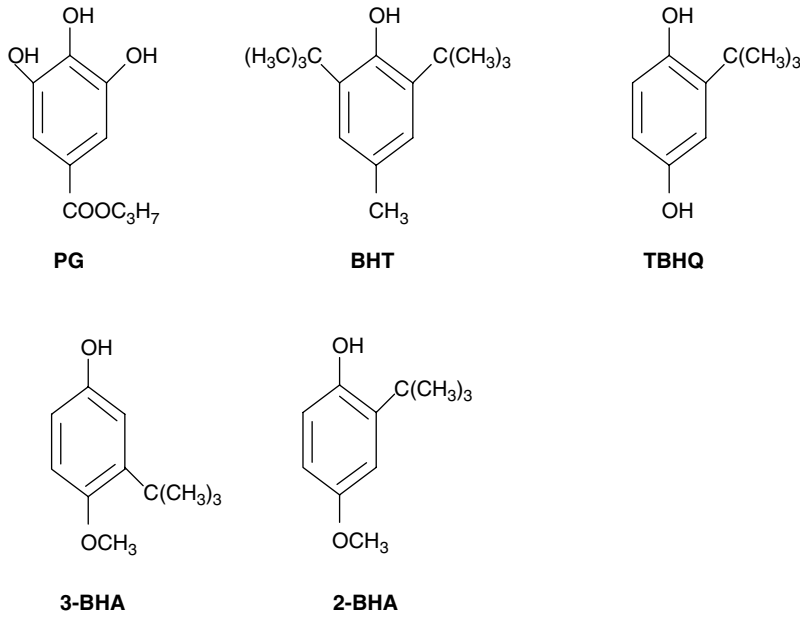
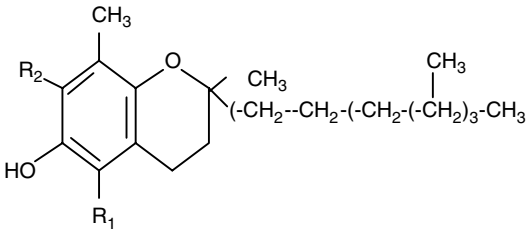


FIGURE 8.7 Structures of various synthetic antioxidants.

but residual BHA does protect fried foods. BHA is a mixture of two isomers (Figure 8.7). The $-C(CH_3)_3$ group on the conjugated ring increases oil solubility and enhances resonance stabilization of the phenoxy radical. This alkyl group on the conjugated ring also enhances hydrogen-donating properties. Butylated hydroxytoluene is highly soluble in oil due to its two $-C(CH_3)_3$ groups and single methyl group (Figure 8.7). TBHQ has two hydroxy groups and significant solubilities in a wide range of fats, oils, and solvents. The order of antioxidant efficacy in fish oil stored at 60°C was TBHQ> PG=BHA>BHT (87).

C. Tocopherols and Tocotrienols

Tocopherols are of plant origin and exist in four forms (α, β, γ, δ). The structures of the isomers are illustrated in Figure 8.8. Tocopherols are soluble in oils and ethanol. When tocopherol reacts with a peroxyl radical, at least five resonance structures of the tocopherol radical can form (83). BHA, BHT, and PG are considerably more stable to heat treatment than α-tocopherol (86). α-, β-, γ-, and δ-tocopherol inhibited formation of cholesterol oxidation products to different degrees in metal-induced oxidation of unilamellar phospholipid-cholesterol liposomes (88). In beef muscle, tocopherolquinone and 2,3-epoxy-tocopherolquinone were the dominant tocopherol oxidation products and lower amounts of 5,6-epoxy-tocopherolquinone and tocopherolhydroquinone were detected (89). This was consistent with mainly a peroxyl radical scavenging function of tocopherol but also some scavenging of other free radicals. Predominant amounts of the 2,3- and 5,6-epoxy-tocopherolquinone products would suggest a nearly exclusive mechanism of peroxyl-radical scavenging. When examining Atlantic mackerel, a substantial amount of tocopherol was present in stored muscle that was highly rancid (90). This suggested that tocopherol was not an effective antioxidant in the mackerel muscle. Tocotrienols are similar in structure to tocopherols but contain three unsaturated units in the isoprenoid chain. γ- and δ-tocotrienols extended shelf life of coconut fat better or in a manner similar to their corresponding tocopherols during 60°C storage and exposure to frying temperatures (91).



Type	R1	R2
Alpha	$-CH_3$	$-CH_3$
Beta	$-CH_3$	-H
Gamma	-H	$-CH_3$
Delta	-H	-H

FIGURE 8.8 Structures of tocopherols.

D. Plant Phenolics

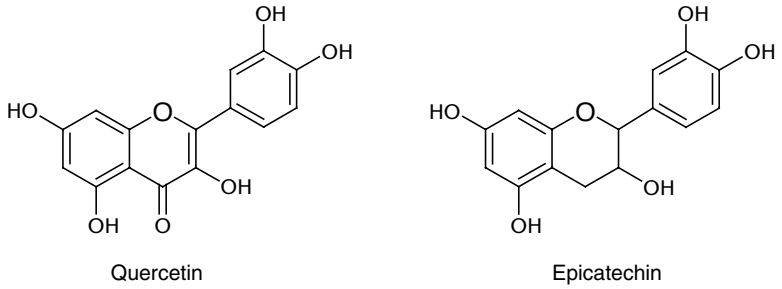
Simple plant phenolics contain a single conjugated ring with various substitutions. These compounds are usually water soluble and examples are gallic acid and hydroxycinnamic acid. Anthocyanidins are 3-ringed structures that exist as protonated cations at acidic pH values, are colorless open-ringed structures at intermediate pH values, and are anions at higher pH values. Glycosylated anthocyaninidins are termed anthocyanins and are the common red pigments in fruits. Flavan 3-ols are colorless compounds that are common in tea. Epicatechin is an example of a flavan-3-ol (Figure 8.9). Quercetin is a flavonol and is one of the most abundant flavonoids (Figure 8.9). Flavonoids is a general term that includes anthocyanins, flavonols, flavones, isoflavones, and chalcones. A quercetin metabolite was found to have antioxidant properties in a liposomal membrane (92). Linked flavan-3-ol repeated molecules are high molecular weight, generally poorly soluble in water, and referred to as proanthocyanidins, procyanidins, tannins, or heteropolyflavans (93). Extensive structural diversity exists in different plant phenolics. In rosemary leaf extract, carnosol, carnosic acid, rosmarinic acid, and rosmaridiphenol have antioxidant potency (86).

The rate of peroxyl radical scavenging by quercetin and epicatechin was greater in non-polar solvents compared to hydrogen bonding solvents (94). In a liposomal model system that generate free radicals during metal-induced peroxidation, 1) antioxidant activity increased with increasing hydroxy substitutions present on the B ring for anthocyanidins but the opposite was observed for the flavan-3-ol, catechin, 2) substitution by methoxyl groups decreased antioxidant activity of anthocyanidins, and 3) substitution of a galloyl group at position 3 of the flavonoid moiety decreased antioxidant activity of the catechin (95).

Many phenolic antioxidants have been characterized in grapes, berries, teas and spices. Beet root pigments were found to have free radical scavenging properties (96). Betanidin 5-O-beta-glucoside in beet was found to inhibit lipid oxidation at low concentrations (97). In pineapple juice, phenolic compounds containing cysteine, glutamyl, and glutathione linkages were identified (98). Proteins (casein or albumin) decreased antioxidant efficacy of tea flavanols (99). Freezing and storage had negligible effects on antioxidant capacity of raspberry phenolics (100). Ferryl myoglobin, a possible pro-oxidant in muscle tissue, was reduced by epigallocatechin gallate from green tea (101). The antioxidant effects of tea catechins in raw chicken muscle were attributed to free radical scavenging ability and iron chelating effects (102).

E. Carotenoids

Carotenoids are fat-soluble pigments. Canthaxanthin and astaxanthin possess oxo groups at the 4 and 4′-positions in the β-ionone ring (Figure 8.10). β-carotene and zeaxanthin do not contain oxo groups and were found to be less inhibitory to methyl linoleate peroxidation than canthaxanthin or astaxanthin (103). β-carotene, however, can scavenge free radicals. Peroxyl radicals either add directly to the hydrocarbon portion of the molecule displacing an unsaturation site or add to the β-ionine ring forming a β-carotene cation radical; these oxidation products, however, are



Quercetin Epicatechin

FIGURE 8.9 Structures of the flavonoids quercetin and epicatechin.

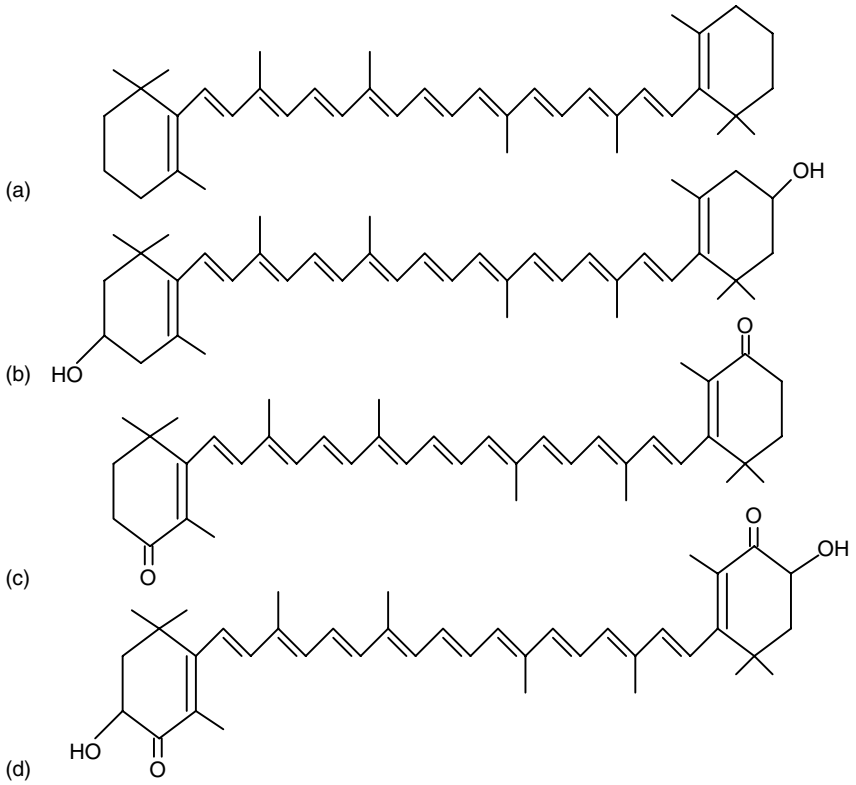


FIGURE 8.10 Structures of various carotenoids. (a) β-carotene, (b) zeaxanthin, (c) canthaxanthin, (d) astaxanthin.

susceptible to breakdown that results in formation of alkoxyl radicals (83). There is evidence that carotenoids are effective antioxidants at low oxygen concentrations but not higher oxygen concentrations (57). Carotenoids including lycopene can inactivate singlet oxygen by physical quenching of the activated oxygen specie (104).

F. Other Free Radical Scavengers and Reductants

Uric acid is present in plasma and can inhibit lipid oxidation by scavenging free radicals or singlet oxygen (105). Ascorbate is believed to scavenge tocopheroxyl free radicals thereby regenerating tocophopherol (17). Ascorbate can also scavenge various free radicals such as $^{\bullet-}O_2$, $^{\bullet}OOH$, and $^{\bullet}OH$ (48). Like flavonoids, ascorbate reduces hypervalent forms of heme proteins to potentially inhibit lipid oxidation in muscle foods (106). Heme oxygenase converts heme into bilirubin. Bilirubin is believed to scavenge free radicals, which results in formation of biliverdin that is reduced back to bilirubin by NADH and biliverdin reductase. This redox cycle was used to explain the high antioxidant power of bilirubin *in vivo* (107). It is not known how effective bilirubin inhibits lipid oxidation in food systems. Ubiquinol is a phenolic compound that is conjugated to an isoprenoid chain and is associated with mitochondrial membranes. Oxidation of ubiquinol results in formation of semiubiquinone radical. Dietary ubiquinone increased ubiquinol levels in lipoproteins and decreased lipid oxidation rates (108). Ubiquinol is considered a weak free radical scavenger due to internal hydrogen bonding that interferes with abstraction of its phenolic hydrogen by free radicals (109).

A potent natural antioxidant from shrimp was tentatively identified as a water-soluble, polyhydroxylated derivative of an aromatic amino acid (110).

G. Metal Inactivators

Ethylenediamine tetraacetic acid (EDTA) can inhibit lipid oxidation by forming an inactive complex with metals. EDTA can either promote or inhibit lipid oxidation depending on the iron/EDTA ratio, which modulates the effective charge in the system (111). EDTA is approved for use in foods at low concentrations. It is poorly soluble in fats and oils but only small amounts are needed for maximum activity. EDTA protected lard better than a combination of BHT and citric acid (86). It should be noted that EDTA indirectly acts as a free radical scavenger. Jimenez and Speisky (112) showed that glutathione scavenged free radicals less effectively in the presence of copper than when EDTA was mixed with copper prior to addition of gluathione. The ability of EDTA to tie up copper or form a chelate with glutathione apparently increased the free radical scavenging ability of glutathione. The carboxylic acid groups of EDTA are protonated at low pH values

(i.e., when pH is below the pKa for the acid groups of EDTA). This interferes with the ability of EDTA to complex metals or other cellular components.

Desferrioxamine is often used as a "metal chelator" in research studies, but this can lead to errant results since desferrioxamine can also act as a free radical scavenger (113). EDTA, tartaric acid and citric acid are other commonly used metal chelators in the food industry. Citrate esters improve oil solubility but at least two free carboxyl groups are needed for effective metal inactivation. Propylene glycol increases solubility of citric acid in oils and fats (86). Sodium tripolyphosphate can act as an antioxidant via metal chelation (114). A disadvantage of using metal chelators in general is that iron bioavailability during digestion may be compromised.

Ceruloplasmin inhibits metal-catalyzed oxidation via its ferroxidase activity. The ferroxidase converts Fe^{2+} to Fe^{3+}, a less catalytic form of iron (115). Transferrin (plasma protein) and ferritin (muscle cell protein) can inactivate metals by chelation of iron but also can release iron causing a pro-oxidant effect; lipolysis and ascorbate, respectively are capable of triggering the iron release (22, 116). Carnosine is a B-alanylhistidine dipeptide found in skeletal muscle at high concentrations. It is capable of chelating copper, scavenging peroxyl radicals, and forming adducts with aldehydes (117). Histidine was found to inhibit non-enzymatic iron mediated lipid oxidation apparently due to formation of an inactive chelate but histidine was also found to activate enzymic pathways of lipid oxidation (118).

H. Enzymes That Inactivate Oxidation Intermediates

Superoxide anion ($^{-\bullet}O_2$) can be produced by heme protein autoxidation or by any process that causes addition of an electron to oxygen (119). Superoxide can reduce Fe^{3+} to Fe^{2+}, the more pro-oxidative form of iron. In addition, the pKa of $^{-\bullet}O_2$ is around 4.5. Thus at pH values below 4.5, the conjugate acid $^{\bullet}OOH$ is the predominant form which can directly initiate lipid oxidation (84). Superoxide dismutase is present in cells and extracellular fluids to remove $^{-\bullet}O_2$ resulting in formation of oxygen and hydrogen peroxide.

Hydrogen peroxide (H_2O_2) can react with either low-molecular-weight iron or heme proteins to form free radicals that initiate/propagate lipid oxidation processes. Biological systems are equipped with antioxidants to deal with this stress. Catalase, a heme-containing enzyme reacts with H_2O_2 to form water and oxygen (120). In plants and algae, ascorbate peroxidase removes H_2O_2 and forms monodehydroascorbate and water. Glutathione peroxidase removes H_2O_2 and forms water and oxidized glutathione. The reaction of glutathione peroxidase with lipid hydroperoxides results in formation of an alcohol, water, and oxidized glutathione. Compounds such as methionine and thiodipropionic acid can also decompose peroxides but at much slower rates than the enzymes.

I. Scavenging of Lipid Oxidation Breakdown Products

Lipid oxidation breakdown products (e.g., aldehydes, ketones, hydrocarbons) form a mixture of volatiles that causes objectionable flavors and odors. Carnosine, anserine, histidine, lysine, albumin, and sulfur or amine containing compounds have the ability to bind aldehydes and therefore decrease rancidity in foods (83). These "scavengers" should be examined in relation to browning of beef considering that lipid oxidation derived aldehydes accelerated the conversion of oxyMb to metMb and hence have the capacity to accelerate browning in beef (121).

J. Other Mechanisms of Antioxidant Action

Spermine was found to inhibit lipid oxidation in hepatocytes of CCL_4-treated rats; a possible mechanism was formation of polyamine-phospholipid complexes (122). Conjugated linoleic acid (CLA) has been shown to decrease rates of lipid oxidation in muscle tissue (123). The mechanism may be related to the ability of dietary CLA to decrease polyenoic fatty acid concentrations in the muscle (124). Organosulfur compounds such as diallyl sulfide and N-acetyl cysteine may exert their antioxidant protection by modulating antioxidant enzymes such as catalase and glutathione-s-transferase (125).

K. Interfacial, Charge, and Location Effects

Deciding which antioxidant(s) to utilize in a particular food system is a formidable task. Having water and lipid soluble antioxidants was found to maximize extension in shelf life of mayonnaise prepared from fish oil (126). However, cost limitation is a factor that limits amounts of antioxidant addition. Most foods have a water phase, lipid phase and water-lipid interface. Location of different antioxidants should affect antioxidant potency. Membrane phospholipids are believed to be more prone to lipid oxidation than triacylglycerols in muscle foods so protecting membrane lipids is desired (127). δ-Tocopherol could be preferentially incorporated into isolated membranes compared to triacylglycerols by proper selection of antioxidant solvent (ethanol instead of corn oil) (128). In minced chicken muscle containing added triacylglycerols, δ-tocopherol could be preferentially incorporated into the membrane fraction if the antioxidant was added to the lean muscle before addition of TAG lipids (129). Hydrophilic antioxidants (trolox and ascorbic acid) were generally more effective than more hydrophobic compounds (tocopherol and ascorbyl palmitate) in bulk oils while the hydrophobic compounds were more effective in oil-in-water emulsions (130, 131). However, when comparing carnosic acid to the more hydrophobic methyl carnosate, the latter was a more effective antioxidant in both bulk oils and emulsions (132). Benzoic acid, a water-soluble phenolic, partitioned into the oil phase of a whey-protein

stabilized emulsion more than could be explained by oil/water partitioning alone (133). This suggested that benzoic acid bound to protein adsorbed at the interface. In oil-in-water emulsions, excess surfactant solubilized phenolic antioxidants into the aqueous phase but the removal of antioxidants from the oil or oil interface phases did not accelerate lipid oxidation (134). The ability of excess surfactant to cause lipid hydroperoxides and iron to partition into the aqueous phase (away from oil droplets) may explain the ability of excess surfactant to inhibit lipid oxidation in oil-in-water emulsions (50, 135). Positively charged protein emulsifiers inhibited lipid oxidation more effectively than negatively charged emulsifiers in oil-in-water emulsions (136). This was attributed to the ability of the positive charge of the protein interface to repel iron away from the oil phase. The ability of Trolox to inhibit lipid oxidation in liposomes was least when the membrane bilayer and trolox molecule were negatively charged and removing the repulsive forces by altering membrane type or pH increased antioxidant efficacy (137). More studies are needed to evaluate the distribution of antioxidants in different phases in conjunction with lipid oxidation kinetics during storage.

VI. PRODUCTION OF FATS AND OILS

Production of fats and oils from plant, animal, fish, and dairy lipids can be broken into four classifications: recovery, refining, conversion, and stabilization. Pressing or solvent extraction are common processes to liberate oil from plant seeds. Care should be taken during transportation of seeds to prevent cell rupture prior to oil extraction. Lipases and lipoxygenases in the cytosol that mix with TAG prematurely due to decompartmentation will be detrimental to oil quality (i.e., formation of free fatty acids and peroxidized lipids prior to extraction will reduce TAG purity and hence yields). Heating during or prior to the pressing step (115°C for 60 min) inactivates lipases and lipoxygenases. Other benefits of heating are rupturing of cell wells, decrease in oil viscosity, and coagulation of proteins. Elevated moisture levels are discouraged due to the ability of excess water to facilitate hydrolysis of esterified lipids. Recovery of animal fat and marine oil is also a high temperature process called rendering. Trimmings, cannery waste, bones, offal, tallow and lard can be subjected to rendering to produce valued added oils and fats.

Refining is the removal of non-TAG components including free fatty acids, phospholipids, pigments, protein, and wax. The degumming step is a water wash that removes phosphatides (e.g., lecithin, phospholipids). Hydration in the presence of heat makes phosphatides insoluble in the oil allowing removal by centrifugation. Heating of oil contaminated with phosphatides can result in foaming and even fire due to the surfactant properties of the phospholipids. The next step in refining is neutralization. Free fatty acids and phosphatides react with sodium hydroxide to form a soap (e.g., saponified material). Subsequent bleaching removes undesirable pigments typically by use of neutral clays. Waxes are then removed by cooling the oil to around 7°C for 4 hours and filtering at 18°C. The final step in refining is deodorizing, which removes hexane, pesticides, and peroxide decomposition products that can potentially impart off-odors and off-flavors. Deodorization is accomplished by steam distillation at high temperatures (180°C to 270°C) under vacuum. Freshly deodorized oils should have a peroxide value of zero and a free fatty acid content of less than 0.03% (138). Ideally fat-soluble antioxidants such as tocopherols are retained in the purified oil. Unfortunately, refining strips antioxidants from the TAG which often requires post-processing addition of antioxidants to pure oil.

The conversion processes winterization and fractional crystallization are physical processes that alter the lipids and thus are out side the scope of this chapter. Various chemical processes of conversion (e.g., interesterification) and stabilization (e.g., hydrogenation) are described later in this chapter. Stabilization techniques for fats and oils are also discussed in the preceding section on antioxidants.

VII. MODIFICATION OF LIPIDS AND PRODUCTION OF SPECIALTY FATS

This section describes the numerous chemical processes that are available to modify the functional properties of food lipids. Functional properties include 1) oxidative stability, 2) plastic range, 3) flavor properties, 4) nutrient content, 5) health promoting effects, and 6) caloric value. Increasing fatty acid saturation or redistributing fatty acids on the glycerol backbone to improve functionality can be accomplished in bulk oils by treatment with low-molecular-weight catalysts. In other cases, more specific alteration of lipids is accomplished through the use of enzymes to improve functionality. Endogenous enzymes in yeasts, molds, and bacteria utilize nonlipid or lipid containing carbon sources to produce a wide array of different specialty lipids (e.g., cocoa-butter substitutes, triacylglycerols rich in omega-3 fatty acids, biosurfactants, polyunsaturated fatty acids, wax esters, and hydroxy fatty acids). A thorough description of the emerging fields of "lipid biotechnology" and "structured lipids" is available (139, 140). Some specific examples that utilize lipases to produce specialty lipids are cited in Section VII.C of this chapter. The opportunity to modify lipids "pre-harvest" is addressed in Section VII.D.

A. Hydrogenation

Hydrogenation is done for two important reasons: 1) provides a semi-solid fat at room temperature from an oil source and, 2) increase oxidative stability during storage. Hydrogenation involves mixing oil with a catalyst such as nickel at elevated temperatures (140°C to 225°C).

Hydrogen gas is then introduced with agitation. Once the desired saturation is obtained, the material is cooled and the catalyst is removed by filtration. Typical products that result include shortenings and margarine. A disadvantage of this process is the formation of trans fatty acids that are considered unhealthy.

B. Non-Enzymatic Interesterification

Factors that contribute to textural properties of fats include not only degree of fatty acid unsaturation and the chain length but also the location of fatty acids on the glycerol backbone. Chemical interesterification "randomizes" the location of the different fatty acids, thereby improving the utility of the fat. Spreadability, melting point, and solid-fat content temperature profile are modified by the randomization. This process typically involves the use of sodium methoxide (0.1%) as a catalyst. The catalyst should function at low temperatures (around 50°C) to avoid polymerization and decomposition of lipids during interesterification. Moisture inactivates the catalyst. Therefore, the water content must be below 0.01%. Free fatty acids and lipid peroxides must be below 0.1% and 1%, respectively. The catalyst must be soluble in the lipid.

The mechanism of interesterification using alkaline bases involves nucleophillic attack by the catalyst towards the slightly positive carbonyl carbon. This attack liberates a fatty acid methyl ester and a resulting glycerate anion (Figure 8.11). The glycerate anion is the nucleophile for subsequent carbonyl attacks. This process continues until all the available fatty acids have exchanged positions. Sodium methoxide also removes an acidic hydrogen from the carbon alpha to the carbonyl carbon. The carbanion produced is a powerful nucleophile.

On occasion randomness is not desirable. If the fat is maintained below its melting point, interesterification proceeds with the formation of more saturated triacylglycerols. This "directed interesterification" produces a product with a higher solids content at higher temperatures, which extends its plastic range.

A practical application of interesterification involves the modification of lard. In its native form, lard has negative attributes including grainy texture, poor appearance, poor creaming capacity, and limited plastic range (141). The graininess is due to a preponderance of palmitic acid at the sn-2 position. Randomization decreases the amount of palmitic acid and the sn-2 position and hence decreases graininess. Directed interesterification resolves the plastic range problem. Improvement in plasticity and stability is due to alterations in the polymorphic behavior. The interesterified lard crystallizes into a β'-2 form that promotes the improved functionality (8).

Fish oils are liquid at room temperature due to their high content of polyunsaturated fatty acids including

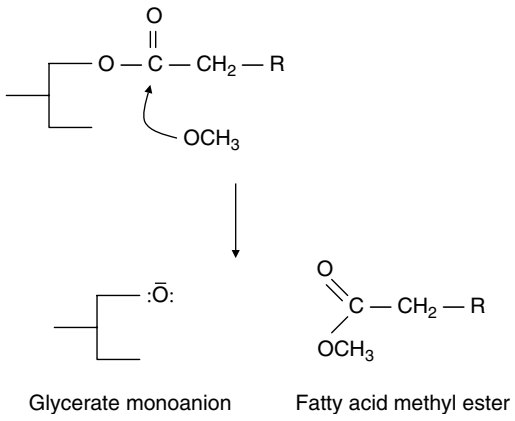


FIGURE 8.11 Proposed mechanism of chemical interesterification. Adapted from Ref. 141.

omega-3 fatty acids (e.g., 22:6 and 20:5). Ingestion of omega-3 fatty acids are noted for their ability to decrease incidences of various diseases but are also highly susceptible to lipid oxidation in foods, which causes off-flavors and off-odors. A possible route to increased consumption of these fatty acids with less quality loss during storage is chemical interesterification. Interesterification of a hydrogenated vegetable oil and the fish oil will produce a mixture of fatty acids on the glycerol backbone ranging from highly saturated to highly unsaturated. The saturated TAGs can be removed by low temperature fractional crystallization and centrifugation. The fraction obtained with intermediate unsaturation (moderately higher temperature crystallization) comprises TAGs containing both saturated fatty acids and the highly coveted omega-3 fatty acids. Compared to the starting fish oil, this results in triacylglycerols 1) with a greater plastic temperature range increasing product applications, 2) more resistance to lipid oxidation due to the incorporation of the saturated fatty acids, and 3) a relatively more stable source of omega-3 fatty acids for incorporation into foods. An area of concern would be the stability of the omega-3 fatty acids at the temperatures used during chemical interesterification. An alternative process that requires lower reaction temperatures is enzymatic interesterification.

C. Enzymatic Modification of Lipids

Enzymatic interestification is accomplished using lipases from bacterial, yeast, and fungal sources. The regio- and stereospecificity obtained through the use of lipases is a marked advantage over chemical interesterification. Enzymatic interesterification requires less severe reaction conditions, products are more easily purified, and produces less waste than chemical interesterification. Enzymatic interesterification is more expensive at the present time although advances are expected to lower costs. In any event, certain processes can be accomplished

with lipases that are not achievable via chemical interesterification. For example, it is optimal to incorporate stearic acid (18:0) at the sn-1 or sn-3 position because stearic acid is least absorbed at these positions compared to the sn-2 position (142). This is advantageous since caloric value is decreased while maintaining a long chain saturated fatty acid that expands the plastic range. An sn-1,3 lipase facilitates the regioselectivity desired whereas chemical interesterification cannot. Since fatty acids at the sn-2 position are more efficiently absorbed than those at the sn-1,3 positions, the ideal location for essential fatty acids is at the sn-2 position. Fatty acids at sn-2 will be shuffled to other positions on the triacylglycerol in chemical interesterification, which is undesirable. The sn-1,3 lipases, however, allow those endogenous fatty acids to remain at the sn-2 site.

The major triacylglycerols in cocoa butter all contain oleic acid at the sn-2 position (1-palmitoyl-2-oleoyl-3-stearoyl-glycerol, 1,3-dipalmitoyl-2-oleoyl-glycerol, and 1,3-distearoyl-2-oleoyl-glycerol). Palm oil is rich in palmitic and oleic acid but lacks appreciable amounts of steric acid. Thus, palm oil has been reacted with stearic acid and an sn-1,3 specific lipase; replacement of palmitic acid with stearic acid at the sn-1 or sn-3 position produced an effective cocoa butter substitute. Chemical interesterification will randomize the location of all the fatty acids and produce a less effective substitute. Cocoa butter is an expensive material due to its limited quantities and unique melting properties (hard and brittle at room temperature but melts as it is warmed in the mouth).

The reaction of fatty acids with esters such as those found in triacylglycerols is termed acidolysis. Another acidolysis reaction involves incorporating capric acid (10:0) and caproic acid (6:0) into an oil stock. This is beneficial since these fatty acids are readily oxidized in the liver and therefore are excellent sources of energy as opposed to normal storage fat for individuals having deficiencies in fat absorption.

Transesterification is the exchange of acyl groups between two esters, specifically between two tricacylglycerols. Mixtures of hydrogenated cotton oil (rich in stearic and palmitic acid) and olive oil (rich in oleic) can be reacted in the presence of the proper lipase and minimal water to create a cocoa butter substitute. To separate the desired TAG from the undesired TAG, trisaturated TAG can be removed by crystallization in acetone or temperature differentials that crystallize out the more saturated triacylglycerols. This process can also be performed using sodium methoxide catalyst instead of lipases but again the randomization of oleic acid from the sn-2 position in the chemical interesterification should produce a less effective substitute than a sn-1,3 lipase-driven interesterification that regiospecifically alters the starting oil stocks, thereby maintaining oleic acid at the sn-2 position.

Alcoholysis is the esterification reaction between an alcohol and an ester. The most common alcoholysis is the production of mono- and diacylglycerol surfactants (e.g., emulsifiers) by reacting glycerol with triacylglycerols. Specifically, this reaction is termed glycerolysis and is usually performed using nonspecific lipases. The newly formed mono- and diacylglycerols are isolated by temperature-induced crystallization. In glycerolysis, Tc is defined as the critical temperature below which monoacylglycerols crystallize out of the reaction mixture (143). This pushes the equilibrium of the reaction to produce more monoacylglycerols. Vegetable oils have low melting points and hence low Tc due to the abundance of polyunsaturated fatty acids compared to animal fats. By reducing the temperature below the Tc for vegetable oils (Tc = 5°C to 10°C), yields of monoacylglycerols can be increased.

D. Modification of Lipids Prior to Harvest

Genetic manipulation of lipid biosynthesis is a possible route to improve functionality of TAG and phospholipids (144). For example, the overexpression of cis-9 desaturase in transgenic tomato results in increases in 16:1(cis 9), 16:2(cis 9,12), and 18:2(cis 9,12) fatty acids, which enhance positive flavor attributes in the fruit mediated by a lipoxygenase/hydroperoxidelyase/isomerase/reductase enzyme system (145, 146). Apparently the enhanced fatty acids are precursors for the desirable flavor compounds in tomato. In cell membranes, phosphatidyl glycerol containing two saturated fatty acids is correlated with decreased chilling injury in plants. Incorporating plastidic sn-glycerol-3-phosphate acyltransferase (GPAT) from a chilling-insensitive species (spinach) into a moderately chilling sensitive species (tobacco) increased disaturated phosphatidyl glycerol and was successful at decreasing chilling injury in the tobacco (147). Oxidative stability of transgenic canola oil was improved by decreasing the activity of a cis 15-endogenous desaturase using antisense technology (148). This lowered the 18:3 (cis 9,12,15) content from 6.9% to 1.4% in the oil. Although lipid stability was improved by this technique, 18:3 is an essential fatty acid so functionality is improved at the expense of nutritional quality. A thorough review of genetic engineering of crops that produce modified vegetable oils is available (149).

E. Fat Replacers

Fat replacers can be primarily carbohydrate, protein, or lipid based. Protein or carbohydrates replacers are called *mimetics* and tend to absorb water readily but cannot carry lipid-soluble flavor compounds. The other category of fat replacers, called *substitutes*, will typically contain fatty acids esterified to a carbohydrate. The fatty acids provide desirable physical properties of fats but are not readily cleaved by lipases during digestion. In other words, the lipids are not metabolized and therefore caloric intake is

reduced per gram or fat ingested. An example of a fat substitute is raffinose polyester. Raffinose is made up of galactose-glucose-fructose units. The eleven available hydroxy groups can potentially be esterified with fatty acids. As the degree of substitution increases the susceptibility to hydrolysis and absorption will decrease (150). Sucrose fatty acid esters act as emulsifiers, texturizers, and protective coatings in various foods products.

Benefat™ consists of short chain fatty acids (e.g., 2:0, 3:0, 4:0) and a long chain saturated fatty acid (stearic acid, 18:0). Short chain fatty acids have low caloric value because they are easily hydrolyzed by digestive lipases and readily converted to carbon dioxide (151). Stearic acid is only partially absorbed, especially if located at the sn-1 or sn-3 position. Benefat is around 5 kcal/g while typical fats are 9 kcal/gram. Currently, Benefat is produced by base-catalyzed interesterification of hydrogenated vegetable oils with TAGs of acetic, propionic, and/or butyric acids (152). The ratios of the short chain fatty acids and the long chain saturated fatty acid can be varied not only providing a low caloric intake but also the physical properties required for specific food applications. Benefat can be used in cookies, baked goods, dairy products, dressings, dips, and sauces (150).

VIII. CHEMISTRY OF FRYING

Frying of oils results in distinctive fried flavors and undesirable off-flavors if the oil is overly deteriorated. Off-flavors are manifested via 1) hydrolysis, 2) oxidation, and 3) polymerization reactions. The interaction of steam, water, and oil will hydrolyze TAG into mono-acylglycerols, di-acylglycerols, and free fatty acids. With increased time even glycerol will be produced due to complete hydrolysis of an individual triacylglycerol. Little glycerol can be detected in frying oils since glycerol volatilizes around 150°C and frying temperatures are typically higher. Factors that control hydrolysis are oil temperature, interface area between oil and aqueous phases, water level, and steam level (153). Metals that contaminate the oil interact with lipid hydroperoxides to form free radical species that initiate and propagate oxidation reactions in the presence of oxygen. Frying temperatures will greatly increase the rate of these fundamental lipid oxidation processes and stimulate reactions that may not occur at lower temperatures resulting in an array of oxidation products including aldehydes, ketones, alcohols, esters, hydrocarbons, and lactones. These low-molecular-weight compounds that form due to degradation of the frying oil are considered "volatile," contributing desirable and undesirable flavors. Polymerization is common in frying where molecules cross-link often as a free radical-free radical reaction. As polymerization increases so too does viscosity of the oil. Most polymerized products are nonvolatile (e.g., dimeric fatty acids, TAG-trimers) and hence do not produce flavor. However, with further heating these non-volatile compounds can be degraded to off-flavor and toxic products. Degradation products negatively affect not only flavor and safety but also color and texture of the fried products.

Antioxidants and antifoam are added to frying oil to extend frying life. Other measures of delaying degradation of oil quality include utilizing fresh oil, using an oil low in polyunsaturated fatty acids and contaminating metals, filtration of oil with adsorbents, turnover of oil, and decreasing exposure of oil to oxygen. Antifoam will aid in reducing exposure of oil to oxygen. Continuous heating is better than discontinuous heating in extending frying life of the oil (153). Not all oxidation that occurs with frying is negative. For instance, 2-4-decadienal is considered a positive flavor compound. Often a preliminary batch of fried foods is prepared and discarded so that the subsequent batches have a desired flavor profile. More unsaturated oils oxidize faster than less saturated oils which decreases the amount of time needed to obtain a proper frying flavor in the food.

Free fatty acid content is an unreliable measure of frying oil quality. There still is not a fully appropriate single test to assess frying oil quality. The FoodOil sensor (FOS) (Northern Instruments Corp., Lino Lakes, MN) measures dielectric constant of frying oil compared to fresh oil and has had some success. The dielectric constant increases with increasing polarity so that once a certain value is reached the oil needs change.

IX. FOOD IRRADIATION

The purpose of food irradiation is to destroy microorganisms and hence extend shelf life. Lipids can be adversely affected. Typical dosages range from 1 to 10 kGy. Sterilization is achieved at doses of 10–50 kGy. When ionization radiation is absorbed by matter, ions, and excited molecules are produced. These ions, and excited molecules can dissociate to form free radicals. Reactions induced by irradiation prefer to react near the oxygen portion of TAG (154). Reaction occurs preferentially near the oxygen due to the high localization of electron deficiency on the oxygen atom. This explains the preponderance of aldehydes with the same chain length as the most abundant parent fatty acid (cleavage at location b) (Figure 8.12). Cleavage at locations c and d results in hydrocarbons that have one and two carbons less, respectively, than the parent fatty acid, which also is more common than a random assortment of hydrocarbons. Alternatively, free radicals can combine. For instance, two alkyl radicals react to form a dimeric hydrocarbon; acyl and alkyl radicals result in a ketone; acyloxy and alkyl radicals produce an ester; alkyl radicals can react with various glyceryl residue radicals to form alkyl glyceryl diesters and glyceryl ether diesters.

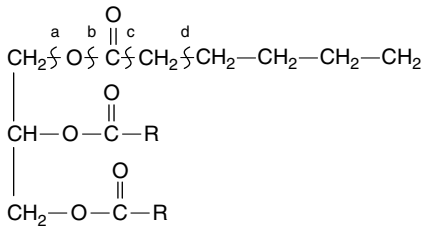


FIGURE 8.12 Cleavage sites on a triacylglycerol due to radiolysis. Adapted from Ref. 8.

Irradiation was found to accelerate lipid oxidation in raw pork patties and raw turkey breast that was aerobically packed (155, 156). Lipid oxidation was accelerated by irradiation (3 kGy) in aerobically packed, pre-cooked chicken (157). Irradiation caused formation of a brown pigment in raw beef and pork, but not turkey (158). Carbon monoxide was implicated as the cause of pinking in irradiated raw turkey breast muscle (159).

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9 Fats: Physical Properties

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CONTENTS

I. INTRODUCTION

The physical properties of fats and oils are of great practical importance so it is necessary to understand the makeup of these materials and how they should be used (1–9). Thus, many technical applications of fatty materials, including their uses in edible products, depend on the oiliness, surface activity, solubility, melting behavior, or other physical properties peculiar to long-chain compounds (10). Because fats and oils are mainly composed of mixtures of triacylglycerols, the physical properties of these molecules are going to determine the physical characteristics of the oil or fat. Thus, these characteristics are dependent on such factors as seed or plant source, degree of unsaturation, length of carbon chains, isomeric forms of the constituent fatty acids, molecular structure of the triacylglycerols, and processing. This chapter will review the most important physical properties of triacylglycerol molecules as well as of the most common edible fats and oils.

II. CRYSTALLIZATION AND POLYMORPHISM

Crystallization from solution is usually a slow process that first requires supercooling and then leads to nucleation and crystal growth. A high degree of supercooling will be conductive to nucleation, and very small crystals will be formed. At temperatures closer to the crystallization point, crystal growth will be favored and large crystals will be formed (2). Once formed, crystals, which may be stable or metastable, are able either to modify their habit or undergo phase transitions, respectively. Both processes result in polymorphic behavior, a behavior common to fats and other lipids (11–22).

A. Crystalline Structure of Triacylglycerols

In the solid state, molecules adopt the ideal conformation and arrangement in relation to their neighbors in order to optimize intra- as well as intermolecular interactions and achieve close-packing. The smallest building unit of a crystal, the repeating unit of the whole structure, is called the unit cell (Figure 9.1). The crystal structure is obtained by repetition of this unit in the three axial directions (5). Only seven different cells are necessary to include all possible point lattices. These correspond to the seven crystal systems into which all crystals can be classified (Table 9.1).

Of these seven crystal systems, it is now accepted that three predominate in crystalline triacylglycerols (23). Usually, the most stable form of triacylglycerols has a triclinic subcell with parallel hydrocarbon–chain planes ($T_{\parallel}$). A second common subcell is orthorhombic with perpendicular chain phases ($O_{\perp}$). The third common subcell type is hexagonal (H) with no specific chain plane conformation (24). This hexagonal form exhibits the lowest stability.

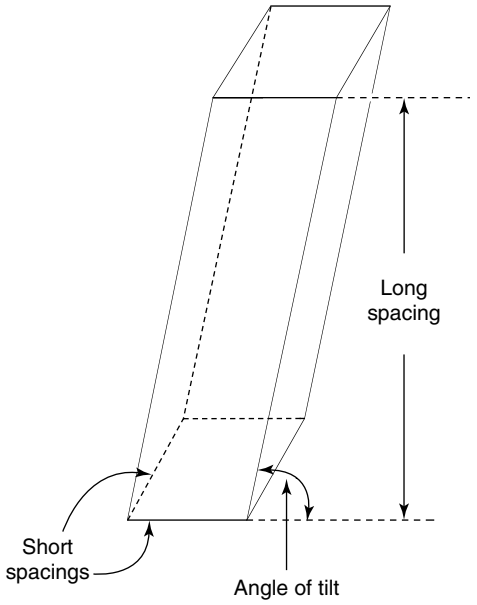

FIGURE 9.1 The triclinic unit cell for long-chain compounds.

TABLE 9.1
The Seven Crystal Systems

System	Angles and Axial Lengths
Cubic	All axes equal and all at right angles $a = b = c$ and $\alpha = \beta = \gamma$ and = 90º
Tetragonal	Two of three axes equal and all at right angles $a = b \neq c$ and $\alpha = \beta = \gamma$ and = 90º
Rhombohedral	All axes equal and none at right angles $a = b = c$ and $\alpha = \beta = \gamma$ and ≠ 90º
Hexagonal	Two axes = 120º and the third at 90º relative to them $a = b \neq c$ and $\alpha = \beta = 90º$ and $\gamma = 120º$
Orthorhombic	All axes unequal and all at right angles $a \neq b \neq c$ and $\alpha = \beta = \gamma$ and = 90º
Monoclinic	Three unequal axes having one pair not equal to 90º $a \neq b \neq c$ and $\alpha = \gamma = 90º \neq \beta$
Triclinic	All axes unequal and none at right angles $a \neq b \neq c$ and $\alpha \neq \beta \neq \gamma$ and ≠ 90º

Source: Ref. 11.

Interpretation of X-ray crystallography data from trilaurin and tricaprin resulted in representation of triacylglycerols in a tuning fork conformation when crystalline (Figure 9.2). The fatty acids esterified at the *sn*-1 and *sn*-2 positions of glycerol are extended and almost straight. The *sn*-3 ester projects 90º from *sn*-1 and *sn*-2, folds over at the carboxyl carbon, and aligns parallel to the *sn*-1 acyl ester. Molecules are packed in pairs, in a single layer arrangement, with the methyl groups and glycerol backbones in separate regions. The main cell is triclinic centered and contains two molecules; the subcell is also triclinic.

In addition to these bilayer structures, triacylglycerols may also be arranged in trilayers (Figure 9.2) (25–27).

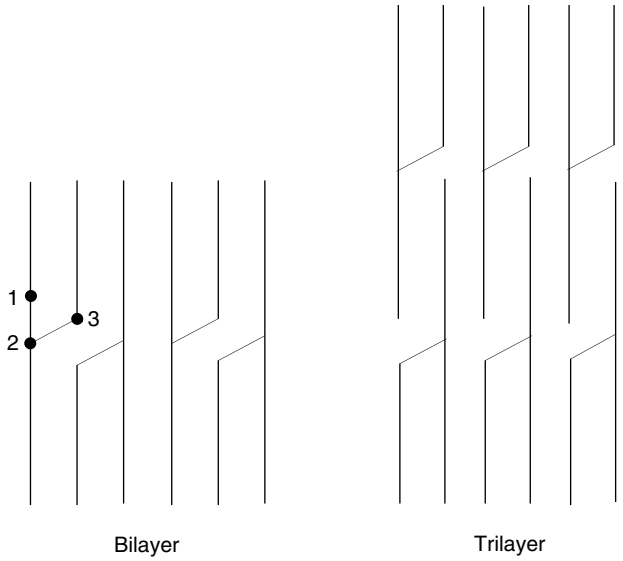

FIGURE 9.2 Double and triple chair arrangements of β form.

Thus, a trilayer structure occurs when the *sn*-2 position of the triacylglycerol contains a fatty acid that is either *cis*-unsaturated or of a chain length different by four or more carbons from those on the *sn*-1 and *sn*-2 positions (28). Also, a trilayer structure has been predicted to arise if the *sn*-2 position contained a saturated acyl ester with unsaturated moieties occupying the *sn*-1 and *sn*-3 positions (29). When unsaturation results in a *trans* configuration around the carbon–carbon double bond, the crystal structure exhibits the normal bilayer appearance (12).

B. Polymorphism and Phase Behavior of Natural Fats

Polymorphism is the ability of fat crystals to exist in more than one crystal form or modification. In the case of natural fats, these crystal forms are α, β′, and β, in order of increasing stability (Table 9.2). The changes among these phases are monotropic, and, therefore, proceeds in the solid phase from lower to higher stability. The forms differ in crystalline structure and in melting points, and correspond to the crystal structures described for natural fats in Section II.A. Thus, the most stable and with highest melting point $T_{\parallel}$ is the β polyform. Another polyform, with variable stability and a melting point lower than β, is β′, which has orthorhombic subcell packing ($O_{\perp}$). Finally, phases with the hexagonal subcell have the lowest melting point and represent the α polymorph.

In addition to these three basic polymorphs, other polymorphs showing subtle differences may be observed. Within groups having the same subcell, lower melting polymorphs are designated with a progressively higher subscript. In addition, the bilayer or trilayer structure of the triacylglycerol is designated with 2 or 3 following the polymorph description. Thus, β'_2-2 designates a bilayer of a β′ polymorph with the second highest melting point.

TABLE 9.2
Characteristics of the Polymorphic Forms of Monoacid Triacylglycerols

Characteristic	α Form	β′ Form	β Form
Chain packing	H	$O_{\perp}$	$T_{\parallel}$
Short spacing (Å)	4.15	4.2, 3.8	4.6, 3.9, 3.7
IR bands (cm-1)	720	727, 719	717
Density	Least dense	Intermediate	Most dense
Melting point	Lowest	Medium	Highest
Morphology	Amorphous-like	Rectangular	Needle shaped

Source: Refs. 10, 12, 15.

The fatty acid makeup and position in the glycerides of the fat solids and temperature history are the two main factors in determining polymorphic behavior (1). Other factors include kind and quantity of impurities, nature of possible solvent, and degree of supercooling. A high level of fatty acids of identical chain length results in a slow conversion rate of β′ to β and a coarsening of crystal structure. The more heterogeneous of fatty acid makeup, the more likely it will be β′ and fine-grained or needlelike crystals. Thus, mixed fatty acid triacylglycerols, such as those in lauric fats, tend to be β′-stable.

If a fat is cooled rapidly, the tendency is to form the small, α-crystals. These generally do not last long and convert rapidly to the β′ needlelike crystals. These β′ crystals are considered highly stiffening and, hence, are the form of choice for plastic shortenings (1). Depending on the glyceride composition and the temperature history, the β′-form may convert to the most stable β-form. This form has large, coarse, platelike crystals. These are not stiffening; hence, those hydrogenated fats exhibiting this behavior are the choice for the solids in fluid shortenings (1). Generally speaking, β′-forms melt about 5–10°C higher than the α-forms, and the β-forms also melt about 5–10°C higher than the β′-forms. Fats that tend to crystallize in β-forms include soybean, corn, olive, sunflower, and safflower oils, as well as cocoa butter and lard. On the other hand, cottonseed, palm, and rapeseed oils, milk fat, tallow, and modified lard tend to produce β′ crystals that tend to persist for long periods.

1. Milk Fat

As with many natural fats, the temperature at which crystallization occurs influences milk fat firmness, crystalline conformation, and percentage of solid fat. Hardness variability in milk results from different thermal treatments and may be better understood considering the presence of three milk fat fractions, which are observed by using differential scanning calorimetry. These fractions are defined as high-, middle-, and low-melting fractions (HMF, MMF,

and LMF, respectively). LMF is liquid at ambient temperature. Stable polymorphs of MMF and HMF were found to be a mixture of β′-2 + β′-3, and β-2, respectively (30,31).

2. Palm Oil

Palm oil is expressed from the pulp of the oil palm (*Elaeis guineensis*) fruit and is unique among vegetable oils because of the large percentage (10–15%) of saturated acyl esters at the *sn*-2 position of the triacylglycerols. In addition, it has almost 5 % of free fatty acids that play a role in the hardness. At room temperature, the oil appears as a slurry of crystals in oil. Three polymorphs have been determined: β'_2, α-2, and the stable β'_1 form (32). The β′ stability has resulted in the addition of palm oil to oils destined for shortening or margarine, since β-tending fats can result in gritty textures.

3. Lauric Fats

Lauric fats are those fats rich in laury acyl esters in the triacylglycerol molecule, mainly coconut oil and palm kernel oil. Two polymorphs have been identified in these fats: α and β′-2. The α form is fleeting and can be recognized only after rapid cooling, as it quickly transforms into the β′-2 polymorph (33–35). The melting point of these fats is sharp at 22°C for coconut and 25°C for palm kernel oil (36).

4. Liquid Oils

Evaluations of polymorphism in fats that are liquid at room temperature are limited. Cottonseed and peanut oils crystallize in a β'_2 form that is transformed into a stable β'_1-2 form. Four other oils (corn, safflower, sunflower, and soybean) show polymorphism similar to that of peanut and cottonseed, but these four fats developed a stable β-2 form (12).

5. Hydrogenated Fats

Complete hydrogenation eliminates the asymmetry, often leading to β stable polymorphs. Thus, soybean, peanut, sunflower, corn, and sesame oils, are converted to hydrogenated fats having stearoyl esters and consequently show the stable β-2 form. For oils rich in palmitic, hydrogenation leads to fats containing a high proportion of 1,3-dipalmitoyl-2-stearoyl-*sn*-glycerol (PStP). Because the rearrangement of PStP into a stable β form is hindered by misalignment of the methyl end plane of the β′ unit cell (23), a fat rich in this triacylglycerol will stay in the β′ form. On the other hand, a hydrogenated fat rich in StPSt can transform into a stable β form. The high PStSt fats have equally stable β′ and β forms, and any transformation to the β form occurs over a long period of time (12).

6. Cocoa Butter

Cocoa butter occupies a special place among natural fats because of its unusual and highly value physical properties. Products containing cocoa butter, such as chocolate, are solid at room temperature; have a desirable "snap"; and melt smoothly and rapidly in the mouth, giving a cooling effect with no greasy impression on the palate. The main characteristic of cocoa butter is the presence of a high content of symmetrical monounsaturated triacylglycerols (1-palmitoyl-2-oleyl-3-stearoyl-*sn*-glycerol (POSt), 1,3-distearoyl-2-oleyl-*sn*-glycerol (StOSt), and 1,3-dipalmitoyl-2-oleyl-*sn*-glycerol (POP) account for about 80% of the total). The polymorphic behavior of cocoa butter is more complex than that of its component glycerides, and a specific system for cocoa butter is often used. This was introduced by Wille and Lutton (37) and recognizes six different polymorphs –I, for the lowest melting form, through VI, for the highest melting form (Table 9.3). Another system in use recognizes only five polymorphs, designated γ, α, β′, β_2, and β_1, in order of increasing stability and melting point (42–45).

The desirable physical properties of cocoa butter and chocolate – snap, gloss, melting in the mouth, and flavor release – depend on the formation of polymorph V or β_2, which has to be obtained under controlled temperature conditions (41,46). After a long storage or unfavorable storage conditions such as extreme temperatures, chocolate may show "bloom." This is a grayish covering of the surface caused by crystals of the most stable β phase (phase VI) (41). Eventually the change progresses to the interior of the chocolate and the resulting change in crystal structure and melting point makes the product unsuitable for consumption.

7. Confectionery Fats

Cocoa butter is the primary fat used in chocolate. Its expense has led to the development of other fats, used alone or in combination, to replace some or all cocoa butter in cocoa-containing confections. These confectionery

TABLE 9.3
Nomenclature and Melting Point (°C) of Cocoa Butter Polymorphs

Form	Melting Point[a]	Form	Melting Point[a]
I	17.3–17.9	γ	16–18
II	23.3–24.4	α	21–24
III	25.5–27.7		
IV	27.3–28.4	β′	27–29
V	33.0–33.8	β_2	34–35
VI	34.6–36.3	β_1	36–37

[a] Values correspond to the range of the different values described in the literature.

Source: Refs. 12, 37–45.

or specialty fats can be classified into cocoa butter equivalents (CBE) and cocoa butter substitutes (CBS) (47). Essentially, a CBE is a mixed fat that provides a fatty acid and triacylglycerol composition similar to those of cocoa butter. A CBS is a fat that provides some of the desired physical characteristics to a confection independent of its dissimilar chemical composition to that of cocoa butter.

Miscibility is an important characteristic of confectionery fats. When fats of different composition are mixed, the melting point or the solid fat content of the blend may be lower than that of the individual components (eutectic effects). This happens when cocoa butter is mixed with a CBS that may lead to unacceptable softening. Mixing of cocoa butter and CBE gives no eutectic effect, and this type of fat can be used in any proportion with cocoa butter, analogously to milk fat (33). However, it has been reported that minor components of milk fats exert a significant influence on the crystallization behavior when milk fat is mixed with cocoa butter and other confectionery fats, having, for instance, a softening effect and antibloom properties (8,48,49). CBEs are generally based on three raw materials – shea oil, illipe butter, and palm – oil and processed by fractionation. CBEs also require the same tempering procedures as cocoa butter, since they will exhibit polymorphism similar to that of cocoa butter. It is also possible to tailor make CBEs to higher solid content and melting point than some of the softer types of cocoa butter. These fats are described as cocoa butter improvers (CBIs).

CBSs are available in two types, lauric and nonlauric. Lauric CBSs are based on palm kernel oil or coconut oil and are not compatible with cocoa butter. They do not need tempering, and the crystals formed are stable. Nonlauric CBSs are produced by hydrogenation of liquid oils, frequently followed by fractionation and/or blending. These products, especially those made from palm olein, are very stable in the β' form. Nonfractionated CBSs are used in compound-coating fats for cookies. The fractionated, hydrogenated CBSs have better eating quality and can tolerate up to 25% cocoa butter when used in coatings.

C. Techniques to Determine Crystallization and Polymorphism

The techniques used to elucidate crystal structures are either spectroscopic or microscopic. Spectroscopic techniques include X-ray diffraction and Raman and infrared spectroscopy. Microscopic techniques include polarized light and electron microscopy (50).

1. Infrared and Raman Spectroscopy

The region of major interest in an IR spectrum for the study of fat polymorphism is the methylene rocking vibration mode which appears between 670 and 770 cm^{-1}. The spectra vary according to the polymorphic form. The nomenclature for polymorphs is as follows: a doublet at 728 and 718 cm^{-1} indicates the presence of the β' form, a singlet at 720 cm^{-1} the α form, and a singlet a 717 cm^{-1} the β modification (51). In addition, vibrational (infrared and Raman) spectra have given fruitful information on molecular conformations of aliphatic chains and olefinic groups, and methyl end packings (52–54).

Raman spectroscopy is also used to identify the different states of order of lipids (55). Two regions are of interest: the C-C stretching vibration region at about 1100 cm^{-1}, and the C-H stretching vibration region at about 2850 cm^{-1}. Lipids with crystalline chains show two sharp peaks near 1065 and 1130 cm^{-1}, whereas these peaks are shifted towards a broad band near 1090 cm^{-1} when the chain melts. The ratio 1080/1130 can be taken as a measure of the degree of a liquid-type order (56). In addition, a peak at 2850 cm^{-1} corresponds to symmetric vibrations of methylene groups characteristic for the liquid state, whereas a peak at 2890 cm^{-1} is caused by antisymmetric vibrations of methylene groups and dominates when hydrocarbon chains are crystalline (57).

2. X-Ray Diffraction

The principle of X-ray diffraction is to excite an anticathode which will emit X-rays being diffracted by the crystal structure at a specific angle. The angle depends on the distance between two crystal planes, d, and d is different for each crystal structure. The chain packing of the triacylglycerol molecules determines the spacing between adjoining molecules. The cross-sectional structures determine the short spacings (Figure 9.1). Each of the chain-packing subcells is characterized by an unique set of X-ray diffraction lines in the wide-angle region between 3.5 and 5.5 Å. The nomenclature used to identify lipid crystal forms was proposed by Larsson (40) and is based on the following criteria: a form that gives only one strong short-spacing line near 4.15 Å is termed α; a form that gives two strong short-spacing lines near 3.80 and 4.20 Å and also shows a doublet in the 720 cm^{-1} region of the infrared absorption spectra is termed β'; a form that does not satisfy criterion two is termed β (Table 9.2).

X-ray diffraction is a powerful analytical technique to identify polymorphic phases unambiguously in both pure triacylglycerol systems and edible fats (43,58–60). In addition, recent developments in high-energy accelerators and X-ray detectors have reduced the exposure times of the sample to the order of milliseconds. Thus, with synchrotron radiation X-ray diffraction, the kinetics of rapid triacylglycerol polymorphic transformations has been elucidated under both isothermal and nonisothermal conditions in pure and mixed triacylglycerol systems (18,61–63).

The complex structure of the crystal network is determined by the fractal dimension, D, which describes the

relation between the number of crystals in a crystal aggregate and its radius, R (64,65). In general, the higher the D value, the more compact the crystal dispersion and, in the case of triacylglycerols, its value changes with ageing of the system after crystallization. In turn, the magnitude of D affects the value of the elastic modulus and, therefore, the texture of the crystal dispersion at a given temperature (e.g., mouthfeel, spreadability). Although the rheology of fat crystal dispersions determines important properties in vegetable oil system (i.e., texture, sedimentation), still more research is needed to understand the structure-property relationship (65).

3. Microscopic Techniques

Polarized light spectroscopy allows under certain circumstances to differentiate the α (platelets), β′ (small needles), and β (larger and growing in clump) forms. This technique makes it possible to view crystals in the range of 0.5–100 μm (66).

In contrast to polarized light microscopy, electron microscopy resolves details in areas of 0.1 μm. However, since fine structures of fats are temperature sensitive, special techniques such as freeze-fracture or freeze-etching are required (66). Structures of liquid and crystallized fat in systems such as butter and margarine can be characterized in micrographs due to the amorphous appearance of the originally liquid fat (67).

III. THERMAL AND RHEOLOGICAL PROPERTIES, AND OTHER PHYSICAL CONSTANTS

A. Melting

1. Melting Points

The melting point is the temperature at which a solid fat becomes a liquid oil. Thus, an individual fatty acid or triacylglycerol has a specific complete melting point for each polymorphic form (Table 9.4 collects the melting points of common triacylglycerols in their three polymorphic forms). Complications arise in fats and oils because they are essentially mixtures of mixed triacylglycerols which crystallize in several crystal forms (Table 9.5 collects triacylglycerol composition of some common fats and oils). These molecules, although of the same chemical structure, differ in chain length, unsaturation, and isomerism. Each component in these products has its own melting point. Fats, therefore, do not have sharp melting points, but a melting range. What is commonly known as the melting point of a fat is in reality the end of the melting range. Table 9.6 collects melting and solidification points of some common fats and oils. In addition, softening points (74) and congeal points (75) are sometimes reported.

TABLE 9.4
Melting Points (°C) of Common Triacylglycerols

	Form		
TAG[a]	α	β′	β
LaLaLa	15.0	34.5	46.5
MMM	33.0	46.0	58.0
PPP	45.0	56.6	66.1
StStSt	54.9	64.1	73.4
AAA	62.0	69.0	78.0
PStP	47.0	68.9	65.5
PPSt	47.4	59.9	62.9
PStSt	50.6	60.8	65.0
StPSt	51.8	64.0	68.5
POP	18.1	30.5	35.3
PPO	18.5	35.4	40.4
POSt	18.2	33.2	38.2
StPO	25.3	38.7	40.5
PStO	25.5	37.4	–
StOSt	23.5	36.6	41.2
StStO	30.4	42.2	42.1
StESt	46.0	58.0	61.0
StRSt	25.8	48.0	–
PLP	–	18.6	–
OPO	–	–	18.7
POO	−4.0	2.5	19.2
StOO	−1.5	8.6	23.0
OOO	−32.0	−11.8	5.1
EEE	15.5	37.0	42.0
LLL	−33.7	−21.0	−10.0
LnLnLn	−44.6	–	−24.2

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: A, arachidic; E, elaidic; L, linoleic; La, lauric; Ln, linolenic; M, miristic; P, palmitic; R, ricinoleic; and St, stearic.
Source: Refs. 7, 8, 41, 68.

The more complex and diversified the mixture of triacylglycerols in the fat, the greater the melting range. If the melting range is less than 5°C, the fat is considered to be non-plastic (cocoa butter, for example). If the melting range is significant (in certain cases it may exceed 40°C) the fat is called plastic. This happens for the majority of natural and processed fats.

The temperature at which a fat or oil is completely melted depends on various factors (12), including the average chain length of the fatty acids (in general, the longer the average chain length, the higher the melting point); the positioning of the fatty acids on the glycerol molecule (as an example, safflower oil, which has a long average chain length, will melt like a medium chain length triacylglycerol); the relative proportion of saturated to unsaturated fatty acids (the higher the proportion of unsaturated fatty acids, the lower the melting point); and the processing techniques employed, for example the degree and selectivity of hydrogenation and winterization.

TABLE 9.5
Triacylglycerol Composition of Common Fats and Oils

	TAG																				
Fat or Oil	**PPSt**	**PStSt**	**PPO**	**PStO**	**StStO**	**PPL**	**StStL**	**PStL**	**POO**	**StOO**	**OOO**	**POL**	**StOL**	**OOL**	**PLL**	**PLLn**	**StLL**	**OLL**	**OLLn**	**LLL**	**LLLn**	**Others**
Cocoa butter			16.0 [b]	36.0	25.0		< 2	3.0	4.5	5.5	< 2	< 3	< 1	< 0.2	< 3							
Corn			< 1			2.1			4.8 [d]		2.6	12.3 [c]	[d]	9.9	16.8		[c]	22.9		26.5	< 2	0.2
Cottonseed			3.4			9.0		< 2	2.5		< 1	17.1	< 2	5.7	24.5		2.1	13.1		13.5		5.1
Grapeseed												15.9 [c]		10.3	17.0		[c]	21.0		35.7		0.1
Hazelnut			< 0.3	< 0.1					9.0	4.0	52.0	3.0		20.5	< 1			5.5		< 2		
Lard	2.3	6.1	7.7	18.8	< 2			4.8	24.5	5.6 [e]	5.8	9.2		3.5	< 1							8.7
Olive [f]	< 1		2.9	< 1		<1			23.1	3.6	43.1	4.5		10.4	< 1			< 1				
Olive [g]	< 1		5.3	< 2		2.1			20.0	3.7	21.8	12.3		18.2	2.8			5.8		< 1		
Peanut			2.3	2.2		2.9			6.7	< 2	11.8	12.9	3.0	19.4	5.1		< 2	18.3		2.0		10.5
Soybean			< 2	< 1		< 2			< 2		< 2	7.8	3.0	7.6	13.1	2.8	4.2	20.4	5.0	19.3	7.5	2.9
Sunflower			< 1			< 1			< 1		< 1	4.0	2.1	6.5	11.3		7.5	29.1		36.3		1.0
Tallow	5.2	11.1	11.2	13.8	4.1				22.8	11.2 [e]	6.9 [d]	2.1	[d]	< 2								9.7

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: A, arachidic; E, elaidic; L, linoleic; La, lauric; Ln, linolenic; M, miristic; P, palmitic; R, ricinoleic; and St, stearic.

[b] Also includes MStO. [c] Also includes StLL. [d] Also includes StOL. [e] Also includes PPP. [f] From Spain. [g] From Tunisia.

Source: Refs. 8, 41, 68, 69.

TABLE 9.6
Melting Points (°C) of Common Fats and Oils

Fat or Oil	Melting Point (°C)	Solidification Point (°C)
Butter	36	
Castor		−12 to −18
Cocoa butter	31–35	28–29
Coconut	24–26	14–23
Corn	−10 to −20	−10 to −18
Cottonseed	10–15.5	−6 to 4
Grapeseed		−11 to −17
Hazelnut		−18 to −20
Lard	32–38	27–32
Linseed		−18 to −27
Olive	−9 to 0	−9 to −2
Palm	30–37	20–40
Palm kernel	23–30	20–24
Peanut	8–13	0–3
Rapeseed		−8 to −18
Safflower (high linoleic)		−15
Sesame		−3 to −6
Soybean	−15	−10 to −16
Sunflower	−15	−16 to −18
Tallow	40–50	27–38

Source: Refs. 68–73.

2. Specific Heat and Heat of Fusion

Specific heat, c_p, is defined as the amount of heat required to increase the temperature of 1 g of material by 1°C. It is believed to be largely independent of the molecular weight for oils, but it does increase with unsaturation (10). In addition, the specific heats of the solid and liquid states of fatty compounds are different. Fats at temperatures just above their melting points have specific heats of ~0.5 cal/g. Their solid forms have lower values. The specific heat of oils has often been observed to increase linearly with temperature (76). Other workers, however, have observed nonlinear increases at higher temperatures (77,78), although these increases may be due to autoxidation reactions (79). Specific heat data for some simple triacylglycerols are given in Table 9.7. Specific heat data of common fats and oils are given in Table 9.8.

The transformation of solid to liquid releases the latent heat of crystallization. Transformation of a lower to a higher polymorphic form also is an exothermic reaction. The heat of fusion (Hf) of a fatty material includes the amount of energy required to melt a gram of material and the heat of crystal transition. Heat (with negative sign) is released when a fat crystal is transformed from a less stable form to a more stable form. Heat of fusion data for several monoacid triacylglycerols are given in Table 9.9 and they increase with chain length. The corresponding values for common fats and oils are collected in Table 9.10.

TABLE 9.7
Specific Heat (c_p) of Triacylglycerols

	c_p (cal/g · °C) [b]	
TAG[a]	Liquid	Solid
LaLaLa	0.510 (66)	–
	0.530 (97.1)	
MMM	0.518 (65.3)	–
	0.534 (91.9)	
PPP	0.519 (65.7)	–
	0.539 (96.0)	
StStSt	0.530 (79.0)	0.259 (−77.1, β)
	0.542 (98.5)	0.310 (−30.3, β)
		0.273 (−81.0, α)
		0.346 (−36.5, α)

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; and St, stearic.
[b] Numbers in parentheses indicate the temperature in °C.
Source: Ref. 80.

TABLE 9.8
Specific Heat (c_p) of Common Fats and Oils

Fat or Oil	Temperature (°C)	c_p (cal/g · °C)
Castor	20	0.435
Coconut	20–30	0.511
Corn	20–30	0.415
Cottonseed	19.3	0.475
Grapeseed	20–30	0.395
Lard	20–30	0.483
Linseed	40	0.48
Olive	20–30	0.475
Palm	20–30	0.5
Peanut	20–30	0.490
Rapeseed	20–30	0.469
Sesame	20–30	0.478
Soybean	19.7	0.458
Sunflower	20–30	0.430
Tallow	30–38	0.55

Source: Refs. 70, 73, 76–78, 81.

B. Plasticity

Plasticity is defined as the capability of fats of being molded by largely maintaining the deformation due to a stress after removal of the stress. This is a consequence of the semisolid state of fats at room temperature. Their solid character is the result of the presence of a certain proportion of crystallized triacylglycerols. As the temperature drops, more of the triacylglycerols solidify and the fat becomes progressively firmer. On the other hand, as the temperature rises, most solid triacylglycerols melt and the fat becomes progressively softer until it has practically no "body" or plasticity at all, and eventually it becomes completely melted (82).

TABLE 9.9
Heat of Fusion (ΔH_f) and Transition of Triacylglycerols

TAG [a]	Transition	Temperature (°C)	ΔH_f (cal/g)
LaLaLa	β→Liq	46.3	46.2
MMM	β→Liq	57.0	50.3
	α→Liq	32.3	34.6
	α→β	32.2	−12.6
PPP	β→Liq	65.7	53.1
	α→Liq	44.7	37.4
	α→β	44.7	−13.3
StStSt	β→Liq	72.5	54.5
	α→Liq	54.0	38.9
	α→β	54.0	−13.7

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; and St, stearic.
Source: Ref. 80.

TABLE 9.10
Heat of Fusion (ΔH_f) of Common Fats and Oils

Fat or Oil	ΔH_f (cal/g)
Butter	24.5
Cocoa butter	35.0
Coconut	30.6
Cottonseed	16.8
Lard	29.2
Linseed	18.2
Olive	20.3
Palm	22.6
Palm kernel	32.0
Peanut	21.7
Rapeseed	19.8
Sesame	16.3
Soybean	17.9
Sunflower, crude	19.0
Tallow	27.3

Source: Refs. 69, 70, 76.

If a fat or a shortening that is workable over a wide temperature range is desired, then it should be made up of a combination of triacylglycerols ranging widely in melting points. By the same reason, when a fat or shortening with a narrow temperature range of workability is needed, it is made up of a greater amount of triacylglycerols of similar melting points.

One of the most important properties of fats is the solid fat content (41). It is dependent on temperature and temperature history, and it is related to different physical properties of the fats, including hardness, heat resistance, waxiness, coolness, and flavor release (83). Fats may retain their solid character with solid fat contents as low as 12 to 15%. Below this level, fats become pourable and lose their plastic character. The relationship between hardness and solid fat content indicates that there is only a range of solids that results in a product being neither too hard nor too soft. This is called the plastic range of fats. Shortening is an example of a product that requires an extended plastic range. The solid fat content (SFC) as a function of temperature of some natural fats is collected in Table 9.11.

Plastic fats combine the physical properties of both solids and liquids. This is caused by the presence of a three-dimensional network of crystals in which a considerable amount of liquid oil is immobilized. A plastic fat is usually workable at room temperature when the solid fat content lies between 20 and 40%. Under the influence of weak attractive forces between crystals, mostly due to van der Waals forces, a three-dimensional structure is formed that lends the product a good deal of resistance to deformation. As a result of the presence of this fat crystal network, plastic fats exhibit a yield value. Thus, the product behaves like a rigid solid until the deforming stress exceeds the yield value and the fat start flowing like a viscous liquid. The application of a shear stress to such product is accompanied by structural breakdown and a decrease in strength. This is also known as work softening. The consistency of plastic fats is determined by a number of factors, including solid fat content, crystal size and shape, and polymorphic form.

The earliest developed technique for solid fat content determination was dilatometry. It remains a recommended method of the American Oil Chemists' Society (84) but it has been largely replaced by various nuclear magnetic resonance methods (85,86). Lipid crystallization and, therefore, changes in solid fat content, can also be measured by differential scanning calorimetry (87) or by using ultrasounds (88).

TABLE 9.11
Solid Fat Content (SFC) of Common Fats and Oils

	SFC[a]				
Fat or Oil	10	30	50	70	90
Butter	28.0°C	16.0°C	10.5°C	2.0°C	−12.0°C
Cocoa butter	32.5°C	30.5°C	29.0°C	25.5°C	8.0°C
Coconut	23.0°C	20.5°C	16.0°C	10.0°C	−1.0°C
Cottonseed	3.5°C	−6.0°C	−11.0°C	−23.0°C	−28.5°C
Lard	40.0°C	29.5°C	17.5°C	2.5°C	−7.5°C
Linseed, crude	−8.5°C	−13.5°C	−15.5°C	−17.0°C	−23.0°C
Olive	6.0°C	−2.0°C	−5.5°C	−8.0°C	−11.0°C
Palm, crude	31.5°C	16.5°C	12.0°C	7.5°C	−6.0°C
Palm kernel	26.0°C	23.0°C	18.5°C	8.5°C	−6.5°C
Peanut, crude	−3.0°C	−7.5°C	−12.5°C	−15.5°C	−19.5°C
Rapeseed	3.0°C	2.0°C	1.0°C	−1.0°C	−4.5°C
Sesame	−4.0°C	−10.0°C	−15.5°C	−19.5°C	−22.5°C
Soybean	−5.5°C	−11.5°C	−13.0°C	−14.0°C	−20.0°C
Sunflower, crude	−10.0°C	−13.0°C	−14.5°C	−15.5°C	−18.0°C
Tallow	46.5°C	41.0°C	31.0°C	15.5°C	5.0°C

[a] Data indicate the temperature at which the SFC shown at the top of the column is achieved.
Source: Ref. 70.

C. Viscosity

Viscosity is a measure of internal friction of a liquid to resist flow. It is usually denoted by η, and defined as the ratio between the shear stress (in force per unit area) and the shear rate (the velocity gradient between the moving and stationary phases). The viscosity, η, carries a dimension of poise (P) or dyne/($cm^2 \cdot s$). Water has a value of 1 centipoise (cP) at 20°C. The viscosities of triacylglycerols or oils (Tables 9.12 and 9.13, respectively) are much higher than that of water, which can be attributed primarily to the intermolecular attractions of the long-chain fatty acids of their triacylglycerols. This is one of the reasons why the oils exhibit their unique oily characteristics and have the ability to form oily or lubricating films. This lubricating action is very important in the preparation of some foods, such as grilled foods (1).

Vegetable oils follow Newtonian flow behavior, i.e., at a given temperature a constant value of viscosity is obtained independent of the force applied to the oil. As a result, oil viscosity (η) is defined as the slope of the shear stress-shear rate curve, also known as the flow curve (65).

In general, the viscosity of oils decreases slightly with an increase in unsaturation, and, therefore, hydrogenated oils are slightly more viscous than original oils (94). In addition, for an equivalent degree of unsaturation, oils and fats containing a greater proportion of fatty acids of relatively low molecular weight are slightly less viscous than those containing a higher proportion of high-molecular-weight acids. Most common oils range in viscosity from 20 to 50 cP at ambient temperature. An exception is castor oil, which has a viscosity exceptionally high because of its high content of ricinoleic acid.

The viscosity of highly polymerized oils is much greater than that of normal oils. For this reason, viscosity is occasionally referred to in determining the conditions of fats used in deep frying. During use in the frying kettle, the viscosity of a frying fat or oil will tend to increase as oxidation and polymerization increase (95). This can be related to polymer development and tendency toward foaming.

Viscosity is also an important parameter in the process of melt crystallization. The dry fractionation of oils to produce a solid and a liquid fraction is greatly influenced by the viscosity of the oil at the fractionation temperature. Toro-Vazquez and Gallegos-Infante (96) have described the relationship between viscosity and crystallization in a system containing saturated triacylglycerols and liquid oil.

TABLE 9.12
Viscosities of Triacylglycerols

	Viscosities (cP)				
TAG[a]	60°C	70°C	75°C	80°C	85°C
LaLaLa	13.59	10.30	9.11	8.09	7.22
MMM	17.71	13.42	11.70	10.35	9.20
PPP		16.79	14.67	12.92	11.44
StStSt			18.50	16.21	14.31

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; and St, stearic.
Source: Refs. 89, 90.

TABLE 9.13
Viscosities of Common Fats and Oils

	Viscosity (cP)				
Fat or Oil	20°C	30°C	40°C	50°C	60°C
Castor	950–1100	453	232	128	
Cocoa butter			42		
Coconut		34	24	17–20	13
Corn	56–66		31		18
Cottonseed	65–69		33		
Grapeseed	53–58				
Hazelnut	66–76				
Lard			35	25	17–20
Linseed	42–47	33	24	18	
Olive	75–79		42		
Palm			40	25–31	
Palm kernel		36	25	17–20	
Peanut	68–82	49	33	24	
Rapeseed	86–97	56	38	27	
Safflower[a]	52–54				
Safflower[b]	70–75				
Sesame	64–67	42	29	21	
Soybean	53–58	43	32	23	
Sunflower	51–57	40	28	20	
Tallow			42	30	19–20

[a] High linoleic.
[b] High oleic.
Source: Refs. 10, 69, 70, 76, 91–93.

D. Vapor Pressure

The vapor pressure, defined as the pressure associated with the vapor in equilibrium with a condensed phase, is very low in triacylglycerols of long-chain fatty acids. Therefore, they can only be satisfactorily distilled by molecular or short-path distillation. These values decrease as chain length increases (Table 9.14). Analogous values and behavior are also observed in fats and oils (Table 9.15).

E. Smoke, Flash, and Fire Points

The smoke, flash, and fire points are important properties when oils or fats are heated in contact with air, as in frying operations. These indices are often used as a quick quality control means for a critical process step such as deodorization. In this case, lower than normal smoke, flash, or fire points indicate the presence of an excess of residual nontriacylglycerol impurities, such as free fatty acids, monoacylglycerols, and other volatiles, which should be largely removed during steam deodorization.

TABLE 9.14
Vapor Pressure of Triacylglycerols

	Temperature (°C)	
TAG[a]	0.05 mm Hg	0.001 mm Hg
LaLaLa	244	188
MMM	275	216
PPP	298	239
StStSt	313	253
StOSt	315	254
MPSt	297	237
PLaSt	290	232
MLaSt	282	223

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; and St, stearic.
Source: Ref. 97.

TABLE 9.15
Vapor Pressure of Fats and Oils

	Temperature (°C)	
Oil	0.05 mm Hg	0.001 mm Hg
Cottonseed	250[a]	
Olive	308	253
Peanut	250[a]	
Soybean	308	254

[a] Values at 0.04 mm Hg.
Source: Refs. 68, 91.

The smoke point is the temperature at which smoking is first detected in a laboratory apparatus protected from drafts and provided with special illumination (98). The flash point is the temperature at which volatile products are evolved at such rate that they are capable of being ignited but do not support combustion. The fire point is the temperature at which the volatile products will support continuous combustion. Smoke, flash, and fire points of common fats and oils are shown in Table 9.16.

The smoke and flash points of fats and oils are greatly dependent on content of free fatty acids and to a lesser degree on partial glycerols. The influence of degree of unsaturation is minimal, but chain length has an important effect. Oils containing short-chain fatty acids (e.g., the lauric acids) have lower smoke and flash points than oils with predominantly longer fatty acids. According to Formo (10), the smoke points of corn, cottonseed, and peanut oils vary from about 232°C at a free fatty acid content of 0.01% to about 94°C at 100% free fatty acid content. The flash points correspondingly decrease from about 329 to 193°C and the fire points from 362 to 221°C.

F. Heat of Combustion

The heat of combustion is defined as the amount of heat released when a definite quantity of a substance is completely oxidized at constant pressure or constant volume. All fats and oils have very similar heat of combustion values and they are about 9.4 kcal/g. These values are collected in Table 9.17.

TABLE 9.16
Smoke, Flash, and Fire Points of Common Fats and Oils

Fat or Oil	Free Fatty Acids (%)[a]	Smoke Point (°C)	Flash Point (°C)	Fire Point (°C)
Castor	n. s.	200	298	335
Coconut	0.2	194	288	329
Corn	n. s.	227	326	359
Cottonseed	0.04	223	322	342
	0.18	185	318	357
Lard	2.30		282	352
Linseed	n. s.	160	309	360
Olive (virgin)	n. s.	199	321	361
Palm	0.06	223	314	341
Peanut	n. s.	229		
	0.09	207	315	342
	0.11	198	333	363
Rapeseed	0.08	218	317	344
Safflower, crude	1.7	159	317	362
Sesame	n. s.	165	319	
Soybean	n. s.	234	328	363
	0.04	213	317	342
Sunflower	n. s.	253		
	0.1	209	316	341
Tallow	2.5		263	332
	0.5		324	357

[a] Abbreviation: n. s., not specified.
Source: Refs. 70–73, 99.

G. Thermal Conductivity

Thermal conductivity describes the ease with which heat passes through a material. Specifically, it quantifies the

TABLE 9.17
Heat of Combustion of Common Fats and Oils

Fat or Oil	Heat of Combustion (Kcal/g)
Castor	8.880
Coconut	9.020
Corn	9.413
Cottonseed	9.447
Lard	9.449
Linseed	9.364
Olive	9.456
Peanut	9.410
Rapeseed	9.680
Sesame	9.394
Soybean	9.478
Sunflower	9.499
Tallow	9.485

Source: Refs. 10, 69, 70, 73.

rate of flow of thermal energy through a material in the presence of a temperature gradient, given by the amount of heat transfer across a unit area in a unit amount of time, divided by the negative of the space rate of change in temperature in the direction perpendicular to the unit area. Fats are relatively poor conductors of heat (10) and the thermal conductivity decreases with the temperature (100). Thermal conductivity data for common fats and oils are given in Table 9.18.

H. Thermal Diffusivity

Thermal diffusivity is the thermal conductivity divided by the product of specific heat capacity and density. It is more generally applicable than thermal conductivity in most heat transfer problems. Thermal diffusivity values for some fats and oils are given in Table 9.19.

TABLE 9.18
Thermal Conductivity (cal/cm · s) of Common Fats and Oils

	Temperature				
Fat or Oil	**20°C**	**40°C**	**60°C**	**80°C**	**100°C**
Butter	$4.02 \cdot 10^{-4}$ [a]				
Castor	$4.00 \cdot 10^{-4}$			$3.85 \cdot 10^{-4}$ [c]	
Corn	$4.14 \cdot 10^{-4}$	$4.06 \cdot 10^{-4}$	$4.00 \cdot 10^{-4}$	$3.83 \cdot 10^{-4}$	$3.72 \cdot 10^{-4}$
Cottonseed	$4.00 \cdot 10^{-4}$	$3.92 \cdot 10^{-4}$	$3.89 \cdot 10^{-4}$	$3.81 \cdot 10^{-4}$	$3.72 \cdot 10^{-4}$
Grapeseed	$4.03 \cdot 10^{-4}$	$3.89 \cdot 10^{-4}$	$3.81 \cdot 10^{-4}$	$3.69 \cdot 10^{-4}$	$3.57 \cdot 10^{-4}$
Lard	$5.55 \cdot 10^{-4}$				
Olive	$4.00 \cdot 10^{-4}$			$3.85 \cdot 10^{-4}$ [c]	
Peanut	$4.02 \cdot 10^{-4}$ [b]				
Rapeseed	$3.79 \cdot 10^{-4}$				
Sesame	$4.31 \cdot 10^{-4}$	$4.19 \cdot 10^{-4}$	$4.08 \cdot 10^{-4}$	$3.97 \cdot 10^{-4}$	$3.83 \cdot 10^{-4}$
Soybean	$4.21 \cdot 10^{-4}$	$4.11 \cdot 10^{-4}$	$3.97 \cdot 10^{-4}$	$3.88 \cdot 10^{-4}$	$3.76 \cdot 10^{-4}$
Sunflower	$4.00 \cdot 10^{-4}$	$3.92 \cdot 10^{-4}$	$3.89 \cdot 10^{-4}$	$3.81 \cdot 10^{-4}$	$3.72 \cdot 10^{-4}$
Tallow	$8.33 \cdot 10^{-4}$		$4.17 \cdot 10^{-4}$		

[a] Data at 10°C.
[b] Data at 4°C.
[c] Data at 71°C.
Source: Refs. 70, 100, 101.

TABLE 9.19
Thermal Diffusivity (m^2/h) of Common Fats and Oils

	Temperature				
Fat or Oil	**20°C**	**40°C**	**60°C**	**80°C**	**100°C**
Corn	0.391	0.385	0.366	0.351	0.338
Cottonseed	0.403	0.388	0.372	0.359	0.345
Grapeseed	0.401	0.382	0.367	0.356	0.342
Sesame	0.364	0.349	0.334	0.319	0.304
Soybean	0.380	0.363	0.350	0.334	0.320
Sunflower	0.340	0.322	0.310	0.295	0.281

Source: Ref. 70.

I. Thermal Expansion

The thermal expansion is defined as the increase in the size of a substance when the temperature of the substance is increased. The thermal expansion coefficients are the proportionally constants that related these increases. Fat and oils have thermal expansion coefficient values in the range $6.6 \cdot 10^{-4}$–$7.8 \cdot 10^{-4}$ mL/g · °C (Table 9.20). These values increase approximately linearly with temperature for liquid oils (79).

J. Dielectric Constant

The dielectric constant is the property of a material that determines how much electrostatic energy can be stored per unit volume of the material when unit voltage is applied. It is quantified as the ratio of electric flux density produced in a material to the value in free space produced by the same electric field strength. In fats and oils, most values are in the range of about 3.0–3.2, and, therefore, dielectric constants are not a particularly distinguishing characteristic. Castor and oiticica oils, however, which contain oxygenated fatty acids (ricinoleic and licanic acids, respectively), have higher dielectric constants (around 4.0). Oxidation increases the dielectric constant of oils by the introduction of polar groups (104). On the other hand, thermal polymerization without considerable oxidation has relatively little effect (10). Dielectric constants for some common fats and oils are given in Table 9.21.

K. Density

The density of a material is a measure of the mass per unit volume. Liquid oils have a density between 0.91 and

TABLE 9.20
Thermal Expansion of Common Fats and Oils

Fat or Oil	**Thermal Expansion Coefficient (mL/g · °C)**
Butter	$6.64 \cdot 10^{-4}$
Castor	$6.90 \cdot 10^{-4}$
Cocoa butter	$7.72 \cdot 10^{-4}$
Coconut	$6.77 \cdot 10^{-4}$
Corn	$7.22 \cdot 10^{-4}$
Cottonseed	$6.75–7.30 \cdot 10^{-4}$
Linseed	$6.90 \cdot 10^{-4}$
Olive	$7.19–7.27 \cdot 10^{-4}$
Palm	$7.24 \cdot 10^{-4}$
Palm kernel	$7.02 \cdot 10^{-4}$
Peanut	$6.52–6.75 \cdot 10^{-4}$
Rapeseed	$6.71 \cdot 10^{-4}$
Sesame	$6.87 \cdot 10^{-4}$
Soybean	$6.70–7.24 \cdot 10^{-4}$
Sunflower	$6.61–7.46 \cdot 10^{-4}$
Tallow	$7.26 \cdot 10^{-4}$

Source: Refs. 70, 79, 102, 103.

TABLE 9.21
Dielectric Constants (λ) of Common Fats and Oils

Fat or Oil	Temperature (°C)[a]	λ
Castor	11	4.62
Cocoa butter	40	2.99
Coconut	n. s.	3.44
Cottonseed	20	3.15
Linseed	20	3.19
Olive	21	3.11
Peanut	11	3.03
	20	3.05
Sesame	13	3.02
Sunflower	20	3.11

[a] Abbreviation: n. s., not specified.
Source: Ref. 70.

0.92 g/mL at 25°C, and this value decreases as the temperature increases (Table 9.22). Oil density generally increases with lower molecular weight fatty acids and by hydrogenation (76,94,107). The densities of solid fats are generally higher than those of their liquid states. In addition, the various types of crystal forms, α, β', and β, melting at progressively higher temperatures, also show increasing densities (Table 9.23).

TABLE 9-22
Density of Common Fats and Oils

	Density (g/mL)		
Fat or Oil	20°C	40°C	60°C
Canola	0.914–0.917		
Castor	0.955–0.968	0.942–0.952	
Cocoa butter		0.906–0.909	
Coconut	0.926	0.908–0.920	
Corn	0.917–0.925	0.905–0.911	
Cottonseed	0.917–0.925	0.905–0.908	
Grapeseed	0.923–0.926	0.896–0.920	
Hazelnut	0.912–0.915	0.899–0.904	
Lard	0.916	0.896–0.906	
Linseed	0.928–0.933	0.914–0.922	
Olive	0.910–0.916	0.899–0.905	
Palm	0.922	0.895–0.900	
Palm kernel	0.930	0.899–0.913	
Peanut	0.914–0.920	0.906–0.912	
Rapeseed	0.910–0.916	0.897	
Safflower[a]	0.922–0.927		
Safflower[b]	0.910–0.916		
Sesame	0.915–0.923	0.910–0.913	
Soybean	0.921–0.924	0.906–0.912	
Sunflower	0.920–0.925	0.906–0.910	
Tallow	0.936–0.952[c]	0.893–0.904	0.885–0.887

[a] High linoleic.
[b] High oleic.
[c] Data at 15°C.
Source: Refs. 10, 69, 70, 72, 73, 102, 105, 106.

TABLE 9.23
Density of Solid Triacylglycerols

TAG or Fat[a]	Polymorphic Form	Temperature (°C)	Density (g/mL)
LaLaLa	β	−38.6	1.057
MMM	β	−38.4	1.050
PPP	β	−38.2	1.047
StStSt	β	−38.6	1.043
	β'	−38.0	1.017
	α	−38.0	1.014
OOO	Highest m. p.	−38.7	1.012
Lard	Highest m. p.	−38.6	1.005

[a] Abbreviations: m. p., melting point; TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; and St, stearic.
Source: Ref. 108.

L. Solubility

Fats and oils are almost completely insoluble in water. When they are held together in systems such as cake batters or butterfat in milk, these systems require the use of food emulsifiers and/or mechanical means such as homogenizers. These same fats, oils, and fatty acids are completely miscible with most organic solvents, such as hydrocarbons, ethers, esters, and so on, at temperatures above their melting points. The solubility of fats in organic solvents increases with temperature, decreases with increasing mean molecular weight, and increases with increasing unsaturation. This last is the basis for fractional crystallization of fats into a number of fractions based on molecular weight and unsaturation. Alcohols from methanol to octanol have the property of being miscible with the liquid portion of plastic fats but not the solid. This has been used as a method for the separation of solid fat (109). The Crismer value is sometimes used to measure the solubility of an oil in a standard solvent mixture, composed of t-amyl alcohol, ethyl alcohol, and water in volume proportion 5:5:0.27. This value is characteristic within a narrow limit for each kind of oil (110). Examples of such values are 68.5–71.5 for olive oil, 67–70 for canola (low erucic rapeseed oil) and 76–82 for high erucic rapeseed oil (111,112). The miscibility of an oil is related to the solubility of glycerides and is affected mainly by the unsaturation and chain length of the constituent fatty acids.

Water is slightly soluble in oil at 0°C, with solubility amounting to about 0.07 and 0.14% at 32°C (2).

Some selected solubility data of nitrogen, oxygen, hydrogen, and carbon dioxide are shown in Table 9.24. With the exception of carbon dioxide, the solubilities of these gases in oil usually increase with temperature.

M. Surface Tension, Interfacial Tension, and Emulsification

Surface tension is the force acting on molecules at the surface of the oil that tends to pull them into the bulk of the

TABLE 9.24
Solubility of Gases in Oils

Fat or Oil	I. V.[a]	Temperature (°C)	Solubility (mL gas/100 mL Oil)			
			Nitrogen	Hydrogen	Oxygen	Carbon Dioxide
Butter	n. s.	40	10.1[b]	5.4	14.2	109.5
	n. s.	60	9.6[b]	6.8	12.7	91.0
Cottonseed	n. s.	40	8.7[b]	4.7	12.7	87.6
	104.3	30.5	7.1	4.6		
	104.3	49.6	7.8	5.4		
	104.3	147.8	11.8	10.2		
Lard	n. s.	40	8.8[b]	5.0	11.5	100.3
	70.1	41.5	7.7	5.2		
	70.1	147.3	12.1	10.4		
	1[c]	64.3		6.1		92.0
	1[c]	67.0	8.4		14.5	
	1[c]	84.7			15.4	
	1[c]	88.0				79.1
	1[c]	139.4	11.7	9.8		61.9
Soybean	n. s.[d]	22.5			3.2	
	n. s.[e]	22.5			1.3	
Sunflower	n. s.[d]	22.5			2.9	
	n. s.[e]	22.5			1.9	

[a] Abbreviations: I. V., iodine value; n. s., not specified.
[b] Data correspond to the solubility of air.
[c] Hydrogenated lard.
[d] Data correspond to the crude oil.
[e] Data correspond to the refined oil.
Source: Refs. 10, 68, 70, 113.

liquid. The interfacial tension is the surface tension at the surface separating two non-miscible liquids. Surface and interfacial tensions against water for different oils are given in Tables 9.25 and 9.26, respectively, and are similar among them (114).

Interfacial tensions may be decreased by the use of emulsifiers (115). Thus, the presence of monoacylglycerols and lecithin decreases the interfacial tension between oil and water from 30 to <10 dyne/cm. This reduction of interfacial tension lowers the energy required to homogenize the oil/water mixture, thus making possible the fine oil-in-water or water-in-oil dispersions.

Acylglycerols with three fatty acids attached to a glycerol molecule have minimal emulsification properties. However, fats and oils are important constituents of emulsions (116–124). An emulsion consists of a three-phase system composed of a continuous phase (the phase or medium in which the disperse phase is suspended), a disperse phase (the phase which is disrupted or finely divided within the emulsion), and an emulsifier (16). The emulsifier is present at the interface between the dispersed phase and the continuous phase, and keeps them apart; it reduces the interfacial tension between the two liquids, enabling one liquid to spread more easily around the other; and it forms a stable, coherent, viscoelastic film that prevents or delays coalescence of the dispersed emulsion droplets.

TABLE 9.25
Surface Tension of Oils

Fat or Oil	Surface Tension (dyne/cm)				
	17°C	20°C	50°C	80°C	130°C
Castor	34.9	34.0	32.2	30.0	
Coconut		21.4[a,b]		28.4	24.0
		33.6[b]			
Corn		34.8[b]			
Cottonseed		33.2[a]		31.4	27.5
		35.5			
Linseed		34.6[a]			
		36.4			
Olive	36.0–36.8	32.6	30.5	29.1	
Palm kernel		32.4[a]			
		33.5			
Peanut	44.4	31.6[a]			
		34.6			
Sesame	36.3				
Soybean		34.1[a]			
		35.8			
Rapeseed	35.3	34.5[a]	29.6	28.1	
		32–34			

[a] Data correspond to crude oil.
[b] Data at 25°C.
Source: Refs. 2, 68, 70, 71, 93.

TABLE 9.26
Interfacial Tension of Oils

Oil	Interfacial Tension (dyne/cm) at 70°C
Cottonseed	29.8
Peanut	29.9
Soybean	30.6

Source: Ref. 68.

These properties of the emulsifiers are a consequence of their structure: the molecules contain two distinct sections, one having polar or hydrophilic character, the other having nonpolar or hydrophobic properties. The relative sizes of the hydrophilic and hydrophobic sections of an emulsifier mostly determines its behavior in emulsification. To make the selection of the proper emulsifier for a given application and to predict the type of emulsion that will be formed, the so-called hydrophile-lipophile balance (HLB) system has been developed. It is a numerical expression for the relative simultaneous attraction of an emulsifier for water and for oil. Emulsifiers with HLB in the range 2–6 tend to form water-in-oil (W/O) emulsions, those with HLB in the range 7–9 are good wetting agents, and those with HLB in the range 10–18 tend to form oil-in-water (O/W) emulsions (115). HLB values of some commercial nonionic emulsifiers are given in Table 9.27. These values can be estimated from experimental measurements of its cloud point or can be calculated from a knowledge of the number and type of hydrophilic and lipophilic groups that it contains, according to the equation:

$$\text{HLB} = 7 + \Sigma \text{ (hydrophilic group numbers)} - \Sigma \text{ (lipophilic group numbers)}$$

Hydrophilic and lipophilic group numbers have been tabulated (115,116,125).

Foods contain many natural emulsifiers, of which phospholipids and proteins are the most common (126,127). Mono- and diacylglycerols are examples of emulsifiers that are added to products in order to provide ease of mixing. They absorb at the interface, reducing interfacial tension, and increasing the spreadability of the continuous phase, or the wettability of the dispersed phase.

Many natural and processed foods exist either partly or wholly as emulsions, or have been in an emulsified state at some time during their existence (117,128–132). Milk is the most common example of a naturally occurring food emulsion (133). Mayonnaise, salad dressing, cream, ice cream, butter, and margarine are all examples of manufactured food emulsions. Powdered coffee whiteners, sauces, and many desserts are examples of foods that were emulsions at one stage during their production but subsequently were converted into another form (116).

TABLE 9.27
HLB Values of some Commercial Nonionic Emulsifiers

Emulsifier	HLB
Sorbitan trioleate	1.8
Sorbitan tristearate	2.1
Mono- and di-acilglycerols	3.2–3.5
Glycerol monostearate	3.8
Sorbitan monooleate	4.3
Sorbitan monostearate	4.7
Sorbitan monopalmitate	6.7
Sorbitan monolaurate	8.6
Polyoxyethylene sorbitan monostearate	9.6
Polyoxyethylene sorbitan monooleate	10.0
Polyoxyethylene sorbitan trioleate	11.0
Glycerol monostearate	11.0
Polyoxyethylene monostearate	11.1–16.0
Polyoxyethylene monolaurate	12.8
Sodium oleate	18
Sucrose monoester	20
Potassium oleate	20
Sodium stearoyl lactylate	22
Sodium lauryl sulfate	40

Source: Refs. 41, 115.

N. Ultrasonic Properties

Ultrasonic velocity in triacylglycerols or oils is a measure of the speed of sound in these food components. It is related to its fatty acid composition and the supramolecular lipid structure. This is one of the most important variables in predicting the velocity of sound in an emulsion. The speed of sound in oils decreases monotically with temperature (134), is similar for oils and water at 20°C (79), and is lower in solid fats than oils. Ultrasonic velocities for some triacylglycerols as well as for some common oils are given in Tables 9.28 and 9.29, respectively.

The ultrasonic attenuation coefficient is a measure of how much ultrasound is dissipated in an oil per unit distance. This coefficient has little direct value beyond predicting the ultrasonic properties of an emulsion. However, in this application, a precise value is essential. The attenuation coefficient increases with frequency at a single temperature (79). Attenuation and its frequency dependence tend to decrease with increased temperature. Ultrasonic attenuation coefficients for some common oils are given in Table 9.29.

IV. OPTICAL AND SPECTROSCOPIC PROPERTIES

A. Color

Fatty acids and triacylglycerols, are colorless and essentially transparent to visible light. Natural fats and oils, however, often contain pigments that partially absorb transmitted light. Most of these pigments are removed from fats and oils by the refining and bleaching process,

TABLE 9.28
Ultrasonic Properties of Triacylglycerols

	Ultrasonic Velocity (m/s)		
TAG [a]	20°C	40°C	70°C
LaLaLa		1357	
PPP			1290
PSP			1292
SSS			1301
POP		1389	1293
PPO		1390	1295
POS		1392	1297
PSO		1393	
SPO		1394	
SOS			1302
OOO	1463	1397	1304
LLL	1474	1407	

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; and St, stearic.
Source: Refs. 108, 135, 136.

TABLE 9.29
Ultrasonic Properties of Fats and Oils

	Ultrasonic Velocity (m/s)			Ultrasonic Attenuation Coefficient (at 20°C)	
Fat or Oil	20°C	40°C	70°C	2 MHz	5 MHz
Butter		1359			
Castor	1494	1457			11.0
Coconut		1362			
Corn	1470	1403	1308		
Cottonseed		1405			
Grapeseed			1309		
Linseed		1414			
Olive	1466	1401	1302	6.5	1.94
Palm	1459	1399	1298		
Palm kernel		1368			
Peanut	1466	1405	1308	3.6	1.0
Rapeseed	1468	1411	1308	3.6	1.0
Safflower	1472	1408	1310	4.0	1.18
Sesame		1403			
Soybean	1470	1405	1309	4.9	
Sunflower	1472	1407	1311		

Source: Refs. 79, 135–139.

but some of them remain and most oils have their specific color, which is a consequence of the type and amount of natural pigments present. Therefore, some oils are naturally darker than others (68).

Carotene is the predominant red/yellow color pigment in soybean, safflower, and sunflower oils, among others. Carotene becomes colorless when subjected to the temperatures encountered in the edible oil processing steps. The oxidation products and other pigments found in these refined oils can be removed by adsorption on diatomaceous earth in the edible oil processing step referred to as bleaching. Most of the red color found in cottonseed oil, on the other hand, comes from a minimal residual level of gossypol and gossypol derivatives, especially a complex gossypurpurin. While some of the pigments that contribute to oil colors can be removed by adsorption on bleaching earths and any carotene is rendered colorless by heating, the gossypol can only be removed by alkali refining. The level of color removal that can be achieved is, to a great extent, dependent on the handling and storage of the seed and crude oil prior to refining.

In addition, poor grade of crude oil or improper processing and handling may produce oils and fats which are darker than usual. Thus, insufficient refining may leave residual phospholipids that will darken the oil during deodorization. Vegetable oils and shortening will also darken after being stored for a long time or at elevated temperatures due to the oxidation of tocopherol to tocoquinones. During frying, oil darkening is further complicated by the polymerization of oil and interaction between the oil and other components of the food being fried (95).

Color may be determined by a number of procedures. The Lovibond method determines color by matching the color of the light transmitted through a specific depth of liquid fat or oil to the color of the light originating from the same source, transmitted through glass color standards (140). Results are given in red and yellow units describing the combination that matches the sample color. By using this methodology, the maximum accepted values for edible oils are collected in Table 9.30. Other methods that also determine color by comparison with permanent color standards or glasses of known color characteristics have also been described (142,143).

Color can also be determined spectrophotometrically. In this case the oil or fat is dissolved in the required solvent and the transmittance or the extinction of the solution is then determined at the specified wavelengths with reference to pure solvent (144). These absorptions may be expressed as specific extinctions (the extinction of 1% solution of the oil in the specified solvent, in a thickness of 1 cm), conventionally indicated by K (145). K values are usually employed to define olive oil quality (146).

TABLE 9.30
Lovibond Colors of Edible Oils[a]

Fat or Oil	Red	Yellow
Coconut	1.0	10
Cottonseed	2.5	
Palm	3.0	
Palm kernel	1.5	
Peanut	2.0	25
Safflower	1.5	15
Soybean	1.0	
Sunflower	2.0	20

[a] Maximum accepted values are indicated.
Source: Refs. 71, 73, 99, 105, 106, 141.

B. Refractive Index

The refractive index of a fat is a measure of the relative velocities of light in air and in the material to be tested. It is defined as the ratio of the sine of the incident angle of light from air and the sine of the refractive angle in the medium. This index, which can be easily measured, is influenced by wavelength, temperature, density, and constitution, and it is employed to examine the purity and concentration of a liquid sample, and to control the progress of some reactions such as hydrogenation and isomerization. For lipids, the refractive index normally increases with the hydrocarbon chain length and with the number of double bonds and conjugation, and decreases with an increase in the temperature (the refractive index for an oil drops by 0.00035 per °C). The refractive indices of some triacylglycerols and common fats and oils are given in Tables 9.31 and 9.32, respectively.

TABLE 9.31
Refractive Indices of Triacylglycerols

TAG[a]	Temperature 60°C
LaLaLa	1.440
MMM	1.443
PPP	1.445
StStSt	1.447
StLaLa	1.444
LaStLa	1.444
StPP	1.447
PStSt	1.447
LaStSt	1.445
StLaSt	1.446
PStSt	1.446
StPSt	1.448
StMP	1.444
StLaP	1.443
StPM	1.444
StPLa	1.443
StMLa	1.442
LaLaO	1.446
LaOLa	1.446
PPO	1.448
POP	1.448
StStO	1.449
StOSt	1.449
LaOO	1.450
MOO	1.451
POO	1.451
StOO	1.452
OOO	1.455
LLL	1.465
LnLnLn	1.474

[a] Abbreviations: TAG, triacylglycerol. Fatty acids in TAG: La, lauric; M, miristic; P, palmitic; St, stearic; O, oleic; L, linoleic; and Ln, linolenic.
Source: Refs. 89, 147.

TABLE 9.32
Refractive Indices of Common Fats and Oils

	Temperature		
Fat or Oil	20°C	40°C	60°C
Butter		1.455	1.447
Canola	1.465–1.467		
Castor	1.476–1.481	1.466–1.473	
Cocoa butter		1.456–1.458	
Coconut	1.454 [c]	1.448–1.450	1.441
Corn	1.474–1.477	1.465–1.466	> 1.447
Cottonseed	1.470–1.473	1.464–1.468	
Grapeseed	1.473–1.476	1.464–1.471	
Hazelnut	1.470–1.471	1.462–1.463	
Lard		1.457–1.461	1.451–1.453
Linseed	1.479–1.484	1.472–1.475	
Olive	1.468–1.471	1.461–1.462	
Palm		1.453–1.458	
Palm kernel	1.457 [c]	1.450–1.452	1.443
Peanut	1.470–1.474	1.461–1.463	
Rapeseed	1.472–1.473	1.464–1.466	
Safflower [a]	1.474–1.478		
Safflower [b]	1.470–1.474		
Sesame	1.474–1.477	1.465–1.468	
Soybean	1.473–1.477	1.465–1.469	> 1.447
Sunflower	1.474–1.476	1.466–1.468	
Tallow		1.448–1.460	1.450–1.454

[a] High linoleic.
[b] High oleic.
[c] Data at 25°C.
Source: Refs. 69–73, 93, 99, 105, 106.

C. Ultraviolet Spectroscopy

Monoene and methylene-interrupted polyene acids and their triacylglycerols absorb ultraviolet light at wavelengths too low for convenient study. Therefore, the use of ultraviolet spectroscopy in the study of fats and oils is confined to systems containing or generating conjugated unsaturation. Thus, ultraviolet spectroscopy is a valuable tool and it is broadly employed for detecting fatty acids and their corresponding triacylglycerols with conjugated double bonds. Conjugated dienes show a single absorption peak at 230–235 nm, whereas conjugated trienes show three peaks at ~260, 270, and 280 nm. Methylene-interrupted polyenes undergo double bond migration to produce compounds with conjugated unsaturation in reactions such as oxidation or hydrogenation and the appearance of absorption at appropriate wavelength has been employed in the study of such processes. Thus, the edible oil industry estimates spectrophotometrically at 233 nm the amounts of conjugated dienes in the finished fats and oils (148), and the K234 and K270 are indexes of olive oil quality (146,149).

D. Infrared (IR) Spectroscopy

The infrared spectra has been applied to solid lipids to provide useful information about polymorphism, crystal

structure, conformation and chain length of fats and oils, and it is also frequently employed for identification and quantitative analysis of fats and oils. Most oils containing the usual mixture of saturated and unsaturated acids have similar infrared spectra. Superimposed on this there may be additional absorption bands associated with less common functional groups. A selection of the bands of interest in the study of fats and oils is collected in Table 9.33.

The most frequent use of infrared spectroscopy is in the recognition of *trans* isomers. The *trans*-double bond

TABLE 9.33
Infrared Absorption Bands of Interest in the Study of Fats and Oils

Functional Group	Absorption (cm^{-1})	Functional Group	Absorption (cm^{-1})
O–H stretching		**C=C stretching**	
Free O–H	3640–3600	$-CH=CH_2$	1645
Bonded O–H (single-bridged dimer)	3600–3500	–CH=CH– (cis)	1660
		–CH=CH– (trans)	1675 weak
Bonded O–H (double-bridged polymer)	3400–3200	–CH=CH–CH=CH–	1650, 1600
Hydroperoxide (O–O–H)	3560–3530	**C–H bending**	
Acid (O–H)	3000–2500	$-CH_3$	1460 (δ_{as})
			1380 (δ_s)
C–H stretching		$-COOCH_3$	1440–1435
$=CH_2$	3080 (ν_{as})		1365–1356
	2975 (ν_s)	$-CH_2-$	1470
=CH–	3020	$-CH=C_2$	1420
$-CH_3$	2960 (ν_{as})	–CH=CH–	1415
	2870 (ν_s)		
$-CH_2-$	2925 (ν_{as})	**C–O stretching and C–O bending**	
	2850 (ν_s)	Ester	
–CHO	2820, 2720	–CO–O– two bands at	1300–1050
		R–CO–O–R	1190
C=O stretching		$R-CO-O-CH_3$	1165
Ester		–C=C–CO–O–	1300–1250
–CO–O–	1735		1200–1050
–C=C–CO–O–	1720		
–CO–O–C=C–	1760	Acid	
νC=C	1690–1650	–COOH	1420
			1300–1200
Acid			
Saturated		Carboxylate	
monomer	1760	$-COO^-$	1400
dimer	1710		
α,β-unsaturated		Alcohol	
monomer	1720	Free OH	1250 (δ)
dimer	1690	Associated OH	1500–1300 (δ)
		Primary OH	1050
Carboxylate		Secondary OH	1100
$\nu_{as}CO$	1610–1550	Tertiary OH	1150
ν_sCO	1400		
		C–H bending (out of plane)	
Aldehyde		–CH=CH– *(cis)*	730–675
–CHO	1725	–CH=CH– *(trans)*	968
α,β-unsaturated	1685		
α,β-γ,δ-unsaturated	1675	**Skeletal and "breathing"**	
		Epoxide	
Ketone		*trans*	916–880
–CO–	1715	*cis*	838–829
α,β-unsaturated	1675		
νC=C	1650–1600		
α,β-γ,δ-unsaturated	1665		

produces a characteristic absorption at 968 cm^{-1} that does not change for additional double bonds unless these are conjugated, when there are small changes from this value. Therefore, this absorption can be employed to estimate the percentage of *trans* of the total amount of double bonds (146,150,151). There is not a similar diagnostic infrared absorption band for *cis* unsaturation, but Raman spectra show strong absorption bands at 1665 ± 1 cm^{-1} (*cis*-olefin) and 1670 ± 1 cm^{-1} (*trans*-olefin).

Carbonyl compounds have a strong absorption band in the region 1650–1750 cm^{-1}. The wavelength varies slightly with the nature of the carbonyl compounds (Table 9.33) and this may be of diagnostic value. Thus, this band has been suggested for free acidity determination in oils (152) and it is usually employed for measurement of fat content in automatic analyzers (153). Other applications of infrared spectra are the determination of iodine value, saponification value, free acid, oxidative stability, and carotene content, among others (3,154–156). By using Fourier transform infrared spectrometers (FTIR), these parameters can be measured by simple menu-driven procedures in a few minutes. The results are not only obtained more quickly, but without recourse to solvents or laborious titrimetric methods.

Near infrared spectroscopy, covering the region 800–2500 nm, is also being employed in fat and oil analysis. Thus, methods to determine fatty acid composition, peroxide value, oil content of individual seeds, recognition of individual vegetable oils, oil oxidation and in-line measurement of tempered cocoa butter and chocolate, among others, have been developed (3,157–160).

E. Raman Spectroscopy

As commented above, Raman spectroscopy may be appropriate to use for the simultaneous analysis of the *cis* and *trans* content of oils, since *cis* and *trans* carbon-carbon double bond stretching bands can be observed simultaneously. By using this technique, the *cis*/*trans* isomer ratios were determined with a precision of 1% (161). The carbon-carbon double bond stretch and the methylene scissor intensity ratios have also been used to determine iodine values. In addition, the total unsaturation in oils and margarines can also be determined (162).

F. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is used in two ways in the study of fats and oils. With low-resolution instruments, it is possible to determine the proportion of solid and liquid in a fat and the content of oil in a seed. High-resolution spectrometers, on the other hand, are used to mainly examine solutions and give information about the solute.

1. Low-Resolution NMR

Differently to high-resolution NMR, broad band NMR does not distinguish among hydrogen nuclei in different atomic environments. Two techniques can be employed, namely wide-line NMR or pulsed NMR, and both distinguish between hydrogen atoms in liquid and solid environments. Nowadays, the most widely used technique is pulsed NMR. By using this technique, a measurement related to the total number of hydrogen nuclei is followed by a second measurement after 70 μs to determine only those hydrogen nuclei in a liquid environment. This determination depends on the fact that the signal for hydrogen nuclei in solid triacylglycerols decays much more quickly (less than 1% remains after 70 μs) than that of hydrogen nuclei in a liquid environment (which requires about 10000 s).

These measurements require only about 6 seconds and are used routinely for the study of margarine and other confectionery fats and of cocoa butter and similar substances. However, though the measurement is so quick, it may have

TABLE 9.34
Chemical Shift Assignments of Main ^1H NMR Signals of Fats and Oils[a]

Chemical Shift (ppm)	Assignment
	Glycerol and Unsaturated Protons
5.40–5.26	O9/O10/L9/L10/L12/L13/Ln9/Ln10/Ln12/Ln13/Ln15/Ln16
5.26–5.20	β-Glycerol
4.32–4.10	α-Glycerol
	Saturated Protons
2.75	Ln11/Ln14
2.72	L11
2.34	L2/Ln2
2.23	O2
2.22	S2
2.03	Ln17
2.00	L8/L14
1.99	Ln8
1.95	O8/O11
1.57	L3/Ln3
1.56	O3
1.55	S3
1.28	L chain/Ln chain
1.23	O chain
1.19	S chain
0.93	Ln18
0.84	L18
0.82	O18/P16/St18

[a] Assignments are abbreviated by fatty acid and carbon number. 1(3)- and 2-Positions of glycerol are designated by the Greek symbols α and β, respectively. Labeling of acyl chains: S, saturated; P, palmityl; St, stearyl; O, oleyl; L, linoleyl; Ln, linolenyl chain. Depending on the oil composition and experimental conditions, a lower number of signals is usually observed.

TABLE 9.35
Chemical Shift Assignments of Main ^{13}C NMR Signals of Fats and Oils[a]

Chemical Shift (ppm)	Assignment	Chemical Shift (ppm)	Assignment	Chemical Shift (ppm)	Assignment
Carbonyl Carbons			**Aliphatic Carbons**	29.22	L5β
176–174	Fatty acids	34.25	P2β/St2β	29.21	O5α/Ln5β
173.29	P1α	34.23	O2β	29.19	L5α/Ln5α
173.26	O1α	34.20	L2β/Ln2β	29.17	O6β/St4α
173.25	St1α	34.09	P2α/St2α	29.16	P4α
173.22	L1α/Ln1α	34.06	O2α	29.15	O6α/L6β
172.88	P1β	34.04	L2α/Ln2α	29.14	L6α/Ln6β
172.85	O1β/St1β	31.98	St16αβ	29.13	O4α/St4β/Ln6α
172.82	Ln1β	31.96	P14αβ	29.12	P4β
172.81	L1β	31.94	O16αβ	29.11	L4α
Olefinic Carbons		31.55	L16αβ	29.10	Ln4α
131.96	Ln16αβ	29.80	O12αβ	29.09	O4β
130.23	Ln9α	29.76	O7β/St11αβ/St12αβ/St13αβ/St14αβ	29.07	L4β
130.22	L13β	29.74	O7α/P11αβ/P12αβ/St10αβ	29.06	Ln4β
130.21	L13α/Ln9β	29.73	P10αβ	27.26	O11αβ
130.06	O10β	29.72	St9αβ/St8αβ	27.23	L14αβ/Ln8αβ
130.04	O10α	29.70	P8αβ/P9αβ/St7β	27.21	O8αβ/L8αβ
130.01	L9α	29.68	P7β/St7α	25.66	L11αβ
129.98	L9β	29.66	P7α	25.65	Ln11αβ
129.74	O9α	29.65	L7β	25.56	Ln14αβ
129.71	O9β	29.63	L7α	24.95	P3β/St3β
128.32	Ln12β	29.62	Ln7β	24.92	O3β
128.31	Ln12α	29.61	Ln7α	24.91	St3α
128.26	Ln13α	29.56	O14αβ/St5β	24.90	L3β/P3α/Ln3β
128.25	Ln13β	29.54	P5β	24.88	O3α
128.12	L10β	29.53	St5α	24.86	L3α/Ln3α
128.10	L10α	29.52	P5α	22.73	St17αβ
127.93	L12α	29.42	St15αβ	22.72	P15αβ
127.92	L12β	29.40	P13αβ	22.71	O17αβ
127.80	Ln10β	29.37	L15αβ	22.59	L17αβ
127.79	Ln10α	29.36	O13αβ/O15αβ	22.57	Ln17αβ
127.15	Ln15α	29.35	St6β	14.29	Ln18αβ
127.14	Ln15β	29.33	P6β	14.13	P16αβ/St18αβ
Glycerol Carbons		29.32	St6α	14.12	O18αβ
68.93	β-Glycerol	29.31	P6α	14.08	L18αβ
62.13	α-Glycerol	29.24	O5β		

[a] Assignments are abbreviated by fatty acid and carbon number. 1(3)- and 2-Positions of glycerol are designated by the Greek symbols α and β, respectively. Labeling of acyl chains: S, saturated; P, palmityl; St, stearyl; O, oleyl; L, linoleyl; Ln, linolenyl chain. Depending on the oil composition and experimental conditions, a lower number of signals is usually observed.

to be preceded by a lengthy tempering routine. Without controlling tempering the results would not be reproduced from day to day or between laboratories. The tempering regime varies with the kind of fat but a typical procedure for cocoa butter involves melting at 100°C then holding at 60°C (1 h), 0°C (1.5 h), 26°C (40 h), 0°C (1.5 h), and finally at the measuring temperature for 1 h. For many fats the long tempering at 26°C can be omitted (3).

For oilseed breeders, the NMR method is often used to estimate the oil content of oilseeds in a nondestructive manner. This information is of commercial value and can assist seed breeders and agronomists in their studies to develop improved varieties. Pulsed NMR is also employed in lipid crystallization studies (163).

2. High-Resolution ^1H NMR

The use of ^1H NMR in the study of oils, fats and food lipids has increased particularly because of the great amount of information that high field instruments can provide in a very short period of time (164). The NMR spectrum consists of a series of sharp signals whose frequencies and multiplicities can be related to the chemical nature of the different hydrogen atoms (methyl, methylene, olefin, etc.) and whose intensities are directly related to the number of hydrogens producing the signal (165,166). In this spectrum, all hydrogen atoms having the same chemical surroundings produce signals at the same frequency. The position of a resonance signal in the

spectrum is called the chemical shift (δ). In the ^{1}H NMR spectra of fats and oils the resonances appear between δ 4.10 and 5.40 ppm for glycerol and unsaturated protons, and saturated protons signals appear between δ 0.80 and 2.80 ppm. Table 9.34 collects the assignation of most common signals.

By using the information contained in the spectra, ^{1}H NMR has been employed, among others, to determine the iodine value, number of double bonds, average molecular weight, proportion of acyl groups in the triacylglycerol molecule, *n*-3 polyunsaturated fatty acid proportion, and docoxahexahenoic acid content (167–176). Some attempts to apply ^{1}H NMR spectroscopy to oil authenticity have also been carried out (177).

^{1}H NMR spectroscopy may also be employed to determine minor oil components, but the signals of these components should not overlap with those of the main components, their concentration be high enough to be detected, and high field equipment be employed. Thus, the determination of saturated and unsaturated aldehydes in virgin olive oils as well as diacylglycerols have been described (170,177,178).

3. High-Resolution ^{13}C NMR

High-resolution ^{13}C NMR spectra are more complex than ^{1}H spectra and they do not provide quantitative information so easily. Nevertheless, they contain much more structural information (chemical shifts and intensities) if this can be teased out of the data provided with each spectrum. ^{13}C NMR resonances of fats and oils can be grouped into four well-defined spectral regions: carbonyl carbons ranging from 173.3 to 172.8 ppm; unsaturated carbons ranging from 132.0 to 127.1 ppm; glycerol carbons ranging from 69.1 to 61.6 ppm; and aliphatic carbons ranging from 34.3 to 14.0 ppm. The assignation of the different signals has been the objective of many studies and it is nowadays clearly resolved (179–181). The main resonances observed in the ^{13}C NMR spectra of fats and oils are collected in Table 9.35.

Information contained in these spectra has been employed for edible oil authenticity determination and quality controls, including the analysis of fatty acid composition and distribution of fatty acids in the triacylglycerol molecule, the free fatty acid, iodine value, and diacylglycerol determination, the analysis of minor components, the oil stability prediction, the determination of polar components and oil colors, etc. (182–198). All these data suggest that with only one analysis, NMR allows the determination of a large number of components with very little or without any manipulation of the oil samples that nowadays need many different analyses. In addition, the application of multivariate statistics to NMR spectral data increases considerably the potential of the technique. However, and because minor components of the oils are playing an essential role in defining oil authenticity and quality, concentration of these compounds (either by using a chromatographic procedure or by the use of unsaponifiables) or their observation during routine analysis by using special probes seem to be a necessary requisite to achieve a routine application of NMR to most aspects of oil analysis.

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I. INTRODUCTION

The water-soluble vitamins consist of a mixed group of chemical compounds. Their classification into specific chemical groups depends on both chemical characteristics and functions. The letter designations (vitamins B_1, B_2, B_3, etc., C) represent in part remnants from the past as the discovery of given dietary growth or curative factors were given letter designations.

Vitamins are novel in their roles as "external" or dietary regulatory agents. They have largely evolved to serve: 1) specific cofactor and/or co-substrate functions, 2) as regulatory agents, or 3) as antioxidants. All of the vitamins undergo specific and metabolically controlled modifications before activation or conversion into their functional forms. The most limiting events that control function are often a specific step(s) in cofactor formation, e.g., a phosphorylation reaction or ATP addition.

The use of broad functional categories as headings to organize this chapter was chosen. For example, niacin, riboflavin, and ascorbic acid serve primarily as redox cofactors. The roles of thiamin, pyridoxine (vitamin B-6) and pantothenic acid (as a component of coenzyme A) are distinguished because of their importance to carbohydrate, protein and amino acid, and acyl and acetyl transport, respectively. Folic acid, vitamin B-12 (cobalamin), and biotin will be discussed in relationship to their roles in single-carbon or CO_2 transfer reactions.

II. VITAMINS IMPORTANT IN REDOX REACTIONS

A. Ascorbic Acid (Vitamin C)

Ascorbic acid functions primarily as a cofactor for microsomal mono-and dioxygenases (hydroxylases) and oxidases. In most animals, ascorbic acid is synthesized from glucose in the liver. In birds and reptiles, ascorbic acid synthesis takes place primarily in the kidney. In animals that require ascorbic acid, e.g., humans, it is a deficiency of gulonolactone oxidase, the last step in ascorbic acid synthesis, which results in the need for a dietary source (Figure 10.1; 12).

1. Chemistry

Ascorbic acid (2,3-enedial-glulonic acid) is a powerful cellular reducing agent and is of general importance as an antioxidant because of its high reducing potential. Both of the hydrogens of the enediol group dissociate, which result in the acidity of ascorbic acid ($pK_1 = 4.2$). When ascorbic acid plays a role in reductions, the reaction usually occurs in a stepwise fashion with monodehydroascorbic acid, as a semiquinone intermediate. This intermediate then disproportionates to ascorbic acid, and dehydroascorbic acid (Figure 10.1).

2. Absorption, Tissue Distribution, and Metabolic Functions

Dietary ascorbic acid is absorbed from the duodenum and proximal jejunum. Measurable, albeit small, amounts of ascorbic acid also cross the membranes of the mouth and gastric mucosa. Although some controversy exists regarding the relationship between dietary intake and the intestinal absorption of ascorbic acid, studies indicate that within the physiological ranges of intake (40–200 milligrams per day for humans or ~5–25 mg/kJ of diet); 80–90% of the vitamin is absorbed. Uptake at apical membranes involves a specialized Na^+-dependent, carrier-mediated system. Exit of ascorbic acid from enterocytes utilizes a Na^+-dependent carrier system. To date, two Na^+-dependent L-ascorbic acid transporters have been cloned and partially characterized. The preference for these transporters is for L-ascorbic acid. Dehydroascorbic acid uptake is facilitated by hexose transporters (3, 12).

In tissues, the highest concentration of ascorbic acid is found in the adrenal and pituitary glands followed by the liver, thymus, brain, and pancreas. In diabetic animals, the ascorbic acid content of tissue is often depressed, which may be due to competition for uptake between dehydro forms of ascorbic acid and glucose (3).

Ascorbic acid is maintained in cells by several mechanisms. Ascorbic acid reductases maintain L-ascorbic acid in the reduced form, which is less susceptible to easy diffusion. Within most cells, measurable amounts of ascorbic acid are also maintained as the 2-sulfate derivative. The ability to maintain ascorbic acid in the reduced state and as ascorbic acid-2-sulfate appear important in maintaining cellular ascorbic acid levels (12).

In the neonate, glutathione is also very important to ascorbic acid recycling. In this regard, an argument can be made for a dietary need for ascorbic acid in the neonates of some animal species in which the adult does not have a dietary requirement. For example, the levels of glutathione are relatively low in neonate rat and mouse tissues. As ascorbic acid is oxidized to dehydroascorbic acid, there is the need for conservation, because dehydroascorbic acid is easily degraded. Glutathione in its

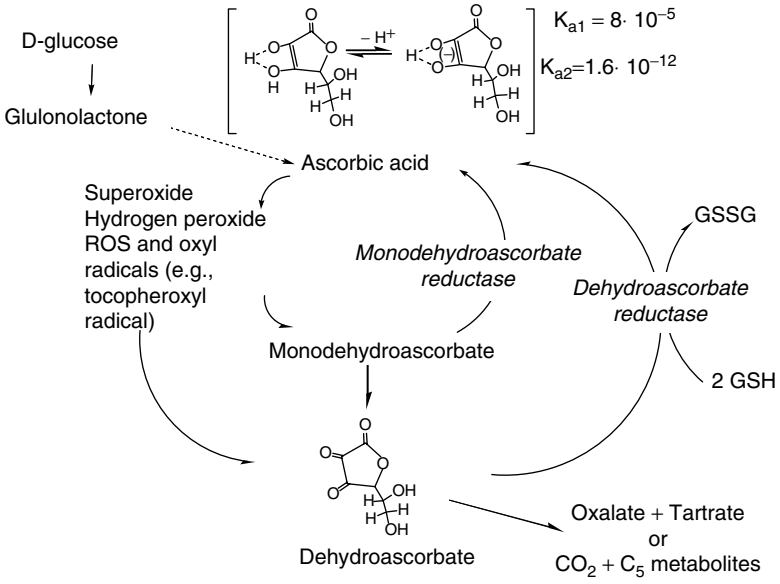

FIGURE 10.1 Ascorbic acid. In most animals ascorbic acid is derived from glucose. Gulonolactone is produced from glucose and its oxidation to ascorbic acid is catalyzed by gulonolactone oxidase (dashed line). The K_as for the two dissociable protons in ascorbic acid are indicated. At pH 7, dehydroascorbic acid can be reduced to ascorbic acid with a $Eo' = 0.08$, e.g., dehydroascorbate $+ 2e^- + 2H^+ \leftrightarrow$ ascorbate. In a biological system, reduction of the monodehydroascorbic acid to ascorbic acid occurs by the NADH-requiring enzyme, monodehydroascorbic acid reductase. Reduction of dehydroascorbic acid utilizes reduced glutathione and is catalyzed dehydroascorbic acid reductase, also known as glutaredoxin.

reduced form serves as the reducing substrate for glutaredoxin, an enzyme that can maintain ascorbic acid in a reduced state (3, 12).

As a cellular reducing agent, ascorbic acid plays a number of very important roles. It serves as a cofactor for mixed-function oxidations that result in the incorporation of molecular oxygen into various substrates. Examples include the hydroxylation of proline in collagen, elastin, C1q complement, and acetylcholine esterase. Some of the P-450-dependent hydroxylases that carry out the hydroxylation of steroids, drugs, and other xenobiotics also have a need for ascorbic acid (12).

Ascorbic acid-dependent hydroxylation steps also occur in the biosynthesis of carnitine and the hydroxylation of tyrosine in the formation of catecholamines. Most of the enzymes involved in these processes are metal-requiring enzymes, in which the role of ascorbic acid is to maintain the metal (usually Cu or Fe) in a reduced state.

3. Requirement, Deficiency, and Toxicity

Most animals with the exception of primates, guinea pigs, some snakes, fruit-eating bats, birds such as passerines, and salmonid fish synthesize ascorbate (Figure 10.1). Impaired collagen synthesis is a principle feature of ascorbic acid deficiency, which is evidenced in capillary fragility, bleeding gums, delayed wound healing, and impaired bone formation. Connective tissue lesions are primarily a result of under-hydroxylated collagen (at specific prolyl and lysyl residues). This can result in abnormal collagen degradation and, as a consequence, decreased collagen production. In addition, the inability to deal with metabolic stress requiring normal adrenal gland function and abnormal fatty acid metabolism (carnitine synthesis) can be signs of scurvy.

The current requirement for humans is 75 mg per day (U.S. RDA for the healthy adults) or 30–60 mg per 1000 kcal or 4 MJ of diet (4; Table 10.1). Because of the mechanisms in place to homeostatically regulate ascorbic acid, evidence of toxicity, other than gastric upset, is seldom reported (e.g., diarrhea, gastric irritation). Evidence of toxicity, however, may be manifest, when ascorbic acid is consumed in gram quantities per day. Of interest, ascorbic acid intake in this range can result in decreased histamine production and facilitate reduction of nitrosamines and other putative cancer promoting agents with similar chemical characteristics. The interaction with nitrosamines occurs primarily in the stomach, and is chemical in nature owing to ascorbic acid's potential to function as an excellent reducing agent. The anti-histamine effects from pharmacologic exposure of ascorbic acid are in part the basis for its use as an analgesic in the treatment of "common colds."

When fed in excess of metabolic need, tissue levels of ascorbic acid are homeostatically maintained. Homeostasis occurs by the induction of ascorbic acid decarboxylases, which initiates enhanced degradation of ascorbate to CO_2 and C-4 or C-5 fragments (12).

TABLE 10.1
The Daily Reference Intakes for the Water-Soluble Vitamins (Part 1)[a]

Vitamin	Age Condition	EAR[b]	RDA[c]	AI[d]	UL[e]	Vitamin	Age Condition	EAR	RDA	AI	UL
Ascorbic Acid	Years	mg/day	mg/day	mg/day	mg/day	Niacin	Years	mg/day	mg/day	mg/day	mg/day
	0–0.5	–	–	40	–		0–0.5	–	–	2.	–
	0.5–1	–	–	50	–		0.5–1	–	–	4.	–
	1–3	13	15	–	400		1–3	5	6	–	10
	4–8	22	25	–	650		4–8	6	8	–	15
	9–13	39	45	–	1200		9–13	9	12	–	20
	14–18	63	75	–	1800		14–18	12	14 (M), 16 (F)	–	30
	19–>70	75	90	–	2000		19–>70	11	14 (M), 16 (F)	–	35
	19–30	70	85	–	2000		19–30	13	17	–	35
	Pregnancy						Pregnancy				
	19–30	100	120	–	2000		19–30	13	17	–	35
	Lactation						Lactation				
Riboflavin	Years	mg/day	mg/day	mg/day	mg/day	Thiamin	Years	mg/day	mg/day	mg/day	mg/day
	0–0.5	–	–	0.3	–		0–0.5	–	–	0.2	
	0.5–1	–	–	0.4	–		0.5–1	–	–	0.3	
	1–3	0.4	0.5	–	–		1–3	0.4	0.5	–	
	4–8	0.5	0.6	–	–		4–8	0.5	0.6	–	
	9–13	0.8	0.9	–	–		9–13	0.7	0.9	–	
	14–>70	0.9 (M), 1.1 (F)	1.1 (M), 1.3 (F)	–	–		14–>70	0.9 (M), 1.0 (F)	1.1 (M), 1.2 (F)	–	
	19–30	1.2	1.4	–	–		19–30	1.2	1.4	–	
	Pregnancy						Pregnancy				
	19–30	1.3	1.6	–	–		19–30	1.2	1.5	–	
	Lactation						Lactation				
Pantothenic Acid	Years	mg/day	mg/day	mg/day	mg/day	**Vitamin**	Years	mg/day	mg/day	mg/day	mg/day
	0–0.5	–	–	1.7	–	B-6	0–0.5	0–0.5	–	–	0.1
	0.5–1	–	–	1.8	–		0.5–1	0.5–1	–	–	0.3
	1–3	–	–	2	–		1–3	0.4	0.5	–	30
	4–8	–	–	3	–		4–8	0.5	0.6	–	40
	9–13	–	–	4	–		9–13	0.8	1.0	–	60
	14–>70	–	–	5	–		14–>70	1.1	1.3	–	80
	19–30	–	–	6	–		19–30	1.1	1.3	–	100
	Pregnancy						Pregnancy				
	19–30	–	–	7	–		19–30	1.1	1.3	–	100
	Lactation						Lactation				

The Daily Reference Intakes for the Water-Soluble Vitamins (Part 2)[a]

Biotin	Years	µg/day	µg/day	µg/day	µg/day	Folic Acid	Years	µg/day	µg/day	µg/day	µg/day
	0–0.5	–	–	5.0	–		0–0.5	–	–	65	–
	0.5–1	–	–	6.0	–		0.5–1	–	–	80	–
	1–3	–	–	8.0	–		1–3	120	150	–	300
	4–8	–	–	12	–		4–8	160	200	–	400
	9–13	–	–	20	–		9–13	320	400	–	1000
	14–>70	–	–	25	–		14–>70	320	400	–	1000
	19–30	–	–	30	–		19–30	520	600	–	1000
	Pregnancy						Pregnancy				
	19–30	–	–	30	–		19–30	520	600	–	1000
	Lactation						Lactation				

(*Continued*)

TABLE 10.1 (*Continued*)

Vitamin	Age Condition	EAR[b]	RDA[c]	AI[d]	UL[e]
Vitamin	Years	µg/day	µg/day	µg/day	µg/day
B-12	0–0.5	–	–	0.4	–
	0.5–1	–	–	0.5	–
	1–3	0.7	0.9		–
	4–8	1	1.2	–	–
	9–13	1.5	1.8	–	–
	14–>70	2	2.4	–	–
	19–30	2.2	2.6		–
	Pregnancy				
	19–30	2.2	2.6	-	–
	Lactation				

[a] Dietary Reference Intakes (DRI) — Reference values that are quantitative estimates of nutrient intakes to be used for planning and assessing diets for apparently healthy people. There are 4 reference values: the EARs, RDAs, AIs, and UIs.

[b] Estimated Average Requirement (EAR) — The nutrient intake value that is estimated to meet the requirement of half of the healthy individuals in a group. The EAR is used to assess the adequacy of dietary intakes within a population and it is used to develop the RDAs.

[c] Recommended Dietary Allowances (RDA) — The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a designated group.

[d] Adequate Intake (AI) — AI is used when the RDA for a nutrient is not available. It is a recommended daily intake that is based on observed or experimentally determined approximations of nutrient intake by a group of healthy people. An AI is developed when there is no EAR or research to help develop the RDA for a nutrient.

[e] Tolerable Upper Intake Level (UL) — Highest level of daily nutrient intake that is likely to pose no risks of adverse health effects to almost all individuals in the general population.

4. Food Sources and Stability

Ascorbic acid is widely distributed in fruits and vegetables, with foods such as citrus fruits, berries, kiwi, peppers, Brussels sprouts, and broccoli being particularly high in this vitamin. Ascorbic acid is relatively unstable and easily destroyed during processing and storage of foods. It is labile to neutral and alkaline conditions, heat, light, and exposure to oxygen. The rate of decomposition is accelerated by the presence of metals, especially iron and copper, and by enzymes such as peroxidases. Food preservation techniques that act to limit losses of ascorbic acid are blanching of vegetables, pasteurization, and deaereation of juices to limit oxygen and inactivate the enzymes. Additionally, packaging in containers that limit exposure to light and oxygen will maintain ascorbate concentrations. Erythorbic acid, a biologically inactive analog of ascorbic acid, is often added to foods as an antioxidant to preserve freshness. A summary of chemical characteristics for ascorbic acid and other B-vitamins is given in Table 10.2 (4, 13).

III. THE B VITAMINS

A. Niacin

Throughout the 18th and 19th centuries, the disease pellagra was prevalent in Western Europe and the southern region of the United States. This disease would eventually became associated with the vitamin, niacin, and the consumption of corn (maize), as a principle source of dietary energy. The nutritional availability of niacin in corn is relatively low bioavailability. Corn also has a low content of tryptophan. As shown in Figure 10.2, niacin can be generated upon tryptophan degradation.

Normally, niacin is derived from foods, such as corn, by hydrolysis of NAD (nicotinamide adenosyl dinucleotide) and NADP (nicotinamide adenosyl phosphodinucleotide) by the action of pancreatic or intestinal nucleosides and phosphatases. As most human diets now contain adequate tryptophan, and available NAD and NADP, niacin deficiency is seldom observed (6).

1. Chemistry

NAD and its phosphorylated form, NADP, are two coenzymes derived from niacin. Both contain an unsubstituted pyridine 3-carboxamide that is essential to function in redox reactions with a chemical potential near –0.32 V. Virtually all cells are capable of converting niacin to NAD. Most enzymes that require NAD are oxidoreductases (dehydrogenases).

NAD catalyzes a diverse array of reactions, such as the conversion of alcohols and polyols to aldehydes or ketones. The most common mechanisms involve the sterospecific abstraction of a hydride ion [H: (-)] from the substrate with its subsequent transfer. It is of interest that cells generally delegate NAD to enzymes in catabolic

TABLE 10.2
Stability of Water-Soluble Vitamins

	Effect of pH					
Vitamin	**Acid**	**Neutral**	**Alkaline**	**Oxygen**	**UV Light**	**Heat**
Ascorbic Acid	S	U	U	U	U	U
Biotin	S	S	U	S	S	S
Cobalamin (B-12)	S	S	S	U	U	S
Folic acid	S	U	U	U	U	U
Niacin	S	S	S	S	S	S
Pantothenic acid	U	S	U	S	S	U
Pyridoxine (B-6)	S	S	S	S	U	U
Riboflavin (B-2)	S	S	U	S	U	S
Thiamin (B-1)	S	S	U	U	S	U

S=Stable, U=Unstable.

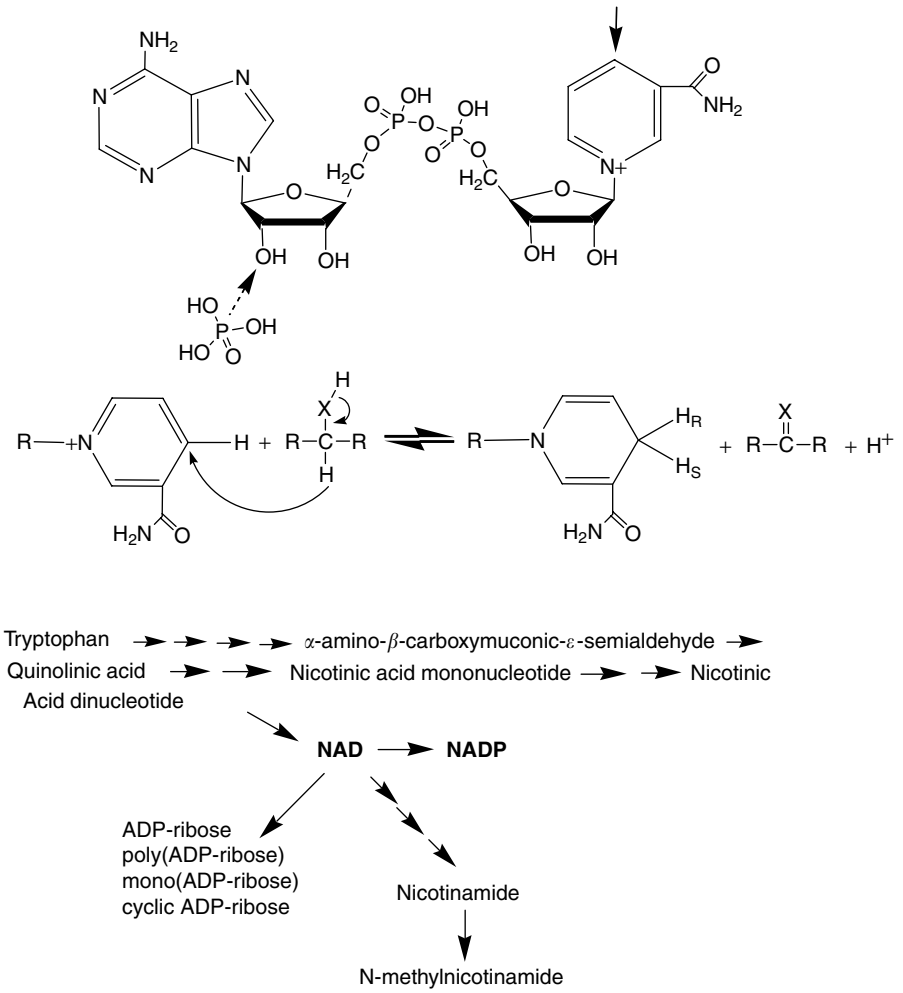

FIGURE 10.2 NAD and NADP. The structures for NAD and NADP (via ATP-derived phosphate addition to NAD, cf. dashed arrow) and their site of hydrogen transfer (solid arrow) are indicated along with a mechanism of hydrogen transfer. The HR and HS designations are shown to indicate that the transfer occurs with stereospecificity. NAD and NADP are products of tryptophan degradation through the quinolate portion of the tryptophan degradation pathway. In addition to acting as a reductant, NAD is precursor to ribosylations and cyclic ribosylated products that are important to cellular regulation.

pathways, whereas NADP is utilized in synthetic pathways. An additional and equally important function of NAD is its role as a substrate in poly- and monoribosylation reactions. Mono and polyribosylation post-translational chemical modifications are important in many cellular regulatory functions (see next section).

2. Absorption, Tissue Distribution, and Metabolism

Transporters for niacin uptake have been identified, but are not as well characterized as some of the other vitamin transporters. As noted, NAD may be derived from tryptophan through the so-called quinolinate pathway (Figure 10.1). About half the niacin as NAD or NADP is associated with enzymes; the remainder is available as a substrate for mono- and polyribosylation reactions that are important in the regulation of a broad range of enzymes. In the nuclei of cells, polyribosylation of specific histones precedes the normal process of DNA repair. When niacin is in excess, most mammals convert it to N-methylnicotinamide, which has a low renal threshold and is excreted (6).

3. Requirements, Toxicity, and Pharmacology

Niacin is needed in amounts corresponding to 14 to 16 mg per day or 4–6 mg per 1000 kcal or 4 MJ of diet (4; Table 10.1). Niacin requirements are often expressed as equivalents, where one equivalent corresponds to 1 mg of niacin. The conversion of tryptophan to niacin produces about 1 mg or equivalent of niacin for every 60 mg of tryptophan degraded (Figure 10.2).

There are a number of therapeutic uses for pharmacologic doses of niacin-derived compounds, when increased blood flow is desirable. Nicotinic acid can cause vasodilatation when consumed in amounts of 100 mg or more. Niacin in gram quantities is an effective lipid-lowering agent (increases HDL). However, in some individuals, flushing, gastric irritation, and other similar side effects caused by pharmacologic doses of nicotinic acid or niacin may preclude use (6).

4. Food Sources and Stability

Niacin is found in high levels in animal tissues (chicken, fish, beef – especially liver), and peanuts, and in moderate amounts in whole grains such as wheat and barley, enriched cereal products, mushrooms, and some vegetables such as corn and peas. Niacin is very stable in a variety of conditions. Indeed some treatments such as moderate heat and alkali treatment of corn can result in greater niacin availability (Table 10.2). An excess of water used in cooking and processing of foods can result in leaching of the vitamin, as is similar for all water-soluble vitamins (4).

B. Riboflavin

Riboflavin was one of the first of the B vitamins identified (10). Originally, it was thought to be the heat stable factor responsible for the prevention of pellagra. Riboflavin is present in tissue and cells as FAD (flavin adenine dinucleotide) and FMN (flavin adenine mononucleotide). FAD and FMN are cofactors in aerobic processes, usually as cofactors for oxidases, although FAD can also function in anaerobic environments as a dehydrogenase cofactor.

1. Chemistry

Flavins, such as riboflavin derivatives (Figure 10.3), are ideally suited to catalyze one electron, one-proton transfer reactions. Oxygen is often utilized as a co-substrate in the redox reactions catalyzed by FAD- and FMN-containing enzymes. Carbon-carbon double bond formation and redox of sulfur-containing compounds, e.g., lipoic acid, are catalyzed also by flavoproteins. $NADP(H) + H^+$ commonly serves as a reductant in processes to regenerate FAD and FMN. Riboflavin has a number of designations, some of which are still in use (vitamin B-2, vitamin G, ovoflavin, uroflavin, lactoflavin, heptoflavin).

Riboflavin was separated and identified from other B vitamins, because it is heat stable, but light instable and fluorescent. The chemical name for riboflavin is 6,7-dimethyl-9-(d-1′-ribityl) isoalloxazine. Deviations in structure can markedly affect activity (10). For example, the polyol side-chain must be in the D-form and substitution in other positions causes loss of activity or gives rise to compounds that have inhibitory activity, e.g., 5,6-dimethyl (isoriboflavin).

2. Absorption, Tissue Distribution, and Metabolism

Like most water-soluble vitamins, riboflavin is absorbed in the proximal part of the small intestine by a carrier-mediated process. Riboflavin is not particularly soluble. There are upper limits to riboflavin's availability, which are dictated in part by solubility. In this regard, an important chemical characteristic that is attributed to the ribose moiety is improved water solubility. Isoalloxazines are not particularly water-soluble.

Intestinal riboflavin uptake is adaptively regulated by dietary levels, and by specific intracellular protein kinase-mediated regulatory pathways. Over-supplementation with pharmacological amounts of riboflavin can lead to a significant and specific down-regulation in riboflavin uptake; riboflavin deficiency causes a significant and specific up-regulation in intestinal riboflavin uptake. Factors that have been shown to interfere with the normal intestinal riboflavin uptake process include alcohol and certain tricyclic drugs (e.g., chlorpromazine).

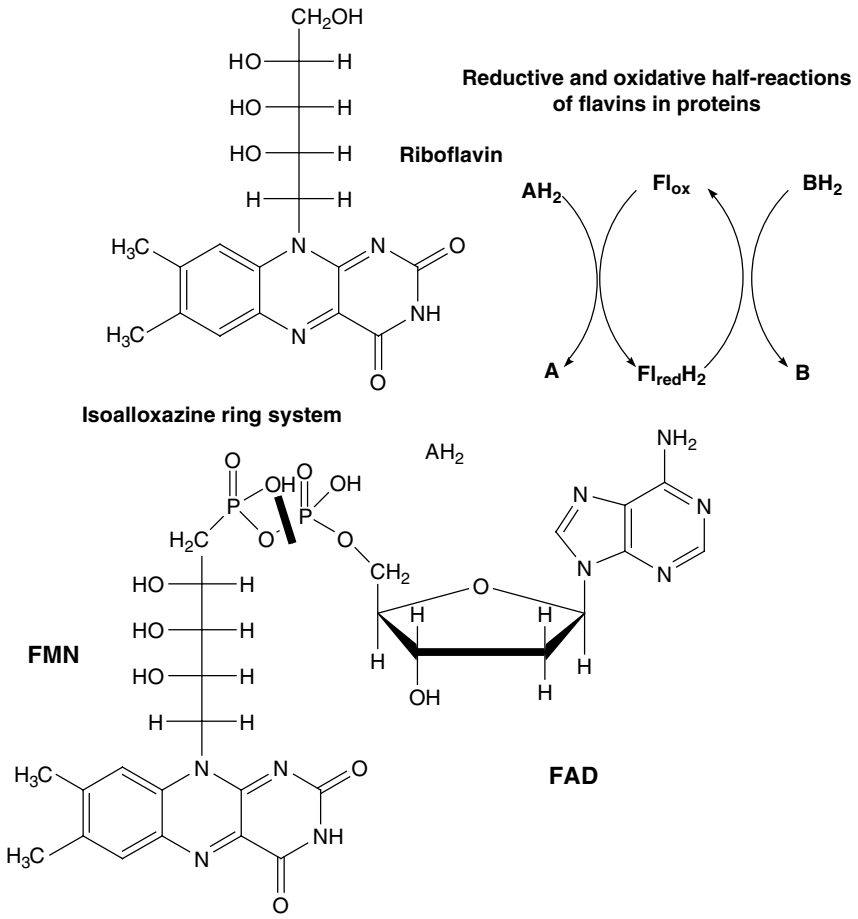

FIGURE 10.3 Riboflavin, FMN, and FAD. When associated with flavoproteins, the reductive and oxidative half-reactions for FMN and FAD occur wherein the nitrogens at positions 1 and 5 are involved. Redox of the isoalloxazine ring involves the following transition: $-N^1H-C=C-N^5H- \leftrightarrow -N^1=C-C=N^5-$.

Transport to tissues can be perturbed by boric acid and certain drugs, e.g., theophylline and penicillin, which displace riboflavin (or FMN) from known binding proteins (e.g., albumin > globulins > fibrinogen).

Once in cells, riboflavin is phosphorylated to FMN. In most cells about 20% of the riboflavin is present as FMN and of the remainder, over 70% is present as FAD with 1 or 2% as free riboflavin. Urine is the major route of excretion for riboflavin, although some FMN is excreted in bile (10 and refs. cited therein).

3. Metabolism and Requirements

The requirements for riboflavin, 1.1 to 1.3 mg/day, are lower than those for niacin or ascorbic acid because riboflavin is tightly associated with the enzymes that serve as a cofactor, and accordingly turns over much slower than niacin or ascorbic acid (Table 10.1; 4). In some cases, FMN is covalently bound (e.g., as in succinic dehydrogenase). Riboflavin is not toxic, primarily because of its regulated uptake at the intestinal level, and its relatively low solubility compared to other B-vitamins.

In humans, signs of riboflavin deficiency include lesions of the oral cavity, the lips, and the angle of the mouth (cheilosis), inflammation of the tongue (glossitis) and accompanying seborrhic dermatitis. Like other severe B-vitamin deficiencies, the filiform papillae of the tongue may also be lost with changes in color from pink to magenta. Anemia and increased vascularization of the eye are present in some animals with riboflavin deficiency.

4. Food Sources and Stability

The best sources of riboflavin are milk and dairy products. Other good sources are meats, especially liver, eggs, dark green leafy vegetables, and fortified breakfast cereals (4). Today, milk and breakfast cereals make the greatest contributions to riboflavin intake in the U.S. and the U.K. Riboflavin is stable to acid pH, heat, and oxygen, but is very susceptible to destruction by light, resulting in the inactive compounds lumiflavin and lumichrome (Table 10.2; 13). These compounds and other oxidative products can contribute to off-flavors due to oxidative

damage to milk lipids. Milk exposed to visible spectrum light will lose 50% of its riboflavin content after two hours, thus the importance of packaging milk in opaque containers.

IV. B-VITAMINS IMPORTANT TO SPECIFIC FEATURES OF CARBOHYDRATE, PROTEIN, OR LIPID METABOLISM

A. Thiamin

Studies on thiamin played an important role in the development of early concepts on the function and importance of vitamins. For example, the demonstration by the Dutch medical officer, Christian Eijkman, that polyneuritis could be produced in an experiment animal by dietary manipulation was conceptually very important. Eijkman and his colleagues fed a diet of polished rice, presumably low in thiamin, to chickens (one of the first uses of an animal model to study disease) and observed characteristic features of beriberi, such as head retraction, a common neurological sign in animals. The focus on rice and the observation that there appeared to be a curative principle in rice bran led to the eventual isolation of thiamin. This sequence of events and experimental protocols provided the underpinnings that led to the discovery of vitamins as precursors to cofactors (19).

1. Chemistry

The five-member (thiazole) ring of thiamin contains an arrangement of atoms (-N=CH-S-) called an *ylid*. The central carbon has carbanion character that acts as an electron-rich center for reactions that are commonly characterized as decarboxylations and transketolations (Figure 10.4). Thiamin is not stable to heat or alkali.

2. Absorption, Tissue Distribution, and Metabolism

Thiamin occurs in the human body as free thiamin and its mono-, di-, and triphosphorylated forms: thiamin monophosphate, thiamin pyrophosphate (TPP), which is designated as thiamin diphosphate, and thiamin triphosphate (TTP), which is found mostly in neural tissue. TPP and TTP are the cofactor forms of thiamine.

Thiamin is essential to the utilization of carbohydrates as energy sources, because of roles in TCA cycle regulation, and perturbations in the pentose phosphate – related carbohydrates pathways. In the TCA cycle, the principle enzymes are pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. These enzymes catalyze the decarboxylations of pyruvate and α-ketoglutarate. TPP is also a cofactor for transketolase reactions. Transketolation is a central activity in the metabolic sequence known as the pentose phosphate pathway. One of the most important intermediates of this pathway is ribose-5-phosphate, a phosphorylated 5-carbon sugar, required for the synthesis of the high-energy ribonucleotides, ATP, GTP, the nucleic acids, DNA and RNA, and the niacin-containing coenzyme NADPH, which is essential for a number of biosynthetic reactions. A deficiency of thiamin can lead to decreased production of NADPH, which impacts synthetic processes, such as fatty acid biosynthesis. In addition, it is evident from the neurological disorders caused by thiamine deficiency that thiamin plays a vital role in nerve function. In the brain, TTP is proposed to function in ion transport. The concentration of thiamin in the brain is resistant to changes in dietary concentration. Stimulation of nerves results in the release of thiamine monophosphate and free thiamine with accompanying decrease of cellular thiamine pyrophosphate and thiamine triphosphate and changes in Na^+ and K^+ gradients (19).

Human thiamin deficiency, beriberi, is termed dry, wet, or cerebral, depending on the systems that involved. The main feature of dry (paralytic or nervous) beriberi is peripheral neuropathy, including abnormal (exaggerated) reflexes, diminished sensation, and weakness in the legs and arms. Muscle pain and tenderness are also features. When cardiovascular manifestations occur, the term wet beriberi is used. Signs include a rapid heart rate, cardiac enlargement, and edema. Ultimately, congestive heart failure may be the cause of death. Cerebral beriberi can lead to Wernicke's encephalopathy and Korsakoff's psychosis. Signs include abnormal eye movements, stance and gait abnormalities, and abnormalities in mental function, e.g., confusion (19).

3. Requirements, Pharmacology, and Toxicity

The requirement for thiamin is 1.1 to 1.2 mg per day or 0.5 mg per 1000 kcal or 4 MJ of diet (4; Table 10.1). Factors that influence requirements are inadequate intake, strenuous physical exertion, fever, pregnancy, breastfeeding, and adolescent growth, or exposure to antagonists, such as tannins, alcohol (sufficient to cause inflammatory bowel conditions), and thiaminases, enzymes in many foods, particular mollusks, and the muscle of fish. Individuals who habitually eat raw freshwater fish, raw shellfish, and ferns are at higher risk of thiamin deficiency because these foods contain a thiaminase. For example, an acute neurological syndrome (seasonal ataxia) in Nigeria has even been associated with thiamin deficiency precipitated by a thiaminase in African silkworms, a traditional high-protein food for some Nigerians.

Antagonists include pyrithiamine and oxythiamine, which act to inhibit the phosphorylation of thiamin. Amprolium (coccidiostat) inhibits thiamin absorption.

4. Food Sources and Stability

Thiamin is present in most animal and plant foods, but is especially prominent in whole grains, organ meats, pork,

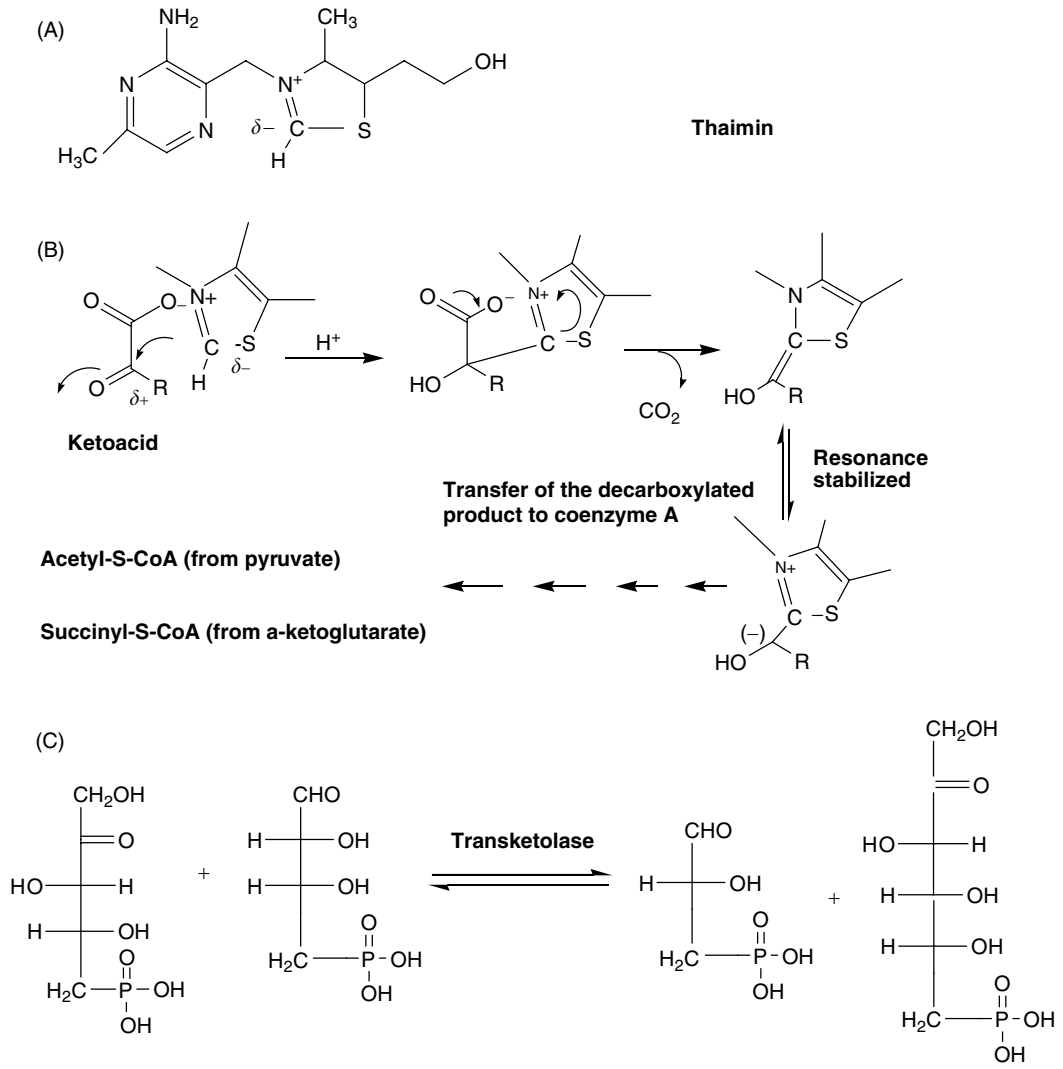

FIGURE 10.4 Thiamin. The carbanion character of thiamin **[A]** facilitates the non-oxidative and oxidative decarboxylation of α-ketoacids **[B]** and transketolase reactions **[C]**.

eggs, nuts, and legumes. Many food cooking and processing conditions will negatively affect thiamin content, as it is unstable in neutral and alkaline conditions, and with heat, oxygen, and sulfur exposure (Table 10.2). Milling of whole grains will remove the bran where thiamin and other B vitamins are in greatest concentrations; however, the current practice of fortification has restored thiamin levels in flours and grain-based products (13).

B. Pantothenic Acid

Roger William discovered pantothenic acid in 1933, and observed that it was an essential growth factor for yeast and lactic acid bacteria. Over the next 15 years, pantothenic acid was shown to be essential in animals. Pantothenic acid serves as a component of coenzyme A (Figure 10.5) and as a part of phosphopantotheine, e.g., at the acyl carrier site in acyl carrier protein (ACP) (18). Recently, pantothenic acid has been shown to be present in a number of other enzymes where thioesters facilitated transfer reactions are essential (5).

1. Absorption, Tissue Distribution, and Metabolism

Both Coenzyme A (CoASH) and ACP are present in foods. Consequently, absorbed pantothenic acid must first be released from CoASH and ACP, steps that involve the actions of peptidases and nucleosidases. The mechanism of absorption of pantothenic acid in the small intestine involves the same carrier-mediated Na^+-dependent system that transports biotin (15). Similarly, colonic absorption of pantothenic acid was found to involve the biotin-Na^+-dependent, carrier-mediated system. The interaction between biotin and pantothenic acid transport has also been described in other tissues such as the blood brain barrier, the heart, and the placenta, but the importance of this interaction is not understood.

2. Metabolic Functions and Requirements

CoASH is the principle moiety for the vectoral transport of acyl and acetyl groups in synthetic and catabolic reactions. A deficiency is characterized by impaired acetyl and acyl metabolism. The ability to utilize fatty acids as fuels is clearly compromised in experimental pantothenic acid deficiency. There is also an increased production of short chain fatty acids and ketone bodies, which can lead to severe metabolic acidosis. Dermal lesions occur because of impaired fatty acid metabolism. Selected aspects of pantothenic acid utilization and CoASH regulation are given in Figure 10.5.

The requirement for pantothenic acid is about 5 mg per day or 2 mg per 1000 kcal (~4 MJ) of diet. Even at gram intakes, pantothenic acid does not appear to be toxic (4).

3. Food Sources and Stability

Good dietary sources of pantothenic acid are meats, organ meats, eggs, nuts and legumes, cereals and some vegetables such as broccoli. The vitamin is stable in neutral pH, but unstable outside of this range, and also susceptible to degradation by heat treatment.

C. Pyridoxine

Pyridoxine or vitamin B-6 is a collective term for pyridoxine, pyridoxal, and pyridoxyl amine. Pyridoxine is most abundant in plants; pyridoxal and pyridoxyl amine are most abundant in animal tissues. The active form of vitamin B-6 is phosphorylated at the 5 position, e.g., pyridoxal-5-phosphate (7).

1. Chemistry

The most common of the reactions catalyzed by vitamin B-6 containing enzymes is the transaminase reaction. Transaminations are essential to the interconverson of amino acids to corresponding α-keto acids. The mechanism is also useful for reactions important to producing racemic amino acid mixtures (e.g., the conversion of L-alanine to D-alanine) and α, β–addition or elimination reactions. An example of an α, β–elimination reaction is the conversion of serine to pyruvic acid or the conversion of homocysteine plus serine to cystathionine. The basic feature of the transamination mechanism is electron withdrawal from the α-carbon resulting in a proton liberation, which sets the stage for subsequent substitution and additional reactions (Figure 10.6; 7).

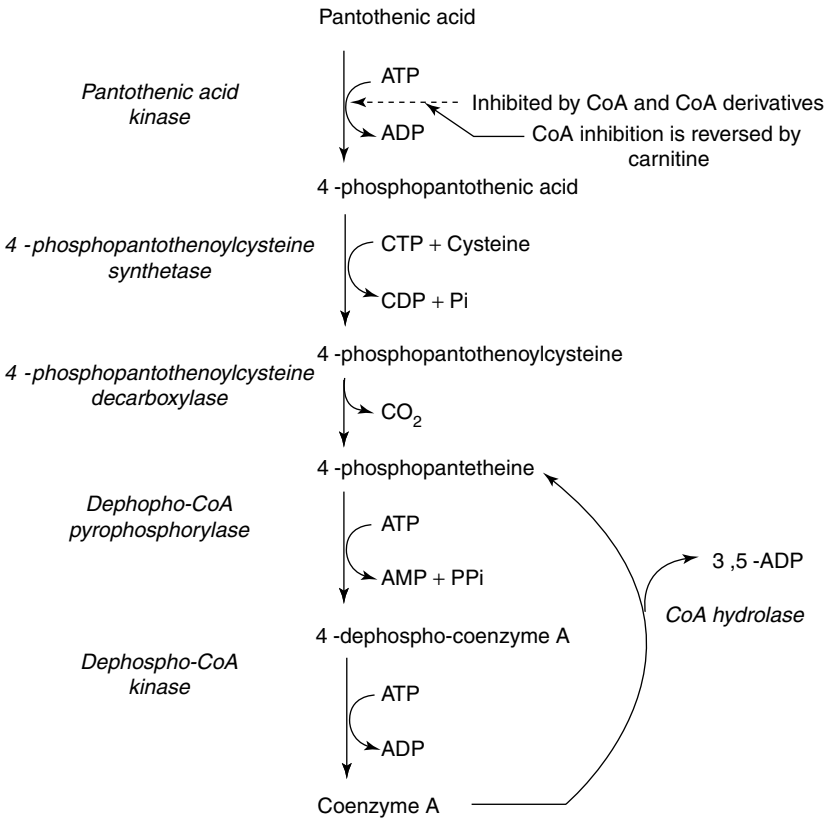

FIGURE 10.5 Synthesis of Coenzyme A (CoASH) from pantothenic acid. The synthesis of CoASH from pantothenic acid is regulated in part by feedback inhibition, e.g., by CoA derivatives at the step catalyzed by pantothenic acid kinase. Carnitine also plays a role in regulation.

The second most common reaction involves electron withdrawal from the α-carbon and carboxylic acid group carbon, which results in decarboxylation (Figure 10.6). Decarboxylation reactions include the conversions of tyrosine to tyramine, 5-hydroxytryptophan to serotonin, histidine to histamine, and glutamate to γ–aminobutyric acid (GABA). Signs of convulsions associated with vitamin B-6 deficiency are attributed to insufficient activity of pyridoxal-5″-phosphate-dependent, L-glutamate decarboxylase.

A third type of reaction involves electron withdrawal from the β,γ-carbons of amino acids (Figure 10.6). This sets the stage for hydride condensations or aldol reactions. A good example of an aldol reaction is the conversion of serine to glycine with the transfer of the β-carbon (as formaldehyde) to another vitamin cofactor, tetrahydrofolic acid in single carbon reactions (see the next section). Another example of a hydride condensation is the formation of α-aminolevulinic acid, the first step in heme biosynthesis.

Another important function of vitamin B-6 (as pyridoxal-5′-phosphate), independent of amino acid metabolism, is its role in glycogen phosphorylase. Glycogen phosphorylase catalyzes the hydrolysis of ether (α-1 to 4 C-O-C) bonds in glycogen to form glucose-6-phosphate. Ether bonds are best catalyzed through acid-mediated mechanisms. The acid proton in this instance is derived from the phosphate group of pyridoxal-5′-phosphate. This important mechanism was only elucidated recently. Previously, it was speculated that the association of vitamin B-6 with glycogen phosphorylase was primarily some type of storage mechanism for B-6.

2. Absorption, Tissue Distribution, and Metabolism

Vitamin B-6 is absorbed in the upper gut by energy-dependent pathways. Muscle, kidney, and liver are rich in pyridoxal enzymes, e.g., glycogen phosphorylase (in

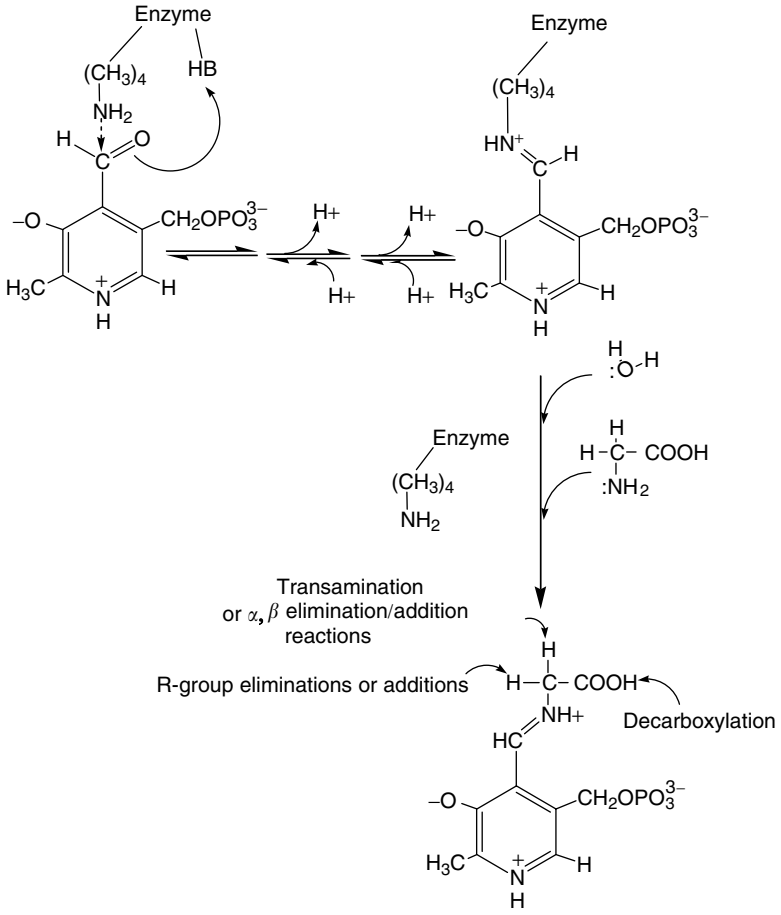

FIGURE 10.6 Pyridoxal-5′-phosphate. Vitamin B-6, as pyridoxal-5′-phosphate, is linked to pyridoxal-5′-phosphate containing enzymes by an dissociable imine linkage with a lysyl residue at the enzyme's active site. By a process of transamination given amino acids are transferred to pyridoxal-5′-phosphate. Depending on the enzyme and nature of the enzymatic process, transamination reactions, α,β-elimination/addition, R-group elimination/addition, or decarboxylation reactions can occur.

muscle) and amino acid catabolizing enzymes and transaminases (in liver and kidney).

The major form of vitamin B-6 in cells is pyridoxal –5′-phosphate. The conversion of the pyridoxal form of the vitamin to pyridoxic acid signals elimination from the body (7 and refs. cited).

3. Requirements, Pharmacology, and Toxicity

Vitamin B-6 deficiency is rarely seen as most diets provide adequate amounts. Normally, the vitamin B-6 is met at 1.3 or more mg per day or about 0.3 mg per 1000 kcal (4MJ) of diet (Table 10.1; 4). Drug-induced vitamin B-6 deficiency can occur following administration of the tuberculostatic drug, isoniazid (isonicotinic acid hydrazide). This drug forms hydrazone derivatives with pyridoxal or pyridoxal phosphate, which can then act to inhibit pyridoxal-containing enzymes. Patients receiving long-term isoniazid therapy respond to the administration of supplemental vitamin B-6. Penicillamine (β-dimethylcysteine) used in the treatment of Wilson's disease also can interfere with normal B-6 metabolism due to the formation of thiazole derivatives. A naturally occurring antagonist to vitamin B-6, linatine (1-amino-D-proline) is present in flax seed, which forms a stable product with pyridoxal phosphate (7).

The most important signs of B-6 deficiency relate to defects in amino acid metabolism. Neurologic signs occur because of reduced synthesis of important biogenic amines from amino acid precursors (7), and anemia is a consequence of decreased heme synthesis. Adverse effects have only been documented from vitamin B-6 supplements and never from food sources. Chronically high doses of pyridoxine can result in painful neurological symptoms known as sensory neuropathy. Symptoms include pain and numbness of the extremities, and in severe cases difficulty walking. Sensory neuropathy typically develops at doses of pyridoxine in excess of 1,000 mg per day. At intakes below 200 mg/day, sensory neuropathy is not observed.

4. Food Sources and Stability

The richest sources of vitamin B-6 are meats and whole grains. Heat and light are the two primary treatments that negatively affect the stability of the vitamin (Table 10.2). Similar to other B vitamins, milling of whole grains and removal of the bran will result in significant losses of pyridoxine. Fortification of cereal products will restore B vitamin levels (4).

V. B-VITAMINS INVOLVED IN SINGLE CARBON AND CO_2 TRANSFER REACTIONS

A. Biotin

Biotin was discovered following a long search for factors important to the understanding of the condition, egg white poisoning, which was associated with poor growth, alopecia, and dermatitis in animals exposed to raw eggs and egg whites as a primary source of dietary protein. It was eventually found that biotin-binding factors in eggs could reduce biotin availability (see below).

1. Chemistry

Biotin functions in enzymatic carboxylation reactions as a cofactor for CO_2 – fixing enzymes. Examples include: acetyl CoA carboxylase, which is essential for fatty acid synthesis; propionyl CoA carboxylase, which participates in odd chain fatty acid metabolism; pyruvate carboxylase, which is involved in the formation of oxaloacetate, an important obligatory step in reverse glycolysis and gluconeogenesis; and β-methylcrotonyl CoA, important in leucine metabolism (9).

Biotin is covalently bound in carboxylases and transcarboxylases by peptidyl linkage between the carboxylic acid moiety of biotin and the ε-amine function of peptidyl lysine (extends from the side chain associated with the sulfur-containing ring of biotin, which is only partially depicted in Figure 10.7). The biotin-lysine adduct is called biocytin. Biocytin can be cleaved by the enzyme biocytinase to generate free biotin.

2. Metabolism and Requirements

Biotin is found in highest concentrations in the liver. In plant foods, biotin is present in relative high concentrations in most cereals. Dietary biotin exists in free and protein bound forms. The latter form is digested first by gastrointestinal proteases and peptidases to generate biocytin (N-biotinyl-L-lysine) and eventually free biotin plus lysine. Like the other water-soluble vitamins, biotin is taken up by specialized Na^+-dependent carrier-mediated mechanisms. At the basolateral membrane, biotin exits cells via a Na^+-dependent carrier system. In adult humans, biotin uptake is significantly higher in the duodenum and jejunum compared to ileum. As noted previously, biotin shares the transporter with pantothenic acid (15, 18).

When biotin-containing carboxylases are degraded in cells, biotin is also released as biocytin. Cellular biocytinases catalyze its cleavage to release free biotin for reutilization. In the absence of this enzyme, biotin reutilization is compromised. Re-biotinylation requires ATP to catalyze the formation of a peptide bond between biotin and a lysyl group at the active center of targeted carboxylases (9).

The nutrition need for biotin is expressed as an "Adequate Intake" and amounts to ~25 micrograms per day (Table 10.1; 4). Reasons for the relatively lower requirements are: biotin is covalently attached to a limited number of enzymes that it serves as a cofactor; biotin is extensively reutilized; significant amounts of biotin are produced by the gut microflora.

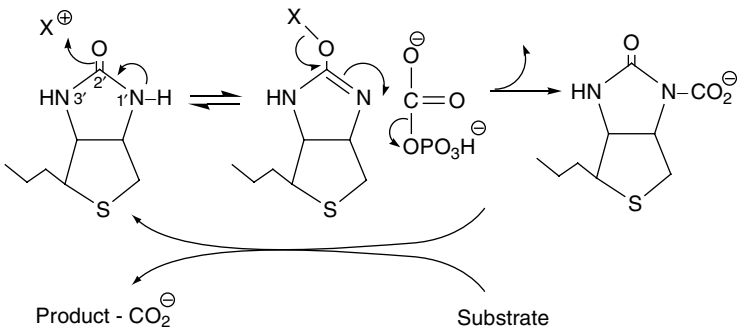

FIGURE 10.7 Biotin.

Nevertheless, nutritional problems associated with biotin status can occur. Biotin and biocytin have a high affinity for certain proteins, particularly avidin in egg white. The consumption of raw egg albumin can induce biotin deficiency, due to the strong association of biotin with avidin rendering dietary biotin unavailable. Ingestion of significant quantities of raw egg white by fur-bearing animals and pigs has led to the condition initially described as "egg white injury."

Biotin deficiency leads to impairment of gluconeogenesis and fat metabolism. Biotin deficiencies can also induce severe metabolic acidosis. The inability to carry out fat metabolism, also markedly affects the dermis. Alopecia and dermatitis are characteristics of biotin deficiency in most animals (9).

3. Food Sources and Stability

Biotin in animal foods is found in highest concentrations in the liver, but also in meats, milk, and egg yolk. In plant food, biotin is present in relative high concentrations in most cereal grains, as well as nuts, mushrooms, and some green vegetables. It is relatively stable to most treatments, with the exception of heat and alkaline conditions, which result in minor losses.

B. Folic Acid and Vitamin B-12

Vitamin B-12 and folic acid will be discussed together, because their functions and metabolism are uniquely linked (Figure 10.8). Knowledge regarding folic acid and B-12 evolved from efforts to better understand macrocytic anemias and certain degenerative neurologic disorders (8). The Scottish physician, Combe, recognized in the early 1800s that certain forms of macrocytic anemia appeared related to a disorder of the digestive organs. In classic studies by Minot, Murphy, Castle, and others, it became clearer that the disorder was associated with gastric secretions and in some cases could be reversed by consuming raw or lightly cooked liver.

In parallel studies, folic acid was also associated with macrocytic anemia. Large-scale efforts throughout the 1940s and 1950s and careful clinical and basic studies eventually led to the isolation of folic acid and vitamin B-12.

1. Chemistry Folic Acid

The folates are a group of heterocyclic compounds composed of 4-(pteridin-6-ylmethyl)-aminobenzoic acid conjugated with one or more L-glutamate units. Folate and folic acid are the preferred synonyms for pteroylglutamate and pteroylglutamic acid, respectively, and designate any members of the family of pteroylglutamates having various levels of reduction of the pteridine ring, one-carbon substitutions, and differing numbers of glutamate residues (Figure 10.8; 2).

The reactions that involve folic acid include the generation and utilization of formaldehyde, formimino, and methyl groups. For these conversions to occur, folic acid must be reduced in the form of tetrahydrofolic acid (THFA). Reduction brings the nitrogen at positions 5 and 10 closer together and changes their electrochemical properties, which facilitates the formation of the various THFA single carbon derivatives.

The formyl, methanyl, and methylene forms of THFA are utilized for purine synthesis and in thymidylate (i.e., DNA-related) synthesis. These reactions are essential to cell division and proliferation. Folate also participates in reactions involved in the interconversion and catabolism of amino acids. Much of the folate in the body is also in the form of methyl-THFA for eventual methyl transfer reactions. Removal of the methyl group from methyl-THFA and its eventual irreversible transfer to methionine by the vitamin B-12 requiring enzyme, methionine synthetase, represents an important interaction between folic acid and vitamin B-12 (1, 2).

2. Chemistry: Vitamin B-12

Vitamin B-12 consists of a porphyrin-like structure of tetrapyrrole rings with a monovalent cobalt metal at the center (Figure 10.9; 1). A 5′,6′-dimethylbenzimidazolyl nucleotide is also linked to the tetrapyrrole rings via a phosphate sugar linkage. Vitamin B-12 catalyzes the

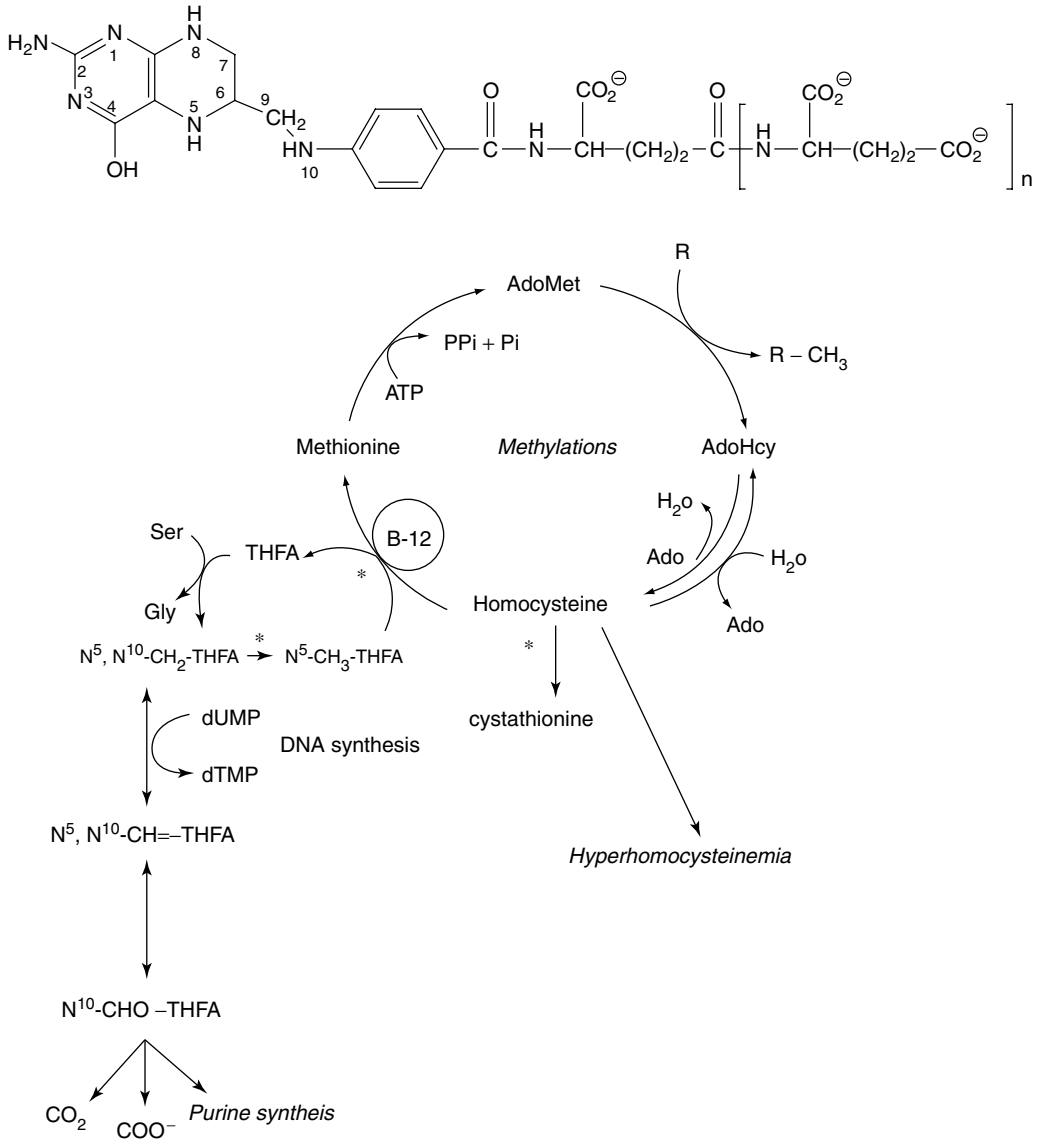

FIGURE 10.8 Folic acid. Basic features of the folic acid structure are shown. Folate is essential to: 1) methylation reactions (via the vitamin B-12-dependent methionine synthetase step), 2) DNA synthesis (via single carbon transfer from N^5, N^{10}-methylene-THFA in the conversion deoxyuridylate to deoxythymidylate), 3) purine synthesis (via transfer of a single carbon from N^{10}-formyl-THFA into the purine pathway). Not shown are reduction steps that result in the regeneration of THFA (at positions 5 and 8) from oxidized folic acid.

conversion of homocysteine to methionine via transfer of a methyl moiety derived from methyl-THFA. Vitamin B-12 in the form of the adenosyl derivative can also catalyze the conversion of methylmalonyl CoA to succinyl CoA. Deficiency of vitamin B-12 leads to intracellular accumulation of metabolites of these two reactions, homocysteine and methylmalonic acid (1, 2, 8, 13, 16).

3. Digestion, Metabolism, and Tissue Distribution

Folate absorption requires processing of food-derived folates from poly- to monoglutamyl forms. The proximal part of the small intestine is the main site of absorption of dietary folate. A specialized carrier-mediated system is involved in folate uptake across the apical membrane of the absorptive cells, as well as other cells. The factors that negatively affect intestinal folate absorption are alcohol consumption and the use of certain pharmacological agents like sulfasalazine and phenytoin (Dilantin). An important step in the digestive process is the conversion of polyglutamyl folate to monoglutamyl folate by intestinal polyglutamyl hydrolase.

There are a number of substances in unprocessed foods (e.g., uncooked beans) that act as folate hydrolase inhibitors. In plasma, there are specific carrier proteins, which take folic acid to targeted cells. In this regard,

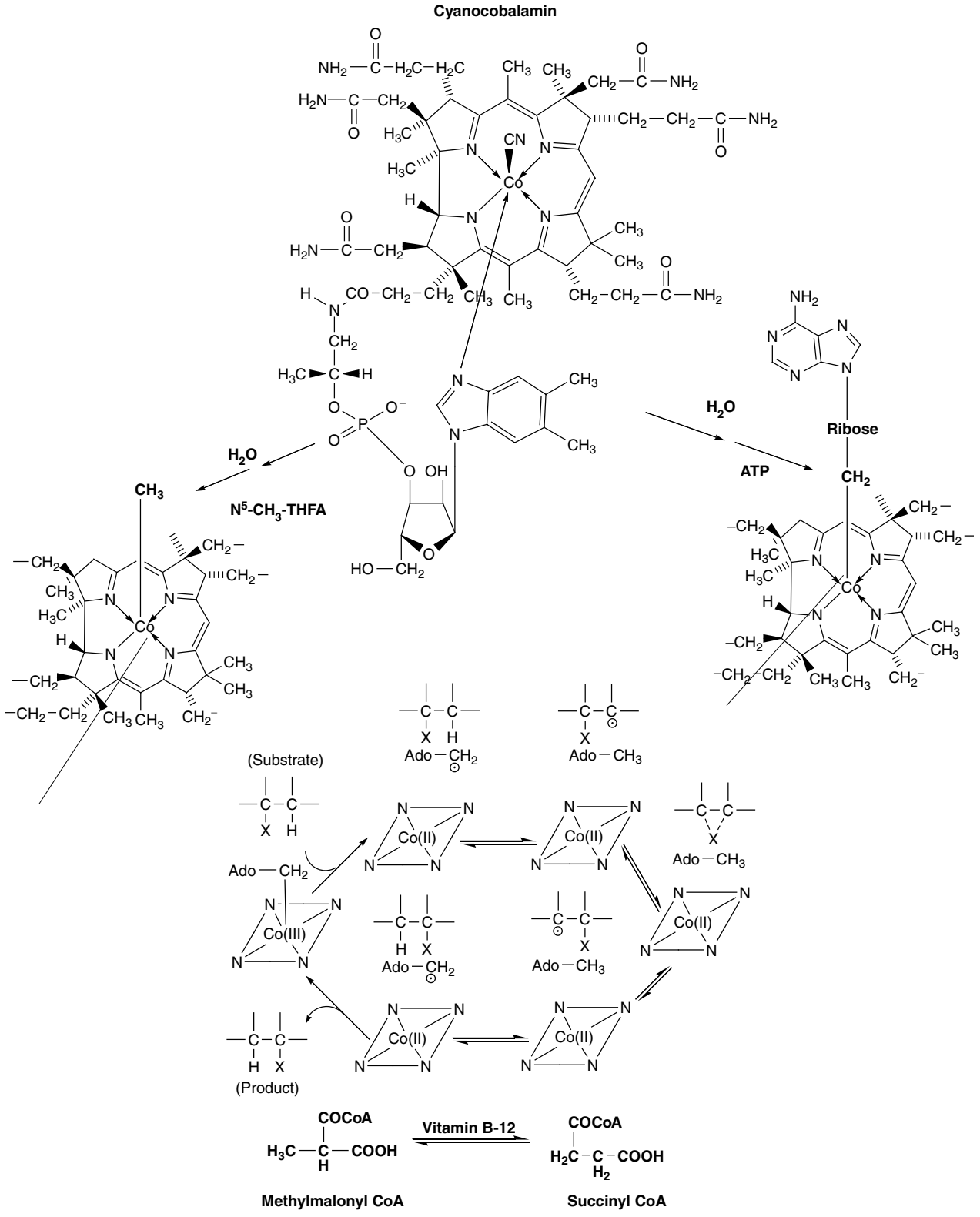

FIGURE 10.9 Cyanocobalamin. Commercial preparations of vitamin B-12 usually have a cyano group coordinated with the cobalt (associated with the corrin ring of vitamin B-12). In an aqueous environment the cyano group can be displaced by water for the eventual transfer of a methyl group (donated by N5-methyl-THFA) or an adenosyl moiety (from ATP). Methylated vitamin B-12 serves as a cofactor for methionine synthetase (see Figure 10.7). Adenosylated vitamin B-12 serves as a cofactor for unusual isomerase reactions, such as the conversation of methylmalonyl CoA to succinyl CoA. A mechanism for this process is shown, which involves the redox of cobalt in the corrin ring of cobalamin (Co+3«Co+2).

ethanol can also markedly decrease folate biliary secretion, thereby reducing availability to body tissues from liver. In tissues, the distribution of folates is largely dependent on the concentration of folate dependent enzymes. Methyl-THFA mono glutamate is rapidly transported (bound to carrier proteins) to liver. Uptake proceeds through a receptor-mediated endocytotic process and is then quickly converted to folate in various polyglutamyl forms (2).

Steps important to processing of vitamin B-12 involve first release from foods under acidic conditions. Vitamin B-12 then binds to proteins produced by cells of the gastric fundus, pancreas, and salivary gland. Two proteins have been identified, which are designated as R protein and intrinsic factor. Vitamin B-12 first binds to R protein and is released in the intestinal lumen by the action of pancreatic and intestinal proteinases and peptidases, to allow vitamin B-12 to bind to intrinsic factor. Intrinsic factor is a small-molecular-weight glycoprotein, which is made in stomach parietal cells (1). The vitamin B-12 – intrinsic factor complex then interacts with receptors on the intestinal brush border localized in the midgut (i.e., ileum). Interference with R protein or intrinsic factor production, or inflammatory disease affecting the ileum, or overproduction of intestinal microflora can adversely affect the availability of vitamin B-12 (1).

After vitamin B-12 is taken up by luminal cells, it is transported into the lysosomes where the vitamin B-12 – intrinsic factor complex is degraded and the vitamin B-12 is released and vectorally directed for release into plasma. Vitamin B-12 is transported in plasma by one of three specific transport proteins, transcobalmin I, II, or III. Transcobalmin I carries vitamin B-12 to the liver. Transcobalmin II carries vitamin B-12 from the liver to peripheral tissues. Note that the processing of folate and vitamin B-12 in and out of tissues differs from that for other water-soluble vitamins. The process is receptor mediated and endocytotic, in contrast to involvement of an active sodium-dependent transporter (1).

4. Requirements, Pharmacology, and Toxicity

Decreased production of methanyl and methylene forms of folate can result in decreased purine synthesis and thymidylate formation from uridylate. The latter can cause replicating cells to arrest in the S-phase of the cell cycle. Epithelial cells are often most affected by folate deficiency (2, 8). The consequences range from megaloblastic anemia to growth retardation. Accordingly, aggressive supplementation of folic acid has been associated with a decrease in the appearance of certain developmental defects, e.g., abnormal neutral tube closure (2, 11, 13, 16).

As noted, the most important link between folate and vitamin B-12 occurs at the methionine synthetase step, where 5-methyl THFA serves as a substrate with homocysteine to form methionine. In this regard, recent studies have suggested that low folate levels may play a role in the etiology of coronary artery disease because of the association of elevated homocysteine as a risk factor in vascular disease (8).

The movement of single carbon units from folate through methionine synthetase in the formation of methionine is also important for S-adenosylmethionine (SAM) formation. SAM is essential to methylation of phospholipids, and production of methylated forms of various amino acids and carbohydrates. SAM also serves as the methyl source in DNA methylation.

Deficiencies of both vitamin B-12 and folic acid produce clinical signs of macrocytic anemia and disynchronies in growth and development owing to the importance of folic acid to purine and DNA synthesis. Chronic deficiencies of either folic acid or B-12 can also promote fatty liver disease and indirectly influence extracellular matrix maturation stability by causing abnormal elevations in homocysteine (1, 2, 8, 11, 13, 16). Such signs and symptoms are attributable to both THFA and B-12 deficiencies, because of the integral relationship of vitamin B-12 to THFA regeneration. Dietary intakes of folic acid, sufficient to maintain functional THFA levels, can mask the initial signs of vitamin B-12 deficiency (e.g., macrocytic and megaloblastic anemia). Prolonged vitamin B-12 deficiency in humans results in serious neurologic disorders due to degeneration of the myelin sheath (1).

The requirement for folic acid is 400 µg per day (Table 10.1; 4). However, there are some conditions in which the folic acid requirements are conditionally high, e.g., when either natural or pharmacological folic acid agonists are present in the diet. A range of genetic polymorphisms in proteins important to folate metabolism can also influence the folic acid requirement. Alterations in folate hydrolase, folate reductase, or methionine synthetase that cause changes in the affinity for folate binding are all known to impact folate requirements (2).

The requirement for vitamin B-12 is 2.4 µg per day or 1 µg per 1000 kcal (~4 MJ) of diet (Table 10.1; 4). Although vitamin B-12 deficiency is rare, disease of the stomach, proximal duodenum, or ileum and pancreatic insufficiency can affect folic acid and vitamin B-12 absorption, respectively.

5. Food Sources and Stability

Folic acid: Foods that are particularly high in folate are oranges, green leafy vegetables, and whole grains. Fortification with folic acid to all enriched cereal grain foods was begun in the U.S. in 1998 by mandate of the Food and Drug Administration (11). The primary objective of this program was to provide increased folic acid to women of childbearing age in order to decrease the occurrence of neural tube defects (NTD). The fortification program has successfully decreased NTDs by 19%, but any longer term health benefits on other diseases is unknown at this time. Folic acid is stable in acid pH, but is rapidly

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destroyed in neutral or alkaline conditions and is unstable to prolonged heat, oxygen, and light exposure.

Dietary sources of B-12 are meats with liver being the highest followed by dairy products and some seafoods (1, 2, 4). B-12 is also present in yeast and fortified cereals. The reliance on primarily animal products for vitamin B-12 means that strict vegetarians are potentially at risk for B-12 deficiency. B-12 is relatively stable and destroyed only by extended heat treatments and exposure to light and oxygen (Table 10.2).

VI. CONCLUDING COMMENTS

Additional compounds, mostly derived from carbohydrate and amino acid metabolites, are listed with the vitamins, because of conditional dietary needs or functions in metabolic regulation. Many of these compounds typically perform specialized transport function, particularly in relation to fatty acids. Examples include choline (a major source of methyl groups in the diet), inositol (important in signal transduction), and carnitine (required for the transportation of fatty acids from the cytosol into the mitochondria). Taurine, queuine, the ubiquinones (coenzyme Q), the tetrahydrobiopterins, pyrroloquinoline quinone (17), and lipoic acid could also be added to the list, because of their novel roles in metabolism.

Regarding the need for vitamins, acute deficiencies for most water-soluble vitamins can be induced under experimental conditions. Frank deficiency diseases are rarely seen in most populations unless specific food sources are severely limited due to food distribution problems, environmental problems such as drought, or severe economic constraints that limit access to food. However, marginal deficiencies can be observed when individuals consume a monotonous diet in which relatively few foods constitute a source of calories. Genetic polymorphisms in proteins involved in vitamin metabolism may also affect vitamin nutriture and result in increased needs. When a defect is associated with a vitamin deficiency, what often defines why the defect occurs is the K_m for the association or binding of the vitamin with proteins associated with the defect (e.g., a specific enzyme). In such cases, the K_m or related constants is usually high. In addition, disturbances of absorption (e.g., pancreatic insufficiency, biliary obstructions, alcohol, enteropathies), antagonists (e.g., antibiotics, tannins, caffeic acid, alcohol), or metabolic conditions (e.g., pregnancy, diabetes) may contribute to the increased need for given vitamins (14).

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11 Fat-Soluble Vitamins

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I. INTRODUCTION

The fat-soluble vitamins include vitamin A, vitamin D, vitamin E, and vitamin K. Knowledge of their roles in metabolism and maintenance of health has greatly expanded over the recent past decades. Consumer interest has been energized by many diet-health relationships that, in some cases, have been well documented. Such interest and the expanding knowledge base has led to rapid growth of the use of fat-soluble vitamins in supplements and functional food products, increased fortification of food, the need to improve global food composition data banks, technology improvements in synthesis and food technological aspects, and increased need for accurate analytical methods both for scientific and regulatory use. Recent publication of the Dietary Reference Intakes (1–3) has added new perspectives on human requirements and diet planning. This chapter provides an overview of chemistry and nutrition of the fat-soluble vitamins.

II. VITAMIN A AND CAROTENOIDS

Night blindness resulting from vitamin A deficiency represents one of the earliest recognized dietary deficiency diseases (4). Scientific studies began in the early 20th century, and McCollum and Davis reported (1913) the presence of a lipid-like substance in butter and egg yolk required for the growth of young rats. The factor was named fat-soluble A in 1916, and the name vitamin A was first used in 1920 to emphasize the discovery of the first fat-soluble vitamin, differentiating it from the water-soluble vitamins. The structure of vitamin A was determined in 1931. The vitamin A activity of β-carotene was identified in 1929, and the term "provitamin A" was then used to differentiate carotenoid precursors of vitamin A from carotenoids without potential to form vitamin A. Plant carotenoids are, therefore, the precursors of all vitamin A found in the animal kingdom. Dietary vitamin A, by convention, is called preformed vitamin A when all-trans-retinol or its esters are consumed as a dietary constituent of animal products or through use of supplements (5). Identification of roles for vitamin A in gene expression, embryonic development, and immunological function (1) has greatly expanded interest in the nutritional significance of vitamin A to human well-being. Likewise, identification of functions other than as a precursor for vitamin A for both provitamin A carotenoids and other carotenoids such as lutein and lycopene have confirmed their significance as health-promoting components of a well-balanced diet (6).

A. CHEMISTRY

1. Vitamin A

Vitamin A refers to all isoprenoid compounds from animal products with the biological activity of all-trans-retinol (Figure 11.1). All-trans-retinol is the parent structure to most retinoids and contains a substituted β-ionone ring (4-{2,6,6-trimethyl-2-cyclo-hexen-1-yl}-3-buten-2-one) (5) with a side chain of three isoprenoid units linked at the 6-position of the β-ionone ring. The conjugated double-bond system includes the 5,6-β-ionone ring carbons and the isoprenoid side chain. Retinoids include all substances with vitamin A activity and many structurally similar synthetic compounds without vitamin A activity (7). Various retinoids are identified as significant for cell differentiation, immunity, and embryonic development. All-trans-retinoic acid, retinol esters, and various cis-isomers are significant metabolites (Figure 11.1). 13-cis-Retinol is frequently found in food extracts as a result of isomerization of all-trans-retinol during the extraction process (8). Acetate and palmitate esters of all-trans-retinol are the primary commercial forms of vitamin A used by the pharmaceutical and food industry in supplements and fortified foods. Esterification stabilizes the vitamin toward oxidation. Availability of the esters in beadlet or encapsulated products adds further stability during processing and marketing of supplements and fortified foods. The USP standard is retinyl acetate. Physical properties of all-trans-retinol, retinyl esters, and closely related retinoids are provided in Table 11.1.

2. Carotenoids

Carotenoids comprise an extensive family of plant pigments, and as many as 1000 occur naturally in the plant kingdom (6, 9). In the plant, carotenoids function in photosynthesis as light harvesting pigments that supplement the ability of chlorophyll to capture light energy (10, 11). They protect the photosynthetic apparatus by quenching reactive oxygen species ($^1O_2$, $^3O_2$, O_2^{2-}) and acting as free radical interceptors, thus preventing oxidative events which can irreversibly damage cell membranes and DNA (11).

Carotenoids are formed by the head-to-tail linkages of eight isoprene units to provide a C_{40} skeleton. Nomenclature for the carotenoids specified by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB) was reviewed by Weedon and Moss (12). Structures of common provitamin A carotenoids and other significant carotenoids to the human are given in Figure 11.2. Lycopene, which shows the acyclic hydrocarbon backbone chain, is considered the parent compound (12). Modifications of lycopene produce the diverse family of the carotenoids found throughout the plant kingdom. β-carotene (Figure 11.2) is characterized by the presence of β-ionone rings on both ends of the hydrocarbon chain. Oxygenation produces the xanthophylls. Oxygen functions include hydroxylation at the 3- or 4-position to produce lutein and β-cryptoxanthin, respectively, and ketolation to yield canthaxanthin. Other structural variants include aldehydes, epoxy, carboxy, methoxy, and

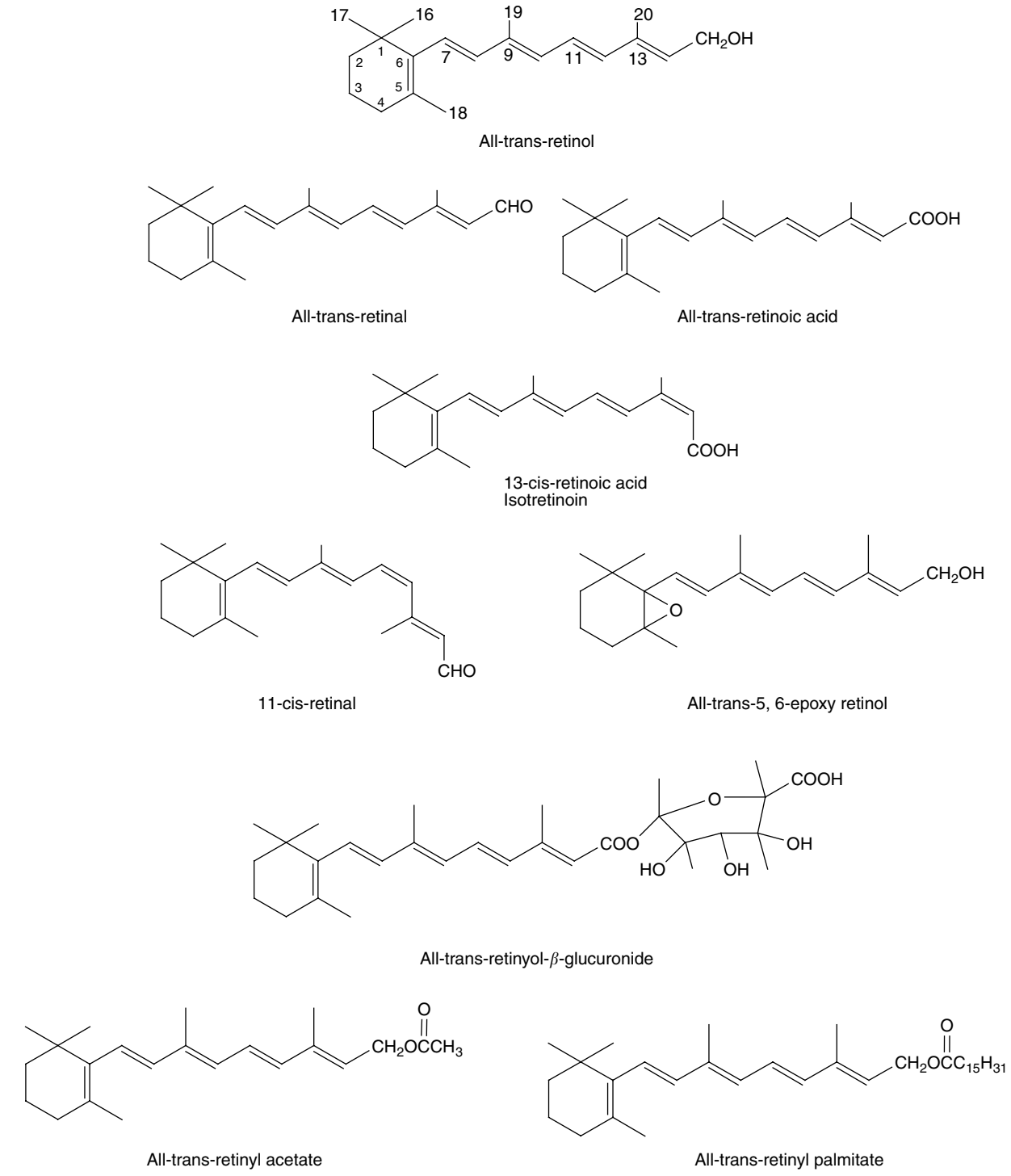


FIGURE 11.1 Structures of vitamin A, its metabolites and esters.

various other oxygenated forms. Some of the most frequently occurring carotenoids include the hydrocarbons lycopene, phytoene, and phytofluene, carotenal esters, and carotenol fatty acid esters (5).

The most commonly occurring provitamin A carotenoids in plant foods are β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin. cis-Isomers are biologically not as active compared to the all-trans naturally occurring carotenoids. Vitamin A activity depends on the presence of one, non-hydroxylated β-ionone ring in the provitamin A structure. Presence of the β-ionone ring allows conversion to all-trans-retinol (6). Acyclic hydrocarbons (lycopene,

TABLE 11.1
Physical Properties of Retinol, Other Retinoids, and Selected Carotenoids

Substance[a]	Molar Mass	Formula	Solubility	Melting Point, °C	Crystal Form	UV Absorption[b] λ max nm	UV Absorption[b] $E^{1\%}_{1cm}$	UV Absorption[b] ε × 10^3	UV Absorption[b] Sol[c]	Fluorescence[d] Ex nm	Fluorescence[d] Em nm
All-trans-retinol	286.45	$C_{22}H_{32}O_2$	Soluble in abs alcohol, methanol, ether, chloroform, fats, oils; practically insoluble in water or glycerol	62–64	Yellow prisms	325	1845	[52.8]	EtOH	325	470
CAS No. 68-26-8						**325**	**1810**	**[51.8]**	H		
10073											
13-cis-retinol	286.45	$C_{20}H_{30}O$		58–60	Pale yellow needles	328	1689	[48.4]	EtOH		
CAS No. 2052-63-3											
6495											
11-cis-retinol	286.45	$C_{20}H_{30}O$				319	1220	[34.9]	EtOH		
						318	**1200**	**[34.4]**	H		
All-trans-retinyl acetate	328.5	$C_{22}H_{32}O_2$		57–58	Pale yellow prismatic	325	1560	[51.2]	EtOH	325	470
CAS No. 127-47-9						**325**	**1590**	**[52.2]**	H		
10073											
All-trans-retinyl palmitate	524.86	$C_{36}H_{60}O_2$		28–29	Amorphous or crystalline	325	940	[49.3]	EtOH	325	470
CAS No. 79-81-2											
10073											
All-trans-retinal	284.43	$C_{20}H_{28}O$	Soluble in ethanol, chloroform, cyclo-hexane, petr ether, oils; practically insoluble in water	61–64 (trans)	Orange crystals	383	1510	[42.9]	EtOH		
CAS No. 116-31-4											
8249						**368**	**1690**	**[48.1]**	H		
13-cis-retinal	284.44	$C_{20}H_{28}O$				375	1250	[35.6]	EtOH		
						363	**1365**	**[38.8]**	H		
11-cis-retinal	284.44	$C_{20}H_{28}O$		63.5–64.4	Orange prisms	380	878	[25.0]	EtOH		
8249						**365**	**928**	**[26.4]**	H		
All-trans-retinoic acid	300.43	$C_{20}H_{28}O_2$	Soluble in DMSO; practically insoluble in water, mineral oil, glycerin	180–182	Crystals	350	[1508]	45.3	EtOH		
CAS No. 302-79-4											
8251											
13-cis-retinoic acid	300.44	$C_{20}H_{28}O_2$		174–175	Reddish-orange plates	354	[1325]	39.8	EtOH		
CAS No. 4759-48-2											
5249											
9-cis-retinoic acid	300.43	$C_{20}H_{28}O_2$		190–191	Yellow fine needles	345	[1228]	36.9	EtOH		
CAS No. 5300-03-8											
244											
Provitamin A Carotenoids											
β-carotene	536.87	$C_{40}H_{56}$	Soluble in CS_2, hexane, chloroform	183	Red rhombic square leaflets	425	–	–	LP		
CAS No. 7235-40-7						453	2592	139			
1866						479	–	–			
						452	2505	134			
α-carotene	536.87	$C_{40}H_{56}$	Freely soluble in CS_2, chloroform; soluble in ether, hexane	187.5	Deep purple prisms	422	–	–	LP		
CAS No. 7488-99-5						444	2800	150			
1865						474	–				

β-cryptoxanthin	552.87	$C_{40}H_{56}O$	Freely soluble in chloroform, CS_2, benzene, pyridine	158–159 (racemic)	Red plates with metallic luster				EM
CAS No. 472-70-8				169		452	2380	[132]	
2636				(natural)		480	2080	[115]	
γ-carotene	536.87	$C_{40}H_{56}$	Somewhat less soluble than β-carotene	152–153.5 (synthetic)	Red plates (synthetic)	437	2055	[110]	PE
CAS No. 472-93-5						462	3100	[166]	
1867				177.5 (natural)	Deep-red prisms (natural)	494	2720	[146]	
						508.5			C
						475			
						446			
β-Apo-8′-carotenal	416.65	$C_{30}H_{40}O$	Freely soluble in chloroform; sparingly soluble in acetone	136–142 (decomp)	Powder with dark metallic sheen	457	2640	110	LP
CAS No. 1107-26-2									
Other Carotenoids									
Phytoene	544.95					275			LP
						285	1250	68	
						296			
						286	915	50	H
Phytofluene	548.97	$C_{40}H_{68}$	Freely soluble in petr ether, ether, benzene; practically insoluble in water, methanol, ethanol	B.P. 140–185	Pale orange viscous oil	331			LP
CAS No. 540-05-6						348	1350	73	
7473						367			
Lycopene	536.87	$C_{40}H_{56}$	Soluble in chloroform, hexane; almost insoluble in methanol, ethanol	172–173	Long, deep red needles	446	2250		PE
CAS No. 502-65-8						472	3450	185	
5640						505	3150		
Lutein	568.87	$C_{40}H_{56}O_2$	Soluble in fats and fat solvents; insoluble in water	183	Yellow prisms with metallic luster	421			EtOH
CAS No. 127-40-2						445	2550	145	
10120						475			
Zeaxanthin	568.87	$C_{40}H_{56}O_2$	Slightly soluble in petr ether, ether, methanol; soluble in CS_2, hexane, chloroform, pytidine, ethyl acetate	207 (Zechmeister) 215.5 (Kuhn)	Yellow rhombic plates with steel-blue metallic luster	426	–		LP
CAS No. 144-68-3						452	2348	133	
10171						479	–		
						450	2540	144	EtOH
						450	2480	141	
Canthaxanthin	564.84	$C_{40}H_{52}O_2$	Soluble in chloroform, oil; very slightly soluble in acetone	217 (decomp)	Violet crystal	466	2200	124	LP
CAS No. 514-78-3						470	2250	127	Cy
1758									
Violaxanthin	600.87	$C_{40}H_{56}O_4$	Soluble in alcohol, methanol, ether; almost insoluble in petr ether	200	Orange prisms	420	–		EtOH
CAS No. 126-29-4						443	2550	153	
10059						470	–		
Neoxanthin	600.87	$C_{40}H_{56}O_4$				416	–		EtOH
						439	2243	135	
						467	–		
						438	2470	148	
Astaxanthin	596.84	$C_{40}H_{52}O_4$		182–183	Needles	503			CS_2
CAS No. 472-61-7						472	2135	124	EtOH
860						470	2100	125	H
						485			C

[a]Common or generic name, CAS No. – Chemical Abstract Service number, bold print designates the Merck Index monograph number.

[b]Values in brackets are calculated from corresponding ε or $E^{1\%}_{1cm}$ values.

[c]Solvent: EtOH=ethanol, H=hexane, LP=light petroleum, EM=ether+methanol, PE=Petroleum ether, Cy=Cyclohexane, C=Chloroform, CS_2=Carbon disulphide.

[d]In isopropanol.

Source: Refs. 16–19, 22, 29.

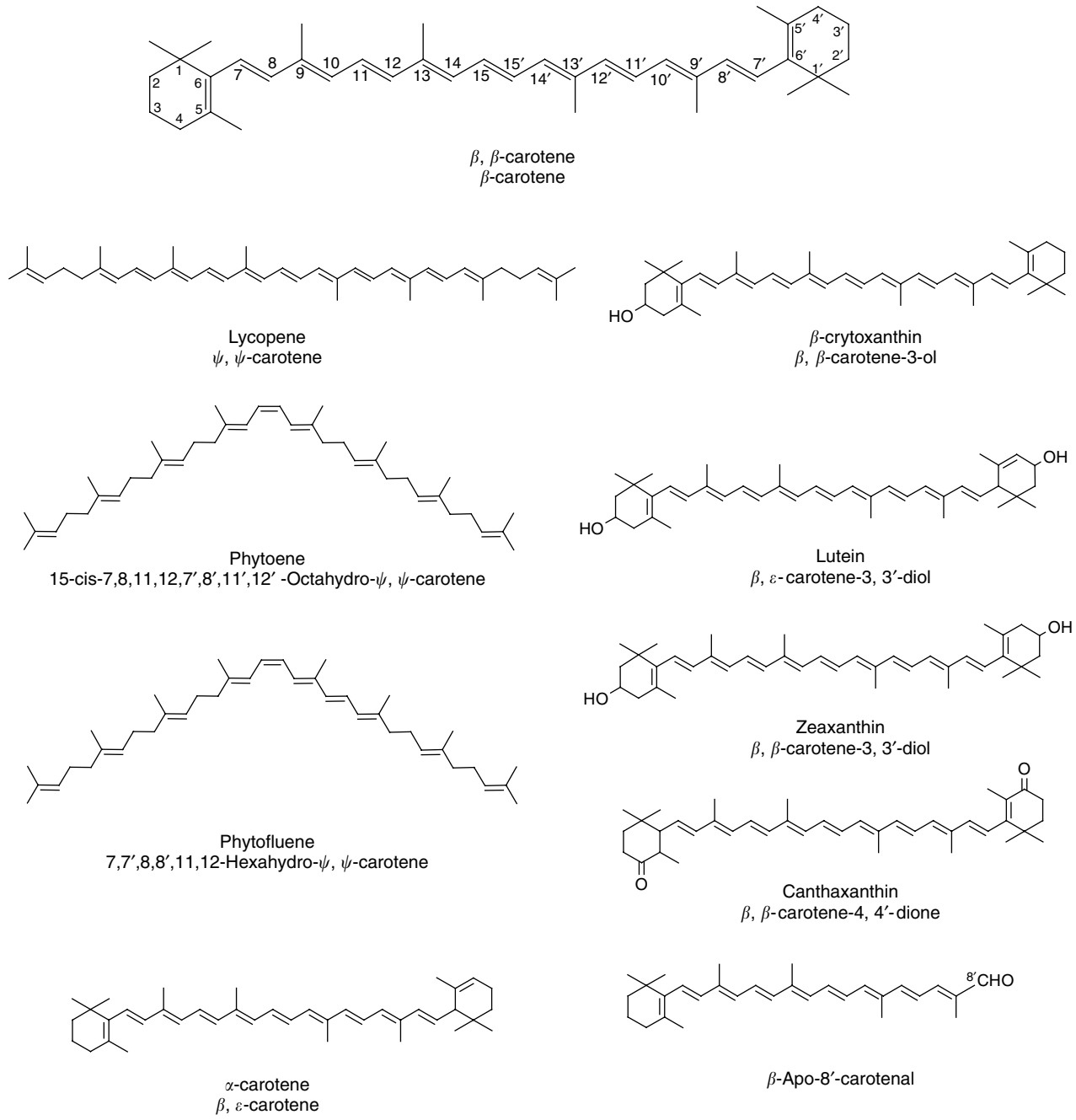


FIGURE 11.2 Structures of commonly occurring carotenoids.

phytoene, phytofluene), therefore, are not provitamin A carotenoids. Carotenoids with one hydroxylated ring or other oxygen function on one ring usually possess less than 50% of the biological activity of β-carotene (β-cryptoxanthin). Hydroxylation of both β-ionone rings (lutein) leads to a complete absence of vitamin A activity. Conversion of the provitamin A carotenoids to all-trans-retinol can occur by central oxidative cleavage at the 15,15′ double bond to yield two moles of vitamin A per mole of β-carotene or by random oxidative cleavage that yields only one or two moles of vitamin A per mole of β-carotene, depending on the point of cleavage (13–15). Central cleavage is accomplished by β-carotene-15,15′-dioxygenase within the intestinal absorptive cells (1).

Physical properties of various carotenoids are provided in Table 11.1. β-Carotene and β-apo-8′-carotenal are added to margarines, salad dressings, and other foods to enhance color as well as to fortify the products. In the

poultry industry, xanthophyll concentrates derived from the marigold are added to rations to enhance yellow to red pigmentation in the skin and in egg yolks. The xanthophylls deposited in the skin and yolks do not add to the vitamin A activity of the products, since xanthophylls lack vitamin A activity.

3. Spectral Properties

The conjugated double bond system of the retinoids gives the compounds strong UV absorption properties. UV absorption maxima (Table 11.1) vary with structural modification of the all-trans-retinol. Effects on UV absorption by structural variations of the retinoids indicate that maximal absorption ranges from 318 to above 360 nm. Absorption maxima vary with solvent and the presence of cis-isomers (Z-). Isomerization to the cis-form decreases the absorption maxima and $E^{1\%}_{1cm}$ values relative to all-trans-retinol (Table 11.1) (17, 20).

All-trans-retinol and retinyl esters fluoresce strongly at λ_{ex} from 325 to 490 nm. Fluorescence intensity is greater in non-polar solvents; therefore, fluorescence is an ideal detection mode for normal-phase liquid chromatography-based analytical systems compared to UV detection (5, 21). Most other retinoids other than retinol and its esters do not fluoresce and oxidation of the alcohol results in lower fluorescence (20).

Carotenoids contain eleven π electron conjugated double bonds and, thus, possess strong primary absorption in the visible region due to the long conjugated double bond system. The bright and varied pigmentation of the carotenoids characterize the strong, visible region absorption. Published absorbance maxima are given in Table 11.1. Spectra commonly show two to three absorption maxima between 400 and 500 nm. A characteristic UV absorption peak occurs in cis-isomers approximately 142 nm below the longest-wavelength absorption maxima of the all-trans carotenoid (22). cis-Isomers show UV maxima between 330 to 340 nm and a downward shift of the entire spectrum (20, 22). Absorption intensity is affected by solvent or mobile phase composition (23, 24). Detection at 450 nm is commonly used as a detection mode for β-carotene after LC resolution.

4. Stability

Eitenmiller and Landen (5) summarized studies that exist in the literature on the stability of all-trans-retinol and β-carotene in foods during processing and storage. Little information exists on the stability of other retinoids or carotenoids such as lycopene and lutein. However, due to similarities in chemical properties, stability characteristics would be somewhat similar. Ball (25) provided an excellent review of the stability of all-trans-retinol and β-carotene with many specific literature citations. Significant facts regarding the stability of all-trans-retinol and carotenoids presented by Eitenmiller and Landen (5) include the following:

1. Conversion of all-trans to cis-isomers is easily induced by light, acids, metals, lipoxygenase action, and heat processing, acting independently or synergistically.
2. All-trans-retinol and the carotenoids are oxidatively unstable. Autoxidation of lipids can induce rapid loss of vitamin A activity. Some carotenoids are active singlet oxygen quenchers. This ability is directly related to the number of double bonds in the conjugated double bond system. β-Carotene, lutein, and other carotenoids preferentially react with singlet oxygen, converting it to the triplet state (26). The excited triplet state carotenoids can thermally disperse excess excited state energy through chemical reactions that destroy the carotenoids (27). Degradation products include various cleavage products.
3. Carotenoids scavenge free radicals at low oxygen pressures (<155 mm Hg) and act as primary chain-breaking antioxidants. Carotenoids, like β-carotene, trap peroxyl radicals through the addition of the radical at the 5,6-double bond of the carotenoid with conversion to peroxides (27).
4. All-trans-retinol and the carotenoids become more unstable as the food matrix is disrupted or the compounds are removed from the matrix by extraction prior to analysis. Destruction of the food matrix can liberate lipoxygenases that catalyze isomerization.
5. Blanching of plant products prior to freezing inactivates lipoxygenase and removes oxygen from the tissue. Thus, carotenoids are protected from lipoxygenase-initiated oxidative degradation during freezer storage.
6. Air drying or freeze drying can produce large losses in all-trans-retinol or the carotenoids. Storage of freeze-dried foods, because of the open, porous nature of the product, requires removal of oxygen with inert gas or vacuum to stabilize the vitamin A activity. Losses can be considerable during the drying process.
7. All-trans-retinol and the carotenoids are relatively stable at alkaline pH. Therefore, saponification can be used for sample extraction if the reaction vessel is evacuated and protected from light. Saponification at ambient temperature can be used to slow isomerization reactions that are more predominant at elevated temperatures.
8. All-trans-retinyl palmitate and all-trans-retinyl acetate are the commonly available commercial

forms of vitamin A used for food fortification and by the pharmaceutical industry. The ester forms are more stable to oxidation. However, conversion of all-trans to cis-isomers can readily occur. Most LC procedures will resolve the all-trans from the cis-isomers, which is essential to accurately assess biological activity. Encapsulation of concentrates in a matrix of gelatin, microcrystalline cellulose, or modified starch inhibits oxidation and isomerization. Antioxidants including tocopherols and other free radical interceptors are usually added to vitamin A preparations to inhibit oxidation.

Due to the lability of all-trans retinol and the carotenoids to oxidation and isomerization, precautions must be taken during analysis of foods and other biological samples to avoid conditions adverse to the analytes. Precautions to minimize destruction during extraction and determinative chromatography include the following (5, 28):

1. During all phases of sample handling, exclude oxygen. Air should be replaced by vacuum or inert gas.
2. Addition of antioxidants such as butylated hydroxytoluene (BHT), pyrogallol, ascorbic acid, or combinations is necessary prior to saponification. Low levels of antioxidants in extracting solutions and mobile phases are often added to protect the retinoids and carotenoids from oxidation.
3. Trans- to cis-isomerization ($E \rightarrow Z$) is promoted at elevated temperatures. Therefore, use of the lowest practical temperature is recommended. Use of solvents with low boiling points is preferred. For rotary evaporation, 40°C should not be exceeded. Solutions should be stored at −20°C, preferably lower.
4. All sunlight should be avoided. Analytical steps and extractions should be completed in dim light, diffused sunlight, or under gold fluorescent light. Solutions of sample analytes and standards should be stored in low actinic glassware whenever possible. Isomerization rapidly occurs through light activation and is a common source of cis-isomer formation in biological sample extracts.
5. Acid must be avoided. All solvents must be acid free. Addition of Triethylamine (TEA) at 0.001% is useful to neutralize low acid levels in some solvents.
6. Alkaline conditions can lead to base-catalyzed isomerization, especially if exposure to light occurs.

B. Nutrition and Biochemistry of Vitamin A

1. Functions of Vitamin A

Functional roles of vitamin A continue to be identified as the scientific basis of its relationship to cell growth and differentiation and immunological function becomes better understood. Specifically, vitamin A is required for normal vision, gene expression, reproduction, embryonic development, growth and immunological function.

The role of vitamin A in maintenance of eye health and the visual cycle is quite well understood. Retinoic acid is required for normal differentiation of the cornea, conjunctival membranes, and photoreceptor rod and cone cells of the retina (1, 7). In the visual cycle, all-trans-retinol is isomerized to 11-cis-retinol which is oxidized to 11-cis-retinal (29). In the rod cells, 11-cis-retinal is bound to opsin to form the visual pigment, rhodopsin. Absorption of light by rhodopsin isomerizes 11-cis-retinal to all-trans-retinal which triggers signaling to neuronal cells in the visual cortex of the brain (1). All-trans-retinal is released from the protein, converted back to all-trans-retinol, and stored as all-trans-retinyl esters, completing the cycle (1, 7, 29).

Identification of two families of nuclear receptors, retinoic acid receptors (RAR) and retinoid receptors (RAX), has helped explain the role of vitamin A in cell differentiation. In the nucleus, all-trans- and 9-cis-retinoic bind with RAR and 9-cis retinoic acid binds with RAX. The activated receptors regulate gene expression significant to embryonic development and integrity of epithelial cells (1, 7, 29).

The relationship of vitamin A deficiency to decreased disease resistance and increased child mortality in areas of the world lacking proper food supplies has been recognized for decades. Vitamin A influences immune response through maintenance of circulating natural killer cells with anti-viral and anti-tumor activity, by increasing phagocytic activity, and by increasing production of cytokines which regulate production of T and B lymphocytes (1). Fortunately, in vitamin A deficient individuals, immune function can be improved through supplementation of the diet with vitamin A.

2. Vitamin A Deficiency

Vitamin A deficiency is characterized by changes in the eye that can cause irreversible blindness. Clinical symptoms are referred to as xerophthalmia. The initial stage of development of xerophthamia is night blindness or the inability to adapt to dim light due to decreased ability to regenerate rhodopsin. At this stage, addition of supplementary vitamin A will reverse the disease. Progression of the disease leads to corneal and conjunctival xerosis (dryness) due to a decreased amount of globlet cells and to the appearance of Bitot's spots on the conjunctiva. Final stages are referred to as keratomalacia or ulceration and

scarring of the cornea that leads to loss of vision. Other symptoms include skin lesions, loss of appetite, epithelial keratinization of epithelial tissue, impaired embryonic development, lack of growth and increased susceptibility to infections (1, 30–32).

While vitamin A deficiency in developed countries is not prevalent and is limited to individuals with fat absorption abnormalities, chronic liver disease, and alcoholics (32), it continues to be the leading cause of blindness world-wide and greatly contributes to infant and child mortality. Recent estimates indicate that 250,000 to 500,000 children go blind annually due to lack of vitamin A (1, 33, 34). Although fortification programs can be successful in improving vitamin A status at low cost, large segments of the world's population can still benefit from fortification of staple foods or through provision of supplements. The role of the food industry and public health organizations in this regard can still dramatically improve human well-being.

3. Hypervitaminosis A

Vitamin A toxicity can occur from high intake of foods high in vitamin A or high potency supplements. Toxicity to retinoids has been classified as acute, chronic, and teratogenic (1, 29). Acute toxicity results from a single dose or a limited number of large doses over a short time period. A single dose greater than 200 mg (>200,000 RAE (retinol activity equivalent), >660,000 IU or 0.7 mmol) or all-trans-retinol can result in acute toxicity in adults. For children, 50% of the adult dose can cause acute toxicity (29). Symptoms include nausea, vomiting, headache, increased cerebrospinal fluid pressure, vertigo, blurred vision, muscle incoordination, and bulging fonanel in infants (1). Chronic toxicity results from ingestion of large doses at or above 30 mg (30,000 RAE) per day for months or years (1). Symptoms of chronic toxicity include alopecia, ataxia, liver abnormalities, membrane dryness, bone and skin changes, visual impairment, and nervous system effects (1, 29). Most symptoms are reversible when vitamin A intake is decreased (29). Teratogenic effects can result from single, large doses (30–90 mg) or long-term intakes that result in chronic toxicity (29). Common teratogenic defects include physical malformations, heart, kidney, and thymic disorders, and central nervous system disorders (29).

4. Dietary Reference Intakes

Based on newer information on absorption of dietary carotenoids, the Institute of Medicine (1) established the retinol activity equivalent (RAE) to replace the retinol equivalent (RE) as a measure of the vitamin A activity of dietary provitamin A carotenoids. One RAE is defined as 1 μg of all-trans-retinol, 12 μg of all-trans-β-carotene and 24 μg of other provitamin A carotenoids (usually limited to α-carotene and β-cryptoxanthin). International units (IU) are used for labeling pharmaceuticals, supplements, and foods. One microgram of all-trans-retinol equals 3.33 IU of vitamin A activity. The Reference Daily Intake (RDI) set by the Nutritional Labeling and Education Act of 1990 (NLEA) is 5000 IU.

Establishment of the RAE was based on the accepted carotene: retinol equivalency ratio (μg) of a low dose of purified β-carotene in oil of 2:1, indicating that 2 μg of β-carotene in oil yields 1 μg of retinol (1). Differences between RE conversion factors and RAE factors stem from absorption studies that show that 6 μg of dietary β-carotene is equivalent to 1 μg of purified β-carotene in oil (1). Since previous data indicated 3 μg of dietary β-carotene was equal to 1 μg of β-carotene in oil ($6 \times 2{:}1 = 12{:}1$), the RAE calculation doubles the amounts of dietary provitamin A carotenoids required to provide 1 μg of retinol or 1 RAE as compared to calculation of RE values. The Institute of Medicine report (1) emphasizes that vitamin A intake from provitamin A carotenoids has been overestimated by previous assumptions made on carotenoid absorption from mixed meals including fruits and vegetables. Implications to the presentation of food composition databank information are significant in that reliable data on carotenoid composition of fruit and vegetables are still somewhat limited.

The ability of the scientific community to reliably quantify carotenoids from fruits and vegetables has greatly improved with the introduction of a C_{30} stationary phase designed to provide high absolute retention, enhanced shape recognition of carotenoid isomers, and to moderate silanol activity of the support (35). This LC support has gained wide acceptance, with utilization for multi-analyte analyses including the tocopherols (36, 37). With a LC reversed-phase method based on the C_{30} support, Darnoko et al. (37) quantified tocopherols and 13 carotenoids in red palm oil. The C_{30} support adds a new dimension to LC assay of fat-soluble vitamins from foods and supplements and expands the capability to use reversed-phase systems, in general, for fat-soluble vitamin assay.

Dietary Reference Intake (DRI) values for vitamin A range from an Adequate Intake (AI) of 400 μg RAE/day (RAE, retinol activity equivalents) for the 0- to 6-month infant to Recommended Dietary Allowance (RDA) values of 700 μg RAE/day for adult women and 900 μg RAE/day for adult men. The RDA increases to 1300 μg RAE/day for lactating women (19–50 years) (1). The Tolerable Upper Intake Level (UL) is 3000 μg RAE/day for adults (Table 11.2).

5. Food Sources and Dietary Intake

Significant dietary sources of preformed vitamin A include organ meats (liver), fish oils, butter, eggs, whole milk, and fortified reduced fat and skim milk, other dairy products, and high fat species of fish including tuna and sardines (8). Fortification of foods with retinyl palmitate and/or

TABLE 11.2
Dietary Reference Intake Values for the Fat-Soluble Vitamins

	EAR[a]		RDA[b]		AI[c]		
Life Stage Group	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**UL[d]**
	Vitamin A (μg RAE/d)						**Preformed Vitamin A (μg/d)**
0 through 6 mo					400	400	600
7 through 12 mo					500	500	600
1 through 3 y	210	210	300	300			600
4 through 8 y	275	275	400	400			900
9 through 13 y	445	420	600	600			1,700
14 through 18 y	630	485	900	700			2,800
>19 y	625	500	900	700			3,000
Pregnancy							
14 through 18 y		530		750			2,800
19 through 50 y		550		770			3,000
Lactation							
14 through 18 y		885		1,200			2,800
19 through 50 y		900		1,300			3,000
	Vitamin D (μg/d)						**(μg/d)**
0 through 12 mo					5	5	25
1 through 50 y					5	5	50
51 through 70 y					10	10	50
>70 y					15	15	50
Pregnancy							
14 through 50 y						5	50
Lactation							
14 through 50 y						5	50
	Vitamin E (mg α-T/d)						**Any Form of Supplementary α-T (mg/d)**
0 through 6 mo					4	4	
7 through 12 mo					5	5	
1 through 3 y	5	5	6	6			200
4 through 8 y	6	6	7	7			300
9 through 13 y	9	9	11	11			600
14 through 18 y	12	12	15	15			800
19 through 70 y	12	12	15	15			
> 70 y	12	12	15	15			
Pregnancy							
14 through 18 y		12		15			800
19 through 50 y		12		15			1,000
Lactation							800
14 through 18 y		16		19			1,000
19 through 50 y		16		19			
	Vitamin K (μg/d)						
0 through 6 mo					2	2	
7 through 12 mo					2.5	2.5	
1 through 3 y					30	30	
4 through 8 y					55	55	
9 through 13 y					60	60	
14 through 18 y					75	75	
19 through 70 y					120	90	
>70 y					120	90	

(*continued*)

TABLE 11.2 (*Continued*)

Life Stage Group	EAR[a] Male	EAR[a] Female	RDA[b] Male	RDA[b] Female	AI[c] Male	AI[c] Female	UL[d]
			Vitamin K (μg/d)				
Pregnancy							
14 through 18						75	
19 through 50						90	
Lactation							
14 through 18						75	
19 through 50						90	

[a]EAR=Estimated Average Requirement. The intake that meets the estimated nutrient needs of half of the individual in a group.

[b]RDA=Recommended Dietary Allowance. The intake that meets the nutrient need of almost all (97–98%) of individuals in a group.

[c]AI=Adequate Intake. The observed average or experimentally determined intake by a defined population or subgroup that appears to sustain a defined nutritional status, such a growth rate, normal circulating nutrient values, or other functional indicators of health. The AI is needed if sufficient scientific evidence is not available to derive an EAR. The AI is not equivalent to an RDA.

[d]UL=Tolerable Upper Intake Level.

Source: Refs. 1–3.

β-carotene helps to prevent vitamin A deficiency in countries that require fortification. Commonly used food vehicles for delivery of vitamin A include fluid milk, dry milk, margarine, and some edible oils. Choice of the proper food for delivery of vitamin A depends on which foods are consumed by the target population. In the United States, milk is fortified with not less than 2,000 IU of vitamin A (retinyl palmitate), and margarine is fortified with no less than 15,000 IU per pound (by a combination of retinyl palmitate and β-carotene) (38).

Provitamin A carotenoids are widely distributed throughout the plant kingdom. However, reliable quantitative data only exist for β-carotene, α-carotene, and β-cryptoxanthin in some fruit and vegetables. Minor carotenoids with provitamin A activity provide only small amounts to the total vitamin A in human diets because of lower natural levels compared to the primary provitamin A carotenoids. Color or color intensity cannot be used to predict the vitamin A activity of a food due to the complexity of carotenoid profiles and the fact that most carotenoids such as lycopene have zero vitamin A activity. The red color of ripe tomatoes is primarily due to lycopene and most tomato cultivars are quite low in vitamin A activity (5). An excellent database exists that was formulated through efforts of the United States Department of Agriculture and the National Cancer Institute. The database contains compositional information on 2400 fruits and vegetables for β-carotene, α-carotene, β-cryptoxanthin, lutein plus zeaxanthin, and lycopene (39–41). The database provides the most easily applied tool to estimate dietary intake (42). Estimation of dietary intake of non-provitamin A carotenoids has become more significant to public health as new, functional roles are identified for carotenoids such as lutein and lycopene. Although β-carotene and several other carotenoids are clinically associated with lower risk of several chronic diseases, the DRI Panel on Antioxidants and Related Compounds (3) supported increased consumption of fruits and vegetables but did not set DRIs for β-carotene or other carotenoids. The Panel did not recommend use of supplemental β-carotene other than as a source of provitamin A for the control of vitamin A deficiency.

The Institute of Medicine report on DRIs for vitamin A (1) gives quite good, documentable data on vitamin A intake in the U.S. based upon the Third National Health and Nutrition Examination Survey (NHANES) (43). The following facts pertaining to vitamin A intake were delineated:

1. The median dietary intake of vitamin A is 744 to 811 μg RAE for men and 530 to 716 μg RAE for women.
2. For adults, 25 to 50% had vitamin A intakes less than the estimated average requirement (EAR) of 500 μg RAE/day for women and 625 μg RAE/day for men.
3. The data suggest that many adults in the U.S. have lower liver stores of vitamin A than is considered optimal. However, intake is sufficient to avoid clinically measurable deficiency.
4. Conversion factors based upon the RAE factors give dietary intake levels that are lower compared to older estimates calculated using retinol equivalent (RE) factors. Therefore, greater amounts of provitamin A carotenoids are needed to meet vitamin A requirements.
5. Carrots provide 25% of the β-carotene in the U.S. diet.

6. Cantaloupe, broccoli, squash, peas, and spinach are major contributors of β-carotene.
7. Carrots contribute 51% of the α-carotene in the U.S. diet.
8. Fruits are the only sources of β-cryptoxanthin.
9. All provitamin A carotenoids contribute 26 and 34% of vitamin A consumed by men and women, respectively. Previous estimates indicated that approximately 75% of the vitamin A in the U.S. diet originated as preformed vitamin A from animal products.

III. VITAMIN D

Rickets was initially identified in 1919 as a deficiency of a fat-soluble factor in dogs fed fat-free diets and kept indoors in the absence of sunlight (44). The deficiency state was cured by feeding cod liver oil. The fat-soluble, antirachitic factor was named vitamin D in 1925 by McCollum's research group and proven to be produced in the skin by ultraviolet irradiation. Vitamin D was isolated as ergocalciferol or vitamin D_2 from irradiated ergosterol from yeast in 1931. The structure was identified in 1932. Cholecalciferol or vitamin D_3 was later characterized and shown to be the antirachitic factor in cod liver oil. Better understanding of the intermediary metabolism of vitamin D occurred in the 1970s with the identification of the hydroxylated metabolites, 25-hydroxyvitamin D_3 ($25(OH)D_3$) and 1 α, 25 dihydroxy D_3 (1 α, $25(OH)_2D_3$). Biologically, 1 α, $25(OH)_2D_3$ is the primary metabolically active form known as calcitriol. Vitamin D, as a general term, is referred to as calciferol (2).

A. Chemistry

Vitamin D refers to steroids that are antirachitic. Structures of vitamin D_3 (cholecalciferol) and the steroid nucleus are given in Figure 11.3. The International Union of Pure and Applied Chemists – International Union of Biochemistry (IUPAC-IUB) nomenclature rules for steroid structure are used to characterize the ring system (45, 46). The rings (A, B, C, D) are derived from the cyclopentanoperhydrophenanthrene steroid structure. Cholesterol serves as the parent compound (Figure 11.3) (46). In nature, 7-dehydrocholesterol and ergosterol are the provitamin forms for cholecaliferol (D_3) or ergocalciferol (D_2). Previtamin D_2 requires opening of the D ring at the 9,10 bond. The open-ring vitamin D forms are secosteroids. Accepted IUPAC-IUB systematic names are 9,10-seco(5Z,7E)-5,7,10(19) cholestatriene-3β-ol for vitamin D_3 and 9,10-seco(5Z,7E)-5,7,10(19),22 ergostatetraene-3β-ol for vitamin D_2. Conversions of the provitamins to previtamins D_2 and D_3 by irradiation to vitamin D_2 and vitamin D_3 are shown in Figure 11.4 along with the structures of the hydroxylated vitamin forms ($25(OH)D_3$ and 1 α, $25(OH)_2D_3$). Vitamin D_2 and vitamin D_3 structurally vary by a double bond at C-22 and a methyl group at C-24

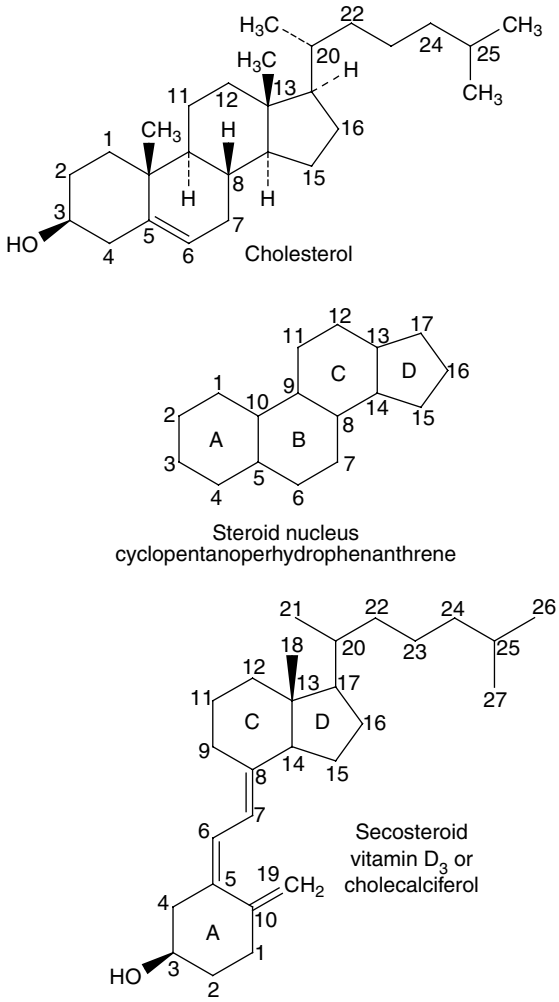


FIGURE 11.3 Structures of vitamin D_3, cholesterol, steroid, nucleus.

in vitamin D_2. Since the structures are quite similar, chemical and physical properties are similar (Table 11.3).

1. Spectral Properties

Vitamin D has a characteristic broad UV spectrum with maximum absorption near 264 nm and a minimum near 228 nm (47). The vitamin does not fluoresce. Because vitamin D is present, even in fortified foods, at quite low concentrations, most methods of quantification by LC rely on extract clean-up and concentration prior to determinative chromatography (5). With proper sample treatment, UV detection at 264–265 nm is sensitive and specific enough to provide reliable data. UV detection is generally not sensitive enough to quantify hydroxylated metabolites in serum or biological tissues.

2. Stability

Stability of vitamin D is excellent in the absence of water, light, acidity, and at low temperatures (47). The

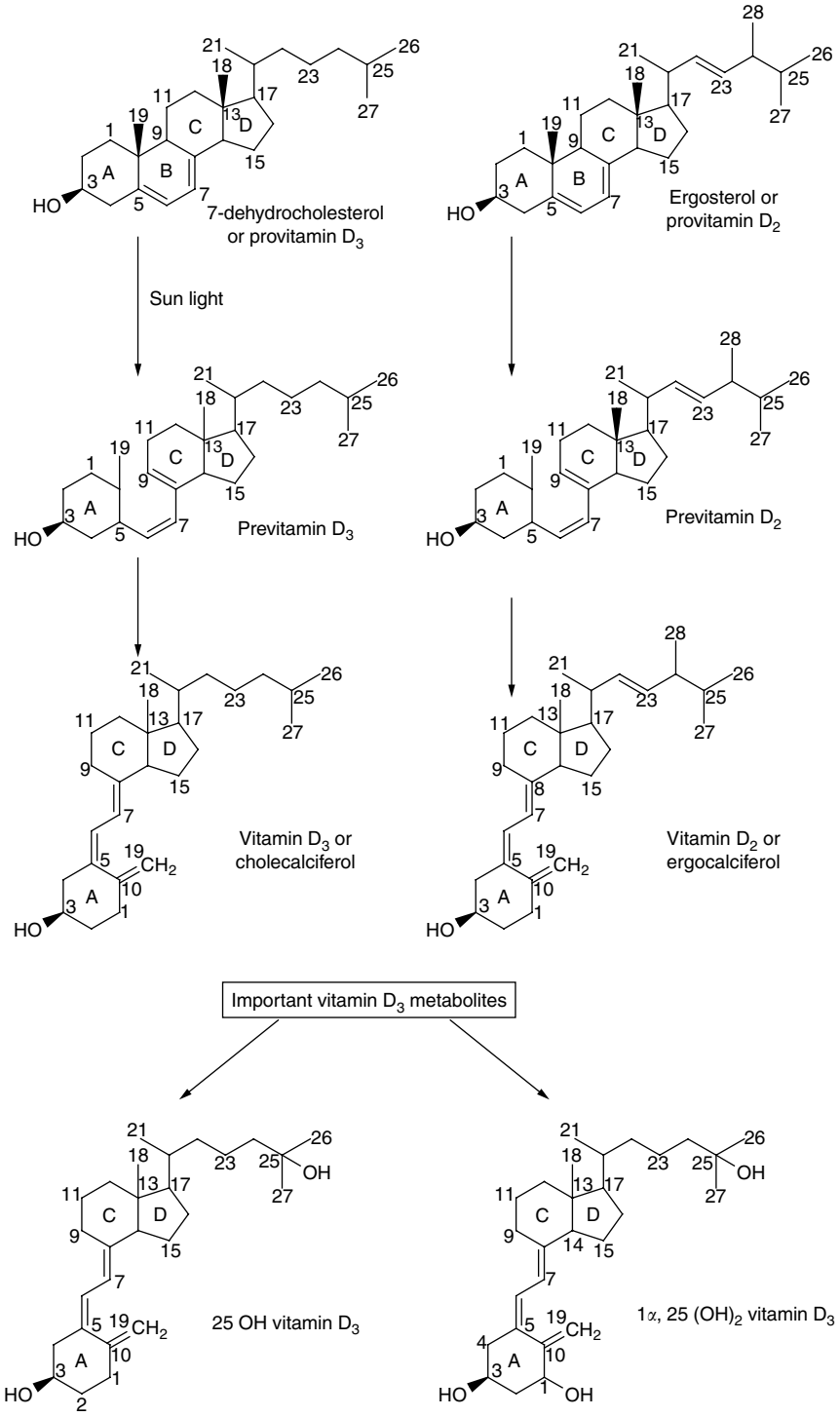


FIGURE 11.4 Structures of vitamin D, its precursors and metabolites (from Ref. 8).

5,6-trans-isomer and isotachysterol can form when exposed to acid or light (48). For food samples, saponification provides a convenient first step in extraction of vitamin D, since the stability under alkaline conditions is quite good. Precautions discussed in the vitamin A-carotenoid section of this chapter must be adhered to for extraction of vitamin D even though vitamin D is considered to be much more stable to oxidation compared to vitamin A. Oxidation can be a predominate route for decomposition at the conjugated double bond system at the 5,6 and 7,8 positions of the secosteroid structure. However, vitamin D is less susceptible to oxidative losses than vitamin A, carotenoids or vitamin E (47). Since various environmental conditions can lead to isomerization of vitamin D_2 and vitamin D_3 to the previtamin

TABLE 11.3
Physical Properties of Vitamin D, Hydroxylated Forms, Vitamin E and Vitamin K

						Spectral Properties[b]				
									Fluorescence[c]	
Substance[a]	Molar Mass	Formula	Solubility	Melting Point EC	Crystal Form	λ max nm	$E^{1\%}_{1cm}$	ε×10^{-3}	λ_{Ex}	λ_{Em}
Vitamin D_3 CAS No. 67-97-0 **10079**	384.64	$C_{27}H_{44}O$	Soluble in most organic solvents; insoluble in water	84–85	Fine needles	264	485	18.3		
Vitamin D_2 CAS No. 50-14-6 **10078**	396.65	$C_{28}H_{44}O$	Soluble in most organic solvents; insoluble in water	115–118	Prisms yellow to white	264	462	19.4		
25(OH) vitamin D_3 CAS No. 19356-17-3 **1638**	400.64	$C_{27}H_{44}O_2$	Soluble in most organic solvents; insoluble in water	82–83		265	[454]	18.2		
1α,25$(OH)_2$ vitamin D_3 CAS No. 32222-06-3 **1643**	416.63	$C_{27}H_{44}O_{33}$	Slightly soluble in methanol, ethanol, and ethyl acetates; insoluble in water	111–115	White crystalline powder	264	[418]	19		
α–T CAS No. 59-02-9 **9571**	430.7	$C_{29}H_{50}O_2$	Freely soluble in oils, fats, acetone, alcohol, chloroform, ether	2.5 ~ 3.3		292	75.8	[3265]	295	320
β–T CAS No. 16698-35-4 **9572**	416.68	$C_{28}H_{48}O_2$	Freely soluble in oils, fats, acetone, alcohol, chloroform, ether	296		296	89.4	[3725]	297	322
γ–T CAS No. 54-28-4 **9573**	416.68	$C_{28}H_{48}O_2$	Freely soluble in oils, fats, acetone, alcohol, chloroform, ether	−2 ~ −3		298	91.4	[3808]	297	322
δ–T CAS No. 119-13-1 **9574**	402.65	$C_{27}H_{46}O_2$				298	87.3	[3515]	297	322
α–T3 CAS No. 58864-81-6 **9576**	424.66	$C_{29}H_{44}O_2$				292	91.0	[3864]	290	323
β–T3 CAS No. 490-23-3 **9577**	410.63	$C_{28}H_{42}O_2$				294	87.3	[3585]	290	323
γ–T3 CAS No. 59-02-9	410.64	$C_{28}H_{42}O_2$				296	90.5	[3716]	290	324
δ–T3 CAS No. 59-02-9	396.61	$C_{27}H_{40}O_2$				297	88.1	[3494]	292	324
α-tocoperyl acetate CAS No. 58-95-7 (l) 52225-20-4 (dl) **9571**	472.74	$C_{31}H_{52}O_3$	Freely soluble in acetone, chloroform, ether	26.5~ 27.5 −27.5		286	40-44	[1891–2080]	285	310
α-tocopheryl succinate CAS No. 4345-03-3 **9571**	530.79	$C_{33}H_{54}O_5$	Practically insoluble in water	76 ~ 77		286	38.5	[2044]	–	–
Phylloquinone Vitamin K_1 CAS No. 84-80-0 **7465**	450.69	$C_{31}H_{46}O_2$	Sparingly soluble in methanol; soluble in ethanol, acetone, benzene, petr ether, hexane, dioxin, chloroform, ether	–	None yellow viscous oil	242 248 260 269 325	396 419 383 387 68	[17.8] [18.9] [17.3] [17.4] [3.1]		

(*continued*)

TABLE 11.3 (*Continued*)

Substance[a]	Molar Mass	Formula	Solubility	Melting Point EC	Crystal Form	Spectral Properties[b] λ max nm	$E^{1\%}_{1cm}$	ε×10⁻³	Fluorescence λ_{Ex}	Fluorescence λ_{Em}
Menaquinone-4	444.65	$C_{31}H_{40}O_2$		35	Yellow	248	439	[19.5]		
Vitamin $K_{2(20)}$					crystals					
CAS No. 863-61-6										
MK-4										
5855										
Menaquinone-6	580.88	$C_{41}H_{56}O_2$		50	Yellow	243	304	[17.7]		
					crystals					
Vitamin $K_{2(30)}$						248	320	[18.6]		
CAS No. 84-81-1						261	290	[16.8]		
MK-6						270	292	[16.9]		
5855						325–328	53	[3.1]		
Menaquinone-7	649	$C_{46}H_{64}O_2$		54	Light	243	278	[18.0]		
Vitamin $K_{2(35)}$					yellow	248	195	[19.1]		
CAS No. 2124-57-4					micro-	261	266	[17.3]		
MK-7					crystalline	270	267	[17.3]		
5855					plates	325-328	48	[3.1]		
Menadione	172.18	$C_{11}H_8O_2$	Insoluble in water;	105–107	Bright					
Vitamin K_3			moderately soluble		yellow					
CAS No. 58-27-5			in chloroform,		crystals					
5853			carbon tetrachloride							

[a]Common or generic name; CAS No. – Chemical Abstract Service number, bold print designates the Merck Index monograph number, l=Natural form, dl=Synthetic form.

[b]In ethanol (in petroleum ether for vitamin K), values in brackets are calculated from corresponding $E^{1\%}_{1cm}$ or ε value.

[c]In hexane.

Source: Refs. 16, 25, 86–88, 131–133.

forms, analytical methods must be capable of measuring all biologically active forms, including the previtamins, to accurately assess vitamin D activity. Thermal interconversion is difficult to completely avoid during sample preparation prior to quantification.

Vitamin D is quite stable to food processes used for fluid milk or in the production of nonfat dry milk. Research has shown that light and air exposure of fluid milk in the marketing channel results in only small losses of vitamin D (48). Documentation of degradation during the production of spray-dried, fortified whole milk showed that vitamin D was stable to the preheating by direct steam injection to 95°C, five-stage evaporation and spray-drying. No significant losses were noted (49).

B. Nutrition and Biochemistry of Vitamin D

1. Functions of Vitamin D

Vitamin D functions as a steroid hormone. In this respect, vitamin D_2 and D_3 undergo conversion through hydroxylation to the biologically active 1 α, $25(OH)_2D$ form. 25(OH)D is initially formed in the liver by the action of vitamin D 25-hydroxylase. The 25(OH)D constitutes the primary circulating form of vitamin D, and circulating levels can be used as an indication of overall vitamin D status. In the kidney, 25(OH)D is hydroxylated by 25-hydroxyvitamin D-1 α-hydroxylase to 1 α, $25(OH)_2D$ (50, 51). The dihydroxy form is then transported to target tissues where receptor binding occurs. Hydroxylation in the kidney also forms 24R, $25(OH)_2D$, although actions for this dihydroxy form of vitamin D are not clearly established (51). Overall, the actions of 1 α, $25(OH)_2D$ include maintenance of serum calcium and phosphorous concentrations and mobilization of monocytic stem cells in the bone marrow to become mature osteoclasts (2). In this regard, 1 α, $25(OH)_2D$ regulates mineral homeostasis by stimulation of the intestinal lumen-to-plasma flux of calcium and phosphorous, stimulation of renal resorption of calcium and phosphorous, and stimulation of bone resorption to increase calcium and phosphorous levels in the serum (51). Osteoblast formation is controlled at the bone cell differentiation level. In the kidney, regulation of

the overall vitamin D endocrine system occurs through control of activity of the $25(OH)_2D$-1 α-hydroxylase. Other accepted roles for 1 α, $25(OH)_2D$ include general effects on cell regulation and differentiation, regulation of protein synthesis, essentiality for insulin secretion, neural function and brain metabolism, immunological function, estrogen synthesis (52), and antiproliferative effects on various cancers (53–60). Detailed discussion on the complex vitamin D endocrine system has been presented by Norman and colleagues (44, 51).

2. Vitamin D Deficiency

Inadequate intake of vitamin D, lack of exposure to sunlight or metabolic failure to convert vitamin D to 1 α, $25(OH)_2D$, or a combination of the factors leads to deficiency (2). Deficiency states are characterized by inadequate mineralization or demineralization of the skeleton. These states are referred to as rickets in children and osteomalacia in adults. Deficiency due to inadequate intake or exposure to sunlight responds to supplementation of the diet but vitamin D-resistant rickets does not. Vitamin D-resistant rickets arises through genetic disorders and includes loss of the renal resorption system for phosphate, absence of the 25-hydroxy-vitamin D-1 α-hydroxylase in the kidney and through disruption of the vitamin D receptor gene (61). Rickets in children produces widening at the end of long bones, rachitic rosary, deformations in the skeleton (bowed legs, knocked knees, curvature of the spine, and others) (2). Osteomalacia in adults results in loss of calcium from the bone with bone pain, muscular weakness, and development of a porous bone structure (2, 44). Absence of sufficient 1 α, $25(OH)_2D$ leads to decreased circulating calcium levels and an increased production of parathyroid hormone (PTH). PTH stimulates calcium mobilization from the bone, conserves calcium excretion and increased excretion of phosphorous with the effect of bone demineralization and osteoporosis (62–64).

3. Hypervitaminosis D

Hypervitaminosis D can occur through improper use of supplements, excessive intake of foods fortified with vitamin D or rarely through manufacturing errors of supplements or food resulting in improperly labeled products with excessively high vitamin D levels. Current Upper Tolerable Intake Levels (UL) range from 25 to 50 μg/day (Table 11.2); however, severe effects have been noted at intakes of 250–1,250 μg/day or higher (2). Symptoms include increased intestinal absorption of calcium and increased resorption of calcium from bone resulting in hypercalcemia. Hypercalcemia can lead to loss of renal function. Other symptoms include anorexia, nausea, vomiting, thirst, polyuria, muscular weakness, joint pain, and general disorientation with eventual death (44).

4. Dietary Reference Intakes

Dietary Reference Intakes (DRIs) for vitamin D are limited to Adequate Intakes (AI) and Tolerable Upper Intake Levels (UL) (2). AIs range from 5 μg/day for infants through 50 year adults and 15 μg/day (600 IU/day) for older adults (>70 year). The AI recommendations assume that no vitamin D is available from synthesis in the skin by exposure to sunlight (2). UL values range from 25 μg/day for infants (0–6 months) to 50 μg/day for all other age groups (Table 11.2). Regarding development of hypervitaminosis D, only a small degree of safety exists between the AIs and the levels that can produce symptoms of hypervitaminosis D. For infants (AI-5 μg/day) 45 μg of vitamin D per day has been established as the NOAEL (highest level at which no adverse effects have been observed) (2). This level represents an intake only 9 times higher than the AI. The UL for infants is set at 25 μg/day (5 times the AI). For adults, the UL is 50 μg/day or 10 times the AI.

5. Vitamin D Sources for the Human

a. Synthesis in the Skin

The availability of vitamin D from unfortified foods is limited, and the major source to the human is synthesis in the skin upon exposure to sunlight. Synthesis is completely dependent upon exposure to the sun and varies with season, climate, and environmental conditions that can limit exposure to the sun (65). 7-Dehydrocholesterol from cholesterol is photoconverted to previtamin D_3 by exposure to UV irradiation between 290 and 315 nm (Figure 11.4). The previtamin D_3 is then isomerized to vitamin D_3. Likewise, ergosterol can be converted to previtamin D_2 by photoconversion. However, plant foods are generally devoid of vitamin D activity. Variability of sunlight exposure, particularly during winter months, makes dietary supply of vitamin D essential to avoid deficiency.

b. Food Sources and Dietary Intake

Vitamin D content of food and supplements is reported on an international unit (IU) or microgram basis (2). One IU of vitamin D is defined as the activity of 0.025 μg of cholecalciferol (vitamin D_3) measured by the rat or chick bioassay. The United States Pharmacoepial (USP) standard is either vitamin D_2 or vitamin D_3 since biological activity for the human is equal. Applications are developing for the use of 25(OH)D in supplements and for food fortification (66). The activity of 25(OH)D is 5 times that of vitamin D_3 (1 IU=0.005 μg) (2).

Fortified foods represent the only concentrated dietary sources of vitamin D other than some fatty fish (salmon), liver of some aquatic mammals, some fish oils and eggs from hens that have been fed rations containing high levels of vitamin D (2). The lack of many naturally occurring significant vitamin D sources, and the fact that consumption of higher vitamin D-containing foods

requires large intake levels to meet the vitamin D requirement places a significant emphasis on the role of food fortification in ensuring an adequate dietary supply of vitamin D. In the United States, the primary vehicle for food fortification is fluid milk. Although fortification of fluid milk is optional (38), most fluid dairy products are fortified with 400 IU/quart (10 μg/quart or 9.6 μg/L). Margarine fortification in the United States is optional, and most commercial margarines are only fortified with retinyl palmitate and β-carotene (8). Vitamin D_2 and vitamin D_3 can be used interchangeably for food fortification; however, for fluid milk products, most are fortified with vitamin D_3. Synthetic vitamin D_2 or D_3 is readily available to the food and supplement industries.

Since food fortification with vitamin D requires the addition of μg levels to the food on a per serving basis, problems have occurred with both over- and underfortification of fluid milk and infant formulas which must contain 40 IU/100 kcal. Surveys have noted wide variability in vitamin D content of milk and infant formula products arising from processing errors (67–69).

Dietary intake in the United States was estimated at a median level of 2.9 μg/day for young women from the second National Health and Nutrition Examination Survey (NHANES II) (2). However, it is recognized that reliable intake data do not exist (2). Lack of reliable data results from analytical difficulties, variable composition of fortified foods, and because food intake surveys have not emphasized vitamin D (2). Recommendations pertaining to the dietary reference intakes (DRI) were limited to average intake values (AI) due to the lack of reliable data required to set estimated average requirements (EARs) and recommended dietary allowances (RDAs). Average intake values range from 5 μg/day for infants to 15 μg/day for adults 70 years or older with the assumption that no vitamin D is available from sunlight exposure. Further, it is recognized that older individuals are more susceptible to conditions leading to vitamin D deficiency (2).

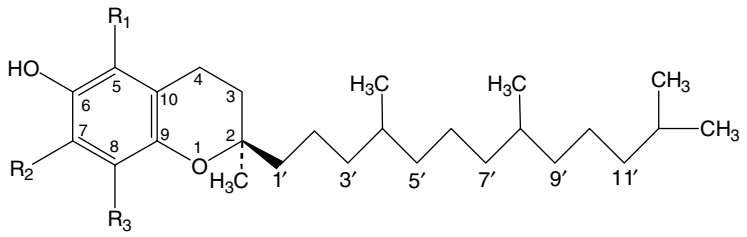
IV. VITAMIN E – TOCOPHEROLS AND TOCOTRIENOLS

Evans and Bishop discovered and characterized a fat-soluble nutritional factor necessary for reproduction and fetal death in rats (70). Published in 1922, the factor was designated "Factor X" and the antisterility factor. The name, vitamin E, was given since its discovery closely followed the discovery of vitamin D. A vitamin E active compound was isolated from wheat germ oil by the Evans' research group and named α-tocopherol (α-T) from the Greek words *tocos* (birth) and *ferein* (bringing) to denote the essentiality of the vitamin to reproduction in rats (71). The "ol" ending denoted that the compound was an alcohol (72). Other significant early historical events of vitamin E research include the isolation of β- and γ-tocopherol (β-, γ-T) from vegetable oil in 1934 (73), determination of the structure of α-T in 1937 (74, 75), synthesis of α-T in 1938 (76), recognition of the antioxidant activity of the tocopherols (77), recognition that α-T was the most effective tocopherol in prevention of vitamin E deficiency (73), isolation of δ-tocopherol (δ-T) from soybean oil in 1947 (78), and identification of the four naturally occurring tocotrienols (α-T3, β-T3, γ-T3, δ-T3) (79, 80). Quantification of the vitamin E content of the diet was initiated in the late 1940s. Publication of the paper "Vitamin E Content of Foods" in 1950 (81) represents one of the earliest documentations of dietary levels.

A. Chemistry of Vitamin E

Vitamin E is the collective term for fat-soluble 6-hydroxychroman compounds that exhibit the biological activity of α-T measured by the rat resorption-gestation assay. Tocol (Figure 11.5) (2-methyl-2-(4′,8′,12′-trimethyltridecyl)-chroman-6-ol) is generally considered the parent compound of the tocopherols. Accepted nomenclature has been set by the IUPAC-IUB Joint Commission on Nomenclature (82–84). Naturally occurring vitamin E consists of α-, β-, γ-, and δ-T and the corresponding α-, β-, γ-, and δ-T3 (tocotrienol), (Figure 11.5). The tocopherols are characterized by the 6-chromanol ring structure methylated to varying degrees at the 5-, 7-, and 8-positions. At position 2, there is a C16 saturated side chain. The tocotrienols are unsaturated at the 3′, 7′, and 11′ positions of the side chain. The specific tocopherols and tocotrienols, therefore, differ by the number and positions of the methyl groups on the 6-chromanol ring. α-Tocopherol and α-T3 are trimethylated; β-T, β-T3, γ-T, and γ-T3 are dimethylated; and δ-T and δ-T3 are monomethylated (Figure 11.5). Trivial and chemical names are given in Figure 11.5.

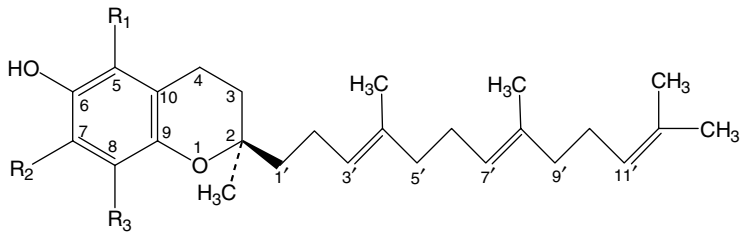
The tocopherols possess three asymmetric carbons (chiral centers) at position 2 of the chromanol ring and at positions 4′ and 8′ of the phytyl side chain. Synthetic α-T (all-rac-α-T) is a racemic mixture of equal parts of each stereoisomer. Therefore, each tocopherol has eight (3) possible optical isomers. Only RRR-tocopherols are found in nature. The eight isomers of all-rac-α-T (RRR-, RSR-, RRS-, RSS-, SRR-, SSR-, SRS-, and SSS-) are depicted in Figure 11.6. Only the 2R-stereoisomeric forms (RRR-, RSR-, RRS-, and RSS) of α-T are considered active forms of vitamin E for the human (3). The tocotrienols arising from 2-methyl-2-(4′,8′,12′-trimethyltrideca-3′,7′,11′-trienyl) chroman-6-ol (non-methylated ring structure) have only one chiral center at position 2. Consequently, only 2R and 2S stereoisomers are possible. Unsaturation at position 3′ and 7′ of the phytyl side chain permits four cis/trans geometric isomers. The eight potential tocotrienol isomers are given in Table 11.4. Only the 2R, 3′ trans, 7′ trans isomer exists in nature. Isolation and elucidation of the structural properties of the tocotrienols



Tocopherols

Trivial name	Chemical name	Abbreviation	Ring position R^1	R^2	R^3
Tocol	a	-	H	H	H
α-tocopherol	5,7,8-trimethyltocol	α-T	CH_3	CH_3	CH_3
β-tocopherol	5,8-dimethyltocol	β-T	CH_3	H	CH_3
γ-tocopherol	7,8-dimethyltocol	γ-T	H	CH_3	CH_3
δ-tocopherol	8-methyltocol	δ-T	H	H	CH_3

a = 2-methyl-2-(4′,8′,12′-trimethyltridecyl) chroman-6-ol



Tocotrienols

Trivial name	Chemical name	Abbreviation	Ring position R^1	R^2	R^3
Tocol	b	-	H	H	H
α-tocotrienol	5,7,8-trimethyltocotrienol	α-T3	CH_3	CH_3	CH_3
β-tocotrienol	5,8-dimethyltocotrienol	β-T3	CH_3	H	CH_3
γ-tocotrienol	7,8-dimethyltocotrienol	γ-T3	H	CH_3	CH_3
δ-tocotrienol	8-methyltocotrienol	δ-T3	H	H	CH_3

b = 2-methyl-2-(4′,8′,12′-trimethyltrideca-3′,7′,11′-trienyl) chroman-6-ol

FIGURE 11.5 Structural interrelationship of tocopherols and tocotrienols.

was accomplished in the 1960s (80, 85). Physical properties of various vitamin E forms are given in Table 11.3.

1. Spectral Properties

UV and fluorescence properties of vitamin E compounds are given in Table 11.3. Maximal UV absorption for tocopherols, tocotrienols and their esters occurs between 292 and 298 nm. Minimum absorption is between 250 and 260 nm (25, 89–92). Esterification at the C-6 hydroxyl shifts the absorption to shorter wavelengths. For example, all-rac-α-tocopheryl acetate has maximal absorption at 286 nm (88, 91, 92). Intensity of absorption decreases with esterification. Reported $E^{1\%}_{1cm}$ values for all-rac-α-tocopheryl acetate range from 40 to 44 compared to 75.8 to 91.8 for the tocopherols and tocotrienols (88).

Vitamin E alcohols possess strong native fluorescence that provides an ideal and very specific mode of detection for LC-based methods. Excitation of the chroman ring near or at maximal absorption produces maximal emission at 320 nm or slightly higher wavelengths. Many quantitative methods are based on excitation at 292 nm and emission at 320 nm (88). Vitamin E esters show only weak fluorescence compared to the alcohols. Older literature (prior to 1985) often states that α-tocopheryl acetate does not fluoresce. However, the ester shows weak fluorescence sufficient to quantify the ester that is easily detected by currently available fluorescent detectors. By measuring the α-tocopheryl acetate as the ester and avoiding saponification of the sample, accurate measure of the biological activity is possible. Biological activity of all-rac-α-tocopheryl acetate is lower than that of RRR-α-T.

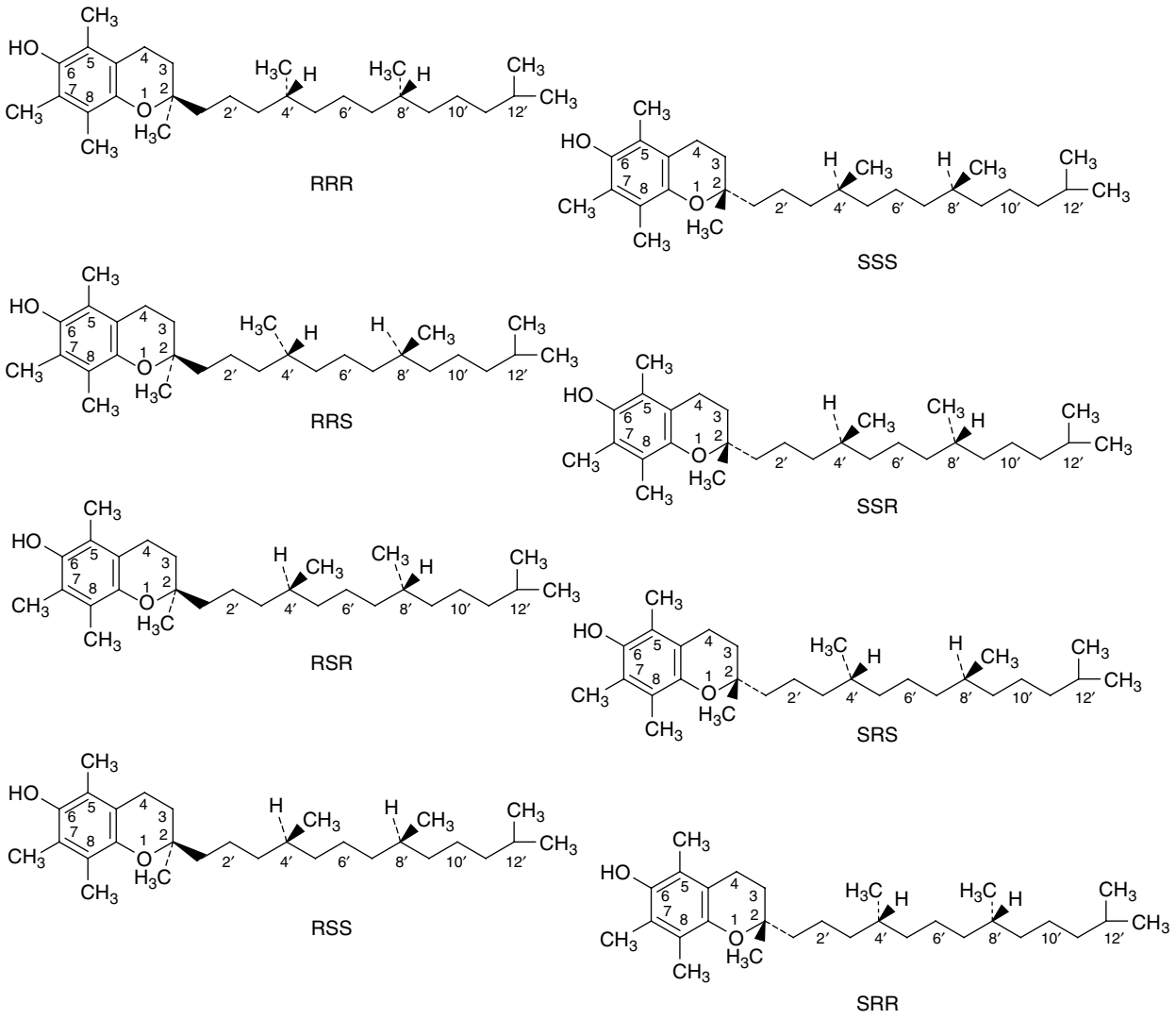


FIGURE 11.6 Stereoisomers of α-tocopherol (from Ref. 8).

TABLE 11.4
The Eight Possible RS, Cis/Trans Isomers of the Tocotrienols

R Configuration Position 2	S Configuration Position 2
2R, 3′cis, 7′cis	2S, 3′cis, 7′cis
2R, 3′cis, 7′trans	2S, 3′cis, 7′trans
2R, 3′trans, 7′cis	2S, 3′trans, 7′cis
2R, 3′trans, 7′trans	2S, 3′trans, 7′trans

2. Stability

Vitamin E is a natural antioxidant, acting as a chain-breaking, primary antioxidant to intercept peroxyl free radicals in biological systems. As an antioxidant, α-T, or other vitamin E alcohols, is, thus, converted into the α-tocopheryl radical and to termination products consisting of dimers and trimers and various oxygenated forms. Since it is active in any fat system undergoing oxidation, oxidative losses can become substantial quite rapidly. Losses are accelerated by light, heat, irradiation, alkali pH, lipoxidase activity, metals, and by the presence of other proxidants in the fat system, including pre-formed free radicals (88). Tocopherols and tocotrienols are stable to heat and alkaline conditions necessary for saponification of lipids. Therefore, saponification is routinely used for extraction of vitamin E prior to analysis by LC.

Frying of foods normally results in loss of the native vitamin E components in the edible oil. Heat combined with incorporation of air with the food, the polyunsaturated nature of the oil and introduction of proxidants into the oil provides an ideal environment for oxidation. Vitamin E through its antioxidant action, combined with some loss through volatilization, leads to rapid loss of vitamin E from the frying oil. Refining of edible oil produces some loss of native vitamin E, primarily at the

deodorization stage. However, loss is not enough to destabilize the oil to oxidation. Deodorizer sludge, once a by-product of edible oil refining with little value, now represents the raw material source for isolation of natural vitamin E. Natural vitamin E is in demand world-wide for use in supplements, feeds, and cosmetics (72).

B. Nutrition and Biochemistry of Vitamin E

1. Functions of Vitamin E

Vitamin E is the primary, lipid-soluble, chain-breaking antioxidant that combines actions with other lipid and water-soluble antioxidants to provide cells an efficient defense against free radical damage. Free radicals are chemical species capable of independent existence that contain one or more unpaired electron (93). Free radical generation occurs when organic molecules undergo homolytic cleavage of covalent bonds and each fragment retains one electron of the original bonding electron pair. Two free radicals are produced from the parent molecule with net negative charges. The free radicals have the ability to react with an electron of opposite spin from another molecule. Free radical generation also occurs when a nonradical molecule captures an electron from an electron donor. During normal metabolism, many reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced (94). ROS and RNS include both radicals and oxidants capable of generation of free radicals (95, 96). Common ROS and RNS are given in Table 11.5.

Oxidants and oxygen radicals formed from triplet oxygen by reaction with other radicals or by photoexcitation, metabolism, irradiation, metal catalysis, or heat are the major inducers of oxidative stress in living systems. They also initiate antioxidative events in raw and processed foods. RNS, particularly nitric oxide ($NO^{\cdot}$) contributes to oxidative stress along with ROS. Nitric oxide acts as a biological messenger with regulatory functions in the central nervous, cardiovascular, and immune systems (97). It is synthesized through the oxidation of arginine to $NO^{\cdot}$ by nitric oxide synthetase (NOS:EL 1.14.13.39). Nitric oxide synthetase is highly active in macrophages and neutrophilis where $NO^{\cdot}$ and superoxide anion ($O_2^{\cdot -}$) are produced during the oxidative burst triggered by inflammation (98).

TABLE 11.5
Reactive Oxygen and Nitrogen Species

Radicals	Nonradicals
ROS	
Superoxide, $O_2^{\cdot}$ Γ	Fe-oxygen complex
Hydroxy, $OH^{\cdot}$	Hydrogen peroxide, H_2O_2
Alkoxy, $LO^{\cdot}$	Singlet oxygen, $^1O_2$
Hydropheroxyl, $HO_2^{\cdot}$	Ozone, O_3
Peroxy, $LO_2^{\cdot}$	Hypochlorous acid, HOCl
RNS	
Nitric oxide, $NO^{\cdot}$	Nitrous acid, HNO_2
Nitrogen dioxide, $NO_2^{\cdot}$	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peroxynitrate, ONOOΓ
	Peroxynitrous acid, ONOOH
	Nitronium cation, NO_2^+
	Alkyl peroxynitrates, ROONO

α-Tocopherol is located in the cell membranes and protects lipoproteins. It scavenges peroxy free radicals, protecting unsaturated fatty acids. Lipid-generated free radicals have greater affinity for reaction with α-T than with unsaturated fatty acids located within the cell membrane. It is an efficient chain-breaking antioxidant since it can rapidly transfer the phenolic H^+ at C-6 to lipid peroxy radicals. The α-T becomes the α-tocopheroxyl radical, which is stabilized by resonance.

Potency of α-T as an antioxidant depends upon its molecular properties and orientation within the cell membrane. In the membrane, the phytyl side chain is embedded within the bilayer (Figure 11.7) with the chromanol ring and the 6-hydroxyl positioned toward the surface of the membrane. Hydrogen bonding and hydrophobic interactions between the chromanol ring, the phytyl tail, and fatty acids stabilize the membrane and position the chromanol ring to facilitate hydrogen atom donation to lipid peroxy radicals. The α-tocopheroxyl radical migrates from the lipid bilayer to the surface of the membrane, facilitating regeneration of α-T by ascorbic acid and other water-soluble reducing agents that act as hydrogen donors to the α-tocopheroxyl radical.

Non-autoxidative roles for α-T have been recently delineated which cannot be fulfilled by other tocopherols or tocotrienols. These functions at the molecular level appear to be highly significant to understanding the onset of many chronic diseases. Several critical reviews exist that indicate that the molecular control aspects of vitamin E are just beginning to be understood (98–103). α-Tocopherol acts as a cell signaling molecule at the posttranscriptional level or at the gene expression level. Many of these cell signaling functions of α-T are operative through inhibition of protein kinase C (PKC). PKC enzymes are phospholipid-dependent serine/threonine kinases that participate in regulation of cell growth, death, and stress responsiveness (104). α-Tocopherol acts at the posttranscriptional level by activating protein phosphatase PP_2A which dephophorylates PKC (105, 106). Some specific physiological responses regulated by PKC include cell proliferation, platelet adhesion and aggregation, immune response, free radical production and gene expression. Now regulation of gene expression at the transcriptional stage is accepted as a primary regulatory function of α-T.

Specific non-antioxidant functions for α-T3 have been identified that are not fulfilled by α-T. Hendrich et al. (107)

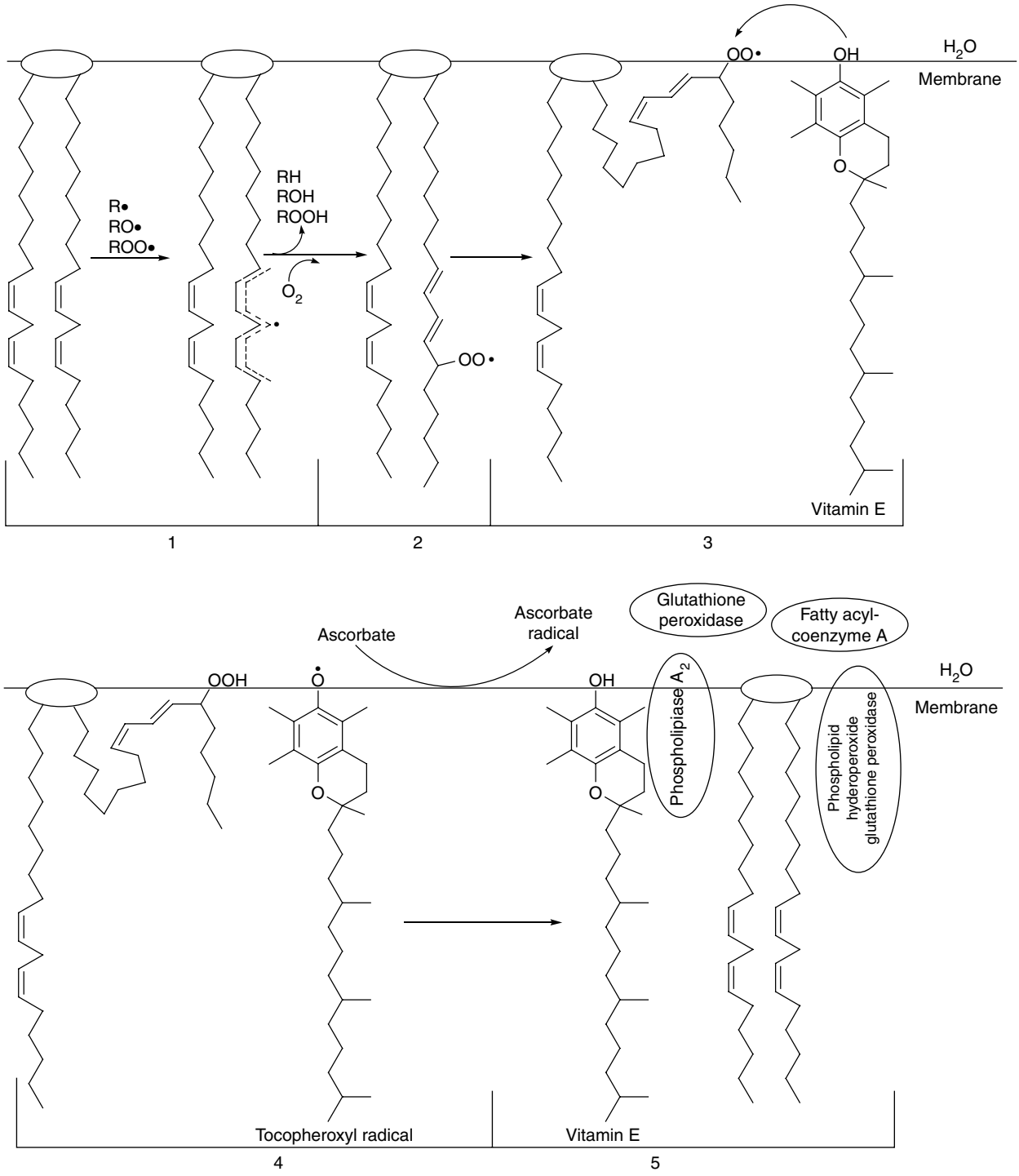


FIGURE 11.7 Representation of the positions of α-T and ascorbic acid at the membrane-water interface.

suggested that tocotrienols should be considered as a specific group of food components independent of the tocopherols due to specific differences in action that differ from actions of α-T. Such specific actions include posttranscriptional suppression of 3-hydroxy-3-methylglutaryl Co A reductase (HMG-Co A reductase) through a cell signaling event (108, 109). The ability of tocotrienol supplements to lower human total serum cholesterol levels is currently controversial (110).

2. Vitamin E Deficiency

Vitamin E deficiency in humans is almost always due to factors other than dietary insufficiency. Deficiency results from

genetic abnormalities in production of the α-tocopherol transfer protein (α-TTP), fat malabsorption syndromes, and protein-energy malnutrition (3). Fat-malabsorption can be related to pancreatic and liver abnormalities that lower fat absorption, abnormalities of the intestinal cells, length of the intestine and defects in the synthesis or assembly of the chylomicrons (61). Genetic abnormalities in lipoprotein metabolism can produce low levels of cylomicrons, very low density lipoproteins (VLDL) and low density lipoprotein (LDL) that affects absorption and transport of vitamin E (111). Abetalipoproteinemia is an autosome recessive genetic disorder leading to mutations in the microsomal triglyceride transfer protein (112). The disease is associated with ataxia and impaired intestinal absorption of lipids and vitamin E, since the triglyceride transfer protein participates in the transport of lipids and other fat-soluble substances. Defects in the gene that codes for α-TTP leads to an inherited autosomal recessive disease referred to as familial isolated vitamin E deficiency or ataxia with vitamin E deficiency (AVED) (112–114). Changes in α-TTP result in inefficient transfer of RRR-α-T from the liver and recycling of plasma RRR-α-T.

Clinical symptoms include many neurological problems stemming from peripheral neuropathy with degeneration of the large-caliber axons in the sensory neurons (3). Common symptoms are ataxia, muscle weakness and hypertrophy, neurological abnormalities, reproductive disorders, abnormalities of the liver, bone marrow, and brain (61). At the cellular level, increased oxidation can occur due to increased oxidative stress.

3. Dietary Reference Intakes

When establishing the dietary reference intakes (DRIs) for vitamin E, the Institute of Medicine, Panel on Dietary Antioxidants and Related Compounds decided that human requirements should only be based on the 2-R isomers of α-T (RRR-, RSR-, RRS-, RSS-) (3). This decision stems from the accumulated evidence of the strong selectivity of α-TTP for the 2-R isomers in the liver which leads to preferential secretion of the 2-R isomers into nascent VLDL. Other forms of vitamin E (2-S isomers of α-T, β-T, γ-T, δ-T, tocotrienols) are primarily secreted into the bile and excreted. The selection process is not 100% effective; however, only small amounts of other dietary and supplemental vitamin E forms are delivered to the cells.

For adults, EAR and RDA are 12 and 15 mg of α-T per day, respectively (Table 11.2). The UL value includes all forms of α-T from supplemental intake of all-rac-α-T. The UL is 1000 mg/day.

Since the DRI values refer only to 2R-α-T forms, discussion is necessary on the units used to report vitamin E concentrations in foods and pharmaceuticals. Currently used units include the following:

1. International Units (IU) and USP Units

$$\textit{Molar Conversion Factor (μmol/IU)} = \frac{\textit{USP Conversion Factor (mg/IU)} \times \textit{1000 (μmol/mol)}}{\textit{Molecular Weight (mg/mol)}}$$

Calculation for RRR-α-tocopheryl acetate:

$$\textit{Molar Conversion Factor (μmol/IU)} = \frac{\textit{USP Conversion Factor (mg/IU)} \times \textit{1000 (μmol/mol)}}{\textit{Molecular Weight (mg/mol)}}$$

$$= \frac{\textit{0.735 (mg/IU)} \times \textit{1000 (μmol/mol)}}{\textit{472 (mg/mol)}} = \textit{1.56 (μmol / IU)}$$

$$\textit{α-T Conversion Factor (mg/IU)} = \frac{\textit{Molar Conversion Factor (μmol/IU)} \times \textit{430 (mg/mol)}}{\textit{1000 (μmol/mol)} \times R}$$

Where R=2 for synthetic vitamin E and esters, R=1 for natural vitamin E and esters.
So, the α-T conversion factor for RRR-α-tocopheryl acetate is determined as follows:

$$\textit{α-T Conversion Factor (mg/IU)} = \frac{\textit{Molar Conversion Factor (μmol/IU)} \times \textit{430 (mg/mol)}}{\textit{1000 (μmol/mol)} \times R}$$

$$= \frac{\textit{1.56 (μmol/IU)} \times \textit{430 (mg/mol)}}{\textit{1000 (μmol/mol)} \times \textit{1}} = \textit{0.067 (mg/IU)}$$

The United States Pharmacopeia (USP) (115) defined the IU of vitamin E as 1 mg of all-rac-α-tocopheryl acetate based on biological activity measured by the rat fetal absorption test. Biological activities of various vitamin E forms in relation to the activity of all-rac-α-tocopheryl acetate are given in Table 11.6. After 1980, the USP discontinued use of the IU and replaced it with USP units based on the same basis of biological activity as the IU (3). One USP unit is defined as the activity of 1 mg of all-rac-α-tocopheryl acetate. Therefore, USP units and IUs are equivalent (116).

The Institute of Medicine, Panel on Dietary Antioxidants and Related Compounds (3) recommended that USP units be redefined by USP to take into account the fact that all-rac-α-T has only 50% of the activity of RRR-α-T present in nature or with other 2R stereoisomers found in all-rac-α-T preparations that are used for food fortification and in supplements (3). Factors to convert USP units (IUs) to RRR-α-T or other 2R-isomers of α-T are given in Table 11.7.

Derivation of the conversion factors given in Table 11.7 follows the general formula:

2. α-Tocopherol Equivalents

TABLE 11.6
Biological Activity of Natural and Synthetic Vitamin E Forms[a]

Vitamin E Forms	Biological Activity	
	USP Units (IU)/mg	Compared to RRR-α-T (%)
Natural Vitamin E (RRR-)		
α-Tocopherol	1.49	100
β-Tocopherol	0.75	50
γ-Tocopherol	0.15	10
δ-Tocopherol	0.05	3
α-Tocotrienol	0.75	50
β-Tocotrienol	0.08	5
γ-Tocotrienol	Not known	Not known
δ-Tocotrienol	Not known	Not known
Synthetic		
2R4′R8′R α-tocopherol	1.49	100
2S4′R8′R α-tocopherol	0.46	31
All*rac*-α-tocopherol	1.10	74
2R4′R8′S α-tocopherol	1.34	90
2S4′R8′S α-tocopherol	0.55	37
2R4′S8′S α-tocopherol	1.09	73
2S4′S8′R α-tocopherol	0.31	21
2R4′S8′R α-tocopherol	0.85	57
2S4′S8′S α-tocopherol	1.10	60
RRR-α-tocopheryl acetate	1.36	91
RRR-α-tocopheryl acid succinate	1.21	81
All*rac*-α-tocopheryl acetate	1.00	67
All*rac*-α-tocopheryl acid succinate	0.89	60

[a]*Source*: Ref. 8.

α-Tocopherol Equivalents (α-TEs) were defined for recommending dietary intakes of vitamin E based on biological activity of tocopherols and tocotrienols determined by the rat fetal absorption test (Table 11.6) (117). One mg of α-TE is the activity of 1 mg of RRR-α-T. Total α-TEs (mg) of mixed diets containing only RRR-isomers is determined by multiplying the amount (mg) of α-T by 1.0, β-T by 0.5, γ-T by 0.1, α-T3 by 0.3, and γ-T3 by 0.05. In fortified foods, the conversion factors for all-rac-α-T and all-rac-α-tocopheryl acetate are 0.74 and 0.67, respectively. Use of the α-TE unit has been the accepted way of reporting vitamin E concentration in foods for approximately the past two decades. The Panel on Antioxidants and Related Compounds (3) determined from USDA food intake survey data that 80% of the α-TE from foods arises from RRR-α-T. Therefore, to convert α-TE mg to RRR-α-T, the conversion factor is 0.8.

The following conversions are fully explained in the Dietary References Intake report (3):

a. mg of α-T in a meal = mg of α-TE × 0.8
b. mg of α-T in a food, fortified food, or multivitamin = IU (USP unit) of RRR-α-T × 0.67 or IU (USP Unit) of all-rac-α-T × 0.45

Anytime both natural and synthetic forms of α-T are present, analytical procedures must be capable of resolution of the specific compounds in order to apply the above formulas. Almost always in a fortified food, both RRR-α-T and all-rac-α-tocopheryl acetate will exist together.

4. Food Sources and Dietary Intake

The second National Health and Nutrition Examination Survey (NHANES II) has been extensively evaluated to show dietary sources of vitamin E in the United States. Major food groups contribute the following percentages of total vitamin E: fats and oils, 20.2; vegetables, 15.1; meat, poultry, and fish, 12.6; desserts, 9.9; breakfast cereals, 9.3; fruit, 5.3; dairy products, 4.5; mixed main dishes, 4.0; nuts and seeds, 3.8; soups, sauces, and gravies, 1.7 (3, 117). Data collected from the Continuing Survey of Food Intakes by Individuals (CSFII, 1994) are given in Table 11.8 (D. Haytowitz, personal communication, 2003). The tabulation shows that high oil content foods are major sources but cereals fortified with α-tocopheryl acetate are also significant sources. Raw tomatoes and tomato products, due to high consumption, are significant sources of vitamin E in the U.S. diet.

Using the CSFII and NHANES data as well as other studies, the DRI committee estimated the median intake of α-T from food and supplements at 9.8 mg for men and 6.8 mg for women (3). It was emphasized that data on vitamin E intake from food intake surveys might be low due to potential for underreporting of energy and fat intake, problems with assessment of fats and oils added during food preparation, uncertainty about the types of fats added, and the variability of food composition tables.

TABLE 11.7
Conversion Factors to Calculate α-Tocopherol from International Units or USP Units to Meet Dietary Reference Intakes for Vitamin E[a]

	USP Units (IU)/mg	mg/USP Units (IU)	μmol/USP Unit (IU)	α-Tocopherol mg/USP Unit (IU)
Natural Vitamin E				
RRR-α-tocopherol	1.49	0.67	1.56	0.67
RRR-α-tocopheryl acetate	1.36	0.74	1.56	0.67
RRR-α-tocopheryl acid succinate	1.21	0.83	1.56	0.67
Synthetic Vitamin E				
All-*rac*-α-tocopherol	1.1	0.91	2.12	0.45
All-*rac*-α-tocopheryl acetate	1	1	2.12	0.45
All-*rac*-α-tocopheryl acid succinate	0.89	1.12	2.12	0.45

[a] *Source*: Ref. 8.

TABLE 11.8
Significant Sources of Vitamin E in the Diet in the United States[a]

		% Vitamin E in U.S. Diet[b]
1	Margarine, regular stick, 80% fat	5.5
2	Salad dressing, mayonnaise, soybean oil, with salt	4.3
3	Oil, soybean, salad or cooking	3.1
4	Cereals, ready-to-eat, Total	2.8
5	Oil, corn, salad or cooking	2.7
6	Shortening, composite, household	2.5
7	Salad dressing, Italian, commercial, regular, with salt	2.4
8	Peanut butter, smooth, with salt	2.3
9	Snacks, potato chips, plain, salted	2.3
10	Eggs, whole, raw, fresh, frozen	2
11	Sauce, pasta, spaghetti/marinara, ready-to-serve	1.6
12	Oil, canola	1.4
13	Tomato products, canned, sauce	1.2
14	Shortening, composite, institutional	1.1
15	Rolls, hamburger or hot dog, plain	1
16	Margarine-like spread, tub, composite, 60% fat, with salt	1
17	Milk, cow, whole, fluid, 3.3% fat	1
18	Oil, cottonseed, salt or cooking	0.9
19	Tomato products, canned, puree, without salt	0.9
20	Fast foods, chicken, breaded, fried, boneless, plain	0.9
21	Broccoli, cooked, boiled, drained	0.9
22	Tomatoes, red, ripe, raw	0.7

[a] *Source*: D. Haytowitz, 2003.

[b] Calculated on the basis of mg α-tocopherol equivalents (mg α–TE).

Although the estimated median intakes for men and women are below the RDA of 15 mg of α-T per day (Table 11.2) and many clinical studies exist that indicate beneficial responses to supplemental vitamin E, the panel believed that clinical evidence was too limited to recommend use of vitamin E supplements for the general population.

V. VITAMIN K

Vitamin K was characterized through the efforts of several research groups in the 1930s. Observations by Dam in Denmark showed that chicks developed blood with poor clotting properties and fatal hemorrhages when fed diets extracted with ether (118). Addition of the ether extract back to the diet alleviated the deficiency. The fat-soluble factor was named vitamin K by Dam in 1935 based on the word "koagulation." In 1939, the vitamin K form (2-methyl-3-phytyl-1,4-naphthoquinone) was isolated by research groups led by Dam and Doisy (119, 120). The vitamin K_1 designation was given along with the generic name of phylloquinone. Vitamin K_2, the menaquinone-n (MK-n) form, was isolated from fermented fish meal by Doisy's group in 1939. Vitamin K_1 was synthesized by Doisy's group in 1939. In 1943, both Dam and Doisy were awarded Nobel Prizes for their work on isolation and synthesis of vitamin K.

A. CHEMISTRY

Vitamin K compounds consist of 2-methyl-1,4-naphthoquinone and all derivatives providing antihemorrhagic activity of vitamin K_1 (phylloquinone). 2-Methyl-1,4-naphthoquinone (Figure 11.8) is the parent compound of the family but does not occur in nature (120). It is commonly used in animal rations and is commercially referred to as menadione (vitamin K_3, MK-0) and, formerly, as menaquinone. Naturally occurring vitamin K forms include vitamin K_1 and the vitamin K_2 series. Vitamin K_1 is phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) (Figure 11.8). Vitamin K_1 is synthesized in the plant kingdom and constitutes the primary food source of vitamin K activity (120). Alkylation at carbon-3 of the 2-methyl-1,4-napthoquinone ring with five-carbon isoprenoid units produces the vitamin K series. Vitamin $K_{1(20)}$, the most common phylloquinone, contains four isoprenoid units of which three are reduced. The reduced side chain or phytyl side chain at carbon-3

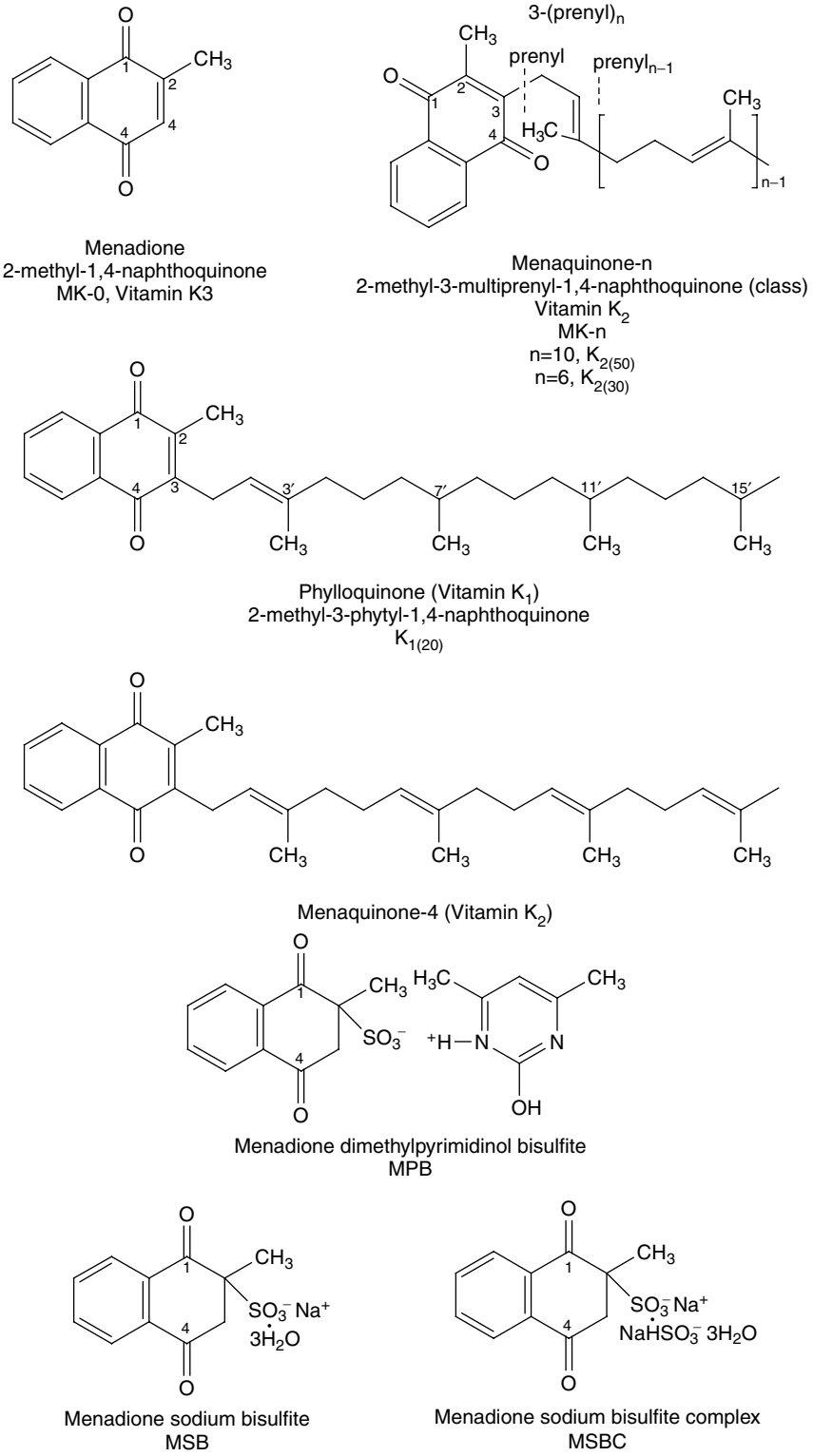


FIGURE 11.8 Structures of vitamin K and water-soluble menadione forms.

of the 2-methyl-1,4-naphthoquinone ring with one double bond characterizes the phylloquinones. The United States Pharmacopeia (USP) designates vitamin K_1 as phytonadione. USP phytonadione is a mixture of E and Z isomers with not more than 21% Z isomer content (115). Natural phylloquinone is 2′-E, 7′R, 11′R (118).

Vitamin K_2 (menaquinone-n series, MK-n) (Figure 11.8) have polyisoprenoid side chains at carbon-3 of the

2-methyl-1,4 naphthoquinone ring. Vitamin K_2 compounds are synthesized by bacteria except MK-4 is synthesized by birds and animals from menadione (119). Side chains are unsaturated and usually contain 4–13 isoprenoid units. The MK-n designation gives the number of prenyl groups in the side chain. One or more of the isoprenoid units may be reduced. Reduction usually occurs at the second isoprenoid units from the naphthoquinone ring (118). Physical properties of vitamin K compounds are given in Table 11.3.

Vitamin K compounds other than menadiones that are modified to increase water solubility are lipid-soluble and soluble in non-polar solvents. Vitamin K_1 (20) is synthesized and used by the food and supplement industries and for use in olestra-containing products (121). Menadione is toxic and not used for human supplements. It is an important nutritional additive to poultry and animal rations. Vitamin K_1 is not used in animal feeds due to cost (25). In poultry rations, chemotherapeutic agents inhibit intestinal synthesis of vitamin K and, therefore, increase dietary requirements (25). Stabilized forms of menadione with increased water solubility are available to the feed industry. These include menadione sodium bisulfate (MSB), menadione sodium bisulfite complex (MSBC), and menadione dimethyl-pyrimidol bisulfite (MPB) (Figure 11.8). The menadione salts are absorbed more efficiently than menadione and show greater stability due to their water solubility.

1. Spectral Properties

Both phylloquinones and menaquinones show UV spectra characteristic of the naphthoquinone ring. Phylloquinone shows absorption maxima at 242, 248, 260, 269, and 325 nm in hexane. The UV spectrum of menadione in hexane has an absorption maximum at 252 nm (25). Vitamin K compounds do not fluoresce. Conversion of the quinone to the hydroquinone induces strong fluorescence. Post-column reduction to the hydroquinone forms the basis of excellent quantitative procedures currently used for assay of vitamin K in foods, supplements, and other biological samples (122). The hydroquinone shows maximal fluorescence at $\lambda_{ex} = 244, \lambda_{em} = 418$.

2. Stability

Vitamin K is quite stable to oxidation and food processing and preparation conditions (122). It is unstable to light and alkalinity. Instability to alkalinity eliminates the use of saponification for extraction from foods; so, it is difficult to include extraction of vitamin K with multi-analyte procedures that rely on saponification as the initial step of the assay. Reducing agents destroy the biological activity of vitamin K_1. Isomerization of trans- to cis-isomers leads to loss of biological activity. Presence of variable quantities of the cis-isomer in vitamin K concentrates requires that quantitative methods be capable of resolving cis- and trans-forms to accurately estimate biological activity (122).

B. Nutrition and Biochemistry of Vitamin K

1. Functions of Vitamin K

Vitamin K is a cofactor for vitamin K-dependent carboxylase required for the posttranslational conversion of glutamic acid to gamma-carboxy-glutamyl residues (Gla) (120, 123). Numerous Gla-containing proteins are known. These include the blood coagulation proteins consisting of prothrombin (factor II, factors VII, IX, and X, and proteins C, S, and Z) (120). Three Gla proteins including osteocalcin, matrix Gla protein, and protein S (120, 123) are bone matrix components. Various other Gla proteins have been identified, but the functions for such vitamin K-dependent proteins are not clearly understood (120).

2. Vitamin K Deficiency

Deficiency of vitamin K in the adult is rare. It is defined as vitamin K-responsive hypoprothrombinemia characterized by an increase in prothrombin time (PT) (1). It usually results from fat malabsorption syndromes, liver disease, and antibiotic treatments that inhibit synthesis of vitamin K_2 by the gut microflora. Anticoagulant treatment with coumarin produces a secondary deficiency by disruption of vitamin K function and inhibition of synthesis of vitamin K-dependent clotting proteins.

In human infants, deficiency of vitamin K commonly occurs and is named hemorrhagic disease. The deficiency occurs through poor placental transfer of vitamin K from the mother to the fetus and lack of bacterial synthesis in the gut of the newborn baby. Other factors include the low concentration of vitamin K in breast milk and low concentrations of blood clotting factors at birth (120). Hemorrhages occurs in the skin, subcutaneous tissue, GI tract, umbilical cord, and intracranially. Central nervous system disorders can occur if untreated. Hemorrhagic disease is prevented by intramuscular injection of vitamin K_1 (0.5–1 mg) or oral dosage (2.0 mg) within 6 hours of birth (120). Breast-fed infants develop hemorrhagic disease more frequently than formula-fed infants because human milk is low in vitamin K. Infant formula is fortified at a minimum of 4 μg per 100 kcal as specified by the Infant Formula Act of 1980 (124).

3. Dietary Reference Intakes

The panel on DRIs for vitamin K was unable to establish average requirements for vitamin K because of lack of data; therefore, only Adequate Intake (AI) values were set (1). The AI levels are 120 and 90 μg/day for adult men and

women, respectively (Table 11.2). Tolerable Upper Intake Levels (ULs) were not established, since no adverse effects have been reported at high intake levels (1).

4. Food Sources and Dietary Intake

Leafy green vegetables, certain legumes, and vegetable oils are considered good sources of vitamin K. Vitamin K_1 is the primary food source, and green leafy vegetables provide 40 to 50% of the total intake (125). Milk and dairy products, meats, eggs, cereals, fruits and vegetables, are low but consistent sources of vitamin K to the U.S. diet (122). Reliable data did not exist for the vitamin K content of the diet until excellent LC procedures were developed during the 1990s by Sadowski and colleagues (126–129). Establishment of methodology and collection of reliable data led to the development of a reliable database to estimate intake of vitamin K_1 (130). The database was based on vitamin K_1 intake in women's diets collected from the 1990 Food and Drug Administration Total Diet Study (130). Major food sources were spinach, collards, broccoli, iceberg lettuce, and coleslaw with salad dressing. Addition of fats and oils to mixed dishes is a significant source of vitamin K_1 (125). The panel on DRIs for vitamin K estimated the median intakes of vitamin K_1 for men and women to be 89 to 117 μg and 79 to 88 μg per day, respectively (1). Data from the Third National Health and Nutrition Examination Survey (NHANES III) (43) were used to estimate the intakes.

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12 Fundamental Characteristics of Water

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CONTENTS

I. INTRODUCTION

Water, a natural occurring and abundant substance that exists in solid, liquid, and gas forms on the planet Earth, has attracted the attention of artists, engineers, poets, writers, philosophers, environmentalists, scientists, and politicians. Every aspect of life involves water as food, as a medium in which to live, or as the essential ingredient of life. The food science aspects of water range from agriculture, aquaculture, biology, biochemistry, cookery, microbiology, nutrition, photosynthesis, power generation, to zoology. Even in the narrow sense of food technology, water is intimately involved in the production, washing, preparation, manufacture, cooling, drying, and hydration of food. Water is eaten, absorbed, transported, and utilized by cells. Facts and data about water are abundant and diverse. This chapter can only selectively present some fundamental characteristics of water molecules and their collective properties for readers when they ponder food science at the molecular level.

The physics and chemistry of water are the backbone of engineering and sciences. The basic data for the properties of pure water, which are found in the CRC Handbook of Chemistry and Physics (1), are useful for food scientists. However, water is a universal solvent, and natural waters contain dissolved substances present in the

environment. All solutes in the dilute solutions modify the water properties. Lang's Handbook of Chemistry (2) gives solubility of various gases and salts in water. Water usage in the food processing industry is briefly described in the Nalco Water Handbook (3). For water supplies and treatments, the Civil Engineering Handbook (4) provides practical guides. The Handbook of Drinking Water Quality (5) sets guidelines for waters used in food services and technologies. Wastewater from the food industry needs treatment, and the technology is usually dealt with in industrial chemistry (6). Most fresh food contains large amounts of water. Modifying the water content of foodstuffs to extend storage life and enhance quality is an important and widely used process (7).

A very broad view and deep insight on water can be found in "Water – A Matrix of Life" (8). Research leading to our present-day understanding of water has been reviewed in the series "Water – A Comprehensive Treatise" (9). The interaction of water with proteins (10, 11) is a topic in life science and food science. Water is the elixir of life and H_2O is a biomolecule.

II. WATER AND FOOD TECHNOLOGY

Water is an essential component of food (12). Philosophical conjectures abound as to how Earth evolved to provide the mantle, crust, atmosphere, hydrosphere, and life. Debates continue, but some scientists believe that primitive forms of life began to form in water (13). Complicated life forms developed, and their numbers grew. Evolution produced anaerobic, aerobic, and photosynthetic organisms. The existence of abundant life forms enabled parasites to appear and utilize plants and other organisms. From water all life began (14). Homo sapiens are integral parts of the environment, and constant exchange of water unites our internal space with the environment.

The proper amount of water is also the key to sustaining and maintaining a healthy life. Water transports nutrients and metabolic products throughout the body to balance cell contents and requirements. Water maintains biological activities of proteins, nucleotides, and carbohydrates, and participates in hydrolyses, condensations, and chemical reactions that are vital for life (15). On average, an adult consumes 2 to 3 L of water: 1–2 L as fluid, 1 L ingested with food, and 0.3 L from metabolism. Water is excreted via the kidney, skin, lung, and anus (16). The amount of water passing through us in our lifetimes is staggering.

Aside from minute amounts of minerals, food consists of plant and animal parts. Water is required for cultivating, processing, manufacturing, washing, cooking, and digesting food. During or after eating, a drink, which consists of mostly water, is a must to hydrate or digest the food. Furthermore, water is required in the metabolic process.

Cells and living organisms require, contain, and maintain a balance of water. An imbalance of water due to freezing, dehydration, exercise, overheating, etc. leads to the death of cells and eventually the whole body. Dehydration kills far more quickly than starvation. In the human body, water provides a medium for the transportation, digestion, and metabolism of food in addition to many other physiological functions such as body temperature regulation (17).

Two thirds of the body mass is water, and in most soft tissues, the contents can be as high as 99% (16). Water molecules interact with biomolecules intimately (9); they are part of us. Functions of water and biomolecules collectively manifest life. Water is also required for running households, making industrial goods, and generating electric power.

Water has shaped the landscape of Earth for trillions of years, and it covers 70% of the Earth's surface. Yet, for food production and technology it is a precious commodity. Problems with water supply can lead to disaster (5). Few brave souls accept the challenge to stay in areas with little rainfall. Yet, rainfall can be a blessing or a curse depending on the timing and amount. Praying for timely and bountiful rainfall used to be performed by emperors and politicians, but water for food challenges scientists and engineers today.

III. WATER MOLECULES AND THEIR MICROSCOPIC PROPERTIES

Plato hypothesized four *primal substances*: water, fire, earth, and air. His doctrine suggested that a combination and permutation of various amounts of these four *primal substances* produced all the materials of the world. Scholars followed this doctrine for 2000 years, until it could not explain experimental results. The search of fundamental substances led to the discovery of hydrogen, oxygen, nitrogen, etc., as chemical elements. Water is made up of hydrogen (H) and oxygen (O). Chemists use H_2O as the universal symbol for water. The molecular formula, H_2O, implies that a water molecule consists of two H atoms and one O atom. However, many people are confused by its other chemical names such as hydrogen oxide, dihydrogen oxide, dihydrogen monoxide, etc.

A. Isotopic Composition of Water

The discoveries of electrons, radioactivity, protons, and neutrons implied the existence of isotopes. Natural isotopes for all elements have been identified. Three isotopes of hydrogen are protium (^1H), deuterium (D, ^2D or ^2H), and radioactive tritium (T, ^3T or ^3H), and the three stable oxygen isotopes are ^{16}O, ^{17}O, and ^{18}O. The masses and abundances of these isotopes are given in Table 12.1. For radioactive isotopes, the half-lives are given.

Random combination of these isotopes gives rise to the various isotopic water molecules, the most abundant

TABLE 12.1
Isotopes of Hydrogen and Oxygen, and Isotopic Water Molecules Molar Mass (amu), Relative Abundance (%) or Half Life

Isotopes of Hydrogen			Stable Isotopes of Oxygen		
^{1}H	^{2}D	^{3}T	^{16}O	^{17}O	^{18}O
1.007825	2.00141018	3.0160493	15.9949146	16.9991315	17.9991604
99.985%	0.015%	12.33 years	99.762%	0.038%	0.200%

Isotopic water molecules molar mass (amu) and relative abundance (%, ppm or trace)

$H_2{}^{16}O$	$H_2{}^{18}O$	$H_2{}^{17}O$	$HD^{16}O$	$D_2{}^{16}O$	$HT^{16}O$
18.010564	20.014810	19.014781	19.00415	19.997737	20.018789
99.78%	0.20%	0.03%	0.0149%	0.022 ppm	trace

one being $^{1}H_2{}^{16}O$ (99.78%, its mass is 18.010564 atomic mass units (amu)). Water molecules with molecular masses about 19 and 20 are present at some fractions of a percent. Although $HD^{16}O$ (0.0149%) is much more abundant than $D_2{}^{16}O$ (heavy water, 0.022 part per million), D_2O can be concentrated and extracted from water. In the extraction process, HDO molecules are converted to D_2O due to isotopic exchange. Rather pure heavy water (D_2O) is produced on an industrial scale especially for its application in nuclear technology, which provides energy for the food industry.

A typical mass spectrum for water shows only mass-over-charge ratios of 18 and 17, respectively, for H_2O^+ and OH^+ ions in the gas phase. Other species are too weak for detection, partly due to condensation of water in mass spectrometers.

The isotopic composition of water depends on its source and age. Its study is linked to other sciences (18). For the isotopic analysis of hydrogen in water, the hydrogen is reduced to a hydrogen gas and then the mass spectrum of the gas is analyzed. For isotopes of oxygen, usually the oxygen in H_2O is allowed to exchange with CO_2, and then the isotopes of the CO_2 are analyzed. These analyses are performed on archeological food remains and unusual food samples in order to learn their origin, age, and history.

B. Structure and Bonding of Water Molecules

Chemical bonding is a force that binds atoms into a molecule. Thus, chemists use H-O-H or HOH to represent the bonding in water. Furthermore, spectroscopic studies revealed the H–O–H bond angle to be 104.5° and the H–O bond length to be 96 picometers (pm = 10^{-12} m) for gas H_2O molecules (19). For solid and liquid, the values depend on the temperature and states of water. The bond length and bond angles are fundamental properties of a molecule. However, due to the vibration and rotational motions of the molecule, the measured values are average or equilibrium bond lengths and angles.

The mean van der Waals diameter of water has been reported as nearly identical with that of isoelectronic neon (282 pm). Some imaginary models of the water molecule are shown in Figure 12.1.

An isolated water molecule is hardly static. It constantly undergoes a vibration motion that can be a combination of any or all of the three principal modes: symmetric stretching, asymmetric stretching, and bending (or deformation). These vibration modes are indicated in Figure 12.2.

Absorption of light (photons) excites water molecules to higher energy levels. Absorption of photons in the

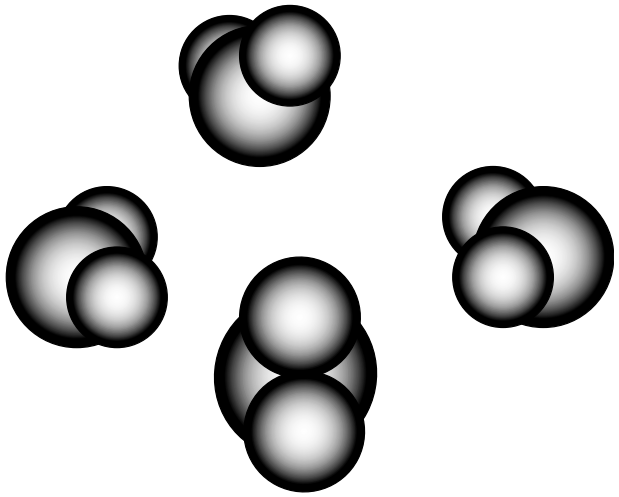

FIGURE 12.1 Some imaginative models of water molecules.

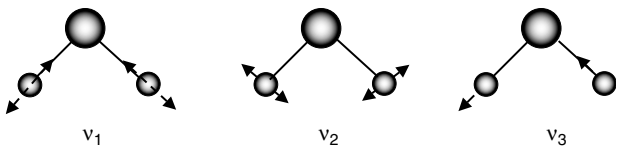

FIGURE 12.2 The three principal vibration modes of the water molecule, H_2O: ν_1, symmetric stretching; ν_2, bending; and ν_3, asymmetric stretching.

infrared (IR) region excites the vibration motion. Photons exciting the symmetric stretching, bending, and asymmetric stretching to the next higher energy levels have wave numbers 3656, 1594, and 3756 cm^{-1}, respectively, for H_2O (20). These values and those for other water molecules involving only ^{16}O are given in Table 12.2.

The spectrum of water depends on temperature and density of the gaseous H_2O. A typical IR spectrum for the excitation of only the fundamental vibration modes consists of three peaks around 1594, 3656, and 3756 cm^{-1}. Additional peaks due to excitation to mixed modes appear at higher wave numbers.

Rotating the H_2O molecule around the line bisecting the HOH angle by 180° (360°/2) results in the same figure. Thus, the molecules have a 2-fold rotation axis. There are two mirror planes of symmetry as well. The 2-fold rotation and mirror planes give the water molecules the symmetry point group C_{2v}.

Rutherford's alpha scattering experiment in 1909 showed that almost all atomic mass is in a very small atomic nucleus. In a neutral atom the number of protons in the nucleus is the same as the number of electrons around the nucleus. A proton and an electron have the same amount, but different kinds of charge. Electrons occupy nearly all of the atomic volume, because the radius of an atom is 100,000 times that of the nucleus.

Electrons, in quantum mechanical view, are waves confined in atoms, and they exist in several energy states called orbitals. Electrons in atoms and molecules do not have fixed locations or orbits. Electron states in an element are called electronic configurations, and their designation for H and O are $1s^1$, and $1s^2 2s^2 2p^4$, respectively. The superscripts indicate the number of electrons in the orbitals 1*s*, 2*s*, or 2*p*. The electronic configuration for the inert helium (He) is $1s^2$, and $1s^2$ is a stable core of electrons. Bonding or valence electrons are $1s^1$ and $2s^2 2p^4$ for H and O, respectively.

The valence bond approach blends one 2*s* and three 2*p* orbitals into four bonding orbitals, two of which accommodate two electron pairs. The other two orbitals have only one electron each, and they accommodate electrons of the H atoms bonded to O, thus forming the two H–O bonds. An electron pair around each H atom and four electron pairs around the O atom contribute stable electronic configurations for H and O, respectively. The Lewis dot-structure, Figure 12.3, represents this simple view. The two bonding and two lone pairs are asymmetrically distributed with major portions pointing to the vertices of a slightly distorted tetrahedron in 3-dimensional space. The two lone pairs mark slightly negative sites and the two H atoms are slightly positive. This charge distribution around a water molecule is very important in terms of its microscopic, macroscopic, chemical, and physical properties described later. Of course, the study of water continues and so does the evolution of bonding theories. Moreover, the distribution of electrons in a single water molecule is different from those of dimers, clusters, and bulk water.

The asymmetric distribution of H atoms and electrons around the O atom results in positive and negative sites in the water molecule. Thus, water consists of polar molecules.

The **dipole moment, μ**, is a measure of polarity and a useful concept. A pair of opposite charge, *q*, separated by a distance, *d*, has a dipole moment of $\mu = d\ q$ with the direction pointing towards the positive charge as shown in Figure 12.4.

The dipole moment of individual water molecules is 6.187×10^{-30} C m (or 1.855 D) (21). This quantity is the vector resultant of two dipole moments due to the O–H bonds. The bond angle H–O–H of water is 104.5°. Thus, the dipole moment of an O–H bond is 5.053×10^{-30} cm. The bond length between H and O is 0.10 nm, and the

TABLE 12.2
Absorption Frequencies of D_2O, H_2O, and HDO Molecules for the Excitation of Fundamental Modes to a Higher Energy Level

	Absorption Energies in Wave Numbers (cm^{-1})		
Vibration Mode	**H_2O**	**HDO**	**D_2O**
Symmetric stretching	3656	2726	2671
Bending	1594	1420	1178
Asymmetric stretching	3756	3703	2788

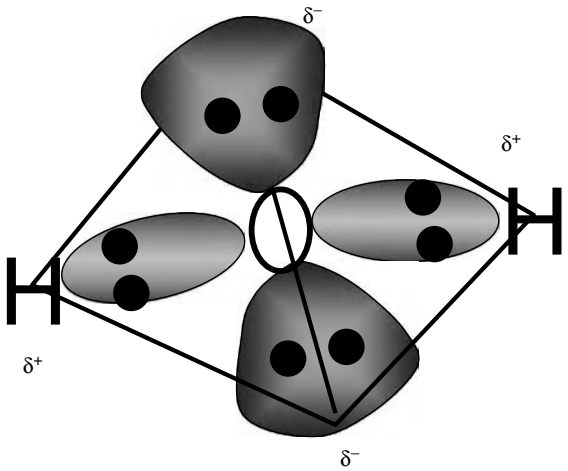

FIGURE 12.3 A tetrahedral arrangement of the Lewis dot-structure and charge distribution of the H_2O molecule.

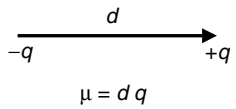

FIGURE 12.4 Separation of a positive and a negative charge *q* at a distance *d* results in a dipole, $\mu = d\ q$.

partial charge at the O and the H is therefore $q = 5.053 \times 10^{-20}$ C, 32% of the charge of an electron (1.6022×10^{-19} C). Of course, the dipole moment may also be considered as separation of the electron and positive charge by a distance 0.031 nm.

It should be pointed out that the dipole moments of liquid and solid water appear to be higher due to the influence of neighboring molecules. For the liquid and solid, macroscopic properties need be considered.

C. Hydrogen Bonds

Attraction among water molecules is more than polar-polar in nature. The O atoms are small and very electronegative. As a result, the positive H atoms (protons) are very attractive to the negative O atoms of neighboring molecules. This O- - -H – O strong attraction is called a **hydrogen bond**, a concept popularized by L. Pauling (22). Furthermore, hydrogen atoms bonded to atoms of N and F, neighboring elements of O in the periodic table, are positive, and they form hydrogen bonds with atoms of N, O, or F. The strength of hydrogen bonds depends on the X–H - - - Y (X or Y are N, O, or F atoms) distances and angles; the shorter the distances, the stronger are the hydrogen bonds.

When two isolated water molecules approach each other, a dimer is formed due to hydrogen bonding. The dimer may have one or two hydrogen bonds (Figure 12.5). Dimers exist in gaseous and liquid water. When more water molecules are in close proximity, they form trimmers, tetramers and clusters. Hydrogen bonds are not static, they exchange protons and partners constantly. Hydrogen bonding is a prominent feature in the structures of various solid phases of water usually called ice as we shall see later.

Water molecules not only form hydrogen bonds among themselves, they form hydrogen bonds with any molecule that contains N–H, O–H, and F–H bonds. Foodstuffs such as starch, cellulose, sugars, proteins, DNA, and alkaloids contain N–H and O–H groups, and these are both H-donors and H-acceptors of hydrogen bonds of the type N- - H–O, O- -H–N, N- -H–N, etc. A dimer depicting the hydrogen bond and the van der Waals sphere of two molecules is shown in Figure 12.6 (23).

Carbohydrates (starch, cellulose and sugars) contain H–C–O–H groups. The O–H groups are similar to those of water molecules, and they are H-acceptors and H-donors for hydrogen bonds. Proteins contain O–H, R–NH_2 or $R_2 >$ NH groups, and the O–H and N–H groups are both H-donors and H-acceptors for the formation of hydrogen bonds. Thus, water molecules have intimate interactions with carbohydrates and proteins.

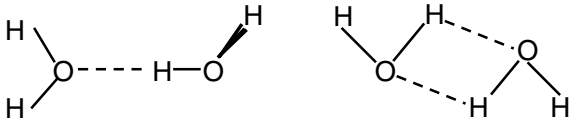

FIGURE 12.5 Two possible structures of the water dimer, $(H_2O)_2$.

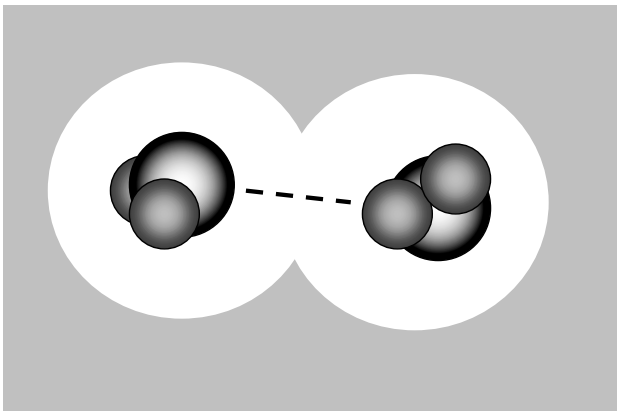

FIGURE 12.6 Hydrogen bond and the van der Waals sphere of two water molecules, after M.F. Martin (23).

IV. MACROSCOPIC PROPERTIES OF WATER

Collectively, water molecules exist as gas, liquid, or solid depending on the temperature and pressure. These **phases** of water exhibit collective or macroscopic properties such as phase transitions, crystal structures, liquid structures, vapor pressures, and volume-pressure relationships of vapor. In addition, energies or enthalpies for melting, vaporization, and heating are also important for applications in food technology.

Thermodynamic constants for phase transitions given in Table 12.3 are those of pure water. Natural waters, of course, contain dissolved air, carbon dioxide, organic substances, microorganisms, and minerals. Water in food or used during food processing usually contains various organic and inorganic substances. These solutes modify the properties of water and caution should be taken to ensure proper values are applied in food technology.

The triple point of water defines the temperature of 273.16 K in the SI unit system. The division of 1/2 73.16 in thermodynamic temperature scale is approximately 1°C.

Water has many unusual properties due to its ability to form hydrogen bonds and its large dipole moment. As a result, the melting, boiling, and critical points for water are very high compared to substances of similar molar masses. In general, the higher the molar masses, the higher are the melting and boiling points of the material. Associated with these properties are its very large heat of melting, heat capacity, heat of vaporization, and heat of sublimation. Moreover, its surface tension and viscosity are also very large. Thermodynamic energies and volume changes for phase transitions of H_2O are summarized in Table 12.3. These data are mostly taken from the Encyclopedia of Chemical Technology, Vol. 25 (1991) (24).

TABLE 12.3
Thermodynamic Constants for Phase Transitions of H_2O (molar mass, 18.015268 g mol^{-1}) at 101.314 kPa Pressure

	Fusion (Melting)	Vaporization	
		Boiling	Sublimation
Temperature K	**273.15**	**373**	**273.16**
ΔH kJ mol^{-1}	6.01	40.66	51.06
ΔS J mol^{-1} K^{-1}	22.00	108.95	186.92
ΔE kJ mol^{-1}	6.01	37.61	48.97
ΔV L mol^{-1}	–1.621	30.10	–

Energy change of phase transition: ΔH, enthalpy; ΔS, entropy; ΔE, internal energy. ΔV: volume change of phase transition.

TABLE 12.4
Properties of Ice at 273.15 K

Heat of formation ΔH_f	292.72 kJ mol^{-1}
Density	0.9168 g cm^{-3}
Heat capacity	2.06 J g^{-1} K^{-1}
ΔH_{fusion}	6.01 kJ mol^{-1}
Dielectric constant at 3 kHz	79
Thermal expansion coefficient	
Volumetric	120×10^{-6} cm^3 g^{-1} K^{-1}
Linear	52.7×10^{-6} cm g^{-1} K^{-1}

A. Crystal Structures and Properties of Ice

Hydrogen bonding is prominent in the crystal structures of various solid phases of H_2O. The triple point of water is at 273.16 K and 4.58 torr (611 Pa). The melting point at 1.00 atm (760 torr or 101.325 kPa) is 273.15 K in the Kelvin scale. When water freezes at these temperatures and atmospheric pressure or lower, the solids are hexagonal ice crystals usually designated as I_h. Properties of I_h are given in Table 12.4. Snowflakes have many shapes because their growth habit depends on temperature and vapor pressure, but they all exhibit hexagonal symmetry, due to the hexagonal structure of ice (25).

However, from a geometric point of view, the same bonding may also be arranged to have cubic symmetry. The existence of cubic ice has been confirmed. When water vapor deposits onto a very cold, 130–150 K surface or when small droplets are cold under low pressure at high altitude, the ice has a cubic symmetry usually designated as I_c. At still higher pressures, different crystal forms designated as ice II, III, IV, … etc., up to 13 phases of cubic, hexagonal, tetragonal, monoclinic, and orthorhombic symmetries have been identified (26). The polymorphism of solid water is very complicated. Some of these ice forms are made under very high pressures, and water crystallizes into solid at temperatures above the normal melting or even boiling temperatures. Ice VII is formed above 10 G Pa (gigapascal) at 700 K (26).

When liquid water is frozen rapidly, the molecules have little chance of arranging into crystalline ice. The frozen liquid is called **amorphous ice** or **glassy ice**.

The basic relationships between nearest neighboring water molecules are the same in both I_h and I_c. All O atoms are bonded to four other O atoms by hydrogen bonds, which extend from an oxygen atom towards the vertices of a tetrahedron. A sketch of the crystal structure of hexagonal I_h is shown in Figure 12.7 (27). In I_h, hydrogen positions are somewhat random due to thermal motion, disorder, and exchanges. For example, the hydrogen may shift between locations to form H_3O^+ and OH^- ions dynamically throughout the structure. In this structure, bond angles or hydrogen-bond angles around oxygen atoms are those of the idealized tetrahedral arrangement of 109.5° rather than 104.5° observed for isolated molecules. Formation of the

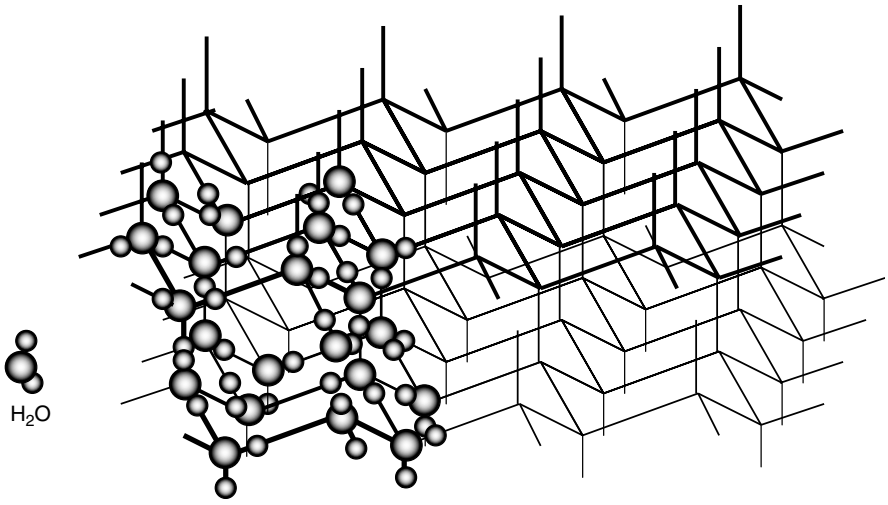

FIGURE 12.7 Ordered crystal structure of ice, bond distance: O–H, 100 pm; H- - -O, 175 pm; and O–H- - -O, 275 pm.

hydrogen bond in ice lengthens the O–H bond distance, 100 pm compared to 96 pm in a single water molecule. The diagram illustrates a crystal structure that is completely hydrogen bonded, except for the molecules at the surface.

Each O atom of hexagonal ice I_h is surrounded by four almost linear O–H - - O hydrogen bonds of length 275 pm, in a tetrahedral fashion. Each C atom of cubic diamond is also surrounded by four C–C covalent bonds of length 154 pm. Thus, the tetrahedral coordination can either be cubic or hexagonal, from a geometrical viewpoint. Indeed, the uncommon cubic ice and hexagonal diamond have been observed, giving a close relationship in terms of spatial arrangement of atoms between ice and diamond (26). Strong hydrogen bonds make ice hard, but brittle. The structure is related to its physical properties, which vary with temperature.

The pressure of H_2O vapor in equilibrium with ice is called the **vapor pressure of ice**, which decreases as the temperature decreases. At the triple point or 0°C, the pressure is 611.15 Pa. When ice is slightly overheated to 0.01°C, the pressure increases to 611.657 Pa. However, at this temperature, the vapor pressure of liquid water is lower. The vapor pressures of ice between 0°C and – 40°C are listed in Table 12.5 at 1°C interval. Various models can be used to estimate the vapor pressure at other temperatures. One method uses the Clausius-Clapeyron differential equation

$$\frac{dp}{dT} = \frac{H}{T\,\Delta V}$$

where p is the pressure, T is the temperature (K), H is the latent heat or **enthalpy** of phase transition, and ΔV is the difference in volume of the phases. The enthalpy of sublimation for ice depends on the temperature. At the freezing point, the enthalpy of sublimation for ice is 51 (51.06 in Table 12.3) kJ mol^{-1}, estimated from the vapor pressure at 0 and –1°C. The enthalpy of sublimation is required to overcome hydrogen bonding, dipole, and intermolecular attractions. The energy required in freeze-drying processes varies, depending on temperature and other conditions. Water in solutions and in food freezes below 0°C.

The number of hydrogen bonds is twice the number of water molecules, when surface water molecules are ignored. The energy required to separate water molecules from the solid is the enthalpy of sublimation (55.71 J mol^{-1}). Half of this value, 26 kJ mol^{-1}, is the energy to separate the H- -O linkages, and it translates into 0.26 eV, per H- -O bond. These values are close to those obtained by other means (25, 26, 28–30). Several factors contribute to this linkage, and the hydrogen-bond energy is less than 0.26 eV.

TABLE 12.5
Aqueous Vapor Pressure (Pa) in Equilibrium with Ice between 0 and – 40°C (at 0.01°C, *p* is 611.657 Pa)

t (°C)	– 0 ■	– 1 ■	–2 ■	–3 ■	– 4 ■
– ■ 0	611.15	259.90	103.26	38.01	12.84
– ■ 1	562.67	237.74	93.77	34.24	
– ■ 2	517.72	217.32	85.10	30.82	
– ■ 3	476.06	198.52	77.16	27.71	
– ■ 4	437.47	181.22	69.91	24.90	
– ■ 5	401.76	165.30	63.29	22.35	7.20
– ■ 6	368.73	150.68	57.25	20.04	
– ■ 7	338.19	137.25	51.74	17.96	
– ■ 8	309.98	124.92	46.73	16.07	
– ■ 9	283.94	113.62	42.16	14.37	

Missing digits of *t*, ■, in the row are found in the column, and vice versa. These values are taken from the CRC Handbook of Chemistry and Physics (1).

B. Properties of Liquid Water

The macroscopic physical properties of this common but eccentric fluid at 298 K (25°C) are given in Table 12.6. Water has unusually high melting and boiling points for a substance of molar mass of only 18 daltons. Strong hydrogen bonds and high polarity account for this.

The heat of formation is the energy released when a mole of hydrogen and half a mole of oxygen at 298 K and 1.00 atm react to give one mole of water at 298 K. This value differs from that for ice in Table 12.4 due to both temperature and phase differences. As temperature increases, the average kinetic energy of molecules increases, and this affects water's physical properties. For example, surface tension of water decreases, whereas the thermal conductance increases as the temperature increases. Heat capacity at constant pressure (C_p), vapor pressure, viscosity, thermal conductance, dielectric constant, and surface tension in the temperature range 273–373 K (0–100°C) are given in Table 12.7.

Liquid water has the largest heat capacity per unit mass of all substances. Large quantities of energy are absorbed or released when its temperature changes. The large heat capacity makes water an excellent reservoir and transporter of energy. A large body of water moderates climate. The heat capacity C_p of water varies between 4.1 to

TABLE 12.6
Properties of Liquid Water at 298 K

Heat of formation ΔH_f	285.89 kJ mol^{-1}
Density at 3.98°C	1.000 g cm^{-3}
Density at 25°C	0.9970480 g cm^{-3}
Heat capacity	4.17856 Jg^{-1} K^{-1}
$\Delta H_{vaporization}$	55.71 kJ mol^{-1}
Dielectric constant	80
Dipole moment	6.24×10^{-30} C m
Viscosity	0.8949 mP? s^{-1}
Velocity of sound	1496.3 m s^{-1}
Volumetric thermal expansion coefficient	0.0035 cm^3 g^{-1} K^{-1}

TABLE 12.7
Properties of Liquid Water in the Range 273–373 K (0–100°C)

Temp. t (°C)	Heat Capacity Cp (J g^{-1} K^{-1})	Viscosity (mPa s)	Thermal Conductance (W K^{-1} m^{-1})	Dielectric Constant	Surface Tension (mN m^{-1})
0	4.2176	1.793	561.0	87.90	75.64
10	4.1921	1.307	580.0	83.96	74.23
20	4.1818	1.002	598.4	80.20	72.75
30	4.1784	0.797	615.4	76.60	71.20
40	4.1785	0.653	630.5	73.17	69.60
50	4.1806	0.547	643.5	69.88	67.94
60	4.1843	0.466	654.3	66.73	66.24
70	4.1895	0.404	663.1	63.73	64.47
80	4.1963	0.354	670.0	60.86	62.67
90	4.2050	0.315	675.3	58.12	60.82
100	4.2159	0.282	679.1	55.51	58.91

More detailed data can be found in the CRC Handbook of Chemistry and Physics (1).

4.2 J g^{-1} K^{-1} (74 to 76 J mol^{-1} K^{-1}) even at temperature above 100°C and high pressure. The enthalpy of vaporization for water is also very large (55.71 kJ mol^{-1} at 298 K). Thus, energy consumption is high for food processing when water is involved.

Water and aqueous solutions containing only low molar-mass solutes are typical Newtonian fluids for which the **shear stress** is proportional to **shear strain rate**. Viscosity is the ratio of shear stress to shear strain rate. On the other hand, viscosity of solutions containing high molar-mass substances depends on shear strain rate. For pure water, the viscosity decreases from 1.793 to 0.282 mPa s (millipascal seconds; identical to centipoise (cp)) as temperature increases from 0 to 100°C. Thus, the flow rate through pipes increases as water or solution temperature increases.

The dielectric constant of water is very large, and this enables water to separate ions of electrolytes, because it reduces the electrostatic attraction between positive and negative ions. Many salts dissolve in water. When an electric field is applied to water, its dipole molecules orient themselves to decrease the field strength. Thus, its dielectric constant is very large. The dielectric constant decreases as temperature increases, because the percentage of molecules involved in hydrogen bonding and the degree of order decrease (28, 29). The measured dielectric constant also depends on the frequency of the applied electric field used in the measurement, but the variation is small when the frequency of the electric field is less than 100 MHz. The dielectric behavior of water allows water vapor pressure to be sensed by capacitance changes when moisture is absorbed by a substance that lies between the plates of a capacitor. These sensors have been developed for water activity measurement (31).

The light absorption coefficients are high in the infrared and ultraviolet regions, but very low in the visible region. Thus, water is transparent to human vision.

The variation of vapor pressure as a function of temperature is the bases for defining **water activities** of food. Liquid water exists between the triple-point and the critical-point temperatures (0–373.98°C) at pressures above the vapor pressures in this range.

As with ice, the vapor pressure of liquid water increases as the temperature increases. Vapor pressures of water (in kPa instead of Pa for ice in Table 12.5) between the triple and critical points, at 10°C interval, are given in Table 12.8. When the vapor pressure is 1.00 atm (101.32 kPa) the temperature is the boiling point (100°C). At slightly below 221°C, the vapor pressure is 2.00 atm. The critical pressure at the critical temperature, 373.98°C, is 217.67 atm (22,055 kPa). Above this temperature, water cannot be liquefied, and the phase is called **supercritical water**.

The partial pressure of H_2O in the air at any temperature is the **absolute humidity**. When the air is saturated with water vapor, the **relative humidity** is 100%. The unsaturated vapor pressure divided by the vapor pressure of water as given in Table 12.8 at the temperature of the air is the **relative humidity**. The temperature at which the vapor pressure in the air becomes saturated is the **dew point**, at which dew begins to form. However, when the dew point is below 273 K or 0°C, ice crystals (frost) begin to form. Thus, the relative humidity can be measured by finding the dew point. Dividing the vapor pressure at the dew point by the vapor pressure of water at the temperature of the air gives the relative humidity. The transformations between solid, liquid, and gaseous water play important roles in hydrology and in the transformation of the environment on Earth. Phase transitions of water combined with the energy from the sun make the weather.

TABLE 12.8
Vapor Pressure (kPa) of Liquid H_2O between Triple and Critical Points at Every 10°C

t°C	0■■	1■■	2■■	3■■
■00	0.6113	101.32	1553.6	8583.8
■10	1.2281	143.24	1906.2	9860.5
■20	2.3388	198.48	2317.8	11279
■30	4.2455	270.02	2795.1	12852
■40	7.3814	361.19	3344.7	14594
■50	12.344	475.72	3973.6	16521
■60	19.932	617.66	4689.4	18665
■70	31.176	791.47	5499.9	21030
■80	47.375	1001.9	6413.2	22055*
■90	70.117	1254.2	7438.0	

* Critical pressure at 373.98°C.

Missing digits of t, ■, in the row are found in the column, and vice versa. Values from the CRC Handbook of Physics and Chemistry (2003) (1), which lists vapor pressure at 1° interval.

TABLE 12.9
The Density of Water (g/mL) as a Function of Temperature between 0 and 39°C (31)

t°C	0 ■	1 ■	2 ■	3 ■
■ 0	0.9998426	0.9997021	0.9982063	0.9956511
■ 1	0.9999015	0.9996074	0.9979948	0.9953450
■ 2	0.9999429	0.9994996	0.9977730	0.9950302
■ 3	0.9999627	0.9993792	0.9975412	0.9947971
■ 4	0.9999750*	0.9992464	0.9972994	0.9943756
■ 5	0.9999668	0.9991016	0.9970480	0.9940359
■ 6	0.9999430	0.9989450	0.9967870	0.9936883
■ 7	0.9999043	0.9987769	0.9975166	0.9933328
■ 8	0.9998509	0.9985976	0.9962370	0.9929695
■ 9	0.9997834	0.9984073	0.9956511	0.9925987

Missing digits of t, ■, in the row are found in the column, and vice versa.

Density is a collective property, and it varies with temperature, isotopic composition, purity, etc. The International Union of Pure and Applied Chemistry (IUPAC) has adopted the density of pure water from the ocean as the density standard. The isotopic composition of ordinary water is constant, and the density of pure water between 0 and 39°C extracted from (32) is given in Table 12.9.

The density of cage-like ice I_h, due to 100% of its molecules involved in hydrogen-bonded is only 9% lower than that of water. This indicates that water has a high percentage of molecules involved in the transient and dynamic hydrogen bonding. The percentage of hydrogen-bonded water molecules in water decreases as temperature increases, causing water density to increase. As temperature increases, the thermal expansion causes its density to decreases. The two effects cause water density to increase from 0 to 3.98°C, reaching its maximum of 1.0000 g mL^{-1} and then decrease as temperature increases.

Incidentally, at 8°C, the density of water is about the same as that at 0°C. At 25°C, the density decreases 0.3% with respect to its maximum density, whereas at 100°C, it decreases by 4%. Dense water sinks, and convection takes place when temperature fluctuates at the surface of lakes and ponds, bringing dissolved air and nutrients to various depths of waters for the organism living in them. On the other hand, the pattern of density dependence on temperature of water makes temperatures at the bottoms of lakes and oceans vary little if the water is undisturbed. When water freezes, ice begins to form at the surface, leaving the water at some depth undisturbed. Water at the bottom remains at 4°C, preserving various creatures living in water.

When hydrogen bonded to tissues and cells or in food, water has a unique order and structure, and the vapor pressure and density differ from those of pure water. Yet, the collective behavior of water molecules sheds some light regarding their properties in food, cells, tissues, and solutions.

V. CHEMICAL PROPERTIES OF WATER

Water is a chemical as is any substance, despite the confusion and distrust of the public regarding the term "chemical." Thus, water has lots of interesting chemical properties. It interacts intimately with components of food particularly as a solvent, due to its dipole moment and its tendency to form a hydrogen bond. These interactions affect the chemical properties of nutrients, including their tendency to undergo oxidation or reduction, to act as acids or bases, and to ionize.

A. Water as a Universal Solvent

Water is dubbed a universal solvent because it dissolves many substances due to strong interactions between water molecules and those of other substances. Entropy is another driving force for a liquid to dissolve or mix with other substances. Mixing increases disorder or entropy.

1. Hydrophobic Effect and Hydrophilic Effect

Because of its large dielectric constant, high dipole moment and ability to donate and accept protons for hydrogen bonding, water is an excellent solvent for polar substances and electrolytes, which consist of ions. Molecules strongly interact with water-loving molecules are **hydrophilic**, due to hydrogen bonding, polar-ionic or polar-polar attractions. Nonpolar molecules that do not mix with water are **hydrophobic** or **lipophilic**, because they tend to dissolve in oil. Large molecules such as proteins and fatty acids that have hydrophilic and hydrophobic portions are **amphipathic** or **amphiphilic**. Water molecules strongly intermingle with hydrophilic portions by means of dipole-dipole interaction or hydrogen bonding.

The lack of strong interactions between water molecules and lipophilic molecules or the nonpolar portions of amphipathic molecules is called the **hydrophobic effect**, a term coined by Charles Tanford (33). Instead of a direct interaction with such solutes, water molecules tend to form hydrogen-bonded cages around small nonpolar molecules when the latter are dispersed into water. Hydrogen-bonded water molecules form cages, called **hydrates** or **clathrates**. For example, the clathrate of methane forms stable crystals at low temperatures (34).

Nonpolar chains in proteins prefer to stay together as they avoid contact with water molecules. Hydrophilic and hydrophobic effects play important roles in the stability and state of large molecules such as enzymes, proteins, and lipids. Hydrophobic portions of these molecules stay together, forming pockets in globular proteins. Hydrophilic and hydrophobic effects cause nonpolar portions of phospholipids, proteins, and cholesterol to assemble into bilayers or biological membranes (34).

2. Hydration of Ions

Due to its high dielectric constant, water reduces the attractions among positive and negative ions of electrolytes and dissolves them. The polar water molecules coordinate around ions forming hydrated ions such as $Na(H_2O)_6^+$, $Ca(H_2O)_8^{2+}$, $Al(H_2O)_6^{3+}$, etc. Six to eight water molecules form the first sphere of hydration around these ions. Figure 12.8 is a sketch of the interactions of water molecules with ions. The water molecules point the negative ends of their dipoles towards positive ions, and their positive ends towards negative ions. Molecules in the hydration sphere constantly and dynamically exchange with those around them. The number of hydrated-water molecules and their lifetimes have been studied by various methods. These studies reveal that the hydration sphere is one-layer deep, and the lifetimes of these hydrated-water molecules are in the order of picoseconds (10^{-12} s). The larger negative ions also interact with the polar water molecules, not as strong as those of cations. The presence of ions in the solution changes the ordering of molecules even if they are not in the first hydration sphere (9).

The hydration of ions releases energy, but breaking up ions from a solid requires energy. The amount of energy depends on the substance, and for this reason, some are more soluble than others. Natural waters in the ocean, streams, rivers and lakes are in contact with minerals and salts. The concentrations of various ions depend on the solubility of salts (35) and the contact time.

Drinking water includes all waters used in growth, processing, and manufacturing of food. J. De Zuane divides ions in natural water into four types in The Handbook on Drinking Water Quality (5).

Type A includes arsenic, barium, cadmium, chromium, copper, fluoride, mercury, nitrate, nitrite, and selenium ions. They are highly toxic, yet abundant.

Type B includes aluminum, nickel, sodium, cyanide, silver, zinc, molybdenum, and sulfate ions. Their concentrations are also high, but they are not very toxic.

Type C consists of calcium, carbonate, chloride, iron, lithium, magnesium, manganese, oxygen, phosphate, potassium, silica, bromine, chlorine, iodine, and ozone. They are usually present at reasonable levels.

Type D ions are present usually at low levels: antimony, beryllium, cobalt, tin, thorium, vanadium, and thallium.

Most metals are usually present in water as cations, with a few as anions. However, some chemical analyses may not distinguish their state in water. The most common anions are chloride, sulfate, carbonate, bicarbonate, phosphate, bromide, iodide, etc. Toxicity is a concern for ions in water, but some of these ions are essential for humans.

Pure water has a very low electric conductivity, but ions in solutions move in an electric field making electrolyte solutions highly conductive. The conductivity is related to total dissolved solids (TDS), salts of carbonate, bicarbonate, chloride, sulfate, and nitrate. Sodium, potassium, calcium, and magnesium ions are often present in natural waters because their soluble salts are common minerals in the environment. The solubilities of clay (alumina), silicates, and most common minerals in the Earth's crust, are low.

3. Hard Waters and Their Treatments

Waters containing plenty of dissolved CO_2 (H_2CO_3) are acidic and they dissolve $CaCO_3$ and $MgCO_3$. Waters with dissolved Ca^{2+}, Mg^{2+}, HCO_3^-, and CO_3^{2-} are called **temporary hard waters** as the hardness can be removed by boiling, which reduces the solubility of CO_2. When CO_2 is driven off, the solution becomes less acidic due to

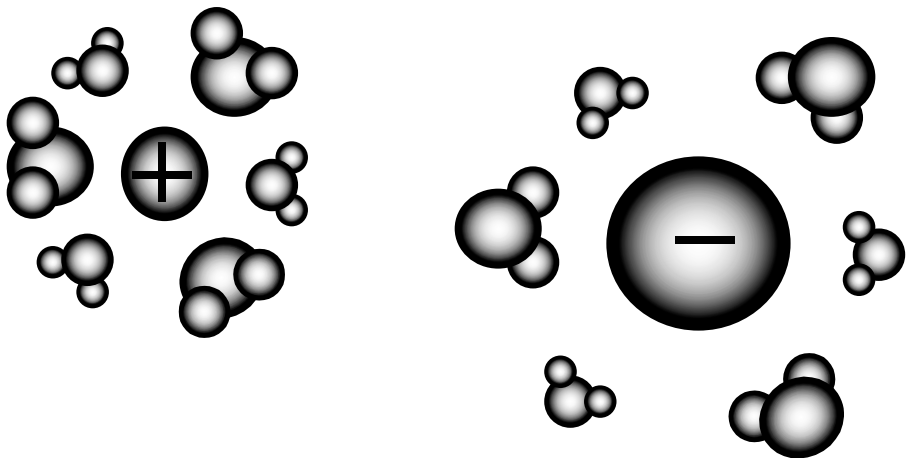

FIGURE 12.8 The first hydration sphere of most cations $M(H_2O)_6^+$, and anions $X(H_2O)_6^{-1}$. Small water molecules are below the plane containing the ions, and large water molecules are above the plane.

the following equilibria (the double arrows, ⇆, indicate reversible reactions):

$$H^+ (aq) + HCO_3^- (aq) \leftrightarrows H_2CO_3 (aq) \leftrightarrows H_2O + CO_2 (g)$$

$$HCO_3^- (aq) \leftrightarrows H^+ (aq) + CO_3^{2-} (aq)$$

Reducing the acidity increases the concentration of CO_3^{2-} and solids $CaCO_3$ and $MgCO_3$ precipitate:

$$Ca^{2+} (aq) + CO_3^{2-} (aq) \leftrightarrows CaCO_3 (s)$$

$$Mg^{2+} (aq) + CO_3^{2-} (aq) \leftrightarrows MgCO_3 (s)$$

Water containing less than 50 mg L^{-1} of these substances is considered soft; 50–150 mg L^{-1} moderately hard; 150–300 mg L^{-1} hard; and more than 300 mg L^{-1} very hard.

For the **lime treatment**, we determine the amount of dissolved Ca^{2+} and Mg^{2+} first and then add an equal number of moles of lime, $Ca(OH)_2$, to remove them by these reactions:

$$Mg^{2+} + Ca(OH)_2(s) \rightarrow Mg(OH)_2(s) + Ca^{2+}$$

$$Ca^{2+} + 2\,HCO_3^- + Ca(OH)_2(s) \rightarrow 2\,CaCO_3(s) + 2\,H_2O$$

Permanent hard waters contain sulfate (SO_4^{2-}) ions with Ca^{2+} and Mg^{2+}. Calcium ions, Ca^{2+}, of the sulfate solution can be removed by adding sodium carbonate:

$$Ca^{2+} + Na_2CO_3 \rightarrow CaCO_3\,(s) + 2\,Na^+$$

Hard waters cause scales or deposits to build up in boilers and pipes, and they are usually softened by ion exchange with resins or zeolites. In these processes, the calcium and magnesium ions are taken up by the zeolite or resin that releases sodium or hydrogen ions into the water. Reverse osmosis has also been used to soften hard water.

However, water softening replaces desirable calcium and other ions by sodium ions. Thus, soft waters are not suitable drinking waters. Incidentally, bakers use hard water because the calcium ions strengthen the gluten proteins in dough mixing. Some calcium salts are added to dough to enhance bread quality.

4. Properties of Aqueous Solutions

Waters containing dissolved substances are aqueous solutions; their physical properties differ from those of pure water. For example, at the same temperature, the H_2O vapor pressures of solutions are lower than those of pure water, resulting in **boiling point elevation** (higher), **freezing point depression** (lower), and osmotic pressure.

Concentrations can be expressed in several ways: part per million (ppm), percent, moles per liter, mole fraction, etc. The mole fraction of water is the fraction of water molecules among all molecules and ions in the system. The vapor pressure of an ideal solution, P_{solution}, is the vapor pressure of water (at a given temperature), P°_{water}, modified by the mole fraction X_{water}.

$$P_{\text{solution}} = X_{\text{water}}\, P^\circ_{\text{water}}\ (X_{\text{water}} < 1)$$

If the solute has a significant vapor pressure, P_{solute} is also modified by its mole fraction,

$$P_{\text{solute}} = X_{\text{solute}}\, P^\circ_{\text{solute}}$$

For non-ideal solutions, in which water and solute strongly interact, the formulas require modifications. A practical method is to use an **effective mole fraction** X defined by:

$$P_{\text{solution}} / P^\circ_{\text{water}} = X$$

In any case, the vapor pressures of solutions containing nonvolatile electrolytes are lower than those of pure water at their corresponding temperature.

Phase transitions take place when the vapor pressures of the two phases are the same. Because vapor pressures of solutions are lower, their melting points are lower but their boiling points are higher. The difference in temperature, ΔT, is proportional to the concentrations of all solutes, $m_{\text{all-solute}}$ (molality),

$$\Delta T = K\, m_{\text{all-solute}}$$

where K is either the molar boiling point elevation constant, K_b, or the molar freezing point depression constant K_f. For water, $K_f = 1.86$ K L kg^{-1}, and $K_b = 0.52$ K L kg^{-1}. Due to ionization of electrolytes, positive and negative ions should be treated as separate species and all species should be included in $m_{\text{all-solute}}$.

The tendency of water molecules from a dilute solution to diffuse into a more concentrated solution, through semipermeable membranes, has a measurable quantity called **osmotic pressure**, π, which is proportional to the concentration (mol per kg of water) of all dissolved species, $m_{\text{all-solute}}$ in mol kg^{-1} and temperature T in K,

$$\pi = -\, m_{\text{all-solute}}\, R\, T$$

where R is the gas constant 8.3145 J mol^{-1} K^{-1}. Water molecules diffuse from pure water ($\pi = 0$) into the solution, and the osmotic pressure is therefore given as a negative value here. Theoretically, a solution with $m_{\text{all-solute}} =$ 1.0 mol kg^{-1} of water, $\pi = -$ 2477 J kg or -2.477 kJ kg at 298 K. Note $m_{\text{all-solute}} = \Sigma m_i$ (m_i being the concentration of ion or molecule i) in the van't Hoff equation, which is often used in other literature.

Solutions having the identical osmotic pressure are isotonic. Applying more pressure to a solution to compensate for the osmotic pressure causes water molecules to diffuse through membranes, generating pure or fresh

waters. This process is called **reverse osmosis**, and it has been used to soften water or desalinate seawater, converting it to fresh water.

The lowering of vapor pressure and the osmotic pressure of solutions play important roles in hydration and dehydration of food and in living cells. Solutions containing proper concentrations of nutrients and electrolyte have been used to medically treat dehydrated patients. J.R. Cade and his coworkers applied these principles to formulate drinks for athletes; he and his coworkers were credited as the inventors of the sports drink Gaterade (36). The concept of a balanced solution for hydration became a great business decade after its invention.

B. Acidity and Alkalinity of Water

Acidity and alkalinity are also important characteristics of water due to its dynamic self-ionization equilibrium

$$H_2O\ (l) \leftrightarrows H^+ + OH^-,$$

$$K_w = [H^+][OH^-] = 1 \times 10^{-14} \text{ at 298 K and 1 atm}$$

where $[H^+]$ and $[OH^-]$ represent the molar concentrations of H^+ (or H_3O^+) and OH^- ions, respectively, and K_w is called the **ion product of water** (see tables in Refs. 1 and 36). Values of K_w under various conditions have been evaluated theoretically (37, 38). Solutions in which $[H^+] = [OH^-]$ are said to be neutral. At 298 K, for a neutral solution,

$$pH = -\log [H^+] = pOH = -\log [OH^-] = 7 \text{ (at 298 K)}$$

The H^+ ions or protons dynamically exchange with protons in other water molecules. The self-ionization and equilibrium are present in all aqueous solutions, including acid and base solutions, as well as in pure water. Water is both an acid and a base.

Strong acids such as $HClO_4$, $HClO_3$, HCl, HNO_3, and H_2SO_4 ionize completely in their solutions to give H^+ (H_3O^+) ions and anions: ClO_4^-, ClO_3^-, Cl^-, NO_3^-, and HSO_4^-, respectively. Strong bases such as NaOH, KOH, and $Ca(OH)_2$ also ionize completely giving OH^- ions and Na^+, K^+, and Ca^{2+} ions, respectively. In an acidic solution, $[H^+]$ is greater than $[OH^-]$. In a 1.0 mol L^{-1} HCl solution, $[H^+] = 1.0$ mol L^{-1}, pH = 0.

Weak acids such as formic acid HCOOH, acetic acid (CH_3COOH), ascorbic acid ($C_6H_8O_6$), oxalic acid ($H_2C_2O_4$), carbonic acid (H_2CO_3), benzoic acid (C_6H_5-COOH), malic acid ($C_4H_6O_5$), lactic acid H_3CCH-(OH)COOH, and phosphoric acid (H_3PO_4) also ionize in their aqueous solutions, but not completely. The ionization of acetic acid is represented by the equilibrium

$$CH_3COOH\ (aq) \leftrightarrows H^+\ (aq) + CH_3COO^-\ (aq),$$

$$K_a = 1.75 \times 10^{-5} \text{ at 298 K}$$

where K_a is the **acid dissociation constant**.

The solubility of CO_2 in water increases with its (CO_2 partial) pressure, according to Henry's law, and the chemical equilibria for the dissolution are

$$H_2O + CO_2\ (g) \leftrightarrows H_2CO_3\ (aq)$$

Of course, H_2CO_3 dynamically exchanges H^+ and H_2O with other water molecules, and this weak diprotic acid ionizes in two stages with their acid constants, K_{a1} and K_{a2}.

$$H_2O + CO_2\ (aq) \leftrightarrows H^+\ (aq) + HCO_3^-\ (aq),$$

$$K_{a1} = 4.30 \times 10^{-7} \qquad \text{(at 298 K)}$$

$$HCO_3^-\ (aq) \leftrightarrows H^+\ (aq) + CO_3^{2-}\ (aq),$$

$$K_{a2} = 5.61 \times 10^{-11}$$

Constants K_{a1} and K_{a2} increase with temperature. At 298 K, the pH of a solution containing 0.1 mol L^{-1} H_2CO_3 is 3.7. Acidophilic organisms may grow, but most pathogenic organisms are neutrophiles and they cease growing. Soft drinks contain other acids – citric, malic, phosphoric, ascorbic acids, etc. – which lower the pH further.

Ammonia and many nitrogen-containing compounds are weak bases. The ionization equilibrium of NH_3 in water and the **base dissociation constant** K_b are

$$NH_3 + H_2O \leftrightarrows NH_4^+\ (aq) + OH^-$$

$$K_b = 1.70 \text{ x } 10^{-5} \qquad \text{at 298 K.}$$

Other weak bases react with H_2O similarly.

The ionization or dissociation constants of inorganic and organic acids and bases are extensive, and they have been tabulated in various books (39–41).

Amino acids and proteins contain acidic and basic groups. At some specific pH called the **isoelectric point**, they carry no charge, but exist as zwitterions. For example, the isoelectric point for glycine is pH = 6.00 and it exists as the zwitterion $H_2C(NH_3^+)COO^-$.

C. Oxidation-Reduction Reactions in Water

Oxidation of hydrogen by oxygen not only produces water, but also releases energy. At the standard conditions, the electrochemical half reaction equations are:

$$H_2 = 2\ H^+ + 2\ e^- \qquad E° = 0.000 \text{ V (defined)}$$

$$O_2 + 4\ H^+ + 4\ e^- = 2\ H_2O \qquad E° = 1.229 \text{ V}$$

The cell reaction and the cell potential at the standard condition for it are:

$$2\ H_2 + O_2 = 2\ H_2O \qquad \Delta E° = 1.229 \text{ V}$$

Proper setups for harvesting this energy are the goals of hydrogen-fuel-cell technology. The cell potential ΔE for non-standard conditions depends on pH and temperature. Its value is related to the energy released in the reaction. A plot of ΔE versus pH yields a Pourbaix diagram, which is useful to evaluate the stability of various species in water. Water can be a reducing or oxidizing reagent, because it offers protons or electrons. Applying a voltage to pass electrons through a chemical cell decomposes water by electrolysis.

Waters containing dissolved oxygen cause additional reactions, for example:

$$2\ H_2O + 2\ e^- = H_2 + 2\ OH^- \qquad E° = -0.828\ V$$

$$O_2 + 2\ H^+ + 2\ e^- = H_2O_2 \qquad E° = 0.682\ V$$

$$O_2 + H_2O + 2\ e^- = HO_2^- + OH^- \qquad E° = 0.076\ V$$

$$O_2 + 2\ H_2O + 2\ e^- = 4\ OH^- \qquad E° = 0.401\ V$$

At the proper conditions, a suitable chemical reaction driven by the potential takes place.

Oxidation-reduction reactions involving water usually are due to proton or electron transfer. These oxidation-reduction reactions occur for the growth, production, manufacture, digestion, and metabolism of food.

Water participates in oxidation-reduction reactions in many steps of photosynthesis, resulting in the fixation of CO_2 into biomolecules, releasing oxygen atoms of water as O_2. Engineering a new generation of plants with greater photosynthetic capacity facing lack of waters challenges geneticists (42) and botanists. We now understand photosynthesis in great detail from the studies by many scientists. Photosynthetic reactions are related to food production, but they are so complex that we can only mention them here (43).

The oxidation-reduction reactions of water cause corrosion on metal surfaces. Not only is deterioration of facilities very costly for the food industry, corrosion of pipes results in having toxic metal ions Cu^{2+} and Pb^{2+} in drinking water. The concern about lead ions in drinking water led the Environmental Protection Agency to ban the use of high-lead solders for water pipes. These reactions are electrochemical processes. Galvanic effects, high acidity, high flow rate, high water temperature, and the presence of suspended solids accelerate corrosion, as do lack of Ca^{2+} and Mg^{2+} ions in purified waters. The formation of scales protects the metal surface. However, balancing the clogging against surface protection of pipes is a complicated problem, requiring scientific testing and engineering techniques for a satisfactory solution.

D. The Hydrogen Bond and Chemical Reactions

Enzymes are mostly large protein molecules, and they are selective and specific catalysts responsible for most reactions in biological bodies. Folding of the long protein provides specific 3-dimensional selective pockets for their substrates. The pockets not only fix the substrates in position, they also weaken certain bonds to facilitate specific reactions. This is the mechanism by which enzymes select their substrates and facilitate their specific reactions.

Hydrogen bond strength is stronger in nonaqueous media than in aqueous solutions as the charge densities on the donor and acceptor atoms increase (44). Hydrogen bonds between the enzyme and its substrate can be stronger than those in an aqueous environment, thus speeding up the reaction rate even further.

The hydrolysis of peptide linkage is the reaction of a protein with water:

$$\text{R-C(=O)-NH-R}' + H_2O \rightarrow \text{R-C(=O)OH} + H_2N\text{-R}'$$

This type of reaction can be catalyzed by acids, bases, and enzymes.

VI. WATER ACTIVITY

Water is a nutrient and a component in food groups: grains, meat, dairy, fruits and vegetables. Furthermore, major nutrients such as carbohydrates, proteins, water-soluble vitamins, and minerals are hydrophilic. Even parts of fat or lipid molecules are hydrophilic, but the alkyl chains of fats and proteins experience the hydrophobic effect in an aqueous environment (45).

Foodstuffs interact with water by means of polar, hydrogen-bonding, and hydrophobic interactions. The results of these interactions change the chemical potential (properties) of water. Foodstuffs dissolve in or absorb water. Thus, water within food may be divided into bound water, affected water, and free water in the order of their interaction strength. The bound water molecules are similar to those in the first hydration sphere of ions, and those close to the first sphere are affected water molecules. Further away from the interface are free water molecules. The structure and properties of the first two types change. Interaction of water with dietary fiber is an example (46). Thus, properties of water in food are different from those of pure water.

Water molecules in both liquid and vapor phases can participate in hydration reactions. At equilibrium in a system with two or more phases, their vapor pressure or chemical potential, μ, must be equal. The chemical potential, μ, of a solution or water-containing foodstuff must be equal at a given temperature T, and

$$\mu = \mu_w + R\,T \ln (p/p_w),$$

where R is the gas constant (8.3145 J mol^{-1} K^{-1}), p is the vapor pressure of the solution or of water in foodstuff, and p_w is the vapor pressure of pure water at the same temperature. The ratio p/p_w is called the **water activity**

a_w (= p/p_w), which is related to the water chemical potential of water in solutions or in the foodstuff. For ideal solutions and for most moist foods, a_w is less than unity, $a_w < 1.0$ (31).

Both water activity and relative humidity are fractions of the vapor pressure of pure water. Methods for their measurements are the same. We have mentioned the measurement by changes in capacitance earlier. Water contents have a sigmoidal relationship with water activities, $a_w = 1.0$ for infinitely dilute solutions, $a_w > 0.7$ for dilute solutions and moist foods, and $a_w < 0.6$ for dry foods. Of course, the precise relationship depends on the material in question. In general, if the water vapor of the atmosphere surrounding the food is greater than the activity of the food, water is absorbed. Otherwise, dehydration takes place. The water activity reflects the combined effects of water-solute, water-surface, capillary, hydrophilic, and hydrophobic interactions. The water activity of a foodstuff is a vital parameter, because it affects its texture, taste, safety, shelf life, and appearance.

Furthermore, controlling water activity rather than water content is important. When $a_w < 0.9$, most molds are inhibited. Growth of yeasts and bacteria also depends on a_w. Microorganisms cease growing if $a_w < 0.6$.

VII. WATER POTENTIAL

Similar to water activity in food, **water potential** is a term used in plant, soil, and crop sciences. Water potential, represented by Ψ (psi) or Ψw, is a measure of the free energy of water in a system: soil, material, seeds, plants, roots, leaves, or an organism. Water potential is the difference between the chemical potential of pure water and water in the system at the same temperature. Pure water has the highest free energy: $\Psi = 0$ for pure water by convention, and $\Psi < 0$ for solutions. Water diffuses from high potential to low potential. Physiological processes decrease as the water potential decreases.

In general, water potential, Ψw, is a combined effect of osmotic (Ψs), matrix (interface and water binding Ψm), turgor (Ψt) pressures, and gravity (Ψg).

$$\Psi w = \Psi s + \Psi m + \Psi t + \Psi g$$

Osmotic pressure, Ψs, is always present due to solutes in the fluids. The metric pressure, Ψm, is related to bound-, affected-, and free-waters in the system. The outwardly directed pressure extended by the swelling protoplast against the wall is called **turgor pressure**, Ψt. Usually, this term is insignificant until the cell is full, and at such point, Ψt increases rapidly and stops when Ψw = Ψt. Otherwise, the cell ruptures. The mechanical rigidity of succulent plant parts, the opening of stomata and the blossom are usually the results of turgor pressure. In systems such as tall plants and soil science, pressure due to the gravitational pull of water, Ψg, is also included in the water potential.

For example, the water potential of potato tissues can be measured by incubating them in a series of solutions of known osmotic pressures. The potato will neither lose nor gain water if the osmotic pressure of the solution equals the water potential of potato tissues. The osmotic pressure ($\pi = -m\,R\,T$) may be evaluated from a known concentration m, using the equation given earlier. Instead of energy units, water potential is often expressed in units of pressure (megapascal, MPa), which is derived by dividing the energy by the molar volume (0.018 L mol^{-1} for H_2O) of water (47).

There are many other methods for water potential measurements depending on the system: soil, leaf, stem, organism, etc. The soil water potential is related to the water available for the plants growing on the soil. Water potential of a plant or leaf indicates its health or state with respect to water. Thus, water potential is a better indicator for plant, agriculture, irrigation, and environmental management than water content. Water moves through plants because

$$\Psi_{water} = 0 > \Psi_{soil} > \Psi_{root} > \Psi_{stem} > \Psi_{leaf}$$

Thus, the concept of water potential and water activity are very useful in growth, manufacture, handling, storage, and management of food.

VIII. LIVING ORGANISMS IN WATER

The closer we look, the more we see. Living organisms on Earth are so complicated that their classification and phylogeny are still being studied and revised. New relationships are proposed to modify the five kingdoms proposed by Robert Whittaker in 1969. Nevertheless, most of the earliest unicellular living organisms in the Monera and Protista kingdoms are still living in water. Both the numbers of species and individuals are staggering. For example, photosynthesis by algae in oceans consumes more CO_2 than that by all plants on land. Algae were probably present on Earth before other organisms. Many phyla (divisions) of fungi, plantae, and animalia kingdoms also make water their home. Both numbers and species of organisms living in water are probably more than those on land. The subject of living organisms in water is fascinating, but we can only mention some fundamentals about their relationships to water here. Certainly, every aspect of living organisms in water is related to food, because Homo sapiens is part of the food chain, if not at the top of it.

All life requires energy or food. Some living organisms receive their energy from the sun whereas others get their energy from chemical reactions in the aquatic media. Chemical reactions are vital during their lives. For example, some bacteria derive energy by catalyzing the oxidation of iron sulfide, FeS_2, to produce iron ions $Fe(H_2O)_6^{2+}$ and elemental sulfur. Water is the oxidant, which in turn reduces oxygen (48). Chemical reactions provide energy for bacteria to sustain their lives and to reproduce. Factors

affecting life in water are minerals, solubility of the mineral, electrochemical potentials of the material, acidity (pH), sunlight, dissolved oxygen level, presence of ions, chemical equilibria, etc. Properties of water influence life in general, and in the aquatic system in particular. As the population grows, aquaculture probably will be seen as a more efficient way of supplying protein for the ever-increasing population.

Regarding drinking water, we are concerned with aquatic organisms invisible to the naked eye. Pathogenic organisms present in drinking water cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera, and other illnesses. Pathogens are usually present in waters contaminated with human and animal wastes that enter the water system via discharge, run offs, flood, and accidents at sewage treatment facilities. Insects, rodents, and animals can also bring bacteria to the water system (49, 50). Testing for all pathogenic organisms is impossible, but some organisms share common living conditions with some pathogenic bacteria. Thus, water testing can use these harmless bacteria as indicators for drinking water safety.

IX. WATER RESOURCE, SUPPLY, TREATMENT, AND USAGE

About 70% of the Earth's surface is covered with water, but only about 2% is covered by fresh water. Ocean waters are salty; only a small percentage is fresh water resources (lakes, rivers, and underground). Fresh water is needed for drinking, food, farming, washing, and manufacturing.

When salty water freezes, the ice so formed contains very little salt, if any. Thus, nearly all ice, including the massive ice at the polar cap, is fresh water. In fact, the ice cap in the Antarctic contains a lot of fresh water ice, but that cannot be considered a water resource.

Hydrologists, environmentalists, and scientists, engineers, sociologists, economists, and politicians are all concerned with problems associated with water resources. Solutions to these problems require experts and social consensus.

X. SUBCRITICAL AND SUPERCRITICAL WATERS

Waters at temperatures between the normal boiling and critical points (0 to 373.98°C) are called **subcritical waters**, whereas the phase above the critical point is **supercritical water**. In the 17th century, Denis Papin (a physicist) generated high-pressure steam using a closed boiler, and thereafter pressure canners were used to preserve food. Pressure cookers were popular during the 20th century. Analytical chemists have used subcritical waters to extract chemicals from solids for analysis since 1994 (51).

Water vapor pressures up to its critical point are given in Table 12.8, but data on polarity, dielectric constant, surface tension, density, and viscosity above 100°C are scarce. In general, these properties decrease as the temperature increases. In fact, some drop dramatically for supercritical water. On the other hand, some of them increase with pressure. Thus, properties of sub- and supercritical waters can be manipulated by adjusting temperature and pressure to attain desirable properties.

As the polarity and dielectric constant decrease, water becomes an excellent solvent for non-polar substances such as those for flavor and fragrance. However, foodstuffs may degrade at high temperatures. Applications of sub- and super-critical water are relatively recent events, but applications of supercritical CO_2 (critical temperature 32°C) for chemical analyses started in the 1980s, and investigations of supercritical water followed. However, research and development have been intensified in recent years (52). Scientists and engineers explore the usage of supercritical water for waste treatment, polymer degradation, pharmaceutical manufacturing, chromatographic analysis, nuclear reactor cooling, etc. Significant advances have also been made in material processing, ranging from fine particle manufacture to the creation of porous materials.

Water has been called a green solvent compared to the polluting organic solvents. Sub- and super-critical waters have been explored replacements of organic solvents in many applications including the food industry (53). However, supercritical water is very reactive, and it is corrosive for stainless steels that are inert to ordinary water. Nevertheless the application of sub- and super-critical waters remains a wide-open field.

XI. POSTSCRIPT

Water, ice, and vapor are collections of H_2O molecules, whose characteristics determine the properties of all phases of water. Together and in concert, water molecules shape the landscape, nurture lives, fascinate poets, and captivate scientists. Human efforts to understand water have accumulated a wealth of science applicable in almost all disciplines, while some people take it for granted.

Water molecules are everywhere, including outer space. They not only intertwine with our history and lives, they are parts of us. How blessed we are to be able to associate and correlate the phenomena we see or experience to the science of water.

An article has a beginning and an end, but in the science of water, no one has the last word, as research and exploration on water continue, including its presence in outer space (54). Writing this chapter provoked my fascination on this subject, and for this reason, I am grateful to Professors Wai-kit Nip, Lewis Brubacher, and Peter F. Bernath for their helpful discussions and encouragement.

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13 Bioactive Amines

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CONTENTS

I. DEFINITION, CLASSIFICATION, AND PHYSICO-CHEMICAL CHARACTERISTICS

It has long been known that certain amines fulfill a number of important metabolic and physiologic functions in living organisms. They are formed during normal metabolic processes and are, therefore, present in foods.

Bioactive or biologically active amines (Figure 13.1) are aliphatic, cyclic, and heterocyclic organic bases of low molecular weight (74, 102, 165). They are derivatives of ammonia in which the hydrogen atoms are replaced by one, two, or three alkyl moieties to give, respectively, primary, secondary, or tertiary amines. Many different structures of varying complexity are found (166).

Most of the amines have been named after their precursor amino acids, e.g., histamine originates from histidine, tyramine from tyrosine, tryptamine from tryptophan, and so on. However, the names cadaverine and putrescine are associated with decomposition and putrefaction, and spermine and spermidine with seminal fluids where they were found for the first time (59, 102).

Bioactive amines can be classified on the basis of the number of amine groups, chemical structure, biosynthesis, or physiological functions (9, 160, 165). According to the number of amine groups, they can be monoamines (tyramine, phenylethylamine), diamines (histamine, serotonin, tryptamine, putrescine, cadaverine), or polyamines (spermine, spermidine, agmatine). Based on the chemical structure, amines can be aliphatic (putrescine, cadaverine, spermine, spermidine, agmatine), aromatic (tyramine, phenylethylamine), or heterocyclic (histamine, tryptamine, serotonin). They can also be classified as indolamines (serotonin) and imidazolamines (histamine). According to the biosynthetic pathway, amines can be natural or biogenic. Natural amines – spermine, spermidine, putrescine and histamine – are formed during *de novo* biosynthesis, e.g., *in situ* as required from their precursors (9, 74). Biogenic amines are formed by bacterial decarboxylation of free amino acids. Histamine can be either natural (stored in mast cells or basophils) or biogenic. Based on the physiological functions, amines are classified as polyamines and biogenic amines. Polyamines play an important role in growth while biogenic amines are neuro- or vasoactive (9). This is the most widely used classification and will be the one considered throughout this chapter.

The common and chemical names, the molecular formulas and weights, and the physico-chemical characteristics of some bioactive amines are summarized in Table 13.1. The molecular weights are usually low, varying from 88.15 (putrescine) to 202.34 (spermine). The amines behave as

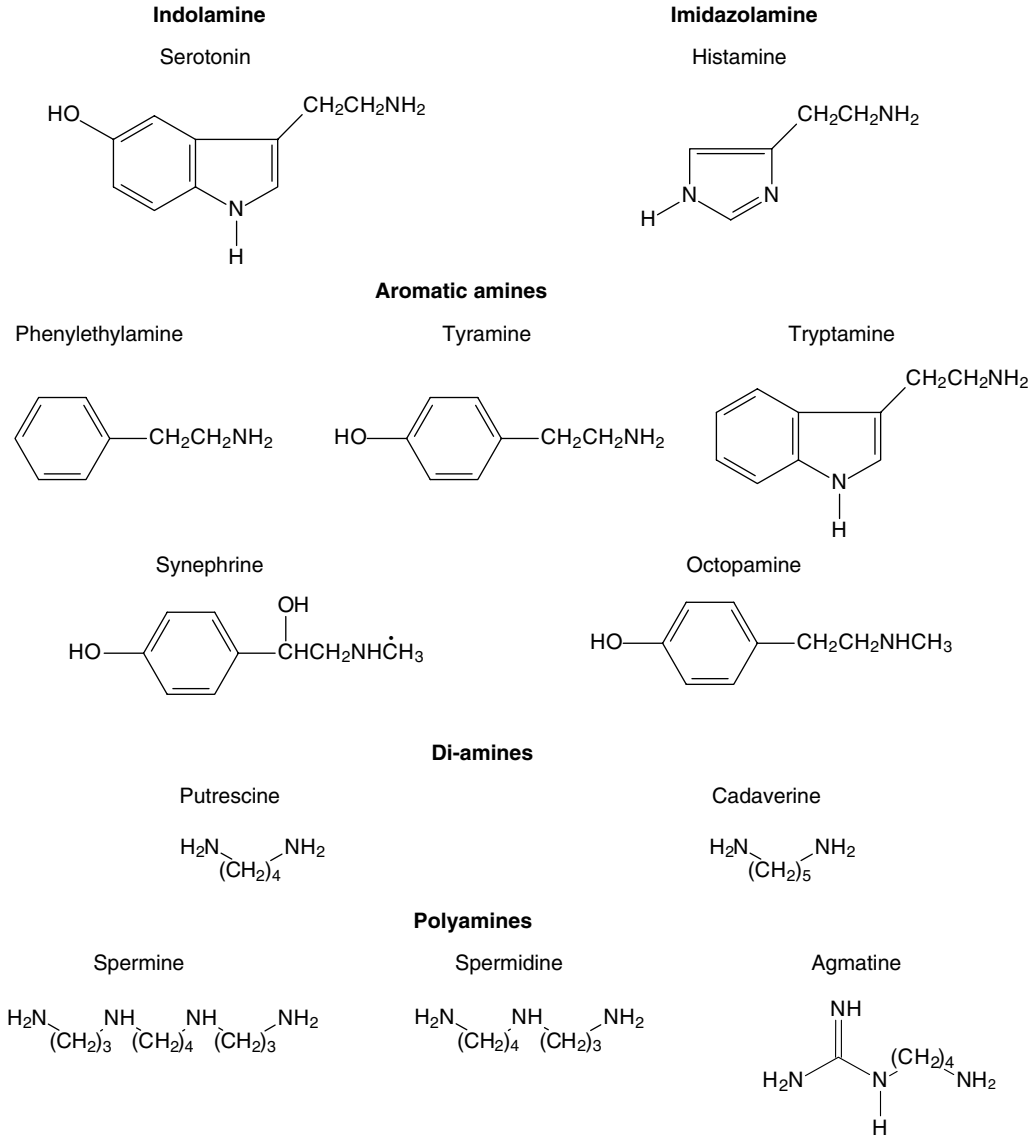

FIGURE 13.1 Classification and chemical structures of some bioactive amines.

cations, being protonated at physiological pH values (123). The pK_a for amines lies between 6 and 11 (41, 101, 166).

At room temperature, the free amines are either liquid, syrupy liquid, crystal, or needle. The boiling points vary from 128 to 210 °C, and the melting points from 9 to 231 °C. The solubility varies widely depending on the amine and on its form – salt or free base (101, 213). At high pH values, dissociation of amine salts into free amines may occur. The refractive index has been reported by Lide (101) for putrescine (1.4969), cadaverine (1.463), and phenylethylamine (1.5290). Density of cadaverine is 0.873 g/mL. Maximum UV absorptions for tryptamine are 222, 282, and 290 nm in ethanol; 224 and 278 nm in acetonitrile:water, 20:80; and 225 and 278 nm in methanol: water, 15:85. Maximum UV absorptions for tyramine and histamine are, respectively, 226 and 275 nm and 225 nm in acetonitrile:water, 20:80; and 228 and 275 nm and 228 nm in methanol:water, 15:85 (34). For synephrine and octopamine, maximum UV absorption values are 231 and 272 nm, and for tyramine 231 and 275 in acetate buffer (pH 4,9):acetonitrile, 9:91 (200, 213). The stability varies with the amines. Some amines can absorb CO_2. Synephrine is stable to light and air. Serotonin hydrochloride is sensitive to light and aqueous solutions are stable at pH 2.0 to 6.4. Decomposition of octopamine hydrochloride can occur at 170 °C (213).

Few sensory data on amines are available. This represents an area that deserves further attention. However, a few amine-based compounds can be used by the flavor compounder. For example, phenylethylamine, is recognized by the Flavor Extract Manufacturers' Association as FEMA # 3220, and has a fishy odor (108).

TABLE 13.1
Common and Chemical Names, Molecular Formulas and Weights, and Physico-Chemical Characteristics of some Bioactive Amines

Amine	Chemical Name	Molecular Formula	M W[1]	Melting Point °C	Boiling Point °C	pK_a	Solubility[2]
AGMATINE	4-(aminobutyl) guanidine; 1-amino-4-guanidobutane	$C_5H_{14}N_4$	130.19	231 ($C_5H_{14}N_4.H_2SO_4$)	—	—	1 – ethanol ($C_5H_{14}N_4{\cdot}H_2SO_4$) 2 – H_2O ($C_5H_{14}N_4{\cdot}H_2SO_4$)
PUTRESCINE	1,4-butane-diamine; tetramethylenediamine	$C_4H_{12}N_2$	88.15	23–24 280	158–160	pK_{a1} = 9.35 pK_{a2} = 10.80 (20 °C) ($C_4H_{12}N_2{\cdot}2HCl$)	1 – benzene; diethyl ether 3 – H_2O 4 – H_2O ($C_4H_{12}N_2{\cdot}2HCl$ & $C_4H_{12}N_2{\cdot}2HCl$); ethanol
SPERMIDINE	*N*-(3-aminopro-pyl)-1,4-butane-diamine; N-(γ-amino-propyl)	$C_7H_{19}N_3$	145.24	256–258 ($C_7H_{19}N_3.3HCl$)	128–130	pK_{a1} = 8.15 pK_{a2} = 9.74 pK_{a3} = 10.24	3 – H_2O; ethanol; diethyl ether
SPERMINE (gerontine, musculamine, neuridine)	*N,N′*-bis(3-aminopropyl)-1,4-butane-diamine; N,N´-bis(3-aminopropyl) tetramethylenediamine	$C_{10}H_{19}N_3$	202.34	55–60	141–142	pK_{a1} = 7.91 pK_{a2} = 8.68 pK_{a3} = 10.21 pK_{a4} = 10.56	1 – benzene; petroleum ether; diethyl ether 3 – H_2O; lower alcohols; chloroform
CADAVERINE (animal coniine)	1,5-pentanediamine; pentamethylenediamine	$C_5H_{14}N_2$	102.18	9 225–230 ($C_5H_{14}N_2.2HCl$)	178–180	pK_{a1} = 10.05 pK_{a2} = 10.93 (25 °C)	1 – absolute alcohol ($C_5H_{14}N_2{\cdot}2HCl$) 2 – diethyl ether 3 – H_2O; etanol; H_2O ($C_5H_{14}N_2{\cdot}2HCl$)
PHENYLETHYL-AMINE	benzene ethanoamine	$C_8H_{11}N$	121.18	—	197.5	pK_{a1} = 9.84 (25 °C)	3 – H_2O; CCl4 4 – diethyl ether
HISTAMINE	1H-imidazole-4-ethanamine; 2-(4-imidazolyl)-ethylamine	$C_5H_9N_3$	111.15	83–84 244–246 ($C_5H_9N_3.2HCl$)	209–210	pK_{a1} = 6.04; pK_{a2} = 9.75 (25 °C)	2 – diethyl ether 3 – H_2O; ethanol; chloroform 4 – H_2O ($C_5H_9N_3{\cdot}2HCl$) methanol
OCTOPAMINE (norsympatol, norsynephrine)	α-(aminomethyl)-4-hydroxy-benzene-methanol; α-(aminomethyl)-p-hydroxybenzyl alcohol	$C_8H_{11}NO_2$	153.18	160	—	—	4 – H_2O ($C_8H_{11}NO_2{\cdot}HCl$)
SEROTONIN	5-hydroxy-tryptamine; 5-hydroxi-3-(β-amino-ethyl) indol	$C_{10}H_{12}N_2O$	176.21	167–168 ($C_{10}H_{12}N_2O.HCl$)	—	pK_{a1} = 9.8 pK_{a2} = 11.1 (25 °C)	1 – 100% ethanol; acetone; pyridine; chloroform; ethyl acetate; diethyl ether; benzene 2 – metanol; 95% ethanol 3 – H_2O ($C_{10}H_{12}N_2O{\cdot}HCl$)
SYNEPHRINE (analeptin, ethaphene, oxedrine, p-sympatol, simpalon)	4-hydroxy-α[(methylamino)methyl]-benzene-methanol; 1-(4-hydroxiphenyl)-2-methylamino-ethanol	$C_9H_{13}NO_2$	167.2	184–185 151–152 ($C_9H_{13}NO_2.HCl$)	—	—	3 – ethanol 4 – H_2O ($C_9H_{13}NO_2{\cdot}HCl$ & $C_9H_{13}NO_2{\cdot}$tartrate)
TRYPTAMINE	1H-indole-3-ethanamine; 3-(2-aminoethyl) indol	$C_{10}H_{12}N_2$	160.21	118 ($C_{10}H_{12}N_2.HCl$)		pK_{a1} = 10.2 (25 °C)	1 – H_2O; diethyl ether; benzene; chloroform 3 – etanol, acetone
TYRAMINE	4-(2-aminoethyl)phenol; 2-*p*-hydroxy phenyl-ethyl amine	$C_8H_{11}NO$	137.18	164–165 269 ($C_8H_{11}NO.HCl$)	205–207	pK_{a1} = 9.74 pK_{a2} = 10.52 (25 °C)	2 – H_2O; benzene; xylene 3 – boiling ethanol

[1] MW = Molecular weight.

[2] Solubility: 1=insoluble, 2=fairly soluble, 3=good, 4=very good.

Source: Refs. 41, 101, and 213.

Wang et al. (206) investigated the odor threshold of polyamines. They reported that free polyamines have an unpleasant, almost putrid, ammoniacal odor. In food, because of their basic groups, polyamines normally bind with the acidic groups of other food components, notably those in nucleic acids or lipids. However, at high pH, free polyamines could result. The determination of odor thresholds for putrescine, spermidine, and spermine by an informal panel of nine people provided odor descriptions which varied widely from sodium hypochlorite bleach, dusty, and musty, to putrid, amine, and ammoniacal. Using a formal panel of 15 members, the odor threshold of the polyamines, e.g., polyamine concentration at which 50% of the panelists gave a positive response, was determined. Spermine and putrescine had odor thresholds in water of the same magnitude (2.4 and 2.2 mg/100 g, respectively), while those of cadaverine (19.0 mg/100 g) and spermidine (12.9 mg/100 g) were approximately six to nine times higher. However, when 2% soybean flour was added, odor thresholds increased significantly, from five to ten times greater than those in water, reaching values of 10.9 g for putrescine and 102.5 mg/100 g for spermidine.

II. PHYSIOLOGICAL IMPORTANCE

Bioactive amines participate in important metabolic and physiological functions in living organisms. Biogenic amines are generally either psychoactive, neuroactive, or vasoactive. Psychoactive amines, such as histamine and serotonin, affect the nervous system by acting on neural transmitters in the central nervous system. Vasoactive amines act directly or indirectly on the vascular system. Pressor amines – tyramine, tryptamine, and phenylethylamine – cause a rise in blood pressure by constricting the vascular system and increasing the heart rate and force of contraction of the heart. However, tyramine does it indirectly by causing the release of noradrenaline from the sympathetic nervous system (158, 165). Serotonin and histamine are also strongly vasoactive (139).

Histamine is a powerful biologically active chemical that can exert many responses within the body. Although mast cells and blood basophiles contain large amounts of histamine stored in special granules, the effect of histamine does not appear unless special reactions (i.e., an allergic reaction) release it into the bloodstream. Histamine exerts its effects by binding to receptors on cellular membranes which are found in the cardiovascular system and in various secretory glands (87). Histamine is a strong capillary dilator and can produce hypotensive effects. It can directly stimulate the heart, cause contraction or relaxation of extravascular smooth muscle (excite the smooth muscle of the uterus, intestine and respiratory tract), stimulate both sensory and motor neurons, and control gastric acid secretion. It also mediates primary and immediate symptoms in allergic responses (139, 158, 165, 173, 177).

Serotonin is a vaso- and bronco-constrictor, reduces the volume and acidity of the gastric juice, has anti-diuretic effect, stimulates smooth muscle, and affects carbohydrate metabolism (63). When introduced into the afferent circulation, serotonin causes a release of prostaglandin and other vasoactive substances. Serotonin is a neurotransmitter, particularly in the central nervous system. It is involved in the regulation of a number of important functions, including sleep, thirst, hunger, mood, and sexual activity (42).

Tyramine is taken up into adrenergic nerves and causes a massive release of noradrenaline. It has a marked effect on the release of the putative neurotransmitter amines dopamine, noradrenaline, and serotonin from nerve terminals (42).

Phenylethylamine and tryptamine are endogenous constituents of many tissues, including brain. Both can cross the blood-brain barrier with ease. It is conceivable that large amounts of phenylethylamine and tryptamine from foodstuffs may enter the central nervous system and lead to side-effects of psychiatric nature. It has been reported that phenylethylamine and tryptamine can inhibit uptake and stimulate release of catecholamines and serotonin from a variety of preparations of brain and heart tissues. It has also been demonstrated that phenylethylamine can affect binding of serotonin to its receptor sites in the human and rat brain. Introduction of tryptamine into the afferent circulation can cause a release of prostaglandin and other vasoactive substances into the systemic circulation (42).

There is evidence, particularly with respect to the invertebrate nervous system, suggesting that octopamine may be a neurotransmitter. Less is known about synephrine, although it has been shown to be a constituent of various tissues and body fluids. Synephrine is used pharmacologically as a stimulant, decongestant, and in the treatment of hypotension in oral form (42, 165).

Polyamines are indispensable components of all living cells, where they play essential roles in cell metabolism, growth, and differentiation (11). During normal and adaptive growth, polyamines are involved in a variety of growth-related processes which reflect their multifunctional character. Polyamines have various electrostatic interactions with macromolecules especially DNA, RNA, and protein and are involved in the regulation and stimulation of their synthesis (104).

Diverse effects of the polyamines on cell replication have been identified, such as the control and initiation of translation and regulation of its fidelity, stimulation of ribosome subunit association, enhancement of RNA and DNA synthesis, and reduction in the rate of RNA degradation. Polyamines stabilize the structure of tRNA and help condense DNA and covalently modify proteins (9, 10). Polyamines stimulate the rate of transcription, the amino acid activation, and the rate of translation during the course of protein synthesis (148). Polyamines also stimulate cell differentiation and interact and module various intracellular

messenger systems (104). The most important function of polyamines, one in which they cannot be replaced by any other positively charged molecule, is to act as second messengers, thereby mediating the action of all known hormones and growth factors (9).

Polyamines interact with different components of the cell membrane thus modulating its functions. They are, therefore, important in the permeability and stability of cellular membranes (9, 48, 104, 160). During the period that follows birth, antigenic macromolecules can penetrate the small intestinal mucosal membranes in quantities that may be of immunologic importance. Polyamines reduce mucosal permeability to macromolecules and prevent food allergies by decreasing mucosal permeability to allergenic proteins (27, 147).

According to Drolet et al. (48) and to Bardocz (9), spermine and spermidine, as well as the diamines putrescine and cadaverine, are efficient free radical scavengers in a number of chemical and *in vitro* enzyme systems. They could inhibit lipid peroxidation and prevent senescence. Levels of superoxide radical formed either enzymatically with xanthine oxidase or chemically from riboflavin or pyrogallol were significantly inhibited. The more reactive hydroxyl radical generated by the Fenton reaction was also effectively scavenged by spermine and spermidine. The efficacy of polyamine scavenging appears to be correlated with the number of amino groups (48, 168).

Polyamines are essential for the maintenance of the high metabolic activity of the normally functioning and healthy gut. They are involved in the repair of gut damage caused by the deleterious components of the food and/or bacteria (11, 104). The development of gastrointestinal tract in ruminants and non-ruminants is dependent upon the increases in ornithine decarboxylase (ODC) activity and in polyamines content in the mucosa (123). Studies performed with rats indicated that during the third postnatal week, rapid intestinal maturation occurs and this period is characterized by an increase in epithelial cell proliferation and differentiation, resulting in histological and enzymatic maturation of the small bowel epithelium, marked increase in mucosal thickness, and increase in mature mucosal enzyme activities. Prior to this process of epithelial maturation, a ten- to twenty-fold increase in mucosal ODC activity and a concomitant increase in *S*-adenosyl-L-methionine decarboxylase (SAMDC) activity as well as putrescine, spermidine, and spermine content were found (104, 123). Simultaneous administration of α-difluoromethyl ornithine (DFMO), a potent irreversible ODC inhibitor, to the nursing mother or directly to the newborn rat pups, resulted in a marked attenuation of the increase in mucosal ODC and polyamine content which was followed by a significant delay in biochemical and histological maturation of the intestinal epithelium (123).

According to Löser (104), polyamines in milk exert various direct and indirect trophic effects on the immature intestine and play an important role as luminal growth factors for intestinal maturation and growth. The protective effect of milk against a newborn's allergies could be explained by its high levels of polyamines. They bring about a decrease in protein permeability, diminishing the amount of allergens reaching the intestinal submucosa, and thus allow a better maturation of the immune system in susceptible newborn (106, 147).

Because of the diversity of the roles of polyamines in cellular metabolism and growth, they are important in health and disease. The requirement for polyamines is particularly high in rapidly growing tissues, e.g., in the young during periods of intense growth and in the mucosa of the gastrointestinal tract. Similarly, during periods of wound healing, post-surgery recovery, liver regeneration, and compensatory growth of the lung, requirements are also high. In healthy adults, however, requirements should not be as high because polyamines are only needed to replace cells and mediate the action of hormones and growth factors (9, 123). Studies are needed to determine the daily requirements.

Three sources of polyamines have been identified: biosynthesis *in situ* from amino acids, direct ingestion from the diet and synthesis, and release by the bacterial flora resident in the gastrointestinal tract of the individual. It is now clear that the diet is an important source of polyamines and it is likely to supply at least a part of the polyamines required to sustain normal metabolism (9).

III. SYNTHESIS

The synthesis of the biogenic amines histamine, tyramine, tryptamine, phenylethylamine, and cadaverine occur through decarboxylation of the precursor amino acids histidine, tyrosine, tryptophan, phenylalanine, and lysine, respectively. In the synthesis of serotonin, tryptophan is transformed by tryptophan hydrolase in 5-hydroxitryptophan, which is decarboxylated by aromatic amino acid decarboxylase in 5-hydroxytryptamine or serotonin. Tyrosine is the precursor of the phenolic amines octopamine and synephrine in citrus (165).

The generalized reaction for amino acid decarboxylation is indicated in Figure 13.2. Aldehyde amination can be another route of synthesis, carried out by aldehyde transaminases (166).

Prerequisites for the formation of amines in foods are the availability of free amino acids, high processing temperatures, or the presence of decarboxylase-positive microorganisms and favorable conditions for microbial growth and decarboxylase activity. Free amino acids occur as such in foods, but may also be released from proteins as a result of proteolytic activity or thermal degradation. The formation of amines by high processing temperatures has been demonstrated by Cirilo et al. (38) during coffee roasting. Longer roasting time (12 compared to 6 minutes) at

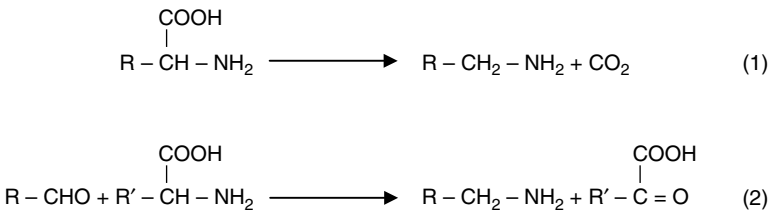

FIGURE 13.2 Formation of amines by amino acid decarboxylation (1) and by aldehyde amination (2).

300 °C in a Probat roaster caused significant increase in spermidine and serotonin levels and formation of agmatine. Decarboxylase-positive microorganisms may constitute part of the associated population of the food or may be introduced by contamination before, during, and after processing (74, 108).

Two mechanisms of action for amino acid decarboxylation have been identified: a pyridoxal phosphate dependent and a non-pyridoxal phosphate dependent reaction. Pyridoxal phosphate joined in a Schiff base linkage to the amino group of a lysil residue forms the active site of the enzymes. The carbonyl group of pyridoxal phosphate reacts readily with the amino acid to form Schiff base intermediates, which are then decarboxylated with the elimination of water to yield the corresponding amines and the original pyridoxal phosphate moiety. Non-pyridoxal phosphate-catalyzed decarboxylation involves a pyruvoyl residue instead. The pyruvoyl group is covalently bound to the amino group of a phenylalanine residue of the enzyme and is derived from a serine residue. The pyruvoyl residue acts in a manner similar to pyridoxal phosphate in the decarboxylation reaction (158)

Regarding the formation of amines by microorganism decarboxylase activity, several studies have been performed to investigate the production of amines by species isolated from fish, cheese, meat products, vegetables, and alcoholic beverages. These studies indicated that many species of *Escherichia*, *Enterobacter*, *Salmonella*, *Shigella*, and *Proteus* are active biogenic amines formers. Certain species of *Achromobacter*, *Lactobacillus*, *Leuconostoc*, *Pseudomonas*, *Pediococcus*, *Streptococcus*, *Micrococcus*, and *Propionibacterium* are also capable of amine production (16, 23, 51, 82, 149, 195).

In fermented foods, the applied starter cultures may also affect amine production. Straub et al. (174) investigated the production of putrescine, cadaverine, histamine, tyramine, and phenylethylamine by 523 strains of 35 species of food fermentation microorganisms. These authors observed that some strains of carnobacteria, *Lactobacillus buchneri*, *L. curvatus*, *L. reuteri*, and *Staphylococcus carnosus* were proficuous amine producers. However, some strains of *L. alimentarius*, *L. brevis*, *L. bavaricus*, *L. delbrueckii* ssp. *Lactis* and *Micrococcus* spp. were capable of producing amines at smaller quantities. The importance of selecting starter cultures based on their potential for amine production is emphasized.

The amino acid decarboxylating activity of some food microorganisms is indicated in Table 13.2. The production of amines by bacteria is affected by pH, temperature, oxygen tension, presence of vitamins and cofactors, and availability of free amino acids and of fermentable sugars. In pH values of 2.5 to 6.5, the production of amines by the bacteria is stimulated as a protection against the acidic environment (201). The activity of decarboxylases is dependent on the microorganism's growth phase, being higher at the stationary phase. With regard to the temperature, decarboxylases are more active at temperatures lower than 30 °C and without action at temperatures above 40 °C. However, at temperatures between 0 and 10 °C, the activity will depend on the microorganisms present (7, 74).

Polyamine synthesis is a more complex process, although the first few steps also include decarboxylation reactions. There are several proven biosynthetic pathways responsible for polyamine production (Figure 13.3). Putrescine is an obligate intermediate in polyamine synthesis. In animals the first step is decarboxylation of ornithine by ODC (EC 4.1.1.17). In plants and some microorganisms, an alternative pathway exists to produce putrescine from arginine via agmatine by arginine decarboxylase (ADC; EC 4.1.1.19). An additional path for the formation of putrescine is via citrulline (9, 59, 168, 202).

Both ODC and ADC are dependent on pyridoxal phosphate. Agmatinase is the enzyme that converts agmatine to putrescine. Agmatine is metabolized to putrescine in a two-step conversion. Agmatine iminohydrolase (AIH) catalyzes the formation of N-carbamoylputrescine, which is converted to putrescine by N-carbamoylputrescine amido hydrolase (NCPAH). N-carbamoylputrescine can also be formed from citrulline by citrulline decarboxylase (CDC). The arginine pool can be increased through protein degradation. Ornithine and urea formation from arginine allows recovery of nitrogen and carbon (59).

Spermidine and spermine are formed by the subsequent addition of an aminopropyl moiety to putrescine and spermidine, respectively. These reactions are catalyzed by the aminopropyltransferase enzymes spermidine synthase (SpdS, EC 2.5.1.16) and spermine synthase (SpmS, EC 2.5.1.22). The aminopropyl group is derived from methionine via *S*-adenosyl-L-methionine (SAM) in a reation

TABLE 13.2
Amino Acid Decarboxylating Activity of Food Microorganisms

Amine	Microorganism	Ref.
HISTAMINE	*Achromobacter histaminum*	7
(Histidine decarboxylase)	*Acinetobacter calcoaceticusvar, A. lwofii, A. nitratum*	33
	Aeromonas spp. *A. hydrophila*	36
	Alteromonas putrefaciens	49
	Betabacterium spp.	51
	Citrobacter diversus, C. freundii	72
	Clostridium perfringens, C. bifermentans, C. fallax, C. novyi	117
	Edwardsiella spp.	122
	Enterobacter aerogenes, E. agglomerans, E. cloacae, E. intermedium	139
	Escherichia coli, E. freundii	140
	Hafnia alvei	142
	Klebsiella oxytoca, K. pneumoniae	144
	Lactobacillus 30a; L. acidophilus, L. alimentarius, L. arabinose, L. bavaricus, L. buchneri, L. bulgaricus, L. casei, L. delbrueckii, L. fermentum, L. helveticus, L. plantarum, L. reuteri, L. sanfrancisco	145
	Morganella morganii (Proteus morganii)	146
	Oenococcus oeni (Leuconostoc oenos)	159
	Pediococcus cereviseae	160
	Photobacterium histaminum, P. phosphoreum	173
	Proteus mirabilis, P. reptilivora, P. rettgeri, P. stuartii, *P. vulgaris*	174
	Providencia alcalifaciens, P. stuartii	178
	Pseudomonas aeruginosa, P. fluorescens, P. putida, P. putrefaciens, P. reptilvora	182
	Ristella spp.	
	Salmonella enteriditis, S. paratyphi, S. schottmuelleri, S. typhi	
	Serratia liquefaciens, S. odorifera	
	Shigella spp.	
	Staphylococcus xylosus	
	Streptococcus faecalis, S. faecium, S. lactis, S. mitis	
	Vibrio alginolyticus	
TYRAMINE	*Betabacterium* spp.	33
(Tyrosine decarboxylase)	*Carnobacterium divergens, C. Galinarum, C. maltaromicus, C. piscicola*	51
	Clostridium aerofoetidum, C. sporogenes	53
	Enterococcus faecalis, E. faecium	58
	Escherichia coli	71
	Lactobacillus brevis, L. buchneri, L. bulgaricus, L. curvatus, L. delbrueckii ssp. *lactis, L. hilgardii, L. paracasei, L. pentoaceticus* Rudensis, *L. pentosus, L. plantarum, L. rhamnosus*	114
	Lactococcus lactis	121
	Leuconostoc mesenteroids, L. paramesenteroids	122
	Micrococcus varians	132
	Oenococcus oeni (Leuconostoc oenos)	139
	Proteus mirabilis	140
	Pseudomonas fluorescens, P. reptilivora	159
	Serratia liquefaciens	160
	Staphylococcus carnosus, S. piscifermentans, S. saprophyticus	173
	Streptococcus faecalis, S. faecium, S. durans (group D)	174
CADAVERINE	*Edwardsiella cloacae, E. hoshinae, E. ictaluri*, *E. tarda*	72
(Lysine decarboxylase)	*Enterobacter aerogenes, E. gergoviae*	112
	Escherichia coli, E. blattae	142
	Fusobacterium varium	145
	Hafnia alvei	159
	Klebsiella planticola, K. pneumoniae, K. oxytoca, K. terrigena	174
	Kluyvera ascorbata	183
	Lactobacillus acidophilus, L. bavaricus, L. brevis, L. casei, L. curvatus, L. hilgardii	
	Micrococcus spp.	

(*continued*)

TABLE 13.2 (*Continued*)

Amine	Microorganism	Ref.
	Obesumbacterium aeruginosa, O. proteus	
	Pseudomona aeruginosa, P. fluorescens	
	Salmonella choleraesuis, S. gallinarum, S. pullorum, S. typhi	
	Serratia fonticola, S. liquefaciens, S. marcescens, S. odorifera	
	Staphylococcus carnosus	
	Vibrio alginolyticus, V. campbelli, V. cholerae, V. harveyi, V. parahaemolyticus, V. vulniticus	
	Yersinia ruckeri	
PUTRESCINE	*Cedecea davisae*	6
(Ornithine decarboxylase)	*Citrobacter amalonaticus, C. diversus, C. freundii,*	50
	Edwardsiella hoshinae, E. tarda	72
	Enterobacter cloacae, E. aerogenes, E. gergoviae, E. intermedium, E. sakazakii	112
	Escherichia coli, E. blattae	142
	Hafnia alvei	159
	Klebsiella pneumoniae	174
	Kluyvera ascorbata, K. cryocrescens	183
	Lactobacillus bavaricus, L. brevis, L. buchneri, L. casei, L. curvatus, L. plantarum, L. hilgardii	
	Micrococcus kristinae, M. varians	
	Morganella morganii	
	Obesumbacterium proteus	
	Proteus mirabilis	
	Pseudomonas aeruginosa, P. aureofaciens, P. fluorescens, P. putida	
	Salmonella choleraesuis, S. paratyphi, S. pullorum	
	Serratia fonticola, S. liquefaciens, S. marcescens	
	Shigella sonnei	
	Staphylococcus carnosus, S. epidermidis	
	Vibrio alginolyticus, V. cholerae, V. harveyi, V. parahaemolyticus, V. vulnificus	
	Yersinia enterocolitica , Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. ruckeri	
PHENYLETHYLAMINE	*Enterococcus* spp.	71
(Phenylalanine decarboxylase)	*Lactobacillus curvatus, L. delbrueckii* ssp. *lactis, L. farciminis, L. hilgardii, L. brevis*	121
	Leuconostoc mesenteroids, L. paramesenteroids	122
	Micrococcus spp.	174
	Staphylococcus carnosus, S. epidermidis, S. piscifermentans	
AGMATINE (Arginine decarboxylase)	*Lactobacillus hilgardii*	6
TRYPTAMINE (Tryptophane decarboxylase)	*Lactobacillus bulgaricus*	33

catalyzed by the enzyme SAMDC (AdoMetDC; EC 4.1.1.50). The 5′-methyltioadenosine, resulting from the liberation of the aminopropyl group by SAM is converted in methyltioribose and in methionine, recycling the $–SCH_3$ group which warrants the synthesis of polyamines (59, 168, 202).

The rate-limiting enzymes for polyamines synthesis are ODC and SAMDC. DFMO is an irreversible inhibitor of ODC (26). A similar difluoromethyl analog of arginine (DFMA) can also inhibit putrescine formation from arginine. Both inhibitors are highly specific and in most cases DFMA can also be partially metabolized to urea and DFMO, thereby indirectly inhibiting ODC. Methylglyoxal bis-guanylhydrazone (MGBG) is the most common inhibitor of SAMDC, which catalyzes the step committing SAM to the synthesis of polyamines (59).

The regulation of polyamine biosynthesis is complex and precise control prevents overproduction and deficiency of polyamines in animals (130). Several regulatory mechanisms contribute to the regulation of intracellular polyamine homeostasis. Intracellular polyamine concentrations are primarily regulated by intracellular polyamine *de novo* synthesis, conversion and degradation as well as uptake of extracellular polyamines. Preliminary regulatory mechanisms are intracellular *de novo* synthesis via ODC, reconversion of polyamines via interconversion pathway (spermine/spermidine N-1-acetyltransferase and polyamine oxidase) and oxidative degradation of polyamines (46, 104).

Uptake of extracellular polyamines from the gut lumen was found to be a further important regulatory mechanism of polyamine metabolism. It is well established that the

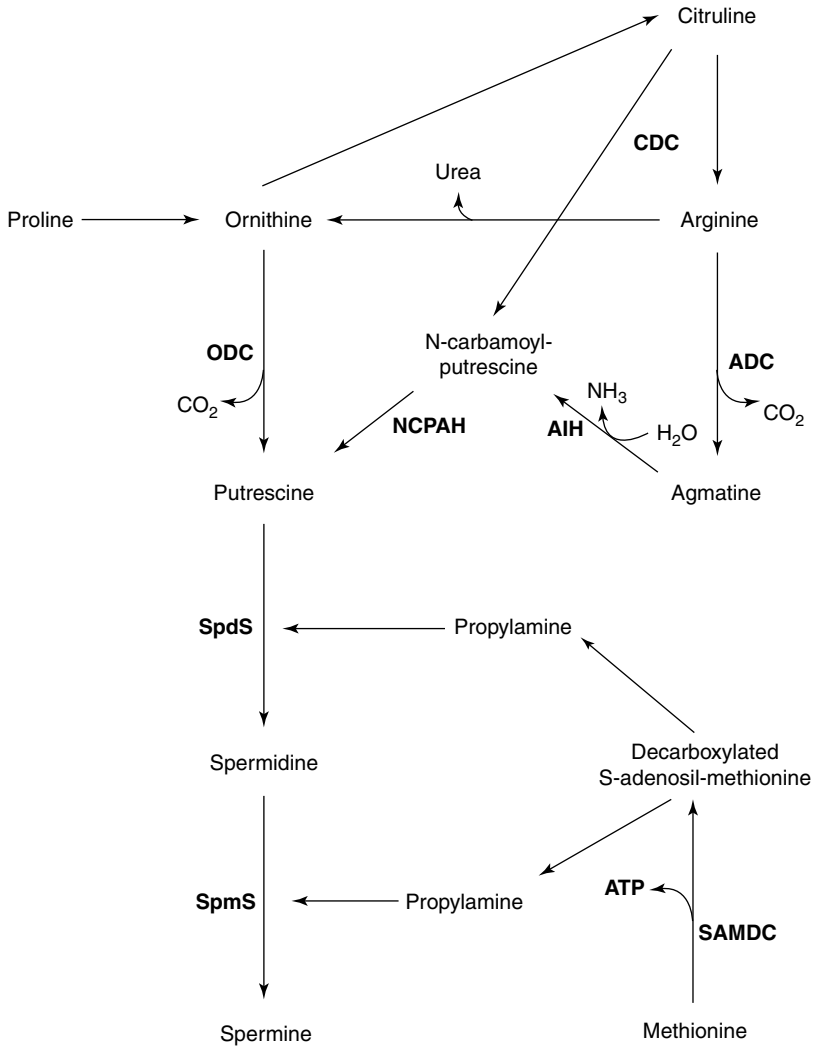

FIGURE 13.3 Pathways for the synthesis of polyamines.

alimentary tract is an important source of polyamines and that dietary as well as to some extent gut bacterial-derived polyamines significantly contribute to the total polyamine body pool. These findings stress the importance of a rapid uptake of dietary, luminal (brush-border membrane) polyamines by the intestinal mucosa upon demand with a consecutive passage through the blood stream (basolateral membrane) to the body (104).

IV. CATABOLISM OF BIOACTIVE AMINES

Healthy individuals can metabolize amines present in foods by acetylation and oxidation (9, 165). Biogenic amines are oxidized by monoaminoxidases (MAO; EC 1.4.3.4) and diaminoxidases (DAO; EC 1.4.3.6). Polyamines are usually acetylated first, then oxidized by polyaminoxidases (PAO; EC 1.5.3.11).

The general equation for the oxidation of amines by amine oxidases (166) is shown in Figure 13.4 and results

$$RCH_2NH_2 + O_2 + H_2O \rightarrow H_2O_2 + RCHO + NH_3 \quad (1)$$

$$R\text{-}CH_2\overset{+}{N}RR'R'' + \tfrac{1}{2}O_2 \rightarrow RCHO + \overset{+}{N}H_2R'R'' \quad (2)$$

FIGURE 13.4 Equations for the oxidation of amines by amine oxidases in the (1) absence and (2) presence of catalase.

in aldehydes, ammonia, and hydrogen peroxide (H_2O_2). According to Marley and Blackwell (113), it is the ionized form of the amine, i.e., the form that is not readily absorbed from the gut, that reacts with the enzyme. Amines that are not ionized to any extent are not oxidized. The overall reaction in the presence of catalase is also shown in Figure 13.4. The aldehyde formed may be oxidized further to the corresponding carboxylic acid.

Amine oxidases belong to a large class of deaminating oxidases (amine:oxygen oxidoreductases) widely distributed among all living organisms. This class of enzymes is

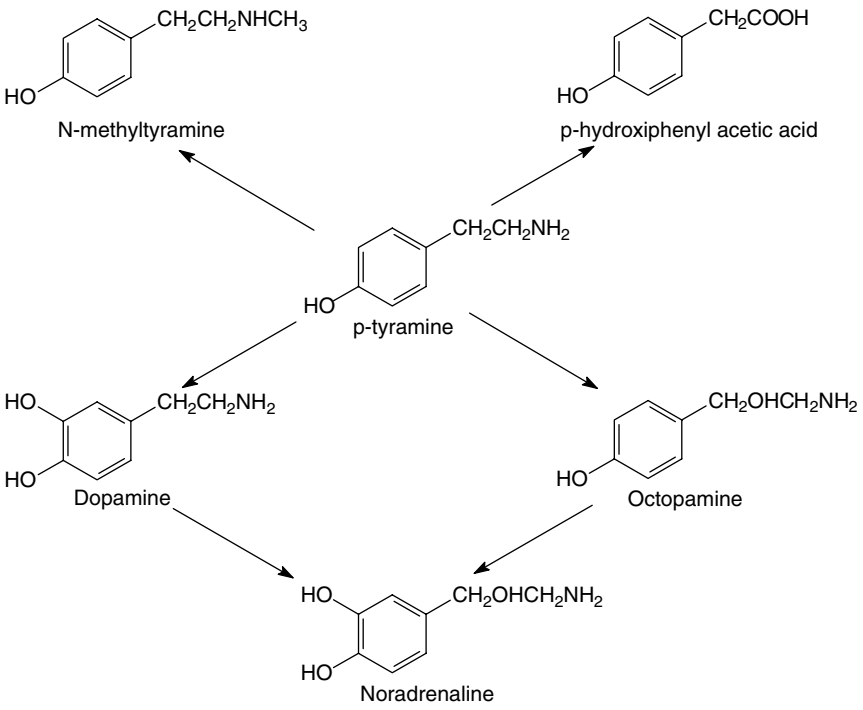

FIGURE 13.5 Tyramine metabolism in man.

divided into two subclasses: copper- and FAD-containing amino oxidases. The copper containing are active on primary amino groups and the FAD-dependent cleave secondary amino groups (134). The amine oxidases contain copper and are sensitive to carbonyl groups. Enzyme activity is usually inhibited by carbonyl reagents and metal chelators (166). Many aliphatic amines of the series $CH_3(CH_2)_nNH_2$ are substrates for the enzyme. The rate of oxidation and affinity vary with the number of carbon atoms in the carbon chain. With increasing length of chain, the rate of oxidation increases, a maximum being reached with 5 or 6 carbon atoms (113). Any substrate of amine oxidase, because it has affinity for the enzyme, may interfere with the oxidation of another substrate.

Tyramine may undergo one of several different catabolic reactions (Figure 13.5). According to Bieck & Karl-Heinz (17), tyramine is extensively metabolized in the gastrointestinal mucosa and liver. The major route is the oxidative deamination to p-hydroxyphenylacetic acid. It is catalyzed by MAO. Other pathways are oxidation to octopamine by dopamine-β-hydroxylase and methylation to N-methyltyramine by N-methyltransferase. Conjugation with either sulphate or acetate groups is also possible. Sulfate conjugation to the o-sulfate accounts for 10–15% of the total dose in urine after oral tyramine administration (17, 60, 87, 115, 139).

Serotonin is oxidized by MAO in man to 5-hydroxyindolacetic acid (5-HIAA). Deamination of tryptamine by monoamine oxidase has also been demonstrated. These amines compete with tyramine as substrate for the enzyme. Such substrate competition could be relevant with a food that contains a number of amines deaminated by amine oxidase (113).

Histamine can also be catabolized by several different catabolic reactions (Figure 13.6). The two main routes are oxidation to imidazole-acetaldehyde and methylation to 1,4-methylhistamine. Indeed, in mammals, 60 to 80% of the metabolites of histamine are derived from oxidative deamination. Amine oxidases are involved in both routes. They convert 1,4-methylhistamine to the corresponding aldehyde and play a minor role in the conversion of histamine to imidazole-acetaldehyde. Inhibitors of monoamine oxidase could therefore interfere with either of these stages, although the metabolism of 1,4-methylhistamine is the one most likely to be affected since diamine oxidase is primarily involved in the conversion of histamine to imidazole-acetaldehyde (113). Percentage recovery of histamine and its metabolites in the urine 12 hours after intradermal ^{14}C-histamine administration in human males indicated that the majority of the radioactivity was recovered as N-methylimidazole acetic acid (42–47%), followed by imidazole acetic acid conjugated with ribose (16–23%), as imidazole acetic acid (9–11%), as N-methylhistamine (4–8%), and as histamine (2–3%) (211).

DAO is a non-selective enzyme and also oxidizes putrescine and cadaverine. In fact, histamine is not the

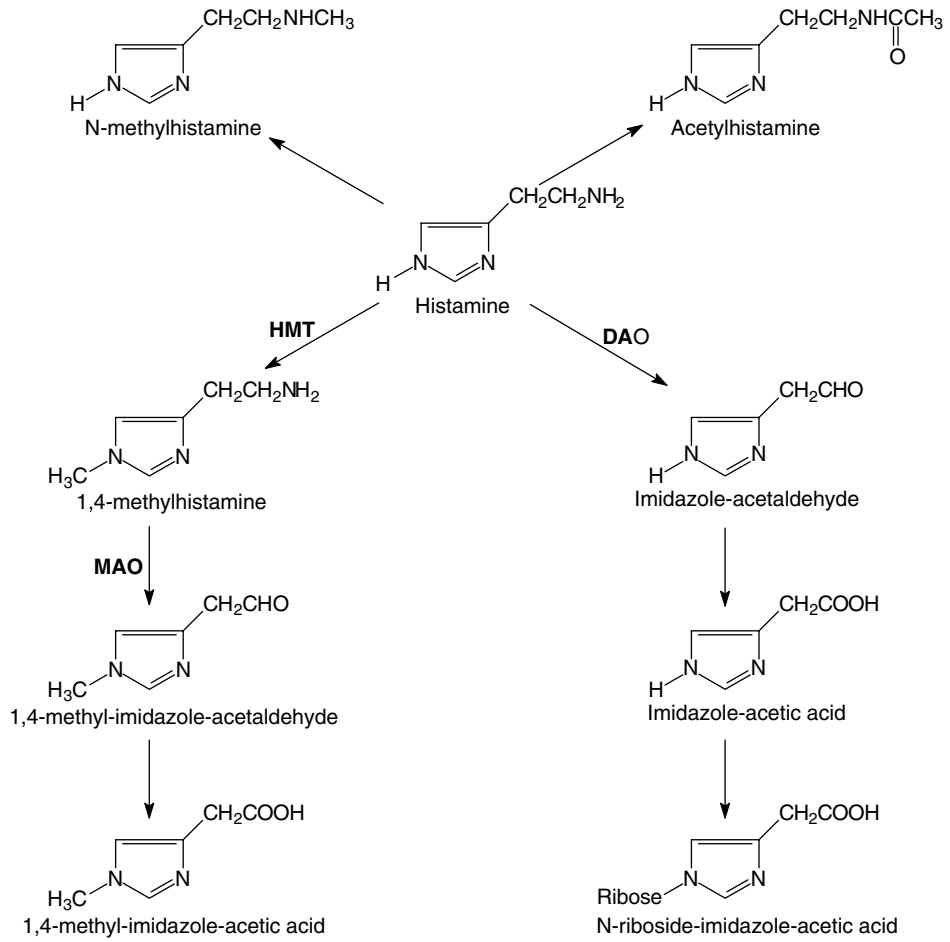

FIGURE 13.6 Histamine metabolism in man.

favored substrate of DAO, being deaminated at about one-third the rate of putrescine and cadaverine. Histamine N-methyl transferase (HMT) is very selective for histamine and requires S-adenosylmethionine as a methyl donor (183).

Several studies were undertaken to assess the potential inhibitory effect of various chemicals on histamine metabolizing enzymes. Taylor and Lieber (181) investigated 37 chemicals and observed that seven strongly inhibited rat jejunal mucosa HMT, while eight inhibited DAO. The most potent inhibitors of HMT activity were tyramine (99%), phenylethylamine (99%), tryptamine (98%), octopamine (94%), agmatine (87%), aminoguanidine (81%), and nicotine (78%), whereas cadaverine, indole, tartrazine, theophylline, thiamin, and trimethylamine gave intermediate levels of inhibition. The most potent inhibitors of DAO activity were aminoguanidine (100%), anserine (100%), carnosine (100%), histamine (99%), agmatine (97%), thiamin (92%), cadaverine (87%), and tyramine (77%). Caffeine, hypoxanthin, indole, 1-methylhistidine, phenylethylamine, piperazine, spermidine, spermine, synephrine, theobromine, theophylline, tryptamine, and xanthine gave intermediate levels of DAO inhibition.

Acidic but not basic drugs are rapidly absorbed from the stomach. This is because the gastric mucosa is selectively permeable to undissociated forms of drugs. Consequently, basic substances such as histamine, serotonin, or tyramine present in foods would be ionized at the acid pH of the stomach and therefore not absorbed whereas in the duodenum and distal gut the alkaline pH would convert them to the un-ionized form which is lipid soluble and more easily absorbed. Once the amines have crossed the intestinal wall, they are carried in the portal blood to the liver and then to the lungs before having access to the systemic circuit (113).

Putrescine and cadaverine are oxidatively deaminated by the reaction of DAO (EC 1.4.3.6). DAO converts putrescine yielding 4-aminobutyraldehyde after cyclization to form Δ^1-pyrroline, with the release of ammonia and hydrogen peroxide (Figure 13.7). Cadaverine is converted via oxidation and cyclization to the 6-membered piperidine ring of anabasine and other alkaloids (62).

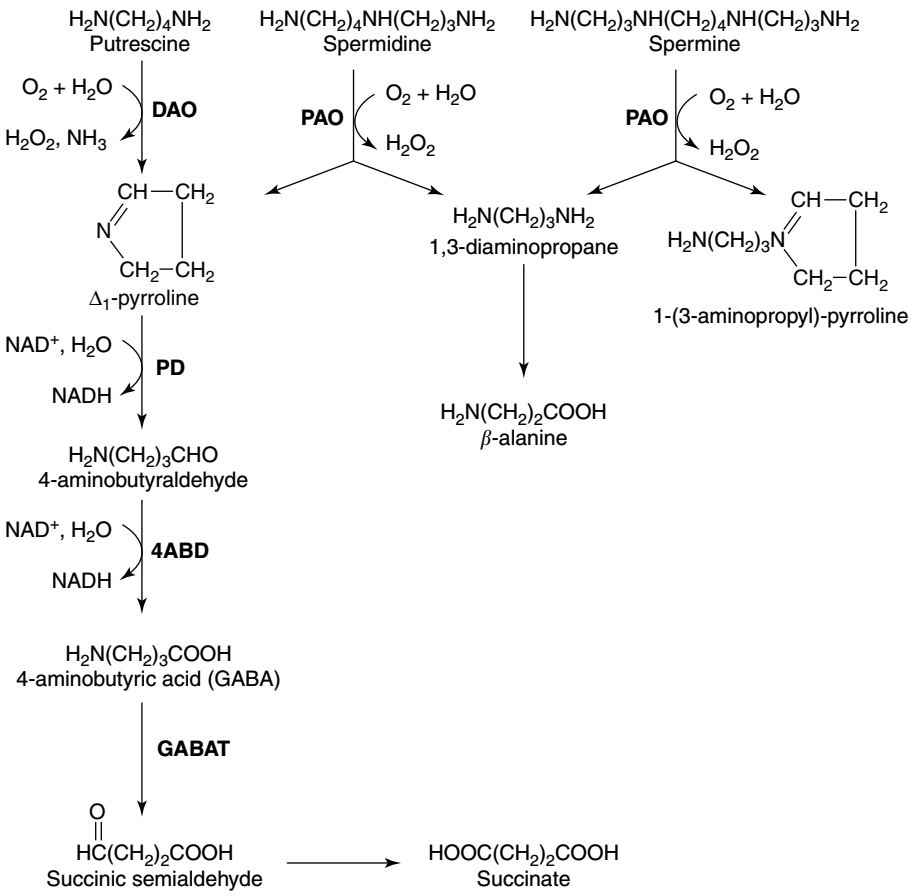

FIGURE 13.7 Putrescine, spermidine, and spermine metabolism in man.

The polyamine oxidases (PAO) are specific for polyamines, and they have FAD as a cofactor (59). Polyamine oxidases degrade spermidine and spermine via the oxidative cleavage at the secondary amino group (153). Degradation of spermidine by PAO yields Δ^1-pyrroline, 1,3-diaminopropane, and hydrogen peroxide, while spermine oxidation yields 1,3-aminopropylpyrroline along with diaminopropane and hydrogen peroxide. Diaminopropane can originate β-alanine (62, 168, 202).

The 4-aminobutyraldehyde formed by the oxidation of putrescine and spermidine can be further oxidized to 4-aminobutyric acid (GABA) by a NAD-dependent pyrroline dehydrogenase (4-aminobutyraldehyde dehydrogenase, 4ABD). This enzyme has been found where DAO or PAO activity is present. The product of this reaction, GABA, can be transaminated and the resulting succinic acid incorporated into the Krebs cycle (Figure 13.7). This metabolic sequence can account for the complete recycling of the carbon and nitrogen from di/polyamines. It may also explain the fact that, in at least some cases, di- and polyamines can be used as organic nitrogen sources by some cells (59, 202).

It has been demonstrated that there is a conversion of spermine and spermidine back into putrescine. The enzymatic reaction of such a reversal pathway has been established; it involves two enzymes acting sequentially, spermidine/spermine N-1-acetyltransferase (SAT; EC 2.3.1.57) and polyamine oxidase (PAO, EC 1.5.3.11) (57).

The amounts of polyamines reaching the body are different for individual polyamines. One hour after rats were intubated with ^{14}C-labeled putrescine, only 29–39% of the label was found as polyamines, with 11–15% remaining as putrescine. Spermidine and spermine were better conserved: 79% of spermidine and 72–74% of spermine were recovered in the original form. If conversion to the other two natural polyamines is also taken into account, 87–96% of the radioactive spermidine and 79–82% of spermine was conserved in polyamine form (10). Since spermidine and spermine are better preserved for further utilization in the body, they are considered the right polyamines to be absorbed from foods.

Catabolism of histamine or tyramine can also be achieved by bacteria. Tyramine oxidase from *Sarcina lutea* was observed to oxidatively deaminate tyramine in the presence of oxygen and to form p-hydroxyphenylacetaldehyde, ammonia, and hydrogen peroxide. *Aspergillus niger* and *Trichosporon* sp. possess amine oxidases that oxidize a wide range of primary amines. Numerous bacteria possess diamine oxidase and are able to degrade histamine, including *Pseudomonas aeruginosa*,

E. coli, *Proteus vulgaris*, *Serratia flava*, and *Clostridium feseri*. In the intestine of man, *E. coli* and *E. aerogenes* are capable of acetylating histamine (124, 139).

V. TOXICOLOGICAL ASPECTS

Amines in foods do not usually represent any health hazard to individuals unless excessive amounts are ingested or the natural mechanism for their catabolism is genetically deficient or impaired by diseases or pharmacological agents (45, 139). Individuals with respiratory and coronary problems, hypertension, or vitamin B_{12} deficiency are at risk because they are sensitive to lower amounts of amines. Men with gastrointestinal problems (gastritis, irritable bowel syndrome, Crohn's disease, stomach, and colonic ulcers) are also at risk since the activity of oxidases in their intestines is usually lower than that in healthy individuals. Patients taking medication that are inhibitors of MAO, DAO, and PAO activity can also be affected, as such drugs prevent amine catabolism. These MAO and DAO inhibitors are used for the treatment of stress, depression, Alzheimer's and Parkinson's diseases, pulmonary tuberculosis, malaria, panic syndrome, and social phobia (60, 61).

Bioactive amines have been implicated as the causative agents in a number of food poisoning episodes (Table 13.3), particularly histamine and tyramine toxicity (158, 177). Tryptamine and phenylethylamine are associated with migraine (87). Furthermore, the importance of polyamines in tumor growth is widely recognized (9, 137).

The most frequent food-borne intoxication caused by amines involves histamine**.** Histamine intoxication is also referred to as "scombroid poisoning" due to its association with the consumption of scombroid fish; however, non-scombroid fish, cheese, and other foods have also been implicated in some cases (173). The disease manifests several minutes to a few hours after ingestion of the histamine containing food (79). At first, a flushing of the face and neck is usually observed, accompanied by a feeling of heat and general discomfort. Often it is followed by an intense throbbing headache. Other symptoms may be cardiac palpitations, dizziness, faintness, thirst, swelling of the lips, urticaria, rapid and weak pulse, and gastrointestinal complaints (abdominal cramps, nausea, diarrhea). However, the most common symptoms are rash, diarrhea, sweating, and headache. In severe cases, bronchospasms, suffocation and severe respiratory distress are reported. Recovery is usually complete within 8 h (12, 139, 158, 165).

TABLE 13.3
Toxic Effects of Bioactive Amines

Toxic Effects	Amines Involved	Food Associated	Symptoms
Histamine intoxication or scombroid poisoning	Histamine (toxic effect is potentiated by putrescine, cadaverine, spermine, tryptamine, tyramine, phenylethylamine, ethanol)	Scombroid fish: tuna, bonito, mackerel, skipjack, herring Other fish: sardines, anchovy, mahi-mahi Cheese: Swiss, Gouda, Cheddar, Gruyere, Cheshire; Sauerkraut; sausage; wine	**Gastrointestinal:** nausea, vomiting, diarrhea, abdominal cramps **Neurological:** throbbing headache, palpitation, flushing, burning throat, itching, rapid and weak pulse, dizziness, faintness, tingling **Hemodynamic:** hypotension, capillary dillatation **Cutaneous:** rash, urticaria, edema, localized inflamation **Severe cases:** bronchospasms, suffocation, severe respiratory distress
Tyramine intoxication	Tyramine	Cheese Beer Wine	Headache, fever, increased blood pressure, vomiting, perspiration, pupils and palpebral tissue dilatation, salivation, lacrimation, increased respiration, palpitation, dyspnea
Cheese reaction[1] or hypertensive crisis (associated with patients under MAOI drugs)	Tyramine 2-phenylethylamine	Cheese: Gouda, Swiss, Gruyere, Cheddar Beer, wine, yeast extract, Chocolate, pickled herring, Dry sausage, broad beans	Hypertensive crisis, severe headache, cerebral hemorrhage, neuronal sequel, cardiac failure, pulmonary edema, visual alterations, palpitation, nausea, sweat, vomit, muscle contractions, excitation, mental confusion, high blood pressure, fever, perspiration
Migraine	Tyramine 2-phenylethylamine tryptamine serotonin	Cheese Chocolate Beer Wine	Throbbing headache, migraine attack
Increased growth hyperplasia	Spermine spermidine	Meat	Increased growth of tumors

[1] MAOI-monoaminoxidase inhibitors.

Source: Refs. 9, 139, and 177.

TABLE 13.4
Histamine Poisoning Episodes in Different Countries

Country	Years	No. of Outbreaks	No. of Cases*	Implicated Foods*
Canada	1975–1981	6		Fish (tuna, mahi-mahi, mackerel)
				Cheese (cheddar)
Denmark	1976–1982	33	–	Tuna, mackerel
	1993–1998	13	–	–
Finland	1993–1998	9	> 772	–
France	1980–1983	10	> 500	Fish (albacore, tuna, sardines, herring)
				Cheese (Gruyere)
				Meat (ham)
	1993–1997	38	–	–
Germany	1971–1982			Fish (mackerel, sardine, tuna)
				Sauerkraut
Japan	1950–1954	14	1215	Dried saury, canned mackerel, Iwashi sakuraboshi, frigate tuna
	1970–1980	42	4122	Fish (tuna, mackerel, sardine, dorado, marlin, kamaboko, anchovy)
				Chicken
The Netherlands	1967			Gouda cheese
New Zealand	1973–1975			Skipjack tuna, mackerel, kahawai, kingfish, trumpeter fish
Sweden	1993–1998	4	12	–
United Kingdom	1976–1982	136	439	Mackerel, bonito, sprats, pilchard, sardine, tuna, anchovy, kipper, gefilte fish
	1987–1996	105	405	Tuna, mackerel, salmon
United States of America	1968–1981	110	888	Fish (tuna, mahi-mahi, mackerel, bonito, albacore, jack, blue fish, snapper, kumu, skipjack, yellow tail, anchovy)
				Cheese (Swiss)
	1973–1987	202	1216	Finfish
	1988–1998	5	31	Tuna, mahi-mahi, yellow fin tuna, yellow tail

* Information not available.

Source: Refs. 15, 31, 152, 177, and 212.

Cases of histamine intoxication have been reported in the United States, Japan and United Kingdom. A few incidents have also been reported in several other countries as indicated in Table 13.4. However, outbreaks and cases of histamine poisoning are often not reported since it is relatively mild, has short duration and many patients do not seek medical attention. Furthermore, many physicians remain unaware of histamine poisoning and do not consider it as a possible diagnosis. Even when medical attention is sought and a correct diagnosis is made, only a few countries keep official records of incidents (177). Therefore, it would be safe to assume that the true incidence of histamine poisoning is unknown.

Fish incriminated in outbreaks of histamine poisoning included scombroid (tuna, bonito, mackerel, skipjack, albacore, bluefin tuna, herring and saury) and non-scombroid fish (mahi-mahi, sardines, pilchards, herring, bluefish, jack mackerel, anchovies, kahawai, black and striped marlin), which contained high histamine levels (12, 139). Misidentification of the fish involved in outbreaks may occur on occasion due to different common names used in different places. The type of fish consumed, the method of harvesting, and the consumption patterns are important factors in determining the likelihood of histamine poisoning (177).

Cases of cheese-related histamine poisoning were also reported. The first case was reported in the Netherlands in 1967 and was associated with Gouda cheese. Since then, several cases have been reported after consumption of Gouda, Swiss, Gruyere, Cheddar, and Cheshire cheeses (158, 173, 177, 180). Other foods have also been implicated in incidents of histamine poisoning, among them chicken, sauerkraut, and ham. Fermented foods and proteinaceous foods subject to spoilage are particularly likely to contain large amounts of histamine. Sauerkraut, yeast extract, wine, and fermented, dry sausages can, on occasion, have sufficient histamine levels to cause a toxic response if enough quantities are consumed (66, 139, 177).

Outbreaks of histamine poisoning have also been observed in patients on antituberculosis therapy. Such outbreaks were associated with interaction of fish or cheese

containing high histamine levels with isoniazid and other nicotinamide derivatives (154, 179).

Marley and Blackwell (113) reported that following intravenous injection of 0.1 mg of histamine phosphate, facial flushing, pulse quickening, fall in the blood pressure and rise in cerebrospinal fluid pressure occurs within 20 seconds and the onset of histamine headache occurs 1 min after injection. They also reported that less than 225 mg of histamine taken orally usually does not produce symptoms, although susceptible subjects (allergy, asthma, or peptic ulcers) might be adversely affected by smaller quantities. According to Taylor (177), some mild effects of histamine poisoning were observed in humans by orally administrating 100 to 180 mg of histamine in combination with good quality tuna. The paradox between the lack of toxicity of pure histamine and the apparent toxicity of smaller doses in fish or cheese could explain the existence of potentiators of histamine toxicity. These compounds would act to decrease the threshold dose of histamine needed to provoke an adverse reaction. They would enable absorption of amounts of histamine larger than could be achieved in their absence.

Two hypotheses have been used to justify the effect of potentiators on histamine poisoning: barrier disruption and inhibition of histamine metabolizing enzymes (179). In the first hypothesis, potentiators would interfere with the protective actions of intestinal mucin, which binds to histamine and prevents its passage across the intestinal wall. According to Chu and Bjeldanes (37) and Shalaby (158), such compounds are spermine, spermidine, cadaverine, and putrescine.

In the case of inhibition of histamine metabolizing enzymes, potentiators would inhibit DAO or HMT. DAO is not selective towards histamine but also oxidizes other diamines. Inhibitors of DAO include agmatine, cadaverine, tyramine, putrescine, phenylethylamine, anserine, carnosine, thiamin, semicarbazide, carbonyl reagents, imidazole derivatives, and the pharmacological agents: aminoguanidine and isoniazid (79, 139, 154, 183). Many antihistaminic drugs are DAO inhibitors. Some inhibitors of MAO are also somewhat effective as DAO inhibitors (177). The inhibitors of HMT include agmatine, tyramine, cadaverine, putrescine, tryptamine, and phenylethylamine (183). This enzyme is also inhibited by antimalarial drugs, among them, quinacrine, chloroquin, and amodiaquin (79, 173, 177). According to Hui and Taylor (79), amines were less potent inhibitors of DAO and HMT, compared to pharmacological compounds.

The toxic effect of histamine can also be potentiated by the presence of ethanol**,** which potentiates the effect of amines by directly or indirectly inhibiting amine oxidase (69, 138).

The threshold toxic dose for histamine in foods is not precisely known since the existence of potentiators could dramatically influence it. Based on experience acquired in the investigation of hundreds of histamine poisoning incidents, the U.S. Food and Drug Administration established 10 mg/100 g as the hazard action level for histamine in tuna (56).

Tyramine is the second type of amine involved in intoxication. When foods containing high tyramine levels are ingested, large amounts of non-metabolized tyramine can reach the blood stream. This causes release of noradrenaline from the sympathetic nervous system, leading to a variety of physiological reactions (Table 13.3). There is an increase in blood pressure by peripheral vasoconstriction and by increasing the cardiac output (87, 158). Tyramine can also dilate the pupils and the palpebral tissue, cause lacrimation, salivation, fever, vomit, and headache, and increase respiration and blood sugar (51, 60, 87, 113, 115). When consuming foods rich in tyramine, about 30% of individuals with classical migraine can have headaches. These tyramine-sensitive patients appear to suffer from a deficiency of the enzyme responsible for the formation of the sulfate conjugate of tyramine (42).

Ingestion of foods rich in tyramine by individuals under MAOI treatment results in a dangerous illness known as "cheese reaction." Such a name was given since most of the cases involved cheese. However, it is not the only type of food incriminated, since cases have also been reported with yeast extracts (marmite), pickled herring, dry sausage, alcoholic beverages, broad beans, chicken liver, beer, among others (60, 68, 87, 113). Cheese reaction consists in a hypertensive crisis, usually accompanied by severe headache. The attacks last from 10 minutes to 6 hours, during which hypertension and headache fluctuate. There can be visual alterations, nausea, vomit, muscle contraction, mental confusion, or excitation. Neck stiffness and photophobia can occur. Chest pain simulating angina pectoria, acute heart failure, pulmonary edema, neuronal sequel, and cerebral hemorrhage have also been described. Fatal incidents have been reported in the literature (60, 61, 87, 113, 158).

MAOI drugs have been widely used due to their efficacy in the treatment of malaria, tuberculosis, depression, atypical depression, social phobia, panic and anxiety syndromes, bulimia, Parkinson's and Alzheimer's (60, 94, 139). They include antidepressants, antihypertensive, antimicrobial, and antineoplastic drugs, among them isoniazid, tranylcypromine, isocarboxazid, pargyline, phenelzine, procarbazine, furazolidone, moclobemide, and ipronazide (87, 113, 115, 165). In order to prevent hypertensive attacks when prescribing these drugs, it is necessary to warn patients to avoid foods containing tyramine (42, 60, 139, 165). Foods that require absolute restriction on an MAOI regimen include aged cheese, smoked or pickled fish, cured or spoiled meat, yeast extract, marmite, sauerkraut, and broad beans. Foods that should be avoided unless tyramine contents are known are red wine, meat extracts, soy sauce, dry sausage, and draft beer (60, 64, 115).

In man, 20–80 mg of tyramine injected intravenously or subcutaneously causes a marked elevation of blood pressure. In individuals on MAOI, as little as 6 mg taken orally within a 4 h period can be deleterious (139, 176).

Tryptamine has pharmacological action similar to tyramine. High levels of tryptamine can exert direct effect on smooth muscles, cause headache, and increase blood pressure by constriction of the vascular system (87, 139, 165).

Phenylethylamine, like tyramine, causes an increase in blood pressure by liberating noradrenaline from tissue stores. Phenylethylamine may be the precipitant of migraine headache (87, 139, 165). Levels of 3 mg of phenylethylamine cause migraine headaches in susceptible individuals. Migraine has been observed about 12 h after ingestion of chocolate which contained phenylethylamine. Chocolate has also been implicated in a well-documented hypertensive attack in a patient taking the MAOI pargyline (139).

Even though putrescine and cadaverine have less pharmacological activity than the aromatic amines, after ingestion of very large amounts of these compounds, toxic effects can be observed. Intoxication symptoms reported are hypotension, bradycardia, dyspnea, lockjaw, and paresis of the extremities. However, the most important consequence of these compounds in food is probably the potentiation effect of the toxicity of other amines (66, 87).

Serotonin has been widely discussed as a possible cause of migraine headache, but the evidence is not clear. High levels in foods have been responsible for intestinal discomfort and fibrosis of the myocardium. Serotonin is a powerful vasoconstrictor and vasopressor when 1 mg is injected, but an oral dose of 20 mg has no effect. Although there are no definite reports of intoxication due to serotonin, elevated levels of its metabolites are biochemical signals of pheochromocytoma and malignant carcinoid. Therefore, plant products rich in serotonin like banana, pineapple, fig, walnut, and tomato should be excluded from the diet before attempting to diagnose carcinoid tumor (139, 165).

High spermine levels can cause kidney toxicity, and can affect blood coagulation and pressure, heart beat, and respiration (108).

Due to the diversity of the roles of polyamines in cellular metabolism and growth, rapidly growing tissues require very large amounts of polyamines. Accordingly, the importance of putrescine, spermine, and spermidine in tumor growth is widely recognized, and the inhibition of polyamine biosynthesis in tumor-bearing individuals is a major target of cancer therapy research (9, 11, 137). The capacity of tumor cells to synthesize polyamines by the decarboxylation of ornithine is higher than that of non-transformed tissues (10). Polyamines also have an effect on the non-specific immune system specialized in tumor killing, on the plasma concentration of interleukin-1 and -6, and on the tumor necrosis factor concentration (54). Polyamines absorbed from exogenous sources, mainly food, and the gastrointestinal tract, also play an important role in tumor growth (10).

In order to stop the formation of polyamines, the enzyme ODC can be inhibited. Although DFMO is an efficient and irreversible blocker of ODC, it does not completely stop growth as polyamines are also formed by alternative routes. A polyamine deprivation regimen in combination with polyamine oxidase and antibiotics suitable for the partial decontamination of the gastrointestinal tract, have been shown to reduce growth of tumor and to enhance the efficacy of chemotherapy (10, 54, 137).

Some amines can be nitrosated, or act as precursors for compounds capable of forming nitrosamines, some of which are carcinogenic to several species of animals. Putrescine and cadaverine, upon heating, can be converted to pyrrolidine and piperidine, respectively, from which N-nitrosopyrrolidine and N-nitrosopiperidine are formed upon heating and reaction with nitrite or nitric oxide (158). Spermidine, spermine, and agmatine can also react with nitrite to form nitrosamines in foods or *in vivo* in the gastrointestinal tract over a wide range of biological and environmental conditions (68, 74). A reaction product of tyramine and nitrite, 3-diazotyramine, induced oral cavity cancer in rats (47).

The determination of the exact toxicity threshold of amines is extremely difficult. The toxic dose is dependent on the efficiency of the detoxification mechanism of different individuals. Upper limits of 10 mg of histamine, 10 mg of tyramine, and 3 mg of 2-phenylethylamine in 100 g of foods have been suggested (74). In the case of alcoholic beverages the proposed limits are 2 to 8 mg of histamine and 8 mg tyramine per liter. However, ingestion of foods containing 6 mg of tyramine can cause migraine and 10 to 25 mg can cause hypertensive crisis in individuals taking MAOI drugs (35, 60).

Til et al. (186) investigated the acute and subacute toxicity of five amines in Wistar rats. The approximate LD_{50} values observed were as follows. Tyramine and cadaverine showed the lowest acute toxicity of more than 2000 mg/kg body weight. The approximate LD_{50} of putrescine was 2000 mg/kg, while spermidine and spermine showed the highest acute toxicity, namely 600 mg/kg body weight. The no-observed-adverse-effect level (NOAEL) was 2000 ppm (180 mg/kg body weight/day) for tyramine, cadaverine, and putrescine, 1000 ppm (83 mg/kg body weight/day) for spermidine, and 200 ppm (19 mg/kg body weight/day) for spermine.

VI. OCCURRENCE OF AMINES IN FOODS

Bioactive amines are inherent to living organisms and, therefore, are present in plants, meat, and dairy products. The amount and type of amines in foods depend on the nature and origin of the commodity. However, they can change during production, processing, fermentation, and storage. It can also be affected by hygienic conditions.

Amines are resistant to heat treatment employed in food processing. Based on these findings, amines have been considered good indicators of the freshness, spoilage, and of the degree of quality of fresh and processed food products, reflecting the quality of the raw material used and of hygienic conditions prevalent during processing.

It is evident that all types of food, whether originated from plants or animals, contain putrescine, spermine, and spermidine. In addition to these most common compounds, other amines may also occur naturally. During fermentation or spoilage, spermine content can decrease, because it can be used as a nitrogen source by some microorganisms. There is also formation and accumulation of different types of biogenic amines.

Much still remains unknown about the concentration of amines in food products. Most data available relate to specific amines, mainly histamine and tyramine in fish, cheese, meat, and alcoholic beverages. Comprehensive studies on the levels of all amines are useful from the toxicological, sensorial, and technological points of view. Recent work has focused on the role of polyamines in plant physiology and also on animal health and growth. However, bioactive amines is obviously an area deserving further detailed investigation.

A. Fruits and Vegetables

The polyamines spermidine and spermine occur ubiquitously in the plant kingdom, together with their diamine precursor putrescine. Polyamines are required for normal development and can be used as organic nitrogen sources. Work on higher plants suggests that polyamines play a critical role in several processes, among them, root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development, and response to abiotic stress, such as potassium deficiency, osmotic shock, drought, and pathogen infection. They are also important in the synthesis of secondary metabolites of biological interest, for example, nicotine and alkaloids (59, 129, 168, 202).

A relation has been found between the synthesis of polyamines and the inhibition of ethylene biosynthesis. This has been interpreted as a result of metabolic competition by the same precursor, i.e., *S*-adenosylmethionine. Furthermore, polyamines can scavenge free radicals involved in the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Therefore, exogenous application of polyamines to delay fruit ripening has been proposed and used with promising results (55, 95).

Polyamines are associated with cell walls and cell membranes. They modulate pectinesterase and bind to pectin, delaying fruit softening and senescence (93, 99). The firming effect of the polyamines is similar to that of calcium chloride, and may be due to its ability to bind to cell walls and membranes, stabilizing them, or by making cell walls less accessible to wall-softening enzymes (93, 135). Another hypothesis is that polyamines inhibit the action of enzymes involved in softening or wall degradation. For instance, Kramer et al. (93) found polyamines to have inhibitory effect on polygalacturonase extracted from apples that had been inoculated with fungus. Exogenous application of polyamines to microsomal membranes resulted in a reduction in fluidity at the membrane surface through association of the polyamines with membrane lipid. Polyamines can also delay membrane deterioration by interaction with anionic components of the membrane and stabilizing the bilayer surface. The radical scavenging properties of polyamines have also been suggested to be involved in protecting membranes from lipid peroxidation and other oxidative stresses (70).

In higher plants, polyamines occur in the free form, electrostatically bound to negatively charged molecules or conjugated to small molecules, such as cinnamic acids, e.g., p-coumaric, ferulic, and caffeic acids, resulting in hydroxycinnamic acid amides (HACCs). These compounds have been implicated in a variety of plant growth and developmental processes. More recently, HACCs have begun to attract renewed attention with several studies suggesting an important role in plant responses to pathogens (202).

Besides the polyamines spermidine, spermine, and putrescine, other amines are also naturally present in fruits and vegetables, among them phenylethylamine, serotonin, tryptamine, histamine, cadaverine, agmatine, adrenaline, noradrenaline, octopamine, synephrine, agmatine, and aliphatic amines. Some amines may have a protective role in deterring predators. Serotonin is one of the active principles that occurs in the stinging hairs of *Mucuna pruriens*, *Girardinia heterophylla*, and *Urtica* spp. (167). Histamine occurs in stings of *Jatropha urens* and *Urtica dioica* (166). Some amine conjugates are important as antifungal and antiviral agents. Some aliphatic amines simulate the smell of rotting meat, thereby attracting pollinating insects (165, 167). Some amines are quite important as precursors of compounds of biological significance. For instance, the plant hormones indol-3-yl-acetic acid (IAA) and phenylacetic acid are derived from tryptamine and phenylethylamine, respectively. The tryptamines are also precursors of the tricyclic beta-carboline alkaloids formed by condensation with one or two carbon moiety (42, 167).

Aliphatic volatile amines are widespread in food plants. In the late 1960s and early 1970s several studies were conducted to identify these compounds. Dimethylamine, ethylamine, propylamine, isopropylamine, and butylamine were found in most of the products analyzed, among them apple, banana, potato, cocoa, coffee, oat, soybean, and tea. Other amines detected included hexylamine in apple, isoamylamine in banana and cocoa, ethylmethylamine in coffee, trimethylamine in cocoa and tea, and triethylamine in cocoa (108, 165).

The occurrence of bioactive amines in different fruits and vegetables is indicated in Table 13.5. Tyramine has been

TABLE 13.5
Occurrence of Amines in Fruits and Vegetables

Common Name	Amine Present	Reference
Almond	PHM	165
Apple	DM, E, P, B, HX, IP, PUT, SPD	11, 165
Apricot	SRT	42
Avocado	TYM, DOP, SRT	40, 139, 165, 188
Banana	DM, E, IB, IA, PR, TYM, DOP, NADR, SRT, OCT, TRM, HIM, PHM, SRT, PUT, SPD	2, 40, 42, 159, 165, 188, 210
Barley	DM, E, IB, IA, TYM, HOR, TRM, SRT, GRA	165, 167
Bean (sprout)	SPD, SPN, PUT, CAD, HIM	172
Bean (red, kidney)	PUT, SPD, SPN	11
Beet (leaf)	DOP, HIM, PHM	125, 165
Bell pepper	OCT	210
Broccoli	SPD, SPN, AGM, PUT	172
Cabbage	PHM, PUT, SPD, SPN	11, 125
Cabbage, Chinese	SPD, SPN, PUT, HIM, TYM	163
Cabbage, red	PHM	125
Calamondin	SYN	165
Capers	SPD, AGM, PUT	172
Carrots	PHM, PUT, SPD, SPN	11, 125
Cassava	SPD, PUT	172
Cauliflower	SPD, SPN, AGM, PUT, PHM	11, 125, 172
Cherry	SRT	42
Citrange	TYM, SYN	165
Cocoa	DM, TM, E, TE, B, IB, IA, TYM, TRM, SRT, PHM	42, 86, 165
Coffee	DM, EM, E, P, IB, SRT	38, 165
Collard greens	SPD, SPN, AGM, PUT	199
Cucumber	PUT, SPD, SPN	11
Date	SRT	165
Eggplant	TYM, TRM, SRT, SPD, SPN, PUT, HIM	42, 141, 159, 188
Elderberry	TYM	210
Endive	SPD, SPN, PUT, TYM	163
Fig	SRT	165
Grapefruit	PUT	11
Green beans	PUT, SPD, SPN	11
Green gram (seed)	TYM	165
Green onion	SPD, SPN, AGM, PUT, TYM	172
Hearts of palm	SPD, SPN, PUT	172
Hop	HIM, GRA, HOR	165
"Jilo"	SPD, SPN, AGM, PUT, HIM, TYM	172
Kale	PHM	125
Lemon	TYM, SYN, OCT	42, 165
Lettuce	PUT, SPD	11
Lettuce, American	SPD, PUT, AGM	39
Lettuce, iceberg	SPD, SPN, PUT, TYM	163
Maize	TYM, HIM, PHM	165
Mandarin	OCT, SYN	42
Millet (seed)	TYM, HOR	165
Oat	DM, E, P, IP, B, IB, A	165
Onion	PUT, SPD, SPN	11
Orange	TYM, OCT, SYN, NADR, TRM, OCT, PUT, SPD	11, 40, 42
Parsley	SPD, SPN, PUT, TYM	172
Passion fruit	SRT	165
Papaya	SRT	165
Peach	SRT	42
Pears	PUT, SPD, SPN	11
Peas	PUT, SPD, SPN	11
Pineapple	TYM, SRT	42, 165

(*continued*)

TABLE 13.5 (*Continued*)

Common Name	Amine Present	Reference
Potato	TYM, TRM, DOP, NADR, DE, P, IB, PUT, SPD, SPN	11, 42, 165, 188
Plantain	SRT	113
Plum	TYM, NADR, TRM, SRT	40, 42, 165, 188
Purslane	DOP, NADR	165
Radicchio	SPD, SPN, PUT, TYM	163
Radish	PHM, PUT, SPD, SPN	11, 125
Raspberry	TYM	40, 165
Rhubarb	PHM	125
Rice	PUT, SPD, SPN	10
Soybean	DM, EM, E, P, IB, PUT, SPD, SPN	11, 165
Spinach	SPD, PHM, AGM, PUT, HIM, TYM, DOP	125, 159, 165, 172, 188
Swede	PHM	125
Tangerine	TYM, HOR, OCT, SYN	42, 165, 210
Taro	TYM	165
Tea	PHM, DM, TM, E, P, IB, IA	165
Temple	TYM, OCT, SYN	165
Tomato	TYM, TRM, SRT, HIM, SPD, PUT, HIM	11, 42, 159, 165, 167, 172, 188
Walnut	SRT, TYM	165, 210
Watermelon	TRM	165

A=amylamine, ADR=adrenaline, AGM=agmatine, B=butylamine, CAD=cadaverine, DE=diethylamine, DM=dimethylamine, DOP=dopamine, E=ethylamine, EM=ethylmethylamine, GRA=gramine, HIM=histamine, HOR=hordenine, HX=hexilamine, IA=isoamylamine, IB=isobutylamine, IP=isopropylamine, NADR=noradrenaline, OCT=octopamine, P=propylamine, PHM=phenylethylamine, PR=propanolamine, PUT=putrescine, SPD=spermidine, SPN=spermine, SRT=serotonin, SYN=synephrine, TE=triethylamine, TM=trimethylamine, TRM=triptamine, TYM=tyramine.

detected in cabbage, lettuce, chicory, radish, tomato, potato, green onion, spinach, eggplant, elderberry, avocado, banana, plum, grapes, raspberry, pineapple, orange, lemon, and tangerine (40, 115, 188). Other phenolic amines such as octopamine and synephrine occur in citrus. Octopamine was also found in banana (165). Histamine has been found in cabbage, eggplant, tomato, beet, and spinach. Tryptamine was reported in tomato, eggplant, barley, orange, plum, and watermelon (59, 164, 188). Cadaverine is naturally found only in plants of the *Leguminosae* family (168, 172). Phenylethylamine was detected in beet, cabbage, cauliflower, rhubarb, carrots, radish, maize, and banana (165). Serotonin has been most frequently reported in banana, plantain, pineapple, plum, peach, avocado, tomato, apricot, eggplant, coffee, and walnut (40, 59, 188, 197). Due to the specific occurrence of bioactive amines in the plant kingdom, they have been used as a tool for taxonomic studies. However, some of them can also be formed by decarboxylases from contaminant microorganisms (59, 168, 172).

Most of the studies on polyamines in fruits and vegetables were performed recently, in the last decade. It is well known that spermidine is the prevalent amine followed by putrescine. Most fruits and vegetables contain only small amounts of polyamines; however soybean and chicory were found to contain the largest amounts (130).

As indicated in Table 13.6, very few studies investigated several amines simultaneously in fruits and vegetables. Therefore, information is still lacking on the levels of amines and on the influence of many factors including plant species and variety, type of tissue, conditions of growth, stage of development, degree of ripening, processing, and storage conditions (164, 188).

The tyramine content of fruits is generally low. Raspberries contain the highest reported levels, averaging 4.8 mg/100 g. Similar levels have been found in ripe banana pulp. Red plum, orange, and avocado contain between 0.6 and 2.3 mg/100 g, but most fruits contain 0.1 mg/100 g or less (42). With a few exceptions, fruits and vegetables contain very low levels of histamine. Histamine concentration in fresh tomatoes and maize is less than 0.4 mg/100 g, while levels in spinach and eggplant vary from 3 to 6 mg/100 g. Baking reduced histamine content in spinach and eggplant. Fresh fruits and vegetables generally contain less than 1 mg of phenylethylamine per 100 g. The highest level (4 mg/100 g) was found in swede. It was also reported in grapes (42, 125). The tryptamine content of plants has been reported to be less than 0.5 mg/100 g in several fruits and vegetables (42). Synephrine is most prevalent in citrus fruits. The juices of tangerines and Cleopatra mandarins contain 13 and 28 mg/100 mL, respectively, while orange and lemon juice contain 1.9 mg/100 g (42, 210). Generally, low quantities of octopamine have been detected in orange, lemon, tangerine (0.1 mg/100 g), mandarin (0.2 mg/100 g), and banana, with similar levels reported in their juices. Octopamine has been reported in bell pepper at levels of 23.4 mg/100 g (210). Serotonin has been detected in some fruits at levels of 0.1 to 0.2 mg/100 g in papaya, 0.1 to 0.4 mg/100 g in

TABLE 13.6
Types and Levels of Bioactive Amines in Fruits and Vegetables

	Amine Levels (mg/100 g)[1]										
Product	**SPD**	**SPN**	**AGM**	**PUT**	**CAD**	**HIM**	**TYM**	**TRM**	**PHM**	**SRT**	**Source**
APPLE	0.14–0.28	nd	0.04–0.17	—	—	—	—	—	—	—	11, 130
BANANA											3
Green	1.04	nd	nd	0.75	nd	nd	nd	nd	nd	1.73	
Ripe	1.07	nd	nd	0.83	nd	nd	nd	nd	nd	1.22	
Overripe	0.95	nd	nd	0.33	nd	nd	nd	nd	nd	0.71	
BEAN, broad											157
Fresh	1.67	1.79	—	0.72	0.87	0.85	0.62	2.43	0.43	—	
Germinated	0.62	1.43	—	5.56	3.26	1.72	1.36	13.6	1.03	—	
BEAN, green	0.77–0.88	0.46–0.55	—	0.43–0.54	—	—	—	—	—	—	11
BEAN, kidney	1.90–2.00	2.28–2.57	—	0.04	—	—	—	—	—	—	11
BEAN, sprout	1.37–2.83	0.12–0.43	nd	1.84–5.90	0.58–12.0	0.49–8.75	nd	nd	nd	nd	172
BROCCOLI	1.98–15.2	nd–1.60	0.72	0.34–3.17	nd–0.12	nd	nd	nd	nd	nd	54, 129, 172
CABBAGE	0.32–0.36	0.32–0.36	—	0.04–0.16	—	—	—	—	—	—	11
CAPERS	0.19–0.57	nd	nd–0.20	0.13–0.24	nd	nd	nd	nd	nd	nd	172
CARROT	0.36–1.19	nd–0.38	—	0.07–0.35	nd	—	—	—	—	—	11, 54, 130
CASSAVA, cooked	0.16–0.27	nd	nd	0.08–0.61	nd	nd	nd	nd	nd	nd	172
CAULIFLOWER	0.47–3.93	nd–1.29	nd–1.89	0.13–0.89	nd–0.10	nd	nd	nd	nd	nd	11, 54, 172
CHICKPEA											157
Fresh	30.7	1.82	—	0.79	1.05	0.58	0.37	3.02	0.19	—	
Germinated	1.84	1.7	—	6.28	3.95	1.78	1.21	29.9	0.48	—	
CHICORY	39.2–75.4	nd	—	15.0–120	—	—	—	—	—	—	95
COFFEE											38
Green	0.6	0.44	nd	1.03	nd	nd	nd	nd	nd	1.13	
Roasted, 6 min	0.12	nd	nd	nd	nd	nd	nd	nd	nd	0.16	
Roasted, 12 min	0.2	nd	0.12	nd	nd	nd	nd	nd	nd	0.29	
COLLARD GREENS	2.28	0.38	0.24	0.67	nd	nd	nd	nd	nd	nd	199
CORN	4.85	nd	—	8.62	—	—	—	—	—	—	130
CUCUMBER	0.15–1.03	nd–0.28	—	0.32–0.87	nd–0.06	—	—	—	—	—	11, 54
EGGPLANT											172
Whole	0.66–1.15	0.12–0.90	nd	1.22–4.04	nd	3.69–12.5	0.12–0.47	nd	nd	nd	
Peel	1.15b	0.51	0.33	3.4	nd	10.1a	14.1	nd	nd	nd	
Pulp	0.87b	0.19	nd	4.42	nd	0.29b	nd	nd	nd	nd	
Seed	2.11a	0.23	0.18	5.1	nd	8.20a	nd	nd	nd	nd	

HEARTS OF PALM, canned	0.52–0.94	0.17–0.55	0.07–0.18	nd	nd	nd	nd–0.78	nd	nd	nd	172
JILO	0.37–0.91	nd–0.25	nd–0.25	0.27–2.70	nd	0.18–1.20	0.14–0.50	nd	nd	nd	172
LENTIL, sprout	6.44	1.59	6.04	2.19	15.4	—	—	—	—	—	162
LETTUCE	0.42–1.03	nd–0.18	0.03	0.12–0.73	0.07	nd	nd	nd	nd	nd	39
LUPINE											157
Fresh	3.67	4.06	—	0.82	0.52	0.86	0.78	1.17	0.12	—	
Germinated	0.53	0.63	—	8.46	2.69	1.64	1.22	56.7	0.64	—	
MUNG BEAN, sprout	3.39	0.42	5.26	nd	1.56	—	—	—	—	—	162
ONION GREENS	0.26–1.23	nd–0.09	nd–0.48	nd–0.14	nd	nd	0.23–0.63	nd	nd	nd	172
ONION	0.55–0.81	0.08–0.12	—	0.55–0.72	—	—	—	—	—	—	11
ORANGE	0.04–1.16	nd–0.12	—	9.51–15.3	nd	—	—	—	—	—	11, 54, 130
ORANGE, mandarin	nd–0.45	nd–0.30	—	6.73–20.0	nd–2.16	—	—	—	—	—	54
PARSLEY	0.79–3.77	nd–0.71	nd	0.30–0.87	nd	nd	nd	nd	nd	nd	172
PEAR	3.02–7.60	0.81–4.93	—	2.36–2.42	—	—	—	—	—	—	54
PEAS											11, 90
Cooked	6.21–6.82	3.35–7.17	—	0.54–0.59	—	—	—	—	—	—	
Frozen	0.29–8.84	0.16–0.85	—	1.17–10.7	0.19–0.42	0.23–0.38	0.41–1.65	0.13–2.44	—	—	
POTATO	0.83–1.88	nd–0.40	—	0.58–1.76	—	nd–0.13	—	—	—	—	11
RADISH											11, 162
Fresh	0.04–0.06	0.12–0.16	—	0.02–0.03	—	—	—	—	—	—	
Sprout	12.5	1.76	1.29	4.92	—	0.91	—	—	—	—	
RASPBERRY	—	—	—	—	—	—	1.28–9.25	—	—	—	40
RICE, polish	0.55	0.29	—	0.09	—	—	—	—	—	—	130
SOYBEAN	3.32–28.9	2.97–4.93	—	0.16–4.14	—	—	—	—	—	—	130
SPINACH											90, 130, 172
Fresh	0.75–2.42	0.06–0.19	0.35–1.22	0.11–0.80	nd	nd	0.06–0.11	0.33–0.77	nd	nd	
Puree	0.13–1.54	0.14–0.38	—	0.25–11.9	—	0.19–0.77	0.21–0.98	0.35–3.18	0.14–1.43	—	
STRAWBERRY	0.38–0.67	0.03–0.13	0.11–0.33	—	—	—	—	—	—	—	135
TOMATO											11, 54, 90, 130, 172
Fresh	0.16–0.70	nd–0.14	nd	0.19–12.2	nd–0.05	nd–0.31	nd–0.09	nd–0.03	nd–0.01	nd–0.12	
Paste	0.27–1.58	0.21–0.29	—	0.79–4.11	0.14–1.76	0.20–10.1	0.30–3.11	0.26–1.98	—	—	
Ketchup	0.11–3.34	0.14–1.21	—	0.24–16.5	0.14–13.1	0.20–1.80	0.45–14.9	0.13–7.25	—	—	
WHEAT, flour	1.33	0.38	—	0.15	—	—	—	—	—	—	130

[1] nd=not detectable; —=not determined. **SPN=spermine**, SPD=spermidine, AGM=agmatine, PUT=putrescine, CAD=cadaverine, HIM=histamine, TYM=tyramine, TRM=tryptamine, PHM=phenylethylamine, SRT=serotonin.

passion fruit, 1.0–1.2 mg/100 g in avocado and fig, 1.2 to 7.8 mg/100 g in pineapple and banana. The serotonin content in plantain is substantially higher. Banana peel contains higher serotonin levels than the pulp (3, 165, 188). Serotonin has also been detected in green and roasted coffee. Green beans of *Coffea arabica* L. cv red catuaí contained 0.94 mg/100 g of serotonin, while American (300 °C/6 min) and French (300 °C/12 min) roasted beans contained 0.16 and 0.29 mg/100 g, respectively (38).

Spermidine levels are high in tissues undergoing growth and in seeds, which are responsible for the preservation of the specie (59, 168, 172). Studies by Starling (172) corroborated this distribution pattern of spermidine in vegetables of the *Solanaceae* family. Moreover, this investigator detected higher histamine levels in the peel of these vegetables, which reinforces their role as plants protectors.

Studies on the influence of polyamines during development of fruits such as avocado, mandarin, apple, tomato, pear, pepper, litchi, and olive, revealed peak levels of putrescine and spermidine and rapid cell proliferation during early stages of growth and, then, decreasing values as the fruits grow and become ripe (129, 141, 155, 187). However, different results were observed during eggplant development and ripening (141). The serotonin content in bananas is affected by the degree of ripening. As the fruit ripens, serotonin in the peel increases steadily while in the pulp it increases and then decreases (188, 197). However, the levels of serotonin and putrescine decreased as the fruit changed from ripe to over-ripe (3).

Changes in bioactive amine levels and profiles at preharvest stage have been reported in response to various kinds of stress, including those from water (39, 204), mineral deficiency (13, 168), acid, herbicide, ozone-caused damages (41), osmotic shock, temperature or altitude variation (165), and chilling injury (129, 203). These reports have demonstrated that most of the stress conditions resulted in an increase of polyamine levels, particularly putrescine.

In a variety of fruits and vegetables, chilling injury is manifested by significant increases in polyamine levels. Putrescine accumulated in different products exposed to chilling temperature, among them lemon (116, 190), broccoli (129), grapefruit, pepper (116), and zucchini squash (92, 156, 203). Chilling injury caused a decrease of spermidine and spermine levels in zucchini squash (92, 156), but an increase of spermidine in peaches (191). Pretreatment of zucchini squash with CO_2 alleviated chilling injury and caused a smaller increase in putrescine (156). Infiltration of zucchini squash with spermidine prior to storage at 2.5 °C was efficient in reducing chilling injury (92). Acording to Serrano et al. (156) polyamines can prevent chilling injury by protecting membrane lipids from peroxidation.

Many researchers studied the relationship between food freshness and amine levels. In this respect, bioactive amines may be indicators of the freshness and quality of fruits and vegetables. During storage at 5 °C for 6 days, total amine content increased in Chinese cabbage from 2.0 to 3.4 mg/100 g, in chicory from 1.5 to 2.1 mg/100 g, in iceberg lettuce from 1.4 to 2.4 mg/100 g, and in radishes from 1.9 to 2.6 mg/100 g fresh weight. Among the amines detected, only putrescine concentration markedly and continuously increased during storage. Putrescine levels increased 3- to 8-fold depending on the type of vegetable. Spermidine and tyramine contents varied randomly and probably resulted from endogenous plant cell metabolism. The total bacterial numbers reached a maximum of 4×10^7 CFU/g at the end of storage, with *Enterobacteriaceae* representing ca 90 to 99% of the total microbial population. A relationship was established between *Enterobacteriaceae* population and putrescine concentration (164). Storage temperature affected markedly putrescine and spermidine contents of broccoli buds packed in polyethylene pouches. Spermidine levels decreased with storage time; however, at 2 °C the rate was slower compared to 23 °C. At 2 °C there was a significant increase in putrescine levels, whereas at 23 °C the levels decreased (129).

There is evidence that direct application of polyamines can have beneficial effects on the shelf life of fruits. During controlled atmosphere storage of apples, reduced rates of softening correlated with increased levels of endogenous polyamines (93). Pressure infiltration of polyamines caused an immediate increase in apple firmness and a decrease in softening rate at 0 °C. In addition, vacuum infiltration of polyamines in tomatoes resulted in firmer fruits than water-infiltrated ones (135).

The exogenous application of putrescine was shown to increase fruit firmness and to retard softening during storage (135, 205). However, vacuum-infiltrated spermine and spermidine (10 mM or 100 mM) significantly increased firmness of strawberry slices, whereas putrescine was not as effective. The greater ability of spermidine and spermine to bind isolated polysaccharides may explain why putrescine was less effective at increasing firmness of strawberry slices than spermidine and spermine at the same concentrations (93, 135).

Processed vegetables usually contain higher biogenic amine levels compared to the fresh product (90). During processing, the incorporation of ingredients can alter amine profile and levels. Furthermore, microorganisms with amino acid decarboxylase activity can be introduced either by addition of starter cultures or by contamination (74). Cocoa beans contain relatively low quantities of phenylethylamine and tyramine. During fermentation there was an increase in the levels of these amines, while roasting increased phenylethylamine levels substantially (42, 86). During coffee roasting there was a significant change in amine profile with total loss of putrescine and spermine, and decrease of spermidine and serotonin levels (38).

During germination of legume grains, protein is synthesized rapidly and increased levels of polyamines are

expected. Furthermore, there is development of biogenic amines due to physiological changes in the tissues during sprouting and/or the activity of bacterial decarboxylase enzymes. The warm and moist environment is conducive to the rapid proliferation of microorganisms including *Enterobacteriaceae* and *Pseudomonas* spp., known to produce amino acid decarboxylases. During germination of *Phaseolus mungo*, putrescine content sharply increased, spermine levels decreased, and spermidine fluctuated, resulting in an overall increase in polyamines. The increase of putrescine levels was also observed in the germination of pea and maize, in bean and soybean seedlings, and in lentil sprouts (157, 162).

B. Fermented Beverages

Bioactive amines are among the major factors determining the quality of fermented beverages such as wine and beer (151). High amine levels can make the product unfit for consumption due to toxicological aspects. Furthermore, amines can also be significant in terms of aroma and flavor. In general, a weakening of the flavor impression is attributed to amines, whereby an unpleasant, bitter aftertaste has been noted and described as off-taste in wine and mousy in beer (45). Although tyramine and histamine are the major amines investigated in fermented products, several different amines have been detected. The types and concentrations of amines in fermented beverages vary widely (Table 13.7).

The origin of bioactive amines in wines is still a matter of controversy. Some amines are normal constituents of grapes with amounts varying with variety, soil type and composition, fertilization and climatic conditions during growth, and degree of maturation. Putrescine and spermidine are usually abundant in grapes, whereas agmatine, cadaverine, spermine, histamine, tyramine, and phenylethylamine have been found in small amounts (69, 73, 131, 151, 198). Lower amine levels in grapes were observed to be formed in cooler and rainier seasons (151). The degree of maturity of grapes is important since nitrogen content in the pulp is two to five times higher in ripened grapes (216).

Several amines can be formed and accumulate during wine making, among them putrescine, tyramine, histamine, and phenylethylamine, while spermidine levels decrease (73, 151). Reports are contradictory on the origin of biogenic amine during wine making. According to Vidal-Carou et al. (198), there was a slight formation of tyramine and no histamine formation during alcoholic fermentation of Spanish wines. On the contrary, Buteau et al. (25) found histamine production during alcoholic fermentation. However, in these studies there was no control of the microbial population present in the must; therefore, the formation of amines could not be attributed solely to the yeast.

Most researchers attribute the formation of biogenic amines, especially tyramine and histamine, to the action of bacteria involved in malolactic fermentation (25, 170, 198). The rates of formation and the levels of amines accumulated vary widely according to the type of microorganisms and vinification practices. One evidence of amine formation during malolactic fermentation was reported by Soufleros et al. (170). During malolactic fermentation carried out by indigenous lactic acid bacteria, amino acid concentrations decreased significantly while biogenic amines increased. Another evidence was the negative correlation with malic and citric acids content (146). Delfini (44) compared the ability of several strains of *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp. to produce histamine, and observed that *Pediococcus damnosus* (*P. cerevisiae*) could produce significant amounts of histamine while *Leuconostoc oenos* (*O. oeni*) strains were poor producers of biogenic amines. Lafon-Lafoucade (96) suggested that histamine build-up occurred mainly as a result of bacteria growth in poor media. Lonvaud-Funel & Joyeux (105) showed that histamine production by *O. oeni* was stimulated in media without glucose or malic acid and depended particularly on the histidine concentration. Under those conditions, histidine decarboxylation contributed an additional energy source for the bacteria as already demonstrated for other microorganisms (120). Moreno-Arribas et al. (122) isolated *Lactobacillus brevis* and *L. hilgardii* capable of tyramine and phenylethylamine formation from wines containing high amine levels. They observed that the factors affecting tyramine formation were tyrosine levels in the must and also the presence of sugars, mainly glucose. However, malolatic fermentation does not necessarily result in the formation of biogenic amines (198).

Biogenic amines can also be formed in wine by the action of contaminant microorganisms, e.g., enteric bacteria. In this case, formation of amines has been related to the lack of hygiene during wine making. Based on this assumption, histamine alone or together with other amines can be an indicator of the quality of raw materials employed or unsanitary conditions prevailing during wine production (25, 170, 198).

Other factors during the vinification process can affect amine formation, among them must treatment, length of fermentation in the presence of pulp and skin, alcohol content, sulfur dioxide concentration, added nutrients, pH, temperature, use of proteolytic enzymes, length of maturation or aging step, and quantity and type of finings and clarification agents (6, 25, 69, 73, 98, 102, 151, 169, 171, 194, 198, 216).

According to Battaglia and Frolich (14), concentrations of 5 mg/L of histamine may provoke headache after the consumption of 0.5 L of wine. Therefore, the content of bioactive amines in wines may be regulated in the future following the implemented regulations by FDA for fish (56). Moreover, some countries have established limits for histamine in wines. Switzerland recommends 10 mg/L as maximum level, Germany 2 mg/L, Belgium 5 mg/L, and France 8 mg/L (97).

TABLE 13.7
Types and Levels of Bioactive Amine in Wines and Beers

Beverage (number of samples)	Range of Amine Levels (mg/L)[1]								
	SPD	SPN	AGM	PUT	CAD	HIM	TYM	TRM	PHM
WINE									
Pinot noir (36)	nd–2.35	nd–2.38	nd–8.37	2.43–203	nd–2.07	nd–23.98	nd–8.31	nd–5.51	nd–0.89
Cabernet									
Sauvignon (53)	nd–4.03	nd–1.17	nd–1.61	3.15–23.6	nd–1.51	nd–10.10	nd–7.53	nd	nd–1.37
Cabernet Franc (30)	0.07–0.30	nd	nd	0.77–1.43	nd	nd–1.37	0.30–0.83	nd	0.17–0.50
Merlot (30)	0.03–0.23	nd	nd	0.97–1.10	nd	0.07–1.67	0.33–0.50	nd	0.20–1.13
Bordeaux (25)	—	—	—	4.03	0.88	4.91	7.31	—	—
Canadian									
Red wines (26)	—	—	—	2.19	0.32	3.66	4.27	—	—
White wines (12)	—	—	—	1.25	nd	1.86	nd	—	—
Porto wines (17)	—	—	—	3.33	0.23	3.48	2.17	—	—
BEER									
Lager (46)	nd–6.00	nd–1.41	2.10–46.8	0.85–9.80	0.15–2.60	nd–0.90	0.30–3.10	nd–0.80	nd–0.70
Ale (18)	—	—	1.10–15.7	2.60–9.70	nd–4.20	0.50–2.00	1.90–17.4	—	—
Stout (10)	0.31–1.38	nd–2.05	2.80–16.8	1.99–5.84	0.30–1.37	nd–0.85	0.48–36.8	nd–10.1	nd–0.69
Ice (5)	0.60–0.80	nd–0.30	3.20–4.00	3.90–4.50	0.10–0.20	nd	0.70–1.40	nd	nd
Bock (23)	0.25–2.10	nd–1.73	4.80–35.1	1.55–6.30	0.15–1.72	nd–1.46	0.81–5.05	nd–3.50	nd–1.72
Non-alcoholic (7)	1.35–2.30	nd–1.20	6.35–8.59	2.30–4.95	nd–0.50	nd–0.62	0.60–3.30	nd–1.41	nd–0.32
Kriek (9)	—	—	1.10–3.40	3.50–5.10	1.90–15.2	1.60–14.0	6.70–36.4	—	—
Spontaneous									
fermentation (12)	—	—	1.00–18.8	2.80–15.2	0.40–39.9	nd–21.6	0.8–67.6	—	—

[1] nd=not detected; —=not determined. SPD=spermidine, SPN=spermine, AGM=agmatine, PUT=putrescine, CAD=cadaverine, HIM=histamine, TYM=tyramine, TRM=tryptamine, PHM=phenylethylamine.

Source: Refs. 68, 69, 83, 171, and 216.

The types and levels of amines detected in beers are indicated in Table 13.7. Agmatine and putrescine were often detected in beer, being the prevalent amines. Spermidine, spermine, cadaverine, histamine, tryptamine, and phenylethylamine occurred sporadically (68, 83). Kriek and spontaneous fermentation beers showed the highest histamine and tyramine levels (83). Amine levels in beers varied widely, being affected mainly by raw materials, brewing techniques, and microbial contamination during brewing (45, 85, 215).

Some amines at low levels are normal constituents of the raw materials for beer production, such as putrescine, agmatine, and spermidine. Malt can contribute significantly to amine contents in wort and beer. High levels of putrescine, agmatine, spermine, and spermidine and low levels of histamine, phenylethylamine, tryptamine, and cadaverine are usually found in malt. Tyramine, putrescine, spermine, spermidine, and agmatine have been detected in barley. Relatively high levels of tyramine, phenylethylamine, putrescine, spermine, spermidine, and agmatine have been detected in hops; however, its contribution to amine levels in the beer is not significant since the amount used is very small. The use of judicious choice of adjuncts (corn syrup, corn, wheat, barley, or rice) could result in lower amine levels. Rice was observed to be useful in reducing biogenic amines levels in worts and beers (68, 84, 89, 166, 215).

During beer production and storage several amines can be formed and accumulate. The formation of amines during brewing occurs principally during main fermentation (89). High amine production has also been observed during malting (84). During mashing and wort boiling, some amines such as tyramine and agmatine can accumulate. Their formation is attributed to thermal amino acid decarboxylation and possibly to enzymatic activity in the malt (45, 84). Acidified compared to original wort showed increased amine levels (45). During fermentation, *Saccharomyces uvarum*, a top or ale yeast, and *Saccharomyces cereviase* var. *uvarum*, a bottom or lager yeast, did not produce histamine or tyramine (85, 215). *Saccharomyces cereviase* var. *uvarum* recycling with phosphoric acid treatment did not influence biogenic amine formation (82).

During fermentation, wort contamination by amino acid decarboxylase bacteria such as lactic acid bacteria, might occur, leading to the formation of biogenic amines, mainly histamine, tyramine, tryptamine, phenylethylamine, and cadaverine (45, 83, 215). Therefore, biogenic amines could be associated with hygienic conditions during brewing (32, 45, 68, 82, 215). *Lactobacillus brevis* was responsible for tyramine and putrescine production in wort

fermented with a top-fermenting yeast (215). *Pediococcus* spp. produced tyramine during beer fermentation and the amount produced depended on the contaminants counts (84). Experiments with beer spoilage microorganisms showed that *Lactobacillus frigidus*, *L. brevissimilis*, *L. brevis*, and *L. casei* produced higher amine levels compared to *L. plantarum*, *L. lindneri*, and *Pediococcus damnosus*. Moreover, commercial samples with *lactobacillus* contamination, had higher levels and different amine profiles (45). Wort contamination with *Enterobacter agglomerans*, *E. cloacae*, *E. intermedium*, and *Serratia marcescens* produced increased levels of putrescine and cadaverine. Wort contaminated with *Serratia marcescens* incubated at 27 °C produced higher amine levels compared to 6 °C (65). Therefore, biogenic amines could be used as indicators of microbial contamination.

C. Milk and Dairy Products

The types and levels of bioactive amines found in milk and dairy products are indicated in Table 13.8. In general, amine content in milk and dairy products is quite low, except for cheeses which can contain very high levels. Polyamines are naturally present in cow's milk. Spermine is the prevalent amine (34%), followed by putrescine (17%), spermidine (15%), and agmatine (3%) (11, 150). Serotonin and phenylethylamine were also detected in fresh milk corresponding to 16 and 15% of total amine levels, respectively (150). In reconstituted powdered milk (149), UHT milk (4), and yogurt (128) the amine profile changed, keeping similar total levels. There was loss of spermine and putrescine. Cadaverine, which is not inherent to milk, was also detected. Its presence could be correlated with *Enterobacteriaceae* counts, suggesting contamination of the product during processing (112).

A variety of amines has been found in different types of cheese (Table 13.8). In general, spermine and spermidine are present at low levels. The levels of other types of amines vary widely, depending on the cheese, ripening time or aging, and type of microorganisms present. However, high histamine and tyramine levels have been reported in several types of cheese (81, 173, 189).

Cheeses represent an ideal environment for biogenic amine production. Whether or not amines accumulate and

TABLE 13.8
Types and Levels of Bioactive Amines in Milk and Dairy Products

	Range of Amine Levels (mg/100 g)[1]									
Product	**SPD**	**SPN**	**AGM**	**PUT**	**CAD**	**HIM**	**TYM**	**TRM**	**PHM**	**SRT**
MILK[2]										
Fresh	0.01–0.16	0.13–0.26	nd–0.03	nd–0.21	nd	nd	nd	nd	0.04–0.17	nd–0.28
Powdered	0.21–0.29	0.07–0.10	0.59–0.64	nd	0.03–0.04	nd	nd	nd	nd	nd
UHT	0.05–0.13	0.07–0.15	nd	0.04–0.08	0.02–0.08	nd	nd	nd	nd	nd–0.39
YOGURT[2]	nd–0.04	nd–0.02	nd–0.04	nd	nd–0.03	nd	nd	nd	nd	—
CHEESE										
Blue cheese	—	—	—	9.6–23.7	42.3–227	nd–409	2.2–166	nd–110	—	—
Camembert	—	—	—	nd–60.5	nd–118	nd–48.0	2.30–200	nd–6.00	nd	—
Cheddar	—	—	—	nd–99.6	nd–40.8	nd–154	nd–153	nd–30	—	—
Feta	—	—	—	0.16–19.3	0.03–8.28	nd–8.46	nd–24.6	0.22–0.57	0.08–0.70	—
Gorgonzola	0.13–3.23	0.07–0.55	nd–1.79	1.2–124	5.8–428	1 .7–191	8.9–255	2.4–43	0.07–1.03	nd–1.81
Gouda	nd–1.35	nd–1.13	nd–1.34	nd–107	nd–99.5	nd–30.5	nd–67	nd–88	nd–1.92	nd–3.04
Grated	nd–1.23	0.07–0.80	nd–1.41	nd–6.30	0.07–13.4	nd–8.80	nd–21.3	nd–0.34	nd–1.98	nd–1.27
Minas	nd–2.10	0.07–2.58	nd–0.04	nd–2.64	nd–0.30	nd–2.50	nd	nd–0.72	nd–0.64	nd–0.31
Mozzarella	nd–1.06	nd–1.31	nd–0.13	nd–1.37	nd–2.34	nd–11.3	nd–1.56	nd–0.35	nd–0.26	nd–0.47
Parmesan	nd–0.15	0.07–0.09	nd	nd–1.36	nd–0.25	nd–27.2	nd–29	nd–1.70	nd–0.04	nd–0.23
Processed	nd	nd–10.0	—	nd–6.00	nd–12.0	nd	nd–16.0	nd	nd–40.0	—
Provolone	nd–2.38	0.07–0.97	nd–0.18	nd–8.17	nd–111	nd–6.04	nd–0.44	nd–1.08	nd–1.40	nd–1.40
Swiss	—	—	—	—	—	nd–250	nd–180	nd–1.60	—	—
Montasio										
60 days	—	—	—	0.06–1.01	0.02–0.40	0.55–1.82	0.21–3.51	0.01–0.36	0.07–0.44	—
90 days	—	—	—	0.06–3.84	0.07–1.00	1.63–6.43	1.32–8.63	0.02–0.23	0.04–1.97	—
150 days	—	—	—	12.9–110	0.50–3.02	10.1–37.8	12.8–37.4	0.18–0.81	0.77–2.00	—

[1] nd=not detectable; —=not determined. SPD=spermidine, SPN=spermine, AGM=agmatine, PUT=putrescine, CAD=cadaverine, HIM=histamine, TYM=tyramine, TRM=tryptamine, PHM=phenylethylamine, SRT=serotonin.

[2] Unit=mg/L.

Source: Refs. 4, 11, 81, 128, 149, 150, 173, 189, and 192.

persist in cheese depends on a number of factors. Biogenic amine formation can be affected by the availability of free amino acids and the presence of microorganisms, both added or contaminant, capable of amino acids decarboxylation. Conditions favorable for microbial growth and decarboxylase activity are also important, e.g., pH, salt concentration, temperature, water activity, ripening temperature and time, storage temperature, presence of suitable cofactors, and amine catabolism by means of mono- and diaminoxidase (51, 58, 72, 143, 173, 189).

Cheese is a protein-rich food, and amino acid is mainly brought about by the action of proteolytic enzymes from the starter and the rennet which contribute to the breakdown of casein (87). Amino acid decarboxylating enzymes show optimum activity at acid pH as a mechanism of environment neutralization against an excessive pH decrease which is incompatible with the bacteria's growth. The pH of cheese, 5.0–6.5, is the optimum range for most decarboxylase activity (5, 51, 143). The production of histamine and other biogenic amines is accelerated by high temperatures during production and manufacture of cheese (51, 87, 173). The increase in the concentration of salt can decrease amine formation, but salt-tolerant, amine-producing bacteria need to be controlled (51, 87, 143, 173, 175). The cofactor piridoxal phosphate, necessary for decarboxylase activity mainly over tyrosine, lysine, and ornithine, is present in milk and cheese at levels of 42 to 215 μg/100 g, which is sufficient to saturate the decarboxylases required for amine production (51, 143). However, there can be a decrease in biogenic amine levels due to catabolic activity of some microorganisms. Bacteria capable of metabolizing histamine (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Serratia flava*, *Clostridium feseri*) and tyramine (*Sarcina lutea*, *Aspergillus niger*, *Trichosporum* spp.) have been reported in the literature (139, 143).

Numerous bacteria, both intentional (starter cultures) and adventitious, have been reported as being capable of amine production at levels depending on bacterial densities and synergistic effects between microorganisms (81). The decarboxylase-positive microorganisms can be part of the natural population of the milk, or can be introduced as starter culture or by contamination, before and during cheese making and after processing. According to Joosten and Northold (88), it is necessary to have densities of 10^7 to 10^9 CFU/g in order to produce high concentrations of amines.

The hygienic quality of milk is very important in the formation of biogenic amines in cheese (143, 173). Cheese made with milk of poor microbial quality contains higher amine levels than cheese made with high quality milk (5, 87, 133). Among contaminating microorganisms, *Enterococcus* ssp. are notorious tyramine formers. *Enterococcus faecalis* was associated with considerable production of biogenic amines including phenylethylamine (126). *Enterobacteriaceae* species, even at low densities, have been observed to produce and accumulate histamine, tyramine, putrescine, cadaverine, and phenylethylamine (51, 88, 112, 126, 173). *Clostridium* can produce histamine, tryptamine, and tyramine (51). Gram-negative microorganisms can increase the concentrations of putrescine and cadaverine in cheese (87, 112, 133).

According to Fernández-García et al. (58) and Greif et al. (72), lactic starter cultures used in the production of cheese can be an important critical point in the production of biogenic amines. The production of acid is the first manifestation of their growth. A second major change is the alteration of proteins. Starter cultures hydrolyze proteins and increase free amino acids levels which can be used for their growth (143). Several starter cultures have been observed to possess amino acid decarboxylase activity (87, 173). According to Straub et al. (174), several fermentation organisms failed to produce amines in phosphate buffer. However, in milk, *Lactobacillus* ssp. play a major role in histamine, tyramine, and putrescine accumulation. Various species of *Lactobacillus* produce histamine, e.g., *L. bulgaricus*, *L. casei*, *L. acidophilus* (51, 133, 173). *Lactococcus lactis* was observed to produce histamine, tyramine, and tryptamine (33). Wild lactococcal and leuconostoc strains were capable of tyramine formation (71). According to González de Llano et al. (71), Halász et al. (74), and Straub et al. (174), the amine forming ability should be taken into account when selecting starter cultures.

The influence of temperature, rennet, and NaCl on bioactive amines formation in milk by a commercial starter culture containing *Lactococcus lactis* ssp. *cremoris* and *L. lactis* ssp. *lactis* was investigated by Santos et al. (149). Sterilized reconstituted dry milk was inoculated with 1% culture in the presence or not of rennet and NaCl and incubated at 20 and 32 °C/24 h. There was a change in the levels of amines natural to milk. Histamine, serotonin, phenylethylamine, and tryptamine were also formed. Tyramine was only formed at 32 °C. Addition of rennet favored the formation of putrescine, tyramine, and tryptamine. Incorporation of NaCl in the milk for cheese manufacture decreased agmatine, spermidine, putrescine, histamine, and tyramine levels. By decreasing incubation temperature and adding NaCl, it was possible to minimize bioactive amine formation.

Pasteurization of milk can affect amine formation in cheese. In cheeses made from pasteurized milk, decarboxylase-positive bacteria usually remain at low levels, with reduced risk of biogenic amine formation even if the substrate is enriched in free amino acids by proteolytic enzymes. However, in cheeses made from raw milk, where a considerable population of decarboxylase-positive lactic acid bacteria is more likely to occur, formation of biogenic amines was enhanced by the higher levels of free amino acids (58). Pasteurization can be alternatively

achieved by high pressure treatment of milk. Besides destroying pathogenic flora of milk, cheese made with high-pressure-treated milk ripens faster as it promotes higher proteolysis, without increasing levels of biogenic amines (128).

Another factor that may affect amine formation during cheese manufacture is the aging process. Tyramine concentration increased in Gouda cheese from milk with added tyrosine or with proteolysis faster than normal (87). Indeed, incorporation of proteinases to accelerate the ripening process of raw milk Hispanico cheese resulted in higher tyramine concentration, which increased with cheese age. However, it did not affect histamine formation (58). The longer the aging process, the higher the content of some biogenic amines as indicated for Montasio cheese (Table 13.8).

Although it is very difficult to elaborate cheese free of biogenic amines, efforts should be made to elucidate amine formation in cheese in order to optimize technology and secure low amine levels, mainly of the most dangerous ones (143). Use of raw material of good hygienic quality, pasteurization of cheese milk, use of hygienic practices during manufacture, prevention of temperature abuse throughout production, and selection of starters with low decarboxylase activity are measures to avoid the accumulation of these undesirable compounds (126, 127, 189).

D. Fish

Small amounts of bioactive amines occur naturally in fish (Table 13.9). Under normal physiological conditions, fish muscle contains high levels of spermine and spermidine and low levels of histamine and putrescine (9, 68, 118).

Several factors have been observed to affect amine levels in fish. Fish-to-fish and seasonal variations are observed due to genetics, environment, sex, physiological stage, and tissue sampled (1, 43, 53, 117, 195). Scombroid fish, such as tuna, bonito, mackerel, yellowfin, and bluefin, are particularly susceptible to histamine formation as they contain large amounts of free histidine (7, 31, 177). However, fish from other families (Scomberesocidae, Pomatomidae, Coryhaenidae, Carangidae, Clupeidae, and Engraulidae) are also susceptible. Data are contradictory with respect to both histidine and histamine levels in light and dark muscles (7, 68, 208). However, no significant difference in biogenic amines levels was found between samples from different parts along the light muscle of *Thunnus thynnus* and upper and lower loin light muscles of *T. alalunga*, respectively (68, 195).

The microbial flora of the fish also plays an important role in the formation of amines. However, it may be transient, varying with the environment, diet and hygienic conditions during capture, handling, processing, and storage (29). Several species, either natural or contaminant, are capable of producing histamine, such as *Morganella morganii, Klebsiella pneumonia, K. oxytoca, Hafnia alvei, Clostridium perfringens, Lactobacillus delbrueckii, Escherichia coli, Citrobacter freundii, Enterobacter cloacae, E. aerogenes, E. agglomerans, Proteus mirabilis, P. vulgaris, Serratia liquefaciens, Acinetobacter lwoffi, Pseudomonas putrefaciens, P. putida, P. fluorescens, Vibrio alginolyticus,* and *Aeromonas hydrophyla* (1, 7, 23, 103, 117, 142, 195, 214). However, *Morganella morganii*, *Hafnia alvei,* and *Klebsiella pneumoniae* are the main histamine-producing bacteria which have been isolated from fish implicated in histamine poisoning outbreaks (177).

Other factors that influence the levels of amines in fish include place of capture, air, water and fish temperature, post-capture handling practices, chilling and freezing systems and rates, and storage conditions (7, 56, 68, 136). Several handling techniques have been used in commercial fishery. According to Dawood et al. (43) and Hardy and Smith (75), eviscerated fish contained lower concentrations of amines than whole samples. However, Price et al. (136) did not find significant differences in the quality and histamine levels of round, bled, or dressed albacore. Several investigations have demonstrated that storage temperature is a critical factor influencing histamine and other amine formation in fish muscle (49, 67, 182, 195). According to Ababouch et al. (1), freshly caught sardines contained high levels of bacteria located on the skin and the gills. These bacteria invaded and grew rapidly in sardine muscle, reaching 5×10^8 and 6×10^8 cfu/g, respectively, after 24 h at room temperature and 8 days in ice. Histamine, cadaverine, and putrescine accumulated to levels of 235, 105, and 30 mg/100 g, respectively, after 24 h storage at room temperature and after 8 days storage in ice. The requirement for rapid and uninterrupted refrigeration after catch can never be over-emphasized. The United States Food and Drug Administration (FDA) established a guidance level for histamine in fish at the dock or before processing of 5 mg/100g. The FDA also states that rapid chilling of fish immediately after catch is the most important strategy to limit histamine formation. The internal temperature of the fish should be brought to 10 °C or below within 6 hours of death. Chilling from 10 to 4.4 °C or less should not take longer than 18 hours. Any time above 4 °C significantly reduces the expected safe shelf life (56).

Studies have indicated that the levels of spermine and spermidine decrease and those of putrescine and histamine increase during storage and deterioration of fish such as tuna, rainbow trout, salmon, sardine and some fresh water fish, e.g., snapper, carp, catfish, "tambaqui," "lambari," and "tilápia" (23, 43, 67, 118). There is also formation and accumulation of cadaverine, tyramine, tryptamine, and phenylethylamine. The rates of change, however, are affected by storage temperature, pH, oxygen, nutrients presents, incorporation of additives, and packaging system (1, 23, 117, 118, 142, 195, 207). Studies on the influence of temperature on histamine formation by *Morganella morganii* and *Hafnia alvei*, prolific histamine

TABLE 13.9
Mean Levels of Bioactive Amines in Different Tissues of Tuna, in Tuna of Different Quality and in Tuna Affected by Storage Temperature and Canning

	Mean Amine Level (mg/100 g)[1]						
Fish	**SPD**	**SPN**	**PUT**	**CAD**	**HIM**	**TYM**	**PHM**
ALBACORE TUNA							
light muscle							
upper loin	0.26b	0.68	0.22	0.13	nd	nd	nd
lower loin	0.25b	1.21	0.14	0.11	nd	nd	nd
dark muscle	0.79a	2.5	0.06	0.07	nd	nd	nd
TUNA FISH							
good	0.44	0.95	0.12	0.15	0.38	—	—
borderline	0.36	0.67	0.23	1.03	2.36	—	—
decomposed	0.07	0.12	0.25	1.93	25.3	—	—
TUNA							
fresh	—	—	0.04	0.02	nd	nd	nd
0 °C/21 days	—	—	0.52	2.44	10.8	1.38	nd
8 °C/9 days	—	—	1.11	5.62	368	3.25	0.69
20 °C/3 days	—	—	0.45	10.84	687	1.71	0.81
ALBACORE TUNA							
before canning	0.43	0.73a	0.20a	0.06	0.07b	0.04a	0.01b
after caning	0.49	0.35b	0.09b	0.04	0.11a	0.00b	0.24a

[1] nd–not detectable, —=not determined. Mean values with different letter in the same group differ statistically ($p < 0.05$, Tukey test). SPD=spermidine, SPN=spermine, PUT=putrescine, CAD=cadaverine, HIM=histamine, TYM=tyramine, PHM=phenylethylamine.

Source: Refs. 68, 118, and 195.

formers, indicated that at 1 °C, no histamine was formed, whereas at 19 and 30 °C, the highest levels were found (7). Veciana-Nogués et al. (195) investigated the influence of temperature on bioactive amine formation in tuna and observed similar evolution profile at the three temperatures, although the highest amounts were achieved in samples stored at the higher temperature. There was a great increase in histamine followed by cadaverine. Formation of histamine, tyramine, and cadaverine were related to mesophilic microorganisms, *Enterobacteriaceae*, and coliforms. According to Du et al. (49), as histamine levels exceeded 5 mg/100 g in tuna fillets stored at 4, 10, and 22 °C, the total bacterial counts reached 10^7 cfu/g and the histamine producers reached 10^2 to 10^3 cfu/g. Histamine production by *Klebsiella pneumonia* was reduced as the concentration of NaCl increased, with marked inhibition occurring at 5.5% NaCl (184). Use of antimicrobial agents may provide an additional impediment to bacterial histamine production. Potassium sorbate at a concentration of 0.5% inhibited growth and histamine production by selected strains of *Morganella morganii* and *K. pneumonia* (182). Sodium chloride at 1% stimulated amine formation; however, levels above 3% or 2% combined with 0.5% clove completely inhibited growth and amine production by *Enterobacter aerogenes* in mackerel broth (209). Vacuum packaging did not show any beneficial effect in controlling bacterial growth and histamine production on tuna samples (207). Canning of fish does not affect significantly histamine levels. However, significant losses are observed for spermine, putrescine, and tyramine and accumulation for phenylethylamine (68, 80).

Based on the changes observed in bioactive amines, Mietz and Karmas (118) proposed a chemical index to evaluate the quality of tuna. It is calculated by the sum of the levels of putrescine, histamine, and cadaverine divided by the sum of spermine and spermidine added by 1. Nominal cutoff values were 0 to 1 for good quality, 1 to 10 for borderline, and higher than 10 for decomposed tuna fish. This index compared favorably to sensory and authentic pack value scores. It was considered adequate to evaluate the quality or freshness of tuna and other types of fish and seafood, among them salmon, rockfish, snapper, lobster, and shrimp (119). In some species of fish, the presence of tyramine can also be detected during storage and deterioration. Based on these findings, Veciana-Nogués et al. (195) proposed for the quality assessment of tuna the use of an index calculated from the sum of the contents of histamine, tyramine, cadaverine, and putrescine. According to these authors, it showed

good correlation with both storage time and organoleptic assessment and the level of 5 mg/100 g could be a guiding limit value for tuna and anchovy acceptance.

A maximum average histamine content of 10 mg/100 g has been established by several countries and communities (European Community and Mercosur) for acceptance of canned tuna and other fish belonging to the Scombridae and Scomberesocidae families (56). Because of the potentiating effect of other amines on the toxic effect of histamine, European regulation recommends the use of HPLC technique for the determination of the amines (107).

E. Meat and Meat Products

Spermine, spermidine, and putrescine occur naturally in meat. Meat is very susceptible to chemical and physical changes during storage of fresh meat or during processing of meat products. It is also susceptible to proteolysis by microorganisms and endogenous or microbial enzymes, which can liberate amino acids. The biogenic amines histamine, putrescine, tyramine, tryptamine, 2-phenylethylamine, and cadaverine can, then, be formed (76, 102, 196). Since biogenic amines are metabolites of microbial activity and are resistant to heat treatment (67, 102), they have been considered to be a useful index of quality of fresh and processed meat, reflecting the quality of the raw material used and the hygienic conditions prevalent during its processing. Amine levels in different types of fresh and stored meat are indicated in Table 13.10.

Immediately after slaughter, high levels of spermine and spermidine and traces of putrescine and cadaverine have been detected in red and white chicken meat. Spermine is the prevalent amine contributing with 70% of total amine levels. Low levels of histamine are also detected in thighs. During storage at 4 ± 1 °C, there was a decrease in spermine, spermidine levels remained constant, and putrescine, cadaverine, histamine, and tyramine were formed. At 15 days, higher levels of amines were found in breast compared to thigh. An index based on the ratio of the polyamines spermidine/spermine levels was considered appropriate for the evaluation of chicken meat quality (161).

Amine types and levels detected in chicken-based products, mortadella, frankfurters, sausage, meatballs, hamburger, and nuggets varied widely, indicating that ingredients added and processing affected amine profiles. Nuggets were the only products with an amine profile similar to that of fresh chicken meat. There was prevalence of spermidine over spermine for most of the products, suggesting the incorporation of significant amounts of vegetable protein in the formulations. Significantly higher biogenic amine levels were observed in sausages, which could indicate the use of low quality raw material or of contamination during processing and storage (161).

In fresh pork meat, spermine was the prevalent amine followed by low levels of spermidine and agmatine. No significant difference was observed between loin and leg tissues. During storage of pork loin at 5 ± 1 °C, mean spermine and spermidine levels remained constant, and putrescine, cadaverine, and histamine were formed. Cadaverine was detected on the 4th day of storage when mesophilic and psychrotrophic counts reached 4.9×10^5 and 7.9×10^5 cfu/g, respectively. Putrescine and histamine were detected only on the 12th day, with counts of 1.3×10^6 and 1.9×10^8 cfu/g for mesophilic and psychrotrophic microorganisms, respectively (193). Small changes in spermine and spermidine levels and formation and accumulation of putrescine, cadaverine, histamine, and tyramine were also observed by Hernández-Jover et al. (78). They suggested that the sum of these four biogenic amines could be a useful index to evaluate meat freshness, with values below 5 mg/kg indicating high hygienic quality meat.

Spermine was also the prevalent amine in fresh beef, followed by spermidine. Low levels of histamine were also detected in some samples (30, 76). At 4 °C, vacuum-packed beef had three times the shelf life of the unpacked product and lower levels of histamine, putrescine, and cadaverine (91).

Cooked meat products such as ham and mortadella showed prevalence of spermine over spermidine as observed for fresh meat; however, levels were lower compared to fresh products due to dilution of meat with fat and other ingredients used in the manufacturing process. The levels of biogenic amines varied widely (76, 78).

Much higher amounts of biogenic amines have been reported in dry fermented sausages. Tyramine was the prevalent amine, followed by putrescine, but their contents fluctuated widely. The presence of tryptamine and phenylethylamine was found to be common in the majority of the ripened products, although at low levels (28, 52, 76). Although the contents reported for certain amines are occasionally higher than those reported for fish products, there is still a lack of information on the incidence of histamine and tyramine intoxication with these products. However, sausages have been incriminated in migraine headaches and in hypertensive crisis episodes (60, 76).

Production of dry sausages offers favorable conditions for the formation of biogenic amines. There is growth of microorganisms for several days, and a certain degree of proteolysis takes place giving rise to the presence of free amino acids, which favors amino acid decarboxylase activity. Furthermore, raw material quality, thawing conditions, ripening temperature, and addition of a properly selected starter culture can affect formation and accumulation of biogenic amines. Therefore, these are important critical control points in amine formation (18, 111).

Good quality raw material only contributes with spermine and spermidine to the sausage. However, high

TABLE 13.10
Types and Levels of Bioactive Amines in Different Fresh, Stored, and Deteriorated Meat and Meat Products

	Amine Levels (mg/100 g)[1]									
Product	**SPD**	**SPN**	**AGM**	**PUT**	**CAD**	**HIM**	**TYM**	**TRM**	**PHM**	**SRT**
CHICKEN										
Fresh breast	0.73	1.79	nd	nd	nd	nd	nd	nd	nd	nd
Fresh thigh	0.72	1.62	nd	nd	nd	0.07	nd	nd	nd	nd
Breast storage/4 °C										
4 days	0.77a	1.72a	nd	nd	nd	nd	nd	nd	nd	nd
10 days	0.60a	1.25b	nd	nd	nd	nd	nd	nd	nd	nd
15 days	0.87a	1.12b	nd	2.04	0.43	1.03	1.74	nd	nd	nd
Products										
Hot dog	1.19–2.66	0.06–1.71	nd	nd–0.14	nd–0.15	nd–0.12	nd–0.14	nd	nd	nd–0.09
Mortadella	0.49–2.43	0.64–1.59	nd	nd–1.92	nd–0.54	nd–0.72	nd–0.14	nd	nd	nd–0.08
Sausage	0.34–1.11	0.60–1.46	nd	0.08–8.20	nd–6.68	nd–4.66	nd–3.36	nd–2.08	nd	nd–0.67
Meatball	0.35–1.86	0.51–1.10	nd	nd–0.16	nd–0.10	nd	nd–0.14	nd	nd	nd–0.13
Hamburger	0.42–2.44	0.45–1.56	nd	nd–0.19	nd–0.41	nd–0.08	nd–0.27	nd	nd	nd–0.22
Nugget	0.29–0.81	0.54–1.28	nd	nd	nd	nd	nd	nd	nd	nd
PORK										
Fresh loin	0.22	3.03	0.09	nd	nd	nd	nd	nd	nd	nd
Fresh leg	0.22	3.37	0.11	nd	nd	nd	nd	nd	nd	nd
Loin storage/5°C										
4 days	0.41	1.67	nd	nd	0.13b	nd	nd	nd	nd	nd
8 days	0.1	1.94	nd	nd	0.24b	nd	nd	nd	nd	nd
12 days	0.89	2.65	nd	0.14b	0.33b	0.36	nd	nd	nd	nd
16 days	0.63	3.36	nd	0.47a	2.02a	0.67	nd	nd	nd	nd
BEEF										
Fresh	0.19–0.42	1.80–4.46	—	nd	nd	nd–0.20	—	nd	—	—
Unpacked 12 days	—	—	—	1.04a	0.52	nd	—	—	—	—
Vacuum 12 days	—	—	—	0.11b	0.31	nd	—	—	—	—
COOKED										
Ham (20)	0.14–0.35	0.64–3.57	—	nd–1.24	nd–2.88	nd–0.09	nd–7.81	—	—	—
Mortadella (20)	0.10–0.89	0.76–3.22	—	nd–0.57	nd–4.00	nd–0.48	nd–6.70	nd–0.50	nd–0.50	—
DRY SAUSAGE										
Italiano (21)	0.06–1.29	1.67–5.93	0.05–1.01	5.33–11.8	0.68–12.8	0.09–12.1	12.6–23.0	0.02–1.16	0.61–3.10	nd–0.94
Milano (15)	0.04–0.29	0.61–3.01	0.05–0.16	3.42–6.23	0.08–9.50	nd–0.28	5.87–14.3	0.07–1.67	0.02–0.84	nd
Hamburguês (9)	0.12–1.40	2.55–3.33	0.04–1.57	3.72–6.73	1.98–5.38	nd–1.61	11.9–15.5	0.07–0.77	0.18–0.43	nd–0.01
Salaminho (9)	0.07–0.17	0.55–1.39	0.12–0.19	3.99–4.46	0.73–2.73	0.01–0.33	5.84–10.6	0.02–0.26	0.04–0.42	nd
Chorizo (20)	0.19–1.00	1.38–4.35	—	0.26–41.6	nd–65.8	nd–31.4	2.92–62.7	nd–8.78	nd–5.15	—
Salchichón (22)	0.07–1.38	0.69–4.25	—	0.55–40.0	nd–34.2	nd–15.1	5.33–51.3	nd–6.51	nd–3.47	—
Fuet (11)	0.09–1.10	0.94–3.01	—	0.22–22.2	0.54–5.13	nd–35.8	3.18–74.3	nd–6.78	nd–3.37	—
Sobrasada (7)	0.24–0.70	1.03–1.78	—	0.18–50.1	0.30–4.16	0.28–14.3	5.76–50.1	nd–6.48	0.02–3.85	—

[1] nd=not detectable; —=not determined. SPD=spermidine, SPN=spermine, AGM=agmatine, PUT=putrescine, CAD=cadaverine, HIM=histamine, TYM=tyramine, TRM=tryptamine, PHM=phenylethylamine, SRT=serotonin.
Source: Refs. 28, 30, 76, 91, 161, and 193.

amounts of biogenic amines in sausages can be related to the use of low hygienic quality meat (76, 77, 132). The choice of good quality raw materials helps minimize the number of amine-producing bacteria. Furthermore, biogenic amine formation may be affected by the thawing time and storage temperature of raw material (110). Fresh lean meat stored for 5 days at −20 °C maintained hygienic quality and low tyramine, cadaverine, and putrescine content. However, when stored for 5 days at 4 °C, sausages formed amines earlier and accumulated up to 50-fold higher amounts. Storage temperatures of 15 °C, compared to 4 °C, favored proteolytic and decarboxylase reactions, resulting in increased amine concentration (20). Isolates obtained from raw sausages showed histamine-forming activity, most of them belonging to the *Enterobacteriaceae* family, *Klebsiella oxytoca*, *Enterobacter aerogenes*, and *E. cloacae* (144).

Intense proteolysis takes place during the dry curing process, mainly due to muscle proteinases (76). Proteolysis during fermentation is favored by the denaturation of proteins as a consequence of acidity, dehydration, and action of sodium chloride. The activity of endogenous but also microbial proteases modifies the composition of the non-protein nitrogen fraction, including the production of free amino acids (18).

Dry sausages can be spontaneously fermented by wild microbial flora. However, tyramine and putrescine are usually produced and accumulated. The use and selection of starter cultures can reduce the production of these and other amines (77, 110). Starter culture shortens the fermentation process and also reduces the growth of deleterious microorganisms (76). They usually consist of one or several strains of lactic acid bacteria and the group of catalase-positive cocci, including micrococci and staphylococci, or a combination of both (18). Lactic acid production by lactic acid bacteria has a preservative effect, and facilitates the drying process, the development of the typical curing color, and the cohesion of the sausages. However, the production of biogenic amines in meat and meat products has often been related to lactic acid bacteria, *Enterobacteriaceae* and *Pseudomonas*. Micrococci and staphylococci also contribute to sausage ripening by enhancing the development of the characteristic flavor and color. Catalase production protects against color changes and rancidity, while nitrate and nitrite reductase aid reddening and reduce residual nitrite content (21).

Several reports on the effect of different combinations of starter cultures on biogenic amines formation and accumulation *in vitro* and in sausage are available. *Staphylococcus carnosus* and *S. xylosus* were considered safe starter cultures according to their tyramine production *in vitro* (114). However, *S. carnosus* was observed to have a remarkable potential to form biogenic amines, but not *S. xylosus* (174). Indeed, *S. carnosus* did not prevent the formation of tyramine and phenylethylamine by contaminant lactic acid bacteria during sausage ripening (110). Sausages made with *S. carnosus* and *S. xylosus* showed strong proteolysis but had lower amounts of tyramine compared to naturally fermented sausages (18).

The use of *Lactobacillus sake*, *Pediococcus pentosaceus*, *Staphylococcus carnosus*, and *S. xylosus* inhibited formation of putrescine but not tyramine during ripening of Turkish "soudjoucks" (8). *L. sakei* and *Staphylococcus* spp. drastically reduced tyramine, cadaverine, and putrescine accumulation (21, 22). *L. curvatus* only attenuated tyramine content compared to *L. sakei*; however, both were able to limit production of putrescine and cadaverine and to prevent tryptamine and phenylethylamine formation by the wild flora (22). Mixtures of *Pediococcus cereviseae* and *L. plantarum* were unable to produce significant levels of histamine and tyramine (140). Histamine production was lower in sausage fermented by mixed starter cultures of *Lactobacillus plantarum*, *Pediococcus acidolactici*, and *Micrococcus roseus* compared to those fermented by natural microflora (16). Some strains of *Enterococcus*, *L. curvatus*, or *S. carnosus* produced tyramine and phenylethylamine (121).

The technological conditions used to produce sausages can also affect amine formation since they affect the growth and activity of the microorganisms present. The largest diameter contained higher amines levels than thinner sausages. Histamine levels in "sobrasada" and tyramine, histamine, putrescine, and cadaverine levels in sausages were higher in the central part compared to the edges (19, 196). The choice of optimal processing temperature for the amine-negative starter culture is an important critical control point in the formation and levels of biogenic amines (111). Addition of glucono-delta-lactone (GLD) to sausage causes a faster decrease in pH, stabilizes the color, simplifies production, and suppresses microorganisms which cause spoilage (streptococci, coliforms, and total plate counts), thereby decreasing significantly the levels of histamine and putrescine (109). However, sausages produced with 0.3% GLD contained higher histamine and tyramine levels and less acceptable organoleptic evaluation (24). Moreover, tyramine degradation by microorganisms with tyramine oxidase activity, such as *Micrococci* during ripening, can be used (100).

A recommended upper limit of 10 to 20 mg/100 g for histamine in meat products has been proposed by the Netherlands Institute of Dairy Research and by the Czech Republic (121, 185). A recommendation should be extended for tyramine in meat products. Furthermore, the addition of selected starter culture, the use of proper technological conditions favoring starter development, and the utilization of raw materials with good hygienic quality make it possible to produce fermented sausages nearly free of biogenic amines (21).

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14 Pigments in Plant Foods

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I. INTRODUCTION

The appearance of foods, especially color, is one of the major contributors to consumers' food choices. Color level also influences the sweetness and flavor of foods (1–3). Pigments in plants are responsible for the coloration of fruits and vegetables. Naturally occurring pigments from plants are generally considered safe due to their presence in edible plant materials and are exempt from the toxicological testing that synthetic dyes must undergo. Consumers' preferences are shifting from synthetic food dyes to natural food colorants due to a perceived health concern and potential health benefit of some pigments: β-carotene is a nutrient precursor of vitamin A, chlorophyll

derivatives are potential chemo preventatives, lycopene is associated with decreasing the risk of several types of cancer, and phenolic anthocyanins in wines have been implicated in reducing heart disease (4, 5).

Color perception of food is the result of the pigments' ability to reflect or emit the electromagnetic spectrum of light energy and stimulate the retina in eye. The majority four pigments from plant foods can be classified into four groups depending on their structures: chlorophylls (tetrapyrroles), carotenoids (tetraterpenoids), anthocyanins (*O*-heterocyclic), and betalains (*N*-heterocyclic) (4, 6). Generally, chlorophylls and carotenoids are lipid-soluble pigments and flavonoids and betalains are water-soluble pigments (2). Chlorophylls are green pigments ubiquitously found in photosynthetic tissues of higher plants. Carotenoids are a large group of compounds that provide yellow to red coloration in plants. Flavonoids, especially anthocyanins, provide red, blue, and violet colors of many fruits and vegetables. Betalains are responsible for the red and yellow colors in beef root and amaranth (4, 7).

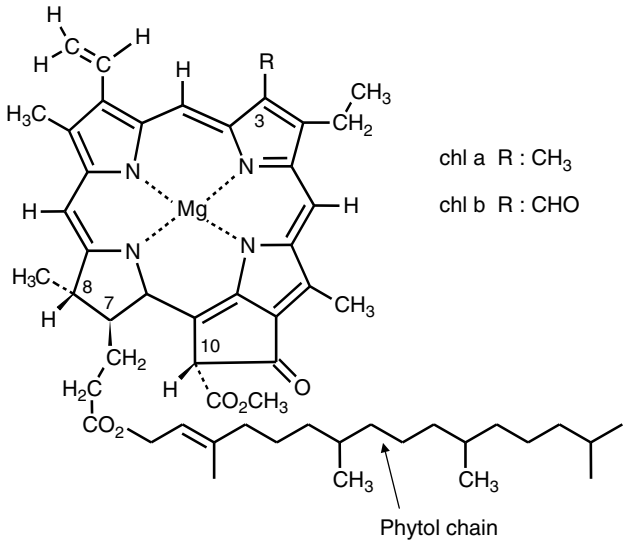


FIGURE 14.1 The structure of chlorophyll *a* and *b*.

II. CHLOROPHYLL

Chlorophylls are present in green plants capable of photosynthesis. Chlorophylls are the most abundant pigments among biologically produced pigments (8, 9). In foods, chlorophylls are widely distributed in green leafy edible plants and vegetables.

A. Structures of Pigment and Its Derivatives

Chlorophylls are derived from porphyrin complexed with magnesium. The porphyrin ring is a fully unsaturated and conjugated macrocyclic structure with tetrapyrrole units in which the pyrrole rings are joined by methylene bridges. Several chlorophylls are found in plants. Chlorophyll *a* (blue-green) and *b* (yellow-green) are present in green plants in the ratio of 3:1 (Figure 14.1). The magnesium in chlorophylls can be removed easily by acids and chlorophyll *a* and *b* form pheophytins *a* and *b* (olive-brown color), respectively. Hydrolysis of the phytol side chain forms the water-soluble chlorophyllide derivative. Both the removal of phytol group and magnesium can generate pheophorbide, which is also water soluble (Figure 14.2) (4, 7, 10). The C10 carbomethoxy groups (CO_2CH_3) of pheophytins and pheophorbide can be removed by thermal processing and pyropheophytin and pyropheophorbide are formed, respectively (Figure 14.2).

B. Chemical Properties

1. Thermal Processing

Thermal processing can isomerize the C10 carbomethoxy groups (CO_2CH_3) of chlorophyll *a* and *b*, and produce chlorophyll *a′* and *b′*, respectively (11). The colors of epimers are the same as that of parent chlorophylls due to their identical spectral properties. Thermal processing enhances removal of Mg in the tetrapyrrole center in weakly acidic conditions forming the olive-brown colored pheophytins. Chlorophyll *b* is more stable to thermal processing than chlorophyll *a* due to the electron withdrawing effects of its C3 formyl group. Thermal degradation of chlorophylls in vegetable tissue is affected by pH. In alkaline condition (pH 9.0), chlorophyll is stable to

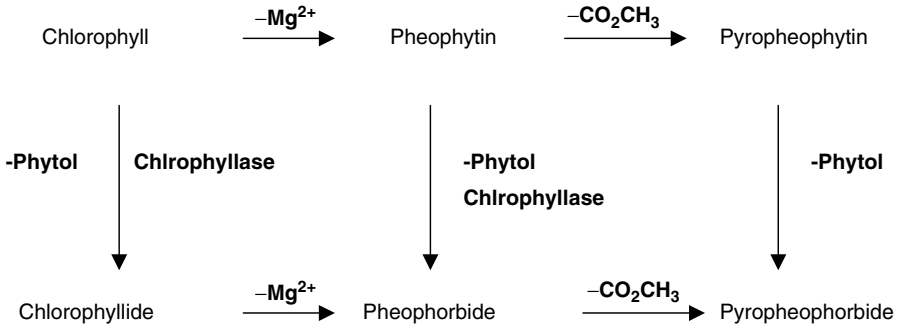


FIGURE 14.2 Reaction of chlorophylls and derivatives.

heat treatment while it is unstable in acidic condition (pH 3.0). Thermal processing for chlorophylls is sequential: Chlorophyll changes to pheophytin by first removing Mg and finally to pyropheophytin by loss of the C10 carbomethoxy groups (12). Canned products of vegetables contain mainly pheophytin and pyropheophytin, which are responsible for the olive-brown color in many canned vegetables (11, 12).

2. Allomerization

Chlorophylls in alcohol or other solvents exposed to oxygen can be oxidized and produce blue-green color compounds, which is called allomerization. The major allomerization products are 10-hydroxychlorophyll and 10-methoxylactones. The main allomerization product of chlorophyll *b* is 10-methoxylactones (8, 13).

3. Photodegradation

Chlorophylls in plant tissue are protected from degradation during photosynthesis when located within the plant matrix. However, when exposed to light chlorophylls in solution are light sensitive and degrade into colorless chlorophyll derivatives. It is believed that photodegradation of chlorophylls is due to the opening of tetrapyrrole rings and fragmentations of chlorophylls into small molecules (14). Chlorophylls are well-known photosensitizers, which can be excited by light absorption and generate many reactive oxygen species, including hydroxyl radicals and singlet oxygen. Singlet oxygen and hydroxyl radicals can react with the tetrapyrrole ring of chlorophylls and produce peroxides and more free radicals, which eventually degrade chlorophylls into colorless photodegradation products (7, 15).

4. Enzymatic Decomposition

Chlorophyllase is an esterase and can catalyze the removal of the phytol group from chlorophylls and pheophytins to form chlorophyllides and pheophorbies, respectively (Figure 14.2). Chlorophyllase can be active only on porphyrins with a C10 carbomethoxy group (CO_2CH_3) and hydrogens at the C7 and C8 positions (16). The activity of chlorophyllase is optimum at the temperature between 40°C and 82°C (7, 17) and some mild heat treatment can activate the chlorophyllase enzyme (18). Chlorophyllase in vegetable tissue loses its activity when vegetables are heated over 100°C.

C. Physical Properties

Chlorophyll is located in chloroplasts of green plants. Approximate chlorophyll contents of selected green vegetables are shown in Table 14.1. Due to the biological variability and differences among varieties, chlorophyll content in Table 14.1 should be used as a general guide (18). Chlorophylls can absorb visible light between 600 and 700 nm (red region) and between 400 and 500 nm (blue region). Depending on the presence of the metal atom in the tetrapyrrole center, the color of chlorophylls can change. Replacement of magnesium with Sn^{2+} and Fe^{3+} give a grayish-brown color while copper and zinc ions retain a green color (10). The stability of chlorophylls can be increased by substituting magnesium with copper. Copper chlorophyll complexes have better stability to oxygen, light, and alkaline conditions. Chlorophylls in leaves are broken down during senescence. Chlorophylls in fruits are often present in an unripe state such as in tomatoes and gradually disappear during ripening (19). In

TABLE 14.1
Approximate Chlorophyll Content in Selected Vegetables

		Chlorophyll Content (μg/g plant tissue)		
Vegetables		***a***	***b***	**Total**
Spinach	Fresh tissue	1380	440	1576
	Dry tissue	6980	2490	
	Canned-dry tissue	830 (Phe *a*) 4000 (Pyro *a*)		
Peas	Fresh tissue	106	12	
	Dry tissue	34 (Phe *a*) 33 (Pyro *a*)	13 (Phe *b*) 12 (Pyro *b*)	
Lettuce	Fresh tissue	334	62	
Kale	Fresh tissue	1370	464	1870
Celery	Leaves-fresh tissue	1143	225	
	Stalk—fresh tissue	29	7	
Asparagus	Fresh tissue	139	74	180–300
	Dry tissue	180 (Phe *a*) 110 (Pyro *a*)	51 (Phe *b*) 30 (Pyro *b*)	
Beans	Fresh tissue	54	17	
	Dry tissue		230–870	
	Canned-Dry tissue	340 (Phe *a*) 260 (Pyro *a*)	180 (Phe *b*) 95 (Pyro *b*)	

Chl = Chlorophyll, Phe = Pheophytin, Pyro = Pyropheophytin.
Adapted from Ferruzzi and Schwartz (18).

Europe, copper chlorophyll derivatives have been used as colorants in sugar confectionary, ice cream, dessert mixes, and cheeses with a combination of other colorants while in the U.S. the use of the derivatives is limited toothpaste and tooth powder (9).

III. CAROTENOIDS

Carotenoids are the most widely distributed pigments in nature with over 700 carotenoids identified. Carotenoids are located in the chloroplasts of plants and often masked by more dominant chlorophyll pigments. In humans, some carotenoids can act as precursors of vitamin A and have been associated with biological activities to inhibit specific chronic diseases (7, 20).

A. Structures of Pigment and Its Derivatives

Carotenoids are a group of tetraterpenoids. The basic carotenoid structural backbone consists of isoprenoid units formed either by head-to-tail or by tail-to-tail biosynthesis. There are primarily two classes of carotenoids: carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls contain oxygen in the form of hydroxyl, methoxyl, carboxyl, keto, or epoxy groups. Lutein in green leaves and zeaxanthin in corn are typical xanthophylls. The structures of carotenoids are acyclic, monocyclic, or bicyclic. For example, lycopene is acyclic, γ-carotene is monocyclic, and α- and β-carotenes are bicyclic carotenoids (4). Double bonds in carotenoids are conjugated forms and usually the all *trans* forms of carotenoids are found in plant tissues (Figure 14.3).

Carotenoids are distributed in a wide range of fruits and vegetables containing chlorophyll pigments. The most common carotenoid in plant tissue is β-carotene, which exhibits a yellow color. Tomatoes (lycopene), carrots (α- and β-carotenes), pumpkin (β-carotenes), corn (lutein and zeaxanthin), and sweet potatoes (β-carotenes) are other typical examples. Annatto, which is the seed of the tropical bush *Bixa orellana*, is in the class of carotenoids and has a yellow to orange color. The major coloring annatto pigment is *cis*-bixin used in dairy products such as cheese (7) (Figure 14.3).

B. Chemical Properties

1. Thermal Processing

Carotenoids are relatively stable during typical thermal processing. However, severe heat treatment can induce isomerization of carotenoids and even fragmentation of carotenoids into volatile compounds. Isomerization of carotenoids can be induced by heat, acid, or light.

2. Oxidation

Carotenoids are easily oxidized due to the presence of conjugated double bonds in molecules. In the case of β-carotene, epoxides and carbonyl compounds are first formed. Further oxidation results in the formation of short-chain mono- and deoxygenated compounds including epoxy-β-ionone. Extensive oxidation causes the loss of color in carotenoids (4, 7).

3. Enzyme Effects

Lipoxygenase indirectly accelerates the oxidation of carotenoids and loss of pigments. Lipoxygenase first catalyzes the formation of peroxides of unsaturated double bonds in fatty acids and the generated peroxides of fatty acids react with carotenoids. The coupled reactions are efficient enough to be used for measuring the activity of lipoxygenase in plant tissue (21).

C. Physical Properties

Red, yellow, and orange fruits, root crops, and vegetables are rich in carotenoids. The color of carotenoids ranges from yellow to red depending on the number of conjugated double bonds. Carotenoids show three distinct maxima in the visible spectrum (Table 14.2) (10). The absorption of light of carotenoids is between 430 and 480 nm. A minimum of 7 conjugated double bonds is needed to produce the characteristic yellow color. Currently β-carotene, β-apo-8′-carotenal, and canthaxanthin have been synthesized and used in food industry. Carotenoids from plant extracts and/or individual compounds have been used to impart a color to foods products, including margarine, ice cream, cheese products, beverages, and bakery products (22).

D. Nutritional and Functional Properties

1. Nutritional Effects

Carotenoids can serve as precursors of provitamin A. Provitamin A activity requires that the carotenoids possess a retinoid structure (β-ionone ring). β-Carotene possesses the highest provitamin A activity due to the two β-ionone rings in the structures (Figure 14.4). *Cis* forms of carotenoids have been reported to decrease provitamin A activity (23).

2. Antioxidant Effects

Carotenoids are known to serve as efficient singlet oxygen quenchers and free radical scavengers due to the many double bonds in the molecule. As the number of double bonds increases in carotenoids, the singlet oxygen quenching ability increases (20, 24). However, some carotenoids, such as β-carotene, may act as either an antioxidant or prooxidant depending on the conditions, especially oxygen concentration (20).

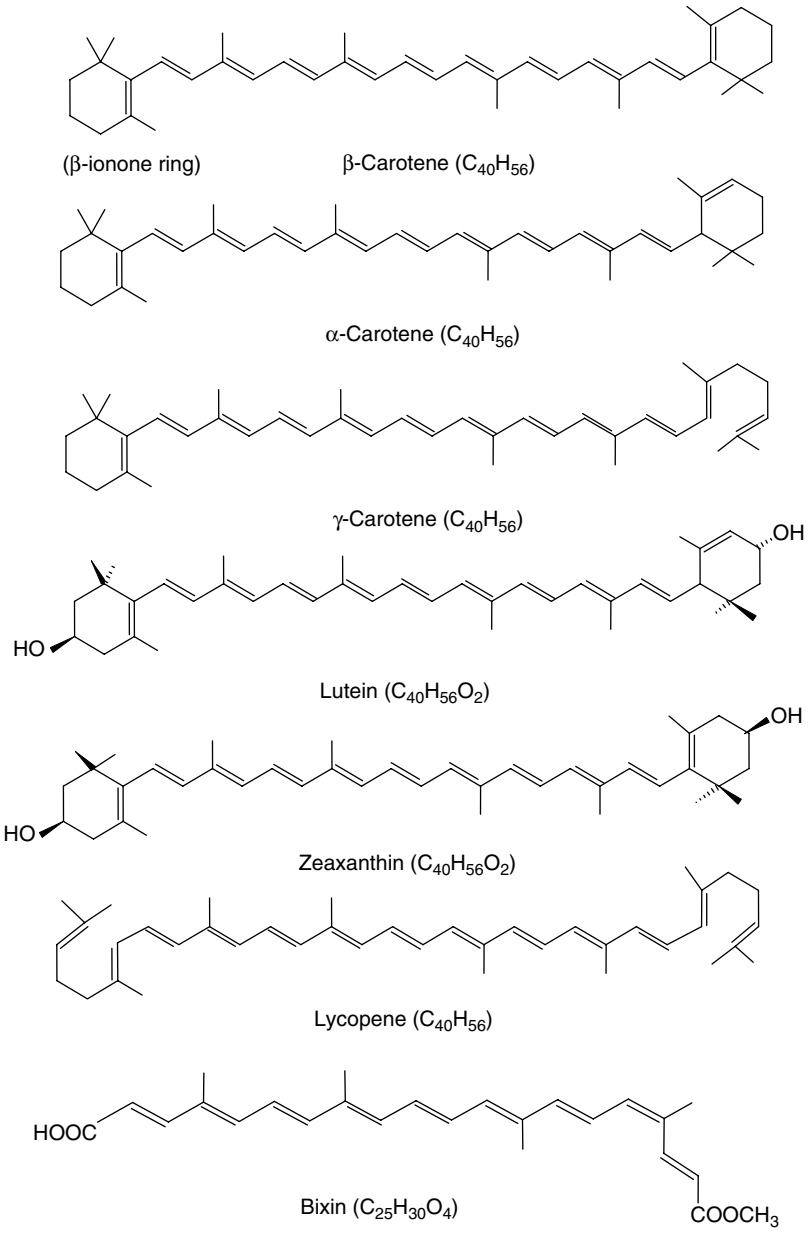


FIGURE 14.3 Structures of carotenoids.

TABLE 14.2
Absorption Wavelength Maxima for some Carotenoids

Compounds	Conjugated Double Bonds	Wavelength, nm (in petroleum ether)		
A. Effects of the number of conjugated double bonds				
Phytoene	3	275	285	296
Phytofluence	5	331	348	367
ξ-Carotene	7	378	400	425
Neurosporene	9	416	440	470
Lycopene	11	446	472	505
B. Effects of the ring structure				
γ-Carotene	11	431	462	495
β-Carotene	11	425	451	483

IV. FLAVONOIDS

The red and blue colors in flowers and fruits are due to the presence of flavonoid pigments especially anthocyanins. Other groups of flavonoids are responsible for the yellow or white color of flowers. Some brown or black colors in nature arise from either oxidation of flavonoids or chelation with metals (25). Flavonoids are polyphenolic compounds. Approximately 5,000 flavonoid compounds are reported in the literature (26).

A. ANTHOCYANINS

Anthocyanins are generally located in petal epidermal cells in the plant vacuoles (27). Anthocyanins give a wide

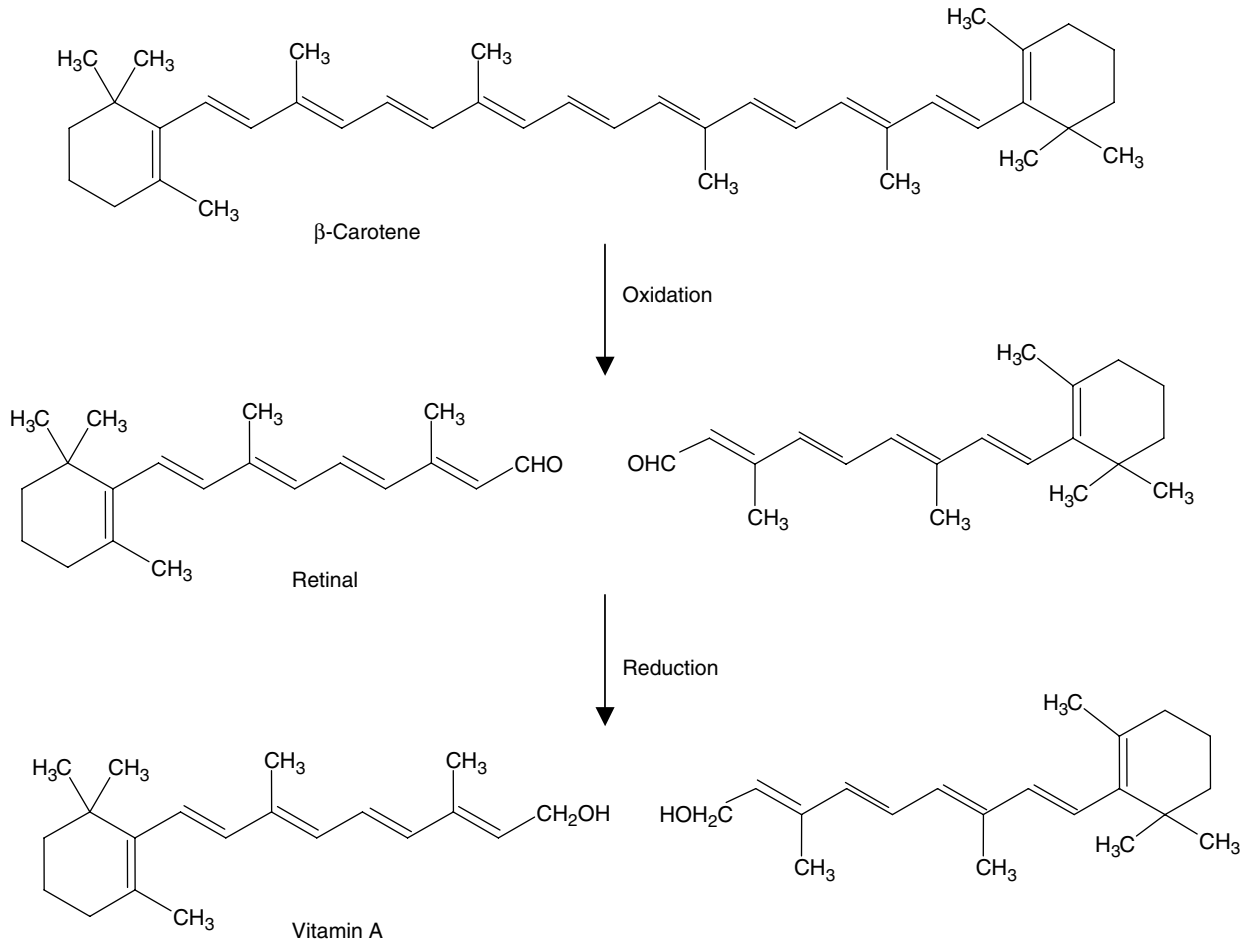


FIGURE 14.4 Formation of retinal and vitamin A from β-carotene.

range of colors with red, blue, purple, violet, magenta, and orange in many fruits and vegetables. The word anthocyanin comes from two Greek words: *anthos*, flower, and *kyanos*, blue. More than 300 anthocyanin pigments have been identified in nature (7, 26, 28).

1. Structures of Pigment and Its Derivatives

The chemical structures of anthocyanins and flavonoids are *O*-heterocyclic compounds (Figure 14.5). The basic carbon skeleton structure of anthocyanins is $C_6C_3C_6$. Anthocyanins are present in polyhydroxy and/or polymethoxy derivatives of salts. The types of anthocyanins differ depending on the number of hydroxy or methoxy groups, the type, number, and sites of bound glycosidic sugars, and the type and number of aliphatic or aromatic acids attached to the sugars (Figure 14.6). Some anthocyanins exist complexed with metals, such as Fe, Al, and Mg (28, 29).

Anthocyanins always exist as glycosides in nature. The aglycone of anthocyanin, a sugar free form, is known as anthocyanidins. Anthocyanidins are very unstable. Glycosidic substitution of anthocyanins increases the stability and water solubility of these pigments. Common glycosidic sugars in anthocyanins are one or two molecules of glucose, galactose, arabinose, xylose, and rhamnose. The sugar moiety is usually attached to the C3-position of the hydroxyl group. Increasing the number of sugar residues within the anthocyanin molecule tends to increase the stability. Acylation of the sugar residues in anthocyanins can also increase the stability of these pigments (26, 30). Acylated anthocyanins are highly colored above pH 4.0, while conventional anthocyanins are colorless above pH 4.0 (26). Acids found in acylated anthocyanins are cinnamic (*p*-coumaric, caffeic, ferulic) and aliphatic (malic, succinic, and acetic) acids. Only 6 anthocyanins are commonly found in foods out of known 22 aglycones, including pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Figure 14.6) (26). As the number of hydroxyl groups of anthocyanins increases, the blue color of anthocyanins increases (pelargonidin → cyanidin → delphinidin). As the number of methoxyl group or glycoside linkages within anthocyanins increases, the red color of anthocyanins increases (cyaniding → peonidin; pelargonidin → pelargonidin-3-glucoside) (10).

2. Chemical Properties

Anthocyanin pigments are relatively unstable and influenced mainly by pH and temperature. Other factors

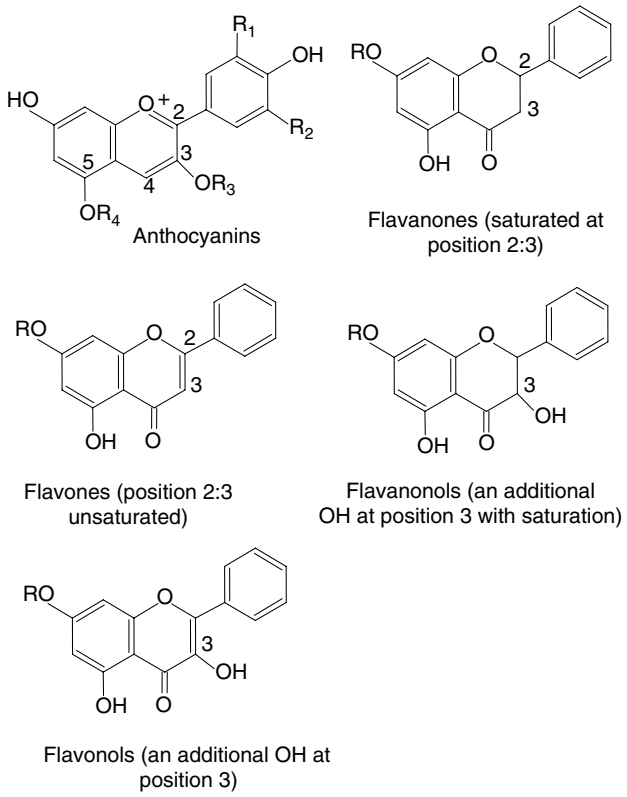


FIGURE 14.5 The chemical structures of flavonoids and anthocyanins. R_1 and R_2 = −H, -OH, or $–OCH_3$, R_3 = − glycosyl, R_4 = −glycosyl or –H.

influencing the stability of anthocyanin pigments are enzymes, oxygen concentration, ascorbic acid, sulfur dioxide, metal ions, and sugars.

The color stability of anthocyanins also depends on the substituents. Increased hydroxylation decreases stability, while methylation tends to increase pigment stability, which is due to the blocking of reactive hydroxyl groups.

a. pH effects

Color and structures of anthocyanin pigments in an aqueous medium changes depending on pH: the blue quinonoidal base, the red flavylium cation (R^+), the colorless carbinol base, and the colorless chalcone (Figure 14.7). Two compounds, red flavylium cation and colorless carbinol base, are important during pH changes from 1 to 6. At low pH, red flavylium cation dominates while at pH 4–6, the colorless carbinol base prevails. As the pH increases, the carbinol base increases and the color becomes weaker. The loss of color is due to a pH-dependent hydration at the C2 position of red flavylium cation.

$$R^+ + H_2O \leftrightarrows ROH + H^+$$

In alkali solutions (pH 8–10), highly colored ionized anhydro bases are formed. At pH 12, the fully ionized chalcones are the predominant pigments of anthocyanins. The major limitation of anthocyanin pigments as food colorants is the reduction of color intensity, changes in hue, and instability at upper pH (31, 32). However, anthocyanins in plants have vivid color and are quite stable within the plant matrix. The color of anthocyanins in plants may be stabilized through the self association of cationic forms, copigmentation with other flavonoids, formation of metal complexes with Fe, Al or Mg, and/or intramolecular interactions between acyl groups and phenolic groups (31).

b. Thermal processing

Thermal processing may easily degrade anthocyanin pigments. Thermal processing can change the equilibrium of the four types of anthocyanins toward a colorless chalcone derivative because all the reactions are heat sensitive (31).

Quinonoid ⇆ Flavylium ⇆ Carbinol base ⇆ Chalcone

c. Enzyme effects

Glycosidases and polyphenol oxidases can cause the loss of color from anthocyanin pigments. Glycosidases hydrolyze glycosidic linkages and produce sugars and aglycone. Anthocyanidins are less water soluble than their corresponding anthocyanins and can transform to colorless products. Polyphenol oxidase oxidizes anthocyanins in the presence of oxygen and *o*-diphenols. The enzyme first oxidizes *o*-diphenols into *o*-benzoquinone, which reacts with anthocyanins to form oxidized anthocyanins and degradation products (7, 29).

d. Other factors affecting stability of anthocyanin

Oxygen can react with double bonds of anthocyanin pigments resulting in loss of color. The color stability of anthocyanins in many fruit products, such as grape and cranberry juices, can be increased by removing oxygen using vacuum processing or adding nitrogen headspace (7, 32). It has been known that ascorbic acid and anthocyanin pigments disappear simultaneously in fruit juice. Oxidation of ascorbic acid produces hydrogen peroxide, which cleaves the pyrylium ring and produces colorless esters and coumarin derivatives (29).

Generally, light accelerates the decomposition of anthocyanin pigments especially in fruit juices and red wines. Anthocyanins with C5 hydroxyl groups are more susceptible to photodegradation than without C5 hydroxyl groups. In red wine, acylated and methylted diglycosides are more stable than nonacylated diglycosides, which are more stable than monoglycosides (26, 33, 34).

High concentrations of sugar can stabilize the anthocyanin pigments, which may be due to the lowering of water activity in the system. Water can react with flavylium cation at the C2 position and form colorless carbinol base. At low concentration of sugars, not only water but sugar itself can accelerate the degradation of anthocyanins. Brown pigments in fruit juices can be formed from anthocyanins and furfural or hydroxylmethylfurfural, which are Maillard browning reaction products (29).

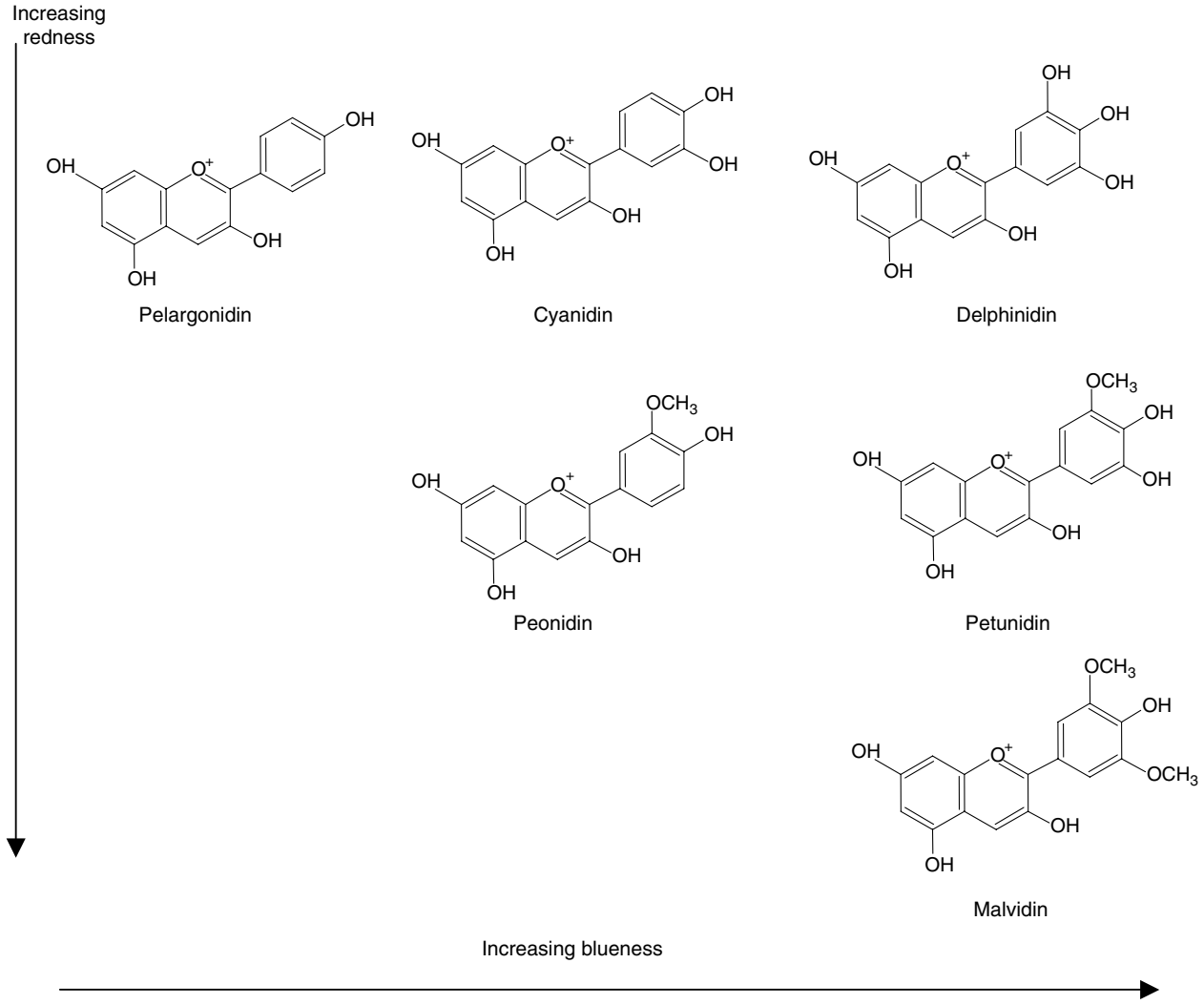


FIGURE 14.6 The most common anthocyanins in foods.

Metal complexes of anthocyanins are common and stabilize the color of anthocyanin-containing foods. Metals, including Ca, Fe, Al, and Sn, have protective effects on the anthocyanin stability in cranberry juices (29). However, a fruit discoloration, or "pinking" in pears and peaches, is believed to come from a metal anthocyanin complex.

Sulfur dioxide (SO_2), an antimicrobial agent in some fruit products, can react with anthocyanins at the C4 position and produce colorless pigments. This bleaching process can be reversible by desulfuring using thorough washing procedures before further processing.

The more concentrated anthocyanins show higher stability than less concentrated pigments (26). Copigmentation of anthocyanins with other polyphenolic compounds and metal ions can improve the stability and intensity of the color (29, 35).

3. Physical Properties

The color of anthocyanins and anthocyanidins arises from the excitation of many double bonds through absorbing light energy. Anthocyanidins have two absorption maxima: one in the visible light spectrum at 500–550 nm and a second in the ultraviolet spectrum at 280 nm. Anthocyanins in selected fruits, vegetables, and food products are shown in Table 14.3.

Currently, grapes and red cabbage are commercially available major anthocyanin sources (26). Fruit juices, vegetable juices, jellies, jams, ice cream, yogurts, canned candies, and bakery fillings are the main food products where anthocyanin pigments are applied. Europe, Asia, and South America use anthocyanin pigments to a less limited extent compared to the U.S. (28).

B. Other Flavonoids

Flavonoids include flavones, flavonols, flavanones, flavanonols, and anthocyanins, which are all based on a common structure (Figure 14.5). The flavones have a double bond between C2–C3, while flavanones are saturated at C2–C3. Flavonols possess an additional hydroxyl group at the C3 position and flavanonols are

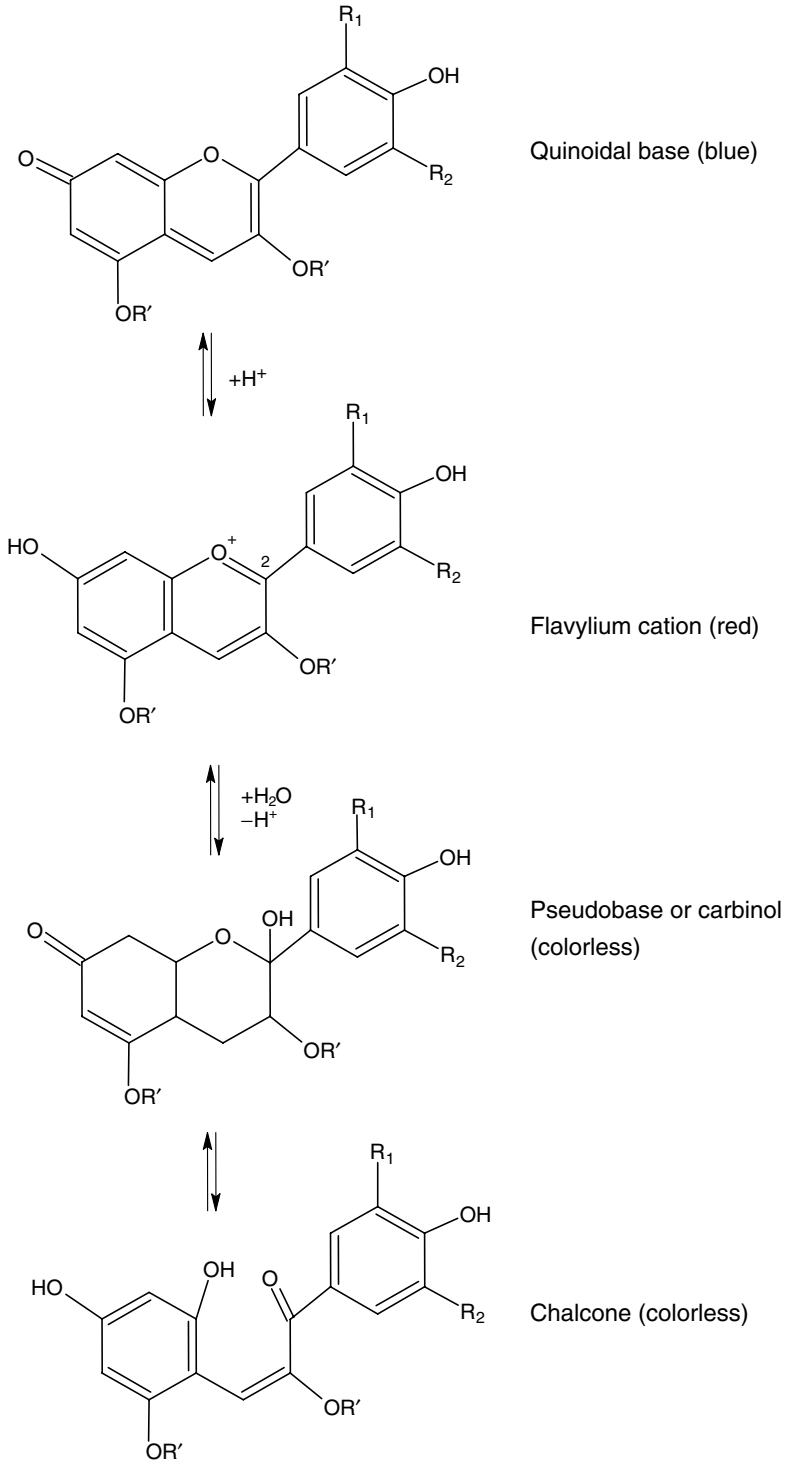


FIGURE 14.7 Structure changes of anthocyanins at 25°C with pH. R = sugar moiety.

saturated between C2 and C3 with hydroxyl group at the C3 position. Each flavonoid group is different depending on the number of hydroxyl, methoxyl, and other substituents on the two benzene rings. The most ubiquitous flavonoid is quercetin, 3, 5, 7, 3′, 4′-pentahydroxy flavone (4).

The spectral absorption of flavonoids occurs between 280 and 390 nm depending on the substituents and chelation with metals. Metals such as iron and aluminum increase the yellow color of flavonoids. Flavonoids play important roles in the formation of color in processed foods through metal chelation and copigmentation with other pigments (35).

Tannins are water-soluble polyphenolic compounds that can react with protein and other polymers such as polysaccharides. The color of tannins ranges from

TABLE 14.3
Anthocyanins in Selected Fruits, Vegetables, and Food Products

Fruit or Vegetable	Anthocyanins
Apple	Cy-3-gal, Cy-3-ara, Cy-7-ara
Pear	Cy-3-gal
Peach	Cy-3-glc
Plum/prune	Cy-3-gal, Cy-3-rut, Peo-3-glu, Peo-3-rut
Cherry	Cy-3-sop, Cy-3-gal, Cy-3-rut, Cy-3-glc, Cy-3-glc-rut
Raspberry	Cy-3-glc, Cy-3-glc-rut, Cy-3-rut, Cy-3-sop, Cy-3-glc-sop
Strawberry	Pg-3-glc, Pg-3-gal, Cy-3-glc
Grapes	Cy-3-glc, Del-3-glc, Peo-3-glc, Pet-3-glc, Mv-3-glc, Mv-3,5-diglc
Orange	Cy-3-glc, Del-3-glc
Cabbage(red)	Cy-3-glc
Banana	Pet-3-gly
Fig	Cy-3-gly
Wines	Cy-, Del-, Peo-, Pet-, and Mv-3-glc, and 3,5-diglc
	Cy-, Del-, Peo-, and Mv acyl derivatives
Potato	Pg-, Peo-, Pet-, and MV-3-rut-5-glc-coumaric esters and Pg, Cy-, Del- and Pet-3-rut

Cy=Cyanidin, Del=Delphinidin, Mv=Malvidin, Peo=Peonidin, Pet=Petunidin, Pg=Pelargonidin, ara=arabinoside, gal=galactoside, glc=glucoside, gly=glycoside, rut=rutinoside, sop=sophoroside.

yellowish-white to light brown, and tannin contributes to the astringency in foods. Proanthocyanidins, condensed tannins, are colorless compounds and can convert to colored compounds during food processing. Proanthocyanidins are present in cocoa beans, apples, pears, and other fruits (36).

V. BETALAINS

Betalains are found in beetroot and amaranth, and provide red and yellow colors to these plants. The red betalains are known as betacyanins and the yellow betalains are betaxanthins. Betalains are water-soluble and located in the vacuoles of plant cells (37, 38).

A. Structures of Pigment and its Derivatives

General structures of betalains, *N*-heterocyclic compounds, are the condensation of a primary or secondary amine with betalamic acid (Figure 14.8). Betacyanin pigments contain 1, 2, 4, 7, 7 pentasubstituted 1, 7-diazaheptamethin system and exhibit a light absorption maximum at 540 nm. Betaxanthins do not have 1, 2, 4, 7, 7 pentasubstituted 1, 7-diazaheptamethin system and the light absorption maximum is approximately 480 nm. Depending on the substituents, various betacyanins can be present such as betanidin, betanin, amaranthin, isobetanidin, isobetanin, and isoamaranthin. Isobetanidin, isobetanin, and isoamaranthin are the epimers of betanidin, betanin, amaranthin, respectively, at C15 positions (Figure 14.8). Betanin from red beet and amaranthin from amaranth are major sources of betalain pigments. Betaxanthins possess an amino acid substituents rather than the indole nucleus found in betacyanins (Figure 14.8). Indicaxanthin, vulgaxanthin-I, and vulgaxanthin-II are betaxanthins with proline, glutamine, and glutamic acid, respectively, as the amino acid substituent. Betacyanins can be converted into betaxanthins in the presence of excessive amino acids, which indicates that betacyanins and betaxanthins are structurally related.

B. Chemical Properties

1. Thermal Processing

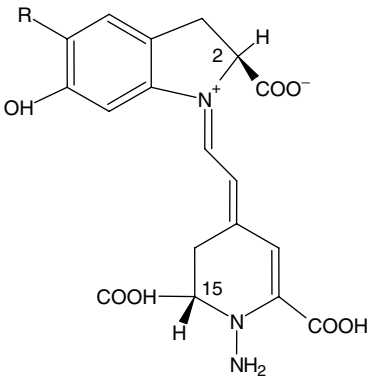
Betanin, the major pigment found in beetroot, degrades to betalamic acid and cyclopa-5-*O*-glucoside either under mild alkaline condition, heating of alkaline solutions, or during thermal processing (Figure 14.9). This reaction requires water, indicating that water activity affects the stability of betanin in food products. Low water activity helps to retain their pigments. This reaction is reversible during thermal processing and betanin can be regenerated through Schiff-base condensation between the aldehyde group of betalamic acid and amine of cyclopa-5-*O*-glucoside. Heat treatment increases the isomerization of betanin into isobetanin (38).

2. Other Factors Affecting Betalain Stability

Betalain can be oxidized in the presence of oxygen and degrades the color of its pigments. Light exposure also accelerates the oxidation of betalain. The presence of antioxidants, such as ascorbic acid and isoascorbic acid, can improve stability (38).

C. Physical Properties

Betalain solutions show a maximum light absorbance at 537–538 nm between pH 3.5 and 7.0. Below pH 3.5,

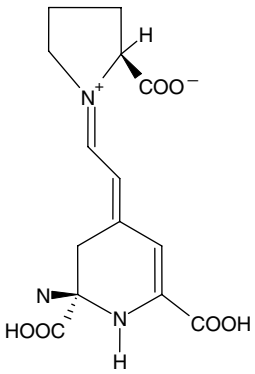


Betanidin, R = OH
Betanin, R = glucose
Amaranthin, R = 2′-glucuronic acid-glucose

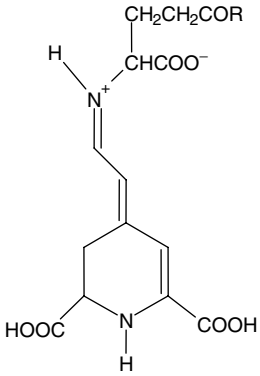
Epimers at C15 positions

Isobetanidin, R = OH
Isobetanin, R = glucose
Isoamaranthin, R = 2′-glucuronic acid-glucose

Betacyanins



Betaxanthins
(Indicaxanthin)



Betaxanthins

Vulgaxanthin-I, R = NH_2
Vulgaxanthin-II, R = OH

FIGURE 14.8 General structures of betalaines (betacyanins and betaxanthins).

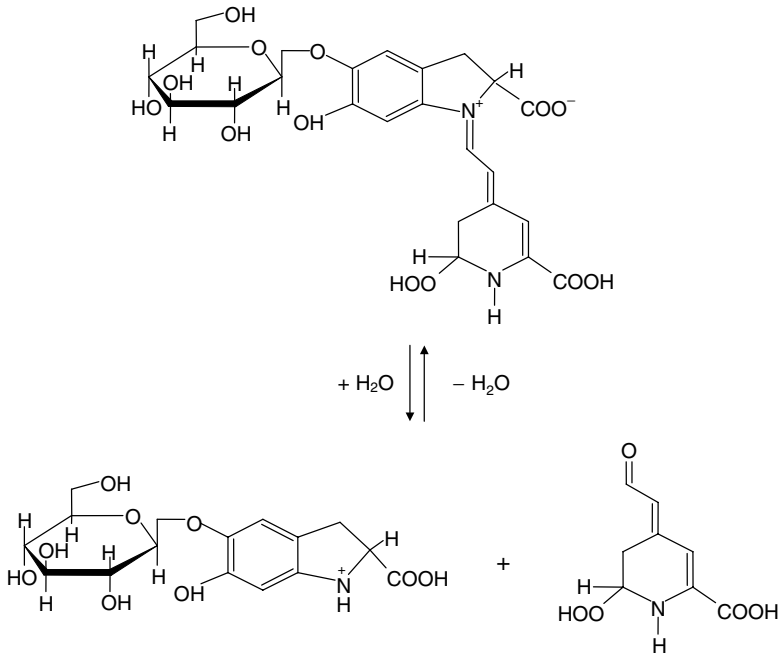


FIGURE 14.9 Betalain degradation mechanism.

maximum absorption of betalain shifts towards shorter wavelengths (535 nm at pH 2.0), while above 7.0, the maximum absorption shifts to longer wavelengths (544 nm at pH 9.0). The antioxidant effects of betalain on lipid peroxidation have been reported (39). Extracts of beetroot containing these pigments are used in foods, particularly confectionary products (29, 38).

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Part B

Food Categories

15 Carbonated Beverages

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CONTENTS

I. BACKGROUND INFORMATION

A. History of Soft Drinks

The first carbonated beverage, of sorts, was provided by nature, and dates back to antiquity, when the first carbonated natural mineral waters were discovered—although they weren't usually used for drinking. Instead, they were used for bathing by the ancient Greeks and Romans, owing to their purported therapeutic properties. It wasn't until thousands of years later, in 1767, that the British chemist, Joseph Priestley, was credited with noticing that the carbon dioxide he introduced into water gave a "pleasant and acidulated taste to the water in which it was dissolved" (1). The history of carbonated soft drinks (CSDs) is somewhat sparse during its early evolution, but most agree that development of CSDs is due, in large part, to pharmacists.

Today, carbonated beverages are primarily recognized for their refreshing and thirst-quenching properties. In the early to middle 1800s, however, it was these pharmacists who experimented with adding "gas carbonium," or carbon dioxide, to water, and supplementing its palatability with everything from birch bark to dandelions in the hopes of enhancing the curative properties of these carbonated beverages (2). "Soft drinks," a more colloquial yet very common name for carbonated beverages, distinguish themselves from "hard drinks," since they do not contain alcohol in their ingredient listing (3). This is in clear contrast to other beverages, such as distilled spirits, beer, or wine. These non-alcoholic, carbonated beverages are also called "pop" in some areas of the world, due to the characteristic noise made when the gaseous pressure within the bottle is released upon opening of the package (4). Figure 15.1 provides a brief illustration of the major milestones in the history of American soft drinks.

CSDs, "pop," soda—whatever the moniker given to these beverages, one thing is clear: they have been an important part of our popular culture for decades, and will continue to be for many years to come.

B. Soft Drink Facts and Figures

Few people consciously consider how something as ostensibly simple as "soda pop" can markedly affect the economy on several fronts. The National Soft Drink Association (NSDA), founded in 1919 as the American Bottlers of Carbonated Beverages (ABCB), today represents hundreds of beverage manufacturers, distributors, franchise

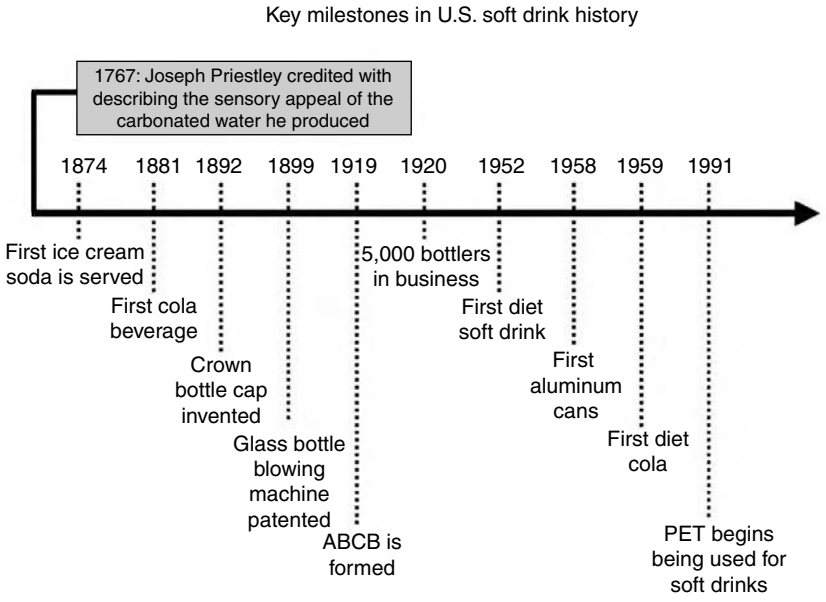

FIGURE 15.1 Key milestones in the U.S. beverage industry.

companies and support industries in the United States. According to the NSDA, Americans consumed nearly 53 gallons of carbonated soft drinks per person in 2002, and this translated into retail sales in excess of $61 billion. Nearly 500 bottlers operate across the United States, and provide more than 450 different soft drink varieties, at a production speed of up to 2,000 cans per minute on each operating line! Figure 15.2 summarizes the apportionment of total soft drink production in the year 2000.

Finally, as an industry, soft drink companies employ more than 183,000 people nationwide, pay more than $18 billion in state and local taxes annually, and contribute more than $230 million to charities each year. Few could argue that the soft drink industry has earned its place in the history of the American (and global) economy!

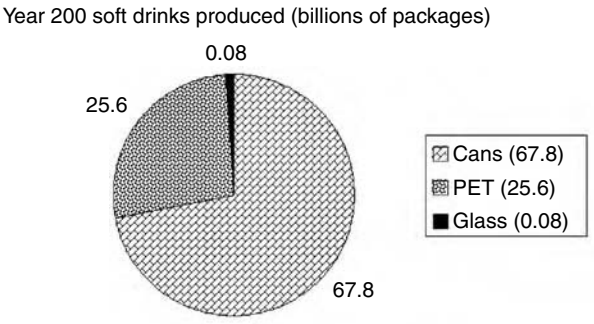

FIGURE 15.2 Distribution of cans, PET, and glass CSD packages.

C. Carbonation Science

Before discussing the process of manufacturing carbonated soft drinks, it is important to establish some fundamental chemical/physical concepts with regard to the carbonation process itself. Simply put, in the beverage industry, "carbonation" is the introduction of carbon dioxide gas into water, as depicted in Figure 15.3.

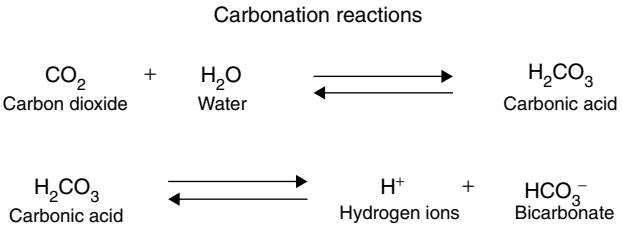

FIGURE 15.3 Carbonation reactions.

The favorable results of this simple combination are many: (1) the carbonation provides the characteristic "refreshing" quality for which carbonated beverages are most popular; (2) the dissolved carbon dioxide acts as both a bacteristat and a bactericide; and (3) the carbon dioxide dissociates in aqueous medium to form carbonic acid, which depresses the pH of the solution, thereby making the product even more protected from microbial harm (5). All in all, from a microbiologic perspective, carbonated soft drinks are innately very safe beverages.

Once the carbon dioxide is introduced into the water, which will ultimately join with flavors and sweeteners to form the complete beverage, the beverage technologist must understand how to measure and express the level of carbonation. The accepted convention in the beverage industry is *not* to measure carbon dioxide as a true concentration,

expressed in parts per million, or milligrams per liter. Instead, carbonation is expressed in units called "volumes." The concept is ultimately based on the physical gas laws of Henry, Boyle, and Charles, wherein pressure, temperature, and volume are closely interdependent. The colder the liquid, the more gas can be dissolved within it. Even within the industry, however, there is some confusion over what the exact definition of a "volume" is (6), usually arising from the temperature included in the definition. For our purposes, we will define one "volume" based on the Bunsen coefficient, described by Loomis as, "The volume of gas (reduced to 0°C and 760 mm) which, at the temperature of the experiment, is dissolved in one volume of the solvent when the partial pressure of the gas is 760 mm" (7). More informally, and to put this concept in perspective, consider a 10-ounce bottle of carbonated beverage, representing roughly 300 ml of liquid. If this carbonated beverage is prepared at one "gas volume," the package would contain approximately 300 cc of carbon dioxide. We would consider this very low carbonation from a sensory perspective, and would have a barely noticeable "fizz" upon removal of the closure. Imagine, however, for the same 300 ml of liquid, we carbonate to four gas volumes (a level typical of many products on the market today). This means that roughly 1200 cc of carbon dioxide has been introduced into the same 300 ml volume of liquid. More gas, into the same amount of liquid, and the same vessel size—imagine the increase in pressure contained within the bottle. This example explains why the characteristic "pop" of soda pop is heard when a bottle is uncapped, or a can is opened!

For the purposes of this text, the discussion of carbonation has been somewhat oversimplified, in order to make the concept more easily understood. As with any industry, the more one investigates any given topic, the more complicated and scientifically intense the subject usually becomes. Carbonation, for example, can be affected by a variety of factors, including other solids present in the liquid being carbonated, temperatures of the gas and the liquid, atmospheric pressure/altitude, and how far carbon dioxide varies from ideal gas behavior (8). These are cited merely for consideration, but are outside the scope of this chapter.

D. Process Overview

The process of manufacturing carbonated beverages has remained fundamentally the same for the last several decades. Certainly, new equipment has allowed faster filling speeds, more accurate and consistent fill heights, more efficient gas transfer during carbonation, and other improvements, but the process remains one of cooling water, carbonating it, adding flavor and sweeteners, and packaging it in a sealed container. Figure 15.4 illustrates the overall process in somewhat more detail, as we continue to build upon the basic foundation we will be discussing throughout this chapter. As we proceed, the figures depicted will become more complete, as each critical process to carbonated beverage manufacture is explained.

Carbonated beverage production begins with careful measurement of the formula quantities of each component into the syrup blending tank. Critical components include the concentrate, which contains the bulk of the flavor system; the sweetener, which typically includes the nutritive sweeteners high-fructose syrup or sucrose (in the case of

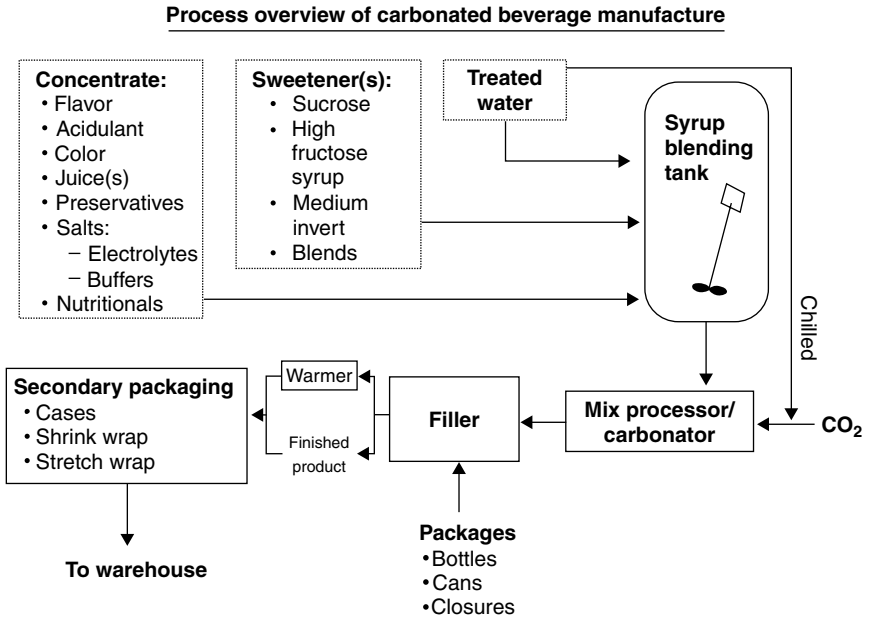

FIGURE 15.4 Process overview of carbonated beverage manufacture.

diet beverages, these are replaced with one of the high-potency sweeteners available); and water, which generally begins as municipal drinking water, and is further purified within the beverage plant. These are then blended to ensure homogeneity of the batch according to carefully prescribed standard operating procedures.

Once blending in the syrup tank is complete, the "finished syrup" is tested for correct assembly, then pumped to the mix processor, where the syrup is diluted to finished beverage level with chilled, carbonated, treated water (often a 1:6 dilution of syrup:treated water, although this varies by product). After this, the now carbonated beverage-level solution proceeds to the filler, where it is fed (usually volumetrically, by gravity) into bottles or cans, then sealed (capped in the case of bottles, seamed in the case of cans). Then, the finished product is either passed through a warmer, in order to avoid excessive condensation from forming (depending on the type of secondary packaging used), or sent directly to secondary packaging. This can include plastic or cardboard cases, shrink wrap, stretch wrap, or even more innovative devices. After packaging, the product is palletized, and stored in the warehouse until it is ready for distribution.

II. RAW MATERIALS PREPARATION

A. Concentrate

In the carbonated soft drink industry, "concentrate" is the name given to a mixture of many different categories of ingredients, illustrated in Figure 15.5.

The most notable of these, and, indeed, the topic of many urban legends surrounding its utter secrecy, is the flavor component. This is where the proprietary formulations of essential oils are found, which combine to form the characteristic flavor of the trademark beverage. Flavor components can include a single, "primary" component, or be distributed in various ways among multiple components—for example, a high-potency sweetener supplied as a dry salt as part of a secondary flavor component. In general, the majority of flavor systems include primary flavor components, and these fall into three broad categories: (1) simple mixtures, (2) extracts, or (3) emulsions.

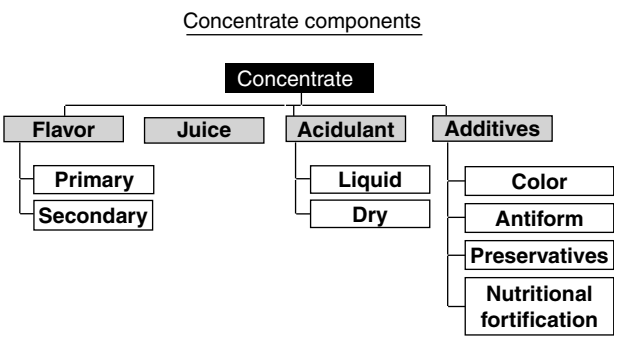

FIGURE 15.5 Concentrate components.

Simple mixtures. These are perhaps the simplest of the flavor categories to understand, but they also represent the minority of those in existence. Here, a combination of miscible liquids, or easily soluble solids, are blended together to form a homogenous aqueous mixture. Because so many essential flavor oils are not readily water soluble, the beverage technologist must abandon the idea of the simple mixture for one of the other, more flexible categories of flavors.

Extracts. As the name implies, this category of flavors involves extracting the desired flavor constituents from essential oils. Simply put, the extraction solvent—usually ethanol (although sometimes propylene glycol is used)—is used to partition those flavor constituents which are soluble in the solvent, but not freely soluble in the water directly. In this way, these flavor compounds become fully dissolved in the ethanol first. Then, this ethanolic "extract" (which is, in effect, an ethanolic solution of the flavor compounds) is added to water. Since ethanol is freely miscible with water, it acts as a carrier vehicle to help dissolve or disperse the otherwise water-insoluble flavor constituents (9). Today, equipment for both batch and continuous liquid extraction of flavor oils is available, and more novel approaches have also been developed (for example, gas extraction, super-critical fluid extraction, and other patented processes).

Emulsions. This third category is likely the largest, encompassing the bulk of the flavor systems available today. In the carbonated beverage industry, oil-in-water (or o/w) emulsions are the standard. This model involves an oil (lipophilic) internal phase, and an aqueous (hydrophilic) external phase, being made "compatible" by the use of a surfactant, or emulsifier. Surfactants are compounds that are amphiphilic; that is, there are both hydrophilic and lipophilic portions of the same molecule! This facilitates a decrease in the surface tension when oil and water are mixed together, and also allows the lipophilic portion to align with the oil, and the hydrophilic portion to align with the water (10). In so doing, the emulsifier forms a bridge, of sorts, between the two phases, and allows them to be dispersed, without gross separation, for the desired length of time (generally at least as long as the technical shelf life of the beverage).

Since carbonated beverages are of low pH, owing in part to the carbonic acid from the dissolved carbon dioxide, but also from the acid components of the formulae, acid hydrolysis is one of the major concerns to the beverage flavor developer. By positioning itself between the oil and water phases, the emulsifier protects the sensitive flavor oils from chemical degradation in this acidic environment. In addition, the emulsifier protects the flavors oils from oxidation from the naturally dissolved oxygen in the water which constitutes the aqueous phase. So, a well-designed

and prepared emulsion can dramatically extend the sensory shelf-life of the flavor system, and the overall physical stability of the beverage.

In addition to the flavors, Figure 15.5 also depicts a variety of other components which may be part of the concentrate. These include juices, which must be handled and stored carefully in order to preserve their quality, acidulants (both liquid and dry), and a host of other additives, depending on their desired function (for example, antifoam, preservative, nutrients, etc.).

B. WATER

Water is the major component in carbonated beverages, and represents anywhere from 85 to near 100% of the finished product. Interestingly, it is unlike any other ingredient, since we rarely have the number of options for water supply that we have with other raw materials! Obviously, then, particular diligence must be employed when selecting a water supply. Beverage plants use water from ground supplies, surface supplies, or both. Ground supplies include springs, deep and shallow wells, and artesian aquifers. Surface supplies include rivers, lakes, streams, and reservoirs. Within these sources, there is wide variation in type and content of inorganic (for example, metals, minerals, sulfate, chloride, nitrate), organic (for example, volatile organics, natural organic matter), microbiologic (bacteria, viruses, protozoa), and radiologics (radionuclides, alpha- and beta-activity). Table 15.1 provides a relative comparison of some characteristics of ground and surface supplies (11).

One critical point of which to be aware is that municipal treatment plants should not normally be depended upon to consistently supply water suitable for the needs of most carbonated beverage manufacturers. While the municipality treats the water so that it is safe to drink, and aesthetically pleasing to the consumer (potable and palatable), they cannot afford to consider the needs of all industrial end users, so they may not consistently supply a water of the high quality needed for producing our finished product and ensuring the beverage a long shelf life. There is also the possibility of contamination of the city water as it passes through the distribution system from the municipal treating plant to the beverage plant. This is particularly true with respect to organic matter and metal content, such as iron. The quality of the water used for carbonated soft drinks must be considered from several perspectives:

(1) *Regulatory Compliance*: The water used must be in compliance with all presiding local and national laws and guidelines. This jurisdiction is generally clear in the United States, between the Environmental Protection Agency and the Food and Drug Administration. However, as you consider the international beverage locations, the regulatory picture sometimes becomes more cloudy.

(2) *Beverage Stability*: Intuitively, as the major ingredient in carbonated soft drinks, the constituents in water can have a profound impact on the overall quality and shelf life of our beverage products. For example, if alkalinity is not

TABLE 15.1
Comparison of Ground and Surface Water Supplies

Parameter	Ground Water	Surface Water
Total dissolved solids	Higher	Lower
Suspended solids	Lower	Higher
Turbidity and color	Lower	Higher
Alkalinity	Higher	Lower
Total organic carbon	Lower	Higher
Microbiology		
Protection from bacteria and viruses	Highly protected	Highly susceptible
Protection from protozoa	Almost completely protected	Highly susceptible
Presence of iron and/or manganese bacteria	Common	Rare
Hydrogen sulfide gas	Common	Uncommon
Aeration/dissolved oxygen	Lower	Higher
Temperature	More consistent	More variable
Flow rate	Very slow (1 m/day)	Very fast (1 m/sec)
Flow pattern	Laminar	Turbulent
Susceptibility to pollution through surface run-off	Low	High
Time for a contaminant plume to resolve	Very long—often decades, potentially centuries	Usually short—days/months; sometimes years

Source: Bena, D. 2003. Water Use in the Beverage Industry, in Food Plant Sanitation (YH Hui, ed.). Marcel Dekker, New York.

controlled, the acidic profile of the beverage formulae will be compromised, making the beverage more susceptible to microbial growth and spoilage.

(3) *Sensory*: Many contaminants, even at levels within drinking water standards, may adversely affect the finished beverage. For example, some algae produce compounds (geosmin and methyl isoborneol) which are sensory active at levels as low as nanograms per liter (12). These can result in "dirty, musty" flavor and aroma in finished products.

(4) *Plant Operations*: Water for non-product (auxiliary) uses must also meet the performance standards of the carbonated soft drink producer. These standards and guidelines are usually enacted to prevent corrosion (for example, from high chloride content in heat exchangers) and scaling (for example, from hardness salts in boilers), which may result in premature equipment failure and/or loss of operational efficiency.

Whether the beverage plant has its own well, or the water supply comes from a modern municipal treatment plant, each individual water supply presents its own particular problems. In most, if not all cases, the incoming raw water which supplies a beverage plant already meets the applicable standards for potability of drinking water. The beverage producer then further purifies the water to meet the quality necessary for its products. This treatment can take many forms, but the three largest categories of in-plant beverage water treatment are (1) conventional lime treatment systems (CLTS), (2) membrane systems (including reverse osmosis, nanofiltration, and ultrafiltration), and (3) ion-exchange. Volumes have been written about each treatment modality, and a detailed discussion is beyond the focus of this chapter. However, a brief summary of each treatment category is provided below (13).

(1) Conventional lime treatment systems (CLTS). This treatment chain represents the majority of most beverage treatment armadas worldwide, although the balance is quickly shifting in favor of membrane technologies. CLTS involves the addition of a coagulant (as an iron or aluminum salt), hydrated lime (for pH control), and chlorine (for oxidation and disinfection) to a reaction tank. The agitation is gently controlled over the course of a two-hour retention time, during which a floc begins to form, grow, and settle, bringing contaminants with it to the bottom of the tank, where they await discharge. Figure 15.6 illustrates what happens in this reaction vessel.

Historically, and as little as twenty-five years ago, conventional lime treatment was regarded as the "ideal" treatment for raw water of virtually any quality. Indeed, this system, coupled with the required support technology—fine sand filtration, granular activated carbon, polishing filtration, and ultraviolet irradiation—does address a broad range of water contaminants. The advantages and

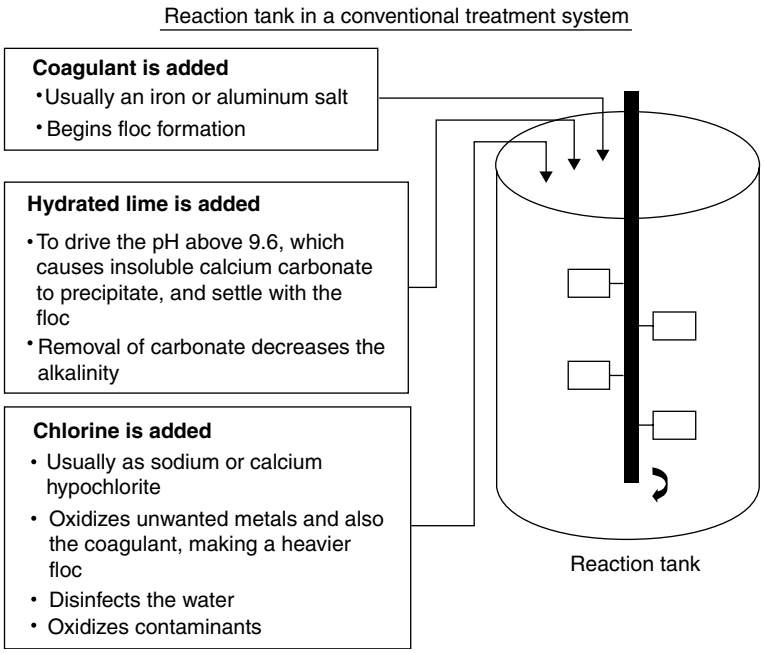

FIGURE 15.6 Reaction tank in a conventional lime treatment system.

disadvantages of conventional lime treatment are summarized in Table 15.2.

(2) Membrane technology. Clearly, this has seen the most growth in recent years with the advent of more resistant membrane materials of construction and more flexible rejection characteristics. Included in this category is the prototype of the cross-flow, polymeric membrane filtration systems—reverse osmosis, along with nanofiltration and ultrafiltration (both polymeric and ceramic). By carefully controlling the membrane pore size during manufacture, and the applied pressure during operation, reverse osmosis membranes can effectively remove in excess of 99% of many dissolved species—down to the ionic level (for example, dissolved calcium or sulfate). Table 15.3 illustrates the relative capabilities of the three major membrane processes with regard to a variety of constituents possible in the incoming water (14).

Since reverse osmosis is often the cited "membrane standard" against which the performance of others are judged, the advantages and disadvantages of reverse osmosis are listed in Table 15.4.

Also worth mentioning, though not discussed, among this group are the "hybrid" technologies, which include novel membrane and ion-exchange utilization. Examples are electrodialysis technology for removal of ionic species in water, and continuous electrodeionization.

(3) Ion-exchange. This technology is routinely utilized for partial or complete demineralization of the water supply, softening, or dealkalization, or it can be customized for selective removal of a specific contaminant (for example, denitratization). In simplest terms, ion-exchange involves using a selective resin to exchange a less desirable ion with a more desirable ion. Of course, a great deal of chemical research goes into the development of these selective resin materials, but the functional outcome remains

TABLE 15.2
Advantages and Disadvantages of CLTS

Advantages	Disadvantages
Removes alkalinity and hardness	Does not effectively reduce nitrate, sulfate, or chloride concentration
Removes organic debris, particulates, and natural organic matter (NOM)	Sludge formation and disposal requirements
Reduces metal concentrations (iron, manganese, arsenic, others) and some radionuclides	May promote the formation of disinfection by-products (trihalomethanes) under certain conditions
Reduces some color compounds (tannins), off-tastes, and off-odors	Often difficult to operate consistently in waters with very low dissolved solids
Reduces bacteria, virus, and protozoan populations	Relatively large space requirements on plant floor ("footprint")

Source: Bena, D. 2003. Water Use in the Beverage Industry, in Food Plant Sanitation (YH Hui, ed.). Marcel Dekker, New York.

TABLE 15.3
Relative Comparison of Reverse Osmosis, Nanofiltration, and Ultrafiltration

Component	Reverse Osmosis	Nano-Filtration	Ultrafiltration
Alkalinity	95 to 98%	50 to 70%	None
TDS	95 to 98%	50 to 70%	None
Particulates	Nearly 100%	Nearly 100%	Nearly 100%
Organic matter	Most > 100 MW	Most > 200 MW	Some > 2000 MW
THM precursors	90 + %	90 + %	30 to 60%
Sodium	90 to 99%	35 to 75%	None
Chloride	90 to 99%	35 to 60%	None
Hardness	90 to 99%	50 to 95 + %	None
Sulfate	90 to 99%	70 to 95 + %	None
Nitrate	90 to 95%	20 to 35%	None
Protozoa	Near 100%	Near 100%	Near 100%
Bacteria	Near 100%	Near 100%	Near 100%
Viruses	Near 100%	Near 100%	Near 100%
Operating pressure	200 to 450 psi	100 to 200 psi	80 to 150 psi

Approximate removal percentages. Actual performance is system-specific.

Source: Adapted from Brittan, PJ. Integrating Conventional and Membrane Water Treating Systems. International Society of Beverage Technologists Short Course for Beverage Production, Florida, 1997.

TABLE 15.4
Advantages and Disadvantages of Reverse Osmosis

Advantages	Disadvantages
Removes nearly all suspended material, and greater than 99% of dissolved salts in full-flow operation	Pretreatment must be carefully considered, and typically involves operating costs for chemicals (acid, antiscalant, chlorine removal)
Significantly reduces microbial load (viruses, bacteria, and protozoans)	Does not produce a commercially sterile water
Removes nearly all natural organic matter (NOM)	Membranes still represent a substantial portion of the capital cost, and may typically last 3–5 years
May be designed as a fully automated system with little maintenance	Low solids water may be aggressive toward piping and equipment, so this must be considered for downstream operations
Relatively small space requirements on the plant floor ("footprint")	High pressure inlet pump is required

Source: Bena, D. 2003. Water Use in the Beverage Industry, in Food Plant Sanitation (YH Hui, ed.). Marcel Dekker, New York.

straightforward. For example, softening resins are often employed to remove hardness (calcium and magnesium) from the water entering boilers and heat exchangers. In this application, the hardness ions are not wanted. The softening resin (for example, a sodium zeolite clay) is charged with active and replaceable sodium ions. When the "hard" water passes across the softening bed, the resin has a selectivity for calcium and magnesium, so it replaces them for sodium. The result is that the water exiting the softener is virtually free of calcium and magnesium (since they were replaced by sodium), and is safe to use in boilers and other equipment, since it will no longer have the tendency to form scale.

To supplement the major treatment systems mentioned above, the carbonated beverage producer often utilizes a host of other "support technologies," including activated carbon filtration (to remove organic contaminants and chlorine), sand filtration (to remove particulates), and primary and secondary disinfection (using chlorine, ozone, ultraviolet, heat, or a combination). By the time the treated water is finished, it is microbially and chemically safe, clear, colorless, and ready to be used for syrup and beverage production.

C. Sweeteners

The two major categories of sweetener types are nutritive (that is, they provide some caloric value) and "high potency" (that is, the type used in diet beverages, since they are many times sweeter than sucrose, and generally non-caloric). There are several high potency sweeteners available to the worldwide beverage developer (aspartame, acesulfame potassium, and others), and they are almost exclusively, if not always, included as part of the "concentrate" flavor system as a dry substance package. As such, their quality can be more easily controlled by the vendor, as with any of the other concentrate ingredients, and minimal intervention is needed at the carbonated soft drink manufacturing facility. These high potency sweeteners, therefore, will not be addressed in this chapter. A concise treatise on the topic, however, is provided by the International Society of Beverage Technologists (15).

Next to water, however, the nutritive sweeteners represent the second most prevalent ingredient in the finished beverage. The most common nutritive sweeteners used in the carbonated soft drink industry are sucrose and high fructose syrups, with sucrose (from cane or beet) being the most common internationally. Within the United States, nearly all of the nutritive sweetener used in carbonated beverages is high fructose corn syrup (HFCS, either 42 or 55%). In 1996, the U.S. corn refining industry produced over 21 billion pounds of high fructose corn syrups, representing only about 12% of the total corn crop (16).

Although high fructose syrups may be obtained from other starting materials, like wheat or tapioca starch, corn remains the most prevalent starting material. A starch slurry is first digested by the addition of alpha-amylase enzyme, which results in gelatinization and ultimate dextrinization of the starting starch. Then, glucoamylase enzyme is added to result in an enriched glucose syrup (95% glucose). After this the glucose syrup is purified via particle filtration, activated carbon adsorption, and both cation and anion exchange. Then, evaporation brings the solids content within range for effective passage through the isomerization column containing the glucose isomerase enzyme. This enzyme converts much of the 95% glucose syrup to fructose, which is again purified as before and evaporated. The result is HFCS-55 of high quality. In some formulae and/or markets, HFCS-42 is used, which is simply a blend of the HFCS-55 with the 95% glucose stream to result in a product which is 42% fructose. The generic process by which corn starch is transformed to high fructose corn syrup is illustrated in Figure 15.7 (17).

In general, HFCS-55 (55% fructose) is a highly pure ingredient, due, in large part, to the activated carbon, cation, and anion exchange steps required of the process. However, the most recent research highlights the occurrence of potent sensory-active compounds which could form via chemical or microbial pathways in HFCS,

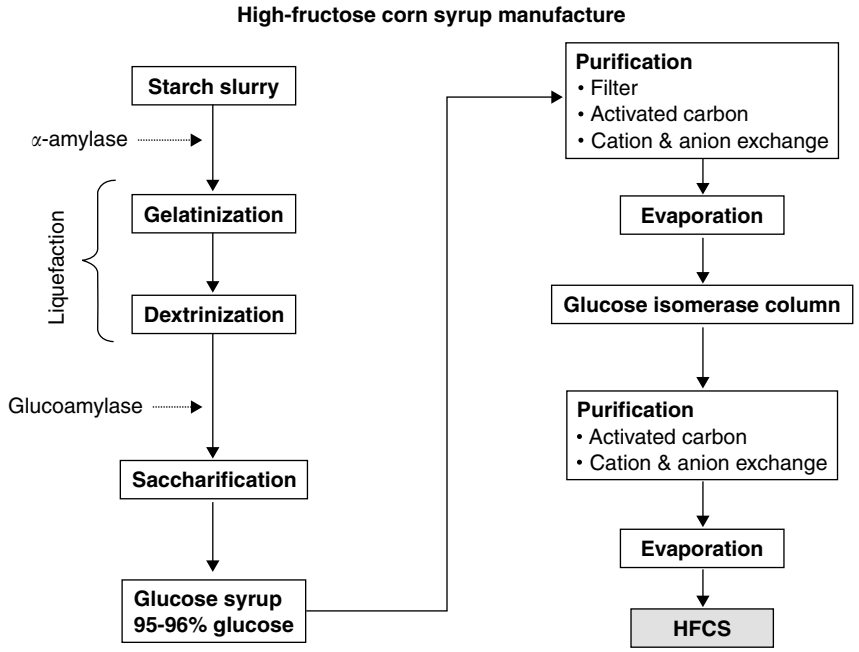

FIGURE 15.7 High fructose corn syrup manufacture.

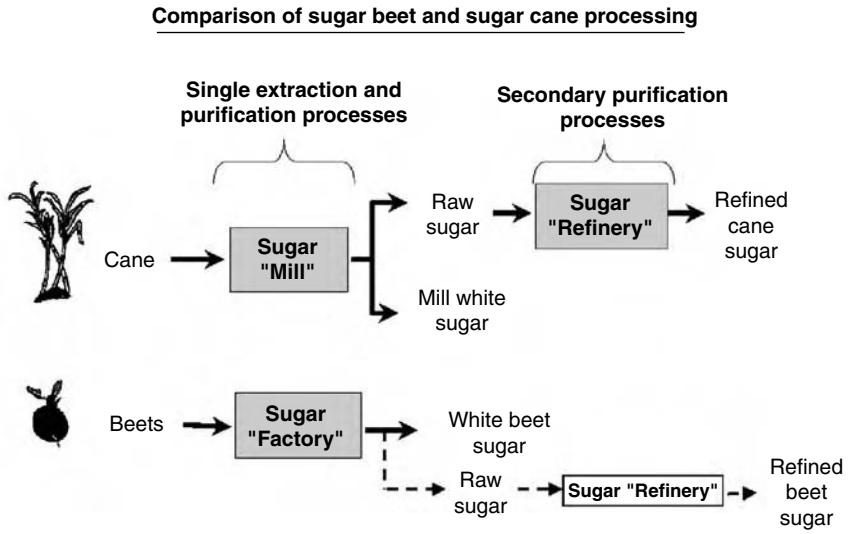

FIGURE 15.8 Cane vs. beet sugar process flow.

including isovaleraldehyde, 2-amino acetophenone, and maltol (18). When properly produced and stored, no additional treatment is necessary at the beverage plant.

Sucrose, though the clear exception in the North American beverage industry, continues to be the mainstay for international beverage markets. It may be obtained from sugar cane or sugar beet, following two distinct separation and purification schemes, as depicted in Figure 15.8 (19).

The three indicators of sucrose quality generally recognized by the sugar industry are color, ash, and turbidity. Internationally, depending on the quality of the available sucrose, it is not uncommon to subject the incoming granular or liquid sucrose to additional treatment at the beverage plant. Ash, or residual inorganic minerals, remains difficult to adequately treat at the carbonated soft drink plant, so great effort is made to source sucrose with an acceptable ash content (as defined by the individual company specifications). Turbidity is easily remedied at the beverage plant via an in-line filtration step, often incorporating diatomaceous earth as a filter aid. Color, considered by some as the primary indicator of sucrose quality, is also able to be treated at the beverage plant, but typically

requires hot treatment through activated carbon. This removes color and many sensory-active compounds, and also serves to render the sucrose free of most viable microorganisms. Figure 15.9 (19) briefly summarizes the handling and treatment of sucrose at the carbonated soft drink facility.

Liquid sucrose, usually commercially available at 67 Brix concentration (67 Brix is equivalent to 67% sucrose, by weight), is sometimes used for the production of carbonated soft drinks. Two distinct disadvantages of using liquid sucrose instead of granulated sucrose include the following: (1) the end user ultimately pays for shipping 33% water, since the ingredient is only 67% sucrose solids, as compared to granulated sucrose, which is 100% sucrose solids, and (2) this water also means that the liquid has a higher water activity than granulated sucrose, making it much more susceptible to microbial spoilage. With liquid sucrose operations, absolutely diligent transport and handling procedures are imperative.

The last, less common type of nutritive sweetener used in this industry is medium invert sugar. Chemically, this product has similarities to both sucrose and high fructose corn syrup. With medium invert sugar, or MIS, the starting material is liquid sucrose, which is then treated with one of three processes: (1) heat and acid, (2) ion-exchange, or (3) invertase enzyme. The end result of any of these processes is that roughly 50% of the starting sucrose is transformed into "invert sugar," an equimolar mixture of glucose and fructose. At this point, the inversion process is stopped, and the final commercial product contains 50% sucrose, 25% glucose, and 25% fructose. This gained favor over liquid sucrose in the beverage industry for two main reasons: (1) the finished material is 76 Brix, vs. 67 Brix for liquid sucrose, so less water is shipped, and (2) MIS has a much lower water activity, and is, therefore, much more microbiologically stable.

In summary, the producers of carbonated soft drinks have several options at their disposal for providing the sweetness to the consumer, which is so characteristic of these products. Internationally, sucrose is the major sweetener used, while in the United States, high fructose corn syrup is preferred. Irrespective of the type of sweetener, the beverage industry has treatment methods at its disposal to ensure that this ingredient consistently meets the high standards of chemical and microbial quality necessary to be used in the production of syrup and beverage.

D. Carbon Dioxide

At normal temperatures and pressures, carbon dioxide is a colorless gas, with a slightly pungent odor at high concentrations. When compressed and cooled to the proper temperature, the gas turns into a liquid. The liquid in turn can be converted into solid dry ice. The dry ice, on absorbing heat, returns to its natural gaseous state.

We learned a little of the history of carbonation earlier in this chapter, since the concept is so critical to the production of carbonated soft drinks. Just as critical is the

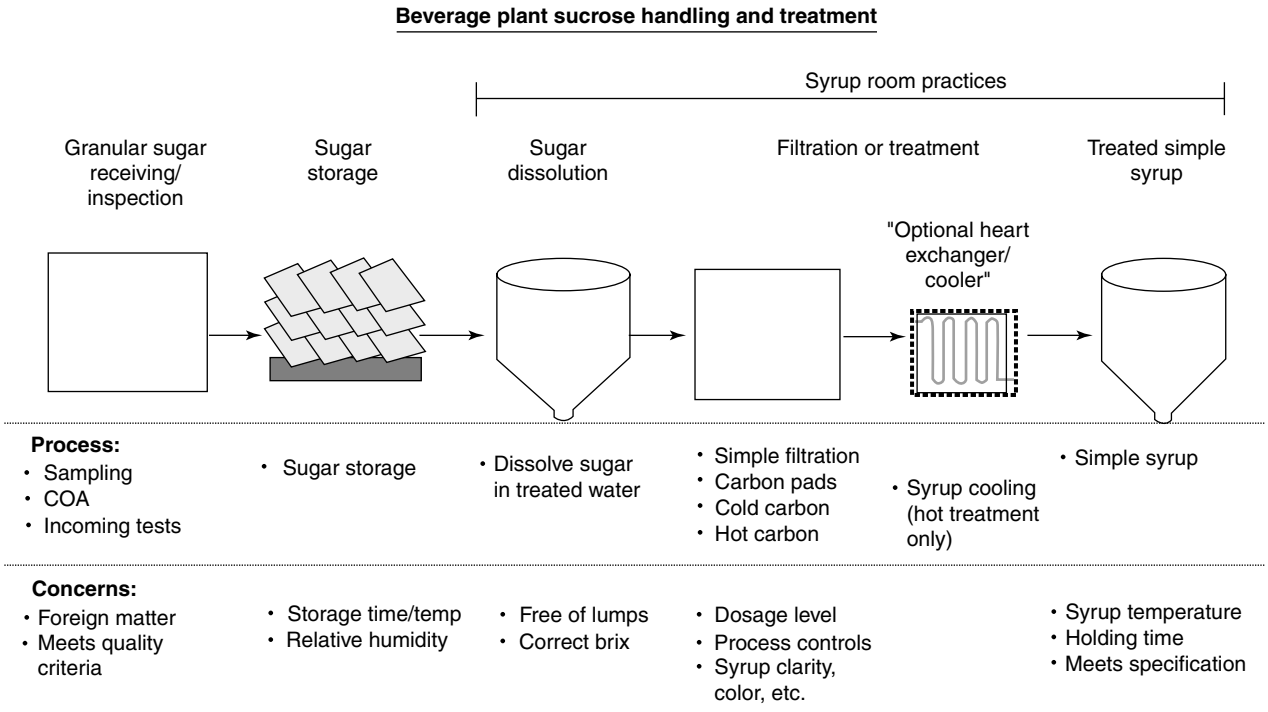

FIGURE 15.9 Sucrose handling and treatment at the beverage plant.

quality of the carbon dioxide used in this application. For many years, the quality of carbon dioxide was minimized, largely because there were no uniformly available methods with which to test the gas, as an ingredient. Those procedures that were available required special expertise to properly sample and handle this cryogenic gas. The standards of quality of the carbon dioxide used in beverages were traditionally relegated to the U.S. Compressed Gas Association (CGA), whose "quality verification levels" were incorporated into the beverage company's specification system. Then, in 1999, the International Society of Beverage Technologists (ISBT) developed the Quality Guidelines and Analytical Procedure Bibliography for Bottlers' Carbon Dioxide (20). This was a cross-corporate effort by carbon dioxide suppliers, end-users, testing labs, and allied businesses to completely update the obsolescent guidelines which had been recognized for decades. The guidelines are only available for purchase through ISBT (www.bevtech.org); they include parameters related to health/safety, sensory, and good manufacturing practices at the supplier.

Carbon dioxide may be obtained and purified from a number of different "feed gas" sources, the majority of which are listed in Table 15.5.

There are other more "exotic" sources which are often the result of carbon dioxide being generated as a by-product during an organic chemical synthesis. In addition to commercial supplies, some carbonated beverage plants produce and purify their own carbon dioxide. The most common feed gas sources for these applications are combustion (where the flue gas is recovered, concentrated, then purified) and breweries (where the CO_2 generated from microbial metabolism is recovered and purified). Whether supplied commercially or in-house, the carbon dioxide used in carbonated soft drinks is of high quality (greater than 99.9% CO_2); in most cases, it even exceeds that of medical grade gas.

The liquid carbon dioxide which is delivered to beverage plants is generally stored in large "bulk receivers," which are vertically or horizontally oriented steel tanks with urethane foam or vacuum insulation. In the most common arrangement, carbon dioxide is withdrawn from the liquid phase at the bottom of the tank, and vaporized by one of several methods. Due to this withdrawal, the equilibrium between vapor and liquid in the tank remains dynamic. The air gases (oxygen, nitrogen) partition into the vapor phase of the vessel, and are routinely purged to maintain the purity of the carbon dioxide within the bulk receiver. Similarly, some components preferentially partition, in trace amounts, into the liquid phase of the carbon dioxide (liquid CO_2 is an excellent solvent). Many beverage plants choose to subject the freshly vaporized carbon dioxide to one final step of purification just prior to the point of use. This is usually a simple filtration through activated carbon alone, or through a mixed adsorbent bed of carbon (to remove organic contaminants); a silica-based desiccant (to remove moisture); and a molecular sieve (to remove sulfur compounds and some oxygenates).

TABLE 15.5
Feed Gas Sources for Carbon Dioxide

- ✓ Combustion
- ✓ Wells/Geothermal (Natural CO_2 Wells)
- ✓ Fermentation (Breweries, ethanol plants, etc.)
- ✓ Hydrogen or Ammonia Plants
- ✓ Phosphate Rock
- ✓ Coal Gasification
- ✓ Ethylene Oxide Production
- ✓ Acid Neutralization

Adapted from CGA-6.2. 2000. Commodity Specification for Carbon Dioxide. U.S. Compressed Gas Association, Table 3, Page 5.

In addition to the quality considerations already discussed, carbon dioxide safety is a key consideration for beverage industry technologists. Carbon dioxide is not usually considered to be a toxic gas in the generally accepted sense of the term (that is, poisonous) and is normally present in the atmosphere at a concentration of approximately 0.03% (300 ppm). Under normal circumstances, carbon dioxide acts upon vital functions in a number of ways, including respiratory stimulation, regulation of blood circulation, and acidity of body fluids. The concentration of carbon dioxide in the air affects all of these. High concentrations are dangerous upon extended exposure, due to increased breathing and heart rates and a change in the body acidity. OSHA (Occupational Safety and Health Administration) establishes regulations governing the maximum concentration of CO_2 and the time-weighted average for exposure to CO_2. These regulations should be reviewed before installation of any CO_2 equipment, and the requirements fully met during operation and maintenance.

Since carbon dioxide is heavier than air, it may accumulate in low or confined areas. Adequate ventilation must be provided when carbon dioxide is discharged into the air. At lower levels where carbon dioxide may be concentrated, self-contained breathing apparatus or supplied-air respirators must be used. Filter type masks should not be used. Appropriate warning signs should be affixed outside those areas where high concentrations of carbon dioxide gas may accumulate, and lock-out/tag-out procedures should be followed, as appropriate (21).

III. SYRUP PREPARATION

Most carbonated beverage formulae begin with a "simple syrup," which is usually a simple combination of the nutritive sweetener (sucrose, HFCS, MIS) and treated water. In some cases, it may also contain some of the salts outlined in the specific beverage document, depending on the order of addition which is required. Once the sweetener is completely dissolved, and the simple syrup is a

homogenous batch, then the flavor and remaining components are added to form the "finished syrup." All simple syrup should be filtered before being pumped to the finished syrup blending/storage tanks.

Using Granulated Sucrose. Accurate weighing of granulated sugar is important. Granulated sugar is normally received in bulk form or in bags. Internationally, receipt in 50- or 100-pound jute or paper bags is not uncommon. It is extremely important that the sugar received by either means should be dry and free of lumps. Moist sugar creates two immediate and serious problems: (1) moist sugar can have high microbial counts, much of which will be yeast. Yeast is a serious problem for carbonated beverages, since it can lead to fermentation and eventual spoilage of the finished product. (2) Moist sugar makes accurate measuring difficult, since the moisture content is being weighed, in addition to the sucrose solids. This makes final control of the batch difficult and inconsistent.

Sugar in lumps will create difficulties in making simple syrup and will take longer to dissolve. Lump sugar is usually an indication that the sugar was not fully dried during refinery production or was stored improperly (22). Never use bulk sugar systems when faced with wet or even slightly moist sugar. It will cause "bridging" (flow restriction) in silo storage and make effective handling impossible. It is critical that any bulk sugar supply is consistently dry and that the storage environment can be controlled to ensure constant low humidity. Even the most modern silo can "bridge" when faced with a moisture problem.

Granulated sugar should always be added slowly into the treated water already measured into the tank. While sugar is being added, the tank agitator should be in constant operation. The agitation should continue until the sugar is completely dissolved. After the sugar has been completely dissolved, and the simple syrup has been filtered into the blending/storage tank, the syrup is checked for sugar content (Brix). Table 15.6 outlines intuitive, but useful, reasons for off-target Brix readings.

Using Liquid Sugars. There are three main types of liquid sugars that are used for syrup production, as discussed earlier: liquid sucrose, medium invert sugar, and high fructose syrups. Making simple syrup from liquid sucrose is similar to the procedure employed when using granulated sugar. The first step is to check the Brix of the liquid sucrose to find out how much water must be added to the batch to bring the Brix of the simple syrup to the level required by the formula. Most companies beverage documents include a table that specifies how much of the liquid sucrose and additional treated water should be added to the batch based on Brix. When liquid sucrose supplies are received at the plant, they should be accompanied by an analysis sheet comparing the tank load against the company standards.

TABLE 15.6
Possible Brix Errors during Simple Syrup Production

High Brix	Low Brix
Weighing error - excess sugar	Weighing error - short sugar
Faulty scale	Faulty scale
Instrument error	Not weighing sugar bags
Too little water	Too much water
	Instrument error
	Moist sugar

Source: Delonge, H. 1994. Pepsi-Cola Production Manual, Volume 2, Sugar and Sugar Handling.

Medium Invert Sugar is resistant to microbial spoilage when being transported from supplier to plant, and while in storage. However, good sanitation procedures are still required, as well as special precautions to prohibit secondary infection. When liquid invert shipments are received at the plant, they should be accompanied by an analysis sheet comparing the tank load against standards. The formula document should include a table that specifies how much of the sweetener and additional treated water should be added to the batch based on Brix and the percent inversion. When testing for Brix in MIS samples, a correction factor must be used on refractometer readings to compensate for the non-sucrose solids as a result of inversion.

Using High Fructose Syrups. For liquid sugars, in general, a sample should be taken before the sugar is accepted, and the analysis should confirm that the material is within standards. The installation, including receiving station, pumps, air blower/ultraviolet lamp, tanks and piping/fittings, should be of approved materials (stainless steel) and in accordance with the individual beverage company's design guidelines. High fructose syrup is subject to crystallization, so storage temperatures should be controlled (generally maintained between 75°F/24°C and 85°F/29°C), by the use of indirect heating. The receiving station is a critical point and should be fully cleaned and hot sanitized before every delivery. As with MIS, when testing for Brix in HFS samples, a correction factor must be used on refractometer readings to correct to true Brix and compensate for the non-sucrose solids.

No matter what type of nutritive sweetener is used, once the simple syrup has been correctly prepared in the mixing tank, it should be pumped through the syrup filter into the storage tank so that the other concentrate components may be added. Most simple syrups will be in Brix range between 60–65, which makes them extremely susceptible to microbial spoilage, again, with yeast the most likely culprit. Be sure to recognize and respect any time constraints included in the syrup preparation instructions. For example, a general "rule of thumb" is that simple syrup should not be kept longer than four hours before converting it to finished syrup. If hot sugar processing is used, remember to atemperate the simple syrup to

ambient temperature prior to the addition of concentrate. This will help minimize thermal degradation of the flavor oils. Also, it is very important to add the individual components in the specific order detailed in the syrup preparation instructions. Incorrect order of addition can lead to a variety of problems, including changes in viscosity, flavor degradation, nutrient breakdown, and precipitation of insoluble materials in the syrup tank.

IV. CARBONATION

Earlier in this chapter, we discussed the history, theory, and principle of introducing carbon dioxide gas into water to produce a carbonated beverage. We also addressed the importance of the quality of this carbon dioxide, as well as of the treated water used to dissolve it. In this section, we will discuss the practical aspects of carbonation control.

"Mix processing" refers to the process of combining the finished syrup, treated water, and carbon dioxide in the correct proportions to meet beverage specifications. In addition to the proportioning function, mix processing will usually incorporate deaeration, mixing, carbonating, and cooling, depending on the manufacturer's design and the type of products being handled. The design of mix processing systems will vary from one manufacturer to another, incorporating the features that the manufacturer feels are advantageous to controlling production.

The primary function of the carbonating unit or the carbonator is to add carbon dioxide to the product. It must be carbonated to the level that, after filling and closing, results in a product within the standards for beverage carbonation. Some carbonating units incorporate cooling in the same tank or unit. The product can be slightly pre-carbonated with CO_2 injection and then exposed to a CO_2 atmosphere directly where cooling is in progress. Other systems separate the carbonating and cooling steps. The three most common forms of carbonating technology incorporate one or a combination of the following: (1) conventional (atmospheric exposure) introduction, (2) CO_2 injection, or (3) CO_2 eduction.

The ability of water, or beverage, to absorb carbon dioxide gas, is largely dependent on the efficiency of the carbonating unit (23). Other factors that influence CO_2 absorption include (1) product type, (2) product temperature, (3) CO_2 pressure, (4) time and contact surface area, and (5) air content. If the water temperature rises, the gas pressure must be increased if the same absorption of CO_2 is to be maintained. Conversely, if the temperature of the water or beverage entering the carbonating unit drops, the CO_2 becomes more soluble, and the pressure must be decreased to keep the volumes of carbonation within standards. Automatic CO_2 controls compensate for fluctuations in temperature, pressure, and flow. This allows the carbonating unit to produce a constant CO_2 gas absorption. Such controls are standard in modern processing units, which are available as basic units, or with computer interfaces to track the variation in product temperature, pressure, flow, and final CO_2 gas volumes absorbed during operating hours.

In many ways, this is a gross oversimplification of a process which, to this day, sometimes eludes strict control. Certainly, equipment has dramatically improved over the years, but loss of carbon dioxide remains a significant issue in terms of overall plant productivity. New membrane carbonation systems hold great promise for continuing this evolution, by helping to carbonate, at least in theory, more precisely and accurately than ever before. It is yet to be seen if these systems will endure the economic challenges, industry acceptance, and rigors of time.

V. FILLING, SEALING, AND PACKING

In the most fundamental terms, this section will address the introduction of the now freshly prepared and carbonated finished beverage into the package, and sealing it in a manner so as to preserve its integrity: simple in theory, sometimes challenging in application. The bottle filling unit includes bottle handling/transfer components, a filling machine, and a capper/crowner.

The purpose of the filler is to fill returnable and nonreturnable bottles to a predetermined level. It should do this efficiently while minimizing foaming and deliver the bottle to a crowner or closure machine to be sealed, or, in the case of cans, to the lid seamer. A discussion of the design and engineering of filling machines is beyond the scope of this chapter, and is normally relegated to the specific operating manuals supplied by the respective equipment vendor.

Carbonated beverage fillers, to prevent the loss of carbon dioxide from the freshly carbonated beverage, must be counter-pressured. The advantage in using CO_2 gas for counterpressure purposes at the filler bowl is to reduce product air content. With can fillers, this is possible because the counterpressure gas is normally purged from the can to the atmosphere as part of the filling process. Most bottle fillers presently in use vacate the counterpressure gas back into the filler bowl as the bottle is being filled. The empty bottle moving into the sealing position (at the filling valve) already contains air. Even if the counterpressure gas is CO_2, vacating this mixture (air and CO_2) back into the filler bowl assures that the bowl will contain (predominantly) air. This can negate the advantage of CO_2 as a counterpressure gas, and actually be wasting CO_2 to the point of an economic disadvantage. In place of carbon dioxide, air or nitrogen is sometimes used as the counterpressure gases.

Imagine what happens when a carbonated beverage is agitated, and then quickly uncapped. Sometimes, this same type of foaming that results can occur during filling. Foaming at the filler, even in small amounts, can cause a number of problems. Some of these deal with product

TABLE 15.7
Problems Resulting from Foaming at the Filler

Quality	Economics/Operations
✓ Underfilled package	✓ Impact on filling speed
✓ Product residue on bottle	✓ Loss of CO_2 and product
✓ Incorrect CO_2 level	✓ Increased BOD (biochemical oxygen demand) to the drain (sewer surcharge)
	✓ Increased cost of clean-up

Source: Delonge, H. 1994. Pepsi-Cola Production Manual, Volume 2. Carbonation.

quality, others with economics or plant operation, and are summarized in Table 15.7 (24).

The cause(s) of foaming in a filling operation can range from a simple problem, that can be corrected quickly, to one requiring extensive trial and error testing. Many times, the troubleshooting exercise requires a combination of technical skill, creativity, and experience. Some causes of foaming at the filler are summarized in Figure 15.10.

When the problem is a single valve, or occurs for a short period of time, it is usually easy to troubleshoot and correct. On-going foaming problems can be extremely difficult to correct. Manuals supplied by the manufacturer of the filler/mix processor usually address troubleshooting foaming problems in detail, and should be consulted. If the problem persists, contact the filler manufacturer.

One of the problems which can result from excessive foaming at the filler, aside from the poor aesthetics of "sticky" packages, is the formation of mold colonies on the external walls of the package. This might also be evident in the thread areas of bottles when the cap is removed. Proper sealing of the newly filled package is a critical step in the processing of carbonated soft drinks. The closure can be a variety of different types, including crimp-on metal crowns on glass bottles, screw-on metal or plastic caps on plastic bottles, or a seamed lid onto a can body. Each of these applications requires different equipment, but the over-riding objectives are the same: (1) withstand the pressure from the carbon dioxide in this closed system, (2) provide the consumer with a safely sealed product, and one with tamper evidence, (3) prevent leakage of product out of the package, and (4) help contribute to the visual appeal of the overall package.

After proper application of the closure or lid, some beverage manufacturing plants pass the bottles and cans through a warmer, which is a tunnel of water sprays of carefully controlled temperature. The purpose is to bring the temperature of the filled packages (still cold from the

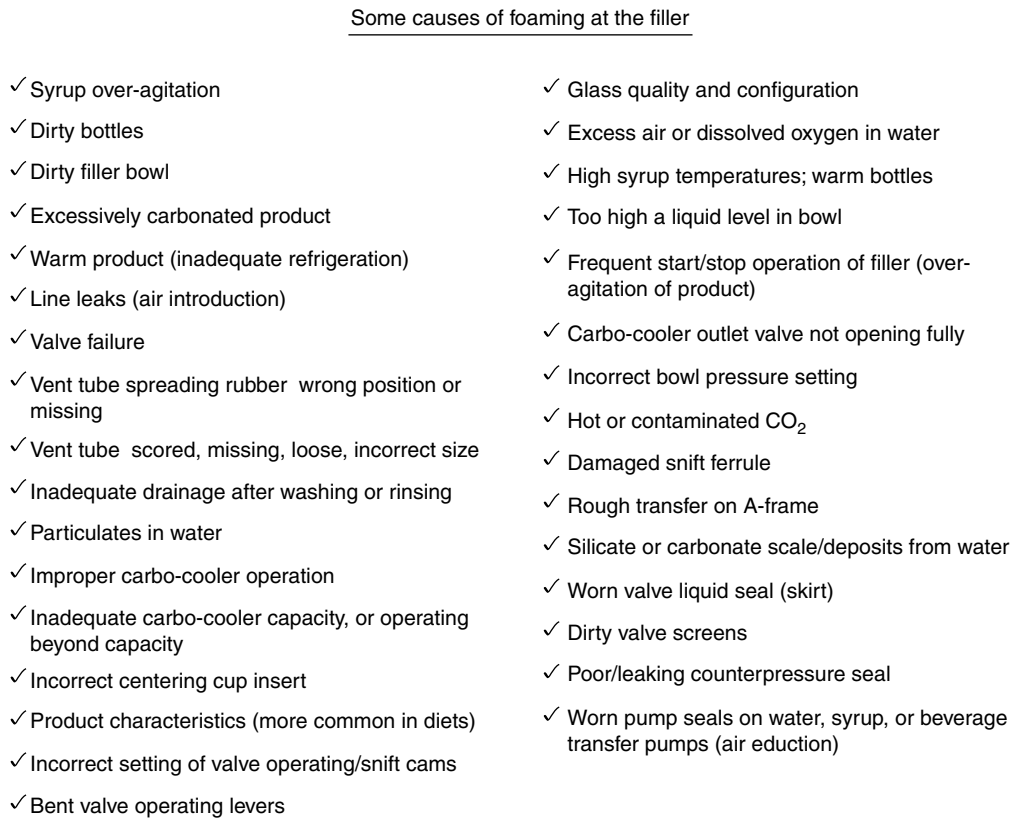

FIGURE 15.10 Some causes of foaming at the filler. Source: Bena (25).

chilled carbonated water introduced at the mix processor) up to close to ambient. The main reason for this is to prevent excessive condensation, which can lead to problems, depending on the secondary and tertiary packaging that are used.

For example, in the U.S. and in many countries internationally, it is common to place bottles of carbonated beverage into rigid plastic crates for transport to a retail outlet. In these instances, warming is not usually needed, since the plastic crates are essentially inert, and allow for adequate air flow and ventilation of the product. Some products, however, perhaps because of a particular marketing promotion, will shrink wrap multiple bottles together, then place them in a cardboard case box, and then stack them on a pallet which is stretch-wrapped for structural stacking integrity. In the second example, if the bottles were not warmed after filling, there is a high probability that the excess condensation would be trapped (by the shrink wrap), absorbed by the cardboard (presenting a mold risk), and then subjected to a "green house effect" from the poor ventilation of the stretch wrap. It becomes quickly evident that a beverage producer's job is not complete simply because the product makes it safely to a sealed container!

VI. QUALITY CONTROL AND ASSURANCE

In this section, we will distinguish quality "control" from quality "assurance" by having control refer to testing typically performed by the beverage plant, either immediately on-site or at a local contract lab. "Assurance" will refer to the subject of a broader, usually centrally managed program (for example, frequent testing of the product from the trade by a central corporate laboratory). Typically, the bulk of testing performed in a carbonated beverage facility falls under the category of quality control. Each company prescribes its own specific testing protocol, including the parameters to test, analytic test methods to apply, and frequency. In addition, a rigorous quality program would clearly outline the actions to be taken (and by whom) in the event that this testing demonstrates an out-of-specification situation.

Since there is no single protocol for all plants to follow, Figure 15.11 summarizes the major categories of testing to consider when evaluating a beverage plant's quality monitoring scheme. This list is by no means exhaustive, but it does provide an idea of how rigorous the monitoring and control in a beverage plant should be.

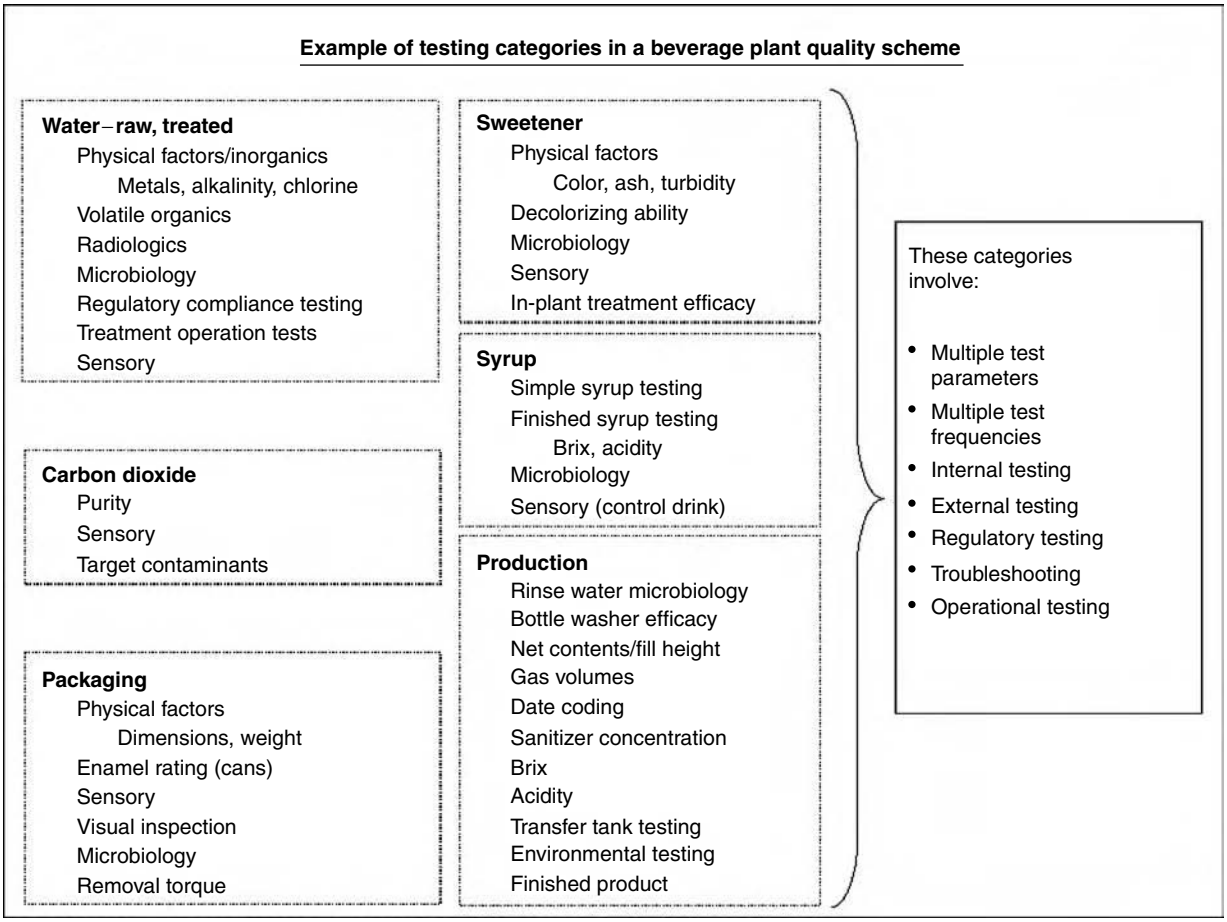

FIGURE 15.11 Example of testing categories in a beverage plant quality scheme.

In addition to this quality control scheme, most larger beverage companies have developed formalized quality assurance schemes, which are usually under a centralized corporate management. The programs generally include some auditing function to visit the production plants for compliance to standards and guidelines, and sampling of finished products from the trade. These programs vary in terms of their focus and rigor, but trade sampling provides perhaps the best representation of what the consumers in a particular market are receiving. From this perspective, the data obtained are of extreme value, and must be reviewed in concert with in-plant and external data, in order to provide the best overall picture of quality performance.

VII. FINISHED PRODUCT

Low pH, high acidity, carbonation, and often ingredients that provide some natural antimicrobial activity (for example, d-limonene in citrus oils) all combine to make carbonated soft drinks a robust category of beverages. Of course, "robust" is a relative term, so as not to imply that carbonated beverages are completely immune to problems in finished product. The formulae, however, go a long way in providing a margin of "designed" product safety.

In fact, for non-fruit-juice-containing carbonated beverages, the types of problems which are typically encountered in the trade are relatively few, and rarely, if ever, present a health or safety threat to the consumer. Microbiologically, we have already mentioned the possibility of having mold form where the overall moisture in the environment is not controlled. For example, remember the scenario of freshly filled bottles, moist with condensation, then shrink wrapped, palletized, and stretch wrapped. The resulting "green house" effect could easily provide the necessary conditions in which mold could grow. In finished product, however, these beverages might contain a variety of organisms, but they will not remain viable under the conditions of the beverage. Only aciduric organisms can multiply, and these include some molds, yeasts, lactic acid bacteria, and acetic acid bacteria (26). Of these, the clear majority of microbial problems are caused by spoilage yeast. This "spoilage" normally refers to any condition which affects the design appearance, flavor, or aroma of the product, and is usually a problem of aesthetics where carbonated soft drinks are concerned.

In addition, as with any packaged products, the packaging materials can be the source of finished product problems. For example, misapplication of closures may occur, where removal torque is so high that consumers have difficulty opening the bottles. In areas of the world where returnable bottles are used, depending on their handling, they can become badly scuffed, presenting an unappealing look to the consumer.

Many problems with finished product can be—and are—averted before the product ever leaves the beverage facility. This is due, in large part, to the diligent monitoring of the soft drink manufacturing process from beginning to end. We have already learned that the raw materials are held to high standards of quality upon receipt, and some—like water, sucrose, and carbon dioxide—are often further purified within the beverage plant itself. Then, these raw materials are combined into a finished syrup, and it is checked against standards of assembly and quality. This finished syrup is then diluted and carbonated, filled, then sealed to form the final beverage. The final product is tested chemically, microbially, and sensorially, to ensure that it meets the highest standards of its trademarked brand.

This said, the summary above represents only a small portion of the quality systems that overarch most finished products, and which are clearly beyond the scope of this chapter. Suffice to say that many beverage companies begin to control quality as far back in the supply chain as possible, so far, that some companies own their own citrus groves in order to strictly control the quality of the orange juice used in their orange-juice containing carbonated beverages! In addition, as the principles of HACCP (Hazard Analysis and Critical Control Points) become more commonplace in the beverage industry, many bottlers and canners are voluntarily formulating their own HACCP plans to formalize the monitoring and control of their processes. All of this is done with a single, predominant end-goal in mind, to provide the consumer with consistently high-quality, great tasting, refreshing beverages.

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16 Muffins

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CONTENTS

I. BACKGROUND INFORMATION

A. History of Muffins

English muffins originating in London were made from yeast dough in contrast to quick-bread muffins served in early America. Muffins are described as a quick bread since "quick-acting" chemical leavening agents are used instead of yeast, a "longer-acting" biological leavening agent. Muffins have become increasingly popular as a hot bread served with meals or eaten as a snack. Freshly baked muffins are served in restaurants and bakeries and consumers can buy packaged ready-to-eat muffins from grocery stores and vending machines. It is possible for restaurants and small bakeries to serve a muffin of a consistent high quality with the availability of dry mixes, frozen muffin batter, and pre-deposited frozen muffins available on the wholesale market.

B. Health Concerns

The economic burden of chronic disease is a worldwide problem. Chronic diseases contributed to 60% of the deaths worldwide in 2001 (55). The increasing rate of obesity and the ageing of the population are expected to impact the burden of chronic disease. Those with obesity are at greater risk and have an earlier onset of the chronic diseases of diabetes, cardiovascular diseases, cancer, and stroke. Ageing increases the risk for all chronic diseases. Nearly one-quarter of the population in developed countries is made up of those above 60 years of age, with expectations for the numbers to increase to one-third of the population by 2025 (55).

The problems of overweight and obesity are growing rapidly around the world and co-exist with malnutrition in developing countries (55). Surveys of U.S. adults done in 1999–2000 showed that 64% of adults were overweight and 30% were obese (21). The percentage of children and adolescents in the U.S. who are overweight has tripled in the past 30 years with 15% of 6–19 year olds being overweight in 1999–2000 (41).

Obesity rates have increased threefold or more in some parts of North America, Eastern Europe, the Middle East, the Pacific Islands, Australasia, and China since 1980 (54). The prevalence rates of overweight and obesity are growing rapidly in children and adults in such countries as Brazil and Mexico where malnutrition and obesity co-exist in the same household (9). Countries with the highest percentage (5% to 10%) of overweight preschool children are from the Middle East (Qatar), North Africa (Algeria, Eqypt, and Morocco), and Latin America, and the Caribbean (Argentina, Chile, Bolivia, Peru, Uruguay, Costa Rica, and Jamaica) (10).

Globalization of food and the availability of energy-dense snack and "fast foods" have had a significant impact on dietary patterns and the incidence of chronic disease in both developing and developed countries (27). For example, Coca-cola and Pepsi soft drinks and McDonald's, Pizza Hut, and Kentucky Fried Chicken fast foods are now available worldwide (27). Changes in dietary patterns combined with a sedentary lifestyle have increased the rates of obesity and chronic disease. Dietary factors related to chronic disease are excessive intakes of calories, fat—especially saturated fat—and sodium, and low intakes of fruits and vegetables and whole-grain breads and cereals (52).

National dietary guidelines recommend limiting intakes of total fat, saturated fat, trans fat, cholesterol, free sugars, and sodium, and promote dietary fiber from whole grain breads and cereals and fruits and vegetables (56). In 2002, consumers in the U.S. reported making food choices in an effort to avoid fat, sugar, calories, and sodium, and to increase fiber intake (39). Consumers chose fat free foods or foods low in fat 74% to 80% of the time and selected low calorie foods and low sodium foods 76% and 67% of the time, respectively. High fiber foods were chosen 75% of the time and 40% reported using organic foods (39).

The food industry has responded to concerns of consumers and public health officials by developing "healthy" food products, lower in saturated fat, trans fat, cholesterol, sodium, sugar, and calories. New ingredients have been developed by food scientists in government and industry to use as fat replacers and sugar replacers to use in preparing baked products that are lower in calories and saturated and trans fat (Table 16.1, Table 16.2). The newest category of ingredients is concentrated bioactive compounds with specific health benefits (Table 16.3) (43). These ingredients are added to formulations during food processing to enhance the health benefits of specific food products or to develop "functional foods." Individual foods, such as apples, blueberries, oats, tomatoes, and soybeans, are being marketed as functional foods because of the health benefits of components of these foods. For example, diets that include oat fiber and soy protein lower serum cholesterol, and lycopene in tomatoes reduces the risk of prostrate cancer. Apples and blueberries contain unique antioxidants shown to reduce the risk for cancer (42). Examples of bioactive ingredients available to the baking industry are OatVantage™ (Nature Inc., Devon, PA) a concentrated source of soluble fiber, and FenuPure™ (Schouter USA, Minneapolis, MN), a concentrated source of antioxidants from fruits and vegetables.

C. Food Labeling and Health Claims

The Nutrition Labeling and Education Act (NLEA) issued by the Food and Drug Administration (FDA) in the U.S. in 1990 required food labels to include nutrition content on all packaged foods to be effective in 1994 (11). Information required on the Nutrition Facts portion of the food label are the serving size and the amount per serving

TABLE 16.1
Ingredients Used as Fat Replacers in Baked Products

Brand Name	Composition	Supplier
Carbohydrate Based		
Beta-Trim™	Beta-glucan and oat amylodextrin	Rhodia USA, Cranbury, NJ
Fruitrim®	Dried plum and apple puree	Advanced Ingredients Capitola, CA
Just Like Shorten™	Prune and apple puree	PlumLife division of TreeTop, Selah, WA
Lighter Bake™	Fruit juice, dextrins	Sunsweet, Yuma City, CA
Oatrim®	Oat maltodextrin	Quaker Oats, Chicago, IL
Paselli FP	Potato maltodextrin	AVEBE America, Inc., Princeton, NJ
Z-Trim	Multiple grain fibers	U.S. Department of Agriculture
Low and Noncaloric Lipid Based		
Enova™	Diglycerides	Archer Daniels Midland/Kao LLC, Decatur, IL
Benefat®	Triglycerides modified by substituting short or medium chain fatty acids	Danisco Culter, New Century, KS
Salatrim/Caprenin		Proctor & Gamble, Cincinnati, OH
Olestra/Olean®	Sucrose polyester	Proctor & Gamble, Cincinnati, OH

TABLE 16.2
Ingredients Used as Sugar Replacements in Baked Products

Sweetener	Brand Name	Sweetness Compared to Sucrose	Supplier
Acesulfame-K	Sunett®	200% sweeter	Nutrnova, Somerset, NJ
Sucralose	Splenda®	600% sweeter	Splenda, Inc., Fort. Washington, PA

TABLE 16.3
Ingredients Marketed for Specific Health Benefits

Brand Name	Composition	Health Benefit	Supplier
Caromax™ Carob Fiber	Carob fruit fiber; soluble fiber, tannins, polyphenols, lignan	Lower serum cholesterol	National Starch & Chemical, Bridgewater, NJ
FenuPure™	Fenugreek seed concentrate; galactomannan	Regulate blood glucose; lower serum cholesterol	Schouten USA, Inc., Minneapolis, MN
Fibrex®	Sugar beet fiber; soluble fiber, lignan	Lower serum cholesterol; regulate blood glucose	Danisco Sugar Malmo, Sweden
MultOil	Diglycerides + phytosterols	Lower serum cholesterol	Enzymotec Migdal HaEmeq, Israel
Nextra™	Decholesterolized tallow and corn oil; free of trans fat	Reduce the risk for coronary heart disease	Source Food Technology, Durham, NC
Novelose 240	Corn fiber; high amylose, resistant fiber	Reduce risk for colon cancer	National Starch & Chemical, Bridgewater, NJ
Nutrifood®	Fruit and vegetable liquid concentrates; source of antioxidants–carotenoids, anthocyanins, polyphenols	Reduce risk for chronic diseases–cancer, diabetes, and cardiovascular disease	GNT USA, Inc., Tarrytown; NYGNT Germany, Aachen, Germany
OatVantage™	Beta-glucans, a soluble fiber	Lower serum cholesterol	Nurture, Inc., Devon, PA

of calories, protein, fat, saturated fat, cholesterol, carbohydrates, fiber, sodium, calcium, vitamins A and C, and iron. A 1993 amendment to the NLEA authorized food manufacturers to add health claims related to specific food components (11) (Table 16.4). However, many "functional foods" lack the scientific evidence to meet FDA criteria to make health claims (50). A 2003 amendment to NLEA requires that trans fatty acids be listed under saturated fat on the Food Facts label by January 1, 2006 (11).

The Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO) Codex Guidelines on Nutrition Labeling adopted in 1985 are similar to the

TABLE 16.4
Health Claims Approved for Food Labeling in the U.S.[1]

Food Component	Health Claim
Calcium	Osteoporosis
Dietary fat	Cancer
Dietary saturated fat and cholesterol	Coronary heart disease
Fiber-containing grain products, fruits and vegetables	Cancer
Sodium	Hypertension
Folate	Neural tube defects
Dietary sugar alcohol	Dental caries
Fruits, vegetables and grain products that contain fiber, particularly soluble fiber	Coronary heart disease
Soy protein	Coronary heart disease
Whole-grain foods	Heart disease and certain cancers
Plant sterols/stanol esters	Coronary heart disease
Potassium	High blood pressure and stroke

[1] FDA/CFSAN. 2002b. Food labeling and nutrition. Information for industry. A food labeling guide. Appendix C. Health claims. http://www.cfsan.fda.gov/~dma/flg-6C.html. Accessed on July 3, 2003.

NLEA implemented by the FDA in 1994 (13). The Codex Alimentarius Commission adopted the Codex Guidelines for the use of Nutrition Claims on food labels in 1997 (14). Codex standards are voluntary and each country within the United Nations is free to adopt food-labeling standards. The European Union that includes 15 member states in Europe also sets guidelines for nutrition labeling and nutrition claims, subject to requirements of individual member states.

The Food Standards Agency of the United Kingdom (U.K.) was established in 2000 as the regulatory agency to set policy for food labeling in Great Britain and Northern Ireland (23). The Food Standards Australia New Zealand (22) specifies the requirements for food labeling in these countries (22). Health Canada published new food labeling regulations January 1, 2003, making nutrition labeling mandatory for most foods and allowing diet-related health claims on food labels for the first time (26).

D. Food Labeling Standards for Organically Grown Foods

The Organic Foods Production Act of 1990 passed by the U.S. Congress required the U.S. Department of Agriculture to develop certification standards for organically produced agricultural products (2). Producers who meet the standards may specify the percentage of the product that is organic on the food label if 70% or more of the ingredients in the product are organically grown (2). The Codex Alimentarius Commission has also published standards for labeling organically grown foods (15). Organic fruits and vegetables are produced without using conventional pesticides, petroleum-based fertilizers, or sewage sludge-based fertilizers. Animal products identified as organic come from animals given organic feed without antibiotics or growth hormones. Food products that have been developed through genetic modification cannot be labeled as organically grown foods (2,15).

E. Ingredient Labeling for Possible Allergens

The Codex Alimentarius Commission of FAO/WHO and FDA/CFSAN require that food labels list all ingredients known to cause adverse responses in those with food allergies or sensitivities (11,20). The FDA requires listing ingredients from eight foods that account for ~90% of all food allergies. These foods are peanuts, soybeans, milk, eggs, fish, shellfish, tree nuts, and wheat (20). Codex standards require listing ingredients from these same eight foods plus all cereals that contain gluten—rye, barley, oats, and spelt—lactose, and sulphite in concentrations of 10 mg/kg or more (11). Gluten, lactose, and sulphite are listed on food labels because these substances cause distress for some, even though these substances are not considered allergens. Individuals with celiac disease or gluten intolerance eliminate all sources of gluten from the diet. A small percentage of individuals lack lactase, the enzyme needed to digest lactose, and avoid dairy products and all other foods with lactose additives.

Food processing plants are required to follow Good Manufacturing Practices (GMP) to avoid possible cross-contamination with trace amounts of allergens during processing. An example of possible cross-contamination is using the same plant equipment to prepare "nut free" muffins after the equipment was used to prepare muffins with nuts (49). An example of GMP is dedicating food-processing plants to the production of allergenic free foods (49).

Small bakeries, defined by the number of employees or annual gross sales, and restaurants are exempt from the FDA food labeling requirements. Food labeling to identify foods that have been genetically modified through bioengineering (GM) is voluntary (16). However, because of consumer concerns about GM foods, managers of bakeries may choose to include a statement on the ingredient label such as "we do not use ingredients produced by biotechnology" (16,17). Consumers with food allergies have learned to read the list of ingredients on the food label to identify any possible sources of allergens. Managers of small bakeries that use nuts or soy flour in their operation, but unable to follow GMP because of the added cost, may choose to alert consumers with a statement on the ingredient label, such as "this product was made on equipment that also makes products containing

tree nuts." Making a decision to sell bakery products made with organic ingredients requires assessing the market for these products, the availability of organic ingredients, and the expected income from the operation.

II. RAW MATERIALS PREPARATION

A. Selection and Scaling of Ingredients

Muffins made by large commercial bakeries are cake type muffins while those made in the home or small institutions are bread muffins. The differences between cake and bread muffins are that cake muffins are higher in fat and sugar and use soft wheat flours. A common problem encountered in bread type muffins is tunnel formation resulting from overdevelopment of gluten. However, this problem is avoided in cake muffins since sugar, fat, and soft wheat flours interfere with gluten development and prevent tunnel formation. Bread muffins contain 12% of both fat and sugar compared to 18% to 40% fat and 50% to 70% sugar in cake muffins (5).

Formulas for a standard cake muffin and bran muffin are shown in Table 16.5. Ingredient formulas used by commercial bakeries are based on the weight of flour at 100% (25). The amounts of other ingredients are a percentage of flour weight (Baker's percent). For example,

$$\frac{\text{total weight of muffin ingredient}}{\text{total weight of flour}} \times 100\% = \% \text{ of the ingredient}$$

If the weight of another ingredient is the same weight as flour, the percent for that ingredient is also 100%. The advantage of using Baker's percent is that batch sizes can be easily increased or decreased by multiplying the percent for each ingredient by the same factor. Weighing all ingredients, including liquids, is faster and more accurate than using measurements, especially in large commercial bakeries.

1. Flour

Flour is the primary ingredient in baked products. Flour represents 30% to 40% of the total batter weight in most cake muffins (5). Most muffin formulas contain a blend of cake or pastry flour and a high protein flour such as bread flour, or all bread flour (51). The protein in flour is needed to provide structure in quick breads made with limited amounts of sugar. Flour contains starch and the proteins, glutenin, and gliadin, which hold other ingredients together to provide structure to the final baked product. Hydration and heat promote gelatinization of starch, a process that breaks hydrogen bonds, resulting in swelling of the starch granule, which gives the batter a more rigid structure (27).

Substituting whole-wheat flour, wheat germ, rolled oats, or bran for part of the flour is an excellent way to increase fiber. Other flours used in muffins include cornmeal, soy, oat, potato, and peanut. An acceptable product is possible when cowpea or peanut flours are substituted for 25% or when whole-wheat flour or corn meal is substituted for 50% of all-purpose flour (29). Acceptable muffins have been prepared when soy protein flour was substituted for 10% to 20% (47) or 100% of all-purpose flour (6). None of these flours contains glutenin or gliadin except whole wheat, and large pieces of bran in whole-wheat flour cut and weaken gluten strands. Thus, there is minimal gluten development when these flours are used; however, the muffins tend to be crumbly and compact unless other modifications are made in the formula.

2. Sugar

Amounts of sugar in muffins range from 50% to 70% based on flour at 100% (5). Sugar contributes tenderness, crust color, and moisture retention in addition to a sweet taste. Sucrose promotes tenderness by inhibiting hydration of flour proteins and starch gelatinization. Sugar is hygroscopic (attracts water) and maintains freshness. Corn syrup, molasses, maple sugar, fruit juice concentrates, and honey are used as sweeteners for flavor variety. Honey or molasses is often used as a sweetener in whole wheat or bran muffins to cover the bitter flavor of the bran (51). The quantity of liquid will need to be decreased if these sweeteners are used instead of sucrose because of the high water content in these syrups.

Chemical changes in sugars during baking contribute characteristic flavors and browning. Carmelization of sugar is responsible for the brown crust of muffins. Carmelization involves dehydration and polymerization (condensation) of sucrose (35). Reducing sugars such as dextrose, corn syrup, or high fructose corn syrup are often added to muffins at levels of 1% to 3% to increase crust color (51). Reducing sugars react with amino acids in flour, milk, and eggs to form a complex responsible for the flavor and brown crust of muffins.

The reaction between the aldehyde or ketone group in reducing sugars and the amino acids in protein is described as the Maillard reaction (37). This Maillard reaction together with carmelization contributes to the characteristic flavor and color of the crust of a baked muffin. Crust temperatures reach 100°C and above, which lower water activity. Both the high temperature and low water activity are necessary for the Maillard reaction to occur (38).

Sugar replacers such as acesulfame-K and sucralose (Table 16.2) can be substituted for all or part of the sugar. Sugar replacers, however, do not contribute to tenderness, browning, or moisture retention, thus other formula modifications are necessary for an acceptable product. For example, adding a small amount of molasses or cocoa for

TABLE 16.5
Muffin Formulas Listed by Baker's Percent and Weight[1,2]

Ingredient	Basic Cake Muffin Baker's %	Weight gm	Bran Muffin Baker's %	Weight gm
Flour	100.00	990	–	–
Bread flour	–	–	50.0	4545
Cake flour	–	–	18.75	1704
Bran	–	–	31.25	2842
Sugar	60.00	5455	31.25	2842
Baking powder	5.00	455	1.50	136
Baking soda	–	–	2.20	220
Salt	1.25	114	1.50	136
Milk powder	7.50	682	12.50	1136
Molasses	–	–	37.50	3409
Shortening	40.00	3636	18.75	1704
Whole eggs (liquid)	30.00	2727	12.50	1136
Honey	–	–	19.00	1727
Water	60.00	5455	100.00	990
Raisins	–	–	25.00	2273
Total	303.75	27616	316.70	32790

Mixer: Hobart N-50 with 5 quart bowl and paddle agitator.
Directions for basic cake muffin formula:
Blend dry ingredients together by mixing for 1 minute at low speed.
Add shortening and eggs and mix for 1 minute at low speed.
Add water and mix for 1 minute at low speed.
Scaling Weight: 2.5 ounces batter.
Yield: 2–1/2 dozen muffins.
Bake: at 205°C for 19–21 minutes in a gas-fired reel oven.
Directions for bran muffin formula:
Blend dry ingredients and mix for 1 minute at low speed.
Add shortening, eggs, honey, molasses, and 50% (4.5 kg) of the water and mix for 1 minute at medium low speed.
Add the remaining water and mix for 1 minute at low speed.
Add raisins and mix at low speed for 3 minutes or until raisins are dispersed.
Scaling Weight: 3 ounces batter.
Yield: 3 dozen muffins.
Bake: at 193°C for 20–25 minutes in a gas-fired reel oven.

[1] Benson RC. 1988. Technical Bulletin. Muffins. Manhattan, KS: American Institute of Baking 19(6):1–4.
[2] Doerry W. 1995. Chapter six: Cake muffins. In: Breadmaking. Vol 2: Controlled Baking. Manhattan, KS: The American Institute of Baking. pp. 208–213.

color to substitute for color from carmelization of sucrose. The shelf life of muffins prepared without sugar would be very limited.

3. Fat

Muffins contain 18% to 40% fat based on flour at 100% (5). Fat contributes to the eating qualities of tenderness, flavor, texture, and a characteristic mouthfeel. Fat keeps the crumb and crust soft and helps retain moisture, and thus contributes to keeping qualities or shelf life (36). Fat enhances the flavor of baked products since flavor components dissolve in fat. Both shortening and vegetable oils are used in muffins.

To meet the demands of the consumer, muffin formulas are being modified to reduce total, saturated fat, trans fat, and calories, and to increase the amount of monounsaturated and polyunsaturated fat. Canola oil and flaxseed meal are being added to muffins to increase the proportion of monounsaturated fat. Muffins made with reduced fat and polyunsaturated fatty acids (13% safflower oil) were comparable in sensory and physical characteristics to the standard muffin made with shortening at 20% (8). Low fat and fat free muffins are available ready to eat and as frozen batters or dry mixes for bakeoff.

Various fat replacers have been classified by their macronutrient base (Table 16.1). Carbohydrate- and lipid-based fat replacers can be used to prepare muffins acceptable to the consumer. Lipid-based fat replacers that have the same chemical and physical characteristics of triglycerides are described as fat substitutes (1). These products

provide the same characteristics as fat but with fewer calories. Monoglycerides, diglycerides, and modified triglycerides are examples of fat substitutes that replicate the mouthfeel and sensory qualities of baked products made with shortening.

Enova™ (Archer Daniels Midland KAO LLC, Decatur, IL) is an example of a diglyceride that is lower in calories than other oils and being marketed as beneficial in weight management (45). Benefat® (Danisco Culter, New Century, KS) and Caprenin (Proctor & Gamble, Cincinnati, OH) are examples of triglycerides modified by substituting shorter chain fatty acids (1). Sucrose polyesters of six to eight fatty acids are marketed as Olean® (Procter & Gamble, Cincinnati, OH), a fat substitute with the same physical qualities as shortening without the calories since sucrose polyesters are not digested or absorbed in the human intestinal tract.

A commercial shortening product (Nextra™) (Source Food Technology, Durham, NC) made from decholesterolized tallow and corn oil is being marketed to the baking industry as a trans-free fat to replace shortening (44). Other methods used by the food industry to decrease the amount of trans fat are 1) blending hydrogenated fat high in stearic acid with unhydrogenated oils, and 2) interesterifying (rearranging) unhydrogenated oils with saturated-fat-based oils (30).

Carbohydrate-based fat replacers are described as fat mimetics. These include cellulose, corn syrup, dextrins, fiber, gum, maltodextrins, polydextrose, starches, and fruit based purees. Z-trim, developed by a U.S. Department of Agriculture scientist, is a mixture of plant fibers (31). Fat mimetics replicate the mouthfeel and texture of fat in baked products and extend the shelf life by binding water and trapping air (3). Acceptable low fat cake muffins (5% fat) used 2% pregelatinized dull waxy starch and corn syrup (3.6%) to replace fat (28).

Fruit purees or pastes of one or more fruits—apples, dates, figs, grapes, plums, prunes, and raisins—are being promoted as fat replacers. Just Like Shorten™ is a mixture of dried prunes and apples. The fruit purees have humectant properties, promote tenderness and moistness, increase shelf life, and can replace some of the sugar and/or fat in muffins and cakes.

Formulas will need to be developed based on adjustments in ingredients when fat replacers are substituted for all or part of the fat in the formula. New formulas need to be prepared and the muffins evaluated using the muffin scorecard (Table 16.6) as well as evaluating the shelf life. Several formula adjustments may be necessary before an acceptable muffin is developed.

4. Leavening Agents

The amount of baking powder used in muffins varies between 2% and 6% based on flour at 100% with lower amounts in muffins with ingredients that increase acid (5). Gases released by a leavening agent influence volume and cell structure. During baking, heat increases gas volume and pressure to expand cell size until proteins are coagulated (35). Stretching of the cell walls during baking improves texture and promotes tenderness (35).

The quantity of leavening used in a baked product depends on the choice of leavening agent as well as other ingredients. Formulation of baking powders considers the amount of leavening acids needed to neutralize baking soda or sodium bicarbonate, an alkaline salt. Double-acting baking powder (most commonly used in muffins) contains both slow- and fast-acting acids (37). Fast-acting acids are readily soluble at room temperature while slow-acting acids are less soluble and require heat over extended time to release carbon dioxide. Formulations of slow- and fast-acting acid leavening agents control the reaction time and optimize volume (7). An example of a formulation to neutralize sodium bicarbonate is a mixture of slow- and fast-acting acids—monocalcium phosphate monohydrate (a fast-acting acid) combined with sodium aluminum sulfate (a slow-acting acid). Development of baking powder requires consideration of the unique neutralizing value (NV) and the rate of reaction (ROR) or the percent of carbon dioxide released during the reaction of sodium bicarbonate with a leavening acid during the first eight minutes of baking (4,7).

Baking soda is used in addition to double-acting baking powder when muffins contain acidic ingredients such as sour cream, yogurt, buttermilk, light sour cream, molasses, and some fruits and fruit juices (37). Baking soda in the amount of 2% to 3% in addition to baking powder is added to acidic batters (5).

Sodium carbonate is a product of an incomplete reaction in formulas with excess sodium bicarbonate. Excess sodium carbonate results in a muffin with a soapy, bitter flavor, and a yellow color because of the effect of an alkaline medium on the anthoxanthin pigments of flour (38). Also, formulas with too much baking powder or soda results in a muffin with a coarse texture and low volume because of an overexpansion of gas, which causes the cell structure to weaken and collapse during baking. Inadequate amounts of baking powder will result in a compact muffin with low volume. Figures 16.1 and 16.2 show different chemical reactions for fast-acting and slow-acting baking powders (37).

5. Whole Eggs

Liquid eggs contribute 10% to 30% of muffin batter based on flour at 100% and dried eggs contribute 5% to 10% (5). Eggs provide flavor, color, and a source of liquid. Upon baking, the protein in egg white coagulates to provide structure. Adding egg whites to muffin batter provides structure to the finished product and a muffin that is

TABLE 16.6
Scorecard for Muffins[1]

Evaluator	Product	Date
External Qualities		
1a. Volume	Score	
Specific Volume: $\pi r^2 \times$ height = weight in grams (cm) 1=low volume, compact cells; 5=light with moderate cells; 7=large volume, large cells and/or tunnels		
1b. Contour of the surface		
1=absolutely flat; 3=somewhat rounded; 5=pleasingly rounded; 7=somewhat pointed; 9=very pointed		
1c. Crust color		
1=much too pale; 3=somewhat pale; 5=pleasingly golden brown; 7=somewhat too brown; 9=much too brown		
Internal Qualities		
1d. Interior color		
1=much too white; 3=somewhat white; 5=pleasingly creamy; 7=somewhat too yellow; 9=much too yellow		
1e. Cell uniformity and size		
1=much too small; 3=somewhat thick; 5=moderate; 7=somewhat too large; 9=numerous large tunnels		
1f. Thickness of cell walls		
1=extremely thick; 3=somewhat thick; 5=normal thickness; 7=somewhat too thin; 9=much too thin		
1g. Texture		
1=extremely crumbly; 3=somewhat crumbly; 5=easily broken, 7=slightly crumbly; 9=tough, little tendency to crumble		
1h. Flavor		
1=absolutely not sweet enough; 3=not nearly sweet enough; 5=pleasingly sweet; 7=somewhat too sweet; 9=much too sweet		
1i. Aftertaste		
1=extremely distinct; 3=somewhat distinct; 5=none		
1j. Aroma		
1=lack of aroma; 5=sweet and fresh aroma; 9=sharp, bitter, or foreign aroma		
1k. Mouthfeel		
1=gummy, cohesive; 3=somewhat gummy; 5=tender, light and moist; 7=somewhat dry and tough; 9=tough and hard to chew		
Overall Acceptability		
1=very unacceptable; 3=somewhat acceptable; 5=very acceptable		

[1] Modified from McWilliams M. 2001. Chapter 3: Sensory evaluation. In: Foods: Experimental Perspectives. 4th ed. Upper Saddle River, NJ: Prentice Hall. pp. 33–57.

easily broken without excessive crumbling (48). Substituting egg whites for whole eggs, however, will result in a dry, tough muffin unless the formula is adjusted to increase the amount of fat (48). Fat in the yolk acts as an emulsifier and contributes to mouthfeel and keeping qualities.

6. Nonfat Dry Milk Powder

Milk powder represents 5% to 12% of the muffin batter based on flour at 100% (5). Milk powder is added to dry ingredients and water or fruit juice is used for liquid in muffin formulas. Milk powder binds flour protein to provide strength, body, and resilience—qualities helpful in reducing damage during packing and shipping (51). In addition, milk powder adds flavor and retains moisture. The aldehyde group from lactose in milk combines with the amino group from protein upon heating, contributing to Maillard browning.

7. Sodium Chloride

The amount of salt in muffins is 1.5% to 2% based on flour at 100% (5). The function of sodium chloride is to enhance the flavor of other ingredients. Sodium chloride may be omitted from the formula without compromising flavor, if other ingredients such as dried fruit or spices are added for flavor.

$$3CaH_4(PO_4)_2 + 8NaHCO_3 \rightarrow Ca_3(PO_4)_2 + 4Na_2HPO_4 + 8CO_2 + 8H_2O$$

Monocalcium Phosphate; Sodium Bicarbonate; Tricalcium Phosphate; Disodium Phosphate; Carbon Dioxide; Water

FIGURE 16.1 Formation of bicarbonate of soda from a fast-acting acid salt.

Step 1.

$$Na_2(A1)_2(S0_4)_4 + 6H_2O \rightarrow 2Al(OH)_3 + Na_2SO_4 + 3H_2SO_4$$

Sodium Aluminum Sulfate; Water; Aluminum Hydroxide; Sodium Sulfate; Sulfuric Acid

Step 2.

$$3H_2SO_4 + 6NaHCO_3 \rightarrow 3Na_2SO_4 + 6H_2CO_3 \rightarrow 6CO_2 + 6H_2O$$

Sulfuric Acid; Sodium Bicarbonate; Sodium Sulfate; Carbonic Acid; Carbon Dioxide; Water

FIGURE 16.2 Formation of bicarbonate of soda and carbon dioxide from from a slow-acting acid salt.

8. Liquids

Liquids perform several functions in baked products (5). These include dissolving dry ingredients and gelatinization of starch and providing moistness in the final baked product. Insufficient liquid results in incomplete gelatinization of the starch and a muffin with insufficient structure to support expansion of air volume. The muffins will have non-uniform cell structure, overly crumbly texture, low volume, and a dip in the top.

9. Additional Ingredients

Other ingredients are often added to muffins for variety in flavor, texture, and color, and to increase the specific nutrients or health components such as fiber, vitamins and minerals, or antioxidants from fruit and vegetable extracts. Part of the flour may be replaced with cornmeal, bran, whole-wheat, oat, or other flours to increase the fiber content. Adjustments in the amount of water in the formula are necessary when whole-wheat flour, bran, or other concentrated sources of fiber are added because fiber absorbs a great deal of water (51). An example of a concentrated source of fiber is Caromax™ (National Starch & Chemical, Bridgewater, NJ)(42). Nutrifood® (GNT USA, Tarrytown, NY), a liquid concentrate marketed as a blend of the antioxidants carotenoids, anthocyanins, and polyphenols is an example of a bioactive ingredient (43).

Other ingredients can be substituted for part of the liquid. For example, applesauce, bananas, shredded carrots, or zucchini. Variations in texture are achieved by adding fresh fruit such as apples or blueberries or dried fruit such as dates, raisins, or apricots. Nuts and poppy seeds complement the flavor of sweet muffins while grated cheese, whole-kernel corn, green peppers, chopped ham, and bacon add interest to corn muffins. Added flavorings include cinnamon, nutmeg, allspice, cloves, and orange or lemon zest. Topping mixtures such as chopped nuts, cinnamon, and sugar are added to the batter after depositing.

III. PROCESSING STAGE 1

A. Mixing

There are two primary methods for mixing muffins—the cake method and the muffin method. The cake method involves creaming sugar and shortening together, followed by adding liquid ingredients with the final addition of dry ingredients. The muffin method of mixing involves two to three steps. First, dry ingredients are mixed together; second, shortening or oil and other liquids are mixed together; and third, the liquids are added to the dry ingredients and mixed until the dry ingredients are moistened. Additional ingredients are added at the end of the mixing cycle or after depositing the muffin batter. Institutional or commercial bakeries use a mixer on slow speed for three to five minutes. Inadequate mixing results in a muffin with a low volume, since some of the baking powder will be too dry to react completely.

IV. PROCESSING STAGE 2

A. Depositing

The traditional size of muffins is two ounces although today muffins are marketed in a wide range of sizes from one-half ounce mini-muffins to muffins five ounces or larger in size (51). For institutions or bakeries, small batter depositors are available that will deposit four muffins at a time. Also available are large piston type depositors that maintain accurate flow of the batter (5).

V. PROCESSING STAGE 3

A. Baking

Many physical and chemical changes occur in the presence of heat to transform a liquid batter into a final baked muffin. Solubilization and activation of the leavening agent generates carbon dioxide that expands to increase the volume of the muffin. Gelatinization of starch and

coagulation of proteins provide permanent cell structure and crumb development. Carmelization of sugars and Maillard Browning of proteins and reducing sugars promote browning of the crust. Reduced water activity facilitates Maillard Browning as well as crust hardening (38).

The choice of oven, baking pans, and baking temperature influence the final baked product (5). A good flow of heat onto the bottom of the pan is necessary to produce a good product. Muffin tins are usually placed directly on the shelf or baking surface. The appropriate oven temperature is related to scaling and the type of oven. Standard two-ounce muffins are baked at 204°C or slightly higher in a deck oven. Deck ovens may be stacked and are often used in small retail bakeries since these are less expensive and easier to maintain than reel or rotary ovens. Reel ovens consist of an insulated cubic compartment six or seven feet high. A Ferris wheel type mechanism inside the chamber moves four to eight shelves in a circle, allowing each shelf to be brought to the door for adding or removing muffin tins from the shelves (32). Retail bakers often prefer the reel oven since several hundred to several thousand pounds of batter can be baked each day. Rack ovens may be stationary or the racks may be rotated during baking.

VI. PROCESSING STAGE 4

A. Cooling

Products should be cooled prior to wrapping. This allows the structure to "set" and reduces the formation of moisture condensation within the package. Condensed moisture creates an undesirable medium that promotes yeast, mold, bacterial growth, and spoilage.

VII. PROCESSING STAGE 5

A. Packaging

Muffins may be wrapped individually, in the tray in which they are baked, or transferred into plastic form trays for merchandizing (5). The shelf life of muffins is three to five days for wrapped muffins, and four to seven days for those packaged wrapped in foil or plastic wrap. The storage life of muffins is significantly influenced by exposure to oxygen and moisture (46). Cake muffins have a longer shelf life than bread muffins because of the high sugar content and lower water activity (51). Added ingredients such as cheese, ham, and dried fruits, high in sodium or sugar content, reduce water activity and increase the shelf life.

VIII. FINISHED PRODUCT

A muffin fresh out of the oven will vary in appearance based on the formula, whether the formula is for a cake or bread muffin; the size of the muffin, mini-muffin or mega-muffin; and the desired shape from flat or mushroom shaped tops to the traditional bell-shaped muffin (51). In general, a desirable muffin product has a symmetrical shape, a rounded top golden brown in color, cells that are uniform and moderate in size, a sweet flavor and pleasant aroma, is tender and moist, is easily broken apart, and is easy to chew with a pleasant aftertaste.

A. Muffin Evaluation

Bakers can use the Table 16.6 Scorecard for Muffins to evaluate muffins in the process of developing or modifying muffin formulas. Large commercial bakeries may use more sophisticated methods to evaluate bakery products, such as gas chromatography to evaluate flavor components.

1. Volume

Compact muffins with small cells or large muffins with peaked tops and tunnels are undesirable in all types of muffins. Diameter is a more important criterion than volume for evaluating mushroom and flat topped muffins. For bell shaped muffins, volume can be evaluated objectively by measuring the height and width of the muffin ($\pi r^2 \times$ height). The volume can be determined indirectly by measuring the circumference of a cross section of the muffin in cubic centimeters and dividing by the weight in grams. This can be done by measuring the height of the muffin at the highest point, then slicing off the top of the muffin and measuring the diameter of the muffin.

2. Contour of the Surface

The muffin should be rounded with a pebbled surface.

3. Color of Crust

Crust color should be a pleasing golden brown, not pale or burnt.

4. Interior Color

Crumb color should be a pleasant creamy color, not white and not too yellow. Crumb color will be darker with whole grain flour or added ingredients such as nuts or dried fruits, or spices.

5. Cell Uniformity and Size

Cell structure can be evaluated by making a vertical cut in the muffin to form two equal halves and then making an ink print or photocopy (34). A desirable muffin should have a uniform cell structure without tunnels.

6. Thickness of Cell Walls

Uniform thick-walled cells are desirable. Coarseness, thin-cell walls, uneven cell size, and tunnels indicate poor grain.

7. Texture

Texture depends on the physical condition of the crumb and is influenced by the grain. A desirable muffin should be easily broken, and slightly crumbly. Extreme crumbling, or toughness with lack of crumbling, are undesirable characteristics.

8. Flavor

An acceptable muffin should have a pleasingly sweet flavor. Flat, foreign, salty, soda, sour, or bitter tastes are undesirable.

9. Aftertaste

An acceptable muffin should have a pleasant, sweet aftertaste, not bitter or foreign.

10. Aroma

Aroma is recognized by the sense of smell. The aroma may be sweet, rich, musty, or flat. The ideal aroma should be pleasant, fresh, sweet, and natural. Sharp, bitter, or foreign aromas are undesirable.

11. Mouthfeel

Mouthfeel refers to the textural qualities perceived in the mouth. Characteristics can be described as gritty, hard, tough, tender, light, and moist. A desirable muffin is tender, light, and moist, and requires minimal chewing.

ACKNOWLEDGMENTS

The information in this chapter has been derived from a chapter in *Food Chemistry Workbook*, edited by J.S. Smith & G.L. Christen, Science Technology System, West Sacramento, California, 2002. Used with permission.

The author acknowledges Ron Wirtz, Ph.D., former Library Director, AIB, and currently Head, Education & Information Services, Greenblatt Library, Medical College of Georgia.

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17 Cereals–Biology, Pre- and Post-Harvest Management

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CONTENTS

I. INTRODUCTION

Cereals are the most important crops in the world. They dominate world agricultural production since they directly or indirectly provide a large proportion of the human sustenance. About half of the plowed land in the world is used for growing the principal cereals. Cereal grains commonly contain 60–70% starch and 7–14% protein. They are the most important source of carbohydrates for humans and domestic animals, and also provide a substantial proportion of protein. Cereals are the staple foods for many people everywhere on the globe, particularly in the developing countries, because they are a relatively cheap source of calories and protein compared to meat.

Grain is a collective term applied to cereals. The main cereal grains include wheat, rice, maize, barley, sorghum, rye, oats, and millets. The majority of the world population subsists mostly on wheat, rice, and maize. These three cereals account for more than 80% of total cereal grain yield in the world. Other cereals belong to minor cereal crops called coarse grains. Most cereals can be used as staple grains for humans, but maize, barley, oats, and grain sorghum can also be used as feed grains for livestock, particularly in developed countries.

Cereals are members of the grass family Gramineae, being monocotyledonous angiosperms. They show great genetic diversity and are ubiquitous, ranging in adaptation from the semi-arid and humid tropics to both wet and dry temperate zones, and even to very cold climates (e.g., rye and oats). Typical characteristics include fibrous root systems, an upright stalk with nodes and internodal spacing and a pithy stalk, and narrow long blade leaves with parallel veins. Cereal grains are single-seed fruits called caryopses. The seed consists of seed coat, embryo (germ) and endosperm. Cereal grains contain starch, protein, lipid, fiber, vitamins, and minerals.

Cereals are comparatively easy to grow and harvest, to store, and to process into versatile and popular foods. The chemical, physical, and biological properties of cereal grains determine their suitability for a specific market. Grain yield and quality are genetically controlled and climatically influenced, and also are dependent on field, pre- and post-harvest management during the growing, harvesting, drying and storage periods. Cereal grain losses occur throughout production, harvesting, threshing, drying, storage, marketing, and distribution. These losses are estimated to be nearly 20–30% of grain yield. Therefore, it is important for proper field, pre- and post-harvest managements to reduce or eliminate losses of cereal grains and provide high quality product to market.

II. BIOLOGICAL CHARACTERISTICS

Cereals are usually referred to their common names, such as durum wheat and common wheat. In botanical classification, cereals are monocotyledonous plants belonging to the Gramineae family. Each cereal has a genus, and the genus contains more than one species or subspecies. Each species of cereals has different varieties. Table 17.1 shows the genus, species, and common names of major cereal crops. Figure 17.1 shows the morphology of major cereal plants (1). General biological traits of different parts in cereal plants are described in the following sections.

TABLE 17.1
Scientific and Common Names of Major Cereals in the Grass Family (Gramineae)

Common Name	Scientific Name (genus and species name)
Common wheat	*Triticum aestivum*
Durum wheat	*Triticum durum*
Rice	*Oryza sativa*
Maize (corn)	*Zea mays*
Barley	*Hordeum vulgare*
Grain sorghum	*Sorghum vulgare*
Common oats	*Avena sativa*
Rye	*Secale cereale*
Foxtail millet	*Setaria italica*
Pearl millet	*Pennisetum glaucum*

A. ROOTS

Cereal plants have seminal or primary roots and coronal roots. Seminal roots produce when the seed germinates. Wheat, barley, rye, and maize commonly develop 3, 5, or 7 seminal roots, whereas rice and sorghum produce only a single branched seminal root. Seminal roots may function until the plant matures. After the young cereal plant unfolds a few leaves, coronal roots arise from stem nodes underground and develop into an elaborate root system. Coronal roots normally grow below soil surface, but sometimes arise from the nodes above soil surface as aerial roots (e.g., maize and sorghum). The root systems are responsible for absorption of water, fertilizers and mineral nutrients, and also for anchor and support of the plants.

B. STEMS

Stems of cereals are divided into nodes and internodes, being cylindrical, or nearly so. The length of internodes varies with position and by species. The basal internodes generally remain short. In addition to main stem, branches called tillers may grow from subterranean nodes in most cereals. A number of tillers can be produced from the primary stem, and they in turn develop others. The number of tillers varies significantly among different species and varieties of cereals. Generally 5–40 tillers may arise from a single seed under favorable planting conditions. Most cereals (e.g., wheat, rice, barley, oats, rye, and finger millet) are thin-stemmed grassy plants, but maize, sorghum, and pearl millet have thick stems more similar to sugar cane than grass.

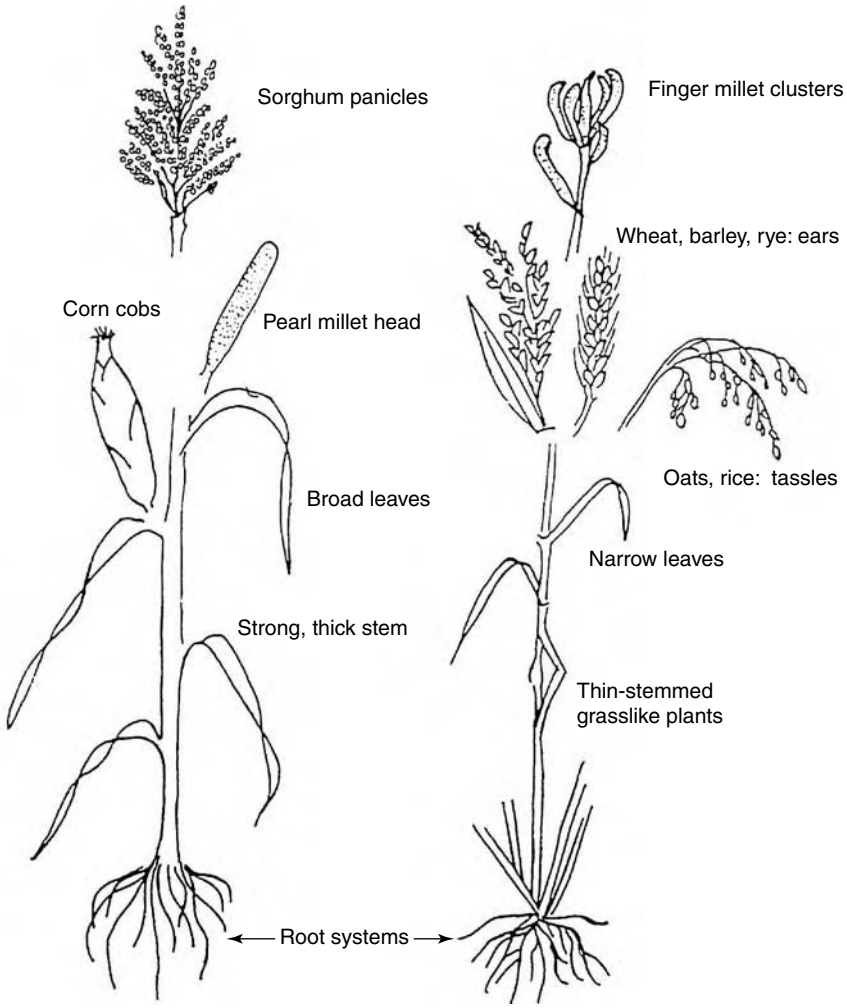

FIGURE 17.1 Morphology of major cereal plants. (Adapted from Ref. 1.)

The functions of stems are to support the plants and be responsible for transportation of water, fertilizers, and mineral nutrients. Resistance to lodging, the capacity of stems to withstand the adverse effects of rain, wind, and even diseases and pests, is an important characteristic of cereals because plant lodging usually results in serious loss of yield. Short, thick, heavy stems with thick walls are considered to be the best insurance against lodging. Excessive soil nitrogen may accelerate plant growth, which easily leads to increased lodging. A suitable balance of nitrogen, phosphorus, and potassium in the soil reduces lodging to a minimum (2).

C. Leaves

Leaves of cereals consist of sheath and blade (lamina), arising from buds that are lateral appendages of the stems. Cereal leaves are characterized by long, narrow, flat blades that taper to a point. The leaves of maize, sorghum, and pearl millet are broader and larger than those of other cereals. The leaf veins usually run parallel. At the junction of the sheath and blade is a thin membranous outgrowth called ligule that often is of taxonomic value. Lateral outgrowths called auricles occur above the ligule. Rice and barley have long clasping smooth auricles, while wheat has shorter hairy auricles, and rye has very short auricles. Oat sheath has no auricles. The sheaths of rye and oats and the leaf margins of oats have hairs (2). Furthermore, the shapes of the shieldlike ligule at the base of each leaf blade are also different. These leaf traits are very helpful to distinguish between cereal plants at the earlier developmental stages.

Leaves are mainly responsible for photosynthesis and transpiration. In photosynthesis, carbon dioxide and water react in the presence of light and chlorophyll in leaves and produce food and oxygen. In order for photosynthesis to take place, the stomata must be open, exposing the mesophyll tissues to carbon dioxide in the air. With the stomata open, the tissues are also exposed to the drying influence of air, resulting in evaporation. Evaporation of water through open stomata of leaves is called transpiration.

D. Inflorescences

Flowers of cereals are grouped on an axis of the inflorescence. The inflorescence of wheat, barley, and rye is a spike in which the sessile spikelets are attached along a rachis. Inflorescence of rice, oats, sorghum, and most of the millets is a branched panicle bearing spikelets on pedicels. A spikelet contains one or more florets subtended or enclosed by two bracts (lemma and palea) known as glumes. In the floret are the stamens, pistil, and lodicules. There are three stamens in each floret, except for rice which has six. Most cereals have perfect or bisexual flowers which contain both stamens and pistils. Maize and wild rice have separate staminate and pistillate flowers borne on the same plant. The staminate and pistillate flowers of maize are borne in separate inflorescences on the same plant. The staminate flowers are borne in the tassel at the top of the stalk, while the pistillate flowers are located in spikes which terminate lateral branches arising in the axils of lower leaves.

Cereals may be naturally self-pollinated (wheat, rice, barley, and oats), cross-pollinated (sorghum and proso millets), or naturally cross-pollinated (maize, rye, and pearl millet) (2, 3). Reproduction of cereal crops is by means of inflorescences which develop grains or seeds through pollination and fertilization.

E. Grains

The structure, chemical composition, and physical properties of cereal grains determine their suitability for a specific market. Knowledge of grain structure, composition, and physical properties is essential to drying, storage, milling, and even food processing of cereal grains.

1. Grain Structure

Cereal crops, like other members of the grass family, produce one-seeded fruit which is a caryopsis, but generally called a grain or kernel. Botanically, caryopsis contains a seed and a fruit coat (pericarp) which surrounds the seed and adheres tightly to a seed coat. The seed consists of a seed coat, an embryo (germ), and an endosperm. All cereal grains have these parts in approximately the same relationship to each other. The caryopsis of all cereals develops within floral envelopes which are actually modified leaves. These are called chaffy parts (glumes), and constitute the hulls or husks of cereal grains. In the threshing process, hulls are separated from the naked caryopsis, such as wheat, maize, sorghum, millets, rye, and naked barley, but not from rice, oats, and most cultivars of barley because their floral envelopes cover the caryopsis so closely and completely that they remain attached to the caryopsis after threshing. In addition, the kernels of wheat, rye, barley, and oats usually have a longitudinal crease (ventral furrow).

Cereal grains include pericarp, seed coat, endosperm, and embryo. The pericarp is composed of several layers and surrounds the entire seed. The starch-rich endosperm contains simple or compound starch granules, and it is the principal portion of the grains serving as food reserve for the embryo. The outermost layer of endosperm is called the aleurone layer, consisting of a single layer or more layers. Peripheral to the aleurone is a series of highly compressed remnant cell layers comprising the nucellus, seed coat, and pericarp. These, in combination with the aleurone layer, are usually referred to as the bran.

Grain size and proportions of the major parts of mature kernels from eight cereals are shown in Table 17.2 (4–6). Table 17.3 compares the major structural characteristics of

TABLE 17.2
Grain Size and Proportions of the Major Parts of Mature Cereal Kernels

Cereal	Germ (%)	Pericarp (%)	Aleurone (%)	Endosperm (%)
Wheat	2–3	12–15	—81–86—	
Rice	3.5	1.5	4–6	89–92
Maize	8.4–10	5.5–8		82–84
Barley	3.4	18.3	—79—	
Sorghum	7.8–12.1	—7.3–9.3—		80–85
Oats	3.7	—28.7–41.4—		54.9–67.6
Rye	3.5	12	—85—	
Pearl millet	17.4	—7.5—		75

Source: Adapted from Refs. 4–6.

TABLE 17.3
Structural Characteristics of Cereal Grains from Commerce

Cereals	Caryopsis Type	Ventral Furrow	Aleurone Thickness	Starch Granules
Wheat	Naked	Prominent	Single cell	Simple
Rice	Covered	Absent	Multiple	Compound
Maize	Naked	Absent	Single	Simple
Barley	Covered	Present, not prominent	Multiple	Simple
Sorghum	Naked	Absent	Single	Simple
Oats	Covered	Present, not prominent	Multiple	Compound
Rye	Naked	Prominent	Single cell	Simple
Pearl millet	Naked	Absent	Single	Simple

Source: Adapted from Refs. 4 and 7.

the cereal grains of commerce (4, 7). Longitudinal cross sections of the kernels from the three major cereals (wheat, rice, and maize) are shown in Figures 17.2, 17.3, and 17.4 (4, 5, 8, 9).

a. Wheat

Wheat kernels are 5–8 mm in length, 2.5–4.5 mm in width, and 30–45 mg in weight. The kernel size and shape of the wheat vary significantly depending on cultivars and kernel position in the spike. Also, there is wide variation in endosperm texture (hardness) and color of the wheat kernels. Wheat pericarp comprises about 12–15% of the kernel weight, removed in the milling process along with the aleurone layer of endosperm. Wheat endosperm constitutes 81–86% of the kernel weight, being the major end product of a wheat flour mill, whereas germ accounts for only about 2–3%.

Wheat pericarp surrounds the whole seed and includes outer and inner layers. The seed coat has three layers, a thick outer cuticle, a thin inner cuticle, and a pigment layer (only for colored wheat). The aleurone layer is the outermost layer of endosperm, containing thick-walled cells. Wheat germ is composed of two major parts, embryonic axis (rudimentary root and shoot) and scutellum (cotyledon). The starchy endosperm of wheat contains starch granules embedded in or surrounded by an amorphous protein matrix. Three types of cells (peripheral, prismatic, and central) make up most of the wheat endosperm. Wheat endosperm usually varies in hardness (texture) and appearance (vitreousness) among different cultivars. Generally, high-protein hard wheat varieties

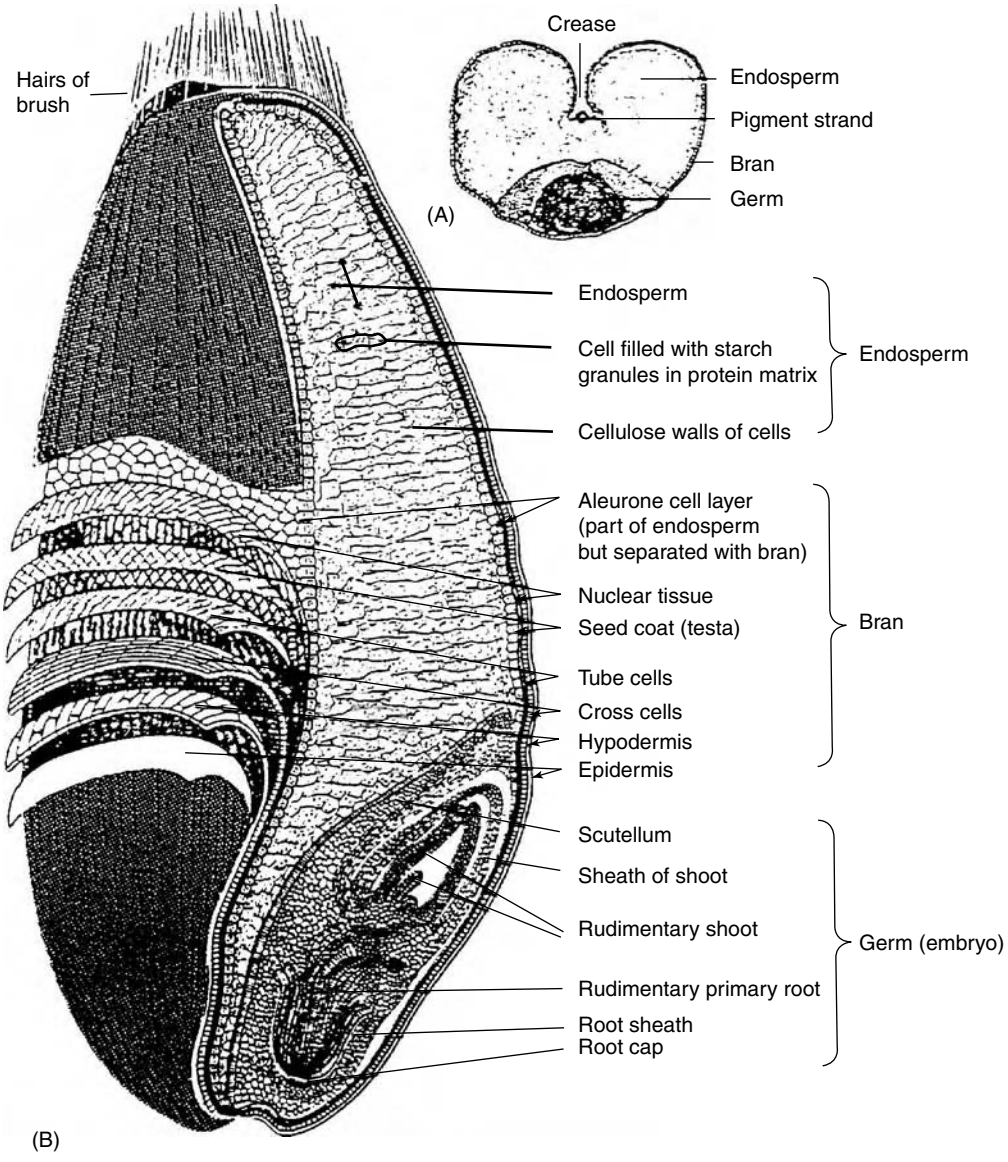

FIGURE 17.2 Diagram of a wheat kernel in (A) cross and (B) longitudinal sections. (From the Wheat Flour Institute, Washington, D.C.)

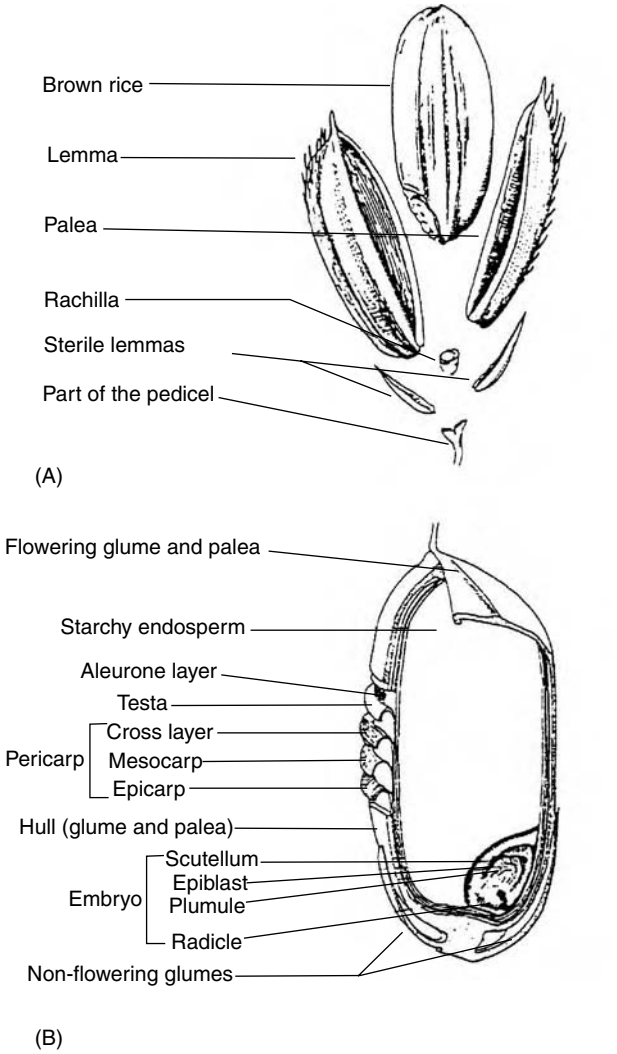

FIGURE 17.3 Structure of a (paddy) rice grain (A) and mid-longitudinal section (B). (Adapted from Ref. 9.)

tend to be vitreous, whereas low-protein soft wheat varieties tend to be opaque.

b. Rice

Rice grains are also called paddy or rough rice because of attached husks. Based on the length-to-width ratio, rice grains are divided into three grain types, long-grain, medium-grain, and short-grain. The grain is about 5–10 mm in length, 1.5–5.0 mm in width, and 20–35 mg in weight. A paddy rice grain consists of the husk (lemma and palea) (~20% in weight) and the brown rice kernel. Brown rice (rice after removing husk) contains pericarp (about 2%), seed coat and aleurone layer (about 5%), germ (3–4%), and endosperm (89–92%). After removing the pericarp during milling, brown rice produces white rice (milled rice). Rice endosperm is both hard and vitreous. Some rice cultivars have opaque areas called white belly, resulting from air spaces in the endosperm. The thin-walled endosperm cells are tightly packed, with polygonal compound starch granules and protein bodies. Protein bodies are more numerous in the cells just inside the aleurone layer than in cells near the center of endosperm. In addition to oats, rice is the only cereal with compound starch granules.

c. Maize

Compared to other cereal grains, maize has a unique shape and low specific gravity. Maize kernels are the largest cereal grains, weighing 250–300 mg each (wider variation 150–600 mg), 8–17 mm in length, and 5–15 mm in width. They are flat because of pressure during growth from adjacent kernels on the cob, have a blunt crown and a pointed conical tip cap. Maize kernel is composed of four parts, tip cap, pericarp, germ, and endosperm. The tip cap is often separated from the kernels along with the shelling process. Pericarp makes up about 5–7% of the kernel weight and germ constitutes about 8–11%. The endosperm comprises 82–84% of the kernel weight, being the major end product of maize flour mill.

There are seven major types of maize kernels, including dent, flint, flour, sweet, pop, waxy, and pod maizes (10). Their major differences are based on quality, quantity, and pattern of endosperm composition. Dent maize is characterized by the presence of corneous, horny endosperm at the sides and back of the kernels, whereas the central core is soft and floury. The indented crown is peculiar to dent types and is the basis for the term "dent corn." Flint maize has a thick, hard, vitreous endosperm layer surrounding a small, soft, granular center. The kernels are smooth and rounded. Popcorn is the most primitive race of maize, characterized by a very hard, corneous endosperm, essentially a small-kernelled flint. Flour corn is one of the oldest maizes, characterized by soft endosperm throughout the kernel. The endosperm of flour corn is almost floury, with little or no corneous endosperm. Sweet corn is believed to have originated from a mutation of a Peruvian race. The sugary gene of sweet corn retards normal conversion of sugar to starch, and the kernel accumulates a water-soluble polysaccharide, making up about 12% of the dry weight of sweet corn (but only 2–3% in other types). The kernel starch of waxy maize is almost amylopectin, a branched-chain α-D glucose polymer. Waxy maize is used to manufacture starch. Pod corn is an ornamental type with long glumes enclosing each kernel except husks covering the ear, but not grown commercially.

2. Grain Composition

High level of starch (starchy seeds) is the most important characteristic of all cereal grains. The starch contents of cereals usually account for 55~75% of total grain weight. Protein is another important component in cereal grains, consisting of 6~14%. Most cereal grains contain a low level of lipids, normally below 2–3%. Of all cereal grains, maize contains the highest level of oils; oil content of some varieties reaches about 6%. Additionally, moisture contents of cereal grains commonly range from 12 to 15%. Cereal fiber is an important source of human dietary fiber. Typical composition of three major cereals (wheat, rice, and maize)

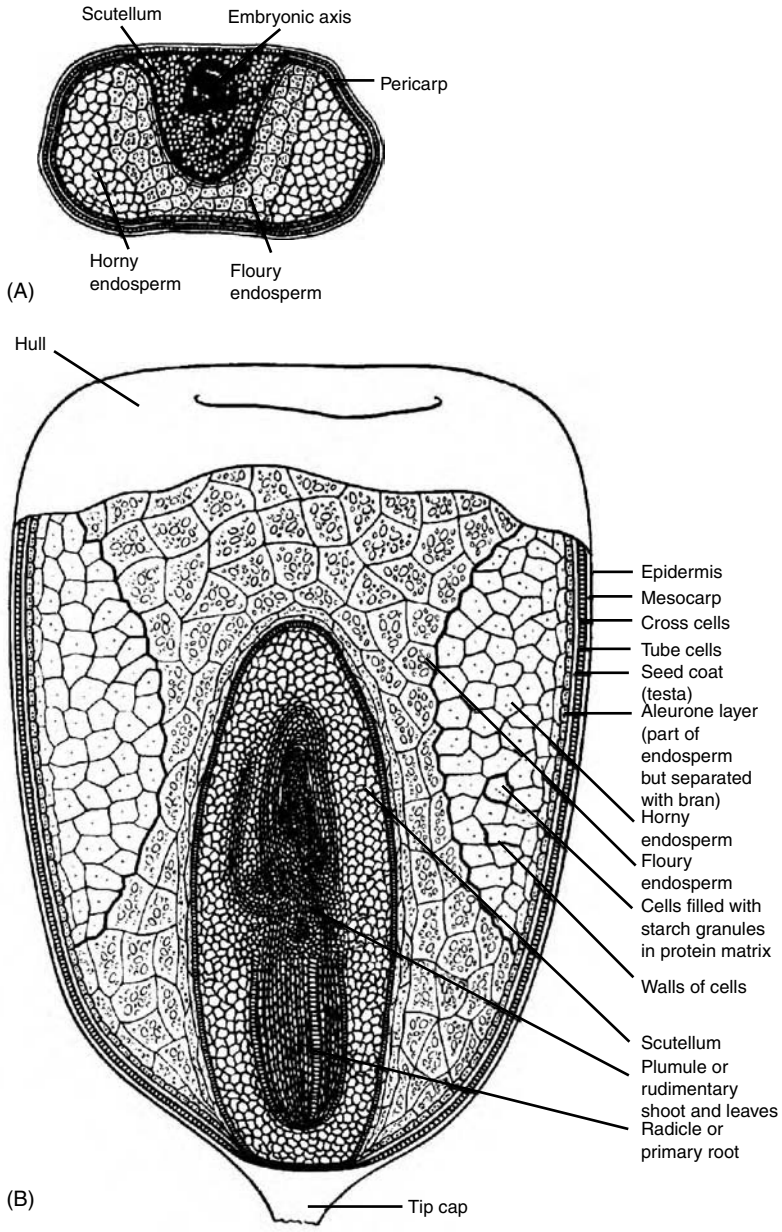

FIGURE 17.4 Diagram of a corn kernel in (A) cross and (B) longitudinal sections. (From the Corn Refiners Association.)

TABLE 17.4
Typical Composition of Three Major Cereal Grains[a]

Cereals	Starch (%)	Protein (%)	Fat (%)	Fiber (%)
Wheat	69.7	10.6	1.9	1.0
Rice (brown)	64.3	7.3	2.2	0.8
Maize	63.6	9.8	2.0	4.9

Source: Data from Refs. 5 and 11.

[a] At 14% moisture content.

is shown in Table 17.4 (5, 11). Other nutritional constituents of cereals have also been extensively investigated, including minerals and vitamins (10, 12, 13).

The chemical composition of the different parts in cereal grains varies widely, depending on position within the grain and also between grains of different species and varieties. Table 17.5 shows the composition distribution of endosperm, germ, and bran in wheat grain (14). Starch is mostly distributed in the endosperm. Protein and lipids normally occur in germ. Bran and germ contain more pentosans (cellulose) and minerals (14).

3. Physical Properties of Grains

Physical properties of cereal grains commonly include 1000-kernel weight, bulk density or test weight, repose angle,

TABLE 17.5
Composition Distribution of Endosperm, Germ, and Bran in Wheat Grain (%, dw)

Grain Parts	Total Weight (%)	Starch (%)	Protein (%)	Fat (%)	Pentosans (%)	Mineral (%)
Endosperm	82–85	70–85	8–13	1–1.6	0.5–3.0	0.3–0.8
Bran	15	0	7–8	1–5	30–40	3–10
Germ	3	20	35–40	15	20	5–6
Total Grain	100	60–70	10–14	1.5–2.5	5–8	1.6–2.0

Source: Adapted from Ref. 14.

porosity, thermal conductivity, grain hardness, etc. (4, 5). These properties are important in the design of handling equipment, drying and storage facilities, and influence drying and storage of cereal grains. Principal physical properties of the three major cereals are shown in Table 17.6 (5, 15).

The weight of grain is ordinarily given as the 1000-kernel weight. The 1000-kernel weight of maize is significantly higher than that of wheat and rice, which can translate to a lower drying rate for maize kernels. The weight of a given volume of grain including the voids is the bulk density, a widely used property which usually determines the bin volume required to store a certain mass of grain, and also affects the grain velocity in a continuous-flow grain dryer at a certain capacity. Wheat has the highest bulk density, while rice has the lowest. The void space in a mass of grain is expressed as a percentage of the total volume of the grain, and is called the porosity. The porosity of the three grains varies between 40% and 48%, typical of cereal grains. Resistance of bulk grain to airflow is, in part, a function of the porosity and the grain size.

When poured on a level surface, grain forms a pile whose outer edge makes an angle to that surface. The value of the angle is specific to the grain and is called the angle of repose which determines the maximum angle of a pile of the grain with the horizontal plane. It is important in the filling of a flat storage facility when grain is not piled at a uniform bed depth but rather is peaked. The slope of a pile of grain may be less than the angle of repose but not greater.

The specific heat expresses the energy required by a unit mass of grain to increase in temperature by 1°C. Of the three cereal grains, maize requires the most energy to reach the desired temperature at which the optimal evaporation rate of water occurs. Thermal conductivity is a determination of the resistance to the conduction of thermal energy within individual cereal grains. In grain with high conductivity, the thermal gradients in the kernels disappear faster during drying than in equal sized kernels of another grain with lower conductivity. The specific surface area refers to the kernel area per unit volume of cereals which exchanges energy and moisture with air during the drying process. For same volumes, there is more heat and mass transfer area for wheat and rice than for maize.

TABLE 17.6
Major Physical Properties of Wheat, Rice, and Maize (Typical Grains) at 12–16% Moisture Content (wb)

Physical Properties	Wheat	Rice	Corn
1000-kernel weight (g)	32	27	325
Bulk density (kg/m^3)	805	590	745
Test weight (kg/L)	77	58	72
Porosity (%)	41	48	40
Repose angle (°)	31	36	35
Specific heat (J/kg · °C)	1.67	1.51	2.01
Conductivity (W/m · °C)	0.137	0.106	0.159
Specific surface area (m^2/m^3)	1181	1132	784

Source: Adapted from Refs. 5 and 15.

III. GROWTH PROCESSES AND PRODUCTION

A. Growth Cycles and Habits

In general terms, the completed life cycle of plants refers to the stages that occur from the time a seed is planted until a new seed is produced, including seed sowing, germination, vegetative growth, reproductive growth, production of seeds, and death. Cereal plants pass through a typical vegetative growth and reproductive growth cycle (16). They grow primarily in a vegetative stage and then go into a reproductive stage. Stalks and leaves grow first and then start flowering and producing grains. Vegetative growth still continues during the reproductive stage, but at a much slower rate than prior to the start of reproduction.

Higher plants can be classified as annuals, winter annuals, biennials, or perennials (3). All cereals are normally annuals; that is, they complete the life cycle in one season. However, certain rice and sorghum species (e.g., wild rice) are able to live as perennials for several years in mild climates. Except in very cold climates, some cereals behave as winter annuals; that is, they live through the winter as small plants in the vegetative stage, and send up stalks and flower in the spring. There are spring and winter types of wheat, barley, oats, and rye. Winter varieties are planted in the fall, vernalized (require freezing temperature) during the fall and winter, and produce seeds and are harvested in the next summer. Spring varieties usually live in cold on mild climates, and are sown in the spring and harvested in the late summer and fall.

The length of the vegetative and reproductive period of any cereals or cereal varieties is greatly determined by temperature, photoperiod, plant nutrition, and growth

hormones, and also by genetic factors that affect reactions to these conditions. Each cereal species has an approximate minimum, optimum, and maximum temperature at which growth occurs, although varieties of a species may differ somewhat in their temperature reaction. When the mean temperature is 10°C below the optimum, the time required to grow a crop to maturity is about doubled. All cereal varieties with a winter growth habit, as well as some spring small-grain varieties, require cold for initiation of the flower primordia. Warmer temperatures favor rapid growth and flowering after the initial floral structure is formed.

Photoperiod is also an important factor affecting growth habits. When nights (daily dark periods) are short and days (photoperiods) are long, long-day cereals (wheat, barley, oats, and rye) can flower normally, otherwise their growth period will delay. Some can flower even under continuous light. However, when nights are long and days are short, short-day cereals (rice, maize, sorghum, oats, and millets) can flower normally, otherwise their growth period will postpone. They need a daily dark period for 3–5 weeks to initiate flowering. Additionally, soil rich in nitrogen will keep down the carbon-nitrogen ratio and delay flowering in wheat, barley, oats, and rye, known as nitronegative cereals, but favor early flowering in maize, sorghum, rice, and millets, called nitropositive cereals, unless the nitrogen supply is excessive. Therefore, wheat, barley, oats, and rye are cool-season, long-day, nitronegative, winter or spring habit plants, whereas rice, maize, sorghum, and millets are warm-season, short-day, nitropositive, spring habit plants.

B. Distribution and Production

Cereals grown in temperate climates are wheat, maize, barley, rye, grain sorghum, oats, and some millets. Important cereals grown in hot climates are rice, sorghum, pearl millet, and finger millet. Winter rye, wheat, barley, and oats can be suitable for cold climates. The world production of major cereals is shown in Table 17.7 (1). Wheat, rice, and maize are the three most important cereal crops, accounting for 70–80% of total area and total yield in the world. Major cereals are grown and distributed in about 170 countries and regions. The principal areas of cereal production are located in North America, China, Europe, India, Argentina, Australia, and Northern Africa (2, 17).

Wheat, the most important cereal crop in the world, is grown widely in the northern hemisphere in Europe and North America, but large quantities are also planted in China (the largest production of any country), Australia, India, Russia, and South America (Table 17.8) (14). About 95% of rice in the world is produced in Monsoon Asia, an area that extends across the southeastern part of Asia from India to Japan and includes most of the adjacent tropical and subtropical island countries. Maize is the highest yield cereal crop, distributing mainly in the United States, Canada, China, Brazil, Europe, and Africa.

TABLE 17.7
World Production of Major Cereals (1996)[a]

	Area		Yield			Countries	
Cereals	M Ha	%	M T	%	T/Ha	Number	%
Wheat	230	32.3	584	28.5	2.54	120	71
Rice	151	21.3	562	27.4	3.73	112	66
Maize	140	19.7	577	28.1	4.12	149	88
Barley	67	9.4	155	7.6	2.33	98	58
Sorghum	47	6.6	69	3.4	1.46	93	55
Oats	17	2.4	31	1.5	1.79	71	42
Rye	11	1.5	23	1.1	2.05	52	31
Millets	37	5.2	30	1.5	0.81	68	40
Total	710	100	2050	100	2.89	170	100

Source: Data from Ref. 1.

[a]M Ha, million hectare; M T, million tonnes; T/Ha, tonnes per hectare.

TABLE 17.8
Major Wheat-Producing Countries and Regions of the World (1997–1998)

Country/Regions	Yield (million tonnes)	Percentage (% total)
China	121.0	20.1
EU	95.8	15.9
USA	68.6	11.4
India	68.7	11.4
Russia	44.0	7.3
Europe	34.6	5.7
Canada	23.5	3.9
Australia	17.5	2.9
Pakistan	17.0	2.8
Turkey	16.0	2.6
Argentina	12.7	2.1

Source: Data from Ref. 14.

C. Factors Affecting Grain Production and Quality before Harvest

In addition to climate (days of sunshine, temperature, and precipitation), many other factors influence grain production and quality of cereal crops, including varieties, cropping systems (e.g., rotation), soils, fertilizers, water, weeds, diseases, insect pests, and so on. Grain yield and quality are formed and determined during the growing season. Thus, varietal selection, suitable rotation arrangement, and favorable field management are essential to cereal production.

The great contribution of good varieties to profitability of cereal production and quality is well recognized. Good varietal selection and utilization is the most important strategy to increase yield and improve quality. Numerous breeders and farmers are devoting to the breeding and improvement of new cereal varieties. Many excellent varieties with high yield, good quality, and resistance to diseases and pests, have been selected and bred for

extensive production in many countries including some developing countries. Modern biotechnology will play a more important role in these aspects.

Principal field managements include seed sowing, cultivation, fertilizers and water management, weed control, and disease and insect control. In addition to varieties, the yield and quality of all cereal crops are strongly dependent on the availability of an adequate supply of soil mineral nutrients and water throughout the growing season. The higher the yield potential, the higher the nutrient demand is. Nitrogen (N), phosphorus (P), and potassium (K) are three major nutritional elements required by cereal crop growth, largely affecting both grain yield and quality. Crop nutrition demand is normally met through the application of inorganic fertilizers (N, P, and K), although in organic cereal systems additional nutrients can only be supplied from manures and other organic sources. During major fertilizer application, an adequate ratio of N, P, and K should be controlled, normally around 3:1:1 (various ratio for different crop species/varieties); otherwise it will cause abnormal growth and affect grain quality. Other essential trace elements include sulfur, calcium, magnesium, iron, zinc, boron, and so on, normally being supplied by the fertile soil (17).

Water is also essential to the life processes of cereal plants. Water shortage occurs at the vegetative growth stages, which frequently makes shorter plants and earlier maturity. Water shortage at flowering and maturity periods usually causes serious decrease of grain yield and produces many bad grains. For different cereals, irrigation and drainage in the field should be conducted at the adequate growth stages. For instance, rice needs more water during tillering stage and flowering period, and irrigation should be done. At late tillering stage it is required to drain water for controlling tillers, and at late maturity drainage should be done again for full maturity and drying field for harvest.

Additionally, the presence of weeds, diseases, and insect pests during growth obviously reduces cereal grain yield, and influences grain quality. Control of weeds, diseases, and pests is also an important field management measure to maintain and protect grain quantity and quality during the growing season. In addition to using varieties with strong resistance to diseases and pests, pesticides/fungicides and bio-insecticides are usually used. Herbicides are widely applied to kill or control weeds in the field. Appropriate cultivations and rotations not only reduce the loss and damage from diseases and pests, but also control weed growth and decrease weed competition on cereals.

IV. HARVESTING AND THRESHING

Harvesting, threshing, drying, and storage are the important steps of pre- and post-harvest management between producing cereal grains and becoming various cereal food products. Successful, suitable, and efficient harvesting and threshing of cereal crops is one of the key steps to keep grain quality and quantity. It is desirable to know when to harvest, to understand harvesting and threshing machines and their operation, and to minimize losses during harvesting and threshing.

A. Harvesting Time

Cereal grains normally stop growing and gaining in dry weight when they approximately reach the hard-dough stage, or when the moisture content drops below 20~40%. Further ripening is only a desiccated process of the kernels, not accompanied by transport of nutrients into the grains. Ripening is not entirely uniform among different heads or different grains within a head. Most cereals at the hard-dough stage have a moisture content of 25~35%, the heads are usually light yellow, and the kernels are too firm to be cut easily with the thumbnail. Grain quality normally starts to deteriorate in the field prior to harvest. Rainfall prior to and during the harvest period may induce ear diseases and premature sprouting, and high grain moisture contents may necessitate increased drying costs. Cereal grains should be harvested as near maturity as possible to avoid losses of prematurity and overmaturity (3, 18).

Premature harvest reduces yield and quality of cereal grains. Underdeveloped grains are low in test weight, starch content, and market value. Cereal crops are usually harvested 7 days or more before they are ripe and allowed to dry under cool humid conditions, or 3 to 4 days early under warm dry conditions, without appreciable loss in yield and quality (3). Wheat grain sometimes draws material from the straw after it is cut when nearly ripe. Considerably more growth has occurred when immature barley grains were left to dry in the head than when they were threshed immediately.

Delayed harvest also influences grain yield and quality. Losses from the delayed harvest are caused by shattering, crinkling, lodging, and leaching. For instance, delay in rice harvesting leads to lower yield because of lodging and shattering, and overmaturity may induce the formation of longitudinal and multiple transverse cracks in rice grains. Formation of such cracks leads to milling losses. Delaying wheat harvest may result in grains with high α-amylase content, lower specific weight and protein content, which seriously reduce grain quality for breadmaking. Cereal stems may crinkle down or break over soon after maturity, especially in damp weather. Oats are more susceptible than barley to crinkling, while barley is more susceptible than wheat or rye. Most tall-stalked grain sorghum varieties go down soon after maturity or after a frost. Weathered or sun-bleached grains are unattractive and often bring a lower grade and price on the market. Overmature grains exposed to wet and dry weathering for long periods in the field are

TABLE 17.9
Moisture Contents for Wheat, Rice, and Maize at Harvest and for Safe Storage (%, wb)

Cereals	Maximum Harvest Moisture	Optimum Harvest Moisture	Moisture for Safe Storage	
			6–12 months	Over 1 year
Wheat	20	18	14	13
Rice (paddy)	38	22	14	13
Corn	25	23	14.5	13

Source: Data from Ref. 5.

lowered in test weight because the grains swell when damp and do not shrink to their original volume after drying (3).

Moisture contents of wheat, rice and maize at harvest are shown in Table 17.9 (5). Grain moisture content at harvest is an important consideration, and usually varies with seasons and geographic regions. In wheat, grain moisture at harvest should be about 10~20%. Rice is harvested at a moisture content of 20~30%. Maize is harvested at considerably higher moisture levels than wheat, especially at higher latitudes. Maize is often harvested at 30~35% moisture in the northern Corn Belt of the United States, and at 35~45% in Eastern Europe (5). In Britain, cereal grain is often harvested at moisture contents of about 16~20%, whilst in exceptionally late seasons in northern regions grain may be harvested at around 25% (18).

Barley is commonly harvested at about 15% moisture, but sometimes producers started harvesting at 30~40% moisture, resulting in a higher yield because of reduced losses at harvest. The fresh barley grain is either artificially dried for food use or stored in silage-type bins for livestock feed. Oats should be at around 13~14% moisture for harvest; however, since oats shatter easily, it is common practice to cut and windrow them when the grain contains about 20% moisture. When it dries to a moisture content of 13~14%, the grain is combined. Sorghum should be harvested at 18~20% moisture if it is to be dried, but at around 13% moisture if it is to go directly to storage (19).

B. Methods and Machines of Harvesting and Threshing

Nowadays, all cereal crops can be harvested by modern machines that replace traditional harvesting tools, such as hand sickle, scythe, and cradle. Because of high working efficiency, machine harvesting and threshing saves time and labor, reduces cost, and is extensively used in developed countries (17). However, manual harvesting and threshing is still widely used in most developing countries. For example, rice harvesting is mostly done manually in Asia and Africa. The rice plants are cut near the ground with a sickle, allowed to dry in the field, collected, stacked, and threshed. In Asia and Africa, paddy rice is traditionally threshed by manually beating the straws against a slat or stone, or by trampling under feet of animals, bullock draw carts, tractor wheels, or tractor-drawn roller. However, the drum thresher is also widely used nowadays for rice threshing in Japan, China, India, and others.

There are many types of machines for crop harvesting and threshing, such as harvester-threshers, mowers, windrowers, strippers, pickers, choppers, balers, rakes, cubers, stackers, and conditioners (3, 17). The word combine is used to describe machines that combine several harvesting and threshing operations into one. Grain combine is usually adapted to each cereal crop by making small changes in the cutter bar, the reel, or the thresher (cylinder or similar mechanism). For example, for maize, the cutter bar-reel combination used for wheat is replaced by a header which strips the ear of maize off the stalk.

The most common combine is the harvester-thresher combination used to harvest most cereal crops. Bishop et al. (19) summarized the basic operation of the combine, including the following procedures: 1) the grain is cut and conveyed to a thresher; 2) the thresher removes the grain from the head, or stalk, or cob; 3) the grain is separated from the hulls, straw, cob, pods, leaves, or similar plant materials; 4) the grain is cleaned by removing the rest of the chaff, dirt, and remaining trash; and 5) the grain is transferred to a bin. Additionally, in operating a combine, the various parts must be properly adjusted. The reel and cutter bar should be adjusted to the correct height, and the correct cylinder speed in the thresher should be employed for different grains.

The harvest-thresher or combine is most extensively and the earliest used to harvest wheat and barley. Maize is commonly harvested with a combination picker-sheller or a combine with a special header. These two machines can remove ears of maize from the stalk. The ears are then shelled by the machine, the grain is sent to the bin, and the rest of the plant material is discarded back onto the field. Combine harvesters are also used for rice harvesting in some developed countries. Sorghum may be harvested with a simple combine. Sorghum grain is relatively soft, and speed of the cylinder that threshes the grain has to be reduced to prevent grain damage.

Conditions of cereal crops at harvest have a significant influence on combine performance, e.g., severely lodged cereals not only produce inferior quality grains, but also reduce combine speed and efficiency. The presence of weeds also interferes with harvesting and often leads to higher grain moisture contents and contamination with weed seeds which increase cleaning costs.

C. Harvesting and Threshing Losses

Losses in yield can be due to improper harvesting techniques or harvesting too early or too late. If harvest begins too early, plants have not reached full maturity, causing shrinkage of the grain. If harvested too late, plants can

lodge or stalks can break, grain can shatter or ears can drop, and quality will deteriorate.

Factors affecting harvester efficiency and yield losses include combine adjustments, field speed, kernel moisture levels, and lodging. Suitable adjustment and operation of harvest equipment helps minimize harvest losses, and losses should not exceed 3% from a properly adjusted and operated combine.

Threshing losses are caused by incomplete threshing, physical damage by beaters in the machine, or even by hand spillage and cleaning losses in the winnowing and screening processes, which immediately follow threshing. Incomplete stripping is common in areas of high labor cost, causing paddy losses up to 12%. When harvesting takes place in the rainy season, the paddy is wet and can easily choke cleaners on mechanical threshers and cause losses. Maize shelling losses are also caused by incomplete stripping of grain from cob, kernel breakage in the machine, and kernel scratching. Small-toothed shellers give low damage with 3% loss, and threshing machines and combine harvesters can give more than 5% broken grains (20).

V. DRYING AND STORAGE

Drying and storage are important post-harvest managements for achieving high quality grain to meet market requirements. After harvesting, it is necessary to clean, dry, and store cereal grains and protect their quality and quantity properly until cereals are processed into food products. To protect cereal grains appropriately after harvesting, producers need to know how to clean, dry, and store grains, to regulate storage conditions (temperature and moisture), and to control insects, microorganisms, and rodents.

A. Grain Cleaning

For drying and good storage, chaff, dust, straw particles and broken grains should be removed from harvested grains. Weeds and other impurities (e.g., mud, stone, and sand) must be also separated from grains. For good quality to market, the grain must be as near 100% as possible of one variety free from broken, discolored grains and shriveled, undersized grains of the same variety. A typical grain cleaner often consists of two parts, a shaking screen and a fan driven from the same motor. Straw and other large objects are removed by screening, and dust and other lightweight materials are blown out by aspiration. Machines used to remove unwanted grains include three types: slotted cylindrical grader, indented cylinder, and optical sorter (20). Bailey (21) also introduced grain cleaning methods and equipment. Three common cleaning and separating methods are used in elevators, those using air streams, perforated screens, and indents.

B. Principles of Drying and Storage

Moisture is one of the major factors contributing to the deterioration of cereal grain quality during storage. Moisture content of grains is defined as the amount of water that can be removed without alterations in chemical structure, which varies depending on the types of grains (e.g., covered husk, naked, or pearled kernels), moisture at harvest, harvesting method, chemical composition, relative humidity of atmosphere, and seasonal fluctuations. Safe storage moisture content of grains is commonly defined as the amount of water at which the rate of respiration is low enough to prevent generation of heat and consequent deterioration (22, 23). For safe storage, wheat, rice, and maize grains have to be dried to 14% moisture or lower (Table 17.9) (5). For long-term storage (over 1 year), lower moisture contents are recommended to prevent microbial growth and insect reproduction. For short-term storage (1–3 months), moisture can be slightly higher (above 15%).

Equilibrium moisture content (EMC) is also important to grain drying and storage. EMC is defined as the moisture content of material after exposed to a particular environment for an infinite period of time, usually depending on humidity and temperature of the environment (5). Moisture content of cereal grains tends to equilibrate with the moisture of surrounding air. Wet grains lose moisture to dry air, and dry grains absorb moisture from wet air. Also, the amount of water held by a volume of air depends on temperature. When drying with air of a specified temperature and humidity, grain can dry to no lower than the EMC. Once the grain has reached the EMC, no further moisture transfer will occur. Dry grain will absorb moisture when exposed to air leading to an EMC higher than current moisture content of the grain (23). As shown in Table 17.10, EMC values increase with increase of relative humidity and decrease slightly with increase of storage temperature (5, 23). Furthermore, EMC values usually vary with differences in grain structures and maturity of various cereal grain species/varieties.

Infestation of insects and microorganisms is affected by interaction of grain moisture, relative humidity, and storage temperature (21, 22). Insects attack grains at moisture content above 8% at all ranges of relative humidity, if the storage temperature is above 19°C. Molds harm grains at above 60~65% relative humidity and above 12% moisture, irrespective of temperature at 20–40°C. To control both insects and molds, grains should be stored at or below 19°C and 60~65% relative humidity. This basically coincides with 12~14% EMC in the grains. However, it may not be economically feasible to maintain storage temperature at or below 19°C. Thus, safe storage moisture of grains is usually based on EMC at 27°C and 70% relative humidity, particularly in developing countries (Table 17.11) (22–24). Drying cereal grains to safe moisture levels and storing in moisture-proof structures sufficiently

TABLE 17.10
Equilibrium Moisture Contents (EMC) of Cereal Grains (%, db)

		Relative Humidity (%)									
Cereals	**Temp (°C)**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Barley	10	4.7	6.8	8.6	10	12	13	15	18	21	30.6
	20	4.6	6.6	8.4	10	11	13	15	17	21	29.9
Maize (yellow)	10	5.0	7.5	9.7	11	13	16	18	21	26	38.3
	20	4.6	6.9	8.9	10	12	14	17	20	24	35.2
	27[a]	4.2	6.4	7.9	9.2	10.3	11.5	12.9	14.8	17.5	–
	50[a]	3.6	5.7	7.0	8.1	9.3	10.5	11.9	13.8	16.3	–
Rice (rough)	10	6.3	8.6	10.4	12	13	15	17	19	22	29.5
	20	5.9	8.0	9.7	11	12	14	16	18	20	27.7
Sorghum	10	6.4	8.7	10.5	12	13	15	17	19	22	29.5
	20	6.2	8.4	10.2	11	13	14	16	18	21	28.6
Wheat (durum)	10	5.9	8.3	10.3	12	13	15	17	20	23	32.7
	20	5.6	7.9	9.7	11	13	14	16	19	22	31.0
Wheat (soft)	10	6.4	8.6	10.3	11	13	14	16	18	21	28.1
	20	6.1	8.2	9.8	11	12	14	15	17	20	26.7
Oats[a]	25	4.5	6.6	8.2	9.4	10.3	11.4	12.8	15.0	18.2	23.9
Rye[a]	25	5.3	7.4	8.8	9.8	10.8	12.2	13.9	16.3	19.6	25.7

Source: Adapted from Refs. 5 and 23.
[a] EMC (%, wb).

TABLE 17.11
Recommended Storage Moisture Content of Cereals Grains (%, wb)

	Duration of Storage in Midwest United States			Storage at 27°C and 70% Relative Humidity[a]
Cereals	**Through Winter**	**Through Summer**	**More Than 1 Year**	
Barley	14	13	13	
Maize	15	14	13	13.0
Oats	14	13	13	
Rye	14	13	13	
Sorghum	15	14	13	13.5
Wheat	14	13	13	13.5
Wheat flour				12.0
Paddy rice				14.0
Milled rice				12.0
Millets				15.0

Source: Adapted from Refs. 22–24.
[a]This storage condition is particularly suitable for the developing countries.

resistant to insects, molds, and rodents would greatly reduce storage losses.

C. Drying Methods and Facilities

1. Sun Drying or Natural Air Drying

In ancient times, harvested grains were usually sun-dried or air-dried. This traditional drying method is still widely used by farmers in developing countries and some developed countries. The bulk of the grain is still dried by spreading the wet grain in thin layer on the ground in open air to be dried by the sun. Sun drying is a very simple and cheap method of drying cereal grains harvested in the warm part of the year, but is not suitable for large quantities of grains, and not under wet weather conditions.

2. Mechanical Drying

Since industrialized times, mechanical drying has gradually replaced traditional drying methods. Mechanical drying essentially involves forcing or sucking ambient or heated air through the mass of grains. The major features of mechanical drying methods are: 1) drying rate can be controlled by regulating air temperature ventilating through the grains; 2) grains can be dried irrespective of weather, and does not depend on natural resources like solar energy; 3) losses due to insects, birds, rodents, or rains are eliminated; 4) it requires little space for drying; 5) turnover is high; and 6) drying cost is much higher than sun drying (22).

a. Drying systems

Mechanical drying systems can be divided into on-farm drying and off-farm drying (5). On-farm driers have three categories: bin, non-bin, and combination driers. Bin driers are normally low-capacity and low-temperature systems, able to produce excellent quality grain. Non-bin driers, the most popular drier type in the United States, are high-capacity and high-temperature systems that frequently overheat and overdry the grain, thus easily causing grain quality deterioration. Combination drying uses the non-bin high-temperature drier to dry the grain from harvest moisture content to 16–20% and completes the drying process

with in-bin drying. Combination drying has the advantages of both systems (i.e., high capacity and good quality), but requires additional investment and is more complicated.

Off-farm or elevator grain driers also have three categories: crossflow, concurrent flow, and mixed flow, which are high-capacity and high-temperature units. Crossflow models dry grain nonuniformly, causing considerable stress cracking of the kernels. Mixed-flow driers dry the grain more uniformly; the dried grain is normally of higher quality than that dried in crossflow models. Concurrent-flow driers have counterflow coolers and produce the highest quality grain; their disadvantages are relatively high capital cost and complexity of the technology. A significant improvement in grain quality can be obtained with off-farm driers by optimizing the operating parameters and by installing automatic drier controllers.

Salunkhe et al. (22) summarized the two major types of mechanical drying systems advocated for either batch or bulk drying process in developing countries, i.e., low-temperature drying and medium-temperature drying systems. In low-temperature drying, there is an increase in airflow through the grains by means of a fan. The grains gradually reach a moisture level in equilibrium with the relative humidity of ambient air which must be below 70% for such drying. Various drying-cum-storage structures employed for such process are on-the-floor drying, in-bin drying, or tunnel drying. Medium-temperature drying is needed when air humidity is above 70%, initial grain moisture is too high, or ambient temperature is too low to effect drying before deterioration begins. Warm air is blown through a bed of grain of controlled depths, and the drying process ceases before EMC is reached. This can be applied for both batch and bulk drying. Medium-temperature driers have several types, such as tray drier, radial-flow drier, sack drier, and multi-duct ventilated flow drier.

Brook (23) comprehensively described the mechanical grain drying systems commonly used in the United States, including 1) Fans: two typical fans, axial-flow fan and centrifugal-flow fan, are used for forcing drying air through a mass of grain. 2) Low-temperature bin driers: drying with natural air or air with temperature increased by up to 5°C. Drying and storage occur in the same bin, called an in-bin drier which consists of high-capacity fans, grain unloading equipment, perforated drying floor, heater, spreader, and grain stirrer (Figure 17.5) (23). These are low initial investment and low-drying-capacity systems best for grains with low moisture levels at harvest and for drier geographical areas with low humidity. 3) High-temperature bin-batch driers: can dry batches of grains with heated air and remove moisture in a shorter time, with drying temperatures of 35–60°C. In these systems, drying and storage occur in separate bins. They require moderate initial investment and medium energy use, and often cost less per unit drying capacity but require more labor because of transfer of each batch to storage. Two typical high-temperature bin-batch

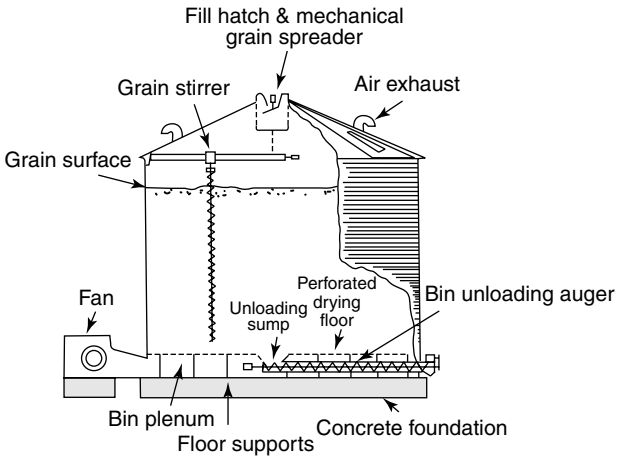

FIGURE 17.5 Low-temperature bin drier. (From Ref. 23.)

driers include on-floor bin-batch driers and roof bin-batch driers. 4) High-temperature column driers: characterized by multiple vertical columns that hold grains while the air is forced horizontally through grains, with airflows of 80–100 m^3/min·t and with drying temperatures up to 100°C. These are advanced driers which require moderate to high initial investment and high energy use. Three common types of high-temperature column driers are manual batch column driers, automatic batch driers, and continuous-crossflow column driers. 5) Continuous-flow bin driers: have a bin with a perforated drying floor, fan, heater, grain spreader, grain-unloading equipment, and an auger to transfer grain to storage. They unload hot and dried grains semicontinuously from the bottom of the drying bin to storage bins for cooling. Grain flow is automatically controlled to limit overdrying, with typical airflows of 10–25 m^3/min·t and with drying temperatures of less than 80°C. 6) Others: concurrent-flow driers, fluidized-bed driers, spouted bed driers, rotary drum driers, and so on. These belong to high-investment and high-drying-capacity drying systems. New drying technologies, such as differential grain-speed crossflow drying, multistage concurrent-flow drying, multistage mixed-flow drying, and cascading-rotary drying, have the potential to decrease energy consumption and grain deterioration by about 50%.

b. Cooling equipment

Cooling equipment is one part of the drying systems. Grains dried through any heated air driers should be cooled in time, otherwise causing quality deterioration. Delayed-cooling methods are usually used to decrease energy consumption during high-temperature drying, increase drier throughput, and reduce stress cracks and breakage susceptibility.

The three most commonly used delayed-cooling methods are in-bin cooling, dryeration, and combination high- and low-temperature drying (23). The in-bin/in-storage

cooling is the simplest delayed-cooling method suitable for any type of high-temperature driers. Dryeration is an energy-efficient method of delayed-cooling and drying completion, and can cut 15–30% off energy consumption of high-temperature drying while increasing drying capacity, although dryeration requires more management. With dryeration, hot grains are transferred to the cooling bin immediately after drying. Cooling is delayed at least 4 hours for steeping or tempering. After grains are cooled in the dryeration bin, they should be moved to storage. Combination high- and low-temperature drying is most suitable for crops harvested too wet for safe low-temperature bin drying, particularly wet maize (>22% moisture content). A combination system normally costs more than a single drying method, but can give high drying capacity, flexibility of high-temperature drying, and energy savings and superior grain quality of low-temperature drying.

3. Drying Losses

Drying losses are caused by underdrying or overdrying, from pre-harvest to post-storage. Two kinds of losses often occur. The first is actual removal of moisture from the grain system, and the second is damage to the grain during drying, leading to subsequent loss during milling (15).

Modern drying systems normally cause lower losses to most cereal grains. However, drying of rice or maize by mechanical driers requires more care to avoid losses in processing properties. In developing countries, threshed grains or harvested ears are usually spread on a hard surface in the farmyards for sun drying, which easily causes higher grain losses. Part of the grains may be eaten by birds and other animals, or may be blown away. If grains are rewetted in the drying yard by a sudden storm, they will be damaged.

D. Storage Methods and Facilities

Since ancient times, peasants have used traditional methods and containers to store cereal grains, such as small buildings, earthenware pots, small wooden containers, underground pits, outdoor piles, woven bags and baskets. Most traditional methods and containers are still employed in many developing countries. In recent centuries, advanced storage technologies and facilities have been developed to provide safe storage environments of grains and to maintain grain quality and quantity more efficiently.

Various types of storage facilities have been introduced (15, 21, 22), including piles of unprotected grain on the ground, underground pits or containers, piles of bagged grain, storage bins of many sizes, shapes, and types of construction. Major classifications are farm storage, bin sites, country elevators, and terminal elevators. Storage structures and facilities should meet the following major requirements: 1) provide maximum protection from moisture, insects, molds, and rodents; 2) have aeration design for regulation of storage temperature and relative humidity; 3) allow smooth in-and-out movement of grains; 4) be sufficiently airtight for fumigation; and 5) have capacity to protect grains from fire.

1. Farm Storage Facilities

Harvested cereal grains are often or temporarily stored at the farm in developing countries. Farm storage structures are constructed from locally available materials which are simple and cheap, and are based on the principle of hermetic storage with aeration facilities. Major structure types of farm storage in India include circular steel bin, plastic bin, pre-fabricated steel bin with hopper bottom, aluminum bin, reinforced cement concrete bin, cement masonry bin, welded wire-mesh bin, ferro-cement bin, and so on (22). In China, simple storage structures are built with local cheap materials, e.g., sticks, mud, stalk or straw, timber.

Modern farm silo is basically a large cylinder of corrugated galvanized steel, with means of access for cleaning, input, and extraction of grain and, sometimes, a built-in drier consisting of a fan with air heater and a means of distributing the warm air through the grain so that moisture can be removed (15). Since modern silo is with good storage conditions, and is durable and secure from attack by predators and even thieves, it has replaced other storage facilities for farm storage in developed countries.

2. Commercial Storage Facilities

Commercial storage systems involve storage of large quantities of cereal grains, with better storage structures and functions. In China, storage facilities of town-level and county-level, normally constructed of concrete, are the important commercial storage systems, with moisture- and rodent-proof structures, and with an arrangement for controlled aeration as well as fumigation.

Elevators are the most common, advanced commercial storage facilities in developed countries. In addition to storage, elevators have other functions such as sorting, cleaning, sizing, drying, and fumigation. They serve the marketing system by equating supply with demand, by providing convenient means for transferring title by endorsement of warehouse receipts, and by transferring grain from one transportation facility to another such as trucks to cars, cars to barges, and barges to ocean vessels.

Country elevators receive grains directly from producers. These storage facilities are usually constructed of wood, concrete, or steel. Their main function is to accumulate grains from nearby farms, to reload them into transportation facilities such as trucks, railroad cars, or barges, and to send them to market. Country elevators were originally intended to serve an area limited by horse and wagon delivery, and their storage capacity was very small. Truck transport from farms has broadened access areas, and elevator sizes have increased accordingly. The

largest elevators approach the size of terminal elevators and are often referred to as subterminals.

Terminal elevators are ordinarily constructed in transportation terminals and larger markets. Their storage capacity is very large, normally ranging in total storage space from 5,000 to 500,000 tons or more (200,000 to 20 million bushels). They receive grains from country elevators by truck or railroad and transfer them to storage or into other transportation equipment such as barges or other vessels. Terminal elevators also equip with high-capacity facilities for cleaning, drying, and conditioning of grains.

Flat bins are auxiliary bins of large capacity in connection with terminal elevators. The pressure of grain surpluses requiring safe storage for long periods leads to the construction of flat bins. These bins are built wider and lower than common silo storage bins to reduce costs and side-pressures, thus can provide ample storage space at the lowest possible cost. Floors are directly on the ground, handling equipment is kept at a minimum, and the roof tends to follow the slope of the pile of grains. Large bins may be directly attached to the elevator or may be built adjacent to it.

Additionally, the slip-form storage bins (silo-type bins) for construction of round concrete bins were developed in about 1900 and are still in commercial use. The slip-form consists of a concentric, double-ring form into which concrete is poured. Often two or more rows of round bins are built side by side to form a block of bins. The areas between the circles also become bins, and are called interstitial or star bins. Concrete silos may be as high as 40 m. Although other shapes such as oval or hexagonal have been tried with concrete, the round form gives the greatest strength with the least material.

In developed countries, grain is almost completely handled and stored in bulk, but in developing countries bag storage is still practical and common, since farms are often scattered and labor for filling and loading bags costs is low. Bag is made of plant fiber or woven polymer threads. Air can pass through this barrier, and moisture can migrate from or into the grain, but grain in bags can normally be accepted for storage at 1–2% higher moisture content than for bulk storage. Bags with grains are usually stored in a well-sealed and secure warehouse with regulating openings for controlled ventilation and complete building fumigation. Openings can be used for both natural air and fan-controlled aeration. Bags should be carefully stacked so that alternate layers are placed that can be covered with a tarpaulin for fumigation. A one-meter corridor is left between stacks for the operatives to pass when examining the storage state.

E. Aeration and Stored Grain Management

Dried grains should be aerated as soon as possible after they are put into storage. Frequent aeration during storage is necessary to control grain temperature, to avoid formation of hot spots due to insect and mold activity, to reduce the risk of grain damage or spoilage, and to maintain grain quality (22, 25).

1. Effects of Aeration

Aeration is the process of moving air at ambient temperature through stored grains in order to decrease or increase grain temperature to desired level (5). The major effect of aeration is to control and maintain a uniform temperature in stored grains and to keep that temperature as low as is practical. Also, aeration can equalize grain moisture and remove odors from stored grains. Use of fumigants with aeration systems can permit their distribution through the grain in deep bins and silos.

2. Aeration Systems

An aeration system mainly consists of fan and duct systems. The major considerations in the design of aeration systems are airflow rate, fan selection, and air distribution (25). The airflow rate recommended depends on purpose of aeration, type of stored grains, storage structures, and climate conditions. Fan selection is based on airflow rate and type and depth of stored grains. There are two common kinds of fans used for aeration, axial-flow fans which are suitable for horizontal storage structures, and centrifugal fans suitable for vertical storage structures. Other fans, such as radial-bladed fans, are suitable for vertical low-capacity bins. Occasionally, aeration fans are mounted on top of storage bins or silos. Some aeration systems are equipped with two fans, a pressure fan on the bottom and a suction fan on top. Airflow is usually downward in a suction system and upward in a pressure aeration system.

Duct systems are used for air distribution. Most aeration systems use perforated ducts to distribute the air and provide for air movement whether out of the duct into the grain in a pressure aeration system or into the duct from the grain in a suction system. Many farm bins are equipped with full-perforated false floors or partial perforated floors. In flat storages, large bins, and tanks, more than one ventilating duct is required, and the layout of the duct system is also important.

3. Operation and Management of Aeration Systems for Stored Grains

Foster and Tuite (25) described how to operate aeration systems, including seasonal aeration operating schedules, daily fan operation, and airflow direction regulation. Aeration practices depend on local climate, e.g., the mean temperature difference between the warmest month and coldest month is 28°C in the Corn Belt of the United States. The mean daily humidity of the air is usually below that in equilibrium with grain at safe storage

moisture content, except in winter. Under mean climatic conditions, grain can be aerated continuously without undesirable moisture increase.

Once grains are put into storage, they should be aerated immediately to remove harvest or dryer heat. The temperature should be uniformly reduced to 15–20°C for summer-harvested grains and to 10–15°C for fall-harvested grains. Grains are aerated in late fall and winter to cool them to below 5°C. Weather in late November and early December is usually suitable for cooling grains to near mean winter temperatures. Once this is completed, no further aeration is recommended until spring. In March or April, grains may be aerated to warm them to above 10°C. After spring aeration is started, it should continue until grains are uniformly warmed to near mean outdoor temperature. As the warming front moves through, moisture condenses on the cold grains, providing ideal conditions for rapid mold growth. Continued aeration keeps the condensation area moving and helps avoid serious spoilage.

Most seasonal aeration schedules described above are based on daily 24-hour fan operation. In much of the United States, mean humidity in the fall cooling and spring warming periods permits continuous fan operation without increasing moisture of cereal grains above the safe level (13–15%). However, in rainy seasons or regions with higher humidity, fan operation should be limited or even prohibited in high-humidity weather. For instance, in Central, Eastern, and Southern China, aeration is limited or prohibited in rainy weather of late spring and summer.

4. Losses during Storage

Insects, microorganisms, and rodents or birds cause major losses and deterioration of cereal grains during storage, especially in developing countries. Losses during storage may increase if condition of the received grains is unsatisfactory, if the storage sites and facilities are unsuitable for safe storage, or if there is poor management during storage or deterioration in storage environment. Transportation is temporary storage and may also cause losses under unsuitable environments, such as dirty and already infested railcar, or improper supervision (20).

Deterioration caused by insects, microorganisms, and rodents during storage is usually affected by the storage structures and environments. Higher storage temperature and grain moisture content may lead to multiplication and growth of insects and microorganisms. Poor storage management and unsuitable storage structures easily cause serious damage from rodents and birds. Additionally, cracked, broken, damp, and dirty grains with foreign materials (e.g., straw and trash) are more easily infested by insects and molds. Drying and cleaning of cereal grains before storage and appropriate storage management are important for reducing storage losses.

F. Control of Insects, Microorganisms, and Rodents during Storage

Principal measures for decreasing storage losses are to prevent and control insects, microorganisms, and rodents. When grain storage starts, destruction from insects, microorganisms, and rodents may begin, with the rate of grain losses depending on storage conditions. The growth rate of microorganisms usually depends on grain moisture content and storage temperature, and that of insects mainly on storage temperature.

1. Insects

Insects are the major cause of losses of stored grains. Beetles, moths, and certain mites are serious pests of stored grains. Table 17.12 lists the major insects of stored cereal grains and their optimum growth temperatures (15, 22, 26). There are two major groups of storage insect, external and internal infesters. Almost all are either beetles (*Coleoptera*) or moths (*Lepidoptera*) (15). The nature of insect damage is classified into three types: 1) grains are bored inside, leaving the outer coat practically intact; 2) only the germ of grains is damaged; 3) a part of the grains, including the outer coat, is eaten (22). Rice weevil, maize weevil, granary weevil, lesser grain borer, and Angoumois grain moth develop and feed inside kernels. Infestation remains hidden until the adults emerge from the kernels. Other beetles feed primarily on broken and whole kernels. Grain-infesting insects are very sensitive to temperature, but insensitive to moisture. Generally, they grow slowly or not at all below 16°C, and cannot survive in temperatures of 42°C or above, and appear to thrive best at about 29°C. Storage pests are well adapted to live in a very dry environment.

Insects not only consume large quantities of grains but also decrease the quality by the presence of insect fragments, feces, and unnatural odors. Insects consume nutritious components, encourage moisture uptake by the infested grains, and promote growth of microorganisms. When insect population reaches a certain level, temperature of stored grains will increase rapidly. If insects are at over-growth, temperature may increase up to 45°C. When microorganisms are associated, temperature may rise even up to 75°C, causing massive spoilage of grains. Thus overgrowth of insects usually results in serious losses of grain quality and quantity.

2. Microorganisms

Molds are also important damaging agents of stored grains. Under favorable moisture, temperature, and relative humidity, many molds can grow on grain surface. Common molds founds in grains belong to the genera *Aspergillus*, *Fusarium*, *Penicillium, Alternaria*, *Cladosporium*, and *Helminthosporium* (22).

TABLE 17.12
Insects of Stored Cereal Grains and Their Optimum Growth Temperature

Common Name	Scientific Name	Optimum Temp. (°C)	Minimum Temp. (°C)
Rice weevil	*Sitophilus oryzae* L.	27–31	17
Maize weevil	*Sitophilus zeamais* Motsch.	28	17
Grain weevil	*Sitophilus granaries* L.	26–30	15
Angoumois grain moth	*Sitotroga cerealella* Ol.	26–30	16
India meal moth	*Plodia interpunctella* Hub.	28–30	–
Almond moth	*Ephestia cautella* Walk	25	10
Rice moth	*Corcyra cephalonica* Staint	28–30	10
Lesser grain borer	*Rhizopertha dominica* F.	32–35	23
Larger grain borer	*Prostephanus truncatus*	–	–
Rust red grain beetle	*Tribolium castaneum* Herbst	32–35	22
Confused flour beetle	*Tribolium confusum* J. du Val.	30–33	21
Drug store beetle	*Stegobium paniceum* L.	–	–
Saw-toothed grain beetle	*Oryzaephilus surinamensis* L.	31–34	21
Khapra beetle	*Trogoderma granarium* Everts	33–37	24
Flat grain beetle	*Laemophiloeus pusillus* Schonherr.	–	–
Long headed flour beetle	*Latheticus oryzae*	35	–
Australia spider beetle	*Ptinus tectus*	25–35	10
Cadelle beetle	*Tenebroides mauritanicus* L.	–	–
Cigarette beetle	*Lasioderma serricorne* F.	–	–
Flour or grain mite	*Acarus siro* L.	21–27	7
Warehouse moths	*Ephestia* spp.	–	–

Source: Adapted from Refs. 15, 22, and 26.

The most dangerous and important fungi are from three genera: *Aspergillus*, *Fusarium*, and *Penicillium* (15). Most storage fungi cannot survive at grain moisture content below 17%. They can survive at 50°C and above 65% relative humidity, but are most destructive below 25°C and above 80–85% humidity. Fungal damage to stored grains includes losses of seed viability, nutritional and apparent quality, processing properties, and production of mycotoxins (e.g., aflatoxins and ochratoxin), heating, mustiness, and decay. *Aspergillus flavus*, *Furarium graminearum*, and *Penicillium verrucosum* produce alflatoxins, zearalenones, and citrinin/ochratoxins, respectively. These toxins are important from the point of health hazards. Aflatoxins are carcinogenic agents and ochratoxin can cause liver and kidney damage. Although mycotoxins-induced diseases are rare in developed countries, they commonly occur among rural populations in developing countries.

Bacteria are also found in stored grains, mainly belonging to the families Bacillaceae, Pseudomonadaceae, and Micrococcaceae. Activities of bacteria are normally affected by temperature and moisture content of the grains during storage. Bacteria require higher moisture content (>20%) and higher humidity (>90–95%) for their growth than fungi. Thermophilic bacteria can proliferate at 55°C and raise the temperature of infected grains to as high as 70–75°C. Bacterial damage to stored grains is similar to that of fungi, but occurs more rarely.

3. Rodents

Vertebrates (rodents and birds) cause extensive damage to grain in both field and store. Damage from rodents is a serious problem at farmer's and trader's level. Commensal rats and mice are the most destructive vertebrates on Earth. There are 21 common species of rodents that attack stored grains in different sites throughout the world (22). In addition to house mice, Norwegian rats and mice are conventional rodents.

Not only do rodents eat large quantities of grains, they also contaminate grains with urine, hairs, and excreta. Rodents are energetic creatures and eat about 10% of their body weight of food per diem. One rat will consume around 10 kg of grain each year and excrete 2–5 kg of droppings (15). Apart from consuming and contaminating grains, certain rodents can damage containers (e.g., bags and wooden, bamboo or mud bins, and make burrows in walls, warehouse floor, and cables) and spread diseases.

4. Control Methods and Measures

a. Control of storage temperature and moisture

An important measure of controlling insects and microorganisms is to keep a safe storage environment with cool temperature and low moisture. The recommended safe storage moisture contents of most cereal grains range from 13 to 15% (Table 17.11) (22–24). Lower temperatures increase shelf life of grains by decreasing respiratory

rate and inhibiting activities of insects and microorganisms. When grains are cooled below 15°C, most insects and fungi cannot grow. In developing countries, control of such low temperature will greatly increase storage costs. Generally, maintenance of temperature at about 27°C and relative humidity below 70% during storage are important. Frequent aeration during storage is necessary to avoid formation of hot spots due to activities of insects and microorganisms and to control uniform temperature throughout storage environments.

b. Cleanliness

Cleanliness is considered the most common means of reducing or eliminating pest infestation. It is important to thoroughly clean storage facilities before new grains are stored. Old infested grains, empty bags or sacks, chaff, webs, and other junk that may harbor insects must be cleaned out. The floor, walls, and roof structure should be brushed and sprayed with insecticide before storage. Cleaning of harvesting equipment and transportation tools is also required. Also, clean conditions around storage area and facilities can help keep rodent numbers down.

c. Use of fumigants and insecticides

Fumigants are quick-acting respiratory poisons which can be applied to static grains. Commonly used fumigants are methyl bromide, carbon disulfide, carbon tetrachloride, ethylene dichloride, ethylene dibromide, chloropicrin, hydrogen cyanide, and phosphine (3, 15). The effectiveness of each fumigant is dependent on species of insects, stage of development, temperature, humidity, application method, fumigant concentration, storage facilities, etc. Some recommendations for fumigation of stored grains are shown in Table 17.13 (15). Because chemical fumigants are also highly poisonous to man, precautions given on the labels of the chemical containers must be strictly followed. Great care is required in their application, and staff must be trained well. Gas masks should be worn during the use of fumigants. Storage bins and buildings that have been fumigated must be opened for ventilation for several hours or days before people can enter.

Insecticides that kill insects of stored grains belong to the contact type which can penetrate the insect cuticle and enter body tissue. Common contact insecticides include pyrethrum (pyrethrins), chlorinated hydrocarbon insecticides, organophosphorus insecticides, etc. (22). For example, pyrethrins can be used to kill insects on walls and floor of storage bins during cleaning, and can also be used as protecting agent by spraying on the grains.

d. Control measures of rodents

Rodents cause serious storage losses of cereal grains, particularly in developing countries. Relevant control measures include sanitation, removal of foods easily accessible to rodents, proofing, trapping, use of ultrasonic devices, biological control, single- or multi-dose poisons, tracking poisons, fumigations, and the use of chemosterilants, attractants, and repellants (22). The most common control measures are sanitation, use of anticoagulants, and fumigation of live burrows. Fumigation of live burrows with aluminum phosphide is a cheap and effective method with usually 100% killing after second fumigation. Anticoagulants were tested and also found to be effective in controlling rats.

e. Other control methods

Other promising control methods have been investigated, including modified atmosphere, irradiation, use of insect sex attractants, insect growth regulators, insect pathogens, parasites and predators, etc. (15, 22). As an alternative to fumigation, grain storage atmosphere can be made lethal or inhibitory to insects and microorganisms by reducing oxygen concentration, increasing carbon dioxide or nitrogen concentrations, or creating a partial vacuum (tight-air packaging). Radiation treatments usually cause mutation, sterilization, or death of insects and microorganisms, and are considered a control method for stored grains. The use of sex attractants produced by insects has been investigated to control stored-grain insects. Insect growth regulators can interfere with either morphogenesis or molting of insects. These compounds have intense biological activity, specificity, and little or no vertebrate toxicity. Because of economic factors or chemical toxicity, these promising control methods have, however, not yet been commonly used for stored grains.

TABLE 17.13
Some Recommended Fumigants and Used Dosages of Stored Cereal Grains[a]

Storage Structure or Situation	Application	Fumigants	Dosage (g/tonne)	Treatment Time (days)
Silos, deep bins	Admixture during filling	Phosphine	3–4	5
Flat bulk storage	Admixture by probe	Phosphine	3–4	5
Flat bulk storage	Surface application	Methyl bromide	34	1
Rail cars in transit	Gas jets at top of stack	Methyl bromide	58	1
Stacks under tarpaulins[b]	Gas jets at top of stack	Methyl bromide	28–54	1

Source: Adapted from Ref. 15.

[a] Fumigating is conducted at 20–25°C.

[b] Different cereals require various dosage: wheat, 34 g/tonne; rice, 28 g/tonne; maize, 36 g/tonne; and sorghum and millets, 54 g/tonne.

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18 Legumes: Horticulture, Properties, and Processing

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CONTENTS

I. INTRODUCTION

In terms of agricultural production, cereals are the most important source of human food and animal feeds but the legume family, Leguminosae, encompasses an extremely diverse group of herbs, vines, shrubs and trees (1). The subfamily Papilionoideae has 600 genera and 13,000 species of plants which have fruits enclosed in a specific pattern in elongated pods and whose roots form symbiotic relationships with nitrogen-fixing bacteria in nodules. Over 80 legume species are consumed world-wide and represent the second most important food source. About 20 legume species are cultivated on an appreciable hectareage. These legume crops are primarily herbaceous annuals that can be consumed directly as mature dry seeds, as immature green seeds, or as whole green pods.

The most important food legumes are listed in Table 18.1 with their botanical names, global distribution, and approximate annual production. Soybean and peanut are grown widely for extraction of refined vegetable oils and high protein meals, but also have important whole seed uses. About one-half of the world production of peanut is crushed for production of edible oil and meal for animal feeds; much of the large U.S. crop is processed into peanut butter or roasted for direct consumption. On the other hand, over 80% of the world soybean production is pressed for the edible oil and valuable soybean meal, and only about 15% is processed directly into food products.

The low alkaloid sweet lupin *(L.angustifolius*) is a protein supplement in ruminant feeds and, after removal of 25% hull, in pig and poultry rations in Australia (Table 18.1). *Lupinus albus*, with 12% lipid, is grown in other

TABLE 18.1
Scientific and Common Names and Annual Production of Important Food Legumes

Name	Scientific Name	Common Names	Distribution	Production (mt)
Proteinaceous Oilseeds				
Peanut	*Arachis hypogaea* L.	Groundnut, goober	India, China, USA	23
Soybean	*Glycine max* (L) Merr.	Soyabean, Japan pea	USA, Brazil, China	175
Lupin	*Lupinus albus, luteus* and *angustifolius* L.	Sweet lupin, tarwi	Australia, Russia, Mediterranean	1
Starchy Pulses				
Common bean	*Phaseolus vulgaris* L.	Haricot, dry bean	Brazil, India, China	20
Dry pea	*Pisum sativum* L.	Field pea, garden pea	Russia, China, France	10
Chickpea	*Cicer arietinum* L.	Bengal gram, chana	India, Turkey, Pakistan	8.5
Fababean	*Vicia faba* L.	Broad bean, horse bean	China, Ethiopia, Egypt	3.5
Lentil	*Lens culinaris* Medik.	Masur, red dhal	India, Turkey, North America	3
Pigeon pea	*Cajanus cajan* (L) Millsp.	Red gram, Congo pea	Asia, Africa, South America	3
Cowpea	*Vigna unguiculata* (L) Walp.	Black-eyed pea, kaffir bean	Africa, Asia, Nigeria	1
Mung bean	*Vigna aureus, mungo,* and *radiata* (L.) Wilczek	Green, black, and golden gram	India, China, Thailand	1
Lima bean	*Phaseolus lunatus* L.	Butter bean, sieva bean	USA, Central and South America	<1

parts of the world. In efforts to breed an oilseed crop, lines with 16% oil have been reported.

Pulses have limited industrial applications and are mainly consumed directly as whole or dehulled split seeds. Common beans and dry peas constitute about one-half of global pulse production of about 58 mt, and production is widely distributed among countries in the Americas, Indian sub-continent, Asia, and Europe (Table 18.1). However, about 90% of global pulse utilization is concentrated in developing countries. In these low-income food-deficit areas, pulses contribute about 10% of the daily protein and 5% of the energy intake of human diets.

Driven by increasing demand from West Asia, North Africa, Central and South America, and India, production and export of the principal pulses has expanded in North America, Australia, China, Turkey, and Argentina. With respect to world trade, common beans and dry peas are the major imported pulses while lentils, chickpeas, and broad beans are also purchased by low-income countries.

II. MORPHOLOGY AND MICROSTRUCTURES OF SEEDS

Compared to cereal grains, legume seeds are medium to large in size (0.3–2.5 cm diameter), rounded in shape, with relatively hard texture. Seeds vary from white to colored or mottled in appearance and surfaces can be smooth, textured, or wrinkled (1). The proportions of the three major seed components are 8–18% seedcoat, 80–90% cotyledons (Figure 18.1), and 1–2% embryonic axis (not shown) (2). The seedcoat or testa protects the embryonic structures from water absorption, microbial and insect invasion. In lentils, the seedcoat exhibits a pattern of conical papillae on the surface, about 3 μm at the base (Figure 18.2C). The outermost layer of the seedcoat is a thin waxy cuticle that serves as a hydrophobic barrier (Figures 18.1 and 18.2C). The major structure of the seedcoat is a single layer of vertical, elongated palisade cells. The thick walls of the palisade cells provide strength and rigidity to the seedcoat; the wall thickness and depth determine the proportions of 'hull' that is characteristic of each species. The thin layer of hourglass cells (5–6 μm thick) occurs, with interconnecting air spaces, below the palisade layer.

Between the hourglass cells and the cotyledon is a separate layer of flattened parenchyma cells (Figure 18.2C) that constitute the residue of the once prominent endosperm in the fertilized ovule (See Section IV.F, Dietary Fibre). When seeds are decorticated during processing, the endosperm layer separates with the seedcoat. The seed embryo consists of the embryonic axis and two cotyledons or seed leaves. The embryonic axis has a small plumule (foliage leaves) with shoot-tip, an embryonic stem (hypocotyl), and a radicle or root that are attached to, and enclosed by, the cotyledons.

There are two openings in the legume seedcoat (2). The small micropyle is the site where the pollen tube entered the valve, and is closed in some species. The large oval hilum is the scar where the seed was attached to the pod and is large enough to be a major factor in regulation of seed drying or rehydration (Figure 18.2A, B). Along the hilum length, but behind the opening, is the tracheid bar with numerous ducts that interconnect the pod and seed vascular systems. Although not an opening, there is a linear ridge on one end of the hilum, called the raphe, that arose from the fusion of the pod to the seedcoat during seed development (Figure 18.2A).

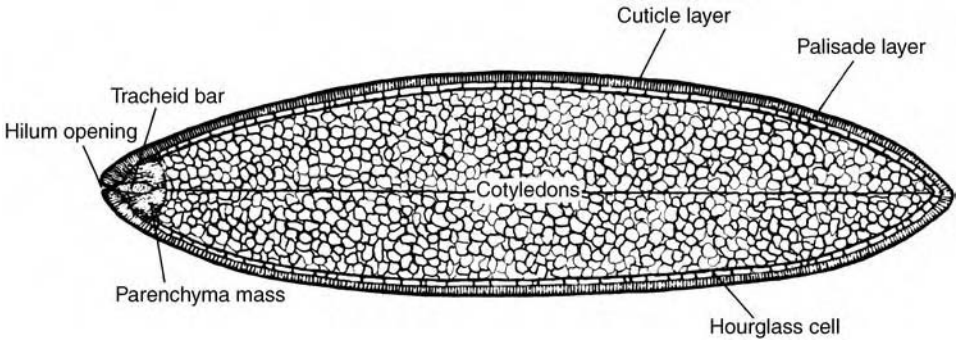

FIGURE 18.1 Schematic diagram of the structure of a cross section through a lentil seed. (From Ref. 2.)

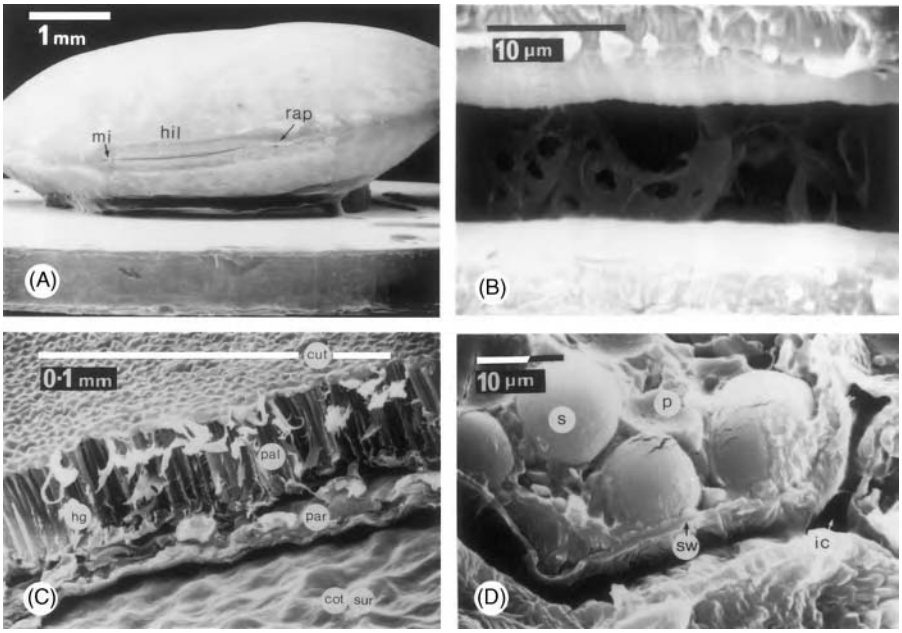

FIGURE 18.2 SEM micrographs of: (A) a gold-coated lentil seed illustrating the micropyle (mi), hilum (hil), and raphe (rap); (B) the hilum opening exposing the tracheid bar; (C) the lentil seedcoat showing the cuticle (cut) layer on the surface of the palisade (pal) cells, traces of hourglass (hg) cells in open spaces, parenchyma (par) layer, and the cotyledon surface (cot sur); (D) an exposed portion of the cotyledon with the storage cell wall (sw) enclosing several starch (s) granules in the protein matrix (p), and the intercellular spaces (ic). (From Ref. 2.)

The two large cotyledons contain nutrient reserves to support early shoot and root growth during germination (Figure 18.1) (2). Their large parenchyma cells (100–130 µm diameter) have thin tough cell walls (1–2 µm thick) that are cemented together by a layer of pectic substances called the middle lamella (Figure 18.2D). There are intercellular spaces between the cells, primarily at the junctures of three or more adjacent cells that facilitate water uptake and hydration. Each cotyledonary cell is packed with round or oval starch granules (10–30 µm dia) that have smooth surfaces, unlike the tightly packed angular granules in cereal endosperms. The legume starch granules are embedded in a thick protein matrix which also contains small protein granules.

III. THE HARD-TO-COOK PROBLEM

Most pulses are consumed directly as cooked whole or split (dehulled) seeds, and long cooking times are characteristically required to achieve the desired degree of softness, digestibility, and inactivation of heat-labile antinutrients. Slow water uptake by seeds has been associated with long cooking times, but dehulling and presoaking of seeds overnight can reduce cooking time, often by as much as 75%. Soybean and dry pea have only 5–8% hulls that are easily removed; other pulses contain 10–18% hulls that are removed by abrasive dehullers, or after soaking or boiling.

Tang et al. (3) found that the initial moisture contents of lentils markedly affected the route of moisture migration,

rate of water absorption, imbibition time, and proportion of hardshell lentils. At moisture contents of 16–24%, moisture diffused mainly through the large surface of the seedcoat. The permeability of lentil seedcoats decreased with decreasing moisture content because of reduced pore and fissure sizes in the cuticle and palisade layers. When lentil moisture content was at 12%, the hilum opening became the dominant route for moisture to enter the seed. The width of lentil hilum openings also decreased with moisture content, and some hila were closed at 12% moisture content. Impermeable seedcoat and concurrent closing of the hilum opening resulted in hardshell lentils. The complexity of the hardshell and hard-to-cook phenomena in other legumes has been reviewed by Swanson et al. (4) and Stanley (5).

IV. CHEMICAL AND NUTRITIONAL COMPOSITION

A. Energy

The metabolizable energy contents of legume oilseeds are proportional to their oil or lipid contents (Table 18.2). With over 50% lipid, peanuts average 2255 kJ/100 g while soybean, lupin, and chickpea have 1695, 1565, and 1530 kJ/100 g, respectively. The starchy pulses contain about 1440 kJ/100 g of metabolizable energy which is similar to those of whole grain cereals but 5–7% less than in milled cereal flours. The metabolizable energy in pulses arises from their high compositions of starch and protein, but is subject to the slow rates of digestion described below.

B. Lipids

Most pulses are low in lipid, only 1–2% (Table 18.2), of which one half are neutral lipids (mono-, di-, and triacylglycerides) and the remainder are metabolic polar lipids (phospholipids, glycolipids, sterols, sterol esters, and lipoproteins) (6). On the other hand, the proteinaceous oilseeds like peanut and soybean are rich in neutral lipids and are commercial sources of edible oils. These neutral lipids are stored in the cotyledons as oil bodies or spherosomes and contain primarily unsaturated and polyunsaturated fatty acids in the triacylglycerols which have important functional and nutritional attributes. Many legumes contain linolenic acid as a component in the lipids, as well as the bound enzyme lipoxygenase, so that oxidative rancidity and off-flavor development are a problem once seed contents are mixed by grinding.

C. Starch

While the oilseeds are nearly devoid of starch, it is the main component in pulses (Table 18.2). The large starch granules in the cotyledon cells are composed of a combination of two glucose polymers (7). Inside cell organelles these polymers are synthesized into interwoven strands to form the semicrystalline granules. Amylopectin, the larger polymer, is an α-1,4-linked glucose chain with α-1,6 branches, with molecular weights in the millions. Amylose is a smaller linear polymer composed of α-1,4-linked glucose units, and few branches. The number of glucose units in the amylose chain range from 1300 to 1900 among pulse species and the average molecular weight is 177,000. In pulses, the proportion of amylose to

TABLE 18.2
Energy and Chemical Constituents in Food Legumes

Common Name	Energy kJ/100 g	Protein*	Lipid	Starch	Sugars	Ash	Total Dietary Fiber
		% Dry Weight Basis					
Proteinaceous Oilseeds							
Peanut	2255	28	53	1	5	2	8
Soybean	1695	39	20	2	8	5	22
Lupin	1565	38	10	2	10	4	33
Starchy Pulses							
Common bean	1468	24	2	42	5	4	21
Dry pea	1418	20	1	52	5	3	17
Chickpea	1520	19	6	50	7	3	18
Fababean	1430	28	2	45	4	3	17
Lentil	1442	24	1	52	6	2	13
Pigeon pea	1443	21	2	46	6	4	19
Cowpea	1442	24	1	47	7	4	15
Mung bean	1445	25	1	47	4	4	23
Lima bean	1420	21	1	43	5	5	23

* N × 5.7.

amylopectin by weight is about 33:67, but amylose contents can vary from 20–65%. Tuber starches contain up to 20% amylose. Genotypes of the major cereals vary from 0–85% amylose, but 25% is normal for corn and wheat starch. As a class, pulse starches are considered to be high in amylose content.

The shape and size of starch granules are also characteristic of the plant source, those of legumes being oval, round, spherical, or kidney shaped with smooth surfaces and no apparent fissures (7). The starch granules in pulses vary widely in width (10–55 μm) and length (8–70 μm). Most of the pulse starches are simple granules, the exception being wrinkled pea starch, which appears to be a mixture of simple and compound granules, the latter being composed of 3–10 subunits joined together.

D. Proteins

The proteinaceous oilseeds are rich sources of protein, and the defatted meals are major protein supplements for cereal-based animal feeds (Table 18.2). Similarly, the pulses at 18–30% protein are major sources of protein for human nutrition in low-income countries.

There are enzymatic, structural, and globular storage proteins in pulses. Storage proteins account for 70–80% of seed nitrogen and occur within the cell as matrix or discrete protein bodies (1). The major storage proteins are classified as either legumin or vicilin, their subunit compositions and molecular weights being characteristic of the species. These are reported elsewhere under the appropriate section, for the legume species. (see Chapters 5–7, 19.)

While pulses are considered to be good sources of protein for human nutrition, their utilization may be quite inefficient. The digestibilities of pulse proteins can vary between 65 and 88%, depending on the structure and composition of storage proteins (8). Most pulse proteins are rich in essential amino acids, especially lysine and threonine. However, they are deficient in sulphur-containing amino acids, methionine, and cystine. The biological value of legume proteins, as measured by protein efficiency ratio (PER), may vary from 0.2 to 2.0 where a balanced protein like casein has a PER = 2.5. By blending a low PER pulse with a source of cereal protein rich in sulphur amino acids, PER values in the range of 1.8–2.0 can be obtained.

The Kjeldahl method for determination of total organic nitrogen (N) is a widely accepted procedure. However, the selection of the appropriate nitrogen-to-protein conversion factor (N:P factor) for calculating total protein content has been a point of controversy, and practices are not consistent among laboratories. Based on amino acid composition, the true N:P factors for common bean and dry pea were found to be 5.4, and the mean N:P factor for 23 diverse food products was 5.7 ± 0.3 (9). The latter factor was recommended for all mixed or blended food diets instead of the common factor of 6.25 which exaggerates the true protein content.

E. Toxins and Antinutritive Factors

Food legume seeds may contain a wide range of constituents which have adverse effects on digestive enzyme activity, digestibility, nutrition, and health (10). Most commercial cultivars have been selected for reduced levels of these factors and heat processing inactivates the proteinaceous factors. Protease inhibitors in soybean, lima bean, common bean, and chickpea retard proteolytic enzyme activity (11). Lectins are polymeric proteins in soybean and common bean that bind to monosaccharides in glycoproteins of the cell membranes, causing lesions in the intestinal mucosa and reduced nutrient absorption. The presence of saponins in some cultivars of common bean and cowpea can reduce feed intake and cause growth depression, but the concentrations are usually too low for significant effects. Goitrogenic substances in soybean and peanut cause enlargement of the thyroid gland, an effect that is counteracted by administrating iodine. Certain cultivars of lima bean grown in some tropical countries contain a cyanogenic glycoside that is enzyme-hydrolyzed to release hydrogen cyanide during processing, causing outbreaks of cyanide poisoning. Lupin cultivars have been bred to eliminate alkaloids which are particularly poisonous but can be detected by their bitter taste. Vicine and convicine in fababean can cause a haemolytic anemia called favism in genetically susceptible individuals. Fortunately, the broad bean/fababean cultivars are consumed primarily as whole seeds and so can be readily avoided if one is susceptible.

F. Dietary Fiber

Recent definitions of dietary fiber include all components of plant material that are resistant to the digestive secretions of the human upper gastrointestinal tract. These components are predominantly non-starch polysaccharides and lignin associated with the structure of cell walls. In legumes the main dietary fiber constituents are cellulose, xyloglucans, galactomannans, pectic polyuronides, and glycoproteins. While peanut is relatively low in total dietary fiber, other legumes contain 13–23% (Table 18.2), and are viewed favorably in control of many diseases associated with Western diets.

The cell walls in legume cotyledons are relatively thick (2) and resist physical disruption during processing and cooking, and subsequently during their passage through the alimentary tract (12). The cell walls constitute a barrier to digestive enzymes and delay the rate of starch and protein digestion. Thus legumes are an important component of low calorie diets. A slow rate of glucose absorption has been recorded for beans and other pulses,

a favorable result for diabetics where high blood sugar levels are a problem. Beans and lentils in Western diets give relatively flat postprandial blood-glucose curves, often expressed numerically as the "glycaemic index." The slow digestion of legume starch has been attributed to the survival of intact cell walls after cooking, but evidence is presented in a later section on slow digestibility of starch granules as well (Section V.C).

Cell wall polysaccharides are also implicated in binding of essential minerals such as iron, zinc, and calcium when diets contain a high proportion of legumes (12). This adverse effect on mineral absorption in humans is exacerbated by the mineral binding effects of phytate (myo-inositol hexaphosphate) that is associated with legume cell walls. Mineral bioavailabilty can be greatly improved by commercial dephytinization procedures in the preparation of special dietary foods that are rich in fiber.

Most pulses are not rich sources of soluble fiber such as gums, pectins, and β-glucans which have an important role in controlling gastrointestinal and metabolic diseases. Legumes with fully developed endosperms as the storage organ, and reduced cotyledons in the seeds, have galactomannans as the reserve carbohydrates. Guar and locust bean contain water soluble gums with unique thickening properties that have many uses in ice cream, cheese, sauces, salad dressings and, industrially, in paper making, drilling muds, etc.

G. Sugars

Food legumes in general contain significant concentrations of free sugars (4–12%) (Table 18.2), which are composed of about 40% disaccharides (sucrose mainly) and 60% α-galactosides (raffinose, stachyose, and verbascose) (13). The latter group represents a problem for consumers since the human digestive system lacks the enzyme α-galactosidase. Thus this raffinose family of oligosaccharides passes into the large intestine where sugars are fermented anaerobically to produce gas.

H. Ash and Minerals

The ash content of pulses varies from 2–5% (Table 18.2). Quantitatively, pulses contain significant amounts of potassium, phosphorus, magnesium, and calcium (10). The high potassium-low sodium ratios of pulses are desired for people with hypertension. Pulses could be good dietary sources of calcium and phosphorus but the ratio is 1:2 while the desired balance is 2:1. Also, a high proportion of phosphorus in pulses is present in phytic acid bodies in the cotyledons which, during digestion, bind calcium as an insoluble salt that is not absorbed in the digestive tract (12). Pulses can provide a high proportion of daily requirements of iron, magnesium, manganese, and copper, but soluble minerals can leach into the cooking water and be lost during drainage.

I. Vitamins

Pulses are excellent sources of the water-soluble B vitamins, particularly thiamin, folic acid, and pantothenic acid, and contain significant quantities of nicotinic acid, riboflavin, and vitamin B6 (1,10). Differences between pulse species and environmental effects are large, and losses during domestic and commercial processing are significant. Pulse seeds are low in vitamin C and fat-soluble vitamins, although green seeds and pods can be good sources of carotenes, vitamin C and E.

J. Tannins and Polyphenols

The hulls of pulses contain about 50% cellulose, 20% hemicellulose, 20% pectin-like and water-soluble carbohydrates, and 2–12% lignin, condensed tannins, and procyanidin. The hydroxyl groups of the latter compounds form cross-links with proteins to cause seed hardening during storage and decreased protein digestibility during cooking (5).

The combination of slow or incomplete cooking, digestive enzyme inhibitors, low protein digestibility, and oligosaccharides are limiting factors in pulse utilization and contribute to the flatulence problem. Techniques for reducing the content of flatulence-producing constituents include breeding of low-oligosaccharides cultivars, germination before cooking, and use of hydrolytic enzymes.

V. PULSE PROCESSING AND UTILIZATION

A. Harvest and Storage

Some pulse species have an indeterminate growth habit; new flowers and pods are produced continuously until plant growth ceases due to heat, drought, or nutrient stress (14). Early harvested seeds at high moisture content are difficult to thresh, while low-moisture seeds from delayed harvest are susceptible to shattering and mechanical breakage. The optimum moisture content for threshing pulses is between 14% and 19%, wet basis. Variable topography in a field can result in seed moisture contents ranging from 18–55% (15). For some pulses, it is a general practice to swathe or chemically dessicate the crop so that seeds are dried uniformly in the field prior to threshing. Dessication results in more rapid seed drying, but longer cooking times have been reported.

Moisture content of seed has a marked influence on the storage stability of pulses. Thus, it is desirable to adjust the freshly harvested seeds from 19–20% to $<$14% moisture (14, 15). Artificial drying at maximum temperatures of $<$50°C will minimize seed breakage during subsequent handling, while heat treatments above 75°C will decrease seed germination.

Moisture content of the seeds continues to be an important factor influencing the quality of pulses during

storage. Aeration of seeds during storage will reduce heat pockets and mold development. In tropical countries where seeds are stored at high temperature (> 30°C) and relative humidity (> 80%), pulses develop a hard-to-cook (HTC) condition where seeds imbibe water slowly and fail to soften upon cooking. Investigators now distinguish between "hardshell" seeds that fail to absorb water and soften during soaking and those which take up water readily but fail to soften during cooking (HTC). Seedcoat and hilum characteristics determine the development of the hardshell condition while the properties of the cotyledon cell walls affect cooking properties. Postharvest losses due to pests, molds, cooking quality, and nutrition are substantial in tropical countries, often up to 50% of the stored pulse crop.

B. Traditional Processing

Pulses have the advantage over many crops in their simplicity of preparation and in the variety of edible forms (1, 10). In many low-income countries, most pulses are consumed in the home by soaking the dry seeds and cooking in boiling water until soft. The cooking time should be sufficient to gelatinize the starch granules in the cotyledons, denature the proteins, and inactivate antinutritional factors.

Traditionally, pulses are soaked in cold water overnight (8–24 h) (10). Hydration can be enhanced by heating but microbial growth is also favored at elevated temperatures. As well, leaching or diffusion of low-molecular-weight constituents from the seed is accelerated by heat. Pulses with thick, tough seedcoats are abraded and cracked mechanically before soaking to promote moisture uptake. If seedcoats are unpalatable or contain undesirable constituents like tannins, the soaked seeds are hand-rubbed to remove the seedcoat and provide faster cooking and more digestible cotyledons.

In many African and Asian countries, pulses are commonly dehulled (dehusked) by cracking or milling the seeds, splitting the cotyledons, and even polishing the abraded surfaces (10). Both dry and wet decortication methods are practiced to produce dhal. Unfortunately, the yields of split dhal at the rural level may be as low as 65%. Industrial dehulling equipment to produce split peas, pigeon peas, lentils and chickpeas is available commercially. These units are generally expensive because the technical requirements include high yield of splits with minimal abrasion on the cotyledon surfaces. Polishing with organic solutions is necessary for commercial trade.

Dhals have a short cooking time and so are suitable for cooking with vegetables, condiments, and spices (10). Commonly, the dhals are cooked to a soft texture, mashed, and then boiled again with added water and other ingredients. Other home and industrial processing methods involve roasting, puffing, or parching the pulse seeds or dhal by applying dry heat at temperatures of 100–200°C for 1–5 min. There are efficient roasters designed to apply direct heat with hot sand or ceramic beads. Roasted whole seeds or dhal have an appealing nutty flavor along with improved protein quality.

In Asian countries, food legumes are soaked and held at ambient temperatures for several days to facilitate germination (10). Enzymes activated during germination partially hydrolyze (digest) proteins, starch, and oligosaccharides, inactivate antinutrients, release minerals, and synthesize many vitamins. The sprouted grains may be consumed directly, or dehulled, roasted, and ground for use in blends with other foods.

The Japanese make extensive use of fermented soybean and soy flours for preparing a variety of nutritious food products. There is a similar range of Indonesian and Indian dishes prepared from germinated or fermented soybean, peanut, mung bean, and chickpea.

Canning greatly increases the convenience of using pulses in urban areas but the cost of industrial processing may be too high for low-income consumers. The dry peas, beans, lentils, chickpeas, and cowpeas may be soaked in brine or hot-water hydrated to inactivate enzymes, partially cooked, blended with sauce combinations, canned, sealed, and autoclaved, depending on the final product (10). Pork and beans are popular in North America while refried beans are a staple in Mexico.

C. Recent Advances in Starch Functionality

Pulses are occasionally ground into a flour for use in soups, gruels, infant foods, porridges, and blended food products. Unfortunately, the pulse flours hydrate poorly and form viscous, lumpy slurries that do not cook uniformly. For this reason, pulses are frequently cooked as whole seed or dhal before grinding to a paste and consuming after further steaming, frying, deep-fat frying, or boiling to give a product of uniform consistency. Also, when raw pulses are ground into flour and stored, they soon develop undesirable beany odors and flavors. Grinding releases lipoxygenases that catalyze the formation of peroxides from unsaturated fatty acids, especially linolenic acid, that degrade the fatty acid to low-molecular-weight reactive and volatile compounds. Applying dry heat to seeds for 6–8 min at 105°C will inactivate this enzyme, obviating the need for blanching or steaming that require immediate drying to control microbial contamination.

Refined pulse starches have few industrial or food applications, such as in mung bean noodles or in carbonless paper, because the large granules, often 20–40 μm in length, have a highly ordered crystalline structure with amylose molecules being uniformly distributed throughout the granule (16). In cereals and tubers, amylose occurs in micelles that permit entry of water into the central cavity of the granule and rapid water uptake, granule swelling, and elution of starch molecules. But the pulse

starches exhibit restricted swelling power and solubility and are resistant to α-amylase attack. The high stability of pulse starches to thermal processing results in long cooking times for pulse flours as well as refined starches.

Cooking properties of starches can be evaluated experimentally in a recording viscoamylograph. Dilute slurries of refined native starch granules (6–12%) are stirred and heated at 1.5°C/min from 20–95°C and held at 95°C for 30 min before cooling. Cereal and root starches take up water rapidly, swell to high slurry viscosities, but the open granule structure progressively releases the amylose and amylopectin molecules into solution. Then viscosities drop rapidly as a free molecular slurry of starch is formed. The exposed hydroxy groups on the starch molecules are then free to interact and bond in various food and industrial applications.

The typical lack of strong functional properties in pulse starch products is illustrated in Figure 18.3 (17). The scanning electron micrographs of samples taken from the amylograph slurry of dry pea starch show slow granule swelling and water uptake during heating from 30–95°C. Only at near boiling are the granules expanded enough for amylose molecules to diffuse out. After 30 min at 95°C the slurry is still a mixture of partially disintegrated granules and free molecular starch. On cooling, this mixture forms a firm gel due to intermolecular bonding, but water is not held firmly and syneresis occurs during storage or in food preparations. Therefore, flours or refined starches from corn, waxy corn, wheat, and potato are preferred over legume starches for most food and industrial applications.

Considerable variability in amylograph viscosity curves among pulses has been noted by several investigators. For example, comparisons of the pasting viscosities for navy, northern, kidney, pinto, and black bean (*Phaseolus vulgaris*) identified black bean starch as being similar to cereal starches (16). The somewhat smaller granules of black bean exhibited a substantial increase in granule size and viscosity in the initial sampling at 30°C (Figure 18.4). Between 60°C and 75°C, the elution of molecules, and granule degradation, was substantial. At

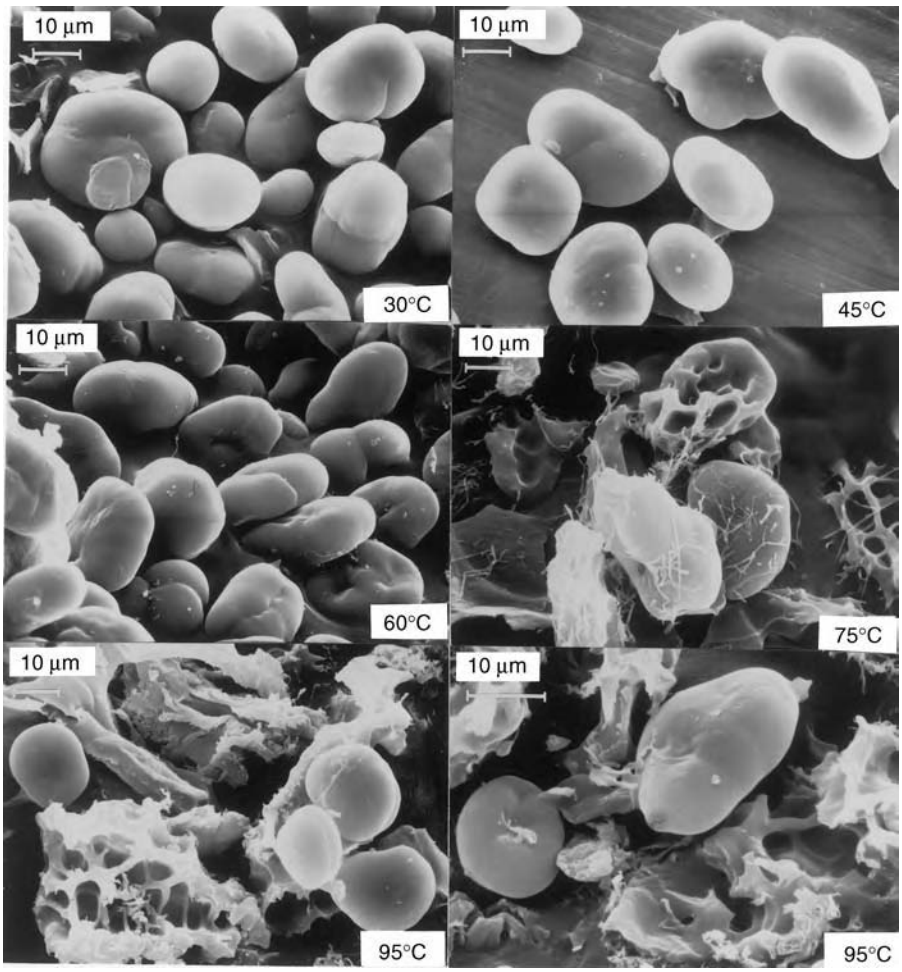

FIGURE 18.3 SEM-micrographs for an 8% slurry of native pea starch sampled during recording of the amylograph at 30°C, 45°C, 60°C, 75°C, 95°C, and after 30 min at 95°C. (From Ref. 17.)

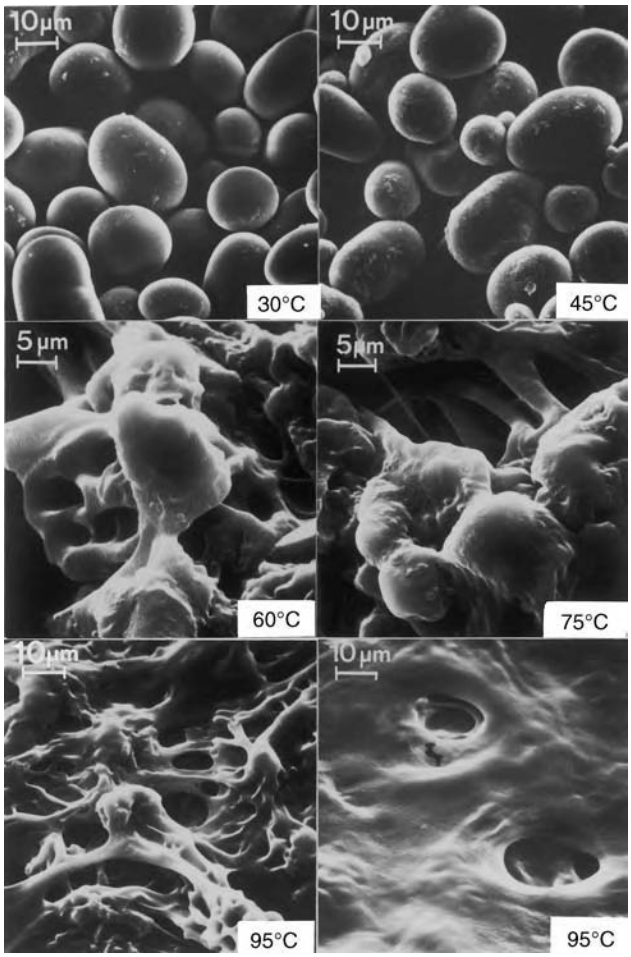

FIGURE 18.4 SEM-micrograph for a 9.5% (w/v) slurry of native black bean starch taken from the amylograph at 30°C, 45°C, 60°C, 75°C, 95°C, and after 30 min at 95°C. (From Ref. 16.)

95°C, there were only remnants of granules remaining, and after 30 min at 95°C, a slurry of free amylose and amylopectin was achieved. Further evaluations of pulse biotypes and cultivars are needed to find genotypes that exhibit better cooking properties in order to open the potential for starch extraction and utilization.

To expand the range in functional properties available for modern food and industrial applications, cereal and tuber starches are modified chemically by substituting low concentrations of reactive groups onto starch chains within the intact starch granule. Among the potential substitution groups used to modify corn starch (Figure 18.5), acetylation and hydroxypropylation improved the cooking properties of pulse starches but cross-linking with phosphorus oxychloride decreased water uptake and granule dissociation (7). The most effective agent for improving the cooking properties of native pulse starches is 3-chloro-2-hydroxypropyltrimethylammonium chloride (17). The formation of the cationic starch ether in a pulse starch results in rapid granule dispersion at near 50°C, yielding a complete molecular dispersion on heating to 95°C (Figure 18.6). On cooling, the gel structure was firm and syneresis was eliminated after storage of the gels at 4°C and −15°C. Cationic starches are widely used as wet-end additives in paper making to improve sheet strength, give better retention of fines and as fillers. Cationization opens the way for pulse starches to compete functionally with modified corn, waxy corn, wheat and potato starches in a variety of industrial applications. However, the markets for these expensive modified starches are limited and highly competitive.

D. Recent Advances in Protein Functionality

Characteristically, legume seeds are rich in protein and contain intermediate to high levels of lysine and threonine which are important in balancing deficiencies of these essential amino acids in cereal diets (Table 18.3) (18). Certain legume proteins, such as soybean, also exhibit strong functional properties, especially water solubility, water and fat binding, and emulsification. Thus soybean flours, protein concentrates, and isolates have been used widely as nutritional supplements and functional ingredients in processed and blended foods.

Due to the structural features of the cell walls and starch granules, starchy legume flours have weaker functional properties than defatted soybean or peanut flours. To overcome the adverse effects of the starch component, wet and dry milling processes have been developed to separate the protein and starch fractions in pulses to better assess the properties and potential uses of the protein.

The impact milling and air classification system (19) is a dry method involving mechanical dehulling on a resinoid disc, abrasive dehuller, followed by fine grinding on an impact mill that reduces the particle size to less than 325 mesh (Figure 18.7). Then the impact-milled flour is fractionated into light (protein) and dense (starch) particles by a single pass through an air classifier at a cut point of 15 micron (800 mesh) diameter between the two fractions. Usually the separated starch granules have considerable protein matrix adhering to their surfaces, in which case the starch fraction can be remilled and classified. The two protein fractions differ in composition, but are usually combined. The second milling will increase starch damage and may not be advisable in certain applications for this major component.

The wet milling process for pulses is adapted from corn and soybean technology (Figure 18.8). The coarsely ground flour is dispersed in dilute alkali, hydrated, screened to remove fiber, and then passed through a wet mill (20). Centrifugation of the slurry separates the starch granules, and the solubilized protein can be precipitated at its isoelectric pH to yield a protein curd. Washing the starch and protein isolates adds to the whey and wash losses.

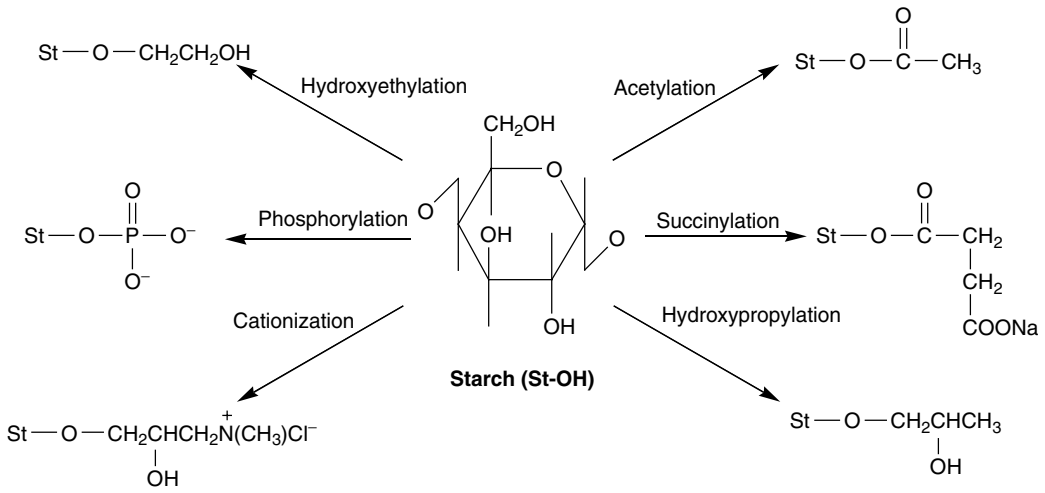

FIGURE 18.5 Chemical modifications of starch with substitution and cross-linking agents that alter the functional properties of starch for food and industrial applications.

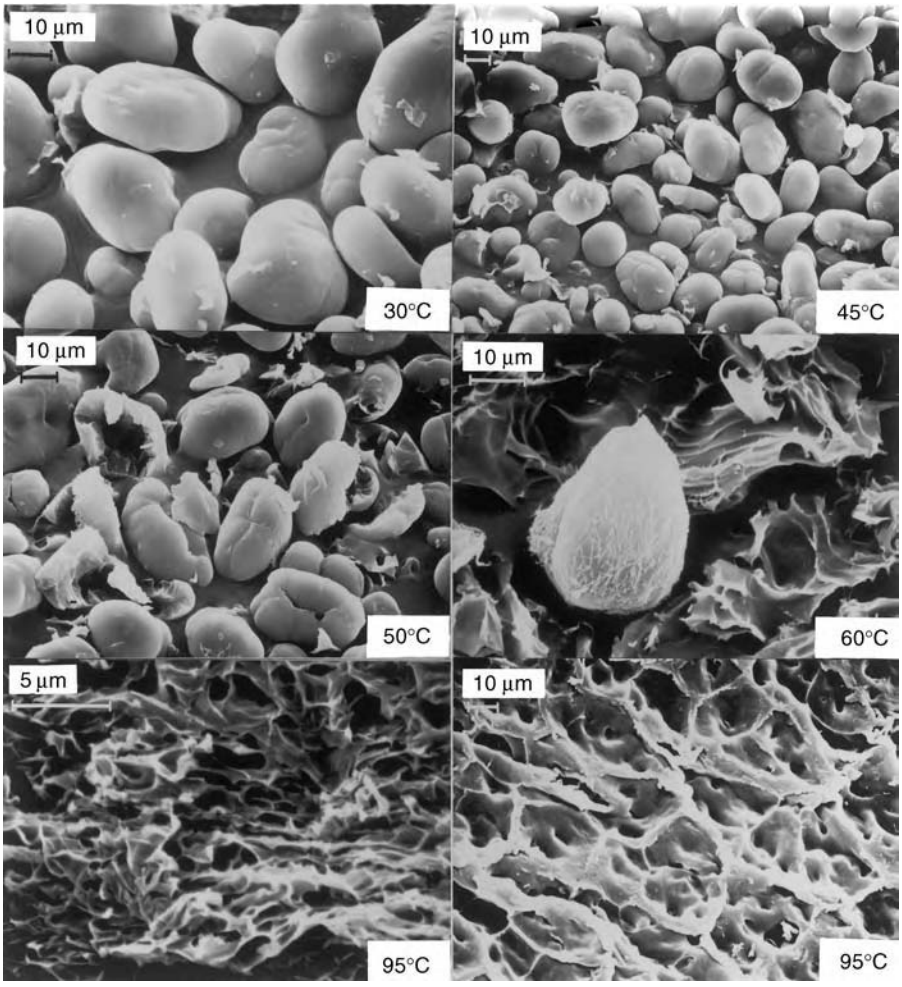

FIGURE 18.6 SEM-micrograph for an 8% (w/v) slurry of cationic pea starch (degree of substitution = 0.042) sampled from the amylograph at 30°C, 45°C, 50°C, 60°C, 95°C, and after 30 min at 95°C. (From Ref. 17.)

TABLE 18.3
Functional Properties of Flours, Protein Fractions, and Protein Isolates from Soybean and Dry Peas, Dry Basis

Legume and Product	Protein[a] %	Lysine	Methionine	Threonine	Nitrogen Solubility Index %	Water Holding Capacity g/g sample	Oil Absorption Capacity g/g sample	Oil Emulsification Capacity ml/g sample	Foam Volume at 60 min ml	Dry Product Color[b] Units
		g amino acid/100 g protein								
Soybean										
Flour	48	6.8	1.6	4.3	21	1.8	0.6	372	280	86
Isolate	82	6.3	1.5	4.1	31	3.2	1.1	451	95	85
Dry Pea										
Flour	25	7.7	1.3	3.8	80	0.9	0.4	346	180	91
Protein fraction	47	7.5	1.2	3.7	65	1.4	0.7	372	280	88
Isolate	80	7.2	0.9	3.3	38	2.7	1.0	366	210	80

[a] N × 5.7.
[b] 100 = white, 0 = black.
Source: Ref. 18.

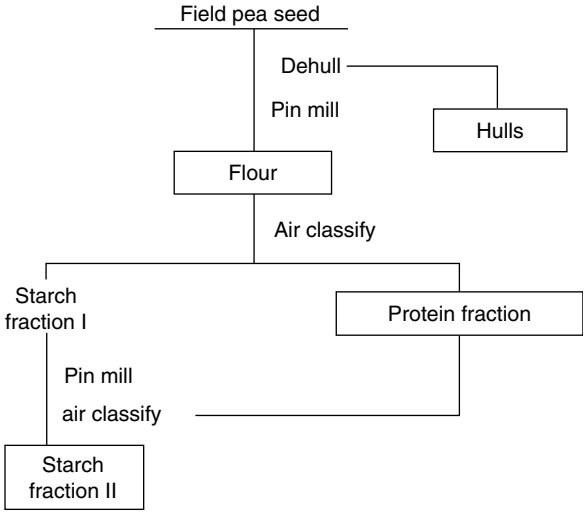

FIGURE 18.7 Flow diagram for pin milling and air classification of pulses into protein and starch fractions. (From Ref. 20.)

In a comparative study of dry and wet milling (20), pea flour containing 22% protein and 55% starch yielded an air-classified fine fraction containing 53% protein and a coarse fraction with 83% starch, dry basis (Figure 18.9). The fines also contained some broken starch granules plus most of the lipid, ash, sugars, flavour and colour compounds in the flour. The recovery of starch in the coarse fraction was very high (93%) but the starch content of 83% was not comparable to commercial corn starch at 95–99%.

The protein isolate from wet milling contained 88% protein, like soy protein isolate, and a refined starch containing <1.0% protein, as in corn starch (Figure 18.9). The refined fiber was light colored and relatively free of other constituents. The principal drawback of the wet milling system was the substantial losses of protein and starch in the whey and washes, and expensive effluent recovery procedures would need to be implemented.

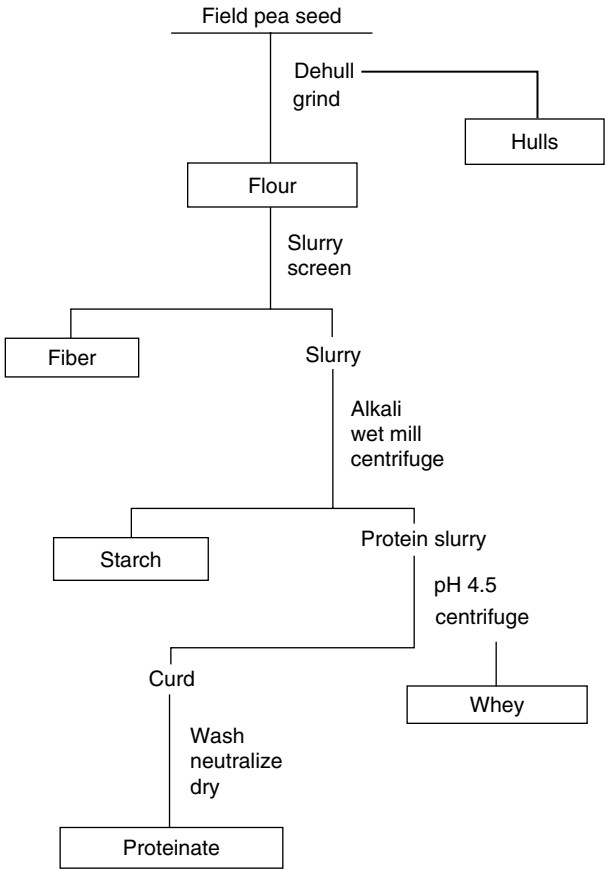

FIGURE 18.8 Flow diagram for preparation of refined fiber, refined starch and proteinate from pulses by wet milling techniques. (From Ref. 20.)

Direct comparisons of functionality in dry pea and soybean proteins have been made (18, 20). The protein fraction from air classification of pea flour was similar in protein content to soybean flour and was very similar to soybean in all functional properties (Table 18.3). The soybean isolate

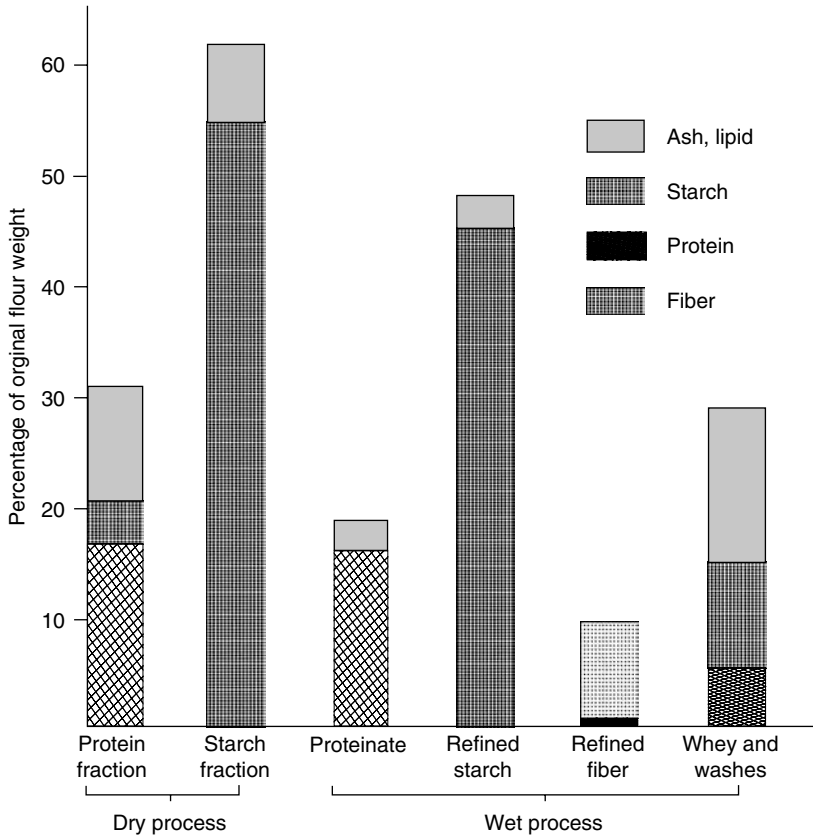

FIGURE 18.9 Yields of products from dry and wet milling of dehulled dry peas. (From Ref. 20.)

was superior to pea isolate in oil emulsification but inferior in foaming properties. The dominant color in all legume products was creamy-yellow and only the pea isolate was slightly darker than the other products.

Natural pea flour exhibited an exceptionally high nitrogen solubility (NSI) at the pH 6.6 used in this test while that of defatted soybean flour was particularly low (Table 18.3). Also the protein isolation procedure decreased the solubility of pea protein quite severely. To assess the effect of impact milling and the alkaline extraction/isoelectric precipitation treatments on nitrogen solubilities of these legumes, the pH dispersibility curves were determined from pH 2–11 (Figure 18.10). The soybean flour and isolate gave almost complete recovery of the product nitrogen as insoluble curd at pH 4–5, indicating the near absence of nonprotein nitrogen material. In dry pea flour and protein (concentrate) fraction, 10–15% of the total nitrogen was soluble protein and nonprotein compounds. During protein isolation, this soluble nitrogen appeared in the whey (Figure 18.9).

At pH levels above and below the isoelectric range, the soybean proteins in both flour and isolate showed only gradual increases in solubility (Figure 18.10). However, the native proteins in pea flour and protein fraction gave high nitrogen solubility values on both sides of the isoelectric pH 4–5. The restricted protein solubility of both isolates between pH 6–9 might be due to the alkaline extraction treatment, acid denaturation, binding with phytin and, in the case of soybean, solvent extraction and desolventizing. For the pea, the alkaline and acid denaturation effect could be minimized by extracting protein at pH 7–8, an advantage over soybean.

In conclusion, the starch in pea flour had an adverse effect on functionality. By removing the starch component, the protein fraction exhibited comparable or superior functional properties to those of soybean flour, and should have potential applications in meat emulsions, bakery and beverage products. Pea protein isolate would also compete favorably with soybean isolate if pea starch has high value uses.

E. Potential for Air Classification

Air classification offers several advantages over wet milling. Only hulls, protein, and starch fractions are manufactured. No water or chemicals are added, so there is no effluent for disposal and microbial problems would be minimal. Electricity is the only essential utility for the two pieces of equipment, and the requirement for engineering skills among operators would be minimal. A seed cleaner

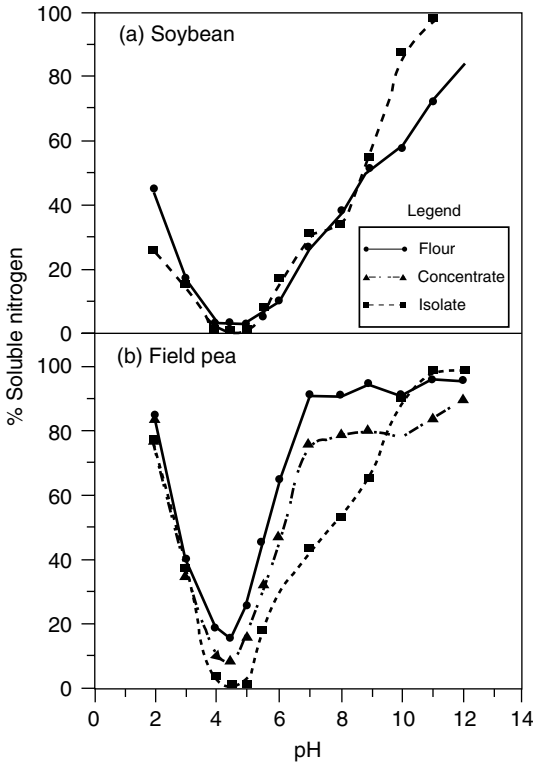

FIGURE 18.10 Nitrogen solubility curves for flours, air classified protein fraction and isolates of a) soybean and b) field pea. (From Ref. 18.)

and grain dryer would be useful optional equipment as well as baggers and loaders.

Since most of the pulses are grown and utilized in low-income tropical and semi-tropical countries, the air classification of pulses should be particularly applicable in these areas. There is great market potential for the high lysine protein fraction in formulated nutritional foods, especially for infants. Also the wet milling of corn into refined corn starch is not done commonly in tropical countries. Therefore a legume starch fraction, manufactured locally, should find markets where a 95% pure starch at $<1.0\%$ protein is not essential.

Numerous studies have shown that nearly all pulses which contain starch can be air classified into fine and coarse fractions. Soybean and lupine yield no coarse fraction (21). Also, the lipids in chickpea interfered with fractionation efficiency and cause plugging of ducts during product transfer. Common bean is the main legume grown in low-income regions and has been evaluated extensively by air classification. As shown in Table 18.4, the intermediate protein level in beans still gives a high yield of fines (40%) with intermediate protein content (53%). The low starch content of the bean results in a low yield of starch fraction, which may be desirable if markets are limited. The starch granules in dry peas and lentils separate fully into high yields of starch fraction with high starch contents and less protein contamination. The dry pea and lentil fines are high in protein content although the yields are low. Fababean is a high protein pulse that gives consistently superior results in protein content of fines. Unfortunately, the vicine and convicine in the flour (about 0.7%) separated into 1.4% in the fines and 0.3% in the coarse fraction. When utilized as a supplement and functional ingredient in foods, susceptible individuals would not easily avoid the bean.

Among more tropical pulses, mung bean gave high yields and composition of protein and starch fraction while cowpea and lima bean were intermediate to the other legumes in air classification efficiency.

F. Applications for Air-Classified Fractions

Characteristically pulses have beany, bitter flavors which are usually removed effectively by aqueous extraction during soaking and cooking or wet milling. Unfortunately, the adverse flavors are concentrated into the protein fractions during air classification. Therefore, any potential applications would need to include appropriate wet heat

TABLE 18.4
Yield and Chemical Composition of Fine and Coarse Fractions from Air Classification of Impact-Milled Pulse Flours, % Dry Basis

	Flour		Fine Protein Fraction			Coarse Starch Fraction		
Legume	Protein[a] %	Starch %	Yield %	Protein %	Starch %	Yield %	Protein %	Starch %
Common bean	25	46	40	53	1	60	7	76
Dry pea	22	55	34	55	3	66	6	84
Fababean	31	52	37	70	2	63	8	81
Lentil	23	55	37	53	5	63	5	85
Cowpea	25	48	39	49	3	61	9	77
Mung bean	27	53	40	60	5	60	5	84
Lima bean	23	50	39	48	1	61	8	80

[a] N × 5.7.

Source: Ref. 21.

treatments to remove steam-volatile constituents. For example, blends of pea and fababean protein fractions with wheat flour at the 15% substitution level were mixed into dough, proofed, and baked by normal bread-making procedures (22). Despite the high levels of dilution with wheat flour, beany and grassy flavors did develop during dough fermentation, but these were driven off during baking. Loaf volumes and crumb textures were adversely affected by the supplements, but the addition of 2% vital gluten and 1% of dough conditioner per 100 g flour gave satisfactory bread volume and structure. Glycolipids like sucrose monolaurate and polyoxyethylene-8-stearate were the most effective conditioners.

Numerous attempts to prepare high-protein snack foods and breakfast cereals by extrusion cooking have not had commercial success. The protein fractions gave limited expansion, and the heat treatments in the barrel failed to fully debitter the product. With a lower flavor profile, extrusion of the starch fraction appeared feasible. Recently, Wang et al. (23) used a twin-screw co-rotating extruder to develop a pasta-like product from the pea starch fraction. Screw configurations were designed to gelatinize the starch, denature residual proteins, vent off adverse flavors, and texturize the extrudate. The product exhibited superior integrity, flavor, and texture after cooking than wheat semolina pasta, although cooking losses were higher. This product made entirely from the pea starch fraction by twin-screw extrusion would be a nutritional and functional alternative to wheat-based products for consumers who cannot tolerate wheat gluten.

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19 Asian Fermented Soybean Products

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CONTENTS

I. INTRODUCTION

It is well known that soybean is originated in China, and has been cultivated there for over 5,000 years. Soybean is considered one of the five basic grains, along with rice, wheat, barley, and millet, which are essential to the Chinese diet and civilization. During the Qin Dynasty (about 200 A.D.), soybean was introduced to Korea from Northern China, and then to Japan and Southeast Asia. Soybean did not appear in Europe and North America until the 18th and 19th centuries, respectively. It is now cultivated all over the world (1).

Fermented soybean foods use either whole soybeans or soybean products as substrates, sometimes along with other cereals. Fermented soyfoods are produced by specific microorganisms or by several microorganisms acting in a sequential manner. The ultimate aim of fermentation is to develop food products having certain desirable characteristics such as flavor, aroma, texture, and keeping quality. During fermentation, protein and carbohydrate fractions are hydrolyzed into smaller constituents, which increase digestibility. Vitamins are also accumulated in the fermented products (2).

Despite the widespread cultivation of soybean, traditional fermented foods based on soybean are limited to the Orient. Most traditional soyfoods are known to have originated in China, and were then introduced to other Asian countries. After centuries of preparation, considerable modification and adaptation in processes had been made to suit specific environmental conditions and cultural practices. These traditional foods are increasingly popular in many Asian countries, and have been extensively developed and consumed in China, Korea, and Japan, and to a lesser extent in Indonesia, India, Malaysia, and Thailand. Table 19.1 lists some common indigenous fermented soyfood products consumed in various parts of Asia (2, 3).

Since fermented soyfoods are nutritious and highly digestible, they have played an important nutritional role in the Oriental population in the past, and will continue to do so in the future. Today, the preparation of many traditional fermented soybean products remains a household art, and only a few (such as soy sauce and *natto*) have evolved with modern biotechnological development and are manufactured on a large scale. In this chapter, various fermented soybean products are discussed with respect to their preparation methods and utilization. Only products that have an important influence and are produced in large quantities are described.

II. SOY SAUCE (*JIANGYOU*)

Soy sauce is a dark brown liquid extracted from a fermented mixture of soybean and wheat or soybean only, with aroma and flavor similar to that of *miso*. First produced in the Zhou Dynasty (684–705 A.D.) in China, soy sauce is now widely consumed in China, Japan, Korea, and other Oriental countries, and is also used in North America and Europe as a condiment and coloring agent (4). Soy sauce is known as *jiangyou* (Mandarin) or *chiangyu* (Cantonese) in China, *shoyu* in Japan, *kanjang* in Korea, *Tao-yu* or *kecap* in Indonesia, and *tayo* in the Philippines (5).

A. *Qu* (*Koji*), *Koji* Starter, and Inoculum

Before describing the methods of preparing soy sauce, it is necessary to introduce some terms commonly used in fermented soybean products.

1. *Qu* (*Koji*)

Qu is the Chinese word for *koji*, and is an intermediate product for making soy sauce, soy paste, *douchi* (soy nuggets), *kecap*, and other similar products. *Qu*, which contains a large variety of enzymes, is made by growing molds on rice, barley, wheat, soybean, or a combination.

2. *Koji* Starter

Koji starter, also known as seed *koji*, *koji* seeds, or *tane-koji*, provides spores of microorganisms to make *koji*. Traditionally, wild spores of different molds were used as the starter for soy sauce, soy paste, and soy nuggets. However, in modern manufacturing, the making of *koji* starter begins with the growth of a selected mold strain, such as *Asperigillus oryzae* or *A. sojae*, in pure culture.

TABLE 19.1
Traditional Fermented Soybean Foods in Asia

Product	Region	Substrates	Microorganism(s)	Nature of Product	Product Use
Douchi (*Hamanatto, tao-si, tao-tjo*)	China, Japan, Philippines, East India	Whole soybean, wheat flour, salt, seasoning	*Mucor* spp., *Aspergillus oryzae, Bacillus subtilis, Pediococcus* spp., *Streptococcus* spp.	Solid beans, retain individual form, soft, raisin-like	Seasoning, snack
Kecap	Indonesia and vicinity	Soybean, wheat	*Aspergillus oryzae*, *Hansenula* spp., *Lactobacillus* spp., *Saccharomyces* spp.	Liquid	Condiment, seasoning
Kinema	Eastern Nepal, India (Sikkim, Darjeeling)	Soybean	*Bacillus subtilis*	Solid	Fried curry, side dish
Meitauza	China, Taiwan	Soybean	*Actinomucor elegans*	Solid	Fried in oil or cooked with vegetables
Meju	Korea	Soybean	*Asperigillus oryzae, Rhizopus* spp.	Paste	Seasoning
Fermented soybean paste *(Doujiang, miso, chiang)*	China, Japan other cereals, salt	Soybean and rice or *Lactobacillus* spp.	*Aspergillus oryzae, Torulopsis etchellsii,*	Paste	Soup base, seasoning
Natto	Japan	Whole soybean	*Bacillus natto*	Solid beans	Snack
Fermented soybean milk	China, Japan	Soybean	Lactic acid bacteria	Liquid	Drink
Soy sauce (*Jiangyou, shoyu, kecap*)	China, Japan, the Philippines, other parts of the Orient	Soybean and wheat, salt	*Aspergillus oryzae or A. soyae, Lactobacillus* spp., *Zygosaccharomyces rouxii*	Liquid	Seasoning for meat, fish, cereals, vegetables
Sufu (Doufuyu, furu, taokaoan, tao-hu-yi)	China	Soybean curd, salt, seasoning, pigment	*Actinomucor elegans, Mucor hiemalis, M. silvaticus, M. subtilissimus*	Solid	Soybean cheese, condiment
Tauco	West Java (Indonesia)	Soybean, cereals	*Rhizopus oligosporus, Aspergillus oryzae*	Liquid	Drink
Tempeh	Indonesia and vicinity, Surinam	Soybean	*Rhizopus* spp., *principally R. oligosporus*	Solid	Fried, roasted, used as meat substitute in soup.

Different raw materials are used and sterile conditions are needed to avoid contamination.

3. Inoculum

Traditionally, in the making of soy sauce, soy paste, and soy nuggets, fermenting product from a previous batch was used as an inoculum to be mixed with salted *koji* and cooked soybeans. In recent years, pure cultures of *Zygosaccharomyces rouxii*, *Torulopsis* sp., and certain lactic acid bacteria such as *Pediococcus halophilus* and *Streptococcus faecalis* were used as inoculum, which speeds up fermentation and reduces contamination by weed yeasts and bacteria.

B. Classification of Soy Sauce

Differences among soy sauce samples are due to many factors, such as raw ingredients, microorganisms used in fermentation, processing conditions, and additives such as sweeteners and preservatives (6). According to the preparation principles, soy sauce can be divided into three groups: fermented soy sauce, chemical soy sauce, and semi-chemical soy sauce. It can be further divided based on differences in raw ingredients, methods of preparation, or duration of aging. There are two major kinds of fermented soy sauce in China according to fermentation procedures: 1) high-salt-high-water fermentated soy sauce, in which soybeans or defatted soybean flakes and wheat flour are used as raw materials; and 2) low-salt-semidried-state fermentated soy sauce, in which defatted soybean flakes and wheat bran are used as fermentation substrates. Chemical soy sauce is made by acid hydrolysis and will not be described in this chapter. To improve quality, chemical soy sauce is often blended with fermented soy sauce to semi-chemical products before being sold.

Five types of *shoyu* are available in Japan: *koikuchi*, *usukuchi, tamari*, *shiro*, and *saishikomi*. Of all the *shoyu* consumed in Japan, 85% is of the *koikuchi* type (7). *Koikuchi* is made from a mixture of soybean and wheat kernels in almost equal amount and is characterized by a deep reddish brown color and a strong, pleasant aroma (7). *Usukuchi*, the second most popular type of *shoyu*, is commonly used to preserve the original flavor and color of foods. The remaining three types of *shoyu* are produced and consumed only in isolated localities for special uses. *Tamari shoyu* is similar to traditional Chinese soy sauce, with a soybean:wheat ratio of about 9:1 to 8:2. In contrast, *shiro shoyu* is made with a soybean:wheat ratio of 1:9 to 2:8, and fermentation is controlled to prevent color development. *Saishikomi shoyu* is produced similar to *koikuchi*, but the *koji* is mixed with raw soy sauce instead of a brine solution.

Traditional Indonesian *kecap* is a Chinese style soy sauce. However, industrial Indonesian *kecap* is a Japanese-type product (8).

TABLE 19.2
Sugar Contents in Soy Sauce

Sugar	Content (% w/w)
Glucose	2.05
Galactose	0.17
Mannose	0.06
Xylose	0.06
Arabinose	0.08
Unidentified sugars	0.23
Disaccharides	0.65
Polysaccharides	1.15
Total	4.45

Source: Adapted from Ref. 28.

C. Preparation of Soy Sauce

1. Chinese *Jiangyou*

Traditionally, Chinese soy sauce is produced from soybean only, but for industrial preparation, wheat flour or bran is added.

a. Traditional household method

The following is a typical method used in Northern China. The raw materials for *jiangyou* making are soybean, salt, and water. Soybean is selected and washed, and then boiled until the beans are soft enough to mash. The mashed soy mud is shaped into bean loaves of about 1–4 kg. The loaves are often made around the Chinese Spring Festival, acting as *qu*. They are placed in a warm room for spontaneous fermentation until the beginning of summer. The fermented loaves are crushed into lumps and mixed with water and salt in earthen vats. The vats are placed outdoors and exposed to sunlight to increase the temperature. The later stage fermentation lasts for at least 30 days, and the mixture is stirred thoroughly daily. The *moromi* becomes thick and dark after fermentation, and has a pleasant fermentative aroma and taste. The liquid is separated and transferred to another jar, and exposed to sunlight for several weeks. A premium grade soy sauce can then be made. Fresh brine can be added to the mash two or more times to extract the second and third grades soy sauce. This is also followed by several weeks of exposure to the sun.

b. Modern methods

Defatted soy meal and wheat bran are mixed at a ratio of 8:2 to 6:4, and the mixture is soaked in water and steamed. After cooling, the mixture is inoculated with a small amount of seed spores of *Aspergillus sojae* and/or *A. oryzae* to make *qu* in about 22–30 hours at 35°C.

For low-salt-semidried-state fermented soy sauce, the *koji* is mixed with 12–13°C Bé brine to make a *moromi*, with a water content of about 50% and a salt content lower

than 10%. The *moromi* is fermented at a higher temperature (40–45°C) for half a month, and mixed with additional brine solution to increase the salt content to at least 15%. The *moromi* is in a semi-liquid state. Lactic acid bacteria and yeasts are added to the brine solution, and fermentation is allowed to continue for half a month or longer at 30–35°C.

For high-salt-high-water fermented soy sauce, the *koji* is mixed with 18°C Bé brine, and fermentation is carried out at 30°C or at room temperature for 3–6 months. This method is adopted by most manufacturers.

A combination of the above two methods has also been used. The low-salt-semidried-state *moromi* is first fermented at 40–42°C for 2 weeks, and then mixed with brine to form a high-salt-high-water *moromi*, fermented at 35–37°C for 15–20 days, followed by fermentation at 28–30°C for 30–100 days. In order to save rice and wheat grains, liquid hydrolysates of broken rice or wheat flour, produced by α-amylase and β-amylase from wheat bran, are used by some producers. The fermented mash is heated to more than 80°C and the liquid product is separated by gravity. The soy sauce may be pasteurized or mixed with benzoic acid before being clarified, bottled, and shipped to the market.

2. Japanese *Shoyu*

Although there are some variations in making different types of Japanese soy sauce, the basic steps are the same, including the treatment of raw materials, *koji* making, brine fermentation, pressing, and refining (Figure 19.1).

a. Treatment of raw materials

Soybeans or defatted soybean flakes are soaked in water to increase the moisture content to about 60% and then cooked with steam at 0.8–1.0 kg/cm^2 gauge pressure for 40–45 minutes, or by a high temperature short time (HTST) process (6–7 kg/cm^2, 20–30 seconds). The wheat kernels are heated with hot air at 150°C for 30–45 seconds at atmospheric pressure, and the roasted kernels are cracked into 4 to 5 pieces. When wheat flour and wheat bran are used, they are steamed after being moisturized (5), then the cooked soybeans are mixed with the roasted wheat (9).

b. Preparation of koji

The treated raw materials are inoculated with 0.1–0.2% seed spores of *Aspergillus sojae* and/or *A. oryzae*. After 2 to 3 days of solid culture at 25–28°C, the mixture becomes a greenish yellow mass as a result of mold growth and sporulation, and is called *koji*.

c. Brine fermentation

Matured *koji* is mixed with an equal amount of brine to make *moromi*. In general, the salt content is kept at around 17% (10). At the initial stage, *Penicillum halophilus* is added to the mash to produce lactic acid which lowers the pH, and to prepare the appropriate conditions for yeast fermentation to produce ethanol. The microorganism also plays an important role in the quality control of the mash, and has an influence on *shoyu* flavor and taste. To control growth rate, it is necessary to keep the fermenting mixture at 15°C in the first month, allowing the pH of the mash to decrease slowly from 6.5 to 5.0. Then *Zygosaccharomyces rouxii* and *Candida* species are added as starters. The temperature of the *moromi* is allowed to rise slowly from 15°C to nearly 28°C for vigorous alcoholic fermentation, and this temperature is kept for 4 months. After the completion of alcoholic fermentation, the temperature is kept at 15°C for 1 month (9, 10).

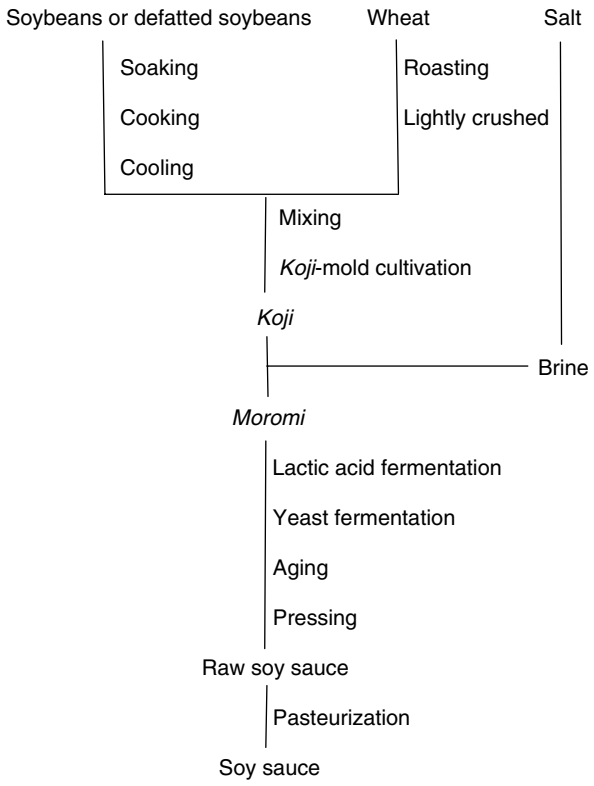

FIGURE 19.1 Flow chart of soy sauce manufacturing.

Lactic acid bacteria and yeast cells interact in a mash, and this affects the production of both lactic acid and ethanol (11). Immobilization of lactic acid bacteria and yeast cells in separate fermentors has been found to be effective for speeding up the production of *shuyo* (12, 13).

d. Pressing and refining

The next step in *shoyu* processing is pressing and refining, involving filtration, pasteurization, and packaging. The aged *moromi* is pressed to separate the soy sauce from the residue. The residue can be extracted with additional 20% brine to increase the yield. After pressing, the filtered raw soy sauce is pasteurized at 70–80°C for a few minutes to inactivate residual enzymes and undesirable microorganisms. It may be necessary to clarify the soy sauce further by centrifugation or sedimentation. The product is treated with caramel as a coloring agent and then packaged (9).

3. Indonesian *Kecap*

Kecap is a liquid seasoning widely used in Malaysia and Indonesia. In Indonesia, *kecap* is usually made by small-scale producers in a traditional manner. Black soybeans are used as the raw material (14). Commercial soy sauce factories have been established in Indonesia, and they apply modern Japanese technology for *kecap* production. Defatted soybean flakes and wheat are used instead of black soybeans (15).

In *kecap* processing, the cooked soybeans are made into *koji* by mixing with mold inoculum consisting of various species of *Aspergillus*. The *koji* is incubated for 48 hours in a room with controlled temperature and relative humidity. At the end of the solid-state fermentation, the *koji* is transfered to a fermentation tank containing brine at a concentration of 20–25%. This process, *moromi* fermentation, lasts at least 2 months, and the pleasant aroma of *kecap* is formed. *Pediococcus halophilus* is the dominant microorganism during the brining stage in *kecap* fermentation (16). *Kecap* liquid is withdrawn at the end of *moromi* fermentation and transferred to a sedimentation tank to precipitate the dispersed particles of raw *kecap*. Based on the formulation, liquid *kecap* can be blended with other ingredients to produce condensed *kecap*. The product is pasteurized for 15 minutes to destroy spoilage microbes. Finally, bottling and labeling of the end product are carried out.

D. Chemical and Biochemical Changes during Soy Sauce Processing

Soy sauce is made by a two-step fermentation process with a mixture of molds, yeasts, and bacteria using soybean and wheat flour as raw materials. The first step involves fermentation with *Aspergillus sojae* and/or *A. oryzae* to make *koji* (9). During this period, enzymes from *koji*, such as proteinases, peptidases, and amylases, hydrolyze most of the proteins to peptides and amino acids, and almost all of the starches to simple sugars. These become the nutrients for the yeasts and lactic acid bacteria in the subsequent step of brine fermentation.

The second stage of fermentation involves *Pediococcus halophilus*, *Zygosaccharomyces rouxii, Candida versatilis*, and *Candida ethellsii*. All the microorganisms can tolerate a salt concentration of 18–20%. However, the brine effectively prevents the growth of undesirable microorganisms. Sugars are fermented by the salt-tolerant microorganisms into lactic acid, ethanol, and various aroma components (10). *Z. rouxii* produces ethanol and 4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2H)-furanone (HEMF). The latter is considered one of the important compounds for soy sauce flavor (12), reaching a maximum concentration at 16% NaCl when fermented by *shoyu* yeasts (13). Ethanol (2–3%) and many aromatic components are produced by *Z. rouxii*. The *Candida* yeasts produce phenolic compounds such as 4-ethylguaiacol and 4-ethylphenol, which add characteristic aroma to soy sauce (10).

The yeasts and *koji* culture have strong esterase activities which could be responsible for the decomposition of the flavor esters during soy sauce brewing, lowering ester flavor in the product. Glutamic acid and aspartic acid are the major amino acids present in soy sauce, and the levels of arginine, tryptophan, and cystine are decreased during fermentation. The total acid content in soy sauce is about 0.95%, dominanted by lactic and acetic acids (9). The glucose content in Japanese soy sauce is about 2.05%, different from the Chinese traditional soy sauce which has little fermentable sugars left for yeast growth after the growth of *Tetragenococcus halophilia* cells (18).

III. Fermented Soybean Paste (*Doujiang* and *Miso*)

Fermented soybean paste is a popular traditional seasoning in Asia, especially in East Asia. It is commonly known as *jiang* or *doujiang* in China, *miso* or *misho* in Japan, *jang* in Korea, *tacho* in Indonesia, and *taosi* in the Philippines. Chinese *jiang* is believed to be the oldest form of fermented soy paste. According to the ancient Chinese book, *Analects of Confucius*, it has a history of more than 3,000 years (19). *Miso* and *jang* are considered to be derived from *doujiang*.

Fermented soybean paste is a light yellow to dark brown thick paste. It has a very salty taste due to high salt content (normally 12–15%, and ranges from 5% to 20%), and has a special flavor of fermented soybean. It is usually served with other foods as a seasoning or used as a condiment in food preparation.

The process of *doujiang* making is very similar to that of soy sauce preparation. The main steps include the preparation of starter, *qu or koji*, mixing the starter with salt and water, and fermentation. Figure 19.3 shows the flow chart of *doujiang* making using pure culture starter.

A. Preparation of *Doujiang*

1. Traditional Household Method

The traditional method of making *doujiang* is almost the same as making *jiangyou*, but the liquid is not separated from the paste. Another household method of *doujiang* making differs from the former one mainly in the preparation of *qu*. Boiled soybean is mixed with a small amount of wheat flour. The mixture is then spread onto a bamboo tray and kept in an incubation room for a week to make *qu.*

2. Pure Culture Method

a. Preparation of starter

A mixture of soybean and wheat flour (in 2:3 to 3:2 ratios) is used for making *qu*. The wheat flour is usually cooked

by baking or steaming, which is labor and energy intensive. Many producers therefore use raw flour directly. The washed, soaked, steam-cooked, and cooled soybean is mixed with the flour, and inoculated with 0.15–0.3% seed spores. The process of *qu* making is the same as that in soy sauce processing (Figure 19.2).

b. Fermentation

Figure 19.3 shows the fermentation steps involved in *doujiang* making. Matured *qu* is poured onto a fermentation container and pressed slightly. The temperature will soon increase spontaneously to 40°C, and brine (14.5°C Bé) at 60–65°C is added. After the brine penetrates evenly into the *qu*, small amount of fine salt is dusted on the surface. The temperature of the *qu* will reach 45°C, and fermentation is carried out at this temperature for 10 days. Brine (24.0°C Bé) and fine salt are added, and the mixture is thoroughly stirred with a mechanical mixer or by compressed air. After fermentation for another 4–5 days, the *doujiang* can be bottled and marketed.

B. Preparation of *Miso*

The basic steps of *miso* manufacturing are similar to those of *doujiang* making, including treatment of raw materials, preparation of *koji*, mixing of ingredients, and fermentation. However, *miso* is quite different from *doujiang* in terms of raw materials, sensory characteristics, and usage (20). The raw materials for *miso* making can be soybean alone or soybean with rice or barley. The taste of *miso* is sweeter than *doujiang*. *Miso* is often used to prepare various types of soup, whereas *doujiang* is usually used as a seasoning.

There are three kinds of *miso*, based on the use of raw materials: soybean *miso*, rice *miso*, and barley *miso*. Rice *miso* is used to illustrate the processing steps involved in *miso* making (Figure 19.4). Unlike Chinese *qu*, rice is used as the raw material for *koji* making. The washed and soaked rice is steam-cooked and cooled. The cooked rice is inoculated with *tane-koji* (*Aspergillus oryzae*). The soybeans are washed, soaked, steam-cooked (or boiled), mashed, and cooled. The matured rice *koji* is mixed with the cooked soybean paste, together with salt and other species of microorganisms in the fermentation containers. The mixture is fermented for more than 6 months, and the rice *miso* is ready to be packed.

Since excessive salt intake is regarded as one of the causes of hypertension, there has been increasing interest in developing low-salt fermentated soy pastes. A salt-free *miso* can be prepared by supplementing the *koji* with ethanol (21), or ethanol with sugars and polyols (22). Supplementation of ethanol in *miso* not only enables low-salt fermentation but also enhances flavor formation (23).

C. Chemical and Biochemical Changes during *Miso* Processing

Due to enzymatic hydrolysis of macromolecules such as polysaccharides and proteins, fermented soy paste contains digestible, small-molecular-weight components. Unpasteurized *miso* has been found to have a vitamin B_{12} content ranging from 0.15–0.25 μg/100 g (24). During fermentation, complex metabolites are formed and contribute to the aroma and taste of the product. One of these metabolites is HEMF, formed by *Zygosaccharomyces rouxii*, with a strong cake-like aroma and a threshold value of less than 0.04 ppb, and is regarded as the most effective component in enhancing the aroma of *miso* (25).

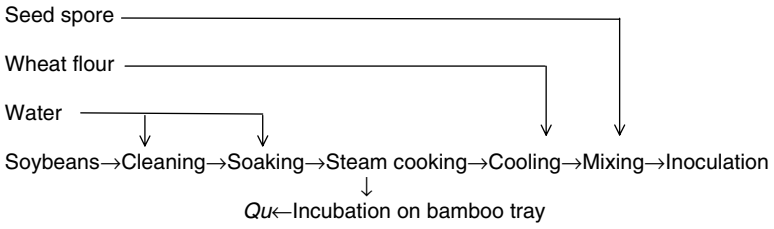

FIGURE 19.2 Pure culture method to make *qu*.

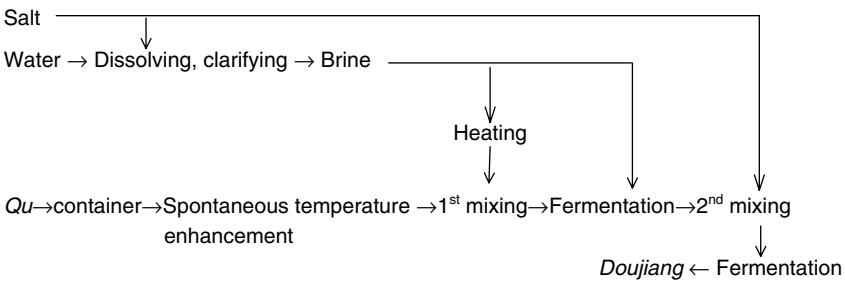

FIGURE 19.3 Pure culture method for *doujiang* making.

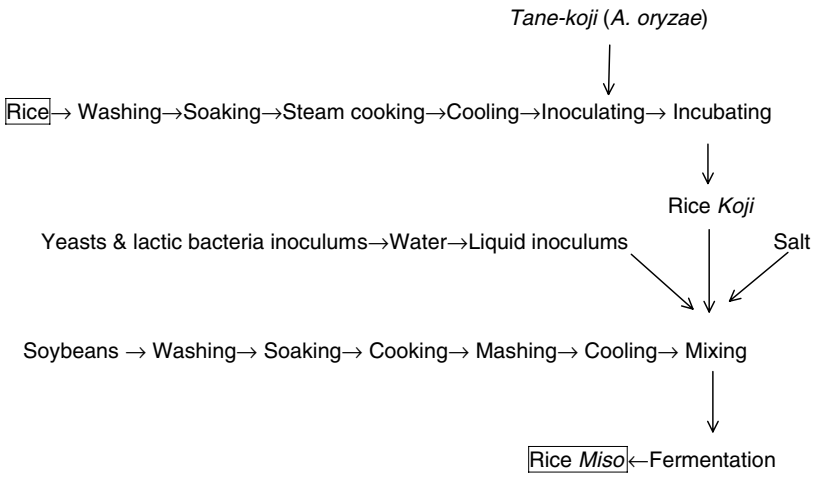

FIGURE 19.4 Flow chart of rice *miso* manufacturing.

Free amino acids are also very important components contributing to the taste of fermented soy paste.

IV. *SUFU (DOUFURU)*

Sufu is a soft cheese type product made from soymilk curd (*tofu*) by the action of microorganisms (26). It has a long history and written records date back to the Wei Dynasty (220–265 A.D.) in China (27). *Sufu* has been widely consumed by Chinese in a way similar to cheese by Westerners, mainly as an appetizer or a side dish, e.g., with breakfast rice or steamed bread (26).

Because of the numerous dialects in China and difficulties of phonetic translation from Chinese to English, *sufu* has appeared in the literature under many different names, such as *tosufu, fu-su, fu-ru, toe-fu-ru, tou-fu-ru, dou-fu-ru, teou-fu-ru, fu-ju, fu-yu, jiang-dou-fu, and foo-yue* (27). Officially, *sufu* should be named *doufuru* or *furu* in Chinese.

There are many types of *sufu* prepared by various processes in different parts of China. Three main types of *sufu* can be identified according to processing methods and fermentation microorganisms: mold-fermented *sufu*, bacteria-fermented *sufu*, and enzymatically ripened *sufu*. *Sufu* can also be classified based on color and flavor, due to different dressing mixtures used in the ripening stage. According to the color and flavor of the products, *sufu* can be classified as red *sufu*, white *sufu,* grey *sufu*, sauce *sufu*, and colored *sufu* (28). Figure 19.5 shows the different types of commercial *sufu* products.

FIGURE 19.5 Commercial *sufu* products.

A. Manufacturing Processs of *Sufu*

1. Mold-Type *Sufu*

Four steps are involved in the manufacturing of mold-type *sufu*: preparation of soybean curd (*tofu*), preparation of *pehtze* with a pure mold culture (fermentation), salting, and ripening. Figure 19.6 shows the flow chart of *sufu* manufacturing.

a. Preparation of tofu

Soybeans are washed, soaked in water overnight or at 25°C for 5–6 hours, and ground. After removing the residue by filtering, soybmilk is collected. In order to inactivate the growth inhibitors and to remove some of the beany flavor, the soymilk is heated to a boil. The curdling process is initiated by the addition of salts, such as calcium sulfate and magnesium chloride when the milk has

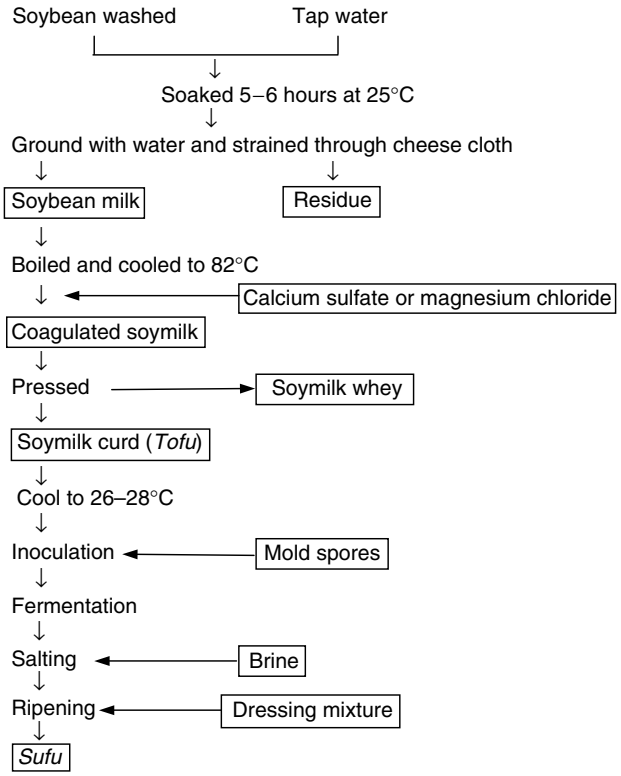

FIGURE 19.6 Flow chart of *sufu* manufacturing.

been cooled to about 82°C. Generally, 20% more coagulant is used to produce *tofu* for *sufu* than for regular *tofu* production. Coagulant is added at a concentration of 2.5–3.5% (coagulant/dry soybean, w/w). The mixture is agitated vigorously to obtain a homogenous coagulum, and then set aside for 15 minutes to complete the coagulation. The coagulum is pressed to remove excess whey, and the resulting *tofu* is cut into cubes of desired sizes.

b. Preparation of pehtze (pizi)

The *tofu* cubes (2.5 × 3 × 3 cm) are placed in a tray with pinholes at the top and bottom to aid air circulation. They should be separated from one another since the mold mycelia must develop on all sides of the cubes. When the cubes are cooled to 35–40°C, they are inoculated by spraying on their surfaces with a pure culture of an appropriate fungus. The inoculated cubes, *pehtze* (or *pizi*) are usually incubated at 20–24°C for 3 to 7 days, with temperature and time dependent on the fungal starin used. For *Actinomucor taiwanensis* and some *Mucor* strains, the best fermentation temperature is above 30°C (29, 30). During incubation, the *pehtze* cubes are covered with white mycelia (Figure 19.7).

c. Salting

After the *pehtze* cubes are separated from each other and the mycelia on the surface are rubbed down, they are piled up in a jar with salt spread between layers. During the salting period, the *pehtze* absorbs salt (not over 15%) and loses water, resulting in shrinkage and increase in hardness. Normally, the salting period lasts for 7–12 days, and the salted *pehtze* is washed with salty water. After being air-dried, it is transferred to another jar for further processing. The added salt not only retards mold growth, but most importantly, the salt solution releases mycelia-bound proteases which are not extracellular and can be easily eluted by salt or other ionic solutions, but not by water (31).

FIGURE 19.7 *Pehtze.*

d. Ripening

Ripening involves a series of biochemical reactions catalyzed by microbial enzymes. The differences among various types of *sufu* are mainly due to the use of different dressing mixtures during ripening. The basic and most common brine contains 12% NaCl and rice wine with about 10% ethyl alcohol. Other ingredients include yellow wine, red kojic rice, soy mash, pimiento, fennel, garlic, shallot, ginger, etc. The ripening time varies for different *sufu* types with different dressing mixtures. Normally it is allowed to age for 2 to 6 months. The product is then bottled with brine and marketed.

2. Bacterial-Type *Sufu*

The preparation of *tofu* is the same as in the mold-type *sufu*. The *tofu* cubes are steamed at 1 kg/cm^2 for 20 minutes. After cooling to 20–30°C, the cubes are salted for 24 hours and washed with water, put into fermented trays, and incubated at 28–30°C for 5 to 6 days after inoculation. When the *pehtze* is covered with bacteria and secretes a yellow liquid, it is dried at 50–60°C for 8 to 10 hours. The dried *pehtze* is placed into jars with a dressing mixture of salt, red kojic rice, rice wine containing 50% ethyl alcohol and other seasonings. The jars are sealed and incubated at 35°C for 20 days. More dressing mixture is then added and a further 50 days of fermentation is carried out, resulting in the production of a red *sufu* (32).

B. Microorganisms for Fermentation

Usually, the microorganisms for mold-type *sufu* are *Mucor* spp. (33), and for bacteria-type *sufu Bacillus* spp. or *Micrococcus* spp. (34). The organisms must develop enzyme systems having high proteolytic and lipolytic activities, and also have white or light yellowish mycelium to ensure that the final product has an attractive appearance. The texture of the mycelial mat should also be dense and thick to prevent any distortion in the shape of the fermented cubes. Of course, the mold growth should not develop any disagreeable odors, astringent tastes, or mycotoxin. *Actinomucor elegans* (35), *Mucor hiemalis*, *M. silvaticus*, *M. prainii* (36), *M. sufu* (35), and *M. subtilisimus* all possess these characteristics. Among them, *Actinomucor elegans* and *A. taiwanensis* seems to be the best organisms and are used commercially in Beijing and Taiwan, respectively.

In addition to *Mucor* spp., *Rhizophus* spp., *Aspergillus oryzae* and *Monascus* spp. which are found in red *sufu*, many other species of microorganisms have been isolated, such as *Penicilium*, *Cladosporium*, *Alternaria*, *Bacillus*, *Staphylococcus*, *Micrococcus*, and *Corynebacterium*. Some researchers suggested that yeasts and bacteria play a role in the flavor and texture of *sufu*, and the predominant halophile is identified as *Tetragenococcus halophila*. Microorganisms isolated from *sufu* made in different regions of China are listed in Table 19.3.

Han found that *Bacillus cereus* in a few *sufu* samples was over 10^5 CFU/g, and *Staphylococcus aureus* enterotoxin A was detected in some of the white and grey *sufu* samples, indicating a potential hazard to consumers (37).

C. Chemical and Biochemical Changes during *Sufu* Processing

During *sufu* manufacturing, glycerol esters are hydrolyzed to fatty acids and glycerol, and glycerol is transformed into organic acids by bacteria. Glutamic acid and leucine are notably present in the water-soluble fraction. Alcohol is produced during the brining and ripening process, and esters are then chemically or enzymatically synthesized from alcohol and fatty acids. Nucleic acids, such as guanidine nucleotide, are the degradation products after the thalli have autolyzed, providing the delicious taste and aroma of *sufu* along with the free fatty acids, organic acids, esters, and seasonings in the dressing mixture such as pimiento, pepper, curry, and sesame oil. The complex flavor has been reported to contain 22 esters, 18 alcohols, 7 ketones, 3 aldehydes, 2 pyrazines, 2 phenols, and other volatile compounds (38). The sugar content of ripen *sufu* is about 5%, so it has a somewhat sweet taste. The added salt imparts a salty taste to the products.

In spite of the differences in color and flavor, most types of *sufu* have a similar proximate composition. As shown in Table 19.4, *sufu* is a nutritious food. The vitamin B_2 content in red *sufu* is 0.42–0.78 mg/100 g, and in grey *sufu*, it is up to 9.8–18.8 mg/100 g, lower only than that in animal livers. Moreover, *sufu* contains vitamin B_1 at 0.04–0.09 mg/100 g and nicotinic acid at 0.5–1.10 mg/100 g. Minerals are abundant in *sufu*, especially calcium, iron, and zinc (39).

V. SOY NUGGETS (*DOUCHI*)

Soy nuggets (*douchi* in Chinese) are made by fermenting soaked and boiled whole yellow soybeans or black

TABLE 19.3
Major Microorganisms Isolated from *Sufu* Made in Different Regions of China

Microorganism	Regions Where *Sufu* is Made
Mucor sufu	Shaoxing, Suzhou, Zhengjiang
Mucor rouvanus	Jiangsu
Mucor Wutongqiao	Wutongqiao in Sichuan
Mucor feavus	Wutongqiao in Sichuan
Mucor racemosus	Taiwan, Niuhuasi in Sichuan
Mucor hiemalis	Taipei
Mucor spp.	Taiwan, Guangdong, Guilin, Hangzhou
Avtinomucor elegans	Beijing, Taipei, HongKong
Rhizopus liquefiems	Jiangsu
Aspergillus oryzae	Jiangsu, Wutongqiao in Sichuan
Penicillium spp.	Jiangsu
Alternaria spp.	Jiangsu
Cladosporium spp.	Jiangsu
Bacillus spp.	Wuhan
Micrococcus luteus	Kedong in Helongjiang
Saccharomyces	Jiangsu, Wutongqiao in Sichuan

Source: Adapted from Ref. 33.

TABLE 19.4
Nutrient Composition of *Sufu*

Component	Content (per 100 g)
Water	56.3 g
Protein	15.6 g
Fat	10.1 g
Carbohydrate	7.1 g
Crude fiber	0.1 g
Ash	1.12 g
Cholesterol	Not detected
Caloric	703.4 KJ
Calcium	231.6 mg
Phosphorus	301.0 mg
Iron	7.5 mg
Zinc	6.89 mg
Vitamin B_1	0.04 mg
Vitamin B_2	0.13 mg
Nicotinic acid	0.5 mg
Vitamin B_{12}	1.77 mg

Source: Adapted from Ref. 43.

soybeans with microorganisms, and are consumed as seasonings (40). *Douchi* production involves two distinct steps of fermentation. The first, which occurs during *koji* making, is by fungi or bacteria (similar to *natto* making), leading to enzyme production. The second is by yeast and lactic acid bacteria, with added salt and seasonings such as shallot, ginger, or garlic, and results in the production of a delicious flavor.

Douchi is the first traditional fermented soyfood to be described in written records. It originated in China before the Han Dynasty (206 B.C.). The first record of *douchi* was in *Si Ji*, which was written by Si Maqian (about 104 B.C.). In *Ben Cao Gang Mu* (Chinese Materia Medica), written by Li Shizhen in the Ming Dynasty (1368–1644 A.D.), some health-enhancing functions of *douchi* were recorded, such as the enhancement of appetite, promotion of digestion, inducement of sweat and recovery from fatigue, soothing of the mood, and prevention of asthma, etc. Products similar to *douchi* are also produced in other Asian countries, for example, *hamanatto* in Japan, *tao-si* in the Philippines, and *tao-tjo* in Eastern India (5).

A. Douchi Varieties

There are various types of *douchi* produced in China (see Figures 19.8 and 19.9). According to the water content, *douchi* can be classified into dry *douchi* and water *douchi*. Dry *douchi* is fermented loose whole soybeans, produced mainly in Southern China. Water *douchi* is fermented soybeans with additional water, and the final products are sticky, somewhat like *natto*. It is made in Northern China and the family-made products (Figure 19.9) belong to this type (40). According to the selection of fermentation microorganisms, *douchi* can be classified into three varieties: *Aspergillus*-type, *Mucor*-type, and bacteria-type. Among them, the *Aspergillus*-type is the earliest and most common type.

B. Preparation of *Douchi*

Douchi has been produced in China for a long time. Because of the diversity of climate, some famous brands are produced in various regions of China using different processing methods. Figure 19.10 shows the flow chart for the preparation of *Aspergillus*-type *douchi*.

1. Pre treatment of Raw Materials

Soybeans are cleaned to remove foreign matter and damaged or decomposed beans. They are soaked in water for 2–7 hours at a temperature below 40°C, until the water content reaches 45% (41). Boiling of soybeans is necessary to destroy contaminating bacteria that could interfere with fermentation, and to release nutrients required for mold growth. Traditionally, soybeans are boiled at 100° for 5 hours. For industrial preparation, soybeans are steamed at 0.1 MPa for 45 minutes to 1 hour. For bacterial-type *douchi*, soybeans are boiled at 100°C for 30–40 minutes.

The water content of soybeans should be controlled after boiling, so that the microorganisms can multiply and the beans remain intact. The best water content differs for different *douchi* varieties, about 50% for *Aspergillus*-type *douchi* and 45% for *Mucor*-type *douchi* (41).

2. Making of *Qu*

a. Aspergillus qu

Boiled soybeans are cooled to 35°C and inoculated with 0.3% *Aspergillus oryzae* or other *Aspergillus* spores, then incubated at about 25°C. If the temperature of soybeans is increased to 35°C, they should be cooled down by turning them over. When the soybeans are covered with yellow-greenish mycelium, usually the fermentation time is about 72 hours, and the *qu* is matured.

FIGURE 19.8 Commerical *douchi* products.

FIGURE 19.9 Homemade *douchi.*

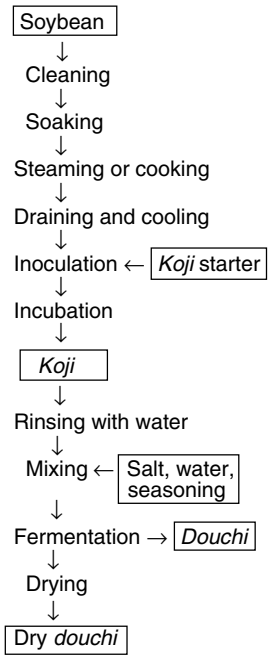

FIGURE 19.10 Flow chart of *Aspergillus*-type *douchi* manufacturing.

b. Mucor qu

Boiled soybeans are cooled to 30°C and inoculated with 0.5% *Mucor* strain spores, then incubated at 2–7°C, keeping the internal temperature of the beans below 18°C by ventilation. After 10–12 days, when the mycelium color changes to grey with a few black spores, the product is ready for harvest (41).

c. Bacteria qu

After boiling and draining, the soybeans are sealed and incubated at a high temperature (over 25°C) for 3–4 days. When the soybeans are covered with mucilage with a special flavor, the *qu* is matured.

3. Fermentation

Washing with water is a special processing step for the preparation of *Aspergillus*-type *douchi*. The *koji* is washed with water to remove the spores, mycelia, and part of the enzymes. Spores will impart a bitter and astringent flavor to the final product. Too much enzymes on the surface tend to hydrolyze the macromolecules excessively, causing soluble materials to increase, and the surface texture becomes coarse. Ordinarily, the washing time is about 10 minutes and the water content of the washed beans is 33–35%. After washing, the soybeans are stacked in containers without cover, and water is sprinkled occasionally to adjust the water content to about 50%.

Salt (18% w/w, soybean weight basis), a small amount of sugar, and a selected flavoring such as capsicum paste are mixed with the *koji* in order to obtain the desirable flavor (42). The mixture is put into jars, and one layer of soybean is loaded on top of another up to about 80% by volume. The jars are then sealed with plastic film.

The *Aspergillus*-type *douchi* is fermented in the jars at 30–35°C for 4–6 months, traditionally under sunlight. After fermentation, *douchi* are dried to 30% water content.

For *Mucor*-type *douchi*, the soybeans are not washed with water, but mixed with salt and flavoring directly. Fermentation is carried out at about 20°C for 10–12 months.

Salt is added to the soybeans to prevent metamorphose and to improve flavor, but the enzyme activities are inhibited, with an increase in fermentation time. Non-salt fermentation, carried out at a higher temperature (55–60°C), can shorten the fermentation time to 3–4 days.

C. Chemical and Biochemical Changes during *Douchi* Processing

The protein, fat, carbohydrate, and vitamin contents in *douchi* (Table 19.5) indicate that it is a nutritive food (43). During fermentation, the levels of free amino acids increase gradually (43). Glutamic acid is the predominant amino acid, contributing to the delicious flavor of *douchi*. Other types of flavor components are organic acids. In *douchi*, lactic acid content is high, followed by acetic and L-pyroglutamic acids (Table 19.5) (43).

VI. *Natto* and *Kinema*

Natto is a whole or fractional, slimy, fermented soybean product (Figure 19.11) which has a characteristic aroma. It is made with soaked and steamed soybeans fermented by *Bacillus* species. *Natto* was originated from China in the Tang Dynasty, and was introduced to Japan along with Buddhism (44). In Northeastern Japan, it has been produced for about 1000 years. It is now widespread in Japan, and is also prepared in Korea, Thailand (*thua-nao*), and India (*kinema*) (45). *Natto* is served for breakfast and dinner along with rice, and is sometimes consumed with

TABLE 19.5
Nutrient Composition of *Douchi*

Components	Content (per 100 g)
Water	35–50 g
Protein	20 g
Fat	7.1 g
Carbohydrate	21.4 g
Total acid	1.5 g
Formal nitrogen	0.7–1.0 g
Calcium	184 mg
Phosphorus	198 mg
Iron	5.5 mg
Vitamin B_1	0.13 mg
Vitamin B_2	0.23 mg
Nicotinic acid	3.2 mg

Source: Adapted from Ref. 43.

FIGURE 19.11 *Natto.*

shoyu and mustard. It can also be used as an ingredient for sauce production or as a flavoring agent.

A. Preparation of *Natto*

Natto preparation is relatively simple when compared to other fermented soybean products which employ a series of microorganisms, such as *sufu* and soy sauce. Only one organism, *Bacilli subtilis,* an aerobic Gram-positive rod bacterium, is involved in the fermentation of *natto*. *Bacillus natto*, classified as a related strain of *B. subtilis*, is the most commonly used strain. Many new strains have been bred and are used to make *natto*, such as strains with high γ-glutamyltranspeptidase (GTP) activity (46), high thrombolytic activity (47), and high elastase activity (48), or strains that produce highly viscous products.

The traditional and industrial manufacturing processes for *natto* are shown in Figure 19.12. Round and small soybeans with high content of soluble sugars are the ideal raw materials to produce *natto*. Since these soybeans have a small diameter, the steaming time can be shortened. Meanwhile, soluble sugars provide a carbon source for microbial growth, as well as a sweet taste to the final product. Soybeans are cleaned and soaked overnight in water, then cooked at 1.5 kg/cm^2 pressure for 30–40 minutes. Traditionally, the cooked soybeans are wrapped with rice straw, and kept in a warm place for 1–2 days. The rice straw provides *B. subtilis* and absorbs the unpleasant ammonia odor. In the industrial process, soybeans are inoculated with 1–3% pure culture suspension of *B. natto* and then packed in trays. After fermentation at 40°C for 18–24 hours, the beans are covered with a white sticky coating, and the products are ready for harvesting. In addition to refrigeration, drying at a low temperature is another effective method for prolonging the shelf life of *natto*.

B. Chemical and Biochemical Changes during *Natto* Processing

One characteristics of *B. subtilis* is that it can secrete many extracellular enzymes such as proteases, amylases, GTP (49), levansucrase, and phytase (50). During fermentation, the quantity of soluble and dialyzable matters increases from 22% and 6% to 65% and 40%, respectively (51). Some chemical reactions are catalyzed by these enzymes, with the formation of flavors and sticky materials, including 2, 3-butanediol, acetic acid, propionic acid, iso-butyric acid, and γ-polyglutamic acid (52). A 60-fold increase in free amino acids was observed during fermentation, which accounted for about 26% of the total amino acid content (53). Since there is no lipase, the fat and fiber content of soybeans are kept almost constant (54). The content of soluble sugars, the carbohydrate source for *B. natto*, is very low. The levels of oligosaccharides such as sucrose, raffinose, and stachyose decreased during soaking, boiling, and fermentation (55).

C. *Kinema*

Kinema is an indispensable dietary component in Nepal and the hilly regions of the Eastern states of the Indian subcontinent, where it has long been used as a meat substitute. *Kinema* is fried in oil, then cooked in water with vegetables and spices to prepare a thick curry, and eaten as a side dish with rice (56).

1. Preparation

To make traditional *kinema*, soybeans are washed, soaked overnight in water until soft and crushed lightly to dehull, boiled for about 90 minutes, crushed to grits, wrapped in fern leaves and sackcloth, and left to ferment for 1–3 days in a warm place (25–30°C) until the beans have a typical alkaline, *kinema* flavor and a desirable sticky consistency (57).

TABLE 19.6
Organic Acid Content in Different *Douchi* Products

Douchi Type	Product	Acetic Acid	L-Pyroglutamic Acid	Lactic Acid
Aspergillus type	Tianmashan *koji*	8.70	3.61	9.41
	Tianmashan *douchi*	38.94	7.98	88.89
	Yipinxing *koji*	5.87	1.10	2.94
	Yipinxing *douchi*	19.24	5.10	80.82
Mucor type	Yongchuan	3.40	5.10	12.89
	Black soybean	1.51	4.22	1.80

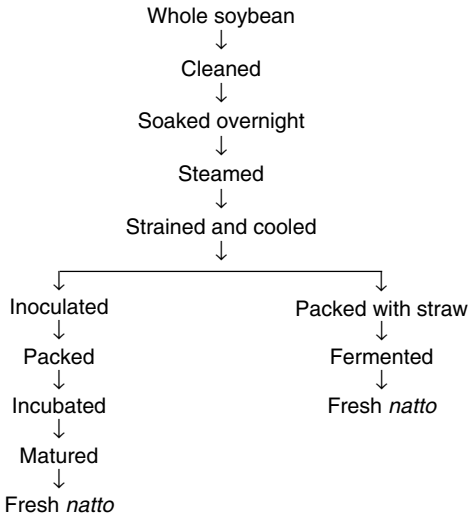

FIGURE 19.12 Traditional and industrial processes of *natto* making.

2. Microorganisms

Bacillus subtilis is the dominant microorganism and the sole fermentation organism found in *kinema*. *Enterococcus faecium, Candida parapsilosis*, and *Geotrichum cadidum* are the accompanying flora in *kinema* samples (58, 59). Among all starter cultures isolated from *kinema*, *Bacillus subtilis* KK2:B10 and GK-2:B10 are the best strains for improved *kinema* production (60). Organoleptically, the monoculture fermentation of soybean by *B. subtilis* produced the best quality *kinema* (61).

3. Microbiological Safety

Although *Staphylococcus aureus* has not been detected in any of the tested *kinema* samples, *Bacillus cereus* and *Escherichia coli* were present in several market samples, and some of them have the ability to produce diarrhoeal enterotoxin. *Enterobacteriaceae* and *Coliform* bacteria exceeded 10^5 CFU/g in most samples. It has been concluded that the traditional way of making *kinema* and its culinary use in curries is safe. However, for novel applications of *kinema*, safety precautions are advisable (58).

VII. *TEMPEH*

Tempeh is a fermented soyfood consisting of dehulled and tender-cooked soybeans bound together by a dense cottony mycelium of fragrant white *Rhizopus* mold into compact cakes or patties (62). It is a popular traditional Indonesian food produced by solid state fermentation (SSF). The technology was introduced by immigrants from Southeast China, and the product is similar to the Chinese *douchi*. *Tempeh* was then introduced to Malaysia, the Netherlands, and North America by Indonesian immigrants. It has now become quite popular in the United States and attracts attention in Japan and many European countries, particularly in the vegetarian market as a substitute for meat (63).

A. Types of *Tempeh*

Depending on the raw materials, several types of *tempeh* are produced in Indonesia, including the soybean *tempeh*, okara *tempeh*, velvet *tempeh*, and coconut or peanut presscake *tempeh,* or mixtures of these. In other countries, *tempeh* is made with other raw materials, such as common bean, lupin, horsebean, chickpea, and wheat.

Sold fresh, refrigerated, or frozen, *tempeh* is usually sliced and fried until its surface is crisp and golden brown, and it has a nice flavor and texture, which can be compared to those of fried chicken or fish sticks. Hence, *tempeh* is often consumed as a main dish or as a meat substitute, and has become a key source of protein in vegetarian diets. Most of all, the high vitamin B_{12} content led to its popularity among vegetarians in the Western world since plants do not contain this type of vitamin (64).

Tempeh can be served with grains and eggs for breakfast, or after frying, in salads, sandwiches, burgers, sauces, and soups for lunch or dinner.

B. Manufacturing Process of *Tempeh*

Tempeh can be homemade or produced in factories. There are two dehulling methods, namely wet dehulling and dry dehulling. Figure 19.13 shows the flow chart of *tempeh* production in temperate climate regions (62).

1. Pretreatment of Raw Materials

Soybeans are cleaned and dehulled. Cleaning will remove damaged beans and foreign matter. Since the fermentating microorganism *Rhizopus oligosporus* cannot grow on whole soybeans, dehulling is essential. Wet dehulling is performed by hands or foots after precooking and the hulls are separated by skimming (65). Dry dehulling is performed by cracking the seeds with a stone or burr mill, followed by separation of the hulls with an air stream or by gravity separation (66).

2. Acidification and Cooking

Acidification lowers the pH, normally to 4.8–5.0, favoring mold growth and restricting the growth of potential spoilage bacteria (67, 68). Traditionally, soybeans are soaked in hot (initially at 100°C) or room temperature water for 8–22 hours, and bacterial fermentation results in acidification. Nowadays, acidification of soybeans is done by the addition of an acidulant such as vinegar, lactic acid, or acetic acid to the cooking water (66).

A basic boiling period, typically 40–60 minutes, is required to soften the beans and to kill spoilage bacteria and their spores. The cooked soybeans are drained, cooled to about 38°C, and dried till the moisture content reaches 45–55% (66, 69).

3. Inoculation and Incubation

After cooling, the cooked soybeans are inoculated. Usually 10^6 viable spores of *Rhizopus oligosporus* per 100 g of cooked soybeans gave optimal mold growth (70). Then the soybeans are packed and placed in a warm place for fermentation. Traditionally, banana leaves or other large leaves are used as *tempeh* containers. Nowadays, perforated polyethylene bags, *tofu* tubs, aluminum trays, or slatted wooden trays covered with perforated polyethylene sheets are used (71).

Temperature, time, and relative humidity are the three crucial factors in *temeph* fermentation. The best relative humidity is between 70 and 85%. Fermentation can be carried out at a temperature ranging from 25 to 37°C. The standard procedure in Indonesia is fermentation at 25°C for 44–52 hours, but moderate temperature fermentation (31°C) and high temperature fermentation (37°C) are recommended as well (69, 71). High temperature fermentation favors growth of *R. oligosporus* and *Klebsiella pneumoniae*, and the products contain more vitamin B_{12} than those with moderate temperature fermentation, but the shelf life is shortened and temperature control is more difficult (62).

4. Harvesting, Storage, and Preservation

When soybeans are covered with and bound together by white mycelia, fermentation is completed. Fresh *tempeh* can last for several days at room temperature without obvious changes, and its shelf life can be prolonged by freezing, drying, frying, dehydration, canning, or other preservation methods. In addition, blanching or steaming is also an effective means to extend shelf life.

C. Microorganisms for *Tempeh* Making

Wet dehulling *tempeh* production involves two distinct stages of fermentation. The first stage, which occurs during soaking, is bacterial, resulting in acidification and the growth of *Bacillus cereus* is prevented. The second stage

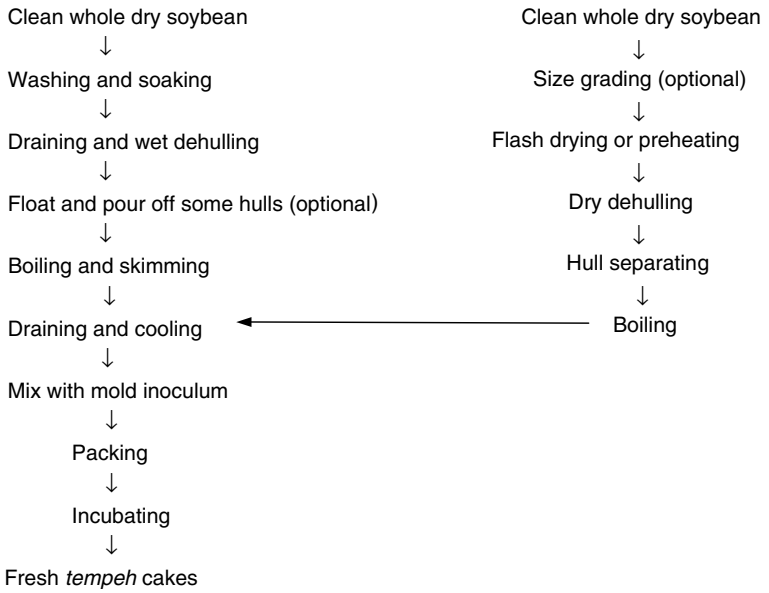

FIGURE 19.13 Wet and dry dehulling methods of *tempeh* making in temperate climate regions

is fungal, resulting in the growth of white mycelium on the bean cotyledons (68).

1. Microorganisms for Acidification

Lactic casei, *Enterococcus faecium*, *Streptococcus dysgalactiae*, and *Staphylococcus epidermidis* are the predominant species responsible for acidification (72), and *Citrobacter diversus*, *Enterobacter agglomerans*, *E. cloacae*, *Klebsiella pneumoniae*, and *K. oacenae* are present during soaking (73). *Lactic* and *Streptococci* spp. dominate the flora in both the unacidified and the acidified soaking water, while yeasts and coliforms are found only in unacidified water (74).

2. Microorganisms for Solid State Fermentation

There are three basic types of starters for *tempeh* fermentation: 1) pure culture mold starters typically containing only *Rhizopus oligosporus* spores, 2) mixed pure culture starters such as *R. oligosporus* and *Klebsiella pneumoniae,* and 3) mixed culture starters for traditional *tempeh*, containing a mixture of molds, bacteria, and/or yeasts. Because of its strong protease and lipase activity, *R. oligosporus* is the principal mold species used to make *tempeh*, and *R. oligosporus NRRL 2710* is the recommended commercial strain (72). Other *Rhizopus* species such as *R. oryzae*, *R. chinensis*, *R. arrhizus*, and *R. stolonifer* are also considered suitable for *tempeh* making (75, 76).

The *Rhizopus* strains form riboflavin, nicotinic acid, nicotinamide, ergosterol, and vitamin B_6, but do not produce physiologically active vitamin B_{12} (75, 76). Vitamin B_{12} is synthesized by bacterial strains of *Propionibacterium, Pseudomonas, Clostridium,* and *Streptomyces* (77), and include *Klebsiella pneumoniae*, *K. terigena, K. planticola*, and *Enterobacter cloacae* (72). *K. pneumoniae* and *Citrobacter freundii* show the best vitamin B_{12} formation capability. In order to increase vitamin B_{12} content, the mold and bacterium mixed pure culture starters have been used (69).

3. Microbiological Safety

Rhizopus oligosporus inhibited the growth, sporulation, and aflatoxin production of *Asperigullius flavus*, and no botulinal toxins were produced during the normal fermentation time of 24 hours or less (67). In early commercial *tempeh* production, *Enterobacteriaceae* and lactic acid bacteria were found in a majority of the samples, and *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* were found in some samples at a level of 10^5 CFU/g. *Yersinia enterocolitica* was found in several samples, whereas *Salmonella* was not observed (63). Regardless, traditional *tempeh* is regarded as a safe food (69).

D. Chemical and Biochemical Changes during *Tempeh* Processing

The composition of soybean is significantly altered by the physical and chemical treatments and the action of enzymes during fermentation in the making of *tempeh* (Table 19.7). *Rhizopus* spp. produces a variety of enzymes, including carbohydrases, lipases, proteases, endocellulase, xylanase, arabinase, α-D-galactosidases, etc. (72). Solid and nitrogen losses occur mainly during dehulling, soaking, and cooking, with little loss during fermentation. The initial pH increases to a high level after fermentation, hence biological acidification does not resulted in a sour tasting product (68). *Tempeh* is a nutritive food, as shown by its nutrient composition (Table 19.7). There is no marked difference in protein content between *tempeh* and unfermented soybeans, but an increase in free amino acid content can be observed during fermentation.

Tempeh is virtually non-flatulent since raffinose, stachyose, and other flatulence-causing carbohydrates found in soybeans are reduced. *Tempeh* is easily digested and can be tolerated by patients suffering from dysentery and nutritional edema (78).

R. oligosporus and *R. oryzae* have strong lipase activities and can break down soybean lipids into stearic, oleic, linolenic, linoleic, and palmitic acids, with linoleic acid predominating (79). The concentrations of calcium, phosphorus, iron, copper, zinc, magnesium, and manganese tend to be increased during fermentation, but potassium concentration decreases to a large extent (80). The bioavailability of zinc, calcium, iron, and magnesium increases due to their strong chelating properties, leading to a decrease in phytic acid content.

TABLE 19.7
Chemical Compositions of *Tempeh* Products

Tempeh	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)	Ash (%)
Fresh	60.4	19.5	7.5	9.9	1.4	1.3
Deep-fried	50.0	23.0	18.0	8.0	2.0	1.0
Freeze-dried	1.9	46.2	23.4	25.8	2.7	2.7
Fermented with *Rhizopus* sp. T-3	8.3	40.8	19.7	19.4	5.4	6.4
Cooked soybean	63.5	16.0	9.0	7.6	2.1	1.8

Source: Adapted from Refs. 2 and 73.

Riboflavin, pyridoxine, and nicotinic acid concentrations increase significantly over the 72-hour fermentation period as a result of their synthesis by different strains of *Rhizopus*, but the thiamine concentration decreases slightly (81). The highest reported value of vitamin B_{12} content in *tempeh* was 6.2 μg/1370 g. During fermentation, the total amount of vitamin E remains constant but the content of free tocopherols decreases (81).

VIII. HEALTH BENEFITS OF FERMENTED SOYBEAN PRODUCTS

Fermented soyfoods have some beneficial effects on human health, arising mainly from certain metabolically transformed components. Some examples of the health benefits of fermented soyfoods are illustrated below.

Angiotension inhibitory enzyme (ACE) activity has been detected in soy sauce, leading to reduced blood pressure in hypertensive rats after oral administration (82). Peptides with ACE activities were also found in water soluble fractions of *sufu* (83), water extracts of *douchi* (43), and *natto* extracts (84).

Isoflavones exist as aglycon in fermented soybean products. Isoflavones, genistein in particular, inhibit the activity of protein tyrosine kinases, thus reducing the risk of cancer (85). 8-Hydroxydaidzein, 8-hydroxygenistein, and syringic acid isolated from *miso* had high DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activities (86). 6, 7, 4′-Trihydroxyisoflavone, and glycitein (87), 6, 7, 4′-trihydroxyisoflavone, daidzein, and genistein may be responsible for the antioxidant activity of *tempeh* (88). *Natto* also has antioxidation activity on unsaturated fatty acids, and shows protective effects on the free radical-mediated cellular damage induced by cumene hydroperoxide (89). Certain components such as saturated hydrocarbons (C30–C32, especially hentriacontane) are present in *natto* and they have possible antitumor-promoting activity (90). Lactic bacteria from *miso* could bind hereroccyclic amines, which are mutagenic and exist in cooked foods, especially fried and toasted foods rich in protein (91).

The insoluble peptides in *sufu* can lower cholesterol level in blood serum (85). Moreover, peptides with the capacity of binding cholic acid were found in water-insoluble fractions of *sufu*. A water-solube extract of *douchi* was found to exert a strong inhibitory activity against rat intestinal α-glucosidase (92). It was found that *douchi* has an anti-hyperglycemic effect and may have potential uses in the management of type-2 diabetic patients (93).

A strong fibrinolytic enzyme, nattokinase, and at least three pro-urokinase activators (PUA) which are different from nattokinase, have been purified from *natto* (94, 95). An enzyme with a strong fibrinolytic activity was purified from *douchi* (96). *Natto* is the only food known that naturally contains vitamin K2 (97), which may contribute to the relatively lower fracture risk in Japanese women (98). *Bacillus natto* is effective in restraining the growth of *Salmonella* spp., *Escherichia coli* O157:H7, and microorganisms that can induce typhoid fever and diarrhea. Staphylococcal enterotoxin A (SEA) was fragmented into small peptides by subtilisin, an extracellular proteinase produced by *Bacillus natto* (99).

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20 Vegetables: Types and Biology

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CONTENTS

I. DEFINITION OF VEGETABLES

A vegetable is defined as "an edible, usually succulent plant or a portion of it eaten with staples as main course or as supplementary food in cooked or raw form" (1).

II. IMPORTANCE OF VEGETABLES

More than 10,000 plant species are eaten as vegetables worldwide. Among these species, only 50 or so are commercially important (2). Vegetables contribute to humans essential minerals, vitamins, dietary fibers, proteins, fats, starches, and energy. Vegetables are a major source of vitamin C. The amounts of carotenes in pumpkins, capsicum peppers, and tomato are useful to mankind. Dietary fibers in vegetables include cellulose, hemicellulose, pectic substances, and lignin that are important in preventing several human diseases. Vegetables also neutralize the acid substances produced by other high-energy food (3). While organic acids and volatile compounds are responsible for flavor and aroma, chlorophyll, carotene, and anthocyanin make up the colors. Vegetables not only form an essential part of a well-balanced diet, but the flavor, aroma, and color also make them important in human diet and appetite (4).

III. DOMESTICATION OF VEGETABLES

All modern crops had their earliest beginnings as wild plants. These wild plants with specific characteristics attracted humans to harvest them for medicinal (5), herbal, or food purposes. Then the seeds and other plant parts were dispersed. It was the beginning step during plant domestication. Vegetables were brought into cultivation 10,000 years ago, so humans produced sufficient vegetables of their own. The following artificial selection creates gene recombination of higher yield and better quality (6). Evolution of consumption, production techniques, socio-economic interest, general political climate, production policy, international competition, and trade agreements made the structure of today's vegetable exploitation heterogeneous (7).

IV. CLASSIFICATION OF VEGETABLES

Vegetables are commonly grouped according to botany, edible parts, life cycle, sensitivity to temperature, family grouping, or accepted use (2). Other classification schemes include sensitivity to soil pH and chilling damage, tolerance to nutrient levels and salt, and depth of rooting (2–4, 8–10).

A. Botanical Classification

Botanical classification is based on morphology, anatomy, embryology, physiology, biochemistry, etc. The successive groupings of plants are kingdom, division, subdivision, phylum, subphylum, class, subclass, order, family, genus, and species (2, 8), Predominant vegetables belong to the class Angiospermae that is grouped into subclasses Monocotyledoneae and Dicotyledoneae. Most vegetables belong to the Dicotyledoneae. There are fewer monocot vegetables, i.e., sweet corn, asparagus, yam, onion, etc. The genus and species make up the scientific name that is accepted worldwide. The climate requirements, the use for economic purposes, the disease and insect controls of a particular family or genus are often similar. Well-known families of vegetables are Solanaceae, Brassicaceae, Fabaceae, Alliaceae, and Apiaceae (10).

B. Classification Based on Edible Part

Classification of vegetables by edible part informs a grower or handler about specific cultural or handling techniques. Common groupings includes root, stem, leaf, immature flower bud, fruit, and sprout. Root crops include carrot, radish, beet, turnip, and sweet potato. Stem vegetables are asparagus and potato. The yield and quality of root and stem vegetables are affected by soil texture, fertility, and irrigation. Leafy crops include lettuce, cabbage, celery, spinach, kale, and mustard that are very perishable. Edible parts of cauliflower, broccoli, and artichoke are immature flower buds. Immature fruits are harvested from pea, snap bean, lima bean, summer squash, cucumber, okra, sweet corn, and eggplant, but actually we eat the immature seeds of lima bean and sweet corn. Edible parts of cucurbits (pumpkin, white gourd, squash, muskmelon, and watermelon), tomato, and pepper are mature fruits (1–3, 8–10).

C. Classification Based on Temperature

Vegetables are separated into warm-season and cool-season vegetables based on temperature requirements for optimum growth and development. Warm-season crops are adapted to 18~29°C, intolerant to frost and mostly grown for edible fruits. Exceptions are sweet potato for storage root and New Zealand spinach for leaves. Cool-season vegetables have optimum growth at cooler temperature and are shallower rooted and smaller sized. Cool-season crops are grown for edible stems, leaves, roots, and immature flower parts. Asparagus, Brussel sprouts, broccoli,

cabbage, celery, garlic, onion, pea, radish, artichoke, and spinach are cool-season vegetables. Harvested parts are usually stored near 0°C except potato (2, 8–10).

The sub-groupings of cool-season crops into hardy and half-hardy vegetables, and warm-season crops into tender and very tender vegetables, are based on the ability of young plants to withstand frost, and the ability of seeds to germinate at low temperatures. Hardy vegetables generally tolerate moderate frost without injury. Tender vegetables are susceptible to damage during cold weather. The very tender vegetables are easily damaged by light frost (10).

D. Classification Based on Life Cycle

Vegetables are also classified based on their life span. Most vegetables are annuals that complete life cycles within one growing season. Biennial vegetables require two seasons for completing their life cycle. Many cole crops such as broccoli, cauliflower, cabbage, and root crops such as carrot are biennials but grown as annuals. Perennial vegetables complete their life cycle in more than two years. Rhubarb, globe artichoke, and asparagus are grown commercially as true perennials. Tomato, pepper, eggplant, potato, and sweet potato are perennials in their native environments but are grown as annuals for production in temperate regions (2, 3, 8–10).

V. TYPES OF VEGETABLE GROWING

There are several types of vegetable growing such as home gardening, commercial production, and processing production. Commercial vegetable production, includes at least three categories: fresh market, processing, and controlled environment production (5, 11).

A. Home Gardening

People grow vegetables in their own gardens to save money, for outdoor leisure, for fresher tasting and better quality vegetables, and for better nutrition and improved health (2).

B. Commercial Production

The goal of commercial production of vegetables, either for fresh use or processing is only for economic profit.

Fresh Market. The harvested vegetables are sold for fresh use (2, 5, 11).

a. **Market Garden.** Market gardens are located near but on the outskirts of population centers. A wide variety but small scales of high-profit crops are grown intensively and year-round. The harvested vegetables are for local consumption (2, 5, 11).
b. **Truck Farm.** Truck farms are often located in inexpensive rural areas and near transportation systems. One or two crops are grown on a large acreage for distant markets. Transport over large distances permits specialization and the de-localization of production (2, 11).
c. **Controlled Environment Production.** Vegetables are grown in the modified environment for optimal plant growth. Light, temperature, humidity, nutrients, and even composition of atmosphere may be controlled. Investment and production costs including heating and cooling are expensive (5).

C. Processing Production

Vegetables are highly perishable. Post-harvest decay is estimated to be more than 20~50% in the tropics and subtropics. Processing is one of the various feasible technological measures to reduce high post-harvest losses of vegetables (3). Now in processing production, vegetables are grown in the field as raw materials for processing, usually on large acreage, harvested by machines and through contracts. The contract specifies some production techniques, price at a given quality, and standards for acceptance of harvest. Growers usually have a low margin of profit (11).

VI. CONSUMPTION OF VEGETABLES

The per capita consumption of vegetables varies among countries and regions, according to people's eating habits and the supply. The average world consumption of vegetables is around 85 kg per person per year, with around 120 kg per capita in industrialized countries (12), and around 30 kg per capita in developing countries such as in sub-Saharan Africa and 150 per capita in China (7).

VII. COMMERCIALLY IMPORTANT VEGETABLE CROPS

A. Root Crops

Several root crops are grown especially for their edible storage roots. They belong to different botanical families. Only one enlarged (fleshy) underground root is produced per plant for carrot (Apiaceae), table beet (Chenopodiaceae), radish, turnip, and rutabaga (Brassicaceae). Several fleshy roots are produced from one plant for sweet potato (Convolvulaceae). They are consumed either fresh or in processed forms. Most root crops have long storage life and extend the market supply over a long period. There are other minor root crops produced more on a regional basis, such as salsify (*Tragopogon porrifolius*) and black salsify (*Scorzonera hispanica*) of Compositae, parsnip (*Pastinaca sativa*) and celeriac (*A. graveolens* var.

rapaceurm) of Apiaceae, yam bean (*Pachyrrhizus erosus*) of Fabaceae, and horseradish (*Armoracia rusticana*) of Brassicaceae (13, 14).

The carrots, table beet, radish, turnip, and rutabaga are all direct-seeded to well-prepared seedbeds. After emergence, plants are thinned to desired population density. The crops are established more easily under cool-moist conditions (14).

1. Carrots [*Daucus carota* L. ssp. *sativus* (Htoffm.) Arcang]

Cultivated carrots, originating in Afghanistan and central Asia, became popular in Europe around the 13th century. European settlers brought carrots to the U.S. in the 17th century (15, 16). Carrots are now mainly grown in Asia and Europe. The Eastern/Asiatic carrots have reddish purple (anthocyanin-containing) or yellow roots, pubescent leaves, and a tendency for early flowering. Western carrots have orange, yellow, red, or white roots, less pubescent leaves and also a tendency to bolt. The western orange type developed as selections from yellow carrots for high carotenoid content were developed into modern cultivars. In the U.S. carrots are mainly grown for the fresh market with California being the leading state of acreage. For processing, Washington State leads the production (14).

Carrot is a cool-season crop with optimal mean growing temperatures ranging between 16 and 21°C. At these temperatures, root color and shape are also optimized. At a mean temperature of 12~13°C, roots tend to grow relatively long and slender, whereas at a constant 24°C, roots are shorter and thicker. Alternating low night and moderate day temperature also tends to produce long and slender roots. Temperature greater than 30°C, particularly in later stages of development, induces undesirable strong flavor and coarseness in the roots (16).

Carrot cultivars are classified by root shape and date of maturity (14):

a. Danvers: roots medium to long with broad shoulders, tapering toward the tip (tapered tips).
b. Imperator: roots slender, slightly longer and smoothly tapered, late maturing, good for storing, grown for winter market consumption.
c. Nantes: roots nearly cylindrical shaped, medium to long, early maturing, eaten fresh in summer.
d. Chatenay: medium to short and tapered with blunt end, maturing by mid-summer.

Carrots are usually mechanically harvested in 90~120 days of planting. Large-scale carrots are eaten raw, cooked, or processed into juice. The harvest stage is judged by suitability, before carrots achieve their full potential size or weight. Fresh carrots are marketed either topped or bunched with attached tops. Fresh cut-and-peel baby carrots are also available; they may also be cut into short pieces from mature carrots. Carrots are a good plant source of provitamin A, containing about 5~8 mg/100 g of β-carotene (14).

2. Radish (*Raphanus sativus* L.)

Radish is unknown in the wild state. Its origin may be in the eastern Mediterranean or in China, with a long history of cultivation. Now radishes are grown worldwide but consumed in great quantities by the Chinese, Korean and Japanese. Large variations exist in the shape, size, and color of the roots. Radishes were among the first European crops introduced into America by the Spaniards and grown by the early colonists (14, 16).

Radish is a cool-season vegetable crop with optimum growing temperature ranging between 15 and 20°C. At higher temperature, the enlargement of roots is retarded and results in coarseness and pungency (14).

Radishes are commonly grouped into four types (16):

a. Western or small radishes, usually consumed raw as relishes.
b. Oriental radishes, with mild-flavored large roots, usually cooked or pickled in the East.
c. Leaf radishes, consumed as greens by the Chinese, also cultivated for fodder.
d. Rat-tailed radishes, cultivated in Asia with young pods consumed raw, cooked, or pickled.

In the U.S. garden radishes are very popular in home gardens because of short growth cycle and easiness to grow. The white, long-rooted types are also popular in many regions.

3. Table Beets (*Beta vulgaris* L. Crassa Group)

Originating in Europe and Western Asia, the garden beet or table beet is one of the various forms of *Beta vulgaris* of the Chenopodiaceae family (17). It is closely related to Swiss chard, sugar beet, and fodder beet. The leaf beets were developed before the root beets, but the red root beets were cultivated by the Romans. The root beet is grown throughout Europe and America. The red pigment, betanin (a nitrogen-containing anthocyanin), can be used for food coloring. The table beet, introduced in 1800, is one of the most popular home garden crops in the U.S.

Table beets prefer a cool climate with sunny days. Temperatures for optimum growth range between 16 and 19°C. During hot weather, the roots may become tough. Beets are very sensitive to soil acidity, and require a pH of 6.2 to 6.8 (17).

Beet roots may vary in color and shape. The oblate to globe-shaped, red-rooted types are most popular. Most of the commercial production is for processing (14).

4. Turnip [*Brassica rapa* L. var. *rapa* (DC.) Metzz.] and Rutabaga [*B. napus* L. var. *napobrassica* (L.) Reichb.]

Both turnip and rutabaga are members of the Brassicaceae family. They are similar in plant size and general characteristics. Turnip is an ancient crop with its exact origin unknown while rutabaga is a crop of European origin known as Swede in Europe (13, 14). The turnip roots have little or no neck and a distinct taproot, while rutabagas have a thick neck bearing a number of leaf-base scars, and roots containing the taproot and those originating from the underside of the edible root.

Turnips and rutabagas are both cool-season crops, requiring 15~18°C for best root growth. Turnips are easy to grow and require a 2-month growth period. Rutabagas grow less rapidly and require an additional 4 weeks of growth.

Both turnip and rutabaga have swollen roots of different colors or shapes. However, most turnip cultivars are round and white-fleshed, and rutabaga cultivars are globe-shaped with yellow flesh. There are also turnip cultivars grown for green foliage (14).

5. Sweet Potato [*Ipomoea batatas* (L.) Lam]

Sweet potato, a member of the Convolvulaceae family, was originated from tropical America. It was grown for its storage roots in the New World long before Columbus arrived. Storage root of sweet potato is a major carbohydrate source in developing nations. It contains about 27% carbohydrate, provitamin A, vitamin C, calcium, and iron. Tender leaves and shoot tips are also used as vegetables in Southeast Asia. Other than food, sweet potato has industrial applications: as a source of starch, glucose, syrup, and alcohol. It is also used as livestock feed. Older vines are fodder for cattle, swine, and fish. Some vining cultivars can be used as ground cover or for ornamental purposes (18).

Sweet potato is a tender, warm-season crop. The best growing temperatures are 29°C days and 21°C nights, with an optimum mean of 24°C (14, 18). It is a perennial but commonly grown as an annual. Adventitious buds arise from fleshy storage roots, and develop into branching vines quickly covering the ground (18).

Sweet potato is vegetatively propagated by slips or vine cuttings. Thin skin of storage roots is easily broken. Four to seven days of 26.6~29.4°C and 85~90% RH curing promotes the formation of cork layers on wounded surfaces that prevent decay (18).

Storage roots of sweet potato as food have two types: soft-fleshed and firm-fleshed (18):

a. Soft-fleshed (wet): sweeter, softer, medium to deep orange flesh, commonly used for baking.
b. Firm-fleshed (dry): yellow skin with white, yellow, or light orange flesh, mostly used for boiling and frying.

B. Stem and Tuber Crops

Stem vegetables are those grown for their succulent tender shoots (asparagus, bamboo shoots), fleshy stems (kohlrabi, celtuce, strumous mustard), starchy underground tubers (potato), corms (taro), and succulent rhizomes (ginger). Among them, kohlrabi, celtuce, strumous mustard, and asparagus are seed propagated, and others are asexually propagated. The latter have low multiplication rate and need reliable sources for healthy growing materials. In the United States, asparagus and potatoes are of more commercial importance.

1. Potato (*Solanum tuberosum* L.)

The potato, native to the Andean regions of Peru and Bolivia, has been cultivated since early civilization (19). It is one of the most important food crops in the world. The Spaniards introduced it to Europe and Irish immigrants brought potatoes to New England in 1718. The potato is referred to as the "Irish potato" because of its association with the potato famine in Ireland in 1845–1846. Idaho and Washington State are the largest producers of potatoes in the United States (20).

Potatoes are grown for their tubers, the enlarged underground storage stems. In addition to their starch content, the tubers serve as a good source of vitamin C. The potato is also a source of moderate levels of protein and minerals. The protein of potato is richer in lysine than that of cereal, and its biological value is high. Potatoes can be cooked in a great variety of ways. They can also be processed into chips, French fries, flakes, and dehydrated products. French fries and potato chips are popular food items worldwide (19).

Potatoes are asexually propagated by healthy tubers, which are obtained from certified disease-free stocks grown in favorable cool areas. The young shoots develop from the buds or "eyes" of the seed tubers. It is a cool-season crop. The interaction of photoperiod and temperature are the most important factors affecting plant and tuber development. Long days delay the start of tuberization, and temperatures above 30°C prevent tuber initiation. Tubers are usually initiated about 45 days after planting. Following tuberization, tuber enlargement is ideal at mean temperatures of 17°C (19).

Based on skin color and texture, potato cultivars are classified as white, red, or russet. Russet tubers tend to be oblong and relatively dark colored and thick skinned at maturity. There are early, midseason, and late cultivars according to maturity time. Based on starch content or specific gravity, potatoes are grouped into baking, boiling,

and processing types. Russet Burbank, the leading cultivar grown in the U.S., is excellent for frying and baking. Kennebec is an excellent all-purpose potato (20).

2. Asparagus (Asparagus officinalis L.)

Asparagus, a dioecious perennial monocot, is a member of the Liliaceae family (21). The region between the eastern Mediterranean and eastward to the Caucasus Mountains is the center of origin of asparagus (22). It has been cultivated for medicinal and food use for more than 2,000 years. In the 1600s it was introduced to America. Commercial production is centered in California, Washington State, and Michigan (23).

Priced as a gourmet item, asparagus produces tender spears, unexpanded shoots each year. Nutritionally, asparagus is a source of vitamins A and C (22). The plant is composed of ferns, a crown, and a root system. The fern is a photosynthetically active modified stem called a cladophyll. The crown is a series of rhizomes (underground root-like stems) attached to the plant base. Upper portions of the horizontal rhizome contain the buds from which spears arise. Fleshy and fibrous roots develop from the lower portion of the rhizome. The fleshy roots act as storage organs. The carbohydrates stored in crown and roots support spear growth in the spring.

Asparagus grows best under conditions of high light intensity, warm days, cool nights, low relative humidity, and adequate soil moisture. Optimum productivity occurs at 25~29°C during the day and 13~19°C at night (22).

Female plants generally produce larger spears than males, but the males produce more smaller-diameter spears. All male lines are developed for superior productivity with reduced seed production (23).

C. Bulb Crops

Bulb crops are all herbaceous monocot species of Allium and are members of Alliaceae family. The genus Allium contains about 500 species, mostly wild. The few species cultivated as vegetables are grown for their fleshy leaf bases and/or tender leaves. Only onions and garlic have prominent bulbs; all others have "pseudostems." All bulb crops contain the thio-allyl compound alliin which breaks down to give a number of volatile sulfur-containing compounds and give the characteristic odor and pungency of the crop. The chemical substances in onion and garlic especially are believed to be associated with reduced risks of cardiovascular diseases and certain cancers (21, 24).

The bulb crops are propagated by seeds (onion, Welsh onion, and Chinese chives), cloves (garlic), or division (Welsh onion and Chinese chive).

1. Onion (*Allium cepa* L. Cepa Group)

Originating in Central Asia, the common onion has been cultivated for more than 4,000 years (24, 25). As an important flavoring, onion is a very popular crop worldwide. Asia is the largest producer, with Japan and China taking a share of 27%. Columbus introduced the onion to America and it soon spread to all parts of the Americas. Onions have many culinary uses. They can also be processed into dry products such as rings, flakes, and powder for the food processing industry. Quercetin, a flavonoid in onions, provides the protective effect against cancer. Onions can be planted using sets (small bulbs produced in previous season), transplants, and seeds.

Onions are cool-season crops with optimum growth temperature ranging between 13 and 24°C. Onion bulbing is usually favored by long days. However, the length of day required for specific cultivars is different. Under favorable day length, temperatures of 21~27°C are favorable for bulb development. Low relative humidity extending into the harvest and curing periods are desirable (24, 25).

Onions are usually grouped by their day length requirement for bulbing. Within the group, there are early and late maturing cultivars. Onions can also be grouped into mild or pungent (26):

a. Short-day type (European onion): bulbing in response to 10~11 h of day length, mild, soft-fleshed bulbs for fresh use.
b. Intermediate-day type: bulbing in response to 12~13 h of day length, pungent, soft-fleshed for fresh use.
c. Long-day type (Spanish onion): bulbing in response to 14 or more hours of day length, pungent, hard, good for storage.

Bulbs vary in skin color, shape, and size. There are more yellow or brown onion cultivars than red or white cultivars.

2. Garlic (*Allium sativum* L.)

Originating in Central Asia, garlic has been cultivated from at least 2000 B.C. Widely grown in Asia, garlic is eaten not only for the bulbs, but also for its foliage and flower stalks. Each plant develops the bulb underground, and 8~20 cloves together form a cluster covered by white or purplish papery sheath. Garlic has long been believed to have medical advantages in addition to its flavoring use. It is dehydrated to produce garlic powder and garlic oil capsules made of garlic extracts as a diet supplement. In the U.S. most garlic is produced in California for the bulbs. The planting is carried out in late summer and fall from clean and healthy cloves. The plants overwinter in the field and resume rapid top growth after spring. Large cloves produce greater yields than small cloves.

Garlic is a cool-season crop with cloves germinating best in temperatures of 20~25°C. The optimum temperatures for plant growth and bulb development are 18~20°C and 20~25°C, respectively. Bulbing is initiated as temperatures and day length increase (24, 25).

Garlic cultivars can be grouped by their day length requirement for bulbing (26). Into "Late" and "Early" types. They can also be grouped into hardneck and softneck types. In the Orient, the softneck type is preferred for foliage production (27).

D. Cole and Related Crops

Cole crops originated along the East Mediterranean and Asia Minor. They are all members of the species *Brassica oleracea* of the family Brassicaceae. During domestication, many cultivated types with distinct edible parts have formed, including cabbage and Brussel sprouts (head), kohlrabi (thickened stem), cauliflower and broccoli (inflorescence), and kale and Chinese kale (foliage) (28–31).

1. Cabbage (*Brassica oleracea* L. *Capitata* Group)

Cabbage has been used as food for more than 3,000 years. The ancient Greeks held cabbage in high esteem. Cabbage was probably introduced by the Romans or Celts from the coastal regions of the Mediterranean Sea to the chalky coasts of England and northwestern France. Present-day cultivars most likely originated from wild non-heading types. Now cabbage is very popular worldwide and is grown extensively in Eastern Europe and the Far East (30).

Cabbage is a herbaceous biennial but grown as an annual. During vegetative development, the plant produces a succession of out-spreading leaves on a stem with very short internodes. About 20 new leaves incurve, overlap, and form a compact head. Leaves are broad, thick, fleshy, heavily veined, and covered with wax. Cabbage is durable for storing and shipping. It ranks higher than tomato but lower than spinach in mineral content (30).

Cabbages are grown for three types of markets: fresh market, late or stored market, and the sauerkraut market. There are several types of cabbage head (31):

a. Wakefield: pointed, small, pointed head, early maturing.
b. Copenhagen market: round, medium-large head, early maturing.
c. Flat Dutch: large, flat, very solid head.
d. Danish Ballhead: round-oval, medium-sized head, relatively late maturing, storable.
e. Savoy: medium-large, flat-globe-shaped head, crinkly leaves, good quality for fresh market.
f. Red: round, medium-sized heads, reddish-purple leaf.

2. Cauliflower (*Brassica oleracea* L. var. *botrytis* L.)

Both cauliflower and broccoli are of the cabbage family with cauliflower being more exact in environment and cultural requirement. Cauliflower is grown for the curd (head), which is the shortened shoot with bracts and undifferentiated flower parts at the terminal end of the plant axis. The curd may be white, creamy, yellowish green, purple or orange. However, pure white curds are preferred. Cauliflower was first mentioned in the U.S. in 1806. California leads in commercial production (30).

Optimum temperatures for growth are 15~20°C with an average maximum of 25°C and minimum of 8°C. Many tropical cultivars are early matured and require higher temperature and long days for good vegetative growth before forming curd. After the white head has developed to 5~7.5 cm, it is protected from sunburn and turning green by tying the outer leaves together over the head center or just bending a few outer leaves to cover. In Asia, blanching is achieved by covering the developing head with a piece of spun-bonded material which can be reused (28, 29).

Cauliflowers are generally grouped into three major types by maturity (30):

a. Super Snowball (early): dwarf with medium sized leaves and somewhat flattened, and maturing in 50~55 days after transplanting.
b. Snowball (mid-season): larger and later, large rounded and very dense curd, maturing 70~80 days after transplanting.
c. Winter (later): grown where winters are mild, maturing 150 or more days after transplanting.

The later the maturity is, the larger the curd. In California cauliflowers may also be grouped by curd size and density.

3. Broccoli (*Brassica oleracea* L. var. *italica* Plenck)

Broccoli evolved from wild cabbage earlier than cauliflower and was cultivated by the ancient Romans (28). However, it was relatively unknown in England until the 18th century. It was grown in the U.S. in the early 1800s (28–30), but its popularity came much later.

Broccoli is similar to cauliflower in the structure of its flower head. Unlike cauliflower, the edible plant portion is the inflorescence consisting of fully differentiated immature flower buds and the tender portion of the upper stem. These flower buds form a compact head. If the terminal inflorescence is removed, secondary inflorescences may develop in the axils of lower leaves.

Broccoli is the most nutritious of the cole crops in vitamin content, calcium, and iron. Its anticancer properties have been often reported. Per capita consumption

continues to increase. California is the largest producer in the U.S. (30).

Broccoli is adapted to a range of soil types and can tolerate heat to a greater degree than cauliflower. The optimum temperatures for plant growth are 20~22°C and 18°C for head development. It is sensitive to boron deficiency (28, 29).

There is no major subgroup for broccoli. Cultivars of Calabrease, Green Comet, Green Duke, and Premium Crop are popular (30).

E. Other Leafy Vegetables

Greens are grown for leafy portions both for cooking and salads. They are high in mineral and vitamin contents. All greens are more of specialty crops except spinach, which is produced on a large commercial scale. All greens in North America are cool-season crops except New Zealand spinach which is a warm-season crop (32).

Lettuce, endive-escarole, and chicory are leafy salad vegetables. Their tender leaf blades with a little petiole and stem are used fresh or raw in salads. They are excellent dietary sources of bulk and fiber. Only lettuce is grown on a large scale (34).

1. Chinese Cabbage, Pe-tsai (*Brassica rapa* L. Pekinensis Group)

Chinese cabbage is native to China and eastern Asia (32). Its recent popularity has resulted in a considerable increase in production in Europe and the U.S. It produces an elongated head. Moderate day and cool night temperatures are essential for productivity and quality. Temperatures ranging between 13~21°C are suitable for its growth (28).

High temperature during head formation causes a loose head and increased incidence of tipburn. There are several types of head: elongated, shorter, tall, and short and compact (28, 32).

2. Spinach (*Spinacia oleracea* L.)

Spinach is thought to be native to Central Asia. It ranks second only to broccoli in total nutrient concentration. Spinach is used fresh and for canning, freezing, and pureed baby food. Due to a short growth period of 30~50 days, annual spinach is cultivated between planting of other vegetable crops (33).

Spinach is usually dioecious, rarely monoecious. Dioecious types produce extreme male and vegetative male. The extreme male plant is small. While vegetative male and female plants produce more foliage and flower later, they are the preferred types for commercial production (33).

Spinach is a hardy, cool-season vegetable. It prefers 15~20°C for growth, 15°C for seed germination. Spinach is direct-seeded. Sized seed and specialized belt seeders are used to reduce seeding rate (33).

Cultivars of spinach are classified into three types (33):

a. Savoy type: large, fresh market use, suitable for long distance shipment for less anaerobic respiration.
b. Smooth-leaved: mostly for processing, preferred for easy washing of leaves.
c. Semi-savoyed type: for both fresh market and processing into frozen packs.

3. Lettuce (Lactuca sativa L. var. capitata)

Lettuce is native to the Mediterranean and inner Asia Minor. Until the 18th century, lettuce was widely used in the Americas. Now the U.S. leads in the production and consumption of lettuce in the world. Lettuce cannot be processed. It is a leafy salad vegetable (34).

Lettuce prefers 24°C for seed germination, and 18~23/7~11°C of day/night temperatures for growth. Lettuce is direct-seeded or transplanted. Coated or pelleted seeds are direct-seeded by seeders. Osmo-conditioning of seeds and fluid drilling are also used (34).

There are four distinct types of lettuce (34):

a. Crisphead type (Iceberg): large and solid head, usually over 0.9 kg and 15.2 cm in diameter, brittle and crisp leaves with prominent veins and midribs, very large outer leaves in medium to dark green, inner leaves tightly folded in light color, most durable for shipping and handling.
b. Butter-head type (Boston, Bibb, or semi-heading): smooth, soft, pliable leaves forming a loose head, better table quality and more delicate flavor than crisp-head type, leaves easily torn and bruised, mainly for local markets, often for greenhouse production.
c. Cos type (Romaine): long and narrow leaves, upright plant, long and somewhat loose heads, more tolerant to stress, best for local markets.
d. Loose-leaf type (bunching): not heading, early, easy to grow, popular in home garden, not suitable for long distance shipment because of its short market life, produced primarily in greenhouses in winter.

Stem lettuce or celtuce (*L. sativa* var. *asparagina* Bailey) is grown for its thick, succulent stem. It is usually cooked in stews and other dishes, or pickled. Stem lettuce is popular in the Far East, but not widely grown in the U.S.

4. Celery (Apium graveolens L. var. dulce)

Celery originated in Sweden. It was initially used for medicinal purposes. Now the long, fleshy, but low nutritive content petiole is harvested for its flavor and texture,

mostly for the fresh market. It is used mainly as a salad crop, in soups, and a small portion is dehydrated. The seeds are also used as a condiment for flavor (35).

Celery is a biennial but grown as an annual. Outer ribs along the petiole's abaxial length are composed mainly of thick-walled collenchyma cells responsible for mechanical strength and stringiness. Celery demands a particular climate. It prefers 15.6~18°C for growth. The production costs per acre are the highest among all vegetable crops (35).

Celery cultivars are classified into two types (2).

a. Golden (yellow or self-blanching) type: golden foliages, earlier, less vigorous, thinner petioles, more sharply ribbed, stringy, more inferior in eating and keeping quality, primarily for specialty markets.
b. Green type: green foliages.
   (i) Utah type: predominate, many attractive and well-overlapped petioles, a well-developed heart.
   (ii) Summer Pascal type: excellent eating quality, generally lacking compactness, few petioles, poor heart development, less affected by cold, less likely to bolt in early planting.
   (iii) Slow Bolting type: less affected by cold, less likely to bolt in early planting.

Celeriac or knob root celery (*Apium graveolens* var. *rapaceum*) is grown for its enlarged roots. Smallage (*A. graveolens* var. *secalinum*), grown long before celery, is most popular in Asian and Mediterranean regions. It produces rosettes of long, thin petioled leaves (16, 36).

F. Fruit Vegetables

Fruit vegetables are grown for their fruits for consumption. They are mainly grouped into cucurbits, legumes, and Solanum fruits. Within the same botanical family, different crop species have similar cultural requirements and pest problems. Other fruit vegetables include okra and sweet corn.

1. Cucurbitaceae

The Cucurbitaceae, a very important food crop family, has been consumed and utilized by human beings for more than 10,000 years. The gourd family consists of 118 genera, only 9 of which are used as vegetables. Among them, three genera, i.e., *Cucumis, Citrullus*, and *Cucurbita,* are of more commercial importance in the world; however, the others are of greater importance in Asia or other regions. These include the genera *Benincasa, Lagenaria, Luffa, Momordica, Sechium*, and *Trichosanthes*. The genera *Cucurbita* (pumpkin and squash) and *Sechium* (chayote) were domesticated in the Americas while the others were of old World origin (originating in Asia and Africa). All are warm season crops and very susceptible to cold injury. However, some types adapt to cool and dry climates. They are herbaceous annuals except chayote which can be grown as perennial. These cultivated species of the Cucurbitaceae family have similar plant habits and cultural methods. They are also known as cucurbits or vine crops. The plant is either a climbing or trailing vine or a bush type. The bush type is of determinate growth and usually bears earlier than vine type. The root system consists of a deep taproot and highly branched short laterals with horizontal distribution similar to the range of plant canopy. They are grown mainly for their fruits. Other parts of the plant may also be consumed for food such as the seeds of watermelon and squash, the flowers of squash and luffa, and the shoots of chayote. Indeterminate vines continue to grow until the plant dies. Side shoots emerge from the leaf axils. Large leaves are borne singly and alternate (38–41).

Most cucurbits are monoecious, producing female and male flowers at separate nodes in the same plant. Usually female flowers are borne singly and male flowers either singly or in cluster in the leaf axils. Melons have andromonoecious type, producing perfect flowers and male flowers in the same plant. The gynoecious types, producing only female flowers, are also available in cucumber. The sex expression, a genetic trait, can be modified by environmental factors and growth regulators. High temperature and long days favor male blooms, while low temperature and short days favor female flowers. The use of ethephon induces female flowering while gibberellic acid and silver nitrate promote male flowers. The plants can be manipulated for the purpose of seed production (38–41).

Most cucurbits can be grown from direct seeding or by transplanting; however, special care is required for the latter practice (43). The seedlings are grown in individual containers to 3~4 true leaf stage to be transplanted. Most crops are direct-seeded in the field in the U.S. The plant spacing varies according to the plant types, with closer in-row spacing for small vined and bush types than for large vined ones. The plastic mulch can be used in the field to raise the temperature for early plantings. A critical period for water occurs during blooming and early fruit set. For sufficient pollination of the plants, the beehives may be brought into the field after female flowers bloom. Cultivation, weed control, irrigation, and pest control are managed similarly to all vine crops (39, 42).

a. Bitter melon (Momordica charantica L.)

Bitter melon, indeginous to the tropics of India or Southeast Asia, is very popular in tropical areas. It is distinct from other vegetable cucurbits by its delicate foliage, slender stems, and simple tendrils. The fruit surface is studded with protuberance. The bitterness of the fruit is due to momordicosides, glycosides of tetracyclic triterpenoids. Immature fruits are less bitter and can be

eaten raw or cooked. They may also be picked or dried for later use. At maturity the fruits turn orange and split open at the blossom end, to expose the bright red fleshy arils surrounding the seeds. The sweet arils can be eaten. Young shoots, leaves, and flowers are eaten as potherbs in India and southeast Asia. All parts of the whole plant are employed as folk medicine. The fruit of bitter melon is a good source of vitamin C, and has been also investigated as an agent inhibiting growth of the HIV virus.

Bitter melon requires warm, sunny areas with fertile, high water-retaining soils of pH 5.5~6.5. The optimum temperatures for seed germination are 30~35°C, for vine growth and fruiting, 25°C. Commercial crops are produced either on ground or on support in arch shape or in triangle shape. The fruits are usually protected from light and fruit flies with paper bags or crude fibers (42, 44).

The cultivated forms of bitter melon are grouped by fruit color into white, light green, and green; by fruit shape into spindle, pear-shaped, or elongated; by fruit size shape into regular (up to 30 cm long and 10 cm in diameter) and wild (up to 8 cm long and 4 cm in diameter); and by the shape of protuberence.

b. Pumpkins and squashes (Cucurbita spp.)

Pumpkins and squashes consist of four of the five cultivated species of the genus *Cucurbita*. They originated in tropical and subtropical America and have been cultivated for thousands of years. However, different types of pumpkins and squashes are not easily distinguished by botanical names or by morphological characteristics. The inter-crossability among different types and among species makes clear classification difficult. The classification is largely based on culinary use and stage of maturity. *C. moschata* is believed to have originated in Central America or northern South America, *C. maxima* in the Andes Mountains of South America, and both *C. pepo* and *C. argyrosperma* in northern Mexico and the southwestern U.S. After pumpkins were introduced to China and Japan, they became important vegetable crops there and many Oriental types were derived. This gives much diversity in fruit shape color and size to the crop (42, 44).

The crop requires a warm season with temperatures between 18 to 30°C for optimum growth. Optimum temperatures for seed germination are 25~30°C, and for fruit development, 25~27°C. Growth period from planting to harvest ranges from 40~60 days for summer squash to 80~140 days for pumpkins and winter squash. For early production, a light loamy soil is desired. Sunny, dry weather is important for successful pollination by honeybees and good fruit development (39, 42, 43).

C. maxima, in general, has better fruit quality in flavor and texture than *C. moschata*. But *C. moschata* has disease resistance and with stands high temperature better than *C. maxima*.

In the U.S. *Cucurbita* species with round and orange fruits are called pumpkins, while those that have fruits of other colors and shapes are called squashes (39, 43):

(i) Summer squash: Commonly *C. pepo*, grown for their immature fruits with soft skin, including yellow crook neck and straight neck, scallop squash, cocozelle, and zucchini.
(ii) Winter squash: including all four *Cucurbita* species, grown for mature fruits usually with hard rind, such as acorn, hubbard, butternut, banana, and orange marrow.
(iii) Pumpkins: mostly *C. pepo* and *C. moschata*, grown for their ripe fruits used as an ingredient in pies.

The naked-seeded pumpkins are grown for their seeds to be roasted for snacks. *C. maxima* can grow to jumbo size (22~45 kg) for exhibition purposes.

c. Cucumber (Cucumis sativa L.)

Both cucumber and melon are members of genus *Cucumis*; each belongs to a different subgenus. Cucumber is indigenous to India and has been cultivated for more than 3,000 years. It also has a long history in China where it is considered a secondary center of genetic diversification. Early travelers brought cucumber to Mediterranean countries (42). In the early 14th century, cucumber was cultivated in the U.K. It was introduced to the U.S. by 1539. Now cucumber is widely used as fresh and processed products. The leading U.S. States for fresh market cucumbers are Georgia and Florida, while Michigan, North Carolina, and Texas lead in processing type production (43).

Cucumber requires temperature of 30°C during the day, 20°C at night for optimum plant growth. The seeds germinate best in 25~30°C, and in this range, fruits develop rapidly. The crop growth rate increases steadily as the temperature increases to 32°C (42, 43). Glasshouse cultivation is also common for the cucumber in Northern Europe, Asia, and the Middle East.

Cucumbers are divided by use into slicing type and pickling type, by culture into outdoor type and greenhouse type (39, 43):

(i) Pickling cucumber: fruits cylindrical in shape with blocky ends and a medium green color.
(ii) Slicing cucumber: smooth, symmetrical, and white-spine fruits longer than pickling type, with glossy, dark-green skin.
(iii) Greenhouse cucumber: mostly parthenocarpic (set fruit without pollination).

d. Wax gourd (Benincasa hispida (Thunb.) Cogn)

Wax gourd is the only species of the genus *Benincasa*, which is named for Italian botanist Count Benincasa. The

species name refers to the pubescence on the foliage and immature fruit. Of Indo-Malayan origin, wax gourd is an important vegetable in India, China, the Philippines, and elsewhere in Asia. Both mature and immature fruits are consumed, either cooked or picked. The mature fruits harvested in summer can be stored at 13~15°C and 70~75% RH for over 6 months. The name winter melon refers to its long storage life. The fruits are especially valued for their high water content, bland taste, and cooling properties. The sliced pulp is dried for later use, and sometimes candied in sugar syrup. The candied fruits are boiled to make the popular summer drink, wax gourd tea, served as a seasonal and festival specialty. The Chinese steam the entire mature fruit as a soup tureen with various stuffed ingredients. In addition to the food value, wax gourd is also important in traditional medical practices. The rinds and seeds are part of various medications throughout Southern Asia. The fruit wax is sometimes collected to make candles. Mo-kwa (*B. hispida* var. C*hieh-qua* How.), a botanical form of wax gourd, is usually grown for its immature fruit which is about 0.4~0.7 kg and 18~25 cm long. It is high yielding and more heat tolerant.

Wax gourd grows best in sunny, moderately dry areas. It requires fertile, well-drained soils of pH 5.5~6.4. The optimum temperatures for seed germination are 30~32°C and for plant growth and fruiting 24~27°C (42, 44). The cultivated forms of wax gourd can be divided by fruit shape into long cyclindrical and short cylindrical; by fruit size into small (1.5~5 kg) and large (7~20 kg); and by skin color and the presence of waxy white bloom on fruits into dark green, light green, and waxy.

e. *Other Cucurbits*

As many Asian immigrants moved to the U.S. other cucurbits of Asian or African origin become common in areas with large Oriental populations. These crops include immature fruits of smooth luffah (*Luffa aegyptaca* Miller), angled loofah [*L. acutangula* (L.) Roxb.], bitter melon (*Momordica charantia* L.), bottle gourd [*Lagenaria siceraria* (Mol.) Standl.], wax gourd or Mo-kwa, the immature fruits of *Benincasa hispida.* Among these, bitter melon also has medical use (42, 44).

2. Legumes

Vegetable legumes, all dicotyledonous annuals, are members of the Leguminosae (Fabaceae) family. The immature fruits are important vegetables and the dry seeds are an important staple food. In some, the leaves, tender shoots, or the roots are harvested and used as vegetables (47). Legumes may be classified according to the position of the cotyledons in the germinated seedlings. Epigeal is the type where cotyledons are above the ground; hypogeal is the type where cotyledons remain underground. Many legumes can assimilate their own source of nitrogen as a result of a symbiotic relationship with bacteria of *Rhizobium* in their root nodules. However, before successful symbiotic relationship is established, the crop still needs adequate supply of nitrogen in the soil for growth (46, 47).

Peas and broad beans are cool season crops while beans are warm season crops, intolerant of frost. Each legume species has strains or varieties adapted to a particular range of conditions. All vegetable legumes are direct-seeded to the field. The pole type or the tall cultivars are usually supported or trained to poles. Generally bush types require less time for flowering than the pole cultivars (48).

a. *Common bean, Snap bean (Phaseolus vulgaris L.)*

Common bean is the most widely cultivated bean in the genus *Phaseolus*. Over 7,000 years ago, on common bean originated in Central and South America. It was introduced to Europe in the 16th century and soon spread to other parts of the Old World (47). Beans are marketed fresh, canned, or frozen. Wisconsin is the largest bean-producing state for processing and Florida leads in fresh market production (48, 49).

The optimum soil temperature range for germination is 25~30°C. The optimum temperature range for growth is 16~30°C. Temperature above 30°C at flowering can cause flowers to abort. Vine types are adapted to cooler temperatures than bush types. The desirable maturity characteristics are undersized seed development and low sidewall fibers (47–49).

Common beans are divided into bush and vining types according to growth habits, and fresh market and processing cultivars according to uses. Based on pod color, common beans are either green-podded or yellow-podded, sometimes even purple-podded. Beans may also be classified by shape of the pod or color of the seeds (48).

b. *Lima bean (Phaseolus lunatus L.)*

There are two types of lima beans, the large-seeded and small-seeded types. They originated in both Central and South America before 5000 B.C. (47). The Native Americans spread the crop and it became an important vegetable crop in the U.S. It can be consumed fresh, frozen, or canned. The lima bean growth habit is similar to the common bean. California is the only state to harvest dry lima beans, both baby lima beans and large-seeded ones (48, 49).

Lima beans require a slightly warmer climate than common beans (47). They germinate at 15~30°C with an optimum soil temperature of 25°C. Mean monthly temperatures of 15~24°C are necessary to grow the crop. The small seeded type, being less restrictive, can tolerate hotter and drier conditions than the large-seeded one (47).

The U.S. cultivars have seed coats of white, creamy, buff, or light green. The large-seeded type is narrowly adapted, but is of better quality. The small-seeded type is grown widely (48):

(i) Bush type: productive with small seeds, ex. Henderson, Early Thorogreen.

(ii) Pole type: largely for home gardens (Pierce), producing small seeds.

c. *Peas (Pisum sativum L. ssp. sativum)*

As one of the most ancient crops, peas originated in the eastern Mediterranean region and Near East. Peas can be dated to 7,000~9,000 years ago (47, 48). Dry peas were utilized as food in Europe from very early days and green peas were not used until the 16th century (46). The edible podded types evolved in more recent times. In the Orient, the tender shoots of peas are used as greens (50). The term English pea reflects many cultivars of peas developed and grown in England. Columbus brought the pea to North America where it quickly spread to all parts. Commercial production in the U.S. is primarily for processing, including canning and freezing. Seeds are either smooth or wrinkled; the former is starchy and the latter is sweeter (48, 49).

Peas thrive in cool and moist weather. The crops grow best at mean temperatures of 13~18°C. Long days and cool temperature accelerate flowering. The smooth-seed types are more adapted to cool weather conditions than the wrinkle-seeded types. The edible podded types are more adapted to warm conditions than the green pea types. Heat units are commonly used to predict harvest dates for the processing industry (47).

There are several types of peas, including shelled peas, edible podded peas, and dry peas. Pea cultivars are classified by seed color, growth habit, seed quality, and pod appearance (48, 49):

(i) Dry peas: light green seed color, starchy.
(ii) Canning peas: most determinate, light green and sugary seeds.
(iii) Freeze peas: most determinate, dark green and sugary seeds.
(iv) Edible podded peas: pods lacking the stiff, papery inner parchment, the whole pods consumed.
  (a) Snow peas: sugary seeds slightly enlarged.
  (b) Snap peas: sugary seeds more developed and pod wall thickened.

d. *Southern pea [Vigna unguiculata (L.) Walp. ssp. unguiculata (L.) Walp.]*

The origin of Southern pea is rather obscure. It is possibly of tropical African origin (47) or from India (47, 48). There are three distinct cultivar groups in *Vigna unguiculata*, characterized by growth habit and pod character (48). The immature pods of all three are used as vegetables. The yard-long bean is more popular to the Southeast Asians with pods ranging from 30 to 75 cm in length. Catjang cowpea has erect small pods 7~12 cm long with small and cylindrical seeds. It is more common in India. Southern peas, also known as black-eye peas, are mostly confined to the southern U.S. They are grown primarily for the green shelled seeds, a large amount of which are processed and canned or frozen (48).

Southern pea grows best in hot, dry climates. Optimum temperatures for growth are 27~30°C during the day and 17~22°C at night. It tolerates heat better than common beans or lima beans. Fresh market peas may be harvested 16~17 days after bloom (47).

Cultivars of southern pea differ in maturity, in pod color, crowding of seeds in the pods. Black-eye peas refer to those having a dark outline or eye around the hilum. Other major types include purple hulled and creamy yellow. The large cultivars are well suited for pick-your-own and local sale (49).

3. Solanum Fruits

Solanum fruit crops, all of tropical origin, are members of the Solanaceae family. They include tomato, peppers (both bell pepper and chili), and eggplant, all grown as annuals for commercial production. Tremendous phenotype variations are available in fruit shape, size, and color. They are warm season crops with eggplant being more heat tolerant than pepper and tomato. Chili pepper can grow in higher temperature than bell pepper. The potato, in the same genus as the eggplant, is discussed under stem and tuber crops (20, 52).

These crops share similar disease problems. In some countries, grafting culture of tomato and eggplant on resistant stocks is used to combat soil-borne diseases. Early production can be forced by planting transplants to fields mulched by plastics. Direct seeding can be successfully used for later, especially processing, production. Transplants are usually greenhouse grown in plugs. Tomato and bell pepper are grown in glasshouses or other protective structures in some regions where climate limits field production (20).

a. *Tomato [Lycopersicon esculentum (L.) Mill]*

The tomato originated in the Andes of South America and evolved from the cherry tomato (*L. esculentum* var. *cerasiforme*). However it was introduced from Mexico to Europe early in the 16th century (37, 52). Early use of tomato was hampered by the belief that it was poisonous, because many of the Solanum species contain alkaloids. The tomato was introduced to the U.S. in 1710, and was produced in New Orleans in 1779 (51, 52).

On a worldwide basis, tomato is one of the most important vegetables or salad plants (37). The acid sweet taste and unique flavor account for its popularity and diverse usage. Although not among the most valuable crops in nutrient contents, tomato is an important source of vitamins A and C because of the substantial per capita consumption. The U.S. leads in total tomato production and processing. California

and Florida lead the nation in fresh market and processing tomato, respectively (20).

Tomato requires temperatures of 25~30°C for optimum germination. Day temperatures of 25~30°C with night temperatures of 16~20°C are optimal for growth and flowering (20, 51, 52). Fruit set is best between 18~24°C with night temperatures more critical than day temperatures. Cultural practices of pruning, staking, and caging are used to increase light interception and aeration for production enhancement.

Tomatoes are classified as determinate and indeterminate in growth. Higher planting densities are given to the former type to compensate for their lower yield potential. Tomatoes are grouped into fresh market, processing, and home garden types according to use. Processing cultivars are usually determinate and ready for harvest in 75 days after field setting. The greenhouse tomatoes are indeterminate and the home garden types may be either determinate or indeterminate. Variation in shapes (globe to pear), fruit color (yellow, pink, and red), and size (cherry, beef) exist in different cultivars (20).

b. Pepper (Capsicum annuum L.)

With a long history of cultivation of more than 7,000 years, peppers are native to tropical and subtropical America (52). Among the five domesticated species in the genus *Capsicum*, *C. annuum* is the most widely cultivated and economically important species and includes both bell pepper and chili pepper. *C. frutescens* has small fruits. Tabasco is the best known cultivar and is grown commercially for making Tabasco sauce (20, 54).

Columbus introduced peppers to Europe, and subsequently to Africa and to Asia. Peppers were soon integrated into people's cuisine due to the characteristic flavor and pungency. In addition to use as food or spices, peppers are also used in pharmaceutical products such as pepper plaster. The pepper is an indispensable food in many countries. Asia is the largest producer with China leading in world production (53). In the U.S. New Mexico leads in chili pepper production while California leads in bell pepper production (55). Peppers are good sources of vitamins C and A.

The pepper is a warm-season crop. The optimum temperature for pepper growth and development is higher than that for tomato. The seeds germinate rapidly in 25~30°C. The base growing-degree-days temperature is 18°C. The average temperature for optimum growth is 21~30°C, for fruit set 20~25°C, and for color development 18~24°C. The plants are not photoperiod sensitive for flowering. The small fruit cultivars are more tolerant to high temperature extremes (51–53).

Peppers are classified into two main types, the sweet-fleshed fruit and the pungent fleshed fruit. They can also be grouped by fruit appearance and use. Classified by pod type, there are bell, pimiento, cheese, ancho, cayenne, Cuban, jalapeno, serrano, wax, and cherry, among others. Important commercial types are listed as follows (20, 54):

(i) Bell type: large blocky fruit with three to four lobes and thick flesh, mature green fruits harvested for fresh market, mature colored fruits in red, orange, or gold also in commercial markets.
(ii) Cherry peppers: round or slightly flattened fruits, orange to deep red when harvested, sweet or hot, small or large.
(iii) Chili type: pungent and thin flesh fruit, from cherry size to slender fruit up to 20 cm long.
(iv) Pimento peppers: fruit sweet with thick wall, conical or heart-shaped, turning red at maturity.
(v) Tabasco peppers: fruits 2.5~7.5 cm long, slim, tapered, and highly pungent.

Peppers can be fresh, canned, brined/pickled, frozen, fermented, dehydrated, and extracted for oleoresin (53).

c. Eggplant (Solanum melongena L.)

Native to India, eggplant is not as popular as tomato or pepper as a vegetable. However, it is widely used in China, India, Japan, and many Mediterranean countries. The name eggplant is believed to reflect the early forms with small, white fruits resembling eggs. There are in general three forms of eggplants: round egg-shape fruits, long slender fruits, and dwarf plant form (20, 51, 52). Fruit tissues contain high levels of phenolics; upon cutting they are quickly oxidized by enzymes, resulting a brown discoloration of the flesh (51).

Introduced to American gardens in 1806, the eggplant was primarily an ornamental curiosity until the 20th century. Commercial eggplant acreage is primarily located in Florida and New Jersey (20).

Eggplant is very sensitive to low temperatures and it requires a relatively long, warm growing season. Optimum temperatures for seed germination are 24~32°C, for growth and development, day temperatures of 22~30°C and night temperatures of 18~24°C. The elongated fruit type tends to be more resistant to high temperature than the round fruit type. To have high yield, plants require high light intensity (51, 52).

There are two basic types of eggplants based on fruit shape (52):

(i) Standard oval shaped type: large, smooth, glossy purplish-black fruit.
(ii) Oriental type: long slender purple-black fruit.

There are also types with white, yellow, or apple-green-colored fruits.

4. Sweet Corn (*Zea mays* L.)

Sweet corn is a monocot of the Poaceae (Gramineae) family. An ancient crop dating from about 5,000 B.C., corn originated in the highlands of central and southern America. Sweet corn originated from a mutation of grain corn (56). Corn was not popular initially because it was difficult to store. Commercial sweet corn production began about 200 years ago in the U.S. The U.S. leads the world in sweet corn production. In addition to fresh use, a considerable volume is processed by canning and freezing of kernels after removal from the cob (57).

Corn plants are monoecious with male flowers borne as the terminal inflorescence on the main stem and female flowers borne as lateral ear shoots. The basal nodes of an ear shoot are concentrated with little internode space. They form a tight husk around the developing ear. Each individual kernel is a single-seeded fruit composed of a small embryo and a large endosperm. Sweet corn kernels contain less starch, and more sugar and water-soluble polysaccharides which are responsible for the creamy texture (56).

The optimum temperature range for corn growth is 24~30°C. In general, the warmer the air temperatures, the faster the corn will grow to maturity (57). However, moderate temperatures are optimum for carbohydrate accumulation. Cool nights are particularly important at harvest time. Plants flower sooner when days are short. The crop is sensitive to soil acidity, requiring a soil pH of 6.0~6.8. It requires high water, but not waterlogging and high fertilization.

Sweet corn cultivars are classified by kernel color (yellow, white, and bicolor), maturity date (early and late), and use (market, freezing, canning, or shipping) Classified by kernel sweetness, there are the following (57):

(a) Standard sweet corn: containing the recessive *su 1*, traditional sweet corn flavor and texture.
(b) Modified sugary sweet corn: containing the sugary enhancer gene *se*, high sugar, thin pericarp.
(c) Supersweet corn: containing recessive *sh* 2 gene, higher in sugar, lower in starch, tougher skinned.

G. Bean Sprouts

Bean sprouts are produced mostly from germinated seeds of mungbean [*Vigna radiata* (L.) Wilcz] and soybean [*Glycine max* (L.) Merr]. The use of bean sprouts has long history in China. The seeds are soaked for 24 h, sprouted, and allowed to grow in the dark for several days before harvest for consumption. The etiolated hypocotyls and young cotyledonary leaves are eaten with other vegetable dishes (58). The sprouts are a good source of vitamins B_1 and B_2 and dietary fiber. More than 20 kinds of sprouts have been developed. Among them, pea sprouts and alfalfa sprouts are popular vegetable sprouts grown from legume seeds. Light exposure a few days before harvest produces pea sprouts green in color. Sprouts are grown under light to harvest green sprouts. Daily watering is necessary. The growth of sprouts does not require supplemental nutrients but depends on the storage reserve of the seeds. Given optimum temperature for seed germination, water for sprouts to grow, and proper aeration, bean sprouts can be produced year round in controlled environments (58).

ACKNOWLEDGMENT

The information in this chapter from "Vegetables: types and biology," by J.S.J. Tsao and H.F. Lo, in *Handbook of Vegetable Preservation and Processing*, edited by: Y.H. Hui et al., Marcel Dekker, New York 2004.

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21 Nutritional Value of Vegetables

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CONTENTS

I. INTRODUCTION

Vegetables are considered a significant part of all major dietary guidance systems. Their many chemical elements and compounds are known to affect thousands of physiological functions and to promote health (1). This chapter provides an overview of nutrients and non-nutrient phytochemicals commonly found in vegetables, along with a description of the basic nutrient profile for vegetables in general. Factors affecting nutrient variations, both naturally occurring and due to processing, are summarized. Lastly, this chapter reviews many of the purported health benefits derived from various vegetable phytochemicals.

II. NUTRIENTS

A. Nutrients

About a century ago, researchers observed that growth and survival of animals was directly affected by various individual components in foods. These components were termed nutrients and were to be considered required for normal growth and health. In the early 1900s, researchers began to focus on the disease-preventing properties of vegetables. McCollum and colleagues realized that the addition of vegetables to a seed diet was necessary to prevent deficiency conditions in omnivore species (2). Researchers then realized that a diet deficient in even a single essential nutrient (required from food), could result in a dietary deficiency disease or even death (3).

Strictly speaking, nutrients are compounds that cannot be synthesized by the human body from other chemicals or cannot be synthesized rapidly enough to meet the needs of the body. Thus, by the classical definition of nutrient, the term "essential nutrient" is redundant. However, the terms "non-essential nutrient" or "dispensable nutrient" are sometimes used to describe chemical compounds that are contained in foods and have a function in the body, but are typically synthesized by the body in adequate amounts.

As nutrition science evolved, a third category of nutrients has been identified as "conditionally essential." This terminology is used to describe substances that may become essential under specific conditions that reduce the body's capability to synthesize adequate amounts of the compound. This may be caused by changes in physiological demands due to a genetic defect, a disease condition,

the stress of surgery, or the use of certain drugs such as statins and Co-enzyme Q10 (4,5).

Presently, there are approximately 50 individual food elements and chemical compounds identified as essential nutrients. These nutrients are classified into six broad chemical categories based on chemical structure and functions including water, proteins, fats, carbohydrates, vitamins, and minerals. Table 21.1 lists nutrients by essentiality category and the basic physiological functions of providing energy, structure, or regulation of the body's thousands of chemical reactions.

Nutrients frequently act in concert to regulate specific physiological functions. For example, calcium, magnesium, and potassium regulate muscle contraction and relaxation (7,8). Vitamins B-6, B-12, and folate function in concert to prevent the excessive accumulation of homocysteine, which in turn reduces the risk of coronary artery disease (9).

The proper proportion of specific nutrients is also important in maintaining health. For example, the ratio of omega-3 fatty acids (primarily from fish oils and some vegetable oils) to omega-6 fatty acids (primarily derived from many vegetable oils) is an example of the essentiality of correct nutrient proportions. Both types of fatty acid are essential for regulating eicosanoid synthesis which in turn affects physiological functions such as blood pressure, inflammation, and blood clotting. The appropriate proportion of omega-3 to omega-6 fatty acids is 1 to 4 up to 1 to 10. An imbalance of these fatty acids appears to be related to various chronic health problems (10).

Nutrients, even essential nutrients, are known to be harmful in excessive amounts. The Institute of Medicine has published both the recommended levels of intake and the tolerable upper intake levels for many nutrients. These recommendations provide guidelines on what constitutes generally safe ranges of intake and what levels of a nutrient may be excessive and even harmful to humans (11–14).

The use of certain drugs may require nutrient intake to be maintained within a more narrow range. For example, excess vitamin K intake, even from naturally occurring plant sources, can interfere with the function of common blood anticoagulant drugs (15).

B. Nutrient Profiles

Foods from various common food groups (meat and poultry, milk products, fruits, vegetables, grains, and beans) have classic nutrient profiles or distinctive nutrient fingerprints. Typically, these nutrient profiles are expressed as the amount of various key nutrients typically contained in 100 grams of edible portion.

Compared to other food group nutrient profiles, vegetables provide the nutrient characteristics that most consumers perceive as health promoting (Table 21.2). Vegetables contain no cholesterol, very little fat, sugar, and sodium, yet provide concentrated sources of many vitamins and minerals. There are literally hundreds of vegetables, with the majority of these cultivated in Asia (17). Table 21.3 presents representative nutrient ranges and means for 38 of the more commonly consumed vegetables in America (18).

C. Factors Affecting Nutrient Composition of Vegetables

Biological, chemical, and physical factors all affect the nutrient composition of vegetables. For these reasons, nutrient values for any particular vegetable may differ significantly from published values commonly used in databases. Table 21.4 presents a partial list of factors that can significantly affect variability of nutrient content in foods. Comprehensive texts and research papers (as well as references cited within) are referenced in this table for those interested in a deeper understanding of any single production factor.

Because of the virtually endless possibilities of factor combinations, there is no definite set of rules dictating how the exact nutrient composition of any single plant may vary from the usual. Vitamins and minerals, rather than protein, fat, and carbohydrates, are the nutrients likely to have the greatest variation even within a single plant species.

Vitamins, functioning as cell regulating cofactors, will continue to be utilized by the plant even after being harvested (24). Other factors such as heating, acid, or alkaline exposure, and processing techniques that cause oxidation can decrease the vitamin concentration to a fraction of the initial value (25).

The content of some minerals in plants is dependent upon the amount of a particular element available in the soil for the growing plant. For example, depending upon the selenium content in the soil, plants may have very low levels of selenium or contain toxic amounts.(26).

D. Bioavailability

Even though vegetables may contain ample quantities of nutrients and phytochemicals, some vegetables also contain chemicals that bind with nutrients and phytochemicals making the beneficial compounds unavailable for absorption. An understanding of these bioavailability considerations is essential to avoid using nutrient data of vegetables in a misleading way (27,28).

Nutrient antagonists can significantly decrease the bioavailability of nutrients from foods. High levels of one mineral may competitively reduce the absorption of another mineral element. For example, magnesium interferes with calcium absorption (29). Zinc also is known to interfere with magnesium absorption (30). Phytochemicals, like

TABLE 21.1
Human Essentiality and Physiological Function of Nutrients Found in Plants

Nutrient	Essentiality	Physiological Function: Energy	Structure	Regulation	Examples of Key Specific Functions
Water	Essential		x	x	Provides fluid structure for every cell
PROTEIN—AMINO ACIDS:					Source of kilocalories; protein
Histidine, Isoleucine, Leucine, Lysine, Methionine	Essential	x	x	x	structure of all cells; regulate
Phenylalanine, Threonine, Tryptophan, Valine	Essential	x	x	x	chemical reactions through
Alanine, Arginine, Asparagine, Aspartic Acid	Dispensable	x	x	x	enzymes; necessary for
Glutamic Acid, Glycine, Proline, Serine	Dispensable	x	x	x	DNA systhesis
Cysteine, Glutamine, Tyrosine	Conditional	x	x	x	
LIPID—FATTY ACIDS:					Source of calories; cell membrane
Linoleic: omega-6, alpha-Linolenic: omega-3	Essential	x	x	x	structure; eicosanoid synthesis;
Other Fatty Acids	Dispensable	x	x		
CARBOHYDRATES:					Energy source especially essential
Glucose	Dispensable	x			for red blood cells and brain
MINERAL ELEMENTS:					
Calcium, Fluoride, Magnesium, Phosphorus	Essential		x	x	Bone and tooth structure
Chloride, Chromium, Copper, Iodine	Essential			x	Miscellaneous physiological and
Iron, Manganese, Molybdenum	Essential			x	regulatory functions including: energy
Potassium, Selenium, Sodium, Zinc	Essential			x	metabolism; synthesis and transport of red blood cells and hormones; water balance; and immune system functions
Arsenic, Boron, Nickel, Silicon	Essential			x	Functions unclear
VITAMINS:					
Biotin, Choline, Folate, Niacin (B-3), Pantothenic acid	Essential			x	Miscellaneous regulatory functions including: preventing oxidation;
Riboflavin (B-2), Pyridoxine (B-6), Thiamin (B-1)	Essential			x	energy metabolism; blood clotting; and eye health
Vitamin A (carotenoids), Vitamin C (ascorbic acid)	Essential			x	
Vitamin E (tocopherol), Vitamin K	Essential			x	

Essential nutrients not present in vegetables include Vitamin D and Vitamin B12. Dietary fiber is commonly classified as a non-digestible form of carbohydrate. However, it is not a nutrient and has been included in Table 5 as a phytochemical.
Source: Ref. 6.

TABLE 21.2
Nutrient Profile of "Classic" Food Groups Based on 100 gram (g) Edible Material as Raw or Minimally Prepared for Consumption; Information is Based on USDA Nutrient Database Series 14 and Presented as Unrounded Range and Rounded Mean for Each Food Group

	Water (g)	Calories (kcal)	Total Fat (g)	Saturated Fat (g)	Cholesterol (mg)	Sodium (mg)	Carbohydrates (g)	Fiber (g)	Sugar (g)	Protein (g)	Vitamin A (IU)	Vitamin C (mg)	Calcium (mg)	Iron (mg)
Vegetables	64–96	5–145	0.04 –0.7	0.0 –0.17	0	2–201	2–24	0–5	0–6	0–5	0–19000	3–93	0–119	0.1–3.3
	90	35	0.25	0	0	35	7	2	1.5	2	1770	24	50	0.9
Fruits (not including	70–95	27–92	0.09–0.96	0–0.22	0	0–20	6–23	0–4	1–18	0–1	10–3800	4–98	4–40	0.1–0.6
avocado)	85	50	0.25	0	0	2	12	1.8	10	1	550	28	15	0.3
Grains—Cooked	65–90	43–135	0.16–1.08	0.2–2.0	0	1–35	9–30	0–7	0–1	1–4	0–98	0	1–13	0.2–1.5
	76	96	0.5	1	0	15	20	2.2	0.5	2.5	10	0	8	0.8
Beans—Cooked	61–70	118–173	0.38–8.98	0.05–1.32	0	1–13	10–28	2–9	0–3	7–17	0–9	0–2	14–142	1.1–5.2
	65	139	2.2	0.5	0	3	21	6	2	10	3	1	62	2.8
Nuts	2–47	224–718	1.12–76.5	0.16–29.7	0	0–38	12–73	0–11	0–13	3–26	0–1091	0–36	1–234	0.9–9.2
	12	533	45	7	0	9	28	5	4	13	130	8	74	3.2
Meat	47–76	110–274	1.25–21	0.33–8.54	41–440	39–102	0–6	0	0	17–32	0–35350	0–34	4–11	0.7–8.6
	66	173	8.5	3	100	68	0	0	0	22	2165	3	11	2.2
Eggs	70–75	149–185	10.0–13.8	3.1–3.7	425–933	125–150	0–1	0	0–1	13–14	300–1328	0	50–100	1.4–4.1
	73	170	12	3.5	790	140	1	0	1	13	820	0	67	3.3
Milk	81–91	35–108	0.18–7.01	0.12–4.6	2–27	44–52	4–5	0	4–5	3–6	126–205	1–4	119–193	0.0–0.1
	87	64	3	2	11	50	5	0	5	4	180	2	138	0.1

Source: Ref. 16.

TABLE 21.3
Vegetable Composition Based on 100 g Edible Portion as Raw or Minimally Prepared for Consumption. Range Information Based on Various Sources Including USDA Nutrient Database Series 14 and Others

Vegetable	Water (g)	kilocalories	Fat (g)	Protein (g)	Total Carbohydrates* (g)	Sugar (g)	Fiber (g)
Artichoke (globe)	80–86	17–70	0.3–0.4	0.5–4.5	13	2	0.8–5.4
Asparagus	92–93	9–27	0.2	2.2–3.9	4.6	1.3–2.3	0.0.7–2.1
Beet root	83–89	44–58	Tr - 0.7	1.3–1.8	10	6–7.3	0.6–3.1
Broccoli	89–91	28	Tr - 0.3	3.1–4.0	5.3	0.4–2	1.3–3
Brussels sprouts	84–89	16–58	Tr - 0.5	2.4–4.4	8.7	3.6–4	1.3–4.6
Cabbage	86–93	8–36	Tr - 0.7	1.4–3.3	5.4	2.7–3.8	0.6–3.4
Carrot	84–95	19–47	Tr - 0.7	0.6–2.0	10.1	5.4–7.5	0.6–2.9
Cassava	50–74	120–153	Tr - 0.7	0.7	27	1.2	0.6–1.7
Cauliflower	84–92	11–34	Tr - 0.3	1.8–3.4	5.2	2.4–2.6	0.8–2.4
Celery	89–96	5–22	Tr - 0.5	0.7–2.0	3.7	1–1.2	0.7–2.7
Chard	91–94	16–19	0.2–0.4	1.5–2.6	3.8	0.8–1.1	0.6–1.6
Chayote	74–95	24–29	0.1	0.8	5.1	–	0.4–0.6
Cucumber	91–97	9–16	Tr - 0.2	0.6–1.4	2.8	1.8–2.6	0.3–0.7
Eggplant	89–94	15–38	Tr - 0.7	0.7–2.4	6.7	2.1–4.2	0.9–2.5
Endive	93–94	11–24	Tr - 0.2	1.6–1.8	3.4	0.3–1.0	0.8–2.2
Leek	71–92	25–52	Tr - 0.4	1.3–2.5	7.6	1–4	1.0–3.3
Lettuce	92–97	11–27	Tr - 0.5	0.8–1.6	2.2	1.1–2.2	0.3–1.4
Mustard	68–89	10–28	Tr - 0.3	1.6–2.4	2.1	0.4–0.9	1.8–3.7
Onion	81–93	13–49	Tr - 0.35	0.9–2.2	8.6	5.2–6.7	0.5–1.7
Parsley	68–89	21–60	Tr - 1.0	3.7–5.2	6.9	Tr	0.9–9.1
Parsnip	79–83	56–83	Tr - 0.5	1.5–1.7	19.5	5.5–9.5	2.2–4.4
Pea	65–81	49–138	Tr - 0.8	4.6–8.2	15.6	2.3–7.4	1.8–5.5
Peppers	70–93	27–37	0.1–0.7	1.2–2.0	6.4	1.7–13.9	0.5–2.7
Plantain	58–74	116–128	0.05–0.8	1	31.2	5.6	0.3–2.3
Pumpkin	80–96	15–36	Tr - 0.2	0.6–1.8	4.9	2.5–3.2	0.5–1.3
Potato	71–85	75–109	Tr - 0.1	1.6–2.3	25.2	0.3–1.6	0.3–2.4
Radish	92–95	15–22	Tr - 1.1	0.7–1.2	3.6	2.0–3.4	0.5–1.0
Spinach	91–93	16–35	0.3	2.3–5.1	3.5	0.3–0.4	0.6–2.7
Summer squash	86–95	19–44	0.03–0.3	0.6–1.5	4.4	1.0–3.9	0.3–1.9
Sweet corn	57–80	86–142	0.8–2.1	2.9–4.5	19	3.2–5.2	0.6–3.2
Sweet potato	60–80	98–125	0.04–0.7	1.4–2.8	24.3	5.4–11.6	0.5–2.3
Taro	54–83	111–142	0.1–0.5	0.5–2.9	34.6	1	0.4–5.1
Tomato	90–96	14–23	Tr - 1.26	0.7–1.2	4.7	1.2–3.4	0.4–1.8
Turnip	87–93	11–35	Tr - 0.2	0.6–1.1	6.5	3.8–4.6	0.7–2.8
Watercress	90–94	11–29	Tr - 0.6	1.7–3.1	1.3	0.2–0.6	0.5–3.3
Yam	54–84	104–116	0.03 - 4	1.5–2.4	27.6	0.5	0.4–3.9
Zucchini	95–98	7–16	Tr	0.4	4	1.3–2.2	0.6–1.4

(*Continued*)

dietary fiber and phytic acid, can reduce the bioavailability of minerals such as iron or calcium.

Minerals are the nutrient class most commonly affected by decreased bioavailability. Most commonly, the low availability of the mineral to the body is due to the mineral's chemical form or to other components in the diet. Bioavailability for each mineral can vary extensively. For example, on the average, a human absorbs from the diet about 1 to 10% of the iron and manganese, 1 to 20% of the zinc, and 15 to 40% of the magnesium and calcium. These percentages will vary based on the quantity of food components such as dietary fiber and oxalic acid which bind with minerals making them unavailable for absorption. Spinach is a good example of a vegetable with relatively high levels of calcium that are virtually unavailable to the body due to the high oxalate concentration in the spinach. Consequently, listing spinach as a food that is high in calcium is technically correct, however, it is deceiving because it is not a good source of calcium for humans (31).

Another factor that can affect the bioavailability of minerals is the physiological status of a person. For some minerals, the efficiency of absorption is increased (within certain limits) during times of dietary deficiency and the absorption efficiency is decreased during times of high intake (32).

TABLE 21.3
(*Continued*)

Vegetable	Ascorbic acid (mg)	Vitamin A (IU)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Folic Acid (μg)	Calcium (mg)	Iron (mg)
Artichoke (globe)	5–33	16	0.07–0.2	0.01–0.17	0.1–1.3	66	19–74	0.02–1.0
Asparagus	11–41	100–550	0.1–0.23	0.08–0.3	0.8–1.1	25–156	13–28	0.5–2.0
Beet root	Tr - 6	Tr - 35	0.01–0.03	0.01–0.06	0.06–0.4	20–80	15 32	0.4–2.8
Broccoli	40–93	700–1550	0.06–0.07	0.12–0.2	0.6	50–71	48–160	0.9–1.5
Brussels sprouts	35–128	50–720	0.06–0.13	0.08–0.19	0.4–1.04	14–86	10–53	0.1–2.5
Cabbage	20–220	Tr - 1330	0.03–0.17	0.03–0.21	0.15–1.55	20–37	30–204	0.5–1.9
Carrot	4–58	1660–28130	0.04–0.1	0.03–0.06	0.2–1.16	10–14	27–57	0.2–1.2
Cassava	9–48	10–240	0.04–0.23	0.02–0.1	0.25–1.4	22	23–91	0.6–3.6
Cauliflower	8–114	Tr - 20	0.04–0.13	0.04–0.06	0.25–0.89	30–48	13–43	0.2–1.9
Celery	5–15	Tr - 220	0.02–0.5	0.02–0.5	0.2–0.4	7–28	31–53	0.4–9.9
Chard	30–72	390–3300	0.04–0.07	0.06–0.14	0.61–1.14	14	51–176	1.4–4.0
Chayote	8–20	Tr - 50	0.01–0.03	0.02–0.04	0.4–0.45	18	12–13	0.3–0.4
Cucumber	5–19	Tr - 215	0.02–0.1	0.02–0.11	0.1–0.6	6–13	14–23	0.3–0.8
Eggplant	1–5	Tr - 65	0.05–0.08	0.02–0.04	0.5–0.8	14	6–36	0.4–1.0
Endive	7–12	560–2050	0.06–0.08	0.1–0.8	0.4	142	44–52	0.8–2.8
Leek	4–32	10–405	0.03–0.8	0.02–0.1	0.2–0.5	24	30–85	1–2
Lettuce	3–33	40–2200	0.04–0.14	0.03–0.1	0.2–0.5	20–73	17–107	0.05–4.0
Mustard	25–102	970–3030	0.04–0.09	0.06–0.2	0.36–0.8	29–73	65–220	0.7–4.5
Onion	6–10	Tr - 170	0.02–0.03	0.02–0.04	0.1–0.2	19	20–52	0.2–0.5
Parsley	90–200	1220–5200	0.08–0.2	0.11–0.6	0.53–1.8	40–183	130–325	2.3–19.2
Parsnip	10–18	Tr	0.07–0.11	0.05–0.09	0.2–0.7	20–58	36–57	0.6–1.5
Pea	12–35	50–600	0.25–0.52	0.06–0.15	1.3–3.3	8–63	13–52	1.2–3.6
Peppers	73–342	40–750	0.03–0.1	0.02–0.18	0.3–2.17	22	9–29	0.5–1.5
Plantain	6–54	15–910	0.02–0.1	0.01–0.1	0.16–1.4	26	2–23	0.1–2.1
Pumpkin	4–20	50–10820	0.03–0.05	0.03–0.08	0.4–0.9	9	15–66	0.3–0.8
Potato	8–64	Tr - 10	0.04–0.16	0.02–0.04	0.8–5.1	6–11	4–13	0.5–1.4
Radish	6–43	Tr - 10	Tr - 0.04	0.01–0.05	0.2–0.65	10–27	21–52	0.3–1.9
Spinach	1–59	800–6720	0.05–0.15	0.08–0.24	0.35–0.75	53–194	60–595	0.8–4.5
Summer squash	3–46	Tr - 1200	0.02–0.1	0.01–0.4	0.2–1.4	8–26	9–40	0.2–2.4
Sweet corn	7–10	Tr - 170	0.15–0.2	0.06–0.1	1.7	46	2–9	0.5–0.7
Sweet potato	7–68	3340–17050	0.1–0.15	0.03–0.14	0.41–1.56	10–11	14–45	0.6–1.3
Taro	3–8	Tr - 5	0.03–0.27	0.03–0.1	0.06–1.16	19	18–150	0.7–2
Tomato	19–48	50–625	0.04–0.11	0.02–0.12	0.45–0.91	5–15	5–14	0.4–1.2
Turnip	17–37	Tr - 5	0.03–0.07	0.03–0.06	0.4–0.94	4–15	30–65	0.01–0.5
Watercress	37–153	450–4700	0.05–0.2	0.09–0.3	0.2–1.38	9–50	63–222	1.3–5.1
Yam	Tr - 13	Tr - 200	0.05–0.15	0.01–0.03	0.1–0.55	16	8–37	0.5–1.2
Zucchini	2–5	240	Tr - 0.04	0.04	0.2–0.4	17	13–14	0.2–0.4

* USDA database values only — expressed as total carbohydrate by difference; includes sugar, starch, and dietary fiber. Tr = trace amount.
Source: Refs. 16, 18.

III. NON-NUTRITIVE PHYTOCHEMICALS

A. PHYTOCHEMICALS

Many components of foods are not strictly required by the body for growth and daily maintenance, yet some of these components may promote health and help prevent disease. It has become common to call these compounds by the general term phytochemicals when present in plant foods or zoochemicals when present in animal foods. Some phytochemicals are conspicuous by their colors (carotenoids) or flavors (tannins); however, the presence of many other phytochemicals is not as evident.

In 1919, E.V. McCollum wrote in his book *The Newer Knowledge of Nutrition*, "A plant structure, or an animal body is an exceedingly complex mixture of chemical substances many of which are themselves individually as complicated in their structure as the most complex machine. The first step in the direction of reaching an understanding of the chemistry in the living mass, must involve the separation and the study of the structural units of which the tissues are composed" (2, p. 2).

Although McCollum was likely writing this about nutrients, his statement is as true today as nearly a century ago. Presently the field of nutrition is identifying and quantifying thousands of non-nutritive phytochemicals.

TABLE 21.4
Factors That Can Affect Nutrient Content of Foods or Reported Nutrient Values

General References Relating to Multiple Factors (19–22)

Agriculture Production
Genetics: species and plant variety

Harvesting, Shipping, and Storage
Plant maturity, harvesting time and method; Ripeness of plant, Harvesting time and temperature, Time before, during, and after processing

Level and Type of Processing
Fresh, Canned, Frozen, Concentrated, Dehydrated, Dried, Fermented, Salted, Smoked, With/without sweeteners, salt, fat, added liquid

Heat Processing
Pasteurization, Irradiation, Ultra-high temperature, High-temperature short term, Microwave, Pressure

Environmental
Geography, Altitude, Climate, Pest control, Season, Sunlight, Soil composition/fertilization, Water

Added Food Ingredients and/or Supplemented with Nutrients

Preparation Methods
Whole or cut, Cut/grind size, Mixed/whipped/blended, hot/cold preparation, Dry or moist heat frying, Cooled/frozen

Laboratory Analysis (23)
Sampling scheme, Chemical analysis methods, Laboratory procedures, Use of calibration standards, Intra-laboratory variation, Inter-laboratory variation, Data transcription

Source: Refs. 18–23.

Many of these plant chemicals have been identified as having health-promoting qualities. A partial list of vegetable phytochemicals linked to beneficial biological activities is presented in Table 21.5. Other phytochemicals have negative effects upon health either by inhibiting specific nutrient utilization or by being toxic (57).

B. Phytochemical Profiles

The development of chemical profiles for vegetable phytochemicals is in its infancy. Due to the enormity of the task, it will likely be decades before good phytochemical profiles exist.

C. Factors Affecting Phytochemical Composition of Vegetables

Many of the factors that affect nutrient composition (i.e., genetics, environmental factors, and processing) likely also affect the phytochemical composition of vegetables. This is especially true for the phytochemicals functioning as antioxidants. However, information on this topic is extremely limited.

IV. HEALTH BENEFITS DERIVED FROM VEGETABLES

Modern society has dramatically affected how we eat. Since the introduction of T.V. dinners, it appears that convenience greatly influences people's food choices. Researchers have found that monkeys or apes foraging in the wild appear to get far higher levels of many essential nutrients and beneficial phytochemicals relative to their body weight than the average American (58).

Without a doubt, vegetables can be considered more nutrient dense (nutrient content per kilocalorie of food) than foods from other food groups. However, the phytochemicals in vegetables may provide equally important benefits for the prevention of chronic diseases like cancer and heart disease (59).

Research on the purported health benefits of vegetables focuses on two main areas of study:

1) Maintenance of gastrointestinal tract health and
2) Reduction of chronic disease risk

A. Gastrointestinal Tract Health

The gastrointestinal tract (GI-tract) is the gateway through which the body transports nutrients and phytochemicals into the circulation for delivery to body cells. A complex network of internal organs and tissues is responsible for the digestion or breakdown of food components into compounds and elements that can be absorbed into the body. In addition, the GI-tract serves as a protective barrier to prevent some substances from entering the body (60).

Due to the extremely active and chemically hostile internal environment necessary to accomplish the digestive process within the GI-tract, cells along the 25–30 feet of intestine are exposed to a great deal of chemical and physical damage. Consequently, many of these cells have only a three- to five-day life span. This constant turnover of GI-tract cells results in continuous cellular replacement and repair of damage.

TABLE 21.5
Some Important Non-Nutrient Phytochemicals in Vegetables

Phytochemical	Anti-cancer	Antioxidant	Anti-inflamatory	Blood Clotting	Detoxifi-cation	Eye Health	G.I.-Tract Health	Heart Health	Immune System	Osteo-porosis	Examples of Food Sources	Ref.
Capsaicin	+/−			+							Hot chile peppers	33
Carotenoids alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, lycopene, zeaxanthin	+/−	+				+		+			Orange, yellow, & green vegetables	34–36
Curcumin (phenolic)	+	+	+						+		Turmeric, mustard	37
Coumarins				+							Vegetables, tonka bean, sweet clover, licorice	38,39
Dietary fiber	+						+	+			Wide variety of vegetable sources	33,40–43
Glucosinolates (glucobrassicin) Isothiocyanates (sulphorophane) Indoles (indole-3-carbinol)	+	+									Cruciferous vegetables, broccoli sprouts	36,37,40, 44–46
Inositol phosphates (phytate)	+	+									Whole grains, beans	47
Monoterpene (limonene)	+										Citrus fruit peel	36,37,40
Organosulfur compounds diallyl sulfide, allyl methyl disulfide, allyl methyl trisulfide, S-allyl cysteine, diallyl trisulfide, and others	+/−										Garlic, onions, and other allium vegetables; mustard and horseradish	33,48,49
Protease inhibitors	+/−										Legumes, cereals, vegetables	36,50
Tannins	+/−	+							+		Grapes, tea, lentils, wine	51,52
Flavonoids apigenin, catechins, chrysin, kaempferol, myricetin, quercetin	+	+	+		+			a		+	Green vegetables, onions, garlic, tea	40,47,48, 53
Isoflavonoids, biochanin A, daidzein, formononetin, genistein, glycitein	+/−							+		+	soybeans, clover sprouts, alfalfa sprouts	54,55
Lignans	+										flaxseed, berries, whole grains	54,56
Coumestans	+										soybeans, clover sprouts, alfalfa, beans	54,56

There is a significant amount of nutrient recycling from injured GI-tract cells which allows many nutrients to be digested and absorbed along with new food components. This combination of recycled and dietary nutrients is utilized to support adequate replacement of the cell lining of the GI-tract.

Over the last decade there has been an increase in the number of reported cases of various gastrointestinal diseases. Two of the most common GI-tract diseases are diverticulitis (inflammation of small pouches formed along the gastrointestinal tract) and gastro-esophageal reflux disease (GERD), the common cause of indigestion which causes the pain popularly called "heartburn" (61). In fact, GERD is so common and chronic in the United States that Prilosec® was one of the world's top-selling drugs in the year 2000.

Diverticulitis and GERD have one significant dietary factor in common. Both of these conditions appear to be related to years of inadequate dietary fiber intake (61,62). Ironically, fiber is the most abundant phytochemical in vegetables.

Vegetables contain both soluble and insoluble forms of dietary fiber. The physiological effects of these two dietary fiber types have both similarities and some significant differences.

Both soluble and insoluble fiber types hold water and create bulk inside the GI-tract. Soluble fibers slow the rate of stomach emptying into the small intestine. It is thought that the stomach distension caused by fiber bulk and the slower stomach emptying produces an extended feeling of satiety after a meal (63,64). Intake of high soluble fiber also tends to decrease the overall nutrient absorption rate and may also reduce the amount of nutrients and phytochemicals absorbed. This can benefit those with problems in the management of blood glucose and may reduce the absorption of cholesterol (65). Excessively high intake of dietary fiber can interfere with the absorption of minerals.

B. Immune System Health

The immune system is part of the body's natural defense system against disease and disease-producing conditions. Approximately 80 percent of the immune system is located directly adjacent to the GI-tract. Ordinarily, undigested food molecules, microorganisms, and many toxins cannot readily cross through the intestinal lining and do not enter the circulatory system. However, disruptions to the integrity of the GI-tract can challenge the immune system beyond its capacity to maintain health (60).

C. Chronic Disease

Many phytochemicals have been associated with preventing or decreasing the incidence of disease. Disease conditions and related mechanisms that are purported to be affected by various phytochemicals are summarized in Table 21.5.

No doubt there are numerous triggers in the initiation of cancer and heart disease. And although there may be many varied mechanisms of phytochemicals with anticancer and heart promoting properties, one mechanism may be related to the antioxidant property shared by many of these compounds. The process of normal cellular metabolism produces chemicals that are reactive oxygen species like hydrogen peroxide and the superoxide anion free radical. It is thought that free radical production causes a secondary oxidative stress whenever there is an imbalance of antioxidants to oxidants. This can occur with an excess of oxidation stress or an inadequate amount of antioxidants in the diet.

Research has shown that antioxidants are involved in delaying many diseases and conditions that are associated with aging such as cancer, heart disease, decreased immune functioning, and visual and cognitive impairment (66). A number of vitamins, minerals, and phytochemicals provide antioxidant protection in the body. Based on a per gram or per kilocalorie basis, vegetables contain significant amounts of antioxidants.

Dietary phytoestrogens may help reduce the risk of developing certain hormone-stimulated cancers such as breast and prostate cancers. However, much more research on this relationship is needed since some studies indicate that phytoestrogen compounds may stimulate progression of some types of cancer (67).

Flavonoids such as those found in onions, tea, and red wine also are under study for potential cancer prevention. The possible mechanisms of action may vary from one flavonoid to another. They may prevent cancer cell proliferation through specific enzyme inhibition (68).

V. SUMMARY

As a major category of foods, vegetables have a variety of qualities and characteristics that supports common recommendations to include them as a significant part of a balanced and varied diet. They serve as important sources of a wide variety of vitamins and minerals essential for normal human nutrition.

Vegetables supply these nutrients in forms that are generally low in energy and fat, making them more "nutrient dense" than most other food sources. The nutrient content of a particular vegetable can vary, with the extent dependent on the nutrient, and a variety of factors including plant genetics, agricultural factors, storage and handling, processing, packaging, and preparation. Nutrient content values in databases generally reflect averages.

The extent to which a vegetable food is a good source of a nutrient also depends on type of processing and the bioavailability of the nutrient. In some cases, a vegetable can contain high levels of a mineral such as calcium or iron, but the form of the mineral or interfering compounds in the vegetable allow very little of the mineral to be

absorbed into the body. Some nutrients are not found in vegetables, including vitamins D and B-12 and the long chain omega-3 fatty acids commonly found in fish oils and some species of algae.

In addition to nutrients, vegetables provide a great variety of non-nutrient chemical compounds commonly called phytochemicals. The potential benefits and risks of various phytochemicals found in vegetables represent an increasingly active area of nutrition research. The body of scientific research to date supports the inclusion of a wide variety of vegetables in the human diet for reducing the risk of developing a number of disease conditions that tend to develop with age. Additional research is needed to clarify more specific risks and benefits of various types of chemical compounds found in vegetables.

ACKNOWLEDGMENT

The information in this chapter has been derived from "Nutritional Value of Vegetables," by C.A. Titchenal, J. Dobbs. In *Handbook of Vegetable Preservation and Processing*, edited by: Y.H. Hui et al., Marcel Dekker, New York, 2004.

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22 Canned Vegetables: Product Descriptions

Peggy Stanfield
Dietetic Resources

CONTENTS

I. INTRODUCTION

This book is not the proper forum to present the manufacturing process for all categories of canned vegetables being marketed in the United States. This chapter provides a short description of requirements for the major commercial canned vegetables.

In the United States, two federal agencies have the responsibility to make sure that the canned vegetables in the market are safe and do not pose any economic fraud. The U.S. Food and Drug Administration issues regulations to achieve both goals. The U.S. Department of Agriculture issues voluntary guides to achieve the same goals. The information in this chapter has been modified from such regulations and guidelines.

II. CANNED CORN

Canned sweet corn is the product prepared from clean, sound kernels of sweet corn packed with a suitable liquid packing medium which may include water and the creamy component from corn kernels. The tip caps are removed. The product is of the optional styles.

III. CANNED GREEN BEANS AND CANNED WAX BEANS

A. General Description

Canned green beans and canned wax beans are the foods prepared from succulent pods of fresh green bean or wax bean plants conforming to the characteristics of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. Such food is so processed by heat, in an appropriate manner before or after being sealed in a container as to prevent spoilage.

Requirements are provided for optional color and varietal types; styles of pack; ingredients; and labeling.

Optional color types are Green or Wax. Optional varietal types include:

1. Round. Beans having a width not greater than 1-1/2 times the thickness of the bean; or
2. Flat. Beans having a width greater than 1-1/2 times the thickness of the bean.

Optional styles of pack:

1. Whole. Whole pods of any length.
2. Shoestring or sliced lengthwise or French style. Pods sliced lengthwise.
3. Cuts. Transversely cut pods not less than 19 mm (0.75 in) long as measured along the longitudinal axis, which may contain the shorter end pieces that result from cutting such pods.
4. Short cuts. Pieces of pods cut transversely of which 75 percent, by count, or more are less than 19 mm (0.75 in.) in length and not more than 1 percent by count are more than 32 mm (1-1/4 in) in length.
5. Diagonal cuts. Pods cut in lengths as specified, except the pods are cut at an angle approximately 45 deg. to the longitudinal axis.
6. Diagonal short cuts. Pods cut in lengths as specified, except the pods are cut at an angle approximately 45 deg. to the longitudinal axis.
7. Mixture. Any mixture of two or more of the styles specified.

Optional ingredients are:

1. Salt.
2. Monosodium glutamate.
3. Disodium inosinate.
4. Disodium guanylate.
5. Hydrolyzed vegetable protein.
6. Autolyzed yeast extract.
7. Nutritive carbohydrate sweeteners.
8. Spice.
9. Flavoring (except artificial).
10. Pieces of green or red peppers or mixtures of both, either of which may be dried, or other vegetables not exceeding in total 15 percent by weight of the finished product.
11. Vinegar.
12. Lemon juice or concentrated lemon juice.
13. Glucono delta-lactone.
14. Mint leaves.
15. Butter or margarine in a quantity of not less than 3 percent by weight of the finished product.
16. When butter or margarine is added, emulsifiers or stabilizers, or both, may be added.
17. No spice or flavoring simulating the color or flavor imparted by butter or margarine is used.

B. Labeling

The name of the food is "green beans" or "wax beans" as appropriate. Wax beans may be additionally designated "golden" or "yellow."

A declaration of any flavoring that characterizes the product:

1. A declaration of any spice, seasoning, or garnishing that characterizes the product, e.g., "with added spice," or, in lieu of the word "spice," the common name of the spice, e.g., "seasoned with green peppers."
2. The words "vacuum pack" or "vacuum packed" when the weight of the liquid in the container is not more than 25 percent of the net weight, and the container is closed under conditions creating a high vacuum in the container.
3. The name of the optional style of bean ingredient, if a product consists of a mixture of such styles, the words "mixture of" the blank to be filled in with the names of the styles present, arranged in the order of decreasing predominance, if any, by weight of such ingredients.
4. If the product consists of whole beans and the pods are packed parallel to the sides of the container, the word "whole" may be preceded or followed by the words "vertical pack," or if the pods are cut at both ends and are of substantially equal lengths, the words "asparagus style" may be used in lieu of the words "vertical pack."
5. If the product consists of short cuts or diagonal short cuts, a numerical expression indicating the predominate length of cut in the finished food may be used in lieu of the word "short," e.g., "1/2 inch cut."

The following may be included in the name of the food:

1. The word "stringless" where the beans are in fact stringless.
2. The name of the optional varietal type, or the specific varietal name, e.g., "Blue Lake Green Beans," or both.
3. If a term designating diameter is used, it should be supported by an exact graphic representation of the cross section of the bean pod or by a statement of the maximum diameter in common or decimal fractions of an inch and, optionally, by the millimeter equivalent stated parenthetically. The diameter of a whole, cut, diagonal cut, or short cut is determined by measuring the thickest portion of the pod at the shorter diameter of the bean perpendicular to the longitudinal axis.

IV. CANNED MUSHROOMS

A. Legal Requirement

The FDA has established a standard of identity for canned mushrooms. Some aspects are presented below.

1. Definition

Canned mushrooms is the food properly prepared from the caps and stems of succulent mushrooms conforming to the characteristics of the species *Agaricus (Psalliota) bisporus* or *A. bitorquis*, in one of the optional styles, packed with a suitable liquid medium which may include water; and may contain one or more safe and suitable optional ingredients. The food is sealed in a container and, before or after sealing, is so processed by heat as to prevent spoilage.

2. Styles

The optional styles of the mushroom ingredient are:

1. Buttons—consisting of whole mushrooms with attached stems not exceeding 5 millimeters (0.2 inch) in length, measured from the bottom of the veil.
2. Whole—consisting of whole mushrooms with attached stems cut to a length not exceeding the diameter of the cap, measured from the bottom of the veil.
3. Quarters—consisting of buttons or whole style cut into four approximately equal parts.
4. Slices or sliced—consisting of buttons or whole style of which not less than 50 percent are cut parallel to the longitudinal axis of the stem and 2 millimeters to 8 millimeters (0.08 inch to 0.32 inch) in thickness.
5. Random sliced—consisting of buttons or whole style sliced in a random manner.
6. Pieces and stems—consisting of pieces of caps and stems of irregular shapes and sizes.

3. Optional Ingredients

Optional ingredients are:

1. Salt.
2. Monosodium glutamate.
3. Disodium inosinate.
4. Disodium guanylate.
5. Hydrolyzed vegetable protein.
6. Autolyzed yeast extract.
7. Ascorbic acid (vitamin C) in a quantity not to exceed 132 milligrams for each 100 grams (37.5 milligrams for each ounce) of drained weight of mushrooms.
8. Organic acids (except no vinegar is permitted), only where the inside metal of the container is fully enamel-lined and in glass containers with fully enamel-lined caps. Ascorbic acid is an example.
9. Calcium disodium ethylenediaminetetraacetate ($CaNa_2$ EDTA) in a quantity not to exceed 200 parts per million for use to promote color retention.

4. Fill of Container

1. The fill of the mushroom ingredient and packing medium container is not less than 90 percent of the total capacity of the container.
2. The drained weight of the mushroom ingredient is not less than 56 percent of the water capacity of the container.

B. General Processing

Canned mushroom is considered a low acid canned food and there are stringent regulations govering its manufacture. This section provides some background information. A commercial processor must comply with basic federal requirements.

1. Types and Varieties

All cultivated mushrooms belong to the species *Agaricus campestris*. Various bracket fungi, puffballs, and other fungi have been used as food, but none has been grown commercially in this country. Mushrooms are classified as white, cream, or brown, depending on the color of the cap. Those grown for canning are almost exclusively of the white variety. In the East and Midwest the white variety is also the principal kind grown for fresh mushroom

consumption, while in the West and in Canada a fair quantity of the cream variety is used. Of course, in the last two decades or so, many varieties of mushrooms are available from imports, e.g., countries in the Pacific Rim.

2. Growing Requirements

Since mushrooms, like all fungi, do not possess chlorophyll with which to manufacture carbohydrates from CO_2 absorbed from the air, they must obtain carbohydrates and other nutrients by growing on organic material containing these ingredients. Compost is the favorite growing medium in commercial mushroom houses.

Unlike plants possessing chlorophyll, mushrooms can grow in tare in darkness. A cool, moist atmosphere is most favorable for their development. Caves and abandoned mines have been used extensively for the growing of mushrooms.

Most mushrooms are grown in houses constructed especially for the purpose. Cinder blocks are a favorite construction material. The houses should be well insulated against cold in winter and heat in summer, and should have heating facilities for use in winter and means for keeping the air moist when the outside humidity becomes low.

Most houses produce two distinct crops a year. Each crop consists of several "breaks." After the mushrooms are harvested the north beds are covered with a new layer of "casing" soil and watered down for the next growth of mushrooms. After several such "breaks," the beds are cleaned out and filled with fresh compost. The entire cycle area may be completed in as little as three months or even less, up to as long as seven months, depending largely on temperature.

Higher temperatures usually mean a greater proportion of small land mushrooms.

Artificial air conditioning enables some growers to obtain three crops a year.

The months of October to May, inclusive, are the months of heaviest production. Harvesting is light during the warm weather months. A few growers maintain production during the summer by means of artificial air conditioning.

3. Harvesting and Delivery

Most canners grow the greater part of the mushrooms they use, purchasing the remainder of their requirements from other growers. Some canners grow for canning exclusively; others grow both for the fresh market and for processing.

Mushrooms are pulled from the beds with roots attached before the "veil" or membrane breaks open and exposes the "gills." Depending upon the contract between the packer and grower, the mushrooms may be delivered to the plant either with or without roots attached. In the latter case, the roots are cut from the mushrooms in the growing houses by the harvesters. In either case they are placed in baskets holding from three to as much as ten pounds for delivery to the plant. Obviously, unlike small growers, large corporations use a more advanced system to handle this harvesting and delivery.

Freshly harvested mushrooms with the root portion attached will remain fresh longer than if the root portion has been removed. Mushrooms frequently grow in clusters, which may contain from three to five or more units. The units may vary in size from tiny to large in the same cluster that developed from one root.

Mushrooms deteriorate rapidly after picking, becoming discolored and wilted. They should be delivered to the cannery or processor promptly after picking. When mushrooms cannot be processed promptly after delivery to the cannery, they should be placed in a refrigerated room at a temperature of 36° to 37°F until needed. Refrigeration permits the supplies to be carried overnight to begin canning operations the following morning, or late weekend deliveries may be carried over to Monday morning.

Mushrooms must be handled carefully at all times to avoid bruising, which results in dark discolored areas.

C. Preparation and Canning

The following description of a canned mushroom operation is reasonably typical. The order, methods, and equipment may vary from plant to plant, especially a small versus a big one.

After delivery to the plant, mushrooms which cannot be processed immediately are placed in refrigerated storage until they can be processed.

The baskets of mushrooms are taken to the cutting line for removal of root stubs and stems. In most plants the cutting operation is performed mechanically, although some plants may still be cutting by hand. The stem may undergo one or two cuttings, depending on the style of mushrooms desired, whether whole or button.

In the case of whole mushrooms, only the root portion of the stem is removed by the mechanical cutter. If the style of buttons is desired, the cutters first remove the root portion of the stem which is carried away for waste. The rest of the stem is then removed by cutting immediately below the veil. This portion of the stem is used in the style of *stems and pieces*.

1. Washing, Trimming, and Sorting

After cutting, both the caps and stems are conveyed to a spray washer which removes the clinging bits of casing soil or other dirt. The mushrooms then pass over an inspection belt where seriously blemished mushrooms may be trimmed or sorted out. Misshapen, blemished, trimmed, and broken mushrooms are sorted out and placed with other mushroom material for the stems and pieces style.

Mushrooms with partially open veils may be placed with the pieces delivery material, or be added to the buttons or whole mushrooms intended for one of the sliced styles.

2. Sizing

Mushrooms intended for whole mushrooms or buttons are conveyed by means of water flume or other techniques to the sizers.

The mushrooms may be sized in a revolving drum sizer submerged in water. Rotary size graders which are not immersed in water may also be used. A submerged sizer minimizes bruising and gives the mushrooms an additional washing. The caps float upward from the sizer and each size is floated off into a separate holding tank.

The buttons may be separated into six different sizes. The larger sizes are generally sliced and the smaller sizes packed as buttons. Price lists of packers may quote sizes in terms of number of buttons per 8-ounce can as 20/40, 40/60, 60/80, or l00/120. The same size designations apply when the mushrooms are packed in smaller sized cans. For example, the 40/60 size would pack between 20 to 30 buttons in a 4-ounce can.

3. Slicing

Mushrooms intended for slicing are generally sliced prior to blanching; however, slicing may be performed after blanching. The mushrooms are passed through a mechanical slicer with circular knives which cut them into slices of predetermined thickness. A shaker screen removes the small pieces after slicing.

Three styles of sliced mushrooms are produced: 1) Sliced Whole; 2) Sliced Buttons; and 3) Random Sliced Whole.

"Sliced Whole" style is prepared by aligning the mushrooms prior to slicing so that the mushroom is sliced lengthwise from stem to apex.

"Sliced Buttons" style is prepared by positioning the mushrooms prior to slicing so that the mushroom is sliced parallel to the longitudinal axis.

"Random Sliced Whole" style is prepared by slicing the mushroom in any direction.

4. Blanching

The mushrooms are flumed from the holding tanks or slicer to the blancher. The purpose of blanching is to shrink the mushrooms in order to obtain the proper fill. Shrinkage is due to loss of mushroom juice. Mushrooms may shrink as much as 30 to 40 percent in size in blanching

Mushrooms may be blanched by immersing in water at a temperature of 200°F or more. The usual method, however, is to pass the mushrooms through a continuous steam blancher where they are exposed to live steam for a period of 5 to 8 minutes. In some cases the mushrooms may be filled into the cans and then blanched in the cans. Since iron tends to discolor mushrooms, the blancher should be made of stainless steel or other non-corrosive metal. During blanching, the color of some white varieties may change from near-white to a light tan or buff color.

5. Filling and Weighing

Whole or Button styles are generally filled into the can by hand while a semi-automatic filler is usually used for slices and piece pack. After filling, the cans are moved by belt conveyor in front of the weighers, who weigh the individual cans and adjust the fill so that the finished product will meet the required minimum drained weight.

As a safety factor, weighers generally overfill the cans, usually in accordance with a schedule of overfill weights which vary according to can size and style of pack.

Cans may vary considerably in weight, particularly in the smaller sizes. Deficient drained weights have been found to be due in some cases to the use of an unusually light can as tare by the weigher. The weigher should choose a tare can of average weight.

6. Brining and Cooking

After weighing, a salt tablet which may also contain ascorbic acid is added and cans are moved under taps of hot water, the temperature of which may range from 190° to 200°F. The taps are adjusted to fill cans to overflowing. A hot brine solution is sometimes used instead of the water and salt tablet. It is generally unnecessary to use an exhaust on the filled cans since a sufficiently high vacuum is obtained for most purposes by the addition of hot water.

After closing, the cans are processed in retorts under steam pressure. After processing, the cans may be cooled by water in the retort or a cooling tank. The cans should be cooled to 90° to l00°F, in order to check the cook but leave cans warm enough to dry off readily and prevent rusting.

7. Modern Technology and FDA Regulations

The information in this section on mushrooms must be interpreted in terms of the following premises:

1. Depending on the size and operation of a processor, the use of new equipment and machinery has eliminated a lot of problems that used to accompany the processing of mushrooms.
2. If used properly, food additives can contribute to the proper processing of mushrooms.
3. Always be concerned that mushrooms in a container are subject to the important regulations promulgated by the FDA for low acid canned and acidified foods.

V. CANNED ONIONS

A. Production

1. Varieties

Yellow globe onions, a commercial term applied to several different varieties and strains of onions, are preferred by processors of onions. The more acceptable varieties are the Southport yellow globe and the yellow globe Danver. Western processors for the most part use only the yellow globe Danver.

2. Harvesting

The customary period at which to harvest onions for canning is in the fall when the tops of the onions have begun to turn greenish yellow. Usually the crop is dug by a hoe or an implement which turns the ground exposing the onions to the surface. After the onions are taken from the ground they are thrown in windrows or piles in the field, where they remain until the tops are completely dry. After curing sufficiently, the tops are cut or pulled off close to the bulbs. The onions are then placed in sacks or crates and are placed in storage or shipped.

B. Preparation and Canning

1. Receiving

Onions are usually delivered to the cannery in sacks or crates, and are placed in storage until used. Well-cured onions will keep for several months if stored in a well-ventilated place. In some areas it may be necessary to store them in an enclosed shed for protection against freezing. Onions will sprout within a few months if stored in a warm place. Badly spoiled onions become soft. The fresh fruit and vegetable inspection service inspects a large volume of onions for shippers and buyers at time of shipment or in receiving markets. Onions delivered to canners may or may not have been officially inspected.

Generally the onions are emptied from sacks onto a belt conveyor, carrying them to a sizer which eliminates over- and under-sized onions. From the sizer the onions are placed in buckets or pans on a "merry-go-round" sorting table where ends of the onions are trimmed and onions possessing rot, decay, or other serious defects are discarded.

2. Peeling

From the "merry-go-round" sorting table, the onions are conveyed to a carborundum peeler which tends to loosen the outer skin of the onion bulb. As the onions leave the carborundum peeler they pass through a continuous lye peeler containing a three to ten percent lye solution, depending upon the variety and character of the onions, which further loosens the outer scales of the onion bulb.

3. Washing

Following the peeling process, the onions pass through a rotary screen washer where adhering portions of the outer loosened scales are washed off under a strong spray of water. A closely controlled check is necessary to assure complete removal of the lye solution from the onion bulbs.

4. Pre-Removing of Blemishes and Defects

After washing, the onions are moved by conveyer belt to an inspection table where onions containing blemishes are removed.

5. Sizing

Normally onions are separated into three size classifications: tiny, small, and medium. Each processor has developed his own particular sizing operation. However, for the most part onions exceeding an inch and one-half in diameter and those with a diameter of less than 5/8 of an inch are not used for canning.

6. Final Removing of Blemishes and Defects

As the onions come from the sizer, they are conveyed onto a final inspection table where loosened scales, loose centers, onions possessing blemishes, or excessive discolored onions are removed.

7. Filling

The onions are then filled into cans or glass jars and a sufficient amount of hot brine is added for proper fill. After the cans or glass jars are filled they are quickly closed. In packing onions, the product is acidified to assure sterilization and to prevent spoilage. A pH of 4.5 is most desirable. The acid solution may be used as a dip, or may be added direct to the containers in the filling operations.

8. Defects

a. General

Immediately after ascertaining the uniformity of size and shape, segregate any defects in the following groups in accordance with the definitions outlined by the USDA.

b. Extraneous vegetable material

Remove from each container the pieces present and arrange all the extraneous material such as loose skins and dried onion tops. Extraneous vegetable material refers to harmless vegetable material and the material falling into this category is evaluated with respect to its effect on the overall requirements for the grade classification. No tolerance is provided for extraneous vegetable material of a different origin than the onion plants such as weeds and weed seeds. When such extraneous material is found the supervisor should be contacted.

c. Blemished onions

A blemished onion is one that is affected by surface or internal discoloration to such an extent that the appearance or eating quality is materially affected. The following are examples of onions scoreable as being blemished:

1. **Staining**. Onions that show brown or streaked discoloration from lying on the ground.
2. **Seed Stems**. Onions often throw up stalks on which to bear seeds during the later part of their growth. When harvested the seed stems are cut or broken off leaving thick tough stems extending through the centers of the onions. Onions possessing tough or woody seed stems should be considered blemished onions.
3. **Sunburn**. Sunburn is a green discoloration caused byexposure of the bulb to the sun and is normally present only on the outer scale of the onion bulb. This condition should not be confused with the natural greening of certain varieties of onions wherein the green color may be present in the outer scales of the onion bulbs.
4. **Sunscald**. This injury takes place at harvest time when the bulbs are exposed to heat and bright sunlight. The tissue of the exposed area of the bulb will scald and become soft and slippery. When temperatures are reduced and the onions are exposed to the air, the scalded tissue loses moisture by evaporation and leather-like areas are produced which may be bleached almost white.
5. **Freezing injury**. This injury is recognized by the watersoaked appearance, soft feel, and discoloration appearing in a portion of the scales or scales. The affected area normally has a translucent or paper-like appearance.
6. **Smudge**. Smudge is characterized by black blotches or aggregations of minute black or dark-green dots on the outer scales. These dots are often arranged in concentric rings. Generally the lesions are on the outer scales but they may be found on inner scales. On the fleshy scale of the bulb the fungus produces sunken yellowish spots.
7. **Surface Molds**. Surface molds may be black, blue, or gray in color and may be found growing either on the outer scales or frequently between the outer scales of the bulb.
8. **Rot**. Several types of rot may be present in onions, some of which are bacterial soft rot, blue mold rot, fusarium rot, and green mold rot. Normally onion bulbs affected by rot have a water-soaked appearance with various discolorations of outer or inner scales. Canned onions should not contain any units showing rot other than an accidental unit.

d. Seriously blemished onion

A seriously blemished onion is an onion that is affected by surface or internal discoloration to such an extent that the appearance or eating quality is seriously affected. Insect injury, wherever the insect bite extends through the scale of an onion bulb, is very noticeable and should be considered seriously blemished. Dark pathological areas which are unsightly are considered seriously blemished.

9. Mechanical Damage

Onion bulbs mechanically damaged by crushing, gouging, or trimming should be classified as damaged only when the condition materially affects the eating appearance or quality of the bulb.

10. Loose Scales or Pieces of Scales

Loose scales or pieces of scales are those not attached to an onion bulb. Do not aggregate pieces of scales to give the equivalent of one loose scale.

11. Detached Center

Detached center is when the center portion of the onion bulb has become detached. The onion bulb thus damaged is scored as a defect and the loose centers which have become detached are disregarded.

12. Well Trimmed

Determining whether onions are well trimmed is judged entirely on an appearance basis. In meeting the requirement for well trimmed the top and root of the onion should be neatly removed. Onion bulbs with off-slant cuts that materially affect the appearance of the unit are not considered well trimmed.

VI. CANNED PEAS AND DRY PEAS

Canned peas is the food prepared from fresh or frozen succulent seeds of the pea plant of the species *Pisum sativum* L. but excluding the subspecies *macrocarpum*. Only sweet wrinkled varieties, smooth-skin varieties, or hybrids thereof may be used. The product is packed with water or other suitable aqueous liquid medium to which may be added one or more of the other optional ingredients. Such food is sealed in a container and, before or after sealing, is so processed by heat as to prevent spoilage.

In addition to the optional packing media, the following safe and suitable optional ingredients may be used:

1. Salt.
2. Monosodium glutamate.
3. Disodium inosinate.

4. Disodium guanylate.
5. Hydrolyzed vegetable protein.
6. Autolyzed yeast extract.
7. One or any combination of two or more of the dry or liquid forms of sugar, invert sugar syrup, dextrose, glucose syrup, and fructose.
8. Spice.
9. Flavoring (except artificial).
10. Color additives.
11. Calcium salts, the total amount of which added to firm the peas should not result in more than 350 milligrams/kilogram (0.01 ounce/2.2 pounds) of calcium in the finished food.
12. Magnesium hydroxide, magnesium oxide, magnesium carbonate, or any mixture or combination of these in such quantity that the pH of the finished canned peas is not more than 8, as determined by the glass electrode method for the hydrogen ion concentration.
13. Seasonings and garnishes.
14. Pieces of green or red peppers or mixtures of both, either of which may be dried, or other vegetables not exceeding in total 15 percent of the drained weight of the finished food.
15. Lemon juice or concentrated lemon juice.
16. Mint leaves.
17. Butter or margarine in a quantity not less than 3 percent by weight of the finished food, or other vegetable or animal fats or oils in a quantity not less than 2.4 percent by weight of the finished foods.
18. When butter, margarine, or other vegetable or animal fats or oils are added, emulsifiers or stabilizers or both may be added, but no color, spice, or flavoring simulating the color or flavor imparted by butter or margarine may be used.

A. Labeling

The name of the food is "peas" and may include the designation "green." The term "early," "June," or "early June" should precede or follow the name in the case of smooth-skin peas or substantially smooth-skin peas, such as Alaska-type peas or hybrids having similar characteristics. Where the peas are of sweet green wrinkled varieties or hybrids having similar characteristics, the name may include the designation "sweet," "wrinkled," or any combination thereof. The term "petit pois" may be used in conjunction with the name of the food when an average of 80 percent or more of the peas will pass through a circular opening of a diameter of 7.1 millimeters (0.28 inch). If any color additive has been added, the name of the food should include the term "artificially colored."

The following should be included as part of the name or in close proximity to the name of the food:

1. A declaration of any flavoring that characterizes the food.
2. A declaration of any spice, seasoning, or garnishing that characterizes the product, e.g., "seasoned with green peppers," "seasoned with butter," "seasoned with _____ oil," the blank to be filled in with the common or usual name of the oil, "with added spice," or, in lieu of the word spice, the common or usual name of the spice.
3. The words "vacuum pack" or "vacuum packed" when the weight of the liquid in the container is not more than 20 percent of the net weight, and the container is closed under conditions creating a high vacuum in the container.

VII. CANNED PUMPKIN AND CANNED SQUASH

A. Production

1. Varieties

The names pumpkin and squash are popularly applied to the fruits of the species of the genus *Cucurbita*, namely *C. pepo*, *C. maxima*, and *C. moschata*. In general, the term pumpkin is applied to the late maturing or fall varieties of *C. pepo* and *C. maxima*. The principal varieties of *C. pepo* and *C. maxima* used for canning are the Connecticut field pumpkin, Dickinson pumpkin, Kentucky field pumpkin, the Boston marrow squash, and the Golden Delicious squash.

2. Harvesting

Pumpkin and squash should not be harvested for canning until fully matured. Harvesting is usually done after the leaves begin to turn yellow. Mature pumpkin or squash have a hard rind which can be dented only with difficulty with the thumbnail. If picked too green the under portions of the pumpkin will have a greenish color and this may be carried over into the finished product. Pumpkin and squash are usually harvested starting approximately September 15 in the Midwest and Northeast States, and October 1 in the Pacific Northwest.

B. Preparation and Canning

1. Receiving

Pumpkin and squash are usually delivered as harvested and stored at the cannery until used. Well-matured pumpkin or squash will keep for several weeks if stored in a well-ventilated place. In some areas it may be necessary to store them in an enclosed shed for protection against freezing. Normally no inspection is made of pumpkin or squash received by the plants.

2. Washing

Whole pumpkins or squashes are fed by hand or conveyed into a combined tank and spray washer, consisting of a rotary drum partially submerged in a tank of water. The combined soaking and rotary motion loosens adhering dirt which is removed by strong sprays of water. Grit sometimes becomes embedded in the rind, necessitating thorough washing.

3. Trimming

From the washer the pumpkins or squash pass to the inspection belt where stems are knocked off by hand and blossom ends, scar tissue rot, and other blemishes are trimmed out.

4. Cutting

In some canneries the trimmers also cut the pumpkin or squash into halves or quarters with long knives and scrape out the seeds and stringy pulp by hand. In many plants mechanical cutters are used into which the whole units (or halves) are fed by a conveyor cutting the units in pieces. Strong sprays of water help knock out most of the seeds, which drop from the cutter through small perforations.

Where the units are cut and the seeds and pulp removed in separate operations, the cut pieces pass to a revolving drum where they are tumbled under strong sprays of water which remove most of the seeds and pulp.

Where seeds are to be saved for planting, they may undergo further washing to separate them from the pulp.

Cut pieces are in some cases passed over an inspection belt where imperfect pieces and internal rot, not visible from the outside, may be picked out by hand.

5. Wilting (Steaming)

The cut pieces are cooked in live steam until they are tender all the way through. The length of time necessary depends upon the size of the pieces and the nature of the equipment in which the steaming is done. The following are examples in which the wilting or steaming may be accomplished:

1. In metal baskets in retorts, either under pressure or at atmospheric pressure.
2. In continuous metal box wilters. The pieces are carried through on a continuous belt and are subjected to live steam.
3. In wilting towers. These are tall cylindrical silo-like structures into which the cut pieces are fed continuously at the top by conveyor and removed at the bottom by a screw conveyor. The pumpkin or squash is continuously treated with live steam as it passes through the wilting tower.

6. Pressing

The wilted pumpkin or squash is soggy with liquid which is a mixture of condensed steam and pumpkin or squash juice. The product is treated to remove excess liquid in order to attain the desired consistency in the canned product. This is done by putting the wilted pumpkin or squash through an adjustable press. In certain plants the pressing and wilting are done simultaneously by the use of augers inside of cone-shaped perforated screens.

7. Pulping

The pressed pumpkin or squash goes to the pulper or cyclone to remove hard particles, pieces of stems, seeds, fiber, and other extraneous material. In some cases the product is first put through a coarse, heavy cyclone to remove the bulk of extraneous material and an ordinary cyclone to reduce the size of the particles. For the latter a screen with perforations 1/8 inch in diameter is commonly used.

Some processors use what is commercially known as a Fitz mill. This machine, constructed with hammer and knife edges on opposite sides, reduces or pulps pumpkin by a combined impact mashing action.

8. Finishing

From the pulper the product goes to the finisher which removes the finer bits of seeds or other material and gives the final product the desired physical character. There is a difference of opinion among canners as to the most desirable size of the particles of pumpkin in the finished product. Some prefer a very smooth product which can be obtained by using a very fine finisher; others feel that the canned pumpkin or squash should have a noticeable amount of grainy structure and, therefore, use a finisher that is relatively coarse.

9. Preheating

The filling temperature of the prepared pumpkin or squash is an important part of processing. Heat penetration of the product is very slow because of its physical character, and the temperature at the beginning of the process is correspondingly important. By use of the preheater, it is possible to fill all of the cans at a uniform high temperature.

The preheater is usually a straight piece of pipe surrounded by a larger pipe. The product is pumped through the smaller internal pipe and the space between the two pipes is filled with steam, the temperature of which can be controlled. The rate of flow of the product through the pipe and the temperature of the steam determines the temperature at which it goes to the filler. The preheater normally raises the temperature of the product to 190–200°F. To prevent scorching the preheater is usually constructed so that it will shut off automatically if, for any reason, the flow through the inside pipe is stopped.

10. Filling

The hot pumpkin or squash goes directly to the filler and into cans. If the thickness of the product at this point is too great, the product may not be handled properly by the filler. If, for any reason, the filler or closing machine is forced to stop for any length of time all of the pumpkin material should be put back with the material going through the preheater. Pumpkin or squash has a corrosive action on tin-plate and should be packed in enamel-lined cans. It is important to fill the cans completely so that the product is in contact with the entire inner surface of the cover when the can is sealed. Even a small headspace may result in some discoloration after processing.

11. Processing

The filled cans should be processed promptly. High closing temperature may be offset by undue delay between closing and starting of the process time. A partially filled crate of cans should be sent at once to the retort rather than waiting any length of time for additional cans.

12. Cooling

Prompt and adequate cooling is especially important since canned pumpkin has a slow heating and cooling rate. Failure to cool the product promptly may result in overcooking and loss of color and may be directly responsible for spoilage by thermophilic bacteria. Cooling is usually accomplished by moving the metal retort baskets through a long tank of water by means of an overhead endless chain. The speed of the chain is regulated according to the degree of cooling desired. Cold water is kept continuously flowing into the tank to hold down the temperature. Immediately after cooking, while the cans are distended by heat and internal pressure, minute openings in the double seams may be present. Cooling water contaminated with bacteria may be drawn through seam openings as pressure in the cans is replaced by vacuum; therefore, water in the cooling tank should be kept clean. Some canners cool by means of a water spray in order to reduce the contamination hazard.

VIII. CANNED VEGETABLES

Additional production descriptions are provided for the following vegetables: artichokes, asparagus, bean sprouts, shelled beans, lima or butter beans, beets, beet greens, broccoli, Brussels sprouts, cabbage, carrots, cauliflower, celery, collards, dandelion greens, kale, mustard greens, leaves of the mustard plant, okra, onions, parsnips, peas, black-eye or black-eyed peas, field peas, green sweet peppers, red sweet peppers, pimientos (pimentos), potatoes, rutabagas, salsify, spinach, potatoes, sweet, Swiss chard, truffles, turnip greens, turnips.

Table 22.1 provides a basic description of each canned vegetable. Column I describes the canned vegetable. The vegetable ingredient in each such canned vegetable is obtained by proper preparation from the succulent vegetable described in column II of the table. If two or more forms of such ingredient are designated in column III of the table, the vegetable in each the form is an optional ingredient. To the vegetable additional ingredients (required or permitted) are added, and the food is sealed in a container and so processed by heat as to prevent spoilage.

Water is added to the vegetable ingredient may be added except that pimientos may be canned with or without added water, and sweet potatoes in mashed form are canned without added water. Asparagus may be canned with added water, asparagus juice, or a mixture of both. Asparagus juice is the clear, unfermented liquid expressed from the washed and heated sprouts or parts of sprouts of the asparagus plant, and mixtures of asparagus juice and water are considered to be water when such mixtures are used as a packing medium for canned asparagus. In the case of artichokes, a vinegar or any safe and suitable organic acid, which either is not a food additive as defined or if it is a food additive as so defined, is used in conformity with regulations established, is added in such quantity as to reduce the pH of the finished canned vegetable to 4.5 or below.

The following optional ingredients may be added in the case of the vegetables in Table 22.1.

An edible vegetable oil, in the case of artichokes and pimientos, and snaps, in the case of shelled beans, black-eyed peas, and field peas.

In the case of all vegetables (except canned mashed sweet potatoes), one or more of the following optional seasoning ingredients may be added in a quantity sufficient to season the food.

1. Refined sugar (sucrose).
2. Refined corn sugar (dextrose).
3. Corn syrup, glucose syrup.
4. Dried corn syrup, dried glucose syrup.
5. Spice.
6. A vinegar.
7. Green peppers or red peppers, which may be dried.
8. Mint leaves.
9. Onions, which may be dried.
10. Garlic, which may be dried.
11. Horseradish.
12. Lemon juice or concentrated lemon juice.
13. Butter or margarine in a quantity not less than 3 percent by weight of the finished food. When butter or margarine is added, safe and suitable emulsifiers or stabilizers, or both, may be added. When butter or margarine is added, no spice or flavoring simulating the color or flavor imparted by butter or margarine is used.

TABLE 22.1
Canned Vegetables, Source, and Optional Forms of Vegetable Ingredients

I—Name or Synonym of Canned Vegetable	II—Source	III—Optional Forms of Vegetable Ingredient
Artichokes	Flower buds of the artichoke plant	Whole; half or halves or halved; whole hearts; halved hearts; quartered hearts
Asparagus	Edible portions of sprouts of the asparagus plant, as follows:	
	3 and 3/4 in. or more of upper end;	Stalks or spears
	3 and 3/4 in. or more of peeled upper end	Peeled stalks or peeled spears
	Not less than 2 and 3/4 in. but less than 3 and 3/4 in. of upper end	Tips
	Less than 2 and 3/4 in. of upper end	Points
	Sprouts cut in pieces	Cut stalks or cut spears
	Sprouts from which the tip ha been removed, cut in pieces	Bottom cuts or cuts—tips removed
Bean sprouts	Sprouts of the Mung bean	
Shelled beans	Seed shelled from green or wax bean pods, with or without snaps (pieces of immature unshelled pods)	
Lima beans or butter beans	Seed shelled from the pods of the lima bean plant	
Beets	Root of the beet plant	Whole; slices or sliced; quarters or quartered; dice or diced; cut; shoestring or French style or julienne
Beet greens	Leaves, or leaves and immature root, of the beet plant	
Broccoli	Heads of the broccoli plant	
Brussels sprouts	Sprouts of the Brussels sprouts plant	
Cabbage	Cut pieces of the heads of the cabbage plant	
Carrots	Root of the carrot plant	Do
Cauliflower	Cut pieces of the head of the cauliflower plant	
Celery	Stalks of the celery plant	Cut; hearts
Collards	Leaves of the collard plant	
Dandelion greens	Leaves of the dandelion plant	
Kale	Leaves of the kale plant	
Mustard greens	Leave of the mustard plant	
Okra	Pods of the okra plant	Whole; cut
Onions	Bulb of the onion plant	Do
Parsnips	Root of the parsnip plant	Whole; quarters or quartered; slices or sliced; cut; shoestring or French style or julienne
Black-eye peas or black-eyed peas	Seed shelled from pods of the black-eye pea plant, with or without snaps (pieces of immature unshelled pods)	
Field peas	Seed shelled from pods of the field pea plant (other than the black-eye pea plant), with or without snaps (pieces of immature unshelled pods)	
Green sweet peppers	Green pods of the sweet pepper plant	Whole; halves or halved; pieces; dice or diced; strips; chopped
Red sweet peppers	Red-ripe pods of the sweet pepper plant	Do
Pimientos or pimentos	Red-ripe pods of the pimiento, pimento, pepper plant	Whole; halves or halved; pieces; dice or diced; slices or sliced; chopped
Potatoes	Tuber of the potato plant	Whole; slices or sliced; dice or diced; pieces; shoestring or French style or julienne; French fry cut

(*Continued*)

TABLE 22.1
(*Continued*)

I—Name or synonym of Canned Vegetable	II—Source	III—Optional Forms of Vegetable Ingredient
Rutabagas	Root of the rutabaga plant	Whole; quarters or quartered; slices or sliced; dice or diced; cut
Salsify	Root of the salsify plant	
Spinach	Leaves of the spinach plant	Whole leaf; cut leaf or sliced; chopped
Sweet potatoes	Tuber of the sweet potato plant	Whole; mashed; pieces or cuts or cut (longitudinally cut halves may be named on labels as halves or halved in lieu of pieces or cuts or cut)
Swiss chard	Leaves of the Swiss chard plant	
Truffles	Fruit of the truffle	
Turnip greens	Leaves of the turnip plant	
Turnips	Root of the turnip plant	Whole; quarters or quartered; slices or sliced; dice or diced; cut

In the case of all vegetables, the following optional ingredients may be added:

1. Salt.
2. Monosodium glutamate.
3. Disodium inosinate.
4. Disodium guanylate.
5. Hydrolyzed vegetable protein.
6. Autolyzed yeast extract.

In the case of all vegetables flavoring (except artificial) may be added.

In the case of bean sprouts, lima beans, carrots, green sweet peppers, red sweet peppers, and potatoes, any safe and suitable calcium salts may be added as a firming agent.

In the case of canned artichokes packed in glass containers, ascorbic acid may be added in a quantity not to exceed 32 milligrams per 100 grams of the finished food.

In the case of canned asparagus, ascorbic acid, erythorbic acid, or the sodium salts of ascorbic acid or erythorbic acid may be added in an amount necessary to preserve color in the "white" and "green-tipped and white" color types.

In the case of canned asparagus packed in glass containers, stannous chloride may be added in a quantity not to exceed 15 parts per million calculated as tin (Sn), except that in the case of asparagus packed in glass containers with lids lined with an inert material, the quantity of stannous chloride added may exceed 15 parts per million but not 20 parts per million calculated as tin (Sn).

In the case of canned black-eyed peas, disodium EDTA may be added in a quantity not to exceed 145 parts per million.

In the case of potatoes, calcium disodium EDTA may be added in a quantity not to exceed 110 parts per million.

A vinegar or any safe and suitable organic acid for all vegetables (except artichokes, in which the quantity of such optional ingredient is prescribed by the introductory text) in a quantity which, together with the amount of any lemon juice or concentrated lemon juice that may be added, is not more than sufficient to permit effective processing by heat without discoloration or other impairment of the article.

The name of each canned vegetable is designated in column I of the table.

When two or more forms of the vegetable are specified in column III of the table, the label should bear the specified word or words, showing the form of the vegetable ingredient present, except that in the case of canned spinach, if the whole leaf is the optional form used, the word "spinach" unmodified may be used in lieu of the words "whole leaf spinach."

If the optional ingredient specified is present, the label should bear the statement "_____ oil added" or "With added _____ oil," the blank being filled in with the common or usual name of the oil.

If asparagus juice is used as a packing medium in canned asparagus, the label should bear the statement "Packed in asparagus juice."

If the optional ingredient specified is present, the label should bear the statement "With snaps."

The name of the food should include a declaration of any flavoring that characterizes the product as specified, and a declaration of any spice or seasoning that characterizes the product; for example, "with added spice," "seasoned with red peppers," "seasoned with butter."

Wherever the name of the vegetable appears on the label so conspicuously as to be easily seen under customary conditions of purchase, the words and statements specified should immediately and conspicuously precede or follow such name, without intervening written, printed, or graphic matter, except that the varietal name of the vegetable may so intervene.

IX. CANNED CHILI SAUCE

Chili sauce is the product prepared from mature, clean, sound, tomatoes of the red or reddish varieties which are peeled and chopped or crushed, or all (or a portion) of the tomatoes may be chopped, crushed, or macerated and the peelings screened out in a manner so that at least a substantial portion of the seed remains in the product, to which is added salt, spices, vinegar, nutritive sweetening ingredients, and to which may be added vegetable flavoring ingredients such as chopped onion, chopped green or red pepper, chopped green tomatoes, chopped celery, and sweet pickle relish in such quantities as will not materially alter the appearance of the product with respect to the predominance of the tomato ingredient, and any other ingredients permissible under FDA regulations and standards. The chili sauce is processed in accordance with good commercial practice; is packed in hermetically sealed containers; and is sufficiently processed by heat, before or after sealing, to assure preservation of the product. The refractive index of the filtrate of the chili sauce at 20°C is not less than 1.3784.

A. Ingredients

1. Tomato Pulp

The primary ingredient in chili sauce is red tomatoes. The tomatoes may be hand peeled and broken up by stirring or the tomatoes may be prepared by any one of a number of machines especially designed for the purpose, or there may be a combination of whole peeled tomatoes and more or less macerated tomatoes from which the peelings have been removed by screening. These machines are usually similar to the tomato pulper or finisher, except that the holes through which the tomato material is forced are usually quite large. They are not nearly as efficient as the usual finishing machine at removing the peeling and defects from the tomatoes. For economic reasons some manufacturers have resorted to using a large amount of cyclone pulp and a small amount of hand-peeled or mechanically peeled tomatoes or tomatoes which have been forced through small openings. In general the more of the larger pieces of tomato material present, the better the pulp is for chili sauce.

2. Sugar

The use of any of the nutritive sweetening ingredients is permitted in this product. The usual sweetener is sugar (sucrose). The solids of chili sauce are usually brought up higher by the use of sugar and lowered by concentrating the tomatoes than is usual with catsup. Some manufacturers use as much as 1/2 more sugar than they do with catsup yet finish at about the same point with respect to soluble solids.

3. Spices, Salt, and Acids

Most of the same spices are used in chili sauce as in the manufacture of catsup, except that garlic is seldom used in chili sauce. The proportions of the various spice ingredients are not standardized between manufacturers. Salt and vinegar are used in about the same proportions as with catsup.

4. Other Ingredients

The other ingredients, such as onion, bell peppers, celery, and sweet pickle relish, contribute to the flavor of the product and also provide body to the finished product; that is, they provide part of the consistency and most of the chewiness of the finished chili sauce. The ingredients used and the proportions of the ingredients used vary widely from packer to packer; therefore, quite a variety of flavors can be expected in chili sauces. The onion ingredient is often dehydrated onion flakes. Red or green diced dehydrated peppers are often used.

5. Manufacture

Most important in the manufacture of chili sauce is the preparation of the tomato pulp.

6. Tomato Pulp

Where peeled tomatoes are used for a part or all of the tomato pulp they are usually taken from the regular canning lines after at least a partial preparation by mechanical or hand peeling, trimming, and coring. There is usually some selection of the raw tomatoes that are to be run through chili sauce preparation machines. Some packers divert very ripe tomatoes that need no trimming to the chili sauce lines while others box-sort or load-sort the tomatoes and trim them on the conveyer belts. This trimming removes defective parts and stems that would become defects in the sauce. The trimmers may or may not remove most of the green portions of the tomatoes depending on the machinery used and the manufacturer's desires with respect to the appearance of chili sauce. If green pepper, pickle relish, or green tomatoes are added green shouldered tomatoes are not usually trimmed. Manufacture consists of combining the ingredients in a manner so that the finished chili sauce will have the desired qualities of color, consistency, finish, absence of defects, and flavor. The tomato pulp, whether from broken peeled tomatoes or from special chili sauce machines, or cyclone juice to which some tomato material containing tomato seed is added, is run to kettles, usually steam jacketed, and reduced by boiling to about one-half the original volume. Concentration in vacuum pans is particularly satisfactory for this operation.

7. Adding Other Ingredients

Onion is often added at the beginning of the boil. The other ingredients may be added at any time but the

sugar is usually added late to prevent caramelization. Spices, if in the form of oils or cream of spice, are usually added late to prevent evaporation of the flavor ingredients.

8. The Finishing Point

Because of the nature of the ingredients there is no accurate means of determining a correct finishing point which will apply to all formulae. The first batches when starting, or after any major change in formula, are dropped when they appear to be about correct. As with tomato catsup the consistency of the hot sauce may be measured by any suitable device and the refractive index may be taken. Succeeding batches can then be adjusted by these instruments by increasing or decreasing the boiling or by adjusting the amounts of pulp and sugar to the desired result.

9. Processing

Chili sauce is usually closed at about 180°, at which temperature further processing is usually not necessary. Foaming may occur at higher temperatures and an additional process is usually given if the sauce is closed at lower temperatures.

X. CANNED TOMATOES

Canned tomatoes is the food prepared from mature tomatoes conforming to the characteristics of the fruit *Lycopersicum esculentum* P. Mill, of red or reddish varieties. The tomatoes may or may not be peeled, but should have had the stems and calicies removed and should have been cored, except where the internal core is insignificant to texture and appearance.

Canned tomatoes may contain one or more of the safe and suitable optional ingredients, be packed without any added liquid or in one of the optional packing media, and be prepared in one of the styles. Such food is sealed in a container and before or after sealing is so processed by heat as to prevent spoilage.

One or more of the following safe and suitable ingredients may be used:

1. Calcium salts in a quantity reasonably necessary to firm the tomatoes, but the amount of calcium in the finished canned tomatoes is not more than 0.045 percent of the weight, except that when the tomatoes are prepared in one of the styles specified the amount of calcium is not more than 0.08 percent of the weight of the food.
2. Organic acids for the purpose of acidification.
3. Dry nutritive carbohydrate sweeteners whenever any organic acid is used, in a quantity reasonably necessary to compensate for the tartness resulting from such added acid.
4. Salt.
5. Spices, spice oils.
6. Flavoring and seasoning.
7. Vegetable ingredients such as onion, peppers, and celery, that may be fresh or preserved by physical means, in a quantity not more than 10 percent by weight of the finished food.

A. Packing Media

Packing media includes:

1. The liquid draining from the tomatoes during or after peeling or coring.
2. The liquid strained from the residue from preparing tomatoes for canning consisting of peels and cores with or without tomatoes or pieces thereof.
3. The liquid strained from mature tomatoes (tomato juice).
4. Tomato paste, or tomato puree, or tomato pulp complying with the compositional requirements.

1. Styles

Styles may be:

(a) Whole.
(b) Diced.
(c) Sliced.
(d) Wedges.

2. Name of the Food

The name of the food is "tomatoes," except that when the tomatoes are not peeled the name is "unpeeled tomatoes."

The following should be included as part of the name or in close proximity to the name of the food.

(a) A declaration of any flavoring that characterizes the product as specified.
(b) A declaration of any added spice, seasoning, or vegetable ingredient that characterizes the product, (e.g., "with added _____" or, "with _____" the blank to be filled in with the word(s) "spice(s)," "seasoning(s)," or the name(s) of the vegetable(s) used or in lieu of the word(s) "spice(s)" or "seasoning (s)" the common or usual name(s) of the spice(s) or seasoning(s) used) except that no declaration of the presence of onion, peppers, and celery is required for stewed tomatoes.
(c) The word "stewed" if the tomatoes contain characterizing amounts of at least the three optional vegetables listed.

(d) The styles: "Diced," "sliced," or "wedges" as appropriate.
(e) The name of the packing medium: "tomato paste," "tomato puree," or "tomato pulp" or "strained residual tomato material from preparation for canning." The name of the packing medium should be preceded by the word "with."
(f) The following may be included as part of the name or in close proximity to the name:

The word "whole" if the tomato ingredient is whole or almost whole, and the weight of such ingredient is not less than 80 percent of the drained weight of the finished food as determined in accordance with the method prescribed.

The words "solid pack" when none of the optional packing media are used.

The words "in tomato juice" if the packing medium is used.

The name of each ingredient used should be declared on the label as required.

The standard of quality for canned tomatoes is as follows:

The drained weight is not less than 50 percent of the weight of water required to fill the container

The strength and redness of color is not less than that of the blended color of any combination of the color discs.

Blemishes per kilogram (2.2 pounds) of the finished food cover an area of not more than 3.5 cm (0.54 square inch) which is equivalent to 1.6 cm (0.25 square inch) per pound based on an average of all containers examined.

If the quality of canned tomatoes falls below standard with respect to only one of the factors of quality specified, there may be substituted for the second line of such general statement of substandard quality ("Good Food—Not High Grade") a new line to read as follows:

"Poor color" or
"Excessive peel" or
"Excessive blemishes."

3. Fill of Container

The standard of fill of container for canned tomatoes is a fill of not less than 90 percent of the total capacity of the container.

If canned tomatoes fall below the standard of fill of container, the label should bear the general statement of substandard fill

XI. CANNED TOMATO JUICE

Tomato juice is the food intended for direct consumption, obtained from the unfermented liquid extracted from mature tomatoes of the red or reddish varieties of *Lycopersicum esculentum* P. Mill, with or without scalding followed by draining. In the extraction of such liquid, heat may be applied by any method that does not add water thereto.

Such juice is strained free from peel, seeds, and other coarse or hard substances, but contains finely divided insoluble solids from the flesh of the tomato in accordance with current good manufacturing practice. Such juice may be homogenized, may be seasoned with salt, and may be acidified with any safe and suitable organic acid. The juice may have been concentrated and later reconstituted with water and/or tomato juice to a tomato soluble solids content of not less than 5.0 percent by weight.

The food is preserved by heat sterilization (canning), refrigeration, or freezing. When sealed in a container to be held at ambient temperatures, it is so processed by heat, before or after sealing, as to prevent spoilage.
The name of the food is:

1. "Tomato juice" if it is prepared from unconcentrated undiluted liquid extracted from mature tomatoes of reddish varieties.
2. "Tomato juice from concentrate" if the finished juice has been prepared from concentrated tomato juice as specified or if the finished juice is a mixture of tomato juice and tomato juice from concentrate.

Each of the ingredients used in the food should be declared on the label as required.

The standard of quality for tomato juice is as follows:

The strength and redness of color complies with specific requirement.

Not more than two defects for peel and blemishes, either singly or in combination, in addition to three defects for seeds or pieces of seeds, defined as follows, per 500 milliliters (16.9 fluid ounces):

(a) Pieces of peel 3.2 millimeters (0.125 inch) or greater in length.
(b) Blemishes such as dark brown or black particles (specks) greater than 1.6 millimeters (0.0625 inch) in length.
(c) Seeds or pieces of seeds 3.2 millimeters (0.125 inch) or greater in length.

If the quality of the tomato juice falls below the standard, the label should bear the general statement of substandard quality.

In lieu of such general statement of substandard quality when the quality of the tomato juice falls below the standard in one or more respects, the label may bear the alternative statement, "Below Standard in Quality _____," the blank to be filled in with the words:

(i) "Poor color"
(ii) "Excessive pieces of peel"
(iii) "Excessive blemishes."
(iv) "Excessive seeds" or "excessive pieces of seed."

The standard of fill of container for tomato juice is not less than 90 percent of the total capacity, except when the food is frozen.

If the tomato juice falls below the standard of fill, the label should bear the general statement of substandard fill.

XII. CANNED TOMATO CONCENTRATES, "TOMATO PUREE," "TOMATO PULP," OR "TOMATO PASTE"

Tomato concentrates are the class of foods each of which is prepared by concentrating one or any combination of two or more of the following optional tomato ingredients:

1. The liquid obtained from mature tomatoes of the red or reddish varieties (Lycopersicum esculentum P. Mill).
2. The liquid obtained from the residue from preparing such tomatoes for canning, consisting of peelings and cores with or without such tomatoes or pieces thereof.
3. The liquid obtained from the residue from partial extraction of juice from such tomatoes.

Such liquid is obtained by so straining the tomatoes, with or without heating, as to exclude skins (peel), seeds, and other coarse or hard substances in accordance with good manufacturing practice. Prior to straining, food-grade hydrochloric acid may be added to the tomato material in an amount to obtain a pH no lower than 2.0. Such acid is then neutralized with food-grade sodium hydroxide so that the treated tomato material is restored to a pH of 4.2 $\pm$ 0.2. Water may be added to adjust the final composition. The food contains not less than 8.0 percent tomato soluble solids as specified.

The food is preserved by heat sterilization (canning), refrigeration, or freezing. When sealed in a container to be held at ambient temperatures, it is so processed by heat, before or after sealing, as to prevent spoilage.

One or any combination of two or more of the following safe and suitable ingredients may be used in the foods:

1. Salt (sodium chloride formed during acid neutralization should be considered added salt).
2. Lemon juice, concentrated lemon juice, or organic acids.
3. Sodium bicarbonate.
4. Water.
5. Spices.
6. Flavoring.

The name of the food is:

1. "Tomato puree" or "tomato pulp" if the food contains not less than 8.0 percent but less than 24.0 percent tomato soluble solids.
2. "Tomato paste" if the food contains not less than 24.0 percent tomato soluble solids.
3. The name "tomato concentrate" may be used in lieu of the name "tomato puree," "tomato pulp," or "tomato paste" whenever the concentrate complies with the requirements of such foods, except that the label should bear the statement "for re-manufacturing purposes only" when the concentrate is packaged in No. 10 containers (3.1 kilograms or 109 avoirdupois ounces total water capacity) or containers that are smaller in size.
4. "Concentrated tomato juice" if the food is prepared from the optional tomato ingredient described and is of such concentration that upon diluting the food according to label directions as required, the diluted article will contain not less than 5.0 percent by weight tomato soluble solids.

The following should be included as part of the name or in close proximity to the name of the food:

1. The statement "Made from" or "Made in part from," as the case may be, "residual tomato material from canning" if the optional tomato ingredient is present.
2. The statement "Made from" or "Made in part from," as the case may be, "residual tomato material from partial extraction of juice" if the optional tomato ingredient present.
3. A declaration of any flavoring that characterizes the product and a declaration of any spice that characterizes the product, e.g., "Seasoned with _____," the blank to be filled in with the words "added spice" or, in lieu of the word "spice," the common name of the spice.

The label of concentrated tomato juice should bear adequate directions for dilution to result in a diluted article containing not less than 5.0 percent by weight tomato soluble solids, except that alternative methods may be used to convey adequate dilution directions for containers that are larger than No. 10 containers (3.1 kilograms or 109 avoirdupois ounces total water capacity).

A. Fill of Container

The standard of fill of container for tomato concentrate, as determined by the general method for fill of container, is not less than 90 percent of the total capacity, except when the food is frozen.

XIII. TOMATO CATSUP

Catsup, ketchup, or catchup is the food prepared from one or any combination of two or more of the following optional tomato ingredients:

1. Tomato concentrate as described.
2. Lemon juice, concentrated lemon juice, or safe and suitable organic acids may be used in quantities no greater than necessary to adjust the pH.

3. The liquid derived from mature tomatoes of the red or reddish varieties *Lycopersicum esculentum* P. Mill.
4. The liquid obtained from the residue from preparing such tomatoes for canning, consisting of peelings and cores with or without such tomatoes or pieces thereof.
5. The liquid obtained from the residue from partial extraction of juice from such tomatoes. Such liquid is strained so as to exclude skins, seeds, and other coarse or hard substances in accordance with current good manufacturing practice. Prior to straining, food-grade hydrochloric acid may be added to the tomato material in an amount to obtain a pH no lower than 2.0. Such acid is then neutralized with food-grade sodium hydroxide so that the treated tomato material is restored to a pH of 4.2 ± 0.2.

The final composition of the food may be adjusted by concentration and/or by the addition of water. The food may contain salt (sodium chloride formed during acid neutralization should be considered added salt) and is seasoned with optional ingredients (see above).

The food is preserved by heat sterilization (canning), refrigeration, or freezing. When sealed in a container to be held at ambient temperatures, it is so processed by heat, before or after sealing, as to prevent spoilage.

One or any combination of two or more of the following ingredients in each category is added to the tomato ingredients:

1. Vinegars.
2. Nutritive carbohydrate sweeteners.
3. Spices, flavoring, onions, or garlic.
4. Labeling.
5. The name of the food is "Catsup," "Ketchup," or "Catchup."

The following should be included as part of the name or in close proximity to the name of the food:

1. The statement "Made from" or "Made in part from," as the case may be, "residual tomato material from canning" if the optional tomato ingredient or tomato concentrate containing the ingredient is present.
2. The statement "Made from" or "Made in part from," as the case may be, "residual tomato material from partial extraction of juice" if the optional tomato ingredient or tomato concentrate containing the ingredient is present.

The name "tomato concentrate" may be used in lieu of the names "tomato puree," "tomato pulp," or "tomato paste" and when tomato concentrates are used.

A. Fill of Container

The standard of fill of container for catsup is not less than 90 percent of the total capacity except when the food is frozen, or when the food is packaged in individual serving-size packages containing 56.7 grams (2 ounces) or less.

ACKNOWLEDGMENT

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23 Frozen Vegetables: Product Descriptions

Peggy Stanfield
Dietetic Resources

CONTENTS

I. INTRODUCTION

This book is not the proper forum to discuss the manufacture of every processed vegetable available in the market. However, regulatory agencies such the Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have issued some minimal criteria for each processed vegetable such as what they are, what types and styles are available and so on. The information in this chapter describes each available frozen vegetable product and has been modified from the product grades (USDA) and product standards (FDA). Product standards and product grades are established to achieve two objectives: assure product safety and minimize economic fraud.

The information provided here has one major objective: to remind a commercial processor what each frozen vegetable is and the applicable criteria for particular products.

II. FROZEN ASPARAGUS

Frozen asparagus consists of sound and succulent fresh shoots of the asparagus plant (*Asparagus officianalis*). The product is prepared by sorting, trimming, washing, and blanching as necessary to assure a clean and wholesome product. It is then frozen and stored at temperatures necessary for preservation.

1. TYPES

1. Green or all-green consists of units of frozen asparagus which are typically green, light-green, or purplish-green in color.
2. Green-white consists of frozen asparagus spears and tips which have typical green, light-green, or purplish-green color to some extent but which are white in the lower portions of stalk.

2. STYLES

Spears or stalks style consists of units composed of the head and adjoining portion of the shoot that are 3 inches or more in length. Tips style consists of units composed of the head and adjoining portion of the shoot that are less than 3 inches in length. Center cuts or cuts style consists of portions of shoots (with or without head material) that are cut transversely into units not less than one-half inch in length and that fail to meet the definition for cut spears or cuts and tips style.

Cut spears or cuts and tips style consists of the head and portions of the shoot cut transversely into units 2 inches or less but not less than one-half inch in length. To be considered this style head material should be present in these amounts for the respective lengths of cuts:

1. 1-1/4 inches or less. Not less than 18 percent (average), by count, of all cuts are head material.
2. Longer than 1-1/4 inches. Not less than 25 percent (average), by count, of all cuts are head material.

III. FROZEN LIMA BEANS

Frozen lima beans are the frozen product prepared from the clean, sound, succulent seed of the lima bean plant without soaking, by shelling, washing, blanching, and properly draining. They are then frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

1. TYPES

1. Thin-seeded such as Henderson, Bush, and Thorogreen varieties.
2. Thick-seeded Baby Potato such as Baby Potato, Baby Fordhook, and Evergreen. Thick-seeded, such as Fordhook variety.

IV. FROZEN BEANS, SPECKLED BUTTER (LIMA)

Frozen speckled butter (lima) beans are the frozen product prepared from the clean, sound, freshly-vined (but not seed-dry) seed of the speckled butter (lima) bean plant (*Phaseolus limensis*). The skins of the seed are pigmented and the external colors range from variegated speckling of green, pink, red, and/or lavender to purple. The product is prepared by shelling the pods; by washing, blanching, and properly draining the seeds that have been sorted and blended or otherwise prepared in accordance with good commercial practice. They are frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

V. FROZEN BROCCOLI

Frozen broccoli is the product prepared from the fresh, clean, sound stalks or shoots of the broccoli plant [*Brassica oleracea* (Italica group)] by trimming, washing, blanching, sorting, and properly draining. The product is frozen in accordance with good commercial practice and maintained at temperatures necessary for its preservation.

1. STYLES

1. Spears or stalks are the head and adjoining portions of the stem, with or without attached leaves, which may range in length from 9 cm (3.5 in.) to 15 cm (5.9 in.). The spears or stalks may be cut longitudinally.
2. Short spears or florets are the head and adjoining portions of the stem, with or without attached leaves, which may range in length from 2.5 cm (1 in.) to 9 cm (3.5 in.). Each short

spear or floret must weigh more than 6 g (0.2 oz). The short spears or florets may be cut longitudinally.

3. Cut spears or short spears are cut into portions which may range in length from 2 cm (0.8 in.) to 5 cm (2 in.). Head material should be at least 62.5 g (2.2 oz) per 250 g (8.8 oz) and leaf material should not be more than 62.5 g (2.2 oz) per 250 g (8.8 oz).
4. Chopped spears or short spears are cut into portions which are less than 2 cm (0.8 in.) in length. Head material should be at least 12.5 g (0.4 oz) per 50 g (1.8 oz) and leaf material should not be more than 12.5 g (0.4 oz) per 50 g (1.8 oz).
5. Pieces or random cut pieces are cut or chopped portions of spears or short spears or other units which do not meet the requirements for cut or chopped styles.

VI. FROZEN BRUSSELS SPROUTS

Frozen Brussels sprouts are the frozen product prepared from the clean, sound succulent heads of the Brussels sprouts plant (*Brassica oleracea* L. var. *gemmifera*) by trimming, washing, blanching, and properly draining. The product is frozen in accordance with good commercial practice and maintained at temperatures necessary for its preservation.

VII. FROZEN CARROTS

Frozen carrots are the clean and sound product prepared from the fresh root of the carrot plant (*Daucus carota*) by washing, sorting, peeling, trimming, and blanching, and are frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

1. Styles

Wholes (or whole carrots) retain the approximate confirmation of a whole carrot.

Halves or halved carrots are cut longitudinally into two units.

Quarters or quartered carrots are cut longitudinally into four approximately equal units. Carrots cut longitudinally or cut longitudinally and crosswise into six or eight units approximating the size and appearance of quartered carrots are also permitted in this style.

Slices or sliced carrots are sliced transversely to the longitudinal axis.

Diced carrots consist of approximate cube-shaped units.

Double-diced carrots consist of approximate rectangular shapes that resemble the equivalent of two cube-shaped units.

Strips are carrots that consists of approximate French-cut shapes, with flat-parallel or corrugated-parallel surfaces, one-half inch or more in length.

Chips are carrots that consist of predominately small-sized units (such as less than one-half cube) and variously shaped pieces or slivers in which the longest-edge dimension approximates not more than one-half inch.

Cut carrots consist of cut units that do not conform to any of the forgoing styles.

VIII. FROZEN CAULIFLOWER

Frozen cauliflower is prepared from fresh flower heads of the cauliflower plant (*Brassica oleracea botrytis*) by trimming, washing, and blanching, and is frozen and maintained at temperatures necessary for preservation of the product.

1. Styles and Requirements

1. Clusters are individual segments of trimmed and cored cauliflower heads, which measure not less than 20 mm (0.75 in.) in the greatest dimension across the top of the unit. A maximum of 10%, by weight, of clusters less than 20 mm (0.75 in.) in the greatest dimension across the top of the unit are allowed.
2. Nuggets or small clusters are individual segments of trimmed and cored cauliflower heads, which measure from 6 mm (0.25 in.) to less than 20 mm (0.75 in.) in the greatest dimension across the top of the unit. A maximum of 20%, by weight, of clusters, 20 mm (0.75 in.) or greater, and a maximum of 10%, by weight, of clusters less than 6 mm in the greatest dimension across the top of the unit are allowed.

IX. FROZEN CORN ON THE COB

Frozen corn on the cob is the product prepared from sound, properly matured, fresh, sweet corn ears by removing husk and silk, by sorting, trimming, and washing to assure a clean and wholesome product. The ears are blanched, then frozen and stored at temperatures necessary for the preservation of the product.

1. Styles

1. Trimmed. Ears trimmed at both ends to remove tip and stalk ends and or/cut to specific lengths.

2. Natural. Ears trimmed at the stalk end only to remove all or most of the stalk.

2. Lengths

1. Regular. Ears which are predominantly over 3-1/2 inches in length.
2. Ears which are predominantly 3-1/2 inches or less in length.

Colors of frozen corn on the cob: Golden (or yellow); white.

X. FROZEN LEAFY GREENS

Frozen leafy greens are the frozen product prepared from the clean, sound, succulent leaves and stems of any one of the plants listed below by sorting, trimming, washing, blanching, and properly draining. The product is processed by freezing and maintained at temperatures necessary for its preservation. Any functional, optional ingredient(s) permissible under the law may be used to acidify and/or season the product.

1. Types

Beet greens.
Collards.
Dandelion greens.
Endive.
Kale.
Mustard greens.
Spinach.
Swiss chard.
Turnip greens.
Any other "market accepted" leafy green.

2. Styles

1. Leaf consists substantially of the leaf, cut or uncut, with or without adjoining portion of the stem.
2. Chopped consists of the leaf with or without adjoining portion of the stem that has been cut into small pieces less than approximately 20 mm (0.78 in.) in the longest dimension but not comminuted to a pulp or a puree.
3. Pureed consists of the leaf with or without adjoining portion of the stem that has been comminuted to a pulp or a puree.

XI. FROZEN OKRA

Frozen okra is the product prepared from the clean, sound, succulent, and edible fresh pods of the okra plant (*Hibiscus esculentus*) of the green variety. The product may or may not be trimmed, is properly prepared and properly processed, and is then frozen and stored at temperatures necessary for preservation.

1. Styles

1. Whole okra consists of trimmed or untrimmed whole pods of any length that may possess an edible portion of the cap. The length of a whole pod is determined by measuring from the outermost point of the tip end of the pod to the outermost point of the stem end of the pod, exclusive of any inedible stem portion that may be present.
2. Cut okra is trimmed or untrimmed whole pods, which may possess an edible portion of cap, and which have been cut transversely into pieces of approximate uniform length. The length of a unit of cut okra is determined by measuring the longitudinal axis of the unit.

XII. FROZEN ONION RINGS, BREADED, RAW OR COOKED

Frozen breaded onion rings, hereinafter referred to as frozen onion rings, is the product prepared from clean and sound, fresh onion bulbs (*Allium cepa*) from which the root bases, tops, and outer skin have been removed. The onion bulbs are sliced and separated into rings, coated with batter (or breaded), and may or may not be deep fried in a suitable fat or oil bath. The product is prepared and frozen in accordance with good commercial practice and maintained at temperatures necessary for the proper preservation of the product.

1. Types

The type of frozen onion rings applies to the method of preparation of the product, and includes:

1. French fried onion rings that have been deep fried in a suitable fat or oil bath prior to freezing.
2. Raw breaded onion rings that have not been oil blanched or cooked prior to freezing.

XIII. FROZEN PEAS

Frozen peas is the food in "package" form, prepared from the succulent seed of the pea plant of the species *Pisum sativum* L. Any suitable variety of pea may be used. It is blanched, drained, and preserved by freezing in such a way that the range of temperature of maximum crystallization is passed quickly. The freezing process should not

be regarded as complete until the product temperature has reached −18°C (0°F) or lower at the thermal center, after thermal stabilization. Such food may contain one, or any combination of two or more, of the following safe and suitable optional ingredients:

XIV. PEAS, FIELD AND BLACK-EYE

Frozen field peas and frozen black-eye peas, hereafter referred to as frozen peas, are the frozen product prepared from clean, sound, fresh, seed of proper maturity of the field pea plant (*Vigna sinensis*), by shelling, sorting, washing, blanching, and properly draining. The product is frozen and maintained at temperatures necessary for preservation. Frozen peas may contain succulent, unshelled pods (snaps) of the field pea plant or small sieve round type succulent pods of the green bean plant as an optional ingredient used as a garnish.

XV. FROZEN PEPPERS, SWEET

Frozen sweet peppers are the frozen product prepared from fresh, clean, sound, firm pods of the common commercial varieties of sweet peppers, which have been properly prepared, may or may not be blanched, and are then frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

1. TYPES

Type I, green.
Type II, red.
Type III, mixed (green and red).

2. STYLES

1. Whole stemmed: whole unpeeled pepper pods with stem and core removed.
2. Whole unstemmed: whole unpeeled pepper pods with stems trimmed to not more than 1/2 inch length.
3. Halved: whole stemmed, unpeeled pepper pods which have been cut approximately in half from stem to blossom end.
4. Sliced: whole stemmed, unpeeled pepper pods or pieces of pepper pods which have been cut into strips.
5. Diced: whole stemmed, unpeeled pepper pods or pieces of pepper pods which have been cut into approximate square pieces measuring 1/2 inch or less.
6. Unit: a whole unpeeled pepper pod or portion of a pepper pod in frozen sweet peppers.

XVI. FROZEN POTATOES, FRENCH FRIED

Frozen French fried potatoes are prepared from mature, sound, white or Irish potatoes (*Solanum tuberosum*). The potatoes are washed, sorted, and trimmed as necessary to assure a clean and wholesome product. The potatoes may or may not be cut into pieces. The potatoes are processed in accordance with good commercial practice which includes deep frying or blanching in a suitable fat or oil and which may include the addition of any ingredient permissible under the law. The prepared product is frozen and is stored at temperatures necessary for its preservation.

1. TYPES

Frozen French fried potatoes are of two types, based principally on intended use, as follows:

1. Retail type. This type is intended for household consumption. It is normally packed in small packages that are labeled or marked for retail sales. It may be otherwise designated for such use.
2. Institutional type. This type is intended for the hotel, restaurant, or other large feeding establishment trade. Primary containers, usually 5 pounds or more, are often not as completely labeled as for retail sales.

2. STYLES

Styles of potatoes are grouped into general, strips, slices, dices, and Rissolé.

The style of frozen French fried potatoes is identified by the general size, shape, or other physical characteristics of the potato units. Styles with cut units may be further identified by substyles as follows:

1. Straight cut refers to smooth cut surfaces and
2. Crinkle cut refers to corrugated cut surfaces.

The strips style consists of elongated pieces of potato with practically parallel sides and of any cross-sectional shape. This style may be further identified by the approximate dimensions of the cross-section, for example:

1/4 × 1/4 inch
3/8 × 3/8 inch
1/2 × 1/4 inch, or
3/8 × 3/8 inch

Shoestring refers to strip, either straight cut or crinkle cut, with a cross section predominantly less than that of a square measuring 3/8 × 3/8 inch.

Slices is a style that consists of pieces of potato with two practically parallel sides, and which otherwise conform generally to the shape of the potato. This style may also contain a normal amount of outside slices.

Dices consists of pieces of potato cut into approximate cubes.

Rissolé is a style that consists of whole or nearly whole potatoes.

Any other individually frozen French fried potato product may be designated as to style by description of the size, shape, or other characteristic that differentiates it from the other styles.

3. Length Designations

The length designations described in this section apply to strip styles only.

Frozen French fried potato strips are designated as to length in accordance with the following criteria. Percent, as used in this section, means the percent, by count, of all strips of potato that are 1/2-inch in length, or longer.

1. Extra long. Eighty (80) percent or more are 2 inches in length or longer; and 30 percent or more are 3 inches in length or longer.
2. Long. Seventy (70) percent or more are 2 inches in length, or longer; and 15 percent or more are 3 inches in length or longer.
3. Medium. Fifty (50) percent or more are 2 inches in length or longer.
4. Short. Less than 50 percent are 2 inches in length or longer.

XVII. FROZEN POTATO, HASH BROWN

Frozen hash brown potatoes are prepared from mature, sound, white or Irish potatoes (*Solanum tuberosum*) that are washed, peeled, sorted, and trimmed to assure a clean and wholesome product. The potatoes so prepared are blanched, may or may not be fried, and are shredded or diced or chopped and frozen and stored at temperatures necessary for their preservation.

1. Styles

1. Shredded. Shredded potatoes are cut into thin strips with cross-sectional dimensions from 1 mm by 2 mm to 4 mm by 6 mm and formed into a solid mass before freezing.
2. Diced. Diced potatoes are cut into approximate cube shape units from 6 mm to 15 mm on an edge and loose frozen. They contain not more than 90 grams, per sample unit, of units smaller than one-half the volume of the predominant size unit.
3. Chopped. Chopped potatoes are random cut pieces predominantly less than 32 mm in their greatest dimension and loose frozen.

XVIII. FROZEN VEGETABLES, MIXED

Frozen mixed vegetables consist of three or more succulent vegetables, properly prepared and properly blanched; may contain vegetables (such as small pieces of sweet red peppers or sweet green peppers) added as garnish; and are frozen and maintained at temperatures necessary for the preservation of the product.

1. Kinds and Styles of Basic Vegetables

It is recommended that frozen mixed vegetables, other than small pieces of vegetables added as garnish, consist of the following kinds and styles of vegetables as basic vegetables:

1. Beans, green or wax: Cut styles, predominantly of 1/2 inch to 1-1/2 inch cuts.
2. Beans, lima: Any single varietal type.
3. Carrots: Diced style, predominantly of 3/8 inch to 1/2 inch cubes.
4. Corn sweet: Golden (or Yellow) in whole kernel style.
5. Peas: Early type or sweet type.

2. Recommended Proportions of Ingredients

It is recommended that frozen mixed vegetables consist of three, four, or five basic vegetables in the following proportions:

1. Three vegetables. A mixture of three basic vegetables in which any one vegetable is not more than 40 percent by weight of all the frozen mixed vegetables.
2. Four vegetables. A mixture of four basic vegetables in which none of the vegetables is less than 8 percent by weight or more than 35 percent by weight of all the frozen mixed vegetables.
3. Five vegetables. A mixture of five basic vegetables in which none of the vegetables is less than 8 percent by weight or more than 30 percent by weight of all the frozen mixed vegetables.

ACKNOWLEDGMENT

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24 Fruits: Horticultural and Functional Properties

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CONTENTS

I. INTRODUCTION

Fruits, either fresh or processed, form an important part of our daily diet, and demand is increasing in all affluent countries of the world. This has become possible because of the availability of the improved quality and extended variety of fruits throughout the year. Recent advances in agricultural technology have contributed significantly to the improved production of fruits throughout the world. In addition, the critical advances in fruit processing technologies, refrigeration, transportation, storage and distribution have made it possible for consumers to enjoy these products year-round. The emphasis in fruit processing is shifting now from traditional methods like canning, freezing and dehydration to "fresh cut" or "minimal processing." Because present-day consumers prefer food products that contain fruit, food processors are using more fruits as value-added ingredients (fruit concentrates, pulps, candied pieces, etc.) in their formulations, like snacks, baby foods, baked goods and many other processed foods. At the same time, the variety of fruits available for extended periods throughout the year in developed countries has increased. Many tropical fruits that were earlier considered exotic and expensive are now being commonly consumed as fresh produce.

Fruit consumption has witnessed unprecedented growth during the past few decades. Current nutrition studies are making consumers more informed about the health benefits of various fruit constituents. The importance of vitamins, minerals, antioxidants, dietary fiber and a long list of phytochemicals present in fruits are being discussed daily in the print and television media. Now, the idea of nutrient clusters also supports the need for a varied healthy diet that contains recommended servings of fruits and vegetables (1). A diet that lacks fruits and vegetables rarely supplies the levels of nutrients needed for optimal health. The world population is also ageing and life expectancy is increasing with every passing decade. This prompts us to understand the relationship of nutrition to ageing and to study the effect of dietary requirements on an aged population. The role of phytochemicals present in fruits and vegetables in promoting health and reducing the risk of various chronic diseases among the older adults is now attracting the attention of health professionals (2).

II. BIOLOGY OF FRUITS

Fruit, in strict botanical terms, is the fleshy or dry ripened ovary of a plant, which encloses the seed or seeds. Therefore, mangoes, apricots, bananas, grapes, bean pods, almonds, tomatoes and cucumbers are all technically fruits. However, the term fruit is restricted to the ripened ovaries that are sweet and either succulent or pulpy. A fruit, when developed from only the carpellary structures, is called a true fruit or, if it is developed from a superior ovary, a superior fruit. Fruits with accessory parts are called accessory fruits (grapes, dates and plums), or because of their frequent development from inferior ovaries, are called inferior fruits (bananas, pears and walnuts). Invariably, the true fruits develop from the superior ovaries of hypogynous or perigynous flowers, but the accessory fruits usually develop from the inferior or part-inferior ovaries of epigynous or semiepigynous flowers. The number of ovaries and flowers that constitute the fruits also characterizes fruits. A simple fruit is derived from one ovary (e.g., apples), whereas an aggregate fruit is developed from several ovaries of one flower (e.g., strawberries). A multiple or collective fruit is developed from the structures of ovaries and accessories of several flowers consolidated into a mass (e.g., figs and pineapples). Simple fruits are either true or accessory fruits, but the aggregate and multiple fruits are always accessory fruits.

The classification of fruits is based on numerous criteria, e.g., dehiscence versus indehiscence, dry versus fleshy, textural quality, morphology, development, relationship of ovary to other reproductive parts of the plant and number of carpels and seeds present therein. Fleshy fruits are generally indehiscent, but with a few exceptions. The major types of simple fleshy fruits include a pome with a thin inner part (endocarp) and fleshy outer part (e.g., pear and pomegranate); the latter is a leathery pome. The drupe (stone fruit, e.g., cherry, peach, plum) has an outer exocarp (skin) in the fruit wall, a central fleshy mesocarp (flesh) and an inner stony endocarp or stone. The berry has the entire center of fruit as fleshy (e.g., grapes, bananas, kiwi), whereas hesperidium, a modified berry, has a thick, leathery separate outer rind (e.g., citrus fruits).

Fruits are very perishable in nature because these are living and respiring tissues. Based on the post-harvest patterns of respiration, fruits are classified as climacteric and nonclimacteric (3). Climacteric fruits, such as mango, banana, pear, apricot, guava, kiwi, papaya, peach, fig, plum and apple, produce large amounts of carbon dioxide and ethylene; the production rates of these gases coincide with ripening. These fruits can be harvested at an early mature stage and ripened artificially with ethylene gas (4). The nonclimacteric fruits, such as the orange, strawberry, grape, lime, lemon, pineapple, grapefruit and cherry, show no change in their low production rates of these two gases during ripening and post-harvest storage. Nonclimacteric fruits, if picked too early, are generally sour and of poor quality because they ripen gradually over a long period of time. Environmental factors, like temperature, relative humidity and atmospheric gaseous composition, greatly influence the respiration rate and shelf life of fruits during post-harvest storage. Handling methods should be chosen carefully so that they maintain fruit quality and chemical composition. The delay between harvesting, cooling, processing and consumption may lead to direct losses in

quality (water loss and decay) and indirect losses in flavor and nutritional quality. The harvesting at an appropriate stage of maturity is, therefore, important for better post-harvest shelf life and final fruit quality (5).

III. EDIBLE FRUITS OF THE WORLD

Fruits are grown in temperate, subtropical and tropical climates of the world. Among the fruits grown in temperate and subtropical regions, oranges are the leading groups of fruit in terms of production, which are mainly processed into juice (Table 24.1). Bananas are the second leading fruits and are consumed mainly in fresh form. The major portion of grapes the third leading fruits, goes into juice, wine production and dried fruits, i.e., raisins (6). The major grape-growing countries of the world are Italy, France, the United States, Spain and Russia. The fourth leading group, apples, are mostly eaten as fresh, but reasonable quantities are also processed into juice, dried or canned. Kiwi is another fruit that has become increasingly popular in the international market. The production of fruits is increasing rapidly throughout the world and fruits are becoming great foreign exchange earners for the developing countries.

TABLE 24.1
World Production of Major Fruits (1000 MT)

Fruit	Production	Fruit	Production
Oranges	63,838	Plums	7,836
Bananas	58,975	Peaches	10,923
Grapes	58,466	Papayas	5,024
Apples	56,087	Strawberries	2,682
Mangoes	23,428	Apricots	2,295
Pineapples	12,794	Avocadoss	2,342
Pears	13,318	Grapefruits & Pomelos	5,038
Plantains	29,501	Raspberries	306

Source: Ref. 6.

Banana, mango, papaya and pineapple are the important tropical fruits that have gained commercial importance in international trade (7). Mangoes and lychees are becoming immensely popular fruits among the Asian population all over the world. The United States is the leading producer of fruits in the world, is followed by Brazil, Italy, India, France, Russia and China (6, 8). A few of the important examples of fruits grown in each of the climatic regions of the world (9) are presented in Table 24.2.

IV. COMPOSITION OF FRUITS

Fruits and their processed products are not only colorful, flavorful and appealing to the eye, but are also vital sources of essential vitamins, minerals, dietary fiber and a host of important health-promoting phytochemicals (10). In addition to these nutrients, they also contain complex carbohydrates and proteins (Tables 24.3–24.5). Fruits are good sources of calcium, phosphorus, iron and magnesium. They also supply a major portion of vitamin C in our diets. Green-, yellow- and orange-colored fruits are rich sources of β-carotene (a provitamin A). A substantial portion of the complex carbohydrates in fruits is present as cellulose, hemicellulose, pectic substances and lignins, which serve as dietary fiber in our diets. Dietary fiber content and composition of some of the important fruits, determined by the Association of Official Analytical Chemists (AOAC) method (11) as well as the Uppsala method (12), are presented in Table 24.6. The dietary fiber constituents neutralize the acid produced by meats, milk products and other high protein foods in our diets. The presence of polyphenols, anthocyanins and phytochemical constituents is now being examined for their protective role against cardiovascular diseases and certain types of cancer (13).

Water is the most abundant constituent of most fresh fruits, at usually around 80%, but some fruits, like apples and oranges, may contain more than 90% water (15). Some

TABLE 24.2
Classification of Fruits Based on Climatic Region

Temperate Region	Subtropical Region	Tropical Region
Pome fruits: Apple, Asian pear (*Nashpati*), European pear, quince	Citrus fruits: Orange, grapefruit, tangerine, mandarin, lemon, lime, pomelo	Major tropical fruits: Banana, mango, papaya, pineapple
Stone fruits: Peach, plum, nectarine, apricot, cherry, almond, chestnut, lychee, walnut, pecan	Non-citrus fruits: Kiwifruit, figs, olive, pomegranate, cherimoya, ber (*Zizyphus*), bael (*Aegle marmelos*), date fruit (*Phoenix dactylifera* L.), loquat (*Eribotrya japonica* Lindl.), persimmon (*Diospyros*), phalsa (*Grewia subinaegualis* L.), jamun (*Syzygium cuminii* Skeels)	Minor tropical fruits: Cashew apple, guava, carambola, durian, longan, passion fruit, mangosteen, rambutan, tamarind, sapota, coconut, amla (*Phyllanthus emlica* L.), jackfruit (*Artocarpus heterophyllus*)
Small fruits and berries: Grape, strawberry, raspberry, cranberry, blueberry, blackberry, currant, gooseberry		

Source: Ref. 9.

TABLE 24.3
Proximate Composition (%) of Major Fruits Grown in the World (Based on Total Edible Parts)

Fruit	Water	Energy	Protein	Fat	Carbohydrate	Minerals
Grapes	81.6	67	1.3	1.0	15.7	0.4
Oranges	86.0	49	1.0	0.2	12.2	0.6
Bananas	75.7	85	1.1	0.2	12.6	0.6
Apples	84.4	58	0.2	0.6	14.5	0.3
Mangoes	83.4	59	0.5	0.2	15.4	0.4
Pineapples	85.4	52	0.4	0.2	13.7	0.3
Pears	83.2	61	0.7	0.4	15.3	0.4
Plums	81.1	66	0.5	0.2	17.8	0.4
Peaches	89.1	38	0.6	0.1	9.7	0.5
Papayas	90.7	32	0.5	0.1	8.3	0.4
Apricots	85.3	51	1.0	0.2	12.8	0.7
Avocados	74.4	80.5	1.8	20.6	–	1.2
Strawberries	89.9	37	0.7	0.5	8.4	0.5

Source: Refs. 7, 8 and 10.

TABLE 24.4
Mineral Contents (mg/100 g of Total Edible Parts) of Major Fruits Grown in the World

Fruit	Calcium	Phosphorus	Iron	Magnesium
Grapes	16	12	0.4	13
Oranges	41	20	0.4	11
Bananas	8	26	0.7	33
Apples	7	10	0.3	8
Mangoes	12	12	0.8	–
Pineapples	18	8	0.5	–
Pears	8	11	0.3	7
Plums	18	17	0.5	9
Peaches	9	19	0.5	10
Papayas	20	13	0.4	–
Apricots	17	23	0.5	12
Avocados	14	27	0.7	23
Strawberries	21	21	1.0	12

Source: Refs. 7, 8 and 10.

fruits, like bananas, jackfruit, dates and grapes, are rich in carbohydrates (mainly sugars), whereas others, like nuts, dried apricots and figs, are good sources of proteins and amino acids. A few fruits, like avocados, olives and nuts, are exceptionally rich in oils and fats. In general, most fruits typically contain between 10 to 25% carbohydrates, less than 1% proteins and very small amounts (less than 0.5%) of fat. Some exceptions like dates have about 77.97 to 79.39% total sugars at the *kimri* stage of maturity (16), avocados have a fat content ranging from 15.5 to 23.4% (17) and olives have a fat content ranging from 15 to 30% (18).

Sucrose, glucose and fructose are the main sugars found in most fruits, although sorbitol is present in reasonable amounts in some fruits (cherry, plum and pear). The total sugars are present mainly in the cytoplasm, and range from 0.9% in limes to 16% in fresh figs. Sucrose

Table 24.5
Vitamin Content (Per 100 g of Total Edible Parts) of Major Fruits Grown in the World

Fruit	Vitamin A (IU)	Thiamine (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Ascorbic acid (mg)
Grapes	100	0.05	0.03	0.3	4
Oranges	200	0.10	0.04	0.4	50
Bananas	190	0.05	0.06	0.7	10
Apples	90	0.03	0.02	0.1	4
Mangoes	630	0.05	0.06	0.4	53
Pineapples	15	0.08	0.04	0.2	61
Pears	20	0.02	0.04	0.1	4
Plums	300	0.08	0.03	0.5	5
Peaches	1330	0.02	0.05	1.0	7
Papayas	110	0.03	0.04	0.3	46
Apricots	2700	0.03	0.04	0.6	10
Avocados	–	0.07	0.12	1.9	11
Strawberries	60	0.03	0.07	0.6	59

Source: Refs. 7, 8 and 10.

content could be in traces in cherries to more than 8% in ripe bananas and pineapples. Fructose, being sweeter than sucrose and glucose, has a desirable influence on the taste of fruits. Starch is present as small granules within the cells of immature fruits, which is converted to sugars as the fruit matures and ripens. Cellulose, hemicellulose, pectic substances and lignins are the other polysaccharides, which are the major constituents of cell walls. These compounds are broken down into simpler and more soluble components during fruit ripening (19).

Fruits contain less than 1% proteins, except in fruit nuts, such as almonds, pistachios and walnuts (~20% protein). It is mainly the enzymes that constitute this 1% protein, and these enzymes are responsible for catalyzing various metabolic processes involved in fruit ripening and senescence viz. polygalacturonase hydrolyzes pectic substances, resulting in fruit softening, and polyphenoloxidase catalyzes oxidation of phenolics, leading to browning of cut apples. Ascorbic acid oxidase catalyzes oxidation of ascorbic acid, and thus lowers the nutritional quality of fruits. Chlorophyllase removes the phytol ring from chlorophyll, which leads to loss of green color during ripening of fruits.

Most fresh fruits are low in lipids (0.1 to 0.2%), except avocados, nuts and olives. Lipids are present in the cell membranes, as a part of surface wax and cuticle. The surface wax contributes to fruit appearance, and the cuticle protects against water loss and pathogens. The type of fatty acids present determines the flexibility of cell membranes, with higher saturation resulting in rigid cells.

Organic acids are major intermediary products of metabolism and are further oxidized through the Krebs cycle to provide energy for the maintenance of cell integrity. The acidity of most fresh fruits is due to these organic acids. Certain fruits, like lemons, may contain as

TABLE 24.6
Dietary Fiber Content and Composition of Fruits (g/100 g of Total Edible Parts)

			Uppsala Method[b]							
			Soluble Fiber				Insoluble Fiber			
Sample	Dry wt., FW	AOAC[a] Total fiber, FW	Total Fiber, FW	Neutral Sugars, DW	Uronic Acids, DW	Total, DW	Neutral Sugars, DW	Uronic Acids, DW	Klason Lignin, DW	Total, DW
Apples										
Applesauce, canned	11.5	1.4	1.2	1.0	1.5	2.5	6.5	1.3	0.4	8.2
Macintosh, unpeeled	15.3	2.3	1.8	0.4	1.7	2.1	7.0	1.7	1.3	10.0
Apricots										
Dried	69.4	7.7	7.1	0.9	2.1	3.0	5.3	1.3	0.7	7.3
Fresh, unpeeled	11.3	1.6	1.5	0.7	3.5	4.2	6.4	1.3	1.0	8.7
Berries										
Blackberry, frozen	17.9	7.0	6.5	0.9	1.9	2.8	13.5	3.3	16.5	33.4
Cranberry sauce, frozen	39.4	1.4	1.1	0.2	0.5	0.7	1.2	0.1	0.7	2.0
Raspberry, red, fresh	12.3	4.4	4.1	1.1	2.4	3.5	9.9	1.7	18.7	30.3
Strawberry, frozen	10.7	1.9	1.7	1.2	2.6	3.8	5.7	1.4	5.1	12.2
Grapes										
Black, seeded	21.5	1.0	0.9	0.2	0.1	0.3	1.3	0.9	1.5	3.7
Red, seedless	21.3	1.3	1.0	0.1	0.2	0.3	1.5	0.8	2.1	4.4
Oranges										
Mandarin, canned	16.1	0.3	0.2	0.2	0.1	0.3	0.6	0.4	0.2	1.2
Temple, fresh	14.7	1.7	1.5	0.7	1.5	2.2	4.8	2.8	0.5	8.1
Valencia, fresh	13.1	1.6	1.5	0.9	1.1	2.0	4.5	3.7	0.8	9.0
Peaches										
Canned in fruit juice	15.8	1.5	1.4	1.1	2.0	3.1	3.9	1.1	0.9	5.9
Fresh, unpeeled	13.3	1.9	1.7	1.5	3.0	4.5	6.5	1.0	1.1	8.6
Fresh, peeled	12.4	1.6	1.3	1.1	2.7	3.8	4.5	0.9	1.0	6.4
Plums										
Canned in heavy Syrup	27.0	2.1	1.8	1.0	1.5	2.5	2.9	0.5	0.8	4.2
Prunes	65.8	8.0	7.3	1.3	2.6	3.9	4.3	1.0	1.8	7.1
Prunes, fresh	16.6	2.2	1.9	0.7	2.1	2.8	4.3	1.4	2.6	8.3

[a]Mean of four measurements, [b]Mean of two measurements, FW = Fresh weight, DW = Dry weight.

Source: Ref. 14.

much as 4.17 to 4.64% acidity, citric acid being the predominant one (20). Malic and citric acids are the most abundant in most fruits, except the tartaric acid in grapes and the quinic acid in kiwi fruits. The titratable acidity of fruits plays an important role in determining the maturity of most of these fruits. Acid content of fruits usually decreases during the ripening process as these are used in respiration or converted to sugars (21).

Pigments are the chemicals responsible for the characteristic color of skin and the flesh of fruits. In general, peel tissues of white and yellow flesh nectarines and peaches, and yellow and red plums, contain higher levels of phenols; anthocyanins and flavonols are almost exclusively located in this tissue (22). Many changes occur in these pigments during maturation and ripening of fruits. Fruit color is, therefore, used as an index of maturity and stage of ripeness in many fruits. The color of fruits also changes during the period of post-harvest in cold storage. Changes in the color and anthocyanin content have been investigated during the development and ripening of strawberries and pomegranates (23, 24). An increase in phenylalanine ammonia lyase (PAL) activity correlates with anthocyanin production in strawberries. The anthocyanins increase in pomegranate and low bush blueberry fruits during cold storage (24, 25). PAL activity and anthocyanin concentration in the skin of harvested apples increases after irradiation with UV and white light (26). The anthocyanin content in apples is adversely affected by higher carbon dioxide levels (73%) during cold storage (27), but a moderate carbon dioxide atmosphere (10 kPa) prolongs the storage life and maintains the quality of pomegranate, including adequate red color intensity of the arils (28).

β-Carotene is a precursor of vitamin A, and not only imparts color to fruits, but is also important in terms of

nutritional quality. Most of the carotenoid pigments are quite stable and remain intact in fruit tissues, even after senescence, processing and storage. The elucidation of biosynthesis pathway in plants has opened up new possibilities to manipulate/engineer fruit cultivars rich in provitamin A carotenoids (29). A combination of biochemical and genetic approaches has led to the isolation of a key gene, lycopene-epsilon-cyclase, responsible for the relative proportions of β-carotene and lycopene in tomato fruits (30). Among the phenolics, anthocyanins occur as glycosides and are water soluble, unstable during processing as well as to changes in pH. These glycosides are readily hydrolyzed by enzymes to free anthocyanins, which may be further oxidized by phenoloxidases to yield brown-colored oxidation products. The green color imparted to fruits by chlorophyll is influenced by pH changes, oxidation and the action of the enzyme chlorophyllase.

The plant polyphenols occur naturally in plant foods, like teas, fruits, juices and grape seeds. Grape seed tannins are known to exhibit remarkable antioxidant effects (31). The total phenolic compounds are higher in immature fruits than in the mature fruits, and usually range between 0.1 and 2% on a fresh weight basis. These include chlorogenic acid, catechin, epicatechin, leucoanthocyanidins, flavonols, cinnamic acid derivatives and simple phenols. Chlorogenic acid occurs widely in fruits and is responsible for the enzymatic browning of cut or damaged fruit tissues. The phenolic content of red grapes is about 20–50 times higher than of white grapes (32). Apart from nutritional significance, these polyphenols are associated with color in fruits and vegetables and contribute to flavor sensations like astringency and bitterness (33). Anthocyanins are responsible for the bright red and purple colors of fruits and flowers (34). The astringency is directly linked to the phenolic content and decreases during fruit ripening, as the phenolics are converted from soluble to insoluble, nonastringent form (35). These phenolic compounds are plant secondary metabolites and play an important role in plant-derived food quality, as they affect various quality attributes, such as appearance, flavor and health-promoting properties (36).

Fresh as well as processed fruits are important sources of vitamins in the human diet. Apricot, peach, papaya, cherry, orange, cantaloupe and watermelon are good sources of β-carotene (provitamin A), whereas Indian gooseberry, strawberry, orange, grapefruit, kiwi, papaya and cantaloupe are important sources of ascorbic acid. Peach, banana, orange, apricot, avocado, grapefruit and apple also contain appreciable amounts of certain B-complex vitamins. The levels of some of these vitamins may be reduced during processing into various products, as some of these are sensitive to heat, oxygen, light, pH and certain trace minerals. Banana, peach, orange and apple are rich in potassium, whereas orange, banana, peach, raisin and fig are rich in phosphorus. Citrus fruits, like tangerine, grapefruit and orange, contain good amounts of calcium, and strawberry, banana, apple and orange are rich in iron. On a fresh weight basis, the dietary fiber content of fruits ranges from 0.5 to 1.5%. The dietary fiber comprises cellulose, hemicellulose, lignin and pectin, which are derived from the cell wall and skin of fruits (9).

Chemical composition of fruits is affected by a number of preharvest and post-harvest factors. Among the preharvest factors, genetics (like cultivars and rootstocks) are known to influence raw fruit composition, durability and response to processing. As an example, several reports are available on the genetic improvements of grape vines (37, 38). Similarly, climatic factors, like light intensity, significantly affect the concentration of certain vitamins, and temperature influences mineral uptake and metabolism due to its effect on transpiration rate. Cultural practices like fertilizer application affect the mineral content of fruits while pruning/thinning influences nutritional composition by changing fruit density and size (39).

V. STRUCTURAL PROPERTIES OF FRUITS

Fruits are consumed not only for their nutritional value, but also for pleasure. The type of sensations one experiences as the fruit deforms and fractures during the initial stage of chewing determines our acceptance or rejection. Over the years, food processors have developed many methods to evaluate structural properties (such as modulus, fracture stress and strain) and to relate these parameters to the textural quality of fruits. Fruit firmness is another important mechanical property in determining the quality and ripeness behavior of fruits. A more fundamental understanding of the relationship between structural properties and microstructure of fruits is a prerequisite for any improvements in the textural qualities of these food products. Single edge notch bend tests were employed to relate mechanical properties to food texture (40). These workers reported good agreement between the measured (3.4 ± 0.3 MPa) and calculated (3.0) Young's modulus (E values) for apples. The fracture toughness, K_{Ic}, fracture energy, G_c, and yield stress, σ_y, values for apples were reported to be 10.1 ± 1.4 kPa $m^{1/2}$, 39.6 ± 10.5 Jm^{-2} and 0.3 ± 0.1 MPa, respectively. The firmness of pear fruit is a function of the mechanical properties of cell wall, cell properties like turgor and bonding between neighboring cells (41). A sigmoidal relationship between fruit firmness and tensile strength of tissue soaked in isotonic solutions is observed. The major mechanism of tissue failure involves cell wall failure and cell fracture at high firmness and intercellular debonding at low firmness. The stress-hardening of the cell wall in response to an increase in cell turgor increases the cell wall elastic modulus. Fruit firmness decreases from 100 to 20 N and from 60 to 25 N during the ripening of European and Asian pears, respectively. Tissue and cell

extension at maximum force declines as fruit softens. During fruit ripening, the hydrolysis of pectins present in the middle lamella leads to tissue breakdown by cell-to-cell debonding rather than the cell wall rupture.

As the mechanical properties are a function of ripening, these parameters can be used to discriminate between batches of certain types of fruits based on their degree of ripeness. Mechanical parameters can be used in assessing the optimum stage of ripening for harvest of Burlat sweet cherries using a penetration test, a compression test between two plates, and a compression-relaxation test (42). Based on compression-relaxation data, the calculated "apparent secant modulus" seems to be the most useful single mechanical parameter to clearly classify batches and to distinguish different varieties. Data in Table 24.7 show that the maximum penetration force ranges from 6.2 N (for very green cherries) to 1.8 N (for purple cherries). The hardness decreases as the cherries mature, but a sharp reduction in force occurs between stages red and very red, which are used as optimum stages of harvesting.

Firmness is a critical parameter of textural quality of apples. Information on the mechanical properties of apple flesh and intact apples is required to develop suitable methods for the sorting of whole apples according to firmness. Apple firmness is affected by many factors such as pre-harvest factors (genetics, fertilizers and other nutrients, breeding for fruit size, use of plant regulators); and post-harvest factors (maturity at harvest, pre-storage treatment with calcium, heat, plant regulators, storage atmosphere) (43). Calcium chloride treatment (2%) is effective in firming the apples (44). After six months of storage, treated samples had textural characteristics values equal to or better than the control treated with water only. The involvement of calcium ions in the maintenance of apple tissue during storage is suggested (45). The infiltration with calcium ions increases the tensile strength of tissue from air-stored apples to 85% of that of untreated controlled atmosphere-stored fruits. Both the movement of calcium ions from the middle lamella and loss of its binding sites occur during fruit softening, thus affecting the fruit texture. The softening of apricots during canning is accelerated when chelators, such as organic acid anions, remove structural calcium from the cell wall once the cell membrane is lyzed during heat processing (46). Infiltration of these apricot fruits, susceptible to softening, with calcium chloride before processing gives firmed canned products.

The stone cells also influence the firmness of Asian, European and Chinese pears (47). Fruit firmness increases with the increase in weight of stone cells, but soluble solid content is not affected. Weight of stone cells (per g of fruit) is negatively correlated with fruit size. Different cultivars show differences in the weight and size of stone cells. The degree of ripeness, specimen orientation and location within the apple tissue affect the failure stress, strain and energy and apparent modulus of elasticity (Young's modulus). Failure stress is the highest in vertical direction and lowest in tangential direction (48). Young's modulus is significantly ($p < 0.05$) higher in radial samples than in tangential and vertical samples. The bottom sections give the largest values for failure stress and Young's modulus, and the top sections give the lowest values for Young's modulus. A non-destructive method has been developed to determine fruit elasticity using the coefficient of elasticity (49). The coefficient of elasticity is the ratio of compressive stress to deformation. While using different types of penetrometers, coefficient of elasticity shows a close correlation with compressive stress and an acceptable correlation with rupture stress for apples. The coefficient of elasticity can be used to characterize stress-strain behavior of fruits, as it is a good indicator of firmness for both scientific and practical evaluation.

TABLE 24.7
Mechanical Parameters for Burlat Sweet Cherry with Skin (Mean Values)

Parameter	Very Green	Green	Red	Very Red	Purple
Penetration Test					
Max. penetration force, N	6.2 (1.1)	4.3 (0.6)	4.2 (0.6)	2.3 (0.4)	1.8 (0.2)
Compression test					
Force to bioyield, N	51.0 (10.6)	30.0 (5.3)	19.3 (1.7)	14.1 (1.9)	13.5 (1.2)
Terminal slope, N cm^{-1}	1.5 (0.4)	0.8 (0.2)	0.4 (0.1)	0.3 (0.0)	0.2 (0.0)
Compression-Relaxation Test					
Maximum force, N	6.1 (0.7)	4.7 (0.4)	3.5 (0.5)	2.0 (0.3)	1.4 (0.3)
Residual force, N	3.7 (0.5)	2.8 (0.3)	2.1 (0.3)	1.2 (0.2)	0.9 (0.2)
Residual force/Maximum force	0.61	0.60	0.60	0.60	0.64
Relaxed force, N	2.4 (0.2)	1.9 (0.1)	1.4 (0.2)	0.8 (0.1)	0.6 (0.1)
Apparent secant modulus, N	76 (8.6)	59 (5.0)	43 (5.8)	25 (3.6)	18 (3.6)

Mean values of 20 cherries, and, in parentheses, 95% confidence interval.

Source: Ref. 42.

Size of individual fruits among their class is an important parameter that determines the textural attributes and consumer acceptability. Among the three size classes, ≤ 8 mm, 9–10 mm and 11–12 mm of Maine wild blueberries, consumers prefer the largest size (50). The compression test using an Instron testing machine measuring force and deformation to the point of rupture of individual blueberries relates to consumer acceptance. The consumers prefer larger 11–12 mm berries as these show lower stress and strain values, i.e., easier to chew. The compression tests reveal significant differences among all three size classes with respect to apparent modulus. A linear relationship is observed between the sensory texture and the elastic modulus (Tables 24.8 and 24.9).

The curve generated by the texturometer by plotting force as a function of time is known as a texture profile. This curve when analyzed in conjunction with the sensory texture parameters defined by Szczesniak (51), such as fracturability, hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness, is known as texture profile analysis technique and still used for food samples. Mechanical parameters of food samples measured by a texturometer correlate well with the sensory scores obtained by using a trained texture profile panel. This correlation indicates that the food texturometers have the capability to measure certain characteristics in a similar manner to those perceived by the human mouth. This texture profile analysis technique has been employed in a number of fruits such as pears (52), peaches (53) and apples (54). A typical texture profile curve is presented in Figure 24.1. Five measured and two calculated parameters, originally suggested by Szczesnaik (55) and Friedman et al. (56), modified by Bourne (57) are described as follows:

1. *Fracturability* (earlier called *brittleness*) is defined as the force at the first significant break in the first positive bite area (PA1).
2. *Hardness* is defined as the peak force (PP1) during the first compression cycle.
3. *Cohesiveness* is defined as the ratio of the positive force area during the second compression cycle to the positive force area during the first compression cycle, or PA2/PA1.
4. *Adhesiveness* is defined as the negative force area for the first bite (NA1), representing the work required to pull the plunger away from the food sample.
5. *Springiness* (originally called *elasticity*) is defined as the height to which the food recovers during the time that elapses between the end of the first bite and the start of the second bite.
6. *Gumminess* is defined as the product of hardness and cohesiveness.
7. *Chewiness* is defined as the product of gumminess and springiness.

Texture profile analysis (TPA) is a useful technique to evaluate such structural properties of fruits. For more information on TPA, the reader is referred to the book by Rao and Rizvi (58).

TABLE 24.8
Means Separation of Mechanical Tests for Engineering Stress and Strain and Modulus of Elasticity by Berry Size

Berry Size	Mechanical Test*		
	Peak Force, mN	Deformation, mm	Modulus of Elasticity, mN/mm²
8 mm	0.323a	0.00481a	25.72a
9–10 mm	0.307a	0.00455a	17.70a
11–12 mm	0.304a	0.00395b	14.49b

*Means with different letters are significantly different ($p \leq 0.05$).

Source: Ref. 50.

TABLE 24.9
Regression Model Results of Panelist and Berry Size with Respect to Sensory and Mechanical Attributes

Attribute	Panelist	Berry Size	Model R-Square
Sensory Attribute			
Flavor	Y	Y	0.597
Texture	Y	Y	0.559
Overall	Y	Y	0.635
Mechanical attribute			
Peak force	Y	N	0.539
Deformation at peak force	Y	Y	0.561
Modulus of elasticity	Y	Y	0.685

Y indicates significance at the 0.05 level.

Source: Ref. 50.

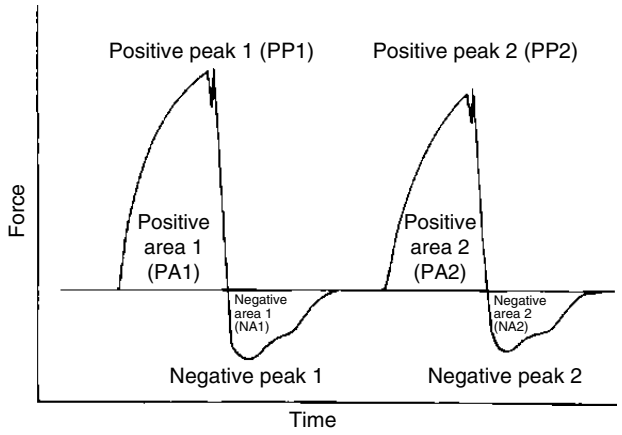

FIGURE 24.1 A typical texture profile curve with a two-bite compression cycle. (*Source*: Ref. 58.)

Fresh-cut fruit slices have become quite popular among consumers in the U.S. Apart from type of cultivars and storage conditions, fruit size and firmness are the other two important parameters that affect the quality of fresh-cut fruits. Among the factors affecting the quality of fresh-cut pears, fruit ripeness based on flesh firmness of 44 to 58 N is optimal for fresh-cut pear slice processing (59). The pear slices obtained from smaller size fruits (122–135 g) undergo a greater cut surface discoloration and deteriorate faster than slices obtained from larger fruits (152 g). Similarly, the optimal ripeness for preparing fresh-cut peach slices is the ripe stage having greater than 13–27 N flesh firmness (60). However, the optimal ripeness for preparing fresh-cut nectarine slices is the partially ripe (greater than 27–49 N) or ripe (greater than 13–27 N flesh firmness) stages. The size of fruits also determines their acceptance among consumer populations. Hampson et al. (61) reported that age groups differed slightly in apple fruit size preference. Fruit diameter considered ideal for dessert use ranged from 7.4 to 7.6 cm among various age groups.

Volatile compounds extracted from hyuganatsu fruits (*Citrus tamurana* Hort. ex Tanaka) vary with a fruit size of 100 to 350 g (62). Sesquiterpene contents are the highest in larger fruits, trans-beta-farnesene is lower in middle range fruits and l-carvone tends to increase with fruit size. As fruit size has a great influence on market price, processing quality and consumer acceptance, fruit breeders aim to produce larger fruits with better quality through the application of various plant growth regulators (63). The application of thidiazurin (TDZ) increases the fresh fruit weight and yield of kiwifruit nearly 13 and 22% with 2 and 10 ppm TDZ, respectively (64). A higher dose (10 ppm) gives slightly rounder fruits than the control and during ripening, no significant change in soluble solids, flesh firmness, glucose, fructose, sucrose and starch is observed. The application of CPPU [N-(2-chloro-4-pyridyl)-N′-phenyl urea] at concentration ranging from 4 to 8 mg/l increases maximum fruit size without causing fruit asymmetry in apples (65).

A number of chemical thinning treatments for apples are known to influence their ripeness and processing quality (66). Carbaryl alone, 2-1-naphthylacetic acid (NAA) and ethephon improve fruit size, but the benzyladenine/gibberellic acid is ineffective as a thinner and fruit size is not altered at harvest. Although ethephon shows promise as a fruitlet thinner and a promoter of ripeness in Paula red cv, these beneficial effects must be balanced against possible adverse effects on storage life of this fruit. The application of NAA (30 ppm) plus ethephon (500 ppm) is also beneficial in advancing fruit maturity, enhancing fruit color, increasing fruit size and providing better sensory quality with higher soluble solids and low acidity in pear (*Pyrus communis* L.) cv LeConte (67). As the source-sink balance is critical in the papaya fruit set, fruit development and sugar accumulation, defoliation and fruit removal can be employed to alter fruit size and quality (68). Fruit thinning increases the new fruit set and ripe fruit total soluble solids in papaya. Larger fruit size, faster fruit development and higher sugar contents are observed in immature fruits when old fruits are removed.

Porosity is another important physical property that plays a significant role during fruit processing operations, such as impregnation of fruit chunks in sugar syrups or their own juices. The porosity behavior of a few fruits (apple, strawberry, mango, peach and kiwi fruit) during vacuum impregnation treatments to determine deformation and impregnation levels through the hydrodynamic mechanism (HDM) and the deformation-relaxation phenomena (DRP) is important (69). Porosity and other physical characteristics of these fruits are presented in Table 24.10. The ratio of effective porosity (ε_e) to fruit porosity (ε) obtained from the density data is lower than 1 in almost all the fruits. This indicates that only a fraction of fruit pores is available to HDM action. DRP affects the volume

TABLE 24.10
Some Physical Characteristics of Fruits

Characteristic	Apple, cv Golden	Mango, cv Tommy Atkins	Strawberry, cv Chandler	Kiwi Fruit, cv Hayward	Peach, cv Miraflores
Fruit density[1]	0.787±0.014	1.022±0.005	0.984±0.009	1.051±0.006	1.038±0.005
Solid/liquid density[1]	1.0548±0.0008	1.13±0.02	1.05±0.009	1.076±0.006	1.0654±0.001
Fruit porosity	25.4±1.4	9.9±1.3	6.3±1.6	2.3±0.8	2.6±0.5
Water activity	0.985±0.00	0.9895±0.0007	0.9923±0.0015	0.992±0.000	0.9920±0.0010
Moisture content[2]	0.835±0.016	0.7868±0.0007	0.911±0.007	0.815±0.011	0.820±0.002
Ripeness index[3]	44.1±1.9	43.69±0.16	48.0±1.3	10.0±0.3	31±2
Soluble solids[4]	15.3±0.4	17.8±0.5	7.2±0.6	14.28±0.13	15±2
pH	3.833±0.012	4.29±0.00	3.36±0.02	3.527±0.008	3.91±0.14
Fruit acidity[5]	0.346±0.009	0.408±0.009	0.149±0.010	1.43±0.05	0.49±0.04

[1] (Kg/m^3), [2](Kg water/Kg of sample), [3](Soluble solids/fruit acidity), [4](°Brix), [5](Kg prevailing acid/100 Kg of sample).

Source: Ref. 69.

fraction of impregnated liquid in all fruits. Post-harvest fruit density can also be used in sorting fruits according to taste, because density is an indicator of dry matter, starch and sugars in unripe and in kiwifruit ripened during storage (70). The soluble solids (being mainly sugars) correlate to fruit density, and can be used for sorting kiwi fruit according to taste. The floating or sinking of this fruit in saline solutions could be used as a rapid method for this purpose.

VI. MORPHOMETRIC PROPERTIES

Fruits come in various shapes, ranging from round to oval, oblique, oblong, oblate, obovate, elliptical, truncate, unequal, ribbed, regular, irregular, cylindrical, conical or pyramidal (71). The morphology of fruits can be evaluated by considering either bulk or individual units, but it is important to have information on the accurate estimate of shape, size, volume, specific gravity, surface area and other physical characteristics. Specific charts can be prepared for different fruits and using these charts, the shape of the fruit can be described either by a number on that chart or by the above-mentioned descriptive terms. The shape of fruits has significance in harvesting, handling, grading, processing and packaging operations. Most of the harvested fruits are graded roughly according to size and appearance. The size and shape of fruits are variable and depend upon the cultivars, climate and agricultural practices followed during growth of crops. Figure 24.2 shows an example of a standard chart describing shapes of fruits and vegetables (72).

VII. MICROSCOPIC PROPERTIES

The study of physical properties of fruits requires some knowledge of their structure as well as their relationship to the chemical composition. As an example, citrus fruit consists of three distinctly different parts: the epicarp consists of the colored portion of the peel, called flavedo, and contains carotenoid pigments as well as oil glands (73). These oil glands contain essential oils characteristic of each citrus cultivar. Immediately under the epicarp is the mesocarp or albedo, a thick, white, spongy layer, rich in pectic substances and hemicelluloses. The combined albedo and flavedo are called the pericarp, but is commonly known as peel or rind. Next to the albedo is the edible portion of citrus fruits or the mesocarp. The mesocarp consists of many segments and, inside each segment, is located juice sacks or vesicles (Figure 24.3).

Many chemical components are distributed among various tissues of the citrus fruit. For example, flavanone glycosides are present in higher concentrations in the albedo than the juice sacks or the flavedo (74), and the bitter principles (limonin) are the highest in seeds and membranes (75). In contrast, grape berries always occur in clusters. A cluster consists of peduncle, capstem, rachis and berries. The grape bunch shape could be cylindrical, conical, pyramidal or globular. The grape berry consists of skin, pulp and seed. The seeds may vary from 0 to 4 per berry and are rich in tannins (5–8%) and oil (10–20%). The skin constitutes 5–12% of the mature grape berry and contains most of the aroma, coloring and flavoring components. The juice accounts for 80–90% of the grape berries.

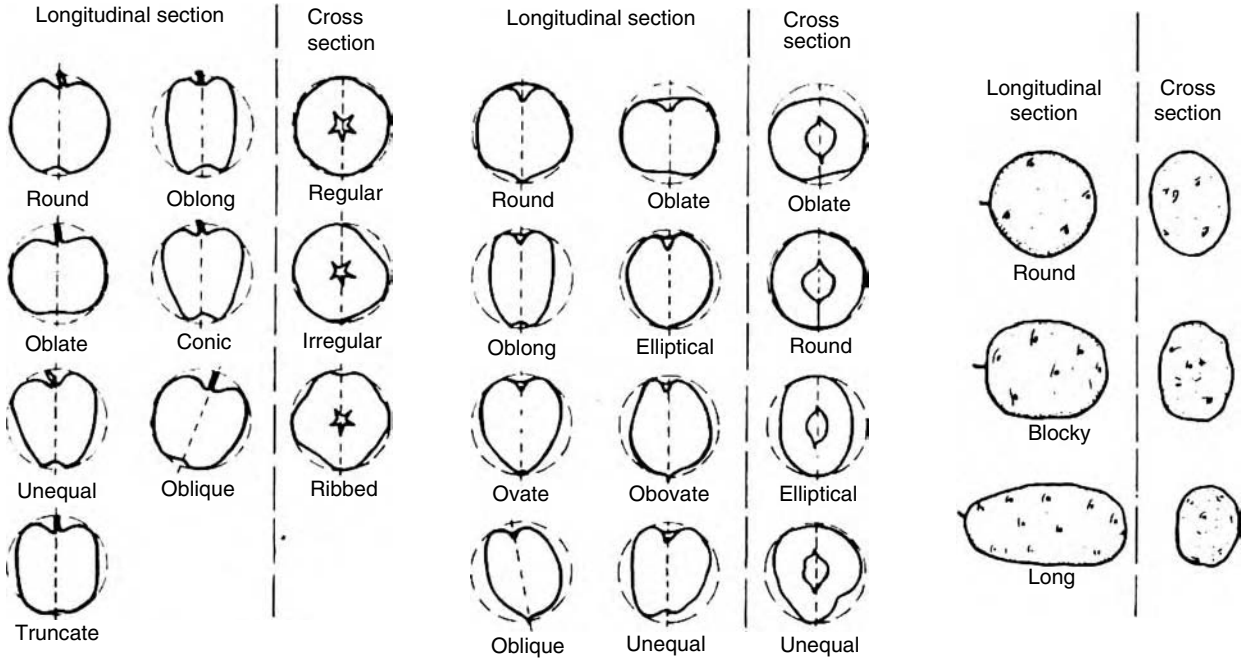

FIGURE 24.2 Example of charted standard for describing shape of fruits and vegetables. From left to right: apples, peaches, potatoes. (From Ref. 72.)

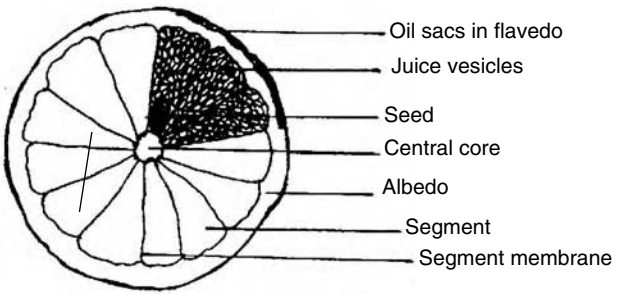

FIGURE 24.3 Cross section of a citrus fruit. (*Source:* Ref. 10.)

In physiological terms, a fruit can be defined as a structural entity arising from the development of the tissues that support the ovule. The basic function of fruit is the dispersal of seeds for the propagation of the species. Unripe fruits generally have rigid, well-defined structures, whereas ripe fruits have soft and diffused cell walls. An early model of the plant primary cell wall, in which cellulose fibrils are coated with hemicellulose and embedded in a matrix composed of pectin and protein (Figure 24.4), has been proposed by Keegstra et al. (76). This model has been further developed over the years and it provides an adequate basis to investigate fruit ripening.

The plant cell consists of a cytoplasm surrounded by a cell wall. Each cell is attached to adjacent cells by a pectin-rich middle lamella. The cytoplasms of these cells are interconnected with each other through plasmadesmata, which, in ripe fruits, provide a degree of cohesion. As long as the cell continues to register growth, its cell walls remain thinner. At this stage of fruit development, it is known as the primary cell wall and consists of roughly 90% polysaccharides and 10% protein. The primary cell wall components can be further classified into pectic polysaccharides (34%), hemicellulose (24%), cellulose (23%) and hydroxy proline-rich glycoprotein (19%). The ratio of these constituents varies in cell walls of different plants (77). The way in which various components of the cell wall are linked with each other is not exactly known yet. It is believed that the various domains of pectic substances are covalently linked together to form complex molecules. It is suggested that the xyloglucan, which is the major hemicellulose in primary cell walls, is strongly held to the surface of cellulose fibers through hydrogen bonds. The glucuronoarabinoxylan may bind to themselves as well as to the cellulose in cell walls (78). Such interactions would help in the formation of cross-links of polymers in primary cell walls.

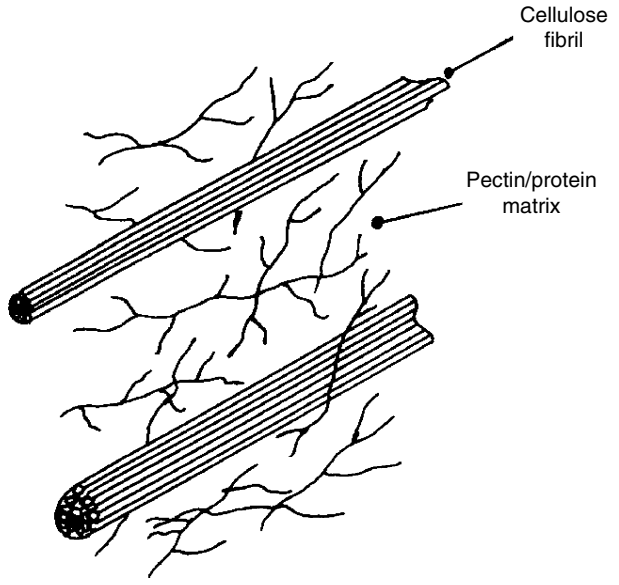

FIGURE 24.4 Idealized cell wall model. (*Source*: Ref. 76.)

The hydroxyproline-rich glycoprotein has regions of helical conformation, which are likely to give a rod-like molecule to serve structural function in plant cells (79). It has also been proposed that glycoproteins could be held in the cell walls by phenolic cross-links, which may be glycoprotein-protein or glycoprotein-polysaccharide in nature (80). A number of phenolic materials, like ferulic acid, p-coumaric acid and other unidentified phenols, have been isolated from plant cell walls. Ferulic acid alone accounts for 0.5% of the cell wall and is suggested to be linked to the nonreducing termini of arabinose and/or galactose-containing regions of pectic substances. Such residues can cross-link to form diferuloyl bridges, which would reduce extensibility of plant cell walls and could also play a role in resistance to fungal pathogens (81).

In addition, a number of cell wall models have been proposed to account for some of the cell wall properties viz. their strength in withstanding turgor pressure; their ability to grow without loss of strength; and their behavior under chemical and enzymatic attack. A modified updated cell wall model is presented in Figure 24.5 (82). The major features of this model indicate that several layers of pectin form an outer network around the cell wall constituting the middle lamella. The pectic molecules are interconnected through covalent bonds as well as calcium bridges. Some regions of pectic molecules are strongly hydrogen bonded with xyloglucans, but the pectins also have some covalently linked glucose and xylose sugars. Under these pectin layers are several layers of cellulose fibers, which are noncovalently associated with xyloglucans. Xyloglucan-associated cellulose is cross-linked through hydroxyproline-rich glycoproteins in a noncovalent manner involving isodityrosine bridges (83).

Cell number, volume and weight that determine fruit weight are hormonally controlled during fruit growth. Most of the fruits develop by cell enlargement accompanied by cell division, which may continue briefly (e.g., in

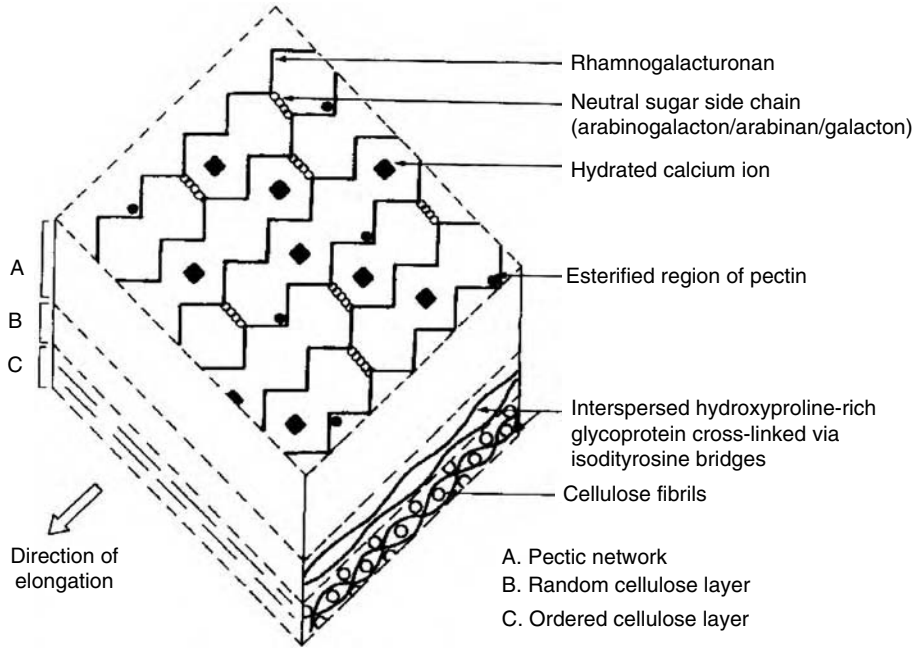

FIGURE 24.5 Modified model of primary cell wall. (*Source*: Ref. 82.)

peaches and other stone fruits) or until the end of fruit maturity (e.g., citrus fruits). Usually, all parts of the fruits do not grow at the same rate. In the case of apples, 25% of the total volume of fruit is contributed by the expansion of intercellular spaces. In the case of kiwi, the cells may enlarge more than 10–15 times. Some fruits (e.g., avocado) may increase 300,000 times in size compared with their flowers. Large fruits generally require additional anatomical features for nutrition or support or both for the developing seeds. Extra phloem in fruit vascular bundles and the increased amount of vascular tissues in the fruit wall and septa supply these nutrients to the developing seeds in large fleshy fruits. Collenchyma and sclerenchyma are common supportive and protective tissues, and the long axes of these cells are oriented crosswise to provide a mechanically strong structure to the fruits.

As large-sized fruits bring higher prices for the growers, the fruit breeders direct their research programs to achieve this objective (84). After the cessation of cell division, peach fruit growth depends mainly on cell enlargement. Mesocarp cells do not grow uniformly during the rapid fruit growth and maturation stage, and the length of radial cells near the stone is greater than that of the outer areas of the peach fruit. Flesh cell division continues 4–5 weeks after blooming, and increases in size only thereafter. In the case of the wild peach Ohatsumomo, cells divide more slowly than the commercial cultivars. This wild peach has the fewest and the smallest cells, thereby, giving a very small fruit. Similar observations by Cano-Medrano and Darnell (85) on blueberry fruits indicated that the differences in final fruit size between pollinated and GA-induced parthenocarpic blueberry fruits were due to differences in cell enlargement rather than increase in cell numbers. Similarly, the size of apple fruits of Jonagold and Fuji cultivars is observed to be a function of cell numbers rather than cell diameter (86). However, in the case of early maturing American Summer Pearmain, Tsugaru and Senshu cultivars, fruit size correlates positively with cell diameter.

The texture of fruits is also affected by the changes in cell wall components that may take place during chilling or heat processing treatments. Various cytochemical and structural changes during cold storage affect the quality of peaches (87). Mealiness in fruits develops due to chilling injury as a result of separation of mesocarp paremchyma cells resulting in increased intercellular spaces and accumulation of pectic substances in the intercellular matrix. No structural change is observed in the cellulosic component of cell walls of peach fruit. In peaches having a leathery texture, mesocarp parenchyma cells collapse, the intercellular spaces increase and the pectin-positive staining in the intercellular matrix is significantly higher. As the internal breakdown progresses, dissolution of middle lamella, cell separation, irregular thickening of the primary cell wall and plasmolysis of the mesocarp parenchyma cells are observed. Addition to cell wall degradation leading to fruit softening, starch hydrolysis (possibly producing cell turgor changes) might also be involved in the softening of kiwifruit during storage at

0°C (88). As the softening of kiwi fruit progresses, more uronic acids and neutral sugars (galactose, arabinose and rhamnose) usually associated with pectic substances are detected.

Cell structure of fruits is related to their fracture behavior. Cell size and intercellular spaces influence the values of fracture toughness and fracture energy (40). In addition to cell size, cell wall chemistry also influences the mechanical properties of fruits and, hence, texture. Currently, there is a lot of interest in the mechanical properties of plant cell walls. Fruits derive their textural qualities from the strength and stiffness of these cell walls, bonding between the adjacent walls and the turgor pressure. Deformation of plant cells by a fine probe provides a measure of cell wall stiffness that is responsible for many of the mechanical properties of fruits (89).

Fruits are generally harvested at the fully mature stage, when the development and growth in size have ceased. In fully mature fruits, many physical as well as chemical changes take place that convert these fruits to a more palatable form. Astringency decreases due to reduced tannin content. Specific flavors are developed due to increased sweetness and lower acid content. The softening caused by breakdown of the cell wall structure in the pulp is often accompanied by a change in coloration. Chlorophyll in the chloroplasts of the outermost cells decreases while the carotenoids and anthocyanins develop in the fully mature fruits. Most of these changes are brought about by the coordinated action of a group of hydrolytic enzymes on the cell wall constituents. Apart from protein (mainly enzymes) synthesis, starch and pectic substances hydrolysis are the major biochemical changes occurring during fruit ripening. During normal ripening, polygalacturonase (PG) activity increases while the pectin esterase (PE) activity decreases. Any imbalance in their activity could lead to inadequate hydrolysis of pectic polymers, with increased accumulation of higher- molecular-weight pectin with low levels of esterification. The woolliness defect in nectarines is possibly due to the accumulation of these pectic polymers (90). Changes in cellulase (endo-β-1, 4-D-glucanase) activity are also associated with the modifications in cell wall structures during fruit ripening (91).

For further detailed description of the physical properties of fruits and food materials, the reader is referred to two excellent books in this area by Mohsenin (72) and Rao and Rizvi (58).

VIII. PROCESSING AND FUNCTIONAL PROPERTIES

Processing and functional properties of fresh fruits are of paramount importance because these affect the acceptability of fresh or processed fruit products by consumers. A set of quality characteristics important for consumer acceptance is associated with each fresh fruit or its processed products. A list of characteristics identified as important for consumer acceptance of fruits and vegetables is reviewed by Kader (92). Unfortunately, no single parameter/property can establish consumer acceptance forever since consumer tastes and preferences change with time and are also influenced by cultural factors. Careful evaluation of fruits in terms of consumer acceptance criteria is essential to identify target markets. As an example, German consumers prefer a fuller-flavored grapefruit, whereas French consumers prefer the one that is sweeter (93). Similarly, regional preferences exist for apples in the United States; New Englanders look for a tart taste, whereas Southerners prefer a sweeter one (94). Most packers and wholesale and retail dealers buy fruits on the basis of grades and standards established by the concerned agencies in each country.

IX. MATURITY INDICES

Maturity at harvest is one of the most important factors affecting the rate of change of quality during post-harvest handling and processing into finished products. Various biochemical and physiological changes occurring at the final stages of fruit development result in making these fruits palatable and acceptable to consumers. Maturity indices for fruits can be determined by estimating duration of development, measuring size, weight or density; physical attributes like color, firmness, moisture or total soluble solids content; chemical attributes like starch, sugar, acidity, flavor and aroma constituents; or the morphological characteristics (95, 96).

A. Size, Mass and Density

Size, mass and density can easily be employed as maturity indices. Each fruit becomes larger as it matures on the plant, although other production factors like moisture and soil fertility also affect the size of fruits. As an example, fruit size is considered the most effective method of segregating strawberries into different maturity classes (97). Similarly, specific gravity is used as an index of maturity in pineapples (98).

B. Color

Color is another useful measure of maturity in many fruits. Uniformity and intensity of color is important for appearance qualities and processing properties. Chlorophyll tends to disappear as the fruit matures on the plant as well as during handling and storage. On the other hand, anthocyanins are synthesized in light while fruits are attached to the plant, but are not affected greatly during post-harvest handling and storage. The pigments of grapes, anthocyanins (red, blue, purple and black), are modified by attachment of a glucose molecule. The basic part of grape

pigments is made up of the five anthocyanins, namely, cyanidin, peonidin, delphinidin, petunidin and malvidin. The red pigments in grapes (anthocyanins) reach their full potential at complete maturity (99). Total anthocyanin content shows the greatest potential as a maturity index for raspberries (100). For the color measurement of horticultural crops, a number of techniques, like visual color matching charts, guides and dictionaries to match, light reflectance meter, light transmission meter, delayed light emission measurement and determination of various pigments (e.g., chlorophyll, carotene, lycopene, xanthophylls, anthocyanins), are now available. Standard color chips are now available for assessing the maturity of peaches (101). Colorimeters can also be used for this purpose (102). The technique of delayed light emission has the advantage of not being affected by orientation of multicolored fruits like papaya (103), cantaloupes (104) and peaches (105).

C. Firmness

Firmness or softness of fruit is an important processing property that is affected by the stage of maturity and storage. Firmness is also an important textural quality attribute and is usually combined with other attributes, such as color and flavor, for use as an index of both maturity and processing quality of fruits. Use of firmness and total soluble solids as an index of maturity of kiwifruit, have been recommended by Crisosto et al. (106). Firmness can be measured using penetration force (Magness-Taylor pressure tester, Effegi penetrometer, Instron universal testing machine); fibrousness and toughness by shear force measurement using Instron, or resistance to cutting by a fibrometer or chemical analysis for crude fiber and lignin content; measurement of water content as an indication of succulence; measurement of extractable juice as an indication of juiciness; or by sensory evaluation for grittiness, crispness, mealiness, chewiness and oiliness. A nondestructive firmness tester is available for assessing the maturity of avacados (107). Firmness is a simple, rapid technique for measuring the harvest maturity of apples (108) and pears (109).

D. Solids Content

Moisture or solid contents are being used effectively as maturity indices of fruits for processing purposes. Iodine staining of starch has been used as a measure of assessing maturity in apples (110). Sugars are the industry standard to assess the maturity of cantaloupe and other melons. Soluble solids have been suggested as a maturity index for sweet cherries (111), and Brix-to-acid ratio for citrus fruits (112), whereas both soluble solids and acidity are used to assess maturity in grapes (113). Mango is another popular specialty fruit of great economic importance to the tropical regions, in which sugars and acids influence its consumer acceptability (114, 115). As a result of starch hydrolysis from increased amylase activity (116), sucrose is the major sugar in ripe mango fruits. Among the reducing sugars, glucose is reported to be the major sugar by Selvaraj et al. (117), whereas fructose was identified as a predominant sugar by Medlicott and Thompson (118) and attributed this contradiction to the varying cultivars and storage conditions used by these workers.

E. Flavor

Flavor is another important processing property and consumption attribute critical to consumer acceptability and hidden attributes of fruits (119). Sweetness is a function of sugar concentration and sourness is a function of acidity, and their ratio determines the flavor of fruits because consumers perceive sweetness or sourness in terms of sugar:acid ratio. Sugar concentration is usually estimated by measuring the percentage of soluble solids (°Brix) using a refractometer (120) and acidity by titration against a standard base. The Brix-to-acid ratio (BAR) is used to assess the relative sweetness or sourness for most of the fruits for processing purposes. Lack of attention to flavor compounds in breeding programs has led to flavor mediocrity in many fruits. The lack of information on flavor compounds available to breeders on this complex trait is the main reason for this. Using gas chromatography, as many as 225 volatile compounds have been reported for grape cultivars (99, 121), although every particular cultivar emits a special aroma. Many fruits contain a single volatile compound, described as character impact compound, that imparts the flavor message by itself. Some examples of these character impact compounds given by Shewfelt (122) are ethyl-2-methylbutyrate in apples, γ-decalactone in peaches and isoamylacetate in bananas. However, the full aroma of any fruit is a subtle combination of many compounds, which makes it difficult for food scientists to duplicate fruit flavors in artificial beverages. A number of volatile compounds have been identified to be associated with the distinctive aroma of fresh as well as processed fruits. These aroma compounds, in combination with sweet, sour, salty, astringent and bitter sensations, endow characteristic flavors to various fruits and their products. The type of cultivars, post-harvest processing and storage treatments are some of the factors that affect the flavor properties of various fruits and their processed products (123, 124). The task of preserving and improving the aroma of fruits will probably be difficult. For example, some treatments currently used to prevent decay or to preserve texture and color can damage aroma quality. A higher concentration of carbon dioxide is usually used to reduce decay in strawberries and other similar crops, but it can induce fermentation (125). Similarly, lower oxygen levels, while extending the shelf life of apples and other pome fruits, can reduce aroma at stress levels, and may even cause off-flavors (126).

X. PROCESSING OF VARIOUS FRUITS

A. Grapes

The grape cultivars are classified according to table, juice, wine and raisin purposes. Table grapes are consumed as fresh fruit or processed into juice. They should have large clusters and berries of appealing appearance, fine flesh with low acidity and high sugar content, and a few or no seeds (99). In the case of colored grapes, heating the crushed berries for 10–15 min at 60–63°C is necessary to extract the coloring matter (127). This heating step is not required in white grapes. Frozen grape juice concentrates are available commercially, but their production is less compared with orange juice concentrate. Wine grapes are processed into juice and fermented by yeast to make wine, or distilled to make a number of beverages (e.g., brandy). The wine grapes should have small clusters with round berries set compactly, with soft, juicy flesh of high acidity and low pH (128). After wine, raisins are the second most important product made from grapes (129). Raisin grapes are a special category of table grapes, having thin skin, firm flesh with higher sugar content and moderate to low acidity, with berries loosely attached in the clusters.

B. Citrus Fruits

Apart from consumption as fresh fruits, citrus fruits are processed mainly into juice, frozen concentrate, jam, marmalade, squash, candied peel and certain by-products, such as pectin, citric acid and peel oil. Juice is the most popular product prepared from citrus fruits and may be canned, frozen or chemically preserved (130). Frozen concentrated orange juice is by far the most important processed product from citrus in the U.S. The popularity of this product has been decreasing recently, as consumers prefer natural or minimally processed food products (131). Sound and mature fruits with the highest possible juice quality are desirable for producing orange juice. The color, flavor, yield and total soluble contents of the fruit increase with the maturity of oranges. The best quality orange juice can be produced if the Brix-acid ratio is between 13 and 19, 15 being the preferred one. Mid-season or late-season oranges give better yield and quality of juice than the early-season fruits (132). An excessive amount of peel oil in juice is undesirable. However, a certain amount of peel oil (0.01–0.02%) is considered to be necessary for obtaining maximum flavor (133).

C. Date Fruit

Date palm (*Phoenix dactylifera* L.) is an important food crop in the Middle Eastern countries. Rygg (134) reviewed the detailed description of the date growing regions of the world, date varieties and general cultivation practices. Four maturity stages have been recognized in date fruits, i.e., *kimri, khalal, rutab* and *tamer* (135). At the *kimri* stage, the fruit is hard in texture, green in color and can be used for making pickle, relish and chutney (16, 136). Depending upon the cultivar, the *khalal* stage fruit develops a yellow, red, purple or yellow-scarlet color, but retains a firm texture and can be used for making jam, butter, dates-in-syrup or for eating as fresh dates (137, 138). The *rutab* stage is characterized by softening of the half portion of the fruit, developing a dark brown color and increased sweetness and can be used for jam, butter, date bars, date paste or eaten as fresh (139). During the *tamer* stage, the whole of the fruit becomes soft in texture, wrinkled, relatively drier, and dark in color and attains maximum sweetness (140). The *tamer* fruits can be further dried for prolonged shelf life during storage. The chemical composition of date fruit varies with maturity. The total sugars in date fruits at the *kimri* stage range from 32.99 to 38.20%, but are increased to 77.97 to 79.39% at the *tamer* stage of maturity. The ranges of protein, fat, ash, pectin, tannin and crude fiber contents of *tamer* stage date fruits are 2.1–2.4, 0.1–0.2, 1.6–2.0, 1.3–1.9, 0.4–0.4 and 2.5–2.9%, respectively (141).

The majority of the date fruit is harvested at the *tamer* stage and enters the trade for consumption as such (142). Recently, the quantities of processed dates have been increasing rapidly as a result of encouragement and support being provided to the date fruit processing industry by governmental agencies. The development of newer processed date fruit products would increase the economic value of this crop immensely (143). Due to increased production and a decreased tendency towards direct consumption of date fruits, the introduction of processed products holds a great future for this important food crop of this arid region of the world.

D. Banana

Bananas are one of the most important fruits grown in the tropics. About 50% of the bananas produced are consumed in Africa as a cooked vegetable and are often called plantains. A number of processed products, such as juice, pulp, puree, concentrate, canned slices, fruit bars, powder, wine, brandy and deep-fried chips, have been prepared from ripe as well as unripe bananas (144). Among all these products, banana puree is by far the most important processed product prepared from the pulp of ripe fruit. Puree finds uses in dairy desserts, bakery items, mixed fruit drinks, sauces and as a part of special diets in hospitals and nursing homes.

E. Apple

Apples are processed into a variety of products, such as juice, concentrate, sauce, butter, preserve, vinegar, cider, wine, brandy, candy, jam, jelly and canned products. Apples are also dried as rings, chips or cubes. Edible pectin is also produced from the waste of the apple processing

industry. Apple juice, in the form of clarified apple juice (made using pectinol enzymes), natural apple juice, pulpy apple juice and as fruit cocktail blends, is second only to orange juice in Europe and North America (145–147). Apple juice contains most of the soluble components of original apples, such as sugars, acids (mainly malic acid) and various other carbohydrates.

F. Mango

India contributes about 64% of the world production of mangoes. The mango fruit is highly perishable, highly susceptible to disease, extremes of temperature and physical injury, and international trade in fresh mango has been limited. However, a number of products, such as canned mango pulp, juice, nectar, squash, beverage, jam, chutney, pickle, mango leather and raw mango powder, are routinely prepared (6). Mango is one fruit that is processed and used at almost every stage of its growth. Raw mango fruit is mainly used for the preparation of raw mango powder, chutney and pickle (148, 149). Mango puree made from ripe mango is one of the most important processed products, which finds further utilization in various other products, such as nectar, squash, jam and ready-to-serve beverages. Puree is canned for long-term storage and marketing worldwide (150). Mango pulp can also be preserved using 350–700 ppm of sulfur dioxide, but there are reservations among consumers towards sulfited foods due to objectionable odor and toxicity (151). Mango fruit beverages are very nutritive drinks and are extremely popular in India and other Asian countries (152). Mango beverages with 20% puree in the formulation and with 20°Brix taste very sweet, so most of these mango drinks are adjusted to only 15°Brix, because less than 15% puree adversely affects the color and flavor of the mango beverages during storage.

Mango, being rich in a desirable strong flavor of its own, lends itself to be blended with a number of other fruits for the preparation of fruit juice cocktails (153). Blends of mango puree with papaya, orange, kinnow, apple, guava, pear, peach and apricot could be used in many foods (154). Peeled slices/cubes obtained from ripe or semiripe mango fruits can be preserved in sugar syrup, especially from some Indian mango cultivars, such as *Saheb Pasand*, having a TSS of 30°Brix that has bright-colored pulp and a firm texture (155). Mango leather, another popular product, is prepared by drying mango pulp to a final moisture content of about 15%. For the preparation of mango leather, an ideal ratio of 25:0.3 for TSS:acid has been suggested (156). Pectin when used at a rate of 0.5 to 0.75% improves the texture of mango leather.

G. Pineapple

The pineapple is one of the most important commercial fruits of the world, and is mainly used in processed form as dessert fruits, or as canned pineapple in the form of slices, rings, chunks, fruit cocktails or in the preparation of juice and jams. Most of the world's pineapple production is canned, largely as slices, juice, chunks and diced pineapple (157). Pineapple juice contains neutral polysaccharides (mainly galactomannan), which are removed using pectinase, cellulase and hemicellulase enzymes (158). Pineapple juice stored at higher temperature (37°C) loses its color rapidly, but it can be stored at room temperature for 12–15 months without any serious loss in quality or nutritive value (159). Pineapple concentrate can also be prepared, which can be stored satisfactorily at 10–38°C by adding sulfur dioxide without any significant loss in color or flavor (160). Although attempts have been made to prepare pineapple juice powder by freeze-drying, the product has not become commercially viable (161).

H. Pear

Pears have been consumed primarily as fresh fruit, although a part of pear production has been processed into juice, wine, candy, dried and canned products (162). Pears are a good source of pectin, sugars and thiamine. Pear fruit is reported to help in maintaining a desirable acid/base balance in the human body. Because of its low sucrose content, pear fruit is also recommended for diabetics. The pear fruit is either canned as juice, or as chunks in fruit cocktail along with other fruits. Puree prepared from pear fruit finds uses in baby foods, pear butter and jams. Pear juice concentrate is finding wider applications in beverages and fruit spreads. Pear candy prepared from sand pear and Bagygosha cultivars are reported to retain about 50% of the ascorbic acid during 40 weeks of storage at room temperature (163). The stone pear is suitable for jam, chutney, clarified juice and preserve (164).

I. Plum

Plums are generally consumed as fresh fruit, and only a small quantity is used for canning, dehydration and beverages. Whole plums fruits with higher sugar content and firm flesh are dried and are called prunes. More than 50% of the total world production of plums comes from the U.S., the former U.S.S.R., China and Romania (6). Apart from canning, plums are used for the preparation of jam, jelly, beverages, wines and brandy. Prune juice produced from dried plums is rich in minerals and acts as a mild laxative. Use of pectolytic enzymes increases the yield of prune juice and aids in easy filtration (165). Pectolytic enzymes not only increase plum juice yield, but also drastically decrease the apparent viscosity and improve the color and clarity without affecting the flavor (166). A natural plum juice containing pulp has also been prepared recently (167). Being high in acidity, no more than 40% of plum juice can be used in the preparation of beverages like sweetened plum juice. About 20% of enzymatically

extracted plum juice with 15°Brix has been found to be optimum for preparing nectar (168).

Preparation of plum juice concentrate is one of the best options of utilizing fruit during glut seasons. Plum juice can be concentrated under vacuum to 73°Brix. This juice concentrate has higher total soluble solids, reducing sugars, total solids, browning and viscosity, but the pectin content and acidity are slightly reduced (169). Firmer fruits are generally preferred for canning than softer fruits. Softening of ripe plums during canning can be prevented using 500 ppm of calcium chloride (170). About 75% of the world supply of dried prunes comes from California and the Pacific Northwest. Dried prunes, prune flakes, nuggets and granules are also produced commercially for use as fillings in bakery products. Low-moisture prune powder is also available for use as a sweetening and flavoring agent in whole wheat and rye bread formulations. The plum pulp is also utilized in ice cream mix, confectionery items and meat sauces (171). Wine or brandy prepared from fruits other than grapes are referred to by the name of that fruit used. Plum wines are quite popular in Germany and many Pacific coastal states of the U.S. The method for making plum wine or brandy is similar to that of grapes (172).

J. Peach

Peach and its smooth-skin mutant nectarine are some of the most important stone fruits in temperate regions of the world. Apart from consumption as fresh fruit, peaches are usually canned. Only a small portion is utilized as frozen or dried or used as jams, peach conserve, marmalade, peach butter, fruit bar, pickle and beverages (173). Peach puree is the basic starting material for the preparation of many other products, such as jam, nectar, juice, baby foods and ready-to-serve beverages. Initial color and viscosity of puree have been considered as important quality characteristics for further processing (174). Commercial canning of peaches is the major industry in the world. Peaches can be canned as whole, halves, quarters or slices, usually in sugar syrup or juices of other fruits (175). The peach fruit for canning should be picked at or near optimum maturity and should be of uniform large size (a dia of 6.03 cm or more), good yellow color and of good cooking quality (176). Steep preservation is another simple technique that could be used for peaches (177).

K. Berries

Juices from berries are widely used for the manufacture of jams, jellies, beverages, wine, fruit yogurt and ice cream (178). To increase juice yield from berries, pectolytic enzymes are added to the pulp (0.1g/kg of fruit). The enzymes are allowed to act on the cell wall pectic substances to release juice. The juice could be preserved by the addition of antimicrobial agents (potassium benzoate or sodium sorbate) followed by pasteurization (179). The juice can also be concentrated, but the susceptibility to undesirable changes in flavor during storage has hindered its application in food and beverages (180). Wines from strawberry, raspberry and kiwifruit are also prepared (181–183). Use of pectolytic enzymes for increasing juice extraction yield is essential for making wine with desirable clarity, color and flavor.

L. Apricot

All of the mechanically harvested apricots (roughly more than 50% of total production) in the U.S. are used for processing, mostly for canning. About 84% of the total production of apricots in the U.S. is canned, dried and frozen (184). The canning process for apricots is the one typically used for most stone fruits. The textural quality of canned apricots is affected by a number of factors, such as sterilization conditions, maturity of fruits and storage conditions. Even immature fruits can be canned after treating them with ethylene for two days (185). Dried apricots are another important snack, becoming popular with present-day consumers because of their desirable color, flavor and nutritive value. Dried apricots are produced from fully ripe and plump fruits (186). High natural sugar level and low pH of dried apricots prevents microbial spoilage and enzymatic deterioration in quality during storage. The apricot fruits unsuitable for canning are utilized for the preparation of juice and concentrates. The juice concentrate can be used for the preparation of nectar and baby foods. The concentrated juice can also be dried to obtain powder that can be used in bakery products, beverages, jellies and desserts (165). Because of excellent natural flavor, apricots are also processed to jams, jellies and preserves. Due to higher susceptibility to browning and oxidation during freezing, frozen apricots are not very popular.

M. By-Products of the Fruit Processing Industry

Fruits are marketed either as fresh produce or processed as frozen, dehydrated, canned, pureed, juices, jams, jellies, marmalades, pickles, chutney and in many more forms. During these processing operations, a large proportion of fruit ends up as residue that needs to be handled properly to avoid environmental pollution while generating additional revenue for the processing industry. The wastes arising out of the fruit processing industry can be utilized for the preparation of human food, animal feed, fertilizers, soil conditioners or for landfill (187).

Dietary fiber is extracted from apple and pear pomace to obtain a product containing 56 and 77% fiber, respectively. This product has the consistency of whole wheat flour with a bland taste and is utilized in baked goods,

breakfast cereals, granola products, laxatives, pharmaceutical preparations and pet foods (188). Peels and cores from apples can be utilized to produce vinegar and jelly juice stock. Apple pomace can be extracted with liquid carbon dioxide to produce an intensely flavored fraction. Pectin is an important by-product from the apple processing waste. Different apple cultivars are reported to vary in pectin yield, jelly grade and other qualities of the extracted pectin (150). Pectin extracted with alcohol from apple pomace can be dried to 5% moisture content and used for jam and jelly-making. About 250 g of citric acid can be obtained by growing *Aspergillus niger* on apple pomace (one kg solids) under controlled conditions. Around 30–35% of the pear fruit ends up as waste during canning operations. The waste from pear fruit processing industries has been used in the preparation of vinegar, brandy, denatured alcohol and sugar syrup or has also been dried for use as cattle feed (170). Kiwifruit peel is another suitable source of citric acid production. In the presence of 2% methanol at 30°C, about 100 g of citric acid per kg of kiwifruit peel can be produced by solid-state fermentation using *Aspergillus niger* (193). Apple pomace can be used as animal feed, either fresh or as dried product (150).

Citrus fruit processing waste can be utilized for the production of citric acid, but the process is uneconomical because citric acid now produced by microbial fermentation is much lower in cost. Citrus peels contain 2.5 to 5.5% pectin, which can be extracted with acidified water. After centrifugation, the clarified pectin solution is dried to obtain pectin powder. By the cold pressing method, fresh orange peel also yields about 0.54% superior quality oil, which finds uses in juice and squash manufacture (199). Guava fruit is another important source of food-grade pectin. During the extraction of pectin by hot water-boiling, use of sodium hexametaphosphate or 1:1 mixture of ammonium oxalate and oxalic acid at 0.25–0.75% concentration gives higher yields of pectin with high jelly grade (200).

About 40 to 60% of the fruit intake ends up as waste in the mango processing factories. This fruit waste consists of 12–15% peel, 5–10% pulp waste and 15–20% mango seed kernel (160). The pectin obtained from mango peel is comparable with the pectin extracted from citrus peel (161). Mango peel is a very good source of sugars (48.1%), pectin (12.9%), protein (3.9%), fiber (8.4%), tannins (2.3%) and minerals (2.9%; all values on dry basis) and constitutes about 13% of the mango fruit (161). Good quality pectin obtained from mango peel can be used in the manufacture of jam, jelly, marmalades and many pharmaceuticals. Depending upon the cultivars, mango kernel (or stone) is about 45.7 to 72.8% of the mango fruit and is a good source of starch (57.8%), fat (13.7%), tannins (10.6%) and protein (7.1%) on a dry basis (162). Among the other by-products, mango kernel fat has attracted major attention because it could be a very good substitute for tallow and cocoa butter in soups and confectionery products (201, 202). Cake left after the extraction of kernel oil could substitute for wheat and maize flour in animal feed as it is a good source of many essential amino acids. Mango peel is also suitable as a supplement in fish feed. The waste from mango processing plants can be used for biogas production. Recently, a mixture of 5-(12-*cis*-hepta decenyl) and 5-pentadecyl resorcinol has been isolated from mango peel, and this has been found to be effective against *Alternaria alternata*, a fungus that causes black spot disease in mango fruit (163).

Guava seeds are usually discarded during processing, but these seeds are rich in oil (5–13%), which can be utilized in salad dressing. Guava seed oil is especially rich in essential fatty acids, such as oleic (54%) and linoleic (29%) acids (203).

Green banana fruit, pseudostems and foliage are good sources of energy and serve as cattle feed after supplementation with a protein source. About 1000 banana plants can yield 20–25 tonnes of pseudostems, which contain about 5% edible starch that finds a number of industrial uses (145). Banana stem waste is another important waste product that can be used for growing food yeast (146). The pineapple processing industry waste can be utilized for the preparation of several products, such as citric acid, cattle feed, sugar syrup, wine, bromolain enzyme, wax and sterols (169). Peach processing generates a lot of waste in the form of peel, seeds and trimmings, which contain proteins, polysaccharides, sugars, amino acids and pectin. After enzymatic treatment, peach solid waste may be utilized for ethanol production (186).

Production of citric acid from fruit processing solid wastes using *Aspergillus niger* in solid-state fermentation has been suggested (193). Apart from these specific by-products of commercial value, the waste from most of the fruit processing plants could be utilized in animal feeds and production of ethanol, biomass and many other chemicals.

XI. NUTRITIONAL AND HEALTH PROPERTIES

Consumption of fruits and vegetables by adults in the U.S. has not reached the recommended level suggested by nutritionists and other health professionals. Unfortunately, children consume even less. Various governmental agencies, professional associations, food processors and health organizations are making efforts to encourage increased consumption of fruits and vegetables (204), because intake of fruits and vegetables has an inverse association with the risk of cardiovascular diseases and mortality in the U.S. population (205). Fruits are rich not only in dietary fiber, vitamins and minerals, but a number of bioactive compounds present therein are strong antioxidants and function to modify the metabolic activation/detoxification of carcinogens.

A. Fruits and Dietary Fiber

A low intake of dietary fiber is associated with a spectrum of degenerative diseases, such as constipation, diverticular disease, coronary heart disease, hiatus hernia, appendicitis, varicose veins, piles, diabetes, obesity, bowel cancer and gallstones (206). Soluble dietary fiber, including pectic substances and hydrocolloids, is present in fruits, vegetables, legumes and oat bran. By-products from fruit processing industries are potential sources of both soluble and insoluble dietary fiber in our diet (207). Association between fruit, vegetable and dietary fiber consumption and colorectal cancer risk in the U.S. population has been observed (208). The total fruit and vegetable consumption is inversely associated with colorectal cancer risk, but subanalyses show this association is largely due to fruit consumption. The association is stronger and the dose-response effect is more evident among those individuals who consume the lowest amounts of fruits and vegetables (less than 1.5 servings) compared with those who consume greater amounts (more than 2.5 servings/d).

To assess the role of certain tropical fruits, such as lychee, guava and ripe mango, in the prevention of cardiovascular diseases, contents of total and water-soluble dietary fiber are determined by Gorinstein et al. (209). Lychee, guava and ripe mango had a total fiber content of 2.2, 5.6 and 3.1 g/100 g fresh fruit and soluble fiber of 1.05, 2.7, 1.51 g/100 g fresh fruit, respectively. Use of lychee, guava and ripe mango could be suitable for the prevention of cardiovascular disease through dietary methods. The non-starch polysaccharides in Starkspur Supreme Delicious apples from Arkansas ranges from 1.41 to 1.98 g/100 g fresh fruit (210). Total fiber contents in four berries (blackberries, cranberries, red raspberries, strawberries) ranges from 1.0 to 7.0% fresh weight (14). Peeling and canning changes the uronic acid and neutral sugar contents of soluble and insoluble fiber fractions of several fruits.

Rosado et al. (211) analyzed 24 fruits for dietary fiber contents. The total dietary fiber (wet weight basis) ranged from 0.3 to 7%, and soluble fiber contents (% of total dietary fiber) ranged from 6 to 44%. The total dietary fiber contents in 47 Italian fruits range from 0.22 to 6.47 g/100 g for fresh fruits and 5.0 to 30.0 g/100 g for dry fruits (212). Marlett (213) analyzed 23 fruits and reported a mean dietary fiber content of 1.4 ± 0.7 g/100 g fresh weight, and 13 to 20% of this was soluble fiber fraction. Pectin constituted 15 to 30% of the total dietary fiber contents among fruits. The total dietary fiber contents of five fruits that are most commonly consumed in Malaysia range from 1.5 to 3.6% on a fresh weight basis (214). The incorporation of prunes in the diet as a source of dietary fiber lowers plasma low-density-lipoprotein cholesterol more significantly (3.9 mM) than when grape juice (4.1 mM) is consumed (215). Prunes also significantly lower fecal bile acid content of lithocholic acid. Fecal output is nearly 20% higher after consuming prunes, but the total bile acid content is unchanged.

B. Phytochemicals in Fruits

Oxygen during normal metabolism yields a number of reactive oxygen species (ROS), most of which are free radicals having an odd number of electrons. These reactive species initiate lipid peroxidation, a chain reaction that can oxidize DNA, proteins (enzymes) and cell membranes (216). It is now proposed that oxygen free radicals are involved in several pathological conditions, such as oxidation of LDL and development of atherosclerosis, different stages of cancer development, autoimmune destruction of β cells leading to diabetes, inflammatory damage in asthma, in rheumatoid arthritis, age-related macular degeneration (AMD) and oxidation of lens proteins leading to cataracts (217). To neutralize these reactive species, the body's defense system is equipped with a few enzymes, some high-molecular-weight antioxidant proteins, and some low-molecular antioxidants, like ascorbate, vitamin E, β-carotene, glutathione and uric acid. If this balance is overwhelmed, oxidative stress builds up and this becomes the underlying cause of aging and some of the age-related diseases, such as cancer, cataracts, AMD and cardiovascular disease (218). Fruits have the ability to protect us against this free radical damage if consumed regularly. Fruits, like prunes, raisins, blueberries, cranberries and blackberries, have the highest antioxidant capacity, followed by strawberries, raspberries, plums, oranges, red grapes, red cherries, kiwi fruit, pink grapefruit, bananas and apples (219).

Many bioactive phytochemicals (or neutraceuticals) present in fruits have the ability to provide immense health benefits to us. These phytochemicals include terpenoids, phenolics, alkaloids and fiber. The role of these compounds against cancers, coronary heart disease, diabetes, hypertension, inflammation, microbial, viral and parasitic infections, ulcers, etc., has been recently reviewed (220). In addition to primary antioxidants, such as ascorbate, vitamin E and carotenes, most of the other phytochemicals present in fruits act as antioxidants to reduce the oxidative damage caused to lipids, proteins, nucleic acids and other cellular targets by reactive oxygen species (ROS). The effect of these antioxidants on changes in oxidative stress status associated with aging and other chronic conditions, like diabetes, cardiovascular diseases, cataracts and age-related macular degeneration, is being actively explored at Tufts Nutrition Antioxidant Research Laboratory (2). Although many other foods (such as vegetables, whole grains and whey proteins) also play an important role, the contribution of fruits to human nutrition and health is equally important.

C. Fruit Intake and Cancer Risk

The relation between cancer risk and the consumption of fruits and vegetables is now gaining a lot of attention

(221). Diets rich in fruits and vegetables are more strongly related to a lower risk of epithelial cancers of the respiratory and digestive tract. However, the intake of fruits and vegetables does not show a strong association with hormonally related cancers such as breast, endometrium, ovary and prostrate. No information is available on the total intake of phenolic phytochemicals. If this group includes anthocyanins, flavanols, isoflavones, phenolic acids and tannins, the total phenolic phytochemical intake may be up to hundreds of milligrams per day (222). This intake could be even higher in populations where red wine, soy products, lentils and beans are consumed. This information on the intake of phenolic phytochemicals would be useful because most of these phenolics have antioxidant properties with similar biological activities. Some of these phenolics trap nitrates and prevent the formation of mutagenic N-nitroso compounds in foods (223). The intake of these N-nitroso compounds is known to induce cancers of the nasopharynx, esophagus and stomach (224). In a recent study, regular consumption of fruits was strongly associated with reduced risk of many types of cancers (225). The strongest evidence correlates with the reduced risk of the mouth, esophagus, pharynx, lung, stomach and colon, whereas moderately strong evidence exists for cancers of the breast, pancreas and bladder.

D Antioxidants/Phytochemicals in Fruits

Until recently, fruits were known only as a source of certain vitamins, minerals and dietary fiber, but now a number of nonessential bioactive compounds (phytochemicals) are attracting the attention of food scientists and health professionals. Among the vitamins present in fruits, ascorbic acid has been investigated extensively. Ascorbic acid is a water-soluble antioxidant, which can easily be oxidized to form a free radical semidehydroascorbic acid that is quite stable. As ascorbic acid can lose an electron very easily, it is a very effective antioxidant in cytoplasm of biological systems (226). Blood levels of vitamin C above 49 μmol/L have been associated with a 64% reduced risk of cataracts in a Mediterranean population (227). Although more than 600 carotenoids have been identified so far, only about 50 of these possess vitamin A activity. Carotenoids, being lipid-soluble, act as antioxidants to protect the cell membranes of biological systems by quenching singlet oxygen and scavenging free radicals. Lycopene has the greatest antioxidant activity, followed by α-carotene, β-carotene, lutein and cryptoxanthin (228). Autumn olive (*Elaeagnus umbellata* Thunb.) bears yellow or brilliant red berries, which are very rich in lycopene (229) containing 15 to 54 mg from naturalized plants and 17 to 48 mg/100 g of fresh fruit from four cultivars with red-pigmented fruit. In contrast, the fresh tomato fruit had only about 3 mg of lycopene per 100 g. In addition, this fruit also contained α-cryptoxanthin, β-carotene, lutein, phytoene and phytofluene. This newly identified fruit (Autumn olive) can become an excellent source of lycopene and other carotenoids in our diet.

Lutein is a well-known carotenoid, whose intake has been shown to be inversely associated with ocular diseases, such as cataracts and age-related macular degeneration (230, 231). Lutein offers protection to the cells of the macula by acting as an antioxidant against the reactive oxygen species (232). The consumption of carotenoids from various sources does not reduce plasma carotenoid concentrations in the medium term, thus suggesting the eating of a diet rich in a variety of fruits and vegetables (233). Limonoids (terpenes) present in citrus fruits are shown to inhibit Phase I enzymes and induce Phase II detoxification enzymes in the liver and provide protection against cancer (234).Vitamin E is another major lipid-soluble antioxidant that protects the polyunsaturated fatty acids in cell membranes against free radicals and singlet oxygen species. Glutathione, selenium and flavonoids are the other water-soluble antioxidants. About 2000 chemical distinct flavonoids (flavonols, flavones, flavanones, isoflavones and flavanols) are present in fruits that may have antioxidant activity.

Phenolics, such as tannins and flavonoids, present in fruits have also been studied extensively as antioxidant protectants for humans. Plant phenolics are mostly produced through phenylpropanoid pathways and comprise a variety of compounds, such as cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. They are known to be strong antioxidants and prevent oxidative damage to DNA, lipids and proteins, which may play a role in chronic diseases like cancer and cardiovascular disease (235). Ferulic acid (a phenolic compound), a component of the plant cell wall, has the ability to mop up damaging free radicals and reactive oxygen species (236). One possible use for ferulic acid may be as a stimulant for sperm motility in fertility treatments or during *in vitro* fertilization. The polymeric tannins from grape seeds function as antioxidants and neutralize the effects of oxidative stress induced both by the deficiency of other vitamins and an atherogenic diet (31). Flavonoids are known to perform against free radicals, free radical-mediated cellular signaling, allergies, inflammation, platelets aggregation, ulcers, tumors, microbe viruses and hepatotoxins (237). Catechins and gallic acid present in grapes and berries have free radical scavenging activity, and inhibit eicosanoid synthesis and platelet aggregation.

Anthocyanidins are water-soluble flavonoids that are the principal pigments in fruits and are known to have antioxidative properties (238). Among the flavonoids, anthocyanins are reported to possess higher antioxidant activity as measured by oxygen radical absorbance capacity (ORAC) equivalents (239). On a fresh weight basis, the anthocyanin content in fruits, such as blackberries,

blueberries, cranberries, raspberries, strawberries and boysenberries, may range from 200 to 4950 mg/kg. An intake of 100 to 150 g of these fruits could easily give us 100 to 200 mg of anthocyanins, which are well above the suggested levels (240). Intake of two to five servings of fruits and vegetables per day would result in 1200 to 1640 ORAC equivalents for the person and may be responsible for the health benefits achieved with increased consumption of these foods. The polyphenols from blueberries and cranberries possess antioxidative and anti-inflammatory activities in endothelial cells and are reported to be beneficial in reducing initiation or development of cardiovascular diseases (241).

E. Stability of Phytochemicals during Processing

Food technologists are currently busy adding bioactive phytochemicals for developing new food products. These formulated foods will not only meet the nutritional needs of growth and maintenance, but are also expected to provide additional health benefits to consumers. Some of these targeted benefits are preventing disease and improving physical performance and the overall quality of life. The biochemical aspects, physiological effects and health benefits of phytochemicals in fruits were reviewed recently (242). The topics covered are characteristics of major fruit phytochemicals (phenols and carotenoids); physiological effects of phenols, carotenoids and other bioactive compounds (limonoids, ascorbate, limonene and folate) found in citrus fruits; evidence of health benefits, antioxidative and anticancer properties of bioactive compounds present in berries; and composition and physiological effects of phytochemicals in strawberries, apples, melons and some other fruits.

The antioxidant capacity of these phytochemicals is affected not only by various horticultural factors, but also by the food processing practices employed in the industry. The antioxidant capacity due to phenols in blueberries is decreased by food processing practices, such as heating and aeration (243). The composition, antioxidative capacity and levels of anthocyanins and total phenols in cultivated blueberries (*Vaccinium corymbosum* L.) and wild blueberries (*V. augustifolium* Aiton) are influenced by the method of extraction (244). The highest anthocyanin, total phenolic contents and antioxidative capacity are observed when aqueous methanol is used as a solvent. Irrespective of the solvent used, wild blueberries give higher values for these three parameters than the cultivated blueberries.

The temperature, pH and oxygenation during extraction of anthocyanins affect the antioxidative activity of extracted blueberries (245). Extraction of fruits at 60°C gives higher recovery of anthocyanins and antioxidant capacity than the extract obtained at 25°C, but subsequent loss in anthocyanins during room temperature storage occurs only in the former. Antioxidant capacity is higher in pH 1 extracts than those at pH 4 and 7. Oxygenation is detrimental to both the anthocyanins and antioxidant capacity. The antioxidant capacity of these processed products is positively correlated with anthocyanin ($R=0.92$) and phenolic ($R=0.95$) contents, and negatively correlated with percentage polymeric color ($R=0.64$). In general, the higher the extent of processing treatment, the lower is the antioxidant capacity. Simple colorimetric tests for anthocyanins and phenols are available to evaluate their antioxidant capacity in processed food products.

The antioxidant capacity, ascorbic acid, phenolics and anthocyanins of small fruits (strawberries, raspberries, cultivated and wild blueberries) during storage at 0, 10, 20 and 30°C for up to eight days are investigated (246). These four fruits vary significantly in total antioxidant activity, which is strongly correlated with the total phenolics ($R=0.83$) and anthocyanins ($R=0.90$). Antioxidant capacity is three times higher in blueberry species than either strawberries or raspberries. Ascorbic acid contents differ significantly among these fruit species, strawberries and raspberries having almost four times more ascorbate than blueberries. No loss of ascorbate is observed after eight days of storage at any of the temperatures in strawberries or cultivated blueberries, but there are losses in two other fruit species. Storage at a temperature higher than 0°C increases antioxidant capacity of strawberries and raspberries, and it is accompanied by increases in anthocyanins in strawberries, and increases in anthocyanins and total phenolics in raspberries. Ascorbic acid contributes very little (0.4–9.4%) to the total antioxidant capacity of these fruits. The increase in antioxidant capacity through post-harvest phenolic synthesis, therefore, indicates the possibility of enhancing the health benefits of these small fruit crops.

XII. FUTURE CONSIDERATIONS

Fruits are grown universally in almost every part of the world and serve as vital sources of essential vitamins, minerals, dietary fiber and many other bioactive compounds. Fruits are highly perishable in nature, and the growth of post-harvest technology is essential to minimize losses during their production, processing, handling and transportation. Fruits are processed into a variety of products, and are consumed either alone or in combination with grains, milk and milk products to provide maximum nutritional advantages to consumers.

Apart from a botanical basis, fruits have been classified solely on color or other physical properties. Most of these physical properties (viz. size, mass, total solids, firmness, density, color, specific gravity and porosity) are well correlated with the chemical composition, nutritional value and processing quality of finished products. The classification based on color serves as a useful indicator, not only for the fruit breeders, but also for the food scientists to look for

many phytochemicals (e.g., carotenoids from yellow, orange fruits, anthocyanins from colored fruits). This has opened up a vast opportunity for fruit biotechnologists to pursue a significant area of health-based fruit quality modification to selectively increase levels of different vitamins and other phytochemicals in various fruit species in coming years. This will give another boost to the fruit-based agroprocessing industry around the world. Considering the scope of functional foods based on phytochemicals, nowhere is the marketing transformation more visible than among the fruit growers and processors. As most fruits contain bioactive compounds that have extraordinary health-promoting characteristics, the growers, cooperatives and processors are quick to move these products to the frontline of their marketing programs.

While phytochemicals in fruits clearly show promise to prevent many diseases, important scientific questions are yet to be answered. We have to look for the answers to some of the questions like "Which of these phytochemicals are more effective, what is the mechanism of their action, and more importantly, how to preserve these bioactive compounds during processing?" We also need to identify and quantify these bioactive compounds in foods, their bioavailability, pharmacokinetics and metabolism in humans, determination of the relationship between molecular structure and antioxidant capacity, mutual interaction of these phytochemicals in the human digestive tract when consumed together, doses required for their effectiveness, and their stability during processing, storage and distribution of these food products right up to our dining table.

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25 Frozen Fruits: Product Descriptions

Peggy Stanfield
Dietetic Resources

CONTENTS

I. FROZEN FRUITS

A. APPLES

Frozen apples are prepared from sound, properly ripened fruit of *Malus sylvesfris (Pyrus malus);* are peeled, cored, trimmed, sliced, sorted, and washed; are properly drained before filling into containers; may be packed with or without the addition of a nutritive sweetening ingredient and any other legally permissible ingredients and are frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

The term "slices" means frozen apples consisting of slices of apples cut longitudinally and radially from the core axis.

B. APRICOTS

Apricots are the food prepared from mature apricots of one of the optional styles specified, which may be packed as solid pack or in one of the optional packing media specified.

Such food may also contain one or any combination of two or more of the following safe and suitable optional ingredients:

1. Natural and artificial flavors.
2. Spice.
3. Vinegar, lemon juice, or organic acids.
4. Apricot pits, except in the case of unpeeled whole apricots and peeled whole apricots in a quantity not more than 1 apricot pit to each 227 grams (8 ounces) of finished frozen apricots.
5. Apricot kernels, except in the case of unpeeled whole apricots and peeled whole apricots, and except when an optional ingredient is used.
6. Ascorbic acid in an amount no greater than necessary to preserve color.

Such food is sealed in a container and before or after scaling is so processed by heat as to prevent spoilage.

1. Optional Styles of the Apricot Ingredients

The optional styles of the apricot ingredients arc peeled or unpeeled: (i) whole, (ii) halves, (iii) quarters, (iv) slices, (v) pieces or irregular pieces.

Each such ingredient, except in the cases of unpeeled whole apricots and peeled whole apricots, is pitted.

2. Packing Media

The optional packing media are (a) water, (b) fruit juice(s) and water, (c) fruit juice(s).

Such packing media may be used as such, or any one or any combination of two or more safe and suitable nutritive carbohydrate sweetener(s) may be added. When a sweetener is added as a part of any such liquid packing medium, the density range of the resulting packing medium expressed as percent by weight of sucrose (degrees Brix) should be designated by the appropriate name for the respective density ranges, namely:

When the density of the solution is 10 percent or more but less than 16 percent, the medium should be designated as "slightly sweetened water" or "extra light syrup," "slightly sweetened fruit juice(s) and water" or "slightly sweetened fruit juice(s)," as the case may be.

When the density of the solution is 16 percent or more but less than 21 percent, the medium should be designated as "light syrup," "lightly sweetened fruit juice(s) and water," or "lightly sweetened fruit juice(s)," as the case may be. When the density of the solution is 21 percent or more but less than 25 percent, the medium should be designated as "heavy syrup," "heavily sweetened fruit juice(s) and water," or "heavily sweetened fruit juice(s)," as the case may be.

When the density of the solution is 25 percent or more but not more than 40 percent, the medium should be designated as "extra heavy syrup," "extra heavily sweetened fruit juice(s) and water," or "extra heavily sweetened fruit juice(s)," as the case may be.

3. Labeling Requirements

The name of the food is apricots. The name of the food should also include a declaration of any flavoring that characterizes the product and a declaration of any spice or seasoning that characterizes the product; for example, "Spice Added," or in lieu of the word "Spice," the common name of the spice, e.g., "Seasoned with Vinegar" or "Seasoned with Apricot Kernels."

When two or more of the optional ingredients specified are used, such words may be combined, as for example, "Seasoned with Cider Vinegar, Cloves, Cinnamon Oil, and Apricot Kernels."

The style of the apricot ingredient and the name of the packing medium preceded by "In" or "Packed in" or the words "Solid Pack," where applicable, should be included as part of the name or in close proximity to the name of the food, except that pieces or irregular pieces should be designated "Pieces," "Irregular Pieces," or "Mixed Pieces of Irregular Sizes and Shapes."

The style of the apricot ingredient should be preceded or followed by "Unpeeled" or "Peeled," as the case may be. "Halves" may be alternatively designated "Halved," "Quarters" as "Quartered," and "Slices" as "Sliced." When the packing medium is prepared with a sweetener(s) that imparts a taste, flavor, or other characteristic to the finished food in addition to sweetness, the name of the packing medium should be accompanied by the name of such sweetener(s), for example, in the case of a mixture of

brown sugar and honey, an appropriate statement would be "_____syrup of brown sugar and honey" the blank to be filled in with the word "light," "heavy," or "extra heavy," as the case may be.

When the liquid portion of the packing media consists of fruit juice(s), such juice(s) should be designated in the name of the packing medium as follows.

In the case of a single fruit juice, the name of the juice should be used in lieu of the word "fruit."

In the case of a combination of two or more fruit juices, the names of the juices in the order of predominance by weight should be used in lieu of the word "fruit" in the name of the packing medium.

In the case of a single fruit juice or a combination of two or more fruit juices any of which are made from concentrate(s), the words "from concentrate(s)" should follow the word "juice(s)" in the name of the packing medium and in the name(s) of such juice(s) when declared as specified.

Whenever the names of the fruit juices used do not appear in the name of the packing medium, such names and the words "from concentrate," should appear in an ingredient statement.

4. Label Declaration

Each of the ingredients used in the food should be declared on the label.

Frozen apricots are prepared from sound, mature, fresh, peeled or unpeeled fruit of any commercial variety of apricot, which are sorted, washed, and may be trimmed to assure a clean and wholesome product. The apricots are properly drained of excess water before filling into containers; may be packed with the addition of nutritive sweetening ingredient(s) (including syrup and/or syrup containing pureed apricots) and/or suitable antioxidant ingredient(s) and/or any other legally permissible ingredients(s).

The apricots are prepared and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product.

5. Styles of Frozen Apricots

(a) Halves are cut approximately in half along the suture from stem to apex and the pit is removed.
(b) Quarters are apricot halves cut into two approximately equal parts.
(c) Slices are apricot halves cut into sectors smaller than quarters.
(d) Diced are apricots cut into approximate cubes.
(e) Cuts are apricots that are cut in such a manner as to change the original conformation and do not meet any of the foregoing styles.
(f) Machine-pitted means mechanically pitted in such a manner as to substantially destroy the conformation of the fruit in removing the pit.

C. Berries

Frozen berries are prepared from the properly ripened fresh fruit of the plant (genus *48 Rubus);* are stemmed and cleaned, may be packed with or without packing media, and are frozen and stored at temperatures necessary for the preservation of the product.

1. Types of Frozen Berries

(a) Blackberries
(b) Boysenberries
(c) Dewberries
(d) Loganberries
(e) Youngberries
(f) Other similar types, such as nectar berries

2. Blueberries

Frozen blueberries are prepared from sound, properly ripened fresh fruit of the blueberry bush (genus *Vaccinium),* including species or varieties often called huckleberries, but not of the genus *Gaylussacia;* they are cleaned and stemmed, are properly washed, are packed with or without packing media, and are frozen and maintained at temperatures necessary for the preservation of the product.

Types of frozen blueberries are (a) native or wild type; (b) cultivated type.

D. Red Tart Pitted Cherries

Frozen red tart pitted cherries are the foods prepared from properly matured cherries of the domestic *(Prunes cerasus)* red sour varietal group that have been washed, pitted, sorted, and properly drained; they may be packed with or without a nutritive sweetened packing medium or any other substance permitted under federal regulations and are frozen and stored at temperatures necessary for the preservation of the product.

II. FROZEN JUICES

A. Apple Juice

Frozen concentrated apple juice is prepared from the unfermented, unsweetened, unacidified liquid obtained from the first pressing of properly prepared, sound, clean, mature, fresh apples, and/or parts thereof by good commercial processes. The juice is clarified and concentrated to at least 22.9° Brix. The apple juice concentrate so prepared, with or without the addition of legal ingredients, is packed and frozen in accordance with good commercial practice and maintained at temperatures necessary for the preservation of the product.

The Brix value of the finished concentrate should not be less than the following for the respective dilution factor of frozen concentrated apple juice:

Dilution Factor Value of Concentrate: minimum Brix (degrees)

1 plus 1 22.9
2 plus 1 33.0
3 plus 1 42.2
4 plus 1 50.8
5 plus 1 58.8
6 plus 1 66.3
7 plus 1 73.3

B. Lemon Juice (for Preparing Frozen Concentrate for Lemonade)

Lemon juice is the unfermented juice, obtained by mechanical process, from sound, mature lemons (*Citrus limon* (L.) Burm. f.), from which seeds (except embryonic seeds and small fragments of seed which cannot be separated by good manufacturing practice) and excess pulp are removed. The juice may be adjusted by the addition of the optional concentrated lemon juice ingredient in such quantity that the increase in acidity, calculated as anhydrous citric acid, does not exceed 15 percent of the acidity of the finished food. The lemon oil and lemon essence (derived from lemons) content may be adjusted in accordance with good manufacturing practice. The juice may have been concentrated and later reconstituted. When prepared from concentrated lemon juice, the finished food contains not less than 6 percent, by weight, of soluble solids taken as the refractometric sucrose value (of the filtrate), corrected to 20°C, but uncorrected for acidity, and has a titratable acidity content of not less than 4.5 percent, by weight, calculated as anhydrous citrus acid.

The food may contain one or any combination of the safe and suitable optional ingredients. Lemon juice may be preserved by heat sterilization (canning), refrigeration, freezing, or by the addition of safe and suitable preservatives. When sealed in a container to be held at ambient temperatures, it is preserved by the addition of safe and suitable preservatives or so processed by heat, before or after sealing, as to prevent spoilage.

1. Optional Ingredients

The optional safe and suitable ingredients are (i) concentrated lemon juice (lemon juice from which part of the water has been removed), (ii) water and/or lemon juice to reconstitute concentrated lemon juice in the manufacture of lemon juice from concentrate, and (iii) preservatives.

2. Labeling

The name of the food is "lemon juice" if the food is prepared from unconcentrated, undiluted liquid extracted from mature lemons; or if the food is prepared from unconcentrated, undiluted liquid extracted from mature lemons to which concentrated lemon juice is added to adjust acidity.

The name is "lemon juice from concentrate" or "reconstituted lemon juice" if the food is prepared from concentrated lemon juice and water and/or lemon juice; or if the food is prepared from lemon juice from concentrate and lemon juice.

Frozen concentrate for lemonade is the frozen food prepared from one or both of the lemon juice ingredients together with one or any mixture of safe and suitable nutritive carbohydrate sweeteners. The product contains not less than 48.0 percent by weight of soluble solids taken as the sucrose value.

When the product is diluted according to directions for making lemonade which should appear on the label, the acidity of the lemonade, calculated as anhydrous citric acid, should be not less than 0.70 gram per 100 milliliters, and the soluble solids should be not less than 10.5 percent by weight.

3. The Lemon Juice Ingredients

Lemon juice ingredients are lemon juice or frozen lemon juice or a mixture of these or concentrated lemon juice or frozen concentrated lemon juice or a mixture of these. For this purpose, lemon juice is the undiluted juice expressed from mature lemons of an acid variety, and concentrated lemon juice is lemon juice from which part of the water has been removed. In the preparation of the lemon juice ingredients, the lemon oil content may be adjusted by the addition of lemon oil or concentrated lemon oil in accordance with good manufacturing practice, and the lemon pulp in the juice as expressed may be left in the juice or may be separated.

Lemon pulp that has been separated, which may have been preserved by freezing, may be added in preparing frozen concentrate for lemonade, provided that the amount of pulp added does not raise the proportion of pulp in the finished food to a level in excess of that which would be present by using lemon juice ingredients from which pulp has not been separated. The lemon juice ingredients may be treated by heat, either before or after the other ingredients are added, to reduce the enzymatic activity and the number of viable microorganisms.

C. Frozen Concentrate for Artificially Sweetened Lemonade

Frozen concentrate for artificially sweetened lemonade conforms to the description for frozen concentrate for lemonade, except that in lieu of nutritive sweeteners it is sweetened with one or more of the artificial sweetening ingredients permitted by law, and the soluble solids specifications do not apply. When the product is diluted according to directions that should appear on the label, the acidity of the artificially sweetened lemonade, calculated

as anhydrous citric acid, should be not less than 0.70 gram per 100 milliliters. It may contain one or more safe and suitable dispersing ingredients serving the function of distributing the lemon oil throughout the food. It may also contain one or more safe and suitable thickening ingredients. Such dispersing and thickening ingredients are not legal food additives.

The name of the food is "frozen concentrate for artificially sweetened lemonade." The words "artificially sweetened" should be of the same size and style of type as the word "lemonade." If an optional thickening or dispersing ingredient is used, the label should bear the statement "_____ added" or "with added _____," the blank being filled in with the common name of the thickening or dispersing agent used. Such statement should be set forth on the label with such prominence and conspicuousness as to render it likely to be read and understood by the ordinary individual under customary conditions of purchase.

D. Frozen Concentrate for Colored Lemonade

Frozen concentrate for colored lemonade conforms to the description for frozen concentrate for lemonade, except that it is colored with a safe and suitable fruit juice, vegetable juice, or any such juice in concentrated form, or with any other legal color additive ingredient suitable for use in food, including legal artificial coloring.

The name of the food is "frozen concentrate for_____lemonade," the blank being filled in with the word describing the color, for example, "frozen concentrate for pink lemonade."

E. Grapefruit Juice

Grapefruit juice is the unfermented juice, intended for direct consumption, obtained by mechanical process from sound, mature grapefruit (*Citrus paradisi* Macfadven) from which seeds and peel (except embryonic seeds and small fragments of seeds and peel that cannot be separated by good manufacturing practice) and excess pulp are removed and to which may be added not more than 10 percent by volume of the unfermented juice obtained from mature hybrids of grapefruit. The juice may be adjusted by the addition of the optional concentrated grapefruit juice ingredients specified, but the quantity of such concentrated grapefruit juice ingredient added should not contribute more than 1 percent of the grapefruit juice soluble solids in the finished food. The grapefruit pulp, grapefruit oil, and grapefruit essence (components derived from grapefruit) content may be adjusted in accordance with good manufacturing practice. The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining essential composition and quality factors of the juice. It may be sweetened with the dry nutritive sweeteners. If the grapefruit juice is prepared from concentrate, such sweeteners in liquid form also may be used. When prepared from concentrated grapefruit juice, exclusive of added sweeteners, the finished food contains not less than 10 percent, by weight, of soluble solids taken as the refractometric sucrose value (of the filtrate), corrected to 20°C, and corrected for acidity by adding $(0.012 + 0.193x\text{-}0.0004x)$, where x equals the percent anhydrous citric acid in sample, to the refractometrically obtained sucrose value. Grapefruit juice, as defined in this paragraph, may be preserved by heat sterlization (canning), refrigeration, or freezing. When scaled in a container to be held at ambient temperatures, it is so processed by heat, before or after scaling, as to prevent spoilage.

1. Optional Ingredients

The optional ingredients are (a) concentrated grapefruit juice (grapefruit juice from which part of the water has been removed); (b) water and/or grapefruit juice to reconstitute concentrated grapefruit juice in the manufacture of grapefruit juice from concentrate; and (c) one or any combination of two or more of the dry or liquid forms of sugar, invert sugar syrup, dextrose, glucose syrup, and fructose.

The name of the food is "Grapefruit juice" if the food is prepared from unconcentrated, undiluted liquid extracted from mature grapefruit, or if the food is prepared from unconcentrated, undiluted liquid extracted from mature grapefruit to which concentrated grapefruit juice is added to adjust soluble solids.

"Grapefruit juice from concentrate" is the name if the food is prepared from concentrated grapefruit juice and water and/or grapefruit juice; or if the food is prepared from grapefuit juice from concentrate and grapefruit juice. The words "from concentrate" should be shown in letters not less than one-half the height of the letters in the words "grapefruit juice."

If any nutritive sweetener is added, the principal display panel of the label should bear the statement "sweetener added." If no sweetener is added, the word "unsweetened" may immediately precede or follow the words "grapefruit juice" or "grapefruit juice from concentrate."

F. Orange Juice

Orange juice is the unfermented juice obtained from mature oranges of the species *Citrus sinensis* or of the citrus hybrid commonly called "Ambersweet" [1/2 *Citrus sinensis* × 3/8 *Citrus reticulata* × 1/8 *Citrus paradisi* (USDA Selection: 1-100-29: 1972 Whitmore Foundation Farm)]. Seeds (except embryonic seeds and small fragments of seeds that cannot be separated by current good manufacturing practice) and excess pulp are removed. The juice may be chilled, but it is not frozen.

The name of the food is "orange juice." The name "orange juice" may be preceded on the label by the varietal name of the oranges used, and if the oranges grew in a

single State, the name of such State may be included in the name, as for example, "California Valencia orange juice."

G. Pasteurized Orange Juice

Pasteurized orange juice is the food prepared from unfermented juice obtained from mature oranges, to which may be added not more than 10 percent by volume of the unfermented juice obtained from mature oranges of the species *Citrus reticulata* or *Citrus reticulata* hybrids. Seeds (except embryonic seeds and small fragments of seeds that cannot be separated by good manufacturing practice) are removed, and pulp and orange oil may be adjusted in accordance with good manufacturing practice. If the adjustment involves the addition of pulp, then such pulp should not be of the washed or spent type. The solids may be adjusted by the addition of one or more of the optional concentrated orange juice ingredients. One or more of the optional sweetening ingredients may be added in a quantity reasonably necessary to raise the Brix or the Brix-acid ratio to any point within the normal range usually found in unfermented juice obtained from mature oranges. The orange juice is so treated by heat as to reduce substantially the enzymatic activity and the number of viable microorganisms. Either before or after such heat treatment, all or a part of the product may be frozen. The finished pasteurized orange juice contains not less than 10.5 percent by weight of orange juice soluble solids, exclusive of the solids of any added optional sweetening ingredients, and the ratio of the Brix hydrometer reading to the grams of anhydrous citric acid per 100 milliliters of juice is not less than 10 to 1.

The optional concentrated orange juice ingredients are frozen concentrated orange juice and concentrated orange juice for manufacturing when made from mature oranges; but the quantity of such concentrated orange juice ingredients added should not contribute more than one-fourth of the total orange juice solids in the finished pasteurized orange juice.

The optional sweetening ingredients referred to are sugar, invert sugar, dextrose, dried corn syrup, and dried glucose syrup.

The name of the food is "pasteurized orange juice." If the food is filled into containers and preserved by freezing, the label should bear the name "frozen pasteurized orange juice." The words "pasteurized" or "frozen pasteurized" should be shown on labels in letters not less than one-half the height of the letters in the words "orange juice."

If the pasteurized orange juice is filled into containers and refrigerated, the label should bear the name of the food, "chilled pasteurized orange juice." If it does not purport to be either canned orange juice or frozen pasteurized orange juice, the word "chilled" may be omitted from the name. The words "pasteurized" or "chilled pasteurized" should be shown in letters not less than one-half the height of the letters in the words "orange juice."

H. Frozen Concentrated Orange Juice

Frozen concentrated orange juice is the food prepared by removing water from the juice of mature oranges, to which may be added unfermented juice obtained from mature oranges of the species *Citrus reticulata,* other *Citrus reticulata* hybrids, or of *Citrus aurantiurn,* or both. However, in the unconcentrated blend, the volume of juice from *Citrus reticulata* or *Citrus reticulata* hybrids should not exceed 10 percent, and from *Citrus aurantium* should not exceed 5 percent. The concentrate so obtained is frozen. In its preparation, seeds (except embryonic seeds and small fragments of seeds that cannot be separated by good manufacturing practice) and excess pulp are removed, and a properly prepared water extract of the excess pulp so removed may be added. Orange oil, orange pulp, orange essence (obtained from orange juice), orange juice and other orange juice concentrate or concentrated orange juice for manufacturing (when made from mature oranges), water, and one or more of the optional sweetening ingredients may be added to adjust the final composition. The juice of *Citrus reticulata* and *Citrus aurantium,* as permitted by this paragraph, may be added in single strength or concentrated form prior to concentration of the *Citrus sinensis* juice, or in concentrated form during adjustment of the composition of the finished food. The addition of concentrated juice from *Citrus reticulata* or *Citrus aurantium,* or both, should not exceed, on a single-strength basis, the 10 percent maximum for *Citrus reticulata* and the 5 percent maximum for *Citrus aurantiumi* prescribed by this paragraph. Any of the ingredients of the finished concentrate may have been so treated by heat as to reduce substantially the enzymatic activity and the number of viable microorganisms. The finished food is of such concentration that when diluted according to label directions the diluted article will contain not less than 11.8 percent by weight of orange juice soluble solids, exclusive of the solids of added optional sweetening ingredients. The term "dilution ratio" means the whole number of volumes of frozen per volume of frozen concentrate required to produce orange juice from concentrate having orange juice soluble solids of not less than 11.8 percent by weight exclusive of the solids of any added optional sweetening ingredients.

The optional sweetening ingredients are sugar, sugar syrup, invert sugar, invert sugar syrup, dextrose, corn syrup, dried corn syrup, glucose syrup, and dried glucose syrup.

If one or more of the sweetening ingredients are added to the frozen concentrated orange juice, the label should bear the statement "_____added," the blank being filled in with the name or an appropriate combination of names of the sweetening ingredients used. However, the name "sweetener" may be used in lieu of the specific name or names of the sweetening ingredients.

The name of the food concentrated to a dilution ratio of is "frozen concentrated orange juice" or "frozen orange juice concentrate." The name of the food concentrated to a dilution ratio greater than 3 plus 1 is "frozen concentrated orange juice, _____plus l" the blank being filled in with the whole number showing the dilution ratio; for example, "frozen orange juice concentrate, 4 plus 1." However, where the label bears directions for making 1 quart of orange juice from concentrate (or multiples of a quart), the blank in the name may be filled in with a mixed number; for example, "frozen orange juice concentrate, $4^1/_3$ plus 1." For containers larger than 1 pint, the dilution ratio in the name may be replaced by the concentration of orange juice soluble solids in degrees Brix; for example, a 62° Brix concentrate in 3% gallon cans may be named on the label "frozen concentrated orange juice, 62° Brix."

I. Reduced Acid Frozen Concentrated Orange Juice

Reduced-acid frozen concentrated orange juice is the food that complies with the requirements for composition and label declaration of ingredients prescribed for frozen concentrated orange juice except that it may not contain any added sweetening ingredient. A process involving the legal use of anionic ion-exchange resins is used to reduce the acidity of the food so that the ratio of the Brix reading to the grams of acid, expressed as anhydrous citric acid, per 100 grams of juice is not less than 21 to 1 or more than 26 to 1.

The name of the food is "reduced acid frozen concentrated orange juice."

J. Orange Juice for Manufacturing

Orange juice for manufacturing is the food prepared for further manufacturing use. It is prepared from unfermented juice obtained from oranges as provided earlier, except that the oranges may deviate from the standards for maturity in that they are below the minimum for Brix and Brix-acid ratio for such oranges, and to which juice may be added not more than 10 percent by volume of the unfermented juice obtained from oranges of the species *Citrus reticulata* or *Citrus reticulata* hybrids (except that this limitation should not apply to the hybrid species). Seeds (except embryonic seeds and small fragments of seeds that cannot be separated by good manufacturing practice) are removed, and pulp and orange oil may be adjusted in accordance with good manufacturing practice. If pulp is added it should be other than washed or spent pulp. The juice or portions thereof may be so treated by heat as to reduce substantially the enzymatic activity and number of viable microorganisms, and it may be chilled or frozen, or it may be so treated by heat, either before or after sealing in containers, as to prevent spoilage.

The name of the food is "orange juice for manufacturing."

K. Orange Juice with Preservative

Orange juice with preservative is the food prepared for further manufacturing use. It complies with the requirements for composition of orange juice for manufacturing as specified, except that a preservative is added to inhibit spoilage. It may be heat-treated to reduce substantially the enzymatic activity and the number of viable microorganisms.

The preservatives referred to are any safe and suitable preservatives or combinations thereof.

The name of the food is "orange juice with preservative." Each of the ingredients used in the food should be declared on the label as required by regulations. In addition, the name of each preservative should be preceded by a statement of the percent by weight of the preservative used. If the food is packed in container sizes that are less than 19 liters (5 gallons), the label should bear a statement indicating that the food is for further manufacturing use only.

Wherever the name of the food appears on the label so conspicuously as to be easily seen under customary conditions of purchase, the statement for naming the preservative ingredient used should immediately and conspicuously precede or follow the name of the food, without intervening written, printed, or graphic matter.

L. Concentrated Orange Juice for Manufacturing

Concentrated orange juice for manufacturing is the food that complies with the requirements of composition and label declaration of ingredients prescribed, except that it is either not frozen or is less concentrated, or both, and the oranges from which the juice is obtained may deviate from the standards for maturity in that they are below the minimum Brix and Brix-acid ratio for such oranges: However, the concentration of orange juice soluble solids should not be less than 20° Brix.

The name of the food is "concentrated orange juice for manufacturing, _____" or "_____ orange juice concentrate for manufacturing," the blank being filled in with the figure showing the concentration of orange juice soluble solids in degrees Brix.

M. Concentrated Orange Juice with Preservative

(a) Concentrated orange juice with preservative complies with the requirements for composition and labeling of optional ingredients prescribed for concentrated orange juice for manufacturing by Sec. 146.153, except that a preservative is added to inhibit spoilage. (b) The preservatives referred to in paragraph (a) of this section are any safe and suitable preservatives or combinations thereof. (c) The name of the food is "concentrated orange juice

with preservative, _____," the blank being filled in with the figure showing the concentration of orange juice soluble solids in degrees Brix. (d) Label declaration. Each of the ingredients used in the food should be declared on the label as required by regulations. In addition, the name of each preservative should be preceded by a statement of the percent by weight of the preservative used. If the food is packed in container sizes that are less than 19 liters (5 gallons), the label should bear a statement indicating that the food is for further manufacturing use only.

N. Pineapple Juice

Pineapple juice is the juice, intended for direct consumption, obtained by mechanical process from the flesh or parts thereof, with or without core material, of sound, ripe pineapple *(Ananas comosus* L. Merrill). The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining essential composition and quality factors of the juice. Pineapple juice may contain finely divided insoluble solids, but it does not contain pieces of shell, seeds, or other coarse or hard substances or excess pulp. It may be sweetened with any safe and suitable dry nutritive carbohydrate sweetener. However, if the pineapple juice is prepared from concentrate, such sweeteners, in liquid form, also may be used. It may contain added vitamin C in a quantity such that the total vitamin C in each 4 fluid ounces of the finished food amounts to not less than 30 milligrams and not more than 60 milligrams. In the processing of pineapple juice, dimethylpolysiloxane may be employed as a defoaming agent in an amount not greater than 10 parts per million by weight of the finished food. Such food is prepared by heat sterilization, refrigeration, or freezing. When sealed in a container to be held at ambient temperatures, it is so processed by heat, before or after sealing, as to prevent spoilage.

The name of the food is "pineapple juice" if the juice from which it is prepared has not been concentrated and/or diluted with water. The name of the food is "pineapple juice from concentrate" if the finished juice has been made from specified pineapple juice. If a nutritive sweetener is added, the label should bear the statement "sweetener added." If no sweetener is added, the word "unswcetened" may immediately precede or follow the words "pineapple juice" or "pineapple juice from concentrate."

Each of the ingredients used in the food should be declared on the label.

O. Quality

The standard of quality for pineapple juice is as follows: (a) The soluble solids content of pineapple juice (exclusive of added sugars) without added water should not be less than 10.5° Brix as determined by refractometer at 20°C uncorrected for acidity and read as degrees Brix on International Sucrose Scales. Where the juice has been obtained using concentrated juice with addition of water, the soluble pineapple juice solids content (exclusive of added sugars) should be not less than 12.8° Brix, uncorrected for acidity and read as degrees Brix on the International Sucrose Scales. The acidity is not more than 1.35 grams of anhydrous citric acid per 100 milliliters of the juice. The ratio of the degrees Brix to total acidity is not less than 12. The quantity of finely divided "insoluble solids" is not less than 5 percent or more than 30 percent.

P. Blended Grape and Orange Juice

1. Product Description

Frozen concentrated blended grapefruit juice and orange juice is the frozen product prepared from a combination of concentrated, unfermented juices obtained from sound, mature grapefruit (*Citrus paradisi*) and from sound, mature fruit of the sweet orange group (*Citrus sinensis*) and Mandarin group (*Citrus reticulate*), except tangerines. The fruit is prepared by sorting and by washing prior to extraction of the juices to assure a clean product. The juices may be blended upon extraction of such juices or after concentration, and fresh orange juice extracted from sorted and washed fruit, as aforesaid, is admixed to the concentrate. It is recommended that the frozen concentrated blended grapefruit juice and orange juice be composed of the equivalent of not less than 50 percent orange juice in the reconstituted juice; however, in oranges yielding light-colored juice it is further recommended that as much as the equivalent of 75 percent orange juice in the reconstituted juice be used. The concentrated juice is packed in accordance with good commercial practice and is frozen and maintained at temperatures necessary for the preservation of the product.

2. Styles of Frozen Concentrated Blended Grapefruit Juice and Orange Juice

There are two styles: (a) Style I, without sweetening ingredient added. The Brix value of the finished concentrate should be not less than 40° or more than 44°; and (b) Style II, with sweetening ingredient added. The finished concentrate, exclusive of added sweetening ingredient, has a Brix value of not less than 38°; and the finished concentrate, including added sweetening ingredient, should have a Brix value of not less than 40° but not more than 48°.

ACKNOWLEDGMENT

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26 Milk Proteins

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CONTENTS

I. INTRODUCTION

Bovine milk contains approximately 3.5% protein, and is an important protein source for both man and developing neonate. The protein concentration of milk may vary quite considerably due to a variety of factors; breed and age of cow, stage of lactation, number of lactations and diet of cow exert influence upon both the overall protein concentration of milk and the proportion of the individual milk proteins.

About 100 years ago it was shown that the proteins in milk could be fractionated into two well-defined groups. When the pH of raw milk is adjusted to 4.6 at ~30°C, a precipitate containing 80% of the total milk protein is formed. This fraction is called casein and the remaining soluble material under these conditions is referred to as whey or serum proteins or non-casein nitrogen. Both casein and whey proteins are quite heterogeneous, as outlined in Figure 26.1. These two classes of milk proteins are considered separately due to the large differences in their structures and physico-chemical properties. Many reviews and monographs on the structures and properties of casein and whey proteins have been published (1–6). Most of the existing information on milk proteins has been compiled in the recently published Encyclopedia of Dairy Sciences (7).

This chapter provides an overview of the properties and structures of the main milk protein components. These topics have been covered in a greater detail in several text and reference books mentioned throughout this chapter.

II. CASEINS

For more than 50 years, it was believed that the casein fraction was a pure single entity. The application of moving boundary electrophoresis and sedimentation techniques in the late 1930s demonstrated the heterogeneity of the casein fraction. Three components were demonstrated and named α-, β- and γ-caseins in order of decreasing electrophoretic mobility and represented 75, 22 and 3%, respectively, of whole casein. In 1956, the α-casein

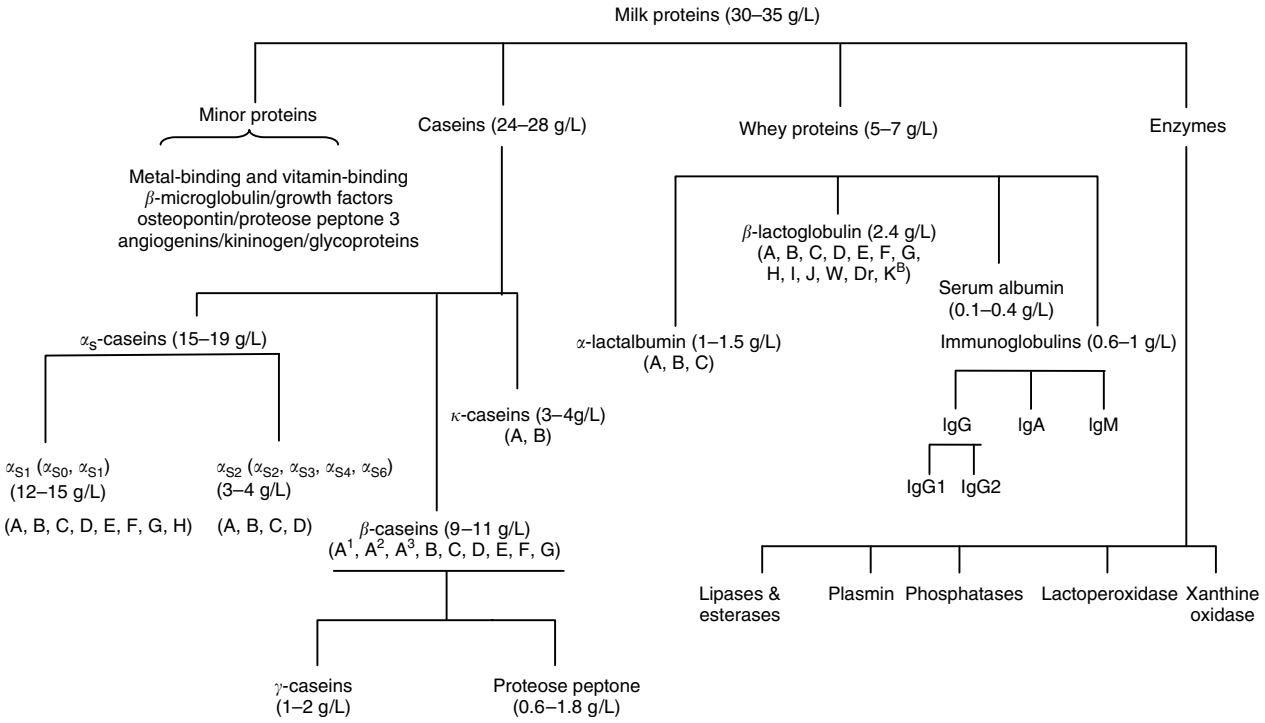


FIGURE 26.1 Distribution of fractions, and approximate concentrations and genetic variants of the major proteins in bovine milk. (Adapted from Ref. 3.)

fraction was shown to contain two proteins, one of which was precipitated by low Ca^{2+} concentration and was called α_s-casein, while the other, which was insensitive to calcium, was called κ-casein (8). α_s-Casein was later shown to consist of two proteins which are now called α_{S1}- and α_{S2}-caseins. Thus, bovine milk contains four major caseins, denoted as α_{S1}-casein, α_{S2}-casein, β-casein and κ-casein, which represent approximately 37, 10, 35 and 12% of the whole casein, respectively (9).

Minor components of the casein system are a heterogeneous group of proteins known as γ-caseins. These caseins occur as a result of limited proteolysis of the C-terminal of β-casein, caused by the action of the indigenous proteinase, plasmin (10). The γ-caseins are classified as γ^1-(β-casein f29-209), γ^2-(β-casein f106-209~11) and γ^3-(β-casein f108-209). The corresponding N-terminal portion of β-casein is the source of the so-called proteose peptones; PP5, PP8 fast and PP8 slow, i.e., β-CN f1-105/7, f1-28 and f29-105/7, respectively (see Figure 26.2). The proteose peptones elute in the whey fraction of milk.

Each of the four major caseins exhibits variability in the degree of phosphorylation and glycosylation. All caseins are phosphorylated: most of the α_{S1}-casein molecules contain 8 PO_4 residues but some contain 9; α_{S2}-casein contains 10, 11, 12 or 13 mol PO_4/mol; β-casein usually contains 5 mol PO_4/mol but occasionally 4 mol PO_4/mol; κ-casein contains 1 mol PO_4/mol but some molecules of κ-casein may contain 2 or 3 PO_4/mol. κ-Casein is the only casein which is normally glycosylated and contains galactose, galactosamine and N-acetyl neuraminic acid which occur as either trisaccharides or tetrasaccharides attached to threonine residues in the C-terminal region. κ-Casein may contain zero to four tri- or tetrasaccharide moieties, resulting in nine forms of κ-casein (4).

A further heterogeneity in caseins arises from the occurrence of genetic polymorphism, which is due to either substitutions or, rarely, deletions of amino acids in the caseins as a consequence of mutations causing changes in base sequences in the genes. Among the 21 variants in caseins (Figure 26.1), two are due to deletion of a segment in the α_{S1}-casein. The variants A and H of α_{S1}-casein differ from variant B by the absence of amino acid sequences 14–26 and 51–58, respectively. The deletion of residues 51–59 in α_{S2}-casein A leads to the occurrence of variant D. All other genetic variants of casein involve amino acid substitutions. As the casein system is very complex and heterogeneous, a logical nomenclature, recommended by the Nomenclature Committee of the American Dairy Science Association, has been adopted. The casein family is indicated by a Greek letter with a subscript (i.e., α_{S1}) followed by CN. The genetic variant is indicated by a Roman letter (A, B, C, etc.) with a superscript, if necessary (e.g., α_{S1}-CN A^2). The degree of phosphorylation is indicated as the number of phosphate residues, e.g., α_{S1}-CNB - 8P.

A. Casein Structures and Properties

The primary structures of the major caseins have been known since the early 1970s and are shown in Figures 26.3 a–d. In comparison to typical globular proteins, the structures of caseins are quite unique. Each of the caseins has distinct areas of positively and negatively charged groups in their primary structures resulting in the amphiphilic nature of the individual proteins (Figure 26.4). In addition, the hydrophobicity of each of the caseins varies considerably as a function of position on the peptide chain (Figure 26.4).

The caseins, compared to typical globular proteins which have mainly α-helical and β-sheet structures, contain less secondary and tertiary structures. Most of the secondary structure is likely to be present in the hydrophobic domains.

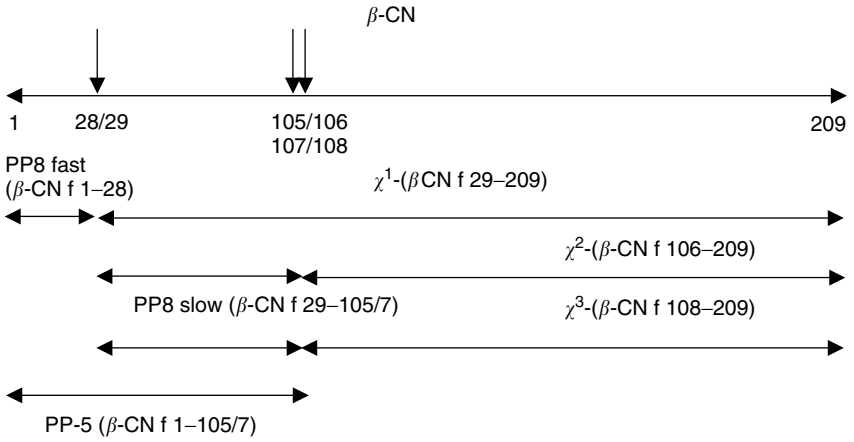


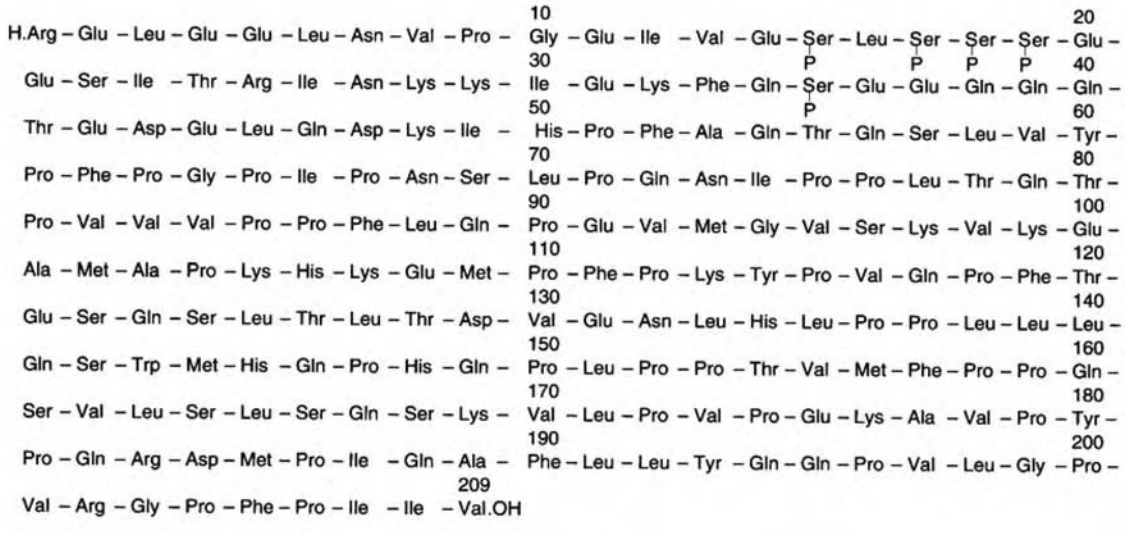
FIGURE 26.2 Principal plasmin cleavage sites on β-casein with resulting peptides.

FIGURE 26.3 (a) Primary structure of bovine α_{S1}-casein B. (Source: Mercier et al., Structure primaire de la caséine α_{S1}-bovine. Eur J Biochem 25:505–514, 1972, Blackwell Publishing Ltd.); (b) Primary structure of bovine α_{S2}-casein A. (Source: Federation of the European Biochemical Societies, from: Complete amino acid sequence of α_{S2}-casein, by Brignon et al., FEBS Lett 76:274–279, 1977.)

1. α_{S1}- and α_{S2}-Caseins

The primary structure of α_{S1}-casein is shown in Figure 26.3a. The α_{S1}-casein molecule contains 199 amino acids and 8 phosphate groups that are esterified to serine groups. It has no cysteine residues. The molecule has a net charge of about −24 at pH 6.7; the sequence 45 →89 which contains 8 phosphate groups and 12 carboxyl groups, has a net charge of about −23 at pH 6.7. Three hydrophobic regions are located in the sequences 1–44, 90–113 and 132–199 (4). The molecule contains 17 proline residues which are almost randomly distributed within the hydrophobic portion of the molecule and these residues disrupt the formation of α-helices and β-sheet structures. Physical methods, such as circular dichroism (CD) or Raman spectral analysis indicate that there is a low level of either α-helix or β-sheet (about 30–40%) (16). Sequence predictions suggest that the likely positions for helix formation are near residues 60, 100 and 125.

α_{S2}-Casein contains 207 amino acids, including 10 proline residues and two cysteines at positions 36 and 40 (Figure 26.3b). Among the caseins, it is the least hydrophobic and most highly and variably phosphorylated. The degree of phosphorylation ranges from 10 to 13 phosphate groups, and these casein forms have been identified as α_{S2}-, α_{S3}-, α_{S4}-, α_{S5}- and α_{S6}-caseins (α_{S5}- is a dimer of α_{S3}- and α_{S4}-). These phosphate groups are located in three regions (7–31, 55–66 and 129–143) of the molecule. There are two large hydrophobic regions: residues 90–120 and 160–207 (4). The C-terminal 47-residue sequence has a net charge of +9.5 while the N-terminal 68-residue sequence has a net charge of −21. The C-terminal half probably has a globular conformation while the N-terminal region probably forms a randomly structured hydrophilic tail.



(c)

(d)

FIGURE 26.3 (c) Primary structure of bovine β-casein A[2]. (Source: Ribadeau-Dumas et al., Structure primaire de la caséine β-bovine: séquence complète. Eur J Biochem 25:505–514, 1972, Blackwell Publishing Ltd.) (d) Primary structure of bovine κ-casein B. (Source: Mercier et al., Structure primaire de la caséine κ-bovine: sequence complète. Eur J Biochem 35:222–235, 1973. Blackwell Publishing Ltd.)

2. β-Casein

β-Casein is made up of 209 amino acids and contains a high proportion of proline residues (35 residues), which are randomly distributed throughout the molecule. The molecule contains no cysteine residues and 5 phosphate groups each as a serine phosphate ester. There are 6 forms of β-casein with 0–5 phosphate groups attached to serine residues (3). It is the most hydrophobic of the intact caseins and has two large hydrophobic regions (55–90 and 130–209). The N-terminal 21-residue sequence has a net charge of −12, while the rest of the molecule is very hydrophobic and has no net charge (4). Consequently, this molecule is very amphipathic with a polar domain comprising one-tenth of the chains but carrying one-third of the total charge and a hydrophobic domain consisting of the C-terminal three-fourths of the molecule.

Theoretical calculations suggest that β–casein could have 10% of its residues in the α-helices, 17% in the β-sheets and 70% in unordered structures (17).

3. κ-Casein

κ-Casein consists of 169 amino acids and is the only protein of the casein family that is glycosylated. About half of the κ-casein molecules are glycosylated at positions 131, 133, 135 or 142, and most of the κ-casein molecules are phosphorylated at Ser 149. Like β-casein, the structure of κ-casein is highly amphipathic. The N-terminal domain comprising residues 1–105 (para-κ-casein) is highly hydrophobic and carries a net positive charge. The

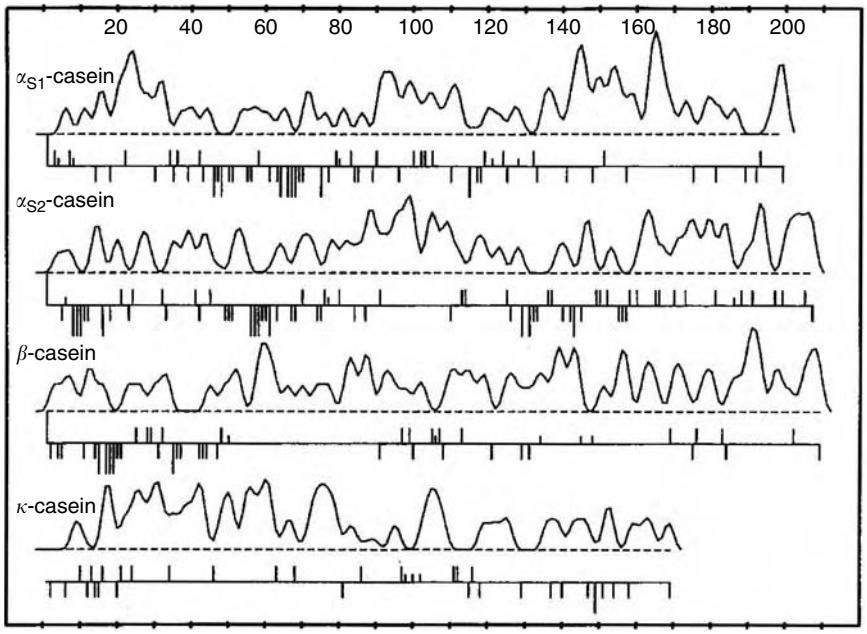


FIGURE 26.4 Hydrophobicity (curved lines with dashed line as baseline) and distribution of charged residues (vertical bars above and below the solid lines) as a function of sequence position of α_{S1}-casein B, α_{S2}-casein A, β-casein A^2 and κ-casein B. The vertical bars below the solid line indicate the phosphoserine (long bars), glutamic and aspartic acid side chains and the C-terminal group. The vertical bars above the solid line indicate the histidine (shorter bars), lysine and arginine side chains and the N-terminal groups (note that κ-casein has a pyroglutamate terminus). (Source: Casein and Caseinates, Swartz et al. In: Encyclopedia of Food Science and Technology. Copyright © (Wiley 1991). Reprinted by permission of John Wiley & Sons, Inc.)

C-terminal 53-residue sequence carries a net negative charge, with a preponderance of polar residues. These two domains are joined by a peptide (residues 96–112) that carries a net positive charge, which is generally predicted to be β-strand and contains a motif that is readily recognised by chymosin (4). Chymosin is able to cleave specifically the Phe105-Met106 bond to form two peptides: a large hydrophobic peptide (para-κ-casein, residues 1–105) and a smaller hydrophilic peptide (caseino-macropeptide, CMP, residues 106–169).

κ-Casein appears to be the most highly structured of the caseins, with 10–20% α-helix, 20–30% β-structure and 15–25% turns (16, 18). Several structural motifs have been suggested including possible anti-parallel and parallel β-sheets or βαβ structure in the hydrophobic domain and a β-turn-β-strand-β-turn motif centered on the chymosin sensitive residues 105–106 (18).

B. Self-Association of Caseins

Due to their peculiar charge distributions and large size of hydrophobic domains, the casein monomers cannot sufficiently remove their hydrophobic surfaces from contact with water. Consequently, the caseins associate with themselves and with each other. The association behavior of caseins has been covered in more detail in a number of reviews, including Rollema (19) and de Kruif & Holt (20).

At low ionic strength (0.003–0.01 M) and neutral or alkaline pH, α_{S1}-casein occurs as a monomer. As the pH is decreased and the ionic strength is increased, this protein shows progressive self-association to dimers, tetramers, hexamers, etc. Dynamic and static light scattering studies indicate that a rod-like chain polymer is formed (21).

The association behavior of monomeric α_{S2}-casein is similar to that of α_{S1}-casein, except for the effect of ionic strength. With increasing ionic strength, the extent of association first increases, but above 0.2 M, the degree of association decreases with a further increase in ionic strength (19). This peculiar behavior has been attributed to the non-uniform distribution of positive and negative charges along the polypeptide chain.

The self-association of β-casein is described by a monomer-polymer equilibrium:

$$n\beta\text{-}CN \rightleftharpoons \beta\text{-}CN_n$$

The association is characterized by formation of detergent-like micelles, with the critical micelle concentration ranging from 0.3 to 0.7 mg mL^{-1} depending on the temperature and ionic strength. The driving forces in micelle formation are the hydrophobic interactions between the C-terminal segments. At 4°C and low ionic strength, β-casein in solution exists as a monomer, but as the

temperature and/or ionic strength is increased, the monomers associate to form polymers, with *n* ranging from 20–60. For the shape of the polymer, a thread-like structure, a spherical particle and an oblate ellipsoid approaching spherical shape have been proposed (22).

In unreduced form, κ-casein is present largely as disulfide-linked polymers. In a reduced form, κ-casein associates according to the monomer-polymer equilibrium in a similar manner to β-casein. At low protein concentrations, a critical micelle concentration (typically 0.2–0.5 mg mL^{-1}) is obser-ved which decreases with increasing ionic strength. At pH 7 and 20°C, the degree of polymerization is independent of temperature between 4 and 20°C and ionic strength between 0.1 and 1.0 M (23). In contrast to β-casein association, κ-casein polymers are of a fixed size irrespective of temperature or ionic strength.

C. Calcium Binding and Precipitation of Caseins

All caseins are able to bind calcium, and the extent of binding is directly related to the number of phosphoserine residues in the molecule. Thus the calcium binding capacity follows the order: α_{S2}- > α_{S1}- > β- > κ-casein (19). Binding of calcium reduces the negative charge on casein molecules, diminishing electrostatic repulsions, and inducing precipitation.

At all temperatures, α_{S1}-casein B, α_{S1}-casein C and α_{S2}-caseins are insoluble in Ca^{2+} concentrations above about 4 mM. α_{S1}-Casein A, in which a relative hydrophobic region is deleted, is soluble at Ca^{2+} concentrations up to 0.4 M and temperatures below 33°C. Above 33°C, it precipitates but redissolves on cooling to 20°C. β-Casein is soluble at high concentrations of Ca^{2+} (0.4 M) at temperatures below 18°C, but above 18°C β-casein is very insoluble, even in the presence of low concentrations of Ca^{2+} (4 mM). κ-Casein, with only one phosphoserine, binds little calcium and remains soluble in Ca^{2+} at all concentrations. κ-Casein is also capable of stabilizing α_{S1}-, α_{S2}- and β-caseins against precipitation by Ca^{2+} and restricts the growth of the aggregates to colloidal dimensions (19).

D. Casein Micelles

In normal milk, about 95% of the casein proteins exist as coarse colloidal particles, called micelles, with diameters ranging from 80 to 300 nm (average ~150 nm). These particles are formed within the secretory cells of the mammary gland and undergo relatively little change after secretion. On a dry weight basis, the micelles consist of ~94% protein and ~6% of small ions, principally calcium, phosphate, magnesium and citrate, referred to collectively as colloidal calcium phosphate (CCP). The κ-casein content of casein micelles is inversely proportional to their size, while the content of CCP is directly related to size. The composition and some of the physico-chemical characteristics of casein micelles are presented in Table 26.1. The structure and properties of casein micelle have been reviewed recently by de Kruif and Holt (20) and Horne (25).

TABLE 26.1
Some Physicochemical Characteristics of Casein Micelles

Diameter	50–300 nm
Surface Area	8×10^{-10} cm^2
Volume	2×10^{-15} cm^3
Density	1.063 g cm^{-3}
Molecular weight (hydrated)	1.3×10^9 Da
Voluminosity	4.4 cm^3 per g protein
Hydration	2 g H_2O per g protein
Water content (hydrated)	63%

Source: Adapted from McMahon and Brown. J Dairy Sci 67:499–512, 1984. American Dairy Science Association.

The micelles are very open and highly hydrated structures containing about 2–4 g H_2O per g protein, depending on the method of measurement. The apparent zeta potential for casein micelles is about −19 mV at 25°C. The structure of the micelle is dynamic, e.g., cooling the milk to about 4°C causes solubilization of a significant proportion of β-casein and some κ-casein and much lower levels of α_{S1}- and α_{S2}-caseins. Almost complete disintegration of micelles occurs by addition of a calcium sequestrant such as EDTA or through the addition of high levels of urea or SDS (20).

The precise structure of the casein micelle is a matter of considerable debate at the present time. A number of models have been proposed over the past 40 years, but none of them can describe completely all aspects of casein micelle behavior. The models include coat-core models which postulate that the interior of the micelle is composed of proteins that are different from those on the exterior (26,27) and sub-unit structure models to which the term sub-micelle is attached (1,28,29).

In the sub-unit models (29), caseins are aggregated to form sub-micelles (10–15 nm in diameter). It has been suggested that sub-micelles have a hydrophobic core that is covered by a hydrophilic coat. The polar moieties of κ-casein molecules are concentrated in one area. The remaining part of the coat consists of the polar parts of other caseins, notably segments containing their phosphoserine residues. The sub-micelles are assumed to aggregate into micelles by CCP which would bind to α_{S1}-, α_{S2}- and β-caseins via their phosphoserine residues. Sub-micelles with no or low κ-casein are located in the interior of the micelle whereas κ-casein rich sub-micelles are concentrated on the surface.

Other models consider the micelle as a porous network of proteins (of no fixed conformation); the calcium phosphate nanoclusters are responsible for crosslinking the

protein and holding the network together (2). More specifically there are no subunits because individual polypeptide chains with two or more phosphate centers provide a network of strong interactions that link together most of the Ca-sensitive caseins in a micelle. The surface layer is a natural extension of the internal structure. A recent model proposed by Horne (30) assumes that the assembly of the casein micelle is governed by a balance of electrostatic and hydrophobic interactions between casein molecules. As stated earlier, α_{S1}-, α_{S2}- and β-caseins consist of distinct hydrophobic and hydrophilic regions. Two or more hydrophobic regions from different molecules form a bonded cluster. Growth of these polymers is inhibited by the protein charge residues, the repulsion of which pushes up the interaction free energy. Neutralization of the phosphoserine clusters by incorporation into the CCP diminishes that free energy as well as producing the second type of cross-linking bridge. κ-Casein acts a terminator for both types of growth, as it contains no phosphoserine cluster or another hydrophobic anchor point.

A common factor in all models is that most of the κ-casein appears to be present on the surface of casein micelles. The hydrophilic, C-terminal part of κ-casein, is assumed to protrude 5 to 10 nm from the micelle surface into the surrounding solvent, giving it a "hairy" appearance and providing a steric stabilizing layer. The highly charged flexible "hairs" physically prevent the approach and interactions of hydrophobic regions of the casein molecules.

E. Casein Micelle Stability

The micelles are stabilized by two principal factors: (1) a surface potential of C. −20 mV at pH 6.7 which alone is insufficient for colloidal stability and (2) steric stabilization due to protruding κ-casein hairs. Casein micelles can be caused to aggregate by several factors. Much attention has been focussed on the curd formation during cheese making brought about by the action of chymosin which destroys the stabilizing effect of κ-casein. Chymosin is highly specific in its action, splitting the κ-casein at the Phe105-Met106 bond, releasing the hydrophilic peptide and destabilising the micelles. This action results in a decrease in the micellar zeta potential from about −20 to –10 mV, and prior to aggregation a decrease in micellar hydrodynamic size as the hairy layer is cleaved off (20). Many other proteases with a more general action can also hydrolyze a specific bond of κ-casein, resulting in micelle aggregation.

Casein micelles aggregate and precipitate from solution when the pH is lowered to about pH 4.6. When the pH of milk is reduced, CCP is dissolved and the caseins are dissociated into the milk serum phase (31,32). The extent of dissociation of caseins is dependent on temperature of acidification; at 30°C, a decrease in pH causes virtually no dissociation; at 4°C about 40% of the caseins are dissociated in the serum at pH ~5.5 (32). Aggregation of casein occurs as the isoelectric point (pH 4.6) is approached. Apparently little change in the average hydrodynamic diameter of casein micelles occurs during acidification of milk to pH~5.0 (31). The lack of change in the size of micelles on reducing the pH of milk to 5.5 may be due to concomitant swelling of the particles as CCP is solubilized. The mobility of casein micelles measured by nuclear magnetic resonance spectroscopy does not change with pH (33).

Casein micelles are very stable at high temperatures, but they can aggregate and coagulate after heating at 140°C for 15–20 min. Such coagulation results from a number of changes in milk systems that occur during heating, including a decrease in pH, denaturation of whey proteins and their association with κ-casein, transfer of soluble calcium and phosphate into colloidal state, dephosphorylation of caseins and a decrease in hydration (34,35).

The micelles are also destabilized by addition of about 40% ethanol at pH 6.7 and by lower concentrations if the pH is reduced. This is due to the collapse and folding of the hairy layer of κ-casein in the non-solvent mixture, allowing micelles to interact and aggregate (36).

Freezing of milk has been shown to cause destabilization of casein micelles which is due to a decrease in pH and an increase in the Ca^{2+} concentration in the unfrozen phase of milk.

III. WHEY PROTEINS

The whey protein fraction accounts for approximately 20% of total protein. Whey proteins are an even more heterogeneous group of proteins than the caseins, containing a greater number of individual proteins. The principal fractions of whey proteins are β-lactoglobulin, bovine serum albumin, α-lactalbumin and immunoglobulins which account for more than 95% of the proteins in the whey fraction (Figure 26.1). These proteins have been well characterized. Major reviews covering structures and properties of whey proteins have been published (5,6,9,37). Unlike the caseins, the whey proteins possess high levels of secondary, tertiary and in most cases, quaternary structures. Most are typical globular proteins and are denatured by heat treatments. Both β-lactoglobulin and α-lactalbumin are synthesized in the mammary gland whereas serum albumin is transported to the mammary gland via the blood serum.

A. β-Lactoglobulin

β-Lactoglobulin is the most abundant whey protein which represents about 50% of the total whey protein in bovine milk. There are 13 known genetic variants of β-lactoglobulin: A, B, C, D, E, F, G, H, I, J, W, Dr and K^B (38). The A and B genetic variants are the most common and exist in almost the same frequency. The primary structure of

FIGURE 26.5 (a) Primary structure of bovine β-lactoglobulin B. (Source: Braunitzer et al., Automatische sequenzanalyse eines proteins (β-lactoglobulin AB). H-S Z Physiol Chem 353: 832–834, 1972, Walter de Gruyter GmbH & Co. KG.) (b) Primary structure of bovine α-lactalbumin B. (Source: Brew et al., The complete amino acid sequence of α-lactalbumin. J Biol Chem 245:4570–4582, 1970, The American Society for Biochemistry and Molecular Biology.)

β-lactoglobulin is shown in Figure 26.5a. The β-lactoglobulin monomer comprises 162 amino acids with one free thiol group (Cys 121) and two disulfide bridges (Cys 106-Cys119 and Cys 66-Cys160) and has a molecular weight of 18,000 Da. The A and B variants differ at positions 64 and 118, where Asp and Val in β-lactoglobulin A are replaced by Gly and Ala in β-lactoglobulin B.

β-Lactoglobulin is a highly structured protein; optical rotary dispersion, circular dichroism, infrared spectroscopy and nuclear magnetic resonance show around 10% α-helix, 50% β-sheet and 40% unordered structure including β-turns. The tertiary structure of β-lactoglobulin consists of nine anti-parallel β-strands, of which eight are wrapped into a β-barrel (Figure 26.6) (40,41). β-Strands A-D form one side of the calyx, and β-strands E-H are also part of strand A from the opposite side. The ninth strand, I, is on the outside, on the opposite side of strand A to strand H. The three-turn α-helix is located on the outside of the barrel and aligned along strands A, G and H.

In milk, native β-lactoglobulin occurs as a dimer. The association behavior of this protein is dependent on several parameters, including pH, temperature, protein concentration and ionic conditions. Below pH 3.5, β-lactoglobulin dissociates into its monomers, whereas between pH 3.5 and 5.2 it reversibly forms tetramers/octamers. Above pH 7.5, it starts to unfold, with a concomitant increase in the reactivity of thiol group. The proportion of dimer increases with increasing protein concentration and ionic strength. At temperatures between 30 and 55°C, the β-lactoglobulin dimer dissociates into monomers and at higher temperature (above 60°C), the monomer unfolds resulting in an increased reactivity of the free thiol group (38).

The biological function of β-lactoglobulin remains unclear but it appears to have at least two roles. Firstly, β-lactoglobulin is capable of binding several hydrophobic molecules, including retinol (vitamin A) (38). Retinol is bound in a hydrophobic pocket of β-lactoglobulin and transported to the small intestine where it is transferred to a retinol-binding protein, which has a structure similar to β-lactoglobulin. This binding also protects retinol against oxidation. Secondly, through its ability to bind fatty acids, β-lactoglobulin stimulates lipase activity.

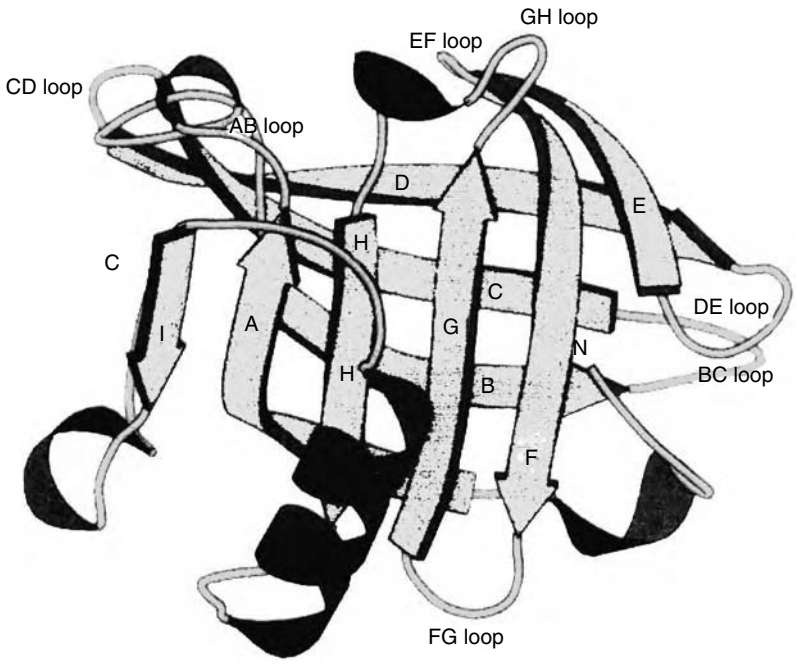


FIGURE 26.6 Ribbon diagram of a single subunit of β-lactoglobulin lattice X, with nine anti-parallel β-strands and with joining loops labeled. (Source: Structure, 5, Brownlow et al., Bovine β-Lg at 1.8Å resolution — still an enigmatic lipocalin, pp 481–495. Copyright (1997) Elsevier.)

B. α-Lactalbumin

α-Lactalbumin accounts for about 20% of whey protein and has three known genetic variants. The primary structure of α-lactalbumin is shown in Figure 26.5b. α-Lactalbumin has a molecular weight of about 14,000 Da and contains four intrachain disulphide bonds. It is relatively rich in tryptophan (four residues per mole). There is a considerable similarity between the primary and tertiary structure of α-lactalbumin and chicken egg white lysozyme (43). Its secondary structure at physiological pH consists of 26% α-helix, 14% β-structure and 60% unordered structure. The approximate globular structure of α-lactalbumin (dimensions 23Å × 26Å × 40Å) includes three regular α-helices, two regions of 3_{10} helix, and a small 3-stranded anti-parallel β-pleated sheet separated by irregular β-turns. The bilobal structure is formed by segregation of α-helices in one lobe and a small β-sheet and irregular structures in the other (Figure 26.7).

α-Lactalbumin binds one Ca^{2+} per mole in a pocket containing four Asp residues (45). At pH below 5.0, the Asp residues become protonated which results in the loss of bound Ca^{2+} which makes this protein susceptible to denaturation. Under acidic conditions, α-lactalbumin undergoes a transconformation to a non-native (A state) with altered spectroscopic properties (46). In the A state, the secondary structure of α-lactalbumin is nearly as compact as the native protein but lacks a fixed tertiary structure; this state is commonly referred to as the molten globule state.

The biological function of α-lactalbumin is to modulate the substrate specificity of galactosyltransferase in the lactose synthetase complex which is responsible for the synthesis of lactose in the lactating mammary tissue (45). The concentration of lactose in milk is directly related to the concentration of α-lactalbumin; the milk of some marine mammals which contains no α-lactalbumin, co tains no lactose.

C. Serum Albumin

Serum albumin isolated from milk is identical to the serum albumin found in the blood and represents about 5% of the total whey proteins. The protein is synthesized in the liver and gains entrance to milk through the secretory cells. The protein has the longest single polypeptide chain of the proteins found in milk consisting of 582 amino acid residues and has a molecular weight of 66,000 Da. It has 17 disulphide linkages, which hold the protein in a multi-loop structure, and one free thiol group. The secondary structure of serum albumin has 55% α-helix, 16% β-pleated sheet and 29% unordered structure. The current view of the three-dimensional crystal structure of serum albumin is that the molecule exists in three major domains, each consisting of two large double loops and a small double loop with an overall elliptical shape (47).

Serum albumin appears to function as a carrier of small molecules, such as fatty acids, but any specific role that it may play is unknown.

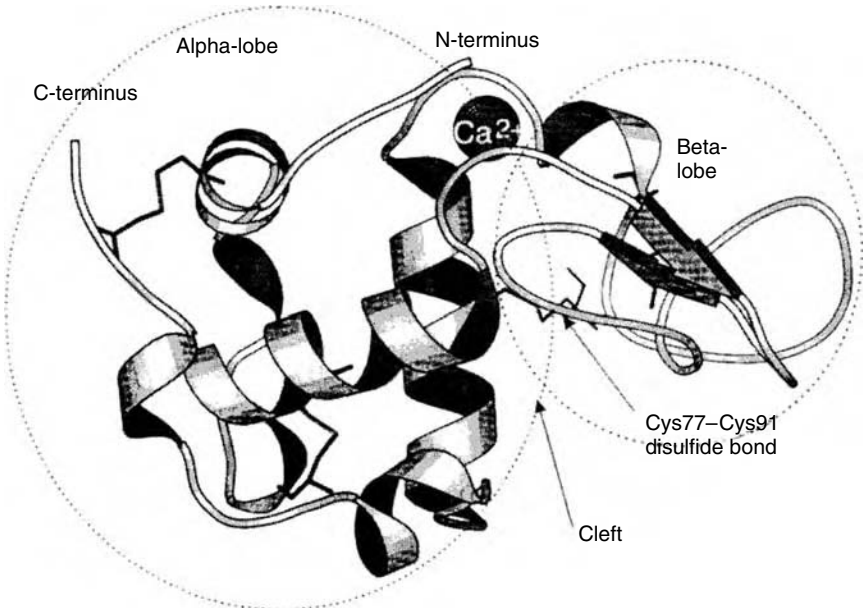


FIGURE 26.7 Tertiary structure of bovine α-lactalbumin, showing bilobal structure displayed using MOLSCRIPT. (Source: Brew, α-Lactalbumin, pp 387–421, 2003. In Advanced Dairy Chemistry Volume 1: Proteins, 3rd ed., Part A: Kluwer Academic/Plenum Publishers.)

D. Immunoglobulins

Milk-borne immunoglobulins (Igs) provide the offspring with an immunological protection against microbial pathogens and toxins and protect the mammary gland against infection. Igs are divided into classes and subclasses; essentially there are 5 classes of Ig: IgA, IgG, IgD, IgE and IgM. IgA, IgG and IgM are present in milk. These occur as subclasses, e.g., IgG occurs as IgG_1 and IgG_2. IgG consists of two light (L) polypeptide chains and two heavy (H) polypeptide chains, of molecular weights 22,400 and 50–60,000 Da, respectively; these are linked together by disulphide bonds. IgA consists of two such units (i.e., 8 chains) linked together by a secretory component (SC) and a junction component (J) while IgM consists of five linked 4-chain units. The concentration of different Ig classes in milk and colostrum differs considerably depending on species, breed, age, stage of lactation and health status. As ruminant neonates are born virtually without Ig, ingested Igs are necessary for survival. Consequently, ruminants' colostrum contains considerably higher concentrations of Igs than human milk.

In addition to antigen binding, all Igs exhibit one or more effector functions by linking various parts of the immune system. For example, Igs may bind to leucocytes (which are an integral part of milk and of vital importance in defending the mammary gland against pathogens) or to host tissues. In addition, Igs can prevent the adhesion of microbes to surfaces, inhibit bacterial metabolism, agglutinate bacteria and neutralize toxins and viruses. Milk Igs have also been found to exert a synergistic effect on the activity of non-specific antimicrobial factors such as lactoferrin and lysozyme as well as lactoperoxidase. A more detailed discussion on the structure, origin, transfer and function of Igs has been published by Hurley (48).

IV. Minor Proteins

Several proteins are found in relatively small quantities in the whey fraction of milk. These proteins are referred to as minor protein. They include metal- and vitamin-binding proteins, β_2-microglobulin and growth factors.

A. Metal Binding Proteins

Human milk contains 2–4 mg mL^{-1} of lactoferrin which is specifically involved in metal-binding. Lactoferrin, which appears identical to transferrin found in blood, has also been detected at quite low concentrations in bovine milk (49). Lactoferrin has the ability to bind iron very strongly, indicating that the protein is involved in iron absorption and protection against enteric infection in the neonate (49). Considerable interest has been expressed in supplementing bovine milk-based infant formulae with lactoferrin, as bovine milk contains much lower levels of lactoferrin than human milk and lactoferrin, isolated from human milk, can bind 2 moles of iron per mole of protein (50). The biological importance of lactoferrin has been reviewed recently by Lönnerdal (51).

B. Vitamin Binding Proteins

Milk contains an array of vitamin-binding proteins, including Vitamin B_{12}-binding protein, folate-binding

protein, Vitamin D-binding protein and riboflavin-binding protein. These proteins occur at low concentrations, but may play a significant role in the uptake of vital vitamins from the diet (52,53).

Three different proteins are required for the uptake of Vitamin B_{12} in the gastro-intestinal tract. The milk of several mammalian species (human, rat, pig and rabbit) has been shown to contain one of these proteins (haptocorrin), whereas bovine milk contains a different Vitamin B_{12}-binding protein (transcobalamin) (54).

Milk also contains folate-binding proteins; these proteins are specifically involved in the uptake of folate from the intestine. *In vivo* studies on rats have shown that protein-bound folate is absorbed at a lower rate than free folate, resulting in increased retention time of folate, allowing it to reach its target tissues. Folate-binding protein also reduces the availability of folate to bacteria in the gut and hence may have antibacterial properties (55).

Raw bovine milk contains a riboflavin binding protein (56) and riboflavin bound to this milk protein has been shown to have similar antioxidant activities to riboflavin bound to egg white riboflavin binding protein (57).

C. Growth Factors

Highly potent hormone-like polypeptides, termed growth factors, which play a significant role in the regulation and differentiation of a variety of cells, can be found in milk. These growth factors may modulate growth and differentiation of a variety of cell types, modulate mammary development, and may also modulate neonatal development of the gastrointestinal (GI) tract. The growth factors are predominantly found in developing glands and colostrum, while milk may also contain comparatively lower concentrations of growth factors, depending on stage of lactation.

Growth factors and hormones identified in mammary secretions include lactoferrin (58), epidermal growth factor (EGF), transforming growth factor α (α-TGF) and β (β-TGF), insulin-like growth factor (IGF), insulin (59), basic fibroblast growth factor (basic FGF) (60) and prolactin (61). It is unclear whether many of these growth factors are targeted to promote growth of the mammary gland or the intestinal cells of the recipient neonate.

D. Other Minor Proteins

Several other minor proteins, including β_2-microglobulin, osteopontin, proteose peptone 3, angiogenins, kininogen and milk glycoproteins, have been identified in the whey fraction of milk. At present, relatively little information is available on the biological activities and functions of these proteins.

V. MILK ENZYMES

Milk contains in the region of 60 indigenous enzymes, approximately 20 of which have been isolated and characterized in great detail. The remaining 40 enzymes, whose presence has been demonstrated via their activity, are of little or no technological interest. The principal milk enzymes of technological importance are discussed below, and the details of these and other milk enzymes are detailed in Table 26.2. The reader is directed to Chapter 27 (this volume, "Enzymes of Significance to Milk and Dairy Products") for a comprehensive review of this area.

A. Lipases and Esterases

The ability of milk lipase (EC 3.1.1.) to hydrolyze triglycerides to form fatty acids is well known, and is responsible for the onset of rancidity in milk and milk products. Milk lipase is a lipoprotein lipase, and is similar to the lipoprotein lipase found in the mammary gland, suggesting that it may be transferred to milk from the mammary gland. In bovine milk, about 80% of milk lipase is associated with the casein micelles by electrostatic bonding

TABLE 26.2
Some Enzymes Present in Bovine Milk

Name	EC Number	Optimum		Inactivation[1]
		pH	Temperature (°C)	
Xanthine oxidase	1.1.3.22	~8	37	7 min 73°C
Sulphydryl oxidase	1.8.?	~7	~45	3 min 73°C
Catalase	1.11.1.6	7	37?	2 min 73°C
Lactoperoxidase	1.11.1.7	6.5	20	10 min 73°C
Superoxidase dismutase	1.15.1.1	?	?	70 min 76°C
Lipoprotein lipase	3.1.1.34	~9	33	30 s 73°C
Alkaline phosphatase	3.1.3.1	~9	37	20 s 73°C
Ribonuclease	3.1.27.5	7.5	37	?
Plasmin	3.4.21.7	8	37	40 min 73°C

[1] Heat treatment required to reduce activity to approximately 1%.

Source: Ref. 62, page 92, by courtesy of Marcel Dekker, Inc.

between negatively charged phosphates on the caseins and positively charged amino acid residues in the heparin-binding sites of milk lipase (63).

Milk lipase is a glycoprotein which is optimally active at pH 9.2 and 37°C, and has a native molecular mass of 100 kDa. Milk lipase is strongly inhibited by the products of its activities (64), i.e., long-chain fatty acids; therefore blood serum albumin (BSA) and Ca^{2+} enhance the activity of milk lipase as BSA and Ca^{2+} bind free fatty acids. The enzyme is inactivated by ultraviolet light, heat, acid and oxidizing agents. Less than 10% of milk lipase remains active after pasteurization.

In milk triglycerides, long-chain fatty acids are attached to the glycerol at the 1 and 2 positions, while shorter-chain fatty acids are found at the 3 position. Milk lipase liberates fatty acids from the 1,3 positions in milk triglycerides. Lipolysis increases the levels of short-chain, volatile fatty acids present in milk. This results in rancid, butyric, bitter, soapy or astringent flavors that render milk and milk products unacceptable for consumption.

Increased lipolysis can result from vigorous agitation or homogenization of milk which causes damage to the milk fat globule membrane and leaves it vulnerable to the action of milk lipase. The extent of lipolysis may be reduced on strict temperature control (< 5°C) and avoiding excessive agitation prior to pasteurization (64).

Milk also contains three types of esterases. These are A-type carboxylic ester hydrolase (which hydrolyzes aromatic residues); B-type esterase (which hydrolyzes aliphatic esters rapidly and aromatic esters slowly); and C-type esterase (which are active on alkaline esters) (65).

B. Plasmin

Plasmin (EC 3.4.21.7), the principal proteolytic enzyme in milk, is mainly found in the form of the inactive zymogen, plasminogen. Through a system of plasmin inhibitors and plasminogen activators (along with their associated inhibitors), plasminogen is converted to active plasmin (10). Plasmin is a serine proteinase with a high specificity for peptide bonds to which lysine or arginine supplies the carboxyl group. β- and α_{S1}-caseins are most susceptible to plasmin hydrolysis; α_{S2}-casein is also attacked, while κ-casein is relatively resistant to hydrolysis. Plasmin cleavage of β-casein yields γ-casein and proteose-peptones (66,67) (see Figure 26.2).

Severe heat treatment of milk, such as ultrahigh temperature (UHT), reduces plasmin activity due to inactivation of plasmin by thiol-disulphide interchange reactions between plasmin and the highly reactive thiol groups of β-lactoglobulin (68). However, high-temperature-short-time (HTST) pasteurized milk stored at 20–37°C displayed significantly increased plasmin activity as storage time increased (69). This increase in plasmin activity, concomitant with a decrease in plasminogen activity, is due to denaturation of inhibitors of plasminogen activator (10).

Plasmin activity may influence the quality of dairy products, such as cheeses, UHT-treated milk products and milk protein products. Increased plasmin activity in cheese has been shown to improve the flavor and overall quality of certain cheeses. Plasmin may also play a role in the age gelation of UHT-treated milk.

C. Phosphatases

Bovine milk contains a number of indigenous phosphatases; the two principal types which have been shown to have a technological significance in milk are milk alkaline phosphatase (EC 3.1.3.1) and milk acid phosphatase (EC 3.1.3.2).

1. Alkaline Phosphatase (ALP)

ALP occurs in all mammalian milk; in bovines milk levels vary between cows and also within the lactation period. The activity of ALP is used as an index of the efficiency of milk pasteurization, because ALP is slightly more resistant to heat than *Mycobacterium tuberculosis* (70). However, the activity of ALP may not always be an accurate indicator of HTST pasteurization of milk for a number of reasons. Under certain conditions, reactivation of ALP may occur, complicating interpretation of test results. The enzyme also appears to be fully inactivated at sub-pasteurization conditions and in addition the relationship between $\log_{10}$ % initial activity and pasteurization equivalent (PE) is less linear than the relationship between PE and lactoperoxidase or γ-glutamyl transpeptidase activities in milk (53). Furthermore, ALP is only partially inactivated during ultra-high pressure treatment of milk, rendering the ALP test an unsuitable indicator of effectiveness of the ultra-high pressure treatment process.

2. Acid Phosphatase (ACP)

The concentration of ACP in milk is dependent on stage of lactation and increased levels have been observed in mastitic milk; however, the activity of ACP is much lower than that of ALP. ACP is a very heat stable enzyme which is not affected by HTST pasteurization, but is completely inactivated following UHT treatment (72).

ACP may play a role in the heat stability of dairy products. As the caseins are phosphoproteins and are good substrates for ACP, the micellar integrity of caseins may be lost on dephosphorylation of casein serine residues. ACP may also influence cheese flavor; several partially dephosphorylated phosphopeptides have been isolated from different cheese types and have been attributed to ACP activity (73,74).

D. Lactoperoxidase

Lactoperoxidase (LPO; EC 1.11.1.7) is a broad specificity peroxidase which is present in high concentrations in bovine milk compared to human milk. LPO exhibits

antibacterial activity in the presence of H_2O_2 and thiocyanate (SCN^-), in which SCN^- is converted to hypothiocyanate ($OSCN^-$) (75). Inactivation of eight different bacteria including *E. coli*, *Pseudomonas fluorescens*, *S. aureus*, *Enterococcus faecalis*, *Listeria innocua* and *Lactobacillus plantarum*, has been shown by LPO (76). LPO may also be used as an indication of mastitic activity, as LPO levels increase during mastitis. Research has focused on activation of the indigenous enzyme for cold pasteurization of milk and protection of the mammary gland against mastitis, and also on addition of isolated LPO to calf or piglet milk to protect against enteritis.

E. Xanthine Oxidase

Bovine milk xanthine oxidase (EC 1.1.3.22) is a dimeric metallo-flavoprotein with a molecular mass of approximately 300 kDa and is a major component of the milk fat globule membrane. It is a non-specific oxidoreductase that plays a metabolic role in purine catabolism, catalyzing the oxidation of hypoxanthine to xanthine, and xanthine to uric acid, with the concomitant reduction of O_2 to H_2O_2. Activity levels of xanthine oxidase differ between species; human milk contains approximately 1–6% of the activity of bovine milk (35 mg/L) (77). About 33% of the iron and all of the molybdenum in human milk is found in xanthine oxidase.

VI. INDUSTRIAL MANUFACTURE OF MILK PROTEINS

A range of protein products can be obtained from milk, and the more commonly used methods for obtaining these products are described in Figure 26.8. The production and utilization of milk proteins has been reviewed recently by Mulvihill and Ennis (78).

A. Casein Products

The manufacture of this protein family of products from milk involves centrifugal separation of the skim milk from the cream, followed by pasteurization. The caseins are then precipitated either by the action of a coagulant such as rennet or by a reduction in pH to the isoelectric point (4.6) by fermentation or direct addition of mineral acid. The coagulated protein is heated to form the curd, and the curd is then separated from the whey by filtration or centrifugation, in combination with countercurrent washing with water. The curd may then be dried as insoluble casein, or first be reacted with alkali, e.g., sodium hydroxide, followed by drying to produce a water-soluble caseinate. A protein product including both the casein and the whey proteins, known as total milk proteinate, may be manufactured by a variation of the process described in Figure 26.8.

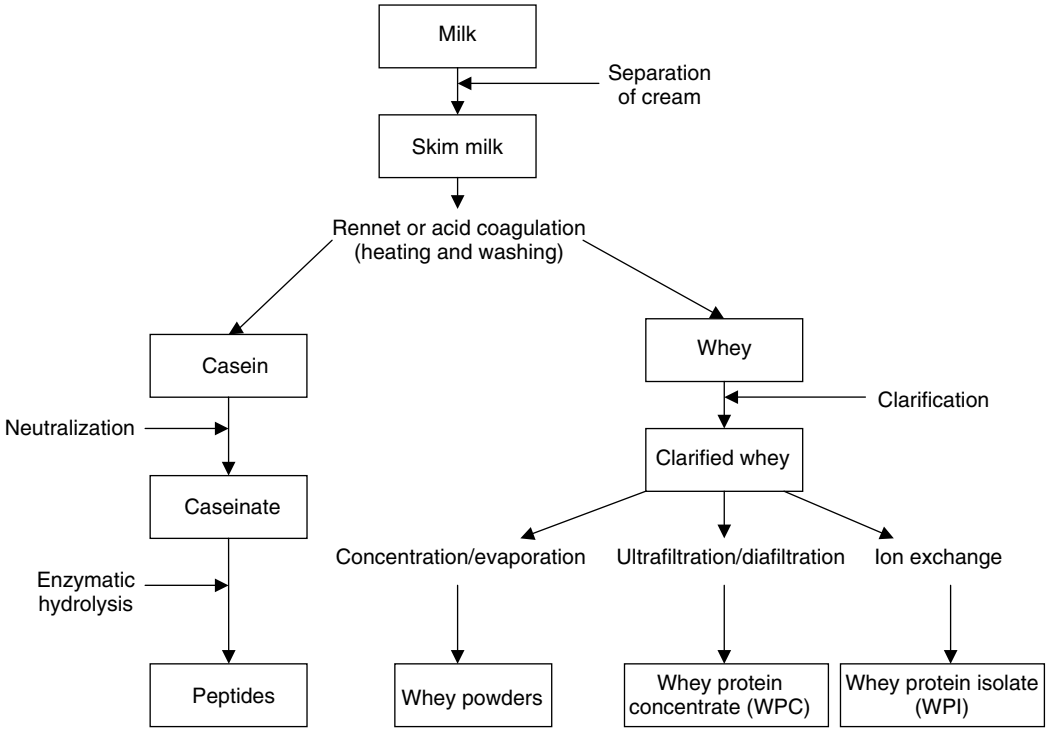


FIGURE 26.8 Outline of principal processes used for the manufacture of casein and whey proteins products.

B. Whey Products

Whey is produced as a by-product of cheese and casein manufacture. There are many possible products and manufacturing processes, some of which are outlined in Figure 26.8. The first step involves separation and selective concentration of residual fat and casein by centrifugation. This is followed by concentration of the whey proteins by the use of membrane separation (ultrafiltration and diafiltration). The protein stream is further concentrated by evaporation and then spray-dried to produce whey protein concentrate, with an approximate protein concentration of 85%. Alternatively, further fractionation and concentration of the whey proteins may be performed using ion exchange to produce whey protein isolates (containing approximately 95% protein).

The protein products described have a wide range of food ingredient and industrial applications, many utilizing the emulsifying, water- and fat-binding, and nutritional properties of the proteins. The decision to incorporate whey protein isolate ahead of whey protein concentrate into a food matrix is based on a balance of cost, fat content, and functional properties differences, among others, between the products.

VII. FUNCTIONAL PROPERTIES OF MILK PROTEINS AND FOOD USES

Protein functionality has been broadly defined as any physiochemical property which affects the processing and behavior of proteins, as judged by the quality attributes of the final product (79). These attributes may include structure, appearance, texture, viscosity and mouthfeel (80). Milk proteins possess functional properties which provide desirable textural or other attributes to the final product and for this reason have found numerous applications in traditional dairy products and in other foods (Table 26.3).

The functional properties of proteins are governed by intrinsic properties, i.e., their structural characteristics, e.g., size and surface hydrophobicity. These intrinsic properties are themselves affected by many extrinsic or environmental factors, such as pH, ionic strength and temperature, and also by interactions between the proteins and other materials in the food system. Not only does milk comprise various proteins with unique intrinsic properties, the milk proteins themselves are commercially available in many different forms. Furthermore, milk proteins may be modified by physical, chemical or enzymatic means, possibly resulting in significant improvements in one or more functional properties (Table 26.4). However, physical and enzymatic methods of modification are currently preferred over chemical methods, mainly due to consumers' concerns over "added chemicals" and the potential risk of occurrence of toxic side-reactions.

A. Solubility

Protein-water and protein-protein interactions in aqueous systems are vitally important and control the dispersibility, viscosity and solubility properties of proteins (81). A sensitive balance between repulsive and attractive intermolecular forces, which are in turn controlled by protein and water structures and affected by environmental factors, controls solubility (82). Generally, proteins are soluble in water and electrostatic and/or hydration repulsion between proteins is greater than the driving forces for hydrophobic interactions. Thus, the polar and ionizable groups of proteins largely confer water solubility (79).

Solubility is often used as a key indicator of a protein's ability to exhibit other functional properties such as

TABLE 26.3
Functional Properties of Milk Proteins Exploited in Food Systems

Functional Property	Potential Food Applications
Solubility	Nitrogen fortification of low pH beverages
Emulsification	Pastries, coffee creamers, milk beverages, milk shakes, high fat powders, butter-like spreads, cheese fillings and dips
Foaming	Egg replacer, whipped toppings, fizzy drinks, ice cream, mousses, meringues, sponge cakes
Heat stability	Imitation pasta, meat products, marshmallow and nougat
Aggregation/Coagulation/Gelation	Cultured milk products, comminuted meat products
Viscosity	Cake mixes, milk-based flavored beverages, frozen desserts
Water-binding and hydration	Imitation cheeses, toffees, fudges

TABLE 26.4
Methods Used to Improve the Functionality of Milk Proteins

Method	Treatments
Physical	Heat, pH, high pressure
Chemical	Phosphorylation, deamidation, glycosylation, covalent attachment of hydrophobic groups, acylation, reduction of disulphide bonds
Enzymatic	Hydrolysis, cross-linking with transglutaminase, glycosylation, phosphorylation, deamidation

emulsifying, foaming and gelation properties, although this general rule may not always apply, particularly in the case of highly hydrolyzed milk proteins. Solubility may be generally defined as the amount of protein that goes into solution or into colloidal dispersion under specified conditions (e.g., pH, ionic strength, protein concentration and temperature) until a maximum concentration is reached, after which the soluble concentration remains constant and a solid phase appears (83).

Solution conditions, such as pH, ionic strength and temperature, also affect the amount of water associated with proteins (82). Solution pH, for example, affects the amount of water associated with proteins by influencing the net charge of the protein. Ionic strength affects the solubility of proteins depending on the number of hydrophobic groups exposed on the protein's surface.

Unlike whey proteins, which exhibit excellent solubility over the entire pH range, caseinates (alkali dispersions of casein which are used in commercial applications) are almost completely insoluble in the region of their isoelectric point (pI: pH 4.0–5.0). However, outside of this region, caseinates possess excellent solubility, resulting in a U-shaped pH-solubility profile (84). Enzymatic hydrolysis has been frequently applied for improving solubility of caseinates in the pI region. Improved solubility is mainly due to a reduction in the molecular weight, increased exposure of polar groups and decreased secondary structure of the peptides formed upon hydrolysis of the intact protein. Enzymatic hydrolysis of food proteins has been thoroughly described by Adler-Nissen (85).

Casein and caseinates are utilized for their high solubility at highly acidic pH values (pH 2–3.5) in fruit juices and carbonated beverages (86), and may also be used for the nitrogen fortification of low pH beverages.

B. Interfacial Properties

Milk proteins have found numerous applications in food systems due to their ability to form and stabilize emulsions. Caseins possess high surface hydrophobicity with a well-balanced distribution of hydrophilic and hydrophobic domains and possess a high degree of conformational flexibility, which allows them to interact strongly at the oil-water interface (87). However, the caseins have high aggregative tendencies and low solubilities around their pI (88). Whey proteins also adsorb rapidly to, unfold and reorientate at oil-water and air-water interfaces, forming emulsions which are only slightly less stable than those formed with casein under the same conditions. Emulsions formed with pure β-lactoglobulin may increase in stability over time, due to intermolecular linking of disulphide groups which are in close proximity at the oil-water interface.

Due to the excellent surface activity of milk proteins, they have found numerous applications in several food products in which incorporation of oil/water/air into the continuous system is required. Examples of these include salad dressings, mayonnaise, liqueurs, meringues, soufflés, whipped toppings, confectionary, sponge cakes, etc. Caseinates generally give emulsions with increased droplet coverage and high foam expansion but produce less stable emulsions and foams than those formed with whey protein concentrate (WPC). Preheat treatment may be used to improve the emulsifying and foaming characteristics of WPC, while enzymatic hydrolysis to different extents has been shown to significantly improve both the emulsifying and foaming properties of sodium caseinate-stabilized emulsions and foams at certain pH values (84). However, extensive hydrolysis generally results in the loss of emulsifying and foaming activity. Combinations of enzymatic hydrolysis and cross-linking, using a novel cross-linking enzyme transglutaminase, have resulted in products which exhibit significantly greater emulsifying properties than the products of hydrolysis or cross-linking per se (89).

The foaming and emulsifying properties of milk proteins are also affected by many extrinsic or environmental factors, such as protein concentration, energy input, state of protein aggregation, pH, ionic strength, temperature, calcium ion concentration and the presence of contaminants which may destabilize the oil-water or air-water interface. In addition, the ratio of oil:water phase used during emulsion formation is of major importance to emulsion properties.

C. Heat Stability

Due to their limited secondary structure, the caseins possess excellent heat stability. Na, K and NH_4 caseinates are extremely heat stable and aqueous solutions of Na caseinate may be heated up to 140°C for 60 min without precipitation; however, Ca caseinate has much lower heat stability by comparison. Consequently, caseinates may be used in food products which experience thermal treatments, such as soups and sauces. The effects of heating on sodium caseinate have been extensively studied (90–92). Whey proteins, on the other hand, are readily denatured above 70°C, leading to aggregation and, depending on pH and

protein concentration, precipitation or gelation. Factors such as pH, protein concentration, total solids content and the presence of sugar may influence the denaturation of milk proteins.

A common method for measurement of heat stability involves heating a protein solution of known concentration and pH, in an oil bath or autoclave for a given period of time at defined temperatures. Changes in free amino nitrogen, soluble ammonia, turbidity, and trichloroacetic acid soluble and pH 4.6 soluble amino nitrogen have been attributed to the effects of heating on protein solutions. Enzymatic modification with transglutaminase has been shown to improve the heat stability of both reconstituted skim milk (93) and Na caseinate (94).

D. Aggregation/Coagulation/Gelation

Milk proteins also have the ability to form rigid, heat-induced irreversible gels that hold water and fat, and provide structural support (80). Coagulation and/or gelation are required in some cultured milk products, and may be brought about on addition of acid or rennet to milk to form casein gels. Caseinates per se are rarely used as gelling agents in formulated food products; however, the ability of whey proteins to form gels under a range of conditions have found many applications in food products.

Gelation of whey proteins occurs on heating (usually in the range 80–100°C) depending on the concentration (normally above 8%) and purity of the whey protein. The initial steps of gelation include unfolding of the globular protein molecules, followed by aggregation in the aqueous medium. Once aggregation exceeds a certain level, a three-dimensional self-supporting network is formed which traps the solvent within the system, resulting in an irreversible thermo-induced gel.

While Ca caseinate solutions at concentrations greater than 15% form reversible gels on heating to 50–60°C, Na caseinate may also be used to form gels in conjunction with κ-carrageenan (95). The introduction of covalent cross-links using transglutaminase has also been used to induce gelation in Na caseinate (96,97).

Important attributes of gels are gelling time, opacity (or lack of) and gel hardness and strength which may be measured using rheological methods; these are affected by extrinsic factors such as pH and ionic salt concentration. Lipids and lactose may adversely affect whey protein gelation.

Further applications of milk protein gels include the immobilization of enzymes or entrapment of drugs or bioactive peptides for the purpose of oral drug delivery systems.

E. Viscosity

Milk proteins, either in solution or incorporated into a food matrix, provide physical stability to emulsions and to other suspended particles in foods by influencing the viscosity of the system, whilst also contributing to mouthfeel (98). The viscosity of a protein system depends on the proteins' intrinsic properties such as heterogeneity, size, shape and charge; these properties are further affected by extrinsic conditions, such as temperature, concentration, pH, ionic strength and previous processing history. Randomly coiled structures, such as caseins, generally display greater viscosity in solution than whey proteins which have a compact globular structure.

Na caseinate solutions are highly viscous at 15% concentrations and display pseudoplasticity above this concentration. Viscosity of Na caseinate solutions increases exponentially with protein concentration, and generally decreases with increasing temperature, depending on concentration. Viscosity of Na caseinate solutions is also highly dependent on pH, with minimum viscosity observed at pH 7. Native whey protein solutions have very low viscosities compared to caseinates; however, viscosity increases at higher temperatures (>70°C) due to unfolding and aggregation of the globular whey protein. The viscosity of milk protein solutions is usually expressed as relative or apparent viscosity and may be measured using a variety of viscometers.

F. Water-Binding and Hydration

The ability of proteins to hydrate and entrap water without syneresis is utilized in many food applications. Water molecules bind to both polar and non-polar groups in proteins to different extents, depending on the charge and polarity of the amino groups. Due to the globular tertiary structure of the whey proteins, the majority of the amino acid residues are buried within the protein interior. Thus, the hydration capacity of the whey proteins arises predominantly from binding of water to amino acid residues on the protein surface. Hydration values for native individual whey proteins range from 0.3 to 0.6 g H_2O per g protein (99,100). Casein micelles, on the other hand, can bind up to 4 g H_2O per g protein, mainly due to the enormous amount of void space within the casein micelles' structure and binding of water to the hydrophilic surface of the micelles.

Solution conditions, such as pH, ionic strength, and temperature, affect hydration of proteins (101). Hydration capacity is minimal at the pI, where protein-protein interactions are favored over protein-water interactions. Low salt concentrations can also increase the hydration capacity of proteins.

Milk proteins have found numerous applications in food products due to their ability to bind water and swell, thereby resulting in an increase in viscosity in the food system. Whey proteins are used as water-binders in food systems which will receive sufficient heat treatment to denature the globular protein and increase its hydration capacity. These food systems include meat patties,

sausages, bread and cakes. Caseins and caseinates have found application in products such as imitation cheeses, confectionary and comminuted meat products.

Aside from hydration capacity of proteins, large amounts of water may also be held within a food matrix such as a gel. This water mainly includes physically entrapped water and it is this water which provides juiciness and tenderness to the final product.

VIII. MILK PROTEIN-DERIVED BIOACTIVE PEPTIDES

In addition to the biologically active proteins indigenous to milk, all the principal milk proteins contain sequences which, when released on proteolysis, exhibit biological activity. These biological activities include opiate, antithrombotic, antihypertensive, immunomodulatory, metal-binding and antimicrobial activities (Table 26.5).

A. Opioid Activities

Opioid peptides are short peptides (5–10 amino acids) termed casomorphins or exorphins for their ability to bind opioid receptors on intestinal epithelial and other cells (103) and have an opiate-like effect. The major opioid milk peptide β-casomorphin is a fragment of the β-casein sequence 60–70, while α_{S1}-casein (α-casomorphin), β-lactogobulin (β-lactorphin) and α-lactalbumin (α-lactorphin) also contain peptides which display opiate-like activity. Although opioid peptides derived from milk may be generated by proteolytic digestion of milk proteins *in vitro*, only β-casomorphin has been found in digesta after *in vivo* digestion of milk in calves (104) but not in humans (105). Milk protein-derived opioid peptides prolong gastrointestinal transit time (106), exert anti-diarrhoeal action (107), modulate intestinal transport of amino acids (108) and influence postprandial metabolism by stimulating secretion of insulin (109) and somatostatin (110).

TABLE 26.5
Bioactive Peptides Derived from Milk Proteins

Bioactive Peptide	Protein Precursor	Bioactivity
Casomorphins	α_{S1}-, β-Casein	Opioid agonist
α-Lactorphin	α-Lactalbumin	Opioid agonist
β-Lactorphin	β-Lactoglobulin	Opioid agonist
Lactoferroxins	Lactoferrin	Opioid antagonist
Casoxins	κ-Casein	Opioid antagonist
Casokinins	α_{S1}-, β-Casein	ACE-inhibitory
Lactokinins	α-Lactalbumin, β-Lactoglobulin, serum albumin	ACE-inhibitory
Immunopeptides	α_{S1}-, β-Casein	Immunomodulatory
Lactoferricin	Lactoferrin	Antimicrobial
Casoplatelins	κ-Casein	Antithrombotic
Phosphopeptides	α_{S1}-, β-Casein	Mineral binding

Source: Fitzgerald and Meisel, Milk protein hydrolysates and bioactive peptides, pp 675–698, 2003. In Advanced Dairy Chemistry Volume l: Proteins, 3rd ed., part A. Kluwer Academic/Plenum Publishers.

B. Opioid Antagonist Activities

Opioid antagonistic peptides are also found within the structure of milk proteins. Termed casoxins and lactoferroxins, they are derived from peptic digestion of κ-casein and lactoferrin, respectively. Opioid antagonists may antagonize the inhibition of gut motility as induced by casomorphins (111). Opioid antagonists have been found in bovine and human κ-casein (111) and in human α_{S1}-casein (112).

C. Inhibition of Angiotensin-I-Converting Enzyme (ACE)

The casein-derived ACE inhibitors, or casokinins, have been isolated from bovine α_{S1}- and β-casein and from human β- and κ-casein (103,114). Opioid fragments relating to β-casomorphin-7 (115) and β-lactorphin (116) have also been identified as ACE inhibitory peptides with moderate activities. ACE is a multifunctional enzyme that is located in different body tissues and is associated with the renin-angiotensin system, which regulates peripheral blood pressure. Inhibition of ACE has been shown to block the conversion of angiotensin-I to angiotensin-II, a potent vasoconstrictor.

D. Immunomodulatory Effects

α-Lactalbumin, β- and α_{S1}-casein-derived immunopeptides have been found to enhance phagocytosis and modulate proliferation and differentiation of lymphocytes (117). Immunomodulation appears to be a particularly important bioactivity of the developing, involuting or inflamed mammary gland. Hormones, growth factors and cytokines found in milk also appear important in immunomodulation and immuno-development (111).

E. Antithrombotic Effects

Casoplatelins, peptides derived from the C-terminus of bovine κ-casein, have been shown to exhibit antithrombotic activity, i.e., inhibition of blood clotting. These peptides function by inhibiting the aggregation of ADP-activated platelets as well as binding of human fibrinogen γ-chain to a specific receptor site on the platelet surface (118).

F. Mineral Binding Properties

While intact casein has been shown to bind zinc and calcium, tryptic hydrolysates of α_{S1}-, α_{S2}-, β- and κ-casein also display mineral binding properties. Termed caseinophosphopeptides (CPPs), these peptides can bind and solubilize high concentrations of calcium due to their highly polar acidic domain. Consumption of high concentrations

of calcium in early life contributes to development of maximal bone density, which in turn can prevent osteoporosis in later life (102). In addition, calcium-binding CPPs can have an anticariogenic effect in that they inhibit caries lesions through recalcification of the dental enamel (119).

G. Antimicrobial Activity

Lactoferrin is an iron-binding glycoprotein present in the whey fraction in milk from which antimicrobial peptides have been derived (120). It is considered to be an important component of the host defense against microbial infections. The antimicrobial ability of lactoferricin may be correlated with the net positive charge of the peptides. These cationic peptides kill sensitive microorganisms by increasing cell membrane permeability (121). Lactoferricin displayed antimicrobial activity against yeast, filamentous fungi (122), *Escherichia coli, Listeria monocytogenes* (123) and clinical isolates of *E. coli* 0157:H7 (124). Recently, fragments from α_{S1}- and α_{S2}-casein have been found to inhibit the growth of *E. coli* (125) and *Staphylococcus aureus* (126).

IX. CONCLUSION

Bovine milk protein fulfills important nutritional, functional and physiological roles in both the developing neonate and man. From a nutritional viewpoint, milk protein is a balanced source of all the essential amino acids. The functional properties of milk proteins are exploited in many food products and the ability of milk protein to act as surface active agents, contribute to texture or to bind water or fat, is determined, in part, by its unique structure. Milk also contains an array of enzymatic activities; these enzyme activities are important from both a functional and physiological perspective. Finally, all the principal milk proteins contain sequences which, when released on proteolysis, may confer beneficial physiological effects.

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27 Enzymes of Significance to Milk and Dairy Products

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CONTENTS

I. INTRODUCTION

When considering the processing and quality of milk and dairy products, a number of enzymes are of significance. These fall broadly into 3 categories:

1. Indigenous enzymes: Fresh raw bovine milk contains a heterogeneous group of enzymes, derived from various sources. While different authors may use different nomenclature to indicate the origin of enzymes, in this chapter, enzymes of bovine origin that are found in milk will be referred to as indigenous. While the complement of milk enzymes has not been completely classified, and gaps in knowledge and understanding clearly occur, the principal classes of enzymes include proteolytic and lipolytic enzymes, phosphatases, antimicrobial enzymes, e.g., lysozyme and lactoperoxidase, and a number of other enzymes, such as xanthine oxidase. Approximately 60 indigenous enzyme activities have been reported in bovine milk, of which ca. 20 have been characterized (1, 2). The milk of mammals other than the cow probably contains similar enzyme profiles, although most have not been studied in detail (2).
2. Exogenous enzymes: A number of enzymes are added to milk during processing (3); these will be referred of hereafter as exogenous. The oldest example of such use of an enzyme in the dairy industry is the application of extracts of stomachs of calves, kids or lambs (containing chymosin) to coagulate milk in cheesemaking. Today, a range of milk coagulants is used (e.g., rennet extracts, recombinant calf chymosin, microbial rennets, etc.). Other enzymes that are commercially available today can be used to manipulate and accelerate cheese ripening (e.g., added protease and lipase preparations), to hydrolyze lactose in milk (lactases or β-galactosidases) and, most recently, to manipulate the

texture of milk products through the creation of new covalent inter-protein cross-links (transglutaminase).

3. Endogenous enzymes. Endogenous enzymes in this case refer to those that are present in a food but are not part of it, e.g., enzymes produced by microorganisms that grow in milk, either as natural contaminants (e.g., psychrotrophic bacteria, mastitis pathogens) or following deliberate inoculation (e.g., the lactic acid bacteria used in the manufacture of fermented dairy products). While such microorganisms possess a wide range of enzymes, which can contribute either negatively (e.g., lipases and proteases of *Pseudomonas*) or positively (e.g., the proteolytic system of *Lactococcus*) to the characteristics of dairy products, they will not be considered in this chapter. The reader is directed to reviews in this area (4–8).

In this chapter, the indigenous and exogenous enzymes of importance to milk and dairy products will be discussed. The significance of these enzymes to dairy product manufacture and quality will be emphasized.

II. INDIGENOUS ENZYMES

It has long been recognized that there is a significant and heterogeneous mixture of enzymes in fresh milk. Today, many of these enzymes have been characterized but our understanding of the significance of all these enzymes for product quality is incomplete.

In terms of indigenous enzymes, the key questions to be considered may be summarized as follows:

(i) What enzymes are present and from where do they originate?

As stated already, a number of enzyme activities have been identified in milk, which will be listed and described in detail below; however, it is unlikely that all enzymes in milk have been identified. Indigenous enzymes differ in their origin (e.g., blood or lysosomes of somatic [white blood] cells) and distribution in milk. Indigenous enzymes in milk originate from:

(a) Blood (via leaky junctions between mammary secretory cells)
(b) Somatic cells, particularly during mastitic infection
(c) Mammary cell cytoplasm, as a result of the way in which milk is expressed in the udder

Indigenous enzymes in milk are found in, or associated with, casein micelles, the milk fat globule membrane (most enzymes), milk serum or somatic cells.

One of the major challenges in studies of indigenous milk enzymes is the requirement for suitable methodologies and assays to allow evaluation of their nature and significance.

(ii) At what level are these enzymes found, and what factors affect their activity?

The enzyme complement in raw milk is far from constant, and may vary qualitatively and quantitatively due to several factors, including individual variation between cows, health status (particularly incidence of mastitis), stage of lactation, nutritional status, breed and physiological or nutritional stress. Thus, fresh milk as a raw material for production of dairy products may exhibit considerable, potentially significant, variation in its complement of indigenous enzymes. For some indigenous enzymes (e.g., plasmin), the factors affecting its activity in milk are quite well defined; in the cases of many other enzymes, such as the acid protease cathepsin D, this information is incomplete. Many of these factors, where known, will be discussed in the relevant section for each enzyme hereafter.

(iii) What is their significance in dairy products?

The most significant features of indigenous enzymes with respect to dairy products are (2):

(a) Indices of animal health (especially mastitis)
(b) Indices of the thermal history of milk
(c) Deterioration of product quality
(d) Desirable changes in dairy products
(e) Protective (antibacterial) effects.

This review will concentrate on the effects of indigenous enzymes on the technological properties of milk and dairy products.

Perhaps the principal issue to be addressed in terms of evaluating the significance of indigenous milk enzymes for the majority of modern dairy products is whether the activity remains following pasteurization, which is the primary processing step commonly applied to most milk. Many studies have shown that milk is not rendered enzymatically inert by such thermal processing. Indeed, the activity of the principal proteolytic enzyme in milk, plasmin, may be increased during storage of milk post-pasteurization, as will be discussed below. Post-pasteurization, the residual activity of indigenous enzymes have been linked to a wide range of positive or negative effects in dairy products (e.g., in the case of plasmin, from the ripening of cheese to coagulation and instability of UHT milk products). Furthermore, when it occurs, inactivation of enzymes by pasteurization or other processing steps may not be sufficient to eliminate completely the activity of certain enzymes in milk; in many cases, the enzymes already have had significant opportunity to act on milk

constituents (e.g., to hydrolyze casein in such a manner as to affect product quality) prior to pasteurization, either rapidly at temperature near their optimum (i.e., in the udder at 37°C, prior to milking) or more slowly during refrigerated storage at the farm or factory.

In the following sections, the significance of indigenous enzymes will be discussed in the above light, under each of the major groups known to be present in raw milk.

A. Indigenous Lipase Activity in Milk

Lipoprotein lipase (LPL) is found as an indigenous enzyme in the milk of all mammals, although its level varies from species to species; LPL is the only indigenous lipase in bovine milk (9–11). It plays an important role in milk production in the udder, as the products of its action on triglycerides in lipoproteins, i.e., fatty acids and monoglycerides, are taken into the cell for the production of energy, synthesis of tissue components or milk lipids, or storage. LPL activity in the mammary gland is low before and during pregnancy but increases soon after parturition and remains high throughout lactation (12). This elevated LPL activity means that triglycerides are diverted to the mammary gland for the synthesis of milk lipids. LPL is probably synthesized mainly and secreted into milk together with the casein micelles; some transfer of LPL to the milk fat globules may occur, particularly after milking (9). To date, no role has been proven for LPL in milk utilization by the neonate; Olivecrona et al. (9) speculated on possible roles for this enzyme, including assisting with the binding of milkfat to the intestinal mucosa, catalysis of the transfer of lipids into cells, facilitating the action of pancreatic lipase by partial hydrolysis of milkfat and perhaps an anti-parasitic function.

Bovine LPL is a non-covalent homodimer of glycosylated subunits containing 450 residues, 5 disulphide bridges and 2 oligosaccharide chains. The structure of LPL has been deduced by comparison of its sequence with that of the closely related pancreatic lipase (9). The active site of the enzyme is in a hydrophobic pocket protected by a surface loop ("lid"). LPL requires apolipoprotein CII (apoCII) for activity on lipoprotein substrates; apoCII is not related to the co-lipase needed for the action of pancreatic lipase. The exact mechanism by which apoCII activates LPL is unknown, but it has been speculated that it helps to orientate the lipase molecule at the oil-water interface at which the enzyme acts (9).

LPL binds strongly to heparin and related polysaccharides (13) *via* a groove on the side of the enzyme opposite the active site. The role of heparin binding appears to be to anchor the enzyme to heparin sulphate proteoglycans of the vascular endothelium (9). The catalytic mechanism of LPL is similar to that of pancreatic lipase and it preferentially releases fatty acids from the *sn*-1 and *sn*-3 positions of mono-, di- and triglycerides in emulsified lipid substrates. Since short- and intermediate-chain fatty acids are esterified mainly at these positions, the action of LPL results, in particular, in the release of these fatty acids. The apparent pH optimum of LPL on milk fat globules as substrate is ca. pH 8 (9).

In common with many lipases, LPL is subject to strong product inhibition (9). Since LPL can also catalyze the synthesis of ester bonds between fatty acids and partial glycerides, if the products of the hydrolysis (fatty acids) are not removed or complexed, LPL will spend much time producing fatty acids by hydrolysis and then using them as substrates for synthesis. In addition, fatty acids interfere with the binding of the enzyme to lipid droplets (14).

LPL in milk is associated mainly (>80%) with the casein micelles and its presence is of significance for lipolysis in milk and dairy products (9). Lipolysis occurs slowly in milk during storage, principally due to the action of LPL; it is only on prolonged storage or in cases of poor hygiene that bacterial lipases become important. Under ideal conditions (37°C, pH 7, in the presence of activators), raw milk should become rancid within a few minutes (9). Lipolysis in milk may be classified as spontaneous (i.e., without mechanical damage) or induced. Most milks do not exhibit spontaneous lipolysis. The incidence of induced lipolysis in milk has been reduced by improvement in design and operation of mechanical pipeline milking systems to avoid foaming or excessive shear forces during pumping. However, lipolysis may remain a problem if the milk is held for a long period before being transported to the dairy for processing.

LPL may cause lipolysis in raw milk but, since this enzyme is largely inactivated by pasteurization, its contribution to lipolysis in products made from pasteurized milk is minor. It is essential to pasteurize milk either before, or immediately after, homogenization, as the changes to the milk fat globule membrane would facilitate rapid lipolysis unless LPL is inactivated (9, 11).

LPL plays a role in lipolysis during ripening of cheese made from raw milk, or from sub-pasteurized milk (15). The presence of active LPL in raw milk cheese is one of the factors responsible for the difference in flavor between cheeses of the same variety made from raw or pasteurized milk, although heat-induced changes to the indigenous microflora of the cheesemilk are probably of greater importance (16).

A second lipase, bile salt-stimulated (or activated) lipase (BSSL), is found in the milks of humans and some primates, but not in those of domesticated milk-producing animals. BSSL is also present in pancreatic juice where it is referred to as "non-specific lipase" or "cholesteryl ester hydrolase." BSSL is found at high concentrations in human milk, where it is thought to improve the utilization of milk lipids by the neonate. Efforts are under way to produce this enzyme by fermentation with a view to supplementing infant formulae (9).

B. Indigenous Proteinases in Milk

1. The Plasmin/Plasminogen System

Babcock and Russel (17) first recognized the presence of indigenous proteolytic activity in raw milk. Today, the principal indigenous proteinase in milk is recognized to be the alkaline proteinase, plasmin (EC 3.4.21.7), which was first identified in milk by Kaminagowa et al. (18). Several reviews of the nature and significance of plasmin in milk have been published in recent years (19–23).

Plasmin is identical to the enzyme of the same name (which is also known as fibrinolysin) found in blood. That enzyme is a key element of the blood clotting mechanism (its role is in dissolving blood clots), and in that role its activity is, perhaps unsurprisingly, under the control of a complex and interconnected system of activators and inhibitors (24). It is thought that plasmin in milk originates from blood through leakage across the mammary gland secretory cell membranes, and to date almost all of the components of the blood plasmin system have also been found in milk, presumably through leakage (23). The principal elements of the plasmin system in milk are:

- Plasmin, the active enzyme, a serine proteinase of molecular weight 88,092 Da
- Plasminogen, its inactive zymogen (precursor)
- Plasminogen activators (PA), which proteolytically cleave plasminogen to yield active plasmin; there are two classes of PA in milk: tissue-type PA (tPA) and urokinase-type PA (uPA)
- Inhibitors of plasminogen activators (PAI)
- Inhibitors of plasmin (PI)

The properties of these elements of the plasmin system, where known, are summarized in Table 27.1. All the parts of the plasmin system listed above are indigenous to milk. In addition, some pathogenic bacteria associated with mastitis, i.e., infection of the mammary gland, such as *Streptococcus uberis*, possess PA activity, which they use to accelerate proteolysis in the infected udder (25). Furthermore, the proteolytic activity of some psychrotrophic bacteria can release plasmin from the casein micelles, where it is normally located, into the serum phase of milk, or whey (26, 27).

TABLE 27.1
Principal Constituents of the Plasmin System in Milk

Constituent	Function	Location
Plasmin	Active enzyme	Casein micelle
Plasminogen	Inactive zymogen	Casein micelle
Plasminogen activators (PA)		
Tissue-type (tPA)	Convert plasminogen to plasmin	Milk serum
Urokinase-type (uPA)	Convert plasminogen to plasmin	Somatic cells
Plasminogen activator inhibitors (PAI)	Inhibition of PA	Milk serum
Plasmin inhibitors (PI)	Inhibition of plasmin	Milk serum

a. *Proteolysis of Milk Proteins by Plasmin*

There are two principal classes of proteins in milk. The caseins (α_{s1}-, α_{s2}-, β- and κ-) are relatively hydrophobic proteins which occur, in milk, in the form of spherical micelles containing thousands of molecules of each casein, plus nanoclusters (small particles) of (colloidal) calcium phosphate; the structure of the caseins has been described as rheomorphic, which means that they have a high degree of conformational flexibility (28). The casein micelles are stabilized in the aqueous environment of milk by a "hairy layer" comprising the hydrophilic C-terminal glycoprotein moiety of κ-casein. The second class of milk proteins, the whey proteins, are globular proteins, the principal of which are β-lactoglobulin and α-lactalbumin.

Plasmin is an alkaline serine proteinase, with a temperature optimum of 37°C and a pH optimum of 7.5. It preferentially hydrolyzes peptide bonds of the type Lys-X and, to a lesser extent, Arg-X; of the caseins (CN), β-CN is its preferred substrate. Hydrolysis of this protein by plasmin yields three polypeptide products known as the γ-caseins (β-CN f29-209, f106-209, f108-209), as well as proteose peptones (PP) PP 5 (β-CN f1-105/7), PP8 fast (f1-28) and PP8 slow (f29-105/7) (29). In solution, plasmin also readily degrades α_{s2}-CN (30, 31), but peptides derived from it have not been identified in milk. While plasmin acts more slowly on α_{s1}-CN (32), a number of α_{s1}-CN-derived peptides, collectively known as the λ-caseins, have been identified in milk (33). κ-CN is resistant to hydrolysis by plasmin. The whey proteins are also resistant to proteolysis by plasmin, and β-lactoglobulin can, in fact, act as an inhibitor of plasmin activity.

b. *Factors Affecting Plasmin Activity in Milk*

Plasmin activity is usually measured in a citrate dispersion of milk or cheese, using either a fluorogenic or chromogenic substrate (34–36). Total activity (plasminogen plus plasmin) is measured after activation of plasminogen by added urokinase, and the level of plasminogen calculated by difference. Assays have also been described for activity of PA (37); the ratio of plasminogen to plasmin in milk is also frequently used as an indication of the extent of plasminogen activation.

One problem with enzymatic assays for plasmin is the presence of protease inhibitors in milk. Some methods for assaying this activity avoid such interference by centrifugation of plasmin, bound to casein, from milk and re-suspending the pellet in a buffer containing ε-amino-caproic acid, which releases the enzyme from the micelles, prior to assay (37). A new modified method uses a clarifying

reagent to reduce the turbidity of milk before fluorimetric analysis, without the need for more complex sample preparation (38). Alternatively, ELISA methods have been used to quantify plasmin levels in milk (39). However, different approaches will yield different information, for example, the exact concentration of plasmin in milk versus its actual proteolytic potential, as determined by all inhibitory substances present.

The activity of plasmin in milk is not constant, but varies due to a number of factors. Furthermore, there are several mechanisms by which plasmin activity can be modulated in milk, which leads to a complex multi-factorial system influencing the net plasmin activity in fresh raw milk.

The two principal routes by which plasmin activity in milk may be increased are (40):

- Increased transport of active plasmin from blood
- Increased conversion of plasminogen to plasmin by PA

These two routes are not independent; the increased permeability of the blood/milk barrier which facilitates the first route also increases the levels of PAs (41, 42). For example, somatic cells in milk possess PA activity, and can thereby activate plasminogen.

The principal factors which affect plasmin activity in milk are summarized in Table 27.2.

When milk is refrigerated, proteolysis is reduced greatly, although β-casein dissociates from the casein micelles into the serum (43), which should, in theory, facilitate proteolysis. However, at 4°C, no activation of plasminogen occurs and the activity of plasmin decreases over time (44).

Plasmin activity is further affected by the processing of raw milk. The effect of pasteurization on plasmin activity in milk is complex. The heat treatment per se (typically heating to 72–74°C for 15–30 s in a plate heat exchanger) slightly reduces the activity of plasmin. However, inhibitors of PA are believed to be inactivated by pasteurization, which accelerates the subsequent conversion of plasminogen to plasmin (in the absence of down-regulation by these inhibitors), and eventually causes a net increase in plasmin activity in pasteurized milk relative to raw milk (45).

More severe heat treatments than pasteurization result in far more significant inactivation of plasmin in milk. In general, the inactivation of plasmin in milk is thought to be linked to the denaturation of the whey protein, β-lactoglobulin (β-lg); thermal denaturation of the latter protein exposes a highly reactive sulphydryl group, which can undergo disulphide-sulphydryl interchange reactions with disulphide bonds which are key structural features of the molecular conformation of plasmin. In the absence of β-lg, plasmin is very heat resistant, and will refold to an active state after thermal unfolding; however, in the presence of β-lg, heat-induced formation of heterologous disulphide-linked complexes prevents such refolding, and leads to loss of enzyme activity. In ultra-high-temperature (UHT)-treated milk, for example, plasmin activity is very low (46, 47).

In fact, heating milk to a temperature sufficient to denature β-lg reduces proteolysis therein by two mechanisms: enzyme inactivation, as discussed above, and complexation of denatured β-lg with casein micelles, which may sterically hinder access of the enzyme to cleavage sites on the caseins (48).

c. Significance of Plasmin in Milk and Dairy Products

In pasteurized liquid milk, proteolysis of casein by plasmin during storage has little direct impact: while, in principle, such action could result in physical instability (as will be discussed below for UHT milk) or development of bitterness (through the liberation of small hydrophobic peptides), the growth of psychrotrophic bacteria, such as *Bacillus* species, is generally the principal determinant of shelf life (49).

However, in long shelf-life milk products (such as UHT milk), where bacterial growth and activity do not occur, and the product is often stored at ambient temperature, even the low residual activity of plasmin remaining

TABLE 27.2
Farm-level Factors Affecting Plasmin Activity in Milk

Factor	Comments on Known Effects
Stage of lactation	Plasmin activity generally increases in late lactation
Mastitis	Plasmin activity is generally well correlated with milk somatic cell count (SCC); levels of protease inhibitors in milk also increase mastitis
Diet	Restricted diets increase plasmin activity, especially in late lactation
Breed of cow	Milk from Fresian cows has higher plasmin activity than that of Jersey cows
Age of cow	Plasmin activity is higher in milk from older cows
Genotype	Milk with different genotypes of β-lactoglobulin can vary in plasmin activity
Hormone usage	Somatotrophin suppresses plasmin in milk; estrogen increases activation of plasminogen to plasmin.

after the severe heat treatment applied (typically, 135–140°C for 2–4 sec) can contribute to proteolysis, albeit at a very low level, during storage. Furthermore, it has been suggested that such slow hydrolysis of caseins may be linked to the occurrence of physical changes in the milk, such as irreversible gelation during storage (46, 47).

Plasmin activity can potentially influence cheese quality in two main ways. Firstly, pre-manufacture hydrolysis of the caseins has been shown to affect the rennet coagulation properties of milk (50, 51); while rennet coagulation times can actually be reduced by plasmin action, gels formed from hydrolyzed milk are weaker than those from control milk (although these effects may be confounded by other changes in milk with elevated plasmin activity, such as late lactation or mastitic milk). Secondly, plasmin in curd is an important contributor to primary proteolysis of the caseins during cheese ripening. The activity of plasmin in cheese is directly affected by the cheese manufacture protocol (e.g., a high curd cooking temperature increases the relative contribution of plasmin to ripening) and the physicochemical environment of the cheese (e.g., pH, temperature, etc.). Studies involving either inhibition or augmentation of plasmin activity in cheese (the latter to investigate the possibility of using plasmin to accelerate cheese ripening) have shown that it probably does not contribute directly to cheese flavor, being principally involved in production of large polypeptides, rather than amino acids (52).

2. Other Indigenous Proteinases in Milk

Milk from a healthy cow contains a low level (<200,000 cells/ml) of white blood (somatic) cells. On mastitic infection, the somatic cell count (SCC) of milk increases rapidly and markedly, principally due to a massive influx of one type of white blood cell, polymorphonuclear leucocytes (PMN). Somatic cells in milk possess lysosomes which contain a range of proteolytic and other enzymes, the significance of which for the quality of dairy products is ill-defined (21).

Proteolytic activity in milk is correlated with its SCC; while part of this is unquestionably due to increased plasmin activity (Table 27.2), it is acknowledged that lysosomal proteinases contribute to proteolysis. This can be estimated by using specific assays for a range of proteolytic enzymes, or by examining the nature and origin of hydrolytic products in milk samples with different SCC (53, 54).

The second proteinase to be identified conclusively in milk was the lysosomal aspartic proteinase cathepsin D (55, 56). This enzyme is present in a number of different molecular forms in milk, not all of which are proteolytically active, and is located in the serum phase of milk (57). Cathepsin D activity in milk may be assayed using haemoglobin or synthetic chromogenic peptides as substrate; its activity is actually quite low, relative to that of plasmin (proteolysis patterns in fresh milk quite closely reflect those of the latter enzyme). Cathepsin D at least partially survives pasteurization (58, 59).

The proteolytic activity of cathepsin D and chymosin on α_{s1}-casein are very similar; cathepsin D is quite active on this substrate (60, 61). β-Casein is also hydrolyzed readily by cathepsin D, yielding a pattern similar, but not identical, to those produced by chymosin (60, 61). The action of cathepsin D on bovine α_{s2}-casein produces a hydrolysis pattern quite different from that produced from this substrate by chymosin (60, 61). As for plasmin, the whey proteins are relatively resistant to the action of cathepsin D, compared with the caseins (60).

κ-Casein, the micelle-stabilizing protein, is hydrolyzed by cathepsin D at the Phe_{105}-Met_{106} position, the bond cleaved by chymosin during the rennet coagulation of milk (60, 61). Thus, it might be expected that cathepsin D could coagulate milk; however, exogenous cathepsin D coagulates milk very slowly (60, 61). The ability of cathepsin D to coagulate milk is strongly pH-dependent and is faster at lower pH, reflecting the low pH optimum of the enzyme. The level of indigenous cathepsin D in milk (ca. 0.4 μg ml^{-1}) is much lower than that required to cause coagulation of milk within a reasonable time, even under ideal conditions; thus, the action of indigenous cathepsin D probably does not significantly influence rennet coagulation, particularly if cheese is made from pasteurized milk (60). However, the enzyme may contribute to the degradation of κ-casein in other dairy products during prolonged storage.

In terms of the significance of cathepsin D for other products, most studies to date have focused on cheese, where it is extremely difficult to discern its contribution due to its close resemblance to chymosin. However, evidence of its activity has been reported in Swiss cheese and, most significantly, in Feta-like cheese made from thrice-pasteurized milk without the use of rennet (59) and in Quarg made from pasteurized milk without use of rennet (62).

Cathepsin D is probably only one of a number of lysosomal proteolytic enzymes in milk; it seems almost inevitable that other enzymes from this source are present in milk, at levels proportional to the SCC of milk. However, this area has received little attention to date. Many lysosomal proteinases are cysteine proteinases (including cathepsins B, L and H); cysteine proteinase activity in milk, due to the presence of at least two separate enzymes, and strongly correlated with SCC, has been reported by two independent groups (63, 64). The presence of immunoreactive cathepsin B in normal milk has been demonstrated (63). The specificity of cathepsin B on the caseins has also been determined recently (65).

The principal serine proteinase in the polymorphonuclear leucocytes (PMN), the principal type of somatic cell found in milk during mastitis (66), is elastase, an inhibitor of which has been found in milk (67); another important

somatic cell proteinase is cathepsin G. There have also been sporadic reports of other indigenous proteolytic enzymes in bovine milk, such as an indigenous lysine aminopeptidase (68), but the presence of this enzyme has not been confirmed.

In general, it may be argued that the levels of proteinases other than plasmin in normal milk are too low to have an influence on the quality of dairy products; however, this area warrants further attention.

C. Enzymes Involved in Phosphoryl Transfer

Four categories of enzymes are involved in the transfer of the phosphoryl group: phosphatases (EC 3.1.3…), phosphodiesterases (EC 3.1.4…), kinases (EC 2.7.1…– EC 2.7.6…) and phosphorylases (EC 2.4.1… and EC 2.4.2…). The most important phosphohydrolyases in milk are two types of phosphomonoesterases (phosphatases), alkaline phosphatase and acid phosphatase. A number of other activities have been found in milk, including 5′-nucleotidase, glucose-6-phosphatase, phosphodiesterase I, deoxyribonuclease (DNase), ribonuclease (RNase), inorganic pyrophosphatase, adenosine triphosphatase and nucleotide pyrophosphatase. This class of enzymes in milk has been reviewed regularly (69–71).

The major indigenous phosphomonoesterase in milk is alkaline phosphatase (EC 3.1.3.1). This enzyme is a dimeric glycoprotein (85 kDa subunits), requiring 4 Zn atoms per molecule for activity; it is activated by divalent metal ions (Ca^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Mg^{2+}) and inhibited by metal chelators and orthophosphates. The enzyme is present mainly in the milk fat globule membrane. Alkaline phosphatase is optimally active in the pH 9–10.5 range and at 37°C (70).

The thermal inactivation kinetics of alkaline phosphatase have been studied thoroughly as residual alkaline phosphatase activity is widely used as an index of pasteurization (72). The enzyme is slightly more heat-resistant than *Mycobacterium tuberculosis*, which for many years was the most heat-resistant vegetative pathogen known in milk and the target organism for pasteurization. Results of "phosphatase tests" below certain limits are required for certification of pasteurized milk in many countries. However, the use of alkaline phosphatase as a marker enzyme for pasteurization suffers from a number of drawbacks (73):

- Some microorganisms in certain types of cheese (e.g., some lactic acid bacteria and *Penicillium roqueforti*) have alkaline phosphatase activity and thus certain cheeses, made from properly pasteurized milk, may exhibit a positive phosphatase test.
- Reactivation of alkaline phosphatase can occur following certain heat treatments equivalent to, or greater than, pasteurization (e.g., temperatures of 82–180°C for milk or 74–180°C for cream for a short time).
- The enzyme appears to be inactivated fully by sub-pasteurization conditions (70°C × 16 s).
- The relationship between pasteurization equivalent and log (% residual activity) is less linear for alkaline phosphatase than for some other indigenous enzymes in milk (e.g., lactoperoxidase or γ-glutamyl transpeptidase).

The major technological importance of alkaline phosphatase is its use as a marker enzyme for pasteurization; no other technological roles have been confirmed (70).

An indigenous acid phosphatase (EC 3.1.3.2) in milk was reported first in the 1940s. A high proportion of the total acid phosphatase activity in milk is in skim milk, although cream contains a higher specific activity. This enzyme is a glycoprotein with a molecular mass of ca. 42 kDa and a pI of 7.9, containing a high level of basic amino acids but lacking methionine. It is inhibited by several heavy metals, F^-, oxidizing agents, orthophosphates and polyphosphates, activated by thiol reducing agents and ascorbate and is unaffected by metal-chelating agents (70). The enzyme is active against phosphoproteins, including the caseins, and is quite heat-stable, surviving HTST pasteurization but being inactivated by UHT treatments. Although a lower level of acid phosphatase activity than alkaline phosphatase activity is found in milk, acid phosphatase may be technologically important, due to its lower pH optimum (ca. 3.5–5), which is closer to the pH of many fermented dairy products (70).

There have been reports that milk contains more than one indigenous acid phosphatase (70), although it is likely that this heterogeneity is due to leukocyte enzymes from somatic cells, some of which are always present in milk.

As discussed by Shakeel-Ur-Rehman et al. (70), several small partially dephosphorylated peptides have been isolated from a number of cheese varieties, although it is not entirely clear whether indigenous or endogenous (starter) phosphatases are responsible for their production. However, the activity of the indigenous acid phosphatase is thought to be important.

Milk also contains two indigenous acid phosphatases of leukocyte (somatic cell) origin; the acid phosphatase activity of milk increases 4–10 fold during mastitis (74–76).

Nucleases are particular types of phosphodiesterases which hydrolyze the phosphodiester linkages in nucleic acids. As reviewed by Stepaniak et al. (77), milk is a rich source of RNase, which is almost entirely located in the serum phase, and is optimally active at ~pH 7.5. A number of isoenzymes of RNase are present in milk, principally RNase A (which is identical to pancreatic RNase A) and RNase B, in a ratio of about 4:1. Bovine milk contains approximately 3 times as much RNase as human, ovine or

caprine milk. Most of the RNase activity in milk survives HTST pasteurization, although activity is essentially completely lost on UHT treatment. There has also been one report of indigenous DNase activity in milk (77).

D. Lactoperoxidase

Milk contains natural inhibitors ("lactenins") of the growth of susceptible strains of starter bacteria. Further study has shown the lactenins to be antibodies (which cause susceptible bacteria to aggregate and localized acid production) and lactoperoxidase. Lactoperoxidase, an oxidoreductase, is a glycoprotein consisting of a single peptide chain with a molecular weight of 78,000 Da; it is a haem protein, with one iron molecule bound per mole of enzyme, and its stability depends on binding of a chelated calcium ion (78–80).

This enzyme catalyzes the following reactions:

(1) $$H_2O_2 + 2HA \rightarrow 2H_2O + 2A \qquad (27.1)$$

where the substrate, HA, can be one of several compounds, including aromatic amines, phenols, vitamin C.

(2) oxidation of thiocyanate (CNS^-) by H_2O_2 (27.2)

The products of reaction 27. 2, particularly the hypothiocyanate anion ($OSCN^-$), inhibit many bacteria; this inhibition is particularly effective against bacteria which produce H_2O_2, such as lactic acid bacteria. It is believed that hyopthiocyanate can oxidize vital bacterial enzymes and sulphydryl groups in the cytoplasmic membrane, impairing its transport properties (81). Lactoperoxidase itself has no antibacterial action, but merely catalyzes the formation of antibacterial substances (e.g., the hypothiocyanate anion) from precursors.

The inhibition may be temporary (especially for Gram-positive bacteria such as streptococci and lactobacilli) or permanent and lethal (for Gram-negative catalase-positive bacteria such as *E. coli*, *Salmonellae* and *Pseudomonas* spp.) (78, 82).

However, while there is always a relatively constant and high level of lactoperoxidase in milk (30 mg/L, a 30-fold excess of the minimum required to achieve microbial inhibition), the concentrations in milk of other critical components and substrates of the system can vary widely (79). For example, the level of thiocyanate is generally limiting and depends on the availability of precursor substances (such as cyanoglucosides) in the feed (plants of the brassica family, e.g., cabbage, are rich in these); the origin of the very low level of H_2O_2 naturally present in milk is unclear, but may be related to the action of milk leucocytes and mammary tissues, the presence of catalase-positive microorganisms or the action of xanthine oxidase (79). The total antibacterial action is proportional to the production of the inhibitory end-products, and hence this can also vary (78).

The lactoperoxidase system can be exploited to prevent the spoilage of raw milk by adding thiocyanate (typically 10 mg kg^{-1} of a powdered preparation), followed by thorough mixing and addition of a small amount of H_2O_2 (often in the form of granular sodium carbonate peroxyhydrate) (83). This method has particular applications in countries with a warm climate, where it can allow milk to be stored at a relatively high temperature (e.g., 30°C for 7–8 hours, or 20°C for 16–17 hours) (84). This allows producers without cooling facilities to transport milk to central cooling and processing centers, and has significant economic implications for milk-producing farmers in developing countries. A Code of Practice for the application of this method for preserving milk was approved by the FAO/WHO Expert Committee on Food Additives in 1989 and by the Codex Alimentarius Commission in 1991. Ideally, the natural thiocyanate content of the milk should be determined, in a centralized testing facility, before determining the level of thiocyanate to be added.

The effectiveness of the lactoperoxidase system can also be enhanced by adding xanthine oxidase to milk, an indigenous oxidizing enzyme which can produce H_2O_2 from substances such as xanthine and hypoxanthine.

Lactoperoxidase treatment of milk is not regarded as a treatment suitable for liquid milk, but rather as a processing aid which merely enhances a naturally occurring functionality of the milk; quality of the raw milk must still be good. The lactoperoxidase system has also been exploited in mastitis therapy during the dry period, control of post-fermentation acidification of yoghurt, preservation of HTST pasteurized milk and stabilization of dairy emulsions. The system has also been applied in cosmetics, ophthalmic solutions, dental and wound treatments, toothpastes and mouth rinses, and anti-tumor, anti-bacterial and anti-viral products (for a review of applications of lactoperoxidase, see Ref. 85).

Lactoperoxidase is considerably more heat-resistant than, for example, alkaline phosphatase, having a D-value at 80°C of 4 sec, and a very high Q_{10} value (72, 86).

E. Other Indigenous Enzymes

Approximately 60 enzyme activities have been identified in milk, although most have not been studied in detail. As discussed by Fox (2) and Farkye (87), some indigenous enzymes present at low levels in milk have an established function for the development of the neonate, while others may be present in milk simply because of the way in which it is synthesized in the udder or through leakage from the blood. A partial list of minor enzyme activities identified in milk is shown in Table 27.3; most of these enzymes have no technological significance.

Putative roles have been suggested by Farkye (87) for some indigenous enzymes in milk present at low concentrations, although it should be stressed that many of these

TABLE 27.3
Partial List of Minor Enzyme Activities in Milk (Modified from Ref. 87)

EC No.	Enzyme	Reaction Catalyzed	Location in Milk
1.1.1.14	L-Iditol dehydrogenase	L-Iditol + NAD^+ ⇆ L-sorbose + NADH	SM
1.1.1.27	L-Lactate dehydrogenase	L-lactate + NAD^+ ⇆ pyruvate + NADH + H^+	?
1.1.1.37	Malate dehydrogenase	Malate + NAD^+ ⇆ oxaloacetate + NADH	SM
1.1.1.40	Malic enzyme (oxaloacetate-decarboxylating) ($NADP^+$)	Malate + $NADP^+$ ⇆ pyruvate + CO_2 + NADPH	SM
1.1.1.42	Isocitrate dehydrogenase ($NADP^+$)	Isocitrate + $NADP^+$ ⇆ 2-oxogluterate + CO_2 + NADPH	SM
1.1.1.44	Phosphoglucuronate dehydrogenase (decarboxylating)	6-Phospho-D-gluconate + $NADP^+$ ⇆ D-ribulose 5-phosphate + CO_2 + NADPH	SM
1.1.1.49	Glucose-6-phosphate dehydrogenase	D-Glucose-6-phosphate + $NADP^+$ ⇆ D-glucono-1,5-lactone 6-phosphate + NADPH	SM
1.1.3.22	Xanthine oxidase	Xanthine + H_2O + O_2 ⇆ uric acid + $O_2^{(-\cdot)}$ Xanthine + NAD^+ + H_2O ⇆ uric acid + NADH	FGM
1.4.3.6	Amine oxidase (Cu-containing)	RCH_2NH_2 + H_2O + O_2 ⇆ RCHO + NH_3 + H_2O_2	SM
_	Polyamine oxidase	Spermine → spermidine → putrescine	SM
_	Sulphydryl oxidase	2RSH + O_2 → RSSR + H_2O_2	SM
_	Fucosyltransferase	Catalyzes transfer of fucose from GDP L-fucose to specific oligosaccharides and glycoproteins	SM
1.6.99.3	NADH dehydrogenase	NADH + acceptor ⇆ NAD^+ + reduced receptor	FGM
1.8.1.4	Dihydrolipomide dehydrogenase (diaphorase)	Dihydrolipomide + NAD ⇆ lipoamide + NADH	SM/FGM
1.11.1.6	Catalase	$2H_2O_2$ ⇆ $2H_2O$ + O_2	SM/FGM
1.11.1.9	Glutathionine peroxidase	2GSH + H_2O_2 → GSSH + $2H_2O$	SM
1.15.1.1	Superoxide dismutase	$2O_2^{(-\cdot)}$ + $2H^+$ → H_2O_2 + $O_2^{\bullet}$	SM
2.3.2.2	γ-Glutamyl transferase	(5L-Glutamyl)-peptide + amino acid ⇆ peptide + 5-L-glutamyl-amino acid	SM/FGM
2.4.1.22	Lactose synthetase A protein UDP-galactose: D-glucose, 1-galactosyl-transferase B protein: α-lactalbumin	UDP galactose + D-glucose ⇆ UDP + lactose	SM
2.4.1.38	Glycoprotein 4-β-galactosyltransferase	UDP galactose + *N*-acetyl-D-glucosaminyl-glycopeptide ⇆ UDP + 4-β-D-galactosyl-*N*-acetyl-D-glucosaminylglycopeptide	FGM
2.4.1.90	*N*-Acetyllactosamine synthase	UDP galactose + *N*-acetyl-D-glucosamine ⇆ UDP + *N*-acetyllactosamine	
2.4.99.6	CMP-N-acetylneuraminate-galactosyldiacyl-glycerol α-2,3-sialyltransferase	CMP-*N*-acetylneuraminate + β-D-galactosyl-1,4-*N*-acetyl-D-glucosaminyl-glycoprotein ⇆ CMP + α-*N*-acetylneuraminyl 1-2,3-β-D-galactosyl-1,4-*N*-acetyl-D-glucosaminyl-glycoprotein	SM
2.5.1.3	Thiamin-phosphate pyrophosphorylase	2-Methyl-4-amino-5-hydroxy-methylpyrimidinediphosphate + 4-methyl-5-(2-phosphonooxyethyl)-thiazole ⇆ pyrophosphate + thiamine monophosphate	FGM
2.6.1.1	Aspartate aminotransferase	L-Aspartate + 2-oxoglutarate ⇆ oxaloacetate + L-glutamate	SM
2.6.1.2	Alanine aminotransferase	L-Alanine + 2-oxoglutarate ⇆ pyruvate + L-glutamate	SM
2.7.7.49	RNA-directed DNA polymerase	n Deoxynucleoside triphosphate ⇆ n pyrophosphate + DNA_n	SM
2.8.1.1	Thiosulphate sulphurtransferase	Thiosulphate + cyanide ⇆ sulphite + thiocyanate	SM
3.1.1.8	Cholinesterase	An acylcholine + H_2O ⇆ choline + a carboxylic acid anion	FGM
3.1.3.4	Phosphatidate phosphatase	A 3-*sn*-phosphatidate + H_2O ⇆ a 1,2-diacyl-*sn*-glycerol + orthophosphate	FGM
3.1.3.5	5′-Nucleotidase	5′-Ribonucleotide + H_2O ⇆ ribonucleoside + Pi	FGM
3.1.3.9	Glucose-6-phosphatase	D-Glucose 6-phosphate + H_2O ⇆ D-glucose + Pi	FGM
3.1.4.1	Phosphodiesterase I	Removes 5′-nucleotides successively from the 3′-hydroxy terminal of 3′-hydroxy-terminated oligonucleotides	FGM
3.1.6.1	Arylsulphatase	A phenol sulphate + H_2O ⇆ a phenol + sulphate	
3.2.1.1	α-amylase	Hydrolyzes 1,4-α-D-glucosidic linkages in polysaccharides containing at least three 1,4-α-linked D-glucose units	?

(*Continued*)

TABLE 27.3 (*Continued*)

EC No.	Enzyme	Reaction Catalyzed	Location in Milk
3.2.1.17	Lysozyme	Cleaves 1,4-β-linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues in peptidoglycan	?
3.2.1.21	β-Glucosidase	Hydrolysis of terminal non-reducing β-D-glucose	FGM
3.2.1.23	β-Galactosidase	Hydrolysis of terminal non-reducing β-D-galactosides	FGM
3.2.1.24	α-Mannosidase	Catalyzes the hydrolysis of terminal, non-reducing residues from α-D-mannosides	SM
3.2.1.30	*N*-Acetyl-β-D-glucosaminidase	Hydrolysis of terminal non-reducing *N*-acetyl-β-D-glucosamine residues from glycoproteins	SM??
3.2.1.31	β-Glucuronidase	β-D-glucuronoside + H_2O ⇆ alcohol + D-glucuronic acid	?
3.2.1.51	α-L-Fucosidase	An α-L-fucoside + H_2O ⇆ alcohol + L-fucose	—
3.4.11.1	Cystol aminopeptidase (leucine aminopeptidase)	Aminoacyl-peptide + H_2O ⇆ amino acid + peptide	SM
3.4.11.3	Cystinyl-aminopeptidase (oxytocinase)	Cystinyl-peptide + H_2O ⇆ amino acid + peptide	SM
3.4.21.4	Trypsin	Hydrolyzes peptide bonds, preferentially Arg-X, Lys-X	SM
3.6.1.1	Inorganic pyrophosphatase	Pyrophosphate + H_2O ⇆ 2 orthophosphate	SM/FGM
3.6.1.3	Adenosine triphosphatase	ATP + H_2O → ADP + Pi	FGM
3.6.1.6	Thiamine pyrophosphatase (nucleoside diphosphatase)	A nucleoside diphosphate + H_2O ⇆ a nucleotide + orthophosphate	FGM
3.6.1.9	Nucleotide pyrophosphatase	A dinucleotide + H_2O ⇆ 2 mononucleotides	SM/FGM
4.1.2.13	Fructose-biphosphate aldolase	D-Fuctose-1,6-biphosphate ⇆ glycerol phosphate + D-glyceraldehyde-3-phosphate	SM
4.2.1.1	Carbonate dehydratase	H_2CO_3 ⇆ CO_2 + H_2O	SM
5.3.1.9	Glucose-6-phosphate isomerase	D-Glucose 6-phosphate ⇆ D-fructose 6-phosphate	SM
6.4.1.2	Acetyl-CoA carboxylase	ATP + acetyl-CoA + HCO_3^- ⇆ ADP orthophosphate + malonyl-CoA	FGM

SM = skim milk; FGM = fat globule membrane.

hypotheses are highly speculative and that further research is necessary for their validation.

Some of these are summarized below:

- L-Lactate dehydrogenase, catalase, β-glucuronidase and *N*-acetyl-β-D-glucoseaminidase have been suggested as marker enzymes for mastitis.
- Inactivation of α-mannosidase has been suggested as a method to monitor heat treatment of milk at a temperature between 80 to 90°C.
- Glutathione peroxidase is a selenoenzyme and thus is a source of Se in milk.
- Indigenous lysozyme may have an antibacterial effect. Lysozymes (muramidase, peptidoglycan-*N*-acetylmuramoyl hydrolase) cleave the glycosidic linkage between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine in the peptidoglycan of bacterial cell walls.
- Sulphydryl oxidase in milk produces H_2O_2, which may be involved in the antibacterial lactoperoxidase system in milk.
- Superoxide dismutase may play a role in maintaining the oxidative stability of milk by scavenging the superoxide ions produced by xanthine oxidase.
- Xanthine oxidase, one of the most well-studied indigenous enzymes in milk, plays a role in the metabolism of purines, catalyzing the oxidation of hypoxanthine to xanthine, of xanthine to uric acid and the superoxide anion. It has been suggested that xanthine oxidase might be involved in the development of a spontaneous oxidized flavor in milk, although studies disagree on its role in this defect. It had been suggested that xanthine oxidase plays a role in the development of atherosclerosis, but this hypothesis has been disproved.
- Catalase, which contains iron, may act non-enzymatically to promote lipid oxidation.

III. EXOGENOUS ENZYMES

A. Milk Coagulants

It is speculated that rennet-coagulated cheese developed accidentally, due to the separation into curds and whey of milk stored in bags made from animal stomachs. Scientific study of this phenomenon revealed that such stomachs contain enzymatic activity with milk-clotting activity (MCA). The nature and composition of this activity changes with age of the cow; an extract of calf contains predominantly

the aspartic proteinase, chymosin, with a small amount of pepsin. With increasing age, however, the proportion of chymosin decreases, while that of pepsin increases.

While many proteinases will coagulate milk under suitable conditions, many decades of investigation of other enzymes suitable for coagulation of milk for industrial cheesemaking failed to uncover an enzyme better than chymosin. The first commercially available standardized calf-derived coagulant preparation was produced by Christian Hansen in 1874. Rennet was the first enzyme industrially produced and sold with a standardized enzyme activity. The characteristics of chymosin as a milk coagulant have been reviewed (88–93).

Calf chymosin has a very high specificity for the Phe_{105}-Met_{106} bond of bovine κ-casein; this is thought to be partly due to electrical attraction between the positively charged region of the protein from residues 98 to 111 and the negatively charged active site of the enzyme, and also to structural conformation of the binding pocket of the enzyme relative to this region of the substrate protein. Cleavage of this bond removes the glycosylated region of the protein (glycomacropeptide, GMP); the remainder of the molecule is referred to as para-κ-casein (κ-CN f1-105). The enzymatic reaction is first-order and diffusion-limited in nature; the velocity of the reaction depends greatly on pH and temperature.

The portion of the molecule removed by chymosin, known as the caseinomacropeptide (CMP), stabilizes the hydrophobic caseins as micelles in the aqueous environment of milk, by mutual electrostatic and steric repulsion of individual micelles. Removal of the GMP reduces the zeta potential of casein micelles, and greatly destabilizes them. When denuded of a critical level (~80%) of this protective coating, in the presence of a sufficient level of calcium and at a temperature above 18°C, the casein micelles begin to aggregate, at first into chains and clusters, but finally coalescing into a three-dimensional network, which results in the gelation of the milk (for a review of the manner in which coagulants destabilize the casein micelle, see Ref. 94).

Chymosin is an aspartic proteinase (MW 35,600 Da) with a pH optimum for proteolytic activity around 4. Chymosin has been very well characterized at the enzymatic and molecular levels. While it exhibits a very high specificity for the Phe_{105}-Met_{106} bond of κ-casein, chymosin is also generally proteolytically active on the caseins. However, during the coagulation of milk, the reaction which causes milk coagulation occurs at such a relatively rapid rate that very little additional proteolysis occurs during the course of cheesemaking; hence, the loss of peptides in whey is very low. This unusually low level of non-specific proteolysis during cheesemaking (in comparison to other milk coagulants) is the main reason why chymosin is regarded as such an ideal milk coagulant (95).

While most of the chymosin added to milk in cheesemaking is lost in the whey, some adsorbs onto the caseins and is incorporated into the cheese curd; in addition, some is retained in the serum phase (whey) within the cheese (96). Along with plasmin, chymosin is one of the most significant agents during the early stages of ripening of many cheese varieties, hydrolyzing the caseins to large polypeptides that are further degraded by starter bacterial proteinases and peptidases. Chymosin hydrolyzes α_{s1}- and β-caseins quite readily (95). The hydrolysis of β-casein at pH 6.5 is strongly inhibited by increasing salt concentration, due to hydrophobic interactions, which shield chymosin-sensitive peptide bonds; this inhibitory effect is reduced at lower pH values. Chymosin principally hydrolyzes α_{s1}-casein at the Phe_{23}-Phe_{24} bond, yielding the peptides α_{s1}-CN (f1-23) and α_{s1}-CN (f24-199; sometimes known as α_{s1}-I-casein); this cleavage reaction plays a role in the initial softening of cheese texture during ripening (97). However, several other bonds in α_{s1}-casein are also cleaved by chymosin in cheese. α_{s2}-Casein is relatively resistant to hydrolysis by chymosin (95).

The activity of chymosin is generally evaluated by activity tests which measure the ability of an extract or preparation containing the enzyme to coagulate milk under controlled conditions (temperature, pH, etc.). More recently, a more sensitive and objective method was developed in the authors' laboratory; this test uses reversed-phase high-performance liquid chromatography (RP-HPLC) to measure the hydrolysis of a synthetic heptapeptide substrate by chymosin (98).

The optimum temperature for the coagulation of milk by chymosin is 45°C, but at temperatures above 50°C the enzyme is rapidly inactivated (99). This inactivation is responsible for the low chymosin activity in Swiss-type cheese, which is cooked during manufacture at around 55°C; the resultant lack of primary proteolysis of α_{s1}-casein in these cheeses during ripening contributes to their rubbery and elastic texture. Pasteurization of whey almost completely inactivates the enzyme, facilitating further processing of this by-product of cheese manufacture.

In the 1970s, the availability of calf stomachs became limited due to increased demand for rennets and reduction in the number of calves slaughtered, leading to an intense search for novel milk coagulants. An ideal alternative to chymosin as a coagulant for milk would:

- Be an acid proteinase capable of hydrolyzing κ-casein at approximately the same position as chymosin
- Possess a similar ratio of MCA to overall proteolytic activity
- Have an MCA that is not very pH dependent in the region 6.5–6.9
- Have thermostability comparable to that of calf rennet at the pH values and temperatures used during cheesemaking

- Have low thermostability during whey processing
- Possess the ability to produce desired flavor, body and texture characteristics in the finished cheese (100)

One result of this search was the identification of a number of microbial coagulants, as will be discussed below; the other was the application of the developing discipline of biotechnology to the production of pure chymosin (fermentation-produced chymosin, FPC) from genetically manipulated microorganisms, such as *E. coli*, *Aspergillus niger* and *Kluyveromyces lactis* (101, 102). More recent public concerns over bovine-related health issues (such as BSE and foot-and-mouth disease) have further increased demand for FPC and other alternatives to traditional rennets.

Among the principal advantages of FPC today are:

- Constant, controlled activity of a very pure enzyme
- Guaranteed supply
- Suitability for vegetarian, Kosher or Halal cheese

FPCs are identical in amino acid sequence to calf chymosin, and differences from the latter enzyme may occur only due to differing degrees of glycosylation by the host microorganism; such changes, however, have not been found to influence the properties of the enzyme. FPCs have been intensively studied in cheesemaking trials and generally found to give very satisfactory results (103), and are now widely used commercially in many countries.

Substitutes for chymosin used as coagulants include pepsins (bovine, porcine and to a lesser extent, chicken) and microbial proteinases from *Rhizomucor miehei*, *R. pusillus* and *Cryphonectria parasitica*. However, the fungal rennet substitutes are now used most commonly. Interestingly, *C. parasitica* proteinase cleaves κ-casein at Ser_{104}-Phe_{105} rather than at the Phe_{105}-Met_{106} bond, which is cleaved by chymosin, pepsins and the other fungal proteinases. *C. parasitica* proteinase has relatively high heat stability. The use of coagulants more heat stable than calf rennet should be avoided; otherwise, excess proteolytic activity may remain in the curd and may result in excessive proteolysis and bitterness unless ripening times and/or cooking temperatures are changed to compensate for the more rapid rate of proteolysis (96).

Plant rennets have a long history; the use of fig rennet was mentioned in Homer's *Iliad*. However, most plant coagulants are too proteolytic relative to their MCA, resulting in a reduced yield of curd and the development of bitterness during ripening. An exception is a protease from dried flowers of the cardoon thistle, *Cynara cardunculus*, which contain two enzymes, cardosins A and B, and which have been used successfully for many centuries in the Iberian Peninsula for the manufacture of some traditional cheeses, e.g., Serra da Estrela, La Serena, Los Pedroches (104).

Commercial rennet extracts are free from lipolytic activity. However rennet paste, which does contain lipolytic activity, is used in the manufacture of some hard Italian varieties (e.g., Provolone, Pecorino Romano and many Pecorino cheeses). Rennet pastes are prepared from the abomasa of calves, kids or lambs slaughtered after suckling. The abomasum and contents are partially dried and ground into a paste, which is slurried in milk before being added to the milk from which cheese is to be made. Rennet paste contains a lipase, pregastric esterase (PGE), which is highly specific for short-chain acids esterified at the *sn*-3 position. Suckling stimulates the secretion of PGE by glands at the base of the tongue, and it is washed into the abomasum with the milk. Due to concerns regarding the hygienic quality of rennet pastes, research has been focused on exogenous lipases which could be blended with rennet extracts to produce substitutes for rennet pastes; certain fungal lipases may be acceptable alternatives, as are semi-pure preparations of PGE (100).

B. Acceleration of Cheese Ripening and Enzyme-Modified Cheeses

In addition to their use as coagulants, exogenous enzymes have been investigated as possible agents to accelerate the ripening process of cheese. Cheese ripening is a slow, and consequently expensive, process in which the bland-flavored immature curd develops the flavor and texture characteristic of the mature cheese. Although soft (high moisture) varieties ripen quickly, the ripening period of hard cheeses is quite long (e.g., 6–24 months for Cheddar). A number of strategies have been investigated to reduce this ripening period, including the use of an elevated temperature, exogenous enzymes, slurry systems, adjunct cultures and high pressure treatment (105–108).

Proteinases, peptidases and lipases have been added individually, or in various combinations, to cheese with the objective of accelerating ripening. However, the use of exogenous enzymes to accelerate ripening suffers from a number of drawbacks, which have limited the commercial use of this approach. Probably the most serious limitation of the use of exogenous enzymes to accelerate ripening is that cheese flavor is the result of a wide range of sapid compounds produced by a number of enzyme-catalyzed pathways. Thus, addition of specific enzymes will not accelerate all pathways simultaneously and equally and thus can result in unbalanced flavor. In the case of exogenous proteinases, reduction in yield, textural problems and the development of bitterness have been reported (although the last defect can be ameliorated by the use of selected peptidase preparations). A second major

drawback to the use of exogenous enzymes is that most of the enzyme added to the cheesemilk is lost in the whey. Enzymes can be added more efficiently to varieties such as Cheddar by mixing with the dry salt, but problems with uniform distribution of enzyme have been reported. Encapsulation of enzymes (e.g., in milk fat or liposomes) has been used in attempts to overcome some of these problems, but with limited success (106).

Enzyme-modified cheeses (EMCs) are concentrated cheese flavors produced by the enzyme-catalyzed hydrolysis of cheese curd or other ingredients (109, 110). EMCs are produced by adding exogenous enzymes, e.g., exogenous proteinase, peptidase and/or lipase preparations, to a pasteurized slurry of emulsified cheese curd and incubating under controlled conditions (typically 30–45°C for 24–72 h). The product is then heat treated (70–85°C) to inactivate the added enzymes. EMCs are sold as pastes (40–60% moisture) or, after drying, as powders. EMCs may be manufactured using one-step or component approaches; in the latter approach, several different flavor fractions (e.g., produced by lipolysis or proteolysis) are then blended to give the final product.

Flavor generation in EMCs is principally *via* lipolysis and proteolysis of the base material using exogenous enzymes. EMCs contain high levels of free fatty acids, peptides and amino acids, far in excess of those of natural cheese. The extensive proteolysis of the caseins during the manufacture of EMCs often leads to bitterness unless proteolytic enzymes are carefully selected or other debittering strategies are adopted. The flavor of EMCs may be augmented by the addition of flavor enhancers such as monosodium glutamate, yeast extract, NaCl, organic acids or starter distillates.

A wide range of exogenous lipases, proteinases (e.g., from *Bacillus* or *Aspergillus* spp.) and peptidases (e.g., from *Aspergillus*, *Rhizomucor* or *Lactococcus* spp.) are used in the manufacture of EMCs. Most lipases used are derived from animal or microbial sources; the most commonly used animal lipase is PGE. PGE preferentially liberates strongly flavored fatty acids. A range of microbial lipases is also available; they tend to be cheaper than animal lipases and may have broader specificity. In addition, microbial lipases have vegetarian status and are free from amylase activity, which otherwise may cause problems in foods to which EMCs are added, many of which may contain starch.

Proteinases used in the manufacture of EMCs are generally derived from microbial sources (e.g., *Bacillus* spp. or *Aspergillus* spp.) and are used to develop rapidly an intense savory background flavor. Peptidase preparations, derived from fungal or bacterial sources, are used to enhance flavor and/or to control bitterness. Peptidase preparations contain a range of enzymes, including endopeptidases, aminopeptidases and proline-specific peptidases.

EMCs have the advantages over other sources of cheese flavors in the intensity and range of flavors available, reduced production costs and extended shelf-life. Because of their high flavor intensity, only small amounts of EMCs (ca. 0.1%, w/w) are needed to impart a cheese flavor to products in which they are used (e.g., processed cheese, cheese analogues, cheese spreads, snack foods, soups, sauces, cookies, dips and pet foods).

C. β-Galactosidase

Hydrolysis of the milk sugar, the disaccharide lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose), yields two monosaccharides, D-glucose and D-galactose (111). Hydrolysis of lactose may be achieved either chemically, by acidification to low pH values, or enzymatically. The enzymes capable of catalyzing the latter hydrolysis reaction are collectively known as β-galactosidases or, more simply, lactases (although, strictly speaking, many β-galactosidases, especially those of plant origin, cannot degrade lactose) (112, 113).

A large proportion of the world's population suffers from lactose intolerance, leading to varying degrees of gastrointestinal distress if dairy products are consumed. The two products of the hydrolysis of lactose, however, are more readily metabolized; glucose and galactose are also sweeter than the parent sugar.

Lactose also has a number of other properties that cause difficulty in the processing of dairy products. Among these is its tendency to form large crystals on cooling of concentrated solutions of the sugar; galactose and, to an even greater extent, glucose, are more soluble than lactose (112). Thus, for example, hydrolysis of whey concentrates offers the possibility of preserving such products through increasing osmotic pressure while maintaining physical stability. Industrial applications of lactose hydrolysis have, in fact, focused to a large extent on treatment of whey to produce lactose-hydrolyzed whey concentrates or syrups (112).

Other applications of lactose hydrolysis include production of low-lactose market milk (114); this may even be achieved domestically by addition of a commercial preparation of β-galactosidase to fresh milk and incubation in the refrigerator. For long shelf-life products (such as UHT milk), β-galactosidase may be added to the package at a very low level and allowed to work slowly during storage. Lactose hydrolysis also has applications in ice cream manufacture, where it can reduce the incidence of sandiness (due to lactose crystallization) during storage and, due to the enhanced sweetness, permit the reduction of sugar content. Use of lactose-hydrolyzed milk can also accelerate the acidification of yoghurt (112).

Several sources of β-galactosidase (including yeasts, fungi and bacteria) have been identified, including *E. coli, Aspergillus niger, A. oryzae* and *Kluyveromyces lactis*

(111–113). The enzyme from the latter yeast is active at 4°C, which raises interesting possibilities in dairy processing (for example, it could be added to milk before cold storage in silos, removing the need for holding at a higher temperature with concomitant problems of microbial control). There is also much current interest in the use of β-galactosidase from thermophilic bacteria, which could permit operation of reactors at a high temperature, resulting in high rates of hydrolysis without the risk of growth of contaminating microorganisms (115). The principal criterion for acceptability of a microbial β-galactosidase is that the source should be safe and acceptable to regulatory authorities; genes for some microbial β-galactosidases have been cloned into safe hosts for expression and recovery for this reason.

The activity of β-galactosidase is generally monitored either by measuring disappearance of lactose or production of galactose and glucose (measurement of product monosaccharides is easier and more common, e.g., by HPLC or commercial enzymatic assay kits) (112). Synthetic substrates may also be used for assays of activity. In milk, simple and rapid estimation of hydrolysis of lactose may be achieved by monitoring the freezing point: freezing point; depression is linearly correlated with the degree of hydrolysis. However, many assay methods are complicated in cases where production of side-products (oligosaccharides) is significant.

For hydrolysis of lactose in milk, β-galactosidase may simply be added in free solution, allowed sufficient time to react typically, for most enzymes, at a temperature from 10 to 35°C, and inactivated by heat-treatment of the product. In an attempt to control the reaction and prevent uneconomical single-use of the enzyme, immobilized enzyme technologies have been studied widely (116, 117). In such systems, β-galactosidase may be immobilized on inorganic supports (which can change its kinetic properties) or entrapped within gels or fibers, such as those of cellulose acetate. One of the earliest systems, developed by the Corning Glass Company in the U.S., used covalent enzyme attachment to microporous silica beads (112). In entrapment-based systems, the porosity of fibers allows inward and outward diffusion of reaction products, but does not allow the enzyme to escape. The enzyme is, in effect, in solution within the fibers, and its enzymatic characteristics are not affected. Batchwise systems based on cellulose acetate membranes capable of processing 10,000 L milk per day have been developed. Overall, however, few immobilized systems for lactose hydrolysis are used commercially, the main limitations being the poor mechanical properties of supports and other technological problems with the process.

In an alternative approach, after hydrolysis of lactose, β-galactosidase may be recovered from milk or whey by ultrafiltration and reused, which combines the kinetic advantages of using the soluble enzyme with the economical benefits of recycling of the enzyme.

A new technique with potential for application in lactose hydrolysis processes is the use of permeabilized cells of bacteria or yeast (e.g., *Kluyveromyces lactis*) with β-galactosidase activity (118). These cells are treated, e.g., with ethanol, to allow diffusion of substrate and reaction products across the damaged cell membrane; the cell itself becomes the immobilization matrix and the enzyme is active in its natural cytoplasmic environment. Permeabilization may provide a crude but convenient and inexpensive enzyme utilization strategy.

Lactose hydrolysis reactions rarely lead to complete conversion of lactose to monosaccharides, for two reasons (112). Firstly, the galactose produced in the reaction can inhibit the enzyme in a feedback inhibition mechanism. Secondly, side-reactions (such as transferase reactions) often occur, resulting in the production of isomers of lactose and oligosaccharides. While initially thought to be an undesirable by-product of lactose hydrolysis, galactooligosaccharides, it is now recognized, may actually act as bifidogenic factors, enhancing the growth of desirable probiotic bacteria in the intestines of consumers and depressing the growth of harmful anaerobic colonic bacteria.

Overall, despite considerable interest, industrial use of lactose hydrolysis by β-galactosidases has not been widely adopted (119).

D. Transglutaminase

Transglutaminase (TGase) can modify the properties of many proteins through the formation of new cross-links, incorporation of an amine or deamidation of glutamine residues (120). To create protein cross-links, TGase catalyzes an acyl-group transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues and the primary amino group of a variety of amine compounds, including lysine residues in proteins. TGase (protein-glutamine: amine γ-glutamyl-transferase) type-enzymes are widespread in nature. For example, blood factor XIIIa, or fibrinoligase, is a TGase-type enzyme, and TGase-mediated cross-linking is involved in cellular and physiological phenomena such as cell growth and differentiation, as well as blood clotting and wound healing. TGase enzymes have been identified in animals, plants, fish and microbes (e.g., *Streptoverticillium mobaraense*); the latter is the source of much of the TGase used in food studies. TGase from different sources may either be calcium-independent (most microbial enzymes) or calcium-dependent (typical for mammalian enzymes) in mode of action.

TGase-catalysed cross-linking can alter the solubility, hydration, gelation, rheological, emulsifying, rennetability and heat stability properties of a variety of food proteins. Not all proteins are similarly affected by the action

of TGase; the structure of individual proteins determines whether cross-linking by TGase is possible.

Perhaps not surprisingly, in recent years a number of studies have explored the potential applications of TGase in dairy protein systems. The caseins are good substrates for TGase, due to their open structure (121, 122). Treatment of casein micelles with TGase stabilizes them to the action of dissociating agents, such as calcium chelators or urea. In contrast, the whey proteins, due to their globular structure, require modification, for example heat-induced denaturation, to allow cross-linking.

A number of studies have shown clear effects of TGase treatment on the properties of milk and dairy products (123, 124). For example, TGase treatment of fresh raw milk increases its heat stability (125); however, if milk is pre-heated under conditions where whey protein denaturation occurs prior to TGase treatment, the increases in heat stability are even more marked. This is probably due to the formation of cross-links between the caseins and unfolded denatured whey proteins, brought into close proximity by formation of disulphide bridges between β-lactoglobulin and micellar κ-casein. When the whey proteins in milk are in the native state, the principal cross-linking reactions involve the caseins; heat-induced denaturation renders the whey proteins susceptible to cross-linking, both to each other and to casein molecules.

There has been considerable interest in the effects of TGase treatment on the cheesemaking properties of milk, in part due to the potential for increasing cheese yield. Some commercial reports have indicated that treatment, either of milk before renneting, or of curd if TGase is added during the cheesemaking process itself, can achieve this effect. However, a number of recently published studies (124, 126) have indicated that the rennet coagulation properties of milk and syneretic properties of TGase-crosslinked rennetted milk gels, as well as the proteolytic digestibility of casein, are impaired by cross-linking of proteins, which may alter cheese manufacture and ripening. Further studies are required to evaluate whether limited, targeted cross-linking may give desirable effects.

Other potential benefits of TGase treatment of dairy proteins include physical stabilization and structural modification of products such as yoghurt, cream and liquid milk products, particularly in formulations with a reduced fat content, and the production of protein products, such as caseinates or whey protein products, with modified or engineered functional properties (127–129).

Overall, it appears clear that TGase may have commercial applications in modifying the functional characteristics of milk and dairy products. However, some issues remain to be clarified; for example, control of the reaction in milk, to fix the exact extent of cross-linking of proteins to achieve desired properties, requires understanding of the heat inactivation kinetics of TGase in milk, which have not been reported. Further research is also required on the effect of variables such as temperature, pH and other operating conditions on the nature and rate of cross-linking reactions. Finally, a clear commercial advantage of using TGase over other methods of manipulating the structure and texture of dairy products (such as addition of proteins or hydrocolloids) must be established definitively.

E. Miscellaneous Exogenous Enzymes

There are also some additional enzymes that may be added exogenously to milk (130), although their commercial application is limited. These are summarized below.

(i) Since human milk contains significantly more indigenous lysozyme than bovine milk, the supplementation of milk-based infant formulae with lysozyme has been proposed. It has been claimed that supplementation of such formulae with egg-white lysozyme gives beneficial results, especially with premature infants, but results are equivocal. Lysozyme is effective at killing *Clostridium* cells and preventing the outgrowth of their spores and thus it also has been studied as an alternative to nitrate for preventing the growth of *C. tyrobutyricum* in cheese and the butyric acid fermentation which leads to late-gas blowing. Lysozyme has also been shown to be quite effective against *Listeria monocytogenes* and other bacteria involved in food-borne illness and spoilage (130).

(ii) Glucose oxidase (GO) catalyzes the oxidation of glucose to gluconate *via* gluconic acid-δ-lactone (Figure 27.1). Glucose oxidase has four principal uses in the food industry, viz, removal of trace levels of glucose, removal of trace levels of O_2, generation of H_2O_2 *in situ* and production of gluconic acid *in situ*. However, GO is not commercially very significant, particularly in the dairy industry (131).

(iii) Superoxide dismutase (SOD) catalyzes the reduction of superoxide anions to H_2O_2 and O_2. It has been suggested that SOD together with catalase may be useful as an antioxidant in dairy products, although as far as we know, it is not used commercially for this purpose (130).

(iv) Sulphydryl oxidase, which catalyzes the oxidation of sulphydryl groups to disulphides

$$\text{i.e., } 2RSH + O_2 \rightarrow RSSR + H_2O$$

has been shown to ameliorate the cooked flavor of UHT milk, although, as far as we know, the enzyme is not used commercially for this purpose (132).

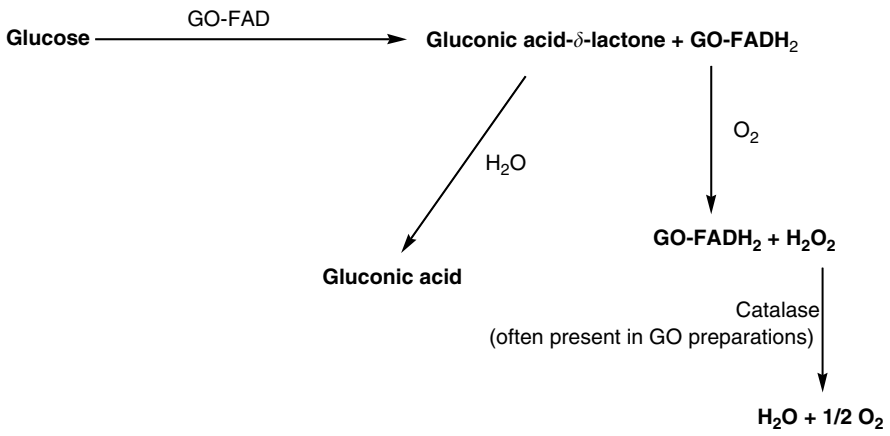

FIGURE 27.1 Reactions catalysed by glucose oxidase (GO).

(v) Catalase catalyzes the decomposition of H_2O_2 to H_2O and O_2. Produced H_2O_2 may be used for the cold-sterilization of milk and excess H_2O_2 may be destroyed by the use of exogenous catalase (130).

(vi) Exogenous β-lactamase, which degrades β-lactam antibiotics (e.g., penicillin), has been shown to be able to render milk contaminated with penicillin suitable for cheese manufacture (133).

IV. CONCLUSIONS

In summary, the two classes of enzymes considered in this chapter, i.e., indigenous and exogenous enzymes, are of great significance to the dairy industry, and an area of ongoing research and study, for two major reasons.

Firstly, milk is not an enzymatically inert raw material; on the contrary, it possesses a heterogeneous and highly variable complement of enzymes of many different types, the exact definition of which remains unclear, as does the significance of many of these enzymes for dairy product quality.

Secondly, the dairy industry is and remains a significant consumer of large quantities of commercially produced enzymes (e.g., the world market of commercial rennet is ~ 30×10^6 liters per annum, representing one of the largest commodity enzymes in use today). Identification of novel sources of enzymes (e.g., the search for more thermostable β-galactosidases, which would allow operation at temperatures which would inhibit microbial growth), and optimization of the application and control of the activity of existing enzymes remain key food biotechnological goals. In parallel, demand for new dairy products drives the study of novel enzymatic reactions that can be harnessed to manipulate the flavor, texture or other characteristics of milk and dairy products.

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28 Meat: Chemistry and Biochemistry

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CONTENTS

I. INTRODUCTION

Meat quality is very important for all segments of the industry from producers to consumers. There are many aspects such as source, cost, ethical factors, religion, production systems and safety that affect meat acceptability by consumers. Other quality factors perceived by consumers are related to sensory characteristics (i.e., color, tenderness and flavor), nutritional properties (i.e., calories, vitamins content, fatty acids profile, etc.) and appearance (i.e., exudation, marbling, amount of fat, etc.). One of the major concerns for consumers is the variability in meat quality; this constitutes a problem to solve within the meat industry. The composition, structure and metabolic status of the muscle have a great influence on numerous chemical and biochemical changes affecting meat quality characteristics such as tenderness in beef or exudation in pork and poultry. This chapter summarizes the chemistry and biochemistry of meat, taking into account how all these factors interact during postmortem stages and thus how they affect the final meat quality. A better understanding of these reactions and changes is essential for the standardization and improvement of production processes to obtain a high quality meat.

II. MUSCLE STRUCTURE

Skeletal, smooth and cardiac constitute the main types of muscles. However, from the point of view of meat, skeletal muscle is the most important because it represents a high percentage of the total body weight. Skeletal muscle is voluntary (the organism can contract it voluntarily), striated (cross striations may be observed under the microscope due to the alternance of dark and light bands) and multinucleated (several nuclei located peripherally in the cell). The muscle is covered by a sheath of connective tissue, the epimysium. There are several bundles of fibers inside the muscle which are covered by thin connective tissue layers, the perimysium, and each individual fiber is wrapped by thin collagen, the endomysium (see Figure 28.1). Each muscle fiber may contain about 1,000

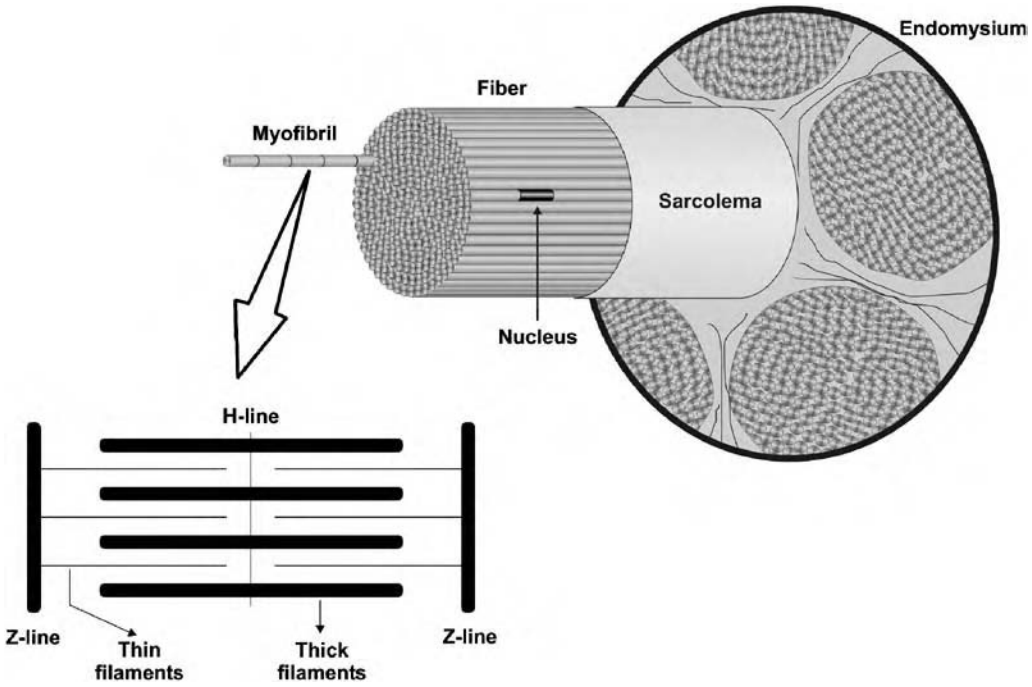

FIGURE 28.1 Scheme of the organization and structure of skeletal muscle.

myofibrils, which are arranged in a parallel way, and are responsible for the contraction of the muscle (1). Fibers have an approximate diameter of around 50 μm and may vary in length from a few milimeters up to several centimeters (2). They contain myofibrils, composed of thick and thin filaments arranged in an array and responsible for muscle contraction and relaxation, giving rise to alternating dark (A) and light (I) bands. The Z and M lines bisect each I and A band, respectively (see Figure 28.1). The M lines connect the center of the thick filaments to keep the structure (3). The Z line appears as a zigzag structure and has diverse properties such as resistance to physical forces but, on the other hand, it is susceptible to protease action (4). The sarcomere, usually in the range of 2–3 μm in length, is defined as the distance between two consecutive Z lines. The thick and thin filaments are composed of myofibrillar proteins (see Table 28.1). Actin filaments extend into Z lines at one end and extend between thick filaments at the other end (5). Thick filaments are mainly composed of myosin with minor presence of other proteins. The fibers are embebbed in a liquid or cytoplasm, also known as sarcoplasm, that contains mitochondria, lysosomes, enzymes, lipids, glycogen, myoglobin, ATP and creatine. As most of these proteins are soluble in water and are present in the sarcoplasm, they are known as sarcoplasmic proteins. The scanning electron microscopy of skeletal muscle reveals details of the muscle fiber structure in a three-dimensional image. An example of a micrograph from the scanning electron micro-scope is shown in Figure 28.2.

Muscles present different appearances and have been traditionally classified as red or white based on the color properties that are dependent on the proportion of the muscle fiber types. Other classifications based on the amount of released water have not been used widely. The red color is related to the content in myoglobin, naturally present in the muscle, and hemoglobin from remaining blood. Differences between red and white muscles are especially evident in pork and poultry while differences in color are relatively masked in beef due to its higher content in myoglobin. Based on histochemical properties, muscle fibers have been classified as red, intermediate and white (6). Red fibers contain higher amounts of myoglobin, are better capillarized, are oxidative in metabolism, and can function over a long period of time but are slower contracting than white fibers that are mainly glycolytic (7). Intermediate fibers have oxidative and glycolytic activity. Thus, red muscles contain a higher proportion of red fibers and are mostly related to locomotion while white muscles have a higher proportion of white fibers and are mostly related to support tasks (8). Other classifications of muscle fibers are based on the contraction speed (9): slow twitch oxidative (type I), fast twitch oxidative type (IIA) and fast twitch glycolytic type (IIB). So, the response of slow twitch muscles to a stimulus is slower and once the stimulus is finished the relax is also slower than fast twitch muscles.

Differences between red and white muscles are also significant during meat processing because large variations are found in sensory properties such as tenderness, juiciness, flavor (10) as well as physicochemical (11) and

TABLE 28.1
Location and Functions of Major Meat Proteins (Adapted from Refs. 2, 9, 24, 151)

Protein	Type	Function
Myosin	Myofibrillar	Major contractile
Actin	Myofibrillar	Major contractile
Tropomyosin	Myofibrillar	Regulatory
Troponins T, C, I	Myofibrillar	Regulatory
α and β actinin	Myofibrillar	Regulatory
Titin	Myofibrillar	Cytoskeletal
Nebulin	Myofibrillar	Cytoskeletal
Filamin,synemin, vinculin, zeugmatin, Z nin	Myofibrillar	Z-line
C, H, X, F, I proteins	Myofibrillar	Thick filaments
Desmin	Myofibrillar	Myofibrils union at Z-line level
Creatin kinase	Myofibrillar	M-line
Myomesin	Myofibrillar	M-line
M protein	Myofibrillar	M-line
Mithocondrial enzymes	Sarcoplasmic	Respiration
Lysosomal enzymes	Sarcoplasmic	Intracellular digestion
Other cytosolic enzymes	Sarcoplasmic	Glycolysis, gluconeogenesis, citric acid cycle, neutral proteolysis
Myoglobin	Sarcoplasmic	Natural pigment
Hemoglobin	Sarcoplasmic	Pigment from residual blood
Cytochrome	Sarcoplasmic	Respiratory pigment
Collagen	Connective	Structure resistance
Reticulin	Connective	Elasticity
Elastin	Connective	Structure resistance
Proteoglycans and glycoproteins	Connective	Ground substance

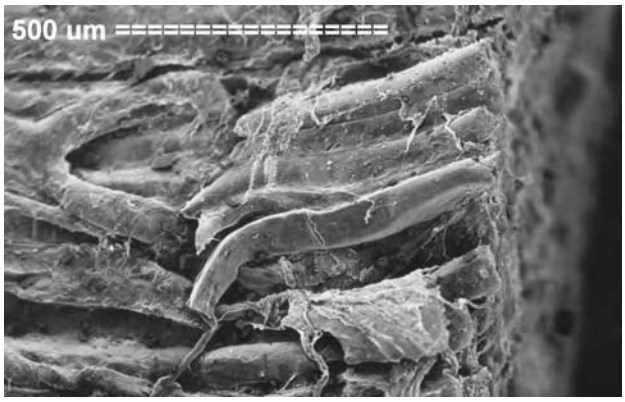

FIGURE 28.2 Scanning electron micrograph of porcine muscle fibers in porcine muscle (Toldrá and Voyle, 1987, unpublished).

biochemical characteristics like enzyme activity (12, 13), lipid composition (14, 15) and content in free amino acids and dipeptides (16–18). These variations may be attributed to certain differences in the biochemical and functional properties of myofibrillar proteins associated with the type of fiber (9, 19).

III. MEAT COMPOSITION

Meat presents a complex structure with water, proteins and lipids present in higher amounts. Water is the most abundant compound in meat (within the range of 65–80%) and has an important influence on juiciness, color, texture and surface appearance. The rest of the composition of meat can be divided into nitrogenous and non-nitrogenous compounds. Nitrogenous compounds are mainly composed of proteins (myofibrillar, sarcoplasmic and connective), peptides (carnosine, anserine and balenine), free amino acids, nucleotides/nucleosides (ATP-derived compounds) and water-soluble vitamins (group B vitamins). In addition to water, non-nitrogenous compounds include lipids (triacylglycerols, phospholipids and cholesterol), minerals (iron, phosphorus, sodium, potassium, etc.), trace elements (zinc, selenium, etc.), carbohydrates (glycogen, glucose, etc.) and fat-soluble vitamins (vitamins A, D, E and K). The main groups of compounds are described below.

A. PROTEINS

After water, protein is the major component of meat, in the range of approximately 15–22 g/100 g. Proteins are very important in postmortem conversion of muscle to meat, in changes affecting tenderness during meat processing and for the nutritional quality of the diet. Meat proteins are considered of high biological value because they provide relatively high percentages of all the essential amino acids (20). There are three categories in meat: myofibrillar, sarcoplasmic and connective (listed in

Table 28.1), in an approximate ratio 60/30/10, with each category having specific roles (7).

Myofibrillar proteins are the main constituents of the structure of the myofibrils. They are soluble in high ionic strength buffers and its content is around 9.5 g/100 g. Myosin and actin provide the structural backbone of the myofibril. Myosin is the major myofibrillar protein and, in fact, is the predominat protein of thick filaments. It is a large molecule composed of two heavy chains of 220 kDa and four light chains within the range of 16–22 kDa (9). The actin monomer, G-actin, has a globular shape and, in the presence of ATP, polymerizes to form filamentous F-actin. The thin filaments ensure by the helical arrangement of F-actin molecules twisted around each other (21). Two extremely large proteins, titin and nebulin, also known as gap filaments, ensure the longitudinal continuity and integrity of the structure (22, 23). Proteins in the Z-line area are involved in the linkage of thin filaments of adjacent sarcomeres while desmin connects adjacent myofibrils at the level of the Z-line. Regulatory proteins like tropomyosin and troponin, which has three subunits (Tn-T, Tn-C and Tn-I), are involved in the muscle contraction mechanism (9).

Sarcoplasmic proteins, around 9 g/100 g, constitute a varied group consisting of proteins soluble in low ionic strength buffers or even water. Myoglobin is the major sarcoplasmic protein responsible for the red color of meat. Myoglobin content in meat is variable, depending on the animal species (high in beef or lamb, low in pork and very low in poultry) and age (increases with the age of the animal). More or less hemoglobin may remain in meat depending on the efficiency of blood draining from muscle during bleeding. The rest of sarcoplasmic proteins consist of metabolic enzymes located in mitochondrias, lysosomes, microsomes, and nucleus and free in the cytosol.

Connective proteins, around 3 g/100 g consisting basically of collagen and elastin, are insoluble and contribute to the skeletal framework (strength, support and shape) of the muscle but also to certain toughness of the meat when consumed. In fact, collagen becomes tougher with the age of animal due to the progressive increase in the number of cross-links. Collagen is rich in hydroxyproline, a low biological value amino acid. There are different types of collagen (8). Type I is found in tendons, type II in cartilages and type III in skin and vascular tissues. Types IV and V form fine networks in the basement membranes around the muscle (24). Elastin is more elastic and is mainly involved in tissues requiring a certain degree of flexibility such as skin, tendons, ligaments, muscle and the walls of large arteries (24).

B. Lipids

The lipids content of muscle is variable (in the range of 1.5–17 g/100 g) and mainly depends on the degree of fattening and amount of adipose tissue. The main classes of lipids are non-hydrolyzable lipids (fatty acids, aldehydes, aliphatic hydrocarbons, sterols), neutral fats (mono, di and triacylglycerols and sterol esters) and phospholipids. The relative proportion varies depending on the species, the developmental stage and physiological status of the animal (8). Main locations of lipids are intramuscular, intermuscular and in adipose tissue. Intramuscular lipids are mainly composed of triacylglycerols, stored in fat cells, and phospholipids, located in cell membranes. Cholesterol is the only sterol found in meat; the cholesterol ester comprises about 90% of the total cholesterol content (25). Intermuscular and adipose tissue lipids are mainly composed of triacylglycerols and contain a small amount of cholesterol (below 60 mg/100 g).

Triacylglycerols are major constituents of reserve fats and have fatty acid composition that varies widely with species and diet. There are three possible pathways for the biosynthesis of triacylglycerols: the glycerol-3-phosphate, the dihydroxyacetone phosphate and the monoacylglycerol pathway (8). The type and fatty acid composition of feeds has a strong influence on the fatty acids profiles of fats, especially in monogastric animals (26, 27). Thus, the effects of different feeds on fatty acid composition of pork and poultry fats have been extensively reported in the literature (27–32) following consumer demands for higher unsaturated fats (26). In the case of ruminants, fat hydrolysis and hydrogenation of unsaturated fatty acids by the microbial population of the rumen somehow tend to standardize the final composition in fatty acids and certain changes in the diet fed to cattle may also change the content of the fat in the meat. For instance, dietary full-fat canola or even full-fat soybeans have been reported to alter the fatty acid composition of intramuscular and subcutaneous lipids of beef (33). The type of diet may have important consequences for the sensory quality of meat, especially its flavor (34). So, changes in the fatty acid composition of phospholipids have been reported to give significant differences in flavor characteristics of forage and grain-fed beef (35). It must be pointed out that meat from ruminants represents a major dietary source of conjugated linoleic acids (CLA), a group of C18 isomers of linoleic acid with conjugated double bonds in the cis or trans configurations at positions 10 and 12 or 9 and 11 (36). CLA content in meat is in the range of 3–6 mg/g of fat. CLA have been reported to have some health-promoting biological activity such as inhibition of tumor growth and reduction of atherosclerotic risk (36, 37). The composition in fatty acids determines main fats properties. So, fats with excessive polyunsaturated fatty acids, like linoleic (typical of feeds rich in corn, for instance) and linolenic acids, tend to be softer, have an oily appearance and are more susceptible to oxidation. This is the reason why meats with a high percentage of polyunsaturated fatty acids are not preferred for further meat processing.

Phospholipids are present in minor amounts but due to their particular fatty acid composition, which is richer in polyunsaturated fatty acids, they have an important role in flavor development and oxidation in postmortem meat. Major constituents are phosphatidylcholine (lecithine) and phosphatidylethanolamine while phosphatidylserine and sphingomyelin are present in minor amounts. Some variability in phospholipids has been reported depending on the genetic type of the animal and anatomical location of the muscle (38). For instance, the amount of phopholipids tends to be higher in red oxidative muscles than in white glycolytic muscles (15).

C. Minor Compounds

Meat constitutes a good source of group B vitamins that, although present in low concentrations, contribute significantly to the daily intake requirements (39, 40). Pork meat is particularly rich in thiamin. However, the amount of fat-soluble vitamins in meat is rather poor or even almost negligible. Only vitamin E is significant in those animals with specific supplementation in the diet. Feed enrichment with vitamin E, at levels above dietary requirements (i.e., 200 mg/kg feed), is a relatively new practice, expanded in recent years, to protect unsaturated lipids from oxidation and obtain other benefits like better color stability of fresh beef (41, 42) and improved water retention in pork meat (43). The content in carbohydrates is rather poor, around 1.5 g/100 g, although it depends on the previous exercise of the animal prior to slaughtering. Meat also constitutes a good source of dietary iron, with the additional advantage that a good percentage is in the absorbable form of heme iron (44), and trace elements like zinc, selenium, magnesium, manganesum, etc. (40, 45–48). The mineral content is around 1 g/100 g.

IV. THE MEAT ENZYME SYSTEMS INVOLVED IN MAJOR BIOCHEMICAL CHANGES

Skeletal muscle contains a wide variety of enzymes involved in multiple metabolic pathways. Some of the most important, shown in Figure 28.3, are related to carbohydrate metabolism (glycolytic enzymes), protein breakdown (endo-peptidases), generation of small peptides (tri and dipeptidylpeptidases) and free amino acids (aminopeptidases and carboxypeptidases), hydrolysis of triacylglycerols and phospholipids (lipases and phospholipases) and transformation of ATP into numerous derived compounds. Most of these enzymes remain active in postmortem muscle, playing important roles in glycolysis, proteolysis, lipolysis and transformation of nucleotides that are essential for the development of meat quality. The location of these enzymes may vary. Some are located in organules like lysosomes while others are bound to membranes or free in the cytosol (49). A brief description of the proteolytic and lipolytic enzymes that play an important role in meat quality is given below.

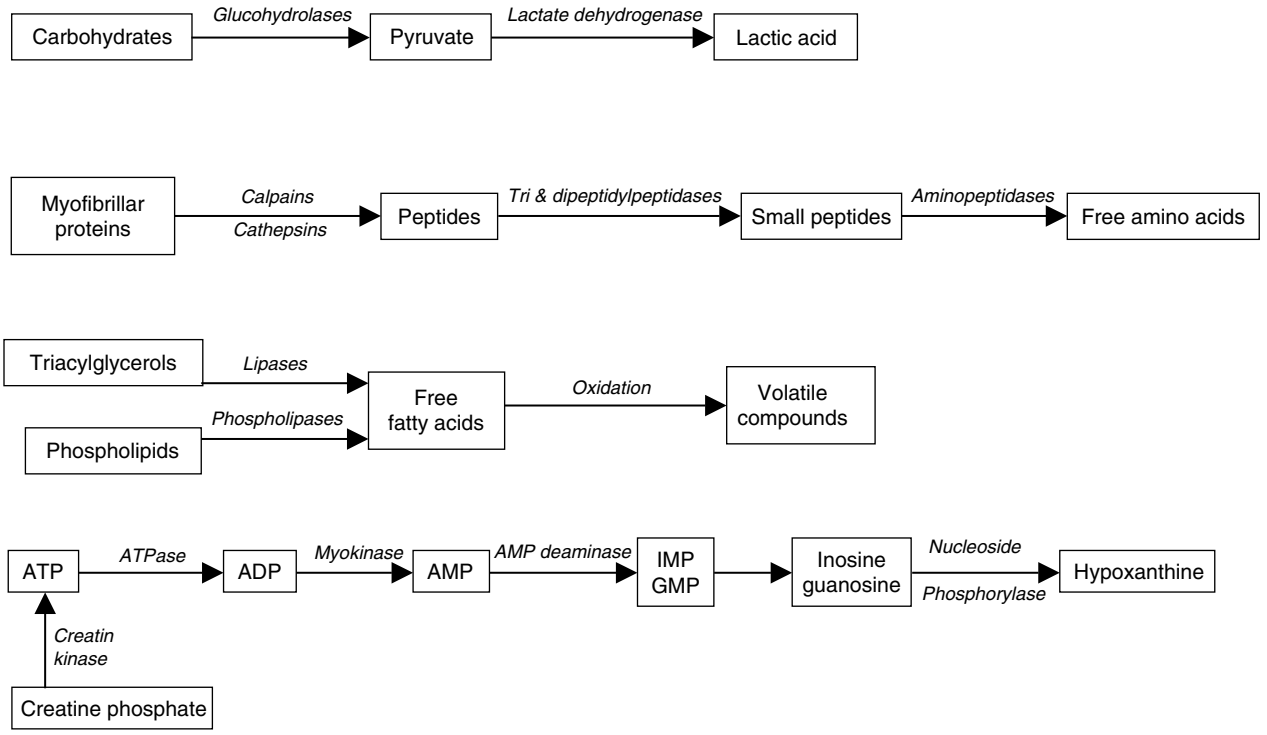

FIGURE 28.3 Scheme showing some of the most important enzymatic reactions affecting meat quality.

A. Proteases

Lysosomes are organules containing a large number of hydrolytic enzymes, including proteases. Cathepsins B, H and L, which are cysteine proteinases, and cathepsin D, an aspartate proteinase, are the main lysosomal proteinases. In general, these enzymes are small, 20–40 kDa (see Table 28.2), which allow their penetration into the myofibrillar structure. However, although the anaerobic glycolysis in postmortem muscle generates the adequate environment, such as pH drop, for cathepsin activity (50), the enzymes probably play a minor role in meat texture development during meat ageing as there is little or no degradation of myosin and actin, two important proteins very sensitive to cathepsins (51).

Cathepsins D and L are able to degrade myosin heavy chains, titin, M and C proteins, tropomyosin and troponins T and I (52–54). Cathepsin L is very active on both titin and nebulin. Cathepsin D may be quite inactive at refrigeration tenperatures (53) but can act during cooking because of its thermostability (55). On the other hand, cathepsin B is able to degrade myosin heavy chains, actin and even collagen but has no effect on myosin light chains and troponin C (56, 57), and cathepsin H shows both endo and aminopeptidase activity, being classified as an aminoendopeptidase (58). Cathepsin activity is regulated by cystatins, a family of cysteine peptidase inhibitors which are predominantly extracellular and have been isolated from different animal species (59, 60). Four families of cystatins have been reported in the literature based on their primary structure (51). Optimal pH for cathepsin D is acid (in the range of 3.0–5.0), slightly acid for cathepsins B and L (around 6.0) and near neutrality (6.8) for cathepsin H (61–64).

Calpains, a group of cystein endopeptidases, are located in the cytosol especially in the Z-line area. They have different names in the literature such as calcium-activated neutral proteinase, calcium-dependent protease or

TABLE 28.2
Main Characteristics of Meat Proteolytic Enzymes (Adapted from Refs. 51–84)

Enzyme	Classification	Location	Substrates	Optimal pH
Cathepsin B (EC 3.4.22.1.)	Cystein	Lysosome	Myosin, actin, collagen	5.5–6.0
Cathepsin D (EC 3.4.23.5.)	Aspartyl	Lysosome	Myofibrillar proteins	4.0
Cathepsin H (EC 3.4.22.16.)	Cystein	Lysosome	Myofibrillar proteins	6.8
Cathepsin L (EC 3.4.22.15.)	Cystein	Lysosome	Myofibrillar proteins, collagen	5.5–6.0
μ-Calpain (EC 3.4.22.17.)	Cystein/metallo	Z-line	Myofibrillar proteins	7.5
m-Calpain (EC 3.4.22.17.)	Cystein/metallo	Z-line	Myofibrillar proteins	7.5
20S proteasome	Ntn-hydrolase	Cytosol	Myofibrillar proteins	7.0–7.5
DPP I (EC 3.4.14.1)	Cystein	Lysosome	Polypeptides	5.5
DPP II (EC 3.4.14.2.)	Serin	Lysosome	Polypeptides	5.5
DPP III (EC 3.4.14.4.)	Serin	Cytosol	Polypeptides	8.0
DPP IV (EC 3.4.14.5.)	Serin	Membrane	Polypeptides	7.5–8.0
TPP I (EC 3.4.14.9.)	Serin	Lysosome	Polypeptides	4.0
TPP II (EC 3.4.14.10.)	Serin	Cytosol	Polypeptides	6.5–7.5
Alanyl aminopeptidase (EC 3.4.11.14.)	Cystein/metallo	Cytosol	Amino terminus of peptides	6.5
Arginyl aminopeptidase (EC 3.4.11.6.)	Cystein/metallo	Cytosol	Amino terminus of peptides	6.5
Methionyl aminopeptidase (EC 3.4.11.18.)	Cystein	Cytosol	Amino terminus of peptides	7.5
Leucyl aminopeptidase (EC 3.4.11.1.)	Metallo	Cytosol	Amino terminus of peptides	9.0
Pyroglutamyl aminopeptidase (EC 3.4.19.3.)	Cystein	Cytosol	Amino terminus of peptides	8.5
Carboxypeptidase A (EC 3.4.16.1.)	Serin	Lysosome	Carboxy terminus of peptides	5.2–5.5
Carboxypeptidase B (EC 3.4.18.1.)	Cystein	Lysosome	Carboxy terminus of peptides	5.0

calcium-activated factor. Calpain I or μ-calpain requires 50–70 μM of Ca^{2+} for activation and calpain II or m-calpain requires 1–5 mM of Ca^{2+}. Optimal pH for calpain activity is 7.5 (see Table 28.2) but poor activity is observed below pH 6.0, reaching negligible activity at pH 5.5 (65). Calpains are heterodimers of 110 KDa composed of an 80 KDa catalytic subunit responsible for the peptidase activity and a 30 KDa regulatory subunit which is common for both enzymes (66). Calpain I can be autolyzed in the presence of Ca^{2+} and usually shows a poor stability in meat while calpain II may be stable for a few weeks (67). Calpains have shown ability to degrade titin, nebulin, troponins T and I, tropomyosin, C-protein, filamin, desmin and vinculin but cannot degrade myosin, actin, α-actinin and troponin C (68, 69). The endogenous inhibitor calpastatin regulates calpain activity in postmortem muscle. Calpastatin is destroyed by autolysis after a few days postmortem (67).

The proteasome complex is a large protease with multiple catalytic sites such as a chymotrypsin-like activity, a trypsin-like activity and a peptidyl-glutamyl hydrolyzing activity (70, 71). The proteasome 20S can be associated with large regulatory complexes, like one or two 19S complexes, or one or two 11S activator complexes or even other activator complexes like PA700 or PA28 (51). The 20S proteasome can degrade myofibrils and affect M and Z lines, especially in high pH meats and slow-twitch oxidative muscles and thus could have a role in tenderness in those specific muscles (72, 73).

Peptidases constitute a large group of proteases. The most important found in meat are tripeptidylpeptidases (TPP) and dipeptidylpeptidases (DPP). TPP I, a lysosomal enzyme, shows optimal activity at acid pH and TPP II is most active at neutral pH. Both enzymes are able to hydrolyze different tripeptides from the amino termini of peptides, like Gly-Pro-Phe and Ala-Ala-Phe, respectively (74, 75). DPP I and II are located in the lysosomes and have optimal acid pH. DPP I has special preference for hydrolyzing dipeptides Ala-Arg and Gly-Arg from the amino termini of peptides and DPP II for Gly-Pro. DPP III is found in the cytosol and DPP IV is linked to the plasm membrane, both enzymes having optimal pH in the range of 7.8–8.0 (76–79). DPP III has a preference for hydrolyzing dipeptides Arg-Arg and Ala-Arg and DPP IV for Gly-Pro. Dipeptidases catalyze the hydrolysis of dipeptides and their names vary depending on the preference for certain amino acids. For instance, cysteinylglycine dipeptidase is specific for the dipeptide Cys-Gly and arginin dipeptidase has a special preference for basic amino acids (80).

Aminopeptidases constitute a group of exopeptidases able to release a free amino acid from the amino termini of peptides and proteins. They have a large molecular mass and a complex structure (62). Major aminopeptidases in skeletal muscle are: arginyl, alanyl, pyroglutamyl, leucyl and methionyl aminopeptidases. All of them are active at neutral or basic pH (see Table 28.2) and their names are related to the preference or requirement for a specific N-terminal amino acid although they can hydrolyze other amino acids at slower rates. Alanyl aminopeptidase, which mainly hydrolyzes alanine, is considered the major aminopeptidase in postmortem muscle. This enzyme is able to hydrolyze a wide spectrum of amino acids such as aromatic, aliphatic and basic aminoacyl-bonds (81). Arginyl aminopeptidase, also known as aminopeptidase B, hydrolyzes basic amino acids such as arginine or lysine (82). Methionyl aminopeptidase, a calcium-activated enzyme, has a wide spectrum of activity with preference for methionine, alanine, lysine and leucine (83). Leucyl and pyroglutamyl aminopeptidases are active at basic pH and play a minor role in meat (84). Carboxypeptidases are lysosomal enzymes with optimal activity at acid pH and able to generate free amino acids from the carboxy termini of peptides and proteins. Carboxypeptidase A has a preference for hydrophobic amino acids while carboxypeptidase B has a wider spectrum of activity (80).

B. Lipases

Major lipolytic enzymes in muscle are lysosomal acid lipase and phospholipase A (see Table 28.3). Both enzymes

TABLE 28.3
Main Characteristics of Meat Lipolytic Enzymes (Adapted from Refs. 61, 62, 86, 88, 89, 92)

Enzyme	Location	Substrate	Optimal pH
Lysosomal acid lipase	Lysosome	Long chain tri and diacylglycerols	5.0
Neutral lipase	Membrane	Long chain tri and diacylglycerols	7.5
Phospholipase A	Lysosome	Phospholipids	5.0
Muscle acid esterase	Lysosome	Short chain tri and diacylglycerols	5.0
Muscle neutral esterase	Cytosol	Short chain tri and diacylglycerols	7.5
Hormone-sensitive lipase	Adipose tissue	Long chain tri, di and monoacylglycerols	7.0
Monoacylglycerol lipase	Adipose tissue	Long chain monoacylglycerols	7.0
Lipopotein lipase	Adipose tissue	Lipoproteins	8.5
Acid esterase	Adipose tissue	Short chain tri and diacylglycerols	5.0
Neutral esterase	Adipose tissue	Short chain tri and diacylglycerols	7.5

are located in the lysosomes and are responsible for the generation of long chain free fatty acids in meat. Lysosomal acid lipase hydrolyzes primary ester bonds of tryacylglycerols at acid pH (4.5–5.5). This enzyme can also hydrolyze di and monoacylglycerols although at a slower rate (85, 86). Phospholipase A hydrolyzes phospholipids, at positions 1 or 2. The order of preference for fatty acids, especially polyunsaturated C18, esterified to phospholipids as a result of *in vitro* assays, is as follows: Linoleic acid > Oleic acid > Linolenic acid > Palmitic acid > Stearic acid > Arachidonic acid. The activity of lipases, phospholipase A and lysophospholipases is quite a bit higher in oxidative than in glycolytic muscles (15, 87). Acid and neutral esterases, able to hydrolyze short chain fatty acids from tri, di and mono acylglycerols, have been identified in the lysosomes and cytosol, respectively (88). In general, lipases prefer long chain fatty acids while esterases are especially active against short chain fatty acids.

There are three lipases in adipose tissue (see Table 28.3): hormone-sensitive lipase, monoacylglycerol lipase and lipoprotein lipase with optimal pH in the neutral/basic range (62, 89). The hormone-sensitive lipase (HSL) has a high specificity for the hydrolysis of diacylglycerols. The triacylglycerol hydrolysis by this enzyme is the rate-controlling step in the lipolysis phenomena in adipose tissue (90). The monoacylglycerol lipase (MGL) hydrolyzes 1 or 2 monoacylglycerols with no positional specificity, releasing medium and long chain monoacylglycerols (91). Lipoprotein lipase is located in the capillary endothelium and hydrolyzes the acylglycerol components at the luminal surface of the endothelium (92). This enzyme has a preference for fatty acids at position 1 over those at position 3 (93, 94). Acid and neutral esterases are also present in adipose tissue (62, 88).

V. CHEMISTRY OF THE CONVERSION OF MUSCLE TO MEAT

Multiple reactions involved at the early postmortem are briefly summarised in Figure 28.4. It is important to emphasize that not all muscles, or even parts within the same muscle, change in an uniform way because these changes will depend on the respective ratios of white and red fibers. Additionally, the rate of these changes also depends on the temperature achieved within each muscle. Twelve moles of ATP are produced per mole of glucose under aerobic conditions like those usually found in living muscle. The first important change in the muscle following death consists of its inability to synthesize or to remove certain metabolites (see Figure 28.5). The supply of oxygen is cut off once the blood circulation ceases and then there is a progressive decrease in oxygen concentration in the muscle cell and a reduction of the redox potential towards anaerobic values (95). The lack of available oxygen stops the activity of the mitochondria system and cell respiration is progressively stopped. Once in anaerobic

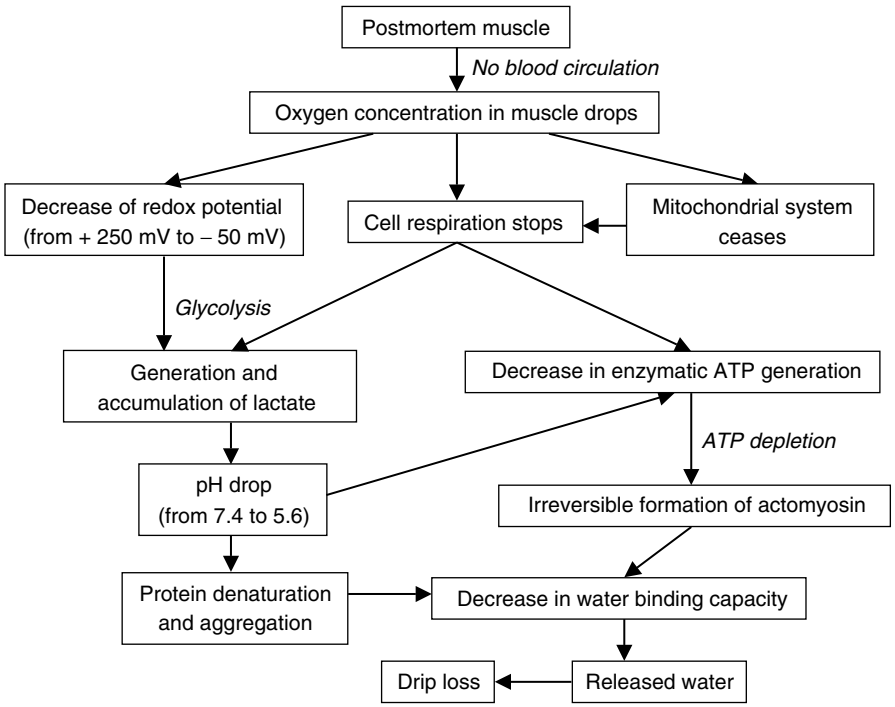

FIGURE 28.4 Scheme summarizing early post-mortem changes in muscle. From Toldrá, "Muscle foods:Water, structure and functionality," Food Sci Tech Int 9, 173–177, 2003.

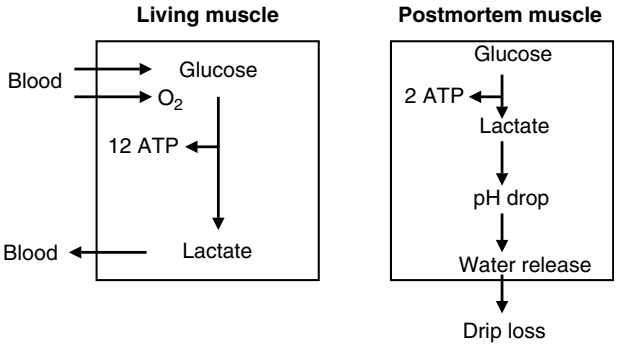

FIGURE 28.5 Main differences between living and postmortem muscle. From Toldrá, "Muscle foods:Water, structure and functionality." Food Sci Tech Int 9, 173–177, 2003.

conditions, lactic acid is produced from glucose through glycolysis, but the efficiency is lower than under aerobic conditions because only 2 moles of ATP are produced per mole of glucose. The generation of ATP is necessary in the muscle to provide the required energy to drive the Na/K pump of the membranes, to drive the calcium pump in the sarcoplasmic reticulum and to provide energy for muscle contraction. ATP may also be formed from ADP and creatine phosphate through the action of the enzyme phosphocreatin kinase (96). ATP is also necessary for muscle contraction-relaxation where it acts as a lubricant but, once ATP is fully exhausted, the muscle remains contracted (rigor mortis). This is related to the formation of actomyosin and the loss of extensibility (97).

An important consequence of lactic acid accumulation is the pH drop to acid values (5.6 to 5.9) within a few hours postmortem. The pH drop rate depends on the metabolic status of the muscle and glycogen availability. As pH approaches the isoelectric point of myofibrillar proteins (pH values around 5.0), their charges move towards neutrality and water binding decreases rapidly, the structure tightens and myofibrillar proteins are partially denatured (98). Thus, the water binding depends on the ultimate pH in the muscle and the amount of released water, lost as dripping, increases when the ultimate pH is lower (99). Important muscle soluble compounds like myoglobin, lysosomal enzymes, nucleotides and nucleosides, free amino acids, vitamins and minerals are partly lost in the drip, affecting the final quality. Pork carcasses during early postmortem are shown in Figure 28.6.

There are many and relevant biochemical changes during early postmortem and ageing of meat with important consequences for quality. Most of these changes are enzymatic in nature either affecting meat tenderness due to structural protein breakdown or generating a substantial number of new compounds with direct influence on taste and/or aroma. The trend towards rapid lean growth in cattle, pigs and poultry increases the relative proportion of meat quality defects, mainly related to color and water-holding capacity (100).

FIGURE 28.6 Pork carcasses at early postmortem in the slaughterhouse. Image courtesy of Industrias Cárnicas Vaquero SA, Madrid, Spain.

A. Glycolysis

Main reactions for glycolysis are schematized in Figure 28.3. Key enzymes in the glycolytic chain are phosphorylase, phosphofructokinase and pyruvic kinase. Lactate dehydrogenase is involved in the last step, consisting of the conversion of pyruvic acid into lactic acid. The generation and accumulation of lactic acid in the meat causes the pH to drop from neutral (pH around 7.2) to acid values (below 5.8). If the reserves of glycogen are rather poor prior to slaughter, the final pH will not experience such an intense drop and will remain above 6.0. The pH limit for the DFD pork meat condition (dark, firm and dry) is considered around 5.9 at 24 h. The ultimate pH depends on the type of muscle, animal species and physiological state. The rate of glycolysis also depends on the temperature. Minimum pH drop is observed at 10–12°C but if temperature decreases down to 0°C too early, there is a release of calcium ions into the myofibrillar space that activates ATPase. Under these circumstances and if there is still some available ATP, an extra contraction of actomyosin is

produced, resulting in a tight structure phenomenon known as cold shortening (97, 101). Cold shortening is mainly observed in beef, lamb and turkey, and with less intensity in pork (102). Prevention of cold shortening may be achieved by keeping carcasses above 15°C until the pH reaches 6.0. Another important alteration, known as thaw rigor or thaw shortening, is produced when thawing meat that was frozen in the prerigor state (still keeping a substantial amount of ATP). Under these circumstances, calcium is released and moves is into the intracellular spaces, causing an extensive contraction (101).

Exudation constitutes one of the major problems in pork quality. This condition, is known as pale, soft and exudative (PSE), is characterized by its pale color, soft texture and high drip loss (103). Another type of exudative meat, known as red, soft and exudative (RSE), is characterized by similar defects although this meat keeps its normal color (104), making it difficult to differentiate from normal meats (105). Finally, another defective meat with lower incidence is known as dark, firm and dry (DFD) due to its darker color, firm texture and dry appearance on the external surface. Pork meat classification is usually based on pH, color and drip loss (106, 107). Exudative (PSE and RSE) meats are characterized by a low pH value at early postmortem (i.e., pH below 5.8 at 2 h), a pale color reflected in a L value higher than 50 (only for PSE) and drip loss higher than 6%. Figure 28.7 shows a typical pH drop profile for normal, DFD and PSE pork meats. The negative effects of exudative meats may be reduced significantly with some preventive measures such as appropiate pre-transport handling, lairage, stunning, postmortem temperature and chilling rate. The incidence of exudative pork meat is still significant. For instance, a 1992 survey of the pork supply in the U.S. revealed that 16% was PSE and 10% was DFD (108). Only 16% was considered to be of ideal quality (RFN) while the remainder was found to be of somewhat questionable quality (RSE), indicating very little progress in the elimination or minimization of the problem (108).

B. Nucleotide Breakdown

ATP is the main source of energy for the biochemical reactions in postmortem muscle. However, its content rapidly decreases from an initial 5–8 μmol/g muscle to final negligible values (rigor state). Initially, some ATP may be formed through creatin kinase acting on creatin phosphate and through anaerobic glycolysis (96) but, once creatin phosphate and glycogen are exhausted or the involved enzymes inactivated, ATP drops in a few hours to near zero values through conversion into ADP, AMP and other derived compounds (see Figure 28.10 for an example on pork meat). The concentrations of ADP and AMP decrease to negligible values after 24–48 hours postmortem. 5′-inosin-monophosphate (IMP) is formed from AMP deamination by the action of AMP deaminase, an enzyme very active at pH near 6.2. Some IDP, generated from ADP, may be temporarily detected although it is depleted in a few days. IMP can be further degraded into inosine and hypoxanthine, both compounds experiencing a substantial increase as meat is aged (see Figure 28.8). Similar reactions, although at minor concentrations, are also observed for 5′-guanosin monophosphate (GMP). The rate for all these reactions varies depending on the metabolic status of the animal prior to slaughter as well as the pH and temperature of the meat (109, 110).

C. Proteolysis

Proteolysis consists of the progressive enzymatic degradation of major meat proteins, especially myofibrillar

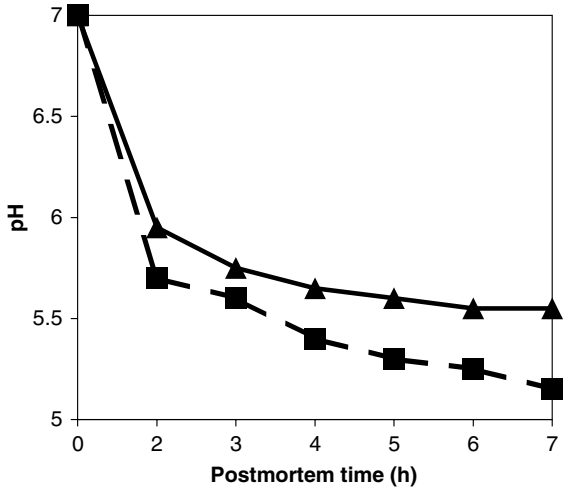

FIGURE 28.7 Example of pH fall of normal (—■—) and pale, soft, exudative (PSE) (▲) pork meat (Toldrá, 2000, unpublished).

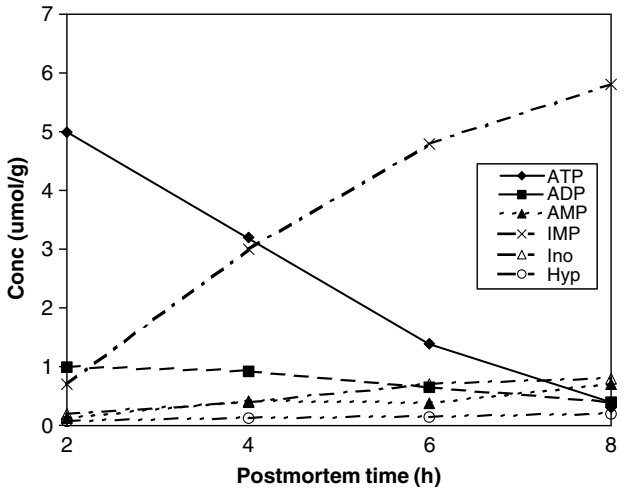

FIGURE 28.8 ATP depletion and evolution of ATP-derived compounds in aged pork meat (adapted from Ref. 109).

proteins, and the subsequent generation of peptides and free amino acids. The breakdown of structural proteins results in a weakening of the myofibrillar network and a sensible improvement in meat tenderness. There are many muscle endopeptidases (mostly calpains and cathepsins) and exopeptidases (mostly tri and dipeptidylpeptidases and aminopeptidases) involved in proteolysis (see Figure 28.3). Main properties of these enzymes have been described above (see Table 28.2). The extent of proteolysis and thus the tenderness and generation of taste compounds depend on many factors (111). The activity of endogenous muscle enzymes may vary depending on the genetics (112, 113) and the age of the animals (114, 115). Furthermore, the action of these enzymes also depend on the processing technology. For instance, major or minor action of the enzymes may be expected depending on the temperature and time of ageing. These enzymes are also regulated by certain agents like salt (63, 64, 116, 117).

In general, the amount of peptides increases during meat ageing as reported for beef, pork, chicken and rabbit (118). Some characteristic peptides have been described in postmortem meat and some of them are related to meat tenderness or have flavor properties, for instance, the 30 kDa peptide originating from troponin T through the action of calpain (119) which is related to meat tenderization (120–124), a 110 kDa polypeptide from C protein (125) and some peptides corresponding to 1282.8 Da from the sarcoplasmic protein glyceraldehyde 3-phosphate dehydrogenase, 1734.8 Da from troponin T and 5712.9 Da from creatine kinase (125). Recently, a 32 kDa peptide derived from troponin T has been reported to increase during 20 days of ageing (111). Several small peptides were also isolated from pork meat and some of them were proposed as predictors of pork meat quality (126–128). The generation of these peptides may be depressed if some salt, which inhibits muscle peptidases, is added (62). The activity of endogenous muscle aminopeptidases is quite relevant and, in fact, a significant amount of released free amino acids that contribute to meat taste may be found after several days of meat ageing (129–132).

D. Lipolysis

Lipolysis consists of the breakdown of triacylglycerols by lipases and phospholipids by phospholipases resulting in the generation of free fatty acids (see Figure 28.3). Some of these fatty acids may contribute to taste but, most important, unsaturated fatty acids may contribute to the generation of aroma compounds through further oxidative reactions. Main lipolytic enzymes, located in muscle and adipose tissue and involved in these phenomena, are listed in Table 28.3. These enzymes show good stability and, although their activity depends on pH, salt concentration and water activity, the conditions during meat ageing favor their action (88, 133). The generation rate of free fatty acids increases with length of ageing. In the case of intramuscular lipids, most of the released fatty acids proceed from phospholipid degradation while in adipose tissue they proceed almost exclusively from triacylglycerols (134, 135).

The generated mono and polyunsaturated fatty acids are susceptible to further oxidative reactions to give volatile compounds (136). The beginning of lipid oxidation is correlated with flavor development but an excess of oxidation may lead to off-flavors, one of the main mechanisms responsible for quality deterioration in meat and meat products. Warmed-over flavor (WOF) is recognized as a sensory defect that has been described as the rapid onset of off-notes developed in cooked meat after refrigeration storage (137). WOF is characterized by a decrease in fresh meatiness and the development of cardboard and rancid/painty notes in beef, pork and chicken, that may be detected even after 48 hours (137).

The beginning of lipid oxidation consists of the formation of free radicals, reactions catalyzed by muscle oxidative enzymes, like peroxydases and ciclooxygenases, external light, heating and the presence of moisture and/or metallic cations. The next step in oxidation is the formation of peroxide radicals (propagation), by reaction of free radicals with oxygen. The hydroxyperoxides (primary oxidation products) formed are flavorless but very reactive (136) giving secondary oxidation products that contribute to flavor (138). The oxidation is finished when free radicals react with each other. Unsaturated fatty acids, especially those of phospholipids, are prone to oxidation. The oxidation rate depends on the level of unsaturated fatty acids and number of unsaturations. For instance, arachiconid acid (C20:4) is oxidized faster than linolenic acid (C18:3), and this faster than linoleic acid (C18:2) and oleic acid (C18:1). An example of the evolution of oxidation during refrigerated storage of pork meat is shown in Figure 28.9. Typical volatile compounds resulting from lipid oxidation (139) are aliphatic hydrocarbons (poor contribution to flavor), alcohols (high odor threshold), aldehydes (low odor threshold) and ketones.

VI. DEVELOPMENT OF SENSORY QUALITY

The chemical and biochemical changes taking place during the refrigerated storage of meat are intimately linked to the development of specific sensory properties of meat like color, texture and flavor which are basic to quality. An example of meat conditioning is shown in Figure 28.10.

A. Color

The protein myoglobin constitutes the major pigment in meat, from 50 to 80% of the total pigment content (Miller, 1994). Myoglobin is composed of globin, a protein moiety,

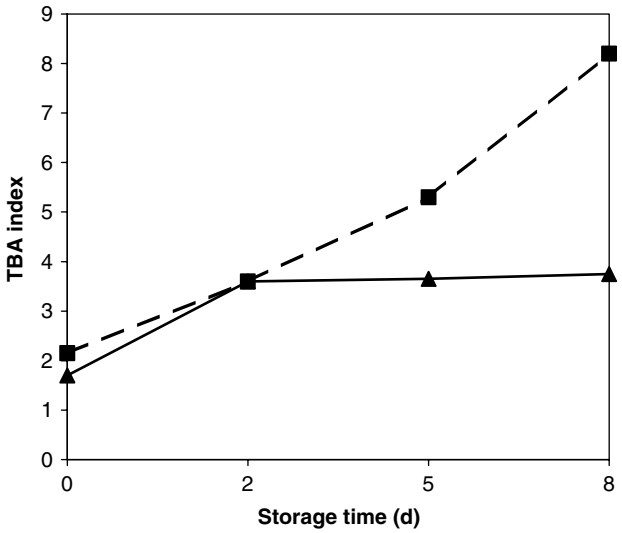

FIGURE 28.9 Example of lipid oxidation, measured through the thiobarbituric acid (TBA) index, during the refrigerated storage of pork meat (▲) and adipose tissue (—■—) (Toldrá, 2000, unpublished).

and an iron-containing heme group. When the heme iron exists in a reduced ferrous form, it is called deoxymyoglobin and gives a purplish-red color (140). When oxygen occupies the sixth ligand by oxygenation under high oxygen tension, it is called oxymyoglobin, and gives a cherry-red color. These two myoglobin forms may be oxidixed to metmyoglobin, which gives an undesirable brownish-red color (140). Metmyoglobin may be formed at high temperature, low pH and under exposure to UV light. Some bacteria may also favor its formation (97) while some lactic acid bacteria have shown ability to reduce metmyoglobin to myoglobin (141). When oxygen tension is low, oxygen dissociates from the heme and gives myoglobin. The content in myoglobin varies depending on the species. So, beef meat (containing about 5 mg of myoglobin per g of muscle) has the darkest color, followed by lamb (about 2.5 mg/g) and pork (about 1 mg/g) (142). Myoglobin content also varies depending on the physiological role (muscles with higher content of red fibers are darker) and age (myoglobin content increases with age) (16). In general, a better red color may be found in meats from mature animals than in meats from younger animals.

FIGURE 28.10 Pork meat (loins) at slaughterhouse before distribution to retailers. Image courtesy of Industrias Cárnicas Vaquero SA, Madrid, Spain.

Color stability is very important for fresh meat marketability. Color may be influenced by drop in pH rate and is more stable at relatively higher ultimate pH values. The oxidation of myoglobin is accelerated by increases in temperature. The partial oxygen pressure is very important to the balance between the three myoglobin forms. Finally, light may produce meat discoloration as well as bacterial contamination (140).

B. Texture

Calpains play an important role in proteolysis and tenderness development during early postmortem while cathepsins play a minor role (143). Calpains are activated in postmortem meat by the increased calcium concentration as a consequence of calcium release from the sarcoplasmic reticulum and mitochondria and cause an intense breakdown of the myofibrillar structure, especially around the Z-line area. Further evidence has been obtained by addition of specific cathepsin inhibitors, observing a similar degree of meat tenderization and myofibrillar fragmentation (144) or even acceleration of the tenderization rate when calcium salts are added (145). The need for increased tenderness and optimized ageing processes prompted the development of tenderization models based on the activity of calpains (146). However, other factors must be considered when comparing proteolytical enzyme levels in different species (beef, pork, lamb and poultry) and respective rates of tenderization (147). Even non-enzymatic mechanisms regarding depolymerization of intermediate filaments under non-physiological conditions have been proposed for meat tenderization (148). Calpastatin and cystatins regulate the activity of calpains and cathepsins, respectively, and their content varies with species. For instance, pork muscle has lower calpastatin level (149) and lower calpain II activity than beef and lamb but at 1 day postmortem pork meat was the most tender (150). Thus, calpastatin and cystatins, or even the enzyme/inhibitor ratios, appear to be better predictors of meat quality than the assays of enzyme activity alone (151).

Changes in rate of tenderization may be due to different ultimate pH values (value at 24 h postmortem). So, preslaughter stress and consequent glycogen depletion may raise the ultimate pH to intermediate values (around 6.0) and give tougher meat at first day postmortem while lower and higher ultimate pH give tender meat (152). Myofibrillar protein extractability is lower in PSE pork meat than in normal meat and this may change the susceptibility of myofibrils to proteolysis (153). In fact, major changes by calpains are observed in DFD and normal meats while the action of cathepsins remains similar in both normal and PSE types of meats (154). Proteolysis and ultrastructural breakdown has been reported to be faster in fast glycolyzing muscles. Higher calpain I activity and lower calpastatin activity (high enzyme/inhibitor ratio) confirms that calpain I has an important role in tenderness even in meats with lower pH values (155).

C. Flavor

Raw meat has little aroma but meat flavor is developed as a consequence of a good number of compounds produced in postmortem muscle. The most important nonvolatile compounds with taste properties are free amino acids, peptides, inosine and hypoxanthine, lactic and succinic and other acids, sugars, and the sodium salts of glutamic and aspartic acids (156). Flavor enhancers such as 5′-inosin- and 5′-guanosin-monophosphate, glutamic acid and monosodium glutamate are also important for the final flavor of meat (157, 158). Free amino acids contributing to meat taste have been reported in beef, rabbit and pork (130, 131, 159, 160). Peptides generated in aged meat also make an important contribution to taste. An octapeptide, Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, was isolated from beef after digestion with papain (161, 162). This peptide was named the beefy meaty peptide (BMP) or delicious peptide because of its taste-enhancing properties. This octapeptide exhibits sourness and astringency at pH 3.5 and umami at pH 6.5 which is enhanced in the presence of salt and monosodium glutamate (163). Other tripeptides like Ala-Asp-Glu, Ala-Asp-Glu, Asp-Glu-Glu and Ser-Pro-Glu and dipeptides like Glu-Glu and Glu-Val, with taste-enhancing properties, have been isolated from chicken meat treated with bromelain (164). The production of peptides with desirable flavors is favored within the pH range 5.0–6.0 in beef (165). The division of generated peptides in aged beef into three fractions revealed that those with molecular mass of 500–1000 kDa and higher than 10,000 kDa had a negligible effect on taste while those within the range 1,000–10,000 kDa suppressed sourness, probably by inhibition of the binding of lactic acid to membranes, and enhanced umami taste (118). In a similar way, small peptides in the range 2,700 to 4,500 Da or even below 2,700 Da, have been detected in postmortem pork meat and some of them gave characteristic brothy and umami tastes (166).

Volatile compounds are generated with aroma properties belonging to the following groups: aldehydes, ketones, esters, hydrocarbons, acids, alcohols, lactones, furanes, pyrroles, pyridines, pyrazines, non-heterocyclic sulphur compounds, thiophenes and thiazoles (167). Further generation of these taste and aroma compounds and interactions among them take place during meat processing and cooking (157, 168). For instance, the optimal flavor quality of aged top round beef meat was found after 4 days of ageing (165). The main reactions involved during cooking are: pyrolysis of amino acids and peptides, carbohydrate degradation, Maillard reactions, Strecker degradations,

degradation of thiamin and lipid degradation (157). Depending on the balance and relative intensity of all these reactions, the flavor may change. For instance, some pyrazines, thiophenes and thiazoles are responsible for the roasted flavor. Thus, the different flavors for different meats depend on the balance of the non-volatile and volatile compounds and the way they interact (167, 169).

In summary, the chemistry and biochemistry of meat are quite complex and involve numerous chemical reactions (i.e., lipid oxidation, Maillard reactions, Strecker degradations, etc.) and biochemical reactions related to indigenous enzymes (i.e., glycohydrolases, proteases, lipases, etc.). Knowledge of these reactions is very important in order to obtain standardized high quality meats because all related chemical and biochemical changes have a strong effect on final quality and consumer acceptability.

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29 Chemical Composition of Red Meat

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CONTENTS

I. INTRODUCTION

Red meat refers to the meat from mammalian skeletal muscle tissue with distinctive red color including beef, pork, and lamb. Although muscle and meat are used interchangeably on many occasions, there are differences between the two terms. Muscle refers to a tissue responsible for contraction and movement in live animals, while meat is a broad term referring to edible tissues from animals consisting of muscle, adipose, and other connective tissues (1). The major components of meat include water, proteins, lipids, carbohydrates, minerals, soluble non-protein substances, vitamins, and some incidental compounds such as feed additives or contaminants (Table 29.1). The composition of meat is dependent on the species, breed, sex, age, activity, the anatomical location of the cut, the relative proportions of tissues in the retail cut, and other factors. However, the composition of lean meat is fairly consistent even across different species (Table 29.2). Hence, in later discussions, the chemical composition of red meat is not separated into species.

II. WATER IN MEAT

Generally, water is the most abundant component in lean meat, ranging from 65% to 80%. However, if the meat contains excessive adipose tissue, fat may be the predominant component (Table 29.3). Water is not randomly distributed in meat. At the time of slaughter, approximately 85% water in muscle is located intracellularly. The remaining 15% water is located in the extracellular spaces. The water in the muscle is withheld through different mechanisms, less than 15% tightly bound to proteins, another 15% loosely bound to proteins, and the remaining 70% by capillary forces (6). Since most of the water is distributed in the spaces among myofibrils, swelling or shrinkage of myfibrils alters the distribution of water within the muscle but does not necessarily affect the muscle volume as a whole (7).

The level of water in meat has a direct impact on cooking yield as well as on tenderness and juiciness of meat products. Hence, it is desirable to improve water binding capacity of meat through breeding, slaughtering, post mortem handling, formulation, and further processing such as mixing, tumbling, and other procedures.

TABLE 29.1
Composition of Typical Mammalian Muscle*

Component	Wet %
Water	75.0
Protein	19.0
Lipids	2.5
Carbohydrates	1.2
Non-protein nitrogenous compounds	1.65
Inorganic	0.65
Vitamins and other minor components	Minimal

* Compiled from Ref. 2.

TABLE 29.2
Composition of Separable Red Lean Meat from Different Species (%)

	Water	Protein	Lipids	Ash
Beef	71	21	5.8	1.0
Pork	72	21	5.9	1.0
Lamb	73	20	5.2	1.0

Compiled from Refs. 3–5.

TABLE 29.3
Composition of Red Meat with Adipose Tissues (%)

	Water	Protein	Lipids	Ash
Beef	60	18	20	1
Pork	42	12	43	1
Lamb	62	17	18	1

Compiled from Refs. 3–5.

III. MEAT PROTEINS

For lean meat, proteins constitute 16% to 22% of the meat mass. Meat proteins can be divided into three groups based on their solubility: sarcoplasmic proteins (soluble in aqueous solutions with an ionic strength of 0.15 or less), myofibrillar proteins (soluble in aqueous solutions with an ionic strength of sodium or potassium ions at least 0.3), and stromal proteins (insoluble even in high ionic strength solutions of sodium or potassium ions) (2).

A. Sarcoplasmic Proteins

The sarcoplasmic proteins contribute to about 30% of the total protein, corresponding to 5.5% of lean meat. The sarcoplasmic proteins include a mixture of about 50 components. The major components are glycolytic enzymes, myoglobin, cytochromes, and flavoproteins (2). It should be noted that glycolytic enzymes are not randomly suspended in the sarcoplasm. Instead, they are bound to the myofibrillar protein (actin). This feature may assist in the muscular metabolism and physiological function. In addition, the proportion of the bound glycolytic enzymes increases in electrically stimulated glycolysis and decreases when the stimulation stopped (2). Glycolytic enzymes also bind to other sites in muscle cell, including the sarcolemma, the sarcoplasmic reticulum, and the membranes of nuclei and mitochondria. These glycolytic enzymes play a critical role during post mortem glycolysis. The myoglobin, and to a lesser extent, hemoglobin, are responsible for the typical red color of meat. The amount of myoglobin is determined by the metabolic characteristics of the muscle. For instance, red meat contains high levels of myogolbin to provide more oxygen for the synthesis of ATP. In contrast, white meat contains low levels of myoglobin because its energy is generated primarily through glycolysis. Generally, sarcoplasmic proteins have limited functionalities in water-binding, gel-forming, and emulsifying capacity.

B. Myofibrillar Proteins

Myofibrillar proteins constitute about 60% of total muscle proteins, corresponding to about 11.5% of lean meat. Myofibrillar proteins can be further divided into three subgroups including contractile proteins, regulatory proteins, and cytoskeletal proteins (Table 29.4). The major contractible proteins are myosin and actin. The major regulatory proteins are tropomyosin, troponin, actinins, and other minor regulatory proteins. Cytoskeletal proteins include a diverse group of proteins such as titin, nebulin, desmin, filamin, vinculin, and synemin. The myofibrillar proteins construct the thick filament, thin filament, Z-line, and M-line which can be observed under the microscope or electron microscope.

1. Myosin

Myosin is the most abundant myofibrillar protein, contributing to abut 40% of the total myofibrillar protein or about 5.5% of lean meat. Myosin contains six subunits with two identical heavy chains and four light chains (2). There are three kinds of light chains, i.e., DTNB light chain (MW 18,000 D), alkaline light chain 1 (MW 25,000 D), and alkaline light chain 2 (MW 16,000 D). The names of the light chains come from the reagents used to release them from myosin, either sulfhydryl agent 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) or alkaline solution. One myosin molecule contains two identical DTNB light chains and two identical alkaline light chains (either 1 or 2). The six subunits form two identical units which form a complete myosin molecule with a length of 1500 Å (2). A whole myosin molecule looks like a golf club with the N-terminus in the head region. The four light chains and ATPase activity are located at the head. Many myosin molecules aggregate at the tail to form myosin filament (thick filament) which in turn forms myofibrils with other proteins (2, 9). Another protein component for thick filament, titin, will be discussed later.

TABLE 29.4
Some Characteristics of Myofibrillar Proteins from Vertebrate Skeletal Muscle

Protein	MW (KD)	%*	Location	Function
Contractile				
Myosin	500	43	Thick filament	Contraction
Heavy chain	220		(A-band)	
Light chain	16/18/25			
Actin (G-form)	42	22	Thin filament (I-band)	Contraction
Regulatory				
Tropomyosin	70	8	Thin filament	Regulates contraction
α-chain	34			
β-chain	36			
Troponin	74	5	Thin filament	
Troponin-C	18			Ca^{2+}-binding
Troponin-I	21			Inhibits myosin-actin interaction
Troponin-T	35			Binds to tropomyosin
α-actinin	95	2	Z-disk	Fastens thin filaments to Z-disk
β-actinin	130	0.1	Thin filament	Regulates thin filament length
γ-actinin	35	0.1	Thin filament	Inhibits G-actin
Eu-actinin	42	0.3	Z-disk	Density
C-protein	140	2	Thick filament	Adheres to thick filament
M-protein	165	2	M-line	Binds to myosin
X-protein	152	0.2	Thick filament	Binds to myosin
H-protein	69	0.18	Thick filament	Adheres to C-protein & myosin
Paratropomyosin	35	0.15	Edges of A-band	Involving postmortem changes
Creatine kinase	42	0.1	M-line	Binds to M-protein
Cytoskeletal				
Titin	>1000	8	Entire scaromere	Holds thick filaments & links them to Z-disks
Nebulin	600	3	N-line	Binds & holds thin filaments
Desmin	550	0.18	Z-disk	Links neighboring Z-disks
Filamin	230	0.1	Z-disk	Binds & holds titin
Vimentin	58	0.1	Z-disk	Links Z-disk in periphery
Synemin	220	0.1	Z-disk	Binds to desmin & vimentin
Zeugmatin	550	0.1	Z-disk	Links thin filaments to Z-disk

*Percentage of total myofibrillar protein
Compiled from Ref. 8.

2. Actin

The other important contractile protein is actin, contributing to about 20% of total myofibrillar proteins or about 2.5% of lean meat (Table 29.4). Actin contains a single polypeptide chain. Each actin molecule forms a globe that is called G-actin. A series of G-actin aggregate linearly to form a fibrous structure that is called F-actin. F-actin has a double stranded right-handed helical structure with a half pitch consisting of 13 monomers (10). The polymerization of G-actin to F-actin (G-F transformation) is thought to be similar to the process of crystallization (11). It occurs only above a critical concentration of actin at which F-actin is in equilibrium with G-actin. F-actin, tropomyosin, troponin, and nebulin are the major components for the thin filaments in myofibrils.

3. Tropomyosin

Tropomysoin contributes to about 8% of myofibrillar proteins. It is an α-helical molecule approximately 41 nm long. It is so named because its amino acid composition is similar to that of myosin (12). Tropomyosin consists of two subunits with a molecular weight of approximately 37,000 daltons. The two subunits are α and β which can be separated by sodium docecyl sulfate gel electrophoresis or by ion-exchange chromatography.

4. Troponin

Troponin is a protein of MW 80,000 consisting of three subunits that are named I, T, and C. Troponin C is an acidic protein that can bind Ca^{++} with four calcium

binding sites on each troponin C (13). Troponin I is a basic protein that inhibits the actin-myosin interaction in the presence of ATP. The presence of tropomyosin greatly enhances this inhibition (14). Troponin T binds strongly to tropomyosin periodically at 40 nm intervals along the entire length of the thin filament (15). Since each troponin molecule must regulate seven actin molecules, it is believed that this must be mediated via a dislocation of the filamentous tropomyosin molecule along the groove of actin filaments (16).

5. Actinins

Actinins are proteins that play a role in regulating the physical state of actin. Four classes of actinins are known, α-actinin, β-actinin, γ-actinin, and eu-actinin. α-actinin is the major actinin found in skeletal muscle, contributing to about 1–2% of myofibrillar proteins. It is an acidic protein located in Z-lines, probably participating in cementing the components of the Z-line (17). Mild treatment of myofibrils with proteases results in a marked decrease in the density of Z-lines with a release of intact α-actinin. The decrease in Z-line density and the loss of register of Z-lines in adjacent myofibrils are characteristic of postmortem muscle. It is believed that the release of α-actinin contributes to the tenderness of postmortem meat (18). β-actinin is a protein contributing to less than 0.01% of myofibrillar proteins. β-actinin is present at the free end of actin filaments to prevent actin from binding to another actin filament (19). γ-Actinin is a protein contributing to less than 0.01% of myofibrillar proteins. It inhibits the polymerization of G-actin. Eu-actinin contributes to about 0.3% of myfibrillar proteins. It is located at Z-lines to interact with both α-actinin and actin.

6. Other Regulatory Proteins

Other regulatory proteins include M-protein, C-protein, and F-protein, among other minor components. M-protein contributes to about 0.5% of myfibrillar proteins. It is located at the M-line of the thick filaments. Other proteins found at the M-line include myomesin and creatine kinase (20–22). C-protein comprises 2% of the myofibrillar protein with a relatively high proline content. C-protein copolymerizes with myosin filaments. F-protein binds to myosin filament. I-protein is located at the A-band. It is suggested that I-protein inhibits the unnecessary splitting of ATP in relaxed muscle (9).

C. Cytoskeletal Proteins

Cytoskeletal proteins include titin (connectin), nebulin, desmin (skeletin), and other minor components. Titin is the largest protein molecule with a MW of over 1,000,000. Titin contributes to about 10% of myofibrillar proteins. It seems that titin forms a three-dimensional net of very thin filaments to link the neighboring Z-lines (23). It extends longitudinally in each half sarcomere from the M-line to the Z-disk as a third filament of the myofibril. Titin and C-protein bind to the outside shaft of the thick filament. They encircle and stabilize the thick filament and hence should be considered components of thick filament. It is believed that titin is responsible for the resting tension associated with each sarcomere. Nebulin constitutes approximately 4% of myofibrillar proteins. It is located close and parallel to the thin filament. Nebulin extends longitudinally along the entire length of the thin filament from the A-band to the Z-disk. In mature muscle, it serves as a template for assembly and/or scaffold for stability of thin filaments. It may also help to anchor thin filaments to Z-disks (1). Desmin contributes to less than 0.2% myofibrillar proteins. It is located at the periphery of the Z-disk and also in the filaments that link neighboring Z-disks. Other minor proteins contributing to the cytoskeleton include filamin, vimentin, synemin, and paranemin (1).

D. Stromal or Connective Tissue Proteins

Stomal proteins include collagen, elastin, and other insoluble proteins. Connective tissue proteins play an important role in physiological functions as well as in determining the eating quality of meat. Their physiological functions include covering the body, protecting the body from damage, and connecting muscles, organs, and other structures to the skeleton and to each other (24).

Collagen is a major structural component of all connective tissues including tendon, bone, cartilage, skin, vascular tissues, and basement membranes. It is the most abundant protein in animal bodies constituting 20–25% of the total protein. Different tissues have different types of collagens (Table 29.5). Distribution of collagen is not uniform among skeletal muscles with the amount generally paralleling physical activity of the muscle. Hence, muscles of limbs contain more collagen than those around the spinal column. Consequently, the former are tougher than the latter. At a level of 33%, glycine is the most abundant amino acid in collagen, followed by proline and hydroxyproline comprising another one-third of the amino acids of collagen. Since hyroxyproline is a relatively constant component of collagen (13–14%) and does not occur to a significant extent in other animal proteins, chemical analysis of hydroxyproline is commonly used to determine the amount of collagen in tissues. In addition, collagen is a glycoprotein in that it contains a small quantity of galactose and glucose (24).

Tropocollagen is the structural unit of the collagen fibril. Tropocollagen molecules are composed of three α-chains that form a triple helix, with each α-chain forming a left-handed helix and three of the left-handed α-chains form a right-handed supercoil. Following synthesis of tropocollagen in fibroblasts, they are secreted

TABLE 29.5
Distribution and Molecular Properties of Genetically Distinct Collagens

Type	Molecular composition	Tissue location
I	$[\alpha1(I)]_2\alpha2(I)$	Skin, tendon, bone, dentine
II	$[\alpha1(II)]_3$	Cartilage, disc, vitreous, notocord
III	$[\alpha1(III)]_3$	Vascular system, skin, intestine
IV	$[\alpha1(IV)]_2\alpha2(IV)+\alpha3(IV)$	Basement membrane
V	$[\alpha1(V)]_2\alpha2(V)+\alpha1(V)\alpha2(V)$	Embryonic tissue, skin, vascular system
	$\alpha3(V)+$	Other combinations
VI	$[\alpha1(VI)_2\ \alpha2(VI)\alpha3(VI)]$	Vascular system
VII	$[\alpha1(VII)]_3$	Skin amniotic membrane
VIII	$[\alpha1(VIII)]_3 + [\alpha2(VIII)]_3$	Aortic intima
IX	$[\alpha1(IX)\alpha2(IX)\alpha3(IX)]$	Cartilage
X	$[\alpha1(X)]_3$	Cartilage
XI	$\alpha1(XI)\alpha2(XI)\ \alpha3(XI)$	Cartilage

Compiled from Refs. 24–27.

into the intercellular matrix where they are assembled into collagen fibrils. During fibril assembly, tropocollagen molecules are aligned longitudinally end to end and laterally in a slightly less than one-fourth overlapping stagger. The overlapping stagger gives rise to the unique striated appearance of collagen fibrils. It should be pointed out that only type I and type III collagen fibrils can form collagen fibers. Type I collagen forms large fibers while type III collagen forms fine fibers. In fibrous collagens, cross-linkages are formed via intermolecular or intramolecular cross-links. The number of cross-links depends on the load and stress of the muscle and also on the age of the animal. Limb muscle and aged animals generally have more crosss-links, resulting in tougher meat than meat from loin or young animals. The cross-links result from covalent bonding of hydroxyprolines, deaminated lysine, hydroxylysine, or sulfhydryl groups. The enzymes responsible for the modification of proline and lysine are located in fibroblasts and the modifications occur post-translation of the polypeptides.

Elastin is a much less abundant connective tissue protein than collagen. Its ultrastructural characteristics are not well known. Elastin is a rubbery protein present throughout the body in ligaments and arterial walls, as well as in the framework of a number of organs including muscle. Elastin fibers can be easily stretched and, when tension is released, return to their original length. Elastin contains eight amino acid residues with glycine being the most abundant. There are two unique amino acid residues in elastin, desmosine and isodesmosine. Elastin is highly resistant to digestive enzymes and cooking has little solubilizing effect on it. Thus, elastin contributes little to the nutritive value of meat (1).

IV. LIPIDS

Lipids is the component with the highest variability for meat, ranging from 1.5% to 13.0% (28), but lipids content can be as high as 43% if the meat contains excessive adipose tissue (3–5). The variability of lipids arises primarily from the differences in neutral fat, or triacylglycerides. Other lipid components such as phospholipids, cerebrosides, and cholesterol are fairly constant among different meat cuts. The differences in triacylglycerides reside in the fatty acids. The common fatty acids found in meat include stearic, palmitic, oleic, and linolenic (Table 29.6). Feed has an impact on the composition of fatty acids in meat. This is particularly obvious for pork. Bacon and other meat products from hogs that have ingested flax seed or fishy residue may develop painty or fishy flavors. Dietary fatty acids have less impact on the fatty acid profile of ruminant animals because the ruminant bacteria convert unsaturated fatty acids to saturated fatty acids through hydrogenation (28).

The level of phospholipids in muscle tissues is generally in the range of 0.5–1%. Most of the phospholipids are phosphoglycerides, i.e., phosphotidylcholine, phosphotidylethanolamine, and phosphotidylserine. Since phospholipids contain relatively high levels of polyunsaturated fatty acids, they tend to be oxidized to generate off-flavor. Meat also contains a small amount of cholesterol, generally in the range of 100 mg/100 g (3–5).

V. CARBOHYDRATES

Carbohydrates and related compounds contribute to about 1.0% of meat weight (22). The major components are glycogen and glucose. Other components such as triose

TABLE 29.6
Fatty Acids Composition of Animal Fats

Fatty Acids	Pork Lard	Beef Tallow	Mutton Tallow
Lauric (C12:0)	Trace	<0.2	Trace
Myristic (C14:0)	0.7–4.0	2–8	1–4
Palmitic (C16:0)	26–32	24–33	20–28
Stearic (C18:0)	12–16	14–29	25–32
Arachidic (C20:0)	Trace	0.4–1.3	Trace
Total saturated	*40–48*	*40–56*	*46–60*
Myristoleic (C14:1)	<0.3	<0.6	Trace
Palmitoleic (C16:1)	2–5	1.9–2.7	Trace
Oleic (C18:1)	41–51	39–50	36–47
Linoleic (C18:2)	3–14	<5	3–5
Linolenic (C18:3)	<1	<0.5	Trace
Arachidonic (C20:4)	<3	<0.5	Trace
Total unsaturated	*52–60*	*44–60*	*40–54*

Compiled from Refs. 3–5, 28.

phosphates and lactic acid arise from the metabolism of glucose or glycogen. The level and metabolism of carbohydrates determine, to a great extent, the eating quality of meat because during postmortem glycolysis, glucose is converted to lactic acid and ATP, both of which have significant impact on postmortem changes. Other important carbohydrates are bound to proteins which are called proteoglycans (protein accounting for 5–15% and carbohydrate accounting for 85–95%) and glycoproteins (protein being the major component and carbohydrate being the minor component).

VI. OTHER COMPONENTS

The mineral content in meat is generally low, with potassium 0.355%, sodium 0.07%, and phosphorus 0.19% (Table 29.7). However, meat provides some essential trace elements (Fe, Zn, Cu, etc.) that have higher absorption than food of plant origins.

Non-protein nitrogenous (NPN) substances contribute to about 1.5% of meat weight. These substances include creatine, creatine phosphate, nucleotides, free amino acids, peptides, creatinine, etc. The level of NPN may have a significant impact on the flavor of meat. Meat also contains significant amount of vitamins, especially vitamin B1 and niacin (Table 29.8).

TABLE 29.7
Mineral Content of Separable Red Lean Meat (mg/100 g)

	Beef	Pork	Lam
Potassium	350	384	280
Phosphorus	200	220	170
Sodium	63	54	66
Magnesium	22	23	26
Calcium	6	17	10
Zinc	4.0	2.0	4.0
Iron	2.0	0.9	1.8

Compiled from Refs. 3–5.

TABLE 29.8
Vitamin Content of Separable Red Lean Meat (mg/100 g)

	Beef	Pork	Lamb
Vitamin B1	0.1	0.98	0.13
Vitamin B2	0.18	0.27	1.23
Niacin	3.59	4.8	6.0
Pantothenic acid	0.36	0.79	0.70
Vitamin B6	0.44	0.51	0.16
Folic acid	0.007	0.005	0.023
Vitamin B12	0.003	0.0007	0.003

Compiled from Refs. 3–5.

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30 Meat Species Identification

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CONTENTS

I. INTRODUCTION

Meat species identification has been an active area of research for the past three decades. Identification of the animal origin is important for fair trade, for ensuring compliance with labeling regulations, and for other issues such as wild life management and conservation. Initially, method development for species identification was mainly for the legal enforcement of regulations governing the import of raw meats at ports of entry, since species substitutions, such as the substitution of horse or kangaroo meat for beef (1, 2), and pork for beef or sheep meat (3), had been reported in several countries. Methods that give a clear qualitative result, verifying and identifying the species of origin, were sufficient at the time. However, as processed meat and prepared ready-to-eat products have become increasingly available to consumers, the possibility of fraudulent adulteration and substitution of the expected species with other meats has also increased. Widespread problems due to mixing undeclared meat species have been documented among fresh and processed ground meat products in retail markets as either a consequence of improper handling or economic fraud (4, 5). Whether this is due to accidental contamination of low levels of undeclared meat or intentional adulteration involving significant amounts and/or multiple species, such practices pose a substantial concern to consumers in terms of economic loss, food allergies, religious observance, loss of traceability, and food safety (6). It has been observed that the adulteration problem occurs more frequently in precooked meats than in fresh meat, possibly due to the lack of reliable and economical analytical methods for cooked meats (7). Wherever the law enforcement is inadequate with regard to meat species monitoring programs, the rate of adulteration increases substantially. Therefore, the recent focus of species detection has been on the content of various processed and precooked ground and comminuted meat products. The development of

reliable analytical methods that can sensitively identify and quantify the unknown species in processed and composite mixtures has thus become more important and, at the same time, more complicated. Research efforts have concentrated on searching for species markers that are not only unaffected by the processing conditions, such as cooking temperature, but can also be used to quantitatively assess the proportion of the target tissue present in meat samples (6).

Recently, an emerging issue concerning the transmission of the fatal neurodegenerative disease, Bovine Spongiform Encephalopathy (BSE, commonly known as mad cow disease), has made the science of species detection even more demanding and challenging. Most scientists agree that the BSE epidemic originated from the practice of feeding cattle with rendered animal protein derived from scrapie-infected sheep tissue, and the disease is further spread by recycling meat and bones from infected animals back into cattle feed (8). Etiologically linked to BSE, the recent occurrence of a new variant of Creutzfeldt Jakob Disease (nv CJD) in humans is believed to result from consumption of beef from BSE-infected cattle (9). Ultimately, food safety is the threshold issue for consumers' fear of BSE. To prevent the spread of the disease in animals and reduce the potential risk of BSE transmission to humans, stringent feed control is essential. The use of rendered protein supplement derived from ruminants was banned in ruminant feed in the European Union (EU) in 1994 (10) and in the U.S. in 1997 (11). Since 1996 the World Health Organization (WHO) has recommended that all countries ban the use of ruminant tissue in ruminant feed (12). The EU has introduced a total ban on the feeding of processed animal protein to all animals which are for human consumption under the new processed Animal Protein Regulations, while blood, milk and gelatin are exempt from the feed ban (13). This total feed ban has been in force since 2000. While the practice of adding rendered animal by-products, such as meat and bone meal (MBM) as a protein supplement to non-ruminant animal feed and pet food continues in many BSE-free countries, different regulatory emphases are placed on specific animal species and the materials that are permissible for use or prohibited. Therefore, methods that can reliably detect prohibited animal species in MBM and feedstuffs are crucial for effective implementation and enforcement of the preventive measures for BSE, as well as other Transmissible Spongiform Encephal-opathies. The requirement of adequate sensitivity and reliability of the assay to not only detect prohibited species but also differentiate the prohibited tissues from allowed animal materials, such as blood, milk and gelatin of the same species, and without cross reaction with plant materials in severely heat-processed and highly complex feed samples has greatly increased the level of the challenge involved in method development.

In this chapter, the author presents a brief overview of the techniques that are currently used to identify meat species and/or quantify the species content. These methods are summarized in Figure 30.1 and the currently available commercial assays for meat species identification are listed in Table 30.1. The term "meat species" is used in this chapter to refer to a broad range of animal species including mammalian, avian and marine animals. The intent is not to provide an exhaustive review of the analytical methods in this area but to describe the trend of method development and to discuss the principles, usefulness and limitations of the most commonly used techniques illustrated by using exemplary studies. More detailed reviews of the methods used for meat species identification in food or feed products at various developmental stages can be found in a number of articles in the literature (6, 14–22). The classic methods such as electrophoresis, chromatography and immunodiffusion usually depend on the stability of the soluble proteins and are generally applied to raw meat products, although some have also been applied to cooked meat with limited success. Since the recent development of new techniques has centered on protein-based enzyme immunoassay and DNA-based Polymerase Chain Reaction (PCR) analyses in heat-processed products, essential factors and considerations important to their assay development are discussed. An overall comparison between these two approaches is made in terms of specificity, sensitivity, quantitative measure and convenience of the assay.

II. CURRENTLY AVAILABLE METHODS

A. ELECTROPHORESIS

Electrophoresis is a powerful technique for the separation of soluble proteins into distinctive banding patterns. In general, most electrophoretic methods use sarcoplasmic proteins for species identification in raw meat, because heat denatures and insolubilizes most of the native sarcoplasma proteins, protein extracts of heat-processed meat result in only a few faint bands. Analysis of meat samples by electrophoretic methods must be made by comparing the patterns obtained to those of authentic samples run simultaneously. Usually, the complex protein banding fingerprint makes electrophoresis unsuitable for the analysis of meat mixtures. Several electrophoretic techniques are described below. They are commonly used to differentiate animal species, mostly in raw samples, although a few heat-processed sample treatments are also included.

1. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE is the most widely used technique for the separation of soluble proteins based on their molecular sizes.

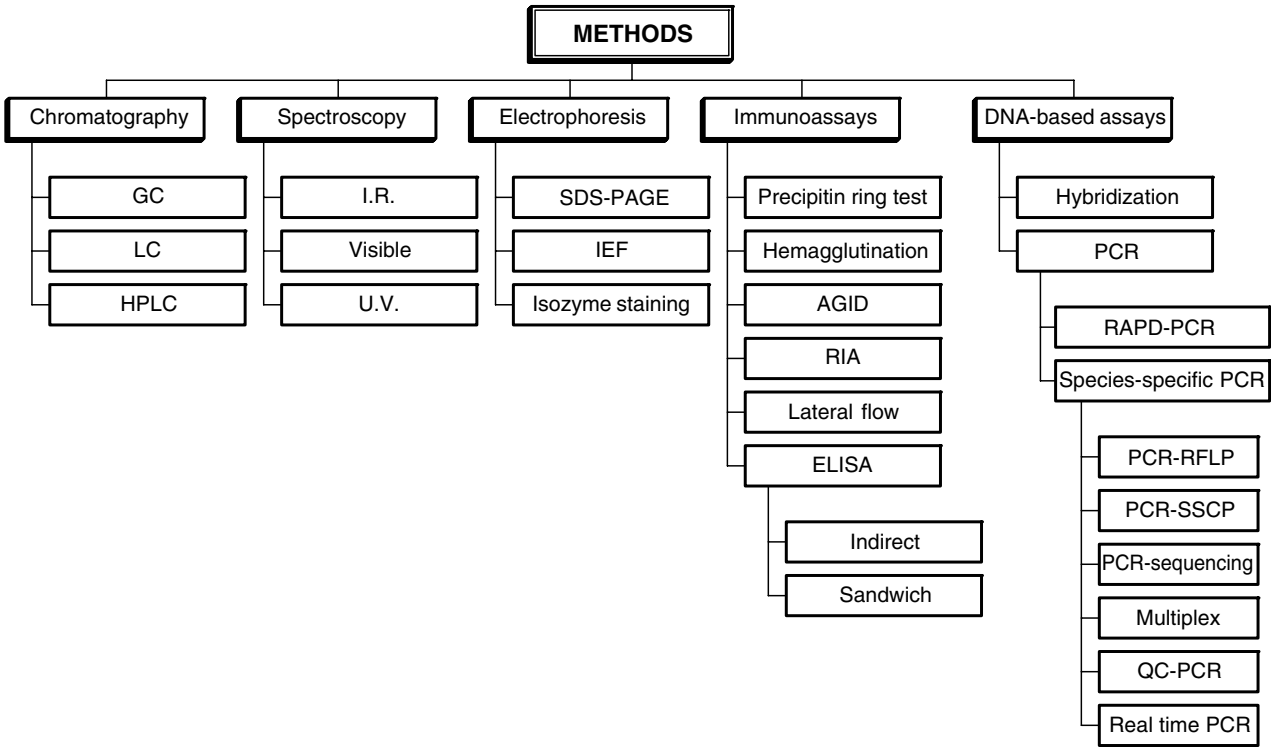

FIGURE 30.1 Current methods for meat species identification.

This technique has been successfully used to detect differences in native and heat-treated muscle proteins of various animals (23–25). Separation of different muscle proteins by SDS-PAGE usually results in relatively uniform patterns, therefore, this method is less reliable for meat species identification (26–28). Using more sensitive silver staining for visualization of the SDS-PAGE protein bands, Zerifi et al. (29) reported that this method detected species adulteration in cooked meat at concentrations of 10% (w/w) in a binary meat mixture. However, many factors other than the animal species, such as the amount of protein loaded on the gel, the freshness of the meat, the age and sex of the animal, the residual blood content, the degree of heat processing and the staining techniques, may all affect the protein-banding patterns. Thus, the lack of sensitivity and reproducibility of the assay and the difficulty of the gel interpretation are the main drawbacks limiting the use of SDS-PAGE for meat species identification.

2. Isoelectric Focusing (IEF)

IEF separates proteins into characteristic patterns based on differences in the isoelectric points of their protein bands. IEF was first used by Tinbergen and Olsman (30) for species identification. It has since been extensively used and officially recommended for the identification of fish species (31–34). The IEF protein banding profiles for many species are available from the U.S. Food and Drug Administration (http://vm.cfscan.fda.gov). It is also used in identifying animal species in either raw or cooked meats (35–39). Although the resolution is better than SDS-PAGE, the patterns of the total protein profiles obtained by IEF are much more complicated and less consistent (40). The technique may be useful for identifying individual species within families or genera, but the large number of protein bands makes the interpretation of the results impossible in meat mixtures or in uncommon meat samples.

3. Isozyme Staining

Another possible approach is to use isozyme staining to improve the sensitivity of electrophoretic assays in raw meat. Isozymes are different enzyme proteins that catalyze the same reaction. After electrophoretic separation of meat protein extracts, the gel is stained for a particular enzymatic reaction. The staining makes the isozymes catalyze the formation of colored compounds via a set of coupled reactions. For most meat extracts, isozyme staining will lead to several different protein bands on the gel at different positions, which correspond to the different enzyme proteins that are capable of catalyzing the same enzymatic reaction. The relative positions of the isozyme markers are characteristic of the species. Several staining systems using isozymes, such as esterase (41, 42), phosphoglucomutase (43), creatine kinase (44, 45), lactate dehydrogenase (40, 46) and peroxidase (47) have been employed for species

TABLE 30.1
Commercial Assay Products for Meat Species Identification

Company	Product Name	Type of Test	Species	Conditions	References
Adgen	DNAnimal Consensus	PCR	B, C, S, P or T	Raw, cooked, boiled, smoked, gelatin, processed	http://www.adgen.co.uk/index.php?area=foodfeed&kword=meat%20speciation&formaction=get_products&referrerArea=speciation&intro=meat_spec
	BOS Ident		B		
	BOS Quant		B		
	Ruminant MBM tests (Neogen tests)	Lateral flow strip tests	Rum	MBM and feed	
Alcum	Standard Animal-Kits	PCR	C, T, B, P, Go, Do or S	Raw, boiled, cooked and sausage	http://www.alcum.de/engl/index.html
	Extra Animal-Kits		C, T, P, B or H	Highly heated canned foods or products	
Biotools B&M Labs, S.A.	Biofood identification kit	PCR-RFLP	Vertebrates (e.g. Fish, P, B, C, T, S, Go, R, H, Dog, Cat, Du or G)	Raw and processed	http://www.biotools.net/eng/productos/f12.htm
	Biofood mixed kit	PCR	H, S, B, Go, C, or P	Raw and processed	http://www.biotools.net/eng/productos/f13.htm
Cibus Biotech	CIB-A-Kits (standard)	PCR	C, B, Do, Go, P, S or T C-T-B-P (mix kit)	Raw, boiled and cooked	http://www.cibus-biotech.de/products/products.htm l#2.1
	CIB-A-Kits (extra)	PCR	C, B, H, O, P or T C-T-B-P (mix kit)	Extremely processed and canned	
Coring System Diagnostic GmbH	PCR-standard-kits	PCR	Do, C, T, B, S, P or Go B-P-C-T (mix kit)	Raw and cooked	http://www.coring.de/eng/pcr_uk.htm#tierart
	PCR-extra-kits	PCR	C, H, T, O, P or Rum B-P-C-T (mix kit)	Processed products	
ELISA Technologies	ELISA-TEK microplate kits	ELISA	C-P-Po or C-T-S B-P-Po or S-H-D	Raw Cooked	http://www.elisa-tek.com/
	MELISA-TEK Meat Species Kit	ELISA	Rum or P	MBM, feed, cooked and raw	
	DTEK immunosticks	Immunosticks	B, P, S, H, Go, R, K, Wb or Po	Raw	
r-Biopharm	Surefood	PCR/ELISA	B, P, S, Go, C, G, T, Du or D B-S-Go, C-G-T-Du, B-P-T-C, B-P, C-T or C-P	Raw and processed	http://www.r-biopharm.com/food-andfeed/pdf/surefood_elisa.pdf
Neogen	Reveal ruminant feed Reveal ruminant MBM	Lateral flow strip tests	Rum	MBM and finished feed	http://www.neogen.com/bse.htm
Strategic Diagnostics Inc.	FeedChek	Lateral flow test	Mam/avian/fish, Mam only	Raw and finished feed	http://www.sdix.com/ProductSpecs.asp?nProductID=40
Tepnel	BioKits Animal Speciation Testing	ELISA/ Immunosticks	B, P, S, H or Po B, P, S or Po	Cooked Raw	http://www.tepnel.com/ag_bio_and_food_testing/animal_speciation_testing.asp
		Immunosticks	C, T, K, R, H, Go or Bu	Raw	
		PCR	B, P, S, C, H, T, R or Go	Cooked and raw	

B = Beef
Bu = Buffalo
C = Chicken
D = Deer
Do = Donkey
Du = Duck
G = Goose
Go = Goat
H = Horse
K = Kangaroo
Mam = Mammalian
MBM = Meat & bone meal
O = Ostrich
P = Pork
Po = Poultry
R = Rabbit
Ru = Ruminant
S = Sheep
T = Turkey
Wb = Water buffalo

identification of meat after electrophoresis. Although isozyme patterns are essentially the same for all members within a species, there are distinctive patterns between each species (40) and some variations within species age and sex groups may also occur (41). Since the proteins must retain their activity for the staining process, it is essential that the isozyme markers do not denature during extraction and electrophoresis (43, 46). As a result, this method is less reliable due to the difficulty of controlling the enzyme activity of the unknown samples and its application is thus limited to the identification of species in raw meat.

4. Capillary Electrophoresis (CE)

Capillary electrophoresis is a sensitive separation technique that combines the electrophoresis principle with chromatographic methods for protein analysis. A sodium dodecyl sulfate polymer-filled capillary gel electrophoresis (CE-SDS) method was developed by Cota-Rivas and Vallejo-Cordoba (48) for species identification of raw beef, pork and turkey meat. The CE-SDS sarcoplasmic protein profiles that resulted were specific for each animal species both qualitatively and quantitatively. The same authors (49) later developed a pattern recognition statistical model that utilized linear discrimination analysis to interpret the CE-SDS meat protein profiles. This method is sensitive enough to differentiate between mechanically recovered chicken meat and hand-deboned chicken breast meat based on the difference in their hemoglobin (50). In general, however, this level of sensitivity is not desirable for routine meat species identification but only serves to complicate the results. Although fast separation, automation and on-line data analysis are major advantages of the technique, investment in an expensive instrument is required. It is also extremely difficult to interpret the results, especially when mixtures of multiple species or protein additives are involved.

B. Chromatography

Chromatographic methods such as gas chromatography (GC), liquid chromatography (LC) and high performance liquid chromatography (HPLC) have been applied to identify species in meat samples based on an examination of their fatty acid composition (51), histidine dipeptides (52, 53) or protein profiles (54, 55). Among these techniques, HPLC has been the most frequently used method by researchers. For example, a reverse phase high-performance liquid chromatography (RP-HPLC) method has been used to identify several animal species in raw and heated meat based on species-dependent histidine peptides, specifically the ratio of carnosine to serine (C/A) (53). In another study a size-exclusion HPLC column was used to examine differences in the myofibrillar protein profiles of fresh meat samples (55). Although the chromatographic patterns were similar for different muscles within species, there were several unique proteins and the percentage area under several protein peaks varied (54, 55), which may present difficulties for data interpretation. Based on the rationale that fat is not affected by heating, an LC method was developed for the detection of pork and lard in fresh and heat-processed meats based on the increased ratio of the triglyceride containing saturated fatty acid to the triglyceride containing unsaturated fatty acid at the C-2 position after derivatization (56). However, since the fat content in meat products varies widely, it is difficult to quantify meat adulteration from the fat profiling methods alone. As with electrophoretic methods, chromatographic methods may be capable of differentiating between individual meat species, but they are less effective in detecting adulterated species in meat mixtures or cooked meat because of the increased complexity of the chromatographic patterns. In addition, the requirements of expensive instruments and laborious sample preparation procedures have restricted their use for regulatory purposes.

C. Spectroscopy

Spectroscopic methods are based on the light absorption at selective wavelength of the electromagnetic spectrum by the molecules in the samples. Spectroscopies using mid-infrared (2000–800 cm^{-1}), near-infrared (750–2498 nm) and visible (400–750 nm) reflectance spectra of meat samples for both discrimination and quantification of species content have been reported (57, 58). These spectral ranges can be used alone and in combination. Data in each spectral region are related but not identical and their combination may provide a synergistic advantage to spectroscopic analysis (59). A near-infrared spectroscopic technique was developed to distinguish between beef and kangaroo meat (60) and detect beef hamburgers adulterated with 5–25% mutton, pork, skim milk powder or wheat flour with an accuracy of up to 92.7% (61). A feasibility study on the application of mid-infrared spectroscopy has been used for quantification of chicken/turkey blends (62). A combined visible and near-infrared spectroscopy has been applied to identify species in raw homogenized meats (63) and to detect MBM in fish meal (64). When an adulterant is detected, the adulteration level can be predicted by using calibration equations for each adulterant. However, the performance of the calibration equations may be affected by several factors, including spectral scatter, derivative treatment and even sample presentation methods. The position of the spectral peaks of absorption bands may shift not only due to adulteration with other meat, but also with different food additives, fat contents, residual blood and moisture levels and cooking methods (61). In addition, this spectroscopic technique requires advanced knowledge of using statistical models for data processing and different models may be required for each possible blend of meat (58). Thus, the application of this technique to identify

meat species in highly processed products presents serious difficulties and it is neither a common nor a convenient method for the routine analysis of meat origin.

D. Immunoassays

Ever since the beginning of the 20th century, immunological approaches based on specific antibody-antigen reactions have been applied in meat species identification (65). Classical immunological procedures that use antispecies antisera include the precipitin ring test (66), hemagglutination inhibition tests (67) and the agar gel immunodiffusion (AGID) technique (68). Advances in immunochemical techniques have led to new types of immunoassays, including radioimmunoassays, enzyme immunoassays and non-enzymatic chromatographic immunoassays (lateral flow), with greatly improved sensitivity and accuracy. Radioimmunoassay is commonly used only in clinical settings, as the hazardous radioactive materials involved and the requirement for expensive and delicate scintillation counting equipment have restricted its use in routine and field tests of meat species. Two immunoassays, agar gel immunodiffusion (AGID) and the more recent enzyme-linked immunosorbent assay (ELISA), both of which are widely used for meat identification, are described in more detail in the following sections.

1. Agar Gel Immunodiffusion

Agar gel immunodiffusion (AGID), a double immunodiffusion technique originally described by Ouchterlony (68), involves the diffusion of antigens and antibodies in a semisolid agar gel. After overnight incubation, a visible opaque band is formed at the point where the antibody and its correspondent antigen have met. The modification of this technique using stabilized reagent paper discs and pre-prepared agar plates has led to the development of convenient field test kits such as ORBIT (Overnight Rapid Bovine Identification Test, 69), PROFIT (Poultry Rapid Overnight Field Indentification Test, 70), PRIME (Porcine Rapid Overnight Field Identification Method, 71), SOFT (Serological Ovine Field Test, 72), REST (Rapid Equine Serological Test, 73), DRIFT (Deer Rapid Identification Field Test, 74) and MULTI-SIFT (MultiSpecies Identification Field Test) for meat speciation by USDA-FSIS meat inspectors (75). These field tests are mainly based on the detection of serum proteins and are suitable for testing intact pieces of raw meat. Antisera against plasma proteins for raw meat species identification are available commercially. The detection limit of the assay for the target species in a meat admixture relies on the quality of the antispecies antisera. With traditional AGID, the detection limit of the assay is usually in the region of 3–10% (17, 76). In most cases, standardization of the commercial antisera against each type of sample is required. Although AGID is relatively simple to perform, it requires a substantial amount of antisera and the variable sensitivity, possible interferences by non-meat ingredients and the requirement for overnight incubation have restricted AGID to qualitative assays of whole pieces of fresh meat.

2. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA has gained widespread acceptance for meat speciation applications due to its simplicity, specificity and sensitivity and is now recognized as a suitable analytical method for quality control and enforcement of muscle food safety and labeling regulations. It can be used for assaying various analytes in complex mixtures with minimal sample preparation requirements. ELISA does not need major scientific equipment, is easy to perform, uses only small quantities of immunoreagents, and has large-scale screening and field test capacity. However, the success of the assay primarily relies on the quality of the detecting antibodies that capture the target protein antigen in a sample extract. Both polyclonal (Pabs) and monoclonal (Mabs) antibodies have been employed in various formats of ELISA for the detection of species adulteration. Traditionally, Pabs obtained from the antisera of immunized animals have been used in ELISA. More recently, however, several Mabs against muscle proteins have been developed for the identification of raw meat (77–81) and for heat-processed meats and feedstuffs (6, 28, 82–85). A comprehensive review of studies published up to 1998 of ELISAs that utilize Pabs and Mabs for species identification of raw and cooked meat is provided in Hsieh et al. (6).

The two ELISA formats most commonly used for meat speciation are indirect ELISA and double sandwich ELISA. Indirect ELISA involves the adsorption of a fixed amount of meat proteins that may or may not contain the antigen molecules of a target species onto a microtiter plate. An antispecies antibody is then used to bind the antigen to the solid phase. The immunoreaction between the bound antigen and the antibody can be detected by a secondary anti-immunoglobulin enzyme conjugate, and subsequently color is developed by the addition of the enzyme substrate. The color intensity is proportional to the amount of antigen present in the protein extract. Indirect ELISA is often used to determine the titers and specificity of an antibody reagent. However, it is neither a convenient nor a reliable assay format for the routine analysis of unknown samples because the protein concentration for each meat extract must be determined before the sample can be properly diluted and coated on the microtiter plate. The dilution and composition of the meat extract affects the antibody binding, and thus the ELISA results. Sandwich ELISA employs an antibody immobilized on the solid phase as the capture antibody

and another antibody as the detecting antibody to form a sandwich with the antigen in the middle and two antibodies attached on the different epitopes of the antigen molecule. This is a more user-friendly format that is widely used for commercial test kits because properly diluted sample extracts can be directly added to the assay plates which are pre-coated with the first capturing antibody reagent. To develop a sandwich ELISA, if Pabs are used in the assay, the same Pab reagent can be used for both the capturing and detecting antibodies due to its heterogenous nature. If Mabs are used, however, two different antibodies with similar specificity and affinity that bind to different epitopes are required. A combination of Pab and Mab is also commonly used in a sandwich ELISA.

3. Factors to Be Considered in Immunoassay Development

a. Species markers

To identify an animal species using an immunoassay, a suitable species marker must be chosen as the antigen for antibody development. For most raw meat identification, serum proteins have been used as antigens to develop the immunoassays (2, 86–88). Serum proteins are water soluble and easy to extract, thus the sample preparation is simple. However, the presence of serum proteins may not necessarily denote the presence of muscle tissues due to variations in the residual blood content of the muscle tissues. Such assays would not be reliable for quantifying adulteration levels of extraneous muscle tissues (89). Moreover, serum proteins are susceptible to heat denaturation; the reliability of using serum proteins for detecting species origin would be greatly reduced in cooked meats (90). Any antigens used for cooked meat identification must either be able to withstand cooking or maintain their antigenicity after heating.

Assays based on the detection of heat-resistant, species-specific muscle proteins are more indicative for a quantitative measure of lean meat, as well as supporting the assay in both raw and cooked meat mixtures (77, 82). Several researchers have attempted to resolve the thermal-stable components of different meat species. Milgrom and Witebsky (91) described antigens from the adrenal gland that are suitable for species identification. Those antigens, designated as BE, retained their immunogenic properties even after being subjected to boiling and autoclaving, and can be precipitated with ethanol. Hayden (92) developed polyclonal antisera to adrenal BE antigens for species identification using an immunodiffusion assay, which enabled the detection of meat from different species at 5–10% levels in cooked beef sausages. Using isoelectric focusing, Jones and Mortimer (37) demonstrated that for the thermal-stable muscle proteins (TSMP) eluted in a low pH range (3.5–6.5), the characteristic pattern of thermal-stable proteins from different species were not notably different. Sherikar et al. (93) later reported that "troponin T," with a molecular weight of 36 kD, was the specific antigenic fraction of the TSMPs. Assays based on these TSMP markers showed more or less cross-reactivity to certain non-specific proteins. ELISA protocols currently used by the USDA-FSIS for the identification of cooked meats utilize Pabs against heat-resistant muscle glycoproteins (94, 95). Several commercial sandwich ELISA kits for cooked meat speciation have also been produced based on the detection of the glycoproteins (Table 30.1). These commercial kits are able to analyze meats cooked under normal conditions, although the ELISA signal decreases rapidly when the meat is cooked above 100°C.

Antisera raised against crude thermal-stable muscle proteins according to the method of Kang'ethe and Gathuma (96) have been used to detect ruminant proteins in rendered animal materials heated to >130°C (97). However, the ELISA based on these antisera requires a lengthy procedure to remove gelatin, which interferes with the measurement, and to concentrate sample proteins in order to boost the assay sensitivity. The assays also exhibit cross-reactivity with plant protein present in the feedstuff (98, 99). In our own laboratory, troponin I (TnI), a subunit of troponin which is a contractile regulatory protein in the muscle thin filament, has been identified as a suitable species-specific and thermal-stable biomarker for meat species identification in raw, cooked and autoclaved meats (100, 101). Mabs developed against TnI could detect and quantify muscle protein heated up to 132°C for 2 hours without compromising any of the reaction signals (85). An indirect ELISA based on the Mabs raised against porcine TnI was developed to quantify pork skeletal muscle tissue in a meat admixture, with a detection limit of 0.5% (w/w) (28). Sandwich ELISAs based on TnI-specific Mabs have also been developed for the detection of prohibited animal proteins, such as ruminant, mammalian and all animal species, in rendered MBM and/or in feeds with a detection limit of 5 ng/ml of TnI, or at least 0.05% of the adulteration meat species in feed (102). In addition, the abundance of TnI in muscle tissue and its excellent extractability, even after severe heating, offers advantages for a sensitive detection method with a simple sample extraction procedure. The lateral flow strip test kits marketed by a U.S. company (Table 30.1) are based on the detection of the ruminant TnI antigen, and are currently being used internationally for BSE surveillance.

b. Antibodies

Once a suitable antigen has been selected, the most important criterion for the successful development of enzyme immunoassays for meat speciation is the production of species-specific antibodies raised against the antigen. The antibodies are the key immunoreagents that differentiate individual species. The performance of ELISA depends on the nature, quality and availability of the antibody used

and both Pabs and Mabs have been used in immunoassays for species identification. Pabs are antibodies obtained from the antiserum produced by an immunized animal in response to an antigen. They are relatively quick and easy to develop and are heterogeneous in nature, with subpopulations binding to either species-specific or non-species-specific epitopes; hence cross-reactivity of the antisera with closely related species is commonly observed (103). However, the preparation of species-specific antisera requires immunoaffinity adsorption of cross-reactivity, which is a costly and time-consuming procedure. Moreover, variations in specificity and affinity between batches of antisera are the major concerns when developing standardized procedures (15). In contrast, Mabs are produced by a chemical fusion of antibody-producing cells (B-lymphocytes) and myeloma cells. They offer advantages over Pabs due to their homogeneous nature and biologically well-defined characteristics. Although the initial development costs of Mabs are higher than for Pabs, the use of Mabs in immunoassays ensures a continuous supply of a uniform immunoreagent and will eventually reduce the cost of the analysis (6, 77). Since every Mab has unique binding properties, ELISA procedures for each Mab must be optimized individually to achieve the most accurate analysis and the lowest detection limit.

Usually, IgG is the class of Mabs that is preferred for use in immunoassays, as it has been reported that IgM Mabs frequently participate in unexpected cross-reactions (104). IgM Mabs that are high-molecular-weight molecules are also more difficult to purify and store than IgGs (105). The most convenient way to avoid obtaining IgMs is to screen hybridoma clones with detection antibodies specific to the IgG γ-chain.

4. Specificity

Immunoassays for species identification are typically developed for a specific animal species and cross-reactivity of the antibody reagent to closely related species is often a major concern. As mentioned previously, the preparation of species-specific Pab reagents requires immunoaffinity absorption of the cross reactive components. Usually, pure antigen is required to immunize animals in order to increase the chance of producing more specific Pabs in the antiserum. Developing Mabs does not require the use of a pure antigen and crude antigens could theoretically be used for the production of species-specific Mabs. However, we found this to be ineffective, as the chance of selecting a specific clone by immunizing and screening with crude antigen is remote. The development of mono-specific Mabs is much more difficult than that of group-specific Mabs. This is mainly because species-specific antigenic determinants on the crude protein antigens are much rarer than those determinants shared by zoologically related species. The use of a pure antigen with known species-specific antigenic determinants substantially increases the chances of eliciting specific antibodies.

Developing antibodies with broad specificity is also important for the initial screening of samples for the detection of species adulteration or substitution in meat products. Currently available commercial ELISA kits for meat species identification are designed for qualitative tests of a single species at a time. For regulatory purposes, it is very expensive and time-consuming to test a large number of samples for multiple species using these ELISA kits, and it would be much more cost- and labor-effective for a single screening to be used to distinguish between different classes of meat. Mabs developed to recognize common mammalian species in cooked poultry products (83) and to detect poultry meat in cooked mammalian meat (84) have been reported. More recently, Chen et al. (85, 102) developed ruminant-specific, mammalian-specific and all animal-specific Mab-based immunoassays for the detection of ruminant, mammalian and animal proteins, respectively, in feedstuffs. These assays do not differentiate between individual species within the group but detect the presence of animal proteins of the target group.

The specificity requirement of an assay to differentiate allowed animal proteins from prohibited tissue proteins to ensure compliance with the BSE surveillance regulations presents fresh challenges for the development of species identification immunoassays. Specificity of the antibodies to muscle proteins seems to be a prerequisite for the development of such assays. Immunoassays using skeletal-muscle-specific Mabs that are able to detect severely heat-treated target meat with no cross-reactivity to blood and food proteins, including milk proteins, egg albumin, soy proteins and gelatin, have been reported (102, 106). The muscle-specificity of the assay is also important for food testing because food proteins are frequently used as additives in commercially prepared meat products.

E. DNA-Based Assays

Immunoassays have been extensively used for authentication of animal species in raw meat for decades, but the non-availability of antibodies or cross-reactions of the antibodies to proteins from closely related animal species in highly processed and heat-treated meat is the major reason behind recent efforts to develop nucleic acid-based methods as a alternative approach. In general, DNA is both more stable and more heat-resistant than proteins and the information content of DNA is greater than that of proteins due to the degeneracy of the genetic code. For these reasons, DNA-based methods are now being developed to counter the challenges that currently limit immunoassay techniques. DNA hybridization and polymerase chain reactions (PCR) are the two major DNA-based analytical approaches to meat species identification. DNA hybridization techniques are generally time-consuming and complicated. In contrast,

the more recent PCR techniques, which easily amplify target regions of template DNA with or without prior knowledge of the genetic information, have proved to be more sensitive and rapid than DNA-based techniques for meat species identification. Lockley and Bardsly (20) have reviewed DNA-based methods for food authentication.

1. Hybridization

The initial studies using DNA for meat speciation were based on hybridization of labeled DNA probes to samples. Either genomic DNA or cloned DNA was hybridized with target genomic DNA that had been covalently attached to nylon membranes in a slot- or dot-blot format and detected by color development or autoradiography (107–111). This had the advantage that preparation of probes from genomic DNA did not require prior knowledge of the DNA sequences. Utilizing hybridization based on a total genomic probe, a cross-reaction between cattle, sheep and goats was observed, reflecting some degree of homology in the sequences of the satellite DNA in these closely related species (109). Modified DNA hybridization for reducing cross-reaction by the addition of unlabeled DNA from the cross-hybridizing species enabled differentiation between sheep and goat with about a 10% detection limit (111). Improved assay specificity was also achieved by using probes derived from published satellite sequence information. Wintero et al. (108) compared a probe based on a 2.7 kb porcine specific satellite fragment (112) with labeled total genomic DNA, and found the former to be more specific. The use of species-specific satellite DNA probes for the unequivocal identification of raw meat from pigs, cattle, deer, chickens, turkeys, rabbits, sheep and goats has been reported (113–115). As the species-specific probes tend to be relatively short oligonucleotides of less than 100 bases, hybridization is possible even after DNA degradation has occurred. These methods are specific and useful for the identification of cooked meat species, but the procedure is complex, time-consuming and inadequate for complex sample matrices or for quantification of adulteration levels.

2. Polymerase Chain Reaction (PCR)

PCR methods have gained tremendous popularity in recent years because the amplification power of the PCR technique enables the detection of target DNA sequences when sample quantity is limited. A wide variety of PCR-based methods for the identification of meat species in food and feed products have been developed in the past decade. All of these PCR methods can be categorized into two approaches, Random Amplified Polymorphic DNA (RAPD) and species-specific PCR. RAPD amplifies multiple unidentified sequences using non-specific primers, leading to species-specific fingerprints of PCR products visualized by gel electrophoresis. No prior knowledge of the DNA sequences is required. Species-specific PCR uses either conserved or specific oligonucleotide primer pairs targeting species-distinguishing sequences in genomic or mitochondrial DNA. The prior knowledge on the DNA sequences is required for primer design. The amplified PCR products can then be discriminated according to their sizes, sequencing or further analyses such as Restriction Fragment Length Polymorphism (RFLP) or Single Strand Conformational Polymorphism (SSCP). Advances in modern PCR techniques have emphasized improving the efficiency, reproducibility and speed of detection.

a. Random Amplified Polymorphic DNA (RAPD)

Using arbitrary primers based on short oligonucleotide sequences, the RAPD method amplifies numerous unidentified sequences simultaneously. After gel electrophoresis, the PCR products generate fingerprint patterns that contain features unique to a particular species. The discriminating ability of the RAPD-PCR is virtually unlimited because there is an unlimited choice of arbitrary primers. However, not all random primers can adequately distinguish between species. A careful screening and selection of the primer used is important for the successful application of the technique (116). RAPD has been employed to differentiate species in domestic animals (117), various meat and meat products (116, 118) and feedstuffs (119). RAPD has also been shown to produce clear fingerprints from processed products in which DNA has been slightly degraded, such as smoked or salted fish products (120). Although the technique generates informative fingerprints in a relatively short time, the reproducibility of the patterns is unpredictable due to various factors, such as changes in cycling conditions or intra-species polymorphisms. Use of longer primers and higher stringency amplification conditions may improve the assay reproducibility and resolution (121). Therefore, RAPD assay protocols must be optimized for each application and must be stringently followed to ensure assay repeatability. The DNA molecules are usually degraded into smaller fragments in severely processed products; RAPD analysis thus cannot reliably be used to analyze canned or sterilized samples. In addition, RAPD may not be practical for identifying species of origin in products containing 50–50% mixtures of species that can be interbred, because the pattern of a 50–50% meat mixture cannot be distinguished from that of a hybrid (120). Thus, RAPD is useful as a rapid, qualitative method for meat speciation, but known standards must be run simultaneously each time a sample is tested (117). A review of the principles and applications of RAPD assay for genetic analysis of livestock species was published by Cushwa and Medrano (16).

b. Species-specific PCR

Species-specific PCR for species identification has been developed to target a DNA segment with sufficient species-specific variation. The forward and reverse primers are designed to be specific to each species based

on the alignment of available DNA sequences from selected genes. Under stringently defined reaction conditions, such primers generate an amplicon only in the presence of DNA from a given species. Complete sequence information permits the size of the product to be predicted, so that the identification can be confirmed if an appropriately sized amplicon appears on the gel. Mitochondrial genes such as the ATPase subunit 8 and subunit 6 (122, 123), D-loop (124) and cytochrome b (Cyt b) genes (125–129), satellite DNA (130), actin genes (131, 132), Art2 short and CR1 long interspersed repetitive elements in genomic genes (133) and growth hormone gene (134) have been studied for the purpose of identifying animal species, with most of the methods concentrating on the Cyt b gene as a target sequence. The specificity of PCR primer pairs that encoded the Cyt b gene sequence has permitted the amplification of degraded tuna DNA for the identification of cooked and canned tuna fish species in commercial preparations (135). Tartaglia et al. (122) developed a bovine-specific PCR assay using bovine-specific mitochondrial DNA sequence, which allowed the detection of bovine meat and bone meal in feedstuffs at the 0.125% level. By amplifying the satellite DNA using a bovine-specific primer pair, Guoli et al. (130) was able to identify raw, cooked (100°C, 30 min) and autoclaved (120°C, 30 min) beef without cross-amplification with other animal species tested. Multiplex PCR allows simultaneous identification of multiple species with one PCR by using one universal primer from a conserved DNA sequence in the gene paired with multiple primers target to hybridize on species-specific sequences for each species (127, 136). All these species-specific PCR products were obtained without the need to adopt other secondary associated techniques such as, RFLP, SSCP or DNA sequencing.

More recently, several real-time PCR methods have been developed in order to shorten the assay time by eliminating the need for post-PCR processing, such as electrophoresis associated with conventional PCR. For real-time PCR using TaqMan™ technology (137), one of the primer probes is labeled with a reporter fluorescence dye and a quencher dye. When both dyes are attached to the probe, the fluorescence is quenched by the quencher dye so that negligible fluorescence from the reporter dye's emission is observed. Once the PCR amplification begins, DNA polymerase cleaves the labeled probe and the reporter dye is released from the probe. The separation of the reporter dye from the quencher dye during each amplification cycle generates a sequence-specific fluorescent signal. The signal increases in real time as the PCR cycles progress, as the intensity of the fluorescence is a function of the number of cycles. Real-time PCR, therefore, can be used to monitor the PCR product accumulation as the amplification cycle proceeds, allowing the detection of the target sequence with a high sensitivity. Since the analysis and detection take place in a closed reaction vessel, this has the advantage of minimizing carry-over contamination and reducing the incidence of false-positive results. TaqMan™ technology amplifying species-specific primers has been developed for the detection of beef or pork in foods with a detection limit of 0.1% to 0.01% of the adulteration level (138–140). An alternative real-time PCR system using LightCycler™ technology has been used for the detection of trace amounts of tiger bone, with a detecting power of 10 substrate molecules (141). In this system, fluorescence is emitted when labeled probes are hybridized to newly synthesized DNA (142).

3. Factors to Be Considered in the Development of PCR Methods

a. DNA probes

The specific interaction between the sequence of the DNA probe and the complementary sequence in the DNA segment of interest determines the assay outcome. PCR allows a particular sequence of DNA in samples to be targeted by primers and amplified. Selection of the primer sequence for PCR amplification depends on the sequence variation in primers that subsequently and adequately produce amplicons unique to each animal species. Primers used will affect the sensitivity and accuracy of the PCR assay. Total genomic probes can be used to identify distantly related species, but tailored oligonucleotide probes need to be designed for differentiation of closely related species (17). Most strategies to date have targeted the Cyt b gene in the mitochondrial DNA sequences because of the high number of copies per cell in animal tissues (143), inherent variability (144) and the difference in its gene arrangement between plants and animals (145). It has been shown that each cell may contain up to 1000 copies of Cyt b locus and Cyt b sequences differ by at least a few nucleotides even in very closely related species (146). PCR assays based on the amplification of Cyt b have the advantage of increased sensitivity compared to single or low copy nuclear DNA targets (147). The repetitive sequence in genomes can also be markers for sensitive species identification because these repetitive elements are present in more copies than mitochondrial DNA. Using short and long interspersed nuclotide elements, Tajima et al. (133) reported a PCR assay with improved sensitivity. Their primers were able to detect DNA in feed containing 0.01 to 0.001% commercial MBM. However, the quantity of genomic DNA or mitochondrial DNA per gram present in different types of tissues varies (148), which may affect the detection limit of the assay or a quantitative estimation of the target species in a meat mixture (136). For identification of hybridized animals, assays based on the maternally transmitted mitochondrial DNA are invalid because only the maternal lineage can be identified. Species origin of a hybrid animal can only be verified by analysis of nuclear DNA (149).

Although DNA is more heat stable than most proteins, DNA is also degraded by heat or the action of radicals. Severe heat treatment or prolonged processing of the meat product will increase the degradation of DNA; thus the recovery of analyzable DNA can be difficult or uneven from different species (127). The fragment sizes of DNA from meat heated to 100°C is reduced from ~1100 bp to ~300 bp (110), while the DNA extracted from canned tuna meat was degraded to an average size of ~100 bp. In order to confirm the utility of the PCR method for analyzing heat-processed tissues, the amplification should be carried out on DNA derived from larger portions that were subject to longer heating times (147). In other words, the length of the DNA fragments should be long enough to distinguish species variation and at the same time short enough to allow amplification of highly degraded DNA. RAPD analysis does not seem to be reliable for analyzing canned or sterilized samples. The specie-specific oligonucleotide probes which recognize relatively short sequences of DNA with adequate sequence variation between species, on the other hand, are able to accommodate a considerable amount of DNA degradation without influencing assay specificity. In addition, PCR fragments generated from mitochondrial DNA seem to be more successful than nuclear DNA for speciation of heat-processed samples because mitochondrial DNA, being circular in shape, is more resistant to heat disintegration (150). Most recently developed PCR methods (123, 138, 151–154) for the detection of bovine tissue in feedstuffs are based on the amplification of the ATPase subunits 8 and 6 of the bovine mitochondrial DNA sequences, which was firstly described by Tartaglia et al. (122).

b. Secondary analyses of PCR products

For species identification, several secondary analyses have been applied to PCR amplified products of which each targets a conserved region of the DNA sequence. These methods vary in their complexity, utility and cost due to the nature of each assay.

1. PCR-RFLP

PCR analysis with subsequent restriction fragment length polymorphism is the most widely used method of identifying meat and fish species (19). PCR-RFLP allows the genetic variation between species to be distinguished by digesting PCR amplified fragments from a conserved region of DNA with restriction enzymes, which act on DNA at specific sites. Based on the sequence variability among species, these enzyme-digested products can be visualized by agarose or polyacrylamide gel electrophoresis as a DNA fingerprint unique to each species. The banding pattern varies when different restriction enzymes are employed. It is important to use appropriate restriction enzymes and to ensure the complete digestion of all the DNA fragments analyzed. The enzymes selected should be available commercially, economically priced, reasonably active in the PCR mix and able to maximally differentiate between the species analyzed. PCR-RFLP has been extensively used for meat species identification in food and feed samples (149, 154–160). Partis et al. (147) evaluated this DNA fingerprinting method for determining the species origin of raw and cooked meats by amplifying a fragment within the Cyt b gene, and concluded that although the PCR-RFLP is a suitable method for identifying species in raw and cooked pure animal tissues, the assay is not suitable for analyzing mixtures since the results may not be representative of the true components present in the mixture due to differential template amplification. The general applicability of RFLP may also be hampered by intraspecies DNA sequence polymorphism or point mutations that affecting the typical RFLP pattern of a species. To overcome this drawback, the use of two restriction enzymes for the identification of an unknown sample may be sufficient because no individual identified as having two different RFLP patterns compared to other individuals of the same species has been observed (156).

2. Sequencing

If information on the DNA sequence is available from databases, individual species may be identified through a given sequence of digestions with specific restriction enzymes (155). Direct sequencing of the PCR products is an ideal confirmation test and allows an assignment to a species even if there is no reference material available. Comparing Cyt b sequences from the database Brodmann et al. (5) reported that vertebrates of different classes showed correspondence of at the most 80%, and animals of the same genus showed correspondence above 94%. Correspondences of more than 99% generally indicate the same species. Traditionally, sequencing has been considered to be time-consuming and expensive and to require data handling skills, but due to recent advances in the nucleotide sequencing process, this method is nowadays relatively simple and fast. Since sequencing PCR fragments has become a standard procedure in laboratories working with recombinant DNA technologies, it has been suggested that a DNA sequence database for unambiguous authentication of animal species should be constructed (19). However, it is still difficult to apply sequencing to mixtures of different species.

3. PCR-SSCP

In comparisons involving high homology DNA sequences, further analysis of PCR products may be accomplished using Single Strand Conformational polymorphism (SSCP), which can be used to analyze the polymorphism at single loci. The PCR-SSCP technique is based on the principle that single-stranded DNA molecules take on specific sequence-dependent secondary structure under non-denaturing conditions. After performing the PCR, the amplicons are denatured to single strands. Single-stranded molecules differing by as little as a single base substitution will form different conformers and migrate differently in a non-denaturing gel

electrophoresis (161). This technique has been applied to identify fish species (162–165) as well as differentiate between samples of species of pig and wild boar meat (166). Stringent conditions must be maintained for intrabase pairing, as this affects the electrophoretic mobility and reference material must be treated in the same way and run alongside the unknown sample. Intraspecies variability of single-strand DNA patterns may also cause some confusion in identifying an unknown sample. In such cases, different regions of the DNA probe can be used for SSCP, or use of RFLP analysis or sequencing of the amplicons (163).

F. Overall Comparison of Immunoassay and PCR Methods

Immunoassay systems are suitable for use in the laboratory for routine analyses or large-scale sample screening, as well as for development into convenient field test kits. These assays usually only require simple sample preparation and need no major instrumentation. The overall assay time can be as short as 30 minutes. However, immunological approaches depending on antibodies often suffer from loss of reactivity due to heat treatment of the sample and cross-reactions of proteins from closely related species. Availability of suitable antibody reagents is the major limiting factor for the application of immunoassay methods. Immunoassays based on the detection of serum proteins are limited to qualitative analysis only because the amount of residual blood does not parallel the quantity of meat tissue present in a sample. In order to achieve a quantitative analysis, the assay must target an abundant antigen with an even distribution in muscle tissues from different parts of the animal, so that the amount of antigen measured by the assay corresponds to the amount of the target muscle tissue in the sample, regardless of the location of the cut. Once developed, the antibodies can be applied in various immunoassays for maximum success.

It is a common belief that PCR-based techniques offer a high detection sensitivity and specificity. They can be applied in both raw and highly processed products even with extensive protein denaturation; thus most of the recently developed methods for meat species differentiation in feedstuffs are PCR-based techniques (see Table 30.2). Since DNA can potentially provide more information through the acquisition of sequence database, differentiation of closely related species is less difficult than in an immunoassay, which relies on the availability of specific antibodies. However, PCR-based methods are technically demanding and prone to contamination; they also require expensive instruments and dedicated facilities. Moreover, PCR based methods for quantification of the extent of sample adulteration are complex and the data interpretation is difficult. Current DNA-based techniques provide no satisfactory answer to the proportional amount of a particular species for the following reasons: 1) the difference in DNA quantity and extractability in different tissues (113); 2) differential template amplification resulted from primer mismatches (147); and 3) the ubiquity of DNA in all types of cells. Although quantitative PCR detection of porcine DNA has been reported (167, 168), the results only refer to the DNA content over a certain limited range, which cannot be translated into the content of fat or lean meat. DNA based methods are not able to distinguish between different tissues or materials (21). Therefore it is impossible to use DNA-based methods to differentiate prohibited animal tissue from the allowed blood or milk in feedstuffs. In addition, most PCR methods require the extraction of nucleic acids from the sample tissue, which limits its application for large-scale practice and the development of field testing kits. For all the above reasons, PCR-based techniques have not been adopted for routine analysis, while faster and more specific DNA-based technologies are developing rapidly. Accumulation of an extensive database of DNA sequences from different animal species will facilitate the design of primers, and thus the development and usefulness of PCR methods.

III. FINAL CONSIDERATIONS

Reliable analytical methods are indispensable for the accurate labeling of food and feed products. Detection of species adulteration requires an assay capable of working in complex and variable matrices. Hence, the challenges in developing antibody-based immunoassay or PCR methods for meat species identification lie in overcoming the potential cross-reactivity with other species and food additives. Usually, the judgment of the violation samples is limited to determining the minimum level that the method could achieve. For practical purposes, it is necessary to set a cutoff value for judging violations. Hsieh et al. (4, 7) applied 1% as their criterion for reporting the violation rate of species adulteration in official meat samples, because adulteration at such a low level would not confer any economic incentive. An assay capable of detecting down to the 1% adulteration level thus seems to be adequate for routine analysis. The assay sensitivity of both modern immunoassays and PCR methods are comparable, and either can achieve this generally acceptable detection limit of 1% or below for the adulteration level in food products. As the minimum safe level to prevent transmission of BSE in feedstuffs has not been established, no agreed sensitivity value of the assay has been established. A detection limit at a lower level than 1% is generally expected for safety reason. It is also crucial that the assay conditions are properly controlled in order to preclude incidences of false positive and false negative results.

It is necessary to optimize and standardize the conditions for protein or DNA extraction from a variety of samples. Due to the variation in sample composition, factors affecting the recoveries of antigen or DNA from samples,

TABLE 30.2
PCR and ELISA Methods Used to Identify Animal Species in Feedstuffs

References	Methods	Target Species	Probes	Detection Limit	Test of Industrial Samples No. Correctly Identified/No. Tested
159	PCR-RFLP	Dog or cat	Cyt b mt DNA	0.01% in rendered meat mixtures (121°C, 30 min)	
123	PCR	Bovine, ovine, swine or chicken	mtDNA*	0.01% in ref feedstuffs	
134	TaqMan™ real-time PCR	Mammal orbBovine (cross react w/*Cervidae*)	Growth hormone genes	0.02 ng or 0.01% DNA 1–5% in MBM	4/7 bovine in MBM 5/7 mammalian in MBM
158	PCR-RFLP	Cattle, sheep, goats, deer, elk	mtDNA	Not reported	
139	Real-time PCR	Cattle or pig	mtDNA	0.1–0.5% in feed	
168	PCR	Beef	Satellite DNA	1% bMBM (120°C, 30 min) in feed	
138	Real-time PCR	Bovine	mtDNA*	0.001% in LSR	4/6
133	PCR	Ruminants, pigs, chickens	Genomic DNA	0.01% in feed	
154	PCR-RFLP	Sheep, pork, poultry	mtDNA*	0.125% in LSR 1–5% in industrial MBM	3/7
157	PCR-RFLP	Ruminant or nonruminant	Cyt b mtDNA	Not reported	
153	PCR	Bovine	mtDNA*	0.125% in feed	False-negative 1.25% (3/240) False-positive 0.83%
152	PCR	Bovine, ovine or porcine	mtDNA*	0.3% (bovine/ovine) 1% (porcine) in LSR	Amplified 5/7 samples with accuracy of 72.5% and 60% for 2 extraction methods, failed to amplify 2/7 samples
151	PCR	Bovine	mtDNA	0.125% bMBM in feed	
122	PCR	Bovine	mtDNA*	0.125% bMBM in feed	
102	Sandwich ELISA	Ruminant	Tn I-specific Mabs	5 ng/ml TnI or 0.05% in feed	
85	Indirect ELISA	All animal, mammalian and ruminant species	Tn I-specific Mabs	0.1% (all animal) 0.3% (mammalian) 2% (ruminant) in feed	
98	Sandwich ELISA	Ruminant and porcine	Rabbit antisera	1250 ppm proteins after gelatin removal	
97	Sandwich ELISA	Ruminant	Rabbit antisera	166 ppm proteins after gelatin removal	

* Primer design according to Tartaglia et al., 1998 (Ref. 122)

bMBM: bovine meat and bone meal
Cyt b: cytochrome b
LSR: laboratory scale rendered samples
Mabs: monoclonal antibodies
MBM: meat and bone meal
mtDNA: mitochondrial DNA
PCR: polymerase chain reaction
RFLP: restriction fragment length polymorphism
TnI: troponin I

such as the extraction method, pH, salt content and heating conditions of the sample and sample matrices, need to be carefully controlled, studied and considered in interpretation of results. For validation of an assay, the lab-formulated meat mixtures might not sufficiently represent the vast variety of commercial products. Therefore, conducting trials on a variety of commercially available meat and feed products is important for assessing the validity of a new assay.

Ideal analytical methods for meat species identification and detection of species adulteration need to be specific, sensitive, rapid, affordable, of high throughput, able to analyze heat-processed as well as raw products and provide quantitative measures. No single method can meet all these requirements for all types of samples at all times. Selection of an assay depends on many factors, such as the purpose of the assay, nature of the sample, sample size and numbers, specificity and sensitivity required, turnaround time, cost, availability of facilities and equipment, etc. Although many challenges have yet to be fully met by any laboratory or diagnostic service, most assays mentioned in this chapter may be used complementarily to each other. To safeguard consumers, the integrity of the food chain is non-negotiable. As the problems and trends of species adulteration change in the meat and/or feed industries, in order to ensure compliance with the corresponding regulations, our analytical skills will continue to face challenges in this area. New techniques emphasizing faster and more convenient assays, such as one-step assays, biosensors, microarray technology, and automated systems based on antibodies, DNA or both, will provide the weapons for the next generation's fight to protect consumers and safeguard our food supply.

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31 Poultry: Chemistry and Biochemistry

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CONTENTS

I. INTRODUCTION

The conversion of muscle into meat is a complex process that involves an understanding of muscle components as well as metabolic reactions. This chapter will focus on the chemical components of the live muscle and chemical and biochemical reactions leading to the conversion of muscle into meat.

II. COMPOSITION AND CHEMISTRY

A. Water

One of the most abundant and important components of meat is water. Poultry meat contains approximately 75% water by weight. Water has several functions within meat, one of which is a medium for transportation of nutrients, hormones, metabolites, and waste to and from cells. It also provides a medium for chemical reactions and metabolic processes. Three states of water exist in muscle: bound, immobilized, and free. Bound water is very tightly bound to proteins and can only be removed by ashing. Immobilized water has weaker interactions than the bound water and is associated with ions and proteins. This water, held by ionic interactions, may be released during cooking or heat application. Free water is loosely held in the cell, and is susceptible to loss known as drip or purge.

B. Carbohydrates

Skeletal muscle does not have an abundant supply of carbohydrates. Glycogen, the storage form of glucose, is the major carbohydrate found in muscle and can be up to 0.5 to 1.3% of the weight of the muscle (1). It is important in providing energy for metabolism, contraction, and relaxation. Other carbohydrates include intermediate substances and products of cell metabolism.

C. Lipids

Poultry muscle contains several types of fatty acids including saturated, unsaturated, acylglycerols or neutral lipids, and phospholipids. The neutral lipids are the most abundant. These fats are composed of fatty acids and a glycerol backbone. They consist of mono-, di-, and triglycerides, depending upon how many fatty acids are bound to the glycerol backbone. Phospholipids are mainly found in the cell membranes and contain both hydrophobic and hydrophilic sites. Lipids have many functions including providing energy for the muscle cell, metabolic functions, and cell membrane structure and function.

D. Proteins

Protein constitutes approximately 16% to 22% of the muscle, second in abundance only to water (1). Muscle contains several classes of proteins including myofibrillar, sarcoplasmic, and connective tissue proteins. The myofibrillar proteins are long, fibrous, charged, and are salt soluble in elevated ionic concentrations of 0.3 M or greater (<1.5% NaCl) (2). These myofibrillar proteins are responsible not only for contraction and relaxation, but also water holding capacity and protein functionality. The myofibrillar proteins can be further divided into three functional categories: contractile (myosin, actin), regulatory (tropomyosin, troponin) and cytoskeletal proteins (titin, connectin, C-protein, and desmin). Of these proteins, myosin and actin are considered to be the most abundant and the most functional, especially in food complexes.

Sarcoplasmic proteins are mainly globular and function as enzymes and cofactors involved in energy metabolism. Many of these proteins share common properties such as high isoelectric points, globular structures, and low molecular weights. With these properties, the sacroplasmic proteins are water soluble and can be extracted in low salt solutions with an ionic strength of less than 0.15 M.

Connective tissue proteins provide strength and support for the muscle in such ways as tendons and ligaments. Within the interstitial space of the muscle, the connective tissues consist of collagen, reticulin, and elastin along with other supporting proteins termed ground substance. Connective tissue proteins are very fibrous and insoluble in high ionic strength solutions.

E. Inorganic Substances

Most of the inorganic constituents consist of cations and anions used in muscle metabolism, contraction, and relaxation. Calcium is required for contraction to occur and is stored within the muscle cells. Other inorganic substances are potassium, sodium, magnesium, chloride, and iron.

III. SKELETAL MUSCLE

A. Ultrastructure

Skeletal muscle is a complex structure that is a major component of the body and has been reviewed by several authors (3–7). The muscle is surrounded by a thick connective tissue sheath known as the epimysium which is continuous with the tendon (Figure 31.1). The muscle is then divided into bundles of fibers surrounded by the perimysium. The connective tissue surrounding individual muscle fibers is the endomysium.

Avian skeletal muscle fibers are long, unbranched, narrow, and multinucleated cells (4–6). These fibers can vary in length from several millimeters to more than 30 cm and have a diameter of 10 μm to 100 μm. Surrounding the muscle fiber is the sarcolemma, an elastic plasma membrane composed mainly of proteins and lipids. The sarcolemma not only surrounds the contractile units of the muscle, but also regulates uptake and release of molecules by the cell. The most distinguishing feature of the sarcolemma is its ability to depolarize during a nerve impulse. Invaginations of the sarcolemma form a network of tubules known as the transverse tubule (t-tubule) system. When an action potential reaches the myoneural junction, the t-tubule system aids in the progression of this impulse longitudinally along the sarcolemma in both directions along the entire length of the fiber.

The sarcoplasmic reticulum (SR) is an intracellular membrane structure separate from the sarcolemma and t-tubule system. The SR is responsible for the release and sequestration of calcium involved in contraction (3). Small structures called “feet” bridge the gap between the t-tubules and the SR. When the muscle cell is stimulated, the action potential reaches the sarcoplasmic reticulum through the t-tubule system causing the release of stored Ca^{+2} for muscle contraction initiation (4).

The basic contractile unit of the muscle is the sarcomere which is mainly composed of the myofibrillar proteins myosin and actin (Figure 31.2). These proteins are arranged parallel to the muscle fiber and overlap in certain regions. This overlap accounts for the banding pattern of the myofibril. The protein-dense bands where actin and myosin overlap are referred to as the A bands because they are anisotropic in polarized light. The less dense area is known as the I band since it is isotropic in polarized light.

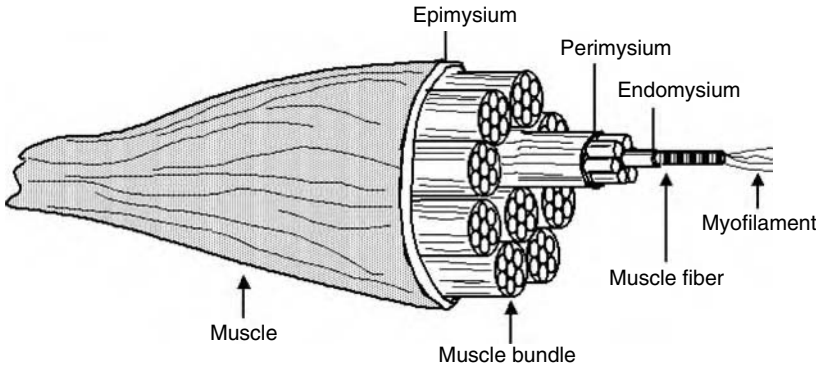

FIGURE 31.1 Diagram of gross muscle structure indicating muscle and connective tissue components. (Modified from Bechtel, 1986, and Gualt, 1992.)

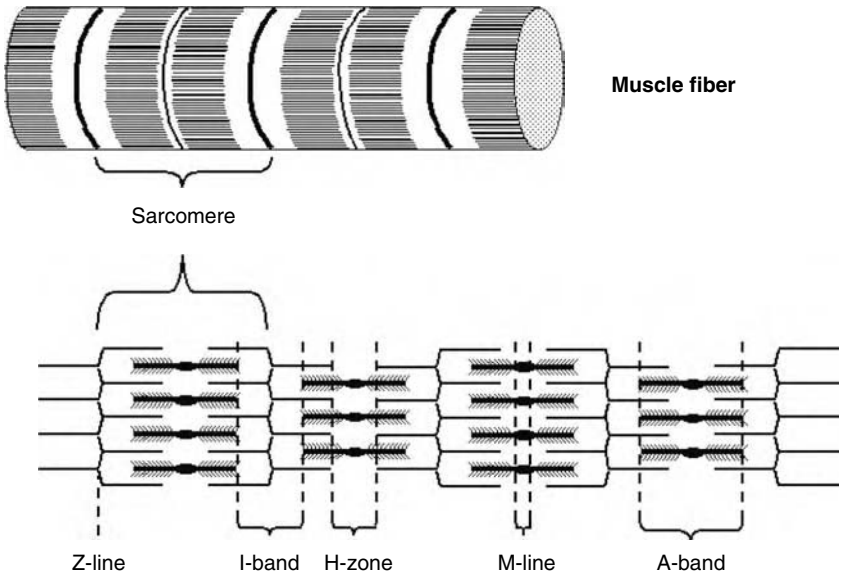

FIGURE 31.2 Diagram of the striated muscle fiber and the structure of the sarcomere.

B. Muscle Proteins

The muscle is made up of three major types of proteins: myofibrillar (or contractile), sarcoplasmic, and stromal (or connective). The myofibrillar proteins make up approximately 50–60% of total muscle protein and include proteins that are involved in the contraction-relaxation process (5, 6, 9). Sarcoplasmic proteins comprise approximately 30–35% of the muscle proteins and include the enzymes involved in muscle metabolism (5, 9). Connective tissue is made of stromal proteins and comprises approximately 3–6% of total muscle protein (9).

1. Thick Filament

Myosin is a globular and filamentous protein, which is the basis for the thick myofilament. Myosin makes up approximately 45–50% of total myofibrillar protein (6, 10). It is also one of most important proteins, along with actin, that is involved in muscle contraction. The protein consists of two globular heads attached to a long α-helical-like filamentous tail (Figure 31.3). Myosin is a large molecule of approximately 520 kD and is made up of six subunits. There are two heavy chains, approximately 220 kD, that make up the fibrous portion of the molecule, and two sets of light chains, approximately 17–22 kD, that are found in the globular regions of the molecules (5). Furthermore, the myosin molecule can be divided into two sections: heavy meromyosin (HMM) and light meromyosin (LMM). The globular heads, located in HMM, contain the adenosine triphosphate (ATP)-binding site and the actin-binding region, which are necessary for muscle contraction. The HMM section also contains a portion of the fibrous myosin. The LMM section, a portion of the fibrous myosin, is the area of the molecule that is packed into the thick filament. It is thought that the myosin molecule has two hinge points, one between

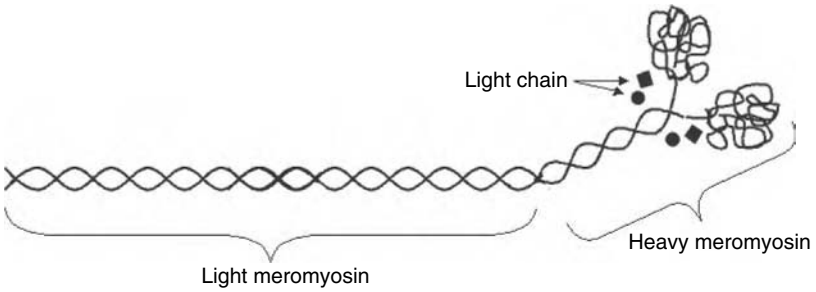

FIGURE 31.3 Diagram of myosin, a component of the thick filament. (Modified from Vander et al., 2001.)

HMM and LMM and the other between the fibrous myosin and globular myosin, so that it can function properly and effectively in the contraction-relaxation process. Approximately 200 myosin molecules aggregate along with other structural proteins to form the thick myofilament (7, 8). During aggregation, the myosin heads point toward the Z-lines and the tails toward the M-line. The thick filament is approximately 1.5 μm in length and 14–16 nm in diameter (4). The primary structural proteins are called C-proteins and M-proteins. The C-proteins are thought to hold the myosin filament together by wrapping around the myosin filament (11). There are seven bands of C-protein around the thick filament on each side of the H-zone (4, 5). The M-protein and myomesium are thought to adjoin the two myosin filaments at the tail region of myosin in the center of the sarcomere, or at the M-line (3). At the M-line, creatine kinase is also present, but its role in the structure of the thick filament is not known (3, 11). Creatine kinase is important to muscle metabolism. It is used in the formation of ATP from creatine, which is a short term energy supply for the muscle. Other proteins are also associated with the thick filament including F-protein, H-protein, and I-protein although their role in structural integrity of the thick filament is not known. It is thought that the I-protein inhibits myosin ATP-ase activity, an enzyme involved in ATP hydrolysis (11).

2. Thin Filament

The actin protein is made up of globular molecules, approximately 42 kD, that form a filamentous protein which is the basis for the thin filament (Figure 31.4). Actin comprises approximately 20% of the total myofibrillar proteins and is essential to the muscle contraction process (5). Approximately 200 globular molecules, G-actin, are bound together by ionic and hydrophobic bonds to form each filamentous actin, or F-actin. Two strands of F-actin along with two strands of filamentous tropomyosin and associated troponin (globular) are twisted together to form the thin (actin) filament. There are approximately 13 G-actin molecules per strand per twist of F-actin. The tropomyosin protein is made up of two α-helical polypeptide chains that twist around each other forming a filamentous rope-like structure. Tropomyosin, approximately 41 nm in length and 71 kD, lies near the groove of the F-actin filament (11). In conjuction with tropomyosin is the globular protein, troponin, which appears every seven G-actin molecules. Troponin is made up of three subunits: troponin I (21 kD), troponin T (37 kD), and troponin C (15 kD). Each subunit has a different function that is important in the contraction process. Troponin-T binds to tropomyosin, troponin-I inhibits actomyosin interactions by binding to actin, and troponin-C binds to calcium. These functions will be discussed later in this chapter. The thin filament, made of actin and the tropomyosin-troponin complex, is approximately 6–8 nm in diameter and extends approximately 1 μm from the Z-line toward the thick filament where a portion of the thin filament overlaps with the thick filament at the A band. The I band of the sarcomere consists of the thin filament alone.

In addition to actin, tropomyosin, and troponin, there are other proteins also present that are associated with the thin filament. γ-Actinin and β-actinin are present along the thin filament and at the end of the filament, respectively. It is thought that these proteins aid in regulating the length of the thin filament, thereby stabilizing the structure. Furthermore, β-actinin may inhibit interfilament intereactions thereby allowing it to smoothly slide during contraction and relaxation (5).

3. Z-Line and Gap Filaments

Each sarcomere is made up of thick filaments, thin filaments, and other proteins located near or at the Z-line and either as an integral part or on the periphery, the beginning and end of each sarcomere. Some of the Z-line proteins include desmin, vimentin, synemin, α-actinin, Eu-actinin, vinculin and zeugmatin (5, 10). Nebulin is also present near the Z-line in the N-line. Other myofibrillar proteins that are present include Z-protein and tensin, located in the integral, and filamin, plectin, spectrin, and ankyrin, located in the periphery; however, the role of these proteins in muscle is not fully understood.

Desmin is generally located around the periphery of the Z-line, running transversely, indicating that this protein acts

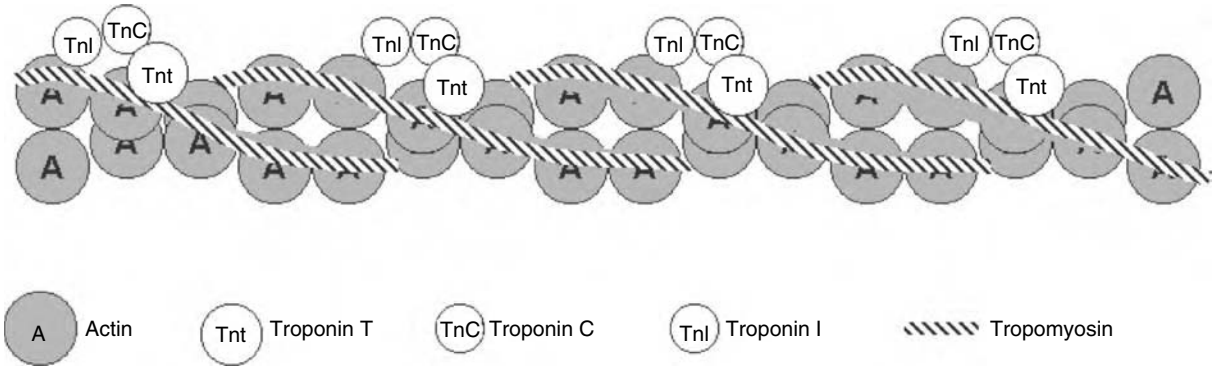

FIGURE 31.4 Diagram of thin filament. Actin, tropomyosin, and troponin make up the thin filament (Drawn by E. M. Hirschler; Modified from Cohen, 1975).

to tie the adjacent myofibrils together by forming a filamentous network. Furthermore, desmin may also link myofibrils to subcellular organelles, nuclei and mitochondria, and to the cellular membrane (10). Vimentin and synemin, like desmin, are also involved in forming a network around and between adjacent myofibrils. The role of these proteins is to provide structural integrity to not only the single myofibril, but also to a group of myofibrils, or muscle fiber. α-Actinin is a protein that ties actin into the Z-line, or Z-disk. This protein is an integral component of the Z-disk and is located in the interior. In addition to its role in structural integrity, α-actinin is thought to play a role in the formation of actin (i.e., direction of forming filament, length of filament). Although not well understood, zeugmatin and Eu-actinin are thought to link actin to the Z-disk (5). Vinculin is thought to attach the myofibrils to the sarcolemma; however, its function is not fully understood (5, 11). Nebulin was previously thought to be associated with the N-lines within the sarcomere and to control the geometric organization of the thin filament (12). However, more recent evidence suggests that nebulin is an elongated filamentous protein that runs parallel to the thin filament binding to both F-actin and α-actinin, thereby suggesting that nebulin aids in stabilizing the thin filament (10, 12, 13). Nebulin is also thought to serve as a template for the thin filament during formation. In addition to these proteins, there is another major protein present in the muscle that aids in structural integrity known as titin. Titin, a gap filament, is the third most abundant protein in the muscle, comprising approximately 8% of the myofibrillar proteins. Titin is a highly elastic filamentous protein that is located throughout the sarcomere, extending longitudinally from the Z-line to the M-line (10). Titin is thought to serve as a template for the formation of thick and thin filaments. It also serves to keep the thick filaments aligned in striated muscle (5, 10).

4. Stromal Proteins (Connective Tissue)

There are two types of connective tissue found in the body: connective tissue proper and supportive connective tissue. While these types differ in their function, they are very similar in composition. Connective tissue that covers the muscle, muscle bundle, and muscle fiber (epimysium, perimysium, and endomysium, respectively) is known as connective tissue proper (4). It is this type of connective tissue that can influence the tenderness of meat. In contrast, connective tissue in bones and cartilage provides structural support; therefore this type is known as supportive connective tissue. Connective tissue is made up of a non-structured ground substance which contains carbohydrates, proteins, and lipids (14). There is also an extracellular matrix which is fibrous in structure and made up of a class of proteins called stromal proteins (5). The stromal proteins consist primarily of collagen and elastin, and aggregation of these proteins make up the extracellular fibers that are embedded in the ground substance. Unlike any other aggregation of proteins, these have high tensile strength once a fiber is formed. Several authors have compiled a comprehensive review of connective tissue (5, 15, 16).

5. Collagen

Collagen makes up the majority of the stromal proteins and is considered the most abundant protein in the body, comprising up to one third of the total body protein (3, 14). It is not only found in muscle, but also in bone, skin, tendons, cartilage, and the vascular system (4, 14). Collagen has a unique structure. The collagen molecule is made up of three polypeptide chains that form into a triple helix known as *tropocollagen,* the structural unit of a collagen fibril (Figure 31.5). Globular domains also exist on the collagen molecule and are referred to as non-triple helical domains (15). These domains are important in the association of multiple collagen molecules. The polypeptide chains are made up of repeating amino acid sequences of Gly-X-Y, where X and

FIGURE 31.5 Diagram of tropocollagen, the collagen molecule made up of three polypeptide chains that form a triple helix.

Y can be any amino acid except tryptophan. Most often the X and Y are proline and hydroxyproline, respectively (5, 15). The amino acid glycine comprises one third of the amino acids found in collagen. Hydroxyproline and hydroxylysine are also present in high proportions within collagen, and are generally only found in collagen. There can be at least 19 variations of the polypeptide chains. Fifteen identified types of collagen can form from various combinations of the individual polypeptide chains that make up the tropocollagen molecules (16). Types I, III, IV, V, and VII are associated with connective tissue in skeletal muscle (4, 14). Type I collagen is located in the epimysium and perimysium, type III in the perimysium and endomysium, and types IV and V in the endomysium (16). It is thought that type III collagen plays a major role in meat tenderness when connective tissue is a factor (14).

Tropocollagen molecules, 300 nm in length, are bound together to form *collagen fibrils* with a striated appearance in types I, II, III, V, and XI. Fibrils are assembled by the tropocollagen molecules aligning adjacently end to end. The molecules align in a quarter-stagger parallel pattern with an end overlap of 25 nm, and are stabilized by ionic and hydrophobic interactions (5, 16). This quarter-stagger and overlapping pattern that repeats every 67 nm results in the striated appearance of the *collagen fiber* (Figure 31.6). The collagen fibers have a distinctive crimp (fold) that is observed once the fiber is formed; however, the crimp disappears when the muscle is under tension (Figure 31.7). It is thought that this *planar crimping* serves as a shock absorber to take up strain before tension is applied to the muscle (15, 16).

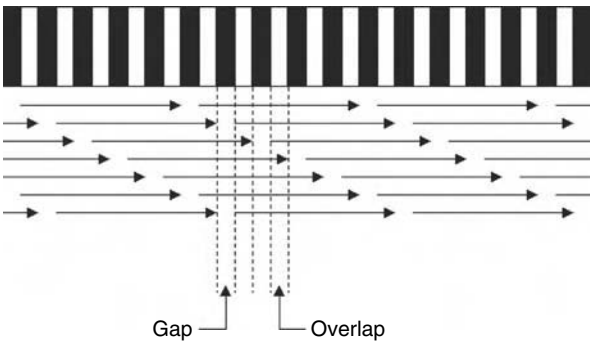

FIGURE 31.6 Diagram of collagen fibril where the light segments represent overlapping collagen molecules and the dark segments represent areas where a gap is present. (Modified from Sims and Bailey, 1992.)

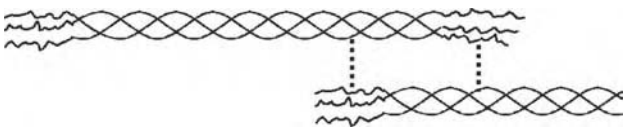

FIGURE 31.7 Diagram of intermolecular crosslinking between two collagen molecules. (Modified from Bailey and Light, 1989.)

The fibrous structure of collagen has high tensile strength due to its enzyme-induced intermolecular (between molecules) crosslinks (Figure 31.7). These crosslinks are covalent bonds that can occur in the carboxy- and amino-terminal ends of the tropocollagen, depending on the collagen type. Because type III collagen is the predominant collagen type that can play a role in meat quality, only the crosslinks of this type will be discussed. However, the crosslinking mechanism is similar in other collagen types.

Three types of crosslinks can occur in the formation of stable collagen fibers. First, disulfide bonds are formed in type III and IV collagen. These types of collagen contain the amino acid, cysteine, whereas the remaining types do not. The disulfide bonds are formed near the carboxy-terminal end of a tropocollogen molecule and can occur both intra- (within molecule) or intermolecularly between α-chains of tropocollagen. Secondly, lysine-derived reducible divalent crosslinks can be formed during fiber synthesis, both intra- and intermolecularly. The third type of crosslink that can occur in collagen is a trivalent crosslink that forms as the animal ages. This type of crosslink is usually referred to as *mature crosslinks*. Mature crosslinks are heat stable and are the cause of toughness due to connective tissue associated with mature animals. In the latter two types of crosslinks, the enzyme lysyl oxidase plays a major role in the crosslink formation.

Lysyl oxidase binds to α-chains in the tropocollagen molecule in either the amino- or carboxy-terminal end. Lysyl oxidase reacts with and converts the lysyl group into an aldehyde group. Two aldehyde groups can further react to form either an intramolecular crosslink, known as an aldol, or the aldehyde can react with hydroxylysine to form an aldimine or oxo-imine (or keto-imine) bond (15). The aldimine bond is heat labile while the oxo-imine is heat stable. Both of these bonds form intermolecularly at either the amino- or carboxy-terminal end of the tropocollagen molecule. As the animal ages, nonreducible trivalent bonds can form from these existing divalent bonds. There is much less information available on the mechanism of mature crosslink formation. However, it is known that the trivalent bonds increase the tensile strength of collagen. Furthermore, the mature crosslinks are heat stable. Therefore, upon cooking of meat from a mature animal, the crosslinks will not break and the network of connective tissue remains intact resulting in decreased tenderness of the meat.

The tropocollagen molecules found in type IV collagen do not form collagen fibrils, but form a more open network of tropocollagen that is hexagonal in shape, resembling chicken wire. The molecules found in type IV collagen are 400 nm in length and, due to the location of the glycine residues and the charge profile, the molecules are more flexible and form a thin open structure upon aggregation (16). Type IV collagen is often found in basement membranes. In skeletal muscle, it is one of the types of collagen found in the endomysium.

6. Elastin

In addition to collagen, elastin also makes up the stromal proteins of connective tissue. However, elastin is much less abundant than collagen and plays less of a role in meat quality except in instances where elastin content is high in certain muscles. Elastin is generally found in arterial walls and ligaments in the body. It is a rubbery protein that is insoluble in water or salt solutions, is very heat stable, and is resistant to digestive enzymes.

C. Contraction/Relaxation

Contraction and relaxation of the muscle are both active processes which have been reviewed by several authors (4–6, 17, 18). The act of contraction begins when an action potential received from a motor neuron at the motor end plate depolarizes the sarcolemma, allowing a rapid influx of Na^+ and efflux of K^+, and causing a membrane polarity reversal. When a threshold of -70 mV is reached, this action potential is propagated in both directions along the sarcolemma down the length of the entire muscle, finally reaching the sarcoplasmic reticulum through the t-tubule system. Once the action potential reaches the sarcoplasmic reticulum, the sequestered Ca^{+2} is released into the sarcoplasmic fluid. This release increases the free Ca^{+2} concentration from 10^{-7} moles/L normally present within the muscle to 10^{-6} or 10^{-5} moles/L (4). The free Ca^{+2} then binds to the regulatory protein troponin-C causing a change in configuration of the troponin-I subunit, which releases the inhibition of tropomyosin, exposing the actin-binding site to myosin. This release of tropomyosin allows myosin to bind to actin and form actomyosin crossbridges. The myosin ATPase is then activated and hydrolyzes ATP into ADP and inorganic phosphates, thereby releasing the energy required to tilt the myosin head (Figure 31.8). This action pulls the actin filament toward the center of the sarcomere, resulting in a shortened sarcomere (Z-lines are closer together). During the resulting contraction, actomyosin crossbridges are continually broken and formed to allow for sliding of the filaments. In cases of severe muscle contraction, the actin filaments may overlap. However, it is important to note that during contraction, the actual length of the myosin and actin filaments do not change.

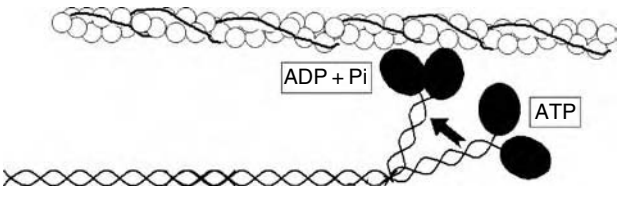

FIGURE 31.8 Diagram of myosin heads coupling with actin and ratcheting releasing ADP and Pi.

Relaxation is measured by a decrease in muscle tension (4). In order for relaxation to occur, the membranes must become repolarized, Ca^{+2} must be resequestered within the sarcoplasmic reticulum, and ATP must be regenerated through a series of reactions. Membrane repolarization is due to the Na^+/K^+ ATPase pump re-establishing a resting membrane potential of -90 mV through the active transport of Na^+ out of the cell and K^+ into the cell (17). The resequestration of Ca^{+2} is accomplished by activation of an ATPase, which aids in pumping Ca^{+2} back into the sarcoplasmic reticulum.

Both muscle contraction and relaxation require energy in the form of ATP for re-establishment of the electrochemical gradient through the Na^+/K^+ pump, for breaking and forming actomyosin bonds, and for pumping Ca^{+2} into the sarcoplasmic reticulum (4). Normally, muscle contains approximately 2 to 4 mM of ATP (18). Due to the fact that muscle cannot store a sufficient amount of ATP for the above conditions to occur, a rapid source and an efficient method of rephosphorylating ADP to ATP must be available.

D. Energy Metabolism

The synthesis of ATP required for these processes to occur has been reviewed by several authors (3, 4, 6). The most rapid source of energy is through phosphocreatine, a sarcoplasmic component, which, when combined with ADP, is converted to ATP and creatine by the catalytic enzyme creatine kinase. During extensive use of ATP within the muscle, creatine is depleted and must be regenerated at the mitochondrial membrane during rest.

$$\text{ADP} + \text{Phosphocreatine} \xrightarrow[\text{Creatine Kinase}]{} \text{ATP} + \text{Creatine}$$

Aerobic metabolism is the method that generates the most ATP (Figure 31.9). This series of reactions utilizes carbohydrates, proteins, and lipids, which are degraded to carbon dioxide and water while releasing energy as ATP. Glucose is broken down into pyruvate through glycolysis, netting three ATP molecules per molecule of glucose. In the presence of oxygen, pyruvate then enters the tricarboxylic acid cycle (TCA) in the mitochondria, releasing hydrogen ions. These ions then enter into the electron transport chain and through oxidative phosphorylation net 34 ATP molecules per molecule of glucose (4). Therefore a total of 37 ATP molecules are produced during aerobic metabolism.

Anaerobic glycolysis is a less efficient method of producing ATP, with three molecules of ATP produced per glucose molecule. In anaerobic metabolism, there is a lack of oxygen and pyruvate cannot enter into the TCA cycle. Instead, the combination of pyruvic acid and H^+, both by-products of glycolysis, form lactic acid. In a normal functioning homeostatic system, lactic acid is removed

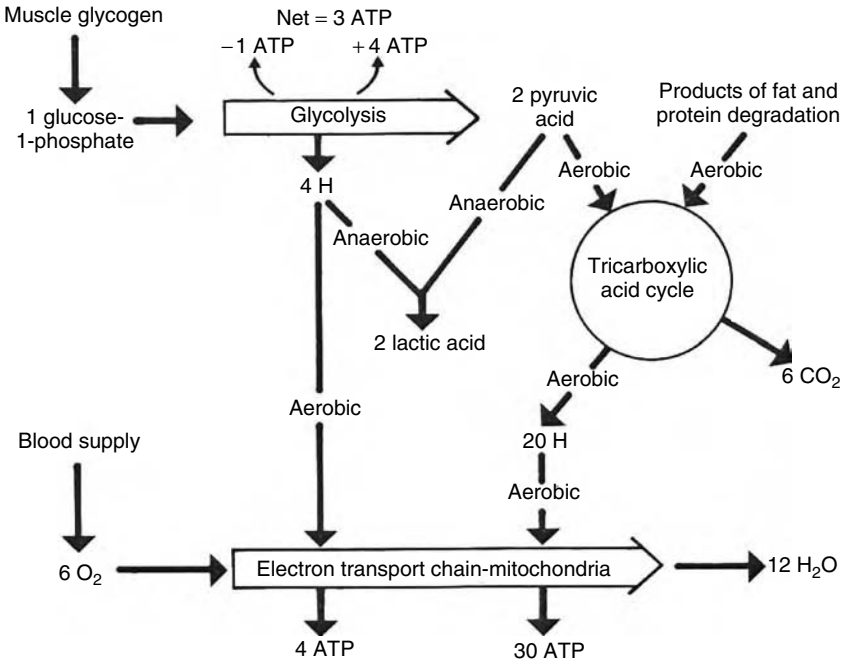

FIGURE 31.9 Pathways that supply energy for muscle function. One molecule of glucose 1 – phosphate is split from glycogen and is degraded to CO_2 and H_2O in the aerobic pathway (sarcoplasm). Then energy yield in terms of ATP is indicated at each step. When oxygen is limited, energy is supplied by glycolysis and conversion of pyruvic acid to lactic acid. (Courtesy of W. H. Freeman and Company.)

through the blood stream. However, during poultry processing, exsanguination, occurs, leading to a build-up of lactic acid in the muscle. This increase in lactic acid postmortem is important in the development of rigor mortis as will be discussed later.

E. Glycogen Catabolism

Homeostasis can be defined as the state of equilibrium in an organism. Homeostasis is maintained by the cyclic nature of providing energy to the muscle cells as well as removal of waste (Figure 31.10). Glucose is absorbed by the gastrointestinal tract into the blood stream where it is either absorbed by the liver for storage as glycogen or carried to the muscle cell for utilization or storage as glycogen. Glycogen is the major storage form of glucose in the muscle and can be 0.5 to 1.3% of the weight of the muscle (4).

In times of high muscle activity, liver glycogen can be hydrolyzed into glucose and transported via the blood for utilization by the muscle cell (Figure 31.10). The regulation of glycogen breakdown into glucose in the muscle cell is controlled through phosphorylase (19, 20). Phosphorylase is the enzyme controlling the conversion of glycogen into glucose-1-phosphate for ATP generation in glycolysis. In resting muscle, phosphorylase activity is low. However, in an active muscle, phosphorylase is activated through a process of phosphorylation to allow breakdown of glycogen for ATP production.

There are several different control mechanisms for phosphorylase, including external regulators, hormones and calcium concentration. Phosphorylase can be activated by an increased amount of ATP breakdown products (inorganic phosphate, AMP, and IMP) which are indicative of an active muscle. Hormones such as epinephrine and adrenaline can also activate this cascade. During stress, glycogen breakdown is required for generation of ATP for the "fight or flight" response.

Calcium concentrations can also stimulate glycogen catabolism. Calcium is required for muscle contraction. When the muscle receives a nervous impulse, Ca^{+2} is released from the sarcoplasmic reticulum increasing the cellular concentration to 10^{-6} M. This concentration is required not only for contraction via myofibrillar ATPase activity, but is also the same concentration required for activation of phosphorylase. Therefore, both nervous and hormonal changes aid in concert to increase the breakdown of glycogen to ATP for energy.

Once glycogen has been catabolized into glucose, the muscle cell utilizes glucose to form ATP. Glycolysis yields 3 ATP, pyruvate and two molecules of lactic acid. Pyruvate can then enter the TCA cycle in the mitochondria and yield CO_2 and H^+. The H^+ then enters into the mitochondrial electron transport chain yielding water, oxygen, 34 ATP, and heat.

Metabolic waste such as lactic acid, heat, CO_2, and water are removed from the cell by the circulatory system

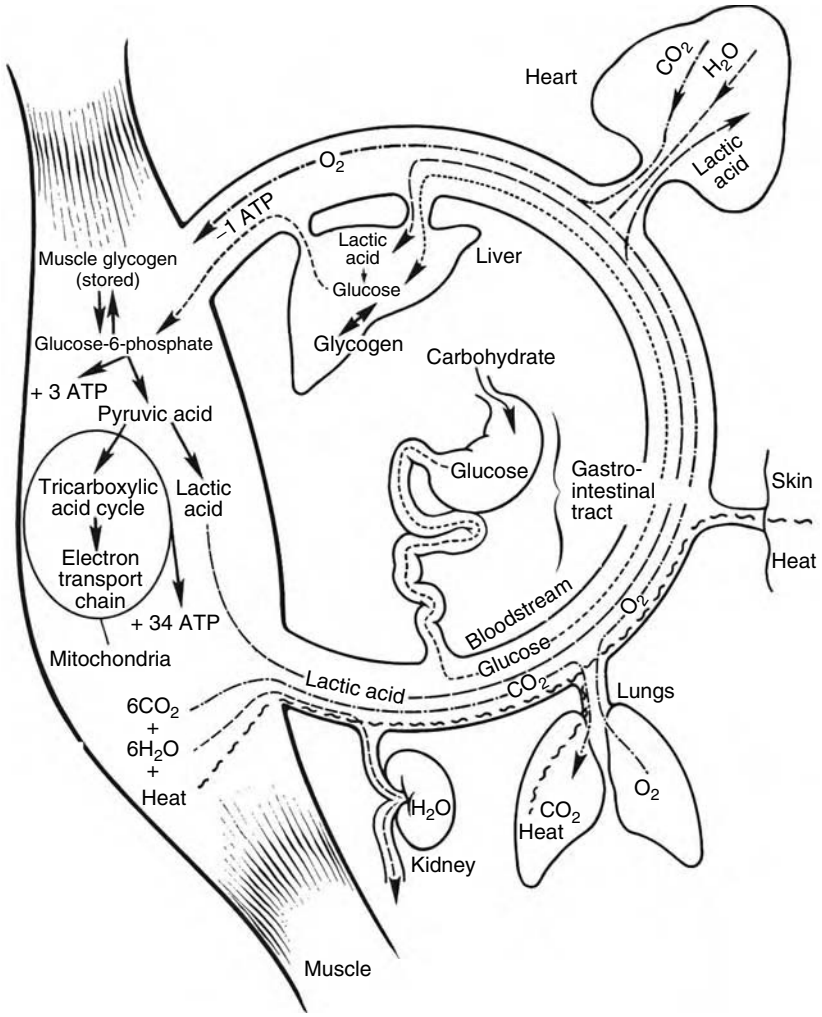

FIGURE 31.10 Cyclic pathways that provide energy for muscle contraction and relaxation. (Courtesy of W. H. Freeman and Company.)

and removed from the body via the lungs and kidneys. Lactic acid can be metabolized into CO_2 and water by the liver or it can be re-synthesized into glucose for storage. During times of increased activity, a build-up of lactic acid can occur and can cause acidosis. However, following a rest period, the lactic acid is removed via the blood and the muscle can return to its resting state.

F. Rigor Mortis

Rigor mortis or "stiffness of death" is the process of converting muscle into meat through a series of biochemical, physical, and structural changes. These changes begin with exanguination at which time there is a loss of both oxygen transportation to the muscle and a removal of metabolic waste such as lactic acid from the muscle. When stores of creatine phosphate have been depleted, glycogen stores are used for further phosphorylation of ADP. Aerobic metabolism continues until oxygen is depleted. Anaerobic metabolism is then utilized for the production of ATP until lactic acid accumulates and pH decreases to a level (approximately pH 5.9) that prevents glycolysis. However, ATP is still present within the muscle cell. Therefore, any stimulation to the muscle such as deboning prior to the completion of rigor mortis will increase actomyosin bond formation and sarcomere shortening, resulting in decreased tenderness. Due to this objectionable toughness associated with early deboning, poultry carcasses must be aged for 4–6 hours to allow for rigor mortis development (21, 22). This phase of oxygen depletion and muscle extensibility is referred to as the onset of rigor mortis (Figure 31.11).

Reduced extensibility begins the onset phase and continues until the muscle is completely inextensible and rigor mortis is complete. During the completion phase, 90% or more available actomyosin bonds are formed compared with only 20% in the living muscle (4). An ATP concentration of 1 μM/g is required for muscle function to occur. When the concentration of ATP decreases to below 1 μM/g, the muscle is no longer extensible (Figure 31.12).

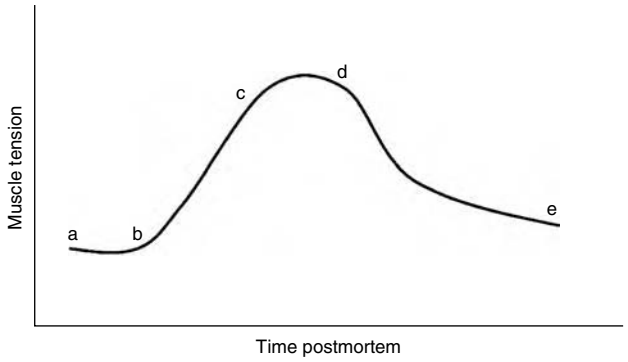

FIGURE 31.11 Phases of rigor mortis development. a–b delay phase; b–c development of rigor; c–d completion phase; d–e resolution phase. (Reprinted with permission from S. Barbut, Poultry Processing Products, An Industry Guide. Boca Raton, FL: CRC Press, 2002, p. 58.)

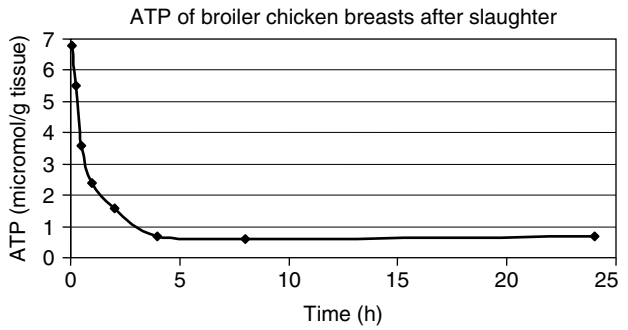

FIGURE 31.12 ATP concentration in broiler muscle during rigor mortis development.

The final stage of rigor, resolution, is characterized by a decrease in structural integrity of the muscle during postmortem storage. This decrease in structural integrity is due to proteolytic degradation of certain myofibrillar Z disk proteins such as desmin, titin, and nebulin by proteases such as calpain. Calpains improve meat tenderness by degrading Z disk proteins, thus increasing myofibrillar fragmentation. Also, ionic interactions may affect the postmortem tenderization of meat. Ionic strength increases in postmortem muscle due to the release of Ca^{+2} from the sarcoplasmic reticulum and the accumulation of phosphate from ATP degradation. This increased ionic strength decreases the sphere of charge surrounding each protein and weakens the structural integrity of the myofibrils, resulting in decreased toughness (23).

G. Metabolic Differences between Red and White Fibers

Muscle fiber types differ among species and also among muscles within a single animal due to genetic expression, function, and stage of growth. The four basic fiber types include: slow-twitch oxidative (type I, red), fast-twitch oxidative and glycolytic (type IIA, red), fast-twitch glycolytic (type IIB, white), and intermediate (type IIC). Broiler pectoralis muscles, for example, contain 100% white fibers, while Pekin duck pectoralis contain around 84% red fibers (24–26). Intermediate muscles contain a mixture of both red and white fibers.

Red fibers contain more myoglobin, are more vascularized, and are able to utilize oxidative metabolism as a source of energy, while white fibers mainly utilize glycolytic enzymes (Table 31.1) (5, 27). As a result of the increased oxidative metabolism in the red fibers, they contain not only more but larger mitochondria. When comparing red muscles of the broiler leg to white muscles of the broiler breast during rigor development, the white muscles have an increased accumulation of lactic acid due to their ability to use stored muscle glycogen in the glycolytic pathway for energy (Figure 31.13). Without oxygen, the red fibers cannot utilize the oxidative pathways and energy replenishment decreases.

White fibers are able to contract more rapidly and in short bursts since they mainly utilize glycogen rather than fat stores and have a more developed sarcoplasmic reticulum and t-tubule system (6). White fibers are also more easily fatigued compared to red fibers, which contract slower and for a longer duration without fatigue (27).

Red fibers develop rigor mortis at a faster rate compared to white fibers. No biochemical changes occurred after 2 hours postmortem in red muscles; however, the white muscle fibers display metabolic changes up to 8 hours postmortem. The red muscle fibers of the duck pectoralis have a faster rate of pH decline than the white muscle fibers of the broiler pectoralis, indicating a more rapid onset of rigor mortis (24). Red fibers are generally smaller in diameter when compared to white fibers, and have

TABLE 31.1
Relative Comparisons of Red and White Fibers in Poultry Muscle

Parameter	White	Red
Myoglobin	Low	High
Color	White	Red
Contraction Speed	Fast	Slow
Fatigue Resistance	Low	High
Contractile Action	Phasic	Tonic
Capillary Density	Low	High
Energy Source	Glycolytic	Oxidative
Mitochondrial Size	Small	Large
Mitochondria Number	Low	High
SR and T-tubules	More Developed	Less Developed
Fiber Diameter	Larger	Smaller
Connective Tissue	More	Less
Rigor Development	Slow	Fast
Glycogen Content	High	Low
Lipid Content	Low	High

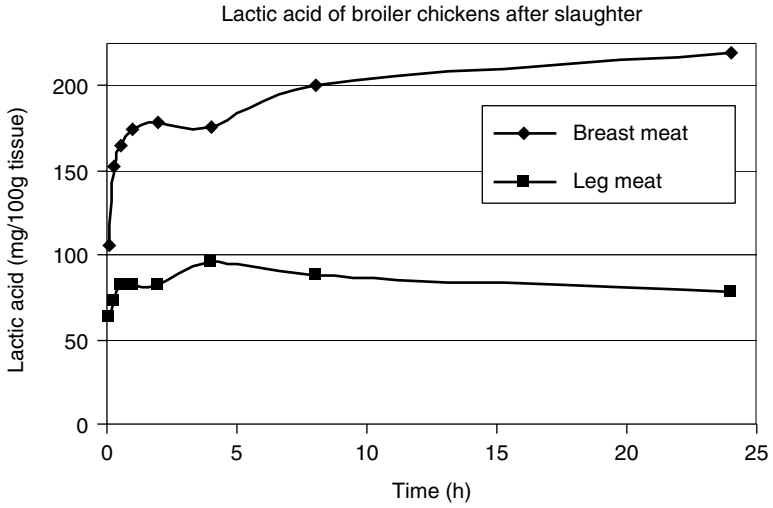

FIGURE 31.13 Comparison of lactic acid accumulation in red- and white-fibered muscles in broilers during rigor development.

proportionally more connective tissue surrounding each fiber as compared to white fibers (5). Thus, tenderness decreases with an increase in red fiber content within specific muscle groups.

IV. MEAT QUALITY CHARACTERISTICS

The inability of the muscle cells to rid themselves of metabolic by-products such as lactic acid causes several postmortem metabolic and structural changes within the muscle, the most important of which is a decrease in pH (28). This decrease in pH is the most significant postmortem change and can affect important meat quality attributes such as color, water holding capacity, and texture (29, 30). These three attributes are used to evaluate meat quality by both processors and consumers. Two conditions caused by the rate of pH decline include dark, firm, and dry (DFD) and pale, soft, and exudative (PSE) meat.

Dark, firm, and dry meat develops when muscle glycogen is depleted prior to slaughter, resulting in high muscle pH from reduced postmortem glycolysis (4, 6, 31). Dark, firm, and dry meat is characterized as having a high water holding capacity, even though the meat appears dry (5).

Pale, soft, and exudative meat is characterized as having a pale color, soft texture when cooked, and a low water holding capacity. This condition develops due to an accelerated postmortem metabolism which leads to a rapid decline in postmortem muscle pH (4–6). Rapid postmortem glycolysis has been observed in both swine and turkeys in which pH of PSE muscles was less than 5.8 within 45 min postmortem in swine and 15 min postmortem in turkeys. This would be compared to normal muscle pH at this postmortem time of greater than 6.0 (32, 33). This rapid decline in pH occurring while muscle temperatures are still elevated denatures proteins and causes a decrease in solubility and enzymatic activity (34, 35). Porcine muscles with pH values below 6.0 at 45 min postmortem had a higher ATPase activity, indicating a more rapid glycolytic rate, and were found to produce poor meat quality (36). Authors have reported that rabbit muscle kept at 37°C for 6 hours postmortem resulted in a rapid pH decline to below 5.8 and a loss of half Mg-ATPase activity compared to muscles incubated at 34°C (37).

A. Color

Color is an important quality attribute as consumers are often willing to pay more for poultry products based on color. Several factors can influence meat color including pH, myoglobin concentration, and nitrites (4). Even though myoglobin has been shown to be important in determining raw meat color, it is less important in broiler and turkey breast meat meat simply because there is less myoglobin than in other meats (beef, pork).

Pinking is an important problem in turkey and broiler meat. Pinking can result from several factors such as undercooking meat, contamination with nitrites, addition of ingredients such as peppers, improper stunning, and several antemortem stressors. There are several problems that can arise from pinking such as food safety issues with undercooked meat, consumer complaints, and a decreased purchasing of poultry products by consumers. Probably one of the main reasons for pinking in white poultry meat is the addition of nitrite. Nitrite is an ingredient that is normally added to cured products and produces a characteristic pink cured color upon heating. Normally, nitrite is not added to products made with white meat but can inadvertently be added through contamination from water sources or human error.

In cooked products, several pigments have been linked to pinking in poultry white meat. Myoglobin facilitates the

transfer of oxygen and waste to and from muscles from the blood. Denaturation of myoglobin is related to pH, time, and cooking temperature. Undenatured myoglobin due to improper heating of high pH that prevent denaturation at adequate cooking temperatures can reault in pinking of white meat. This is especially true in turkey breast meat, which has a higher concentration of myoglobin than broiler white meat. Cytochrome c is another important pigment in meat which can lead to undesired pinking since it has a higher denatutaion point that myoglobin. Cytochrome c is a heme protein responsible for transport of electrons in the mitochondria. Levels of this protein have been elevated in stressed animals.

B. Water Holding Capacity

The state of water in striated muscle has been reviewed by several people (3, 4, 38). Striated muscle contains approximately 75% water, which exists as three forms in the muscle: bound, immobilized and free. Since water is a polar compound, it can bind with other water molecules as well as charged protein groups. Bound or constitutional water is less than 1% of muscle water and is located within the protein molecules. This water has such a strong protein-water interaction that it cannot be lost except during ashing of the meat. The second form of water is immobilized or interfacial water and is approximately 10–15% of water in meat. Immobilized water is attracted to the bound water layer, creating multilayers of water each more loosely bound as the distance from the bound water increases. Due to the strong water-water and water-protein interactions, immobilized water is typically lost with cooking. Free water accounts for the remaining portion of water in meat tissues and is associated with the extracellular space. Free water is held loosely through capillary forces and can be lost easily through mechanical actions such as cutting, grinding, cooking, and storage of meat.

Water binding capacity of meat is altered in two ways: by the net charge effect and the steric effect. As a polar molecule, water can bind to other charged amino acid side groups. The amount and type of charge on a protein changes with pH. As pH decreases, the number of reactive groups available for water binding decreases. The point at which water binding is minimal on a protein is referred to as the isoelectric point or pI. Since actin and myosin are the most predominant proteins in the muscle and since they are most responsible for water holding capacity, their pI is the pH at which the meat's water holding capacity is the least. The pI of muscle is approximately 5.1 where the number of positive charges equals the number of negative charges resulting in a net zero charge or the pH at which the meat's water holding capacity is minimal (17).

The steric effect or degree of contraction has the greatest effect on the water holding capacity of meat. As the amount of space between the muscle protein structures decreases with contraction, less space is available for water around the proteins. Contractile state and pH of the muscle can influence the amount of interstitial space. If the muscle is in a contracted state, there is less space within the muscle to hold water intracellularly due to the shortening of the sarcomeres. Therefore, water is expelled into the extracellular space.

Muscle pH can also affect the amount of space to bind water molecules. If repulsion between charged groups is increased, as occurs in higher pH muscle, the protein network is enlarged and allows an increased amount of water holding capacity. In PSE meat, when the pH is closer to the pI of the myofibrillar proteins, attraction between charged groups increases and the protein network shrinks. Therefore, part of the immobilized water becomes free water, which may be lost as drip (39).

Myofibrillar proteins such as myosin, the predominant muscle protein, have a large amount of water holding capacity. Any changes to this protein can decrease the ability of the muscle to retain water. Denaturation of myosin has been found to decrease water holding capacity (35, 40). In PSE muscle the myosin head shrinks from 19 nm in normal muscle to 17 nm (40). This shrinkage of the protein lattice causes expulsion of intracellular water into the extracellular space and decreases water holding capacity. The extent of myosin shrinkage is also dependent upon the rate of pH decline, ultimate pH, and temperature during chilling with the faster rate of decline and higher temperatures early postmortem, leading to increased denaturation and shrinkage.

C. Texture

Protein gels are formed by intermolecular crosslinks resulting in an ordered three-dimensional network of proteins. The two primary steps involved in thermal gelation are denaturation of native myosin and aggregation of the partially unfolded myosin molecules to form the protein gel. Myosin denaturation can decrease the strength of heat-induced gelation. There are two transition temperatures (T_m) of myosin that are important in gel formation. Chicken breast myosin Tm_1 at 47.5°C is associated with the aggregation of the globular heads through disulfide bond formation while Tm_2 at 54°C is more associated with the network formation by the unfolding of the helical tail portion (41). However, turkey breast myosin is more temperature sensitive with the Tm_1 in turkey breast being only 40°C at pH 6–12 (42). These researchers also found that there was full reversibility of turkey breast myosin denaturation when heated for 5–30 min at 40°C and 5 min at 50°C followed by incubation at 4°C for 24 hours.

Gel strength is negatively correlated with cook loss and meat color, with higher cook loss and paler meat resulting in decreased gel strength. This negative correlation is probably due to the decreased solubility of myosin in PSE meat as well as fewer charges available for water-

water and protein-protein interactions. Extraction of salt soluble proteins from PSE meat is decreased compared to that of normal meat and gel strength is significantly less from product made with PSE meat. It has been postulated that the precipitation of phosphorylase onto the myofibrils at the Z line and A band results in a decreased solubility of myosin (43).

V. CONCLUSION

Muscle is a complex and highly specialized tissue that is able to convert chemical energy into mechanical energy for locomotion. The biochemical reactions that allow the muscle to complete these highly specialized tasks are the same reactions which cause the conversion of muscle into meat. The loss of the circulatory system is responsible for the anoxic conditions and allows the build-up of lactic acid which begins the process of rigor development. Meat quality characteristics can be affected by the rate of rigor mortis development. Once rigor mortis is complete, the muscle has been converted into meat.

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32 Chemical Composition of Poultry Meat

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CONTENTS

Poultry, especially chicken broilers and turkey, is one of the most widely consumed muscle foods in the world. It is an important source of dietary energy and nutrients, such as high quality proteins, essential fatty acids, vitamins and highly bio-available minerals [1]. In south-east and east Asia, as well as in eastern and western Europe, ducks and geese also play an important role because of their tasty meat. The world consumption of poultry meat in the year 2001 exceeded 69 949 thousand tons, ranked between production of beef and veal (56 647 thousand tons) and pig meat (91 188 thousand tons) [2]. The increase in muscle proportion and the reduction in carcass fat content by selection and careful restriction are major attempts to improve carcass quality of poultry and the nutritional value of meat [3–7]. However, concern has been raised over its high consumption and the fat content, in particular saturated fatty acids, which can greatly increase the risk of cardiovascular diseases and some type of cancer [8,9].

The chemical properties of poultry meat (or muscle) have been studied intensely. These studies were primarily carried out on whole poultry carcasses and/or specific muscles and included the examination of variables such as breed (genetics), diet formulations, age, gender, housing (live bird production) and general management practices (short-term antemortem stress) on composition, nutritive value, and quality of meat [10–13]. The present review will focus on the impact of muscle type on the nutritional value of poultry meat.

I. CHEMICAL COMPOSITION

Information about body composition can be important for those producing and selling animal products and nutritionists. Producers need information about body composition to produce leaner meat animals, sellers need information to determine how much of the energy that an animal eats is captured as growth or other forms of production [10].

The chemical composition of poultry breast (B) and thigh/leg (T/L) muscles is presented in Tables 32.1 through 32.3. The richest in protein were breast muscles of turkeys, broilers, geese, and ducks. The T/L muscles of

TABLE 32.1
Approximate Chemical Composition Profile for Breast (B) and Thigh/Leg (T/L) Poultry Muscles

Poultry Type	Moisture (%)		Protein (%)		Total Lipid (%)		Ash (%)		Reference
Muscle/Meat	B	T/L	B	T/L	B	T/L	B	T/L	
Chicken									
Broiler (6 wk) M	74.36	73.21	22.80	19.14	1.58	6.65	1.26	1.05	[11,12,14–18]
	(0.52)	(0.41)	(0.92)	(1.05)	(0.61)	(1.23)	(0.27)	(0.09)	
Broiler (8 wk)	75.37	74.66	22.39	19.00	1.48	5.33	0.61	0.51	[13*,19]
	(0.24)	(1.92)	(0.01)	(0.01)	(0.03)	(1.23)	(0.00)	(0.00)	
Hens (64 wk)**	73.21	74.04	–	–	1.51	4.49	1.08	1.06	[20]
Turkey (22 wk) M	72.74	72.24	23.36	19.54	1.63	4.84	1.18	1.09	[21–23]
	(2.10)	(1.03)	(1.37)	(0.08)	(0.35)	(1.09)	(0.02)	–	
Turkey (15 wk) F	73.51	72.91	23.29	19.52	1.25	6.28	1.17	1.06	[21,22,24]
	(0.56)	(24.0)	(1.14)	(0.31)	(0.59)	(0.80)	(0.05)	(0.02)	
Duck									
Pekin (7 wk) M	76.82	75.80	21.20	20.90	1.31	2.00	0.99	0.80	[25–27]
	(1.32)	(1.41)	(1.57)	(1.65)	(0.82)	(0.62)	(0.13)	(0.00)	
Pekin (8 wk) M	77.17	76.85	20.87	19.35	1.60	3.45	–	–	[12,26,28,29]
	(1.53)	(0.49)	(0.40)	(0.35)	(0.87)	(2.47)	–	–	
Muscovy (12 wk) M	76.43	76.06	20.89	20.31	1.43	3.43	1.07	–	[29–33]
	(0.39)	(0.48)	(0.67)	(0.27)	(0.47)	(0.61)	(0.08)	–	
Mule (12 wk)	74.55	74.50	21.78	21.40	2.41	2.70	1.15	1.20	[4,34,35]
	(0.67)	(0.00)	(1.29)	(0.00)	(0.38)	(0.00)	(0.07)	(0.00)	
Geese									
White Italian (16 wk) M	71.07		22.26		4.84		1.50		[36,37]
	(0.96)		(0.65)		(0.48)		(0.16)		
White Italian (17 wk)	72.36	71.55	22.48	20.38	3.11	6.51	1.18	1.00	[38,39]
	(0.08)	(2.81)	(0.62)	(0.49)	(0.18)	(3.27)	(0.11)	(0.03)	

M = male, F = female, * = drumstick, ** = white, dark muscles.

all kinds of poultry contained approximately 0.4–3.8% less protein (Table 32.1). Some authors found higher differences in protein content between breast and thigh muscles of broilers (23.80 vs. 18.30% [16]), 22 wk male turkeys (24.51 vs. 19.60% [22]), 16 wk female turkeys (25.24 vs. 21.29% [43] or 23.96 vs. 18.92% [45]) (Table 32.3), and 14 wk geese (23.02 and 19.39% [46]).

The fat content of breast muscles, in ascending order, was turkey females, Pekin and Muscovy ducks, broilers, hens, turkey males, Mule ducks, and geese; for T/L muscle, it was Pekin, mule, and Muscovy ducks, hens, turkey (Table 32.1). The fat content for the breast muscles from all poultry types was lower than for the L/T muscles.

Moisture content for Pekin and Muscovy ducks was greater than for other kinds of poultry. Moisture was comparable for turkey and geese and it was lower than in broilers, hens, and Mule ducks. Broilers, Pekin ducks, and goose breast muscles had higher percentage of moisture than the T/L muscles. The higher content of moisture in broiler breast muscles than in thigh muscle (73.74 vs. 73.22%) was also found by Gornowicz and Dziadek [15] but it was not significantly different (Table 32.3). However, Biliński et al. [38] found about 1% lower moisture content in 17 wk goose breast muscle compared to thigh muscle (72.31 vs. 73.54%).

The content of ash in breast muscle of chickens, Pekin ducks, and geese was higher than in thigh muscles while it was comparable both brest & thigh muscles for hens, turkeys and Mule ducks.

A. Chemical Composition—Age, Sex, Strain

The influence of age and sex on chemical composition of poultry meat is presented in Tables 32.2 and 32.3. In earlier studies as the broiler increased in age from 6 to 8 wk protein content in light male and female meat increased by 0.52 and 0.80% and fat in male white and dark meat increased by 1.32 and 1.66% [47]. The protein content increased with age to the detriment of water content in 12 vs. 14 wk female turkey breast and thigh muscles (23.60 vs. 24.80% and 20.70 vs. 22.40% [41]) and Pekin and Mule duck breast muscles [4,26] (Table 32.2). In 16–18 wk female turkey muscles the protein (breast) and moisture (breast and thigh) contents were comparable, while in thigh muscles protein content decreased after 16 wk [41]. During the growth from 2 to 5 wk Pekin duck breast muscles decreased in protein content from 21.5 to 20.0% and then after 7 wk increased to 21.9% while the leg muscle protein content was not influenced by age [25]. With advancing age increased fat content was observed in turkey breast muscle [40]; female turkey

TABLE 32.2
Influence of Age on Approximate Chemical Composition Profile for Poultry Breast (B) and Thigh/Leg (T/L) Muscles

Poultry Type	Moisture (%)		Protein (%)		Total Lipid (%)		Ash (%)		Reference
Muscle/Meat	B	T/L	B	T/L	B	T/L	B	T/L	
Turkey (16 wk)	73.74		23.98		0.18		1.04		[40]
Turkey (20 wk)	73.52		23.85		0.47		1.00		[40]
Turkey (12 wk) F	74.70	75.20/	23.60	20.70/	1.50	3.70/			[41]
Turkey (16 wk) F	73.60	72.80/	24.30	20.10/	1.90	6.30/			[41]
Turkey (18 wk) F	73.50	72.90/	24.10	20.60	1.90	5.50/			[41]
Duck									
Pekin (7 wk)	77.50	76.80/	19.40	19.00/	0.40	1.50/			[26]
Pekin (8 wk)	76.00	76.50/	21.30	19.60/	0.60	1.70/			[26]
Pekin (9 wk)				21.30	20.20/	1.50	3.20		[6]
Pekin (12 wk)				22.00	19.60	2.10	3.20		[6]
Muscovy (10.5 wk)			19.90	20.30/	1.40	1.90/			[6]
Muscovy (15 wk)			21.00	20.50/	1.30	1.70/			[6]
Mule (8 wk)			19.20	20.40/	1.00	1.60/			[6]
Mule (14 wk)			22.00	20.40/	1.40	1.80/			[6]
Mule (10 wk) M	75.60		21.84		2.09		1.10		[4]
Mule (12 wk) M	74.10		22.53		2.83		1.20		[4]
Geese (10 wk)	75.14	75.78/	20.77	19.27/	2.61	3.45/	0.99	0.79/	[38]
Geese (17 wk)	72.31	73.54/	22.04	20.73/	3.24	4.20/	1.10	0.98/	[38]
Geese (33 wk)	72.13	73.32/	20.92	19.57/	4.12	5.73/	1.19	1.09/	[38]
Geese (15 wk)*	71.69	/70.73	21.53	/20.39	5.87	/8.61	1.18	/1.01	[42]
Geese (24 wk)**	71.56	/71.35	21.67	/20.34	5.17	/7.12	1.06	/1.06	[42]

M = male, F = Female, * = intensive, ** = semi-intensive system.

thigh muscle [41]; Pekin breast [6]; Pekin thigh muscles [26]; and Mule duck breast and thigh muscles [4,6]. However, less fat content was found in 15 vs. 10.5 wk Muscovy ducks [6] and 24 vs. 15 wk geese [42]. According to Bieliński et al. [38] the protein and fat content in goose breast and thigh muscles increased with age from 10 to 17 wk, remained comparable between 17 and 25 wk, and after 33 wk protein content decreased and fat content increased, respectively. Moreover, in muscles of 10 wk geese the moisture content was the highest and the ash content was the lowest and they did not change with age (Table 32.2). Friend et al. [48] found no effect of age on geese breast muscles protein and fat contents.

The differences in chemical composition between males (M) and females (F) were found for broilers, turkeys, and ducks (Table 32.3). Evans et al. [47] found that fat content was higher in male broiler light (white, breast) meat than in light females (16.18 vs. 14.66%) and the protein content of dark meat of males compared with females was higher (18.32 vs. 17.60%) but the moisture (64.37 vs. 66.62%) and fat (11.85 vs. 13.99%) contents were lower. Gornowicz and Dziadek [15] found only a higher content of moisture and ash in broiler male muscles compared to female muscles (Table 32.3). The breast muscles of 22/24 wk male turkeys contained less moisture [22,23,45] and more fat than those of 15/16 wk females [21–23,43,45]. However, Faruga et al. [43] noticed higher amounts of moisture in 24 wk turkey males both in breast and thigh muscles than in 16 wk females. Lower moisture content and higher fat content were also observed in male Mule duck breast compared with females [4]. The reverse trend, i.e., higher moisture and lower lipid content, was found for male and female breast muscles of Pekin ducks [28]. Muscovy duck [28] and goose [36,44] male breast muscles were characterized by higher fat content than females. The thigh muscles of turkey and male Pekin duck had lower lipid levels than female thigh muscle [22,23,27,43,45] (Table 32.3). At the same age (14 wk) male turkeys deposited in breast and thigh muscles less total lipid content than females, i.e., 0.96 vs. 1.32% and 2.26 vs. 2.88%, respectively [49]; this was in line with lower fat content in male turkey thigh muscles than female at different ages (22/24 vs. 15/16 wk). Male Turkeys had higher ash content in the breast muscles compared to females (1.11 vs. 0.93% [40]).

Other authors found no differences in moisture, fat, and protein contents based on sex for broiler muscles [15] and Pekin duck [25] and goose [48] breast and/or leg muscle protein and fat; broiler breast [14], turkey breast [40] and Muscovy duck [29] and 12/16 wk goose [50]

TABLE 32.3
Influence of Sex on Approximate Chemical Composition Profile for Poultry Breast (B) and Thigh/Leg (T/L) Muscles

Poultry Type	Moisture (%)		Protein (%)		Total Lipid (%)		Ash (%)		
Muscle/Meat	B	T/L	B	T/L	B	T/L	B	T/L	Reference
Broiler (6 wk) M	73.74	73.22/	21.49	18.34/	0.88	6.73/	1.79	1.15/	[15]
Broiler (6 wk) F	70.44	70.17/	21.67	18.15/	0.71	6.67/	1.35	0.91	[15]
Turkey (22wk) M	73.95		21.85		1.57		1.20		[21]
Turkey (15 wk) F	74.13		22.43		0.88		1.22		[21]
Turkey (24 wk) M	72.35	72.51/	24.64	22.31/	2.09	4.39/	1.12	1.09/	[43]
Turkey (16 wk) F	70.24	68.65/	25.24	21.29/	1.17	7.42/	1.12	1.06/	[43]
Turkey (22 wk) M	70.32	72.97/	24.51	19.60/	1.32	4.07/	1.17	1.09/	[22]
Turkey (15 wk) F	73.04	72.74/	24.58	19.74/	0.93	5.72/	1.13	1.08/	[22]
Turkey (22 wk) M	73.96	71.52/	23.73	19.49/	2.01	5.61/			[23]
Turkey (16 wk) F	74.60	71.61/	23.96	18.92/	0.76	8.61			[23]
Turkey M	73.70		23.81		0.31		1.11		[40]
Turkey F	73.56		24.02		0.34		0.93		[40]
Duck									
Pekin (7 wk) M	75.30	74.80/	22.30	21.70/	1.50	2.70/	0.90	0.80/	[27]
Pekin (7 wk) F	75.70	75.10/	21.00	20.50/	2.10	3.40/	1.10	1.00/	[27]
Pekin (8 wk) M	76.60		20.80		2.10				[28]
Pekin (8 wk) F	74.00		20.50		2.60				[28]
Muscovy (12 wk) M	76.13	/75.72	21.32	/20.12	2.09	/3.86			[29]
Muscovy (10 wk) F	76.63	/75.62	21.20	/19.95	1.90	/3.96			[29]
Muscovy (11 wk) M	76.50		20.20		2.50				[28]
Muscovy (9 wk) F	76.30		19.60		1.60				[28]
Mule (10 wk) M	75.60		21.84		2.09		1.10		[4]
Mule (10 wk) F	76.35		21.66		1.69		1.11		[4]
Mule (12 wk) M	74.10		22.53		2.83		1.20		[4]
Mule (12 wk) F	75.02		22.27		2.26		1.24		[4]
Geese (16 wk) M	70.39		22.72		5.18		1.39		[37
Geese (16 wk) F	70.45		21.64		4.32		1.38		[37]
Geese (16.6 wk) M	73.10		22.70		6.10		1.60		[44]
Geese (16.6 wk) F	73.70		22.60		5.30		1.40		[44]

M = male, F = Female.

muscles; turkey thigh [22,23,45] and goose [36] breast muscle protein and moisture; and Mule duck breast muscle [4] mineral content.

Differences in the moisture, protein, and fat content of pullet and cockerel breast and thigh muscles or broiler breast muscles were associated with strain crosses [13,15]. Strain also influenced the protein percentage and fat in broiler thigh muscles but no strain-related differences were found in moisture content. The small differences in muscle chemical composition among strain crosses may slightly alter the nutritional value of broiler meat [13]. Puchajda et al. [22,45] found that turkey medium-heavies' genetic lines influenced the differences in breast protein content of 22 wk males and in thigh muscle fat content of 15/16 wk females. However, another survey found no influence of genetic lines of heavies turkey males (22 wk) on muscle protein and fat content [23]. Filus et al. [21] did not show any significant indication of the dependency of hybrid turkey heavies and medium-heavies on the chemical composition of meat.

Górska and Górski [26] reported differences among duck crossbreds in breast and thigh muscle moisture and protein contents with no apparent species-fat content relationship in the case of both muscles. Nutritive values of breast and thigh muscles of SW (Small White), Synta (Pekin) ducks and their reciprocal crosses showed a tendency of paternal effect on protein and fat contents in breast and thigh muscles; in ash this was only evident in thigh muscles [27]. The strain had also a significant impact on the protein [39,50], moisture, and/or ash content in goose breast and leg muscles [39]. According to Friend et al. [48] strain influenced the fat content in goose breast muscles but had no influence on protein content.

II. AMINOACIDS AND COLLAGEN

Poultry meat is an important source of high biological value proteins providing all of the indispensable (essential) amino acids [44,51,52]. On average, 40% of the amino acids present in meat are indispensable for human health [9]. Dietary proteins are the only source of amino acids that we need. Our bodies require nine essential amino acids that animal protein contains in the best "proportion" [1]. The high lysine content of meat protein is important nutritionally because it counter-balances the low lysine content of cereal proteins when they are eaten together in a mixed diet [9].

The content, type, and cross-links in collagen influence nutritional and dietetic values of meat and its tenderness [53,54]. Poultry breast muscle was found to contain less collagen than thigh muscle (Table 32.4). The same trend was observed when collagen was expressed in relation to total protein content in the particular kind of poultry meat with the exception of 8 week Pekin ducks for which breast protein contained 1% more collagen than thigh muscle protein. As a protein of lower value collagen was the lowest in turkey and geese, and higher in broiler an Pekin duck muscles.

III. HEME PIGMENTS

Primary heme pigments in poultry meat include myoglobin, hemoglobin, and cytochrome c. The quantity of myoglobin greatly influences the appearance of poultry meat. Hemoglobin is another pigment of importance since about 20 to 30% remains in the carcass of a well-bled bird, thus influencing final carcass coloration [57]. Heme pigment content and "free iron ions" were associated with occurrence of lipid oxidation [19,58].

Table 32.5 shows total heme and heme pigments in poultry muscles. Goose and duck breast muscles contained considerably more heme pigments than the breast muscles of turkeys, hens, and broilers. The same trend was observed in T/L muscle; however, differences in heme pigment content (except for young and adult layer) were less pronounced among different poultry types. Nishida et al. [62] found different amounts of myoglobin in adult layer leg muscles, i.e., from 4.44 to 5.82 mg/g in adductores, 2.53 mg/g in sartorius, and 0.57 mg/g in biceps femoris, which was greater than for young layer, amounted to 0.33 mg/g of myoglobin in biceps femoris. The broiler, hen, and turkey breast muscles had from 1.4 to 6.4 times lower concentration of heme pigments, hemoglobin, myoglobin, and cytochrome (broilers) than those for T/L muscles (Table 32.5). These differences were smallest broilers and greatest for hens. In duck and goose breast muscles the heme pigments content was 14 to 79% greater than that in the leg muscles. However, total pigment concentration in Muscovy ducks and hemoglobin content in Pekin ducks were not significantly different in both muscles.

The mg of total pigment per gram of muscle increased in the following order: broiler breast, hen breast, turkey breast, broiler thigh/leg, hen leg, turkey leg, duck leg, goose leg, duck breast, and goose breast.

A. Heme Pigments—Age, Sex, and Strain

With advancing age (18 vs. 28 wk) myoglobin concentration significantly increased in male turkey breast muscles

TABLE 32.4
Collagen Content for Poultry Breast (B) and Thigh (T) Muscles

Poultry Type	Collagen		Collagen/Protein (%)		
Muscle	B	T	B	T	Reference
Broiler	3.12 (mg/g)				[55]
Broiler	1.27 (mg/g)				[12]
Broilers	0.54 (%)	1.05 (%)	2.34	5.31	[53]
Broilers M	0.733 (%)	1.04 (%)			[56]
Broilers F	0.62 (%)	0.783 (%)			[56]
Turkey (22 wk) M	385 (mg%)	572 (mg%)	1.63	2.94	[23]
Turkey (16 wk) F	429 (mg%)	484 (mg%)	1.80	2.55	[45]
Turkey (16 wk) F	358 (mg%)	839 (mg%)	1.48	4.17	[41]
Duck					
Pekin	1.75 (mg/g)				[12]
Pekin (8 wk)			5.0	4.05	[26]
Muscovy (12 wk)	2.52 (%)				[33]
Geese					
White Italian (14 wk)	340 (mg%)	670 (mg%)	1.45	2.83	[36]
White Italian (17 wk)	400 (mg%)	600 (mg%)	1.81	2.89	[38]

M = male, F = Female.

TABLE 32.5
Total Heme, Hemoglobin, and Myoglobin Content in Poultry Breast (B) and Thigh/Leg (T/L) Muscles

Poultry Type	Total Pigment (mg/g)		Hemoglobin (mg/g)		Myoglobin (mg/g)		Cytochrome (mg/g)		
Muscle/Meat	B	T/L	B	T/L	B	T/L	B	T/L	Reference
Broiler*	0.32	0.59/	0.17	0.38/	0.15	0.21/	13.71	37.38/	[59]
Broiler**	0.44	0.79/	0.28	0.48/	0.16	0.30/	27.15	65.99/	[59]
Broiler	0.24	/1.09	0.24	0.75	–	0.34			[60]
Broiler (8 wk)	0.43	/1.75	0.12	/0.58	0.31	/1.17			[61]
Hen (60 wk)	0.46	/2.49	0.13	/0.80	0.33	/1.69			[61]
Turkey	0.51		0.06		0.45				[40]
Turkey (24 wk) M	0.74	/2.66	0.16	/1.02	0.58	/1.64			[61]
Duck									
Pekin (8 wk)	3.59	/2.91	1.19	/1.17	2.40	/1.74			[61]
Pekin (8 wk)	4.31	/3.54							[29]
Muscovy (12 wk) M	3.90	/3.02							[29]
Muscovy (10 wk) F	3.68	/2.79							[29]
Goose (16 wk)	6.47	/3.80	1.71	/1.14	4.76	/2.66			[61]

M = male, F = Female, * = ice slush, ** = air chilling.

and male and female thigh muscles with no changes in the breast muscles from female turkeys [63].

Male turkeys exhibited significantly higher myoglobin concentration than females [63] and male Muscovy ducks had higher total heme pigment concentration than females (Table 32.5) [29].

IV. VITAMINS

Poultry meat is rich in niacin (PP), an essential vitamin that plays a role in many metabolic cycles. Moreover, it is also an important source of vitamins from the B group, like thiamine (B_1) and riboflavin (B_2), both important in energy metabolism, and B_{12}, which is necessary to the nervous system and exclusively present in foods of animal origin (not available in plants) [1]. Depending on the kind of diet, broiler and turkey muscles, hens, ducks, and goose meat can be also a supplementary source of vitamins diluted in lipids, i.e., vitamins A, D, and E [64,65].

The content of vitamins in poultry muscles is presented in Table 32.6. Thiamine content (B_1) was the highest in duck flesh and substantially lower, 2.79 times, in goose flesh, 5.1 times in broiler breast, and 5.6 times in turkey white meat [52]. The content of vitamin B_1 was comparable in broiler breast and T/L muscles and turkey white and dark meat, respectively. The increasing order of the amount of riboflavin (B_2) in poultry meat was as follows: broiler breast, turkey white, turkey breast, hen breast, broiler leg, turkey dark, duck and goose flesh. The broiler muscles had two times more niacin (PP) than turkey light and dark meat and 2–3 times more than hen, duck, and goose flesh. The richest in vitamin B_6 were broilers, then geese and turkey, while duck content of this vitamin was the lowest.

A. Vitamins—Age, Sex, and Strain

Riboflavin (B_2) content in breast broiler muscles decreased with age from 7 to 12 wk (0.093 vs. 0.086 mg/100 g) with no changes in niacin (PP) and vitamin B_6 [66]. In contrast, Singh and Essary [67] reported that with advancing age of broilers from 8 to 10 wk the content of thiamine (B_1) and riboflavin (B_2) increased in breast (0.032 vs. 0.045 and 0.040 vs. 0.050 mg/100 g) and thigh (0.059 vs. 0.066 and 0.096 vs. 0.113 mg/100 g) while the amount of niacin (PP) decreased in thigh muscles (6 vs. 5.6 mg/100 g) and did not change in breast muscles (10.4 vs. 10.2 mg/100 g).

Ang and Hamm [14] stated no significant differences due to sex in riboflavin (B_2), niacin (PP), and vitamin B_6 in the breast of 7 wk broilers. It was in line with results of Singh and Assary [67] who found that only male broiler thigh contained significantly more thiamine (B_1) and riboflavin (B_2) than female thigh muscles, i.e., 0.065 vs. 0.060 and 0.111 vs. 0.101 mg/100 g, respectively.

V. MINERALS

Minerals influence palatability, acidity of meat, biological activity of different enzymes, and osmolarity of meat. Iron is an important nutrient for human beings because it plays a part in the process of oxygen transport from lungs to various tissues by means of hemoglobin to which it is linked. Iron functions as a catalyst in many metabolic reactions. Its absence provokes anaemia [1]. Preponderance of haem

TABLE 32.6
Approximate Analysis of Vitamins in Poultry Breast (B) and Thigh/Leg (T/L) Muscles

Poultry Type	Thiamine (B_1) (mg/100 g)		Riboflavin (B_2) (mg/100 g)		Niacin (PP) (mg/100 g)		B_6 (mg/100 g)		Reference
Muscle/Meat	B	T/L	B	T/L	B	T/L	B	T/L	
Broiler			0.093		12.12		0.84		[66]
Broiler			0.086		11.98		0.824		[14]
Broiler	0.070	0.076/	0.092	0.188/	11.194	6.328/	0.55	0.33/	[52]
Broiler	0.090	/0.080	0.153	/0.226	12.44	/2.78			[65]
Hen**	0.083		0.159		5.16				[65]
Turkey*	0.064	0.081	0.122	0.221	5.844	3.075	0.56	0.36	[52]
Turkey	0.036		0.150		4.92				[65]
Duck**	0.360		0.450		5.300		0.34		[52]
Duck**	0.177		0.226		3.45				[65]
Goose**	0.129		0.377		4.278		0.64		[52]
Goose**	0.120		0.330		6.40				[65]

* = light and dark meat, ** = flesh.

iron in meat, which is absent from plant sources, makes the iron from meat more bioavailable [9]. Zinc is important because it is involved in several metabolic reactions and meat is its main source. Its deficiency reduces the immunological defenses of the organism [1,68].

Table 32.7 shows the mineral content in poultry meat. The broiler breast muscles contained more Mg, K, P, and Li (18.2 vs. 5.8 μg/g [69]), less Cu, Fe, Zn, and Na, and a comparable level of Ca than T/L muscles. Goluch-Koniuszy et al. [70,71] found no difference in Cu and Mg content between broiler breast and leg muscles and Kunachowicz et al. [65] found lower Ca content in broiler breast than thigh muscles. Turkey breast muscles were characterized by higher content of K and P and lower content of remaining minerals when compared with thigh muscles [41]. However, Posati [52] showed higher Mg content in light than in dark turkey muscles. Goose breast muscles had a higher content of Fe and lower Ca than thigh muscles. The higher content of Fe in broiler and turkey T/L muscles as well as in goose breast muscle was related to their greater content of myoglobin and hemoglobin than in corresponding breast and thigh muscles, respectively (Table 32.5). Higher content of Zn in T/L muscles can be explained by their greater metabolic activity.

The iron content in goose muscles was substantially higher than in broiler and turkey muscles and in hen, duck, and goose flesh (Table 32.7). The richest in zinc were turkey thigh muscles. The average zinc content was in duck flesh and broiler T/L muscles, and the lowest was in turkey and broiler breast muscles. The Mg content was rather uniformly distributed in broiler and turkey muscles and its content in duck, hen, and goose flesh was between that of broilers and turkeys. It was difficult to estimate the order of Na, K, and P contents in poultry meat on the basis of different author results presented in Table 32.7.

A. Minerals—Age, Sex, and Strain

In broiler breast and thigh muscles sex influenced Fe, Zn, and Na levels. Fe content was lower in breast and thigh muscles of broiler males than females while Zn content was higher in males than females, but differences were not so high as for Fe content [16]. According to Goluch-Koniuszy et al. [71], sodium content was higher in female breast muscles and in male leg muscles, respectively. Other authors found no sex influence on the level of nutrients (Cu, Fe, Zn, Mg, Ca, Na, K, P, and Li) in broiler cockerels and pullets [69,70].

Strain influenced the level of Mg, Ca, and K [16] as well as Ca and Li levels [69] in broiler breast and thigh muscles.

VI. LIPID FRACTIONS CONTENT

Lipids play a key role in many quality traits of meat products including nutritional value and sensory properties, mainly flavor because they are both solvent and precursors of aroma compounds. Intramuscular lipids refer to lipids contained in both intramuscular adipose tissue and muscle fibers. The intramuscular adipose tissue comprises cells located along the fibers and in the interfascicular area. The fat cells contain almost exclusively triacylglycerols (TAG). The lipids of the fibers consist of cytosolic droplets of TAGs and membrane lipids, phospholopids (PL), and cholesterol. The amount of TAGs in the fibers only accounts for a small part of the total intramuscular TAGs [74].

TABLE 32.7
Minerals in Poultry Breast (B) and Thigh/Leg (T/L) Muscles (Wet Weight Basis)

	Minerals (μg/g)																	
Poultry Type	**Cu**		**Fe**		**Zn**		**Mg**		**Ca**		**Na**		**K**		**P**		**Mn**	
Muscle	**B**	**T/L**	**B**	**T/L**	**B**	**T/L**	**B**	**T/L**	**B**	**T/L**	**B**	**T/L**	**B**	**T/L**	**B**	**T/L**	**B/T**	**Reference**
Broiler	0.51	0.68	6.81	10.70	6.65	15.27	247	216	39.00	42.00	456	620	2099	1835	2479	1829		R
	(0.03)	(0.02)	(1.70)	(3.28)	(0.07)	(1.01)	(73.76)	(60.62)	–	–	(88.84)	(39.00)	(627)	(461)	(339)	(127)		
Broiler	0.41	0.65	7.20	10.30	8.00	19.85	280	235	110	105	650	860/	2550	2300	1960	1675	0.18/0.20	[52]
Broiler			4.00	7.00/			330	230/	50.00	90.00/	550	850/	3850	3000/	2400	1960/		[65]
Turkey F 0.41	0.96/	5.01	11.87/	9.08	26.03	97.99	129/	40.95	51.83/	630	880/	3780	3410/	2830	2630/	0.19		[41]
Turkey	0.57	0.98/	8.44	16.40/	9.23	24.27												[73]
Turkey*	0.75	1.47	11.90	17.50	16.20	32.2	270	220	120	170	630	770	3050	2860	2040	1840	0.19/0.22	[52]
Turkey			5.00				350		20.00		470		4600		2380			[65]
Goose			55.00	50.00/					155	230								[37]
Flesh																		
Hens			16.00				190		110		660		3070		1620			[65]
Duck	2.53		21.00–24.00		19.00		140–190		80–110		660–740		2420–2710		1490–2030		[65–52]	
Goose	3.06		24.00–25.70		–		180–240		50–130		480–870		2430–4200		1520–3120		[65–52]	
Influence of Sex																		
Broiler M 0.53	0.67	4.46	6.13	6.70	14.10	162	/153			363	/581	1626	/1462				[70,71]	
Broiler F 0.57	/0.73	4.10	/5.89	6.63	13.12	164	/167			399	/552	1634	/1474				[70,71]	

M = male, F = Female, * = Light, Dark; R = [11,58,68–72].

TABLE 32.8
Total Lipid Content and Lipid Class Composition of Poultry Breast (B) and Thigh/Leg (T/L) Muscles

Poultry Type	Total Lipids (g/100 g)		TAG* (g/100 g)		PL** (g/100 g)		FFA (g/100 g)		Reference
Muscle/Meat	B	T/L	B	T/L	B	T/L	B	T/L	
Broiler			0.24	/0.70	0.50	/0.64	0.024	/0.033	[75]
Broiler		0.90	2.20	0.37	0.73/	0.61	0.26/		[76]
Broiler M	0.90	2.30/	0.35	0.76/	0.62	0.23/			[77]
Broiler F	0.90	3.20/	0.43	0.83/	0.55	0.16/			[77]
Broiler (11 wk)	0.97	/2.10	0.22	/1.08	0.68	/0.90			[78]
Broiler (11.7 wk)	0.87	2.60/	0.35	1.84/	0.52	0.76/			[64]
Turkey	1.21	2.03/	0.58	1.14/	0.50	0.76/	0.015	0.026/	[79]
Duck									
Muscovy (12 wk) M	1.82		0.56		1.12				[32]
Mule (10/12 wk) M	2.09/2.83		0.88/1.65		1.12/1.09				[4]
Mule (10/12 wk) F	1.69/2.26		0.58/1.11		0.98/1.0				[4]
Landaise Goose (24 wk)			3.88		0.98		0.04		[80]

M = male, F = Female, * = TAG (triacylglycerols), ** = PL (phospholipids).

Table 32.8 shows that the TAG content was the lowest in broiler breast, average in turkey and Muscovy duck breast, highers in male mule duck, and the highest in goose breast muscles. The PL content in broiler and turkey breast muscles was lower by nearly half of its level in duck and goose breast muscles.

Broiler, hen [81], and turkey breast muscles contained less total lipids, TAGs, and PL than thigh muscles. The high level of PL is characteristic of red, oxidative muscles. Therefore breast, glicolytic muscles of broilers and turkeys contained less PL than T/L contra partners as well as breast muscles of ducks and geese which mainly comprised red fibers [12,82–84]. Surprisingly, some authors found that PL was higher in the lipids of broiler breast than thigh muscle lipids [76,77]. In both broiler and turkey muscle types, free fatty acids were present in only trace amounts. The FFA concentration was higher in broiler leg muscles by 37% [85] and turkey thigh muscles by 73% [79] compared to corresponding breast muscles.

Broiler muscle lipid fraction contained more FFA amounts than turkey muscles. The broiler and Muscovy and 10 wk Mule duck breast muscles contained more PL than TAG. The reverse was true for goose breast and 12 wk male Mule duck muscles while in turkey breast and 12 wk mule female duck the level of the main components of lipids was comparable. On the other hand, in broiler and turkey T/L muscles PL content was lower than the TAG content.

The fatty acid composition of the different lipid classes of broiler and turkey muscles is shown in Tables 32.9 and 32.10. There were only small differences between broiler and turkey breast and leg TAG fatty acid composition (Table 32.9). According to Pikul et al. [78] in broiler muscles palmitic (C16:0), oleic (C18:1), linoleic (C18:2n6), and linolenic (C18:3n3) acids in TAG accounted for about 26%, 37%, 22%, and 1%, respectively. Ahn et al. [85] and Sklan and Tenne [75] found that broiler breast TAG contained less oleic acid (C18:1) and more linoleic acid (C18:2n6) and linolenic acid (C18:3n3) than leg muscles. Sklan and Tenne [79] found lower content of palmitic acid (C16:0) and higher content of linoleic acid (C18:2n6) in turkey breast TAG than in turkey thigh TAG. There were no arachidonic acid (C20:4n6) and polyunsaturated PUFA n-3 in turkey muscle TAGs fraction. The decreasing percentage of the fatty acid groups in TAGs was monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), and PUFA for broiler muscles [64,75,78,85] and SFA, MUFA, and PUFA for turkey muscles [79].

The PL fatty acid composition of broiler and turkey breast muscles was different from T/L muscles (Table 32.10). The PL fraction of broiler and turkey breast contained more palmitic acid (C16:0) and oleic acid (C18:1) and less stearic acid (C18:0) and linoleic acid (C18:2n6) than PL from T/L muscles [64,75,78]. The above pattern was also valid for phosphatidyl choline (one apart phosphatidyl ethanolamine major PL) with the one exception of similar or higher values of linoleic acid (C18:2n6) in breast and T/L broiler muscles [75,85]. The broiler breast and T/L muscles contained a higher level of arachidonic acid (C20:4n6) in PL fraction than corresponding turkey muscles by 6.5 and 7.0%, respectively. The relative percentage of PUFA was the same in the PL from broiler breast and leg muscles while for turkey PL the percentage of PUFA was lower in breast than thigh muscles. Compared to phosphatidyl choline (PC) phosphatidyl ethanolamine (PE) in broilers contained more PUFAs. Turkey muscles lacked PUFA n-3 in the PL fraction, likewise in the TAGs fraction.

TABLE 32.9
Fatty Acid Composition (%) of the TAG Fraction of Lipids from Broiler and Turkey Breast (B) and Thigh/Leg (T/L) Muscles

Reference	[75]	[85]	[78]	[75]	[85]	[78]	[79]	[79]
Poultry Type	Broiler			Broiler			Turkey	
Muscle	B	B	B (11 wk)	T	L	L (11 wk)	B	T
FFA profile (%)								
C14:0		0.80	0.82		1.02	0.83		
C16:0	25.10	21.42	26.01	25.20	21.22	25.65	29.30	32.60
C18:0	6.50	5.96	6.36	5.80	5.45	5.81	9.00	9.40
C20:0		0.81	0.28			0.28		
C22:0			0.28			0.39		
C14:1			0.20			0.12		
C16:1	8.00	4.93	5.21	7.90	5.60	5.73	3.10	5.00
C18:1	43.00	46.35	37.31	44.60	52.08	36.41	30.20	30.30
C20:1			0.72			0.44		
C18:2n6	17.30	17.15	21.39	16.20	13.03	22.12	27.60	23.80
C20:2n6		0.41	0.21		0.29	0.32		
C20:4n6	0.10		0.70	0.30		1.08		
C18:3n3		1.46	1.25		0.69	1.24		
C20:5n3	0.00	0.00			0.19			
SFA	31.60	28.98	33.75	31.10	27.68	32.96	38.30	42.00
MUFA	51.00	52.00	42.72	52.50	58.13	42.26	33.30	35.30
PUFA	17.40	19.02	23.55	16.50	14.18	24.76	27.60	23.80
n-6	17.40	17.56	22.30	16.50	13.31	23.52	27.60	23.80
n-3		1.46	1.25		0.87	1.24		
n-6/n-3		12.03	17.84		15.30	18.97		

The decreasing percentage of the fatty acids groups in PL for broiler muscles, i.e., PUFA, SFA, and MUFA (The reverse when compared with TAGs) differed from turkey muscles: SFA, PUFA, and MUFA [64,75,78].

The fatty acid composition of TAGs was different from the fatty acid composition of PL (Tables 32.9 and 32.10). The PL fraction from broiler and turkey muscles contained more PUFA and SFA and less MUFA than the TAGs fraction. The arachidonic acid content in broiler breast and leg muscles was substantially higher in PL than in TAG. The broiler breast and leg PUFA n-6/n-3 ratio in PL was two times lower than in TAG (2.04 and 2.11) [78].

A. Lipid Fractions Content—Age, Sex, and Strain

The TAG content of Mule duck increased with age and this correlated with the increase in lipid content (Table 32.7) [4].

There was no change in the content or class composition of the lipids with sex of broilers [77]. At any given age, the TAG content was higher in male Mule duck breast compared with the females but this difference was not significant [4].

VII. CHOLESTEROL

Determination of cholesterol in food is important for consumer health [86]. High dietary cholesterol intake is considered to be one of the risk factors for human atherogenesis [87]. The higher cholesterol concentration reduces membrane fluidity, lowers Ca^{+}-ATPase activity, and regulates contraction and relaxation rates [88]. Cholesterol content in chicken tissue is possible to be influenced by fatty acid composition of the diet [3] or by the dietary garlic and copper content [88].

Table 32.11 presents cholesterol content expressed in mg/100 g of poultry meat. The cholesterol content in poultry breast white muscles, in ascending order, was as follows: broilers, hens and turkeys, Muscovy ducks, goose, Pekin and Mule ducks, and ranged from 41.0 to 123 mg/100 g. The cholesterol content in different poultry T/L muscles was on similar level with one exception of higher content in hen dark meat, and ranged from 64.0 to 101 mg/100 g. Cholesterol is solely a membrane lipid in meat, and is also found in cell membranes of adipose tissue and fatty tissue [91]. Cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in broiler and turkey breast muscles (Table 32.1). Moreover,

TABLE 32.10
Fatty Acid Composition (%) of the PL Fraction and Its Classes (PE* and PC) of Lipids from Broiler and Turkey Breast (B) and Thigh/Leg (T/L) Muscles**

Reference	[75]	[75]	[85]	[85]	[75]	[75]	[85]	[85]	[78]	[78]	[79]	[79]
Poultry Type	Broiler										Turkey	
Muscle	B/PE	B/PC	B/PE	B/PC	T/PE	T/PC	L/PE	L/PC	B/PL	L/PL (11 wk)	B/PL	T/PL
FFA profile (%)												
C14:0			0.80	0.48			0.51	0.48	0.15	0.18		
C16:0	16.00	35.00	7.61	26.28	16.70	18.90	7.96	23.9	20.36	16.82	25.60	19.70
C18:0	26.60	11.00	19.21	10.58	24.10	33.70	19.99	15.99	12.76	20.09	22.50	24.30
C20:0			0.24	0.25			0.32	0.47	0.24	0.32		
C22:0									1.37	1.09		
C24:0									0.40	0.61		
C14:1									0.06	0.07		
C16:1	2.10	1.70	0.58	0.85	2.80	1.30	0.48	0.82	0.45	0.71		
C18:1	17.60	24.00	20.06	26.06	17.80	16.60	16.57	23.18	18.06	13.67	21.20	13.60
C20:1			0.72	0.46			0.76	0.38				
C22:1			0.29	0.51			0.45	1.83	2.52	1.92		
C18:2n6	6.80	13.60	14.54	19.15	9.10	10.80	15.61	19.54	10.95	14.99	16.70	26.00
C20:2n6			2.00	1.98			1.39	1.64	0.37	0.40		
C20:3n6			2.27	2.21			1.28	1.34				
C20:4n6	12.60	5.20	17.07	6.21	11.10	10.80	20.13	5.98	20.02	19.71	13.50	12.70
C22:2n6			0.52	0.21								
C22:4n6			3.93	1.31			4.73	1.05	5.03	4.98		
C18:3n3			0.47	0.19			0.34	0.21	0.09	0.31		
C20:3n3			0.35	0.24			0.30	0.25				
C20:5n3	0.20	2.40	1.28	0.42	2.40	1.40	0.93	0.42				
C22:3n3			1.59	0.47			1.54	0.54				
C22:5n3	6.20	3.30	2.73	0.92	4.00	3.10	3.07	0.75	2.27	1.39		
C22:6n3	11.90	3.70	3.78	1.24	11.80	3.40	3.66	1.29	2.71	1.76		
SFA	42.60	46.00	27.86	37.58	40.80	52.60	28.78	40.88	35.28	39.11	48.10	44.00
MUFA	19.70	25.70	21.64	27.88	20.60	17.90	18.25	26.21	21.09	16.37	21.20	13.60
PUFA	37.70	28.20	49.69	34.55	38.40	29.50	52.98	32.92	41.44	43.54	30.20	38.70
n-6	19.40	18.80	39.80	31.07	20.20	21.60	43.14	29.54	36.37	40.08	30.20	38.70
n-3	18.30	9.40	10.69	3.48	18.20	7.90	9.84	3.38	5.07	3.46		
n-6/n-3	1.06	2.00	3.72	8.93	1.11	2.73	4.38	8.74	7.17	11.58		

* = PE (phosphatidyl ethanolamine), ** = PC (phosphatidyl choline).

slow-twitch fibers of thigh muscles have many more mitochondria, their mitochondria are bigger, and the metabolic rate in muscles is faster in comparison to the fast-twitch fibers of breast muscles [88]. Therefore, the cholesterol content in T/L or dark meat was higher by 17.0–48.3% in broilers, by 50.2% in hens and by 20.3–37.3% in turkeys (range from 63.7 to 78.9 mg/100 g) compared to breast or white meat (range from 40.9 to 58.2 mg/100 g). In contrast, the cholesterol level in goose breast muscles was higher by 15.3% than in thigh muscles.

The cholesterol content expressed as a percentage of total lipids in broiler breast muscles (2–2.7%) was two to three times higher than in thigh muscles (1.0%) [17,77]. In contrast, the cholesterol concentration in total lipids of turkey male breast and thigh muscles was not significantly different (4.07 vs. 3.10%) [90]. Irrespective of the differences between muscles, cholesterol concentration was substantially higher in total lipids of turkey muscles than in chicken muscles. This can be explained by lower total lipid content in turkey breast and thigh muscles (1.47 and 2.45%) (Table 32.1) than in breast and thigh broiler muscles (1.80 and 7.30%) [17,90].

A. Cholesterol—Age, Sex, and Strain

The cholesterol content decreased with age: in 7 vs. 9 wk broiler breast muscle by 33.30% [66], in 5 vs. 13 wk broiler breast muscle by 8.40%, and in thigh muscle by 23.57% [92], in 10 vs. 25 wk male turkey breast muscles by 20.97%, and in thigh muscles by 23.76% [49], and in

10 vs. 12 wk male Mule duck breast muscles of males by 13.22% and females by 13.68% [4]. In 9–11 wk broiler breast muscles there was no age dependency on cholesterol content and after 12 wk its content increased to the level of 7 wk broiler breast muscle [66]. The cholesterol content in breast and thigh muscles of female turkeys (10 vs. 18 wk) was entirely independent of age [49].

At the comparable age (14 wk) male turkeys had higher cholesterol content than females in breast and thigh muscles (Table 32.11) [49]. The same trend was observed for goose and male and female Mule duck breast muscles but differences were not significant [4].

VIII. FFA

To prevent serious chronic diseases of humans, it is necessary to consider both the balance intake of fatty acids [8,93] and the intake of cholesterol [86] in poultry meat. A substantial reduction in carcass fats and cholesterol and improvement of the fatty acid make-up in poultry muscles, such as omega-3 fatty acids, bring about nutritional and economic benefits to consumers and producers alike [3]. Fat deposition is controlled by the calorie: protein ratio of the diet, and the fatty acid composition is controlled by type and amount of dietary fat by adding linoleic (LA, C18:2n6) and linolenic (LNA, C18:3n3) acids, vegetable oils, fish meal, fish oils, beef tallow and marine algae [3,8,85,87,94–99]. For example, the composition of fatty acids in chicken carcass can be modified through feeding because in the digestive system of chickens, lipids are absorbed and deposited in tissue in unchanged form. The concentration of fatty acids in tissue is closely correlated with their dietary content, in contrast to SFA and MUFA acids which can be synthesisted de novo from simple precursors such as glucose or amino acids [5,8,94,100]. Linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3), the precursors of the n-6 and n-3 family of fatty acids, respectively, are essential fatty acids and have to be supplied by the diet [100].

TABLE 32.11
Cholesterol Content in Poultry Breast (B) and Thigh/Leg (T/L) Muscles

Poultry Type	Cholesterol (mg/100 g)		
Muscle/Meat	**B**	**T/L**	**Reference**
Broiler	42.40	63.70/	[17]
Broiler	43.40	/84.00	[89]
Broiler	47.41		[12]
Broiler	51.00		[66]
Broiler (11 wk)	40.90	/77.50	[78]
Broiler*	59.40	71.60	[3]
Average	47.42 (6.93)	74.20 (8.64)	
Hens*	50.07	100.61	[20]
Turkey	45.10	/71.90	[89]
Turkey (20 wk) M	47.40	70.00/	[49]
Turkey (20 wk) M	58.20	73.00/	[90]
Turkey (14 wk) M	52.70	78.90/	[90]
Turkey (14 wk) F	48.20	72.30/	[49]
Average for M	50.85 (5.84)	73.45 (3.84)	
Pekin duck	123		[91]
Pekin duck	99.11		[12]
Average	111.06 (16.89)		
Muscovy duck (M)	67.00		[32]
Mule duck (10/12 wk) M	121/105		[4]
Mule duck (10/12 wk) F	117/101		[4]
Goose	80.70	70.00	[89]
Goose (16.6 wk) M/F	88/82		[44]
Average	84.35 (5.16)	70.00	

M = male, F = female, * = white, dark muscles.

The fatty acid composition of muscle tissue plays a major role in lipid stability and product quality. Lipid oxidation is one of the main causes of deterioration in the quality of meat during storage and processing. Lipid oxidation is a major problem in poultry meat due to the high content of polyunsaturated fatty acids (PUFA) and low level of natural antioxidants such as tocopherol [8,64,85,94,101]. However, introducing antioxidants to the diet, especially vitamin E, efficiently protects the carcass from this process [102,103].

Okuyama et al. [104] recommended the decrease of polyunsaturated (PUFA) n-6/n-3 ratio in diet and the separate evaluation of the content of α-linolenic acid (LNA, C18:3n3; the basic fatty acid and precursor of PUFA n-3 family), arachidonic acid (AA, C20:4n6; a key metabolite in the PUFA n-6 family), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) in foods, because the physiological activity of EPA+DHA is five times higher in comparison with LNA. Another dietary factor influencing the risk of atherosclerosis in humans is the ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids [8].

Some long chain PUFAs (i.e., dihomo-α-linolenic acid, AA and EPA) are precursors of eicosanoids — important metabolites in the control of cell metabolism [105]. In contrast to PUFA n-6 arachidonic acid (C20:4n6), dietary PUFA n-3 decrease production of pro-inflammatory eicosanoids and have a positive effect on cardiovascular–coronary heart disease, autoimmune, and other chronic diseases, including cancer [8,100,106]. The incidence of coronary heart disease has been said to be dependent upon two different processes – atherosclerosis and trombosis – where atherosclerosis relates to the thickening of the arterial wall and trombosis relates to formation of a clot that occludes the artery, causing heart attack [8,107]. The DHA has an important role in the development of the central nervous system of the newborn while the EPA is involved in blood clotting and the inflammatory response [9]. In view of physiological effects of arachidonic acid (AA), i.e., an increased synthesis of pro-inflammatory prostaglandins,

TABLE 32.12
Free Fatty Acid (FFA) Profile of Broiler Breast (B) or White (W) and Thigh/Leg (T/L) or Dark (D) Muscles

Reference	[3]	[111]	[101]	[85]	[17,112]	[105]	[77]		[3]	[111]	[101]	[85]	[17,112]	[105]	[77]	
Muscle	**W**	**B**	**B**	**B**	**B**	**B**	**B (M/F)**	**Average**	**D**	**T**	**L**	**L**	**T**	**L**	**T (M/F)**	**Average**
FFA (%)																
C12:0		0.47						0.47		0.31						0.31
C14:0		0.82		0.61	0.50	0.66		0.65		1.05		0.37	0.60	0.69		0.68
C15:0		0.14						0.14		0.13						0.13
C16:0	18.10	23.43	20.40	19.46	22.80	22.62	23.8/21.9	21.08	18.40	21.77	19.30	18.92	22.80	22.00	22.60/22.00	20.83
C17:0		0.30				0.93		0.61		0.30				1.18		0.74
C18:0	12.50	13.88	10.40	8.32	6.85	9.92	7.50/7.00	9.91	10.90	9.90	9.70	6.68	6.00	9.75	7.60/6.30	8.65
C20:0		0.14		0.27		0.93		0.45		0.16		0.21		2.04		0.80
C22/24:0		0.30						0.30		0.19						0.19
C16:1		1.52	2.45	4.47	5.80	3.96	4.50/4.60	3.78		3.44	3.35	5.88	6.90	4.61	6.30/7.30	5.08
C18:1	33.50	26.02	24.55	39.97	38.90	36.66	29.10/28.00	32.67	37.40	31.98	28.25	44.17	41.15	36.35	32.00/36.10	35.90
C20:1		0.34		0.66	0.50	0.59	0.50/0.60	0.52		0.47		0.63	0.50	0.56	0.50/0.60	0.53
C22:1							0.40/0.50	0.40							0.60/0.20	0.60
C24:1			1.73					1.73		0.46						0.46
C18:2n6	18.40	13.38	17.20	17.88	18.60	14.33	17.80/17.00	16.80	18.50	17.23	20.20	18.43	18.55	14.41	18.30/18.90	17.95
C18:3n6			0.5/0.6			0.17		0.33					0.18			0.18
C20:2n6			0.67		0.74		0.14	0.52		0.52		0.31	0.36			0.40
C20:3n6					0.92		0.83	0.87				0.28		0.55		0.41
C20:4n6	8.00	3.65	5.45	3.15	2.10	3.11	5.00/4.00	4.35	5.70	2.10	3.90	1.24	0.90	3.06	3.70/2.50	2.94
C22:4n6			0.76	0.51	1.79			1.02				0.31	0.21	0.95		0.49
C22:5n6						0.10		0.10					0.05			0.05
C18:3n3	1.20	0.52	3.40	1.21	1.35	1.38	0.70/0.80	1.39	1.20	0.82	5.25	1.76	1.55	1.31	0.70/0.90	1.80
C20:5n3 (EPA)	0.80	0.62	1.50	0.28	0.30	0.59	0.90/1.70	0.71	0.50	0.48	0.9	0.09	0.10	0.63	0.60/0.30	0.47
C22:5n3 (DPA)	2.00	1.91	3.10	0.59	0.60	0.86		1.51	1.50	0.96	1.90	0.23	0.20	1.06	0.50/0.40	0.91
C22:6n3 (DHA)	2.50	2.95	2.25	0.72	0.90	0.69	1.80/3.20	1.68	2.30	1.45	1.20	0.30	0.30	0.49	1.00/0.60	1.01
SAT	30.80	39.46	35.05	28.67	30.20	34.13	33.50/31.20	33.12	29.60	33.81	31.65	26.38	29.35	34.48	32.20/30.10	31.07
MUFA	35.60	29.62	27.45	45.10	45.20	41.21	34.50/34.50	36.95	40.10	35.20	32.10	50.67	48.55	41.52	39.40/44.20	41.08
PUFA	33.10	23.19	36.00	26.25	24.60	23.73	32.00/34.30	28.41	30.20	23.08	35.25	22.95	22.05	22.82	28.50/25.60	26.41
Total n-6	27.10	17.69	25.75	23.45	21.45	20.21	27.40/27.20	23.29	24.80	19.85	25.95	20.57	19.90	19.33	25.10/23.30	22.21
Total n-3	6.40	5.50	10.25	2.80	3.15	3.52	4.50/7.10	5.16	5.40	3.23	9.30	2.38	2.15	3.49	3.40/2.40	4.19
n-6/n-3	4.23	3.22	2.51	8.38	7.25	5.74	6.09/3.83	5.35	4.59	6.14	2.80	8.64	9.25	5.54	7.38/9.71	6.33

M = male, F = female.

leukotrienes and platelet-activating factor which is connected with cancer and autoimmune diseases, the decrease of its intakes is recommended [108–110]. Generally, high intakes of EPA+DHA and AA are considered important positive and negative aspects for human nutrition, respectively [49,87,109,110].

The percentage representation of fatty acids in lipids from poultry breast and T/L muscles is presented in Tables 32.12–32.15. The main SFA fatty acids in poultry breast and T/L muscles were palmitic acid (C16:0) and stearic acid (C18:0), with minor quantity of myristic acid (C14:0), and for duck and goose breast muscles also lauric acid (C12:0). The stearic acid (C18:0) is not thought to raise blood cholesterol level while the myristic acid (C14:0), appears the most atherogenic and has four times the cholesterol-raising effect of palmitic acid [107]. Kelly [115] showed that stearic acid (C18:0) in the diet had beneficial effects on thrombogenic and atherogenic risk factors in males. The predominant MUFA fatty acids in the poultry for both muscles were oleic acid (C 18:1) and palmitoleic acid (C16:1). In the PUFA fatty acids group linoleic acid (C18:2n6) dominated followed by arachidonic acid (C20:4n6), α-linolenic acid (C18:3n3) and DHA (C22:6n3, except duck muscles and goose leg muscles). In all tissues the principal fatty acid was oleic (C18:1), followed by palmitic acid (C16:0) and/or linoleic acid (C18:2n6).

Broiler, hen and goose breast, or white muscle lipids contained more SFA, PUFA n-6, and PUFA n-3, and total PUFA but less MUFA than T/L or dark muscle lipids (Tables 32.12, 32.13, and 32.15). However, some authors found in broiler breast muscles, when compared with T/L muscles, less PUFA n-6 by 2.16% [111] or almost the same percentage of SFA [3,17,105], MUFA, and PUFA n-3 [105]. The nutritionally significant PUFA n-6/PUFA n-3 ratio was lower (more favorable) in broiler breast muscle (5.35) compared with thigh muscle (6.33). For hens the ratio of PUFA n-6/PUFA n-3 was slightly higher (by 0.32%) in white than in dark smuscles.

TABLE 32.13
Free Fatty Acid (FFA) Profile of Hen and Turkey Breast (B) or White (W) and Thigh (T) or Dark (D) Muscles

Reference	[20]	[20]	[90]	[49]	[49]	[90]	[49]	[49]
Muscle	Hen W 64 wk	Hen D	Turkey B 20 wk M	B 20 wk M	B 14 wk F	Turkey T 20 wk M	T 20 wk M	T 14 wk F
FFA (%)								
C14:0	0.67	1.53	0.99			0.99		
C16:0	23.01	21.38	24.32	22.40	20.80	24.41	22.20	20.20
C18:0	10.32	8.87	9.95	7.70	11.30	10.49	9.80	8.90
C16:1	2.48	3.78	6.14			5.84		
C18:1	32.38	39.26	28.48	36.60	32.10	28.45	32.70	32.60
C20:1	0.28	0.36	0.30			0.33		
C18:2n6	12.62	15.36	21.54	21.30	21.00	22.58	22.50	24.90
C18:3n6			0.11			0.12		
C20:2n6	0.39	0.24						
C20:3n6	0.45	0.26						
C20:4n6	8.11	3.27	4.15	1.40	3.00	3.59	2.60	2.30
C22:4n6	0.76	0.50	1.03			0.74		
C22: 5n6			0.27			0.19		
C18:3n3	0.75	1.99	0.89	2.80	2.00	0.94	2.50	2.70
C20:5n3 (EPA)	0.00	0.03	0.19			0.18		
C22:5n3 (DPA)	0.52	0.18	0.63			0.43		
C22:6n3 (DHA)	1.58	0.56	1.01	1.60*	3.20*	0.72	2.10*	2.20*
SAT	34.01	31.79	35.26	30.10	32.10	35.89	32.00	29.10
MUFA	35.13	43.40	34.92	36.60	32.10	34.62	32.70	32.60
PUFA	25.57	22.38	29.82	27.10	29.20	29.49	29.70	32.10
Total n-6	22.71	19.63	27.10	22.70	24.00	27.22	25.10	27.20
Total n-3	2.86	2.75	2.72	4.40	5.20	2.27	4.60	4.90
n-6/n-3	8.01	7.69	9.96	5.16	4.62	11.99	5.46	5.55

M = male, F = female, * = EPA + DHA.

TABLE 32.14
Free Fatty Acid (FFA) Profile of Duck Breast (B) and Thigh (T) Muscles

Reference	[12]	[32]	[113]	[113]	[6]	[6]	[6]
Muscle	B Pekin 7 wk	B Muscovy 12 wk	B A44 8 wk	T A44 8 wk	T Pekin 9/12 wk	T Muscovy 10.5/15 wk	T Mule 8/14 wk
FFA (%)							
C12:0	1.72			1.09/0.94	1.20		1.18
C14:0	1.51	1.20	1.20	0.90	0.70/0.67	0.55/0.53	0.63/0.57
C15:0		1.80	0.80	0.30			
C16:0	28.23	22.40	27.80	23.90	23.80/23.10	25.00/22.00	25.70/21.60
C17:0			0.30	0.20			
C18:0	14.01	13.30	11.40	7.50	5.40/5.40	8.00/7.40	6.90/6.90
C14:1		0.50	0.20	0.10			
C16:1	2.20	1.90	3.40	4.50	3.50/2.20	2.10/1.50	3.00/1.70
C17:1			0.40	0.10			
C18:1	32.41	28.40	34.80	48.40	48.80/45.40	41.30/40.60	43.90/43.80
C20:1			0.60	0.70	0.81/0.93	0.52/0.62	0.56/0.77
C18:2n6	13.12	14.90	11.40	10.10	11.50/17.50	17.20/20.00	14.90/19.10
C20:2n6		1.20					
C20:3n6		0.50					
C20:4n6	3.91	12.60	6.90	2.50			
C18:3n3		1.30	0.60	0.80	0.94/0.69	0.88/0.91	0.91/0.77
SAT	45.46	38.70	41.50	32.80	30.99/30.11	34.75/29.93	34.41/29.07
MUFA	34.61	30.80	39.40	53.80	51.11/48.53	43.92/42.72	47.46/46.27
PUFA	17.03	30.50	18.90	13.40	12.44/18.19	18.08/20.91	15.81/19.87
Total n-6	17.03	29.20	18.30	12.60	11.50/17.50	17.20/20.00	14.90/19.10
Total n-3	1.30		0.60	0.80	0.94/0.69	0.88/0.91	0.91/0.77
n-6/n-3		22.46	30.5	15.75	12.23/25.36	19.55/21.98	16.37/24.81

Broiler, hen, duck and goose breast muscles were characterized by a higher proportion of stearic acid (C18:0) and lower proportion of oleic acid (C18:1) than thigh muscles. Lipids associated with broiler or hen breast meat contained less linoleic acid (C18:2n6) and linolenic acid (C18:3n3), but more arachidonic acid (C20:4n6) and eicosapentaenoic EPA (C20:5n3—no difference in hens), docosapentaenoic DPA (C22:5n3) and docosahexaenoic DHA (C22:6n3) than lipids associated with T/L muscles. The level of EPA, DPA, and DHA in broiler breast lipids was at least one and a half that of thigh muscles lipids and DHA exceeded EPA in both breast and thigh muscles lipids by a factor of two and a half. Kralik and Ivankovic [111] found higher content of EPA, DPA, DHA, and total n-3 fatty acids but less n-6 fatty acids in broiler breast muscles compared with thigh muscles which indicated that the percentage of PUFA in both kinds of muscles was comparable. Others found no difference in linoleic acids (C18:2n6) [3,17] or in fatty acid profiles between broiler white and dark muscles [105]. The level of DPA and DHA in hen breast muscles was 2.8 times higher than in thigh muscles [20]. In goose breast muscles, likewise in broilers and hens, the proportion of linolenic acid (C18:3n3) was lower than in leg muscles [42]. In contrast, goose breast muscle lipids were characterized by the presence of higher levels of linoleic acid (C18:2n6) and the same values of arachidonic acid (C20:4n6) as in leg muscles, and according to Biesiada-Drzazga et al. [42], by the absence of EPA, DPA, and DHA in both muscles. The proportion of linoleic acid (C18:2n6) in Pekin [6,12] and in Muscovy duck [6,32] breast muscles compared with thigh muscle was higher and lower, respectively.

Breast and thigh muscle lipids of 20 wk male turkeys were comparable in total values of the main fatty acids fractions [49] or were different in fatty acid composition [90]. Therefore, turkey breast muscles were characterized by a higher proportion of linolenic acid (C18:3n3) and lower proportions of SFA, linoleic acid (C18:2n6), and arachidonic acid (C20:4n6) compared to thigh muscles [49,79] which was in accordance with data for chicken and hen only for linoleic acid (C18:2n6). According to Komprda et al. [90], turkey breast muscles had slightly higher arachidonic acid (C20:4n6) and DHA (C22:6n3) percentages than thigh muscles which corresponded with broilers. However, higher or

TABLE 32.15
Free Fatty Acid (FFA) Profile of Goose Breast (B) and Leg (L) Muscles

Reference	[80]	[114]	[114]	[42]	[42]
Muscle	B 24 wk	B 16 wk M/F	B 26 wk M/F	B 15*/24** wk	L 15*/24** wk
FFA (%)					
C12:0	0.13				
C14:0	0.67	2.20	1.40	0.07/0.08	0.06/0.06
C15:0	0.82				
C16:0	24.87	24.50/26.40	25.10/26.20	24.76/27.47	24.27/20.40
C18:0	6.82	10.60	8.80	3.16/4.35	3.02/2.58
C20:0	0.15				
C22:0	0.09				
C24:0	0.17				
C16:1	3.96	4.10	4.60	1.55/2.05	1.73/1.96
C18:1	43.40	49.80/46.30	50.30/50.20	61.19/58.30	63.20/67.89
C20:1	0.22	0.80-0.90	0.80-0.90	0.06/0.08	0.07/0.07
C22:1	0.02				
C18:2n6	15.00	6.90	6.10	8.76/7.38	7.40/6.76
C20:2n6	0.29	0.02/0.05	0.01/0.02		
C20:3n6				—/0.03	0.03/—
C20:4n6	2.31	0.70	0.50	0.24/0.12	0.07/0.12
C18:3n3	0.66	0.50-0.60	0.50	0.08/0.06	0.07/0.07
C22:5n3 (DPA)	0.13				
C22:6n3 (DHA)	0.25				
SAT	32.31	37.00/40.30	36.10/36.40	27.99/31.90	27.35/23.04
MUFA	47.60	55.00/51.30	56.30/57.00	62.80/60.43	65.00/69.92
PUFA	18.64	8.10	7.10	9.10/7.64	7.58/6.97
Total n-6	17.60	7.60	6.60	9.02/7.58	7.51/6.90
Total n-3	1.04	0.50	0.50	0.08/0.06	0.07/0.07
n-6/n-3	16.92	15.20	13.20	112.7/112.6	107.3/98.57

M = male, F = female, * = intensive system, ** = semi-intensive system.

the same MUFA percentage and lower or the same PUFA percentage in male turkey breast than thigh muscles were not in line with broilers and hens. The PUFA n-6/n-3 ratio was comparable in turkey muscles and hens. A comparison of fatty acid composition in male turkeys and broiler breast and thigh muscles revealed that in turkey muscles the ratio of SFA was higher by 5.06/6.59%; MUFA was lower by 10.28/14.08%; PUFA was higher by 4.60/7.49%; and arachidonic acid (C20:4n6) was higher by 2.05/2.69% [17,90]. Therefore, turkey meat provided substantially less MUFA (a desirable fraction from the viewpoint of human nutrition) and more arachidonic acid (undesirable fraction) compared to broiler meat.

Duck breast muscles contained more SFA than other kinds of poultry and less PUFAs than broilers, hens, and turkeys. The fatty acid contents of duck and goose muscles were very similar and contained approximately 50% SFA, 33% MUFA, and 16% PUFA (Tables 32.12–32.15).

The interpretation of fatty acid composition in poultry meat depends on the presentation method. As a percentage of total fatty acids, broiler thigh SFA were not different (30.60 vs. 31.27%), MUFA was higher (49.06 vs. 43.31%), and PUFA was lower (15.51 vs. 18.87%) than breast muscles of the corresponding fatty acid groups [58]. In contrast, when fatty acid content was expressed in mg/100 g of muscles, broiler thigh muscles contained more SFA (1530 vs. 300 mg/100 g), MUFA (2460 vs. 410 mg/100 g) and PUFA (780 vs. 180 mg/100 g). Likewise, in turkey thigh muscles the sum of SFA, MUFA (725 vs. 441 mg/100 g), PUFA, and EPA and DHA (18.2 and 14.6 mg/100 g) and arachidonic acid (C20:4n6) (72.7 vs. 50.3 mg/100 g) was substantially higher than in breast muscles, due to the higher total lipid content in thigh compared to breast muscles (2.45 vs. 1.47%) [90]. These values differ from that expressed as a percentage of total fatty acids for turkey muscles as shown in Table 32.12. Turkey breast muscles contained 5% less SFA, nearly 40% less MUFA, and approximately the same amount of PUFA compared to broiler breast muscles [17,90]. The EPA+DHA values for turkey and chicken breast muscles

were similar. However, turkey thigh muscles contained less EPA+DHA than broiler thigh muscles (18.2 vs. 31 mg/100 g) due to the substantially lower total lipid content, i.e., 2.45 vs. 7.30%, respectively. The content of arachidonic acid (C20:4n6) in turkey breast muscles (50.3 mg/100 g) was higher by nearly 60% in comparison to chicken breast muscles (32.1 mg/100 g).

A. FFA—Age, Sex, and Strain

Fatty acid composition of broilers, turkeys, ducks, and geese was affected by age and sex. A comparison of 13 wk fast growing broilers with 5 wk broilers showed that older birds had significantly lower MUFA content in breast muscles, and the levels of PUFA and arachidonic acid (C20:4n6) were higher in breast muscles and lower in thigh muscles, respectively [92]. Advancing age of male and female turkey was accompanied by increased MUFA and decreased PUFA percentage in breast and thigh muscles while the percentage of SFA decreased in breast muscles and increased in thigh muscles, respectively (Table 32.13) [49]. Moreover, with increasing age the content of sum of EPA+DHA decreased in both male muscles, but did not differ in female muscles. The arachidonic acid (C20:4n6) content decreased with age in breast muscles of turkeys of both sexes, but not in thigh meat. With age duck thigh muscle lipids had a lower level of MUFA and higher levels of PUFA and linoleic acid (C18:2n6). Age had no influence on SFA content in Pekin duck thigh muscles, but in Muscovy and Mule duck the SFA level decreased with age (Table 32.13). During the time of rearing of geese from 6–30 wk the fat of male and female breast, muscles decreased gradually with the content of SFA by 3.0–3.4% and stearic acid (C18:0) by 4.9%. The share of MUFA and palmitoleic acid (C16:1) increased by 1.1–3.0% and by 1.1%, respectively (Table 32.14) [114]. However, the PUFA, linoleic acid (C18:2n6), and arachidonic acid (C20:4n6) were not influenced by age. Friend et al. [48] found no differences due to age in goose fatty acid composition.

The male broiler breast muscles concentration of total PUFA and PUFA n-3 was lower than in female breast muscles while the male thigh muscle concentration of MUFA was lower and total PUFA and PUFA n-3 were higher than in female thigh muscles. The level of arachidonic acid (C20:4n6) in male broiler muscles was higher than for female muscles [77]. At the same age, male turkey breast muscles were characterized by lower MUFA content and thigh muscles had lower contents of both MUFA and PUFA than corresponding female muscles [49].

The breast muscle lipids of 16 wk male geese males were characterized by more favorable composition of fatty acids compared to females due to the higher share of MUFA and lower level of SFA (Table 32.14) [114].

Geese strains influenced the composition of the following fatty acids: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2n6), and arachidonic (C20:4n6) [48]. Batura et al. [50] also found geese strain dependent on differences in the level of myristic acid (C14:0), linoleic acid (C18:2n6), and arachidonic acid, (C20:4n6).

IX. CONCLUSIONS

Muscles highest in protein content were turkey, broiler and goose breast muscles while the lowest in fat were breast muscles of turkeys, broilers, and Pekin-Muscovy ducks. The best proportion of protein to fat was in the duck thigh muscles. Goose and turkey muscles had the lowest moisture content.

The broiler muscles were the highest in niacin. Duck and goose flesh were the best sources of thiamine (B_1) and riboflavin (B_2). The broiler and turkey thigh/leg or dark muscles contained more riboflavin (B_2) and less niacin (PP) and vitamin B_6 than breast/light muscles. Poultry meat is also an important source of several minerals, especially iron (goose muscles, turkey dark), zinc (turkey dark), potassium and phosphorus (goose flesh), and other trace elements such as copper.

Lipids of white poultry meat (broilers, hens, geese) were richer in SFA and PUFA and the lipids of red meat were richer in MUFA. Therefore, poultry consumption, especially breast muscle, could provide an important additional source of nutritionally important unsaturated fatty acids (PUFA).

Poultry breast muscles have higher nutritional and dietetic values than thigh muscles (lower lipid and collagen content and higher protein content).

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33 Poultry Processing Quality

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CONTENTS

I. INTRODUCTION

Per capita consumption of poultry has increased dramatically during the last 40 years with poultry meat ranking as the most consumed muscle food per capita in the United States. This increased consumption in poultry compared to other meats can be attributed to vertical integration, an increase in value-added product offerings, and nutritional quality. In vertically integrated companies, the same company owns several or even all of the process from live production through slaughter, and distribution to retail or foodservice. Vertical integration has improved product uniformity and increased profit margins for the poultry industry compared to other meat industries. The poultry industry has also increased the production of value-added poultry products to meet consumer demands for convenience, versatility, and variety. Also, nutritionally, the fat in both chicken and turkey meat is easily removed compared to other meats, enabling consumers to adopt a more low-fat type of meat into their diets. The process of converting the live animal into meat is complex. Many steps are implemented to ensure uniformity and high quality for the consumer. This chapter will detail the steps required to convert a live bird into a convenient and versatile product for consumers.

II. SLAUGHTER

Poultry slaughter involves six primary unit operations: preslaughter, immobilization, feather removal, evisceration, chilling, and further processing. In each of these processes, bird uniformity and automation can affect both quality and efficiency (yield). Normally, plants process birds at 70–140 birds per minute depending on the inspection system used, the type of birds (broilers versus turkeys), and the uniformity of the birds.

A. PRESLAUGHTER

The United States Department of Agriculture (USDA) has a "Zero Tolerance" for fecal contamination of poultry

carcasses in the Pathogen Reduction/Hazard Analysis and Critical Control Point System (HACCP) (1). Therefore, birds are removed from feed, but allowed access to water 8–12 hours prior to slaughter to allow for clearing of the intestinal tract (2). This time for clearing of the gastrointestinal tract helps prevent some fecal contamination of carcasses due to intestinal rupture by the automated evisceration/processing equipment. Feed withdrawal fewer than eight hours can result in fecal contamination and full crops, which can lead to contamination of the carcass during evisceration and possible condemnations (3). Feed withdrawal greater than 12 hours prior to slaughter can result in loose feces, green gizzards, and bloated intestines, which leads to downgrades and meat yield loss for processors. Also, the longer the feed withdrawal period, the more water weight is lost, resulting in a decreased yield for processors. Therefore, feed withdrawal should be within the 8–12 hours prior to slaughter to allow for the clearing of the intestinal tract, and to minimize yield losses (4).

Antemortem handling of the birds preslaughter can affect meat quality and consumer acceptability of the product. The largest meat quality determinants at this antemortem stage are handling and stress to the bird. Improper handling can result in bruises, broken and dislocated bones, and variation in breast meat color, all resulting in decreased yield for the processor through carcass downgrading. The areas most frequently bruised are the breast, wings, and legs (5). An estimate of 90–95% of all bruises found on broiler carcasses occurs during the final 12 hours prior to slaughter (6). This period is normally when the birds are caught, cooped, transported to the plant, and unloaded for slaughter. Ages of bruises are easily determined and can be used at the plant to determine problem areas either at the farm or during catching, transportation, or unloading. During the initial formation of a bruise, the area appears bright red, followed by purple, yellow, green, and orange before returning to the normal color of the tissue (7, 8).

Increased stress due to catching and transportation may also lead to poor meat quality and reduced yield. Most birds are usually caught and cooped during the evening to early morning hours to ensure cooler temperatures and to reduce stress. Several broiler companies use automated methods to catch birds. These machines "scoop" birds from the floor of the house into coops minimizing bruising and broken bones. However, most turkey facilities are still completely manual when catching birds due to their large size. Transportation of the birds may lead to increased stress and can result in poor meat quality. This will be discussed in more detail later in the chapter.

B. Immobilization – Stunning, Exsanguination

Following arrival at the processing plant, the birds are unloaded from the transportation trucks onto a conveyor line through an automated "dumping" mechanism. However, turkeys are normally unloaded from the trucks by hand. Both methods can cause bruising and broken bones if the animal is handled improperly. Once on the conveyor, the birds enter the plant and are hung on shackles prior to slaughter. The hanging room is usually a dark room with red or black lights to offer a calming effect on the birds to reduce stress. Once shackled, the birds are electrically stunned in a saline (approximately 1% NaCl) water bath to render the bird unconscious. Electrical stunning is the most common method of bird immobilization in the U.S. because it is inexpensive, convenient, and safe (9, 10).

Electrical stunning produces a uniform heartbeat for better bleeding, immobilizes the bird for slaughter, and relaxes the feather follicles for better picking. Normally, the birds are stunned for 10–12 seconds (10–20 mA per broiler; 20–40 mA per turkey) to produce a state of unconsciousness for 60–90 seconds (11). Stunning parameters with excessive amperage can cause downgrades, while too low of an amperage can cause poor bleeding, poor meat quality, and improperly killed birds. Therefore, proper stunning amperages are important determinants of good quality meat.

Stunning parameters and methods vary from country to country. For example, the European Economic Community requires birds to be irreversibly stunned by using saline bath electrical stunning methods that deliver approximately 100 mA per bird. This high amperage can cause broken clavicles, blood splash, rupture femoral arteries, and other downgrades (12). Brazil and some European countries have replaced high amperage electrical stunning with gas stunning. Gas stunning utilizes argon and carbon dioxide mixtures to displace oxygen and cause hypoxia in birds. Meat quality from gas stunned birds and low amperage stunned birds is better in comparison to high amperage electrical stunning because of the prevention of blood splash and broken bones (13, 14).

To reduce blood splash and ruptured arteries, bleed out must be performed within 10 seconds of low amperage electrical stunning. Exsanguination (or bleed out) is the period of blood removal which usually lasts 1.5 to 3 minutes. Following stunning, the head of the bird is guided through a set of bars for proper presentation to the cutting blade (Figure 33.1). Most of these automated cutting blade machines use a single unilateral neck cut which severs the right carotid artery and jugular vein. However, some processors use a rotating blade to sever both carotid arteries and jugular veins for a more complete bleeding efficiency. Either way, the blood volume lost is 30–50%, mainly from the major arteries and veins (11). This loss of blood volume causes brain failure and eventually death to the bird. Insufficient bleeding can result in poor meat quality and discoloration of the skin during scalding. Flavor is the most affected quality parameter associated with improper bleeding with the development of a gamey flavor due to the residual blood remaining in the muscles.

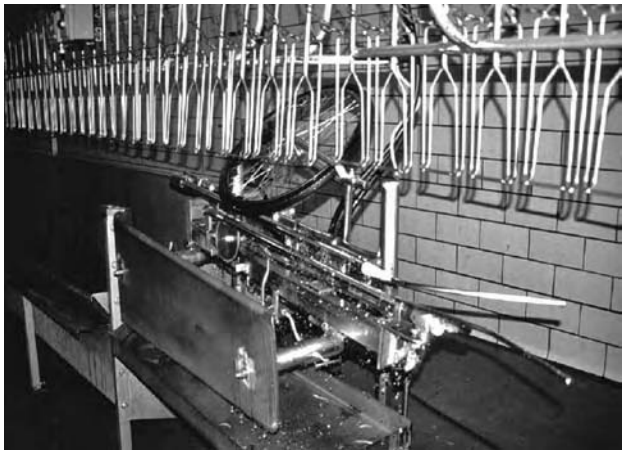

FIGURE 33.1 Killing machine indicating the bicycle wheel and guide bars used for alignment. Reprinted with permission from Poultry Meat Processing, 2002. Ed. AR Sams, Copyright CRC Press, Boca Raton, Florida.

C. Feather Removal – Scalding, Picking

Feather removal is a two-step process – scalding and picking. Scalding is time and temperature dependent with two variations, soft scald and hard scald, depending upon the intended use of the carcass or parts. Soft scald is immersion of the carcasses in 53.35°C (128°F) water for 120 seconds (11). Soft scalding loosens the feathers but does not disrupt the outer skin layer or the cuticle (waxy, yellow-pigmented outer layer). Thus, a yellowish coating is retained on the skin of the bird. Soft scalded birds can be difficult to pick because the feather follicle is not loosened fully. Also, a quality problem can exist in further processed products with soft scalded birds because the cuticle prevents coating adhesion on batter and breaded products.

A hard scald is immersion of the carcass in 61–63°C (140–145°F) water for 45 seconds. The cuticle layer is removed through this hard scald process. Feathers are also easier to remove compared to the soft scald. Also, there are fewer quality problems with hard scalding since coated products have better batter adhesion without the cuticle layer. However, appearance may be a problem for those consumers that prefer the "yellow" tinted soft scalded birds.

Feather removal (picking) is completed by rotating rubber fingers which grasp the feathers and remove them (Figure 33.2). Proper picking is dependent upon the time the carcass spends in the picker as well as the speed of the rotating fingers. Over picking may cause downgrades and mutilations (carcass that have no salvageable parts) while under picking may leave feathers on the carcass resulting in downgrading further down the processing line. Therefore, feather removal is important in maximizing quality and yield of the carcass.

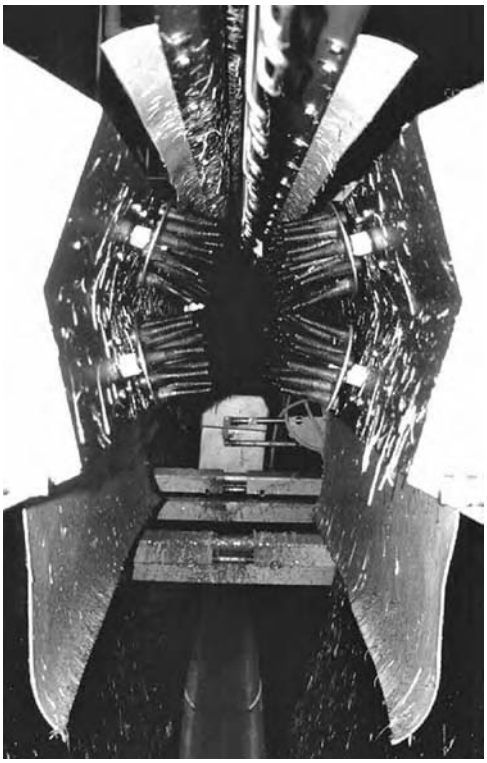

FIGURE 33.2 Picking cabinet with rubber fingers used to remove feathers. Reprinted with permission from Poultry Meat Processing, 2002. Ed. AR Sams, Copyright CRC Press, Boca Raton, Florida.

D. Evisceration – Viscera Removal, Inspection

Prior to actual evisceration, the head, feet, and preen gland (oil gland located at the base of the tail) are removed. Evisceration, or the process of removing viscera from the carcass, is normally automated in chicken plants but is still performed manually in most turkey plants. With either method, the viscera is exposed and removed from the internal body cavity. Once exposed, the USDA inspector examines the carcass for field or plant condemnations and fecal contamination. Condemned birds are usually those with obvious signs of disease as well as cadavers (birds that have died for reasons other than exsanguinations). These birds are disposed of and do not enter the production chain. Fecal contaminated birds must be reprocessed and or trimmed prior to re-entering the production chain. The inedible viscera is then removed from the carcass and discarded while the edible viscera (heart, liver, and gizzard) are collected, cleaned, chilled, and packaged for retail or fast food restaurants. Necks are removed from the birds and lungs are vacuumed out of the body cavity by automated machines. Birds without their viscera are then visually inspected (final inspection) by trained plant personnel for such things as surface lesions, tumors, and bruising. Any imperfections will be trimmed and washed prior to the bird re-entering production.

To ensure high quality of meat, the most important parameter in evisceration is dependent upon the uniformity of the birds and proper machine adjustment. Improperly adjusted machines or flocks with very little uniformity in size or shape can cause an increase in number of mutilations and downgrades. Both of these scenarios cause decreased yield and profit margins for the processor. For this reason, processors utilize specific genetic lines of broilers or turkeys to minimize size variation within the plant.

E. Chilling

Poultry meat must be chilled to 4.4°C within 4 hours of slaughter for 4 lb. broilers, 6–8 hours for 4 to 8 lb broilers and within 8 hours for broilers greater than 8 pounds and for turkeys. In order to accomplish this, two methods are utilized, immersion or air chilling. Air chilling is not the most common method in the United States for poultry carcasses; however, it is used extensively in Europe, South America, and Canada. With air chilling, the birds remain on the shackles following evisceration and enter a room with temperatures ranging from −7°C to 2°C (15). The carcasses remain in this room for 1–3 hours and may have a continual spray mist to help reduce temperatures of the carcass as well as prevent extra moisture loss and reduction in carcass yield. Even with misting, air chilled birds normally have a dry appearance to their skin which disappears during rehydration. However, air chilled birds normally have a lower pathogen load and have an increased shelf-life when compared to immersion chilled carcasses (16, 17).

Immersion chilling is the most common method of chilling poultry in the U.S. Carcasses are removed from the shackle following evisceration and are placed in staged water tanks for a period of about 80 minutes (sometimes longer for turkeys). During immersion chilling, the carcasses are moved through a continuous chilling system by paddles or an auger, through increasingly colder and cleaner water (counter-current flow system) (Figure 33.3). An air bubble agitation system is also employed that decreases the thermal layering effect cooling the carcasses at a faster rate (Figure 33.4). This counter-current flow method of chilling increases water uptake, increases heat exchange, and provides a method of cleaning the bird. Immersion chilled birds have a lower total microbial count than air chilled carcasses due to the washing effect of the immersion. However, there are an increased number of pathogen-positive birds due to cross-contamination issues related to tank sharing during the production day. It is important to note that antimicrobials are used during the chilling process to decrease bacteria, especially pathogens. Chlorine is the most common antimicrobial used in immersion chilling at a level of 20–50 ppm as allowed by FSIS.

FIGURE 33.4 Water immersion chilling tank showing air hoses used to agitate water. Reprinted with permission from Poultry Meat Processing, 2002. Ed. AR Sams, Copyright CRC Press, Boca Raton, Florida.

Immersion chilling involves three steps. The first stage is referred to as pre-chilling and can be a separate tank in some processing plants. This separate tank acts as a "bird wash" to decrease microbial load and debris in the subsequent tanks. The temperature of the pre-chill is around 12°C (55°F) while the other stages are 4–7°C (40–45°F) and finally 1°C (30–34°F) prior to exit of the birds from the chiller and into second processing. During the pre-chill stage, birds absorb some amount of water in their skin while the final stage allows pores in the skin to close trapping the water inside and increasing the weight of the bird.

Postmortem temperature is an important processing factor involved in determining meat quality. Improper chilling of the bird can cause color defects in the meat

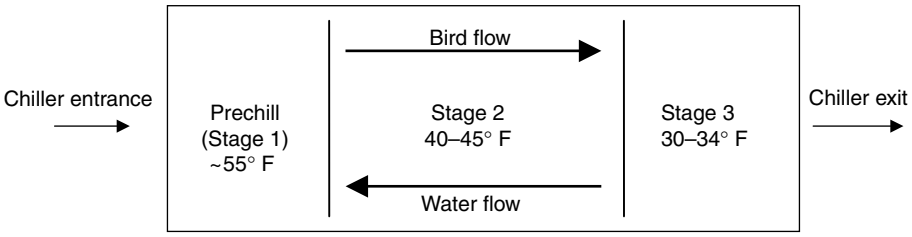

FIGURE 33.3 Immersion chiller system showing the counter-current flow of water and birds.

know as pale, soft, and exudative (PSE) meat. The development of PSE meat is caused by protein denaturation resulting from a rapid rate of pH decline while carcass temperatures are still elevated. The elevated carcass temperatures may have a more damaging effect when meat pH is below 6.2 (18). Carcasses with a normal pH decline may develop PSE meat if improperly chilled (19, 20). Rapid chilling decreases protein denaturation and can significantly reduce the development of PSE meat. Therefore, processors should chill carcasses rapidly early postmortem to prevent poor meat quality problems. Achieving a breast muscle temperature of less than 36°C (95°F) by 60 minutes postmortem and 28°C (82°F) by 90 minutes postmortem may reduce the PSE-like characteristics in turkeys (21).

A recent change in the poultry industry has occurred with a new regulation regarding moisture absorption and retention. The Food Safety Inspection Service has issued a final rule requiring that plants produce poultry products with either no retained water or only the minimum amount required to meet food safety requirements (22). The reasons for this rule deals with both complaints from consumer groups and inconsistencies with red meat processing. Consumer groups have long argued that retained water in poultry meat and subsequent leakage from the meat into packages could lead to spillage and contamination in the home. This increased contamination could result in an increase in food-borne illnesses from *Salmonella* and *Campylobacter.* The inconsistencies in meat processing have focused on the different methods of chilling carcasses. Meat carcasses (beef, pork, and lamb) are air chilled not immersion chilled so water is not retained in these meat products as in poultry carcasses. Actually, water (moisture) is reduced due to shrinking that occurs with air chilling these carcasses. Therefore, the red meat industry has argued that poultry meat is "adulterated" because water has been added to increase its bulk weight and therefore consumers are paying for water along with meat weight. If water is not regulated to be at a minimum uptake during chilling, then the product is not only adulterated but misbranded. Therefore, FSIS has proposed this regulation to help make the meat processing and poultry processing more consistent.

F. Second Processing

Following temperature reduction of the carcasses, the birds are hung on shackles and either packaged as whole birds or cut into parts through automated processes. Adding value to carcasses by cutting them into parts has revolutionized the poultry industry (Table 33.1). In 2000, the predominant form of chicken marketed in the United States was parts (11). This trend started more than 30 years ago when consumers demanded more versatility and variety in chicken meat. At this time, consumers were mostly made up of two income families and there was less time for preparation of meals and cutting up of whole birds. Therefore, as we have entered into the 21st century, this trend of increasing versatility and variety has continued. From a processor stand point, adding value by cutting whole carcasses into parts increases profit margins and consumers are willing to pay an increased price for convenience.

TABLE 33.1
Commonly Used Configurations for Parts of a Chicken Carcass in the U.S.

Part	Description
Half Carcass	Carcass split evenly into right and left halves
Breast Quarter	Anterior right or left quarter containing half of the spine, the ribs, the pectoralis muscles (major and minor), and the attached wing
Leg Quarter	Posterior right or left quarter containing half of the spine, the thigh, and the drumstick
Wing	The three segments of the wing with a variable amount of the breast meat (depending on the customer)
Breast	The major and minor pectoralis muscles with or without rib and sternum bones or skin
Thigh	The upper part of the leg containing the femur
Drumstick	The lower part of the leg containing the tibia and fibula
Drumette	The inner portion of the wing
Wing Portion	The middle section of the wing, with or without the outer "flipper or wing tip" portion still attached
Whole Breast	The anterior half of the carcass without wings, with both breasts still connected in front and with or without the spine connecting them in the back
Keel Piece	The pointed posterior tip of the whole breast before splitting (approximately one third of the whole breast)
Breast Piece	After the removal of the keel piece from the whole breast, the remaining part is split into right and left halves
Whole Leg	Drum and thigh with no spine
Back or Strip Back	Spine and pelvis, production of quarters puts the back as part of the respective quarters
Breast Half or Front Half	The entire, intact, anterior half of the carcass
Leg Half, Back Half, or Saddle	The entire, intact, posterior half of the carcass

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G. Deboning

Following second processing, any birds not sold as whole carcasses or parts for retail are usually stored in tubs on ice in a 4°C cooler for 8–12 hours. This storage time allows for the development of rigor mortis and helps to decrease the toughness associated with early deboning. Once aged, the carcass can be either deboned manually or automatically by specialized equipment. Deboning method is the same for either automatic or manual operations: the breast fillet is stripped from the carcass. The wings are then sold as parts and the butterfly can either then be sold or split into two fillets as a less expensive part.

Deboned broiler beast meat is the most valued portion of the carcass and has the highest scrutiny from the consumer. Since this product is normally free of skin, all discolorations and blemishes (bruises) are evident and highly scrutinized by consumers. The three toughest challenges to the processor with deboned broiler breast fillets are portion control, uniformity, and toughness. Portion control is important in the food service industry (restaurants, hotels, etc.) where every consumer should receive the same amount of meat to allow for better cost estimation and meat ordering. In order to accomplish this portioning, scales are used in the deboning section of plants so all deboned broiler breast fillets are automatically weighed and sold based on uniformity of weight.

To ensure uniformity of size, the breast fillets can also be hand trimmed of excess fat, connective tissue, and even meat. Several restaurants require the meat to be a specific shape and size, so, either manually or by specialized equipment, the breast fillet is cut either horizontally or vertically to achieve the desired shape as defined by the customer. Automated equipment is now available to quickly create specific dimensions of the fillets by water knives. This specialized sizing is costly to the consumer because it is very labor intensive.

Uniform tenderness is a very challenging consumer concern for the poultry industry. Rigor mortis development is discussed in detail in other chapters. However, the development of rigor mortis takes 4–6 hours in broilers. Any stimulation to the muscle prior to the development of rigor mortis can cause an increase in toughness. For this reason, carcasses, or even front halves, are aged on the bone in a cooler for a minimum of 4 hours to overnight to prevent toughening. This aging time is expensive and labor intensive and can even cause a decrease in yield from water loss up to 2–3% (23).

To alleviate this toughness associated with early deboning, several plants have implemented electrical stimulation of the carcass following death or marination techniques following chilling. Electrical stimulation (ES) is different from stunning in that ES requires higher amperage and it is applied to the carcass immediately after death. When applied, electrical stimulation causes muscle contractions which uses energy stored in the muscle and allows rigor mortis to develop at a faster rate. Therefore, storing carcasses to prevent toughness associated with early deboning is no longer needed. Another method used to alleviate toughness is marination or the addition to salt and phosphates to increase juiciness of the meat. This increase in juiciness results in a more tender product. Marination will be discussed further in this chapter.

III. GRADING

A. Classification

Each species of poultry (chickens, ducks, geese, guineas, and pigeons) is divided into classes by their physical appearance mainly associated with age and sex. Based on these classes, quality grades are then established based on the quality of individual carcass and parts as will be discussed later in the chapter.

B. Standards for Grading

One way to add value other than cutting whole carcasses into parts is to grade carcasses or sort them by specific conditions and quality characteristics. The Agricultural Marketing Service (AMS) is a branch of the USDA responsible for poultry grading. Following mandatory inspection for wholesomeness (Figure 33.5), each bird is examined to determine eating quality and uniformity. Grading is voluntary, unlike inspection by FSIS. Therefore, the processing plant must pay for personnel from AMS to grade carcasses, thus increasing the price of the graded product for consumers. However, consumers are willing to pay for the added increase to ensure good eating quality and uniformity with tenderness, juiciness,

FIGURE 33.5 USDA inspection shield for wholesomeness.

color, and meatiness. Grading has become an industry standard and is almost expected from consumers.

Once graded by an authorized grader, the official USDA grade mark is used on packages and is easily identified by consumers (Figure 33.6). The highest quality grade is USDA Grade A, followed by USDA Grade B and then USDA Grade C. The standards for USDA Grade A, B, and C carcasses and bone in parts are listed in Table 33.2 (24). USDA Grade A is usually in retail packages and consumers are mostly familiar with this grade. Downgraded carcasses, USDA Grades B and C, are usually further processed because of some deformities or defects. One way to add value to downgraded carcasses is to cut them into parts for retail.

IV. QUALITY CHARACTERISTICS

Three main characteristics of poultry meat determine overall meat quality: tenderness (texture), water holding capacity, and color.

A. Tenderness

Tenderness is one of the most important meat quality characteristics. Several factors influence the tenderness of whole muscle meat products. These factors include the contractile state of the muscle, the amount of connective tissue, and juiciness. In market age broilers (6 weeks), probably the most important influence is the contractile state of the muscle. Most of the tenderness variation due to contractile state in meat from young broilers is related to the conversion of muscle to meat (rigor mortis development). During this conversion, which begins at the time of slaughter, muscle pH declines due to an accumulation of lactic acid in the muscle. In addition, ATP content declines as the muscle shifts from aerobic to anaerobic metabolism. This rigor mortis period takes approximately 4–6 hours in broilers (25, 26). If the muscle is stimulated by deboning or extreme cold temperatures (cold shortening) prior to rigor completion, the muscle can have some degree of contraction possibly without enough energy present for muscle relaxation (depending on what time the stimulation occurred). Therefore, the muscle would shorten thus altering the state of contraction; the denser the muscle, the tougher it will feel to consumers.

FIGURE 33.6 Grade mark from USDA Agricultural Marketing Service.

The connective tissue component of meat tenderness does not play a major role in young broilers. However, it does affect older animals (such as spent fowl) to a greater extent because as the animal ages, heat-stable crosslinks in the collagen form that will not melt during cooking. Juiciness is important in meat tenderness as well because it provides lubrication and mouth feel which are important from a consumer sensory perception. Cooking method is the primary factor impacting juiciness. For example, overcooking which can cause excess moisture loss can decrease tenderness or perceived tenderness by consumers. Any cooking method in which the product can lose excess moisture (example: uncovered grilling compared to covered baking) can decrease the juiciness and therefore decrease tenderness of the product.

B. Water Holding Capacity

Water holding capacity (WHC) is another important characteristic of meat quality. Water holding capacity can be defined as the ability to retain inherent water in the meat or added water (observed in further processed items). Water can be lost from meat through gravitational (storage, drip loss), mechanical (cutting), or thermal (cooking) forces. Differences in meat quality are directly related to how the meat can handle the application of these forces.

Water exists as three forms in the muscle: bound, immobilized, and free. Bound water is closely associated with the muscle proteins. Because water molecules are polar, they associate with the electrically charged reactive groups of the proteins. This water layer is so tightly bound that it can only be removed by incineration (high temperatures). The second form of water is known as immobilized. This water layer is attracted to the bound layer of water, but the attraction is much weaker. The attraction also decreases as the distance to the proteins increase. The third form of water is known as free water and accounts for the majority of the water in meat. Free water is loosely held by capillary forces and is easily lost during processing procedures such as cutting, grinding, storage (drip), and cooking.

Water holding capacity is affected by pH (net charge effect) and the amount of space between the muscle protein structures (steric effect). The postmortem decline in pH results in a loss of reactive groups to bind water due to reduced net protein charge and reduced protein solubility. As pH decreases in meat, it nears the isoelectric point (pI) of muscle proteins (approximately 5.1). The closer the pH is to the pI of the meat proteins, the number of charged sites on the proteins that normally bind water decreases. Examples of changes in WHC due to pH are pale, soft,

TABLE 33.2
Summary of Specifications for Grading A, B, and C Grade Carcasses

Ready-to-Cook Poultry — A Quality
Summary of Specifications for Standards of Quality for Individual Carcasses and Parts
Effective April 29, 1998 (Not All Inclusive — Minimum Requirements and Maximum Defects Permitted)

A Quality

Conformation:	Normal
Breastbone	Slight curve or dent
Back	Slight curve
Legs and Wings	Normal
Fleshing:	Well fleshed, considering kind and class
Fat Covering:	Well-developed layer — especially between heavy feathers tracts

Defeathering:	**Turkeys** (feathers less than 3/4 in.)		**Ducks and Geese**[1] (feathers less than 1/2 in.)		**All Other Poultry** (feathers less than 1/2 in.)	
Free of protruding feathers and hairs	**Carcass**	**Parts**	**Carcass**	**Parts**	**Carcass**	**Parts**
	4	2	8	4	4	2

Exposed Flesh:[2] Weight Range		**Carcass**		**Large Carcass Parts**[3] (halves, front and rear halves)		**Other Parts**[3]
Minimum	Maximum	Breast and Legs	Elsewhere	Breast and Legs	Elsewhere	
None	2 lbs.	1/4 in.	1 in.	1/4 in.	1/2 in.	1/4 in.
Over 2 lbs.	6 lbs.	1/4 in.	1 1/2 in.	1/4 in.	3/4 in.	1/4 in.
Over 6 lbs.	16 lbs.	1/2 in.	2 in.	1/2 in.	1 in.	1/2 in.
Over 16 lbs.	None	1/2 in.	3 in.	1/2 in.	1 1/2 in.	1/2 in.

Discolorations: Carcass		**Lightly Shaded**		**Moderately Shaded**[4]	
		Breast and Legs	Elsewhere	Hock of leg	Elsewhere
None	2 lbs.	3/4 in.	1 1/4 in.	1/4 in.	5/8 in.
Over 2 lbs.	6 lbs.	1 in.	2 in.	1/2 in.	1 in.
Over 6 lbs.	16 lbs.	1 1/2 in.	2 1/2 in.	3/4 in.	1 1/4 in.
Over 16 lbs.	None	2 in.	3 in.	1 in.	1 1/2 in.

Discolorations: Large Carcass Parts (halves, front and rear halves)		**Lightly Shaded**		**Moderately Shaded**[4]	
		Breast and Legs	Elsewhere	Hock of leg	Elsewhere
None	2 lbs.	1/2 in.	1 in.	1/4 in.	1/2 in.
Over 2 lbs.	6 lbs.	3/4 in.	1 1/2 in.	3/8 in.	3/4 in.
Over 6 lbs.	16 lbs.	1 in.	2 in.	1/2 in.	1 in.
Over 16 lbs.	None	1 1/4 in.	2 1/2 in.	5/8 in.	1 1/4 in.

Discolorations: Other Parts		**Lightly Shaded**	**Moderately Shaded**[4]
None	2 lbs.	1/2 in.	1/4 in.
Over 2 lbs.	6 lbs.	3/4 in.	3/8 in.
Over 6 lbs.	16 lbs.	1 in.	1/2 in.
Over 16 lbs.	None	1 1/4 in.	5/8 in.

Disjointed and Broken Bones:	Carcass—1 disjointed and no broken bones. Parts—Thighs with back portion, legs, or leg quarters may have femur disjointed from the hip joint. Other parts—none.
Missing Parts:	Wing tips and tail. In ducks and geese, the parts of the wing beyond the second joint may be removed if removed at the joint and both wings are so treated. Tail may be removed at the base.
Freezing Defects:	Slight darkening on back and drumstick. Overall bright appearance. Occasional pock-marks due to drying. Occasional small areas of clear, pinkish, or reddish-colored ice.

[1] Hair or down is permitted on the carcass or part, provided the hair or down is less than 3/16 inch in length, and is scattered so that the carcass or part has a clean appearance, especially on the breast and legs.

[2] Maximum aggregate area of all exposed flesh. In addition, the carcass or part may have cuts or tears that do not expand or significantly expose flesh, provided the aggregate length of all such cuts and tears does not exceed a length tolerance equal to the permitted dimensions listed above.

[3] For all parts, trimming of skin along the edge is allowed, provided at least 75 percent of the normal skin cover associated with the part remains attached, and the remaining skin uniformly covers the outer surface and does not detract from the appearance of the part.

[4] Moderately shaded discolorations and discolorations due to flesh bruising are free of clots and limited to areas other than the breast and legs except for the area adjacent to the hock.

Source: USDA, 1998.

TABLE 33.2 (*Continued*)

Ready-to-Cook Poultry — B Quality

Summary of Specifications for Standards of Quality for Individual Carcasses and Parts
Effective April 29, 1998 (Not All Inclusive — Minimum Requirements and Maximum Defects Permitted)

	B Quality
Conformation:	Moderate deformities
Breastbone	Moderately dented, curved, or crooked
Back	Moderately crooked
Legs and Wings	Moderately misshapen
Fleshing:	Moderately fleshed, considering kind and class
Fat Covering:	Sufficient fat layer—especially on breast and legs

Defeathering:	**Turkeys** (feathers less than 3/4 in.)		**Ducks and Geese**[1] (feathers less than 1/2 in.)		**All Other Poultry** (feathers less than 1/2 in.)	
A few scattered protruding feathers and hairs	**Carcass**	**Parts**	**Carcass**	**Parts**	**Carcass**	**Parts**
	6	3	10	5	6	3

Exposed Flesh: Weight Range			
Minimum:	Maximum:	**Carcass**	**Parts**
None	2 lbs.	No part on the carcass (wings, legs, entire back, or entire breast) has more than 1/3 of the flesh exposed	No more than 1/3 of the flesh normally covered by skin exposed
Over 2 lbs.	6 lbs.		
Over 6 lbs.	16 lbs		
Over 16 lbs.	None		

Discolorations:[2] **Carcass**		**Carcass** Lightly or Moderately Shaded Discolorations	
		Breast and Legs	Elsewhere
None	2 lbs.	1 1/4 in.	2 1/4 in.
Over 2 lbs.	6 lbs.	2 in.	3 in.
Over 6 lbs.	16 lbs.	2 1/2 in.	4 in.
Over 16 lbs.	None	3 in.	5 in.

Discolorations:[2] **Large: Carcass Parts** (halves, front and rear halves)		**Large Carcass Parts** Lightly or Moderately Shaded Discolorations	
		Breast and Legs	Elsewhere
None	2 lbs.	1 in.	1 1/4 in.
Over 2 lbs.	6 lbs.	1 1/2 in.	1 3/4 in.
Over 6 lbs.	16 lbs.	2 in.	2 1/2 in.
Over 16 lbs.	None	2 1/2 in.	3 in.

Discolorations:[2] **Other Parts**		**Other Parts** Lightly or Moderately Shaded Discolorations
		Breasts, Legs, and Parts
None	2 lbs.	3/4 in.
Over 2 lbs.	6 lbs.	1 in.
Over 6 lbs.	16 lbs.	1 1/2 in.
Over 16 lbs.	None	1 3/4 in.

Disjointed and Broken Bones:	Carcass—2 disjointed and no broken bones, or 1 disjointed and 1 non-protruding broken bone. Parts—may be disjointed, no broken bones; wing beyond second joint may be removed at a joint.	
Missing Parts:	Wing tips, 2nd wing joint, and tail.	
Trimming:	**Carcass**	**Parts**
	Slight trimming of the carcass is permitted provided the meat yield of any part on the carcass is not appreciably affected. The back may be trimmed in an area not wider than the base of the tail to the area halfway between the base of the tail and the hip joints.	A moderate amount of meat may be trimmed around the edge of a part to remove defects.
Freezing Defects:	May lack brightness. Few pockmarks due to drying. Moderate areas showing a layer of clear, pinkish, or reddish-colored ice.	

[1] Hair or down is permitted on the carcass or part, provided the hair or down is less than 3/16 inch in length, and is scattered so that the carcass or part has a clean appearance, especially on the breast and legs.

[2] Discolorations due to flesh bruising shall be free of clots and may not exceed one-half the total aggregate area of permitted discoloration.

TABLE 33.2 (*Continued*)

Ready-to-Cook Poultry — C Quality
Summary of Specifications for Standards of Quality for Individual Carcasses and Parts
Effective April 29, 1998 (Not All Inclusive) (Minimum Requirements and Maximum Defects Permitted)

	C Quality
Conformation:	Abnormal
Breastbone	Seriously curved or cooked
Back	Seriously crooked
Legs and Wings	Misshapen
Fleshing:	Poorly fleshed
Fat Covering:	Lacking in fat covering over all parts of carcass

Deathering:	**Turkeys**		**Ducks and Geese**[1]		**All Other Poultry**	
Scattering of protruding	(feathers less than 3/4 in.)		(feathers less than 1/2 in.)		(feathers less than 1/2 in.)	
feathers and hairs	**Carcass**	**Parts**	**Carcass**	**Parts**	**Carcass**	**Parts**
	8	4	12	6	8	4

Exposed Flesh: Weight Range			
Minimum:	Maximum:	**Carcass**	**Parts**
None	2 lbs.		
Over 2 lbs.	6 lbs.	No limit	
Over 6 lbs.	16 lbs		
Over 16 lbs.	None		

Discolorations:[2]		**Carcass**	
Carcass		Breast and Legs	Elsewhere
None	2 lbs.	No limit	
Over 2 lbs.	6 lbs.	on size, number of areas, or intensity of discolorations	
Over 6 lbs.	16 lbs	and flesh bruises if such areas do not render	
Over 16 lbs.	None	any part of the carcass unfit for food.	

Discolorations:[2]		**Parts**
Parts		Breasrs, Legs, and Parts
(includes large carcass parts)		
None	2 lbs.	No limit
Over 2 lbs.	6 lbs.	on size, number of areas, or intensity of
Over 6 lbs.	16 lbs.	discolorations and flesh bruises if such areas
Over 16 lbs.	None	do not render any part unfit for food.

Disjointed and Broken Bones:	No limit
Missing Parts:	Wing tips, wings, and tails. Backs shall include all meat and skin from pelvic bones, except that the meat contained in the ilium (oyster) may be removed. The vertebral ribs and scapula with meat and skin and the backbone located anterior (for ward) of ilia bones may also be removed (front half of back).
Trimming:	**Carcass** **Parts** Trimming of the breast and legs is permitted, but not to the extent that the normal meat yield is materially affected. The back may be trimmed in an area not wider than the base of the tail and extending from the tail to the area between the hip joints.
Freezing Defects:	Numerous pockmarks and large dried areas.

[1] Hair or down is permitted on the carcass or part, provided the hair or down is less than 3/16 inch in length, and is scattered so that the carcass or part has a clean appearance, especially on the breast and legs.

exudative (PSE) meat, and dark, firm, and dry (DFD) meat conditions. Because the pH of PSE meat is closer to pH 5.1, it will have lower water holding capacity partly due to its lower pH resulting in fewer charges to bind water; however, DFD has high water holding capacity because its high pH is further away from the pI. Meat with high pH has a pH that is further away from the pI of myofibrillar proteins increasing the number of charged groups; therefore, the proteins have more reactive groups available to hold water.

Water holding capacity is also affected by a steric effect, or the amount of interstitial space between muscle proteins. The amount of this space can be influenced by pH and contractile state of the muscle. At higher muscle pH, further away from the pI, there are more charged ions in the muscle. These charged ions separate muscle proteins by repulsive force which then allows more physical space available to hold water. The contractile state of the muscle can also affect this space. For example, if muscle is in a contracted state, there is less interstitial space available to hold water. Any factor contributing to muscle shortening in the prerigor state such as early deboning can affect WHC.

C. Color

Color is an important meat quality attribute that can be affected by several factors including myoglobin concentration, oxidation state of the iron within the myoglobin, and muscle pH. Pinking in poultry has become a problem in recent years because consumers seem to associate the "pink" color to a food safety problem (undercooking of the chicken or turkey meat). However, pinking can occur as a result of addition of ingredients such as nitrite, improper stunning causing petechial hemorrhaging, and undenaturated myosin following improper heating. Nitrites are normally added to further processed products to add color and for food safety measures. However, during heating, a characteristic pink color develops in the product, which can sometimes be confused with undercooking. Insufficient heating can also cause some undenatured myoglobin pigments to retain a "pinkish" color which could present a quality problem to consumers. Another common reason for pinking is contamination of the meat with nitric dioxide and carbon dioxide from oven gasses. These combinations can cause pinking in meat often leaving the consumer wondering if the product is fully cooked. Pinking in poultry meat can be avoided by cooking meat to an internal temperature of 71–73°C, adding compounds that scavenge undenatured myoglobin components that cause pinking, and keeping burners cleaned and running efficiently (27).

Another common quality problem with color in poultry meat is pale, soft, and exudative meat. Denaturation of proteins, specifically the sarcoplasmic proteins, can cause increased scattering of light in the muscle resulting in lighter (or paler) meat. Shrinkage of myofibrils at low muscle pH levels causes greater scattering of light at the myofibril surface. The transmittance of individual muscle fibers is decreased at low pH resulting in less light absorption and paler meat. Furthermore, myofibrils are birefringent, or have two refractive indices, which cause light to take two different paths as it travels through the myofibrils. Muscle pH strongly affects the difference between the two paths with increased light scattering at low pH levels.

D. Relationship between Color and Water Holding Capacity

Color is also related to water binding capacity of the meat. Color of meat is discussed further in Chapter 31, "Poultry: Chemistry and Biochemistry." In general, pale, soft, and exudative (PSE) meat has a low pH; therefore, water binding is low because more water is contained in the extracellular space rather than intracellular space. With more water in the extracellular space, light will be reflected rather than absorbed. In poultry, muscle pH is highly correlated with L* value, a measurement of lightness. In the case of dark, firm, and dry (DFD) meat, meat color is darker due to the high pH. The high pH causes the meat to bind more water, and because of the high water binding, more light is absorbed rather than reflected and results in a darker color.

V. MARINATION

The easiest way to improve quality for consumers and improve the profit margins for processors is to marinate poultry parts, especially breast fillets. Marination of poultry breast meat continues to grow as the demand for further processed products increases. Marinades can be incorporated into meat by tumbling, mixing, or massaging the meat at low temperatures, thereby facilitating tenderization through disintegration of the muscle fiber sheath and stretching of the myofibrils. Commercial methods used to marinate meat including tumbling, blending, and injection. Each method has its advantages and disadvantages. Tumbling promotes rapid and consistent pickup at controlled temperatures, the ability to marinate large quantities, and the capability to handle many different products and sizes. Blending allows for finer control of product mixing as compared to tumbling, and the ability to directly apply refrigerant. Injection marination is beneficial for its relative consistency of marinade application on large and complex products, and reduction of labor and speed of marination.

The two key ingredients in commercial poultry marinades are salt and phosphate. Marination with a variety of combinations of salt and phosphates has been used as means to improve taste, tenderness, and protein functionality of broiler breast fillets. Marination has also been used to improve protein functionality losses imparted by the PSE condition (28).

One of the most important properties of poultry meat is its water holding capacity (ability to bind and retain innate water, as well as added water during marination). Pale, soft and exudative meat has poor water holding capacity which results in an economic loss for processors, as well as a decrease in consumer acceptance. Marination has been used as a method to increase the water holding capacity, thereby improving the quality and increasing the

yield of the meat. This increase in functional property is due to the marinade solution maintaining a higher pH postmortem.

Myofibrillar proteins, such as actin, myosin, and the actomyosin complex, are primarily responsible for WHC and marinade pick-up and retention. The salt and sodium tripolyphosphates commonly used in poultry meat marination work synergistically to increase water binding by increasing pH and ionic strength, combined with the dissociation of actomyosin, exposing more water binding sites (29). Basically, the salt and phosphate marinade uptake causes the tissue fibers to swell, resulting in decreased cooking loss and increased juiciness and tenderness of the meat.

Salt can affect proteins in many ways. At a low concentration, salt increases the WHC of proteins due to hydrated salt ions binding to charged groups of proteins. This resultant increase in water binding can be attributed to the water associating with bound ions. When salt binds to proteins, electrostatic repulsion causes the protein structure to loosen and allow more water binding. Salt also increases marinade pick-up by solubilizing the actin and myosin proteins which increases the space between the thick and thin filaments to pick up and retain marinade solution. Phosphates also increase WHC by increasing the number of charged sites on the protein for water binding to bind. The most common phosphates used in the poultry industry are sodium tripolyphosphates. However, several other phosphates are available with differing solubility, pH, and functional properties. An increased WHC allows for better uptake and retention of the marinade solution itself, as well as increasing the retention of the meat's own moisture, consequently increasing yield in an inexpensive manner.

The effect of various polyphosphate and salt (NaCl) solutions on myofibril protein extraction has been studied (30). In the absence of phosphates, no significant swelling or any other changes were noted at salt concentrations of 0.4 M or less. When salt concentrations were increased to 0.5 M and 0.6 M, myofibrils started to enlarge transversely and protein extraction became significantly noticeable.

The addition of polyphosphates induces changes in the protein extraction pattern. It is thought that polyphosphates possess an ATP-like property that allows polyphosphates to extract myosin and disintegrate part of the myofibril (30). Actomyosin is formed during rigor mortis and creates a state of permanent muscle contraction that leads to tough meat. Polyphosphates have the ability to dissociate this actomyosin complex, enabling myofibril lattices to expand laterally, resulting in an increase in water uptake. Tripolyphosphates were also shown to have similar effects. The researchers concluded that both poly- and tripolyphosphates are ionic species that dissociate myofilaments, depolarize thick filaments, and remove the actomyosin structural barricade, thereby enhancing cooking yield and juiciness.

VI. CONCLUSION

The process of converting muscle into meat is highly complex. The poultry industry has reduced this complexity some by vertically integrating the production and processing stages. This integration has helped improve uniformity and quality in poultry meat. However, as discussed in this chapter, there are very intricate steps in the conversion of the live animal into meat for consumers and quality can be affected at each step.

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34 Fats and Oils: Science and Applications

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CONTENTS

I. DEFINITIONS OF FATS, OILS, AND LIPIDS

In the scientific and even professional literature, the term lipids is more and more widely used. Lipids are classes of fatty acid derivatives. Their nomenclature is defined by the joint IUPAC-IUB standard (1). Fatty acids (aliphatic monocarboxylic acids) of 4 and more carbon atoms are usually bound as esters in plant and animal tissues, and only rarely as amides or free acids. Triacylglycerols, waxes, phospholipids, glycolipids, mucolipids, and lipoproteins are the most important lipid representatives. For many authors, especially in medical or biological sciences, lipids are exclusively naturally occurring fatty acid derivatives, but this definition is not useful for food science and technology because many closely related compounds, such as hydrogenated oils, structured lipids, fatty acid methyl or ethyl esters, and many oleochemicals do not occur in nature, but have been produced industrially. Aliphatic fatty acids are not the only organic acids bound in lipids because alicyclic or even phenolic acids were detected in natural lipids.

Substances like psychosin or lysoplasmalogens are included as lipids even when they do not contain any fatty acids. Long-chain fatty alcohols naturally occur in the liposoluble fraction of foods. Their properties and chemical structures are close to those of fatty acids, and therefore they are often treated as lipids. The same is true of sterols, but it is preferable to classify steroes as terpenic derivatives or as a separate class of compounds. Sterols are often esterified with fatty acids so that sterol esters are formed. They are classified as lipids because they contain bound fatty acids. Ethyl or higher esters of fatty acids (4–12 carbon atoms) are often present as natural components in fruits or are produced by fermentation in alcoholic beverages. They have specific aromas. They are, however, not considered lipids. It is evident from this discussion no generally accepted definition of lipids exists.

Fats and oils are lipophilic products obtained by processing natural materials of both plant and animal origin. Fats are usually of animal origin, and most oils are of plant origin, but animal oils such as fish oils exist, too. Fats are solid at room temperature while oils are liquid. The classification depends on the ambient temperatures; some products of tropical trees, such as palm or coconut oils, are liquid in the country of origin, but are solid in a temperate climate. Some semisolid lipid products are called butters, such as cocoa butter or shea butter, because their consistency is similar to butter in the country of origin. Officially, only a product of milk fat may be called butter; butters of vegetable origin should be better called fats.

Fats and oils are not pure compounds because they consist of all liposoluble substances extracted during processing of biological materials. They mostly consist of triacylglycerols (more than 95%), which are lipids, but they are accompanied by other liposoluble nonlipidic products, such as sterols, hydrocarbons, or liposoluble vitamins. Essential oils may be present in traces, but they are not lipids as they mainly consist of terpenes. For this reason, it would be preferable to use the terms fats and oils only for industrial products, and to use the term lipids for classes of pure substances. Of course, professionals in English-speaking countries often are not very careful concerning terminology.

II. FATTY ACIDS

A. Definition

Fatty acids bound in lipids are mostly monocarboxylic acids with an aliphatic straight hydrocarbon chain. Branched fatty acids are rare, and are present almost exclusively as minor components. Odd carbon number fatty acids are also present only exceptionally. Fatty acids differ in the number of carbon atoms and double bonds. Some fatty acids are substituted by oxygen groups and even by other functional groups. Fatty acids with less than 4 carbon atoms have not been found in natural lipids.

B. Saturated Fatty Acids

Saturated fatty acids have no double bond in their hydrocarbon chain. They have both systematic and trivial names, usually reflecting their origin (Table 34.1). The most common saturated fatty acid is palmitic acid, present in all lipids, at least in a small amount. Even carbon-number saturated fatty acids are mostly present in natural lipids, while odd carbon-number acids are present only as traces or as no more than minor acids in some fats. Short chain acids (1–3 carbon atoms) occur in nature, but are not bound in natural lipids. They may be found in lipids from some industrial products. Medium chain saturated acids (4–10 carbon atoms) are liquid at room temperature. Higher saturated acids are solid with their melting points increasing with the increasing carbon number.

C. Monounsaturated Fatty Acids

Monounsaturated fatty acids contain a double bond in their hydrocarbon chain. They have both systematic and trivial names. In the case of unsaturated fatty acids, isomerism is possible. The double bond exists in *cis* (*Z*) or trans (*E*) configuration, and the double bond may be located at different carbon atoms (Table 34.2). Natural unsaturated fatty acids are mostly of the *cis*-configuration. Traces of trans-unsaturated fatty acids (0.04–0.05%) are detected even in cold-pressed edible oils (2). The position of double bonds is usually counted from carboxyl carbon, but in texts dealing with nutrition, calculation is often from the final methyl group. In such cases, they are defined, e.g., as an n-6 or ω-6 acid, if the double bond is located at the 6th carbon atom from the final methyl group. The most common unsaturated fatty acid is oleic acid (an n-9 fatty acid), which is present in nearly all lipids at least in small amounts. Monounsaturated acids

TABLE 34.1
The Most Important Saturated Fatty Acids (Compiled from Beilstein and Chemical Abstracts Data Bases)

Systematic Name	Trivial Name	Number of Carbon Atoms	Molecular Weight	Melting Point [°C]
Butyric	Butyric	4	88.1	−4.6
Hexanoic	Caproic	6	116.2	−1.5
Octanoic	Caprylic	8	144.2	16.3
Decanoic	Capric	10	172.3	32.4
Dodecanoic	Lauric	12	200.3	43.8
Tetradecanoic	Myristic	14	228.4	54.4
Hexadecanoic	Palmitic	16	256.4	62.6
Octadecanoic	Stearic	18	284.5	70.4
Eicosanoic	Arachic	20	312.5	75.8
Docosanoic	Behenic	22	340.6	80.2
Tetracosanoic	Lignoceric	24	368.6	84.2
Hexacosanoic	Cerotic	26	396.7	87.7

TABLE 34.2
The Most Important Monounsaturated Fatty Acids (Compiled from Beilstein and Chemical Abstracts Data Bases)

Systematic Name	Trivial Name	Number of Carbon Atoms	Molecular Weight	Melting Point [°C]
9-*cis*-Hexadecenoic	Palmitoleic	16	254.4	34.5
6-*cis*-Octadecenoic	Petroselinic	18	282.5	31.5
9-*cis*-Octadecenoic	Oleic	18	282.5	16
9-*trans*-Octadecenoic	Elaidic	18	282.5	45.5
11-*trans*-Octadecenoic	Vaccenic	18	282.5	39
13-*cis*-Docosenoic	Erucic	22	338.6	33.5
13-*trans*-Docosenoic	Brassidic	22	338.6	60.6

containing a triple bond are very rare, and are found only in fats for nonedible uses.

Unsaturated fatty acids have lower melting points than the respective saturated fatty acids, and are better soluble in organic solvents. Trans unsaturated acids have higher melting points than the respective *cis* acids (Table 34.2).

D. Polyunsaturated Fatty Acids

Polyenoic unsaturated fatty acids contain 2–6 double bonds. Many isomers are possible, but only a few are really found in nature. The majority among them belong to essential fatty acids as they cannot be synthetized in the human body [3]. Most natural polyunsaturated fatty acids have trivial names, which are more widely used than the systematic names (Table 34.3). Melting points of polyenoic fatty acids are lower than 0°C; therefore, they are not given in Table 34.3. An exception is eleostearic acid – because of the presence of trans double bonds (α-eleostearic acid = 49°C, β-eleostearic acid = 71°C). The allylic (pentadienoic) configuration is most frequent, -CH=CH-CH_2-CH=CH-, where the double bonds are separated by a methylene group. Their structures (4) are shown in Figure 34.1. Both n-6 and n-3 polyunsaturated fatty acids belong to the essential fatty acids. They are enzymatically transformed into eicosanoids (5). The n-3 polyenoic acids are appreciated as they neutralize unfavorable effects of the excessive n-6 polyunsaturated fatty acid intake in modern diet of developed countries [6]. The balance between n-6 and n-3 polyunsaturated fatty acids should be maintained in the diet (7).

Conjugated unsaturated acids, -CH=CH-CH=CH- are rather rarely found, e.g., in milk fat or in some nonedible oils. In the most common fatty acids, the first double bond is located at the 9th or 6th carbon atoms (from the carboxyl group) or at the 3rd or 6th carbon atom (from the methyl group). Pentaenoic and hexaenoic n-3 fatty acids are typical for marine oils. They belong to the important essential fatty acids.

III. LIPIDS

A. Esters of Glycerol

Fatty acids are mostly bound as glycerol esters in natural material. Glycerol (propantriol, Figure 34.2.A) contains three hydroxyl groups, each of which could be esterified with a fatty acid residue. Monoacylglycerols (formerly monoglycerides) contain only one fatty acid residue in the molecule, and exist in two isomeric forms (Figure 34.2.B and 34.2.C). The former one is more stable. The 1-acyl isomer has an unsymmetrical carbon atom, so that two optical isomers exist. Diacylglycerols (formerly diglycerides) are formed by substitution of two hydroxyl groups with an acyl group (Figure 34.2.D and 34.2.E). The 1,2-isomer has an asymmetrical carbon atom. It is easily isomerized into its more stable 1,3-isomer. The most common glycerol esters are triacylglycerols (formerly triglycerides), where all hydroxyl groups of glycerol are substituted with fatty acids (Figure 34.2.F). Monoacyl and diacylglycerols are present only in small amounts as intermediary metabolic products.

In simple triacylglycerols, all hydroxyl groups may be substituted with the same fatty acid (Figure 34.2.F), but in most cases, mixed triacylglycerols exist in nature, where two or three different fatty acids are bound on the same

TABLE 34.3
The Most Important Polyunsaturated Fatty Acids (Compiled from Beilstein and Chemical Abstracts Data Bases)

Systematic Name	Trivial Name	Number of Carbon Atoms	Number of Double Bonds	Melting Weight
9-*cis*, 12-*cis*-Octadecadienoic	Linolenic	18	2	280.4
9-*cis*, 12-*cis*, 15-*cis*-Octadecatrienoic	Linolenic	18	3	278.4
6-*cis*, 9-*cis*, 12-*cis*-Octadecatrienoic	γ-Linolenic	18	3	278.4
9-*trans*, 11-*trans*, 13-*trans*-Octadecatrienoic	Eleostearic	18	3	278.4
5,8,11,14-*all-cis*-Eicosatetraenoic	Arachidonic	20	4	304.6
5,8,11,14,17-*all-cis*-Eicosapentaenoic	EPA	20	5	302.4
4, 7, 10, 13, 16, 19-*all-cis*-Docosahexaenoic	DHA	22	6	328.5

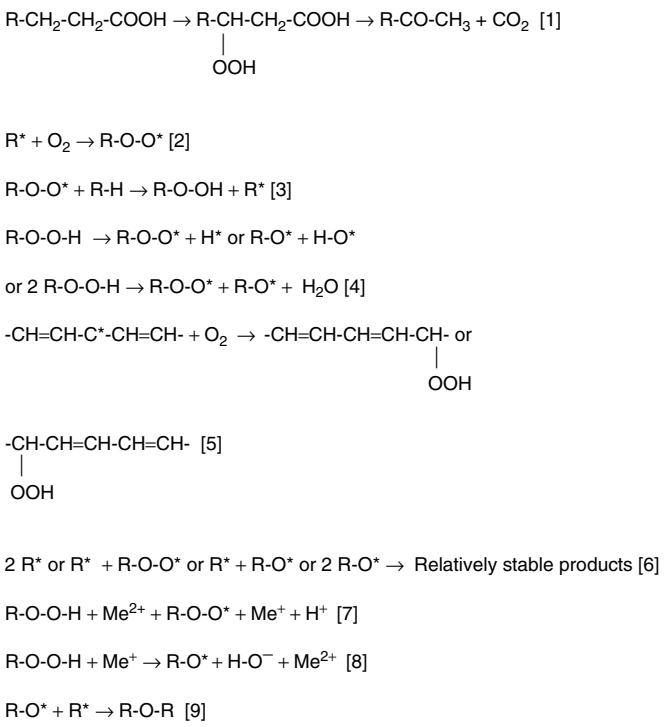

FIGURE 34.1 Chemical structures of polyenoic fatty acids.

glycerol molecule. If the carbon atoms 1 and 3 are occupied by different fatty acids, the carbon atom 2 becomes asymmetrical; if the stereospecificity has to be evident, it is expressed by means of the *sn* system. The carbon numbers of glycerol are numbered stereospecifically (1). The carbon atom that appears on top in that Fischer projection that shows a vertical carbon chain with the hydroxyl group at carbon-2 to the left is designated as C-1. To differentiate such numbering from the conventional numbering, the prefix *sn* is used, e.g., *sn*-1-palmitoyl, 2-linoleoyl, 3-oleoylglycerol. Physical properties of optical isomers are not very different.

The number of triacylglycerol isomers is theoretically very high, but it is usually substantially restricted in natural fats and oils. Triacylglycerols are formed in natural food raw materials by esterification of glycerol under catalytic action of lipases. Lipases possess certain selectivity so that in most natural vegetable fats and oils the carbon atom *sn*-1 is occupied by palmitic acid or other saturated acids, and the carbon atom *sn*-2 by linoleic acid or linolenic acid, less often by oleic acid. The carbon atom *sn*-3 may be substituted with any fatty acid, most often unsaturated fatty acids.

In some plant or animal raw materials fatty acids are bound to monohydroxylic aliphatic alcohols or sterols. They are called waxes (8). The most important wax is bees wax. Their importance in the human diet is low.

Fatty esters of sugars, e.g., sucrose, are produced as fat replacers [9] as they are not cleaved by natural lipases, and cannot thus be utilized as a source of energy by humans.

More information on physical and chemical characteristics of oils, fats, and waxes are obtained from the literature (10).

B. Phospholipids

Phospholipids consist not only of fatty acids and an alcohol, but also contain a phosphoryl group, usually ionized (1). The most common phospholipids are esters of glycerol; therefore, they are called glycerophospholipids. They are derivatives of 1,2-diacylglycerol, where the phosphoryl group is bound to the carbon atom *sn*-3. Such a derivative is called a phosphatidyl (Figure 34.3.A). If no other residue is bound to the phosphoryl group, phosphatidic acids are formed (Figure 34.3.B). They may exist as calcium or magnesium or other salts.

Very often, the aminoalcohol choline is bound to the phosphoryl group, and the resulting compound is phosphatidylcholine (earlier lecithin) (Figure 34.3.C), most often existing as an inner salt. The phospholipid containing another amino alcohol — ethanolamine — is called phosphatidylethanolamine — earlier known as colamine (Figure 34.3.D). Another important phospholipid class contains bound amino acid serine — phosphatidylserine (Figure 34.3.E). Other phospholipids may not contain any nitrogen, e.g., they contain bound myoinositol (hexahydroxycyclohexane); such phospholipids — phosphatidylinositols (Figure 34.3.F) — may be esterified by other phosphoryl residues. The phosphoryl group may be substituted by a glycerol (Figure 34.3.G) or a diacylglycerol (Figure 34.3.H).

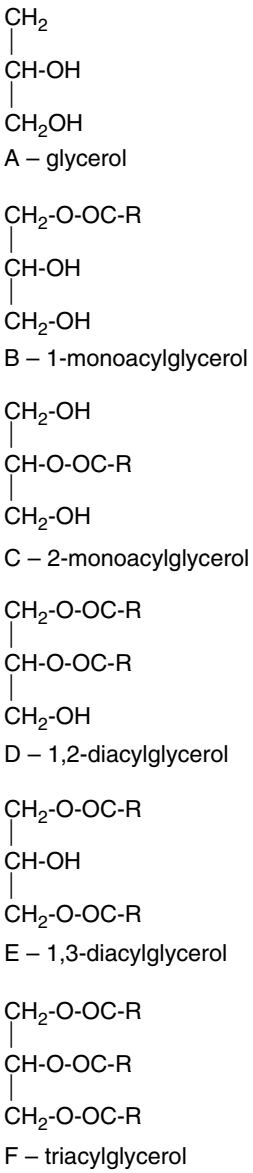

FIGURE 34.2 Chemical structure of glycerol and glycerol esters.

Phosphatidylinositols, among other functions, diminish the postprandial oxidation of triacylglycerols, thus increasing the feeling of satiety, and decreasing the weight gain (11).

In natural phospholipids the fatty acid residue in the position *sn*-2 may be cleaved off by action of phospholipases, and lysophospholipids are formed (Figure 34.3.J). Plasmalogens are phospholipids, where a fatty acid is replaced by an aldehyde in the form of a hemiacetal, usually in its dehydrated form as the respective 2-unsaturated alcohol (Figure 34.3.K).

Some phospholipids do not contain any bound glycerol. Fatty acids are bound to a long-chain alcohol, such as sphingosine in animal tissues (Figure 34.3.L) or phytosphingosine in plant tissues (Figure 34.3.M). They are not bound as esters but as amides, and the hydroxyl group is esterified by a molecule of phosphoric acid, which may contain bound choline or other compounds. Such lipids are called sphingolipids (Figure 34.3.N).

Phospholipids are present in all food materials at least in small amounts (12). Their content in oilseeds varies between 1–3% of the oil basis. The phospholipids fraction is present in crude oils obtained by extraction of oilseeds with nonpolar. It is precipitated from crude oils by action of water, or better by phosphoric or citric acid. The precipitate contains about 60% phospholipids. It is called lecithin. Lecithins of acceptable sensory properties, good functional properties, and reasonable availability are obtained only from soybeans or egg yolk, but the latter are more expensive. For most edible purposes, it is bleached and/or modified, e.g., by addition of free fatty acids. Lecithins enriched in phospholipids or phoshatidylcholine are available on the market. Lecithin is highly appreciated as an emulsifier or for many other purposes. Lower quality products are used for nonedible purposes. For more information about phospholipids see References 13–15.

C. Glycolipids

Glycolipids are fatty acid derivatives containing bound sugars, most often D-galactose or D-glucose. The simplest glycolipids are derived from 1,2-diacylglycerols by substitution of the remaining hydroxyl group with a galactosyl (Figure 34.4.A) or a digalactosyl (Figure 34.4.B) residue. More complicated derivatives were identified, e.g., in oat lipids (16).

Galactosyldiacylglycerols are present in plants as a part of membranes for storage of starch (17). Emulsions containing galactolipids are components of fat replacers as they evoke the sense of fullness.

Cerebrosides are glycosides derived from sphingosine amides — ceramides (Figure 34.4.C).

Glycolipids contain sulphuric acid, sometimes bound to the glycoside residue (Figure 34.4.D). Gangliosides are complex phospholipids containing bound sialic acid (Figure 34.4.E), and usually also one or several residues of bound sugars. They were earlier called mucolipids.

D. Lipoproteins

Lipids form macromolecular complexes with protein, where lipids are bound to proteins by multiple physical forces. They are present either as dispersible aggregates, soluble in water, or as membranes. Covalent bonds are found only exceptionally in natural lipoproteins, but may be formed by interactions of oxidized lipids with proteins.

In water-soluble lipoproteins, the lipid moiety is surrounded by a layer of hydrated proteins (Figure 34.5.A). The contact between the lipid and the protein moieties is effected by a layer of emulsifiers, chiefly sterols and phospholipids. The lipophilic, hydrophobic hydrocarbon chains of the emulsifiers are oriented inside the oil droplet while the hydrophilic parts are oriented towards the relatively polar and hydrophilic protein layer. The stability of

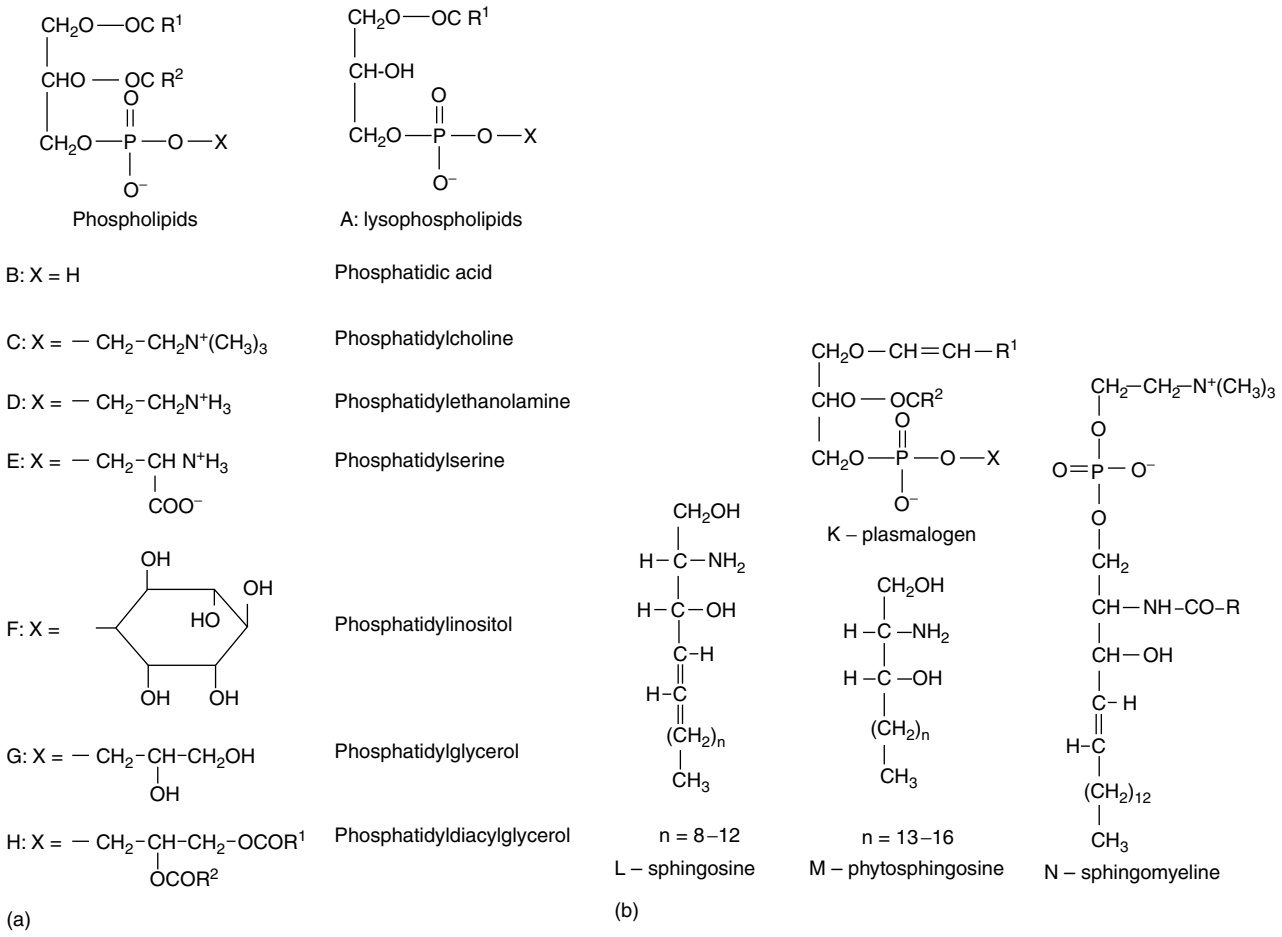

FIGURE 34.3 Chemical structures of phospholipids.

the dispersions of lipoproteins in water depends on the ratio of lipids:proteins. More information on lipoproteins is available from specialized literature (18, 19).

The most widely studied dispersed lipoproteins are those of blood plasma, which belong to the following classes according to their behavior under centrifugation, where they are separated on the basis of their density (specific gravity):

a. VLDL — very low density lipoproteins;
b. LDL — low density lipoproteins;
c. HDL — high density lipoproteins.

The content of hydrophilic components and the stability of lipoprotein dispersion increases with their increasing density.

Lipoproteins forming membranes regulate the permeation of water molecules into cells and subcellular particles. They mostly consist of a double layer of lipoproteins. The polar moieties are oriented towards the water phases on both sides of the membrane, while the non-polar moieties form multiple physical bonds between the two lipid layers of the membrane (Figure 34.4.B). Both phospholipids and sterols are important parts of membrane lipoproteins.

Lipids form complexes with starch and other macromolecular carbohydrates as well as with proteins (20). Three types of these interactions exist:

a. Complexes consisting of lipid inclusion compounds inside an amylose helix or short helices inside amylopectin straight-chain oligosaccharide branches.
b. Complexes, where lipids are sorbed on the surface of carbohydrate particles.
c. Complexes, where lipids are located in capillaries between carbohydrate particles.

IV. OCCURRENCE OF LIPIDS IN PLANT AND ANIMAL FOOD MATERIALS

Lipids are present in almost all food materials of both animal and plant origin (21). They are necessary for the existence of living tissues, even when mostly only in small amounts of 0.5 to 3.0%. These lipids are mainly located in

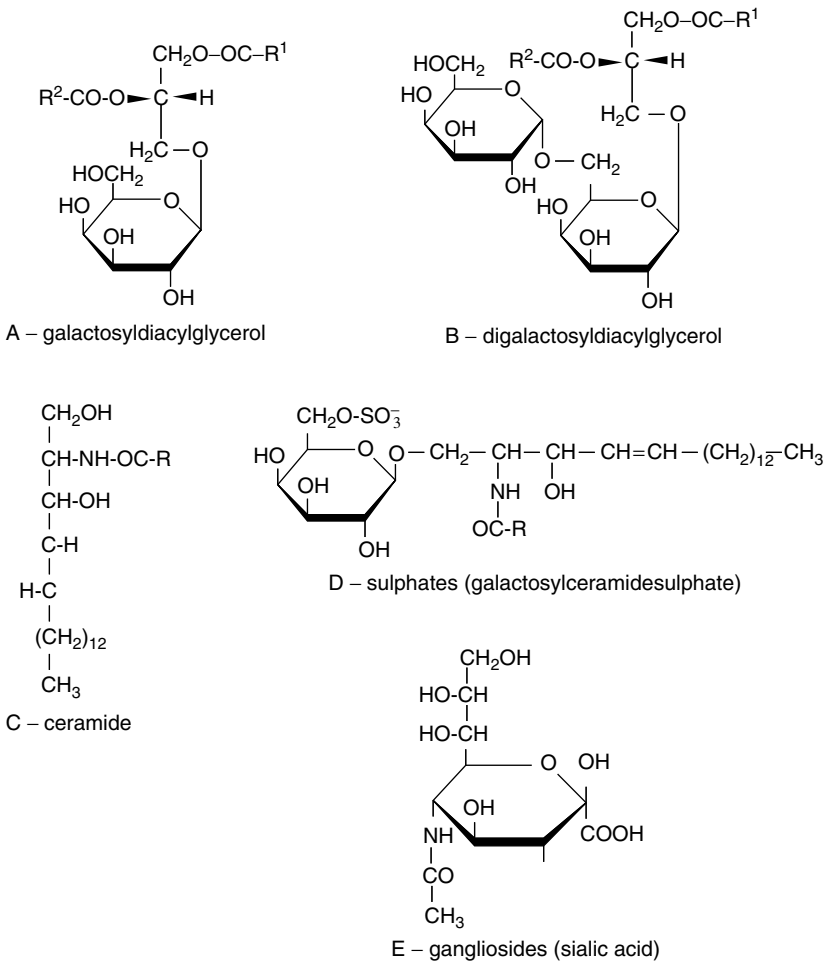

FIGURE 34.4 Chemical structures of glycolipids.

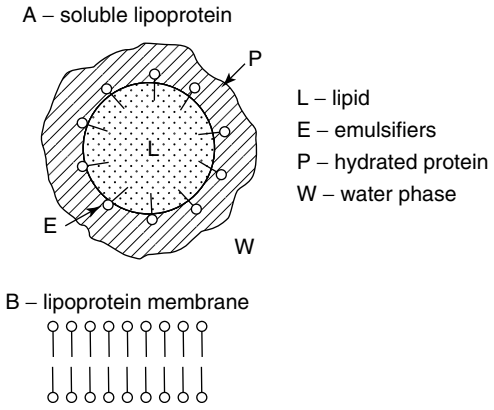

FIGURE 34.5 Chemical structures of lipoproteins.

cell membranes and in intracellular membranes, and mainly consist of phospholipids, glycolipids, and lipoproteins. The triacylglycerol fraction is relatively low in these tissues. The lipids are mostly bound to proteins and other hydrophilic components so that they are extractable only with solvent mixtures containing polar components, such as mixtures of chloroform and methanol or diethyl ether and ethanol.

In addition to their function in the metabolism of living organisms, lipids are deposited as energy reserves. The reserve lipids are very important raw materials in the food industry. They consist mostly of relatively non-polar triacylglycerols. Therefore, they are extractable with hydrocarbon solvents. Plant seeds contain either macromolecular carbohydrates or lipids as a source of energy for the germinating organism. In those plants, where the energy is stored in lipids, the content of seed lipids (called mostly oils) may be 30–60% dry weight (Table 34.4). In some plants, energy is stored both as lipids and as starch, such as in soybeans, where the lipid content is about 20%. In some seeds, lipids are stored also in the pericarp, e.g., in olives, avocado, or oil palm fruits. The fatty acid composition of storage lipids may be very different from that of membrane lipids.

Seed oils have different fatty acid composition from pericarp oils, e.g., palm oil has a fatty acid composition close to that of lard, while the palm kernel oil has the fatty acid composition similar to that of coconut oil (Table 34.5).

TABLE 34.4
Content of Plant Lipids (15)

Plant Name	Systematic Name	Lipid Source	Lipid Content [% Dry Weight]
Soya	*Soja hispida*	Beans	16–22
Sunflower	*Helianthus annuus*	Seed	42–62
Peanut	*Arachis hypogaea*	Seed	45–50
Rape	*Brassica napus*	Seed	40–48
Cocoa	*Theobroma cacao*	Seed	53–59
Almonds	*Prunus amygdalus*	Seed	60–65
Sesame	*Sesamum indicum*	Seed	50–55
Cotton	*Gossypium hirsutum*	Seed	20–24
Oil palm	*Elaeis guineensis*	Pericarp	30–40
Cocos palm	*Cocos nucifera*	Copra	63–70
Olive	*Olea europaea*	Pericarp	13–50
Rice	*Oryza sativa*	Bran	8–15
Wheat	*Triticum aestivum*	Germ	7–12
Corn	*Zea mays*	Germ	14–19

Examples of fatty acid composition of the most important fats and oils are shown in Table 34.5 (trace fatty acids are not included).

Even in plants using starch as a source of energy for germinating organism, at least the germ is rich in lipids so that it may be used for industrial processing, e.g., in the case of corn or wheat germs (Table 34.4). During the germination, oil is gradually used up as a source of energy, or it is converted into other compounds, chiefly cellulose.

Animal tissues also consist of two types of lipids. Lipid membranes and dispersible lipoproteins are present only in limited amounts of about 1–2% dry weight in the respective tissue. They consist mainly of phospholipids and glycolipids. Animals store energy in carbohydrates only in very small amounts; therefore, only energy reserves stored as triacylglycerols are important for the organism and in the industry. Surprisingly, in some tissues the amount of lipids may be much higher than the amounts of proteins. The fatty acid composition of reserve (depot) fat is different from the composition of polar lipids as polar lipids are rich in linoleic acid and more unsaturated polyenoic acids. The fatty acid composition of depot fat in farm animals depends partially on the composition of feed lipids, and similar relations exist in other animals. A typical example are lipids of fish and other aquatic animals as the fatty acid composition is influenced by that of algae and small crustaceans, which are their main source of nutrition. Polar lipids bound in membranes are not substantially affected by fatty acid composition of feed lipids.

The fatty acid composition of the triacylglycerol fraction of fats and oils is very different, and it is specific for each species. Examples of fatty acid composition of the most important fats and oils are shown in Table 34.5. (Trace fatty acids are not included and may be found in the original literature). The fatty acid composition of vegetable oils is very variable. In some species oleic acid prevails, in other it is linoleic acid, and in another group linolenic acid is present, too. In palm seed lipids the major fatty acids are lauric, myristic, and palmitic acids. Triacylglycerols of land animals mostly consist of palmitic, stearic, oleic, and linoleic acids while polyunsaturated fatty acids are major components of marine oils (22).

The traditional fatty acid composition may be substantially modified by breeding, genetic manipulations, or even climatic conditions. Modified soya cultivars yield oil with high saturated fatty acid content, suitable as trans-free replacers of hydrogenated oils, or canola oil with 40% lauric acid is suitable for nonedible uses (23). In animals it is affected by feed lipids. The novel modified oils usually have higher oleic acid content and lower polyunsaturated acid content so that they are more resistant against oxidation on heating or long storage.

V. CHEMICAL CHANGES DURING INDUSTRIAL FAT AND OIL EXTRACTION AND PROCESSING

A. Fat and Oil Extraction from Natural Sources

Oilseeds are the most important raw material for the production of edible oils. Soybeans, followed by palm fruits, are the chief oilseeds worldwide, but rapeseed and sunflower seed are also important in Europe, followed by olives and animal raw materials (24). Seeds are first crushed, and the resulting meal is heated by steam application. During the process, heat denaturation of the protein moiety destroys lipid membranes and other lipoproteins so that the yield of oil substantially increases in comparison to the cold extraction. This process is called conditioning.

Conditioned oilseed meals of oilseeds containing 40–60% oil are then fed to expeller presses, where oil is extracted from the meal under high pressure and temperatures up to 100°C. Crude oil is removed and collected in a tank, and the pressed material (cakes), still containing about 12–20% oil, is cooled to the ambient temperature. Soybeans have only about 20% oil so that the cake has a lower concentration of oil or is treated by solvent extraction before the previous expeller pressing. Rice brans or corn germs are not expeller pressed because of low oil content, but are treated directly with solvents. Olives are cold-pressed in special crushers at temperatures below 40°C with production of virgin olive oils (25, 26).

The cakes are then extracted using solvents, most often hexane, pentane, and their isomers, or other hydrocarbons of similar boiling temperatures. Continuous processes are mostly used, and the resulting extracted meal is then separated from the solution of oil in the extraction solvent (micella). Crude oil is then obtained by

TABLE 34.5
Fatty Acid Composition of Fats and Oils (66)

A. Linoleic Acid Oils

Fatty Acid Code	Sunflower Oil	Peanut Oil*	Safflower Oil	Sesame Oil	Cottonseed Oil**
16:00	5–8	8.3–14	5.3–8.0	7.9–10.2	21.4–26.4
16:01	<0.5	<0.2	<0.2	0.1–0.2	<1.2
18:00	2.5–7	1.9–4.4	1.9–2.9	4.8–6.1	2.1–3.3
18:01	13–40	36.4–67.1	8.4–21.3	35.9–42.3	14.7–21.7
18:02	40–74	14.0–43.0	67.8–83.2	41.5–47.9	46.7–58.2
18:03	<0.3	trace	trace	0.3–0.4	<0.4
20:00	<0.5	1.1–1.7	0.2–0.4	0.3–0.6	0.2–0.5
20:01	<0.5	0.7–1.7	0.1–0.3	<0.3	trace
>20	<1.5	3–6	<2.0	<0.5	<0.1

Note: * = also 22:0, 2.1–4.4%, and 24:0, 1.1–2.2%; ** = also 14:0 0, 6–1.0%.

B. Oils Containing Linolenic Acid and Cereal Oils

Fatty Acid Code	Soybean Oil	Rapeseed (Canola) Oil	Linseed Oil	Corn Oil	Rice Bran Oil
16:00	9.7–13.3	3.3–6.0	7	10.7–16.5	16–28
16:01	<0.2	0.1–0.6	trace	<0.3	0.5
18:00	3.0–5.4	1.1–2.5	4	1.6–3.3	2–4
18:01	17.7–25.1	52.0–66.9	20	24.6–42.2	42–48
18:02	49.8–57.1	16.1–24.8	17	39.4–60.4	16–36
18:03	5.5–9.5	6.4–14.1	52	0.7–1.3	0.2–1.0
20:00	0.1–0.6	0.2–0.8	trace	0.3–0.6	0.5–0.8
20:01	<0.3	0.1–3.4	trace	0.2–0.4	0.3–0.5
>20	<1.2	<1.3*	trace	<0.9	<1.0

Note: * = mostly erucic acid.

C. Oils from Palms, Examples of Vegetable Butters and Oleic Acid Oils

Fatty Acid Code	Palm Oil	Palmkernel Oil	Coconut Oil	Cocoa Butter	Olive Oil*
8:00	0	2.1–4.7	4.6–9.4	0	0
10:00	0	2.6–4.5	5.5–7.8	0	0
12:00	trace	43.6–53.2	45.1–50.3	0	0
14:00	0.7–1.3	15.3–17.2	16.8–20.6	0.1	trace
16:00	40.1–46.3	7.1–10.0	7.7–10.2	25–27	7.5–20
18:00	4.0–6.5	1.3–3.0	2.3–3.5	33–37	0.5–5.0
18:01	36.7–40.9	11.9–19.3	5.4–8.1	34–35	55–83
18:02	9.4–12.1	1.4–3.3	1.0–2.1	3–4	3.5–21
20:00	0.1–0.7	<0.7	<0.2	0.2–1.0	<0.8
>20	<0.4	<0.8	<0.2	trace	<0.3

Note: * = also 16:1 0.3–3.5% and 18:3 <1.5%.

D. Fats of Farm Animals

Fatty Acid Code	Cow Milk Fat*	Chicken Fat	Pork Lard	Beef Tallow	Mutton Tallow
12:00	2.2–4.5	0.1	0	0	0
14:00	5.4–14.6	0.9	trace	1–6	2–4
14:01	0.6–1.6	trace	0.5–2.5	<1	trace, low
16:00	25–41	22	20–32	20–37	20–27
16:01	2–6	6	1.7–5.0	1–9	1.4–4.5
18:00	6–12	6	35–62	25–40	22–34
18:01	18.7–33.4**	37	35–62	31–50***	30–42***

(*Continued*)

TABLE 34.5 (*Continued*)

D. Fats of Farm Animals					
Fatty Acid Code	**Cow Milk Fat***	**Chicken Fat**	**Pork Lard**	**Beef Tallow**	**Mutton Tallow**
18:02	0.9–3.7	20	3–16	1–5	1.9–2.4
20:00	1.2–2.4	trace	<1.0	trace	trace
>20	0.8–3.0	trace	<3.0	trace	trace

Notes: * = also 4:0, 2.8–4.0%; 6:0, 1.4–3.0%; 8:0, 0.5–1.7%; 10:0, 1.7–3.2%; ** = Including 2–3% trans fatty acids; *** = Including minor trans fatty acids.

E. Fish Oils				
Fatty Acid Code	**Cod Liver Oil**	**Herring Oil**	**Carp Oil**	**Menhaden Oil***
14:00	3–5	3–10	3	6–12
16:00	10–14	13–25	17	14–23
16:01	6–12	5–8	17	7–15
18:00	1–4	1–4	4	2–4
18:01	19–27	9–22	28	6–16
18:02	1–2	1–2	13	1–2
18:04	–	1–5	–	1–5
20:01	7–15	9–15	4	0.5–2
20:2–4	0.8–3.0	0.8–1.2	3	1–4
20:05	8–14	–	3	12–18
22:01	4–13	12–27	–	0.2–0.4
22:06	6–17	4–10	–	2–4

Note: * = also 16:2–16:4, 1.8–6.2%; 24:0, 4–15%.

Expressed as weight % total fatty acids; trace fatty acids are omitted; fatty acid codes: number of carbon atoms: number of double bonds.

distilling off the solvent. The last solvent residues are removed by distillation with steam. The resulting crude oil is stored in a separate tank or mixed with oil obtained by expeller pressing.

During the industrial extraction of fats and oils, other lipophilic substances, such as sterols or tocopherols, come into the extract, even when they may be originally not present in oil droplets of seed cells but in other parts of the seed (27).

The extracted meal from the solvent extraction is treated in a toaster at temperatures of about 100°C or higher to liberate the residual solvent, to deactivate enzymes, and to detoxicate extracted meal. The last solvent traces are removed by steaming. Meal is then cooled down and stored in elevators. The extracted meal is used for feeding purposes.

B. Crude Oil Refining

Crude vegetable oil contains various minor components, such as oxidation products of triacylglycerols and terpenes, polar lipids, trace metal derivatives, which deteriorate its sensory and functional properties. Therefore, it is processed by refining. Good detailed information on vegetable oil processing techniques for human nutrition is available (28–30). The first refining step is the degumming. Oil is treated with hot water, usually containing phosphoric or citric acid. Most phospholipids present in crude oil are hydrated and become insoluble in oil. Some phospholipids are not precipitated unless special procedures are used, such as superdegumming. The precipitate, containing coprecipitated components other than phospholipids and some oil, is removed, dried, and used as commercial lecithin. The membrane filtration is another approach to degumming (31).

The degummed product may be treated in two ways. If the free fatty acid content is high, it is alkali refined by a solution of sodium hydroxide or, less often, by sodium carbonate.

Free fatty acids are converted into water soluble sodium salts, which are washed out of oil with hot water. Natural pigments and other impurities are partially removed, too. After the alkali refining, oil is dried by heating at reduced pressure, and treated with bleaching earth or a mixure of bleaching each and with active carbon. In the process of bleaching, natural pigments (mainly carotenoids, pheophytins and chlorophylls) and the last traces of sodium salts are removed. Dimers of polyunsaturated fatty acids or sterols and trans-isomers may be formed in the course of bleaching (32). More information on bleaching is available (33).

Bleached oil is deodorized by treatment with overheated steam under reduced pressure in nitrogen at temperatures of 220–250°C. Volatile substances, formed by oxidation of unsaturated fatty acids during seed storage or seed processing, are removed during the process, and both the sensory value and the resistance against oxidative

sensory deterioration (called rancidity) are thus substantially improved. Other relatively volatile substances are also removed at least partially, such as sterols or tocopherols. The deodorized oil has a bland neutral flavor.

Crude oils containing less than about 1% free fatty acids may be refined in a simpler way, i.e., by physical refining (34). After degumming, crude oil is deodorized in a similar way as in the case of alkali refined oils to remove all relatively volatile components, including free fatty acids. The resulting oil is bleached. The alkali refining step is thus eliminated, which is advantageous from the environmental standpoint. The resulting refined oil is of about the same quality as alkali-refined oil, e.g., as in the case of rapeseed oil (35).

During the process of deodorization *cis,cis*-diunsaturated fatty acid bound in triacylglycerols is partially isomerized into *cis,trans* and *trans,cis*-dienoic isomers in the case of linoleic acid (36). Several other trienoic isomers are formed in oils containing linolenic acid, such as soybean or rapeseed oil. As the nutritional value of these isomers is doubtful, the deodorization temperature should be lower than 240°C to minimize the isomerization. The best results are obtained using thin-film deodorization. In this process the temperature may be lower and the heating time shorter (37).

Vapors removed during deodorization are collected as deodorization sludges. They contain free fatty acids, sterols, and tocopherols so that they can be used for the production of tocopherol concentrates suitable as antioxidants. Sterols may be isolated, too, and used either directly or after hydrogenation in stanols in margarines as an additive lowering blood pressure and decreasing the cholesterol content in blood plasma (38).

Refined oils are either used directly as salad oils or frying oils, or after emulsification as mayonnaise and salad dressings, or are added to margarines (39). If fresh fish oils are properly refined (40), they are acceptable for margarines even without hydrogenation (41), even when their oxidation stabiliity may still be a problem (40).

C. Production of Lecithin

Crude lecithin is obtained in the process of crude oil degumming (39) as explained in Section 34.V.B. It is mostly used for feed or other nonedible purposes; only soybean or egg lecithin are used for edible purposes. Typical properties of soybean lecithin are shown in Table 34.6. They are used as natural surface active agents for many purposes in the food industry (42). Lecithin may be modified in different ways (43). For use in light-colored food products, it is bleached by hydrogen peroxide. For some purposes concentrated phospholipids are necessary; therefore, oil is removed from commercial lecithin by membrane filtration or selective extraction, e.g., with acetone or propane, which is a more acceptable solvent for environmental reasons (44). The most important component of soy phospholipids is phosphatidylcholine. Soy phospholipid concentrate may be enriched to high levels of phosphatidylcholine by interesterification with choline in the presence of phospholipases (45). Fractions enriched with phosphatidylcholine and in phosphatidylinositol may be obtained from lecithin by fractionation with 90% aqueous ethanol (46).

TABLE 34.6
Properties of Food Lecithin (8)

Component	Content [%]
Acetone insolubles	≥ 65
Toluene insolubles	max. 0.3
Moisture	max. 0.3
Free fatty acids*	max. 15

Note: Free fatty acids may be added to change the consistency.

D. Interesterifiction, Ester Interchange, and Saponification

Fats and oils are essentially mixtures of triacylglycerols. They may be converted into other esters by interesterification (47) with methanol or higher alcohols (Figure 34.6.A). The process is called alcoholysis, and is catalyzed by small amounts of alkaline hydroxides or alcoholates, produced *in situ* by dissolution of metallic sodium in alcohol. The conversion of triacylglycerols into methyl esters is used for analytical purposes and for diesel fuels.

The alcoholysis of triacylglycerols with glycerol — glycerolysis — is of technical importance. Solid fats, such as fully hydrogenated oils, are commonly used. They are heated with glycerol to high temperatures in the presence of small amounts of sodium hydroxide. A mixture of monoacylglycerols and diacylglycerols results (Figure 34.6.B), which are used as emulsifiers. They may be fractionated into the monoacylglycerol fraction and the diacylglycerol fraction using short space vacuum distillation. The monoacylglycerol fraction is particularly useful as an emulsifier for the production of margarines and other emulsified fats and oils.

Another procedure for production of partial glycerol esters is the treatment of triacylglycerols with water in the presence of lipases, most often immobilized on a solid support (45).

Tricylglycerols of natural fats and oils possess specific distribution of fatty acids. The distribution may be randomized by interesterification of the particular fat and oil or a mixture (ester interchange, Figure 34.6.C) or of a mixture of different tricylgycerols (Figure 34.6.D). The reaction is carried out again in the presence of sodium hydroxide, metallic sodium, or sodium methoxide as a catalyst. Immobilized lipases may be used as catalysts, too. The reaction proceeds at ambient or moderately increased temperatures. The resulting products may be tailored to specific properties, especially rherological properties (sensory texture or consistency). Low-caloric structured lipids are produced in this way, containing

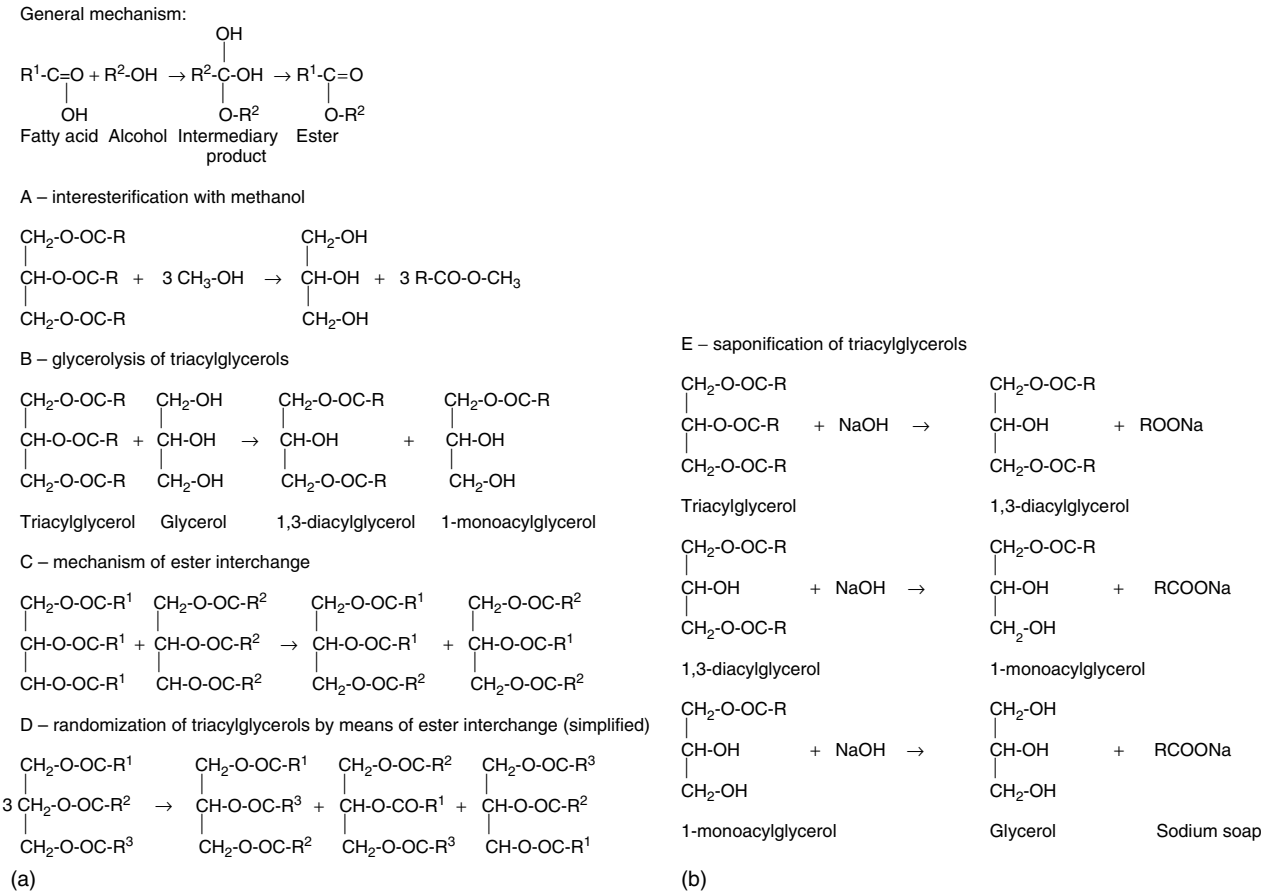

FIGURE 34.6 Esterification reaction of fats and oils.

long-chain, medium-chain, and short-chain fatty acids (48). In contrast, the stability against oxidative rancidity is often decreases (49).

Natural fats and oils may be fully hydrogenated and the resulting trisaturated triacylglycerols are then interesterified with non-hydrogenated oils (50). Plastic solid fats are thus produced, containing no *trans*-unsaturated fatty acids. The topic of structurally modified food fats has been reviewed (51).

As some consumers would like to decrease their energy consumption of fats without affecting the sensory properties of food and decreasing the agreeable fatty taste, some undigestible fat replacers have been introduced to the market, such as Olean, Salatrim, and Caprenin. In these products, esterified fatty acids cannot be split off by lipases (52).

Fats and oils are very rich sources of energy; therefore, their consumption may result in obesity. Fats with lower energy content are produced by interesterification of natural oils with stearic or behenic acid, which are not completely utilized in the metabolism, and medium-chain fatty acids, which have moderately lower energy content and are not deposited in the fatty tissues. These products are marketed as structured fats.

E. Hydrolysis of Fats and Oils

Triacylglycerols are hydrolyzed into fatty acids and glycerol. The reaction with sodium hydroxide was used to produce soap; therefore, the process is still called saponification (Figure 34.6.E). Saponification proceeds by mixing fat with a solution of sodium hydroxide at the boiling temperature. The first stage, i.e., the cleavage of a triacylglycerol molecule into a diglycerol and soap, is slow as the reactants are immiscible and the reaction proceeds only on the interphase. The reaction products act as emulsifiers so that the second stage of saponification is fast. After saponification, sodium chloride is added to the reaction mixture in order to decrease the solubility of soaps in the aqueous phase. The lower aqueous layer also contains glycerol, which is isolated, and distilled from impurities, especially water. The separation of soaps from the water phase containing glycerol by salting is still not complete.

Saponification is now carried out on the plant scale mostly by simply heating fats or oils with water under pressure to high temperature, sometimes in the presence of metallic oxides as catalysts. Fatty acids are gradually cleaved from the original glycerol esters. Free fatty are then purified by vacuum distillation. They are then used for soap

production, production of metallic soaps, or for other purposes. Glycerol obtained in this way is less contaminated than in the case of saponification with sodium hydroxide.

Lipids may be hydrolyzed with immobilized lipases, too (45). Depending on the origin of lipase, the reaction may be stereospecific.

F. Hydrogenation of Fats and Oils

At the beginning of the 20th century, there was an excess of oils on the market, but consumers required solid fats, similar to lard or butter. Hydrogenation was a suitable solution. The unsaturated fatty acids bound in triacylglycerols are converted into less unsaturated or even saturated fatty acids by the addition of hydrogen. Triacylglycerols of saturated fatty acids are solid, unlike triacylglycerols of unsaturated fatty acids. However, triacylglycerols containing three saturated fatty acids in the molecule have too high melting points (about 70°C). They remain solid at body temperature; therefore, they are not attacked by lipases, and their digestibility is only very low. They have a strong tallowy taste, and are thus unsuitable for human nutrition. Therefore, only one or two fatty acids in a triacylglycerol molecule should be converted into a saturated fatty acid. Solid fats with melting points corresponding to lard are thus obtained.

Prerefined edible vegetable oils or fish oils are used as raw material. They are heated to 160–180°C in the atmosphere of hydrogen using nickel as a catalyst. Nickel is deposited on the surface of kieselguhr or another suitable inert substrate; mixtures of nickel with nickel oxide are preferred. Other metals, such as palladium, rhodium, cobalt, or mixtures of nickel with copper also have catalytic qualities, but nickel is usually used. The pressure may be between 0.15–0.5 MPa. Free fatty acids, phospholipids, soap or other surface active compounds, and sulphur derivatives act as catalytic poisons, so they should be removed from oil before the reaction, usually by refining processes. More information is available on both the theory and practice of hydrogenation (53). Partially hydrogenated oils are chiefly solid; therefore, they can be used for the production of shortenings (cooking fats). Fluid shortenings are available on the market, mainly for use in the baking industry (54).

During the hydrogenation reaction polyunsaturated fatty acids are first converted into monounsaturated fatty acids (Figure 34.7.A). The formation of saturated fatty acids (Figure 34.7.B) is slower. The ratio of reaction rates k_1:k_2 is the selectivity of hydrogenation (55), which depends on the catalyst and the reaction conditions. The reaction rate constant k_1 defines the rate of hydrogenation of dienoic acids into monoenoic acids, while the constant k_2 defines the rate of hydrogenation of monoenoic fatty acids into saturated acids. Low hydrogen pressure, low temperature, and low catalyst content determine the selectivity. Because hydrogenation is an exothermic reaction, heat is produced and the reactor has to be cooled. The reaction is usually stopped at the stage when the melting point reaches the required value, usually between 30–45°C, depending on the purpose of the product.

An important side reaction takes place during hydrogenation, i.e., the isomerization of the original *cis* double bond into a *trans* double bond. The *trans* monounsaturated fatty acids have higher melting points than the corresponding *cis* fatty acids so that the desired melting point is already attained at a higher degree of unsaturation. The low hydrogen pressure, lower content of the catalyst, and higher temperature favor the formation of *trans* fatty acids. The *trans* fatty acids are now considered undersirable for human nutrition (36, 56). Therefore, hydrogenation conditions have to be adapted to obtain products with low content of *trans* fatty acids or hydrogenated oils are replaced with other solid fats. The *cis,trans* isomerization is accompanied by positional isomerization of double bonds.

Hydrogenated oils are cooled after the reaction, filtered to remove the catalyst, and refined to remove impurities. They may be used as shortenings or cooking fats, or added to emulsified fats, such as margarines. There is now a tendency to replace hydrogenated oils with palm oil fractions or other solid fats free of *trans* fatty acids.

Free fatty acids may be fully hydrogenated too, e.g., for the production of metallic and alkaline soaps or solid free fatty acids, but higher hydrogen pressure and higher content of catalyst are needed.

At very high hydrogen pressures of 20–50 MPa, higher temperature, and using special catalysts containing copper and chromium, not only double bonds, but also the carbonyl double bond in the carboxyl or ester group are hydrogenated, and fatty alcohols result which are useful for the production of surfactants.

G. Oxidation Reactions

Unsaturated fatty acids bound in lipids are oxidized on storage using several mechanisms (58). Different types of oxidation processes occur in unsaturated fats and oils (59). The spontaneous oxidation by air triplet oxygen is called autoxidation, and it is usually initiated by free radicals or by singlet oxygen. Singlet oxygen is produced in course of photooxidation of unsaturated lipids in the presence of photosensitizers. Several enzymes present in oilseeds or animal tissues, such as lipoxygenases, catalyze oxidation reactions.

The most important oxidation mechanisms are shown in Table 34.7. Enzyme-catalyzed oxidation occurs even in seeds or fruits and other edible parts of raw materials of both plant and animal origin. Lipoxygenases are the most important representatives of this group of enzymes (60). For this reason, even fresh edible oils contain at least minute traces of oxidation products. The oxidation occurring spontaneously in the presence of oxygen is autoxidation. In both cases unsaturated hydroperoxides are the primary reaction products.

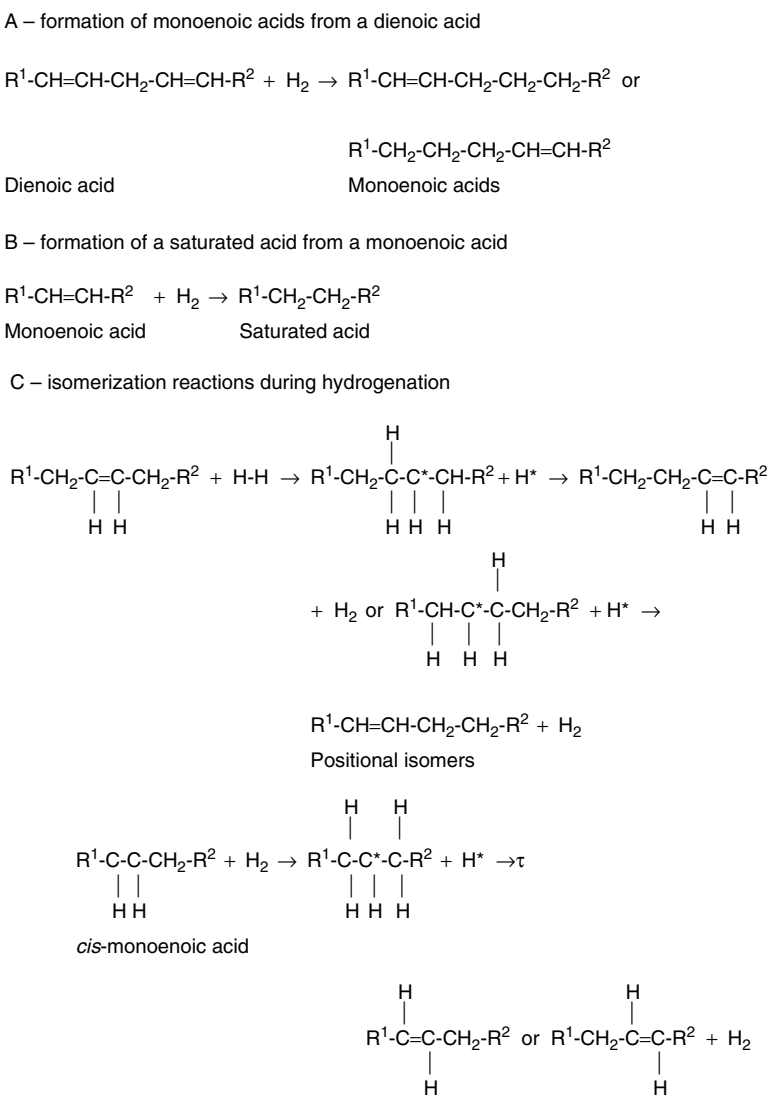

FIGURE 34.7 Hydrogenation of fats and oils.

TABLE 34.7
Mechanism of Lipid Oxidation

Type of Oxidation	Precursors	Primary Products
Lipoxygenase catalyzed	Linoleic, linolenic acids	Conjugated hydroperoxides
Autoxidation	Unsaturated fatty acids	Hydroperoxides
Photooxidation	Unsaturated fatty acids	Hydroperoxides
In vivo oxidation	Essential fatty acids	Eicosanoids
Hydrogen peroxide oxidation	Unsaturated fatty acids	Hydroxy acids

The oxidation is a free radical chain raction, proceeding after the following mechanism (Figure 34.8). The methylene groups adjacent to a double bond or located between two double bonds are the primary site of attack (Figure 34.8.A). Free radicals produced in this way add a molecule of oxygen with formation of free peroxy radicals (Figure 34.8.B).

These radicals can abstract hydrogen from another molecule of an unsaturated fatty acid with formation of a hydroperoxide and another free radical (Figure 34.8.C). Such a reaction chain can be repeated up to several hundred times before the reaction is stopped by a termination reaction (Figure 34.8.D). The reaction is efficiently terminated by interaction of free R-O* or R-OO* radicals with

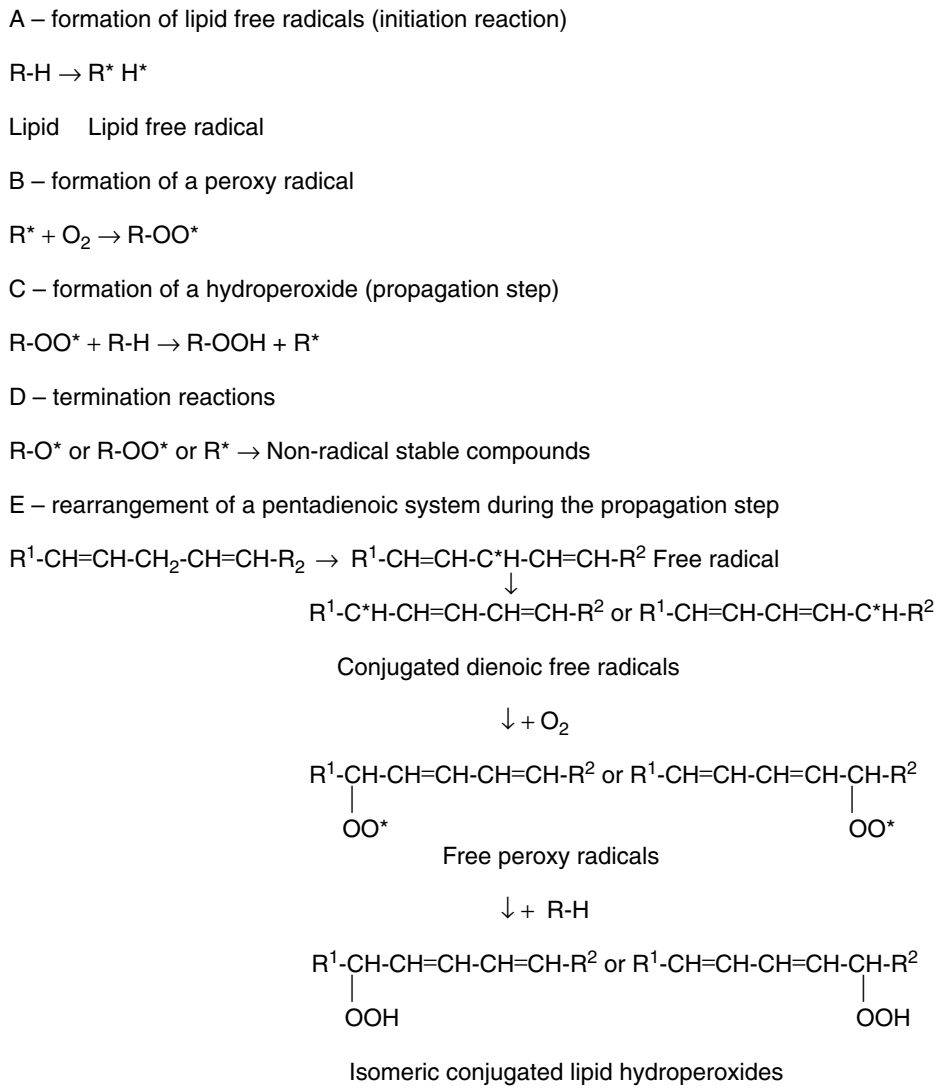

FIGURE 34.8 Mechanism of lipid autoxidation.

antioxidants (61), which are discussed in detail elsewhere in this book. Rearrangement of the double bond and the pentadienoic double bond systems (Figure 34.8.E) accompanies the formation of free radicals. Products with a conjugated double bond system are produced, which contain *trans* double bonds, at least partially. The reaction mixture consists of two to four isomers, the proportions of which depend on reaction conditions. In the case of enzyme-catalyzed reactions, selective oxidation takes place so that certain isomers prevail.

The autoxidation rate increases with increasing temperature. At room temperature linoleic and linolenic acids are oxidized at a much faster rate than monoenoic fatty acids, but at high temperatures, monoenoic fatty acids and even saturated fatty acids can be oxidized, especially in the presence of metallic catalysts. The relative reaction rates depend on the reactive system, e.g., the stability of fatty acids in bulk oils decreases with increasing degree of unsaturation. In contrast, the oxidation stability increases with the increasing degree of unsaturation in water micelles (62).

The lipid hydroperoxides can be oxidized into diperoxides of different structures, usually peroxohydroperoxides. Hydroperoxides are very unstable, particularly at higher temperatures, being dissociated after two mechanisms (Figure 34.9.A,B). The cleavage into alkoxy free radicals is more probable. The degradation is accelerated in the presence of heavy metal compounds with transient valency, such as copper or iron ions (Figure 34.9.C,D). During the reaction the metal ion is reduced into its lower valency state and oxidized again into its higher valency state (61). As the decomposition is again a chain reaction, one metal ion can catalyze the decomposition of many hydroperoxide

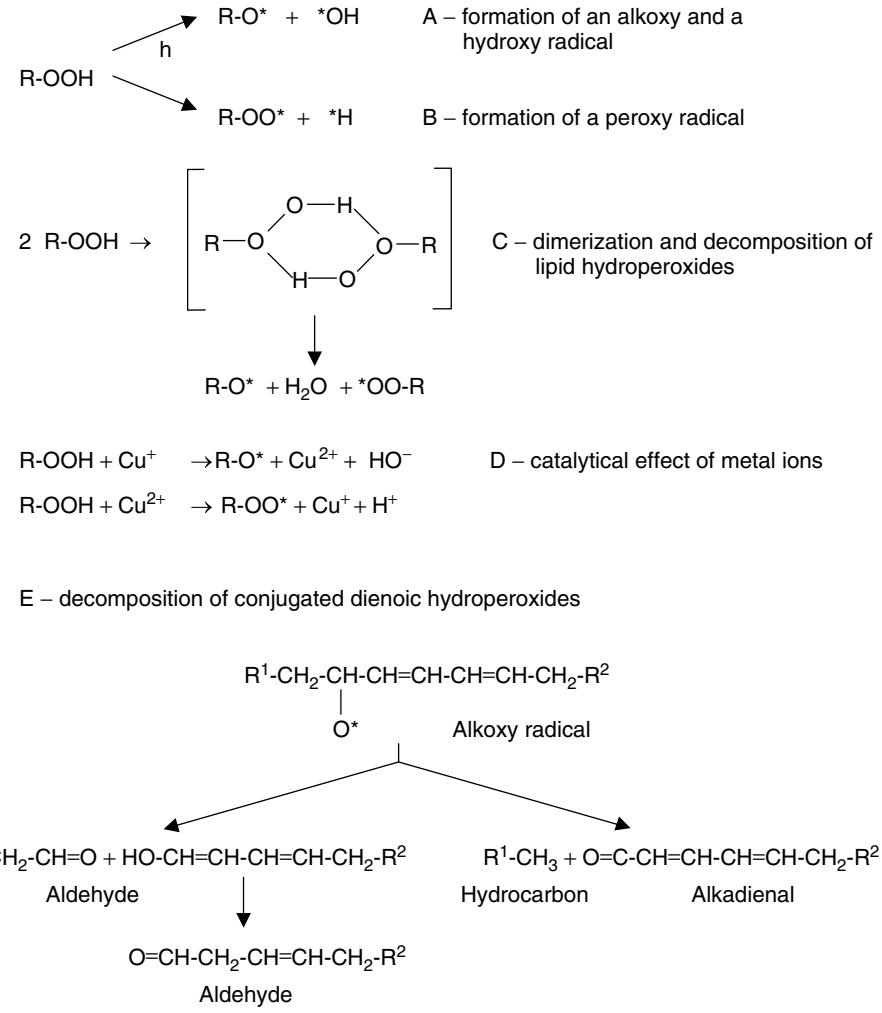

FIGURE 34.9 Decomposition of lipid hydroperoxides into nonvolatile compounds.

molecules. Each reaction produces two free radicals, initiating further oxidation chain reaction. Therefore, heavy metals are very efficient oxidation catalysts.

Free radicals are eliminated by reaction of two free radicals, e.g., with formation of a dimer (Figure 34.9.E), or by reaction with antioxidants, as explained in another Chapter. The dimeric compounds are transformed in free antioxidant radicals of very low reactivity. However, large excess of free radicals becomes harmful in biological systems (63).

Polymers produced during the autoxidation are mainly dimers, where the fatty acid chains are bound by C-C, C-O-C, or C-O-O-C bonds, their ratios depending on the access of oxygen during oxidation. Multiple bonds may be formed in case of polyenoic fatty acids so that lipids oxidized to an advanced degree always contain cyclic compounds. In addition to cyclic oligomers, cyclic monomers are also produced by oxidation (64). The amount of polymers may be high, up to 10%, in frying oils used for a repeated deep frying.

Another reaction of unsaturated lipid hydroperoxides is their decomposition by rearrangement and chain cleavage with formation of both volatile and non-volatile compounds, such as shown in Figure 34.10.A. Aldehydes, alcohols, hydrocarbons, and ketones are produced in this way. They are very sensory active, especially the unsaturated derivatives, and impart to fats and oils a characteristic off-flavor called a rancid flavor. Hexanal, 2-hexenal, 2-octenal, or 2,4-decadienal (Figure 34.10.C) are typical rancidity products.

Another type of oxidation proceeds on light in the presence of photosensitizers, such as chlorophylls or pheophytins. They convert the ordinary triplet oxygen present in air into singlet oxygen, which is several hundred times more reactive than triplet oxygen. It is added to double bonds with the formation of hydroperoxides. Edible oils and foods nearly always contain minute traces of chlorophyll pigments; therefore, the exposure of oils to light should be avoided. The concentration of chlorophylls is particularly high in virgin olive oil which is rather sensitive to singlet oxygen oxidation.

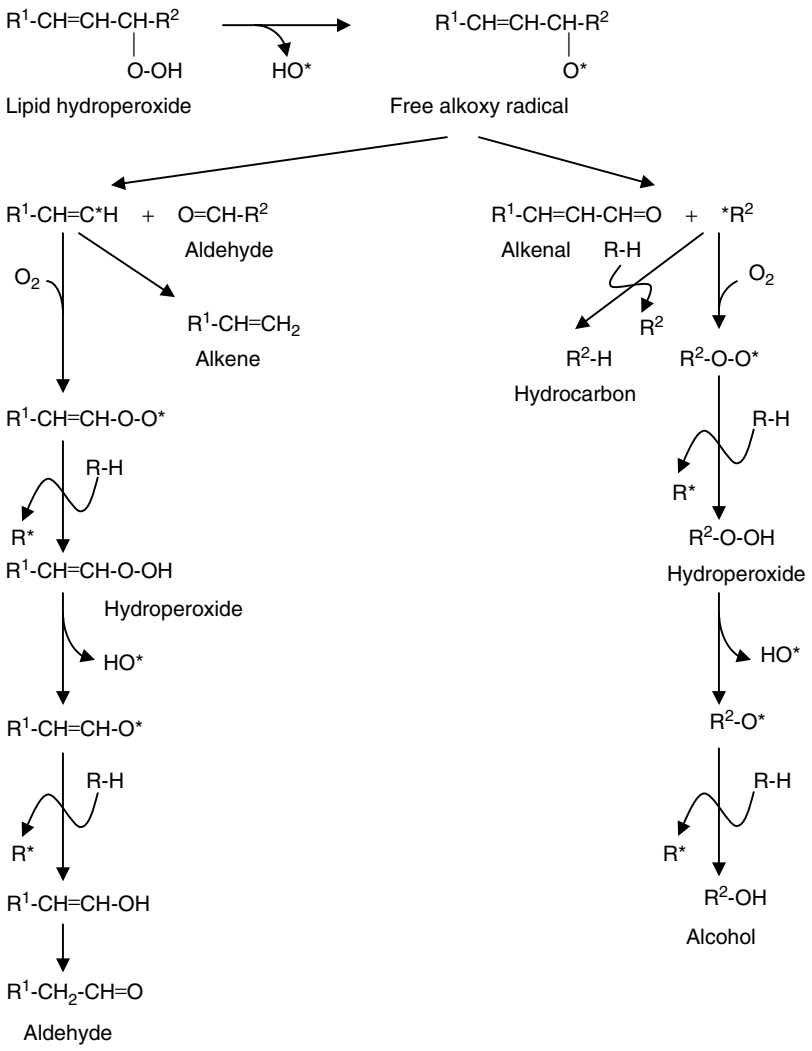

FIGURE 34.10 Cleavage of monoenoic lipid hydroperoxides into volatile compounds.

Oxidation reactions proceed rapidly at high temperatures, such as during deep fat frying (65, 66) or microwave heating (67).

Oxidation is desirable for some industrial purposes. Dry films are formed from thin layers of oil applied on a metallic or wooden surface. Such oils are called drying oils, e.g., linseed or tung oil belong to this group. In the formation of coatings containing linseed oil, and in artistic painting, metal salts are often added to hasten the drying reaction.

H. Chemical Changes in the Production of Oleochemicals from Fats and Oils

Lipids are important sources of oleochemicals. Alkaline soaps have been already mentioned. Salts of other metallic cations, such as calcium salts, are also produced for various purposes, and are called metallic soaps. They are usually produced from solutions of alkaline or ammonium salts of fatty acids by precipitation with metallic anions. Metallic soaps are used for many purposes. Aluminium or zinc stearates are used as lubricants, as paint thickeners, or in the cosmetic and pharmaceutical industries. Salts of heavy metals (such as manganese or cobalt) are used in paint dryers and as additives in plastic materials.

Fatty acids are used as raw materials in other products, mainly surfactants, such as copolymerates with ethylene oxide (Figure 34.11.A) or propylene oxide.

Very important intermediary products are fatty alcohols, produced by high pressure hydrogenation of fats or fatty acids (explained in Section 34.V.E). They may also form copolymers with ethylene or propylene oxides (Figure 34.11.B) or they are sulphated with sulphuric trioxide into sulphates. Alkaline salts of alkyl sulphates are used as detergents.

Fatty acids of fats can be converted into amides by reaction with amoniac (Figure 34.11.C). Amides are dehydrated into nitriles (Figure 34.11.D), which are hydrogenated into alkyl amines (Figure 34.11.E). Salts of

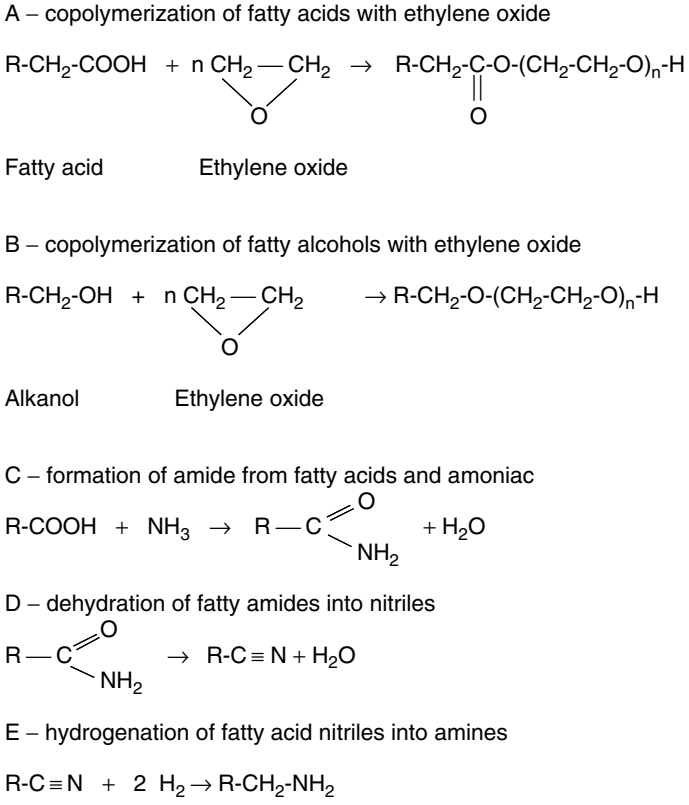

FIGURE 34.11 Formation of surfactants from fats, oils and fatty acids.

alkyl amines (usually alkylated) are technically important surface active agents — inversion salts. Quarternary ammonium salts are particularly useful.

Many other oleochemicals can be manufactured from fats and oils, but they are a topic for special monographs.

VI. PHYSICAL CHANGES OF FATS AND OILS

A. EMULSIFICATION

Fats and oils are insoluble in water, but may form emulsions. Two types of emulsions occur in foods: oil-in-water emulsions (O/W), where water is the continuous phase and oil is the dispersed phase, and water-in-oil emulsions (W/O), where water is the dispersed phase and oil is the continuous phase. Mayonnaise and cream belong to the O/W type, while butter and margarine belong to the W/O type. Mixed emulsions also exist (31), e.g., in margarines or cosmetic emulsions, where drops of the dispersed aqueous phase may contain dispersed tiny oily droplets.

Emulsions are unstable unless they are protected by emulsifiers. Emulsifiers are semipolar compounds, consisting of a polar group and a non-polar hydrocarbon chain. On the water and oil interface they are oriented towards the water phase with their polar groups and towards the oil phase with their hydrocarbon chains (Figure 34.12). The phases are thus protected against coalescence. Even stabilized emulsions are not equilibriated systems, and still have a tendency to partial coalescence, especially if the size of droplets of the dispersed phase is large (68).

The choice of suitable emulsifers is limited; they should be natural food components and harmless to human health. Phospholipids are natural emulsifiers as they act as emulsifiers in blood plasma and other tissues in the organism, and in cream or butter. However, the amount of phospholipids — mostly soybean lecithin — available on the market is not sufficient for the industrial production of food emulsions. Another choice are monoacylglycerols. They are natural metabolites of fats and oils easily produced by glycerolysis of fats and oils in the industry, and the reaction mixture may be used either directly or after fractionation into monoacylglycerols and diacylglycerols. Most margarines and bread spreads contain monoacylglycerols as emulsifiers. The amount of the emulsifier depends on the composition of the water and oil phases, on their ratio, and on the size of dispersed particles. The chemical structures of emulsifiers are similar, consisting of a polar functional group and a long nonpolar chain. The ratio of the polar and the nonpolar moieties indicates the emulsifying capacity, and is defined as the hydrophilic-lipophilic balance (HLB). A higher HLB

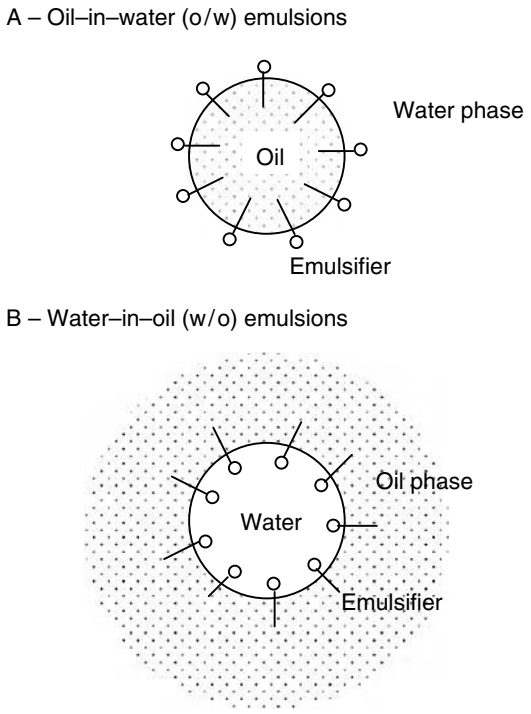

FIGURE 34.12 Role of emulsifiers in the stabilization of an emulsion.

value of an emulsifier enhances the formation of O/W emulsions. In low-fat emulsified products the emulsion stability may be enhanced by additions of ingredients increasing the viscosity of the aqueous phase, such as modified starches or carbohydrate gums. Other emulsifiers are manufactured for nonedible uses. More information may be obtained from specialized literature (69–71).

B. Fractionation of Fats and Oils

Physical properties, mostly the texture (formerly consistency), and the nutrition value, are changed by fractionation into a higher melting and a low melting (or even liquid) fraction. Either dry process or fractionation in the presence of emulsifiers may be used. The proper procedure of crystallization and the crystalline modifications obtained are of great importance (72). The process is used for the preparation of stearin and olein, fractionation of milk fat (73) or palm oil, e.g., for cocoa butter replacements (74). Palm stearin and palm olein are important ingredients for the production of margarines (75).

VII. CONCLUSIONS AND FUTURE TRENDS

New raw materials for edible and speciality fats and oils are constantly being sought, and other new oils are developed by conventional breeding or genetic manipulation from traditional oilseeds. They have fatty acid composition and properties tailored for specific uses. Another trend is to modify the fatty acid and triacylglycerol composition of conventional fats and oils or phospholipids by treatment with lipases or phospholipases or by ester interchange. Minor components may be removed from natural fats and oils by modern technologies, such as membrane filtration. New culinary equipment such as microwave heating affects fats and oils in ways not completely understood, and should be studied. The stability of fats and oils against oxidation is still a problem, and new methods of stabilization, especially new natural antioxidants, are being studied. Fats and oils may be stabilized against oxidation, too, by preventing the air access.

VIII. SUGGESTIONS FOR FURTHER STUDY OF FATS AND OILS

More systematic information on fats and oils may be obtained in general compendia on fats and oils (76–79), or in specialized monographs, such as on soybean processing (39), applications to functional foods (80), and lipid chemistry (81). Tables provide useful information about lipid analysis (82, 83). Data on fatty acid composition of natural lipids have been reviewed recently (21, 82, 84).

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35 Fish Biology and Food Science

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CONTENTS

I. INTRODUCTION

The science of fish as food usually concentrates on the deterioration that occurs after death. Even when packed in ice, the dead fish are still attacked by spoilage bacteria. If they are frozen immediately after catching and cold-stored, the texture of the thawed, cooked product gradually toughens with storage time, while the flavour will become rancid unless special precautions are taken.

While investigating cold-storage phenomena, the writer became aware that natural variations in the raw material could sometimes be more significant than the changes resulting from refrigeration, which had been the focal point of the study. This finding indicated a prior need for an investigation of 'variation,' the role of season and fishing ground, and ultimately their effects on the suitability of a batch of fish as a foodstuff.

Anecdotal evidence existed long before any systematic work had been undertaken in this field. For example, fishermen from the Moray Firth (north of Aberdeen, Scotland) used to risk damaging their nets by fishing as close as possible to stony ground, since experience had taught them that fish caught there were firm-fleshed. Those caught on a soft ground 'had a soft texture,' which precluded their being transported to Aberdeen because they decayed so rapidly. Similar conclusions were derived from the nature of the diet of the fish at the time. Cod (*Gadus morhua* L.) which were consuming soft and fatty sand-eels (*Ammodytes* spp.), 'became soft themselves,' while those with shellfish in their stomachs ('hard-feeding') were firmer.

These observations, based on a lifetime's experience at sea, were of course reliable; the interpretations were not. In fact, the basis for the observations was almost certainly the nutritional status of the fish. Most species starve during the winter, and those that spawn in late winter, developing gonads while starving, drain their resources further. Since they deplete their own body proteins, the flesh becomes soft. When feeding resumes, the fish cram themselves with whatever food has become available — in the case of cod, fatty sand-eels. However, at this point they have not yet recovered, and when the fishermen examine them, they are still soft. Additionally, digestive enzymes are found in abundance in stomachs full of food. If the fish are not sufficiently washed after gutting, some autodigestion could also take place, leading to further softening. Later in the summer, the fish become more choosey and prefer shrimps — and their flesh by now is firmer.

Nearly 50 years ago, a scientific report (1) hinted that the fishing grounds themselves might influence the quality of the fish. It was stated that cod caught in the Faroes kept better in melting ice than did those from other grounds. This is now known to be a pH effect, governed by the carbohydrate reserves of the fish, and will be fully described later.

In the following account, we shall describe some individual features of the fish and how or why they change with the seasons and the locality. Shulman (2) described them collectively as the 'syndrome' of the fish: it is the integrated features which govern the overall acceptability of the fish as food. The fish type used for study has mostly been the Atlantic cod along with lesser consideration of other gadoids, fatty fish, and salmonids. Observations made with one species do not necessarily apply to others.

II. FEATURES OF THE FISH

A. Skin Colour

The skin colour of many species of fish reflects that of the sea-bottom. Thus, cod from the north of Iceland, where the bottom is mostly of black volcanic ash, are the darkest of all that we have encountered. Cod from the fjords along the coast of Norway live amongst weeds and are often of a rich gold colour. Those from the Faroe Bank (60–53 N 08–20 W), which is composed of gleaming white shells, are extremely pale, as are all other species from that ground.

The fish change their colour according to stimuli received from their eyes, so if there is a black fish in a shoal of normally-coloured fish, that fish is blind. An experienced buyer on the fish-market can often pin-point where any batch was caught, so is able to choose those which he most favours.

There are so many variables involved in animal biology that apparently simple relationships do not necessarily apply at all times. In the present instance, the adaptability of fish skin colour to background colour is limited. When some pale-coloured cod from the Faroe Bank were brought back alive to Aberdeen (3) and placed in an aquarium together with darker-coloured cod from the Aberdeen Bank (57–05 N 01–15 W), the skins of the two groups of fish tended to change towards the intermediate colour of their new surroundings, *but not completely*. After as much as 16 weeks in the same environment, the two groups of fish could still easily be distinguished by colour, though not as strikingly as at first. This could indicate either a genetic difference between the two stocks, or different, limited ranges of colours being assigned to the two groups early in life as, for example, are the numbers of vertebrae in herrings (*Clupea harengus* L.), which become established in the young fry of a specific age according to the environmental temperature (4).

B. Flesh Colour

1. Non-Fatty Fish

Figure 35.1 shows the appearance of a cod with the skin removed. The lateral dark area represents 'red muscle,' the remainder 'white muscle.' The former is more fatty and more vascular than the latter and is rich in myoglobin and mitochondria, characteristics typical of very active muscle. Its metabolism is aerobic, and its purpose is to enable the fish to cruise for long periods without resting. The white muscle operates anaerobically and is used for a sudden dash in pursuit or escape; its poor vascularity and lack of haem pigments result in the need for a recovery period between bursts of activity to restore the energy

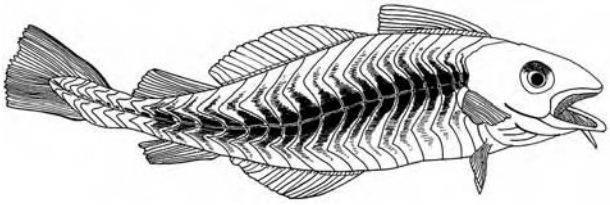

FIGURE 35.1 A cod with skin removed, showing the lateral red muscle. (Drawn by Eva Parsons. Crown copyright.)

compounds. The actual proportion of red muscle between different species of fish reflects their customary physical activity; flat fish which spend much of their time at rest have hardly any of it.

The most active species of the gadoid family is the saithe (*Pollachius virens* L.), so its musculature contains a greater proportion of red muscle than that of cod or whiting (*Merlangius merlangus* L). It can make the fillet look rather dirty, for which reason it fetches a lower price on the market. This can have important commercial consequences, as the following account shows.

A certain company bought the total catch of cod from a trawler, without prior inspection. When filleted, the flesh was found to be unacceptably dark for processing into the frozen cod fingers for which they had been purchased. It was realised that there would be complaints and possible litigation so, rather than scrap the whole batch, they made it into fish-cakes, a lower-priced product. The exact source of the fish was unknown, so an extensive survey of cod from different grounds was undertaken at Torry Research Station (5).

The actual *proportion* of red muscle was found to differ little between cod caught on different grounds, but the *intensity* of the red colour was greater in the fish from two localities at Svalbard, the most northerly grounds investigated. The Svalbard cod are unique in swimming great distances each year, from the Lofoten Islands off Norway, where they spawn in February, to Svalbard, where they arrive in summer to feed. Another feature which makes the Svalbard cod unique (Figure 35.2) is the distribution of red muscle colour between the individual fish of each sample. In the fish from grounds other than Svalbard, the distribution was Gaussian — a symmetrical shape, with values greater and less than the median tapering off in each direction. No such shape was seen in the samples from the two Svalbard grounds, where values ranged haphazardly from pale to intense. Examination of the same race during the spawning season off Lofoten again revealed deep chocolate-brown lateral muscle.

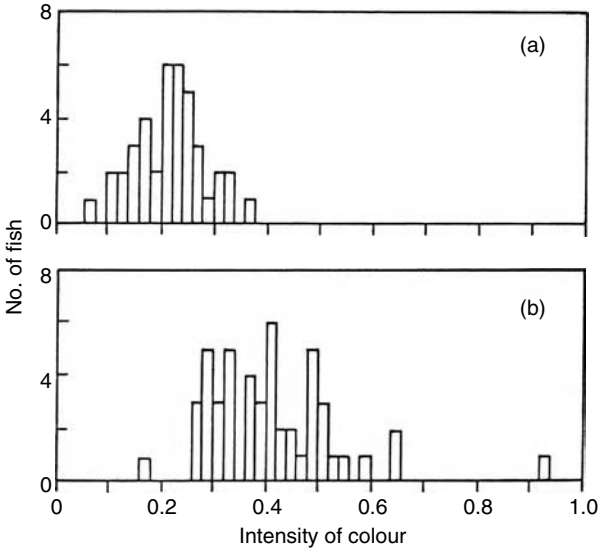

FIGURE 35.2 Distribution of intensity of red muscle colour in batches of 50 cod caught (a) on the Faroe Bank and (b) off West Svalbard. Faroe Bank fish are a stationary stock, Svalbard fish are migratory. The values on the abscissa are optical densities at 512 nm of a 4 cm light path of acid-acetone extracts. (Reference 5.)

The most reasonable interpretation of Figure 35.2 is that, while the red muscle in cod from Svalbard was on average darker than that from other grounds, the fish with the most intense colour had just arrived from their long journey, while the pale ones had reached Svalbard some time previously and were now less active. Subsequent experiments with cod caught near Aberdeen showed that after 28 days of activity, swimming round a circular tank (5), their red muscle had become significantly darker than that in fish from the same batch, which had been rested for the same period; during this resting time, the colour had faded appreciably. Thus, the intensity of colour in red muscle adapts to activity level, rather than being a fixed characteristic of a species.

In a seasonal survey of cod from Aberdeen Bank, the red muscle colour showed a smooth variation during the year (Figure 35.3), the minimum value in April being just 63% of the maximum in August. Since changes in pigmentation follow physical activity, a 'swimming activity curve' would probably lie somewhat to the left. Be that as it may, the more deeply pigmented cod must have been more energetic than usual, presumably because of increased feeding activity.

2. Fatty Fish

All species of fish carry triacyl glycerols as reserves of energy, but non-fatty fish, such as cod, carry virtually all of it in the liver, and fatty fish, such as herring (*Clupea* spp.) and salmonids, distribute it throughout the flesh. Since fatty species are active swimmers, their musculature is usually dark-coloured, but this fact does not actually worry the consumer. The point is that, because of its content of flavourous substances, the red-coloured muscle is more tasty than the white muscle anyway. In a trial at sea with two senior Japanese fishery scientists (6), European species of non-fatty fish were tasted, immediately after catching, as *O-Sashimi*, i.e., raw, with *Wasabi* sauce. The somewhat despised saithe was here considered the most desirable, better even than prime fish such as lemon sole (*Microstomus kitt* Walbaum).

The lipid content of the muscle of fatty fish is the major factor influencing quality. Since it decreases markedly at and following spawning, there is therefore a seasonal variation in the acceptability of these fish. When the proportion of lipid is low, the cut surface of the fillet is matt instead of

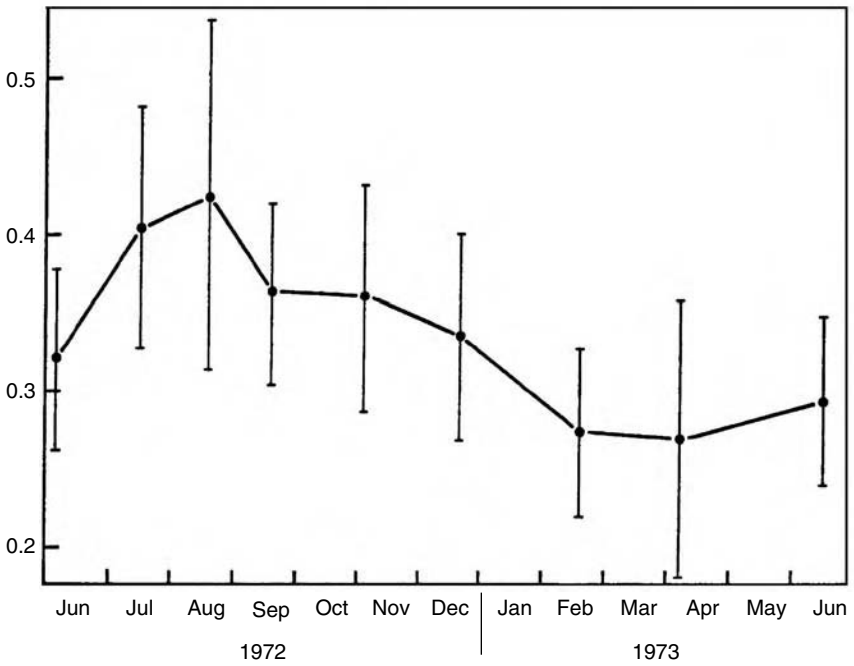

FIGURE 35.3 Changes in the concentrations of total haem pigments in the red muscle of cod over a 12-month period. Units as for abscissa in Figure 35.2. (Reference 5.)

glossy, it does not smoke satisfactorily, and the texture is 'dry' or fibrous in the mouth. The colour is not affected.

3. Salmonids

The pink colour of salmon and trout flesh reveals the presence of carotenoids, mostly astaxanthin and canthaxanthin. As far as the consumer is concerned, the colour is a desirable feature, but why is it present in these species and not in others? For a long time, carotenoids were considered to be a 'playful diversion of Nature' (7) of no real use to the fish except as precursors of vitamin A, but in salmonids they are closely tied to the reproductive cycle. The fish obtain them from their food, some being deposited unchanged while others are converted into other carotenoids before being laid down in the tissues (8). During the feeding season, they accumulate in the liver, flesh, skin, and fins, but accumulation in the flesh is negligible until maturation has definitely started (P.N. Lewtas, personal communication, 1977). As the gonads grow larger, their demand for carotenoids exceeds the dietary intake, and pigment is then increasingly transferred to them from the flesh. The process is induced by 17-methyl testosterone (9) and halted if the fish are castrated (10).

There is therefore a seasonal variation in the amount of pigment in salmonid flesh, which drains almost to zero around the spawning time. Figure 35.4 shows the changes in farmed rainbow trout (*Salmo gairdnerii* Richardson), which occur despite the fact that because the fish were farmed, they were receiving a continuous supply of

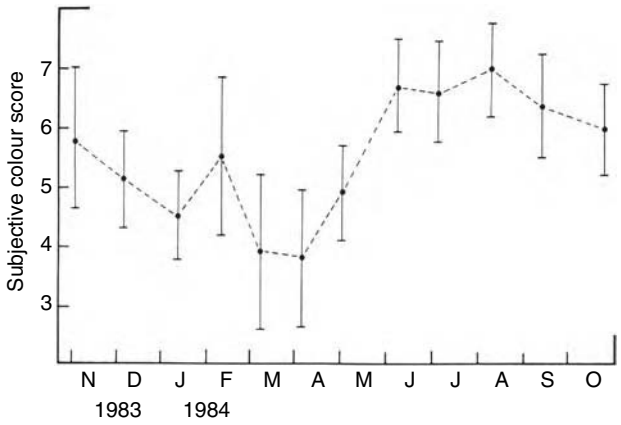

FIGURE 35.4 Seasonal variation in the flesh colour of rainbow trout from one farm, assessed by a panel on a subjective scale. (After Mochizuki and Love, unpublished. Crown copyright.)

canthaxanthin. Studies on chum salmon (*Oncorhynchus keta* Walbaum) during their spawning migration (11) showed that the carotenoid concentration in the blood serum ('in transit') rises steeply in the pre-spawning and near-spawning phases, but drops sharply when spawning actually starts.

A number of functions has been suggested for carotenoids in salmonids: as hormones and as assistants for fertility and embryonic development among other ideas. However, trout embryos with the orange oil droplet removed can develop normally (summarised 7). M. Hata

(personal communication, 1977) successfully reared rainbow trout embryos which were free from carotenoids. The main function of carotenoids may well be to protect the eggs from the harmful effects of sunlight, since salmonids lay them in shallow water.

C. Changes in the Main Constituents

1. Lipids

All mature fish undergo a regular cycle of depletion and restitution. This is the background to most of the phenomena, important in food science, described in the present account. It is based on the fact that ripe gonad tissue in most fish, particularly the females, occupies a relatively large part of the body cavity, and represents a huge synthesis of new protein and transfer of lipid. In most cases, the amount of food that the fish eats is quite inadequate to supply all this material plus the energy required for swimming, so the fish must perforce break down much of its own musculature ('endogenous feeding' - 12). As triacyl glycerols in the muscle of fatty fish are used up for gonad synthesis, the relative proportion of water in the tissue rises. This inverse relationship was called the 'fat-water line' by Brandes and Dietrich (13), who found that the percentage of muscle lipids in fatty fish could be assessed more conveniently just by measuring the water content.

In cod and similar low-fat fish, triacyl glycerols are virtually absent from the white muscle. The total lipid content is only between 0.5% and 0.6%, made up of 'structural' components, phospholipids and cholesterol and derivatives (14), the latter being about 10% of the total lipid. These membrane lipids are not available for mobilisation unless the muscle itself is broken down. Stored triacyl glycerols are removed from the liver instead.

There is some evidence that maturation, as distinct from simple starvation, mobilises polyunsaturated fatty acids selectively from the reserves for transfer to the gonads. An early report (15) stated that the lipids remaining in the bodies of herrings at maturity were less unsaturated than those from fish in the early stages of maturation. Studies on capelin (*Mallotus villosus* Müller) also showed (16) that polyunsaturated fatty acids tended to be mobilised from the body lipids and the more saturated fatty acids tended to be left behind. Neither study compared the changes in maturing fish with those which were not maturing under the same conditions. However, more fatty acid C22:6 (docosahexaenoic acid) has been found to be removed from the livers of starving cod which were actively maturing than from similar fish which had been castrated (17). This fatty acid is the most important one in the gonads of both sexes.

Some activities, such as maturation and migration which deplete the lipid levels in the fish, actually require a certain level of lipid reserves before they can take place. Thus, when young Atlantic salmon are starved on alternate weeks in the springtime, reducing their lipid stores, maturation is suppressed (18). Knowledge of this phenomenon can help the economics of fish farms, since the expensive fodder is directed towards profitable body growth, rather than to gonads which represent waste.

Anchovy (*Engraulis encrasicholus* L) overwinter by migrating from the Sea of Azov in September to warmer water, but must first accumulate a critical level of lipid. This 'triggers' migration at a particular range of water temperatures. Those which fail to exceed 14% lipid during their summer feeding do not migrate at all and die when this shallow sea freezes (19, 20).

2. Proteins

a. *Muscle Tissue*

In the muscle of non-fatty fish there is a significant 'protein-water line' (21), but in this case the water content is not just relative: extra water has entered the tissue. Figure 35.5

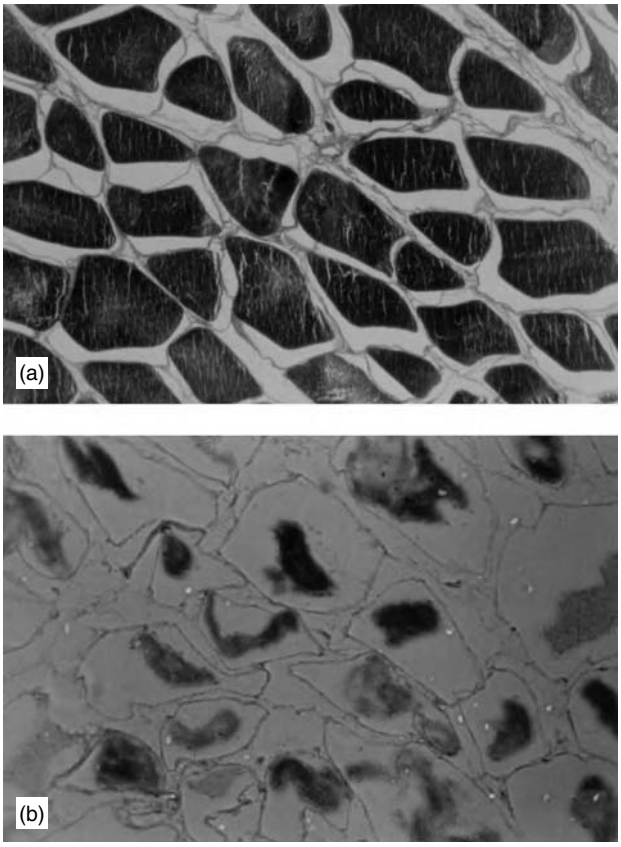

FIGURE 35.5 Cross sections of the white muscle of starving cod. (a) Moderate starvation, water content 83.3%. Some diminution of the solid contractile tissue can be seen, and the collagen sheath of each cell is separated from it by a layer of fluid. (b) Very severe starvation, water content 95.3%. The original outlines of the cells are still delineated by the thin lines of connective tissue but most of the contractile tissue has disappeared. Magnification: 120 times. (After Lavéty, unpublished. Crown copyright.)

shows a cross section of the muscle of a much-depleted cod, compared with a less-depleted counterpart. It will be seen that, while much of the contractile muscle tissue has been removed, the cells still retain roughly the same shape as before, the vacant space now being occupied by fluid.

This observation shows the main weakness of the classical 'condition factor,' used extensively at one time to assess the nutritional status of fish. The weights of fish of the same length were compared, using the formula (W/L^3), where W is the weight of the fish and L the length. Figure 35.5 shows that measuring the loss in weight during starvation or maturation under-estimates the actual loss of protein. In the case of extreme emaciation, cod do actually appear thinner (22) as seen in Figure 35.6, but the loss of protein in the earlier stages is not revealed. The concurrent loss of lipids from the liver can be seen in its diminishing size and its change of colour from creamy-yellow to red.

More than 40 years ago, a simple survey of the proportion of water in the white muscle of cod during the year (23) provided more information than expected (Figure 35.7). The water content of sexually immature cod was seen to increase in the spawning season, though to a lesser extent than in mature fish. This shows that the shortage of food in winter depletes every fish, not just those which synthesise gonads. In addition, fish which have spawned several times are more depleted than first-time spawners. In the author's laboratory (unpublished), it was later shown that the water content of the white muscle of cod held without food in an aquarium could increase to 86% in young fish before death ensued. That of large cod, however, could rise to as much as 96%, so it is clear that larger fish adapt to the greater drain on their resources. As fish grow, the proportion of gonads produced as a percentage of the body weight increases steadily. For example, the burden of spawning in hake (*Merluccius merluccius* L.) doubles for every 10 cm increase of body length (24). Eventually, the fish become so depleted that they cannot recover from spawning and die (25).

The relevance of these observations to food science is that while many species of fish go through a season of wateriness, which makes them unsuitable as food, small fish are less likely to be affected.

b. *Connective tissue*

The glistening sheets of material which bind individual muscle cells together and join blocks of muscle to the vertebral column are constructed of collagen, a protein rich in proline and hydroxyproline. It appears not to be mobilised to supply energy during starvation but, as described later, its physical properties change with the season.

3. Carbohydrates

a. *Their role and origins*

Carbohydrates are stored in the liver as glycogen, a 'polyglucose,' which is released into the blood as free glucose and transported to the muscle as required. On arrival, it is either used immediately to supply energy, or resynthesised into muscle glycogen for temporary storage. Although carbohydrates make up only a small percentage of the weight of the fish at any time, they are probably the most important of all constituents in the context of food science, since they are responsible for changes in the texture of the cooked product and in the way the fillets hold together. Their behaviour will therefore be described in some detail.

Carnivorous species of fish can acquire appreciable quantities of glycogen from the livers of their prey. Vegetarian fish on the other hand receive little or none of it in this manner, and indeed all species continually synthesise most or all of what they need from protein and lipid precursors ('gluconeogenesis'). They do, however, maintain certain levels of it which vary according to the general nutritional status of the fish, the glycogens of liver and muscle varying in tandem with each other (26). Figures 35.8 and 35.9 illustrate these relationships. There have been particular problems in studying the glycogen of white muscle. While there is enough of it in the liver to be measured by standard methods, this is not possible in white muscle because of the low levels present and their

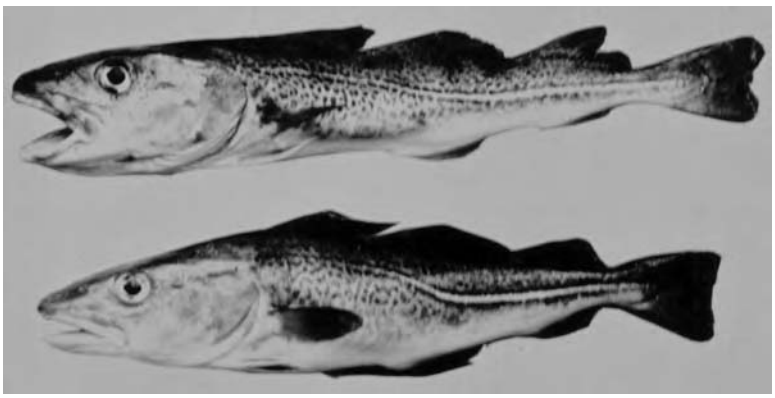

FIGURE 35.6 Severely starved cod (upper), showing overall change in shape compared with a fed specimen (lower). (Reference 21.)

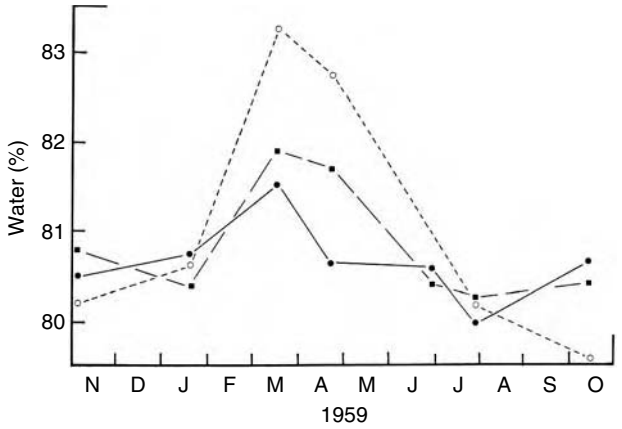

FIGURE 35.7 Changes in the water content of cod white muscle during a year in the wild. Solid circles: immature fish. Squares: first-time spawners. Hollow circles: spawned more than once. Large fish not available in June. (Reference 22, redrawn in Reference 41.)

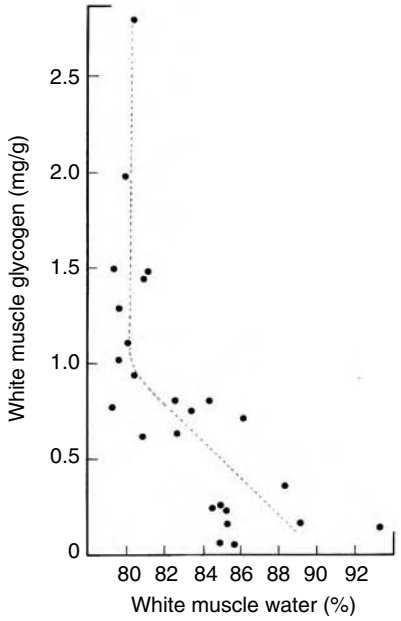

FIGURE 35.8 Relationship between the glycogen content of the white muscle of cod and its nutritional state as indicated by the water content. (Reference 25.)

ephemeral nature. Glycogen fuels all muscular activity, so it is rapidly consumed by the thrashing about which accompanies capture, and indeed its concentration in the muscle can be reduced by half after only 15 seconds of struggling (reviewed p 423 of Reference 8). Attempts to measure it in the muscle of newly-captured or frozen fish are therefore pointless.

The best that can be done in the way of direct assessment of glycogen in muscle is to stun rested fish by a blow on the head before they have had time to struggle. Small

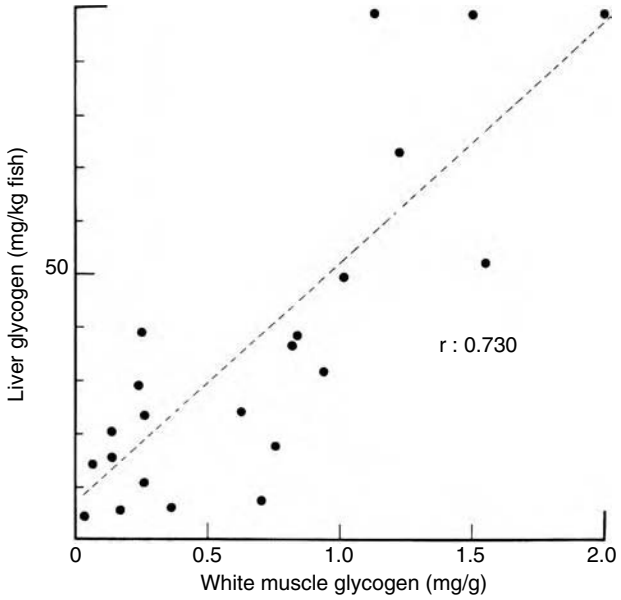

FIGURE 35.9 Relationships between the glycogen contents of liver and muscle in rested cod of various nutritional states. (Reference 25.)

samples of muscle are then rapidly excised and dropped into liquid air for subsequent analysis. As it is not possible even with these precautions to avoid all struggling, the levels as measured vary more than those from the liver.

b. The pH of muscle after death

i. Its derivation and measurement

As soon as a fish dies, oxygen no longer circulates to the muscle and glycogen breakdown stops at the lactic acid stage, through the Embden-Meyerhof-Parnas pathway. Some of it takes another path and is broken down into glucose by the action of an amylase present in the muscle (reviewed 27), so any variation in the proportion of the lactic acid and amylolytic pathways would affect the 'final' pH of the muscle (the pH after carbohydrate degradation is complete, usually measured 24 h after death), since lactic acid lowers it and glucose does not. It has been shown that the initial glycogen concentration strongly influences the 24 h post-mortem pH value of the white muscle of cod, whether fully fed, starved, or starved and refed. Possible effects of variations in the pathway therefore seem unimportant (28).

One question remains. Does the amount of struggling undergone by the fish at death influence the final pH? While the lactic acid formed during exercise is rapidly released into the blood system from the muscle of mammals (29), very little is released from fish muscle (30). The stress which accompanies some modes of exercise seems to cause the fish muscle to retain its lactic acid, probably under the influence of the catecholamine hormones (31). The lactic acid generated by violent exercise will therefore

supplement that generated spontaneously after death, so that the 24 h post-mortem pH values should be the same, however long the fish struggled before death. Experiments have confirmed this, the final pH of the muscle of cod removed quickly from a tank and stunned being similar to that of those previously exercised stressfully (32).

The measurement of pH after the completion of glycolysis therefore constitutes a simple method of assessing the carbohydrate level of the flesh (28) and, by the relationship shown in Figure 35.9, that of the liver also.

Struggling can, arguably, confer a certain benefit. *Rigor mortis* sets in as soon as the residual glycogen falls below a critical level after death. Since struggling greatly reduces the level of glycogen in the muscle, the fish enters *rigor* more quickly, and the condition lasts for a shorter time, than in a fish which dies without stress (33). This benefits farmed salmon, which should not be handled or filleted in *rigor* because it causes gaping; when they have become flaccid again after *rigor* (the 'resolution of *rigor*') they are suitable for filleting. It may take several inconvenient days before this state is reached, so some struggling before death can save money. There seem to be no advantages to processing or handling salmon by prolonging the times before or in *rigor* after harvesting, except where the salmon are to be sold intact to a customer, where the state of *rigor mortis* indicates freshness (34).

ii. Seasonal variation

It is reasonable to expect that the final pH of fish muscle should be high during the winter, because food is scarce and little carbohydrate is present in the muscle to form lactic acid after death. The fish feed continuously during the summer, so the final pH should then be consistently low. However, experimental findings do not bear this out. Figure 35.10 shows that, over a wide range of fishing grounds, the pH of cod muscle after death is high during most of the year, but that around June most of the fish exhibit low values for a short period, after which high values are restored. The approximate date when low values occur varies somewhat from year to year, but the principle is the same (35). The phenomenon appears not to occur in haddock (*Melanogrammus aeglefinus* L.) (31), but a short-term minimum pH value has been observed in June in farmed Atlantic salmon (36).

iii. Experimental starvation and refeeding

Studies of the three main constituents of cod tissues during starvation (37) have shown that they are mobilised for energy purposes in a definite order (Figure 35.11). The integrity of white muscle is more important to the survival of the fish than are the lipid reserves of the liver, so its proteins are not mobilised to supply energy until most of the liver lipids have disappeared. There is therefore a hiatus of several weeks, longer at lower temperatures and *vice versa*, before any change is seen in the protein content of the white muscle during starvation. Red muscle is generally more important to the fish than is white muscle, since it enables the fish to 'cruise' without fatigue. It therefore retains its glycogen for several weeks after the beginning of starvation, while depletion begins immediately in white muscle.

It is again reasonable to expect that, with the decline of available carbohydrate in starving cod, the activities of the enzymes that convert protein and lipid to glycogen (gluconeogenesis) would become more active, to redress the situation. However, it has been shown (38) that in fact the enzymes involved, phosphoenol pyruvate carboxykinase and alanine aminotransferase in the liver, and the fructose diphosphatase in liver, red muscle and white muscle, become steadily *less* active during starvation, though there can be a transient increase in activity during the early stages.

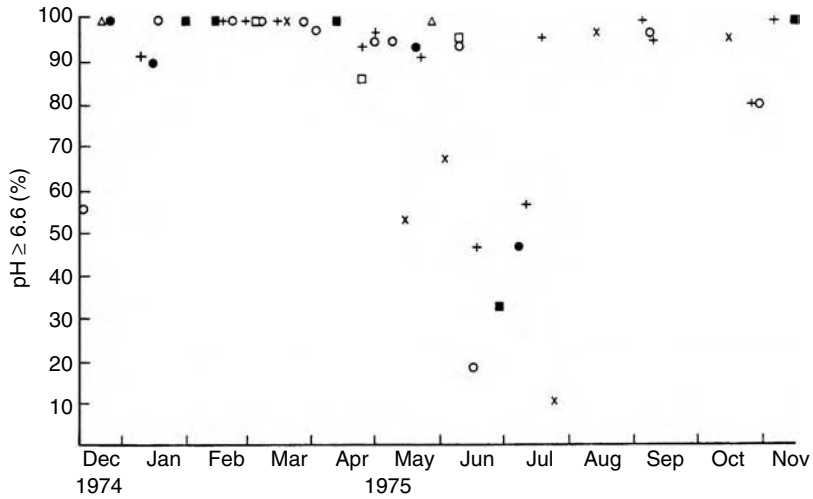

FIGURE 35.10 Proportions of batches of 20 cod, caught on various northern grounds, with post-mortem pH in the white muscle of 6.6 or more. (Reference 34.)

The starving cod adapt to decreasing glycogen resources by reducing their swimming activity, spending long hours motionless on the bottom of the aquarium. In contrast, starving rainbow trout (*Salmo gairdnerii* Richardson) do increase the activity of their gluconeogenetic enzymes, so that reasonable levels of glucose in the blood and muscle are maintained. The trout is a more physically active fish and continues to swim during starvation.

On refeeding, the sequence of change illustrated in Figure 35.11 is reversed. The muscle proteins are restored first, red muscle before white. Only after this do the liver lipids return to normal values (26).

The restoration of glycogen, however, is remarkable. There is enormous over-compensation, both in liver and muscle, during refeeding. A 20-fold increase above non-starving values of liver glycogen is possible (39). Figure 35.12 shows the effect in cod liver (26). It is noteworthy that the value after 195 days has dropped spontaneously to normal, despite the continuation of the feeding regime. Similar rises and subsequent falls are shown in red and white muscle (Figure 35.13).

The large increases in the levels of glycogen in the three tissues of starving-refed fish, compared with the level in fish fed continuously during the experiment, almost seem to indicate a loss of control or a metabolic imbalance, but studies with the DNA/RNA ratio, which varies in proportion to the vigour of protein synthesis, suggest that the over-compensation is a necessary part of the restorative process (26). The glycogen level in the red muscle reaches a maximum value after 60 days of refeeding, compared with 105 days in white muscle. The DNA/RNA ratios reach maxima at the same two points. Perhaps the energy required for protein resynthesis comes from this glycogen. The restoration of red muscle protein has priority over that of white muscle.

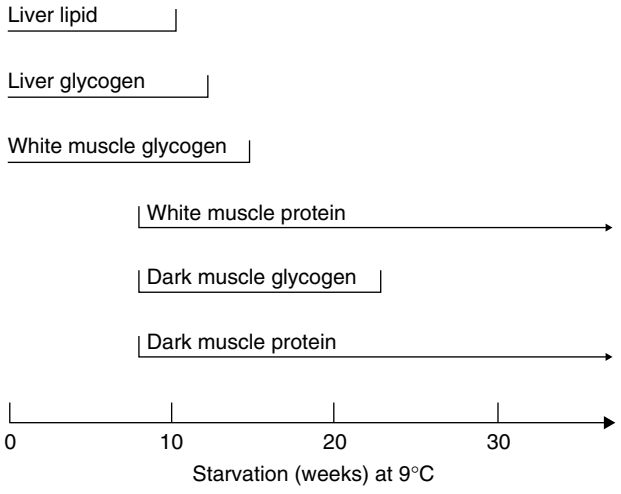

FIGURE 35.11 Diagrammatic representation of the sequence in which the principal constituents of liver and muscle are mobilised during the starvation of cod at 9°C. Time values are approximate. (Reference 25.)

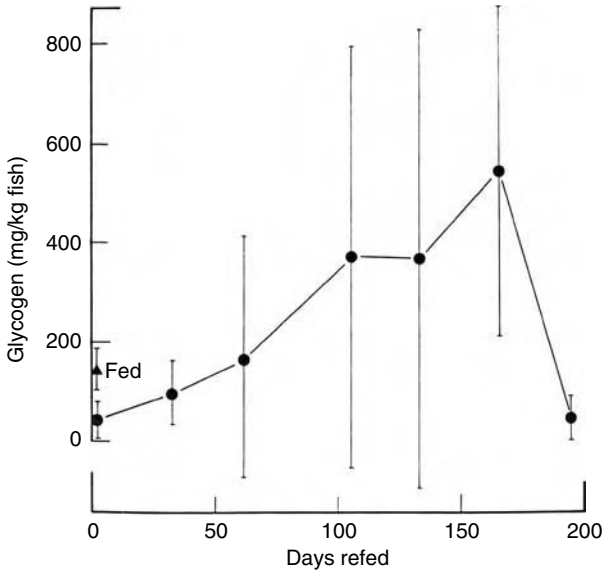

FIGURE 35.12 The excessive increase in liver glycogen during refeeding after starvation. The triangular point shows the level in fish fed throughout the experiment (never starved). (Reference 25.)

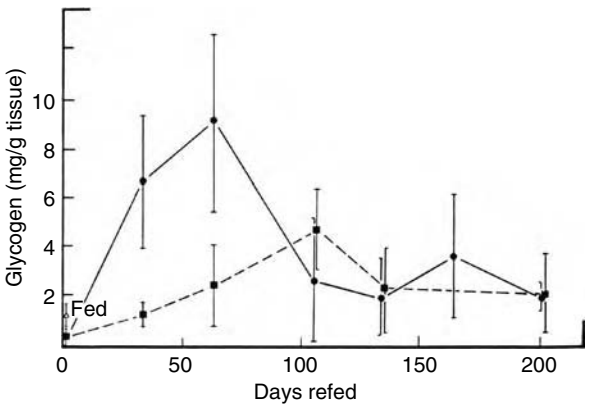

FIGURE 35.13 The excessive compensation of glycogen in red muscle (circles) and white muscle (squares) of cod, refed after starvation. Note how the maxima differ between the two types of muscle and (Figure 35.12) the liver. (Reference 25.)

A smaller glycogen overshoot is seen in fish previously starved for a shorter period, and the type of food proffered for refeeding also influences the phenomenon (26). Figure 35.14 shows that a diet of squid during refeeding results in a bigger overshoot than that resulting from a diet of herrings.

Figure 35.15 shows the corresponding final pH of the white muscle during starving and refeeding. A refeeding period of around 100 days was chosen to correspond with the glycogen maximum (Figure 35.13). A final pH lower than that usually found in fish from the

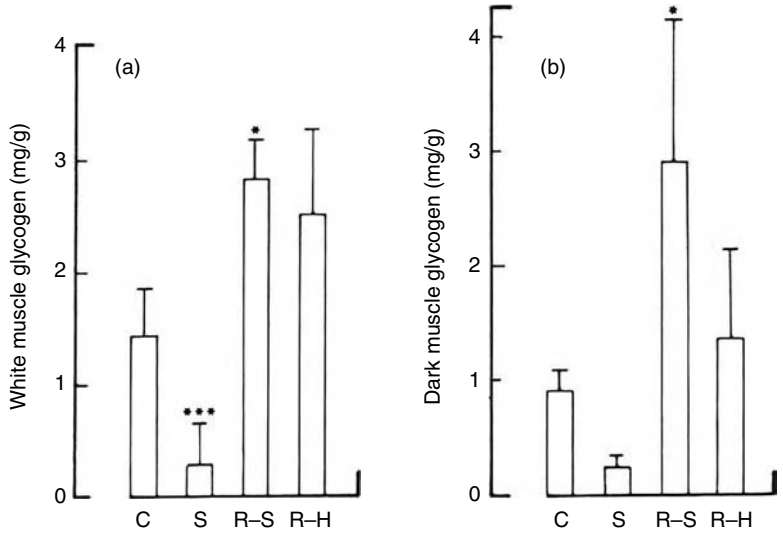

FIGURE 35.14 The effect of diet on the concentrations of glycogen in (a) white muscle and (b) red muscle in cod starved for 77 days and refed for 97 days. Asterisks show the degrees of significance from fed controls. C = control; S = starved; R-S = refed on squid muscle; R-H = refed on herring muscle. (Reference 25.)

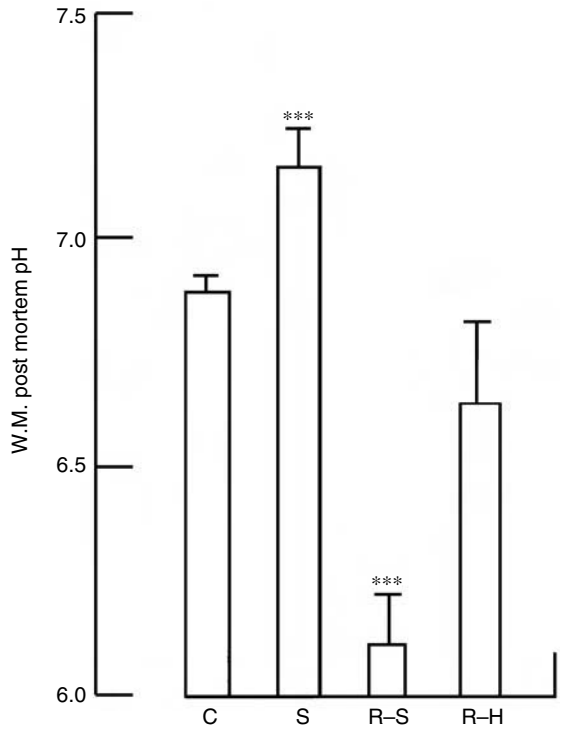

FIGURE 35.15 The final pH of white muscle in cod. Conditions and key as in Figure 35.14. (Reference 25.)

sea can be seen in the fish refed on squid. This result is thought to mirror the phenomenon seen in Figure 35.10 in free-living cod caught around June. Using the period of 100 days, one could speculate that these fish had started to feed in March (the stock used spawns around the second week in March). However, the results show too much scatter to predict the date of resumption of feeding from year to year.

III. BIOLOGICAL PHENOMENA AND FOOD SCIENCE

A. Gaping

1. Anatomical Background

Figure 35.16 shows the appearance of the cut surface of a cod fillet. The pattern of lines shows the cut edges of connective tissue sheets (*myocomma*; plural: *myocommata*) which merge with the skin on the lower surface and are joined to the vertebrae on the upper. They are made of the protein collagen (non-contractile) and bind blocks of muscle tissue (*myotomes*) together, transmitting the force of the muscular contractions used in swimming.

In the muscle tissue of warm-blooded animals such as beef, the connective tissue toughens as the animal ages, eventually resulting in a material which is a challenge to eat, even after long cooking. Fish connective tissue is much more 'frail,' and dissolves into gelatin at temperatures well below that of boiling water. It is therefore never a problem as regards the texture of the cooked product. Most of the potassium of muscle tissue is found within each contractile cell, while sodium is principally in the spaces between the cells and in the connective tissue. Figure 35.16 shows that there is a far greater proportion of connective tissue at the caudal end of the fillet, so consumers on a low-sodium diet should perhaps avoid this part.

Figure 35.17 shows (magnified) the junction between the ends of some white muscle cells on each side of a sheet

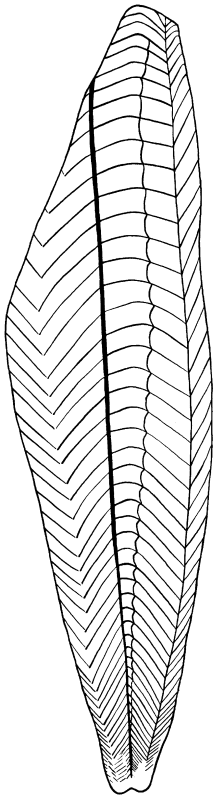

FIGURE 35.16 Drawing of the cut surface of a cod fillet (musculature next to the bone). The thin lines indicate the cut ends of the myocommata. (Reference 39.)

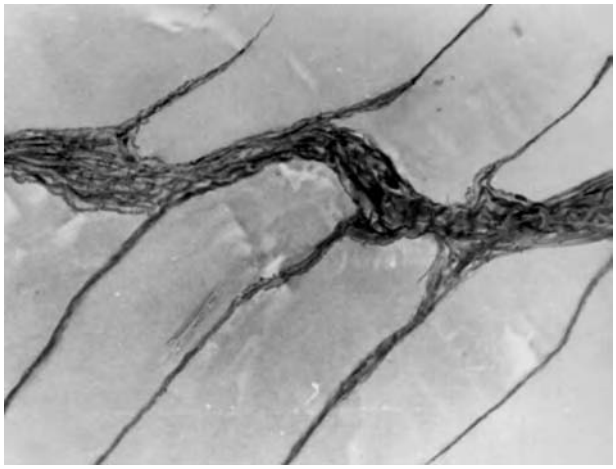

FIGURE 35.17 Photomicrograph of the junction of 4 individual muscle cells (pale areas) with each side of a myocomma (thick black stripe). The cells are about 100 microns across, and the connective tissue tubes separating each from its neighbour are shown as thin dark lines. (Author's picture. Crown copyright.)

of connective tissue, shown in black. The light grey areas are longitudinal sections of individual muscle cells, and it can be inferred that each cell is surrounded by a 'tube' of connective tissue which merges with the myocomma itself.

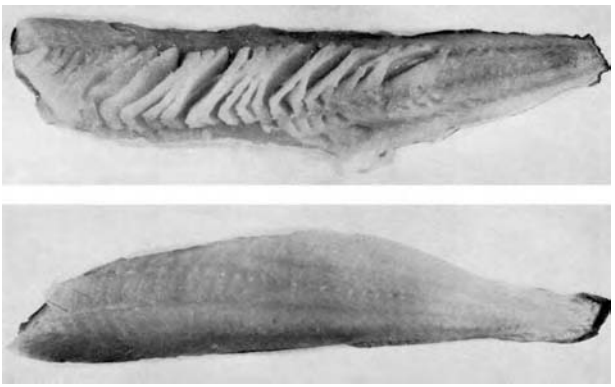

FIGURE 35.18 A bad case of gaping (upper), compared with a normal (lower) fish. Both fish were frozen 'round,' thawed and filleted, but the good specimen was frozen before *rigor mortis* and the gaping one had been preserved for 8 days in melting ice before freezing. (Crown copyright.)

It is an elegant mode of attachment, but when this junction breaks, gaping occurs (Figure 35.18).

2. Causes of Gaping

As a general rule, gaping is a problem of frozen fish. More specifically, it can be seen in fish which have been frozen intact, with or without guts in, then thawed and filleted. It is not usually a problem when they are filleted after the resolution of *rigor mortis* before freezing. Gaping is of great economic significance: a gaping fillet cannot be sliced, smoked — or sold. The difficulties associated with gaping and its commercial significance were brought home to the writer at a large processing plant, the owners of which owned the fishing boats which supplied it. At the time (June), their haddock were gaping so badly after filleting that they had to be minced and sold as a low-priced product which represented financial loss for the company. The management were in a position to control all handling procedures aboard the ships, but nothing they requested improved the situation. This fact triggered an eight-year investigation at Torry Research Station, Aberdeen.

Folk-lore from the fishermen has it that fish stuffed with food ('feedy fish') gape. This suggests that the soft flesh seen in early summer contains weak connective tissue, but experiments again showed otherwise (40). Figure 35.19 shows that it is the best-nourished fish which gape, the starved ones do not. The gaping score was assessed subjectively: a value of '1' signified a single longitudinal split in the fillet, and higher scores were based on the number of gaps between myotomes (41). The score figures are not mathematically related to one another, but the method is still a useful research tool. Figure 35.20 suggests that pH could be the factor responsible rather than softness as such, because the gaping follows the seasonal pattern of pH (42). The relationship was confirmed when

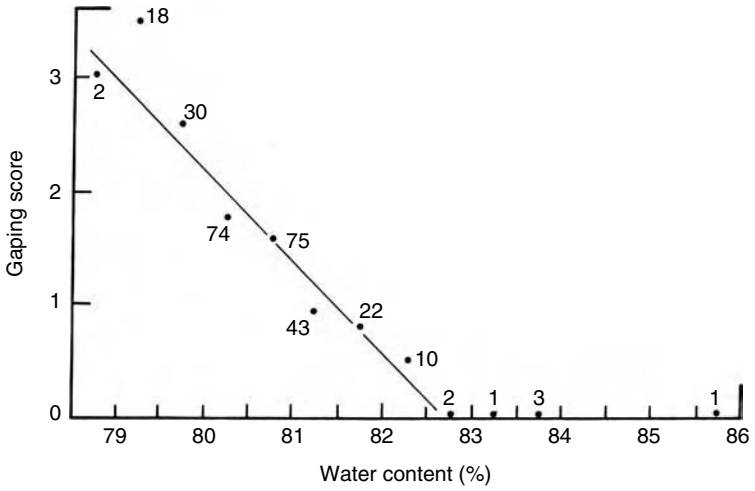

FIGURE 35.19 The relationship between the gaping of cod, frozen whole, thawed and filleted, and the water content of the white muscle. Numbers show the size of each sample wherein the water contents differed by not more than 0.5%. (Reference 41, redrawn in Reference 39.)

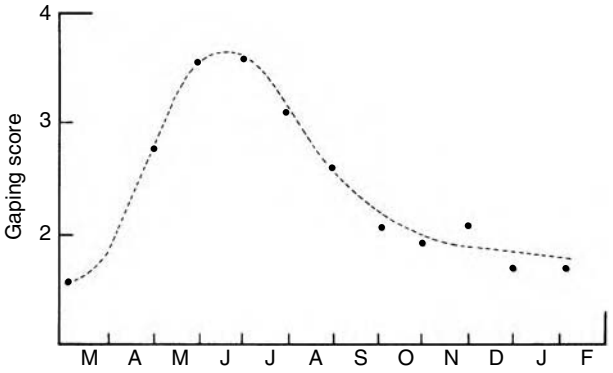

FIGURE 35.20 Seasonal variation in the gaping of cod muscle (1969–1970). Note that the peak corresponds with the period of minimum pH. (Reference 41.)

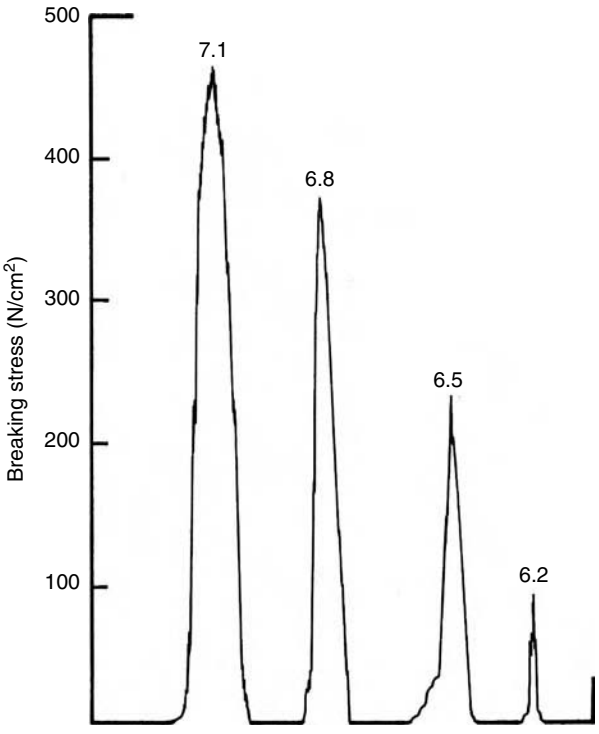

FIGURE 35.21 Tracings showing the force required to break strips of myocomma buffered at various pH values (shown above each peak) at 0. (Reference 42.)

isolated myocommata, suspended in buffers of various pH values and subjected to increasing tension until they broke, became increasingly frail as the pH decreased (Figure 35.21). The effect was reversible: myocommata which had been weakened in a buffer of low pH, then transferred to one of neutral pH, were found to have regained their strength (43). A similar relationship between season, pH and gaping has been found in farmed Atlantic salmon, the highest gaping score being in June and the lowest in January (36).

B. The Freezing Process Itself

The time between the death of the fish and freezing is important. Figure 35.22 shows that gaping is minimal in whole fish, frozen before the onset of rigor mortis and thawed before filleting. It rises when the fish have been frozen *in rigor* and rises further when frozen after the fish have become limp again (41).The phenomenon has been seen in several species of fish, but there are differences in degree (41). Haddock (*Melanogrammus aeglefinus* L.) gape the most, followed in sequence by cod, saithe (*Pollachius virens* L.), redfish (*Sebastes marinus* L.), halibut (*Hippoglossus hippoglossus* L.), and lemon sole (*Microstomus kitt* Walbaum).

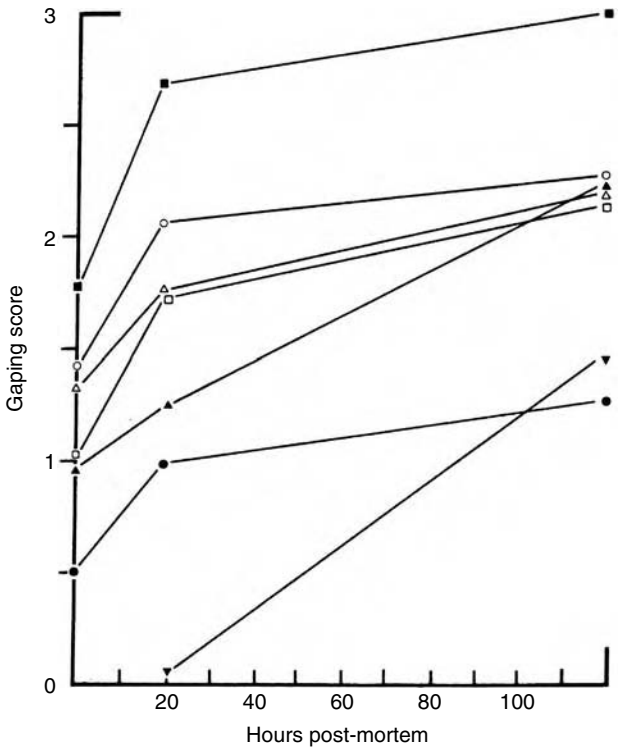

FIGURE 35.22 Gaping in fillets made from different fish species frozen at different times after death. All species were pre-*rigor* at 0 h, in *rigor* at 20 h and post-*rigor* at 120 h. Species from the top downwards: haddock, cod, saithe, redfish, halibut, lemon sole, plaice. (Reference 40.)

The lesson is clear: freeze whole fish as soon and as quickly as possible after death, bearing in mind that if they are frozen slowly, the effect is the same as that of prolonging the period preceding freezing.

The mechanism of gaping from this cause is interesting. The surface of a fillet cut before the onset of *rigor mortis* is dry to the touch, while if it is taken from a fish actually in *rigor*, it feels and looks wet. Freezing results in the solidification of this 'free' water, together with some of the water more closely associated with the contractile proteins. The frozen water becomes physically separated from the muscle cells, now dehydrated, in bodies of protein-free ice, faster freezing generating more numerous, smaller ice crystals and *vice versa*. Some tissue water is so tightly bound to the protein that it never freezes, but the proportion of freezable water is smaller in *pre-rigor* muscle than in muscle in or beyond *rigor*. Measurements on cross-sections of frozen cod muscle (44) show that the area attributable to ice is 46% of the total area in cod rapidly frozen *pre-rigor*, while in that frozen in *rigor* or after its resolution the figure is 57%. In addition, when fish are kept for longer periods in melting ice, water from outside steadily diffuses into the muscle tissue (45), resulting in further break-up of the myocommata on freezing (Figure 35.23).

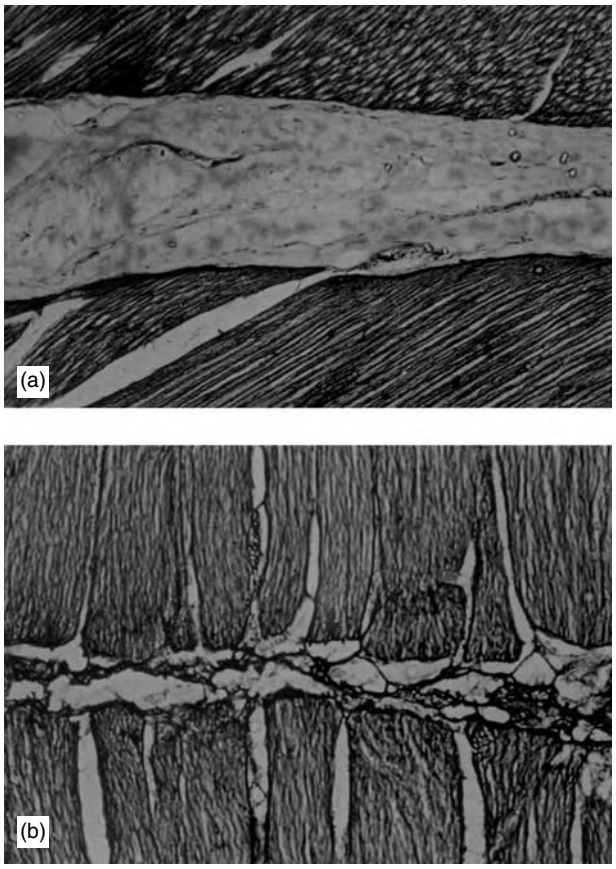

FIGURE 35.23 Sections through frozen cod muscle (small pale areas represent ice crystals). Both specimens frozen very rapidly at –80°C, but specimen (a) frozen 1 day after death, (b) after the whole, gutted fish had been held in melting ice for 8 days before freezing. The band across the middle is the myocomma, with muscle cells on either side. The tissues in (a) appear almost as fresh muscle. The myocomma in (b) is completely disrupted by ice crystals; fish in this condition gape badly. (Reference 44, Crown copyright.)

C. Handling

pH is not the sole arbiter of gaping. The intrinsic strengths of the collagen membranes vary between species. In a comparison of hake (*Merluccius merluccius* L.), cod and catfish (*Anarhichas lupus* L.), hake were found to gape more readily than the other two species and catfish did not gape under any experimental conditions, including very rough handling (46). The mean breaking stresses of myocommata were found to be 30 (hake), 87 (cod) and 142 (catfish) N/cm^3, the final pH values of all the fish being similar. The breaking stresses of skin samples were greater, but differed between the species in the same sequence as above.

In *rigor mortis* of whole fish, the muscle masses on each side of the fish contract strongly against each other, resulting in an unyielding rigidity. If the fish is curved as it enters *rigor*, this shape becomes fixed, and clearly any

attempt to straighten it before filleting would cause severe gaping in species other than catfish. Similarly, throwing rigid fish to other operatives or dropping them from a height would also cause gaping. This is common sense, but it still happens in practice.

Incidentally, if a fish is filleted before *rigor mortis* has set in, the musculature is now free to contract as it enters *rigor*. The shortened fillets, which take on a ribbed appearance, are tough to eat after cooking. If the *pre-rigor* fillet is dropped straight into a hot pan, it contracts even more and is virtually inedible because of its very rubbery texture.

D. Temperature

As stated, fish collagens dissolve as gelatin at much lower temperatures than the collagens of warm-blooded animals. The effect can be seen in Figure 35.24, where gaping increases to a maximum value when freshly-killed cod enter *rigor mortis* at 25°C. Controls at iced temperature do not gape at all (47). During heavy fishing in the summer months, the temperature of the fish could easily rise sufficiently to cause ruinous gaping if freezing were delayed. It may, however, be possible to retrieve the situation (43). Figure 35.25 shows that myocommata break more at higher temperatures, but that they are less easily broken if they are cooled to 0°C before measurement. Hence, it is vital to cool fish before further handling if they have been lying in the sun.

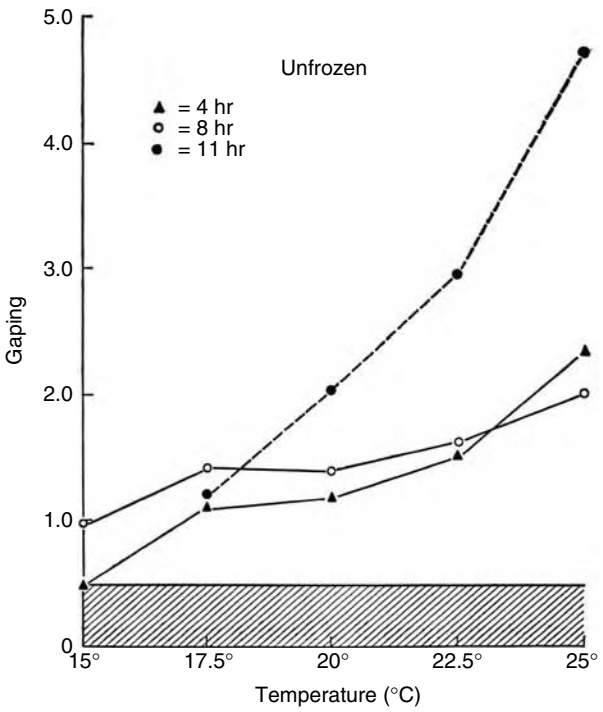

FIGURE 35.24 Gaping after filleting whole, gutted cod kept at different temperatures after death. The hatched area shows the unchanging state of controls kept in melting ice throughout the same period. (Reference 46.)

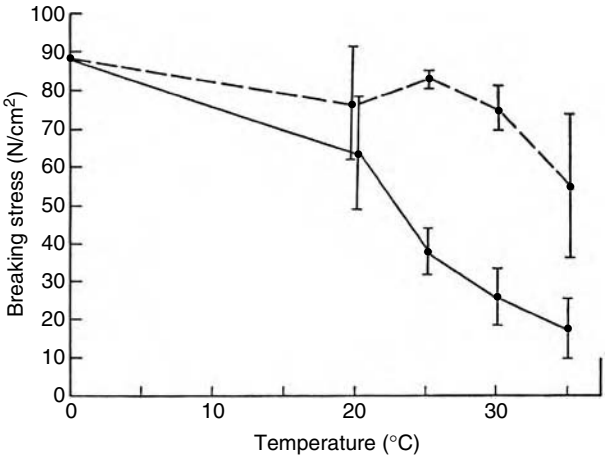

FIGURE 35.25 Breaking stress of isolated myocommata from cod kept at various temperatures for 1 h. Continuous line: measured at the actual experimental temperature. Broken line: collagen specimen cooled to 0°C before being stressed. (Reference 42.)

Rainbow trout also gape more as they enter *rigor* at higher temperatures, but *rigor* at 26°C in this species does not cause gaping: high scores require temperatures at or above 35°C. The higher water-temperatures tolerated by this species during life seem to engender a greater thermal stability in their collagen (36).

E. Size

Figure 35.26 shows that small cod gape more than large. The relationship is seen only in summer and autumn, however, disappearing or even being reversed in the winter and spring (48). Two factors are at work here: the pH of larger cod can be lower than that of smaller, varying with the season, and can over-ride the size effect.

Do any of these observations help to explain the industrial problem with haddock, described earlier? Fish of this

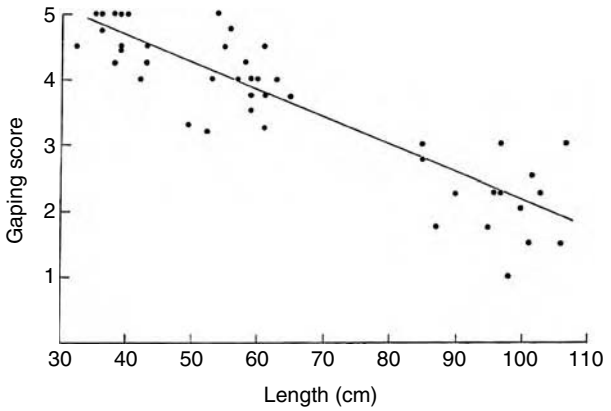

FIGURE 35.26 Influence of body length on gaping in cod caught in June 1968, frozen, thawed and filleted. r = 0.899. (Reference 47.)

species have been found to gape more than other species investigated (41), and the industrial problem was encountered in June, when the final pH values are often low. Isolated myocommata from haddock are in fact stronger than those of cod (43), but the final pH of this species has been observed to be intrinsically low, even as late as October (49), almost certainly accounting for their greater tendency to gape. The reason for the low pH is unknown.

There is scant advice for mitigating severe gaping in haddock. The fish should be put into the freezer before the onset of *rigor* if at all possible and any additional fish chilled *at once* and handled gently. Otherwise, it is better not to freeze them whole around June — though such advice may be impractical.

F. Texture

1. pH

Although the tough texture sometimes found in cooked beef is never found in fish, the latter, stored in the frozen state for too long or at too high a temperature (or both), yields a product which, after cooking, is somewhat difficult to swallow and can leave fibrous strands stuck between the teeth of the consumer. The sensation is of a dry, fibrous product and is unpleasant, especially if coupled with a rancid taste. Here we examine the texture of fish that has not been frozen, which can range between sloppy and firm.

The relationship (50) between the final pH of cod muscle and the texture as eaten is shown in Figure 35.27. The water contents of the muscle of the different fish fell within the narrow range of 80.0 to 80.9%, which is normal for well-fed cod (23). The softest fish were therefore not watery. This finding casts an interesting light on the texture of acutely starving fish, which are also sloppy and can, after cooking, be sucked through the teeth (not pleasant). The very slight effect of water content at constant pH on texture is shown in Figure 35.28. It is clear that pH is the over-riding factor governing the texture of the cooked product. Its importance in assessing the suitability of fish for processing was recognised earlier by Cowie and Little (51), who selected fish on the market for processing solely on the basis of pH, using a probe pH electrode pushed straight into the wet tissue. The dividing line between tough and acceptable frozen cod fillets has been found to apply also to fish *minces* from 16 Australian species caught by mid-water trawling and cooked after storage for up to a year at –18°C (52).

2. Cold Storage

There is little to add to what has been said already. Free water and some water associated with the protein structures of the muscle separate out as ice as the fish freezes. As cold storage continues, the individual muscle cells are less and less able to resorb the melt-water, after thawing. More water therefore drips out of the thawed fillets and the sensation of the cooked product becomes increasingly dry or fibrous.

The rate of deterioration is governed by the storage temperature; it is progressively slower at lower temperatures, but also varies with the species. Studies carried out at –14°C showed that the whiting (*Merlangius merlangus* L.) deteriorated the most quickly, reaching its limit after 10 weeks (53), whereas the lemon sole (*Microstomus kitt* Walbaum) required over 17 years to reach the same degree of deterioration (54). For this to happen, the muscle fibres must be dehydrated by the formation of ice bodies within the tissue. Frozen cod muscle deteriorates very quickly at -1.5, but no change occurs in supercooled muscle stored at the same temperature with no ice present (55).

The consequence of these phenomena is that fish which are already firm-textured through having a low final pH soon become unacceptable as a foodstuff if they are subsequently cold stored. More interestingly, high-pH fish which are sloppy-textured can actually be improved by a short period of frozen storage.

IV. THE SIGNIFICANCE OF GEOGRAPHY

A. Introduction

We have seen earlier that the colour of the red muscle of one particular stock of cod which swim great distances (the Svalbard-Lofoten stock) is more intense than that of

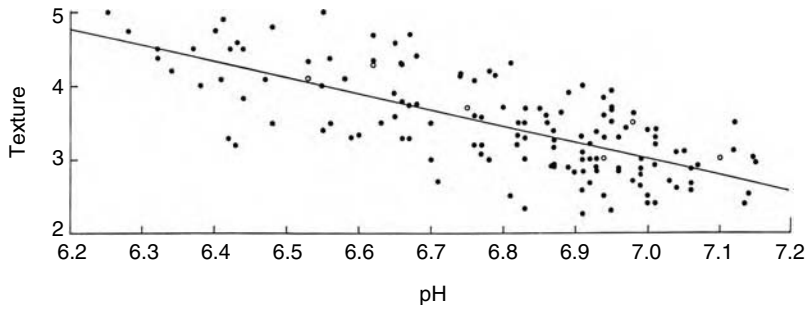

FIGURE 35.27 Influence of final pH on the texture of cooked cod fillets. Water contents of all samples limited to 80.0–80.9%. Texture scores above 3 represent firm or tough flesh, below 3 soft or sloppy. Two identical values are shown as hollow symbols. (Reference 49.)

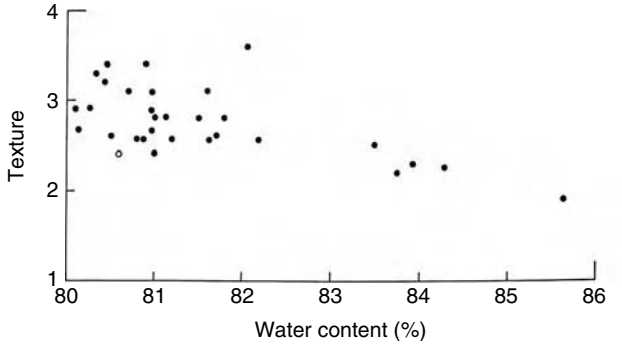

FIGURE 35.28 The influence of muscle water content on the texture of cooked cod muscle as eaten. All pH values were within range of 7.0–7.1. (Reference 49.)

more sedentary stocks. As regards food science, the effect is largely cosmetic but can lead to financial loss.We now consider whether the characteristics of any grounds are such as to affect the desirability of the fish as food.

It is easy to imagine that fish from widely-spaced enclosed bodies of water might differ from one another because of disparities in temperature, water-chemistry, identity of creatures consumed, etc. The chances of finding important differences between fish from widely spaced oceanic locations seemed much less promising, and a detailed survey was undertaken without much optimism (56). However, the results amply justified the enterprise, some characteristics of cod from the Faroe Bank (60-53 N 08-20 W) being significantly different from those of cod from the other grounds investigated.

B. The Faroe Bank Cod

1. The Fish Themselves

Figure 35.29 shows the fine appearance and corpulence of a Faroe Bank cod, quantified in Figure 35.30. Only the neighbouring Faroe Plateau cod are comparable. The livers were very large and creamy, showing how well the fish had been nourished. Early work on cod from Aberdeen Bank (57-05 N 01-15 W) showed that the water content of the muscle ranged from about 80% to 80.9% in well-fed fish during a span of twelve months (Figure 35.7), a value of about 81% or more signifying early stages of starvation (23). The white muscle of spring-caught Faroe Bank cod seems to be unique in having less than 80% of water (Figure 35.31) and more protein nitrogen (Figure 35.32) than cod from other grounds. Values for liver glycogen are high though not remarkable (56), but the final pH of the muscle is lower than in any other group (Figure 35.33). These fish were caught in the spring of 1966. Those caught in the autumn of 1968, when all the fish were sated, still maintained the uniquely low level of muscle water content, but the protein nitrogen and pH values were not remarkable. The others had caught up. The low value of muscle

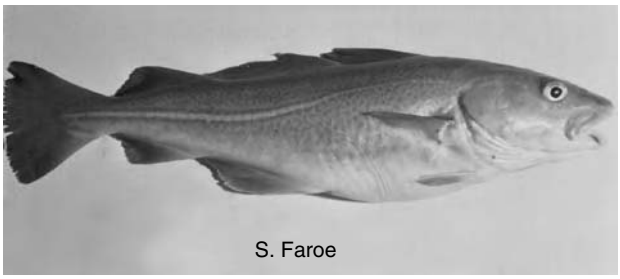

FIGURE 35.29 A cod from the Faroe Bank (length: 51 cm). (Author's picture. Crown copyright.)

water in Faroe Bank cod was observed again during surveys in September 1971 and September 1972.

In a small independent experiment (57), the total lipid content of the white muscle of Faroe Bank cod caught in the spring was 0.63%, compared with a value of 0.55% in Aberdeen Bank cod, while in the autumn the corresponding figures were 0.78% and 0.67%. This small difference appears to be important, as we shall see.

2. Consequences for Cold-Stored Cod

It has been concluded in a well-reasoned article (58) that the term 'quality' is often over-used and made to cover a variety of observations. Preferably, one should specify the parameter studied. Here we study the texture, flavour and appearance of the thawed, filleted and cooked product. Just looking at the superb appearance of the fish lying on the deck at 3 A.M. or so (night-fishing is the rule on the Faroe Bank), one instinctively feels that their overall 'quality' as an item of food must be at the top of the scale. In our survey (56), the fish were frozen whole very soon after capture, stored at −30°C for three months, thawed at low air temperatures, filleted and examined before and after cooking. These conditions are as near ideal as one can provide — but the quality scores for all three parameters were uniformly low.

a. Texture and Gaping

Because of the strong link between pH and texture, it has been suggested that the fish most suitable for long-term storage should have a pH not less than 6.6 (59) or 6.7 (60). In many of the Faroe Bank cod caught in the spring, the pH was lower than 6.6. As a result, the texture of the cooked product as tasted by a panel was firm, but that of the same fish after 3 months at −30°C, thawed and cooked, was judged to be unacceptably tough. (50). Similarly, because of the low pH of the muscle, the thawed fillets gaped. The fact of unacceptability in each case casts doubt on the suitability of these fish for freezing and cold-storage.

b. Flavour

The off-flavour developed by fish muscle during cold-storage (rancidity) has been likened to wet cardboard, boiled

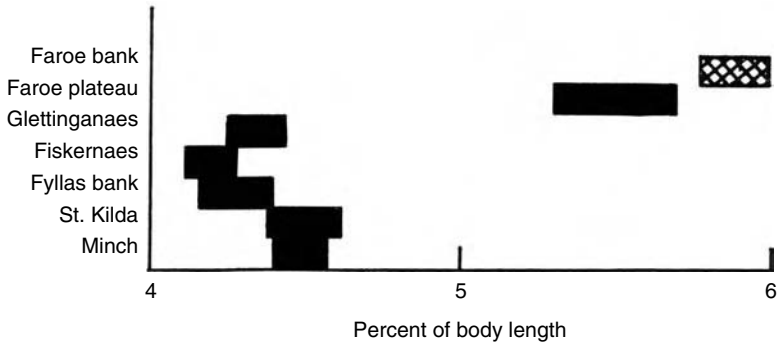

FIGURE 35.30 Corpulence of cod caught on different grounds in spring 1966, shown as the maximum diameter of the caudal peduncle expressed as a percentage of body length. Bars represent 95% confidence limits. (Reference 54.)
Map references: Faroe Bank: 60-53N 08-20W, Faroe Plateau: 62-34N 06-24W, Glettinganaes (SE Iceland): 65-27N 13-08W, Fiskernaes (Greenland): 63-15N 52-40W, Fyllas Bank: 63-55N 52-53W, St. Kilda (Scotland): 57-45N 08-40W, Minch: 58-13N 05-38W

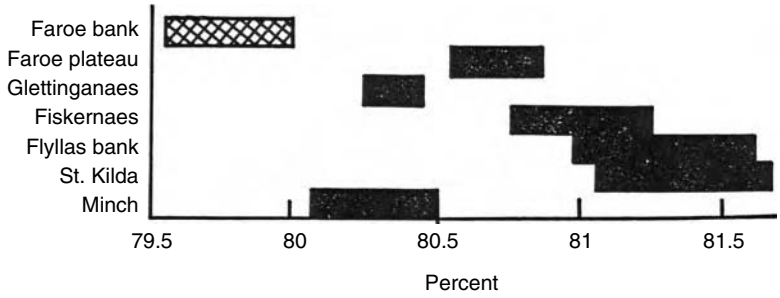

FIGURE 35.31 Water content of the white muscle of cod caught on different grounds. Legend as in Figure 35.30. (Reference 54.)

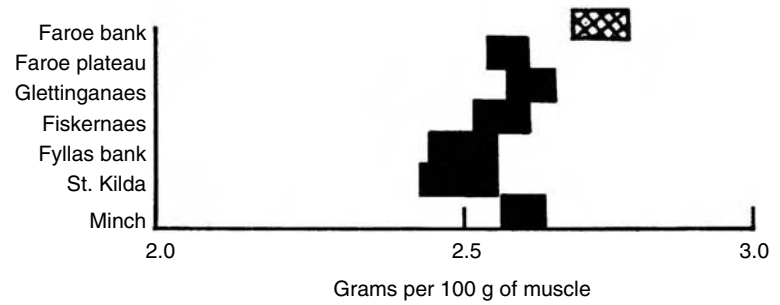

FIGURE 35.32 Total protein nitrogen in the white muscle of cod. Legend as in Figure 35.30. (Reference 54.)

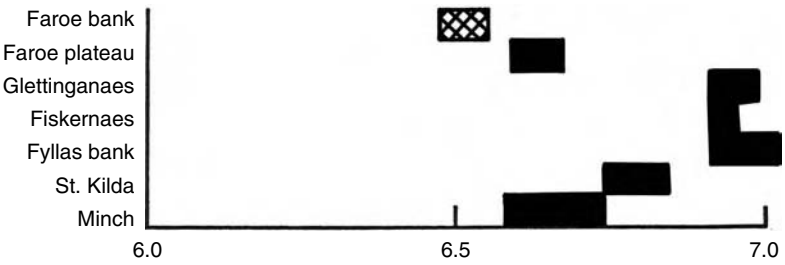

FIGURE 35.33 Final pH of white muscle of cod. Legend as in Figure 35.30. (Reference 54.)

TABLE 35.1
Taste-panel assessment of cold-store off-flavour and off-odour of cod caught in spring 1970 on different grounds, gutted and stored for 3 months at −30°C. The fish were filleted and steam-cooked without seasoning before tasting. 0 = absent; 5 = very strong, the upper limit of commercial acceptability being about 3. Comparing the five grounds by analysis of variance by the *F* test showed that the mean results for the Faroe Bank differed significantly from the others for flavour ($P = 0.01$) and odour ($P = 0.05$). (Reference 64.)

Fishing Ground	Map Reference	Cold Store Flavour		Cold Store Odour	
		X	SD	X	SD
Aberdeen Bank	57-05 N 01-15 W	1.68	0.60	1.32	0.51
Faroe Bank	60-53 N 08-20 W	3.02	0.95	2.29	0.84
Faroe Plateau	62-34 N 06-24 W	1.45	0.63	0.91	0.51
SE Iceland	65-27 N 13-08 W	1.70	0.49	1.04	0.35
NW Iceland	65-35 N 25-00 W	1.37	0.37	0.84	0.24

clothes and other poetic imagery. It results mostly from the oxidation of polyunsaturated fatty acids located primarily in the phospholipids, and has been identified as hept-cis-4 enal (61–63). Many factors govern the rate of the reaction, and the chemistry is complex: the subject has been reviewed recently in detail (64). Since the phospholipids are part of the structure of muscle cell membranes, they present a large surface area for oxidation, compared with the neutral lipids (triacyl glycerols) which are more 'compact' and contain a smaller proportion of polyunsaturated fatty acids. They are the main constituents of the fatty deposits used as stores of energy. The spatial distribution of the two types of lipid is important (64) and may help to explain inter-species differences.

Since the lipid content of cod muscle is usually less than 1%, it might be supposed that it would develop negligible rancidity compared with that found in more fatty species on cold-storage, but this is not necessarily so. The most likely reason is that the lipids of cod muscle consist almost entirely of phospholipids — triacyl glycerols make up only about 1% of the total (65).

In small samples of Faroe Bank cod, the white muscle was found to contain about 20% more total lipid than that in Aberdeen Bank cod, and this probably explains the fact that cold-storage off-flavour (and off-odour) was significantly greater in Faroe Bank cod than in cod from four other grounds (66) and considered unacceptable by the tasters (Table 35.1). Might it be possible to reduce the cold-storage off-flavour of cod by reducing their total lipid content? A further factor which may govern the propensity of frozen fish to become rancid is geographic latitude, which affects the temperature of the sea-water. The lipids of fish become progressively more unsaturated as the water becomes colder (more likely to become rancid), and *vice versa* (21, 67, 68). The purpose appears to be to ensure that the melting-points of structural lipids are below that of the environmental temperature so that they remain flexible.

Experimental starvation of Aberdeen Bank cod for 2 months (69) resulted in a water-content of 84% and some

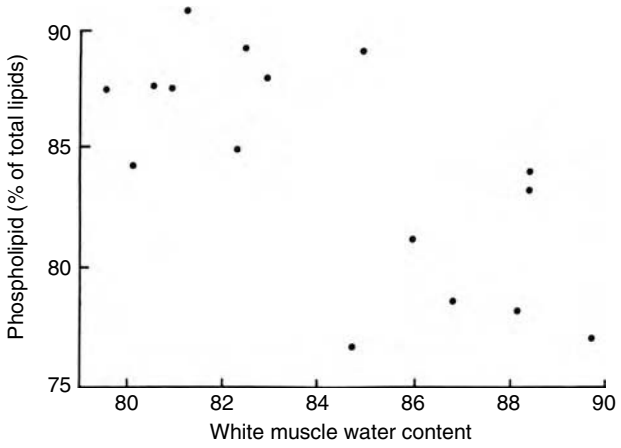

FIGURE 35.34 The decrease in proportion of phospholipids in the lipids of cod white muscle during starvation, shown by the increase in water content. (Reference 65.)

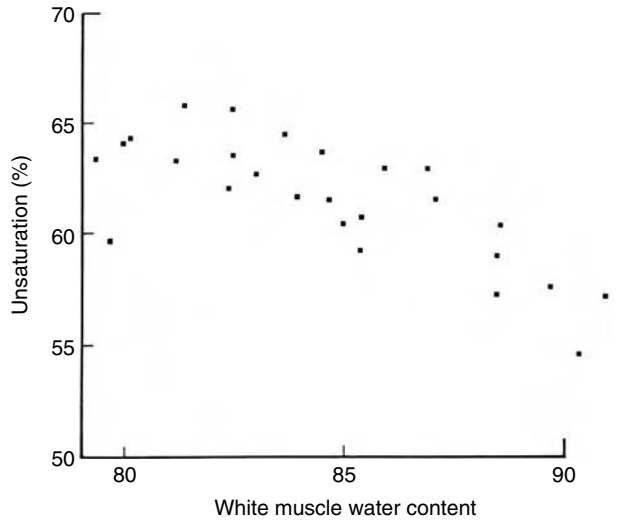

FIGURE 35.35 Decrease in the total polyunsaturation of cod white muscle lipids during starvation. Unsaturation represented as the sum of fatty acids 22:6, 22:5, 20:5, and 20:4 (% of total fatty acids). (Reference 65.)

TABLE 35.2
The effect of moderate starvation of cod on the development of off-odours and off-flavours during cold-storage at −10°C. (Reference 65.)

	Odour		Flavour	
	5 weeks	10 weeks	5 weeks	10 weeks
Fed controls (5 fish)	1.5	1.55	3.43	3.55
Starved fish (5 fish)	0.55	0.4	1.28	1.8
Difference	0.95	1.15	2.15	1.75
Significance level	1%	5%	0.1%	5%

Cis-4-heptenal values were as follows: starved cod muscle, 3.5 nmol/1000g wet muscle; fed cod muscle, 23.0 nmol/1000 g wet muscle.

reduction in the lipids of the muscle. The proportion of phospholipids (Figure 35.34) and the degree of unsaturation in the residual lipids (Figure 35.35) were also reduced. Freezing followed by cold-storage at −10°C resulted in a much reduced development of off-flavour and off-odour (Table 35.2). Analysis of hept-cis-4-enal confirmed the finding. Several factors may be involved here, although the results appear to be a simple cause and effect.

It is improbable that deliberate starvation will ever be used in practice to reduce the deterioration of flavour in cold-stored cod, but, as a water-content of 84% or more is often found naturally in late winter in this species, it suggests that advantage might be taken of the likely seasonal variation in off-flavour.

It should now be obvious that the difference between Faroe Bank cod and the others originates in an unusually rich food supply. There is a deep oceanic channel which keeps the fish separate from neighbouring stocks. We found that they were not eating particularly fatty prey, but that the stomachs were filled with echinoids, crustaceans, and sometimes fish.

3. Chilled Fresh Fish

One feature of Faroe Bank cod does favour them as an item of food, when compared with cod from at least one other ground. It keeps for a longer period in melting ice (1). This is clearly a result of the lower pH, which inhibits the activity of spoilage bacteria (70).

V. ACKNOWLEDGEMENT

The complex interactions described in this chapter have been supplemented and confirmed in species other than gadoids in a large number of reports published over 24 years by Professor Anna Kolakowska and her co-workers (71). It is a pleasure to pay tribute to the fine work of a colleague whom I have never met.

VI. CAVEAT LECTOR

The influences of geographical location on cod regarded as a foodstuff seem to be straightforward enough, but one can never assume that other species will behave in the same way. Cold-storage off-flavours develop strongly in herrings (*Clupea harengus* L.) but the off-flavour in cold-stored Atlantic salmon, another fatty species, seems to develop more slowly — salmon keep well in the deep-freezer. Triacyl glycerols in salmon muscle are found in the myocommata, so perhaps they protect the phospholipids within the myotomes from oxidation: it would be difficult to prove. Again, the starvation of rainbow trout results in a net increase in the unsaturation of the fatty acids of the flesh, but the flesh of the starving fish containing the highest concentration of docosa-hexaenoic acid does not develop more cold-storage off-flavour than those containing less (72). However, the apparent rises and falls of docosahexaenoic acid in this species are merely the results of outflow and inflow of triacyl glycerols.

There are usually traps for the unwary who try to derive generalised relationships from individual dynamic interactions. This is the way of much biological research.

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36 Edible Shellfish: Biology and Science

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CONTENTS

I. INTRODUCTION

Commercially important edible shellfish include marine and freshwater animals that belong to two major taxonomic groups: the Mollusca and the Crustacea. The hard external shell is a common external characteristic that often results in the two groups being given the common name shellfish. Both groups are invertebrates that have no internal skeleton or vertebrae, and in most cases the external shell provides protection, support to soft body tissues, and a mechanism to deal with changes in the environment. The cephalopods (squid, cuttlefish, and octopus) do not possess an external shell, but are grouped with the shellfish by virtue of their molluscan taxonomic status.

This chapter will describe the general biology of edible shellfish species that are commonly eaten. The two major taxonomic groups will be described separately because their biology is so different. Given the importance of these species in harvest and aquaculture industries this provides the focus of research activities. The range of topics covered particularly relate to those aspects of the biology that are relevant to these animals as food, in particular the lifecycle, reproductive and feeding biology, movement, and behaviour.

II. MOLLUSCAN SHELLFISH

Molluscan shellfish have no typical shape or form and the body plan is changed and adapted to the environment (1). Edible mollusc predominantly belong to three broad groups: bivalves (e.g., oysters, mussels, and scallops), gastropods (e.g., abalone and *Trochus*), and cephalopods (e.g., squid, cuttlefish, and octopus), according to the number and arrangement of the shell(s). Bivalve molluscs have two symmetric or asymmetric shells joined by a hinge, while the gastropod molluscs have a single shell, and most of the cephalopods have no external shell. Most of the edible

molluscan shellfish are aquatic, mainly marine or estuarine, and relatively sedentary. Commonly harvested and cultured species include the ubiquitous Pacific oyster (*Crassostrea gigas*), the flat oysters (*Ostrea* spp.), scallops (*Pecten* and *Astropecten* spp.), a variety of mussel species (*Perna* and *Mytilus* spp.) and the small burrowing clam species (*Mercenaria* spp. and *Mya arenaria*), abalone (*Haliotis* spp.) and snails (*Trochus* spp.). The cephalopods either have an internal calcareous cuttlebone (cuttlefish), an internal chitinous pen (squid), or no shell (octopus). The only terrestrial edible mollusc is the land snail (*Helix*).

A. General Biology

The crystalline calcareous shell of the bivalves and gastropods is present from the larval form and provides protection from environmental changes, e.g., temperature, salinity and aerial exposure, and predators. Some species are attached to a hard substrate as adults by either a cement-like substance (e.g., oysters) or a byssus thread (e.g., mussels). The size and extent of musculature in bivalve molluscs is varied; oysters have small amounts of muscle tissue while scallops have a very large adductor muscle. Bivalves that burrow in soft sediments have an extendable muscular foot which they use to pull themselves into the sediment. Some species have limited mobility, for example, scallops can rapidly clap the two shells together which can result in the rapid ejection of water and using this jet propulsion small distances (<1 m) can be moved. Small clam species that burrow in soft sediment move by rolling around in the waves and then re-burrow using the muscular foot. Gastropods, which are more mobile than bivalves, have a large powerful muscular foot beneath the single shell which is used to pull the animal along hard substrates. The muscle tissue of scallops and abalone is the primary tissue eaten, with the gonad and digestive tissue removed, although the scallop may be marketed as "roe-on." In contrast oysters and mussels are eaten whole, typically with the gonad and digestive system as well as the small muscle. The athletes of the molluscan world are squid, which may undertake extensive migrations; the muscular body is essential for movement, respiration, and structural support.

B. Life-History

Most molluscs have a bipartite lifecycle with a relatively short planktonic free-living veliger followed by the benthic adult phase (Figure 36.1). Individuals hatch as a trochophore and within 24 hours a larval shell develops. This is followed by the development of a veliger (also referred to as D-veliger due to the distinctive D shape), which lasts between several days to a month. A short planktonic phase (<5 days) is typical of abalone species and the veligers are non-feeding. A longer veliger stage (c. 20 days) is more typical of many bivalve molluscs and the veligers actively feed on phytoplankton using the velum, a fringe of beating cilia. The velum also provides some mobility in the water column, allowing the veligers to adjust their position in the water column. The planktonic phase is important for a number of reasons as it allows offspring to colonise new areas and also for these individuals to avoid the risk of predation close to the reef. Once the veliger is ready to settle onto the substrate, a change in morphology begins and a 'pediveliger' develops, which has a foot that extends out of the shell and is able to test the substrate before deciding to settle (Figure 36.1). Once a suitable place for settlement has been selected the individual secretes either a cement or byssal threads to attach itself. The pediveliger finally undergoes metamorphosis into the adult form and takes up a sedentary lifestyle.

In some species the female brood the veligers inside her shell, e.g., the flat oyster (*Ostrea* sp.). Fertilisation occurs within the shell environment of the female, using sperm released into the water by males. Veligers are attached by the velum to the female ctenidia (gills) and they use the food filtered by the mother's ctenidia. Shortly before metamorphosis veligers are dislodged and expelled out of the mother.

The selection of settlement sites by the larvae is critical for species with a sedentary adult phase, as conditions for feeding and reproducing must ensure survival. The site must provide water currents for delivery of food and removal of faecal wastes and there must be individuals of the same species in close proximity for reproduction. Selection of settlement sites can range from general to specific, and is generally species-specific. The presence of conspecific adults can be important as this suggests a suitable environment for growth and reproduction (2). Characteristics of the rock surface, e.g., rugosity or presence of algal and bacteria

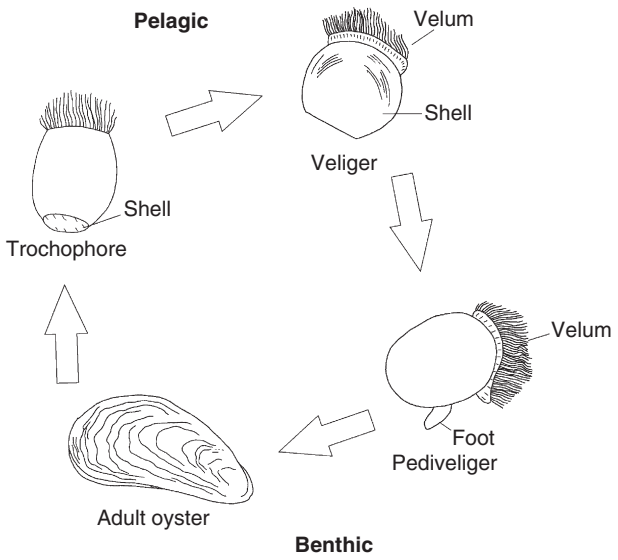

FIGURE 36.1 Lifecycle of an oyster.

species (3), or sediment characteristics of soft shores may be cues for settlement. Factors affecting settlement are of interest to ecologists, particularly with respect to human impacts on shore environments that may modify the substrate and make it become less suitable for settlement by juveniles. Many aquaculture ventures rely on collection of wild spat for on-growing to adults and are exploring suitable materials to enhance settlement rates (4). Hatcheries also need to use suitable surfaces to maximise settlement rates of juveniles. It is possible to induce settlement of pediveligers in culture conditions by exposing them to neuroactive compounds, e.g., L-DOPA. This induces settlement and the metamorphosis of the pediveliger into a spat, without the pediveliger going through the process of selecting a suitable substrate (5). The use of these compounds by shellfish hatcheries allows efficient management of stock by forcing settlement to occur when the hatchery is ready to deal with large numbers of juveniles and it also promotes synchronous settlement of larvae.

C. Reproduction

All molluscs reproduce sexually, with separate sexes in most species (dioecious), e.g., mussels and abalone. However, some species have both sexes present simultaneously (monoecious or hermaphrodism), e.g., scallops have a creamy coloured testis and orange coloured ovary present at the same time. In a few species sex change can occur (sequential hermaphrodism), with sperm being produced in smaller younger individuals, then a change to female in some bigger and older individuals. Many *Crassostrea* oyster species are hermaphroditic or will undergo sex change, which is a function of both secondary genes and/or environmental conditions (6). In oysters and mussels the reproductive tissue is not contained within a discrete organ or region of the body. Instead, gametes are produced in tissue surrounding the digestive system. Other shellfish, e.g., abalone and scallops, have a discrete organ for gamete production which is clearly identifiable.

Spawning in most temperate molluscs is seasonal, either in the spring/summer, e.g., oysters and scallops, or winter, e.g., mussels. Many tropical species may not show distinct spawning seasons, but a component of the population will always have some individuals that are reproductively mature and spawning. Prior to egg and sperm production individuals build up a store of energy reserves, usually glycogen. At some point during the year an environmental trigger, usually water temperature or day length, will stimulate the use of these glycogen reserves to produce gametes (7). Once gametes start to be produced individuals undergo a period of maturation before being ready to spawn and at this point the gonad may constitute most of the individuals' body mass. Gametes are released either in a single spawning episode or in a series of spawning episodes over several days to weeks. After the release of gametes there is a dramatic decrease in the mass of soft tissue and the flesh is flaccid and watery. As the tissue is unsuitable for consumption animals are not harvested for approximately a month after spawning to allow accumulation of carbohydrate reserves to begin.

As many molluscs are sedentary, gametes are released into the water column and fertilisation of eggs by sperm is external; this is referred to as broadcast spawning. Spawning in the population is co-ordinated to ensure that males and females release gametes simultaneously and that the density of gametes in the water is high enough to ensure high rates of fertilisation. The co-ordination of broadcast spawning within the population may be triggered by factors such as changes in water temperature or salinity, lunar and tidal cycles, and increasing or decreasing day length.

The squid, octopus, and cuttlefish have a more complex reproductive system in which, often following extensive courtship, the male passes packages of sperm (spermatophores) to the female (8). The female fertilises each egg and packages it in a jelly-like substance that provides protection for the developing embryo. Eggs are either attached to the reef or macroalgae individually (e.g., octopus and cuttlefish), laid in finger-like strands with multiple eggs in each strand (e.g., reef-associated squid) or unattached large balloon-like masses that float in ocean currents (e.g., oceanic squid) (8). Female octopus will guard and care for the eggs until they hatch, as miniature adults that are capable of jetting, inking, capturing prey, and avoiding predators. The squid and cuttlefish do not provide any parental care of the eggs or the juveniles.

D. Triploidy Production

Oysters, mussels, and clams are eaten whole without the gonad and digestive tissue removed prior to consumption. Taste tests have demonstrated that consumers prefer to eat oysters when the tissue is full and creamy and full of glycogen reserves, but before the animal is ripe with gametes (9). Given that individuals have high levels of glycogen for relatively short periods this means that marketable stock may only be available seasonally. Over the past 10 years there has been interest in the production of sterile oysters, i.e., individuals that are unable to produce gametes. In sterile individuals glycogen reserves are accumulated but not used for reproduction, resulting in the production of stock that are suitable for consumption over an extended period of the year. An additional benefit of producing sterile animals is that exotic aquaculture species may be farmed in new areas without the risk of introducing a species that may be detrimental to native flora and fauna (10).

In molluscs sterile animals have been obtained through the production of triploid individuals (11), which have 50% more chromosomal material than ordinary diploid individuals. Currently, triploid Pacific oysters are being produced by the aquaculture sector in the U.S. and

Australia (see review 12). Triploid oysters usually exhibit reduced gonad development and acceptable meat condition through the inhibition of spawning and faster growth rates (12). Currently the most popular and successful method of producing triploid molluscs is by blocking the formation and release of a polar body in the newly fertilised egg (11). The additional chromosomal material in cells prevents or limits the process of cell division associated with gamete production (meiosis), thereby causing an extra set of chromosome to be retained. Methods used to induce triploidy in oysters are either physical, e.g., temperature and pressure, or chemical, e.g., caffeine. Immersion of newly fertilised eggs in cytochalasin B, a fungal antibiotic, is currently the most effective method and is commonly used by commercial hatcheries.

Methods of triploidy induction in the aquaculture industry need to ensure a high percentage of triploids (ideally 100%), high survival rates and low rates of abnormality in the veligers, and consider operator safety and consumer acceptance. Unfortunately cytochalasin B is classified as a carcinogen and is a potential hazard to operators at bivalve hatcheries. The future use of cytochalasin B is in doubt, both because of its toxicity and because triploidy induction is not 100% effective, usually about 80%. Current research is exploring methods of producing triploids commercially that are potentially less hazardous and more reliable. One recent approach has been to cross tetraploids, individuals with double the normal genetic complement, with diploids (13). This method has successfully produced 99.9% triploids and the veligers have survivorship rates that are comparable with normal diploid oysters. Furthermore, these triploid individuals are produced without the use of artificial treatments and toxic substances.

E. Feeding

Bivalve shellfish (oysters, mussels, clams, and scallops) are filter feeders, using specialised gill structures (ctenidia) to selectively remove phytoplankton and organic matter from the water. The ctenidia have cilia that generate current and the movement of water into the animal as an inhalant flow. Each species is able to select particles within a very narrow size range (3–24 μm). Particles that are too small or too large are rejected by the ctenidia, bound in mucus, and expelled in a strand through an exhalent flow as pseudofaeces. Particles that are suitable for ingestion are passed to the mouth (labial palps) using the cilia on the surface of the ctenidia. Ingested food is bound in a strand of mucus and enters the digestive system by being drawn in by a crystalline style, which releases digestive enzymes. Once the digestive process has been completed faeces are released into the exhalent current and moved away from the animal.

Oysters can filter several litres of seawater daily and the advanced filter feeding mechanism allows bivalves to regulate the efficiency of particle retention and absorption. The rate of pumping and retention of food particles is modified in response to the density of particles in the water while maintaining respiratory functions. If the density of particles in the water is too high the ctenidia become clogged and rates of pseudofaeces production increases. Other factors that affect feeding rates in bivalves are water current, water temperature, salinity, turbidity, and dissolved oxygen (14).

Most edible gastropod molluscs are herbivorous grazers (e.g., abalone), but there are a small number that are carnivorous predators (e.g., trochus, conch, and whelks). Grazing molluscs use a radula, a protrusible tongue-like structure with a complex ribbon of teeth, with which they scrape algae and biofilm off rocks or tear and cut macroalgae. As teeth at the front of the radula wear out they are replaced by secretion of new teeth at the back of the radula. Some of the carnivorous molluscs have specialisations of the radula that allow them to bore holes through the shell of prey or harpoon prey. After maceration of food the radula carries particles of food backwards and into the digestive tract. Digestion is usually extracellular in the stomach and digestive gland.

Squid, octopus, and cuttlefish are voracious carnivorous predators. Squid and cuttlefish predominantly eat crustaceans and fish which are captured using contractile tentacles (the longest of the arms). Octopus species eat predominantly crustaceans and molluscs and they are able to deal with the hard outer parts by either drilling through the shell or in the case of bivalves pulling apart the two valves (15). Members of this group have a parrot-like beak which is used to tear tissue into small pieces and the salivary gland secretes enzymes that are used to break down flesh prior to ingestion. Once in the stomach enzymes secreted by the digestive gland continue digestive processes.

F. Shellfish-Associated Disease Outbreaks

Ingested particles, including bacteria and viruses, are digested by bivalves and then either absorbed into the tissues or excreted. As result of pathogenic micro-organisms being retained and absorbed molluscan shellfish become vectors of bacterial and viral pathogens. The rate of pathogenic micro-organism uptake and retention is a function of the density of micro-organisms in the water being filtered. Oysters may accumulate pathogens to levels as much as four times greater than levels in the water from which food is being removed (16). If shellfish (e.g., oysters) are consumed raw and whole with the digestive system still intact the pathogenic microbes present in the tissue and gut pose a health risk to the consumer. In the U.S. from 1991–1998 the consumption of raw or partially cooked shellfish resulted in more than 2,000 illnesses.

Viral agents that are of chief concern in world shellfish industries are human enteric virus, Norwalk virus,

and hepatitis A. All cases of food-borne viral diseases from shellfish have occurred due to human faecal contamination in areas where shellfish are harvested and the consumption of shellfish that is raw, steamed, incompletely cooked, or frozen. Bacterial agents, particularly *Vibrio* spp. have been identified as being pathogenic or potentially pathogenic to humans (17). Unlike virally associated disease outbreaks, contamination by *Vibrio* tends to be more sporadic and involve individual cases (18). Where outbreaks have occurred they have been associated with *V. vulnifucus* septicaemia with 50% mortality rates (19), or epidemics of cholera caused by consumption of raw shellfish contaminated with *V. cholerae* 01 (20). *Vibrio* spp. naturally occur in estuarine and marine waters and are not associated with faecal contamination; however, numbers of *Vibrio* in shellfish can increase in improperly stored shellfish (21). Some pathogenic bacteria may not naturally occur in marine and estuarine environments, but are associated with incorrect storage, faecal pollution, or infected food handlers may contaminate shellfish. Enteric bacterial pathogens that have been implicated in shellfish associated disease outbreaks are *Clostridium perfringens*, *Salmonella,* and *Shigella* (22).

A factor influencing the timing and occurrence of shellfish-related outbreaks is the ability of shellfish to selectively accumulate viruses. Selective accumulation can be attributed to the feeding mechanism when there is ionic bonding of viral particles to the shellfish mucus. There is evidence that the seasonal occurrence of shellfish-related illnesses by enteric viruses may be due to oysters undergoing seasonal physiological changes that affect their ability to accumulate viral particles (16).

Current practice to remove pathogenic micro-organisms is to put shellfish through a depuration process after harvest. This process provides conditions for shellfish to purge their digestive system of their gastrointestinal contents, thereby supposedly removing the micro-organisms that had been taken up during filter feeding. Depuration is typically conducted in an isolated system, with temperature controlled re-circulating seawater. The seawater should be continually cleaned, e.g., using a UV steriliser attached to the re-circulating system. The temperature of the system needs be stable and set at a level that encourages the shellfish to filter the clean seawater.

The ability of depuration to remove pathogens is dependent upon a number of factors. (1) The molluscs must feed normally in the system; if active pumping and filtering of seawater do not occur then microbes present in the gut will not be purged. (2) Variability in the loads of micro-organisms among individual animals may result in differential removal of pathogens. (3) The depuration conditions (water temperature and salinity) that are suitable for normal feeding are species specific. (4) Microbes that have been incorporated into body tissues will not be removed, only those pathogens currently resident in the gut and on the gills will be removed. (5) The ability of the disinfection technique to inactivate the purged microbes; if purged microbes are not killed by the disinfection system then re-infection of animals in the depuration system is likely.

G. Shellfish Toxicity

Paralytic shellfish poison (PSP), amnesic shellfish poison (ASP), and diarrheic shellfish poisoning (DSP) are caused by naturally occurring biotoxins that accumulate in molluscan shellfish (review 23). These biotoxins are caused by microscopic toxin-producing dinoflagellate phytoplankton that naturally occur in marine waters, normally in amounts too small to be harmful. However, on occasion, a combination of warm temperatures, sunlight, and nutrient-rich waters can cause rapid plankton reproduction, or "blooms." The toxins in the microalgae ingested by shellfish build up and become concentrated in the gut and somatic tissues. This may result in mass mortalities either through toxins affecting the shellfish, or high densities of phytoplankton clogging the ctenidia and causing asphyxia. The toxins also pose a health hazard to humans consuming contaminated shellfish. As cooking does not break down the toxins and many shellfish are eaten raw, the toxin is ingested by humans and other predators. Although first recognised and described in Canada, PSP and ASP have been found worldwide (24).

Paralytic shellfish poisoning is caused by a suite of neurotoxins (saxitoxins) produced by species such as *Alexandrium* spp. and *Gymnodinium catenatum*. The type and storage of paralytic shellfish toxins in bivalves species vary considerably. Mussels, clams, and scallops appear to accumulate high levels of saxitoxins, while in oysters the levels of the toxins are low or absent (23). Amenesic shellfish poisoning is linked to the presence of domoic acid produced by the diatoms *Pseudonitzschia* spp. and *Nitschia* (24). Shellfish appear to concentrate the toxin and again the response to the toxin is species specific. Scallops and mussels appear to be unaffected, while oysters become physiologically stressed. Diarrheic shellfish poisoning occurs when shellfish containing okadaic acid are eaten. Okadaic acid and its derivatives are produced by the dinoflagellates *Dinophysis* spp. and *Prorocentrum lima* (25). As with other toxins there are differential effects among the bivalve species and seasons and the length of time the toxins remain in the tissue. Blue mussels in Sweden can retain toxins for up to five months after accumulation, although high levels of toxins are not necessarily associated with any known health effects (23).

The economic cost of both human pollutants and naturally occurring biotoxins is high for countries that have shellfish harvest and aquaculture industries. The presence of toxic algae has been responsible for the collapse of bivalve populations in Alaska, and fisheries and aquaculture are closed for extended periods in countries such as Sweden,

Norway, the U.S., Spain, and Australia (23). The costs incurred with the occurrence of toxic algae blooms are associated with (1) the need to have expensive shellfish monitoring programs to assess the presence of contaminants, (2) the cessation of harvesting and selling of produce, and (3) the development and running of depuration systems. Current research is focussed on how effective the depuration process is at removing the toxins and how the toxins are removed from different organs. Understanding how toxins are accumulated, transformed, and eliminated allows the time course of the toxic episode to be predicted and hence how long harvest of animals should be suspended. It is possible that toxins are transformed in the body tissues and then excreted from the animal over time, although there is evidence that metabolism of the PSP toxins is more complex than just a transformation of toxic compounds (26).

H. Bioaccumulation and Biomonitoring

As a result of the relatively sedentary lifestyle of many shellfish and their modes of feeding (filter feeding and deposit feeding) many species ingest or uptake and retain trace metals present in the environment. Molluscan shellfish can accumulate heavy metals (e.g., copper) and organic contaminants (e.g., pesticides) in body tissues. Marine molluscs tend to accumulate arsenic, cadmium, copper, lead, mercury, silver, and zinc, but have a low tendency to accumulate chromium. As a result of the accumulation, concentrations of these trace metals in body tissues can be many times higher than present in the environment (27). This accumulation of heavy metals and contaminants has two problems. The first is that many of these animals are prey of higher order predators and as a result serious pollutants enter the food chain and are present in higher concentrations further up the food chain (biological amplification). The second problem is that many shellfish species and their predators are a food source for humans and as a result are a health hazard.

One advantage of sedentary molluscs accumulating heavy metals, viruses, and bacteria is that they provide a time-integrated measure of the bioavailability of these contaminants (see review 28). As such, some bivalves are used as sentinel species to monitor or assess concentrations of contaminants available for uptake in the environment. Such biomonitoring involves assessing the effects and levels of contaminants in the environment on animals. Uptake of contaminants varies among and within species as a function of factors such as size, age, sex, and among geographic locations due to environmental factors such as temperature and salinity. However, it is possible to move healthy individuals into cages at sampling locations and assess the levels of contaminants after a period of time. For example, mussel species (*Mytilus edulis* and *M. californicus*) are commonly used around the U.S. coastline as sentinel species for biomonitoring because they are endemic, abundant on many coastlines, and tolerate translocation. Another example, is the use of oysters to assess the bioavailability of tributyltin (29), a compound has been implicated as a cause of shell thickening in many oyster species (e.g., 30).

III. CRUSTACEAN SHELLFISH

Edible crustaceans all belong to a group known as decapods which are the largest and most specialised of the crustacean species. They are characterised by a heavily segmented body and a chitinous exoskeleton which provides an outer protective shell that is articulated like body armour. All edible crustaceans are aquatic, with most occupying marine and estuarine habitats, but some crayfish and prawns are found in freshwater. Crustaceans are benthic but highly mobile, e.g., prawns swim regularly and some lobsters undertake long migrations. Commonly harvested and cultured edible crustaceans include prawns (shrimps), both freshwater (e.g., *Macrobrachium* spp.) and marine (e.g., *Penaeus* spp. *Metapenaeus* spp.), crabs (e.g., *Callinectes sapidus, Scylla serrata*), clawed lobsters (e.g., *Homarus* spp. *Nephrops* spp.), spiny lobsters (e.g., *Palinurus* spp., *Panulirus* spp., and *Jasus* spp.) and crayfish (crawfish and yabbies).

The common names used to describe edible crustaceans are often confusing. The distinction between prawns and shrimps may be based upon the taxonomic group and the habitat of the adults (31). Freshwater species, e.g., *Macrobrachium,* are often referred to as prawns, while marine and estuarine species (e.g., penaeids) are shrimps. Alternatively, size of the adults is often used to distinguish among the two names; with shrimps being small species and larger species prawns. Similarly, there is often confusion over use of the common names lobster and crayfish. Again the distinction is made taxonomically, but generally lobsters are marine (this includes both clawed and non-clawed species), and crayfish or crawfish are freshwater.

A. General Biology

The general body plan is similar among the decapod crustaceans with two sections making up the body: the fused head and thorax (cephalothorax) and the tail (abdomen). Each section is made up of a series of segments, but the number of segments and specialisation of segments varies among species. The cephalothorax is completely fused with no external segmentation visible, the shell (carapace) surface of the cephalothorax may be smooth or covered with spines depending on the species. In contrast, the abdomen is distinctly segmented. Attached to both sections are specialised paired appendages, the number of which depends on taxonomy and function of the appendage. Around the head and mouth appendages are modified for food capture and feeding (mandibles, maxillae, and maxillipeds). The head also has antennae, antennules, and a pair

of stalked compound eyes. The corneal surface of the eyes is convex and, when on a stalk, the cornea may cover an arc of >180°. The remaining appendages (pereiopods) on the cephalothorax are used in crawling or walking. The first pair of pereiopods in lobsters and crabs is modified as a claw (chela), which may be large and distinctively coloured. Appendages attached to the abdomen (pleopods) are used for swimming, as well as egg attachment in some species, e.g., crabs and *Macrobrachium*. The last pair of pleopods (uropods) and the telson, at the terminal end of the abdomen, forms a tail fan that can be used for locomotion using a rapid flexing motion. Appendages may be specialised in some species for fighting, courting, and egg handling.

Prawns in the genus *Penaeus* and *Macrobrachium* are commercially the most important in the world. They are cylindrical with a well-developed abdomen and an exoskeleton that is thin and flexible. Many penaeid shrimps use the pleopods to excavate shallow burrows in soft bottoms during periods of inactivity. Shrimps move predominantly by rapidly beating the large fringed pleopods, but rapid flexion of the abdomen and the tail fan will allow fast backward movement.

Lobsters and crabs have a dorso-ventrally flattened body, with a heavier and stronger exoskeleton, and the strong claws (chelipeds) have a lot of musculature which is a commercially valuable part of the animal. The abdomen in crabs is reduced and folded under the cephalothorax, the uropods are absent, but large and strong legs on the cephalothorax are used for walking which is the primary mode of locomotion. Reduction of the abdomen allows rapid crawling and they commonly move sideways. In contrast to crabs, lobsters have a very well-developed tail (abdomen), which has a high monetary value given the high tail to body ratio. The legs allow lobsters to crawl while the muscular tail allows fast flexing of the abdomen resulting in rapid backward movement. Spiny lobsters (*Jasus, Palinurus,* and *Panulirus* spp.), present in both tropical and temperate waters, do not have chelipeds, and it is the meat in the tail that is prized. The shovelnose or slipper lobsters (*Scyllarus* and *Thenus* spp.) have a dorso-ventrally flattened body with short, flattened antennae, compared with the cylindrical body and long antennae in clawed and spiny lobsters.

B. Life History

Crustaceans have a more complex life history than molluscs and each stage has distinct behavioural and ecological characteristics (32). Most crustaceans have separate sexes and generally hold their eggs, either in brood sacs or attached to the abdominal appendages. Like many marine invertebrates the crustaceans have a bipartite life history, with a free-living, planktonic larval phase, followed by a benthic adult phase. The stages, forms, and time spent as larvae are highly variable among the decapod crustaceans, often depending on how much development occurs in the egg. However, all the decapod larvae undergo a series of developmental stages during which the larvae metamorphoses into a more advanced form until the final adult form is developed. The different stages of the life history are often associated with different and distinct habitats.

Penaeid prawns undergo several distinct larval and juvenile phases that are associated with different habitats (33). The embryonic phase is short and, as a result, the newly hatched larvae (nauplii) are less morphologically developed than some of the newly hatched forms of other decapod crustaceans (Figure 36.2). The nauplius is the first larval phase and it has only three pairs of appendages which are all used for swimming. Furthermore, the nauplii do not feed and are supported by the maternal yolk reserves that are present when they hatch. The nauplii metamorphose first into feeding protozoea and then a mysis (Figure 36.2), both of which live in offshore waters. Metamorphosis of the mysis produces a megalopa which migrates to brackish inshore waters. The megalopa undergoes metamorphosis into a benthic juvenile which uses estuaries as a nursery habitat. Adults then move from the estuaries back to deeper and full salinity water offshore.

Freshwater prawns, such as *Macrobrachium*, and crabs hatch out as planktonic zoea, advanced larval forms that use thoracic appendages to swim upside down (Figure 36.3). The freshwater *Macrobrachium* have a zoea that lives in estuarine environments as it needs brackish water (34); they are carnivorous, eating zooplankton, worms, and larval forms of other invertebrates. Zoea undergoes a series of developmental stages before becoming a postlarva (*Macrobrachium* prawns) or megalopa (crabs). *Macrobrachium* pelagic postlarvae move upstream towards less saline water swimming with the dorsal side uppermost. During this migration the postlarvae start to undergo the transition to the adult benthic habit. The size at which postlarvae start the upstream

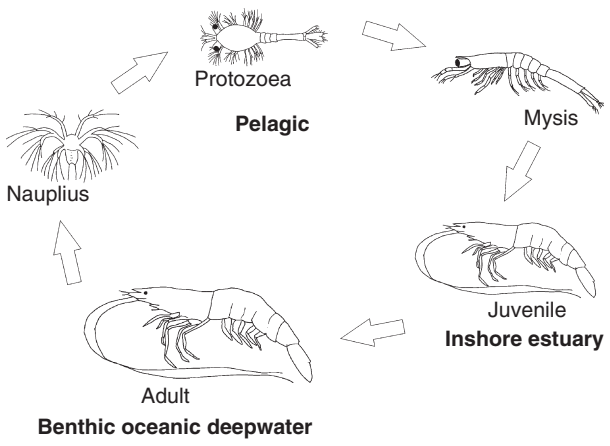

FIGURE 36.2 Lifecycle of a penaeid prawn.

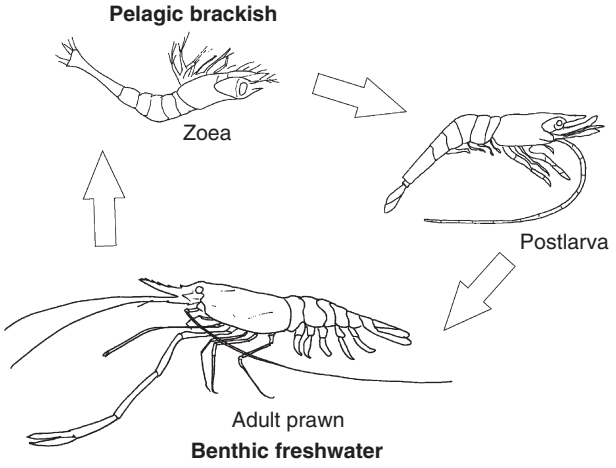

FIGURE 36.3 Lifecycle of the freshwater prawn *Macrobrachium.*

migration is thermally driven and low temperatures can bring about migration in small individuals.

The larval and post larval phase of *Homarus* lobsters is about 6–8 weeks long. They have a very short nauplius phase and quickly metamorphose into a larval form; the first three larval stages are very similar to the zoea phase of the crabs and are sometimes called mysid larvae (35). The zoea of *Homarus* lobsters undergoes a series of developmental stages, ultimately becoming a postlarva (equivalent to the megalopa). Postlarvae undertake the transition from a planktonic to a settling and finally a benthic juvenile through behavioural response to light, gravity, and water pressure. The early benthic juvenile phase is highly cryptic as these individuals use shelters until they are big enough to avoid predation.

The life history of spiny lobsters (palinurids) differs from the other decapod Crustacea in that the nauplius stage is very short (several hours) before development into a larvae (phyllosoma). The phyllosoma is dorso-ventrally flattened, transparent, and very thin (36), a body form that is suitable for the passive dispersion of these larvae in ocean water currents. The phyllosoma phase is very long, lasting as much as 391 days and potentially larvae are carried considerable distances (>1000 km). This extended dispersal phase makes it difficult to locate and collect the phyllosoma in oceanic waters and as a result little is known about their biology. There are 7–13 phyllosoma stages and the last phyllosoma stage metamorphoses into a non-feeding, transparent, and free-swimming puerulus (postlarvae). Puerulus use energy stores acquired as phyllosoma until metamorphosis into a feeding post-puerulus or juvenile. Benthic puerulus are vulnerable to predation and therefore initially hide in cracks and crevices, or in burrows. As a result of this cryptic behaviour it has been difficult to determine the cues important in the selection of settlement sites and habitats. Once juveniles are large enough to protect themselves from predators they begin to adopt a more typical adult lifestyle and a scavenging and omnivorous diet.

C. Growth and Ecdysis

The hard exoskeleton of the crustaceans is a non-living layer that plays a role in protection, gaseous exchange, and support. However, it is unable to increase in size, and therefore growth is constrained by this hard exoskeleton. An increase in size occurs only after the old exoskeleton has been shed (moult) and a new exoskeleton has formed and hardened. The process of moulting is called ecdysis and as a result the process of growth is incremental rather than continuous. During the moulting process the animal increases in size by absorption of water, but somatic growth occurs between moults. Many aspects of the life of crustaceans, including growth and reproduction, are directly under the control of the physiological changes associated with the moulting cycle.

The process of moulting can be divided into five distinct stages (37):

1. Premoult. Animals are getting ready to discard the old exoskeleton by starting to break down the exoskeleton. Enzymes are released that start dissolving the old skeleton, and calcium and other materials are absorbed from the old shell. The epidermal cells beneath the cuticle increase in size and begin to secrete the new exoskeleton which is deposited beneath the old exoskeleton.
2. Moult. As the animal increases in size through ingestion of water the internal pressure causes the weakened old exoskeleton to split at the membrane between the cephalothorax and the abdomen. The animal pulls itself out of the old exoskeleton and emerges out of the original shell. The new exoskeleton is present, but is soft and pliable and the animal is defenceless until it hardens. Water continues to be absorbed to increase body size.
3. Postmoult. The new exoskeleton hardens by the re-deposition of calcium and other soluble materials that were stored from the dissolution of the old endoskeleton. The exoskeleton is still soft and animals are vulnerable to predation.
4. Intermoult. This is the period between moults during which time the water absorbed by the animal during the moult is replaced by somatic tissue. In the early stages of the lifecycle, when the larvae and juveniles are growing quickly and undergoing metamorphosis, the intermoult periods are relatively short. However, once the adult phase is reached, moulting occurs less frequently and the intermoult periods are longer.

Crustaceans may lose appendages through accidents or shed appendages voluntarily in a process called autotomy (38). In the decapod crustaceans there is a specific site on an appendage at which autotomy can occur. This means that, regardless of where the damage has occurred, the appendage will always be dropped off at that point. Once an appendage has been shed cells at the breakpoint begin to enlarge and a regenerated appendage is formed, complete with exoskeleton. Regeneration can only occur during the premoult phase; therefore the animal cannot form a new limb until it is passes through the next moult cycle. If an individual has lost a large number of appendages then the intermoult phase may be shortened and the next moult cycle will start earlier (38).

Growth rates of many crustacean species have largely been described under culture conditions. However, models of population dynamics and stock assessment required for fisheries management rely heavily on accurate estimates of growth rates of wild animals. In fish, hard structures like scales and ear bones (otoliths) grow in such a way as to leave a record of somatic growth. However, the lack of permanent hard structures in crustaceans that contain a record of somatic growth is problematic when estimates of age are need. Tagging studies on wild populations have been used to estimate growth rates (e.g., 39); however, this relies on adequate recovery rates of tagged individuals and retention of tags during moulting cycles. Recently, it has been discovered that both freshwater and marine crustaceans have deposits of an autofluorescent neurolipofuscin that accumulates in the eyestalk ganglion and the concentration of which is correlated to the physiological age of the individual (40, 41). The neurolipofuscin pigment can be extracted from the tissue using solvents and the intensity of fluorescence is used as a measure of neurolipofuscin concentration (42). Alternatively, tissue sections prepared histologically can be viewed under a specialised microscope and the density of fluorescing granules estimated (41). As deposition rates of neurolipofuscin are related to the physiological, not chronological, age of individuals the rates of deposition are modified by environmental factors such as temperature (43). Despite this lipofuscin concentration has the potential to be used to estimate growth rates and determine year-class strength in populations for fisheries management.

D. Hormonal Control of Moulting and Reproduction

The cyclic processes of moulting and reproduction have a major impact on adult physiology and are under the control of hormones (44). The neurosecretory X-organ, located in the eyestalk of many decapod crustaceans, produces moult-inhibiting hormone (MIH) and vitellogenesis-inhibiting hormone (VIH). These hormones once released by the X-organ are sent to a sinus gland, also in the eyestalk, and then released into the hemolymph (blood). If the concentration of moult-inhibiting hormone decreases in the hemolymph this promotes the release of moulting hormones (ecdysteroids) from the Y-gland, located near the mandible muscles. Low concentrations of moulting hormones are present during postmoult and intermoult periods, but they increase during early premoult which starts the processes of moulting, followed by a decrease prior to moulting. Likewise, low concentrations of VIH result in the release of reproductive hormones and the process of egg production begins.

Although the moulting cycle is controlled hormonally factors that initiate moulting are often external, e.g., food, temperature, and day length. Given that aquaculture requires the control of all aspects of an animal lifecycle, including growth and reproduction, there is some understanding of the hormonal control of moulting and how this can be manipulated. In the 1970s it was found that destroying the eye stalk (ablation) will induce non-gravid female shrimp to mature and produce eggs. Eyestalk ablation will reduce the intermoult phase, induce stimulation of ovarian development, and accelerate the rate of ovarian development due to the absence of the moult-inhibiting and vitellogenesis-inhibiting hormones (45). Today eyestalk ablation is used in almost every marine and seawater shrimp aquaculture facility to shorten the intermoult phase, accelerate growth rates, and to produce animals with soft shells for market. In aquaculture the ability to control reproductive activities and ensure the production of eggs and sperm when required has many advantages; (1) it allows development of selective breeding programs, (2) it is possible to promote reproductive development and not be seasonally dependent on natural reproductive cycles, and (3) control spawning activities which can increase the proportion of females that spawn at any time. Therefore, hatcheries use eyestalk ablation as a way to advance reproductive activities and synchronise spawning.

E. Reproduction

The sexes are separate in the decapod crustaceans; in females a pair of ovaries and in males a fused pair of testis is located in the cephalothorax. In some species, shortly after the female becomes sexually mature she must undergo a moult at which time she is able to mate and receive sperm from males. Males have an opening (gonopore) on the last pair of walking legs through which sperm enclosed in a package (spermatophore) is deposited between the fourth pair of walking legs on the female thorax. In prawns, females have a spermatophore receptacle called a thelycum. Females lay eggs through a gonopore after sperm deposition and the sperm is used to fertilise the eggs.

Penaeid prawns release the fertilised eggs into the ocean, but most species transfer eggs to the underside of the abdomen. The egg brood is kept aerated and clean of debris by the fanning movement of the pleopods. The eggs

develop here until the larvae hatch out. A female carrying a brood of eggs is described as being in berry and the eggs can be seen as a mass of orange due to the presence of carotenoids. As the eggs develop they change colour from orange to brown and then finally grey several days before hatching. Larvae hatch from the eggs and remain attached to the brood chamber for up to 24 hours. The female dislodges the larvae by lifting her tail and vigorously beating the pleopods. Larvae are usually released at night to minimise risk of predation and on a high tide to ensure the transport of larvae away from the reef. Generally newly released larvae respond negatively to gravity and positively to light such that they move up in the water column into surface waters.

F. Feeding

Larvae and postlarvae are predators, feeding actively on phytoplankton and invertebrate zooplankton (46). They are able to feed on suspended particles and will actively chase and capture zooplankton. However, they are largely opportunistic feeders and may be cannibalistic when held in high densities. Among the adult forms there is a diversity of feeding modes, but many of the edible crustaceans are scavengers and/or predators and many species are omnivorous. The diet of scavengers (e.g., prawns) includes a range of dead plant and animal matter including detrital aggregates. The prey of predators (e.g., lobsters and crabs) includes worms, crustaceans, molluscs, and fish. Cannibalism may occur at very high densities in culture conditions, particular in crabs and especially when individuals have just moulted and are soft-shelled. Food detection is primarily through chemosensory structures on the head. The antennae and antennules can detect low concentrations of chemicals and are used for distance chemoreception, while appendages around the mouth are used for contact chemoreception and are able to detect high concentrations of organic compounds (33).

Once food is captured or found the maxillipeds are used to hold and crush, and the mandibles and maxillae are used for ingestion. At this point food is broken into fine pieces before being passed into the foregut. In decapod crustaceans the muscular foregut is divided into two chambers; a cardiac stomach and a pyloric stomach. In the cardiac stomach a gastric mill with chitinous teeth is used to further grind food down before it is passed into the pyloric stomach. Digestive processes are initiated in the foregut with the release of enzymes secreted by a digestive gland. Partially digested food is then passed from the foregut through to the mid-gut which is the primary site of enzyme activity and nutrient absorption and the final stages of digestion occur. Enzymes are produced by digestive tubules that form a glandular appendage (digestive gland), which is involved in enzyme synthesis and secretion, nutrient absorption, and lipid storage.

Although toxic poisoning in humans through the consumption of crustaceans is not typically associated in the same way that molluscan shellfish are, there is evidence that crustaceans may accumulate toxins in their tissues (review 47). Uptake of toxins by crustaceans occurs when they consume bivalves that have toxins and toxins are accumulated in the hepatopancreas (48). However, poisoning of humans rarely occurs because the muscle tissue of crustaceans is predominantly eaten, but if the whole animal is cooked (e.g., crab soup) or the digestive gland is eaten then toxins accumulated in this gland can be transferred to humans. In crabs there is evidence that toxicity may be the direct result of the consumption of the macroalgae *Jania* (47).

G. Movement and Migration

For many crustaceans planktonic larvae are typically the dispersal phase of the life history, in those species which have a very long larval phase, e.g., spiny lobster, phyllosoma, can be dispersed considerable distances. Movement patterns of phyllosoma are poorly known because of difficulties in sampling animals that occur at low densities in large oceanic water masses. More recently the approach to studying movement patterns of these larvae has involved integrating oceanographic circulation patterns with larval distribution. In some cases there is evidence that phyllosoma may be retained within gyre systems ensuring that they return to the reef. Although dispersion of larvae and phyllosoma is affected by current systems, they can actively orientate themselves in the water column and effectively control some of their movement. Many species undertake vertical migrations in the water column, thereby using water currents at different depth strata to allow some control in their return to the reef (49). For example, the Western Australian spiny lobster early in the larval phase stays in surface waters which ensures movement offshore away from the reef, but when mid- and late-stage phyllosoma avoid surface waters they are more affected by subsurface circulation which returns them back to the coast (49).

Adult lobster species undergo movements that can occur on a range of spatial scales from 100s of metres to 100s of kilometres (32). Some of these movements are temporally and spatially predictable, with directional migration involving thousands of individuals as seen in some of the spiny lobsters (50). The reasons for the movements are varied and in many cases unknown (36). However, they may be related to spawning activities, redistribution of juveniles, movement out of sub-optimal environments, facilitating exchange of genetic material, or environmental factors, e.g., temperature. In some cases during the migrations lobsters follow predictable routes in single file for 2–4 days, which can make them vulnerable to over-fishing. On the Scotian shelf tagged adult *Homarus* lobster have recorded migrations in excess of

200 km over a year, but they often return to the same area, suggesting long-range homing behaviour (32). The mechanisms used by spiny lobsters to orientate themselves and be able to undertake directional migrations and display homing behaviour are unclear. Recent work has explored the use of magnetic orientation by spiny lobsters through experiments that relocate animals and use magnets to modify the magnetic field around the individual. It appears that spiny lobsters (*Panulirus argus*) do use magnetic information to orientate themselves and be able to return home. Interestingly, individuals appear to use their relative position to the home site to orientate; in other words, they use a geomagnetic map (51).

H. Behaviour

Unlike largely sedentary molluscan shellfish, many edible crustaceans, particularly lobsters and crabs, display advanced social and reproductive behaviour (32). However, much of what is understood about decapod behaviour has been obtained in aquaculture conditions, where animals are held in high densities and inter-individual interactions are frequent. In nature most decapods are largely solitary, nocturnal, and remain hidden. Spacing and occupancy of shelters is a major aspect of their social behaviour (52). Lobsters at high densities will obtain and defend mates and shelters through dominance hierarchies. In particular, dominant males are able to evict subordinate males and are able to gain and retain mates. In contrast, at low densities dominance hierarchies are not formed, but individuals are highly territorial. Although the differences in behaviour under conditions of high and low density are evident in culture conditions, these may also be seen in the wild associated with ontogenetic changes in behaviour. For example, juvenile spiny lobsters are solitary and cryptic, but intermediate-sized individuals will aggregate together in shelters.

The use of the claws in behaviour is diverse, e.g., male fiddler crabs wave their claw to entice females into the burrow for mating, and in contrast, some lobsters and crabs will use the well developed claws in aggressive interactions. Some factors that affect aggressive interactions and their outcome are size, moult stage, and gender of the individuals involved (53). The nature of dominance interactions can range from ritualised displays to physical fights which can result in the loss of limbs. Typically individuals that are bigger and with larger claws will have a greater chance of winning encounters. However, there are distinct changes in aggression during the moult cycle. An individual may maintain a high level of aggressiveness until the point that the old shell has been moulted, possibly so shelter that will provide protection during the soft-shell state can be obtained and maintained. Recently moulted individuals will be less aggressive as the soft-shell provides little protection during interactions.

IV. CONCLUSION

The shellfish represent a biologically diverse group of marine invertebrates that includes very important harvest and aquaculture species world-wide. The sedentary lifestyle of many species makes populations vulnerable to changes in the environment that will affect recruitment of juveniles into adult populations and the quality of adults as a food source. Aspects of biology, ie growth, ecology, reproduction, feeding, and movement that have been studied in detail are often related to our need to manage wild stocks and for the successful culture of new species. Given that these are invertebrates and many of these species are low in the food chain, it may be considered that they will be less vulnerable to over-exploitation. However, the advanced behaviour and extended pelagic larval phase of some lobster species, about which we know little, means that over-fishing is possible and aquaculture of these species will be challenging.

ACKNOWLEDGMENTS

Thanks to Dr Danielle Johnston for providing invaluable expertise and discussion about crustacean biology and Dr Keith Martin-Smith for constructive review of the chapter.

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37 Aquaculture of Finfish and Shellfish: Principles and Applications

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CONTENTS

I. INTRODUCTION

Aquaculture has been described as both the art and science of growing aquatic animals and plants. It has been practiced for thousands of years by different societies using a wide variety of approaches to growing aquatic organisms in water. The origins of aquaculture will probably never be known because there are no ancient aquaculture-specific artifacts or even the remains of ponds or dams that can be distinguished from other uses such as water storage or for growing crops (1). However, there is some historical evidence from around the world that shows aquaculture has existed for thousands of years and in several different places. There is a bas-relief of fishing, probably for *Tilapia*, from fish ponds in ancient Egypt 4000–4500 years ago (1). Several types of integrated aquaculture/agriculture systems were known in South America (1) and there is the 1500–1800-year-old Ahupua'a marine aquaculture in Hawaii (2). Fish ponds were well known in Europe during the Roman Empire and Middle Ages (3). In many ways China could be considered the head and heart of traditional aquaculture, certainly in freshwater, since it is here that large scale aquaculture has the longest history and has shown the most evolution. Pond culture of common carp was recorded over 3000 years ago and about 2500 years ago Fan Li, considered the father of Chinese fish culturists, wrote "Yang Yu Ching" or a "Treatise on Pisciculture" to detail practical methods for freshwater aquaculture (4, 5). Recently, within the last half of the twentieth century, aquaculture has shifted into an industrial phase and global production has increased dramatically. Currently, aquaculture of finfish, molluscs and crustaceans (shellfish) produces 37.5 million tonnes per year and accounts for 29% of the total global fishery harvest (6). Aquaculture continues to expand and has increased its share of the global fishery from less than 4% in 1970 (6). Aquaculture has great potential to expand further and to meet an increasing human demand for protein. To accomplish this dramatic changes in current structures and practice may be necessary. The debate on sustainable aquaculture is only just beginning (7–10).

There are many ways to farm fish: they may be housed in ponds, tanks, cages and raceways; held inside or outside in salinities from freshwater through to seawater; and grown with or without other fish, animals or plants that are also available for harvesting. Aquaculture can be divided very broadly into extensive and intensive forms. It can be argued that intensive aquaculture is distinct from other forms, whereas there is a continuum from extensive to semi-intensive systems. Under intensive aquaculture, water provides physiological support for the fish but all other means of sustaining life are provided from external sources, the most obvious being the supply of nutritionally

complete feeds. Extensive aquaculture is viewed as a system which manages the natural productivity of water to grow products that can be used. The level of management, the types and magnitude of inputs and the diversity of production all contribute to the complexity of non-intensive systems. Thus, Chinese polyculture of up to eight species of finfish is a good example of semi-intensive aquaculture at one end of the continuum. Annual gross yields in Chinese polyculture are reported to reach 20 tonnes per hectare although the median is six to eight tonnes (11). In contrast, extensive systems at the other end of the continuum, such as managed lakes, would rarely exceed 0.5 tonnes per hectare per year (see p. 37-10).

Carp species account for by far the largest portion of global finfish production with the majority of these grown in China (6). Production is based around polyculture within ponds and their integration within wider agriculture systems (11). Outside of China, carp are produced in many countries and systems, including intensive pond and tank production of common carp in Europe and Israel (12) and semi-intensive pond polyculture of common, Indian and Chinese major carp in India. Aquaculture of salmonid fishes, including Atlantic salmon, rainbow trout and Pacific salmon, is the largest intensive finfish aquaculture system in the world and has now overtaken channel catfish aquaculture, one of the first intensively farmed finfish species. Many countries have some salmonid aquaculture and the major producers are Norway, the United Kingdom, Canada and Chile. Nevertheless, the various carp species with more than 15 million tonnes per year dwarf salmonid culture with one tenth of this production (Figure 37.1). The other most important finfish are *Tilapia* and milkfish (6). *Tilapia* species (1.05 million tonnes) are cultured around the world under different systems: they are the mainstay of aquaculture in Africa where they are mainly grown extensively in small ponds; they are used in semi-intensive freshwater polyculture in China, as well as being grown intensively in tanks and ponds in Israel and the United States. Milkfish (0.46 million tonnes) is a major brackish water aquaculture species in South East Asia and a large expansion occurred after the development of commercially effective artificial propagation techniques in Taiwan (13). Species new to aquaculture are constantly under development with considerable focus on higher value marine species. The major hurdle to overcome with most marine species is closure of the life-cycle, which is often technically difficult due to the small size, fragility and unknown feeding and husbandry requirements of marine larvae. Wild-caught fry or juveniles are still used in the aquaculture of many species but the long-term sustainability of this practice is questionable. This is a major challenge that may take many years to solve for some species (see p. 37-6).

Finfish account for approximately half of aquaculture production with crustaceans and molluscs accounting equally for the majority of the remainder (6). Of the top 29 aquaculture species three bivalve molluscs are in the top ten by production with over 8 million tonnes whereas the predominant crustaceans are prawns with about 1 million tonnes per year (Figure 37.1). The monetary value reflects the socioeconomic and technical basis of particular aquaculture systems so that high value prawns and salmonids have a far larger share of the total monetary value of aquaculture production than of the production weight (Figure 37.2). Although aquaculture production is based on at least 210 different plant and animal species the majority comes from the few groups discussed above (6). In 2000 (6) nine countries produced more than half a million tonnes of aquaculture product, including plants, and were headed by China (32 million tonnes), India (2 million tonnes), Japan (1.3 million tonnes) and the Philippines (1 million tonnes).

The aim of this chapter is to provide an overview of the range of aquaculture species and systems found. A more detailed discussion of the principles governing aquaculture practice will use selected examples taken from intensive and semi-intensive aquaculture.

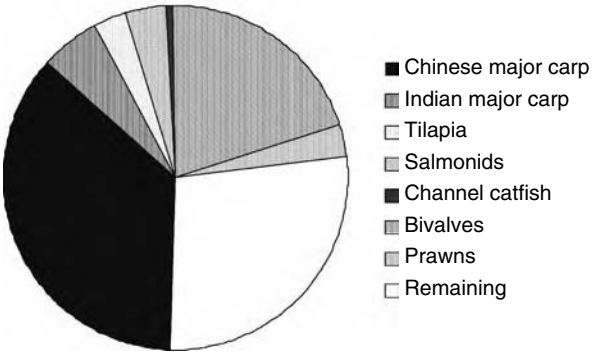

FIGURE 37.1 Distribution (% of total production weight) of major aquaculture groups in 2000 (35). Categories detailed in Table 37.1.

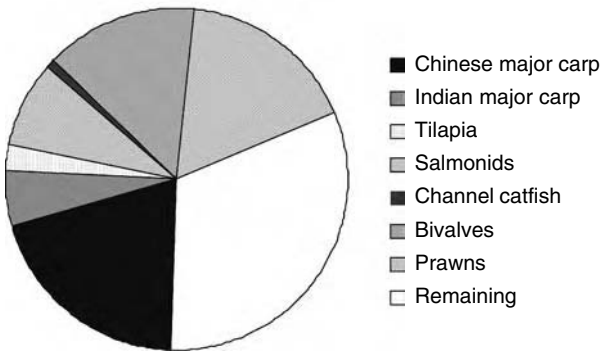

FIGURE 37.2 Distribution (% of total US$ value) of major aquaculture groups in 2000 (35). Categories detailed in Table 37.1.

II. INTENSIVE AQUACULTURE SYSTEMS

The principles that drive intensive aquaculture are based around maximising production in the shortest length of time. Intensive aquaculture is most often based on monoculture of high value species in developed countries. Animals may be contained in a wide variety of structures including net-cages (Figure 37.3), tanks, raceways and earthen ponds (Figure 37.4). Fish are held at high densities and fed nutritionally complete feeds, usually at maximum rations. As has been found in intensive agriculture industries, fish are sensitive to disease and periods of high mortality have been associated with many intensive aquaculture industries.

Reproduction and propagation of major freshwater finfish (i.e., salmonids, carps and catfish) is relatively straightforward and achieved with a high success rate. One of the important features of these finfish groups is the large

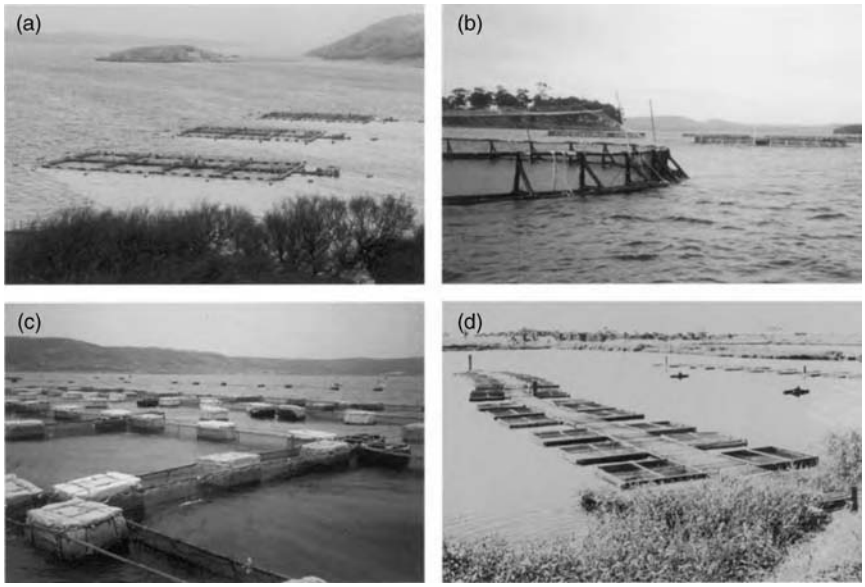

FIGURE 37.3 Use of cages in different forms of aquaculture a) 24-m^2 cages for marine Atlantic salmon farming, b) ring-cages for marine Atlantic salmon farming, c) small 4-m^2 cages for grouper farming, d) small 2-m^2 cages for barramundi grow-out in freshwater ponds.

FIGURE 37.4 Fish holding in (a) concrete tanks used for marine finfish, (b) concrete raceways for rainbow trout, (c) indoor fibreglass tanks for rainbow trout and (d) a drained earthen pond for freshwater silver perch.

size and robust nature of eggs and the ease of feeding compound feeds immediately at, or soon after, first-feeding. This is in marked contrast to marine species in which far greater investment in technologies for rearing larvae and for supplying live feeds has been made. It should be pointed out that some industries, such as Asian sea bass (14), are adopting extensive practices to produce feeding fry for intensive grow-out (see p. 37-6).

Nutrition and feed development are critical issues at present. Aquafeeds use large amounts of valuable fish meal and fish oil and considerable effort is being directed at developing alternative ingredients (15–17). The proportion of the global fish meal supply used in aquafeeds is reported to have increased from 10% (1988) to 35% (1998) (9). These authors also point out that this is due to a reallocation of a relatively static global harvest of fish meal from other feeds to aquafeeds; aquaculture has not caused any increase in fish meal production. Successful experimental feeds that are free of marine products have been developed for larger channel catfish, and following on from this the total animal meal now used in commercial catfish feeds is less than 2% (18). However, the omnivorous channel catfish is one of the few non-carnivorous finfish farmed intensively and the complete removal of marine ingredients from feeds for carnivorous species poses more difficulties. For example, salmonids have limited ability to use dietary carbohydrate (19), and this places constraints on the type of plant-based ingredients that can be used since raw meals contain large amounts of carbohydrates. A likely trend will be the development of high protein ingredients from plant and microbial sources. Products from grains and legumes such as soybean, lupins, canola and peas and microbial sources such as bacteria are currently being investigated (20, 21). Commercial Atlantic salmon feeds can contain over 38% fish oil, making the sector the major user of global fish oil production. This level of use and the probability of increased competition for fish oil from other sectors (*e.g.,* nutraceuticals) have the potential to severely restrict further expansion of salmon aquaculture. Fortunately, it now appears feasible to replace up to 50% of the fish oil in Atlantic salmon feeds with plant oils (16), although there may be salmon health implications under some circumstances (22).

Maintaining animal health and disease control are of major importance in all sectors of aquaculture. There are examples of severe disease outbreaks that have led to huge losses in production. The effects of viruses, including white spot syndrome, monodon baculovirus and yellow head virus, have almost decimated the marine prawn industry; losses up to 1996 were estimated to have reached US$ 3 billion (23). The Atlantic and Pacific salmon industries have suffered from major viral (*e.g.,* IPN, infectious pancreatic necrosis; ISA, infectious salmon anaemia), bacterial (*e.g.,* furunculosis; bacterial kidney disease) and parasitic (*e.g.,* sea lice; ameobic gill disease) diseases. There was a major outbreak of furunculosis in the early 1990s that resulted in yearly losses of US$ 77 million. Fortunately, the disease has declined since 1993 due to the use of effective vaccines (24). Vaccination against a variety of potential pathogens is now a major management procedure in the Norwegian salmon industry and over 90% of all stocks are vaccinated by injection into the intraperitoneal (visceral) cavity (24). Vaccination is only a part of the health care management programs now in place in the salmon industry. These recognise the need for trained personnel, routine monitoring and quick diagnosis; to minimise stress through appropriate stocking densities, careful and minimal handling; site rotation to break the disease cycle; cooperation between farms to ensure single-year class stocking at a location in order to prevent vertical transfer and break disease cycles (24). Aquaculture is also vulnerable to environmental impacts that include predators, blooms of toxic algae, contamination from land run-off and disasters such as oil spills. Aquatic animals are bathed in the contaminated water and it is often much more difficult to move them away from an aquatic threat compared to terrestrial situations.

Offshore marine finfish aquaculture is now being developed in response to human-related pollution and other stresses on the marine coastal environment as well as user conflicts with commercial and recreational groups (25). Technology required for holding and servicing stocks must be far more resilient than for inshore farming and greater mechanisation is needed to reduce the need for manual intervention (25). Assessment of health and growth status, removal of dead fish and feeding are required on a regular basis; grading and harvesting should be possible in any weather. This involves greater use of technology, it is more expensive to purchase and has higher running costs which must be balanced by increased production efficiency (26). There is increasing evidence that growth is faster and more efficient in larger cages (27). Large submersible cages for offshore locations are under development for several aquaculture sectors including salmon and flatfish (28). One example was the Tension Leg Cage, which could be submerged in rougher weather to reduce wave damage at the surface (28). In addition to these open production systems there have been significant advances in closed production system technology. Closed systems are usually land based, use tanks and rely on technology and management to maximise water quality and minimise water use through reuse. This is achieved with water reticulation and physical filtration systems that aid the removal of solid and suspended organic material, heat exchangers to maintain water temperature and reduce heating costs, biofilters containing a microbial flora that removes various harmful nitrogenous compounds and UV filters to reduce bacterial loads.

A. Salmonids

Around ten salmonid species (members of the family Salmonidae) are farmed in 60 countries (29) and over 90% of aquaculture is based on Atlantic salmon, rainbow trout and coho salmon. Salmonid species of interest are naturally found in temperate areas of the northern hemisphere, *Oncorhynchus* species from the Pacific and *Salmo* species from the Atlantic. Demand from recreational fisheries and aquaculture places them as global species with a considerable southern hemisphere presence (30). Rainbow trout have been cultured for many years. Denmark pioneered pond-based production of plate-sized fish at the end of the nineteenth century (30). In the last ten years Atlantic salmon has become the major salmonid species due to a huge expansion in sea-cage farming in Norway followed by Scotland, Canada and Chile. Small juvenile Atlantic salmon, called parr, have brown sides with complex patterns of spots and darker stripes. Adults are typically streamlined and silver scaled with prominent dorsal, pectoral and pelvic fins; the tail should be distinct and slightly forked although farmed fish often have worn fins. Rainbow trout has been described as the Pacific cousin of Atlantic salmon due to the similarities in shape, patterns and silvery colour as well as similar reproductive characteristics (31). Coho salmon are also known as silver salmon. Immature fish have a metallic blue dorsal surface that silvers on the sides; mature males have bright red sides and bright green heads and backs (32).

Atlantic salmon are typically anadromous (return from the sea to spawn in freshwater) with freshwater and seawater phases in their life-cycle. Mature males and females return to freshwater sometime in the 12 months before they spawn. These large impressive fish of up to 30 kilogram famously migrate up the rivers and streams that they last swam down as immature parr several years previously. Once in the spawning grounds the female makes a hollow (redd) in gravelly stream beds where the male fertilises the eggs as they are released. The buried eggs hatch into a yolk-sac stage (alevin) and emerge as fry that start feeding on animal prey. The fry grow into parr that then undergo smoltification, a metamorphosis taking several months, to enable migration into the sea. After two to five years at sea (sea-winters) the fish return as mature adults. Some fish mature and return after one sea-winter and are called grilse. Aquaculture initially recreated this natural life-cycle but varying degrees of manipulation are now used to promote rapid and uniform growth, minimise maturation and maximise harvest over as much of the year as possible.

Broodstock are often removed from a production run on the basis of size and external appearance. They are then conditioned prior to spawning. Selective breeding is used. In Norway it is estimated that 65% of the Atlantic salmon and rainbow trout produced originate from the National Breeding Program (33). This program has incorporated growth, age at sexual maturation, disease resistance and flesh quality as selection characteristics (33). Broodstock conditioning is best in freshwater and synchronous maturation is achieved through the use of hormonal preparations as well as keeping the fish under the same conditions, sometimes males and females together or sometimes in recirculating water allowing chemicals released by the fish to act as they would in the wild (29). Hand "stripping" of eggs and milt, by applying firm pressure along the abdomen, followed by gentle mixing, fertilises the eggs. Hand-stripped Atlantic salmon and rainbow trout can be re-conditioned for the next season. When larger numbers of eggs and milt are required they are removed directly following euthanasia. This procedure has to be adopted with Pacific salmon which only spawn once (30). Sperm are inactive until they touch water so contact is minimised until mixing with eggs. Eggs absorb water on fertilisation, become larger and "sticky" and then harden. Straightforward procedures are followed to ensure maximum fertilisation rates that typically exceed 95%. Fertilised eggs are then incubated under dim or red lights in gently flowing water. Adequate water flow is required to ensure good supply of oxygen and the removal of soluble wastes as well as debris. Incubators are of different designs that vary between deeper cylindrical upwellers to shallow troughs. The California or undercurrent system uses hatching trays positioned in shallow troughs and is considered the most effective design (30). On hatching the alevins use their yolk-sac before being weaned directly onto compound feeds and the fry stage is reached on loss of the yolk-sac. There is considerable individual variation in feeding success and growth rate, so size-grading is used to control size distribution and prevent cannibalism (amongst the smaller stages). Development now proceeds from the parr to the smolt stage. Attention has been focused on controlling this process in order to manage the supply of fish into the major production stage. All in all there has been a highly effective transfer of scientific and technical principles reflected by 80–90% survival from hatch to smolt (30).

Smolt usually have to be transported from the hatchery to the grow-out site; this can be by land, air or sea and care is taken to reduce stress by providing dark aerated conditions and to transfer smolt via pipes directly into the cages. The "smoltification window" describes a period during the season when parr must be of a certain size to start the process of smoltification. It is normally in the spring for Atlantic salmon but more variable for Pacific salmon. In contrast, successful sea-water transfer of rainbow trout, a non-smolting species, is related to larger size (29). The majority of Atlantic salmon on-growing is conducted in sea-cage systems. These are essentially large bag-nets held on floating frames that are anchored to ensure the frames stay in the same place. Bag-nets are five to 20 metres deep and their shape is maintained by devices such as metal

rings around the circumference of the net, weights and lead-core ropes (29). Groups of cages are usually held and anchored together and, depending on the design, they can be joined by walkways or only accessed individually by boat. Galvanised steel square-cages are typically available in 12 to 24 metre square sections. A 24 metre section would consist of a 15 by 15 metre net surrounded by walkways (Figure 37.3a). Each section incorporates floats under the walkways and sections are hinged together to expand a system and to provide flexibility in rougher seas. The other main type of sea-cage is the circular ring-cage in which a buoyant circular ring structure is used to hold a circular bag-net, mooring cables and other ancillary structures such as bird netting or feeders (Figure 37.3b). These structures can range in circumference from 60 to over 120 metres. Compared to square-cages they are usually placed further apart, allow greater water flow through a group of cages and are easier to handle independently of each other. There are a number of commercial variations of square- and ring-cages holding from a few tonnes to over 150 tonnes, approximately 40000 fish (29).

Considerable intervention has been used to manage smoltification and maturation. Controlling the supply of smolt enables stocking over more of the year and more even production throughout the year. This is particularly important when a large proportion of fish mature within one sea-winter as grilse which are smaller than older salmon (29). One-year smolt is the normal age category for smolt but half-year, one-and-a-half and two-year smolt are also produced and used (30). One-year smolt (S1) are exposed to a natural photoperiod and are transferred in spring after one year in freshwater; one-and-a-half year smolt are essentially delayed S1 and transferred in the autumn rather than in the spring like S1; Half-year smolt are transferred in autumn prior to S1 and are produced by photo-manipulation of the photoperiod that accelerates "spring." Mature salmon are not desirable in a production run. Mortality is higher in seawater; they partition feed resources into unwanted reproductive growth rather than somatic growth and develop many poor external and flesh quality characteristics that reduce marketability (29). Whether maturation occurs is highly influenced by size and nutritional status so that faster growing fish, often in warmer temperatures, will mature relatively quickly but be of a smaller size. Strategies to control the effects of maturation include the production of sterile triploid fish or the use of all female stock, since females show lower rates of maturity than males.

Selective breeding has been used as a strategy to improve Atlantic salmon and rainbow trout in Norway since 1971 (33). Initially selection was based on growth, then the frequency of grilse was incorporated to increase the age at maturation. More recent selection strategies have used disease challenge to increase disease resistance and measurements of flesh colour, fat content and fat distribution to improve meat quality (33). There has also been development and testing of genetically modified or transgenic fish. Genetically modified Atlantic salmon parr containing a growth hormone transgene (chinook salmon growth hormone gene attached to an antifreeze protein promoter sequence from ocean pout) grew at over two and half times the rate of control fish due mainly to increased feed intake (34).

B. Marine Finfish

In the majority of developed countries that use intensive aquaculture there is a premium on marine rather than freshwater finfish. Of course, not all marine finfish command the same price or kudos associated with yellowtail, tuna, halibut or grouper, for example. As yet, there is no intensive aquaculture of high value marine finfish on the scale or with the current rate of increase of Atlantic salmon. The large and important Japanese yellowtail industry produced 137000 tonnes in 2000 (35) but this was below production at the start of the 1990s. Farming yellowtail in Japan developed from holding wild-caught fish and intensified in the 1970s. Too rapid expansion and limitations due to disease and markets have led to greater controls on the industry and a reduction in production (36). Although hatchery-based propagation is possible the Japanese industry is still largely based on small (<10 gram) wild-caught fingerlings that are grown to 50–100 grams before being sold for on-growing for about 12 months to about three kilograms (37). Bluefin tuna are very valuable fish and attempts at forms of aquaculture have been under way for many years in Japan, North America, Europe and Australia (38–40). The few commercial tuna farms, mainly located in Australia and the Mediterranean, on-grow large wild-caught juvenile tuna. In Australia, for example, juvenile southern bluefin tuna shoals are caught using a purse-seine, towed back in specially designed ring-cages and transferred to moored cages for a four- to eight-month grow-out (40). The tuna are around 20 kilograms at catch and controlled grow-out allows a high level of value adding to a fishery resource. farmed tuna can treble in weight and larger tuna are more valuable per kilogram as well as on a whole-fish basis. Recently there are indications that aquaculture based on a closed life-cycle of bluefin tuna is possible; successful larval production from northern bluefin tuna that had themselves been grown in culture from larvae has been achieved (41). The research program was started over 25 years prior to successful spawning of the wild tuna in 1995–96 and indicates the high level of complexity and need for a considerable economic and time investment to achieve success. Further development will be required because there may be key differences between wild-caught and domestic broodstock that could result in poor larval quality and a low success rate in the production of viable juveniles from this second generation.

Good examples of intensive marine aquaculture in which the life-cycle is closed and that have a high production are the European sea bass and gilthead sea bream in the Euro-Mediterranean region (42), red drum in the southern United States (43), red sea bream in Japan and the Asian sea bass in South East Asia (14). European sea bass production has increased steadily over the last ten years and all aspects of production, from larval rearing to broodstock management, are considered to be under control (44). Commercial hatcheries produce in excess of 180 million juveniles for sea-based grow-out. Propagation is based on a relatively standard larval rearing protocol supplying a sequence of live feeds, rotifer followed by brine shrimp, before weaning onto a compound feed (44). European sea bass larvae first feed four or five days after hatching when they are between four and six millimetres in length. After three to four weeks they reach ten millimetres and weaning onto a high protein compound feed begins and is usually complete by 40 days. An interesting development is the successful use of a compound feed immediately at first-feeding and for the entire larval period. Consideration of supplying essential nutrients in a form that can be readily assimilated is the key to this success and achieved using ingredients such as phospholipids and hydrolysed proteins (44). Rotifers and brine shrimps form the basis of most larval rearing protocols. Rotifers are smaller and always fed first, then with brine shrimp in order to provide food for smaller individuals before only brine shrimp are used. Other variations include "greenwater" techniques, whereby microalgae are also grown because they appear to benefit both the live feed and the finfish larvae. Red sea bream are fed microalgae (day three to 25), rotifers (day three to 33), brine shrimp (day 20 to 40) and compound feed (day 20 to 60). Live feeds are often "enriched" by feeding them commercial preparations to increase concentrations of specific nutrients, particularly polyunsaturated fatty acids and vitamins.

The aquaculture of Asian sea bass or barramundi provides an interesting view of how one species has been highly adaptable and successful under many systems (14). Its natural range encompasses the northern Indian and tropical western Pacific Oceans and it is found from Iran to Australia (14). Although aquaculture production is relatively small (20000 tonnes in 1999) there is great potential for a species that can be cultured using intensive and extensive methods, indoors or outdoors, and that can be grown over the full range of salinity (from fresh to seawater) and even in brackish bore waters far from the sea. Tucker *et al*. (2002) have recently reviewed barramundi aquaculture: barramundi are catadromous (opposite to salmon) and sea water is needed for the broodstock, eggs and the first half of the larval rearing cycle. The species is also a protandrous hermaphrodite so that individuals first mature into males (after two years) and then into females (after three to five years). Barramundi can be spawned in tanks or strip spawned and this can be achieved with or without hormonal manipulation. Ambient conditions are very important for maturation and spawning. Spawning will occur regularly under summer photoperiod and temperatures: in Australia these parameters are artificially controlled in enclosed tanks to produce a regular supply of larvae from a small broodstock population (14). In more tropical locations farmed broodstock kept under natural conditions at high temperatures (27–34°C) will spawn regularly in tanks. Spawning occurs on the full moon and can be enhanced by first decreasing (also increasing temperature) and then increasing tank water depth (decreasing temperature) to recreate a high tide. Barramundi are very fecund and females produce hundreds of thousands of eggs per kilogram on each spawning. Males are stocked with females and the floating fertilised eggs are collected by nets or from the outflow water. The larvae are 1.5 millimetres when they hatch and have a yolk sac and oil globule as food reserves that are fully depleted after six days. Typically, larvae first-feed after two to three days (2.6 millimetres length) and take around 25 days to metamorphosise into juveniles (17 millimetres length). There are many approaches to larval rearing that range from intensive to extensive. Intensive procedures can be carried out in small tanks using a rotifer and brine shrimp sequence, rotifers from day two to day 15 and brine shrimp from day ten. Production methods may also incorporate a greenwater approach with the use of microalgae over the first part of larval rearing. Extensive procedures are carried out in large earthen ponds relying on natural plankton blooms initiated by fertilising the pond. Juveniles are easily weaned onto compound feeds and transferred from the nursery to grow-out stage. In Southeast Asia grow-out uses small ponds (0.08 to two hectares), coastal impoundments or smaller cages (one to 300 m^3). Several approaches to grow-out are used in Australia. These include open freshwater ponds, small cages held in freshwater ponds (Figure 37.3d), large marine cages and, because barramundi command a high market price, indoor tanks in more southerly non-tropical regions. Production is based on plate-size fish of around 400 to 700 grams and larger fish of around two to three kilograms for fillets or as "banquet" fish.

C. Abalone

Abalone are herbivorous gastropod molluscs that graze, usually nocturnally, on microalgae (attached to surfaces) and macroalgae (seaweed) (4). They have one shell with several holes along the edge for water exchange and a famously pearly inner surface. Their large muscular foot is an expensive delicacy around the world. Aquaculture started in Japan about 50 years ago and numerous *Haliotis* species are now grown commercially around the world. Production is led by China and Taiwan with Australia considered to be leading industry development through

research into areas such as feeds and nutrition, health and grow-out technologies (45). Abalone culture has been reviewed recently by Viana (2002). Farm-raised broodstock are readily available and synchronous reproduction is usually managed through the control of water temperature, water quality and high feed availability. Spawning can be induced by manipulation of temperature and light: temperature shock stimulation of spawning is created by raising water temperature to a target level and then dropping it suddenly and shifting to a dark period. Fertilised eggs hatch into non-feeding larvae which then undergo metamorphosis and leave the water column to settle on the tank floors. Diatoms (microalgae) are grown on hard surfaces and supplied for several months for the post-larvae to graze on before they grow larger and the radula becomes hard enough to graze on macroalgae. This switch takes place at about one centimetre length and abalone from one to three centimetres are then ready for grow-out to about seven centimetres. Broodstock and post-larval stages are often maintained in land-based facilities whereas grow-out may be sea- or land-based. Sea-based systems are designed to provide shelter and good water flow but feeds still have to be supplied. Abalone in land-based farms can be held in ponds, tanks, raceways or other types of enclosure. Shallow tanks with laminar water flow to minimise water use are suitable in conjunction with compound feeds but greater tank depth and water volumes are required when macroalgae are the food. Large sectors of the industry are based on the use of macroalgae as the principal and only food source for grow-out and the regular provision of large volumes of macroalgae is a major operational issue. However, abalone can still be grown intensively on macroalgae. For example, in some intensive Taiwanese farms abalone are housed in banks of many small containers that are lifted out from the water by overhead cranes, packed by hand with macroalgae and returned. Abalone are slow growing and will take up to five years to reach market size. In Australia the use of macroalgae is not thought economic and there has been a focus on developing a complete nutritionally balanced feed. As a result the time taken to reach market size has been reduced from five to less than four years. Abalone are slow feeders and feeds remain in the water for long periods of time which means significant leaching of nutrients can occur (46). A variety of technologies have been explored and include the use of high concentration of ingredients that act as binders and as nutrients (*e.g.,* wheat and maize gluten), combinations of binders and the use of microencapsulation for highly soluble nutrients (46). Growth variation is large and selective breeding programs for growth and growth efficiency are seen to be an important area for future development (45). A variety of traits such as growth rate, growth efficiency and marketable characteristics have been suggested and research has already established that heritability for survival and shell length is high enough for use in selective breeding programs (47).

III. SEMI-INTENSIVE AND EXTENSIVE AQUACULTURE SYSTEMS

Semi-intensive and extensive culture of fish in ponds is fundamentally different from intensive aquaculture. This form of pond culture uses a more holistic approach by exploiting the biological characteristics of the immediate aquatic ecosystem in fish production (48). The principles are based on the management of a pond's trophic web through alterations to the natural progression of the species balance in order to maximise conditions for fish production (48). Such alterations include efficient use of external inputs, principally organic fertilisers, the removal of species to shorten food chains or that are trophic "dead-ends" (*e.g.,* macrophytes), supplementary feeding and complex harvesting and re-stocking protocols. Pond-based polyculture, of mainly carp species, that has been refined in China and the pond-based monoculture of prawns provide examples of semi-extensive aquaculture. Large-scale changes in the types of aquaculture practiced in China are under way and intensive systems are being developed for freshwater and marine species (personal observation). Prawns are also farmed under intensive, extensive, polyculture and integrated aquaculture systems (49).

A. Chinese Finfish Polyculture

As described above the most significant contribution to global aquaculture production is made by finfish cultured using Chinese pond-based polyculture systems. Although largely based on knowledge handed down by farmers without them necessarily understanding the scientific basis of management strategies (50) there has been considerable work by provincial fisheries bureaus to document information as a basis for making improvements through a scientific approach (51). There have been dramatic increases in production over recent years. Although aquaculture is thousands of years old it is only since 1957 that Chinese major carp fry have been artificially propagated. Apart from increases in the number of fish and ponds available other areas where huge improvements have been made include training and technology transfer, effective pond design, placing greater emphasis on polyculture, increasing stocking density and increasing production through multiple harvesting and re-stocking (51, 52).

The finfish used in polyculture can be divided into four groups based on their principal food and feeding habits: plankton, macrophytes, detritus or aquatic animals (53). The major species used in polyculture are silver carp (phytoplankton), bighead carp (zooplankton), grass carp (macrophytes), mud carp (detritus) and black carp (molluscs); common carp (omnivorous feeding) are also used in high numbers. The former group of carp species are known as the Chinese major carp (Table 37.1). Other species such as the blunt nosed bream or Wuchang fish (macrophytes),

TABLE 37.1
Major Aquaculture Species with Top 20 Rank by Production Weight in 2000 (35)

Group	Name (Rank)	Scientific Name	Water, Feeding Mode
Finfish			
Chinese major carp	Silver carp (2)	*Hypophthalmichthys molitrix*	FW, filter feeder
	Grass carp (3)	*Ctenopharyngodon idella*	FW, herbivore
	Bighead carp (6)	*Hypophthalmichthys nobilis*	FW, filter feeder
	Mud carp	*Cirrhinus molitorella*	FW, detritivore
	Black carp	*Mylophryngodon piceus*	FW, carnivore
Indian major carp	Rohu (11)	*Labeo rohita*	FW, omnivore
	Catla (12)	*Catla catla*	FW, filter feeder
	Mrigal (13)	*Cirrhinus mrigala*	FW, detritivore
Other carp	Common carp (4)	*Cyprinus carpio*	FW, detritivore
	Crucian carp (7)	*Carassius carassius*	FW, detritivore
Other finfish	Tilapia (9)	*Oreochromis niloticus*	FW, omnivore
	Milk fish (16)	*Chanos chanos*	B, herbivore
	Channel catfish (20)	*Ictalurus punctatus*	FW, omnivore
	Yellowtail	*Seriola quinqueradiata*	M, carnivore
Salmonids	Atlantic salmon (10)	*Salmo salar*	M, carnivore
	Coho salmon	*Oncorhynchus kisutch*	M, carnivore
	Rainbow trout (18)	*Oncorhynchus mykiss*	FW, carnivore
Shellfish			
Bivalves	Pacific oyster (1)	*Crassostrea gigas*	B, filter feeder
(Molluscs)	Small neck clam (5)	*Ruditapes philippinarum*	M, filter feeder
	Giant ezo scallop (8)	*Patinopecten yessoensis*	M, filter feeder
	Blue mussel (17)	*Mytilus edulis*	M, filter feeder
Prawns	Tiger (14)	*Penaeus monodon*	B & M*, detritivore
(Crustaceans)	Oriental	*Penaeus chinensis*	B & M*, detritivore
	White	*Penaeus vannamei*	B & M*, detritivore
	Giant freshwater	*Macrobrachium rosenbergii*	FW & B*, detritivore

FW = freshwater, B = brackish, M = marine, * = during reproduction and larval stages.

crucian carp (detritus) and *Tilapia* species (plankton and detritus) are also important in more complex polyculture systems. Chinese finfish polyculture has been divided into two main types: the "feeding model" and the "filter-feeding model" (51). The feeding model is based on grass, black and or common carp whereas the filter-feeding model makes more use of silver and bighead carp and rarely uses black carp. Although both models are widely used the balance differs between provinces. The feeding model is prevalent in Zhejiang (89%) and Jiansu (89%) compared to Hubei (25%) and Shandong (25%) (51). The number and mix of the principal species and the use of additional species is dependent on the province and on the farm (11). Polyculture in provinces such as Zhejiang has been practiced for thousands of years. The climate is ideal for extended production over a large part of the year and the province is wealthy and very close to the huge marketplace of Shanghai. Consequently, conditions favour the feeding model and the production of species (grass, black and common carp) that are preferred in the market place. Thus, the complexity of the polyculture practiced depends on geographical location, which influences the growing season; on the historical and technological basis of aquaculture, which influence expertise and technical support; as well as on socioeconomic factors, which influence the mix of species and the inclusion of more valuable species (11).

Broodstock are grown in special ponds for four to seven years and to weights in excess of five to ten kilograms depending on the species (50). Broodstock are placed in monoculture for a pre-spawning phase where water quality and feeding are managed. Species other than common carp normally spawn in flowing rivers so the maintenance of good water quality with high oxygenation is important in these ponds. Hormonal injections are used to induce spawning and male and females are placed together in circular concrete tanks where spawning and fertilisation occur (50). The fertilised eggs sink and are carried out of the tanks to be collected from the outflow and placed into incubation systems. Large circular incubation tanks, constructed from bricks and cement, are stocked at around 800000 eggs per cubic metre of water. Once the swim bladder is formed the larvae can be removed and stocked into the nursery ponds. Rearing progresses over the summer and the first stage lasts for around 30 days. Different carp are usually kept under monoculture due to the similarity in larval diets. Soya

milk is supplied directly to the pond for feeding the larvae and early fry. Limited polyculture may be used in the later phases of fry production. Polyculture protocols use three to eight centimetre fry in different combinations of the main carp species. Stimulation of phyto- and then zooplankton blooms is managed through the application of organic fertilisers to provide food for the growing fry.

Rectangular ponds are recommended and are of different dimensions depending on the production stage: for example, fry ponds are 1.2 metre deep and less than a fifth of a hectare in area; fingerling ponds are 1.5 metre deep and up to a third of a hectare in area; grow-out ponds are two to three metre deep and up to two thirds of a hectare in area. The most efficient grow-out systems follow multiple-batch harvesting and re-stocking protocols, sometimes called "multi-grade conveyor culture," which coordinate several harvests and restocking of both fingerlings and of larger fish over the growing season. For example, in Guangdong Province 35% of ponds may be allocated to fingerling production and the rest to grow-out. The grow-out ponds will be divided for different sizes of fish. The final or "fattening" pond contains mainly bighead, grass, mud and silver carp, to be grown up to harvest size over different periods of time, as well as some common and black carp, Wuchang fish, tilapia and even snakehead (Table 37.2). In hilly regions where there are fewer ponds there is an emphasis on mixed-age culture within the same ponds. Chinese finfish polyculture is dependent on large inputs of organic fertilisers (mainly manure and composts) as well as some feed sources not available in the pond, especially terrestrial plant material for grass carp. Traditional fish farms are usually part of a larger integrated aquaculture-agriculture farm. Integrated farming, the integration of agriculture and aquaculture activities (Figure 37.5a), has several forms in China involving combinations of fish with ducks, pigs, chickens, cows and silk-worms.

TABLE 37.2
Stocking and Harvest Weights of "Fattening" Ponds in a Polyculture System in Use in Guangdong Province, China

Species	Stock Weight (Grams per Fish)	Harvest Weight (Grams per Fish)	Density* (Fish per Hectare)	Rearing Period (Days)
Bighead carp	500	1000–1500	330	40
Grass carp	250–500	1300–1500	600–1200	60–180
Mud carp	50	125	14250	180
Silver carp	250–600	700–1000	300–600	90–180
Common carp			300	
Tilapia			7500–15000	
Wuchang fish	100–250		750	
Black carp			75–150	

* = Based on 15 mu per hectare.

The integration of fish with pigs and with ducks are considered the most successful systems (54). In one documented example (55) 70 hectares of ponds produced 800 tonnes of fish (based on eight species polyculture), 400000 ducks, 1060 pigs, 123 cows and chickens to give an overall annual production of 13.5 tonnes per hectare. In fish-silk culture mulberry bushes (Figure 37.5b) are grown by fish ponds and provide leaves for silk-worms which produce manure and protein from the pupa.

Variations of finfish polyculture are found throughout the world and suit different socioeconomic situations. For example, a system of polyculture was developed in India around the Indian major carp (Table 37.1) and it now incorporates other carp species. In Bangladesh, Indian and Chinese major carp are grown in ponds under polyculture systems and sold as a cash crop. It has been shown that if smaller pond fish species are also included they provide a vital protein source for the farmer's family whereas the carp are treated only as a cash crop (56).

B. Prawns

Penaeid shrimps are the most important farmed "prawns" and over twenty species are farmed (4). The majority of production is of *Penaeus monodon* (570000 tonnes) with a sizable contribution from *P. chinensis* (219000 tonnes) and *P. vannamei* (144000 tonnes) (35). The giant freshwater prawn, *Macrobrachium rosenbergii*, is the fourth most important prawn (119000 tonnes), the majority of production being from China (48%) and Bangladesh (37%) (49). There are many similarities in aquaculture techniques for the different prawn species although there are also important differences, not least of which between the freshwater and marine species (54).

The current status of freshwater prawn aquaculture and techniques have been recently detailed in depth (49). Wild female *M. rosenbergii* migrate into estuaries where the fertilised eggs they are carrying hatch. Larvae metamorphosise through 11 stages before the post-larvae settle on the estuary bottom and migrate into freshwaters. The discovery that the larvae needed a brackish water environment for successful development is considered a major advance in the aquaculture of this species (4). Reproduction and larval rearing are now well understood. Seawater can be trucked inland to hatcheries and siting farms away from coastal areas is viewed as a major advantage of the industry (49). World production has stabilised but aquaculture may yet have great potential because there has been limited development in South and Central America or in Africa and the species characteristics are more favourable to lower intensity farming and to developing polyculture with finfish. Polyculture with finfish in ponds offers an opportunity to increase pond yields with little increased cost. Prawns feed on bottom detritus and do not compete with the finfish (49). A range of production

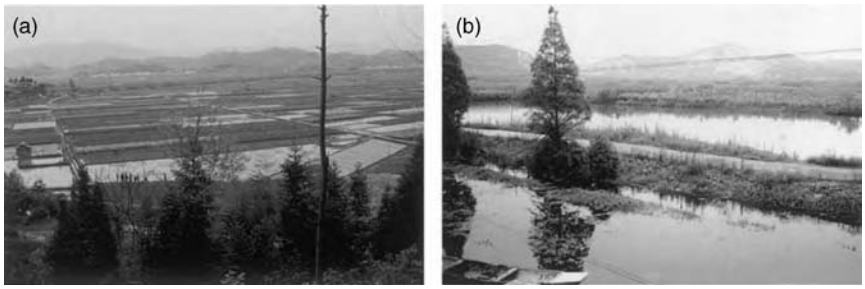

FIGURE 37.5 Chinese aquaculture showing a) view of integrated fish ponds and agriculture (photograph by Z-Y He), b) municipal fish ponds in Zhejiang Province with mulberry bushes on pond banks.

systems from extensive to intensive are used although the majority of the industry is based on semi-intensive grow-out. *M. rosenbergii* aquaculture provides a good example where the different aquaculture systems can be related to key aspects of farming. Extensive farming uses either dedicated ponds or impounded areas where there is another primary purpose such as rice fields or irrigation ditches; no feed is added; there is no control of water quality, predator or competitor species; stocking is 1–4 per square metre and production is less than 500 kilograms per hectare per year; harvesting is difficult because the water cannot be drained or seined. Intensive farming uses small concrete ponds; high water quality is maintained through high water exchange and continuous aeration; predator and competitor species are removed; nutritionally complete compounded feeds are used; stocking density is high at 20 per square meter and production is in excess of five tonnes per hectare per year; ponds are drain harvested (there are few commercial examples of intensive aquaculture). Semi-intensive aquaculture relies on daily management of water quality and water productivity to supply natural food sources; compound feeds are used to supplement pond-based food; stocking density is four to 20 per square meter and production is up to five tonnes per hectare per year (57). However, *M. rosenbergii* is territorial and density has a major effect on growth. Under semi-intensive conditions densities from four to 12 per square metre resulted in the most marked reduction in growth (57). Consequently, there has to be a balance between average growth rate and the total biomass available for harvest. An efficient semi-intensive operation would reach market weight of 30 to 60 grams in six to eight months with a survival of 50 to 80% (57).

Much of the research and development in marine prawn farming has been related to controlling spawning and larval rearing (54). Maturation, spawning and early larval stages of marine prawn species occur during the marine phase of the life-cycle, whereas the brackish waters of estuaries act as feeding grounds for post-larval and juvenile stages (4). Penaeid aquaculture was originally based on collection from the wild of either gravid females, which had mated and would then produce larvae, or post-larvae and juveniles. Hatchery techniques that allow the control and management of maturation, spawning and the fertilisation of eggs are now established (54). One of the key techniques is the removal of one eyestalk (unilateral ablation) from females. This decreases the amount of gonad-inhibiting hormone produced and therefore promotes maturation and spawning, particularly in females. The level of management is species dependent so that *P. monodon* requires ablation. Ablation is used with *P. vannamei* but it is not essential and species such as *P. japonicus* are responsive to environmental manipulation and ablation is not used (4). Broodstock diet is important and although compound feeds could be used there is a strong belief that factors contained within fresh or frozen animal food and not in compound feeds are required. Broodstock diets often contain polychaete worms, squid or bivalves, trash fish or crustacean waste. Males and females are stocked in equal numbers in spawning tanks. Eggs are collected and removed to the hatchery. Larval rearing relies on a controlled environment and maintenance of high quality seawater. A sequence of live-food starting with algae, rotifers and then brine shrimp is used. The "Galveston" method was developed in the U.S. and is more intensive; small larval rearing tanks are stocked at high densities and live-feeds supplied from separate tanks. Post-larval stages are thinned down and transferred to larger tanks or ponds. The "community culture" method originates in Japan. It is based on larger tanks and relies on promoting the growth of live feed species within the same body of water as the larvae.

Grow-out may also be conducted under intensive, semi-intensive and extensive systems: intensive (>200 prawns per square metre) practices tend to use tanks, raceways or smaller concrete ponds whereas extensive (<1 prawn per square metre) practices usually rely on larger earthen ponds or impounded areas (4). Semi-intensive management strategies are broadly similar to those described above for *M. rosenbergii* and typical prawn ponds are rectangular and of one to three hectares, although some ponds in South America are as large as 20 hectares, and around one metre deep (54). Water circulation is vital to managing water quality and food supply. Regular water exchange is practiced along with direct aeration and circulation of pond water to

ensure nutrients are re-suspended for assimilation by plankton. Production is around two to four tonnes per hectare per year. In contrast, intensive farming can be as high as 28 tonnes per hectare per year but has proved very vulnerable to disease problems (54).

IV. PACIFIC OYSTER FARMING

Pacific oyster farming has the highest production of any single aquaculture species. Production in 2000 was nearly four million tonnes and about 11% of total global aquaculture (35). Pacific oysters, originally from Japan, are very adaptable to different environmental conditions and are farmed throughout the world, having been introduced to the United States, Europe, South America, Africa and Australasia (4). They are bivalves so that a soft body is enclosed within two shells (valves) that are joined at a pointed end. The lower valve is cupped and normally the site of attachment to a substrate. The shape of the shell is very variable and heavily influenced by the environment: smooth and elongated shells result from growth on a soft substrate whereas a hard or gravelly substrate will cause flutes and corrugations as well as a deeper rounder shape (58). They inhabit brackish waters such as estuaries and feed by filtering nutrients out of the water. Consequently growth is highly dependent on the nutrient supply in the waters of the farm as well as on water temperature and quality. It takes fifteen to eighteen months to reach market size (about 100 grams) under natural conditions.

Males and females are separate and discharge sperm or eggs into water where fertilisation takes place. Between seasons they may changes sex from male to female (58). Spawning is stimulated by warming waters in the spring and summer and it may occur throughout the year at high temperatures (*e.g.,* 30–33°C in the Philippines). The larvae feed on microalgae in the water column before they metamorphosise into spat that settle out. Traditional Japanese oyster culture relied on the collection of wild spat on bamboo sticks and then on collectors suspended from rafts. Hatchery production has become more prominent although wild spat are still used. An important advantages of a hatchery-based industry is the relative ease of organising a genetic improvement program, as has been initiated in Tasmania (Australia) (59). The spat are grown until winter when they are graded and taken for one-year or two-year grow-out. Suspension of oysters in cultches and bags from floating rafts is the most common method in Japan, suspension from long-lines is more common in rougher deeper water. One-year oysters are transferred to productive waters after winter and grow rapidly. The two-year oysters are "hardened" over the first season on wooden racks positioned intertidally so that oysters are subjected to waves and air exposure. They are transferred to the grow-out areas the next season and are larger than the one-year oysters on harvest (4). Due to economic and legal restrictions on floating structures many farms still adopt bottom culture, in which the oysters are grown directly on the sea bottom (54). Off-bottom structures that are located intertidally and built into the bottom include stakes (placed vertically) or sticks (placed horizontally), stone or concrete slabs placed in bridges and wooden rack systems that support trays and bags or are used to suspend ropes carrying the oyster cultch. Suspended systems such as rafts or long-lines are placed further offshore and carry suspended trays, lines and bags. The location of oyster farms is crucial and efficient grow-out depends mainly on understanding the carry capacity of the water to maximise the use of nutrients and avoid overstocking. Minimising grading and replanting is also important. Depuration is particularly important to ensure oysters are safe to eat. They are eaten raw and being filter feeders can concentrate pathogenic bacteria, viruses, natural toxins (notably from microalgae) and pollutants.

V. SUMMARY

On the order of 300 aquatic species are cultured throughout the world and many species that are new to aquaculture will be under investigation at any one time (and often rejected after some period of research and development). In this overview I have concentrated on major species and groups, particularly on a few finfish, bivalve molluscs and decapod crustaceans (prawns). Large amounts of seaweed are also farmed. There is significant commercial farming of other vertebrate (*e.g.,* frogs, soft-shelled turtles) and invertebrate (*e.g.,* echinoderms, sea cucumbers) groups. Intensive and semi-intensive approaches were described and they emphasise the scope of aquaculture practice as well as how systems need to be appropriate to specific socioeconomic circumstances. Important challenges face aquaculture and ensuring it is sustainable. These will partly be met by increased understanding through research and the development of technological solutions. Best practice clearly does not imply only the use of intensive aquaculture; considerable improvements can be made to all systems and approaches can be transferred. Whichever system is appropriate, more emphasis will be placed on increasing efficiency and maximising the use of valuable resources. A broader view of resources will encompass multiple use of location and the environmental impact of aquaculture, disease prevention rather than control and limiting the use of chemical or antibiotic solutions, the sustainable use of feed ingredients and decreasing reliance on marine products. Biotechnology may offer increased opportunity; there is already perceived but uneven market resistance to genetically modified products. Global forces such as climate change will have important consequences in relation to which species are farmed and where species are farmed. Increased water temperatures may allow species to be grown in new regions but also end the culture of other species. The

importance of the contribution that aquaculture makes to human nutrition will continue to increase and considerable work is being done to ensure its effectiveness.

ACKNOWLEDGEMENTS

My sincere thanks go to Z.-Y. He, Zhejiang Provincial Fisheries Bureau for providing detailed information on Chinese aquaculture and for hosting my visits to his wonderful province.

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38 Frozen Seafood Products: Description

Peggy Stanfield
Dietetic Resources

CONTENTS

This book is not the proper forum to discuss the manufacture of every frozen seafood product available on the market. However, regulatory agencies such as the National Marine Fisheries Service (NMFS) have issued some minimal criteria for several frozen seafood and seafood products: what they are, what types and styles are available, and so on. This chapter describes each available frozen seafood product based on the product grades issued by the NMFS. A product grade is established to achieve two objectives: ensure product safety and to minimize economic fraud.

I. FROZEN HEADLESS DRESSED WHITING

A. Description of the Product

The product described in this section consists of clean, wholesome whiting (silver hake) *Merluccius bilineraris, Merluccius albidus*, completely and cleanly headed and adequately eviscerated. The fish are packaged and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product.

B. Grades of Frozen Headless Dressed Whiting

U.S. Grade A is the quality of frozen headless dressed whiting that possesses a good flavor and odor.

U.S. Grade B is the quality of frozen headless dressed whiting that possesses at least reasonably good flavor and odor.

Substandard or Utility is the quality of frozen headless dressed whiting that otherwise fails to meet the requirements of U.S. Grade B.

C. Determination of the Grade

Good flavor and odor (essential requirements for a U.S. Grade A product) means that the cooked product has the typical flavor and odor of the species and is free from rancidity, bitterness, staleness, and off-flavors and off-odors of any kind.

Reasonably good flavor and odor (minimum requirements of a U.S. Grade B product) means that the cooked product is lacking in good flavor and odor but is free from objectionable off-flavors and off-odors of any kind.

Arrangement of product refers to the packing of the product in a symmetrical manner, bellies or backs all facing in the same direction, and fish neatly dovetailed.

Condition of the packaging material refers to the condition of the cardboard or other packaging material of the primary container.

If the fish is allowed to stand after packing and prior to freezing, moisture from the fish will soak into the packaging material and cause deterioration of that material.

Dehydration refers to the presence of dehydrated (water-removed) tissue on the exposed surfaces of the whiting. Slight dehydration is surface dehydration which is not color-masking. Deep dehydration is color-masking and cannot be removed by scraping with a fingernail.

Minimum size refers to the size of the individual fish in the sample. Fish 2 ounces or over are considered acceptable. Smaller fish cannot be cooked uniformly with acceptable size fish.

Heading refers to the condition of the fish after they have been headed. The fish should be cleanly headed behind the gills and pectoral fins. No gills, gill bones, or pectoral fins should remain after the fish have been headed.

Evisceration refers to the cleaning of the belly cavities of the fish. All spawn, viscera, and belly strings should be removed.

Scaling refers to the satisfactory removal of scales from the fish.

Color of the cut surfaces refers to the color of the cut surfaces of the fish after heading and other processing.

Bruises and broken or split skin refers to bruises over one-half square inch in area and splits or breaks in the skin more than one-half inch in length which are not part of the processing.

Texture defects refers to the absence of normal textural properties of the cooked fish flesh, which are tenderness, firmness, and moistness without excess water. Texture defects are dryness, softness, toughness, and rubberiness.

II. FROZEN HALIBUT STEAKS

A. Product Description

Frozen halibut steaks are clean, wholesome units of frozen raw fish flesh with normally associated skin and bone and are 2 ounces or more in weight. Each steak has two parallel surfaces and is derived from whole or subdivided halibut slices of uniform thickness which result from sawing or cutting perpendicular to the axial length, or backbone, of a whole halibut. The steaks are prepared from either frozen or unfrozen halibut (*Hippoglossus* spp.). They are processed and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product.

B. Styles of Frozen Halibut Steaks

The individual steaks of Style I, random weight pack, are of random weight and neither the weight nor the range of weights is specified.

All steaks of Style II, uniform weight or portion pack, in the package or in the lot are of a specified weight or range of weights.

C. Recommended Dimensions

(a) The recommended dimensions of frozen halibut steaks are not incorporated in the grades of the finished product since dimensions, as such, are not factors of quality for the purpose of these grades. However, the degree of uniformity of thickness among units of the finished product is rated since it is a factor affecting the quality and utility of the product.

(b) It is recommended that the thickness (smallest dimension) of individually frozen halibut steaks be not less than ½ inch and not greater than inches.

Percentage glaze on halibut steak means the percent by weight of frozen coating adhering to the steak surfaces and includes the frost within the package.

Uniformity of thickness means that the thickness is substantially the same for one or more steaks within a package or sample unit.

D. Color Defects

(a) Discoloration of drip liquor means that the free liquid which drains from the thawed steaks is discolored with blood residue usually from the dorsal aorta of the halibut.

(b) Discoloration of light meat means that the normal flesh color of the main part of the halibut steak has darkened due to deteriorative influences.

(c) Discoloration of the dark meat means that the normal color of the surface fat shows increasing degrees of yellowing due to oxidation.

(d) Non-uniformity of color refers to noticeable differences in color on a single steak or between adjacent steaks in the same package.

(e) Dehydration refers to the appearance of a whitish area on the surface of a steak due to the removal of water or drying of the affected area.

(f) Honeycombing refers to the visible appearance of numerous discrete holes or openings of varying size on the steak surface.

(g) Workmanship defects refers to appearance defects that were not eliminated during processing and are considered either objectionable or poor commercial practice.

(h) Texture defect refers to an undesirable increase in toughness and/or dryness, fibrousness, and watery nature of halibut examined in the cooked state.

III. FROZEN SALMON STEAKS

A. Product Description

Frozen salmon steaks are clean, wholesome units of frozen raw fish flesh with normally associated skin and bone and are 2.5 ounces or more in weight. Each steak has two parallel surfaces and is derived from whole or subdivided salmon slices of uniform thickness which result from sawing or cutting dressed salmon perpendicularly to the axial length, or backbone. The steaks are prepared from either

frozen or unfrozen salmon (*Oncorhynchus* spp.) and are processed and frozen in accordance with good commercial practice and are maintained at temperatures necessary for the preservation of the product. The steaks in an individual package are prepared from only one species of salmon.

B. Species

Frozen salmon steaks covered huiby are prepared from salmon of any of the following species:

Silver or coho (*0. kisutch*)
Chum or keta (*0. keta*)
King, chinook, or spring (*0. tshawytscha*)
Red, sockeye (*0. nerka*)
Pink (*0. gorbuscha*)

C. Styles

The individual steaks of Style I, random weight pack are of random weight and neither the individual steak weight nor the range of weights is specified. The steaks in the lot represent the random distribution cut from the head to tail of a whole dressed salmon.

The individual steaks of Style II, random weight combination pack, are of random weight and neither the individual steak weight nor the range of weights is specified. The steaks in the lot represent a combination of cuts from selected parts of the whole dressed salmon.

All steaks of Style III, uniform weight or portion pack, in the package or in the lot are of a specified weight or range of weights.

D. Recommended Dimensions

It is recommended that the thickness (smallest dimension) of individually frozen salmon steaks be not less than 1/2 inch and not greater than 1 1/2 inches.

General appearance defects refer to poor arrangement of steaks, distortion of steaks, wide variation in shape, between steaks greater than normal number of head and/or tail pieces, imbedding of packaging material into fish flesh, inside condition of package, frost deposit, excessive or nonuniform skin glaze, and undesirable level of natural color.

Dehydration refers to the appearance of a whitish area on the surface of a steak due to the evaporation of water or drying of the affected area.

Uniformity of thickness means that the steak thickness is within the allowed manufacturing tolerance between the thickest and thinnest parts of the steaks within a package or sample unit.

Workmanship defects refers to appearance defects that were not eliminated during processing and are considered objectionable or poor commercial practice. They include the following: Blood spots, bruises, cleaning(refers to inadequate cleaning of the visceral cavity from blood, viscera and loose or attached appendages), cutting (refers to irregular, inadequate, unnecessary, or improper cuts and/or trimmings), fins, foreign material (refers to any loose parts, of fish or other than fish origin), collar bone, girdle(refers to bony structure adjacent to fin), loose skin, pugh marks, sawdust, and scales.

E. Color Defects

Discoloration of fat portion means that the normal color of the fat shows increasing degrees of yellowing due to oxidation.

Discoloration of lean portion means that the normal surface flesh color has faded or changed due to deteriorative influences.

Nonuniformity of color refers to noticeable differences in surface flesh color on a single steak or between adjacent steaks in the same package or sample unit. It also includes color variation of the visceral cavity and skin watermarking.

Honeycombing refers to the visible appearance on the steak surface of numerous discrete holes or openings of varying size.

Texture defect refers to an undesirable increase in toughness and/or dryness, fibrousness, and watery nature of salmon examined in the cooked state.

IV. FROZEN FISH FILLET BLOCKS

A. Product Description

Frozen fish blocks are rectangularly shaped masses made from a single species of fish flesh. They are made from fillets or fillet pieces that are either skin-on and scaled or skinless. Blocks processed from skin-on fish flesh should be so labeled. The blocks should not contain minced or comminuted fish flesh. The blocks should not be made by restructuring (reworking) pieces of fish blocks into the shape of a fish block.

Dehydration is a defect that refers to loss of moisture from the surface of a fish block during frozen storage. Affected areas have a whitish appearance.

Moderate dehydration masks the surface color of the product and affects more than 5 percent up to and including 15 percent of the surface area. If more then 15 percent of the surface area is affected, each additional 15 percent of surface area affected is another instance. Moderate dehydration can be readily removed by scraping with a blunt instrument.

Excessive dehydration masks the normal flesh color and penetrates the product. It affects more than 5 percent up to and including 10 percent of the surface area. If more than 10 percent of the surface area is affected, each additional 10 percent of surface area affected is another instance. Excessive dehydration requires a knife or other sharp instrument to remove.

Uniformity of block size. This defect refers to the degree of conformity to the declared size. It includes

deviations from the standard length, width or thickness. Only one deviation for each dimension should be counted.

Moderate means a deviation of length and width of 1/8 inch (0.32 cm) or more up to and including 1/4 inch (0.64 cm). A deviation of thickness of 1/16 inch (0.16 cm) or more up to and including inch 1/8 (0.32 cm).

Excessive means if over ¼ inch (0.64 cm), each additional inch (0.32 cm) of length and width is another instance. If over (0.32 cm), each additional 1/16 inch (0.16 cm) of thickness is another instance.

Underweight refers to underweight deviations from the stated weight.

Slight means from 0.1 ounce (2.84 g) up to and including 1.0 ounce (28.35 g).

Moderate means over 1.0 ounce (28.35 g) up to and including 4.0 ounces (113.4 g).

Excessive means if over 4.0 ounces (113.4 g), each additional 1.0 ounce (28.35 g) is another instance.

An acceptable edge angle is an angle formed by two adjoining surfaces whose apex (deviation from 90 degrees) is within 0.95 cm off a carpenter's square placed along its surfaces. An acceptable corner angle is an angle formed by three adjoining surfaces whose apex is within 0.95 cm of a carpenter's square.

Improper fill is a defect that refers to voids, air packets, ice pockets, ragged edges, bumps, depressions, damage, and embedded packaging material, each of which is greater than 1/8 inch (0.32 cm) in depth, and which would result in product loss after cutting. It is estimated by determining the minimum number of 1-ounce (28.35 g) model units that could be affected adversely. For the purpose of estimating product loss, the 1-ounce (28.35 g) model unit should have the dimensions $4 \times 1 \times 5/8$ inch ($10.16 \times 2.54 \times 1.59$ cm). The total number of model units that would be affected adversely is the number of instances.

Belly flaps (napes) may be either loose or attached to a fillet or part of a fillet. The maximum amount of belly flaps should not exceed 15 percent by declared weight of the block if this amount does exceed 15 percent, each additional 5 percent by declared weight is another instance.

Each lump or mass of clotted blood greater than 3/16 inch (0.48 cm) up to and including 3/8 inch (0.95 cm) in any dimension is an instance of a blood spot. If a blood spot is larger than 3/8 inch (0.95 cm), each additional 3/16 (0.48 cm) is another instance.

Bruises include distinct, unnatural, dark, reddish, grayish, or brownish off-colors due to diffused blood. Each instance is each bruise larger than 0.5 square inch (3.32 cm^2) and less than 1.5 square inch (9.68 cm^2). For each bruise 1.5 square inch (9.68 cm^2) or larger, each additional complete 1.0 square inch (6.45 cm^2) is another instance.

Discoloration refers to deviations from reasonably uniform color characteristics of the species used, such as melanin deposits, yellowing, rusting or other kinds of discoloration of the fish flesh.

Moderate discoloration is a noticeable but moderate degree which is greater than 0.5 square inch (3.23 cm^2) up to and including 1.5 square inch (9.68 cm^2) is one instance. If the discoloration is greater than 1.5 square inch (9.68 cm^2), each additional complete 1.0 square inch (6.45 cm^2) is another instance.

An excessive degree of discoloration is greater than 0.5 square inch (3.23 cm^2) up to and including 1.5 square inch (9.68 cm^2). If the discoloration is greater than 1.5 square inch (9.68 cm^2) each additional complete 1.0 square inch (6.45 cm^2) is another instance.

Viscera and roe refer to any portion of the internal organs. Each occurrence of viscera and roe is an instance. Lace (frill) is a piece of tissue adhering to the edge of a flatfish (Order Pleuronectifonnes) fillet. For each lace, each 1/2 inch (1.27 cm) is an instance.

In skinless fish blocks, each piece of skin larger than 0.5 square inch (3.23 cm^2) up to and including 1.0 square inch (6.45 cm^2) is an instance. For each piece of skin that is larger than 1.0 square inch (6.45 cm^2), each additional complete 0.5 square inch (3.23 cm^2) in area is another instance. For pieces of skin smaller than 0.5 square inch (3.23 cm^2), the number of 0.5-square-inch (3.23 cm^2) squares fully or partially occupied after collecting these pieces on a grid is the number of instances.

Each piece of membrane (black belly lining) larger than 0.5 square inch (3.23 cm^2) up to and including 1.5 square inch (9.68 cm^2) is an instance. For pieces of membrane (black belly lining) that are larger than 1.5 square inch (9.68 cm^2), each additional complete 0.5 square inch (3.23 cm^2) in area is another instance.

For skin-on fillets that have been scaled, an instance is an area of scales over 0.5 square inch (3.23 cm^2) up to and including 1.5 square inch (9.68 cm^2). If the area is greater than 1.5 square inch (9.68 cm^2), each additional complete 1.0 square inch (8.45 cm^2) is another instance. Loose scales are counted and instances are deducted in the same manner as for skinless fillets.

For skinless fillets, the first five to ten loose scales is an instance. If there are more than ten loose scales, each additional complete count of five loose scales is another instance.

Any harmless material not derived from fish, such as packaging material, is foreign material. Each occurrence is an instance.

Bones (including pin bone and fin bone):

(i) Each bone defect to a bone or part of a bone whose maximum profile is 3/16 inch (0.48 cm) or more in length, or at least 1/32 inch (0.08 cm) in shaft diameter or width, or, for bone chips, a longest dimension of at least 3/16 inch (0.48 cm).

(ii) An excessive degree of bone defect is each bone whose maximum profile can not be fitted

into a rectangle, drawn on a flat, solid surface, that has a length of 1 3/16 inch (3.02 cm) and a width of ? inch (0.95 cm).

Fins or part fins is a defect that refers to two or more bones connected by membrane, including internal or external bones, or both, in a cluster.

(i) Moderate occurrence: Connected by membrane in a cluster, no internal bone.
(ii) Excessive occurrence: Connected by membrane in a cluster with internal bone.

Parasites are of two types:

(i) Metazoan parasites. Each such parasite or fragment of such a parasite that is detected is an instance.
(ii) Parasitic copepods. Each such parasite or a fragment of such a parasite that is detected is an instance.

Texture means that the cooked product has the textural characteristics of the indicated species of fish. It does not include any abnormal textural characteristics such as mushy, soft, gelatinous, tough, dry or rubbery.

(i) Moderate means moderately abnormal textural characteristics.
(ii) Excessive means excessively abnormal textural characteristics.

V. FROZEN MINCED FISH BLOCKS

A. Product Description

Frozen minced fish blocks are uniformly shaped masses of cohering minced fish flesh. A block may contain flesh from a single species or a mixture of species with or without food additives. The minced flesh consists entirely of mechanically separated fish flesh processed and maintained in accordance with good commercial practice. This minced flesh is made entirely from species which are known to be safe and suitable for human consumption.

B. Product Forms

Types are as follows.
Unmodified means food additives are used.

(i) Single species
(ii) Mixed species

Modified means contains food additives.

(i) Single species
(ii) Mixed species

Color classifications include:

(1) White
(2) Light
(3) Dark

Texture is described as:

(1) Coarse—Flesh has a fibrous consistency.
(2) Fine—Flesh has a partially fibrous consistency because it is a mixture of small fibers and paste.
(3) Paste/Puree—Flesh has no fibrous consistency.

C. Definitions of Defects

Deteriorative color refers to discoloration from the normal characteristics of the material used. Deterioration can be due to yellowing of fatty material, to browning of blood pigments, or other changes.

(i) Slight deteriorative discoloration—refers to a color defect that is slightly noticeable but does not seriously affect the appearance, desirability, or eating quality of the product.
(ii) Moderate deteriorative discoloration—refers to a color defect that is conspicuously noticeable but does not seriously affect the appearance, desirability, or eating quality of the product.
(iii) Excessive deteriorative discoloration—refers to a defect that is conspicuously noticeable and that seriously affects the appearance, desirability, or eating quality of the product.

Dehydration refers to a loss of moisture from the surfaces of the product during frozen storage.

(i) Slight dehydration—is surface color masking, affecting more than 5 percent of the area, which can be readily removed by scraping with a blunt instrument.
(ii) Moderate dehydration—is deep color masking penetrating the flesh, affecting less than 5 percent of the area, and requiring a knife or other sharp instrument to remove.
(iii) Excessive dehydration—is deep color masking penetrating the flesh, affecting more than 5 percent of the area, and requiring a knife or other sharp instruments to remove.

Uniformity of size refers to the degree of conformity to the declared contracted dimensions of the blocks. A deviation is considered to be any deviation from the contracted length, width, or thickness; or from the average dimensions of the blocks, physically determined, if no dimensions are contracted. Only one deviation from each dimension may be assessed. Two readings for length, three

readings for width, and four readings for thickness will be measured.

(i) Slight—two or more deviations from declared or average length, width, and thickness up to ± 1/8 inch.
(ii) Moderate—two or more deviations from declared or average length, width, and thickness from ± 1/8 inch to ± xx inch (variable, depending on products).
(iii) Excessive—two or more deviations from declared or average length, width, and thickness over ± 3/8 inch.

Uniformity of weight refers to the degree of conformity to the declared weight. Only underweight deviations are assessed.

(i) Slight—any minus deviation of not more than 2 ounces.
(ii) Excessive—any minus deviation over 2 ounces.

An acceptable edge angle is an angle formed by two adjoining surfaces of the fish block whose apex is within 3/8 inch of a carpenter's square placed along the surfaces of the block. For each edge angle, three readings will be made and at least two readings must be acceptable for the whole edge angle to be acceptable. An acceptable corner angle is an angle formed by 3 adjoining surfaces whose apex is within 3/8 inch of the apex of a carpenter's square placed on the edge surfaces. Any edge or corner angle which fails to meet these measurements is unacceptable.

(i) Slight—two unacceptable angles.
(ii) Moderate—three unacceptable angles.
(iii) Excessive—four or more unacceptable angles.

Improper fill refers to surface and internal air or ice voids, ragged edges, or damage. Improper fill is measured as the minimum number of 1-ounce units that would be adversely affected when the block is cut. For this purpose, the dimensions of a 1-ounce unit are 4 × 1 × 5/8 inch.

(i) Slight—1 to 3 units adversely affected.
(ii) Excessive—over 3 units adversely affected.

Blemishes refer to pieces of skin, scales, blood spots, nape (belly) membranes (regardless of color), or other harmless extraneous material. One instance means that the area occupied by a blemish or blemishes is equal to a 1/4 inch square. Instances are prorated on a per pound basis.

(i) Slight—5 to 15 instances per pound.
(ii) Moderate—more than 15 but less than 30 instances per pound.
(iii) Excessive—30 or more instances per pound.

Bones refer to any objectionable bone or piece of bone that is 1/4 inch or longer and is sharp and rigid. Perceptible bones should also be checked by their grittiness during the normal evaluation of the texture of the cooked product (10). Bones are prorated on a five pound sample unit basis.

(i) Slight—1 to 2 bones per five pound sample unit.
(ii) Moderate—3 to 4 bones per five pound sample unit.
(iii) Excessive—over 4 bones, but not to exceed 10 bones, per five pound sample unit.

Flavor and odor are evaluated organoleptically by smelling and tasting the product after it has been cooked.

Good flavor and odor (essential requirements for a Grade A product) means that the cooked product has the flavor and odor characteristic of the indicated species of fish and is free from staleness, bitterness, rancidity, and off-flavors and off-odors of any kind.

Reasonably good flavor and odor (minimum requirements of Grade B product) means that the cooked product is moderately absent of flavor and odor characteristic of the indicated species. the product is free from rancidity, bitterness, staleness, and off-flavors and off-odors of any kind.

Minimal acceptable flavor and odor (minimum requirements of a Grade C product) means that the cooked product has moderate storage induced flavor and odor, but is free from any objectionable off-flavors and off-odors that may be indicative of spoilage or decomposition.

Texture defects are judged on a sample of the cooked fish.

(i) Slight—flesh is fairly firm, only slightly spongy or rubbery. It is not mushy. There is no grittiness due to bone fragments.
(ii) Moderate—flesh is mildly spongy or rubbery. Slight grittiness may be present due to bone fragments.
(iii) Excessive—flesh is definitely spongy, rubbery, very dry, or very mushy. Moderate grittiness may be present due to bone fragments.

D. Additives

Minced fish blocks may be modified with food additives as necessary to stabilize product quality in accordance with the federal requirements.

The fish material should be processed and maintained in accordance with federal hygiene requirements.

VI. FROZEN RAW FISH PORTIONS

A. Description of the Product

The product described in this section consists of clean, wholesome, shaped masses of cohering pieces (not

ground) of fish flesh. The fish portions are cut from frozen fish blocks, and are packaged in accordance with good manufacturing practice. They are maintained at temperatures necessary for the preservation of the product. All fish portions in an individual package are prepared from the flesh of one species of fish.

B. Style

Style I, Skinless portions, means portions prepared from fish blocks which have been made with skinless fillets.

Style II, Skin-on portions, means portions prepared from fish blocks which have been made from demonstrably acceptable skin-on fillets.

C. Types

Type I, Uniform shaped, means all portions in the sample are uniformly shaped.

Type II, Specialty cut, is all portions not covered in Type I.

Dehydration refers to the presence of dehydrated (water-removed) tissue in the portions. Slight dehydration is surface dehydration which is not colormasking. Deep dehydration is color masking and cannot be removed by scraping with a blunt instrument.

Uniformity of size refers to the degree of uniformity in length and width of the frozen portions. Deviations are measured from the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest in the sample.

Uniformity of weight refers to the degree of uniformity of the weights of portions. Uniformity is measured by the combined weight of the two heaviest portions divided by the combined weight of the two lightest portions in the sample. No deductions are made for weight ratios less than 1.2 for Type I.

Blemishes refers to skin (except for Style II), blood spots or bruises, objectionable dark fatty flesh, or extraneous material. Instances of blemishes refer to each occurrence measured by placing a plastic grid marked off in 1/4-inch squares 1/16 square inch) over the defect area. Each square is counted as 1 whether it is full or fractional.

Bones means the presence of potentially harmful bones in a portion. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

Texture defects of the fish flesh and texture of skin in Style II refers to the absence of the normal textural properties of the cooked fish flesh and to the absence of tenderness of the cooked skin in Style II.

Normal textural properties of cooked fish flesh are tenderness, firmness, and moistness without excess water. Texture defects of the cooked flesh are dryness, mushiness, toughness, and rubberiness. Texture defects of the cooked skin in Style II are mushiness, rubberiness, toughness, and stringiness.

D. General Definitions

Small (overall assessment) refers to a condition that is noticeable but is only slightly objectionable.

Large (overall assessment) refers to a condition that not only is noticeable but is seriously objectionable.

Minor (individual assessment) refers to a defect that slightly affects the appearance and/or utility of the product.

Major (individual assessment) refers to a defect that seriously affects the appearance and/or utility of the product.

The net weight of the portions if glazed should be determined by the following method:

(i) Weigh the portions with the glaze intact, which gives the gross weight.
(ii) Thaw the glaze from the surfaces of the product with flowing tap water.
(iii) Gently wipe off the excess water from the surfaces with a single water saturated paper towel.
(iv) Weigh the deglazed portions, which gives the net weight.

VII. FROZEN RAW BREADED FISH STICKS

A. Description of the Product

Frozen raw breaded sticks are clean, wholesome, rectangular-shaped unglazed masses of cohering pieces (not ground) of fish flesh coated with breading. The sticks are cut from frozen fish blocks; are coated with a suitable, wholesome batter and breading; are packaged; and frozen in accordance with good commercial practice. They are maintained at temperatures necessary for preservation of the product. Frozen raw breaded fish sticks weigh up to and including 1½ ounces; are at least 3/8 inch thick; and their largest dimension is at least 3 times the next largest dimension. All sticks in an individual package are prepared from the flesh of one species of fish.

Frozen raw breaded fish sticks should contain 72 percent by weight of fish flesh determined by the official end-product method. Fish flesh content may be determined by the on-line method provided, That the results are consistent with the fish flesh content requirement of 72 percent by weight when verified by the official end-product method. Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors which may affect the relative fish flesh content.

B. Definitions

Selection of the sample unit: The sample unit should consist of 10 frozen raw breaded fish sticks taken at random

from one or more packages as required. The fish sticks are spread out on a flat pan or sheet and are examined.

Examination of sample, frozen state:

Condition of package refers to the presence in the package of loose breading and/or loose frost.

Ease of separation refers to the difficulty of separating sticks from each other or from packaging material that are frozen together during the freezing.

Broken stick means a stick with a break or cut equal to or greater than one-half the width of the stick.

Damaged stick means a stick that has been mashed, physically or mechanically injured, misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of squares ¼ inch (that is, squares with an area of 1/16 square inch each) to measure the area of the stick affected. Deductions are not made for damage less than 1/16 square inch.

Uniformity of size refers to the degree of uniformity in length and width of the frozen sticks. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest. Deductions are not made for overall deviations in length or width up to 1/4 inch.

Uniformity of weight refers to the degree of uniformity of the weights of the sticks. Uniformity is measured by the combined weight of the two heaviest sticks divided by the combined weight of the two lightest sticks. No deductions are made for weight ratios less than 1.15.

Cooked state means the state of the product after cooking in accordance with the instructions accompanying the product. However, if specific instructions are lacking, the product for inspection is cooked as follows: Transfer the product, while still in frozen state, into a wire mesh fry basket large enough to hold the fish sticks in a single layer and cook by immersing 2–3 minutes in 375°F. liquid or hydrogenated cooking oil. After cooking, allow the fish sticks to drain 15 seconds and place the fish sticks on a paper napkin or towel to absorb excess oil.

Examination of sample, cooked state:

Distortion refers to the degree of bending of the long axis of the stick. Distortion is measured as the greatest deviation from the long axis. Deductions are not made for deviations of less than 1/4 inch.

Coating defects refers to breaks, lumps, ridges, depressions, blisters, or swells and curds in the coating of the cooked product. Breaks in the coating are objectionable bare spots through which the fish flesh is plainly visible. Lumps are objectionable outcroppings of breading on the stick surface.

Ridges are projections of excess breading at the edges of the fish flesh.

Depressions are objectionable visible voids or shouldow areas which are lightly covered by breading. Blisters are measured by the swelling or exposed area in the coating resulting from the bursting or breaking of the coating. Curd refers to crater-like holes in the breading filled with coagulated albumin. Instances of these defects are measured by a plastic grid marked off in ¼-inch squares (1/16 square inch). Each square is counted as 1 whether it is full or fractional.

Blemishes refers to skin, blood spots or bruises, objectionable dark fatty flesh, or extraneous material. Instances of blemishes refer to each occurrence measured by placing a plastic grid market off in ¼-inch squares (1/16 square inch) over the defect area. Each square is counted as 1 whether it is full or fractional.

Bones means the presence of potentially harmful bones in a stick. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

Texture defects of the coating refers to the absence of the normal textural properties of the coating which are crispness and tenderness. Coating texture defects are dryness, sogginess, mushiness, doughyness, toughness, pastiness as sensed by starchiness or other sticky properties felt by mouth tissues and/or mealiness.

Texture defects of the fish flesh refers to the absence of the normal textural properties of the cooked fish flesh which are tenderness, firmness, and moistness without excess water. Texture defects of the flesh are dryness, mushiness, toughness, and rubberiness.

VIII. FROZEN RAW BREADED FISH PORTIONS

A. Description of the Product

Frozen raw breaded portions are clean, wholesome, uniformly shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading. The portions are cut from frozen fish blocks; are coated with a suitable, wholesome batter and breading; and are packaged and frozen in accordance with good commercial practice. They are maintained at temperatures necessary for the preservation of the product. Frozen raw breaded fish portions weigh more than 1-1/2 ounces, and are at least 3/8-inch thick. Frozen raw breaded fish portions contain not less than 75 percent, by weight, of fish flesh. All portions in an individual package are prepared from the flesh of one species of fish.

B. Styles

Style I, Skinless portions, are portions prepared from fish blocks which have been made with skinless fillets.

Style II, Skin-on-portions, are portions prepared from fish blocks which have been made with demonstrably acceptable skin-on fillets.

Composition of the product.

(a) Frozen raw breaded fish portions should contain 75 percent by weight of fish flesh. Fish flesh content may be determined by the on-line method provided that the results are consistent with the fish flesh content requirement of 75 percent by weight, when verified by the official end-product method.
(b) Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors which may affect the relative fish flesh content.

Examination of sample, frozen state:

(1) Condition of package refers to the presence in the package of loose breading and/or loose frost.
(2) Ease of separation refers to the difficulty of separating the portions from each other or from the packaging material.

Broken portion means a portion with a break or cut equal to or greater than one-half the width or length of the portion.

Damaged portion means a portion that has been mashed, physically or mechanically injured. misshaped, or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of squares ¼ inch × ¼ inch (that is, squares with an area of 1/16 square inch each) to measure the area of the portion affected. No deductions are made for damage of less than 1/16 square inch.

Uniformity of size refers to the degree of uniformity in length and width of the frozen portions. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest portions in the sample. Deductions are not made for overall deviations in length or width up to 1/4 inch.

Uniformity of weight refers to the degree of uniformity of the weights of the portions. Uniformity is measured by the combined weight of the two heaviest portions divided by the combined weight of the two lightest portions in the sample. No deductions are made for weight ratios less than 1.2.

Cooked state means the state of the product after being cooked in accordance with the instructions accompanying the product.

C. Examination of Sample, Cooked State

(1) Distortion refers to the degree of bending of the long axis of the portion. Distortion is measured as the greatest deviation from the long axis. Deductions are not made for deviations of less than ¼ inch.
(2) Coating defects refers to breaks, lumps, ridges, depressions, blisters, or swells and curds in the coating of the cooked product. Breaks in the coating are objectionable bare spots through which the fish flesh is plainly visible. Lumps are objectionable outcroppings of breading on the portion surface. Ridges are projections of excess breading at the edges of the portions.

Depressions are objectionable visible voids or shouldow areas that are lightly covered by breading. Blisters are measured by the swelling or exposed area in the coating resulting from the bursting or breaking of the coating. Curd refers to crater-like holes in the breading filled with coagulated white or creamy albumin. Instances of these defects are measured by a plastic grid marked off in 1/4-inch squares (1/16 square inch). Each square is counted as 1 whether it is full or fractional.

(3) Blemishes refers to skin (except for Style II), blood spots or bruises, objectionable dark fatty flesh, or extraneous material. Instances of blemishes refers to each occurrence measured by placing a plastic grid marked off in 1/4-inch squares (1/16 square inch) over the defect area. Each square is counted as 1 whether it is full or fractional.
(4) Bones means the presence of potentially harmful bones in a portion. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.
(5) Texture defects of the coating refers to the absence of the normal textural properties of the coating which are crispness and tenderness. Defects in coating texture are dryness, sogginess. mushiness, doughyness, toughness, pastyness, as sensed by starchiness or other sticky properties felt by mouth tissues and/or mealiness.
(6) Texture defects of the fish flesh and texture of skin in Style II refers to the absence of the normal textural properties of the cooked fish flesh and to the absence of tenderness of the cooked skin in Style II.

Normal textural properties of cooked fish flesh are tenderness, firmness, and moistness without excess water. Texture defects of the cooked fesh are dryness, mushiness, toughness, and rubberiness. Texture defects of the cooked skin in Style II are mushiness, rubberiness, toughness, and stringiness.

Minimum fish flesh content—End-product determination refers to the minimum percent, by weight, of the average fish flesh content of three frozen raw breaded portions (sample unit for fish flesh determination).

IX. FROZEN FRIED FISH STICKS

A. Description of the Product

Frozen fried fish sticks are clean, wholesome, rectangular-shaped unglazed masses of cohering pieces (not ground) of fish flesh coated with breading and partially cooked. The sticks are cut from frozen fish blocks; are coated with a suitable, wholesome batter and breading; are fried, packaged, and frozen in accordance with good manufacturing practices. They are maintained at temperatures necessary for preservation of the product. Frozen fried fish sticks weigh up to and including 1½ ounces; are at least three-eighths of an inch thick; and their largest dimension is at least three times the next largest dimension. All sticks in an individual package are prepared from the flesh of one species of fish.

Frozen fried fish sticks should contain 60 percent by weight of fish flesh. Fish flesh content may be determined by the on-line method provided, that the results are consistent with the fish flesh content requirement of 60 percent by weight, when verified by the official end-product method.

Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors which may affect the relative fish flesh content.

Definitions of factors for point deductions are as follows:

B. Examination of Sample, Frozen State

(1) Condition of package refers to the presence in the package of free excess oil and/or loose breading and/or loose frost.

(2) Ease of separation refers to the difficulty of separating sticks from each other or from packaging material that are frozen together after the frying operation and during the freezing.

(3) Broken stick means a stick with a break or cut equal to or greater than one-half the width of the stick.

(4) Damaged stick means a stick that has been mashed, physically or mechanically injured, misshaped or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of squares ¼ inch (that is, squares with an area of 1/16 square inch each) to measure the area of the stick affected. Deductions are not made for damage less than 1/16 square inch.

(5) Uniformity of size refers to the degree of uniformity in length and width of the frozen sticks. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest. Deductions are not made for overall deviations in length of width up to ¼ inch.

(6) Uniformity of weight refers to the degree of uniformity of the weights of the sticks. Uniformity is measured by the combined weight of the two heaviest sticks divided by the combined weight of the two lightest sticks. No deductions are made for weight ratios less than 1.15.

Examination of sample, cooked state:

Distortion refers to the degree of bending of the long axis of the stick. Distortion is measured as the greatest deviation from the long axis. Deductions are not made for deviations of less than 1/4 inch.

Coating defects refers to breaks, lumps, ridges, depressions, blisters, or swells and curds in the coating of the cooked product. Breaks in the coating are objectionable bare spots through which the fish flesh is plainly visible. Lumps are objectionable outcroppings of breading on the stick surface.

Ridges are projections of excess breading at the edges of the fish flesh.

Depressions are objectionable visible voids or shouldow areas which are lightly covered by breading. Blisters are measured by the swelling or exposed area in the coating resulting from the bursting or breaking of the coating. Curd refers to crater-like holes in the breading filled with coagulated albumin. Instances of these defects are measured by a plastic grid marked off in ¼-inch squares (1/16 square inch). Each square is counted as one whether it is full or fractional.

Blemishes refers to skin, blood spots, or bruises, objectionable dark fatty flesh, carbon specks or extraneous material. Instances of blemishes refers to each occurrence measured by placing a plastic grid marked off in ¼ inch squares (1/16 square inch) over the defect area. Each square is counted as one whether it is full or fractional.

Bones means the presence of potentially harmful bones in a stick. A potentially harmful bone is one that after being cooked is capable of piercing or hurting the palate.

Texture defects of the coating refers to the absence of the normal textural properties of the coating which are crispness and tenderness. Coating texture defects are dryness, sogginess, mushiness, doughiness, toughness, pastiness, as sensed by starchiness or other sticky properties felt by mouth tissues; oiliness to the degree of impairment of texture; and/or mealiness.

Texture defects of the fish flesh refers to the absence of normal textural properties of the cooked fish flesh, which are tenderness, firmness, arid moistness without excess water. Texture defects of the flesh are dryness, softness, toughness, and rubberiness.

X. FROZEN FRIED FISH PORTIONS

A. Description of the Product

Frozen fried fish portions are clean, wholesome, uniformly shaped, unglazed masses of cohering pieces (not ground) of fish flesh coated with breading and partially cooked. The portions are cut from frozen fish blocks; coated with a suitable, wholesome batter and breading; are fried, packaged, and frozen in accordance with good manufacturing practices. They are maintained at temperatures necessary for preservation of the product. Frozen fried fish portions weigh more than 11/2 ounces and are at least three-eighths of an inch thick. All portions in an individual package are prepared from the flesh of one species of fish.

Frozen fried fish portions should contain 65 percent by weight of fish flesh. Fish flesh content may be determined by the on-line method, provided, that the results are consistent with the fish flesh content requirement of 65 percent by weight, when verified by the official end-product method.

Production methods employed in official establishments should be kept relatively constant for each production lot so as to minimize variation in any factors which may affect the relative fish flesh content.

Ease of separation refers to the difficulty of separating portions from one another or from packaging material that are frozen together after the frying operation and during the freezing.

Broken portion means a portion with a break or cut equal to or greater than one-half the width or length of the portion.

Damaged portion means a portion that has been mashed, physically or mechanically injured, misshaped or mutilated to the extent that its appearance is materially affected. The amount of damage is measured by using a grid composed of squares 1/4 inch (that is, squares with an area of 1/16 square inch each) to measure the area of the portion affected. Deductions are not made for damage less than 1/16 square inch.

Uniformity of size refers to the degree of uniformity in length and width of the frozen portions. Deviations are measured from the combined lengths of the two longest minus the combined lengths of the two shortest and/or the combined widths of the two widest minus the combined widths of the two narrowest. Deductions are not made for overall deviations in length or width up to ¼ inch.

Uniformity of weight refers to the degree of uniformity of the weights of the portions. Uniformity is measured by the combined weight of the two heaviest portions divided by the combined weight of the two lightest portions. No deductions are made for weight ratios less than 1.20.

XI. FRESH AND FROZEN SHRIMP

A. Product Description

The products are clean wholesome shrimp that are fresh or frozen, raw or cooked. Product forms are:

1. Types

(1) Chilled, fresh (not previously frozen).
(2) Unfrozen, thawed (previously frozen).
(3) Frozen individually (IQF), glazed or unglazed.
(4) Frozen solid pack, glazed or unglazed. (b) Styles. (1) Raw (uncoagulated protein).

2. Blanched (Parboiled)

Blanched means heated for a period of time such that the surface of the product reaches a temperature adequate to coagulate the protein.

Cooked-heated for a period of time such that the thermal center of the product reaches a temperature adequate to coagulate the protein.

3. Market Forms

(1) Heads on (head, shell, tail fins on).
(2) Headless (only head removed: shell, tail fins on).
(3) Peeled, undeveined, round, tail on (all shell removed except last shell segment and tail fins, with segments unslit].
(4) Peeled, undeveined, round, tail off (all shell and tail fins removed, with segments unslit).
(5) Peeled and deveined, round, tail on (all shell removed except last shell segment and tail fins, with segments shouldowly slit to last segment).
(6) Peeled and deveined, round, tail off (all shell and tail fins removed, with segments shouldowly slit to last segment).
(7) Peeled and deveined, fantail or butterfly, tail on (all shell removed except last shell segment and tail fins,with segments deeply slit to last segment).
(8) Peeled and deveined, fantail or butterfly, tail off (all shell and tail fin removed, with segments deeply slit to last segment).
(9) Peeled and deveined, western (all shell removed except last shell segment and tail fins, with segments split to fifth segment and vein removed to end of cut).
(10) Other forms of shrimp as specified and so designated on the label.

B. Examination in the Frozen State

Dehydration refers to a general drying of the shrimp flesh that is noticeable after any glaze and shell are removed. It

includes any detectable change from the normal characteristic, bright appearance of freshly caught, properly iced or properly processed shrimp.

Slight dehydration means scarcely noticeable drying of the shrimp flesh that will not affect the sensory quality of the sample.

Moderate dehydration means conspicuous drying of the shrimp flesh that will not seriously affect the sensory quality of the sample.

Excessive dehydration means conspicuous drying that will seriously affect the sensory quality of the sample.

Examination in the fresh or thawed state indicates the following.

Uniformity of size refers to the degree of uniformity of the shrimp in the container to determine their conformity to the declared count.

Black spots, improperly headed (throats), and improperly cleaned ends refer to the presence of any objectionable black or darkened area that affects the desirability or sensory quality of the shrimp, whether the market form is shell-on or peeled. Objectionable black spot refers to more than three instances of penetrating black spot that is visible but difficult to measure because of its small size (approximately the size of a pencil point): or any areas larger than a pencil point that penetrates the flesh: or aggregate areas of non-penetrating surface black spot on the shell or membrane that is equal to or greater than 1/3 the area of the smallest segment.

Assessments are made on individual shrimp:

Throats are those portions of flesh and/or extraneous material from the head (cephalothorax) that remain attached to the first segment after heading.

Pieces of shrimp, broken or damaged shrimp:

Piece means for a count of 70 or less unglazed shrimp per pound (0.45 kg) any shrimp that has fewer than five segments, with or without tail fins attached: or, for a count of more than 70 unglazed shrimp per pound (0.45 kg), any shrimp that has fewer than four segments: or, any whole shrimp with a break in the flesh greater than 2/3 of the thickness of the shrimp where the break occurs.

Broken shrimp means a shrimp having a break in the flesh greater than 1/3 of the thickness of the shrimp.

Damaged shrimp means a shrimp that is crushed or mutilated so as to materially affect its appearance or useability.

Unusable material includes the following:

Legs refer to walking legs only, whether attached or not attached to the body (heads-on market from excepted).

Loose shell and antennae are any pieces of shell or antennae that are completely detached from the shrimp.

Flipper refers to any detached tail fin with or without the last shell segment attached, with or without flesh inside.

Extraneous material means any harmless material in a sample unit that is not shrimp material.

Unacceptable shrimp refers to abnormal or diseased shrimp.

Head refers to the cephalothorax, except for heads-on shrimp.

Inadvertently peeled and improperly peeled shrimp refer to the presence or absence of head, shell segment, swimmeret, or tail fin, which should or should not have been removed of certain market forms. (Shell-on shrimp with tail fins and/or telson missing is inadvertently peeled, but if the last segment of flesh is missing, the shrimp is damaged.)

Improperly deveined shrimp refers to the presence of dark vein (alimentary canal) containing sand or sediment or roe which should have been removed for peeled and deveined market forms. For shrimp of 70 count per pound (0.45 kg) or less, aggregate areas of dark vein or roe is a defect that are longer than one segment is a defect. For shrimp of 71 to 500 count per pound (0.45 kg), aggregate areas of dark vein or roe defect that are longer than two segments are a defect.

Note: This does not pertain to the last segment. For shrimp of over 500 count per pound (0.45 kg), dark vein or roe of any length is not a defect.

Examination in the cooked state:

Texture. The texture of cooked shrimp should be firm, slightly resilient but not tough, moist but not mushy. Texture as a defect refers to an undesirable toughness, dryness, or mushiness which deviated from the normal characteristics of the species when freshly caught, properly processed, and cooked.

Slight means slightly tough, dry, but not mushy.

Moderate means moderately tough, dry or mushy.

Excessive means excessively tough, very dry or very mushy.

XII. FROZEN RAW BREADED SHRIMP

The U.S. FDA has provided the following standards for frozen raw breaded shrimp.

A. Description

Frozen raw breaded shrimp are whole, clean, wholesome, headless, peeled shrimp which have been deveined where applicable of the regular commercial species, coated with a wholesome, suitable batter and/or breading. Whole shrimp consist of five or more segments of unmutilated shrimp flesh. They are prepared and frozen in accordance with good manufacturing practice and are maintained at temperatures necessary for the preservation of the product.

Frozen raw breaded shrimp is the food prepared by coating one of the optional forms of shrimp with safe and suitable batter and breading ingredients. The food is frozen.

The food tests not less than 50 percent of shrimp material as determined by prescribed method

The term shrimp means the tail portion of properly prepared shrimp of commercial species. Except for composite units, each shrimp unit is individually coated. The optional forms of shrimp are:

(1) Fantail or butterfly: Prepared by splitting the shrimp; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.
(2) Butterfly, tail off: Prepared by splitting the shrimp; tail fins and all shell segments are removed.
(3) Round: Round shrimp, not split; the shrimp are peeled, except that tail fins remain attached and the shell segment immediately adjacent to the tail fins may be left attached.
(4) Round, tail off: Round shrimp, not split; tail fins and all shell segments are removed.
(5) Pieces: Each unit consists of a piece or a part of a shrimp; tail fins and all shell segments are removed.

The above information is categorized as follows.

1. Styles

Style I, Regular Breaded Shrimp, are frozen raw breaded shrimp containing a minimum of 50 percent of shrimp material.

Style II, Lightly Breaded Shrimp, are frozen raw breaded shrimp containing a minimum of 65 percent of shrimp material.

2. Types

Type I-Breaded fantail shrimp subtypes are:

Subtype A. Split (butterfly) shrimp with the tail fin and the shell segment immediately adjacent to the tail fin.

Subtype B. Split (butterfly) shrimp with the tail fin but free of all shell segments.

Subtype C. Split (butterfly) shrimp without attached tail fin or shell segments.

Type II-Breaded round shrimp subtypes are:

Subtype A. Round shrimp with the tail fin and the shell segment immediately adjacent to the tail fin.

Subtype B. Round shrimp with the tail fin but free of all shell segments.

Subtype C. Round shrimp without attached tail fin or shell segments.

Type III-Breaded split shrimp.

3. Definitions and Methods of Analysis

(a) Fantail shrimp:

This type is prepared by splitting and peeling the shrimp except that for Subtype A the tail fin remains attached and the shell segment immediately adjacent to the tail fin remains attached.

For Subtype B, the tail fin remains, but the shrimp are free of all shell segments.

For Subtype C, the shrimp are free of tail fins and all shell segments.

(b) Round shrimp: This type is the round shrimp, not split. The shrimp are peeled except that for Subtype A, the tail fin remains attached and the shell segment immediately adjacent to the tail fin remains attached.

For Subtype B, the tail fin remains, but the shrimp are free of all shell segments.

For Subtype C, the shrimp are free of all shell segments and tail fins.

B. Composite Units

Each unit consists of two or more whole shrimp or pieces of shrimp, or both, formed and pressed into composite units prior to coating; tail fins and all shell segments are removed; large composite units, prior to coating, may be cut into smaller units.

The batter and breading ingredients referred to are the fluid constituents and the solid constituents of the coating around the shrimp. These ingredients consist of suitable substances which are not food additives as defined by regulations. If they are food additives as so defined, they are used in conformity with established regulations. Batter and breading ingredients that perform a useful function are regarded as suitable, except that artificial flavorings, artificial sweeteners, artificial colors, and chemical preservatives, other than those specifically permitted are not suitable ingredients of frozen raw breaded shrimp. Chemical preservatives that are suitable are:

1. Ascorbic acid, which may be used in a quantity sufficient to retard development of dark spots on the shrimp; and
2. The antioxidant preservatives listed in the regulations that may be used to retard development of rancidity of the fat content of the food, in amounts within the limits prescribed.

The label should name the food, as prepared from each of the optional forms of shrimp specified, and following the numbered sequence of the following data

(1) "Breaded fantail shrimp." The word "butterfly" may be used in lieu of "fantail" in the name.
(2) "Breaded butterfly shrimp, tail off."

(3) "Breaded round shrimp."
(4) "Breaded round shrimp, tail off."
(5) "Breaded shrimp pieces."
(6) Composite units:

If the composite units are in a shape similar to that of breaded fish sticks the name is "Breaded shrimp sticks"; if they are in the shape of meat cutlets, the name is "Breaded shrimp cutlets."

If prepared in a shape other than that of sticks or cutlets, the name is "Breaded shrimp _____," the blank to be filled in with the word or phrase that accurately describes the shape, but which is not misleading.

The word "prawns" may be added in parentheses immediately after the word "shrimp'' in the name of the food if the shrimp are of large size; for example, "Fantail breaded shrimp (prawns)." If the shrimp are from a single geographical area, the adjectival designation of that area may appear as part of the name; for example, "Breaded Alaskan shrimp sticks.''

The names of the optional ingredients used should be listed on the principal display panel or panels of the label with such prominence and conspicuousness as to render them likely to be read and understood by the ordinary individual under customary conditions of purchase. If a spice that also imparts color is used, it should be designated as "spice and coloring,'' unless the spice is designated by its specific name. If ascorbic acid is used to retard development of dark spots on the shrimp, it should be designated as "Ascorbic acid added as a preservative'' or "Ascorbic acid added to retard discoloration of shrimp.''

If any other antioxidant preservative is used, such preservative should be designated by its common name followed by the statement "Added as a preservative.''

Frozen raw lightly breaded shrimp complies with the provisions of frozen raw breaded shrimp except that it contains not less than 65 percent of shrimp material and that in the name prescribed the word "lightly'' immediately precedes the words "breaded shrimp.''

Factors affecting qualities that are measured on the product in the unbreaded or thawed debreaded state are degree of deterioration, dehydrations, sand veins, black spot, extra shell, extraneous material, and swimmerets.

Dehydration refers to the occurrence of whitish areas on the exposed ends of the shrimp (due to the drying of the affected area) and to a generally desiccated appearance of the meat after the breading is removed.

Deterioration refers to any detectable change from the normal good quality of freshly caught shrimp. It is evaluated by noting in the thawed product deviations from the normal odor and appearance of freshly caught shrimp.

Extraneous material consists of nonedible material such as sticks, seaweed, shrimp thorax, or other objects that may be accidentally present in the package.

Slight: Slight refers to a condition that is scarcely noticeable but does affect the appearance, desirability, and/or eating quality of breaded shrimp.

Moderate: Moderate refers to a condition that is conspicuously noticeable but that does not seriously affect the appearance, desirability, and/or eating quality of the breaded shrimp.

Marked: Marked refers to a condition that is conspicuously noticeable and that does seriously affect the appearance, desirability, and/or eating quality of the breaded shrimp.

Excessive: Excessive refers to a condition that is very noticeable and is seriously objectionable.

Halo: Halo means an easily recognized fringe of excess batter and breading extending beyond the shrimp flesh and adhering around the perimeter or flat edges of a split (butterfly) breaded shrimp.

Balling up: Balling up means the adherence of lumps of the breading material to the surface of the breaded coating, causing the coating to appear rough, uneven, and lumpy.

Holidays: Holidays means voids in the breaded coating as evidenced by bare or naked spots.

Damaged frozen raw breaded shrimp: Damaged frozen raw breaded shrimp means frozen raw breaded shrimp that have been separated into two or more parts or that have been crushed or otherwise mutilated to the extent that their appearance is materially affected.

Black spot: Black spot means any blackened area that is markedly apparent on the flesh of the shrimp.

Sand vein: Sand vein means any black or dark sand vein that has not been removed, except for that portion under the shell segment adjacent to the tail fin when present.

Extra shell: Extra shell means any shell segment(s) or portion thereof, contained in the breaded shrimp except the first segment adjacent to the tail fin for Type I, Subtype A, and Type II, Subtype A.

XIII. FROZEN RAW SCALLOPS

A. Description of the Product

Frozen raw scallops are clean, wholesome, adequately drained, whole or cutadductor muscles of the scallop of the regular commercial species. The portion of the scallop used should be only the adductor muscle eye whichcontrols the shell movement. Scallops should be washed, drained, packed, andfrozen in accordance with good manufacturing practices and are maintained attemperatures necessary for the preservation of the product. Only scallops of asingle species should be used within a lot.

1. Styles

Style I are solid pack scallops are frozen together into a solid mass.

(1) Substyle a. Glazed.
(2) Substyle b. Not glazed. (b) Style II. Individually quick frozen pack (IQF) scallops are individually quick frozen. Individual scallops can be separated without thawing.

(1) Substyle a. Glazed.
(2) Substyle b. Not glazed.

2. Types

(a) Type 1. Adductor muscle.
(b) Type 2. Adductor muscle with catch (gristle or sweet meat) portion removed.

Dehydration refers to the loss of moisture from the scallops surface during frozen storage. Small degree of dehydration is color-masking but can be easily scraped off. Large degree of dehydration is deep, color-masking, and requires a knife or other instrument to scrape it off.

Extraneous materials are pieces or fragments of undesirable material that are naturally present in or on the scallops and which should be removed during processing.

An instance of minor extraneous material includes but is not limited to each occurrence of intestines, seaweed, etc., and each aggregate of sand and grit up to ½-inch square and located on the scallop surface. Deduction points should be assessed for additional instances of intestines, seaweed, etc., and aggregates of sand and grit up to ½-inch square.

An instance of major extraneous material includes but is not limited to each instance of shell or aggregate of embedded sand or other extraneous embedded material that affects the appearance or eating quality of the product.

Texture refers to the firmness, tenderness, and moistness of the cooked scallop meat, which is characteristic of the species.

Net weight means the total weight of the scallop meats within the package after removal of all packaging materials, ice glaze, or other protective materials.

XIV. FROZEN RAW BREADED SCALLOPS FROZEN FRIED SCALLOPS

A. Product Description

Frozen raw breaded scallops are:

(1) Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading;
(2) Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation; and
(3) Composed of a minimum of 50 percent by weight of scallop meat.

Frozen fried scallops.

Frozen fried scallops are:

(1) Prepared from wholesome, clean, adequately drained, whole or cut adductor muscles of the scallop of the regular commercial species, or scallop units cut from a block of frozen scallops that are coated with wholesome batter and breading;
(2) Precooked in oil or fat;
(3) Packaged and frozen according to good commercial practice and maintained at temperatures necessary for preservation; and
(4) Composed of a minimum of 50 percent by weight of scallop meat.

The styles of frozen raw breaded scallops and frozen fried scallops include the following.

Style I Random pack:

Scallops in a package are reasonably uniform in weight and/or shape. The weight or shape of individual scallops is not specified.

Style II Uniform pack:

Scallops in a package consist of uniform shaped pieces that are of specified weight or range of weights.

1. Types

(a) Type 1. Adductor muscle.
(b) Type 2. Adductor muscle with catch (gristle or sweet meat) portion removed.

Appearance refers to the condition of the package and ease of separation in the frozen state and continuity and color in the cooked state.

"Condition of the package" refers to freedom from packaging defects and the presence in the package of oil, and/or loose breading, and/or frost. Deduction points are based on the degree of the improper condition as small or large.

"Ease of separation" refers to the difficulty of separating scallops that are frozen together after the frying operation and during freezing.

"Continuity" refers to the completeness of the coating of the product in the cooked state. Lack of continuity is exemplified by breaks, ridges and/or lumps of breading. Each 1/16 square inch area of any break, ridge, or lump of breading is considered an instance of lack of continuity. Individual breaks, ridges, or lumps of breading measuring less than 1/16 square inch are not considered objectionable. Deduction points are based on the percentage of the scallops within the package that contain small and/or large instances of lack of continuity.

Workmanship defects refer to the degree of freedom from doubled and misshaped scallops and extraneous material. The defects of doubled and misshaped scallops are determined by examining the frozen product, while the defects of extraneous materials are determined by

examining the product in the cooked state. Deduction points are based on the percentage by count of the scallops affected within the package.

Doubled scallops. Two or more scallops that are joined together during the breading and/or frying operations

Misshaped scallops. Elongated, flattened, mashed, or damaged scallop meats.

Extraneous material. Extraneous are pieces or fragments of undesirable material that are naturally present in or on the scallops and which should be removed during processing.

Examples of minor extraneous material include intestines, seaweed, and each aggregate of sand and grit within an area of 1/2-inch square.

Examples of major extraneous material include shell, aggregate of embedded sand or other extraneous embedded material that affects the appearance or eating quality of the product.

Texture in the cooked state

Texture of the coating:

Firm or crisp, but not tough, pasty, mushy, or oily

Moderately tough, pasty, mushy, or oily

Excessively tough, pasty, mushy, or oily

Texture of the scallop meat

Firm, but tender and moist

Moderately tough, dry, and/or fibrous or mushy

Excessively tough, dry, and/or fibrous or mushy

Character.

Character refers to the texture of the scallop meat and of the coating and the presence of gristle in the cooked state.

(1) *Gristle.* Gristle (type 2 only) is the tough elastic tissue usually attached to the scallop meat. Each instance of gristle is an occurrence.
(2) *Texture* refers to the firmness, tenderness, and moistness of the cooked scallop meat and to the crispness and tenderness of the coating of the cooked product. The texture of the scallop meat may be classified as a degree of mushiness, toughness, and fibrousness. The texture of the coating may be classified as a degree of pastiness, toughness, dryness, mushiness, or oiliness.

XV. FROZEN NORTH AMERICAN FRESHWATER CATFISH AND CATFISH PRODUCTS

A. Scope and Product Description

The descriptions apply to products derived from farm-raised, or from rivers and lakes, North American freshwater catfish of the following common commercial species and hybrids thereof:

(1) Channel catfish (*Ictalurus punctatus*)
(2) White catfish (*Ictalurus catus*)
(3) Blue catfish (*Ictalurus furcatus*)
(4) Flathead catfish (*Pylodictis olivaris*)

Fresh products will be packaged in accordance with good commercial practices and maintained at temperatures necessary for the preservation of the product. Frozen products will be frozen to 0°F (−18°C) at their center(thermal core) in accordance with good commercial practices and maintained at temperatures of 0°F (−18°C) or less.

The product may contain bones when the principle display panel clearly shows that the product contains bones.
Product presentation.

Catfish products may be presented and labeled as follows:

1. Types

(1) Fresh, or
(2) Frozen.

2. Styles

(1) Skin on, or
(2) Skinless.

Market forms include but are not limited to the following:

(1) Headed and gutted.
(2) Headed and dressed are headed and gutted usually with fins removed. This form may be presented with or without the dorsal spine and with or without the collar bone.
(3) Whole fillets are practically boneless pieces of fish cut parallel to the entire length of the backbone with the belly flaps and with or without the black membrane.
(4) Trimmed fillets are whole fillets without belly flaps.
(5) Fillet strips are strips of fillets weighing not less than ¾ ounce.
(6) Steaks are units of fish not less than 1 1/2 ounces in weight which are sawn or cut approximately perpendicular (30 degrees to 90 degrees) to the axial length or backbone. They have two reasonably parallel surfaces. The number of tail sections that may be included in the package must not exceed the number of fish cut per package).
(7) Nuggets are pieces of belly flaps with or without black membrane and weighing not less than ¾ ounce.

3. Bone Classifications

(1) Practically boneless fillet.
(2) Bone-in (fillet cut, with bones).

Dehydration applies to all frozen market forms. It refers to the loss of moisture from the surface resulting in a whitish, dry, or porous condition:

Slight: surface dehydration which is not color masking (readily removed by scraping) and affecting 3 to 10 percent of the surface area.

Moderate: deep dehydration which is color masking, cannot be scraped off easily with a sharp instrument, and affects more than one percent but not more than 10 percent of the surface area.

Excessive: deep dehydration which is color masking, and cannot be easily scraped off with a sharp instrument and affects more than 10 percent of the surface area.

Condition of the product applies to all market forms. It refers to freedom from packaging defects, cracks in the surface of a frozen product, and excess moisture (drip) or blood inside the package. Deduction points are based on the degree of this defect.

Slight refers to a condition that is scarcely oticeable but that does not affect the appearance, desirability or eating quality of the product.

Moderate refers to a condition that is conspicuously noticeable but that does not seriously affect the appearance, desirability, or eating quality of the product.

Excessive refers to a condition that is conspicuously noticeable and that does seriously affect the appearance, desirability or eating quality of the product.

Discoloration applies to all market forms. It refers to colors not normal to the species. This may be due to mishandling or the presence of blood, bile, or other substances.

Slight: 1/16 square inch up to and including one square inch in aggregate area.

Moderate: greater than one square inch up to and including 2 square inches in aggregate area.

Excessive: over 2 square inches in aggregate area. Also, each additional complete one square inch is again assessed points under this category.

Uniformity will be assigned in accordance with weight tolerances as follows:

Weight of portion: 0.75 to 4.16 ounces

Moderate: Over 1/8 ounce but not over 1/4 ounce above or below declared weight of portion

Excessive: In excess of 1/4 ounce above or below declared weight of portion 4.17 to 11.20 ounces

Moderate: Over 1/8 ounce but not over 1/2 ounce above or below declared weight of portion

Excessive: In excess of 1/2 ounce above or below declared weight of portion 11.21 to 17.30 ounces

Moderate: Over 1/8 ounce but not over 1/8 ounce above or below declared weight of portion

Excessive: In excess of 1/8 ounce above or below declared weight of portion

Skinning cuts apply to skinless market forms. It refers to improper cuts made during the skinning operation as evidenced by torn or ragged surfaces or edges, or gouges in the flesh which detract from a good appearance of the product.

Slight: 1/16 square inch up to and including 1 square inch in aggregate area.

Moderate: Over one square inch up to and including 2 square inches in aggregate area.

Excessive: Over 2 square inches in aggregate area. Also, each additional complete one square inch is again assessed points under this category.

Heading applies to the presence of ragged cuts or pieces of gills, gill cover, pectoral fins or collar bone after heading. Deduction points also will be assigned when the product is presented with the collar bone and it has been completely or partially removed.

Slight: 1/16 square inch up to and including one square inch in aggregate area.

Moderate: Over one square inch up to and including 2 square inches in aggregate area.

Excessive: Over 2 square inches in aggregate area. Also, each additional complete one square inch is again assessed points under this category.

Evisceration applies to all market forms. It refers to the proper removal of viscera, kidney, spawn, blood, reproductive organs, and abnormal fat (leaf). The evisceration cut should be smooth and clean. Deduction points are based on the degree of defect.

Slight: 1/16 square inch up to and including 1 square inch in aggregate area.

Moderate: Over 1 square inch up to and including 2 square inches in aggregate area.

Excessive: Over 2 square inches in aggregate area. Also, each additional complete one square inch is again assessed points under this category.

Fins refer to the presence of fins, pieces of fins or dorsal spines. It applies to all market forms except headed and gutted or headed and dressed catfish or catfish steaks. Deduction points also will be assigned when the product is intended to have the dorsal spine but it has been completely or partially removed.

Slight: Aggregate area up to including one square inch.

Moderate: Over one square inch area up to and including 2 square inches.

Excessive: Over 2 square inches in aggregate area. Also, each additional complete one square inch is again assessed points under this category.

Bones (including pin bone) apply to all fillet and nugget market forms. Each bone defect is a bone or part of a bone that is 3/16 inch or more at its maximum length or 1/32 inch or more at its maximum shaft width, or for bone chips, a length of at least 1/16 inch. An excessive bone defect is any bone which cannot be fitted into a rectangle, which has a length of 1 9/16 inch and a width of 1/8 inch. In market forms intended to contain bones, the

presence of bones will not be considered a physical defect.

Skin refers to the presence of skin on skinless market forms. For semi-skinned forms, a skin defect is the presence of the darkly pigmented outside layers. Points will be assessed for each aggregate area greater than 1/2 square inch up to and including one square inch.

Bloodspots refer to the presence of coagulated blood.

Bruises refer to softening and discoloration of the flesh. Both bloodspots and bruises apply to all market forms. Points will be assessed for each aggregate area of bloodspots or bruises greater than 1/2 square inch up to and including one square inch.

Foreign material refers to extraneous material, including packaging material, not derived from the fish that is found on or in the sample. Each occurrence will be assessed.

Texture applies to all market forms and refers to the presence of normal texture properties of the cooked fish flesh, i.e., tender, firm, and moist without excess water. Texture defects are described as dry, tough, mushy, rubbery, watery, and stringy.

Moderate: Noticeably dry, tough, mushy, rubbery, watery, stringy.

Excessive: Markedly dry, tough, mushy, rubbery, watery, stringy.

ACKNOWLEDGMENT

The information in this chapter is based on *Food Safety Manual*, published and copyrighted by Science Technology System, West Sacramento, California, 2004. Used with permission.

39 Freezing Seafood and Seafood Products: Principles and Applications

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CONTENTS

GENERAL PRINCIPLES FOR THE FREEZING, STORAGE AND THAWING OF SEAFOOD

I. INTRODUCTION

Fish and shellfish are perishable and, as a result of a complex series of chemical, physical, bacteriological, and histological changes occurring in muscle, easily spoil after harvesting. These interrelated processes are usually accompanied by the gradual loss or development of different compounds which affect fish quality. The quality changes are highly influenced by many factors, the most important of which is temperature. If fresh fish is not properly stored, exposure to ambient temperature can cause serious deterioration in fish quality. Commercially, icing or chilling continues to play a major role in slowing down bacterial and enzymatic degradation of fish muscle. However, this process is not designed to totally eliminate changes in quality, since it only offers protection for 2–3 weeks, depending on the species.

Freezing preservation of food is an excellent method of preservation with wide applications. Freezing inhibits the activity of food spoilage and food poisoning organisms and the low storage temperature greatly slows down the enzymatic and biochemical reactions which normally occur in unfrozen foods. Freezing accomplishes these objectives in two ways: the lowering of the temperature of the food and the removal of water by converting it into ice. Lowering the temperature to below freezing point inhibits the growth and activity of many, but not all, microorganisms. Converting most of the water into ice with the concomitant increase in concentration of the dissolved substances reduces the water activity of the food to the point where no microorganisms can grow. Although biochemical reactions slow down at lower temperature, they will, unlike microbiological activities, progress even at low commercial freezer storage temperatures. In addition, conversion of water into ice initiates complex physical and physicochemical changes that can cause general deteriorative quality changes not ordinarily occurring in fresh foods. Pre-freezing processing, such as blanching, freezing and storage conditions should therefore be selected, individually, for each product to minimize the effect of these deteriorative reactions.

In most foods frozen commercially, water is the major component. Most of the water in the tissue dissolves soluble cell components, while a small part is bound up in hydrates and in macromolecular colloidal complexes.

In addition, much of the aqueous solution is part of the gel-like or fiber-like structures in the cell. The most obvious change that occurs on freezing is the solidification of water, which means that water is removed from its normal position within the tissues. It appears that removal of water from its normal position is only partly reversible upon thawing, leading to "drip" and other changes. "Drip" is the exudate from thawed tissue which is difficult in practice to distinguish from any superficial moisture or "glaze." There is sometimes an apparent enhanced susceptibility to invasion by microorganisms owing to the moist surfaces which occur during thawing. The conversion of water into ice increases the concentration of soluble cell components (in some cases to the point where they become saturated and precipitate), changes the pH of the aqueous solution and consequently affects the amount of water that is involved in the colloidal complexes and in the gel-like and fiber-like structures. The concentration of cell components leads to a high concentration of electrolytes, some of which interpose themselves in the polypeptide chains of proteins, leading to protein denaturation. In living cells this often leads to death (for example, freezing and frozen storage causes a slight reduction in number of most microorganisms), but in foods, which usually consist of dead tissue prior to freezing, it can lead, during storage, to irreversible changes in texture (e.g., toughness in fish) and to undesirable biochemical reactions (enzymatically produced off-flavors). An understanding of freezing therefore involves physical, physico-chemical, and biochemical aspects.

As mentioned above, freezing is an excellent process for keeping the original quality of foods, such as fish, for longer periods of time (commercially, up to 12 months or more). Freezing and subsequent frozen storage are particularly useful in making seasonal species of fish, like herring and mackerel, available all year round. In addition, freezing preservation is also applied in a number of different products made from various fish species. For example, tuna is frozen on board large commercial fishing vessels, brought to land, and then thawed for canning process. In the production of various value-added fish products, freezing is applied to breaded and battered fish sticks, fillets, steaks, or nuggets. Likewise, high-quality fish are usually filleted, frozen, and eventually sold to consumers.

Ideally, there should be no distinguishable differences between fresh fish and frozen fish after thawing. If kept under appropriate conditions, fish in the frozen state can be stored for several months or more without appreciable changes in quality. However, it is now well recognized that deteriorative changes take place in fish and seafood during freezing, frozen storage and thawing, which influence the quality of final products. Considerably more knowledge of the basic structure of fish muscle and its chemical composition is essential to understanding these changes that occur during processing.

II. NATURE OF FISH MUSCLE

Fish muscle has a unique arrangement of muscle fibers. It is divided into a number of segments called myotomes, which are separated from one another by a thin sheath of

connective tissue called the myocomma or myoseptum. The number of myotomes in fish is dependent on the size of fish, while their diameters vary from head to tail (1). There are two major types of fish skeletal muscles, white and red. The red or dark muscle lies along the side the body next to the skin, particularly along the lateral lines, and may comprise up to 30% of fish muscle, depending on the species (2). Cells in red or dark muscle contain more lipids than those in white muscle (3, 4); they are basically employed for sustained swimming activities, functioning aerobically using lipids for fuel. In addition, red muscle has more mitochondria (5) but less sarcoplasmic reticulum than white muscle (6). It has a large supply of oxygen and a high content of myoglobin, the colored compound that gives its red color. These characteristics, coupled with the presence of the large amount of lipid, particularly among the fatty species, present a serious problem on preservation because of increased susceptibility of this muscle to lipid oxidation. The red muscle of some species has also been reported to contain enzymes that are responsible for chemical reactions such as lipid oxidation and the conversion of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA) (7, 8). The shape of red muscle area in different species varies considerably. Lean fish such as flounder, hake, sole, cod, pollock, and whiting have a very small amount of red muscle, which lies along the fish skin, whereas the fatty and semi-fatty fish species have larger areas of red muscle.

White muscle, on the other hand, constitutes the majority of fish muscle. Unlike red muscle, it has minimal myoglobin and a restricted blood supply (5). Often referred to as the "fast" tissue (9), it is used for anaerobic activities such as short bursts of swimming activity. This muscle exhibits rapid, powerful contractions, the energy for which is produced by reducing glycogen to lactic acid anaerobically (5).

Intermediate between these two types of muscle are intermixed red and white muscle, commonly referred to as "mosaic" muscle (10). In some fish, this is a thin layer of muscle that separates the red from white muscle. However, in other fish, such as salmon, carp, and trout, this muscle is scattered throughout the body of the fish.

The chemical composition of fish varies, depending on several factors, such as age, species, gender, maturity, method of catch, fishing grounds, and other seasonal and biological factors. Even within the same species, chemical composition may vary significantly. Generally, fish contain a considerable amount of protein, lipid, and water, and small amounts of vitamins and minerals. Other components such as non-protein nitrogenous compounds are also present in the muscle. These include urea, taurine, peptides, free amino acids, and nucleotides such as inosine and hypoxanthine (11). These compounds, together with the macronutrients found in fish muscle, may be particularly important to fish processors, since they are frequently used as spoilage indices.

III. PHYSICAL ASPECTS OF FREEZING

A. Formation of Ice

From a physical point of view, fish, land animal, and vegetable tissues can be roughly considered as dilute aqueous solutions. When they are chilled below 0°C, ice crystals form at a temperature characteristic of the product and the initial freezing point (FT), which is also the temperature at which the last ice crystals melt on thawing. The freezing point directly depends on the molar concentration of dissolved substances presented, but not on the water content. Fruits, for example, have high water content and a freezing point of -2 to -3°C, while fish contain less water, yet have a freezing point of about -1 to -2°C. The difference is due to the high sugar and acid content in fruits as compared with the low solute content of fish meat. Ice formation occurs during freezing only after a certain degree of supercooling (supercooling is the phenomenon of reducing the temperature of a solution or material below its freezing point without crystallization occurring) has been achieved, and the formation of ice is accompanied by a heating up of the supercooled product close to the freezing point. In commercial practice, the amount of supercooling is usually insignificant.

As the products are progressively cooled below their initial freezing point, more and more water will be turned into ice and the residual solutions will become more and more concentrated. If, at any time, the products are heated, some of the ice will be turned to water that will then dilute the residual solutions. The ratio of ice to residual solution in frozen foods is a function of temperature and initial concentration of solutes. At a temperature lower than -40°C, there is little or no measurable change in the amount of ice presented in most frozen foods. The percentage ratio of freezing (RF) of frozen foods is usually estimated as follows:

$$\text{RF}\ (\%) = 100 - [(\text{FT/temp. frozen food}) \times 100]$$

where RF represents the percentage ratio of freezing and FT represents the freezing point of the frozen food.

B. Ice Crystal Size

Once water has started to freeze, the rate of ice formation is a function of the rate of heat removal, as well as of the rate of diffusion of water from the surrounding solutions or gels to the surface of the ice crystals. At slow rates of cooling few crystallization centers are formed and the ice crystals grow to a relatively large size. The water in the cell diffuses through the cell wall, leaving the cells in a collapsed condition. Very large ice crystals (few crystals) can lead to mechanical damage to the food product. The cells become physically separated over relatively long distances. As the freezing rate increases, the number of ice crystals increases

while their size decreases. Many studies have been done on the effects of size and location of ice crystals on the quality of frozen food. It appears that, for most foods, the size and distribution of ice crystals, encountered in commercial practice, have relatively little effect on organoleptic quality. However, very slow freezing results in undesirable effects like "drip" on thawing, while very fast freezing may improve the texture of some products.

C. Dimensional Changes

The volume change accompanying the conversion of pure water into ice is about 9%. The volume change of foods as a result of ice formation is less, about 6%, because only part of the water present is frozen and because some foods contain spaces. This volume change has to be taken into account in equipment design. In very fast freezing (for example, immersion of large items in liquid nitrogen), it can lead to the build-up of excessive pressure inside the product, causing breaking and shattering.

D. Completion of Freezing

The freezing process is, for practical purposes, completed when most of the freezable water at the thermal center of the product has been converted into ice, which coincides for most products with the temperature at the thermal center becoming colder than −10°C. Removal of the product from the freezing equipment before this point is reached may result in slow freezing at the thermal center. It is preferable to freeze the product until the equilibrium temperature (average temperature) is −18°C or colder.

E. Desiccation of Frozen Foods

1. During Freezing

It is inevitable that a proportion of water of a product without packaging will evaporate during freezing. The faster the freezing, the smaller the amount of evaporated water. If the product is enclosed in a water-vapor-proof package before freezing, the moisture that escapes from the packet will be nil, but when there is an air gap (on the order of millimeters) between the surface of the product and the internal surface of the package frost may be deposited inside the package to the same extent as moisture evaporates from the product.

For products frozen unpackaged, moisture loss varies from 0.5 to 1.5% or more, depending on the temperature, rate, and method of freezing, as well as the type of product. The colder the air temperature, the less moisture the air can absorb before it is saturated. Faster freezing methods lower the surface temperature of the product quickly to a value where the rate of moisture evaporation or sublimation is small. Where the surface of a product consists of a moisture-resistant layer (skin of the fish or fat on beef, for example), moisture losses are reduced in comparison with products with cut surfaces (fish fillets, hamburger patties, for example). Proper freezer design for a given product is, therefore, an important factor in minimizing moisture loss during freezing.

2. During Storage

Moisture loss during frozen storage is a more serious problem because of the length of storage usually involved. Tight-fitting water-vapor-proof packaging avoids all apparent moisture loss. Many frozen foods, however, are still stored unpackaged or packaged in water-vapor permeable materials. In these cases, moisture loss depends on the average ambient temperature and the temperature of the evaporator, as well as on the temperature fluctuations occurring during storage, and increases with increasing storage temperature, increasing frequency, and amplitude of temperature fluctuations, and with increasing difference between the storage temperature and the lower temperature reached by the evaporator. It should be noted that the saturated vapor pressure of frozen foods is equal to that of pure ice at the same temperature.

If a water-vapor-proof packaging does not fit tightly around the product, desiccation of the product still occurs, but the water removed remains inside the package as frost. The mechanism appears to be as follows:

a. The layer of air between product and packaging is subject to temperature variations. As the outside temperature decreases, the temperature of the inside surface of the packaging at a certain moment drops below the product surface temperature and ice on or in the product will sublime and condense on the inside of the package.
b. When the ambient temperature increases, the process is reversed. However, the water vapor will condense on the product surface rather than in the cellular structure from which it evaporated.
c. As the cooling-heating cycle recurs, the crystals on the product surface tend to follow package temperature more closely than the mass of the product, and this results in further sublimation of ice from the product.

Frost in packages of frozen foods can amount to several percent of the product weight. Because it leads to ready access of oxygen into the product, frost formation may, with some foods, increase quality deterioration.

The effects of temperature fluctuation depend on the average storage temperature. At warmer storage temperatures, a given temperature fluctuation results in a much larger change in ice vapor pressure than at colder temperatures. As a result the effect of temperature fluctuations on desiccation increases with a warmer storage temperature.

Excessive drying, in addition to leading to undesirable loss of weight, can accelerate oxidative changes by causing the loss of an added glaze, and also by causing the removal of ice from the superficial parts of the frozen products, thus allowing a free access of oxygen to the internal tissues. Some parts of the surface of protein foods may be highly desiccated and their structure even irreversibly deteriorated; light spots known as "freezer burn" occur on the surface and the appearance of produce may become unacceptable. Animal foods (fish, poultry, game) in particular can be affected severely by freezer burn.

F. Change in Ice Crystal Size in Frozen Foods during Storage

The changes in shape and size of the ice crystals in frozen foods are caused by periodic variations in temperature experienced during storage; the greater the amplitude of these variations, the greater will be the changes.

G. Thermal Radiation in Frozen Food Storage

Thermal radiation has a significant effect on frozen foods in open display cabinets. In these, the top layer of packages may reach a temperature up to 10°C warmer than the average cabinet temperature leading to quality losses. The temperature of the packages in the top layer represents a thermal equilibrium between the energy transferred by radiation and the energy transferred by conduction and convection. The effect of radiation depends on the emissivity of the radiating surfaces, e.g., the package and the ceiling or walls. (Emissivity characterizes the surface state as far as radiation is concerned. Emissivity reaches a maximum equal to 1 in the case of an ideally absorbing and emitting body (black body) and is zero in the case of a perfect reflector; the latter does not emit or absorb any radiative energy.) The important radiation is that in the far infrared range (wavelength 8 to 10.10 m) and not that of visible light. A reflecting canopy placed about the open area of the cabinet or packaging in bright metallic foil reduces radiation energy gain markedly. Bright metallic foil packaging may reduce product temperature at the top layer of display cabinets by as much as 6–8°C.

IV. PHYSICO-CHEMICAL ASPECTS OF FREEZING

A. Composition and pH Changes during Freezing

Freezing converts a large proportion of the water present in foods into ice and hence makes the remaining solution more concentrated in dissolved, colloidal, and suspended substances. This increased concentration causes a change in acid-base equilibrium (pH) important in the stability of many colloids and suspensions. Shifts in pH (usually towards the acid side) of up to 1 pH unit have been observed under these conditions.

A second result of this increased concentration is the precipitation of salts and other compounds that are only slightly soluble, such as phosphate. This can result in drastic pH changes (up to 2 pH units) and changes the salt composition of the aqueous solution in foods. These changes often affect the physico-chemical systems in food irreversibly. It has been shown, for example, that lactic dehydrogenase, a muscle enzyme, and lipoproteins, an important egg yolk constituent, are irreversibly damaged by a pH decrease from 7 to 5 and by increased phosphate concentration during freezing.

B. Physico-Chemical Changes in Frozen Foods

Textural properties and the initiation and acceleration of several biochemical reactions depend on the physical chemistry of food constituents and hence are affected by the physico-chemical changes brought about by freezing. Loss of water binding properties, resulting in drip, is an example of textural changes, while removal of enzymes from cell particles, allowing them free access to substrates in other parts of the cell, is an example of biochemical reactions initiated and accelerated by freezing. Other physico-chemical changes in frozen foods are actomyosin changes in muscle, leading to toughening (fish) or dryness (poultry), loss of turgor in fresh fruits and vegetables, and gelation of egg yolk.

Many physico-chemical changes increase with increased salt concentration in the unfrozen phase, but will decrease with decreasing temperature as a result of the lower mobility of the salt in the unfrozen phase and the general effect of temperature on chemical reactions. Consequently, physico-chemical changes are most damaging in the range between the freezing point of a food and about −10°C. It is important therefore to expose frozen foods for as short a time as possible to this temperature range, both during freezing and during thawing.

C. Effects of Preparation and Packaging on Frozen Fish

Product preparation and packaging significantly affect the quality and shelf life of frozen fish. If not properly controlled, these processes result in some deleterious effects after prolonged storage.

1. Product Preparation

Product preparation, in particular, produces a considerable effect on shelf stability of frozen fish. Whole and eviscerated fish have longer shelf stability than fillets, while minces can usually only be stored for a much shorter period of time. Crawford et al. (12–14) observed

this difference during several studies using hake. Minced blocks exhibited reduced quality and accelerated deterioration during storage when compared to intact fillets. This characteristic of minces, which is more apparent among the gadoids, is probably due to the mincing action applied to the fish flesh, which results in tissue damage and subsequently more rapid deterioration. In addition, mixing of red and white muscle during mincing may also result in the dispersion of lipids and some of the enzymes present in the red muscle, leading to greater susceptibility of the minced tissue to deteriorative changes.

2. Packaging Materials and Methods

An efficient packaging system is essential to offset the detrimental quality changes that occur during frozen storage. Packaging materials and methods are obviously designed not only to protect the product from microbial and chemical contamination, dehydration, and physical damage, but also to protect the environment from the packaged product. Fish and seafood can leak gases or unsightly fluids, which may have unpleasant odors. Therefore, the choice of appropriate packaging materials and methods for frozen fish is a critical factor in terms of shelf-life extension.

Studies have shown that packaging systems affect the quality and shelf stability of frozen fish. For instance, vacuum packaging is well established as a method to provide an oxygen-free environment to minimize the problems associated with lipid oxidation and dehydration during frozen storage.

Several studies have shown the effectiveness of this method for frozen storage of some species of fish. For example, it has been reported that frozen blocks of fillets vacuum packed in moisture-proof films showed high degrees of acceptance and desirable frozen characteristics (12). Likewise, Santos and Regenstein (15) reported the effectiveness of vacuum packaging for inhibiting lipid oxidation in frozen mackerel fillets. Ahvenainen and Malkki (16) examined the influence of packaging on frozen herring fillets stored at different temperatures. They found that vacuum-packed product covered with metallized cardboard had a longer shelf life than a product vacuum packed and stored without cardboard. Vacuum packaging, on the other hand, need not be used if lipid oxidation is not the limiting factor affecting the shelf life of a product. Although the effect or absence of oxygen in packages on some fish species must be considered, other packaging methods such as glazing and the use of heat-sealable packaging films should also be considered. Pacific hake minced blocks stored in moisture-proof, vapor-proof packaging films exhibited superior quality over glazed samples (12). Likewise, Colokoglu and Kundacki (17) observed frozen mullet packed in plastic films with low permeability to oxygen and moisture to have a longer shelf life than when unpacked in the glazed form. However, it should be noted that glazing is still considered to be the cheapest means of protecting frozen fish during storage and transport. Glazing provides a continuous film or coating that adheres to the frozen product, which retards moisture loss and the rate of oxidation.

Many different glazes are available, including (a) those with inorganic salt solutions of disodium acid phosphate, sodium carbonate, and calcium lactate, (b) alginate solution, otherwise known as the "Protan" glaze, (c) antioxidants such as ascorbic and citric acids, glutamic acid, and monosodium glutamate, and (d) other edible coatings such as corn syrup solids (18). Ice glaze is particularly important in handling frozen fish in developing countries. For products intended for short-term storage, glazing can be practically utilized as a viable alternative to storage without a protective covering. For instance, Jadhav and Magar (19) concluded that glazing was a cheaper alternative to expensive packaging systems for glazed Indian mackerel (*Rastrelliger kanagurta*) stored at −20°C. Glazed samples had a shelf life of 6 months, while samples without a protective covering lasted only 3–4 months.

3. Effects of Freezing, Frozen Storage, and Thawing on Color, Appearance, and Consumer Acceptance

One problem encountered during handling, freezing, and storage of fish is the difficulty in retaining the color and appearance of the meat. Changes in color and appearance of fish occur even immediately after catch. Blood pigments become noticeably discolored to various degrees after some period of time. The natural oils in fish play an important role in these color changes. The color of these oils is produced by the colored pigments dissolved in them which vary from one species to another. These pigments are subjected to considerable oxidation when the fish is frozen and stored. This then results in meat color darkening to either dark brown or, in some cases, black. This discoloration occurs especially when the fish is stored for an extended period of time. Some fish, like tuna, develop discoloration during frozen storage, reportedly due to oxidation of myoglobin to met-myoglobin in fish blood (20). Other species, such as salmon, swordfish, and shark, also exhibit color changes during storage. Salmon has a pink meat, but when subjected to oxidation its color slowly fades and, in extreme cases, may completely disappear after prolonged storage. Swordfish, on the other hand, develops green discoloration beneath its skin during frozen storage which, according to Tauchiya and Tatsukawa (21), is due to the development of sulfhemoglobin, a product of oxidation. Shark flesh also discolors and occasionally develops off-odors during storage, most probably as a result of the presence of high amounts of trimethylamine oxide. Interestingly, these

marked differences in the color and appearance of frozen fish are quite noticeable in fish sold either as steaks or as fillets, especially when cross-sectional cuts of the fish are made, which permits a comparison of the color of the exposed fish surface with that of the inner portion.

In shrimp, the rapid formation of black pigments, widely known as "melanosis," occurs within a few hours after death and is enhanced by exposing the shrimp to air (oxygen). It can occur within just 2–12 hours of exposure. The oxidation reaction leading to the formation of these black pigments can occur at 0°C; however, at −18°C, no visible spots were detected at up to 3 months of storage (22). Below this temperature, it is believed that melanosis can still positively occur. It should be noted, however, that although black spots do not necessarily make shrimp unfit for human consumption, such discoloration is usually associated with spoilage, resulting in a decrease in market value. In other shellfish, such as crab and lobster, the development of blue or black discoloration, otherwise known as "blueing," is one of the most troublesome problems. Blueing may occur after freezing or during frozen storage, or it may appear after thawing and subsequent air exposure or even shortly after cooking. Needless to say, these changes in color and appearance of fish and shellfish significantly affect consumer acceptance. When consumers select frozen fish, if these products can be seen through the packaging material used, the color and appearance of the frozen product provide an indication as to its degree of quality. As shown in Table 39.1, undesirable appearance and discoloration of samples have been observed in different frozen whole fish and fillets obtained from Singapore supermarkets (23). Preventing such quality changes is of great commercial importance, since they detract not only from the consumer acceptability of the products but their shelf stability as well.

Thawing also influences the color and appearance of frozen fish and, inevitably, its consumer acceptability. Depending on the thawing technique used, discoloration may occur in fish and other seafood. For instance, when shrimp are thawed at temperatures higher than 0°C, black discoloration or melanosis may occur. This is due to the unnecessary exposure of the shrimp to air, leading to oxidation. A phenomenon known as "shimi" occurs in frozen-thawed fish meat. Shimi are the undesirable blood spots observed in the belly portion of carp on thawing and are also the distinguishable spots tainting frozen-thawed tuna meat (24). The latter condition is probably due to the blood vessels that remain in unbled tuna meat prior to freezing. When thawed, these blood vessels produce unsightly spots in the meat.

It is possible to determine if the product has been properly thawed and then refrozen. This is particularly noticeable in packaged frozen fish, where spaces on the sides of the package may be filled with a frozen cloudy liquid, known as thaw drip. Such muscle drip was originally attributed to the rupturing of cell walls caused by ice crystal formation during freezing, resulting in excess drip during thawing. However, it has been postulated that drip or exudate formation is directly related to the capacity of the fish protein to hold moisture (25). This unsightly exudate from fish muscle indicates, among other things, inappropriate handling, prolonged ice storage prior to freezing, frozen storage at inappropriate cold-storage temperatures, or improper thawing. If not properly controlled, freezing, frozen storage, and thawing generally result in quality changes in fish and seafood that in most cases render the product unacceptable to consumers.

TABLE 39.1
Characteristics of Some Frozen Fish Purchased in Singapore Supermarkets

Fish	Thawed-State Characteristics
Herring, whole	Skin and meat show rusting
Mackerel, whole	Surface dehydrated, skin and meat show rusting, spongelike meat
Mackerel, whole	Rancid smell in skin and meat
Chinese Pompret, whole	Dehydration at lower part of belly and fin
Chinese Pompret, whole	Head and belly parts yellowish discolored, spongelike meat
White pompret, whole	Skin and meat show rusting, spongelike meat
Jew fish, whole	Slight rancid smell in skin
Lemon sole, whole	Surface dehydrated, spoiled and rancid smell, spongelike meat
Haddock, fillet	No smell, spongelike meat, cracks
Cod, fillet	No smell, spongelike meat
Flounder, fillet	No smell

Source: Ref. 23.

4. Effects of Freezing, Frozen Storage, and Thawing on Palatability Attributes

Changes in the texture, odor, and flavor of fish and seafood affect their palatability. Fresh fish have a distinct succulence and a delicate odor and flavor, which are characteristic of the species. These attributes change noticeably when fish is frozen and stored for prolonged periods of time. Interestingly, the changes that influence the palatability of frozen fish and seafood can all be measured organoleptically and, to some extent, chemically.

a. Change in texture

Frozen fish gradually loses its juiciness and succulence after freezing and subsequent frozen storage. Such textural changes, reportedly caused by protein denaturation (26–29), are more pronounced in some species of fish, specifically the gadoids. In these species, the chemical breakdown of TMAO to DMA and FA and the subsequent cross-linking of FA to muscle protein (30) produce the

textural breakdown in the gadoids and result in a "cottony" or "spongy" texture. Fish muscle that has undergone such changes tends to hold its free water loosely like a sponge. When eaten, the fish muscle loses all its moisture during the first bite, and subsequent chewing results in a very dry and cottony texture.

In some species devoid of TMAO-degradation products, muscle fibers also tend to toughen and to become dry during freezing and storage. This is particularly true for most of the nongadoid species and for crab, shrimp, and lobster when stored for prolonged periods. In contrast, the effect of the thawing method on the texture of fish muscle basically depends on the product form. For instance, whole fish, when thawed, exhibits less textural change than filleted fish, basically as a result of the presence of the backbone, which serves as structural support for the flesh. In terms of the effect of the thawing method, it has been reported that microwave thawing results in higher gel strength of minced samples, when compared to samples thawed under running water (20°C) and samples thawed at room temperature (31). Consequently, the extent of textural changes depends upon the species of fish and upon the condition of handling, freezing, duration of frozen storage, and the thawing method used.

Several methods have been developed to objectively measure such textural changes, in addition to the gathering of comparative data from sensory evaluations. From texture analysis of minced fish, Borderias et al. (32) concluded that hardness as measured by the Kramer Shear cell and puncture (penetration) tests was highly correlated with the sensorial perceived firmness of raw samples, while a compression test was found to be a valuable technique for characterizing the cohesiveness and elasticity of both raw and cooked fish minces.

Alterations in the texture of frozen fish fillets, on the other hand, are difficult to measure objectively, mainly due to the textural variability that exists within the fish fillets, which is associated with the flakiness and the orientation of the muscle fibers (33). Several attempts have been made to determine the extent of textural changes in fish fillet, including those tested using fish minces (32). However, significant correlations were not obtained.

An instrumental method that may work on fish fillets is the deformation test using the Instron Universal Testing Machine equipped with a flat compression plate. As a nondestructive test (34), it can potentially be modified to conform to the irregular shape and the segmented structural orientation of fish fillets.

b. Changes in odor and flavor

Other important changes that affect the palatability of frozen fish include changes in the flavor and odor of fish and seafood. Fish are often described as having a "fishy" odor and flavor. Although the term sounds unpleasant, it can also be used to describe the pleasing taste and odor characteristics of freshly caught fish. Such pleasant, palatable characteristics may be retained as long as the fish are promptly and properly frozen, stored, and thawed. However, the transformation of these attributes to unpleasant and unacceptable traits occurs very rapidly in some fish species, particularly the fatty fish species.

Changes in the delicate flavor of fish and seafood generally occur in three distinct phases during frozen storage: (a) the gradual loss of flavor due to loss or decrease in concentration of some flavor compounds (35, 36), (b) the detection of neutral, bland, or flat flavor, and (c) the development of off-flavors due to the presence of compounds such as the acids and carbonyl compounds that are products of lipid oxidation. These phases, however, only apply to those species with originally delicate, sweet, and meaty flavors. Other species, such as hake, have an originally bland flavor (35), but develop off-flavors during prolonged frozen storage.

Changes in odor occur in two phases: the loss of characteristic odor and the development of off-odors, which render the frozen product unacceptable. Generally, fish and seafood initially have a fresh, seaweed odor, which can be retained even after freezing and frozen storage. However, gradually such odor is lost, and eventually an unpleasant odor is given off, particularly when abused with inappropriate storage temperature. The development of unpleasant odor is due either to lipid oxidation, a reaction more apparent among the fatty fish species that results in the production of a strong oily, blow oily, or rancid odor, or to the degradation of TMAO, which leads to the production of an unpleasant ammonia odor. Other species such as white hake (*Urophysus tenuis*) initially give off weak odors of sweet, boiled milk, but when frozen storage is extended, hake assumes weak off-odors (often described as milk jug odor) followed by a sour milk odor.

V. BIOCHEMICAL ASPECTS OF FREEZING

A. Post Mortem Glycolysis and Lipid Oxidation

Fish muscle obtains energy by hydrolyzing adenosine triphosphate (ATP). At any one moment, its concentration is relatively small. During life it is quickly re-synthesized using the energy produced when glycogen is oxidized to carbon dioxide and water. On death of the fish metabolism in the muscle continues for some time. Post mortem glycolysis, however, is a relatively inefficient process and it cannot maintain ATP at its *in vivo* level. Once ATP has fallen to a critical concentration it can no longer prevent the major proportion of the muscle actin and myosin from cross-linking. This causes the loss of elasticity known as rigor mortis and usually a slow irreversible contraction. The continuing production of lactate and H^+ ions causes the pH of the muscle to fall from its *in vivo* value of about

7.2 to the so-called ultimate pH, which is usually about 5.5. A pH of 5.5 is near the isoelectric point of the muscle proteins, at which they have minimum water holding capacity and a consequent relatively high tendency to drip on thawing. A higher ultimate pH, therefore, means a greater water holding capacity than at a lower pH. The quantity of glycogen present in the muscle at the moment of slaughtering will clearly determine how far the pH will fall during post mortem glycolysis. Like most chemical reactions post mortem glycolysis is temperature dependent. It is generally found that the lower the temperature at which this process occurs, the slower is its rate. Thus if the carcass is maintained at body temperature after death, the rate of pH fall, of ATP depletion, and of rigor mortis onset is fast. If, however, the muscle is chilled quickly, these changes are slowed down and the water holding capacity of the muscle remains relatively high.

There is no practical "cold shortening" problem with fish properly chilled after catching. Fish muscle shows the least shrinkage if held at about 0°C. At warmer temperatures the shrinkage and weight loss are greater and they may be quite substantial for a fillet removed from the skeleton pre-rigor and kept at room temperature. One qualitative difference between fish and meat is the generally lower glycogen content in fish than in meat animals rested before death. Consequently, the post mortem fall of pH in fish is smaller and the resistance against surface bacterial growth less than in meat. In many fish species, therefore, bacterial spoilage is an overwhelming factor.

The fat composition of fish differs markedly from that of meat because fish fat contains a higher proportion of polyunsaturated fatty acids. Although this factor may vary with species and is also influenced by dietary fat intake, it nevertheless implies that fish, in particular fatty fish, are very prone to development of rancidity by auto-oxidation. Such rancidity may even develop in fatty fish held before freezing, but it is particularly during storage of frozen fish that great care in packaging and the use of low temperatures are necessary to preserve quality.

B. Denaturation of Muscle Proteins

The proteins of fish muscle differ from those of meat especially in their higher susceptibility to cold store damage. Frozen storage of fish causes an increase of drip loss on thawing, toughness, coarseness, and dryness on cooking, and loss of the desired glossy pellicle on smoking. These changes are highly associated with the so-called protein denaturation caused by freezing and subsequent storage. They are temperature dependent, with the maximum rate of development being in the range −1 to −5°C. They are considerably slowed down by colder storage temperatures. Many techniques have been used to measure these changes, such as extractable protein in salt solutions (ionic strength 0.5–1.0) which has been most widely used. The changes are mostly in the myofibrillar protein of fish muscle. In general, the sarcoplasmic proteins seem to be more stable on freezing and subsequent storage. This kind of "protein denaturation" is associated with the reaction of certain free fatty acids or their oxidized products on the myofibrillar proteins. Recently it has been found that the ultimate pH attained by fish can considerably affect texture. Thus, low pH in cod is associated with more pronounced toughness and larger drip loss on thawing.

Drip loss, some changes in flavor and taste, and an undesirable softening occur in freeze-thawed fish muscle. When the frozen and thawed fish was cooked, the succulence and water holding capacity greatly decreased and some undesirable changes in texture such as toughness, coarseness, and dryness occurred. Compared with unfrozen fresh meat, the functional properties such as emulsifying capacity, lipid binding properties, water holding or hydrating capacities and gel forming ability were lower in the frozen stored fish muscle. Most of the studies indicated that denaturation of muscle proteins plays a dominant role in the quality changes of frozen stored fish muscle. The fish muscle proteins have been found to be much less stable than those of beef, pig, and poultry muscles (38). The amount of extractable actomyosin decreased with the duration of storage, while no significant change in sarcoplasmic proteins was observed during frozen storage of cod and other fish (36–38). Since the decrease in soluble actomyosin correlated well with palatability scores, it was proposed that denaturation of actomyosin is the major cause for the decrease in eating quality of frozen fish. Although the change in extractable actomyosin is regarded as the primary criterion of freeze denaturation, it still must be noted that extractability data cannot indicate precisely how much protein is denatured and how much is native. According to previous studies, results from electron microscopic analyses (39), decreases in actomyosin peak (20s–30s) areas on ultracentrifugal analysis (40, 41) and viscosity of soluble actomyosin with duration of storage (41, 42) suggested the aggregation of muscle proteins occurred during frozen storage. In addition to aggregation, dissociation of f-actomyosin into f-actin and myosin also occurred. It appeared that the dissociated F-actin, as thin filaments, became entangled and aggregated and that the dissociated myosin monomers folded into globular form. At advanced stages of freeze denaturation, large masses with diffuse outlines were frequently found, indicating the formation of aggregation complex of actin and myosin (43).

ATPase activity of actomyosin and myosin, another property of myosin related to its contractile function, also decreased with the increase of frozen storage (40–42, 44–47). During frozen storage, changes in isolated actomyosin and myosin have been sought in the number of –SH groups (40–42, 44–47), titratable acid groups (48), and net charge (49), and in the salting-out profiles (50, 51). Connell (52) attributed the insolubilization of frozen

stored cod actomyosin to the denaturation of myosin rather than actin. However, isolated carp actin denatured progressively with myosin during frozen storage as demonstrated by SDS-PAGE (53). During the initial frozen storage, it appears that both myosin and actin undergo denaturation, while denaturation of tropomyosin and troponin was observed during elongated frozen storage (53).

Decreases in the solubility, viscosity, ATPase activity, and number of SH groups of frozen stored rabbit, mackerel, milkfish, amberfish, tilapia, and trout were observed (44–47, 54, 55). Although Connell (56) ascribed the intramolecular aggregation of muscle proteins to the formation of non-covalent bonds (57) rather than to the formation of disulfide bonds, the involvement of SH group in the denaturation of muscle proteins during frozen storage has been emphasized by Buttkus (54, 55) and Jiang et al. (44–47). From the studies thus far reported, the crosslinkage of myosin is ascribed to the formation of disulfide bonds, hydrophobic bonds and hydrogen bonds during frozen storage. Free SH groups are firstly oxidized to disulfide bonds. However, only a small decrease was found in the number of free SH groups during frozen storage. Therefore, the changes appear to be the result of rearrangements of disulfide bonds from intra-molecular to intermolecular through a sulfhydryl-disulfide interchange reaction.

Myofibrils, a systematically organized complex of myofibrillar proteins, undergo some structural changes during frozen storage of fish. The most noticeable change is the fusion of the myofibrils as illustrated by cell fragility method (58, 59) and fragmentation into short pieces at the Z-bands (60–63). More recently, studies have been done on the denaturation of enzymes during frozen storage (64, 65). Inactivation of enzymes with globular molecule was considered to be due to the unfolding of intra-molecular structure (66).

Many hypotheses have been proposed to explain the denaturation of muscle proteins (67–70). They include: 1) the effect of inorganic salts concentrated into the liquid phase of the frozen system; 2) water-activity relations; 3) reactions with lipids; 4) reaction with formaldehyde derived from trimethylamine (in fish); 5) auto-oxidation; 6) surface effects at the solid-gas interface; 7) effects of heavy metals; and 8) effects of other water-soluble proteins (such as protease). Among these hypotheses, effects of lipids (67–72), formaldehyde (73–77), and gas-solid interface of myofibrillar proteins caused by free fatty acids and/or lipid peroxides must occur during frozen storage. Jarenback and Liljemark have shown by electron microscopy that, in muscle frozen stored with added linoleic and linolenic hydroperoxides, myosin became resistant to extraction with salt solution (78). However, recent studies on isolated muscle protein indicate that proteins undergo denaturation in the absence of lipids, formaldehyde, heavy metals and water-soluble proteins. Another popular view is the so-called "salt-buffer hypothesis" which gives attention to the effects of highly concentrated salt solution in the unfrozen phase of frozen muscle proteins. The concentrated salt solution may denature the proteins (67–72).

One of the most prevalent chemical reactions to occur in fish muscle during freezing and frozen storage is the complex phenomenon of protein denaturation. It has been postulated that the rupturing of different bonds in the native conformation of proteins in frozen fish is followed by side-by-side aggregation of myofibrillar proteins, specifically myosin, brought about by the formation of intermolecular cross-linkages (27, 79). It is also believed that the significant decrease observed in the center-to-center distance between the thick filaments of the A-band of the sarcomere after prolonged frozen storage favors the formation of cross-linkages between molecules and stiffens the fibers (78). Such intermolecular cross-linkages result in aggregation (30), which leads to the formation of high-molecular-weight polymers (80, 81) and subsequent denaturation of myosin during frozen storage.

Several relevant theories on protein denaturation in relation to fish moisture and freezing damage have been formulated. One theory worthy of note is that of protein denaturation being affected by the freezing out of water. The conformation of most native proteins has the hydrophobic side chains buried inside the protein molecule. However, some of these hydrophobic side chains are exposed at the surface of the molecule itself. It has been suggested that the water molecules arrange themselves around these exposed hydrophobic side chain groups so as to minimize the energy of the oil/water interface and, at the same time, act as a highly organized barrier, which mediates the hydrophobic/hydrophilic interactions between protein molecules (81). These water molecules form a network of hydrogen bonds, which contribute to the stability of the highly organized three-dimensional structure of the proteins. As water molecules freeze out, they migrate to form ice crystals, resulting in the disruption of the organized H-bonding system that stabilizes the protein structure. As the freezing process continues, the hydrophobic as well as the hydrophilic regions of the protein molecules become exposed to a new environment, which may allow the formation of intermolecular cross-linkages (30), either within the same protein molecule, causing deformation of the three-dimensional structure of the protein, or between two adjacent molecules, leading to protein-protein cross-links.

Freezing also concentrates solids, including mineral salts and small organic molecules, within the remaining unfrozen aqueous phase in the cell (82), which results in changes in ionic strength and possibly pH, leading to the denaturation of the protein molecule (83). Love (84) considered this concentrated salt in the unfrozen phase to be the main protein denaturant in the frozen muscle system. If proteins are denatured over time in the presence of concentrated solutes, it is reasonable to believe that longer exposure of protein molecules to these denaturants (e.g., slow

freezing) should be avoided. However, further work must be conducted to determine the effect of the rate of freezing on shelf life of frozen fish as related to the solute concentration effect.

Several methods have been established to determine the extent of protein denaturation during frozen storage of fish and seafood. According to Jiang and Lee (85), protein quality is more sensitively reflected by the enzymatic activities in the muscles than by its extractability, since small microstructural changes in protein molecules can cause more alterations in the enzymatic activities than in extractability. For example, the actomyosin Ca ATPase, which measures the activity of myosin, can be used as an index of protein quality. Since this ATPase is capable of hydrolyzing the terminal end phosphate group of ATP to give ADP (68), this particular enzymatic activity can be determined by measuring changes in the amount of inorganic phosphate present in the muscle. The loss of enzymatic activity reflects the extent of freeze damage and alteration of the protein structure in the muscle system. Connell (79) reported a loss in Ca ATPase activity in muscle during frozen storage. In a more recent study using mackerel, Jiang and Lee (85) observed a loss of ~66% of the original Ca ATPase activity of actomyosin after 6 weeks of storage at –20°C.

Visual examination under a transmission or scanning electron microscope is a powerful technique used in the determination of textural changes in fish muscle due to denaturation. The electron microscopic studies of Matsumoto (30) were able to detect damage to the native structure of the protein: aggregation and an entangled mass were observed. However, results from this technique have to be interpreted cautiously since the fixing processes of tissue or any tissue section may create artifacts by altering the ultrastructural images or by masking the microchanges in the muscle tissue.

The extent of protein denaturation in frozen fish muscle can also be determined by conducting several tests of protein functionality. The physicochemical properties that affect the behavior of protein molecules during processing are defined as the functional properties of the fish myosystem, which include protein homogenate solubility, emulsifying and water-retention properties, gelation, and viscosity (86). The most popular tests to determine the extent of protein denaturation during frozen storage of fish, in relation to its functionality, are determination of the loss in solubility or extractability of proteins and measurement of the water-retention properties of the fish muscle system.

C. Effects of Freezing, Frozen Storage, and Thawing on Nutritional Value

Considerable emphasis has been given to the influence of freezing, frozen storage, and thawing on quality indices such as appearance/color, texture, flavor, odor, and the chemical reactions that accompany such organoleptic changes. Less attention has been given to yet another useful area, i.e., the influence of such treatments on the nutritional value of frozen fish and seafood.

Put simply, considerable attention is given to sensorial perceived attributes because if consumers reject a frozen product on display, it is not purchased or eaten regardless of its nutritional value. Conversely, if consumers are attracted to a frozen product, they tend to buy it whether it has the needed nutrients or not. However, as the market shifts to the development and merchandising of products to meet the demands of health-conscious consumers, the nutritional value of frozen fish becomes of great importance.

When fish and seafood are frozen, and subsequently stored and thawed, protein denaturation occurs in muscle tissues. As a result, formation of thaw drip becomes apparent and consequently leads to the leaching out of dissolved materials. Likewise, there is an increase in the release of a watery "cook liquor" when the product is heated. Such water losses result in the loss of water-soluble proteins; however, such losses do not result in any measurable decrease in the nutritive value of the protein (87). However, such losses lower the proportion of sarcoplasmic proteins in the fish tissue and may also lead to a small loss of water-soluble vitamins and minerals.

Other quality changes, such as lipid oxidation, can also influence the nutritional value of frozen products. Oxidized fish lipids, such as lipid hydroperoxides, may induce oxidative changes in sulfur-containing proteins, producing significant nutritional losses (88).

D. Effects of Freezing, Frozen Storage, and Thawing on Intrinsic Chemical Reactions

When frozen fish are subjected to excessively prolonged cold storage at temperatures above –30°C, a series of intrinsic chemical reactions occurs in fish tissues. These reactions include protein denaturation, breakdown of TMAO, and lipid oxidation.

1. Breakdown of Trimethylamine Oxide

Quite obviously, protein denaturation during frozen storage produces extensive textural changes and deterioration in fish. These changes are more pronounced in some species of fish, specifically the gadoids, and are related to another intrinsic chemical reaction, the breakdown of TMAO.

TMAO is commonly found in large quantities in marine species of fish. It is believed that these species use TMAO for osmoregulation (89). Among the marine species, the elasmobranches contain more TMAO than the teleosts. Among the teleosts, the gadoids have more TMAO than the flatfish. Except for burbot, freshwater species have a negligible amount of TMAO in their muscles, since they

do not take in TMAO in their diet beyond their bodies nutritional requirements, and they promptly excrete any excess.

After death, TMAO is readily degraded to DMA and FA through a series of reactions. This conversion of TMAO to DMA and FA is typically observed in frozen gadoid species such as cod, hake, haddock, whiting, red hake, and pollock (7).

The presence of air (oxygen) affects DMA and FA formation. It has been suggested that oxygen may actually inhibit the reaction by interacting with metal ions, which otherwise would accelerate the TMAO degradation (90). Lundstrom et al. (91) observed that red hake (*Urophysis chuss*) minces stored in the absence of oxygen showed more rapid DMA and FA formation than red hake fillets stored in air. Likewise, the presence of air (oxygen) in packaged white hake (*Urophysis tenuis*) significantly prolonged the shelf life of the frozen samples (15).

TMAO degradation to DMA and FA was enhanced by the presence of an endogenous enzyme (TMAOase) in the fish tissues, as observed in cod muscles by Amano and Yamada (92). They also found an enzyme in the pyloric ceca of Alaskan pollock (*Pollachius virens*), which was believed to cause DMA and FA formation in this species (93). However, evidence also exists which demonstrates that breakdown of TMAO to DMA and FA is nonenzymic in nature (94, 95). The breakdown of TMAO, whether enzymatically or nonenzymatically induced, is believed to produce destabilization and aggregation of proteins.

TMAO has been postulated to be responsible for stabilizing proteins against conformational changes and thermal denaturation (96). However, the conversion of TMAO to DMA and FA has been implicated in gadoid textural problems during frozen storage (12, 97, 98).

It has been suggested that TMAO's breakdown product, FA, may produce cross-linking of muscle proteins (30) due to its high reactivity: FA can covalently bond with various functional groups of proteins, such as the amino, imino, guanido, phenolic, imidazole, and indole residues. This reaction induces both intra- and intermolecular cross-linkages of the molecules, thus producing conformational changes.

However, textural changes may also occur during frozen storage for fish species devoid of the TMAO-enzyme system (99). Such textural changes must then be attributed to another type of mechanism that does not involve the cross-linking of protein molecules due to the presence of FA. Gill et al. (99) reported that the presence of FA in red hake resulted in the covalent cross-linking of troponin and myosin light chains, forming high-molecular-weight aggregates. However, when haddock, a species that does not produce FA, was examined, the same cross-linkages were not found at the molecular level, although textural toughening was observed, but not as pronounced as that in red hake. Based on these observations, they suggested that textural changes in haddock were probably due to secondary bonds, such as hydrogen or electrostatic bonds, and not due to FA cross-links.

Clearly, the presence of FA is not the only factor involved in textural changes during frozen storage. However, with certain species of fish, it appears to be of primary importance.

To objectively determine the extent of textural deterioration due to DMA and FA formation and subsequent reactions, the measurement of DMA content is recommended. Due to the equimolar formation of DMA and FA in fish muscle and the observed high reactivity of FA, DMA content is routinely used as an index. Consequently, the DMA test indirectly measures the FA value in fish muscle. However, use of this test is limited to those species known to produce DMA and FA during frozen storage.

2. Lipid Oxidation

Another chemical reaction generally associated with quality changes during freezing, frozen storage, and thawing is lipid oxidation. This phenomenon most commonly occurs in fatty fish and is considered one of the major causes of frozen shelf-life reduction.

Lipid oxidation results in the development of a condition described as "oxidative fat rancidity." The extent of oxidation in fish lipids varies with the quantity and the type of lipids in the fish muscle, i.e., fatty species are more prone to oxidation than lean species, and species with more highly unsaturated fatty acids are less stable than the other species. When oxidative rancidity progresses sufficiently, it leads to the development of obvious off-taste and odor, resulting in reduced shelf life.

Changes in fish lipids may be related to changes in protein during frozen storage. Several reports indicate that the unstable free radical intermediates formed during autoxidation attack the protein molecules, leading to the formation of protein free radicals (88). These protein free radicals may cross-link with other proteins to form protein-protein aggregates and with lipids to form protein-lipid aggregates (7).

Another possible mechanism for reaction between oxidized lipids and proteins occurs through stable oxidation products such as malonaldehyde, propanal, and hexanal (26), which covalently react with specific functional groups on protein side chains, including the –SH group of cysteine, the amino group of lysine, and the N-terminal amino group of aspartic acid, tyrosine, methionine, and arginine (11). Such interactions increase the hydrophobicity of proteins, making them less water soluble. Free fatty acids (FFA) formed during autoxidation produce indirect effects on textural degradation by promoting protein denaturation (86). FFA are believed to bind myofibrillar proteins, specifically actomyosin, rendering it unextractable (26, 29). According to Sikorski et al. (29), when the hydrophobic sites of FFA interact with protein molecules, the protein molecules

become surrounded with a more hydrophobic environment, which subsequently results in a decrease in protein extractability. This interaction may occur through hydrophilic and hydrophobic forces (29).

Several techniques have been developed to assess the extent of lipid oxidation in fish muscle. The most common techniques include (1) the peroxide value (PV) test, which measures the amount of hydroperoxides or peroxides formed during autoxidation (this test provides only a means for predicting the risk of rancidity development) and (2) the thiobarbituric acid (TBA) test, which measures the amount of malonaldehyde formed upon the decomposition of hydroperoxides during the second stage of oxidative rancidity. Other methods are also undoubtedly available. Therefore, the choice of techniques depends on several factors, such as the accuracy required and the availability of equipment.

VI. MICROBIOLOGY

Most matters of animal and plant origin used as human food are subject to microbiological attack as well as chemical, biochemical, and physical changes. At room temperatures the microbial attack is often so rapid that all the other changes play only a minor role. Microorganisms in all food raw material release enzymes into substrates during their growth. Changes brought about by the activities of these microbial enzymes will alter the odor, flavor, texture, and appearance of the product. Occasionally this is advantageous but in general it causes deterioration and spoilage. The purpose of preservation, however it is accomplished, is to prolong the storage life of the particular food and this is done either by killing the microorganisms or by inhibiting their activity and multiplication.

Freezing and subsequent storage will kill some of the microorganisms present in the unfrozen material, but this is a slow and variable process depending, in part, upon the nature of the food. Thus freezing cannot be relied upon to substantially reduce bacterial contamination present in the foodstuff. The hygienic state of the product before freezing is consequently all-important. Storage at temperatures colder than −12°C inhibits microbial growth and therefore is one effective method of preserving food against microbial spoilage.

Three aspects of microorganisms in frozen foods will be considered.

A. The Resistance of Microorganisms against Freezing and Frozen Storage

Some pathogens are more resistant to freezing than are ordinary spoilage organisms. A direct examination for common or expected pathogens should therefore be carried out. Most of the common pathogenic bacteria are Gram-negative. This group is more sensitive to freezing, frozen storage, and thawing than are the Gram-positive spoilage organisms.

B. Multiplication of Microorganisms in Frozen Foods

Even when microbial growth is completely inhibited, the frozen product can still deteriorate due to the activity of the released microbial enzymes which can still catalyze undesirable biochemical reactions in the food. When the handling of fish before freezing is improper, there is the danger that microorganisms may have released sufficient enzymes and toxin to affect the quality of the frozen product. For example, if lipases are produced before freezing, they can cause marked hydrolysis of fats in fatty fish even when stored at −30°C. If fish has been held at relatively high temperatures before freezing, any pathogens present could multiply and some may produce toxins. The latter will survive freezing and constitute a health hazard. A product destined for freezing should receive the same degree of hygienic handling as that which is to be stored at chill temperature.

Several psychrotrophic microorganisms can multiply at freezing temperatures. In practice, bacterial growth does not occur below temperatures of −10°C. This is probably due to the increasing concentration of soluble salts and organic compounds in the unfrozen water which will decrease the water activity of this fraction. Only the most drought-resistant microorganisms such as fungi and yeasts can grow in these physiologically very dry substrates and this is why these organisms are the ones which are still capable of growing at temperatures colder than those at which bacteria can grow. Yeasts are not reported to multiply below −12°C and fungi not below −18°C. It must, however, be noted that microbial growth is extremely slow at these temperatures and, for practical purposes, can be disregarded below −10°C. During any long retention in the upper freezing range down to −10°C yeasts or fungi may develop and form visible colonies on the surface of the frozen substrate.

C. Microbiology of Thawed Foods

On thawing, frozen foods will spoil almost at the same rate as would be expected from unfrozen products with the same microbial population maintained at similar temperatures. Condensation of moisture on the surface of the product should be avoided as during thawing it may cause a speeding up of microbial growth.

Pathogenic organisms may grow and produce toxin in food without rendering the food unpalatable. They will be occasionally observed in any food even hygienically prepared. Contamination even with small numbers of pathogenic bacteria during preparation of foods for freezing should therefore be avoided as far as practicable.

Competition between different types of microorganisms is important if food that will be further prepared after thawing is stored in the thawed state before such preparation. A food lacking a normal flora of spoilage organisms but contaminated with a few pathogens is more likely to present a health hazard than the same food contaminated to the same degree with normal spoilage flora. In the case of frozen foods allowed to thaw slowly, the psychrotrophic flora is likely to dominate and may so alter the substrate as subsequently to inhibit or slow down the multiplication of any pathogens present when thawing is complete. Packaging exerts little effect on the spoilage pattern; even vacuum packing causes a negligible increase in the growth rate of anaerobes like *Clostridium botulinum* during storage after thawing.

D. Effects of Freezing, Frozen Storage, and Thawing on Microbiological Quality and Safety

It is readily apparent that spoilage changes in fresh fish occur most commonly as a result of bacterial activity. The species of bacteria vary according to storage temperature. In fish stored in ice, *Alteromonas*, *Achromobacter*, and *Flavobacter* spp. predominate. At temperatures between 35 and 55°C, *Micrococcus* and *Bacillus* spp. constitute the main microflora. Some of these microorganisms produce very active proteolytic enzymes, which produce odor, flavor, and textural problems.

When fish and seafood are frozen, the microorganisms present in their tissues are generally inactivated. Thus, during frozen storage, microbiological changes in fish tissue are usually minimal. Microorganisms not destroyed by the freezing process generally do not grow and in some cases die off slowly. Although some microorganisms survive storage at very low temperatures, their activities are suppressed, and bacterial numbers may be considerably reduced if recommended temperatures are maintained (100). The temperature below which microbial growth is considered minimal ranges from −10 to −12°C (101). Microorganisms, however, that survive and remain inactivated during frozen storage resume growth when the fish is thawed and may then lead to microbial spoilage of the thawed product.

Frozen fish are far from sterile and cannot therefore be considered a microbiologically safe product. The microbial activities in fish after thawing depend on the degree of freshness of the raw material, the natural microflora in the fish tissues, and the thawing technique utilized.

1. Stability of Frozen Products

The effects of various freezing conditions on quality and shelf stability of frozen fish and seafood have received considerable attention recently. Studies have dealt with either the stability of the frozen product as related to storage temperature and fluctuation in storage temperature or the effectiveness of food additives in providing shelf stability to frozen products.

a. Effects of storage temperature

The apparent effects of storage temperature on shelf-life stability of frozen fish are related to protein denaturation and lipid oxidation. The effects of temperature on protein denaturation have been comprehensively studied (37, 102). Maximum denaturation is reported to occur at −4°C in cod muscle (36), while changes in extractable proteins in haddock have been found to be greatest at −2 to −6°C (103).

The rate of lipid oxidation and the accumulation of FFA were observed to increase with temperature (77). In a study using various species of fish, it was observed that maximum production of FFA due to enzymic activities of lipases occurred at −12 to −14°C (104), while the maximum rate of lipid hydrolysis was detected at temperatures just below freezing (105).

Storage at much lower temperatures can, therefore, prolong the shelf life of frozen fish. For example, cod stored at −160°C showed no detectable deterioration after 6 months of storage (77). Even at −65 and −50°C, frozen samples exhibited very few changes after 9 months of storage. Such observations also suggest that low storage temperatures limit the problems associated with protein denaturation and lipid oxidation during frozen storage.

Several studies have been conducted in an attempt to determine the shelf life of frozen fish at different temperatures and to establish storage temperatures that can minimize quality deterioration in specific groups of fish. Poulter (106) reported that *Rastrelliger brachysoma* (club mackerel) stored at −10°C remained acceptable until the ninth month of storage, whereas samples kept at −30°C were rejected after 12 months of storage. *Scomber scombrus* (Atlantic mackerel) stored unwrapped at −18°C were rejected after 3 months of storage, while samples at −26°C remained acceptable until the sixth month of storage (107). Early rejection of fatty species at relatively low temperatures is reportedly due to the development of rancid flavor and odor. Several studies have also reported the same dependence of shelf life for different fish species on temperature (15, 108, 109).

Clearly, fish composition has an appreciable effect on shelf-life stability of frozen fish. For instance, in a comprehensive study using different fish species, it was found that fatty fish such as mackerel, salmon, herring, sprat, and trout had a shelf life of 2–3 months at −18°C, whereas lean fish such as cod, flounder, haddock, ocean perch, and pollock exhibited storage stability of up to 4 months at the same storage temperature (110).

Based on several studies, it is recommended that those species most susceptible to oxidative rancidity be stored at very low temperatures (at least −29°C) while species less susceptible to rancidity should be stored at temperatures between −18 and −23°C (18). For species with textural

problems due to the TMAO breakdown, the storage temperature must be below −30°C.

b. Effects of fluctuations in temperature

Fluctuations in storage temperatures affect the shelf-life stability of frozen products due to an increase in the size of the ice crystals formed in fish tissues (26). With slight increases in temperature, small ice crystals melt faster than larger ones, so that when the temperature drops again, the melted ice refreezes around the large ice crystals, forming larger crystals. These large crystals accelerate freezing damage, leading to shorter storage stability.

c. Use of food-grade additives

The effectiveness of different food-grade additives has also received considerable attention recently. The most commonly used types of additives for fish and seafoods function either as antimicrobial agents or as antioxidants.

i. Antimicobial agents

Additives are commonly used in the food industry to prevent the growth of bacteria, yeast, and molds. The selection of an antimicrobial agent or any combination of agents is rather complicated, especially when dealing with fish. The effectiveness of an antimicrobial agent depends on several factors, such as the moisture content of the product and the presence of other microbial inhibitors like smoke and salt.

Several antimicrobial agents have been tested for fishery products. For instance, the sorbic acid salt, potassium sorbate (KS), has been found useful in extending the shelf life of fresh fish. Studies have demonstrated that KS, when applied as part of the ice, increased ice storage stability of red hake and salmon up to 28 and 24 days, respectively (111, 112). KS, in combination with modified atmosphere packaging (MAP), was also determined to be an effective method for prolonging the shelf life of fresh whole and filleted haddock on ice (113).

The shelf life of fresh fish may also be extended under refrigerated conditions with the use of Fish Plus, which exhibits its preservative effect due to the combined action of components such as citric acid, polyphophates, and potassium sorbate. Citric acid lowers the muscle pH, which consequently creates an optimum environment for potassium sorbate to exhibit its antimicrobial effects. Dipping in Fish Plus has been found to extend the shelf life of lingcod on ice to as much as a week (114). Fish Plus may also be used on frozen fish.

ii. Antioxidants

An antioxidant is a substance capable of delaying or retarding the development of rancidity or other flavor deterioration due to oxidation. It is normally used in conjunction with freezing to reduce the rate of autoxidation during frozen storage. Antioxidants delay the development of rancidity by either interfering with the initiation step of the free radical reaction or by interrupting the propagation of the free radical chain reaction (115).

The kinetic of antioxidative action was considered to be that antioxidants act as hydrogen donors or free radical acceptors (AH) and react primarily with ROO· , not with R· radicals.

$$\mathrm{ROO\cdot} + \mathrm{AH} \longrightarrow \mathrm{ROOH} + \mathrm{A\cdot}$$

A low concentration of this chain-breaking antioxidant (AH) can interfere with either chain or initiation, producing nonradical products:

$$\begin{matrix} \mathrm{A\cdot} + \mathrm{ROO\cdot} \longrightarrow \\ \mathrm{A\cdot} + \mathrm{A\cdot} \longrightarrow \end{matrix} \text{nonradical products}$$

Different versions of the antioxidant mechanism have been suggested by different authors (100, 115).

Other antioxidants may function as metal-complexing agents, which partly deactivate the trace metals, often present as salts of fatty acid (100), which would otherwise promote the oxidative reaction. Citric, phosphoric, ascorbic, and erythorbic (isoascorbic) acids are typical metal-chelating agents.

Among these antioxidants, erythorbic acid was used in studies by Kelleher et al. (116) and Licciardello et al. (35) of shelf-life stability of frozen fish. This antioxidant was emphasized due to encouraging results with the use of its salt, sodium erythorbate, in retarding oxidation in whiting, chub mackerel, and white bass fillets (35, 117, 118). Licciardello et al. (35) demonstrated the effectiveness of erythorbic acid in the retardation of oxidative rancidity in fillet blocks of Argentine hake stored at −18°C.

However, the use of erythorbic acid is limited to fish species in which rancidity is the main problem. Kelleher et al. (116) demonstrated the effect of this compound on the frozen storage of red hake (*Urophysis chuss*), a gadoid species, in which lipid oxidation is not the limiting factor for shelf-life extension. They found that the rate of DMA formation at −18°C in samples dipped in erythorbate solution was significantly greatly than the rate in untreated samples. Such effect of erythorbic acid on DMA formation may be explained by the fact that this acid acts as an alternative and preferred scavenger of oxygen, leaving metal ions that would otherwise bind to oxygen and be inactivated, available to catalyze the degradation of TMAO to DMA and FA (119).

APPLICATIONS

I. HYGIENE IN THE PREPARATION OF FROZEN SEAFOOD

Most foods and food products are susceptible to attack by microorganisms and they are always contaminated by a variety of such organisms present in the food production chain. Foodstuffs are subjected to further contamination

during preparation for freezing as a result of contact with the hands of factory staff during preparation, packaging and transport, and with air or water.

In view of the hazards to health and the effect of microbial contamination on quality, every effort must be made to reduce such contamination to a reasonable level during the preparation of foodstuffs for freezing. Throughout the world frozen foods have very seldom been the cause of food poisoning incidents.

A. Human Contamination

As pathogenic bacteria like *Salmonella* and *Staphylococcus* frequently derive from human sources, it is of vital importance that factory employees are aware of the basic concepts of good personal hygiene: the need for frequent washing and the wearing of clean clothes, overalls, hair covering, etc. The use of rubber gloves and a protective mask for the mouth may be desirable in some instances. When gloves are worn they must be thoroughly cleaned and inspected before use. Medical supervision is advisable and in some cases should be made compulsory. Notices in lavatories should draw attention to personal hygiene, especially the need for hand washing. Soaps, hand creams, or dips containing antiseptic agents should be readily available.

B. Buildings and Equipment

Design of the building should ensure that both the buildings and drains are vermin proof. Interior walls, floors, and ceilings should be finished with a non-flaking surface capable of withstanding detergents and sanitizers. All corners should be rounded to facilitate cleaning. The building should be large enough to house production equipment so that all sides of the equipment are accessible for cleaning.

Entrance to the process area should be supplied with adequate washing facilities with foot-operated taps. Wood, which is almost impossible to sanitize, should not be used in contact with food. All windows should be both bird and fly proof.

Equipment usually becomes soiled with organic residues, which act as carriers of microorganisms. It should be designed and constructed to prevent hygienic hazard and permit easy and thorough cleaning. All surfaces should have a smooth, hard, waterproof finish. Cutting boards should be of a hard material; plastic is preferable to wood. Cleaning and disinfection of the food handling area, including equipment and utensils, should be carried out at frequent and regular intervals. Waste materials should be frequently removed, in covered containers, from the working area during factory operation. Processes should be so separated — either in space or time — as to avoid recontamination of products in which the "bacterial load has already been reduced." For cleaning purposes an ample supply of potable water should be available. Chlorinated water is effective both for in-plant use (when concentrations of around 5 to 10 ppm are appropriate) and for sanitation of equipment and surfaces (when concentrations of around 100 to 200 ppm are used) but this should be followed by a rinse. Organic residues inactivate chlorine which, therefore, should only be used to sterilize already clean surfaces. Chlorine can be responsible for flavor loss or taint (due to the formation of chloramines or chlorophenols). Removal of the chlorine (by thiosulphate addition, often combined with treatment in activated carbon towers) is practiced, especially in ice cream producing plants.

C. Cross-Contamination

During the preparation of food for freezing, all efforts should be taken to avoid a build-up of an undesirable microbial population. For some foods, handling should take place at sub-ambient temperature in temperature-regulated rooms, and where a heat treatment is a part of the processing this should be so severe that most of the microorganisms are killed. After heat treatment, the food should be promptly cooled to avoid multiplication of the surviving bacteria in the critical zone between 50 and 10°C. Cooling water, if used, should be chlorinated.

D. Bacteriological Control

Proper organization of the various processes from the hygienic point of view is essential and a constant watch should be kept for lapses in hygiene. This should include a bacteriological control of the various stages in the processing line. Bacteriological methods are now available that give a good estimate of the bacterial load of the raw material and of food contact surfaces. Methods that give a rapid result are especially useful, as they supply plant management with information on the bacteriological state of products actually under preparation.

The results of the examinations should be shown to the factory staff in order to make them comprehend the vital importance of hygiene in food production. Preferably, courses in food hygiene should be held at regular intervals for employees. The aim should be to give those engaged in food production a thorough understanding of the hazard involved.

The above considerations concerning personal hygiene, equipment, and preparation of foods also applies to handling of frozen foods in catering establishments. Thawing of frozen foods in these establishments should be completed as quickly as possible, and any storage of the food after thawing should be in a refrigerator.

II. PACKAGING

A. General Requirements

Not only must packaging used for frozen foods meet all the requirements of normal packaging but it must

also meet requirements of packaging suitable for food such as:

- Chemical inertness and stability;
- Freedom from taint and odor;
- Freedom from toxic materials which may migrate into the food;
- Impermeability, or nearly so, to water vapor and other volatile constituents as well as to any odors from the surroundings;
- Suitability for use in automatic packaging systems;
- Suitable size and shape for display in retail cabinets;
- Protection from bacterial contamination and filth;
- Ease of opening; and
- An attractive appearance.

In addition to these general requirements for food packages, frozen food packages should also:

- Be of such shape as to allow rapid freezing except for I.Q.F. (Individually Quick Frozen);
- Permit volume expansion in the freezing process;
- Be impermeable to liquids and have good wet strength and resistance to water and weak acid;
- Be able to withstand low temperatures, not becoming excessively fragile at cold temperatures encountered during the freezing process;
- Not adhere to the contents in the frozen condition;
- Have a high reflectivity to reduce heat gain by radiation during display in retail cabinets;
- Be impervious to light as far as practicable; and
- Surround the product closely, leaving the minimum of air entrapped, thus limiting sublimation during storage.

B. Packaging Materials

A wide variety of materials have been used in devising packaging systems for various frozen foods, e.g., tinplate, paper, paper-board with a wax or plastic coating, aluminum foil, plastic film, thermoformed plastics, and laminated combinations of these materials.

Low permeability to water vapor is an important characteristic of packaging materials for frozen foods. Table 39.2 compares the permeability of commercially available packaging materials.

1. Paper-Board Packages

Paper-board package for foods is generally in the form of folded cartons, either directly printed or provided with printed wrappers on the outside and in some cases with plastic coated liners. The following coating or laminating materials are commonly used:

- Wax blends (paraffin and micro-crystalline compositions);
- Plastics, e.g., polyethylene or polypropylene (on one or both sides);
- Aluminum foil.

TABLE 39.2
Water Vapor Permeability*

Film Type, 0.025 mm (1 mil)	Transmission Rate at 38°C (100°F), 90% RH
Polyvinylchloride	120–190 g/m^2.24 h
Polyamid (Nylon)	120g/m^2.24h
Polyester (Mylar)	25 g/m^2.24 h
Polyethylene, low density	19 g/m^2.24 h
Polyethylene, high density	6g/m^2.24h
Cellophane MST-type	5–23 g/m^2.24 h
Polyvinylidene chloride (Saran)	1.5–5 g/m^2.24 h

* The data given in this table refer to a test temperature of 38°C and the ranking order between different materials in respect to water vapor permeability. Water vapor permeability of a good package should not exceed about 0.2–0.5g/m^2.24h at −20°C and 75% RH. The important factor, however, is not just the water vapor permeability of the packaging material, but that of the complete package.

2. Wrappers and Bags

Materials most commonly used are: waxed paper; hot melt or plastic coated paper; aluminum foil; coated cellulose films such as MSAT (moisture-proof, scalable, anchored, and transparent); and plastic films, such as polyethylene (PE), polypropylene (PP), and polyvinylidene chloride (PVDC) films. Also of importance are the laminated materials built up from two or more of the above-mentioned materials or other films. Common combinations are cellulose and PE films, sometimes with a PVDC coating.

These materials are used as over wraps, liners, and as single or double wall bags. The bags are either of the prefabricated type or are formed from roll stock on a filling machine.

Particularly important are shrink-wrap materials because of their ability to adhere close to the product, leaving few if any air pockets. Shrink-wrap bags require evacuation of entrapped air before shrinking. Some of these bags can withstand boiling water and, therefore, the package can be used for end-cooking of the product before serving.

3. Wooden Boxes

Often used for fish, wooden boxes require an inner liner or glaze on the product to guard against desiccation.

4. Rigid Aluminum Foil Packaging

These packages, in the form of trays, dishes, and cups, are generally covered with a crimped-on aluminum sheet or a sheet of aluminum foil laminated to paper-board. Normally used for prepared foods and pastries, etc., they allow rapid heating of the product in the package before serving.

5. Semi-Rigid Plastic Packages

These are mostly manufactured from high density PE or PP in the form of trays and plates, covered by a lid; as with aluminum foil, these can also be used for prepared foods requiring heating before serving, providing only gentle heat, such as steaming, is employed.

6. Tin and Composite Containers

These are used mainly for frozen juices which often have a mobile liquid phase even at cold store temperatures. A more recent development is to use coated paper-board in the body and aluminum for the ends, coupled with an easy opening device.

7. Shipping Containers

These are normally manufactured from different materials such as solid, corrugated fiber-board or vulcanized fiber-board paper, and plastics. They are often good heat insulators.

C. Packaging Machinery/Packaging Systems

An essential requirement of any package used in modern industry is that it should form a part of a system which enables the packages to be formed, filled, sealed, and handled mechanically on an integrated packaging line.

1. Form-Fill-Seal Machines

These machines form pouch-shaped or tray-shaped packages from heat-scalable plastic films or laminates or plastic-coated papers in roll form. The packages are formed and filled in the machine simultaneously or consecutively; these machines can work either in a vertical or a horizontal plane.

2. Cartoning System

These can be top filled, end filled, or side filled but irrespective of these differences each machine should perform the operations of erecting, filling, and sealing.

3. Shrink Film Wrapping Equipment

These machines apply shrink film materials from rolls around a given number of consumer-sized packages to form a unit, often replacing cases or boxes. After the application of the film, mostly in the form of a sleeve wider than the width of the contents, the unit is passed quickly through a hot air oven which shrinks the film tightly around the unit.

III. BULK PACKAGING

The practice of storing frozen food in bulk has increased considerably for the following reasons:

- The economy of storage space thus achieved;
- The ability to separate an intricate labor-intensive further processing packaging operation from the essential processing and freezing operation carried out when the raw material is available;
- Flexibility in final package sizes of various produces.

Bulk packaging is extensively applied to fruit and vegetables and to a lesser extent to meat, fish and poultry. Individually frozen products, the form of freezing particularly applied to vegetables, is most suitable for bulk storage. Products may also be bulk frozen into blocks, this method is used primarily for fish and meat.

After freezing, vegetables such as peas, corn, beans and sprouts can be stored on site in silos holding one or more hundreds of tons. Bins of corrugated board, metal or timber construction, with polyethylene liners providing a moisture vapor barrier and protection against dirt, can be used for storage or shipping. Smaller containers may be multi-wall paper sacks, corrugated boxes or fiber drums provided with polyethylene liners. Storage temperatures should be constant to prevent formation of clumps which have to be removed during repacking. This operation normally consists of tipping the contents out of the bulk container, breaking any clumps before passing the product over a screen or through an air-cleaner to remove small pieces prior to visual inspection and repacking, care being exercised over the hygiene requirements of this operation.

Frozen-at-sea whole fish is frozen in blocks or individually. In both cases, the fish, if not packaged, should be glazed prior to storage to minimize desiccation. Some fish is slabbed as skin-on fillets or made into blocks of skinned and boned fish for later cutting; both packs should be cartoned or bagged to minimize desiccation.

IV. FREEZING

Freezing is simply the crystallization of ice in muscle tissue and includes the consecutive processes of *nucleation* and *growth*. These processes are central to the effects of different freezing rates and subsequent effects on meat quality. Meat does not freeze immediately when its temperature drops below the freezing point and the latent heat (i.e., heat required during the phase change during crystal

formation) has been removed. In other words there is a degree of *supercooling*. The greater the supercooling, the greater the number of nuclei formed. The number of nuclei is greatest in the extracellular space, and they are only formed within the cell when the rate of heat removal is higher. As soon as the nuclei form, they begin to grow by the accumulation of molecules at the solid/liquid interface. However, the way they grow depends on the microgeometry and the temperature distribution ahead of the freezing front, in a complex way, as a consequence of dendrite formation with supercooling in front of the dendrite growth. An important concept is *characteristic freezing time*, which is a measure of the local freezing rate and is defined as the time during which the point under consideration decreases from −1°C (freezing commences) to −7°C (when 80% of the water is frozen). The growth of extracellular ice crystals also takes place at the expense of intracellular water. This leads to partial dehydration of the muscle fibers and subsequent distortion. At high characteristic times (slow freezing), the ice crystals are larger and the tissue distortion is greater.

The freezing process can rapidly minimize physical, biochemical, and microbiological changes in the food. This preservative effect is maintained by subsequent storage of the frozen food at a sufficiently cold temperature.

A. Freezing Process

The freezing process can be divided into three stages:

Stage 1: Cooling down from the initial temperature of the product to the temperature at which freezing begins. It must be borne in mind that the act of placing a product in a freezing apparatus does not render it "safe." Time will elapse before warm food passes out of the microbiologically hazardous temperature zone; this is particularly the case if freezing is carried out in slowly moving air as in the freezing of bulk products such as berries for subsequent jam manufacture.

Stage 2: This step covers the formation of ice in the products and extends from the initial freezing point to a temperature about 5°C colder at the center of the product. The major part of the freezable water will be converted to ice and this quite small reduction in temperature is accompanied by a massive enthalpy change.

Stage 3: Cooling down to the ultimate temperature for storage. When leaving the freezer, the frozen product will have a non-uniform temperature distribution: warmer in the center and coldest at the surfaces. Its average temperature will correspond to the value reached when the temperature of the product is allowed to equalize. In general, it is recommended to cool the product in the freezer to an equilibrium temperature of −18°C or colder. Product leaving the freezer with a warmer temperature will be stored for some time in relatively unfavorable conditions. Cooling down to storage temperature may take days or weeks.

B. Freezing Time

The effective freezing time is determined not only by the initial and final temperature of the product and its change in enthalpy but also by the temperature of the heat transfer medium. The dimensions (especially the thickness) and shape of the product unit affect the overall heat transfer, which includes the surface heat transfer coefficient α and the heat conductivity λ characteristic of the product. When freezing by air blast α depends on the air velocity and the shape of the product. In an air blast freezer, the rate of freezing increases with increasing air velocity to an optimum value. The refrigeration load necessary to remove the heat produced by the fans increases with the cube of the air velocity; this factor should be taken into consideration when designing air blast freezers. It is important to direct the air circulation in such a way that all the product is equally exposed to the air current. In packaged food the packaging material presents a resistance to the heat transfer, depending on its thickness and conductivity. This resistance is increased considerably if air is trapped between the package and the product.

The freezing time as a function of the thickness of fish fillets in packages frozen in a plate freezer (α=200 kcal/m^2.°C including the packaging) and in a blast freezer with medium air velocity (α=20 kcal/m^2.°C) with heat removed from both sides of the package, is shown in Figure 39.1(a) (120). This graph indicates that during plate freezing the heat conductivity of the product is the main factor determining freezing time; it also shows that during air freezing the heat resistance of the surface (including packaging material) plays a dominant role for the product thickness encountered in practice.

Figure 39.1(b) (120) shows the freezing time for 450 g fish fillet packs as a function of the overall heat transfer, including the influence of some types of packages used in contact freezing. This graph indicates that the type of packaging alone may increase the freezing time in a plate freezer by 2.5 times, and the surface heat transfer resistance by some 4 times. The freezing of packaged foods takes longer in an air blast freezer than in a plate freezer under comparable conditions. In the freezing of packaged food, where the λ-value is influenced by the air inside the package, the difference in freezing rate between plate and blast freezing diminishes as does the influence of packaging material.

C. Freezing Rate

Freezing must always be fast enough to minimize the development of microbiological and enzymatic changes in the product. A freezing process which occupies a matter of days will, in most cases, lead to deterioration in the frozen foodstuffs.

In the past, the beneficial effect of very rapid freezing on the quality of frozen foods has been overestimated: within certain limits the rate of freezing does not

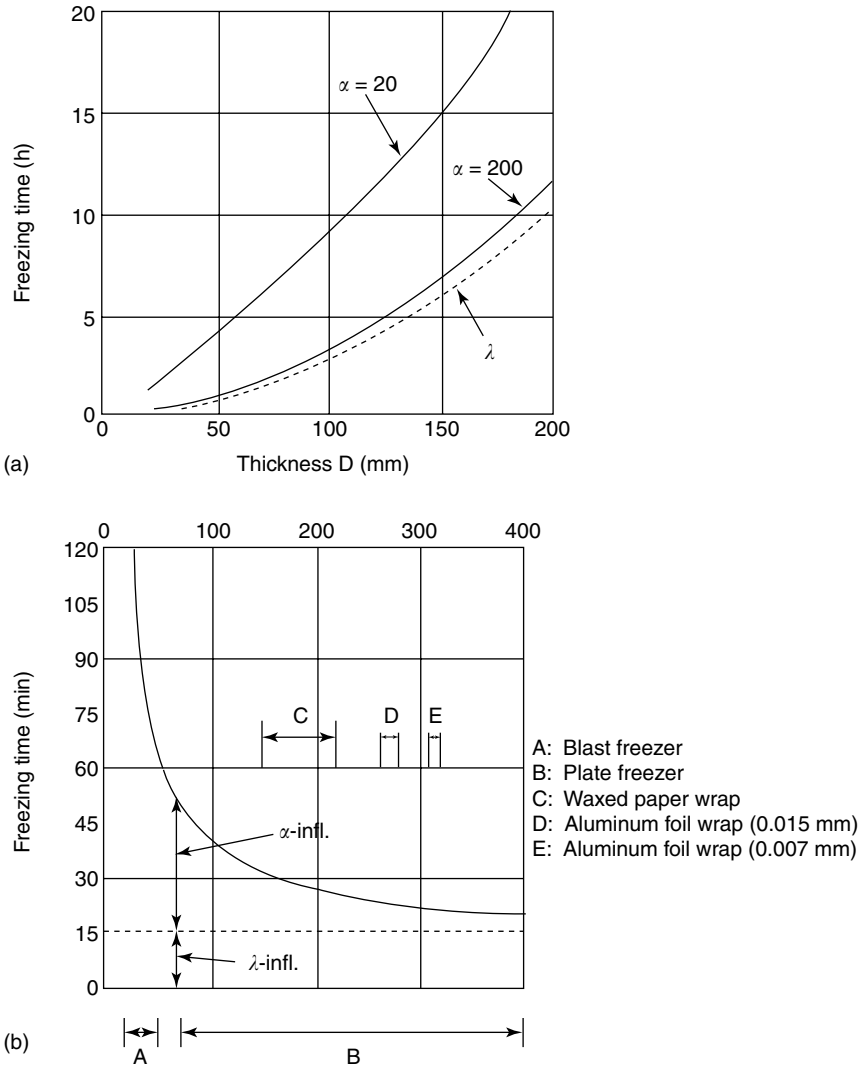

FIGURE 39.1 (a) Freezing time in hours as a function thickness D, of a slab of fish fillets frozen by removal of heat from two opposite sides of the package, for two values of the surface heat transfer coefficient $\alpha=20$ and $\alpha=200$ kcal/m^2. °C. A temperature of -30°C is assumed for the cooling medium (air, cold plates) and the process is considered to be completed when a temperature at the thermal center of -15°C is reached. The total freezing time is divided into two parts, one due to the influence of the internal conduction resistance ("λ-infl."), the other to the surface heat resistance, including package ("α-infl."). (b) Freezing time in minutes for a 32 mm thick 450 g package of fish fillets as a function of the surface heat transfer coefficient. The total time is divided according to the influence of conduction resistance ("λ-infl.") and surface resistance, including package ("α-infl."). The normal range of blast freezers and plate freezers is indicated. The influence of some types of packages in plate freezing is also shown.

materially affect the quality of most foods. This should not be interpreted to mean that the rate of freezing has no effect on the quality of frozen foods: most foods suffer from being frozen very slowly; a few foods demand ultra-rapid freezing. Fish and poultry seem to be more vulnerable to very slow freezing than most other foods, and meat (beef, pork, lamb) rather less. Strawberries and beans have a better texture and water holding ability if frozen ultra-rapidly while fruits and vegetables with a higher starch content such as peas are not as sensitive to freezing speed.

In commercial practice mean freezing rates vary between 0.2 and 100cm/h; 0.2cm/h (slow freezing) for bulk freezing in blast rooms, 0.5 to 3 cm/h (quick freezing) for retail packages in air blast or plate freezers, 5 to 10 cm/h (rapid freezing) for individual quick freezing of small-sized products, e.g., in a fluidized bed and 10 to 100 cm/h (ultra rapid freezing) by spraying with or immersion in liquid gases. For freezing of retail packages freezing rates faster than 0.5 cm/h and for I.Q.F. products rates faster than 5 cm/h are considered satisfactory in most cases. Only very susceptible foods (such as tomatoes)

may be improved by increasing the freezing to above 10 cm/h. At these rates care should be taken to avoid cracking. When freezing larger units, such as beef quarters, with a mean freezing rate of 0.1 cm/h a freezing time of 3 to 5 days is unavoidable and times up to 5 days are quite common.

The rate at which freezing takes place can be considered both at micro and macro levels. At the micro level, freezing rate is described in terms of the speed with which the freezing front moves through the freezing object. At the macro level, the rate at which any given part of the object is cooled determines the temperature profile for that part, and thus has an important bearing on the biochemistry and microbiology of that part.

The undesirable changes in meat during freezing are associated with formation of large ice crystals in extracellular locations, mechanical damage by the ice crystals to cellular structures through distortion and volume changes, and chemical damage arising from changes in concentrations of solutes. The fastest freezing rates are associated with the least damage (121). Differences in freezing rate modify meat properties. Ice crystallization and its growth in meat tissues are discussed by Calvell (122).

Freezing commences when the surface temperature of the meat reaches its freezing temperature. A continuous freezing front forms and proceeds from the exterior to the interior. Extracellular water freezes more readily than intracellular water because of its lower ionic and solute concentration. Slower freezing favors the formation of pure ice crystals and increases the concentration of solutes in unfrozen solutions. Intracellular solutions are often deficient in the nucleation sites necessary to form small ice crystals. Such conditions favor the gradual movement of water out of the muscle cells, resulting in a collection of large extracellular ice crystals and a concentration of intracellular solutes. Freezing damage arises from massive distortion and damage to cell membranes. Such effects have implications during thawing as the large extracellular ice crystals produce drip during thawing. The structural changes that occur also obliterate the recognizable muscle structure.

Fast freezing results in small ice crystal formation in both intracellular and extracellular compartments of the muscle and very little translocation of water. Drip loss during thawing is thus considerably reduced, and the surface reflects more light than that of slowly frozen meat. Consequently, the cut surface appearance is more acceptable.

D. Freezing Methods and Equipment

Freezing equipment may be divided into the following main groups with regard to the medium of heat transfer: the metal group includes plate freezers and air (gaseous medium) blast freezers the liquid group includes immersion freezers and the evaporating liquid group includes liquid nitrogen and liquid fluorocarbon equipment.

While blast freezers are used for all kinds of products, packed or unpacked, blocks or I.Q.F. products, the Plate freezer and Immersion freezer accept only packaged product, and evaporating liquid freezers are used only for I.Q.F. products.

1. Plate Freezers

In a plate freezer the product is pressed by a hydraulic ram between metal plates which have channels for the refrigerant. This arrangement gives very good heat transfer of metal contact. This high thermal efficiency is reflected in short freezing times, provided the product itself is a good heat conductor, as is the case with fish fillets or chopped spinach. It is important that the packets are well filled and the metal trays that are used to carry the packets are not distorted.

The advantage of good heat transfer at the surface is gradually reduced with increasing thickness of the product. For this reason the thickness is often limited to a maximum of 50 mm.

The pressure from the plates maintained throughout the freezing process practically eliminates the "bulging" that may occur in air blast tunnels; the frozen packets will maintain their rectangular shape within close tolerances.

There are two main types of plate freezer: horizontal plate freezers and vertical plate freezers.

a. Horizontal plate freezer

Usually this type has 15–20 plates (Figure 39.2). The product is placed on metal trays, which are pushed in between the plates manually. This calls for a high labor content in the loading and unloading operation.

In order to obtain automatic operation of a horizontal plate freezer, the whole battery of plates is movable up and down in an elevator system. At the level of a loading conveyor the plates are separated. Packages which have been accumulated on the conveyor are pushed in between these plates, simultaneously discharging a row of frozen packages at the opposite end of the plates. This cycle is repeated until all frozen packages have been replaced. Then the space between the plates is closed and all plates are indexed up.

b. Vertical plate freezer

The vertical plate freezer was developed mainly for freezing fish at sea. It consists of a number of vertical freezing plates forming partitions in a container with an open top. The product is simply fed from the top. The frozen block is discharged either to the side, upwards, or down through the bottom. Usually this operation is mechanized; the discharge of product often being assisted by a short hot gas defrost period at the end of the freezing cycle and the use of compressed air to force the product out.

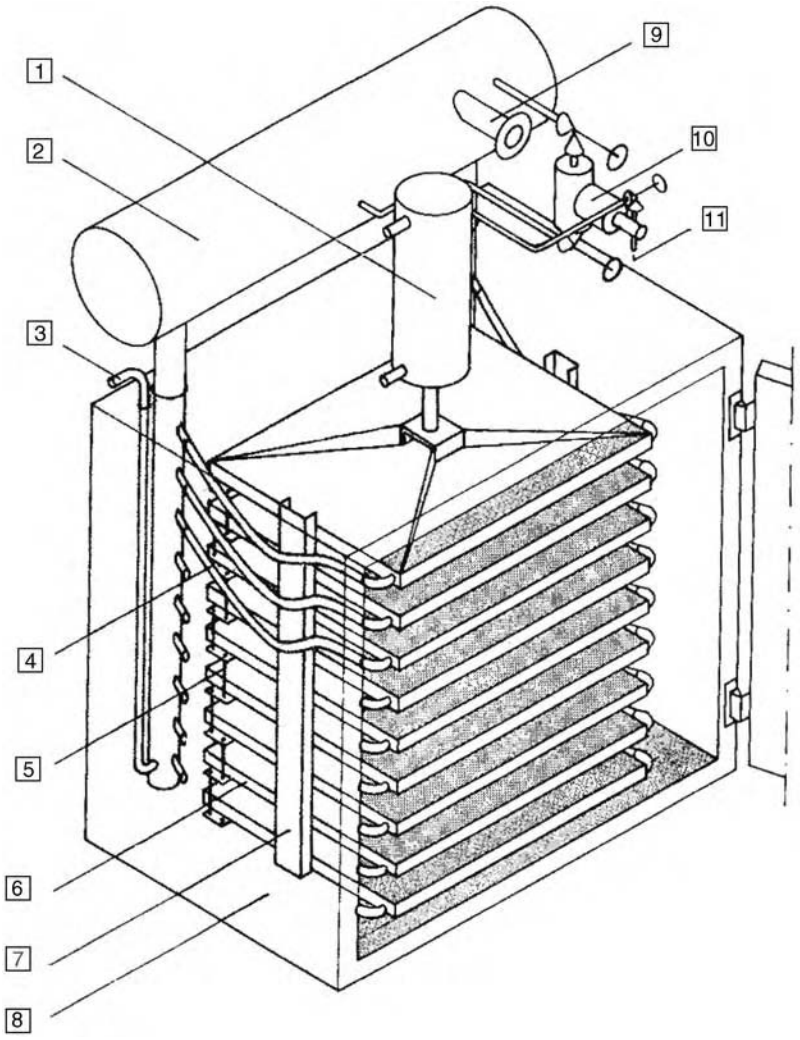

FIGURE 39.2 Horizontal plate freezer. (1. Hydraulic cylinder, 2. Liquid separator, 3. Hot gas defrost, 4. Flexible hoses, 5. Link bolts, 6. Freezing plate, 7. Guide, 8. Insulated cabinet, 9. Suction outlet, 10. Float valve, 11. Liquid inlet.)

2. Air Blast Freezers

Some foods, mainly bulk products such as beef quarters and fruits, for further processing are frozen in rooms with or without forced air circulation. Unless the room has been designed for freezing and equipped with suitable coolers and fans the freezing rate is very slow, resulting in an inferior quality for practically all products. If the room is also used for storage of frozen products the temperature of these products may rise considerably and the evaporators may frost up so quickly that the total refrigeration capacity is reduced below what is required to maintain the temperature of the store.

Good commercial practice for freezing in air blast uses include tunnel freezers, belt freezers, and fluidized bed freezers.

a. Tunnel freezers

In tunnel freezers the product is placed on trays, which stand in or pass through the tunnel in racks or trolleys one behind the other or singly. An air space is left between the trays.

The racks or trolleys are moved in and out of the freezer by manual power or by a forklift truck (stationary tunnels), pushed through the tunnel with a pushing mechanism (push-through tunnel) or are carried through by driving equipment, chain drive, etc. (carrier freezer) or slid through (sliding tray freezer). Tunnels are also used for freezing hanging meat carcasses mostly carried on a suspension conveyor.

Tunnel freezers are equipped with refrigeration coils and fans which circulate the air over the product in a controlled way (see Figure 39.3). Guide devices, properly locating the trays of food, lead to uniform freezing.

Tunnel freezers are very flexible freezers. Products of every size and shape, packaged or unpacked, can be frozen in stationary and push-through tunnels. They are used primarily for freezing packaged products. Unpacked products tend to stick to the trays, which may cause

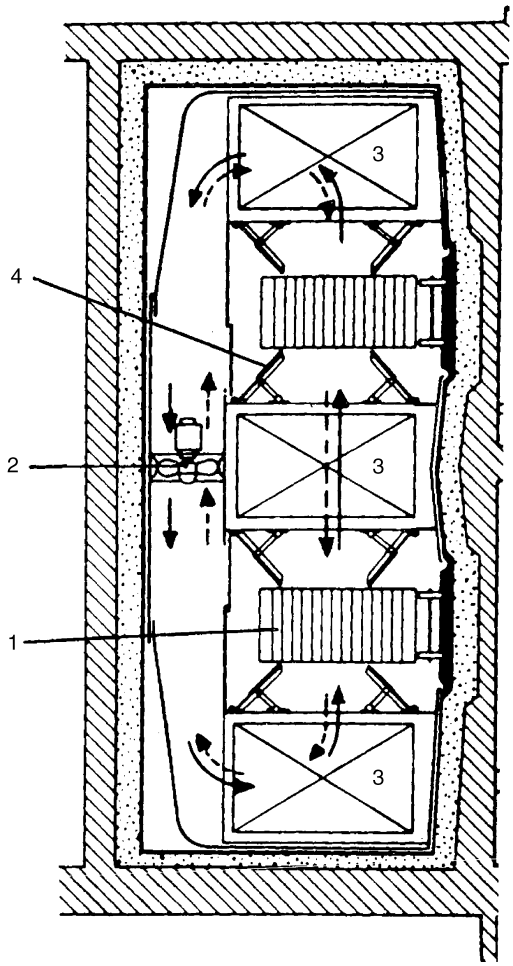

FIGURE 39.3 Sectional drawing of a push-through tunnel freezer. (1. Trolleys with trays, 2. Reversing fans, 3. Air coolers, 4. System of baffles which allows adjustment of the air flow.)

weight losses and time-consuming handling in releasing, cleaning, and transport of the trays. To obtain free flow products, improved handling, and an increase in the freezing rate, individual quick freezing (I.Q.F.) is preferred.

b. Belt freezers

Belt freezers are provided with a single belt (single-belt freezers, see Figure 39.4) or with belts positioned above each other which may run in the same or in opposite directions to increase throughputs and to reduce floor space (multi-belt-freezers) or as a spiral belt wound round a rotation drum stacking up to 30 tiers of the belt above each other (spiral-belt freezers). The belt, generally made of wire mesh, remains inside the freezer so that ancillary equipment for in and out feeding is necessary. Alternatively, the belt is carried to the outside as in some spiral-belt freezers. This arrangement has the advantage that the products can be placed on the belt in the processing room, where the operation can be supervised, before entering the freezer, and the product will remain undisturbed until removed at the outlet (Figure 39.4). The belt is supported by rails and driven by passing around the rotating drum.

Modern belt freezers have vertical air flow so that the air is forced through the product layer. In freezing small products such as beans or cherries good contact with all product particles is thus created. In single-belt freezers with high air velocities the products may agitate. In all belt freezers care should be taken to spread the product uniformly across the total belt width to avoid "channeling," where the air stream bypasses the product.

Belt freezers are used mainly for freezing unpackaged products, e.g., I.Q.F. products. They are especially suitable for foods that need careful handling.

c. Fluidized bed freezer

Fluidization occurs when particles of fairly uniform shape and size are subjected to an upward air stream. At an air velocity depending on the characteristics of the product, the particles will float in the air stream, each one separated from the other, but surrounded by air and free to move. In this state the mass of particles behaves like a fluid. If the product is contained in an inclined trough which is fed at the higher end the fluidized mass moves towards the lower end, as long as more product is added. The product is thus frozen and simultaneously conveyed by air without the aid of a mechanical conveyor (Figure 39.5).

The use of the fluidization principle has the following advantages when compared with a belt freezer:

1. The product is always truly individually frozen (I.Q.F.). This applies even to products with a tendency to stick together, e.g. French style (sliced) green beans, sliced carrots, and sliced cucumber.
2. Independence of fluctuations in load. If partly loaded the air distribution will be the same as for full load, i.e., no hazard of channeling. If over-loaded no product flows onto the floor.
3. Reliability is improved when freezing wet products because the deep fluidized bed can accept products with more surplus water.

An important factor in the overall operation economy of a blast freezer is the weight losses during freezing. Improperly designed equipment will have losses of 5% or more whilst a "well-designed" freezer normally operates with only 0.5 to 1.5% loss for unpackaged products. Part of the weight losses are dehydration losses, which require particular consideration. Weight loss is minimized by low air temperatures and good heat transfer, i.e., high air velocities.

A freezing tunnel that is intended for packaged products should not, without due consideration, be used for thin unpackaged products, e.g., fillets of fish. The result may be that the relation of coil surface to product surface is put out of balance so that air temperature in the tunnel rises with resulting high weight losses. The coil may not be able to accommodate sufficient quantities of frost

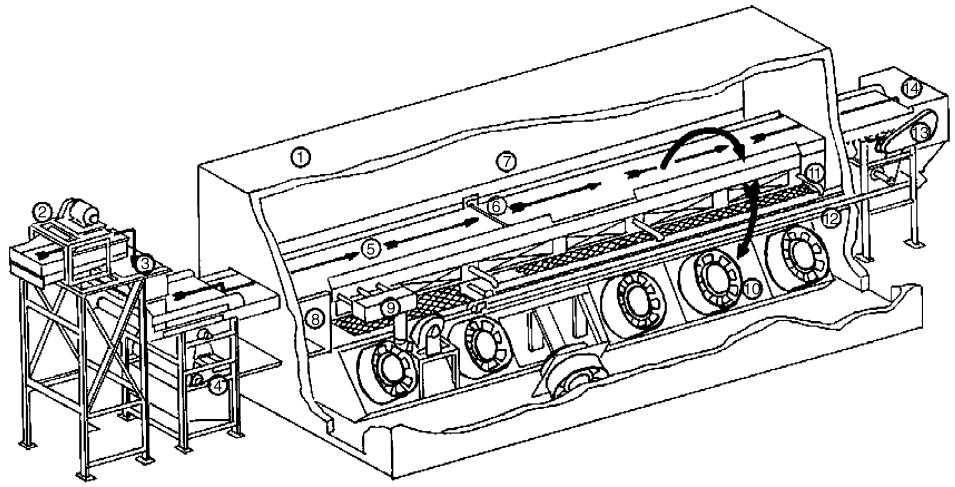

FIGURE 39.4 Single belt freezer. 1. Insulated wall of the tunnel, 2. De-watering vibrator, 3. Loading hopper, 4. Belt drying system, 5. Variable speed belt (open mesh belt), 6. Product spreader, 7. Air agitation zone, 8. Evaporator, 9. High velocity air, 10. Variable air flow fans, 11. Defrost water, 12. Refrigerant piping, 13. Belt speed changer, 14. Unloading hopper.

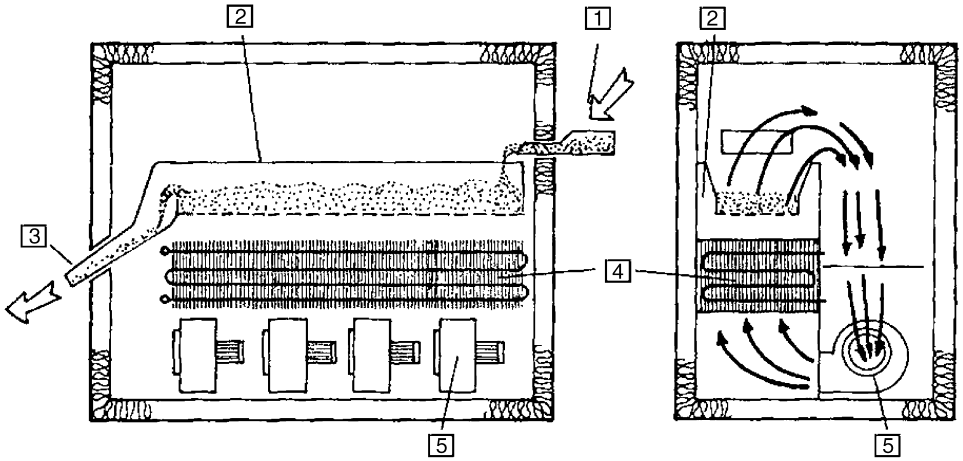

FIGURE 39.5 Fluidized bed freezer. (1. Unfrozen product conveyor, 2. Product trough, 3. Frozen product discharge, 4. Air coolers, 5. Fan.)

which results in reduced heat transfer or reduced air flow, both contributing to high weight losses.

It is important to note that in a vapor tight package containing a product that is not homogeneous, e.g., beans or broccoli, the heat transfer inside the package is carried out by air. The heat transfer is very poor, because there is no air circulation. The result is evaporation of moisture, which actually may be greater than it would have been without the package. This moisture remains as frost on the inside surfaces of the package, so that it is not usually recorded as a weight loss. The influence on product quality is, of course, the same whether the dehydration is recorded or not.

Higher velocities of air give better heat transfer. However, it is not sufficient just to increase the fan power. The most important factor is to direct the air circulation in such a way that every product particle is efficiently and equally exposed to the air current. It is also important to study the conditions of the individual particles because a close study may reveal surprisingly uneven air flows.

3. Immersion Freezer

For irregularly shaped products, e.g., chicken, the best heat transfer is achieved in an immersion freezer. This consists of a tank with a cooled freezing medium, e.g., a salt or propylene glycol solution. The product is immersed in this brine or sprayed while being conveyed through the tank.

Immersion freezers are most commonly used for surface freezing of poultry to obtain a good color. The final freezing is effected in a separate blast tunnel or cold store.

The latter alternative, however, involves quality hazards because of slow freezing of the core.

The product must be protected by an absolutely tight, high quality packaging material. The brine on the package is washed off with water at the exit of the freezer.

4. Evaporating Liquid Freezers

Mainly two liquids or freezants are used: liquid nitrogen (LN2) and liquid fluorocarbon freezant (LFF).

a. LN2 freezer

Liquid nitrogen at −196°C is sprayed onto a single belt freezer. The nitrogen evaporates and is allowed to escape to the atmosphere after the vapors have been used for precooling of the products (Figure 39.6).

The very high freezing rate results in improved textures, particularly in certain fruits and vegetables while with other products there seems to be little quality advantage compared with other freezing methods. LN2-freezing may result in cracking of the product surface if sufficient precautions are not taken.

Like immersion freezers LN2 freezers are often used only for surface freezing. If final freezing is to be carried out the LN2 consumption is of the order of 1.0–1.5 kg per kg of product which makes the operation rather expensive. In spite of this, the low investment and simple operation make this method economical for certain productions, especially in-line processes.

b. LFF system

The freezant is a specially purified dichlorodifluoromethane (fluorocarbon) which has a boiling point of −30°C at atmospheric pressure. The equipment consists of a container with openings at the top. The product is introduced into the container and dropped into a flowing stream of freezant (Figure 39.7). Owing to the extremely good heat transfer the surface is frozen instantaneously so the product may be stacked on the horizontal freezing belt, where it is sprayed with freezant until finally frozen. A discharge conveyor brings the product up and out of the freezer. It is claimed that fluorocarbon leaves only small residues in most products. Experiments in this area are continuing.

On contact with product the freezant evaporates. The vapors are recovered (with only a slight loss) by condensation on the surfaces of the refrigerator, the latter remaining in the container with only small losses to the atmosphere. There is no measurable product weight loss due to dehydration using this method.

V. STORAGE

If the quality of frozen food is to be maintained during its storage life, the correct temperature must be selected for the expected period of storage. During the storage period, the following hazards to quality must be avoided:

1. A low relative humidity in the cold store.
2. Retention beyond the expected storage life.
3. Fluctuations in temperature (both during storage and in the process of loading, unloading, and dispatching vehicles).
4. Physical damage to the product or packaging during the course of storage or handling.
5. Contamination of the product by foreign bodies or vermin.

These hazards can be avoided by ensuring:

a. That the design of the cold store is appropriate to the duty it will be required to perform, and is such that these hazards are, to the greatest extent possible, eliminated at the design stage.

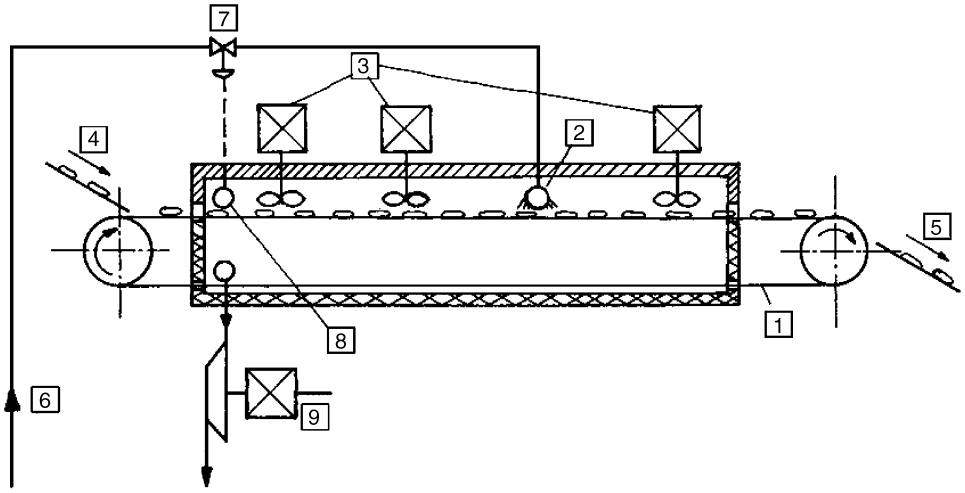

FIGURE 39.6 Liquid nitrogen freezer. (1. Belt, 2. Spraying nozzles, 3. Fans, 4. Inlet, 5. Outlet, 6. Nitrogen tank supply line, 7. Regulating valve, 8. Temperature sensing unit, 9. Nitrogen gas exhauster.)

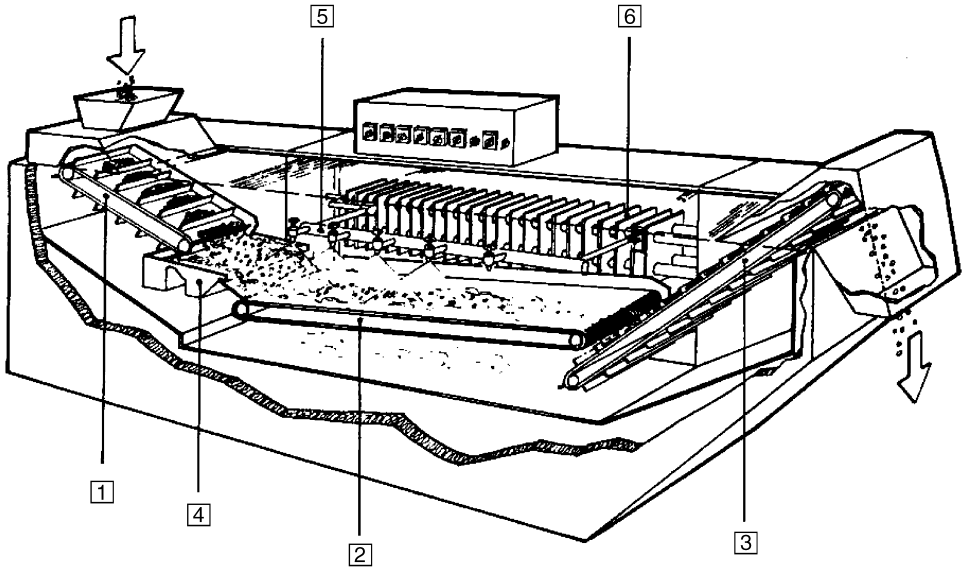

FIGURE 39.7 Liquid fluorocarbon freezer. (1. Product input conveyor, 2. Freezing conveyor, 3. Frozen product exit conveyor, 4. I.Q.F. bath, 5. Spray nozzles, 6. Condenser.)

b. That operation methods, designed to avoid such dangers, are laid down and strictly adhered to.

Small fluctuations in temperature are normal and unavoidable. They should, however, be kept at a minimum both in amplitude and duration in order to minimize the amount of weight loss by drying and in-package desiccation.

A. Cold Store Design

The general design of a cold store is determined by the requirements for effective and safe handling of the merchandise, and a suitable storage climate for the products. The normal arrangement is that rooms are built side by side between road and railway loading banks; thus, all rooms can communicate directly with the loading banks and traffic yards. Today cold stores are frequently built with prefabricated concrete or steel structures. The insulation can be placed on the outside or the inside of the structure. Internal structure means that the insulation will form an unbroken envelope around the building. The insulation is well protected by the structure, internal installations are easy to fix, there is no hung insulated ceiling which can cause problems, and extensions are very simple to carry out.

It is essential that considerable thought should go into the definition of the duties the cold store has to perform. A clear statement has to be prepared of the maximum and average daily activity expected to take place in the cold store:

1. Quantity of each product to be received.
2. Temperature at which each product will be received.
3. Maximum number of operatives and trucks working in the cold store at any one time.
4. Number of anticipated door openings.
5. Maximum quantity to be out-loaded at any one time.

These considerations must be taken into account when calculating the maximum expected heat load. The temperature difference between the surface area of the cooling coils and the required room temperature should be as small as possible and not more than about 6°C.

1. Frost Heave

Frost heave under cold stores is prevented by a special under-floor heating system or a ventilated space under the floor. The heating system may consist of an electrical mat or a pipe grid cast into the sub-floor; glycol or oil is circulated in the pipes. The liquid is often heated by surplus heat from the refrigeration plant.

2. Insulation

The insulation represents a large percentage of the total cost for a normal cold store. It is, therefore, very important that it is designed from an economical point of view. However, one must also consider that the insulation value has an influence on the storage climate in that the transmission losses mean dry heat is entering the cold room. The choice of insulation system must be carried out carefully. The vapor barrier on the warm side must be completely water vapor tight, the insulation should contain no heat bridges, and the internal cladding must be hard, hygienic, and give

a pleasant appearance. Today most cold store insulations are carried out with prefabricated panels, slabs of polyurethane foam, expanded polystyrene, mineral fiber, or cork. Where special attention must be given to the risk of fire, the insulation is combined with a special fire wall or the insulation is carried out with fiber glass between special insulation studs. The vapor barriers may consist of thin gauge aluminum, galvanized steel sheets, or heavy gauge polyethylene sheets. The joints are sealed with special sealing compounds. The internal cladding may be either profiled plastic, laminated galvanized steel sheet, or aluminum sheet. Good concrete kerbing and, in some cases, dunnage battens protect the internal finish and ensure that the merchandise is not stacked directly against the wall.

3. Refrigeration System

The refrigeration system must be designed with regard to the requirements of the climatic conditions for the stored merchandise. It must be adequate to allow for sufficient safety on peak days and summer conditions. The air coolers must be designed and located so that an even temperature can be maintained throughout the cold store even under severe conditions and without generating high air velocities in the cold store. Large evaporator surfaces and air distribution through air ducts or false ceilings will normally ensure this. Air ducts may be omitted if the cooling surface is divided on several cooler units distributed in the room so that the air velocity from the cooler fans is kept at a moderate level.

The most common refrigeration system for large cold stores is a two-stage compressor system with pump circulation of the cold refrigerant to the air coolers. The most common refrigerant is ammonia but halogenated hydrocarbons have also been used in some cases. For small cold stores a direct expansion, one-stage compressor system using halogenated hydrocarbons is widely used. In order to improve safety and make control easier and cheaper most modern refrigeration plants are automated. The degree of automation may vary but normally the room temperature, compressor capacity, lubrication, cooling water, defrosting, pumps, fans, current and voltage of the main supplies, etc. are controlled and supervised by a central control panel in the engine room.

4. Lighting

A cold store is a working place for fork lift drivers and others concerned with the handling of the products. Thus the lighting in the cold store must be good but at the same time it must be remembered that the lighting is adding to the heat load in a cold store. Lamps with a very high power/lighting ratio should be used. Mercury lamps are superior from this point of view and they are often used even if they can cause a slight discoloring of meat products during long storage. A normal cold store should have an average lighting of 100 lux at floor level and of 200 lux in break-up areas.

5. Layout

The layout of the storage space should be such as to reduce to the maximum extent possible the ingress of warm air and the exposure of product to atmospheric temperatures. Where possible, product should be conveyed from factory areas into cold store by means of conveyors in insulated tunnels. There should be no facility for any accumulation of product in ambient temperature. If the operation is a palletized one, then palletization of the product should take place in the cold store in an area set aside for this purpose. Port doors should be provided so that the maximum amount of traffic in and out is handled in this fashion and the product is completely protected from temperature changes during loading/unloading operations.

For safety reason, no glass should be allowed inside the cold store in any unprotected position. Translucent plastic visors should be placed around lamps or any other essential glass.

6. Jacketed Stores

Jacketed stores allow storage at near 100% relative humidity and at uniform and constant temperatures. These conditions, which greatly reduce weight losses of unpackaged foods and frost formation inside packaged frozen foods, are obtained by circulating the refrigerated air in a jacket around the load space to absorb the heat conducted through the insulation before it can enter the load space (Figure 39.8). This technique also increases the life and reduces the maintenance of the structure by preventing condensation and frost formation in the insulation.

7. Equipment

In equipping the store care should be taken to choose equipment which is suitable to the product being handled and which minimizes the possibility of damage or contamination; thus, timber pallets are suitable for properly packed products but lightly packed semi-processed stock may need a pallet constructed in metal or some similar washable and less easily damaged material.

It is likely, for economy reasons, that the store will be designed to maximize the use of height, and to this end some means of support for the product must be provided, e.g., racks, pallet posts, or pallet cages. The layout of pallets in the cold store should be such that damage to the products is minimized. Whilst accepting the need for maximum utilization of space, gangways and turnings should be wide enough for product to be moved without damage, while space should be allowed between lanes of pallets to permit the withdrawal or placing of a pallet when the adjacent lanes are full.

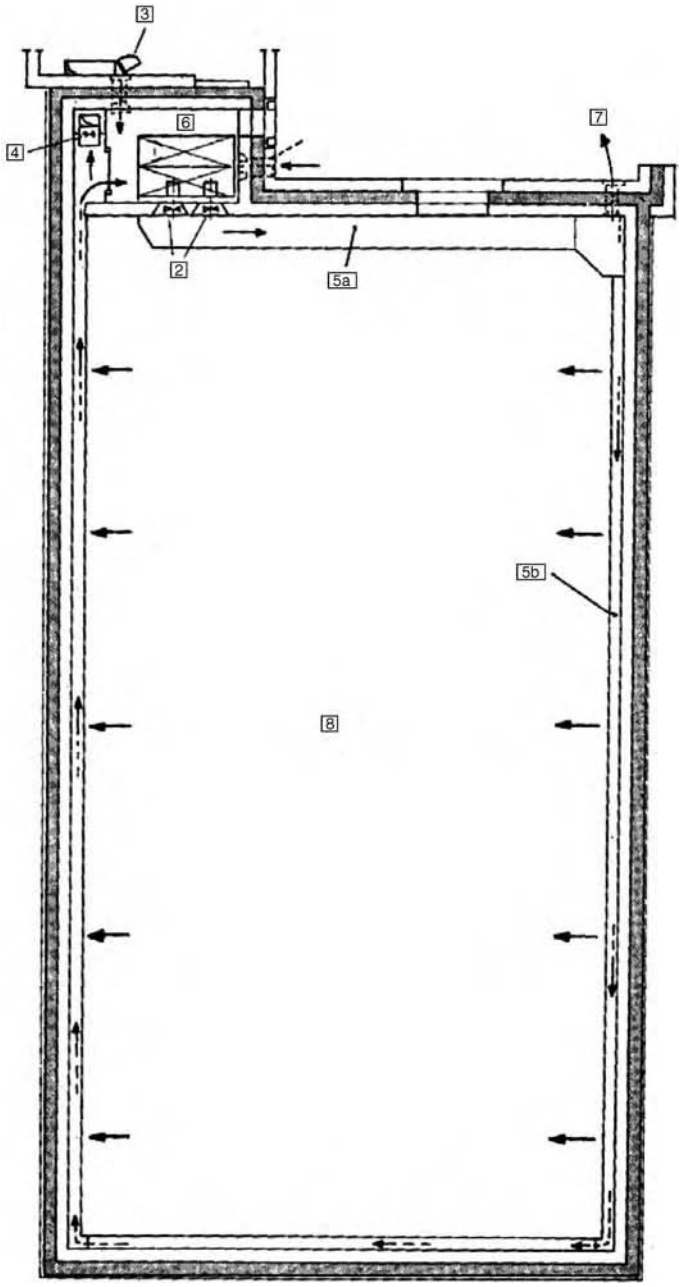

FIGURE 39.8 Jacketed store. [1. Air coolers, 2. Fans, 3. Fresh air duct, 4. Air vent, 5a. Air ducts, 5b. Jacket, 6. Air cooler enclosure, 7. Cooling orifice to antechamber, 8. Cold room. This type of jacketed store serves two purposes thanks to the two-speed fans: (a). At the lower speed, the air only circulates in the jacket. This is the classic operation of jacketed stores (arrows marked thus). (b). At the higher speed, the air enters the store through a system of movable vents which open via the effect of the increased air pressure. Through this means, one can complete the cooling of foodstuffs which may have been subjected to warming up prior to being introduced into the cold store (arrows marked thus).]

8. Operating Methods

It is important that everyone working in a cold store always bear in mind the prime objective of minimizing the exposure of products to ambient temperatures. Methods of handling and routes should be laid down which do not permit the product being placed in ambient temperature. If it is not possible to load a vehicle by means of a port door or some similar method which gives complete protection and the only alternative is to traverse a loading bank, then the complete vehicle load should be assembled inside the cold store and conveyed direct to the vehicle without being placed on the loading bank. A similar procedure should apply in reverse for unloading. Doors

should never be left open other than when personnel or goods are passing through them, and duration should be as short as practicable.

VI. THAWING

Most frozen food processors find it necessary to thaw stock of frozen material in some of their operations, and if thawing is not carried out carefully, quality and yield can suffer. This section sets out the broad principles involved in the thawing techniques available, and indicates some of the problem areas. Irrespective of the procedure involved, heat energy must be supplied, most of it being required to melt the ice in the food. About 300 kJ are required to thaw 1 kg of white fish from a starting temperature of −30°C. The figure for fatty herrings is smaller, about 250 kJ, because of the lower water content of herrings. Thawed materials spoil in the same way as their unfrozen equivalents and must be kept chilled until required; this can often be achieved conveniently by removing the product from the defroster just before thawing is complete so that the product has its own small reserve of "cold."

There are two principal groups of thawing methods: those in which heat is conducted into the product from the surface and those in which heat is generated within the product. In the first group, heat is applied to the surface of the product by exposing it to sources such as hot radiating surfaces, warm air, warm water, heated metal plates, or steam under vacuum. In the second group, heat is generated within the product by such means as electrical resistance, dielectric, or microwave heating. Surface heating methods are much more commonly used than are internal heating methods.

A. Surface Heating Methods

When using surface heating methods, thawing time for a product decreases with:

a. Decrease in physical size of the product;
b. Increase in thermal conductivity (or, more precisely thermal diffusivity) of the thawed product;
c. Increase in temperature difference between the surface of the product and its surroundings;
d. Increase in the movement of the surrounding medium relative to the product surface; and
e. Increase in humidity of the surroundings.

Since the thermal diffusivity of thawed product is less than that of frozen, surface thawing methods suffer from the inherent disadvantage that resistance to heat transfer increases progressively once thawing has started.

All surface heating methods can take advantage of a programmed temperature difference between the surface and the surroundings; the temperature of the surroundings is arranged to start high, and to decrease as the surface warms up to a predetermined level, usually below the temperature where bacterial activity or surface damage could be a hazard to the quality of the food. Thawing times are greatly increased if the product is encased in packaging material. Thus packaging material should be removed where possible.

B. Still Air Thawing

If thawing is conducted in still air, the air temperatures should not exceed 20°C and facilities for supplying heat to the room in which the product is laid out to thaw may be required. Air temperatures greatly in excess of the above should be avoided since the outer layer will warm up and spoil before the center is completely thawed. A typical block of sea frozen whole cod 9 cm thick will take up to 20 h to thaw under these conditions. This time can be reduced by separating the fish as soon as they can be separated without damage. Single fish, 10 cm thick, will take about 8 to 10 h to thaw.

1. Air Blast Thawing

A relative humidity of greater than 90% is advantageous both in reducing weight loss or shrinkage and also in obtaining a high level of heat transfer. Air velocities of 12-18 m/min at a temperature of 6–8°C for 3–5 days or 100 m/min for 2–2.5 days are needed for packages of boneless meat, whereas pork sides are normally thawed at 4–5°C for 2–4 days. Air speeds of the order of about 300 meters per minute at temperatures not exceeding 20°C with the air fully saturated with moisture will thaw sea frozen fish blocks 10 cm thick in 4 h. Higher air velocities at cool temperatures lead to surface desiccation while any higher temperature will lead to microbial growth on the heated surface.

C. Water Thawing

This method is not normally applicable to meat and with fish there is a risk that fillets or cut surfaces will become waterlogged and lose flavor but water thawing can be used satisfactorily for frozen whole fish, even though there may be a slight loss of pigments. Usually there is a slight gain in weight, which is lost again when the fish is filleted.

The temperature of the water being circulated around the frozen fish or sprayed onto the fish should be no warmer than 20°C and the water itself should flow at not less than 30 cm per minute so as to obtain rapid thawing. The thawing time for a block of whole cod 10 cm thick in water at 20°C moving at about 120 cm per minute is similar to that in an air blast defroster using humid air at the same temperature, i.e., about 4 h.

When frozen fish blocks are thawed in still water, water temperatures may, in the initial stages, marginally exceed 20°C, but the thawing should be arranged so that the fish surface temperature does not exceed 20°C.

D. Vacuum Thawing

In this method the product lies on racks inside a container from which the air has been evacuated. Water, usually at about 18°C, is allowed to evaporate freely from heated vessels inside the container. In the absence of air, transfer of water vapor from a heated vessel to the product occurs readily, the water vapor giving up its latent heat on condensation. The advocates of this method claim faster thawing than other surface heating methods for products less than about 10 cm thick.

E. Double Contact Thawing

Plate frozen raw material lends itself particularly to plate thawing in an arrangement similar to that of a multi-plate freezer, with a liquid circulating through the plates (at a temperature not exceeding 20°C providing the necessary heat. A 10 cm block of whole cod when thawed between plates at 20°C for 5 h is ready for filleting 3.5 h later, making a total of 8.5 h for complete thawing. Care must be taken not to allow distortion of the blocks to occur during cold storage since this will lead to poor contact with the plates during thawing. Thawing fluid or semi-fluid food in a vertical plate apparatus may be carried out using temperatures as high as 40–50°C as long as the melted material is allowed to run away from the plates continuously.

F. Internal Heating Methods

Internal heating methods rely on the use of applied electric fields which cause movement of the electric charges inherent in all products. The molecules of the product take up this energy of movement and the food warms up. The amount of heat generated in this way is strongly dependent on the electrical characteristics of the product. Since food is not usually homogeneous, there can be marked variations in the rate of heating of different parts of the food. Furthermore, for any particular component in the food, the rate of heating usually increases as the product thaws, making runaway heating a hazard. If these factors can be accommodated, the great advantage to be gained is extremely rapid, uniform thawing.

G. Electrical Resistance Thawing

In this method, the product is sandwiched between electrodes and an electric current is passed through the product. Some preliminary warming is usually necessary to achieve good electrical contact and a satisfactorily high starting current. In practice this method has so far been used for thawing blocks of fish fillets up to 5 cm thick and weighing about 3 kg but it has not been found suitable for thicker blocks of fillets, blocks of whole fish (except herring), or single whole fish.

H. High Frequency Thawing

In the dielectric method, a high frequency electric field is applied to electrodes astraddle the product, but not physically in contact with it. The commonly used frequencies are either in the range 27 to 100 MHz (dielectric or high frequency heating) or 915 to 2450 MHz (microwave heating, where the energy is directed at the product enclosed in a chamber). The product must be reasonably homogeneous and regular in shape to achieve uniform heating. If the block is not homogeneous, or is irregular in shape, some parts may become overheated before the remainder is thawed. It has been found that if irregular blocks of fish are first immersed in water, thawing becomes uniform. The time taken for a 10 cm block of whole cod is typically just less than 1 h. Partial thawing by microwave is also used, thereby increasing the capacity of the thawing equipment. By going to the higher frequencies, the field strength can be substantially increased and thawing time reduced to a matter of minutes. Penetration into the food mass decreases so that the thickest block of meat which can be completely thawed at 2450 MHz is 3 to 4 cm.

In summary, freezing, frozen storage, and thawing affect the quality and shelf stability of fish and seafood. If kept under appropriate conditions, fish and seafood can be stored in the frozen state for several months without appreciable changes in quality. During frozen storage, microbiological changes in fish and seafood are very minimal. On the other hand, a series of changes such as protein denaturation, lipid oxidation, texture deterioration, loss of fresh odor and flavor, various enzymatically induced reactions, loss of volatile constituents, nutritional losses, and changes in moisture take place in fish and seafood when subjected to excessively prolonged frozen storage. Likewise, such changes may also occur in freeze-thawed fish and seafood. Quantitative evaluation of the influence of freezing, frozen storage, and thawing on fish and seafood is rather complex. The different variables that influence quality are related to one another. Therefore, it becomes almost impossible to describe some quality changes without actually discussing the other related changes that occur in fish tissues.

ACKNOWLEDGMENT

The information in this chapter is based on "Freezing seafood and seafood products: Principles and applications," by S. T. Jiang and T. C. Lee, in *Handbook of Frozen Foods*, edited by Y. H. Hui et al., Marcel Dekker, New York, 2004.

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40 The Application of Gene Technology in the Wine Industry

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CONTENTS

I. INTRODUCTION

Wine plays a major role in the economies of many nations. The world's annual wine production from about 8 million hectares of vineyards totals around 27 billion litres (1). Shifting consumer preferences, globalisation and other economic factors have forced an evolution of the international wine industry from a "cottage industry" of independent producers to global networks of consumer-conscious enterprises (2,3).

An increasing gap between wine production and wine consumption has given rise to an annual oversupply of 15–20% in the global market. There is a growing demand for cost-effective production of wine with minimised

resource inputs, improved product quality, increased health benefits and low environmental impact. It is widely expected that advances in molecular biology and bioinformatics will help equip the wine industry with the tools to tailor grapevines and microbial starter strains, enhancing wine quality, purity, uniqueness and diversity (1,2,4).

Grape and wine research, fueled by greater understanding of basic cellular and molecular biology governing targeted traits and by considerations of future use, promises to be a powerful dynamo of technological progress in this increasingly market-oriented industry (2). It is therefore crucial that basic and applied research not be treated as separate ventures. Rather, grape and wine research, both in problem selection and experimental design, should be aimed at increasing fundamental understanding in a context responsive to the applied needs of producers and consumers.

This review passes familiar light through new prisms to demonstrate that ignoring gene technology imperils the international wine industry, despite the current vocal opposition to genetically modified organisms (GMOs) and products (1–5). This perspective is supported by examples of genetically improved grapevine prototypes showing increased productivity, efficiency, sustainability and environmental friendliness, particularly with regard to improved pest and disease control, water use efficiency and grape quality. Examples of genetically tailored microbial starter strains include those that could play a role in improving the fermentation, processing and preservation of wines, as well as those with the capacity to enhance the wholesomeness and sensory quality of wine.

II. THE GENETIC IMPROVEMENT OF GRAPEVINES FOR WINE PRODUCTION

A. Grapevine Species and Cultivars

Grapevine belongs to the genus *Vitis*, consisting of two sub-genera, *Euvitis* and *Muscadinia*. The preferred wine grape species, *Vitis vinifera*, originated in Europe and consists of about 5000 cultivars (6). However, only a few select and ancient cultivars are relied on for commercial wine production. As a result of the wine industry's reliance on established varietal names and predictable wine styles to sell its products, there has not been a great incentive to breed new *V. vinifera* cultivars. Nevertheless, breeding programmes significantly impacted on the development of rootstock varieties resistant to soil-borne pests and pathogens, as well as to negative abiotic conditions (7). Improvements to rootstock and scion cultivars initially relied largely on arbitrary selections of natural mutations that enhanced cultivation or some aspect of fruit and/or wine quality and were later followed by the more directed, clonal selection schemes (5,8).

B. Genetic Features and Techniques for the Analysis and Development of Grapevines

A dramatic new day in plant improvement is dawning, warmed by molecular biology, genetic transformation and functional genomics. Several plant genomes have now been fully sequenced, and though the accessibility of the grapevine genome is restricted by size and complexity, there are now intense multinational studies underway of the *Vitis* genome.

A growing number of plant species are becoming viable candidates for genetic transformation through *Agrobacterium*-mediated and biolistic bombardment technologies. The grapevine, however, has been resistant to genetic transformation: difficulties include finding tissue culture systems that can withstand the new techniques and subsequent selection regimes (5,8,9). Nevertheless, there are today a few grapevine scion and rootstock cultivars of commercial importance that can be genetically transformed with relatively little difficulty. The emphasis is gradually shifting from developing the technology to implementing it to generate useful plant lines.

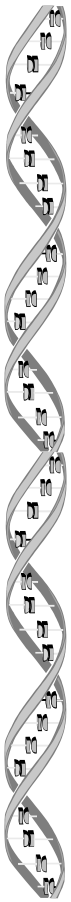
C. Targets for the Genetic Improvement of Grapevine Varieties

The genetic improvement of grapevine cannot occur in the absence of knowledge of the fundamental processes supporting the physiological responses to be altered. In the early days of limited genetic resources, genes with known functions were introduced into plant species in the hope of developing improved phenotypes. This approach, although problematic, demonstrated the value of adding application to theory. It is an exciting time, as the plant sciences enter a new era and the list of gene sequences, including those of grapevine, grows and becomes available. Potential areas for the genetic improvement of grapevine are discussed below and summarised in Figure 40.1.

1. Improving Disease and Pest Resistance

As genetic engineering of crop plants evolves, its primary focus remains upon enhanced disease tolerance. Single genes can confer disease resistance in plants, making individual gene transfers into plant genomes perhaps the best approach to control fungal, viral, bacterial and insect pathogens. Almost all of the approaches to enhanced disease tolerance in plants take advantage of the natural interactions between host and pathogen (10). In the battle for survival, host and pathogen evolve simultaneously, making those interactions fluid and complex.

There are currently two major approaches to manipulating disease tolerance in plants. The first is introducing a gene product with known anti-pathogen activity at high



Disease and pest resistance

Fungal tolerance
- Chitinases and glucanases
- Thaumatin-like proteins - *Vvtl1*
- Stillbene phytoalexins - *stsy, vst1* and *vst2*
- Copper/zinc superoxide - *Cu/Zn SOD*
- Ribosome inactivating proteins (RIPs)
- Detoxifying enzymes (NADPH-dependent aldehyde reductase, Vigna radiata-Eutypine reducing enzyme)
- Polygalacturonase-inhibiting protein (PGIP)
- Antifungal peptides (plant and insect)

Bacterial tolerance
- Antimicrobial peptides - lytic peptides, Shiva-1, defensins
- Disfunctional import and integration protein encoding gene - virE2delB from *Agrobacterium*

Viral tolerance
- Virus coat proteins
- Virus movement proteins
- Replicases
- Proteinases
- 2,5 Oligoadenylate synthase

Grapevine cultivation

Resistance to water stress
- Aquaporins - TIPs (tonoplast integral proteins); PIPs (plasma membrane integral proteins)

Oxidative damage
- Carotenoid biosynthesis genes
- Adh (alcohol dehydrogenase) genes
- SOD (cystosolic CuZnSOD, chloroplast-residing CuZnSOD, mitochondrial residir MnSOD)

Osmotic stress
- Proline accumulation - Vvp5cs (1-pyrroline-5-carboxylate)
- Polyamines -Vvoat (γ-ornithine aminotransferase)

Cold tolerance
- Antifreeze from fish

Vitis vinifera

Grapevine quality

Sugar accumulation and transport
- Phloem loading and unloading - plant and yeast invertases
- Sugar transport - *Vvsuc11, Vvsuc12, Vvsuc27, Vvht1* and *Vvht2*

Colour development
- Ripening related processes - Myb, auxin and ABA
- Anthocyanins - Ufgt (UDP-glucose:flavanoid 3-*O*-glucosyltransferase)

Reduced browning
- Silencing of polyphenol oxidase

Seedlessness
- Baransae gene

FIGURE 40.1 Methods for the improvement of *Vitis vinifera*.

copies or in an inducible manner into the host to optimise the plant's innate defence responses. The other approach relies upon pathogen-derived resistance (PDR): a pathogen-derived gene is expressed inappropriately in time, form or amount during the infection cycle, preventing the pathogen from maintaining infection. PDR is at the root of most antiviral strategies and much of the activity in the genetic transformation of grapevine varieties.

Transgenic grapevines have been generated for providing viral resistance (10); in most of these cases expressing the virus coat protein achieved PDR. More recently, resistance to virus infection has also been achieved by expressing anti-sense to virus movement proteins (11) and replicase proteins.

Various studies have examined the expression of grapevine chitinases, β-1,3-glucanases, thaumatin-like proteins and stillbene synthetic enzymes with regard to fungal infection and grape ripening (12–14). Several of these enzymes have been shown to possess antifungal activity (15–18) and it has been demonstrated that in some cases, transgenic expression of genes encoding the proteins leads to increased resistance (18,19). Research has also shown that transgenic expression of a detoxifying enzyme enhances resistance to *Eutypa lata* (20). In addition, a gene from the American wild grape species *Muscadinia rotundifolia* has been introgressed into a succession of pseudo back crosses of *V. vinifera*. Using this strategy, resistance to *Oidium tuckerii* (powdery mildew) has continued to segregate as a single gene, thereby making the gene an important candidate for the development of a transgenic method to confer powdery mildew resistance in *V. vinifera*.

Obtaining transgenic plants resistant to pathogenic bacteria has been less successful; the expression of the lytic peptide Shiva-1 has been reported (21), and induced expression of several defence genes have been detected in response to bacterial pathogens (13,22).

We are witnessing the onset of a new era in plant cultivation, as old problems are being addressed in new ways. Transgenic grapevines expressing heterologous antiviral and antifungal genes are currently undergoing field testing, the first 'prototypes' of manipulated disease tolerance in grapevine. The technology will undoubtedly become more sophisticated, raising the possibilities of multiple gene transfers and long-term and stable expression of transgenes along with the use of highly specific inducible regulatory sequences.

The process of generating and analysing transgenic plants has yielded considerable knowledge about the nature of plant-pathogen interactions and the disease resistance pathways that operate in plants. Model plants transformed with the various targeted genes become important resources when the nature of the manipulations and their effect *in planta* are further analysed with state-of-the-art technologies, such as transcriptomics, proteomics and metabolomics (23). A range of *Arabidopsis thaliana* mutants blocked in certain pathways of pathogen defence also provide extremely valuable information regarding the functions of genes (24). The disease pathways are now fairly well characterised and much emphasis is currently being placed on clarifying the trigger systems of defence and the subsequent signal transduction processes leading to the various forms of defence.

2. Improving Grapevine Cultivation

Genetic transformation technology carries with it the tantalising prospect of developing plant lines able to adapt to adverse climatic conditions, enhanced by greater understanding of stress tolerance in plants and basic knowledge of key aspects of plant growth and development. Ongoing efforts to develop transgenic grapevines with improved cultivation prospects are focussing on processes such as carbon-partitioning, modes of sugar translocation, water transport and the role of aquaporins, as well as the regulation of these processes. These efforts also deal with important limitations to cultivation such as drought and salt stress, photo-damage and freezing tolerance (25).

To date, a transgenic grapevine expressing antifreeze genes from Antarctic fish has been reported as a mechanism to provide cold tolerance (25). However, plant stress responses are complex pathways of interacting proteins driven by a range of signals attenuated or amplified by equally complex processes. This biological interaction is more difficult to manipulate with single or even multiple gene additions; knowledge of the control mechanisms and alterations thereof might prove more feasible. With this view, the accumulation of proline and polyamines are two examples in grapevine that have been studied. The regulation of key genes in these biosynthetic pathways is providing insight into their involvement in abiotic stress (26,27). Furthermore, the expression of several stress related genes has been shown to be activated during the grape ripening (28,29).

Crop prediction is another area of attention. One of the most difficult issues facing viticultural production is the understanding and management of crop load and quality in the face of seasonal and environmental variation and change in market demand. Most recently, a study of how related genes in grapevine are organised and interact was made possible by the identification of the genes involved in flowering and fruitfulness in *A. thaliana*. For example, the chimera that results in pinot meunier can be separated into a conventional pinot and a mutant pinot form in which fruitfulness is dramatically increased even in juvenile plants. This dramatic change is the result of a single DNA base change in a single gene affecting vegetative and floral development of the vine (30).

3. Improving Grape Quality

Winemakers want small grapes of specific colour and ripeness as measured by the indicators of sugar, acids and phenolics. Researchers are investigating the basic processes of berry ripening and, particularly, ripening signals. Grapevine, a non-climacteric fruit, is studied for the biochemical, hormonal and environmental signals that influence ripening processes such as pigment production, sugar accumulation, transport and the formation of aroma components. The means of glucose/fructose accumulation during ripening is coming to light with the identification and analysis of grape berry genes encoding invertases and sugar transporters (31). The expression of flavenoid biosynthetic genes, responsible for colour development, coincide with hexose accumulation in berries (32–34). The importance of UDP glucose flavanoid-3-glucosyl transferase (UFGT) in the control of berry colour has been highlighted by comparison of gene expression in red and white grapes (35,36), and recent results indicate that Myb genes are involved in regulating UFGT (37). A number of genes that influence grape berry softening have also been isolated (38–40). Auxin and abscisic acid (natural hormones regulating growth and physiology) affect the expression of genes involved in the ripening process and have been implicated in the control of grape berry ripening (41). The aim of this approach is to meet the quality parameters of grapes with the formation of desirable or novel products by changing the metabolic flux through the important biosynthetic pathways active in the ripening berry.

To reach these goals grapevine biotechnology will have to draw upon considerably more knowledge of the underpinning processes as well as upon improvements in transformation technology. Targeted gene insertion and deletion technologies will make these and other innovative prospects more feasible.

III. THE GENETIC IMPROVEMENT OF WINE YEAST FOR ALCOHOLIC FERMENTATION

A. Yeast Species and Strains

Saccharomyces cerevisiae is unquestionably the most important yeast species in winemaking, but it competes with many other species for dominance. The ubiquitous fruit yeast, *Hanseniaspora uvarum* (anamorph *Kloeckera apiculata*), is the most common yeast isolated from grapes,

and many other non-*Saccharomyces* species are also present in the initial stages of spontaneous alcoholic ferments. As the ethanol concentration increases and other conditions change, various yeasts prevail in sequence, until *S. cerevisiae* multiplies to be the dominant species (42).

Not surprisingly, in such a complex environment the yeast ecology is highly variable and dependent on geographic location, viticulture and winemaking practices. Over the last decade, the pace of microbial ecology research has been accelerated by the application of molecular methods. A widely used rapid species identification method is PCR-RFLP (*p*olymerase *c*hain *r*eaction-derived *r*estriction *f*ragment *l*ength *p*olymorphism) of the ribosomal RNA genes (43). Using this method highlights the unpredictability of wine yeast ecology. In some wines for example, there is an almost total absence of apiculate yeast (*Hanseniaspora* sp.), yielding to *Candida stellata, Metschnikowia pulcherrima* or even *S. cerevisiae* during the initial stages of fermentation (44). In other cases non-*Saccharomyces* species persist to the end of fermentation (45). Molecular methods have also begun to accent the role of other *Saccharomyces* sensu stricto species, as well as interspecific hybrids of *Saccharomyces* yeasts in vinification (46–48).

Recently developed molecular methods, in particular mitochondrial DNA RFLP, have been used to analyse fermentation ecology at the species as well as strain level. The most unexpected finding is the number of strains of the same species coexisting during fermentation, with some wines showing over 100 different *S. cerevisiae* strains at the end of fermentation (44,45). Other technologies such as denaturing gradient gel electrophoresis (DGGE) have been developed that no longer require the plating of yeast prior to identification. Using this method new non-culturable species have been discovered in wine (49).

Wines made from spontaneous ferments are often described as more complex and fuller than inoculated wines. It is common practice, nevertheless, in many wineries to inoculate with a specific strain of *S. cerevisiae*, for rapid fermentation and a more consistent product with a reduced possibility of microbial spoilage. It also allows winemakers to use preferred strains to produce wines of a chosen style. With the future availability of yeast strains that can improve multiple facets of the winemaking process, the advantages offered to winemakers performing inoculated ferments with both *Saccharomyces* and non-*Saccharomyces* strains will expand.

B. Genetic Features and Techniques for the Analysis and Development of Wine Yeasts

Several genetic methods are available for the improvement of wine yeast. These include the selection of mutants with the desired properties, the combination of traits by mating and sporulation/dissection of *S. cerevisiae* strains, and the use of protoplast fusion to introduce novel traits of other species (1). Although these classic genetic methods have been used successfully for the development of new strains, they lack the precision and capability of gene cloning.

Specific characteristics of *S. cerevisiae* make it a model experimental organism (50). *S. cerevisiae* multiplies rapidly, shares many cellular regulatory mechanisms with humans and has a stable haploid and diploid life cycle. It has numerous suitable markers, plasmids and promoters for the regulated expression of chosen genes and undergoes efficient homologous recombination, permitting the precise deletion of selected genes. The *S. cerevisiae* genome was the first eukaryotic genome to be completely sequenced and this, along with its facile genetics, has made it an excellent research organism in the post-genomic era (51,52).

There have been major technical developments since the complete *S. cerevisiae* DNA sequence was published. Yeast deletion libraries are available in different mating types and ploidy that possess a set of approximately 6000 yeasts strains, each with a different single gene deleted. Phenotypic and chemical screens of these libraries make clear the biological function of each of the 6000 genes (53,54). The transcriptional response of each gene to different environmental conditions using microarrays is identifying genes that are important to wine fermentation [reviewed in (55)]. These conditions include the response to ethanol, osmotic stress, high and low temperature, high and low pH, copper, limited nitrogen and sugar, anaerobicity and progression into stationary phase (56–61). Microarray experiments are also being used to compare genome structure and gene expression in different grapevine isolates and commercial wine yeast (62–64). Proteomic approaches are being used in parallel to uncover the post-transcriptional changes that occur in different environmental conditions and genetic backgrounds in yeast (65). Of particular importance to wine is the emergence of metabolomics (66,67), which attempts to analyse all the metabolites produced by an organism in response to environmental or genetic changes. This will be fundamental to further understanding of how genetic and environmental changes impact on flavour and aroma.

The opportunity now exists to exploit these research findings and technological developments to produce genetically improved wine yeast strains of great value to the wine industry.

C. Targets for the Genetic Improvement of Wine Yeast Strains

Molecular genetics in wine yeast is being used to improve several aspects of winemaking, including fermentation performance, processing efficiency, sensory attributes and wine wholesomeness. These are summarised in Figure 40.2. Many of these aspects have been described in recent reviews (1,3,68), and the emphasis in this section is on current advances in this area as detailed in the latest literature.

Fermentation performance

Dessication tolerance

- Increased trehalose and glycogen accumulation - *nth1*Δ, *ath1*Δ and ↑*GSY2*
- Increased expression of aquaporins - ↑*AQY1* and ↑*AQY2*
- Increased accumulation of proline and charged amino acids - *put1*Δ, ↑*PRO1* and *car1*Δ

Fermentation stress

- Osmotic tolerance by modifying glycerol metabolism - ↑*GPD1,* ↑*GPD2,* ↑*GPP2*
- Ethanol tolerance by increasing sterol accumulation and ATPase activity - ↑*SUT1,* ↑*SUT2,* ↑*PMA1,* ↑*PMA2*

Nutrition

- Improved sugar transport - ↑*HXT3,* ↑*HXT6,* ↑*HXT7,* ↑*SNF3* and *FSY1*
- Improved nitrogen utilisation - *ure2*Δ, ↑*PUT1* and ↑*PUT4*

Control of indigenous microorganisms

- Increased sulfur dioxide - *met2*Δ, *met10*Δ and ↑*MET14*
- Hydrogen peroxide production - *GOX1*
- Expression of zymocins and bacteriocins - *PED1* and *LCA1*
- Expression of lysozyme, chitinases and glucanses - *HEL1*, *CTS1* and *EXG1*

Processing

Protein clarification

- Expression of yeast haze protection factors - invertase, *HPF1* and *HPF2*

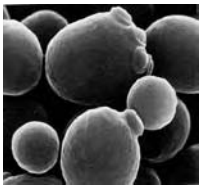
Cell floculation and flotation

- Controlled cell sedimentation by regulating expression of flocculins *FLO1* and *FLO11*
- Vellum formation - *FLO11*/*MUC1*

Polysaccharide clarification

- Glucanases, pectinases, xylanases, arabinofuranosidases - *END1*, *EXG1*, *CEL1*, *BGL1*, *PEL5, PEH1, XYN1–5*, *ABF2* and ↑*PGU1*

Saccharomyces cerevisiae



Wine wholesomeness

Resveratrol

- Increased resveratrol by grape stillbene synthesis - *4CL9/216*, *Vst1*

Ethyl carbamate and biogenic amines

- Decreased ethyl carbamate by decreasing urea formation - *car1*Δ, *URE1*
- Decreased biogenic amines by inhibiting bacterial growth

Sensory Attributes

Grape derived flavours

- Release of colour and flavour trapped in skin by expressing polysaccharide-degrading enzymes
- Release of terpenols by the expression of glycosidases - *END1*, *EXG1* and *BEG1*
- Modified terpenol concentrations by altering ergosterol biosynthesis and terpenol biotransformation
- Release of volatile thiols

Yeast secondary metabolites

- Enhanced production of desired esters by modifying transferases and esterases - ↑*ATF1* and ↑*IAH2*
- Optimised higher alcohol production modification of *BAT1*, *BAT2, ILE*, *VAL* and *LEU* genes
- Low ethanol wine yeast - ↑*GPD1,* ↑*GPD2, ald6*Δ and *GOX1*
- Modified sulfur metabolism - *met10*Δ *met14*Δ, *mxr1*Δ, ↑*MET25,* ↑*MET17* and ↑*SSU1*
- Malolactic fermentation - *mleS*, *mae1* *mae2* and *LDH1*
- Optimised phenol production - *PAD1*

FIGURE 40.2 Methods for strain development of wine yeast. Photograph kindly provided by Dr Alan Wheals.

1. Improving Fermentation Performance

Sluggish and stuck ferments remain a major concern of the wine industry. The wine environment is very stressful for yeast and harsh conditions must be overcome to produce high-quality wines (69). Like all living organisms, yeast has mechanisms in place allowing it to respond quickly to a changing environment. A general environmental stress response (ESR) participates in a wide range of cellular functions, including cell wall modification, fatty acid metabolism, protein chaperone expression, DNA repair, detoxification of reactive oxygen species and energy generation and storage (57). In addition to the general stress response, specific strategies to combat distinct stresses are essential for the survival of yeast in the fermenting grape must environment.

a. Desiccation stress

The desiccation process, part of the production of active dried yeast, challenges the wine yeast to a mixed stress that, although not well defined, appears to share some properties with freezing and osmotic stress. Several metabolites increase the survival of *S. cerevisiae* cells exposed to physical or chemical stresses and have been implicated in the efficiency of reactivating dried wine yeast starter cultures. As a result, there is strong incentive to develop wine yeast strains that accumulate these metabolites.

Disabling the trehalose hydrolysis pathway, thereby increasing cellular trehalose concentrations by deleting the *NTH1* or *ATH1* (neutral and acidic trehalase) genes has been shown to increase freezing and dehydration tolerance in a laboratory and commercial baker's yeast (70,71). The build-up of a second carbohydrate storage molecule, glycogen, is thought to provide the yeast with a readymade energy source upon reactivation of dried yeast. It has been reported that a wine strain overexpressing the *GSY2* (glycogen synthase) gene accumulates glycogen and has enhanced viability under glucose limitation conditions (72). Overexpression of the *AQY1* and *AQY2* (aquaporin) genes also confers freeze tolerance upon both laboratory and commercial baking strains (73). Whether these changes prove to be an advantage to active dried yeast remains to be determined.

It has been shown that proline and charged amino acids provide desiccation and freeze tolerance. Deletion mutations in the *PUT1* gene, encoding proline oxidase, and a dominant mutation in the proline biosynthetic *PRO1*

gene (γ-glutamyl kinase), increase intracellular proline and show higher desiccation, freeze and/or osmo-tolerance (74,75). A deletion mutation in the arginine degrading enzyme *CAR1* (arginase) increases arginine and glutamic acid levels and provides freeze tolerance (71).

b. Fermentation stress

The high concentration of sugar in grape juice imposes an osmotic stress on yeast. As indicated above, proline and trehalose accumulation both provide osmotolerance to yeast. The main osmolyte in yeast, however, is glycerol. In response to an osmotic stress, a MAPK cascade is activated and the two key genes in glycerol biosynthesis *GPD1* (glycerol-3-phosphate dehydrogenase) and *GPP2* (glycerol-3-phosphatase) are rapidly and transiently induced greater than 50-fold (61). Mutations in these genes are osmosensitive whereas mutations that increase their expression enhance osmotolerance (76). It has been reported that commercial wine yeast strains overexpressing either *GPD1* or *GPD2* have a slight growth advantage at the beginning of fermentation (77,78).

The primary cause of most sluggish and stuck fermentations is a high concentration of ethanol, an effect that can be augmented by a number of intrinsic and environmental factors. The physiological basis of ethanol toxicity is complex and not fully understood (59). A major target appears to be cell membranes, in which ethanol increases membrane fluidity and permeability, and impairs the transport of sugars and amino acids. The modification of *SUT1*, *SUT2*, *PMA1* and *PMA2* genes results in increased sterol accumulation and ATPase activity, thereby increasing the resistance to ethanol.

Zymocidal wild yeast may also inhibit the growth of the inoculated wine yeast strain and contribute to sluggish or stuck fermentation (79). Rapid progress in understanding sensitivity to killer toxins is being made, encouraging the development of broad-spectrum zymocidal resistance in wine yeast (80–82).

c. Utilisation of nutrients

Sugar uptake appears to limit complete sugar utilisation during vinification and is strongly influenced by conditions such as ethanol concentration and nitrogen availability. The low affinity hexose transporter Hxt3p and high affinity transporters Hxt6p and Hxt7p play particularly important roles in wine fermentation (83). It is possible that increased expression of these transporters will decrease the occurrence of stuck fermentation. Two hexose transporter homologues, Snf3p and Rgt2p, are required for glucose sensing. Snf3 is specifically required for inducing a number of *HXT* genes under low glucose conditions. Dominant *SNF3* mutants constitutively express hexose transporters and are resistant to translational inhibition upon glucose withdrawal (84,85), providing a potential mechanism for wine yeast improvement.

Nitrogen is the nutrient most often limiting wine fermentations; its deficiency can lead to cessation of fermentation as well as to the production of off-flavours such as hydrogen sulfide. A wine yeast strain carrying a mutation in the *URE2* gene, which represses expression of proline transport and metabolic genes, increases the availability of proline and arginine as nitrogen sources (86,87). However, *ure2Δ* strains possess pleotropic phenotypes. A more specific method may be to specifically target the proline permease and utilisation proteins (encoded by the *PUT* genes) (88). Global gene expression studies of wine yeast growing under differing nitrogen conditions will be important for identifying other genes that can improve fermentation under limiting nitrogen conditions (56,58).

An alternative source of nitrogen in vinification is from autolysis of the yeasts themselves (89). It is possible that a subset of yeast could be targeted to lyse toward the end of fermentation by adjusting the regulation of the cell integrity pathway (90).

d. Control of indigenous microorganisms

Uncontrolled microbial growth can alter the chemical composition of wine and detract from its sensory properties. The excessive use of chemical preservatives to control the growth of unwanted microbes can affect the quality of wine; it also faces increasing consumer resistance. As a response, wine yeast starter cultures that express antimicrobial enzymes or peptides are being developed.

Wine yeast producing increased sulfur dioxide (SO_2) concentrations could be useful for suppressing the growth of indigenous microbes, providing a means of reducing SO_2 as an antimicrobial and antioxidation agent in wine. Decreasing Met10p (sulfite reductase) and Met2p (serine acetyl transferase) activity or increasing Met14p (adenosine 5′ phosphosulfate kinase) activity leads to increased SO_2 production and has been shown to provide flavour stability to beer (91–93). Expression of hen-egg lysozyme or the *Aspergillus niger* glucose oxidase gene (*GOX1*) in yeast offers additional alternatives (94). The expression of *GOX1* in a wine yeast resulted in the production of hydrogen peroxide that can lead to oxidation toxicity of bacteria (95).

An alternative method is the use of a wine yeast with broad spectrum zymocidal activity. Whereas *S. cerevisiae*'s zymocins have a narrow killing range, the zymocins of *Pichia anomala*, *Willopsis saturnus* and *Kluyveromyces lactis* have been shown to be active on many species common in grape juice, including spoilage yeasts such as *Dekkera bruxellensis* (96). Several of the zymocins have been cloned in *S. cerevisiae;* the mechanisms of action are being established (80,97). Although these zymocins are generally less active at low pH, continuing advances in understanding the pH dependence of protein stability should make it possible to engineer more suitable zymocins for winemaking (98).

The zymocin zygocin, produced by the salt-tolerant yeast *Zygosaccharomyces bailli*, is also active against

filamentous fungi (99). However, heterologous expression in *S. cerevisiae* causes the yeast to commit suicide. Some success in producing yeast that kill filamentous fungi by attacking the cell wall has been achieved by expressing the *CTS1* chitinase and the *EXG1* endoglucanase genes (100). Although zymocidal yeasts have also been shown to be active on bacteria, the focus in wine research has been on the production of strains that secrete bacteriocins or bacteriolytic enzymes (e.g., lysozyme) to suppress growth of undesirable bacteria. Secretion of the *Pediococcus acidilactici* and *Lactobacillus plantarum* bacteriocins in *S. cerevisiae* produced a yeast that killed sensitive bacteria (101,102).

2. Improving Wine Processing

Following alcoholic fermentation wine is further processed to achieve clarity and physiochemical stability. The fining and clarification processes often involve expensive and laborious practices that generate large volumes of lees for disposal. This causes wine loss and may remove important aroma and flavour compounds from the remaining wine. To minimise the impact of these practices an increasing spectrum of commercial enzyme preparations are being added to grape must and wine. Generating yeast that remove proteins and polysaccharides and causes cells to flocculate offers an alternative strategy to the addition of costly enzymes.

a. Protein clarification

Heat-induced protein haze is caused by thaumatin-like proteins and chitinases, which are pathogenesis-related grape proteins (103). These proteins slowly denature and aggregate, resulting in light dispersing particles forming in wine. Under winemaking conditions, proteases have been unsuccessful in degrading haze proteins (104). However, a number of glycoproteins have been found to visibly reduce haze formation in wine, including yeast invertase (105) and two mannoproteins from *S. cerevisiae* known as haze protection factors (HPF) (106).

b. Cell flocculation and flotation

Rapid settling of yeast is advantageous to post fermentation processing. Yeast flocculation, which entails an interaction between cell surface lectins and mannans, involves many genes in *S. cerevisiae* (107). *FLO1* has been the best studied and shown to impart flocculation to beer and wine strains (108,109). Given that the timing of flocculation is important, *FLO1* and *FLO11* were expressed from the *HSP30* promoter, linking flocculation to a heat shock towards the end of fermentation (110).

In sherry, the yeast trap carbon dioxide and float to the surface forming a vellum. Several cell wall proteins have been implicated in vellum formation but the process appears complex and is likely to be multigenic (111). The similarities between vellum and biofilm formation point to Flo11p/Muc1p as a candidate gene in the process (112).

3. Improving Wine Sensory Attributes

The endless variety of wine flavours stems from complex interactions among hundreds of metabolites. *S. cerevisiae* accounts for the major changes between grape must and wine, modifying the chemical, colour, mouth-feel and flavour complexity of wine by assisting in the extraction of compounds from solids present in grape must, modifying grape derived molecules, and producing yeast metabolites (113).

a. Grape derived flavours

(i) Polysaccharide degrading enzymes. Polysaccharide degrading enzymes facilitate wine clarification and increase juice yield; they can also lead to the release of colour and flavour compounds trapped in the grape skins. A wide variety of heterologous genes encoding polysaccharide degrading enzymes have been expressed in *S. cerevisiae* (114). Pectin degradation was increased by yeast co-expressing two bacterial pectinase genes, pectate lyase from *Erwinia chrysanthemi* and polygalacturonase from *Erwinia carotovora* (115), or the pectate lyase gene from *Fusarum solani* (116). The overexpression of the endopolygalacturonase-encoding gene (*PGU1*) from *S. cerevisiae* in a wine yeast strain resulted in a higher concentration of terpenols (nerol and geraniol) in the wines, which displayed sensory differences to wines made with the parent yeast (117).

A number of glucanase genes from bacteria, yeast and filamentous fungi have been expressed, either on their own or together in commercial wine strains (114). These yeasts were able to degrade glucans effectively. Similarly, a number of xylanases have been expressed in *S. cerevisiae.* Although the levels of secreted enzymes are still below the concentrations required for the effective removal of polysaccharides, a degree of clarification has already been achieved. Preliminary trials also indicate that some of the modified yeast lead to the release of colour and flavour compounds entrapped in the grape skins.

(ii) Glycosidases. Yeast can increase wine flavour by releasing terpenols present as non-volatile glycosides in grapes. Expression of a β-1,4-endoglucanase gene from the filamentous fungus *Trichoderma longibrachiatum* in a wine yeast produced a wine with increased aroma intensity (118). The impact on flavour release of co-expressing three glucanase genes from *S. cerevisiae* (*EXG1*), *Bacillus subtilis* (*END1*) and *Butyrivibrio fibrisolvens* (*BEG1*) in a yeast remains to be determined (119).

Yeast can influence the concentration of terpenols by two other means: first, strains containing mutations in ergosterol biosynthesis have been shown to produce geraniol, citronelol and linalool (120), and second, it has been shown that yeast are able to convert one terpenol into another. As the terpenols have distinct aromas and sensory thresholds, these biotransformations might have a significant effect on wine sensory properties (121,122).

(iii) Volatile thiols. A number of potent thiol compounds have been shown to provide the basis for the varietal aromas of Sauvignon Blanc and Scheurebe wines (123), and are present in wines made from other cultivars. The sulfur-containing volatiles are present as a cysteine conjugate in grape juice and are released during fermentation by the action of yeast. Release is strain dependent, indicating that the concentrations of the thiols in wine can be regulated by genetically modifying the yeast (124).

b. Yeast secondary metabolites

(i) Esters. The characteristic fruity aromas of wine are caused mainly by the acetate esters of higher alcohols and the C_4-C_{10} ethyl esters. Overexpression of alcohol acetyl transferase genes (*ATF*) has a marked affect on ester formation (125–127). In wine, the concentrations of ethyl acetate, iso-amyl acetate, and 2-phenylethyl acetate increased as much as 10-fold, and the acetic acid concentration decreased by more than half in an *ATF1* overproducing strain (126). In saké strains, isoamyl acetate (banana-like aroma) concentrations have been modified by adjusting the *ATF1*/*IAH2* (isoamyl acetate esterase) balance (128).

(ii) Alcohols. Higher alcohols can contribute positively to wine complexity, but at excessive levels are regarded to have a negative influence on wine quality. They are also important precursors to ester formation. They are produced either anabolically or catabolically, by the breakdown of branched chain amino acids (129). The role of the anabolic pathway has been recently studied by deleting the aminotransferase genes *BAT1* and *BAT*2, the mutations leading to a decreased formation of isobutanol, isoamyl and active amyl alcohols (127,130).

(iii) Low ethanol wine yeast. High ethanol can affect the sensory properties of wine, giving it a perceived 'hotness' as well as suppressing overall aroma and flavour. Moreover, health-conscious consumers are demanding lower alcohol content in wines. Diverting the flow of sugar away from ethanol synthesis and into the glycerol pathway has been achieved by overexpressing the glycerol phosphate dehydrogenase genes, *GPD1* and *GPD2*, in wine strains (77,78,131). Acetic acid (vinegar) concentrations in these strains are increased to unacceptable levels. The acetaldehyde dehydrogenase activity encoded by the *ALD6* gene appears to be the main contributor to the oxidation of acetaldehyde during fermentation (132). A laboratory yeast overexpressing *GPD2* and lacking *ALD6* had the desired effect of producing more glycerol and less ethanol, but without the increase in acetic acid (133). A 'metabolic snapshot' (67) of the glycerol overproducing ferments also demonstrated how seemingly unrelated biochemical pathways can be modified by the change in a single gene (133).

A second strategy used to decrease ethanol is the expression of the *A. niger* glucose oxidase gene (*GOX1*) into *S. cerevisiae*, resulting in a 2% decrease in ethanol (95). The glucose in grape juice is thought to be converted to D-glucono-δ-lactone and gluconic acid, and is thus not available for conversion to ethanol.

(iv) Sulfur metabolism. Due to its low sensory threshold, the rotten egg aroma of hydrogen sulfide (H_2S) is highly undesirable in wine. Modification of genes required for sulfur metabolism leads to a significant decrease in hydrogen sulfide production (134–136). In *SSU1* overexpressing strains, a plasma membrane protein specifically for the efflux of sulfite from yeast (137), the sulfite is secreted prior to reduction to sulfide (93). Increases in *SSU1* expression also confer SO_2 resistance to *S. cerevisiae* strains. Many wine yeast strains that show natural resistance to sulfide have undergone chromosome translocation, positioning the *SSU1* gene downstream of a stronger promoter, and consequently causing its increased expression (138).

(v) Malolactic fermentation. During malolactic fermentation (conducted by certain species of lactic acid bacteria) L-malic acid is decarboxylated to L-lactic acid, which reduces wine acidity. *S. cerevisiae* is unable to transport malic acid into the cell and is inefficient in metabolising malic acid. The construction of a *S. cerevisiae* strain which is able to deacidify wine has been successfully demonstrated by expressing the malolactic gene (*mleS*) from *Lactococcus lactis* and the malate permease gene (*mae1*) from *Schizosaccharomyces pombe* (139).

In an attempt to redirect glucose carbon to lactic acid, the lactate dehydrogenase-encoding genes from *Lactobacillus casei*, the filamentous fungus *Rhizopus oryzae* and bovine muscle were expressed in yeast. As much as 20% of the glucose was converted into lactic acid in these strains (140,141).

(vi) Phenols. The decarboxylation of phenolic acids to volatile phenols can make a positive contribution to wine when present in appropriate concentrations. Although *S. cerevisiae* possesses a phenolic acid decarboxylase, *POF1* (*PAD1*), it displays low activity. Expressing the phenolic acid decarboxylase or *p*-coumaric acid decarboxylase from *Bacillus subtilis* and *Lactobacillus plantarum*, respectively, gave an approximate twofold increase in volatile phenol formation in a laboratory strain (142).

4. Improving Wine Wholesomeness

During the last few years it has become widely accepted that wine contains protective compounds that when consumed in moderate quantities reduce the likelihood of contracting certain diseases. However, a number of unwanted compounds are also present in wine. Generating improved yeasts that produce the correct balance of these compounds will be important for enhancing the health benefits of wine.

a. Resveratrol

Resveratrol, a polyphenolic phytoalexin found mainly in grape skins, has been associated with a large number of

health benefits, including the decreased risk of coronary heart disease, cancer prevention and treatment, and neuroprotection (60). Expression of the *Candida molisciana bgiN* gene, encoding a β-glucosidase, showed an increase in resveratrol in white wine. Release of glucose moieties from the glucoside form of resveratrol was the suggested mechanism (116). Also, yeast with altered phenylpropanoid metabolism, expressing the coenzyme A lyase gene and the grape stillbene synthase gene (*Vst1*), can synthesise resveratrol (143).

b. Ethyl carbamate

Ethyl carbamate, a suspected mammalian carcinogen, is formed through the chemical reaction of ethanol and citrulline, urea or carbamyl phosphate. In a saké strain disrupted for the *CAR1* arginase gene, no urea or ethyl carbamate was produced (144).

c. Biogenic amines

Biogenic amines are neurotoxins that can trigger hypotension and migraines, lead to histamine toxicity and produce carcinogenic nitrosamines (145). These biogenic amines originate from decarboxylation of amino acids by bacteria, including malolactic species (146,147). Wine yeast strains that inhibit bacterial growth may offer a solution.

IV. THE GENETIC IMPROVEMENT OF WINE BACTERIA FOR MALOLACTIC FERMENTATION

Malolactic fermentation (MLF), conducted by malolactic bacteria, is an important step in the grape vinification process, particularly in red wines. Most often MLF will occur after the alcoholic fermentation; however, it is not limited to this stage of winemaking. The role of MLF is threefold: wine deacidification by the conversion of L-malic acid to the 'softer' L-lactic acid, microbial stability and wine flavour modification.

This section will not elaborate on the biochemistry of MLF. Rather it will discuss the bacteria involved in the process, summarise the genetics of the malolactic bacteria and postulate potential genes that could be manipulated in order to improve the malolactic reaction, increase stress tolerance of the bacteria, and/or favourably alter the sensory profile of the wine. The ability to genetically alter the malolactic bacterial genome will depend upon the development of a suitable genetic transfer system (transformation, conjugation or transduction), which currently is unavailable.

A. Lactic Acid Bacteria Species and Strains

The wine bacteria associated with MLF belong to the family of lactic acid bacteria (LAB). This group of bacteria is involved with the fermentation of a range of food products, including milk, vegetables, meat and fruit, especially grapes. The malolactic bacteria are encompassed in four genera, *Lactobacillus (Lb.)*, *Leuconostoc (Lc.)*, *Oenococcus (O.)* and *Pediococcus (P.)* from within the larger group of LAB. The species in these genera can be characterised by their ability to tolerate low pH, high ethanol concentration and by their ability to grow in wine. Those most commonly associated with wine are *Lb.brevis*, *Lb. plantarum*, *Lb. hilgardii*, *Lc. mesenteroides*, *O. oeni, P. damnosus* and *P. pentosaceus*. *Oenococcus oeni*, formerly known as *Leuconostoc oenos* (148), is the LAB species most commonly associated with wine, as it is particularly well adapted to the harsh wine environment (low pH, high ethanol content, low nutrients) (149). For a more detailed description of LAB taxonomy the reader is referred to several reviews [(150,151); *Bergey's Manual of Determinative Bacteriology*, 1986)].

Even though it is postulated that species of *Lactobacillus* and to a lesser extent *Pediococcus* species may conduct the deacidification reaction in spontaneous MLF, species of these two genera are more likely to be associated with spoilage of wine than with positive sensory attributes.

B. Genetic Features and Techniques for the Analysis and Development of Lactic Acid Bacteria

The *O. oeni* genome has been sequenced in its entirety by two different groups (University of California, Davis with the group of D. Mills and at ESBANA, Dijon, France with the groups of J. Guzzo and A. Lonvaud-Funel). This sequence information will greatly enhance the initial work on the *O. oeni* genome (150–153) and add to the limited number of genes that have been characterised. Though *O. oeni* is an important organism in winemaking, knowledge of its genetics is limited. The current lack of a genetic transfer mechanism into *O. oeni* further hampers genetic characterisation of this organism.

Considerable research has been done on other LAB, particularly those from the dairy industry. Substantial work has been devoted to the genetics of *Lactobacillus* sp. but to a lesser extent in *Pediococcus* sp. Numerous vectors have been constructed to introduce modified genes into *Lactobacillus* species and strains. These vectors are designed to enable easy movement between Gram-positive and Gram-negative organisms utilising dual replicons (origin of replication) and antibiotic markers. Also available are temperature-sensitive features on a few of these plasmids. Some of these plasmids/vectors also function in *Leuconostoc* sp., which is the closest LAB genus to *Oenococcus*. Many of these vectors may be suitable for *O. oeni*.

Another approach to improving industrially important *O. oeni* strains is the use of a new technology known as genome shuffling (154,155). This technique involves using a classical strain improvement method to generate

populations with subtle improvements. Next, these populations are shuffled by recursive pool-wise protoplast fusions. Genome shuffling has been successfully applied to improve acid tolerance in a poorly characterised industrial *Lactobacillus* strain (156) and appears to be broadly useful for the rapid development of tolerance and other complex phenotypes in industrial organisms.

C. Targets for the Genetic Improvement of Malolactic Bacterial Strains

Potential improvements to MLF by the genetic modification of *O. oeni* are presented in Figure 40.3. It would be of obvious interest to construct an *O. oeni* strain which is able to ward off potential competitors, improve its ability to cope with the harsh wine environment, increase its efficiency in the bioacidification of wine-conversion of malic acid to lactic acid, and/or to provide mechanisms to improve the organoleptic qualities of wine. Such an organism would not necessarily possess all these attributes, but generating a selection of improved *O. oeni* would be of great benefit to the wine industry.

1. Improving Fermentation Performance

a. Adaptation to environmental stress

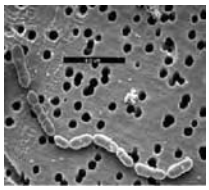
It has been demonstrated that *O. oeni* responds to various environmental stresses, such as high alcohol, acid and sulfur dioxide (SO_2) concentrations, by producing heat shock and stress proteins (*hsp18*, *clpX* and *trxA*) (157). The manipulation of these genes could lead to improved tolerance of *O. oeni* to wines with conditions at the upper limit of *O. oeni* tolerance. The elevated expression of these heat and stress proteins may also aid the commercial production of *O. oeni* for direct inoculation into wine, by better preparing the bacterial cells for the harsh wine environment.

Fermentation performance

MLF efficiency
- Malolactic enzyme
- (*mleR*, *mleA*, *mleP*)
- Increase the efficiency of malate metabolism

Stress
- Heat shock and stress proteins
- (*hsp18*, *clpX*, *trxA*)
- Regulation of production could lead to improved tolerance to wine conditions, especially alcohol (high EtOH, 14–15%)

Oenococcus oeni



Wine wholesomeness

Arginine / Ethyl carbamate
- *Arc* cluster (arginine metabolism)
- Arginine metabolism leads onto ethyl carbamate (animal carcinogen)
- Manipulation of *arc* cluster could lead to ↓ ethyl carbamate production

Biogenic amines
- ↓ histindine decarboxylase (*hdc*) activity to reduce the concentration of histidine in wine

Antimicrobial
- ↑ bacteriocin production (pediocin, lactocin, nisin) might result in↑natural removal of selected spoilage LAB during MLF
- Cyclic peptides produced by *Lb. plantarum* and *P. pentosaceus*; ↑natural removal of selected spoilage fungi during MLF

Sensory attributes

Diacetyl
- Buttery, nutty, butterscotch notes
- *alsS, alsD* (α-acetolactate synthase and decarboxylase)
- ↑↓ synthesis of diacetyl
- Δ *ldh* (drives reaction towards pyruvate and onto diacetyl)
- Modulation of aroma and flavour in wine

Esters
- ↑ acyl transferases (*atf*) and ↓ esterase activity
- ↑ aroma profile of wine

Glycosidase
- *bgl* (β-glucosidase)
- Clone other genes required for sequential release of disaccharide bound aglycon; ↑ aroma and flavour

Mouth-feel
- Polysaccharides β(1→3)glucanase
- Other mouth-feel contributors
- (glycerol, mannoproteins)

FIGURE 40.3 Methods for strain development of lactic acid bacteria. Photograph kindly provided by Dr Jeffery Broadbent.

b. Improved efficiency of malate catabolism

The genes for malate metabolism have been cloned from *O. oeni* and three genes *mleR* (regulator), *mleA* (enzyme) and *mleP* (permease), have been identified (139). In order for malate metabolism to be initiated and to proceed efficiently in wine, a bacterial population density of at least one million cells/mL is needed. To increase the conversion efficiency of L-malate to L-lactate, it is necessary to understand the regulation of *mleA* by *mleR* and determine the rate limiting step for malate metabolism, especially important at low cell density. A rapid adaptation of the *O. oeni* cell to its harsh wine environment may also ultimately enhance the catabolism of L-malate.

2. Improving Wine Wholesomeness

a. Arginine/ethyl carbamate

The role that MLF plays in ethyl carbamate formation remains unclear. Arginine, a quantitatively important amino acid of grape must and wine, is a precursor to ethyl carbamate. Lactic acid bacteria vary in their ability to degrade arginine; experiments conducted in a synthetic and a laboratory vinified wine demonstrated a correlation between arginine degradation, citrulline production and ethyl carbamate formation during MLF conducted by an *O. oeni* and *Lb. buchneri* strain (158–160). The arginine catabolism (*arc*) gene cluster of *O. oeni* (161) has been cloned and characterised, thus providing a basis for manipulating the *arc* genes and reducing the potential of ethyl carbamate production.

b. Biogenic amines

Biogenic amines have undesirable physiological effects when consumed at high concentrations. The major biogenic amines in wine are histamine, phenylethylamine, putrescine and tyramine (162–164). Their concentration is lowest after alcoholic fermentation and increases variably in most wines during MLF (165). Wine-associated LAB, including *O. oeni,* have been shown to decarboxylate amino acids to their corresponding amines (166). This decarboxylation reaction is purported to favour growth and survival in acidic media, since it induces an increase in pH and can also provide energy to the LAB (167). LAB vary in their ability to produce the various amines.

The *O. oeni* histidine decarboxylase gene (*hdc*) has been cloned and characterised (168). A PCR-based test has also been developed for the detection of amino acid decarboxylating genes in LAB (169). The manipulation of the *hdc* gene in *O. oeni* strains with other desirable characteristics would ensure that reduced concentrations of histidine are present in wine.

c. Antimicrobial activity

The use of bacteriocin-producing LAB species/strains in other food industries, especially the dairy industry, has met with considerable success. A pediocin-producing *Lb. plantarum* strain was shown to efficiently combat the spoilage of cheese by *Listeria monocytogenes* (170). Furthermore, the expression of pediocin PA-1 from *Pediococcus acidilactici* or plantaricin 423 from *Lb. plantarum* by the introduction of the respective genes into *S. cerevisiae* has been shown to effectively eliminate LAB from an alcoholic fermentation (101). A similar strategy could be used to introduce the necessary genes into *O. oeni*.

3. Improving Wine Sensory Attributes

a. Diacetyl

One of the most important flavour compounds associated with MLF is diacetyl (a diketone, 2,3-butanedione), which can impart a 'buttery' or 'butterscotch' flavour to wine (171–173). Diacetyl is principally formed during MLF by the bacterial metabolism of citric acid. This diketone is also an important flavour compound in the dairy industry, providing the characteristic 'buttery' flavour of many fermented milk products.

The metabolism of diacetyl is well understood (174–176) and the environmental factors that influence its formation and degradation in wine have been established (177–179). The diacetyl pathway has been amenable to genetic manipulation, leading to overproduction of diacetyl in *Lactococcus lactis* (180). With the inactivation of the lactate dehydrogenase gene (*ldh*) in *L. lactis*, there was an accompanying alteration to the metabolic flux, eliminating lactic acid as a metabolic end product and producing ethanol, formate and acetoin. Acetoin is a degradation product of diacetyl and is considered flavourless in wine because of its high aroma threshold. The overexpression of α-acetolactate synthase (*ilvBN* genes in *L. lactis*) leads to an increased production of acetoin (the reaction is driven towards acetoin and away from diacetyl), whereas the inactivation of the *aldB* gene (encoding α-acetolactate decarboxylase) resulted in an increased production of α-acetolactate and diacetyl at the expense of acetoin. The latter scenario is quite desirable in the dairy industry. The analogous genes [*alsS* (α-acetolactate synthase) and *alsD* (α-acetolactate decarboxylase)] have been cloned and sequenced from *O. oeni* (181), providing a means of altering diacetyl concentrations in wine. An alternative strategy would be to inactivate the citrate permease gene (*citP*), thus removing the initial substrate for diacetyl. Such a natural mutant of *O. oeni* has been isolated and is commercially available (MT01, Lallemand).

b. Glycosidases

Many potential aroma and flavour compounds are found in grapes and wine as glycosidically bound aglycons (monoterpenes, norisoprenoids, benzene derivatives and aliphates). Though β-glucosidase activity has been demonstrated in *S. cerevisiae* and *O. oeni* (182), both have limited ability to release the disaccharide glycosides.

Improving release of glycosides in *O. oeni* strains following MLF using similar methods to those described in yeast could lead to enhanced grape variety aroma in the wine.

c. Esterases

Various sensory studies pre and post MLF have shown that the fruity qualities of a wine may be enhanced after MLF (183,184). An improved understanding of the formation and further metabolism of esters [acyl alcoholtransferase (ester synthesising enzymes) and esterases (ester catabolising enzymes)] in *O. oeni* could lead to the enhancement of specific, desirable esters.

d. Bitterness

Bitterness is a spoilage problem, primarily in red wine, that can be associated with LAB, including *O. oeni* strains. The fermentation of glycerol can lead to the formation of acrolein, which when it reacts with phenolic hydroxyl groups, results in wine bitterness. The manipulation of the pathway of glycerol catabolism, for example the glycerol dehydratase, may lead to reduced acrolein formation.

The presence of certain peptides is also a potential cause of bitterness in wine. Bitterness due to proteolytic action and formation of short peptides has been studied extensively in dairy LAB. There appears to be a minimal proteolytic activity associated with *O. oeni* and the peptide transport system of *O. oeni* is poorly understood. A better understanding of potential bitter wine peptides and their formation could reduce the occurrence of bitterness in some wines.

e. Mouth-feel properties

Numerous MLF sensory studies and anecdotal evidence point to changes in the texture and body of the wine following MLF, with reports indicating a fuller, richer, longer aftertaste (183). The chemical changes contributing to these favourable mouth-feel properties are poorly understood. The deacidification process itself contributes substantially to changes in mouth-feel, with the consequent increase of pH and decrease in titratable acidity. In addition, the L-lactic acid feels softer than L-malic acid. Other contributors to wine mouth-feel or taste perception have been suggested, including polysaccharides, glycerol and mannoproteins. The contribution of MLF in this area remains to be studied.

MLF has great potential to further enhance wine qualities by retaining or altering the aroma/flavour profile of the wine as well as by conferring microbial stability. However, the genetics of *O. oeni* for all these processes are still not well understood. With the availability of the complete *O. oeni* genome sequence, the development of an efficient gene transfer system into *O. oeni* and the understanding of the various pathways, 'tailor-made' *O. oeni* strains will become possible.

V. THE FUTURE OF GENETICALLY IMPROVED GRAPEVINES, YEASTS AND BACTERIA IN THE WINE INDUSTRY

Molecular genetic research has much to offer the wine industry. It is a powerful technology for studying the regulation of pathways in grapevine, yeast and bacteria, thus providing an understanding of how viticulture and winemaking practices impact on grape and wine quality. In some cases, especially yeast and bacteria, identifying the important genes will provide non-GM improvement strategies. Molecular techniques are also starting to dominate systematics, simplifying *Vitis* varietal identification and wine microbial ecological studies.

This review has focused upon the use of molecular techniques to produce GM grapevines and wine microbes that can provide major advantages to the wine industry and wine consumer. Several obstacles, however, must be overcome before these techniques can be put to use. These include scientific and technical issues, a number of regulatory and economic concerns and the public perception of GM foods (5).

A. Scientific and Technical Obstacles

The bacterium *Escherichia coli* and plants such as *Zea mays* and *Arabidopsis thaliana* have been used as experimental model organisms for many decades, but the study of important wine bacterial species and *Vitis vinifera* is much more recent. Similarly, although laboratory strains of *S. cerevisiae* are easily manipulated, wine yeast have characteristics such as homothallism, polyploidy, and the lack of auxotrophic markers that make them more difficult to manipulate. There is increasingly rapid progress, however, in the molecular improvement of these organisms, and as important genes continue to be identified, the potential for use of GMOs in the wine industry will continue to expand.

B. Regulatory, Economic and Political Obstacles

Most countries' legislation and regulations applying to the use of GMOs and the approval of GM foods are now broadly similar and fall into two categories. The approach exemplified by the regulations of the United States of America takes the view that each case of a GMO needs to be considered on its merit and there is no *a priori* reason to assume that the use of GM plants and microbes is of itself more risky than conventional methods of introgressing new genes into organisms and products. The arguments used in this approach are those of 'substantial equivalence' and statutory approval usually requires several obvious assurances, such as that there be no additional threat to human health or the environment. The second approach is exemplified by the European Union's

regulatory system that takes the view that GM material has an intrinsic level of risk beyond that accepted in non-GM products. The regulations are framed around this philosophy and the requirements to demonstrate the properties of GMO-based material are substantially more rigorous than those placed upon the introduction of food material bred by conventional genetic means.

While genetically improved grapevines, yeast and bacteria need some form of economic protection, patents covering many of the tools used in genetic engineering, including the genes themselves, leave little 'freedom to operate' (185). If commercialisation of GMOs is to be viable, it is imperative that intellectual property issues be addressed.

There is also a belief that patents on genetically engineered organisms give an unfair advantage to certain large multinational producers, thereby concentrating economic power in their hands. With the visibility of the anti-globalisation movement and a surplus of wine in some countries, it is possible that the commercialisation of improved GM wines could be misused to justify technical barriers to free trade.

Bottle labelling is another hurdle. The marketing of wine relies to a great extent on label integrity and product identity, and it is hoped that wines exhibiting improved flavour and colour characteristics will be able to retain the established varietal names. It may also become obligatory to label GM wines as such, and it is not clear whether this will be detrimental to the sale of these wines (186). For example, the European Union and Japan have recently introduced new legislation such that all food products with more than 0.9% and 0.5% GM content, respectively, will have to be labelled as GM. Any GM food product derived from GM ingredients but whose presence is undetectable in the product will also have to be labelled.

Much of the wine industry is bound by tradition and regional culture, making it particularly unreceptive to technological change. In addition, there is a concern that GM wines will become standardised, and lose their mystique and romanticism. Quite to the contrary, gene technology will allow winemakers to diversify their products and provide even greater consumer choice.

C. Public Perception Obstacles

The public perception of GM foods has been, so far, that the potential risks outweigh the benefits. Sensationalist reporting has increased public fears of 'Frankenfoods,' which of course spread far more readily than good sense or wise science. Ironically the potential advantages to consumers and the environment are numerous, and include a healthier and better product and agricultural sustainability. To reverse negative perceptions it is essential that scientists ensure the trust of the public by providing balanced, understandable information on the safety and environmental risks associated with GMOs (187).

It should be remembered that the improvement of foods has been going on for many thousands of years, initially by selection and later by selective breeding, and unwanted and indeterminate changes have been routinely transferred along with the desired modifications. These methods are completely acceptable to consumers, if they are even aware of them. Genetic engineering provides a more precise means of bringing about the intended changes, which together with recent developments in the field of metabolomics, offers techniques to produce foods that comply with the test of 'substantial equivalence' (188,189).

Evaluating potential environmental risks of GMOs is more difficult, and will have to be examined on a case-by-case basis (190). Meanwhile, improvements in the technology continue to reduce the potential of risk (191). Guidelines for the release of GMOs in most countries require the absence of any selective advantage conferred by the transgenic organism that could allow it to become dominant in natural habitats. Again, improved molecular techniques such as Real Time PCR should assist in evaluating competitiveness (192,193).

The nature of the modification will also influence consumer acceptance. Whereas some modifications do not add foreign DNA, others require the insertion of antibiotic or herbicide resistant markers often considered undesirable. Some genetic modifications cross species barriers; these are more likely to generate debate and allow objectors to pose religious and moral questions.

In winemaking the yeast and bacteria are not present in the final product, and the clarification of wine removes nucleic acid, including that of the grapes. This should allay consumer fears that they are ingesting modified DNA (194).

VI. CONCLUSIONS AND OUTLOOK

In many respects wine is no longer seen as a dietary beverage, but as a non-essential luxury product, offered to consumers along with many other luxury products. The international wine industry is obviously in business to attract a fair proportion of the consumers' disposable income in competition with other luxury goods and services. To do so it must offer products that exhibit desirable and pleasurable characteristics to the consumer, continue reducing its impact on the environment, and offer a competitive quality/price ratio while remaining profitable for the producers.

Competitors of the wine industry, e.g., producers of other alcoholic beverages, do not hesitate to employ new technology as it becomes available. Winemakers must become more sophisticated about the application of new scientific and technological knowledge while still respecting the cultural roots and traditions associated with winemaking. The ongoing research into the genetic improvement of grapevines and wine microbes as outlined in this chapter

provide the global wine sector with an enormous opportunity to improve the quality/price ratio of wine by embracing biotechnological innovations.

In the medical world the uses of GM products are accepted and widespread. In agriculture the uptake in the field crop industries has been steadily increasing to the degree that in 2002 almost 60 million hectares of GM field crops were being grown worldwide. And this trend toward greater acceptance is likely to continue. With the rapid growing power of bioinformatics and the dawn of the 'omics' era in molecular biology it is widely expected that high quality information and 'precision' gene technology will increasingly allow grape and wine biotechnology to be harnessed for the good of the producer, consumer and environment. That is why some optimists even claim that gen*omics*, transcript*omics*, prote*omics* plus metabol*omics* equal econ*omics*!

Reality looms in the form of agronomic, business, regulatory and social hurdles currently blocking commercial availability of GM grapes and wine. But the smart money is saying that the benefits to the industry such as minimised resource inputs, improved product quality, increased health benefits and low environmental impact will ultimately put gene technology in the winner's circle.

ACKNOWLEDGMENTS

Research conducted at The Australian Wine Research Institute is supported by Australia's grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government.

The information in this chapter is based on "The application of gene technology in the wine industry," in: *Wine Biotechnology Manual*, copyright ©2003 by Isak S. Pretorius. Used with permission.

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Part C

Food Analysis

41 Food Analysis: Basics

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CONTENTS

I. INTRODUCTION

Foods are analyzed for chemical composition and characteristics for a variety of reasons in the food industry, including nutrition labeling and quality control (1–5). The analyses may be performed continuously within the processing line, or samples are collected and analyzed in the quality control laboratory or sent to an outside analytical laboratory. In the latter two cases, samples must be properly selected and prepared for analysis, then analyzed and the data interpreted. Selecting the method of analysis depends on a variety of factors, including the objective of the testing, characteristics of the method and the food products, and needed accuracy and precision. Out of necessity, methods used during processing and on final products are typically rapid, while more time-consuming and accurate methods are specified to suppliers of raw ingredients and used for nutrition labeling of the final product. Whatever the method of analysis, data obtained must be handled appropriately and interpreted as the basis for decision-making. These basics of food analysis are necessary whether the method being utilized is wet chemistry, spectroscopy, chromatography, or some other method.

II. WHY ANALYSES ARE DONE

Demands of consumers, strong competition within the food industry, and government regulations and international standards all contribute to the need for analysis of food products and ingredients.

A. Consumers

Safety, quality, nutrition, and good value are all important to consumers as they select their foods for purchase. It is increasingly a challenge for the food industry to meet the demands of consumers. Consumer interest in food safety has increased the testing for allergens and pesticide residues. Consumer concern about food materials that have been genetically modified using biotechnology has led to testing of foods and raw materials for such modification. Consumer interest in the relationship between diet and health has increased the importance of testing foods to allow for certain nutrient content claims (e.g., low fat) and health claims (e.g., dietary saturated fat and cholesterol, and the risk of coronary heart disease) (1).

B. Food Industry

To manage product quality, food companies must apply analytical methods across the entire food supply chain, from the raw ingredients to the final product (Table 41.1).

TABLE 41.1
Types of Samples Analyzed in a Quality Assurance Program for Food Products

Sample Type	Critical Questions
Raw materials	Do they meet your specifications?
	Do they meet required legal specifications?
	Will a processing parameter have to be modified because of any change in the composition of raw materials?
	Are the quality and composition the same as for previous deliveries?
	How does the material from a potential new supplier compare to that from the current supplier?
Process control samples	Did a specific processing step result in a product of acceptable composition or characteristics?
	Does a further processing step need to be modified to obtain a final product of acceptable quality?
Finished product	Does it meet the legal requirements?
	What is the nutritive value, so that label information can be developed? Or is the nutritive value as specified on an existing label?
	Does it meet product claim requirements (e.g., "low fat")?
	Will it be acceptable to the consumer?
	Will it have the appropriate shelf life?
Competitor's sample	What are its composition and characteristics?
	How can we use this information to develop new products?
Complaint sample	How do the composition and characteristics of a complaint sample submitted by a customer differ from a sample with no problems?

From: SS Nielsen, ed. Food Analysis, 3rd ed. New York: Kluwer Academic, 2003 (Ref. 1).

Analyzing various types of samples in the food processing system can help answer questions critical to the success of the business. Heavy emphasis is placed on the quality of raw ingredients, which reduces the need for extensive testing during processing and on the final product. Responsibility for ingredient testing has been shifted largely from the food companies to the ingredient suppliers. Most food companies have "select suppliers" who are given detailed ingredient specifications. These suppliers are asked to do the analytical testing and provide a "certificate of analysis" to show compliance with these specifications. Limited additional testing of ingredients may be done upon receipt by the food company. In some cases, a food company arranges to receive a small sample of the ingredient to check for particular characteristics before accepting a large shipment of the ingredient (1).

C. Government Regulations

Government regulations dictate certain types of analyses (6). Three examples follow of food analysis required based on federal regulations in the U.S. First, nutrition labeling regulations in the U.S. are a major driver of the analysis of foods (Table 41.2). Chemical analysis of foods is required not only for the nutrition label itself, but also for making nutrient content claims that characterize the level of a nutrient (e.g., low fat, reduced sodium, high calcium) or health claims (e.g., folate and neural tube defects, soluble fiber from whole oats and coronary heart disease). Second, the U.S. Food and Drug Administration (FDA) has established standards of identity for certain food products. These standards often include which ingredients a food must contain, the maximum levels for inexpensive ingredients (e.g., water), the minimum levels for expensive ingredients (e.g., fat), and recommended analytical methods to determine the chemical composition. Table 41.3 summarizes food analysis-related information from the standard of identity for several food products. The recommended methods of analysis specified in standards of identity are typically from the *Official Methods of Analysis* of AOAC International (formerly known as the Association of Official Analytical Chemists, AOAC) (7). Third, grade standards established by the U.S. Department of Agriculture, while voluntary rather than mandatory, are used often as quality control tools. These grade standards classify products in a range from substandard to excellent in quality. This information is useful both to consumers (e.g., Grade A eggs) and to institutions (schools, hospitals, military) that purchase foods. Grade standards often include not only chemical composition characteristics (e.g., soluble solids and titratable acidity distinguish Grade A and B orange juice), but also sensory evaluation and visual appearance.

TABLE 41.2
Typical Methods Used for Nutrition Labeling Tests[a]

Item on Nutrition Label	Typical Analysis
Calories	Calculated from content of protein, total carbohydrate (or total carbohydrate less the amount of insoluble dietary fiber), and total fat (4, 4, and 9 calories per gram, respectively)
Calories from Fat	Calculated from total fat (9 calories per gram)
Total Fat	Extraction with petroleum or diethyl ether (e.g., Soxhlet or Goldfish methods), sometimes preceded by acid hydrolysis; Roese-Gottlieb or Mojonnier methods; Babcock; sum of fatty acid content by gas chromatography
Saturated Fat	Gas chromatography
Cholesterol	Gas chromatography
Sodium	Inductively coupled plasma-atomic emission spectroscopy, Atomic absorption spectroscopy, Mohr or Volhard titration, ion selective electrodes
Total Carbohydrate	Calculated as % Total carbohydrate = 100% − (% Moisture + % Protein + % Fat + % Ash)[b]
Dietary Fiber	Gravimetric-enzymatic method
Sugars	High performance liquid chromatography
Protein[c]	Kjeldahl, nitrogen combustion (Dumas)
Vitamin A	High performance liquid chromatography
Vitamin C	2, 6-Dichloroindophenol titration, microfluorometric assay
Calcium	Inductively coupled plasma-atomic emission spectroscopy, atomic absorption spectroscopy
Iron	Inductively coupled plasma-atomic emission spectroscopy, atomic absorption spectroscopy

[a]Nutrition labeling regulations of the United States Nutrition Labeling and Education Act of 1990.

[b]Moisture content may be determined by methods such as oven (e.g., forced draft oven, vacuum oven, rapid moisture analyzer), distillation, or Karl Fischer titration. Ash content for the purpose of calculating total carbohydrate content is usually done by dry ashing in a muffle furnace.

[c]Protein methods given are for determining content. If protein is expressed as a percent of the Daily Value, protein quality must be determined by either the Protein Efficiency Ratio method (foods for infants or children under four years of age) or the Protein Digestibility-Corrected Amino Acid Score method (foods other than for infants).

D. International Standards

Many food companies must meet not only the regulations of individual governments, but also standards set by international organizations. For example, standards published in the *Codex Alimentarius* by the Codex Alimentarius Commission are intended to facilitate international trade of foods, ensure fair business practices, and protect the health of consumers. These standards make necessary the analysis of raw agricultural commodities and processed food products being imported or exported (6).

III. WHERE ANALYSES ARE DONE

The need continues for collecting samples from the processing line for analysis in the quality control laboratory or by an outside analytical laboratory, but there is increasing interest in continuously monitoring the quality characteristics of foods in line as they are being processed. Such analysis better ensures the quality of the final product and reduces the need for further testing of the final product before distribution. Infrared spectroscopy, one of the main tools used for this application (8), is described in more detail in Chapter 43, Spectroscopy Basics. However, briefly, infrared spectroscopy measures the absorption of radiant energy, in the near- or mid-infrared region of the electromagnetic spectrum, by molecules in foods. The frequency of energy absorbed depends on the functional groups of the molecules being measured, and the amount of energy absorbed is related to the concentration of the food constituent of interest. Other methods of analysis are being used and further studied for on-line analysis applications.

Samples collected from the processing line may be analyzed immediately using equipment stationed very near the processing line (e.g., rapid moisture analyzer to check moisture content of cereals; portable colorimeter to check color). However, often samples collected from the processing line are transported to an established quality control laboratory where generally rapid methods are used to test quality characteristics as part of the quality management plan. For less routine analysis and for nutrition labeling tests, collected samples may be sent to outside analytical laboratories. These analytical laboratories often are certified laboratories with highly trained and experienced personnel, and often are equipped with expensive analytical instrumentation. For example, a typical quality control laboratory would not be set up to do analyses for dietary fiber or certain vitamins, but samples for these analyses could be sent easily to an analytical laboratory.

IV. STEPS IN ANALYSIS

Analysis of foods involves three main steps: 1) selecting and preparing the sample, 2) performing the analysis, and 3) calculating and interpreting the results.

Collecting the sample and preparing it for analysis, which are covered in more detail in the next section, are much more difficult and important than they would appear. Non-homogeneity of food and ingredients, along with uneven distribution of certain constituents, make collecting a representative sample a challenge. Conditions of sample preparation and the instability of certain food constituents make it difficult to ensure that the composition and characteristics of the sample collected are identical to those of the sample subjected to the analytical procedure.

TABLE 41.3
Chemical Composition Requirements of Some Foods and Ingredients with Standards of Identity

Section in 21 CFR[1]	Food Product	Requirement	AOAC METHOD[2] Number in 13th Ed.	Number in 17th Ed.	Name/Description
131.125	Nonfat dry milk	Moisture ≥ 5% by wt.	16.192	927.05	Vacuum oven
		Milkfat ≤ 1.5% by wt.	16.199–16.200	932.06A, 932.06B	Roese-Gottlieb
145.110	Canned applesauce	Soluble solids ≥ 9%, expressed as % sucrose, °Brix	22.024	932.14A	Refractometer
168.20	Glucose syrup	Total solids ≤ 70%,mass/mass (m/m)	31.2088–941.14A, 941.14B 31.209		Vacuum oven, with diatomaceous earth
		Reducing sugar ≥ 20% m/m (dextrose equivalent, dry weight basis)	31.220(a)	945.66(a)	Lane-Eynon
		Sulfated ash ≤ 1% m/m, dry weight basis	31.216	945.63B	Dry ashing
		Sulfur dioxide ≤ 40 mg/kg	20.106–20.111	962.16A–963.20C	Modified Monier-Williams

[1]CFR, Code of Federal Regulations (2004).

[2]*Official Methods of Analysis* of the Association of Official Analytical Chemists (AOAC) International.

Each food or ingredient component or characteristic to be tested may require a unique assay. Descriptions of wet chemistry, spectroscopy, chromatography, and other methods for food analysis are given in subsequent chapters. However, for any particular assay, the analyst doing the assay needs to consult manuals, reference books, and articles for details on the necessary chemicals, reagents, equipment, and step-by-step instructions. It must be stressed that glassware and other labware used must be clean and equipment properly checked and calibrated.

The results obtained from performing the assay of food components and characteristics are utilized to make decisions and take action. A later section of this chapter deals in more detail with data handling and assessing its reliability. The analytical data obtained can be evaluated and integrated with other relevant information to address food quality-related problems. Such data are critical not only for quality assurance, but also in product formulation and process design.

V. SAMPLING AND SAMPLE PREPARATION

A. Sample Collection

Sample collection and sample reduction methods must be developed to ensure a representative sample is being subjected to analysis (9, 10). The choice of a sampling plan is affected by four factors: 1) purpose of the inspection, 2) nature of the product, 3) nature of the test method, and 4) nature of the population being investigated (11). Sampling plans are typically applied to heterogeneous (versus homogeneous) populations, for which it is more difficult to obtain a representative sample. Sampling plans are designed to examine either attributes (i.e., to decide acceptability based on whether the sample possesses a certain characteristic, e.g., *Clostridium botulinum*) or variables (i.e., to estimate the amount of a substance or characteristic on a continuous scale, e.g., color). Variable sampling generally requires a smaller sample size than attribute sampling (12).

Three types of sampling plans exist: 1) single (one sample of a specified size, to allow accept/reject decisions), 2) double (select two samples, then make accept/reject decisions after testing one or both samples), and 3) multiple (reject low-quality lots and accept high-quality lots quickly; do further sampling of intermediate quality lots). No sampling plan is free of risks. The two types of risks associated with sampling are consumer risks and vendor risks. The consumer risk is the probability of accepting a poor quality product. The vendor risk is the probability of rejecting an acceptable product.

Sampling may be done manually by a person or continuously using some mechanical system. If operating properly, the continuous sampling should be less prone to human bias than is manual sampling.

Ideally the sampling method is statistically sound. However, sometimes nonprobability sampling is unavoidable. With such sampling, a representative sample of the population cannot be collected. Preferably one can do probability sampling, which has a statistically sound basis to obtain a representative sample and eliminate human error. Probability sampling ensures random samples, and allows calculation of sampling error and the probability of including any item of the population in the sample.

Samples collected for analysis must be clearly labeled in preparation for sample preparation and then analysis.

Samples collected for official or legal purposes must be sealed to protect from tampering, and the chain of custody must be clearly identified.

B. Preparation of Samples

The *Official Methods of Analysis* of AOAC International (7) gives detailed instructions on preparation of specific food samples for analysis, determined by the nature of the food and the type of analysis to be done. Such instructions include size of samples collected, particle size reduction, and storage of samples.

Samples that are too large in mass must be reduced. The mass can be reduced by spreading the sample on a clean surface, dividing into quarters, and collecting the two opposite quarters. The process can be repeated until a sample of manageable size is obtained. The method can be adapted to liquids by using four containers (9).

Grinding is used commonly to reduce particle size so more homogenous samples can be obtained and samples are suitable for analysis. Grinding procedures often specify the mesh size, meaning the number of openings per linear inch of mesh. A 40-mesh size, meaning very small particles, is used for assays that involve an extraction, such as lipid analysis. A 20-mesh size gives larger particles used for moisture or total protein analyses.

The various types of mills used for grinding differ in their mode of action and appropriate applications. A common concern in grinding is the loss of moisture due to air exposure or heating. Grinders are sometimes water cooled to reduce heating, and samples are sometimes ground frozen.

In addition to protecting samples from moisture loss during grinding, it is necessary to handle and store samples in such a way as to avoid several other potential problems. Enzymes naturally present in foods can degrade the food constituents to be analyzed. Enzyme action can be slowed or stopped by freezing, heat denaturation, or other means appropriate for the specific enzyme. Unsaturated lipids and some vitamins and pigments are especially sensitive to oxygen and light. This makes it necessary to store some samples under nitrogen in the dark, or analyzed as soon as possible after collection. Microbial growth in samples prior to analysis can change the chemical composition. Freezing, drying, and chemical preservatives may be used alone or in combination to prevent microbial problems.

VI. SELECTING THE METHOD AND DOING THE ASSAY

A. Objective

The objective of the measurement is a major factor in determining the choice of the method of analysis (1). For example, time-consuming, accurate, official methods are used for nutrition labeling purposes, while rapid, less accurate methods are applicable for on-line measurements. Well-equipped and well-staffed analytical laboratories are the best to utilize methods referred to as reference, definitive, official, or primary. The manufacturing floor in a food processing facility would more appropriately use rapid secondary or field methods. For example, results from the secondary, rapid refractive index method for sugar analysis used on the manufacturing floor can be correlated to the primary method of high performance liquid chromatography used in the analytical laboratory. Likewise, moisture analysis data from a time-consuming forced draft oven method can be related to that of a moisture balance unit used in a pilot plant.

B. Characteristics

The characteristics described and questions raised in Table 41.4 are useful in choosing a method of analysis for a particular application. The characteristics related to validity of the method (i.e., accuracy, precision, specificity, and sensitivity) are critical, and will be discussed further in the next section on data handling. The time, equipment, and personnel availability are major factors to consider. A careful assessment of advantages and disadvantages of methods for particular applications makes it easier to make an appropriate choice.

C. Food Matrix

Some analytical methods are very robust and can be applied to all food types, but most methods are not so widely applicable (13, 14). The major chemical components of a food, i.e., the food matrix, can affect the performance of many analytical methods. Fat, protein, and carbohydrate are the three major food components expected to have the strongest effect on analytical method performance. For example, the high fat or sugar content of certain foods can cause interferences in some assays. Extraction steps and digestion procedures can be necessary and specific, depending on the food matrix. Because food systems are quite complex and varied, one technique for analysis of a particular component cannot be applied to all foods. Rather, multiple techniques and procedures must be considered for application to any particular food matrix.

D. Official Methods

The availability of official methods has made easier the choice of methods for analyzing specific food components and characteristics (1). Such official methods have been carefully developed, standardized, and compiled by several nonprofit scientific organizations. Official methods make it possible to compare results between different laboratories that use the same procedure. These methods also provide a way to evaluate new and more rapid assays.

TABLE 41.4
Criteria for Choice of Food Analysis Methods

Characteristic	Critical Questions	Characteristic	Critical Questions
Inherent properties			
• Specificity/ Selectivity	Is the property being measured the same as that claimed to be measured, and is it the only property being measured? Are there interferences? What steps are being taken to ensure a high degree of specificity?	• Equipment	Is the method very sensitive to slight or moderate changes in the reagents? Do you have the appropriate equipment? Are personnel competent to operate equipment?
• Precision	What is the precision of the method? Is there within-batch, batch-to-batch, or day-to-day variation? What step in the procedure contributes the greatest variability?	• Cost	What is the cost in terms of equipment, reagents, and personnel?
		Usefulness	
		• Time required	How fast is it? How fast does it need to be?
• Accuracy	How does the new method compare in accuracy to the old or a standard method? What is the percent recovery?	• Reliability • Need	How reliable is it from the standpoints of precision and stability? Does it meet a need or better meet a need?
Applicability of method to laboratory		***Personnel***	Is any change in method worth the trouble of the change?
• Sample size	How much sample is needed? Is it too large or too small to fit your needs? Does it fit your equipment and/or glassware?	• Safety • Procedures	Are special precautions necessary? Who will prepare the written description of the procedures and reagents?
• Reagents	Can you properly prepare them? What equipment is needed? Are they stable? For how long and under what conditions?		Who will do any required calculations?

From: SS Nielsen, ed. Food Analysis, 3rd ed. New York: Kluwer Academic, 2003 (Ref. 1).

AOAC International is a volunteer organization dedicated to providing methods that perform with accuracy and precision under usual laboratory conditions. Members of AOAC International select published methods or develop new methods, then collaboratively test the methods in their own laboratories. The program to validate methods is carefully managed, with a specified number of laboratories involved, samples per level of analyte, controls, control samples, and the review process. If methods are found acceptable, they are adopted as official methods (initially as first action, and then as final action). Adopted methods are published in the *Official Methods of Analysis* (7), which is published about every four to five years and contains methods appropriate for a wide range of products and other materials (Table 41.5). New methods and revisions to current methods are published in supplements to the most recent edition of the book.

Other books of official methods of analysis related to foods and ingredients include the following:

1. *Approved Methods of Analysis*, published by the American Association of Cereal Chemists (AACC) (15); methods are mostly applicable to cereal products (e.g., tests for physical dough properties, baking quality, staleness/texture).
2. *Official Methods and Recommended Practices* published by the American Oil Chemists' Society (AOCS) (16); methods are applicable mostly to fat and oil analysis (e.g., vegetable oil, oilseed by-products, detergents, lecithin).
3. *Standard Methods for the Examination of Dairy Products*, published by the American Public Health Association (17); methods are applicable to milk and dairy products; chemical methods are for acidity, fat, lactose, moisture/solids, added water.
4. *Standard Methods for the Examination of Water and Wastewater*, published jointly by the American Public Health Association, American Water Works Association, and the Water Environment Federation (18).
5. *Food Chemicals Codex*, published by the Food and Nutrition Board of the National Research Council/National Academy of Sciences (19); contains methods for the analysis of food additives.

TABLE 41.5
Table of Contents of Official Methods of Analysis of AOAC International, 17th Edition (Ref. 7)

Chapter	Title	Chapter	Title
1	Agriculture liming materials	26	Distilled liquors
2	Fertilizers	27	Malt beverages and brewing materials
3	Plants	28	Wines
4	Animal feed	29	Nonalcoholic beverages and concentrates
5	Drugs in feeds	30	Coffee and tea
6	Disinfectants	31	Cacao bean and its products
7	Pesticide formulations	32	Cereal foods
8	Hazardous substances	33	Dairy products
9	Metals and other elements at trace levels in foods	34	Eggs and egg products
10	Pesticide and industrial chemical residues	35	Fish and other marine products
11	Waters; and salt	36	Flavors
12	Microchemical methods	37	Fruits and fruit products
13	Radioactivity	38	Gelatin, dessert preparations, and mixes
14	Veterinary analytical toxicology	39	Meat and meat products
15	Cosmetics	40	Nuts and nut products
16	Extraneous materials: isolation	41	Oils and fats
17	Microbiological methods	42	Vegetable products, processed
18	Drugs: Part I	43	Spices and other condiments
19	Drugs: Part II	44	Sugars and sugar products
20	Drugs: Part III	45	Vitamins and other nutrients
21	Drugs: Part IV	46	Color additives
22	Drugs: Part V	47	Food additives: Direct
23	Drugs and feed additives in animal tissues	48	Food additives: Indirect
24	Forensic sciences	49	Natural toxins
25	Baking powders and baking chemicals	50	Infant formulas, baby foods, and enteral products

E. Cautions in Analysis

Basic guidelines for performing analytical methods appropriately caution the analyst with regard to proper use of blanks, sources of contamination, and other potential sources of error (e.g., equipment, analyst technique) (20). Problems with blanks, contamination, and other sources of error greatly affect the accuracy and precision and therefore the usefulness of data obtained.

A reagent blank is a sample that contains the reagents used in the sample analysis, in the appropriate quantities, but without any of the material being analyzed. For example, the reagent blank in the phenol-sulfuric acid method for total carbohydrate would contain sulfuric acid and phenol in the appropriate quantities, but water in place of any carbohydrate-containing sample. That reagent blank would be used to zero the spectrophotometer before reading the absorbance of the carbohydrate-containing samples. Use of a reagent blank is especially important in many mineral analyses to account for any mineral contamination in the reagents.

Contamination can be a source of error in many assays, but is especially a problem when the quantities of the compounds of interest in the food are very small. Such is the case in mineral analysis, because mineral levels in the food can be very low and since glassware and the water used can contain significant quantities of minerals. In the case of mineral analysis, any glassware used must be acid washed and reagents must be of the highest analytical grade possible.

Some of the sources of error in analytical methods are often overlooked. Especially when faced with a set of questionable data, the analyst or supervisor must do some problem solving and identify the factors that contributed to the problem. For example, one must ask if the sample analyzed was representative of the sample as a whole, and whether or not it may have been modified in composition and characteristics during sample preparation. Did the analyst follow in detail the analytical procedure? Was the equipment properly standardized and used? Table 41.6 gives a partial list of sources of error that can cause data gathered to be inaccurate and show low precision.

VII. VALIDITY OF METHOD

Validity of the data obtained using a specific analytical method can be assessed in multiple ways. As a first approach to evaluate the usefulness and validity of an analytical method being related to sensory characteristics,

TABLE 41.6
Sources of Laboratory Error

A. Sample Collection and Handling
1. Representative
2. Proper preparation
3. Stability (time, temperature, light, oxygen)
4. Identification throughout analysis

B. Analytical Errors
1. Sample and reagent measurements (weighing, pipetting)
2. Procedural steps (timing, mixing, order of reagents)
3. Range and sensitivity limitation

C. Reagents
1. Preparation; initial and periodic checking
2. Conditions of use (temperature, mixing)
3. Stability; storage conditions
4. Contamination

D. Instruments
1. Limitations
2. Operation
3. Routing checking

F. Calibration
1. Blanks
2. Linearity limits
3. Standards

G. Data Handling

H. Special Problems
1. Personal attitudes and techniques
2. Laboratory water

one should ask a very practical question. How do the differences detected and the variability seen between samples by the analytical method compare to the detected differences and acceptability to a consumer? Also, how does the variability seen between samples by the analytical method compare to the variability of the specific characteristic inherent in processing of the food (1)?

A common test of validity is assessing the inherent characteristics of the method, including accuracy, precision, specificity, and sensitivity, as described in the next section. Of major assistance in assessing accuracy is the use of standard reference materials, which can be obtained from various organizations (e.g., in the United States from the National Institute of Standards and Technology). Control samples internal to the laboratory also can serve as a standard reference material. Such a sample can be prepared by careful selection of an appropriate type of sample (i.e., closely matching the matrix of the samples to be analyzed by a specific method). These control samples should be gathered in a large quantity, mixed and prepared to ensure homogeneity, then packaged in small quantities and stored properly. The control samples can be analyzed routinely when test samples are analyzed. Besides standard reference materials, another test of method accuracy is using check samples (21). Organizations such as the AACC and AOCS offer check sample services, providing test samples to evaluate method reliability. Subscribing laboratories can check the accuracy of their data for specific types of check samples supplied, comparing their values against the statistical norm for those same samples obtained from other subscribing laboratories.

VIII. RELIABILITY OF ANALYSIS AND DATA HANDLING

A. Accuracy and Precision

Reliability of a method is judged largely on its accuracy and precision (22–24). How close the experimental measure is to the correct or true value is referred to as accuracy. How close replicate measures are indicates reproducibility, which is referred to as precision. Standard reference materials or internal control samples are very useful in assessing accuracy since they should provide the true value. The percent relative error is the value commonly used to assess accuracy, comparing the experimental mean to the true value.

$$\% \text{ Relative error} = [(\text{Experimental mean} - \text{True value})/\text{True value}] \times 100$$

Standard deviation (SD) measures the variability of the experimental values (x_i) around the mean ($\bar{x}$), and is the initial value calculated to assess precision:

$$SD = \frac{\sqrt{\Sigma(x_i - \bar{x})^2}}{n} \tag{41.1}$$

With the mean and standard deviation, one can calculate the coefficient of variation, which relates the size of the standard deviation to the mean. This allows one to compare the precision of multiple sets of data for which the mean values differ.

$$\text{Coefficient of Variation (CV)} = \frac{SD}{\bar{x}} \times 100\% \tag{41.2}$$

Other useful terms associated with precision and accuracy are confidence interval, standard error of the mean, and relative deviation from the mean.

B. Specificity

The specificity of a method is how well the method detects and measures the compound of interest, and only

that compound. Some analytical methods are intended to be nonspecific (e.g., lipid extraction with organic solvents), and others very specific (e.g., enzyme test kit to measure glucose). Both types of methods can be very useful, when chosen appropriately for the particular application (22).

C. Sensitivity and Detection Limit

Sensitivity of a method describes how small of a change can be made in the test material before we see a difference in readout from an instrument. This term relates the size of the change in the measuring device to the change in concentration of the compound of interest. Detection limit, a related but different term, describes the lowest possible increment or amount that we can detect with some degree of confidence. Below that lower limit, we cannot be sure if something is present. If one is working near the detection limit for a particular assay, it may be possible to concentrate the sample (22).

D. Standard Curves

For analytical methods, two variables (e.g., concentration and absorbance) are commonly related to one another using a standard curve. The concentration of analyte in a sample is determined by the response measured, which is related to a known amount of standard. Usually a set of standards is prepared at several concentrations, subjected to the assay conditions, and the appropriate analytical measure is taken (e.g., absorbance, volume of titrant, chromatography peak area). Generally, the concentrations of the standards (the independent variable) are plotted on the x-axis, and the measured values (the dependent variable) are plotted on the y-axis, to generate a standard curve. The best-fit line of this relationship is mathematically determined using a mathematical procedure called linear regression, and the equation of the line can be determined. The equation of a straight line is described by $y = mx + b$, where y is the dependent variable, x is the independent variable, m is the slope of the line, and b is where the point on the line intercepts the y-axis (i.e., y-intercept). Ideally all the data points for a standard curve would fall exactly on a straight line, but various sources of error limit this possibility. Values called the correlation coefficient or coefficient of determination can be calculated to mathematically describe how well the data fit a straight line. The equation of that line can be used to calculate the concentration of an analyte of unknown concentration (x), once the measured value (y) for that sample has been determined (22).

E. Reporting Results

Results from analytical methods are commonly reported as a mean and the standard deviation (or some other measure of precision). To appropriately state the sensitivity of the assay and make the data meaningful, one must report the value with the correct number of significant figures. Detailed guidelines exist to help determine the appropriate number of significant figures to report. Rules also exist for rounding off numbers. Generally for analytical procedures, extra numbers are carried forward in the calculations, and the rounding is done on the final answer. Finally, mathematical tests exist for rejecting data obtained that do not match other data gathered. Rejecting data, and therefore excluding them in the final data calculations, must be very carefully considered. It is not the solution to a problem of consistently poor accuracy and precision (22, 24).

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42 Analysis of the Chemical Composition of Foods

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CONTENTS

I. INTRODUCTION

The chemical composition of a food is of utmost importance from many standpoints, including nutrition and health, toxicology and safety, and stability to microbiological, chemical or physical changes. Analysis of these food components is required for regulatory purposes, to provide the data upon which nutrition labels and health claims are based, and for the information of health-conscious consumers. Food analysis is also used to monitor the stability or, conversely, the deterioration or reactivity of food components as a function of processing or storage conditions throughout the food

distribution chain, which can influence decisions to control parameters for the purpose of quality assurance.

Foods are often characterized by the composition of their chemical components. However, a wide range in the precise levels of those constituents may in fact exist, due to intrinsic biological variability, multiplicity of cultivars and variants of each species, or as a result of geographical location, environmental and seasonal influences, or from the application of genetic engineering. Furthermore, the chemical composition of foods may also be influenced by natural or unintentional contaminants, by processing or upon storage. This potentially wide range of composition and characteristics leads to difficulty in defining the chemical composition of foods, and to issues of validating authenticity or detecting adulteration issues (1).

Given the fact that the constituents comprising foods are very large, both in number and diversity, it is not surprising that the analysis of the chemical composition of foods also encompasses a wide scope as well as a large number of techniques. Chapter 41 describes some key considerations for selecting the most appropriate method, including the objectives of the analysis, nature of the sample, and the available resources. The reported quantity of a food component may depend on the method used for its analysis. There is a need to develop standard specifications for food commodities, as well as more precise nomenclature and detailed description and classification of foods and their components (2), and identification of key foods for food composition research (3). The use of International Network of Food Data Systems (INFOODS) tagnames has been proposed to provide clarity when describing the new nutrient variables (4). Examples of tagnames are described in Tables 42.1 A–D (5).

An overview of the chemical composition of foods can be obtained by conducting proximate analysis, which refers to the measurement of the major components of food, namely moisture, minerals or "ash," lipids, proteins, and carbohydrates. However, even within this simplified classification of food composition, the method of analysis is influential and should be clearly stated when reporting data, as illustrated in the tagnames for fat, protein, and carbohydrate (Tables 42.1 B–D, respectively.).

Further analysis of the composition is often conducted to meet the requirements for nutritional labeling, such as the contents of particular minerals and vitamins, cholesterol, saturated, monounsaturated and polyunsaturated fatty acids, simple sugars, and dietary fiber (6). Table 42.2 lists the nutrients in the United States Department of Agriculture's National Nutrient Database (7), while Table 42.3 shows the nutrients listed in the Canadian "Nutrition Facts Table Core Nutrition Information" as well as the methods of analysis used by the Canadian Food Inspection Agency (8).

More detailed analyses, usually preceded by fractionation and purification steps, are available to determine the precise nature of individual constituents. For example, rapid advances in proteomics have enabled sequence analysis of the multitudes of proteins naturally occurring in a cell, including isoforms or variants that may differ from each other by a single amino acid (9). Similarly, sophisticated instrumental analyses have been developed

TABLE 42.1A
Examples of INFOODS Tagnames Describing Moisture and Ash Contents and Energy Value of Foods

INFOODS Tagname	Component	Comments
<DM>	Dry matter	
<WATER>	Water Synonym: moisture	
<ASH>	Ash Synonym: minerals	
<ENER>	Energy, method of determination unknown Synonyms: kilojoules; kilocalories; calories; food energy	The tagname should be used if it is not known whether the energy value represents gross energy or total metabolizable energy. It should also be used if it is known that the energy value was calculated from the proximate components but the conversion factors used are unknown.
<ENERA>	Energy, gross; determined by direct analysis using bomb calorimetry Synonyms: kilojoules; kilocalories; Calories; food energy	
<ENERC>	Energy, total metabolizable; calculated from the energy-producing food components. Synonyms: kilojoules; kilocalories; calories; food energy	In addition to a value for the quantity of total metabolizable energy, includes a description or listing of the conversion factors used to calculate this energy value from the proximate quantities.

Source: Taken from "Tagnames for food components" on the INFOODS website at http://www.fao.org/infoods/tagnames_en.stm (accessed June 18, 2003), from Klensin et al. (5).

TABLE 42.1B
Examples of INFOODS Tagnames for Selected Food Components Related to Fat and Fatty Acids

INFOODS Tagname	Component	Comments
<FAT>	Fat, total; Synonym: total lipid	
<FATCE>	Fat, total; derived by analysis using continuous extraction	The Soxhlet method has often been used to analyze for total fat using continuous extraction. This method tends to underestimate the total fat value of a food.
<FATRN>	Fatty acids, total trans	
<FASAT>	Fatty acids, total saturated	
<FACID> :	Fatty acids, total	Two data items are required for tagname <FACID>: the total quantity of fatty acids and identification of the conversion factor used to calculate this value from the quantity of total fat.
<FADT>	Fatty acids, total double trans	Some countries are planning actions to limit these acids (not single trans) from foods, e.g., chocolate.
<FAESS>	Fatty acids, total essential	This value is the sum of linoleic acid, linolenic acid, and arachidonic acid.
<FAFRE>	Fatty acids, total free	
<FAMS>	Fatty acids, total monounsaturated	
<FAPU>	Fatty acids, total polyunsaturated	
<FAPUN3>	Fatty acids, total omega-3 polyunsaturated	

Source: Taken from "Tagnames for food components" on the INFOODS website at http://www.fao.org/infoods/tagnames_en.stm (accessed June 18, 2003), from Klensin et al. (5).

for the identification and quantitation of positional and geometric isomers of fatty acids in lipids (10, 11), and the nature of monosaccharide building blocks and their linkages in oligo- or polysaccharides (12).

It is beyond the scope of this chapter to describe all these aspects of food analysis. This chapter will primarily focus on methods for proximate analysis for the composition of major components of foods, and will briefly allude to some of the other components that are part of the nutrition facts listed on food labels in North America. An overview will be given of the principles and considerations behind official methods and some rapid methods of analysis, and some of the recent trends in analysis of chemical composition of foods will be explored.

The reader is encouraged to consult two excellent textbooks by Nielsen (13) and Pomeranz and Meloan (14) for more in-depth discussion on proximate analysis, and other methods of food analysis, as well as the principles behind some of the instrumental methods used in analysis. Primary literature sources such as the *Journal of Food Composition and Analysis* should be consulted for current trends and issues of interest in the analysis of chemical composition of foods. Detailed protocols for official methods of analysis have been compiled by various scientific organizations (e.g., AOAC International, the American Association of Cereal Chemists, the American Oil Chemists' Society, the International Dairy Federation), government agencies (e.g., the Food and Drug Administration in the United States, Health Canada and the Canadian Food Inspection Agency in Canada), and international bodies (e.g., the International Organization for Standardization and the Codex Alimentarius Commission). In addition to publications about methods for analysis, most of these scientific organizations and government agencies have useful information on analysis of chemical composition of foods at their websites. As a good starting point, the reader is encouraged to browse the internet addresses provided in the chapters by Nielsen (15) and Nielsen and Metzger (6). The Compendium of Methods for Chemical Analysis of Foods on the Health Canada website (http://www.hc-sc.gc.ca/food-aliment/csipc/fr-ra/e_chem_analysis_ foods.html) (16) is also a useful resource. Global standards are coordinated by the Codex Alimentarius Commission (for example, see references 17–19), and detailed information such as standards, the current meetings and deliberations of expert committees, etc. are described at its official website (http://www.codexalimentarius.net/index_en.stm#) (20).

II. PROXIMATE ANALYSIS

Pomeranz and Meloan (14) defined proximate analysis as "the determination of the major components (moisture, minerals, carbohydrates, lipids, and proteins)." The data from proximate analysis are typically included in standard tables of composition of foods, such as the U.S. Department of Agriculture Nutrient Database for Standard Reference (7). The following sections provide an overview on the methods that may be used for determining each of the major components in proximate analysis. Many of the methods cited here are official methods of AOAC International (21; accessible also on CD-ROM), and the reader is encouraged to refer to

TABLE 42.1C
Examples of INFOODS Tagnames Describing Protein and Nitrogen Contents of Foods

INFOODS Tagname	Component	Comments
<NNP>	Nitrogen, non-protein	
<NPRO>	Nitrogen, protein	
<NT>	Nitrogen, total	Determined by Kjeldahl method
<PRO->	Protein, total; method of determination unknown	The <PRO-> tagname should be used for a total protein value when it is not known whether the value was the result of a direct analysis or whether it was calculated from total nitrogen, protein nitrogen, or amino nitrogen. The <PRO-> tagname should also be used if it is known that the total protein value was calculated from one of the nitrogen components, but the conversion factor used in the calculation is unknown.
<PROA>	Protein, total; determined by direct analysis	
<PROCN>	Protein, total; calculated from amino nitrogen	Two pieces of data are associated with the tagname <PROCNA>. The first is the quantity of total protein and the second is the conversion factor used to calculate total protein from amino nitrogen. Note: The total protein found in food tables is rarely calculated from amino nitrogen. <PROCNT> is the appropriate tagname for total protein in most cases.
<PROCNP>	Protein, total; calculated from protein nitrogen	Two pieces of data are associated with the tagname <PROCNP>. The first is the quantity of total protein and the second is the conversion factor used to calculate total protein from protein nitrogen. Note: The total protein found in food tables is rarely calculated from protein nitrogen. <PROCNT> is the appropriate tagname for total protein in most cases.
<PROCNT>	Protein, total; calculated from total protein nitrogen	Three pieces of data are associated with the tagname <PROCNT>. The first is the quantity of total protein; the second is a keyword which identifies the source of the conversion factor used to calculate the total protein from total nitrogen; and the third is the actual conversion factor used. If possible, all three pieces of data should be included with <PROCNT>. However, it is acceptable to include only the keyword or the conversion factor (rather than both) with the total protein value if one or the other is unknown. If the conversion factor used was generated from a source other than one of those identified by the available keywords, the conversion factor should be listed without any keyword information.

Source: taken from "Tagnames for food components" on the INFOODS website at http://www.fao.org/infoods/tagnames_en.stm (accessed June 18, 2003), from Klensin et al. (5).

these methods and the original references cited therein for more detail. The reader is also referred to other chapters in this handbook for descriptions of various spectroscopic techniques and other instrumental methods of analysis, as well as more detailed coverage of protein and fat analysis.

A. Water (Moisture)

The moisture content of foods can range from 0 (e.g., for granulated sugar or vegetable oil) to over 90% (e.g., in raw watermelon or cucumbers) (22). The determination of the water or moisture content of a food and, conversely, of the dry matter or total solids (% solids = 100% − % moisture), is important not only to provide a basis for expressing the content of the other components on a wet- or dry-basis, but also as an important factor in food stability and quality. Moisture assays can be one of the most important analyses performed on a food product and yet one of the most difficult in terms of obtaining adequate precision and accuracy (22).

1. Overview of and Considerations for Moisture Determination

Water may exist in foods in free form, as tightly adsorbed water, or as chemically bound water of hydration. The percentage of water that is actually measured is dependent on the method used for its analysis, and it is therefore important to always state the exact methodology used when reporting

TABLE 42.1D
Examples of INFOODS Tagnames for Selected Food Components Related to Total Carbohydrate, Fibre, Starch, and Sugars

INFOODS Tagname	Component	Comments
<CHO->	Carbohydrate, total; method of determination unknown	
<CHOCDF>	Carbohydrate, total; calculated by difference	This value is calculated using the following formula: 100 g minus total grams of water, protein, fat, and ash
<CHOCSM>	Carbohydrate, total; calculated by summation	This value is the sum of the sugars, starches, oligosaccharides, and carbohydrate dietary fibre.
<CHOAVL>	Carbohydrate, available	This value includes the free sugars plus dextrins, starch, and glycogen.
<CHOAVLM>	Carbohydrate, available; expressed in monosaccharide equivalents	This value includes the free sugars plus dextrins, starch, and glycogen.
<FIBAD>	Fibre; determined by acid detergent method	Includes cellulose, lignin, and some hemicelluloses
<FIBADC>.	Fibre, acid detergent method, Clancy modification	
<FIBC>	Fibre, crude	The crude fibre method of fibre analysis is obsolete
<FIBHEX>	Hexoses in dietary fibre	
<FIBINS>	Fibre, water-insoluble	Sum of insoluble components from the AOAC total dietary fibre method; includes primarily lignin, cellulose, and most of the hemicellulose Values for <FIBINS> may also be obtained by subtracting soluble fibre from total dietary fibre, i.e., by subtracting the value of <FIBSOL> from the value of <FIBTG>.
<FIBND>	Fibre; determined by neutral detergent method	Includes lignin, cellulose, and insoluble hemicellulose
<FIBSOL>	Fibre, water-soluble	Sum of soluble components from the AOAC total dietary fibre method; includes primarily algal polysaccharides, gums, pectins, and mucilages
<FIBTG>	Fibre, total dietary; determined gravimetrically by the AOAC total dietary fibre method	Sum of the water-soluble components and the water-insoluble components of dietary fibre; can be calculated by adding the values of <FIBSOL> and <FIBINS>; includes all non-starch polysaccharides and lignin
<FIBTS>	Fibre, total dietary; sum of non-starch polysaccharide components and lignin	Sum of the polysaccharide components of dietary fibre measured sequentially on the same sample (for example, by using the Southgate colorimetric procedure) plus lignin measured gravimetrically
<FIB->	Fibre; method of determination unknown	Note: Tagname <FIB-> is used to identify fibre values which represent unknown fibre components or which were obtained by unknown methods. Additional tagnames for fibre may be created to identify fibre components or specific methods of analysis that are not currently addressed in this listing.
<STARCH>	Starch, total	The sum of all polysaccharides yielding glucose after hydrolysis with suitable enzymes; includes amylose, amylopectin, glycogen, and dextrins
<STARCHM>	Starch, total; expressed in monosaccharide equivalents	The sum of all polysaccharides yielding glucose after hydrolysis with suitable enzymes; includes amylose, amylopectin, glycogen, and dextrins.
<STARES>	Starch, resistant Synonym: retrograded starch	
<SUGAR>	Sugars, total	Sum of free monosaccharides and disaccharides.

Source: Taken from "Tagnames for food components" on the INFOODS website at http://www.fao.org/infoods/tagnames_en.stm (accessed June 18, 2003), from Klensin et al. (5).

TABLE 42.2
Nutrients Listed in the USDA National Nutrient Database, Available Sorted Either by Food Description or in Descending Order by Nutrient Content in Terms of Common Household Measures

Nutrient
Moisture
Protein
Fat
Energy (Calories)
Carbohydrate (by difference)
Total dietary fiber
Total sugar
Calcium
Iron
Magnesium
Phosphorus
Potassium
Sodium
Zinc
Copper
Manganese
Selenium
Vitamin A (IU)
Vitamin A (RAE)
Vitamin E (alpha-tocopherol)
Vitamin K (phylloquinone)
Vitamin C
Thiamin
Riboflavin
Niacin
Pantothenic acid
Vitamin B-6
Vitamin B-12
Dietary folate equivalents
Cholesterol
Total saturated fatty acids
Total monounsaturated fatty acids
Total polyunsaturated fatty acids

Source: USDA 2004 (7).

moisture content. Methods for moisture determination include oven drying, distillation, and titration, as well as methods based on physical properties. Some of these methods are described in further detail in the following sections.

In addition to moisture content, water activity (a_w) is often measured and used as an indicator of the availability of water in food for chemical or enzymatic reactions or microbiological growth. Water activity is defined as the ratio of the partial pressure of water above a sample to the vapor pressure of pure water at the same temperature, and is commonly measured by sensors that measure the relative humidity of the sample atmosphere after equilibration, or by a variation of this method known as the chilled mirror technique (22).

It is important to bear in mind that some foods are prone to either gain moisture from or lose moisture to the surrounding atmosphere, depending on the nature of the food constituents, the relative humidity and temperature of the environment, and other parameters such as surface area. Detailed protocols for sampling and sample preparation have been described for different categories of foods (21), and should be followed to ensure that representative samples reflecting the composition of the food can be obtained for the analysis.

2. Oven Drying Methods

The principle of moisture determination by oven drying is based on evaporation of water from the sample, and calculation of moisture based on loss in weight. Oven drying methods are therefore considered as "indirect" methods, since moisture is not actually determined per se.

While the theory behind moisture determination by oven drying is simple, and routine simultaneous analysis of multiple samples is possible, in practice many factors must be considered. The amount of moisture removed during oven drying is influenced by many variables, including the time and temperature of drying, the type of oven used, and the type of sample.

Although the boiling point of pure water is 100°C, the presence of solutes raises the boiling point. This boiling point elevation continues throughout the drying process, as the sample becomes increasingly concentrated upon the removal of water. In theory, the higher the drying temperature, the more complete the moisture removal and the shorter the time required. It has been reported that a temperature of 250°C is required to obtain the true moisture content, assuming no adsorbed water present at the temperature in question (22). However, in practice, volatilization, deterioration, and chemical reactions of the constituents may occur at high temperatures, and it is prudent to use the minimum temperature that will allow moisture removal at an acceptable rate (14, 22). Drying in a vacuum oven may be preferred for some food samples since the boiling point is lower under vacuum, and the absence of air can minimize oxidative reactions especially of high fat samples during drying.

Using vacuum ovens and forced draft or convection ovens, the temperatures and times specified for various products typically range from 70–130°C and 1–16 hours, respectively. For example, the time-temperature combinations are 1 h at 130±3°C for solids (total) and moisture in flour (AOAC Official Method 925.10), 6 h at 70±1°C in a vacuum oven for moisture in dried fruits (AOAC Official Method 934.06), 16–18 h at 100–102°C in an air oven or 2–4 h at ca 125°C in a mechanical convection or gravity oven for moisture in meat (AOAC Official Method 950.46B(a) and (b)). More rapid drying, on the order of minutes, can be accomplished using infrared or microwave ovens for drying. Official methods stipulating different temperature-time combinations or oven types for the same food material have been approved for some

TABLE 42.3
Methods of Analysis Used by the Canadian Food Inspection Agency for the Nutrition Facts Table Core Nutrition Information

Nutrient	Method Reference	Technique
Calories	Atwater Method (Guide to Food Labelling and Advertising, section 6.4.1)	Application of Atwater factors to fat, carbohydrate and protein content
Fat (Sum of fatty acids expressed as triglycerides)	AOAC 996.06*	Capillary Gas Chromatography using SP2560 100m × 0.25mm, 0.2μm film column
Fatty acids: Saturates (all fatty acids that contain no double bonds) *trans* (unsaturated fatty acids that contain one or more isolated or non-conjugated double bonds in trans configuration)	AOAC 996.06*	Capillary Gas Chromatography using SP2560 100m × 0.25mm, 0.2μm film column
Cholesterol	AOAC 994.10*	Direct Saponification and Capillary Gas Chromatography
Carbohydrate (mono- and di-saccharides + starch+ fibre+sugar alcohols+ polydextrose)	By Difference (100-%ash-%moisture-%protein-%fat)	Determination by applicable AOAC method for Ash, Moisture, Protein, Fat
	HPB FA-78**, available carbohydrate, in special cases	Acid and enzymatic hydrolysis followed by redox titration
Fibre	AOAC 992.16* (Mongeau) or AOAC 985.29* (Prosky) (as in Guide to Food Labelling and Advertising 6.4.4.3)	Gravimetric determination after defatting and enzymatic hydrolysis of protein and carbohydrate (starch). (Results found non-compliant by Mongeau method should be confirmed by Prosky method)
Sugars (all monosaccharides and disaccharides)	AOAC 980.13* (modified HPLC column and mobile phase)	Aqueous food extraction followed by HPLC-RI
Protein	AOAC 981.10* AOAC 993.13*	Nitrogen by Kjeldahl or Combustion
Vitamin A (retinol and derivatives and beta carotene expressed as retinol equivalents, RE)	AOAC 992.04* (Method A-12 (version 3.0) 1993**) Determination of Vitamin A in milk, infant formula, and other complex food commodities. JAOAC. 76: 2, 1993	HPLC determination of vitamin A with UV detection
	Method A-7** HPLC Determination of Vitamin A in margarine, milk, partially skimmed milk, and skimmed milk. JAOAC 63: 4, 1980	HPLC determination of vitamin A with UV detection
	Method LPFC-200** The fluorometric determination of Vitamin A in dairy products. J. Dairy Sci. 55:1077 (1992)	Fluorometric determination of vitamin A
Vitamin C (L-ascorbic acid and L-dehydroascorbic acid and their derivatives, calculated as mg equivalents L-ascorbic acid)	HPLC-C1 (1992)** Determination of vitamin C by HPLC. References: 1) Pelletier, O., and Brassard, R. Determination of Vitamin C in food by manual and automated methods. J. Food Sci., 42:1471–1477, 1977. 2) Behrens, W.A., and Madere, R. Ascorbic and Dehydroascorbic acid content of infant formula J. Food Comp. Anal., 2: 48–52, 1989. 3) Behrens, W. A., and Madere, R. Ascorbic and dehydroascorbic acid contents of canned food and frozen concentrated orange juice. J. Food Comp. Anal., 3: 3–8, 1990. 4) Behrens, W. A., and Madere, R. A Highly sensitive high-performance liquid chromatography method for the estimation of	HPLC determination of vitamin C with electrochemical detection

Continued

TABLE 42.3 (*Continued*)

Nutrient	Method Reference	Technique
	ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. Analytical Biochemistry 165:102–107, 1987.	
Iron	LPFC-137 **	Preparation of samples by calcination to determine different elements by Atomic Absorption Flame Spectroscopy
Calcium	LPFC-137**	Atomic Absorption Flame Spectroscopy
Sodium	LPFC-125** A rapid method for the determination of sodium and potassium.	Preparation of samples by aqueous extraction and flame emission spectroscopy.

* *Official Methods of Analysis of AOAC International* (Ed. W. Horowitz., AOAC International, Gaithersburg, Maryland.

** LPFC are laboratory procedures available from the CFIA or Health Canada websites.

Source: Canadian Food Inspection Agency 2002 (8).

foods, e.g., AOAC Official Methods 925.23, 990.19, and 990.20 for solids (total) in milk. The final choice of the appropriate method may depend on factors such as the need for speed, or further analyses using the dried material, or the availability of equipment. In order to determine the optimal temperature and time for drying of specific food products or using new equipment not described in the official methods published by scientific organizations, analysts should conduct their own trials and establish the appropriate conditions based on the resultant drying curves.

Additional steps conducted prior to oven drying are recommended for some types of samples. For example, a two-stage drying process involving a steam bath for predrying is frequently suggested for liquid products, while air-drying and grinding are performed prior to oven drying for samples such as bread or grain. Since surface area and porosity of the sample influence rate and efficiency of drying, and since surface crust formation, or case hardening, can impede moisture removal, sand or other inert material may be added prior to drying of food materials which have a tendency to lump together or to form a surface crust, such as dehydrated fruits or other foods high in sugar.

3. Distillation Methods

The moisture content of spices, nuts, oils and other foodstuff with relatively low moisture content can be measured directly through distillation methods (e.g., AOAC Official Methods 986.21 and 969.19 for spices and cheese, respectively). Water is co-distilled with an immiscible solvent with a high boiling point; the distilled water is condensed, collected, and measured in a collecting vessel or trap. Since direct measurement of water is involved, distillation methods can provide better accuracy and precision than oven drying methods based on weight loss, especially for low-moisture samples. However, it should be noted that samples are still exposed to high temperatures during distillation, and therefore these methods are not suitable for analysis of heat-sensitive foods. In addition, distillation techniques are not easily amenable to routine testing of multiple samples.

4. Chemical Methods – Karl Fischer Titration

The Karl Fischer titration method is suitable for low-moisture foods that are sensitive to decomposition or volatilization under vacuum or high temperatures, such as dried fruits and vegetables (AOAC Official Method 967.19), candies and chocolates (AOAC Official Method 977.10), roasted coffee beans, oils and fats (AOAC Official Method 984.20). Several modifications of the original Karl Fischer method, which was based on the colorimetric detection of a stoichiometric reaction involving the reduction of iodine by sulfur dioxide in the presence of water, have been proposed over the years, leading to the commercial availability of titration units that automatically dispense the required proportions of reagents and titrants for conductometric or coulometric assay, and provide digital displays of the calculated moisture content.

5. Physical Methods

Physical methods of moisture determination are based on the measurement of various physical properties of food samples, such as dielectric constant, conductivity, specific gravity or density, refractive index, freezing point, or absorption of energy in the mid- or near-infrared spectrum, followed by comparison to the corresponding properties of standards with known moisture content (22). For example, hydrometry using a calibrated lactometer is applied to determine the total solids content of milk

(AOAC Official Method 925.23B), while refractometry is used to determine solids in syrups (AOAC Official Method 932.14C). Near-infrared spectrophotometry is used for water content in dried vegetables (AOAC Official Method 967.19), while mid-infrared spectroscopy forms the basis for using IRMA (Infrared Milk Analyzers) for proximate analysis of milk, including solids, fat, lactose, and protein (AOAC Official Method 972.16).

Physical methods often provide rapid analysis of large numbers of samples, but it is important to bear in mind that the relationship between the measured physical property and the moisture content of the specific food sample must be known, either by reference to published tables of standard composition for that food, or by construction of standard curves by the analyst.

B. Ash

Ash is defined as the inorganic material remaining after oxidation of organic matter, and is used to refer to the total minerals in food. The ash content of unprocessed fruits and vegetables typically ranges from about 0.4% in raw tomatoes (23) to 4% in beans (14). Pure fats and oils contain practically no minerals, while the ash content of salted butter is about 2.1%. Similarly, fresh meat and poultry contain about 1% ash, while dried and salted beef may contain as high as 12% ash (14). Milling of wheat yields white flour with 0.47% ash, or whole wheat flour with 1.60% ash (7). The determination of ash content may thus be used as an indicator of the extent of processing of a food commodity, or of the addition of salt to foods.

1. Overview of and Considerations for Ash Determination

Minerals in foods may occur as complexes with other minerals, or with macromolecules such as proteins. The incineration or oxidation of organic matter during ashing may result in volatilization of some minerals, or formation of new complexes of other minerals, as summarized by Pomeranz and Meloan (14). Both the amount and the composition of ash that remains after incineration or oxidation therefore depend on the nature of the food and the method of ashing. This is an important consideration in selection of the ashing method and conditions, especially if further analysis of specific minerals (e.g., Ca, Zn, Fe, and so on) is required (24).

Since minerals often constitute 1% or less of the total wet weight of a food sample, it is critical that contamination of the food with metals or their salts be avoided. Potential sources of trace metal contaminants include glassware, grinders or metal blades used for sample preparation, and the crucibles used to hold the samples during ashing.

The two main methods of ashing, which are termed dry ashing and wet ashing, are described in the following sections.

2. Dry Ashing

Dry ashing involves the incineration of samples in a muffle furnace at high temperatures ranging from 400–700°C and typically around 525 or 550°C for 12–18 hours, until a light gray or white powder remains (14). The residue after incineration is weighed as a direct measure of the ash content.

Some modifications of the basic dry ashing procedure are required depending on the sample. High fat or moisture content may cause smoking or spattering during ashing of samples. Therefore, liquid samples may be pre-dried, and fat from high fat samples should be extracted with a solvent, prior to ashing. In most cases, dry ashing can be performed using the solids remaining after oven-drying or solvent extraction conducted for moisture or fat determination, respectively. A few drops of ashless olive oil may be added to animal products, syrups, and spices, to allow steam to escape as a crust is formed on the product (23). Incomplete combustion, especially in high sugar samples, is manifested by the appearance of black or carbon residues in the ash, and requires re-suspension in water or acid, followed by re-ashing (e.g., see AOAC Official Method 900.02, for ash of sugars and syrups). Acceleration of dry ashing may be achieved by the addition of an ethanol solution of magnesium acetate. The magnesium acetate method for ash content of flour (AOAC Official Method 936.07) uses a temperature of 700°C for ashing, compared to 550°C for the direct method (AOAC Official Method 923.03). New microwave muffle furnaces with temperatures reaching up to 1200°C can also reduce ashing time to minutes instead of hours (23).

Dry ashing is simple in both theory and practice, and simultaneous analysis of many samples is feasible. However, as noted in the preceding section, the composition of the minerals in the residue is not usually identical to that in the original food sample, and it is assumed that losses due to volatilization or other causes are compensated by gains due to formation of new complexes such as oxides. Formation of fusion complexes between some minerals means that the dry ash is not usually suitable for further analysis of specific mineral content. To address this, a method of dry ashing of different categories of foods for determination of individual contents of Na, K, Ca, Mg, Fe, Zn, Cu, and Mn was established by the Health Protection Branch Laboratories of Health Canada (25). The protocol involves dry ashing at 450°C for 16 hours, followed by addition of water and nitric acid, evaporation to remove the acid, and re-ashing at 375°C for another hour. Lanthanum and cesium solutions are added as ionization suppressors, to allow determination of Ca, Mg and Na, K, respectively.

3. Wet Ashing

Wet ashing is also referred to as wet oxidation or wet digestion. It involves the oxidation or digestion of organic matter by heating in the presence of concentrated acids such as nitric acid, sulfuric acid (usually with hydrogen peroxide) or perchloric acid, either singly or in combination. A typical wet ashing procedure involves heating or boiling of the sample with concentrated sulfuric and nitric acids, at about 200°C, until white fumes from decomposing sulfuric acid are observed; additional nitric acid is then added, and boiling or ashing of the sample is continued until a clear or light yellow solution is obtained (23).

Due to the lower temperatures and shorter times involved in wet ashing, less volatilization or fusion of minerals occurs, compared to dry ashing. The minerals usually stay in solution after wet ashing, and further analysis of the composition of individual minerals may be performed, for example, using atomic absorption spectrophotometry or inductively coupled plasma emission spectrophotometry. Despite these advantages of wet ashing for specific mineral composition, wet ashing is not usually preferred over dry ashing as the method for total ash content determination. This is because of the dangers of using corrosive acids, especially perchloric acid which is potentially explosive, specifically in the presence of undigested biological components, and the relatively limited number of samples which can be analyzed at one time. Combination dry-wet ashing methods have been proposed, for example, for analysis of minerals in infant formula (AOAC Official Method 985.35). Specially designed microwave ovens have also been proposed for safer and quicker wet ashing of sample, using either open-vessel or closed-vessel digestion systems depending on the amount of sample and the temperatures required for digestion (23).

C. Fat

The term "fat" is often used synonymously with "lipid" or "oil" to represent food components that are insoluble in water but are soluble in organic solvents (14). The fat content of foods varies widely, from less than 1% in many fruits and vegetables, to almost 100% in lard, shortening, or vegetable oils (7).

1. Overview of and Considerations for Fat Determination

The methods for fat determination may be generally classified into (i) methods that involve solvent extraction of fat from the food, followed by gravimetric, volumetric, or other means of measurement of the fat, and (ii) instrumental methods that measure the fat content based on its physical properties in the food, in comparison to standards of known composition. Other methods involving extraction of fat without the use of solvents have also been developed.

Methods using solvent extraction in fat analysis are based on the functional definition of fats, as substances that are insoluble in water but soluble in organic solvents. However, this functional definition of fat does not give the true picture of its heterogeneity in terms of chemical composition. Fat in foods consists primarily of highly non-polar or hydrophobic molecules classified as triacylglycerols, with smaller amounts of di- and mono-acylglycerols, sterols, and the somewhat more polar molecules such as phospholipids and sphingolipids. Minor components such as fat-soluble vitamins, pigments, or hydrocarbons are also included as "fat" when defined by the property of solubility, whereas free fatty acids, especially the shorter chain fatty acids, may be water-soluble and therefore not included as fat.

In addition to heterogeneity in the type of molecule, further diversity of fat composition exists as a function of the chain length as well as saturation or degree of unsaturation of the hydrocarbon chain of fatty acid constituents of fats. Depending on the exact chemical composition, the constituents of "fat" in a particular food sample may encompass a wide range on the polarity scale. Consequently, the composition as well as the efficiency of extraction of these different components of fat from food are dependent on the polarity of the solvent used for extraction. Furthermore, fats in foods frequently occur in bound form, for example, as non-covalent complexes with proteins or carbohydrates, or as lipoproteins or glyco-lipids. Organic solvents do not usually extract the fat molecules in these bound forms, unless they are released prior to extraction by pre-treatment with mild acid, alkali, or enzymatic digestion. The fat content determined by direct extraction with non-polar solvents is usually termed "crude fat," while that determined by extraction after a pre-treatment may be referred to as "total lipids."

It is worth noting that the "total lipid (fat)" content reported in the USDA Nutrient Database, and identified as "nutrient 204," was determined for most foods by gravimetric methods such as those using ether or chloroform-methanol solvent extraction, or by acid hydrolysis (7). However, "Nutrient 204 may not be identical to the fat level declared on food labels under the Nutrition Labelling and Education Act of 1990 (NLEA). Under NLEA, fat is expressed as the amount of triglyceride that would produce the analytically determined amount of lipid fatty acids" (7). Similarly, the fat content reported in the "Nutrition Facts Table Core Nutrition Information" on food labels in Canada (Table 42.3) is also based on the sum of fatty acids, expressed as triglycerides, analyzed by capillary gas chromatography (AOAC Official Method 996.06).

2. Solvent Extraction Methods

Solvent extraction may be continuous (e.g., the Goldfish method), semi-continuous (e.g., the Soxhlet method for cereals AOAC Official Method 920.39C), or discontinuous

(e.g., Mojonnier method for milk fat AOAC Official Method 989.05).

In the continuous method of solvent extraction, evaporating solvent from a boiling flask continuously condenses onto the dried sample held in a thimble. The solvent, carrying fat extracted as solvent drips through the sample, is collected back in the boiling flask. After completion of extraction, typically 4 hours to overnight (depending on the rate of solvent dripping through the sample), the solvent is evaporated from the extraction flask by air-drying overnight followed by brief oven drying, and the fat remaining in the flask is weighed. The semi-continuous solvent extraction method is similar except the extraction apparatus is constructed to allow solvent to remain with and soak the sample for 5–10 minutes, prior to returning to the boiling flask. In this manner, the sample is surrounded by solvent, thus avoiding channeling of solvent and providing for a more complete extraction of fat from the sample. For this reason, the semi-continuous methods, particularly the Soxhlet method, are often considered the standard methods for fat analysis of dried samples (26, 27).

For efficient extraction by both the Goldfish and the Soxhlet methods, it is important that samples are dried and ground to small particle size to maximize the surface area for extraction. Efficient extraction by organic solvents can only be achieved on dry samples, due to the inability of the organic solvents to penetrate the water phase of wet samples. Ethyl ether and petroleum ether are the most commonly used solvents for extraction by these methods. Ethyl ether is slightly more polar than petroleum ether and is a better solvent for fat. However, sample drying becomes essential to avoid extraction of non-fat components by ethyl ether; in addition, ethyl ether has a tendency to form peroxides and is more flammable than petroleum ether. Petroleum ether is cheaper, less dangerous and preferred over ethyl ether if a more selective solvent for hydrophobic lipids is desired (26).

The Roese-Gottlieb and the Mojonnier methods (AOAC Official Methods 905.02 and 989.05, respectively) are official methods for analysis of fat in milk, involving the release of bound fat by alkaline digestion with ammonium hydroxide, addition of ethanol, followed by sequential, discontinuous extraction of fat using ethyl ether and petroleum ether. The extractions are repeated three times and carried out in flasks designed to facilitate decanting of the fat-containing organic solvent (top layer) from the aqueous solution (bottom layer) during extraction. The fat-containing solvents from the repeated extractions are pooled, the solvent is removed by evaporation, and the weight of the fat is measured to determine the fat content. The Mojonnier method, using acid instead of alkali pre-treatment, has been modified for determination of fat content in flour (AOAC Official Method 922.06) and pet food (AOAC Official Method 954.02).

As an alternative method to gravimetric measurement (i.e., by weight), the measurement of refractive index has been proposed as a method to determine fat that has been extracted by solvent (14, 26). Fat is extracted using a discontinuous method with a solvent such as bromonaphthalene, which has a refractive index that is different from the fat to be extracted. The fat content is calculated by comparing the refractive index measured for the solvent containing the extracted fat, to the refractive index of solvent alone and that of the standard fat. The refractive index method has been reported to yield values of fat content comparable to the crude fat content measured gravimetrically.

For all of the solvent extraction-based methods, the content of fat analyzed depends on whether or not pretreatments such as addition of acid or alkali to release bound fat have been performed, as well as the polarity of solvent used for extraction. For example, the content of "fat (crude) or ether extract in flour" (AOAC Official Method 920.85) would be expected to differ from that of "fat in flour – acid hydrolysis method" (AOAC Official Method 922.06). Many researchers have reported that the amounts of extracted lipid as well as non-lipid material increase with the polarity of solvents used for extraction. The binding of lipids to other molecules such as carbohydrates and proteins, and the differing abilities of solvent or solvent mixtures to solubilize lipid classes has led to the concept of "total lipid extract" and "extractable lipid" (28). Even with ammonia pretreatment to release bound lipids, the Roese-Gottlieb method was reported to grossly underestimate the total lipid content of samples such as chocolate powder, liquid milk, and eggs (28); this could be due to the use of relatively non-polar solvents, ethyl and petroleum ethers, for extraction after hydrolysis. More polar solvent mixtures such as dichloromethane-hexane (1:4) or acetone-hexane (1:4) have been suggested to improve the efficiency of extraction from dry solid samples using the Soxhlet method (28). For wet or liquid samples, specific ratios of chloroform and methanol are used in combination with water in the Bligh and Dyer method (29) and in AOAC Official Method 983.23 for determining "fat in foods" after enzymatic hydrolysis. In a modified Bligh and Dyer procedure, methanol is replaced by propan-2-ol, and chloroform is replaced by cyclohexane, with a ratio of 11:8:10 for water:propan-2-ol:cyclohexane; the modified procedure has the advantage that the extracted lipid is in the top (cyclohexane) layer of the extraction mixture, compared to the bottom layer when chloroform is used (28).

3. Non-Solvent Separation Methods

The Babcock method for milk fat (AOAC Official Methods 989.04 and 989.10) is the best-known example of a non-solvent wet extraction or separation method for fat analysis. Concentrated sulfuric acid (with specific gravity of 1.82–1.83 at 20°C) is added to a specified weight of milk sample in the Babcock bottle, digesting protein and generating heat, and thus releasing bound fat from the sample. The acid also functions to increase the

density of the aqueous layer. Subsequent centrifugation at 55–60°C and addition of hot water at that temperature cause the fat layer to float on top of the aqueous layer into the calibrated neck of the Babcock bottle, where the percentage of fat content is read directly from the volumetrically calibrated markings.

The Babcock method has been adapted for determination of fat in other dairy products such as cream and cheese, as well as for essential oil in flavor extracts (AOAC Official Method 932.11) and fat in seafood (AOAC Official Method 964.12) (26). Similar to the Mojonnier method, the Babcock method can be applied to wet or liquid as well as dried samples, and includes a pre-treatment to release bound fat. However, it should be noted that fat components with high specific gravity, such as phospholipids, will not float to the top layer and therefore will not be included in the volumetric measurement for fat after centrifugation.

4. Instrumental Methods and Comparison to Standard Solvent Extraction Method

An automated turbidimetric method (AOAC Official Method 969.16) has been developed to determine fat in milk. In this method, EDTA is added to dilute the sample and to eliminate turbidity due to the casein protein. Homogenization is then used to form uniform fat globules, which may then be determined by measurement of turbidity, which is related to fat content through comparison to a standard curve.

Fat content of foods can also be analyzed using various other instrumental methods measuring such properties as density, nuclear magnetic resonance, x-ray absorption, dielectric constant, or infrared spectra (26). The differences in these properties between fat molecules and other food components form the basis for relating the measurement of these properties to the fat content. These methods are usually rapid and do not require extraction of fat from the food prior to analysis. However, it is important to construct standard curves for the specific food being analyzed, in order to determine the quantitative relationship between the particular physical property being measured by the instrumental method, and the fat content of standards with known composition, usually determined by solvent extraction methods such as the Soxhlet method.

Vogt et al. (27) compared four rapid methods to the standard Soxhlet extraction method for fat measurement in fish herring. The four methods were (i) the Torry Fatmeter based on dielectric properties of water, and intercorrelations between fish water and fat contents; (ii) microwave drying and calculation of fat from water content; (iii) near-infrared reflectance (NIR) spectroscopy with partial least-squares regression analysis; and (iv) fexIKA Soxhlet solvent extraction instrument modified to include filtration under reduced pressure. The NIR method gave the best prediction of the standard method (Soxhlet), while other methods gave lower fat content values. However, the NIR instrument is relatively expensive. In comparison, the microwave method is low cost, requiring only a microwave oven, and the Torry meter is moderately priced. The Soxhlet and fexIKA were also both considered to have the disadvantages of being time-consuming and requiring solvents.

D. Protein

Proteins are macromolecules composed of amino acids linked together through amide or peptide bonds to form polypeptide chains, which can range in molecular mass from a few thousand to over a million daltons. Proteins contribute to the nutritional value as well as textural and sensory properties of foods, and they also perform structural and biologically important functions. The protein content of foods ranges from less than 1–2% in fruits and vegetables, to 20–30% in meat, legumes, and dairy products (30).

1. Overview of and Considerations for Protein Determination

The most common official method for determination of protein content in food is based on measurement of total nitrogen content using the Kjeldahl method, followed by multiplication by a conversion factor to calculate the protein content. The Dumas (nitrogen combustion) method is also based on the principle of nitrogen determination and conversion to protein content. A number of other methods, mostly colorimetric or spectroscopic methods, are based on quantifying either the peptide bonds or particular side chains of the amino acid residues, and may be useful as rapid methods for quality control or research. These latter methods require calibration of the measured property, such as absorbance or color intensity, using standards of known composition, usually determined by nitrogen analysis, or sometimes by gravimetric analysis.

Many factors can affect the accuracy of analyzing protein content in food through measurement of its nitrogen content. Proteins are composed of amino acids whose chemical structures include hydrogen, carbon, nitrogen, oxygen, and sulfur atoms. Of these elements, nitrogen is considered most distinctive for proteins in comparison to other components considered in proximate analysis, since each of the twenty amino acid building blocks contains a nitrogen-containing amino (or imino) group. However, the side chains of these amino acids differ greatly, ranging from hydrocarbon chains containing primarily carbon and hydrogen atoms, to aromatic groups and polar or ionic groups that also contain sulfur, oxygen, or nitrogen atoms. Since proteins contain different combinations of the amino acids, each of which contains a different percentage of nitrogen in its chemical structure, the percentage of nitrogen can vary greatly between different proteins. Use of the correct conversion factor is required if an accurate estimation of protein is to be expected.

A nitrogen-to-protein conversion factor of 6.25 is commonly used to calculate the protein content of a food from the nitrogen content determined by Kjeldahl or combustion methods. This factor assumes an average of 16% (= 100 / 6.25) nitrogen content in proteins, i.e., 16 grams of nitrogen per 100 grams of protein. However, depending on the amino acid composition of the particular protein, the actual conversion factor may deviate from 6.25. For example, the conversion factors are 6.38, 5.70, and 6.25 for dairy products, wheat, and almonds, respectively (30), and 4.74, 5.3, 4.38, and 5.7 for chocolate and cocoa, coffee, mushrooms, and yeast, respectively (7). The conversion factor and source of information for the factor should be stated in listing protein content, as illustrated by some of the tagnames listed in Table 42.1C.

The presence of non-protein nitrogen in foods is another important consideration that may affect the accuracy of protein determination that is based on nitrogen measurement. Foods may contain varying levels of free amino acids or small peptides, ammonium salts, urea, uric acid, vitamins, amino sugars, nucleic acids, phospholipids or other constituents that contain nitrogen in their chemical structure. If unaccounted for, the contribution of nitrogen from these non-protein nitrogen sources will result in an over-estimation of protein content. Methods have therefore been established, using trichloroacetic acid or other protein precipitant, to enable distinction between protein and non-protein nitrogen. For example, AOAC Official Methods 991.20, 991.21, 991.22, and 991.23 describe methods for milk total nitrogen, non-protein nitrogen, protein nitrogen by direct method, and protein nitrogen by indirect method, respectively.

Due to the long period of time to perform Kjeldahl analysis and the necessity to determine the difference between non-protein nitrogen and total protein nitrogen, spectrophotometric or colorimetric methods for total proteins are commonly used as rapid methods suitable for research or quality control. However, there are potential interferences and other considerations that must be kept in mind when using these rapid methods (30–32).

An overview of the main methods for protein analysis based on total nitrogen analysis, and spectrophotometric, colorimetric, or other instrumental methods for protein analysis, are described in the following sections. For more details, the reader should refer to the chapter on protein analysis in this Handbook, and to an excellent book on this topic written recently by Owusu-Apenten (31).

2. Methods for Total Nitrogen Analysis

In the Kjeldahl method for total nitrogen analysis, the protein and other organic molecules in foods are digested by concentrated sulfuric acid in the presence of catalysts. The nitrogen is present in the acid digest in the form of ammonium sulfate, and may be analyzed either by an acid-base titration of the nitrogen after neutralization and distillation of the resulting ammonia, or by other techniques including nesslerization or colorimetric reaction with a phenol hypochlorite reagent (30). The Kjeldahl method is described in AOAC Official Method 920.87 for protein (total) in flour or AOAC Official Methods 928.08 and 981.10 for nitrogen or crude protein, respectively, in meat. Various modifications of the original method have been proposed over the years, implementing copper as an alternative catalyst to mercury (AOAC Official Methods 984.13 and 2001.11), semi-automation or automation (AOAC Official Methods 976.05, 967.06, and 990.02), and analysis of microgram quantities of nitrogen (AOAC Official Method 960.52).

In the Dumas (also known as nitrogen combustion or pyrolysis) method, the samples are subjected to very high temperatures up to 1000°C. Nitrogen that is freed by pyrolysis and subsequent combustion at high temperature in pure oxygen, is then quantitated by gas chromatography and thermal conductivity. Official methods based on combustion have been established, for example, for animal feed, meats, cereal grains and oilseeds, and fertilizers (AOAC Official Methods 990.03, 992.15, 992.23, and 993.13, respectively).

Both the Kjeldahl and the combustion methods determine total nitrogen, so the correct nitrogen-to-protein conversion factor and the contribution of non-protein nitrogen to the total nitrogen content must be considered for an accurate determination of protein content. The analyte is referred to as "crude" protein in the AOAC Official Methods, when total nitrogen is determined without correcting for non-protein nitrogen. Combustion methods have the advantages of speed of analysis, and avoidance of corrosive and toxic reagents, compared to the Kjeldahl method, but the expenses of the equipment and high-purity oxygen must be considered.

3. Spectrophotometric Methods

Aromatic amino acids (tryptophan, tyrosine, and phenylalanine) and peptide bonds absorb electromagnetic radiation in the ultraviolet (uv) region near 257–280 nm and 190–205 nm, respectively (33). The intensity of uv absorbance can be related to the content of those groups and, therefore, to the protein content of a solution.

Spectrophotometric methods are simple in principle, but only applicable to samples that are non-turbid solutions of relatively pure protein composition. Since the content of aromatic amino acids differs between proteins, and since the extinction coefficient and wavelength of maximum absorbance are characteristic for each of the three aromatic amino acid types, this method for protein analysis requires standard curves or tables, such as those compiled by Fasman (33), relating the absorbance at a particular wavelength to the concentration of the specific protein. The uv absorbance of the peptide bond is less dependent on the nature of the amino acid side chains.

However, the absorbance of ultraviolet radiation in this wavelength region by other food components, such as organic acids, nucleic acids, triacylglycerols, etc., may result in considerable interference and inaccuracy in relating the absorbance directly to protein content in complex foodstuff.

4. Colorimetric Methods

A number of colorimetric methods have been established for determination of protein content, based on the development of color upon reaction with either the peptide bonds or with particular functional groups of the proteins. Colorimetric methods based on reaction with peptide bonds, such as the biuret, Lowry, and bicinchoninic acid (BCA) methods, show relatively low protein-to-protein variability in the relationship between colorimetric intensity (absorbance) and concentration for different proteins. Protein-to-protein variability is much greater for methods that are based on reaction with specific functional groups on the amino acid side chains, since the amino acid composition of the protein as well as the protein content will affect the resulting colorimetric response. The Bradford or Coomassie Brilliant Blue method is presently the most common example of the latter class of methods, although Acid Orange 12 and Amido Black 10B were established as official dye-binding methods for protein in milk in 1967 and 1975 (AOAC Official Methods 967.12 and 975.17, respectively).

The Bradford method is based on a change in the wavelength and intensity of absorbance (color) of the reagent dye Coomassie Brilliant Blue G-250, when it is bound to proteins, particularly at basic amino acid side chains and enhanced by hydrophobic interactions. The biuret method is based on the development of a blue color upon complex formation of cupric ions with peptide bonds in alkaline solution, while the Lowry method combines the biuret reaction with reduction of the Folin-Ciocalteau phenol reagent by tyrosine and tryptophan residues in proteins, and the BCA method involves the reduction of cupric to cuprous ions by peptide bonds under alkaline solution, followed by development of a purplish color upon formation of cuprous ion complexes with the BCA reagent. The absorbance is read at 595, 540, 700, or 562 nm for the Bradford, biuret, Lowry, and BCA reactions, respectively, and the protein content is determined using a standard curve. As noted above, due to protein-to-protein variability, the selection of the appropriate protein for the standard curve is important, especially in the case of the Bradford method. The Bradford, Lowry, and BCA methods have greater sensitivity and lower limit of detection than the biuret reaction. Selection of the most appropriate method depends on considerations with regard to potential interferences from other constituents in the sample, availability of the appropriate protein standard for calibration, and the sensitivity that is required (30, 31).

5. Infrared Spectroscopy

Infrared spectroscopy may be used to determine the protein content of foods by relating the absorption of infrared radiation at wavelengths assigned to the peptide bond of proteins. This technique is rapid, may be applied to solid as well as liquid samples in the presence of other food constituents, and is not affected by non-protein nitrogen or amino acid composition. However, calibration must be conducted using standard curves relating the infrared absorption to protein content. Examples of mid-infrared and near-infrared spectroscopy for protein content include the official methods for protein (crude) in forages (AOAC Official Method 989.03) and for protein in milk (AOAC Official Method 972.16).

E. Carbohydrate

Carbohydrates in foods include monosaccharides (particularly D-glucose and D-fructose), disaccharides (e.g., sucrose, lactose, maltose), higher oligosaccharides (e.g., raffinose, stachyose and fructo-oligosaccharides) and polysaccharides (e.g., starch, food gums and hydrocolloids, pectin, hemicellulose, cellulose) (34, 35). The sugar alcohols, such as sorbitol, mannitol, xylitol, lactitol, and maltitol, are also considered as carbohydrates (34). Carbohydrates are important from a nutritional point of view, whereby metabolic energy is provided by digestible carbohydrates including simple sugars (monosaccharides, starch) while dietary fiber is obtained from the indigestible fraction. Polysaccharides also play a crucial role in textural properties of foods, while mono- and disaccharides may take part in reactions such as Maillard browning or caramelization, thereby imparting characteristic flavor and color to foods.

The total carbohydrate content of foods ranges from negligible (e.g., in chicken breast meat) to over 80% (e.g., in honey and breakfast cereals such as corn flakes) (35).

1. Methods and Considerations for Total Carbohydrate Determination

In proximate analysis for nutrition labeling, the total carbohydrate content of a food is usually calculated by subtracting the weights of moisture, ash, total fat, and crude protein from the total weight of the food (8, 35). When alcohol is present, it is also subtracted from the total weight in the calculation of carbohydrate by difference (7). It should be noted that protein calculated from total nitrogen, which may contain non-protein nitrogen, is used in determining carbohydrate by difference (7).

Although the calculation of carbohydrates by "difference" has a long history of usage as the method for determining total carbohydrates reported in food tables or nutrition labels, there are several problems with this

approach (34). Firstly, it includes a number of non-carbohydrate components such as lignin, organic acids, tannins, waxes, and some Maillard browning products. Secondly, it incorporates the sum of all of the analytical errors from determination of the other analytes in proximate analysis. Thirdly, a single value for "total carbohydrates" is not very informative with regard to nutritional value, as it fails to distinguish between the distinct physiological properties of different classes of carbohydrates (34).

The phenol-sulfuric acid method may also be used to estimate total carbohydrates (35). Heating of carbohydrates in the presence of strong acids produces furan derivatives and their polymers, which condense with various phenolic substances, including phenol itself, to yield a yellow-orange color. The absorbance at 490–500 nm is related to the concentration of carbohydrate using a standard curve. Although the phenol-sulfuric acid method is applicable to different classes of carbohydrates from monosaccharides to polysaccharides, the relationship between absorbance and concentration, and even the wavelength of maximum color intensity, are dependent on the chemical composition of the carbohydrates. Therefore, accuracy is dependent on selecting the appropriate reference carbohydrate or carbohydrate mixtures to prepare the standard curve, which is a difficult task in reality since most foods are composed of complex mixtures of carbohydrates. Incorporation of additional sample purification steps such as selective precipitation may be helpful to address this problem (e.g., AOAC Official Method 988.12E for dextran in raw cane sugar).

Finally, rather than calculating total carbohydrates by "difference," or by chemical methods for total carbohydrate determination such as the phenol-sulfuric acid method, an alternative approach involves the direct measurement of the individual components, which are then combined to give a value for total carbohydrates (34). In addition to information on total carbohydrate content, the various classes or sub-classes of the individual components may be reported, such as total simple sugars, starch, dietary fiber, etc. Mono- and disaccharides may be analyzed specifically by enzymes, gas-liquid chromatography (GLC), or high performance liquid chromatography (HPLC). Polyols and oligosaccharides are also usually determined by GLC or HPLC methods. Starch analysis is based on enzymatic degradation, followed by determination of the liberated glucose. Non-starch polysaccharide-analysis involves removal of starch enzymatic hydrolysis products and other low-molecular-weight carbohydrates, followed by hydrolysis of the non-starch polysaccharides to its monomeric constituents for analyisis. Although a number of methods have been established in the past for determination of crude fiber, acid-detergent fiber, neutral detergent fiber, etc. (e.g., see Table 42.1D), it is recommended that dietary fiber be determined by the enzymatic-gravimetric methods of Prosky and co-workers or Lee and co-workers, or the enzymatic chemical methods, such as that of Englyst and co-workers (34). Examples of the enzymatic-gravimetric method are AOAC Official Methods 985.29, 991.42, 991.43, and 993.19, for determining insoluble, soluble, and total dietary fiber.

III. OTHER COMPONENTS

As shown in Tables 42.2 and 42.3, in addition to conducting proximate analysis for the major food components described in Section II, further analysis is required to provide information on nutrition labels in the United States and Canada, respectively, and similar regulatory requirements are found in many countries internationally. This additional information includes the energy or caloric content, the contents of subclasses of fatty acids and carbohydrates, and the contents of a number of other components that may be minor in quantity but are of major importance as nutrients.

The specific methods recommended for analysis may vary between different countries. International standards are described in the FAO/WHO Codex Alimentarius Methods of Analysis and Sampling (19). The list of methods used by the Canadian Food Inspection Agency or CFIA (Table 42.3) to verify nutritional information is illustrative of the use of a combination of sources for the methods. As stated at the CFIA website: "The methods of analysis recommended are those published in the most recent version of the 'Official Methods of Analysis of AOAC International' wherever possible. Other collaboratively studied methods such as those published by the American Oil Chemists' Society, American Association of Cereal Chemists, ISO, etc. would also be considered appropriate. In house or journal methods with adequate method validation data are another possible option for method selection" (8). More detailed information on some of these methods is described in various chapters in this Handbook, including those on gas chromatography, HPLC, and mass spectrometry.

IV. TRENDS IN FOOD COMPOSITION ANALYSIS

Some of the trends in the analysis of chemical composition of food, with regard to methods validation, components analyzed, and new techniques, are outlined in the following sections.

A. Validation of Methods of Analysis and Validity of Food Composition Data

Nutrient values in food composition databases may be collected from various sources of information generated by chemical analysis of food samples, by calculations, or by

expert estimation or "imputation" (36). Furthermore, the analytical data may be obtained from chemical analysis of food samples from the food industry, from government agencies, from the scientific literature, or by analytical laboratories that may or may not be ISO-accredited. Since the data are gleaned from various sources and may be of uneven quality and detail of supporting documentation, it is crucial to assess whether or not the data are in fact reliable for inclusion in databases (36). For example, the USDA Nutrient Data Laboratory has been working to establish software for data acquisition, compilation, and dissemination, as well as to facilitate the evaluation of analytical data quality based on five categories – sampling plan, number of samples, sample handling, analytical method, and analytical quality control, leading to a combined rating represented by a quality index and confidence code (36).

There is a continuing need for reliable analytical methods that can be used to determine compliance with national regulations as well as international requirements in all areas of food quality and safety. Some form of validation procedure must be used to determine the reliability of a method. The Codex Alimentarius Commission, for example, requires that in order for a method of analysis to be included in a Codex commodity standard, certain method performance information should be available. This includes specificity, accuracy, precision (repeatability, reproducibility), limit of detection, sensitivity, applicability, and practicability, as appropriate. However, "It is not practical or necessary to require that all analytical methods used for food control purposes be assessed at the ideal level … Limiting factors for completing ideal multi-laboratory validation studies include high costs, lack of sufficient expert laboratories available and willing to participate in such studies, and overall time constraints" (20).

A joint FAO/IAEA expert consultation on "Validation of Analytical Methods for Food Control" was held in Vienna, December 2–4, 1997, to review existing schemes for validation of international methods, to identify requirements for validation of methods for analysis of veterinary drug and pesticide residues, food additives, and environmental contaminants in food, and to recommend alternative approaches which would be practical, cost effective, and considerate of time and human resource constraints (18). Although a collaborative study conducted according to generally accepted international protocols is always the preferred validation procedure, in some cases it is not feasible to do so. In these circumstances, the evaluations may be done in one laboratory, provided they are conducted according to five principles addressing competence and third party review of the testing laboratory, appropriate criteria assessment of the analytical method, careful documentation in an expert validation report, and evidence of transferability (18).

B. The Impact of Health Issues on Analysis of Chemical Composition

Increasing numbers of reports on the associations between consumption of certain foods or nutrients with improved health or reduced risk of disease, have led to an explosion of literature on food composition information on these nutrients, nutraceuticals, and functional foods. However, the validity of the measurements for specific bioactive analytes has not been adequately assessed in many cases. There is a need to apply the quality indices for methods validation described in Section IV.A., and to initiate an informatics approach to developing nutrient databases for these components. Recent trends in this direction include the initiation of a flavonoid database (37) and databases for several bioactive food components including carotenoids, six classes of flavonoids, omega-3 fatty acids, and plant sterols (38).

Increasing interest in the physiological roles of dietary fiber and resistant starch has also led to a renewed need to assess the methods for their determination. Resistant starch is considered to be that starch which resists digestion and absorption in the small intestine and escapes into the colon, where it may be fermented by bacterial microflora. The enzymatic-gravimetric and enzymatic-chemical methods for dietary fiber analysis include protocols for determining the content of resistant starch (35), but it has been reported that some data on resistant starch have only estimated retrograded amylose, which is only one of the 3 or 4 components of resistant starch in the diet (34). Similarly, data on dietary fibre intake around the world are difficult to compare because of methodology differences (34). In fact, a debate is ongoing at the present time on what should be included in the definition of dietary fiber. At the heart of the controversy is the issue of whether total fiber should be classified into dietary fiber (i.e. sources of fiber that are intrinsic or intact in plants) versus functional fiber (i.e., fiber that has been isolated and then incorporated as an ingredient in food products), with associated concerns of how these two classes of fiber might be analytically distinguished (39, 40).

C. Trends in Methodology for Food Analysis

There is a continuing need to explore the possibilities and limitations of rapid methods for analysis of food, and of equipment that will enable their successful application for quality control (41, 42). As reviewed by Ibañez and Cifuentes (43) in an excellent article, there have been many advances in applying new analytical techniques in food science, with an increasing focus on sophisticated techniques. These include nuclear magnetic resonance, capillary electrophoresis, mass spectrometry, and infrared spectroscopy, as well as coupled techniques and new approaches to sample

preparation (43), mass spectrometry, and hyphenated mass-spectrometry techniques (44, 45) and the vibrational spectroscopic techniques of near-infrared, mid-infrared, and Raman spectroscopy (46). Other recent trends include the increased application of enzymes as indicators of food quality, biosensors, immunochemical techniques, DNA probes, the polymerase chain reaction (PCR), rapid methods for microbio-logical analysis, and authentication of foods using isotopic methods (1, 43). There has also been great interest in methods for the detection of genetically modified foods. For example, Dahinden et al. (47) described the application of real-time PCR technology for quantification of the genetically modified organism (GMO) content of soybean (Roundup Ready soybean-RRSoybean) or corn (Bt176, Bt11, Mon810, and T25), and for demonstrating the amplification capacity of DNA from corn (invertase) and soybean (lectin).

V. CONCLUSIONS

This chapter has provided an outline of the basic principles and considerations for proximate analysis of food, and briefly described the analysis of other constituents that are of interest from the viewpoint of nutrition and health. A multitude of techniques based on different principles are available or being developed for the analysis of the chemical composition of foods. Due to the diversity and heterogeneity of these food constituents, and the dependence of the measured content of each constituent on the method used for its analysis, it is imperative that reports on food composition be accompanied by sufficient description of the analytical methods used for obtaining those data, and that these methods have been validated by officially recognized associations. Only then can the data on chemical composition of food analyzed by different sources or agencies be meaningfully compared, interpreted, and applied.

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43 Spectroscopy Basics

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CONTENTS

I. INTRODUCTION TO SPECTROSCOPY

Spectroscopy is the study of the interaction of matter with electromagnetic radiation by absorption, emission, or scattering. Spectroscopic techniques are invaluable for obtaining both structural and quantitative information about atoms and molecules, and are used extensively in the analysis of foods and individual components.

Electromagnetic radiation has a dual nature and acts as if it is a particle (a photon) travelling through space with the characteristics of a wave, with oscillating electric and magnetic fields (Figure 43.1)(1). The energy of the radiation, E, is directly related to the frequency, ν (the number of oscillations per second), by Planck's constant ($h = 6.63 \times 10^{-34}$ J-s),

$$E = h\,\nu \tag{43.1}$$

The frequency, ν, is inversely related to the wavelength, λ, by

$$\nu = c/\lambda \tag{43.2}$$

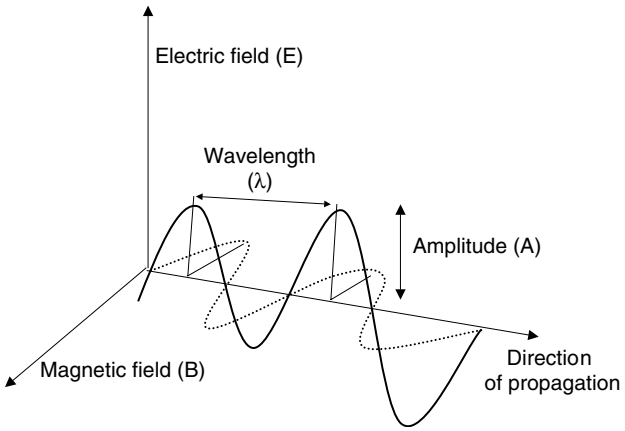

FIGURE 43.1 The characteristics of electromagnetic radiation, with electric and magnetic fields perpendicular to each other.

where c is the velocity of the radiation. The frequency of radiation is determined by the source, and does not change as the wave propagates through different media. However, the velocity, and therefore the wavelength, is affected by the medium. Wavelength is inversely related to energy, so ultraviolet radiation with short wavelengths has more energy than visible light. It should be noted that only those wavelengths that are visible to our eye, from about 400 to 800 nm, are referred to as light. Radiation is the general term used for electromagnetic radiation that ranges from cosmic rays with wavelengths of 10^{-12} m, to radio waves with wavelengths of 10^{4} m.

The charged particles of matter, the nucleus and the electrons, can interact with specific wavelengths of radiation, and the energy from the radiation can be transferred to, or absorbed by, the material. This absorption of energy excites the atom or molecule from a lower energy state (often the ground state at room temperature) to a higher energy state, and is observed as an absorption spectrum. Alternatively, the emission of radiation when the atom or molecule moves from a higher energy state to a lower, more stable, energy state can be monitored as an emission spectrum. There are a limited number of discrete energy levels that a particular atom or molecule can achieve, based on its structure. Therefore, only wavelengths of radiation with the precise amount of energy that exists between two different energy states can be absorbed or emitted. This allows atoms and molecules to be identified by their unique absorption or emission spectra.

The absorption and emission spectra of atoms are less complicated than those of molecules. Atoms can undergo electronic energy transitions, in which electrons (usually valence electrons) move to higher or lower energy states. Electronic transitions associated with excitation of valence electrons can occur with the energy of ultraviolet or visible radiation. Molecules also undergo electronic transitions, but have rotational and vibrational motions as well. For each electronic state, there are several rotational states,

and for each rotational state, there are several vibrational states (Figure 43.2). Vibrational motion occurs from the bending or stretching of bonds between nuclei. The energy required for vibrational motions is much smaller than for electronic transitions, and vibrational transitions are typically excited by infrared radiation. Rotational motion arises from movement of a molecule about its axes or center of gravity. Transitions between rotational states require even less energy than vibrational transitions, and can occur with the energy found in the far infrared to microwave regions of the electromagnetic spectrum.

In addition to absorption and emission, radiation can also be re-directed through scattering, reflection, or rotation due to its interaction with matter. This can occur with or without the transfer of energy, and therefore after interacting with a sample, the radiation may have a different or the same wavelength as the original radiation.

While many forms of spectroscopy are based on the interaction of electrons with electromagnetic radiation, the interaction of nuclei with radiation can also be detected under certain conditions. The nuclei of many elements have magnetic properties, and when they are placed in a strong external magnetic field, they will populate different magnetic states. If the nuclei are then exposed to radiation of an appropriate frequency, they will absorb the radiation and undergo transitions between magnetic states. The energy difference between these magnetic states varies with the strength of the external magnetic field and the chemical environment, but occurs in the radiowave region of the electromagnetic spectrum.

A summary of the spectroscopic techniques that will be explored in this chapter is given in Table 43.1. Spectroscopic techniques of fluorescence, infrared, and mass spectroscopy are discussed in other chapters of this book.

II. ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY

A. Introduction

Spectrophotometry is based on the ability of valence electrons of molecules to absorb specific frequencies of radiation, from the ultraviolet range (200 to 400 nm) through the visible range (400 to 800 nm). The absorption of the radiation can be detected using a spectrophotometer and will yield an absorption spectrum. Spectrophotometry can be used to obtain qualitative information regarding the compounds that are present in a sample extract, but it is not possible to obtain detailed structural information. In molecules, there are simultaneous transitions between different vibrational, rotational and electronic states, resulting in rather broad absorption bands. Spectrophotometry is more commonly used to quantitate the amount of a compound in solution. It is an extremely versatile technique, and spectrophotometric methods of detection and quantitation have been developed for hundreds of compounds. The technique can also be used to detect enzyme activity, and spectrophotometric detectors are commonly used for high performance liquid chromatography (HPLC). Some selected applications of spectrophotometry in food science are given in Table 43.2.

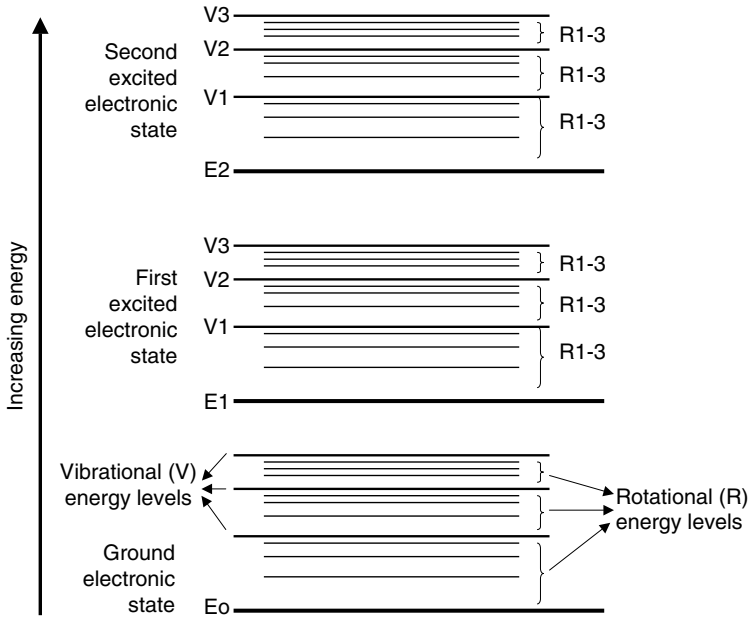

FIGURE 43.2 A partial energy diagram of a molecule, showing the relationship between electronic, vibrational, and rotational energy levels.

TABLE 43.1
Selected Characteristics of Some Spectroscopic Techniques

Spectroscopic Technique	Interacting Radiation	Transition	Interaction
Near Ultra-Violet Spectroscopy	200–400 nm	Valence electrons of molecules	Absorption
Visible Spectroscopy	400–800 nm	Valence electrons of molecules	Absorption
Tristimulus Colorimetry	400–800 nm	—	Reflection
Atomic Absorption Spectroscopy	160–900 nm	Valence electrons of atoms	Absorption
Atomic Emission Spectroscopy	160–900 nm	Valence electrons of atoms	Emission
Polarimetry/			
Optical Rotary Dispersion	200–800 nm Circularly Polarized	Valence electrons of molecules	Rotation
Circular Dichroism	170–700 nm Circularly Polarized	Valence electrons of molecules	Absorption
Nuclear Magnetic Resonance	Radiowaves $>$ 1 mm	Nuclear spin transitions	Absorption and Emission

B. Principles

The energy associated with ultraviolet and visible wavelengths of radiation varies from about 150 to 36 kcal mole^{-1} (15). Energy of this magnitude corresponds to the energy differences between electronic states of valence electrons of molecules. Therefore, electromagnetic radiation in this region absorbed by molecules can promote valence electrons to an excited orbital. The types of electronic transitions that occur with absorption of ultraviolet and visible radiation include sigma bonding (associated with single bonds) to sigma antibonding orbitals (σ-σ^*), non-bonding electrons to sigma antibonding orbitals (n-σ^*), or more commonly, pi bonding orbitals (formed by parallel overlap of p-orbitals) to pi antibonding orbitals (π-π^*) and non-bonding electrons to pi antibonding orbitals (n-π^*). The σ-σ^* and n-σ^* transitions require higher amounts of energy, and therefore usually involve absorption of ultraviolet radiation below 200 nm. These transitions cannot be monitored in commonly used instruments. A π-π^* transition occurs with a carbon double bond and n-π^* transition requires a nitro or carbonyl group with a non-bonded electron as well as a double bond. The π-π^* and n-π^* transitions occur readily with the energy available in the ultraviolet and visible spectrum. Molecules in which these types of electron transitions occur are referred to as chromophores, from *chroma*, the Greek word for color, and *phoros* meaning producer. Many food colorants have these types of bonds, and therefore are capable of absorbing visible light, giving color to our foods. Some typical chromophoric groups are given in Table 43.3. An auxochrome is a compound that does not exhibit absorption itself, but alters the wavelength or the intensity of absorption of a chromophore. If a longer wavelength of radiation is absorbed, it is termed a bathochromic or red shift, while a shift to shorter wavelengths is referred to as a hypsochromic or blue shift.

In addition to excitation of valence electrons, absorption of radiation by a molecule can result in charge transfer reactions involving the movement of an electron from one atom to another. As well, transition metals, with unfilled d- or f-orbitals, can also absorb radiation in the ultraviolet-visible region of the spectrum, corresponding to transitions between different d- or f-electronic states.

TABLE 43.2
Selected Applications of Spectrophotometry

Application	Reference
Quantitation of chlorogenic acid in potatoes	2
Determination of protein and fat in milk	3
Determination of paraquat in food and other samples	4
Determination of sulfite in foods using HPLC and ultraviolet spectrophotometric detection	5
Ultraviolet spectra to assess oxidative stability of corn oils	6
Determination of vitamin C by visible spectrophotometry after derivatization with Folin reagent	7
Determination of histamine in fish meal using visible spectrophotometry	8
Determination of transglutaminase activity	9
Determination of allicin and alliinase activity	10
Derivative spectrophotometry to determine two food dyes simultaneously.	11
Derivative spectrophotometry for determination of *o*- or *p*-nitrophenol, as a marker for some pesticides	12
Derivative spectrophotometry to determine tryptophan in proteins	13
Estimation of specific growth rates and lag times of microbial cultures under different conditions	14

TABLE 43.3
Some Chromophoric Groups

Chemical Group	Structure	Transition
Acetylenic	- C $\equiv$ C -	π-π^*
Amide	- $CONH_2$	π-π^*, n-π^*
Carbonyl	- C = O	π-π^*, n-π^*
Carboxylate	- COO^-	π-π^*, n-π^*
Ester	-COOR	π-π^*, n-π^*
Ethylenic	- C = C -	π-π^*
Nitro	- NO_2	π-π^*, n-π^*
Oxime	- C = N -	π-π^*, n-π^*

Source: Ref. 15.

To obtain the absorption spectrum of a sample, a beam of radiation is passed through the sample, and the power of the incident beam, P_o, is compared to the power of the radiation passing through the sample, P, the transmitted beam (Figure 43.3). The power of the transmitted beam will be reduced from the incident beam due to reflection at the cuvette and sample interfaces, scattering within the sample, and absorption by the analyte. The transmittance of the sample, T, is the ratio of the radiant power transmitted by a sample to the power of the incident beam,

$$T = P/P_o \tag{43.3}$$

Transmittance values range from 0 to 1, or from 0–100% for percent transmission. There is a logarithmic relationship between transmission and concentration of a compound that makes it inconvenient to relate the two. Therefore, another term, absorbance, A, is used, defined as

$$A = \log (1/T) = -\log T \tag{43.4}$$

The relationship between transmission and absorbance with concentration can be seen in Figure 43.4.

It was established, independently by Bouguer in 1729 and Lambert in 1760, that absorbance was directly proportional to the thickness of a sample, when concentration was constant, while Beer in 1852 determined that if the sample thickness was constant, the absorbance was proportional to concentration (15). These observations are combined to derive what is commonly referred to as the Beer-Lambert Law, or simply Beer's law, as

$$A = a \cdot d \cdot c \tag{43.5}$$

where a is absorptivity, a proportionality constant for a molecule at a specific wavelength, d is the sample pathlength, and c is the concentration of absorbing molecules.

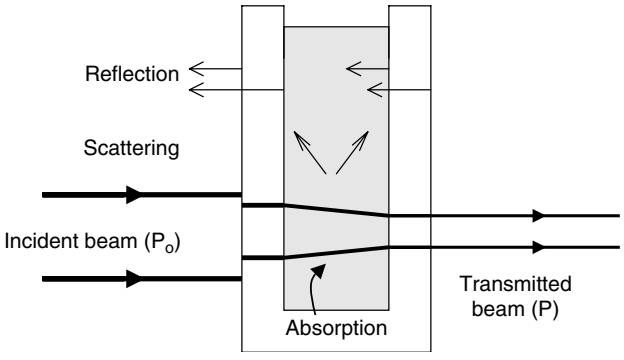

FIGURE 43.3 Attenuation of a beam of radiation by reflection at the cuvette and sample interfaces, scattering by particulates, and absorption by the analyte.

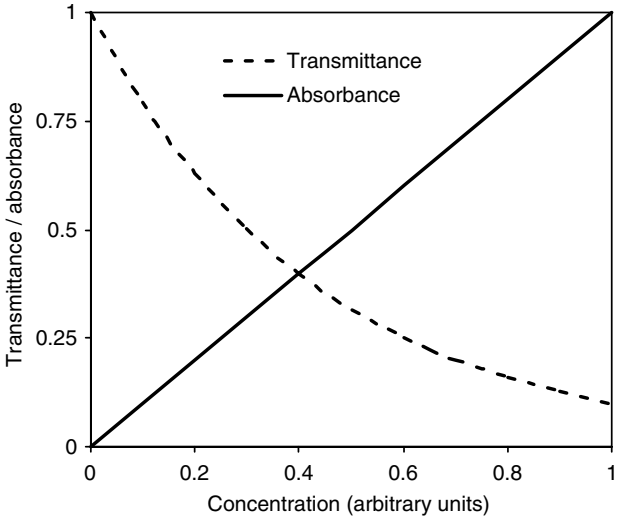

FIGURE 43.4 The relationship between transmittance, T, and absorbance, A, for a hypothetical compound.

The units of absorptivity vary, but reflect the units for concentration and sample pathlength. Molar absorptivity, or the molar extinction coefficient, ε, has units of $M^{-1}cm^{-1}$, while it is typical to give absorptivity constants of some compounds, such as proteins, in terms of a 1% solution at a specified wavelength and a 1 cm pathlength.

Beer's law is used in all absorptive spectroscopies, but is only valid if the following conditions are met: (1) the incident radiation is monochromatic, composed of a single frequency, since each wavelength will have its own absorptivity; (2) the solution is dilute enough that molecules absorbing the radiation act independently of each other; (3) the absorbing sample does not scatter or reflect the incident radiation (some unavoidable effects may be compensated by using a reference cell that contains all the sample components except the analyte); and (4) the absorption occurs in a uniform solution. Beer's law can also be applied to mixtures of molecules, as long as the components of the mixture do not chemically or physically interact. Therefore, absorbance of a mixture is additive, as follows,

$$A_{\text{compounds } 1-\iota} = \varepsilon_1 dc_1 + \varepsilon_2 dc_2 + \ldots. = d\,\Sigma\varepsilon_\iota c_\iota \tag{43.6}$$

C. Equipment

The basic elements of a spectrophotometer consist of a radiation source, a wavelength isolator, a sample container, and a detector.

1. Radiation Source

An ideal radiation source will produce stable, uniform, high intensity radiant energy over the wavelength spectrum of interest. A tungsten filament (or tungsten-halogen) lamp can be used from 320 nm into the infrared region to

2500 nm, but cannot be used in the ultraviolet region. The most common type of lamp for the ultraviolet region is a deuterium electrical discharge lamp with a quartz envelope, which can operate from about 180 to 370 nm.

2. Wavelength Isolator

A wavelength isolation device is an essential component of a spectrophotometer that enhances the selectivity of the analysis and the sensitivity of the instrument by eliminating most of the radiation that is not absorbed by the sample. The benefit of selecting radiation with a narrow range of wavelengths can be explained by the following example. Assume that a radiation source provides a constant 10 units of energy at *every* nm from 350 to 449 nm. A sample placed in the radiation beam absorbs 5 units of energy but only at 400 nm. If no wavelength isolation is used, the spectrophotometer must detect the difference between no sample (1000 units of radiation) and the sample (995 units), a 0.5% difference. However, if a crude wavelength isolation device is used which restricts the incident radiation to 10 nm around the wavelength of interest, 395–404 nm, the difference between the blank and the sample is now 100 versus 95 units of radiation. This is a difference of 5%, resulting in a 10-fold increase in sensitivity. A second important reason for using a wavelength isolation device is to enhance the selectivity of the analysis. If the radiation incident on the sample has a wide range of wavelengths, the observed absorption may be due to the interaction not only of the analyte of interest at the expected wavelength, but also from a contaminant which absorbs at another wavelength.

Using filters that only transmit radiation of certain wavelengths is one of the oldest methods of wavelength selection. Filters, however, have low selectivity and transmit relatively wide ranges of radiation. It is more common to use a monochromator, with a diffraction grating or a prism to separate wavelengths of radiation and focus them on the sample. The monochromator has an entrance slit to allow radiation to enter. The radiation is collected on a concave mirror and can be reflected to a prism or diffraction grating, which physically separates the different wavelengths of radiation. The diffraction grating is a reflective surface with 1200 to 1400 etched grooves per millimeter. The individual wavelengths of radiation are then reflected from another concave mirror to a focal plane aligned with the exit slit. By adjusting the exit slit, the desired wavelength of radiation is passed through the slit and can interact with the sample. In practice, it is not possible to select a single wavelength of radiation, but rather a narrow bandwidth of radiation, centered on the wavelength of interest, is obtained. Many instruments allow the entrance and exit slit widths to be varied, which affects the bandwidth of radiation selected. While a narrower exit slit results in a narrower bandwidth, the radiant power will be decreased. Usually, absorbance peaks in the ultraviolet-visible region are fairly broad, so a relatively wide bandwidth will give good results, and has the added advantage of giving a better signal to noise ratio.

3. Sample Holder

The sample holders used for spectrophotometric measurements are cuvettes. These are often sold in matched pairs that can be used in a dual beam spectrophotometer. Commonly used cuvettes have a pathlength of 1 cm, and may be constructed to hold either 4.5 or 1.5 mL volumes. Specialized cells are also available, with pathlengths varying from 1 mm to 10 cm, and volumes ranging from a few microliters to greater than 30 milliliters. Since the cuvette becomes part of the path that the radiation follows, it is critical that it does not contribute significantly to the absorption at the wavelengths of interest. The materials used for cuvettes vary depending on the wavelength of radiation that must be detected. For visible spectroscopy in the range from 340 nm to 800 nm, glass, quartz, polystyrene, or methacrylate cuvettes may be used. The polystyrene and methacrylate materials will give 75–90% transmission at the lower end of this range. For the ultraviolet region, only quartz or fused silica cuvettes must be used, as the other materials are opaque to ultraviolet radiation. It is important that the cuvette surface is free from scratches, material deposits, or other imperfections that can cause scattering or absorption of the incident radiation. Glass, quartz, or silica cuvettes can be cleaned periodically using 5M nitric acid or special detergents, designed to remove biological deposits, followed by rinsing with copious amounts of distilled-deionized, or reverse osmosis water. Ultrasonic baths for cleaning are not recommended by some manufacturers, as the cuvette may crack if it resonates with the bath frequency. Only lens paper or a soft cloth should be used to wipe the outer surface of the cuvette, as other papers with wood fibers can scratch the surface. The advantage of the plastic cuvettes is that they are inexpensive enough for single use, and therefore, maintenance is not an issue. An alternative to using cuvettes is to carry out analyses in microplates containing 96 wells. The absorbance of each well is obtained using a spectrophotometric reader designed specifically for such plates. Using this format, multiple assays can be performed quickly, with significant savings of precious samples or reagents since the volume of each well is only a few hundred microliters. However, the pathlength, and therefore the absorbance of the sample, varies with the total volume used for the assay and must be determined.

4. Detector

The radiation that is transmitted through a sample is quantified by conversion to an electrical signal by a detector. A

common type of detector is a photomultiplier tube. The photomultiplier tube is made up of a photoemissive cathode, coated with an easily ionized material, and several dynodes in a vacuum. A voltage of 400 to 2500 V exists between the cathode and the anode. When a photon strikes the cathode, electrons are ejected and strike the first dynode and release multiple secondary electrons. These accelerate toward the next dynode, which again multiplies the electrons released in a cascade, with an eventual gain of 10^4 to 10^7. These gains are very useful in detecting low levels of radiation. The amplified electrical signal can be displayed on an analogue meter, through the position of a needle on a meter, or converted to a digital signal and manipulated by a computer and displayed on a numerical read-out.

5. Dispersive Instruments: Single-Beam and Double-Beam Optics

In a single-beam spectrophotometer, the radiation follows a path through a single cuvette to a detector. This type of instrument is calibrated for 0% T by completely blocking all radiation from the detector, and 100% T using a reference sample that, ideally, contains all the components except the analyte. Because the calibration for 0 and 100% T is carried out independent of the sample readings, random fluctuations in lamp intensity, electrical power, and drift over time may introduce error into the sample readings. Repeated calibrations can be used to compensate for instrumental drift over time.

A double-beam spectrophotometer has a design advantage over a single beam instrument in that the radiation emitted by the source is split into two beams of equal intensity (Figure 43.5). This can be accomplished by a rotating sector mirror (chopper), which rapidly focuses the radiation beam sequentially through the sample cell, and then the reference cell, as it rotates. A single detector can receive alternating signals from the sample and the reference cells or there may be separate detectors for the sample and reference. With either approach, any minor fluctuations or drift in the radiation will affect the reference and sample beam identically, and can be ignored. A disadvantage of the double beam configuration is that the power of the incident radiation on the sample is decreased by splitting the beam, and therefore, the signal to noise ratio may be lower than with single-beam optics.

6. Diode Array Instruments

Diode array spectrophotometers have a very different design from dispersive instruments (Figure 43.6). In this type of instrument, the radiation covering all the wavelengths of interest is collimated, passed through the sample, and then separated into its component wavelengths using a fixed grating. The radiation is then projected onto hundreds of diodes that are present on a silicon chip,

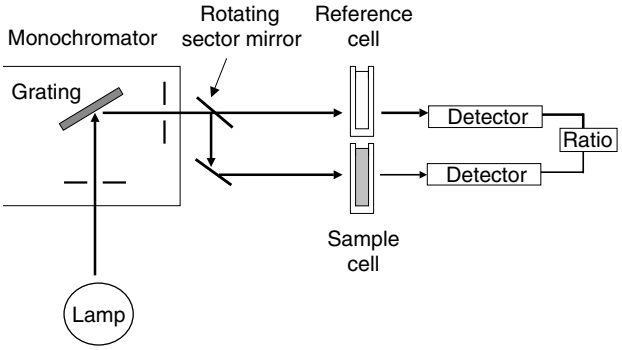

FIGURE 43.5 A schematic representation of a double-beam spectrophotometer.

termed the diode array. Each diode acts as a capacitor, and creates an electrical discharge proportional to the incident radiation. A photodiode is generally made up of a semiconductor and a capacitor to charge the semiconductor. As radiation hits the semiconductor, electrons flow through it, thereby lowering the charge on the capacitor. The intensity of radiation of the sample is proportional to the amount of charge needed to recharge the capacitor at predetermined intervals. This type of instrument allows an absorption spectrum to be obtained in less than a second, since each diode collects radiation at its unique wavelength at the same time; therefore, one of the major advantages of this type of instrument is the rapid analysis.

D. Quantitation

Quantitation is the most common use for spectrophotometry, and is typically carried out using a standard curve. A solution with a known concentration of the analyte is used to make up a series of dilutions. Then a plot of absorbance versus concentration at the appropriate wavelength is made. The slope of this plot can be used to determine the extinction coefficient of the compound. Alternatively, by knowing the extinction coefficient of the compound in the solvent, it is possible to calculate the concentration, using Beer's law, relating the concentration of an analyte in solution to the amount of absorbance of radiation.

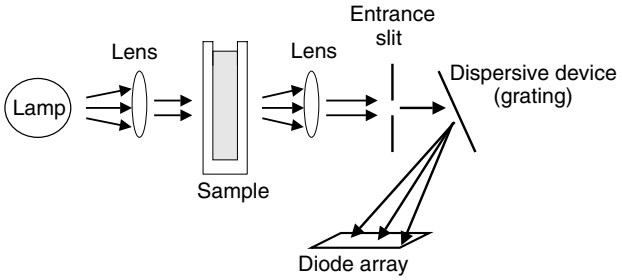

FIGURE 43.6 A schematic representation of a diode array spectrophotometer.

Care should be given to the selection of an appropriate reference sample. As noted above, the ideal reference sample contains all the sample components except the analyte. However, in practice, distilled water or even air is sometimes used to establish 100% transmission, which can cause erroneous absorbance measurements. If the refractive index of the reference and sample are not close, different amounts of reflective radiation loss will occur at the cuvette window. This failure to match the refractive index of the sample and reference can result in a shift of the baseline, with apparently greater than 100% transmission in the sample.

While many spectrophotometers are reported to give linear responses to 3 absorbance units, linearity of absorbance for any compound must be confirmed experimentally. At high concentrations of the analyte, one of the assumptions of Beer's law, the independence of the absorbing species, may not hold. At high concentrations, the analyte may interact with itself or other molecules, which can alter the absorption spectrum. As well, the design of the spectrophotometer dictates that the most accurate data will be obtained from absorbance values in the range from about 0.2–0.8 absorbance units. When sample concentration is very high, most of the radiation incident to the sample is absorbed, leaving only a small percentage traveling to the detector. Therefore, even a small error in determining the transmitted radiation power yields a large relative error, when compared to how much radiation is being detected. When the sample concentration is very low, on the other hand, most of the radiation falls on the detector. Again, a small error in radiation power at the detector translates into a large relative error in the amount of radiation absorbed by the sample.

Detection limits in spectrophotometry depend on the absorptivity of the compound being measured. A compound with a molar extinction coefficient of 10^4 to 10^5 $M^{-1}cm^{-1}$ can be detected in the micromolar concentration range. The wavelength of maximum absorbance (or minimum transmission) is the best to use for the analysis. This wavelength will yield the greatest sensitivity, with the greatest changes in absorbance as concentration changes. The accuracy and precision of a spectrophotometric assay depends on instrumental limitations, control over physical and chemical variables such as temperature, pH, and reagent purity, and operator skill.

In some analyses, the spectrum of the analyte of interest cannot be completely separated from interfering materials in the sample, or the baseline is shifted. In these cases, it may be beneficial to use the first, second, or higher order derivative for qualitative and quantitative analysis of absorption spectra (16). The derivatives can be easily calculated from the absorption spectra with the computers and programs that are standard with many modern instruments. The first derivative of a single peak spectrum is a plot of the gradient $dA/d\lambda$ versus wavelength, with a minimum that occurs at the λ_{max} and a maximum. The concentration of the analyte is proportional to the distance between these. The second derivative is $d^2A/d\lambda^2$ versus wavelength, and has two maxima with a minimum between them at the λ_{max}. The derivative spectra feature sharp bands, compared to the broader bands of the original spectra, which can enhance detection of minor spectral features. If Beer's law is valid for the original spectrum, then the derivative can be used for quantitation as

$$d^n A / d\lambda^n = d^n \varepsilon / d\lambda^n dc \qquad (43.7)$$

Another quantitative application of spectrophotometry is to determine reaction rates, rather than absolute absorbances. The rate of change in absorbance is determined just after mixing all the sample components and well before equilibrium is established. The concentration of the analyte is then proportional to the slope of absorbance as a function of time. Such procedures may be used for determining enzymatic activity. It should be noted that reaction rates can be very dependent on chemical and physical aspects of the reaction, such as pH and temperature, and these factors must be carefully controlled to obtain meaningful results.

Ultraviolet-visible absorption spectra can be used to qualitatively identify the presence of molecular species, by comparing the spectrum of an unknown with a library of possible compounds, or simply observing a characteristic absorption peak. Absorption is proportional to concentration, but spectra obtained at different concentrations can differ due to variations in absorptivity at different wavelengths, making comparisons difficult.

E. Sample Preparation

Clear solutions are required for spectrophotometry. If the analyte is in a suspension with some turbidity, rather than a clear solution, then a significant amount of radiation will be scattered, rather than absorbed, violating one of the assumptions of the Beer-Lambert law. A wide variety of solvents with various additives can be used to enhance solubility of the analyte of interest. The only limitation is that the solvent and additives must not themselves exhibit a significant amount of absorption in the region where the analyte will be monitored. All common solvents are suitable for use in the visible range of the spectrum, while fewer are transparent at the lower end of the ultraviolet range (Table 43.4). For optimal results, the absorbance of the cuvette and solvent should be no more than 0.05 absorbance units.

Turbid suspensions are deliberately used for some specific applications, not involving absorption. Microbial cell growth curves can be determined by monitoring the decrease in transmission of radiation through a sample caused by scattering of radiation, and with a standard

TABLE 43.4
Ultraviolet Absorption Cut-Off of Some Solvents

Solvent	Ultraviolet Cut-Off (nm)
Acetonitrile	190
Cyclohexane	210
Ethane	210
Hexane	210
Methanol	210
Water	210
Glycerol	220
Ethyl ether	220
Chloroform	245
Ethyl acetate	260
Carbon tetrachloride	265
Benzene	280
Acetone	330

curve, microbial cell density in a solution can be estimated. Alternatively, an increase in transmission, as a result of a decrease in the scattering of radiation, of a bacterial suspension can be used as a qualitative indication of cell lysis.

III. TRISTIMULUS COLORIMETRY

A. Introduction

Tristimulus colorimetry is the measurement of the perceived color of objects, based on the reflection of visible light from an object. Three components influence colorimetry readings: the radiant energy spectrum of the light source impinging on the object, the characteristics of the object itself, and the sensitivity of the detector, the human eye (17). A change to any of these three interacting components usually results in a change of perceived color. Color is not a strictly physical characteristic of an object, but is a psychophysical characteristic, since it depends on human perception of light reflected from an object. There are many factors that influence color perception by an individual including the light source, the viewing angle, the background, the size of the object, surface texture, particle size, number of receptors in the eye, and even aging or yellowing of the lens in the eye. These factors, and the ability of the human eye to distinguish subtle color differences, make reproducible color description and color difference estimation by people very difficult. Instruments have been developed that standardize the radiation source, the viewing conditions, and the detector, so that reproducible color parameters can be obtained. This allows color to be unambiguously defined for an object, and color differences between objects to be measured. Colorimetry can be used to establish color standards or color tolerances that are used for quality assessment, to determine the effects of physical and chemical conditions on the color of a sample, and to establish color parameters that can be used as rapid and non-destructive indices of other components in the sample, for example, the amount of a pigment in a sample or the loss of a reactive component. Applications of tristimulus colorimetry to foods are given in Table 43.5.

B. Principles

Colorimetry has the ultimate objective of being able to provide color coordinates that will enable different people to describe and visualize a color unambiguously. This is most easily achieved with a uniform color space, so that the distance between two colors in three-dimensional space correlates with their visual color difference. Numerous color appearance models, or color space models have been developed, and one well-recognized system is the CIE[1] L*, a*, b* system, which provides a reasonably uniform color space (Figure 43.7). The L* value represents lightness and ranges from 0 (black) to 100 (white), the a* value represents red to green (positive to negative values), and the b* value represents blue to yellow (positive to negative values). These values can be manipulated to yield the hue and chroma of the object. Hue is the name given to a color, such as red, blue, or yellow, while chroma is the saturation, or intensity of the color, relative to a white object viewed under similar illumination. The absolute value of a* and b* values indicates the chroma of the object, and can be defined as

$$\text{Chroma} = (a^{*2} + b^{*2})^{0.5} \tag{43.8}$$

while the hue is the angle defined by a point in color space joined to the origin and the positive a* axis. Hue can be calculated in positive degrees as

$$\text{Hue} = \tan^{-1}(b^*/a^*) \tag{43.9}$$

TABLE 43.5
Selected Applications of Tristimulus Colorimetry

Application	Reference
Optimization of color stability of cured hams, determined from tristimulus color parameters	18
Tristimulus colorimetry to assess the color of honey	19
Tristimulus colorimetry to assess color changes in an oil in water emulsion due to Ostwald ripening	20
Tristimulus colorimetry to assess whitening ability of dairy products and coffee whiteners	21
Tristimulus colorimetry to assess relationship between color and flavor of roasted peanuts	22

[1] CIE, the International Commission on Illumination abbreviated from its French title Commission Internationale de l'Eclairage, is an international organization for research and exchange of information on matters relating to the science and art of lighting.

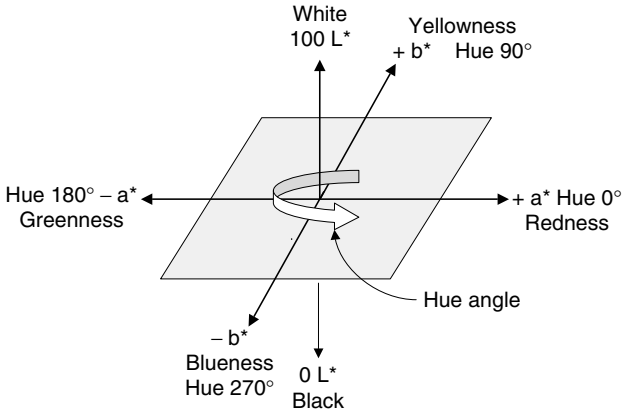

FIGURE 43.7 The CIE L*a*b* color appearance model. Hue is the positive angle calculated from a point in color space joined to the origin with the positive a* axis. Chroma (not shown) has a minimum at the intersection of the three axes and increases with distance from the origin.

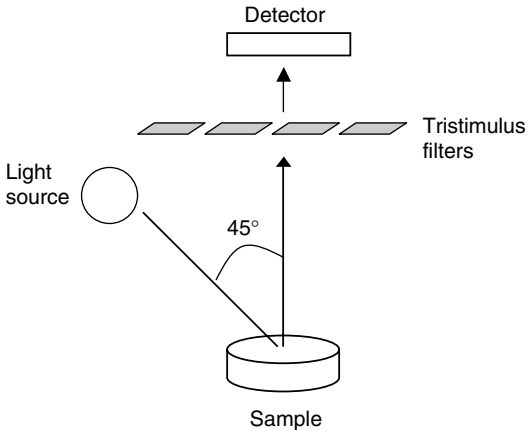

FIGURE 43.8 A tristimulus colorimeter with 45° illumination and 0° viewing geometry.

C. Equipment

Tristimulus colorimeters use a light source with a defined energy spectrum, standardized illumination and viewing conditions, and sensors that simulate the way the human eye perceives color. While different instruments use different color appearance models, values from each model can be interconverted using appropriate equations. Tristimulus refers to the concept that most colors can be matched by addition of different amounts of the three primary color lights—red, green, and blue. The components of a tristimulus colorimeter include a light source, a sample holder, a set of filters, and a detector (Figure 43.8).

1. Light Source

The light source has a significant effect on the perceived color of an object, since the reflected light is dependent on the intensity of the incident light. It is a common experience to observe that the color of an object differs when viewed under different lighting conditions. There are two light sources that are commonly used in tristimulus colorimeters, Illuminant A and D65. Each is defined by the CIE. Illuminant A represents incandescent illumination. D65 mimics daylight, and is characterized by a correlated color temperature of 6500 K. The correlated color temperature of an illuminant is derived from the concept of a black body radiator, a theoretical light source, in which the quantity and wavelengths of energy emitted increase with the absolute temperature. Therefore, as correlated color temperature increases, the emitted radiation becomes more intense, with more blue (shorter) wavelengths. Although D65 is a commonly used illuminant, there are other D illuminates used in colorimeters with different correlated color temperatures.

The angle that the light illuminates the object, coupled with the angle that the reflected light is viewed will also influence the perceived color. Therefore, the CIE has defined two sets of geometries for illumination and viewing that are commonly used in tristimulus colorimeters. In the first set, the sample is illuminated from all angles, using a sphere light source, and viewed at 0° to the surface. Alternatively, the sample can be illuminated at 0°, and the reflected light collected from all angles. Both of these configurations will yield the same tristimulus values. The second set of geometries includes illumination at 45° and viewing at 0° (shown in Figure 43.8), or alternatively, illumination at 0° and viewing at 45°. Again, both configurations will yield the same result. This design eliminates all components of gloss from the color parameters.

2. Filters and Detector

The filters used in a tristimulus colorimeter mimic the color sensitivity of an average person, based on the spectral responsiveness of the three types of cones in the human eye. The response curves are known as the standard observer curves. The concept can be understood as the amounts of red, green, and blue primary lights that are needed to match the colors of an equal energy spectrum by a person with average color vision. However, for calculation purposes, the red, blue, and green primary colors are mathematically converted to X, Y, and Z primaries, as some yellow hues cannot be matched by adding red, green, and blue light. Tristimulus colorimeters may use four filters, rather than three, for the same reason. Phototubes that monitor the light transmitted through each filter are used for detection.

An alternative system to the use of filters is a scanning spectrocolorimeter that uses a monochromator, coupled with diodes for detection. The reflectance data for every 5 or 10 nm are multiplied by the color-matching function of

the standard observer curves, and then integrated across the spectrum to yield values for the color appearance model used by the instrument.

The specification of absolute color of an object requires careful calibration of the light source and detectors of a tristimulus colorimeter, and it is difficult to obtain identical readings between two different instruments. However, very reproducible results can be obtained from determinations of color differences between two objects, and this is a more common and reliable application of a colorimeter. It is important to remember that some color differences, measured instrumentally, may not be distinguished visually. Therefore, instrumental results must always be related to human perception.

D. Sample Preparation

The ideal material for determining surface color is uniform in color, flat, smooth and does not exhibit gloss; however, few food materials have all of these characteristics. Therefore, the characteristics of each sample must be considered to obtain useful color information. Color measurements may be obtained on liquid, solid, or powder samples, and may require very little preparation prior to analysis. The main objective is to evaluate a sample as it would be normally viewed. Color parameters will vary with depth, surface characteristics, and particle size of the sample. Smaller particles will cause more scattering of light, and be perceived as brighter but less colorful than the same material with larger particles. Since color may vary within the sample, on the top or bottom, or depending on orientation, it may be appropriate to take several instrumental readings, turning or moving the sample between readings. The average color parameter can then be calculated. For translucent or transparent samples, a consistent sample depth must be used since color will change with the sample thickness. As well, some light may be transmitted through translucent or transparent samples, and therefore it is necessary to use a consistent background such as a white plate, and to avoid stray light that might be transmitted through the sides of the container holding the sample.

IV. ATOMIC ABSORPTION SPECTROSCOPY

A. Introduction

Atomic absorption spectroscopy (AAS) is a technique used to detect or quantitate the presence of elements, usually metals, at the sub part per million concentration. It can be used to detect elements in almost any solid, liquid, or gaseous sample, although most samples are converted into homogeneous solutions prior to analysis. Usually, only a single element is quantitated in each analysis. AAS is based on the absorption of radiation by ground state atoms, rather than molecules, but obeys the same general principles described for spectrophotometry. Two common types of AAS are flame AAS, and graphite furnace or electrothermal AAS. These methods differ in the way that ground state atoms are produced. Some applications of AAS to determine metals in food samples are given in Table 43.6.

B. Principles

There are two key steps involved in AAS (27,28). The first step is the production of free atoms of the analyte from the sample material, produced by heating the sample using either a flame or a furnace. During heating of a sample, the solvent is evaporated, water of hydration is removed, oxides may be produced, and finally free atoms of the metal are generated. The second step is the excitation of the atoms to a higher electronic energy state, mediated by the absorption of radiation by the valence electrons. The energy associated with the absorbed radiation corresponds to the difference in energy between the ground state and an excited state of the valence electrons, and is characteristic of each atom. There are multiple excited states that an atom can achieve, and therefore the absorbance spectrum of an atom is composed of several absorption lines. However, the resonance transition state, from ground state to the first excited state, is the most intense and therefore used most often as it offers the best sensitivity. Alternative resonances may be used if there is interference at the wavelength of the first excited state. Absorption lines for the different elements occur in the wavelength range from about 160 to 900 nm. The lines of the atomic absorption spectrum are very narrow compared to the absorption spectrum of a molecule obtained in the ultraviolet and visible range because there are no vibrational or rotational transitions for free atoms.

C. Equipment

1. Flame Atomic Absorption Spectroscopy

The basic components of a flame AAS are the radiation source, flame atom cell with a burner, sample introduction system, monochromator, and the detection system (Figure 43.9). A computer is usually used for control of the instrument, and manipulation of the data.

TABLE 43.6
Selected Applications of Atomic Absorption Spectroscopy (AAS)

Application	Reference
Flame AAS to determine Cd, Cu, and Pb in samples	23
Graphite furnace AAS determination of vanadium in foods	24
Graphite furnace AAS determination of Pb in wine	25
Flame AAS simultaneous determination of Cu, Zn, Fe, and Mn, or Na, K, Ca, and Mg	26

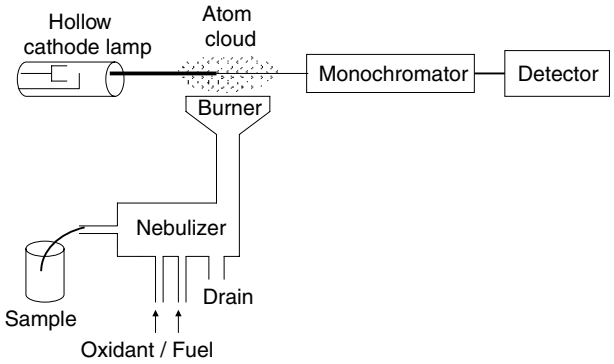

FIGURE 43.9 A schematic representation of a flame atomic absorption spectrometer.

a. *Radiation source*

The most common radiation source is the hollow cathode lamp (HCL), where the cathode is composed of the same metal as the analyte of interest. The HCL contains a glass envelope with a silica window for use in the ultraviolet spectrum. The glass envelope is filled with an inert gas such as neon or argon, at a low pressure between 1 to 5 torr. A voltage applied between the anode (positively charged) and the metal cathode causes the gas to ionize at the anode. The positive ions are accelerated toward the cathode, strike it, and cause some of the metal ions from the cathode to be released in an excited state. The excited ions produce an intense spectrum of radiation, characteristic of the metal when they return to the ground state. These metal atoms then diffuse back to the cathode or deposit on the glass envelope. Therefore, the HCL emits very narrow spectral lines of radiation, some of which are identical to the absorption lines of the analyte (Figure 43.10). The spectral width of atomic absorption lines is about 10^{-5} nm. There is some broadening of these lines to about 0.001–0.005 nm, but even these 'broader' absorption lines are much narrower than the resolution limits of the conventional spectral wave band selector. Therefore, the specific emission lines of the HCL eliminate a large background signal, and inherent loss of sensitivity, that would result from using a broad spectrum radiation source with a monochromator.

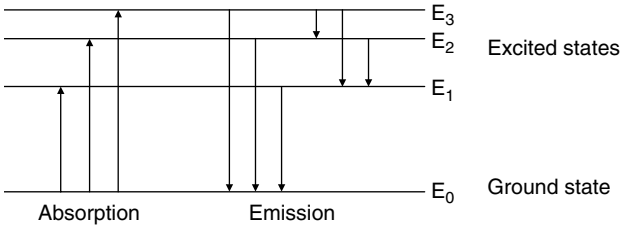

FIGURE 43.10 A comparison of the absorption and emission transitions corresponding to spectral lines for a hypothetical metal with three electronic excitation states. The emission transitions include those for absorption and additional transitions.

Typically, single element lamps are used but there are some multi-element lamps that can generate the absorption spectra of several metals simultaneously. However, usually one element is more volatile than the others, and tends to cover the surface of the other metals, reducing the intensity of radiation from these elements. The advantage of these multi-element lamps is that a single sample may be used to quantitate several elements, which is more time- and cost-efficient. An example of a multi-element lamp is the calcium and magnesium HCL, which has been used extensively for analysis of dairy products, plant extracts, and other samples.

b. *Flame atom cell*

The flame atom cell is where the sample is converted into free atoms by heating in a flame held in the burner. The flame therefore acts as the sample holder. A typical flame uses air as the oxidant and acetylene as the fuel. The ratio of oxidant and fuel can be varied to produce an oxidizing or reducing flame, which is optimized for each element. This flame is hot enough (~2500 K) to dissociate molecules of the sample into atoms for approximately 50 of the elements. However, there are approximately 10 to 20 elements that require a hotter flame, obtained using nitrous oxide-acetylene flame (~3200 K). These include elements that form very stable oxides such as aluminum, barium, and boron, and others such as lanthanum, the rare earth metals, molybdenum, silicon, and vanadium (29). A relatively long flame, several centimeters in length, is used to maximize the pathlength of the sample, and the amount of radiation absorbed by the atoms.

c. *Sample introduction system*

The sample is usually introduced into the flame as a fine mist with an ideal droplet size of 2 to 6 μm. The system should introduce the sample reproducibly, with no interferences, and no cross contamination between samples. Pneumatic nebulizers are the most common method of sample introduction. A jet of compressed air aspirates and nebulizes the solution. In a concentric nebulizer, the oxidant gas surrounds the sample as it emerges from a capillary tube, causing reduced pressure at the tip, and a suction of the sample solution from the container. The high velocity of the sample as it exits from the capillary tube creates a pressure reduction that causes the drop to break into a fine mist. Larger aerosol droplets are removed through a drain tube as the sample passes through an expansion chamber, and the remaining sample is mixed with the gases. Typically only 5 to 10% of the sample is nebulized and flows to the burner. Ultrasonic nebulizers, using high frequency sound between 0.1 to 10 MHz, have also been developed. These have a higher efficiency (~30%) than the pneumatic systems and can generate homogeneous aerosols with droplets smaller than 5 μm. They also allow the aerosol generation rate and gas flow rate to be varied independently. Because the sample is fed into the flame in a steady stream, a steady state signal is produced for each sample, depending on the flow rate.

d. Monochromator and detector

The monochromator is used to isolate the wavelength of interest. However, the inherent selectivity of the hollow cathode lamp means that only a low resolution monochromator is needed. A typical monochromator is composed of entrance and exit slits, and a prism, or gratings, similar to that used for a spectrophotometer. There has been some development of more complex optical systems for multi-element sensing. A photomultiplier tube, similar to those used in spectrophotometry, can be used for detection.

2. Graphite Furnace Atomic Absorption Spectroscopy

Furnace AAS differs from flame AAS in the way that atoms are generated and the sample is introduced into the system. Most designs are based on a heated graphite atomizer, commercially developed by the Perkin-Elmer Corporation. This system is a graphite tube, 50 mm long with a 10 mm diameter, heated by electrical resistance. An inert gas such as argon or nitrogen flows through the tube at a constant rate, and the system is enclosed in a water jacket. Discrete microlitre volumes of sample are deposited through an injection port onto a platform in the center of the tube, and are heated in three stages by applying a variable current. In the first stage, the solvent is evaporated, then the sample matrix is ashed, and finally the elements are atomized. The atom cloud then absorbs radiation from the HCL, and a single signal peak is produced from each sample application. Samples can be manually introduced to the graphite tube with micropipettes, but automatic sample introduction is also used. The technique requires from 2 to 200 µl volume, or a few milligrams of solid sample. While the technique is attractive because of the enhanced sensitivity and the small sample size, it is much more difficult to obtain reproducible results than with flame AAS. Furnace AAS is more prone to physical and chemical interferences that require more complex procedures to correct. As well, the instrumentation is more complex than flame AAS and requires more skill to operate and maintain.

D. Quantitation

Quantitation is achieved with a standard curve, where the relationship between signal intensity and the known concentration of analyte is determined. Usually, at least four concentrations of the analyte and a blank are used to establish the standard curve. The concentration of the unknown sample must fall within the upper and lower limits of the standard curve. The accuracy of the results will decrease, however, if atomization of analyte occurs differently in the standard solution compared to the samples. In cases where the matrix of the unknown cannot be matched with the standards, the method of standard addition may be used to compensate for interferences in the analysis. The signal from an unknown is determined, and then increasing amounts of the standard are added to the sample, and the signal is determined for each addition. The concentration of the original sample can then be determined by back extrapolation.

Flame atomic absorption produces a steady-state signal, depending on how rapidly the sample is introduced into the flame, and has a detection limit of 100 ppb for many elements. Graphite furnace AAS can have detection limits 10 to 100 times lower than flame AAS, achieving sub-ppb detection, and produces a transient signal, similar to a chromatographic peak. Therefore, peak area (recommended for more accurate results) or peak height may be used for quantitation.

E. Sample Preparation

Almost any solid, liquid, or gas sample can be analyzed by AAS, with the appropriate pre-treatment to obtain a homogeneous solution. Liquid samples may be used directly, sometimes only with dilution to an appropriate metal concentration range. For flame AAS, solid samples are dissolved, or digested using a wet or dry ashing procedure, prior to analysis. Care must be taken to avoid losses of volatile metals during dry ashing. Solid samples may be analyzed directly by graphite furnace AAS. Metals can be extracted from a gas sample by passing the gas through a scrubber, followed by solvent extraction. Solvents and chemicals used to prepare the samples may be a source of contamination, so ultra-pure reagents should be used, and possible contamination must be accounted for by analyzing a blank. The actual measurement of a prepared sample may take approximately 10 seconds for flame AAS or 2 minutes for furnace AAS.

During AAS, there can be chemical or physical factors inherent in the sample that interfere with or enhance the production of atoms, causing errors in quantitation. A well-documented case of reduction in volatility occurs with calcium in the presence of phosphate. When these two metals are present together, calcium phosphate forms, reducing the atomization of calcium compared to that achieved from calcium chloride. Releasing agents, or ionization suppressors, are added to samples with this problem, to minimize interferences caused by incomplete or slow breakdown of a molecule to atoms or the formation of ions. Lanthanum and strontium are examples of release agents that release calcium from phosphate interference. Formation of complexes that enhance volatility are less common, although fluoride may have this effect.

V. ATOMIC EMISSION SPECTROSCOPY

A. Introduction

Atomic emission spectroscopy (AES) is used for the qualitative and quantitative analysis of metals, similar to atomic absorption spectrophotometry. While there are some common features between the two techniques, there

are also important differences. Both techniques require the formation of atoms, followed by their excitation to a higher energy state. However, AES monitors the specific wavelengths of radiation emitted by excited atoms or ions, as the excited electrons fall back to lower states. Therefore, the *sample itself* creates the radiation signal in AES, and an external lamp is not required. Because a specific radiation source is not required, multiple elements can be detected in a single AES analysis. Examples of the application of AES to detect elements in food samples are given in Table 43.7.

B. Principles

AES uses thermal energy to form free atoms from a sample matrix, and to promote valence electrons of the atoms into an excited state (28). When the electrons fall back to the ground state, the excess energy is emitted as radiation that can be detected and quantified. Similar to AAS, emission lines occur from about 160 to 900 nm, and are typically very narrow. Each ion or atom will emit a distinct set of spectral lines dependent on the electron structure of the material and the permitted energy states of the electrons that are used for identification. Qualitative analysis can be achieved by monitoring the presence of a spectral line with intensity greater than the background. The intensity of the spectral line is proportional to the concentration of the element, allowing for quantitative analysis.

The emission spectral lines are more complicated than the absorption spectral lines. With atomic absorption, the electronic transitions are usually from the ground state to the first excited state (the resonance transitions), while in atomic emission, more intermediate transitions are detected. However, because of the very narrow and characteristic emission lines from the gas-phase atoms, each element can be detected relatively free of interferences from other elements. Most elements exhibit several spectral lines that have reasonably similar sensitivities. Therefore, if one spectral line exhibits interference, an alternative may be chosen. There are several commonly used variations of atomic emission spectroscopy, depending on the energy source used to excite the samples, including flame AES (also termed flame photometry), arc/spark AES, and inductively coupled plasma (ICP) AES.

TABLE 43.7
Selected Applications of Atomic Emission Spectroscopy (AES)

Application	Reference
Trace element (Ca, Cu, Fe, Mg, Mn, Zn, K, Na) food composition data for 32 different fruits by flame AAS and flame atomic emission spectroscopy	30
Selenoamino acids determined in garlic, onion, and broccoli using gas chromatography with atomic emission detection	31
ICP-AES to determine Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Sr, Tl, V, and Zn contents of food samples	32
ICP-AES of nickel, iron and copper in margarine samples	33
Simultaneous ICP-MS determination of 34 trace elements in the wines (Li, Be, Mg, Al, P, Cl, Ca, Ti, V, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Mo, Ag, Cd, Sb, I, Cs, Ba, La, Ce, Tl, Pb, Bi, Th, and U)	34
Double focusing ICP-MS analysis of Sr, Cd, Hg, and Pb, and Na, Al, Ca, Mg, Cr, Mn, Fe, Ni, Cu, Zn, and Se in milk whey	35

C. Equipment

The instrument used for AES requires an energy source to atomize and excite the analyte, a sample nebulizer and introduction system, a monochromator to select the emission wavelengths, a detector to record the radiation intensity, and data manipulation/readout device. Therefore, except for the radiation source (the HCL), the components are similar to those of an atomic absorption spectrometer (Figure 43.9), and some instruments can be used for both techniques. The liquid sample is aspirated into the energy source, using pneumatic or ultrasonic nebulizers, similar to those used for AAS. As previously described, only a small percentage of the sample reaches the energy source while the rest is lost through droplet condensation. In the energy source, the solvent is evaporated, the sample matrix (if present) is ashed, and the analyte is atomized and electronically excited. The emitted photons are passed through a narrow entrance slit, dispersed using a mono- or poly-chromator, and detected with a photomultiplier. The energy sources and the specific characteristics and applications of the different types of AES are described below.

1. Flame Atomic Emission Spectroscopy

Some of the flames used for flame AES include air-hydrogen (~2300 K), oxygen-hydrogen (~3000 K), and oxygen-acetylene (~3400 K). The flame must supply enough energy to atomize the analyte and to excite the atoms to higher energy states. The alkali metals and alkali earth metals (sodium, potassium, lithium, cesium, calcium, strontium, and barium) are easily excited and amenable to analysis by flame AES. The flame provides relatively low amounts of energy, however, and may result in low intensities of radiation for other metals that require more energy to reach higher excited states. Still, there are more than 70 elements, and some metal oxides, that can be detected and quantitated using flame AES. In theory, using a hotter flame should produce a stronger emission, but this is not always the case since atoms may ionize at higher temperatures. Ionization creates new spectral emission lines, reducing the intensity of the atomic lines.

Because of the low energy flame, resonance spectral lines, arising from the transition between the first excited state and the ground state, are used for quantitation. However, at high concentrations, these spectral lines can self-absorb (i.e., atomic absorption occurs), causing a reduction in intensity and making it difficult to obtain reliable results. In addition, corrections to background emissions must be made for organic solvents which will yield spectral lines from products of complete or incomplete oxidation, and from the combustion fuel of the flame itself.

2. Arc/Spark Atomic Emission Spectroscopy

This type of AES utilizes an electrical discharge to excite the sample of interest. These sources can achieve temperatures of 4000 to 6000 K. Arc/spark emission spectroscopy has several advantages over flame AES. It is more universal in its application in that the higher temperature source can excite most elements, and therefore most metals can be analyzed. As well, the greater energy used in excitation causes transition beyond the first excited state, and allows transitions between two upper excited states to be used for analysis. This can improve quantitation since the number of atoms in the upper excited states is relatively low; therefore there is little self-absorption of these spectral lines, and a linear response between concentration and line intensity can often be obtained. Arc/spark AES is 10 to 1000 times more sensitive than flame AES. However, intense spectral lines that can cause interference can be produced from materials in the air near the electrical discharge.

3. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

A plasma is an ionized gas that is neutral in charge. A plasma can be created starting with a stream of gas (typically argon) that flows through an open tube surrounded by a metal coil, such as copper. The coil transfers power from the radio frequency generator, acting as an inducer, and therefore the source is referred to as an inductively coupled plasma (ICP). The coil sets up an oscillating electromagnetic field. A few charged argon ions are introduced by an igniting device, and are caught in the oscillating field. These ions move rapidly with the field, generating more ions and free electrons from collisions with gas atoms. These secondary ions, in turn, create even more ions and free electrons, resulting in the plasma. Another stream of argon is used to carry the aerosol sample from the nebulizer into the plasma. The aerosol must be dried to remove the large amounts of solvent that would cool or extinguish the plasma. This is accomplished by passing the sample through a heated tube, and then a cooled condenser. When the atoms of the sample are introduced into the plasma, they collide with rapidly moving gas ions, become excited, and then relax to a lower energy state, emitting distinct spectral lines. The plasma source is extremely hot—estimates range from 6500 to 10,000 K—and therefore it is capable of breaking virtually all chemical bonds in the sample, resulting in independent ions and atoms. The method has high sensitivity since a large proportion of most elements will be excited, with little self-absorption and little chemical interference. Linearity can be achieved over four to six orders of magnitude. There are two configurations, radial and axial, for collecting radiation from the plasma. In the radial configuration, radiation is collected from the side of the plasma, across a narrow emitting central channel. An axial configuration views the plasma end-on. This configuration increases the pathlength of the sample, and increases the detection limits 5 to 10 times.

4. Detection Systems

An advantage of AES over AAS is that multiple elements can be detected in a single analysis. There have been several different detection systems designed to accommodate this characteristic (36).

a. Sequential spectrometers

Sequential instruments are the least expensive and most flexible types of emission spectrometers. Most include a single photomultiplier tube that receives wavelengths of emitted radiation in sequential order from a monochromator. This configuration is good to use when the metals to be determined, their concentration, and the sample matrices vary. The major disadvantages of the sequential instruments are the relatively long analysis times, since only one element may be determined at a time, and the greater sample volume required.

Two methods are used to obtain reproducible wavelength selection with this type of instrument. One approach is to rely on the analyte to produce the largest spectral line near the expected wavelength. This can result in selection of an erroneous line if an interfering element is present at relatively high concentrations. Alternatively, a known spectral line, such as the carbon line at 193 nm, can be used for reference, and the wavelength of interest is identified as a predefined number of steps from the reference. This method is less likely to lead to selection of the wrong spectral line, but increases analysis time.

b. Simultaneous spectrometers

Simultaneous spectrometers use multiple detectors, with one or more photomultiplier tubes for each analyte of interest, so that the instrument is configured for a set number of analytes, with known interferences. The radiation of different wavelengths emitted by the sample is dispersed through a monochromator onto the detectors. Because detectors and grating are stationary, there is no need for a peak search routine. Matrix-dependent interferences can be overcome by installing multiple detectors for

a single element, and selecting the one that is most sensitive and interference free. The major disadvantage of these instruments is the lack of flexibility, as they are configured for specific spectral lines of analytes, and the installation of additional detectors after the instrument has been configured can be expensive.

c. Solid-state array detector spectrometers

To overcome the limitation of both the sequential and simultaneous instruments, charge-injection device (CID) or charge-coupled device (CCD) detectors have been introduced to give very high sensitivity and resolution. These detectors can monitor 250,000 or 5,000 spectral lines simultaneously, respectively. These instruments with a CID or CCD can be obtained for the same price as an instrument with a simultaneous configuration and 25 photomultiplier detectors, and therefore are likely to be the configuration of choice.

d. ICP-AES mass spectrometry

Mass spectrometry can be used in conjunction with ICP to detect the atoms and ions, rather than using the radiation they emit (28). The atoms and ions are produced by the plasma as previously described, extracted from the center channel of the plasma, and separated and detected using mass spectrometry, by their mass to charge ratio. The technique is extremely sensitive, with detection at the part per trillion level, and may require a clean room environment to eliminate background interferences. It is the only atomic absorption/emission technique that can separate and quantitate isotopes, but it is by far the most expensive type of AES.

D. Quantitation

AES can be used quantitatively, using a standard curve or by the method of standard addition, as described for AAS; however, careful calibration is required to obtain accurate results. Many different experimental variables affect the intensity of radiation emitted, including spectral line overlap from elements in the samples that cannot always be avoided. In these cases, correction factors are calculated, using pure solutions of each component.

Matrix effects are common for flame and arc/spark AES. If the matrix is more volatile than the analytes, it will cause the signal from the elements to be released over a longer time and with a lower intensity. Ideally, the matrix of the sample and the standards should be identical. If there are substantial differences in the matrix between the samples and standards, background correction must be used.

It is difficult to compare the different atomic absorption or atomic emission techniques for sensitivity and detection limits, since factors such as the equipment configuration, slit width, the sample matrix, and the spectral line used influence the signal obtained. For a particular analysis, a literature search can be carried out to determine the best method. However, some general comparisons of the techniques can be made. Flame AES can detect elements at sub-ppm levels, while arc/spark-AES and ICP-AES have detection limits that are 10 to 1000 times lower, at sub-ppb to several hundred ppb. As mentioned above, ICP-AES mass spectroscopy has a detection limit that is several orders of magnitude lower than this, often at ppt to the sub-ppt level. In relationship to atomic absorption techniques, ICP-AES is between that of flame and furnace atomic absorption spectrophotometry, with flame AAS being the least sensitive method of detection.

E. Sample Preparation

Sample preparation for AES is similar to that required for AAS. Only liquid samples can be applied to AES, and therefore solid samples must be solubilized in an appropriate solvent, and may require wet or dry ashing.

VI. POLARIMETRY

A. Introduction

Anisotropic materials, those that have a lack of symmetry in their molecular or crystalline structure, and nonracemic samples, those containing unequal concentrations of enantiomers of a chiral molecule, have the ability to rotate a plane of polarized radiation. Such substances are said to have optical activity. The measurement of the rotation of plane polarized radiation by an optically active material is called polarimetry. Polarimetry is a nondestructive technique that can be used for studying the structure of anisotropic materials, for checking the concentration and purity of chiral mixtures in solutions, and as an aid in identifying unknown compounds. Chemical or enzymatic reactions may be monitored by the change in optical rotation over time. The technique is used extensively for the analysis of carbohydrates in the sugar refining industry. Some selected applications of polarimetry in food science are given in Table 43.8.

B. Principles

Linearly or plane polarized radiation is made up of equal intensities of left- and right-circularly polarized radiation (40). Optical rotation occurs because the electrical component of left- and right-circularly polarized radiation interact with the asymmetric centers of chiral molecules, differently. This differential interaction causes the two circularly polarized components to travel at different velocities. Therefore, they are not in phase when they exit the sample, and exhibit an overall rotation. Optical rotation occurs for both visible and ultraviolet radiation. The rotation is said to be dextrorotatory (d) if the radiation is rotated clockwise when viewed by an observer looking toward the radiation

TABLE 43.8
Selected Applications of Polarimetry

Application	Reference
Polarimetry in sugar analysis	37
Polarimetry as a quantitative tool for the determination of collagen content of isinglass finings	38
Laser-based polarimetry for the detection of gentamicin analogues separated by reverse phase ion pair chromatography in whole milk	39

source, and the enantiomer is given the (+) designation. Similarly, the enantiomer that rotates radiation to the left, or counterclockwise, is called the levorotatory (l) or the (−) enantiomer. It should be noted that the direction that polarized light will be rotated by a chiral compound cannot be predicted by its stereochemistry (designated *R* or *S*), and must be determined experimentally.

C. Equipment

The simplest polarimeter consists of a monochromatic radiation source, a polarizer, a sample cell, a second polarizer, which is called the analyzer, and a radiation detector (Figure 43.11). The analyzer is oriented 90° to the polarizer so that no radiation reaches the detector. When an optically active substance is present in the beam, it rotates the polarization of the radiation reaching the analyzer so that there is a component that reaches the detector. The angle that the analyzer must be rotated to regain the minimum detector signal is the optical rotation.

D. Quantitation

The amount of optical rotation depends on the number of optically active species, through which the radiation passes, and is therefore dependent on both the sample pathlength and the analyte concentration. Specific rotation, $[\alpha]$, accounts for these factors, and is defined as:

$$[\alpha]_{\lambda} = \alpha / (l \cdot d) \qquad (43.10)$$

where α is the measured optical rotation in degrees, l is the sample pathlength in decimeters, and d is the density if the sample is a pure liquid, or the concentration if the sample is a solution. In either case, the units of d are g/cm^3. As well, optical rotation depends on the wavelength of radiation, λ, and the temperature, t, so both are specified. A sodium lamp that has a spectral emission D-line at 589 nm is often used as a monochromatic source, and the specific rotation is designated as $[\alpha]_D$ to denote this. It can be noted that the specific rotation, $[\alpha]_{\lambda}$, and the measured optical rotation, α, of Equation 43.10 are analogous to the absorptivity, *a*, and the absorbance, A, respectively, of the Beer-Lambert law (Equation 43.5). Therefore, similar to spectrophotometry, the concentration of an analyte can be determined knowing the specific rotation of the compound, and determining the optical rotation at the specified wavelength and temperature. Alternatively, a standard curve relating optical rotation to concentration of a standard compound may be used for quantitation.

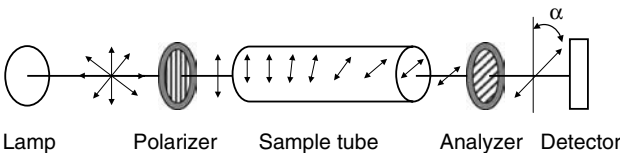

FIGURE 43.11 A schematic representation of a polarimeter.

The optical rotatory dispersion (ORD) is the optical rotation as a function of wavelength. It is recorded using a spectropolarimeter, which has a broad-spectrum radiation source and a scanning monochromator. This technique has largely been replaced by circular dichroism spectroscopy. Additional information on ORD may be found in (40).

E. Sample Preparation

Samples must be liquids or dissolved in an appropriate solvent for analysis by a polarimeter. The solvent used must be specified since it will affect the specific rotation of the analyte.

VII. CIRCULAR DICHROISM SPECTROSCOPY

A. Introduction

Circular dichroism (CD) can be observed when optically active matter both absorbs and rotates left- and right-handed circular polarized radiation slightly differently (40). If the left- and right-circularly polarized radiation has the same amplitude and wavelength, the resultant radiation is observed as plane polarized radiation. However, when the plane polarized radiation passes through a sample that absorbs the two circularly polarized components to different extents, the radiation rotates along an ellipsoid path (Figure 43.12). The difference in the left- and right-handed absorbance, *A*(l)- *A*(r), is very small and will cause an ellipticity of the radiation, ψ, of a few 1/100ths to 1/10ths of a degree. This small ellipticity can be measured accurately, and recorded as a function of wavelength to yield a CD spectrum. CD spectroscopy can be a more informative method than ultraviolet-visible spectrophotometry since it reflects not only the absorption of radiation, but also the chiral centers of the analyte through features termed Cotton bands. CD spectroscopy can be used to establish the stereochemistry of a chiral molecule or confirm chiral purity, and is very useful for obtaining information about the secondary structure of chiral macromolecules such as proteins, peptides, polysaccharides, and nucleic acids. For example,

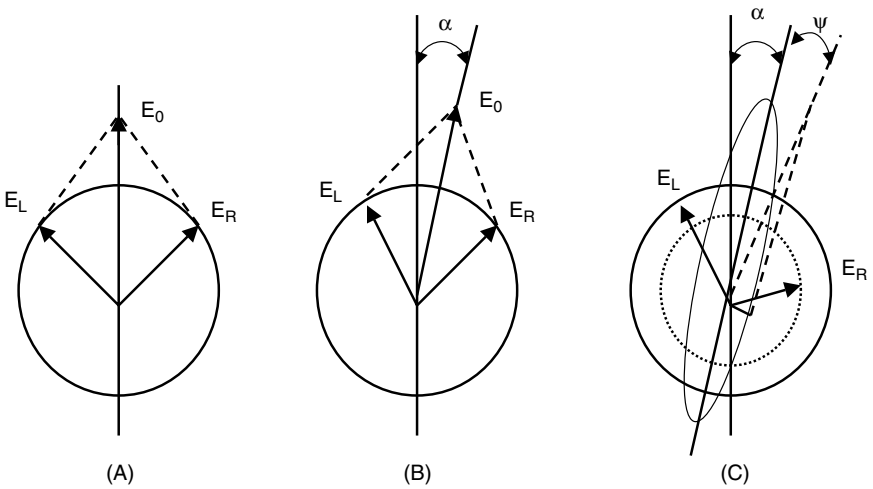

FIGURE 43.12 A. A sample that is not optically active does not preferentially interact or absorb left (E_L) or right (E_R) circularly polarized radiation. The radiation leaving the material is linearly polarized, with no rotation and ellipticity (E_O). B. The preferential interaction of one type of circularly polarized radiation passing through an optically active sample will cause a rotation in the detected radiation of α. C. The preferential absorption of one type of circularly polarized radiation will cause the transmitted radiation to follow an ellipse, with an ellipticity of ψ.

each of the three basic secondary structures of a polypeptide chain (helix, sheet, and coil) shows a characteristic CD spectrum from about 180 to 260 nm (41). Therefore, the spectrum of a protein will be the sum of the helix, sheet, and coil components, and can be deconvoluted to yield the proportion of the protein that is in each secondary structure. Examples of the application of CD spectroscopy in food analysis are given in Table 43.9.

B. Principles

The CD spectrum is recorded in radians of ellipticity, ψ, as a function of wavelength, and can be expressed as

$$\psi = [2.303c \cdot d(\varepsilon_L - \varepsilon_R)] \quad (43.11)$$

where c is the concentration in mol/l, d is the optical pathlength in cm, ε_L and ε_R are the molar absorptivity coefficients of the sample for the left and right hand circularly polarized radiation at each wavelength. It is more convenient to convert ellipticity from radians to degrees, as

$$\theta = \psi(360/2\pi) = [32.09c \cdot d(\varepsilon_L - \varepsilon_R)] \quad (43.12)$$

Molar ellipticity, [θ], can then be calculated to obtain an intrinsic quantity, as

$$[\theta] = (\theta \cdot M)/(l \cdot c' \cdot 100) \quad (43.13)$$

where M is the molecular weight, l is the pathlength in decimeters, and c′ is the analyte concentration in g/ml. For work with proteins, ellipticity is normalized to a mean residue ellipticity, $[\theta]_p$ with units of degree cm^2/decimol residue, to allow comparison between samples, calculated as

$$[\theta]_p = (\theta \cdot M)/(10 \cdot c \cdot d \cdot n_r) \quad (43.14)$$

where n_r is the number of residues in the protein.

TABLE 43.9
Selected Applications of Circular Dichroism (CD) Spectroscopy

Application	Reference
Conformation of wheat glutenin subunits under different chemical conditions	42
CD analysis of the effect of heating on beta-lactoglobulin A, B, and C	43
CD characterization of anthocyanin polymerization in wine liquid chromatographic fractions	44
CD analysis of ketoses, and application of the method to enzymatic hydrolysis and isomerization reactions, and chemical hydrolysis	45

C. Equipment

The components of a CD spectropolarimeter include a radiation source, a monochromator, a polarizer and modulator, a sample holder, and a detector (Figure 43.13). The radiation from the monochromator is first linearly polarized and then passed through a dynamic quarter wave plate that modulates it into left- and right-circularly polarized forms that pass through the sample. One type of quarter wave plate is an isotropic (uniform) crystal that is made to

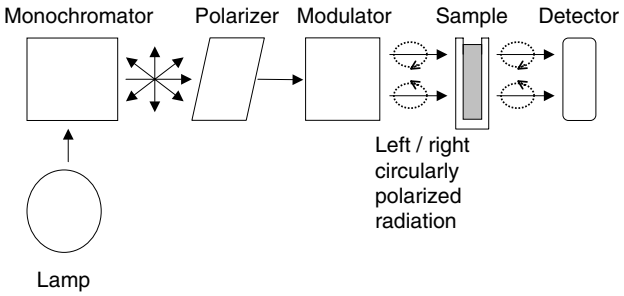

FIGURE 43.13 A schematic representation of a circular dichroism spectrometer.

be anisotropic through the application of an alternating current at high voltage. The signal intensity obtained in CD spectroscopy is small, and therefore the incident power of the radiation source must be high, and a 500 W xenon lamp is often used. As well, oxygen must be removed from the instrument by purging with pure nitrogen to eliminate the production of ozone that would interfere with absorbance at the lower wavelengths, since CD spectra are recorded from ~170 nm.

D. Sample Preparation

The requirements for a compound to be amenable to CD spectroscopy are that it must have ultraviolet or visible absorption bands and be optically active. It should be remembered that a molecule that is chiral may not be optically active if it is present as a racemic mixture. As well, samples that are not optically active may be derivatized with a chiral material prior to analysis. The detection limits for CD spectroscopy can be around the ppm range. Unfolded protein and particulate matter act as scattering particles, and add significant noise to the CD spectrum. Therefore, passing sample solutions through a 0.45 to 0.2 μm filter may improve the signal to noise ratio.

The CD spectrum of a protein can typically be obtained using 20 to 50 μl of a 0.1 to 0.5 mg/ml protein in a low ionic strength buffer (5 to 10 mM), in a cuvette with a 0.1 to 1.0 mm pathlength. Protein concentration has a significant effect on the spectrum, and therefore accurate determination of protein concentration is required. Any compound that absorbs in the region of interest (190 to 250 nm), such as chloride ions, should be avoided, and only additives in the minimum concentration essential to maintaining the solubility of the protein should be present in the sample.

VIII. NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

A. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful, non-destructive analytical tools. It uses low energy radio frequency radiation to induce transitions between different nuclear spin states of a sample in a magnetic field. A single type of nuclei (e.g., ^{1}H, ^{13}C, or ^{31}P) is observed in one analysis. The most common nuclei detected are protons, which have a high natural abundance, and high inherent sensitivity, and carbon-13, which has a low natural abundance (~1.1% of naturally occurring carbon) and a low sensitivity. However, other nuclei of potential interest in food systems, such as ^{14}N, ^{23}Na, and ^{43}Ca, can also be detected. The utility of NMR spectroscopy arises because the same nuclei in different chemical environments experience slightly different magnetic fields. This causes spin state transitions for the same nuclei in different functional groups to occur at slightly different resonance frequencies. NMR spectroscopy can be used for determining the detailed structural features of molecules, quantitative measurement of sample components, and for two- and three-dimensional imaging of solid materials (46). Some selected applications of NMR spectroscopy in food science are given in Table 43.10.

TABLE 43.10
Selected Applications of Nuclear Magnetic Resonance (NMR) Spectroscopy

Application	Reference
High resolution ^{1}H-NMR and MRI to monitor water-soluble and salt-soluble extracts from fish muscle during frozen storage	47
High resolution ^{1}H-NMR to determine the solution structure of *Escherichia coli* heat-stable enterotoxin b	48
High resolution ^{1}H- and ^{13}C-NMR spectroscopy analysis and characterization of carrageenan samples	49
Low resolution ^{1}H-NMR to determine crystallization kinetics of trehalose and trehalose-salt solutions	50
High resolution ^{2}H-NMR and ^{17}O-NMR to study water mobility in multicomponent model media	51
Solid-state ^{13}C cross-polarization, magic-angle spinning NMR to study the effect of post mortem changes on the quality of pork	52
Cross-polarization, magic angle spinning NMR to study glucose rotational mobility under different conditions	53
Site-specific natural isotope fractionation-NMR (SNIF-NMR) method of detection of maple syrup adulteration by beet or cane sugar addition	54
MRI to monitor lipid migration in chocolate confectionery	55
MRI to quantify extent of mixing in a non-reacting 2-component system	56
MRI to monitor the effects of compression on the structure of tomato	57
MRI used to validation thermal processing in food manufacture	58
^{23}Na-MRI to monitor the migration of sodium ions into pork during brining	59

B. Principles

Nuclei with an odd number of protons, neutrons, or both will have an intrinsic nuclear angular momentum or nuclear spin (60). This causes the nucleus to have magnetic characteristics including a magnetic moment and a magnetic dipole. When such a nucleus is placed in a static magnetic field, B_o, the nuclear spin can align in the same direction as the external field, in a low energy state, or in the opposite direction as the external magnetic field, in a less favorable high energy state (Figure 43.14). The difference in energy (ΔE) between the two states increases with the strength of the magnetic field. When the nuclei are exposed to radio frequency radiation that has the same energy as the difference between the two states, they will absorb it and the population of the high energy state will increase slightly, by only a few nuclei for every 10^5 nuclei. This small excess of nuclei in the higher energy state accounts for the relatively low sensitivity of NMR compared to some other spectroscopic techniques. The frequency of radiation that causes the transition is referred to as the chemical shift, δ, and is expressed in parts per million (ppm), calculated relative to a reference signal as

$$\delta = [\nu_{\text{signal}} - \nu_{\text{reference}} / \nu_{\text{reference}}] \times 10^6 \quad (43.15)$$

Using chemical shift allows spectra obtained from instruments with different magnetic strengths to be compared directly. After absorbing energy, nuclei re-emit the radio frequency radiation and return to the lower energy state in a process termed relaxation. There are two types of relaxation, spin-lattice (T_1) that involves energy exchange with the environment, and spin-spin relaxation (T_2) that involves energy exchange between similar molecules. The relaxation times are a characteristic of the chemical and physical environment of the nuclei.

There are several parameters of the NMR spectrum that can yield information about the sample. Different chemical structures can be inferred from specific chemical shifts. Characteristic splitting patterns of the NMR signals known as spin-spin coupling that occur from interactions between nuclei close to each other and the size of the splitting, the J coupling constant, are also indicative of specific structures. The area under the NMR signal peaks is proportional to the number of nuclei causing the signal. As well, the relaxation times, T_1 and T_2, have been used to determine the mobility and physical state of sample components.

Low resolution NMR spectroscopy utilizes magnets with a field strength up to ~2.0 tesla (85 MHz)[2]. An advantage of this technique is the relatively low cost of the equipment, since the magnet strength is low and the magnetic field does not need to be extremely uniform. Low resolution NMR spectroscopy can be used to determine the proportion of the sample that is moisture or lipid, the physical state of a component, and to follow chemical reactions such as crystallization, gel formation, or protein denaturation. It is not possible to obtain detailed structural information using low resolution NMR spectroscopy, as spin-spin coupling and the coupling constants may not be well resolved.

High resolution techniques use magnets of 2.3 to 21 tesla (100 to 900 MHz), with high magnetic field homogeneity. The more powerful the magnet, the greater the resolution between resonance signals. High resolution techniques are used to yield detailed structural information about molecules, and while not routine, it is possible to carry out structural analysis of macromolecules that are greater than 100 kDa (61). High resolution NMR spectroscopy has an advantage over other structure-determining techniques such as x-ray crystallography as the molecule can be analyzed in a more natural state, without the need for crystallization. High resolution NMR spectroscopy has also been used to detect sample adulteration by a technique known as site specific natural isotope fractionation (SNIF) that determines isotope ratios in specific chemical structures.

An extension of NMR spectroscopy is magnetic resonance imaging (MRI) (62). MRI yields two- and three-dimensional images of the internal structure of objects, using proton resonances. Linear radio frequency gradients are applied to the sample, in addition to the static magnetic field, B_o. This makes the resonance frequency of a nucleus dependent on its location in the gradient, and a position-dependent spectrum is obtained. By applying linear radio frequency gradients in several directions, an image based on proton density within a sample is obtained. MRI can be used to understand heat and mass transfer in a sample, water mobility, food stability, and processes such as ripening, drying, crystallization, and gelation.

C. Equipment

The basic components of a NMR spectrometer are a magnet and shim coils, the probe, the radio frequency receiver, and a computer (Figure 43.15). The magnet can be the most expensive component, and produces the principal magnetic field. Magnets greater than 4.7 tesla (200 MHz) are surrounded by liquid helium and liquid nitrogen to maintain the super-conducting conditions. There may be a number of shim coils to modify the magnetic field around the sample so that it is very homogenous, a requirement to obtain high resolution. The NMR probe is positioned in the shim coils, and generates the radio frequency radiation that causes the nuclear spin transitions. Radio frequency coils also act as the receiver to detect the relaxation signals from the sample. The sample is positioned within the radio frequency coil of the probe and is spun to minimize heterogeneity in the sample and the magnetic field. A computer is an integral part of NMR spectrometers, and controls the settings of the components, and data storage and manipulation.

[2] Although magnet strength is measured in tesla, it is common to refer to instruments in terms of the resonance frequency for a proton at the specified magnet strength, expressed in MHz.

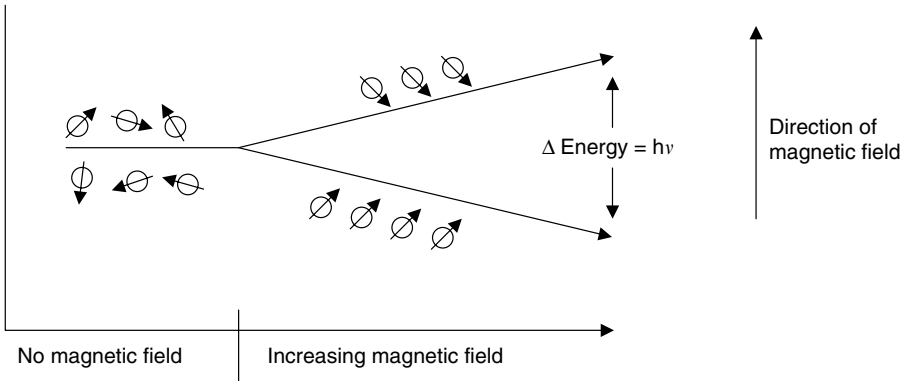

FIGURE 43.14 A schematic illustration of nuclei with a magnetic moment in the absence or presence of an applied magnetic field. The energy difference between the magnetic states of the nuclei (ΔE) is related to the strength of the magnetic field, and is a function of the radiation frequency (ν) and Planck's constant (h).

Fourier transform spectrometers are the most common type of instrument. They emit a short pulse of radiation over a broad range of frequencies, so that the spin state of all the nuclei of interest (i.e., all the protons in a sample) is altered. All of the resulting relaxation signals are also received at the same time, and can be separated mathematically yielding the NMR spectrum. An alternate approach is the continuous wave instrument that emits the range of radio frequencies sequentially, and also received the signal sequentially. Therefore, data collection with a continuous wave instrument takes a significantly longer time.

D. Sample Preparation

Solutions and solid samples may be analyzed by high resolution NMR spectroscopy. Pure samples are dissolved in an appropriate solvent (usually one in which all the hydrogens have been replaced with deuterium) to yield a homogeneous solution and filtered to remove particulate matter that will affect resolution. With the application of more powerful magnets, the resolution between the resonances increases and the amount of sample required for analyses decreases. A few micrograms to milligrams of sample may be required, depending on the instrument, and the molecular weight of the analyte. The ideal solid for high resolution NMR spectroscopy is a uniform powder compacted into a sample holder, that is subjected to 'magic-angle spinning'—a 54.44° angle between the sample spinning axis and the external magnetic field that is used to improve signal resolution.

For low resolution NMR applications, samples can be analyzed without extraction or solubilization because radio frequencies can penetrate solid materials. The sample must only be manipulated to fit in a sample tube. Therefore, valuable information relating to the intrinsic structure and composition of a sample can be determined based on the relaxation parameters, T_1 and T_2. Large samples with no modification can be monitored by MRI, the only limitation being the size of the probe cavity.

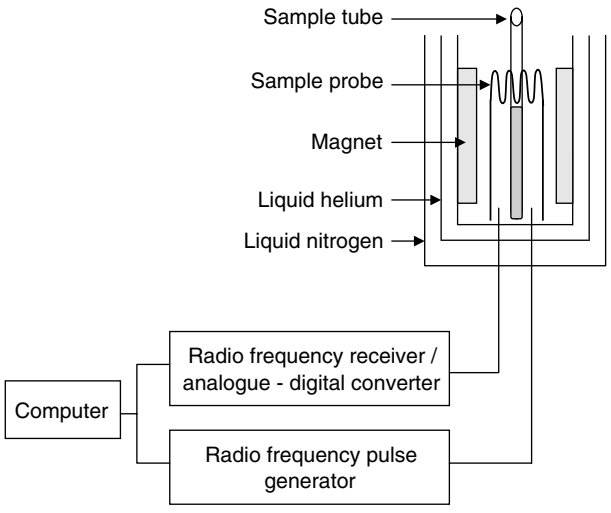

FIGURE 43.15 A schematic representation of a nuclear magnetic resonance spectrometer.

IX. CONCLUSION

This chapter has briefly highlighted the essential features of some commonly used spectroscopic techniques and provided examples of how each technique can be applied to food-related analyses. These techniques are fundamental analytical tools that have allowed us to better understand the individual components of food and foods as complex systems. They have played a key role in the maintenance of food quality and safety. Improvements in instrumentation and advances in the application of these techniques will continue to provide food scientists with more sensitive and informative methods of analysis.

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44 Infrared and Raman Spectroscopy in Food Science

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CONTENTS

I. INTRODUCTION

Infrared (IR) and Raman spectroscopy provide detailed information about both the composition of foods and the molecular structure and the functionality of the components in food systems. Both techniques are based on molecular interactions with electromagnetic radiation that result in transitions between the vibrational energy levels of the ground electronic energy state of the molecule, corresponding to the excitation of various stretching and bending vibrations. However, the nature of these interactions is fundamentally different in these two types of vibrational spectroscopy, and hence they are regarded as complementary techniques. In general terms, as well as specifically in regard to applications to food systems, IR spectroscopy has found much more extensive use than Raman spectroscopy owing to the greater simplicity and lower cost of the instrumentation as well as the greater utility of IR spectroscopy as a quantitative analysis tool. On the other hand, Raman spectroscopy offers certain advantages in relation to sample handling, the study of aqueous systems, and possibilities for on-line process monitoring. Thus, the selection of IR or Raman spectroscopy, or the combined use of both techniques, depends on the nature of the application. Near-infrared (NIR) spectroscopy combines the sample-handling advantages of Raman spectroscopy with the powerful multicomponent analysis capabilities of IR spectroscopy and thus has found widespread application for quality control and process monitoring in the food industry for several decades. However, by comparison with (mid-) IR and Raman spectra, NIR spectra are fairly uninformative, and NIR analysis is largely based on statistical treatment of the spectral data. Entire books have been devoted to NIR

analysis of foods (1, 2) and NIR spectroscopy will not be considered in this chapter.

In the following sections, the principles of IR and Raman spectroscopy and the instrumentation and sample-handling techniques employed to acquire spectra will be briefly described. The utility of these two techniques for investigations of the individual components of food systems will then be illustrated by considering the information that can be extracted from the IR and Raman spectra of food proteins. Finally, a survey of the applications of IR and Raman spectroscopy in the analysis of foods will be presented.

II. FUNDAMENTAL PRINCIPLES OF IR AND RAMAN SPECTROSCOPY

IR and Raman spectroscopy are both based on the excitation of molecular vibrations by interaction with electromagnetic radiation. A nonlinear molecule made up of N atoms possesses $3N - 6$ vibrational modes, each of which has a specific frequency; the number of vibrational modes is reduced by one in the case of linear molecules. The factors determining the frequencies of molecular vibrations can be illustrated by considering the simplest case, a diatomic model AB, which has a single vibrational mode involving the stretching of the bond linking atoms A and B. The frequency at which this bond vibrates can be calculated from the model of a harmonic oscillator, whereby the restoring force (F) on the bond is given by Hooke's law:

$$F = -kx \tag{44.1}$$

where k is the force constant of the bond and x is the displacement from the equilibrium internuclear distance. Under this approximation, the vibrational frequency, ν, is given by

$$\nu = (1/2\pi)(k/\mu)^{1/2} \tag{44.2}$$

where μ is the reduced mass of the system, as defined by the following equation:

$$\mu = m_A \cdot m_B / (m_A + m_B) \tag{44.3}$$

where m_A and m_B are the individual atomic masses of A and B. By convention, vibrational frequencies are given in wavenumbers ($\overline{\nu} = \nu/c$, where c is the speed of light), expressed in units of reciprocal centimeters (cm^{-1}), rather than in units of frequency (s^{-1}), although in older literature, band positions are often reported in units of wavelength (λ), i.e., microns (μ). The frequencies of molecular vibrations fall primarily in the mid-IR region of the electromagnetic spectrum (4000–400 cm^{-1}; 2.5–25 μm).

The quantum-mechanical treatment of molecular vibrations leads to modifications of the harmonic oscillator model as the vibrational energy levels are quantized:

$$E = (\nu + 1/2)h\nu \tag{44.4}$$

where ν is the vibrational quantum number, h is Planck's constant, and ν is the fundamental vibrational frequency. The quantum-mechanical theory predicts that at room temperature, only transitions from the ground-state vibrational level ($\nu = 0$) to the first excited vibrational level ($\nu = 1$) will occur; however, experimentally this is not the case as weak overtone bands, corresponding to transitions to higher energy levels, can be observed, especially in the NIR spectral region.

IR spectroscopy is based on the measurement of molecular absorption of IR radiation of frequencies that match those of the molecular vibrations. However, all of a molecule's vibrational modes do not give rise to IR absorption bands because a vibration will only be IR-active if it results in a change in the dipole moment of the molecule. For example, the stretching vibration of a homonuclear diatomic molecule, such as N_2 or O_2, is not IR-active because the equal displacement of the two atoms from the center of mass does not change the dipole moment of the molecule. However, this is no longer the case when the two atoms in the diatomic molecule have different masses, and hence the stretching vibrations of molecules such as CO are IR-active. Similarly, the symmetric stretching vibration of the CO_2 molecule illustrated in Figure 44.1 is IR-inactive because of the molecule's linear geometry while the corresponding vibration of a nonlinear triatomic molecule such as H_2O is IR-active, as are the asymmetric stretching vibrations of both linear and nonlinear triatomics.

Raman spectroscopy is based on the inelastic scattering of light by the molecules in a sample rather than their absorption of IR radiation. When a sample is irradiated with light, most of the photons are elastically scattered; that is, the wavelength of the scattered photons is unchanged. However, a small fraction (~1 in 10^6 photons) will show a shift in wavelength from that of the incident light. This phenomenon was theoretically predicted by Adolf G. Smekal in 1923 but was first observed experimentally by Sir Chandrasekhara V. Raman in 1928 and hence is called

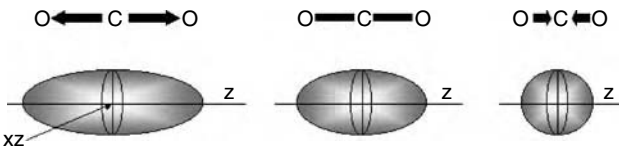

FIGURE 44.1 The symmetric stretch of the CO_2 molecule is a Raman-active but not an IR-active vibration because it results in a change in the molecule's polarizability but does not involve a change in its dipole moment.

the Raman effect. Detection of the inelastically scattered photons requires that the sample be irradiated with monochromatic (i.e., single-wavelength) light. Furthermore, since the Raman effect is very weak, an intense monochromatic light source is required. For these reasons, Raman spectroscopy only became practicable in the 1960s with the development of lasers and highly sensitive photon-counting devices, such as photomultiplier tubes.

In Raman spectroscopy, a sample is irradiated with a laser line of frequency $\overline{\nu}_0$, and the intensity of the scattered light as a function of frequency is then measured, yielding a Raman spectrum (Figure 44.2). Although most of the elastically scattered light is filtered out prior to reaching the detector, the spectrum is still predominated by a broad signal centered at the frequency of the incident light (referred to as the Rayleigh line). The remainder of the spectrum is composed of much weaker bands at lower frequencies, $\overline{\nu}_0 - \overline{\nu}_i$ (referred to as Stokes lines). The frequency shifts $\overline{\nu}_0 - \overline{\nu}_i$ (referred to as Raman shifts) are independent of $\overline{\nu}_0$, the frequency emitted by the excitation source, and correspond to the vibrational frequencies of the molecules in the sample. Even weaker bands occur at higher frequency, $\overline{\nu}_0 + \overline{\nu}_i$ (referred to as anti-Stokes lines). The Stokes and anti-Stokes lines are equally displaced from the Rayleigh line; however, the anti-Stokes lines are rarely observed owing to their very low intensity.

For a molecular vibration to be Raman-active (i.e., show a Raman effect), it must result in changes in the polarizability of the molecule, which is represented by an ellipsoid, and therefore must result in changes in the shape of the electron density cloud around the molecule. For example, as illustrated in Figure 44.1, in the symmetric stretching vibration of CO_2 the shape of the electron cloud is different between the minimum and maximum internuclear distances. Therefore, the polarizability of the molecule changes during the vibration and this vibrational mode is Raman-active. In the asymmetric stretch, the change in the polarizability ellipsoid as one of the bonds extends is cancelled by the opposite change due to the compression of the other bond, and hence there is no overall change in polarizability and the asymmetric stretch is Raman-inactive.

It is important to note that the criteria (referred to as "selection rules") for an IR-active vibration (i.e., a change in the dipole moment of the molecule) and a Raman-active vibration (i.e., a change in the polarizability of the molecule) are different. In fact, for any molecule that possesses a center of symmetry (such as the CO_2 molecule considered above), vibrations that are IR-active are Raman-inactive, and vice versa. In general, vibrations that give rise to weak IR bands often produce intense Raman bands, and vice versa. Hence, IR and Raman spectroscopy are complementary techniques, and it is usually necessary to apply both types of vibrational spectroscopy to obtain the full spectral profile of a sample.

III. INSTRUMENTATION

A. Infrared Spectroscopy

IR spectrometers essentially consist of a broad-band source of IR radiation, a means for resolving the IR radiation into

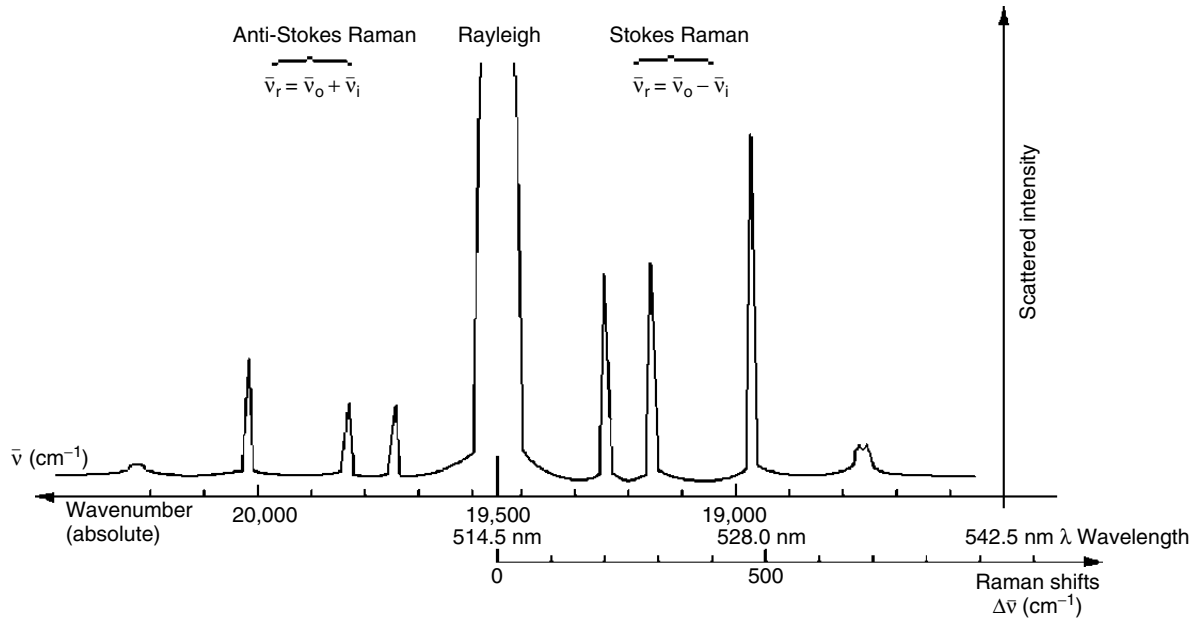

FIGURE 44.2 Schematic representation of a Raman spectrum excited with the 514-nm line of an argon ion laser. Most of the laser light is elastically scattered with the same frequency (ν_0) as the incident light. However, a small proportion is inelastically scattered with frequencies of ($\nu_0 + \nu_i$) and ($\nu_0 - \nu_i$), where ν_i is the frequency of one of the fundamental vibrations of the molecules in the sample.

its component wavelengths, and a detector. Generally speaking, an IR spectrum of a sample can be represented mathematically by the following equation:

$$T(\overline{\nu}) = I(\overline{\nu})/I_0(\overline{\nu}) \quad (44.5)$$

where T is defined as transmittance, I is the intensity of IR radiation reaching the detector when it passes through the sample, I_0 is the intensity of IR radiation reaching the detector with no sample in the beam, and $\overline{\nu}$ is the wavenumber of the radiation. Usually, transmittance is converted to absorbance (A) using the following relationship:

$$A = -\log T \quad (44.6)$$

In the early years of IR spectroscopy, prisms were used to resolve IR radiation from the source into its component wavelengths but they were subsequently replaced by diffraction gratings. In the 1970s, the field of IR spectroscopy was revolutionized when these dispersive IR spectrometers began to be replaced by Fourier transform infrared (FTIR) spectrometers. FTIR spectroscopy is based on interferometry and makes use of a beamsplitter to divide the IR radiation into two beams, with one beam being directed to a fixed mirror and the other to a moving mirror (Figure 44.3). When these two beams are reflected back to the beamsplitter and recombine, they undergo constructive and destructive interference due to the path difference between the two mirrors, yielding an interferogram. The path difference, known as retardation δ, is proportional to time t because the moving mirror travels at a constant velocity, v, i.e., $\delta = 2vt$. Through the use of a fast Fourier transform (FFT) algorithm, the time domain interferogram, $I(\delta)$, is converted into the frequency domain, $I(\overline{\nu})$, according to the following relationship:

$$I(\delta) = 0.5H(\overline{\nu})I(\overline{\nu})\cos 2\pi\overline{\nu}\delta \quad (44.7)$$

where $H(\overline{\nu})$ is a single wavenumber-dependent correction factor that accounts for instrumental characteristics. The interferogram decoded into the frequency domain is in the form of an energy curve called a single-beam spectrum (Figure 44.4). The ratio of the single-beam spectrum of the sample against a background single-beam spectrum recorded with no sample in the optical path results in a transmittance spectrum, in accordance with Equation [44.5], which is then normally converted into an absorbance spectrum by carrying out the mathematical transformation represented by Equation [44.6].

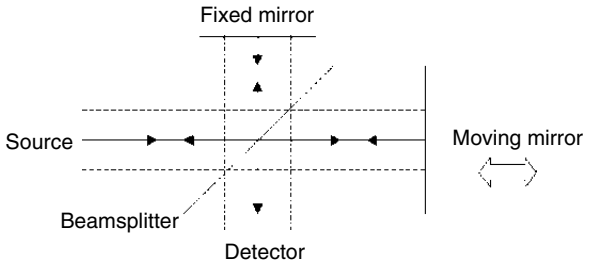

FIGURE 44.3 Schematic diagram of a Michelson interferometer. The IR radiation from the source is split into two beams by a beamsplitter and directed to the fixed and moving mirrors. The two IR beams recombine at the beamsplitter and are directed to the sample and the detector.

FTIR spectrometers have several advantages over conventional dispersive IR instruments, including a dramatic improvement in the signal-to-noise ratio (S/N) obtained by multiplexing (simultaneous detection of all frequencies), reduction in scan time, and higher energy throughput. Another important advantage is the excellent wavelength reproducibility of FTIR spectrometers, owing to the use of an internal reference laser, which allows spectral data manipulations such as spectral subtraction, addition, and ratioing to be performed with a very high degree of accuracy. The advancement of FTIR spectroscopy has been greatly assisted by the availability of increasingly powerful personal computers, which have facilitated the use of sophisticated software packages for both qualitative and quantitative applications.

B. Raman Spectroscopy

The design of traditional dispersive Raman spectrometers differs markedly from that of IR instruments. However, FT-Raman spectrometers, which have become increasingly common in recent years, are similar in design to FTIR spectrometers, and combined FTIR/FT-Raman systems are available from most major FTIR vendors. In dispersive Raman spectroscopy, the source is a high-power laser emitting visible radiation at several discrete frequencies, with filters being employed to select a single laser line; commonly employed laser lines are the 488.0-nm (blue) and 514.5-nm (green) lines of an argon-ion laser. After this monochromatic radiation impinges on the sample, a portion of the scattered radiation is directed to a monochromator to resolve it into its component frequencies and then to a photomultiplier tube. The resulting signal as a function of frequency is plotted as intensity versus Raman shift, yielding a Raman spectrum.

The major drawbacks of traditional Raman spectrometers originate with the use of high-power visible lasers as excitation sources. Samples that are susceptible to either thermal or photochemical degradation may be destroyed by the laser radiation, although various experimental approaches can be taken to minimize such damage. However, a more pervasive problem is the fluorescence that results when species present in the sample are electronically excited by the visible laser radiation. Because the Raman effect is weaker than fluorescence by many orders of magnitude, even the fluorescence from a trace

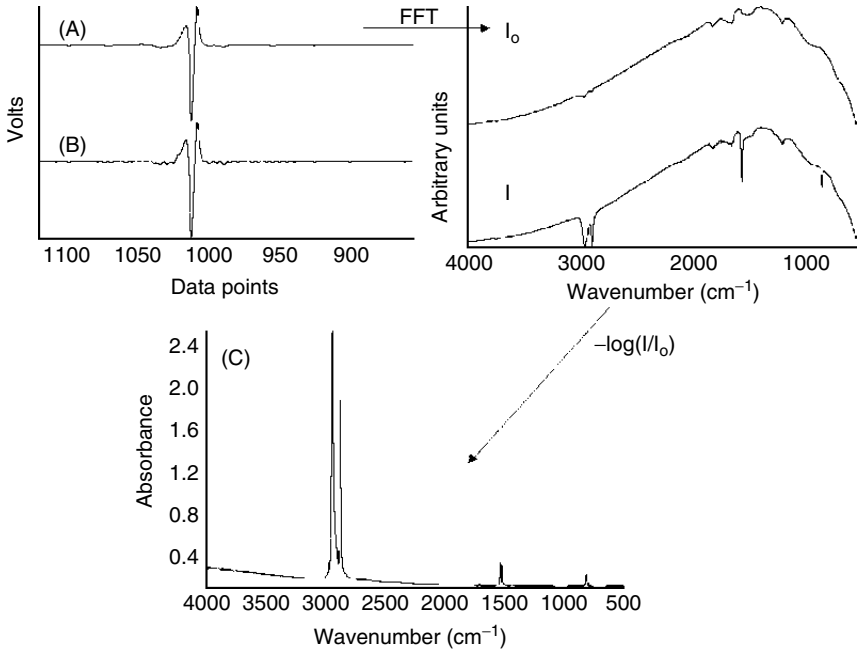

FIGURE 44.4 (A) Interferogram recorded with no sample in the path of the IR beam; (B) interferogram recorded with a thin film of polyethylene (PE) placed in the path of the IR beam. Fourier transformation of the interferogram A and B by a fast Fourier transform (FFT) algorithm yields, respectively, the open-beam spectrum (I_0) and the single-beam spectrum (I) of PE. (C) Absorbance spectrum of PE obtained by the mathematical transformation shown on the figure.

impurity can completely mask the Raman spectrum of the sample. In the past, this phenomenon severely restricted the scope of Raman spectroscopy, particularly in relation to samples of biological origin. However, it has been substantially alleviated by the use of laser excitation lines in the NIR instead of the visible region of the electromagnetic spectrum, such as the 1064-nm line emitted by neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers. Because the use of NIR lasers as excitation sources in conventional dispersive Raman spectrometers is not technically feasible, this option only became practicable with the development of FT-Raman spectrometers (3), which, like FTIR spectrometers, are based on interferometry. In fact, FTIR spectrometers can be modified to acquire Raman spectra by incorporating a Nd:YAG laser and using beamsplitters and detectors appropriate for the NIR region. The availability of these dual-purpose instruments has greatly facilitated comprehensive vibrational spectroscopic studies, although their high cost remains a disadvantage.

IV. SAMPLING METHODS

A. Infrared Spectroscopy

Infrared sample-handling accessories include transmission cells, attenuated total reflectance (ATR) accessories (both multiple- and single-bounce crystals and fiber-optic probes), as well as diffuse reflectance infrared Fourier transform (DRIFT) and photoacoustic spectroscopy (PAS). The latter two techniques, reviewed previously (4), will not be considered here, as they have had limited application in mid-IR food analysis, although DRIFT is widely used in NIR food analysis.

1. Transmission Mode

The transmission mode is the most common and oldest mode of IR sample analysis. The sample is placed in the optical path of the IR beam, and the amount of light transmitted through the sample is inversely proportional to the sample thickness, which can vary from meters for gas samples to microns for condensed-phase samples. The spectra of liquids and solutions are recorded in a transmission cell, composed of two optical windows separated by a spacer that determines the pathlength. The optical windows commonly employed are polished salt crystals (e.g., NaCl or KBr), because all materials containing covalent bonds, including glass, do not transmit IR radiation. There are also severe restrictions on the pathlength that can be employed because of the limited energy available from IR sources. For instance, aqueous solutions are typically measured in cells having pathlengths of 15–40 μm, owing to the intense absorption of IR radiation by water; beyond this thickness, virtually no IR radiation reaches the detector.

Transmission cells can be demountable or sealed, and the use of flow-through cells such as that shown in Figure 44.5 facilitates sample handling. Sealed transmission cells

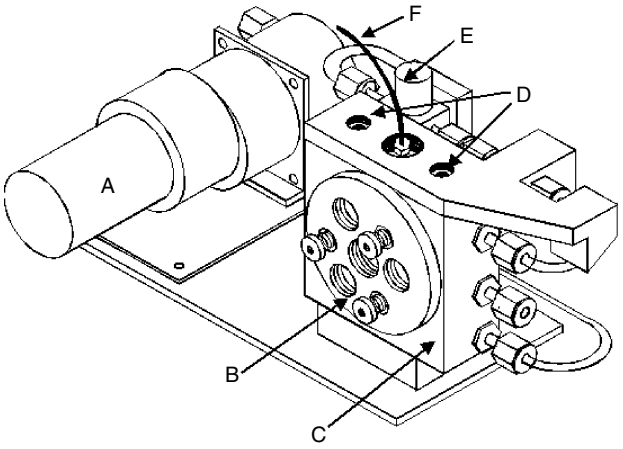

FIGURE 44.5 Schematic diagram of a transmission flow-through cell: (A) micropump, (B) demountable cell insert (transmission cell), (C) cell block, (D) heat cartridges, (E) solenoid valve, (F) thermocouple. The accessory slides horizontally so that the cell can be moved out of the path of the beam to record an open-beam background spectrum.

are preferred for quantitative analysis because a constant pathlength is required to obtain reproducible results. However, if a demountable cell is used because the sample is too viscous to inject or pump through a sealed cell, or the cell cannot be effectively cleaned between samples by simply rinsing with a solvent or detergent solution, an internal standard with a distinct absorption band can be introduced into the sample to compensate for pathlength variations.

In the case of solid samples, light scattering effects are a problem when particle dimensions are comparable to or exceed the wavelengths of mid-IR radiation and, as such, particles must be ground to <3 μm prior to analysis. The sample can then be diluted with KBr, KCl, or NaCl and pressed to form a pellet. An alternative procedure for analyzing solid samples is to make a liquid dispersion using oils, such as mineral oil (Nujol) or paraffin oil. Both the pellet and the dispersion method have the disadvantage of destroying the sample.

2. Attenuated Total Reflectance

The restrictions on sample thickness and particle size imposed by the nature of transmission measurements represent major sample-handling limitations. Attenuated total reflectance (ATR) spectroscopy, developed in the 1960s, provides a more versatile sample-handling approach. ATR spectroscopy is based on total internal reflection of the IR beam in an ATR crystal, which is made of a high-refractive-index material and serves as an internal reflection element (IRE). Total internal reflection gives rise to an evanescent wave that emerges from the surface of the ATR crystal at each point of internal reflection and decays as it propagates away from the surface (Figure 44.6). The

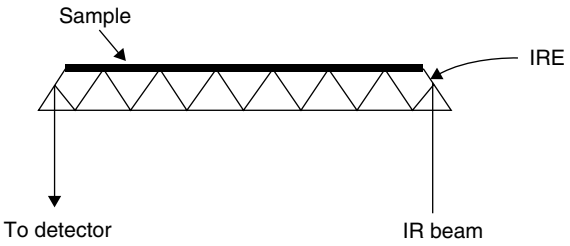

FIGURE 44.6 Schematic of a horizontal multiple-bounce attenuated total reflectance (MB-ATR) accessory.

evanescent wave is attenuated by the absorption of the IR radiation by species on or near the surface of the ATR crystal, and measurement of this attenuation as a function of wavenumber yields the IR spectrum of a sample placed on the surface of the ATR crystal.

The effective pathlength of an ATR accessory is calculated by multiplying the number of internal reflections by the depth of penetration (d_p), which is defined as the distance at which the intensity of the evanescent wave decays to $1/e$ of its value at the surface as given by the following equation:

$$d_p = \lambda/\{2\pi n_1[\sin^2\theta - (n_2/n_1)^2]^{1/2}\} \qquad (44.8)$$

where λ is the wavelength of IR radiation, n_1 is the refractive index of the IRE material, n_2 is the refractive index of the sample, and θ is the angle at which the IR radiation strikes the ATR crystal. Owing to the variation of the depth of penetration as a function of wavelength, the relative intensities of the bands in an ATR spectrum will differ from those in a conventional transmission spectrum, but this effect can be corrected for mathematically.

For a typical ATR accessory, the effective pathlength ranges from a few microns at the high-wavenumber end of the IR spectrum to several tens of microns at the low-wavenumber end. Thus, the ATR technique provides an inherently short effective pathlength without any physical restriction on the sample thickness, such that samples can be simply poured or spread on the surface of the ATR crystal and then wiped off after analysis. In addition, particle size in solid samples is not a concern as, unlike transmission spectra, ATR spectra are not affected by light scattering, and even the spectra of opaque samples can be recorded. The ATR technique is particularly advantageous for analyzing aqueous solutions, because the pathlength is sufficiently short and the common IRE materials (e.g., germanium and ZnSe) are water-insoluble, unlike most of the materials suitable for use as transmission cell windows.

The evident advantages of the ATR sample-handling technique are accompanied by certain disadvantages. The inherently short effective pathlength limits sensitivity. In addition, because the depth of penetration is on the micron scale, any surface contamination on the ATR crystal will

make a major contribution to the spectrum. Furthermore, a spectrum representative of the entire sample will be obtained only if the sample is homogeneous on this scale. In the case of powdered and solid samples, intimate contact between the sample and the ATR crystal is required, and although various clamping devices are available to press the sample against the ATR crystal, it can be difficult to obtain reproducible spectra. Another drawback that limits the utility of ATR spectroscopy is the need for precise temperature control, because the depth of penetration is dependent on the refractive indices of the IRE material and the sample, which in turn are dependent on temperature.

Many of the disadvantages described can be alleviated by employing a single-bounce (SB-ATR) accessory because of the much smaller dimensions of the ATR crystal as compared to those in multiple-bounce (MB-ATR) accessories (Figure 44.7). Although such accessories were rarely employed in the past owing to their very short effective pathlengths, which severely limited sensitivity, improvements in the optical design of SB-ATR accessories in recent years have compensated for this limitation. Because of the small surface area of the ATR crystal, precise temperature control and good optical contact between solid samples and the crystal (particularly with diamond IREs, which can withstand a strong clamping force) can be more readily achieved than with MB-ATR accessories. In addition, only very small amounts of sample are required to cover the ATR crystal (e.g., for liquid samples, <50 μL), and thus cross-contamination between samples can be virtually eliminated by wiping the crystal with the next sample prior to applying it onto the crystal to record its spectrum, provided that the samples are miscible with each other. These advantages make this type of accessory very promising for quantitative analysis of foods, although potential applications are restricted by the short effective pathlength and hence limited sensitivity.

B. Raman Spectroscopy

In terms of sample handling, Raman spectroscopy is a much more versatile and flexible method than IR spectroscopy because it does not suffer from restrictions on sample thickness (or pathlength), the need for specialized sample-handling accessories, or problems caused by light scattering. Thus, Raman spectra can be recorded directly from solid samples, even if opaque, with no sample preparation, and samples can be contained in ordinary glass vials. Thin glass capillaries are ideal for recording Raman spectra of solids or liquids because the laser is a highly focused energy source, allowing spectra to be obtained from samples as small as 100 μm in diameter. The main sample-handling difficulty encountered in Raman spectroscopy is excessive heating of the sample if an intense visible laser is employed as an excitation source. To alleviate this problem, the laser beam may be defocused, or various accessories that are available to prevent heating of the sample may be employed; these range from devices that continuously rotate the sample, which can effectively reduce sample heating in the case of fluid samples, to expensive helium-cooled cryostats, which may be required to prevent burning in the case of thermally unstable solids. The need for such measures has been reduced, however, with the use of NIR lasers in FT-Raman spectroscopy.

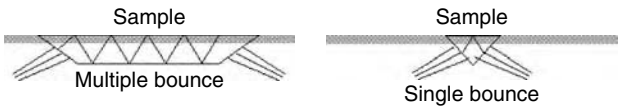

FIGURE 44.7 Illustration of the relative sample dimensions in multiple- and single-bounce horizontal ATR accessories.

V. IR AND RAMAN SPECTROSCOPY OF FOOD COMPONENTS AND FOODS

The vibrational spectra of foods are the superposition of the spectra of all the individual components present and are thus generally very complex. The IR spectra of aqueous systems are dominated by the intense absorptions of water (Figure 44.8A), which can obscure the spectral features of other components in broad regions of the spectrum, as illustrated by the transmission spectrum of milk in Figure 44.8B. Although the spectral contributions of water can be removed digitally by spectral subtraction (Figure 44.8C), information cannot be obtained from regions of intense IR absorption by water except when very short pathlengths are employed; this limitation is often addressed by taking advantage of the inherently short effective pathlengths of ATR accessories (Figure 44.9). This difficulty is absent in Raman spectroscopy because water is a weak Raman scatterer.

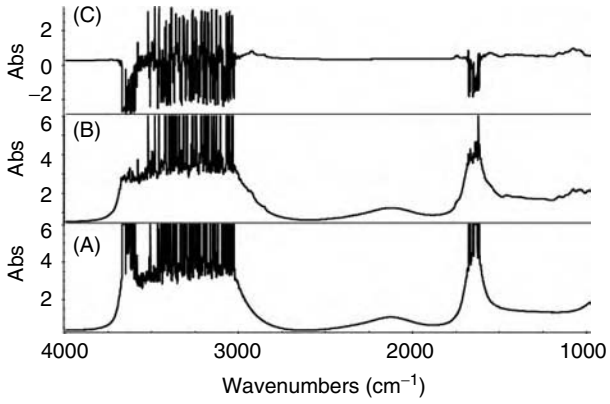

FIGURE 44.8 FTIR spectra of water (A) and milk (B) recorded using a 50-μm transmission cell and difference spectrum (C), obtained by subtraction of spectrum A from spectrum B.

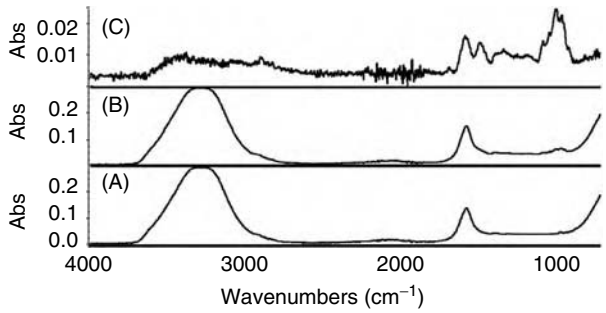

FIGURE 44.9 ATR/FTIR spectra of water (A) and milk (B) recorded using an SB-ATR accessory and difference spectrum (C), obtained by subtraction of spectrum A from spectrum B.

The other major components of foods—fats, proteins, and carbohydrates—give rise to strong bands in both IR and Raman spectra. Although many of the bands due to these individual components overlap with each other, fats, proteins, and carbohydrates each have certain characteristic bands that arise from particular functional groups. The band positions and assignments of some of the major bands of these components are summarized in Table 44.1. However, it should be noted that vibrational frequencies are sensitive to electrical effects, steric effects, the nature, size, and electronegativity of neighboring atoms, phase changes, hydrogen bonding, and solvent polarity. Although this sensitivity may complicate spectral interpretation, it also contributes to the high information content of vibrational spectra. Thus, IR and Raman spectroscopy are valuable techniques for the characterization of materials in terms of their chemical composition, detailed investigations of molecular structure and bonding, and studies of the interactions of molecules with their environment. Furthermore, IR and Raman spectra of substances serve as their "fingerprints," allowing for the identification of substances with a high degree of specificity by comparison of their spectra with those in a spectral database or "library."

The powerful qualitative analysis capabilities of vibrational spectroscopy can be applied to complex samples, such as foods, for purposes of classification and authentication. For such applications, multivariate analysis techniques such as principal component analysis (PCA) are commonly used to reduce the dimensionality of the spectral data (5). Because an FTIR or FT-Raman spectrum is produced by Fourier transformation of an interferogram, as described in Section III.A, the spectra inherently consist of digital data. The dimensionality of the data depends on the spacing between data points, which is a function of the instrument resolution at which the spectra were collected and the level of zero filling employed in the calculation of the Fourier transform. For example, the FTIR spectra of condensed-phase samples are typically collected at 4-cm^{-1} resolution between 4000 and 400 cm^{-1} and thus, with one level of zero filling, each spectrum contains ~1800 data points. Each of these data points is a variable that contains the absorbance at a particular wavenumber, but many of these variables are highly correlated since (a) the inherent width of the bands is usually much greater than the resolution so that a given band contributes to several contiguous data points and (b) any given species present in the sample usually has multiple bands and the intensities of these bands thus change collinearly as a function of change in concentration. By using PCA, most of the variation in the data can be described by a few orthogonal principal components (PCs), or latent variables, which are linear combinations of the original variables. The data are characterized by scores, which are projections of the spectra onto each PC, and loadings, which represent the contributions of the variables to each PC. Cluster analysis based on PCA

TABLE 44.1
Selected Vibrational Bands of Major Food Components[a]

Food Component(s)	Frequency (cm^{-1})	Band Assignment(s)
Water, carbohydrates	3600–3200	O-H stretching vibration; strong water IR absorption band
Fats	3012–3004	C-H stretching of *cis* double bonds
Fats, carbohydrates, and proteins	3000–2800	C-H stretching of CH_2 and CH_3 groups
Fats	1745–1725	C=O stretching, ester linkages in triacylglycerols
Proteins	1700–1600	Amide I band, peptide linkages of proteins
Fats[b]	1678–1665	C=C stretching of *trans* double bonds
Fats	1662–1648	C=C stretching of *cis* double bonds
Water	1650	H-O-H bending vibration; strong IR absorption band
Proteins	1560–1520	Amide II band, peptide linkages of proteins
Proteins	1300–1190	Amide III band, peptide linkages of proteins
Carbohydrates	1250–800	C-O stretching and C-O-H bending vibrations
Fats[b]	971–965	C=C-H bending of isolated *trans* double bonds

[a] Compiled from a review of the literature.
[b] Predominantly hydrogenated fats and oils.

scores may then be performed to group samples according to their degree of spectral similarity, which in turn reflects their degree of compositional similarity. Alternatively, with the application of supervised pattern recognition techniques (e.g., discriminant analysis), the PCA scores may be employed to assign samples to particular classes for purposes of authentication. The capability of distinguishing between different classes of samples on the basis of differences in their vibrational spectra has led to investigations of the potential utility of IR and Raman spectroscopy as a means of detecting adulteration of foods.

Both IR and Raman spectroscopy can also be employed for quantitative analysis. However, although Raman scattering intensity is directly related to concentration, quantitative analysis by Raman spectroscopy has generally been regarded as problematic, and relatively few applications in the area of food analysis have been reported. On the other hand, the scope of reported applications of quantitative IR spectroscopy in food analysis has increased substantially in recent years owing to the advantages of FTIR spectrometers and the new sample handling technologies available combined with the development of a range of sophisticated multivariate analysis methods. As in other types of absorption spectroscopy, IR quantitative analysis is based on the linear relationship between absorbance and concentration, as expressed by the Bouguer-Beer-Lambert law, commonly known as Beer's law:

$$A_\nu = \varepsilon_\nu bc \tag{44.9}$$

where A_ν is the absorbance measured at frequency ν, ε_ν is the absorption coefficient of the absorbing species at the same frequency, b is the pathlength of the cell, and c is the concentration of the absorbing species. Therefore, a calibration curve can be developed by relating changes in absorbance to changes in concentration of a species at a fixed pathlength. It is important to note that IR spectroscopy is a secondary method of analysis, so the development of a calibration requires a set of standards of known composition, prepared gravimetrically or analyzed by a primary reference method. Once a calibration has been developed, it can be used to predict the concentrations of unknowns, provided that the spectra of the unknowns are recorded under the same conditions as the calibration standards and the spectra of the standards are representative of those of the unknowns in the spectral region(s) employed for quantitation. IR spectroscopy can also be employed to directly predict various physical properties and quality attributes of samples, provided that they are dependent on the chemical composition of the substance.

Although simple in theory, implementation of quantitative IR spectroscopy can be complicated by underlying absorptions due to other components as well as inter- and intramolecular interactions. However, these effects can usually be modeled by the application of multivariate analysis methods, such as multiple linear regression (MLR), partial-least-squares regression (PLS), or principal component regression (PCR). A full discussion of the mathematical basis of these multivariate analysis methods is available elsewhere (6) and is beyond the scope of this chapter.

A detailed review of IR and Raman spectroscopic studies of the major components of foods has recently been published (7). Thus, in the following section, the information that can be extracted from the IR and Raman spectra of individual food components will be illustrated by considering only a single class of food components, namely, proteins. In the subsequent section, reported applications of IR and Raman spectroscopy in the qualitative and quantitative analysis of various types of foods will be surveyed. This section will highlight the most significant research findings and practical applications and will focus mainly on IR spectroscopy, owing to its more widespread use in food analysis as well as the much larger amount of research activity in this area. However, certain specific applications for which Raman spectroscopy is particularly well suited will also be discussed.

A. IR and Raman Spectroscopy of Food Proteins

IR and Raman spectroscopy have found extensive application in the study of proteins and are particularly useful techniques for the elucidation of protein secondary structure. The spectra of polypeptides and proteins exhibit nine amide bands that represent different vibrations of the peptide linkage. The wavenumber positions of these bands and their vibrational assignments are listed in Table 44.2 (8). Obtaining information pertaining to protein secondary structure from IR and Raman spectra is based on empirical correlations between the wavenumbers of certain of these bands and the various conformations adopted by the polypeptide chain. The amide I band (in the range of 1700–1600 cm^{-1}) is the most frequently employed in IR spectroscopy, and characteristic amide I band positions have been identified for α-helices, 3_{10}-helices, parallel or antiparallel β-sheets, turns, and unordered or irregular structures (Table 44.3). Using these band assignments, secondary structures of proteins can be deduced, although it is generally advisable to confirm the results with a second technique such as circular dichroism. The amide III band (in the range of 1300–1190 cm^{-1}) can similarly be employed for the estimation of the relative proportions of the secondary-structure components. However, in some cases, the amide III band can be overlapped by bands due to side-chain vibrations of particular amino acid residues—or absorptions from other biomolecules present, for example, in a food matrix—and consequently one must be cautious when assigning bands in this region. In IR spectra, the amide III band is much weaker than the amide I band and hence is much less frequently employed in secondary-structure investigations. However,

TABLE 44.2
Amide Bands of Proteins[a]

Designation	Nature of Vibration
A	N-H stretching
B	N-H stretching
I	80% C=O stretching; 10% C-N stretching; 10% N-H bending
II	60% N-H bending; 40% C-N stretching
III	30% C-N stretching; 30% N-H bending; 10% C=O stretching; 10% O=C=N bending; 20% other
IV	60% O=C-N bending; 60% other
V	N-H bending
VI	C=O bending
VII	C-N torsion

[a] Adapted from Ref. 8.

TABLE 44.3
Approximate Positions (cm^{-1}) of Amide I′ Band Components in IR Spectra of Proteins[a]

Secondary-Structure Component	Frequency (cm^{-1})
Antiparallel β-sheet (intra- or intermolecular)	1695–1675
Loops and turns	1674–1662
α-Helix	1659–1646
Unordered structure (random coil)	1645–1641
3_{10}-Helix	1639–1637
Intramolecular parallel or antiparallel β-sheet[b]	1636–1625
Intermolecular antiparallel β-sheet[c]	1624–1614

[a] Compiled from a review of the literature. The band positions are for proteins in D_2O solution (by convention, termed amide I' band components).
[b] Assigned to antiparallel β-sheet structure when it is accompanied by a high-wavenumber component (1695–1675 cm^{-1}); otherwise assigned to parallel (or extended) β-sheet structure.
[c] Indicative of protein aggregation.

both the amide I and amide III bands are commonly employed in Raman spectroscopy (Table 44.4) (9), as their Raman intensities are comparable.

The IR spectra of proteins are most commonly recorded in aqueous solution in a transmission cell, although ATR accessories have also been employed to study the spectra of protein films and powdered samples (Figure 44.10). The Raman spectra of proteins can also be recorded in aqueous solution but the spectra obtained from solid samples are generally of much higher quality and exhibit sharper bands (Figure 44.11). For IR studies involving examination of the secondary-structure-sensitive amide I band, proteins are commonly dissolved in D_2O because H_2O has a strong band in the amide I region. Since D_2O has no absorptions that overlap with the amide I band (which, by convention, is termed the amide I′ band when D_2O is the solvent), the use of D_2O solutions allows the protein absorptions to be observed without the need

TABLE 44.4
Approximate Positions (cm^{-1}) of Amide I and Amide III Band Components in Raman Spectra of Proteins[a]

Secondary Structure Component	Amide I (cm^{-1})	Amide III (cm^{-1})
α-Helix	1660–16	1300–1275
β-Sheet	1680–1665	1240–1230
Unordered structure (random coil)	1670–1660	1260–1240

[a] Adapted from Ref. 9.

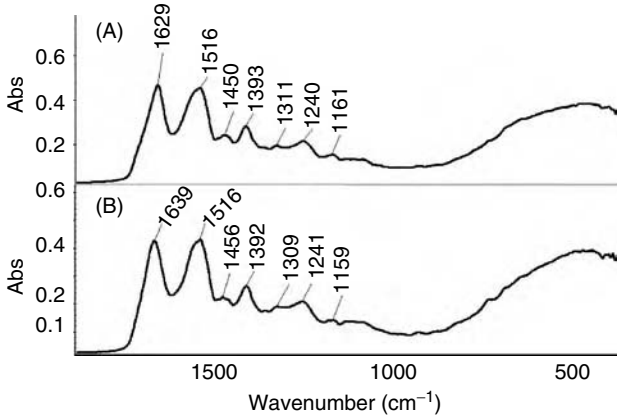

FIGURE 44.10 ATR/FTIR spectra of powdered β-lactoglobulin (A) and α-lactalbumin (B) recorded using an SB-ATR accessory. A pressure device was employed to ensure optical contact between the sample and the ATR crystal.

for subtraction of the spectrum of the solvent. More importantly, it also makes it permissible to use much longer pathlengths (40–80 μm vs. 10 μm for aqueous solutions), thereby yielding a higher signal-to-noise ratio. An additional advantage of dissolving proteins in D_2O is the resulting ability to study the rate at which the hydrogens of the amide groups exchange with the D_2O solvent due to the ~100-cm^{-1} shift of the amide II band (from 1560–1520 to 1460–1420 cm^{-1}) that occurs upon H-D exchange. This rate is indicative of the compactness of the protein since amide groups exposed to the solvent undergo H-D exchange faster than those in the interior of the protein. Thus, increases in the rate of H-D exchange as a result of variations in physicochemical parameters such as pH, temperature, and pressure can be interpreted in terms of the extent of protein unfolding.

Because of the inherent overlap between the amide I′ band components of the various conformations of the polypeptide backbone, computational band-narrowing techniques such as Fourier self-deconvolution (FSD) and derivative spectroscopy are routinely employed. It should be noted that caution must be exercised in applying these techniques because they can distort the shapes of the

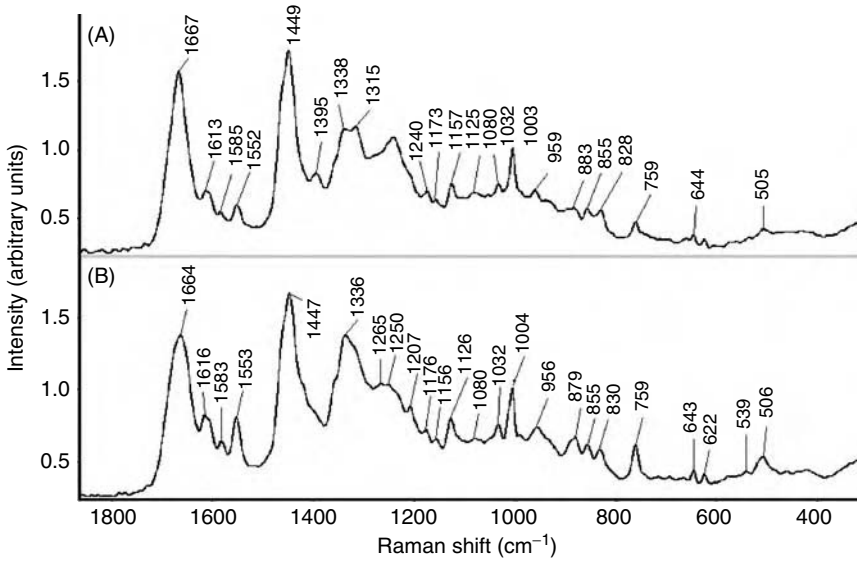

FIGURE 44.11 FT-Raman spectra of powdered β-lactoglobulin (A) and α-lactalbumin (B) in 1-mm glass capillaries recorded with 1064-nm excitation. (Nd:YAG laser). For band assignments, see Ref. 10.

bands and can also decrease the signal-to-noise ratio (11). Furthermore, the relative band intensities in derivative spectra are affected by the inherent widths of the bands, whereas deconvolution has the advantage of preserving the true relative band intensities (11). On the other hand, the results of deconvolution depend strongly on the values of the adjustable deconvolution parameters and are thus more subject to artifacts. For example, as illustrated in Figure 44.12, the selection of inappropriate deconvolution parameters that result in "overdeconvolution" amplifies the spectral noise, which may lead to spurious peaks. Similarly, atmospheric water vapor gives rise to very weak narrow peaks in the amide I region of IR spectra that can also be misinterpreted as amide I′ band components in deconvolved spectra. The latter pitfall is best avoided by thoroughly purging the spectrometer, including the optical compartment, with dry air or nitrogen. Other possible sources of artifacts are absorptions of buffers or contaminants in the protein solution (12).

Additional information about protein structure can be obtained from bands due to side-chain vibrations of amino acid residues, such as aspartic acid, glutamic acid, tyrosine, and tryptophan. For example, the ratio of the intensities of the Raman bands of tyrosine at 850 and 830 cm^{-1}, attributed to ring-breathing and out-of-plane bending vibrations, respectively, provides the following information about the polarity of the microenvironment of tyrosine residues (13):

- High I_{850}/I_{830} (>2.5): Tyr acts as H-bond acceptor
- Low I_{850}/I_{830} (<0.5): Tyr acts as H-bond donor
- $I_{850}/I_{830} \sim 1$: Tyr is exposed to an aqueous environment (i.e., Tyr near or at the protein surface)

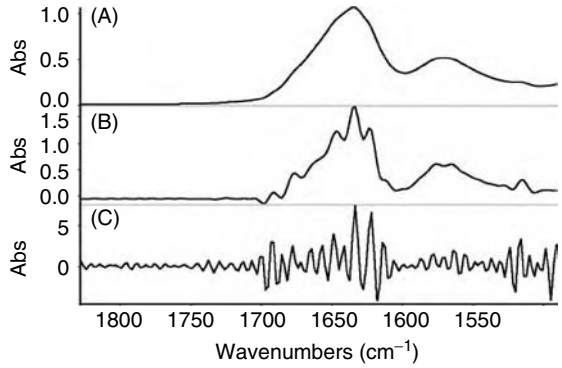

FIGURE 44.12 Raw FTIR spectrum (A), spectrum after Fourier self-deconvolution (FSD) (B), and "overdeconvolved" spectrum (C) of β-lactoglobulin in D_2O (5% w/v). FSD parameters: (B) bandwidth factor of w = 27 and resolution enhancement factor of k = 2.4; (C) w = 27 and k = 4.

Raman spectroscopy is also a very useful tool for probing the formation and rupture of disulfide linkages because the S-S and S-H stretching vibrations can be clearly discerned in the Raman spectra of proteins. The FT-Raman spectra of the amino acids L-cysteine and L-cystine are presented in Figure 44.13 and show, respectively, a band at 2552 cm^{-1} assigned to the S-H stretching vibration and a band at 498 cm^{-1} assigned to the S-S stretching vibration. In the Raman spectra of proteins, these bands are shifted to 2580–2550 and 550–500 cm^{-1}, respectively (14). The corresponding IR bands are not readily discernible, making Raman spectroscopy the technique of choice for the observation of disulfide linkages and examination of thiol-disulfide exchange. For example, in Figure 44.11, the higher content of disulfide linkages in α-lactalbumin as compared

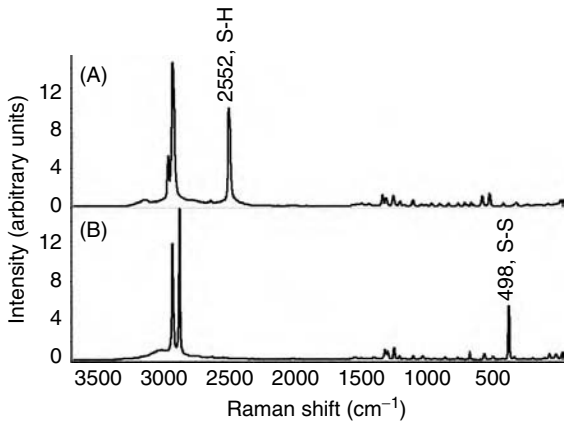

FIGURE 44.13 FT-Raman spectra of L-cysteine (A) and L-cystine (B). The spectra were recorded from solid samples in 1-mm glass capillaries, with 1064-nm excitation (Nd:YAG laser). Laser power was 500 mW; 1024 co-added scans were collected at 8-cm^{-1} spectral resolution.

to β-lactoglobulin is evident from the stronger intensity of the ν(S-S) band at ~ 505 cm^{-1}.

Although many of the proteins that have been studied by FTIR and Raman spectroscopy may be categorized as food proteins, such as whey, soy, and egg proteins, the majority of these studies have not been specifically concerned with the behavior of the proteins as food components. However, some detailed investigations of food proteins have been conducted to gain a better understanding of protein stability and structure-functionality relationships, with the overall objective of obtaining information that can assist the food industry in making more effective use of food proteins (7). Figure 44.14 shows an example from our studies on the thermal denaturation of whey proteins by variable-temperature FTIR spectroscopy (15–18). The spectra in the amide I region (Figure 44.14A) were recorded during heating of a 7% (w/v) solution of β-lactoglobulin in D_2O in a temperature-controlled transmission cell from 25 to 95°C and have been deconvolved by Fourier self-deconvolution. A plot of the changes in intensity of several of the amide I′ band components as a function of temperature is shown in Figure 44.14B. The decreases in band intensity at 1648 (▲) and 1635 cm^{-1} (●), assigned to α-helical and intramolecular β-sheet structures, respectively, and indicative of protein denaturation, occur prior to a sharp increase in the band intensity at 1618 cm^{-1} (■), assigned to intermolecular β-sheet formation and characteristic of protein aggregation. Similar experiments have been performed with the use of a diamond-anvil high-pressure IR cell to monitor denaturation of whey proteins under conditions of applied hydrostatic pressure, similar to those utilized in high-pressure processing, and provided information on the nature of the differences between thermal and pressure-induced denaturation (19, 20). In addition,

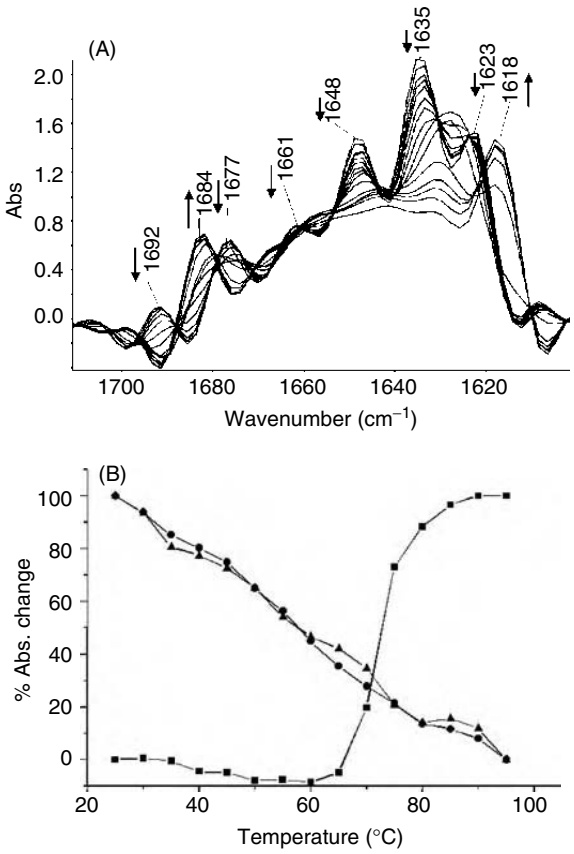

FIGURE 44.14 (A) Spectral overlay plot of the deconvolved amide I′ band in the FTIR spectrum of β-lactoglobulin (7% w/v) as a function of increasing temperature (25–95°C). FSD parameters: w = 27 and k = 2.4. (B) Plot of changes in intensity at 1648 (▲), 1635 (●), and 1618 cm^{-1} (■) as a function of increasing temperature.

multivariate analysis techniques have been employed to correlate FTIR spectral changes observed as a function of changes in physicochemical parameters, such as pH, ionic strength, temperature, and pressure, with rheological data collected under the same conditions (7). Such studies provide a better understanding of the structural changes at the molecular level that govern rheological behavior.

B. Analysis of Foods by FTIR and Raman Spectroscopy

1. Milk and Dairy Products

To date, the utility of IR spectroscopy in the quantitative analysis of foods has been most clearly exemplified by milk analysis. The determination of fat, protein, and lactose in milk by IR spectroscopy is an official method of the Association of Official Analytical Chemists (AOAC) and is used extensively as a basis for milk payment, dairy herd recording, and routine quality control in the dairy industry. High-volume automated instruments allow for

the analysis of 100–500 samples per hour. Dispersive spectrometers and subsequently filter-based instruments were originally employed in commercial IR milk analyzers, but FTIR milk analyzers are now on the market (21). The accepted IR milk analysis methodology employs a 37-μm CaF_2 transmission flow cell, which is kept at a constant temperature (40 ± 0.1°C) to obtain reliable, stable, and accurate measurements. Typically, IR milk analyzers incorporate a high-pressure (150–200 kPa) homogenizer to reduce the size of the fat globules to <1 μm to avoid light scattering effects. The pressure is maintained in the system throughout the analysis to avoid formation of air bubbles in the cell, and the cell is flushed with the next sample at extremely high velocity (30 m/s), eliminating the need for rinsing of the cell. Whereas filter-based IR milk analyzers were restricted to the analysis of fat, protein, and lactose, because the filters employed were specifically selected for the measurement of these components, commercial FTIR milk analyzers can be calibrated to measure other components in a wide range of dairy products, such as infant formula, ice cream, yogurt, and whey and whey concentrates (21).

In transmission measurements of dairy products, light scattering by fat globules must be eliminated in order to obtain quantitative analytical results. In the case of fluid products, this requirement can be addressed, albeit at some cost, by the use of high-pressure homogenizers, as described above. Because ATR measurements are not affected by light scattering, ATR accessories would appear ideal for the analysis of dairy products, including non-fluid products such as butter and cheese. However, the presence of fat globules in dairy products is, in fact, highly problematic because it results in sample inhomogeneity on the scale of the depth of penetration, which is in the order of a few microns or less, and thus the spectrum obtained is not truly representative of the sample composition. In a study of the effect of fat globule size in cream on ATR/FTIR measurements, a nonlinear relationship was found between absorbance at 1744 cm^{-1} (commonly used as a measurement peak for fat) and fat content over the range of 0–48% (by volume) (22). The authors of this study proposed the use of a nonlinear calibration curve for samples having fat globules > 1 μm. In a study of assessing the feasibility of employing ATR/FTIR spectroscopy for fat, protein, and moisture determination in cheese (23), large variations in the ATR/FTIR spectra of replicate samples taken from the center of a cheese were observed owing to both variations in the diameter of the fat globules and inhomogeneous distribution of fat within the cheese. Based on the fairly poor accuracy of PLS calibrations that were developed for the determination of fat, protein, and moisture contents, the authors concluded that the utility of the ATR/FTIR method for the analysis of cheese was quite limited but might be improved by homogenization of the samples prior to analysis; however, this requirement would significantly complicate the procedure. Another approach to overcoming the problem was employed in the development of a rapid FTIR quality control method for fat and moisture determination in butter employing an ATR sample-handling accessory (24). Samples were dissolved in 1-propanol and warmed to 40°C, thereby solubilizing the fat without causing separation of an aqueous phase, and applied to a heated ATR crystal (40°C) for spectral recording. A calibration was developed by employing gravimetrically prepared mixtures of anhydrous butterfat and water in 1-propanol as calibration standards. Analysis of 20 butter samples by this ATR/FTIR method yielded good agreement with reference values obtained by the Mojonnier method (24).

The speed of FTIR analysis (typically, <2 min/sample) is particularly advantageous in a processing environment since it allows the manufacturing process to be monitored and adjusted as it proceeds. A method for the simultaneous determination of sucrose, lactose, total solids, and fat in chocolate milk by FTIR spectroscopy for use in a production environment has been described (25). An ATR/FTIR method for fat and solids determination was developed for quality control purposes during manufacture of sweetened condensed milk (26). The ATR sample-handling technique is highly suitable for this type of viscous product, which would be very difficult to handle if a transmission cell were used, and highly reproducible results were obtained when samples were homogenized at 65°C prior to ATR/FTIR analysis. More recently, an SB-ATR/FTIR method has been developed for monitoring enzymatic hydrolysis of lactose during the production of lactose-reduced milk (27).

2. Beverages

Aside from milk analysis, numerous FTIR methods for the analysis of different types of beverages have been developed. Determination of sucrose, glucose, fructose, and total sugars in juices and soft drinks by ATR/FTIR spectroscopy was investigated by Rambla and co-workers (28, 29). Sinnaeve et al. analyzed apple juice using a 16-μm temperature-controlled transmission cell and successfully quantified malic acid, total sugars, glucose, fructose, sucrose, and specific gravity (30). Flow injection analysis coupled with transmission FTIR spectroscopy was used in the determination of sucrose in fruit juices (orange juice, apple juice, and sports drink) (31). Acetate buffer was used as the carrier solvent, and samples were passed through an enzyme reactor containing β-fructosidase prior to FTIR spectral acquisition. In another study, flow injection analysis/FTIR spectroscopy was employed to quantify malic acid, tartaric acid, and citric acid in juices and soft drinks using pH modulation to eliminate matrix effects (32). In a flow injection analysis/FTIR method for the determination of caffeine in soft drinks, caffeine was extracted by passing

the samples through a solid-phase extraction column (C_{18}), using chloroform as the eluent (33). A rapid ATR/FTIR method was developed for the determination of caffeine in soft drinks without extraction (34). This method allowed caffeine quantitation in soft drinks at levels as low as 0.5 mg/100 mL and was found to produce results similar to, but slightly higher on average than, those obtained from the conventional ultraviolet (UV) method.

Various publications have described methods for ethanol determination in distilled liquors, wines, and beers. Dilution of beverages having ethanol contents of >15% (v/v) is often required for analysis in a transmission cell, but this step can be eliminated when an ATR accessory is employed (35). The use of ATR fiber-optic probes in the quantitation of alcohol in liqueurs has been reported (36).

Recently, commercial FTIR wine analyzers, employing a flow-through transmission cell similar to that incorporated in FTIR milk analyzers, have been marketed (21). Patz et al employed an FTIR wine analyzer to analyze for alcohol, tartaric acid, malic acid, lactic acid, total acidity, pH, volatile acidity, reducing sugars, fructose, glucose, total SO_2, total phenols, and glycerol (37). The calibration and test sets comprised 165 wine samples, preanalyzed by standard methods; because the concentration ranges for glycerol and volatile acidity in these samples were too narrow, standards prepared in water were added to the calibration set. The authors reported that results were acceptable for all parameters except total SO_2 and total phenols. Comparable results were obtained with a standard benchtop FTIR spectrometer equipped with an SB-ATR accessory in a similar study conducted by the McGill IR Group to demonstrate the feasibility of employing SB-ATR/FTIR spectroscopy for the routine analysis of distilled liquors and wines (27). Dubernet and Dubernet used a commercial FTIR wine analyzer to analyze for alcohol, reducing sugars, total acidity, pH, malic acid, tartaric acid, lactic acid, total phenols, volatile acidity, CO_2, glycerol, gluconic acid, and saccharose content in 200,000 wine samples (38). These authors reported that not all wines could be analyzed accurately using the same calibration due to matrix effects and therefore developed separate calibrations for dry and sweet wines as well as for wine musts. Because calibration development may require the accurate analysis of several hundred representative wines for multiple components, which is clearly a major undertaking, an alternative approach based on synthetically prepared mixtures of the major components present in wine has been investigated (39). It was demonstrated that the preparation of large numbers of calibration standards that are often required for multivariate calibration and the acquisition of their spectra may be automated by sequential injection, a form of flow injection analysis. However, when calibrations based on model solutions of nine common components as a substitute for preanalyzed wines were applied to the analysis of wine samples, only limited agreement was obtained between the IR-predicted values for organic acids and sugars and results obtained by high-pressure liquid chromatography (HPLC) (39).

In a different type of application related to wine production, SB-ATR/FTIR spectroscopy in combination with PCA and cluster analysis was used to discriminate between phenolic extracts of different wine cultivars (40). Phenolic extracts were obtained by solid-phase extraction of the wine samples and subsequent elution of the phenols with methanol. Only 1 μL of the methanolic extract was required to cover the SB-ATR crystal and the methanol was allowed to evaporate prior to spectral recording. Almost complete discrimination of all cultivars investigated was achieved. SB-ATR/FTIR spectroscopy was also used in the analysis of white wine polysaccharide extracts to identify the type of wine-making process employed (must clarification versus maceration) based on the polysaccharides present (41).

3. Edible Oil Analysis

In the past decade, a large amount of work has been done on both FTIR and FT-Raman analyses of edible fats and oils. These included the development of both qualitative applications, involving the characterization and classification of oils, and numerous examples of quantitative analysis applications. The literature in this area has been surveyed in a recent review (42), and only selected applications will be highlighted in this section.

Typical FTIR and FT-Raman spectra of an edible oil are presented in Figure 44.15. The FTIR spectra of edible oils and premelted fats in their neat form can be recorded with the use of either a heated transmission flow cell or a heated ATR accessory, making these types of samples fairly ideal from a sample-handling perspective. On the other hand, dispersive Raman spectroscopy is generally unsuitable for the analysis of edible oils owing to background fluorescence arising from colored components present in oils. However, with the recent advent of FT-Raman instrumentation employing NIR excitation sources, the problem of background fluorescence has largely been eliminated, as can be seen from the high quality of the FT-Raman spectrum in Figure 44.15, and thus the potential for practical implementation of Raman analysis of fats and oils has been greatly enhanced.

The most noteworthy application of IR spectroscopy in the analysis of fats and oils is the determination of isolated *trans* isomers, which is an official method of the AOAC, the American Oil Chemists' Society (AOCS), and IUPAC. In the original IR *trans* analysis method, which was developed over 50 years ago with dispersive IR spectrometers, samples were dissolved in carbon disulfide before recording their spectra in a fixed-pathlength transmission cell (43). This dilution procedure was necessary

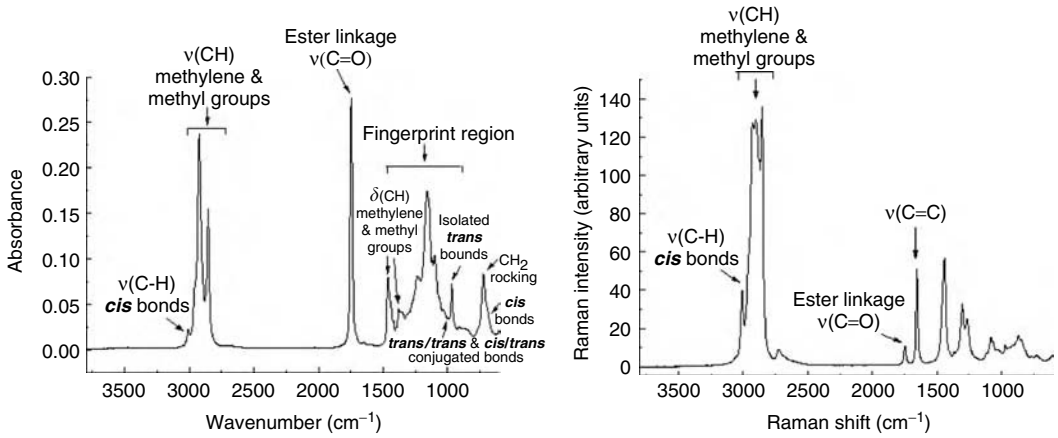

FIGURE 44.15 FTIR spectrum (left) and FT-Raman spectrum (right) of an edible oil. The spectra contain bands characteristic of *trans* double bonds because the sample is a partially hydrogenated oil.

because the analysis of oils in their neat form would require a cell pathlength of 10 μm or less due to the high absorptivity of the major absorption bands. With the development of FTIR spectrometers, however, it became possible to employ transmission cells with somewhat longer pathlengths (25–100 μm) or ATR accessories for the analysis of oils in their neat form. In 1996, Mossoba et al. described a rapid and simple ATR method for the determination of isolated *trans* isomers at levels down to 1% (44), and this method was subsequently adopted as an official method by both the AOAC and the AOCS. The use of an SB-ATR accessory for this analysis is advantageous because the small surface area of the ATR crystal allows for more precise temperature control and minimizes sample cross-contamination (45). Furthermore, the small volume of sample required (<50 μL) is advantageous in the analysis of fats extracted from foods, especially low-fat food products. This sample-handling technique was employed for the determination of *trans* content in hydrogenated vegetable oils in a collaborative study (46). The relative standard deviation between five labs was 1.62% for a sample having a *trans* content of 39.12% and 18.97% for a sample having a *trans* content of 1.95%. The relatively high error at low *trans* levels is attributable to the poor signal-to-noise ratio resulting from the very short effective pathlength of the SB-ATR accessory. Another factor limiting the accuracy of the method is the need for a *trans*-free reference oil that closely matches the fatty acid composition of the sample. If an appropriate reference oil is not available, as generally would be the case in the analysis of fats extracted from foods, the accuracy of the method may be poor, particularly at low *trans* levels (47).

The McGill IR Group extended the SB-ATR method for *trans* analysis to allow simultaneous determination of iodine value (IV), a measure of total unsaturation (45). Calibration models for the prediction of both IV and *trans* content were developed using PLS regression and a calibration set consisting of 9 pure triacylglycerols. Good agreement was obtained between the results obtained by gas chromatography (GC), the FTIR/PLS method, and the AOCS FTIR method for *trans* analysis and between a GC method and the FTIR/PLS method for IV determination (45). Much better precision and accuracy were achieved with the SB-ATR accessory than in previous work with an MB-ATR accessory (48). The McGill IR Group has also done extensive work on edible oil analysis using the custom-designed transmission flow-through cell shown in Figure 44.5, heated to 80°C to avoid fat crystallization during analysis (49). Methods have been developed for the determination of *trans* and *cis* content (50), free fatty acids (51), solid fat index (52), iodine value (53), saponification number (53), anisidine value (54), and peroxide value (PV) (55). While most of these methods are based on the spectral features of neat fats and oils, the PV method employs the stoichiometric reaction of triphenylphosphine with hydroperoxides to produce triphenylphosphine oxide, which has a strong absorption band at 542 cm^{-1} that is used for PV quantitation. Ruiz et al. automated this method through the application of flow injection analysis to allow on-line PV analysis, without the need for manual sample manipulation (56).

The complementary nature of IR and Raman spectroscopy is well illustrated by the spectra of edible fats and oils. By comparison of the spectra in Figure 44.15, it may be noted that the relative band intensities in the IR and Raman spectra differ markedly. Of particular significance in relation to the measurement of unsaturation is the 1700–1600 cm^{-1} region, in which strong C=C stretching absorptions are the dominant features in the Raman spectra of unsaturated oils whereas the corresponding bands in IR spectra are extremely weak. Because the C=C stretching absorptions of *cis* and *trans* double bonds are observed at different wavenumbers (1656 and 1670 cm^{-1}, respectively), Raman spectroscopy allows for the determination of both the degree

and type of unsaturation (57, 58). Thus, the potential utility of Raman spectroscopy, in conjunction with fiber optics, to monitor hydrogenation processes has been suggested (59). Raman spectroscopy has also been reported to provide information about the relative proportions of saturated, monounsaturated, and polyunsaturated fatty acids in a variety of fats and oils of both plant and animal origin (60).

As reviewed in Ref. 42, there have also been many studies during the past decade on the classification of edible oils by vibrational spectroscopy. Although the spectra of most pure fats and oils appear fairly similar visually, they reflect the differences in fatty acid composition that exist among different types of fats and oils. Thus, with the use of chemometric techniques such as PCA and discriminant analysis, different types of oils can be distinguished on the basis of differences in their IR or Raman spectra. The powerful capabilities of these techniques are exemplified by the successful use of FTIR spectroscopy, in conjunction with discriminant analysis, to differentiate between extra virgin and refined olive oils, despite the strong similarities between the spectra of these two types of oils (61). In another study, SB-ATR/FTIR spectroscopy was employed to detect adulteration of sunflower oil in olive oil down to a level of 20 mL/L of extra virgin olive oil (62). Similarly, Raman spectroscopy was shown to allow for the detection of adulteration of virgin olive oil with hazelnut oil, a very chemically similar oil type, at levels down to 5% (63). These examples are illustrative of fairly extensive research that has demonstrated the potential utility of FTIR and Raman spectroscopy for the authentication of edible oils.

Another important qualitative analysis application involves the dynamic monitoring of oil oxidation by FTIR spectroscopy to evaluate the oxidative stability of oils. For example, by monitoring the FTIR spectra of a thin film of oil spread on the heated surface of an ATR sample-handling accessory (64, 65) or on a polymer film (66) and subtracting the fresh oil spectrum ($t = 0$) from those taken subsequently over time, the resulting differential spectra reveal subtle spectral changes that can be employed to track changes in oxidation via "indicator bands" such as the *cis*, isolated and conjugated *trans*, hydroperoxide, and aldehyde absorptions. This approach allows one to develop a "dynamic" oxidation profile (peak height vs. time) based on these indicator bands and evaluate the relative stability of oils in a simple and rapid manner.

4. Syrups and Semi-Solids

ATR/FTIR spectroscopy is particularly useful for the quantitative analysis of viscous samples that cannot be easily introduced into a sealed transmission cell. The potential utility of ATR/FTIR spectroscopy for continuous on-line process monitoring of the conversion of corn starch to corn syrup has been demonstrated (67). Another study used a ZnSe ATR accessory to determine dry substance (glucose, maltose, and fructose) in glucose syrups after sample dilution (1 g/2 mL) (68). Determination of fruit content and detection of adulteration in strawberry jam by ATR/FTIR spectroscopy was investigated (69) but the accuracy of the method was limited by the overlapping of the 1725 cm^{-1} band used for fruit quantitation with the strong water absorption band at ~1650 cm^{-1}. In a later study, 36 jam samples were classified by ATR/FTIR spectroscopy according to their fruit content as "strawberry" or "nonstrawberry," with a correct classification rate of 91% (70). In a similar but much larger study, 95% of over 1000 fruit puree samples were correctly classified as "raspberry" or "nonraspberry," and it was shown that adulteration with sucrose or with apple and plum could be detected at levels of 4% (w/w) and 20% (w/w), respectively (71). The detection of inverted cane sugar adulteration in honey (72) and beet and cane sugar adulteration in maple syrups (73) by ATR/FTIR spectroscopy has also been reported.

5. Solids

Classification and detection of adulteration of solid food products by ATR/FTIR spectroscopy has been investigated. Instant coffee samples were dissolved in distilled water at 50°C at a concentration of 30% (w/v) and applied onto the surface of a horizontal ATR accessory (74). Spectra were recorded immediately to avoid settling out of a small insoluble fraction. Discrimination of *Arabica* and *Robusta* instant coffees was achieved by applying linear discriminant analysis (LDA) to the scores obtained by PCA of the spectral data, and the composition of *Arabica* and *Robusta* blends was determined by multivariate analysis. A similar methodology was employed for the detection of adulteration of instant coffees with glucose, fructose, and xylose, and the carbohydrate profile was also successfully quantified (75). The capability to distinguish between minced chicken, pork and turkey meats as well as fresh and frozen-thawed samples by ATR/FTIR spectroscopy has also been described (76).

For quantitative analysis of solid samples, IR transmission measurements are unsuitable owing to light scattering effects whereas an ATR/FTIR spectrum may not be fully representative of the sample owing to inhomogeneity on the scale of the depth of penetration associated with ATR measurements. In a study of protein-starch mixtures (77), samples were prepared by mixing dry gluten and starch or casein and starch and then adding a gram of water per gram of sample to produce wet mixtures, which varied in consistency from a moist powder at high starch content to an elastic mass at high gluten content. These samples were spread evenly on the ATR crystal to ensure uniform coverage and a constant pressure was applied to achieve good optical contact between the ATR crystal and the sample. However, poor reproducibility was obtained, especially for the casein-starch mixtures due to large particle size of casein.

In general, IR quantitative analysis of solid food products with the use of transmission or ATR sample-handling techniques requires some prior sample preparation. For example, quantitative analysis of sugar powders (glucose, fructose, sucrose) by ATR/FTIR spectroscopy was performed by impregnating the powders with an organic solvent (acetone), thereby forming a film on the surface of the ATR crystal after evaporation of the acetone (78). Determination of total fat and protein in meat by FTIR spectroscopy was carried out on meat samples prepared by suspension in 0.1N NaOH solution at 67°C and homogenized using a high-pressure valve homogenizer, prior to injection into a 37-μm CaF_2 heated (65°C) flow-through cell (79). Total fat determination in food products by transmission FTIR spectroscopy has been performed by extracting the fat in a chloroform/methanol (2:1) solvent (80) or 1-propanol (81).

The need for such sample preparation steps can potentially be eliminated through the use of alternative sample-handling techniques. One technique that has been investigated is photoacoustic (PAS) spectroscopy, which is based on the detection of IR absorption by using sound (82, 83). However, PAS has poor S/N relative to transmission and ATR techniques because the efficiency of energy transfer (absorbance to sound) is poor, and thus it is a relatively insensitive technique. In addition, water vapor gives rise to strong photoacoustic signals, requiring that the sample be very dry during analysis. Finally, the photoacoustic signal is dependent on several factors, such as incident energy, absorption coefficient, surface morphology, and thermal diffusivity, which, in part, are sample dependent. Accordingly, PAS does not appear to have general applicability for quantitative food analysis.

Raman spectroscopy is better suited to the analysis of solid samples than IR spectroscopy. In the area of food analysis, this suitability is best illustrated by various studies on modified starches, in which the degrees of acetylation and succinylation were determined from the FT-Raman spectra of solid samples contained in glass capillary tubes (84). However, no examples of the use of Raman spectroscopy in the analysis of processed foods have been reported.

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45 Application of Gas Chromatography to the Identification of Foodborne Pathogens and Chemical Contaminants in Foods

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CONTENTS

I. INTRODUCTION

Gas chromatography (GC) has been widely used in various applications involving the qualitative and quantitative analyses of foods (1) for several decades. In order to improve food safety and quality, GC techniques and methods have been developed and evaluated for many diverse applications. GC has been an excellent tool for the separation and determination of trace level, thermally stable, volatile organic analytes in complex food matrices.

Many food matrices are analyzed by GC in order to determine nutritional and palatability needs, check food adulteration, engineer novel food products, or meet process-control, quality assurance, food labeling, or other requirements. The widely different components include natural constituents, contaminants, and additives of foods, as well as products that arise from natural reactions and/or industrial processing. Food constituents are primarily water, lipids, proteins, carbohydrates, vitamins, and minerals, and components such as fatty acids, sterols, alcohols, aroma components, and off-flavors, are determined by GC. Contaminants in food products include residues of pesticides, veterinary drugs, and industrial pollutants (such as polyhalogenated hydrocarbons and polycyclic aromatic hydrocarbons), as well as toxins from spoilage, vegetative bacteria, endospores, or fungi (mycotoxins). Additives usually consist of components that are added to food products (such as preservatives, colors, or flavors).

Auto-oxidation and food processing (for example, frying, catalytic hydrogenation, or irradiation) also lead to the production of components (such as cyclic or *trans* fatty acids, histamine, urethane, nitrosamines, or 2-alkylcyclobutanones irradiation markers) that can be identified by GC.

In the past decade, GC advances have been achieved including the development and/or optimization of GC instrumentation components (injectors, detectors) and of portable, miniaturized, high-speed GC systems in which retention times are very short due to high flow rates, short and narrow bore columns, low film thickness, increased diffusivity of a solute in the gas phase, and faster temperature programming. GC detectors with greater selectivity and sensitivity such as mass spectrometers (MS), pulsed flame photometric detectors, and halogen specific detectors proved to be advantageous in food-analysis applications. Fast-GC/MS analyses (1) have been carried out by using micro-bore columns with time-of-flight (TOF)-MS (2–4), low pressure (LP)-GC/MS (5–7), and supersonic molecular beam (SBM)-MS (8–10).

In this chapter, three different areas of research selected from a large field of issues related to food analysis by GC techniques are addressed. The sections describe the instrumentation and applications of fast-GC to the study of food contaminants, the application of high resolution (HR) GC to the analysis of polyhalogenated contaminants in foods, and the GC analysis of foodborne bacterial lipids. Reasons for selecting fast-GC include its future potential in the analysis of many GC-amenable food additives and contaminants. Polyhalogenated aromatic compound determination in foods provides an excellent example of the continued application of gas chromatography to one of the most intractable analytical problems with food analysis. Without the advances in column design, both size and phase, the isomer specific analysis of PCDD/Fs and PCBs would be a much longer and more difficult process. Finally, GC-based methodologies are important analytical chemical tools that have been used increasingly for the speciation of microorganisms.

II. FAST GAS CHROMATOGRAPHY AND FOOD ANALYSIS

A major breakthrough in gas chromatography occurred in 1957 with the introduction of open tubular columns (11). This was followed in 1962 by an infamous demonstration of very fast gas chromatography (12). Utilizing a 120 cm $\times$ 0.035 mm squalene-coated column, H_2 carrier gas, and a hammer to depress the syringe, Desty and coworkers (12) obtained near-baseline separation of 15 hydrocarbons and isomers in under 2 s. In addition to such demonstrations of very fast analysis times, early work in open tubular columns also led to the development of columns with extremely high plate numbers (10^6). In spite of these early breakthroughs, open tubular columns were not rapidly accepted for chromatographic analysis. Small sample capacity, fragile, rigid glass capillaries, difficulty of incorporation into existing instruments and irreproducible column characteristics, attributed to slowing the implementation of open tubular columns. It was not until the late 1970s that the introduction of fused silica columns (13) and improvements in GC instrumentation led to the wider use of capillary columns.

The greater use of capillary columns was not accompanied by a large reduction in analysis time or sudden interest in fast chromatography. Instead, the greater efficiency of capillary columns was used to analyze and separate increasingly more complex mixtures. To ease in the transfer of methods from packed columns, developers chose capillary columns with large diameters, thick films, and excessive lengths. The concept of "killing it with plates" (14) and "over separating" (15) are often used to describe these early methods. Indeed, many of these methods became official or standard methods and the "over separation" still persists. Recently, with the increased cost of analysis, the interest in process control and the desire to develop rapid field-deployable test methods for environmental and security monitoring, there has been a renewed interest in rapid gas chromatographic analyses. The availability of fast chromatographic instrumentation (16) and method translation software will undoubtedly expand the use of the technique.

The terminology "fast gas chromatography" can be extremely subjective. If an analysis normally takes 70 minutes, then a 40-minute run time could be considered "fast." Dagan and Amirav first attempted to quantify the terminology used for Fast, Very Fast, and Ultra-Fast Gas Chromatography by identifying a "speed enhancement factor" (SEF) (17). SEF, the product of column length reduction factor and carrier gas linear velocity increase, constitutes a reduction factor in the flow time of the carrier gas compared to conventional GC. Dagan and Amirav defined a 30-m narrow bore column with a 1 ml/min gas flow rate (34 cm/s helium linear velocity) as the reference conditions for conventional GC-MS. Although SEF is quantitative in nature we find the classifications based on peak widths and total analysis time introduced by van Duersen et al. (1) to be more useful. According to the classification, "fast GC" separations happen in minutes and have peak widths of several seconds. "Very fast GC" describes seconds range separation with 30–200 ms peak widths and "ultra fast GC" is sub-second separations with 5–30 ms peak widths. Hinshaw (18) also "quantified" analysis speed by dividing methods into four levels based on relative speed, column dimensions, and inlet pressures. Hinshaw used the terminology of conventional, rapid, high-speed and very-high-speed for levels 1 through 4, respectively. This discussion will apply the van Duersen criteria for describing applications and will primarily focus on fast GC and the application in food analysis.

A. Chromatographic Theory

Prior to reviewing the practical aspects of fast gas chromatography, a brief review pertaining to resolution and efficiency in chromatographic separation is in order. It should be noted that the following discussion is a brief summary and is not intended as a thorough discussion of chromatographic theory. For the interested reader more in-depth and complete discussions of the equations and theory relating to sample introduction, speed of separation and detection in gas chromatographic analysis can be found in a number of sources (14,19–22).

In chromatographic analysis the goal is to separate the analytes of interest, denoted as the "critical pair," and to do so in the shortest amount of time. By definition, when the pair is separated, all of the peaks in the chromatogram will also be separated. The degree of separation is called the resolution (R_s), which is commonly defined by

$$R_s = \frac{\Delta t_R}{4\sigma_c} \tag{45.1}$$

where Δt_R is the difference in retention time between a critical pair and σ_c is the standard deviation of chromatographic peak broadening. Baseline separation is achieved when peaks of equal size show a resolution of >1.5; however a resolution of > 1.0 is often sufficient. The number of theoretical plates required to produce a given resolution is expressed as:

$$N = 16R_S^2\left[\frac{1+k}{k}\right]^2\left[\frac{\alpha}{\alpha-1}\right]^2 \tag{45.2}$$

where k is the solute capacity factor of the second eluting peak of the critical pair and α is the relative retention or selectivity factor for the two components.

Once resolution of the critical pair is achieved, the time for the chromatographic separation is minimized. The run time can be determined by the column length (L), average linear gas velocity ($\bar{\mu}$), and the solute capacity factor (k) of the last eluting peak using the following equation:

$$t_R = (L/\bar{\mu})(1+k) \tag{45.3}$$

where t_R is the retention time of the last eluting compound. Equation 45.3 exhibits the relationship between analysis time, column length, and linear gas velocity, but is more instructive when written in terms of the number of theoretical plates (N) and the height equivalent to a theoretical plate (H).

$$t_R = N(H/\bar{\mu})(1+k) \tag{45.4}$$

Equation 45.4 demonstrates the direct relationship between run time, plate number, and plate height. By combining equations 45.2 and 45.4 the relationship between analysis time and resolution becomes evident:

$$t_R = 16R_S^2\left(\frac{\alpha}{\alpha+1}\right)^2\frac{(1+k)^3}{k^2}\frac{H}{\bar{\mu}} \tag{45.5}$$

B. Reduction of Chromatographic Analysis Time

Using narrow bore columns is one of the easiest and most common methods for reducing the analysis time, while maintaining resolution. Schutjes et al. (23), although not the first to utilize narrow bore columns, derived the expressions relating a decrease in column diameter to a decrease in analysis time. They also experimentally confirmed the relationships for isothermal and temperature programmed conditions using 30 and 50 μm diameter columns. Klee and Blumberg (13) report nearly a 10-fold increase in "relative speed" by reducing the column diameter from 530 to 100 μm, while keeping "separation power equivalent." These studies demonstrated that decreasing column diameter effectively increases the number of plates per unit length of column (24). However, there are practical limits to the column diameter, Schutjes et al. (23) used 30 and 50 μm columns in their studies; however, due to potential problems with sample capacity, inlet pressure limitations, and maintaining injection efficiency it has been recommended that columns with diameters ≥ 100 μm (13) and ≥180 μm (24) be used in routine analysis.

Along with reducing column diameter, the choice of carrier gas can also impact speed of analysis, without decreasing resolution. By switching from helium to hydrogen as a carrier a 30% reduction in analysis time can be achieved, while switching from nitrogen gives a >3-fold reduction in analysis time (25). Finally, reduced outlet pressures through vacuum-outlet conditions have also been shown to be a useful method for decreasing analysis time at constant resolution (26). However, under certain conditions Cramers et al. (26) did report a 12.5% reduction in column efficiency when using vacuum outlet conditions. Although vacuum outlet conditions are only applicable when utilizing mass spectrometric detectors, an understanding of the effects of high pressure drops is important when developing fast chromatographic methods. A thorough study of the effect of high pressure drop commonly seen in fast chromatography, performed by Blumberg (27–29) and summarized by Klee and Blumberg (13), shows the complex relationship of pressure drop, column efficiency, analysis speed, column length and diameter, and film thickness.

A number of methods are available to the analyst for shortening analysis time through a reduction in resolution. Many of these, shortening column length (30), adjusting carrier gas velocity, and increasing temperature program rates (30), are familiar options during traditional method development. However, as was mentioned previously,

many of the current "official" methods are over separated, and some estimates suggest that up to 75% of the chromatogram contains no useful information (14). Even if that value overstates the extent of "lost time," the analyst should not overlook the ability to gain analysis speed through the optimization of resolution reducing parameters (14,19–21).

The reduction of peak widths and separation times achieved in fast chromatography begin to place stringent requirements on other instrumental parameters, often referred to as "extra-column sources." The Golay model (10), assumes that band broadening occurs only in the column, which is an accurate assumption when describing systems with long columns and low linear gas velocity. However, given the short column and/or high gas velocity in fast chromatography, this assumption is not always valid (20). Therefore, "extra-column sources" in general, and sample introduction specifically, can become an extremely important part of the analysis. The need to introduce the sample, without overloading the column or introducing band broadening, is critical to maintaining fast, highly resolved chromatograms. For fast chromatography conventional split/splitless injectors can be utilized, although the ratio or timing must be adjusted to avoid overloading column capacity (13). A number of other sample introduction systems have been developed for use in fast, very fast, and ultra-fast GC (21,22,31–33). The best method of introduction depends on the application of the chromatographic method.

As with sample introduction, sample detection mechanisms must maintain chromatographic efficiency by limiting band broadening. Additionally, detectors must have a sampling frequency fast enough to provide 15–20 data points across a peak (22). Many of the commonly used detectors including electron capture (EC), photoionization (PI), and thermal conductivity (TC), are sufficiently fast, but traditionally have large dead volumes, which leads to band broadening. The use of make-up gas to offset the large detector volume and limit band broadening leads to a loss of sensitivity with EC, PI, and TC detection (21). Small volume cells have been utilized to reduce band broadening and limit or eliminate the need for make-up gas (22). The use of mass-flow-sensitive detectors such as flame ionization, photometric, and thermionic detectors offer satisfactory data acquisition and sensitivity for use in fast GC (21). Along with the selection of a detection mechanism that has adequate sensitivity and maintains chromatographic efficiency, it is also beneficial to choose a selective detector that does not respond to co-extracted matrix components. Such selectivity will assist in shortening analysis time by reducing the need for increased chromatographic resolution. Perhaps the most common example is the use of mass spectrometric detectors to separate compounds not fully resolved by the chromatographic system (34). Wu and coworkers (35) utilized an element selective detector to assist in decreasing analysis time in their method. Additionally, Mastovska et al. (36) described the benefits in using a flame photometric detector compared to FID when analyzing for organophosphate pesticides in wheat.

Column selectivity changes can also be used to alter the analysis and subsequently gain speed. The choice of stationary phase can have a profound effect on elution order and elution time. Additional changes in temperature rates, gas velocity, or column length can then be used to further increase analysis speed. An extension of the use of a selective stationary phase is the use of two columns (GC × GC) with different retention properties (14). Unfortunately, specialized instruments, data systems, and experience are required to utilize these methods.

C. Applications of Fast Chromatography

In general, our interest in fast chromatography is not concentrated on a specific sub-discipline of the technique, but is in the overall potential benefits offered by the methods. As in any laboratory, the ability to analyze more samples in less time would represent a significant cost and time savings. Conversely, the shortened analysis time would enable us to investigate more samples in a given time period, allowing for a greater cross section of the food supply to undergo analysis. We are also intrigued by the application of fast chromatography to rapid field sampling. Such instrumentation would be useful in greater monitoring of the food supply, or in assisting manufacturers with process control as part of additional QA/QC procedures.

Although there has been an improvement in instrumentation and methods development associated with fast GC, food analysis can pose difficult challenges. Fortunately, the increase in interest in fast GC has produced a number of recent promising publications, which have application in our area of research. The research of Chen and coworkers (37) on the residual solvents is related to the work performed in our laboratories analyzing food and food packaging for volatile compounds (38–40). They were able to shorten the analysis of 40 organic solvents from 45 to less than 5 minutes using a short, narrow bore capillary column. Additional decreases in time were realized, by implementing temperature and pressure programming and limiting the list of analytes. Sample introduction was carried out by headspace analysis and direct injection. Although both showed acceptable reproducibility, the impact of the rapid method and sample introduction on detection limits was not evaluated.

Wang and Burleson (41) reported the development of a fast GC method used in the analysis of pyrolysis products from synthetic polymers. Pyrolysis GC has been shown to be useful in identifying low level additives in synthetic polymers (42) and a fast GC method could be useful in our analysis of polymer packaging materials (43,44). Wang and Burleson successfully transferred the traditional method to a fast GC, achieving a 10-fold increase in analysis speed. They also investigated the effect of head pressure and/or oven temperature on analysis time in conventional GC. Their

findings indicate that pyrolysis-fast GC is a useful method for analyzing synthetic polymers in short time periods.

Although the applications mentioned above have direct implications in the analysis of food and food packaging, they do not represent the same challenges often encountered with direct food analysis. Food is an extremely complex matrix, with a variety of interferences, often at concentrations significantly higher than the analyte of interest. Therefore, the ability to analyze foods by fast GC represents a particular challenge. Reed and coworkers (45) used a 5 m × 330 μm column, combined with fast temperature programming, to analyze for 2,6-di-(tert-butyl)-4-methylphenol (BHT) in chewing gum, breakfast cereal, and granola bars. Realizing that the extraction of BHT is often the rate limiting step in these analyses, Reed et al. utilized microwave assisted extraction (MAE) to further increase the overall processing time (45). Chromatographic analysis of each of the three sample matrices was completed in about 3 minutes with acceptable resolution and reproducibility reported for all samples.

Sandra and David (46) reported a fast gas chromatographic method for the determination of polychlorinated biphenyls (PCBs) in food matrices, and the fatty acid composition (as fatty acid methyl esters, FAME). The method was developed in response to the need to analyze a large number of samples (4000) in as short a time as possible. Initial development and validation was carried out in side-by-side studies using the official method. For the PCB method a shorter, narrow bore column was implemented to achieve a 4-fold increase in analysis speed without sacrificing resolution. Sandra and David (46) also applied a splitless injection to the fast GC, improving the limits of detection. It should be noted that the splitless liner was replaced after every 100 samples. A shorter, narrow bore column was also used for the FAME analysis and it also produced a reduced analysis time. This study was extremely successful at demonstrating the utility of fast GC; additionally, it represents one of the most extensive uses of fast GC that has been reported to date.

Lloyd and Grimm (35) increased the temperature program rates in order to increase analysis speed for sugars and FAMEs in food. For the sugar analysis they report a >7-fold increase in speed, but do note a loss in chromatographic resolution. Such a loss in resolution is to be expected when using temperature program rates as the driving force behind faster separations (22). However, the resolution of the fast separation was still acceptable for this analysis. The FAME analysis showed an increase in speed similar to the sugar analysis; however, separation of several of the compounds was incomplete. The mass selective detector did help distinguish co-eluted analytes, except in the case of positional (*cis, trans*) isomers. Lloyd and Grimm concluded that fast GC was successful in reducing analysis time, but had reservations about the loss of resolution.

Mastovsak et al. (36) evaluated the impact of reducing column length and temperature program rates in their analysis of 15 organophosphate pesticides in wheat. They also compared the performance of conventional and fast GC with flash GC, a commercially available resistive heating device. By reducing the column length from 30 to 5 m, increasing their temperature program rate, but maintaining column diameter and film thickness, Mastovsak and coworkers were able to reduce their analysis time 10-fold. Splitless injections with different splitless timing were used for the conventional, fast, and flash analysis. The researchers found that the narrower peak widths produced in flash GC experiments produced better detection limits, despite the smaller quantities of analyte being introduced to the column. The comparison of fast GC and flash GC methods showed similar run times, but the flash GC produced better retention time reproducibility. Additionally, the flash GC runs had faster cooling rates, leading to faster overall analysis times. The only drawback of the fast and flash GC was the loss of some resolution and subsequent coelution of analytes. However, some of the lost resolution could be attributed to the less selective stationary phase used for the flash GC analysis.

D. Method Translation Software

In creating methods for fast GC, the analyst has two choices; either redevelop the entire method or translate the method from traditional chromatography. Klee and Blumberg (13) offer an in-depth discussion of principles and practices of method translation. Method translation is often the easier of the two choices, especially if retention indices or "fingerprint" patterns need to be retained. To assist in the transfer, it is helpful to use method translation software, which is currently available on Agilent Technologies' internet site (www.chem.agilent.com/cag/servsup/usersoft/main.html#mxlator). A number of researchers (24,47) have evaluated the software's capabilities. David et al. (47) utilized the method translation software in transferring analysis of essential oils, bacterial fatty acids, and other complex mixtures from traditional to fast gas chromatography. By using a shorter, narrow bore column, and switching to hydrogen carrier gas they were able to analyze nutmeg and lemon oils in <13 min, a 6-fold decrease in analysis time. Additionally, the shorter analysis exhibited comparable retention indices and peak area ratios as the longer runs, allowing the "fingerprint pattern" of the separations to be maintained. Wool and Decker (24), by implementing a shorter, narrow bore column and using hydrogen as the carrier gas, observed a 50% reduction in time for analysis of 22 pesticides. Their conclusion was that "Method Translator should be a standard tool for analysts interested in speeding up the analyses done in their laboratories."

Fast gas chromatography is not a new concept, but the research and use appear to be undergoing a recent

renaissance. This renewed interest is probably due to a number of factors, including cost of analysis, interest in field sampling, and availability of adequate instrumentation. Whatever the reasons it is clear from past and recent publications that there are real benefits in developing fast gas chromatographic methods. These benefits will apply to laboratory sampling, process control, and field sampling, and will have an impact on the analysis of food and food packaging.

III. HIGH RESOLUTION GC AND THE ANALYSIS OF CONTAMINANTS IN FOODS

A. Scope of the Problem

This discussion will not be a review of the more than 25-year history of research involving high resolution gas chromatography (HRGC) and its application to the analysis of poly-halogenated contaminants. Rather than be a review, this treatment will examine the current approaches and future directions. The poly-halogenated contaminants of great interest in foods fall into several classes based on their carbon skeleton. A common feature of all the classes is the large number of isomers with similar physical and chemical properties, while conversely possessing widely varying effects on biological systems. These compounds exhibit very similar chemical properties to the analyst needing to detect and identify them, but nevertheless the individual isomers must be identified to determine their potential importance to a biological system. Table 45.1 lists some classes of poly-halogenated aromatic contaminant studied as a result of food contamination incidences or occupational exposures.

Table 45.2 provides a list of some of these classes along with a few chemical properties and their toxic equivalency factors (TEFs) values where known. Estimated toxicities often vary by many orders of magnitude for chemicals with fairly similar boiling points, melting points, vapor pressures, and octanol/water partitioning coefficients.

TABLE 45.1
Classes for Poly-Halogenated Aromatic Compounds, Synthetically and Environmentally Derived Contaminants

Chemical Class	Range(X)	Possible Congeners
Polychlorinated dibenzo-p-dioxins (PCDDs)	1–8(Cl)	75
Polychlorinated dibenzofurans (PCDFs)	1–8(Cl)	135
Polychlorinated naphthalenes (PCNs)	1–8(Cl)	75
Polychlorinated biphenyls (PCBs)	1–10(Cl)	209
Polybrominated biphenyls (PBBs)	1–10(Br)	209
Polybrominated diphenylethers (PBDEs)	1–10(Br)	209
Polychlorinated diphenylethers (PCDEs)	1–10(Cl)	209
Polybrominated dibenzo-p-dioxins (PBDDs)	1–8(Br)	75
Polybrominated dibenzofurans (PBDFs)	1–8(Br)	135

B. HRGC Column Stationary Phases

During the past 20 years, high resolution capillary GC columns have become the standard approach to separation difficulties presented by complex mixtures of polyhalogenated contaminants. Greater toxicological understanding of these chemicals has required increasing amounts of analytical data that report isomer-specific quantifications of these compounds in foods. Column lengths used in these procedures are typically at least 50 m, but most often 60 m, in length. PCDD/Fs provide a good example of separation difficulties with these compound classes. No single column could effectively separate all the isomers with largest TEFs from all others.

Ryan et al. (48) published the separations of all 136 tetrachloro- to octachloro-PCDD/Fs on nine different stationary phases and reported retention times on all the nine column phases. The column stationary phases covered a range of polarity from purely non-polar methyl silicone and the widely used 95% methyl, 5% phenyl polysiloxane (DB-5, RTX-5, equivalent, etc.) to the most polar 90% biscyanopropyl/ 10% 1:1 phenyl/ cyanopropyl polysiloxane (SP-2331) and 100% biscyanopropyl (CP-Sil-88) and the more recently introduced SB-smectic liquid crystalline methyl (80%) diphenyl carboxylic ester (20%) phase. Polar phases generally separated more of the 2,3,7,8-substituted isomers with the fewer numbers of congeners co-eluting, especially with 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF, while leaving some HxCDFs or HxCDDs unresolved. Columns containing DB- 210 (50% methyl/ 50% trifluropropyl polysiloxane) or DB-225 (50% cyanopropylphenyl/50% methyl polysiloxane) equivalent phases are used for resolving the multiple co-elutions with 2,3,7,8-TCDF found on non-polar columns, but also produce co-elutions with 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and certain HxCDFs such that they must be used with a second, usually non-polar, column.

Two medium polarity columns tested produced separations comparable or better to some more polar phases. A DB-17 column (50% phenyl/ 50% methyl polysiloxane) separated 2,3,7,8-TCDF completely and separated most other 2,3,7,8-substituted congeners except 1 isomer co-eluting with 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF, and an incomplete separation of 1,2,3,6,7,8-HxCDF. An OV-17 column still contained an isomer co-eluting with 2,3,7,8-TCDF and with 1,2,3,7,8-PeCDD, while producing more complete separation for HxCDFs. While some column phases performed much better than others, a combination of at least two columns was needed to identify all isomers of the 2,3,7,8-chlorine substituted dibenzo-p-dioxin and –furan isomers in complex mixtures. The polar phases provide separation for all but a few of the 12 2,3,7,8-substituted tetrachloro-hexachloro-DD/Fs, but require hour-long GC run times leaving significant overlaps between chlorination levels. Often the performance of these polar phases

TABLE 45.2
Some Physical Properties for Poly-Halogenated Pollutants and Associated TEFs

Class or Member	MP [a]	BP [a]	VP	log(Kow)	TEF [b]
Polychlorinated Dibenzo-p-Dioxins (PCDDs)					
2,3,7,8-TetraCDD	305	412.2	1.4E–09	6.2–7.3	1
1,2,7,8-TetraCDD	–	–	–	–	0
1,2,3,7,8-PentaCDD	240	–	–	6.8	1
1,2,3,4,7,8-HexaCDD	–	–	–	–	0.1
1,2,3,6,7,8-HexaCDD	285–286	–	–	7.6	0.1
1,2,3,7,8,9-HexaCDD	243–244	–	–	7.6	0.1
1,2,3,4,6,7,8-HeptaCDD	–	–	–	–	0.01
1,2,3,4,6,8,9-HeptaCDD	140	–	–	–	0
1,2,3,4,6,7,8,9-OctaCDD	150	–	–	–	0.0001
Polychlorinated Dibenzofurans (PCDFs)					
2,3,7,8-TetraCDF	219–228	–	0.000002	5.8	0.1
2,3,4,8-TetraCDF	–	–	–	–	0
1,2,3,7,8-PentaCDF	225–227	–	–	–	0.05
2,3,4,7,8-PentaCDF	168–170	–	0.0000011	–	0.5
1,2,3,4,7,8-HexaCDF	226–227	–	–	–	0.1
2,3,4,6,7,8-HexaCDF	–	–	–	–	0.1
1,2,3,4,6,7,8-HeptaCDF	236–237	–	–	–	0.01
1,2,3,4,7,8,9-HeptaCDF	–	–	–	–	0.01
1,2,3,4,6,8,9-HeptaCDF	–	–	–	–	0
1,2,3,4,6,7,8,9-OctaCDF	–	–	–	–	0.0001
Polychlorinated Biphenyls (PCBs)					
Arochlor 1254 or 1260	–	275–420	7.7E–05 [d]	6.8	–
3,3′,4,4′-TetraCB	–	–	1.1E–05 [d]	–	0.0005
3,3′,4,4′,5-PentaCB	–	–	2.1E–06 [d]	–	0.1
3,3′,4,4′,5,5′-HexaCB	–	–	4.0E–07 [d]	–	0.01
2,3,3′,4,4′-PentaCB	–	–	5.3E–06 [d]	–	0.0001
2,3′,4,4,′5-PentaCB	–	–	7.2E–06 [d]	–	0.0001
2,3,4,4′,5-PentaCB	–	–	3.3E–05 [d]	–	0.0005
2′,3,4,4′,5-PentaCB	–	–	6.8E–06 [d]	–	0.0001
2,3,3′,4,4′,5-HexaCB	–	–	5.5E–06 [d]	–	0.0005
2,3,3′,4,4′,5′-HexaCB	–	–	1.0E–06 [d]	–	0.0005
2,3′,4,4′,5,5′-HexaCB	–	–	1.4E–06 [d]	–	0.00001
2,3,3′,4,4′,5,5′-HeptaCB	–	–	1.08E–06 [d]	–	0.0001
2,2′,3,3′,4,4′,5-HeptaCB	–	–	2.8E–06 [d]	–	0.0001
2,2′,3,4,4′,5,5′-HeptaCB	–	–	3.8E–06 [d]	–	0.00001
Polychlorinated Naphthalenes (PCNs)					
1,2,3,5,6,7/1,2,3,4,6,7-HexaCN	–	234/205	–	–	0.002(EROD) [c]
1,2,3,4,5,7/1,2,3,5,6,8-HexaCN	–	–	–	–	0.0002(EROD) [c]
1,2,3,5,7,8-HexaCN	–	148	–	–	0.002(EROD) [c]
1,2,4,5,6,8-HexaCN	–	153	–	0	0.000007(EROD) [c]
1,2,3,4,5,6,7-HeptaCN	–	–	–	–	0.003(EROD) [c]

[a] Ahlborg et al. (1994) (Ref. 94).
[b] EPA Study (1992) (Ref. 95).
[c] Hanberg et al. (1990) (Ref. 96).
[d] Holmes et al. (1993) (Ref. 97).
MP = melting point; BP = boiling point; VP = vapor pressure; Kow = octanol/water partitioning coefficient; TEF = toxic equivalency factor.

will deteriorate faster than non-polar ones which significantly alter the quality of the separation and increase maintenance time and costs. Some phases have been marketed specifically to produce a near-complete separation of the critical 12 2,3,7,8-substituted tetrachloro- through hexachloro-DD/Fs. The "DB-Dioxin" separates all 2,3,7,8-substituted congeners except for 1,2,3,4,7,8-HxCDF, although some congeners were not 100% resolved from other isomers.

Fraisse et al. (49) reported an improved separation using a non-polar phase initially developed for low bleed requirements of ion trap mass spectrometers. The "DB-5ms" phase

used the same composition (95% methyl-5% phenyl polysiloxane) as a DB-5 or RTX-5 equivalent phase, but with phenyl groups pendant on as well as inserted as aryl inclusions in the polysiloxane chain. The resulting phase could easily separate 2,3,7,8-TCDD and 2,3,7,8-TCDF from the other 21 or 37 isomers, respectively, compared with an ordinary DB-5 which could not resolve 2,3,7,8-TCDF from 5 other isomers and produced only a slight separation of 2,3,7,8-TCDD from several closely eluting isomers. In addition, all the hexachlorinated isomers were also separated, except for a poor separation of 1,2,3,7,8,9-HxCDF, compared with separating only 4 of the 7 congeners on DB-5. The 1,2,3,7,8-PeCDD was still resolved well enough, but not to baseline as with DB-5. This column effects a separation for PCDD/Fs from food extracts with near-complete isomer specificity with a very durable high temperature column. There are almost no circumstances (e.g., an old DB-5 ms column measuring high levels of TCDF in a shellfish sample containing all TCDF isomers or where the cleanup was poor) where a second or alternative column is necessary for food analysis in a regulatory setting.

Isomer-specific PCB methods have recently focused on the unique GC separation and mass spectrometry determination of the PCB congeners with established TEFs (see Table 45.2). Frequently, the PCBs are fractionated before GC separations are attempted using non-polar columns (DB-5 equivalent, etc.). The separations greatly simplify the task of the GC so that a single non-polar capillary column with high durability can separate the remaining congeners. PCB congeners with no orthochlorine substitutions are isolated for analysis with the dioxins. Other PCB congeners are analyzed separately or further fractionated by the number of orthochlorine substitutions (e.g., mono-orthochloro and di-orthochloro-). Some methods attempt to determine all of the PCB congeners with TEFs with a single GC injection. EPA 1668 Rev. A has the purpose of separating PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189 from all other congeners in an environmental sample using an SPB-octyl column (Supelco 2-4218). This method requires retention time of decachlorobiphenyl to be greater than 55 minutes (quite long) and analyzes PCB 156 and 157 as a summed peak. The combination of the SPB octyl column and a DB-1 is reported in the method to uniquely separate 180 PCB congeners. Garrett et al. (50) reported that the SPB octyl column produced high bleed, producing unstable retention times within a few days. Some new columns could not meet the specifications of EPA 1668 Rev. A. An RTX-5 sil MS column was suggested as an alternative. Covaci et al. (98) reports separating PCBs in human adipose using a 50 m × 0.22 mm id HT-8 column made with 8% phenyl methyl silicone and dicarbaclosododecarborane that SGE has claimed will separate 192 of the 209 PCB congeners at least partially.

The separation and retention behavior of all 72 polychlorinated napthalenes isomers was investigated on six different HRGC columns phases by Järnberg et al. (51). No column could separate all congeners at any chlorination level from dichloro- to hexachloro-. A pair of hexachloronapthalene isomers (1,2,3,5,6,7 and 1,2,3,4,6,7) known to strongly bioaccumulate, were not separated on any phase. More recently, a complete separation of all 14 pentachloro- and 10 hexachloronapthalene isomers was demonstrated using a proprietary column phase made by Restek that used per-methylated β-cyclodextrin (52).

C. HRGC Column Dimensions

The efficient separation of isomeric mixtures has required relatively long columns (50 m or 60 m). Some better separations are accomplished on polar stationary phases with lower temperature maxima, further slowing the elution of highly chlorinated congeners. Hayward et al. (53,54) demonstrated that a shorter and narrower "minibore" 40 m × 0.18 mm ID DB-5 ms column could produce nearly identical separations as the wider and longer 60 m columns in 40 min and was durable enough for analysis of large numbers of food samples for PCDD/Fs. MacPherson et al. (55) optimized the conditions for PCDD/Fs, coplanar PCBs, and chlorinated pesticides on 40 m × 0.18 mm and 20 m × 0.1 mm DB-5 columns by adjusting temperatures ramps and using higher inlet pressures enabling comparable separations and quantifications using either 60 m, 40 m, or 20 m DB-5 columns. On 20 m columns, PCDD/Fs were eluted in as little as 14 min. This same approach using 20 m columns and fast temperature ramps was applied to a mixture of 56 PCBs and to PAH mixtures (56). Microbore columns have also been tested in dual column applications for determining target PCB congeners and dioxins in the same GC run. MacPherson et al. (57) recently reported the sequential acquisition by HRMS of mono-ortho PCBs (PCBs 105, 118, 156 etc.) eluting from a 20 m × 0.1 mm ID column while PCDD/Fs and non-ortho-PCBs 77, 81, 126, and 169 were being separated on a 40 m × 0.18 mm ID column in the same GC injected immediately after the injection on the 20 m column of the PCB fraction. This way separate PCB fractions could be determined with the same acquisition and into the same data file for processing. Worrall et al. (58) used a similar approach, but delay the injection of the PCDD/F containing fraction on the 40 m column for 2.4 min to help avoid co-determination of chlorinated diphenylethers that would be present in the mono-ortho PCB fraction eluting on the 20 m column.

Dimandja et al. (59) demonstrated a very fast separation of 38 PCBs found in human serum in 5 min using a short 15 m column using time-of-flight (TOF) mass spectrometry for detection. The high spectral acquisition rates inherent in TOF have been used to reduce the time of separations on standard sized columns allowing simultaneous PCB and pesticide determinations in as little as 9.5 min (60).

D. Multi-Dimensional GC (GC^2)

Multidimensional GC or GC^2 refers to the separation of chemicals using two or more independent migrations for a chemical during the same fixed distance (GC) experiment. Multidimensional separations increase the capacity and resolution of a given GC system. The concepts for multidimensional separations are aptly described by Giddings (61). Liu and Phillips (62) reported achieving two-dimensional gas chromatography through use of an on-column thermal modulator system. A narrow bore column of standard length (20 m) is connected through the modulator (short section of column rapidly heated and cooled) to a short (~0.5 m) microbore column that produces a second fast separation of components pulsed to it by modulator from the first column. Two dimensional gas chromatograms could be generated on complex mixes of hydrocarbons using this system. The same approach was reported for pesticides in human serum by Liu et al. (63). Rapid separations of pesticides, PCBs or PCDD/Fs require fast mass spectral acquisition of a TOF instrument for mass spectrometric detection. More recent work has focused on robust modulator designs. Vreuls et al. (64) compared the performance of 4 modulator systems and report separating 91 PCB congeners and all 17 2,3,7,8-PCDD/Fs with the GCxGC/microECD with an ultimate goal of using TOF for the determination of PCBs and dioxins.

IV. APPLICATION OF GC TO THE IDENTIFICATION OF FOODBORNE BACTERIA

Among the U.S. population, it is estimated that millions contract foodborne illnesses each year (65). Currently, there is active surveillance for laboratory-diagnosed cases of 10 foodborne diseases resulting from infection with *Campylobacter, Escherichia coli* O157, *Listeria monocytogenes, Salmonella, Shigella, Vibrio* spp.*, Yersinia enterocolitica, Cryptosporidium parvum, Cyclospora cayetanensis,* and hemolytic uremic syndrome (HUS) (66). Since September 2001, there has been an additional need to identify select agents such as toxins (botulinum toxin and staphylococcal enterotoxin), chemical agents (sarin nerve gas and mustard gas), viruses, and particularly pathogenic bacteria including *Bacillus anthracis*, *variolla* (small pox), *Clostridium botulinum*, and *C. perfringens* (67). This requirement has led to a sudden surge in demand for routine rapid tests as well as fast and accurate microbiological assays and analytical methodologies for bacterial identification.

The most common techniques used for identifying bacteria include polymerase chain reaction (PCR), immunoassay biochemical reaction tests, and classical microscopy (67). Analytical chemical methods such as infrared spectroscopy (IR) (68–71), mass spectrometry (MS) (71,72), and gas chromatography (GC) (72–80) have been also used increasingly for bacterial speciation in research laboratories.

A. Gas Chromatography and Cellular Membrane Components

Early reports demonstrating that GC analysis of cellular fatty acid could be used successfully to identify bacteria were published in the 1960s (74,75). Whole bacterial cells are usually treated with sodium hydroxide and alcohol to cause hydrolysis and release of cellular membrane fatty acids. Sodium salts are formed and subsequently esterified. The resulting mixture of volatile fatty acid methyl esters (FAMEs) are separated by GC, identified and quantified. Derivatization increases volatility and improves chromatographic resolution. It was later recognized that further application of sophisticated multivariate statistical analyses to GC fatty acid profiles would facilitate the identification of microorganisms (75,76). The resulting new chemotaxonomy (75,76) based on multivariate statistical strategies such as principal component analysis differed from traditional microbial taxonomy that did not involve any statistical treatment.

The structures and names of cellular fatty acids are often complex (80). The structures of fatty acids can vary widely and include saturated, unsaturated, and branched fatty acids as well as those with hydroxy groups (e.g., 3-OH-C14:0) and rings. Branched fatty acids include the *iso* series, $(CH_3)_2CH(CH_2)_aCOOH$, and the *antiso* series, $CH_3CH_2CH(CH_3)(CH_2)_bCOOH$ or $CH_3(CH_2)_cCH(CH_3)(CH_2)_dCOOH$ with branching at a carbon in the middle of the fatty acid chain. Using a simplified nomenclature, examples include *iso*-C15:0, *antiso*-C15:0, and 10-Me-C19:0. An example of a fatty acid with a saturated 3-membered cyclopropane ring would be *cis*-11,12-methy- lene-octadecanoic acid (C19:0cyc11,12).

The primary source of cellular fatty acids is the lipid component of the cell membranes (including phospholipids) or the lipid A component of lipopolysaccharides in Gram-negative bacteria and lipoteichoic acid in Gram-positive bacteria (77). Most bacteria synthesize fatty acids with chain lengths having between 10 and 19 carbon atoms, and those with highest frequency are fatty acids with 16 to 18 carbons. Distinctive properties that allow identification of various microorganisms originate from differences in fatty acid composition, a characteristic that includes fatty acid distribution and quantity. Branched structures predominate in some Gram-positive bacteria, while cyclopropane-containing fatty acids and hydroxy fatty acids are often characteristic of lipopolysaccharides of Gram-negative bacteria. Gram-negative bacteria tend to have a greater proportion of saturated and monounsaturated fatty acids with an even number of carbon atoms than Gram-positive bacteria. The latter usually have a larger proportion of saturated branched-chain fatty acids

with an odd number of carbon atoms and low levels of saturated straight-chain fatty acids.

With the advent of long fused silica capillary columns, the application of GC to the determination of cellular FAMEs has become more widely used since the 1980s. An automated commercial system, Microbial Identification System (MIS) (79), that applies a GC procedure with flame ionization detection (FID) and multivariate analysis to the profiling of fatty acids ranging in length from 9 to 20 carbon atoms has been used increasingly by research, government, and commercial laboratories because it offers a FAME database for more than 2000 bacteria. The generally recommended GC-FID procedure (79) is outlined below. GC procedures have been widely used to identify bacterial species and strains primarily from clinical, environmental, plant, and soil, but only to a limited extent from food matrices (80).

A full understanding of this chemical type of bacterial identification requires that analysts and researchers in different disciplines collaborate and/or acquire expertise in all three microbiological (bacterial growth), chemical/analytical (GC), and biometric/chemometric (multivariate analysis) techniques.

B. Microbiological, GC-FID, and Multivariate Procedures

The analytical chemistry of bacterial identification depends on the comparison of the chemical composition of whole cells or their constituents (such as lipids or carbohydrates) that exhibit differences in microbiological characteristics. For the analysis to be reproducible, the various species and strains must be grown strictly under identical conditions to minimize variability.

Before any "fingerprinting" procedures can be used to analyze bacterial samples, the microorganisms must be cultured in order to produce sufficient biomass (*ca.* 40 mg) (79) for GC-FID analysis.

The accurate identification of unknown bacteria using a particular FAME database requires the use of microbiological conditions identical to those used to create that database. These conditions are primarily the temperature used in the cultivation of bacteria, the age of the culture, and the nature of the growth medium (79). It has been well documented (81) that differences in these factors will lead to large variations in the lipid content and composition of bacteria. Most aerobic bacteria are grown on trypticase soy broth agar (TSBA) medium that consists of 30 g trypticase soy broth and 15 g agar. Other common media are also used for aerobic bacteria and depend on the nature of the organism investigated. A temperature of 28°C would allow the growth of a wide range of microorganisms on TSBA. For anaerobic bacteria, agar cultures are grown at 35°C on brain heart infusion (BHI) with supplements. To minimize variability due to age, broth cultures are harvested at a given turbidity, and plate cultures are grown for 24 (aerobes) or 48 hours (anaerobes). Longer incubation times may be used for slow-growing bacteria. Approximately 40 mg of the bacterial cells are needed per test sample. For plate cultures, the physiological age is usually standardized by selecting a particular sector from a quadrant-streaked plate. Bacterial colonies are harvested with a 4-mm loop (or spatula) from the third quadrant. For a slow-growing organism, other quadrants may be used. Oftentimes, more than one quadrant must be harvested to collect as much as 40 mg. The weighed cells are placed in a culture tube with a teflon-lined screw cap and their membranes are prepared for GC analysis.

The preparation of FAMEs from cellular membranes for GC analysis involves several steps that include saponification, methylation, and extraction (79,80). Lipids from approximately 40 mg (79) of bacterial cells are first saponified with 1 mL of a sodium hydroxide (NaOH) solution prepared from 45 mg NaOH, 150 mL H_2O, and 150 mL methanol. Tubes containing bacterial cells and NaOH solution are securely capped, vortexed for 5–10 sec, heated in a boiling water bath for approximately 5 min, vortexed for 5–10 sec again, and returned to the water bath for 25 min. The tubes are allowed to cool down to room temperature and the fatty acids are methylated with 2 mL of an acidic methanol stock solution prepared from 6N HCl (325 mL) and methanol (275 mL). The tubes are vortexed for 5–10 sec, recapped, carefully heated at 80°C ± 1°C for 10 ± 1 min, and rapidly cooled. The resulting FAMEs are poorly soluble in the aqueous phase and are extracted with 3 ml of a 1:1 solution of hexane:*tert*-butyl ether by gently shaking the tubes for 10 min. The lower aqueous phase is pipetted out and discarded. Sample clean up consists of washing the remaining organic phase with 3 mL NaOH solution prepared from 10.8 g NaOH in 900 mL H_2O. This is carried out by gently shaking the recapped tubes for 5 min. Two-thirds of the top organic phase is pipetted into a GC vial that is capped and saved for subsequent GC analysis.

The remaining analytical steps include computer-controlled automation (79). A GC autosampler allows test samples to be chromatographed unattended. GC separation is usually carried out on a 25-m and 0.2-mm-ID phenyl methyl silicone fused silica capillary column. A temperature program that consists of ramping the temperature from 170°C to 270°C at 5°C/min is followed. GC peaks are electronically integrated and fatty acid composition data are stored. GC-FID profiles of FAME test samples are compared to those in the MIDI commercial databases and analyzed by proprietary pattern recognition algorithms. Multivariate algorithms apply statistical techniques to reduce the dimensionality of multivariate data while preserving most of the variance (82).

C. Application of GC-FID Methodology to Foodborne Pathogens

While the vast majority of GC studies involve the identification of clinical, environmental, and other microorganisms (83–86), there is a paucity of publications on the analysis of fatty acid GC profiles of foodborne pathogens (87).

In a validation study (87), the performance of the GC-FID-based MIS was compared to four other commercially available automated microbial identification systems. These four microbial identification systems are based on substrate utilization and bacterial growth; these processes lead to changes in pH, which in turn trigger changes in the color of indicators. All five systems were evaluated for their ability to identify six of the most common foodborne pathogens. The sensitivities, specificities, and repeatabilities of the MIS, the MicroScan WalkAway 40 system (Dade Diagnostics Corp., Mississauga, Ontario, Canada), the MicroLog system (Biolog, Inc., Hayward, CA), the VITEK system (bioMerieux Vitek, Hazelwood, MO), and the Replianalyzer system (Oxoid Inc., Nepean, Ontario, Canada) were tested by identifying food isolates of *Bacillus cereus, Campylobacter jejuni, Listeria monocytogenes, Staphylococcus aureus, Salmonella* spp., and verotoxigenic *Escherichia coli* (VTEC).

In the GC validation study by Odumeru et al. (87), 40 reference positive isolates (RPIs) and 40 reference negative isolates (RNIs) of these six microorganisms were used. Of the 40 RPIs, 35 were obtained from food samples and 5 from the American Type Culture Collection (ATCC). Of the 40 RNIs, 5 were ATCC strains, and 35 were cultured from food, clinical, or environmental samples and consisted of laboratory isolates that were related to but not identical to the bacteria of interest; RNIs showed similarities regarding their biochemical reactions and Gram staining results to those of the microorganism of interest. Sensitivity was defined in this study as the proportion of the RPIs that were correctly identified with an acceptable identification confidence level specified by the system's manufacturer. Specificity was determined by the proportion of RNIs that were not identified as the pathogen of interest with an acceptable confidence rating. Repeatability tests consisted of performing replicate analyses for 20 randomly selected ATCC strains and laboratory isolates from the RPIs and RNIs. Repeatability of identification was defined as the proportion of replicate analyses that generated the same result at similar confidence levels.

Odumeru et al. (87) reported that the sensitivities of the five systems used for the identification of microorganisms ranged from 42.5 to 100%. In particular, the sensitivity of the MIS was 90% for *Listeria* spp., 47.5% for *S. aureus*, 55% for *B. cereus*, 72.5% for *C. jejuni*, 85% for *Salmonella* spp., and 52% for *E. coli.* The authors attributed the lower sensitivities found for some of these pathogens with the MIS to the fact that they were based on fatty acid composition while the reference systems for these species were based on biochemical reactions. The specificities were usually close to 100%; those of the MIS were 100% for *Listeria* spp., 100% for *S. aureus*, 97.5% for *B. cereus*, 32.5% for *C. jejuni*, 100% for *Salmonella* spp., and 97.5% for *E. coli.* The repeatabilities of the MIS for the identification of test organisms were generally lower (30 to 90%) than those of the remaining four systems (60–100%). The repeatability of the MIS for RPIs was 30% for *Listeria monocytogenes,* 60% for *S. aureus*, 90% for *B. cereus*, 90% for *C. jejuni*, 90% for *Salmonella* spp., and 70% for *E. coli,* while the repeatability of the MIS for RNIs was 55% for *Listeria monocytogenes,* 90% for *S. aureus*, 80% for *B. cereus*, 75% for *C. jejuni*, 65% for *Salmonella* spp., and 65% for *E. coli.* According to Odumeru et al. (87), the selection of an automated system for the identification of foodborne bacteria depends on many factors including the nature of the available range of organisms in the system's database and the ability of the system to correctly identify the pathogen of interest.

In a GC-FID application study (88), the sources of *Bacillus cereus* in pasteurized milk were investigated. The MIS was used to determine the incidence and distribution of *B. cereus* vegetative cells and spores in raw and pasteurized milk. The presence of *B. cereus* in pasteurized milk is a concern for the dairy industry because it may lead to off-flavors, sweet curdling, and even outbreaks of food poisoning. *B. cereus* is a Gram-positive, spore-forming microorganism that can produce toxins in pasteurized milk at refrigeration temperatures. In pasteurized milk, *B. cereus* may originate from spores that are present in the raw milk or from the dairy plant environment.

In the study by Lin et al. (88), a total of 232 milk samples from sampling points along milk processing lines and 122 environmental swabs were collected in two dairy plants over several months. The fatty acid composition of each *B. cereus* isolate was determined by GC-FID. Using MIS, a database of *B. cereus* FAME profiles for 229 *B. cereus* isolates from milk samples and environmental swabs was created and used to determine the relationships between *B. cereus* isolate test samples.

Less than 10% of samples were positive for *B. cereus* vegetative cells in raw milk. The average *B. cereus* count in positive samples was less than 50 cfu per ml after enrichment at 8°C for 3 days. The incidence of *B. cereus* spores in raw milk samples was measured by the presence of *B. cereus* in heat-treated (75°C for 20 min) milk and was found to be very high. Of the heat-treated raw milk samples, more than 80% were found to contain *B. cereus,* and the average *B. cereus* count in positive samples was more than 1.1×10^5 cfu per ml after enrichment at 8°C for 14 days.

In pasteurized milk the incidence of *B. cereus* was high. After enrichment at 8°C for 14 days, 76–94% of these samples were contaminated with *B. cereus* and the average count reached 3.7×10^5 cfu per ml. Of the final products (pasteurized milk in cartons or plastic bags) more than 90% contained *B. cereus* and the average count reached 5.5×10^6 cfu per ml after enrichment. Most *B. cereus* isolates obtained from the pasteurized milk and final products belonged to the same sub-groups as the *B. cereus* strains germinated from spores in raw milk. Therefore, the authors (88) concluded that *B. cereus* spores in raw milk were the major source of *B. cereus* contamination in pasteurized milk.

The environmental swabs contained no *B. cereus* vegetative cells after enrichment at 8°C for 14 days. However, the heat-treated swabs had a low incidence (5%) of *B. cereus,* and the positive ones had a low average count for *B. cereus* of 30 cfu per ml after enrichment at 8°C for 14 days. According to Lin et al. (88), the presence of *B. cereus* in environmental swabs suggested that the dairy plant environment was a potential minor source of *B. cereus* in pasteurized milk.

Sundhein et al. (89) studied the contamination and spoilage of cold-stored chicken carcasses by *Pseudomonas* species. The shelflife of fish, red meat, and poultry in air is limited due to the presence of psychrotrophic pseudomonads that cause the formation of slime and production of off-odors. GC-FID traces of cellular FAMEs were analyzed by the MIS and used as an effective complementary technique to carbon source assimilation tests for the identification of pseudomonads from fresh and chill-stored chicken carcasses. Hundreds of bacterial strains were isolated from 18 chicken carcasses and based on results of carbon assimilation tests they were assigned to one of 17 defined groups of chicken pseudomonads. Isolates that had carbon assimilation patterns that could not be matched to any known species exhibited FAME profiles that corresponded to *P. fluorescens, P. lundensis* or *P. fragi.* The *P. fluorescens* biovars had greater levels of *cis* 9–16:1 (21–37%) and *cis* 9–18:1 (10–19%) relative to those of 17:0 cyclo (1–17%) and 19:0 cyclo (0–1%), while the opposite was found for *P. lundensis* and *P. fragi*. The authors concluded that none of the species was dominant and that the relative incidence of the various species may vary with flock or even individual birds.

D. Optimization of Analytical Methodologies

In research facilities, scientists have introduced minor refinements or major modifications to GC-FID analytical procedures, have added to existing commercial databases or created their own databases, and have applied and compared different analytical methodologies and pattern recognition algorithms.

A major drawback of using GC-based methods for fatty acid profiling is the requirement of laborious fatty acid derivatization procedures. To eliminate this manual step, researchers have used supercritical fluid derivatization/extraction and GC-MS (90), *in situ* thermal hydrolysis methylation (THM)-GC-MS (91), and *in situ* THM-MS (72) for the determination of cellular FAMEs. With *in situ* THM-MS, the 60-min extraction/methylation and the 20-min GC separation steps are eliminated, and this approach may potentially be amenable to rapid, single-step, automated analysis (72).

Muller et al. (86) used trimethylsulfonium hydroxide (TMSH) pyrolysis to complement the MIS procedure for the identification of FAMEs. This optimized TMSH procedure was used to transesterify bound fatty acids in phospholipids and triacylglycerol molecules to FAMEs in a rapid single step that can be carried out at room temperature. This reaction also released secondary alcohols and, for mycobacteria, mycolic acid cleavage products with chain lengths of C22 to C26 that were also amenable to GC-FID analysis. A pyrolysis-GC-atomic emission procedure (92) has also been used for identification of microorganisms.

Alternatively, the carbohydrate composition of bacterial cell hydrolysates may be analyzed by using a fully automated alditol acetate derivatization procedure and GC-MS and GC-MS-MS (78).

Selective detectors that require sophisticated instrumentation such as GC-MS/MS have provided high specificity and sensitivity in detecting trace amounts of chemical markers from clinical and environmental bacterial samples (78). However, the profiling of cellular fatty acids from vegetative cells and spores by GC-FID has been a more widely used analytical procedure (88,93). No single technique is suitable for all applications, and the full potential of analytical microbiological methodologies has yet to be reached.

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46 Modern Thin-Layer Chromatography in Food Analysis

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CONTENTS

I. INTRODUCTION

Thin-layer chromatography (TLC) is a well-established and widely used separation technique. Most undergraduate students of chemistry or food science used TLC as a primitive separation tool, which does not need more than small pieces of TLC plates, a glass jar and some solvents. TLC has evolved from a simple separation method of the past into an instrumental technique that offers automation, reproducibility and accurate quantification for a wide variety of applications [1]. The use of modern 10*10 cm TLC plates with narrow particle size distribution is called high performance thin layer chromatography (HPTLC), to distinguish the method from the use of traditional 20 × 20 cm TLC plates.

Numerous applications of TLC in the areas of food analysis, especially food composition, artificial additives, and contaminants have been reported. An excellent overview of modern thin layer chromatography is given by C. F. Poole [1,2], Sz. Nyiredy [3], J. Sherma and B. Fried [4], and also by N. Grinberg [5]. TLC analysis methods of agricultural products, foods, beverages, and plant constituents is comprehensively reviewed by J. Sherma for the period 1995–2000 [6].

There is strong competition among the different separation systems in food analysis. Widely used are GC (gas chromatography), IC (ion chromatography), and CE (capillary electrophoreses). HPLC (high performance liquid chromatography), which is often called the direct competitor of TLC [7], is especially common. The question here is not whether we need TLC beside HPLC but whether it is necessary to discuss how far thin-layer chromatography can be employed as a pilot technique. It is important to determine to what extent TLC can be used for optimization of separation selectivity. New developments show that TLC is not obsolete but has its niche in separation science and that niche is even still expanding. In this sense TLC and HPLC are competitors (S. Ebel from [7]).

II. THEORY OF TLC

HPLC and TLC separations are similar because nearly identical stationary and mobile phases are used. In HPLC reversed phase separations are preferred whereas in TLC the normal phase conditions dominate (the mobile phase is more lipophilic than the stationary phase). TLC has often been used as a pilot method for HPLC because TLC with various mobile phases can be performed much faster. Thin-layer chromatography in general has the advantage

of higher sample throughput by performing separation in parallel. TLC can handle cruder extracts than column techniques because the separation medium is used only once. The experience of the analyst is important in TLC separations because some steps have to be performed by hand. HPLC, in contrast, is fully automated with regard to the sample application, separation, and detection. But nevertheless, pre-cleaning of the analyte is mostly done by hand.

There are principal differences in TLC compared to commonly used column techniques like HPLC, GC, CE, or IC. In all the column techniques mobile and stationary phases have to be in equilibrium conditions before samples are injected. In TLC the sample is applied on the dry plate before the solvent contact starts the separation. It is worth noting that the solvent used in TLC is not identical with the mobile phase of the separation. The mobile phase arises from the equilibrium formation between solvent and TLC plate. When a plate is immersed under capillary flow-controlled conditions in a tank containing a few milliliters of the solvent, the distance moved by the solvent front z_f is related to time by the following equation:

$$z_f = \sqrt{\chi t} \tag{46.1}$$

$$\chi = 2k_0 d_b \frac{\gamma}{\eta} \cos\delta \tag{46.2}$$

t = separation time
χ = velocity constant
k_0 = permeability constant
d_b = particle diameter
γ = surface tension
η = viscosity of the solvent
δ = contact angle

To increase layer efficiency, high performance thin layer plates have been commercially available since the mid-1970s. The layers of these plates consist of fine particles with a very narrow particle distribution [8]. Assuming a narrow particle-size distribution, the velocity constant increases linearly with the average particle size. For coarse-particle layers of TLC plates the solvent front velocity is greater than for HPTLC layers. The velocity of the solvent front depends linearly on the ratio of surface tension and viscosity of the mobile phase. Although for silica gel layers the contact angle of all common solvents is close to zero, for reversed-phase layers containing bonded lipophilic groups the contact angle of the solvent increases rapidly with increasing water content of the mobile phase and cos(δ) will become very small, even zero. In this case the solvent is virtually unable to ascend the plate surface.

The nature of the sorbents, which is fixed as a layer of 100 μm to 250 μm thickness on glass, aluminum, or plastic plates, crucially influences the kind of separation. In TLC mainly strong polar sorbents like silica gel or Al_2O_3 are used. These sorbents have active centers, where sample molecules can be reversibly fixed. During the separation, an equilibrium is established between sample molecules, active centers of the stationary phase, and the solvent. If molecules remain primary in the stationary phase, they will move less than molecules staying mainly in the mobile phase.

The definition of the relative spot movement in comparison to the movement of the solvent front is given by the following formula:

$$R_f = \frac{z_S}{z_f - z_0} \tag{46.3}$$

R_f = retardation factor
z_S = movement distance of the sample spot
z_f = movement distance of the solvent front
z_0 = difference between starting position of solvent and spot

Two extreme situations are possible. If the sample does not pass into the mobile phase, it will not move. The R_f-value of this sample will be $R_f = 0$. If the sample stays constantly in the mobile phase, it will move with the solvent front and will not have any interchange with the stationary phase. In this case the R_f-value will be 1. It is obvious that a sample spending the same time adsorbed at the stationary phase as dissolved in the mobile phase will show an R_f-value of 0.5. If k is the equilibrium factor (often call the capacity factor), which represents the distribution probability between stationary and mobile phase, the expression (1) is valid.

$$R_f = \frac{m_m}{m_s + m_m} = \frac{1}{k + 1} \tag{46.4}$$

m_s = mass of sample in the stationary phase
m_m = mass of sample in the mobile phase
k = capacity factor

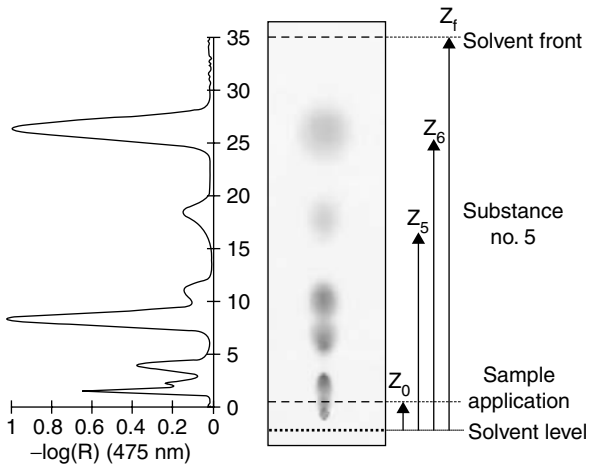

FIGURE 46.1 Separation of a dye mixture (CAMAG dye III, Switzerland) with densitogram (left).

If a sample stays at the point of application, obviously no separation can have taken place. The same situation is valid when all samples move with the solvent front. A sample separation is only possible if the samples change phases to different extents. In formulas, the appropriate k-values of substances must be different for them to separate. Chromatography is a separation technique where molecules can be separated because of their distinctive attraction to mobile and stationary phase. During the time of separation, all molecules in the mobile phase show a randomized diffusion movement. This diffusion causes a gaussian shaped peak with the variance σ_S. Diffusion effects are responsible for peak-broadening, which can be described with the following expression:

$$N' = \left(\frac{z_S - z_0}{\sigma_S}\right)^2 = NR_f' \qquad (46.5)$$

σ_S = variance of the sample spot (peak width)
N' = number of measurable plates
N = number of theoretical plates
R_f' = average R_f-value of all separated substances

The number of theoretical plates (N) is be corrected by an averaged R_f-value of all separated spots, because not all sample spots will, like in column separations, move over the same distance. The product of the theoretical plates number and the averaged R_f-value of all separated substances is a measure for layer efficiency. The basic evidence of the formula is that the squared quotient of the passed distance and the peak width is a constant value for all separated spots.

The baseline width (W_B) of a peak is virtually the spot diameter and this is easy to measure on the plate. In contrast, the peak width at half peak height in a densitogram is much easier to determine than the baseline peak width. The baseline width of a chromatographic peak can be assumed as $W_B = 4\sigma_S$ and the peak width at half peak height ($W_{1/2}$) is $W_{1/2} = 2.354\sigma_S$. For this, the chromatographic law can be expressed as follows:

$$N' = 16\left(\frac{z_S - z_0}{W_B}\right)^2 = 5.545\left(\frac{z_S - z_0}{W_{1/2}}\right)^2 \qquad (46.6)$$

W_B = baseline peak width
$W_{1/2}$ = peak width at half peak height
N' = number of measurable plates

It is worth mentioning that increasing separation distances are gained proportional to the square root of time. To double the separation distance it needs four-fold time! Unfortunately, diffusion is a matter of time and will increase over-proportionally with increasing distance. This is a definite disadvantage of TLC in comparison to column techniques, where the flow velocity is constant. This disadvantage of planar chromatography strongly affects the separation of two substances. The resolution (R_S) of two neighbourhood peaks is defined as the distance between both peak maximums and the averaged baseline width of the peaks.

$$R_S = \frac{z_{S2} - z_{S1}}{\frac{w_{B1} + w_{B2}}{2}} = 2\frac{z_{S2} - z_{S1}}{w_{B1} + w_{B2}} \qquad (46.7)$$

The resolution can be expressed in terms of capacity factors, the number of effective plates, and the R_f-value.

$$R_S = \frac{1}{4}\sqrt{N'}(1 - R_{f2})\left(\frac{k_1}{k_2} - 1\right) \qquad (46.8)$$

$N'(1 - R_{f2})^2$ = number of effective plates
R_{f2} = retardation factor of peak 2
k_1, k_2 = capacity factors of peak 1 and peak 2

The change in resolution of two closely migrated spots as a function of different R_f values is shown in Figure 46.3. Resolution increases only by square root of measurable plate number, which is a measure of layer quality and

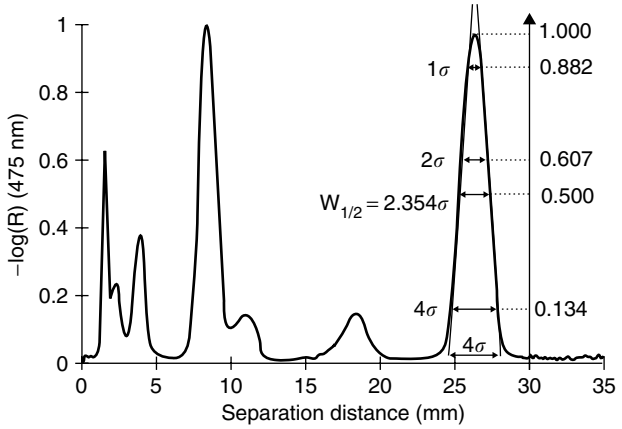

FIGURE 46.2 Gaussian peak of dye no. 6 in a densitogram of several dye spots.

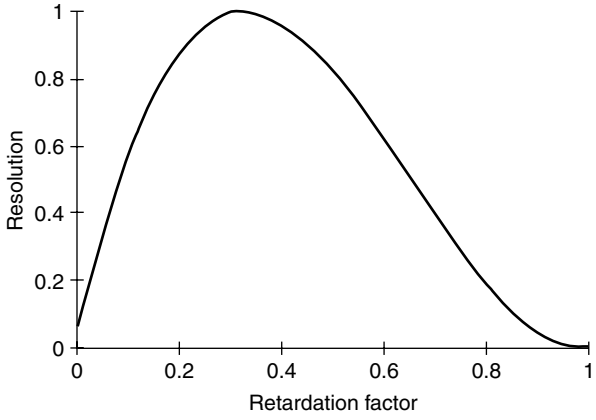

FIGURE 46.3 The plot shows the change in resolution of two peaks, plotted as a function of R_f-values.

efficiency. The influence of peak position on resolution is the opposite behavior to that of the layer quality. At larger values of R_{f2} the term $(1 - R_{f2})$ decreases and in the case of $R_{f2} = 1$ the resolution will become zero [9]. This is reasonable and agrees with the statement that phase changes are essential for separation. Figure 46.3 indicates that a resolution optimum of two closely migrating peaks can be observed at a R_f value of 0.33.

In summary, the selectivity $(k_1/k_2 - 1)$ can easily be increased by a factor of 10 or even 50 by solvent/sorbent changes, while it is difficult to change the layer quality factor $\sqrt{N'}$ by more than 2–3. Therefore, for resolution enhancement it is most rewarding to invest time in solvent optimization and to a lesser extent in sorbent optimization and least in the improvement of layer quality [10].

If the separation system is perfectly chosen, baseline separation of the desired substance can be done even on a few centimeters. In Figure 46.4 the separation of caffeine is shown, separated over a distance of only 11 mm. Samples from coffee, tea, or even strong sugar-containing beverages can be applied without further cleaning steps directly on the plate, after removal of CO_2.

The separation power of TLC or HPTLC plates is normally not sufficient to separate more than ten spots in one run. For the separation of more complicated mixtures, more than one solvent system must be used. However, the planar chromatographic separation power can be increased if a two-dimensional separation mode is used. Several AOAC (Association of Official Analytical Chemistry) methods, for instance, recommend a two-dimensional separation of aflatoxin samples to avoid overlapping spots [80]. For a two-dimensional HPTLC separation, samples have to be spotted at all four edges of a 10 × 10 cm HPTLC plate and four standards are also spotted on each of the four plate sides exactly between the sample spots. After simultaneous development from both plate sides to a distance of 45 mm the plate is dried and, after a turn of 90°, developed once again to a distance of 45 mm by use of a different mobile phase. The separation in two directions using different mobile phases extends the TLC separation power dramatically. A separation of a dye mixture in two dimensions is plotted in Figure 46.5.

In Figure 46.5 a dye mixture consisting of six dyes is shown, which can be separated only by two-dimensional separation using different solvent phases. One spot in the two-dimensional separation is not in a rectangular position between the two single separations. This indicates a compound, which changed its chemical property during the separation. Two-dimensional developments can be beneficially used to check sample decomposition during the separation.

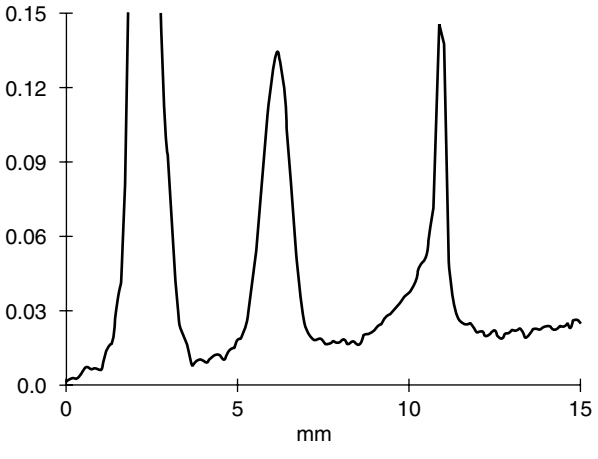

FIGURE 46.4 Plotting the caffeine peak at 6 mm from a well-known sparkling and sugar-containing black beverage. The solvent system isopropanol-cyclohexane-aqueous NH_3 (25%) (7+2+1) has been used in combination with a Si_{60} plate [11].

III. STATIONARY PHASES

TLC and HPLC plates are commercially available as pre-coated layers supported on glass, plastic sheets, or aluminum foil. The classic TLC plates are 20 × 20 cm in size whereas the modern HPTLC plates are smaller (10 × 10 cm or 10 × 20 cm). Compared with TLC, HPTLC-phases provide better separation efficiency, faster separations over shorter distances, and lower detection limits. In Table 46.1 the parameters of both plate types are listed.

The choice of the layer and the development solvent depends on the nature of the sample. In general, more than 75% of all HPTLC separations are done using silica gel as the stationary phase [5]. Silica gel, silica, or kiesel gel are various names for a polycondensation product of orthosilicic acid. It is prepared from silica solution by precipitation with acids. The improvements of HPTLC-plates are still under development. Macherey-Nagel company (Düren, Germany) developed especially hard and waterproof silica gel plates. These materials are commercially available

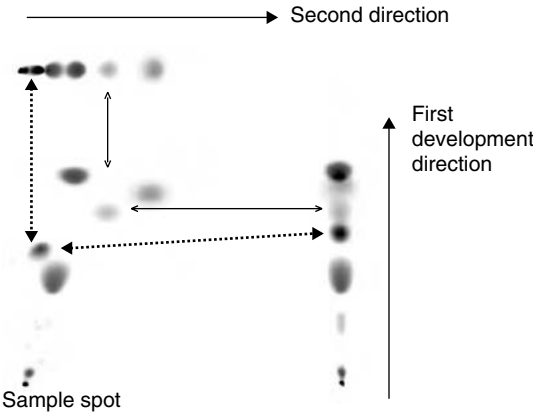

FIGURE 46.5 The plot shows a two-dimensional HPTLC separation of a dye mixture, with two one-dimensional standard separations right and on top.

TABLE 46.1
Comparison of Conventional TLC and Modern HPTLC Parameters

Parameter	TLC	HPTLC
Plate size (cm)	20×20	10×10
Layer thickness (μm)	100–250	100
Particle size (μm)	20	5–15
Sample volume (μl)	1–5	0.1–5
Application distance from plate side (mm)	10–15	5–10
Solvent migration distance (cm)	6–15	3–7
Starting spot diameter (mm)	3–6	1–1.5
Time of development (min)	30–200	3–20
Detection limits in remission (ng)	10–50	5–50
Fluorescence (pg)	500–1000	20–100

under the labels DurasilR and Nano-DurasilR. Merck (Darmstadt, Germany) sells HPTLC-plates with regular, ball-like particles under the name LiChrospher^R. The company claims a shorter developing time and better resolution in comparison to irregular articles. The use of Al_2O_3 as a stationary phase is restricted to mainly pesticide separations. Aluminia and silica gel plates form typical normal phase adsorption systems in retaining substances by active centers of the stationary phase.

Cellulose phases are more often used as aluminia, especially for amino acid separations and basic reacting substances like nucleotides. Polyamides as stationary phases are not often used. This very interesting material is well suited for the separation of acid-reacting substances like phenols, nucleotides, or dansyl derivatives. Bonded phases, based on silica gel, are becoming increasingly important in food analysis. These sorbents are typical for reversed phase separations. HPTLC is mostly performed on chemical bonded C-18, C-8, C-2, C-NH_2, C-CN, and C-$CHOHCH_2OH$ (diol phase) material. Cellulose and chemical bonded stationary phases are typical materials for distribution chromatography.

The flexibility of planar chromatography is a consequence of the absence of restrictions on the choices of mobile and stationary phases. Usually, TLC and HPTLC plates are obtained from commercial suppliers, but home-made layers are recommended for special purposes. Plates containing silver ions, for example, are able to separate compounds differing only in the position of one double bond [12]. The plates are difficult to store and are made by hand, mostly for research purposes. Other customers prefer home-made plates.

Although TLC is an inexpensive separation method, industrial produced plates must be purchased or may not be available everywhere. In developing countries cost and availability might be strong reasons to be independent of plate suppliers. A versatile TLC-layer can be made by mixing 27 g of corn starch with 3 g gypsum in 20 mL water and 10 mL ethanol. Nada Perišić-Janjić et al. [13] published the determination of organic acids like malic acid, citric acid, tartaric acid, and ascorbic acid in fruit juice using the solvent mix ethanol-n-butanol-water-conc. ammonia (40+30+15+15) in combination with the TLC-layer just described. A separation of amino acids with the solvent system 2-propanol-formic acid-water (40+2+10) is also possible as well as the separation of fat-soluble vitamins. For the separation of vitamin E and D_2 a solvent system containing acetone-conc. acetic acid (3+2) was used. Fructose, glucose, sucrose, and galacturonic acid are perfectly separated with the solvent system n-propanol-benzylalcohol-water-formic acid-dioxan-benzene (10+27+5+4+10+20) and anthocyanins from fresh cherries and cherry juice were separated on this unconventional layer using n-butanol-glacial acid-water-benzene (30+20+10+0.5).

IV. MOBILE PHASES

In planar chromatography the mobile phase is not restricted to a special kind of solvent. This makes it possible to separate a wide range of different analytes using only a single plate layer. In HPLC solvents are restricted to substances with low absorption values because analyte and mobile phase both have to pass the on-line detector. In planar chromatography even strong light absorbing solvents can be used because the mobile phase is removed before plate scanning. Nevertheless carcinogenic substances should be avoided. In the older literature (and even modern methods) benzene is often recommended as a solvent, but in this case toluene should be used.

Sometimes it is necessary to use chlorinated substances like chloroform or CH_2Cl_2. The leftover solvents should be collected separately to protect the environment. The mobile phase in planar chromatography is commonly chosen by trial and error, guided by literature descriptions of similar separations. On the basis of a sufficient substance movement in the R_f-range between 0.2 and 0.8, fine-tuning of the mobile phase should be done using different solvent mixtures with nearly the same solvent strengths (P'). The solvent strengths of a mixture can be calculated as the sum of the appropriate solvent strengths of the different mixture shares. A change in selectivity can be achieved if solvents with various selectivity parameters (x_e, x_d, x_n) are used. In Table 46.2 the most important solvents for planar chromatography are listed.

To create a new solvent system, one solvent should be tested from at least each selectivity class. While the velocity of the solvent front depends linearly on the ratio of surface tension and viscosity, this quotient should also be taken into account. Small values indicate fast separations, little peak diffusion, and therefore narrow peaks.

TABLE 46.2
Solvent Parameters for Different Solvents (the Values in Brackets Refer to RP Conditions; the Polarity Index (*P′*) Refers to the R_f-Value, Whereas (x_e, x_d, x_n) Influences the Selectivity of a Separation (+ γ/η[m/s]))

Solvent	P′-Value	x_e	x_d	x_n	Group	γ/η(25°)+
n-Hexane	0.1	–	–	–	0	56
n-Pentane	0.1	–	–	–	0	67
Cyclohexane	0.2	–	–	–	0	28
Dibuthylether	2.1	0.44	0.18	0.38	I	91
Diisopropylether	2.4	0.48	0.14	0.38	I	91
Toluol	2.4	0.25	0.28	0.47	VII	48
Triethylamin	1.9	0.56	0.12	0.32	I	52
Methyl-t-Butylether	2.7	0.49	0.14	0.37	I	72
Diethylether	2.8	0.53	0.13	0.34	I	71
Methylenchlorid	3.1	0.29	0.18	0.53	V	62
1,1-Dichlorethane	3.5	0.30	0.21	0.49	V	41
2-Propanol	3.9	0.55	0.19	0.27	II	8.7
n-Butanol	3.9	0.56	0.19	0.25	II	8.3
THF	4.0 (4.4)	0.38	0.20	0.42	III	56
1-Propanol	4.0	0.54	0.19	0.27	II	11
t-Butanol	4.1	0.56	0.20	0.24	II	7.3
$CHCl_3$	4.1	0.25	0.41	0.34	VIII	47
Ethanol	4.3 (3.6)	0.52	0.19	0.29	II	19
Ethylacetat	4.4	0.34	0.23	0.43	VIa	52
Methylethylketon	4.7	0.35	0.22	0.43	VIa	57
Dioxan	4.8	0.36	0.24	0.40	VIa	26
Chinolin	5.0	0.41	0.23	0.36	III	26
Aceton	5.1 (3.4)	0.35	0.23	0.42	VIa	74
Methanol	5.1 (3.0)	0.48	0.22	0.31	II	38
Pyridin	5.3	0.41	0.22	0.36	III	39
Methoxyethanol	5.5	0.38	0.24	0.38	III	18
Acetonitril	5.8 (3.1)	0.31	0.27	0.42	VIb	75
Acetic acid	6.0	0.39	0.31	0.30	IV	21
DMF	6.4	0.39	0.21	0.40	III	40
Ethylenglycol	6.9	0.43	0.29	0.28	IV	2.3
DMSO	7.2	0.39	0.23	0.39	III	2.4
Formamid	9.6	0.37	0.33	0.30	IV	17
Water	10.2 (0.0)	0.37	0.37	0.25	VIII	73

Sample Pre-Treatments, Application and Plate Development. In food analysis a pre-treatment step prior to separation is usually necessary. The pre-cleaned analyte is then applied on the plate, separated, and either visually detected or scanned by use of appropriate items. The determination of α-solanine and α-chaconine in potatoes is a typical example of a complete HPTLC determination. Solanines are toxic substances because they inhibit cholinesterases and cause gastrointestinal necrosis. An undamaged potato tuber contains 20–150 mg/Kg total solanines. Exposure to light or mechanical damage can increase the content. A routine analytical method to determine solanines is useful [14].

For preparation of potato samples 2 g dehydrated potatoes were extracted with three 15 mL portions of boiling methanol-acetic acid (95+5). The solution is filtered and evaporated under vacuum. The residue is dissolved in 4 mL methanol-acetic acid (99+1). In modern methods very often pre-columns, containing a stationary phase, are used in pre-cleaning. The analyte is accumulated on the stationary phase, cleaned by washing with appropriate solvents, and eluted by a solvent with high solvent strength. This pre-treatment increases the analyte concentration and removes contamination.

The sample application for qualitative purposes is mostly done by use of glass capillaries. If the same capillary is used, even quantitative measurements are possible. Glass capillaries in the size 0.5 μm, 1.0 μm, 2.0 μm, and 5.0 mm are commercially available. The disadvantage of this kind of application is the rather large spot diameter of the starting spots, which restrict the chromatographic resolution. For better resolution an automated application item should be used which is able to bring the sample bandwise on the plate. If a band length

of 7 mm is chosen, even 20 µl analyte can be applied on an HPTLC-plate. In general, an automated application is recommended if low measurement uncertainties are desired. Prior to separation the plates should be pre-washed by development at best with the mobile phase. In the case of solanine separation, the authors recommend pre-washing with methanol, pre-equilibration with the separation solvent for 1 h in a twin-trough chamber, and development up to a distance of 85 mm [14]. The separation conditions are very important, especially with respect to the vapor phase to obtain reproductive results [10]. The use of vapor dry chambers without mobile phase pre-equilibration should be avoided, and therefore the use of twin chamber or a linear chamber is recommended. The linear chamber for sandwich developments (Figure 46.6) is ready to use without time-consuming pre-equilibration because the very small vapor volume, in contrast to trough chambers, is responsible for reliable development conditions. If TLC plates are to be used, the separation distance should not be above 15 cm. For HPTLC-plates the separation optimum is 4.5 cm.

After solanine separation the plate is dried and dipped in a modified Carr-Price reagent containing 70 g antimony (III) chloride in 280 mL acetic acid-CH_2Cl_2 (1+3). After dipping the plate is subsequently heated for 5 min at 105°C [14]. One of the most important advantages of planar chromatography is the huge number of different staining systems [15], which makes planar chromatography very specific. The Carr-Price system, for example, reacts most specifically and sensitivly with the double bond of steroids. If staining is recommended, the plate should be automatically dipped for a defined time instead of spraying with reagent because sprayed plates are useless for quantification purposes.

The solanine peaks are quantified by densitometric reflectance measurement at 507 nm. The range of linearity for α-solanine is 100–2000 ng per spot. The limit of detection (LOD) is 50 ng and the limit of quantification (LOQ) is 100 ng. The LOD is the smallest quantity of the target substance that can be detected as peak, but not precisely quantified in the sample. The LOQ is the smallest quantity of the target substance in the sample that can be assayed under experimental conditions with well-defined precision and accuracy. The analytical precision is determined by measuring the repeatability at different concentrations. The repeatability for 5 determinations within 3 days for 500 ng α-solanine is 3.2%. Accuracy is a measure of agreement between a conventionally accepted value or a reference value and a mean experimental value. The extraction recovery for 900 ng α-solanine is calculated to 98.9% [14]. All these data are necessary to present a complete TLC or HPTLC method in food analysis.

Separations without complicated pre-cleaning steps are superior to those which need a lot of practical work prior to detection. The application of "dirty" samples on the separation medium is only possible in planar chromatography because disposable plates are used. This is a real advantage in comparison to HPLC because the pre-cleaning is simplified and the overall precision can be increased. C. F. Pool and co-workers [16] describe an elegant method to quantify vanillin in chocolate. Although chocolate is a difficult matrix it is only necessary to extract 5–8 g chocolate with 15 ml 70% ethanol in an ultrasonic bath for 15 minutes. After extraction the sample is centrifuged and separated on silica gel HPTLC-plates with chloroform-ethyl acetate-1-propanol (94+2+4) to a distance of 6 cm. Quantification is done by remission measurements at 280 nm.

V. DENSITOMETRIC MEASUREMENTS

In situ densitometry offers a simple way of quantifying by measuring the optical density of the separated spots directly on the plate [17]. During densitometric measurements the illuminating light is either absorbed or scattered. Only the scattered light is reflected from the plate and this light provides the desired information. This reflected light is called remission light. Quantitative evaluation of thin-layer chromatograms by optical methods is based on a differential measurement of light, emerging from the sample-free and sample-containing zones of the plate, although the relationship between detector response and sample concentration is not simple [18–20]. At first approximation a parallel light beam with the intensity I is used for illuminating the HPTLC-plate. To get a lamp-independent spectrum it is recommended to use the quotient of the sample spectrum and the spectrum of the clean HPTLC-plate. Usually the remission values (I_{rem}) are

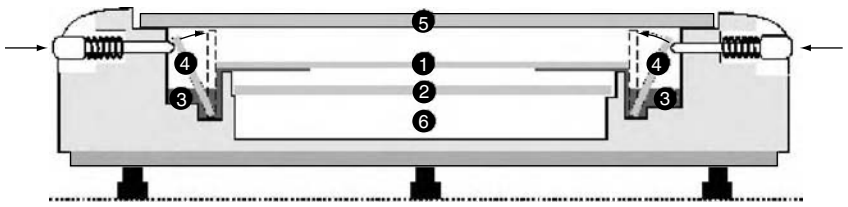

FIGURE 46.6 Sketch of a linear chamber. 1. HPTLC plate; 2. counterplate used only for sandwich development; 3. troughs for eluents; 4. glass strip to regulate eluent flow; 5. covering glass plate. (Reproduced with permission from CAMAG, Switzerland.)

calculated from the measurement values of the HPTLC-track divided by reference values (I_0).

$$R(\lambda) = \frac{I_{rem}(\lambda)}{I_0(\lambda)} \tag{46.9}$$

$R(\lambda)$ = corrected remission light spectrum
$I_{rem}(\lambda)$ = remission light spectrum
$I_0(\lambda)$ = reference spectrum

The proper choice of the reference spectrum $I_0(\lambda)$ is a very effective method for baseline corrections. A convenient way to transform remission data into mass-dependant signals is to use the folowing formula:

$$A(I_{rem},\lambda) = -\ln R(\lambda) \tag{46.10}$$

The logarithm expression sets all intensities smaller than I_0 to positive values. Theoretical predictions indicate that the Kubelka/Munk-equation is the only transformation formula which shows linearity between the remission data and the sample mass (m) [11].

$$KM(I_{rem},\lambda) = \frac{(1 - R(\lambda))^2}{2R(\lambda)} = const.m \tag{46.11}$$

In principle, all light absorbing substances are detectable because a modern diode-array TLC-scanner covers the range from 200 nm to 1100 nm. In Figure 46.7 a typical contour-plot of a dye mixture is shown, which comprises 450 single spectra, evaluated with Equation (46.10). Within less than 1 minute the diode-array scanner measures the track over the distance of 45 mm in the wavelength range of 200 to 600 nm with a spatial resolution of 0.1 mm.

There are numerous examples for *in situ* densitometric determination in food matrices. For example, the quantification of methyl and propyl parabens, benzoic acid, and sorbic acid at 228 nm and 254 nm are published in [21]. Fruit juices and jams are extracted and separated on silica gel TLC-plates, using the solvent mix ethanol-NH_3 aqueous-ethyl acetate-acetone (1+3+3+28) for the parabens and ethyl acetate-acetic acid-hexane (1+4+16) for both acids. The AOAC Official Method Polycyclic Aromatic Hydrocarbons (PAHs) and Benzo[a]pyrene in Food, describes a PAH separation using isooctane on cellulose plates. After separation the spots have to be scraped out and measured externally in UV [22]. In contrast, a modern TLC-scanner can separate 16 PAHs in one run, using the different absorption maxima of the PAHs spectra for quantification. Even spots not separated on the plate can be quantified, because spots can be spectrally distinguished [20].

The Kubelka/Munk equation is the only theoretically founded expression describing all remission processes. If a substance absorbs more light than the clean part of a

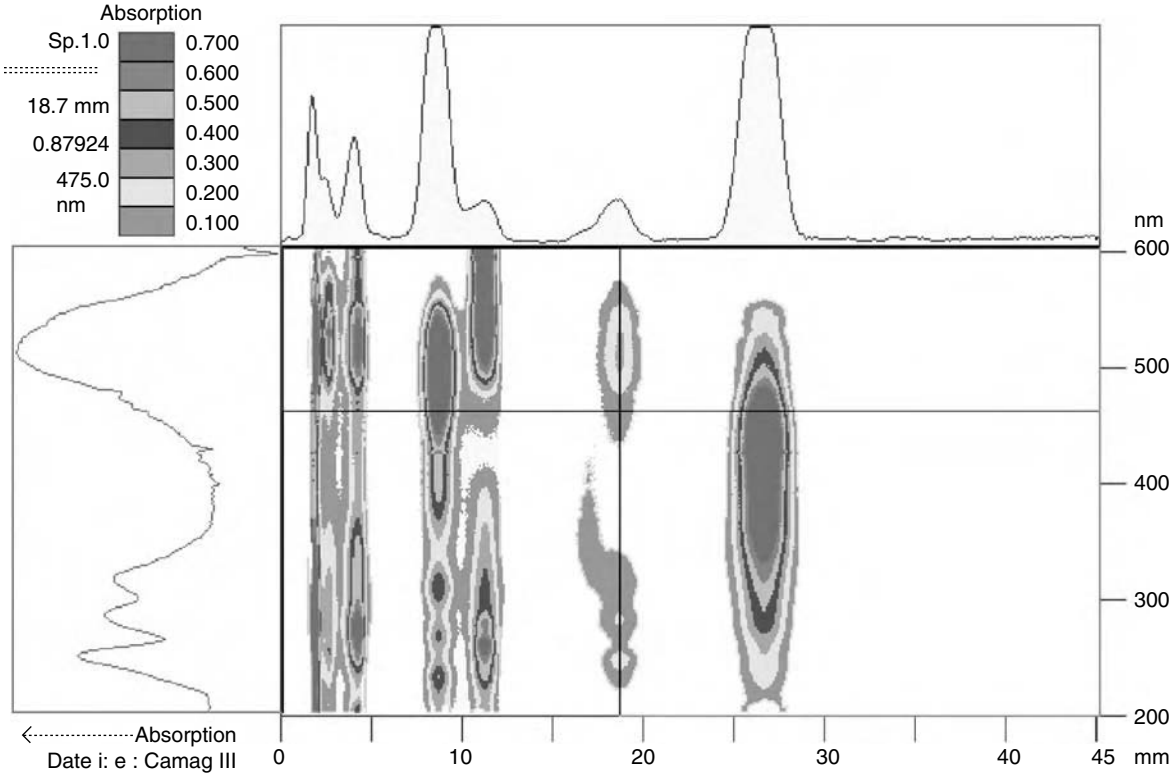

FIGURE 46.7 The plot shows the contour-plot of a CAMAG-III dye mixture. The dyes are separated with toluene over a distance of 45 mm. The spectrum at 18.7 mm (peak 5) is plotted left and the densitogram, taken at 475 nm, is shown on top.

plate, the comparison of sample and reference spectrum using Equation (46.10) or (46.11) can be used for quantification purposes. If the spot emits more light than the reference, the sample shows fluorescence. The fluorescence spectrum is derived from sample and reference spectrum using either Equation (46.11) or Equation (46.12).

$$F(I_{rem},\lambda) = [I_{rem}(\lambda) - I_0(\lambda)]/1000 \qquad (46.12)$$

Quinine is a fluorescent compound which emits light which was absorbed below 380 nm. In Figure 46.8 the densitogram of a beverage sample, applied undiluted on a silica gel HPTLC-plate, is plotted. The mobile phase (the same as shown in Figure 46.4) cannot separate quinine completely from sugars. The absorption densitogram (lower plot in Figure 46.8, evaluated using Equation (46.10) shows an irregular baseline and the quinine signal cannot be used for quantitative determination. If the same data set is evaluated using Equation (46.12) (upper plot in Figure 46.8), the quinine peak can be easily integrated [11].

The AOAC Official Method No. 969.27 [23] describes the separation of the non-nutritive sweeteners calcium cyclamate, sodium saccharin, and dulcine in non-alcoholic beverages on silica-gel plates using n-butanol-alcohol-NH_3-H_2O (40+4+1+9). Saccharin (R_f = 0.5) shows fluorescence at shortwave UV and can be quantified using the fluorescence mode of a diode-array scanner. In the AOAC method, color reagents for cyclamat and dulcine are recommended. These substances are detectable by direct remission measurements.

TLC scanners for *in situ* remission measurements are modern computer-controlled, high-sensitivity instruments that measure absorption and fluorescence. Modern diode-array TLC scanners are able to measure simultaneously at different wavelengths. They allow quick peak identification and peak purity investigation, using the registered spectra. Furthermore, the simultaneous registration at different wavelengths opens the way for chemo metric evaluations, like peak purity monitoring, to improve accuracy and reliability in HPTLC analysis. Diode-array scanners make fluorescent measurements possible without use of edge-filters or other facilities. Even a separate run for fluorescent measurements is not necessary, because the required information can be extracted from the remission data [11, 17–20].

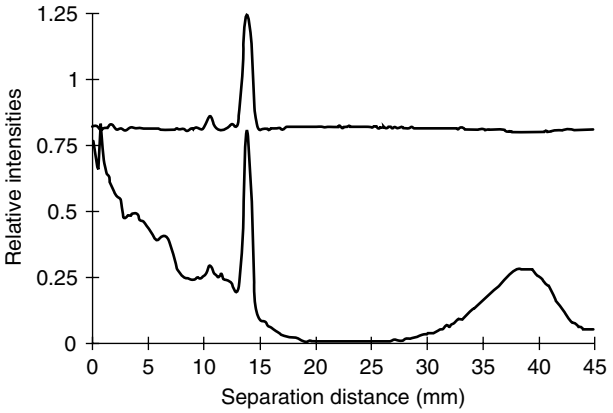

FIGURE 46.8 Densitogram of a bitter lemon sample in absorption (lower graph) and fluorescent mode (upper graph).

VI. SUGAR ANALYSIS AND SEPARATION OF DYES AND FOOD LIPIDS

Planar chromatography offers a wide range of different reagents for specific staining of analytes. The recommended handling of mostly hazard reagents eliminated many TLC methods from official method collections. To avoid plate dipping with mostly hazard reagents, specific plate coatings can be used for staining purposes. In the past, sugars had to be transformed into colored products by use of 4-aminobenzoic acid in glacial acetic or similar reagents [24]. The AOAC official method for glucose in honey or fructose starch sirup in honey recommends sugar detection by spraying with aniline/diphenylamine reagent after separation with n-butanol-acetic acid-water (2+1+1) on silica gel [25, 26]. In modern HPTLC amino phases are used for sugar separation [27, 28]. The separation is possible by using CH_3CN-H_2O solvent systems. After separation the amino plates are placed at 150°C for 15 minutes. All carbonyl-group containing substances will react with the NH_2-group to form colored, strong fluorescent products. Sample pre-treatments usaully are not necessary. In Figure 46.9 the separation of the artificial sweeter Sucralose® after amino plate derivation is shown. The detection limit is 10 ng/spot of Sucralose® [29], which is a typical value for what is termed thermochemical activation in sugar analysis.

Dye separation is one of the basic TLC applications because no scanner and staining reagent are necessary. Companies like CAMAG sell dye mixtures to check the layer quality of TLC and HPTLC plates. In [30] a rapid clean-up procedure for FD&C color additives in foods by use of reverse phase C_{18} cartidges is given. The color additives are separated on silica gel G TLC plates with n-butanol-methyl ethyl ketone-NH_4OH-H_2O (5+3+1+1). Similar systems are used to quantify colours from non-alcoholic and alcoholic beverages [31] and from raw sausages [32]. Detection of natural pigments from red pepper, paprika, and tomato is performed by using different hexane-aceton solvents or by using a mixture of CH_2Cl_2-ethyl ether (9+1) on silica gel [33].

Beside dyes, food lipids are a classic field of TLC analysis. A review of planar chromatography separations of food lipids is given in [34]. An AOAC official method describes the separation of some fatty acids in oils and fats. Erucic acid, cetolic acid, and trans-isomers of docosenoic acid are converted to methyl ester and separated by use of silver impregnated silica gel TLC plates.

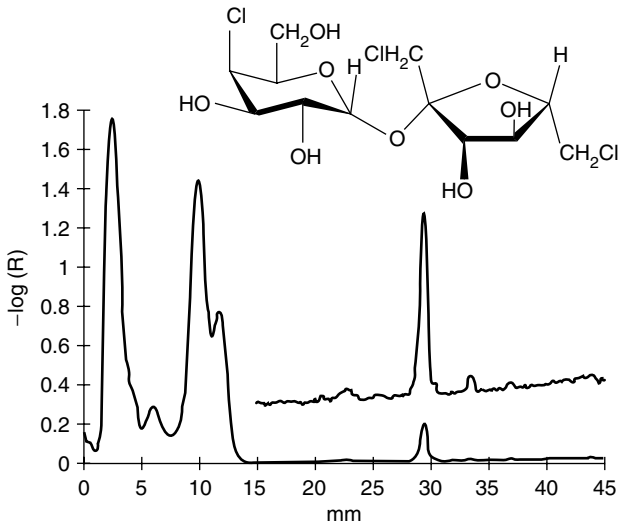

FIGURE 46.9 Densitogram of a "Cola" type softdrink in the absorption mode (1 µl spotted) containing 48 mg/L Sucralose® (peak at 29 mm). The upper signal line is plotted 5-fold enhanced.

The development of the plates is performed at −22°C with toluene-n-hexane (9+1) as mobile phase [35]. For spot visualization the plate is dipped in sulfuric acid-ethanol-hexane (1+35+64) for 2 seconds and then heated to 110°C for 45 min. This treatment turns lipids into fluorescent compounds [36]. Animal fat in vegetable fats and oil is identified by analyzing cholesterol with ether-petrol-ether (1+1) on silical gel. In the AOAC official method, cholesterol is scraped off at R_f: 0.2–0.3 and quantified by use of GC [37]. Charring with 50% sulfuric acid can be used to visualize cholesterol as well as all carbon-containing substances [38]. In this sense sulfuric acid acts as a universal detector in planar chromatography. To visualize oils and fats, plates are often sprayed (or dipped) in 2′7′-dichlorofluorescein reagent (0.5 g/L in 50% aqueous methanol), because the fluorescence in a lipophilic surrounding is mostly enhanced. This can either be used to visualize fats with fluorescing substances, or to enhance fluorescent intensity by dipping in non-polar reagents like Triton-X or parafine.

VII. DRUGS IN FOOD AND FEED

TLC or HPTLC methods are widely used in the screening of drugs in food and feed. The following examples show that in planar chromatography quantification can be done without a special column pre-cleaning. Coccidostats like aklomide or buquinolate are incorporated in the broiler feed as additives to prevent coccidiosis in poultry. To check the legal limits of aklomide in feeds [39] the sample is extracted with methanol and separated on a silica gel G TLC plate in ether [39]. The plate is sprayed with 1 ml 20% $TiCl_3$ to 25 ml p-(dimethyl-amino)cinnamaldehyde (DMC) (0.1 g in 100 ml 1.0 N HCl) solution. Aklomide forms reddish pink spots. Buquinolate can be identified after extraction from feeds with $CHCl_3$, concentrated and separated by TLC with $CHCl_3$ as mobile phase. Busquinolate shows fluorescence under short wavelength UV light with excitation at 265 nm and emission wavelength at 375 nm (R_f: 0.4–0.6) [40]. For quantification purposes the busquinolate TLC zone is transferred to a vial, extracted with 80% alcohol and measured with a spectrophotofluorometer [40]. The busquinolate fluorescence makes the method very specific.

Invermectin is a highly potent antiparasitic drug that is active at extremely low dosage against a wide variety of nematodes and arthropod parasites. The drug is widely used for antiparasitic control in cattle, horses, sheep, and swine. An HPTLC method using UV detection after reaction with acetic anhydride in pyridine was able to detect 5 µg/Kg in cattle blood or plasma [41]. With an improved derivative step (fluoroacetic anhydride instead of acetic anhydride) and a separation on silica gel HPTLC Si60 plates using $CHCl_3$-ethyl acetate (75+25) as the solvent, a visual fluorescence detection of the drug in liver, muscle, and fat as low as 5 µg/Kg was achieved [41]. AOAC methods in particular, often prefer visual detection to keep the method simple. For qualification the spot color and R_f-values are accepted as sufficient. Quantification is done by comparison of sample and standard spot intensity or zone transfer to spectrometric devices. By use of a diode-array scanner, food and feed additives can be easily identified and quantified using *in situ* measured UV/vis-spectra in combination with spectral libraries. Time-consuming transfer steps can be avoided. If fluorescence signals can be used, HPTLC methods are very specific and show detection limits comparable to HPLC.

There are several official AOAC methods for sulfonamide residues in animal tissues. The screening method for sulfabromomethazine, sulfadimethoxine, sulfamethazine, sulfapyridine, sulfaquinoxaline, and sulfathiazole recommends a tissue extraction with ethyl acetate after addition of sulfapyridine as internal standard and a cleanup step by seperating organic and aqueous solvents. Spotting zones on silica gel TLC-plates are focused by developing 1 cm in ethanol followed by 2 developments, 6 cm and 12 cm, in $CHCl_3$-tert-Butanol (8+2). Compounds are derived by dipping the plate in fluorescamine solution (25 mg fluorescame in 250 ml acetone) and scanned in UV [42]. To identify sulfamethazine the plate is sprayed with 1% sodium nitrite, dried at 100°C, and sprayed with NEDA (0.4% 1-naphthyl-ethylendimine in methanol) to form pink spots [43]. The AOAC method for trisulfapyrimidines determination in drugs recommends separation with $CHCl_3$-methanol-NH_4OH (30+12+1) on silica gel. The substances are located under shortwave UV [44]. A simple HPTLC

method to quantify thiamphenicol in bovine plasma has been published by P. Corti. Different phases like NH_2, CN-, and Si_{60} plates are used to increase determination specificity. A detection limit of 180 μg/Kg thiamphenicol is achieved whereas the HPLC detection limit is calculated to 70 μg/Kg [45].

The multiclass and multiresidue qualitative detection of chloramphenicol, nitrofuran, and sulfonamide residues in animal muscle was published by J.-P. Abjean [46]. The identification is done with the help of a specific reaction either with pyridine to identify nitrofurans or with fluorescamine to identify chloramphenicol and sulfonamides. Furans appear as blue spots on Si_{60} plates and were visually quantified. The limit used by the U.S. Food and Drug Administration for enforcement purposes for nitrofurans in 1989 was 100 μg/Kg. The expected limit of sensitivity with the method described is 1 μg/Kg [46]. The HPTLC method allows screening of residues in food with a sample throughput of about 20 samples per analyst per day without requiring expensive apparatus.

The identification and determination of oxytetracycline, tiamulin, lincomycin, and spectinomycin in veterinary preparations separated by two solvent systems is described in [47]. After separation on TLC silica gel aluminum sheets the absorption spectra of the separated substances were recorded by densitometry. The spots are visualized with Ehrlich's reagent (1 g 4-dimethylaminobenzaldehyde in 36% HCl-ethanol, 25+75) or 16% sulfuric acid and heated at 105°C for 10 min. The stained spots were scanned again to confirm the identification. Quantitative analysis achieved quantification limits per spot of 40 ng for tiamulin, 200 ng for lincomycin, 280 ng for oxytetracycline, 500 ng for spectinomycin, and 630 ng for oxytetracycline [47]. All antibiotic determination methods mentioned use specific post-separation reactions, which can be easily performed on the plate. Nearly all compounds can be identified using the R_f-values in combination with appropriate staining reactions. Hundreds of different staining reactions have been published and all act as specific TLC detectors [15].

Most specific is the use of enzymes for detection purposes. Oxytetracycline and chlortetracycline hydrochloride are antibiotics incorporated into feeds, either individually or in combination with other drugs, at concentrations ranging from 10 mg/Kg to 500 mg/Kg [48]. P. K. Markakis developed a simple, reliable, and precise TLC method for both these tetracyclines in animal feeds in the presence of other drugs. He used HPTLC Si_{60} plates and $CHCl_3$-methanol (10+1) as the developing solvent. After separation the appropriate HPTLC-zones were scraped out and the material was incubated for 11 h at 30°C in the presence of *Bacillus subtilis*. A detection limit of 0.625 mg/Kg for chlortetracycline hydrochloride and 1.25 mg/Kg for oxytetracycline was achieved, using the diameter of *Bacillus subtilis* inhibition zones for quantification [48].

The combination of TLC and very specific biology detection shows the great flexibility of planar chromatographic methods; nevertheless, scraping the TLC plate and incubation are time-consuming preparation steps that are difficult to automate. Much more elegant is the paper by H. E. Hauck [49] where an *in situ* bioautography of antibiotics is presented. Methanolic extracts of foods and feeding stuffs are subjected to HPTLC plates und separated using different solvents. After separation the plate is dipped in a bacteria culture (*Bacillus subtilis*) and incubated at room temperature for 19 h. The plate is sprayed with a tetrazonium salt as a reagent to detect active bacteria as purple color. The inhibition zones are recorded with a video system. Detection limits down to 100 μg/Kg were obtained [49]. This kind of detection is useful not on special compounds but on special attributes, for example, antibiotic quality. Attribute identification is also used to identify free radical scavenger capacity (RSC) of foodstuff [50]. Extracts of samples were separated on silica gel plates and after separation the plates were stained with a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl radical. Dots of separated foodstuff extracts with RSC turn yellow, with color intensity depending on the RSC compounds present in the extract. This test is sensitive enough to detect differences of RSC between varieties and brands of water- or methanol-soluble products [50].

VIII. PESTICIDES

Attribute analysis is particularly useful for pesticides which inhibit the enzyme cholinesterase. This is the case for all organophosphates and carbamates [51]. For quantification, pre-cleaned samples were separated on Si60 HPTLC plates using tetrahydofurane-hexane (7+25) as a solvent. The plates were dipped in a solution containing cholinesterase, 1-naphthyl acetate, and Fast Blue salt B. If the enzyme is not inhibited, it will cleave the acetate to 1-naphthyl phenol. The substance will react on the plate surface with Fast Blue salt B to form a violet color. The inhibition spots of organophosphates and carbamates become visible as white zones on a violet background. The spots can be quantified by reflectance spectrometry at 535 nm. Detection limits for different substances were in the range of 13–800 pg per spot [51].

The AOAC standard test to detect organochlorine pesticide contaminations like aldrin, DDT, dieldrin, chlordane, strobane, and toxaphen [52] recommends n-hexane as the solvent and silica gel as the stationary phase. The stationary phase is either mixed with $AgNO_3$ or dipped in 0.1% aqueous $AgNO_3$ after separation. When the plate is radiated with shortwave UV in the presence of chlorine substances, AgCl will be formed, which reacts with light to elementary silver. Reduced silver forms black spots, which can be used for a very sensitive chlorine pesticide screening.

For determination of carbaryl pesticide residues in apple and spinach the samples are extracted with CH_2Cl_2 and separated on Al_2O_3 TLC plates with aceton-benzene (1+4) [53]. The plate is sprayed with 1.0 N alcoholic KOH and then with a solution of diethylene glycol-alcohol solution (1+9) with p-nitrobenzene-diazonium fluoborate (25 mg/100 mL). The diazonium salt forms blue spots in the R_f-range between 0.52–0.6. For semi-quantitative estimation size and intensity of sample and standard spots have to be compared. For the determination of biphenyl pesticide residues in citrus fruits [54] no derivation step is necessary. Biphenyl is extracted from blended peel or pulp by steam liquid-liquid extraction. The extract is separated on a silica gel TLC plate with heptane. Viewed under UV light, biphenyl appears as a blue spot on a yellow background.

TLC or HPTLC is in general well suited to separate a few compounds from a complicated matrix. The resolution in TLC becomes maximal for R_f-values around 0.3. If a special phase system separates substances in the optimal R_f-range of 0.1 and 0.8, only compounds with k-values between 9 and 0.25 will move (4). This is a very narrow polarity window. All other substances outside this window will stay at the point of application or will move with the solvent front. Therefore, only few substances of similar polarity can be separated in one run. But in samples like water or food, pesticides of a wide polarity range can be present. To cover a wide range of polarity a special gradient mode, the automated multiple development (AMD) technique, can be exploited using a great variety of solvents [54, 55]. The principle of AMD is simple. The TLC plate is developed at first only over a very short distance using a solvent of great elution strengths to move all substances. After plate drying another separation step takes place, but now the solvent strength is slightly reduced whereas the separation distance is slightly increased. During the first separation cycle all substances of the sample will move because the highest elution power of the solvent is working. When the solvent front reaches a sample spot the sample will be concentrated in a narrow band because the molecules in the sample zone start their upward moving earlier than those in the upper part of the spot. During every cycle the zones will be concentrated to a narrow band if the substances are moving. Between the developments the solvent is completely removed from the developing chamber and the plate is dried under vacuum. For the next development the layer is conditioned with the vapor phase of the next solvent. Cycle for cycle, the solvent strength is reduced and more and more substances will remain at their plate positions. At the end of the last cycle only the compound with the largest mobility will move. Usually the number of cycles is between 10 and 40 for a separation distance of not more than 8 cm. All the steps in AMD are computer controlled and fully automated. A development of 40 cycles needs between 2 and 4 h. The AMD technique brings substances of a large polarity range to move and compresses substances in very narrow zones. This technique, like all gradient developments, increases the chromatographic resolution dramatically. It should be mentioned that a commercially available AMD system is able to separate more than 40 samples in parallel.

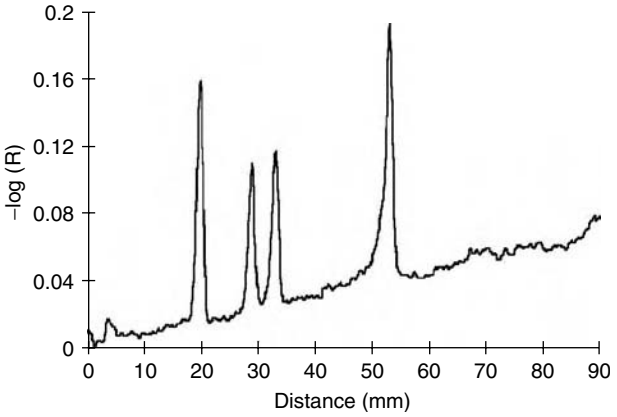

FIGURE 46.10 AMD separation of four pesticides, separated by use of a "universal" gradient [56]. Separated are 2,4-D (Aryloxyalkanoic acid), Methoxuron, Atrazine, and Parathion-ethyl.

The AMD technique is especially suited for pesticide monitoring because several pesticides can be simultaneously determined in the same sample [55–57]. An overview of AMD methods for the determination of pyrethrin and pyrethroid pesticide residues in crops, foods, and environmental matrices is given by Z.-M. Chen [57]. The combination of AMD and diode-array TLC-scanner is a powerful tool especially for pesticide monitoring because the photodiode array technique substantiates the analytical result by providing spectral confirmation for the appropriate substances.

IX. MYCOTOXINS

Mycotoxins are fungal metabolites, which are of great concern as toxic contaminants in food and feeds. Among a number of other techniques, planar chromatography is still very popular in the determination of certain mycotoxins. Sample preparation is necessary and is mostly an extraction step to clean up the sample and to concentrate the mycotoxins is proposed. In general, TLC can cope better with more crude extracts than HPLC where the clean-up step is more important to save the column. An important feature of TLC is its off-line operating principle. For instance, Leming Lin et al. [58] employed two development systems for the determination of zeraleone and patulin in maize. In the first step the lipids in the sample were isolated, and the second development is used to separate the mycotoxins. For mycotoxin visualization on plate two

different methods are frequently applied: the examination under UV light if the mycotoxins show naturally fluorescence and the spraying or dipping with chemical reagents that produce colored or fluorescent products. The spot colors and the R_f-values in combination with *in situ* measured spectra are used for mycotoxin identification. The natural fluorescence of aflatoxins, citrinin, and ochratoxin A makes its identification much more certain. A review from 1998 sums up more than 10 different mycotoxin TLC methods compared with other techniques [59]. The high sample throughput of TLC and HPTLC makes it the most cost-effective analytical technique for mycotoxins screening routine. A drawback of TLC in comparison with HPLC is its untapped potential for automation.

Ochratoxin A is a mycotoxin with nephrotoxic, carcenogenic, teratogenic, and immunosuppressive properties. A review of chromatographic methods for determination of ochratoxin A is given by H. Valenta [60]. The official AOAC method for ochratoxin determination in barley [61] recommends silica gel plates and the solvent mix benzene-methanol-acetic acid (18+1+1) [61]. The solvent system toluene-ethyl acetate-formic acid (5+4+1) can be used as well [62]. The blue-green fluorescence of the ochratoxin A, brought out by long-wave UV light, is used for detection. For densitometric fluorescence measurements an exication wavelength range from 310–340 nm and an emission range from 440–475 nm should be used. A detection limit of 25 μg/Kg rice is achieved [63, 64], whereas the quantification limit for HPLC is 0.2 μ/Kg [61, 63, 64].

A review of the chromatographic determination of patulin in fruits and fruit juices is given by G. S. Shepard [65]. Patulin has no teratogenic or carcenogenic effects but shows embryo toxicity accompanied by maternal toxicity. The official AOAC method of patulin determination in apple juice [66] is that the juice is extracted with ethyl acetate and cleaned up on silica gel column. The silica gel TLC plate is developed with toluene-ethyl acetate-90%-formic acid (5+4+1) and sprayed with 3-methyl-2-benzothiazolinone hydrazone-HCl solution (0.5 g in 100 ml H_2O) before the plate is heated for 15 min to 130°. Patulin appears as yellow brown spots at R_f 0.5 with a detection limit of 20 μg/L whereas HPLC methods show a detection limit of 0.5 μg/L [67] and a quantification limit of 5 μg/Kg [64]. The quantification limit for patulin in HPTLC is 50 μg/Kg [64].

The AOAC official method for zearalenone in [68] corn recommends $CHCl_3$-acetone (9+1) as the mobile phase for separation on a Si_{60} HPTLC plate with a quantification limit of 100 μg/Kg and an overall recovery of 97% [64, 67–69]. The appropriate HPLC quantification limit is 35 μg/Kg [64]. The separation of deoxynivalenol (DON) in wheat on silica gel 60 TLC plates with the mobile phase $CHCl_3$-acetone-isopropanol (8+1+1) is published in [70]. The developed plates have to be sprayed with $AlCL_3$ solution and heated 7 min to 120° to see blue fluorescent spots under long-wave UV at R_f 0.6. The detection limit is 37.5 mg/Kg for TLC [71] and 20 μg/Kg for HPLC [72]. Using toluene-ethyl acetate-formic acid (6+3+1) nephrotoxic citrinin and ochratoxin A can be separated and viewed under UV [73]. Citrinin can be separated from patulin and aflatoxin B_1, B_2, G_1, and G_2 in one run, using oxalic acid impregnated silica gel TLC plates. A limit of detection of 35 μg/Kg for citrin is achievable [74].

Fumonisins B_1, B_2, and B_3 were isolated in 1988 from corn and have been associated with cases of leucoencephalomalacea in equines, pulmonary oedema in swine, and hepatic cancer in rats [75]. A great majority of reports on fumonisin determinations use fluorescent derivatives for sensitive detection [76]. Using an immunoaffinity column for extract purification and an RP-18 HPTLC plate with 4% aqueous KCL-methanol (2+3) as the mobile phase, a fluorescamine derivation detection limit of 2 ng per spot is achievable [74]. This is an overall detection limit of 100 μg/Kg. The appropriate detection limit for HPLC is 10 to 500 μg/Kg [75].

Aflatoxins are considered to play an important role in the high incidence of human hepatocellular carcinoma in certain areas of the world [77]. There are official AOAC methods for aflatoxins in peanut products, corn, milk and cheese, liver, green coffee, and cotton seed. Mostly these methods refer to the TLC separation method for peanuts and peanut products using silica gel TLC plates and the solvent mixture acetone-$CHCl_3$ (9+1) [78]. Due to the natural fluorescence of all aflatoxins the overall detection limit for TLC methods is 5 μg/Kg [79] or better [80]. The HPLC overall detection limit for all four aflatoxins is published as better than 1 μg/Kg [59]. Quantification limits for aflatoxin B_1 are specified with 5 μg/Kg in corn [64] in comparison to 3 μg/Kg (in corn also) for HPLC [66]. Dell et al. [81] reports that for the aflatoxin determination in peanut butter the TLC method was more precise than HPLC. Tosch et al. compared TLC and HPLC methods and showed that TLC appeared to be equivalent to HPLC [82] with respect to precision, accuracy, sensitivity, recovery, and linearity of response.

One of the major advantages of TLC analysis is the simplicity of the separation step, which makes the method very cost effective. If densitometric quantifications are recommended, costs will arise. An interesting method to keep the analytical costs in densitometry low has been published by E. Anklam et al. [83, 84]. A simplified densitometer for the detection of aflotoxins was developed using a low-cost UV-light emitting diode (UV-LED) with a peak emission wavelength of 370 nm [83]. The resulting signal was further amplified by means of a commonly used operational amplifier integrated circuit and directly converted into a digital signal with a simple analogue-digital converter. The signal was recorded at the signal port (RS232) of a portable PC and processed with a widely used spreadsheet program.

The same working group developed a modified computer scanner by substitution of the scanner light tube with a black light tube and additional inclusion of a UV-filter. The modified scanner in combination with a personal computer can be used to determine aflatoxins at low nanogram levels, which, when used in combination with an appropriate TLC method, enables monitoring of the compounds in food and feed at the levels stipulated in European legislation [84]. Both systems are available for less than 2000 Euro and both methods can be expanded in combination with the appropriate TLC system to all mycotoxins that show long-wave UV fluorescence.

TLC is a well-established, easy-to-perform, fast, routine, cost-effective, and suitable method for analysis of a wide range of mycotoxins [59]. TLC will continue to play an important role in mycotoxin analysis because of the worldwide requirement for fungal metabolite control. Mycotoxins occurring in a matrix are usually very low quantity levels and the analysis method must be very sensitive. In general, all mycotoxin TLC methods show detection limits or quantification limits 10-fold higher than HPLC values. Nevertheless the FDA regulatory level for aflatoxin in human food and for feed for immature livestock is 20 μg/Kg. The appropriate value for deoxynivalenol is 1000 μg/Kg. HPTLC is able to check these levels in feed and food as well as HPLC. The advantage of HPTLC methods has been described by B. Renger [85]. An assay and purity testing of phospholipids HPTLC is only 38% of the HPLC costs, a value that can surely be adapted to TLC food analysis.

X. FUTURE OF PLANAR CHROMATOGRAPHY

What is the future of TLC and HPTLC? First of all, TLC methods that are able to check official regulatory levels without using technical equipment will of course remain in use. This simple and inexpensive way of doing analysis is too effective to discontinue. It must also be kept in mind that not all countries have or will have the resources to check official regulatory levels using expensive methods like HPLC. A low-cost flat-bed scanner in combination with a personal computer for documentation and quantification purposes will be used. HPTLC methods are strong in screening and will play an increasing role in the field of pesticides and drug monitoring in feed and food. The combination of AMD and diode-array scanner technology, using spectral libraries for sample identification and checking of regulatory levels, will increase in importance. The quantification ability of HPLC will be used for the determination of critical analyts like sugars, the quantification of which can be easily performed by NH_2 layers. The simple pre-cleanings of caffeine, quinine, and sucralose® in beverages or vanillin in chocolate will probably be used for quantification purposes to keep the overall standard deviation low. All in all, TLC and HPLC methods will find niches where the chromatography is more cost-effective than other methods or the performance is so outstanding that no other assays will show comparable results.

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47 High Performance Liquid Chromatography

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CONTENTS

I. GENERAL

According to the IUPAC definition (1) chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (the stationary phase) while the other (the mobile phase) moves in a definite direction.

The mobile phase is a fluid which percolates through or along the stationary bed. Chromatography is a differential migration technique where the flux of solutes to separate is perpendicular to the deplacement of the mobile phase (2).

Three types of fluid are in current use: liquid, gas, or supercritical. Chromatography is named principally by the nature of the fluid. We can distinguish liquid, gas, or supercritical fluid chromatography.

The stationary phase may be a solid or a liquid. The combination of mobile and stationary phases unambiguously names the chromatographic mode.

Mobile phase	Gas	Liquid	Supercritical
Stationary	Liquid	Liquid	Liquid
Phase	Solid	Solid	Solid

Gas-liquid chromatography (GC) and liquid-solid chromatography are by far the most popular types. A special type of stationary phase consists of bonding chemical moieties onto a solid surface by covalent bonds. It is a bonded phase. Liquid chromatography with alkyl (or any other) bonded phases is considered liquid-solid. Use of liquid-liquid chromatography is rapidly declining and reports on the technique in recent years are scarce.

According to the nature of the stationary phase a chromatographic mode is often named by the chemical species which governs the retention mechanism.

We can thus distinguish in liquid chromatography (LC):

Adsorption chromatography, often referred to as normal phase mode (NP)
Reversed phase chromatography (on alkyl bonded silica) (RP)
Ion chromatography (IC)
Affinity chromatography
Size exclusion chromatography (SEC)
Chiral chromatography
Micellar liquid chromatography

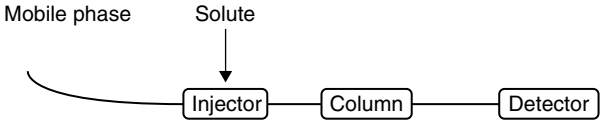
High performance liquid chromatography (HPLC) is a well-established method in modern analysis. The method is simple, robust, and applicable to the majority of components to be analyzed. Low efficiency and small peak capacity are of HPLC when complex mixtures have to be separated. In contrast to GC the diffusion coeficient of the analytes is small and that is the biggest obstacle to efficient separations.

II. BASIC CHROMATOGRAPHIC INSTRUMENTATION

The heart of the separation is the column. Solutes are injected onto the column where they partition between the mobile and the stationary phase. When a solute is flowing off the column it is eluted.

Typically a chromatograph is depicted as follows:

Solutes to separate are placed in an injector, they are driven to the separation column; when they elute, they are monitored by a detector.



There are two ways to perform chromatography: analytical mode and preparative mode. We shall only consider the analytical mode in which solutes are infinitely diluted.

Molecules of solute which do not interact with stationary phase are not retained. Molecules of solute that can partition between both phases are retained. Detection and recording of separated solutes yield a chromatogram (Figure 47.1). On the chart solutes appear as peaks, the shape of which is nearly gaussian.

III. DEFINITIONS

The column volume is Vcol; the volume of the stationary phase inside the column is Vs; the volume of the mobile phase is V_M, which can be divided in extraparticular volume Ve and intraparticular volume Vi according to the type of the packing; and the porosity is $\varepsilon = V_M / Vcol$.

t_M is the mobile phase hold-up time; this is not the same as the retention time of the unretained solute, written as t_0. In fact, t_0 and t_M are not equal (especially in packed columns) and the nature of the unretained (or inert) solute should be mentioned in any reported experimental conditions. t_{Ri} is the retention time of the solute i.

The volume of the mobile phase required to elute an unretained solute is called V_0.

$$t_0 = \frac{V_0}{F}$$

where F is the mobile phase flow rate. $F = u_o \varepsilon S$ where u_o is the linear velocity of the liquid; ε is the porosity; and S is the cross section of the column. $t_0 = L/u_o$. Similarly, V_{Ri} is the retention volume of solute i.

The mobile phase must flow through the column. In packed columns Darcy's law (3) is written

$$u_0 = B_0 d_p^2 \Delta P / \eta L$$

B_0 is the permeability of the column packed with particles of average size d_p; ΔP is the pressure difference between inlet and outlet of the column; η is the viscosity of the mobile phase; and L is the column length.

The retention factor is

$$k_i = \frac{(t_{Ri} - t_0)}{t_0} = \frac{(V_{Ri} - V_0)}{V_0} = \frac{(d_{Ri} - d_0)}{d_0}$$

where d_0, d_R are retention distances measured on the recording chart.

The retention factor is also equal to the ratio of amounts of a sample component in the stationary and mobile phases, respectively, at equilibrium.

$$k = \frac{\text{Number of solute molecules in stationary phase}}{\text{number of solute molecules in mobile phase}}$$

$k = K(Vs/V_M)$ where K is the partition coefficient and Vs/V_M is the phase ratio.

The adjusted retention time is $t'_{Ri} = t_{Ri} - t_0$ and $k = t'_{Ri}/t_0$. Similarly,

$$V'_{Ri} = V_{Ri} - V_0$$

$$d'_{Ri} = d_{Ri} - d_0$$

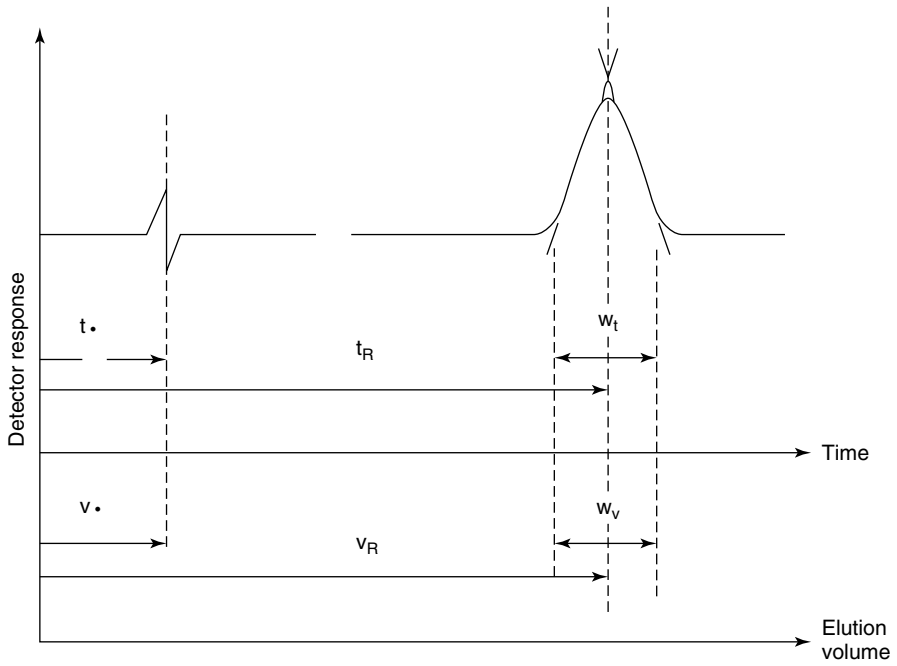


FIGURE 47.1 A chromatogram.

In analytical mode, peaks are presumed to be gaussian and retention parameters are taken at maximum peak heights.

When peaks are not truly gaussian, it is necessary to use statistical moments. The zero moment

$$M_0 = \int_b^a h(t) \cdot dt$$

where h(t) is the peak height at time t yields the peak area.

The first moment $M_1 = (1/M_0) \int_b^a t \cdot h(t) \cdot dt$ expresses the true retention time as it corresponds to the elution time of the center of gravity of the peak.

$$M_2 = \text{peak variance}$$

$$M_3 = \text{peak skew}$$

When two consecutive (i, j) gaussian peaks are close, the

$$\text{resolution is } Rs = 2\,(t_{rj} - t_{ri})/(\omega_i + \omega_j)$$

ω is the peak width in time units (see Figure 47.2).

When peaks are gaussian $\omega = 4\sigma$ (σ is the standard deviation).

When two peaks are well resolved then $Rs \geq 1.25$ (see Figure 47.2).

Resolution can be written $Rs = (\sqrt{N/4})(\Delta u/\bar{u})$ where Δu is the difference in velocity between two solutes i and j, $\bar{u}$ is the average velocity, and N is the efficiency.

Assymetry is determined by the A/B ratio (Figure 47.3).

Selectivity a is defined as $\alpha = k_j/k_i$. In this way $\alpha \geq 1$; when $\alpha = 1$ no separation occurs.

When solutes are late-eluting it is convenient to use gradient elution. The gradient retention factor is

$$k^* = t_G F/\Delta\%B V_M S$$

where t_G is the gradient time in min, F is the flow-rate in ml/min, $\Delta\%B$ is the gradient range (e.g., $\Delta\%B = 0.80$ for a 20–100% gradient), V_M is the column volume in ml, and S is a constant for a given compound (see below).

Sharp peaks are indicative of the efficiency of the chromatographic process since the variance is small. Efficiency is also called plate number.

$$N = \left[\frac{t_R}{\sigma_t}\right]^2 = \left[\frac{V_R}{\sigma_V}\right]^2 = \left[\frac{d_R}{\sigma_d}\right]^2$$

When peaks are gaussian $N = 16(t_R/\omega)^2$:

$$N = 5.54\left[\frac{t_R}{\delta}\right]^2$$

δ is the peak width at mid-height. When peak tailing occurs, it is convenient to use the Dorsey-Foley equation (4) from an exponentially modified gaussian equation.

The area of a gaussian peak is a function of its standard deviation and peak height according to the equation

$$A = \sqrt{2\pi\sigma h_p},\ h_p \text{ is the peak height}$$

$$\text{From } N = \left[\frac{t_r}{\sigma}\right]^2$$

and rearranging

$$N = \frac{2\pi(h_p t_r)^2}{A^2}$$

The plate height H is the column length divided by the plate number N = L/H

$$\text{Number of effective plates } N_{eff} = 16\left[\frac{t'_R}{\omega}\right]^2$$

The above equations can be combined. If $\bar{k} = \frac{(k_i + k_j)}{2}$ and assuming peaks are close enough,

$$Rs = \frac{\alpha - 1}{\alpha + 1} \frac{\bar{k}}{1 + \bar{k}} \frac{\sqrt{N}}{4}$$

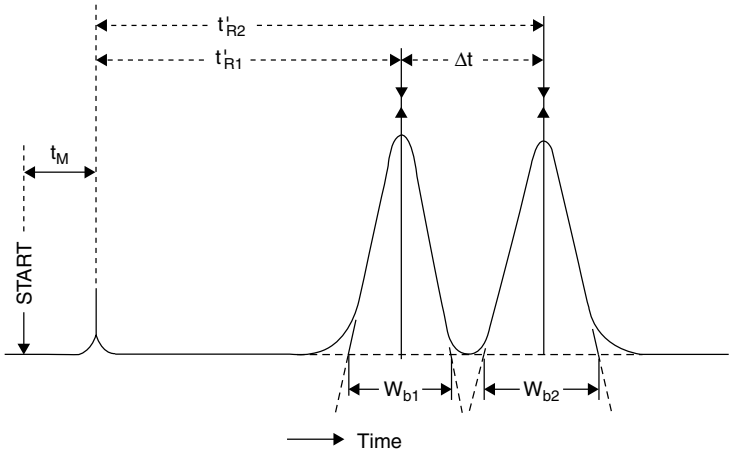


FIGURE 47.2 Resolution between two gaussian peaks: t is retention time, ω is peak width.

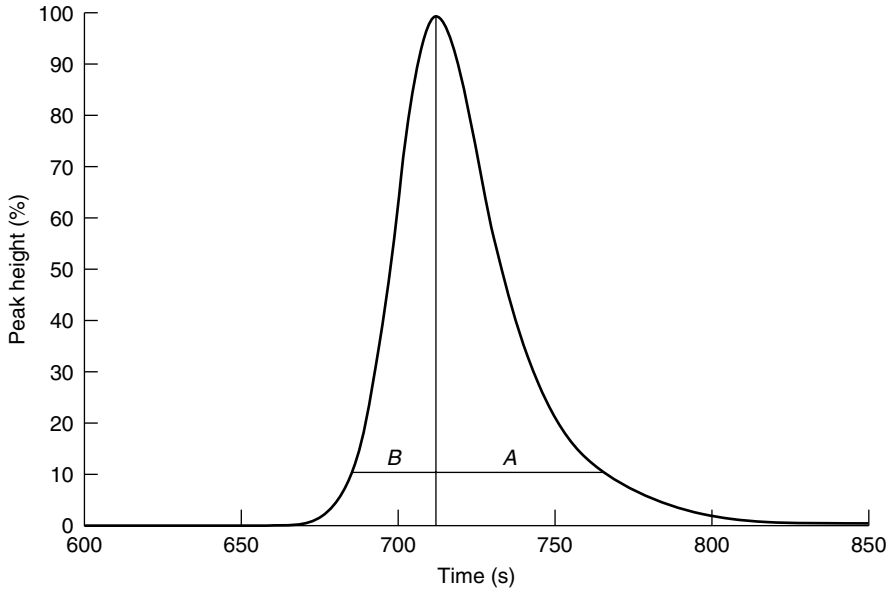


FIGURE 47.3 Asymmetry factor by determination of A/B ratio at 10% height.

A slightly different equation is written as

$$N = 16\,Rs\left[\frac{\alpha}{\alpha - 1}\right]^2\left[\frac{1 + \bar{k}}{\bar{k}}\right]^2$$

The equation permits us to determine the number of plates required to achieve the separation between two solutes of retention factors k_i and k_j, respectively, with a given Rs.

There are three factors in this equation which together determine the chromatographic resolution. The overall resolution is affected (assuming that the column length L is constant) by the selectivity term ($\alpha/\alpha-1$), the retention term factor ($k/1+k$), and the plate height H. Larger k values may lead to an increase in resolution at the expense of longer run times and wider bands. It is best to set k values between 1 and 6. The resolution power is the number of plates necessary to separate two solutes of relative retention α with a resolution Rs.

$$\alpha = [1 - 4Rs/\sqrt{N.1}+k/k]^{-1}$$

The height equivalent to a theoretical plate (HETP) is dependent on the flow rate of the mobile phase. A slightly different equation from the well-known Van Deemter Equation (5) is valid in LC, the Knox Equation (6).

$$h = A\nu^{1/3} + B/\nu + C\nu$$

where h is the reduced plate height $h = H/d_p$; ν is the reduced velocity, $\nu = u_0 d_p/D_m$ where D_m is the diffusion coefficient of the solute in the mobile phase; and d_p is the average particle diameter.

The peak capacity is the number of peaks which can be observed on a chromatogram with baseline resolution (sometimes with unit resolution) assuming the constant N.

$$n_p = 1 + \frac{\sqrt{N}}{4}\log\frac{t_{Rz}}{t_{r1}}$$

In this equation t_{Rz} is the retention time of the last eluted solute. t_{r1} is the retention time of the first eluted solute.

Capacity of a column is the maximum amount of sample that can be injected into a column before significant peak distortion occurs.

Peak distortion is measured by either peak skew or by asymmetry factor.

IV. INSTRUMENTATION

Despite the trend towards miniaturization, most instruments are "conventional." A scheme of a LC instrument is displayed in Figure 47.4.

It consists of:

Solvent reservoirs
A solvent delivery system
An injection device
The column
A detector
A data acquisition system.

A. Solvent Reservoirs

Solvent reservoirs are usually made of glass. They should be equipped with degassing device. Degassing with helium is usually performed but it may form slugs of helium in the tubing.

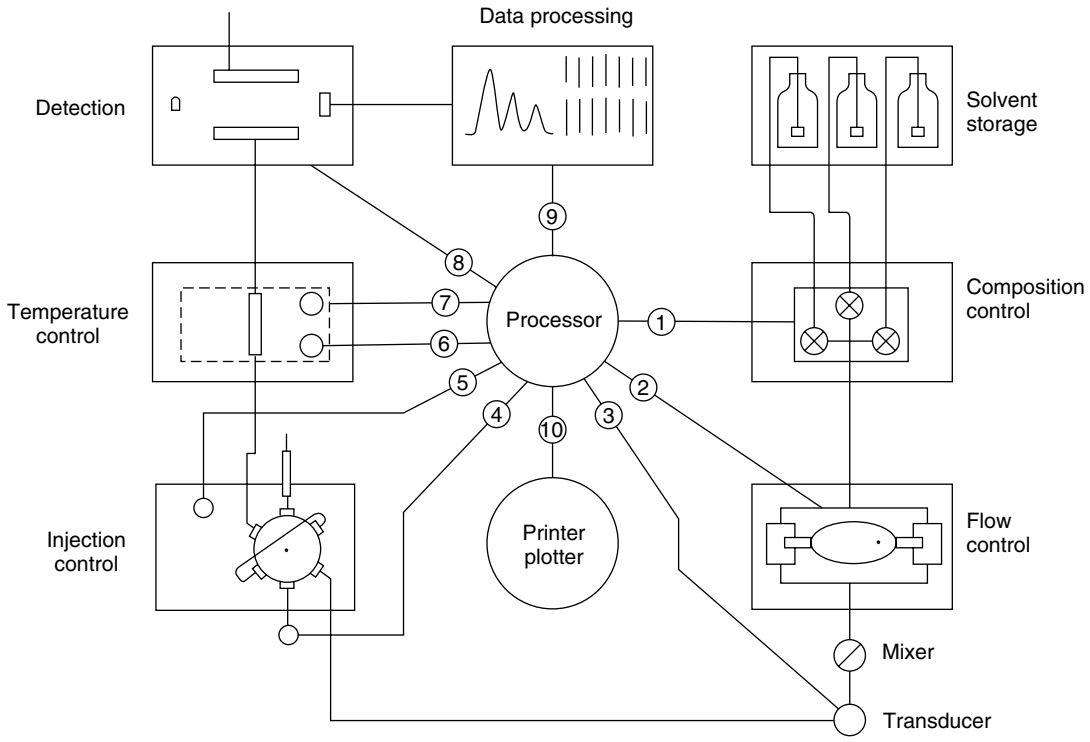


FIGURE 47.4 A scheme of a LC instrument.

Solvents are mixed in a mixing chamber. To account for the desired composition solenoid valves are actuated. Solvents should be HPLC grade. Careful attention should be paid to water and preparation of buffers.

B. Solvent Delivery System

Reciprocating pumps are almost exclusively utilized with conventional columns. They exhibit large column back pressure compensation abilities, flow rates in the 0.1–10.0 ml range with high precision. Precise control of flow rate is of primary importance to ensure reproducibility of retention times. In some cases titanium or ceramic head pumps ensure biocompatibility. With open tubular columns, split flow techniques are probably best. Flow splitting devices are based on the microflow processor concept.

At present, most commercially available pumping units for conventional HPLC are the single or multihead reciprocating piston type. There is a trend towards very high pressures (up to 1200 bar) but that is achieved by modifying a commercially available pump.

Requirements for solvent delivery systems are as follows:

Flow rate stability
Flow rate accuracy (usually ± 0.3%)
Flow rate reproducibility
Large flow rate range (from 0.1 to 10 ml/min in HPLC)
Compatibility with any liquid
Standing with high pressures

In capillary LC, syringe-style, positive-displacement, continuous delivery design delivers solvent at the low-microliter flow rates that are optimal for micro-LC-MS but eluent compressibility and mixing of microflows are serious drawbacks.

1. Gradient Formation

Increasing the solvent strength permits us to achieve elution of highly retained solutes. Moreover, optimization procedures such as Drylab G make use of two gradients.

Two methods can generate binary solvent gradients: low pressure mixing or high pressure mixing. Some manufacturers display both. In the low mixing method, two solvent reservoirs and one single pump are used. In high pressure mixing mode two pumps separately deliver the required volumes of solvents.

The most critical points are the electronic control units and the mixing chamber.

Linear or curved shape gradients are possible but linear gradients are used by the vast majority of chromatographers. There is obviously a time delay between the solvent composition programmed and the actual composition at the column inlet due to the dwell volumes. Gradient delay and rounding are observed. Blank runs are carried out with the injector directly connected to a UV detector. Mobile phase

A is UV transparent and mobile phase B contains a slight proportion of UV absorbing solvent (1% acetone in methanol, for example). The gradient is performed and the delay time is determined. This is of primary importance to obtain reliable retention times.

Gradient delay volume is the volume between the start of the gradient and the top of the column.

Gradient delay time is the time elapsed between the start of the gradient and the time it reaches the top of the column.

C. Column

Most commercially available columns are 4.6 mm inside diameter P (i.d.). They are made of stainless steel and the inner wall of the tubing is electropolished. Stainless steel tubing is easily machined and compression fittings can be affixed readily to ensure high pressure seals. Among the alternatives are polytetrafluoroethylene (PTFE)-lined stainless steel, polyetheretherketone (PEEK), hasteloy, and titanium. Metal-free systems are desirable for ion chromatography and the analysis of trace metals by ion exchange. Variable lengths are available (10-cm-long columns are most popular). Columns or cartridges can be serially connected to increase the plate number. Columns are packed with 3 μm or 5 μm particles either spherical or irregular shaped. Particles are either porous or non-porous. Silicagel is still the mainstay base material for most HPLC packings. Silicagel is rigid, provides excellent efficiency, is reasonably priced, and can be functionalized by virtue of the reactive silanols (SiOH). Most commercial silica-based analytical HPLC materials are made of ultra-pure monodisperse silica. In a porous particle solutes transfer from the moving mobile phase outside the particle into the stagnant mobile phase within the pores to interact with the stationary phase. The slow rate of mass transfer into and out of the porous particles is a major source of band broadening. The non-porous packings are less and less in use due to the limited sample capacity; on the one hand the thin layer allows faster mass transfer; on the other hand, the back pressures are high. Superficially porous packings (e.g., Poroshell from Agilent) are recommended for large biomolecules. Polymeric materials are used by approximately 15–20% of liquid chromatographers. Similar to silicagel, polymeric packings are also available with a variety of particle diameters. Polystyrene-divinylbenzene (PS-DVB) is the most popular with polymethacrylates. The PS-DVB polymers have a wide pH range, high cross-linking, withstand average pressure, and have no silanols to interact with the basic compounds. Conversely, the efficiency provided cannot compare with silica-based material since the rate of mass transfer is slower.

Copolymers of organofunctional silanes and tetraethoxysilane are a new means to synthesize of HPLC packings.

Zirconia provides some interesting features since it can be produced in monodisperse porous spherical particles, withstand a pH of 14, and has no silanols. However, it exhibits hard Lewis acid sites and consequently has a strong affinity towards Lewis bases (hydroxyls, phosphate). To overcome this drawback the analyst must use a competing anion or, even better, a polymer-coated, zirconia-based stationary phase.

Manufacturers provide a test chromatogram and ensure reproducibility from batch to batch.

An increase in column performances can be achieved by increasing the column permeability. The use of monolithic columns is a convenient way to overcome the drawback of the back pressure generated by small particles (see Darcy's equation above). Moreover, in the search for new stationary phase configurations with enhanced mass-transfer properties, the concept of monolithic stationary phases was established in which the separation medium consists of a continuous rod that has no interstitial volume but only internal porosity consisting of micro- and nanopores. Four approaches have been utilized to prepare continuous beds: polymerization of an organic monomer with additives, formation of a silica-based network using a sol-gel process, fusing the porous particulate packing material in a capillary by a sintering process, and organic hybrid materials. Polymer-based monolithic chromatographic supports are usually prepared by the *in situ* polymerization of suitable monomers and porogens within a tube that acts as a mold. Polymer monoliths are Polystyrene-divinyl benzene, polyacrylamide, or polymethacrylate (7–9). Silica-based monoliths were pioneered by Nakanishi and coworkers (10–12). Silica-based monolithic columns were released in 2000 by Merck. They consist of a single rod of silica-based material embedded in a PEEK tubing (13, 14). The new sorbent material consists of monolithic cylindrically shaped rods of highly porous metal-free silica. The defined homogeneous bimodal pore structure, produced during the manufacturing process, means that the columns possess a unique combination of a large internal surface area, over which quick chemical adsorption can take place, together with a significantly higher total porosity.

The pressure drop is dramatically decreased and longer columns can be used. These monolithic columns possess about 15% higher porosity as compared to particulate ones. Monolithic columns are 10 cm long and can be connected in series.

To reduce solvent consumption, there is a trend towards microcolumns, minicolumns, or true capillary columns.

Columns which exhibit internal diameter in the range 0.5–1.0 mm are called microcolumns (they were formerly called microbore). Good efficient microcolumns can be produced at the present time because the packing procedure has been optimized.

Capillary LC columns are of 10–500 μm internal diameter. It seems that 50 μm i.d. packed with 8 μm particles exhibit the best performances.

From theoretical papers published in the 1980s, open tubular columns in LC can only match performances of packed columns if the diameter of the column is of the same order of magnitude of the particle diameter in conventional columns. This conclusion has led to nanoscale LC with 5–11 μm i.d. open tubular columns which are used in research laboratories but not yet available for routine use.

The current trend of lab on a chip will result in new packing materials. Packing a stationary phase in microchannels looks impossible but microfabrication by ablation is promising (15).

Guard columns (1 cm long) must be connected to the analytical column to increase the lifetime, especially when dealing with complex samples. Guard columns, which fit between the injector and the analytical column, collect debris and stongly retained components. Two types of guard columns are available: those that are separate with their own holders and those that are integrated with the analytical column. A recent improvement is the use of guard discs that can be replaced periodically.

D. Injection

For manual and automated injection, the majority of the injection systems consist of injection valves. Sample loops are usually 10 μL for injection with a conventional column. In LC the analyst can increase the volume of injection without disturbing the separation efficiency. Conversely, the analyst must prevent any mass overloading. If so, partition of the solute is not performed in the linear portion of the isotherm with the consequence of peak tailing. Autosamplers are time-saving devices; design and associated problems were thoroughly discussed by Dolan (16,17).

E. Detectors

Some requirements of a LC detector are that it be of such a design that the separated components are not remixed while passing through the detector cell; have a low drift and noise level; have a fast response time; have a wide dynamic range; be relatively insensitive to changes in mobile phase flow rates and temperature; and be easy to operate and reliable.

There are three different types of detector noise: short-term noise, long-term noise, and drift.

1. Photometric Detectors

They rely on Beer's law. The Beer Lambert law is expressed as
where

$$A = \varepsilon \cdot l \cdot c$$

ε = molar absorptivity of the solute
l = path length through the sample
c = concentration of solute
A = absorbance

a. UV-VIS

These detectors measure changes in absorbance of light in the 190–350 nm region or 350–700 nm region.

Basic instrumentation includes a mercury lamp with strong emission lines at 254, 313, and 365 nm, cadmium at 229 and 326 nm, and zinc at 308 nm. Deuterium and xenon lamps exhibit a continuum in the 190–360 nm region which requires the use of a monochromator. A filter or grating is used to select a specific wavelength for measurement. Cut-off filters pass all wavelengths of light above or below a given wavelength. Band-pass filters pass light in a narrow range (e.g., 5 nm). In the single beam mode, the energy from the source lamp passes through the sample flow cell to a photocell via some wavelength selection device. The double beam system is preferred.

The flow cell is typically 8 μL with a 10-mm path length.

The photodiode array (see below) is currently the best sensor.

According to Beer's law, the higher the path length, the higher the transmitted light. For that purpose most cells are Z shaped.

With capillaries such as LC capillaries or CE capillaries there is only limited path length. A free portion of capillary is brought into the light path of a UV absorbance detector. When the aperture of the source is adjusted to the inside diameter of the capillary the effective light path is

$$l_{eff} = 1/2\pi r$$

where r is the radius of the capillary.

To improve the transmitted light a ball is placed after the capillary to focus the beams.

The limit of detection is highly dependent on the molar absortivity of the solutes ε (see Beer's law). UV-VIS detectors must be checked for wavelength accuracy, absorption accuracy, scattered light, and spectral resolution. Derivative spectra are obtained by differentiating the absorbance (A) spectra of the sample with respect to wavelength.

First, second, or higher-order derivatives may be generated. All the derivatives emphasize the features of the original spectra by enhancing small changes in slope.

Derivative spectra are generated optically, electronically, or mathematically. The usual optical method utilizes the wavelength modulation technique where wavelength of incident radiation is rapidly modulated over a narrow wavelength range. Electrically, derivatives are performed by analog resistance capacitance devices. Mathematical methods are obviously the best performing. The Savitsky Golay algorithm generates derivatives with a variable degree of smoothing.

Derivatives are used to

Enhance spectral differences
Enhance resolution
Selective substraction.

A photodiode array (PDA) is shown in Figure 47.5.

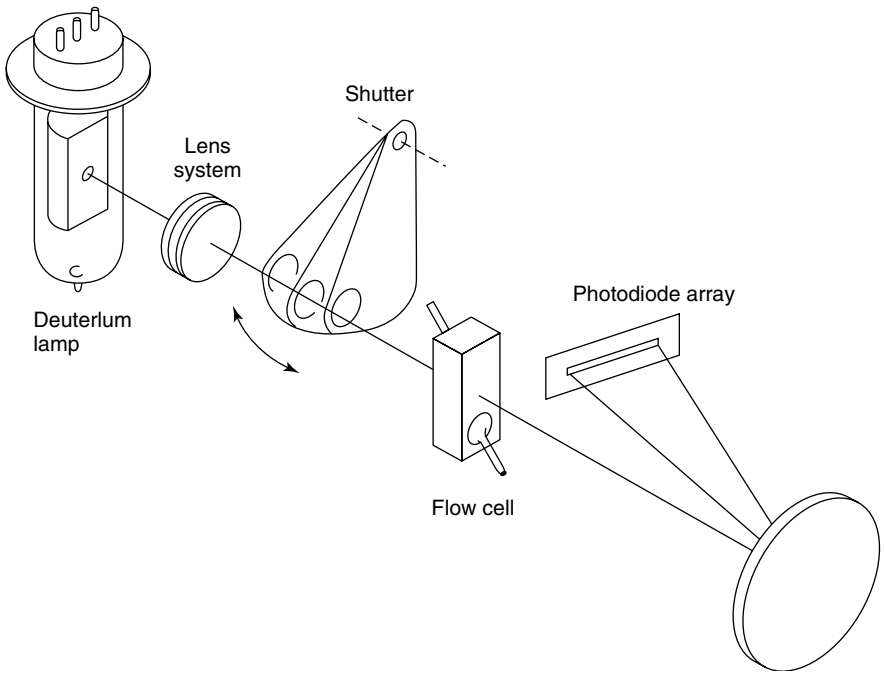


FIGURE 47.5 A photodiode array.

The detection of structurally similar impurities eluting simultaneously with the analytes of interest is a problem. The analyst must detect the existence of peaks of interest, determine the extent of their purity, and confirm their identity.

Photodiode operation relies on the photovoltaic effect. In the typical photodiode there are two components of semiconductor called P and N. P is a very pure silicon with low levels of three valent impurities such as boron or gallium. Each impurity atom can accept an electron from the valence bonds, giving rise to a hole that can take part in the electrical conduction process and an immobile negatively charged impurity ion. Since the hole is positively charged such a material is a P (positive) silicon crystal. If the impurity added is a pentavalent atom (As) the atoms behave as donors of electrons that can move through the entire silicon crystal. It is thus a N (negative) type. A photon of wavelength less than 1.1 nm is able to break a covalent bond between the silicon atoms. The free electron formed is free to move with the missing electron in the broken valence bond which induces electrical conduction by repeated replacement. The PDA detector passes the total light through the flow cell and disperses it with a diffraction grating. The dispersed light is measured by an array of photosensitive diodes. The array of photodiodes is scanned by the microprocessor (16 times a second is usual). The reading for each diode is summed and averaged. A PDA detector can simultaneously measure the absorbance of all wavelength versus time. The amount of data storage is a key feature in PDA. A run can easily take several megabytes of data storage. The dynamic range is usually 0.5 mAU–2.0 AU. Each pixel spans 1.25 nm. Noise is around + or − $1.5*10^{-5}$ absorbance units (AU) with a 2 sec time constant.

Peak purity is based on the proprietary spectral contrast algorithm which converts spectral data into vectors that are used to compare spectra mathematically. This comparison is expressed as a purity angle. The purity angle is derived from the combined spectral contrast angles between the peak apex spectrum and all other spectra within that peak. To determine peak purity, the purity angle is compared to the purity threshold. For a pure peak the purity angle will be less than the purity threshold.

Spectral deconvolution techniques are used when two peaks co-elute.

Identification of peaks is performed by comparison with spectra contained in a library of standards.

b. Fluorescence detection

The quantum yield ϕ is a fundamental molecular property that describes the ratio of a number of photons emitted to the number of photons absorbed.

$$\phi = \frac{k_f}{k_f + \Sigma k_d}$$

k_f is the rate constant for fluorescence emission. Σk_d is the sum of the rate constants for all the nonradiative processes which can depopulate S_1.

The signal intensity I_f is given by Beer's law:

$$I_f = I_o(1 - e^{\varepsilon lC})\phi k$$

When sample absorbance is small this expression is reduced to

$$I_f = I_0 \cdot 2 \cdot 3 \cdot \varepsilon \cdot 1 \cdot C \cdot \phi k$$

k is the instrumental efficiency for collecting the fluorescence emission. I_o is the intensity of the incident radiation.

Fluorescence emission provides more selectivity and increased sensitivity compared to UV absorption. Attomole detection is possible. Xenon lamps are far superior to Hg lamps or D_2 lamps as light sources. Selection of excitation or emission wavelength is done by a monochromator.

Laser-induced fluorescence is in current use. Various lasers are utilized (He-Cd diode, Argon ion). The diode laser seems the best choice. Due to the highly collimating nature of lasers most scattering sources are eliminated. Increased detection is carried out with pre- or post-column derivatization. A three-dimensional spectrofluorometer incorporating a charge-coupled-device has been described (18) that made possible the collection of fluorescence excitation-emission intensity data of polycyclic aromatic hydrocarbons in a flow stream in 0.05 s with ng/milliter limits of detection.

Chiroptical detection provides additional qualitative and quantitative information for photometric detection since only chiral compounds are monitored. A detector provides both the differential absorbance of the circular dichroism and the absorbance of the UV mode simultaneously as a function of time (19).

c. Chemiluminescence method

Oxidative combustion of nitrogen-containing compounds produce nitric oxide. Nitric acid when in contact with ozone produces a metastable nitrogen dioxide molecule

$$NO + O_3 \longrightarrow NO_2^* + O_2$$

which relaxes to a stable state by emitting at a wavelength of 700–900 nm

$$NO_2^* \longrightarrow NO_2 + h\nu$$

Chemiluminescence is used for specific detection of nitrogen.

d. Derivatization

Many solutes do not exhibit UV absorption or fluorescence; they can be converted into UV absorbing or fluorescent derivatives by pre- or post-column derivatization. When precolumn derivatization is carried out the chromatographic system is obviously different fom the one selected for the non-derivatized solutes. A large volume of literature deals with post-column reactions. They can be carried out in coils, in packed bed reactors, or by photolysis. The main requirement is not the completion of the reaction but the reproducibility. Reaction vessels should not produce excessive band broadening. Table 47.1 displays some derivatizing reagents for the fluorescence labeling of functional groups.

2. Electrochemical Detection

a. Conductivity

The detection method is based on the application of an alternative voltage E to the cell electrodes. The cell current is directly proportional to the conductance G of the solution between the electrodes by Ohm's law.

$$G = \frac{1}{k} = \frac{i}{E}$$

The measured conductivity is the sum of individual contribution to the total conductivity of all the ions in solution. Kohlrausch's law states that

$$k = \frac{\Sigma_i \lambda_i^0 C_i}{1000}$$

C_i is the concentration of each ion i, and λ_i^0 is the limiting equivalent conductivity which is the contribution of an ion to the total conductivity divided by its concentration extrapolated to infinite dilution.

Kohlrausch's law is only valid in dilute solutions (chromatography or electrophoresis). The magnitude of the signal is greatest for small high mobility ions with a multiple charge such as sulphate. The conductivity detector was the breakthrough in ion chromatography (IC).

Early IC systems detected ions eluted by strong eluents from high capacity ion exchange columns by measuring changes in conductivity. To achieve reasonable sensitivity, it was necessary to suppress the conductivity of eluent prior to detection in order to enhance the overall conductance of the analyte and lower the background conductance of the eluent. This was achieved by a "suppressor" column in which counter ions were exchanged with H^+ or OH^-. Due to excessive band-broadening column suppressors are no longer in use. Membrane based devices are utilized. The membrane suppressor incorporates two semi-permeable ion exchange membranes sandwiched between sets of screens. The eluent passes through a central chamber. Regenerant flows in a counter-current direction over the outer surfaces of the membranes providing constant regeneration. Electrolysis of water produces hydrogen or hydroxide ions required for regeneration. There is no contamination with carbonate (Figure 47.6).

In conductivity detectors the change in conductivity Δk depends on the concentration of the injected ion (A) and its equivalent ionic conductivity λ_A compared with that of the eluent ion λ_E.

$$\Delta k = (A) \cdot (\lambda_A - \lambda_E)$$

Conductivity detectors usually operate at 10 kHz; scale ranges are 0.01 to 5000 μS in 12 steps.

TABLE 47.1
Derivatizing Reagents for the Fluorescence Labeling of Functional Groups

Reagent	Abbreviation	Functional Group
Aminoethyl-4-dimethylaminonaphthalene	DANE	Carboxyl
4-(Aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole	ABD-F	Thiol
Ammonium-7-fluorobenzo-2-oxa-1,3,diazole-4-sulfonate	SBD-F	Thiol
Anthracene isocyanate	AIC	(Amine), hydroxyl
9-Anthryldiazomethane	ADAM	Carboxyl (and other acidic groups)
Bimane, monobromo-	mBBr	Thiol
Bimane, dibromo-	bBBr	Thiol
Bimane, monobromotrimethylammonio-	qBBr	Thiol
4-Bromo-methyl-7-acetoxycoumarin	Br-Mac	See Br-Mmc
4-Bromo-methyl-7-methoxycoumarin	Br-Mmc	Carboxyl,imide, phenol, thiol
N-Chloro-5-dimethylaminonaphthalene-1-sulfonamide	NCDA	Amine (prim., sec.), thiol
9-(Chlormethyl)anthracene	9-CIMA	See Br-Mmc
7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	NBD-Cl	Amino (prim., sec.), phenol
2-p-Chlorosulfophenyl-3-phenylindone	DIS-Cl	Amino acids, amino sugars
9,10-Diaminophenanthrene	9,10-DAP	Carboxyl
2,6-Diaminopyridine-Cu^{2+}	2,6-DAP-Cu	Amines (prim. aromatic)
4-Diazomethyl-7-methoxycoumarin	DMC	See ADAM
5-Di-n-butylaminonaphthalene-1-sulfonyl chloride	Bns-Cl	See Dns-Cl
Dicyclohexylcarbodiimide	DCC	Carboxyl
N,N′-Dicyclohexyl-O-(7-methoxycoumarin-4-yl)methylisourea	DCCl	Carboxyl
N,N′-Diisopropyl-O-(7-methoxycoumarin-4-yl)methylisourea	DlCl	Carboxyl
4-Dimethylaminoazobenzene-4′-sulfonylchloride	Dbs-Cl	See Dns-Cl
N-(7-Dimethylaminoazobenzene-4′-methyl-3-coumarinylmaleimide	DACM	Thiol
5-Dimethylaminonaphthalene-1-sulfonyl-aziridine	Dns-aziridine	Thiol
5-Dimethylaminonaphthalene-1-sulfonylchloride	Dns-Cl	Amino (prim., sec., tert.), (hydroxyl), imidazole, phenol, thiol
5-Dimethylaminonaphthalene-1-sulfonyl-hydrazine	Dns-hydrazine	Carbonyl
4-Dimethylamino-1-napthoylinitrile	DMA-NN	Hydroxyl
9,10-Dimethoxyanthracene-2-sulfonate	DAS	Amine (sec., tert.)
2,2′-Dithiobis (1-aminonaphthalene)	DTAN	Aromatic aldehydes
1-Ethoxy-4-(dichloro-s-triazinyl)naphthalene	EDTN	Amine, hydroxyl (prim.)
9-Fluorenyl-methylchloroformate	FMOCCl	Amine (prim., sec.)
7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole	NBD-F	Amine (prim., sec.), phenol, thiol
4′-Hydrazino-2-stilbazole	—	α-Oxo acids
4-Hydroxymethyl-7-methoxycoumarine	Hy-Mmc	Carboxyl
4-(6-Methylbenzothiazol-2-yl)-phenyl-isocyanate	Mbp	Amine (prim., sec.), hydroxyl
N-Methyl-1-naphthalenemethylamine	—	Isocyanates (aliphatic, aromatic)
1,2-Naphthoylenebenzimidazole-6-sulfonyl chloride	NBl-SO2Cl	See Dns-Cl
2-Naphthylchloroformate	NCF	Amine (tert.)
Naphthyl isocyanate	NlC	(Amine), hydroxyl
Ninhydrin	—	Amine (prim.)
4-Phenylspiro(furan-2(3H), 1′-phthalan)-3,3′-dione (fluorescamine)	Flur	Amine (prim., sec.), hydroxyl, (thiol)
o-Phthaldialdehyde (o-phthalaldehyde)	OPA	Amine (prim., sec.), thiol
N-(1-Pyrene)maleimide	PM	Thiol

(Reproduced from Journal of Planar Chromatogarphy. With permission.)

b. Amperometric

Electrochemical detection is a concentration-sensitive technique. In amperometric mode compounds undergo oxidation or reduction reaction through the loss or gain, respectively, of electrons at the working electrode surface. The working electrode is kept at the constant potential against a reference electrode. The electrical current from the electrons passed to or from the electrode is recorded and is proportional to the concentration of the analyte present.

A thin layer cell is displayed in Figure 47.7.

A thin gasket with a slot cut in the middle is sandwiched between two blocks: one contains the working electrode, the other contains the counter electrode. The slot in the gasket forms the thin layer channel. The reference electrode is placed downstream from the working electrode.

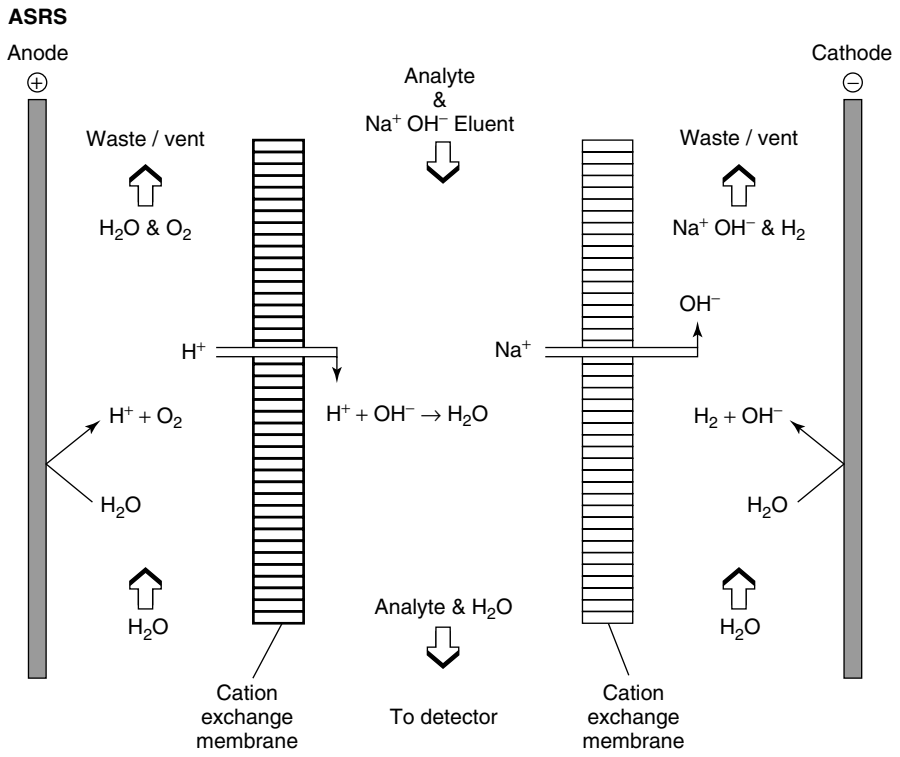


FIGURE 47.6 Anion self-regenerating suppressor for ion chromatography. (Courtesy of Dionex.)

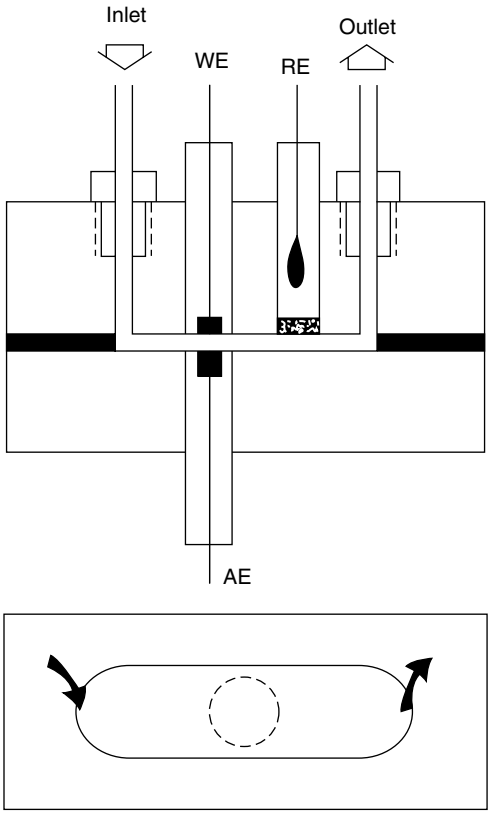


FIGURE 47.7 Electrochemical detector: a thin layer cell. WE: working electrode; AE: auxiliary electrode; RE: reference electrode.

The thin layer design produces high mobile phase linear velocity which in turn produces high signal magnitude.

The intensity of the current is

$$i = f \cdot n \cdot F \cdot u^{1/2} \cdot C \cdot D^{2/3} \cdot A$$

- n = the number of electrons
- F = the Faraday constant
- u = the linear velocity of the mobile phase
- C = the analyte concentration
- D = the diffusion coefficient of the solute
- A = the electrode surface
- f = the geometrical constant of the cell

The quality of the sample clean-up procedure often determines the detection limits. The instability of the reference electrode is the source of voltage noise.

A parallel dual electrode may be used for a number of reasons.

i. With one electrode at a positive and one electrode at a negative potential, oxidizable and reducible compounds can be detected in one single chromatographic run.
ii. When two solutes with different redox potentials co-elute from the column, the potential of one electrode can be selected such that only the most easily oxidized (or reduced) compound is detected while on the other electrode both compounds are converted. The concentration of

the second compound is evaluated by subtraction of the signal.

Series dual electrodes are set up in that one electrode is in oxidative and the other is in reductive mode. The downstream electrode measures the products of the upstream electrode. The second electrode only responds to compounds which are converted reversibly. The redox product is more selectively detected.

Voltammetric analysis is performed by scanning the potential or by applying triangular potential wave form to the electrode. Co-eluting peaks are distinguished if their voltagrams are significantly different.

3. Refractive Index Detector (RI)

This is one of very few universal detectors available. An RI detector monitors both the eluent and the analyte. The output reflects the difference in refractive index between a sample flow cell and a reference flow cell. The measured RI response is determined by the volume fraction of the analyte in the flow cell (x) and the volume fraction of the eluent in the other flow cell (1 − x).

$$\eta - \eta_2 = v_1 (\eta_1 - \eta_2)$$

where

v_1 = volume fraction of the analyte
η_1 = refractive index of pure analyte
η_2 = refractive index of pure solvent (contained in a reference cell)
η = refractive index of solution in sample cell.

$$\text{RI signal} = K_{RI} \cdot dn/dc \cdot C$$

where K_{RI} is an instrument constant, dn/dc is the refractive index increment, and C, the concentration.

There are four types of RI detectors.

- The deflection type is by far the most popular; it relies on Snell's law which governs the angles of incidence and refraction at an interface:

$$\eta_1 \sin \theta_1 = \eta_2 \sin \theta_2$$

where θ_1 is the angle of the beam with respect to the normal of the interface in the medium with RI of η_1.

- The eflection type according to Fresnel's law of reflection; measurement of Δn is a measure of change in reflectivity
- The interference type (utilized in capillary LC)
- The Christiansen effect type

The refractive index is very sensitive to temperature and pressure. For that reason the reference and the measurement cell are close since the difference in temperature between them is critical. Specifications of a RI detector are: refractive index range, linearity range, cell volume, maximum pressure in cell, and temperature control.

$$\frac{d\eta}{dt} \cdot 10^{-4} = 0.67 \text{ for water}$$
$$= 6.84 \text{ for dichloromethane}$$

$$\frac{d\eta}{dp} \cdot 10^{5} = 1.53 \text{ for water}$$
$$= 6.84 \text{ for dichloromethane}$$

He/Ne laser-based RI detection has been developed for nanoscale LC.

4. Light Scattering Detector

The principle of operation is a three-step process. The effluent of the LC column is vaporized in a nebulizer by means of a gas. The droplets pass through a drift tube at a temperature of 40–50°C, and the only particles left are the analyte and the solvent impurities. A laser (typically 1 mV He/Ne) irradiates the particles, and the scattered light is collected by a glass rod and transmitted to a photomultiplier tube (Figure 47.8). The light measured is proportional to the amount of sample in the light scattering chamber.

Parameters affecting the response are particle size, degree of nebulization (most critical), and nature of the solvent. When an LSD detector is used to detect thermally labile compounds the temperature used to evaporate the mobile phase is critical. If the temperature is too high, the compounds of interest can be thermally decomposed and reduce the sensitivity of the assay. The temperature range of the nebulizer is 40°–220°. The design of the evaporation tube is critical. The amount of scattered light depends strongly on the molar absorptivity of the solute. The light-scattering detector is universal detector but not a mass detector. Its response is nonlinear and the calibration curve is log-log. It can easily be used with a gradient. The benefits are low temperature evaporation of the mobile phase, and gradients can be used since the mobile phase is removed. The detector is suited for lipids and sugars. The signal of a light-scattering detector is proportional to the molecular weight of polymers.

V. HYPHENATION

Combining a chromatographic separation system on-line with a spectroscopic detector in order to obtain structural information on the analytes present in a sample has become the most important approach for the identification or confirmation of identity of target and unknown chemical species. In most instances, such hyphenation can be accomplished by using commercially available instruments. For most trace-level analytical problems the LC-MS combination is the accepted approach. However, more information is retrieved from concatenation of LC-MS with FTIR or NMR.

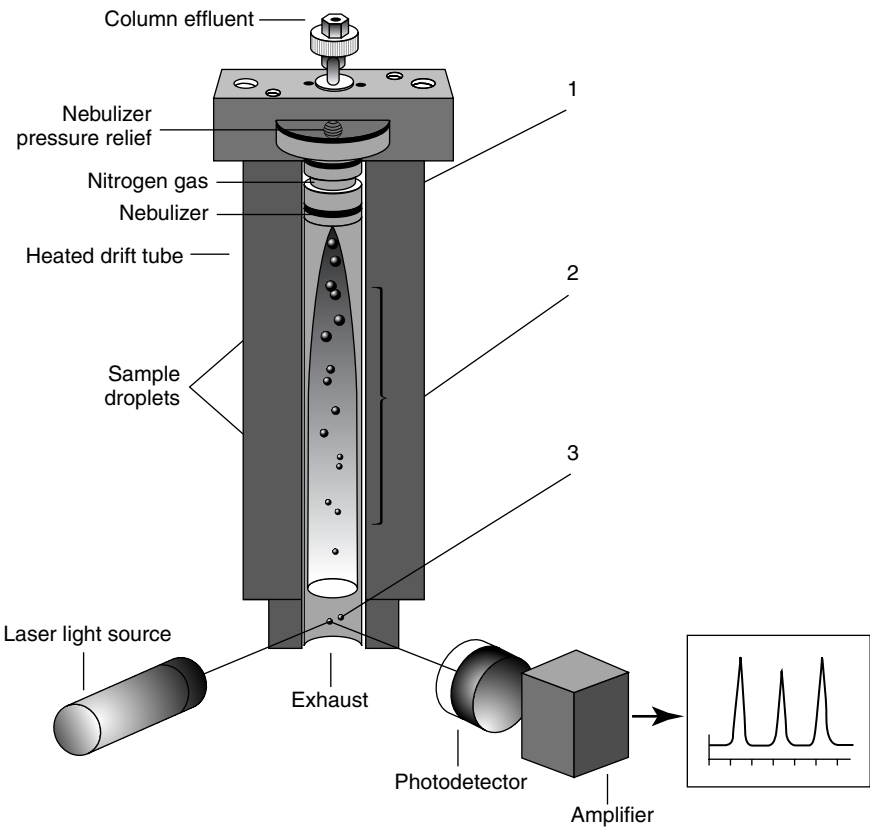


FIGURE 47.8 Light scattering detector: 1: nebulization; 2: mobile phase evaporation; 3: detection. (Courtesy of Alltech.)

The LC-UV-NMR-MS (hypernation) is the best example (Figure 47.9).

A. LC-MS

The mass spectrometer is becoming the detector of choice for many LC methods. In this case it is important in RPLC to select buffers that are MS-compatible such as 0.1% trifluoroacetic acid (pH ≅ 1.9) and ammonium formate for applications at higher pH values.

Interfaces have been developed to solve the problem of handling high LC flow rates (1 mL/min) and the high vacuum required by mass spectrometers.

The mass spectrometer is a mass flow sensitive detector. The enrichment factor is the ratio of the analyte concentration in the MS flow and that in the LC flow. The transfer yield is

$$Y = Q_m/Q_l$$

Q_m is the amount of solute transferred in MS and Q_l is the amount of solute from LC column.

LC is not nearly as compatible with MS as in GC.

Hyphenating LC and MS requires overcoming some difficulties.

- Conventional packed columns are operated at 1 mL/min; to overcome this drawback microbore columns are more and more frequently utilized.
- LC separations make use of non-volatile mobile phases and very often buffer solutions.
- Ionization of non-volatile or thermally labile solutes is difficult. However, difficulties have been overcome and LC-MS has become a robust and routinely applicable tool in environmental laboratories.

Electrospray (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric-pressure photoionization (APPI) are today the ionization techniques enabling robust interfacing of LC to (tandem) MS. Nowadays LC-MS-MS instruments are marketed within the chromatographic community as a laboratory workhouse requiring only basic knowledge about mass spectrometry from the analyst. A wide variety of atmospheric pressure ionization (API) source designs are available from the instrument manufacturers.

1. Ionization

a. Electrospray ionization(ESI)

A typical LC-MS with ESI interface is displayed in Figure 47.10. In ESI the ionization occurs in the liquid phase (in the spray) where ions might already be present or are created by application of a high electrical potential (3–5kV) to the sprayer tip. Evaporation of the liquid is assisted by

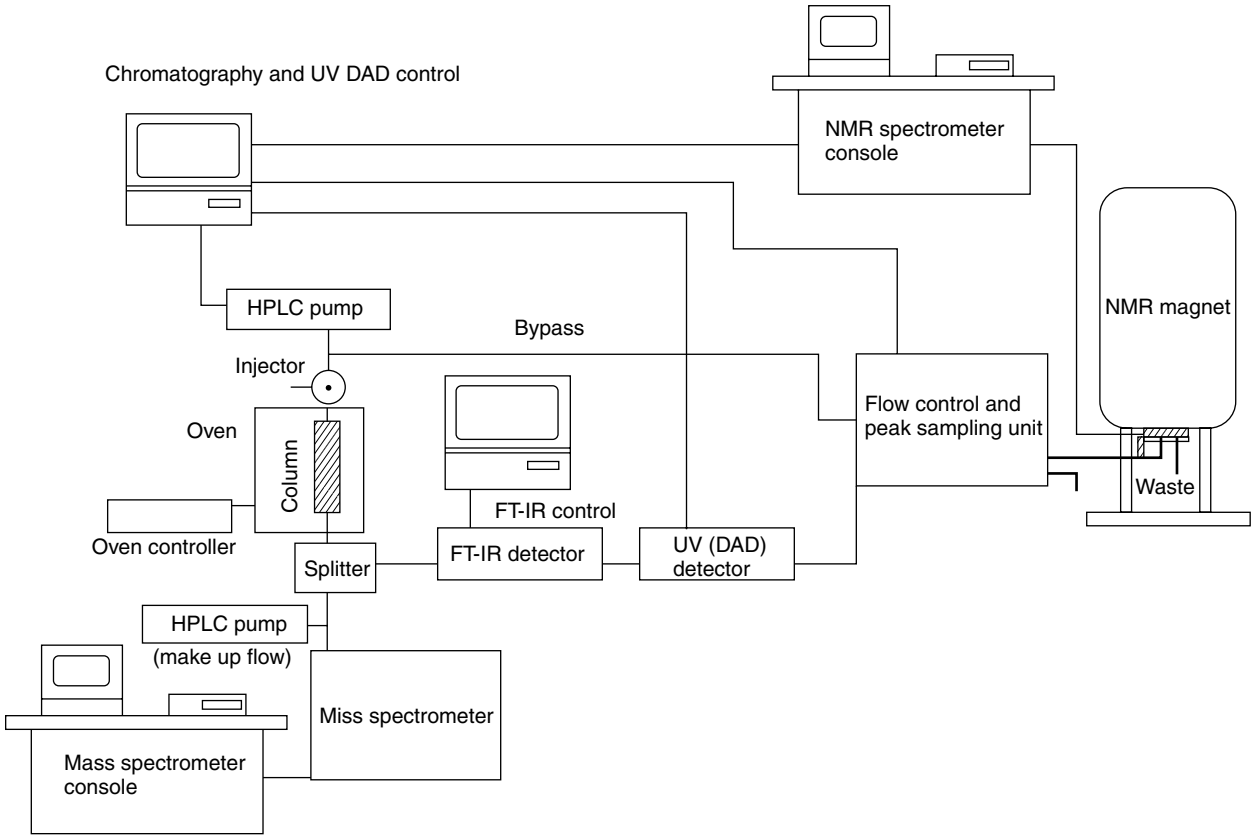


FIGURE 47.9 Hypernation: set up for LC-[DAD UV] –FTIR NMR/MS. (Reproduced from Journal of Chromatography A, Vol. 1000, p. 350. With permission from Elsevier.)

pneumatic pressure and by heat. Orthogonal spray orientation keeps the capillary and ion optics cleaner. The standard ESI sources cover the range from 0.1 to 2 $mLmin^{-1}$ and are therefore compatible with the average flow rate (1 mL/min) from 4.6 mm i.d. columns. ESI is a soft technique well suited to thermally labile molecules. The drawback is the occurrence of matrix effects. ESI is concentration dependent. A great deal of effort is focused on miniaturization of the system.

Two mechanisms have been proposed for the formation of ions by electrosray. The first is ion evaporation, which results in analyte ion desorption from a droplet due to high-field strength generated by charged droplets (20,21) The other relies on Rayleigh fissions to form very small charged droplets followed by solvent evaporation to result in a gas phase analyte ion (22). One characteristic of ESI instrumentation is the large pressure differential which is necessary for the combination of ionization at atmospheric pressure with the low pressures required for mass analysis and ion detection. The pressure differential is usually achieved either with a sampling cone/skimmer or capillary/skimmer arrangements. As the gas expands through the sampling cone into the lower pressure region, it forms a supersonic jet. The position of the skimmer in the source relative to the cone or capillary is extremely important for ensuring minimal ion losses in this region. Potentials applied between the skimmer and the cone/capillary serve both to focus the ion beam through the skimmer orifice and to accelerate ions through the intermediate pressure region.

Most manufactured electrospray interfaces attempt to negate the effects of the charged residues by using off-axis nebulizers, have an arrangement of off-axis skimmers or skimmer capillary sampling orifices, or use a shield in front of the sampling orifice. The disadvantage of ESI is its low tolerance to buffer salts. Nano ESI is more and more in use in protein analysis.

b. Sonic spray ionization (SSI)

In this technique (Figure 47.11), a sample solution is sprayed from a sample introduction capillary by a high speed nitrogen gas flow that is coaxial to the capillary, and ions of the chemical in the solution as well as charged droplets are produced at room temperature and atmospheric pressure. An electric field is applied to the solution in the capillary to increase the charge density of produced droplets, so that multiply charged ions are produced. The most important feature of SSI is that it does not require a heated nebulizer or the application of a high voltage to the sprayer. The range of solution flow rates in SSI is under

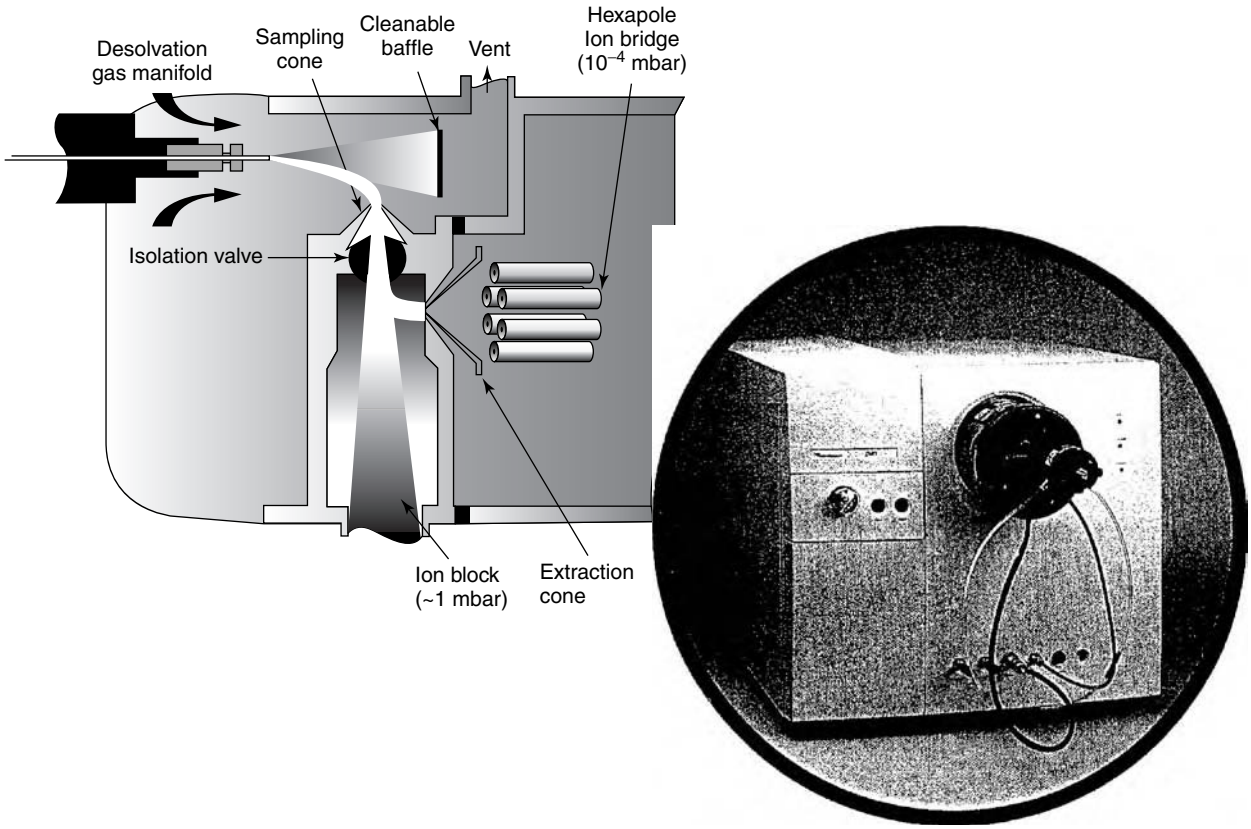


FIGURE 47.10 Schematic of the LC-MS electrospray interface with orthogonal electrospray system. (Courtesy of Agilent.)

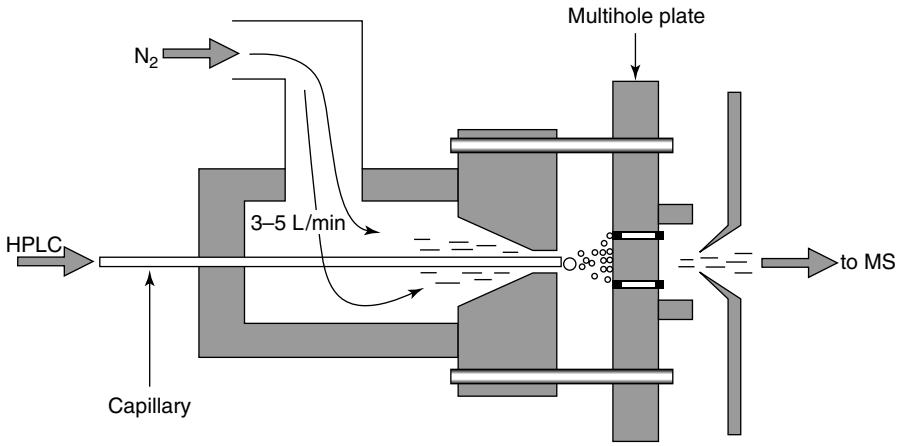


FIGURE 47.11 Schematic of a sonic spray ionization source. (Courtesy of Hitachi.)

0.2 mL/min; SSI is useful for semi-micro LC-MS but not well suited for conventional flow rates of 1mL/min.

c. Atmospheric pressure chemical ionization (APCI)

Atmospheric pressure chemical ionization (APCI) is a three-step process: the liquid flow from the LC is sprayed and rapidly evaporated by a coaxial nitrogen stream and heating the nebulizer to high temperature (350°–500°). Additional ionization is achieved by means of a corona discharge (3–6 keV) producing primary ions (N_2^+ and O_2^+ in positive mode). Interface consists of a concentric pneumatic nebulizer and a heated large-diameter, quartz tube. The primary ions react immediately with the solvent molecules of the mobile phase with the formation of reagent ions (e.g., H_3O^+, $CH_3OH_2^+$ in positive mode). The reagent ions react (e.g., proton transfer) with solute molecules yielding $[M+H]^+$ in positive ion mode or

$[M-H]^-$ in negative ion mode. Processes are separated in APCI which allows the use of some unfavorable solvents. Ionization occurs in the gas phase whereas it occurs in the liquid phase in ESI. APCI is mass dependent and less prone to matrix effects. APCI can withstand high flow rates but miniaturization is more difficult than with ESI.

d. Atmospheric-pressure photoionization (APPI)

Photoionization is induced by means of a xenon lamp (10 eV). A dopant species, with the ionization potential of <10 eV, such as toluene, is utilized. Covaporized dopant molecules are ionized by UV radiation. Photoions thus produced initiate ion molecule reactions with the solute molecules, yielding $[M+H]^+$ and M^+ by proton transfer or charge transfer. The solvents in the mobile phase must have a ionization potential >10 eV to prevent ionization from the xenon lamp with a resulting increase in background signal.

e. Maldi

Matrix-assisted laser desorption ionization (MALDI) is well suited for macromolecules such as peptides, proteins, oligosaccharides, and oligonucleotides. In a Maldi experiment a proper organic matrix (e.g., glycerol) is required for mixing with the analyte in a ratio typically 500/1. The mixture is dried and inserted in a MS. A laser beam will desorb and ionize the matrix species, thus ionizing the analyte. Most Maldi-MS systems are based on time of flight (TOF) mass analyzers. Ions produced by the laser beam are extracted from the source and expelled to the flight tube.

B. Mass Analyzers

The resolving power of a mass spectrometer is a measure of its ability to distinguish between two neighboring masses. Resolution is Δamu/amu (atomic mass unit). Spectrometers easily perform resolutions of 50,000 (i.e., distinguish Δamu = 0.01 when M = 500). Resolution is often written in ppm, Δamu $*10^6$ /M.

The quadrupole mass filter consists of four parallel hyperbolic rods in a square array (Figure 47.12). The inside radius (field radius) is equal to the smallest radius curvature of the hyperbola. Diagonally opposite rods are electrically connected to radio frequency/direct current voltages. For a given radio frequency/current voltage ratio, only ions of a dedicated m/z value are transmitted to the filter and reach the detector. Ions with a different m/z ratio are deflected away from the principal axis and strike the rods. To scan the mass spectrum, the frequency of the radio frequency voltage and the ratio of the ac/dc voltages are held constant while the magnitudes of ac and dc voltages are varied. The transmitted ions of m/z are then linearly dependent on the voltage applied to the quadrupole producing m/z scale which is linear with time. A triple quadrupole instrument uses two quadrupole MS analyzers for the actual MS experiments and a third quadrupole in RF mode which transmits all incoming ions from MS1 to MS2.

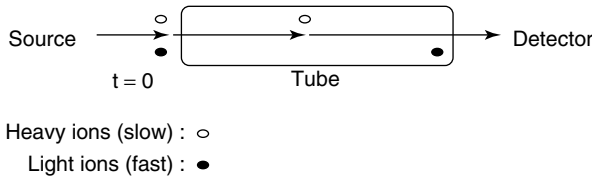
For quadrupole mass spectrometers, selected ion monitoring (SIM) yields significantly enhanced detection limits compared with scanning MS operation because of the greater dwell time for signal acquisition at each selected m/z values.

Ionization steps and ion separation are space separated in a quadrupole system whereas they are time spaced in ion traps. An ion trap (Figure 47.13) is a linear quadrupole bent to a close loop. Typically, three electrodes are a common design of an ion trap: a ring electrode and two end cap electrodes. The outer rods form a ring and the inner rod is reduced to a mathematical point in the center of the trap. End electrodes exhibit a hole in their center to allow for introduction of ions and ejection of these ions towards the detecting device. A radio frequency is applied to the ring electrode and consequently, a quadrupole field is produced which traps ions. Each ion is submitted to an oscillating motion, the amplitude of which depends on the RF and the m/z ratio. Ions of different masses are stored together in the trap and released one at a time by scanning the applied voltages. They can be ejected through the end caps and detected by applying a RF voltage with a frequency corresponding to the characteristic frequency of the ion moving through the ion trap or by scanning the amplitude of the applied RF voltage. With a reduced pressure of gas (He)(10^{-3} torr) the motion of ions in the trap is dampened and the ions move closely around the center of the trap. Ion traps are a powerful tool in elucidating fragmentation mechanisms since it allows stepwise and controlled fragmentation in multistage MS. Ion traps are small benchtop instruments.

Linear two-dimensional ion traps are now commercially available (23).

1. Time of Flight

A scheme is displayed below:



A small number of ions is extracted from the source in a few μseconds, accelerated with few kV, and directed to a tube. The process can be repeated 100,000 times per second. Kinetic energy is similar for every ion and ions with higher velocities (light ions) will reach the end of the tube before heavy ions. Instruments have two tubes with a mirror in the middle and resolution may reach 5000. A schematic diagram of an instrument is displayed in Figure 47.14.

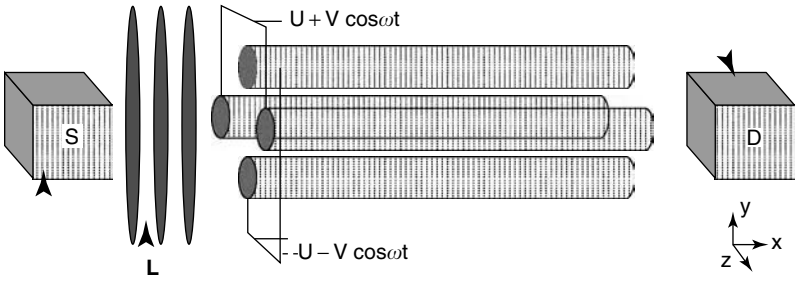


FIGURE 47.12 Scheme of a quadrupole instrument. (Coustesy of Micromass.)

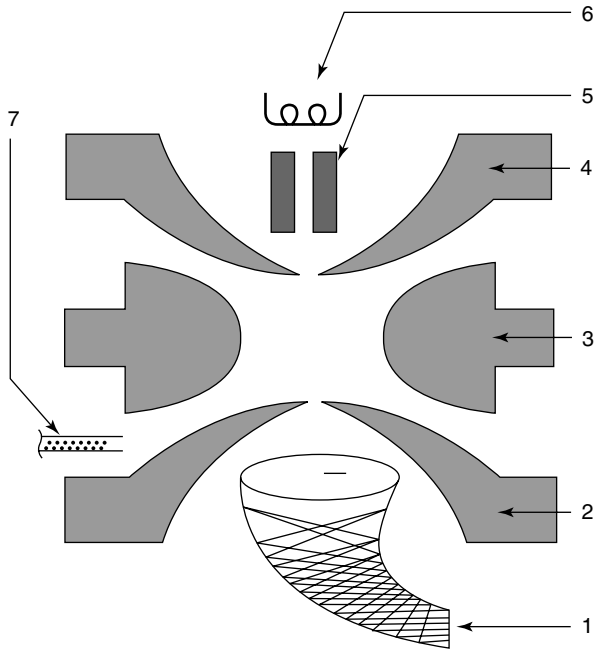


FIGURE 47.13 An ion trap. 1 = channeltron detection; 2 = cap electrode; 3 = ring electrode; 4 = cap electrode, 5 = electron gate; 6 = eletron emitting device; f = column. (Courtesy of Agilent.)

In a tube L the time of flight t is connected to the velocity v

$$t = L/v$$

Ions get a kinetic energy $E_k = 1/2\ mv^2 = 2V$ where V is the voltage, then $t = L[m/2Vz]^{1/2}$. The time of flight is proportional to the square root of the ion mass which allows discrimination according to the m/z ratio.

As an example, if 300V are used to accelerate the ions in the flight tube, an ion with m/z = 1000 needs about 200 μs to reach the detector.

The hybrid quadrupole time of flight mass spectrometer (Q-TOF) was introduced as a mass spectrometer capable of tandem MS with particular emphasis on its applicability for protein and peptide analysis. Key components of the instrument are the quadrupole, hexapole collision cell, and the reflectron-TOF analyzer. In normal mode, the quadrupole region and hexapole collision cell, are operated in a non-mass discriminative wide-band pass mode allowing all ions to pass unperturbated in the pusher of the TOF analyzer. Like all TOF analyzers, the m/z measurement is based on the amount of time required for an ion to travel the distance from the source of entry (pusher on a Q-TOF) to the multi-channel plate (MCP) detector. The signal detected by the MCP is integrated over an unspecified period of time and each integrated spectrum is written to disk.

The electronics of the detector must record the complete mass spectrum within the flight time of the ions (1–100 μs range) with peak widths in the ns range. This is possible since high scan rate (up to 20,000 scans/s) allows for the detection of narrow chromatographic peaks. There is virtually no limit on mass range and no ion loss. The use of time-dependent accelerating fields can improve the resolution of TOF instruments. The technique involves the introduction of an appropriate time-delay between the ion formation and ion acceleration. During this time, ions disperse with their individual kinetic energies. Since the accelerating potential varies linearly with the position of the ions

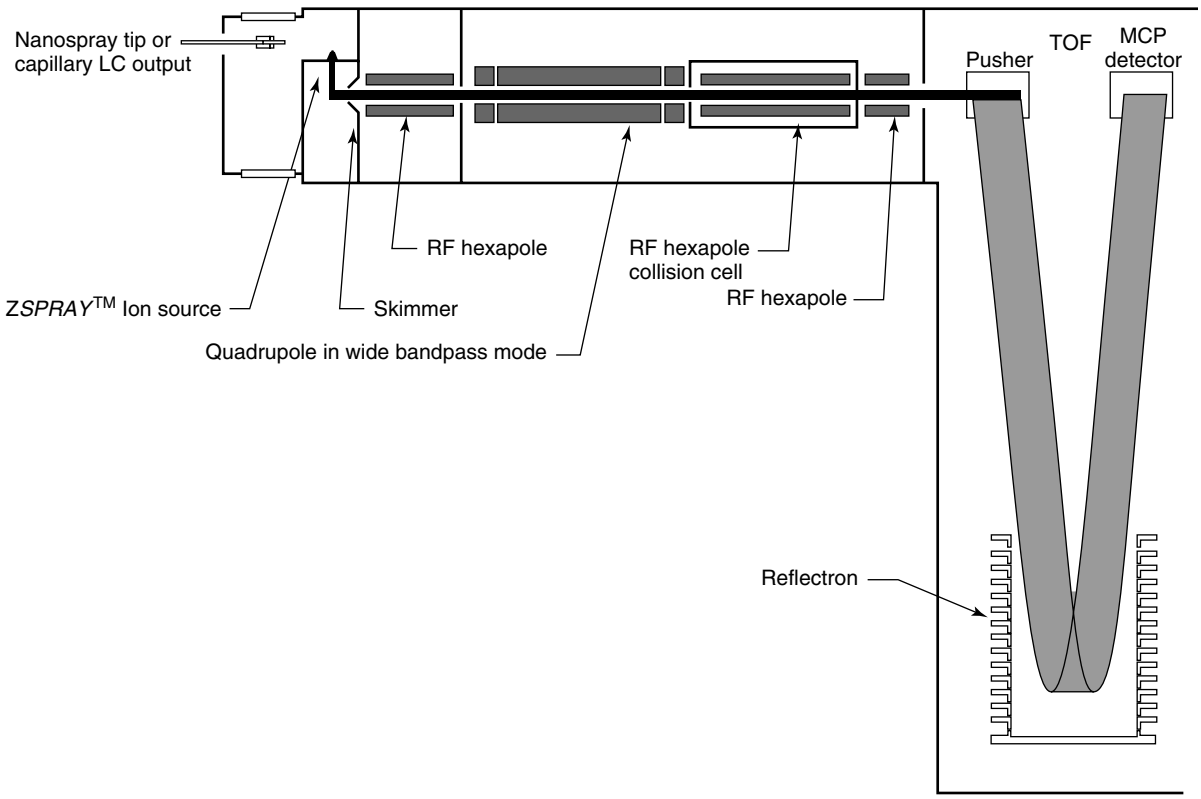


FIGURE 47.14 Schematic of a quadrupole time of flight (Q-TOF) mass spectrometer shown operating in MS mode. (Courtesy of Micromass.)

in the source region, ions with the same m/z ratio but different positions are accelerated through different acceleration voltages. In this way, correction for the initial ion velocity distribution is achieved.

TOF-MS has to a large extent replaced high resolution double-focusing magnetic sector instruments for LC-MS applications. With the fast acquisition capacity of the TOF instrument (>10 spectra/sec), the effluents from four HPLC columns are continuously electrosprayed.

C. ICP-MS

Inductively coupled plasma mass spectrometry is highly selective in ultra-trace-level metal detection technique. Interfacing HPLC to ICP-MS can be achieved with relative ease by means of a length of PEEK tubing connecting the HPLC column to the nebulizer of the ICP-MS. Performances are strongly dependent on the type of nebulizer utilized as the sample introduction device. HPLC-isotope dilution-ICPMS offers considerable potential for speciation studies.

D. NMR

On-line coupling of HPLC with nuclear magnetic resonance spectroscopy (NMR) has proved useful for a wide range of applications. The shortcoming of suppression of eluent signals can be circumvented by use of a capillary separation technique. Capillary concentration techniques are important to take advantage of the miniaturized rf coils. In this mode detection cells with internal volumes in the nanoliter scale and miniaturized probe heads have been developed by Albert and colleagues in Tübingen (24). The system can be used in either HPLC, CE, or CEC and consists of a capillary inserted into a 2.5 or 2.0 mm NMR microprobe equipped with a Helmholtz coil. In experiments a capillary tube of 315 μm can create a detection volume of 900 nL. The flow rate of the capillary HPLC-NMR can be adjusted to 3 μL/min with the help of a T piece inserted between the HPLC pump and the capillary device. The polyimide coating was removed over the length of the NMR coil directly after the outlet frit of the capillary packing. In another design the packed capillary LC column is placed directly below the cryomagnet. With the help of a transfer capillary (400*50 μm) the eluate is transferred to the detection capillary with an internal diameter of 180 μm. The NMR detection volume is thus 200 nL. For example, assignment of vitamin E structures is possible.

VI. PERFORMANCES

The HETP equation with packed columns has been devised by Knox (6) and is written in reduced variables:

$$h = A\,\nu^{1/3} + B/\nu + C\nu$$

where A stands for the anisotropy of flow rate, B is the molecular diffusion, and C is the mass transfer term. With good columns A = 1, B = 2, and C is around 0.01. h is the reduced plate height (h/d_p with packed columns, h/d_c in open tubular columns), and ν is the reduced velocity $\nu = ud_p/Dm$. Small particles yield HETP curves with negligible C term. In this mode, HETP does not vary too much when flow rates are increasing beyond the minimum but back pressures dramatically increase. It must be pointed out that the Van Deemter equation $H = A + B/u + Cu$ applies with monolithic columns.

The pressure used to pack a column can have a dramatic effect on the performance characteristics of the column.

Recorded peak width is the sum of column and extra-column contributions to band spreading. The instrument contribution comes from the injector, connecting tubings, column frits, detector, and data-handling. Since residence times of the solute in all these parts are independent, variances are additive and thus:

$$\sigma^2 = \sigma^2_{\text{injector}} + \sigma^2_{\text{column}} + \sigma^2_{\text{detector}} + \sigma^2_{\text{connecting tubes}} = \sigma^2_{\text{column}} + \sigma^2_{\text{extra column}}$$

There are two methods to obtain the extra column band broadening of the chromatographic system: a linear extrapolation method using σ^2_{peak} as a function of retention time; and a zero length column method where the column is removed from the system and replaced by a capillary.

In the linear extrapolation $N_{th} = (t_r/\sigma_{\text{column}})^2$; hence $\sigma^2_{\text{column}} = t_r^2/N_{th}$.

A plot of σ^2_{peak} versus t_r^2 will be linear with a slope determined by N_{th} and an intercept value on the vertical σ^2_{peak} axis representing $\sigma^2_{\text{extra column}}$.

Column performance is expressed as the number of theoretical plates per unit of time, N/t or per meter N/m or by separation impedance, $E = \Delta P t_o/N^2\eta$ where ΔP is the pressure drop, t_o the retention time of the inert solute, N the plate count, and η the mobile phase velocity. N is around 100,000 plates/m. A new monolith column can produce 38,000 plates/25 cm.

Recently, temperature has attracted attention from LC chromatographers since the eluent viscosity is reduced. The contribution of temperature to the retention is mainly given by the enthalpy term of the Van't Hoff equation

$$\text{Lnk} = -\Delta H/RT + \Delta S/R + \log\beta$$

where ΔH is the enthalpy change associated with the transfer of the solutes between phases, ΔS is the corresponding entropy change, R is the molar gas constant, T is the absolute temperature, and β is the phase ratio of the column. Plots of Lnk versus 1/T are usually called Van't Hoff plots and the slope of the curve yields ΔH. Most studies are related to reverse phase systems. When dealing with small solutes that experience hydrophobic interactions, lower selectivity is observed with elevated temperatures. Solutes with basic groups or ionizable compounds experience increased selectivity at high mobile phase pHs. In the Knox equation the A term is not affected by temperature, the C term is reduced, but the B term increases. The stability of silica-based C18 stationary phases is questionable over 80°C but PS-DVB are resistant up to 220°C. It must be pointed out that the dielectric constant of water is reduced at elevated temperatures giving it some characteristics of an organic solvent. A discussion on temperature effects can be found in (25).

Zhu et al. (26, 27) studied the combined effects of solvent gradients and temperature.

VII. OPTIMIZATION

When dealing with complex samples column switching is the method of choice to focus on solutes of interest. The method is rather simple; it uses two columns packed with different supports but the compatibility of eluent with the two phases is not straightforward.

The best chromatogram is the one which provides the complete resolution ($Rs \cong 1.25$) of all solutes in a minimum time. The large number of chromatographic parameters and the relationships between them rules out the possibility of empirical optimization by trial and error. Many methods have been developed to optimize parameters of interest. These methods were reviewed by Siouffi (28). The analyst either selects a response function (most often the resolution Rs) or tries to predict retention. There are roughly two types of approaches: the chemometrics approach and methods based on models. Because most useful variables in RPLC reside in the mobile phase, they are generally referred to as mobile phase optimization.

Full or fractional factorial designs are useful for screening the effects of a large number of parameters. One does not need to know the retention mechanism. Data from a Central Composite Design can be evaluated and plotted as a response surface which provides a graphical representation of the data over the range of the key parameters to study. It allows us to check the influence of a parameter but it is not predictive. Experimental retention data can be used to train an artificial neural network to enable it to predict the effects of changes in experimental variables.

Softwares and method development in LC separations are readily and widely available. Such products rely on the input of two or more pilot runs to calibrate a retention model. The Drylab computer simulation approach is widespread (29–31). The retention data of two initial gradient runs are used to adjust the steepness and the range of the gradient. In Chromsword from Galushko (32), a

molecule must be translated into volume fragments and bond dipoles

$$\text{Lnk} = a(\Sigma Vi)^{2/3} + b(\Sigma Ge \cdot sj_{H_2O})$$

where Vi are the increments of the partial molar volumes of fragments in water, $Ge \cdot sj_{H_2O}$ are the increments of energy of interaction of bond dipoles with water, and a, b, and c are the parameters of the RP system and the column (polarity, column ratio, etc.).

Baczek et al. (33) performed a comparison of the two softwares. The Prisma optimization model was developed by Nyiredy (34) for the purpose of TLC optimization and was extended to HPLC. It is a three-dimensional geometrical design which correlates the solvent strength with the selectivity of the mobile phase. A software package called Virtual Column enables simulation and optimization of ion chromatography separations (35).

The linear Solvation Energy Relationship advocated by Abraham (36) and extensively studied by Carr and Poole (37,38) relies on the following equation

$$\text{Lnk} = \text{Ln}K_0 + rR_2 + vV_x + s\pi^2_H + a\Sigma\alpha^2_H + b\Sigma\beta^2_H$$

where R_2 is an excess molar refraction of the analyte, V_x is its molar volume (in $cm^3 \cdot mol^{-1}/100$) according to the MacGowan algorithm, π^2_H is the solute dipolarity/polarizability descriptor, $\Sigma\alpha^2_H$ is the analytes ability to donate hydrogen bonds, $\Sigma\beta^2_H$ is the measure of hydrogen bond accepting capability. Lnk_0, r, s, a, and b are fitting coefficients which reflect the difference in specific bulk property between the mobile and stationary phases. The solvation parameter model is a useful tool for evidencing the contribution of defined intermolecular interactions to retention of neutral molecules (or polar but not ionized molecules) in chromatographic systems. The retention process is the sum of the *differential* interactions of a solute with the mobile phase and the stationary phase.

In general, the solute size and the basicity are the most important solute descriptors governing retention.

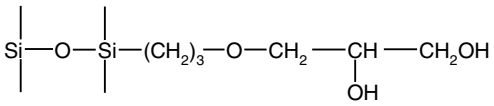
VIII. DIFFERENT MODES OF LC

A. Adsorption or NPLC (Normal Phase Liquid Chromatography)

In this mode the stationary phase is polar. Since only silicagel and alumina were utilized at the beginning of liquid chromatography and hydrophobic alkyl bonded phases were developed later, this mode is called normal phase.

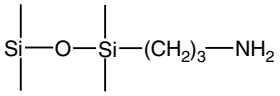
Stationary phases: Bare silicagel and alumina are declining but bare silica is still widely used in thin layer chromatography. Polar bonded phases are now widely used.

Synthesis of bonded silica is performed either by surface modification or bulk modification.



Diol phase Si—O—Si—$(CH_2)_3$—O—CH_2—CH(OH)— CH_2OH

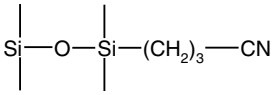
is very similar to bare silica but less retentive.



Si—O—Si—$(CH_2)_3NH_2$

– Amino phase

acts by the lone pair of electrons on the nitrogen atom.



– Cyano phase Si—O—Si—$(CH_2)_3CN$

is less polar and has been advocated in some optimization procedures as the unique phase since it may be used with any solvent.

Other polar phases are available but less often advocated.

Mobile phases: Theoretically any solvent is convenient.

Extensive work has been carried out on adsorption theory and retention mechanism since the pioneering work of Snyder (39). In this chromatographic mode physical adsorption of solute occurs. That means weak interactions with the stationary phase. Adsorption of the solutes occurs via donor acceptor mechanism (hydrogen bonding, charge transfer, etc.). According to Snyder's displacement model a solute S takes place of n molecules of previously adsorbed mobile phase solvent molecules M.

$$nM_S + S_m \overset{K}{\Leftrightarrow} S_s + nM_m$$

The subscripts s and m refer to stationary phase and mobile phase, respectively.

$$K = \frac{(S)_s}{(S)_m} \frac{(M_m)^n}{(M_s)^n}$$

From this starting equilibrium the Snyder-Soczewinski equation (40) yields the retention equation

$$\text{Ln } k = \text{Ln} \frac{V_a W}{Vm} + \alpha(\varepsilon^0 - S^0 A_s) + \Sigma \text{ secondary effects}$$

$V_a W$ is the volume of solvent forming a monolayer coverage on the absorbent surface.

When a solute is strongly adsorbed, eluent must exhibit a strong affinity towards the stationary phase to displace the solute. ε^0 represents the dimensionless energy of solvent adsorption. Ranking ε^0 gives a scale of adsorption strength. It is obvious that n alkanes exhibit weak affinity towards polar stationary phases. Conversely, silicagel and alumina are hygroscopic and exhibit strong affinity towards water. Ranking ε^0 constitutes the eluotropic strength scale (Table 47.2).

Fine retention tuning is somewhat difficult with a single solvent. Mixtures of solvents most often constitute the eluent. Binary mixtures are a blend of apolar diluent (A) and polar modifier (B). Direct calculation of the eluting strength is possible through a formula derived by Snyder (39). The plot of ε^0 versus % of B is displayed in Figure 47.15. These plots are readily available in most manufacturers' softwares. Three (or more) solvent mixtures as eluent are difficult to handle and reproduce. With a binary mixture retention is given by

$$\text{Ln } k = Ct - n \text{ Ln } (X_B)$$

where X_B is the molar fraction of modifier B and Ct is a constant.

Continuous increase of X_B is gradient elution (see below).

Normal phase chromatography is well adapted to separation of polar or moderately polar compounds. NPLC is well suited for the separation of structural isomers.

B. Reversed Phase LC (RPLC)

In this mode the stationary phase is hydrophobic (apolar) and to maximize the difference between the nature of both stationary and mobile phases, the former is hydrophobic, the latter is highly polar. Retention in RPLC is described in terms of free energy change

$$\Delta G^\circ = RT \text{ Log } K + \text{Log } \varphi$$

$$\text{Log } K = -\Delta H^\circ/RT + \Delta S^\circ/R + \log \varphi$$

where T is the absolute temperature, R is the gas constant, φ is the phase ratio, and ΔH° and $\Delta S^\circ$are enthalpy and entropy, respectively, associated with the transfer of a solute from an aqueous mobile phase to a non-polar stationary phase.

TABLE 47.2
Eluotropic Series for Different Adsorbents

	Solvent Strength Parameter					
Solvent	**Alumina**	**Silica**	**Carbon**	**Aminopropyl**	**Cyanopropyl**	**Diol**
Pentane	0.00	0.00				
Hexane	0.01	0.01	0.13–0.17			
Carbon tetrachloride	0.17	0.11		0.069		
1-Chlorobutane	0.26	0.20	0.09–0.14			
Benzene	0.32	0.25	0.20–0.22			
Methyl-tert. butyl ether	0.48		0.11–0.124	0.049–0.085	0.071	
Chloroform	0.36	0.26	0.12–0.20	0.13–0.14	0.106	0.097
Dichloromethane	0.40	0.30	0.14–0.17	0.13	0.120	0.096
Acetone	0.58	0.53			0.14	
Tetrahydrofurane	0.51	0.53	0.09–0.14	0.11		
Dioxane	0.61	0.51	0.14–0.17			
Ethyl acetate	0.60	0.48	0.04–0.09	0.113		
Acetonitrile	0.55	0.52	0.01–0.04			
Pyridine	0.70					
Methanol	0.95	0.70		0.00	0.24	

Buffering Ranges

Buffer	**pK_a**	**Range**
Phosphate	201	1.1–3.1
	7.2	6.2–8.2
Acetate	4.8	3.8–5.8
Citrate	3.1	2.1–4.1
	4.7	3.7–5.7
	5.4	4.4–6.4

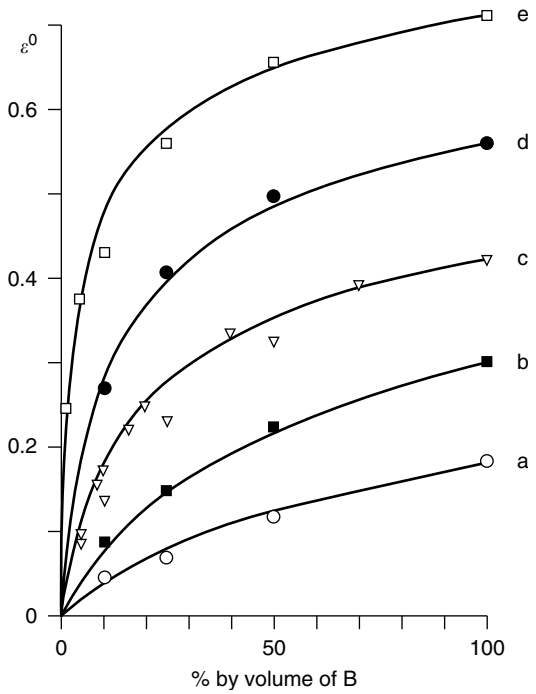
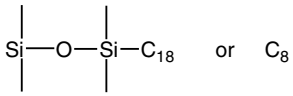


FIGURE 47.15 l Plot of eluent strength versus the % of modifier in a binary mixture (A: diluent; B: polar modifier) in NPLC. (a) carbon tetrachloride, (b) propyl chloride, (c) methylene chloride, (d) acetone, (e) pyridine. (reproduced with permission from Edinburgh University Press, (Scotland.)).

Stationary phases: More than 600 reversed phase columns are commercially available and every year new phases are released (see the annual review in LC-GC magazine). Most are silica bonded of the C_8 or C_{18} type.



In spite of many shortcomings silica backbone is still the material of choice and is considered as unsurpassed by other inorganic oxides such as zirconia or polymers, e.g., polystyrene/divinyl benzene. Hydrophilic solutes are more retained on porous graphite packings than on C_{18} bonded silica. Small, monodisperse, spherical particles are available in a variety of particle diameters (1.5–10.0 μm) and pore sizes (60–4000 Å). Differences exist between type of silica, type of ligand, end capping, bonding density, residual silanols, etc. Selection of the proper packing among the available types from manufacturers is not simple. The overall retention behavior of packed columns depends on the physico-chemical properties of the silica (specific surface area, packing density), the chemical surface properties (type and concentration of surface silanols, surface concentration of metal oxides), the bonding procedure (whether mono, di, or tri alkoxy or chlorosilanes have been used), the surface concentration of bonded groups achieved, and end capping or not. In many cases the manu-facturer's description is insufficient to characterize the stationary phases. ^{29}Si and ^{13}C NMR provide information on ligand attachment to substrate surface.

From the carbon content, %C and the specific surface area Sp the surface concentration (Sco) of the bonded phase is

$$\text{Sco} = \%\text{C}/(100\text{nC}*12\text{Sp})(1-\%\text{C}/100*\text{Ml-}1/\text{nC}*12)$$

Where nC is the number of carbon atoms (typically 8 or 18), Ml is the molecular mass of the ligand. Bonding density is expressed as μmoles/m^2. Silicagel has a maximum silanol density of 8.0 μmoles/m^2 but only part of them can react. With stationary phases with alkyl group surface concentrations below 3.8 μmoles/m^2, silanophilic interactions contribute to retention producing asymmetric peak shapes of basic solutes. At surface coverages of 4.24 μmoles/m^2, ca 85% of the silanol groups are blocked. To ensure that the minimum silanols are remaining manufacturers perform a further reaction with a small size silane (e.g., Trimethyl chloro silane). This is called end capping. Another procedure is to shield silanols with t-butyl groups, for example.

RP phases should be tested for

Efficiency expressed by N/m (plates per meter)
Hydrophobic properties
Steric selectivity
Silanophilic properties
Metal content

The majority of column characterization procedures can be divided into empirical methods, thermodynamically based methods, and model-based methods. A good chromatographic method for the characterization of a RP packing should attempt to measure several parameters. Many tests have been descibed in the literature. In Engelhardt's test (41), toluene, ethylbenzene, phenol, benzoic acid, aniline, NN-dimethylaniline, and p-ethylaniline are injected in methanol/water (49/51) eluent; the retention of toluene and ethylbenzene are measures of the hydrophobicity of a packing while the retention and the tailing factor of the bases are measures of the silanophilic interaction. Hydrophobic properties depend on the level of the surface coverage.

Hydrophobicity, which is usually considered as the ratio of the retention factors of successive homologues (e.g., pentylbenzene/butylbenzene), is a good guide to the carbon content of the column. The measured hydrophobicity is dependent on the mobile phase composition and temperature of the column (42).

All tests give good agreement on the hydrophobic retention, which is proportional to the carbon content per unit column volume, whereas hydrophobic selectivity, i.e.,

separation potential of two analytes differing for one methylene group, hardly shows differences between the various column types. Manufacturers who provide carbon content should also provide the specific surface area. In Tanaka's test (43) the analyst is able to measure the hydrophobic retentivity of the packing, the steric selectivity, the hydrogen bonding, and the ion exchange properties of the packing. Shape selectivity of RPLC phases refers to the ability of a packing to discriminate between conformational differences between molecules; however, it must be kept in mind that the majority of the evaluation methods for HPLC columns have been developed specifically for narrow pore phases using small molecular probes. Silanol activity is checked from the peak shape of benzylamine and phenol. Kele and Guiochon (44) extensively published on the reproducibility and performances of RP phases. Claessens (45) published a review of RP phases' characterization. McCalley (46) evaluated the performance of RP columns for the analysis of basic compounds.

Stationary phases especially developed for the analysis of basic compounds are phases in which the ionic interaction between basic analytes and silanols are minimized. In this mode various approaches of stationary phase manufacturing have been described: high purity silica, end capping procedures, polymer encapsulation, shielding of silanols, bidentate, or surface-modified silica. Polar-embedded phases provide good peak shapes of basic analytes and good compatibility towards highly aqueous mobile phases. To overcome the contamination by metal traces, silica is prepared by a sol-gel method which does not guarantee metal-free silica. It has been observed that basic analytes exhibit variable retention on RP packings; there may be partial or total exclusion effects giving rise to detrimental loss of resolution. At pH 7, which is often used to increase the retention of hydrophilic bases, poor peak shapes are obtained on many phases. The properties of RP columns also substantially depend on the actual eluent pH; addition of organic modifier to an aqueous buffer causes a shift which can be as high as one pH unit in the actual pH of the eluent. According to Neue (47), the pH needs to be controlled to better than 0.01 units, the mobile phase composition to better than 0.1% and the temperature to better than 0.1% if one wants to get reliable data on the batch to batch reproducibility of a packing. Classification and ranking of RP phases can be performed through principal component analysis (48).

Silica bonded phases are not stable over the whole pH range; low pH ($\leq$2) or high pH ($\geq$9) may damage the siloxane bond, and phosphate buffers lead to fast degradation. To overcome this drawback polymeric phases of the polystyrene divinylbenzene (PS-DVB) type have been developed. With a high degree of cross linking they are mechanically stable and can withstand high pressures. Porous glassy carbons (PGC) are pH stable as well but the number of published separations with this support is small compared to silica bonded supports.

On these hydrophobic supports, hydrophilic solutes are not retained and by consequence water is the weakest eluent. The eluotropic strength is thus exactly the reverse of the one observed in NPLC.

Mobile phases are typically water + organic modifier mixtures. Methanol, acetonitrile, and tetrahydrofuran (THF) are the usual organic modifiers.

The mechanism of retention has been a matter of dispute. The volume of "definitive" papers on the topic is impressive. Partition mechanism is generally accepted but some deviations from this mechanism may be observed. A low organic modifier content causes the collapse of the bonded chains that are brought together by the intermolecular C_{18} dispersive interactions while they tend to exclude water. A high organic modifier content induces the rupture of the C_{18} intermolecular interactions via chain solvation by the modifier and the formation of a brush-like structure.

Two features are important in RPLC. In an homologous series (n-alkanols, saturated fatty acids, etc.) linear plots of Ln k versus carbon number of the solutes are observed. Increasing the volume percentage of organic modifier in the mobile phases decreases retention according to

$$\text{Ln } k = a - b\phi - c\phi^2$$

where ϕ is the volume percentage of the modifier in a binary mixture water-organic modifier.

Curvature of the quadratic plot of Ln k versus ϕ is highly dependent on the nature of the solute (Figure 47.16). In a more or less limited range of the volume %, the above equation can be written as

$$\text{Ln } k = \text{Ln } k_w - S\phi$$

where Ln k_w is the retention with pure water as mobile phase. Values of k_w are obtained by extrapolation to $\phi = 0$.

S is the slope of the regression and is characteristic of the solute.

Discrepancy can occur when methanol, acetonitrile, or tetrahydrofurane (THF) is used as a modifier. k_w has often been considered the hydrophobicity of the solute. It comes from the correlation plot of Ln k_w versus Log P octanol-water. Like correlation plots, some discrepancies can occur, depending on the number of solutes in the plot and correlation coefficient values.

Slope (S) of the Ln k_w versus ϕ plot is typical of the solute considered. When considering, for example, two solutes, two situations can occur: either the two solutes have the same S, and in this case, selectivity remains constant whatever the ϕ value, or the two solutes exhibit S_1 and S_2 values which means that slope are different and consequently there exists a ϕ value where no separation occurs.

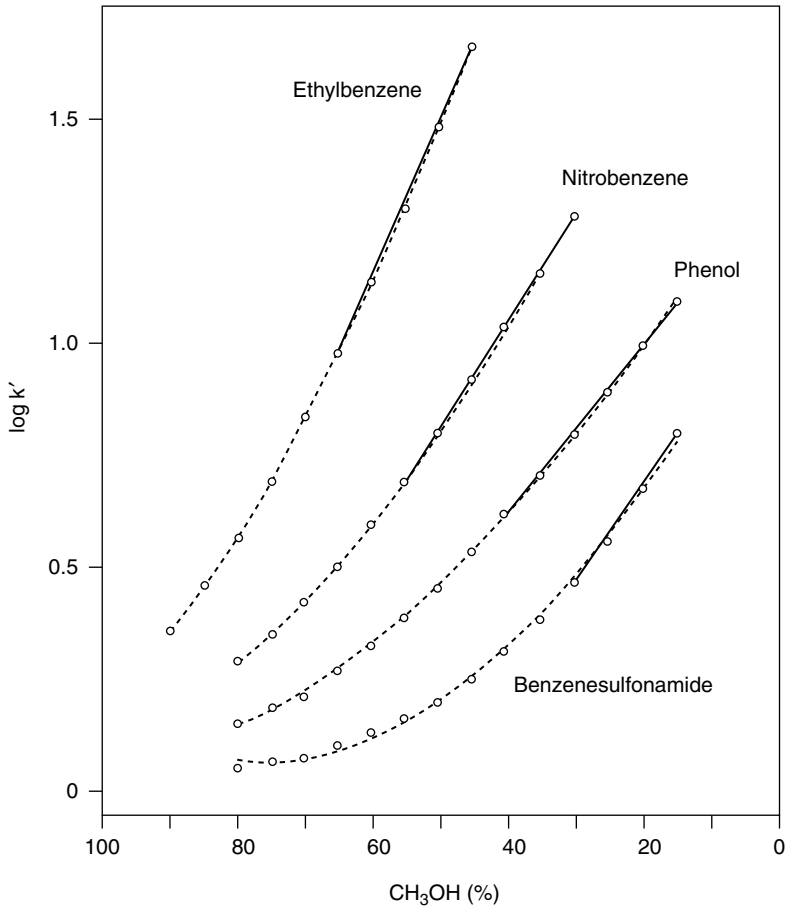


FIGURE 47.16 Plots of solute retention on C_{18} stationary phase (as logk) versus the volume fraction of modifier in RP. The solid line shows the linear region, the dotted line shows the quadratic region. (Reproduced from Chromatographia with permission from Vieweg.)

Beyond this value the order of retention is reversed. Peak crossover occurs when scanning the ϕ values range.

Selection of organic modifier is often achieved through trial and error. Experienced chromatographers are well aware of solute-solvent interactions. Snyder (49) has provided a useful selectivity triangle of solvent where dipole-dipole, proton donor, and proton acceptor interactions are the three apices (Figure 47.17). Solvents are gathered in eight groups. Solvents in a single group exhibit similar interactions. When considering the triangle, it is obvious that acetonitrile and methanol, for example, will interact differently. When solutes are eluted according to the sole hydrophobicity (for example, an homologous n alkanol series) use of either methanol or acetonitrile does not change the order of retention. Conversely, when very different chemical species are separated use of methanol or acetonitrile will yield different retention and selectivities and reversal of retention order is possible. In the same way chloroform and dichloromethane (two modifiers very often advocated in NPLC) may yield different retentions. A discussion on solvent classification has been published by Siouffi (50).

Gradient elution is performed when solutes are strongly retained. To decrease the t_r of these late eluting peaks modifier (stronger eluent) is incrased. From the above linear equation a linear increase in ϕ will result in a linear decrease in Ln k. This is called LSS (linear solvent strength).

The general approach is based on the solution of the basic differential equation $dV = k \cdot dVo$ where dV is the differential increase in the volume of mobile phase that has passed through the column, dVo is a differential fraction of the column hold up Vo, and k is the retention factor that is assumed constant during the migration of the solute band by an infinitely small distance corresponding to dVo. In binary gradients a "weaker" eluent (or diluent) A is associated with a "strong" eluent B. In linear gradient $\varphi = a + bt$ where a is the initial concentration φ of the solvent B in the mobile phase at the start of the gradient, and b is the steepness (slope) of the gradient per time or per volume unit of the eluate. Snyder (51) and Jandera (52) extensively published on gradient and derived equations which can be used for optimization of separation.

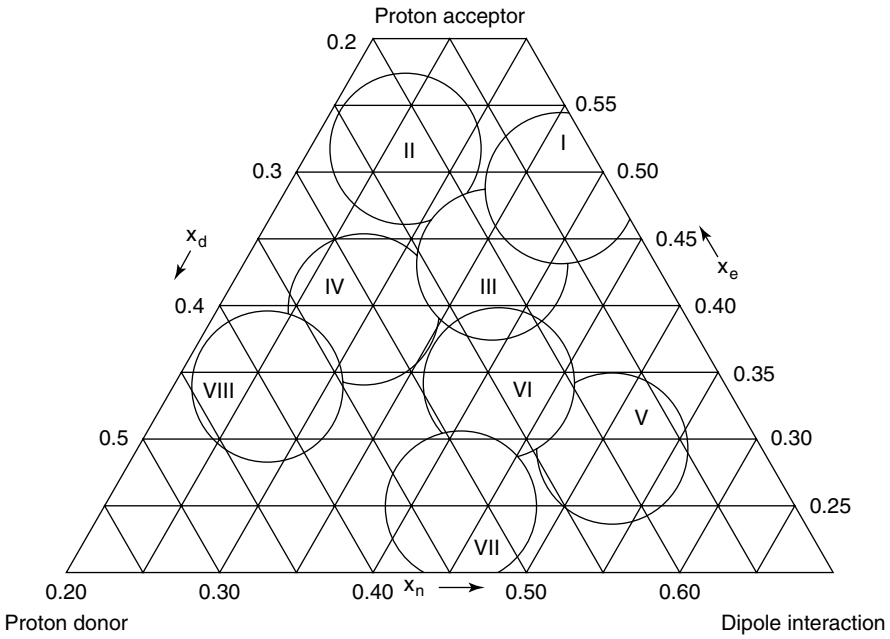


FIGURE 47.17 Selectivity triangle for solvents (Ref. 49). (Reproduced with permission from Preston Publications.)

Classificaiton of solvent selectivity according to snyder

Group Designation	Solvents
I	Aliphatic ethers, *mehtyl t-butyl ether*, tetramethylguanidine, hexamethyl phosphoric acid amide (trialkylamines)
II	Aliphatic alcohols, *methanol*
III	Pyridine derivatives, *tetrahydrofuran*, amides (except formamide), glycol ethers, sulfoxides
IV	Glycols, benzyl alcohol, *acetic acid*, *formamide*
V	Ethylene chloride
VI	a) Tricresyl phosphate, aliphatic ketones and esters, *dioxane*, polyesters b) Sulfones, nitriles, acetonitrile, propylene carbonate
VII	Aromatic hydrocarbons, *toluene*, halosubstituted aromatic hydrocarbons, nitro compounds, *methylene chloride*, aromatic ethers
VIII	Fluoroalcohols, m-cresol, water, (*chloroform*)

Gradient runs can be used to predict either isocratic or gradient separations. First, software was developed for RP separations. The model has been extended to other types. The selection of initial instrument parameters and separation mode are left to the user. Binary systems were first proposed. Extension to ternary mobile phase in RPLC permits a fine selectivity tuning.

RP phases allow a wide range of separation. They are well suited for polar organic solutes soluble in water. A rule of thumb is that retention order is similar to solubility in water or water modifier mixture. Apolar solutes such as polycyclic aromatic hydrocarbons are well separated on C_{18} phases according to their hydrophobicity.

In micellar liquid chromatography the mobile phase consists of surfactants at concentrations above their critical micelle concentration (CMC) in an aqueous solvent with an alkyl bonded phase. Micelles act as a mobile phase modifier. Retention behavior is controlled by solute partitioning from the bulk solvent into micelles and into the stationary phase as well as on direct transfer from the micelles in the mobile phase into the stationary phase. Reference (53) gives a broad overview of the method.

C. Ionizable Solutes

Separation of ionic or ionogenic solutes can be performed with three fundamental methods: ion pair formation, ion exchange, and ion exclusion.

a). Assume a weak acid AH. The dissociation equilibrium is

$$AH \Leftrightarrow A^- + H^+ \quad \text{with the dissociation constant } k_A$$

At low pH in a buffered solution only AH exists. Separation with a RP system is thus possible provided that the acid is not too strong. Alkyl sulfonic acids, for example, may exhibit very low pK_A. Separation of acids in RP occurs according to the degree of hydrophobicity. Knowledge of the analyte pKa can greatly assist in achieving a desired mobile phase pH. A requirement is that the buffer should be soluble in the amount of the organic modifier. This is particularly relevant in the case of gradient methods especially with methanol and phosphate buffer. For UV detection, the

buffer must be sufficiently transparent at the wavelength selected. LC-MS confers its own set of considerations regarding buffer concentration, buffer volatility, and ionization suppression. Ammonium acetate is better suited for LC-MS. Table 47.3 displays some characteristics of common buffers. Conversely, if pH solution is raised, A^- and H^+ are the only existing species. A^- can be chromatographed on an anion exchanger. Another method is ion pairing or ion interaction.

b). Ion pairing

In this technique a reversed phase column (most often RP8) is utilized with a mobile phase consisting of an aqueous organic mixture to which an ion pairing agent is added.

An ion pairing agent may form with analyte an ion pair with increased lipophilicity and consequently is retained on the RP stationary phase. Since the ion pairing agent is continuously fed onto the column it is most often advocated that the ion pairing agent induces a dynamic modification of the surface of the RP stationary phase. The lipophilic part of the ion pairing agent coats the surface, leaving the ionic part to interact with counter ions.

Retention can occur via

i. Retention of the ion pair formed between the cations (or the anions) of the analyte and the anion (or the cation) of the ion pairing agent. The ion pair itself is adsorbed onto the stationary phase.
ii. The analyte is retained through an ion pair complex formed with an amphiphilic ion previously absorbed onto the surface of the hydrophobic material.
iii. The analyte is retained through ion exchange reactions with the adsorbed pair reagent.

Retention increases as concentration of the ion pairing agent increases but decreases beyond a certain concentration; pH is a key parameter.

Ion pair reagents are most often either nitrogen (+) or sulfonate (−) species, e.g., cetyl trimethyl ammonium bromide, diamino dodecane, tributyl ammonium chloride, cetyl pyridinium chloride; sodium dodecyl sulfate, Chap:3-[(3 cholamidoproyl) dimethyl ammonio]-1propane sulfonate, Tris: tris-(hydroxymethyl) aminomethane.

TABLE 47.3
Buffering Ranges

Buffer	pKa	Range
Phosphate	2.1	1.1–3.1
	7.2	6.2–8.2
Acetate	4.8	3.8–5.8
Citrate	3.1	2.1–4.1
	4.7	3.7–5.7
	5.4	4.4–6.4

c). Ion chromatography (IC)

IC is the modern version of the well-known ion exchange chromatography. IC is able to separate any ion when the appropriate column is utilized: bromide, chloride, fluoride, nitrite, phosphate, sulphate, etc. It is able to speciate oxidation states of several metals such as Fe(II) and Fe(III). The challenge to IC comes from capillary electrophoresis.

Instrumentation required for IC simply comprises a metal-free HPLC system and conductivity detection. Since conductivity is a common property of ions this method is universal and quite sensitive.

In IC both mobile and stationary phases are ionic.

a. Ion exchange

Principle of operation: ion exchangers are insoluble solid materials which contain exchangeable cations or anions. These ions can be exchanged for a stoechiometrically equivalent amount of other ions present in an electrolyte solution.

Exchange reactions are written as (for example, in cation exchange)

$$R\text{—}H^+ + Na^+ \Leftrightarrow R\text{—}Na^+ + H^+$$

R— is the matrix of ion exchanger.

The thermodynamic equilibrium constant is

$$K_{H+}^{Na+} = \frac{(R\text{–}Na^+)\cdot(H^+)}{(Na^+)\cdot(R-H^+)}$$

Assume E^+ is a monovalent element cation, and M^{n+} is a sample metal ion

$$R\text{–}nE^+ + M^{n+} \Leftrightarrow R\text{–}M^{n+} + nE^+$$

$$K_{E/M} = \frac{(M^{n+}Rn)\cdot(E^+)^n}{(E^+\text{-}R)^n\cdot(M^{n+})}$$

At low loading of sample ions, the term (E^+-R) is equal to the exchanger capacity Q.

$$K_D = \frac{(M^{n+}Rn)}{(M^{n+})}$$

is proportional to k, the retention factor

and

$$k = K_{E/M}\cdot\frac{(Q)^n}{(E^+)^n}$$

More generally,

$$\text{Ln } k_A = \frac{1}{y}\cdot \text{Ln } K_{A/E} + \frac{x}{y}\cdot \text{Ln}\frac{Q}{y} + \text{Ln}\frac{W}{V_M}\frac{x}{y}\text{Ln}(E_m{}^y)$$

$$\text{Ln } k = \text{Constant} - \frac{x}{y}\text{Log } (E_m{}^y)$$

k_A is the retention factor for a solute A^x
$K_{A/E}$ is the ion exchange coefficient for solute A and eluent E

Q is the ion exchange capacity of the stationary phase
W is the weight of the stationary phase used in the column
V_M is the volume of mobile phase
x is the charge of the solute anion
E_m^y is the concentration of the element ion in the mobile phase.

In IC low capacity ion exchange columns are utilized. Eluents are in the range 1–10 mmol.

Stationary Phases. In ion exchange we can distinguish

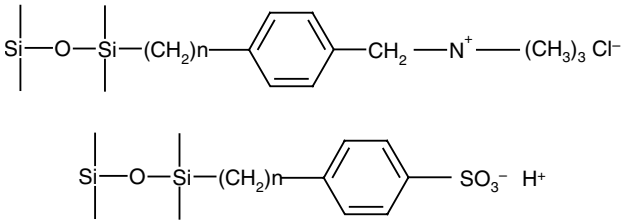
SCX: strong cation exchangers (functional group SO_3^-); in the H^+ form they represent "solid" acids.
WCX: weak cation exchangers (functional group COO^-)
SAX: strong anion exchangers or strong base (functional group alkanol quaternary amine)
WAX: weak anion exchanger (functional group amino)

Amphoteric ion exchangers contain anionic and cationic exchange sites.

Chelating ion exchange groups are: iminodiacetate, 8-hydroxyquinolinol, β diketone, triphenyl methane dyes, carbamates, EDTA, PAR [(4–2 pyridyl-azo)resorcinol]. New packings are compiled in the annual review which appears in LC/GC magazine.

Base materials

i. The majority of stationary phases are agglomerated or pellicular materials consisting of a monolayer of charged latex particles which are electrostatically attached to a functionalized internal core particle. Polystyrene Divinylbenzene (PS-DVB) with different degrees of cross linking is the substrate. Particle diameters are typically in the range of 5–25 μm while adsorbed latex particles are 0.1 μm. This material exhibits very good pH stability and excellent mass transfer properties. For example, analysis of oligosaccharides from honey is performed at pH 11–12.
ii. Microporous methacrylate-based materials are mainly used for anion exchange because of their resistance to high pH eluents.
iii. Silica-based materials are produced by grafting organic moieties according to the procedure utilized in producing reversed phase silica: aminopropyl bonded silica or functionalized silicas such as sulphonate are available. An advantage of silica-based materials is the low probability of secondary interactions between the solute ions and the silica substrate. Use of Silica-based materials is rapidly declining due to their poor hydrolytic stability.



iv. Electrostatic agglomerated films on ultra-wide-pore substrates (1000–3000 A) have been widely used. Wide pores accomodate a coating of ion exchange colloid on the interior or exterior surfaces. Another means of producing base material is the polymer coating of silica material. A layer of polybutadiene-maleic acid (PBDMA) is deposited on silica and then cross linked by peroxide-initiated radical chain reaction.

Mobile phases

Any ion in solution can be used that can compete with the analyte for the fixed ions on the stationary phase. In anion analysis hydroxyde eluents have distinct advantages over the carbonate eluents. These advantages include a wider linear working range for analyte conductivity, increased sensitivity, and the capacity to effectively elute highly retained ionic analytes using gradient capabilities. Conversely, carbonate is a divalent anion that possesses strong pushing properties in comparison to hydroxide eluents. In the EG 40™ eluent generator from Dionex, deionized water is pumped through the KOH generation chamber and a DC current is applied between the anode and the cathode of the cartridge (Figure 47.18). Under the applied field, the electrolysis of water occurs while hydronium ions generated at the anode displace K+ ions in the electrolyte reservoir. The displaced K+ ions migrate across the ion exchange connector into the KOH generation chamber and combine with OH− ions generated at the cathode to produce a KOH solution.

Factors influencing retention and selectivity are: hydration enthalpy, hydration entropy, polarizability, charge, and size and structure of both eluent and solute ions. Concentration of eluent ion (or ionic strength) and pH also play important roles.

A secondary equilibrium occurs according to the nature of the sorbent matrix. Hydrophobic interaction markedly influences retention (a typical example is the well-known aminoacid sequence on cation exchanger which does not follow the pKa sequence). With inorganic ions the perchlorate (ClO_4^-) effect is also well established. Non-ionic eluent modifiers are often used to change the ion exchange affinity of hydrophobic ions.

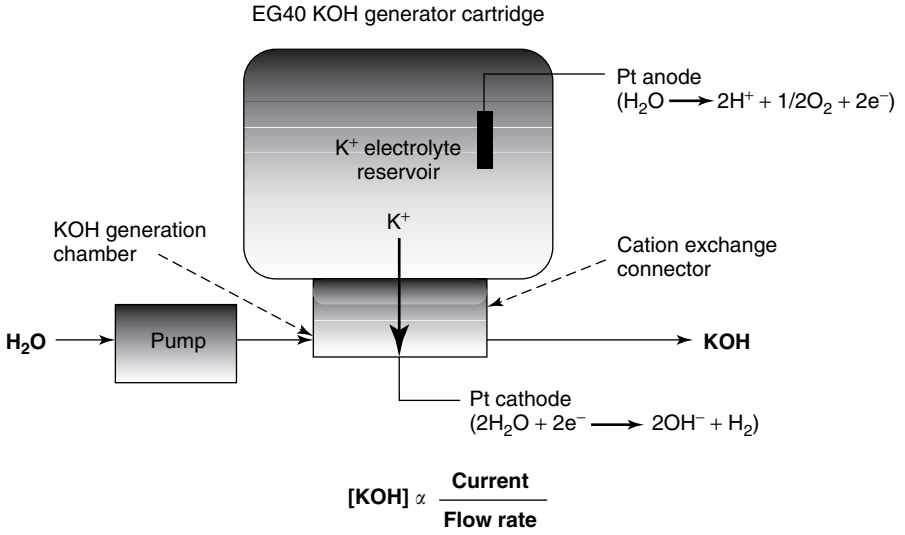


FIGURE 47.18 The EG 40 generator cartridge. (Courtesy of Dionex.)

Gradient elution starts with an eluent of low ionic strength for the resolution of the most weakly retained species. The dependence of the analyte retention on the eluent concentration is a straight line with slope given by the ratio of charges of analyte and eluent. Column switching permits the separation of both inorganic and organic anions in one run. Simultaneous analysis of ions is possible; column switching is configured for anion analysis in column A and cation analysis in column B.

Separation selectivity depends on the degree of electrostatic forces. With a SCX phase the binding force decreases with decreasing diameter.

$$H^+ < Li^+ < Na^+ < NH^{4+} < K^+ < Ag^+ < Be^{2+} < Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$$

With SAX material a selectivity series with respect to binding force also exists:

$$F^- < OH^- < CH_3COO^- < Cl^- NO_2^- < CN^- < NO_3^- < SO_4^{2-}$$

b. Ion exclusion

Ion exclusion relies upon Donnan equilibrium. The main parameter in this mode is the electrostatic interaction of the solute with the charged functional groups on the surface of the stationary phase.

The stationary phase can be considered as a charged membrane separating the flowing mobile phase from the static, occluded mobile phase trapped in the pores of the exchanger. Ionic solutes are rejected because of their inability to penetrate inside the pores and so are eluted at the void volume of the column. Conversely, non-ionic substances may partition between the occluded liquid phase and the flowing mobile phase. The degree of partition determines the extent of the retention. Sample anions are excluded from the resin phase by the fixed charges of the sulfonate groups of the cation exchange resin.

Separations by ion exclusion are usually performed on a cation exchange column with a PS-DVB resin. The solute retention volume is

$$V_R = V_o + K_D \cdot V_i$$

where

- V_o is the interstitial volume of eluent (eluent outside the resin beads)
- Vi is the occluded volume within the resin beads
- K_D is the distribution constant of the solute

When a very large solute or ion cannot enter the stationary phase K_D is 0; when the solute is free to enter $K_D = 1$.

The range of retention volumes is rather small and consequently, ion exclusion columns are rather long.

Acids are found to elute in decreasing order of their acid dissociation constants; the stronger acids elute first.

D. Chiral Stationary Phases

The food and beverage industry is becoming increasingly concerned with the analysis of enantiomers which can affect flavor, fragrance, age, and even the adulteration of products. Most efforts have been directed towards resolution of amino acids either native or derivatized.

In chiral chromatography, an enantioselective molecule (selector) is either immobilized on a surface support as a stationary phase or present as an additive to the mobile phase.

When a chiral selector is used as the stationary phase, the primary retention mechanism is complexation with the surface immobilized chiral selector. For 1:1 solute-selector

complexation, the equilibrium constant (Kcomp) is related to the retention factor k by

$$\text{Kcomp} = \frac{[\text{solute-selector}]}{[\text{solute}]\,[\text{selector}]} = \frac{\text{k}}{\varphi[\text{selector}]}$$

where [solute-selector], [solute], and [selector] represent the equilibrium concentrations of solute selector, free solute, free selector and respectively, and φ is the phase ratio.

Enantioselective ligand exchange chromatography was suggested by Davankov et al. (54) in the late 1960s for the resolution of racemic compounds into stereoisomers of amino acids, amino alcohols, and diamines. Such molecules exhibit a pair of heteroatoms which can form labile chelate type coordination complexes with Cu(II), Zn (II), or Ni(II). A chiral bidentate ligand such as hydroxyproline was attached to a support. Copper (II) was added to the mobile phase and coordinated with both the chiral selector on the stationary phase and the free amino acids in solution, to form a transient diastereomeric complex. This approach is limited to specific chiral molecules which can coordinate with the copper (II).

A discussion of the mixed-ligand ternary complexes between the chiral selector and the enantiomers to be separated can be found in Ref. 55.

The only commercially successful chiral stationary phase for thin layer chromatography is based on the ligand exchange approach (56).

The use of cyclodextrins as chiral selectors has grown rapidly in recent years. Cyclodextrins are cyclic oligosaccharides composed of D-glucose units connected through the 1 and 4 positions by α linkages (Figure 47.19). Those containing 6, 7, and 8 glucose units (i.e., α cyclodextrin, β cyclodextrin, γ cyclodextrin) are most common and available commercially. Cyclodextrins are usually described and depicted as toroidal molecules (conical cylindrical); they exhibit a relatively hydrophobic cavity in the middle and a relatively hydrophilic surface on the outside. They can form host-guest complexes with a variety of molecules. Since the internal cavity of the cyclodextrin tends to be more hydrophobic than either its exterior most non-polar or weakly polar molecules can penetrate and reside in the cavity. Cyclodextrins can be used as a mobile phase additive but the selectivity of covalently bonded cyclodextrins on a support is larger. There are many commercially available "cyclodextrin" stationary phases. Various moieties are utilized to functionalize the cyclodextrins and a huge amount of literature is available on the topic. Commercially available cyclodextrin derivatives are: acetylated, hydroxypropylether, naphtylethyl carbamate, or 3,5 diphenyl carbamate. The various moieties used to functionalize cyclodextrins can alter the enantioselectivity. It is possible to suppress the inclusion complexation by using a non-hydrogen bonding, polar organic solvent such as acetonitrile as the main component of the mobile phase. The acetonitrile occupies the cyclodextrin cavity and enhances hydrogen bonding between the hydroxyl groups on the cyclodextrin and any hydrogen bonding groups on the chiral analyte.

Macrocyclic glycopeptide antibiotics are multimodal as they can be used in the reverse phase mode, normal phase mode, or polar organic mode. The most successful are avoparcin, teicoplanin, ristocetin A, or vancomycin. They exhibit an aglycon "basket" made of fused macrocyclic rings and pendant carbohyhydrate moieties (Figure 47.20). Vancomycin and teicoplanin are the the most interesting. Teicoplanin, for example, (Mw1877) exhibits 2″ stereogenic centers; it has a hydrophobic acyl side chain attached to a 2-amino-2-deoxy-β-D-glycopyranosyl moiety. Teicoplanin has been used successfully in a number of applications (57); it can be used with no buffer in the mobile phase which greatly facilitates the LC/MS.

Protein LC columns have the least capacity of any chiral stationary phases. α1-acid glycoprotein, (AGP), ovomucoid, human serum albumin (HSA), or bovine serum albumin (BSA) are used in the reversed phase mode with aqueous buffers or hydro-organic solvents. HSA-based columns usually present the problem of a significant variation of the chromatographic performances, depending not only on the immobilization procedure but also on the origin of the anchored protein. Recombinant HSA can overcome the problem. AGP is one of the most used protein phases to separate enantiomeric amines. Reversal of elution order is not unusual in RPLC when working with protein-based enantioselective columns.

Historically, cellulose or starch components were the first to be used as chromatographic chiral selectors.

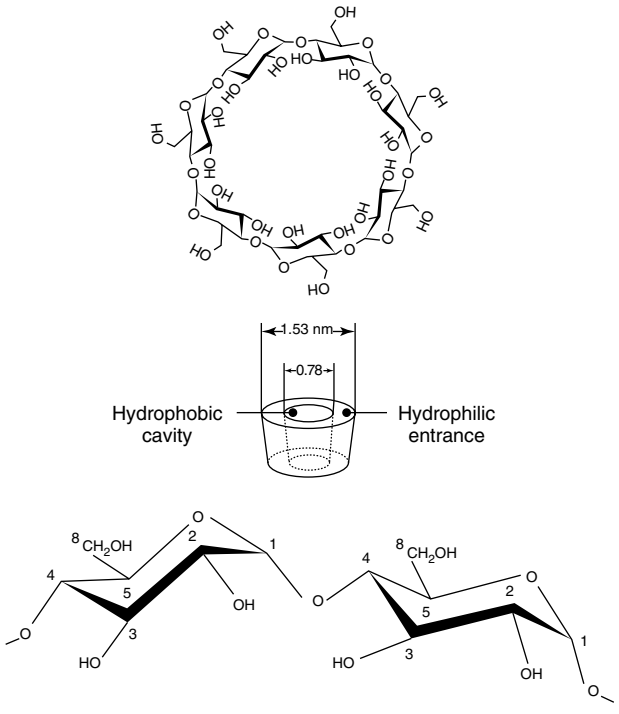


FIGURE 47.19 Schematic of the various cyclodextrins.

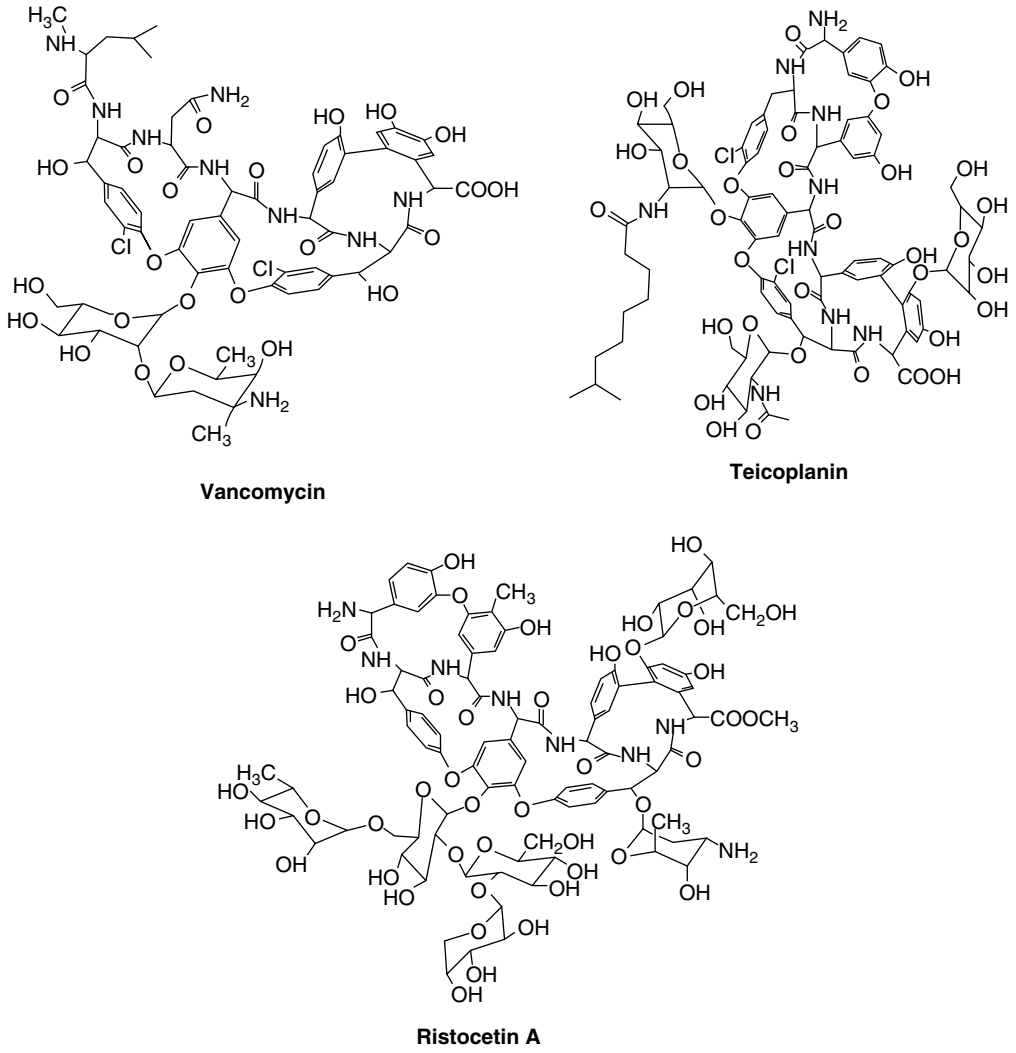


FIGURE 47.20 Structures of Vancomycin, Teicoplanin, and Ristocetin A.

Cellulose and amylose are the most accessible naturally occurring optically active polymers. These polysaccharides themselves show chiral recognition, but do not afford practical chiral stationary phases. Their derivatization brings about practically useful CSPs. Cellulose esters or phenylcarbamates, amylose phenylcarbamates are not covalently attached to the support, but rather are coated on wide pore silica that has first been silanized. Polysaccharide-derived CSPs supported on silica are classically prepared by reaction of the polysaccharide with a benzoyl chloride or a phenyl isocyanate in homogeneous conditions; these derivatives are coated from a solution onto a macroporous γ-aminopropylsilica matrix by evaporation of the solvent. They have good capacity in the normal phase mode. Among the various kinds of cellulose esters, cellulose tribenzoate and its derivatives show high chiral recognition abilities when adsorbed onto macroporous silica. The optical resolving ability of the benzoate or carbamate derivatives are dependent on the substituents on the phenyl group (58). Francotte (59) has demonstrated that many polymeric chains are involved in the chiral recognition mechanism. The supramolecular effect depends on the dissolution solvent which predetermines the geometry of chiral centers. A compilation of enantiomer separation by such phases can be found in Reference 60.

Pirkle and coworkers (61) first employed the concept of reciprocity of chiral recognition in designing effective chiral selectors. The reciprocity of chiral recognition states that if a chiral stationary phase (CSP) derived from an optically pure compound A can discriminate each isomer of compound B, then a CSP derived from an optically pure B may discriminate each enantiomer of compound A. Many CSP were synthesized. Some contain either a π acid or π base moiety (or even both). However, simultaneous interactions must be present, too (hydrogen bonding, dipolar interactions).

Molecular imprinted polymers (MIPs) can selectively recognize the template molecule used in the imprinting

process. In molecular imprinting, monomers, such as methacrylic acid and styrene, are first assembled around the template molecule. This arrangement of monomers is fixed on polymerization. After polymerization, the polymer is dried, ground, and sieved, the linker bond is chemically cleaved, and the templates are freed from the polymer matrix, leaving cavities that are complementary to the template in shape and spatial configuration. For a review, see Reference 62.

Chirbase provides comprehensive structural, experimental, and bibliographic information on successful or unsuccessful chiral separations which have been obtained on CSPs. Data mining and pattern recognition with a decision tree leads to a more rational choice of the CSP or of the mobile phase to a given CSP (63).

E. Size Exclusion

In this mode, the stationary phase is a porous solid support, the mobile phase is the most eluting one e.g. porous silica and water. All molecules are eluted in the void volume. Large molecules cannot enter the pores and travel in the external volume only, they are excluded; small molecules can penetrate into the pores and they travel through both external and internal volumes; by consequence their velocity is lower than the one of large molecules. The relevant retention parameter is the radius of gyration of the molecule which can be obtained by light scattering detection at several angles. Since there is no partition, the entropy is of paramount importance and the column temperature should be thoroughly controlled. The column set is calibrated using a series of polymer standards to determine a relationship between logM and the retention time of the standards. Calibration is typically performed using well-characterized standards (polystyrene, dextrans).

For any polymer, $Hv = M[\eta]$ where Hv is the hydrodynamic volume, M is the molecular weight (Mw), and $[\eta]$ is intrinsic viscosity. The intrinsic viscosity is the value of the reduced specific viscosity at infinite dilution $[\eta] = \lim c \to 0\ \eta_{sp}/c$. The Mark Houwink equation relates intrinsic viscosity and molecular weight for a given polymer/solvent combination.

$[\eta] = K\ M^{\alpha}$ where K and α are the Mark Houwink constants.

A triple detection (differential refractometry, viscosimetry, and light scattering detector) is often used which yields an accurate response and allows the determination of branching distribution of polyolefins, for example.

The number-average M_n, the weight-average M_w, molecular mass values, and polydispersity are defined by

$$M_n = \Sigma n_i M_i / n_i$$

$$M_w = \Sigma n_i M^2 / n_i M_i$$

Polydispersity $= M_w/M_n$ where n_i represents the number of oligomer molecules having a mass of M_i, M_n is the number-average molecular mass, and M_w is the weight-average molecular mass.

IX. SAMPLE PREPARATION

A sample preparation step is often necessary to isolate the components of interest from a sample matrix. Sample preparation is often the major source of error in analytical procedures as practitioners are often required by law to use traditional methods.

Ideal sample preparation technique should be simple, solvent free, efficient, and inexpensive. EPA method 3600C gives general guidance on the selection of clean-up methods that are appropriate for various target analytes.

A sample pretreatment will:

i. Improve accuracy
ii. Improve detectability
iii. Improve selectivity by removal of interfering matrix

A result is reproducible if the sample to be analyzed is fully representative of the material to be tested. That means that the samples taken can be equated with the entire batch.

We can distinguish off-line and on-line procedures.

A. Lyophilization

Relatively large samples containing water-soluble analytes are frozen in a dry ice-acetone bath or in liquid nitrogen. Subsequently, the frozen samples are placed in the freeze dryer where water is removed by vacuum sublimation. After freeze drying, the residues can be dissolved in a suitable organic solvent.

B. Ultrafiltration

Ultrafiltration involves the use of specialized membranes that allow rapid and gentle concentration or removal of molecules based on their molecular weight. Ultrafiltration membranes consist of a very thin and dense layer on top of a macroporous support that has progressively larger open spaces on the downstream side of the membrane. Substances that pass through the membrane will also pass easily through the macroporous support. Concentration can be accomplished because molecules smaller than the molecular weight cut-off of the membrane flow through. Molecules larger than the membrane cut-off will be retained on the sample side of the membrane. Performances are affected by pressure. As the pressure increases so does the flow rate but there is a resistance from the concentrated layer on the surface of the membrane. This phenomenon is called concentration polarization.

Membranes are usually made out of cellulose or polysulfone.

Porosity of membrane determines the size of molecules concentrated. In the supported liquid membrane device analytes are extracted in a flow system from an aqueous sample through a hydrophobic membrane liquid into a second aqueous solution. The impregnated membrane is clamped between two circular PTFE blocks. Two types of transport mechanisms across the membrane are in use: pH gradient or ion pairing formation.

C. Dialysis

Dialysis is a method used to separate molecules through a semi-permeable membrane separating two chambers. The concentration gradient of the components across the membrane drives the separation. Dialysis is used for removal of excess low-molecular-weight solutes. Analytes small enough to diffuse through the pores of the membrane are collected.

Microdialysis probes are implanted into the area of interest and slowly perfused with a solution usually matching the fluid outside. The probe is equipped with a membrane through which substances pass due to the concentration gradient. It can be coupled on line to liquid chromatography (HPLC), capillary electrophoresis (CE), or mass spectrometry.

The flux through a dialysis membrane is described by Fick's law:

$$I = -D \cdot A/\tau * dc/dx$$

where

- I is the flux (mol/s)
- D is the solute diffusion coefficient (m^2/s)
- A is the membrane area (m^2) τ: is the tortuosity of the membrane
- dc/dx is the concentration gradient across the membrane mol/m^4
- D is obtained from the Stocke Einstein equation,

$$D = k \cdot T / 6\pi \eta r$$

- k is the Boltzmann's constant
- T is the absolute temperature (^oK)
- η is the viscosity of the medium
- r is the radius of the molecule

The smaller the molecule, the larger the diffusion coefficient and the higher the flux. The relative recovery or the dialysis factor is the ratio of the dialysate (analyte in the outgoing liquid) to the concentration outside the membrane.

There are three modes of conducting a microdialysis experiment:

Perfusion and stirring are continuous over the monitoring period

Perfusion is carried out only during sampling but the stirring is maintained

Perfusion and stirring are carried out during sampling only

D. Liquid/Liquid Extraction (LLE)

This sample pretreatment is mainly devoted to organic compounds which can be removed from an aqueous solution by extracting them into a water-immiscible solvent. Chelation or ion pairing between large and poorly hydrated ions and chelating agent may form neutral compounds which can be extracted by organic solvents.

When two immiscible solvents are placed in contact, any substance soluble in both of them will distribute or partition between the two phases in a definite proportion. According to the Nernst partition isotherm, the following relationship for a solute partitioning between two phases a and b is

$$\frac{(A)_a}{(A)_b} = K_d \quad K_d \text{ is the partition coefficient}$$

$$(A)_a \Leftrightarrow (A)_b \text{ very often } (A)_{aq} \Leftrightarrow (A)_{org}$$

It assumes that no significant solute-solute interactions or strong specific solute-solvent interaction occurs. The K_d value is constant when the distributing substance does not chemically react in either phase and temperature is kept constant.

The fraction extracted R is related to K_d by

$$\frac{C_o V_o}{C_o V_o + C_w V_w} = K_d V/1 + K_d V$$

C_o and C_w represent the solute concentration in organic (o) and water (w) phases, respectively. V_o and V_w are volumes of organic and aqueous phases $V = V_o / V_w$.

It is possible to increase the extent of extraction with a given K_d by increasing the phase volume ratio. When performing micro LLE the analyst works with an extreme ratio of extracting solvent/extracted liquid (for example, 1/800). Another way is to carry out a second and a third extraction. After n extractions the final concentration of the compound in the aqueous phase is

$$Cw_n = C_w \left[\frac{Vw^n}{V_W + K_d V_0} \right]$$

An extraction process is more efficient if it is performed with several small portions of solvents.

Solubility increases as the values of solubility parameter δ of the solute and the solvent are close.

$$\text{Ln } K_d = \frac{\overline{V}_s}{RT} [(\delta_s - \delta_i)^2 - (\delta_j - \delta_s)^2]$$

$\overline{V}_s$ is the molar volume of the distributing solute, δ_s is its solubility parameter, and δ_i and δ_j are the solubility parameters of the pair of immiscible solvents.

It must be kept in mind that some species may exist under different forms in aqueous media. An acid, for example, must be written in this form:

$$HA \Leftrightarrow A^- + H^+$$

$$K_d = \frac{(HA)_0}{(HA)_w} = \frac{(HA)_0}{(HA)_{aq} + (A^-)_{aq}}$$

P_{ow} is the partition coefficient of a solute between octanol and water. It is a common measure of hydrophobicity. The usual measurement for Log P_{ow} is the shake flask method where a compound is shaken in an octanol-water mixture. After equilibrium the concentration is measured in one or both phases. Reversed phase HPLC has also been extensively used. Correlation plots of Log P_{ow} against retention time or capacity factors are drawn.

High values of Log P_{ow} give guidelines for extraction.

The single drop extraction is a micro LLE method in which a single drop of organic solvent is contained at the end of a PTFE rod. The procedure is as follows: 2 microliters of organic solvent are drawn into a microsyringe; the needle of the microsyringe is passed through the sample via valve and immersed in the aqueous sample; the syringe plunger is depressed to expose the solvent to the sample; the drop is drawn back into the syringe; and the needle is removed from the sample vial. Usually the needle is inserted into the hot injector of a GC instrument (64).

Microporous membrane liquid-liquid extraction (MMLLE) is a continuous liquid-liquid extraction proceeding via a hydrophobic membrane. The microporous membrane is sandwiched between two immiscible liquid phases: one aqueous and the other an organic solvent. The membrane is wetted with a suitable organic solvent that fills the pores of the membrane. The mass transfer process takes place between the two phases via the pores of the membrane (65).

E. Solid-Phase Extraction (SPE)

This is the most widely used method. Analytes (mainly organics) are trapped by a suitable sorbent by passing through a plastic cartridge containing an appropriate support. A selective organic solvent is used to wash out the target analytes. SPE is rapid and relies upon chromatographic retention and Log Pow. It can be easily automated. Off-line procedures are inexpensive. On-line devices are readily available from many companies.

1. Off-Line Methods

A typical SPE cartridge is displayed in Figure 47.21.

Sorbents are very similar to the liquid chromatography stationary phase. The analyst can take advantage of:

i. Non-polar interactions (hydrophobic): typically octadecyl modified silica, polystyrene, -divinyl benzene copolymers, or carbon-based sorbent.
ii. Polar interactions through hydrogen bond, for example. In this mode sorbents are: bare silica, polar-bonded silica, or polyamide.
iii. Ion exchange: benzene sulphonic acid (cation exchange) quaternary amine (anion exchange).
iv. Immuno sorbents: the lack of selective sorbents to trap organic analytes in water is certainly the most significant weakness of the SPE technique.

The most popular format is the cartridge filled with 40–60 μm d_p packing materials. The most second popular format is the disk which allows higher flow rates without channeling effects. One format devoted to high throughput

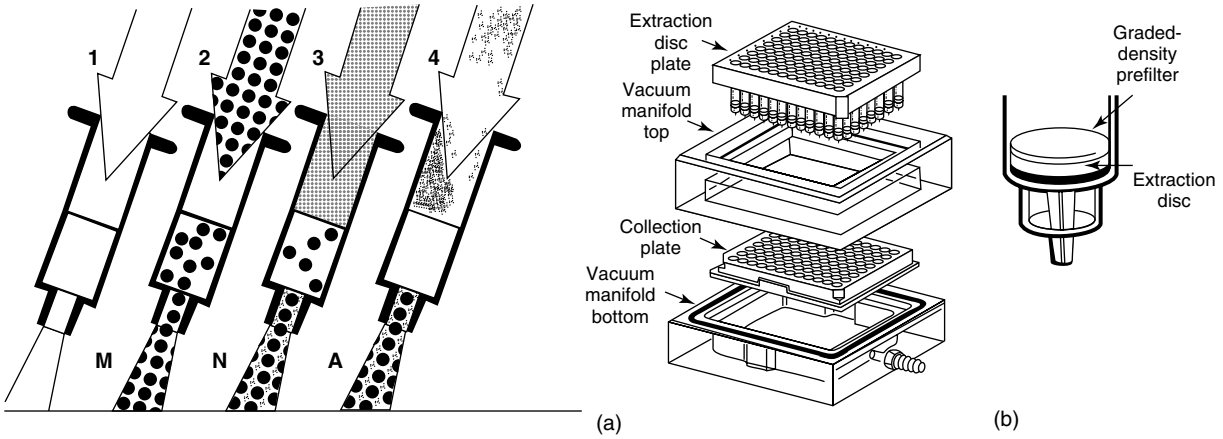


FIGURE 47.21 A SPE cartridge (far left), a 96 well plate format (a), and an extraction disk (b).

applications is the 96 well plate format (Figure 47.21) designed to fit automated plate handling systems.

The SPE technique procedure is as follows: conditioning, sample application, washing, and elution.

The adsorbent must be wetted. With bare silica no problem generally occurs but problems may occur with hydrophobic sorbents. In this case adsorbent must be treated with a suitable solvent. Methanol is preferred for most applications; however, other solvents which are miscible with water such as isopropanol, tetrahydrofuran, or acetonitrile are convenient as well. Conditioning is achieved with about 2–3 column volumes of solvent. Then 1–2 column volumes of the sample solvent are poured through the column. After this step the phase must not run dry. After adsorption of the sample molecules the phase may run dry through either a water jet pump or flushing with inert gas.

Elution is then performed with a strong solvent to elute with the lowest possible volume.

Capacity is the quantity of sample molecules retained per unit quantity of adsorbent. Capacity depends on solute size. It lies in the range of 4 to 60 mg/g of packing.

Breakthrough of solutes occurs when they are no longer retained by the sorbent. Overloading beyond the sorbent capacity may also lead to breakthrough of analytes. The breakthrough volume can be measured from the breakthrough curve obtained by monitoring the signal of the effluent from the extraction column (Figure 47.22).

V_b is usually defined at 1% of the initial absorbance and corresponds to the sample volume that can be handled without breakthrough.

V_r is the retention volume of the analyte.

V_m is defined at 99% of the initial absorbance.

The method is time consuming. Another method is proposed: a small volume spiked with a trace concentration (μg/L) level of all the analytes is percolated through the cartridge and peak areas are recorded. The first volume is selected so that breakthrough does not occur for any solute. The sample volume is then increased and the concentration decreased in order to have a constant amount of analytes in the percolated samples. In this mode peak areas remain constant. The breakthrough volume of an analyte is calculated when the peak area begins to decrease and the corresponding recovery can be also calculated by dividing the peak area obtained for the sample volume by the constant peak area obtained for sample volumes before breakthrough.

Prediction of breakthrough volume is important for selecting a convenient sorbent and consequently the amount of sorbent (66).

i. Hydrophobic sorbents: n alkyl silicas are by far the most utilized. A large number of applications on such sorbents has been published. The drawbacks (as with every bonded silica) are the poor stability in very acidic or basic media, the relatively low capacity for polar solutes, and the low recovery of basic analytes.

   From liquid chromatography we know that in reversed phase mode

$$\text{Ln } k = \text{Ln } k_w - S\,\phi$$
$$\text{or Ln } k = \text{Ln } k_w - b\phi - a\phi^2$$

   where ϕ is the organic modifier volume percent in the binary mobile phase (water/modifier).

   Ln k_w can be estimated by a graphical extrapolation to zero modifier content. Ln k_w represents the hypothetical capacity factor of the solute with pure water as eluent. Since Ln k_w is very often correlated with Log P octanol/water, it is often taken as a hydrophobicity constant. Values of Ln k_w may be as high as 3–4 which means that large sample volumes with trace amount of solutes can be handled.

   Styrene Divinyl Benzene copolymers (PS-DVB), either porous or rigid, are stable over the whole pH range. Calculated Ln k_w values on these sorbents are higher than those on C_{18}. Consequently, moderately polar compounds which are not retained by C_{18} silica are more

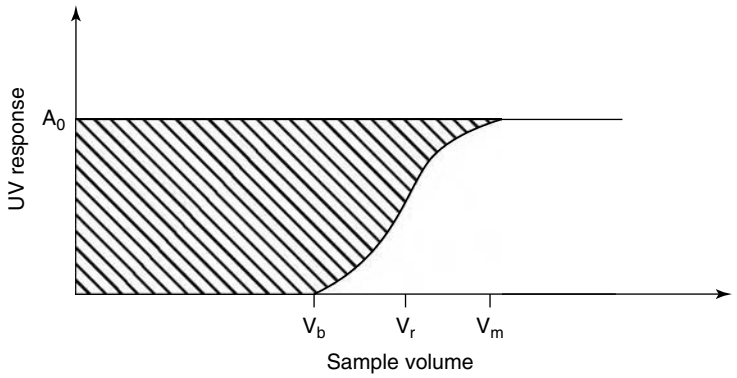


FIGURE 47.22 Breakthrough curve.

readily concentrated on these sorbents. Carbon-based sorbents are gaining acceptance since the availability of porous graphitic carbon. Data obtained with this support demonstrate that it exhibits high retention for apolar compounds but some differences with PS-DVB and C_{18} bonded silica are observed. Hydrophilic-lipophilic polymers do not require equilibration prior to extraction of the analytes.

Selection of eluting solvent is performed through knowledge of eluting strength ε^0 of the solvent in the chromatographic mode.

ii. Use of polar adsorbents is less advocated. Behavior of solutes is well understood since a lot of chromatographic data can be retrieved from TLC (thin layer chromatography) experiments. Flash chromatography is often a new version of silica-based cartridges. Flash chromatography is utilized for laboratory-scale separation. The apparatus is a sintered funnel partially filled with the sorbent (mostly bare silica or reverse phase silica). The mixture of compounds to be purified is adsorbed on the top of the sorbent and the funnel is connected to a flask under vacuum. The vacuum is maintained while the solvent is added to the surface of the column. The solvent is sucked through and collected in the flask.

iii. By pH adjusting many solutes can be ionized (e.g., carboxylic acids) and trapped on ion exchangers. Owing to pH stability resins are widely used. The drawback comes from high amounts of inorganic ions which easily overload the capacity of the sorbent. SPE products of the mixed mode type are gaining acceptance. Most of them are a mixture of hydrophobic and ion exchange materials.

iv. Classical SPE sorbents suffer from a lack of selectivity. Many compounds present in the sample and belonging to the same range of polarity as the target analytes are co-extracted, thus making difficult the determination of analytes of interest. A biological approach consists of developing antibodies against a target molecule. These antibodies are immobilized onto a solid phase to produce an immunosorbent. The most common approach involves covalent bonding of the antibodies onto activated silica or sepharose. Another method is the sol-gel one, which consists of encapsulating antibodies in the pores of a hydrophilic glass matrix. As usual, there are two procedures: off-line and on-line. Main requirements are cross reactivity (affinity towards compounds with a structure similar to the antigen), capacity (defined as the total number of immobilized active antibodies), and extraction recovery. It is now possible to produce antibodies against some target compounds including some small molecules such as pesticides. For example an immunosorbent made with polyclonal anti-isoproturon antibodies covalently bound to a silica sorbent is able to concentrate several phenylureas. Due to the high selectivity, phenylureas can be detected at 0.1 μg/L level in waste waters (67).

2. On-Line Methods

On-line coupling SPE to either LC or GC is easily performed. In the simplest way a precolumn is placed in the sample loop position of a six port switching valve. After conditioning, sample application, and cleaning via a low-cost pump the precolumn is coupled to an analytical column by switching the valve into the inject position. The solutes of interest are directly eluted from the precolumn to the analytical column by an appropriate mobile phase.

The sequence can be fully automated, for example, in the Prospekt system (69).

F. Solid-Phase Micro Extraction (SPME)

This process uses a 1 cm length of focused silica fiber, coated on the outer surface with a stationary phase and bonded to a stainless steel plunger holder that looks like a modified microliter syringe (68). The fused silica fiber can be drawn into a hollow needle by using the plunger. In the first process, the coated fiber is exposed to the sample and the target analytes are extracted from the sample matrix into the coating. The fiber is then transferred to an instrument for desorption (Figure 47.23). Miniaturized SPE devices were recently designed by Millipore. These Zip Tips™ are used with Maldi –TOF.

G. Clean-Up

Extracts obtained from either LLE or SPE contain analytes and other compounds which may interfere in the chromatographic separation. A clean-up is required. The most widely used is fractionation by LC. Extract is loaded onto a chromatographic column packed with an appropriate sorbent (silica, alumina, florisil, bonded silica), and step elution with solvents is carried out. Each fraction is collected and submitted to chromatography. Derivatization prior to fractionation is sometimes performed.

Coupling two sorbents in SPE procedure, for example, hydrophobic sorbent and ion exchange in series, is efficient.

A chart on sample preparation is available upon request from LC-GC International.

X. VALIDATION

Evaluation and validation of analytical methods and laboratory procedures are of paramount importance since the quality of produced chemical information must be

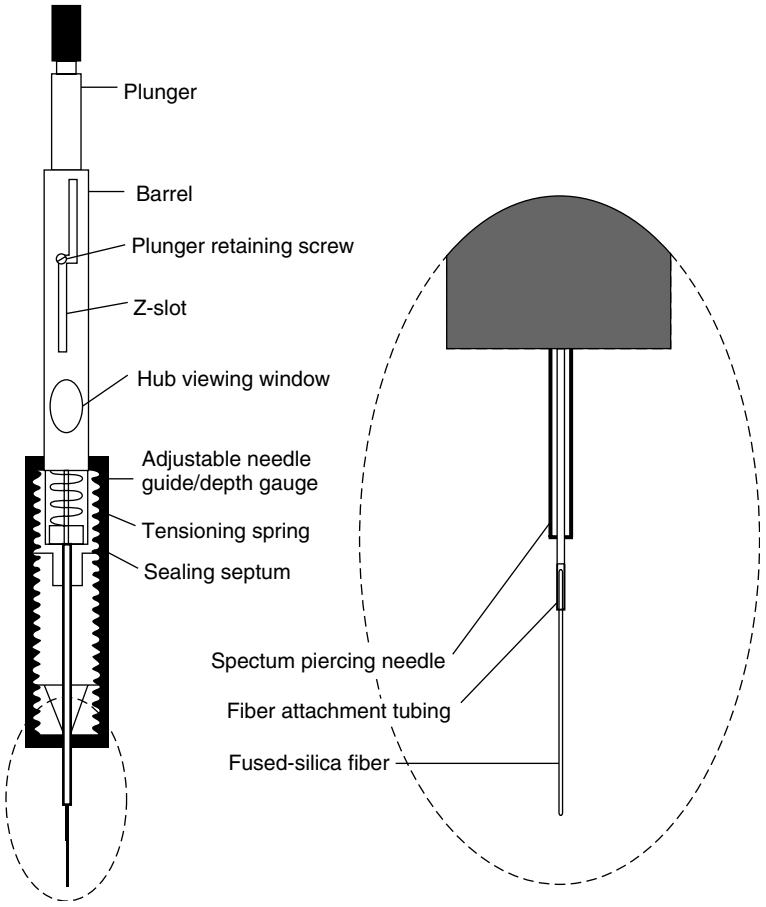


FIGURE 47.23 SPME system.

acknowledged by the customer as the end user of this information. A recognition of quality demands is achieved through accreditation or certification based on international quality standards as issued for example by ISO or OECD. The ideal validated method for food control was defined by the FAO, Food and Nutrition Paper 68, Validation of Analytical Methods for Food Control, Report of FAO/IAEA Expert Consultation FAO/UN Rome, 1998. The ideal validated method is one that has progressed fully through a collaborative study in accordance with internationally harmonized protocols for the design, conduct, and interpretation of method performance studies. This usually requires a study design involving a miniumum of five test materials, the participation of eight laboratories reporting valid data, and most often includes blind replicates or split levels to assess within laboratory repeatability parameters.

Validation begins at the vendor's site, in the structural validation stage. During this stage, the analytical instrument and software are developed, designed, and produced according to Good Laboratory Practice (GLP) and/or the International Organization for Standardization (ISO) guidelines. The FDA has published draft guideline 21 CFR Part 11, which focuses on software validation of computer systems (Draft Guidance for Industry: 21 CFR Part 11 Electronic Records; Electronic Signatures, Validation, U.S. Food and Drug administration, August 2001.

21 CFR Part11 has specific requirements that involve back-up and recovery of chromatographic data.

Calibration is a set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material and corresponding values realized by standards. Calibration constitutes the link between materials and analyzed samples that is necessary for traceability of analytical results.

A. Detection

Dynamic range is that range of concentrations of the test substance over which a change in concentration produces a change in detector signal.

The lower limit of the dynamic range is defined as the concentration producing a detector output signal equal to a specified multiple of the detector short-term noise level.

The upper limit of the dynamic range is the concentration at the point where the slope of the curve obtained

by plotting detector response as a function of concentration becomes zero.

Linear range is that range of concentrations over which the sensitivity (S) is constant to within a defined tolerance (Figure 47.24).

Limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample, below which the analytical method cannot reliably detect a response.

A widely used detection limit technique is the 3 σ approach which is mandated for EPA testing. The standard deviation in concentration units is calculated by computing the standard deviation of blank replicates (≥7) and dividing by the slope of the calibration curve. The number is multiplied by the appropriate value of the Student's t for the chosen α and for n−1 degrees of freedom. The method used to determine LOD should be documented and defined.

Limit of quantification (LOQ) is the smallest quantity of compound to be determined in given experimental conditions with defined reliability and accuracy. A signal to noise ratio of ten is adequate.

$$\text{Limit of detection} = (3Sx/y)/b$$

$$\text{Limit of quantification} = (10Sx/y)/b$$

where b = slope of best fit regression line and Sx/y = estimate of residual standard deviation.

Both LOD and LOQ are affected by chromatographic conditions. Peak height is proportional to the solute concentration in the sample and to the injected volume. If one wants to improve the plate count of the column he or she may lose sensitivity.

Detector's noise is short-term noise in the maximum amplitude of response for all random variations of the detector signal of a frequency greater than 1 cycle per minute. Long-term noise is similar to short-term noise except that the frequency range is between 6 and 60 cycles per hour. Drift is the measure of the amplitude of the deviation of detector response within 1 hour.

B. Repeatability (ISO 3534)

Qualitative means the closeness of agreement between the results obtained by the same method on identical test material under the same conditions (same operator), same laboratory, same apparatus, and short interval of time (same day).

Quantitative is the value below which the absolute difference between two single test results obtained under the above conditions may be accepted to lie with a specified probability (usually 95%).

It is generally assessed by a minimum of nine determinations over the prescribed range for the procedure, e.g., at three concentrations, three replicates each, or by a minimum of six determinations at 100% of the test concentration.

C. Reproducibility (ISO 3531)

Reproducibility expresses the precision between laboratories.

Qualitative means the closeness of agreement between individual results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different times, interday).

Quantitative is the value below which the absolute difference between two single test results on individual material obtained by operators in different laboratories using the standardized test method may be expected to lie within a specific probability (usually 95%).

D. Selectivity

Selectivity is a measure of the extent to which the method is able to determine a particular compound in the matrices

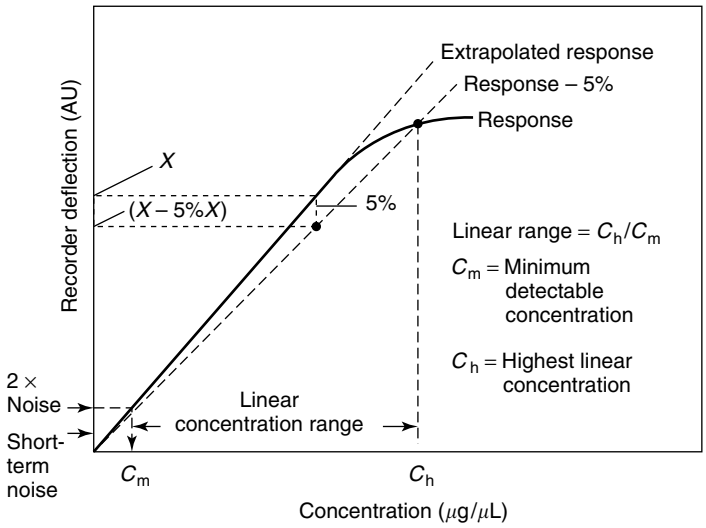


FIGURE 47.24 A calibration curve.

without interference from matrix components. Specificity is often used instead of selectivity.

E. Linearity

Linearity of analytical procedure it is the ability of the method to elicit test results that are directly proportional to analyte concentration (or mass) within a given range.

The analyst must determine:

- linear slope
- y intercept
- correlation coefficient
- relative standard deviation
- normalized intercept/slope.

F. Accuracy

Accuracy measures the difference between the true value and the mean value obtained from repeated analysis. Accuracy can be assessed by analyzing a sample of known concentration (reference material) and comparing the measured value to the true value. One can compare test results from the new method with results from an existing method. Another procedure is to spike the analyte in blank matrices or to perform standard additions. Analysis of variance (ANOVA) estimates the within run precision and the between run precision.

G. Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (RSD) for a statistically significant number of samples.

H. Reliability

Reliability means the probability that the results lie in the interval defined by two selected limits. It gives a rigorous method for evaluating the correctness of a method of analysis in relation to two limits for error.

I. Ruggedness

Ruggedness is the capacity of an analytical method to produce accurate data in spite of small changes in experimental conditions (for example, small flow-rate variations in HPLC). The robustness of the method is evaluated by varying method parameters such as percent of organic modifier, pH of the buffer, column temperature, flow rate, etc.

J. Extraction Recovery

Percentage recovery of the extraction is determined by comparing the detector response of an extracted sample with that of a directly injected standard.

K. Internal Standard

Mostly used in chromatography and capillary electrophoresis, the internal standard monitors the behavior of sample solutes to be analyzed and quantitatively determined.

Internal standard must fulfill some requirements:

- It must exhibit retention behavior similar to the solutes.
- It must exhibit chemical functionalities and structure similar to the solute.
- If a derivatization step is involved in the method, the same reaction must be applied to the internal standard.
- If a sample pretreatment is required, it is better to submit the internal standard to the sample pretreatment and check recovery.

Analysis procedure

A standard solution contains the sample and the internal standard at concentrations C_T and C_E, respectively.

$$m_T = C_T{*}V_T = K_T \cdot A_T$$

$$m_E = C_E{*}V_E = K_E \cdot A_E$$

V_T, V_E are the injected volume of the sample and the internal standard. K_T is the response coefficient of the sample and A_T the peak area. K_E is the response coefficient and A_E the peak area of the internal standard. Usually $V_T = V_E$

The concentration ratio is kept constant whatever the injection volume. A sample solution contains the substance to quality at concentration C_X. Internal standard is added at the same C_E concentration as in the previous standard solution. We thus can write:

$$m_X = C_X \cdot V_{inj} = K_X \cdot A_X$$

$$m_E = C_E \cdot V_{inj} = K_E \cdot A'_E$$

$A'_E \neq A_E$ since two injections are performed. V_{inj} is constant.

$$\frac{C_X}{C_E} = \frac{K_X}{K_E} = \frac{A_X}{A'_E}$$

$$\frac{K_X}{K_E} = \frac{K_T}{K_E}$$

then

$$\frac{C_X}{C_E} = \frac{C_T \cdot A_E}{C_E \cdot A_T} = \frac{K_X}{K_E}$$

$$\frac{C_X}{C_E} = \frac{C_T}{C_E} \cdot \frac{A_X}{A_T} = \frac{K_X}{K_E}$$

$$C_X = C_T \cdot \frac{A_E}{A_T} \cdot \frac{A_X}{A'_E} \text{ if } C_E \text{ is kept constant}$$

It is necessary to check the detector response. Standard solutions are prepared.

$$C_{1T} + C_E \longrightarrow A_{1T} + A'_E$$

$$C_{2T} + C_E \longrightarrow A_{2T} + A''_E$$

$$C_{3T} + C_E \longrightarrow A_{3T} + A'''_E$$

Sample solution $$C_X + C_E \longrightarrow A_E + A_E$$

XI. CONCLUSIONS

The large volume of available literature on HPLC is highly indicative of the wide use of the technique. Columns are being constantly improved and are highly reproducible. Progress in instrumentation is based on the hyphenation with mass spectrometry which can be considered as *the* detector. Looking for a separation, one is 99% sure that it has been carried out previously. The drawback is that the published separation generally does not fit the analyst's purpose since it has been performed on standards or another matrix, but it is of great help. Analysts should not forget that selectivity is cheaper to achieve than efficiency.

The reader can find more information on chromatography in Refs. 70 to 73; on mass spectrometry in Refs. 74 to 77; and on sample preparation in Refs. 78 and 79.

ABBREVIATIONS AND ACRONYMS

%	Percent (parts per hundred)
A	Absorbance (A = log [1/T])
AAS	Atomic absorption spectrometry
ACN	Acetonitrile
amu	Atomic mass unit
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
AU	Absorbance units
AUFS	Absorbance units full scale
CCD	Charge-coupled device
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CL	Chemiluminescence
CLND	Chemiluminescent nitrogen detection
CMC	Critical micelle concentration
CSP	Chiral stationary phase
DAD	Diode-array detection
ESI	Electrospray ionization
FTIR	Fourier transform infrared
GC	Gas chromatography
GLP	Good laboratory practice
HETP	Height equivalent to a theoretical plate
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICP	Inductively coupled plasma
IEC	Ion-exchange chromatography
IR	Infrared
IS	Internal standard
ISO	International Organization for Standardization
LDR	Linear dynamic range
LIF	Laser-induced fluorescence
LIFD	Laser-induced fluorescence detection
LLE	Liquid liquid extraction
LSC	Liquid-solid chromatography
LSD	Light scattering detector
LSS	Linear solvent strength
MALDI	Matrix assisted laser desorption ionization
MCP	Multi channel plate
MIP	Molecular imprinted polymer
MMLLE	Micro membrane liquid liquid extraction
MS	Mass spectrometer or mass spectroscopy
MW	Molecular weight (also mol wt, M)
NMR	Nuclear magnetic resonance
NP	Normal phase
NPC	Normal phase chromatography
PDA-UV	Photodiode-array UV
PS-DVB	Polystyrene-divinylbenzene
Q-TOF	Quadrupole time of flight
RI	Refractive index
RP	Reversed phase
RP-HPLC	Reversed-phase high performance liquid chromatography
SAX	Strong anion exchanger
SCX	trong cation exchanger
SIM	Selected ion monitoring
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SSI	Sonic spray ionization
TLC	Thin layer chromatography
TOF	Time of flight
UV	Ultraviolet
UV-VIS	Ultraviolet-visible

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48 The Use of Mass Spectrometry in Food Analysis

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CONTENTS

Mass spectrometry (MS) is a powerful modern analytical technique which provides detailed structural information for molecules. This capability, and the fact that it easily interfaces with commonly used chromatographic techniques, makes it a very useful tool for food analysis. MS can be used to characterize food composition or to monitor adulteration of foods by residues or other extraneous substances. A recent review by Careri et al. [1] is an excellent reference that demonstrates the wide scope of MS in the analysis of food, including the analysis of natural and xenobiotic substances as well as metals in food products. An earlier review by Careri et al. [2] described the use of LC/MS for residue analysis in food.

This chapter is not meant to be an exhaustive list of all references pertaining to the use of MS for food analysis, but rather is intended to further illustrate the many different applications in which MS has been used to characterize food. First, the types of modern instruments and experiments that may be used to perform MS on food samples are briefly described. An overview of how MS has been used to determine the natural components of food is followed by a description of the use of MS to determine residue level contaminants such as pesticides, animal drugs, mycotoxins, and packaging materials in foods. Further illustration of how MS has been used to study foods for a variety of purposes is given using examples of specific commodities (bovine milk and berries). Finally, some of the emerging trends in the analysis of food by MS are discussed.

I. MASS SPECTROMETRY (MS): INSTRUMENTS AND TECHNIQUES

The MS experiment consists of forming ions, separating those ions based on differences in their mass/charge (m/z) ratio, and detecting (counting) the number of ions at each m/z value. Although MS has changed dramatically in the past few decades, this basic process applies to modern techniques as well as those performed with more traditional MS instruments. A complete review of the fundamentals of MS theory is beyond the scope of this chapter, but many such resources are available [3, 4].

MS is an important modern analytical technique for the analysis of food because of the type of detailed information that is obtained. Traditionally, using an electron impact (EI) ion source, the analyte is introduced in the gas phase into an evacuated chamber and is bombarded with an electron beam of 70 eV. As a result of this interaction, the compound loses an electron and becomes a positively charged radical cation. In addition, the amount of energy used is generally enough to break bonds in the molecule and provide ionized fragments. The resulting fingerprint of ions at different m/z values with varying intensities can be used to determine a probable identity of the original analyte. This process of interpreting mass spectra to deduce molecular structure from EI experiments has been well studied and described [5].

In addition to collision with a high energy electron beam, there are many other methods of ionizing compounds so that they can be analyzed by MS. For example,

[*] The opinions expressed are those of the author and do not necessarily reflect the official views of the U.S. FDA.

positive ion chemical ionization (CI) transfers a proton from an ionized reagent gas, such as methane or isobutane, to an analyte with higher proton affinity to form a protonated (ionized) compound. Alternatively, a highly electronegative compound can capture thermalized (slower) electrons to form a negative ion when a reagent gas is present in the ion source. Desorption from high energy sources such as a lasers, fast atoms, high fields, etc. can also be used to form and eject ions from solids or liquids into the lower pressure region of an MS instrument. Matrix assisted laser desorption ionization (MALDI) has become a very popular method for introducing large biomolecules such as proteins into a mass spectrometer and has helped revolutionize the study of these compounds in many biological applications.

The introduction of two interfaces, electrospray ionization and atmospheric pressure chemical ionization, has allowed LC/MS to become widely used and commercially available. In electrospray ionization (ESI) the effluent from an LC is sprayed, usually pneumatically assisted with nitrogen gas, into a high electric field (3–5 kV) at atmospheric pressure. This process creates a stream of charged droplets that become smaller as they are accelerated into the field. Charged particles in the solution are ejected as the droplets evaporate or are released when the droplets explode as the surface forces become too high. The ionized molecules can then be sampled and accelerated through small orifices into a high vacuum region of the instrument where they can be separated and detected. One advantage of ESI is that multiply charged ions can be formed (e.g., on proteins with multiple sites available for protonation). This allows compounds with very high molecular weight to be analyzed with MS instruments utilizing traditional mass range (m/z 1000–2000) ion separators. With atmospheric pressure chemical ionization (APCI), the LC effluent is again sprayed into an atmospheric pressure chamber with nitrogen gas. However, in this case the LC mobile phase (water, methanol, etc.) is vaporized and ionized by a corona discharge. The ionized mobile phase can then act as a chemical ionization reagent gas and perform proton transfer reactions with the analyte to generate charged molecules. The bulk of the mobile phase must then be thermally vaporized before entering the lower pressure region of the MS instrument.

In contrast to electron ionization, the alternative ionization methods of CI, ESI, and APCI are relatively soft (low energy) ionization techniques. Therefore, not as many fragment ions are formed by the breaking of chemical bonds during these processes. As a result, the mass spectrum obtained may only consist of one peak, the protonated (or deprotonated) molecular ion. While these data give valuable information regarding the molecular weight of an analyte, structural ions are not available. For this reason, tandem MS or "MS/MS" is often used in conjunction with these ionization techniques to yield additional structural details. In the MS/MS experiment, one ion is isolated from all other ions, and a secondary energy source (such as bombardment with energized argon or helium gas) is introduced to break that ion into fragment ions. A second stage of ion separation is then performed to yield another mass spectrum consisting of only ions created from the collision induced dissociation of the initial parent ion. This secondary spectrum is referred to as a product ion spectrum and contains more information regarding fragments of the original molecule. For example, one common MS/MS instrument used is the triple quadrupole. After an ion is formed and introduced into the MS, it can be isolated from others using a quadrupole ion filter. A second quadrupole, which uses only RF frequency, acts as a collision cell, where the ion is impacted with argon. The third quadrupole can then be used to separate ions formed in the collision cell. This instrument can be used in other modes, but this is a common method to obtain structural information for a specific analyte. An ion trap separation device can also be used to obtain information-rich product ion spectra from molecular ions created from soft ionization techniques.

Mass spectrometers can be programmed to optimize for selectivity, specificity and sensitivity for any given compound. For example, when looking for a specific analyte in a complex mixture, an MS method can be programmed to scan for only one ion or set of ions (selected ion monitoring, or SIM). With an MS/MS instrument, specific ion transitions or parent-product ion pairs can be acquired (selective or multiple reaction monitoring, SRM or MRM). These techniques can greatly improve detection and confirmation limits for residues in food. Another valuable MS experiment that has been used in food analysis, for component characterization as well as residue analysis, is the measurement of the exact mass of molecules of interest using high resolution instruments. Time-of-flight analyzers have been used to obtain exact mass information for large biomolecules; these are often coupled to a MALDI MS source.

The commodities included for consideration under the category of food analysis represent a wide variety of complex matrices of plant and animal origin. In order to analyze a specific compound in a food product by MS, extensive sample preparation and analyte isolation is often required. Examples of some of the approaches used to accomplish this are liquid-liquid extraction, solid-phase extraction, and immunoaffinity techniques. The goal of these procedures is to remove as many matrix components as possible, while concentrating the analyte of interest. Often there is a trade-off between maximum recovery of the residue and the time and effort required to perform the analysis. This becomes more of an issue as requirements for high sample throughput increases.

Regardless of the sample preparation and extraction procedures used, a chromatographic separation step is

usually applied prior to MS analysis when analyzing components or residues in food. If the compounds of interest are sufficiently volatile and thermally stable, GC/MS is the preferred technique. Electron ionization is the most common MS technique used with GC because it gives structurally rich spectra. Chemical ionization with either positive or negative ion detection, is also possible with GC/MS. The most common LC/MS methods involve the use of a reverse-phase LC system interfaced to an electrospray or APCI MS source. Tandem MS can then be used to obtain additional structural ions. Often many compounds of interest in food analysis can be ionized using either ESI or APCI, and the best interface for each analyte may have to be determined empirically. Helpful resources detailing many more of the practical aspects for implementation of successful LC/MS analyses are also available [4].

II. MS ANALYSIS FOR DETERMINATION OF FOOD COMPOSITION

MS has been used to characterize the natural composition of food products. Specifically, this can be used to describe the profile of major components in a food product, to differentiate between similar products, or to measure small amounts of naturally occurring substances which are believed to be either beneficial or deleterious. The type of MS analysis used for the characterization of food components depends on the nature of the analytes. Many of the articles cited below are reviews and should serve as a guide to the reader looking for a specific type of analysis or food product.

Several reviews cover MS analysis of major components in foods including proteins and peptides [6, 7]. The analysis of peptides and proteins requires the use of technique that is designed for large non-volatile molecules such as electrospray ionization or MALDI. MS can be used in protein studies to characterize conformational changes or post-translational modifications such as glycosylation or phosphorylation. The information obtained by MS can then be used to help determine how these factors affect food qualities such as flavor, texture and nutritional value. The effect of processing (pasteurization, hydrolysis, etc.) on the structure and function of food proteins can also be examined using MS techniques. Analyses of the proteins associated with milk (casein, whey components lactalbumin and lactoglobulin), cereal (globulins, albumins, glutenins, prolamins), and eggs (ovalbumin, conalbumin, lyzosyme) have been performed using MS. One study characterized the different gluten proteins that are unique to various grains (wheat, rye, barley and oats) in cereal mixtures using MALDI time-of-flight (TOF) MS [8]. A few studies of meat proteins have been performed using MS. In one report, LC/MS using electrospray was used to study the polypeptides from whole and ground meat and how that profile changed during storage of the meat products [9].

The analysis of fatty foods (lipids, oils, fatty acids, etc.) can be accomplished using either GC/MS or LC/MS methods. In one study, GC/MS was used to determine the types and amounts of free fatty acids produced in the ripening of ewe milk cheese [10]. In this study, solid phase microextraction (SPME) was used to extract the fatty acids from the food matrix and the amount of butanoic, hexanoic, octanoic and decanoic acids were measured at part-per-million levels using GC/MS. Alternatively, a direct infusion electrospray MS method was developed to study different nut and vegetable oils and to determine if additional (adulterant) oils were added to olive oil [11]. Razzazi-Fazeli et al. [12] published a determination of cholesterol oxides in processed foods such as butter, lard, and egg powder using LC/MS with APCI and selected ion monitoring.

Characterizing the carbohydrate composition in food is also important. These compounds are difficult to analyze using traditional methods as they are nonvolatile, thermally unstable, and generally do not have a strong UV chromophore. In one food application, the saccharide profiles of caramel from different sources were evaluated using GC/MS after forming trimethylsilyl derivatives. Specific compounds were identified as possible indicators of the authenticity of the caramel product [13]. The carbohydrates found in human milk have been studied after derivatization with 2-aminoacridone using MALDI-TOF and electrospray MS, in addition to spectrofluorimetry [14]. The changes that occur to carbohydrates after processing can also by studied by MS methods. For instance, the starch components in treated potato starch were analyzed using anion exchange chromatography coupled to electrospray MS after enzymatic hydrolysis of the starch polymer [15]. In addition to studying carbohydrate profiles, MS is often used to characterize compounds such as peptides or flavonoids that may also include one or more sugar molecules.

Minor components of food products can also be characterized by MS analysis. Flavor and fragrance components tend to be inherently volatile and thermally stable organic compounds such as ketones, aldehydes, alcohols, and terpenes. GC/MS is the preferred MS analysis technique for these compounds. Isolation from the food product is often accomplished by head-space sampling techniques such as purge and trap or solid phase micro extraction methods [16, 17]. Examples of how these MS techniques are used in flavor analysis for specific food commodities, bovine milk and berries, are illustrated later in this chapter.

Recently there has been a great deal of interest in minor components of foods that may have high nutritional or nutraceutical value. Vitamins such as tocopherols and folates can be measured in food using MS techniques. For example, a method to determine alpha-tocopherol in infant foods (milk and cereal) was developed using

LC/MS (single quadrupole) with an APCI interface [18]. Deuterium- labeled tocopherol was used as an internal standard and detection limits of 2.5 ng/mL were achieved. A LC/fluorescence method for folic acid and 5-methyltetrahydrofolic acid in a variety of foods was validated using a complementary electrospray LC/MS method [19].

Flavanoids, including anthocyanins, may have beneficial antioxidant properties. The identification and quantification of these compounds in different foods is an important aspect of the research to determine the effects these chemicals may have on human health. There are many recent examples of how MS is being used in this emerging area of food analysis including the characterization of anthocyanins in food using LC/MS and capillary electrophoresis [20]. The oxidative phenolic compounds in artichokes have been characterized using electrospray LC/MS [21]. Flavonoid glycosides, such as kaempferol glucoside and others, were studied in almonds [22] using MALDI-TOF MS and in tomatoes using electrospray LC/MS/MS [23]. The distribution of anthocyanins, flavonols, and carbohydrates in different layers of red onion was also studied using electrospray LC/MS [24].

Some minor components in food products are thought to be deleterious and the characterization of these is also important. For example, the health effects of acrylamide residues that can be formed in various fried and baked foods have recently been questioned and can be monitored by LC/MS/MS [25]. Another area of interest is the presence of known cancer causing agents such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines that may occur in food treated in certain ways, such as frying or smoking. These compounds can also be monitored using MS analysis [26]. In one study, polyaromatic hydrocarbons in liquid smoke flavorings were analyzed by GC/MS, and a higher proportion of lower molecular weight PAHs were found; benzo(a)pyrene was present in a few samples, but at fairly low concentrations [27]. The determination of heterocyclic amines in lyophilized meat extracts was performed using LC/MS with an APCI source and ion trap MS/MS detection. Low part-per-billion levels could be detected [28]. Volatile nitrosamines in dry sausages were also monitored using an APCI LC/MS/MS method [29].

III. MS ANALYSIS IN THE DETECTION OF CONTAMINANTS AND RESIDUES IN FOOD

MS in also used to detect, measure, and identify foreign compounds such as pesticide or drug residues and environmental contaminants in food products. Several types of experiments can be performed when using MS to analyze food commodities for residues including screening, determinative or confirmatory analyses. Historically MS, often in conjunction with GC or LC, has been used for confirmatory analysis to positively identify a residue of concern. Mass spectrometry is the ideal tool to confirm the identity of a residue due to its inherent sensitivity and selectivity. There are established guidelines that define how closely the mass spectrum of an unknown or suspect residue must match that of a standard in order to be considered confirmed [30, 31]. MS methods can also be used to quantitate the amount of residue present. Traditionally, this has been more widespread with GC/MS, but is becoming more standard practice with LC/MS as well [32]. Care must be taken to use appropriate internal standards. If available, isotopically labeled analogues are the preferred internal standard because their response and fragmentation will most closely match that the analyte of interest. Because of its universal nature, MS can also be a powerful tool for screening for multiresidues or even multiclass residues in food analysis, and this application is becoming more common as MS is used more routinely.

Antibiotics are the most common type of drugs given to food animals. These are given both therapeutically and subtherapeutically to prevent disease and increase feed efficiency. The possibility of drug residues remaining in the edible product and the potential human health problems associated with exposure to these residues is a concern with widespread drug use in food animals. Mass spectrometry is commonly used to analyze antibiotic residues in foods [33, 34]. MS was traditionally used as a confirmatory analysis method to identify illegal residues in food. Many of the drugs of concern are polar nonvolatile compounds, so while it used to be more common to analyze derivatized residues by GC/MS, many recent reports utilize LC/MS methods. There are many examples of this including beta-lactam and sulfonamides residues in milk [35, 36], tetracycline residues in various foods [37], and fluoroquinolones and phenicols in aquacultured products [38], as well as many others. In addition to antibiotics, other animal drugs such as hormones can be monitored by MS. An example is a method that uses GC/MS not only to confirm, but also to quantitate anabolic steroids in bovine muscle tissue [39]. An upcoming trend is to use the universal response of the mass spectrometer, along with the capability of LC/MS/MS to provide diagnostic information, to screen for many types of residues with a generic extraction procedure [38, 40].

There is a long record of using MS for the determination and identification of pesticide residues in food products, especially in produce commodities. Historically pesticide residues could be monitored by GC/MS analysis only after extensive sample preparation using large sample sizes and liquid-liquid extraction procedures. Because many pesticides are volatile and thermally stable GC/MS is still the technique of choice for many of these compounds. The use of time-dependent selected ion monitoring GC/MS programs allows for the screening, determination and

confirmation of a large number of pesticides from a single analysis. This technique has been used to determine pesticides containing nitrogen, sulfur, or oxygen in a variety of foods [41]. Alternatively, the analysis of pesticides using GC/MS/MS with an ion trap detector to obtain product ion spectra can yield a very selective and sensitive method for a large number of compounds [42]. A new trend in the analysis of pesticides by GC/MS is the use of more rapid extraction methods. One report describes the use of automated solid phase extraction to isolate organochlorine and pyrethroid residues from a variety of fruits for analysis by GC with electron capture and confirmation by GC/MS [43]. In another example solid phase micro extraction (SPME) coupled directly to GC/MS has been used for the analysis of chlorpropham in potatoes and amitraz in honey at ppb levels [44]. Another innovation in this area is to use low-pressure fast GC coupled to MS for pesticide analysis [45]. In this approach a shorter, wider bore GC column with a thicker film was operated at fast flow rates and low pressure allowing for faster analysis, increased injection sample size and reduced thermal degradation.

Some classes of pesticides are not as amenable to GC/MS due to limitations of volatility or thermal stability, and LC/MS may be the technique of choice for these analytes [46]. For example, the determination of aldicarb and its metabolites in various food extracts has been reported using LC-APCI [47]. Carbamates are another class of pesticides that can be best analyzed by LC/MS. One report describes the isolation of thirteen carbamates from orange, grape, onion and tomatoes by matrix solid-phase dispersion with analysis by LC/MS [48]. These authors determined that both electrospray and APCI were suitable for the analysis of these compounds with similar sensitivity (ppb) and structural information obtained.

Dioxins are a class of compounds that create a particular analytical challenge. Formed as breakdown products from industrial chemicals, these compounds are extremely toxic and have a very long residence time in the environment. Because of this, the potential of very low, yet possibly still toxic, amounts of dioxins entering the food supply is a real concern. Analytical methods must therefore be able to detect part-per-trillion (and sub-ppt) amounts of these compounds in a wide variety of environmental and food matrices. Another challenge is that dioxins exist in many similar forms, or congeners. These compounds may have closely related molecular structure but different toxicological properties. Historically, extensive extraction methods (Soxlet, multi-stage column chromatography, etc.) using relatively large sample sizes were coupled with GC/MS detection [49]. The MS instruments used may need to have high enough resolution to distinguish between compounds with the same nominal molecular weight. Magnetic sector or high-resolution ion trap instruments typically have been employed for this purpose. Recently some of the newer MS techniques have been applied to dioxin analysis including the use of time-of-flight instruments and isotopic dilution quantitation [50].

The formation of mycotoxins is a prevalent problem in foods such as grains and peanuts. The use of MS, especially LC/MS, has allowed for much more efficient monitoring of these toxins. For example, the analysis of aflatoxin can be performed with great sensitivity and selectivity by LC/MS. One recent reference describes the use of MS to identify individual peanuts with high levels of aflatoxin in a processing stream by dipping the nuts into an extraction fluid and analyzing the fluid by MS/MS [51]. Other methods describe the optimized LC/MS APCI conditions for the determination of A-trichothecene mycotoxins in grains such as oats, maize, barley and wheat [52]. Mycotoxins can also occur in fruits and their juice products. LCMS and GC/MS have been used for the analysis of the mycotoxins alternariol and patulin in apples and other fruit [53, 54].

Several acute human illnesses can result from the consumption of shellfish containing marine toxins including diarrhetic shellfish poisoning, amnesic shellfish poisoning and azaspiracid poisoning. Diarrhetic shellfish poisoning is caused by compounds such as okadaic acid, dinophysistoxin, pectenotoxin, yessotoxin, dinophysistoxins, among others. MS has played an important role in the analysis of these compounds, as well as the initial characterization of newly discovered compounds [55, 56]. Domoic acid is a toxin which causes amnesic symptoms. Determination and confirmation of this compound can also be achieved using LC/MS methodology [57]. A series of related toxins, the azaspiracids, can also results in human illness after consumption of affected shellfish. These compounds have been characterized by electrospray LC/MS using an ion trap instrument and a method to quantitate the azaspiracids in mussel tissues has been developed [58, 59].

A recent concern involving packaged food products is that potentially harmful substances from the packaging material may migrate into the food and be consumed. MS can be used to characterize possible contaminants from processing or packaging materials or the degradation of those substances. For example the amount of styrene or styrene oxide or other volatile organic compounds from polystyrene containers and the factors affecting the rate of migration into the food commodity using GC/MS have been reported [60]. Plasticizers in food have also been determined by GC/MS with ion trap detection [61]. Bisphenols, which are breakdown products from canned food coating material, have been measured in a wide range of different canned foods including soup, fruit, infant formula, and meat products using GC/MS methods [62].

The potential threat for adulteration of food by deliberate tampering, either at the manufacturing facility, the retail outlet, or by individual consumers, has always been

a concern and has become more so in recent years. MS can be a very valuable tool for determining what foreign compound may have been added to a product, as well as the possible source of the contamination. Due to the sensitive nature of this work and possible legal and regulatory implication, there are not many references on this subject in the published literature. A few references include the determination of a rodenticide in consumer products [63] and the identification of cocaine in fruit (and syringes found at the same location) in a supermarket tampering case [64].

IV. SPECIFIC APPLICATIONS OF MS IN FOOD ANALYSIS

The use of MS to analyze two common food commodities, bovine milk and berries, is examined in more detail. The major components of bovine milk have been characterized by MS methods. The triacylglycerols in milk have been characterized by LC/MS using APCI as well as by GC/MS [65] after initial prefractionation using both TLC and gel permeation chromatography. LC/MS alone could not resolve the complicated mixture of triacylglycerols, and interpretation was difficult because many compounds shared common fragment ions. Fractions of the mixture were separated using either TLC or gel permeation and subsequent analysis of these portions was used to identify individual triacylglyceraols. GC/MS was also performed on the majority of the fractions and yielded complementary data to assist in the identification of individual components. Using these techniques, 120 triacylglycerols were identified. The whey proteins derived from milk have also been better described using LC/MS with an electrospray interface and time-of-flight MS detection [66]. This study found that the principle proteins in whey (lactalbumin and lactaglobulins) can be extensively modified by glycosylation and oxidation upon processing.

The more volatile compounds in foods or beverages often determine their flavor. The use of SPME coupled to GC/MS has become the method of choice to sample the volatile compounds present in milk [67, 68]. For a more rapid analysis, the GC column can be omitted and the compounds can be analyzed directly by electron impact MS. In this case, a short fused silica transfer line can take the place of an analytical GC column. The MS then acts as an "electronic nose" and pattern recognition techniques are required to distinguish significant differences in milk samples [69].

Phytoestrogens, which include isoflavones, lignans, and coumestans, are a class of compounds that are often a minor naturally occurring component of milk. These compounds can potentially act as endrocrine disruptors and are passed into bovine milk from injestion of compounds present in animal feed. A method was developed to analyze phytoestrogens in milk using LC/MS/MS [70]. An electrospray interface (using both positive and negative ionization) was used along with a triple quadrupole MS operated in the selected reaction monitoring mode. Identification limits for these compounds using this method was determined to be less than 1 μg/L. Analysis of milk samples confirmed the presence of the hydroxylated isolflavones genistein and daidzein and also higher amounts of the metabolite equol.

Potential residual compounds that could be present in milk include industrial compounds, mycotoxins, pesticides and animal drugs. Examples of industrial chemicals that have been analyzed in milk using MS include polycyclic aromatic hydrocarbons [71] and polychlorinated biphenyls [72]. Cyclopiazonic acid is a mycotoxin produced by *Aspergillus* and *Penicillium* genuses in milk. This compound has been analyzed using negative ion electrospray LC/MS with ion trap detection [73]. The authors were able to quantitate over three orders of magnitude with a detection limit of 5 ng/mL. Twenty milk samples were analyzed for cyclopiazonic acid by this method, and three of those samples were found to be contaminated at detectable levels.

Multiresidue methods for pesticide residues in milk have been developed. A method for detection, quantitation, and confirmation of over 100 pesticides using GC with ion trap MS/MS was developed and applied to several different types of foods including milk [42]. The advantage of using the ion trap as a detector, in this method or any other application, is the ability to achieve additional selectivity and sensitivity. This is demonstrated in this method by the ability to obtain very clean traces for individual compounds using specific individual acquisition parameters in very complex food matrices. Another method developed more specifically for milk uses GC/MS (single quadrupole detection) as well as GC with flame photometric detection to quantitate several classes of pesticides including organophosphorous compounds, organochlorine compounds, methylcarbamates, as well as others [74]. Residues were confirmed using high-resolution GC/MS and GC/MS-MS with a hybrid magnetic sector/quadrupole instrument.

Residues of animal drugs can be a potential problem in bovine milk because dairy cows may be treated for illness such as mastisis or respiratory disease. Some drugs are approved for treatment of affected dairy cattle, but they may result in violative residues if appropriate drugs, dosages, and withdrawal times are not used. Antibiotics are the most common drug residues found in milk, and MS has been used extensively to monitor for these residues. MS methods for residues in milk include sulfonamides [36, 75], tetracyclines [76], penicillins and related compounds [35, 77], aminoglycosides [78], and many others [33]. Other types of animal drugs that can be analyzed for in milk by MS methods include anthelmintics such as bendazoles [79].

Berries are another type of food product that have been extensively analyzed and characterized using MS

methods. One primary focus of analytical methods using berries as a matrix is understanding which chemical compounds give the fruit the best flavor and odor characteristics. A thorough study of the volatile components that define the aroma and flavor characteristics of strawberries has been reported [80]. This method employed SPME headspace analysis combined with thermal desorption GC/MS to identify and quantitate 23 different compounds. Statistical techniques were then utilized to classify and compare the different varieties of berries. GC/MS with headspace sampling was also used in a study [81] that compared the aroma components, primarily aliphatic esters, of strawberries grown in different conditions.

Another aspect of berry chemistry that has received a great deal of attention is the presence of anthocyanins, procyanidins, and flavanols in these fruits. These compounds are thought to have potential beneficial value due to their antioxidant properties. The flavonal components quercetin, myricetin and kaempferol were studied in 25 berries including cranberries, currants, whortleberries, blueberries and strawberries [82]. A LC/UV method was used to quantitate the amounts of those compounds in the various berries and electrospray LC/MS along with diode array data were used to identify the various compounds. In another study, an MS method using MALDI-TOF was compared to a traditional LC method to measure the amount of anthocyanin in blueberries [83]. While both techniques gave comparable profile results, the MS method was faster and also provided confirmatory information, although the LC method provided better information on isomeric compounds.

The chemical residues in berries that are of most concern are pesticide residues. One report describes the analysis of organochlorine and chlorobenzene pesticide residues in strawberries using GC/MS [84]. The residues were extracted from the fruit using the novel techniques accelerated solvent extraction, SPME, or stir bar sorptive extraction. Detection limits of 1–10 μg/kg were reported. This method was used to monitor contamination of strawberries in an industrial region. Another paper also reports on the use of SPME in conjunction with GC/MS (using selected ion monitoring) for a wide variety of pesticides and fungicides in strawberries [85]. In an alternative approach, capillary electrophoresis using an electrospray interface and selected ion monitoring was used to measure the fungicides procymidone and thiabendazole in strawberries as well as other fruits [86]. A survey of consumer samples using this method found that these residues are present in real samples more than 50% of the time.

V. EMERGING TRENDS IN THE MS ANALYSIS OF FOODS

MS should remain an important analytical tool in the characterization of food, and the application of new technologies will continue to expand. The flexibility of the new MS/MS instruments will allow multi-component methods to be developed with performance (in terms of sensitivity and selectivity) that was previously only possible for single compounds. Just as the advent of practical and commercially available LC/MS/MS instruments has had a great effect on food analysis, the introduction of time-of-flight and other high resolution instruments will also have a great impact. The use of exact mass measurements to identify and confirm food components or residues should increase.

Innovations from other areas of analytical chemistry and biology can be borrowed and adapted for food analysis. For example, the advances made in the area of drug discovery in terms of rapid throughput of compounds (multiplexing, robotics, etc.) should lead to the ability to screen a large number of food samples using some of the same technological advances. The use of MS, as well as other means of structural elucidation, to study a wide variety of proteins has exploded in recent years, giving rise to the whole field of proteomics. Researchers in this area are applying their knowledge to a wide variety of biological systems and disease mechanisms. The same analytical techniques should be able to be applied to food matrices as well, leading to better ways to distinguish species or varieties of commodities. Identification of bacterial food pathogens using MS is already an area of increasing development [87].

In the future, MS will likely become a valuable tool in determining not only what components are present in any given food, but also what role a particular compound may play in the food's esthetic quality, nutritional value, and perhaps even in its ability to mitigate or prevent disease.

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49 Food Analysis: Other Methods

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CONTENTS

I. INTRODUCTION

There is considerable demand in the food industry for analytical methods to quantitatively measure various components in raw materials and finished food products, and to monitor physicochemical changes during and after food processing. This chapter describes a number of classical and newly developed methods for food analysis not covered in other chapters. These techniques have wide application in the food industry due to their simplicity and potential applications to a wide variety of food materials.

II. POTENTIOMETRY

Potentiometry is one of the two main categories of electroanalytical techniques, being classified as voltammetry at zero current. The basic principle of potentiometry involves the use of an electrolytic cell composed of two electrodes dipped into a test solution. A voltage (electromotive force, EMF) develops, which is related to the ionic concentration of the solution. This EMF is measured under conditions such that an infinitesimal current (10^{-12} amperes or less) is drawn during measurement. Hence, the classification voltammetry at zero current is used in potentiometry. When an appreciable current is drawn, changes in solution concentration surrounding the electrodes will be produced, and the measured potential corresponds to a system different from the original. In addition, irreversible changes may occur in either of the two electrodes.

A. Electrode Potential

When an electrode is placed in a solution, it tends to send ions into the solution (electrolytic solution pressure), reacting with the electrode (activity). These two factors, the electrode pressure and the activity of the solution ions, combine to produce an electrode potential. At a given external pressure and temperature, the potential is a constant characteristic of the metal in solution.

The pH meter is a good example of a potentiometer. pH is defined as the logarithm of the reciprocal of hydrogen ion concentration. It may also be defined as the negative logarithm of the molar concentration of hydrogen ions. The four major parts of the pH meter system are: (1) reference electrode, (2) indicator electrode (pH sensitive), (3) voltmeter or amplifier that is capable of measuring small EMF differences in a circuit of very high resistance, and (4) the sample being analyzed.

Hydrogen ion concentration (activity) is determined by the voltage developed between the two electrodes. The Nernst equation relates the electrode response to the activity:

$$E = E^\circ + 2.3\frac{RT}{NF}\log A$$

Where E = actual electrode potential; E° = a constant, the sum of several potentials in the system; R = universal gas constant, 8.313 joules/degree/g mole; F = Faraday constant, 96,490 coulombs per g equivalent; T = absolute temperature (Kelvin); N = charge of the ion; and A = activity of the ion being measured.

At 25°C, the relationship of 2.3RT/F can be calculated as:

$$\frac{23 \times 8\,316 \times 298}{96{,}490} = 0.0591 \text{ volts}$$

Thus, the voltage produced by the electrode system follows a linear function of the pH, with the electrode potential being +60 millivolts for each change of one pH unit. At neutrality (pH 7), the electrode potential is zero millivolt. The above relationship of millivolt versus pH exists only at 25°C.

B. Reference Electrodes

1. Standard Hydrogen Electrode

The universally adopted primary standard reference electrode is the standard hydrogen electrode (SHE). It consists of a platinum wire or foil coated with platinum black, immersed in a hypothetical solution of unit hydrogen ion activity and in equilibrium with hydrogen gas at unit partial pressure. The potential of this standard electrode by convention is defined as zero millivolt at all temperatures. The standard hydrogen electrode is the international standard, but is seldom used for routine work because more convenient electrodes and reliable calibration buffers are available.

2. Saturated Calomel Electrode

The saturated calomel electrode is the most common reference electrode. It is based on the following reversible reaction:

$$Hg_2Cl_2 + 2e^- \leftrightharpoons 2Hg + 2Cl^-$$

Thus, the potential is dependent upon the chloride ion concentration, which is easily regulated by the use of saturated KCl solution in the electrode. A calomel reference electrode has three principal parts: (1) a platinum wire covered with a solution of calomel (Hg_2Cl_2), (2) a filling solution (saturated KCl), and (3) a permeable junction through which the filling solution slowly migrates into the sample being measured. Junctions are made of ceramic material or fibrous material. A sleeve junction may also be used. Because these junctions tend to clog up, causing a slow, unstable response and inaccurate results, one electrode manufacturer has introduced a free-flowing junction wherein electrolyte flowing from a cartridge is introduced at each measurement. The calomel electrode is unstable above 80°C or in strongly basic samples (pH >9), and should be replaced by a silver-silver chloride electrode.

3. Silver-Silver Chloride Electrode

The silver-silver chloride electrode is very reproducible and is based on the reversible reaction:

$$AgCl\ (s) + e^- \leftrightharpoons Ag(s) + Cl^-$$

The internal element is a silver-coated platinum wire with the surface silver being converted to silver chloride by hydrolysis in hydrochloric acid. The filling solution is a mixture of 4 M KCl saturated with AgCl used to prevent the AgCl surface of the internal element from dissolving. The permeable junction is usually of the porous ceramic type. Due to the relative insolubility of AgCl, this electrode tends to clog more readily than the calomel reference electrode. However, it is possible to obtain a double-junction electrode in which a separate inner body holds the Ag/AgCl internal element, electrolyte, and ceramic junction. An outer body containing a second electrolyte and junction isolates the inner body from the sample.

C. Indicator Electrodes

The indicator electrode most commonly used in measuring pH is referred to as the glass electrode. Prior to its development, the hydrogen electrode and the quinhydrone electrode were used. The history of the glass electrode goes back to 1875, when it was suggested by Lord Kelvin that glass was an electrical conductor. The glass electrode potential was discovered 30 years later when it was observed that a thin glass membrane placed between two aqueous solutions exhibited a potential sensitive to changes in acidity. Subsequently, the reaction was shown to be dependent upon hydrogen ion concentration. These observations were of great importance in the development of the pH meter.

The glass electrode has three principal parts: (1) a silver-silver chloride electrode with a mercury connection needed as a lead to the potentiometer, (2) a buffer solution consisting of 0.01N HCl, 0.09N KCl, and acetate buffer used to maintain a constant pH, and (3) a small pH–sensitive glass membrane whose potential varies with the pH of the test solution. Conventional glass electrodes are suitable for measuring pH in the range of 1–9. These electrodes are sensitive to higher pH, especially in the presence of sodium ions. Thus, modern glass electrodes developed are usable over the entire pH range of 0–14, and feature a very low sodium ion error, typically <0.01 pH unit at 25°C.

D. Ion-Selective Electrodes

The glass electrode is generally considered useful only in measuring pH, but if the composition of the glass membrane is changed, this type of electrode can be quite sensitive for detecting other cations. In recent years, much attention has been given to this potential. Many electrodes have been developed for the direct measurement of various cations and anions, such as bromide, calcium, chloride, fluoride, potassium, sodium, and sulfide. There are even electrodes available for measuring dissolved gases, such as ammonia and carbon dioxide. While some of these methods are limited in their application due to interference from other ions, the problem can be overcome by pH adjustment, reduction of the interfering ions, and removal of these ions by complexing or precipitation.

Varying the composition of the glass in a glass electrode is one means of changing the sensitivity of the glass membrane to different ions. For example, an electrode membrane containing 71% SiO_2, 11% Na_2O, and 18% Al_2O_3 is sensitive to potassium.

1. Precipitate Impregnated Membrane Electrodes

According to Rechnitz (1), the success of glass membranes as cation-selective electrodes rests largely on the fact that the hydrated glass lattice contains anionic ‘sites’ that are attractive to cations of appropriate charge-to-size ratio. It is quite easy to construct ion-exchange membranes that permit exchange of either cations or anions; the difficulty is that these membranes show insufficient selectivity among anions and cations of a given charge to be satisfactorily used as practical electrodes. The problem is to find an anion-exchange material that displays appreciable selectivity among anions of the same charge, and also possesses suitable properties to permit its processing into a membrane electrode. In this case silicone rubber has been found to be the most effective material.

An inert, semi-flexible matrix (silicone rubber) is used to hold an active precipitate phase (AgI for an iodide electrode) in place. Such membranes are called heterogeneous or precipitate impregnated membranes. Fisher and Babcock (2) used radioactive tracer materials to show that the electrode potential is determined by the electrical charge on the surface of the inorganic precipitate particles, and that the current is carried by the transport of the counter ions through the membrane. The main advantage of membrane type electrodes over the older metal-metal halide electrodes is their insensitivity to redox interferences and surface poisoning. Electrodes sensitive to chloride, bromide, iodide, sulfide, sulfate, phosphate, and hydroxide ions have been developed.

2. Solid-State Electrodes

The active membrane portion of a solid-state electrode consists of a single inorganic crystal doped with a rare earth. For example, the Orion fluoride electrode is crystalline lanthanum fluoride that has been doped with europium to lower its electrical resistance and to facilitate ionic charge transport. This electrode has approximately tenfold higher selectivity for fluoride than hydroxide, and at least 1000-fold higher selectivity for fluoride over chloride, bromide, iodide, hydrogen carbonate, nitrate, sulfate, and monohydrogen phosphate. The fluoride electrode does not require pre-conditioning or soaking prior to use. Solid-state electrodes are available for detecting the anions Cl^-, Br^-, I^-, and S^{2-}, and the cations Cd^{2+}, Co^{2+}, Pb^{2+}, and As^{3+}.

3. Liquid-Liquid Membrane Electrodes

The range of selective ion-exchange materials could be greatly extended if such materials could be used in electrodes in their liquid state. Liquid ion-exchange materials that possess high selectivity for specific ions may be tailored by appropriate chemical adjustment of the exchanger on the molecular level. The main problems hindering the development of successful liquid-liquid electrodes are mechanical. It is necessary, for example, that the liquid ion-exchanger be in electrolytic contact with the sample solution, yet actual mixing of the liquid phases must be minimal. Electrodes of this type are available for Ca^{2+}, Mg^{2+}, Cu^{2+}, Cl^-, ClO_3^-, and NO_3^-.

E. Enzyme Electrodes

Enzyme electrodes are used to determine certain uncharged molecules. This type of electrode contains an enzyme that can convert the molecule to be measured into an ion that can be detected with a conventional electrode. For example, urea can be determined by coating the end of an ammonium ion electrode with uricase imbedded in a gel. As urea in a sample penetrates the gel, it reacts with the enzyme, which converts part of the urea to ammonia. The ammonia, in the presence of water, becomes ammonium ion which is then detected by an ammonium ion electrode.

An enzyme electrode can be prepared by placing a piece of nylon stocking over the end of an electrode. A thin film of polyacrylamide gel containing the specific enzyme is polymerized onto this network. The gel is then covered with a film of clear plastic such as Saran wrap. Both the stocking and plastic membrane can be held in place with a rubber band placed around the neck of the electrode.

F. Applications

The measurement of pH has many uses in the food industry. The safety of many foods is pH-dependent, and the desired flavor of a food is often determined by the $[H^+]$ concentration. Thus, pH measurements are routinely conducted in both the laboratory and the processing plant.

The chloride and fluoride contents in several types of cheese have been determined by potentiometric titration with silver using ion-selective electrodes (3). The chloride and calcium contents of milk fats were measured by potentiometric titration to detect adulteration (4). A portable meter was developed by Dracheva (5) to determine nitrate in fruits and vegetables.

D-Fructose dehydrogenase was immobilized on an electrode and used for the selective determination of fructose (6). Glucose oxidase was immobilized on an electrode surface and found to be sufficiently fast and reliable to determine sucrose in flow injection systems (7). Sugar beet pulp precipitated with acetone was examined and found to be a suitable biocatalytic layer for a tyrosine tissue-based membrane electrode (8).

III. RADIOACTIVITY AND COUNTING TECHNIQUES

A. Radioactivity and High-Energy Particles

Radioactivity is a general term applied to the emission of high energy particles (e.g., alpha and beta particles) and electromagnetic radiation emanating from the unstable and excited nuclei of atoms. The main advantage of radioanalytical methods is their high sensitivity, allowing determinations to the nanogram (10^{-9}g), pictogram (10^{-12}), and sometimes fantogram (10^{-15}) range. These methods have high specificity and can be used to determine most inorganic elements including trace elements, and organic compounds such as amino acids, fatty acids, vitamins, and hormones.

Alpha particles are helium nuclei moving at high speeds (on the average, 1×10^7 cm/min) emitted from unstable nuclei having large atomic numbers. All alpha particles from a given isotope have the same energy, nearly identical penetration ranges which are very short,

and produce about 25,000 ion pairs/cm while they last. Beta particles are distinguishable from simple electrons only by the fact that they originate in the nucleus and are usually moving at high speed. A beta spectrum is continuous, having energies varying from a few thousand electron-volts to several million electron-volts. An average beta particle produces about 60 ion pairs/cm.

Following nuclear transformation, the formed nucleus is often found in an excited state. When the excited nucleus falls back to its ground state, electromagnetic radiations called gamma rays are emitted. Many equations have been developed to describe the various parts of the radioactive process, but the most widely used is the half-life equation:

$$t_{1/2} = 0.693/\lambda$$

where λ is the radioactive decay constant (sec^{-1}). If A is the count rate and A_0 is the number of counts at zero time then the above equation becomes:

$$A = A_0 \exp(-0.693\ t/t_{½})$$

B. Counting Devices

Although the radiations mentioned previously have high energies in bulk, individually the energies are not sufficient, by a factor of about one million, to permit direct observation and measurement. Detection and measurements are therefore done indirectly by utilizing the effects of interactions produced by these radiations as they traverse matters.

1. Geiger–Muller Counter

The Geiger–Muller (GM) counter or tube is very sensitive to alpha and beta particles (98% efficient) compared to gamma rays (2% efficient). The GM counter is relatively inexpensive and simple to operate, but it does not discriminate between the types of radiation and it has a finite lifetime. It is being steadily replaced by proportional and scintillation counters.

When a ray of radiation comes through the mica window of a GM tube and strikes an argon atom, the argon atom is ionized to produce a positive argon ion and an electron. The positive ion moves toward the cathode at a speed about 1000 times slower than the movement of the electron toward the anode. The electron, attracted by the high potential of the anode, is rapidly accelerated. It has sufficient energy that when it collides with an argon atom another ion and electron can be produced. The two electrons accelerating toward the anode produce 4, then 8, and 16, etc. electrons. The net result is called the Townsend avalanche. Thousands of electrons can reach the anode and a small current is produced and the pulse signal is measured.

In addition, some of the electrons striking the anode may have high enough energy to knock electrons from the anode. These electrons will immediately be re-attracted to the anode and they in turn can knock other electrons loose. This is known as photon spread. The total time it takes for this signal to build up is known as the rise time (t_r) and is usually 2–5 μsec in duration.

While electrons are moving toward the anode, the positive ions move slowly toward the cathode as a positive space charge. If they strike the cathode with their full energy, more photoelectrons will be generated, more than the tube can handle. The net result is that the counter will burn out. Something is needed to dissipate this energy and from photon spread. Molecules, with their many energy levels, are used for this purpose, and ethanol and chlorine are the favorites. The ionized argon atoms will transfer their energy to these molecules which may form ions or free radicals or simply absorb the energy. The energy is so spread out that the cathode has little affinity for these particles, and no photoelectrons are thus produced. Since there is a limit to the amount of quenching gas that can be added to this type of counter, the counter will only work as long as quenching gas is present.

What happens if a second ray of radiation enters the counter before the first ray has completed its reaction? If the second ray ionizes an argon atom at a point between the cathode and the positive space charge, then the electron produced will not "see" the anode but will combine with the argon ion instead. The net result is that the ray of radiation was not counted.

2. Proportional Counters

Geiger–Muller tubes are limited to about 15,000 counts per minute (cpm) because of their long dead time. If the voltage applied to the anode of a GM tube is reduced to a value at which the anode can collect electrons but not produces photon spread, the output pulse is proportional to the energy of the initial ionization, since the number of secondary electrons now depends only on the number of primary ion pairs produced initially. A device operated in this manner is called proportional counter.

The dead times of proportional counters are very short, of the order of 1 μsec, and they can therefore count up to 200,000 cpm. The pulse signal is much weaker than that with a GM tube and better amplification system is needed. It is not possible to make a proportional counter out of a GM tube by simply lowering the anode voltage.

Proportional counters can operate at atmospheric pressure, so the quenching gas can be added continuously. Hence, a proportional counter can count indefinitely. Proportional counters are very good for detecting alpha and beta particles, and because of their ionization efficiency, these counters can easily distinguish the two types of particles.

3. Scintillation Counters

The basic principle behind the operation of the scintillation counter is that an energetic particle incident upon a luminescent material (a phosphor) excites the material, and the photons created in the process of de-excitation are collected at the photocathode of the photomultiplier tube causing the ejection of electrons. The electrons ejected are then led to impinge upon other electrodes, each approximately 100V higher in potential than the previous one. During acceleration from dynode to dynode, more electrons are ejected and a large amplification is obtained.

It would be expected that the greater the energy of the incident particle, the greater the number of electrons is produced. Thus the scintillation counter could be used to measure the energy of the particle. In a scintillation counter, advantage is not taken of the proportional properties of the scintillation process. However, a scintillation spectrometer uses these proportional characteristics to good advantage.

The liquid scintillation counter is particularly convenient for the counting of beta emitters with very low energy, such as tritium, carbon-14, and sulfur-35. The pulses of light emitted by the scintillating solution (caused by the particles in the radioactive decay process) are observed with a photomultiplier tube and counted. It is common practice to cool the photomultipliers to lower their noise, caused in part by thermal emission from the photocathode.

4. Semiconductor Detectors

Germanium, a semi-conducting material, can be made sensitive to gamma radiation by placing small amounts of an impurity, such as lithium, into the germanium crystal structure. When a gamma ray interacts in the crystal, an electrical charge is produced. The charge is collected and amplified to produce a voltage pulse whose height is proportional to the amount of energy deposited in the crystal by the gamma ray.

A single crystal of semiconductor material, such as silicon or germanium, will not make a suitable counter because of the direct current in the crystal. Random variations of this current may produce pulses similar to the radiation-induced pulses. To reduce this current, a p-n (positive-negative) junction in reverse bias is used.

C. Applications

Radioactivity techniques are widely applied in the food industry due to their high sensitivity. A wide variety of food components can be detected and quantified. Positron lifetime spectroscopy has been used to study the structural changes of water in the presence of dissolved sugar molecules such as sucrose, D-glucose, and D-fructose (9). Proton-induced X-ray and radioisotope-induced X-ray fluorescence methods were used to determine trace elements in beef, mutton, and chicken (10). Radioactivity tracing of anthocyanins has been carried out in food and beverages (e.g., red wine) where products are difficult to isolate and analyze (11). Variations in cell wall polysaccharides of ready-to-use grated carrots after treatment with ionizing gamma radiation and $CaCl_2$ were demonstrated (12). Calcium did not modify the polysaccharide composition or the effects of the ionizing radiation. The gamma radiolysis of phenylalanine in aqueous solution has been studied as a model for the formation of o-tyrosine in irradiated food containing phenylalanine (13). Several methods have been developed to detect irradiated foods, including the thermoluminescence technique which appears to originate from mineral contamination in food materials which have a natural thermoluminescence (14).

IV. ENZYMATIC METHODS

Enzymes are protein molecules that catalyze various metabolic reactions in living organisms (15). Enzymes are capable of very high specificity and reactivity under physiological conditions. Enzymatic analysis is the measurement of compounds with the aid of added enzymes or the measurement of endogenous enzyme activity to give an indication of the state of a biological system including foods. The fact that enzyme catalysis can take place under relatively mild conditions allows for the measurement of unstable compounds not amenable to some other techniques. In addition, the specificity of enzyme reactions can allow for measurement of components in complex mixtures without the need of complicated and expensive chromatographic separation.

A. Enzyme Kinetics

Enzymes are biological catalysts which increase the rate (velocity) of a thermodynamic reaction. An enzyme does not modify the equilibrium constant of the reaction and is not consumed in the reaction. To measure the rate of an enzyme-catalyzed reaction, the enzyme is mixed with the substrate under specified conditions (pH, temperature, ionic strength, etc.) and the reaction is monitored by measuring the disappearance of the substrate. An enzyme-catalyzed reaction is represented as:

$$S + E \leftrightharpoons ES \rightarrow P + E$$

where S = substrate, E = enzyme, ES = enzyme-substrate complex and P = product. The formation of the enzyme-substrate complex is very rapid (in millisecond scale) and is not normally seen in the laboratory. The time course of an enzyme-catalyzed reaction is illustrated in Figure 49.1 (15). The brief time in which the enzyme-substrate complex is formed is called the pre-steady state period. The slope of the linear portion of the curve following the pre-steady state period gives the initial velocity (V_0).

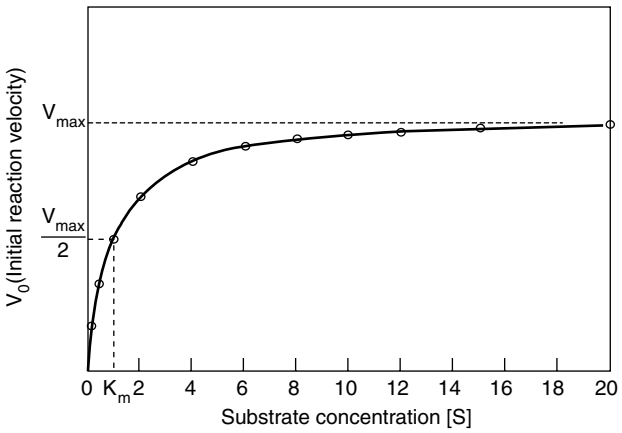

FIGURE 49.1 Effect of substrate concentration on the rate of an enzyme-catalyzed reaction plotted according to the Michaelis-Menten equation.

The rate of the enzyme-catalyzed reactions depends on the concentration of both the enzyme and the substrate. With a fixed enzyme concentration, increasing substrate concentration will result in increased velocity. As the substrate concentration increases further, increase in velocity slows, and at very high substrate concentration, the maximum velocity (V_{max}) of the reaction is reached and no further velocity increase is noted. The substrate concentration at which one-half V_{max} is observed is defined as the Michaelis constant or K_m which is an important characteristic of an enzyme. It is an indication of the relative binding affinity of the enzyme for a particular substrate. The lower the K_m, the greater is the affinity of the enzyme for the substrate.

The Michaelis-Menten equation is derived from a simplified enzyme-catalyzed reaction as:

$$E + S \underset{k_1}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

where k_1, k_{-1}, k_2 are reaction rate constants.

B. Measurement of Enzyme Activity

A wide variety of methods is available to follow enzyme reactions, including spectrophotometry, fluorimetry, manometric methods, titration, and isotope and viscosity measurements. The review by Whitaker (16) provides a comprehensive guide to enzyme methods applicable to foods.

C. Applications

There are several applications of enzyme analyses in food science and technology. In several instances, enzyme activity is a useful measure for adequate processing of a food product. For example, peroxidase activity is used as a measure of adequacy of blanching of vegetable products. Enzyme activity assays are also used by the food technologists to assess potency of enzyme preparations used as processing aids.

Enzymatic analysis is currently being used more often in the determinations of various substrates and enzymes in food products, such as beverages, milk (lactose), fats (cholesterol), and meats (pyrophosphates, creatine, creatinine, gluconate). A few examples of enzymatic analysis of food components are shown.

1. Sulfite

Sulfite is a food additive that can be measured by several techniques, including titration, gas chromatography, and colorimetric analysis. Sulfite can also be specifically oxidized to sulfate by the commercially available enzyme sulfite oxidase (SO). The H_2O_2 product can be measured by several methods, including the use of the enzyme NADH-peroxidase. The reactions are shown as follow:

$$SO_3^{=} + O_2 + H_2O \xrightarrow{SO} SO_4^{=} + H_2O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{NADH-peroxidease}} 2H_2O + NAD^+$$

The amount of sulfite in the system is equal to the NADH oxidized, which is determined by the decrease in absorbance at 340 nm. Ascorbic acid can interfere with the assay but can be removed by using ascorbic acid oxidase (17).

2. Glucose

Glucose in food can be measured by a colorimetric method using glucose oxidase and peroxidase (18). Glucose is preferentially oxidized by glucose oxidase to produce gluconolactone and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide and o-dianisidine react to produce a yellow colored dye that absorbs at 420 nm:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \delta\text{-gluconolactone} + H_2O_2$$

$$H_2O_2 + \text{o-dianisidine} \xrightarrow{\text{peroxidase}} H_2O + \text{oxidized dye (colored)}$$

3. Starch/Dextrin

Starch and dextrin contents in food can be determined by enzymatic hydrolysis using amyloglucosidase, an enzyme that cleaves α-1,4 and α-1,6 bonds of starch, glycogen and dextrins, liberating glucose. The glucose formed can be subsequently determined enzymatically.

V. SEROLOGY, IMMUNOCHEMISTRY AND IMMUNOELECTROPHORESIS

Immunology is both a fundamental medical science and a technology. As a technology it has engendered the development of a whole ensemble of techniques based on the

antigen-antibody (*Ag-Ab*) reaction, categorized under the term *immunochemistry*. These techniques have been highly developed in other domains of biology to seek, identify, quantify, and even purify various molecules or associations of molecules (such as microorganisms) using antibodies.

A. Antigens and Antibodies

An antigen is any substance that, when introduced into a living organism, is susceptible to inducing the production of antibodies that are able to recognize it. Antigens can be molecules (proteins, polysaccharides, etc.) or associations of molecules (viruses, bacteria, etc.). All antigenic molecules are constituted of an assortment of determinants or antigenic sites called epitopes, each possessing a molecular component that is recognized by an antibody. A given antigen can have several identical or different epitopes, with the latter introducing a set of different antibodies. Thus, microorganisms that possess a variety of antigens contain a large number of epitopes.

Antibodies are substances produced by an animal in response to the introduction of a foreign substance (antigen). They react specifically with the antigens that induced their formation. These antibodies (or immunoglobulins) are produced by the B lymphocytes and are present in the blood. There are five classes of immunoglobulins, IgG, IgM, IgA, IgD, and IgE. Immunoglobulins are composed of a pair of long polypeptide heavy chains and a pair of short polypeptide light chains. Both inter- and intra-chain disulfide bonds are present. The heavy chains of different immunoglobulin classes differ chemically, while the light chains are of two types, kappa (*K*) and lambda (λ). All immunoglobulin classes contain either type of light chain. Each immunoglobulin molecule, therefore, consists of a pair of heavy chains specific for its class and a pair of either kappa or lambda light chains (Figure 49.2) (15).

All immunoglobulin molecules have a constant carboxyl-terminal end and a variable amino-terminal end. The amino-terminal end is the "antibody active site" or the "antigen combining site" with an amino acid sequence that varies to correspond with the configuration of challenging antigens. The specificity of antibodies formed in response to an antigen varies. Although antibodies generally react only with the antigen used, cross reactions due to structural similarities between antigens may occur. For example, antibodies to horse serum protein also react with donkey serum protein, and ovalbumin from duck eggs also reacts with antibodies to hen egg albumin. Serum albumins of different mammalian species are antigenically similar, though not identical. The more closely related any two species are, the greater the serological likeness of their corresponding proteins.

The structure of antibodies resembles a "Y" constituted of four polypeptide chains (Figure 49.2) (15). Each

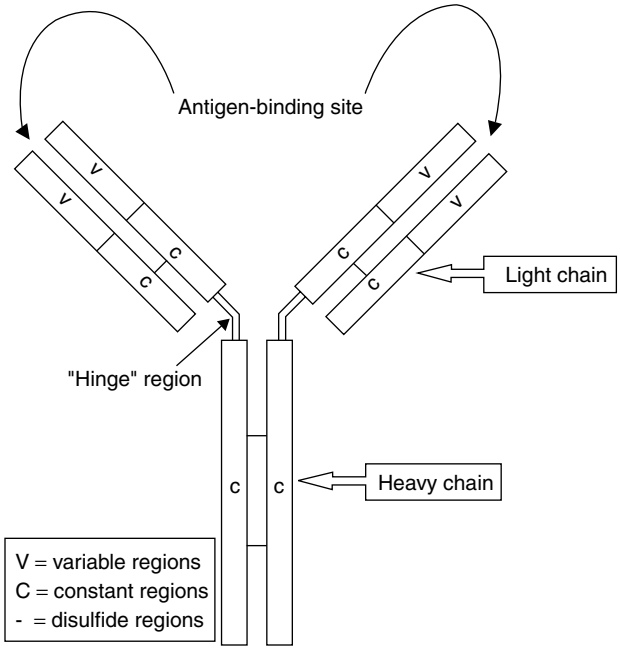

FIGURE 49.2 Diagram of the IgG tetrachain monomer.

branch of the "V" shaped section (or F_{ab} fragment) carries an active site that is complementary to an epitope. Thus, each antibody molecule can bind to two antigenic molecules with identical epitopes. The tail of the "Y" (or the F_c fragment) does not directly participate in binding with the antigen, but can fix to specific cells and activates the complement. Each lymphocyte descendant produces only one type of antibody that recognizes only one type of epitope.

1. Polyclonal Antibodies

After injecting an animal with antigen, antibodies appear fairly rapidly in its blood, and their concentration increases to a maximum and then decreases, constituting the primary response. When the same antigen is then re-injected (booster injection), antibody production is accelerated to higher levels and diminishes more slowly than the first time, constituting the secondary response. This production can be maintained at a relatively high level by re-injecting the antigen at regular intervals. Rabbit is often chosen, but other animals (mice, goats, sheep, etc.) can also be used. Immunization protocols are varied, and the injection can be intravenously (when working with bacteria), intramuscularly, or subcutaneously with the antigen preferably in suspensions (or emulsions) rather than in solution. The quantity of antigen injected (a few μg to a few mg) depends on the nature of the antigen, the animal, and its size.

2. Monoclonal Antibodies

Even if the antigen used to inoculate the animal is pure, the immunoserum collected is composed of a mixture of

antibodies directed against different epitopes. A more recent development involves obtaining a cellular clone that produces only one antibody with a well-defined specificity, known as monoclonal antibody. Kohler and Milstein in 1975 first developed the techniques for the production of monoclonal antibody.

B. Immunodiffusion

Several methods based on specific precipitation of antigen and antibody in agarose gels have been developed (19), and are called immunodiffusion. Single diffusion is a technique in which only one of the two reactants (generally the antigens) migrates in the agarose gel, and double diffusion techniques involve both components migrating simultaneously toward each other. The precipitate forms at a location where the concentrations of the two reactants reach equivalence, leaving a limited precipitation zone for each antigen.

1. Tube Precipitin Test

Qudin (20) devised a method by which complex antigen-antibody systems could be analyzed by allowing them to react in a capillary tube filled with agar. The antibody solution is mixed with warm agar, which is then allowed to harden in the tube. When an antigen solution is added, the antigen reacts with the antibody, forming a precipitation zone. The number of such zones is less than or equal to the number of independent precipitation systems (i.e., antigen-antibody reactions) present in the mixture.

2. Single Radial Diffusion

Mancini (21) developed this method, in which agarose containing the antibody is poured onto a transparent plate. The antigen solutions are deposited in circular wells cut in the gel. The antigens diffuse around the well and form a precipitation ring or halo, and the square root of the inner diameter is proportional to their concentrations. The quantity of antigen contained in the unknown sample is determined by comparing with a range of reference values. The sensitivity threshold is about 3 μg/ml.

3. Double-Diffusion

The double-diffusion method was developed by Ouchterlony (22). It permits both antigen and antibody to diffuse into an agar-filled glass dish that initially contains neither reagents. A few drops each of antigen and antibody solutions are placed separately in small wells in the agar. Antigen and antibody diffuse toward each other at a rate related to their concentrations and their diffusion coefficients. A precipitate line is formed where an antigen interacts with its antibody. Because of differences in diffusion rates, the lines are distinctly separated. The clean separation of lines on these Ouchterlony plates makes it possible to distinguish more reactions than with Qudin tubes. Consequently the plates are more useful in studying complex systems. The line of precipitation formed by an individual reaction can be identified if either the antigen or the antibody is available in relatively pure form.

C. Immunoenzymology

The technique of immunoenzymology was first applied to histochemistry and only assumed its present form as ELISA (Enzyme Linked Immunosorbent Assay) in 1971 simultaneously in France, Sweden, and Holland. The most commonly used enzyme markers are catalase, glucose oxidase, β-galactosidase, alkaline phosphatase, and peroxidase. The enzymatic reaction that allows detection is in the last step of the procedure. The technique involves the addition of a substrate which produces a colored product that can be read visually, or by a simple adapted colorimeter when micro-titration plates are used. The diverse substrates that can be used depend on the enzymes. The most common substrates for peroxidase are orthophenyldiamine (OPD) and 2,2′-di (ethyl 3-benzothiazoline-sulfonic-6) acid (BTSA) coupled to H_2O_2. With certain enzymes, substrates that produce fluorescent or luminescent products can also be used to increase sensitivity, but special measuring devices are required.

ELISA is a heterogeneous assay requiring washing between each step to remove unbound reagents. Widely used enzyme labels include alkaline phosphatase, glucose oxidase, and horseradish peroxidase. These enzymes catalyze reactions that cause substrates to degrade, forming colored products that can be read visually or spectrophotometrically. Depending on the format, either antibody or antigen is adsorbed onto a solid phase, which can be polystyrene tubes, polystyrene microtiter wells, or membranes (nitrocellulose and nylon) (23).

D. Immunoelectrophoresis

To improve the resolution and interpretation of the double-diffusion method, immunoelectrophoresis was developed by Grabar (24).

1. Classical Immunoelectrophoresis

Classical immunoelectrophoresis (25) is performed in two steps. First, there is a classical electrophoresis of the antigen mixture deposited in wells, in which the antigens are separated into small spots as a function of their charge. Customarily, two runs are done in parallel on the same gel, one with the sample mixture, and the other with a standard for comparison (Figure 49.3) (26). Then there is a double diffusion, in which antibodies placed in a central groove between the antigen spots migrate toward the antigens. This produces one or more precipitation lines

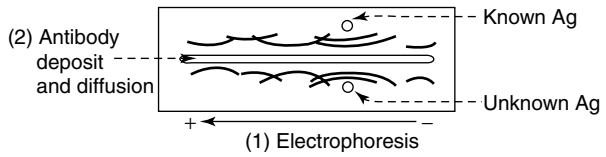

FIGURE 49.3 Classical immunoelectrophoresis (*Source*: From Ref. 26).

depending on whether each electrophoretically separated antigen spot is pure, or is constituted of several antigens that have the same charge. This method is sensitive, reproducible and qualitative in nature (26).

2. Rocket Immunoelectrophoresis

Rocket immunoelectrophoresis is a quantitative technique directly derived from radial immunodiffusion by applying an electric current to the gel (27). The antibodies are distributed evenly throughout the gel, and the sample (antigen) is placed in a small well at one end. As the antigen moves toward the positive electrode, it diffuses outward and reacts with the antibody, forming a precipitate. During migration, the antigen meets more antibodies, but since the antigen is now less concentrated, it will diffuse slower and the line of precipitate is less far away from the center of migration. Eventually, all antigens will have moved forward and reacted, and the resulting shape looks like a rocket (Figure 49.4) (26). The length or the area of the rocket is measured and correlated with standards for quantification.

3. Crossed Immunoelectrophoresis

Crossed immunoelectrophoresis is a two-dimensional separation technique (28, 29). The antigen mixture placed in the well is subjected to simple electrophoresis. The gel band is cut out and placed on a plate, and agarose gel containing antibodies is poured. A second electrophoresis is run perpendicularly to the first. The antigen migrates in the second gel, and produces one or several lines or peaks. This technique is more sensitive than simple immunoelectrophoresis.

E. Applications

The application of immunochemical methods in food analysis has been reviewed by Hitchcock (30). Sinnell and Mentz (31) described the use of electroimmunodiffusion methods for quantitative determination of non-meat proteins added to meat products. Kurth and Shaw (32) reviewed the identification of the species of origin of meat by immunochemical methods. Griffith et al. (33) recommended the use of a commercial ELISA system to measure levels of soya protein in meat. The presence of the major food allergen ovomucoid in human milk was studied

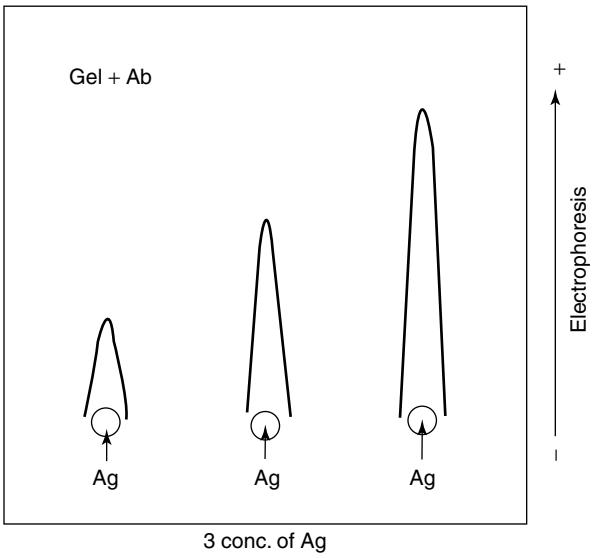

FIGURE 49.4 Rocket immunoelectrophoresis (*Source*: From Ref. 26).

using ELISA (34). ELISA-based methodologies were applied to detect and quantify allergenic proteins in peanut, soy, egg, milk, tree nuts, and crustacea (35).

VI. NUCLEIC ACID-BASED METHODS

Nucleic acids are high-molecular-weight macromolecules that form the genetic material of all living organisms. Two types of nucleic acids are generally encountered, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA serves as the repository for genetic information for both prokaryotes and eukaryotes. RNA serves a functional role in the conversion of genetic information into cellular proteins, and in the case of some viruses RNA serves as the genetic material as well.

Nucleic acids can be considered polymers of deoxyribo- or ribonucleotides. Each nucleotide unit is composed of a nitrogenous heterocyclic base, a sugar residue, and a phosphate group. The heterocyclic bases are either six-membered pyrimidine rings or nine-membered purine rings. Two types of purines and two types of pyrimidines are found in nucleic acids. Both DNA and RNA contain the purines adenine and guanine. DNA contains the pyrimidines cytosine and thymine, whereas RNA contains the pyrimidines cytosine and uracil. The heterocyclic bases are linked to the sugar via an N-glycosyl bond. The resulting compound is called a nucleoside. The nucleoside units in nucleic acids are joined through the 3′-hydroxyl group of one sugar and the 5′-hydroxyl group of the next sugar via a phosphodiester bond. This 3′-5′ internucleotide bond is found in both DNA and RNA. The double-helix model of DNA structure was first deduced in 1953 by James Watson and Francis Crick.

A. Basic Methodology

One of the landmark events in molecular biology was the development in 1975 by Edwin Southern of a DNA:DNA hybridization technique (36). This technique can detect rare DNA sequences in complex populations of DNA fragments generated by restriction enzymes. Nucleic acid strands having complementary base sequences can form double-stranded helices. The process of duplex formation is generally referred to as nucleic acid hybridization. Hybridization can occur between single strands of DNA and RNA or between complementary strands of DNA and RNA. Hybridization also includes the formation of complexes between single-stranded nucleic acids and complementary oligonucleotides. These reactions can be carried out in solution or with the nucleic acid immobilized on solid matrices such as nitrocellulose, nylon, or gels. Hybridization in solution is used to study the sequence complexity of cellular or viral DNA. Radioactively labeled nucleic acid probes (37) or probes capable of being tagged with fluorescent antibodies are used to search for and identify nucleic acid sequences (38). In this case the immobilized nucleic acid on nitrocellulose is bathed in a solution containing the probe.

1. Southern Analysis

Southern analysis is among the most powerful methods for DNA analysis. The first step in Southern analysis is the electrophoresis of DNA. DNA fragments digested with restriction enzymes are fractionated on agarose gels (0.7% to 1%) no more than 6 mm thick to allow for efficient transfer of the separated DNA to the filter membrane. The total amount of DNA loaded per lane of gel is dependent upon the relative abundance of the target sequence that is probed during hybridization.

Prior to transfer, the DNA is usually fragmented into smaller pieces and then denatured. This process can be divided into depurination, denaturation, and neutralization steps. The DNA is then transferred to the membrane using one of several methods: capillary, vacuum-assisted, pressure-assisted, or electrophoretic transfer. The DNA is then hrybridized with a probe.

2. Northern Analysis

Northern analysis involves a three-stage process: gel electrophoresis, gel blotting and hybridization to a labeled probe. Gel electrophoresis separates molecules of RNA on the basis of their size and conformation. Molecular size is the main determinant of migration speed, with smaller molecules moving through the matrix faster than larger molecules. Compact molecules also migrate faster than extended molecules of the same size. However, since RNA aggregation may occur, it is necessary to use denaturing gels to determine the actual size of RNA in the absence of conformational factors, aggregation, and nicks in the RNA. The choice of denaturing conditions depends on the nature of the gel and electrophoretic conditions. Formaldehyde is perhaps the most commonly used denaturant. A fact worthy of consideration during experimental design is that nonlinear RNA migrates more slowly than would be expected from its size since it cannot pass through the pores as easily as linear RNA.

The blotting of gels onto membranes can be achieved by capillary action or by electrophoresis. Nucleic acid transferred to a membrane is fixed into position by baking or UV crosslinking to the membrane. Subsequent hybridization of DNA probes to the bound RNA is carried out under stringent conditions to ensure that only complementary sequences hybridize.

B. Polymerase Chain Reaction

The polymerase chain reaction (PCR) was discovered in 1983 by Kary Mullis. PCR is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. The standard PCR has three steps defined by temperature: denaturation, annealing and primer extension. The first step, denaturation, occurs when the reaction mixture is heated to 92–96°C. At this temperature the template DNA is single-stranded and the enzyme *Taq* (thermostable DNA polymerase isolated from *Thermus aquaticus*) has no measurable synthetic activity and is not denatured by the near-boiling temperature. Successful amplification is dependent upon complete denaturation of the template DNA during this step.

The middle step of the PCR cycle results in the annealing of the oligonucleotide primers to the template DNA. The temperature for this step varies from 45°C to 65°C depending upon the homology of the primers for the target sequence as well as the base composition of the oligonucleotide. The primers hybridize to their target sequences at an annealing rate several orders of magnitude faster than the target DNA duplex can reform. The ideal temperature for annealing oligonucleotides to the target template is 2–5°C below the melting temperature (T_m) for the primers. If the two primers used for a reaction have different T_m, this 2–5°C rule applies for the one with the lower T_m.

The final step is the primer-dependent DNA synthesis by the *Taq* polymerase. This portion of the cycle is carried out at 72°C. The thermal activation of *Taq* is complemented by the use of Tris buffers since *Taq* has a broad pH optimum, with maximum activity at pH 7.3. The pH of the Tris buffer drops from 8.3 at room temperature to 7.3 at 72°C. The increase in temperature leads to maximum enzyme activity. The published rate of DNA synthesis by this highly processive enzyme 72°C is 60 nucleotides per second (39).

A repetitive series of cycles involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by the 5′ ends of the primers. Because the primer extension products synthesized in one cycle can serve as a template in the next, the number of target DNA copies approximately doubles at every cycle. The simplest detection method of amplified product is the standard agarose ethidium bromide stained gel electrophoresis.

C. Applications

Due to recent advances in these nucleic acid-based techniques, numerous food applications have been developed. DNA probes offer an exciting new approach to the highly specific detection of cells in any given environment such as microorganisms in foods. The concept of hybridization and DNA probes, as well as potential applications, have been reviewed (40, 41). The rapid development of biotechnology enables the launching of products and ingredients derived from genetically modified organisms (GMOs) into the food market. Information availability and transparency are essential for the acceptance of these new products by the consumers. A key factor in this issue is the availability of methods to distinguish between GM foods and their traditional counterparts. PCR has been found to be the method of choice for the detection of GMOs in food. PCR methods for the detection of GMOs were developed that can be used for screening purposes and for specific detection of glyphosate–tolerant soybean and insect-resistant maize in foods (42).

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Part D

Food Microbiology

50 Microbiology of Food Systems

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CONTENTS

I. INTRODUCTION: MICROORGANISMS IN FOODS

Microbiology is the study of living organisms that are so small that they can only be observed with the aid of a powerful microscope. In food microbiology, the organisms of concern are usually classified as bacteria, fungi (yeasts and molds), viruses, and parasitic protozoa. Bacteria are single-celled microorganisms found in nearly all environments. Bacterial cells are often classified by their outward appearance, including their size, shape, and arrangement.

Some bacteria have the ability to form spores. Spores can typically withstand adverse environmental conditions better than the vegetative form of the bacteria. For example, spores can be highly resistant to boiling water or chemical sanitizers. Bacterial spores are not a means of reproduction. Fungi include organisms as small as a single cell or as large as a mushroom. True fungi produce large masses of filamentous hyphae that form a mycelium. Yeasts, along with bacteria, reproduce by fission. Other fungi typically reproduce by spores carried on fruiting structures. Viruses are extremely small parasites. They require living cells of plants, animals, or bacteria for growth. The virus is mainly a packet of genetic material which must be reproduced by the host cells. Protozoa are single-celled organisms that can cause disease in humans and animals. Their cell structure is more complex than that of bacteria.

The presence of microorganisms in foods may have adverse or beneficial effects. Some microorganisms may hasten the spoilage of foods, thereby reducing sensory quality and shelf-life. Other organisms may be pathogenic, or could cause human illness. Some species of bacteria, yeast, and molds can be used to ferment foods or to produce desirable changes in quality, especially in taste and texture. These organisms can be intentionally used to process a wide variety of plant or animal foods.

II. HISTORY OF FOOD MICROBIOLOGY

Microorganisms have always been closely related to food and food systems. Authorities believe that approximately 8,000 to 10,000 years ago, humans began to have problems associated with food poisoning and food spoilage. The importance of one's ability to produce and preserve food was recognized early. Both livestock and salt had monetary value and could be compared to today's currency. Wealth and social status in many cases was based upon the amount of success one had at producing in times of plenty and preserving what could not be consumed immediately for times of hardship. Many early religious laws prohibiting the consumption of "impure" or "unclean" foods were based on hygiene, and were similarly seen in countries not conforming to the same religious beliefs.

The first individual to describe microorganisms was Kircher in 1658 who reported "worms" that were undetectable to the naked eye on several decomposing items. Shortly thereafter the theory of spontaneous generation, later known as abiogenisis, became widely accepted. In 1683, Leeuwenhoek's superior knowledge of lens design allowed him to be the first to observe and record yeast cells using a primitive microscope. Spallanzani sought to disprove spontaneous generation in 1765 when his beef broth did not spoil after being boiled in a sealed container for an hour. Critics, however, disagreed, believing his process was void of oxygen, a vital ingredient to spontaneous generation. In 1785, the French government offered a prize to anyone who could develop a practical method to preserve food. In 1809, Nicholas Appert was successful by preserving meats that had been boiled in water in corked glass bottles. At the time, he was unaware of the microbiological logic behind this process. Louis Pasteur, however, was the first to understand the role of microorganisms in food. He was most noted for his heat pasteurization process to destroy deleterious organisms in beer and wine. Pasteur's process was later commercialized in 1867. He also proved that microorganisms caused souring milk. Some other examples of important dates in food microbiology history include:

1825 - U.S. patent issued for food preservation in tin cans.
1840 - Fresh fruit and fish were first canned.
1857 - Milk identified as a vector for typhoid fever.
1874 - Use of ice for transport of meat at sea was widespread.
1880 - Milk pasteurization began in Germany.
1888 - *Salmonella enteriditis* isolated from meat in a food poisoning outbreak.
1895 - First bacteriological study of canning.
1906 - U.S. Congress passed the Federal Food and Drug Act.
1928 - Controlled atmosphere was commercially used for storage of apples.
1929 - Frozen foods introduced into retail markets.
1967 - United States was the first to design a commercial irradiation facility.
1976 - In California infant botulism was identified.
1981 - First outbreak of foodborne listeriosis occurred in the U.S.
1986 - The first diagnosis of Bovine Spongiform Encephalopathy (BSE).

III. SOURCES OF CONTAMINATION: ANIMAL, PLANT, PROCESSING, FOOD HANDLERS

Foods or their ingredients may harbor a wide variety of microorganisms. Some of these microorganisms may be a part of the natural microbiota of raw foods of plant and animal origin, while others may be introduced during production, processing, and preparation for consumption. Microbial contamination refers to the presence or added presence of pathogenic or spoilage microorganisms that were not originally present in or on the food. Microbial cross-contamination refers to the situation where microorganisms are transferred from one surface or food to another food or food contact surface.

Raw and processed foods can be contaminated with many types of bacteria, yeast, or molds prior to consumption. Contamination may occur during all steps of food processing. These steps may include: harvest, slaughter, transportation, processing, packaging, storage, and preparation for consumption. Prior to crop harvest or food animal slaughter, raw agricultural commodities may become contaminated with pathogenic and spoilage organisms from soil, irrigation water, animal feces, insects, manure, and wild or domestic animals. During transport and processing, foods can be contaminated by harvesting and transport equipment. Furthermore, contamination by sorting, packing, cutting, packaging, or other further processing equipment may occur. Also, process water, air or dust, and worker contact may further contaminate food products. Processed or packaged foods can become contaminated by food handlers, including foodservice and retail employees and consumers. Cross-contamination of raw and ready-to-eat foods can occur due to improper sanitation of food contact surfaces or equipment or when

raw and ready-to-eat foods are poorly separated during preparation.

Prevention of contamination or cross-contamination by unwanted microorganisms is a critical element for ensuring the safety and quality of raw and processed foods. At the farm level, biosecurity programs can enhance prevention of microbial contamination. At the processing level food manufacturers can practice regular cleaning and sanitizing of equipment and facilities. Cleaning requires removing visible soil or food residue from equipment, utensils, floors, and walls. Subsequent sanitizing must reduce the number of microorganisms to a very low or safe level. Also, good employee hygiene practices such as handwashing, protective clothing, and prohibiting food and drink consumption in work areas can limit opportunities for microbial contamination of foods and ingredients. During food preparation, workers or consumers should frequently wash their hands and work surfaces. Hands or utensils should not touch raw food prior to ready-to-eat food without washing. Food contact surfaces and utensils must be regularly cleaned and sanitized.

IV. MICROBIAL GROWTH

Microorganisms can grow and reproduce at different rates over time. When bacteria are in a rapid growth phase they may be able to double their population in just 20–30 minutes. Prior to achieving a rapid or exponential growth rate, organisms may need time to adjust to their environment. During food processing, microbes may need time to adjust to changing conditions of acidity, temperature, oxygen level, nutrient levels, or available moisture. The population of bacteria may only marginally increase during the time that the organisms are adjusting to a new environment. After this lag phase of growth, the bacteria may enter into a rapid growth phase that greatly increases their numbers. Eventually the growth rate will slow as a key nutrient becomes exhausted or environmental conditions act to limit growth. When the population or the growth rate is plotted against time, a sigmoid or s-shaped curve often expresses how the growth changes over time. In these plots, a lag phase is followed by a (exponential) growth phase, a stationary phase, and a die-off phase (Figure 50.1).

Microorganism growth rates are influenced by many environmental factors. Similar to other living organisms, they need water and nutrients to grow. Temperature is also a very important factor affecting microbial growth. The time that microorganisms need to grow and reproduce can vary greatly depending on the environmental temperature. Other important factors affecting growth include pH, atmospheric gas concentrations, and available water for growth.

Water is necessary for growth, but microbes cannot grow in pure water. While all foods contain some water, some of this water is not available to the bacteria. Often the water in foods is bound with some of the molecules of the food such as the proteins, fats, and carbohydrates. The bound water may not be accessible to microorganisms to use for growth. Water activity, or a_w, is a measure of the water available to microorganisms in foods. Water activity is measured on a scale of zero to one (pure water). For growth, most bacteria require a a_w of at least 0.90. Yeasts and molds can tolerate a lower water activity, perhaps as low as 0.7. A water activity of less than 0.85 in food will suppress the growth of organisms of public health significance.

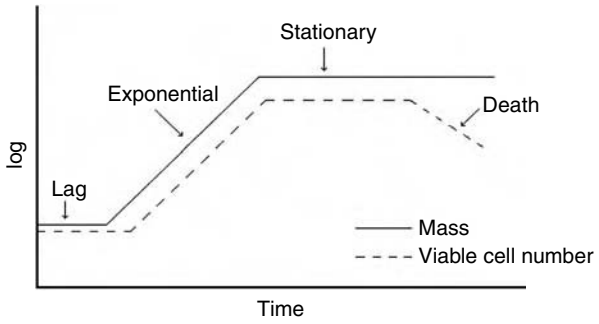

FIGURE 50.1 Bacterial growth curve.

Microorganisms can generally grow over a wide range of temperatures, but their growth rate at different temperatures can vary greatly. Organisms grow fastest at an optimal temperature. The optimum growth temperature for most organisms is near room temperature or body temperature (25–35°C). However, microorganisms may still be able to grow over a temperature range of 10–45°C. Foodborne microorganisms can survive temperatures of 0°C, but typically will not grow at this temperature.

Acidity can be measured with the pH scale where 0 is strongly acidic, 7 is neutral, and 14 is strongly basic. Many bacteria multiply more rapidly when the pH of their surroundings is near neutral, but may be able to grow when the pH ranges from 4 to 9. Yeasts and molds are more tolerant of low pH (high acidity) than bacteria. Many foods have a pH value between 4 and 7 which favors the growth of most pathogenic and spoilage microorganisms. As microorganisms reproduce, their metabolic by-products may alter the pH of their surroundings.

Just as microbial temperature and pH requirements can vary, microbial oxygen requirements can also differ greatly. Bacteria that require oxygen for growth are commonly called aerobes. Anaerobic bacteria do not grow in the presence of oxygen, but can grow in vacuum-packed foods. Some organisms can grow with or without oxygen and are referred to as facultative anaerobes. Finally, some organisms grow favorably under a low oxygen atmosphere. These microaerophiles grow optimally when the oxygen concentration in the atmosphere is 5–10% whereas the concentration in air is 18–20%.

V. MICROBIAL INACTIVATION, SURVIVAL, AND DEATH

Often a correlation exists between the rate and amount of treatment applied during the preserving process and the rate of microbial inactivation, survival, and death. In using heat processing to preserve foods, the temperature denatures proteins, which destroys enzyme activity and enzyme-controlled metabolism in microorganisms. The rate of microbial inactivation follows the first order reaction, by which when the product is heated to a temperature that is high enough to destroy contaminates, the same percentage die in a given time interval regardless of the numbers present initially. This is known as the logarithmic order of death and is described by a death rate curve (Figure 50.2). Microbial inactivation, survival, and death rate of the microorganism can be extrapolated from this curve.

The decimal reduction time, also known as the *D*-value, is defined as the time in minutes to achieve a $1-\log_{10}$ reduction in a specific microorganism at a specific temperature in a specific growth medium. *D*-values are variables for different microbial species and a higher *D*-value indicates greater heat resistance. Since microbial destruction occurs logarithmically, it is theoretically impossible to destroy all cells. The heating process therefore aims to reduce the number of surviving microorganisms by a pre-determined amount, depending on the type of microorganisms that is expected to contaminate the raw product. Usually, foods are processed to ensure at least a 12*D* reduction for *Clostridium botulinum* in low acid foods. This organism is targeted due to its importance in the food industry as well as its spore-forming abilities.

If the destruction of microorganisms is determined by temperature, cells die more rapidly at higher temperatures. The z-value characterizes the change in temperature required to achieve a $1-\log_{10}$ change in the *D*-value. This gives an indication of the temperature dependency of the heat resistance of an organism. Most heat-resistant spores have a z-value of 10°C.

The thermal death time (*F*-value) is used as a basis of comparing heat sterilization techniques. The *F*-value is defined as the equivalency in minutes at a given temperature, of all things considered, with respect to its capacity to destroy spores or vegetative cells of a particular organism. It can also be considered as the time needed to reduce the microbial numbers by a multiple of the *D*-value. When plotted, it is a bell-shaped curve, which is affected by product type, container size, container shape, and method of load of the unit for heat process. $F = D(\log n_1 - \log n_2)$, where n_1 = initial number of microorganisms and n_2 = final number of microorganisms.

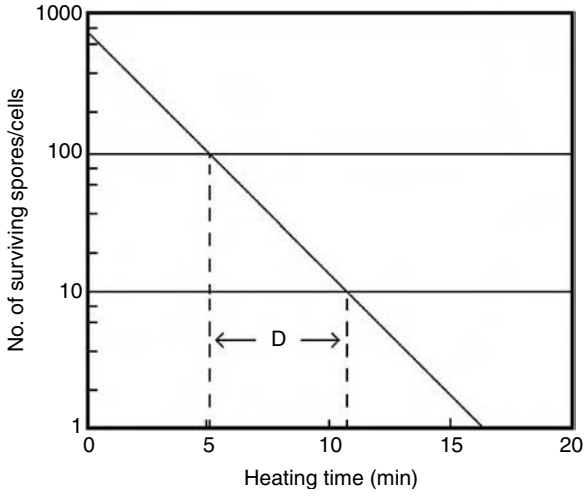

FIGURE 50.2 Death rate curve.

VI. DETECTION OF MICROORGANISMS

Food microbiologists examine foods for the presence, types, and numbers of microorganisms and/or their products. Sampling techniques as well as the enumeration of the affected food product or surface are crucial in obtaining an accurate analysis. In spite of its importance, the enumeration methods used commonly permit only the estimation of microorganisms in a food product or their surrounding environment. Although some analytical methods are more sensitive and more rapid than others, every method has certain inherent limitations associated with its use.

A. Sampling from Foods or the Environment

Many methods exist for sampling the environment of the food plant as well as the foods and air for the presence and relative numbers of microorganisms. Some commonly used methods are described below.

1. Gravimetric and volumetric sampling: Defined portions of food or water samples can be collected and analyzed by numerous microbiological methods. For liquids a volumetric sample of 10–100 mL is typically analyzed. For solids, a gravimetric or weighed sample of approximately 10–50 grams is analyzed. Solid foods may need to be diluted with one of several aqueous solutions during an analysis. In these cases, microbial concentrations can be reported as "per volume of diluent."
2. Swab sampling: Swabbing is the oldest and most prevalently used method for the microbiologic surface examination not only in the food and dairy industries but also in hospitals and restaurants. With this method, surface areas can be sampled corresponding to the size of the

surface with the use of a sterile template. A moistened swab is used to thoroughly rub the exposed surface. After exposure, the swab is returned to its holder containing a suitable diluent which can contain selective media and neutralizers for sanitation chemicals, if necessary. After proper dislodging from the swab, the organisms are then examined by enumeration or qualitative analysis. The swab method is best suited for flexible, uneven, and heavily contaminated surfaces. The ease of removal of the microorganism is dependent on the texture of the surface and the nature and type of flora.

3. Contact plate: The replicate organism direct agar contact (RODAC) method consists of a raised agar surface on the petri plate. When the plate is inverted, the agar surface makes direct contact with the surface. Once exposed, the plate is then covered, incubated, and enumerated for viable colonies. With this method, foods surfaces as well as environmental surfaces can be examined. In testing a surface that may have been cleaned with certain compounds, it may be necessary to add a neutralizer in the medium. This method, however, is not effective for enumerating heavily contaminated surfaces.
4. Air sampling: Numerous methods exist for the sampling of air in food plants for the presence and relative numbers of the microorganism. The most commonly used methods are impingement in liquids, impaction on solid surfaces, and sedimentation. Impingers are advantageous in that a specific quantity of air can be sampled. Filtration, centrifugation, electrostatic precipitation, and thermal precipitation may be used in air sampling. The simplest of air sampling techniques is to open pre-poured petri dishes for specific periods of time in the area to be assessed. The results obtained are influenced by size of particles and speed and direction of air flow.

B. Isolation and Identification

Isolation and identification of microorganisms can be as simple as performing isolation streaks for a colony and gram stains to much more sophisticated procedures using immunologic assays, chemical assays, and physical assays. Many microbiological analytical methods require that samples be immersed into an enrichment media to encourage growth of the target organism and to discourage growth of other naturally occurring organisms. Subsequent transfers to other microbiological media are used to purify food extract and isolate specific bacterial species. Additional confirmatory tests can be used to identify microbial species and biotypes. Selection and use of any microbiological methods should depend on resources available, analysis time, cost, sensitivity, specificity, technical expertise required, etc. In this chapter, it is not possible to go into detail about all of the procedures that may be used to identify microorganisms in foods. Some of these methods are briefly described below.

1. Nucleic acid (DNA) probes: DNA probes have been prepared for a number of foodborne microorganisms such as *salmonellae*, *listeriae*, staphylococcal enterotoxins, *Clostridium perfringens* enterotoxin, *Entamoeba histolytica*, and enterotoxigenic *E. coli*. A DNA probe, which is usually labeled with a radioisotope, consists of the DNA sequence of an organism of interest that can be used to detect homologous DNA or RNA sequences. The probe DNA must hybridize with that of the strain to be sought. Ideally, the DNA sequence that contains the genes that code for a specific contaminant is used.
2. PCR: An increasingly popular and highly reliable method for identification is DNA amplification – polymerase chain reaction (PCR) method. This method is a powerful, rapid system for detecting the presence of foodborne pathogens. This technique, performed in a very clean work environment, employs thermostable DNA polymerase and 5′ and 3′ specified oligonucleotide primers. It enables amplification of a single molecule of DNA to 10^7 molecules after a series of amplification cycles, typically from 20 to 50. The amplified DNA is then detected by use of either agarose gels or Southern hybridization employing a radiolabeled probe. Most PCR reagents now come in kit form.
3. Immunoassays: These methods are based on the interaction of antibodies, and consist of a wide range of rapid detection methods that are relatively simple to detect and identify contaminants associated with food products. Many are readily available in kits. Examples of immunoassays used in the food industry include the use of fluorescent antibodies, immunoaffinity columns, enrichment serology, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, and hemaggultination. The technique of radioimmunoassay (RIA) consists of adding a radioactive label to an antigen, allowing the antigen to react with its specific antibody, and measuring the amount of antigen that combined with the antibody by the use of a

counter to measure radioactivity. Using this method, detection and identification of bacterial cells can be achieved. RIA is used for identifying and enumerating staphylococcal enterotoxins, *V. cholerae* that produce cholera toxin, aflatoxins, and other mycotoxins. Multibacterial species can be detected in one operation when mixtures of homologous antibodies are used. The RIA method can also be used to identify biological hazards such as endotoxins and paralytic shellfish toxins in foods examined.

The ELISA method is similar to that of the RIA but rather than employing a radioactive isotope, it uses an enzyme coupled to antigen. A typical ELISA is performed with a solid phase (e.g., beads, test wells, or dipstick) coated with antigen and incubated with antiserum. Following incubation and washing, an enzyme-labeled preparation of anti-immunoglobulin is added. After gentle washing, the amount of enzyme remaining in the tube or microtiter well is measured colorimetrically. The color change reveals the presence or absence of the contaminant as well as estimation on enumeration. There are many variations of ELISA.

C. Enumeration

Many procedures exist for estimating a microbial population. Ideally, the method used should be accurate, rapid, inexpensive, and standardized. Methods should be selected based on the use of the information that is obtained. Examples of systems used in estimating the number of microorganisms in foods are listed in Table 50.1. The basic methods used in quantitative analysis include standard plate counts (SPC) for viable cells, the most probable number (MPN) method as a statistical determination of viable cells, dye-reduction techniques to estimate numbers of viable cells that possess reducing capacities, and direct microscopic count (DMC) for both viable and non-viable cells.

1. Plate Counts for Viable Cells

The most widely used and basic technique used for enumeration of microorganisms is the standard plate count (SPC). It is relatively simple and consists of plating appropriate dilutions by transferring an aliquot for either pour plating (pouring molten agar onto plate) or spread plating (evenly transferring the aliquot onto a hardened agar surface). The plates are incubated at an appropriate temperature/time and the colonies are enumerated. Use of spiral plates and use of selective media in the agar further enhance this procedure. SPC is considered one of the most precise methods for determining microorganisms such as bacteria and yeast that will grow in an agar medium.

TABLE 50.1
Systems Used in Enumeration of Microorganisms in Foods

Direct microscopic count (DMC)	Electrical
Breed clump count	Conductance
Electronic particle count	Impedance
Howard mold counts	Capacitance
	Voltage drop
Pour plate	Spectrophotometric (optical density)
Spread plate	Adenosine triphosphate (ATP)
Spiral plate	Reductase tests
Drop plate	Easicult-TTC
Plate loop	Immuno-affinity columns
Roll tube	Respiration rates
Oval tube	*Limulus* amoebocyte lysate (LAL)
Burri strip/slant	Chemical indicators
Restriction fragment length polymorphism (RFLP)	(decomposition products)
Little plate	pH
Tube dilution	Agar droplets
Most probable number	Millipore sampler
Membrane filter	Bactoscan
Hydrophobic grid (HGMF)	Microcalorimetry
Direct epifluorescent filter technique (DEFT)	Flow cytometry
	Gas chromatography
Microcolony-DEFT	Radioimmunoassay (RIA)
Agar droplets	Thermostable nuclease
Microtiter-Spot plate	Fluorescent antibody
Dry rehydratable film	Enzyme-linked immunosorbent
Petri film	assay (ELISA)

2. Total Cell Counts

Some methods do not differentiate between viable and non-viable cells, giving total cell counts. These methods are appropriate for use when evaluating the microbial content of a food and/or evaluating the number of body cells in milk such as the detection for mastitis in cows. The value of total cell counts is limited to samples with high cell loads. Total cell counts are useful in determining the quality of pasteurization and recontamination of foods, and are a rapid method to estimate the bacterial load. Examples of total cell counts are direct microscopic count (DMC), spectrophotometric assay, and electronic particle count. In DMC, a suspension of the sample is stained and observed using a counting chamber or spread over a prescribed area on a glass slide and the number of cells calculated per area. Using this method, cell morphology can be seen and it lends itself to fluorescent probes for improved efficiency. However, since it is a microscopic analysis, it is fatiguing to the analyst, and food particles are not always distinguishable from the bacteria.

A rapid method to estimate total cell count is the spectrophotometric analysis. A suspended sample is analyzed by a spectrophotometer, where a light of a specified wavelength is passed through the sample. The amount of

absorbance and percentage of transmittance of the light can then be correlated to an estimated cell count. However, using this method, no selectivity is made to the microorganism or debris in the sample such as food particles. Clumping of cells may also lead to a miscued estimation. Due to the various size of contaminates, adjustments may have to be made to the readings as well as the wavelength used.

Many more methods exist for the enumeration of viable organisms in foods. Most of the basic systems for enumeration of viable organisms are based on the plate count or the tube dilution method. Selective media or environmental conditions are often used if a specific contaminant is targeted. More advanced assays for rapid analysis are based on the metabolic activity of the contaminant on given substrates, measurements of growth response, or the immunologic responses to the microorganism.

Use of nitrocellulose or hydrophobic grid membranes to filter the sample solution through pores that retain bacteria or mycobacterium (usually 0.45 μm) but allow for the suspension to flow through is especially suited for assays when the microbial count of the sample is expected to be relatively low. Following the collection of the contaminant upon filtering a given volume, the membrane is placed on a hardened growth media and incubated at an appropriate temperature. To improve microbial yield and decrease background particles on the filter such as food and sediments, use of prefilters, surfactants and enzymes, and stains are incorporated into the assay. Analysis of the samples can be made after 2–4 hours with a microscope or 24–48 hours similarly to plate counts after incubation.

3. Most Probable Number (MPN) Methods

To estimate a low concentration of organisms in a sample, the most probable number (MPN) method can be used. Several tubes are prepared from consecutive ten-fold sample dilutions and recorded as either positive or negative for growth. In some instances, MPN is easier and simpler to perform than the plate count. It is particularly useful for samples with only a few organisms and can be used to detect organisms in samples larger than 1 g. The relationship between positive and negative tubes has been determined mathematically and tables have been developed for convenient estimations of cell concentrations.

The adenosine triphosphate (ATP) bioluminescence measurement system is relatively simple and rapid, with its results being reliable and acceptable for food and water analysis. The method for determining ATP is based on the reaction of luciferin and luciferase, which when reacted with ATP in the presence of magnesium ions causes a light emission. By using a photometer to measure the light emission when bacterial ATP reacts with the luciferin-luciferase system, quantitative analysis is made of the microbial load. Limitation to the analysis is that, since the luciferin-luciferase system accounts for all ATP in the sample, non-bacteria ATP must be removed prior to analysis.

D. Indicator Organisms

The first use of indicator organisms probably occurred in 1892, when Schardinger tested water for *E. coli* in search of *Salmonella typhi*. He theorized that, while it is difficult to detect *S. typhi* in a water supply, the presence of *E. coli* would indicate that there might have been contamination from sewage and that *Salmonella* or other coliforms might be present. Certain criteria are used to determine the value of an indicator organism in instances where specific pathogens such as fecal coliforms are targeted. Exclusive association with contamination and specificity with the pathogen should be seen. The usual source of the indicator and the significance of it should be known. The indicator should not be present as a natural contaminant of the material being analyzed. Moreover, detection, differentiation from background microorganisms, and enumeration of the indicator should be rapid and accurate. It is also beneficial if a standardized test for detection and enumeration exists. In using indicator organisms to determine product quality or shelf life, it is ideal that the indicator organism used possess qualities such as: presence and detection in all foods to assess quality growth and numbers have a direct correlation with product quality (in spoilage, the indicator organisms can be a natural contaminant of the material being analyzed) detection; and enumeration should be relatively easy, quick, and distinguishable from background microorganisms; growth of indicator microorganisms are not affected adversely by other components of the food flora.

VII. FOOD SAFETY

Ensuring safe food is an important public health priority for our nation. An estimated 76 million illnesses, 323,914 hospitalizations, and 5,194 deaths are attributable to foodborne illness in the United States each year. The estimated cost of foodborne illness is $10–$83 billion annually. For some consumers, foodborne illness results only in mild, temporary discomfort or lost time from work or other daily activity. For others, especially pre-school age children, older adults, and those with impaired immune systems, foodborne illness may have serious long-term consequences or may even be life threatening. The risk of foodborne illness is of increasing concern due to changes in the global market, aging of our population, increasing numbers of immunocompromised and immunosuppressed individuals, and changes in food production practices.

Numerous biological agents other than bacteria are known to cause foodborne illness. Many of these organisms or substances, such as the toxins produced by some

fungi, were identified and confirmed as a causative agent of foodborne illness several decades ago. Others, especially prions and some of the parasitic protozoa, have only been studied during the past 20 years. The etiology of foodborne disease outbreaks is changing and can be a subject of great debate. The majority of illnesses or deaths due to food consumption are grossly underreported worldwide. The ability to link particular food sources, and food production, processing and preparation practices to foodborne illness is a challenge for epidemiologists. In the U.S., fewer than 1 in 1,000 estimated cases of foodborne illness are confirmed. Bacteria are the causative agents in approximately half of the confirmed cases of foodborne illness in the U.S. The remainder are attributed primarily to viruses or to other chemical agents or parasites. In the future, we should expect the proportion of confirmed cases and outbreaks of foodborne illness to increase as improved diagnostic tools are developed and surveillance for foodborne diseases improves.

Over the past two decades, microbiological food safety has received greater attention from regulatory authorities, researchers, public health officials, and consumers. We are continually learning more about the sources of microbial foodborne safety problems and how to prevent them. For several reasons, we may discover new food safety problems that may shift our current priorities for control of microbial foodborne illnesses. For example, we may be able to more easily detect and quantify microbial disease agents due to advances in laboratory methodologies. Also, we may discover new or emerging pathogenic organisms. Many microbial species have been shown to become more or less virulent over time. And, increased global food trade and international travel can lead to rapid transmission of foodborne pathogens around the world.

VIII. SPOILAGE BY MICROORGANISMS

Many species of microorganisms are known to cause spoilage of some foods. Spoiled foods are generally considered unacceptable to eat because of a poor odor, flavor, texture or appearance. Microbiological food spoilage occurs when the growth of bacteria, yeast, or mold leads to the production of microbial metabolic byproducts which make the food product unacceptable to eat.

The shelf life of a food refers to its useful storage life. At the end of shelf life, foods may be perceived as significantly different or unacceptably different from fresh quality. In foods, the shelf life of a perishable item is greatly affected by the initial microbial load present. Generally, if a food contains a large population of spoilage organisms, it will have a shorter shelf life than the same food containing fewer numbers of the same spoilage organisms. Excessive growth of spoilage microorganisms in a food can lead to changes in its composition or structure which may ultimately render the food unacceptable to consumers. For example, many microorganisms produce enzymes that can cause fats or proteins to breakdown into aromatic compounds that can diminish sensory qualities and consumer acceptance. The breakdown of food components may also change the texture or water holding capacity of the food, especially near the surface. High temperature storage of these products can accelerate the rate that these enzymes promote changes in food quality.

Microbiological food spoilage can occur when bacteria, yeast, or mold grows to a sufficient level leading to the production of metabolic by-products that ultimately make the food unacceptable for consumption. Generally, many foods are considered spoiled or at the end of their typical shelf life when their total bacterial concentration is approximately 10^7 per g or per mL. Also, foods may be at the end of their shelf life if the yeast concentration is 10^5 per g or per mL, or if visible mold is present. Since bacteria, yeast, and molds are typically present in some foods and proliferate under different environmental conditions, spoilage of particular foods is usually caused by specific species of bacteria, yeast, or mold.

Generally, spoilage of foods can be delayed by appropriate processing, packaging, and storage of these products. Food processors can also delay microbiological spoilage by using quality raw materials with low microbial loads. Also, strict adherence to industry current Good Manufacturing Practices or processing under sanitary conditions will also prevent the introduction or growth of spoilage microorganisms in processed foods. The processing and packaging techniques used to inactivate or inhibit pathogenic microorganisms may also inhibit spoilage-causing microorganisms.

Microorganisms that may cause spoilage can replicate to unacceptably high concentrations in foods that are stored at higher temperatures and for longer times than recommended. After processing, most foods have optimum or prescribed storage conditions including time, temperature, and humidity. Processors, retailers, and consumers should follow proper storage recommendations for raw and processed foods to ensure optimal product quality.

IX. FOOD PRESERVATION

From the first stage of processing, the quality of food can deteriorate over time. The shorter the amount of time it takes a product or ingredient to be transformed or transported to its final destination, the consumer, the more advantageous. For this reason many foods are kept “alive” and “healthy” as long as possible. Once a food is no longer “alive” the quality decreases at varying rates dependant on the product and its environment. Degradation begins on many physical and chemical levels. This may be caused by microbiological, enzymatic, chemical, or physical means. Environmental

conditions that may increase or decrease the quality of a product include temperature, humidity, handling, packaging, transport, and, of course, time. Product water activity (Table 50.2), pH, amino acid content, fatty acid content, vitamin content, and naturally occurring enzymes may also affect the quality of a product. Atmospheric modification, acidification, temperature reduction, thermal processing, reduction of water activity (Table 50.2), fermentation, and the addition of antimicrobials can combat naturally occurring microbiological processes.

The two major categories of food preservation are chemical methods and physical methods. Chemical methods are chemical preservatives, which include antimicrobials, antioxidants, and anti-browning agents. Physical methods of preservation include low temperature preservation, high temperature preservation, radiation, modified atmosphere packaging, and drying.

A. Antimicrobials and Preservatives

Many antimicrobials are bacteriostatic or fungistatic and can only be added to certain levels within strict accordance with established regulations. Therefore, preservation of the quality of the food will not last indefinitely. Oftentimes use is in conjunction with other methods of preservation in order to provide a "multiple hurdle" defense against microorganisms. Occasionally, preservatives are added to foods for one purpose, but they may also indirectly act as antimicrobials. For many antimicrobials the exact mechanism or component that the chemical affects on the microorganism's cellular level is unknown. In general, particular cellular components such as the cell wall, cell membrane, metabolic processes, and genetic material are targeted for disruption, causing cell death or inhibition. No antimicrobial is the "silver bullet" for all microorganisms. Some may affect several types of microorganism but with only a limited effect; therefore, several different antimicrobials may be applied to help control the multiple microbiological concerns of a single product. A list of several common antimicrobials is provided in Table 50.3.

TABLE 50.2
Water Activity of Microorganisms and Select Foods

Food	Minimal a_w
Fresh fruits, vegetables, meat, and fish	0.98
Cooked meat	0.98–0.95
Cured meat products, cheeses	0.95–0.91
Sausages, syrups	0.91–0.87
Most bacteria can grow	0.91
Most yeasts can grow	0.88
Flours, rice, beans, peas	0.87–0.80
Most molds can grow	0.80
Jams	0.80–0.75
Halophilic bacteria can grow	0.75
Xerotolerant molds can grow	0.71
Dried fruits	0.65–0.60
Spices, milk powder	0.60–0.20

B. Low Temperature Preservation

The ability of low temperatures to preserve foods is based on the principal that all biological systems are controlled by enzymatic reactions including those that control microorganisms and cause quality degradation. The rate of these reactions is directly related to temperature. With a rise or fall in temperature the enzymatic rate of reaction increases or decreases, respectively. In biological systems, for each rise in temperature of 10°C there is approximately a two-fold increase in the enzymatic rate of reaction. Keep in mind that the temperature range and increase must be within appropriate parameters for that particular enzymatic function. Most microorganisms are significantly slowed at temperatures ranging from 0–7°C and many will not replicate with the exception of psychrotrophic organisms that will cause spoilage at these temperatures. Below 0°C all microbiological activity ceases; however, some enzymatic activity may still occur.

TABLE 50.3
Chemical Food Preservatives

Preservatives	Maximum Tolerance	Organisms Affected	Foods
Propionic acids	0.32%	Molds	Bread, cakes, cheeses
Sorbic acid	0.2%	Molds	Hard cheeses, syrups, salad dressings
Benzoic acid	0.1%	Yeasts and molds	Margarine, pickle, relishes, soft drinks, salad dressings
Parabens	0.1%	Yeasts and molds	Bakery products, soft drinks, pickles
Sulfites	200–300 ppm	Insects, microorganisms	Molasses, dried fruits, wines
Ethylene/propylene oxides	700 ppm	Yeasts, molds	Fumigant for spices, nuts
Sodium diacetate	0.32%	Molds	Bread
Nisin	1%	Lactis, clostridia	Pasteurized cheese
Dehydroacetic acid	65 ppm	Insects	Pesticide on strawberries, squash
Sodium nitrite	120 ppm	Clostridia	Meat-curing preparations

C. High Temperature Preservation

Two common types of high temperature preservation are pasteurization and sterilization. These processes are successful due to the general destruction of microorganisms at elevated temperatures. Pasteurization employs a technique of heating a substance, usually a liquid, to temperatures high enough to destroy most bacteria, including pathogens, without major chemical alteration of the product. The organisms that survive fit into either thermoduric or thermophilic categories. Thermoduric organisms, including the genus *Lactobacillus*, can withstand relatively high temperatures but are non-spore forming. Thermophiles, including the genera *Bacillus* and *Clostridium*, are sporeformers that require high temperatures for regular metabolic activity. The complete elimination of all microorganisms is referred to as sterilization. No viable organisms can be detected after this process.

D. Radiation Preservation

Radiation can be defined as the emission of radiant energy in the form of particles or waves. The food industry uses four major forms of radiation, including ultraviolet light, gamma radiation, electron beam radiation, and microwave radiation. The first three types depend on disruption at a molecular level causing lethal mutations in the microorganism's genetic code to destroy microorganisms. Ultraviolet light is most destructive at a range between 240 nm and 280 nm. Gram-negative bacteria are most affected by this kind of radiation. Gamma radiation is radiation emitted from Cobalt or Cesium sources. Electron beam radiation has a lower penetration power and requires a higher dose. Electron beam and gamma radiation are referred to as cold sterilization, achieving no increase in heat to produce to the required bactericidal effect. These methods can, however, cause undesirable color changes in the product at higher dosages. Microwaves use the friction of oscillating molecules to produce heat and thereby destroy microorganisms. Depending upon the radiation source, dose, product, presence or absence of oxygen, condition of food, target organism, microbial load, packaging, and surface cleanliness the effect of radiation varies widely.

In using radiation to preserve foods, the reactive ions produced by irradiating foods injure or destroy microorganisms by altering the cell membrane structure and affecting metabolic enzyme activity. More importantly, the DNA and RNA molecules in cell nuclei, which are essential for growth and replication, are affected. Analogous to heat processing, the rate of destruction of individual cells depends on the rate at which ions are produced and interreact with DNA, whereas the reduction in cell numbers depends on the total dose of radiation received. The rate of reduction is expressed as the D-value, in which the units are expressed in kiloGrays (kGy). A Gray (Gy) is the absorbed dose where 1Gy is the absorption of 1 J of energy per kilogram of food. Like thermal processing, irradiation is a first-order reaction, in which theoretically, a logarithmic reduction in microbial numbers with increasing dose is expected.

E. Drying Preservation

Microorganisms and enzymes require water in order to be active. Low-moisture foods, including freeze-dried foods, and intermediate-moisture foods are the two categories of shelf stable dried food preservation. To be defined as a low-moisture food, the product cannot contain more than 25% moisture and has to achieve a_w less than 0.60. If the product contains between 15–50% moisture and a a_w of between 0.60 and 0.85 it is defined as an intermediate moisture food. During drying a wet hot heat causes more damage to microbial cells than dry heat. Freeze drying employs a vacuum sublimation of the ice content. The lowered a_w and moisture levels produced through these processes are capable of inhibiting any spoilage or pathogenic organisms.

F. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is any package that contains a gas composition deviant from atmospheric air. Typically this is achieved by varying the amounts of oxygen, carbon dioxide, and nitrogen, in effect to increase the amount of CO_2. Carbon dioxide has been known to extend the shelf life of food products for over 100 years. The reduction or removal of atmospheric air does not provide an environment conducive for growth of typical spoilage organisms. Occasionally, as in the case with vacuum packaging, a product may begin with little atmospheric oxygen, which is consumed and converted to CO_2 by normal aerobic microflora. The concentration of CO_2 may reach as high as 30%. Most of the time a known concentration of CO_2 is added to a high barrier plastic film in order to achieve a preservation effect. Many different variations of this general principle are used to achieve the proper effect for different products. Another way to manipulate the atmosphere of a package is to employ the use of different types of plastics for packaging. Depending upon the molecular composition of the plastic and how it is formed the rate at which molecules of oxygen, carbon dioxide, and water vapor pass through it vary. These are referred to as the oxygen transmission rate (OTR), carbon dioxide transmission rate (CO_2TR), and the water vapor transmission rate (WVTR). The units for these measurements are cc/m^2/24 h at 70°F. Transmission rates are also proportional to the thickness of the film. Some plastics may possess a low OTR but a high WVTR; therefore, many manufacturers use several combinations of plastics added in layers to achieve the desired film. No

plastic has a zero transmission rate; therefore, a food package, no matter how well constructed, may start with one type of atmosphere which will change over time.

Unfortunately, there are some problems associated with MAP. Pathogenic organisms compete for nutrients with spoilage organisms. Usually, the consumer would reject a food before pathogenic organisms are in high enough concentrations to cause harm. In the MAP environment the normal spoilage organisms are not there to compete with pathogens. What develops is a food product that smells and looks edible but is in fact riddled with pathogenic organisms. Due to the fear of *Clostridium botulinum* the FDA has recently increased the minimum recommended OTR to 10,000 cc/m^2/24 h at 70°F. An increase in CO_2 also causes some physiological changes such as the exudative loss of water. The binding of CO_2 to moisture in the product forms carbonic acid ultimately changing the pH. This can lead to off-flavors and consumer rejection.

G. Other Processes for Food Preservation

Other methods of preservation are increasingly used by researchers and are commercially available to a limited extent. Destruction of microorganisms through the use of high-power ultrasound has been attempted but thus far has not been made economically feasible. High-intensity pulsed light using high-intensity xenon arc lamps on quick short bursts utilizing a UV affect is currently being studied. Finally, high hydrostatic pressure (300 to 1,000 MPa) is used with products that are liquid or mostly consisting of liquid. The underlying mechanism hinges on the destruction of non-covalent bonds, which in turn disrupt the target organism's cellular function causing deactivation.

X. FERMENTATION

Fermentation is probably the oldest form of food preservation and can be defined as the breakdown of carbohydrates under anaerobic conditions. Fermented foods do not always fall under this strict definition and many times are produced from the breakdown of carbohydrates and carbohydrate-like components under aerobic and anaerobic conditions. Most fermentations, however, seldom break down only carbohydrates. These foods are products of microbial and enzymatic actions affecting many food constituents including fats, carbohydrates, proteins, and many others, each of which contributes to the final product.

Most forms of spoilage are undesirable. Conversely, fermentative spoilage produces favorable benefits of increased shelf life and added flavor. This beneficial spoilage is encouraged by the promotional growth of selected microorganisms for their unique end products and metabolic processes. Most forms of food preservation try to reduce or inhibit growth of microorganisms. Essentially, fermentation is a controlled spoilage using many variables such as atmosphere, temperature, salt content, and starter cultures.

Fermented foods can also be microbiologically safe as well as more nutritious. Many organisms used for fermentation produce acid as a by-product of their metabolic processes. As these organisms increase in number, the amount of acid produced increases, ultimately accumulating in the food and lowering the total pH of the product. Many pathogenic bacteria such as *Clostridium botulinum* cannot proliferate in low acid environments; therefore, the product remains microbiologically safe for extended periods of time. These same organisms can synthesize growth factors and vitamins, release nutrients from indigestible materials, and enzymatically break down indigestible sugars such as hemicellulose into usable sugar derivatives.

A list of several common fermented products, their substrates, and the microorganisms involved can be found in Table 50.4. One classic fermented product is beer. In this fermentation carbohydrates are broken down by the yeast *Saccharomyces cerevisae*, producing alcohol and carbon dioxide as by-products. Dairy fermentations are also very common whereby cheeses, yogurt, buttermilk, and sour cream are produced. For most common cheeses such as blue, cheddar, cottage, and gouda as well as buttermilk and sour cream *Lactococcus lactis* subsp. *cremoris/lactis* are the primary acid producers with a wide variety of secondary microflora.

Vegetable fermentation is also a major industry for food production. Cabbage (sauerkraut), cucumbers (pickles), olives (green olives), and soybeans (soy sauce) are but a few examples of vegetable fermentation. The three major types of vegetable fermentation include lactic acid,

TABLE 50.4
Fermented Food Products

Fermentation Product	Substrate	Microorganism
Cocoa beans	Cacao fruit	*Candida krusei*, *Geotrichum* spp.
Coffee beans	Coffee cherries	*Erwinia dissolvens*, *Saccharomyces* spp.
Miso	Soybeans	*Aspergillus oryzae*
Olives	Green olives	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*
Pickles	Cucumbers	*Lactobacillus plantarum*
Sauerkraut	Cabbage	*Leuconostoc mesenteroides*
Soy sauce	Soybeans	*Rhizopus oligosporus*
Beer and ale	Cereal wort	*Saccharomyces cerevisae*
Bourbon whiskey	Corn, rye	*Saccharomyces cerevisae*
Cider	Apples	*Saccharomyces* spp.
Sake	Rice	*Saccharomyces sake*
Vinegar	Cider, wines	*Acetobacter* spp.
Wines	Grapes	*Saccharomyces* spp.

acetic acid, and alcoholic fermentation. Lactic acid may be produced in fermented vegetables by species including *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus bavaricus*, and *Lactobacillus casei*. Other bacterial species, including *Acetobacter hansenii* and *Gluconobacter oxydans*, may produce acetic acid or vinegar during fermentation. Fermentations that result in alcoholic products may be produced by microbial species including *Saccharomyces cerevisae*, *Kluveromyces marxianus*, and *Aspergillus oryzae*. Meat, fish, and poultry can also be fermented to produce desirable products. For example, meats can be fermented to produce products such as pepperoni and sausage. Although the origins of fermentation may be ancient, the processes are still used today to provide a rich variety of safe and shelf stable foods.

XI. OTHER USES OF MICROORGANISMS IN FOOD PRODUCTION AND PROCESSING

Many bacteria are beneficial and essential to human health. At any time numbers of bacteria on and in a human body outnumber human body cells. Areas that contain consistently large numbers of microflora are the nose, throat, and gastrointestinal tract. When certain amounts of these beneficial bacteria, typically in the live state, are consumed or applied certain health benefits may be experienced. Generally, these microbial cocktails may contain one or many different strains in addition to viable cells, dead cells, and/or certain cellular components, and are loosely defined as probiotics. Prebiotics are indigestible components that influence certain numbers of targeted bacteria in the colon stimulating their activity and/or growth to benefit the host. Synbiotics are prebiotic and probiotic components combined.

Some dairy products contain probiotic bacteria, most commonly from the genera *Bifidobacterium* and *Lactobacillus.* When these harmless types of bacteria establish themselves in the gastrointestinal tract they occupy available space, thereby aiding in the inhibition of colonization of pathogenic bacteria. This concept has been expanded to an on-farm strategy called competitive exclusion. Young animals are given cocktails of nonpathogenic bacteria at a young age to colonize the gut in an attempt to prevent the infection of certain pathogenic strains. It has been successfully achieved in poultry with a combination of 29 species of bacteria and proven effective in cattle with one *Proteus mirabilis* strain and 17 strains of *E. coli*. In addition to reduced numbers of pathogenically infected animals, these treatments allow for faster growth and weight gain. If further developed, probiotic use may ultimately reduce the need for extensive antibiotic treatments for farm animals and aid in the reduction of the appearance of antibiotic-resistant strains of bacteria.

All the benefits from probiotic treatments are not currently known; however, successful treatments do increase the immune response and disrupt metabolic pathways for detrimental flora in the gastrointestinal tract. It is difficult to draw conclusions because key aspects of the studies (i.e., type and amount of cultures used, initial participant health, age, and resident flora) all vary greatly. As we learn more about the intricate relationship between nonpathogenic, pathogenic bacteria, and host response, this new technology will provide a healthier food system.

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51 Microbial Food Spoilage

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CONTENTS

I. INTRODUCTION

Descriptions of the potentially negative influence of microorganisms on food quality often focus on describing potential disease-causing organisms. Typically, many reviews or books on food microbiology contain many sections or chapters describing different pathogenic agents, and often spoilage of all foods is dealt with in a briefer manner. Although microbial spoilage of foods may somehow not be as spectacular as the intriguing behaviour of pathogenic agents, the problem must not be underestimated. Thus, microbial growth and metabolism, which is the major cause of *post-harvest* spoilage of foods, causes an estimated loss of 10–50% of all raw agricultural commodities produced globally (1). In some cases (fruits, vegetables, nuts), spoilage processes may also be initiated *pre-harvest*.

In the context of the present chapter, spoilage will describe changes in a product which render it unaccceptable for the consumer from a sensory perspective. Spoilage is product specific; for instance, formation of ammonia is a sign of spoilage in fresh, chill-stored fish but may be a desired quality in Camembert cheese.

Reactions or events other than microbial growth may lead to spoilage (sensory rejection). This includes phenomena such as lipid oxidation or discolouration, which will not be dealt with in this chapter.

II. CONCEPTS IN MICROBIAL FOOD SPOILAGE

Foods constitute a range of different ecosystems. Some foods are "alive" (fruits, vegetables, grains, nuts) and contain

defense systems which microorganisms need to overcome when growing. Other "dead" foods (meat, fish) are typically preserved by manipulating the physical and chemical environment by cooling, acidifying, salting, etc. (2). Microbial spoilage is almost always a consequence of growth of the spoiling organism to high numbers. Hence, a primary assessment of the microorganisms potentially involved in spoilage of a particular product can be done by matching the growth characteristics of different microorganisms with the preserving characteristics of the product. Although this will not identify the exact cause of spoilage, it will narrow the spectrum of potential culprits.

Microbial spoilage is detectable in many forms: as gas formation causing bombage, as slime formation due to production of extracellular polymers or the degradation of food matrixes, as visible colonial growth, or as off-flavours and off-odours arising from the microbial metabolism of food components. Mostly, growth of microorganisms to high numbers ($>10^5$ yeast cfu/g or $>10^7$–10^8 bacteria cfu/g) is required for spoilage to be noticable. In a few products, enzymes produced by microorganisms may remain active after inactivation of the microorganisms and cause spoilage during storage.

The microbiota on raw foods is very varied but a few species typically become dominant during storage. These are the ones capable of tolerating the specific preservation conditions used. The microbiota on freshly processed foods is also heterogenous and is a function of the microorganisms found on raw materials, on ingredients, and in the processing environment. Logically, the food processing steps, such as heat treatment or acidification, also have a major influence.

The concepts in microbial food spoilage have been outlined (3): the microbiota present when the product is spoiled is called the spoilage association or the spoilage microbiota. To determine the importance of the different microorganisms in the spoilage process, pure cultures are isolated from spoiled foods and tested for their ability to produce the sensory (and chemical) changes associated with the naturally spoiling product. Microorganisms with these abilities are said to posess a spoilage potential. However, it is crucial for the understanding of the spoilage process that quantitative considerations are included. Hence, for each organism with a spoilage potential, it must be evaluated if it is capable of growing in the "spoiling" product and if it reaches cell numbers in the spoiling product that are sufficient for the sensory and chemical changes to take place. Organisms that both qualitatively and quantitatively are linked to the spoiling product are said to have a spoilage activity. Verifying that these organisms are indeed the causative agents of spoilage requires trials with food systems. Thus, the identification of specific spoilage organism(s) of a product will require a combination of sensory, chemical, and microbiological analyses (4). The term spoilage domain is used to describe the range of conditions under which a specific spoilage organism is capable of influencing the spoilage process.

Using such approaches the specific spoilage microorganisms of several food products have been determined (Table 51.1). Gram-negative psychrotrophic bacteria such as pseudomonads are important spoilage agents of most fresh foods. As preservation "pressure" increases, e.g., by adding salt and acid, lactic acid bacteria and other fermentative organisms take over. Filamentous fungi and yeast are the spoilage agents of many products with low water activity or products such as citrus fruits where the fungi tolerate the specific defense components of the food (5). Although the same organisms may be the spoiling agent of different food products, the reactions leading to spoilage can be very different. Thus, pseudomonads are the main spoilage agents of fresh, chilled, aerobically packed meat where they degrade amino acids producing spoilage off-odours (4). In vegetables, where *Pseudomonas* spp. are also identified as one of the main spoilage organisms, they typically degrade the polymer (pectin) by pectinolytic acitivity, leaving a soft rotting liquified product (6).

Different organisms may, as single species, be the cause of spoilage. Hence, both Enterobacteriaceae (mostly *Erwinia* species) and *Pseudomonas* spp. may cause soft rot of vegetables. In some products, it may be difficult identifying one organism as the specific agent of spoilage and here mixtures of organisms may be responsible for spoilage. Examples of such metabiotic relationships include the combination of Enterobacteriaceae and lactic acid bacteria, which in vacuum-packed meat or cold-smoked salmon only produce spoilage metabolites similar to the spoiling products when both groups are present (7–10).

III. EXAMPLES OF SPECIFIC SPOILAGE ORGANISMS

Although many different types of microorganisms grow in different food products, several groups or species often are involved in the spoilage process. The sections below provide examples of the involvement of different microorganisms in food spoilage.

A. Pseudomonads, *Shewanella*, *Photobacterium*, *Pseudoalteromonas*

The psychrotrophic, Gram-negative, non-fermentative bacteria belonging to *Pseudomonas* spp. or identified as *Shewanella* spp. are very often identified as specific spoilage organisms. *Pseudomonas* spp. are the spoilage agents of vegetables where they degrade pectin (6), of aerobically packed meat where they degrade amino acids to volatile compounds such as sulfides (4), and of aerobically stored iced freshwater fish (11) and iced crustaceans where they produce esters and sulphydryl compounds (12). Also, extracellular hydrolytic enzymes of

TABLE 51.1
Typical Spoilage Organisms of Food Products

Organism	Vegetables	Chilled Meat			Chilled Fish			Milk	Flavoured Yogurt	Egg	Fruit	Soft Drinks	Beer	Bakery Goods	Oil-Based
		Fresh	Packed	Cured	Fresh	Packed	Cured								
Pseudomonas	+	+			+			+		+					
Shewanella					+	+									
Photobacterium						+	+								
Enterobacteriaceae	+		+	+			+	+							
Lactic acid bacteria			+	+			+						+		+
Leuconostoc														+	
Brochothrix				+											
Bacillus								+							
Clostridium			+												
Acyclobacillus															
Anaerobes													+		
Halophiles															
Yeasts							+		+			+	+	+	+
Filamentous fungi	+										+	+		+	

pseudomonads may be the cause of milk spoilage where lipolytic and proteolytic activity contributes to off-flavour formation and clotting (13). *Pseudomonas* spp. are also the main cause of rotting in eggs (14) where they cause pink rot (*P. fluorescens*) or fluorescent green rot (*P. putida*) (15). *Pseudomonas* spp. are strict respiratory organisms and are most important in products where oxygen is available.

Psychrotolerant *Shewanella* species are often involved in spoilage of marine products. Their ability to reduce the odourless trimethyl amine oxide (TMAO) to trimethylamine (TMA), which has a fishy smell, and to produce hydrogen sulphide involves them in spoilage of iced marine fish and shellfish. Psychrotrophic *Shewanella* spp. are sensitive to low pH and are not normally associated with spoilage of meat. However, they have been identified as a cause of off-odour in high-pH meat (16) and in chicken (17). The H_2S formation by *Shewanella* spp. in high-pH meat may also cause greening of the meat. Although strictly respiratory, *Shewanella* spp. are capable of utilizing several compounds as electron acceptors in an anaerobic respiration and they are therefore also associated with spoilage of vacuum-packed foods. The type strain of *Shewanella putrefaciens* was originally isolated from tainted butter (18).

In CO_2-packed marine fish, respiratory bacteria are inhibited and a marine, psychrotolerant bacterium *Photobacterium phosphoreum* is selected (19). The organism belongs to the Vibrionaceae family and produces large amounts of TMA (19).

Recently, another marine bacterium, *Pseudoalteromonas,* was identified as part of the spoilage microbiota of iced squid (20). These bacteria are capable of degrading urea, which occurs in high concentrations in squid, and they may be contributing to spoilage through ammonia formation.

B. Enterobacteriaceae

In classical food microbiology, members of the Enterobacteriaceae family are enteric mesophilic organisms and their presence in food products is an indication of hygienic failure. However, the family also covers psychrotrophic environmental strains that may occur in the environment of food raw materials and that may grow on chill-stored products. *Erwinia* species are common spoilage organisms of vegetables where, due to pectinolytic and proteolytic activity, they cause extensive soft rot spoilage (21, 22). Organisms like *Serratia*, *Enterobacter*, *Rahnella*, and *Hafnia* are often isolated in high numbers from fresh proteinaceous products such as milk, meat, or fish. Enterobacteraiceae may spoil liquid milk products during prolonged storage (23, 24) due to formation of off-odours or proteases causing "sweet curdling." Their interactive behaviour with lactic acid bacteria has been suggested to play a role in the spoilage process of vacuum-packed meat (7–9) and cold-smoked salmon (10).

C. Lactic Acid Bacteria and *Brochothrix*

Lactic acid bacteria (LAB) covers a conglomerate of many bacteria. LAB grow well under vacuum-packing and have a relatively high tolerance towards salt and acid. Hence, they must be considered when evaluating spoilage of packed and/or preserved products. Yeasts, as will be discussed below, tolerate many of the same preservation parameters and are often selected in the same products where LAB dominate. Many of the LAB have no effect on food quality and, for instance, some *Carnobacterium* spp. may grow to numbers of 10^8–10^9 cfu/g with no adverse effects on quality (25). Some may even have a preserving effect on the product. However, some species are potent spoilage organisms producing sour off-odours (26, 27), gas (28, 29), or exopolysaccharides (30, 31). Some species (e.g., *L. saké* or *L. curvatus*) are able to produce hydrogen sulphide (32, 33), causing sulphorous off-odours. The green discolouration which may sometimes be seen on meats can be caused by LAB due to hydrogen peroxide oxidation of meat pigment (33). Greening may also be explained by the formation of sulfmyoglobin caused by Gram-negative H_2S-producing bacteria, typically *Shewanella* (34). LAB have also been identified as spoilage organisms of beer and soft drinks. Some species of LAB, such as *Lactobacillus curvatus* and *Lactobacillus saké*, are potent producers of hydrogen sulphide whereas other species produce acid and/or CO_2 in large amounts, causing souring or bombage of the product. Some homofermentative *Lactobacillus* may also form so-called ropy slime due to exopolymer production (9).

If allowed to grow to high numbers, *Leuconostoc* species are often associated with spoilage. They may produce off-odours and off-flavours as well as bombage. However, their most prominent characteristic is their ability to produce slime (exo-polysaccharides), which can be a spoilage sign of cured meat products (9, 35).

Brochothrix thermosphacta is a Gram-positive, catalase-negative bacterium which is capable of growing in chilled, packed products. In vacuum-packed, high-pH meat, *B. thermosphacta* is able to grow (36) and its metabolism (carbohydrate fermentation and lipolytic ability) gives rise to compounds like acetoin and diacetyl, both of which are off-odourous. Acetoin requires glucose as substrate whereas other carbohydrates may be metabolized to, e.g., diacetyl (37). *B. thermosphacta* is also typical of spoilage of cured, packed meat products where it grows to high numbers producing off-odours and then dies off as *Lactobacillus* species become dominant (38). Although *B. thermosphacta* can often be isolated from aerobic packed meat, pseudomonads will eventually dominate and spoil the product (39). In contrast, *B. thermosphacta* is often isolated from meats packed in CO_2-containing atmosphere (40) but does require a limited amount of oxygen to produce spoilage off-odours (41).

D. Sporeformers

Gram-negative, spore-forming organisms, *Clostridium* and *Bacillus*, have been identified as the specific spoilage organisms of several food commodities. Clostridia are typically involved in spoilage of packed (anaerobic) products due to their intolerance to oxygen, whereas *Bacillus* spp. tolerate oxygen and can grow also in non-packed products. Psychrotrophic clostridia have been involved in spoilage of chilled, vacuum-packed raw or cooked meats. Spoilage of vacuum-packed meat may sometimes present itself as a "blown pack" and *Clostridium* species have been identified as the causative agent (42, 43). Several clostridia produce both CO_2, hydrogen, or butyric acid, which can explain the blowing (43) but CO_2 and hydrogen are probably the main causes (44). It appears that several different psychrotrophic clostridia may cause spoilage, including *Cl. gasigenes, Cl. estertheticum* (45), and *Cl. frigidicarnis* (46). Similarly, outgrowth of *Cl. butyricum* can cause so-called late-blowing of cheese (especially hard cheeses) due to fermentation of lactate (47). Sugar-salted, barrel-stored herring may spoil due to growth of clostridial species (48).

Bacillus species spoil different food products which typically have been heat treated as part of processing; hence, spores survive and grow. "Rope" formation in bread is caused by growth of *Bacillus* (49) and "sweet curdling," e.g., clotting, of milk can be caused by *Bacillus* where exoproteases degrade the casein micelles of the milk (50). "Ropiness" is caused by the enzymatic degradation of the bread crumbs and the parallel production of exopolysaccharides by the bacilli (51, 52). *Bacillus stearothermophilus* is a thermophilic organism which may cause a so-called flat-sour spoilage of evaporated milk (53) or some canned products (54).

A unique kind of spoilage has been found in heat-treated fruit juices, where an acid-requiring, spore-forming organism, *Alicyclobacillus acidoterrestris*, has been identified as the cause of a disinfectant taint (2,6 dibromophenol and 2,6 dichlorophenol) (55, 56). The kinetics of spoilage are not completely understood as it appears that very low levels of spores are sufficient to cause spoilage (55).

E. Yeasts

Yeasts are involved in spoilage of a wide array of food products where they are detected due to visible colonial growth, the formation of gas, or production of off-odour (57). A range of pickled products (mayonnaises, ketchups, salad dressings) are preserved with low pH and, often, addition of preservatives. This eliminates most microorganisms but lactic acid bacteria and yeasts may grow. Yeast cells are typically much larger than bacterial cells and hence a lower cell number is required to cause spoilage. Several of the *Zygosaccharomyces* yeasts in particular, and some isolates of *Saccharomyces cerevisiae* are spoilage agents in foods high in sugar, low in pH, and/or containing food preservatives. Also, alcohol-containing products are prone to yeast spoilage. The most important are *Z. rouxii, Z. bailii*, and *Z. bisporus* (58). Foods with low a_w (e.g., high in sugar) are often spoiled by *Z. rouxii* or *Z. mellis* which has been identified in spoiled bakery products (59) and sugars and syrups.

Zygosaccharomyces bailii and, to some extent also *Z. bisporus*, have been involved in spoilage of mayonnaises, ketchups, and salad dressings. *Z. bailii* grows at low pH in acetic acid and salt (60) and may adapt to food preservatives such as sorbate and benzoate. *Z. bailii* is also associated with wine spoilage. At lower temperatures *Z. lentus* is also a spoilage agent in preserved products (61).

Saccharomyces cerevisiae are commonly isolated as wild yeasts in breweries and are potential spoilage organisms (62, 63). Fermented foods may spoil due to yeast metabolism of lactate and acetate or the production of CO_2 and ethanol. Several *Saccharomyces* species have been isolated from spoiled fermented products (64).

Various species of yeast (*Candida*, *Yarrowia*) have been detected at levels up to 10^5 cfu/g in chilled spoiling poultry; however, a role in spoilage has not been elucidated (65, 66).

F. Filamentous Fungi

Filamentous fungi (moulds) may grow in many different types of food products and it is estimated that 5–10% of foods are lost *post-harvest* due to growth of filamentous fungi (2). They may affect sensory quality of the products in several ways: through visible filamentous growth, through production of exo-enzymes which degrade the product, or through production of small off-odourous compounds, such as geosmin (5). They may be involved in spoilage of a very broad range of food commodities but are commonly involved in spoilage of low water activity products (grains, nuts, dried fish, cheeses, fermented meats, bread and bakery products) and in spoilage of fruits and vegetables. Although the production of mycotoxins by filamentous fungi is a food safety issue, companies encountering food spoilage often wish to determine whether mycotoxin production may also have occurred. This often requires specialist advice (5, 67).

Foods spoiled by filamentous fungi are divided into two categories: living products such as fresh fruits and vegetables, cereals and nuts before harvest, and stored, processed, or preserved products (i.e., dried cereals, nuts, meat and fish, bread, bakery products, cheese, juice and beverages) (2).

Living plant foods rely on natural defense mechanisms to prevent microbial growth. Fruits have skins which require penetration, while cereals and nuts may produce phytoalexins or other natural antifungal material. Fresh animal foods are more liable to spoilage by bacteria than fungi. Stored processed and dried products must rely

on classical food preservation techniques such as drying of cereals, nuts, meat, and fish, or heat processing and/or the use of preservatives for juices and beverages. The genera *Aspergillus, Penicillium, Fusarium, Alternaria, Botrytis, Rhizopus*, and *Eurotium* are common causes of food spoilage. Although many species within each genera may have a spoilage potential, only 2–12 species seem to be important in spoilage (5) and for many types of living food, only 1–2 species are of major importance. For instance, some fungi (e.g., *Rhizopus*) have specific enzymes allowing skin penetration whereas others (e.g., *Pencillium*) have mechanisms for overcoming the specific antifungal defenses (2). Although many fungi may potentially spoil citrus fruits, the primary spoilage fungi are *Pen. italicum* and *Pen. digitatum* (2, 5).

An overview of the spoilage mycobiota of different foods is presented in Table 51.2.

G. Others

Several of the groups/species of spoilage organisms described above are involved in spoilage of several foods or products. However, in some products it appears that one single species appears and is involved in spoilage of only one specific product. This may of course just be a matter of understanding the ecology and metabolism of a particular organism to predict if it will have spoilage activity in other products. One such example is the spoilage of flavoured bottled water by species of the aerobic Gram-negative bacteria, *Gluconobacter*, which produces sour off-odours (68, 69). This organism is closely related to the acetic acid bacteria and proliferates at very low pH values (e.g., pH 3.5) (69), hence being adapted to these particular products.

IV. SPOILAGE PATTERNS OF FOODS

A summary of examples of typical spoilage substrates and metabolites is presented in Table 51.3.

A. Vegetables

Vegetables have a high water content (80–90%) and their texture is built by pectins or other carbohydrate polymers such as starch. They typically contain low amounts of protein and simple carbohydrates and many are rich in pigments. pH is around 6 (5.5–7). In some countries, vegetables are distributed at chill temperatures. Increasingly, vegetables are sold processed into ready-to-eat salads (70). Spoilage of vegetables is primarily caused by microorganisms capable of degrading the polymers of the product. The main spoiling organisms are *Pseudomonas* spp., Enterobacteriaceae (mostly *Erwinia*), and filamentous fungi. The bacterial spoilage is evident when high numbers ($>10^8$ cfu/g) are reached. Typically, these organisms are pectinolytic and hence cause softening of the tissue, known as soft-rot. Also, during degradation off-flavours and off-odours are formed. In some bacteria, spoilage is enhanced by proteolytic capability (22), probably because proteolysis causes an increase in pH and some pectate lyases have a higher activity at high pH (8–9) (71).

The expression of hydrolytic enzymes (proteases, pectinases, cellulases) in the plant-pathogenic *Erwinia carotovora* subsp. *carotovora* is regulated in a quorum

TABLE 51.2
Associated Spoilage Mycobiota of Different Foods (Modified from References 2, 5)

	Genera Involved in Spoilage							
Product	***Penicillium***	***Alternaria***	***Fusarium***	***Cladosporium***	***Aspergillus***	***Eurotium***	***Botrytis***	***Rhizopus***
Citrus fruits	+	+						
Pome fruits	+						+	
Stone fruits		+						+
Onions	+				+			
Leafy green vegetables		+					+	+
Potato tubers			+					
Yam tubers	+							
Grain, in field		+	+					
Grain, stored	+				+	+		
Maize			+					
Peanuts					+			
Rye bread	+					+		
Cheese	+			+				
Processed meat products	+					+		
Salted, dried fish					+			
Jams	+					+		

TABLE 51.3
Examples of Typical Spoilage Substrates and Metabolites Found in Microbiologically Spoiled Foods (160)

Sensory Impression	Spoilage Product	Spoilage Substrate	Food Product	Specific Spoilage Organism	Reference
Slime	EPS (dextran)	Sucrose	Kimchi	*Leuconostoc*	31
			Turkey breast	*Leuconostoc*	35
		Sugars	Wine	*Pediococcus damnosus*	29
		Sugars	Bread	*Bacillus*	49
Slime	Hydrolysed polymer	Pectin	Vegetables	*Erwinia, Pseudomonas*	70
Fishy off-odour	Trimethylamine (TMA)	Trimethylamine oxide (TMAO)	Fish	*Shewanella putrefaciens*	161
				P. phosphoresum	19
				Aeromonas spp.	96
Ammonia, putrid	NH_3	Amino acids	Proteinaceous foods	Many microorganisms	
	Biogenic amines	Amino acids	Meat	*Enterobacteriaceae* and LAB	7
			Fish[1]	*Enterobacteriaceae*, LAB	10
			Fish[1]	*P. phosphoreum*	10
Sulhidy off-odour	H_2S	Cysteine	Fish, meat	*S. putrefaciens*	98, 162
				Enterobacteriaceae	4
				Lb. saké, Lb. curvatus	
Greening	H_2S	Cysteine	Meat	*Lb. plantarum*	33
Sulphydryl off-odours	$(CH_3)_2S_2$	Methionine	Fish, meat	*Pseudomonas* spp.	163
				Enterobacteriaceae	164
Acid off-odour	Acetic acid L,D-lactic acid	Glucose, ribose, other CHO	Meat	LAB	165
"Sweet curdling"	Proteinaceus fat particles	Phospholipid	Milk	*Bacillus cereus*	166
Fruity off-odour	Esters		Fish	*Ps. fragi*	167
			Milk	*Ps. fragi*	84
				Ps. putida and *Y. intermedia*	85
Cheesey off-odour	Acetoin, diacetyl, 3-methylbutanoyl	Glucose	Meat	*B. thermosphacta*	4
				Enterobacteriaceae	
				Homofermentative LAB	
Medicine off-odour	2-methoxy-phenol, sediment	Sugars	Juice	*Alicyclobacillus acidoterrestris*	55
Musty odour	Trichloroanisol	2,4,6 Trichlorophenol	Wine	*Penicillium brevicompactum*	5
				Aspergillus flavus	

[1] Biogenic amines may not be the cause of spoilage but can serve as a spoilage index (10).

sensing dependant way. The bacterium produces small hormone-like signal molecules (acylated homoserine lactones, AHLs) that allow the bacterial population to sense its own density (72). Expression of hydrolytic enzymes is up-regulated at high densities. Interestingly, the production of pectin-degrading enzymes in an onion spoiling *Burkholderia* (73) and the production of protease and cellulase in a bean sprout spoiling *Serratia* (22) are regulated also by AHLs. Hence, AHL-negative mutants have a lower spoilage activity.

In vegetables high in simple carbohydrates, such as sugar beets which contain high concentrations of sucrose, levan (slime) formation may cause both spoilage and technical problems during further processing (74).

The spoilage of potatoes is caused by different organisms including *Pseudomonas*, *Streptomyces*, and a range of filamentous fungi (75). Spoilage may present itself as soft-rot, dry-rot, wilt, and blight. It may be initiated pre-harvest but the causative organisms can also attack during storage.

B. Fresh Proteinaceous Foods (Milk, Meat, Fish)

Liquid milk consists of water, protein (casein), carbohydrate (lactose), salts, and lipids in varying proportions. Water activity is high, the pH is between 6 and 7 and preservation parameters include pasteurisation and chill storage. Pasteurised liquid milk products undergo different types of spoilage. Typically, spoilage results from action of *Bacillus*, *Pseudomonas*, or Enterobacteriaceae (76, 77) and is evident when high numbers ($> 10^7$ cfu/ml) are reached (13). Sometimes, degradative enzymes which survive pasteurisation cause spoilage during storage with no bacterial growth (78). Spoilage by *Pseudomonas* and Enterobacteriaceae is the result of post-pasteurisation contamination (79, 80) whereas spores of *Bacillus* may survive pasteurisation and grow during storage. Several types of psychrotrophic *Bacillus* have been involved in spoilage of chill-stored, liquid milk (81, 82). At normal chill storage temperatures, *Pseudomonas* spp. will typically become the dominant

spoilage organism (83). Spoilage is characterized by bitter, sour, or fruity off-flavours (76) and/or by clotting ("sweet curdling"). The bitter off-flavours can be caused by proteolytic action of pseudomonads (78) and protelytic degradation of the casein-micelles leads to clotting of milk. Rancid off-flavours may be caused by lipoytic activity of, for instance, *Pseudomonas* spp. (13), whereas fruity off-flavours arise from esters producted by *Pseudomonas* spp. or Enterobacteriaceae (84, 85).

Water is the main constituent of red meat and poultry. It contains protein, lipid, and several non-protein nitrogen-containing compounds such as amino acids and creatine. Carbohydrates are present as glycogen, glucose, and lactate. In non-stressed animals, pH will decrease significantly (to approx. 5.5) in red meat *post mortem* due to glycogen metabolism. Glycogen depots are depleted in stressed animals *pre mortem* and pH after slaughter reaches 6.0–6.5. These differences can have a profound impact on the spoilage microbiology of vacuum-packed meats since the hydrogen sulphide-producing bacteria *Shewanella putrefaciens* may grow in packed, high-pH meat and cause rapid spoilage (4). The *Shewanella* spoilage is characterised by sulphidy off-odours and H_2S may cause greening of the meat due to conversion of myoglobin to sulfmyoglobin (33, 34). *S. putrefaciens* will not grow at pH 5.0–5.5 and hence packed, normal pH meat has a much longer shelf life. Greening of cured meat is caused by lactic acid bacteria (86) where H_2O_2 converts myoglobin to a green iron (III) complex (87).

"Meat" covers muscle tissue from a range of different animals: beef, veal, lamb, poultry (fatty, lean). Whole meat is typically more stable than ground meat. In aerobic atmosphere, whole meat has a shelf life of approximately 1 week at 5°C whereas ground meat will spoil in 1–2 days depending on the initial bacterial count. The bacterial counts of ground meats are, in general, higher than on non-comminuted meats. Typically, gound meat contains trimmings and has a much greater surface-to-volume ratio allowing a more rapid spoilage (88). Packaging extends shelf life and the atmosphere of packaging will have a marked influence on the extension. Vacuum-packing can extend storage life of beef (held at 0°C) from 2–4 weeks to more than 15 (41). Packing in 100% CO_2 may extend shelf life at 4–5°C to 8–12 weeks (4). Many packaging atmospheres do contain oxygen as this allows retainment of the red oxygenated myoglobin colour.

The microflora on fresh meat is a mixture of many different species originating from hides, environment, and the gastro-intestinal tract. During aerobic storage, psychrotrophic pseudomonads become dominant (4). After 1–2 weeks, counts of 10^8–10^9 are reached and spoilage is detectable as slime and off-odours. Vacuum-packing or CO_2-packing (in O_2-impermeable films) eliminate pseudomonads and the microflora becomes dominated by lactic acid bacteria belonging to *Lactobacillus, Leuconostoc*, and *Carnobacterium* (35, 89). Enterobacteriaceae and *Brochothrix thermosphacta* are often present in quite high proportions reaching 10^3–10^7 cfu/g (4, 9). Depending on the level of oxygen addition to the modified atmosphere packaging, shelf life (at 5°C) may be increased to 3–12 weeks.

Metabolism of glucose and amino acids supports bacterial growth. Off-odours in aerobically packed meat are not detected as long as glucose is metabolised; however, off-odours and spoilage of aerobically packed meat become evident when glucose is depleted and amino acids are metabolised (90). During glucose metabolism, the sweet, fruity off-odours of incipient spoilage are caused by ethyl esters produced by *Ps. fragi*. Degradation of amino acids is the cause of production of sulphur-containing compounds giving the putrid off-flavours in the advanced stages of spoilage.

Packed meats are typically characterised by sour off-odours being produced by the dominant lactic acid bacteria. The so-called cheesey off-odour often detected in vacuum-packed meat products is caused by acetoin and di-acetyl which are produced by *Br. thermosphacta* in packed (high pH) meats (4, 9).

Fresh fish is rich in water, protein and, for some species, lipids. Fish muscle also contains free amino acids, nucleotides and, in some species, trimethylamine oxide (TMAO) (11). TMAO, which is an odourless compound, is typically accumulated in marine fish species, especially the gadoid species, but may also be detected in fish from fresh waters (91). Some bacteria can use the oxygen in TMAO in anaerobic respiration and this reduction results in the formation of trimethylamine (TMA) which has the characteristic "fishy" flavour. As opposed to meat from mammalian animals, fish do not accumulate glycogen and pH therefore does not decrease *post mortem*. As in high-pH meat, this allows psychrotolerant *Shewanella* to grow during storage. Fish are cold-blooded animals and the microorganisms on newly caught fish from temperate waters are adapted to low temperatures. This combination of several factors explains why fish is a very perishable product and should be stored at low temperatures, preferably in melting ice (0°C). The shelf life of iced fish varies from 2 weeks (cod-like fish) to 4 weeks (fish from tropical waters). Some fatty fish species such as herring and mackerel have short shelf lives if left unpacked due to lipid oxidation and development of rancid off-flavours.

During storage of fish in ice, psychrotrophic pseudomonads, shewanellae, and *Neisseria* species become dominant (92–95). *Pseudomonas* spp. spoil freshwater fish when counts reach 10^8–10^9 cfu/g (96). Spoilage is typically characterised by sweet, sulphydryl, rotten off-odours. In contrast, the sensory impression of spoilage of marine fish is characterised as putrid, rotten, fishy due to formation of TMA and H_2S by psychrotrophic *Shewanella* species (97, 98). The off-odourous compounds are typically formed by

bacterial metabolism of low-molecular-weight compounds (99). Spoilage proceeds very rapidly (12–48 hours) if fish is left at ambient temperature and mesophilic *Vibrio* and *Aeromonas* species are causing the spoilage. Packing of fish from marine waters causes selection of the CO_2-resistant, psychrotolerant *Photobacterium phosphoreum* which is the dominat spoilage organism of CO_2 packed fish (19, 100). Fish from fresh waters and fish from tropical waters probably do not harbour this organism and organisms such as lactic acid bacteria become dominant in these products.

C. Cured or Processed Proteinaceous Foods

A wide range of "cured" milk products are produced world-wide. This covers yogurts, soft unripened cheeses, and an enormous range of hard or ripened soft cheeses. Some of these products are acidified by fermenting microorganisms (mostly lactic acid bacteria); in others the curdling is caused by addition of protelytic enzymes (rennet) which degrade the casein-micelles and allow a curd to be formed. Pasteurization, acidification, and, often, chilling, are effective preservation hurdles; however, spoilage of the acidified products can be caused by yeasts and filamentous fungi. In particular flavoured yogurts, where sugar/fruit is added, are good substrates for yeast spoilage organisms (101, 102) and spoiling filamentous fungi (103).

The most important spoilage organisms of hard, semi-hard, and soft cheeses are the filamentous fungi; especially *Pencicillium* species (5, 104, 105). In some cheeses, the development of fungi is part of the processing; however, growth of other filamentous fungi is a sign of spoilage. Fresh, unripened cheeses such as cottage cheese, may also spoil because of bacterial growth and especially *Pseudomonas* spp. and psychrotrophic Enterobacteriaceae produce visible colonies on the surface and cause development of off-odours (106). Bacterial gas formation may be the cause of spoilage of cheeses. So-called "early blowing" can be caused by a range of bacteria producing CO_2 from lactose (107). "Late blowing" is specifically caused by growth and production of butyric acid by clostridia during storage and ripening of hard cheeses (47).

Meat is processed/cured to produce an enormous range of products including bacon, sausages, cooked and sliced product. Raw cured products (ham, fermented sausages) are preserved to an extent where bacteria do not grow well and hence spoilage is typically caused by yeasts or filamentous fungi. Bacterial spoilage by Enterobacteraiceae or clostridia may occur during processing before the salt concentration is high enough (108, 109). Cooked, perishable products include frankfurters and luncheon meat products. *Pseudomonas* are the main spoilage agent of such product-stored aerobically, whereas vacuum or modified atmosphere packaging selects for the same types of organisms as described under fresh meats. The spoilage organisms typically include lactic acid bacteria, *Br. thermosphacta* and Enterobacteriaceae (9, 90, 110). Spoilage may be detectable as slime formation (111) as sour off-odours (26), or as the cheesey off-odours produced by *Br. thermosphacta*.

As meat products, fish are preserved using salting, acidifying, and heat treatments. Lightly preserved products, e.g., cold-smoked fish, typically spoil due to growth and metabolism of lactic acid bacteria and Gram-negative bacteria, either Enterobacteriaceae or *P. phosphoreum* (112–114). *Br. thermosphacta* also may be detected in levels between 10^3 and 10^7 cfu/g (115) and in pure culture it does produce rotten off-odours (116). Growth and spoilage by Gram-negative bacteria are inhibited in semi-preserved products which are salted, lightly acidified, and kept at chill temperatures. Spoilage is caused by growth of lactic acid bacteria or yeasts and may involve souring or gas formation (28). Packed and heated products which are distributed at chill temperatures may spoil due to growth of Gram-positive, spore-forming organisms (117). Heavily salted fish products may spoil due to growth of strictly halophilic bacteria which cause discolouration (red colour) due to their own pigmentation (118) and rotten off-odours due to protelytic degradation. In heavily salted fish which is re-hydrated, bacterial growth resumes and *Psychrobacter* spp. producing musty odours have been identified as spoilage organisms (119). Shelf life of dried fish species may be limitied by fungal growth (2).

D. Fruits

Fruits contain high amounts of easily digestible sugars and the water content of fruits is high. However, bacteria do not grow well because the pH is low (in the range of 2–5) and most bacteria are inhibited under these conditions, so spoilage is caused by filamentous fungi (120). Spoilage manifests itself in many ways as spots, scabs, soft rot, or dry rot. Cucumber, capsicum, and tomatoes are fruits with a pH closer to neutral and spoilage is also often caused by growth of Gram-negative, pectin-degrading bacteria, (*Pseudomonas* spp. or *Erwinia* spp.) (21, 120).

E. Dressings, Ketchup, and High Fat Products

Foods dealt with in this section are typically highly preserved using mayonnaise-based dressings and/or low levels of water activity and/or high levels of preservatives. Typical examples are coleslaw, ketchup, and various dressings. Most Gram-negative bacteria and many Gram-positive bacteria are effectively inhibited by the preserving parameters, and typical spoilage organisms are lactic acid bacteria and yeasts (121, 122). Their spoilage is detected as sour off-odours or swelling of the product (123). Off-odour or slime formation may also characterise the spoilage (124). Many of these types of product contain acetic acid and

only organisms resistant to this acid are involved in spoilage. Several types of *Lactobacillus* have been isolated from spoiled products (124, 125).

Products such as margarine and butter which are low in water activity and high in fat/lipid content are typically stable products but may spoil due to growth of yeasts or filamentous fungi. Thus, *Penicillium* species have commonly been isolated from spoiled margarine (104). Filamentous fungi (e.g., *Chrysosporium* species) have been the cause of chocolate spoilage (126).

F. Beverages

Fruit juices and most soft drinks are high in sugar and typically have a low pH. Hence, yeasts, fungi, and acid-tolerant bacteria can grow in the products and some will ultimately cause spoilage. *Saccharomyces* and *Zygosaccharomyces* strains are common spoilage organisms (127). Also, some lactic acid bacteria have been identified as spoilers of soft drinks (128) and the acid-tolerant, spore-forming *Alicyclobacillus acidoterrestris* has been detected as the cause of medicinal off-odour in pasteurised fruit juices (55).

Wines are typically preserved by the dominance of the fermenting organism and the subsequent production of alcohol. Lactic acid bacteria and yeasts are the major causes of spoilage (57, 63) although strictly anaerobic Gram-negative bacteria have also been the cause of off-odour (129, 130). Beer spoilage organisms may be wild yeasts present in the brewery (63) and can be transferred to the product where they grow and spoil the product (57). Alcohol and hop components are inhibitory to most bacteria. However, several beer-spoiling microorganisms have the ability to grow in hop which will inhibit most microorganisms (129, 131). Sluggish or stuck wine fermentations have sometimes been linked to growth of lactic acid bacteria which may inhibit growth of the desired fermenting organisms (132). Growth of spoilage microorganisms may cause a range of off-odours or may be visible as slime formation (30).

G. Bakery Goods

Filamentous fungi are the most prominent spoilage organisms of bakery products (2, 5, 133). The combination of their ability to grow at low water activities and the easy spreading of spores in the dry baking environment gives them a selective advantage. Another typical spoilage problem is the so-called ropiness caused by *Bacillus* species, especially *B. subtilis* (49, 134).

V. CHEMICAL ANALYSIS OF SPOILAGE

As defined at the beginning of this chapter, "spoilage" is any change in sensory properties that renders a product unacceptable for the consumer. Sensory methods can be developed to a very high degree of standardization using trained panels (135, 136). Using multivariate statistics, sensory analysis may be combined with chemical analysis to determine which compounds are causing specific odours and flavours. Specific electronic chemical gas sensors (so-called "electronic noses") may be used to profile the odours (137) and so-called "electronic tongues" to detect flavours (138).

Gas chromatographic separation followed by mass spectrometry is often used for profiling odours of spoiled foods. Examples include GC-MS studies of bacterial spoilage of prawns (12), cold-smoked salmon (116, 139), and chilled chicken (140). GC-MS analysis also has been used to determine mycological quality of barley grains (141).

Recently attempts have been made to combine non-destructive chemical methods with statistical analyses of complex patterns derived to correlate with spoilage and/or shelf life. An example of such studies include use of sophisticated techniques to determine well-known indicators of spoilage, such as the use of ion mobility spectrometry to determine biogenic amines (142). Sometimes such compounds are indeed only indicative (143) and may not necessarily be the ones responsible for the off-odours and off-flavours. The volatile compounds developing during spoilage of packed, chilled meat were analysed using a so-called electronic nose sensitive to several volatiles (144). Subsequently, multivariate analyses were used to correlate the magnitude of the "nose signals" with sensory impressions. Whilst this allowed an expression correlated to the quantitative degree of spoilage, no information on individual spoilage components was obtained. A somewhat similar approach was described by Ellis et al. (145) that used Fourier transformed infrared spectroscopy data to evaluate spoilage of meat. Different spectra were found in fresh and spoiling meat and peaks indicative of spoilage were identified.

VI. PRESERVATION STRATEGIES

Food preservation in ancient times relied on experience with treatments that ensured lack of spoilage. Examples are heavily dried or salted products and a range of fermented products. Development of more modern food preservation allowed the use of processes eliminating microorganisms, for example pasteurisation or autoclaving, or processes preventing growth completely, for example by freezing. The trend towards so-called milder preservation techniques typically combines a variety of parameters that allow growth to be controlled but not completely abolished. Examples include packaging, chill-storage, mild salting, etc.

Currently a range of new preservation procedures is under investigation. This includes physical treatments such as high hydrostatic pressure which is a promising non-thermal treatment allowing inactivation similar to

heat treatments (146). The high hydrostatic pressure affects the bacterial membrane (147). Other inactivation processes include high-voltage electrical discharges and high-magnetic-field pulses (148).

The use of live bacterial cultures (149) or the antimicrobial peptides (bacteriocins) produced by lactic acid bacteria (150) has been intensively studied, in particular with the purpose of inhibiting pathogenic bacteria. Also, natural antimicrobial enzyme systems (151) or naturally occurring antimicrobials (152–154) are being tested as food preservatives.

Several biopreservation techniques have been assessed for their inhibition of spoilage microorganisms. For instance, Pepe et al. (52) demonstrated that lactic acid bacteria could inhibit rope-producing *Bacillus* in bread. The delay/control of ropiness was seen both when lactic acid bacteria were added to bread inoculated with *Bacillus* and, more importantly, when lactic acid bacteria were used as a starter during dough preparation. Also, a bacteriocin-producing lactic acid bacterium (*Leconostoc* spp.) allowed control of spoilage of vacuum-packed beef by a sulfide-producing lactic acid bacteria (155).

VII. MODELLING GROWTH OF SPOILAGE MICROORGANISMS AND PREDICTION OF SHELF LIFE

Modern "predictive microbiology" was in some ways born with mathematical descriptions of spoilage as a function of, for instance, temperature (156) but has increasingly focused on determining, modelling, and predicting the growth or survival of food-borne pathogenic organisms as a function of changing environmental factors.

Modelling of growth of spoilage bacteria as a function of environmental and preservative parameters allows (i) determination of the range of products in which the particular organism may be imporant (e.g., will have the ability to grow) and (ii) prediction of remaining shelf life of a particular product in which the number of spoilage organisms can be determined in the fresh product. Results as obtained in (i) may reduce the number of challenge trials required to determine shelf life of new products with altered preservation profiles.

For instance, the growth of the soft-drink spoilage organism *Gluconobacter oxydans* under different pH, acidity, sugar, and benzoate concentrations was determined and modelled (157) allowing the effect of change in each parameter on growth to be quantified. Membre and Kubaczka (158) not only modelled the growth of a vegetable juice spoilage bacterium but also described how the actual spoilage process (kinetics of pectin compound degradation) evolved. Parts of the "seafood spoilage predictor" may exemplify (ii) from above. This software includes models for growth of specific spoilage organisms and allows relative rates of spoilage to be predicted based on temperature profiles (159).

VIII. CONCLUDING REMARKS AND PERSPECTIVES

Our understanding of microbial food spoilage is, for a range of products, very detailed. The organisms, their spoilage metabolites, and the conditions under which spoilage is important are known for several product types. This is particularly true for products where one specific organism is important, for instance the spoilage of packed marine fish (from temperate waters) by *P. phosphoreum*. In contrast, our understanding of more complex spoilage processes where several organisms are involved is less developed. Although we have identified the spoilage microbiota and may have some indication of which organisms contribute to the spoilage, we need to further our understanding of these products. In particular, products where "mild" combinations of preservation parameters are used allow the growth of several organisms. These products are often delicatessen products and understanding their spoilage would allow science-based quality indices to be developed and would allow prediction and labelling with appropriate shelf lives.

The evolution of such understanding will be facilitated and must run in parallel with development of methods for specific enumeration of spoilage microorganisms. Very sensitive methods are needed, especially for shelf life predictions based on numbers of specific spoilage organisms. Finally, the understanding of food spoilage and the spoilage process should enable design of more targeted food preservation methods where only the unwanted organisms or their unwanted metabolism is inhibited.

ACKNOWLEDGMENTS

Comments and suggestions from Jesper B. Bruhn on the complete chapter and from Dr. John Pitt on sections dealing with filamentous fungi have been most helpful.

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52 Microbiology of Land Muscle Foods

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CONTENTS

I. INTRODUCTION

Meat and poultry products are highly perishable foods since they support growth of microorganisms associated with spoilage. In addition, by their very nature and origin they may be implicated in the spread of microbial foodborne diseases. During the past twenty years, the increasing consumer demand for a wider variety of convenient

products of high quality and safety along with the producers' desire for economies of scale in production have led to dramatic changes in the processing, storage, distribution and packaging of meat and poultry products. As a consequence, the important areas of meat and poultry microbiology have also changed. Since the vast subject of meat and poultry microbiology has been discussed extensively in various books, book chapters and review papers (1–5), this chapter focuses on recent research interests and matters of current practice used within the meat and poultry production and distribution chain. The topics include sources, type and extent of microbial contamination including spoilage and pathogenic organisms, microbial changes during storage, methods for controlling microorganisms in meat and meat products, and management of processes for the microbiological safety of these foods.

II. MICROBIAL CONTAMINATION OF MEAT FROM LAND ANIMALS

A. General

In general, the muscle tissues of healthy animals and birds, before slaughter, can be considered sterile. Exceptions to this generalization are the lymph nodes and some organs that may carry limited microbial contamination. In contrast, surfaces of the animal exposed to the environment such as hide, pelt, feathers, fleece, the mouth and the gastrointestinal tract may be heavily contaminated (6–8). These parts of the animal are the major sources of meat and poultry carcass contamination. The extent of contamination transfer from the above sources to the carcass is greatly dependent on the conditions under which animals are reared, slaughtered and processed (4,6–8). Hygienic practices, sanitation procedures, product handling and processing procedures, and conditions of storage and distribution are the most important factors that determine the microbiological quality of the final meat and poultry products. Consequently, variations in facilities, raising methods and practices of slaughtering and processing operations may lead to significant differences in the type and extent of meat contamination (4). Sources of contamination during processing, and the factors affecting the type and extent of contamination are discussed in the following sections.

B. Sources of Microbial Contamination

1. Red Meat

a. Live animal

It is generally agreed that the majority of microorganisms on red meat carcasses originate from the live animals, which also serve as sources of environmental and water contamination. These sources introduce contamination in animal products or other foods through cross-contamination and through contamination of equipment and utensils. Contamination from surfaces exposed to the environment is transferred to the underlying sterile carcass tissue during animal slaughter and dressing. The hide of the animal is the most significant contamination source (7,9). During the raising of cattle, pigs and sheep, large numbers of spoilage and pathogenic microorganisms, mainly originating from soil, pastures and feces, can be found on the hides of the animals. For example, the total microflora of cattle hides may reach 10^{12} cfu/100 cm^2 (10), while an incidence of greater than 10% of *Escherichia coli* O157:H7 has been reported for cattle hides in the U.S. (11). The part of the hide with the highest incidence rate of pathogens is the brisket, followed by the flank and the rump (12). In a similar study, however, the part of the hide with the highest incidence of shiga-toxigenic *E. coli* O157 was found to be the back, followed by the neck, flank, ventrum and hock, in decreasing order (13).

A number of factors associated with the type, prevalence and extent of animal hide contamination have been identified in recent years including climate, season of the year, geographic location, and raising, transportation and holding methods (14). Animals raised in feedlots may carry more bacteria of fecal origin while soil microorganisms are usually more common on animals raised in pastures (4,15). During transport from farm to slaughter, animals may be exposed to further fecal, and therefore, pathogen contamination in the transport vehicles. In addition, animal-to-animal transfer of contamination can occur during transport, either directly via body contact or indirectly via contact with contaminated surfaces. Transportation of cattle from the feedyard to a commercial packing facility was found to result in a 14-fold increase in *Salmonella* prevalence on hides, and a 2-fold increase in fecal *Salmonella* levels (16).

Although it may be assumed that the cleanliness of the animals prior to slaughter is a crucial factor that determines the microbiological quality and safety of carcasses after processing, studies have shown this not to be the case (17,18). For example, no association was found between tag (mud, bedding and feces) on the hides of beef cattle and bacterial counts of carcasses (17). Furthermore, preharvest management practices (wool length, use of bedding and wet versus dry pens) appeared to have no effect on the microbiological quality of lamb carcasses (18). However, evaluation of microbial contamination of the stock presented for slaughter may be beneficial for adjustment of the speed of processing lines or the separate processing of highly contaminated animals (19,20).

b. Slaughter and dressing

During the slaughtering process bacteria can enter the tissues through the bloodstream from contamination of the sticking knife. However, bacteria introduced into the bloodstream do not survive for long in the tissues due to the defense mechanisms of the animal that continue for at

least 1 h after death (6). Thus, if high levels of contamination from slaughter instruments are avoided by reasonable hygienic precautions, carcass contamination from this source should not be a problem.

Most of the bacterial contamination of the carcass is acquired during the dressing process. Knife incisions for the removal of cattle and sheep hides introduces microorganisms from the hide onto the underlying tissue. Further bacterial transfer may occur from contact of the hide with exposed carcass tissue or from aerosols, dust, wool and hair from the hide or fleece (7). Unlike cattle and sheep, the skin of pigs is usually not removed during the dressing procedure, although certain facilities process skinned pork carcasses. After slaughter, pigs are scalded to loosen the attachment of the hair to the skin before dehairing (7). Although the scalding operation reduces the number of microorganisms on the skin, recontamination may occur during dehairing due to the high level of contamination of dehairing equipment (21). The numbers of such contaminants can be reduced by the singeing process used to burn hair remaining on the carcass. However, as the singeing of the surface is usually uneven, significant numbers of bacteria, including both spoilage and pathogenic types, can persist on some areas of the carcass or be introduced or spread during carcass polishing operations (8,22,23).

Other significant sources of contamination during the dressing process are the mouth and the viscera of the animals. The mouth harbors large numbers of bacteria including pathogens such as pathogenic *E. coli* and *Salmonella* (13,24). If other areas of the carcass are handled by workers after handling the throat and the tongue, then carcasses may be heavily contaminated with bacteria from the mouth. During evisceration, contamination can occur if the intestinal tract is pierced or if fecal material is released from the rectum during removal of abdominal contents. Careful handling of the head and viscera as well as the use of plastic bags to enclose the head and the end of the cut bung of animals reduces contamination from these sources.

In addition to the hide, head and viscera, bacteria may be introduced onto the carcass during the dressing process from the processing environment such as floors, walls, contact surfaces, knives and workers' hands. Thus, hygienic conditions and sanitation procedures are important factors affecting the microbiological quality of the dressed carcasses.

c. Chilling

Before fabrication, carcasses need to be chilled to adequately low deep muscle temperatures in order to avoid microbial proliferation during and following fabrication (15). Most carcass cooling processes are operated so as to reduce deep muscle temperatures to 7°C or lower within a 12–24 h period (25). However, due to their size, commercial chilling of beef carcass sides requires 18–36 h for temperatures of <7°C to be reached. Traditionally, carcasses were chilled by exposure to a flow of cold air only, which, due to the evaporation of water, resulted in drying of the carcass surface (26). Experimental studies have shown that depending on the temperature, humidity and air velocity, this type of chilling may result in increases, decreases or no changes in total numbers of aerobic bacteria of carcasses (6). Drying of the carcass surface, however, results in loss of carcass weight, which in turn leads to economic losses (27). Hot carcass weight losses of at least 2% during the first 24 h of air chilling have been reported for beef (28,29), pork (30) and lamb (31). In order to circumvent this, most packing plants in North America use intermittent spraying of carcasses with chilled water, especially during the initial stages of chilling, which facilitates carcass temperature decreases without loss of surface moisture. Spray chilling results in carcass weights that are slightly less than those of carcasses entering the chilling process (8). The practice of spray chilling is uncommon outside North America, partly due to concerns that the prevention of drying of the carcass surface would allow growth of pathogenic bacteria (26). Experimental studies, however, have shown that as for traditional air chilling of carcasses, microbial levels on carcasses can increase, decrease or remain unchanged by adjusting air temperatures and speeds, as well as the frequency, intensity and duration of spraying (32–34). The incorporation of chlorine (35,36), acetic and lactic acids (37,38) in the spray chilling process have been evaluated as a means of sanitizing beef carcasses during the chilling process.

d. Fabrication

The chilling process is followed by cutting (fabrication) of the carcass into primal and sub-primal cuts, and trimmings. The meat receives extensive handling during these operations and high levels of cross-contamination may occur by contact of freshly cut meat surfaces with work surfaces, hands and equipment. Factors affecting the extent of contamination during fabrication include temperature of the boning room, the time duration that meat is held there, and the cleanliness of fabrication equipment. Equipment such as cutting tables, conveyor belts, saws and knives can carry high numbers of spoilage and pathogenic bacteria (21,39). In order to minimize cross-contamination and spreading of contamination during fabrication, adequate cleaning of the plant and equipment as well as sanitation practices to prevent microbial contamination and biofilm formation are required. Proper control of the above conditions may maintain bacterial densities on meat after the fabrication process to as close to initial levels of contamination as possible.

2. Poultry

a. Live bird

Although freshly laid eggs rarely contain microorganisms, eggs and ultimately the developing embryo can be

contaminated through two possible routes; by vertical (transovarial) or horizontal transmission of microorganisms. Transovarial transmission occurs when bacteria infect the hen's ovaries or oviducts resulting in possible contamination of the egg during its formation (7,40,41). Of major concern is the vertical transmission of *Salmonella* Enteritidis (42). The more common route of microbial contamination of eggs, however, is via horizontal transmission. This occurs when the natural defenses of the egg, which include the cuticle layer, shell, and outer and inner membranes, are penetrated resulting in contamination of the internal contents of the egg (43,44). Microorganisms contaminating the egg after it has been laid originate from the intestines when the egg passes through the cloaca, nest materials, litter or incubator surfaces (42,45,46).

Newly hatched chicks from uninfected eggs are microbiologically sterile but are readily contaminated with microorganisms present in their environment, for example, from contaminated eggshells, fecal matter, fluff from infected newly hatched chicks, and walls and floors of the incubator (47,48). Healthy chickens carry millions of microorganisms in their intestines and on their skin (49). Sources of microorganisms on the rearing farms are contaminated feed and water, soil, litter, dust and air. Insects, rodents, wild birds, reptiles and other small animals may also act as reservoirs and vectors of microorganisms (41).

During transportation of birds from growing houses to slaughter facilities, contamination and cross-contamination with fecal material may occur. Stress conditions during transport may lead to more frequent excretion of fecal material and cecal contents (50,51), and thus an increase in the level of contamination. Since transport crates become heavily contaminated with fecal material, they need to be thoroughly cleaned and disinfected before being reused to prevent cross-contamination of other flocks (52).

b. Slaughter and processing

Incoming birds are the principal source of most microorganisms found on poultry carcasses. Similar to red meat animals, healthy birds carry extensive microbial contamination on their feathers, skin and intestinal tract (3,49,53). In general, transfer of this contamination to the carcasses occurs during all processing steps including stunning, bleeding, scalding, defeathering, evisceration, washing and chilling (50).

As birds are hung and bled, the flapping of wings may generate dust and aerosols which distribute contamination onto nearby birds or carcasses. After bleeding, poultry carcasses are scalded by submerging in a warm waterbath (scald tank) at temperatures between 50 and 60°C. The accumulation and survival of microorganisms in the scald tank is influenced by the temperature of water and the rate at which fresh water is added (54). The pH of the scalding water and the presence of organic matter are also important for bacterial survival since they affect the rate of inactivation of microorganisms (55). It is obvious that from a hygienic point of view scalding is a hazardous operation. Attempts to avoid the use of immersion scalding have led to the development of alternative methods including spraying systems and division of the scald tank into several smaller ones (56). Although under experimental conditions these methods improve the hygiene of the scalding process, their commercial implementation is, however, limited because most of them have yet to be fully adapted to commercial requirements.

After scalding, the carcasses pass through a series of mechanical plucking machines which remove the feathers. The defeathering process may spread contamination between carcasses or from the defeathering equipment (56). The level of cross-contamination during defeathering is very high since one contaminated bird can cross-contaminate hundreds of others as they pass through the pluckers (50).

Evisceration of poultry carcasses is carried out in several stages including head removal, opening of the body cavity, removal of intestines and cleaning of the carcass. As with red meat animals this process is associated with contamination of the carcass with microorganisms originating from intestines, including pathogens. Contamination during evisceration may occur by bacterial transfer from carcass to carcass by knives, eviscerating implements and worker's hands. The automated transfer of carcasses to the different stages of the evisceration process significantly reduces product handling and potential cross-contamination. However, contamination may still occur due to improper cleaning of the machines involved in the process.

Poultry carcasses are washed and chilled immediately after evisceration. Washing of carcasses removes the organic matter and some of the contaminating microorganisms. Chilling aims to limit multiplication of spoilage bacteria and restrict the growth of pathogens. The two most common chilling methods for poultry carcasses are water (immersion) and air chilling. Carcasses destined for the frozen market are normally water chilled, while fresh, non-frozen carcasses can either be water or air chilled and marketed as 'wet' or 'dry,' respectively (56). Water chilling is almost exclusively used in the U.S. poultry industry, while European processors commonly use air chilling. Water chilling has a washing effect on the carcasses, but if not properly maintained and operated, has the potential for allowing cross-contamination of carcasses with spoilage bacteria, indicator organisms and pathogens. In-plant chlorination of the chiller water at levels >25 ppm reportedly controls or reduces cross-contamination of carcasses with Gram-negative spoilage bacteria and salmonellae (57). Chlorine levels do not normally exceed 50 ppm, and at this level, microbial loads on carcasses are only reduced by 1 log unit (58). Factors affecting the efficacy of chlorine include the initial bacterial load of the water, the water level, organic load, temperature, pH and

trace minerals in the water (59). In some European countries, chlorine addition to the chiller water is prohibited because of the theoretical link to carcinogenesis (60). Other chemicals used or tested in the poultry processing industry to reduce microbial contamination of carcasses during washing and water chilling steps of processing include chlorine dioxide, trisodium phosphate, acidified sodium chlorite, organic acids (lactic and acetic acids), ozone, cetylpyridinium chloride and hydrogen peroxide (59). These are discussed in more detail in a subsequent section of the chapter.

Air chilling of poultry carcasses is basically a dry process, where cold air is circulated, either in a chill room or air-blast tunnel, to chill the carcasses. In order to enhance cooling, the chilling process may be combined with evaporative chilling, which involves the intermittent spraying of carcasses with water, so that the water absorbs heat as it evaporates (61). The latter process is said to minimize carcass weight loss, which contrasts with the weight gains resulting from water chilling (60). In a U.K. study where these three poultry chilling systems were compared, water chilling resulted in a reduction of microbial contamination of carcasses, while air chilling had little effect on microbial numbers recovered from the skin. Microbial levels of the body cavity, however, were reduced by approximately 1 log unit when the dry chilling process was used. Conversely, evaporative cooling through the use of water sprays that were not in-plant chlorinated led to a large (1–2 log units) increase of pseudomonads in the body cavity of the carcasses (61).

After chilling, carcasses are either packaged whole, or are cut into portions, placed in bags or on trays, weighed, and chilled, or more often frozen. The product is thus exposed to extensive cross-contamination through handling and contact with equipment surfaces such as knives, conveyor belts, and weighing and packaging equipment.

C. Type and Extent of Contamination

1. Red Meat

The type and extent of meat carcass and product contamination depends on sanitation, hygienic practices and handling during harvesting and processing, and conditions of storage and distribution. Red meat carcass contamination after processing is usually variable and may consist of 10^1–10^7 aerobic mesophiles per cm^2 (Table 52.1) depending on plant, carcass and site on the carcass sampled (62,63). In general, contamination of pork carcasses is higher than beef (Table 52.1). A nationwide baseline carcass contamination study carried out by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) found the mean level of aerobic plate counts of carcasses after chilling to be 3.69 log cfu/cm^2 for market hogs, 3.05 log cfu/cm^2 for cows and bulls and 2.68 log cfu/cm^2 for steers and heifers (64–66). In an additional study incorporating seven U.S. beef packing plants (four steer and heifer; three cow and bull), mean aerobic plate counts of carcasses after 24 h of chilling was 2.55 log cfu/cm^2 (67). Baseline carcass contamination data from Australia showed mean total viable counts of 2.4 log cfu/cm^2 for beef carcasses (68) and 3.6 log cfu/cm^2 for sheep carcasses (69).

The microflora of fresh red meat carcasses usually consists of Gram-negative rods and micrococci including *Pseudomonas* spp., Enterobacteriaceae, *Acinetobacter* spp., *Alcaligenes* spp., *Moraxella* spp., *Flavobacterium* spp., *Aeromonas* spp., *Staphylococcus* spp., *Micrococcus* spp., coryneforms and fecal streptococci (49,70). In addition, lactic acid producing bacteria, *Brochothrix thermosphacta*, *Bacillus* and *Clostridium* spores, and enteric viruses may be present in lower numbers (49,70). Yeasts and molds rarely contribute to the microflora of fresh meat, and, generally, may be detected only during extended storage and when the surface of the meat becomes dry, which limits

TABLE 52.1
Aerobic Plate Count Distribution on Raw Beef and Pork Carcasses

	Steers and Heifers		Cows and Bulls		Hogs	
Range (cfu/cm^2)	Number of Samples	Percent of Total	Number of Samples	Percent of Total	Number of Samples	Percent of Total
<1	25	1.2	8	0.4	0	0
1–10^1	41	2.0	13	0.6	0	0
10^1–10^2	415	19.9	261	12.4	18	0.9
10^2–10^3	990	47.4	856	40.5	501	23.7
10^3–10^4	474	22.7	651	30.8	954	45.2
10^4–10^5	103	4.9	244	11.6	461	21.8
10^5–10^6	32	1.5	68	3.2	130	6.2
10^6–10^7	9	0.4	9	0.4	43	2.0
$>10^7$	0	0	2	0.1	5	0.2
Total	2,089	100	2,112	100	2,112	100

Source: Refs. 64–66.

bacterial growth and allows yeasts and molds to dominate. They include *Torulopsis, Trichosporon*, *Candida, Rhodotorula, Cryptococcus, Penicillium, Aspergillus, Geotrichum*, *Mucor, Rhizopus, Monillia, Alternaria, Thamnidium* and *Chaeotostylum* (6,70–72).

The prevalence and levels of pathogenic bacteria on red meat carcasses depends on a number of factors including the origin of the animal, sanitation procedures and hygienic practices employed during handling and processing of the product, application of decontamination interventions, and conditions of storage. Based on baseline data collected by FSIS, 14.6% of steers and heifers and 27.2% of cows and bulls are contaminated with one to three different pathogenic bacteria (Table 52.2) (64,65). The baseline data also showed that the prevalence of pathogens on pork carcasses was higher since 52.4% of market hogs were contaminated with one to five different pathogenic bacteria (Table 52.2) (66). The most important pathogens associated with red meat included salmonellae, *Staphylococcus aureus*, verotoxigenic *E. coli, Clostridium perfringens*, *Campylobacter jejuni/coli*, *Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila* (Figure 52.1). Depending on the factors mentioned above the concentration of pathogens on meat carcasses can vary from 1 to >30 most probable number (MPN)/cm^2 (Figures 52.2 and 52.3). Some of these pathogens are more commonly associated with meat from one animal species rather than another. For example, *Y. enterocolitica* (73) and *C. jejuni/coli* (66) are more commonly associated with pork carcasses. In the U.S., the reported prevalence of *C. jejuni/coli* on beef carcasses is ten times less than on pork carcasses (Figure 52.1). *E. coli* O157:H7, on the other hand, is more commonly associated with beef carcasses since cattle and other ruminants have been identified as major reservoirs of this pathogen (11,74,75). The prevalence of pathogens can also differ within the same species. For example, the prevalence of *Salmonella* for cow and bull carcasses is reportedly higher

TABLE 52.2
Number of Raw Meat Samples Containing One or More Species of Pathogenic Bacteria

	Steers and Heifers		Cows and Bulls		Hogs	
Number of Pathogens[a]	Number of Samples	Percent of Total	Number of Samples	Percent of Total	Number of Samples	Percent of Total
0	1,785	85.4	1,538	72.8	1,006	47.6
1	278	13.4	484	22.9	754	35.7
2	23	1.1	82	3.9	265	12.6
3	3	0.1	8	0.4	70	3.3
4	0	0	0	0	15	0.7
5	0	0	0	0	2	0.1

[a] *C. perfringens, S. aureus, L. monocytogenes, C. jejuni/coli, E. coli* O157:H7, *Salmonella* spp.

Source: Refs. 64–66.

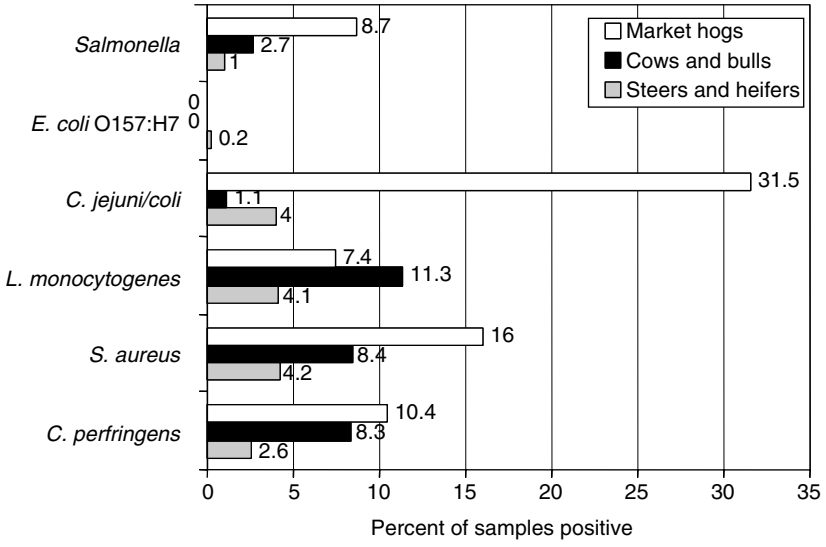

FIGURE 52.1 Prevalence of selected pathogenic microorganisms of raw beef and pork carcass surface samples. (From Refs. 64–66.)

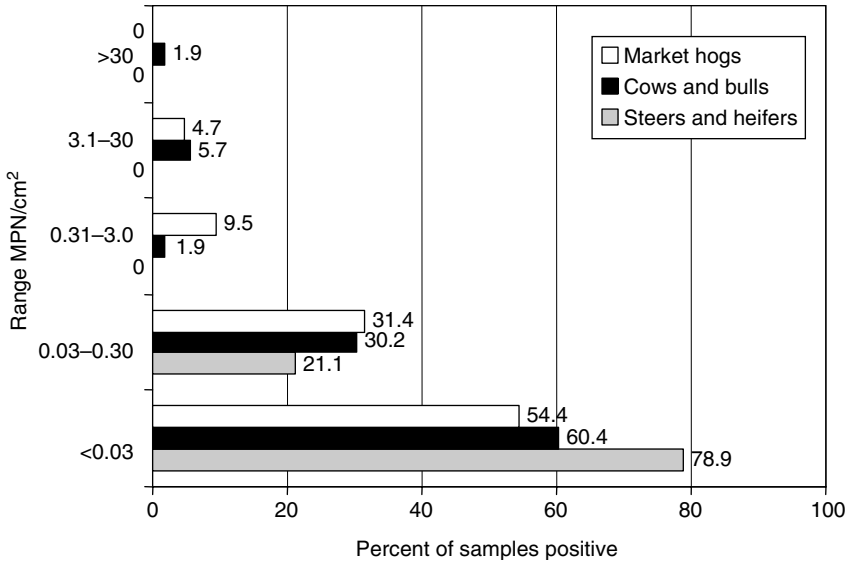

FIGURE 52.2 *Salmonella* distribution on enumerated positive raw beef and pork carcass surface samples. (From Refs. 64–66.)

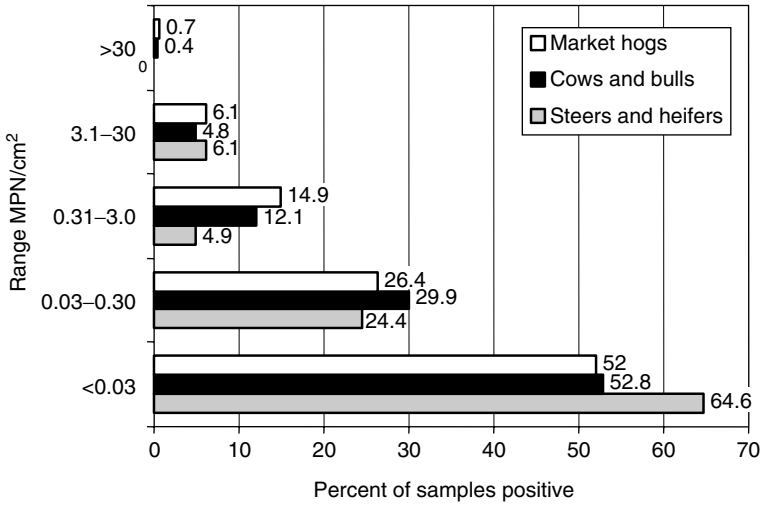

FIGURE 52.3 *Listeria monocytogenes* distribution on enumerated positive raw beef and pork carcass surface samples. (From Refs. 64–66.)

than for steer and heifer carcasses (67,76,77). The season of the year can also affect the prevalence of pathogens on carcasses. Studies carried out on cattle in North America have shown an increased prevalence of *E. coli* O157 during the summer and early fall (11,78).

The extent of contamination and the variety of contaminant types increase with product handling and comminution (70). Comminuted meats present a higher contamination than carcass meat due to an increase in the level of cross-contamination from grinders and utensils, spreading of contamination, and the greater surface area of the trimmings. The mean level of total aerobic mesophiles of ground beef reported in the U.S. during the years 1993–1994 was 3.90 log cfu/g (Table 52.3) (79) compared to 2.68 log cfu/cm^2 reported for beef carcasses.

2. Poultry

The microbial contamination associated with poultry carcasses at the end of processing is a combination of the natural population found on the carcass skin, the transient population that is associated with the skin and feathers at the time of slaughter and the population that is acquired as a result of processing (41). The microbiological condition (numbers and types of microorganisms) of poultry carcasses immediately after processing is thus dependent on factors such as the level of contamination on the live incoming birds, the efficiency of the processing methods, the level of cross-contamination during processing, temperature control, and sanitary and hygienic practices in the abattoir (80). Typically, the number of aerobic mesophilic bacteria associated with

TABLE 52.3
Estimated Prevalences and Mean Levels of Selected Bacteria in Raw Ground Beef

Microorganism	Prevalence %	SE	Mean Log in Positive Samples	SE
Direct enumeration (cfu/g)				
Aerobic plate count	100	NA[a]	3.90	0.12
Total coliforms	92.0	3.9	1.98	0.10
Escherichia coli (Biotype I)	78.6	5.9	1.73	0.13
C. perfringens	53.3	8.7	1.83	0.10
S. aureus	30.0	8.7	1.49	0.06
MPN enumeration (MPN/g)				
L. monocytogenes	11.7	4.1	0.46	0.43
C. jejuni/coli	0.002	0.003	NA	NA
E. coli O157:H7	0.0	NA	NA	NA
Salmonella	7.5	3.1	−1.29	0.89

[a] Not applicable.

Source: Ref. 79.

TABLE 52.4
Aerobic Plate Count Distribution in Chicken Broiler and Turkey Carcass Rinse Fluids

Range (cfu/ml)	U.S. Percent of Total		Canada Percent of Total	
	Chicken Broilers	Turkey	Chicken Broilers	Turkey
<1	0	0.2	0	0.2
1–10^1	0	2.5	0	3.3
10^1–10^2	0.5	29.9	2.7	40.7
10^2–10^3	32.3	53.6	53.4	40.5
10^3–10^4	58.9	13.3	39.7	11.3
10^4–10^5	7.8	0.5	3.7	4.0
10^5–10^6	0.5	0.1	0.5	0.0

Source: Refs. 81–83.

carcasses at the end of processing range from 10^3 to 10^5 cfu/cm^2 (49). Studies in the U.S. and Canada during the last decade showed that the total aerobic mesophiles of chicken and turkey carcasses ranged from 10^2 to 10^6 and from 10^0 to 10^6, respectively (Table 52.4). The reported mean total aerobic mesophile levels on chicken and turkey carcasses were 2.60 log cfu/cm^2 and 2.47 log cfu/cm^2, respectively, in the U.S. and 2.32 log cfu/cm^2 and 2.20 log cfu/cm^2, respectively, in Canada (81–83). Bacterial populations associated with carcasses at the end of processing are predominantly Gram-negative, and include *Acinetobacter*/*Moraxella* spp., Enterobacteriaceae, *Flavobacterium* spp. and *Pseudomonas* spp. (84–88).

Human pathogens that are associated with poultry carcasses are *Salmonella* serotypes, *C. jejuni*, *S. aureus*, *L. monocytogenes*, *C. perfringens*, non-O157:H7 Shiga-like toxin-producing *E. coli*, *Aeromonas* spp. and *Y. enterocolitica* (Figure 52.4) (41,89–94). Poultry meat represents the single most important source of foodborne salmonellosis, with the most common serotypes implicated in outbreaks being *S.* Enteritidis and *S.* Typhimurium (70,95,96). Prevalence levels of *Salmonella* associated with poultry carcasses at the end of processing or at the retail market are reportedly as high as 100% (57,97). The level of *Salmonella* contamination on live birds entering the processing plant is reportedly low, but subsequent cross-contamination during processing procedures results in an increase in the *Salmonella* contamination of carcasses. In a FSIS study, a *Salmonella* incidence level of 3 to 5% was found on live birds entering a processing plant, which increased to 36% on carcasses at the end of processing (98). During the period 1998–2002, the prevalence of *Salmonella* in broiler carcass samples from federally inspected establishments in the U.S. was reduced from a baseline prevalence of 20.0%, to 10.9% (available at: http://www.fsis.usda.gov/OPHS/haccp/ salm5year.htm).

III. MICROBIAL EFFECTS IN MEAT AND MEAT PRODUCTS

A. SPOILAGE

Only 10% of the microorganisms present initially on a muscle food will grow at refrigeration temperatures, and only a fraction of those will eventually spoil the product by means of their biochemical attributes (99,100). A muscle food is characterized as spoiled when it exhibits an offensive appearance and/or has an off-odor or off-flavor. Off-odors are detected first when bacterial numbers exceed 10^7 cells/cm^2, while bacterial slime becomes evident when numbers reach 10^8 cells/cm^2 (101); bacterial colonies coalesce to form slime when the water activity of the product is approximately 0.99 (41). The spoilage microflora that develops during storage is determined by the initial number and types of microorganisms contaminating the product, the processing the product may undergo, the storage temperature and gaseous atmosphere, and composition of the product (102). The types of bacteria that are normally involved in the spoilage of meat and poultry products are *Pseudomonas* spp., psychrotrophic Enterobacteriaceae (*Serratia liquefaciens*, *Hafnia alvei*, *Enterobacter agglomerans*), *Brochothrix thermosphacta*, lactic acid bacteria (*Lactobacillus*, *Carnobacterium*, *Pediococcus*, *Streptococcus*, *Lactococcus* and *Leuconostoc* spp.), *Moraxella* spp., *Psychrobacter* spp., *Acinetobacter* spp., *Aeromonas* spp. and *Shewanella putrefaciens* (70,101). Yeast and mold spoilage of muscle foods can also occur but only under conditions where bacterial competition is reduced (103), such as exposure to ionizing radiation, reduced water activity, the presence of preservatives or antimicrobial treatments (104).

Microbiological spoilage occurs in the aqueous phase of meat (105). Spoilage microorganisms within this phase

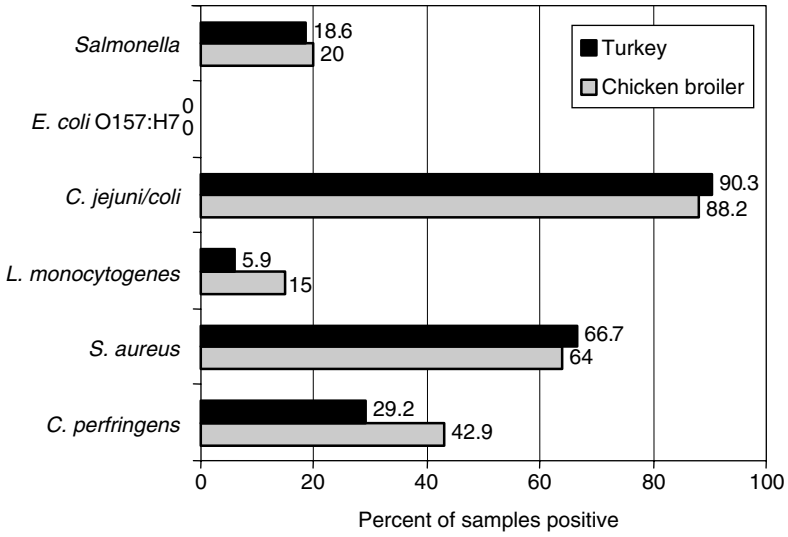

FIGURE 52.4 Prevalence of selected pathogenic microorganisms in chicken broiler and turkey carcass rinse fluids in the U.S. and Canada. (From Refs. 81–83.)

TABLE 52.5
Substrates Used for Growth by Major Meat Spoilage Microorganisms[a]

Microorganism	Substrates Used for Growth[a]	
	Aerobic	Anaerobic
Pseudomonas spp.	Glucose[1], glucose-6-P, D, L-lactic acid[2], pyruvate, gluconate, gluconate 6-P, amino acids[2], creatine, creatinine, citrate, aspartate, glutamate	Glucose[1], acetic acid[1], pyruvate, gluconate, amino acids (glutamate)
Acinetobacter/Moraxella	Amino acids[1], lactic acid[2], glucose[1], amino acids[1,2]	Glucose[1], amino acids[1,2]
Shewanella putrefaciens	Glucose, lactic acid, pyruvate, gluconate, propionate, ethanol, acetate, amino acids (serine)	Formate
Brochothrix thermosphacta	Glucose[1], amino acids[2] (glutamate, L-valine, L-leucine, *iso*-leucine), ribose, glycerol	Glucose[1]
Enterobacter spp.	Glucose[1], glucose-6-P[2], amino acids[3] (lysine, arginine, threonine), lactic acid[4]	Glucose[1], glucose-6-P[2], amino acids[3]
Lactobacillus spp.	Glucose[1]	Glucose[1], lactic acid[2], amino acids[2]

[a] The number in superscript indicates the order of utilization of this substrate.

Source: Ref. 412.

catabolize glucose, lactic acid, certain amino acids, nucleotides, urea and water-soluble proteins (106–108). Table 52.5 shows the substrates utilized by some of the predominant spoilage bacteria and the order in which they are attacked, under aerobic and anaerobic conditions. Spoilage of meat and meat products packaged in air or under modified atmosphere is discussed in the following sections.

1. Spoilage under Aerobic Conditions

Organoleptic changes brought about by aerobic spoilage microorganisms include green, brown or gray discolorations, off-odors and off-flavors, surface slime and taint. Off-odors and slime production may be detected after 10 or 5 days of storage at 0 or 5°C, respectively (109). Off-odors have been described as dairy/buttery/fatty/cheesy at levels of 10^8 bacteria/g, and as sweet/fruity and putrid at levels of $>10^9$ bacteria/g (110).

Meat and meat products stored under aerobic, chill conditions generally succumb to spoilage by strictly aerobic, Gram-negative bacteria, which exhibit faster growth rates under these conditions. *Pseudomonas* spp., and specifically *P. fragi*, *P. fluorescens* and *P. lundensis*, usually predominate (normally >50%) on meat stored at chill temperatures. The pseudomonads preferentially metabolize glucose, resulting in organoleptically innocuous end products. When, however, the diffusion gradient of glucose from the underlying tissue to the surface of the meat can no longer meet the demands of the large numbers of

spoilage bacteria ($>10^8$ cfu/g), amino acids (cysteine, cystine and methionine) and proteins are degraded which result in the formation of ammonia, amines (cadaverine, putrescine, spermidine, spermine, histamine and tyramine) and sulfides (hydrogen sulfide, methylsulfide and dimethylsulfide) (111,112).

Facultative anaerobes belonging to the family of Enterobacteriaceae can also form part of the aerobic spoilage microflora. Like the pseudomonads, these bacteria preferentially metabolize glucose, and some utilize glucose-6-phosphate followed by amino acids. The slower growth rates of the Enterobacteriaceae under chill conditions, however, prevent them from predominating on aerobically stored muscle foods (111). The Gram-positive, facultative anaerobe, *B. thermosphacta*, also usually forms a minor component of the spoilage microflora on aerobically stored meat. Psychrotrophic Enterobacteriaceae and *B. thermosphacta*, however, seem to be more prevalent on lamb and pork, especially on the fat surfaces, and when the meat is stored at 5°C and not 1°C (101,113). Other bacterial groups implicated in the spoilage of meat under aerobic conditions are *Acinetobacter* spp., *Psychrobacter* spp. and *Moraxella* spp. These strict aerobes tend to form a higher proportion of the spoilage microflora on meat of higher ultimate pH (pH >5.8), or when the meat is stored at ambient temperature (114). Unlike pseudomonads, most of these strict aerobic bacteria do not metabolize hexoses, but attack amino acids directly (102).

Meat of high ultimate pH (pH >6.0) and low glucose concentration, like dark, firm, dry (DFD) meat, undergoes spoilage more rapidly than meat of normal pH (pH 5.5 to 5.8). Dark, firm, dry beef stored aerobically at 6°C produced off-odors after 2 days of storage while beef of normal pH produced off-odors after 4 days (115). *Pseudomonas* spp. degrade amino acids on DFD meat without delay and thus onset of spoilage is simply dependent on the time required to accumulate bacterial metabolites responsible for off-odors; this occurs at bacterial levels of $>10^6$ cells/cm^2 (116).

2. Spoilage under Vacuum

The predominant spoilage organisms of meat stored under vacuum and refrigeration conditions are lactic acid bacteria, which are able to metabolize glucose to produce lactic, isobutanoic, isopentanoic and acetic acids (100,117). *Carnobacterium* spp., *Lactobacillus* spp. and *Leuconostoc* spp. are reportedly the dominant lactic acid bacteria isolated from meat and meat products, where levels of 10^7/cm^2 may be reached (118,119). Spoilage is normally characterized by a sour/acid/cheesy odor (101). Growth of *B. thermosphacta*, *Pseudomonas* spp. and Enterobacteriaceae may also occur at levels of 10^3–10^6/cm^2, but is dependent on the oxygen permeability of the packaging film, storage temperature, pH and initial contamination levels (120,121). Higher numbers of *B. thermosphacta* and Gram-negative bacteria are more commonly found on vacuum packaged pork and lamb than on vacuum packaged beef (113,122). The shelf life of vacuum packaged pork and lamb is also reportedly a few weeks shorter than that of beef. Blixt and Borch (123) compared the shelf life of vacuum packed, cold-stored minced pork and beef and found high correlations between intrinsic factors of the two types of meat, such as initial pH values, fat and L-lactate, respectively, and the rate of spoilage. Almost 70% of the variation in the rate of spoilage was explained by changes in the pH and concentrations of L-lactate and glucose-6-phosphate during storage, and not by whether the meat was pork or beef.

Spoilage of vacuum packaged DFD meat occurs within 3 to 6 weeks of storage at 0°C and is characterized by an offensive putrid odor (115,124). The spoilage flora is dominated by *S. putrefaciens*, Enterobacteriaceae and species of *Lactobacillus.* spp. *Shewanella putrefaciens* causes a characteristic green discoloration of vacuum packaged DFD meat which results from the production of hydrogen sulfide from cysteine or glutathione. The green pigment (sulfmyoglobin) is formed by the hydrogen sulfide reacting with the meat myoglobin (125,126).

Certain psychrophilic and psychrotrophic species of *Clostridium* have been characterized as spoilage organisms of vacuum packed meat (beef, lamb, venison and dog rolls) that was stored between −1.5 to 2°C and that exhibited extensive gas production and gross pack distension within 4 to 6 weeks of storage (127–129). Species linked to 'blown pack' spoilage include *C. laramie*, *C. gasigenes*, *C. frigidicarnis, C. estertheticum* and *C. estertheticum*-like strains (128–132). Using restriction fragment length polymorphism analysis of the 16S rDNA genes and 16S–23S rDNA internal transcribed spacer analysis, Broda et al. (132) showed that the source of these organisms on chilled vacuum packed venison was most likely soil particles attached to the hide or present in the feces of the animals.

3. Spoilage in Modified Gas Atmospheres

Lactic acid bacteria and/or *B. thermosphacta* are the predominant spoilage flora of beef, pork and lamb stored in modified atmospheres (133–138). Enterobacteriaceae and *Pseudomonas* spp. reportedly compete more effectively under modified atmosphere packaging conditions than in vacuum packed meat, especially pork that is stored at approximately 5°C and has undergone prior conditioning in air (101). A CO_2 concentration of 100% can be used to extend the storage life of meat of normal and high pH to more than 3 months when stored at 0 to 1°C (101). Pork stored at 0 and 4°C reached total bacterial populations of 10^6 to 10^7 per cm^2 and exhibited off-odors in 119 and 40 days, respectively, with *Lactobacillus* spp. dominating at both temperatures (139).

4. Spoilage of Processed Meats

Chilled comminuted products, including ground meats, burger-type products and raw sausages, in general, have a short aerobic shelf life of 3 to 5 days at 2 to 4°C, due to high initial loads of bacteria (140). The spoilage flora of ground beef stored in air or under modified atmosphere (20% CO_2/80% O_2) is composed of *Pseudomonas* spp., lactic acid bacteria, *B. thermosphacta*, Enterobacteriaceae and yeasts; however, under aerobic conditions *Pseudomonas* spp. are dominant, while under modified atmosphere lactic acid bacteria and *B. thermosphacta* dominate (141,142). The dominant spoilage flora of fresh sausages is dependent on the meat type, storage temperature, gaseous environment, and the presence of preservatives, but in general, it resembles that of ground meat (100).

In general, spoilage of cooked, uncured meat products (whole and restructured joints and poultry, and ready-to-eat meals) is a result of post-processing contamination (100). Gram-negative psychrotrophic bacteria are the main spoilage organisms of aerobically chill-stored sliced cooked meat, while lactic acid bacteria and *B. thermosphacta* predominate on vacuum packed and modified atmosphere packed products (41). Spoilage of meat pies is a result of molds, including *Mucor*, *Penicillium*, *Rhizopus* and *Aspergillus* spp., which usually contaminate the product during cooling (49).

Microbial spoilage of raw, cured meats, like Wiltshire-style bacon, is characterized by surface slime, discoloration, off-odors and off-flavors (143). Major spoilage populations associated with these products are Micrococcaceae, halophilic *Vibrio* spp., lactic acid bacteria, coryneforms and yeasts, which are tolerant to salt and sodium nitrite (113,143). Factors influencing the composition of the spoilage flora are concentration of additives (salt, nitrite and nitrate), temperature, pH and water activity (100).

Cooking of cured meat products inactivates vegetative bacteria but recontamination occurs during post-cooking steps, like slicing, portioning or skinning. Spoilage of these products (cooked ham, corned beef, emulsion-type sausages, luncheon meats) stored at cold temperatures and packed under vacuum or modified atmosphere is predominantly by lactic acid bacteria (99,144–146). *Lactobacillus* spp. (*Lactobacillus sake* and *Lactobacillus curvatus*) and *Leuconostoc* spp. are the main spoilage organisms of cooked, cured meats (99,146–148). Spoilage is characterized by a sour odor and flavor, greening, gas production and slime formation (99,149). The growth rates and identity of the spoilage lactic flora are found to differ depending on the product composition (salt content, nitrite concentration, pH and water activity), processing method, storage temperature and gaseous atmosphere (99,150). The growth of lactic acid bacteria was more delayed in smoked meats (pork loin, bacon, pariza, mortadella and frankfurters) than in non-smoked, boiled whole meats (cooked ham and turkey fillets) (146). Furthermore, characterization of the predominant spoilage lactic acid bacteria showed that the *Lactobacillus sake/curvatus* group predominated on the smoked meats, whereas *Leuconostoc mesenteroides* subsp. *mesenteroides* predominated on the cooked ham and turkey fillets (146).

Canning or commercial sterilization of uncured and cured meats results in a shelf stable product due to the complete elimination of microorganisms. Spoilage rarely occurs due to underprocessing, loss of package integrity and leakage of microorganisms into the package post-processing (4).

Molds are generally responsible for the spoilage of unsmoked, air-dried fermented sausages, some of which may have the ability to produce mycotoxins while growing on the sausage. Furthermore, molds have been reported to grow on the surface of frozen meat stored at temperatures as low as –8 to –10°C (113).

5. Spoilage of Poultry

Poultry spoilage follows the same principles as those involved in the spoilage of red meat products. The bacterial groups most frequently implicated as the main causes of spoilage of refrigerated poultry are *Pseudomonas* spp., and to a lesser extent, *Acinetobacter* spp., *Moraxella* spp. and *S. putrefaciens* (85,151). The *Pseudomonas* population reportedly may increase from $<$10% before storage to 93% of the total carcass bacterial population at the time of spoilage, following storage at 1 to 5°C (84,85,152). The type of spoilage bacteria as well as their growth rate is affected by the numbers and types of spoilage microorganisms present on carcasses at the end of processing, the type of packaging material and the composition of the atmosphere surrounding the packaged product, the storage temperature and the muscle type and pH (85). Poultry leg muscle has a pH of 6.4 to 6.7 while that of breast muscle is 5.7 to 5.9 (153). Spoilage populations of the breast muscle are thus essentially the same as those found on red meat of normal ultimate pH, while populations of the leg muscle mimic those found on DFD meat (126). Poultry leg meat thus spoils more rapidly than breast meat (41).

B. Foodborne Illness

Current estimations indicate that microbially contaminated food causes approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in the U.S., per year. Bacterial agents are responsible for 30% of these illnesses, while viruses and parasites cause 67% and 3% of the illnesses, respectively. The total percentage of deaths caused by each of these agents, however, is the highest for foodborne illnesses caused by bacteria (*ca.* 72%), followed by those of parasitic (*ca.* 21%) and viral (*ca.* 7%) origin (154).

The U.S. Centers for Disease Control and Prevention (CDC) Emerging Infections Program Foodborne Diseases Active Surveillance Network (FoodNet) collects data on 10 foodborne diseases in nine U.S. site areas, equivalent to 37.4 million people. According to preliminary data for 2002, 16,580 laboratory-diagnosed cases were identified, with *Salmonella* (16.1 cases/100,000 population), *Campylobacter* (13.4 cases/100,000 population) and *Shigella* (10.3 cases/100,000 population) having the highest incidence (155). An equivalent network in Australia (OzFoodNet), consisting of seven sites and encompassing 68% (12.9 million) of the Australian population, and monitoring eight foodborne diseases, reported a total of 22,999 cases of foodborne infections for the year 2001, with *Campylobacter* (125.0 cases/100,000), *Salmonella* (34.1 cases/100,000) and *Shigella* (2.6 cases/100,000) again having the highest incidence (156).

During the period of 1993–1997, a total of 2,751 outbreaks of foodborne disease were reported to the CDC, causing a reported 86,058 persons to become ill (157). Bacterial pathogens were responsible for the largest percentage of cases (86%); however, 68% of the reported outbreaks were of unknown etiology (157). Foodservice establishments (restaurants, cafeterias and delicatessens) were responsible for the highest percentage (43%) of the outbreaks, followed by private residences (21%). Contributing factors to the outbreaks during this period were improper holding temperatures (34.1%), poor personal hygiene (17.8%), contaminated equipment (14.5%), inadequate cooking (10.0%) and food from an unsafe source (5.6%) (157). Beef, chicken, turkey, other/unknown meat, pork, ham and sausage accounted for 6.8, 3.1, 2.3, 2.3, 1.4, 1.2 and 0.2%, respectively, of the total number of outbreaks where the vehicle of transmission was known (967 outbreaks). The respective percentage of cases where the vehicle of transmission was known (58,908 cases) for each of the above meat products was 5.4, 1.9, 1.3, 1.1, 1.1, 0.5 and 0.1%, respectively (157). These products thus accounted for 17.3% of the total outbreaks and 11.4% of the total cases of known etiology during the period of 1993–1997. Bacterial pathogens that were involved in the outbreaks through consumption of meat and meat products during this 5-year period were *Bacillus cereus*, *Campylobacter*, *C. botulinum*, *C. perfringens*, pathogenic *E. coli*, *Salmonella*, *Shigella*, *S. aureus* and *Y. enterocolitica* (Table 52.6) (157).

Other documented or suspected bacterial pathogens of potential concern in meat and meat products are *L. monocytogenes* and *Aeromonas hydrophila*, as well as *Arizona hinshawii*, *Bacillus anthracis*, *Brucella* spp., *Chlamydia psittaci*, *Coxiella burnetii*, *Erysipelothrix rhusopathiae*, *Francisella tularensis*, *Leptospira* spp., *Mycobacterium* spp., *Pseudomonas* spp. and *Streptococcus* spp. (158). As it is beyond the scope of this chapter to go into extensive

TABLE 52.6
Number of Reported Foodborne Disease Outbreaks for Meat and Meat Products by Etiology and Vehicle of Transmission – United States (Includes Guam, Puerto Rico, and the U.S. Virgin Islands), 1993–1997

	Vehicle of Transmission						
Etiology	**Beef**	**Pork**	**Chicken**	**Turkey**	**Ham**	**Sausage**	**Other/ Unknown Meat**
Bacterial							
Bacillus cereus	-	1	1	-	-	-	1
Campylobacter	-	1	-	-	-	-	-
Clostridium botulinum	-	-	-	-	-	-	2
Clostridium perfringens	11	1	-	2	-	-	2
Pathogenic *Escherichia coli*	21	-	-	-	-	-	2
Salmonella	14	4	6	6	1	-	6
Shigella	-	-	1	-	-	-	-
Staphylococcus aureus	4	1	1	4	7	-	-
Yersinia enterocolitica	-	2	-	-	-	-	-
Other bacterial	2	-	-	-	-	-	1
Parasitic							
Trichinella spiralis	-	-	-	-	-	-	1
Viral							
Other viral	-	-	1	-	-	-	-
Confirmed etiology	52	10	10	12	8	-	15
Unknown etiology	14	4	20	10	4	2	7
Total 1993–1997	66	14	30	22	12	2	22

Source: Ref. 157.

detail about each of the etiologic agents involved in foodborne illness (159), a few of the important bacterial agents have been chosen and are discussed briefly. They have been separated into common and emerging bacterial pathogens.

1. Common Bacterial Pathogens

FoodNet data for the year 2002 showed that *Salmonella* was responsible for causing the highest total number of cases of gastrointestinal illness among the bacterial agents (155). The *Salmonella* genus comprises two species: *Salmonella enterica*, which is divided into seven subspecies groups (I, II, IIIa, IIIb, IV, VI and VII), and *Salmonella bongori* (160). There are approximately 2,600 serotypes of *Salmonella*, of which *S*. Typhimurium and *S*. Enteritidis are the most prevalent in the U.S. These serovars cause disease in humans, cattle, poultry, sheep, pigs, horses and wild rodents (161). The emergence of the multi-drug-resistant strain, *S*. Typhimurium DT104, has been the cause of widespread concern regarding the use of antimicrobial agents in agriculture (162). Raw meat, and especially poultry, is frequently contaminated with *Salmonella*, which originates from the intestinal tract or fecal material found on the hair, feathers, feet and skin of the animals. Processed meats and ground products reportedly have the highest prevalence of *Salmonella* (162).

Staphylococcal intoxication is one of the most common types of foodborne disease worldwide caused by the ingestion of food contaminated with one or more preformed heat-resistant enterotoxins produced by *S. aureus* (163). This organism is ubiquitous in nature and can be found in the nasal passages, throat and skin of humans and most warm-blooded animals, including food animals; in fact, 30–50% of humans are carriers of *S. aureus* (163,164). The primary source of contamination of foods implicated in foodborne illness are food handlers. Foods are normally contaminated post-processing and then stored at warm temperatures for adequate time to allow for significant growth and production of enterotoxin by the organism (163,164).

Clostridium perfringens forms part of the normal flora of the gastrointestinal tract of humans and animals. Ingestion of $>10^8$ vegetative cells of this organism followed by the production of enterotoxins during sporulation in the small intestine leads to a toxicoinfection (165,166). Cooked meat and poultry that have been cooled over a long period of time at ambient or warm temperatures are common vehicles of foodborne illness (166).

Clostridium botulinum produces neurotoxins which are considered to be the most toxic of all natural substances (164). Seven types (A through G) of *C. botulinum* are currently recognized, based on the antigenic specificity of the toxins. The organism is further divided into four distinct groups based on their DNA homology and reactions to specific substrates (167). Group I includes all of type A and the proteolytic strains of types B and F; Group II includes the nonproteolytic strains of types B and F, and all of type E strains, all of which are able to grow at refrigeration (3.3°C) temperatures; Group III includes all the strains of type C and D; Group IV includes all the strains of type G, which based on their biochemical properties are proposed to form a new species, *C. argentinense* (167,168). The spores of the proteolytic strains (Group I) are more heat resistant than those of the nonproteolytic strains, a fact that led to the development of the 'botulinum process' or '12-D cook' for low acid canned foods (167).

Different types of *C. botulinum* appear to predominate in different parts of the world; for example, the main cause of *C. botulinum* outbreaks in the U.S., China and Argentina is type A, while in central Europe, type B is responsible for most outbreaks. Furthermore, type A is mainly associated with vegetables, while type B is often linked with meats (169). There are different clinical forms of botulism; foodborne, infant, wound and a fourth classification currently called 'adult infectious botulism.' In foodborne botulism, illness is caused due to ingestion of food that has been undercooked or improperly stored or reheated, resulting in toxin production due to growth of the organism (170). With regards to infant and adult infectious botulism, spores that are ingested or inhaled germinate, in the absence of competition in the intestine, resulting in toxin production (167).

2. Emerging Bacterial Pathogens

Outbreaks involving *C. jejuni* are relatively uncommon (157). However, this organism is believed to be one of the most commonly diagnosed causes of sporadic cases of gastroenteritis in the U.S. and other industrialized countries. *Campylobacter jejuni* and other *Campylobacter* spp. are estimated to cause an annual 14.2% of the total foodborne illnesses in the U.S. (154). The infectious dose of *C. jejuni* is low, ranging from 500 to 1000 cells (171). The main vehicle of foodborne illness by *Campylobacter* is poultry, either due to handling raw or eating undercooked poultry, and especially through cross-contamination of other foods. Other sources include raw milk, water, pork, beef, lamb and seafood (172). Of great recent concern is the emergence of fluoroquinolone-resistant strains of *C. jejuni* isolated from patients in Western Europe, shortly after the approval of fluoroquinolones for veterinary use (173).

Escherichia coli is the dominant Gram-negative facultative anaerobe in the gastrointestinal tract of humans and other warm-blooded animals and is generally considered harmless (174). Pathogenic strains of *E. coli*, however, also exist which can cause distinct syndromes of diarrheal disease (159). Currently, at least six categories of pathogenic *E. coli* are recognized; they include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli*

(EHEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (175–177). Unlike most foodborne pathogens, diarrheagenic *E. coli* are tolerant to acidic environments and have been shown to be resistant to acetic, citric and lactic acids applied at concentrations as high as 1.5% (159). More information about each of these diarrheagenic groups can be found in book chapters and reviews by Levine (175), Doyle and Padye (176), ICMSF (164), Donnenberg and Nataro (178), Fratamico et al. (177) and Bacon and Sofos (159).

Enterohemorrhagic *E. coli* causes the majority of the most severe outbreaks associated with pathogenic *E. coli* in the U.S. and other developed countries (177). This group includes *E. coli* serotypes that share the same clinical, pathogenic and epidemiologic features as *E. coli* O157:H7, which is responsible for the greatest proportion of *E. coli*-associated diarrheal disease cases in North America and the U.K. *E. coli* O157:H7 is estimated to cause approximately 73,000 cases of foodborne illness in the U.S. each year (154). Serotypes belonging to the EHEC group produce Shiga toxins, otherwise known as Shiga-like toxins, verotoxins or verocytotoxins, and are associated with hemorrhagic colitis and hemolytic uremic syndrome. Non-O157:H7 EHEC serotypes seem to be more important in some countries in the southern hemisphere like Argentina, Australia, Chile and South Africa (174). *E. coli* O157:H7 was first identified as a pathogen in 1982 when it was epidemiologically linked to the consumption of undercooked ground beef in the U.S. (179). Since then numerous outbreaks associated with this serotype have been documented in Canada, Japan, the U.K. and the U.S. (180). The largest reported outbreak occurred in Japan in 1996, with a total of 9,578 reported cases and 11 deaths, which were linked to the consumption of radish sprouts (181).

Cattle have been identified as a major reservoir of *E. coli* O157:H7. It has been isolated from the oral cavity (13), rumen contents (182), the hide (11,13) and feces (11,13,183,184) of cattle. A study conducted in the U.S. showed that *E. coli* O157:H7 is present in 28% of fecal samples taken from cattle in slaughterhouses during the summer months (11). It is therefore not surprising that undercooked ground beef is implicated in a large number of the cases linked to *E. coli* O157:H7 infection. The FSIS has declared *E. coli* O157:H7 as an adulterant in ground and other non-intact beef products. Results from a FSIS testing program to enforce this regulation showed that 0.78 to 0.86% out of 6,374 to 7,026 samples tested during the period of 2000 to 2002 were positive for this pathogen. Besides foods of bovine origin, outbreaks due to *E. coli* O157:H7 have been linked to raw milk, water, apple cider, mayonnaise, cantaloupe and lettuce (178).

Listeria monocytogenes is the causative agent of approximately 0.02% of all foodborne illnesses in the U.S., annually. Its associated illness, listeriosis, however, accounts for approximately 28% of deaths due to foodborne illness (154). There are two variants of the disease: invasive and non-invasive listeriosis. The invasive form mainly affects specific segments of the population, including the elderly, neonates, pregnant women and immunocompromised individuals (185,186). The non-invasive variant has been recently described and causes febrile gastroenteritis. Among the foods identified as causes of non-invasive listeriosis are cold corn salad, tuna fish and cold-smoked rainbow trout (187,188).

Listeria monocytogenes is widely distributed in nature, including soil, decaying vegetation, animal and human feces, sewage, silage and water. It is a psychrotroph and can grow at temperatures as low as –0.4°C and up to 45°C. Furthermore, it can grow at pH levels of 4.4 to 9.4, a_w levels of >0.92 and NaCl levels of 10% (189). Survival of the pathogen, however, has also been reported at levels of 25.5% NaCl in trypticase soy broth for 24 days at 22°C and >132 days at 4°C (190). Furthermore, *L. monocytogenes* can survive freezing and drying, as well as low or oxygen-free and elevated carbon dioxide atmospheres encountered in MAP (189).

Foods implicated in outbreaks of listeriosis include milk and dairy products, seafood products, vegetables, coleslaw and ready-to-eat meat products (186,191). According to a FSIS monitoring program for ready-to-eat products conducted at FSIS-inspected establishments during the period of 1990 to 1999, *L. monocytogenes* prevalence rates were 5.16% for sliced ham and luncheon meats, 3.56% for small diameter cooked sausages such as franks, 3.25% for fermented sausages (data were only collected from 1997 and onwards), 3.09% for cooked beef, roasted beef or cooked, corned beef, 3.03% for salads and spreads, 2.12% for cooked poultry products, 1.31% for large diameter cooked sausage, and 0.52% for jerky (192). In a more recent study, analysis of 31,705 samples belonging to eight different categories of ready-to-eat foods from retail markets in Maryland and California showed the prevalence of *L. monocytogenes* to be 1.82%, with prevalences ranging from 0.17 to 4.7% among the product categories (193). Furthermore, Wallace et al. (194) found 1.6% of 32,800 packages of frankfurters to be positive for the pathogen. Contamination of ready-to-eat products with *L. monocytogenes* occurs primarily during post-processing steps like slicing, dicing and packaging. Of particular concern is the establishment of virulent strains of the pathogen in the food processing environment which can potentially contaminate multiple lots of product over days or months of production (191). For example, the strain involved in the 2000 multistate outbreak of listeriosis in the U.S. (serotype 1/2a), where turkey deli meat products were implicated, reportedly had persisted in the processing environment for more than 10 years (186,195). Of the 13 serotypes of *L. monocytogenes* that can cause disease, three serotypes (4b, 1/2a and 1/2b)

account for 89 to 96% of the sporadic and outbreak cases of human listeriosis that occur worldwide (196). The most publicized outbreaks in the past 20 years in North America and Europe have involved serotype 4b (186).

In view of the high mortality rate of *L. monocytogenes* (154), as well as outbreaks associated with the consumption of contaminated ready-to-eat meat and poultry products (195,197,198), the FSIS has published an interim final rule to control this pathogen in these products (199). Briefly, according to this regulation, establishments that produce ready-to-eat meat or poultry products that support growth of the pathogen and are exposed to the environment after lethality treatments are required to include in their HACCP plans, or in their sanitation operating procedures or other prerequisite programs, one or more validated measures that prevent product adulteration with *L. monocytogenes*. Three alternative methods to prevent or control *L. monocytogenes* during post-lethality exposure of products are proposed in the regulation: (i) application of both a post-lethality treatment and an antimicrobial agent or process; (ii) application of either a post-lethality treatment or an antimicrobial agent or process; and (iii) require no application of a post-lethality treatment or process, but instead require the combination of a sanitation program with microbiological testing of food contact surfaces to hold products when positive results are obtained (199).

Bacillus cereus causes two types of intoxications: one is diarrheal and the other emetic. In Japan, the occurrence of the emetic syndrome is reportedly ten times that of the diarrheal syndrome. Conversely, in North America and Europe, the diarrheal type is more common (200). Foods usually implicated in the diarrheal syndrome are meats, vegetables, sauces and puddings, while foods containing rice are associated with the emetic syndrome (201). The number of cells of the organism needed to cause illness is $>10^5$/g.

Yersinia enterocolitica is an infrequent cause of foodborne illness; however, it can cause severe foodborne infection and pseudoappendicitis (4,159). Pigs are regarded to be the main reservoir of *Y. enterocolitica* bioserotypes that are pathogenic to humans (164). The most common bioserotypes involved in foodborne illness are O:3, O:8, O:9 and O:5, 27. *Y. enterocolitica* is a psychrotroph and is able to proliferate at temperatures of 0 to 45°C (159). Storage of food at refrigeration temperatures, therefore, should not be considered an effective way to control this pathogen.

3. Viruses and Parasites

Foodborne transmission of viruses has in recent years been recognized as an important cause of foodborne illness. More than 9 million cases of viral foodborne illness of known etiology are estimated to occur annually in the U.S. (154). According to data collected in the U.S., however, outbreaks of viral foodborne illness due to the consumption of land muscle foods are rare (157). Viruses reported to cause foodborne illness include, among others, hepatitis A, Norwalk and Norwalk-like (now known as norovirus), rotavirus, astroviruses and enteroviruses (159,202). In general, transmission of these viruses occurs due to inadequately cooked foods, cross-contamination before consumption and poor sanitation (4,159).

Parasitic agents, on the other hand, are estimated to result in more than 350,000 cases of foodborne illness of known etiology in the U.S. annually (154). Parasites of concern in foods of meat origin include, among others, *Toxoplasma gondii*, *Sarcocystis* spp., *Trichinella* spp. and *Taenia* spp. (203). Treatments that inactivate parasites in foods include proper cooking, freezing, salt, chemicals and ionizing radiation (4,159).

IV. METHODS FOR CONTROL OF MICROORGANISMS IN MEAT AND MEAT PRODUCTS

A. General

Meat and poultry products are highly perishable foods, and, if not properly handled, processed and preserved, may support growth of spoilage and pathogenic microorganisms (4). The major technologies that are employed to preserve the quality and microbiological safety of these and other food products include (204):

a) Procedures that prevent or minimize the access of microorganisms to the product.
b) Procedures that reduce initial contamination by removal or inactivation of microorganisms which have gained access.
c) Procedures that inactivate microorganisms on products.
d) Procedures that prevent or slow down growth of viable microorganisms which have gained access and have not been inactivated.

Procedures that restrict the access of microorganisms include mainly sanitation and hygienic procedures or packaging technologies such as the aseptic packaging of thermally processed foods. Procedures that reduce initial levels of microorganisms, which have gained access to the food, include decontamination technologies such as washing, application of hot water, steam, acids or other chemicals, and their combinations. Procedures that inactivate microorganisms remaining on products include heat or thermal processing, ionizing radiation, high hydrostatic pressure and electric shock treatments. Despite their ability to remove or inactivate the microorganisms, some of the above technologies may also result in the inhibition of microbial growth during storage. Preservation technologies that slow down or

prevent the growth of viable microorganisms rely on the control of factors affecting microbial growth. Microbial growth on meat and poultry products, or other foods, is affected by the type and extent of initial contamination and by factors intrinsic or extrinsic to the product (70). Among these, the most important ones, especially for fresh products, are extrinsic factors such as storage temperature and gas atmosphere, while intrinsic factors such as pH, water activity and antimicrobials become more important in processed products. Major preservation technologies based on the inhibition of microbial growth include temperature control, decreased water activity (curing, drying), acidification, vacuum and modified atmosphere packaging, fermentation-biopreservation, and addition of preservatives. In addition to the extreme modification of a single factor, inhibition of microbial growth is often achieved with a combination of preservation technologies at individually sublethal levels that interact to yield the multiple 'hurdle' effect (205–207). In the next paragraphs the most important traditional and emerging preservation technologies applied to meat and poultry carcasses or products are discussed. The discussion is focused on current technologies and new developments of decontamination and preservation methods including animal carcass decontamination (physical, chemical and combinations), emerging decontamination or preservation technologies (irradiation, high hydrostatic pressure, pulsed-field electricity, ultrasonic energy, UV light, oscillating magnetic fields, controlled instantaneous decompression), preservative packaging (modified atmosphere packaging, active packaging) and biopreservation. Traditional processing and preservation technologies are briefly discussed.

B. Decontamination Technologies

Highly publicized outbreaks of foodborne disease associated with meat and poultry have increased consumer concerns and interest in the safety of these products. In response, the industry is implementing decontamination interventions to improve the microbiological quality of fresh meat and poultry (4,20,208–210). Decontamination treatments can roughly be divided into three types (208): chemical, physical and combinations of the two. Physical treatments are based on the use of knives for removal, by cutting of visually soiled tissue, cold or hot water (wash, rinse, spray), vacuum and/or steam, high hydrostatic pressure, irradiation, pulsed-field electricity, ultrasonic energy and UV light (the latter technologies apply more to processed products than animal carcasses). Chemical decontamination includes treatments with chlorine, organic acids, inorganic phosphates, bacteriocins, organic preservatives and oxidizers. Decontamination treatments may be applied at different stages of production and processing including animal (before slaughter), carcass, primal cuts and final product.

1. Animal Cleaning

As has been mentioned, live animal contamination, in addition to environmental plant contamination, is considered as the most significant source of carcass and meat contamination. One approach to potentially reduce the external animal contamination and subsequently carcass contamination is to clean or wash the hide of the animal with water or chemical solutions before slaughter. There are, however, concerns related to preslaughter washing of animals since fecal material and the microorganisms associated with it may be more readily spread through wet animals. Bell (211) indicated that carcass contamination resulting from contact with a clean hide (i.e., a hide with no visible evidence of fecal contamination) was appreciably lower than that following contact with a fecally soiled hide that had undergone preslaughter washing. Furthermore, a higher contamination level was found on lamb carcasses derived from preslaughter washed animals than on unwashed animals (212,213). In another study carried out in Ireland, it was shown that preslaughter washing of pigs with cold water did not lead to significant changes in total viable counts of the live animals, but *Salmonella* incidence was reduced from 27% on live animals prior to transport to the abattoir to 10% after washing (214). Furthermore, a significant reduction of hide fecal contamination with *E. coli* O157:H7 was obtained on cattle that were washed with a power-hose for 3 min before the slaughter process; a lower recovery of the organism was also found on the resultant carcasses (215). In general, the effectiveness of animal cleaning and washing on the microbiological quality of the carcasses have been variable, while application of these procedures is often difficult due to climate, type of animal and the availability of facilities (20). Preslaughter animal washing has been applied for sheep in New Zealand (212), and for cattle in Australia and by some plants in the U.S., especially in recent years. A means of minimizing accidental transfer of contamination from the exterior of animals that have undergone preslaughter washing to the carcass surface and plant environment is to reduce slaughter speeds, and to modify the steps or the equipment involved in hide removal (15,216,217).

An additional hide decontamination process is chemical dehairing; a patented process (218) for cattle early during slaughter to remove hair, mud, manure and other external contaminants and thus, to minimize carcass and plant contamination from these sources (15,20,217). Application of this process to artificially contaminated beef hide samples resulted in significant reductions of inoculated meat pathogens such as *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* (219,220), and in 1995, FSIS approved commercial trials for testing of the chemical dehairing process. Schnell et al. (221) studied a commercial application of chemical dehairing in a beef

slaughterhouse. Although these authors reported that chemical dehairing resulted in a reduction of the visible contamination of cattle compared to the conventional process, the microbial loads were not significantly different between the two processes. It needs to be noted, however, that in the latter study both dehaired and nondehaired animals were processed in the same facility during the same day and thus the weak effectiveness of chemical dehairing could be attributed to the contamination of the plant environment by the nondehaired animals. In an additional study carried out in a commercial beef slaughtering facility, however, chemical dehairing was found to significantly reduce bacterial levels of carcasses immediately after hide removal (pre-evisceration) by approximately 2 logs (222). Furthermore, carcasses that had undergone chemical dehairing had a significantly lower prevalence of *E. coli* O157:H7 (1%) than carcasses that had not received the chemical treatment (50%) (222).

2. Carcass Decontamination

In general, carcass decontamination technologies in the U.S. are applied immediately after hide removal but before evisceration, as well as at the end of the dressing process, before carcass chilling. Decontamination technologies are based on treatments with water of various temperatures and pressures and other chemical solutions. The effectiveness of these treatments in reducing microbial contamination is affected by a number of factors including water pressure, temperature, chemicals used and their concentration, time duration of exposure (which depends on speed of slaughter and length of the application chamber), method of application and time or stage of application during slaughter and processing (20,217). Decontamination technologies that have been proposed, applied or tested for their effectiveness on the reduction of carcass microbial loads include treatments with water or steam (hot water, steam pasteurization, steam-vacuum), and chemical solutions, mostly organic acids (lactic, acetic and citric), as well as chlorine and chlorine dioxide, trisodium phosphate, acidified sodium chlorite, peroxyacetic acid, cetylpyridinium chloride, hydrogen peroxide, ozone, sodium bisulfate, sodium hydroxide, sodium chloride, and protein compounds such as lactoferrin (15,20,208,210). Today, in the U.S., decontamination systems are approved by FSIS if the agents used: i) are 'Generally Recognized as Safe' (GRAS), ii) do not create an 'adulterant' situation, iii) do not create labeling issues (i.e., added ingredients), and iv) can be supported with scientific studies as being effective (15). Among the agents that have been approved for use in foods by FSIS are acetic, lactic, peroxyacetic, citric, malic, propionic and tartaric acid, chlorine, trisodium phosphate, acidified sodium chlorite, hydrogen peroxide, ozonated water and lactoferrin (available at: http://www.fsis.usda.gov/OPPDE/rdad/FR Pubs/88-026F.htm; http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.htm). Some of these are extensively used in commercial plants in the U.S. In contrast, no chemical or physical treatments of meat and poultry carcasses are allowed by European Union regulations (208).

a. Spot carcass and pre-evisceration decontamination

Under the 'zero tolerance' policy of the U.S., knife-trimming is required to remove visible contamination on carcasses before any washing or other decontamination treatment is allowed. As an alternative, in 1996, FSIS approved the use of steam-vacuuming for removal of fecal and ingesta contamination spots of <2.5 cm in diameter on carcasses, through the use of a hand-held piece of equipment. This method uses hot water and/or steam to loosen soil and inactivate bacteria, followed by removal of the contaminants through application of a vacuum; this process, thus, has a combined effect of removing and/or inactivating surface contamination (15,20,223,224). Kochevar et al. (224) reported that application of steam-vacuuming to beef carcasses resulted in reductions of 1.7–2.0 log cfu/cm^2 in aerobic plate counts and 1.7–2.2 log cfu/cm^2 in total coliform counts. After the approval of the two commercially available steam-vacuuming systems, and as a result of their relatively low cost, steam-vacuuming units became very popular in U.S. slaughterhouses as an alternative method to knife-trimming to remove accidental visible carcass decontamination (20). However, the effectiveness of both knife-trimming and steam-vacuuming in reducing carcass contamination depends on employee diligence of application and the operational status of the equipment (20).

As soon as the hide is removed, whole carcasses are sprayed with water and possibly organic acid solution to remove microbial contamination that may have been acquired during the hide removal stage. In general, water and acid treatments applied soon after slaughter (before evisceration) appear to be effective since bacteria have not yet become firmly attached to the muscle (225–227). This is supported by a study by Cabedo et al. (227), where it was found that the number of bacteria removed or inactivated by the application of different spray-washing treatments decreased, as the length of time between contamination of beef carcass tissue and application of the treatments increased. Pre-evisceration washing of carcasses may also reportedly lead to an alteration of the surface physical characteristics (e.g., contact angle and surface free energy) of the carcass tissue, resulting in less attachment of microorganisms (228). Final washing and decontamination of carcass sides with water, organic acids and other treatments are discussed in the following sections.

b. Water

Water sprays of various pressures are used to remove bone dust and to clean and wash carcasses that have passed the

'zero tolerance' for visible soil inspection at the end of the dressing process in the U.S. Water is also used as a carrier of chemical or physical energy when carcasses are decontaminated with chemical solutions, hot water or steam. The use of hot water as a meat decontamination technology has been studied extensively during the last 30 years. Both laboratory scale and commercial plant evaluation studies have found hot water (74°C) to be an effective method of carcass decontamination (223,224,229–231). The application of hot water for meat decontamination may involve immersion or dipping, deluging, rinsing at low pressures and spraying at higher pressures. Each of these approaches has advantages and disadvantages (20). In all cases, however, the effectiveness of the method is strongly dependent on water temperature. Davey and Smith (232) found a linear relationship between water temperature and *E. coli* numbers on beef carcasses decontaminated with hot water by spraying. In general, effective temperatures exceed 74°C and become more effective as they approach 80–85°C (232,233). Gorman et al. (234) reported that spraying of beef carcasses with hot water at 74°C resulted in higher microbial reductions compared to chemical interventions, while at lower temperatures (16–35°C) chemical decontamination was more effective than water treatment.

Another form of hot water decontamination involves exposure of carcasses to pressurized steam (223,235,236). Davidson et al. (235) reported that exposure of whole and cut-up chicken meat to steam at 180–200°C for 20 sec resulted in 1–3 log reductions in aerobic plate counts and in 50% reduction in *Salmonella* contamination. Morgan et al. (236) applied an ultra-high temperature, ultra-short time steam pasteurization process on beef, pork and chicken samples and found a 4 log reduction in inoculated *Listeria innocua*. A patented steam pasteurization process for carcass decontamination developed by Frigoscandia and Cargill, Inc. (The Frigoscandia Steam Pasteurization System™) has been approved for use in the U.S. (237). Phebus et al. (238) and Nutsch et al. (239) evaluated the Steam Pasteurization System™ using *cutaneus truncii* muscles from steers and beef sides, respectively, and reported significant reductions in aerobic plate counts, *E. coli*, coliforms and Enterobacteriaceae, and inoculated *L. monocytogenes, E. coli* O157:H7 and *S.* Typhimurium. The advantages of using steam rather than water for decontamination purposes are the efficiency of heat transfer, lack of residues, and the reduced water and energy usage (20). However, steam pasteurization requires a major capital investment and may present difficulties in application in a continuous production process.

c. Organic acids

In recent years the use of organic acids such as acetic and lactic, for meat and poultry decontamination, has received considerable attention mainly due to their antimicrobial properties and their characterization as GRAS substances (15,217,226,240–242). Decontamination with organic acid solutions of 1–2% may significantly reduce the bacterial load of meat and poultry carcasses (208,224,243). In addition to the immediate microbial reduction, acid decontamination results in a residual antimicrobial effect during storage, which has been attributed to the residual acids remaining on the meat after treatment as well as the sublethal injury of bacteria caused by the acid treatment (244–246). The effectiveness of acid decontamination can be augmented by increasing the temperature of the solution and the time of application. The stage of processing selected for the application is also important, as mentioned previously. The efficacy of the acid treatments may be further influenced by the type of surfaces to which the bacteria are attached. For example, acid treatment of lean meat surfaces results in higher microbial reductions than on fatty tissues (247).

Organic acid decontamination of carcasses has also been shown to reduce pathogen populations (246,248–250). Decontamination of beef surfaces with 2% lactic or acetic acid resulted in 2–3 log cfu/cm^2 reductions of *S.* Typhimurium DT104 and *E. coli* O157:H7 (250). *Salmonella* is generally more sensitive to organic acid decontamination than *E. coli* O157:H7 (251). This was also shown in a study by Samelis et al. (252), where *S.* Typhimurium DT 104 was found to die off after 2 days incubation, at 4 or 10°C, in lactic acid (pH 2.4) and acetic acid (pH 3.1) fresh beef decontamination runoff fluids (washings), while *E. coli* O157:H7 and *L. monocytogenes* survived in lactic acid washings for at least 2 days, and in acetic acid washings for at least 7 and 4 days, respectively, with better survival at 4 than at 10°C (252). Although decontamination with organic acids has been shown to reduce prevalence and concentration of *E. coli* O157:H7 on meat carcasses (251,253), the potential increase in the acid resistance of the pathogen remains an important concern (254–258), and is discussed in a subsequent section. The potential ability of *E. coli* O157:H7 to resist acidity (254,259) has been attributed, among other mechanisms, to binding of the pathogen to collagen of the meat tissue (260). Recent studies have shown that the latter problem may be reduced by spraying the carcass with a non-ionic surfactant such as Tween 20 prior to spraying with the organic acid (261).

In addition to the microbiological point of view, another important issue regarding the use of acid decontamination on meat and poultry is its effect on the quality of the products. Depending on the concentration and intensity of application, acid treatments may sometimes result in undesirable effects in color and/or flavor of the products. Such effects, however, should be only slight and reversible at acid concentrations below 2% (262,263). Discoloration problems can also be prevented by using buffered acids (264). In general, organic acid (lactic or acetic) spraying finds extensive use as a beef carcass treatment after hot water or steam decontamination.

d. Other chemical solutions

Several non-acid chemical solutions have been tested, and in some instances approved and used in the decontamination of meat and poultry (15,20,208,210,217). Chlorine is a common sanitizing agent used in process water during meat and poultry processing. Application of chlorine (20 ppm) reduced *Salmonella* contamination on chicken carcasses (265,266) while chlorine spray-washing (800 ppm) of beef carcass tissue resulted in a mean reduction of 1.3 log cfu/cm^2 of *E. coli* O157:H7 (267). Chlorine dioxide (ClO_2) has been proposed as an alternative to traditional chlorine sanitizing agents. However, the use of ClO_2 (1.33 mg/l) to control *Salmonella* in poultry chiller water did not result in any significant reduction (<0.5 logs) in bacterial counts on the poultry skin (268). Similar results have been reported by Cutter and Dorsa (1995) who found that spraying beef tissue with ClO_2 (20 ppm) for 10 sec at 520 kPa was no more effective in reducing fecal contamination than spraying with water alone. Mullerat et al. (270) applied Salmide®, a commercial complex mixture of chlorine-based components, and reported that it can reduce the levels of *Salmonella* on poultry skin. Although chlorine has been approved for meat and poultry decontamination in many countries, there are some safety concerns regarding its use related to corrosion of metals and formation of harmful chemical reaction products with organic residue materials (20).

Trisodium phosphate (TSP) has also been approved for treatment of beef and poultry carcasses in the U.S. A patented solution of TSP (AvGard™, Rhone-Poulenc, France) has been shown to reduce both spoilage and pathogenic bacteria including *Salmonella, L. monocytogenes*, *E. coli* O157:H7, *S. aureus* and pseudomonads on meat and poultry tissues (234,271–274). Cabedo (275) reported that TSP may inhibit bacterial attachment and thus allow easier bacterial removal by washing. Studies on the effect of TSP on the sensory characteristics of beef and chicken samples have indicated that a 10–12% solution of TSP does not cause any undesirable sensory effects that are detectable by consumers (276–278).

Acidified (citric acid activated) sodium chlorite (ASC) is another antimicrobial that has received approval from the U.S. federal government for use in beef carcass decontamination systems. Spraying of beef carcass surfaces with ASC, at a concentration of 1,200 mg/l, in combination with a water wash, resulted in a 4.5 log cfu/cm^2 reduction of inoculated (5.5 log cfu/cm^2) *E. coli* O157:H7, compared to a 2.3 log cfu cm^2 reduction obtained with a water wash only (279).

Hydrogen peroxide and ozonated water are also among the chemical agents proposed and tested for meat and poultry decontamination (20), and have recently received approval by FSIS for use on meat and poultry products. The antimicrobial effect of hydrogen peroxide is mainly based on the formation of radicals that damage nucleic acids, proteins and lipids. Treatment of beef and lamb carcass tissue with a hydrogen peroxide solution (5%) has been shown to reduce mean bacteria counts by 1–2 logs (224,227,234). Similar microbial reductions have been observed after spray-washing of beef carcasses with ozonated water (227,230,234). As in the case of chlorine, the use of hydrogen peroxide and ozone, despite their bactericidal effect, may be of concern due to their oxidizing effects on fat and muscle pigments.

Decontamination with cetylpyridinium chloride (CPC) has been shown to be effective in reducing levels of pathogens on meat and poultry carcass tissues. Kim and Slavik (280) reported that CPC spraying or immersion of poultry skin reduced the numbers of *Salmonella* by 0.9–1.7 logs and 1.0–1.6 logs, respectively. Cutter et al. (281) showed that spray washing of beef fat with 1% CPC resulted in significant reductions of *E. coli* O157:H7 and *S.* Typhimurium. In an additional study, 0.5% CPC was shown to reduce *E. coli* O157:H7 levels by 4.8 and 2.1 log cfu/cm^2 on beef carcass surfaces and lean tissue, respectively (282). In a study on the effect of CPC on the sensory characteristics of beef trimmings, the authors indicated that treatments with 0.5% CPC did not affect instrumental color or other sensory characteristics of the samples (278).

Peroxyacetic acid has been recently recognized as an effective chemical agent for meat decontamination. A commercial peroxyacetic acid-based solution (Inspexx 200™, Ecolab Company, St. Paul, MN) for pre-chilling application of carcasses has been approved in the U.S. Ransom et al. (282) reported that 0.02% peroxyacetic acid was effective in reducing the bacterial load of beef samples; however, under the conditions of that study, which did not reflect those recommended by the manufacturer, it was found less effective compared to lactic acid (2%; heated to 55°C).

Naidu and Bidlack (283) reported that lactoferrin, an iron binding protein, presents strong antimicrobial activity and can be used as an alternative to other chemical antimicrobial agents. Activation of bovine lactoferrin, which can be extracted in commercial quantities from skim milk or whey, yields a potent bactericidal peptide that has been shown to inhibit and/or inactivate a wide range of meat pathogenic and spoilage bacteria including *L. monocytogenes, Salmonella*, *Campylobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. (284). The patented process of producing activated lactoferrin involves immobilization of milk lactoferrin, via its N-terminus region, on a food-grade glycosaminoglycan (e.g., galactose-rich polysaccharide or carrageenan), solubilized in a precalibrated citrate/bicarbonate buffer system containing sodium chloride and an excess of unbound lactoferrin (284). The resultant activated lactoferrin reportedly acts as a microbial blocking agent that is able to (i) interfere with the adhesion or colonization of microorganisms to biological surfaces (ii) detach viable or non-viable cells from biological surfaces, (iii) inhibit microbial growth,

and (iv) neutralize the activity of endotoxins (284). The U.S. Food and Drug Administration (FDA) recently accepted activated lactoferrin as GRAS while FSIS approved the compound for use on fresh beef (284).

A variety of other chemical compounds such as polyphosphates, benzoates and propionates, sodium hydroxide, sodium bisulfate, etc. have been tested with various rates of success for the decontamination of meat and poultry (15,20,208,210,217). Application of these or other chemicals as meat and poultry decontaminants in the future will depend on their efficacy, safety, effects on quality and cost (20).

e. Carcass decontamination with multiple processes

The use of more than one treatment may lead to synergistic or additive decontamination effects (20) and could be considered as a multiple hurdle approach (206). In fresh meat decontamination, the multiple hurdle decontamination approach may involve the simultaneous application of treatments (e.g., warm acid solutions) or the sequential application of treatments (e.g., hide cleaning, steam-vacuuming, pre-evisceration washing, hot water or steam treatment, organic acid rinsing). Several studies on the comparison between the effectiveness of single and multiple decontamination treatments have reported that the latter result in significantly higher microbial reductions (Table 52.7) (10,285,286). Bacon et al. (10) studied multiple-sequential decontamination interventions including carcass washing, steam-vacuuming, hot water and organic acid rinsing before and after evisceration in commercial beef slaughter facilities. The authors reported that *E. coli* counts were reduced from an initial range of 2.6–5.3 log cfu/cm^2 to a final range of 0.9–1.3 log cfu/cm^2 after carcass chilling. Graves Delmore et al. (287) used various technologies and interventions to decontaminate beef adipose tissue samples and reported that a higher reduction in *E. coli* counts was achieved by a series of four processes including pre-evisceration washing, warm-water washing and two steps of acetic acid rinsing. In addition to the number and intensity, the sequence of treatments can be very important in a multiple decontamination process (Table 52.7). For example, Castillo et al. (286) reported that a lactic acid rinse, following hot water washing, was more effective than their use in the opposite order. Overall, when the appropriate number and sequence of hurdles are selected, the multiple hurdle decontamination approach can be a very effective tool for improving the safety of meat and poultry carcasses, and may minimize the potential for resistance development and cross protection (258).

f. Concerns of decontamination technologies

Despite the generally accepted effectiveness of decontamination technologies in reducing numbers and prevalence of pathogenic and/or spoilage bacteria on meat and poultry carcasses, there are a number of concerns associated with their use. Application of spraying/rinsing treatments to carcasses may lead to spreading and redistribution of bacteria over the carcass or penetration into the tissue (20). These problems, however, can be avoided by appropriate selection and adjustment of the factors affecting the efficacy of the decontamination treatment. For example, the issue of bacterial redistribution may be addressed by using decontamination interventions that may inactivate (hot water, steam, chemical solutions) rather than remove contamination. As mentioned previously, the period of time before decontamination has an important effect on bacterial attachment and biofilm formation; thus, decontamination treatments applied before evisceration will be more effective since bacterial attachment is still weak.

Another important concern associated with the use of decontamination technologies is the potential development of stress-resistant pathogens (20,242,252,258,288). Heat or acid resistance are important physiological characteristics that may contribute significantly to the behavior of pathogenic microorganisms during meat processing, cooking or in host systems (gastric secretions, phagocytosomal vacuoles) where acidity is the final barrier that the pathogen must overcome before pathogenesis. The potential concern for development of stress-resistant pathogens can be attributed to the 'stress hardening' phenomenon which refers to the increased tolerance of a pathogen to a specific lethal stress after adaptation to the same or a different sublethal stress environment (258,289). Several studies have demonstrated that adaptation of pathogenic bacteria such as *L. monocytogenes, E. coli* O157:H7 and *Salmonella* to a mild

TABLE 52.7
Mean Log Reduction (log cfu/cm^2) of *Salmonella* Typhimurium, *E. coli* O157:H7, Aerobic Plate Count (APC), Total Coliforms and Enterobacteriaceae on Beef Carcass Surfaces as Affected by the Type of Decontamination Treatment

Treatment	*Salmonella* Typhimurium	*Escherichia coli* O157:H7	APC	Total coliforms	Enterobacteriaceae
Water wash	2.3	2.4	1.6	1.8	1.7
Water wash + hot water	4.2	4.0	3.2	4.0	3.8
Water wash + lactic acid	>4.9	4.6	4.6	4.5	4.3
Water wash + hot water + lactic acid	>4.5	4.9	3.6	>4.6	>4.7
Water wash + lactic acid + hot water	4.4	4.4	4.1	>5.0	>4.9

Source: Ref. 286.

stress environment results in increased survival under stress conditions that would be lethal for non-adapted cells (288–293). In addition to increased stress resistance, adaptation may lead to mutations with enhanced virulence (294) since microorganisms may sense the unfavorable conditions as a signal for the expression of virulence factors (295). It needs to be noted, however, that the majority of the studies on the 'stress hardening' phenomenon have been performed in laboratory media and thus more research is needed on the investigation of stress adaptation in actual foods. Nevertheless, an evaluation of the contribution of decontamination interventions to food safety improvement should take into account the potential development of stress-resistant pathogens. It should be stressed, however, that irrespective of potential stress adaptation inducement on survivors, decontamination treatments are highly effective in reducing microbial contamination of carcasses and thus, allowing meat operations to meet regulatory performance standards and industry specifications (15,20,217,258). Proposed strategies to control stress resistance of bacteria involve the continued application of lethal levels of preservatives, or optimization of decontamination interventions, in type, intensity and sequence, to maximize microbial destruction and minimize resistance development (258,296).

C. Meat Processing and Preservation

Processing and preservation technologies involving physical, chemical and biological factors are used by food processors to ensure the safety and stability of foods by inactivating or inhibiting growth of spoilage and pathogenic microorganisms. Physical preservation methods include refrigeration, freezing, heating, drying, smoking, packaging and irradiation. Addition of antimicrobial agents or preservatives to meat products, including sorbates, benzoates and lactates, or curing agents such as sodium nitrite or salt, as well as treatment of products with acidifying agents like acetic and lactic acid, all constitute methods of chemical preservation. An example of biological preservation, or biopreservation, is fermentation where lactic acid bacteria may be used to inhibit the growth of spoilage and pathogenic microorganisms by the production of inhibitory metabolites by the lactic acid bacteria (297). Details of these processing and preservation methods are excellently reviewed by Claus et al. (298), van Laack (299) and Pearson and Gillett (300). Combination of these preservation technologies, applied individually at sublethal levels (hurdle technology), can result in a product that is more microbiologically stable and safe, as well as of higher nutritive and sensory quality (207).

Before discussing some of these technologies, however, another important aspect that can greatly affect the quality and safety of the final product is the cleaning and sanitation operations of a food-processing establishment.

1. Cleaning and Sanitation

During food processing procedures, equipment surfaces and the surrounding environment inevitably become soiled and require cleaning. Failure to effectively remove the soil from these surfaces may result in the soil becoming a niche for microorganisms. These organisms then have the potential to attach to the surface and form biofilms, which upon detachment may cross-contaminate foods. An effective sanitation program includes cleaning and disinfection; however, to ensure good sanitation and to maximize the effectiveness of the cleaning operation, the design and layout of the processing area and food plant are of primary concern (301). The importance of factory design and food processing equipment are described by Forsythe and Hayes (302). According to the same authors, there are three steps involved in cleaning and two steps in disinfection (sanitation). Cleaning involves removal of gross soil or dirt, removal of residual soil with detergent, and rinsing of the cleaned surface to remove detergent and soil; while sanitation, which often follows cleaning, involves the use of an antimicrobial agent to inactivate microorganisms, followed by rinsing off of these agents. Removal of soil normally involves the use of hot or cold water and cleaning aids such as brush bristles. The type of detergent used is important and ideally should be able to disperse insoluble materials, emulsify and saponify fats, rinse well, remove or inactivate calcium and magnesium salts dissolved in hard waters, and should not be corrosive to equipment surfaces (301,302). Detergents may be classified into inorganic alkalis (e.g., sodium hydroxide, sodium carbonate), inorganic and organic acids (e.g., phosphoric and citric acids, respectively), surface-active agents (e.g., synthetic detergents which are either anionic, cationic, non-ionic or amphoteric), and sequestering agents (e.g., sodium polyphosphates and EDTA). Modern detergent formulations contain a mixture of different chemicals, each of which contributes to the desired properties of the detergent (302).

Application of the sanitizer follows rinsing of the detergent from the surfaces. The sanitizer should ideally be able to rapidly inactivate Gram-negative and Gram-positive bacteria, be stable in the presence of organic residues, be able to work in a wide pH range, water hardness, and temperatures, and should be readily soluble in water and readily rinsable. Sanitizers used in the food industry can be classified into chlorine releasing compounds, quaternary ammonium compounds, iodophors and amphoteric compounds (301,302).

2. Processing for Microbial Destruction

Application of heat, or thermal processing, is one of a few preservation methods that result in the inactivation of microorganisms, with time and temperature of cooking determining the number of organisms destroyed. Furthermore, thermal inactivation of microorganisms is

dependent on the level, type and heat resistance of the organisms, as well as the environment (moisture and fat content, pH, salt, nitrite) (298,299). Most spoilage and pathogenic bacteria are sensitive to heat and are destroyed due to inactivation of bacterial enzymes, protein denaturation and increased membrane permeability (299). Most heat labile organisms, like micrococci, pseudomonads and Enterobacteriaceae are inactivated after several minutes at 65°C; destruction of certain spores, however, may require heating to at least 115.6°C for extended periods of time (298). Thermal processing may be classified as 'pasteurization' or 'sterilization,' based on the level of destruction of bacteria and spores. Pasteurization results in the destruction of vegetative cells of pathogenic bacteria; however, heat-resistant spoilage organisms and spores may survive during this process. Commercially sterile products are processed to be free of detectable microbial contaminants, but may contain spores of thermophilic bacteria that do not germinate below 43°C (299). The internal temperature reached in commercially sterile meat products is generally at least 107.2°C, but depending on the salt and nitrite content of the product, the temperature can be as low as 101.7°C (300). These products remain stable and safe even after long-term storage at ambient temperatures in temperate climates.

Other processing technologies that result in inactivation of microorganisms include irradiation and high hydrostatic pressure, and are discussed in a subsequent section.

3. Processing for Microbial Inhibition

As mentioned previously, preservation technologies that inhibit the growth of microorganisms in foods rely on the control of one or more factors affecting microbial growth (e.g., temperature, water activity, pH, antimicrobials). The most important means of preserving fresh unfrozen meat and other perishable meat products is refrigeration at temperatures between –2 and 5°C (299). Although growth of most mesophilic bacteria at these temperatures is prevented, growth of psychrotrophic and psychrophilic bacteria, including pathogens like *L. monocytogenes*, *A. hydrophila* and *Y. enterocolitica*, is only delayed (due to increased lag times and reduced growth rates) (303). Freezing of meat products, however, results in inhibition of microbial growth with some reduction of microbial levels during freezing, and potential increases during thawing (41,299). Preservation of food by drying is brought about by a reduction in the water activity of the product to a level that prevents growth of microorganisms. Drying methods include hot air drying, refrigerated air-drying, freeze-drying and brine-process drying (299,303). Water activity is also reduced with addition of humectants (e.g., salt, sugar) and by freezing. Smoking is also regarded as a preservation method. Many cured meats are smoked, and this process is either carried out directly from smoldering hardwood or hardwood sawdust, or in liquid form that is produced from natural smoke precipitated in water or oil (304). Smoke is composed of many different compounds including alcohol, carbonyls, hydrocarbons and gases; however, the antimicrobial properties of smoke are due to phenols and acids present in the smoke, as well as the heat that is associated with wood smoking (300). Foodborne pathogens, including pathogenic *E. coli* and *S. aureus*, are reportedly inactivated by commercially available liquid smoke products used in processed meats (297).

The use of salt as a preservative serves to inhibit microbial growth by reducing the water activity and increasing the osmotic pressure of the food (70,305). Salt is also the major ingredient, by weight, of curing mixtures; the active curing agent, however, is nitrite, and to a limited extent, nitrate. These curing agents function to stabilize the color of lean tissues, impart the characteristic flavor of cured meat, retard development of rancidity and provide microbial stability by inhibiting the growth of microorganisms, including *Acinetobacter/Moraxella*, *Flavobacterium*, *Pseudomonas*, *Enterobacter*, and *Escherichia* spp., as well as the pathogen *C. botulinum* (49,300,306). The antibotulinal effect of nitrite, however, occurs only in combination with heat, as is the case with canned products. Furthermore, the inhibitory effect of nitrite is reportedly pH dependent, with an approximate tenfold increase of its inhibitory effect for every one pH unit reduction (299).

Addition of antimicrobial agents in meat products is a widely used method of preservation. The use of antimicrobial agents in ready-to-eat meat and poultry products is, in fact, one of the alternatives given to the U.S. food industry by FSIS, as a means to control *L. monocytogenes* in these products (199). Numerous studies have shown the antilisterial effect of GRAS chemical compounds in ready-to-eat meats, including potassium lactate (307,308), sodium lactate and sodium acetate (309–311), sodium diacetate (310,311), and acetic and lactic acid (312). The most widely used chemical preservatives used by the industry, however, are sodium or potassium lactate and sodium diacetate, alone or in combination, at levels of 2% sodium or potassium lactate (on a dry weight basis) and 0.1 to 0.15% sodium diacetate (191). The other alternatives of the final rule for control of *L. monocytogenes* in ready-to-eat meat and poultry products are post-lethality treatments, which may be applied pre-packaging or post-packaging (e.g., radiant heat, hot water or steam pasteurization, high hydrostatic pressure) (313–316) and sanitation measures (199).

4. Preservative Packaging

a. *Modified atmosphere packaging*

Modified atmosphere packaging can be defined as the enclosure of food products in high gas barrier material, in which the gaseous environment has been changed in order to reduce microbial growth and retard enzymatic spoilage, with the intent of extending shelf life (317).

Among the factors limiting the shelf life of meat and poultry products, oxygen can be considered as one of the most important. Consequently, a logical barrier to extend the shelf life of these products is to modify the gaseous atmosphere surrounding them, which is the principle behind modified atmosphere packaging (MAP). A comprehensive list of different gas mixtures and packaging materials with different oxygen transmission rates that have been used to extend the shelf life of different meat types at specific storage temperatures, and the predominant spoilage bacteria isolated from those products can be found in Stanbridge and Davies (138). Four types of MAP can be differentiated by the manner in which spoilage organisms are inhibited (318): (i) in vacuum packaging, the inhibition of aerobic organisms is based on the removal of oxygen from the in-pack environment and the CO_2 production; (ii) In high oxygen MAP, growth of aerobic organisms is inhibited, but not suppressed by moderate concentrations of CO_2; (iii) In low oxygen MAP the low concentrations of O_2 inhibit the growth of aerobic species while the concentration of CO_2 may be sufficiently high to slow down the growth of both aerobic and anaerobic organisms; and (iv) in oxygen-free controlled atmosphere packaging, the absence of oxygen prevents growth of aerobic organisms, while high concentrations of CO_2 inhibit growth of aerobes and organisms tolerant to anaerobic conditions.

b. *Vacuum packaging*

Vacuum packaging is widely used for shelf-ready retail packs of meat products and for primal cuts of raw meat in wholesale storage. Vacuum packaging is not commonly used for display packs of raw meats because the anoxic environments results in a dull, purple color of native myoglobin, which is considered unattractive to the consumers (317,319). The shelf life extension of primal cuts achievable by vacuum packaging can be as long as two- to five-fold compared with aerobic storage depending on their pH, size and film permeability (318,320,321). Beef of pH 5.6 to 5.8, vacuum packaged with film of low oxygen permeability and stored at 0 to 1°C, may have a shelf life of 10 to 12 weeks (144). Growth of *Pseudomonas* under these conditions, where the CO_2 and O_2 concentrations are approximately 20% and $<1\%$ (v/v), respectively, is inhibited.

Vacuum packaging is also applied to cured and other cooked meat (322,323). The major disadvantage associated with the application of vacuum packaging to these products is that both package and meat are subjected to mechanical strain. This may cause product deformations, exudation and even puncture of the pack if bone is present (321). These problems, however, may be overcome with improvements in packaging techniques such as the use of appropriate packaging material, shrink packs, surface sealing and skin packs (4).

c. *High oxygen modified atmosphere packaging*

In high oxygen MAP the gaseous composition is approximately 70% O_2, 30% CO_2 and 0–20% N_2 (318). This type of packaging is used mainly for red meats and it has been introduced to extend color stability and delay microbial spoilage of products on display (319,324). The high oxygen concentration preserves a desirable color by extending the depth of the surface layer of oxygenated muscle tissue while the 30% CO_2 is sufficient to reduce the growth rate of pseudomonads, which dominate the spoilage flora. In general, the storage life of meats in high oxygen MAP can be twice that of meat stored under aerobic conditions. However, spoilage changes of foods stored in high oxygen MAP are comparable to those stored in air, since pseudomonads remain the responsible spoilage organisms.

d. *Low oxygen modified atmosphere packaging*

In low oxygen MAP, the atmosphere is composed almost exclusively of CO_2 and N_2. The exact level of residual oxygen depends on the procedure of air replacement. Without evacuation, oxygen may remain at levels of up to 10% while with evacuation the residual oxygen is about 1% (318). Low oxygen MAP is not commonly used for red meats due to the rapid discoloration of the product. Such packaging is usually applied when product color is not critically dependent on the oxidation state of myoglobin and thus it is more useful for poultry products. The extension of product shelf life achieved with low oxygen MAP can vary significantly depending on the initial atmosphere established in the pack and the extent to which the gaseous composition changes during storage. The spoilage process of low oxygen MAP meats is generally similar to that observed in vacuum packed products since in both cases the limited oxygen concentration inhibits growth of aerobic organisms, and species tolerant to anaerobic conditions dominate the microflora.

e. *Oxygen-free controlled atmosphere packaging*

Oxygen-free controlled atmosphere packaging is based on the complete elimination of oxygen from the package. The strictly anoxic environment may prevent meat discoloration and microbial shelf life may be greatly extended when the air is completely replaced with CO_2 in a controlled-atmosphere package (319). Scientists in New Zealand developed an oxygen-free controlled atmosphere packaging system (Captech system), which is in commercial use with lamb primal cuts and whole carcasses (319,325). The system involves packaging of cuts in heat-shrunk, air-permeable bags, which are then put in a larger pack of highly impermeable film that is flushed with carbon dioxide and sealed. The permeability of the inside packs allows penetration of carbon dioxide during storage and shipment, as well as oxygen after removal from the

external pack and during display for formation of oxymyoglobin, which results in a bright red color. The storage life of such products can be 8–15 times longer than those stored in air depending on their initial contamination, pH and storage temperature. For example, shelf life may be substantially shorter when highly contaminated, high pH products are stored at temperatures above 0°C (318). The success of the packaging method also requires effective evacuation equipment and totally impermeable packaging materials made from aluminium foil laminate with good sealing and puncture-resistant properties. The extended product stability achieved by this type of packaging is likely to ensure its future commercial use with a wide range of meat products.

f. Factors that influence the effectiveness of modified atmosphere packaging

In addition to gas concentration and composition, the packaging material, the storage temperature and the time of MAP application are important factors influencing the shelf life extension conferred by MAP technology (318,321,326–328). Packages are mainly constructed of plastic films, the majority of which are permeable to gases. The extent of gas transmission through a plastic material depends on a number of factors such as type of plastic, thickness of the plastic layer, temperature, humidity and contamination of the plastic material with food components such as fat (318). Thus, proper selection of the plastic film in order to maintain the essential balance of the flushed gaseous atmosphere in the headspace of the pack is crucial. Bacterial inhibition by MAP is also affected by storage temperature. The solubility of CO_2 is inversely proportional to storage temperature and thus low temperatures have a synergistic affect upon its action (327). In addition, the multiple hurdle application (CO_2 and low temperature) leads to a higher inhibition of meat spoilage bacteria (329).

The time of MAP application is another important factor affecting the storage life of the product. If MAP is applied to fresh meat when the spoilage bacteria are still in the lag phase, shelf life may be extended by 50% compared to products stored aerobically, while MAP application during the exponential phase of the spoilage bacteria reduces the shelf life extension to 30% (326). In addition, the effectiveness of MAP is influenced by the extent of contamination at the time of application since high microbial populations result in an increased probability of the presence of some bacteria which are more resistant to the antimicrobial effect of the gaseous atmosphere. In general, the effectiveness of MAP is enhanced with earlier application and lower initial contamination levels.

g. Safety concerns of modified atmosphere packaging

Given that MAP is capable of extending the shelf life of meat products, the major safety concern is whether MAP can inhibit sensory changes by spoilage microorganisms, which ordinarily would warn consumers, while either allowing or promoting the growth of pathogens by limiting the competition with the spoilage flora (321). The impact of MAP on the microbiological safety of meats is not very well defined especially with respect to *C. botulinum*. For example, in sausages and meat sandwiches inoculated with *C. botulinum* (types A and B) and stored at 26°C, toxin was detected at 6 days for aerobically stored sandwiches and at 4 days for anaerobically stored samples (330). The most important observation in the latter study was that anaerobic samples were found organoleptically acceptable when toxic, while the aerobic were not. In a study by Silliker and Wolfe (331), pork cubes inoculated with *C. botulinum* spores and stored at 27°C under MAP were found to contain toxin by day 1 while aerobic samples were toxic by day 2. It is clear that *C. botulinum* may find atmospheric microenvironments allowing growth at highly abusive temperatures and thus the combination of MAP with strict temperature control is of great importance. Even at low storage temperatures, however, it is known that cold-tolerant pathogens such as *Y. enterocolitica, L. monocytogenes* and *A. hydrophila* are able to grow on vacuum-packaged, high pH raw meat (332).

The majority of the available information on the safety of meat and poultry packaged in modified atmospheres is based on traditional inoculation studies. Such data, however, cannot lead to reliable estimations of MAP safety risks since they are not statistically predictive and ignore the relationship between spoilage and pathogenicity. Thus, there is a need for further research information including risk assessment studies in which MAP will be compared against the current distribution and retail practices by taking into account the effect of factors such as temperature, time, pH and atmosphere as well as the relationship between growth of spoilage and pathogenic organisms. Such studies would lead to a better understanding of the action and interaction of the above factors in MAP and identify the potential need for additional antimicrobial hurdles to assure predictable safety.

h. Active packaging

Active packaging is one of the innovative concepts of food packaging that have been introduced as a response to the increasing consumer demands and the continuous changes in market trends. Major active packaging concepts include the use of substances that absorb oxygen, ethylene, carbon dioxide, flavors/odors and substances that release carbon dioxide, antimicrobial agents, antioxidants and flavors (333). Antimicrobial packaging has been characterized as a promising preservation concept for intact meat and poultry products mainly due to the fact that microbial contamination of these products occurs primarily on the surface (334).

The antimicrobial packaging concept is based on the incorporation of antimicrobial agents with polymeric

packaging materials or biodegradable films and coatings. Compared to direct surface application of antimicrobial agents by spraying or dipping, antimicrobial packaging could be more efficient due to the slow release of the agent from the packaging material to the surface of the product, which helps to maintain high concentrations where they are needed. In addition, a long migration period of the antimicrobial agent may extend its activity into all stages of the food chain including transport, storage and distribution.

Non-edible antimicrobial packaging systems may contain different types of food grade additives in their packaging materials. Among compounds that have been proposed and tested in meat and poultry products are organic acids such as sorbate (335), propionate (336) and benzoate (337) or their respective anhydrides, bacteriocins including nisin and pediocin (338,339), enzymes such as lysozyme (340), and natural antimicrobial compounds from aromatic plants (341) (Table 52.8). The antimicrobial activity of triclosan incorporated plastic (TIP) has been recently investigated and the results showed a satisfactory inhibition against both spoilage and pathogenic bacteria associated with meat and poultry products such as *B. thermosphacta*, *S.* Typhimurium, *S. aureus* and *E. coli* (342). Triclosan is a tasteless, odorless and orally non-toxic bacteriostatic agent (343) and its use for food applications has been recently allowed in EU countries. Another interesting application of antimicrobial packaging is the use of polymers with modified surface composition by electron irradiation in such a way that the surface would contain amine groups with antimicrobial activity (344). These surface-bound amine groups have been proven active against pathogenic and spoilage bacteria (345).

Edible coatings and films prepared from polysaccharides, proteins and lipids and supplemented with antimicrobial agents can be considered as another type of antimicrobial packaging application. In this case, however, selection of the active agents is limited to edible compounds. Edible coatings and films with antimicrobial compounds present the following benefits to the meat and poultry industry: (i) inhibit the growth of spoilage and pathogenic bacteria and lead to products with low safety risk and extended shelf life; (ii) help alleviate the problem of moisture loss that leads to texture, flavor and color changes and weight losses; (iii) hold in juices and enhance product presentation; and (iv) reduce the rate of rancidity and brown discoloration caused by lipid and myoglobin oxidation (346). An additional advantage of this application is that the development of films and coatings can be based on the biopolymer by-products of the meat and poultry industry such as gelatin, blood protein and feather keratin.

TABLE 52.8
Applications of Antimicrobial Packaging Tested in Meat and Poultry Products

Product-Substrate	Antimicrobial Agent	Packaging Material
Bologna, cooked ham, pastrami	Acetic acid	Chitosan
Beef muscle	Lactic acid	Alginate
Bologna, cooked ham, pastrami	Propionic acid	Chitosan
Chicken breast	Potassium sorbate	Starch/glycerol
Beef tissue	Lysozyme	Silicon coating
Broiler drumstick skin	Nisin	Polyethylene
Cooked meats	Pediocin	Cellulose

Source: Ref. 334.

5. Biopreservation

Biopreservation refers to extended storage life and enhanced safety of foods using their natural or controlled microflora and/or their antibacterial metabolic end products (347,348). In meats, lactic acid bacteria (LAB) constitute a part of the initial microflora which develops easily during fermentation processing, chill storage or packaging under vacuum or modified atmosphere conditions. Lactic acid bacteria growth in meat can cause interference against spoilage and pathogenic bacteria through several mechanisms including nutrient and oxygen depletion, and production of a wide range of inhibitory metabolic substances such as lactic and acetic acid, acetoin, diacetyl, hydrogen peroxide, reuterin and bacteriocins.

Biopreservation may be applied to food and meat systems by four basic methods (348–350):

a) Adding a pure culture of LAB. This is an indirect way of incorporating the antimicrobial metabolites of LAB (e.g., bacteriocins) in a food product. The success of the method depends on the ability of the culture to grow and produce the metabolites under the environmental and technological conditions of the product (temperature, pH, a_w, additives, etc.).
b) Adding a crude metabolite-preparation obtained by growing the LAB culture on a complex substrate in order to avoid the use of a purified compound.
c) Adding purified or semi-purified antagonistic substances. By using this method the dosage of the antimicrobial metabolite is more accurate and thus more predictable. However, application of this method is limited due to regulations concerning food additives.
d) Adding mesophilic LAB as a protective culture to be activated in the event of potential storage temperature abuse. In this case LAB should remain at low levels in cold environments while under temperature-abuse conditions they should grow competitively with pathogens and thus reduce the safety risk of the product.

a. Lactic acid bacteria

The use of LAB as starter cultures in fermented meat products shortens the fermentation time, ensures product safety, extends shelf life and results in products with desirable, distinct and consistent quality (351,352). In addition to their inhibitory activity against meat spoilage and pathogenic bacteria, the production of lactic acid by LAB denatures meat proteins and affects product texture. Protein denaturation also results in the decrease of a_w which leads to the microbial stabilization of the transformed product.

The starter cultures used predominantly in fermented meat products include strains of *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lactobacillus sake* and *Lactobacillus curvatus*. Other bacteria such as *Micrococcus* (*Micrococcus varians*) and coagulase-negative staphylococci are also used, mostly in Europe, and mainly due to their ability to reduce nitrate to nitrite and the production of specific flavors and catalase, which decomposes hydrogen peroxide and prevents quality defects.

In addition to fermented meat products, the use of LAB is permitted in bacon produced with minimal nitrite levels to reduce formation of nitrosamine during frying; the LAB grow and reduce the pH to inhibit *C. botulinum* growth in the absence of higher nitrite levels if the product is temperature abused (353). Inoculation of selected strains of LAB on fresh meat may suppress other LAB that degrade amino acids to undesirable compounds such as sulfides or biogenic amines (354,355) and contribute to the control of meat pathogens such as *L. monocytogenes* (356).

Addition of LAB to meat products, however, may also impart quality or safety problems. For example, heterofermentative LAB can produce undesirable compounds such as diacetyl, hydrogen sulfide and hydrogen peroxide gases, which affect product flavor and color. In addition, the ability of some LAB to decarboxylate the amino acid, histidine, may result in high levels of histamine, which has been associated with several outbreaks of foodborne illness (357). Thus, starter cultures should be screened for absence of metabolic products that cause quality or safety problems.

b. Bacteriocins

Bacteriocins are ribosomally produced polypeptides or proteins that produce, in their mature form, an antibacterial effect against a narrow spectrum of closely related bacteria (358,359). Bacteriocins exert their inhibitory action by formation of pores in the cytoplasmic membrane of sensitive cells. They are effective inactivators or inhibitors of various Gram-positive bacteria including pathogens, while Gram-negative bacteria are protected by their outer membrane, which prevents bacteriocins from reaching the cytoplasmic membrane (360).

Although several meat-borne LAB have been described as bacteriocin producers (Table 52.9) only a few have been studied as biopreservatives in meat systems. The production and antimicrobial activity of certain bacteriocins in laboratory media does not imply their effectiveness in a food system. Bacteriocin activity may be reduced by the binding of bacteriocin molecules to meat components, by the action of proteases and other enzymes or by an uneven distribution in the food matrix (361). In addition, bacteriocins are not only of narrow activity among microorganisms, but there is also strain variation and their activity in foods may be reduced with storage time. Thus, when evaluating a bacteriocin for meat biopreservation, the influence of the product formulation and processing technology on the antimicrobial performance of the bacteriocin needs to be assayed.

TABLE 52.9
Bacteriocins Characterized from Lactic Acid Bacteria

Lactic Acid bacteria	Bacteriocin
Carnobacterium piscicola	Carnocin U149
	Carnobacteriocin (A and B)
Lactococcus lactis	Nisin (A and Z)
	Lacticin 481
	Diplococcin
	Lactococcin (A, B and M)
Lactobacillus sake	Lactocin S
	Sakacin (A and P)
Lactobacillus curvatus	Curvacin A
Lactobacillus johnsonii	Lactacin F
Leuconostoc gelidum	Leucocin A-UAL-187
Enterococcus faecium	Enterocin 1146
Pediococcus acidilactici	Pediocin (AcH and PA-1)

Source: Ref. 413.

The most studied bacteriocins in meat and meat products include nisin A, A, P and K, leucocin A and pediocin PA-1/AcH (350); nisin, however, is the only bacteriocin approved for use in certain food products. Sprayed nisin has been effective for the decontamination of meat surfaces while its combination with nitrite was effective on *Clostridium* and other Gram-positive pathogens such as *L. monocytogenes* and *S. aureus* in frankfurters, pork slurries and raw meat (362,363). In fermented American-style sausages, pediocin production prevented *L. monocytogenes* growth (364). The activity of pediocin PA-1 was not affected by fat or proteins present in the product while a synergistic effect was observed between the effect of bacteriocin and lactic acid.

6. Emerging Preservation Technologies

a. Irradiation

Food irradiation is generally defined as the process in which foods are exposed to ionizing energy from radioactive sources such as cobalt 60 or with machine sources such as high energy electron beams or X-rays. The role of irradiation as an alternative method to ensure hygienic quality of meat and poultry products is increasingly advocated and

used in some countries (365–367). By properly adjusting the irradiation process it is possible to achieve a specific reduction of the level of microbial contamination commonly found on meat and poultry. Depending on the product and conditions of the process, irradiation treatments at doses of 3–7 kGy can effectively eliminate vegetative cells of pathogens including *Salmonella, S. aureus, Campylobacter, L. monocytogenes* and *E. coli* O157:H7 (368,369). The number of cells that are killed by irradiation depends on various factors such as type of microorganism, type of food, irradiation dose, temperature, oxygen presence and water content (368–372). Table 52.10 provides examples of decimal reduction radiation doses (radiation D_{10} values) which have been determined for various non-spore-forming pathogenic bacteria in meat and poultry products (366). In addition to microbial inactivation, sublethal damage to microorganisms taking place during irradiation can increase their sensitivity to other preservative factors and thus synergistic effects of irradiation and other processes applied in food technology can be encountered (373). Among the benefits of irradiation is its applicability to frozen foods without having to thaw them,

TABLE 52.10
Irradiation D_{10} Values of Some Pathogenic Bacteria in Non-Frozen Meat and Poultry Products

Bacterium	Product	Temp. (°C)	Atmosphere	D_{10} (kGy)
Campylobacter jejuni	Ground pork	NS	Vacuum	0.19±0.01
	Filet americain	18–20	Micro-aeroph.	0.08–0.11
	Ground beef	18–20	Micro-aeroph.	0.14–0.16
	Ground beef	0–5	Air	0.161
	Ground beef	30±10	Air	0.174
	Ground turkey	0–5	Air	0.186
	Ground turkey	30±10	Air	0.162
Escherichia coli O157:H7	Mech. deboned chicken	0	Air	0.26±0.01
	Mech. deboned chicken	0	Vacuum	0.27±0.01
	Ground beef	0	Vacuum	0.27±0.03
	Ground beef (low fat)	4±1	Air	0.241
	Ground beef (high fat)	4±1	Air	0.251
Listeria monocytogenes	Minced chicken	NS	Air	0.417–0.553
	Mech. deboned chicken	2–4	Air	0.27–0.77
	Minced pork	10	Air	0.573–0.648
	Minced pork	10	CO_2:N_2(1:3)	0.602–0.709
	Ground pork	4	Air	0.422–0.447
	Roast beef	NS	Air	0.644±0.061
	Ground beef (low fat)	4±1	Air	0.578–0.589
	Ground beef (high fat)	4±1	Air	0.507–0.574
Salmonella Typhimurium	Filet americain	18–20	Air	0.37
	Ground beef	18–20	Air	0.55
	Ground beef (low fat)	2	Air	0.59
	Minced pork	10	Air	0.403–0.860
	Minced pork	10	CO_2:N_2(1:3)	0.394–0.921
	Roast beef	NS	Air	0.569±0.067
	Mech. deboned chicken	20	Air	0.52–0.56
	Mech. deboned chicken	20	Vacuum	0.52–0.56
	Minced chicken	4	Air	0.436–0.502
	Minced chicken	4	CO_2	0.436–0.502
	Minced chicken	4	N_2	0.550–0.662
Staphylococcus aureus	Minced chicken	4	Air	0.419
	Minced chicken	4	CO_2	0.411
	Minced chicken	4	Vacuum	0.398
	Minced chicken	4	N_2	0.371
	Roast beef	NS	Air	0.387±0.056
	Ground beef (low fat)	4±1	Air	0.437–0.453
	Ground beef (high fat)	4±1	Air	0.443–0.448
	Ground beef (low fat)	2	Air	0.57

NS=Not stated.
Source: Ref. 366.

and in packed products as a terminal treatment, eliminating the possibility of recontamination before consumption.

The main limitations of radiation decontamination are the effect of the process on the nutritional and sensory characteristics of the product (374) and the consumer acceptability of the process. Irradiation of meat and poultry may result in nutrient losses and changes in product odor, flavor and color depending on the radiation dose, dose rate, temperature, packaging and atmosphere during the process (375). Threshold irradiation doses at which detectable sensory changes occur in meat and poultry products are shown in Figure 52.5 (376). However, irradiation at low doses under low or no oxygen, or in the cured and frozen states for low fat products, has no notable sensory effects. A disadvantage of using irradiation at low doses is that bacterial spores, viruses and microbial toxins are not inactivated. Thus, as with other processed foods, irradiated products must be handled in accordance with good manufacturing practices and require appropriate temperature control.

Irradiation treatment of packaged products as a final processing step is a special case since this may cause cross-linking between the packaging material and the food. Irradiation may result in the decomposition of package components to lower-molecular-weight entities with increased migration characteristics (377). Therefore, the FDA requires that packaging that holds food during irradiation complies with specific regulations (21 CFR 179.45) based on appropriate testing.

The safety and effectiveness of irradiation as a method of food processing/preservation have been recognized by the Codex Alimentarius Commission (378). The FDA and FSIS approved a petition from industry to allow radiation decontamination of poultry and non-frozen and frozen red meats with maximum doses of 3 kGy, 4.5 kGy and 7.0 kGy, respectively (379,380). Despite this legislation, however, only a small fraction of the total amount of meat and poultry products produced in the U.S. is irradiated (381). Production of irradiated meat and poultry products is also limited in the EU, where no agreement has been reached on guidelines for regulating food irradiation (381). The limited application of irradiation of foods could be attributed to the widely held opinion of some national authorities and the food industry that consumers would be apprehensive about foods treated with irradiation because of the perceived association with radioactivity (365). Proper information about the safety and the benefits of irradiated foods could increase the level of understanding and acceptance of irradiated products by consumers and lead to a wider application of the process by the food industry (382). Furthermore, problems with the undesirable sensory effects of irradiation as well as investigations on the appropriate packaging materials for irradiated foods need to be addressed before this technology can be extensively used.

b. High hydrostatic pressure

High hydrostatic pressure (HHP) is an emerging non-thermal processing technology whereby foods are subjected to high hydrostatic pressure, generally in the range of 100–600 MPa, at low or moderate temperatures. It has been characterized as a very promising technology for the preservation of sliced cooked and cured meat products mainly due to its potential for the innovative development of new products of low safety risk with a relatively low energy consumption (383). High pressure application is of special interest in food processing technology since pressure represents an alternative to thermal processing, especially for foods such as meat and poultry whose nutritional and sensorial characteristics are thermosensitive. Indeed, HHP can reduce microbial contamination without markedly altering the taste and flavor or the nutrient content of foods (384–386). However, pressure processing also presents some disadvantages since it induces protein denaturation and texture modifications (385,386).

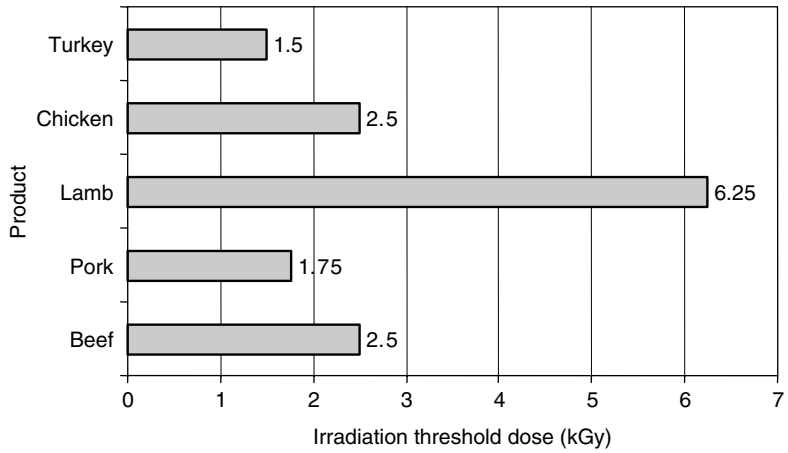

FIGURE 52.5 Irradiation threshold doses of meat and poultry products resulting in detectable sensory changes. (From Ref. 376.)

The mechanisms of inactivation of high hydrostatic pressure can be grouped into cell envelope-related effects, pressure-induced cellular changes, biochemical aspects and effects on genetic mechanisms (available at: http://vm.cfsan.fda.gov/~comm/ift-hpp.html). It has been established that cellular morphology is altered by pressure, and that cell division slows with the application of increasing pressures.

Bacterial spores are the most pressure-resistant life forms known. Thus, unless high hydrostatic pressures in excess of 800 MPa are used, heat in conjunction with HPP is a requirement for effective elimination of bacterial endospores (available at: http://vm.cfsan.fda.gov/~comm/ift-hpp.html). It is well established that the most pressure-resistant pathogen is *C. botulinum*. Spore suspensions of *C. botulinum* strains 17B and Cap 9B tolerated exposures of 30 min to 827 MPa and 75°C (available at: http://vm.cfsan.fda.gov/~comm/ift-hpp.html). Consequently, *C. botulinum* heads the list of most dangerous organisms faced by HPP. Among the vegetative cells, as in the case of other environmental stresses, Gram-positive bacteria are more resistant to pressure than vegetative cells of Gram-negative bacteria. However, there appears to be a wide range of pressure sensitivity among the pathogenic Gram-negative bacteria. Patterson et al. (387) have studied a clinical isolate of *E. coli* O157:H7 that possesses extremely high resistance to HHP.

Microbial inactivation through HHP application on fresh or processed meat has been studied in the last decade (388,389). The results have shown that pressure treatments at 200–600 MPa and 10–25°C for 10–20 min reduced, by at least 5–7 log cycles, the populations of both spoilage and pathogenic bacteria including pathogenic vegetative strains of *E. coli, C. jejuni, Pseudomonas aeruginosa, Y. enterocolitica, S.* Typhimurium*, S. aureus* and *Pseudomonas fluorescens.* The extent of inactivation, however, depends on several parameters such as the type of microorganism, the pressure level, the process temperature and time, and the pH and composition of the product. For example, pressure resistance of vegetative organisms increases at high (35 or 50°C) or low (4°C) temperatures and with decreasing water activity (386,390). Furthermore, the endogenous meat microflora, including *Pseudomonas* spp., appeared to be more resistant to pressure than the artificially inoculated organisms (390). The effect of HHP on microbial inactivation can be increased by combining it with other preservation treatments such as moderate heating or cooling, pH modifications, use of additives or various pretreatments. It may also be of special interest to apply HHP to ready-to-eat, packed products, as a terminal treatment in order to reduce microbial contamination originating from portioning, slicing or comminuting (386).

Other issues related to the application of HHP to meat products are associated with the effect of pressure on meat enzymes and proteins. For example, meat pressurization soon after slaughter usually results in a rapid pH decrease and an intense contraction. This greater rate of pH decrease has been related to changes in the activity of key meat enzymes involved in the regulation of glycogen breakdown. The above issues are considered significant since they may change important quality characteristics such as meat tenderness and structure. The effect of HHP on enzyme activity and meat proteins is extensively discussed in the study by Cheftel and Culioli (386).

Clearly, the benefits of HHP in terms of safety, storage life and quality can be applied to many high-value and heat-sensitive meat products and meat-based, ready-to-eat meals. At present, commercial HHP finds some applications in processed meat products for control of post-processing contamination with *L. monocytogenes*. The high cost of HHP technology and its limitation in terms of capacity could lead to the conclusion that HHP will not replace the conventional preservation and processing technologies. In the future, however, HHP is expected to find its position in the growing market of high-quality, high-priced foods (391).

c. Other technologies

Processing treatments with possible application in the meat industry include pulsed-field electricity, ultrasonic energy, UV light, oscillating magnetic fields (ohmic heating, dielectric heating, microwaves) and controlled instantaneous decompression (208,383,392,393, available at: http://vm.cfsan.fda.gov/~comm/ift-hpp.html). Pulsed field electricity, which is currently used in the meat industry for the electrostimulation of beef carcasses, can also lead to a reduction in the microbial load and extension of the lag phase of bacterial growth (392,394). Ultrasonic energy can be applied to small carcasses such as poultry when they are immersed in water. Research has shown that sonication can reduce the microbial contamination of the carcass especially when combined with appropriate adjustment of pH and temperature or with chlorination (395,396). The use of UV light in meat storage rooms and processing areas may control the bacterial load of the atmosphere (208).

All the above technologies aim at energy saving and being environmentally friendly. They have the same goal of being mild for food and reducing pathogenic and spoilage microorganisms. However, in most countries, these technologies are not approved and thus their application is limited at the present time.

V. MANAGEMENT OF PROCESSES FOR THE MICROBIOLOGICAL SAFETY OF MUSCLE FOODS

Traditionally, the microbiological safety of foods was heavily reliant on end product testing to determine the presence of a pathogen in a batch of product, and in so doing, determine the acceptance or rejection of a specific food lot (397). Limitations are, however, associated with

finished product testing since foodborne pathogens may be distributed sporadically and unpredictably and/or occur at a low incidence within the product (397,398). Thus, the number of samples tested from a specific lot may be insufficient to provide meaningful information, and furthermore, the results obtained may provide a false sense of security (399). A more effective means of ensuring food safety than end product testing is the implementation of food safety management systems that incorporate the principles of good manufacturing practices (GMP), prerequisite programs and hazard analysis critical control point (HACCP) (400–403). To develop an effective HACCP system, a systematic approach is used to identify, evaluate and control food safety hazards. GMP, however, constitute the foundation needed before implementation of HACCP is possible in a food processing operation (403). Prerequisite programs and GMP differ from HACCP in that the former deal indirectly with food safety and are, in general, applicable to the whole processing plant. On the other hand, the sole purpose of HACCP *is* food safety and is product- or line-specific (404). Since its inception, many food industries have adopted HACCP. Countries like the U.S., EU, Canada, Australia and New Zealand have made HACCP compliance mandatory in some or all of their food sectors (405,406). The seafood (since 1997), meat and poultry (phasing in began in 1998), and juice (effective in 2002) industries are currently mandated to implement HACCP in the U.S. (404).

A relatively new concept recommended for adoption into food safety management systems by the International Commission on Microbiological Specifications for Foods, is the food safety objective (FSO) (403,407). The FSO is proposed as a tool to link information from risk assessment and risk management processes with the implementation of measures to control the identified risk(s) (403,407). The FSO refers to the maximum level of a microbiological hazard in a food considered acceptable for consumer protection (403). Once it has been determined that the FSO is technically achievable through the application of HACCP and GMP, the next step is to develop and document strategies to satisfy the FSO, including quantifiable and verifiable performance, process or end product criteria (403). Microbiological criteria differ from FSOs in that they determine the acceptability of a specific food lot. These criteria specify the microorganisms of concern and/or their toxins or metabolites, the sampling plan to be used (number of samples, method of sampling and handling, size of analytical unit), the analytical method to be used, microbiological limits and the number of analytical units required to conform to the limits (403). Sampling plans should be based on statistical probability, and in so doing, provide confidence during interpretation of the results (408).

Microbiological testing programs are thus a necessary part of food safety management systems. The main purpose of testing in a properly implemented HACCP system is to identify hazards and critical control points, and to validate and verify process control measures, and not as a means to assure the microbial safety of the final end product (397–399,401,409,410). As mentioned previously, testing for pathogens during meat and poultry processing operations provides very little information of use for the implementation and maintenance of HACCP systems, since they are generally found infrequently, unpredictably and in low numbers on the animals, and are usually detected rather than enumerated (397). A negative result for the presence of the pathogen could, therefore, be due to process control or simply due to the pathogen being absent at that particular time in that particular sample of product. Testing for pathogens that are found at a low frequency would require larger sample numbers and would thus be costly, and still would not guarantee the safety of the product. Testing for indicator microorganisms, which are indicative of process control, may be more appropriate, as it is generally assumed that the absence or low numbers of an indicator microorganism is a sign of process control and thus a lower likelihood of the presence of the pathogen, with which the indicator microorganism is associated (397,403). Examples of indicator microorganisms that have been used to assess meat plant processes include aerobic bacteria, coliforms, Enterobacteriaceae and *E. coli* biotype I (397,403,411). It should be noted, however, that no microorganisms that can serve as direct indices for pathogens are known. In the U.S., the Pathogen Reduction-HACCP System's Final Rule issued by FSIS in 1996 (400) requires meat and poultry slaughter establishments to conduct microbial testing for generic (biotype I) *E. coli* at regular intervals. Microbial testing for *E. coli* biotype I aims to verify that the establishments' HACCP-based process controls are adequate in preventing and removing fecal contamination, which is also the primary route of contamination of meat and poultry with pathogenic bacteria, like *E. coli* O157:H7, *Salmonella*, and *Campylobacter* spp. (400). Another requirement of the Final Rule is for establishments that produce raw ground products to meet pathogen reduction performance standards for *Salmonella*. These performance standards were issued in order to verify that plant HACCP systems are effective in reducing contamination with pathogenic microorganisms (400). It is expected that improvements in process control that lead to reductions in *Salmonella* will also result in reductions of other enteric pathogens of concern. Reasons for selecting *Salmonella* as the target organism include that it is a major pathogen of concern in the meat and poultry industry, and furthermore, is present in detectable numbers which can be easily tested for with current methodologies, on all types of raw products. Performance standards set by FSIS are based on the most recent U.S. baseline prevalence levels for *Salmonella* (400). Thus, by implementing the requirements of the 1996 Final Rule (sanitation, standard operating procedures, HACCP-based process control, microbial testing and pathogen reduction standards), meat

and poultry establishments are expected to significantly reduce contamination of their products with pathogenic bacteria, which ultimately will reduce the risk of foodborne illness.

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53 Microbiology of Marine Muscle Foods

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CONTENTS

I. INTRODUCTION

This chapter discusses the microbiology of finfish, crustaceans with a chitinous exoskeleton, and molluscan shellfish with a calcareous shell. Bivalve (two-shelled) molluscs are filter-feeding and during this process they concentrate microorganisms from water, which may lead to specific safety issues. Marine muscle foods, or seafood, are produced from a large number of aquatic species that live in habitats as differrent as (i) permanently cold seawater with 3–4% NaCl, e.g., in polar or deep-sea regions, (ii) warm shallow or surface seawaters in the tropics, (iii) estuarine waters or (iv) freshwater of different temperatures. In addition, aquatic animals are captured or reared by different methods and this also influence the microflora of seafoods. The majority of the microorganisms on aquatic animals have no recognized importance in marine muscle foods but spoilage and disease, i.e., infection or intoxication are caused by specific microorganisms.

Spoilage can be considered as any change that renders seafood unacceptable from a sensory point of view. Degradation of lipids by enzymatic hydrolysis or chemical oxidation can result in spoilage of fresh fatty fishes, frozen and dried products as well as fish-oil containing products. However, microbial activity is responsible for spoilage of most fresh and lightly preserved seafood. Globally, the post-harvest losses of food have been estimated to be as high as ~25% due to microbial activity alone (1). At the same time, the world's annual production in capture fisheries has not increased but remained at ~90 million tonnes during the last decade. Only aquaculture production in marine and particularly in fresh water has increased; if reached 35 million tonnes in 2000 (2). Despite the limited increases in fisheries production, international trade in seafood has increased and optimal utilization of available aquatic food resources and prevention of losses during distribution are more important than ever. Historically, seafood spoilage has been inhibited by processes like salting, drying and smoking, developed without knowledge about the microorganisms present in the aquatic raw materials. Today, the interest in fresh and lightly preserved foods is increasing (3) and this represents a challenge

where detailed information about microorganisms in seafoods becomes important to reduce losses due to spoilage and to ensure products are safe at the time of consumption.

This chapter describes the microflora and microbial spoilage of marine muscle foods. The concept of specific spoilage organisms (SSO) is presented and it is shown how it can be applied to determine, predict and extend the shelf life of fresh and lightly preserved seafood. Several previous overview articles concerned the microbiology and microbial spoilage of seafoods. Shewan (4–6) summarized early studies on microbial spoilage, particularly of chilled and aerobically stored fresh fish. Later, Shewan (7) and Liston (8) reviewed the microflora of seafood from different geographical regions. Ashie et al. (9) summarized information on shelf-life extension whereas Dalgaard (10) described the SSO concept and its application with modified atmosphere packed (MAP) seafood. Gram et al. 2002 (11) discussed the importance of microbial interaction in food spoilage. Compared to previous reviews this chapter includes new information on the occurrence and importance of luminous bacteria in marine muscle foods.

Consumption of seafood can result in food-borne human diseases but the more exact numbers of outbreaks and cases are uncertain. In fact, it has been estimated that as little as 1% of the actual cases of food-borne diseases is reported; see (12). The U.S. and the U.K. have well-established reporting systems and some of their epidemiological data from the 1990s are included here to illustrate the importance of marine muscle foods in food-borne disease. In the U.S. finfish and shellfish were responsible, respectively, for 5.1% and 1.7% of the outbreaks and 0.8% and 2.2% of the cases of food-borne disease. For cases due to finfish, the main etiological agents were histamine fish poisoning (47%), ciquatera fish poisoning (24%), salmonellae (18%), *Clostridium botulinum* (3%), *Vibrio cholerae* (2%), *Cl. perfringens* (2%) and viruses (2%). The corresponding agents and figures for molluscan shellfish were viruses (66%), *Vibrio parahaemolyticus* (22%), salmonellae (6%), paralytic shellfish poisoning (3%) and *Cl. perfringens* (2%). In the U.K. cases due to finfish were caused by histamine fish poisoning (70%), salmonellae (16%) and viruses (4%) whereas cases due to molluscan shellfish resulted from viruses (91%) and salmonellae (4%) (12–15). Compared to amounts consumed and relative to other foods the number of cases of seafood-borne disease is high and should be reduced.

This chapter presents information about histamine fish poisoning, *Vibrio* species, *Clostridium botulinum*, *Listeria monocytogenes*, *Salmonella* and viruses. Recent overview articles concerning safety of seafood include (12, 16–18) and these or other chapters in the present volume should be consulted for information about aquatic biotoxins, parasites, toxic algae, and veterinary drugs as well as industrial and environmental contaminants which are hazards of importance in some marine muscle foods but not included in this chapter, which focuses on microbiology.

II. MICROFLORA OF AQUATIC ANIMALS FROM DIFFERENT HABITATS

The microflora of finfish, crustaceans and molluscs depends to a large extent on the microflora of the water in which they live. Microorganisms are found on outer surfaces (e.g., skin, gills and intestine) whereas the muscle tissue of healthy animals is sterile. The concentration of culturable microorganisms is variable and in general 10^2–10^5 cfu/cm^2 are found on skin, 10^3–10^7 cfu/g in gills and $10 - >10^8$ cfu/g in the intestinal content. The variable concentrations of intestinal microorganisms is related to the aquatic animal's intake of food (8, 19, 20). Higher temperatures typically correspond to higher concentrations of culturable microorganisms in water and on aquatic animals. For example, shrimps from cold water harbored 10–10^3 cfu/g, whereas shrimps from warm water had 10^6 cfu/g (8). Similarly, in Japan, seasonal temperature changes had some effect on the total concentration of microorganisms in river, lake and seawater (21–23) and in the intestinal content of wild salmon but no effect was observed for cultured salmon (21). Water salinity has little effect on the total concentration of microorganisms but influences the composition of microbial species in aquatic animals, as discussed in the following section. Organic matter, particularly sewage and land run-off, increases the load of microorganisms, including potentially pathogenic species in water and aquatic animals. Finally, catching methods have an effect; for example, trawled finfish may have 10–100 times higher concentration of microorganisms on skin and gills than similar fishes caught by long line (5).

Heat-labile and sodium-requiring microorganisms are common in sea- and brackish waters as well as on seafood. Thus, for enumeration of microorganisms pour plating with ~45°C hot agar must be avoided as this procedure may kill a major part of the microflora (10). The concentration of microorganisms in deep-water pink shrimp (*Parapenaeus longirostris*) was, for example about twenty times higher when determined by spread plating as compared to pour plating (24). In this respect it may be relevant to note that even in tropical regions where surface seawater is above 25°C, psychrotolerant and heat-labile microorganisms like *Photobacterium phosphoreum* can be present in seawater and aquatic animals at depths below 50–100 m (25–28). Microorganisms on seafood frequently require sodium for growth and although standard plate count agar without NaCl is recommended for many foods this medium is obviously inappropriate for seafood. In addition, bacteria on marine fishes may be fastidious (29) and rich enumeration media are required. For various fresh and lightly preserved seafoods spread plating on pre-chilled plates of Long & Hammer's agar,

modified to contain 1% NaCl, and incubated aerobically during 5–7 days at 15°C have been appropriate for enumeration of the dominant microflora (10, 30). Strictly anaerobic microorganisms are found in low concentrations on skin and gills of newly caught aquatic animals but in some cases these or not-yet-cultured microorganisms dominated the intestinal microflora of marine and freshwater fishes (31–35). In contrast, direct microscopy and both aerobic and anaerobic techniques determined similar concentrations of microorganisms in the intestine of several commercially important fish species (30). Although they may only be important in specific situations, anaerobic or not-yet-cultured microorganisms in the intestinal content of aquatic animals merits further study using culture-independent techniques as increasingly applied within microbial ecology in general.

A. Microflora on Skin, Shell and Gills

The genera or groups of microorganisms found on skin, outer shell and in gills of newly caught or harvested finfish, crustaceans and shellfish have been determined in numerous studies and summarized in several overview articles (8, 17, 19, 20, 31, 36). The dominant groups of Gram-negative bacteria were (i) *Acinetobacter*, *Moraxella* and *Psychrobacter*, (ii) *Pseudomonas* and *Shewanella*, (iii) *Flavobacterium* and *Cytophaga*, (iv) *Vibrio* and *Photobacterium*, (v) *Aeromonas* and (vi) Enterobacteriaceae. The dominant groups of Gram-positive bacteria were cocci primarily *Micrococcus,* coryneforms and rods including *Bacillus*, *Clostridium* and lactic acid bacteria. In some studies the percentage distribution of bacteria in seawater corresponded to the distribution of species on the surface of fish (31). Nevertheless, when data from many studies are compared (8, 17, 19, 20, 31, 36) surprisingly little can be concluded about the effect of water temperature and salinity or about the type of animal, for example dermersal or pelagic on the percentage distribution of genera/groups of microorganisms. However, for animals in freshwater the sodium-requiring species of *Vibrio* and *Photobacterium* are very rarely present whereas *Aeromonas* and Enterobacteriaceae are relatively more important in those habitats. Also, the *Flavobacterium-Cytophaga* group seems less dominant in marine animals. Furthermore, the percentages of *Bacillus*, *Micrococcus* and Enterobacteriaceae tend to be higher in tropical than in temperate regions. Greater differences between the microflora on aquatic animals from warm and cold water could be expected and, as noted by Liston (8), fungi are common on plankton but rarely found on the surface of fishes. Aquatic animals may prevent growth or attachment of microorganisms by antimicrobials like lysozyme in their surface slime, but this area remains little studied. However, the apparent lack of difference between groups of microorganisms on aquatic animals from various habitats may also result from the use of simple identification schemes relying on relatively few phenotypic characteristics. In an attempt to elucidate this problem this chapter will focus on the occurrence of bioluminescent bacteria. Due to a unique ability to glow, these microorganisms have been enumerated and identified in aquatic animals even when they did not quantitatively dominate the microflora. In addition, simple keys for identification of several species of luminous bacteria have been available since about 1970 (37, 38). Luminous bacteria can dominate the microflora in the intestinal content of many aquatic animals; therefore they are discussed in detail in the following section.

B. Intestinal Microflora and Luminous Bacteria

Seafood can be produced from gutted or whole ungutted animals, e.g., products of herring, mussels, oysters, sardines and shrimps. Due to the high concentration of intestinal microorganisms, contamination of seafood during processing of both gutted and ungutted animals is practically impossible to eliminate and the gut microflora has a very direct influence on the microbiology of many seafoods.

Intestinal microorganisms are mainly found in the gut content, although some may be attached to epithelial cells, and concentrations can be orders of magnitude higher than in water, food, skin and gills. This indicates microbial growth within the time it takes food to pass through the digestive tract and under conditions with acid in the stomach and bile, low oxygen and possibly elevated carbon dioxide concentrations in the gut (39, 40). Data from several studies (19, 20, 41) showed Enterobacteriaceae, *Aeromonas* and *Pseudomonas* dominated in the intestinal content of freshwater species, whereas *Vibrio/Photobacterium*, *Pseudomonas* and Enterobacteriaceae dominated in marine species. However, *Acinetobacter/Moraxella*, lactic acid bacteria, yeast and strictly anaerobic microorganisms, including *Bacterioides* and *Clostridium*, can occur in high concentrations. In addition, a *Mycoplasma* phenotype was recently determined in salmon by a culture-independent approach relying on extraction and amplification of 16S rDNA (31, 34, 42–44).

It has been debated if fish has a specific intestinal microflora as found in warm-blooded animals. Sera and Ishida (45) suggested marine fish with a developed digestive tract have a specific gut microflora consisting of marine vibrios, i.e., sodium-requiring species of *Vibrio* and *Photobacterium*, whereas fish with a simple digestive tract, including immature individuals, have more complex intestinal flora that reflect the microflora in water and food. Salmon that return from seawater with an intestinal microflora dominated by marine vibrios can retain this dominating microflora even after three months in freshwater (46) or after migrating 1228 km away from the marine environment up the Yukon River in Alaska (47).

Thus, marine vibrios are either associated with the gut or growing so fast that they avoid 'wash-out.'

In Japan, luminous and non-luminous marine vibrios dominated the intestinal flora of ten commercially important marine fish species including mackerels and tunas (48). In fact, marine vibrios, particularly luminous species of *Vibrio* and *Photobacterium*, frequently dominate the intestinal microflora of marine aquatic animals. The luminous or bioluminescent bacteria are fascinating and they have been extensively studied, but controversy remains about the influence of luminous bacteria on eating quality, shelf-life and safety of marine muscle foods. For example, J. M. Shewan from Torry Research Station in Scotland concluded in an overview manuscript that luminous bacteria were "…of little real importance to the practical fish technologist…" (6) and the opinion that luminous bacteria do not compromise the safety or eating quality of seafood is common. However, it has now been shown that luminous bacteria are responsible for spoilage of different marine muscle foods and due to biogenic amine production may cause histamine fish poisoning (see Sections III and IV).

The luminous marine bacteria occur in light organs in symbiosis with host animals, in seawater and on skin, shell and gills of animals, but, quantitatively, the major habitat is the intestinal content of marine animals where from <10 to $>10^8$ cfu/g can be found (38, 49). At least thirteen species of bacteria contain luminous variants, but only some are important in seafoods. *Photobacterium phosphoreum* and *Vibrio logei* are psychrotolerant bacteria growing at 0°C and are typically inactivated above 25–30°C. *P. leiognathi* and *V. fischeri* grow between ~4°C and 35–37°C, whereas *V. harveyi* grow from 5–10°C and up to ~40°C. Other luminous bacteria include *V. orientalis*, *V. splendidus* biotype I, *V. cholerae*, *V. vulnificus*, *V. salmonicida*, *Shewanella hanedai, Sh. woodyi* and the terrestrial insect pathogen *Photorhabdus luminescens*, but these are less common in seafood (50–53). *P. phosphoreum* dominate in the intestinal content of aquatic animals in cold seawater at ~0°C to ~15°C (28, 29, 54). In coastal waters in California, *V. fischeri* was the dominating luminous bacteria in gut content and in seawater of ~15°C; however, between June and October the water temperature was ~20°C and *V. harveyi* became dominant in both habitats. Interestingly, these studies showed the intestinal microflora of a single fish species in a given location to be dominated by *V. harveyi*, *V. fischeri* or *P. phosphoreum* depending on changes in the water temperature (55, 56). *V. harveyi*-like and *P. leiognathi*-like luminous bacteria dominated in the intestinal flora of a large number of fish species from the Gulf of Oman (57). In the same way, *P. leiognathi*, *V. harveyi* and *V. fischeri* were present in high concentrations and dominated the gut microflora of commercially important crabs from India (58). *V. harveyi* (75–80%) and *V. fischeri* (20–25%) also dominated the intestinal flora of fladhead mullet and sea catfish from India, but the luminous bacteria disappeared when water salinity decreased from 2.17–3.23% to 1.7–1.8% during the monsoon (59). It seems luminous bacteria in aquatic animals are selected by temperature and salinity. Thus, species of luminous bacteria are not specifically associated with intestines of aquatic animals in general, but all luminous marine vibrios are chitinolytic and may contribute to the digestion of the chitin-containing exoskeleton of crustaceans eaten by various marine animals (59–61). Species of non-luminous microorganisms are likely also to be selected by temperature and salinity and the apparent similarity of the dominant microflora of seafoods from different habitats (discussed in section II A) may reflect identification of major groups of microorganisms like *Acinetobacter/Moraxella* or Enterobacteriaceae rather than specific species.

Contamination of seafood with luminous bacteria from the intestinal content is difficult to avoid during processing. *V. harveyi* (80–90%) and *V. fischeri* (10–20%) dominated the luminous microflora on the surface of white seabream caught in the Mediterranean Sea in July but during aerobic storage at 5°C the dominant luminous microflora became *P. phosphoreum* and *V. fischeri* (62). Luminous variants of *P. phosphoreum* and *V. logei* can grow to high concentrations during normal chilled storage of fish and squid from different parts of the world (62–66). In a few studies it has been attempted to measure the light produced by luminous bacteria on seafood. At 10°C and 25°C changes in bioluminescence may be used as an indication of product spoilage, but at 5°C and below bioluminescence in fresh fish was too weak to be useful as an indicator of spoilage (62, 63). From 1989 to 1998 the U.S. Food and Drug Administration (FDA) registered and evaluated 23 luminous seafoods. The majority of the products were cooked and peeled shrimps and imitation crab or lobster meats but glowing red snapper fillet, raw Pacific rockfish, raw herring and raw shrimps were also reported. The dominant luminous bacteria isolated from these products were *P. phosphoreum* and *V. logei* (67). Both *P. phosphoreum* and *V. logei* are heat labile and their presence in cooked seafood must be a result of recontamination. However, bioluminescence of *P. phosphoreum* and *V. logei* is stimulated by the low level of NaCl typically added to cooked shrimps and imitation shellfish products [see Figure 53.1 (38)] and this may explain why processed products have been reported luminous more often than raw fish. In any case, light produced by bacteria in marine muscle food is weak and only visible in a dark room where electric and sunlight are excluded. Thus, common use of refrigerators with inferior electric lights probably masks the bioluminescence of many seafoods. Nevertheless, when glowing seafood is observed it should not be consumed as it is likely to be of poor sensory quality and may be toxic due to histamine formed by luminous bacteria (see Sections III and IV). Finally, when discussing the

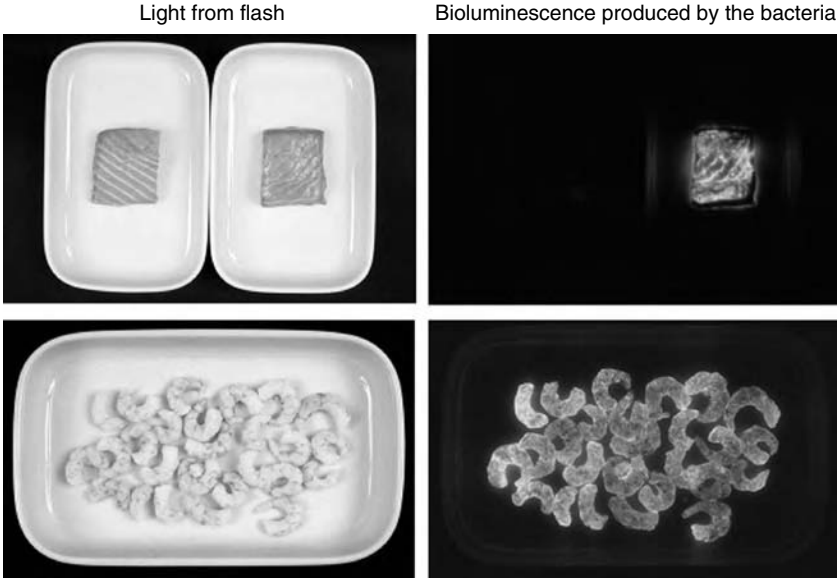

FIGURE 53.1 Bioluminescence by *Photobactrerium phosphoreum* in two pieces of salmon (*Salmo salar*) and in cooked and peeled shrimps (*Pandalus borealis*). Samples were inoculated, incubated aerobically during three days at 5°C and photographed with artificial light (left) and without artificial light (right). One of the two pieces of raw salmon was supplemented with 2% NaCl (w/w) to illustrate how this stimulates bioluminescence. The cooked and peeled shrimps contained 1.5% NaCl which is typical for this product (71).

occurrence and importance of luminous marine bacteria it must be mentioned that non-luminous variants exist for all species of luminous bacteria and the importance of these species of bacteria may be underestimated by enumeration of luminous bacteria only (30, 38, 68–70).

C. Microflora of Farmed Aquatic Animals

The microflora of cultured finfish, crustaceans and shellfish does not as such differ from those of wild aquatic animals. However, cultured aquatic animals are closer to land and human activities than those in the wild and this may influence their environment and microflora. *Listeria monocytogenes,* a psychrotolerant bacteria pathogenic to humans, is absent from clean open waters but found in decaying plant material and indigenous to the general environment. Thus, animals reared in near-shore areas can be contaminated by run-off waters from land and must be expected to contain low levels of *L. monocytogenes* (12). Particularly in Southeast Asia ponds used for shrimp farming may be fertilized by animal excreta and this may result in contamination with *Salmonella, Escherichia coli* and viruses. In warm tropical water the bacteria may persist or even grow and shrimps from some, but not all, areas in Southeast Asia have been contaminated with bacteria potentially pathogenic to humans (72). Mortality of cultured aquatic animals can be a major problem and various diseases are due to bacterial infections. To overcome these problems, antibiotics can be used therapeutically to cure diseases or, on a daily basis, to prevent diseases. Use of antibiotics, particularly prophylatic use in sub-therapeutic concentrations, is problematic because various bacteria develop resistance and the resistance may be transferred by plasmids to other bacteria and thereby reduce efficacy of antibiotic treatment for human and animal diseases. *Vibrio* and *Aeromonas* species, pathogenic to different aquatic animals, have been resistant to several antibiotics and this can make it impossible to control disease in cultured aquatic animals. In Southeast Asia, infection of farmed shrimps by antibiotic-resistant *V. harveyi* has caused so-called luminous vibrioses resulting in significant economic losses during the last 10–20 years. Antibiotic-resistant potential human pathogens, including *Salmonella, V. cholerae*, *Aeromonas hydrophila* and *Plesiomonas shigelloides*, have also been reported in relation to aquaculture production. Human infections by antibiotic-resistant pathogens are most problematic and changes in the use of antibiotics in some aquaculture-producing regions are required. In salmon farming, the use of antibiotics has to a large extent been replaced by vaccination. However, for crustaceans and shellfish with less-developed immune systems, this may not be possible (72–74).

III. MICROBIAL SPOILAGE OF MARINE MUSCLE FOODS

Newly caught fish and shellfish typically have a species-specific flavor that disappears after a few days of chilled storage. Further storage results in development of off-flavors which are often ammonia-like, sulphurous, malt-like or rancid. The off-flavors are typically caused by

microbial metabolites and they increase in intensity during storage resulting in spoilage as determined by sensory methods. The importance of microbial activity in seafood spoilage has been established by comparing off-flavor development in muscle pieces that were (i) sterile, (ii) inoculated with specific microorganisms or (iii) naturally contaminated. Knowledge about spoilage microorganisms facilitates the development of methods to determine, predict and extend product shelf-life and this is particularly important due to the short and variable shelf-life of seafood (10, 75). The short shelf-life of many seafoods is explained by some unique product properties. First, many aquatic animals live in cold waters and their natural microflora include psychrotolerant species able to grow in chilled seafood at temperatures above –2°C. To illustrate the importance of this, it can be mentioned that shelf-life of tropical white-fleshed fish typically is 18–35 days at 0°C, whereas similar coldwater fish spoil after 12–18 days. Second, many finfish, crustaceans and molluscs contain trimethylamine-oxide (TMAO) that stimulates microbial growth and activity. In general, animals from freshwater contain less TMAO than those from seawater, but considerable variation exists between species in both habitats (76, 77). *Aeromonas, Alteromonas,* most Enterobacteriaceae, *Shewanella* and *Vibrionaceae*, including all marine luminous bacteria, reduce TMAO to trimethylamine (TMA) and this anaerobic respiration facilitates their growth under oxygen-limiting conditions, e.g., in vacuum-packed or modified atmosphere-packed products (69, 78–80). TMA contributes to the typical ammonia-like and fishy off-odors in spoiled seafoods, particularly in products with pH above ~6.5 (10). Third, the *post rigor* pH of finfish, crustaceans and molluscs is high compared to beef and pork. White-fleshed dermesal finfish and crustaceans have pH of ~6.5 to above 7, whereas pelagic, dark-fleshed fish like tuna, mahi-mahi, mackerel and garfish, have pH as low as ~5.8. Molluscs have pH similar to finfish, but contain much more carbohydrate (2.5–5.0%) compared to <0.5% for finfish and crustaceans. Consequently, a fermentative type of spoilage with decreasing pH is typical for molluscs but most unusual in other seafoods unless carbohydrates are added (16, 81–84). Finally, high concentrations of free amino acids are present in seafoods and metabolized by spoilage microorganisms, e.g., arginine in shrimps and histidine in dark-fleshed pelagic finfish (85, 86).

Only some of the numerous species of microorganisms in seafoods are important for spoilage and during storage a pattern of microbial growth and activity is frequently observed (Figure 53.2). This pattern is known as the specific spoilage organism (SSO) concept (10). On newly processed, fresh or lightly preserved seafood the SSO are usually present in very low concentrations and constitute only a minor part of the total microflora. During storage and at particular conditions of temperature, atmosphere,

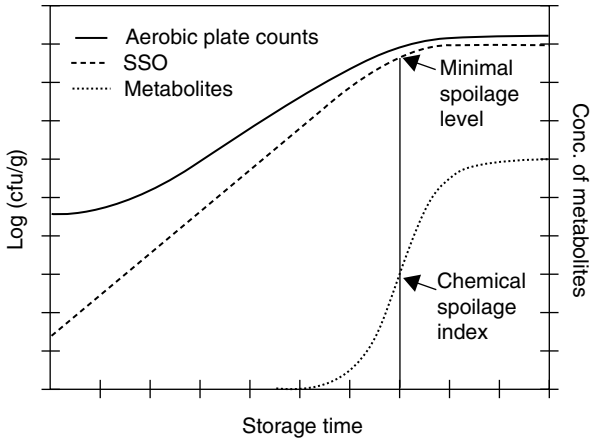

FIGURE 53.2 Specific spoilage organism (SSO) concept. Typical changes in aerobic plate counts, specific spoilage organisms (SSO) and metabolites produced by SSO during storage of fresh seafood (87).

NaCl, a_w, preservatives, etc., SSO, which is often a single microbiological species, grow faster than the remaining microflora, produce metabolites responsible for off-flavors, and finally cause sensory product rejection (Figure 53.2). SSO are typically present in levels of about 10^7 cfu/g when products become sensory spoiled. The importance of an SSO is closely related to its spoilage domain, i.e., the range of environmental parameters within which it is responsible for product spoilage. In contrast to SSO, the spoilage microflora, i.e., the microflora found at the time a product becomes sensorily rejected, can be a mixture of many microbial species, some of which are not important for spoilage.

Identification of an SSO relies on comparison of spoilage characteristics for a naturally contaminated product with those of isolates from the spoilage microflora. Initially, spoilage potential is frequently determined qualitatively as the ability of isolates to produce off-odors and metabolites, e.g., ammonia, TMA or H_2S. This screening technique has been used for decades to separate isolates from spoiled products into potential "spoilers" and "non-spoilers" (88). Isolates of *Shewanella putrefaciens* and *Pseudomonas fragi* often have a strong spoilage potential in seafood. To evaluate if the actual concentration of a microorganism in a product at the time of sensory rejection enables this microorganism to cause spoilage, a quantitative evaluation is required, i.e., determination of the spoilage activity. Yield factors can be used to evaluate if the concentration of cells and metabolites in a seafood corresponds to the spoilage activity of an isolate from the spoilage microflora, i.e., if the isolate is likely to be the SSO (69, 89, 90). The yield factor approach was used to identified *P. phosphoreum* as the SSO in chilled MAP cod fillets and showed this SSO to produce 10–100 times more TMA/cell than *Shewanella putrefaciens.* Despite a strong spoilage

TABLE 53.1
Specific Spoilage Organisms (SSO) in Groups of Fresh and Lightly Preserved Seafoods

Seafood	Typical SSO	Metabolites Produced	Selected References
Fresh chilled products stored in air:			
Various species, particularly those containing TMAO and with pH above ~6;	*Shewanella putrefaciens*-like[a]	TMA, H_2S and other sulphur compounds, hypoxantine	(97–99)
various species, including some with little or no TMAO and low pH ~6	*Pseudomonas* spp.	NH_3, esters, sulphur compounds but not H_2S	(86, 90, 100, 101)
Fresh, chilled products in modified atmosphere packaging:			
TMAO containing species from seawater at temperatures below ~15°C;	*Photobacterium phosphoreum*	TMA, hypoxantine, alcohols and ketones	(24, 69, 92)
species from warmer waters, particularly species with little or no TMAO;	Lactic acid bacteria and *Brochothrix thermosphacta*[b]	Acetic acid, NH_3, tyramine, acetoin, diacetyl, H_2S	(102, 103)
species from tropical freshwater	*Aeromonas* spp.	?	(104)
Fresh and lightly preserved products stored at ambient temperature	*Aeromonas* spp.	TMA, sulphur compounds, biogenic amines	(105, 106)
	Vibrio spp./*Photobacterium* spp.		
	Enterobacteriaceae	NH_3, acetic acid, acetoin, diacetyl, tyramine	(107)
	Enterococcus faecalis		
Lightly preserved and chilled products: Brined, spiced/gravad and smoked products including fish roe	Lactic acid bacteria[c] and *Brochothrix thermosphacta*	Acetic acid, NH_3, tyramine, acetoin, diacetyl, sulphur compounds	(89, 108–113, 130)
	P. phosphoreum, Vibrio and Enterobacteriaceae[d]	TMA, biogenic amines, alcohols, aldehydes, sulphur compounds	

[a] *Shewanella putrefaciens, Shewanella baltica* and other closely related H_2S-producing Gram-negative bacteria.
[b] *Brochothric thermosphacta* is important for products in oxygen-containing modified atmospheres.
[c] Include *Lactobacillus curvatus, Lactobacillus sake* and *Leuconostoc* spp.
[d] Include *Enterobacter agglomerans*, *Hafnia alvei*, and *Serratia liquefaciens*.

potential, *S. putrefaciens* had low spoilage activity and was not important in sensory rejection of MAP cod fillets (Table 53.1). Furthermore, SSO in seafood may be identified by comparison of (i) the profiles of metabolites produced in naturally contaminated products with similar profiles produced by isolated microorganisms and (ii) by evaluating the effect on spoilage when growth of a suspected SSO is specifically inhibited in a seafood (91, 92).

It is well established that many microorganisms from seafoods, including bacteria, yeasts and several SSO (Table 53.1), produce extracellular proteolytic enzymes (93, 94). Nevertheless, seafood spoilage microorganisms typically produce off-flavors from substrates in muscle extractives and proteolytic activity is not important for spoilage of fresh and lightly preserved seafoods (95, 96). The importance of microbial proteolytic enzymes was primarily evaluated for fresh fish, and further research, including lightly and semi-preserved seafoods, seems justified.

A. Specific Spoilage Organisms (SSO) in Groups of Seafoods

Spoilage of fresh chilled and aerobically stored seafood is primarily caused by *Shewanella putrefaciens*-like species and *Pseudomonas* spp. This is well established (Table 53.1), but the taxonomy of these Gram-negative and non-fermentative rods has been changing (114–116). Frequently, *Pseudomonas* were not identified at the species level, but strains similar to *Ps. fragi*, *Ps. fluorescens* and *Ps. putida* seem common in seafood and recently *P. lundensis* dominated the spoilage microflora of chilled aerobically stored marine fish from Greece (114, 117, 118). *Pseudomonas* spp. are unable to reduce TMAO and growth is considerably reduced under oxygen-limited conditions. As an example, *P. fragi* spoiled different species of warm water shrimps stored in ice but spoilage was due to *S. putrefaciens*-like species when shrimps from the same lots were kept in an ice/water slurry (86, 101). Other bacteria may also influence spoilage of fresh chilled and aerobically stored seafood. Lipolytic *Psychrobacter immobilis* can dominate the spoilage microflora in both marine and freshwater fish and, despite a low spoilage potential, they may increase the rancid spoilage of sardines (119, 120). In 1974, van Spreekens (30) suggested *P. phosphoreum* was important for TMA production in iced cod and whiting. More recently, *P. phosphoreum* dominated the spoilage microflora of gutted saithe and plaice (70) and seemed responsible for TMA formation in iced cod fillets and squid (66, 121).

For fresh seafood in modified atmosphere packaging (MAP) with CO_2, luminous and non-luminous variants of *P. phosphoreum* are important spoilage microorganisms

(Table 53.1). Not all studies have detected this SSO in fresh MAP seafoods and in some studies inappropriate enumeration methods and experiments with previously frozen raw material are likely reasons why luminous and non-luminous variants of *P. phosphoreum* were not observed. *P. phosphoreum* is rapidly inactivated by freezing and absent in thawed products (92, 122). The relative importance of *P. phosphoreum* and *Shewanella putrefaciens*-like species in vacuum-packed fresh chilled seafoods probably depends on the initial concentration of the two spoilage bacteria. For shucked bivalve molluscs, i.e., mollusc meat removed from the shells, spoilage is fermentative when stored under vacuum or otherwise with reduced access to oxygen, but the microorganisms responsible remain to be identified (81, 84, 123). Spoilage of chilled MAP seafood from freshwater or products without TMAO need further study, e.g., with respect to identification of lactic acid bacteria and the importance of *Aeromonas* spp. (Table 53.1). High concentrations of *Aeromonas* spp. were found in chilled MAP seafood from tropical regions and they are likely to be the SSO (Table 53.1).

Low dose irradiation changes the spoilage microflora of chilled seafood and *Moraxella* spp. become dominant and probably responsible for spoilage (124). For sous vide cooked and chilled cod fillets, *Clostridium sardiniense*, an anaerobic spore-forming bacteria, caused a most unpleasant type of spoilage due to production of different volatile sulphur compounds (10).

Spoilage of chilled lightly preserved seafoods has been extensively evaluated within the last decade (Table 53.1). In general, lactic acid bacteria seemed to be the most important group of spoilage microorganisms. However, identification of the SSO responsible for spoilage has been complicated and variation in product characteristics, including the initial microflora, NaCl, pH, smoke components, chemical preservatives and packaging, is probably responsible for the various spoilage patterns observed. Thus, the spoilage domain of SSOs identified in several chilled lightly preserved seafood is unclear (Table 53.1), although, with sun-dried tropical fish, the spoilage domains of *Staphylococcus xylosus*, *Halobacterium salinarium* and molds were suggested as a function of temperature and water activity (125). For fermented and semi-preserved seafoods little is known about SSOs, but lactic acid bacteria, yeasts, molds and strict anaerobes seem important in some products.

The SSO concept embodies the hypothesis that in any given product a single species/group of microorganisms is responsible for spoilage. Microorganisms in seafoods interact in several ways, including substrate competition and metabolite inhibition. When the dominating microflora in food reach their maximum population density, i.e., inhibit their own growth, then other groups of microorganisms are frequently inhibited. This has been named the Jameson effect irrespective of the underlying mechanism being substrate competition, bacteriocins, siderophores or other metabolites (126). There is no conflict between the simple SSO hypothesis (Figure 53.2) and the Jameson effect as long as the SSO reaches a high concentration and causes product spoilage before it is inhibited by the dominant microflora. This is typically the case with fresh seafood (Table 53.1). The metabolism of one group of microorganisms may also stimulate the activity of another group and this so-called metabiosis can limit the usefulness of the simple SSO concept for evaluation of microbial seafood spoilage. In cold-smoked salmon, putrescine production by arginine deiminase/decarboxylase negative Enterobacteriaceae increased 10–15 times by co-culturing with arginine deiminase positive lactic acid bacteria. The Enterobacteriaceae and the formed putrescine, however, had little influence on product spoilage (89). In contract, Joffraud et al. (111) found mixtures of spoilage isolates from different microbial species formed stronger off-odors and higher concentration of specific metabolites than mixtures of strains from individual species. The importance of such interactions on spoilage and shelf-life of seafood merits further study.

B. Determination, Prediction and Extension of Shelf-Life

Indices of freshness or spoilage of seafood have been extensively studied and it is generally accepted that there is a poor correlation between remaining shelf-life, as determined by sensory methods, and aerobic plate counts. However, much closer correlations have been observed between remaining shelf-life and concentration of SSOs in different products. Data have been summarized for (i) H_2S-producing bacteria, e.g., *S. putrefaciens*, in different chilled and aerobically stored fish, (ii) *P. phosphoreum* in chilled MAP cod fillets and (iii) *B. thermosphacta* in chilled MAP red mullet and sea bream from Greece. Correlation coefficients between –0.929 and –0.975 were obtained in all cases (102, 127, 128). For several chilled lightly preserved seafoods a close correlation between remaining shelf-life and concentration of any specific group of microorganisms or any specific metabolite has not been determined. Nevertheless, Jørgensen et al. (129) identified a multiple compound quality index (MCQI) to relate sensory quality of sliced and vacuum packed cold-smoked salmon (SVP-CSS) with the product's pH and cadaverine, histamine, putrescine and tyramine content. Furthermore, Leroi et al. (130) related remaining shelf-life of SVP-CSS at 5°C with the products concentration of *Lactobacillus* spp. and concentration of total volatile nitrogen (TVN). Microbiological criteria relying on mesophilic aerobic bacteria in concentrations between 10^5 and 10^6 cfu/g are included in EU Directives [(12), pp. 195–203]. Due to the frequent dominance of heat-labile spoilage bacteria in seafood, it must not be expected that such criteria correspond to sensory spoilage.

Increased transportation of seafood at both national and international levels makes shelf-life prediction important to prevent rejections of products and disappointed consumers. Kinetic models can be used to predict the effect of product characteristics and storage conditions on growth of SSO, but the range of the environmental factors in the models must be within the spoilage domain of the SSO. In fresh seafoods, SSOs often grow without a lag phase and this facilitates shelf-life prediction (Figure 53.2). In fact, kinetic models to predict growth of *B. thermosphacta*, *P. phosphoreum,* psychrotolerant *Pseudomonas* spp. and *S. putrefaciens* as a function of temperature or both temperature and CO_2 concentration in MAP seafoods have been developed. In addition, the models have been incorporated in user-friendly application software and thus the effect of product temperature profiles can be evaluated by electronic time-temperature integration [see (75) for a review or www.dfu.min.dk/micro/sssp/ where the Seafood Spoilage and Safety Predictor (SSSP) software is available]. Other microorganisms, including species of *Aeromonas,* Enterobacteriaceae, lactic acid bacteria and *Vibrio*, are also important in seafood spoilage (Table 53.1) and for these SSO, kinetic models remain to be developed and/or validated in relevant seafoods. Kinetic models are available for some species of Enterobacteri-aceae and lactic acid bacteria (131), but evaluation and validation, particularly with lightly preserved seafood, is lacking. Predictive microbiology is an active research area and in the near future it is likely that more extensive kinetic models can be developed from different existing models and from easily accessible results collected in databases like ComBase (www.combase.cc). Clearly, evaluation of these models' ability to predict growth in specific seafoods will become important. Another and more ambitious future challenge is to predict the species of microorganisms that become SSOs when (i) new product are formulated, (ii) seafoods are processed by a new technology or (iii) seafoods are stored under conditions not previously evaluated. Data obtained within the last decades (Table 53.1) clearly demonstrate that knowledge about the microbial ecology in seafood has been insufficient to make such predictions.

Developments in classical and emerging technologies to extend shelf-life of seafood are numerous and only the effect of a few aspects will be mentioned here. The targeted inhibition of SSOs is interesting as product shelf-life may be extended by mild preservation methods, selected depending on properties of a particular SSO. Mesophilic motile *Aeromonas* spp. spoil Nile perch at ambient temperature and experiments in liquid media showed this SSO could be inhibited by combinations of NaCl, sorbate and smoke components. Relying on this information, a lightly preserved fish product with marked shelf-life extension was developed (132). Other examples include inhibition of *P. phosphoreum* in chilled MAP cod fillet by oregano essential oil (133) or inactivation of this SSO by freezing (92, 122).

IV. MICROBIOLOGY AND SAFETY OF MARINE MUSCLE FOODS

The safety records and etiological agents vary considerably between groups of seafood and between products from different geographical regions. This section includes information concerning the effect of raw material origin, processing, product characteristics, storage conditions and seafood preparation prior to consumption, which are the most important hazards (Table 53.2).

A. Histamine, Biogenic Amines and Histamine Fish Poisoning (HFP)

HFP is a mild disease that occurs rapidly after intake of seafood containing above 500–1000 ppm of histamine. The toxic effect of histamine is probably potentiated by other biogenic amines in seafood, i.e., agmatine, cadaverine, phenylethylmine, putrescine, spermidine, spermine and tyramine. Alone, tyramine may cause migraine headaches in susceptible individuals. Symptoms of HFP can be cutaneous (e.g., rash or inflammation), gastrointestinal (nausea, vomiting, diarrhoea), neurological (e.g., headache, burning or itching) or circulatory (hypotension). Once formed in seafood, biogenic amines are heat stabile and will not be destroyed by cooking, baking or even canning (135–138).

Not all seafoods cause HFP and it has long been know that a high content of free histidine, growth of histidine decarboxylase-producing microorganisms to high concentrations and high activity of microbial histidine decarboxylases are prerequisites for histamine production in significant amounts and thereby HFP. Fish with a sufficient content of free histidine to cause HFP include anchovy, bluefish, bonito, herring, mackerel, mahi-mahi/dolphinfishes, marlin, saury/garfish, swordfish, tuna and yellowtail (135–137). Storage temperature is important for histamine formation and, as a general rule, little histamine is formed below 5–7°C whereas above 7–10°C toxic concentrations are frequently produced. However, toxic concentration of histamine have been observed at 0–4°C, e.g., in mackerel (139), saury (139), sardines (139–141) and tunas (142).

Numerous microorganisms produce histamine but only some species of Enterobacteriaceae, *Vibrionaceae*, and lactic acid bacteria (LAB) can grow to above ~10^7 cfu/g and produce toxic concentrations of histaming in seafood. Some Enterobacteriaceae are strong histamine producers, e.g., *Enterobacter aerogenes*, *E. cloacae*, *Morganella morganii*, *Proteus vulgaris* and *Raoultella planticola.* It seems *Klebsiella* spp. do not produce histamine and that, e.g., strains of *Klebsiella pneumoniae* and *K. oxytoca* previously reported to do so may subsequently have been identified as *Raoultella* spp. (143, 144). Below 7–10°C Enterobacteriaceae did not produce toxic concentrations of histamine (145) but histidine decarboxylase

TABLE 53.2
Seafood Associated Hazards and their Prevention;

Hazard	Safety Concern	Preventive Measures
Histamine and biogenic amines	Microbial formation of >500 ppm histamine in products; of primary concern are Enterobacteriaceae in temperature-abused seafoods and *P. phosphoreum* and possible other bacteria in chilled products	Reduce microbial growth and activity by storage of fresh seafood at <2°C and lightly preserved products at <5°C; critical histamine formation may precede sensory spoilage and storage times should be established accordingly (134)
Vibrio species, primarily hemolysin producing *V. parahaemolyticus,* cholera toxin producing *V. cholerae* and *V. vulnificus*	Growth to high levels in live molluscs, in fresh seafood from warmer waters and in cross-contaminated, ready-to-eat products	Avoid storage of live molluscs and fresh seafood at abusive temperature conditions and avoid consumption of improperly cooked seafood, particularly bivalve molluscs
Clostridium botulinum Group I: Proteolytic, particularly toxin type A and B	Growth and toxin formation in products	Inactivate bacteria and spores by canning (>2.4 min. at 121°C) or prevent growth by chilling (<10°C), acidification (pH <4.5) or salt curing (>10% NaCl)
Group II: Non-proteolytic and psychrotolerant, particularly toxin type E	Growth and toxin formation in ready-to-eat (hot-smoked and fermented) products from temperate or cold regions	3.5% water phase salt prevents toxin formation during 4 weeks storage at 4–5°C
Listeria monocytogenes	Growth in ready-to-eat seafoods	Limit contamination by good hygiene practices, reduce growth through controlled product characteristics and limited product shelf-life *L. monocytogenes* is inactivated by normal cooking
Salmonella spp.	Contamination, even by low levels	Avoid direct or indirect faecal contamination of products; inactivate the bacteria by normal cooking
Viruses, primarily Norwalk-like virus (Noroviruses group I and II) and hepatitis A virus	Presence in seafood particularly in bivalve molluscan shellfish consumed raw or lightly cooked	Avoid harvest of bivalve molluscan shellfish from contaminated waters; avoid consumption of improperly cooked seafood, particularly bivalve molluscs

produced at ≥10°C may remain active after chilling to 0–5°C (146, 147). The quantitative effect of this merits further study in seafoods. Mesophilic *Vibrionaceae*, including *Vibrio alginolyticus* and *Plesiomonas shigelloides*, produce histamine but not in important amounts in seafood. In contrast, the psychrotolerant *P. phosphoreum* (previously N-group bacteria and gut-group vibrios) and the mesophilic *Photobacterium damselae* subsp. *damselae* (previously *P. histaminum* and C-group bacteria) have been important in chilled and temperature-abused seafood, respectively (139). *P. phosphoreum* can produce toxic histamine concentrations at 0–4°C [(148) and unpublished data from the laboratory of the author]. However, the temperature history of seafood causing outbreaks of HFP and the microorganisms responsible for histamine formation typically are not known. Further research, and particularly quantitative studies, e.g., including yield factors (89), are needed to determine the importance of psychrotolerant histamine-producing microorganisms and the risk of HFP from appropriately chilled seafoods. LAB do not produce toxic concentrations of histamine in chilled seafood, whereas formation of tyramine is common, e.g., by *Carnobacterium* spp. (149). A mesophilic LAB, *Tetragenococcus muriaticus*, occurs in salt-fermented fish sauce. At 30°C and with 5–15% NaCl it produced >400 ppm of histamine and the production was markedly increased when pH was reduced from 7.6 to 5.8 and by growth under oxygen-limiting conditions (150). At 20°C, *Lactobacillus buchneri* formed >500 ppm of histamine in herring and tuna salad (151). Interestingly, the optimum pH for histamine production by Enterobacteriaceae, *Vibrionaceae*, and LAB is 5–6, which is much lower than their pH optimum for growth, but similar to the pH in many of the dark-fleshed fish where toxic concentrations of histamine are formed.

EU regulations indicate histamine concentrations in specific seafoods must not exceed 100–200 ppm, whereas the U.S. FDA uses a defect action level of 50 ppm. These relatively low values reflect the fact that histamine concentrations in seafood vary considerably and that a low concentration in one sample suggests a toxic concentration may be present in a different sample from the same lot (137, 152). To control histamine formation in seafood, chilling and storage time are the most important factors (Table 53.2). Freezing can inactivate histamine-producing bacteria but histidine decarboxylase is active during frozen storage and products of dubious freshness should not be frozen. Recently, molecular methods were suggested to identify and detect histamine-producing microorganisms (144, 153). However, quantitative, sensitive and robust methods to enumerate histamine-producing microorganisms in seafood are lacking. This is also

the case for methods to predict histamine formation, although a few simple models to assess the effect of time and temperature on histamine formation in skipjack tuna are available (154, 155) and a semi-quantitative risk assessment tool has been suggested (156).

B. *Vibrio* Species

Within this genus, the most important seafood-borne human pathogenic species are *Vibrio parahaemolyticus*, *V. vulnificus* and *V. cholerae*, but *V. alginolyticus*, *V. fluvialis*, *V. hollisae* and *V. mimicus* have also caused seafood-borne gastrointestinal disease. In Japan, Asian countries and the U.S. outbreaks are common, whereas they are rare in Europe. Disease is primarily due to consumption of temperature-abused raw or inadequately cooked seafood (Table 53.2). Raw fish like sushi and sashimi is increasingly consumed in Europe but this has not yet resulted in outbreaks caused by *Vibrio* spp. Analysis of *Vibrio* spp. is important in Europe and their occurrence has been a major reason to reject seafood imported into the EU (12, 18). As for the luminous vibrios discussed in Section II, occurrence of mesophilic human pathogenic species in water and aquatic animals depends on temperature and salinity. Both their concentration in seawater and outbreaks of seafood-borne disease are highest during in the warmer months of the year. *V. parahaemolyticus* and *V. vulnificus* are typical marine vibrios that grow optimally with 2.0–2.5% NaCl, whereas *V. cholerae* does not require NaCl and growth is optimal with 0.2–0.5%, corresponding to its natural habitat in estuarine waters (12, 157–159).

V. parahaemolyticus causes a gastrointestinal disease characterized by diarrhoea abdominal cramps, nausea, vomiting and headache. Antibiotics, e.g., tetracyclines, can reduce prolonged infections. For clinical isolates, >95% produce a thermostable direct hemolysin (TDH) (causing a positive Kanagawa reaction) or a TDH-related hemolysin (TRH). For seafood isolates, <1–5% contain genes for TDH and/or TRH and the large majority of *V. parahaemolyticus* in seafoods are non-pathogenic (159–161). Pathogenic variants of *V. parahaemolyticus* can be detected by molecular methods, including various PCR approaches, and further development in this area can be expected also for other pathogenic *Vibrio* species (161–164). The infectious dose for *V. parahaemolyticus* is ~10^6 pathogenic cells and growth in seafood is required for disease to occur. In relation to risk assessment activities, there have been attempts to model the effect of water temperature and salinity on the concentration of *V. parahaemolyticus* in live oysters. Furthermore, inactivation in chilled and growth in temperature-abused live oysters have been quantified (157). For processed seafoods, a mathematical model is available to predict the effect of product storage temperature and water activity on growth of *V. parahaemolyticus* (165).

V. vulnificus is an invasive species that may cause septicemia. Symptoms include high fever, chills, nausea and hypotension. The disease can be fatal, with mortality rates as high as 50%, and prompt antibiotic treatment is required. Individuals with liver disorder are particularly at risk and they should never consume raw seafood. Virulence factors and infectious dose remain uncertain for *V. vulnificus* and in contrast to *V. parahaemolyticus*, a high percentage of *V. vulnificus* isolates from oysters seems to be pathogenic (166). *V. vulnificus* is particularly associated with oysters in seawater at above 15°C and models to predict the effect of water temperature and salinity on the concentration in live oysters at harvest and growth at high distribution temperatures post-harvest have been suggested (157, 167, 168).

V. cholerae causes the notorious gastrointestinal disease cholera with severe watery diarrhoea that results in dehydration and can be fatal. For *V. cholerae,* the two serotypes O1 (including the biotype O1 El Tor) and O139 produce cholerae toxin (CT) and they have been responsible for epidemics. However, *V. cholerae* non-O1/non-O139 have also caused sporadic cases of milder gastrointestinal disease and 10–17% of environmental *V. cholerae* non-O1/non-O139 isolates has been found to contain genes for CT production (169). The infectious dose of *V. cholerae* is ~10^6 cells depending on the food matrix (160). Water contaminated with sewage is the major reason for *V. cholerae* O1/O139 in seafood and improved sanitation is the key to solving the problem. *V. cholerae* non-O1/non-O139, however, occur naturally in estuarine waters. In the U.S. 14% of freshly harvested oysters were contaminated with *V. cholerae* non-O1 (18) and *V. cholerae* non-O1/non-O139 were found, e.g., in French mussels (170). In South America, *V. cholerae* 01 was not reported until a major outbreak in the early 1990s caused >400,000 cases and 4,000 deaths. A lightly preserved seafood *ceviche* consisting of raw fish, lime juice, vegetables and salt, was a possible vehicle for *V. cholerae* 01 (171).

Depuration of oysters may not reduce concentrations of pathogenic *Vibrio* spp. and above ~20°C they may even grow. Fortunately *Vibrio* spp. are sensitive to many types of seafood preservation including chilling at 0–5°C, resulting in slow inactivation, and freezing, which inactivates the bacteria more rapidly. The pathogenic *Vibrio* species are heat sensitive and inactivated by normal cooking (12). Recently, high-pressure processing at 250–300 MPa has been used commercially to inactivate *Vibrio* spp. in sucked oysters and concentrations of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* were found to be efficiently reduced (83, 172, 173).

C. *Clostridium botulinum*

Cl. botulinum is a botulinum neurotoxin (BoNT) producing Gram-positive, anaerobic spore-forming bacteria.

After consumption of seafood with BoNT, symptoms of botulism initially include nausea and vomiting, later double vision, inability to focus, difficulty swallowing and speaking and loss of muscular coordination that can lead to death by respiratory paralysis or cardiac arrest. Onset of symptoms after 12–36 hours is typical and without treatment death after 3–6 days is likely. In fact, mortality has been as high as 50–75%, but treatment with antisera and respiratory aid has reduced mortality to 10–15% (174, 175).

The biochemical and phylogenic variability of *Cl. botulinum* isolates does not correspond to a single microbial species (176) and strains have been divided into four groups (I–IV). Based on antigenic properties, seven types of BoNTs have been identified (types A to G). *Cl. botulinum* group I are proteolytic, halotolerant and mesophilic with minimum growth temperature ~10°C. They produce heat-resistant spores ($D_{121°C}$ = 0.21 min) and BoNTs of type A, B and F. Group II consist of non-proteolytic and psychrotolerant strains with minimum growth temperature as low as ~ 3°C. They produce spores that are relatively heat sensitive ($D_{82.2°C}$ = 0.4–2.4 min) and BoNTs of types B, E and F. *Cl. botulinum* group III and IV are not related to food-borne disease, but some strains of *Cl. butyricum* produce BoNT type E and may cause food-borne disease. BoNTs have a very low lethal dose and they are resistant to low pH and NaCl. Fortunately, the proteins are sensitive to heat and pH above 7–7.5 and can be inactivated by 20 min at 79°C or by 5 min. at 85°C (12, 174). BoNTs can be detected by mouse bioassays or enzyme-linked immunosorbent assays (ELISA), whereas enrichment procedures, selective plating media and PCR methods are available for detection of vegetative cells and spores of *Cl. botulinum* (174, 177). *Cl. botulinum* group I occurs frequently in soil, but is also found on aquatic animals, primarily in warm waters. In contrast, group II is found frequently in sediments, water and aquatic animals from freshwater and seawater environments in temperate or cold regions. In agreement with this, numerous studies have found low concentrations of spores of particularly *Cl. botulinum* type E in newly caught or processed seafood. Typically <0.1 spore/g was found, but higher concentrations have been found for pond-raised freshwater fishes. Although seafood is frequently contaminated with *Cl. botulinum*, outbreaks of botulism have primarily been associated with fermented/salted and hot-smoked products (174, 175).

For fresh seafood stored aerobically, vacuum-packed (VP) or modified-atmosphere-packed (MAP) growth and toxin formation by *Cl. botulinum* have been extensively studied. Below ~10°C products become spoiled before toxin can be detected, but with substantial temperature abuse toxin formation is more critical and these storage conditions must be avoided. In general, oxygen delays toxin formation by *Cl. botulinum*, but aerobic storage or modified atmospheres with oxygen cannot totally prevent toxin formation as anaerobic microenvironments are formed in the products (17, 178). Cooking prior to consumption can, to some extent, inactivate BoNT and this may have contributed to the excellent botulinum safety record of fresh seafood irrespective of its storage atmosphere.

The psychrotolerant and non-proteolytic *Cl. botulinum* (group II) is able to grow and produce toxin at ~3°C, with <5% NaCl and at pH > 5.0, but combinations of these parameters are inhibitory, which is most important for the safety of several lightly preserved seafoods. It is generally accepted that 3.5% water phase salt (WPS) is sufficient to prevent toxin formation in seafood during 4 weeks at 4–5°C (175). However, BoNT formation in some seafood was inhibited by considerably lower NaCl concentrations. From a sensory point of view, less than 3.5% WPS is often desirable and further studies to identify product characteristics that may contribute to inhibition of BoNT formation in chilled lightly preserved seafoods is relevant. Mathematical models have been developed to predict the effect of temperature, NaCl, pH, CO_2 and spore concentration on growth or BoNT formation by C*l. botulinum*. These models may assist in identification of safe storage conditions for some types of seafoods (131). Proteolytic *Cl. botulinum* are much more NaCl resistant than the non-proteolytic, but conditions to prevent their growth and BoNT formation in seafood by heating, salt curing or acidification are well established [Table 53.2 (12)]. In fact, recent association of botulism with seafoods has resulted from poorly managed heat treatment or fermentation conditions (174).

D. *Listeria monocytogenes*

Occurrence of *L. monocytogenes* in seafood is a considerable problem with respect to trade, but fortunately it is very rare that seafood consumption results in listeriosis. *Listeria* is Gram-positive, non-spore-forming, motile rods that biochemically resemble lactic acid bacteria. *L. monocytogenes* is typically invasive and causes disease in people who are elderly, pregnant or have predisposing health conditions like organ transplants, diabetes or AIDS. The incubation time can be long (weeks to months) and this complicates tracing of sporadic outbreaks back to the implicated foods. *L. monocytogenes*, however, may also cause a non-invasive febrile gastroenteritis in otherwise healthy people. If diagnosed, listeriosis can be treated with antibiotics, e.g., ampicillin but mortality rates of untreated listeriosis have been high (~30%) and this is a major reason for the extensive studies of *L. monocytogenes* during the last 20 years.

As mentioned in Section II.C, *L. monocytogenes* is indigenous to the general environmant and frequently found in low concentrations on newly caught aquatic animals and in fresh and lightly preserved seafoods (12, 179). Low concentrations of *L. monocytogenes* in seafood are not an important risk to human health and the main safety concern is growth in ready-to-eat products [Table 53.2, (180)]. More than 1000 *L. monocytogenes* per gram or ml

have been found in foods that caused listeriosis (181). Despite this fact, some countries, including the U.S., require absence of *L. monocytogenes* in 25-g samples of products. This zero tolerance is a frequent reason for import refusal of seafood by the U.S. FDA (12).

L. monocytogenes is able to grow between ~0°C and ~40°C, with <10% NaCl corresponding to $a_w > 0.92$, at pH above ~4.5 and it is problematic to control growth in some types of seafoods. In fact, a recent risk assessment found smoked seafood and cooked ready-to-eat crustaceans to be of high risk on a "per serving basis" and of moderate risk when evaluated per annum for the total U.S. population (180).

Models to predict the effect of, e.g., temperature, NaCl/a_w, pH, organic acids, nitrite, smoke components, atmosphere and microbial interaction on growth, survival or inactivation of *L. monocytogenes* have been extensively studied. Some models are included in application software to facilitate prediction of growth in foods (131, 182). However, predictive models developed in liquid laboratory media may not be appropriate for use with seafoods. Thus, users of models should verify that a model has been successfully validated for seafoods with microbial ecology similar to the product to which it will be applied.

L. monocytogenes is inactivated by normal cooking of seafood ($D_{60°C}$ = 2–4.5 min) and inhibited by the high concentrations of acetic acid (~2.5%) used in several semi-preserved seafoods, like marinated herring. However, processing of a number of ready-to-eat seafoods, including cold-smoked products, does not include critical control points for *L. monocytogenes.* To prevent growth to high concentrations the following parameters are of major importance: (i) good hygiene practices with careful cleaning and sanitation of known or likely niches in the processing environment, (ii) controlled product characteristics and (iii) limited shelf-life at specified temperature conditions. Modified atmosphere packing with CO_2-containing atmospheres reduces but does not prevent growth of *L. monocytogenes.* The combined effect of 1–2% lactate and 0.1–0.2% diacetate reduces growth of *L. monocytogenes* in different meat products and use of these organic acids in seafood deserves further study. Addition of lactic acid bacteria can to inhibit *L. monocytogenes* in lightly preserved seafood (183, 184), but this type of biopreservation has not yet been used at industrial scale.

E. Salmonella

Salmonella is responsible for a significant number of the cases of disease caused by finfish and shell-fish, both in the U.S. and in the U.K. (see Section I). In contrast to the etiological agents discussed above, *Salmonella* originate from the human/animal reservoir and therefore good hygienic practices, with focus on clean water and personal hygiene, are essential to control occurrence in seafood.

Salmonella is a mesophilic member of the Enterobacteriaceae family that grows at above ~5°C, pH > 3.8 and with <6% NaCl. The genus consists of only two species: *Sal. enterica* and *Sal. bongori.* The former is divided into six subspecies and the genus includes more than 2400 serovars (185). The infectious dose of *Salmonella* is frequently ~10^6 cells, but it can be much lower (10–100 cells) when the bacteria is protected against stomach acid, e.g., by fat (12). Typhoid and paratyphoid strains can lead to enteric fever syndrome salmonellosis with watery diarrhoea, fever, abdominal pain, headache and nausea occurring 7 to 28 days after intake of the strains. In contrast, symptoms caused by nontyphoid strains appear rapidly after exposure (8–72 hours) and include nausea, abdominal cramps, diarrhoea, fever and vomiting (185). When symptoms disappear, people continue to excrete *Salmonella* for up to several months and this is a potential risk for seafood contamination during processing.

Salmonella is rare in temperate waters, but can be found in tropical estuaries and coastal waters as well as in aquatic animals from these habitats. Compared to other types of foods, seafood has caused relatively few, and no major, outbreaks of salmonellosis, but occurrence of *Salmonella* in seafood has been a major reason to reject products at port of entry into both the EU and the U.S. (12, 185).

Mathematical models to predict growth or inactivation of *Salmonella* as a function of temperature, NaCl, pH and essential oils are available (131) and can be used to facilitate assessment and management of the risk caused by the pathogen in seafoods. *Salmonella* is heat labile ($D_{60°C}$ of 1–3 min) and inactivated by normal cooking and, for example, hot-smoking. Thus, focus on post-processing contamination and reduced consumption of raw seafood are important to limit salmonellosis in seafoods (Table 53.2).

F. Viruses

Viruses cause a higher number of seafood-borne diseases than any other microbial hazard. This is particularly due to filterfeeding molluscan shellfish consumed as raw or lightly cooked products. Seawater contains numerous indigeous viruses in high concentrations but seafood-borne diseses are caused by human enteric viruses and particularly Norwalk-like virus (NLV, also referred to as Noroviruses group I and group II) and to a lesser extent hepatitis A virus (HAV). NLV and HAV do not grow in the environment and the main concerns with respect to seafoods are (i) sewage or faecal contamination of aquatic habitats, particularly shellfish growing areas, where molluscan shellfish through their filtration of water to obtain food can accumulate viruses from the water to concentrations that are infectious to humans and (ii) fecal contamination of seafood during processing as a result of poor personal hygiene (186, 187). Seafood-borne disease due to virus in molluscan shellfish is much more frequent than

due to virus in finfish (Section I), suggesting contamination of aquatic habitatsis relatively more important than contamination during processing.

NLV is a genetically diverse group of virus strains divided into two genogroups (188). NLV causes gastroenteritidis with diarrhoea, vomiting and possibly abdominal pain, nausea and fever. Incubation time is ~24 hours and duration of symptoms ~2 days. The infectious dose is estimated to be low but lack of sensitive enumeration methods has prevented more exact determination (186). Recently, oysters with >1000 virions each were shown by a most-probable-number-reverse-transcriptase-PCR approach to cause an outbreak (189). HAV causes infectious hepatitis with jaundice (yellow coloring of skin and eyes), fever, headace, nausea, malaise/vomiting, diarrhoea and abdominal pian. The disease is occasionally fatal. The incubation period is 2–6 weeks and symptoms can last 2 months (186). Both NLV and HAV contain RNA genomes and a protein cover (capsid).

Viruses, in contrast to bacteria, cannot be cultured in simple laboratory media and NLV and HAV from seafood cannot even grow in cell cultures. In faeces, where high concentrations are precent, virus can be detected by electron microscopy and immunoassays, but the detection limit of these techniques is too high for enumeration of NLV and HAV in seafood. Lack of sensitive methods for detection and enumeration have greatly limited our understanding of the occurrence and ecology of viruses in seafoods. However, application of molecular methods including various PCR approaches is now changing this situation (186, 190–192). Further developments, particularly of sensitive and quantitative PCR-based methods, must be expected in the near future although the genetic variability of NLV and the specific detection of active viruses that cause disease represent challenges. Difficulties with virus detection are reasons why both U.S. and EU use legislative standards for live shellfish that rely on enumeration of faecal coliforms or *Escherichia coli*. In fact, numerous studies have found poor correlation between contamination of shellfish with virus and the coliform indicators of faecal contamination and there is a need for updated standards (186, 191).

To control the presence of human enteric viruses in seafood several options are available. Preventing harvest in contaminated waters is an obvious but not always easy solution. If, e.g., a shellfish harvesting area is contaminated by sewage after heavy rainfall, the virus can remain for a long time in seawater. The decimal reduction time of HAV is 671 days at 4°C and 25 days at 25°C (193). However, overboard disposal of faeces by personnel working in a shellfish harvesting area caused an outbreak of NLV disease. This type of contamination should be easy to prevent (186). Depuration of shellfish by circulation of non-contaminated water for 1–7 days is common and can reduce virus concentrations in the animals but faecal coliforms are eliminated more rapidly and virus removal may require weeks (186, 194). To prevent virus contamination during seafood processing it is important that NLV is shed by a person two days after onset disease whereas HAV is shed in faeces ~2 weeks prior to symptoms and 1–2 weeks after of the disease. In appropriate periods after NLV, and HAV-related disease, seafood industry personnel should not handle products.

RNA has limited stability but viruses seem relatively resistant in seafood although the resistance of pathogenic strains in the low concentrations actually found in seafood merits further study. 90°C during 1.5 min has been recommended for inactivation of virus in seafood (186). High-pressure processing of oysters at 250–300 MPa may reduce levels of NLV but will probably have little effect on HAV (195).

V. CONCLUDING REMARKS

Many aspects of the microbiology of marine muscle foods have been extensively studied and the information obtained has been valuable in the management of product quality and safety. However, occurrence of microbial seafood spoilage and seafood-borne disease remains unacceptably high and further research is needed to improve this situation.

The development and distribution of antibiotic-resistant microorganisms in aquatic animals and seafood needs further study. Concerning SSOs, research is particularly needed for lightly/semi-preserved and fermented seafoods where combinations of several preserving parameters influence microbial growth and activity. It is also relevant to evaluate spoilage caused by mixed groups of microorganisms (metabiosis) and to evaluate how information about SSOs can be used to determine, predict and extend the shelf-life of lightly and semi-preserved seafoods. As a part of this work development of sensitive, specific and rapid methods to detect SSOs is relevant and it seems appropriate to evaluate quantitative PCR approaches. Concerning seafood-borne disease, more precise information is needed to understand the occurrence and inactivation of viruses in seafood. The importance of psychrotolerant histamine-producing microorganisms is not yet completely understood. Finally, improved methods to control the growth of *L. monocytogenes* in lightly preserved seafoods are required.

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54 Microbial Analysis of Foods

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CONTENTS

I. INTRODUCTION

Microbial analysis of foods is important in food safety control. It can be done for many reasons (legislation, trend analysis, outbreak analysis, etc.) and by many participants (governmental agencies, food processors, etc.) (1). An overview of the various categories of microbial testing is given in Table 54.1.

TABLE 54.1
Microbial Testing in Food Microbiology

Categories of Microbial Testing	Definition	User/Purpose
Acceptance/rejection testing	Testing for presence/absence of pathogens in defined amount of sample or determination of plate counts of indicator organisms based on agreed specifications and sampling plans (3)	International trade
End product testing	To document that a batch of food meets legal or internal quality standards	Food producer
Trend analysis	Accumulate data to measure changes in the product rather than in the batch, to verify that the application of HACCP keeps the process under control	Food producer
Statutory testing	To control whether a batch of food meets the legal requirements; duplicate samples are made available for independent analysis by the food producer/outlet	Control authorities
Investigative sampling	To determine the source of contamination of a product by pathogens or spoilage organisms	Food producer
Outbreak analysis	To determine the food which could have caused a problem of food-borne illness	Control authorities
Environmental and hygiene monitoring	To determine the hygienic status of the food plant: swabs or scrapings and agar contact plates from food production infrastructure and equipment	Food producer

The major incentive for testing is legislation. Microbial analyses of end-products are specified and mandatory for specific food categories, especially for foods of animal origin. End-product testing is performed by control authorities to check whether a batch of food meets the legal requirements for such food. Although the limitations of end-product testing of foods at the port-of-entry or elsewhere in the food chain to ensure microbiological safety was recognized, numerous end-product testing is still widely performed. However, the overall framework for microbiological testing and interpretation of results is slowly changing. End-product testing has shifted to a system where the focus is verification of preventive approaches based on the use of good manufacturing practices (GMP) in combination with the Hazard Analysis Critical Control Points (HACCP) systems as more reliable means of assuring product safety in the modern food industry (2). In contrast to original expectations, introduction of HACCP did not lead to a reduction of the number of microbial analyses performed. Effective implementation of HACCP requires knowledge on the incidence of pathogenic bacteria in foods, and the organism's response to conditions in foods. Microbial testing programs have been elaborated by manufacturers to acquire in-house knowledge on the prevalence, persistence and behavior of pathogens in a specific food commodity/production site. In addition, the microbiological analysis of ingredients, half-fabricates and end-products as well as of environmental samples are often part of the monitoring program. Although microbiological analyses included in legislation are especially directed towards investigation of pathogenic bacteria and/or indicator organisms, microbial testing of foods is not restricted to these groups of microorganisms. Microbial analysis is an economically relevant parameter. Many food commodities have a limited shelf life because of microbial proliferation during storage. Microbial analysis to determine the composition and the level of the initial microbial flora is essential to predict typical spoilage patterns and to monitor shelf life in foods.

The present chapter will give an overview of the problems associated with microbial food testing, the classical culture-based methodology available for enumeration, detection and identification of food- and water-borne microorganisms, the microbial parameters under consideration for microbial testing and the conventional applied methodology. For discussion of rapid analysis techniques in food microbiology, the reader is referred to Chapter 55 of this handbook.

II. PROBLEMS ASSOCIATED WITH FOOD ANALYSIS

A. Sampling Plans

The quality and safety of a food product cannot be guaranteed by end-product control. While acceptance or rejection of a food is ideally decided by inspection of 100% of the items of a lot, this is too laborious, time-consuming, costly and destroys the lot. Testing of representative samples has to be undertaken instead. When a batch of food is composed from separate units, a selection of units has to be taken from this lot at random. The sample units so drawn will, after examination, yield results which are compared with defined criteria to reach a decision as to whether the entire lot should be accepted or rejected. The particular choice of sampling procedure and the decision criteria is called the 'sampling plan.' A sampling plan states the number of units required to be randomly collected from a lot and lists the acceptance and rejection criteria. As an example, consider that a lot $N > 1000$ units is to be analyzed for coliforms per gram and a unit is to be called defective if it has ≥ 100 coliforms per gram (rejection criterion) and to be called acceptable or nondefective if it has < 100 coliforms per gram (acceptance criterion). A sampling plan is necessary to define the number of units (n) to sample where the number of defectives (c) equals zero. A sampling plan of $n = 30$ and $c = 0$ may be chosen,

however, unless all units are sampled there is always a probability that a 'bad' lot, when offered, will be falsely accepted (consumer's risk). For each sampling plan (definition of n and c) the probability of lot acceptance in relation to lot quality (usually expressed as percent defective units) can be calculated. Using the above mentioned sampling plan (n = 30, c = 0) the chance (consumer's risk) of accepting a lot with one defective unit out of 40 will be about 50%. With regard to food-borne pathogens, large numbers of samples must be found free of target organisms before any significance can be attached to negative results. No feasible sampling plan can ensure complete absence of a particular organism, e.g., if 60 samples were taken and analyzed there is still one chance in two of being accepted for lots in which 1% of the sample units carry a pathogen. Moreover, the above calculation is based on the assumption that the organisms sought are homogenously distributed in the food. In the vast majority of foods**,** microorganisms are randomly distributed. This makes clear that end-product control alone cannot control the production process but can only help in verification of the good functioning of the preventive modern quality assurance systems, including HACCP. For more information on specific proposals for sampling and sampling plans the reader is referred to the ICMSF publication (3) or to the website www.dfst.csiro.au/icmsf/publications.htm.

B. Dynamic Biological Population

Microbial populations of foods have a dynamic character. Often there is an increase or decrease in numbers of viable cells during processing, storage and distribution. The outcome of a microbiological analysis or a typing method reflects the microbial quality of the food or the type of pathogen present at that particular time of sampling. The microbial condition of the product should not change during sampling. Appropriate measures should be taken to prevent any contamination of the sample units and any microbial growth or death within the sample units during collection, transport to the laboratory and subsequent storage and handling of the samples. Sampling should be performed in an aseptic way using clean, dry, sterile materials. Microbiologically unstable products should be transported and stored at a temperature below 4°C and, in general, the period between sampling and analysis should not exceed six hours. Specific rules for the preparation of test samples are to be found in the ISO 6887 series.

C. Sub-Lethal Damage and the Resuscitation of Damaged Populations

Microorganisms occurring in foods are frequently impaired by sub-lethal injury as a result of having been exposed to adverse conditions. This may be due to processing by mild heat treatment or non-thermal inactivation methods. Exposure to adverse intrinsic conditions including low a_w and pH, or to extrinsic factors such as low temperature, oxidizing agents and modified atmospheres may also lead to sub-lethal injury. Such cells may pose problems of detection because they fail to grow on selective media. An increased lag phase duration in injured cells and difficulty in isolating especially low numbers of stressed cells have been demonstrated (4, 5). However, these sub-lethally injured organisms may be capable of repair in certain food products and may possess the potential for pathogenicity, thus posing a potential public health risk. New improved protocols have been proposed for recovery of injured cells. For enumeration procedures some authors have included a resuscitation step on a solid non-selective medium for a few hours, plates being then overlaid with selective agar. For detection procedures, enrichment broths differed significantly in the ability to detect injured cells. The addition of various components to the media, including sugars, yeast extracts, egg yolk, salt, osmoprotectants, cations, reducing agents and antioxidant enzymes (6), may aid in the recovery of sub-lethally damaged cells.

However, one of the main problems remains the dilemma of promoting resuscitation of injured cells whilst avoiding overgrowth by the competitive microflora. In microbiological food control laboratories the use of sufficiently selective media may even be necessary and give better recovery results when food contains high levels of competing background flora.

D. The Need for Highly Selective Procedures

Raw food products harvested or processed under GMP and under applications of well-functioning quality assurance systems such as HACCP are in general of good microbial quality calling for methods allowing assessment of levels of the order of 100 per gram for indicator organisms to 10^4–10^6 per gram for total viable count depending upon the type of product. Several food-borne pathogens are highly infective and often low numbers of pathogens (0.1–10 cfu per g) are present in a ratio of 10^{-4} amongst physiologically similar organisms. These low numbers may not go undetected in these foods. An example is the enforcement of detection of one to ten colony forming units (cfu) of salmonellae amongst some 10^4–10^5 other Enterobacteriaceae. The detection of low numbers of pathogens amongst high numbers of background flora demands highly selective enrichment and isolation procedures. If the presence of injured cells is suspected prior incubation under non- or less selective conditions may be necessary.

III. MICROBIOLOGICAL BASIC ENUMERATION TECHNIQUES

For more information on basic microbial techniques and modifications thereof the reader is referred to the

Compendium of Methods for the Microbiological Examination of Foods published by the American Public Health Association (7).

A. The Colony Count Method

In the colony count method the total number of bacteria in a product is determined by inoculating dilutions of suspensions of the sample onto the surface of a growth medium that has been solidified by agar-agar (spread-plate method) or by mixing the test portion with the liquefied agar medium in Petri dishes (pour plate method). Enumeration is performed after incubation for fixed periods at temperatures varying from 7 to 55°C in an aerobic, microaerophilic or anaerobic atmosphere. During incubation each individual cell will multiply into a colony that is visible to the naked eye. If several cells are physically connected (e.g., by adsorption to a particle of suspended matter) this will also result in one colony. The result of the viable count technique is therefore expressed as the number of cfu per unit volume. Appropriate dilutions of the food samples should have been made in order to enable counts reported from plates in the range of 25–250 colonies for pour plates and 15–150 colonies for spread plates. The principle of microbial enumeration by the conventional culture-based method is illustrated in Figure 54.1.

B. Culture Media

The culture medium used to cultivate microorganisms contains all necessary components for the growth of the microorganisms. Each medium consists of the following components: water, nitrogen compounds (peptones, amino-acids, etc), an energy source (carbohydrates, proteins, anorganic salts) and sometimes additional growth factors (yeast extract, vitamins, etc.) for nutritionally demanding microorganisms.

By addition or removal of certain components a medium can be made selective. Selective compounds frequently employed in culture media are antibiotics, chemicals (bile salts, azide, selenite, tellurite) and dyes (brilliant green, crystal violet) (Table 54.2). In addition certain extrinsic factors, e.g., increase or decrease of temperature or changes in redox potential can often be used to enhance the selectivity (8).

Designing adequate selectivity into a medium becomes more difficult as the proportion of interfering organisms increases and almost impossible when the background association is more robust than the organisms to be counted or detected. Shigellae, for example, are easily overgrown by other bacteria present in the food. Many of the enterococci are also resistant to many inhibitors used in selective culture media and may pose a seriously interfering background flora. It is often necessary, therefore, to use so-called diagnostic or elective media. Using these, the organisms sought can be distinguished from the background flora on the basis of a specific metabolic activity, e.g., dissimilation of lactose visualized by the incorporation of a pH indicator monitoring acid production, dissimilation of aesculine to aesculitin, iron salts which result in a black precipitate upon production of H_2S by the target organism from a sulphate-containing substrate. For more information on the composition of culture media and their selective and diagnostic agents the reader is referred to the catalogues of the suppliers of these media.

1. Semi-Solid Media

Semi-solid media are media which differentiate microorganisms on the basis of their motility. Due to the low incidence of non-motile *Salmonella* strains, the use of motility enrichment has been found effective for rapid detection and isolation of *Salmonella* (9). As a combined enrichment-isolation medium for *Salmonella*, modified semi-solid Rappaport-Vassiliadis (MSRV) medium compares favorably with standard protocol using RV broth followed by isolation on a selective agar and gives results

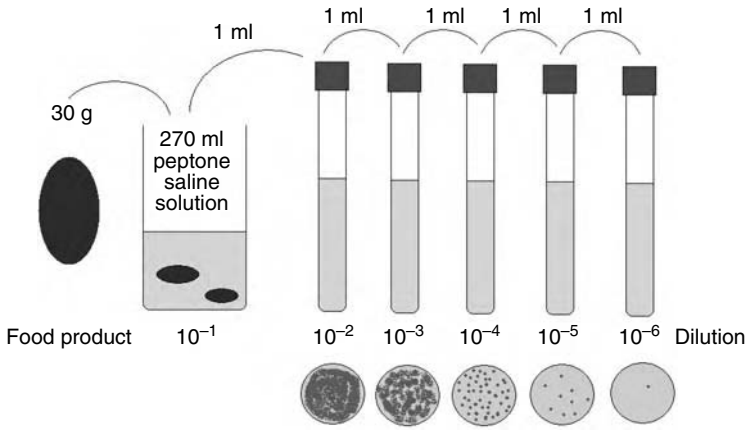

FIGURE 54.1 Lay-out of the conventional procedure for a colony count method.

TABLE 54.2
Review of Selective Agents used in Selective Culture Media

Category of Selective Agent	Examples	Inhibitory Towards	Example of Application in Culture Media (Microbial Parameter)
Dyes	Brilliant green	Gram-positives	Brilliant green agar (*Salmonella*)
	Crystal violet	Gram-positives	Violet red bile glucose agar (Enterobacteriaceae)
Surface active agents	Bile salts	Non-enteros	Violet red bile glucose agar (Enterobacteriaceae)
	Tergitol-7	Gram-positives	Tergitol-7 agar (coliforms)
Inorganic salts	Potassium tellurite	Gram-negatives	Baird-Parker agar (*Staphylococcus aureus*)
	Lithium chloride	Enterococci	(demi)-Fraser broth, Oxford agar (*Listeria monocytogenes*)
Antibiotics	Acriflavine	Gram positive cocci	(demi)-Fraser broth, PALCAM agar (*Listeria monocytogenes*)
	Cycloserine	Broad spectrum	TSC (*Clostridium perfringens*)
	Novobiocin	Gram positives	DIASALM, MSRV (*Salmonella*)
	Oxytetracycline	Broad spectrum	OGY (yeast and molds)
	Polymyxin	Gram negatives	MYP (*Bacillus cereus*)
	Ticarcilline	Broad spectrum	ITC (*Yersinia enterocolitica*)
Anti-fungal agents	Cycloheximide	Moulds	Preston broth, Karmali agar (*Campylobacter*)
	Amphotericin	Moulds	Bolton broth, mCCDA (*Campylobacter*)

one day earlier. Diagnostic semi-solid (DIASALM) agar uses a saccharose/bromocresol purple indicator to observe migrating salmonellae as a pink zone within a green medium. Addition of nitrofurantoin to DIASALM agar favors the isolation of *Salmonella* Enteritidis from poultry samples. In a typical application, three drops of pre-enriched BPW are inoculated in one spot into the center of a semi-solid medium. After incubation, plates are examined for a motility zone. A loopful of the motile zone which is the farthest from the sample inoculum is subsequently subcultured and confirmed as *Salmonella*.

2. Chromogenic and Fluorogenic Media

Chromogenic and fluorogenic media are culture media which have the ability to detect the presence of a specific exclusive enzyme using suitable substrates, a fluorogenic or chromogenic enzyme substrate. The introduction of these media has led to development of improved methods for the identification of microorganisms even in primary isolation media.

Chromogenic and fluorogenic media are well established for detection of indicator organisms but have recently also been accepted in standard methods for detection of food-borne pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes.*

The definition of coliforms is the possession of the β-D-galactosidase gene which is responsible for the cleavage of lactose into glucose and galactose by the enzyme β-D-galactosidase. The determination of β-D-galactosidase can be accomplished by using the chromogenic substrates o-nitrophenyl-β-D-galactopyranoside (ONPG), 6-bromo-3-indolyl-β-galactopyranoside (Salmon-Gal), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). The β-D-galactosidase cleaves the chromogenic substrate and the released chromophore causes distinct and easy-to-read colored colonies, respectively, yellow, red and blue colonies for ONPG, Salmon-Gal and X-Gal.

The new generation of media use β-D-glucuronidase (GUD) as an indicator for *E. coli*. GUD activity is measured by using different chromogenic and fluorogenic substrates such as p-nitrophenyl-β-D-glucuronide rendering yellow colonies or 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc or BCIG) rendering blue colonies. TBX agar is a modification of Tryptone Bile agar to which the substrate BCIG is added. TBX agar complies with the ISO standard 16649 for the enumeration of *E. coli* in food and animal feeding stuffs. The fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) has been incorporated into various liquid media for most probable number detection of *E. coli*. MUG is hydrolyzed by GUD yielding 4-MU, which shows blue fluorescence when irradiated with long-wave UV-light (366 nm).

Commercially available media have been developed which permit rapid simultaneous enumeration of *E. coli* and coliforms in water and foods by their distinctive coloration. These media contain a variety of enzyme substrates for detection of β-D-galactosidase (presence of coliforms) and β-D-glucuronidase (presence of *E. coli*).

For more information on the development in chromogenic and fluorogenic media the reader is referred to Ref. 10.

C. Modifications of the Colony Count Method

Modifications to the format of colony count techniques have been introduced in order to decrease the detection limit (membrane filtration, most probable number method), to decrease the workload (Petrifilm™, spiral plate method) or to monitor environmental contamination (surface contact methods).

1. Membrane Filter Method

For liquid foods, the membrane filtration method enables testing of large volumes by passing them through a bacteriological membrane filter (usually of 0.45 μm pore size) and placing the filter on the growth medium. The method is especially useful for samples that contain low numbers of bacteria. Membrane filtration is the basis of the standard methods for indicator organisms in water analysis.

2. Most Probable Number (MPN) Methods

In this method dilutions of food samples are prepared as for the plate count method. Three serial aliquots or dilutions are then transferred into 9 or 15 tubes of appropriate medium for the three- or five-tube method, respectively. Specific groups of organisms can be determined by use of appropriate selective and differential media. After incubation, the number of positive tubes for each dilution is counted. To confirm the growth in questionable tubes, a loopful of the medium from the tube is transferred to an appropriate culture medium and incubated. On the basis of the number of positive tubes for each dilution a MPN in the original sample is determined by use of standard MPN tables and taking into account the dilution factor. The method was introduced by McGrady in 1915. It is statistical in nature. Unlike the viable count, the MPN does not provide a direct measure of the bacterial count. In addition, the MPN is more variable than the viable count and tends to yield a higher result. Nevertheless, it produces estimates of bacterial concentrations that are below the detectability of most current direct measurement microbiological methods. MPN methods are often the method of choice for detection of low numbers of indicator organisms or pathogens in foods (11).

3. Hydrophobic Grid Membrane Filter (HGMF)

The HGMF can be used to enumerate all or specific microorganisms from a variety of foods. The method employs a specially constructed filter that consists of 1,600 wax grids on a single membrane filter that restricts growth and colony size to individual grids. On one filter, from 10 to 9×10^4 cfu can be enumerated by an MPN procedure, and enumeration can be automated. The procedure consists of pre-filtering homogenized food samples, which traps food particles larger than 5 μm. The sample is then filtered through the membrane followed by placing the filter on a suitable agar medium for incubation, to allow colonies to develop. The grids that contain colonies are enumerated and the MPN is calculated. The method has been given AOAC approval for total coliforms, faecal coliforms, salmonellae and yeast and molds (12, 13).

4. Petrifilm™ Plate Method

An alternative to plating to agar-agar media in Petri dishes is the Petrifilm™ plate method (3M). The Petrifilm™ plate count consists of two plastic films. The bottom film is coated with adhesive, powdered standard methods nutrients, and a dehydrated cold water-soluble gelling agent. The upper film is permeable and fosters oxygen diffusion, although it retains gas formed by, e.g., coliforms during lactose fermentation. An indicator dye, triphenyltetrazolium chloride (TTC), is included. The major advantage of Petrifilm™ is that colony counts can be taken on these small and sample-ready Petrifilm™ plates, without the need for the preparation of culture media. The indicator stains the colonies red and facilitates counting. The Petrifilm™ plate occupies small volume for storage, incubation and disposal and is a user-friendly method to control the microbiological quality of foods (14). The Petrifilm™ plate Aerobic Count method for the enumeration of aerobic bacteria in foods and dairy products has regulatory approval, certification or official recognition in a number of countries. Petrifilm™ products are also available for yeast and mold counts, coliforms, *Escherichia coli*.

5. Spiral Plate Method

Traditional colony count methods are laborious and do not allow for efficient management of numerous samples. The spiral plate method is a variation of an agar spread plate method which automates the sample dilution and agar inoculation steps and provides savings in time and effort. A known volume of sample is dispensed onto a rotating agar plate in an Archimedes spiral. The amount of sample decreases as the spiral moves out toward the edge of the plate. A modified counting grid, which relates the area of the plate to sample volume, is used to count colonies on an appropriate area of the plate and enables the colony count for the sample to be calculated. The primary advantage of this method is that one inoculation can enumerate bacterial densities of, for example, 500–500,000 cfu/ml, depending on the manufacturer. Within that range no additional dilutions are needed and thus savings on materials (pipettes, Petri dishes) can be made. The biggest disadvantage of the spiral plate method is the tendency of food particulates to plug the inoculation stylus. The small volume of sample plated also limits the sensitivity of the method. The spiral plate count is accepted for total enumeration by the U.S. Food and Drug Administration and is an AOAC International Official Method for food testing. The spiral plate method has also been used to test milk samples (15).

6. Surface Contact Methods

An environmental monitoring program may be necessary to assess the microbial contamination of the processing equipment and the plant itself (16) and to verify the effectiveness of cleaning and disinfection cycles. Swab procedures and replicate organism direct agar contact (RODAC) plates are the methods of choice for sampling of surfaces (17).

Swab techniques should be used for flexible, uneven and potentially heavily contaminated surfaces on equipment and utensils. The swab will be more effective than the RODAC plate in recovering organisms from these sites. The swab methods use either cotton or calcium alginate swabs. If one wishes to examine given areas of surfaces, templates may be prepared. The sterile template is placed over the surface, and the exposed area is rubbed thoroughly with a moistened swab. The exposed swab is returned to a test tube containing a suitable diluent and stored under refrigeration until plated. Sponge swab procedures are useful for sampling large areas of food processing equipment and environmental surfaces.

The RODAC procedure should be used only on flat, firm and nonporous surfaces that are relatively easy to clean and disinfect. The RODAC method employs special Petri plates which are poured with an appropriate plating medium resulting in a raised agar surface. When the plate is inverted, the solidified agar makes direct contact with the surface. Plates are incubated under the appropriate time and temperature regime for the microorganism in question and enumerated. Samples taken from heavily contaminated areas will result in overgrowth of the plates.

IV. METHODS FOR IDENTIFICATION AND CHARACTERISATION OF FOOD BORNE MICROORGANISMS

A. Identification Based on Morphological, Physiological and Biochemical Characteristics

Isolates obtained from a food product can be broadly categorized to a group depending on characteristics such as Gram stain, colony characteristics (pigmentation, mucoid colonies, swarming or pin-point), cell morphology (shape, size, motility and flagellar patterns, endospore formation, inclusion bodies), relation to oxygen (aerobic, facultatively anaerobic, anaerobic, microaerophilic), catalase activity, oxidase activity, ability to dissimilate glucose and the type of metabolism (oxidative or fermentative). Most often further identification is based on a number of diagnostic characteristics (18):

- Physiological features such as growth at different temperatures, pH values, salt concentrations, growth in the presence of various substances such as antimicrobial agents
- Biochemical features such as activity of various enzymes, metabolism of carbohydrates

It is essential to start from a pure culture for identification of an organism. Determination of phenotypic characteristics is prone to experimental error. Rigorous standardization of phenotypic methods is of the outmost importance to obtain reproducible results. The results of physiological and biochemical tests may vary depending upon the size of the inoculum, the incubation temperature, length of the incubation period, composition of the medium and the criteria used to define a "positive" or "negative" reaction. Therefore it is advisable to include reference strains, available from culture collections for comparison with, when using a phenotypic identification scheme in order to check the performance of the test under the conditions employed in one's own laboratory.

Several commercial identification kits have been developed to simplify and automate the identification of individual microorganisms, e.g., API galeries (bioMérieux, Marcy-l'Etoile, France), Biolog microplates (Biolog Inc., Hayward, CA, U.S.A.), the BBL Crystal ID system (Becton Dickinson, Meylan Cedex, France). In microbial analysis of foods often a complete identification is not required but only a confirmation of the suspected colony based on a restricted number of typical characteristics which are easily determinable and enable good differentiation between competitive closely related strains. However, miniaturized identification systems are increasingly used because the saving in time (e.g., media preparation, ease of reading) is significant and results are comparable to conventional tests.

B. Identification Based on Chemotaxonomic and Genetic Methods

Phenotypic testing is often insufficient for correct identification of species especially if the taxonomy of the organism is complex, e.g., *Bacillus* spp., lactic acid bacteria. Also the paucity of phenotypic characteristics, in particular bacterial groups, can cause problems for identification, e.g., thermophilic campylobacters.

Chemotaxonomy is the application of chemical and physical techniques to elucidate the chemical composition of whole bacterial cells or parts of cells (19). Amongst the techniques, which can be applied for identification of these difficult taxonomic groups, are comparisons of the fatty acid methyl ester content (FAME) or comparison of the whole-cell protein patterns obtained by highly standardized sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). These techniques are based on the comparison of patterns/fingerprints with a database. FAME makes use of the variability in chain length, double-bond position and substituent groups of the cellular lipids and lipopolysaccharides and has proven to be very useful for the characterization of bacteria, e.g., *Aeromonas* species (20) and *Bacillus* species (21). The comparison of SDS-PAGE profiles has proven to be extremely reliable for comparing and grouping large numbers of closely related strains, e.g., lactic acid bacteria and campylobacters (22, 23) SDS-PAGE yields discriminative information at or below the species level.

The accumulation of bacterial DNA sequences and the introduction of user-friendly PCR protocols provide

the tools for the use of molecular techniques in bacterial diagnostics. For a well-studied genus or species, genus or species-specific primers from the 16 S rDNA sequence may be selected to construct a PCR for identification purposes with the advantage that it is feasible to examine many strains and acquire a rapid reliable identification. PCR assays are useful for correct identification of *Listeria monocytogenes* (24) and thermotolerant *Campylobacter* species (25). Ultimately, identification of strains may be performed by partial or complete sequencing of the 16 S rRNA gene using comparative sequence software and a database. Strains sharing more than 97% of 16S rRNA sequence homology belong to the same species.

For more information on identification of bacteria based on phenotypic and genetic characteristics the reader is referred to Uyttendaele et al. (26).

C. Typing of Food-Borne Microorganisms

Discrimination of strains below the species level is performed in academic and governmental research institutes to assemble epidemiological data on the transmission routes and distribution of food-borne pathogens (27). Moreover, typing of food-borne pathogens is essential to establish the role of a contaminated food as the responsible agent for a food-borne outbreak (28). Typing in microorganisms may also assist in the identification of critical control points of a food manufacturing process by tracking down the sources of product contamination during processing of the food in the manufacturing plant (29).

The relatedness of bacterial isolates may be determined by testing for one or several phenotypic markers using methods such as serotyping, phage typing, biotyping, antibiotic susceptibility testing, bacteriocin typing. These are still the conventional methods for typing of the major food-borne pathogens such as *Salmonella*, *Escherichia coli* 0157, *Listeria monocytogenes* and thermotolerant campylobacters. However, the analysis of DNA by molecular typing methods offers advantages over traditional techniques. DNA can always be extracted from bacteria, thus all bacteria should be typeable. In addition, the discriminatory power of DNA-based methods is greater than that of phenotypic methods. These methods do not require specialized reagents and can be readily performed in molecular biology laboratories (30). Several types of molecular typing techniques can be distinguished: typing techniques based on chromosomal restriction fragment length polymorphism such as PFGE (pulsed field gel electrophoresis) and ribotyping, PCR mediated typing techniques such as RAPD (random amplified polymorphic DNA) and typing techniques combining restriction digestion with selective amplification such as AFLP (amplified fragment length polymorphism). The various molecular typing techniques differ in their simplicity of performance, their reproducibility and their resolution; the latter are sometimes pathogen-dependent. The techniques, which are characterized by their high reproducibility and resolution, are largely also the most complex and laborious ones (PFGE, AFLP). They also tend to be more expensive (cost of reagents and specialized equipment). On the other hand, RAPD is a rapid and simple technique with a good discriminatory power (if a suitable RAPD primer is found) but it often lacks reproducibility (inter- and intra-laboratory). Ribotyping is open to automation and reproducible patterns with a reasonable number of fragments are obtained but it is not as discriminatory as some of the newer molecular methods.

For more information of molecular typing of food-borne pathogens the reader is referred to Heyndrickx et al. (31).

D. Toxin Detection

Apart from establishing the presence of pathogens in foods, it is of concern to determine whether bacterial toxins are produced in the food or if the pathogen is a potential toxin-producing strain. The major food intoxications are caused by *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Clostridium botulinum*. The characteristics and detection of the major bacterial toxins are reviewed in Table 54.3. The immunological methods are still the method of preference for detection of the enterotoxins of *S. aureus*, *Cl. perfringens* and *B. cereus* in foods (Table 54.4) (32). The ELISA (enzyme-linked immuno-sorbent assay) methods use a solid support to which the antibodies are attached. The samples (which may contain the toxin serving as an antigen) are put into contact with the solid support and allowed to react with the antibodies. After the reaction the samples are removed and the plate or strip is washed. The enzyme-antibody conjugate is added and allowed to react with the enterotoxin-antibody complex. The plate or strip is again washed before the substrate is added and subsequently the colorimetric or chemiluminescent reaction is measured. RPLA (Reversed Passive Latex Agglutination) tests are also available. The RPLA technique involves the use of sensitized (antiserum to enterotoxin-treated) latex beads that are exposed to serial dilutions of the extracted enterotoxin. The agglutination titer is determined after overnight incubation. Enterotoxin controls and blanks are included in the test kits. Some kits allow the identification of the type of enterotoxin present, others are limited to demonstration of the presence of enterotoxin (without further indicating the type). For the emetic toxin (cereulide) of *Bacillus cereus* no immunological method is available (no antigenic property assigned to the small cereulide molecule). Cell cultures (33) or a boar spermatozoa test (cytotoxicity) are used to assess the presence of the enterotoxin (34). As an alternative, animal assays may be applied. The mouse lethality assay is still employed as the reference method for botulinal toxins in foods. Immunological

TABLE 54.3
Characteristics and Detection of Toxins Produced by *S. aureus, B. cereus, Cl. perfringens* and *Cl. botulinum*

Staphylococcus	*Staphylococcus aureus* Emetic Toxin	*Bacillus cereus* Diarrheal Toxin	*Bacillus cereus* Diarrheal Toxin	*Clostridium perfringens* Neurotoxin	*Clostridium botulinum*
Toxin type	Enterotoxin (single chain polypeptide, with cystine-lus) MW 26–34 kDa	Enterotoxin Cereulide (cyclic dodecapsipeptide)	Enterotoxin a) Haemolysin BL: tripartite protein complex B, L_1, L_2 b) Non-haemolytic tripartite protein complex	Enterotoxin Single chain protein with MW 36 kDa	Neurotoxin Protein with MW 150–170 kDa complex with non-toxic proteins
Formation of toxin	In the food, during vegetative growth	In the food during the late exponential or stationary phase	During vegetative growth in the food or in the intestines after ingestion of high numbers of cells	In the intestines after ingestion of high number of cells; toxin formed during sporulation	In the food during vegetative growth
Effect of proteolysis	Resistant	Resistant	Activity loss	Increased activity after treatment with trypsin	Activation
Heat stability	Resistant to 100–120°C	Resistant to 90 min at 121°C	Inactivation by 5 min at 56°C	Inactivation by 15 min at 60°C	Inactivation by a few minutes at 75–80°C
Symptoms	Nausea, vomiting (sometimes diarrhea)	Nausea, vomiting	Cramps, diarrhea	Cramps, diarrhea	Gastro-enteritis, trouble vision and finally paralysis
Incubation period	1–5 h (recovery after 24–48 h)	1–5 h (recovery after 6–24 h)	8–16 h (recovery after 12–24 h)	8–24 h (recovery after 24–48 h)	12–36 h (deceased after 3–6 days)
Toxicity (LD_{50})	LD_{50} (monkeys): 25 µg/kg dose causing symptoms in humans: 100–200 ng	Unknown	100 × more toxic than *Cl. perfr.*	1.5 µg for a 20 g mouse	25 pg for a 20 g mouse
Detection methods	Immunological detection (Oxoïd BCET RPLA, Vidas (Biomérieux), Tecria immunoassay)	No immunological detection method Cytotoxicity Animal feed trials	Oxoïd BCET RPLA (L_2 component Haemolysin BL) Tecra immunoassay (Non-haemolytic protein complex) Cytotoxicity	RPLA en ELISA	Mouse bio-assay ELISA

TABLE 54.4
Present Status of ISO Standards for Indicator Organisms in Food and Animal Feeding Stuffs and Water

ISO Standard	Product Group	Title
DIS 21528-1:2003	Horizontal	Detection and enumeration of Enterobacteriaceae – Part 1: most probable number technique with pre-enrichment
DIS 21528-2:2003	Horizontal	Detection and enumeration of Enterobacteriaceae – Part 2: colony-count method
5552:1998	Meat (products)	Detection and enumeration of Enterobacteriaceae without resuscitation – most probable number technique and colony-count technique
7402:1993	Horizontal	Enumeration of Enterobacteriaceae without resuscitation – most probable number technique and colony-count technique
8523:1991	Horizontal	Detection of Enterobacteriaceae with pre-enrichment
4831:1991	Horizontal	Enumeration of coliforms – most probable number technique
4832:1991	Horizontal	Enumeration of coliforms – colony count technique
5541-1:1986	Milk (products)	Enumeration of coliforms – colony count technique at 30°C
5541-2:1986	Milk (products)	Enumeration of coliforms – most probable number technique at 30°C
9308-1:2000	Water	Detection and enumeration of *Escherichia coli* and bacteria of the coli- group – Part 1: membrane filtration
16649-1:2001	Horizontal	Enumeration of β-D glucuronidase-positive *Escherichia coli* – Part 1: colony count at 44°C by membrane filtration
16649-2:2001	Horizontal	Enumeration of β-D glucuronidase-positive *Escherichia coli* – Part 2: colony count technique at 44°C
CD16649-3:2001	Horizontal	Enumeration of β-D glucuronidase-positive *Escherichia coli* – Part 3: most probable number technique
6391:1997	Meat (products)	Enumeration of *Escherichia coli* – colony count technique at 44°C using membranes
11866-1:1997	Milk (products)	Enumeration of presumptive *Escherichia coli* – Part 1: most probable number technique
11866-2:1997	Milk (products)	Enumeration of presumptive *Escherichia coli* – Part 2: most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG)
11866-3:1997	Milk (products)	Enumeration of presumptive *Escherichia coli* – Part 3: colony-count technique at 44°C using membranes
DIS 7251:2002	Horizontal	Enumeration of presumptive *Escherichia coli* – most probable number technique
7899-2:2000	Water	Detection and enumeration of enterococci – Part 2: membrane filtration

Modified from de Boer (7).

methods have been reported for the botulinal toxin but seem to lack sensitivity in comparison to the mouse bioassay. PCR methods targeting the toxin genes have also been developed.

V. MICROBIAL PARAMETERS IN FOOD ANALYSIS

The bacteriological examination of food products falls into one or more of the following categories: total viable count, indicator organisms, specific spoilage organisms, functional flora and food-borne pathogens.

A. "Total" Bacterial (Colony) Count

The total bacterial count or, better, "colony count," is an attempt to measure the total number of viable microorganisms present in a food sample as an indication of the overall microbial quality of the food product. "Total" is often associated with microscopic counts which also include dead cells, whereas these procedures are actually based on the assumption that each viable microbial cell will form a visible, separate colony when mixed with an agar and permitted to grow.

Since microorganisms in foods represent various populations with many different growth requirements, the optimum conditions for determining the total viable count may vary from one food to another. The conventional aerobic plate count for examining frozen, chilled, precooked or prepared foods uses pour plates of Plate Count Agar (PCA) and incubates plates for 72 h at 30°C (35) or 48 h at 35°C (36). For analysis of milk and milk products PCA can be supplemented with milk powder in order to favor growth of nutritional fastidious organisms such as lactic acid bacteria. Generally, the lactic acid bacteria show delayed growth and smaller colony size (pin point colonies) in normal PCA. On the other hand, when high numbers of a functional flora are expected, as is the case in fermented foods, a nutritional poor agar medium such as peptone agar may be used to suppress the growth of this competing functional flora.

For the determination of the colony count of thermophilic and mesophilic bacteria in, for example, canned food, Dextrose Tryptone agar is recommended incubated respectively, for 48 h at 55°C and 72 h at 30°C. Flat sour bacteria (e.g., *Bacillus stearothermophilus*) which are important spoilers in these types of product are on this agar medium typically surrounded by a yellow zone

because of acid production in contrast to the purple medium (pH indicator is bromocresol purple) (7).

For enumeration of psychrotrophs, the predominant spoilage flora of refrigerated minimal processed foods, incubation for 10 days at 7°C for pour plates has been described. Incubation conditions using shorter times at higher temperatures have included 25 h at 21°C for raw and pasteurized milk using Plate Count Milk agar. Under the assumption that most psychrotrophs are Gram-negative bacteria, a selective medium was recommended that contains crystal violet and triphenyl tetrazolium chloride and is incubated for 5 days at 22°C (7).

Aerobic plate counts of water uses pour plates with Yeast Extract agar incubated either for 48 h at 37°C or for 72 h at 22°C (37).

Anaerobic plate count methods use a medium to favor growth of anaerobic organisms. Although thioglycollate is widely used in anaerobic media to lower the redox potential, it has been reported to be inhibitory to some anaerobes. Shaedler anaerobe agar contains cysteine hydrochloride and glucose as reducing substances and has been shown to successfully recover obligate anaerobes under anaerobic incubation (38).

The mesophilic bacterial spore count is determined by previously heating the primary suspension for 10 minutes at 80°C in order to eliminate vegetative cells and subsequently plating on, respectively, PCA (39) and Reinforced Clostridial Agar with incubation under, respectively, aerobic and anaerobic conditions for 48 h at 37°C for the aerobic and anaerobic spore count. A heat treatment for 30 minutes at 100°C is applied before plating and incubation at 55°C for 48 h to determine the thermophilic bacterial spore count (7).

There is no single set of conditions that will allow growth of the whole spectrum of microorganisms present in a natural sample. Nevertheless, in all of the above-mentioned methods, the selectivity is unintentional and merely a consequence of the choice of time, temperature, atmosphere or nutritional composition of the medium. They all aim to maximize the number of bacteria that may be found in a food product. However, once a procedure for a given food is determined, it should be respected and can be very useful for routine microbial analysis of the food.

B. Indicator Organism Count

Specific organisms are sought indicating the standard of hygiene used in the manufacture, storage and handling of the food products. The presence of indicator organisms in given numbers points to failure to comply with appropriate GMP.

The use of Enterobacteriaceae as indicator organisms has gained acceptance now in several countries. In the monitoring of foods processed for safety, e.g., by heat or by a chemical process like chlorination, Enterobacteriaceae are the indicators of choice since all member of the Enterobacteriaceae are known to be eliminated by these treatments. In the case of milk and dairy products, because of the selection of lactose-positive types by the high levels of lactose in the environment, the coli-aerogenes group or coliform bacteria have been used successfully for safeguarding pasteurized products. However, taxonomically, the coliform bacteria are a rather ill-defined group. In general, all Gram-negative bacteria capable of growing on bile salts-containing media, and which produce acid from lactose, are included in the coliform count. In addition, coliforms are not a good indicator in foods where lactose-negative bacteria dominate the microbial ecology of a food.

The entire group of Enterobacteriaceae is of limited use in the examination of foods that have been possibly involved in outbreaks of disease: tests for properly selected pathogens are required here. There is also very limited scope for using the complete taxon of Enterobacteriaceae as an indicator of faecal contamination as not all genera of the Enterobacteriaceae are of faecal origin. The use of an Enterobacteriaceae test on a large number of samples offers considerable advantages for trend analysis in assessment of the overall hygiene during food processing, however, only on the day of production. When raw meats and poultry or vegetables are examined for Enterobacteriaceae in the frame of hygiene monitoring at sampling times during the shelf life, the psychrotrophic types (which grow during refrigerated storage of these products) should not be included in these counts. An alternative approach is to rely on the thermotrophic population fraction of the Enterobacteriaceae also referred to as the 'faecal coliform' group. However, the 'faecal coliform' group is ill-defined as the coliform bacteria having the ability to grow at 44°C and include coliform bacteria which are not of faecal origin and therefore this is also not an absolute marker of faecal contamination. To demonstrate that faecal pollution in a food may have occurred, *Escherichia coli* should be used as the marker organism. It is a well-defined taxonomic unit and ecological investigations have substantiated that *E. coli* originates from the intestinal tract of man and warm-blooded animals.

The Enterococcus group (formerly Lancefield-Group D Streptococci) can be used as an enteric marker organism in specific situations. All streptococci of this group occur in faecal niches. Nevertheless, enterococci possess a high extra-enteric resistance whereas *E. coli* declines quite rapidly in some non-enteric environments, particularly under conditions of reduced a_w. Streptococci are useful marker organisms in certain foods (dry foods, cooked foods) to demonstrate the elimination of the more resistant pathogens (e.g., *Listeria monocytogenes*), an assurance which cannot be provided by the absence of the more fragile Enterobacteriaceae.

Test methods for indicator organisms are also of the utmost importance in water quality assessment. Under European legislation, treated waters should contain (per

100 ml) no coliforms, no faecal coliforms/ *E. coli* and no enterococci. For more information on the use of indicator organisms the reader is referred to Mossel et al. (40).

Violet red bile glucose agar for Enterobacteriaceae and violet red bile lactose agar for coliforms have been widely used in the last 20–30 years for counting and isolating Enterobacteriaceae and coliforms. Enterobacteriaceae and coliforms are determined by incubation at 37°C, faecal coliforms by incubation at 44°C. In fact no new developments in solid media for these bacteria have appeared recently (Table 54.4). Standardized methods for detection and enumeration of *E. coli* comprise a MPN technique, a membrane filtration method and a direct plate count (Table 54.4). The MPN technique comprises, using lauryl sulphate tryptose broth incubated at 37°C and isolation on a chromogenic medium, namely tryptone bile agar with X-glucuronide agar (TBX-agar), and a substrate for β-D-glucuronidase (GUD) and incubated at 44°C. The membrane filtration procedure uses cellulose membranes on minerals modified glutamate agar incubated for 4h at 37°C for resuscitation and subsequent transfer to TBX-agar and incubation at 44°C for enumeration. If high numbers are present, direct enumeration on TBX agar incubated at 44°C can be performed. The introduction of chromogenic media has enabled detection of β-D glucuronidase-positive *E. coli* without the necessity of colony confirmation steps. Over 95% of *E. coli* strains are positive for GUD. The older methodology for detection of *E. coli* relied on used lauryl sulphate trypose broth and subsequent selective enrichment in EC broth (MPN technique) or isolation on tryptone bile agar (membrane filtration method) or violet red bile lactose agar (enumeration) incubated at 44°C and subsequent confirmation of *E. coli* as the only true faecal coliform by indole production from tryptophan at 44°C (41).

Enterococci are enumerated on Slanetz and Bartley agar medium incubated at 37°C for 24–48 h. Kanamycin aesculin azide medium may be used as an alternative (42).

C. Detection of Specific Spoilage Organisms/Functional Flora

Spoilage organisms are usually associated with taints and off-flavours in stored products. They are the major factor in determining the shelf-life of microbiological unstable food products and are considered to be of more relevance than total viable counts to indicate the microbial quality of the product. For prediction of the shelf-life it is of interest to focus microbial analysis on a selected fraction of the microbial community that contributes to the spoilage of the food defined as the specific spoilage group or organism. This microflora is a function of the raw material flora, the processing, preservation and storage conditions. For more information of the spoilage flora associated with different food products is referred to Chapter? The following microbial parameters are often involved in microbial analyses of foods in order to determine the shelf-life of a particular food product: lactic acid bacteria, yeast and moulds, osmophilic yeasts, lipolytic microorganisms, proteolytic microorganisms, sulfide producing spoilers, thermoduric flat sour bacteria, *Pseudomonas* species, *Clostridium tyrobutyricum*, etc. However, this is not a restrictive list.

In contrast to provoking deterioration of the food product, microorganisms (mainly lactic acid bacteria), are sometimes added as deliberate starters or take part in the fermentation processes as a result of their being natural contaminants of the starting substrates. In addition, lactic acid bacteria, predominantly *Bifidobacterium* spp. and *Lactobacillus acidophilus*, are increasingly used in dairy products as probiotic organisms. Microbial analyses may be performed to determine whether the claimed starter of probiotic cultures is, indeed, present in the quantities claimed (43, 44).

Intentionally selective methods have been elaborated to quantify this defined sub-set of the microbial population. Media formulation for the important group of the lactic acid bacteria and the yeasts and moulds is provided beneath. For description of the culture media and incubation conditions of other spoilers used as an indication of the microbial quality of foods the reader is referred to specialized literature.

1. Lactic Acid Bacteria

The MRS formulation was developed by de Man, Rogosa and Sharpe to encourage the growth of lactic acid bacteria which includes species of the following genera: *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc*. The MRS medium is a complex medium with an acid pH (6.2) and includes high concentrations of glucose and the essential growth factors magnesium and manganese. MRS supports rapid growth of the fastidious lactic acid bacteria resulting in a rapid decrease in pH to 5.0 or less because of the lactic acid production thus further enhancing the selectivity of the medium. Growth of lactic acid bacteria is considerably enhanced by micro-aerophilic conditions. MRS is commonly incubated with an agar overlay for 72 h at 30°C. Selection for lactobacilli can be made by pH adjustment. Acidified MRS to pH 5.4 with acetic acid is, for example, used for enumeration of *Lactobacillus bulgaricus* in yoghurt. Vancomycin and thallium acetate can be added to enhance the selectivity towards *Leuconostoc*. Addition of sorbic acid in combination with low pH inhibits growth of competitive yeasts and moulds. However, upon addition a compromise between the combinations and concentrations of substances used has to be reached between selectivity and productivity of the organism sought (38).

2. Yeasts and Moulds

For determination of yeast and moulds several media are available. In general, an antibacterial compound is added

to the culture medium (oxytetracycline, chloramphenicol) or pH of the medium is lowered (pH 5.7) although the latter approach also allows growth of acidophilic bacteria. Dichloran or rose bengal may be added to inhibit spreading and restrict the colony size (45).

VI. DETECTION OF FOOD-BORNE PATHOGENS

Food infection is caused by consumption of food contaminated with the etiological agent, in other words, the pathogenic microorganism. The pathogen develops in the gastro-intestinal system, accompanied or not by toxin production, causing food infection. Food infection can be caused by low numbers of the pathogen. Low numbers can be expected in foods that are correctly processed for safety and not recontaminated or recolonised subsequently. Often detection of single cell in 25 g is required. Virtually all pathogens in foods are sub-lethally stressed. Therefore the detection method of infectious agents encompasses four subsequent steps (46):

- A *resuscitation* procedure or *pre-enrichment* lasting between a few hours to overnight incubation in a non- or half-selective medium to enable recovery and limited outgrowth (10^2–10^4 cfu/ml) of the stressed target organism. Stressed cells are often particularly susceptible to reactive oxygen metabolites. Thus, anaerobic incubation or the addition of free radical quenching agents may be highly effective in resuscitation. Often incubation for a few hours at a more optimum reduced temperature is applied before exposure to the higher selective temperature of incubation (47).
- A period of *enrichment* in a selective medium to suppress the competitive flora and enabling multiplication of the target organism to attain detectable levels of the order 10^5–10^7/ml (48).
- *Isolation* of the pathogen on a selective differential agar medium. Selective agars are streaked with a loopful of enrichment broth and incubated under appropriate conditions. It must be stressed that the ability to grow on specific selective media does not mean that the organism growing is that for which the medium was developed. Differentiation of typical colonies must be regarded as presumptive and confirmatory tests are required.
- Purification of typical colonies and confirmation using a number of morphological, biochemical, and physiological tests.

A few remarks should be made to this general procedure:

- Enrichment is applied where pathogenic microorganisms are present only in small numbers, particularly if large numbers of competing spoilage microorganisms are present. It is not normally applied where the organism is a common contaminant of foods in low (and harmless) numbers, such as the bacterial food intoxicants *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens*. These food borne pathogens are tolerated in the order of 100 cfu/ml or g and only pose a health hazard in numbers above 10^5 cfu/ml or g. In the latter case selective media are used for direct plating without prior enrichment and enumerations may be made.
- Although in many cases enrichment media are used in conjunction with selective plating media, they may be used to enumerate specific microorganisms or groups of microorganisms using the MPN technique. In such circumstances, it is necessary to incorporate a diagnostic (differential) reaction to distinguish the organism being enumerated from other microorganisms, which are able to grow under the imposed conditions. Examples are lactose fermentation in media for 'coliforms' and the 'sulphide' reaction in media for clostridia. Further confirmatory tests are required where reactions are positive.
- The use of culture media with selective agents and the incorporation of an elective system to differentiate the target organism has been discussed above. Numerous selective media are available for the isolation of specific food borne pathogens, and development of media and modification of existing media is a continuous process in microbial food analysis. The choice of media should take into consideration factors such as selectivity and sensitivity of the medium as well as the ease of use. However, another important factor is the type of food for analysis. Food borne pathogens from specific foods may be unusually sensitive to selective agents or competing microorganisms from specific foods may be unusually resistant to selective agents. For example *Listeria monocytogenes* may be sub-lethally stressed and selective agents in enrichment media may interfere with the process of repair in such cells. Consequently, the isolation of stressed *Listeria monocytogenes* from particular types of cheese benefit from a partially non-selective pre-enrichment compared to the completely selective pre-enrichment and enrichment procedure conventionally used (49).

An overview of the recommended procedure according to ISO for detection of the most important pathogenic bacteria implied in food analysis namely *Salmonella* spp.,

Listeria monocytogenes, *Campylobacter* spp. and *Escherichia coli* O157 is shown in Figure 54.2 (a–d). A description of the selective media and recommended confirmation tests for detection of the food-borne intoxicating *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens* is given in Table 54.5.

For more information on approved protocols for the detection of food-borne pathogens the reader is referred to the different national and international standard organizations.

VII. STANDARD METHODS, VALIDATION OF METHODS AND QUALITY ASSURANCE IN MICROBIOLOGY

Microbiological tests are important in governmental food inspection to enforce legal regulations, in international trade to determine compliance with a microbiological standard, in commercial relationships between trade partners to control on agreed microbiological specifications, in the food industry to maintain quality control and process requirements, in

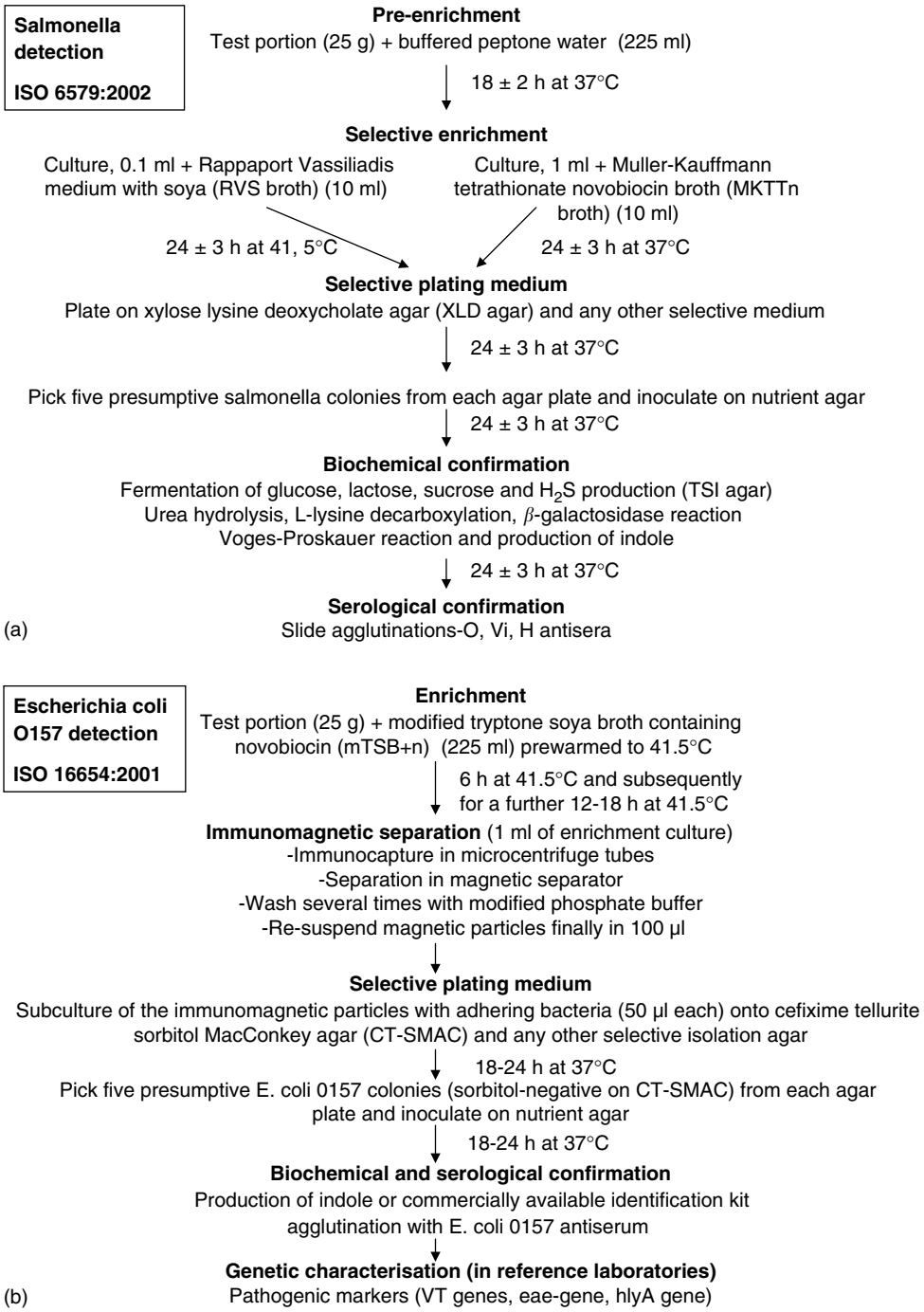

FIGURE 54.2 Schematic lay-out of ISO methods for detection of foodborne pathogens in foods.

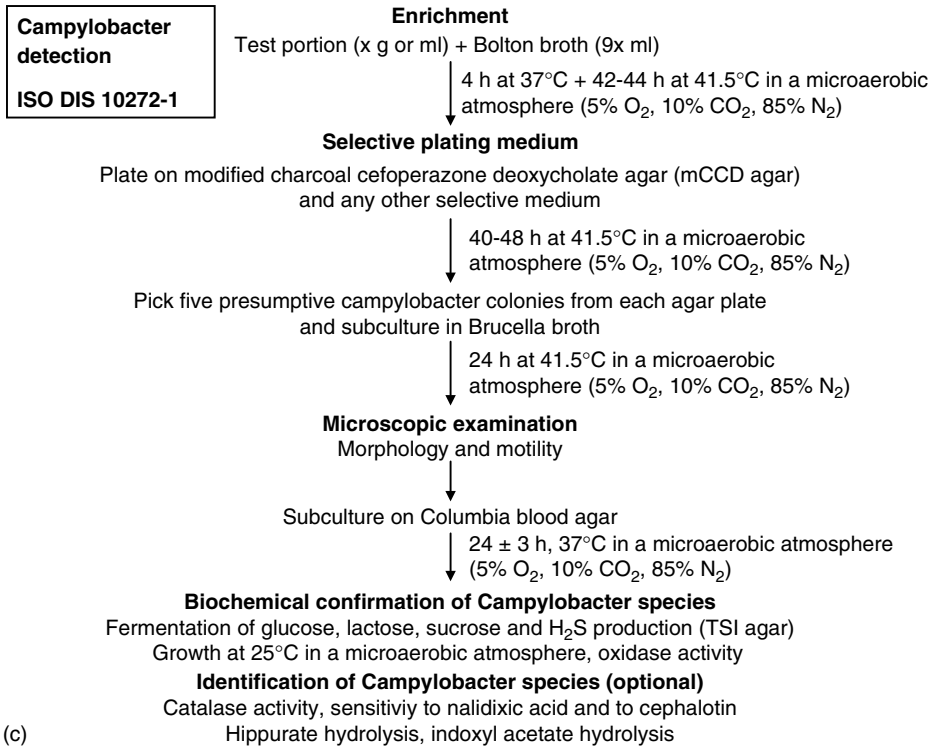

FIGURE 54.2 Continued.

academic laboratories for conducting research, and in reference laboratories to confirm the analyses of another laboratory and to provide surveillance data. The results of these tests should be reliable. It is required that performance characteristics of the method are determined. Apart from these technical characteristics, it is important that all parties involved agree with and accept the methods employed. Mutual recognition of test methods in international trade relations facilitates commerce and exchange of results.

A. Standard Methods

Standard methods have been elaborated by international, national or trade organizations such as ISO (International

TABLE 54.5
Overview of the Standard Media Used for Enumeration of *Staphylococcus aureus, Clostridium perfringens* and *Bacillus cereus* in Foods

	Coagulase-Positive Staphylococci (*Staphylococcus aureus* and Other Species)	*Clostridium perfringens*	Presumptive *Bacillus cereus*
Selective media	Baird-Parker agar medium with egg yolk tellurite emulsion (spread plate)	Egg-yolk-free tryptose-sulfite-cycloserine agar (poured plate + overlay)	Mannitol Egg Yolk Polymyxin agar
Incubation conditions	Aerobic incubation for 48 h at 37°C	Anaerobic incubation for 20 h at 37°C	24–48 h 30°C
Growth promoting compounds	Sodium pyruvate		
Selective compounds	Glycine, tellurite, lithium	D-cycloserine anaerobic incubation	Polymyxin B
Diagnostic compounds	Reduction of tellurite results in gray-black shiny colonies		
	Egg yolk emulsion renders medium yellow and opaque; clear zones around colonies produced by proteolytic action; most strains also form opaque haloes around colonies because of lipase activity	Sodium metabisulphite and ammonium ferric citrate indicate sulphite reduction and produce black colonies	Most strains produce phospholipase C and hydrolyze lecithine in egg yolk resulting in a white precipitate surrounding the colony Failure of *B. cereus* to use mannitol induces alkaline color of the pH indicator phenol red rendering violet/pink background to the rough/dry colonies
Confirmation tests	Coagulase test	Lactose fermentation and gelatine hydrolysis, motility and nitrate reduction	Haemolysis on sheep blood agar and motility
Remarks	Rabbit Plasma Fibrinogen agar (incorporation of rabbit plasma in the basal medium enables direct detection of coagulase-positive staphylococci)	Confirmation in lactose sulfite medium when incubated at 46°C is very specific	
Reference	ISO 6888-1:1999 and ISO 6888-2:1999	ISO/DIS 7937:2002	ISO 7932:1993 and ISO 7392/A1:2002

Standards Organization), AOAC International (formerly Association of Official Analytical Chemists), CEN (Comité Européen de Normalisation), NMKL (Nordisk Metodik-komitté för Livsmeddel), AFNOR (Association Fran-çaise de Normalisation), NNI (Nederlands Normalisatie Instituut), DIN (Deutsches Institut für Normung), IDF (International Dairy Federation), etc. (50).

These standard methods for detection of microbiological contaminants in foods are usually conventional cultural methods. Primarily because the intention is to provide the users of these methods with a reliable and internationally accepted method which enables them to obtain equivalent results in different laboratory settings without having exclusivity of materials related to one manufacturer. Although in essence these standard methods serve only as guidelines for reliable microbial analysis of foods, historically in many countries governmental agencies and trading agencies recommend or accept them as official methods for detection or enumeration of microorganisms in foods (51, 52) they may also recommend their own established methods, e.g., the U.S. FDA compiles its recommended methods in a Bacteri-ological Analytical Manual (US FDA-BAM) (36).

B. Validation of Methods

The confidence in the results produced by a microbiological analytical procedure depends on the reliability of the analytical method. Determination of the performance characteristics of a method by validation will facilitate it to gain acceptance of the test results by international, national or regional regulators and trade partners. Standard methods, which are published by international, national or regional standardization organizations or trade organizations, are considered as validated. In this case the laboratory implementing a new standard method has to demonstrate that criteria for validation indicated in the standard can be achieved. This is done by a systematic research on specific analytical parameters. However, only recently the performance characteristics of six ISO methods, namely *Bacillus cereus* (enumeration), *Listeria monocytogenes* (detection and enumeration), *Staphylococcus aureus* (enumeration), *Clostridium*

perfringens (enumeration) and *Salmonella* (detection), were determined and published in the corresponding ISO methods (53–55). In the present situation, laboratories will have to show that they are capable of performing the standard method of choice correctly on experiments with their own range of samples. This is preferably referred to as a method verification study because it is not a validation study *sensu strictu*. Validation of a method is a formidable collaborative research task that so-called rapid, modern methods are subjected to before they are accepted and will be discussed in Chapter 55. Methods are most effectively assessed with specially designed experiments. Routine analyses usually lack a plan or replications that is are prerequisites for method verification.

For quantitative methods (enumeration) this method verification study should focus on establishment of the trueness (bias) and the precision of the method.

Trueness (*bias*) is defined as the closeness of agreement between the true value or, if not known, the accepted reference value and the mean result which is obtained when the experimental procedure is applied a large number of times (= systematic error) (56, 57). The trueness can be obtained by the analysis of certified reference materials (CRMs), derived from a certifying body (e.g., the Community Bureau of Reference (BCR) of the European Commission (EC)). However, only a limited number of CRMs or stable reference materials from other sources are available for microbiological purposes. The analysis of spiked materials (recovery studies) can be performed as an alternative. Participation in proficiency testing schemes can also help in assessing the laboratory performance for the parameter of trueness.

Precision is defined as the closeness of agreement between independent test results obtained by applying the experimental procedure several times on the same sample under stipulated conditions (= random error) (57, 58). The measure of precision is usually expressed in terms of imprecision and calculated as a standard deviation of the test results. Less precision is reflected by a larger standard deviation. Checks of precision in routinely used methods should be made to ensure that the result does not change with time as a result of changes in reagents, equipment, staff, etc. Distinction is made between:

- Repeatability: indicates the variability observed within a laboratory, over a short time, using a single operator, the same apparatus on identical test material.
- Reproducibility: inter-laboratory reproducibility indicates the variability observed when different laboratories analyse the same sample by use of the same method and may be estimated directly by inter-laboratory study. Intra-laboratory reproducibility relates to the variation in results observed when one or more factors, such as time, equipment and operator, are varied within a laboratory.

For qualitative methods (presence/absence testing) the method verification study should focus on establishment of the detection limit and the specificity and sensitivity of the method.

The *detection limit* is defined as the smallest number of culturable microorganisms that can be reliably detected in the sample. For qualitative methods it can be defined as the smallest number of culturable microorganisms that can be detected on 50% of occasions by the reference method (56).

Both *sensitivity and specificity* relate to the degree to which a method responds uniquely to the specified target organism or group of organisms and relate to the number of false positives and false negatives results that are found with the validated method. Various definitions for sensitivity and specificity are proposed, includig the following (58).

The sensitivity of a method is the proportion of target organisms that can be detected; it can be calculated with the following equation:

$$\text{Sensitivity (\%)} = \frac{\text{number of true positives (P)}}{\text{P + number of false negatives}} \times 100$$

A failure to detect the target when present is a false negative result and will lower the sensitivity of a test. In food microbiology only a very low frequency of false-negative results can be tolerated for safety reasons.

The specificity of a method is the ability to discriminate between the target organism and other organisms; it can be calculated with the formula:

$$\text{Sensitivity (\%)} = \frac{\text{number of true negatives (N)}}{\text{N + number of false positivies}} \times 100$$

A positive result in the absence of the target is a false-positive result and will lower the specificity of a method. For rapid screening methods, a higher false-positive frequency may be acceptable, as positive screening tests are followed by confirmation tests.

In addition to the specificity and sensitivity as described above, the inclusivity and exclusivity of a qualitative method also may be determined, inclusivity being the ability of the validated method to detect a wide range of strains belonging to the target organism, exclusivity being the lack of detection with the validated method of a relevant range of non-target strains (56).

A number of issues need to be addressed in the preparation of a solid method verification scheme. These are listed in Table 54.6.

TABLE 54.6
Considerations in the Preparation of an Experimental Design for Method Verification

Number of food types to be tested	If the method is to be validated for all foods, usually five categories of foods are included
	Food categories are predominantly determined by the origin of the product, e.g., meat products, dairy, fruits and vegetables, etc.
	The modes of processing to increase shelf life may further be used to select food types within a category, e.g., raw, heat-treated, etc.
	The types of food chosen should be relevant to the type of target organism(s) sought and the scope of the laboratory
Number of samples to be analyzed	A sufficient number of samples should be analyzed in order to generate sufficient data to allow the use of appropriate statistics for interpretation
Naturally contaminated food samples versus artificially contaminated food samples	Whenever possible naturally contaminated samples should be used. These represent best real-life encountered samples with the target organism(s) present as a minority (if pathogenic bacteria) in a vast majority of other bacteria and in a non-optimal (stressed) conditions
	When artificially contaminated samples are used, the levels of inoculation and the preparation of the inoculants to achieve this should be similar to those expected to be found in naturally contaminated samples; the background flora also should be representative
The source and number of inoculum strains	Strains that have been isolated from the same type of food product are preferred; if not possible, then inoculants should be fully characterized (reference) cultures
	Cover the recognized range of the target and non-target organism with respect of the geographical distribution, incidence and diversity of the identification characteristics it is biochemical activity, serotype, phage type, etc.

TABLE 54.7
Requirements of the ISO 17025:1999 for the Accreditation of Testing Laboratories

Management requirements	Technical Requirements
Organisation	Personnel
Quality system	Accommodation and environmental conditions
Document control	Test methods and validation, measurement of uncertainty
Review of requests, tenders and contracts	
Subcontracting of tests and calibrations	Equipment
Purchasing services and supplies	Measurement of traceability, control of data
Service to the client, complaints	Sampling
Corrective and preventive actions	Reporting of results
Control of records	
Iternal audits, management review	

By method verification insight is obtained into the possibilities and limitations of the test method under the laboratory's own conditions in the area typically served by the laboratory.

C. Quality Assurance in Microbiology

The performance characteristics of a method are closely related to analyst performance, the equipment and, more generally, the competence of the laboratory to provide accurate, reliable and repeatable test results under controlled conditions. Accreditation of laboratories carrying out microbiological food analysis provides to those relying upon its services the assurance of the reliability of the test results. Accreditation of a laboratory is the formal approval given by a national authorized body (linked by the European co-operation for Accreditation of Laboratories (EAL)) that the laboratory is competent to carry out specific methods of analysis and has a commitment to quality assurance in the lab (59). A detailed account of the requirements that testing labs have to meet is described in ISO 17025:2000 (60) and reviewed in Table 54.7.

It is clear that, to comply with the requirements of the ISO17025:1999, the laboratory should use validated methods which are documented in standard operating procedures and must have a systematic approach of quality control. Of equal importance is the execution of the method by qualified staff with well-maintained and properly functioning equipment and utensils.

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55 Rapid Methods in Food Diagnostics

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CONTENTS

I. INTRODUCTION

The classical procedures for enumeration or detection of microorganisms in foods (Chapter 54) are labor-intensive and time-consuming. Several days are needed to obtain confirmed results. To shorten analysis time, new methods have been developed that can rapidly enumerate or detect low numbers of microorganisms in foods. In this chapter, the term 'rapid' will be used to describe methods which are able to give results within a 24–48 h timeframe, whereas results within 0.5–4 h are considered extremely rapid. In order to allow for immediate corrective action during food manufacturing processes, 'real-time' or 'on-line' detection of microorganisms in foods is sought by manufacturers.

The lack of sensitivity or lack of robustness of most new technologies, however, makes 'real-time' detection in solid foods difficult to obtain. Water and beverages are easier to manipulate and, therefore, more amenable for application of 'on-line' methodology.

A number of instrumental methods have been developed for rapid detection of pathogens using various principles of detection. Direct microscopic methods like DEFT (Direct Epifluorescent Filter Technique) or flow cytometry (FCM) are based on the detection of the actual cells. Indirect methods detect growth and metabolic activity (e.g., conductimetry/impedance) or cellular components (e.g., ATP bioluminescence). Immunological methods have been and still are popular in different formats for rapid detection of pathogens. More recently, nucleic acid-based methods like PCR (Polymerase Chain Reaction) have been advancing rapidly and are promising for rapid screening of foods for presence of pathogens. Finally, the development of biosensors opens new perspectives for 'real-time' or 'on-line' detection of pathogens. Because there are many manufacturers supplying commercially available options for the rapid detection methods discussed and the format in which these techniques are available changes rapidly, no listing of these commercial assays is included. For an up-to-date overview the reader is referred to the internet sites of the manufacturers, or to comprehensive information websites such as http://www.foodhaccp. com or http://www.rapidmicrobiology.com. The present chapter aims to discuss the potential and pitfalls of each of the above-mentioned techniques as a rapid detection method of either spoilage organisms or pathogens in foods. For a discussion of the modified and automated traditional culture-based methods, e.g., spiral plater, chromogenic media, commercial identification kits, etc. the reader is referred to Chapter 54.

II. BENEFITS AND LIMITATIONS OF RAPID METHODS IN FOOD MICROBIOLOGY

Some distinguishing features of both traditional culture-based methods and alternative rapid methods for microbiological analyses of foods are listed in Table 55.1.

Alternative rapid methods are especially cost-effective if large numbers of samples need to be screened for a defined parameter in a short period of time. The reliability of the results is related to the intrinsic performance characteristics of the method and the degree to which operators are trained and familiar with the execution and interpretation of the methods.

Although rapid methods have become increasingly popular in recent years, they also have their limitations:

- Often rapid methods are developed for a specific scope of application (e.g., a defined product group) and linked to strict protocols in order to obtain good performance. They are less flexible than traditional methods which are most of the time horizontal methods that are more amenable to modification depending on the food product to be analyzed.
- Rapid methods may, in many cases, only be able to indicate the presence or absence of target organism(s) but they do not provide an isolated colony, the availability of which may be desirable for epidemiological purposes.
- Novel rapid methods measure parameters (antigen-antibody response, DNA or RNA sequences, metabolic activity) distinct from the classically accepted ones (phenotypic traits). This may complicate the problem of interpretation of the results in comparison studies of the methods.
- Rapid methods that work remarkably well with pure cultures of target organisms fail sometimes when applied to real food specimens which may include inhibitory components. The effect of these may be overcome by previous concentration and purification of target organisms, although at the expense of ease of use and rapid results.
- Often rapid methods still make partial use of the traditional culture-based method. For example, ELISA methods and PCR methods both employ a one- or two-day culture enrichment step to resuscitate sub-lethally damaged cells and enable multiplication of low numbers of pathogens to the intrinsic detection limit of the proper ELISA (10^4–10^6 cfu/ml) or PCR assay (ca 10^2 cfu/ml). As such, these methods do not entirely replace traditional culture-based methods but rather are complementary to the traditional methods in overcoming their lack of speed in reporting results. This also implies that the reliability of the outcome of the ELISA or

TABLE 55.1
Significant Distinguishing Features of Traditional and Rapid Methods

Traditional Culture-Based Methods	Alternative Rapid Methods
Labor-intensive	User-friendly, amenable to automation
Time-consuming	Rapid results
Low investment in apparatus and materials	Often expensive apparatus and consumables needed
Subjective interpretation (linked to personnel observation)	Objective interpretation (print-out)
Moderate reproducible (depends on quality-assurance program in the laboratory)	Better reproducible (quality-controlled reagents, meticulously standardized protocols)

PCR assay not only depends on the assay as such but also on the effectiveness of the enrichment procedure to provide sufficient numbers of the target organism.

III. MICROSCOPIC METHODS

Due to the necessity of incubation to enable multiplication of cells to become visual colonies, the traditional culture-based methods cannot be classified as rapid. Truly rapid methods are based on the detection of target organisms at the single cell level by use of microscopy. In order to visualize cells for microscopical examination, coloring agents are used to provide information on the total level of microorganisms (e.g., acridine orange staining) or specific types of microorganisms (e.g., fluorescent labeled antibodies). In combination with different pre-treatments (e.g., pre-filtration to remove solid particles, enrichment to enhance the number of cells) and detecting principles (epifluorescent microscopy or flow cytometry), microscopic methods have developed into promising rapid methodologies for microbial analysis of particular food categories such as dairy products and beverages.

A. DEFT

DEFT stands for Direct Epifluorescent Filter Technique, a microscopic cell counting method. Originally developed for the determination of total bacterial counts in raw milk, this technique also found application in the fields of meat and fish microbiology. The DEFT procedure consists of four main steps: homogenization and pre-treatment of samples with detergents and proteolytic enzymes, filtration of an aliquot of the sample over a polycarbonate membrane, fluorescent staining of the bacteria retained on the filter and the subsequent microscopic counting of the cells under a fluorescent microscope. This detection can be automated by linking the microscope to an image analyzing system. DEFT differs from conventional light microscopy in that different fluorogenic labels can provide different types of information. Originally, the nucleic acid stain acridine orange was used as the labeling reagent for total bacterial counts. Although it has been said to label viable cells orange and to render dead or impaired cells green, intermediate colors can occur and a clear distinction cannot always be obtained. Alternative fluorescent probes, such as nucleic acid stains like DAPI (4′,6-diamidino-2-phenylindol), indicate total number of bacteria present irrespective of metabolic activity. Viability labels have also been developed, including fluorescein derivatives that are cleaved into intensely green products by viable (metabolically active) cells. Although the actual staining and counting takes less than 0.5–1 h, the total detection time is seriously lengthened by sample pre-treatment steps. The intrinsic detection limit of DEFT is around 10^4–10^5 cells per ml of original sample, and so concentration techniques and enrichment steps usually have to be included in the protocol (1). The inherent lack of specificity in conventional DEFT methodology is a serious drawback in terms of specific pathogen detection. All the above-mentioned fluorescent stains are "universal" ones, incapable of demonstrating specific pathogens. Consequently, fluorescent labeled antibodies are used for specific pathogens in the so-called antibody-DEFT (Ab-DEFT) approach. A protocol using Ab-DEFT has been described for detection of *E. coli* O157:H7 in beef (2).

A novel way of cellular staining in combination with conventional DEFT methodology has been described recently and is termed "oligo-DEFT." It uses the combination of FISH (Fluorescent *In Situ* Hybridisation) with a highly specific oligonucleotide probe for the 16S rRNA, and DEFT. It has been used for the detection of *E. coli* in water, beverages and sprouts (detection limit: 1 CFU per ml) (3).

To conclude, DEFT *in se* allows a near on-line detection of microorganisms, but its practical applicability in food analysis is seriously hampered by the need for sample pre-treatment and concentration/isolation steps. As a result, the 30-min assay time in model systems takes more than 20 h when specific pathogens have to be detected in real food samples.

B. Flow Cytometry

Flow cytometry (FCM) is an optically based method for analyzing individual cells. It comprises the suspension of target cells in a fluid, injection of an aliquot in the detection system (flow cytometer) and the automated real-time analysis by the instrument. Basically, the cells, which can be labeled with a fluorogenic compound prior to the assay, pass a laser beam and scattering of light occurs. The extent and the nature of the light scatter reflects bacterial number, size and shape, and the fluorescence is measured as well by a system of lenses and optical cells (Figure 55.1). The sensitivity of the technique is very high: as few as 10^2 yeast cells and about 10^2–10^3 bacterial cells per ml can be detected with results being obtained in less than 30 minutes. However, as is the case for DEFT, the application in the field of food microbiology is limited by the fact that a homogenous suspension, free of interfering particles, is needed to be injected in the system. This necessitates an appropriate sample preparation method (e.g., enzymatic clearing). As a consequence the application of FCM in food microbiology is mostly limited to the enumeration of total viable counts (TVC) or yeast cells in milk and beverages (4). Flow cytometry is often used in conjunction with the commercially available LIVE/DEAD®BacLight™ bacterial viability kit. The kit was developed to differentiate live and dead bacteria based on plasma membrane permeability. The staining mechanism using LIVE/DEAD kit on bacterial cells is based on the attachment of the non-fluorescent

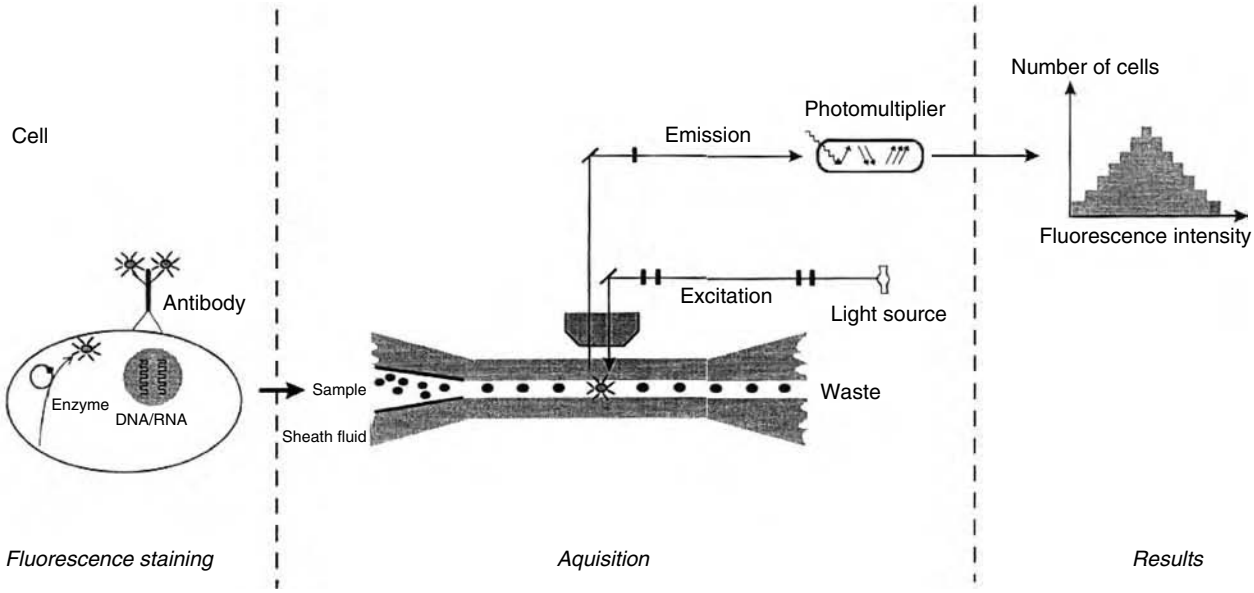


FIGURE 55.1 The principle of flow cytometric analysis. (Ph.D. thesis of C.N. Jacobsen on flow cytometric detection of *Listeria monocytogenes*, courtesy of M. Jakobsen, Dept. Dairy and Food Science, Royal Veterinary and Agricultural University, Denmark.)

agents on nucleic acids. Once the DNA-dye complex is formed fluorescence can be measured. The kit consists of two fluorochromes, which have distinct fluorescent behavior in terms of emission of wavelengths and membrane permeability. The first component is the membrane-permeant stain SYTO9®, which fluoresces green at 530 nm upon excitation at 488 nm. It stains all cells, thus acting as total cell stain. During cell death, accompanied by membrane damage, the second, membrane-impermeant, dye propidium iodide (PI) penetrates into cells and quenches the green SYTO9® fluorescence. PI is able to excite at 488 nm as well and emit red fluorescence at 620 nm. When used in combination, intact cells are labeled green and cells with damaged membranes are labeled red. Raw milk data using LIVE/DEAD staining showed good agreement between FCM and standard plate count methods. The detection limit of the FCM assay was $\leq 10^4$ bacteria per ml of milk. However, this limit is below the level of detection required to satisfy legislation in many countries (5). Sensitivity may be improved by flow cytometry using fluorescein labeled antibodies, or by using immunomagnetic separation as the specific step in combination with a less specific staining such as viability staining for flow cytometric detection of the separated cells (6).

IV. METABOLISM

To permit routine in-process measurements in a production setting to monitor critical control points very rapid methods are needed. ATP bioluminescence and impedance are two methodologies which emerged about a decade ago and that have been extensively evaluated, especially in the dairy and meat industry for fast and reliable microbiological screening of raw material on arrival at the processing plant. Both methods rely on monitoring the metabolic activity of microorganisms. Often, the major drawback of these methods is that the detection limit requires high numbers of bacteria (10^4–10^5 cfu/ml) present in the food matrix because the moment a signal can be measured or detected is in a limited time interval.

A. ATP Bioluminescence

ATP bioluminescence acts by measuring ATP levels in bacterial cells in order to calculate the number of cells. ATP bioluminescence is a natural phenomenon which is responsible for the glowing of fireflies. When the enzyme luciferase and the substrate D-luciferin are added in excess amounts, the light generated is proportional to the ATP present, according to the following reaction.

$$\text{Luciferin} + \text{ATP} + \text{O}_2 \xrightarrow[\text{Mg}^{2+}]{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{CO}_2 + \text{PPi} + \text{light}$$

ATP measurements take about 10–15 minutes. There are two major fields for the application of the ATP bioluminescence in the context of food diagnostics (7):

- Measurement of microbial ATP to determine microbial cell counts
- Measurement of total ATP to test the efficiency of cleaning procedures

1. Measurement of Microbial ATP to Determine Microbial Cell Counts

The problem with ATP bioluminescence is that ATP is the primary energy source of all living cells and the food samples themselves (fat cells in milk, blood, muscle cells) will also contain large amounts of this energy source which have to be destroyed before microbial ATP can be measured. An initial step (enzymatic, physical) for the release and destruction of any somatic cell's ATP should be included before bacterial ATP can be measured.

The use of ATP bioluminescence has been evaluated for the counting of microorganisms present in raw milk (8) or on the surface of raw meat (9, 10) upon delivery to the processing plant. In order to predict CFU values from relative light units (RLU) a correlation needs to be established (Figure 55.2) (8), imposing the necessity of an extensive standardization. This correlation is not universal and should be performed 'in-house.' Indeed the RLU that are measured depend on the actual experimental conditions (type of food matrix) and the type of luminometer for ATP measurement. In general, ATP bioluminescence is not recommended for accurate enumeration of CFU but better suited for a good bacteriological grading of the samples. Each interval of RLU may be considered as a cut-off level, an arbitrary boundary that separates defined levels of microbiological quality. ATP bioluminescence can only be used to control microbial contamination when the food to be tested is expected to have a total viable count above the detection limit of 10^4 cfu/g or cm^2. ATP readings only allow conclusions on the levels of bacterial cells present and not on the composition of the microflora. No correlation can be established between ATP readings and the presence of pathogens.

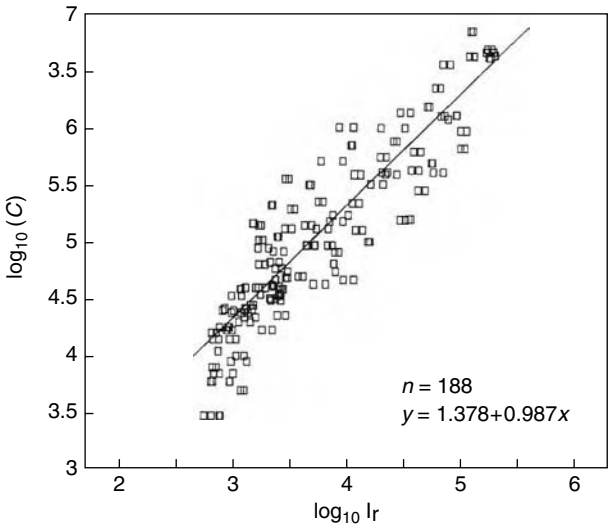


FIGURE 55.2 Relation between relative light intensity, Ir, with an improved ATP filtration method and numerical value of number concentration of bacteria in ml-1, both log-transformed, in 206 raw milk samples. Orthogonal linear regression line calculated for samples with a plate count ≥ 10000 ml-1 (n = 188). (From Reference 8.)

2. Measurement of Total ATP to Test the Efficiency of Cleaning Procedures

Because of the problems described with non-microbial ATP, the measurement of ATP bioluminescence is probably best suited to detect contaminated surfaces on equipment associated with food production. Residues of ATP (microbial or non-microbial) on equipment and working surfaces which have already undergone cleaning procedures suggest insufficient cleaning. As the corresponding tests can be carried out on site with portable luminometers and as results are available within 5 minutes, corrective measures can be taken immediately. Application of ATP determination for hygiene monitoring again necessitates the establishment of cut-off values to be linked to a hygienic standard (dirty, unsatisfactory, tolerable, clean) (Table 55.2) (11). As criteria differ according to the product (e.g., fat remaining in the deep reliefs of the surfaces may produce high RLUs) and kind of surface (e.g., plastic surface yielded higher RLU values than a stainless steel surface), each plant should set its own limits (11).

B. Conductimetry/Impedance

Conductimetry or impedance measurements are based on the detection of electrical current during microbial growth. Changes are caused by bacteria that metabolize uncharged macromolecules in the medium (proteins, carbohydrates, lipids) to mostly charged particles (amino acids, lactate, acetate, etc.), increasing the conductivity of the medium and causing a decrease in electrical impedance. Specially developed media are required for these methods. During the incubation period of the inoculated broth in the instrument, changes in conductance/impedance are measured at regular time intervals. The time taken to reach the threshold value for detection is related to the number of target organisms inoculated in the medium and their subsequent growth. Detection time is inversely related to the bacterial number: the lower the initial content, the longer the detection time (up to 16 h for low numbers ($\pm 10^2$/ml)) (Figure 55.3). The results are presented as a conductance/impedance curve, which is compared with a previously generated calibration curve to estimate the numbers of bacteria. The systems most commonly used to estimate total viable count or indicator organisms include the Bactometer (bioMérieux), Malthus (Malthus Instruments), RABIT (Don Whitley Scientific Ltd.) and Bactrac (Sy-lab). They are fully automated to enable continuous monitoring of changes in electrical current in several samples at the same time. A drawback of

TABLE 55.2
Hygienic Standard of Cleaned and Disinfected Surfaces in Meat Cutting Plants (11)

Material	Dirty RLU/20 cm³	Unsatisfactory RLU/20 cm³	Tolerable RLU/20 cm³	Clean RLU/20 cm³
Stainless steel	>1000	1000-200	199-55	<55
Plastic	>2000	2000-600	599-190	<190

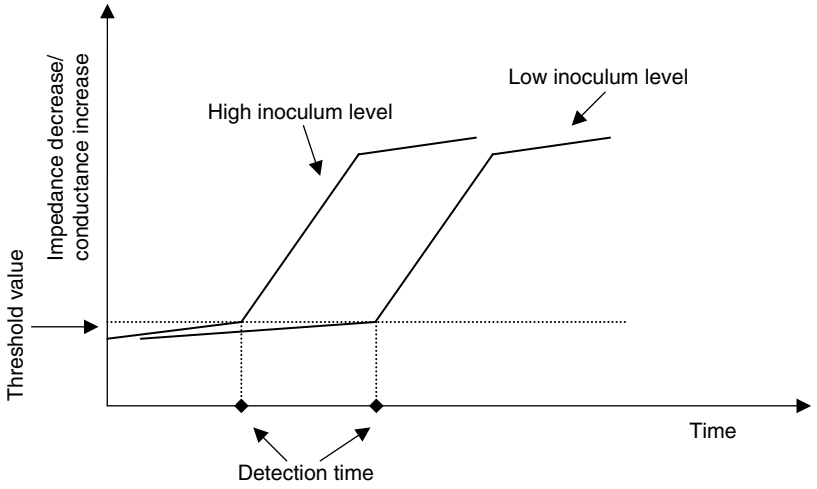


FIGURE 55.3 Graphic presentation of impedance readings, threshold value and detection time.

these electrical measurements is that the food matrix and the degree of sub-lethal stress to which the microflora is exposed may influence the analysis, which necessitates the determination of calibration curves for each food matrix examined.

Indirect conductimetry is based on the production of carbon dioxide by growing microorganisms. The carbon dioxide is absorbed into an alkaline solution and the reduction of conductivity of the solution is measured. This method is suitable for food products with low contamination levels such as juices and beverages, since carbon dioxide formation can be detected much earlier. Specific growth media are not necessary.

Detection of pathogenic bacteria such as *Salmonella* may also be done by the conductance method but usually pre-enrichment is required. Immunomagnetic separation (IMS) to capture and concentrate salmonellae from pre-enriched broth prior to inoculating the impedance medium has been evaluated. Additionally, a reduced pre-enrichment period was proposed since salmonellae from a 6-h pre-incubation period were detected in 5–7 h by impedance. Under these conditions the impedance method can be classified as a rapid method for detection of *Salmonella.*

For more information on the impedance systems available and their use as a rapid method in food microbiology the reader is referred to the reviews of Silley and Forsythe (12) and Wawerla et al. (13).

V. BACTERIAL BIOLUMINESCENCE/ REPORTER BACTERIOPHAGE ASSAYS

Bacterial bioluminescence is a specific bioluminescent assay which shows promise for rapid detection of foodborne pathogens. Many marine microorganisms like *Vibrio* spp. or *Photobacterium* spp. are capable of emitting light due to the presence of so-called *lux*-genes. The introduction of these *lux*-genes into highly specific bacteriophages by genetic engineering has resulted in the development of rapid and sensitive methodologies for pathogens in foodstuffs. Bacteriophages are highly specific for bacteria, usually to the genus level, and as they need host cell machinery to multiply they are good indicators of bacterial viability, too. In the luminescent phage test concept, bacteriophages possessing *lux*-genes infect target bacteria. This infection results in expression of the light-emitting genes which renders the bacteria luminescent upon the addition of an exogenous substrate. Luminescent bacteriophage assays have been developed to detect *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* (14). Loessner et al. (15) described the luciferase reporter bacteriophage A511: *luxAB* to detect viable *L. monocytogenes* in foods, where, without prior enrichment, as few as 500 cells were detected. The presence of less than one cfu/g could be detected within 24 h. Additionally, the assay could detect *L. monocytogenes* cells from naturally contaminated foods, including meat, poultry and various cheeses (16).

Apart from bioluminescent reporter genes, other reporter genes have been implicated in reporter bacteriophage assays. The ability of the *ina*-genes to confer ice nucleation activity on bacteria previously unable to produce ice nuclei, is the basis of a commercially available assay known as the Bacterial Ice Nucleation Diagnostic (BIND) (17). This test uses a genetically modified *Salmonella*-specific phage carrying an *ina*-gene. Due to the synthesis of an ice-nucleating protein *Salmonella*-positive samples will freeze while uncontaminated samples remain in a supercooled liquid state (temperature lowered to −9.3°C or below). It enables the detection of less than 10 *Salmonella* cells per g of food within 2–6 h (18).

The use of reporter bacteriophage assays for the rapid detection of food-borne pathogens is a powerful technique with a strong potential for future applications. Despite this, the technology has not been widely accepted. One potential problem may be the fact that an individual bacteriophage may not possess the host range required to detect all isolates of a bacterial species (14).

VI. IMMUNOLOGICAL METHODS

Over the past two decades, immunological techniques have been developed in a number of different formats for application as rapid confirmation tests (agglutination), as improvements for currently available enrichment and isolation methods (immunomagnetic separation), or as rapid detection methods (ELISA) of food-borne pathogens and their toxins. Immunoassays are based on the specific reaction of an antigen (a specific lipopolysaccharide on the outer cell wall, a protein on the flagella of motile bacteria, or a toxin) with its antibody. Sensitivity and specificity of immunoassays are mainly determined by the antibody used. In this respect the use of well-selected monoclonal antibodies can be of advantage. Each immunoassay may be sensitive to non-specific reactions, for example, *Escherichia hermanni* is known to cross-react with *Escherichia coli* O157 in specific immunoassays. Recognition of false positive results is necessary and supplementary tests such as biochemical tests are recommended to confirm the presence of the target organism.

A. Homogenous Immunoassays

1. Latex Agglutination

Traditional agglutination tests rely on the interaction between soluble antibody (in an antiserum) and a particulate antigen such as a bacterial cell, resulting in visual clumping (agglutination). Agglutination is often used to confirm the serological identity of a pathogenic isolate and is a common test in case of detection of *E. coli* O157 or *Salmonella*.

Latex agglutination uses latex particles to amplify antigen:antibody agglutination reactions. The latex particle is coated with antibody for detection of antigen such as a toxin or a component of a bacterial cell. Upon reaction, cross-linking of the latex particles results in the formation of a lattice structure which is visible to the naked eye as agglutination. If the target antigen is absent or below the detection limit of the test, the lattice structure does not form and the coated latex particles form a homogenous suspension. Latex agglutination for toxins is referred to as Reversed Phase Latex Agglutination (RPLA). Oxoid Ltd (Basingstoke, Hampshire, England) is one manufacturer that provides a range of RPLA kits for the detection of different bacterial toxins.

2. Immunochromatography

Lateral flow immunochromatographic devices were originally introduced, and are now widespread, in clinical diagnosis. They are now being applied for rapid detection of food-borne pathogens. A sample of enrichment culture is added to the sample port of the device. The liquid migrates by capillary diffusion along the surface of a solid support. During this flow, any target organism present reacts with a conjugate (antibody linked to a chromogen) contained in the device. The resulting complex proceeds to move and migrate towards the capture binding protein where it becomes immobilized and forms a visible line in a viewing window (Figure 55.4). For the test to be valid a control line should form in a second viewing window. In an evaluation of rapid methods for detecting *E. coli* O157 on beef carcasses three immunochromatographic methods were compared to the conventional culture method. These assays were all simple and rapid to use, giving a result at least 24 h earlier than culture. However, although one of the assays compared favorably with culture, both other assays lacked the sensitivity of culture and this finding seriously limits their usefulness (19). This was already

FIGURE 55.4 Example of a rapid Listeria test (Clearview™) based on immunochromatography showing test and control lines in the viewing window. (Courtesy of Oxoid, Basingstoke, Hampshire, England.)

mentioned in the study of Johnson et al. (20) who found 18 of the 107 culture-confirmed samples negative with the immunochromatography method for *E. coli* O157. False negatives spanned both high- and low-inoculum subsets.

B. Heterogeneous Immunoassays

The most widely used tests for rapid detection of pathogens, in particular *Salmonella, E. coli* O157 and *Listeria*, in foods are enzyme-linked immunosorbent assays (ELISA) using a sandwich configuration (Figure 55.5). The word 'sandwich' indicates that the assay uses two antibodies which trap or sandwich the target antigen. They use a capturing antibody immobilized on a solid matrix (strip, microtitre tray, dipstick) and enzyme labeling of the secondary antibody to make the primary antigen-antibody reaction detectable. Colorimetric or chemiluminescent substrates are used to visualize the antibody-antigen-antibody sandwich. A range of commercial immunological tests are available in different formats for the common food-borne pathogens. These tests normally require enrichment of the target bacterium to the level of the assay's detection limit (generally 10^4–10^6 CFU per ml). Their dependence on conventional cultural enrichment still remains the most important limiting feature of the majority of these immunoassays. In most cases, a presumed positive result is obtained in approximately 54 h. The magnitude of the resulting reaction will be proportional to the number of bacteria in the broth. However, results are usually converted to a dichotomous scale: results at or above a chosen cut-off value are regarded as positive and results below as negative. A high cut-off value will decrease sensitivity and increase specificity and vice versa. False-positive or false-negative results can be significantly reduced by appropriate antibody selection and preparation. ELISAs are of considerable use in situations where the value of a negative result is high, for example, if a negative is required before a product can be released for distribution or further processing. This explains why these tests are extremely popular for routinely screening food samples following enrichment. In case of a positive ELISA the sample will need to be subjected to further conventional cultural testing to confirm the presence of the pathogen. Many of the ELISAs require the elaboration of a number of steps: capture of the 'target' antigen to the antibody absorbed to the solid phase, washing steps to remove 'non-target' antigens, capture of the secondary labeled antibody (=conjugate), washing to remove excess conjugate, addition of enzyme substrate and reaction which makes the procedure labor-intensive. The introduction of automated plate washers and readers can decrease the hands-on time. Typically these assays take between 1–2 hours, depending upon the format of the test and number and length of incubation steps (21, 22).

Automated immunoassays have also been developed. Vidas (bioMérieux, Marcy-l'Etoile, France) is an automated enzyme-linked immunofluorescent assay (ELFA) based on the detection of specific pathogens using specific antibodies coated on the inner surface of a tip-like disposable pipette which is introduced into the Vidas system along with the Vidas pathogen-specific reagent strip containing the boiled enrichment culture (Figure 55.6). A chemiluminescent substrate is used to visualize the antibody-antigen-antibody sandwich (23, 24). The Vidas system enables high-throughput and rapid screening of large

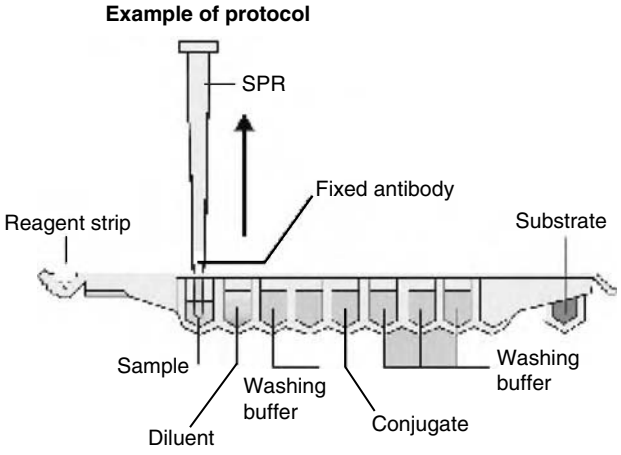


FIGURE 55.5 Principle of ELISA: capturing antibody immobilized in the well of a microtitre tray (a) interacts with target antigen in the sample and enzyme-labeled secondary antibody (conjugate) makes the primary antigen-antibody detectable (b). Washing steps are include to remove unbound conjugate and non-specific sample components (c). Colorimetric or chemiluminescent substrates are used to make the primary antigen-antibody reaction detectable (d).

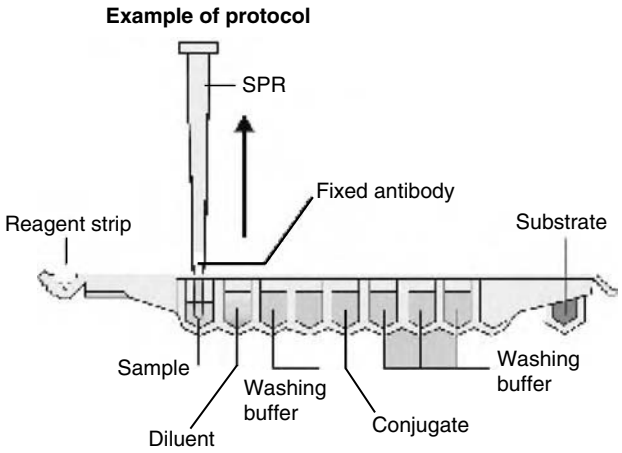


FIGURE 55.6 The Vidas system: an automated ELFA method based on the detection of pathogen-specific antibodies coated on the inner surface of a tip-like disposable pipette which is automatically transferred in the Vidas strip in which the test sample is introduced and which holds the necessary ELISA reagents. (Courtesy of bioMérieux, Marcy-l'Etoile, France.)

number of samples for presence of pathogens. The VIDAS is available for detection of *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, *Campylobacter* spp., *E. coli* O157 and *Staphylococcus aureus* toxins.

C. Immunomagnetic Separation (IMS)

IMS, which employs paramagnetic particles coated with specific antibodies, promises to be a useful tool to remove small food particles from a sample and to selectively enrich and concentrate target bacterial cells by altering the ratio of target to non-target organisms in favor of the target organism. IMS techniques are nowadays widespread in food diagnostics (25). The efficiency and specificity of IMS depends upon the type of magnetic beads, the choice of the antibodies, the target organism, the numbers of the competitive flora and the food matrix. The sensitivity of the IMS technique varies from 10^2–10^4 CFU per ml. In most cases IMS after 24 h pre-enrichment results in an increase in the isolation of the pathogen with concomitant reduction in the number of competitive flora. Thus the use of IMS improves the sensitivity of microbiological tests and eliminates the need for a secondary 24h enrichment, thus reducing the total test time. IMS can be of significant value in conjunction with a number of end-point detection techniques (Figure 55.7). IMS was shown to improve the sensitivity of ELISA, PCR and both used in conjunction enabled detection of *Salmonella* and *E. coli* O157 in foods within 24 h. As such, IMS is an important contribution to the development of a rapid and facile preliminary screening method of food for the presence of pathogens. IMS has become an integral part of the conventional as well as some rapid methods for *E. coli* O157. The use of IMS was shown to increase the isolation rate of *E. coli* O157. The IMS procedure has now been automated (BeadRetriever™, Dynal Biotech Ltd. Wirral, U.K.). Automated immuno-magnetic separation in combination with an enzyme immunoassay complements the conventional culture approach to produce more timely results for the detection of pathogens without significantly increasing the workload of the laboratory (26).

IMS methodology has been coupled to electrochemiluminescence (ECL) detection for rapid detection of *E. coli* O157 or *Salmonella typhimurium* with a commercial immunomagnetic (IM)-ECL sensor. Like other chemiluminescence techniques, ECL offers high signal-to-background ratios and is comparable in sensitivity to radioisotopic methods but has the advantage over other chemiluminescent techniques of being initiated by a voltage potential and thus providing better-controlled luminescence. ECL can be accomplished by heavy metal chelates such as ruthenium(II) trisbipyridal [$Ru(bpy)_3^{2+}$] chelate. Following IMS, [$Ru(bpy)_3^{2+}$]-labeled antibodies were added and after a short incubation period magnetic beads were captured and ECL was measured. The IM-ECL assays were capable of detecting less than 2000 CFU per ml in various food samples (27). The IM-ECL approach proved to be at least 100 times more sensitive than the current ELISA assays and enabled rapid detection (within 24 h) of low numbers (0.05 CFU per g) of *E. coli* O157:H7 in ground beef (28).

An alternative format for IMS, ImmunoFlow, has recently been developed to capture bacteria and eliminates an enrichment step to allow bacteria to be concentrated directly in food and water samples in 30 minutes. ImmunoFlow uses a high-flow-rate fluidized bed with large beads to capture and concentrate bacteria and is volume-independent. Specific antibody is covalently linked to the beads and large beads (>3 mm) are used to prevent sample clogging when testing food samples. Detection of bound targets is done using conventional ELISA protocols. This protocol enabled detection of 10 cells independent of the sample volume (29).

VII. NUCLEIC ACID-BASED METHODS

The application of molecular biological techniques to food microbiology has increased tremendously in recent years. These techniques represent a new generation of rapid methods, based on the primary information contained in the nucleic acid sequences of a particular organism. Indeed, nowadays a bacterial species is defined on

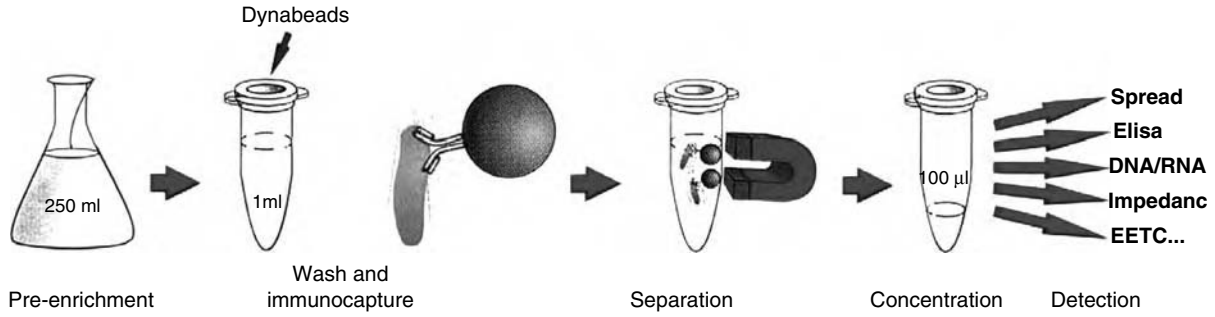


FIGURE 55.7 Immunomagnetic separation uses paramagnetic particles with specific antibodies to selectively concentrate bacterial cells. IMS can be of significant value in conjunction with a number of end-point detection techniques. (Courtesy of Dynal, Oslo, Norway.)

the basis of genetic information combined with a phenotypic description. Detection of pathogens based on specific DNA or RNA sequences thus goes back to the basis of classification of unknown organisms.

A. Oligonucleotide Probes

With the classical DNA probe hybridization assay, microorganisms are collected on a filter, cells are lysed and the liberated DNA or RNA is immobilized in single-stranded form. The nucleic acid is identified by hybridization with radioactively or non-radioactively labeled specific DNA probes. For many pathogens rRNA genes are targeted in hybridization assays. The rRNA gene contains very conserved parts but also variable regions in which specific probes for a certain species can be chosen. In addition, rRNA is present in high numbers in the bacterial cell ($>10^4$/cell). Although all these DNA probes offer good specificity, they are limited in sensitivity. Typically, these assays need about 10^5–10^6 CFU per ml to obtain a reliable result. There are two types of user-friendly nucleic acid probe tests commercially available in routine food laboratories for several pathogens, e.g., *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, etc. The Gene-Trak system (Neogen, Lansing, MI, U.S.A.) uses a dipstick format with a colorimetric detection and is generally applied after 48-h enrichment of the food sample. The AccuProbe test (Gen-Probe Inc., San Diego, CA, U.S.A.) is a chemiluminescent homogenous DNA probe system based on the principle of DNA hybridization protection. It is recommended as a culture confirmation test applied to purified colonies from isolation media, thus providing qualitative results (30).

Recently, PNA probes were developed as an alternative to DNA probes. Peptide nucleic acid (PNA) is a pseudopeptide that binds strongly and specifically to nucleic acids. PNA consists of a non-charged polyamide backbone to which the different nucleo-bases are attached. The neutral PNA backbone confers unique properties on the molecule, relative to oligonucleotides. These include faster hybridization kinetics and the ability to use more stringent hybridization conditions that favor the disruption of the target structure while still promoting strong hybridization of PNA. PNA probes can be used for rapid detection and identification of specific microorganisms (31).

B. Polymerase Chain Reaction

1. Principle and Applications in Food Microbiology

A significant factor in the application of molecular techniques for rapid detection of pathogens lies in the development of the polymerase chain reaction (PCR). Conventional PCR assays incorporate a pair of oligonucleotide primers to amplify by use of the Taq DNA polymerase, a specific gene that is then detected using agarose gel electrophoresis. PCR is based on repetitive cycles of DNA denaturation, primer annealing if they meet a complementary target DNA sequence, and elongation of the primers according to the complementary base sequence given by the target DNA by a thermostable DNA polymerase (Figure 55.8). This results in duplication of the target DNA after each cycle. Thus, during thermocycling, exponential amplification of target DNA occurs. PCR has become an important tool in microbial diagnostics because of the potential for detecting less than 10 copies of a specific gene within a complex sample. However, its sensitivity is also the greatest potential weakness of the method. Cross-contamination with external DNA or carry-over contamination of target DNA produced during prior amplification assays may give false results very easily. Technical improvements (real-time PCR instrumentation, enzymatic decontamination systems), the adherence to careful laboratory procedures and the use of dedicated materials and pre-aliquoted reagents may solve this problem. The reliable identification due to the use of specific DNA or RNA sequences and the intrinsic sensitivity of PCR shows tremendous promise for rapid detection of pathogens in foods. Specific primer sets were developed for almost all established and emerging pathogens in food microbiology (32, 33). Target genes involved for PCR detection of pathogens are numerous:

- rRNA genes or the spacer region between 16 S rRNA and 23 S rRNA genes.
- Virulence genes and toxin genes. Some virulence genes are located on plasmid DNA. A chromosomal location is preferred because of the instability of plasmid during subculturing. By targeting virulence genes (*hlyA*-gene for *L. monocytogenes*, *invA*-gene for *Salmonella*, *eae*-gene for *E. coli* O157:H7, *ail*-gene for *Y. enterocolitica*, etc.) or toxin genes (*vt*-genes for VTEC, *lt*-genes and *st*-genes for ETEC, *ct*-genes for *Vibrio cholera*, BoNT for *Cl. botulinum*, etc.), potential pathogenic strains can be discriminated from non-pathogenic strains belonging to the same species.
- A specific sequence with a known function, e.g., flagellin genes for *Campylobacter*, beta-D-glucuronidase gene for *E. coli,* IS 200 element for *Salmonella*, etc.
- A specific sequence with an unknown function, often derived from species-specific bands in DNA fingerprinting patterns.

Multiplex PCR uses multiple primer sets to amplify two or more target loci in a single reaction. It was described for simultaneous detection of *Listeria* spp. and *Listeria monocytogenes* in poultry products and dairy samples, *Salmonella* spp. and *Shigella* spp. in mussels, *Salmonella* strains and *Escherichia coli* O157 in meat,

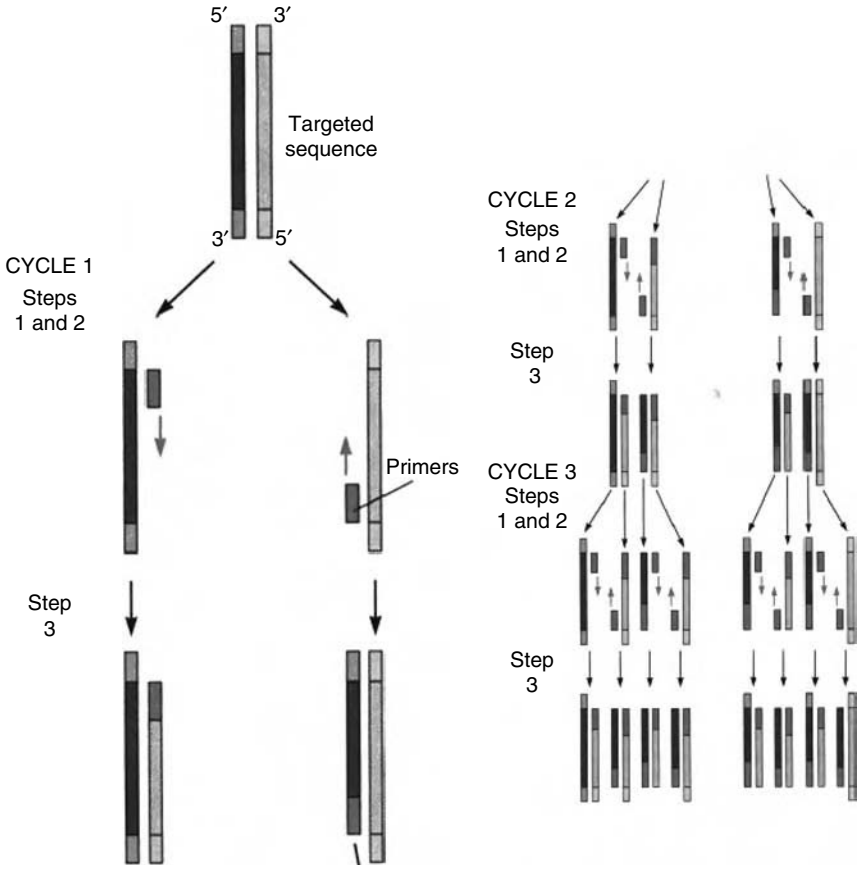


FIGURE 55.8 Principle of PCR. PCR is based on repetitive cycles of DNA denaturation and primer annealing if they meet a complementary target DNA sequence (steps 1 and 2), and elongation of the primers according to the complementary base sequence given by the target DNA by a thermostable DNA polymerase (step 3). This results in duplication of the target DNA after each cycle.

Campylobacter jejuni and *Arcobacter butzleri* in a range of food products (1).

PCR methods are of particular importance in two areas in the field of food microbiology:

1. In the context of rapid detection of food-borne pathogens.
   In accordance with immunoassays, PCR provides a rapid screening method for the presence of pathogens. If a specific primer set is chosen, amplification conditions well optimized, appropriate sampling protocols are applied and proper controls are included, PCR assays may be developed that are highly specific and provide a reliable detection of pathogens in the food sample under examination in a 24-h time period.
2. As a complementary technique in the identification of pathogens where the conventional cultural methodology lacks accuracy.
   Usually, phenotypic characteristics such as morphological, physiological and biochemical features are applied as confirmation tests for food-borne pathogens. However, sometimes the development of simple and restricted confirmation schemes may not be feasible to provide a reliable identification of the pathogenic organism, especially for particular genera or species with a difficult taxonomic position, e.g., identification of *Bacillus cereus* s*enso stricto* being part of the *B. cereus* group including *B. cereus*, *B. anthracis*, *B. mycoides* and *B. thuringiensis* may be accomplished by a PCR method based on the *gyr*B-gene. In the case of emerging pathogens, e.g., pathogenic strains of *Escherichia coli* for which pathogenicity is associated with the presence or absence of certain virulence genes, PCR is an essential part of the full characterization of the strain. For the genus *Campylobacter*, which is characterized by its biochemical inertness, detection of the hippuricase gene by PCR provides a more useful test for confirmatory identification of *C. jejuni* because wrong identification may occur due to non-expression of the gene when

only relying on the hippurate hydrolysis phenotypic confirmation test (34).

Although user-friendly PCR formats are available now, the technique still requires a significant amount of labor with regard to sample preparation and subsequent detection of PCR amplicons.

2. The Necessity of Sample Preparation in PCR-Based Pathogen Detection Systems

Any PCR-based detection system, while potentially very sensitive, must still confront a number of challenges inherent with sampling complex substrates such as food samples. PCR is an *in vitro* enzymatic reaction which only takes place under well-defined conditions of pH and ionic strength and is very susceptible to interfering factors which may be co-extracted from the food sample. If the complexity of the samples adversely impacts the assay, causing reduced amplification efficiency or complete inhibition of the enzymatic reaction, then a negative result may in fact be caused by lack of sensitivity to detect a given pathogen at the minimum concentration that is known to cause health risk. Thus sample preparation needs to be well designed: how much sample is processed, the efficiency of pathogen isolation, the efficiency of nucleic acid extraction, and the effect of co-factors that inhibit PCR. In the context of food diagnostics the following points need to be considered:

1. The PCR technique has the intrinsic sensitivity to achieve the required detection limit for pathogens in food (approximately 5–10 CFU per PCR reaction). However, direct detection of the pathogen in food samples by PCR presents a number of technical difficulties. The relative low levels of pathogens are present in a large amount of food (1–30 g used for food analysis) while only a small sample size (normally one ml of food homogenate) is used for nucleic acid isolation and only a small portion of the DNA extracted (2–10 μl) can be used in the PCR reaction. The pathogen may also be non-uniformly distributed in the food product (35).
2. Several studies indicated the presence of PCR-inhibitory factors in milk (calcium ions), soft cheeses (proteins and fats), red meat (coagulated blood) and various other foods (36, 37). The inclusion of proper controls makes it possible to develop and apply PCR methods for reliable and rapid detection of pathogens in routine laboratories. A positive control (in a separate tube) should be taken along in each PCR run and preferentially an internal control is co-amplified with the target DNA in each PCR tube to check on inhibitory compounds in the sample.
3. From a food safety point of view, care must be taken in interpreting results generated by PCR analysis since this technique cannot distinguish between viable and non-viable organisms. The precise correlation of cell viability with detection of DNA was shown to be poor, with DNA persisting in actively killed cells for significant periods of time (38). The detection of dead cells has to be considered.

The above-mentioned problems can be solved by the execution of PCR after a short enrichment period. Most commonly, the food sample is homogenized in a broth and the number of bacteria is increased *in vivo* by enrichment in order to obtain a sufficient number in a small volume while at the same time diluting out inhibitory components of the food. In addition, previous culture also ensures that only DNA from viable cells is detected (39). Prior enrichment also enables the use of rapid and simple protocols for lysis of bacterial cells and DNA purification. The commercially available PCR systems, the BAX™-assay (DuPont Qualicon, Wilmington, DE, U.S.A.) (20), the Probelia™ assay and its real-time format, the iQ Check PCR assay (Bio-Rad Laboratories, Marnes-la-Coquette, France) (40, 41) and the Taqman™ assay (Applied Biosystems, Foster City, CA, U.S.A) (42) all recommend the application of PCR after a 16–24 h pre-enrichment step.

The use of a shortened pre-enrichment (4–12 h) should be carefully evaluated. In contrast to fully viable pathogenic bacteria used for artificial contamination, bacterial cells in naturally contaminated foods are subjected to stress. The enrichment time needed for a positive PCR detection of stressed cells will be adversely affected. In this state, the microorganisms require a period of recovery before they regain full growth potential and limitation of the enrichment time could lead to an underestimation of the contamination of the food products (43). Often a technical intervention for further concentration of the bacteria is performed after enrichment to increase the sensitivity of the PCR assay and eliminate interfering food components. These include filtration, centrifugation, buoyant density centrifugation, immunomagnetic separation (IMS), surface adhesion or selective adsorption to metal hydroxides, etc. (1, 44). At present several protocols combining a short enrichment, subsequent bacterial concentration, DNA extraction and PCR analysis for rapid (within 24 h) detection of low numbers of pathogenic bacteria in various foods are described (Table 55.3) (20, 40, 42, 45–55). To reduce the time needed for a PCR result the direct application (without prior enrichment) to food is preferred. It must be considered that for application in routine microbiological analysis the complexity of the procedures should be limited. For fluid food products such as milk a single concentration step such as centrifugation or filtration allowed the detection of 1–10 CFU of *L. monocytogenes* per 25 ml

TABLE 55.3
Some Examples of Nucleic Acid Amplification Protocols for Rapid Detection (within 24 h) of Pathogens in Foods

Micro-Organism	Period of Enrichment	Sample Preparation	Detection	Food Types	Ref.
Escherichia coli O157:H7	6–11 h	InstaGene™ Matrix (Bio-Rad laboratories)	PCR + Molecular Beacon based detection	Pasteurized apple juice and milk (I)	Fortin et al. 2001 (45)
Salmonella	16–20 h	InstaGene™ Matrix (Bio-Rad laboratories) EnviroAmp Kit (PE Applied Biosystems)	Taqman PCR assay	Raw meat and shrimp (I +NC)	Kimura et al. 1999 (42)
Salmonella	16–20 h	Multiple protocols	Taqman PCR assay	Chicken carcass rinses, raw meat, raw milk (I+NC)	Chen et al. 1997 (46)
Salmonella	16–20 h	InstaGene™ Matrix (Bio-Rad laboratories)	Probelia™ PCR assay	Various foods (I + NC)	Fach et al. 1999 (40)
Escherichia coli O157:H7	20 h	BAX lysis reagent	PCR + agarose gel detection	Ground beef (I)	Johnson et al. 1998 (20)
Campylobacter spp.	6–24 h	Surface adhesion to a polycarbonate membrane + phenol:chloroform extraction	PCR + agarose gel detection	Meat and poultry (I+NC)	Cloak et al. 2001 (47)
Salmonella spp.	16 h	IMS + heat treatment	PCR + agarose gel detection	Dairy and egg products (I)	Rijpens et al. 1999 (48)
Escherichia coli O157:H7	6 h	Buoyant density centrifugation + boiling	PCR + agarose gel detection	Raw meat (I + NC)	Lindqvist 1997 (49)
Campylobacter jejuni	24 h	GuSCN lysis + silica-based extraction	NASBA + internal enzyme labeled detection probe	Various foods (I), poultry (NC)	Uyttendaele et al. 1995, 1996 (50, 51)
Escherichia coli O157:H7	0 h	Adsorption to titanium hydroxides + GuSCN lysis and phenol-chloroform extraction	PCR + Molecular Beacon based detection	Skim milk (I)	McKillip and Drake 2000 (52)
Listeria monocytogenes	0 h	Centrifugation + IMS + phenol: chloroform extraction	PCR +agarose gel detection	Ham (I)	Hudson et al. 2001 (53)
Campylobacter jejuni	0 h	Mechanical disruption in the presence of DNAzol	PCR+ agarose gel detection	Chicken carcass rinses (I)	Englen and Kelley 2000 (54)
Miscellaneous	0 h	FTA filters	PCR +agarose gel detection	Fresh produce (I), beef (I), apple cider (I)	Lampel et al. 2000 (55)

I = artificially inoculated, NC=naturally contaminated

(56, 57). To obtain this detection limit the filtration step was combined with a complex DNA-purification protocol.

3. Detection of PCR Amplicons/Real-Time PCR

The location of the primers in the selected gene determines the length of the amplification fragment. The detection of the amplicon is usually performed by agarose gel electrophoresis combined with an intercalating dye (e.g., ethidium bromide) and UV light. Electrophoresis makes it possible to estimate the length of the PCR product and length often serves as a proxy for product identification. However, production of non-specific PCR products is known to occur sometimes as the result of priming at loci that share homology with the target sequence or because of primer artefacts. Confirmation of the identity of the amplification product is recommended either by

- Hybridization with a probe internal to the amplification primers (Southern blots or dot-blots)
- Digestion of the amplicon with a restriction endonuclease
- Generation of an internal fragment using nested or semi-nested PCR
- Sequencing to identify the PCR product

In addition to testing the specificity of the product, hybridization with an internal probe or nested PCR can also improve the sensitivity of the assay system.

The application of fluorescent probes to the detection of PCR amplicons, together with suitable instrumentation capable of combining amplification and detection, has led to the development of real-time PCR methodologies. These assays eliminate the need for gel-based detection and yield data amenable to storage and retrieval in an electronic database. Fluorescent real-time detection uses either the SYBR Green fluorescent dye that binds to double-stranded DNA or specific hybridization of fluorescence-labeled probes to the amplicon (44, 58). For the

latter approach three different chemistries are predominantly applied in food microbiology: Taqman probes (Applied Biosystems, Foster City, CA, U.S.A.), molecular beacons (Bio-Rad Laboratories, Marnes-la-Coquette, France) and the LightCycler hybridisation system (Roche Molecular Diagnostics, Basel, Switzerland). These systems are commercially available for routine detection of pathogens in foods. The application of each of these fluorescent real-time PCR techniques is linked to dedicated suitable instrumentation.

a. SYBR Green DNA binding dyes

SYBR Green binds specifically to double-stranded DNA (dsDNA). The unbound dye exhibits little fluorescence in solution in the presence of denatured DNA, but during elongation increasing amounts of dye bind to the nascent dsDNA. When fluorescence is monitored in real time in every cycle at the end of the elongation step, an increase is observed in fluorescence as the PCR progresses and target DNA is exponentially amplified. This method obviates the need for target-specific fluorescent probes, but its specificity is determined entirely by the choice of specific primers. Thus this assay is no more specific than conventional PCR with agarose gel detection. Additional specificity and PCR product verification should be achieved by the generation of melting curves following amplification. Melting curves are generated by slowly ramping the temperature of the sample to a denaturing level (95°C). As the dsDNA denatures, the dye becomes unbound from the DNA duplex, and the fluorescent signal decreases. This change in fluorescence can be plotted against temperature to yield a melting curve waveform. A characteristic melting peak at the melting temperature (T_m) of the amplicon will distinguish it from amplification artefacts.

b. Taqman probes

The Taqman assay utilizes the 5′-nuclease activity of the DNA polymerase to hydrolyse a hybridization probe bound to its target DNA. This probe (the Taqman probe) contains a fluorescent reporter dye at its 5′-end, the emission of which is quenched by a second dye at its 3′-end when the reporter and quencher dye are close to each other. Intact Taqman probes do not generate fluorescence. During the annealing both the primers and the Taqman probe will anneal to its complementary sequence of the target DNA. However, during elongation, when the Taq DNA polymerase is adding bases to the synthesize specific dsDNA, it will degrade the Taqman probe on its way by its 5′-nuclease activity, the quencher and reporter dye will separate from each other and fluorescence will occur (Figure 55.9). By measuring fluorescence in the PCR tube every cycle, the PCR reaction can be followed up in real time.

c. Molecular beacons

Molecular beacons are DNA hybridization probes that form a stem-and-loop structure; the loop portion of the molecule is complementary to the target nucleic acid molecule and the stem is formed by the annealing of complementary arm sequences on the ends of the probe. On one end of the probe a fluorophore is attached and on the other end a quencher of the fluorophore (Figure 55.10). In the absence of the target PCR product the beacon is in a hairpin shape and there is no fluorescence. However, during PCR reactions and the generation of target PCR products, the beacons will attach to the PCR products and cause the hairpin to unfold. As the quencher moves away from the fluorophore, fluorescence will occur and this can be measured. The measurement can be done every cycle of the PCR during the annealing step as the PCR is in progression allowing real-time detection of target PCR products and thus the presence of the target pathogen in the sample. By using molecular beacons containing different fluorophores, one can detect different PCR products in the same reaction tubes, which enables one to perform multiplex tests of several pathogens.

d. The Light Cycler hybridization system

This method uses two hybridization probes. One of the probes carries at its 3′-end a fluorescent donor which emission spectrum overlaps the excitation spectrum of an acceptor fluorophore that is attached to the 5′-end of the probe. Excitation of the donor results in fluorescence resonance energy transfer (FRET) to the acceptor and thus fluorescence. In solution the two dyes are apart, and because FRET depends on the spacing between the two dye molecules, only background fluorescence occurs. During the annealing step, both probes hybridize to their complementary sequence, specific target DNA, in a head-to-tail confirmation. This brings the two dyes in close proximity to one another, promoting FRET and fluorescence is measured. Increasing amounts of fluorescence are measured proportional to the amount of specific target DNA synthesized during the PCR reaction.

There are three competing instruments on the market to perform real-time PCR: the ABI PRISM® Instruments (Applied Biosystems), the iCycler (Bio-Rad Laboratories) and the LightCycler (Roche Diagnostics). All three are run as closed-tube systems requiring no post-amplification manipulations for detection of amplicons. This avoids problems of carry-over contamination. The entire PCR process is amenable to automatization and can handle large amounts of samples making real-time PCR suited for high-throughput screening applications. Although real-time PCR allows quantification, automated fluorescent PCR methods are predominantly used for qualitative pathogen testing (presence/absence in the food sample). However, the real-time monitoring of PCR reactions by tracking a fluorescent signal at every cycle during PCR thermal cycling demonstrates positive results more rapidly (at the cycle at which fluorescence exceeds the statistically determined fluorescence background threshold) thus decreasing the time to detect a positive sample.

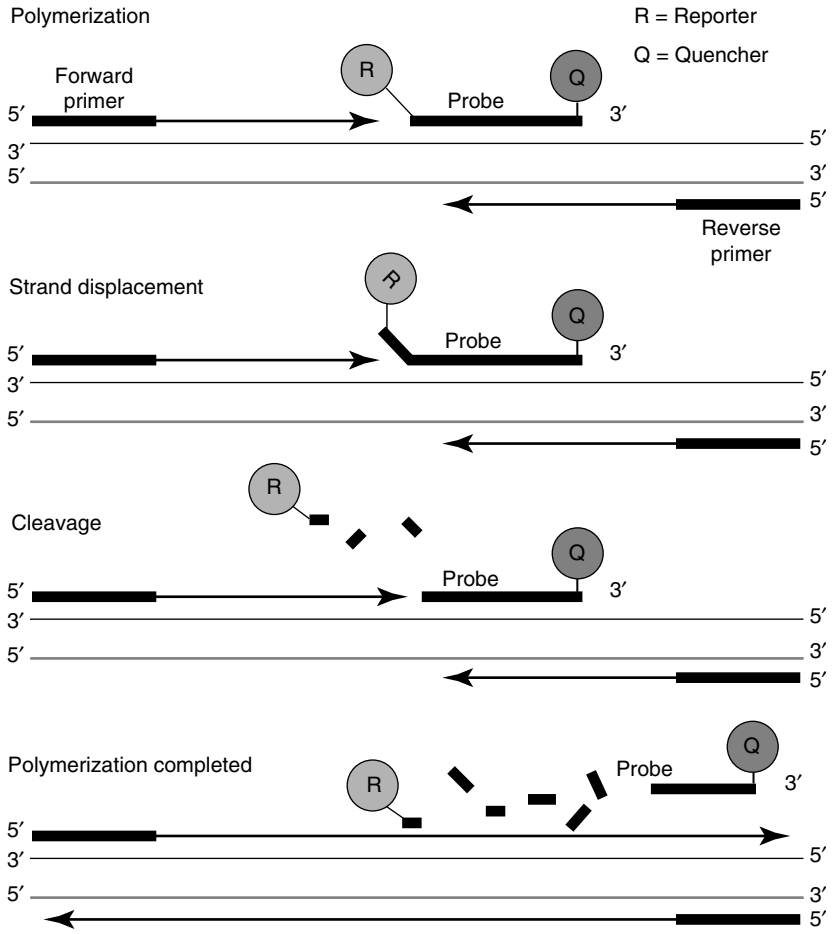


FIGURE 55.9 The Taqman assay utilizes the 5′-nuclease activity of the DNA polymerase to hydrolyze a probe bound to its target DNA. Subsequently, the quencher and reporter dye at the ends of the probe will separate from each other and fluorescence will occur. (Courtesy of Applied Biosystems, Foster City, California, U.S.A.)

4. Quantitative PCR

During real-time PCR, fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The more amplified product appears during PCR amplification, the more fluorescence intensity increases due to hybridization of the fluorescent probe to the PCR product. The instrument-associated software plots automatically during the amplification the RFU (Relative Fluorescent Unit) versus number of cycle which allows the detection in real-time of the PCR product. In the software a baseline for the RFU is calculated as a function of fluorescence noise. When adjusting the graph to have log RFU in Y-axis versus the cycle number in the X-axis it should be verified that the threshold cycle Ct (the cycle at which the fluorescence rises appreciably above background) is located in the linear part of the graph (corresponding to the PCR exponential phase since the scale is in logarithmic units). The combination of a sample preparation method for direct PCR (without cultural enrichment step) with a PCR method using a real-time monitoring fluorescent PCR amplicon detection system allows rapid quantification of pathogens. Quantification is based on the threshold cycle. The higher the level of pathogenic bacteria present in the sample, the higher the input of pathogenic target DNA in the PCR reaction and the faster the threshold cycle is reached. Quantification is performed by an external standard correlating the time to detection to the initial number of pathogens or DNA material used as an input (Figure 55.11). As the real-time quantitative PCR does not use an internal standard but is based on an external scale, quantification demands a good reproducibility of isolation/concentration of bacteria, DNA extraction and PCR. Quantification of *Salmonella, Campylobacter jejuni* and *Escherichia coli* O157 in pure cultures using real-time PCR was reported (59–61). Evaluation of a quantitative real-time PCR for rapid enumeration of *Listeria monocytogenes* showed that log differences in the pathogen added to cabbage could be reliably distinguished. The method produced a linear response over 7 log cycles from 10^2 to 10^9 cfu in 25 g of cabbage (62).

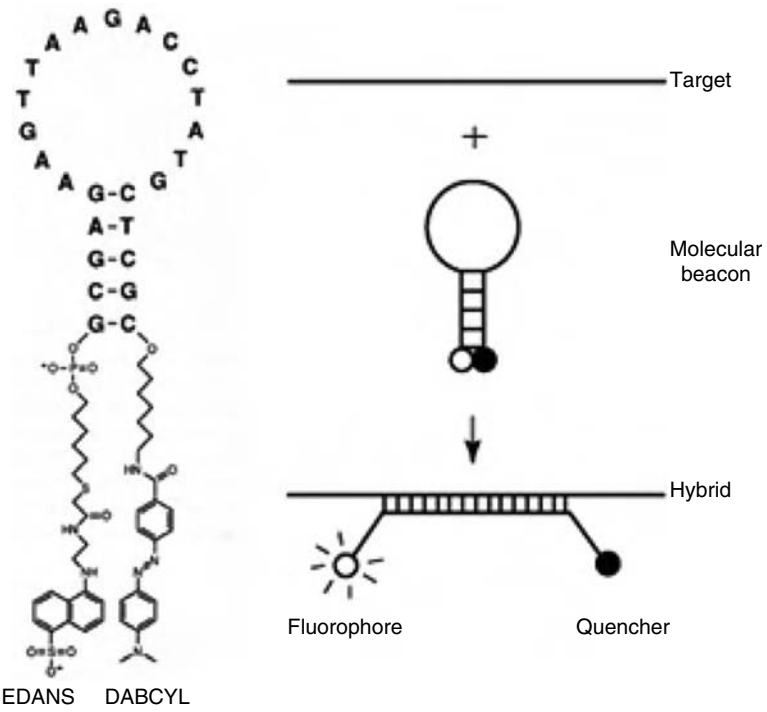


FIGURE 55.10 Molecular beacons are DNA probes that form a stem-and-loop structure. During PCR reactions, the beacons will attach to the PCR products and cause the hairpin structure to unfold. As the quencher moves away from the fluorophore, fluorescence will occur.

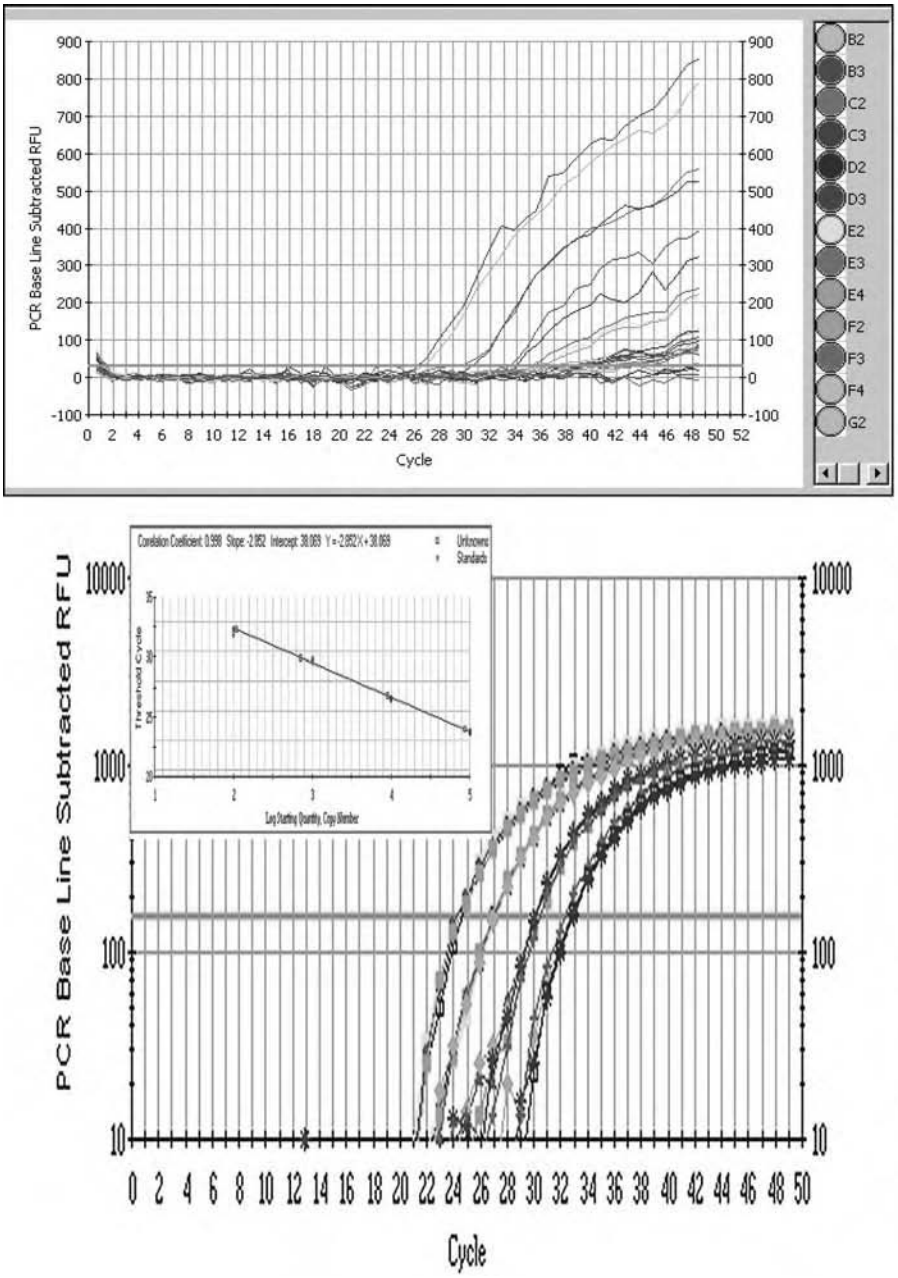
5. Microarrays

The recently developed nucleic acid microarray (oligonucleotide microchip) technology has been introduced to enhance the detection capabilities of PCR. In this context, microarrays serve as a system for rapid detection of multiple amplicons resulting from a multiplex PCR. DNA microarrays are in fact a series of reverse dot-blots for which sequence-specific short oligonucleotide-probes are attached to a substrate in a lattice pattern. Probes appear as "spots" in the final image where each spot represents a unique probe sequence. PCR products can deposit on the surface and if these PCR products specific hybridize to complementary probes, it can be visualized using some type of reporter molecule (either a fluorescent label or biotin that permits subsequent detection with a secondary label). This system enables simultaneous detection of the presence of distinct PCR products based on inter-product DNA sequence differences. Multiplex PCR in combination with microarrays have been used for the detection and genotyping (identification of various virulence factors) of enterohemorrhagic *E. coli*. The microarray technology has great potential for use in diagnostic microbiology, by combination of DNA amplification with a universal primer pair with an oligonucleotide microchip with specific probes for several pathogens (63).

6. RNA Amplification Systems

DNA was also demonstrated to persist in a PCR-detectable form in culture negative samples. However, the presence of intact DNA sequences does not give an indication of cell viability. RNA is chemically and biologically more labile than DNA. The type of target RNA, the primer choice, the inoculum level, the inactivation treatment and the holding conditions of the inactivated culture influence the half-life of the target RNA and as a consequence its (in)ability to be amplified. The longer half-life of rRNA and its variable retention following a variety of bacterial stress treatments make rRNA, under many conditions, a less accurate indicator of viability than mRNA. Studies investigating messenger RNA as a target for amplification showed genes with inducible expression (e.g., the *hlyA*-gene of *L. monocytogenes*) not to be suitable; because of the inducible expression, the extraction efficiency of the transcript is variable. The use of housekeeping genes (e.g., the elongation factor) seems a better target as an indicator for viability.

The most commonly used amplification techniques for detecting mRNA are RT-PCR (reverse transcriptase-PCR) or NASBA (nucleic acid sequence-based amplification). RT-PCR is a two-stage process, in which a target messenger RNA sequence is first transcribed into a complementary DNA (cDNA) sequence, either using random hexanucleotide primers or sequence-specific primers. The



	Threshold Cycle Ct	Identifier
B2	26.6	E1 20000cp/pcr
B3	26.2	E1 20000cp/pcr
C2	29.0	E2 2000 cp/pcr
C3	30.4	E2 2000 cp/pcr
D2	34.0	E3 200 cp/pcr
D3	33.4	E3 200 cp/pcr
E2	36.8	E4 20 cp/pcr
E3	35.4	E4 20 cp/pcr
E4	35.2	E4 20 cp/pcr
F2	40.5	E5 10cp/pcr
F3	38.3	E5 10cp/pcr
F4	37.1	E5 10cp/pcr
G2	39.3	E6 5 cp/pcr
G3	38.7	E6 5 cp/pcr
G4	44.0	E6 5 cp/pcr
G5	39.3	E6 5 cp/pcr
G6	39.1	E6 5 cp/pcr
H2	39.3	E7 2cp/pcr
H3	N/A	E7 2cp/pcr
H4	N/A	E7 2cp/pcr
H5	40.9	E7 2cp/pcr
H6	38.5	E7 2cp/pcr
H7	N/A	E7 2cp/pcr
H8	46.6	E7 2cp/pcr
H9	38.3	E7 2cp/pcr
H10	42.5	E7 2cp/pcr
H11	N/A	E7 2cp/pcr

FIGURE 55.11 During real-time PCR (in the present example using a molecular beacon-based assay performed in an iCycler), fluorescence intensity increases due to hybridization of the fluorescent probe to the PCR product. When adjusting the graph to have log RFU in Y-axis versus the cycle number in the X-axis the threshold cycle Ct (the cycle at which the fluorescence rises appreciably above background) can be derived. Quantification is performed by an external standard correlating the time to detection to the initial number of pathogens or DNA material used as an input. (Courtesy of Bio-Rad, Hercules, California, U.S.A.)

cDNA sequence may then serve directly as a template for PCR. NASBA is a transcription-based amplification reaction that utilizes three enzymes (avian myeloblastosis virus reverse transcriptase (AMV-RT), ribonuclease H (Rnase H) and T7 RNA polymerase) acting together to mimic retroviral replication. A major advantage of NASBA over RT-PCR is that NASBA is performed isothermally which avoids amplification of double-stranded DNA and enables amplification of RNA from a pool of total nucleic acid.

Both methods have been applied for detection of microbial pathogens in food and water samples. NASBA accomplished rapid detection of food-borne pathogens (*Listeria monocytogenes*, *Campylobacter jejuni*) in foods after a preceding 24-48 h enrichment period. RT-PCR has been applied for detection of viable *Listeria monocytogenes* in beef and eggs and *Salmonella* Enteritidis in beef (64, 65) (Table 55.3).

RNA amplification methods offer in many instances the best means of detecting food-borne enteric viruses

(hepatitis A virus, rotaviruses, Norwalk-like virus); other methods such as ELISA or cell culture are too inefficient or slow (66, 67).

7. International Acceptance

Despite rapid advances in the field of molecular-based methods, there is no doubt that the complexity of the food samples will continue to challenge further these rapid detection methodologies. Nucleic acid-based diagnostics, such as PCR, are very young compared to traditional detection methods and although well-established in research laboratories, still need to gain general acceptance as reliable methods in routine food diagnostics. However, it is considered that PCR can be established as a routine reference method, alongside traditional techniques within the next decade. Currently, there are international collaborative efforts to produce PCR-based methods for food-borne pathogens which are suitable for standardization, and thereafter adoption as routine diagnostic procedures. The framework of these efforts is based on three principal steps: demonstration of primer specificity, evaluation of the method through collaborative trial and, finally, proposal to an international standardization body such as CEN (Commission Europeén de Normalisation) (68). At the same time ISO Technical Specifications are under development to formulate some general definitions and recommendations on the requirements for sample preparation, the performance criteria for thermocyclers, and the requirements for amplification and detection in order to control the overall reliability of the PCR methods executed in food diagnostic laboratories.

VIII. BIOSENSORS

Biosensors are defined as analytical devices which combine biospecific recognition systems with physical or electrochemical signalling. The architecture of a generic system comprises three components: the biospecific reaction, the signal emitted when the target is bound, and the platform, which transduces the binding reaction into a machine-readable output signal. Recently new concepts have begun to yield biosensors aimed at addressing pathogen detection in the food industry. They consist of immobilized biologically active material (e.g., enzymes, antibodies or antigens, nucleic acids) in close proximity to a receiving transducer unit. Target recognition results in the generation of an electrical, optical or thermal signal which is proportional to the concentration of target molecules. However, the application of biosensors to the on-line detection of food pathogens is seriously hampered by problems of stability, sterilizability, sensitivity and matrix interferences. Some current papers have reported the detection of common food-borne pathogens like *E. coli, Salmonella* or *Listeria* in model systems within minutes. Only very few papers, however, deal with their direct detection in food samples. Given the complexity of the different types of biosensors and their limited application in food pathogen testing, we refer to excellent reviews by Ivnitski et al. (69) and Hall (70) for further reading.

IX. VALIDATION OF ALTERNATIVE (RAPID) METHODS

In the past, different countries have developed different validation schemes. Several standardization organizations such as AOAC, IDF, AFNOR, NMKL, etc. have expanded their activities and started up a validation protocol for alternative methods. This has frustrated kit manufacturers, as they have to undertake a number of different validations in different countries, in order to get widespread acceptance of their test. There is a need for harmonization in validation schemes. In 2002, the European Standard "Protocol for the Validation of Alternative Methods" was accepted by the CEN. Through the CEN/ISO "Vienna agreement," this European Standard will also be adopted as an ISO standard (ISO 16140) and agreements have been made with AOAC International for mutual recognition of the different validation schemes. A European certification organization named MicroVal for independent validation and approval of alternative methods based on the European standard is set up and pilot validation studies are ongoing. The proposed ISO 16140 standard for validation of alternative methods describes a technical protocol for the validation of qualitative methods and a technical protocol for the validation of quantitative methods which both include a method comparison study and an interlaboratory study. Specific recommendations are given relating to the experimental setup of the measurement protocol and the calculation and interpretation of the data obtained using appropriate statistics. Nevertheless, the acceptance criteria are not clearly defined in the protocol. Results of the alternative method should be 'comparable' to those of the reference method. The actual criteria will depend upon the type of method and the circumstances under consideration.

In the U.S. but also worldwide, AOAC International, which has a long tradition in validation of methods, is the expert organization for validation of alternative (rapid) methods. The collaborative study forms the essence of the AOAC validation process. In this study, competent, experienced analysts, working independently in different laboratories, use a specified method to analyze homogeneous samples for a particular microorganism. An Associate Referee, under the guidance of the General Referee and assisted by a statistical consultant, is responsible for the actual development of the protocol for the collaborative study which should be approved through the Methods Committee on Microbiology and Extraneous Materials. The Associate Referee also conducts ruggedness testing

and a pre-collaborative study to determine the applicability of the method for detection of the target organism(s) in a wide range of food matrices and conditions. The Associate Referee is required to be an expert in a particular target organism or a particular type of method or a food matrix or a combination of these. The General Referee must be a recognized authority in the field of interest (71). For the parameters taken into consideration to evaluate the performance of alternative (rapid) methods the reader is referred to Chapter 54.

X. CONCLUSIONS

Improvements in the field of instrumentation and data processing, immunology and molecular biology have led to the development of rapid (within 24–48 h) methods for the detection of food-borne pathogens. The introduction of these methods for 'real-time' detection of specific bacteria is hampered by the complexity of the food matrix and the low numbers of pathogens present in the sample. One of the most challenging problems is sample preparation. More research is needed on techniques for separating microorganisms from the food matrix and for concentrating them before detection. The potential of combining different methods should be further exploited. Results obtained from alternative (rapid) methods should be carefully interpreted because positive tests are not necessarily linked to biological activity and should be supported by classical cultural methods which are still the reference methods whereas negative test results are only representative for the absence of the pathogen in the sample taken at that time. Anyhow, it should be kept in mind that microbiological monitoring of foods as such cannot control entirely microbiological quality or food safety. It is only a tool to verify the well functioning of preventive measures taken to eliminate, reduce or prevent the contamination from spoilage or pathogenic microorganisms.

ACKNOWLEDGMENTS

Mieke Uyttendaele is indebted to the Fund for Scientific Research, Flanders, for a position as a postdoctoral research assistant.

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HANDBOOK OF FOOD SCIENCE, TECHNOLOGY, AND ENGINEERING

Volume 2

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Taylor & Francis
Taylor & Francis Group
Boca Raton London New York

A CRC title, part of the Taylor & Francis imprint, a member of the
Taylor & Francis Group, the academic division of T&F Informa plc.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-9847-9 (Set)
International Standard Book Number-10: 1-57444-551-0 (Vol 1)
International Standard Book Number-10: 0-8493-9848-7 (Vol 2)
International Standard Book Number-10: 1-57444-552-9 (Vol 3)
International Standard Book Number-10: 0-8493-9849-5 (Vol 4)
International Standard Book Number-13: 978-0-8493-9847-6 (Set)
International Standard Book Number-13: 978-1-57444-551-0 (Vol 1)
International Standard Book Number-13: 978-0-8493-9848-3 (Vol 2)
International Standard Book Number-13: 978-1-57444-552-7 (Vol 3)
International Standard Book Number-13: 978-0-8493-9849-0 (Vol 4)
Library of Congress Card Number 2005050551

Library of Congress Cataloging-in-Publication Data

Handbook of food science, technology, and engineering / edited by Y.H. Hui.
p. cm. -- (Food science and technology ; 148)
Includes bibliographical references and index.
ISBN 1-57444-551-0 (v. 1 : alk. paper) -- ISBN 0-8493-9848-7 (v. 2 : alk. paper) -- ISBN 1-57444-552-9 (v. 3 : alk. paper) -- ISBN 0-8493-9849-5 (v. 4 : alk. paper)
1. Food industry and trade--Handbooks, manuals, etc. 2. Food--Analysis--Handbooks, manuals, etc. 3. Food--Composition--Handbooks, manuals, etc. I. Hui, Y. H. (Yiu H.) II. Food science and technology (Taylor & Francis) ; 148.

TP370.4.H38 2005
664--dc22 2005050551

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Taylor & Francis Group is the Academic Division of Informa plc.

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CONTENTS

I. INTRODUCTION

A. Definition of Consumer Acceptance Research

Consumer acceptance research is the field of study that examines the perceptual, cognitive, social, and attitudinal factors that influence liking of and behavior toward a food product or concept. It involves the study of the intrinsic and extrinsic product factors that influence liking, choice, purchase, or consumption of foods and beverages, utilizing untrained people who are representative of the ultimate users of the product.

B. Importance and Purpose of Consumer Acceptance Research to the Food Industry

The importance of consumer acceptance research to the food industry is reflected in the large number of R&D professionals who are employed by food companies all over the world to conduct consumer-based, sensory research for food product development, optimization, shelf stability analysis and quality control. In addition, another broad range of consumer research activities is performed within marketing research departments in these companies. Considering the cost involved in successfully bringing a new product to market, food manufacturers realize that it is essential to obtain consumer information as early as possible in the product development cycle in order to screen out potential product failures from potential successes. Consumer acceptance research, along with many of the other sensory testing techniques described in Chapter 57, can provide critically important information to decision makers in order to maximize the likelihood of both product and business success.

Since consumer testing is ordinarily more expensive and time consuming than non-consumer sensory testing, it would be inefficient to have consumers evaluate products that have not been previously evaluated by in-house or trained panels to either characterize their sensory properties or to ensure that the samples to be compared differ significantly on some sensory dimension. However, for most of the trained panel techniques discussed in Chapter 57, consumer testing of the liking/disliking of the product(s) will be a necessary follow-on activity. This is especially true, since knowledge about a product's sensory characteristics or the fact that it is discriminably different from another product does not tell the product developer whether it will have any meaningful effect on consumer liking/disliking of the product(s). Without this latter information, product managers risk making decisions that are very costly in time, resources, and money, but without any evidence of their likely impact on consumer liking or purchase. In addition, one of the most important uses of consumer acceptance testing is for product optimization. Without reliable and valid measures of liking/disliking, it is not possible to develop useful relationships between sensory and/or physical variables of the product and consumer acceptance, so that a set of optimum product characteristics can be achieved.

C. Consumer vs. Non-Consumer Sensory Research

1. Differences in Test Populations: Consumers vs. Trained Panels and Experts

The primary difference between consumer research and other sensory research is the test population that is used. A necessary element of consumer acceptance research is the use of untrained people who represent the target population for which the product is intended. This reliance on untrained individuals contrasts with other types of sensory research that is conducted for the purposes of descriptive analysis of products, quality control, sensory-instrumental correlations, and other specialized purposes within specific food commodity areas. The latter research often utilizes trained or expert panels. *Trained panels* are groups of individuals who have been selected for participation on the basis of objective criteria related to their ability to perceive differences in basic sensory attributes and who are trained in specific sensory evaluation techniques and/or the use of a standardized lexicon. *Expert panels* are groups of individuals who have extensive formal experience with the product or the product category, but who may or may not have received training in a specific descriptive methodology. Expert panels are often made up of in-house authorities within an organization. Trained and expert panels should not be used to assess the affective (liking) aspects of foods (1) or to evaluate any aspect of the suitability of the item for its intended purpose. The reason for this is

that the members of these panels are not necessarily the users of the product or product category. In addition, due to their training and experience, these individuals may well have different perceptions of the sensory properties of the product (2), different liking of the products (3), or biased opinions of its suitability for the average consumer. For these reasons, trained and expert panels should not be used in consumer research.

Although it is agreed that consumer research must be conducted using naive users of the product, the extent to which naive consumers can be used to provide descriptive or other, non-affective, sensory data is more controversial (4). Due to the historical evolution of sensory science within the food industry, where commodity experts and highly trained professionals were often employed to perform sensory analysis, the use of consumers to make analytic sensory judgments was often deemed inappropriate. However, as a result of the influence of the fields of psychology and psychophysics, both of which have long relied on the use of naïve subjects to make sensory and perceptual judgments of complex stimuli, a wider acceptance of the use of consumers to make discriminative and descriptive evaluations of products has emerged. In fact, the method of "free-choice profiling" (5, 6) has been developed for the specific purpose of utilizing consumers and consumer terminology for the descriptive evaluation of food products. The pros and cons of the use of consumers for non-affective sensory analysis have recently been debated (7–11) and the utility of having consumers rate descriptive attributes of products in order to segment them on the basis of sensory preferences has been documented (12, 13). At present, it is best said that a consumer panel should be used when affective (liking) data are being obtained or when sensory data are to be generalized to a consumer population. Trained panels should be used when there is a need to maximize sensitivity to differences in the product or when detailed descriptive information is required.

Although trained panels and consumer panels provide different types of sensory data, it is often useful to be able to relate the two. For this purpose, a number of multivariate statistical approaches have been developed. These include multiple regression techniques, principal component analysis, response surface methodology, internal and external preference mapping, and a variety of data clustering techniques. Details and examples of the application of these techniques to consumer acceptance issues can be found in a variety of books and papers on the topic (14–25).

2. Differences in Independent and Dependent Variables

Another common difference between consumer and non-consumer sensory research is the types of variables that are studied. As noted previously, the independent variables of interest in consumer testing include factors that are both intrinsic and extrinsic to the product. Although intrinsic variables, e.g. ingredients, processing variables, and storage conditions, are also studied using non-consumer sensory techniques, the extrinsic properties of the product, e.g. price, packaging, labeling, and product information variables, along with the demographic and psychographic variables of the user, are unique to consumer research. Similarly, while the dependent variables of non-consumer sensory research typically focus on the perception of the product's sensory attributes (its appearance, odor, taste and texture), the dependent variables used in consumer research typically focus on affect (liking or preference), choice, purchase, or consumption of the product.

II. QUANTITATIVE TEST METHODS: MEASURES OF PREFERENCE AND LIKING

A. The Psychological Construct of Affect: Liking and Preference

One of the major dependent variables of interest in consumer research is *affect*, the emotional response or "feeling" toward some object. In operational terms, affect is commonly assessed through judgments of the "hedonic" or liking dimension of a food or through judgments of the relative preference for one item over another. The word hedonic means, "having to do with pleasure" and derives from "hedonism", the philosophy that views pleasure as the ultimate good and the goal of all human behavior.

The affective or hedonic dimension of a food is separate and distinct from its sensory dimensions. It is a conscious experience that accompanies the somatic (physical) effects of emotions. Thus, when someone states that they *like coffee*, this is *not* a descriptive statement regarding anything intrinsic to the coffee itself. Rather, it is an evaluative statement, based on an emotion that is elicited in the observer when he/she drinks coffee. This contrasts with the statement that *the coffee is bitter*, which is a descriptive statement about the sensory properties of the coffee. Descriptive statements can be said to be "objective", i.e. they tell us something about the object's outward manifestations. However, evaluative or affective statements are "subjective", i.e. they tell us something about the subject's private experiences. It is relatively easy to get agreement among individuals about descriptive statements, but it may be impossible to get common agreement about evaluative or affective statements. Similarly, it is sometimes possible to get independent corroboration of descriptive statements through physical tests performed on the food, whereas no such tests are available to corroborate evaluative or affective statements.

In spite of the differences in the underlying nature of sensory versus affective responses, in many ways, affective responses manifest themselves in the same way as sensory

responses. For example, they both have psychological magnitude and duration. They both adapt over time and recover after deprivation. They are both subject to contrast and order effects, and they both can show bipolarity. These similarities between sensory and affective dimensions have led to the development and use of similar methods and approaches for their measurement (26).

B. Measuring Affect

1. Measurement Levels and Scale Types

Measurement is defined as the assignment of numbers to objects according to rules. This definition applies to the measurement of both the physical and psychological attributes of objects, and by extension, to their sensory, cognitive and emotional dimensions. In a classic paper, S.S. Stevens (27), a mathematician and psychophysicist, defined four different levels of measurement and corresponding measurement scales. The four levels of measurement that he identified were *nominal*, *ordinal*, *interval*, and *ratio*, each defined by the rules used to assign the numbers to objects and the allowable transformations of the numbers that keep the scale form invariant.

The most rudimentary form of measurement is nominal measurement. It refers to the use of numbers to name objects, e.g. the numbers appearing on the jerseys of football players or the designation of the symbol "4" to sets of objects containing four elements. Nominal scaling is rarely used in sensory or consumer science, except when simple counts of the number of consumers who like or dislike a product are made. In the latter case, the proportion of consumers who like the product is referred to as the "acceptor set size", and this measure can sometimes be an important measure in food marketing decisions (28).

The second level of mathematical measurement is ordinal measurement. It refers to the assignment of numbers to reflect the *order* of objects along some dimension, e.g. the numbers assigned to the finishing places of racehorses. Ordinal measurement is often used in consumer sensory testing when food samples are compared or ranked for preference (see section IIB2). However, these tests are usually conducted only when a crude measure of the differences among test samples is needed. Most of sensory science, whether consumer or non-consumer oriented, has come to rely on the last two levels of mathematical measurement — interval and ratio measurement.

Interval scaling refers to the measurement of objects in such a way that the differences between adjoining numbers on the scale represent equal intervals along the dimension being measured. Common interval scales include the Fahrenheit and Celsius scales of temperature and the 9-pt hedonic scale for assessing food liking/disliking (29, 30) (see section IIB3a). Although interval scales afford a meaningful degree of quantification along the measured dimension, they lack the precision and mathematical sophistication of ratio scales.

Ratios scales represent the highest level of mathematical measurement and are defined by the fact that the ratios among the numbers reflect the ratios of the magnitudes among the test objects. In order to achieve this, ratio scales require a *true* zero point. [Note that neither the Fahrenheit nor Celsius scales of temperature are ratio scales, because the zeroes on these scales do not represent a true absence of thermal energy and, therefore, 20 degrees Fahrenheit (or Celsius) does not reflect twice as much thermal energy as 10 degrees Fahrenheit (or Celsius)]. Common ratio scales include metric measurements of line length, the Kelvin scale of temperature, and, in sensory science, magnitude estimation and labeled magnitude scales (see section IIB4).

2. Ordinal Methods: Paired Preference and Ranking

a. *Paired preference tests*

The simplest method for assessing the difference in liking associated with two or more food items is to have consumers directly compare the samples and indicate their preferences. The term *preference* refers to the choice of one sample over another. A paired preference test is used when two samples are to be compared. The two samples are presented either sequentially or simultaneously, depending on the test objectives. The order of presentation of the samples is balanced either temporally (sequential test) or spatially (simultaneous test) in order to avoid order and position biases. The task for the consumer is to choose which sample he/she prefers. The test is typically run as a forced-choice task; that is, the consumer *must* choose one sample or the other. The number of consumers who prefer one sample over the other can then be compared to statistical tables found in standard sensory texts (1, 31, 32) in order to determine whether a statistically significant difference exists in consumer preference between the two samples. There has been a recent discussion in the literature concerning the advantage of having a no preference choice for the consumer, since this represents a real possibility; however it has not found wide use by sensory scientists. (33)

b. *Multiple paired preference tests*

When more than two samples are to be evaluated, either a multiple paired preference test or a ranking test can be performed. In a multiple paired preference test, all possible pairs of the samples are presented in a series of paired preference comparisons. If a large number of samples is to be evaluated, a subset of the possible pairings can be used that focuses on comparing one or two samples, e.g. controls, with all of the other samples. The data and conclusions to be drawn from such an iterative set of two-sample comparisons are similar to those that would be obtained

using a ranking test. However, the multiple paired comparison test minimizes the complexity of the choices for the consumer and can produce greater sensitivity when differences among samples are small. The reader is referred to standard texts on sensory analysis to read further about these methods and the statistical procedures that apply to the data (1, 31, 32, 34).

c. *Preference ranking tests*

A ranking test is the most direct way to determine the order of consumer preferences for more than two samples. However, for reasons related to panelist fatigue and memory limitations, the maximum number of samples that should be tested is 5–6 (35). In a ranking test the samples are usually presented simultaneously to the subjects, in order to minimize the variability contributed by memory effects. The samples are presented in a balanced, random order and the consumer assigns numerical ranks to the samples to indicate the order of his/her preferences for the samples. The data can then be then analyzed by nonparametric statistical tests (Friedman's) and appropriate multiple comparisons to determine if a difference in preference exists among the samples, and if so, which samples differ from one another. Alternatively, rank sum statistics can be found in published tables (36–38).

3. Interval Methods: Rating Scales

Ordinal preference testing methods have several limitations. First, they require at least two samples in order to collect the data. Second, they do not provide information about absolute levels of liking or disliking. In a paired preference test showing that Sample A is preferred to Sample B, one still does not know if both samples are liked, if both samples are disliked, or if one is liked and the other disliked. Lastly, paired preference and ranking methods do not provide information about the degree of difference among samples on the affective dimension. In order to obtain information about the absolute level of liking/disliking for a single sample, a rating scale with specific verbal language that addresses the intensity of the liking-disliking dimension is required. In addition, in order to make statements about the degree of difference in liking/disliking between samples, an interval level of scaling is required.

a. *Category rating scales*

Verbal hedonic scales

Perhaps the most common and best-known method for measuring consumer liking/disliking of foods is the 9-pt hedonic scale (30). This scale, shown on the left side of Table 56.1, is designed not merely to determine preferences among samples, but also to index the degree to which the consumer likes or dislikes the sample. The 9-pt hedonic scale was developed at the U.S. Army Quartermaster Food and Container Institute in Chicago in the years immediately following World War II (see (39) for a review of the contributions of this laboratory to consumer research). The goal of the laboratory's research was to maximize the consumption and nutritional intake of soldiers by identifying and/or developing foods that soldiers would readily consume. In order to develop fast and efficient means for predicting the foods that soldiers would eat, the 9-pt hedonic scale was developed.

TABLE 56.1
The 9-Point Hedonic Scale (30) and Geometric Mean Magnitude Estimates of the Semantic Meaning of Each Category Label (48)

9-Point Hedonic Scale	Geometric Mean Magnitude Estimate
• Like Extremely	476
• Like Very Much	360
• Like Moderately	232
• Like Slightly	72
• Neither Like Nor Dislike	0
• Dislike Slightly	−66
• Dislike Moderately	−199
• Dislike Very Much	−347
• Dislike Extremely	−472

Although the 9-pt hedonic scale is commonly treated as an interval scale, this assumption is unwarranted. The scale is simply an ordered metric scale of liking. The reason for this is that the 9-pt hedonic scale represents a scale type frequently used in consumer research and known as a "category scale". A category scale consists of a set of verbal labels that define different "categories" along a measurement dimension. These verbal categories are often assumed to represent equal-sized intervals along the dimension. However, several analyses of these scales have shown that this is rarely the case (see section below).

The 9-pt hedonic scale is only one of many different types of category scales that can be used to assess food liking. Such scales can have varying numbers of categories (typically 3–10), may be unidirectional or bi-directional, and may use variants of the terms "liking and disliking", e.g. "pleasant" and "unpleasant" or variants of the intensity modifiers, e.g. "somewhat", "a little", etc. In one extension of the use of verbal category scales, a scale consisting of a set of verbal labels relevant to children, i.e. "super good", "really bad"; was developed (40). Most category scales of liking may be used to index either overall liking of the product or liking of one of its specific attributes, e.g. its flavor, texture, etc. (see (31) for examples of a wide range of balanced and unbalanced category scales of liking).

Facial hedonic scales

Although the 9-pt hedonic scale is the most commonly used scale for measuring liking, it is often difficult to use with children, with individuals who have reading difficulties, or with the visually or cognitively-impaired. For these

special classes of consumers, facial category scales are often used that place a lower cognitive demand on the respondent. Such scales consist of a set of faces depicting frowns or smiles. Figure 56.1 shows a consumer ballot that uses a 5-pt facial hedonic scale. This ballot was used to assess the liking of foods served in the U.S.D.A. school lunch program (41). Similar facial scales have been developed using either three (42, 43), five (44, 45), or seven points (46), while others have used animal cartoons (47) and adult faces (31).

Limitations of category scales

In spite of the diversity in the nature of category scales and their widespread use in the food industry, all category scales have the same basic measurement properties and all suffer from the same limitations. As stated previously, empirical evidence indicates that the points on the 9-point hedonic scale do not represent equal subjective intervals, i.e. the difference in acceptability between two products rating "1" and "2" on the scale is not the same as the difference between two products rating "4" and "5" on the scale or "8" and "9" on the scale. The right column of Table 56.1 shows data from a study in which a magnitude estimation procedure was used to scale the semantic meaning of the verbal labels of the 9-pt hedonic scale (48). As can be seen, the data show that the differences between points on the scale are not equal. For example, the psychological distance between "like extremely" and "like very much" is larger than the psychological distance between "like moderately" and "like slightly." Other investigators (49–51) have also demonstrated this nonequivalence of scale intervals using similar techniques.

Another problem with category scales is that respondents tend not to use the extreme categories, because they fear that if they use these categories to rate a food item, but a more extremely liked (or disliked) item is presented subsequently, they will have no appropriate category left to assign to it. This central tendency or regression effect means that the 9-point hedonic scale effectively becomes a 7-point scale of liking/disliking. Also, many category scales have a neutral point. The use of a neutral category decreases the efficiency of the scale (52) and may encourage complacency in judgments by providing the consumer a "safe" category into which foods that engender only mild liking or disliking can be placed (53,54).

Rating foods vs. food names

Since the inception of the study of consumer food research, investigators have applied a variety of methods to obtain an index of the liking or "acceptability" of a tasted food. However, for certain practical purposes in the food industry, such as for menu planning, it is often desirable to index consumer liking for a wide array of foods without presenting the actual foods for evaluation. For this purpose, the same methods of analysis used to evaluate the liking/disliking of real foods can be applied to *food names*. Under these circumstances, the term "preference" is sometimes used to describe the nature of the data that are obtained (55). Although many investigators reserve the term *preference* for methods that require the consumer to choose one sample over another (e.g. paired preference methods), the word *preference* is also defined as an underlying "psychological continuum of affectivity" (56). It is in keeping with this latter usage that the word "preference" is often used to refer to affective ratings of food names (a liberal perspective).

Since a food name is not the same as any particular preparation of that food, the correlation between liking/disliking ratings of foods and their corresponding food names is quite variable. In one study where subjects rated both their liking/disliking for food names and for tasted preparations of the same foods, correlations ranged from .02 to .61 across food items (57). This study also showed that "acceptance" ratings of real foods are characterized by a regression toward the mean relative to "preference" ratings for the corresponding food names.

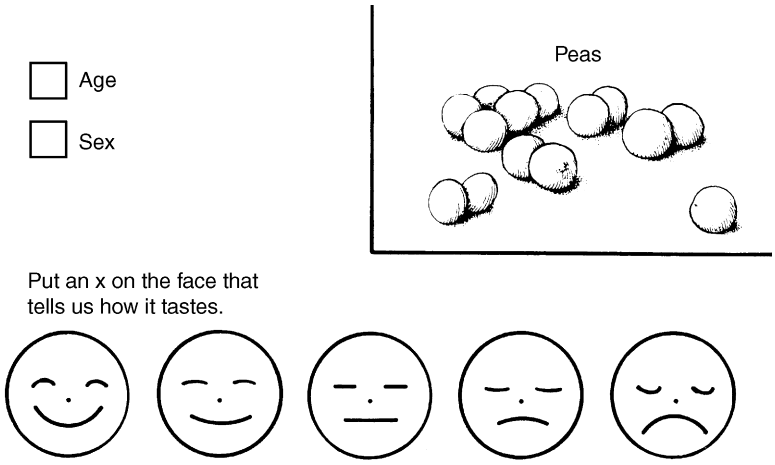

FIGURE 56.1 Facial hedonic scale ballot used to assess food liking/disliking in school lunch programs (41).

That is, foods that are rated as very highly liked, based on ratings of their names, are generally rated lower when actually tasted; whereas, foods that are rated as very disliked on the basis of their names are rated more positively when tasted. This has led to the conclusion that consumers' stated preferences for foods based on their rating of food names reflect a quintessential or idealized image or memory trace of the food, and that actual preparations of the food item are never as good or as bad as this mental image (57).

From a practical standpoint, ratings of affect are also often used to predict future consumer behavior toward a food, e.g. purchase or consumption of it. In a lengthy set of studies conducted by the U.S. military from 1950–1980, soldiers' ratings of liking/disliking in response to food names accounted for 25–50% of the variability in the consumption of these foods (see (57) for a review of these data). More recent studies looking at the relationship between affective ratings of tasted foods and consumption have shown approximately the same degree of predictability (58–65). Although food preference and acceptance measures obtained from affective attitudinal judgments are relatively poor predictors of consumption, this has not interfered with the widespread use of these measures in both industrial and academic settings to predict consumer behaviors toward food. In many cases, affective ratings in response to a tasted food have become the *sine qua non* of consumer behavior toward foods and are used to guide product development, product improvement, and quality assurance throughout the food industry. The continued use of the 9-point scale is a function of its ability to order the liking of both potential new products and products undergoing quality control testing, rather than because it is an accurate predictor of food consumption.

b. Line scales

A different method for measuring the liking and disliking of foods that is not dependent on the use of verbal or pictorial categories is line scaling, also known as linear graphic scaling or visual analogue scaling. A linear graphic rating scale consists of an unbroken horizontal or vertical line that is anchored on both ends with a verbal label. Such a scale may also be anchored in the middle or elsewhere. Figure 56.2 shows a typical linear graphic rating scale used to assess consumer liking. Although line scales have been used to evaluate food quality since World War II (66), they were popularized in the early 1970's due to their use in the procedure known as Quantitative Description Analysis (QDA) (67).

Linear graphic rating scales have been used in a number of studies to rate liking/disliking of foods (68–70). When presented without instructions, linear graphic rating scales are presumed to be interval scales. However, Lawless (71) used a linear graphic rating scale with ratio instructions in order to scale both sensory intensity and affect in response to taste and odor stimuli. In order to enable extremely large ratings of magnitude, Lawless allowed subjects to "extend" the end-point of the line beyond its terminal point. Line scales have also been combined with verbal labels whose semantic meaning have been quantified using direct ratio methods in order to construct a labeled magnitude scale of liking (see below). The latter scales have been shown to produce data comparable to those of ratio methods (48).

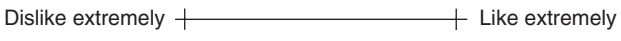

FIGURE 56.2 Typical linear graphic rating scale for assessing liking/disliking.

4. Ratio Methods

a. Magnitude estimation

In the 1950's, S.S. Stevens developed a variety of "ratio" methods to permit subjects to make ratio judgments of the attributes of food and other stimuli. The most widely known of these methods is magnitude estimation (72). It was originally applied to sensory stimuli to establish psychophysical functions relating physical intensity to perceived intensity. Magnitude estimation was probably first applied for the scaling of consumer food liking by Moskowitz and Sidel (49). These authors argued for the practical importance of magnitude estimation as a "supplement" to category scaling, suggesting that a category scale could be used to *label* the perceptual categories of acceptance, but that magnitude estimation should be used to refine the *degree* of acceptability. Later, in several papers and books, Howard Moskowitz, a student of S.S. Stevens, championed the use of magnitude estimation for wide scale use in sensory evaluation of foods (50, 73, 74).

When using magnitude estimation, respondents rate the liking of one product relative to another. If one product is liked twice as much as the other, it is assigned a number twice as large. If it is liked one-third as much, it is assigned a number one-third as large, etc. In one study in which magnitude estimation was used to rate whiskey sour beverages (75), it was found to be more sensitive than the 9-point hedonic scale. However, other studies have found the hedonic scale to be more reliable (76), although both scales were observed to be linearly related to one another. Other investigators (77, 78) have found only minor differences between the two scales. A collaborative study sponsored by the American Society for Testing Materials (ASTM) was carried out to compare the 9-point hedonic scale with both unipolar (only positive numbers) and bipolar (positive numbers are used for liking; negative numbers are used for disliking) magnitude estimation (79). This study found all three scales to rank the test stimuli in the same order, with very similar spread of ratings. Very similar degrees of precision and sensitivity were also found in differentiating samples. Reliability of the category

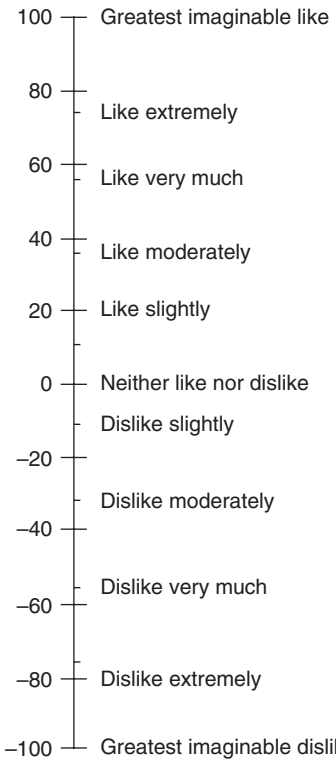

FIGURE 56.3 The Labeled Affective Magnitude (LAM) Scale (48).

scale was higher than for either form of magnitude estimation, although high reliability could be obtained with larger sample sizes. An ASTM method for conducting magnitude estimation has now been published (80).

b. Labeled magnitude scales

Although, magnitude estimation provides valid and reliable data on the sensory and hedonic attributes of food, in practice, it is difficult to use due to the complexity of instructions that are required and certain data analytic demands. This has limited its wide scale use. However, recent work in the development of labeled magnitude scales has combined the practical simplicity of category scales with the quantitative sophistication of ratio scaling for rating both the intensity and affective attributes of tasted stimuli. Green et al. (81) developed the first labeled magnitude scale of oral sensation by placing verbal labels of expressed intensity along a linear numerical scale at specific locations that reflect the numerical ratios among their perceived intensities, as obtained by a magnitude estimation procedure. A critical characteristic of this scale is the presence of a phrase involving the "maximal", "strongest imaginable" or "strongest possible" sensation. The use of this phrase is assumed to serve as a fixed end-point of sensation that places judgments of different subjects on a common sensory "ruler", a scale characteristic that is considered critical for valid sensory results (82–84). Subjects can quickly look at the verbal labels and corresponding numbers, and then place a mark (/) through the line scale to indicate the perceived strength of their sensation. The resulting data have been shown to have ratio properties (81).

More recently, Schutz and Cardello (48) have extended the use of labeled magnitude scales to ratings of consumer liking/disliking. Using the same approach as Green, et al. (81), Schutz and Cardello scaled the semantic meaning of verbal phrases used to describe the liking/disliking of foods. By locating these phrases along a visual analogue scale in accordance with their determined semantic meaning, they created the LAM (Labeled Affective Magnitude) scale of liking (Figure 56.3). As can be seen in Figure 56.3, the non-linearity in the scale is most evident in the middle and at the ends of the scale. During numerous applications of this scale, it was shown that consumers can use this scale to rate the affective aspects of stimuli with equal or better sensitivity, greater reliability and equivalent ease of use as the 9-point hedonic scale (48).

III. QUANTITATIVE TEST METHODS: OTHER AFFECTIVE MEASURES

A. Expected Liking Ratings

Another measure that has become popular in consumer acceptance research on foods, because of its usefulness to understanding the psychological factors that influence consumer food behavior is *expected* liking. Expected liking ratings consist of prospective judgments of the affect that is likely to be associated with a food or beverage. It differs from an affective rating made to a food name, which is primarily retrospective in nature, i.e. past experiences with the food are used to arrive at the liking judgment for the food name. In the case of expected liking ratings, the consumer is asked to assess his/her likely affective response to a food, usually in the context of some information that is presented about the food, e.g. its appearance, brand name, method of processing, etc. Expected liking ratings have often been made using the 9-pt hedonic scale, but any type of affective rating scale can be used. The application of expected liking ratings to consumer food behavior is discussed in section VIIC.

B. Relative to Ideal and Absolute Ideal Ratings

In certain product development situations, it is desirable to know how closely some sensory attribute of a food comes to being the "ideal" level for that product. Such a judgment requires the consumer to integrate an affective evaluation of liking with a sensory judgment of the intensity of the attribute of interest. In such cases, a relative-to-ideal or "just right" scale may be used. Figure 56.4 is an example of a relative-to-ideal scale that one might use to determine if a new formulation of lemonade has the appropriate sweetness

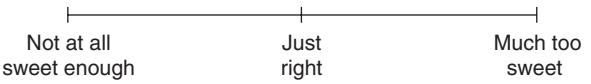

FIGURE 56.4 Relative-to-ideal or "just-right" scale for sweetness.

for consumers. Rather than have consumers rate the sweetness and liking of the product independently in two separate tests, the scale shown in Figure 56.4 can be used. As can be seen, the consumer rates the product from 'not at all sweet enough' to 'much too sweet' on either a linear graphic (shown) or other rating scale. The midpoint of the scale is labeled 'just right'. Such a scale implies that the respondent has an internal ideal for the product against which he/she can compare the sample of interest. Due to the need to have an internal "ideal" for the product, "relative to ideal" scales can only be used for well-established products. It is not possible to use such a scale to assess the ideal level of an attribute for a new or novel food.

A number of researchers have used relative to ideal scales (85–87) and studies have shown good agreement between these scales and hedonic scales. The breakpoint in the hedonic function, i.e. the point at which responses shift from increasing liking to decreasing liking, is highly correlated with the just-right point. Shepherd, et al (88) have argued that relative-to-ideal scales are 'unfolded hedonic scales', i.e., they represent the amount of liking of the product attribute in either direction from the just-right or "bliss" point of the scale. The bliss point in hedonic functions has been documented and studied since the time of Beebe-Center (89), and some investigators (90–92) have formulated psychological theories of food quality that focus on the deviation of sensory attributes from their ideal or bliss-point levels.

It is also possible to have consumers directly rate the ideal levels of all the sensory attributes of a product. In order to discern differences between this "ideal" product and a test product, difference scores are calculated for the attributes of interest. Absolute ideal ratings have been used successfully by a number of investigators (93–95), and strategies for their effective use have been reviewed (96).

C. Multiattribute Preference Ratings: Conjoint Analysis

A common market research tool that has recently been found to have many important applications in consumer acceptance research is known as conjoint analysis (97, 98). Since consumers respond to a combination of variables and levels of variables in making purchase decisions, this procedure allows consumers to make judgments of liking or purchase interest to a "bundle" of attributes. Consumers are presented with a number of conceptual products comprised of different combinations of selected variables and levels (a computer-selected main effects model is ordinarily used to keep the total number of judgments to a manageable level). Consumers rate the product concept on the dependent variable and statistical procedures are then applied that yield both a measure of the "importance" of each variable to the consumer and a measure of the "utility" contributed by each level of the variables. The resulting information allows predictions to be made concerning which product, packaging or other aspect of the product should receive the most attention in the product development and marketing processes in order to maximize consumer liking or purchase.

D. Tailored Questionnaire Items for Survey Research

In addition to the types of rating scales described above, consumer liking and other general or specific attitudes toward food are often assessed using a variety of survey questionnaire formats (99–101), e.g. multiple-choice questions, Likert response scales (agree-disagree), open-ended questions, etc. These techniques are more often used in market research to assess consumer opinions toward new food concepts or for exploring consumer food habits. The required considerations for the proper design, development and implementation of survey research include the design of reliable and valid questionnaire items, proper respondent sampling methods, choice of a viable data collection technique (mail vs. mall intercept vs. internet administration), and analysis of the impact of low response rates on survey validity. The details of these procedures can be found in a variety of standard texts (102,103).

IV. QUANTITATIVE METHODS: BEHAVIORALLY ORIENTED MEASURES

A. Food Frequency Ratings

One practical purpose of any measure of food acceptance is to predict consumer behavior toward the food. However, a simple measure of liking may not suffice to predict the behavior. For example, although a consumer may rate chocolate ice cream an "8" and bread a "5" on a 9-point hedonic scale, this does not mean that the consumer wants to eat chocolate ice cream more often than he/she wants to eat bread. Thus, hedonic judgments of the acceptability of foods are not always a useful tool for menu planning or for predicting food choices. For these purposes, consumer judgments of the desired frequency of consuming the product may be more useful.

Schutz (104) developed the first food frequency scale as a supplement to the 9-point hedonic scale. Known as the FACT (Food Action) rating scale, it consists of a series of statements about the desired frequency of eating an item (Table 56.2). By its nature, the FACT scale provides relative frequency estimates. However, absolute frequency

TABLE 56.2
The Food Action (FACT) Rating Scale (104)

- Eat Every Opportunity
- Eat Very Often
- Frequently Eat
- Eat Now and Then
- Eat If Available
- Don't like, Eat on Occasion
- Hardly Ever Eat
- Eat If No Other Choices
- Eat If Forced

measures can also be obtained by having consumers indicate the number of times during a 30-day period that they would like to consume the item (105). High (>.90) group reliability coefficients have been found for both types of scales (104, 106), while individual reliability coefficients for the latter scale range from .58 to .69 (106, 107). In terms of their ability to predict actual consumer behavior, the correlation of ratings on absolute food frequency scales with food consumption has been reported at .59-.66 (108).

B. Situational Appropriateness Ratings

Appropriateness ratings of food were developed to measure the degree to which a food is appropriate or inappropriate for specific situations or uses. Schutz (109, 110) developed the concept of "situational appropriateness" using a simple category scale that ranges from "never appropriate" to "always appropriate". This scale has been used effectively in a variety of survey formats, and more recently has been shown to be a valuable adjunct to laboratory affective testing of foods (111, 112).

The development of a situational appropriateness scale was one of the first attempts to measure the role of context on consumer food behavior. It is often an overlooked fact that people do not consume foods simply on the basis of how well they are liked or disliked. We eat more or less of foods because it is the right time to eat (breakfast, lunch, etc.), because of health reasons, social constraints, or any one of a number of other factors that are related more to the situation and context of the dining occasion than to any intrinsic aspect of the food itself. This is one important reason why liking ratings account for only about 25-50% of the variance in consumption, as previously noted.

C. Satisfaction Ratings

Another type of attitudinal rating commonly used in consumer research on foods is satisfaction ratings. Satisfaction ratings can be obtained using category, linear graphic, labeled magnitude, or other types of rating scales. In practice, satisfaction ratings are used to assess the post-purchase or post-consumption attitude of the consumer toward a product. When applied to food products, the satisfaction dimension integrates the affective response to a broad range of product attributes. These include, not only its taste quality, but also its price, suitability, convenience, nutritional and health benefits, etc. Moreover, satisfaction with the product is considered to be a function of the consumer's expectations about the product and has been shown to be related to the degree of disparity or disconfirmation between the expected characteristics (or liking) of the product and its actual characteristics (113, 114).

In a recent study, it was found that satisfaction ratings for foods were better predicted from a combination of the expected liking of the food, its appropriateness for the eating situation, and the consumer's preference for the food item than by either liking/disliking ratings or actual consumption (55). Based on these data it appears that the concept of satisfaction takes in a wider range of variables influencing the consumer than simple affect and should be used in those situations where the researcher is interested in the effect of the full range of intrinsic and extrinsic variables on consumer behavior.

D. Behavioral Intent Ratings

One of the traditional methods used by market researchers to predict whether consumers are likely to purchase a particular food is to use a form of "behavioral intent" rating (115). These are ratings by the consumer of his/her future likelihood to behave in some way toward the product. A simple form of behavioral intent measure to quantify purchase intent would utilize a probability-like scale, e.g. "I do not plan to purchase" to "I definitely plan to purchase". Another more sophisticated type of behavioral intent method is known as the "constant sum" technique (116). In this method the consumer is asked to distribute some number of points, e.g. 10, among a set of alternative products or product descriptions in order to reflect the likelihood associated with purchasing each item during their next shopping trip. Unfortunately, neither of these methods is highly predictive of actual purchase behavior. However, they can be used successfully to screen out the best and worst products within a group, and they can serve as a useful adjunct to affective ratings of the products.

E. Purchase Behavior

A more direct behavioral measure of the degree to which a consumer likes or dislikes a product is whether or not he/she actually buys it. Purchase behavior is an important index of liking/disliking for a product, with special relevance to food marketers. Purchase measures include dollar sales, unit sales, and market share. Dollar sales reflect total revenue to the manufacturer and those in the distribution chain, while unit shares index the number of items that consumers have purchased. Market share is a relative index of the manufacturer's sales volume relative to that

of competitors. Of course, pricing heavily influences all purchase indices, so that a very well liked item may not be purchased if it is priced too high, whereas a product that is only slightly liked may be purchased over the highly liked item if it is priced sufficiently low. For this reason, purchase behavior can only serve as a rough approximation of how well liked is the item. The reader interested in measures of purchase behavior should consult standard sales and marketing texts (103, 117).

F. Consumption

Perhaps the most meaningful index of whether or not a consumer likes or dislikes a product is whether or not he/she eats it and how much of it he/she eats. Unfortunately, food consumption measures are difficult, time-consuming, and costly to obtain. To measure food consumption requires that the amount of food eaten by the consumer be quantified by weighing the total amount of food served to the individual, the amount that is left after eating (plate waste), and then subtracting the latter from the former. Less exacting methods require that a visual estimate be made of the amount eaten (as a percent of total served) or that food diaries be maintained. The latter methods are fraught with problems associated with the accuracy of self-reported data. In addition, the hunger or satiety level of the individual influences all consumption measures. Very well liked items will not be consumed if the consumer is full, whereas relatively disliked items may be consumed when the individual is very hungry. As such, consumption measures only serve as an approximation of how well liked or disliked is a food item. It was exactly because of the great difficulty involved in measuring human food consumption that early researchers opted to develop attitude measures to assess liking/disliking of food (30) and to use these measures as predictors of food consumption. Readers interested in further discussion about measures of food consumption are referred to several recent reviews of the topic (118–121).

V. QUALITATIVE CONSUMER TEST METHODS: INTERVIEWS, FOCUS GROUPS

In addition to the quantitative measures described above, a number of qualitative consumer techniques have been developed for use in food acceptance and marketing research. These methods primarily address issues of a creative nature and the motivational factors that drive consumers' purchase behavior. They may be considered exploratory in nature as compared to the confirmatory nature of quantitative consumer research methods, and often allow the investigator to explore more deeply the factors that underly consumer behavior.

A. Personal Interviews

Borrowing from the discipline of psychology, in-depth, personal interviews with consumers can yield valuable information about consumer attitudes and dispositions toward foods. However, this technique has low external validity because the time and effort involved in personal interviews usually results in small numbers of respondents and is unlikely, therefore, to provide representative data about the consumer population to which the investigator would like to generalize. However, personal interviews can be used quite effectively to generate hypotheses that can later be tested using large-scale, quantitative surveys. Recently, personal interview techniques have seen somewhat greater use in consumer acceptance research, due to their use in "laddering" techniques (122) that seek to uncover the cognitive structures that underlie consumer perceptions and behaviors toward foods. Such factors as the use of standardized questionnaire protocols and interviewer training and consistency will affect the reliability and validity of interview information. However, personal interviews do allow for a level of detailed questioning and understanding that is not practical or possible with other, more quantitative techniques, such as written surveys.

B. Focus Groups

Focus groups are a form of group interview. Ordinarily from 8–12 respondents are selected from the community representing some relevant component of the marketplace, either demographically and/or on the basis of product usage. The sessions are held either in homes or in central testing facilities that are designed with one-way mirrors and sound/video recording equipment. A moderator or facilitator who has been trained in group dynamics and who leads the group discussion according to a predetermined script of topics leads the sessions. A typical focus group lasts from 1–2 hours.

Information from focus groups can be used to develop and/or evaluate product packaging concepts, advertising themes, and to help develop appropriate questions for quantitative surveys. Focus groups can also be conducted as a follow-up to a quantitative survey in order to help understand the results (this is typically done using respondents who represent those consumers who either liked or disliked the tested product). The interested reader should refer to other published papers for further details on the use of focus groups in consumer food research (123, 124).

VI. CONDUCTING CONSUMER TESTS: WHO, WHERE, AND HOW

A. Locations for Testing

The location for performing a consumer test ranges widely, from the laboratory to central locations (churches,

shopping malls, etc.), field sites (especially for institutional foodservices), and even the homes of consumers. The choice of which location to use is partly a function of cost and partly a function of the research objective of the study, i.e. whether the objective is exploratory or confirmatory in nature. For simple exploratory or research guidance tests using a small number of respondents, the laboratory is an appropriate choice. As the need for greater external validity arises, such as when significant resources are to be expended based on the results of the study, it is important to have a location that allows for a greater number of respondents and for more normal consumption conditions. As might be expected, as one moves from the laboratory to the field, the opportunity for controlling testing conditions diminishes, making the need for a larger number of respondents necessary to reach statistically significant findings. The appropriate tradeoff is a management decision that must be made during the test-planning phase.

B. Control of Sample Presentation

The presentation of samples in consumer tests should be made in such a way as to minimize the bias produced by the order of testing. This ordinarily means that samples are presented unlabeled, in a random or balanced order, and identified with a non-biasing, e.g. random three-digit, blinding code. The size and temperature of the samples presented is a function of the amount of product that is available, the total number of different products, and the degree to which the researcher wishes to simulate actual eating situations. Ideally, a normal serving size and temperature would be used. However, the problems of sensory adaptation, post-consumption satiety, and practical time constraints usually lead to compromises, so that interpretation of the results must take into account the specific nature of the serving conditions. The location of testing heavily interacts with these decisions, since what is feasible in the laboratory or in the home will argue for one type of presentation over another. In general, it is only possible to approximate the actual consumption conditions, since samples are rarely presented as part of a meal situation. If only food concepts are to be evaluated, such as in conjoint analytic studies, computer presentation can also be utilized.

C. Control over Ambient Conditions

As with sample presentation, control of ambient conditions is dependent upon the testing location. In the laboratory a great deal of control can be gained over lighting, temperature, noise, etc. Figure 56.5 shows a typical laboratory consumer testing situation, in which a consumer is seated in an individualized sensory testing booth that is light, sound and temperature controlled. Food samples are presented to consumers through openings from a kitchen area, which minimizes cooking odors and any visual bias from preparation procedures. The consumer has access to rinse water and a computer terminal/keyboard for viewing questions and entering responses. Of course, less control is possible in central location sites, and control may be minimal in home-use tests or in actual field locations. The consequences of this lack of control will vary depending on the nature and differences among the products evaluated. However, in all cases, it is appropriate to exercise as much control as possible over testing conditions to maximize the likelihood of finding statistically significant and meaningful differences among products. Again there is a tradeoff between being sensitive to possible differences and representing actual eating situations. (The reader is referred to Chapter 57 for further details on sensory testing facilities, controls and procedures.)

FIGURE 56.5 Consumer panelist judging the liking/disliking of foods in a computerized sensory testing booth.

D. Selection of Respondents

The ideal test population for most consumer research is a cross-section of marketplace consumers of the product within the geographical area in which the product is marketed. However, in practice, this target population may be difficult to access. In general, the most common types of consumer groups utilized in testing are 1) shoppers in supermarkets or malls in the geographical area of interest, 2) residents of the local community who volunteer to test products at home or at central test locations and who have been screened to meet certain demographic, psychographic or use criteria, and 3) random samples of employees working in a research or manufacturing facility where the testing is to be conducted. Such consumer samples may be highly stratified and selected to match exacting norms of the target population, e.g. age, gender, education, income, geographic residence, ethnic origin, religious affiliation, or employment, or they may be simply convenience samples, chosen for their ease of access and their presumed similarity to the target population.

Lists of respondents may be obtained from commercially available compilations, telephone directories, and random telephone numbers. Whatever source or method is used, limitations on true randomness or representativeness should always be considered in evaluating the results of a consumer test. Also, in all cases, the individuals must be untrained in sensory methodology and users of the product or product category being tested. A minimum of 50 consumer panelists is required for most consumer testing applications, although the number may be considerably larger when important decisions are to be based on the obtained data (125). The choice of respondents also depends in part on the location of the test. It is not uncommon to simply have a convenience sample in a shopping mall intercept study. In some instances a quota sampling procedure is used so that the number of respondents in each category of interest is represented in a predetermined fashion.

E. Internet-Based Testing

For the evaluation of product concepts, use of the Internet has recently become quite popular (126). Respondents can be obtained from a variety of commercially available sources. Important considerations in the conduct and interpretation of data collected in this manner is the length of time subjects will be willing to spend and the possible non-representativeness of those who have internet access and who volunteer for such testing.

VII. FACTORS INFLUENCING CONSUMER ACCEPTANCE

A. Situation and Context

The study of consumer food acceptability is complicated by the fact that foods are typically eaten within a specific situation and context. The situation and context may include a variety of environmental factors, e.g. where and under what physical conditions the food is eaten, social and cultural factors, e.g. with whom and for what purpose the food is being consumed, and other factors related to the meal context, e.g. what other foods and beverages are being consumed at the time. Such influences can have a profound effect on consumer ratings of the acceptability of foods. Almost all laboratory studies of the consumer responses to food attempt to limit these influences by exerting great control over these "extraneous" variables. This historical approach of conducting sensory consumer research in a sterile laboratory environment has led some investigators to call for more testing of foods in realistic contexts and situations (127, 128).

One obvious factor that can influence consumer liking of foods is the ambient environment in which the food is presented. A cold beverage on a hot day seems to taste better than the same beverage on a cold day. This phenomenon is often explained as being the result of "alliesthesia", a theoretical mechanism by which the perceived pleasantness of a stimulus is influenced by its usefulness to the body, as determined by internal bodily states (129). The effects of alliesthesia have been found for taste, olfactory, and thermal sensation (129–131), and the general conclusion to be reached from these studies is that bodily deprivation levels can modulate the perceived acceptability of food.

Consumer researchers have also studied the influence of a number of situational and contextual variables that operate in naturalistic dining situations for their effect on food acceptance and consumption (132–138). These variables have included the physical dining environment, meal variety, meal cost, and the effort required to obtain and consume the food. Almost all such variables influence consumption of the food, while some also influence acceptance. For example, the effort required to obtain food has been shown to have a large effect on consumer consumption (139–142). However, changes in acceptance have been less reliable (142). Changes in price influence both selection rates and acceptance (143). Further studies have identified a major difference in acceptance ratings of foods depending upon where the foods are served, e.g. in institutional or non-institutional foodservice settings. The same foods served in a pleasant sit-down restaurant environment are better liked than those served in a less pleasant environment, e.g. in a cafeteria (144). In the case of food variety, a number of studies have shown dramatic effects on both food acceptance and consumption in cases where food variety is limited (145–148). The general conclusions that have followed from these studies are that food liking and consumption in a natural eating environment are greatly dependent upon "the situational variables which make it more or less *convenient* for us to eat and which signal mealtime" (136).

B. Cultural and Social Influences

Social and cultural influences can also play a significant role in influencing consumer perceptions and liking of food. One only needs to consider the strict dietary avoidance of pork by Jews and Muslims, the avoidance of beef in India, or the avoidance of eggs by certain African tribes (149) to gain an appreciation of the influences of culture on food likes and behaviors. These differences can also be based on regional variations within the same country. For example, brown eggs are preferred in the Northeastern United States, but in few other areas of the country. Other social and cultural factors, such as income level and status in society can also influence liking/disliking toward certain foods, as reflected in the gourmet and health food preferences of many "upscale" consumers. Other social effects on consumer liking of foods derive from the social influences of parents, peers, leaders, and heroes. For example,

mother-daughter and father-son food preferences are much more alike than those of mother – son or father-daughter (150). Other social imitation effects on food acceptance have been well documented (151, 152) and are the basis for the numerous food advertisements that rely on celebrity endorsements of a product.

C. Information

Information can also have a powerful influence on consumer liking and behavior toward food. Such information can take various forms, including brand and label information, ingredient information, nutrition and health claims, information about processing and preservation techniques, and various forms of product advertising. These influences of information are sometimes referred to as "framing" effects, because they are sensitive to the specific context and wording of the information (153, 154). One hypothesis of how information may come to influence affective ratings of food is through their influence on consumers' expectations of liking. This hypothesis is an extension of expectancy — value theories of consumer behavior that posit that value judgments result from a comparison of the expected performance of a product or service with its actual performance. Thus, the degree to which the product or service confirms or disconfirms expectations about it, will determine the degree of consumer affect. In the marketing and consumer psychology literature, there are a number of models to predict the effect of disconfirmed expectations on perceived product performance or satisfaction. These models have been reviewed and extended to issues of food acceptance by Cardello (113) and Deliza and MacFie (114).

Over the past 25 years, the bulk of empirical research on the role of disconfirmed expectations on product performance has supported an assimilation model (155) of these effects (156–159), that is, consumer acceptance increases in those cases where the expectation for the product is high and decreases in those cases where the expectation is low. Support for such a model has been found in several food-related studies. Figure 56.6 shows data from one such study on cola beverages (160) in which subjects were led to expect that they would receive either their preferred brand of cola beverage or a non-preferred brand (as determined in blind taste tests). In some

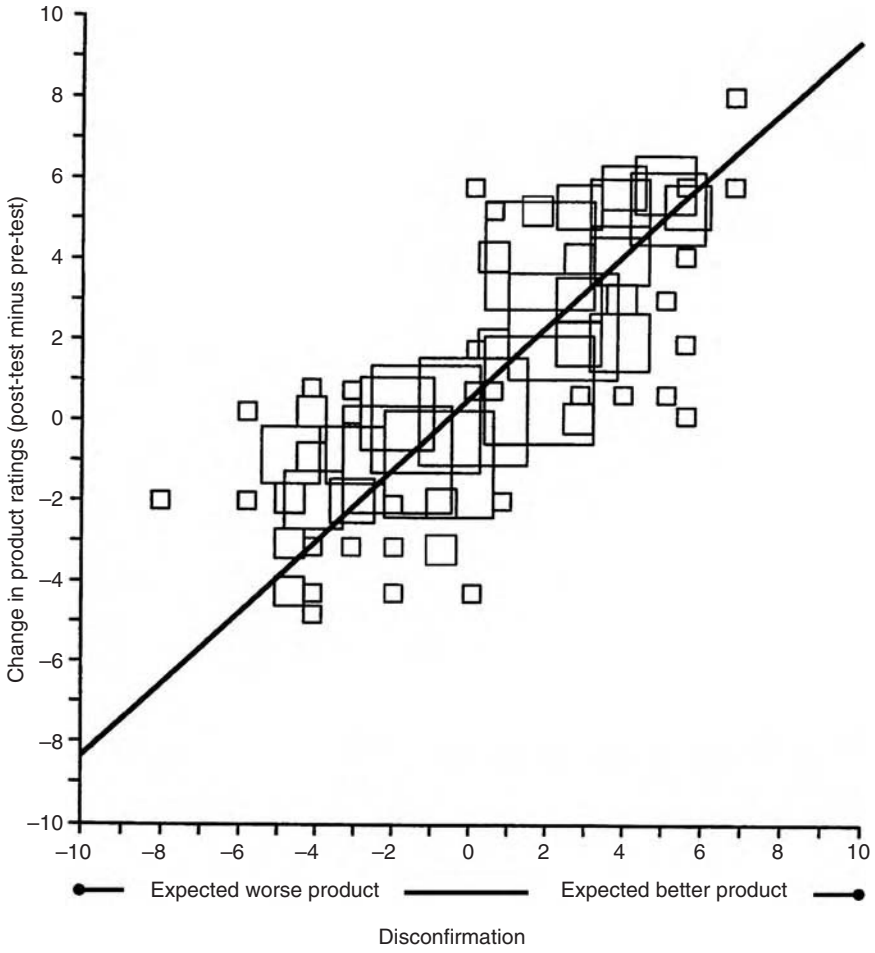

FIGURE 56.6 Consumer data showing how product liking increases as product expectations increase (160). (The size of the boxes reflect the number of data points falling at that location.)

cases, subjects were given the cola that they expected, while in other cases they were given either a *more* preferred or *less* preferred brand. Pre-trial ratings of excepted liking and post-trial ratings of actual liking were obtained from all subjects. Figure 56.6 shows the change in product liking from the baseline level as a function of the direction of disconfirmation experienced by subjects, i.e. whether they expected a better or worse product relative to the one they received. As can be seen, there was a strong linear association ($r^2 = 0.66$) in the data, showing that post-test liking moved in the direction of the expectations. Similar results to these have been found in studies where expectations were manipulated by varying fat-content information (59) and in studies with yogurt (161) and orange juice (162, 163). Similar effects have also been reported using consumption as a dependent measure of product acceptance (164).

D. Perceptions of Risk

With recent advances in food processing techniques, one category of foods for which liking is especially susceptible to the influence of information and other cognitive factors is foods processed by novel and emerging technologies. Like most new products, optimizing the sensory quality of these foods is critical to their success in the marketplace. However, optimal sensory quality, on its own, will not guarantee success. The reason for this is that information about the nature, source, or processing of the food can have a profound effect on consumer liking, choice and purchase decisions. This is especially true when the nature, source or processing of the food is perceived by the consumer to impart some risk to its consumption. e.g. irradiated and genetically modified foods.

The study of consumer risk perception began in the early 1970's with the pioneering work of Starr (165), Fischhoff et al. (166), and Slovic et al. (167). Prior to this time, the risk associated with a new technology was based on expert analyses of risk. These analyses were based on actuarial data related to accidents, fatalities, or losses accountable to the technology. However, this *technical/rational* approach to risk was often found lacking when it came to predicting consumer reactions to novel technologies. Frequently, consumers had great concerns about technologies that had relatively low risk from an objective, i.e. technical/rational, risk analysis standpoint. In response to these discrepancies between consumer perceived risks and expert opinions of risk, researchers adopted a new paradigm for risk perception. This new *normative/value* risk model defined risk as a perceptual construct, one that must be evaluated by lay people on the basis of *subjective* evaluations of risk. In keeping with this focus on perception, the study of risk has adopted many of the same univariate and multivariate psychometric techniques commonly used today to assess consumer perceptions of the sensory qualities of foods.

In a comprehensive study of consumer risk perceptions (168), a factor analytical approach was used to determine the perceived risk associated with a wide range of everyday hazards, including many "technological" hazards. Several of the characteristics found to be important to perceived risk included the degree of control that the consumer has over the risk, whether the risk is voluntary or involuntary, whether the risk is known or unknown, fatal or non-fatal, observable or unobservable, and whether the effects of the risk are immediate or delayed. These hazard/risk characteristics exemplify the kinds of cognitive variables that must be considered when assessing consumer perceptions and acceptability of foods processed by novel or emerging technologies.

E. Consumer Risk Perceptions about Foods Processed by Novel Technologies

Although the mainstream literature on risk perception has served to identify critical variables important to consumer risk perception for many technological and non-technological hazards, consumer perceptions of risk for specific food hazards have been left to other investigators. Over the past several years, numerous investigators have utilized the normative/value approach in surveys and studies that have assessed the perceived risks or "concerns" of consumers toward such specific food safety issues as irradiated foods, genetically engineered foods, foods containing pesticides, foods processed using laser light sources, and microbially contaminated foods (169–188).

In a recently conducted study (189), the effect of consumer concerns and expectations on liking for foods processed by different food processing techniques was examined. In this study, consumers tasted and evaluated chocolate pudding that had been thermally processed. Subjects rated their liking of chocolate pudding (no other information presented) and their level of concern for several different food processing technologies ("irradiation", "high voltage pulses", hydrostatic pressure", "pulsed electric fields", "non-thermal preservation", and "the addition of bacteriocins." Subsequently, they were provided information about the chocolate puddings that they would taste. In one condition, consumers were told that the product they were about to taste had been processed by one of the six food processing technologies, e.g. "this sample was processed by pulsed electric fields". In a second condition, the name of the technology was accompanied by an objective description of that technology, e.g. "in irradiation processing, foods are exposed to a source of ionizing radiation, e.g. cobalt 60, for short periods of time". In the third information condition, consumers were told the name of the technology, given the description of it, and provided a safety benefit statement, i.e. "this process is entirely safe and avoids the thermal damage done to foods by heat pasteurization". After exposure to the information manipulation, consumers rated their expected liking of the product, before and after seeing it, and were then presented the sample to taste and rate for liking.

Results showed a large gender difference, with females showing much greater concern for all the food technologies, than males. Of greater overall importance however, was the fact that correlations of "concern" ratings for the different food processing technologies with expectations of liking for the chocolate pudding that the consumers believed had been processed by that technology were all negative and highly associated for the 3 conditions. These results revealed that as consumers' concerns about the risk of a technology *increase*, expected liking of foods processed by that technology *decrease*. Still more significantly from the perspective of marketing these foods, the diminished expectations were shown to result in systematic decreases in rated liking for the tasted products. On the positive side, this research also demonstrated that factual product and process information, product exposure (merely seeing the product), and a safety/benefit statement all had positive effects on expected liking and acceptance of the tasted product. The data confirm the fact that good communication practices and effective marketing strategies can be utilized to overcome negative images created by risk perceptions or product stereotypes and to significantly improve the probability of consumer market success for foods processed by emerging technologies.

VIII. FUTURE DIRECTIONS IN CONSUMER RESEARCH

As the food industry continues to accelerate its development of novel ingredients, processes and end-uses for foods, so too will the need for new and novel approaches for measuring consumer acceptance accelerate. The dramatic challenges to consumer acceptance that new food processing technologies have raised to date will undoubtedly grow as food science continues to push the frontiers of food processing and preservation. These developments will require new and more powerful methods for assessing the social, psychological, health, and values-based aspects of these new technologies on consumers' liking and willingness to purchase these future foods. In addition, much greater reliance on novel consumer acceptance technologies will likely result from the opportunities that the Internet and other web-based interactive technologies provide for obtaining access to large numbers of consumers with relatively little cost or time-investment. The joint contribution of these influences (advances in food science and web-based testing) will continue to keep an important focus on the theories and methods required to conduct effective consumer acceptance research on foods.

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57 Sensory Science: Methodology

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CONTENTS

I. INTRODUCTION AND OVERVIEW

Measuring the sensory properties of foods and relating these properties to consumer acceptance is a major objective and accomplishment for sensory evaluation. These achievements are possible as a direct result of advances in the science, development of valid and reliable methods, education of research and application professionals, development of cost efficient and well run sensory programs, and institutions and commercial companies that support these programs. The latter is directly related to the expected and realized commercial benefit of sensory tests.

This chapter provides the reader with an overview of sensory evaluation, its uses and limitations. We highlight important developments, current thinking and the current status of the science. The interested reader will find more detail in several books published on the topic, most notably, Lawless and Heymann (1), Stone and Sidel (2), and Amerine, Pangborn, and Roessler (3).

A. Historical Background

The food industry, similar to other consumer product industries, traditionally viewed sensory evaluation in the context of the technical "experts" who through education (e.g., learning to judge the quality of dairy products as part of dairy science education) and years of accumulated experience were able to describe company products and set standards of quality by which raw materials would be purchased and products manufactured and marketed. Three noteworthy developments led companies to evolve away from reliance on the single technical expert, and to reliance on formal sensory panels and procedures. First, products became more complex, and technical experts were less able to predict consumer acceptance. Second, companies could not wait the 2 or more years for an "apprentice" to assume evaluation responsibilities. Thirdly, the emerging field of sensory evaluation was demonstrating the need for, and value of, formal, unbiased, and statistically relevant procedures for qualifying subjects, evaluating products, and communicating actionable results.

During the 1940s and 1950s, sensory evaluation received additional impetus through the U.S. Army's Quartermaster Food and Container Institute, which supported research in food acceptance for the armed forces (4). It became apparent to the military that adequate nutrition, as measured by analysis of diets or preparation of elaborate menus, did not guarantee food acceptance and consumption by military personnel. The importance of the sensory components of food was acknowledged, and resources allocated to studies for determining how best to measure food acceptability. Several psychologists were hired by the military to develop methods for measuring food acceptance, and this practice was continued when the Army's food research laboratories were moved to Natick, MA in the 1960s. The original group of psychologists chose to not participate in the move to Natick. They disseminated their knowledge and interest in sensory evaluation further into the food industry by joining product companies (e.g., Coca Cola, Pillsbury, and General Mills), contract research organizations (e.g., Battelle Memorial Institute) or founding research companies (e.g., Peryam and Kroll Corporation).

Government and industry interest in sensory evaluation encouraged additional developments. For example, in the 1950s the Arthur D. Little Company introduced the Flavor Profile Method (discussed below), a qualitative form of descriptive analysis that did not rely on an individual expert, and formalized subject screening and training procedures. Private contract research companies continue to play an important role through sponsoring workshops, developing proprietary methods, assisting product companies establish internal sensory capabilities, and conducting research studies.

By the mid-1950s, a few universities were offering a series of courses in sensory evaluation; most notably the University of California at Davis (UCD). Many students from the U.S. and abroad introduced the science into companies, while others went on to teach the science at the university level. Currently, a wide range of universities and technical institutes worldwide offer programs in sensory evaluation, and in 2002 UCD initiated a sensory course online: "Applied Sensory Science and Consumer Testing Certificate Program for Distance Learners."

Also during the era of the 1950s and 1960s, various technical and scientific societies organized activities and published procedures focusing on sensory evaluation and the measurement of flavor. These included Committee E-18 of the American Society Testing and Materials, the Food and Agriculture Section of the American Chemical Society, the European Chemoreception Organization, and the Sensory Evaluation Division of the Institute of Food Technologists.

B. Defining Sensory Evaluation

The Sensory Evaluation Division of the Institute of Food Technologists defined sensory evaluation as "a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing" (5). This definition emphasizes several important points, including 1) the scientific focus of the discipline, correctly distancing the discipline from art and opinion; 2) although implied and not directly stated, the foods and materials represent physical, rather than cogitative, stimuli; 3) implicit in the definition is the concept of the measurement and analysis of perceptions from information obtained through the five senses; and 4) the definition forwards a distinction between the perceived characteristics of foods and materials and their actual

physical properties. To this latter point, the definition focuses on the importance of perception for understanding the impact of a product's physical properties; implicit in this is the notion that in sensory evaluation "perception is reality." This has enormous implications on determining how subjects are selected, and, when necessary, trained, what sensory evaluation should measure, and the measurement method.

The evolution of sensory evaluation as an independent discipline in the 1940s and 1950s produced unique methods for determining difference. These and other sensory method developments are a reminder that although they have a common lineage, psychophysics and sensory evaluation have very different objectives. Sensory evaluation focuses on measuring the responses of people to products, and application of findings to action-oriented decisions about products. Psychophysics focuses on the measurement process and explaining the behavioral mechanisms behind sensory responses. Both objectives are important, and each has its own criteria for success.

C. Classification of Sensory Methods

There are several sensory methods, differentiated primarily by objective, measurement procedure, subject qualification, and various combinations of these factors; and new methods continue to be developed. As reported in Stone and Sidel (2), sensory tests are classified primarily by type of test in a three-category classification system, which are: discriminative, descriptive, and affective tests. (We view discriminative and descriptive as analytical tests and thus could consider two categories; analytical and affective tests.) There are several different tests within each class, and they are described in greater detail later. Discrimination tests measure whether there is a perceived difference between products, descriptive tests measure perceived attributes and their intensities for a product, and affective tests measure product acceptance or preference. Affective tests are discussed in the current handbook under the topic of Consumer Testing.

D. Sensory vs. Instrumental Testing

The development of instruments to measure food and beverage characteristics such as appearance, aroma, taste, and texture has a very long history, as has research into correlating instrumental and sensory measures. Interest in this relationship in the food and beverage industry is based in part because it represents a potential means of reducing reliance on the human judgment, which some view as variable and subjective. Value, efficiency, and economy are possible where instrumental measures can be effectively substituted for sensory measures. This is especially true in product quality assessment.

An extensive literature is available describing sensory/instrumental relationships for texture measurement, where mechanical devices for measuring texture have been described in the literature for more than a century. Bourne (6) provided an excellent review of the subject describing mechanical devices to measure product changes, particularly the fruit ripening process, or to simulate the chewing process to measure meat tenderness. The interested reader is also referred to the *Journal of Texture Studies*, especially the publications of A.S. Szczesniak and her collaborators. While such mechanical devices have undergone substantive change in terms of sensitivity, measurement, design, etc., the basic principles have remained unchanged, to wit, creating a mechanical device to measure what humans perceive.

The main difficulty in simulating sensory behavior by an instrument is due to the complex simultaneous activity ongoing in the mouth during eating and drinking. The upper jaw remains stationary while the lower jaw is capable of several different motions to hold, bite, tear, and masticate. At the same time, the highly muscled tongue gathers, moves, and compresses stimuli, the salivary glands contribute moisture and lubricant to the process, and swallowing provides control of volume in the mouth while momentarily limiting intake to the mouth. To our knowledge, this multidimensional activity cannot be reproduced with current instrumentation.

The history of odor detection has always included both human and instrument systems. The earliest instruments began with measuring total volatiles. This was followed by development of chromatographic techniques and gas-liquid chromatography (GLC) which enabled separation of chemicals as well as ability to separate specific chemicals at very low concentrations; e.g., ppm (parts per million), ppb (parts per billion) and less, to facilitate their identification. Subsequent developments enabled integration of the chromatographic separation with even more sophisticated instrumentation such as mass spectroscopy to more precisely clarify chemical structure, and so forth. Amerine et al. (3) describe limitations and early efforts to combine GLC with sensory panel results. Volatiles from foods are trapped and collected for sensory evaluation. Later refinements included development of equipment containing multiple odor ports that delivered the separated odorant at prescribed dilutions simultaneously to sensory panel members. Gas chromatography-mass spectrometry are used to analyze perceived odors, and Noble (7) described the use of instrumental analysis for the sensory properties of foods.

During this same time period, Schutz, Veley, and Iden (8) and Wilkens and Hartman (9) described their efforts to devise other systems for the detection of odorants. Schutz and co-workers investigated use of specific enzyme systems associated with olfactory tissue that were reactive to odorants and correlated results with the physical and chemical properties of those odorants. At the time, the constraints of separation technology, enzyme stability, and

related issues impeded further development of this approach. Wilkens and Hartman, on the other hand, took a very different approach to the problem; they devised an instrument, an electronic analog that relied on microelectrodes made from combinations of metallic wires for the detection of odorants. These authors were able to demonstrate differential response sensitivity to various volatile chemicals with just a few microelectrode metal combinations. Today's "Electronic Noses" and "Electronic Tongues" represent significant advances in electrode specificity, sensitivity to individual chemicals, use of computer chip technology for analyses, and in accessing pattern recognition and neural network statistics as an aid in data interpretation (10, 11, 12, and 13). Bleibaum et al. (11) concluded from their research "the electronic tongue and electronic nose, in combination, can be used to predict the sensory characteristics and their relationship to the quality of apple juices measured by consumers." The degree to which these odor and taste detection instruments, or those yet to be developed, are incorporated into routine product development and quality control activities will depend on how well they correlate to important sensory measures, and are economical and safe to operate in those respective environments.

Correlating sensory flavor attributes and chemical measures has become almost routine in research and quality control, as has correlating sensory and Hunter color measures. Instrumental and sensory correlations also have been described for visual clarity and turbidity (14).

Before leaving this topic, we are reminded that sensory information is uniquely different than information obtained from a physical instrument (a point often overlooked when creating a mechanical system to substitute for a physiological system). It represents the relatively simultaneous integration of information from all the senses, in conjunction with cognitive factors that include learning and expectation. In this sense, sensory information is multidimensional whereas instrumental information is almost entirely one dimensional. Although we can instruct subjects to not pay attention to certain attributes, it is false to assume that those attributes do not influence the subject or his response. Instruments can be useful in sensory evaluation when there is a good understanding of the relationship between those measures, sensory perception, and consumer behavior. At present such relationships are the exception, and considerably more needs to be accomplished before instruments represent a practical alternative to sensory measures.

II. SENSORY EVALUATION FACILITIES

The typical sensory evaluation facility in a R&D center consists of separate areas for testing, panel training, and administration. Satellite facilities are sometimes required to accommodate testing that cannot be accomplished in the immediate vicinity of the research center.

A. Testing

A product evaluation area typically consists of the following three functional areas, adjacent to one another and separated by walls and doors for easy communication and passage. This area is best located for its ease of accessibility to the subjects and for its low level of odors, noise, and vibration associated with more intense R&D and manufacturing activities.

1. Reception Area

The reception area is typically a well-lit and comfortable room located off a main corridor, and offering easy access to the test area. It is equipped with a receptionist desk and chair, telephone for contacting subjects, comfortable chairs where subjects are either seated while receiving instructions or where they wait prior to proceeding to an assigned sensory booth. A blackboard or bulletin board is located on a wall near the receptionist desk and identifies the product category and number of products that are to be tested in the session. A table is also provided for those situations where subjects are offered a reward, or "treat" (juice, coffee, cookies, candy, etc.), immediately following participation in a test. The room should allow easy access to, yet visual separation from, the sensory booth area to minimize distraction from early arriving subjects to subjects still seated in the sensory booths. The dimensions for the reception area depend on the number of subjects it has to accommodate prior to their proceeding to the sensory booths.

2. Test Kitchen Area

The test kitchen area and materials used in this area typically represent a well-equipped commercial, or heavy duty home, kitchen. The area must accommodate preparation and cleanup equipment, equipment and supply storage, product preparation, holding and serving activities, and workstations for kitchen staff. One or two large central islands for product preparation, showings, and holding for serving are recommended, and these must allow for unencumbered foot traffic flow around them during product preparation and serving. Airflow from this area is an important consideration, and food preparation aromas from here must be exhausted away from the sensory booths. Serving counters need to be high enough to minimize posture stress during serving, and wide enough to accommodate products, supplies, and scorecards for subjects seated in sensory booths with pass through into in the serving area. All under- and above-counter space should be utilized for storage, and an adjacent storage room is recommended where possible. A door from the kitchen to the reception area will facilitate any needed communication between the receptionist and kitchen staff, and provide easy access to treats and materials needed to clean and maintain the reception area. Telephones in this area should be equipped with a

visual alert, so that the auditory alert may be turned off during testing.

3. Sensory Booths

Individual sensory booths need to minimize visual and other potential distractions to the subjects from one another, from the kitchen, and from the reception area during the evaluation process. Dimensions, surface colors and materials, lighting, ventilation, pass-through structure, expectoration system, signal system, and direct data entry systems must be considered when designing sensory booths for food research. Clean filtered air should flow into the booth area, and air should flow from the booths to the kitchen, and not the reverse. The interested reader will find useful booth design information in several texts, and is directed to Eggert and Zook (15) and Stone and Sidel (2) for additional detail. Most sensory booths are permanent structures; however, temporary booths can be constructed from sturdy coated art board or plywood, and these can be made collapsible or foldable with hinges or fitted slots for portability. Temporary booths can be moved to off site testing locations.

B. Panel Training

Open discussion is required at various times during panel training, and these discussions can be held in a conference room, focus group facility, or a dedicated training room. The room should be proximal to the test kitchen or product preparation area, be readily accessible by subjects and sensory staff, and free from distraction as described above. Sufficient blackboard, wall, or easel space is required to accommodate attribute lists developed by the panel during training. A one-way mirror to an adjacent viewing room, equipped with video and audio taping capabilities, can accommodate observers without distracting subjects during training.

C. Administration

The staff required to support project planning, report writing, data processing, and general administrative functions should be located in a common area accessible from a main corridor and near to the test kitchen. Access to the administrative area should not require passage through the test area.

D. Satellite Facilities

Most sensory tests are conducted in well equipped laboratories as described above and are associated with company headquarters and a main research center. Tests also may be conducted in locations specific to product quality maintenance and production. Such satellite facilities are smaller, as they require less preparation equipment and space, and fewer booths. Regardless of where done, the same general rules for maintaining these locations apply; that is, a clean, quiet, and distraction-free test environment.

III. ORGANIZING SENSORY PANELS

There are several steps to developing and organizing effective sensory panels. Subjects must be recruited and qualified, must participate in tests, be motivated, and have their performance monitored.

A. Recruiting Subjects

Subjects for sensory panels may be recruited from a general population of consumers local to the sensory facility, or from a company's employee list. Because sensory panel membership is performance related, the decision about which group is best to recruit is influenced by factors of convenience, cost and availability. Subjects may be recruited randomly by telephone, mall intercept, or electronically. Alternatively, recruitment notices can be sent to prospective subjects, or posted in locations frequented by them. The recruitment notice should state that people interested in joining a food evaluation panel are being sought, and individuals who qualify will be required to participate for 1 to 3 hours a day for an extended period of time. People responding positively to the recruitment request are screened further for their availability and willingness to evaluate specific food categories that will be tested. To reduce early panel attrition, it is advisable that subjects are likers and users of foods in the category. Twenty-five to thirty interested and qualified subjects should be invited for sensory screening.

B. Screening and Qualifying Subjects

Sensory panels require subjects who are qualified based on their sensory skills and availability. A variety of tests are used to determine sensory skills, with some being method specific (e.g., Texture Profile®). Most popular, but not most useful, screening methods used by sensory professionals are: basic taste and odor thresholds, basic taste intensity ranking, identification of selected taste and odor compounds, texture stimuli for texture-specific panels, and discrimination of simple compounds and more complex finished products. Successful screening, especially when based on the latter procedure, can eliminate poor performing subjects, but does not guarantee successful performance. No evidence has been provided that demonstrates that threshold testing or use of "standards" actually identifies better performing subjects. However, eliminating subjects who cannot discriminate product difference at better than chance has proven to be a good predictor and is sufficient justification for continuing the latter practice. For discrimination and descriptive panels we recommend using discrimination screening tests within the test product

category. Screening should involve at least ten product pairs, given in replicate, covering all sensory modalities and increasing in difficulty from easy (85 to 100% success rate) to very difficult (50% or less success rate).

C. Scheduling Subjects

Subjects should be scheduled to arrive at the sensory facility at the same time, receive their instructions as a group, proceed to their assigned booth, and begin their evaluation. Scheduling avoids conflicts, assures similar instructions and products are given, provides order, and establishes this as a serious professional activity. Allowing subjects to arrive at the facility and begin testing whenever they like introduces potential error from a variety of sources including product stability, extending the session too long, and traffic congestion in the test area. Most testing is done in the morning from 9:00 to 11:30 and in the afternoon from 1:30 to 4:00. Suppliers that use consumers trained for sensory panels may schedule testing for the evening hours until 9:00 or 9:30 pm.

D. Personal Requirements

Subjects should be informed that they are expected to arrive on time at the test location, and to not have consumed food or flavored beverages, or smoked for approximately ½ hour before testing. They are asked to not use fragranced personal care products, perfumes, and so forth, when reporting to the test site, and they are cautioned to not wear clothing that has retained aromas from previous contact with these products. Subjects are discouraged from discussing product tests other than during sessions conducted by the panel leader. In many cases they are required to sign confidentiality agreements, and in tests involving experimental ingredients or processes may be asked to sign release of liability forms.

E. Test Orientation and Instructions

Subjects reporting to a site are provided with a verbal orientation. The orientation informs subjects about the product category, number of products to be evaluated, the evaluation procedure, and any procedural variations that differ from previous evaluations. Written instructions are posted in each test booth, and subjects are encouraged to read those instructions before evaluating the first product, and again during rest intervals between products.

F. Incentives and Motivation

Positively motivated subjects are more likely to follow test rules, have lower panel absenteeism, remain focused on the task, and are less likely to be negative and disruptive. Although positive motivation does not guarantee satisfactory performance, it increases that likelihood, and is beneficial for the other subjects and for the test staff.

Positive motivation begins with informing subjects that the task is important, maintaining an efficient and professional test program, and providing positive reinforcement where possible, without disclosing information that can influence upcoming tests. Following testing, a daily treat, such as a beverage, baked good, confectionery, fruit, vegetable and dip immediately reinforce subject participation. These items need to be thoughtfully presented and fresh. Once they become routine or are leftovers from previous tests, they lose their reinforcing value. Where there is insufficient space for a treats table, or where one would interfere with testing, a coupon of equal value and redeemable at the company cafeteria can be substituted. Holidays provide the opportunity to incorporate a special theme into the daily treat.

Long term incentives include various forms of recognition rewarding participation and attendance rather than skill. A letter of commendation from a senior executive, company newsletter recognition for the "taster of the month," an annual dinner out or panel party, and accumulating points toward a gift or raffle have proved successful. Incentives that reward the subject and publicize the sensory program are always a good idea. Cash payments are usually reserved for non-employee subjects. Employees should not be compensated for their participation.

G. Performance Records

Sensory panels use a small number of subjects; therefore, the performance of each subject is important. Performance records track the number and type of panels on which the subject participates and notes any sub-standard areas of performance. An individual subject's failure to find differences where the panel result is statistically significant is noted in the performance record. The information will help to identify where additional training is required for the subject or the panel, or when a subject needs to be excused from a panel.

IV. APPLICATIONS

The growth and success of sensory evaluation is directly related to its positive contributions and variety of applications to product development and product quality. The following section describes many of these useful applications.

A. Product Development

Product development is an activity that ranges from new product development to several planned activities associated with changes to a product or process.

1. New Product Development

The new product development cycle begins with a product idea generated from consumer research or technical

discovery, and is complete when marketing research demonstrates sufficient consumer interest in the new product. The amount and detail of direction consumer research will provide about the sensory characteristics for a new product will differ based on a company's ability to obtain that information. Trained panel (e.g., QDA) evaluations in these consumer studies will provide an accurate description of product differences and similarities, and when combined with consumer preferences will identify sensory attributes and intensities to optimize consumer acceptance. The sensory description for the optimized product becomes the development target. Trained descriptive panels and qualified discrimination panels are used to determine when one or more products satisfactorily achieve or approximate a sensory optimized target.

2. Pilot Plant Scale-Up

A pilot plant is often used during product development as a means for providing test products and quantities supporting research and development without interrupting manufacturing or requiring production size quantities. At the conclusion of the R&D development process, the pilot test product (i.e., formulation and process) will need to be converted to a production product, using production equipment and processes. Differences between pilot plant and production product usually is unwanted; however, they are not unexpected. The manufacturer needs to determine that there is a perceived difference between the two products, and if there is, what are the implications of that difference. Where possible, sensory discrimination tests are used to determine whether there is a perceived difference between the pilot and production products, and where a difference is found, trained panel descriptive tests are used to describe those differences. Product Development will need to determine formulation or process variables responsible for the difference and make necessary changes. If the production product is different compared to the pilot plant product and cannot be modified further, it's impact on acceptance will need to be assessed. This is achieved either through comparison to an optimization model, if one was developed during the development process, or in a consumer test of the pilot and production product. Where production product cannot be modified further and it does not satisfy the company's action standard for acceptance for new product introduction, the new product may be abandoned.

3. Cost Reduction

Cost reduction is an ongoing activity, and where possible sensory discrimination tests are used to determine whether there is a perceived difference between the control and cost-reduced product. Where no difference is found, the cost-reduced product is accepted to replace the current product. A difference usually results in rejection of the cost-reduced product. Where a difference is found, descriptive tests may describe differences to determine whether the difference is for an important attribute or one that may be readily corrected. Caution is advisable relative to using a series of discrimination tests where a successful cost-reduced product replaces the control. For example, if A is not significantly different compared to B and B is not significantly different compared to C, and so forth, A very well may be significantly different when compared to Z. This transitivity can occur when a series of non-significant differences result in a product that is significantly different from the original control. This possibility can be minimized through the use of descriptive analysis, comparing the descriptive profile of the proposed new control to that of reproduced original control or to the historical profile for the original control.

4. Ingredient/Process Change

Alternate ingredients, new equipment, and process changes may be introduced at any time. All changes should be evaluated to determine their potential effect on product perception; the evaluation strategy is the same as described for cost reduction. In situations where a change is required by the business, even where sensory differences are found, consumer tests are recommended to estimate the impact of the change.

5. Ingredients/Purchase Specifications

Sensory requirements are designed into the purchase specification for many ingredients. For example, a prescribed perceived heat level is required for hot peppers that will be used in processed salsa.

B. Product Quality

The objective is to provide a consistent product of appropriate sensory quality, where appropriate sensory quality is defined during the product development cycle. The following describes important areas where sensory evaluation supports the quality mission.

1. Sensory Specification

A sensory specification describes the acceptable range for the most important characteristics of a product. Specification development requires products selected to represent the broad range of production product and possible variation. Products are evaluated by a trained panel and by consumers. The trained panel provides sensory descriptions (e.g., QDA) for each product, and consumers, qualified as likers and users of the product, provide the liking scores. The data are analyzed to identify the most important acceptance predictors (usually about 10 to 12) and the tolerance

range for each, and these results are used to train quality control panels at manufacturing sites.

2. Production Benchmarking

Once a new product has been accepted for manufacture and distribution, production samples are evaluated by a trained descriptive panel to establish the sensory profile for the product when first released for sale. The benchmarking documentation is used for future comparisons and references to the original product.

3. Manufacture Quality

In a manufacturing facility, subjects are screened and trained to evaluate a product at one or more stages of production (and where permitted according to GMP and HACCP requirements). These evaluations can include ingredient receipt and storage, during manufacture, and finished product. For finished product, trained sensory panels evaluate attributes included in the sensory specification. A sensory professional coordinates the activity but is not a panel participant. Subjects evaluate attribute intensities, and the sensory professional uses that information to determine whether to recommend accept, hold for further evaluation, or reject the product.

4. Shelf-Life and Stability

The sensory quality of products deteriorates over time, and the rate of deterioration depends on factors related to ingredients, packaging, handling, and storage. Several different sensory related criteria are possible for determining when the end of shelf-life occurs, and no one criterion is universally accepted. Using a qualified descriptive or discrimination panel, any statistically significant difference for a sensory attribute compared to a control product is a popular choice for determining end of shelf-life, albeit a conservative one. Where a sensory specification is available, stored product is evaluated to access whether it deviates from that specification. A loss of consumer acceptance is another popular criterion used to determine end of shelf-life.

5. Distribution Product

Products may experience abuse after leaving the manufacturing facility; therefore, many companies audit product quality at the retail level. Results from qualified descriptive and discrimination sensory panels are used to measure differences compared to data from the product benchmark or sensory specification.

C. Marketing

Sensory evaluation supports marketing and marketing research activities beginning with new product development, continuing through tracking product performance, and contributing to special assignments such as developing tests and data to support or challenge advertising claims. We briefly describe the role of sensory evaluation in those activities.

1. Monitor Competition

Discrimination and descriptive panel information are used to provide immediate intelligence about the similarity and differences between competitive products and also for tracking changes and trends over time.

2. Advertising/Claim Support

Sensory claims often appear on television, radio, and in mass media print and trade oriented publications. Most claims are comparative and preference related, others may identify specific sensory differences. In either case, the claims can be challenged and damages assessed where the claim is not substantiated. All phases of the research, including subject recruitment, selection, training, testing and the data analysis are subject to examination and challenge. Therefore, it is important that sensory claims are supported by appropriate research that can be defended in the legal system. The research must demonstrate that it is free of bias that could influence the outcome, and that sound testing principals are used.

3. Category Review

A broad array of products, often between 12 to 24 and representing the competitive and sensory range for a category, are evaluated by a trained descriptive panel and by consumers. Reviews for a rapidly changing product category may be scheduled as frequently as 6 month or 12 month intervals. The information is used to monitor the competitive market on a broad scale and to track changes and trends over time.

4. Product Optimization

This category of sensory research is conducted for the purpose of optimizing consumer liking for a product. Two different approaches are used for selecting products for this research; one is a design of experiments (DOE), and the other is a category review (CR). The DOE limits products to those the researcher modifies according to a prescribed experimental design (e.g., factorial, RSM); the CR includes a range of products having sensory differences representing the product category. A qualified discrimination panel may be used to eliminate redundant products and a trained sensory panel to provide a descriptive analysis of the products. The statistical analysis relates the consumer, trained panel, and analytical (if included) data sets to determine combinations of sensory and analytical

attributes that best predict optimal consumer liking. The descriptive panel is used for the follow-up research to evaluate test products until one or more satisfy the optimized sensory target.

V. DISCRIMINATION METHODS

Discrimination tests are classified as analytical methods, and are used primarily to determine whether there is a perceived difference between products. The tests may be directional, where the attribute of difference is named, or non-directional, where the attribute of difference is not named. Discrimination tests are relatively uncomplicated to administer (most use a simultaneous sample presentation), analyze, interpret, and report. They are used in sensory studies primarily to screen products as a prelude to other types of tests (e.g., descriptive or consumer), or to screen subjects for participation in descriptive and other sensory analytical panels. Discrimination tests are used in quality control and manufacturing to identify non-conforming product. They may not be the method of choice where inherent variability within samples is large.

Discrimination testing dates back to the psychophysics of the 1800s and procedures used for absolute and difference threshold determination. The following section discusses discrimination tests used by sensory scientists to make product decisions, without reference to competing psychophysical theories or models attempting to explain the discrimination process.

A. General Requirements

Following are some requirements for discrimination tests. We are reminded that these tests are sensory analytical tests, and as such use small numbers of screened and qualified subjects. By small numbers, we mean 20 to 25; however, there is no rule that precludes more or fewer subjects.

1. Subject Selection

Subjects for sensory analytical tests are screened for their sensory skill, and for discrimination tests this means for their ability to detect differences between products. Using consumers or other subjects that have not been screened for sensory acuity is not common practice or is it recommended. Unskilled subjects increase the risk of a Type II error (i.e., missing a real difference); large numbers of subjects, typical for consumer tests, increases the risk of a Type I error (i.e., finding a false difference). A previous section provides a detailed discussion about subject screening.

2. Test Design, *N*, and Replication

Balanced design serving orders are typical for discrimination tests within and between product trials, except for those three sample tests where it is prudent to reinforce the sensory characteristics of a control product that is a benchmark. For each trial in this fixed order scenario, two of the three products are the control and the third is the test product.

Sensory analytical tests typically use small numbers of subjects screened for their sensory skills, and the discrimination model is no exception. About 12 screened and qualified subjects are optimal, and together with a recommended replicate trial provide sufficient data for statistical analysis. Replication is recommended for analytical sensory tests, because each subject contributes a large percent of the data and it is important to know which subjects have difficulty replicating their response. An inability of a subject or a panel to replicate responses can provide important information about problems with the subjects, procedure, and products.

3. Data Analysis

Unless noted otherwise below, statistical testing for discrimination data is typically based on expansion of the Binomial distribution. Published tables are available in the literature for determining the number of correct judgments required for statistical significance (2, 15). These tables provide for 1-tail and 2-tail tests and for chance probability values of 0.5 and 0.33.

B. Non-Directional Discrimination Tests

The following tests are classified as non-directional because subjects are not instructed to select the different product based on any specified attribute.

1. A-Not-A Sequential Presentation

Subjects are provided with two 3-digit coded products, one followed by the other, and indicate whether the second product is the same, or different from the first. The first product is removed prior to serving the second to eliminate a direct comparison of the two. This method is used primarily to minimize observation of unimportant visual differences between products, and to control exposure time between products having significant carryover effects. The probability of a correct judgement is 0.50; data analysis is straightforward and based on the binomial expansion, for which there are several published tables. The A not-A method can be expanded to where two or more coded products are given to the subject, one at a time, without reintroduction of the first product. Although this further reduces carryover effects, selection of this serving option must be balanced against unwanted differences in subject's memory skills.

2. A-Not-A Simultaneous Presentation

Subjects are provided with 2 products, side by side, and indicate whether the products are the same or different. They are instructed to try the product on the left first, and

then the product on the right. This design should include an equal number of pairs in which the products are in fact the same, to reduce the impact of false hits from subjects that always respond that there is a difference.

3. Duo-Trio

Three products are presented simultaneously to the subject, where one is coded "R," for reference, and the other two products have unique 3-digit codes. The subject is instructed to select which coded product is most similar to the reference. Similar to the A-not-A method, it does not require selection based on a named attribute or training with that attribute, has a $p = 0.50$, and one can use published tables for the binomial expansion. As we will see, this method requires less re-tasting and consequent potential for carryover effects than does the Triangle method. A sequential procedure can be used to further reduce direct comparisons and carryover effects; however, it introduces unwanted memory skill issues.

4. Triangle

Three 3-digit coded products are presented simultaneously, and the subject is informed that two of the products are the same and one is different. The subject's task is to correctly select the product most different from the other two. The triangle method requires more tasting to reach a decision than does the duo-trio method. This is because the triangle has 3 pairs of unknown products (A vs. B, A vs. C, and B vs. C) compared to two pairs (Reference vs. A, and Reference vs. B) for the duo-trio. The chance probability for the triangle test is 0.33 to reflect that there are three unknowns, and there are published tables available for assessing significance between products.

5. Matching and Sorting Tests

A variety of non-directional discrimination tests are possible for matching unknowns to two or more controls or standards (e.g., dual-standard method), sorting unknowns into similarity groups, or indicating degree of "sureness" that an unknown matches a previous sample. An example of the latter is the *R*-index described by O'Mahony (17). These methods require more tasting than do the methods described above, and for this reason are not routinely used for product evaluation.

C. Directional Discrimination Tests

For directional difference tests, subjects are instructed to make their choice based on a specified attribute.

1. Paired Difference

Subjects are provided with 2 products, side by side, and indicate which product has more of a specified attribute. They are instructed to try the product on the left first, and then the product on the right. The order of tasting is balanced across subjects and replications.

The primary criticisms of this method, and of all directional discrimination tests, is the time and effort required to train subjects to evaluate the specific attribute, and knowing whether subjects actually selected a product based on the specified criteria. Commercial products are often quite complex, where an ingredient or process difference can result in several sensory differences. Limiting the subject's response to a single attribute may result in a correct choice based on incorrect criteria. For example, different sweetener types and amounts for a beverage or baked product may alter visual and textural characteristics. Products scored different for sweetness actually may differ on some other attribute. Several investigators (1,18,19,20) have convincingly argued that, all things being equal, a directional method is superior to an equivalent non-directional method. The problem is, seldom are all things equal. Where attribute information is required, sensory descriptive methods are recommended.

2. *n*-Alternative Forced-Choice

The directional paired difference test can be expanded to include several samples, and the subject instructed to select the product that has the most, or least, of some attribute. As in all discrimination tests, the probability value must reflect the number of samples from which the subject must select. For example, the probability value is 0.33 for a 3-product test, 0.20 for a 5-product test, and so forth. AFC tests are directional and have the same procedural problems described for the paired difference test. They are multisample tests where all the samples for a trial are simultaneously presented, introducing issues of sensory fatigue for many commercial products.

AFC methods are have been popularized most recently by researchers developing theoretical models to explain sensory discrimination behavior (19,20). Signal Detection, Thurstonian, and other competing approaches may well provide answers to important sensory discrimination issues, and their examination is encouraged. To be useful for routine product evaluation, these approaches will need to offer alternate procedures that are practical in a business environment.

VI. DESCRIPTIVE METHODS

Descriptive analysis is the most sophisticated source of information available about the perceived sensory properties of products. It is particularly important because it provides a focus for development efforts. Descriptive analysis provides a basis for measuring the effects on perception of a process or of ingredients; it is used for correlating instrumental measures, and it is essential for correlating

consumer response behavior and for identifying those product attributes that are most important to consumer preferences. Before discussing specific methods, it is necessary to define what is meant here by descriptive analysis. Descriptive analysis is a sensory methodology that provides quantitative descriptions of products based on the perceptions from a group of qualified subjects. Based on the method, it provides information about a specific sensory modality or provides a total sensory description, taking into account all the sensations that are perceived, visual, auditory, gustatory, olfactory, and kinesthetic — when the product is evaluated. The evaluation is usually (but not always) done in a controlled environment, and can include product handling, and, in this sense, it is a total experience. The evaluation also can be done in a home environment when normal preparation, use, and consumption are required over a period of time.

The origins of descriptive information can be traced to early brewmasters, perfumers, flavorists, and other product specialists. These technical specialists described products, made recommendations about the purchase of specific raw materials, and also evaluated the effect of process variables on product quality (as they determined product quality). Companies used that information to determine that a particular product met their criteria for manufacture and sale to the consumer. These early activities were the basis for the foundation of sensory evaluation as a science, although at the time it was not considered within that context. Formal descriptive analysis received its major impetus from "flavor profile," an approach that demonstrated it was possible to select and train individuals to describe the sensory properties of a product in some agreed sequence, leading to actionable results, without requiring years as an apprentice to an expert. The method attracted considerable interest and was a milestone in the development of the science of sensory evaluation. It also was the source of much controversy, and since then other descriptive methods have been developed.

A. Flavor Profile

The Flavor Profile® method described by Cairncross and Sjöstrom (21), Sjöstrom and Cairncross (22), and Caul (23) was developed at the Arthur D. Little Co. and is the only existing formal qualitative descriptive procedure. The method utilizes a panel of four to six screened and selected subjects who examine, discuss, and evaluate product in an open session. Product attributes are scored for intensity in the order of their perception. The intensity scale was: 0 = none,) (= threshold,) (−1, 1 = slight, 1–2, 2 = moderate, 2–3, and 3 = strong. Once agreement is reached on the description of the product, the panel leader summarizes and reports the results without application of any statistical analysis.

Six subjects are selected for training based on technical background and a series of screening tests, including basic taste recognition and intensity ranking, odor identification for selected chemical compounds, and a personal interview to evaluate interest, attitude and availability. Training takes 6 months or more and includes instructional information about the senses, introduction to the components of the flavor profile, and direct experience evaluating selected products. Training also includes threshold determination for the basic tastes, skills having little or no connection with evaluation of products containing mixtures of supra-threshold stimuli. Selected references are introduced to demonstrate specific sensations, and practice profiles obtained for products evaluated by an experienced panel. During training all subjects serve as panel leader until one is selected. The panel leader is key to this method; they coordinate the testing and report results. This individual assumes a leadership role, directing the conversation and providing a consensus conclusion based on the results. The role of panel leader as defined in the method can have significant consequences without some independent controls. Subjects could be led to a conclusion without being aware this had occurred.

The method had considerable appeal because results could be obtained rapidly. Subjects meet, as a group, for about an hour to evaluate a product, reach a consensus about its sensory properties, and provide the requestor with a result. The developers of the method emphasized that there would be confidence based on the collective professional judgment of the panel and this would obviate the need for statistics. The method is unique in that no direct judgement was made concerning consumer acceptance of the product, although most investigators assumed consumer acceptance based on a result. An early recognized shortcoming of the method was its focus on the single sensory modality of flavor. Subsequent methods would extend screening, training, and evaluation to one or more additional modalities.

B. Texture Profile

Chronologically the next descriptive method of importance was the Texture Profile® method developed at the General Foods Research Center (24, 25, 26). The method was based on Flavor Profile, and extended descriptive analysis into a second modality, texture. Brandt and co-workers (24) defined a texture profile as "the sensory analysis of the texture complex of a food in terms of its mechanical, geometrical, fat and moisture characteristics, the degree of each present and the order in which they appear from first bite through complete mastication." Several of the procedures introduced by Texture Profile raise behavioral issues related to panel training.

If the panel is to be used also for flavor assessment, subject screening includes tasks identical to those described for Flavor Profile. Texture screening tests were devised for hardness and viscosity ranking, and for geometric matching. As many as 40 to 50 subjects may be screened, with 10 to 15 selected for training. Full or partial denture wearers were not considered for panel membership.

Texture training included a two week orientation phase, a three month practice phase, and a three month expansion phase. A standard set of references was used to demonstrate taste, aroma and mouthfeel sensations. The concept of the components of flavor profile was extended to include what were described as mechanical, geometrical, and a category of "other" (moisture and fat) characteristics, intensity, and the order of appearance for the texture properties. An effort was made to describe the physical properties of texture and to provide a sensory definition for each described physical property.

Initially, the texture terms and definitions were compiled, sorted, and categorized from both scientific/technical and popular terms, the latter from laboratory or field situations in which common usage terms were used to describe textural sensations. This led to the development of a classification of terms that were believed to encompass texture sensations. Standardized rating scales were introduced for the mechanical and geometrical properties of texture perception, and these relied on commercially available products to anchor each point of each scale. During training, subjects were expected to learn these definitions and their associations. The product evaluation procedure used a Flavor Profile approach with panelists discussing results in order to reach a conclusion.

Although the method was useful for focusing attention beyond flavor and on the relative importance of texture, it had numerous problems of its own. In developing the method, one objective was to eliminate subject variability through use of a standard vocabulary, and standard rating scales and references as part of the training program. There are inherent risks in experimenter-assigned attributes. While they may be meaningful to a chemist, one might question the perceptual meaning of these technical definitions to a subject. Other subjects could ignore a perception because the characteristic was not among those selected for training. Use of products as scale anchors presents their own set of problems. Products are not invariant; they change over time as a function of marketing and other considerations. Another concern is the separation of texture from other sensory properties of a product such as color, aroma, taste, and so forth. As a rule, perceptions are interdependent, and the exclusion of a sensory modality from a scorecard does not eliminate those perceptions. In effect, the subject is likely to use other attributes to acknowledge these perceptions, and the visible manifestation is increased variability and decreased sensitivity. These other perceptions can influence the responses to the textural perceptions and vice versa.

Profile methods and related "expert" systems treat subject differences as unwanted error, and these training programs resort to behavior modification procedures in an attempt to eliminate this source of variance. The panel leader trains subjects to provide what the leader judges to be the correct response to stimuli, and subjects are trained to agree and repeat those responses in the presence of the stimulus. The approach ignores the reality of the situation, and eliminates the opportunity to assess the importance of individual differences for understanding and generalizing results to consumers.

Interest in eliminating subject variability is, on the surface, a reasonable (although not likely or practical) idea but it must not give way to procedures that sacrifice the validity of the measurement system. The human is a living and changing organism, influenced by physiological and psychological conditions that better support a concept of expected variability. Products also are themselves variable. For these reasons, a panel is used rather than a single subject, and replication has become a mainstay in most current descriptive methods.

C. Quantitative Descriptive Analysis (QDA)

Development of the Flavor and Texture Profile methods demonstrated a need for descriptive information and a formalized procedure for screening and training subjects, and for evaluating products. They also stimulated interest and research on alternative descriptive methods that would correct weaknesses previously identified: the extended amount of time to select and train a panel, reliance on model systems (e.g., compounds in water) for screening subjects, an experimenter-derived descriptive language, the types of scales used, qualitative rather than quantitative information, the role of the panel leader, and the type and role of references. The Quantitative Descriptive Analysis (QDA) method, first described by Stone, Sidel, Oliver, Woolsey, and Singleton (27) and taught by the Tragon Corporation, addressed behavioral, measurement, and quantitative weaknesses of the profile methods, reflecting scientific and technical developments in psychology and the consumer products industry.

The QDA method differs from the profile methods on criteria of subjects recruited, screening procedure and materials, subject training, testing, analysis, and reporting for descriptive information.

1. Subjects

Consumer users in the product category are recruited, and test results are then generalized to the consumer population. Experts and others having technical degrees in the product area are avoided as subjects because of the potential for biased responses based on their technical knowledge of the product category, ingredients, and processes. About 25 subjects are recruited, and it is expected that 12 to 15 will successfully qualify and complete training.

2. Screening

Discrimination tests are used, and screening stimuli are selected from the product category rather than the simple stimuli in water solutions popular with the profile methods. The closer the screening stimuli are to the test stimuli, the more likely that successful performance will be obtained in the actual test. About 10 to 15 replicated pairs of product

stimuli are used, increasing in difficulty from the first pair (easy to discriminate) to the last pair (difficult to discriminate), covering all sensory modalities, and including any differences known to be relevant to consumers. Correct selection of the different product in more than about 65% of the trials, and consistency in correct selection across replications are used as a guide for subject selection.

3. Training

QDA training focuses on language development and the evaluation process, and provides subjects experience with the range of products they will evaluate. Language development is a consensus building process. The panel leader's function is to keep the panel focused on developing objective terms (in contrast to attitudinal terms) that can be measured and obtain agreement amongst subjects on the evaluation procedure. A typical QDA scoresheet includes 30 to 50 or more attributes covering all sensory modalities. The language development process contrasts with the profile methods, which teach a preconceived technical language and use a prescribed set of "universal references" derived from the experimenter. QDA uses references selected directly from the product category, and only on an as-needed basis. Consequently, QDA derived attributes and consumer perceptions take less time to obtain, with the entire training process requiring between 5 to 10 hours, rather than several months.

4. Testing

Subjects evaluate each product individually in a facility equipped with test booths or partitions, or, for an extended use QDA, in their own homes following product usage. Subjects typically evaluate 4 to 6 products, and as many as 24 or more when part of product optimization research. Balanced block serving designs are used and each subject provides 2 to 4 replicate evaluations. Subjects use a semi-structured line scale to score the perceived intensity for each attribute for a product. The scale consists of a 6-inch (~15 cm) line, anchored ½ inch (~1.5 cm) from each end to identify the direction (e.g., weak to strong) for each attribute. This type of scale has no numbers and minimizes word bias. It is very difficult for a subject to memorize the location of previous judgements (assuring greater independence of judgements from replication to replication and product to product), and preserves the interval character of the scale for data analysis.

5. Data Analysis

QDA was the first trained panel method to insist on, and provide for, statistical analysis of subject performance and product differences. Line scale responses are converted to numerical values from 0 to 60 prior to analysis. A unique method of graphing the data, QDA "spider graphs," was developed that readily communicated product similarities and differences, and has become a standard in the food industry. A proprietary statistical package was made available for analyzing QDA data, and included in Tragon's training program for panel leaders. The statistical package uses one-way and two-way Analysis of Variance models in prescribed ways to assess subject performance. Current innovations to the package include algorithms for assessing crossover and magnitude interaction, scale use, and multivariate analysis and mapping techniques. QDA data is well suited to the recent advances in statistical analysis and computer technology.

D. Sensory Spectrum Analysis

This method positions itself as a hybrid of the three methods described previously; however, it is primarily an extension of the profile methods. Concepts first introduced for the Texture Profile method are extended back to the recruiting, screening, training, and testing for flavor panels as well. Separate training programs are required for flavor, texture, and each other modality. Training programs are quite lengthy, a common element for all profile methods. Subjects are taught about sensory processes, similar to the orientation programs found in the other profile methods. They also are taught experimenter-assigned attribute vocabulary and rating scales, the latter anchored at multiple points with experimenter-assigned references. Subjects must learn the required response to selected stimuli, independent of their individual perceptions, as an attempt to produce absolute scales. Teaching identical responses, independent of individual perception, is possible; however, skeptics of the method view this as a form of behavior modification and contrary to understanding and predicting consumer perception. QDA concepts included in the Spectrum method include category-specific discrimination tests for subject screening, intensity scales (although more nominal or ordinal than interval here because of the training method), exposure to a broad range of product in the category, evaluation in individual test booths, and application of statistical analysis. The statistics are generic and less specific than those found in QDA and other recent developments in descriptive methods. Curiously, variance data from panels trained in the Spectrum method has not adequately demonstrated the zero result necessary to support a claim of absolute scales or counter the concept of individual differences.

E. Free-Choice Profiling

Williams and Langron (28) described a radically different descriptive method that required no subject screening and training, where each subject could use their own words and scales to describe products. Generalized Procrustes analysis, an iterative type of factor analysis, is used to force fit individual data into a common space. While this type of summary analysis can show differences between very different products, it misses smaller, and possibly

important, differences. The method does not demonstrate any substantially unique advantages and may be better suited to classification and comparison to other consumer procedures than to trained panel procedures.

F. Other Methods

Descriptive information is recognized as important, if not crucial, as a means for providing a comprehensive product assessment. Descriptive panels are used in several venues including, and not limited to, product development, quality control, advertising claim support, and food science education. Different needs have given rise to descriptive panel variations other than the methods described above. Many descriptive methods are generic and retain in varying degree elements of the core methods; a few other methods have established, or attempted to establish, themselves as different. Larson-Powers and Pangborn (29) introduced the use of a reference combined with a difference from reference scale. The method required simultaneously available stimuli, reducing the number of different products that could be evaluated in a session. The developers of the Flavor Profile introduced a new version called Profile Attribute Analysis (30) that included 7-point intensity scales allowing for statistical treatment of the responses. Stampanoni (31) introduced "Quantitative Flavor Profiling" as a hybrid method based on Flavor Profile and QDA.

VII. TIME-INTENSITY SCALING

The temporal aspects of the sensory experience during food and beverage consumption are apparent to even the casual observer. Not unexpectedly, perceptual changes occur as the physical properties of a food change during chewing, or while in the mouth (e.g., ice cream and butter). Perceptual change also can occur when the chemical properties of a stimulus remain constant, as with taste, odor, and certain mouth sensations (e.g., burning sensation from chemical hot stimuli). Early psychophysical research into taste adaptation included time as a variable, measuring changes in taste threshold over time for simple adapting solutions. Hahn's (32) results demonstrated threshold differences based on the concentration of the adapting solution and for the basic tastes measured (salt, sugar, bitter, and acid). The research also concluded that subjects complete recovery to the adapting solutions was not reached within the 30 seconds allowed, and that there were individual differences. Individual differences in time-intensity research continue to be reported; for example, van Buuren (33), using principal components to analyze time-intensity (TI) curves, concluded "TI curves appear to be determined by characteristics that are related to the judge and that may be unrelated to the product." Given that most time-intensity studies involve small numbers of subjects and data are averaged, individual differences are a vexing concern for generalizing time-intensity results to a larger consumer population.

Time-intensity studies have been reported for the taste, aroma, and texture of foods, and for a variety of products including bread, chewing gum, beer, wine, ice cream, chili pepper, gelatin, and chocolate. Commercial interest in synthetic sweeteners in the 1970s prompted significant published and unpublished research into the temporal effects of these sweeteners compared to sucrose.

A. Measurement

Interest in time and intensity relationships in sensory evaluation is evident by the almost universal practice of measuring aftertaste or aftereffect in descriptive analysis and consumer research. Subjects in time-intensity studies typically receive instruction and practice with the test procedure, scale, and any special apparatus used for data collection, and may be screened for their ability to detect differences for the attribute or attributes measured. Under these conditions, the subjects are viewed as a special type of descriptive analysis panel.

The simplest measurement practice involves scoring the perceived intensity for overall aftertaste or aftereffect (i.e., sensation remaining in the mouth), or for a specified attribute, as a last task during routine product evaluation by an ongoing descriptive panel. The panel is instructed to first wait a prescribed amount of time (30 to 60 seconds or more depending on the product) after completing other attribute evaluations. This procedure can be expanded to include intensity measures for several specific attributes obtained at one or more designated points in time during the evaluation. This approach is appealing because it uses an ongoing panel, can measure several attributes at selected points in time, and special data collection equipment is not required. There is a practical limit to the number of attributes that can be measured during a time interval, as it takes time to score attributes. This and related issues should be resolved during training. The primary disadvantage of this approach it that measurement occurs at discrete points in time, whereas, the perceived change may be continuous. Whether the perceived change is different for different subjects is more a data analysis issue than one of data collection.

Continuous measurement of the change in perceived intensity requires an apparatus that simultaneously tracks time and the subject's intensity scores. While providing the advantage of continuous measurement, the apparatus usually limits the number of attributes that can be measured to one or two, and based on the apparatus requires varying degrees of physical involvement by the subject. Pen type strip-chart recorders were among the earliest devices used for continuous measurement in time-intensity studies (34). The recorder moves chart paper at a constant rate and an intensity scale is fixed on the recorder perpendicular to the direction of chart flow. The subject continuously records perceived intensity for the selected attribute by keeping the pen in contact with the chart paper, moving the pen in the direction of increasing or decreasing intensity until the

perceived intensity for the attribute has returned to baseline. Data from early generation strip chart recorders was handled much like that from similar tests using paper ballots. The subject's intensity score at several points in time were hand measured and converted to numerical value for data analysis. This was a labor-intensive process, depending on the number of time points examined.

Desktop computers linked to a joystick (35), or similar device, for measuring perceived intensity introduced significant improvements in the time required to collect, enter, and analyze data, and simplified the subject's task compared to strip chart recorders. These advances and developments in statistical analysis and graphing software that could be used with time-intensity data encouraged greater use of the method for sensory research.

B. Analysis

Time-intensity studies typically involve one or two attributes, and the results are readily graphed on *x-y* coordinates, with attribute intensity on the *y-axis* and time on the *x*-axis. Curves may represent individual data, averaged data for the panel, or group data for subjects having similar results. Measures of central tendency, such as means or medians, are used to construct data points for selected time periods, or data may be otherwise transformed to reduce or eliminate subject differences. Data points are then connected to create a time-intensity (TI) curve. Selected curve parameters or discrete points are compared directly or analyzed for statistical significance. Maximum intensity (Imax), time to maximum intensity (Tmax), and duration (return time to baseline intensity) are popular discrete points used for comparing TI results for different products, and suitable analysis of variance and multiple range tests are then performed to determine significance for the observed differences. An examination of the literature on this topic reveals views espousing and challenging a variety of curve parameters worthy of consideration, such as area under the curve, peaks, plateaus, slopes, and so forth. Several investigators have proposed combinations of these and other measures, and developments are still forthcoming in this area. The interested reader is directed to Lawless and Heymann (1) for additional detail about TI research in sensory evaluation and the different analysis methods proposed for the data.

One of the biggest challenges facing the experimenter is how to deal with a subject's error of anticipation; i.e., providing responses based on the subject's guessing the purpose of the test.

VIII. DATA ANALYSIS

Statistics is an essential part of the sensory evaluation process, providing a means of summarizing information to reach conclusions about subject performance and product difference. For sensory evaluation there are several issues that make the use of statistics especially challenging. First, there are many different statistical procedures from which to choose, and new tests are regularly introduced and described in the literature. Second, statisticians as well as sensory scientists differ in their view about how responses should be treated, which type of analysis is more or less appropriate and useful, the extent of any data transformation, and so forth. Third, software packages and the power of the PC make all methods for analysis easily obtainable in terms of time and cost, but that does not make them appropriate.

This section provides an introduction to the use of statistics as a guideline for making sensory and business decisions about products of interest. The focus is on statistical applications rather than statistical theory and the proofs, or various algorithms that would take far more than the space allocated here for this topic. The interested reader can find that information and worked examples in books covering the general topic of sensory evaluation and other books that focus on statistics for sensory evaluation (17, 36, 37). Sensory decisions based solely on the statistical conclusions are the exception, not the rule. The statistics that we describe will focus primarily on those analyses found to be useful for the behavioral sciences; e.g., variance measures, and will focus less on statistics more typically used in the engineering and physical sciences.

A. Descriptive Statistics

Sensory responses may take the form of discrete or scaled data. An example of discrete data is found in discrimination results where subjects select a product as similar or different compared to one or more other products. An example of scaled data is found where subjects score individual sensory attributes. In this case scoring may be a ranking, or rating on one form or another of an interval or ratio scale. Descriptive data provides a summary of the individual scores, and as such, is more manageable than the original raw data. However, summary statistics by their very nature can also mask or distort information and the sensory scientist must be aware of this potential problem. Descriptive statistics are grouped into measures for central tendency and dispersion, and are often displayed in tables and graphs.

1. Central Tendency

The mean, mode, and median are important measures for summarizing individual data.

a. Mean

The arithmetic mean for scaled data is the sum of all scores divided by the number of scores that have been summed. The arithmetic mean is the most widely used measure in sensory evaluation. It can be distorted by extreme scores, in which case other means such as the harmonic mean or geometric mean may be better choice. Rank means are possible when rank order scales are used.

b. Mode

The mode is the response category that contains the largest number of responses. This measure is not influenced by extreme scores as is the arithmetic mean.

c. Median

The median is the category containing the 50th percentile, dividing an ordered distribution in half. This measure is also not influenced by extreme scores.

2. Dispersion

The range, variance and standard deviation are the three most frequently used dispersion statistics reported in sensory tests.

a. Range

The range refers to absolute difference between the lowest and highest scores of an ordered distribution. The range is easily influenced by one or more extreme scores, and by itself has limited value. It is more informative when combined with measures of central tendency, variance, and a frequency distribution.

b. Variance

The variance provides a measure of deviation or dispersion from a mean. To calculate the variance for a data set requires that each score is subtracted from the mean, each difference from that operation squared (to eliminate negative numbers), these squared differences summed, and then divided by the number of scores minus 1. The calculated variance resulting from this process is displayed as S^2 to represent sample data rather than population data.

c. Standard deviation

The standard deviation is the square root of the variance and displayed as S to represent sample data. As may be expected, the standard deviation will always be a smaller value than the variance, further reducing the impact of extreme scores.

3. Frequency Distributions

Frequency is the number or percent response for each response category. Frequency data are typically displayed as tables or graphs, which provide useful information about how well other measures, such as the mean and range, represent the data. For example, a flat distribution would indicate that the mean value is not a good summary description for the data. Sensory specialists routinely examine frequency distributions for each product and serving order combination, and different groups of subjects to identify important trends in the data. Products having similar shape distributions may readily be included in a variety of statistical significance tests, whereas those with very different shape distributions may not.

B. Inferential Statistics

This category of statistics is used by the sensory researcher to determine the risk associated with declaring that an observed difference in a test represents a real difference that can be generalized to other test populations, or to a larger consumer population. Using appropriate statistics does not eliminate the risk of making a wrong decision; nonetheless such errors can be minimized through proper experimentation and analysis. There is not sufficient space in this chapter to include discussion about probabilities and hypothesis testing; therefore, the interested reader is directed to the aforementioned publications (17, 33, 34). We will limit our discussion to those inference statistics used most frequently by sensory practitioners.

1. Non-Parametric

This category of statistical tests is used when data from groups or products to be compared are in the form of counts (i.e., frequencies), percents, or ranks. These are distribution free statistics, with no constraint regarding the shape (e.g., symmetrical bell shape Gaussian curve referred to as the normal curve) of the distribution. The number of non-parametric tests is too large to discuss here, and the interested reader will find greater discussion and useful references in the sensory statistics literature cited above. We mention only a few of these tests below.

a. Binomial tests

Data from forced choice tests such as those described for most discrimination tests can be analyzed for statistical significance using tables based on the binomial expansion. This has been described previously in this chapter.

b. Chi-square (χ^2)

This is a useful statistic for determining whether the observed number of responses is significantly different from an expected number (derived from the total number of responses). There are several variations of the formula that can be used based on how the data are collected and the number of classifications being compared. In sensory evaluation the chi-square test is often used to determine whether two response distributions are significantly different, and as a preliminary test for isolating and comparing two response categories from among several. It is used more in sensory consumer tests and less so in sensory discrimination and descriptive tests.

c. Other non-parametric tests

The aforementioned texts include useful descriptions for several other non-parametric tests worthy of note for sensory analytical tests. Those statistical tests include, and are not limited to; the McNemar test, Cochran Q test, Wilcoxon test, Mann-Whitney U test, Friedman test, and the Kruskal-Wallis test.

2. Parametric

This category of statistical tests is reserved for data that satisfies the constraints of the normal curve, which includes equality of the scaled intervals. All things being equal, which they seldom are, parametric tests are viewed as more powerful than non-parametric tests. When response data are clearly nominal (e.g., counting and classification data) or ordinal (e.g., ranks), non-parametric tests are recommended. Parametric tests are appropriate when data are clearly interval or ratio. Having said this, the reader is cautioned that most of the scales used in sensory evaluation have sufficient interval characteristics to permit judicial use of parametric statistics. Underlying statistical assumptions and theory is just that, assumptions and theory. In practice, results may be reliable, valid, and useful even if they do not perfectly satisfy underlying assumptions. In this event, it may be more beneficial to modify the theory and assumptions, rather than forgo the application.

a. t-test

The t-test is used to determine whether the means for two products are significantly different. Different formulae are available to accommodate various conditions such as: independent and dependent observations, large and small N, proportions, and equal and unequal number of observations. The t-test is not the best choice for sensory tests where the same subjects evaluate both products, the analysis-of-variance can be substituted and it extracts more from the error variance. The t-test also is inappropriate where more than two products are to be evaluated and statistically compared to one another. This constitutes multiple t-tests of the same data, a common error.

b. Analysis of variance (AOV)

The analysis of variance is the primary statistical test used for scaled sensory data. It is a versatile statistical procedure that readily accommodates multiple products, treatment conditions and groups of subjects. It is used to determine whether an effect is statistically significant, and when a difference noted is followed by a multiple range test to determine which means are different. Several AOV models are extensively used in sensory evaluation; these include the one-way for independent means, the two-way for dependent means, treatment x levels for different treatment conditions (e.g., x-levels of different variables), and split-plot models for different subject groups evaluating the same and different treatments.

The primary challenges for selecting the best AOV model are to select one that is appropriate for the specific test conditions, to accurately account for all the sources of variance in the test, and to select the appropriate error term for testing the significance of an effect. Statistical significance in the AOV is based on a calculated F ratio consisting of an effect variance in the numerator and an error variance in the denominator. Probability tables for the significance of the calculated F are published in most statistics books; most statistical packages for sensory and behavioral data include the AOV analysis and exact probabilities for the calculated F.

c. Multiple range tests

Multiple range tests are used following an AOV to determine from among a set of means for a significant variable which ones are significantly different. Different range tests reflect what is to be compared and the respective authors' preference for controlling the various error rates possible when making multiple comparisons for a single data set. Too conservative a test will make it difficult to find statistical significance and result in more Type II errors, whereas a less conservative test may result in false differences (i.e., Type I error). The sensory scientist is cautioned here that the underlying theoretical assumptions for a multiple range test may not agree with practical experience using that test. Where this is the case, paying attention to the risk one can still proceed cautiously. Frequently used multiple range tests include; Fisher's LSD (least significance difference), Dunnett's test, Duncan's test, some tests by Tukey, the S-N-K (Student-Newman-Keuls) test, the Scheffé test, and the Bonferroni test. Whichever test is selected, the results must be examined to determine whether they agree with the knowledge and expectation the researcher has about the products and the sensory test.

C. Correlation and Regression

Sensory scientists are often interested in the relationships between different sets of data. The interest may be between different groups of subjects, products, attributes, or combinations thereof. Because correlation does not imply causation, summary statistics and graphs depicting association are useful for data reduction, substitution, prediction, and generally improving understanding about variables. As with inference testing, different statistics are used for different levels of measurement (e.g., nominal, rank order, or continuous).

These measures are used to examine and better understand sensory and instrumental relationships, and in optimization research where sensory attributes are used as predictor variables for consumer acceptance.

1. Correlation

To understand a simple two-variable correlation it is best to first graph results as a scatter plot with the data from one variable on the X-axis and data from the second variable on the Y-axis. For example, descriptive scores for sweetness and bitterness for a set of products can be graphed and a correlation for the relationship between the two attributes calculated. The Pearson product moment correlation coefficient is used for continuous scaled data and is depicted by r. Calculated r-values range from -1 to 0 to $+1$. The

closer to 0, the less is the association between the two variables. The minus sign depicts an opposite relationship, whereas a plus sign depicts a directionally similar relationship; unity represents a perfect correlation. Pearson's is useful only for linear data, curvilinear data can produce low values approaching 0, misleading the casual observer to conclude the variables are unrelated. Correlation can be determined for non-continuous scaled data as well; an example is Spearman's rank order correlation coefficient (ρ Greek lowercase rho).

2. Multiple Correlation (R)

Multiple Correlation is used to assess the degree of association between a dependent variable and a set of predictor variables. In product optimization research several sensory attributes (independent variables) may be included as important predictors of consumer acceptance (dependent variable), and the multiple correlation (R) describes the degree of association between the dependent and independent variables. Several books have been published on the topic of Multiple Regression/Correlation (MRC), and the interested reader is directed to Cohen and Cohen (38) for an in-depth discussion.

3. Regression

Regression is a general term applied to equations that fit a line to observed data points. Simple linear, non-linear, and multiple regression are possible, and the resulting regression line is often used to predict values of the dependent variable (y) from values of the independent variable (x). The sensory scientist will use regression equations, for example, to predict perceived intensity for an attribute based on ingredient concentration. In optimization research, multiple regression is used to predict consumer liking from a combination of sensory attributes and their intensities. Analytical measures may also be used as predictor variables for acceptance in optimization, alone or in conjunction with sensory measures.

D. Additional Multivariate Methods

Several tests in addition to those already described are used in sensory evaluation to simultaneously examine multiple variables. Multivariate analysis often provides understanding of important relationships imbedded within the data and not readily observable by other means. The interested reader will find that the book by Dillon and Goldstein (39) offers a useful integration of theory, explanation and illustration for the topic.

1. Multivariate Analysis-of-Variance (MANOVA)

This analysis is used to test the significance of a set of variables. A good example would be descriptive panel data where several attributes are evaluated for a product set.

2. Discriminate Analysis

Discriminate analysis is a methodology for finding linear combinations of the independent variables that can act as scoring functions to estimate to which of several classification categories an observation belongs. Sensory scientists use this and related procedures (e.g., canonical correlation) in optimization research to identify lifestyle, attitude, and classification information that best identify membership in different consumer preference groups.

3. Principle Components Analysis

This data reduction technique transforms the original set of variables into as few linear combinations as possible to explain as much of the total variation in the original data. The linear combinations, identified as the factors or components, are independent from all other factors. The method is often applied to sensory descriptive panel data to reduce the number of attributes, or to identify a smaller set of independent attributes to include as independent variables in developing multiple regression models for optimization research. It is also used in optimization research to identify products that form independent groups based on sensory attributes.

4. Factor Analysis

Similar to principle components analysis, factor analysis is a data reduction technique, except it focuses on that part of the total variation that a variable shares with other variables in the set. Some sensory researchers prefer to build optimization models using factor scores rather than attributes identified through principle components analysis. However, such a result has limited value when applying the information to make product change, for example.

5. Cluster Analysis

This data reduction technique is used to find a smaller number of groups whose members have elements more similar to one another than to members of another group. Cluster analysis is used in sensory optimization research to identify unique consumer preference groups, and it is used in the same way in descriptive analysis to identify unique subject groups. In the latter situation, uniqueness may indicate the need for additional panel training.

IX. VISUAL PRESENTATION

Visual presentations are an important part of understanding and communicating data. They reveal events and trends in data not readily observed any other way, and they simplify results for less technical and non-statistical audiences (40). Increased availability of desktop computers capable of managing large amounts of data has proven to be of great benefit to sensory scientists. The sensory scientist can stretch,

shrink, rotate, organize and reorganize large and small amounts of data with a simple keystroke or two, and in doing so examine countless relationships among subjects, attributes and products. In addition to the large number of generally available analysis and graphing software programs, such as SAS and SAS/JMP (SAS Institute Inc., Cary, NC), S-Plus (MathSoft Inc., Seattle, WA), SPSS and BMDP (SPSS Inc., Chicago, IL), custom programs have been developed by several companies for application in sensory evaluation. For the latter, the interested reader is directed to: Camo, Inc. Corvallis, OR; Compusense, Guelph, Ontario, Canada; Sensory Computer Systems, Lancaster, PA; and Tragon Corporation, Redwood City, CA. A few examples of graphs useful to the sensory scientist are described below.

A. Histograms

Frequency histograms, together with central tendency and variance measures, provide important information about sensory responses to products and how well the data satisfy assumptions required for other statistical tests, and allow for comparisons between products. Figure 57.1 illustrates a different Product by Serving Order distribution for 24 subjects, where each subject evaluated both products (i.e., 12 subjects per serving sequence). The means are lower for both products in the second serving order, demonstrating a possible order bias, a not uncommon finding in sensory studies, and the primary reason for using balanced serving orders. The bimodal distribution and variance

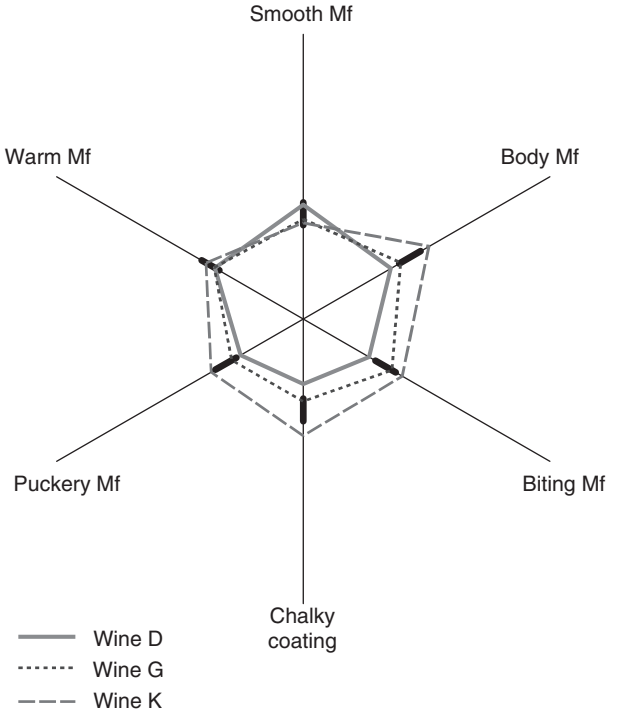

FIGURE 57.2 Typical "spider" plot for selected mouthfeel attributes. Intensity scores are measured from the center out to the point where the product crosses. The attributes are spaced equally around the center point. Bars on spokes represent the magnitude of difference for that attribute to be significant using the LSD test.

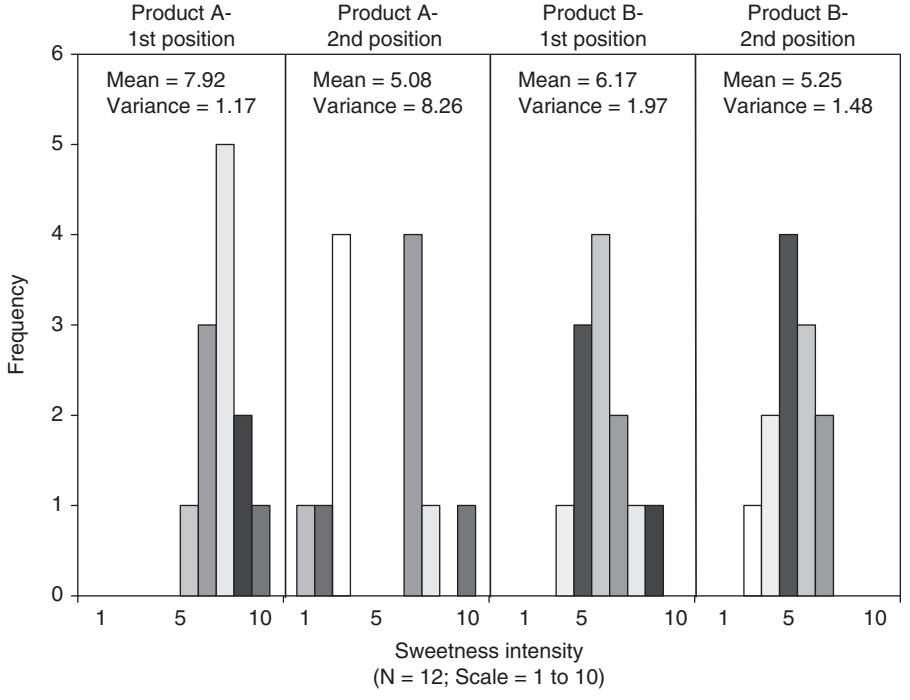

FIGURE 57.1 Illustration of distribution histograms for examining serving order effect. The graph represents 24 subjects that evaluated two products in two serving orders. Twelve subjects evaluated Product A first, and 12 subjects evaluated Product B first.

differences for Product A when served in the second order would alert the sensory researcher that it may be risky, or even inappropriate, to conduct statistical tests for this result if homogeneity of variances is an issue.

B. QDA Spider Charts

Figure 57.2 represents a popular way for graphing descriptive panel results (2,27). Figure 57.2 displays results only for mouthfeel from a study that included additional modalities. Examining Figure 57.2, we readily see product differences for individual attributes, relationships among the attributes, and the overall tendency for a product to score low (Wine D), high (Wine K), or in the middle (Wine G) compared to other products evaluated.

C. Sensory Maps

Mapping is frequently used for displaying results of optimization research involving consumer acceptance and descriptive panel data. The maps often group well liked products together with sensory attributes and consumer information (e.g., lifestyle and attitude) identified as highly related to those product preferences. In the case of descriptive panel data, it is often helpful to visually display products by attribute results. Figure 57.3 is such a graph, and demonstrates how products may differ, or group, based on several attributes across different modalities. Here we see that Wines K and D are scored high for several different attributes; whereas, Wine G falls between the two. We would conclude that Wine K is a strong and high impact product in several areas.

Figure 57.4 is an example of a density map based on a cluster analysis of consumer acceptance scores for a broad range of products of the type typically included in a category review optimization. The cluster map reveals three reasonably unique preference groups embedded within the aggregate population. An appropriate follow-up sensory strategy is to use multiple regression techniques

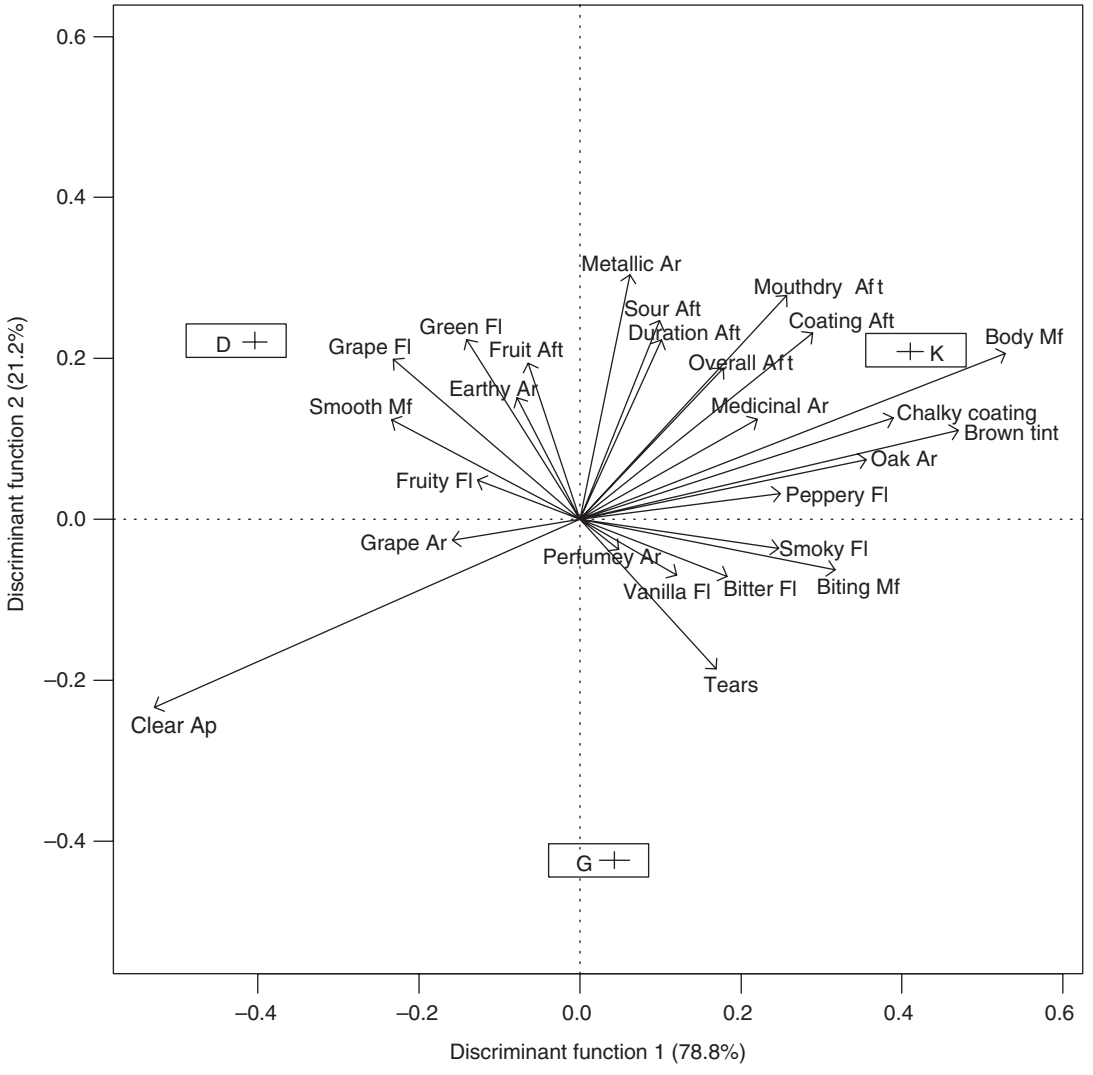

FIGURE 57.3 Attribute factor loading for trained panel evaluation of three wines; D, G, and K.

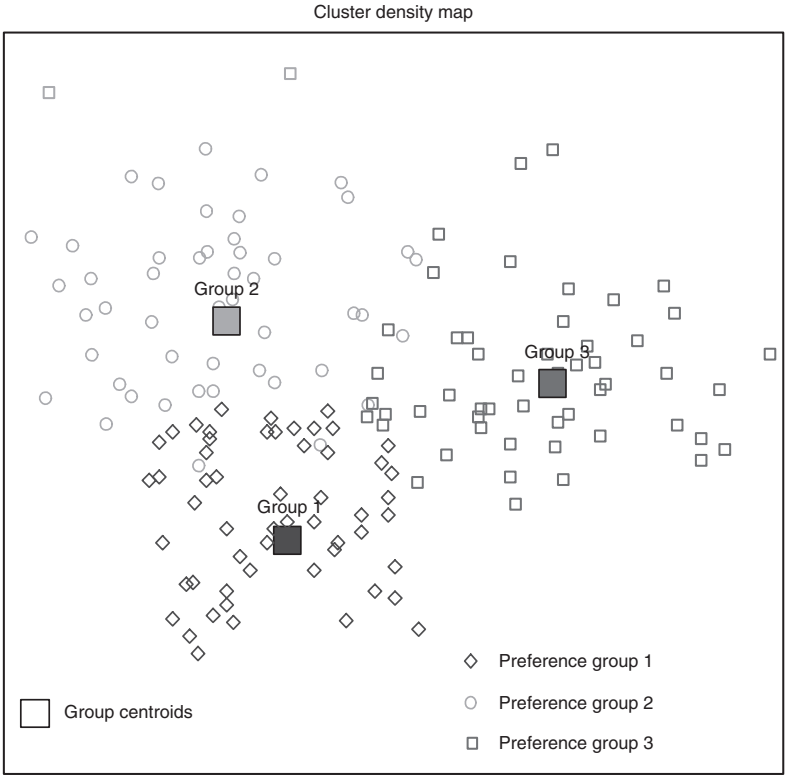

FIGURE 57.4 Cluster Analysis density map for aggregate acceptance scores. Three unique preference groups were identified.

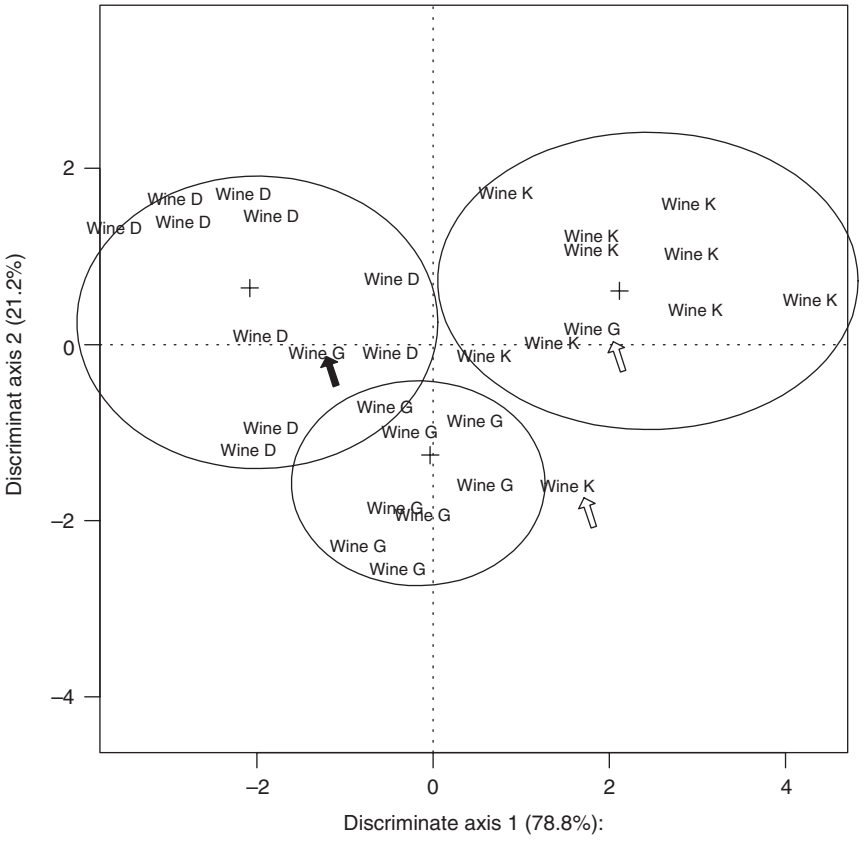

FIGURE 57.5 Discriminate plot for three wines across sensory attributes scored by each of 10 subjects. Arrows show two subjects that differ compared to the other subjects for their evaluation of Products G and K. Filled arrow is Subject #167; open arrow is Subject #542.

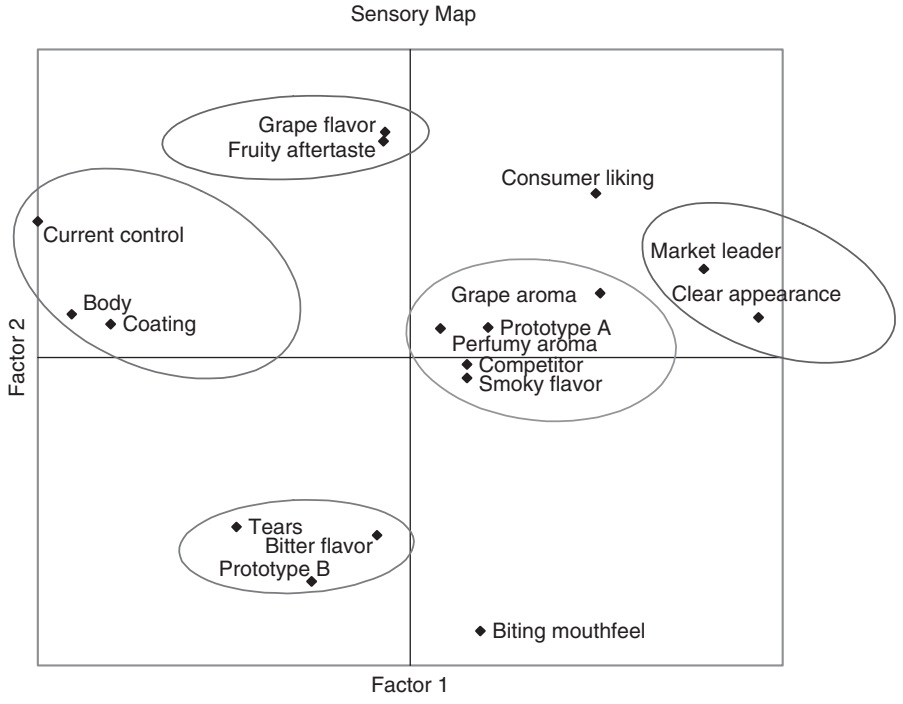

FIGURE 57.6 Sensory map integrating consumer liking and sensory panel attributes for selected products.

TABLE 57.1
Factor Analysis Summary for Consumer Liking and Sensory Attributes

	Factor 1	Factor 2	Factor 3	
Clear Appearance	0.935			
Grape Aroma	0.510			
Coating	−0.804		−0.554	
Body	−0.909			
Grape Flavor		0.732	0.557	
Fruity Aftertaste		0.702		
Consumer Liking		0.533		
Tears		−0.551		
Bitter Flavor		−0.557		
Biting Mouthfeel		−0.888		
Perfumy Aroma			0.950	
Smoky Flavor			0.873	
				Total
% Variance Explained:	41.13	21.92	19.83	82.88

to develop sensory models that best fit each unique preference group as well as one or more models for the aggregate population.

Figure 57.5 is based on a discriminate analysis of individual subject scores for the three wines. This information provides one way to visually identify how well subjects discriminated among the products. Examining Figure 57.5, we see that two subjects differed most among the panel regarding scores for Wines G and K. This information can be used to determine when additional training for subjects is required, or when a subject may be removed from a panel. In either case, these types of visual displays are readily obtainable and provide important insights about products and subjects.

Figure 57.6 is a sensory map that includes consumer acceptance and sensory panel results. The spatial relationships displayed in these types of maps (41) provide useful information to product developers and marketing researchers. In this case, the map identified which sensory attributes were most associated with which products, and that there is a development opportunity to optimize consumer liking for the product category. Sensory maps are typically derived from Factor Analysis (FA) or Principal Component Analysis (PCA) output, similar to that seen in Table 57.1.

X. CONCLUSION

Sensory evaluation is a valuable resource for the food scientist, and has yet to realize its full potential within the food industry. This is partly due to a lack of awareness of what it can do and how to best organize and operate sensory capabilities. The science is dynamic and its applications are almost endless, especially as it relates to its role in consumer research. The sensory sciences outlook is bright as more academic institutions teach and research the topic, and more qualified professionals enter the field. In recent years there has been a significant increase in the use of sensory methods as multinational and domestic companies introduce these procedures on a global basis. This trend will continue, creating new opportunities and demands for the science. Future success will depend in large part on how well sensory professionals and those

that teach and employ them understand that sensory evaluation is about perception; and how best to measure, analyze and communicate sensory information.

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58 Food Sensory Attributes

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CONTENTS

I. INTRODUCTION TO SENSORY EVALUATION

To measure sensory properties and thus determine if the overall objective was achieved is and will continue to be a major accomplishment for sensory evaluation. The development of knowledge and the achievements made possible through this gain regarding the attributes of a product or other pertinent information is another great source layered upon current sensory research and its general knowledge base. The capability of researchers and industry to perform sound sensory science techniques is at all time high. Growth in the area of sensory science has been exceptional during the last decade. Newly applied techniques and development of new modes of observation and data retrieval continue to be implemented and are thrusting sensory science continuously further.

The measurement of human behavior is now directed at a more systematic and professional approach to the testing process. Much of the prior accomplishments were achieved from an indirect method either through marketing services, relations to new product development, or through the dedication of a few select individuals devoted to the sensory profession. Advances in this field were initially slow due to the lack of interest and dedication in sensory science. Today, sensory science is a leading research tool applied to many processes and products.

The importance of the relationship between reliability and validity of results and the credibility of proposed recommendations is often a challenging process in sensory evaluation. Often, evaluations are performed, data is obtained, and results generated which in turn are shuffled into the "meaningless" grouping due to the misunderstandings of and between the separate working group sectors within an organization. Tactics such as these decrease the viability of the sensory group performing the procedures because the achieved information was not properly reported. The success of sensory evaluation, in particular its credibility, begins with an organized effort, and the assurance that the testing process followed accepted procedures and practices in all respected areas of work.

The field of sensory evaluation, being a relatively new discipline, has provoked many divergent opinions and philosophies from both within and outside its parameters. It would be difficult to state the one or two progressive developments that were initially responsible for the emergence of sensory evaluation as a discipline and the limited base of acceptance that ultimately defined the discipline. Agriculture and the international branching of such that began in the 1960's has been seen as one of the major pivotal areas providing growth and interest in sensory evaluation as a necessary function. Also, internationalization of the marketplace as a whole has greatly influenced this growth and interest.

The formal definition of sensory evaluation can be expressed in several different ways. However, the Sensory Evaluation Division of the Institute of Food Technologists (1, 2) has expressed a definition that provides meaningful insight into the subject:

> Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing.

This definition makes it clear that sensory evaluation utilizes all senses in determinations about products. Often, only taste is of consideration and the industry tends to overlook that sensory evaluation is not just "taste testing." Also, this definition emphasizes the many disciplines that are involved with the complete and correct workings of sensory evaluation. Sensory evaluation is a quantitative science in which numerical data are collected to establish specific relationships between product characteristics and human perceptions (3). Sensory evaluation is a science of measurement. Like other analytical test procedures, sensory evaluation is concerned with precision, accuracy, sensitivity, and avoiding false positive results (4). Analysis of data is a critical part of sensory evaluation. Data generated from subjects are often highly variable. There are many sources of variation in individual responses that are extremely difficult to control in sensory testing. Examples include the participants' individuality in personality traits, habits, attitude, acuity of perception, product familiarity and frequency of consumption.

The interpretation of results is one of the most difficult parts of the formal sensory evaluation process. Once the data are obtained and results generated, it is the tedious job of the sensory analyst to carefully write the report and supply the information to the necessary party of interest. Many persons housed in separate departments within a business or research facility take this information and focus on it in an incorrect manner due to the outcome often times not being as they would have liked.

II. PRINCIPLES OF SENSORY EVALUATION

The sensory technique chosen for a particular test is determined by the objective of the study. When testing for acceptability, trained panels should not be used; consumer groups should also not be used to generate descriptive data. Product samples should be labeled with three-digit random codes to alleviate bias. The sample order should be randomized to assist in reducing biases. Panelist influences should be kept to a minimum during evaluation.

A. Sensory Perception

Sensory adaptation, due to continuous exposure of a particular stimulus, will lead to a significant decrease in responsiveness. Foods can be complex mixtures encompassing all attributes. Due to the complexity of a mixture, suppression within modalities will occur. Thus, the perceived intensity will be affected and could possibly be recorded as a lower value than if the intensity were to be recorded as a single attribute, which would represent a truer representation of the value describing intensity.

Taste and smell can be confusing. Many people cannot distinguish between the two due to olfactory sensations arising from volatiles of the food matrix. If subjects are untrained, these sensations can be recorded incorrectly as either representing taste or smell inappropriately.

B. Sensory Testing

Sensory testing was initially developed from an industry standpoint to perform an economic function. It can determine the worth or acceptability of a product as its final result. The principal uses of sensory evaluation are in research, product development and maintenance, quality assurance and as a marketing tool. Sensory testing encompasses many fields other than food. Its primary function is to conduct valid and reliable tests that will in turn provide necessary data upon which sound business decisions can be made.

If sensory analysis is to be dependable, the skill of the sensory analyst in optimizing the proper techniques in sensory testing is of critical importance. There are several general steps that should be followed in sensory testing. The first step is to define the problem, followed by method choice, decision on panel type, experimental design, data analysis, and report generation. The method selection can be a decisive decision. The three categories of sensory techniques are: discrimination procedures, descriptive analysis, and affective testing. The discrimination procedures are designed for difference detection; descriptive analysis is focused on sensory changes; affective testing gathers consumer, preference, or hedonic data.

The selection of the subjects who will be the measuring instruments of the designated study is a critical step in sensory testing. Humans are excellent relative measuring instruments but poor absolute measuring instruments. The basis of qualification of human subjects can be one of the most time consuming sections of the sensory project. Subject or panel maintenance, performance measurement, participation monitoring, and other concerns are necessary duties that must be continually kept in the forefront of the sensory study.

III. SENSORY EVALUATION OF FROZEN FOODS

Before frozen food can be evaluated properly, quality measurements must be determined and properly defined. The definition of the sensory quality of a food or food product is "the acceptance of the sensory characteristics of a product by consumers who are regular users of the product category, or who comprise the target market for the product" (5).

The effects of prolonged storage of foods in the freezing range are varied. In some instances, such as with butter or fatty foods, the odor may become unpleasant by a process such as rancidity. In other foods, such as peaches, the process of oxidation may alter the appearance or color. In some products, such as fish, off-flavors may develop by

both physical and chemical processes. In other cases, such as asparagus, the texture may become undesirable. And in many cases, the nutritive value of the fruit or vegetable may be affected by processes of hydrolysis and oxidation. All these criteria that go into food quality — odor, appearance, flavor, texture, and nutritive value — are important in the defining of "satisfactory" food storage.

For example, frozen peas will lose their desirable bright green color and acquire a yellow to brown tinge at temperatures above 15°F (6), owing to a slow destruction of chlorophyll, the characteristic pigment of green vegetables and other plants. This change is not evident when the storage temperature is maintained at 0°F or less.

Yet tasting panel tests on frozen and stored halibut (7) and salmon (8) have clearly demonstrated that the temperature of storage is more important in maintaining quality than is the freezing rate. Even the texture of twice-frozen foods cannot be the equal of fresh-frozen quality produce. The following sections are brief overviews of selected foods and food groups. The authors selected the most predominant sensory aspects as the primary focus of discussion and review.

TABLE 58.1
USEPA* National Secondary Drinking Water Contaminant Standards (10)

Contaminant	Effects	SMCL** mg/L
Aluminum	Colored water	0.05–0.2
Chloride	Salty taste	250
Color	Visible tint	15 color units
Copper	Metallic taste, blue-green stain	1.0
Corrosivity	Metallic taste	Noncorrosive
Foaming agents	Bitter taste, odor	0.5
Iron	Metallic taste, orange staining	0.3
Manganese	Bitter metallic taste	0.05
Odors	Musty or chemical smell	3 TON***
pH	Low pH- bitter metallic taste, high pH- soda taste	6.5–8.5
Sulfate	Salty taste	250
Total dissolved solids	Salty taste	500
Zinc	Metallic taste	5

* USEPA — United States Environmental Protection Agency.
** SMCL — secondary maximum contaminant level.
*** TON — threshold odor number.

A. Water

The Safe Drinking Water Act (SDWA) establishes regulations for maximum contaminants (primary and secondary). It is not the aim of this section to review primary contaminants that may affect the health of consumers, but to review the secondary contaminants that can cause flavor problems in water itself and/or products in which water with such defects are used. The International Association of Water Quality (IAWQ) specialist group of taste and odors developed a Drinking Water Flavor Wheel that includes the four basic tastes recognizable by the tongue (sour, sweet, salty, bitter) and eight odor descriptors (chemical, medicinal, fishy, fragrant, swampy, grass/woody, chlorinous). Mouthfeel is a descriptor considered as part of the flavor component (9).

Table 58.1 shows the secondary contaminants in water according to the Environmental Protection Agency (EPA). Major problems encountered are the metallic taste cause by copper, iron, manganese, and zinc. Salty taste is caused by chloride, sulfate, and total solids dissolved. Bitter taste is produced by foaming agents, manganese, and low pH (10). Iodinated trihalomethanes, which are disinfection by-products, are considered to cause medicinal odor with odor threshold concentrations of 0.1 to 8.9 g/L. Using flavor profile analysis (FPA), these odorants are described as sweet, solvent, and medicinal (11).

To control taste in water, it is necessary to control inorganic ions. However, organic compounds such as 2-alkyl-5,5-dimethyl-1,3-dioxanes and 2-alkyl-4methyl-1,3-dioxolanes, malodorous compounds have been identified in groundwater as well as in river water and tap water (12). The use of threshold odor number (TON) for odor detection in water seems to not be the most adequate method; therefore, the use of flavor profile analysis (FPA) has been suggested (13,14). Some of the most common odor reference standards used in raw and finished drinking water for FPA are: geosmin (earthy), 2-methylisoborneol (earthy), trans-2-cis-6-nonadienal (cucumber, green vegetation), m-xylene (sweet solventry), benzofuran (mothballs), cis-3-hexe-1-ol (fresh grass, green apple), and dimethyl sulfide (septic, musty) among others (15).

However, especially for drinking water samples from river sources, there may be 200-300 volatile organic compounds present at concentrations greater than 1 ng/L as measured by capillary gas chromatography (GC). Any of these chemicals alone or in combination can cause water sample odor. The odor-causing compounds are often unknown. The concentrations of individual compounds are usually below their odor threshold concentrations (OTCs). Since the identification of all the GC peaks of a typical drinking water sample by GC/MS is usually not possible and the consideration of all compounds is desirable, statistical correlation methods can be used to mathematically describe the relationships between volatile chemicals and tastes and odors (16).

In the food industry, secondary contaminants have a zero tolerance for turbidity, color, odor and taste. To achieve these criteria several water treatments are available. The traditional water treatment is coagulation/flocculation. An alternative is the multiple barrier treatment that includes the use of Ultrafiltration/Reverse osmosis.

B. Bakery Products

Baked goods are highly perishable, and their attractiveness declines rapidly within a few hours of being taken from the cooking mechanism. Freezing is the most-used preservation method that significantly retards quality changes. This is the main reason for the huge market for frozen bakery goods. The history of the frozen food market began with frozen pizzas and doughnuts. The market has expanded tremendously in the last decade.

Like all foods with a significant level of water activity ($a_w > 0.9$), baked foods are subject to microbial contamination and growth during storage. Freshly baked bread is sterile; contamination with mold spores is deposited on the bread surface during cooling and/or packaging. Staling is another limiting factor in the shelf-life of baked goods. During baking, the starch granules present in the flour gelatinize. Upon cooling and storage at ambient temperatures, this gelatinized starch slowly recrystallizes. This retrogradation contributes to an increase in crumb firmness, and leads to a harsh, dry mouth-feel when the product is eaten (17).

Freezing of freshly baked product that has been properly formulated, frozen, stored and refreshed (regardless of the time it has been in the freezer) has sensory properties comparable with the same unfrozen product less than 1 day after baking (17). Quality defects observed in frozen, thawed fully baked products can be traced, for the most part, to migration of water during freezing, storage and thawing. Defects of migrational sorts are: slimy icing, disappearance of icing, wet surface area. Staling is accelerated at low temperatures, which can often lead to toughness of product. This toughness is mostly due to denatured protein. A high-gluten flour gives a tougher product than a low-gluten flour.

The icings, glazes, and fillings of baked goods depend on a fine balance between water, sugars and hydrocolloids to achieve the desired product characteristics (17). These properties include a dry appearance coupled with flexibility and short body, no free water and good suspension of solid fruit pieces for fillings. Freezing can alter the interaction between water and hydrocolloids so that on thawing, free water is present; this results in melting or disappearance of icings and glazes and leads to wet, leathery, unappetizing fillings. Ice crystals formed from free water are prone to sublime during frozen storage and a rim of ice on the inner surface of the packaging indicates that the water has not been adequately bound and stabilized in the icing or filling (17).

The sensory properties of bread and rolls can be greatly altered by the handling of the products. The importance of complete cooling is utmost and greatly affects the quality. Bagels have a chewy texture, and improper freezing techniques can lead to a leather-like texture. Also, soggy dough is a problem with bagels. Croissants are made with layered dough that is rolled into a thin sheet that is spread with shortening or butter. The detrimental freezing attributes here are decreased flakiness, chewiness, and often wetness. Pizza dough is ideally suited for frozen storage. Having a high surface-to-mass ration, the dough freezes quickly and efficiently in a freeze tunnel. Waffles and pancakes are much like bread and rolls regarding proper handling techniques before freezing. Sogginess and wetness is often a problem with waffles and pancakes.

TABLE 58.2
Sensory Attributes in Frozen Bakery (17)

Product	Attribute
Bread	Crumb firmness
	Harshness
	Dry mouthfeel
Icing, glazes	Wetness
	Leathery
	Unappetizing
Croissants	Flakiness
	Chewiness
	Wetness
Pizza	Sogginess
	Wetness
Cakes	Crumbliness

Cakes and other such sweet products have been successfully frozen without loss of sensory attributes. However, it has been noted that loss of volume by as much as 20% during frozen storage has occurred with certain formulations (17). Moister cakes show better volume retention, whereas dry crumb cakes such as chocolate tend to develop a crumblier texture after being frozen. Pastries that are frozen develop a dryness and hardness. If they are filled with filling, the filling will often be gluey or rubbery.

Biscuits, due to their low moisture content, are successfully frozen for lengthy periods of time. Freezing of biscuits imparts flavor retention and is an excellent method for preserving baked goods of this type. Cookies or cookie dough have similar properties of biscuits when frozen. Products of this type have very good retention of sensory attributes when thawed.

Pies are handled very similar to cakes, but they suffer a greater number of detrimental effects when thawed. Syneresis can be a major problem with pie that has meringue, or certain fillings. The fillings of crème pies can develop a rubbery or gluey texture after extended frozen storage and improper handling techniques. The filling must be formulated to withstand freeze-thaw cycles and still retain stability. Custard-type pies freeze well and typically thaw well with minimal loss of sensory attributes.

C. Dairy Products

The changes that occur during freezing vary among dairy products from minimal to extremely severe. The lower the

temperature at which the product is stored, the slower the deterioration rate of the product, and the better the product quality after defrosting. Since the storage temperatures depend on the type of product (frozen or non-frozen), there is a different relationship between the product in question, the storage temperature, and the product quality. Freezing points of dairy products should not be used as the temperature at which to freeze the product. This is due to the fact that storing products at a temperature above which ice is formed may successfully attain preservation, in some cases. As a result, one needs to understand the impact frozen storage has on product quality before choosing freezing as a preservation method. For a number of dairy products the changes during freezing are minimal while for others the changes are very serious. In liquid dairy products freezing causes destabilization of proteins and fat. The destabilization (dehydration) of the protein is accompanied by an increased concentration of mineral constituents and lactose in the unfrozen part. Ice crystals and fat crystals contribute to the destruction of the fat globule membrane leading to the formation of free fat. The lower the temperature, the slower the speed of the deteriorative reactions and the better the quality after defrosting. For every dairy product there is a different relationship between storage temperature and time it takes to undergo a certain amount of quality change (18,19).

1. Milk

Fresh milk may be frozen if storage is required for consumption at a later date or if shipment of the product is required. When thawed, however, the product should still taste relatively "fresh." The flavor of unfrozen whole milk is pleasantly sweet, possessing neither a foretaste nor an aftertaste other than that imparted by the natural richness (20). In addition, it should have no off-colors or lumps visible to the eye. These attributes are often retained better through freezing than any other method. However, if raw or pasteurized milk is improperly frozen, flavor deterioration may occur. Oxidized flavors may appear as a result of the destruction of the milk-fat globule membrane (21). These flavors may increase in intensity the longer the product is frozen. Tressler et al (22) stated that the occurrence of the traditional oxidized flavor defect can be reduced by adding small amounts of ascorbic acid, pasteurizing at relatively high temperatures, homogenizing and controlling the daily ration of the dairy cow.

Preservation of milk by deep-freezing also causes changes in the physicochemical balance, and separation of milk solids occurs upon thawing. Freezing milk at −10°C results in the destabilization of proteins and fat, whereas storage of milk at −30 to −40°C aids in retaining milk's stability longer (23). This destabilization, in turn, increases the mineral constituents and lactose present. By increasing the concentration of these components, tricalcium phosphate is precipitated and the pH value of the milk is reduced. Fat globule aggregation occurs and milky layers and butterfat lumps are noticed (23). Milk fat and total solids are lower in frozen milk samples. Therefore, milk should be stored at lower storage temperatures to minimize separation of the milk components and increase the quality of the product. Milk fat and total solids have been shown to be lower for frozen samples than for those analyzed fresh. Babcock fat test was also affected by rate of freezing and possibly by an interaction between freezing and thawing rates. Milk fat, protein, and total solids in fresh milk can be accurately predicted from determinations on milk which had been frozen and thawed (24).

Thawing of the frozen milk is also critical in minimizing sensory defects. Thawing the container of frozen milk in warm water or in a refrigerator overnight are considered satisfactory methods with minimal adverse defects (22). However, if the product is thawed improperly and lumps or gels are noticed, warming and stirring the milk may aid in redispersing the protein.

2. Cheese

Quality and acceptability defects from freezing are probably most noticed in cheese. Cheeses vary based on the type of milk they are made from, the method of milk coagulation used, amount of whey retained in the curd, whether the curd is ripened or unripened, method of ripening and the type of milk used to make the cheese (goat, cow, sheep). Structure and quality of the cheese are greatly affected by the fat content. The variances among cheese types affect the overall color, texture, odor, and flavor of the product. Freezing is not usually recommended with ripened cheese as a preservation method due to the physical breakdown in the body and structure of the product. However, it is useful in preserving unripened cheese like cottage curd. Previous studies have indicated no differences in the variables between the control and the frozen cheeses. The increase in pH at the end of ripening was less pronounced in the frozen cheese but not significant.

Processed cheese does not freeze well even with the incorporated emulsifiers typically found in these types of cheese foods. Gordon (25) stated that on no account is processed cheese to be stored below freezing point since freezing has a deleterious effect on the cheese structures as in normal cheese. The appearance of structural defects in processed cheese depends on the temperature, the concentration of water-soluble substances in the cheese and its fat content. The physical change of the structure of frozen cheeses does not affect the subsequent quality of the processed cheese manufactured from them. In a time of surplus Gouda and Cheddar cheese can be stored for 1 year (or even longer) at −10° to −20°C and can then be used for the manufacture of a good quality processed

cheese. This method of preservation does prevent the development of off-flavors or unpleasant sharp flavors found in cheese stored for a long time at normal ripening temperatures. According to Burkhalter (26), Emmental and Gruyere cheese used for making processed cheese may be stored frozen (at −20°C), but otherwise untreated, for approximately 18 months, provided that the outer zone (rind) with its tallowy, soapy flavor is removed. Storage at −10°C for 5 months had no significant effect on the structure and quality of processed cheese while the same type of cheese stored at −30°C became mealy. A cheese spread was even stable at −30°C.

Storage experiments carried out on skim milk cheese and cream cheese at −10°C and −25°C showed that these cheeses could be stored for up to 6 months. Apart from a slight whey separation after thawing, the texture remained smooth and the flavor was good. American Cottage became somewhat softer and had a rancid and sharp flavor. The off-flavor was probably due to the poor quality of the cream used for dressing. The results indicated that cream cheese can successfully be stored at −20° to −30°C for 4 to 12 months depending on the initial quality of the cheese (23).

External characteristics of cheese are the first items noticed by a consumer and qualified judge. The color is less intense in frozen cheese. Luck (23) stated this might be due to the microstructural changes of the paste, which occur due to the formation of ice crystals resulting from freezing the product. The structural changes give rise to a more open structure increasing the refraction and clarity of the cheese. In addition, the number, size and distribution of eyes formed in Swiss cheese are reduced and irregularly distributed.

Consumers observe the internal body and structural characteristics next, which include the texture and flavor of the product. This is where the major defects occur in cheese that is frozen. The physical structure of the cheese is broken down during the freezing and thawing stages. The extent to which the structure and body are affected depends on the degree of ripening, amount of salt used in the formulation, and the moisture content in the cheese variety. This in turn affects the degree to which ice crystals are formed when the cheese is stored between −1° to −16°C and the degree to which the defects are noticed. Hydrogen bonds in the polypeptide chains break down causing water to separate from the protein. A crumbly and mealy body is produced. The grainy texture of the body of cheeses is intensified by slower freezing speeds (27). The main defect of cheeses made from frozen curd is in body and texture. The external appearance of cheese pieces is usually normal whereas internally they were made up of layers, similar to those observed by Schulz in soft cheeses. This layered structure may be from ice crystal formation in the serum phase between the casein micelles although the deep-freezing was rapid (30 minutes at −40°C). The method used in France for deep-freezing curd is only for hard cheese. In this case mechanical stirring of curd or its mixing with freshly made curd before further cheese making, can be used to reduce this texture defect. Deep-freezing of curd is applicable for making Teleme from ewe's milk. The cheese produced is not inferior to the cheese made under normal conditions in constituents or sensory qualities. Deep-freezing of curd is applicable for making Teleme from ewe's milk (28).

The odor intensity, overall taste and acidity are also modified by freezing and storage. An increase of hardness and decrease in creaminess in frozen cheeses after 3 months of storage has been reported by (29). However, the acidity, odor, flavor and intensity decrease during storage (29) producing a very mild cheese. Frozen storage did not result in significant alterations in overall compositional, rheological and sensory properties or the level of lipolysis. The extent of proteolysis was slightly lower in the cheeses frozen prior to ripening. Although, some satisfactory results have been achieved when freezing soft cheeses (30).

The grainy aspect of cheese can be significantly affected by storage time. Quality parameters remained relatively constant up to 180 days storage typically following a sharp increase thereafter. This increase is a clear sign that the texture of the cheese is considered altered and granulous properties therefore determined. The graininess of cheeses frozen at slow speeds and for 9 months have been shown to be significantly different to that of the other storage methods of cheeses. Cheese graininess is therefore more intensely affected at slower freezing speeds and when the frozen storage period exceeded 6 months. In this respect, Luck (23) reported that freezing caused a breakdown in texture, prompted by the formation of ice crystals and the subsequent alteration of proteins.

Although some authors have described changes in the texture of frozen cheeses, it is recognized that intensity is greater with slower freezing processes (27). Neither the storage time nor the freezing process significantly modified the hardness and creaminess of the cheeses during storage. Slower freezing processes should be implored to minimize the flavor, odor, aroma and textural defects when processing cheese. The changes of the texture due to freezing can be prevented by storing the cheese at temperatures just above its freezing point (−2° to −5°C). This is a successful method of preservation. Camembert cheeses frozen immediately after making were acceptable after defrosting under normal conditions for up to 3 weeks. Not only does a slower freezing process minimize quality defects, it is more economical from the industry's viewpoint. When the freezing process is implored in this manner it is a practical and economical method in regulating the quality of cheese products, especially that of ewe cheese (29). Consequently, once the cheeses have been frozen, these attributes would not be modified by excessively long storage times. In this respect, Diefes (31) concluded that freezing lead to local protein dehydration, due to the destruction of the protein matrix, giving rise to

greater hardness of cheese. Differences are typically not seen during more prolonged periods of frozen storage in terms of the attributes characteristic of the appearance of the paste, color, appearance and eyes, due to the specific freezing procedure used. Odor, flavor intensity, acidity, and grainy appearance change as a consequence of frozen storage. With up to 6 months storage, practically no differences can be detected with respect to the nonfrozen cheeses; however, after 9 months storage, the acidity, odor intensity, and flavor decreased and the grainy body increases substantially. Saltiness was not affected by freezing nor by frozen storage. The speed of the freezing process only affects the graininess of the cheese, which is slightly greater in slowly-frozen cheeses. From the sensorial standpoint, freezing is therefore a suitable method for regulating the cheese market.

Cheeses could be stored at −20°C for at least 3–6 months, ensuring their availability for sale throughout the year. Slow freezing speeds are recommended, since the small deficiencies in quality detected in cheese frozen at slow speeds are recovered from an economic standpoint, since the faster process is much more costly. Alterations in texture (28, 27) and the presence of oxidized flavors were the main problems detected in cheeses after thawing. However, some of these studies have not reported any deficiencies in frozen cheeses (32), occasionally obtaining a higher sensorial quality with respect to nonfrozen cheeses (33). Odor intensity, intensity taste, and acid taste were modified by freezing and storage, so that to the 270 days of frozen storage were significantly descended. Significant differences have not been detected between salty taste of nonfrozen cheeses and frozen cheeses and stored in freezing.

Texture is substantially altered by freezing and frozen storage, with noticeable increases in the grainy body at 9 months of frozen storage. In the same way, an increase of hardness had been detected and a decrease in creaminess in frozen cheeses from 3 months of frozen storage. The external characteristics of the cheeses also demonstrate important changes after freezing. Color is less intense in frozen cheese. This could be due to microstructural changes in the paste, produced by the formation of ice crystals, giving rise to a more open structure, increasing refraction and making the cheese seem clearer. The number, size and distribution of the eyes are reduced after 90 days of storage in freezing, probably due to the destruction of the structure or wall forming the eyes. These usually decrease in size and in some cases disappear, giving rise to a more irregular distribution. Even though salty flavor is not modified by either storage time or the freezing process used, the intensity of flavor decreases significantly with longer frozen-storage times. Flavor intensity remains practically stable up to 6 months frozen storage, decreasing rapidly thereafter, and regardless of the freezing process use. Some authors have reported no differences in terms of flavor intensity between cheeses prepared from frozen curds and unfrozen cheeses (33), although others have also described very low scores in slowly frozen cheeses and thawed immediately (34). Similarly, other authors have reported no modification in the flavor of Cheddar cheese undergoing frozen storage (35).

As with regards to the intensity of acidity, frozen-storage time has a significant influence on the acidity of the cheese. Texture alteration is one of the adverse effects of freezing in cheese. This quality defect has customarily been attributed to the formation of ice crystals and the resulting mechanical effects. However, other findings suggest that alterations in cheese texture are a consequence of changes in protein structure, such as breaking of hydrogen bonds in the polypeptide chains and changes in water-binding capacity. Such changes lead to more active proteolysis in cheeses that have previously been frozen. In prefrozen cheeses, unordered structures increase immediately after thawing. The proportion of unordered and turn structures is apparently higher in the cheeses frozen more slowly in a plate freezer than in the cheeses frozen more quickly in liquid nitrogen vapor (27).

Slower freezing processes should be implored to minimize the flavor, odor, aroma and textural defects when processing cheese. The changes of the texture due to freezing can be prevented by storing the cheese at temperatures just above its freezing point (−2° to −5°C). This is a successful method of preservation. Camembert cheeses frozen immediately after making were acceptable after defrosting under normal conditions for up to 3 weeks. Not only does a slower freezing process minimize quality defects, it is more economical from the industry's viewpoint. When the freezing process is implored in this manner it is a practical and economical method in regulating the quality of cheese products, especially that of ewe cheese (29).

3. Ice Cream

Freezing is the standard method for processing and preserving ice cream. The quality, palatability, and yield of the finished product are all dependent upon freezing of the ice cream mix. Since no two individuals will execute the details of the freezing operation exactly the same each time, programmed freezers are becoming widely used in the industry to control quality of the product. An ideal ice cream possesses a fresh, clean, pleasant and delicate flavor with a smooth texture. It has a natural color; melts slowly into a liquid state and any particulates present are evenly distributed (36). Regardless, however, of freezer automation, defects can still occur in the flavor, body and texture of the product.

Texture defects are the most common result of improper freezing of ice cream. The most frequently noticed texture defect is referred to as coarse. It is observed by a grainy or icy feeling and accompanied by unusual coldness. Numerous factors contribute to this widespread defect.

TABLE 58.3
Sensory Attributes of Frozen Dairy Products

Product	Attribute
Fresh milk	Sweetness
	Off-colors
	Oxidized flavors
	Lumping
	Gelation
Cheese	Color- intensity
	Odor- intensity
	Flavor- overall, oxidation
	Off-flavor
	Texture- structure, crumbly mealy, grainy, hardness
Ice Cream	Texture- coarse, greasy sandy, gritty

Higher fat mixes decrease the occurrence of this defect in the product because fat globules obstruct the growth of ice crystals. Dipping cabinets are usually kept around −15°C. At this temperature it is reported that ice crystals can double in size increasing the coarseness of the ice cream (36). Therefore, temperature control in dipping cabinets should be more closely regulated to minimize this particular texture defect. A greasy mouthfeel is often associated with the buttery defect. The cause is over agitation of the ice cream mix in the freezer and can be reduced through automated freezing operations. One of the most objectionable defects in ice cream is known as a sandy texture. A gritty mouthfeel results from crystallization of lactose when temperature fluctuations and extended storage times occur. Again, temperature control is critical in controlling texture defects in ice cream.

Flavor and body defects usually result from the addition or absence of ingredients. However, one body defect resulting from temperature fluctuations during storage is that known as shrunken. The ice cream product does not contact fully the sides of the container according to Marshall and Arbuckle (36). When products are frozen to unusually low temperatures or if the product is allowed to warm and then is refrozen, the pressure on air cells changes and results in a shrunken appearance not desired by consumers.

4. Yoghurt, Cultured Milk and Buttermilk

Normal yoghurt, cultured milk and buttermilk are not successfully frozen to prolong their storage life. After a storage period of 3 months at −10°C the flavor is satisfactory but the texture is completely broken. There is an intense whey separation and the products give a mealy sensation on the tongue. Stirred yoghurt may be successfully frozen (−26°C) provided that the total solids content of the yoghurt is sufficiently high as in the case of plain yoghurt. Storage experiments carried out at −25°C on yoghurt of higher solids content did not confirm these results. The texture of the defrosted products was only slightly improved. The quality of the yoghurt was such that it would hardly have a chance on the market. Because it is possible to make frozen yoghurt dessert, it will probably be possible in the future to preserve yoghurt by freezing after adding a suitable stabilizer. At this stage fermented milks are not suitable for preservation by freezing.

D. Fish and Seafood

Human perception of food is complex and may be influenced by the sensory characteristics of the food material but also by the personal experience of the observer, psychological state, ethnic origin, religious conviction (37). Analytical testing to determine differences or similarities in seafood, to identify quality attributes, or to estimate relative intensity of sensory attributes is usually accomplished in a laboratory setting. Difference tests are designed to identify samples that differ in terms of sensory characteristics. Ranking tests (ordinal scaling) are used to determine the rank order of a set of samples for degree of a specified sensory attribute. Scalar testing methods are used to provide information about quantitative relationships among product samples. Descriptive analysis is the most demanding of any sensory method. Panelists determine the qualitative characteristics of aroma, flavor, texture, aftertaste, etc., and they describe a set of descriptors. The traditional flavor profile method requires intensive training (38).

Changes in appearance and eating quality that occur during frozen storage of fish are of great commercial importance. Freeze burn appears on the surface of frozen fish due to loss of moisture, cooked muscle flavor may possess a characteristic “cold store” note and toughness and dryness increase rendering a less acceptable fish. Tasting and determination of sensory attributes still remains the method of choice to detect changes during frozen storage. Sensory methods used to assess frozen fish can be classified into three categories: a) those in which tasters are asked to assess the fish on a scale of acceptability; b) those in which certain diagnostic features are rated on intensity scales; and c) those in which comparisons between two samples are made in order to distinguish the sample under test. Sensory factors that change during frozen storage include toughness and dryness and flavor. Scoring systems based on odor, texture, and flavor have been developed for fish inspectors. Freezing and frozen storage affect the appearance of fish on thawing (39).

The first quality judgment made by a consumer about a fish product at the point of sale is based on its color/discoloration. The next judgment is about the product’s texture: any gaping or ragged fillets. This is followed by odor judgment. A standard sensory evaluation for fish should include color of the flesh, odor and visual texture, absence/presence of blood clots and of bruising and discoloration,

absence/presence of physiological abnormalities, and workmanship (40). Aroma is one of the most important freshness determinants of fresh fish and fishery products. Sensory assessments are desirable because they provide immediate quality information. Initial changes in the aroma of freshly caught fish involve the shift from the fresh, planty aromas to a neutral, flat-sweet odor. This characteristic sweet aroma appears to be contributed by alcohols and 3,6-nonadien-1-ol, which has a sweet melon-like aroma (41).

Frozen storage is an important preservation method for seafood. Freezing and frozen storage contribute to changes in texture as a result of protein denaturation. Also, the thawing method is important since it is recognized that this can affect the sensory attributes of seafood. Myofibrillar proteins undergo denaturation and aggregation during frozen storage. Different species of fish have different degrees of susceptibility. Ingredients such as triphosphate, lecithin and sucrose ester have been used in order to prevent these changes that will affect the textural properties of muscle (42).

Considering all foods under the category seafood, there is a large range in texture. As long as texture falls within the normal range, it does not influence preference as do flavor and appearance. Texture is a critical factor for shellfish, surimi-based products, and squid (43). The texture of fish muscle changes from soft, moist and succulent to unacceptably firm, hard, fibrous and dry. The unacceptability of fish flesh/muscle as food is caused by its increased tenderness due to myofibrillar fragmentation. At −8 and −20°C, carp shows degradation in its myofribrillar fraction after six months (44). Freeze–thaw cycles affect the loss of protein solubility and the physicochemical and enzymatic properties of cod muscle proteins (45).

Sensory analysis is used to assess the quality of fish products. However, it is considered subjective and prompt to error. During frozen storage there is an alteration of functional properties of muscle proteins, loss of water-holding capacity, juiciness, and changes in texture. Mechanical properties can be measured. Shear resistance can be measured using the Kramer shear-compression cell. Correlation between sensory analysis and shear resistance measured by Kramer has been reported in fish hardened as a result of time in storage and also in fish stored at different temperatures. Shear resistance can also be measured using the Warner-Bratzler shear cell. Shear resistance as analyzed by the W-B cell has been found to increase significantly with time in frozen storage under different forms of pre-processing. The puncture test consists of measuring the force required to push a plunger into a food sample. This method assesses changes in texture during storage in ice as associated with the onset of *rigor mortis*. Tension analysis measures the force required to break a sample when is held by two parallel clamps. This test is sensitive enough to measure the viscoelastic properties of fish muscles and has been used to quantify textural changes in the mantle of squid. In compression analysis, the deformation is measured upon application of compression force. This analysis can be performed in one or two successive compression-relaxation cycles. In minced or cooked fish products a deformation of 50% is customary. However, for some mince products and fillets a deformation of 30 and/or 10% is used. Firmness generally tends to increase with time in frozen storage. Correlations between one-cycle compression measurements and sensory parameters such as elasticity in mackerel (*Scomber scombrus*) and hake (*Urophycis tenuis*) have been reported. When the first compression-relaxation cycle is followed by a second compression cycle, the test is known as texture profile analysis (TPA) (46).

Common descriptor terms for cooked seafood include: flavor intensity, fresh fish, gamey, old fish, sweet, briny, sour, seaweed, bitter, fish oil, buttery, nutty, musty, ammonia, metallic, shellfish, flakiness, firmness, moistness, chewiness, mouth-drying, fibrousness, oily mouth coating, cohesiveness, whiteness, and darkness (47). The Flavor Profile™ method is used to describe the aroma, flavor-by-mouth, and aftertaste attributes of cooked fish muscle. Intensity of aroma and flavor character notes are rated on the traditional 4-point profile scale: 0 = just recognizable or threshold; 1 = slight; 2 = moderate; 3 = strong; a designation of 0.5 was used to show an intermediate level of intensity. To describe 17 species of North Atlantic fish the following aroma descriptors were used: briny, sweet, fresh fish, old fish, stale fish, sour, shellfish, gamey fish, fish oil, earthy, nutty-buttery, musty, and scorched. The flavor descriptors were: salty-briny, sour, sweet, fresh fish, old fish, stale fish, shellfish, gamey fish, fish oil, earthy, nutty-buttery, canned salmon, bitter, metallic, mouth drying, and mouth filling (48).

A new seafood nomenclature system, to be used on the sensory or "edibility" characteristics of fish, has been developed by the National Marine Fisheries Service (NMFS) of the U.S. Department of Commerce. The goal of this system is to enable consumers to make educated choices among novel species by providing the comparative sensory data necessary to select a desired flavor, texture, etc. of fish. Standardized methodology for evaluating the sensory properties of cooked fish has been developed (49).

1. Bluefish

Bluefish has a strong total aroma and flavor impact, with a highly aromatic distinctive gamey, fresh fish, sour, fish oil character. The amplitudes (overall impressions) are moderate, with character notes blending together to give a very full aroma and flavor. The aroma of Bluefish has an early impact of a slight to moderate briny, moderate fresh and gamey fish notes, slight fish oil and sour notes, and a very slight sweet. The flavor has an early impact of slight

sweet, moderate fresh fish, slight to moderate gamey fish and sour (sharp), very slight fish oil and salty-briny, and slightly mouth drying. This fish has a distinctive sour (sharp) at a higher intensity than in most fish. In both aroma and flavor, the gamey and fish oil notes seem to be related. They are found at higher intensities in the dark flesh (especially near the skin), but are also present in the lighter flesh. The aftertaste is strong and persistent. The reported Profile integrates proportionate light and dark flesh. The most common sample variation was in the intensity of flavor of the fish oil and sour notes, and sometimes the flesh and gamey notes (48, 49, 37).

2. Blackback Flounder

Blackback flounder has a low total intensity aroma and flavor with a delicate, fresh fish character. The aroma amplitude is just below moderate and the flavor amplitude is moderate. The aroma is slight sweet and briny, slight to moderate fresh fish and very slight sour. The flavor is slight sweet, slight to moderate fresh fish, below slight salt-briny, sour and mouth drying. The aftertaste is a low level fresh, mouth drying and sweet (48, 49, 37).

3. Catfish

Frozen catfish (*Clarias gariepinus* Burchnell) fillets frozen and stored at −20°C for 11 month using an oxygen-permeable packaging (OPP) material and a vacuum packaging (VP) showed no significant differences between any of the aroma, appearance, texture or flavor attributes for the two packaging materials over time during frozen storage. Texture of the catfish fillets changed after 7 months, becoming more flaky with few ice crystals between the fiber, more so in the fillets packed in the OPP. No color changes were observed during the 11 months of storage. After this period of time the frozen catfish fillets were acceptable (50). In another study, channel catfish (*Ictalurus punctatus*) was evaluated during frozen storage concluding that regardless of size, channel catfish is acceptable after 12 months of frozen storage (51). On the other hand, channel catfish fillets treated with ascorbate and kept frozen at −6°C for up to 6 months showed less rancid flavor (52).

4. Cod

The flavor of cod (*Gadus callarias* L.) has been described as sour and slight cooked potato. Common flavor descriptors are: buttery, green, biscuit-like, roasty, mushroom like, cabbage-like, fatty, and metallic. The malty flavor defect is caused by a strong increase of 3-methylbutanal (53). Cod (market) has a moderately low total intensity aroma and flavor, with a low sweet, fresh fish character. The aroma and flavor amplitudes are moderate, as the overall impression of Cod is of a mild fish with an uncomplex aroma and flavor. The aroma is slight to moderate briny and flesh fish, and below slight sweet, sour and shellfish. The flavor is slight sweet and mouth drying, slight to moderate fresh fish, very slight salty-briny and sour, and below slight shellfish. The aftertaste is fresh fish and sour. Occasionally, samples of Cod have a stale fish character note. Cod (scrod) has a moderately low total intensity aroma and flavor, with a low sweet, fresh fish character. The flavor is below slight sweet, slight to moderate fresh fish, very slight salty-briny, sour, and earthy (slight cooked potato), and slight mouth drying. The aftertaste is fresh fish, mouth drying, and sour. Some cod (scrod) samples have produced a stale fish character note and/or a metallic note.

During processing and storage, fish quality may decline due to oxidation of unsaturated lipids causing off-flavors and odors. In lean fish the production of formaldehyde (FA) is important along with protein denaturation and texture changes. Cod (lean fish) and haddock (non-FA-forming) show hydrolytic activity during frozen storage. Storage at −30°C decreases hydrolytic activity (54). For cod, deterioration in texture of the thawed product is a serious problem. The presence of formaldehyde due to enzymatic degradation of trimethylamide oxide (TMAO) into dimethylamine (DMA) and formaldehyde (FA) is an increasing factor of the denaturation of proteins. The quality index method (QIM) is normally used to evaluate fresh and frozen cod. This method is based on a selected number of independent parameters, which describes the quality of thawed cod. The scores for all single parameters are added to give the total quality index. For fillets the parameters are texture, color, bloodstains and gapping, which vary from 0 to 3, and odor and parasites, which vary from 0 to 2 (55).

Cod fillets are generally frozen after catch. It is recommended that fish filleted prior to *rigor mortis* must be frozen to diminish the risk of shortening and deforming. Cod fillets kept at frozen storage for 17 months and evaluated by a trained panel showed that fillets frozen before rigor keep fresher than those frozen after rigor after two months of frozen storage. The attribute 'dry/succulent' is rated in a scale from 0 to 100. Cod receives a score between 60 and 70 after two months of frozen storage. After 12 months fillets are evaluated 'drier' (score = 40). There is a direct relationship between frozen storage and the attribute 'succulent/dry.' The same relationship has been reported for frozen storage and 'tough/tender' (56).

North sea cod kept for 2, 5, 8, 14, and 17 days in ice, filleted and frozen in an air blast at −34°C and stored at −14°, −22°, and −29°C showed a gradual development of unpleasant odor and flavor called 'cold storage odor.' At the same time the fish becomes firmer and drier until it becomes tough and dry. Freshness flavor is unaffected by the freezing and thawing treatment, while firmness and dryness increase significantly (57). During freezing

at −20°C loss of tenderness and of water-holding capacity and fish flavor changes may occur. Prolonged storage of fish causes deterioration of texture as described in increased toughness, chewiness, rubberines, or stringiness. Toughness increases over time, whereas cohesiveness decreases during storage. Higher storage temperature results in faster rates of textural deterioration of cod muscle (58). Frozen cod fillets change texture over frozen storage (59) as a result of muscle proteins denaturation causing toughness of the muscle. Rapidly-frozen and slowly-frozen cod show no more denaturation after one year of storage than newly frozen fillets (60). Sensory attributes of thawed and refrozen cod include color, flavor (strength, fresh fish, saltiness, sweetness, stale, rancid, bitterness, and other flavors), and texture (firmness, flakiness, resilience, juiciness, cohesiveness, fibrousness, roughness, and fiber size). Thawing/freezing cycles do not affect the structure of the muscle even after 9 months of storage. However, slow thawing followed by refreezing resulted in fish that was grayer in color and staler in flavor. Storage time affected the sensorial attributes (61).

Frozen cod can develop off-flavors during storage. The main component for this off-flavor is hept-cis-4-enal. Samples stored for 18 months at −15°C had some of the following compounds: hept-*trans*-2-enal and hepta-*trans*-2,*cis*-dienal. These two compounds were examined and described as cold storage flavor (cardboard, musty). Threshold study showed that hept-*cis*-4-enal is detectable on very low concentrations (0.00004 ppm). Aldehydes present in cod samples originated from lipid oxidation and tend to increase with temperature of storage. The major aldehyde produced was hepta-*trans*-2,*cis*-dienal (62).

5. Cusk

Cusk has a moderately low total intensity aroma and flavor, with a low sweet, fresh fish character. The aroma and flavor amplitudes are below moderate, as this fish lacks fullness and blending, especially in flavor. The aroma is below slight sweet, slight to moderate fresh fish, slight briny and sour, and below slight shellfish. The flavor is below slight sweet and shellfish, slight to moderate fresh fish, slight salty-briny and mouth drying, and very slight sour. The flavor is flat, washes out quickly, and is affected by the chewy, slightly tough texture. The aftertaste is fresh fish, sour, and mouth drying (48, 49, 37).

6. Haddock

Haddock has a moderate total intensity aroma and a moderately low total intensity flavor, with a sweet, fresh fish, shellfish character. The aroma amplitude is just below moderate and the flavor amplitude is moderate, as this fish has a fullness of flavor and an interesting shellfish characteristic. The aroma is slight to moderate briny, and fresh fish slight sweet, shellfish, and mouth drying, fresh fish at slight to moderate, sour at below slight, and salty-briny at very slight. The sweet, fresh fish, and shellfish flavors seem to be related, and the sour taste is fleeting. Earthy (slight cooked potato) and metallic notes, particularly in the dark flesh portion, have been recorded. The aftertaste is fresh fish, sour and shellfish. There was some variation within a session and between sessions in the Haddock samples, particularly in aroma, and was evidence as stale fish character notes (48, 49, 37).

7. Hake

White Hake has a moderately low total intensity aroma and flavor, with sour (dishrag) aroma, and an earthy note in flavor. The aroma and flavor amplitudes are low to moderate due to the old fish, sour (dishrag), and earthy notes, which indicated a lack of blended characters. The aroma of White Hake is a slight to moderate sour (dishrag) and fresh fish and below slight sweet and briny. Aroma notes mentioned by panelists are old fish, earthy (slight cooked potato), and shellfish (clam). The flavor is slight sweet, sour (sharp), and mouth drying, slight to moderate fresh fish, below slight salty-briny and earthy (slight cooked potato). There was an unblended quality to the flavor, and when both earthy and old fish occurred, they seemed related. The aftertaste is fresh fish, mouth drying, sweet, earthy, and sour. This fish often showed great variation in the fresh fish and old or stale fish characteristics.

Quality of hake species (*Merluccius merluccius*, *M. hubbsi*, and *M. capensin*) varies for many reasons ranging from inappropriate fishing and freezing methods to inefficient distribution. Textural (mechanical) analyses such as puncture test, Warner-Bratzler, and Kramer press have been used in order to account for the variance of frozen fillets. As a result of this, four clusters were identified, ranging from excellent quality (low texture, high viscosity) to very poor (high texture, low viscosity). The parameters required for this classification are viscosity, maximum force from Kramer and maximum force and energy from the puncture test (46). Frozen Patagonian hake fillets (*Merluccius hubbsi*) evaluated by using the cook sensory assessment (CSA) shows that fillets remain acceptable for over 12 months both at −20 and −30°C (63).

8. Halibut

Halibut has a moderate total intensity aroma and flavor, with a sweet, fresh fish character, and a very slight gamey fish flavor note. The aroma and flavor amplitudes are below moderate as some samples lack a blended flavor. The aroma is slight briny, slight to moderate fresh fish, below slight sweet and sharp, sour (pungent). The flavor is very slight sweet, slight to moderate fresh fish, above

slight sour, slight salty-briny and mouth drying, below slight gamey and fish oil. The definite sour flavor note is higher than in most fish. The aftertaste is predominantly sour and mouth drying, with some fresh fish and another hard-to-describe note that seems to be a combination of fish oil and gamey (48, 49, 37).

9. Herring

Whole herring (*Clupea harengus*) wrapped in polyethylene film and block-frozen using plate freezers and frozen for 32 days at −24°C were beheaded and salted. Ten trained panelists conducted a sensory analysis to evaluate taste using the following descriptors: ripened, raw, malty/creamy, stockfish, salty, sweet, spicy, and aftertaste. The attributes for texture were: softness, watery and toughness. Spice-salted herring taste characteristics were typical with a dominant malty/creamy taste and a fairly low intensity of stockfish character. The thawed salted herring achieved higher values of the ripened taste during the first few weeks in comparison with the fresh salted herring. Also, the thawed spice salted herring appeared to become soft quickly during storage, indicating a faster rate of ripening. Hardness increased after salting but decreased steadily. Hardness of fresh spice-salted herring at 21 weeks was comparable with thawed herring kept for 16 weeks (64).

Herring is susceptible to lipid oxidation during processing and storage. Pre-freezing storage in refrigerated seawater followed by minimal tissue disruption, removal of oxygen, fast freezing, and low freezer storage temperatures reduces rancidity and gives the longest shelf life. Fillets of herring kept on ice prior to storage at −18°C for up to 84 days show that samples held for 6 days on ice formed oxidation product at the highest rate during frozen storage. Ice storage had a greater impact than frozen storage (65).

10. Grouper

Grouper has a moderately low total intensity aroma and flavor, with a low level of aromatics. The aroma and flavor amplitudes lack full-bodied characteristics, have a low impact of blended character, and low intensity of fresh fish characteristics. The aroma of Grouper has an early impact of slight sweet, and briny notes, and slight to moderate fresh fish, followed by a very slight sour (pungent), and a shellfish note. The flavor is very slight sweet, salty-briny, slight fresh fish, sour (sharp), and shellfish, and slight to moderate mouth drying (the highest intensity for the fish tested). The aroma and flavor shellfish note has a clam character. There are confusing overtones to the sour and shellfish notes that are difficult to describe. The aftertaste is persistent and consists of a moderate level mouth drying and sour, and a low level of fresh fish. The dry tough texture is difficult to ignore. The samples seemed relatively consistent within a session, and the greatest variations between sessions seemed to be in the sour, shellfish notes, with confusing overtones (48, 49, 37).

11. Mackerel

Mackerel has a strong total aroma and flavor impact, with a highly aromatic distinctive gamey, fresh fish, fish oil character. The amplitudes are moderate, with character notes blending together to give a very full aroma and flavor. The aroma of Mackerel has an early impact of moderate gamey and fresh fish notes, a slight to moderate fish oil, and slight sweet, briny and sour notes. The flavor has an early impact of moderate gamey and fresh fish notes, a slight to moderate fish oil, slight sweet and sour (sharp), and a very slight salty-briny and mouth drying. The mouth drying characteristics seem largely overpowered by the oiliness. In both aroma and flavor, the gamey and fish oil notes seem to be related. They are found at higher intensities in the dark flesh, but are also present in the lighter flesh. The reported profile integrates proportionate light and dark flesh. The gamey fish note of Mackerel is heavy and sometimes associated with a burnt or scorched characteristic. Despite the intensity and heaviness of this fish characteristic, it is typical of fresh Mackerel and not indicative of aged fish. The aftertaste is strong and persistent (48, 49, 37).

12. Monkfish

Monkfish has a moderately low total intensity aroma and flavor. Its distinguishing characteristic is a distinctive shellfish aromatic note similar to lobster and clam. The aroma and flavor amplitudes are moderate as this fish is full, complex and blended with interesting notes. The aroma is slight to moderate briny (fresh, seaweed), fresh fish and shellfish, and slight sweet and sour. The dark flesh and gelatinous areas near the skin have more intense briny-seaweed and shellfish-clam character than with flesh. The flavor is slight sweet and sour, slight to moderate shellfish and fresh fish, threshold salty-briny, and very slight mouth drying. The shellfish note adds interest and complexity to the aroma and flavor. The sweet, shellfish, fresh fish and buttery notes seem closely related and give a full flavor. The aftertaste is sweet, fresh fish, and shellfish (48, 49, 37).

13. Pollock

Pollock has a moderately low total intensity aroma and flavor with sweet, fresh fish, low shellfish character. The aroma and flavor amplitudes are moderate, as they are blended with some interest notes. The aroma is slight sweet, briny and sour (sharp), slight to moderate flesh fish, and below slight shellfish-clam. The flavor is slight sweet, sour (sharp) and mouth drying, slight to moderate

fresh fish, below slight shellfish-clam, and very slight salty-briny. Some of the samples had a small amount of dark flesh which a hard to define character related to gamey and fish oil. The aftertaste is sour, fresh fish, and sweet, with a persistent mouth drying. Metallic and stale fish notes can be found with this fish also (48, 49, 37).

14. Salmon

Flavor profiles of fish aroma and intensity differ among species. For fresh salmon, a fish oil characteristic is common. Also, bitter, metallic, nutty/buttery, and sour characteristics are often observed. Homogenates of salmon stored for 26 weeks at −60°C and −13°C and evaluated using aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry (GC-O) gave some of the odor descriptors such as sweet, buttery, vegetable-like, green, cabbage like, mushroom-like, citrus-like, and cucumber-like. Green (Z-3-hexenal) and fatty green (Z,Z-3,6-nonadienal) are responsible for flavor defect in boiled salmon when stored frozen for a long period. The levels of these two compounds are higher in salmon because of its higher content of n-3 unsaturated fatty acids (53).

Components of low volatility can be the key compounds causing off-flavor. Formation of less volatile oxidation products could also explain why the volatile components identified in the stored salmon did not show significant time-temperature interaction effects nor did they correlate to the sensory attributes.

The most noticeable sensory change during frozen storage of salmon is an increase in intensity of train oil taste, bitterness and metal taste. This change is produced by compounds of low volatility but most of all due to free-fatty acids. Palmitoleic acid and linoleic acid, eicosapentaenoic acid, and docosahexanoic acid have high intensity of train oil, bitter and metallic taste (66). Frozen salmon (*Salmo salar*) become rancid after a few months of frozen storage. Also, fat content of fish diet has a direct relation in some sensory attributes such as color (redness) and the taste of salmon. Fish fed with 17% fat diet has shown differences in color (less red) and taste (less strong). Frozen storage temperature also influences color, hedonic consistency, hardness and juiciness (67).

Rancidity in fatty fish such as salmon is not well characterized and has been described as fish oil taste (67, 68) and as fatty and train-oily odors (53). The rancid off-flavor in salmon is caused by formation of volatile oxidation products such as aldehydes and ketones (53). Some of these volatile compounds have very intense odors and flavors and are, even in small concentrations, able to affect the sensory quality. The rancid off-flavor of salmon is mainly caused by an increase in (E,Z)-2,6-nonadienal with a cucumber odor, (Z)-3-hexanal with a green odor, and (Z,Z)-3,6-nonadienal with a fatty odor (53).

The odor of fresh raw salmon is characterized as cucumber-like with weak sweet, sourish, and fish oil notes. For the fresh and cooked salmon the same descriptors were used, but a boiled potato odor has been described as the most pronounced attribute instead of cucumber.

Panelists normally recognize sensory changes of frozen salmon. For train oil, metal, and bitter taste significant time-temperature relations have been determined. The intensity of these attributes increased during storage at −10° and −20°C. Changes in texture are also noticed at −10° and −20°C. Fresh cooked salmon is described as flaky, form, and juicy. After storage, salmon changed to firmer, less juicy and more fibrous (69, 70).

15. Shrimp

Texture, color and flavor of shrimp are influenced by frozen storage. Quality of frozen-stored shell-on and shell-off prawns at −29°C depends on thawing method and frozen storage time. Muscle proteins of freshwater prawn tails are susceptible to freezing-thawing processes, particularly during the first month of frozen storage. Protein destabilization is independent of the freezing method (blast versus still) and the presence or absence of shell, but is affected by thawing rate (71). Quick frozen prawns (*Machrobrachium rosenbergii*) stored at −18°C for up to 225 days and tasted after 1 day of freezing were rated acceptable. At 133 and 225 days, prawns had acceptable texture but thawing time must be increased from 4 hours, for 133 days old, to 24 hours for the 225 days old. Moreover, prawns should be cooked unthawed or after thawing for no more than 4-8 hours to retain its texture (72).

Shrimp are frozen shortly after catch and kept frozen until they reach the consumer. Shrimp kept in frozen storage can develop white spots in the shell, which definitely influence their appearance. During continued frozen storage the spots increase in size; therefore, the quality decreases. White spots are usually developed after 25 days of frozen storage; however, this is a result of the time a shrimp takes to pass through the production process. More important is the storage temperature. Alternating storage at −25°C and −29°C results in increased white spot formation compared to constant storage at −29°C (73).

Sensory variables for cooked, peeled and individually frozen shrimp (*Pandalus borealis*) include evaluation of natural red color, yellowish discolor, dehydration, shininess, smoothness, natural shape, total odor intensity, fresh odor, stale odor, fishy, ammoniacal odor, urine-like odor, sour odor, old seaweed odor, sulfide odor, rancid odor, cardboard odor, mud-like odor, total flavor intensity, fresh, sweet flavor, stale, fishy, sour, metallic, acrid, cardboard, stockfish-like flavor, rancid, mud-like, salty and aftertaste (74).

16. Squid

Studies indicate that squid has excellent freezing characteristics. Glazing or packing of the frozen blocks is essential in order to prevent desiccation during frozen storage.

Quality tests indicate that frozen squid at −18°C or lower can be kept for one year in storage. The sensory characteristics to be assessed in a sensorial examination are: appearance, texture, and odor of raw product, and texture, odor and flavor of cooked product. Freshness of cooked squid can be expressed in a 10-point flavor score scale that includes: fresh (sweet, meaty), slight loss of freshness (creamy, sweet, meaty, metallic), slightly sweet (slightly meaty, creamy, milky), No sweetness, slightly sour, sour (musty, cabbage), slightly bitter (overripe cheese, oily, slight sulphide), bitter (sulphide), and strongly bitter. Texture can be grouped into three categories: 1) normal (firm and rubbery); 2) borderline (mushy, slightly sticky or very firm and rubbery); 3) unacceptable (extremely mushy and sticky or extremely firm and rubbery) (75).

Squid is one of the most highly potential sources of fish protein. Frozen foods and surimi-based products using squid as raw material have become a trend in recent years. The mantle muscle of squid has a specific toughness that is maintained after a second freezing and thawing process. The mantle toughness (hardness) of fresh Argentinian squid (*Illex argentinus*), neritic squid (*Loligo edulis*) and cuttle fish (*Sepia pharaonis*) increase after frozen storage after 120 days. The protein pattern of Argentinian squid mantle refrozen and thawed does not change. Histological samples show that muscle fibers are injured and aggregated over time being that these changes are the ones responsible for toughening of mantle (76).

17. Striped Bass

Striped bass has a total intensity of aroma above moderate and total intensity of flavor at moderate. It is a moderately strong flavored fish with a fresh, gamey, fish oil character. The aroma and flavor amplitudes are moderate, with character notes blended to give a full aroma and flavor. The aroma has an early impact of fresh and gamey fish at slight to moderate, slight sweet, sour and briny, and below slight fish oil. The flavor also has an early impact of fresh, gamey fish, and sour notes at slight to moderate, sweet, salty-briny, and mouth drying at slight, and fish oil at below slight. The sour flavor note is stronger than in most fish. Particularly in flavor, the gamey and fish oil vary, being more intense in the dark flesh. The aftertaste is sweet, sour, and fresh fish, with some gamey fish character (48, 49, 37).

18. Swordfish

Swordfish has a moderately low total intensity aroma and flavor, with a sweet, fresh fish, shell fish character. The aroma and flavor amplitudes are moderate, complex, tightly blended, and the fish has full flavor, with complex first notes, sour (sharp), and mouth filling characteristics. The aroma is just below moderate fresh fish, slight to moderate shellfish, slight sweet and briny, below slight sour, and very slight nutty-buttery. The flavor is just below moderate fresh fish, slight sweet, shellfish, mouth drying, and mouth filling, slight to moderate sour (sharp, citric), very slight canned salmon, salty-briny, and fish oil. The fish oil appears late in the flavor. In aroma and flavor, the fresh fish, shellfish, and nutty-buttery seem rich, closely related, and to have a salmon or tuna character. The canned salmon flavor note is also related and hard to define. The sour (citric) flavor is almost lemon and is stronger than in most of the fish tested. The aftertaste is sour, fresh fish, sweet, and shellfish (48, 49, 37).

19. Tilefish

Tilefish has a moderately low total intensity aroma and flavor, with a sweet, fresh fish, shellfish character. The aroma and flavor amplitudes are moderate, as this fish is rich and full, complex, and blended. The aroma is slight to moderate fresh fish, below slight sweet and sour (pungent), and slight shellfish and briny. The sweet, fresh fish, and shellfish notes seem closely related and associated with a rich character like butter, lobster or scallop. The aftertaste is fresh fish and sweet (48, 49, 37).

20. Trout

Frozen fillets of rainbow trout (*Oncorhynchus mykiss*) stored at −18°C, wrapped with either polyethylene or polyethylene/polyamide (vacuum), and subjected to a light/dark environment have shown that packaging materials have a strong effect on the development of rancid flavor. Also, the fillets stored in darkness generally received better appearance scores than the ones stored in light (77).

21. Tuna

White tuna (*Thunnus alalunga*) possess white color, firm texture, flavorful flesh, and high nutritional content. To extend production, frozen Albacore tuna is used as raw material in the canning industry. Measurement of sensory, chemical, and physical changes has shown that deterioration of fish quality continues during frozen storage. Quality tests include visual appearance evaluation based on general external appearance, eyes, gills, consistency, and ventral cavity. These parameters are used to classify Albacore tuna in four quality categories (highest quality, good quality, fair quality, and rejectable). Frozen tuna quality is good after 12 months of frozen storage. However, after 6 months some specimens developed a slight rancid odor (78).

22. Weakfish

Weakfish has a moderate total intensity aroma and flavor, with a weak fresh fish, gamey, briny (seaweed) character, and a late, low level fish oil flavor. The aroma and flavor amplitudes are below moderate, as the character notes are

TABLE 58.4
Aroma, Appearance, Texture and Flavor Attributes Measured in Selected Fish

	Sensory Attributes			
Product	**Aroma**	**Appearance**	**Texture**	**Flavor**
Catfish (*Clarias gariepinus*) (50)	Earthy fresh Rancid (oil) Off-aroma	Color Juiciness Flakiness Sponginess Stickiness	Juiciness Mushy/soft Gelatinous Sponginess Stickiness	Earthy fresh Sweet Salty Sour Earthy muddy Bitter Rancid Off-flavor Aftertaste
North Atlantic Fish (48)	Briny Sweet Fresh fish Old fish Stale fish Sour Shellfish Gamey fish Fish oil Earthy Nutty-buttery Musty Scorched			Salt-briny Sour (Sharp) Sweet Fresh fish Old fish Stale fish Shellfish Gamey fish Fish oil Earthy Nutty-buttery Canned salmon Bitter Metallic Mouth drying Mouth filling
Salmon (*Salmo salar*) (69)	Boiled potato Train odor	Color	Firmness Juiciness Fibrousness	Earthy taste Fish oil Train oil Bitter Metallic
Cod (*Gadus morhua*) (61)		Color	Firmness Flakiness Resilience Juiciness Cohesiveness Fibrousness Roughness	Strength Fresh fish Saltiness Sweetness Stale Bitterness

not fully blended. The aroma is slight to moderate briny (stronger than in most fish, with a seaweed character), slight fresh fish, gamey and sour, and very slight sweet. The flavor is slight to moderate fresh fish, slight sweet and sour, below slight gamey, and salty-briny, above slight mouth drying, and very slight fish oil. Gamey and fish oil are more intense in the dark flesh. The aftertaste is mouth drying, fresh, gamey fish, with sweet, and sour notes. Compared to other fish, this aftertaste is stronger and more persistent. The character notes of individual samples varied more than in most fish, especially in the levels of gamey and fish oil notes, which depended largely on the amount of dark flesh present (48, 49, 37).

23. Minced Fish

Turbot (*Atheresthes stomias*), grey cod (*Gadus macrocephalus*), Dogfish (*Squalus acanthias*), shortspine thornyhead (*Sebastolobus alascanus*), pollock (*Theragra chalcogramma*), red-banded rockfish (*Sebaster babcocki*), and ocean perch (*Sebaster alutus*) have been evaluated to produce frozen minced fish flesh. Significant differences were found after one month of storage in odor and taste quality. Pollock, pollock/red-banded blend and ocean perch were given relatively high odor and taste scores. After 3 months, no significant differences in odor quality were found among the different fish species. Differences

were found in taste quality, turbot and ocean perch were given the highest scores. After 5 months, there were no differences in odor quality but significant differences were found for taste quality. After this point Pollock was scored unpalatable. Texture of the flesh of the different species can be grouped into three categories: relatively firm, intermediate in firmness, and relatively soft. Color of the minced flesh for all species changes only in the lightness of hue (79).

Atlantic cod (*Gadus morhua*) frame mince without kidney tissue, responsible for 'chemical' and 'petroleum' type flavors, was stored at −14° or −40°C. Aroma, juiciness, texture and flavor of the cooked samples was evaluated. Instron hardness scores were reduced during storage observing a soft, mushy texture. Color is one of the most important factors for mince quality. Panelist scored fillet mince samples juicy with a soft and moderate texture. No off-odors or off-flavors were detected. Frame mince samples were also juicy but more fibrous and firm than fillet mince (80).

Raw, thawed cod mince texture is commonly evaluated for springiness (0 = completely plastic, 10 = very springy) and stickiness (0 = breaks down, 10 = very cohesive). The frozen deterioration of cod mince is reflected in an increase of cohesiveness (81). Texture scores in cod frame mince stored at −7°, −14°, and −40°C for springiness and cohesiveness are higher for mince stored at −7° and −14°C. Changes during frozen storage change sensorial properties of the mince (82).

Quality changes of fish muscle are normally due to autolytic chemical reactions, microbial proliferation, and physical property alterations. Frozen storage of catfish mince at −20°C can increase storage life to at least 3 months (83). Washed and unwashed catfish mince do not change color during frozen storage at −20°C (84). Washing of catfish mince using sodium citrate, sodium erythorbate, sodium citrate plus sodium erythorbate, sodium citrate plus sodium erythorbate, and polyphosphate reduce lipids and increase Hunter 'L' values (85).

E. Fruit and Vegetables

The most common problems in frozen fruits and vegetables are reactions that cause changes in flavor, color, texture, and nutritional value. A major result of freezing on fruits and vegetables is usually a loss of tissue firmness. Freezing causes disruption of the membranes of cells, and excessive softness (86).

Fast freezing rates produce a large number of small ice crystals that causes less damage. Freezing large-size foods involves problems due to thermal gradients. With the high-pressure-shift method, samples are cooled under pressure (200 MPa) to −20°C without ice formation, and then pressure is released to atmospheric pressure (0.1 MPa). This technique leads to uniform and rapid ice nucleation throughout the volume of the specimen. This method maintains the original tissue structure of the sample (87).

Blanching is often used to inactivate undesirable enzymes that cause unfavorable effects on the quality of frozen vegetables and some fruits. The use of peroxidase as an indicator of the blanching process may result in the loss of color, flavor, texture, and nutrients of vegetables and fruits. Off-flavors from the deteriorated vegetables or fruits can result from the oxidation products created by the action of free fatty acids and/or peroxides degrading enzymes (88).

1. Fruits

Fruits as a class are the most difficult of all products to freeze without causing a radical change in their appearance, texture, flavor, and color. Of all the common fruits, the cranberry is about the only one that can be frozen without special treatment and yet retains its flavor, color, and appearance. All other fruits require packing in sugar or syrup, or some other treatment. Without it, most blackberries turn brown and become sour and flavorless (89). Many of the tropical fruits are even more difficult to freeze. Bananas turn black during thawing. Whole oranges may break open, and upon thawing, become very flabby and bitter and lose their characteristic flavor (89).

Raspberries, frozen without sugar or syrup, become very soft and lose their characteristic flavor. Most plums become so sour that they are almost sharp and also become very flabby. Blueberries get very soft and lose much of their flavor and aroma. Unripe fruit, which is still hard, does not yield a product of desirable texture, flavor, aroma, and color. Immature fruit when frozen and thawed is usually very sour and often very bitter; furthermore, color and aroma are usually lacking. Moreover, browning and discoloration are more pronounced and, as a rule, the texture is not good (89). Frozen fruits allowed to stand after thawing are not as palatable as when served while still slightly frosted. They tend to collapse with resultant poor texture. Unless treated to prevent change, they discolor (90). Thus the nutrients, the desirable juices, the sanitary quality, and the firm texture of fresh frozen foodstuff may disappear with varying rancidity once the produce is left in the thawed condition.

Tree or vine-ripened fruit usually yields a better frozen product as it is generally considered to have better color and flavor than fruit that has been picked while still immature and ripened in storage (89). On the other hand, overripe fruit bruises easily and deteriorates rapidly even when handled carefully and can develop undesirable off-flavors in the frozen product (89).

Most blackberries are altered in color, flavor, and texture by freezing and thawing. Most of the common varieties of blackberries and many of the dewberries turn brown and become rather sour; consequently, most varieties are not

suitable for freezing in small packages for use as desserts (89). The English Morello variety is preferred for certain purposes because of its more pronounced flavor. It is much darker in color than the Montmorency and is considered less desirable for freezing because of this characteristic (89). Some authorities recommend the Early Richmond variety for freezing but, although this cherry has a desirable bright red color, it is likely to be soft and lacking in flavor (89). The Royal Duke and May Duke varieties possess a more pleasing cherry flavor than the standard red sour cherry, the Montmorency. They are more meaty and consequently do not collapse on freezing and thawing to the same extent as does the Montmorency (89). Most varieties of sweet cherries brown (oxidize) badly during freezing, storage, and thawing. A marked change in flavor occurs simultaneously. Sweet cherries are mild in flavor; freezing and thawing cause some loss of flavor, consequently frozen sweet cherries are likely to be lacking in flavor (89).

The Mission variety of figs is considered best for freezing because of pronounced flavor, good texture, and attractive color. The Kadota and Adriatic varieties remain unchanged in appearance but not so in the case of flavor. The Calimyrna variety is inferior because of the development of off-flavors in the skin and therefore unsatisfactory unless peeled before frozen. Brown Turkey discolors badly and has inferior flavor after being frozen (89).

New York state grapes retain flavor and other characteristics during freezing and subsequent storage (89). Muscat of Alexandria retains flavor and appearance best after being frozen. The Thompson Seedless had a flavor loss, but remain pleasantly sweet after frozen storage.

Pears become soft and watery during freezing and thawing. They also lose much of their flavor. Because of these undesirable changes, pears are not recommended for freezing (89).

Black raspberries possess a delectable flavor and are not altered appreciably by freezing. Unfortunately black raspberries have a tendency to be seedy; further, the seeds are much more noticeable and objectionable after freezing and thawing (89).

In order to be desirable for freezing preservation, a strawberry should have a pleasing potent flavor and acidity that should be retained during freezing and thawing; it should be a uniformly deep, bright red and should retain this color during a long period of freezing storage. The berries should be of uniformly good size and of firm texture.

Color is one of the most important attributes of food, both for its aesthetic value and for quality judgment. In strawberries, two anthocyanin pigments mainly determine the red color: pelagornidon (Pgd)-3-glucoside and cyanidin-3-glucoside in a ratio 20:1. These pigments are not very stable chemically and may change easily if not properly protected. (89). Freezing is one of the most important unit operations to retain fruit quality during long-term storage. Raspberry fruits (*Rubus idaeus* L.) frozen at −80°C and stored at −20°C retain sensorial (color and aroma) and nutritional qualities after 12 months (91). Freezing does not change the volatile components that contribute to the aroma of raspberry (α-pinene, citral, β-pinene, phellandrene, linalool, α-ionone, caryophyllene, and β-ionone). However, freezing causes some cellular disruption that increases the release of caryophyllene. The volatile constituents of raspberry aroma remain after one year of frozen storage (92).

Papayas have unusually high amounts of bound water. This bound water has a high affinity for polysaccharides and proteins, and remains unfrozen even at −20°C. Frozen storage develops off-flavors and color modification after prolonged storage as a consequence of enzyme action. When Papaya slices are cryogenically frozen at −80°C and stored at −24°C for 12 months, proteins are denatured and the activity of soluble peroxidases increase as a result of mechanical damage produced by the growth of the ice crystal and leading to color and texture modification (93).

Storage of custard apple (*Annona squamosa* L.) has limitations, since it is perishable, and cold storage is not promising due to the development of brown color. The custard apple pulp is frozen at −25°C and stored at −18°C. The sensory attributes were evaluated using a 10-point hedonic scale, and the conclusion formed that frozen pulp quality is affected by freezing as compared to the fresh pulp (94).

The flavor of most fruit juices can be preserved better by freezing than by any other method of preservation. Despite this fact, the freezing of fruit juice has not developed nearly as extensively as the other food freezing industries. The two principal reasons for this are: (1) Properly prepared flash pasteurized juices are nearly as good, and may be kept at ordinary room temperatures without appreciable change of flavor; (2) thawing of juices requires much time, and, if not carefully carried out, the thawed juice may be inferior in quality to a good grade of pasteurized juice.

If berries are pressed without heating or freezing, the yield of juice is low, the juice obtained is pale in color, very mild in flavor, and oxidizes very quickly due to oxidative enzyme action (89).

Freezing and thawing of berries prior to pressing is desirable as it extracts much color and relatively little tannin (89). When Montmorency or English Morello cherries are pitted much juice leaks from the fruit. This juice is usually saved, and some of it is preserved by freezing. Although this juice is of fine flavor, it is very pale in color and so is not considered to be of high quality (89).

In the case of orange juice, slow frozen juice is likely to have an "oxidized" flavor, whereas properly handled quick frozen juice should not have this off-flavor.

The slush-freezing operation requires only six minutes, consequently, the system is very effective in rapidly reducing the temperature of the juice to the point where changes in flavor take place very slowly.

Concentration of fruit juices involves sublimation, it is possible to dehydrate fruit juice to a dry powder without materially changing its flavor, although some of the more volatile components are lost. The process not only produces less change in flavor than any other method of dehydration but also yields a product that rehydrates almost instantaneously.

If the original flavor of the juice is to be retained unimpaired, it is important not only to thaw the juice quickly, but also to keep the juice below approximately 10°C.

The anthocyanin contents of certain juices, i.e. strawberry, significantly decreases during frozen storage and the addition of sugars significantly improves the pigment retention (89).

2. Vegetables

It is more difficult to produce frozen vegetables with textural quality than to produce quality frozen fish and meat. Textural quality of fruits and vegetables lessen upon freezing and thawing, resulting in excessive softening (95, 86, 96, 98). This is the prime defect of frozen fruit and vegetable products.

There is no single key enzyme that is responsible for all the vegetable quality changes during frozen storage. Quality changes include off-flavor development (lipoxygenase, lipase, protease), textural changes (pectinase, cellulose), and color changes (polyphenol oxidase, chlorophyllase, peroxidase, lipoxygenase) (99). In general, if the blanching treatment is not sufficient to inactivate catalase and peroxidase, frozen vegetables will develop off-flavors. The development of hay like flavors, bitterness, and odors in unblanched frozen vegetable may be due to amino-aldehyde reactions (100).

The effect of low-temperature long-time blanching (60°C for 2 hours or 74° to 79°C for 20 to 3 minutes) on improvement of texture of frozen vegetables was observed in several studies on different foodstuffs preserved by different methods, such as canned vegetables (101, 102) and frozen carrots (103, 100, 104).

Processing conditions like blanching and freezing result in substantial dissolution, depolymerization and apparent destruction of cell wall pectins. As a result, pectins are broken down into smaller sized polymers, lost interpolymer associations, or were released from the primary cell walls and middle-lamella, or remained loosely bound to the walls by relatively sensitive bonds (101, 102, 103, 100, 104).

3. Mushrooms

Since most vegetables and fruits consist of many kinds of enzymes, enzymatic reactions such as oxidation reaction, browning reaction and discoloration present a problem to the frozen vegetable industry. In order to keep better quality, including color, flavor, aroma, taste, texture, and appearance of the frozen vegetables, a lot of chemical additives are occasionally used for this purpose. Because mushrooms are one of the easily browning vegetables caused by enzymatic and chemical oxidations, there are many kinds of enzyme inhibitors or antioxidants recommended to prevent those quality lowering. While numerous factors are involved in quality loss in frozen mushrooms, the most detrimental is the enzymatic browning reaction (105).

Problems may occur during freezing and storage of frozen mushrooms, e.g. loss of weight, decrease in nutritive value, tendency of product to stick together, and sometimes development of undesired color and off-flavor. Shelf life of fresh mushrooms (*Agaricus bisporus*) is limited to 1 to 3 days at room temperature, 5 to 7 days at 0 to 2°C. Washing, freezing, and 1 day storage of mushrooms contributed to the loss of 'L' value. No changes have been reported in whiteness of frozen mushrooms after 90 days of storage. However, washing with water increases browning of frozen mushrooms. Immersion in boiling water increases toughness as the storage time of frozen mushrooms increased more than 14 days. The blanching process itself reduces the initial mushroom whiteness (106), and blanched frozen mushrooms show a remarkable toughness after thawing and cooking. Frozen whole mushrooms, blanched for 5 minutes have a tougher texture than those blanched for 1 or 2 minutes.

Mushrooms immersed in boiling water before freezing are quite acceptable for the consumer for all time storage even though there is typically a loss of whiteness as compared with mushrooms washed in water containing sodium metabisulfite. Dipping mushrooms in 0.1% or 0.5% sodium metabosulfite solution for 5 minutes after blanching has resulted in the best color and other quality characteristics after freezing (107) of mushrooms in boiling water before freezing was harmful to their texture. Appearance and color of unblanched frozen mushrooms were most affected by washing them in sodium metabisulftite solution before freezing. Storage time of frozen mushrooms results in lower discoloration of mushrooms washed in water containing sodium metabisulfite or immersed in boiling water for 20 seconds than mushrooms washed in water only. Their texture (shear-press reading) was higher. Immersion in boiling water containing sodium metabisulfite had a dual effect: a marked loss of whiteness and a significant decrease of SO_2 content. Also, the sodium metabisulfite treated mushrooms tend to show a marked toughness during storage.

Chemical dipping methods were effective in maintaining desirable color of frozen whole and sliced mushrooms stored for 3 months (108). Excellent results in stabilization of the color of frozen mushrooms by inhibiting undesired discoloration have been obtained after washing fresh mushrooms in sodium metabisulfite solutions (109). The better quality of frozen mushrooms treated with sodium

bisulfite solution was obtained before and after blanching. EDTA and $NaPO_3$ do not improve the color and other quality such as dripping loss, texture, and sensory evaluation scores.

Sodium bisulfite and sodium diethyldithio carbanate after blanching has a higher ascorbic acid retention and less dripping loss, but its organoleptic evaluation scores were poor. Since sulfites are essential additives with important effects on the whiteness stability of frozen mushrooms, determination of the minimum amount of SO_2 required to maintain optimum of frozen mushrooms quality seems to be necessary.

Loss of frozen mushroom whiteness after 1d has been compared with fresh ones varying an average of 6% to about 17%. No significant change in whiteness of frozen mushrooms during 90 days of storage was detected when short-time dipping in boiling water was applied. Storage time of frozen mushrooms results in lower discoloration (110). Most mushrooms are frozen commercially by air blast or plate freezing methods. However, after freezing some undesirable color and off-flavor may occur. The frozen mushrooms made by individual quick freezing process were better in quality, firmer in texture, and lower dripping loss than that made by air blast freezing process.

The effects of processing vary with the chemical, physical, and sanitary conditions and sensory quality of the frozen mushrooms. IQF mushrooms show better color and higher sensory scores. After 120 days of frozen storage, color and texture of mushrooms changed (107). Frozen whole mushrooms blanched for 5 minutes had markedly tougher texture than mushrooms blanched for 1 or 2 minutes. As the storage time of frozen mushrooms increased from 3 to 6 months, shear press values increased very significantly. The texture of Freon frozen and plate frozen mushrooms was similar after 3 months storage. Blanching time did not affect texture of frozen sliced mushrooms significantly, but storage time did.

4. Leafy Vegetables

Texture is important in frozen leafy vegetables. After freezing-thawing, firmness and crispness decrease and rupture strain increases. The optimum freezing rate of Chinese cabbage has been established at 5°C/minute because of its high moisture, low sugars and thin cell walls; therefore, freezing effects are more notable. Chinese cabbage frozen at 100 MPa and at 700 MPa shows an increase in rupture strain. However, texture of samples frozen at 200 MPa, 340 MPa, and 400 MPa keep intact (111).

The green color of organically grown spinach (*Spinacia oleracea* L.) evaluated by sensory evaluation and Hunter 'L' value after 6 months of storage at −24°C shows that color and sensory attributes of spinach depend on the cultivar and these differences should be considered during freezing (112). Lipid-acyl hydrolases (LAHases) are responsible in lipid degradation during storage of vegetables. LAHases are found in spinach that naturally contains large amounts of galactolipids and phospholipids, substrates for LAHases. Formation of volatiles is the result of oxidation of lipids in vegetables. The enzymes involved in this process are lipases, phospholipids-degrading enzymes, galactolipid-degrading enzymes, and lipoxygenase. In spinach leaves, unsaturated fatty acids are present in large portions and are oxidized to peroxides. The resulting peroxides are decomposed to aldehydes, ketones, and alcohols, causing off-flavors (88).

5. Asparagus

Studies have shown no significant difference in texture, flavor or general acceptability differences in asparagus existed due to blanching treatment (113). Blanching of green asparagus (*Asparagus officinalis* L.) before freezing is necessary to inactivate enzymes, which cause quality changes during frozen storage. One of the changes is the formation of aldehydes such as hexanal, that is described as a green aroma and is a flavor compound formed during auto-oxidation of linoleic acid. There is no difference in texture of asparagus frozen using cryogenic and blast freezing but there are differences on texture when asparagus are kept at −30 or −70°C. During storage, shear force values slightly increased (114). Blanched asparagus stored at −18°C for 4 weeks and evaluated for appearance, color, texture, natural flavor, off-flavor and general acceptability showed that unblanched asparagus scored the highest in appearance, while microwaved and unblanched asparagus had the highest color scores. Flavor of asparagus changed regardless of the blanching treatment. After frozen storage, unblanched asparagus stems were significant darker than blanched stems. Blanching had no effect on lightness of asparagus stems or tips. Asparagus were greener after blanching and less green after frozen storage, also they were yellower after frozen storage (113).

6. Carrots

Freezing destroys the cytoplasmic structure, producing loss of turgor, weakness of the cell wall and some degree of cell separation. All these changes affect texture of the product. The freezing temperature is the most critical factor that changes the cell structure of carrots; and the rate and type of freezing that is the critical factor in tissue damage. Firmness of frozen sliced carrots decreases by about 50% as compared to raw samples (115). Quick freezing results in better texture than slow freezing in carrots (100). The effect of freezing rate on firmness of raw carrots is greater than that on firmness of blanched carrots. The final temperature of freezing does not affect firmness of thawed carrots. As the freezing rate from 0°C to −10°C was quick, firmness of thawed carrots was maintained. The amount of drip increased as freezing rate decreased.

Effect of freezing rate on firmness of blanched carrots has been shown to be the same as that of raw carrots. Consequently, quick freezing (−5°C/min) is best for acceptable texture of frozen carrots (100). Tamura (116) found that when carrots were preheated for 30 minutes at 60°C, frozen at −30°C, thawed in a refrigerator and then cooked, softening of carrots was greatly diminished but cell damage was observed. Carrots frozen at −30°C, had great amounts of cell separation, thus the optimum rate of freezing for carrots has been determined to be −5°C/min (117). Low-temp-blanched carrots frozen at −5°C/min using a program freezer, should have minimization of softening. Preheating increases firmness and rupture strain of carrots, while freezing-thawing decreases firmness and increases rupture strain. Firmness of carrots decreases when frozen and thawed, except for raw carrots frozen in a program freezer and thawed. Raw and preheated disks frozen quickly (−5°C/min) using a program freezer are typically firmer than those frozen at −35°C using a conventional freezer. Differences in firmness of blanched disks as compared to unblanched, have not been noted. Quick-freezing results in better texture (firmness and rupture strain) than slow-freezing, and also improves the texture of frozen carrots. Texture of frozen carrots differs from that of boiled carrots (100). Blanching, High-Temperature-Short-Time (HTST) and rapid freezing at −4.5°C/min is recommended as optimum thermal processing and conditions for improvement of textural quality in frozen carrots (118).

Also, freezing causes extensive degradation of cell wall pectins in carrot tissue. This is evident by the loss of firmness. Freezing at rates of −4.5°C/min causes less softening than slow rates (−0.19°C/min). Slow rates causes loss of pectin material and structural damage due to growing ice crystals (118).

Before freezing, carrots are blanched to inactivate enzymes that cause flavor deterioration during frozen storage. Unblanched carrots develop an off-flavor due to fatty acids released by estarases. The off-flavor is characterized as ‘stearin’, ‘paraffin’, and ‘soap’. Loss of textural quality during blanching, freezing and thawing includes dehydration, damage, drip loss, tissue fractures and mechanical damage from ice crystals during freezing. Steam-blanched carrots subjected to blast or cryogenic freezing and stored at −24°C for five months showed differences in textural quality between blast and cryogenic freezing. Decreasing the temperature from −30° to −70°C resulted in increased toughness but no changes are observed over time (119).

Studies have shown that carrots immersed in liquid nitrogen (LN_2) cracked and became unacceptable in appearance, but those frozen at −5°C/min or −2°C/min using a programmed freezer (final temp, −30°C) had a better texture than those frozen in a conventional freezer at −80°C, −30°C, or −20°C (117).

7. Broccoli

Broccoli (*Brassica oleracea*) is a highly perishable fresh vegetable that, at ambient temperatures, will yellow and become unmarketable in 1 to 3 days. About 37.8% in florets and 61.1% losses of chlorophylls *a* and *b* in stems occurred during freezing storage at −20°C (120). This loss of green color is a major limiting factor in shelf-life of broccoli. Boiling-water blanched broccoli stored at −18°C for 4 weeks has excellent sensory scores for visual appearance and color (121). Blanching has a significant effect on yellow color intensity (b* value, Hunter). Immediately after blanching, stems and florets are more yellow than unblanched samples. These differences remain after frozen storage for florets but not for stems. All blanching treatments increase chroma (color saturation) of broccoli florets and stems compared to unblanched broccoli (122, 121). To avoid the development of dimethyl sulfide (off-aroma) in frozen broccoli, florets must be blanched for 90 seconds before freezing (123). After frozen storage no significant lightness differences due to blanching treatments have been noted in recent findings.

8. Green Beans

Brewer et al (124) reported no difference in either visual color or appearance scores of frozen green beans due to blanching treatment. Brewer et al (124, 121) found that blanching treatment had significant effects on flavor, off-flavor, tenderness and crispness of green beans and broccoli that had been in frozen storage. Brewer et al (124) reported that blanched green beans were greener (higher hue angle) than unblanched beans; this trend was still evident after frozen storage.

Samples of green beans have been shown to be more yellow (higher Hunter b value) after frozen storage than immediately after blanching. Immediately after blanching, blanched asparagus tips were yellower than fresh and blanched tips. These differences were lost after frozen storage. No differences in yellowness of stems existed either immediately after blanching or after frozen storage. Brewer et al (121) reported similar blanching and freezing effects on yellowness of broccoli florets and stems. Similar color changes have been reported in blanched green beans during frozen storage (125). Sensory characteristics, such as color, texture and flavor, are affected by blanching. For green beans, appearance, beany aroma, sweetness, green flavor, aftertaste (metallic/astringent), tenderness, crispness, and wetness are the principal sensory attributes to evaluate in frozen products. After four weeks of frozen storage, color values drop by 50%. Blanching treatment have no effect on visual color, appearance, aroma, sweetness, raw starchy flavor, green bean flavor, overcooked flavor, aftertaste, tenderness, crispness, and wetness of frozen green beans (124). After 12 weeks of frozen storage, panelists detect no differences in color,

sweetness, aroma, firmness, and presence of off-flavors. Off-flavor is detected in frozen cooked samples that are blanched using microwaves. This off-flavor is describes as grassy, bitter and stored (122).

Bennion (126) stated that two results of chemical changes that may occur during frozen storage of foods are off-odors and off-flavors. Campbell et al (127) noted that one of the principal reasons for off-flavors is rancidity of the fatty material in unblanched frozen vegetables. Noble and Winter (128) reported that in unblanched frozen green beans marked off-flavors developed rapidly by the end of four weeks. Off-flavor descriptions for unblanched frozen green beans reported by Moriarty (129) included earthy, sour and bitter.

Bennion (126) suggested that off-odors in frozen raw or insufficiently blanched vegetables may be attributed to the accumulation of volatile carbonyl compounds during frozen storage. Chow and Watts (130) reported that rancid odors in frozen green beans were related to malonaldehyde values, used as an index of unsaturated fatty acid oxidation. Lipid oxidation as determined by both the thiobarbituric acid test and acetaldehyde formation from anaerobic fermentation contributed to flavor deterioration in frozen green beans.

After frozen storage, steam-blanched and boiling-water blanched beans had the lowest shear values. Visual color was poorest for microwave-blanched. Microwave blanching green beans for 3 minutes in a covered container or bag prior to 4 weeks frozen storage resulted in a product that was not different in retention of color from boiling-water blanched beans; however, these bean samples differ in tenderness and crispness from boiling-water blanched beans.

Katsaboxakis and Papanicolaou (125) found that blanching green beans (30–90 seconds) increased negative a_L values and a_L/b_L ratio during frozen storage. Negative Hunter 'a' values measure sample greenness. Unblanched green beans have higher odor and flavor scores before storage but are considered organoleptically unacceptable for the same attributes after the first months of frozen storage and at all subsequent storage times. Blanched samples for 30-90 seconds are slightly superior in sensory qualities compared to those receiving more drastic heat treatment.

Hue angle ($\tan^{-1}(b/a)$) has been shown to be higher for boiling-water, steam, microwave-blanched fresh beans. The same general trend for hue angle has been shown to still be evident after 4 weeks in frozen storage and cooking. Higher hue angles indicate that the sample color was greener.

After 4 weeks in frozen storage and cooking, chroma was greater for steam and microwave-blanched beans than for unblanched green beans. Total color values drop by nearly 50% after storage and cooking for most blanching treatments; total color of microwave-blanched beans has been shown to drop over 70%. Katsaboxakis and Papanicolaou (125) reported significant color loss (Hunter L value, a/b ratio) due to green bean blanching time and time in frozen storage.

After frozen storage, blanching treatment had no effect on visual color, appearance, aroma, sweetness, raw starchy flavor, green bean flavor, overcooked/canned flavor, aftertaste, tenderness, crispness, wetness or degree of liking of cooked green beans. Microwave-blanched beans had more aftertaste than boiling-water blanched beans (125).

Sensory evaluation after 9 months of frozen storage found no significant difference in green bean off-flavor between short and long blanch times at any storage temperature. Off-color was significantly higher in green beans that were blanched for 63 seconds, however, indicating that in this commodity color may be the limiting factor in frozen storage. In the case of green beans, off-flavor is eliminated by a one min blanch, but off-color prevention may require a longer time (125).

9. Peppers

A major problem of jalapeño peppers (*Capsicum annuum*) is the soft texture after exposure to heat and salt brines with a pH less than 3.5. To overcome this problem, jalapeños can be frozen. The maximum firmness of frozen jalapeño pepper is 4.8 times that of unfrozen samples (131).

10. Tomatoes

Sensory characteristics of tomatoes include appearance, red color intensity, tomato flavor intensity, texture, natural-tomato flavor, off-flavor and general acceptability. After 6 weeks of frozen storage, boiling-water blanched tomatoes have the highest scores for appearance, tomato flavor intensity, and natural tomato flavor. Steam blanched tomatoes have the lowest appearance scores. Unblanched frozen tomatoes are usually mushy and least firm after storage (132). Tomatoes blanched (4 minutes) using conventional boiling water, steam, microwaved in a glass container, and microwaved in boilable bags demonstrated that though visual color and sensory attributes were highest for boiling water blanched tomatoes, microwaved-blanched tomatoes retained more nutritive value in the finished product (132). Pectin methylesterase (PME) can cause texture deterioration in processed tomatoes during storage. Blanching, which reduces the loss of product texture, inhibits PME activity as well as the activities of other deteriorative enzymes (132).

Lycopene, the primary pigment in tomatoes, is affected not only by exposure to oxygen from the air, but also by heat during processing, which may cause isomerization of trans-double bonds in the pigment to cis forms (133). This shift in structure can cause a reduction in color intensity of a variety of food products (134). Preliminary acceptance or rejection of a food is usually influenced by the visual

TABLE 58.5
Color, Appearance, Aroma, Flavor and Texture of Selected Vegetables

	Sensory Attributes				
Commodity	**Color**	**Appearance**	**Aroma**	**Flavor**	**Texture**
Green beans (124)	Green	Plump	Grassy/green Beany	Sweet Green bean Overcook After taste	Tenderness Crispness Wetness
Tomatoes (132)	Intensity	Uniformity of color Wholeness	Aroma	Bitter Natural flavor Sweet Off-flavor	Intact Mushy Firm
Broccoli (121)	Green	Plump	Grassy/green	Sweet Starchy Broccoli Overcooked After taste	Tenderness Crispness Wetness

appearance including color and texture (135). Any changes in blanching technique must take into account the alterations in product color that often occur. This reduced, but still substantial, PME activity may be the reason for poor sensory texture of unblanched frozen tomatoes; as PME breaks down pectic substances, tissues lose structural integrity becoming soft and mushy (132).

Immediately after blanching, microwave-blanched tomatoes have been shown to be lightest (Hunter L value) in color. Boiling water-blanched tomatoes are redder (Hunter a value) with a similar trend for yellowness (Hunter b value). However, after frozen storage, steam-blanched tomatoes are lighter in color followed by microwave-blanched and unblanched tomatoes. Lightness of steam and microwave-blanched tomatoes change little during storage. Although total carotene content does not change as vegetables are heated in water, there is a shift in the visual color: orange carrots may become more yellow, and red tomatoes may become more orange-red (135).

Blanched tomatoes have lower hue angles than do fresh tomatoes indicating that blanched tomatoes are more "true red" than fresh tomatoes. Hue angles decrease after frozen storage.

Chroma increases by two-fold as a result of the blanching process. After frozen storage, total color drops significantly. After 6 weeks of frozen storage, boiling water-blanched tomatoes have the highest scores for appearance, tomato taste intensity, and natural tomato flavor followed by microwave-blanched tomatoes. Brewer et al (121) found a similar trend for broccoli. Steam-blanched tomatoes had the lowest appearance scores. Unblanched tomatoes were most mushy and least firm after storage. No texture differences exist among boiling water and microwave-blanched samples.

Begum and Brewer (113) found only moderate acceptability scores for asparagus (3.27–3.48 on 5-point scale) for all blanching treatments and reported that appearance and color appeared to have greater impact on acceptability than did flavor. Unblanched tomatoes had the lowest scores for general acceptability as compared to blanched by various methods. Stone and Young (122) also reported that, in general, microwave blanched vegetables are less acceptable in terms of texture and flavor than those that are steam or water blanched. Some microwave-heated vegetables exhibit toughening and may have grassy or strong off-flavors and aromas (136, 122). These results may be attributed to the amount of water used during blanching, high microwave power level or extended heating time. However, tomatoes are softer products that are likely to soften even more as pectic substances are solubilized.

Individually quick freezing (IQF) versus blast freezing does not influence texture of sweet corn and green peas. Also, no changes in color are observed in green asparagus, green beans and green peas. Asparagus and green beans blast frozen show softening and sweet corn higher color values (137). For sweet corn on the cob (*Zea mays*), frozen using a blast freezer it is recommended to reduce blanching time from the sensory point of view because longer blanching times results in reduced firmness and freshness and also increase cooked flavors (123).

Freezing method does not affect the color of green vegetables but does affect corn. Blast frozen sweet corn has higher blue (less yellow) color than the IQF sweet corn.

In many vegetables the limiting quality attribute during frozen storage is off-flavor development, which is most often catalyzed by lipoxygenase. Williams et al (138) evaluated the sensory character of blanched vegetable purees to which isolated enzymes had been added and found that lipoxygenase was the enzyme most active in aroma deterioration in English green peas and green beans. Lipoxygenase is widely distributed in vegetables

and evidence is mounting to support its involvement in off-flavor development and color loss.

In the case of corn, green beans, and green peas, there is fairly strong evidence that off-flavor development is the limiting factor in frozen storage and lipoxygenase activity is the primary culprit.

Supersweet corn is popular as a fresh, frozen or dried product, however blanching often results in caramelization of these high sugar varieties and formation of an undesirable gray or brown product. Industrial blanch conditions were established based on the requirement for peroxidase inactivation, however off-flavor catalyzed by lipoxygenase is the greatest limitation to storage life.

Sensory results for stored kernel corn indicated that unblanched kernels were significantly less desirable in appearance, texture, flavor, and overall desirability. It would appear that a one min, or possibly shorter, blanch was adequate to ensure the sensory quality of frozen Supersweet kernel corn.

Corn-on-the-cob sensory results show that, although the appearance of both unblanched and 3 minute blanched samples were preferable to those blanched longer, a longer blanch treatment resulted in better flavor and overall desirability after all storage times.

There have been shown to be no significant differences in off-flavor or off-color between the short and long time blanched corn after 9 months storage at +8°C, 0°F, or −8°F.

F. Spices, herbs, and seasoning blends

A spice is any aromatic vegetable substance in whole, broken, or ground form, except for those substances that have been traditionally regarded as foods, such as onions, garlic, and celery; whose significant function in food is seasoning rather than nutritional; that is true to name, and from which no portion of any volatile oil or other flavoring principle has been removed (139).

The shelf-life of spices is greatly extended while kept in their whole form, but when ground the shelf-life is reduced. The ideal condition for storage of spices is to keep them cool, dry, and protected from light. These storage conditions, in addition to airtight packaging, will reduce oxidation. Freezing is a prime choice of storage condition. The freezing of spices has little to no affect on the sensory properties of spices. Freezing is the means in which the "Gold standard" of a spice is typically preserved. The sensory properties of spices and herbs include aroma, appearance, color, flavor, pungency, and in some cases textural aspects. Sensory profiles can be used to define broad parameters, backed by specific and measurable physical parameters such as moisture, particle size, bulk density, color, and where appropriate, heat units. Several of the physical parameters, such as particle size and solubility, although not primarily responsible for flavor, influence this attribute (140).

The shelf-life of a spice, herb or seasoning blend will depend upon many variables that are typically characteristic of either the raw materials or the packaging, the different characteristics of the spice or herb, or possibly the different types of ingredient(s) used singly or in blended form. Typically, the detrimental properties would be loss of flavor and/or product lumping. Flavor deterioration is a problem when the more volatile ingredients are used, especially spice extracts, oils, and liquid flavors, which have been plated onto a non-absorbent base, such as salt, dextrose, malto-dextrin, or possibly starch. There is virtually a non-existent barrier by which to hold these volatiles in the compounded blend into which they have been incorporated. Thus, even to freeze these types of products would be of little benefit for flavor and aroma retention. From an appearance standpoint, the product could appear slightly faded, but could possibly appear quite normal in color, size, shape, texture and so on. Rancidity is another concern for flavor and aroma deterioration. In particular, spices that have high oil contents often develop rancidity if not properly stored. Freezing most definitely delays this deleterious property (140).

Flowability is a concern for spices and spice-herb seasoning blends because loss of moisture in the product causes lumping, as well as moisture absorption causing stickiness with certain spice oils. Free-flow agents are often added to spice blends to assist with this problem. Freezing does not tend to detrimentally affect these types of spice–herb blends. Most seasoning blends typically have a shelf-life of 3–6 months if properly stored. However, if frozen, this timeline can be extended to 6 months to 1 year. Many spice "houses" and distributors store spices, herbs, and seasoning blends up to 2 years without loss of quality and sensory attributes. However, this shelf-life extension becomes an argument for what the end consumer is willing to accept in terms of perceived freshness of the product. Cryogenic milling of certain spices avoids the volatilization, oxidation and enzymatic damage associated with conventional milling techniques. The higher volatile content of these spices (e.g. nutmeg, mace, and cinnamon) provides improved shelf-life and flavor perception. Thus, using cryogenics these can be extended (140).

G. Meats

Preservation by some means is absolutely essential for prolonging shelf-life, and for storage of all fresh meat and most processed meat products. Regardless of the method used, preservation is accomplished by restricting, or in some instances completely inhibiting, microbial activity, as well as enzymatic, chemical, and physical reactions that would otherwise cause deteriorative changes and spoilage.

The purpose of freezing is the preservation of the original characteristics of the product for an extended period of time. The main requirement is that the product before freezing is in an optimal condition from both microbiological and chemical standpoints. The quality of frozen meat is affected by conditions during freezing and subsequent frozen storage, and the length of the storage period. Quality changes occurring during storage at temperatures less than −10°C (14°F) are unrelated to bacterial growth or metabolism and are of (bio)chemical origin (e.g., drying and oxidation). In lean muscle at −5°C (23°F), approximately 85% of the water is frozen; at −30°C (−22°F) nearly 100% of the water is frozen. Due to the presence of solutes, the freezing point of muscle is approximately −2°C (28°F) (141).

The major physical/chemical changes which occur during storage of frozen foods are typically a result of (1) oxidation of lipids, (2) denaturation of proteins, (3) discoloration of product, (4) sublimation of ice, and (5) recrystallization of ice crystals. Chewiness, juiciness, and rancidity are the sensory attributes to be monitored during the storage. Freezing is one of the most effective methods for meat preservation. Low temperature not only protects the product from microbiological spoilage but also slows down the rates of other degradative biochemical reactions. In spite of the many advantages however, freezing causes certain unfavorable changes in meat quality. Ice occupies a greater volume than water, and the exclusion of solutes from ice crystals causes an increase in the ionic strength in unfrozen water. Together these phenomena cause a loss in tissue structure and a partial denaturation of some muscle proteins, in turn reducing protein solubility and gelation capacities (142).

Freezing and frozen storage produce or enable deleterious changes that can reduce meat quality depending on storage time, temperature, freezing rate, and protective packaging used. Secondary breakdown products of lipid oxidation, especially malonaldehyde, have been shown to precipitate on and crosslink muscle proteins, resulting in reduced ability to hold water under physical or thermal. Low-molecular-weight aldehydes and ketones are known to have strong odors and flavors at low concentrations, some of which are objectionable to sensory panels (143).

Color may be the most important characteristic affecting consumer decision-making on acceptability of meat and meat products (143). Such discrimination may be warranted, as meat color is the quality trait most susceptible to alteration by environmental conditions (144).

Consumers associate color with good quality (145, 146). Freezing or frozen storage generally does not affect the color of cooked meat (147). In frozen meat, color changes as a result of storage time, temperature, freeze-thaw cycles, and exposure to light (148, 149).

If flesh tissue is frozen rapidly, the cellular fluids remain in their location and freeze as tiny crystals uniformly distributed throughout the tissue. The faster the transition from 0° to 5°C, the less the translocation of water during freezing (150). Slow freezing results in more thawing drip and less expressible juice than quick freezing (151). Slow freezing causes fluid in the extracellular spaces to freeze first, thus increasing the concentration of solutes and drawing water osmotically from the still-unfrozen cell through the semi-permeable cellular membrane. There is therefore extensive translocation of the tissue fluid such that the fibers appear shrunken and, in some cases, damaged through rupture of the myofibrillar walls. Drip loss is not only disadvantageous economically but can give rise to an unpleasant appearance. Drip loss is due to denaturation of proteins by the high ionic strength of extracellular fluid.

Drip losses, cooking losses, and shear strength increased with time in frozen storage. Samples with 30% fat had greater drip and cooking losses than did samples with 15% fat. Storage temperatures affected drip and cooking losses but not shear strength. Increased storage time also resulted in loss of color.

Protein damage is a function of time and the quantity of drip will tend to increase as with time in storage (152). Furthermore, the free moisture is a medium for potential bacterial spoilage, and also represents loss of flavor in the meat as nutrients such as vitamins, proteins, and minerals are dissolved in the exudate. Slow freezing rates product greater damage to the mitochondria in the cells than fast freezing, and consequently there is a greater release of enzymes (153).

Freezing is recognized as an excellent method for preservation of meat. It results in fewer undesirable changes in qualitative and sensory properties of meat than other methods of preservation. In addition, most of the nutritive value of meat is retained during freezing and through frozen storage. Some loss in nutritive value occurs when water-soluble nutrients are lost in thawing drip, but the amount of drip and nutrients varies with freezing and thawing conditions. Nutrients found in drip include salts, proteins, peptides, amino acids, and water-soluble vitamins. A study by Anon and Calvelo (154) confirmed that drip loss is reduced by shortening the freezing period, although Bechtel reported that protein in the drip was unaffected by the freezing rate. Meat proteins are less affected by freeze concentration; Jalang et al (155) found that regardless of species, frozen muscle at −19°C provided more expressible (press) fluid than fresh muscle, although pork had more press fluid than beef or lamb. Wagner and Anon (156) explained the effect of freezing rate on denaturing by noting that myofibrillar proteins denature in two stages, with an initial rapid reaction followed by a slower, second stage, with the myosin molecule continuing to denature and leading to a decrease in solubility and viscosity (157). None of the nutrients present in meat is destroyed or rendered indigestible by freezing. Dark color

of fresh frozen meat brightens upon exposure to oxygen or air. Thus, qualitative properties of frozen meat approximate those of fresh meat (158).

In general, sensory characteristics such as rancid, acid, sour and bitter odor and flavors, and rubbery texture increase over time in frozen storage when juiciness decreases. Exclusion of oxygen appears to have the greatest impact on preservation of sensory characteristics. Based on sensory data, apparently, frozen storage of ground pork should be limited to 26 weeks even with vacuum-packaging if "fresh pork" sensory and physical characteristics are to be maintained (158).

Apart from temperature, the nature of the muscle food product is a decisive factor in determining storage time. Ground product with its greater surface area and potential for oxygen penetration is more sensitive to oxidation (rancidity) than whole pieces of meat. As pork contains more unsaturated fatty acids, it is more susceptible to oxidative rancidity than beef. Undesirable oxidation and dehydration occurring during frozen storage may be limited by proper packaging (159).

The length of time meat is held in refrigerated storage prior to freezing affects ultimate frozen meat quality. To preserve optimum quality, meat to be frozen must be handled with the same care as refrigerated meat, especially if products are going to be in freezer storage for several months. Some deterioration continues to occur in meat even at freezer storage temperatures. In addition, quality of frozen meat is influenced by freezing rate, length of freezer storage, and freezer storage conditions i.e., temperature, humidity, and packaging materials used. Included among changes that may occur during frozen storage are development of rancidity and discoloration, with the latter change being due to surface dehydration due to microbial and enzymatic activity. At temperatures below about −10°C, most deterioration due to microbial and enzymatic activity is essentially curtailed. On the other hand, in meat with relatively high microbial loads or in improperly chilled meat, slow freezing rates may allow considerable microbial growth (158). The main effect of freezing upon microorganisms is a cessation of growth. Some microbial destruction will occur during the freeze/thaw process, but freezing should not be regarded as a reliable means of destroying microorganisms other than parasites (160).

Tempering produces the most rapid increase in myoglobin and lipid oxidation. This is probably the result of both the thawing process and temperature fluctuations. Thawing provides an ideal environment for the formation of new, large ice crystals and the temperature fluctuations during tempering further enhance ice crystal growth and formation (161). The formation of large ice crystals enhances muscle fiber shrinkage and distortion and increases the solute concentration, which disrupts the integrity of the muscle cells. Such changes permit catalysts of oxidative reactions to come into contact with myoglobin and lipids (162).

Freezing rates affect physical and chemical properties of meat. They may be influenced by temperature of the freezing medium, type and movement of the freezing medium, packaging materials used, and composition of meat products to be frozen. In the latter case, tissues containing fat have lower thermal capacity than lean tissues, and therefore freeze more rapidly (158). A key property for all the components and membranes of muscle foods is their elasticity. The elasticity determines the consequences of freezing and quality of the resulting thawed material (157).

Undesirable physical and chemical effects occurring in meat during freezing are associated with one or more of the following factors: (1) formation of large ice crystals in extracellular locations, (2) mechanical damage to cellular structures resulting from volume changes, and (3) chemical damage caused by concentration of solutes, such as salts and sugars. Nonvolatile solutes lower the freezing point and therefore, meat freezes at approximately −2°C to −3°C. Extent of damage to meat tissues attributable to these three factors is influenced by freezing rate (158).

During slow freezing, temperature of meat products remains near the initial freezing point for an extended time. As a result, a continuous freezing boundary forms and proceeds slowly from exterior to interior. During the process of slow freezing, long periods of crystallization time exist, producing numerous large extracellular masses of ice crystals that are easily lost as drip during thawing. Mechanical damage due to volume changes is more likely to occur during slow freezing because of expansion associated with formation of large ice masses, as well as concomitant shrinkage of muscle fibers that have lost water to extracellular pools. Such muscle tissue has distorted structure in frozen form that completely obliterates normal striations (158).

Cryogenic freezing is a rapid freezing method utilizing condensed gases such as liquid nitrogen, nitrous oxide (N_2O) and dry ice (CO_2). This rapid freezing is primarily the consequence of very low initial temperature. Costs for cryogenic freezing are greater than for conventional freezing. Justification for this method is better achieved quality (163). During cryogenic fast freezing, meat product temperatures fall rapidly below the initial freezing point. Numerous small ice crystals with filament-like appearance are formed both intra- and extracellularly at approximately the same speed. Because of rapid rates of heat transfer, small ice crystals formed have little opportunity to grow in size.

Thus, fast freezing causes spontaneous formation of many small ice crystals resulting in discontinuous freezing boundaries and very little translocation of water. Since most of the water inside muscle fibers freezes intracellularly, drip losses during thawing are considerably lower than in thawing of slowly frozen meat. In addition, muscle fiber shrinkage and distortion are minimized during fast freezing, resulting in near normal ultrastructure in the

frozen state. Volume changes are less and periods of crystallization are shorter than in slowly frozen muscle and, consequently, mechanical damage is correspondingly less. Filament-like ice crystals entrap solutes and thus minimize the ion concentration effect. In addition, smaller and more numerous ice crystals in rapidly frozen meat reflect more light from meat surfaces, resulting in lighter color than that of slowly frozen meat (158).

Conditions under which frozen meat is stored are even more important for maintaining quality. The length of time frozen meat may be successfully stored varies with species, type of product, freezer temperature, temperature fluctuations, and quality of wrapping and/or packaging materials. In general, lowering storage temperatures may extend storage time of all types of frozen meat. Rates of chemical deterioration are greatly reduced by freezing, but reactions such as oxidative rancidity continue slowly even in the frozen state. Most chemical changes could essentially be eliminated by reducing temperatures to −80°C, but such temperatures are not economically feasible in most storage facilities. Growth of putrefactive and spoilage microorganisms, and most enzymatic reactions, are greatly reduced, if not entirely curtailed, at temperatures below −10°C. In general, storage temperatures of less than −18°C are recommended for both commercial and home freezer units. Most of these units operate at temperatures between −18 and −30°C. Although it is expensive to maintain the lower temperatures of this range, length of meat storage life may be significantly extended (158).

Temperature fluctuations during frozen storage should be avoided as much as possible, to minimize the ice crystal growth, formation of large crystals, and associated drip losses. Almost all water in meat is frozen at about −18°C. However, as temperature increases, unfrozen proportions increases and become especially marked above −10°C. Small ice crystals are thermodynamically less stable than large crystals. Water molecules migrate from small crystals through unfrozen pools of water to recrystallize and form large ice crystals. Migration, recrystallization, and ice crystal growth are enhanced by relatively high storage temperatures and by temperature fluctuations. Fluctuating storage temperatures also may cause excessive frost accumulation inside packages, much of which is lost as drip upon thawing (158).

Acceptable quality may be maintained in frozen meat products for several months, only if certain critical packaging requirements are met. These requirements include use of vapor proof packaging material to keep moisture in and oxygen out of packages. Moisture losses, due to improper packaging materials or techniques, result in dehydration and freezer burn, and oxygen from air in packages causes oxidative changes, including rancidity. Another requirement for quality maintenance for extended periods of time, is to eliminate as much air as possible from packages. Other requirements include use of odorless, grease proof packing materials that are strong when wet and resist scuffing, tearing, and puncturing under normal handling conditions (158).

The amount of time meat may be held in frozen storage, while maintaining acceptable quality, depends on degree of saturation of meat fats. Since fish, poultry and pork fats are more unsaturated than beef and lamb fats, they are more susceptible to oxidative changes. Hence, recommended frozen storage times differ for various species. Gradual decreases in flavor and odor acceptability during frozen storage are primarily due to oxidation of lipids (158). The freezing process causes changes in the structure and color of the muscle. Storage temperature, light intensity, type of display area and packaging all affect the rate of deterioration. The color of frozen meat is known to vary with the rate of freezing. Taylor (164, 165) found that, with lower freezing rates, the appearance of the product changed and at very low rates, there was a marked development of translucence leading to a lighter product. Zaritky et al (166) concluded that small crystals, formed by fast freezing, scattered more light than large crystals formed by slow freezing and hence, fast frozen meat was opaque and pale and slow frozen meat was translucent and dark.

Length of frozen storage is influenced by processing state of meat products i.e., fresh, seasoned, cured, smoked, precooked, comminuted, or chemically preserved. Salt enhances development of rancidity, and processed meat products containing salt have limited frozen storage life. It is generally recommended that cured and smoked products not be stored frozen or stored only for very limited time. Sliced meat products such as bacon and luncheon meat, should not be frozen unless vacuum packaged because air incorporated during slicking, together with contained salt, lead to rancid flavor development in a matter of days or weeks. Precooked frozen meat and poultry products lose their "fresh cooked" flavor during frozen storage and develop "warmed over" flavor due to lipid oxidation. These oxidative changes increase with cooking time, but are minimized to some degree by treatment with polyphosphates, bases or gravies. Oxidation is inhibited by pH increases caused by polyphosphates and by natural antioxidants present in many bases. Frozen storage life of precooked meat products also may be extended by packaging in inert gas, such as nitrogen, in which case elimination of oxygen from packages is responsible for inhibition of oxidation (158).

Sensory quality after freezing in restructured meat products can be similar to that of processed meats. In the manufacture of restructured products, there are additional factors which may also accelerate the rate of discoloration, which is the main sensory quality altered by low temperature storage. Of these factors, (1) grinding and mixing (167), (2) addition of sodium chloride (168, 169),

and (3) tempering of frozen meat logs (161) attribute also to loss of sensory appeal. Several serious problems, namely, color instability and fat oxidation, are encountered in restructured meats. Variable color patterns of restructured meat, especially beef products, continue to trouble processors, and color instability is a prime limitation to acceptance of restructure meat products in the retail trade (170).

Cured meats develop rancidity more rapidly than frozen fresh meats. It is customary to freeze bellies or hams only before curing. In addition to rancidity, changes in flavor and texture occur in frozen cured meats. The relative stability of refrigerated cured meats decreases the need for frozen storage; however, the end user may desire to freeze portions of large products, such as ham. This can be done if the meat is properly wrapped and not held in the freezer for more than a few weeks. Longer storage periods lead to flavor changes (163). Mitchell et al (171) found that while freezing and thawing steaks did not seriously affect the quality of the meat, steaks cooked directly from the frozen state were more juicy and had more flavor than those thawed first. However, they found that steaks thawed before cooking were slightly more tender than unthawed steaks, although there was little difference between steaks thawed in a microwave oven, in a refrigerator, or in air, a result also established by Moody et al (172). The lower juiciness ratings for thawed steaks were attributed to drip loss during thawing (173).

The thawing process probably does greater damage to meat than freezing. Several factors are responsible for damaging effects occurring during thawing. Thawing occurs more slowly than freezing, even when temperature differentials are the same. Further, temperature differentials in thawing are generally much less in practice than those used in freezing. During thawing, temperatures rise rapidly to the freezing point, then remain there throughout the entire course of thawing. This situation increases the duration of thawing compared to freezing and provides greater opportunity for formation of new, large ice crystals (recrystallization), for increased microbial growth, and for chemical changes. Thus, time-temperature patterns of thawing are more detrimental to meat quality than those of freezing (158). Time required for thawing frozen meat depends on a number of factors: (1) temperature of meat, (2) thermal capacity of meat (lean products have higher thermal capacities and therefore thaw more slowly than fat products), (3) size of meat products, (4) nature of thawing medium (water provides faster heat transfer than air), (5) temperature of thawing medium, and (6) movement of thawing medium (158).

Factors affecting quality of frozen meat can be ranked, in order of importance as follows: (1) frozen storage conditions, (2) thawing conditions, (3) freezing rate, and (4) prefreezing treatment and handling. Frozen storage, as conducted under commercial and household conditions, is the most damaging phase in handling and processing of frozen meat. Even though damage due to recrystallization of ice occurs during thawing, it is generally less severe than that occurring during storage because of shorter times involved. Original differences in product quality, caused by fast rather than slow freezing rates, gradually diminish during several months of frozen storage. In addition, cooking further reduces differences in quality caused by different freezing rates (158).

The single factor exerting most damaging effects on meat products during frozen storage is temperature, particularly as it fluctuates. Deleterious effects produced by temperature primarily result from ice crystallization. Damage occurring during frozen storage can be minimized, but not entirely prevented, by maintaining low, constant storage temperatures, and by limiting duration of frozen storage (158). During freezer storage an excessive loss of moisture from meat surfaces will result in localized areas of dehydration and discoloration. This condition is called freezer burn and is characterized by cork-like texture and gray to tan coloration. It can result when wrapping and/or packaging materials have been punctures, or when moisture-proof wrapping and/or packaging is not used. Improper maintenance of temperature, with frequent cycles of partial defrosting followed by refreezing during storage, also contributes to freezer burn. During development of freezer burn, proteins become denatured and rehydration is poor. Meat with severe freezer burn is dry and tasteless or, if oxidative rancidity has developed, bitter in flavor (158). Freezer burn can be prevented by a skin-tight covering (moisture-impermeable film or dip or spray coatings) or an ice glaze (163).

Freezing may cause damage to meat structure, particularly if it is frozen too slowly. Water may collect extracellularly and freeze in large pools, forming spikes of ice crystals that puncture muscle fibers, releasing even more moisture. Physical damage caused by slow freezing results in great loss of fluid from meat when it is thawed. This fluid collects in packages upon thawing, and is called drip. Excessive drip results in unattractive packages, loss of nutrients, and dryness in cooked meat (158). Profound effects on the structural and chemical properties of muscle foods, including changes in the muscle fibers, lipids, and proteins result from slow freezing. All of these have the potential for significantly influencing the quality attributes of meat and meat products.

Alterations in protein have been observed in frozen meat. Sarcoplasmic proteins are less soluble after freezing muscle tissue. The total extractable protein, sarcoplasmic protein and actomyosin extractability decrease with frozen storage. Evidence indicates quality of protein and fat deteriorates, and comminuted products manufactured from frozen meat block components gradually decrease in quality characteristics (163). While Miller et al (174) found no acute emulsion instability during 37 weeks of storage, the

trend indicated an eventual failure on further storage. The first signs of change were evident after only one week of freezing and by 25 weeks all observed characteristics showed significant degradative changes.

During refrigerated storage, meat loses moisture from its surfaces, resulting in weight loss called shrinkage. Other than economic losses associated with shrinkage, loss of moisture during the first few days of refrigerated storage seldom has adverse effects on meat acceptability. However, physical changes accompanying shrinkage during prolonged refrigerated storage include surface dehydration and discoloration (158).

The texture and consistency of meat render it highly susceptible to the absorption of volatile materials. Meat tissues readily absorb aromatic compounds from other foods, such as apples or onions. Consequently, off-flavors may occur when meat is stored in the presence of such products (158). The beneficial effect of low temperature storage is the tenderization of meat. Beef increases consistently in tenderness as freezing temperatures are lowered.

The morphology of ice in the frozen tissue and the size and distribution of ice crystals formed in the intra- or extra-cellular spaces are particularly important. These cause changes in the water-holding capacity of the muscles when thawed, in the texture, and in the surface color. Freezing of meat results in an increase in the amount of drip fluid when the meat is thawed, causing changes in the sensory properties of the meat after it is cooked.

The rate of freezing is of great importance, especially between −1° and −4°C (30–25°F). When frozen slowly, ice crystal formation starts in the aqueous intracellular compartments. Subsequently, water in the connective tissue freezes due to a lower salt content than muscle tissue. The remaining water is more concentrated and the higher osmotic value will result in water diffusing out of the muscle cell and condensate on the outer cell ice. Eventually, the intracellular water will freeze. Under improper freezing conditions, ice crystals may grow to 150 μm (diameter) and damage the muscle cell (141). When the critical temperature (−1 to −4°C; 30 to 25°F) is passed rapidly (within 80–120 minutes), the water in the muscle cell freezes before substantial diffusion can occur. The existence of numerous small ice crystals limits the risk for cellular deformation. Fluctuation in storage temperature may permit the small ice crystals to thaw, leading to recrystallization and the formation of large crystals. This may lead to extensive membrane damage and subsequent problems with increased rancidity and drip or purge (141).

Deteriorative changes occurring during freezing are often due to oxidation. The oxidation of meat during frozen storage is largely due to changes in the triglyceride fraction. Susceptibility to lipid oxidation is influenced by species differences, as well as differences between tissues. For example, beef lipids are more stable than those of chicken white meat. Temperature and length of holding can alter the rate of oxidation of lipids in meat. Even at −30°C (−22°F), lipid oxidation may occur. Oxidation during frozen storage is especially a problem with processed meat products, as salt acts as a catalyst in oxidative reactions. Auto-oxidation proceeds faster during storage at high temperatures. The susceptibility of frozen food systems to oxidation is related to low water activity. Whereas fresh meat has a a_w of 0.99, muscle tissue frozen at −18°C (0°F) has a a_w of 0.60. At water activities of 0.60–0.80, heme pigments appear to initiate lipid oxidation. Porcine myoglobin seems less stable to oxidation than myoblobin from other species. Other substances found in meat products are also known to accelerate oxidation. Lipases and liposydases will, especially at a lower pH, induce lipid oxidation and rancidity (141).

Color deterioration of frozen meat can be a problem. Freezing accelerates metmyoglobin formation, causing an undesirable color change. Rapidly frozen meat is lighter in color than slowly frozen meat. Localized tissue dehydration, or freezer burn, may result in a lightening of dark tissue or a browning of light-colored tissues such as chicken skin. Freezer burn is an irreversible condition. It may be prevented by proper packaging. Even with proper freezing techniques, a concentration of soluble salts within the muscle cell will occur. This will cause denaturation of proteins, and the pH of the product will change (141). MacDougall's (147) contention that major color problem during frozen storage was photo-oxidation of pigments indicating therefore that meat was best preserved by aluminum foil packaging. MacDougall's theory (147) that frozen meat oxidized from the surface inwards (compared to fresh meat, which oxidized from the interior outwards) can be demonstrated by the use of packaging techniques.

Freezing of muscle foods should not start before rigor has set in. The primary textural change occurring in properly frozen red meats and poultry is an increase in porosity. Freezing and frozen storage of seafood can result in an undesirable textural change. The muscle may become tougher, drier, and lose its water-binding capacity. The overall decrease in protein extractability is attributed primarily to alterations in the myofribrillar fraction (myosin-actinomyosin binding). The nature of the reactive groups responsible for the aggregation is not well understood. Formaldehyde, a product of decomposition of trimethylamine oxide, is highly reactive with a variety of functional groups and it has been suggested that it is involved in the covalent cross-linking of proteins in frozen-stored fish. The loss of water-binding capacity is probably due to an increase in the number of cross-linkages between myofibrillar proteins. As fish lipids contain highly unsaturated fatty acids, fish muscle is very susceptible to oxidation (141, 175).

Thawing of meats has been a largely ignored subject. Slow thawing will result in a better product than fast thawing, meaning that thawing at refrigeration temperatures is

preferable to thawing at higher temperatures. Thawing time should be too long, as it may lead to microbial growth. An important consideration in a thawed product is the amount of exudate. The exudate, containing soluble proteins, vitamins, and salts, will be increased when cell damage is extensive. Slow thawing allows for reabsorption of the fluids by the tissue (141).

Bulk frozen meat, typically in block form of up to 25 kg, can be processed immediately at temperature of −20°C to −30°C or below. Heavy duty pre-breakers in the form of guillotines, chisel-type implements or strong cutter blades can rapidly reduce whole blocks into pieces suitable for further comminution (157). Similarly, heavy duty mincers or similar machines can operate at very low temperature and effectively reduce meat into small particles. However, the brittle state of meat and the ice contained within the tissues at these temperatures causes considerable shattering of muscle and fat structures, with the consequences of the meat being in a very finely fragmented state. This can be desirable for very fine comminutes of a homogenous character, but undesirable for products where larger particles and retention of muscle structure are required (157). The temperature at which the pre-breaking of frozen meat blocks takes place can have a major influence on the ultimate particle-size distribution of comminuted products, with meat pre-broken at lower temperatures having smaller particles. The perception of particle size is itself a key determinant of burger texture (176). The breakage of meat and the considerable deformation of internal particle structure, often to the structural level of individual muscle fibers, is characteristic of mincing/grinding and in turn determines the particulate character of many burger, sausage, and other meat products (157).

Beef could be satisfactorily preserved in the frozen state for at least 554 days at temperatures below 0.9°C. A later report (177) indicated that uncured pork cuts were in an excellent state of preservation after being stored for 9 months at −35.5 to −36.7°C and that such cuts were still edible after 16 months under these conditions. A recent report (178) indicated that uncured pork chops and roasts could be stored at −30°C for at least 196 days, regardless of the protective wrap used.

Frozen storage does not affect any of the surface or partial compression properties of meat. However, on the first bite fresh samples are slightly more cohesive and during mastication are chewier, require a greater number of chews, and are more cohesive than frozen and thawed samples. However, stored samples release slightly more moisture during mastication. After swallowing, the mouth coating from fresh samples consist of a higher proportion of particles, and the particles from fresh samples consist of a higher proportion of appropriate residual particles described as being fibrous, grainy, crumbly, and mealy, while the particles from stored samples consisted of a higher proportion of appropriate residual particles described as being fibrous and grainy. As a result of these differences, stored samples received higher texture amplitude ratings than their stored counterparts.

Present findings clearly indicated that freezing, frozen storage, and thawing produced beneficial effects on texture properties that enhanced texture amplitude. Such effects resulted in a slight tenderizing effect, a slight improvement in juiciness, and a breakdown to move appropriate particles, as evidenced by stored samples being less cohesive, easier to chew, and releasing more moisture during mastication. However, it is also clear that freezing, frozen storage and thawing produced detrimental effects that detracted from flavor amplitude. For instance, the higher moisture release during mastication observed substantiates previous findings that the juiciness of pork roasts improved slightly with freezing and frozen storage (178) and is on agreement with the results of Tuma (179) on beef. In addition, present findings that pork loins were less cohesive during the first bite and mastication, and were less chewy and required fewer chews during mastication lends support to, and aids in explaining, previous reports that the tenderness of meat improved during freezing and frozen storage (180, 181, 182, 183, 184, 178). However, the low flavor amplitude ratings received by frozen and thawed samples clearly indicates that freezing, frozen storage, and thawing results in a less appropriate and less well balanced and blended flavor. Present findings also appear to be in agreement with previous conclusions that uncured pork palatability can be maintained for at least 6 months (177, 178).

Furthermore, present findings regarding moisture release, cohesiveness, and chewiness coupled with the fact that frozen and thawed samples appeared to break down to more appropriate particles clearly indicated that freezing and frozen storage is beneficial to the textural properties of pork loins. There is some evidence (185) that rapid freezing affects a greater tenderization because the ice crystals are formed within the muscle fibers, causing a disintegration of the fiber with an accompanying increase in tenderness. In one study comparing the eating quality of rib roasts of beef frozen under ten different conditions, it was concluded (186) that the more rapidly the meat was frozen, the better was its all-round quality. It has been claimed (187) that freezing is important in the maintenance of "bloom," or fresh appearance of poultry. This report refers to a "dark color which results from slow freezing." With a lower freezing temperature and hence a faster rate of cooling tenderness appears to be somewhat improved. As far as the authors are aware, there is no satisfactory data to indicate that freezing, regardless of its rapidity, has an important effect on other palatability criteria in meats. Appearance, odor, and taste seem to be unaffected. Some (188) claim that a short interval between formation of the first ice crystals and the complete freezing of the fruit is essential for the maintenance of texture, palatability, and

satisfactory superficial appearance. There is little question that freezing does "tenderize" or soften vegetable tissues. The final test — and the ultimate one, that of appearance, odor, flavor, texture — was equally negative. The slow-frozen vegetables were as acceptable to the palate as the fast-frozen ones. However, it does seem evident that rapidly frozen fish have a higher taste-panel acceptance than very slowly frozen ones. In appearance, odor, flavor, and nutritive value, the home-frozen product can usually vie with the quick-frozen food in commerce as being a quality item.

H. Poultry and Eggs

1. Poultry

Flavor, texture and juiciness of smoked broilers stored at −18°C for up to 12 months does not change (189). When broilers are fed with a diet containing rosemary and sage, and frozen stored at −20°C no rancidity is detected after 4 months (190). Chicken hot-drumettes containing 2% cayenne pepper are scored 2.5 after cooked (1 = no hotness, 5 = extremely hot) after four days of frozen storage (191). A duo-trio sensory evaluation of the flavor of breast meat from broilers fed with marine algae, vacuum packaged, and frozen stored at −23°C showed no changes among fresh breast samples and frozen stored samples after 3 days of storage. Feeding 5.5% marine algae or 2.1% menhaden oil increase the flavor scores after 82 days of storage (192). Leg meat frozen produces more substantial oxidative decomposition of fats (193). The evidence indicates that juiciness, tenderness, and general palatability of broilers are the same whether the birds are frozen with 2 hours of slaughter or are held until the body temperature has dropped to the temperature of the cooler. Furthermore, as the period between slaughter and freezing is increased, the greater is the tendency for the fat in poultry to turn rancid during zero storage (194).

Frozen turkey breast does not show rancidity after 150 days of frozen storage. Oxidized aromas are normally detected. Color scores increase if the level of vitamin E in their diet is increased. In mechanically deboned chicken and turkey meat stored frozen for 6 months, some color is lost but still acceptable for use in emulsion products (195). Chicken thigh meat patties formulated with 5 to 20% fat, cooked and stored for up to 9 months does not show rancidity (196). Patties made with ground, dark turkey meat frozen and kept for 3 weeks are scored low if only polyphosphates or water are added but those with alkaline tripolyphosphates receive higher sensory scores (197).

2. Eggs

Partial freezing, light freezing or super chilling has been used to preserve freshness of food products. Liquid whole egg, liquid yolk and liquid yolk stored at −0.5 and −3°C temperature for up to 50 days length of storage do not affect the palatability test for scrambled eggs (198). Liquid eggs are used as ingredients in candy, baked good, salad dressing, and ice cream. One-third of all pasteurized liquid egg is frozen prior to distribution to extend shelf-life. It has been reported that pasteurization and frozen storage influence liquid egg rheological properties and functional properties such as emulsifying properties, decrease in foaming and whipping. Pasteurized frozen eggs stored for up to 80 days show changes in the solubility of certain proteins such as lipovitellin and livetin, which are responsible for the emulsifying properties of eggs (199, 200). The lumpy character of the thawed yolk is an undesirable quality that generally must be avoided. Egg yolk subjected to linear electron beam irradiation at ~2.5 kGy dosage and frozen stored at −15°C for 60 days show that hue of irradiated samples is more yellow than the nonprocessed samples after 60 days of frozen storage. The saturation index is lower for the irradiated samples. Storage effect in redness and hue are also observed. Color of egg yolk becomes slightly less reddish yellow during storage or the yolk lost pigment during storage (201).

I. Peanuts

Roasted peanut paste kept at −20 and −10°C does not change its sweetness sensory attribute; however, bitterness (cause by saponins) and tongue burn tends to change over frozen storage. The most important sensory attribute that changes over frozen storage is 'staling' that is used to describe light lipid oxidation and the characteristic flavor that results. This attribute has also been described as 'old, cardboardy, strawberry-like note.' As lipid oxidations progresses, changes are described as 'painty.' The fruity attribute in roasted peanut paste develops from abusive environmental and handling exposure but also increases over frozen storage. There is no change for the 'roasted peanut' attribute during storage at low temperature (202).

J. Soybeans and Soy Products

Food companies and consumers alike use the versatile raw ingredient known as soybeans in various food products for numerous reasons. Soy concentrates have the ability to bind and hold natural flavors and moisture, resulting in products that stay moist and flavorful even after reconstitution in conventional, convection, or microwave ovens (203). Soy flour has a blander flavor and is able to retain meat juices; while many lactose intolerant individuals consume soymilk. As a result, soybeans and their constituents are gaining popularity and usage in the food industry.

Soybean producers claim that consumers seek the sweetness, pleasantness and firm, nut-like texture of the soybean. Young et al (204) has reported that females are likely to purchase frozen soybeans to use in recipes as an alternative to meat as a result. However, if improperly processed, soybeans and soy products develop objectionable

off-flavors and off-aromas. Bitter, astringent, and sour flavors are common flavor defects reported in soybean-based products. A chalky texture may be detected in some products such as soymilk due to improper processing. In other soy protein products, the freeze-thaw cycle will result in a gummy and unacceptable product. These off-flavors and textures are important limiting factors in the use of soybeans and soy constituents in products that are shelf stable and/or frozen. These off-flavors may be the result of the high proportion of unsaturated fatty acids found in raw soybeans. There are two major interrelated causes for development of such flavors. One is the high proportion of unsaturated fatty acids, particularly linolenic acid, in the soybean oil fraction, and the other is the abundant presence of lipoxygenases in soybeans. The enzyme catalyzes the oxidation of polyunsaturated fatty acids of soy lipids, producing hyperoxides. Degradation of hyperoxides leads to formation of various types of volatile compounds, many of which have an objectionable odor or flavor, described as beany or greeny.

Soymilk and soy products tend to have an objectionable aftertaste that has been described as sour, bitter, and astringent. These undesirable characteristics probably result from the presence of some minor compounds in soymilk including phenolic acids, saponins, oxidized phospholipids, and isoflavones. Soymilk, if it is not properly processed, tends to have a chalkiness defect. The freezing of this product tends to worsen this defect (205).

There are many different soy derived products that are incorporated into foods for functional, nutritional, processing, and other properties. These ingredients can actually improve the nutrition, flavor, texture, and consumer acceptance of foods. If properly formulated, these types of soy based or enhanced foods freeze well with little detrimental attributes developed in the freezing process.

Soybean isolate is made from a spun soybean oil and is almost flavorless and the taste of which, therefore — exclusive of texture — depends to a large extent upon the quality and expertise involved in adding the flavors. Freezing has the tendency to make soybean isolates gummy. However, depending on the product in which it is incorporated, this may or may not lend the product unacceptable. Soy protein isolates have been perfected that make possible a hot dog that contains only 3% fat, yet cannot be differentiated in flavor and texture from those one might be accustomed to and are competitive in price. These types of soy products tend to freeze well with virtually no loss of flavor or texture quality.

Extruded soybean extender, which can be in dry form as flour or pellets, often has off-flavors and must be blended with products or must have flavors or spices added that are strong enough to override the flavor of soy itself. Typically, this product freezes well and due to the nature of the flavor system, is not seen as being hindered by freezing. For example, there is a great deal of meatless chili made, both frozen and unfrozen, and the strong chili-powder flavor completely dominates the mixture. In this case, the soy extender is added to give the texture of the meat without the much higher cost of meat. There are even "beef" stews of this sort with no beef at all in them, but the generous quantity of spices present in all such stews drowns out the beany flavor of soy (206).

Formulating with textured soy proteins or such extenders is more complex than a direct substitution of another type of muscle food into a food system. Processors sometimes forget that meat is naturally about 80% water and when an extender is used that absorbs too high a quantity of water it changes the texture and flavor of the beef by increasing the percentage of water to solids. If the product is frozen, this leads to an unacceptable product. Often too, saturation occurs, the cells become ruptured and the product becomes separated and soggy. This is an unacceptable quality attribute for products of this type.

Soy meat extender has also proven valuable in imparting proper texture to mechanically de-boned meat. This product tends to freeze well and no "off" flavors or texture attributes are typically observed. Soy flour has the advantage of holding the juices in meats. Its disadvantage is that of taste and physical feel in the mouth.

Soy proteins are used extensively in breads, doughnuts, rolls and sweet goods. They are not necessarily cheaper than the flour they replace, but they hold the moisture levels at an accepted and desired level thereby producing a better-textured product. Some of those using it don't know whether to attribute the better texture to moisture retention or to some interaction of the soy proteins with the other ingredients that has not been adequately researched as yet (203).

Soy-protein burgers, with taste and mouthfeel of beef, are an excellent source of protein. The grain-based burgers are usually higher in dietary fiber and are marketed to people who want a convenient food that has interesting textures and flavors, not necessarily imitating meat. The only caution is to avoid freeze-thaw cycles, because they make soy protein products gummy. However, most of these products are found in the grocer's freezer case, and are accepted well by consumers.

When used as emulsifiers, binders, moisture retainers, and stabilizers, soy proteins can alter or improve the appearance, taste, and texture of the finished product. Flavor-wise, the white flake (soy flour and soy grits) has a bitter, beany taste. The toasted product is darker in color and the enzyme and anti-trypsin activities are destroyed (207). The flavor is also improved to the point where it takes on a sweet nut-like taste (208). The intermediate or cooked product has properties between the two.

Although the cost of soy protein concentrate is more than double that of soy flour (18½–28 cents per lb.), it is rapidly replacing the soy flour used in emulsion products because of its blander flavor. Soy protein concentrates tend to become over-saturated and are unable to retain

moisture when incorporated into certain food systems and then subjected to freezing. Product shelf-life can be shortened if the temperature of the freeze-thaw cycles is not carefully controlled.

ACKNOWLEDGMENT

The information in this chapter has been derived from "Food Sensory Attributes," by P. C. Coggins and R. S. Chamul. In Editors: Y. H. Hui et al *Handbook of Frozen Foods*, Marcel Dekker, New York, 2004.

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59 Large-Scale Concept-Response Databases for Food and Drink Using Conjoint Analysis, Segmentation, and Databasing

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CONTENTS

I. INTRODUCTION: THE NOTION OF A MEGA STUDY AND CONCEPT RESPONSE DATABASE FOR FOOD AND BEVERAGE

One of today's pressing needs is to better understand the mind of the consumer in order to spot newly emerging trends in the market, and to capitalize on them. Data themselves are no longer the choke point in the market. Researchers and businesses are awash in data. Data that cannot be easily obtained by subscribing to information services can be obtained by commissioning a custom study. In one way or another the marketer, the product developer and the researcher can answer most of the questions about new and current products, advertisements, and consumer attitudes.

Given this abundance of information the question one might naturally ask is why bother with more data? What is missing? Why would the rational businessperson invest in new data when there is so much data from which to choose? The answer is quite simple. There are lots of sources of data, but unfortunately there is no systematically developed database about the "mind of the consumer" that can be interrogated to identify patterns of what communications about products drive acceptance. The situation in the food world today can be likened to an amalgam of differently sourced computer programs that perform the gamut of tasks from spreadsheets to presentations to document control and preparation. Until Microsoft came out with its Office Suite® there were many relatively unconnected alternatives for each task. Going from one task to another, e.g., from document preparation to computation

to presentation, meant learning all sorts of new tasks, finding how to do things, and then doing the task. In the meantime the effort was spent on learning the steps to move from one system to another, rather than on the information to be communicated.

There is no systematic knowledge or database available to understand how consumers respond to different brand names, features of products, emotional aspects of products, or aspects of the buying situation. Most of the knowledge resides in unrelated sources, such as corporate offices, trade and academic journals, and the experience of development and marketing professionals. A lot of information resides in disparate documents available to the public and accessed either by some intelligent search engine such as Google®, or by some "pay-as-you-go" system such as Lexis/Nexis®. A cross-sectional *and* longitudinal database to understand the "algebra of the consumer mind" is a major contribution to academic and business oriented product development, marketing, and consumer sciences.

This article presents the structure of the integrated database for food and drink, showing how consumers respond to messaging about food, and how their responses can be better understood by looking at how the respondents profile themselves in a classification questionnaire. The approach is known as the It!™ system. It!™ uses the power of primary research, with a powerful, state of the art research tool (conjoint measurement), executed in-depth for specific categories, and applied to a related set of 20–40 different related products or services. This approach generates one integrated mega-database. Through this integrated database of 20–40 related studies in a specific area it becomes possible to:

1. Identify the features and messages that drive interest
2. Compare these features and messages across different but related product categories
3. Divide people by their profile of attitudes
4. Segment consumers on the basis of the pattern of features and communications that interest them
5. Prescribe new product concepts based upon the winning elements for each segment
6. Assess trends in the population based upon the patterns across years of utilities from the conjoint measure

The "mega-categories" for the food and beverage industries that have been already developed using the approach below are:

1. Food acceptance (reported here as the Crave It!™ Study), for the US and European respondent, and for teenagers
2. Healthful foods (Healthy You!)
3. Beverages (Drink It™)
4. Fast Foods (It's Convenient)

However, the approach to create these "mega-studies" and their databases has further been used for a number of other categories outside of food such as

5. Insurance (Protect It!™)
6. Consumer Health
7. Charity (Give It!™)
8. The shopping situation (Buy It!™)
9. Personal items (mine™)
10. Financial investments (Finance It!™)

II. THE BASIC TOOL FOR CONCEPT RESEARCH — CONJOINT ANALYSIS

Conjoint measurement refers to a set of research procedures, often called trade-off analyses. The idea behind conjoint measurement is that the respondent cannot easily tell the researcher how concept elements might work in combination, but can respond to concepts as a tonality from which the behavior of the individual concept elements can be deduced. For the researcher to understand the dynamics of concept elements requires an experimental design, or systematic combination of different concepts in such a way that the part-worth contribution of each element can be readily measured. Conjoint analysis uses the experimental design as a structure within which to combine the concept elements systematically, and then requires respondents to rate the concept as a totality (1). From the set of ratings assigned to the concepts the researcher deduces the contribution of the component elements. Conjoint analysis has evolved into a widely used, well respected tool for the practical development of concepts in the business community (2, 3).

Conjoint analysis can take several forms, such as the direct selection of alternatives for the concept elements (so-called trade-off analysis), or response to complete concepts (called full profile). No matter which approach the researcher selects, the goal is always to identify the utility or contribution values of the individual elements. Conjoint analysis was developed in the 1960s as a basic measurement technique by mathematical psychologists (4), but soon found a home in the marketing community (5). More recent adaptations have moved the method to handle hundreds of elements (6), and have seen the migration of the approach to the Internet (7).

A. Setting up the Study

The concept elements appear independently, as prescribed by the experimental design. The experimental design ensures that the elements appear statistically independently of each other, allowing regression to be used. Various

TABLE 59.1
Example of an Experimental Design

Concept	A	B	C	D
1	1	5	0	0
2	6	2	0	1
3	3	2	2	3
4	4	5	6	4
5	6	5	3	5
6	6	0	4	4
7	1	3	2	4
8	2	6	4	1
9	1	4	6	1
10	3	4	1	4
11	0	3	3	3
12	0	2	4	0

methods are used to create the design. Elements cannot be randomly combined, but rather appear in a systematic fashion, dictated by the experimental design. Table 59.1 presents a specific experimental design, comprising four variables (A,B,C,D), and six options for each variable (1–6). In some concepts a specific variable may be absent, denoted by the value "0" for the particular variable, for that particular concept (e.g., in Concept #1 variables C and D do not appear).

B. Modeling Considerations

The independence of the concept elements allows the researcher to create a model using ordinary least squares (OLS), which relates the presence/absence of the concept elements to the rating. The OLS modeling follows the convention of market research, which focuses on the *proportion* of respondents who say that they are interested in the concept, rather than focusing on the intensity of interest. To work within the market research framework we re-code the concept ratings, so that ratings of 1–6 (low end of the scale) are changed to the value "0," whereas ratings of 7–9 (high end of the interest scale; so-called "top 3 box") are changed to 100. The equation is expressed as a simple additive model of the form:

$$\text{Top 3 Box} = k_0 + k_1(\text{Element 1}) + k_2(\text{Element 2}) \ldots k_{36}(\text{Element 36})$$

The foregoing equation is written for 36 elements, the usual case for the mega-study. The additive constant, k_0, is the conditional probability that the respondent would be interested in the concept if the elements were not present. The additive constant is a baseline value, estimated purely from the data, since all of the concepts comprised 2–4 elements by design. The coefficients $k_1 \ldots k_{36}$ are the additive or subtractive conditional probabilities that the respondent would be interested in the concept if the element were added to the concept.

The utility values for the additive constant or for a particular element are aggregated across a relatively large number of respondents to arrive at an average value. Typically the data should come from at least 20 respondents, at which point the results begin to stabilize (7).

C. Segmentation

Segmentation refers to the division of respondents according to specific criteria. The criteria can be developed on the basis of how the respondents classify themselves (e.g., sex, market in which they live, etc.). These segments or subgroups of respondents typically generate highly correlated utility values. That is, what one subgroup likes its complementary subgroup will probably like as well. Quite often the correlations across 36 elements for such complementary subgroups are as high as 0.85 or more, and quite often above 0.60.

Another way to look at subgroups identifies groups of respondents in the population with similar response patterns. These are called concept-response segments. Segmentation using utility values is particularly easy using today's statistical computing power. The method used for the mega-studies is known as k-means clustering (8). The ratings for each respondent are subject to a second analysis by ordinary least-squares, this time without any prior transformation to 0/100, but rather using the "raw" ratings assigned by the respondent as the dependent variable. Each respondent generates a second set of utilities, one utility for each concept element. The method of k-means clustering creates an index of "distance" between each pair of respondents by computing the statistic (1-R), where R = Pearson correlation coefficient. All of the utilities for each pair of respondents are used, with the exception of the additive constant. The statistic (1-R) can vary from a low of 0 when the two respondents show perfectly related patterns (what one person likes the other likes as well), through a middle value of 0 (where they are not related), through a high of 2 (what one person likes the other person dislikes, so the patterns are inverse). The clustering divides the respondents so that individuals in the same cluster are "close" to each other based upon the statistic (1-R); i.e., pairs of individuals located in the same cluster have low values for this statistic (9).

D. Establishing Consistency (and Validity) of Conjoint Method Results

In order for results to be believable one must establish a criterion of validity. This is especially the case for conjoint measurement studies executed over the Internet, where there is no way to ensure that the respondent is paying attention, and where the interview length may be as long as 15–20 minutes. Since the conjoint measurement approach uses ordinary least-squares at the individual

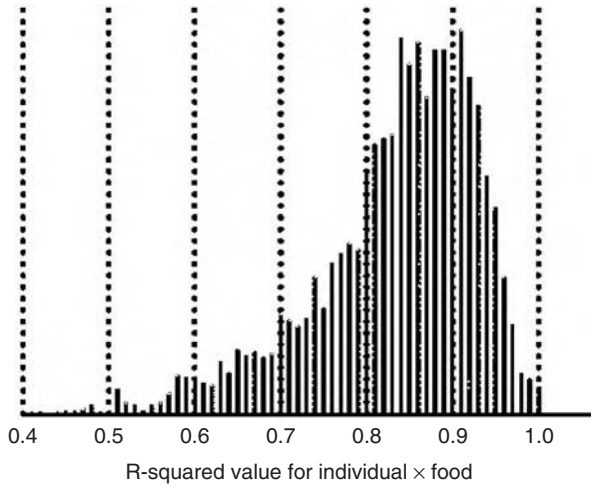

FIGURE 59.1 Distribution of R^2 statistic (consistency and validity measure) from 20 foods, and 4,000+ respondents. An R^2 value exceeding of 0.65 is high significant ($p < 0.05$).

respondent level, it is straightforward to compute a goodness-of-fit measure at the individual level. This measure is the multiple R^2 statistic which shows the proportion of variability accounted for by the equation. The statistic varies from a low of 0 corresponding to random data, up to a high of 1.00 corresponding to a perfect relation between model and data. With 36 elements, the usual number of elements in a mega-study, empirical data suggests that 80% or more of the respondents show consistent results, as shown in Figure 59.1.

III. MEGA-STUDY: A BRIEF OVERVIEW OF THE DATA BASE DESIGN, FIELD EXECUTION, AND ANALYSIS

The approach to create the database uses up to 30–40 linked conjoint studies, following these seven steps:

Step 1 — Identify the general topic area and the specific products for a mega-study. Each mega study comprises 20–40 smaller studies. For example, the aforementioned Crave It! study reported here really comprised 30 different studies. Each study deals with a specific food or beverage (e.g., hamburger, potato chip, coffee). Thus the scope of the mega-study is larger than a typical study that deals with a single product or related set of products. The inspiring metaphor for the mega study is the holistic view afforded by genomics (10).

Step 2 — Develop the experimental design. Conjoint measurement works by main effects experimental design (1). The stimuli or elements are mixed and matched in such a way as to produce different combinations. The statistical objective is to identify the appropriate and efficient experimental design, have a sufficiently large number of independent elements to provide good content (i.e., wide range of concept elements), and enough but not too many concepts to ensure a pleasant interview that does not last too long. The mega-studies use the same type of experimental design, i.e., four independent variables or "categories," and nine alternative concept elements per category. The experimental design dictates the combination of these elements into 60 different combinations. Each combination comprises either one or no element from each of the four categories. The choice of the experimental design is dictated by the desire to minimize the number of combinations that any particular respondent must evaluate, as well as ensure that it is possible to run an ordinary least squares regression on the ratings for the individual. The 60 combinations accomplish both objectives.

Step 3 — Identify the categories and the concept elements. Categories comprise elements that are related to each other. Thus one category might be "primary attributes." The categories are a method for "bookkeeping." The experimental design calls for four "silos" or independent variables, each of 9 elements (E01–E09, E10–E18, E19–E27, E28–E36, respectively). A single category fits into a silo. There may be several categories in a single silo, as Table 59.2 shows. The objective of the bookkeeping approach using silos and categories is to ensure that a concept contains only one message of a certain type, such as emotional reward or product description, so that the concept is logically coherent and does not contain fundamentally conflicting information such as two brand names.

The concept elements in one particular study are comparable to elements in another. The text of the element is appropriate for the particular study, but fundamentally true to the basic overarching design. Furthermore, in quite a number of cases the same text can be used across the studies. This common structure across the studies allowed for meta-analyses, showing patterns transcending a particular study. Emphasis in each study is on the balance of functional and emotional elements (11).

Step 4 — Create a classification questionnaire. The questionnaire deals with different types of issues, such as geo-demographics (age, gender, income, market), what is important in making a decision, state of hunger, time of test, etc. Table 59.3 presents a general set of questions. Where relevant the different answers are given. The classification questionnaire remains virtually the same across individual studies within a mega-study, allowing for comparisons across studies.

Step 5 — Invite respondents to participate. Internet-based research is becoming increasingly easy to execute. More people are obtaining Internet access each week, increasing the number of available respondents. No longer is it a problem to find individuals who have Internet connections, and no longer is the representativeness of respondents on the Internet an issue as it was some years ago. Various service providers have sprung up to deliver either

TABLE 59.2
Example of Categories, Elements, and their Rationales

El	Category	Rationale	Hamburgers	Chocolate Candy
E01	Primary Attributes	Basic physical attributes	Fresh grilled hamburger	A smooth, dense piece of chocolate
E02	Primary Attributes	** (Continuum: basic to complex/detailed physical attributes) in some cases … "healthy"	A chargrilled hamburger with a taste you can't duplicate	Smooth appearance with a light chocolate flavor and a creamy texture
E03	Primary Attributes	** (Continuum: basic to complex/ detailed physical attributes)	A grilled aroma that surrounds a thick burger on a toasted bun	Crispy wafers coated in thin layers of milk chocolate
E04	Primary Attributes	** (Continuum: basic to complex/ detailed physical attributes) in some cases … "real"	Moist bites of bun, burger, and onion	Real chocolate made with ingredients like chocolate, cocoa butter, vanilla, and sugar
E05	Primary Attributes	** (Continuum: basic to complex/ detailed physical attributes)	Juicy burger with the crunch of lettuce and tomato	White chocolate with crunchy cookie pieces throughout
E06	Primary Attributes	** (Continuum: basic to complex/ detailed physical attributes)	Gooey grilled burger with rich sauce and fresh lettuce and tomato	Heavy dense chunk of chocolate with complex flavors, velvet appearance ... enticing aroma
E07	Primary Attributes	** (Continuum: basic to complex/ detailed physical attributes)	Layers of burger, sauce, pickles, and lettuce on a moist sourdough sesame seed bun	Dense chocolate with swirls of dark chocolate and chocolate sprinkles on the surface
E08	Primary Attributes	** (Continuum: basic to complex/ detailed physical attributes)	Lots of crispy bacon and cheese on a juicy grilled hamburger on a lightly toasted bun	Clusters of chocolate and nuts, with caramel & marshmallow throughout
E09	Primary Attributes	Complex physical attributes; details	Burger smothered in onions and cheese	Golden milk nougat with whole almond pieces on top, caramel drizzled over them and enrobed with semi - sweet chocolate
E10	Secondary Attributes/Mood	Party pleaser/inviting	Burgers are a party pleaser	When it's cold outside, chocolate is cozy and inviting
E11	Secondary Attributes/Mood	Beverages	With a chilled glass of water … or carbonated beverage	With a hot cup of coffee, tea, hot cocoa … or carbonated beverage
E12	Secondary Attributes/Mood	With …	With great tasting french fries … and that special sauce	Bite size pieces; ready for a fast taste … with a chocolate truffle filling
E13	Secondary Attributes/Mood	Premium quality/ classic taste	Premium quality … that great classic taste, like it used to be	Premium quality … that great classic taste, like it used to be
E14	Secondary Attributes/Mood	Savor it …	You can just savor it when you think about it during work and school	You can just savor it when you think about it during work and school
E15	Secondary Attributes/Mood	All natural/ changing flavors	100% natural … a real beef burger!	100% natural … and new choices every month to keep you tantalized
E16	Secondary Attributes/Mood	With all the extras you want …	With all the toppings and sides you want … pickles, relish, jalapenos … lettuce, tomato, chips … whatever	With fruit fillings in any flavor you want
E17	Secondary Attributes/Mood	Imagine the taste …	You can imagine the taste as you walk in the door	You can imagine the taste as you walk in the door
E18	Secondary Attributes/Mood	Lick your lips twice …	So tasty & juicy you practically have to lick your lips twice after each bite	So good … you practically have to lick your lips twice after each bite
E19	Emotional	Quick/ fun/ alone	Quick and fun … eating alone doesn't have to be ordinary	Quick and fun … eating alone doesn't have to be ordinary
E20	Emotional	Have to have it … can't stop	When you think about it, you have to have it … and after you have it, you can't stop eating it	When you think about it, you have to have it … and after you have it, you can't stop eating it
E21	Emotional	Fills that empty spot …	Fills that empty spot in you … just when you want it	Fills that empty spot in you … just when you want it

(*Continued*)

TABLE 59.2
(*Continued*)

El	Category	Rationale	Hamburgers	Chocolate Candy
E22	Emotional	Cheers you up …	When you're sad, it makes you glad	When you're sad, it makes you glad
E23	Emotional	Escape routine/celebrations	Now you can escape the routine … a way to celebrate special occasions	Now you can escape the routine … a way to celebrate special occasions
E24	Emotional	Multi-dimensional sensory experience	A joy for your senses … seeing, smelling, tasting	A joy for your senses … seeing, smelling, tasting
E25	Emotional	With family and friends	An outrageous experience … shared with family and friends	An outrageous experience … shared with family and friends
E26	Emotional	Ecstasy …	Pure ecstasy	Pure ecstasy
E27	Emotional	Satisfies hunger …	It feeds THE HUNGER	It feeds THE HUNGER
E28	Brand or Benefit	Basic brands/experiences	At White Castle	From Hershey's
E29	Brand or Benefit	** (Continuum: basic to premium brands)	At Jack-in-the-Box	From M&M/Mars
E30	Brand or Benefit	** (Continuum: basic to premium brands)	At McDonald's	From Nestle
E31	Brand or Benefit	** (Continuum: basic to premium brands)	At Wendy's	From Cadbury
E32	Brand or Benefit	** (Continuum: basic to premium brands)	At Burger King	From Lindt
E33	Brand or Benefit	Premium brands/experiences	At Fuddruckers	From Godiva
E34	Brand or Benefit	Fresh … for you … by you	Fresh from the grill, especially for you … by you	Made fresh … especially for you
E35	Brand or Benefit	Best in world …	Simply the best burger in the whole wide world	Simply the best chocolate in the whole wide world
E36	Brand or Benefit	Safety …	With the safety, care and cleanliness that makes you trust it and love it all the more	With the safety, care and cleanliness that makes you trust it and love it all the more

pre-qualified respondents to the study on a pay-per-respondent basis, or are in the business of sending out invitations to e-mail lists of respondents, and charging only by the number of e-mails. The invitation to the respondent appears in Figure 59.2. An interesting invitation letter generates a large number of respondents, so that it is important at the time of invitation to provide an incentive such as a chance to win a cash prize (sweepstakes).

Step 6 — Execute the interview on the Internet. The respondent is first brought to a "wall," where he can select the study of interest (Figure 59.3). The wall is set up so that the least popular study (fewest respondents participating) is at the top left, and the most popular study (most respondents participating) is at the bottom right. The locations of the studies are dynamically changed at frequent intervals according to this rule. This strategy ensures that the studies are not be biased by location. When the base size reaches a specific cut-off, the study option disappears and the button for the particular project disappears from the wall. After selecting the study, the respondent goes to a particular interview. The interview is structured, beginning with an orientation page (Figure 59.4), proceeding to the conjoint analysis portion which presents the test concepts (Figure 59.5), and finishing with the classification page. At the end of the interview the respondent is shown the winning concept synthesized for the total panel and the winning concept synthesized for the respondent himself (informational feedback; Figure 59.6).

The basic structure of the experimental design driving the combination of concepts remains constant, but the particular combinations of elements vary across respondents by a systematized permutation. In this way each respondent's data can be separately analyzed, but there is no need for concern that a particular combination unduly influences the results. That cannot happen, because each respondent evaluates different combinations. For any particular food, there are 200 different experimental designs created, each comprising 60 combinations of the 36 elements, 2–4 elements at a time. A respondent is randomly allocated to a specific experimental design. No more than three respondents are ever allocated to the same design. This strategy ensures minimal bias due to a particular combination.

The respondent rates the concept (systematic combination of elements) on an evaluative measure. For the Crave It! data base the respondent rates degree of craveability on an anchored 1–9 scale. The anchors are appropriate for each scale, located at the low-scale value of 1 and the high-scale value of 9.

The final portion of the interview comprises the self-profiling classification. The objective here is to have the respondent profile himself after the conjoint measurement, in order to better understand his geo-demographics, values, and patterns of behavior pertaining to the specific food. The information provides a snapshot of how the respondent sees himself. Furthermore, the results can be analyzed from the perspective of each particular food.

TABLE 59.3
Types of Classification Questions Asked in the Mega- Studies

Q1: Tell us your gender
Q2: How hungry are you right now? [check one]
Q3: What time is it? [check one]
Q4: Please tell us your age. [check one]
Q5: How often do you eat <FOOD NAME>? [check one]
Q6: Where do you typically purchase <FOOD NAME>? [check two]
- M1. Food store
- M2. Convenience store
- M3. Warehouse or club stores, like BJ's or Sam's Club
- M4. Superstores, like Wal-Mart or Target
- M5. Quick service restaurant, like McDonald's, KFC, or Taco Bell
- M6. Local chain restaurant, like Applebee's or Friday's
- M7. Local eatery, known only in your area
- M8. Specialty shop
- Etc.

Q7: Which 3 product attributes MOST influence your craving for <FOOD NAME>?
- M1. Appearance
- M2. Aroma
- M3. Texture
- M4. Taste
- M5. Memories
- M6. Associations
- M7. Brand
- M8. Advertising
- M9. Packaging
- M10. Portion size
- M11. Social situation
- M12. Mood
- Etc.

Q8: On what 2 occasions do you typically crave <FOOD NAME>MOST? [check two]
- M1. In the morning or at breakfast-time
- M2. Mid-morning
- M3. Around lunch-time
- M4. Mid-afternoon
- M5. Just before dinner or while dinner is being prepared
- M6. After dinner
- M7. When shopping in the food store
- M8. On the way to or from work or school
- M9. After school
- M10. Watching TV
- M11. When I am alone
- M12. When I am with friends or family
- M13. When I want to celebrate
- M14. When I'm bored and restless
- M15. When my hormones are going crazy
- M16. When my kids/siblings are bugging me
- M17. When I just need to escape

From these self profiling data the researcher can create a profile of foods and occasions/situations when they are most desired and/or consumed. It is important to note, however, that these results are strictly from the self-profiling done in the classification questionnaire.

Step 7 — Analyze the data, set up the database. The data from any single study in a mega-study comprises the utility values from respondents, as well as results from the self-classification questionnaire. The utility values are arranged by total panel and by key subgroups. Subgroups include respondents of different ages, respondents who say that they accept a product for specific reasons, as well as segments of respondents based upon their pattern of utility values. Each product in a particular mega study generates its own database. By creating databases with the same subgroups, and by linking together elements with the same rationale across studies, the researcher ensures that database transcends any particular product.

IV. A SAMPLE OF RESULTS FROM A MEGA-STUDY; THE CRAVE IT! STUDY

In a short article is impossible to do justice to the contents of any of the mega-studies. Thus the remaining part of this article provides the reader with a sense of the type of data that one might obtain by running these studies. The data will be taken from the 2001–2002 Crave It! studies, which dealt with rated craveability of a variety of foods.

a. Number of respondents in a study as a measure of latent interest in the product. The respondents can choose any study that they find interesting. Either the rate at which a study fills up or the total number of respondents in the study gives a sense of latent interest. Respondents tend to choose studies that they find interesting. Table 59.4 shows the final number of respondents, and the proportion of men and women respondents in 19 of the Crave It! studies (2001 data). The study was run until every food had at least 150 respondents. Data of this type show that commonly crave foods include chocolate candy, pizza, and ice cream. Furthermore, by looking at the proportion of men versus women the researcher gets a sense of the type of foods that interest the genders. It is clear from these results that although women participate more, there are gender-linked product preferences driving more men to participate frequently in a study dealing with steak than in a study dealing with a sweet baked product such as cheesecake. The foods differ, with men responding relatively more frequently to protein items, and women responding relatively more frequently to fat/sweet products.

b. What aspects of the food or beverage drive craveability? In the self-profiling questionnaire the respondent was instructed to select three answers from a set of 16. Across the full set of 30 foods, and across all 4700+ respondents, the answers most frequently given were "taste,"

Take our survey and win CASH!! The more surveys you take, the more chances to win!!
Are you the kind of person who loves FOOD and DRINK? Whatever you eat or drink, here is a survey for you! Tell us what kinds of foods and beverages you CRAVE ... choose from up to 32 different kinds ... then qualify to receive one of four CASH PRIZES and join one of The Understanding & Insight Group's exclusive consumer panels.
Simply click on the link below (if your email does not support hotlinks, cut and paste the link into your browser) and choose one of the easy-to-answer surveys.
http://12.109.160.54/uics2y4/craveit2002.asp
You will only have until NOON on XXX to complete this survey, so be sure to respond as quickly as possible. Your opinions are important to us!
Depending on your connection speed, each survey should take between 15 and 20 minutes to complete.
Forward this survey to your friends and family so that we know what kinds of food and beverages they CRAVE, too! Everyone's opinion is important!
Please be assured that any information you provide will be held in the strictest confidence. You will not be contacted by any sales or other research organization as a result of your participation in theis survey.
If you have any difficulty accessing the survey, you can contact Tom Farrar at testmaster@theuandigroup.com.
Thanks in advance for your input, and have fun!
The Crave IT! Team

FIGURE 59.2 Invitation to participate in a "mega study," sent to respondents by e-mail.

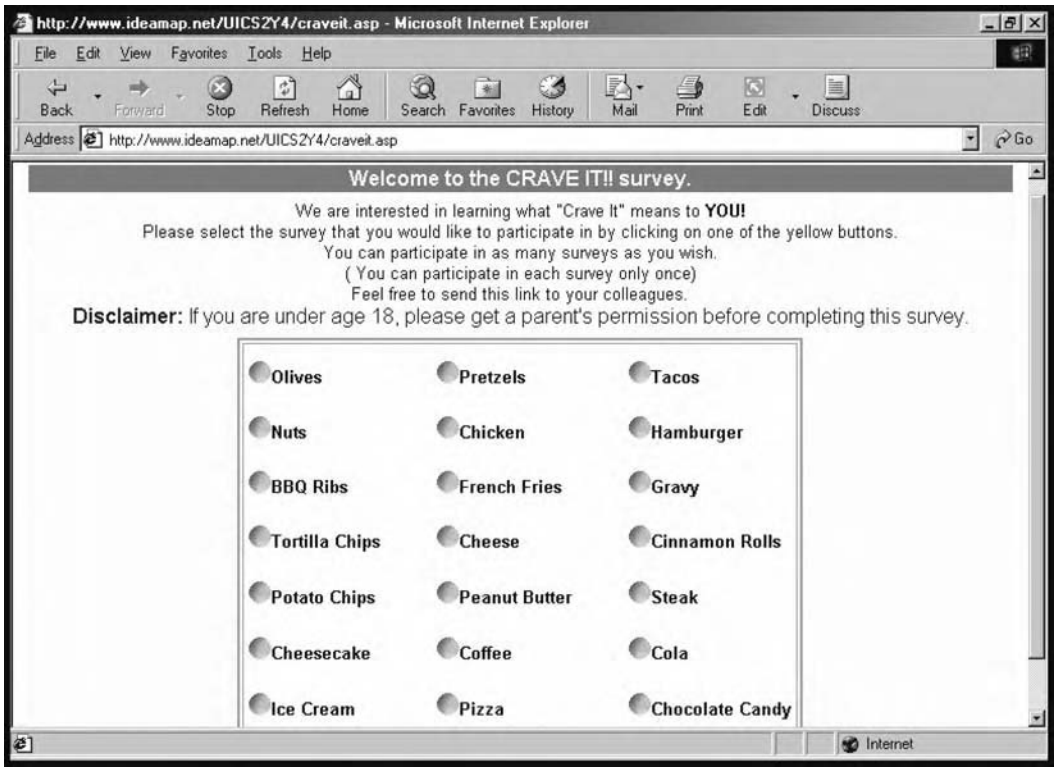

FIGURE 59.3 The "wall" which shows the available studies. The respondent picks a study and proceeds.

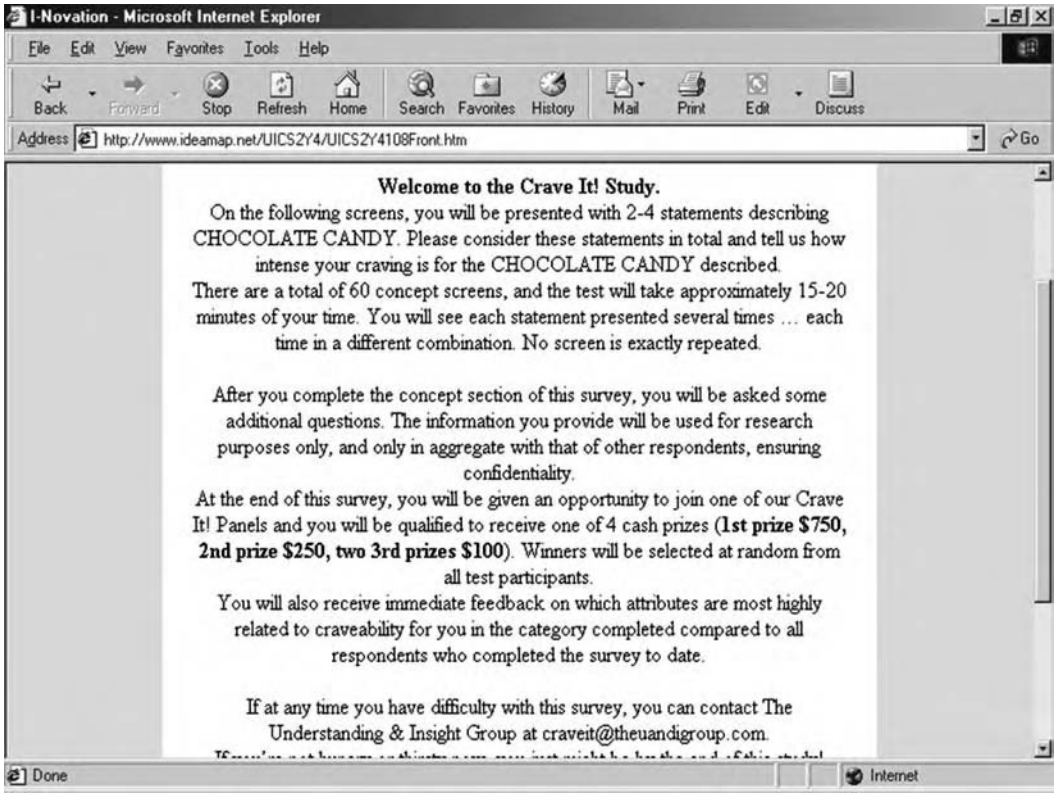

FIGURE 59.4 Orientation to the study.

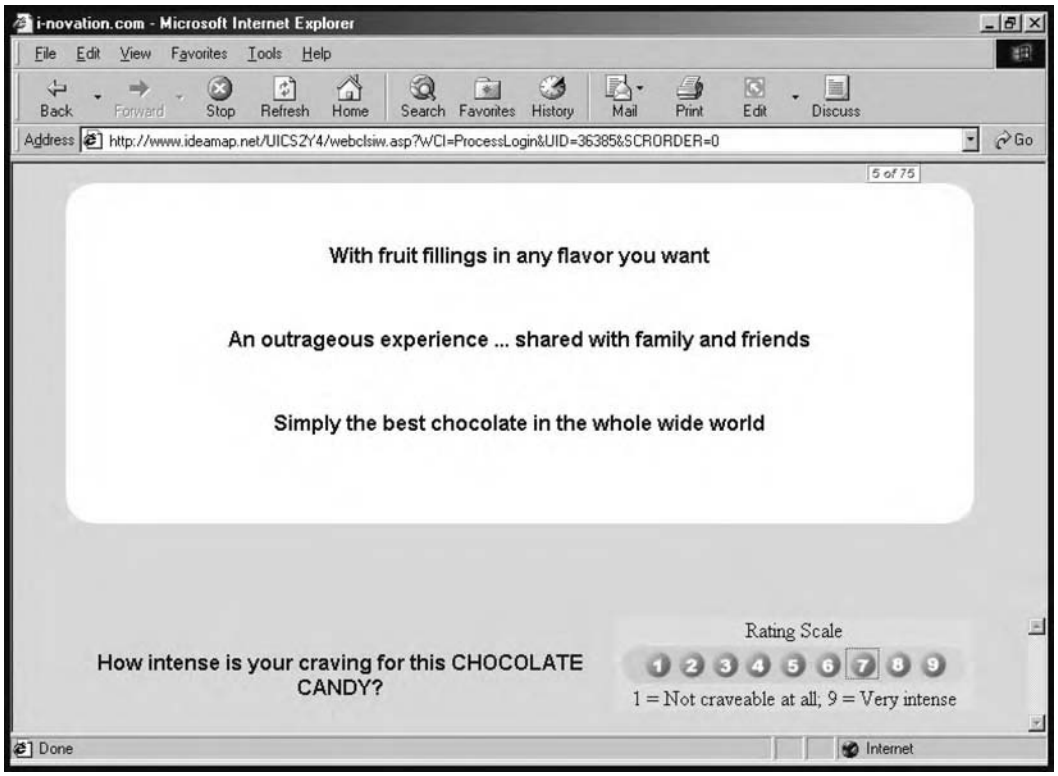

FIGURE 59.5 Example of a test concept.

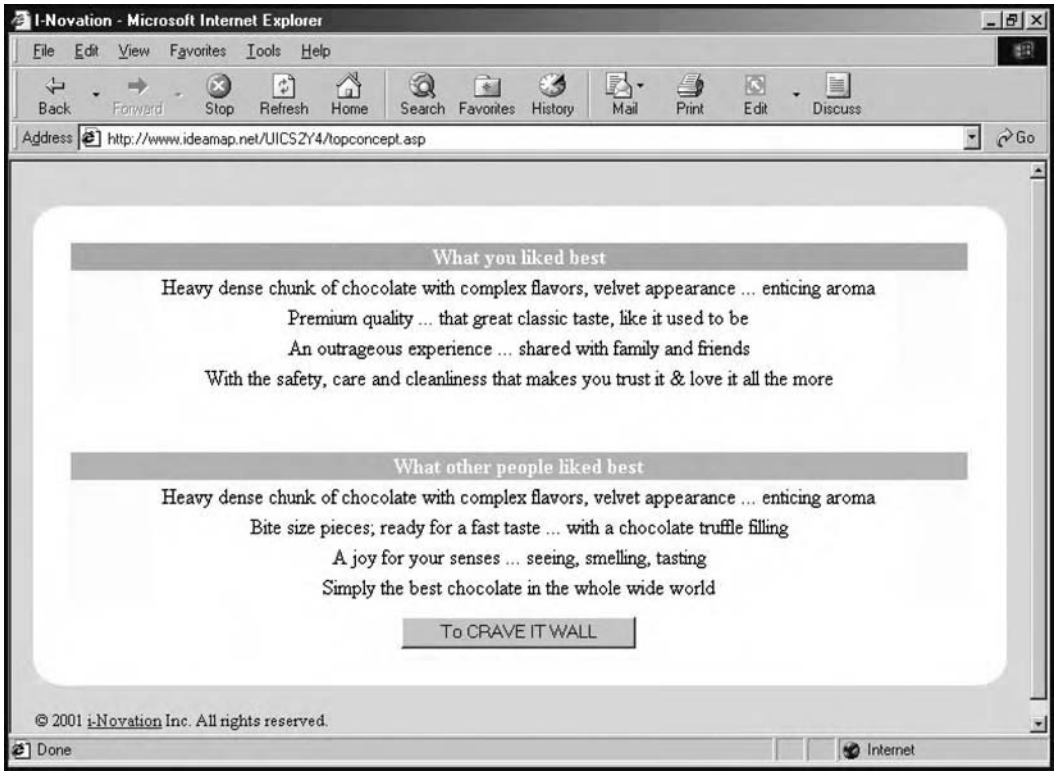

FIGURE 59.6 Winning concept synthesized for the individual respondent versus for the respondents who had previously participated.

TABLE 59.4
Number of Completes, and Proportion of Men and Women Respondents for 19 Foods

Food	Total Respondents	%Men	%Women
Chocolate Candy	478	14	86
Pizza	324	33	67
Ice Cream	321	26	74
Cola	239	26	74
Coffee	208	31	69
Cheesecake	173	16	84
Steak	168	44	56
Potato Chips	153	24	76
Chicken	153	27	73
Cinnamon Rolls	152	20	80
French Fries	151	19	81
Taco	151	21	79
Pretzels	151	25	75
Nuts	151	33	67
BBQ Ribs	151	38	62
Hamburger	151	40	60
Tortilla Chips	150	20	80
Olives	150	24	76
Cheese	150	27	73
Peanut Butter	150	31	69

From the 2001 Crave It! Database

TABLE 59.5
Aspects of the Foods that Drive Craveability

Attribute	Percent
Taste	64
Thirst	44
Aroma	42
General mood/feeling	30
Brand	23
Product appearance	19
Texture	15
Portion size	12
State of being (e.g., relaxed vs. on the go)	7
Environment and social situation	6
Association with family or friends	6
Advertising	4
Season	4
Weather	3
Stress level	3
Packaging	2
None of the above	2

Data from the 2002 Crave It! Study

"thirst," "aroma," and "general feel," as shown in Table 59.5.

c. How the different elements perform for a single product (e.g., chicken). The utilities constitute the heart of the database because they show the reaction of consumer respondents to the different communications. We illustrate the utilities value with data from the 2002 Crave It! database. The study dealt with chicken, comprising 241 respondents and 36 elements. Utilities for the 36 elements for chicken appear in Table 59.6,

TABLE 59.6
Utility Values for the 36 Elements for Chicken from the 2002 Crave It! Database

			Segment 1 of 3	Segment 2 of 3	Segment 3 of 3
		Total Sample	Elaborates	Imaginers	Classics
	Base Size	241	43	101	97
	Constant	47	14	56	53
E01	Classic taste … the way you remember it	−1	−1	1	−2
E02	Fresh grilled chicken	2	9	5	−5
E03	Fresh chicken … slow roasted for added flavor, hot off the rack	6	17	7	0
E04	Everything you want … all in one place … a mixture of tastes and textures	2	6	3	−1
E05	Chicken with a touch of red pepper spice to wake up your mouth	−10	7	−5	−24
E06	Marinated chicken for added flavor and a guaranteed juicy texture	2	11	6	−6
E07	Exactly the way you always imagined it	0	0	2	−3
E08	Char-grilled chicken … cooked over an open fire for a smoky grilled taste	4	16	9	−7
E09	Prepared just to your liking … add whatever your heart desires	4	7	4	1
E10	Premium quality … the best Chicken in the whole world	5	10	2	7
E11	Available at a value price	0	8	−4	1
E12	Cut into strips and served on top of, or mixed into, a bed of crisp salad greens … it's all about crunch	0	16	0	−8
E13	Coated in your favorite batter and spices, then deep fried for a crunchy good taste	7	22	1	6
E14	Cut into strips and served with onions, peppers, cheese, tomatoes, sour cream and guacamole in a soft flour tortilla	−8	32	−15	−18
E15	Baked into a buttery, crispy crust with peas, carrots, and roasted potatoes	−1	30	−17	3
E16	Covered with a delicious wine sauce	−9	11	−3	−24
E17	With great tasting french fries, a baked potato, or creamy mashed potatoes covered in gravy	7	27	2	4
E18	With your favorite beverage	0	0	1	−1
E19	So delicious, just thinking about it makes your mouth water	4	5	4	3
E20	When you think about it, you have to have it … and once you have it, you can't stop eating it	2	4	1	4
E21	You'd drive any distance … at any hour … to get it exactly the way you want it	0	6	−1	−1
E22	Relaxes and refreshes you … inside and out	1	−2	1	3
E23	Reminds you of great home cooking	3	4	3	3
E24	Eating it makes all the stress just melt away	2	−2	1	4
E25	A quick snack for when you're on the run	−1	−3	1	−3
E26	To be enjoyed while surrounded by family and friends	4	3	3	6
E27	A special treat … you will savor every bite	4	3	5	5
E28	At Popeye's	−11	2	−24	−2
E29	At McDonald's	−17	−6	−23	−16
E30	At Wendy's	−10	−1	−17	−6
E31	At KFC	−2	2	−17	10
E32	At Boston Market	−6	1	−13	−2
E33	At TGI Friday's	−6	2	−7	−8
E34	Ready-to-eat, easy to pick up	1	2	−5	7
E35	Certified to be natural and organic	−6	−9	−3	−8
E36	Guaranteed to be safe for you to eat	−2	−1	−4	−1
	Winning elements for total panel and the three segments				
	Top Three Winners — Total Panel				
E13	Coated in your favorite batter and spices, then deep fried for a crunchy good taste	7	22	1	6

(*Continued*)

TABLE 59.6
(*Continued*)

		Total Sample	Segment 1 of 3 Elaborates	Segment 2 of 3 Imaginers	Segment 3 of 3 Classics
E17	With great tasting french fries, a baked potato, or creamy mashed potatoes covered in gravy	7	27	2	4
E03	Fresh chicken … slow roasted for added flavor, hot off the rack	6	17	7	0
	Top 3 Elements — Segment 1 (Elaborates)				
E14	Cut into strips and served with onions, peppers, cheese, tomatoes, sour cream and guacamole in a soft flour tortilla	−8	32	−15	−18
E15	Baked into a buttery, crispy crust with peas, carrots, and roasted potatoes	−1	30	−17	3
E17	With great tasting french fries, a baked potato, or creamy mashed potatoes covered in gravy	7	27	2	4
	Top 3 Elements - Segment 2 (Imaginers)				
E08	Char-grilled chicken … cooked over an open fire for a smoky grilled taste	4	16	9	−7
E03	Fresh chicken … slow roasted for added flavor, hot off the rack	6	17	7	0
E06	Marinated chicken for added flavor and a guaranteed juicy texture	2	11	6	−6
	Top Three Elements — Segment 3 (Classics)				
E10	Premium quality … the best Chicken in the whole world	5	10	2	7
E34	Ready-to-eat, easy to pick up	1	2	−5	7
E26	To be enjoyed while surrounded by family and friends	4	3	3	6

for total panel and for the three concept-response segments. The numbers additive constant shows the predisposition of the respondent to feel that the chicken is craveable. We see at least two simple patterns emerging:

I. The total panel shows only a few elements scoring well. Segmentation reveals more elements that do well, suggesting that in the population there are countervailing forces whose interaction diminishes the utilities of the elements. It is clear from these data and this exercise that for the total panel there are a few winners, but the real information comes from the segmentation. The big opportunities come from identifying the concept-response segments, and building products for them.

II. The additive constants show the predisposition of respondents to find chicken craveable. The Elaborates show the lowest predisposition, with the elements doing the most work. The Imaginers and Classics show much higher predispositions, with the elements doing far less work.

d. How does the same element perform when presented in the context of different foods? By embedding the same element in different studies with various products it becomes possible to determine whether the element performs differently by product. The difference in the performance of a claim, for instance, appears moderate when the claim is "simply the best < FOOD NAME > in the whole wide world."

TABLE 59.7
Utility for the Element "Simply the Best" < FOOD NAME > in the Whole Wide World

	Total	Male	Female
Cinnamon rolls	7	3	8
Ice cream	5	3	6
Hamburger	5	5	5
Tacos	5	7	4
BBQ Ribs	4	6	3
Chocolate Candy	4	5	4
Pizza	4	6	3
Olives	3	6	3
French fries	3	4	3
Cheesecake	3	7	2
Peanut butter	3	0	4
Tortilla chips	3	−4	4
Coffee	2	−1	4
Chicken	2	−1	3
Nuts	2	1	2
Pretzels	1	4	0
Cheese	1	0	1
Potato chips	0	0	0
Cola	0	3	−1
Steak	−1	2	−2

TABLE 59.8
Proportion of Respondents Saying that "Mood" is Key Fact in "Craveability" for 20 of the Foods in the Crave It! Study

	Total Panel	Crave - Mood	Proportion (%)
Chocolate	472	226	48
Ice Cream	316	115	36
Cola	237	86	36
Nuts	149	52	35
Pretzel	148	50	34
Coffee	206	69	33
Olives	147	42	29
Tacos	148	42	28
Tortilla Chips	148	41	28
Potato Chips	151	41	27
Cheesecake	172	45	26
French Fries	148	36	24
Hamburger	150	31	21
Cinnamon Roll	149	29	19
Cheese	149	28	19
Chicken	148	27	18
Peanut Butter	149	27	18
Pizza	318	55	17
Steak	168	22	13
BBQ Ribs	149	17	11

Source: Data from the 2002 Crave It! Database

For cinnamon roles this phrase generates a strong claim (utility = +7), whereas for steak this is a weak claim (utility = −1). Table 59.7 shows these results. Men, in turn, tend to be more swayed by this type of claim than women, but the pattern is somewhat ambiguous. The key here is that the same element can take on different utility values depending upon the context in which it is presented.

e. Foods and mood. Mood is often associated with food, and associated with craveability. The mega study assess the proportion of respondents who feel that a food is associated with mood. Table 59.8 shows these results. The data from the 2002 Crave It! database suggests, without surprise, that chocolate is the food item most frequently associated with mood, whereas pizza, barbecue ribs and steaks are the least associated with mood. It is data like these, cutting across products and respondents that provide the additional insight about how consumers respond to foods.

f. Trends over time. One of the objectives of the mega study was to identify the trends of food acceptance over time, by comparing both the basic level of craveability and the utility values. A sense of changes in craveability over two years comes from Table 59.9. It is clear that some foods, such as pizza, changed more in craveability than did others, such as chips (both tortilla and potato chips). Data of this type developed year after year show changes in how consumers desire food, and may portend trends in the population.

TABLE 59.9
The Additive Constant for Different Foods for 2 Years (2001 and 2002)

	2001	2002	Change
Pizza	30	45	15
French Fries	34	48	14
Pretzels	29	42	13
Hamburger	30	43	13
Tacos	34	47	13
Cheesecake	41	53	12
Nuts	35	45	10
Coffee	38	48	10
Ice Cream	40	49	9
Steak	41	50	9
Cinnamon Rolls	38	45	7
Chocolate Candy	39	46	7
Chicken	40	47	7
Cheese	36	42	6
BBQ Ribs	44	49	5
Tortilla Chips	27	29	2
Potato Chips	40	36	−4

V. USING THE MEGA STUDIES DATABASE TO DRIVE NEW PRODUCT DEVELOPMENT

The mega-study databases can be viewed as both a knowledge building tool and as a practical guide to product development. Most product developers are accustomed to consumer specifications of products in the form of a product concept. Conjoint analysis in particular and the mega study in general provide a blueprint for product development, albeit a blueprint to be used with judgment. Concepts are statements about a specific concept. The output of conjoint analysis is a set of interlocking variables that can be mixed and matched at the end of the study in the same way that they are mixed and matched during the actual consumer research.

To make the best use of the study data requires the researcher to work with the results in the form of a table that can be sorted. The researcher can sort the elements from winning to losing, and select the combinations that make the greatest sense, and which, from the consumer point of view, look most promising. For example, Table 59.10 shows the chicken data, this time sorted to produce a product idea for each segment. It is important to keep in mind, however, that the mega-study does not prescribe simply one product, but rather provides the product

TABLE 59.10
Suggested Starting Concepts for Chicken from the Crave It! Database

			Seg1	Seg2	Seg3
		Total	Elaborates	Imaginers	Classics
	Additive Constant	47	14	56	53
	A product for Segment 1 (Elaborates)				
E14	Cut into strips and served with onions, peppers, cheese, tomatoes, sour cream and guacamole in a soft flour tortilla	−8	32	−15	−18
E06	Marinated chicken for added flavor and a guaranteed juicy texture	2	11	6	−6
E10	Premium quality … the best Chicken in the whole world	5	10	2	7
	Total - Segment 1	46	67	49	36
	A Product for Segment 2 (Imaginers)				
E08	Char-grilled chicken … cooked over an open fire for a smoky grilled taste	4	16	9	−7
E06	Marinated chicken for added flavor and a guaranteed juicy texture	2	11	6	−6
E23	Reminds you of great home cooking	3	4	3	3
	Total - Segment 2	56	45	74	43
	A Product for Segment 3 (Classics)				
E10	Premium quality … the best Chicken in the whole world	5	10	2	7
E34	Ready-to-eat, easy to pick up	1	2	−5	7
E13	Coated in your favorite batter and spices, then deep fried for a crunchy good taste	7	22	1	6
	Total - Segment 3	60	48	54	73

developer with a mechanism by which to estimate whether a specific combination will meet with consumer success.

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60 Flavor of Frozen Foods

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CONTENTS

I. INTRODUCTION

Flavor is one of the main attributes that, together with color and texture, influences the overall acceptability of foods. Generally, flavor is the result of a complex combination of sensations perceived by the chemical senses taste and smell. Taste is perceived by the taste buds on the tongue and other parts of the mouth, and mainly described as sweet, sour/acid, salt, bitter, astringency, metallic, hot, cooling and umami. The sense of smell detects certain odorous molecules in the air above the food that stimulate the olfactory receptors at the top of the nasal cavity. This volatile substances are detected before we eat, as well as during eating as they pass in the breath from the mouth, through the posterior nares into the nasal cavity. Therefore, compounds contributing to flavor can be divided into aroma and taste or non-volatile compounds [1].

Non-volatile precursors include lipids, peptides, amino acids, reducing sugars, vitamins and nucleotides, among others. Interaction of these compounds and/or their breakdown products generates a large number of intermediates and volatiles that contribute to flavor development during processing and storage [2]. Mechanisms by which flavor compounds are formed are important to improve flavor of the starting materials, during processing to gain optimum flavor, and during storage to maintain the flavor quality. This chapter describes the development of flavor precursors during processing, as well as off-flavors derived during freezing storage.

II. NON-VOLATILE CONSTITUENTS

Non-volatile or taste compounds are water-soluble; these can cause salty, sour or acid, bitter, sweet, umami, and hot or cool sensations. Salty taste is caused by the presence of inorganic salts as sodium or potassium chloride, together with monosodium glutamate and monosodium aspartate. Sweetness is produced by sugars (glucose, fructose, ribose) and some L-amino acids (glycine, alanine, serine, threonine, lysine cysteine, methionine, asparagine, glutamine, proline and hydroxyproline). Bitter tastes are generally caused by hypoxanthine, peptides such as anserine and carnosine, and the L-amino acids histidine, arginine, lysine, methionine, valine, leucine, isoleucine, phenylalanine, tryptophan, tyrosine, asparagine and glutamine. While sour and acid tastes are caused by organic acids (lactic acid, and acetic acid), amino acids (aspartic acid, glutamic acid, histidine and asparagine) and acidic phosphates. Hot and cool sensations or trigeminal responses do not contribute to the flavor of unprocessed foods, but addition of spices like chili and pepper usually contributes to the flavor of processed products. Finally, the umami taste has a characteristic savory quality supplied by glutamic acid, monosodium glutamate

(MSG), 5′-guanosine monophosphate (GMP), 5′-inosine monophosphate (IMP) and some other peptides recognized by their flavor-enhancing properties [1, 2].

It has been suggested that amino acids, peptides, proteins and nucleotides are most important for the taste of red meats, fish, poultry and dairy products [3]. Meat flavor compounds are present in flesh and do not require cooking for their generation; but cooking and further processing may affect their concentration. It has been reported that concentrations of reducing sugars decreased by up to 20% during heating, while concentrations of free amino acids increased according to the cooking temperature from 55 to 95°C [4]. Therefore, changes in sugars, amino acids, and nucleotides that occur during processing and storage will affect not only the taste but also the overall flavor, because many of these compounds are precursors of the aroma components [1].

III. AROMA CONSTITUENTS

Aroma compounds are largely formed during processing by several mechanisms; most raw foods have none of the aroma of their processed foods. For instance, the characteristic aroma of cooked meat is largely generated during processing, when heating gives rise to a several reactions that result in the formation of a complex mixture of chemicals. Aldehydes, ketones and sulfur compounds provide the meaty, toasted, roasted, fatty, fruity and sulfurous desirable meat aroma [2].

In the same way, major flavor compounds of sweet corn include 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline, dimethylsulphide, 1-hydroxy-2-propanone, 2-hydroxy-3-butanone, 2,3-butanediol, pyridine, pyrazine, alkylpyrazines, and 2-acetylthiazole. However, concentrations of volatiles in canned corn are many times higher than those present in frozen and fresh sweet corn [5]. Moreover, fermented foods as Manchego-type cheeses develop their characteristic flavor during ripening because of lipoliytic and proteolytic enzyme activity [6]. Furthermore, several routes intervene for the characteristic flavor formation of white bread; first enzymes that regulate the metabolism of the grain produce the flavor precursors, that later are transformed by yeast during dough fermentation. Finally, nonenzymically browning reactions that take place during baking are responsible for the roasty, malty, and caramel notes. If the dough is fermented for longer time, 3-methylbutanol and 2-phenylethanol are formed in high concentrations and responsible for the "yeasty" flavor impression [7].

Aroma profiling is also affected by handling and storage. For instance, more than 20 compounds have been reported to be responsible for the yogurt flavor (acetone, lactic and acetic acids, diacetyl, acetaldehyde, and ethanol); during storage the acetaldehyde content increases, while acetic acid and diacetyl decrease, although acetone is unaffected [8].

In the same way, pasteurized guava puree shows a noticeable increase in ethyl alcohol, n-hexanal, decanoic acid, dodecanoic acid and ethyl acetate when stored at 0 and −10°C, but when stored at −20° a slight decrease in alcohols and hydrocarbons were detected [9].

The study or aroma is a complex task, many aroma compounds have relatively high odor thresholds and make little contribution to the overall flavor; others may be present at very low concentrations but due to their very low thresholds have an enormous effect on flavor. In recent years, developments in analytical instrumentation and methodology have allowed the identification of many aroma compounds for several foods. Techniques for aroma collection include either solvent extraction, concentration by means of adsorption through a static or dynamic gas-purging headspace (DHA) [8, 10, 11, 12], selective extraction using supercritical fluid extraction method (SFE) [13], or solid-phase microextraction (SPME) [14]. Separation and identification is usually performed by gas chromatography-mass spectrometry (GC-MS) or gas chromatography-olfatometry (GC-O), using several ionization tools as electron impact (EI), fast atom bombardment (FAB), field desorption (FL), laser desorption (LD), and electrospray (ESI), among others.

IV. CHEMICAL REACTIONS RESPONSIBLE FOR FLAVOR

Interaction of non-volatile precursors and/or their breakdown products generates a large number of intermediates and volatiles that contribute to flavor. Maillard reaction, lipid oxidation, degradation of thiamine and, proteolytic and oxidative enzyme activities are the main mechanism involved in flavor generation.

A. Maillard Reaction

Maillard reaction also known as nonenzymatic-browning is one of the most important routes that provide a large number of compounds that contribute to flavor in cooked foods. This reaction involves several pathways that result in many aromatic products and high-molecular weight melanoidins; high temperature, low water activity and longer storage favor this reaction. The produced flavors can be pleasant or unpleasant, and typically described as caramelized, bready, nutty, roasted, or meaty. Coffee, roasted meats, bakery, and toasted nuts base their flavor on the Maillard reaction [15].

The initial stage involves condensation of a free amine group and a carbonyl group of a reducing sugar to form a glucosylamine. The glucosylamines from aldoses condensation undergo Amadori rearrangement to yield compounds such as 1-amino-1-deoxy-2-ketoses; while condensation of ketoses is usually followed by the Heynes rearrangement to form 2-amino-2-deoxy-aldolases [16].

The intermediate stage comprises dehydratation, fission, cyclization and Strecker degradation; giving rise to a widely spectrum of compounds that modify color, taste, odor and other properties of foods. The final stage involves condensation into high molecular weight melanoidins.

Concentrations of Amadori and Heyns compounds vary according to the reaction conditions; at pH 4–7, they can deamidate to give carbonyls as deoxyosones, which yield many secondary products. Subsequent stages of the Maillard reaction involve interaction of these secondary products with amines, amino acids, aldehydes, hydrogen sulfide and ammonia, among others. An associated reaction is the Strecker degradation that involves transamination between deoxyosones and amino acids. The amino acid is decarboxylated and deaminated to produce an aldehyde, while deosysones produce a-aminoketones or a-aminoalcohol. If the amino acid is cysteine hydrogen sulfide, ammonia and acetaldehyde are produced [17]. Besides, the amino-ketones can yield pyrazine derivatives that together with aldehydes are powerful aroma constituents [7, 15,16]. Therefore, volatile products formed by fission and aldehydes from Strecker degradation are mainly involved in the production of flavors and off-flavors. Aroma compounds come from simple sugars dehydration and fragmentation (furans, pyrones, cyclopentenes, carbonyls, acids), amino acids degradation (aldehydes, sulphur compounds) or derived from further reactions (pyrroles, pyridines, imidazoles, pyrazines, oxazoles, thiazoles) [18].

B. Lipid Oxidation

There are three classes of lipids in foods: triacilglycerols, phospholipids and cholesterol. All of them contribute to the perception and development of flavor. Phospholipids are located in membranes of cells and subcellular organelles, while triacilglycerols predominate in lipid droplets and fat depots. Proportion of saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) varies within foods and process. For instance poultry meat that contains more polyunsaturated fatty acids than red meat is more susceptible to oxidative changes and fed supplements with high n-3 fatty acids appear to be responsible for variations in the aroma profile of meat [19, 20].

Free fatty acids and triglicerides are capable of being oxidized by autoxidation or by enzymes called lipoxygenases, but polyunsaturated fatty acids are especially susceptible to autoxidation. The initial step of autoxidation involves production of free radicals R° from lipids RH by their interaction with oxygen in the presence of heat, light, high-energy radiation, metal ions or metalloproteins. Particularly, the heme iron of myoglobin and haemoglobin accelerates lipid oxidation in meat and meat products, during cooking as myoglobin denatures and iron is liberated. Furthermore, disruption of lipid membrane as in mechanically separated meat facilitates lipid oxidation reactions [19].

The free radical R° reacts to form a lipid peroxy radical ROO° that can react to give a hydroxyperoxide ROOH. The second step or propagation provides further free radicals in a self-propagating-chain process that can be terminated by combination of two free radicals. The enzyme lipoxygenase is widely distributed in plants and animal foods; it is very specific about the substrate and how the substrate is oxidized leading to hydroperoxides. Lipid hydroperoxides are very unstable and break down to alkoxy free radicals, which decompose, leading to undesirable rancid flavors and odors, mainly aldehyde products, including n-alkanals, trans-2-alkenals, 4-hydroxy-trans-2-alkenals and malonaldehyde [21]. The type and source of lipids, the presence of lipoxygenase and inhibitors also affect the rate of lipid oxidation. Major volatile compounds generated from the oxidation of arachidonic and eicosapentaenoic acids by 12-lipoxygenase are 1-octen-3-ol, 2-octenal, 2-nonenal, 2-nonadienal, 1,5-octadien-3-ol, and 2,5-octadien-1-ol. However, addition of lipoxygenase inhibitors, esculetin, and butylated hydroxyanisole (BHA) reduced formation of volatiles [22]. Susceptibility to oxidation also depends on the molecular differences of isozymes present in foods. As an example, the usual corn genotype and three genotypes varying in sweetness were blanched or unblanched, and stored for up to 12 months at −20°C. All genotypes contained an isozyme of 80 kDa/pI 4.5, sweeter genotypes also contained a peroxidase of 13.8 kDa, but after 12 months of frozen storage, an additional isozyme appeared in some extracts giving rise to differences in the flavor profile [23].

Reactive oxygen species and free radicals such as superoxide anion ($°O_2-$), hydrogen peroxide (H_2O_2), hydroxyl radical (°OH), alkoxyl radical (RO°), peroxyl radical (ROO°) and singlet oxygen ($^1O_2$) are unstable and highly reactive; they can be formed in foods by prooxidative enzymes, lipid oxidation, and irradiation or derived from injured cells. These compounds react easily with lipids, polypeptides, proteins and nucleotides increasing off-flavor development during frozen storage [24].

Additionally, saturated and unsaturated aliphatic aldehydes derived from lipid oxidation may participate in the Maillard reaction to yield long chain alkyl-thiazoles and alkyl-pyridines that contribute to the fatty fried aromas [17].

V. OFF FLAVOR DEVELOPMENT DURING FREEZING STORAGE

During frozen storage and distribution, foods are exposed to a wide range of environmental conditions such as temperature variations, reduced water activity, oxygen, and light that trigger several chemical and physical changes. Deterioration in texture, flavor and color are the most serious problem causing shelf-life reduction, particularly when poor freezing practices are used or when the quality of the starting material is low [25]. Fluctuating temperatures

may cause recrystalization leading to undesirable sandy texture in dairy products, and phase changes involving melting and solidifying of fats on lipid containing foods. In addition, to temperature, other environmental factors such as oxygen, water activity and pH induce chemical and enzymic harmful changes. Deterioration of flavor involves rancidity, bitterness or undesirable fishy taste, due to the formation of low molecular weight compounds from lipid oxidation or protein denaturation. Enzymes such as lipoxygenase, if not denatured during blanching process can also influence food quality even at sub-freezing temperatures [25, 26].

A. Changes in Flavor of Frozen Foods Associated to Cell Damage

Freezing has a negative effect on the textural properties on several fruits and vegetables such as mashed potato, broccoli blueberries and strawberries. Freezing and fluctuation in temperature will produce cell damage by ice recrystalization and dehydration. This may be reflected in a more open structure of the frozen product, which permits a greater ingress of oxygen, thus increasing oxidation, vitamin loss and flavor deterioration [25, 27].

The open structure of frozen foods increases migration of fluids containing cell nutrients that can facilitate loss of micronutrients, microbial contamination, and developing of off-flavors during thawing [28]. As observed in frozen and freeze-dried blueberries that loses several flavor compounds, including the typical blueberry aroma (1,8-cineole) [29]. On the other hand, very low temperature can promote the production of off-flavors in frozen-thawed strawberries when stored at −40 and −80°C; as freezing causes disruption of cells and decreases the pH of the cytosol, thus facilitating the subsequent release of sulphide ion as H_2S, and therefore off-flavors formation [30]. Loss flavor as a result of freezing was associated to a peel injury and cell damage in navel oranges stored at various freezing conditions with large emissions of ethanol, ethyl butanoate, methyl hexanoate and ethyl octanoate [31].

B. Changes in Flavor of Frozen Foods Associated to Lipids

Lipids play a multifunctional role in flavor; they influence both the physical (mouthfeel) and chemical flavor perception, as lipids act as a carrier for lipophilic flavor molecules including off-flavors. Reduction in the fat content will result in higher flavor loss during storage due to flavor volatility. On a molecular level, triglycerides lower the vapor pressure of lipidic flavor compounds, thus increasing their thresholds [32, 33]. It has been reported that the use of fat replacers such as maltodextrine can overcome flavor perception and sensory properties in low fat ice cream [33].

Lipids also bring their inherent flavor and are precursors for flavor development by lipolysis and lipid oxidation [34]; there is a high correlation between reactions involving lipids and the development of off-flavors, during storage. Lipid oxidation is influenced by several factors, such as temperature and the presence of oxygen in the immediate vicinity of foods; but water activity also plays an important role, because at very low values lipid oxidation occurs at high rates as it is found in frozen foods [7, 26].

The presence of unsaturated fatty acids in foods is a prime reason for the development of rancidity and off-flavors during frozen storage as long as oxygen is available. The generation of free radicals during rancidity also leads to undesirable reactions such as loss of vitamins, alteration of color and deterioration of proteins and texture, which modify the whole flavor perception. Warmed-over flavor (WOF) is a flavor defect that occurs in reheated foods, and it is of special concern for precooked frozen foods. This occurs in cooked products that are stored under refrigeration or freezing conditions, where lipid oxidation initiates during storage and warm temperature of the reheating process accelerates the oxidation process with the outcome of rancid flavors, resulting in WOF [28]. Furthermore, NH_3 exposition due to refrigerant leakage is related to rancid flavors in foods with a considerable reduction in the frozen storage life; off-flavors in lamb were detected by consumers after 3 to 6 months storage at −20°C [35].

It is well known that lipid oxidation products as hexanal and TBA reactive substances (TBARS) are highly correlated to the development WOF during frozen storage. Attempts have been made to reduce lipid deterioration during frozen storage by addition of vitamins, antioxidants, or phosphate. Incorporation of a commercial oleoresin rosemary (OR)-coated salt (0.5 or 1 g/kg), sodium tripolyphosphate (STPP; 3 g/kg) or TBHQ (0.2 g/kg) in meat batters decreased hexanal production by 95% [36]. Furthermore, dietary vitamin E (DL-alpha-tocopheryl acetate) equivalent to 5x, 10x and 25x the NRC recommendations levels for the diet improved the oxidative stability and functionality of turkey breast meat, and produced the most typical and acceptable meat flavor with the fewest oxidized off-flavor notes in fresh and frozen turkey meat [37]. Besides, frozen meat from birds feed with docosahexaenoic acid (DHA) was more acceptable and stable due to a higher lipid and flavor stability during storage at −23°C for up to 82 days [38].

Although, ascorbic acid is mainly recognized as antioxidant, due to its radical and O_2 scavenging effect. At low concentrations (800 ppm) ascorbic acid may act as a prooxidant, especially in the presence of metal-catalyzed oxidation. Ascorbic acid is able to reduce Fe^{3+} to Fe^{2+}. Nevertheless, the reduced Fe^{2+} catalyzes the breakdown of hydroperoxides ROOH ($ROOH + Fe^{2+} \rightarrow RO^{\circ} + OH + Fe^{3+}$) to free radicals at a higher rate than Fe^{3+} (ROOH +

$Fe^{3+} \rightarrow ROO^{\circ} + Fe^{2+}$) [39]. However, it has been reported that phosphatidic acid and phoshatidylserine can reduce the lipid oxidation catalytic activity of nonheme iron and hemoproteins [40].

Additionally, cholesterol a monounsaturated sterol present in animal foods such as biscuits, snacks, cheese, raw and cooked meat, egg powder, etc. Cholesterol can be oxidized during storage giving rise to a more than 60 oxidation products such as 7-ketocholesterol through a free radical reaction; cholesterol oxides are toxic and may be involved in flavor deterioration during storage [41].

In order to reduce lipid oxidation and off-flavor development during storage, it is commonly the use of antioxidants such as free radical terminators or metal quelating agents. These compounds can react with peroxyl or alkoxyl radicals and terminate the chain reaction by scavenging chain-propagating radicals. BHA and butylated hydroxytoluene (BHT) are effective free radical scavengers in a wide variety of foods including meat and dairy products. Also natural antioxidants such as sesamol, quercetin, tocopherols, carotenoids, and polyphenols, among others have proved their efficiency to prevent lipid oxidation in foods [24, 42, 43, 44]. On the other hand, addition of phosphates [disodium phosphate (Pi), tetrasodium pyrophosphate (PP), sodium tripolyphosphate (TPP), sodium tetrapolyphosphate (TTPP) and sodium hexametaphosphate (HMP)] or sodium ascorbate monophosphate (SAsMP) in 0.3 and 0.5% levels reduce formation of rancid and soapy flavors in frozen vacuum packaged cooked turkey; with the additional decrease in cooking losses and the increase in moisture retention after thawing [45, 46].

Packaging also plays an important role to control off flavors, in particular modified packaging has been used to improve flavor and aroma of cooked beef, where samples stored under vacuum and N2/CO2 atmospheres were meatier, less warmed-over, less cardboardy, and less oxidized and had lower TBA, than those in air-containing packages [47].

C. Changes in Flavor of Frozen Foods Associated to Proteins

Flavor release and perception is caused by interactions of flavor components with protein molecules by physical adsorption via van der Waals interactions, and by chemical reactions via covalent or electrostatic linkages, including salt, amides, ester formation and condensation of aldehydes with NH_2 and SH groups. The specific distribution of hydrophilic and hydrophobic regions in proteins determines their shape, functionality and the binding of volatile compounds; therefore, alterations in the protein moiety can lead to distortion of the sensory profile [48]. Protein denaturation during freezing storage is due to several factors including changes in moisture, interaction with lipids or products derived from lipid oxidation, and by the activity of specific enzymes as lipoxygenases and TMA-oxidase [25].

First, formation of ice crystals induces dehydration and increases salt concentration that promotes protein aggregation. Distribution of hydrophilic and hydrophobic binding depends on the dielectric constant, pH and ionic strength of the media. Thus, high salt concentrations will disrupt the existing equilibrium leading to denaturation. It has been observed that denatured proteins irreversibly bind sulfur-contain flavors by interchanging protein sulfhydryl groups and disulfides, where disulfides containing allyl or furfuryl groups are more reactive than saturated alkyl disulfides [49].

Free fatty acids derived from lipid hydrolysis can attach themselves hydrophobically or hydrophilically to the protein creating a more hydrophobic regions resulting in a decrease of protein solubility [25]. Moreover, free radicals can abstract a hydrogen from SH-side groups forming protein-free radicals that interact with other proteins or lipids forming protein-protein or protein-lipid aggregates.

Additionally, secondary lipid oxidation products as malonaldehyde, propanal and hexanal can react covalently with side-chain groups of histidine, methionine, cysteine and lysine residues; these interactions increase the hydrophobicity of proteins and may decrease the flavor binding ability [25]. In particular, aldehydes are more stable than free radicals and can diffuse into the cellular media inducing protein modifications [25, 50]. In particular, products derived from lipid oxidation denaturate proteins as myoglobin, where the heme iron is exposed increasing its prooxidant activity [51]. However, it has also been reported that α,β-unsaturated aldehydes enhance oxidation of ferrous oxymyoglobin to ferric metmyoglobin than their saturated counterparts [50, 52].

VI. CONCLUSIONS

Flavor perception and stability is related to proteins, lipids, and carbohydrates as well as the conditions of storage. Although, freezing offers an exceptional food safety and extended storage time, many reactions such as moisture migration, lipid oxidation and protein denaturation promote off-flavor formation. Therefore, stability can be achieved with a more integrated understanding of the flavor interactions within the complexity of foods. Also, attention should be focused on the quality of raw materials and the overall product technology to reduce oxidation and flavor loss.

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61 Frozen Food Texture

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CONTENTS

Freezing is used to preserve and maintain the quality of many foods. These include beef, chicken, pork, fish, and other muscle foods; fruits and vegetables; egg products; dairy foods; doughs and breads; and a wide variety of entrée items (Table 61.1). Relatively low temperatures, the formation of ice as a separate phase, and the freeze concentration of dissolved substances contribute to conditions that limit the growth of microorganisms and preserve quality factors such as flavor and color.

One critical quality factor influenced by freezing is food texture. Texture can be defined as those properties of food determined by the rheological and structural nature of the food and determined by the tactile senses. Many foods are thawed from the frozen state and eaten directly or cooked before consumption. In some cases, the texture of the thawed material is close to that of the fresh and unfrozen food. In other cases, the texture may be changed by the freezing process, yet result in a thawed product that is still acceptable to consumers. For a few foods, freezing results in dramatic changes that are unacceptable to consumers. Yet another class of foods is those consumed frozen, such as ice cream, sherbet, or ice pops. In these foods, the specific composition and freezing process profoundly affect the perceived texture.

TABLE 61.1
Frozen Food Sales in the United States

Type of Frozen Food	Sales ($) 2001
Baked goods	1.4 billion
Breakfast foods	1.05 billion
Fish, Shrimp, Seafood	1.18 billion
Frozen desserts, Fruit, Toppings	786 million
Ice cream, Novelties	6.4 billion
Juices, Drinks	827 million
Meat	333 million
Pizza, Snacks, Prepared foods	12.33 billion
Poultry	890 million
Vegetables	2.9 billion
All Frozen Foods	**28.1 billion**

Source: American Frozen Food Institute (www.affi.com).

In this review, we will cover issues regarding the effects of freezing on the texture of major food groups. We will discuss primary texture attributes of specific foods, as well as the fundamental mechanisms that reduce texture quality, and outline basic procedures before and during freezing that are used to ensure the optimal quality of frozen foods.

I. VEGETABLES

Frozen vegetables make up a significant portion of the frozen food market. Vegetables that are commonly frozen include potatoes, corn, peas, green beans, broccoli, cauliflower, brussels sprouts, and pumpkin. Freezing extends the availability of vegetables and preserves quality during transportation and storage. In some cases, it allows for more convenient processing of the food. For example, pre-cut and treated French fries can be stored frozen for extended periods, with required portions available for immediate baking or frying.

A. Texture Attributes

The textural attributes of importance to most vegetables are firmness, tenderness, and crispness. Firmness implies that there is an immediate resistance to biting. Tenderness indicates that the material can be easily separated into increasingly smaller pieces, particularly when sheared. Crispness is a complex attribute related to the sound and discontinuous breakdown of the product.[1] In vegetables, firmness is directly related to structure of the vegetable cells (Figure 61.1).[2] Turgor arises in cells that are fully hydrated, and, as a result, push the plasma membrane against the surrounding cell wall. Firmness also depends on the cell walls that surround the cells. The cell walls provide a semi-rigid scaffolding, holding the cells in a specific formation. Cell walls are composed of bundled strands of cellulose, along with lesser amounts of lipid, pectin, and proteins. A primary cell wall is formed in young plants. As plants age, one or more rigid secondary cell walls form which incorporate lignin into thick cellulose layers.[2] Adjacent cell walls, and thus cells, are held together at the middle lamella by a sticky, pectin-rich substance.

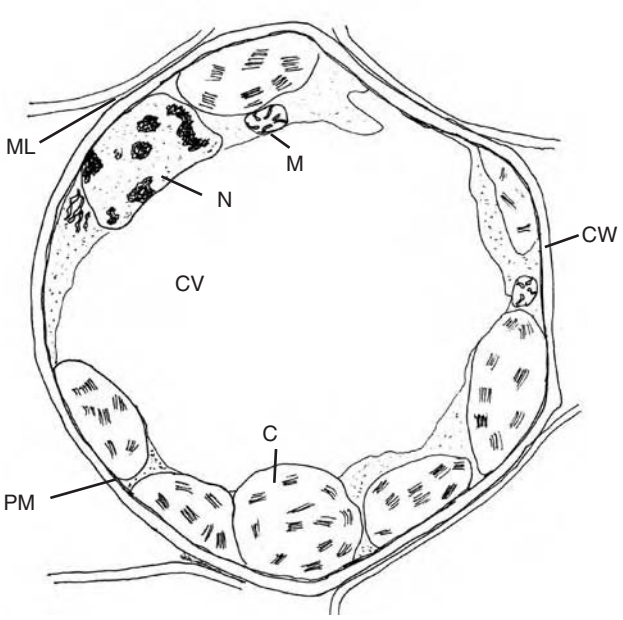

FIGURE 61.1 In the typical plant cell, the intact cell membrane (PM) is pushed back against a rigid cell wall (CW). Internal organelles such as chlorplasts (C), mitochondria (M), and the nucleus (N) are occluded by a large central vacuole (CV).

During freezing, several factors converge to cause degradation of texture related to changes in the cell membrane, cellular contents, cell wall, and middle lamella (Figure 61.2).[3] Particularly during slow freezing, ice forms primarily in extracellular spaces (Figure 61.3). This results in a water activity gradient that causes water to leave the cell. Concurrently, the cell shrinks, the ionic strength of the cytoplasm increases, and the pH changes. These changes may result in protein denaturation, disruption of the plasma membrane, and changes in intracellular organelles. In addition, ice crystals may penetrate the cell wall, and ice formation in the inner lamella may pull cell walls apart.

Several physical tests are available for assessing texture in intact vegetables. Firmness is measured by the force developed as the sample is compressed. Measurements can be made through continuous readings of force and deformation, or through a variety of penetrometers.[4] Tenderness is often measured by the shear force developed when a series of blades is run through the sample. Other specific tests, such as the pea texturometer, may be used.

B. Processing and Storage

The texture of frozen and thawed vegetables will never be better than that of the material before freezing. Vegetables

with good flavor and texture should be used for freezing, and lots should be chosen so as to assure uniform maturity.[5] After initial cleaning, many vegetables may be cut, sliced, or otherwise processed prior to freezing. Such commodities include sliced or diced carrots, French fry cut potatoes, and corn without the cob.

Most vegetables require blanching prior to freezing (Table 61.2). Blanching is the use of hot water or steam to de-activate enzymes in the vegetable.[6] Several enzymes can cause undesirable changes in frozen vegetables. For example, lipases and lipoxygenases lead to the formation of off-flavors.[7] With respect to texture, pectin-hydrolyzing enzymes would cause excessive softening of the tissue during frozen storage. Blanching has the added benefit of reducing microbial load and internal gases.

Unfortunately, the heat of blanching induces significant changes in texture.[8,9] Blanching disrupts the normal cell structure. The cell membrane may be damaged, allowing water to enter the cell. Internal organelles may be distorted and may begin to leak their contents. Heating may also denature proteins, as well as gelatinize starch found in cellular granules. This starch may subsequently retrograde during frozen storage. The major impact of blanching on texture is the reduction of cell turgidity caused by the loss of

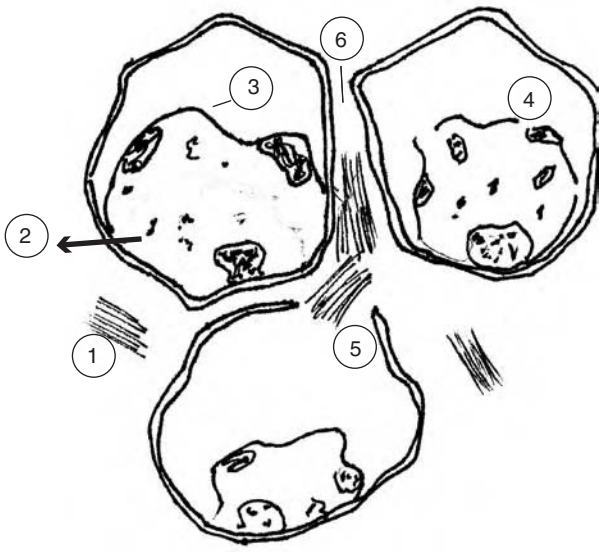

FIGURE 61.2 Formation of extracellular ice (1) causes movement of water from cells (2), resulting in shrinkage of the plasma membrane (3), concentration of internal solutes, and disruption of the membrane (4). Ice may penetrate cell walls (5) or separate adjacent cells at the middle lamella (6).

TABLE 61.2
Advantages to Blanching

1. Deactivate lipases and lipoxygenases which cause off-flavors
2. Deactivate pectinases which soften tissue
3. Reduce microbial load
4. Reduce internal gases

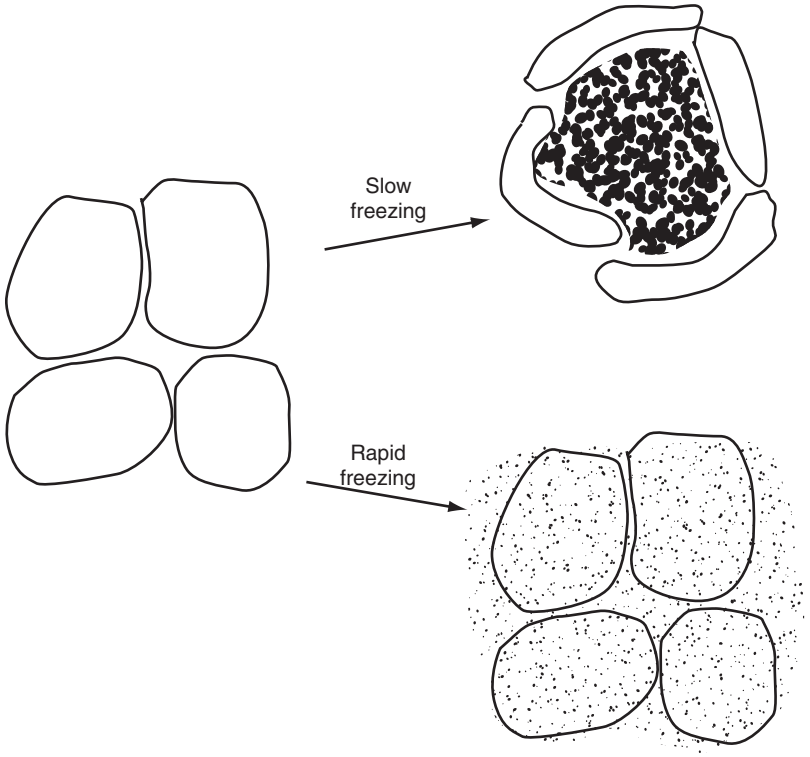

FIGURE 61.3 Large extracellular ice crystals form during slow freezing, while numerous small intra- and extracellular crystals form during rapid freezing. (After Meyman, 1963.)

cell membrane function. When cells do not push up against the cell wall, the tissue becomes flaccid and less firm. In addition, during blanching, pectic substances are released from the cell wall. This decreases cohesion between adjacent cells, and enhances breakdown of the structure.

Blanching methods and times depend on the particular vegetable, and, in some cases, on the particular cultivar. In general, it is advantageous to use cultivars that require minimal blanching, thus reducing textural changes caused by heating.

There is some evidence that the particular blanching regimen affects the texture of the thawed product.[10,11] Brussels sprouts preheated to 52°C subsequently required 20% less blanching.[10] Carrots blanched at 76°C were firmer than those blanched at 100°C.[12] Similarly, green beans blanched at lower temperatures were firmer and exhibited less sloughing.[12]

There have been some studies on changes occurring after blanching. The breakdown of pectins is enhanced in high acid conditions,[13,14] and continues at neutral pH. Firmness may be enhanced by the interaction of demethylated pectins and divalent cations. In addition, added calcium may help increase firmness in some vegetables.[14,15] Low temperature blanching may increase calcium availability or pectin methylesterase activity.[16,17,18] Subsequent linking of demethylated pectin by calcium increases firmness.

For most vegetables, rapid freezing results in optimal texture. Rapid freezing allows less time for osmotic dehydration of cells and the associated freeze-concentration of solutes. The particular freezing method depends on the type of vegetable, its size, and its structural features. Air blast and cryogenic freezing methods have been used successfully with most vegetables. In limited cases, freezing in blocks may be warranted.

II. FRUITS

Many fruits are frozen to extend their availability throughout the year as well as to further shelf life through lengthy distribution. In general, fruits have a less fibrous structure than vegetables, and often suffer more textural changes during freezing and storage (Figure 61.4).[19] Thus, direct consumption of frozen/thawed fruits is not as widespread as that of vegetables. Many frozen fruits are destined for further processing, as for use in muffins, ice cream, yogurt, or jams. In addition, large whole fruits do not freeze well. Fruits such as apples, peaches, melons, or pineapples are typically cut into slices, chunks, or segments prior to freezing. Smaller fruits, such as blueberries, strawberries, blackberries, or grapes, may be frozen whole.

A. Textural Attributes

As with vegetables, firm yet tender fruit is desirable for freezing. In general, freezing does not improve fruit texture, so that fruits that are hard, mealy, too soft, or weepy should not be frozen, particularly if they will be consumed directly. Loss of water holding capacity is also a problem for many frozen fruits. Such fruits exhibit excessive drip loss on thawing, and may lack proper juiciness when chewed.

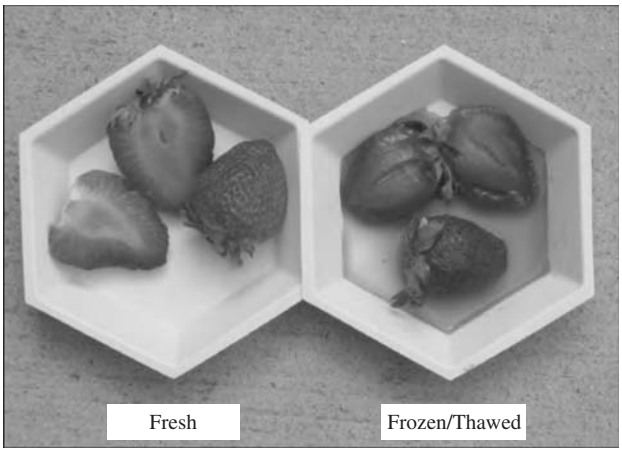

FIGURE 61.4 Fresh strawberries are firm with no juice leakage. Typical of many fruits, frozen/thawed strawberries are flaccid, have poor color, and have drip loss.

B. Processing and Storage

Obviously, the quality of raw ingredients is important to the texture of frozen/thawed fruit. In some cases, the freezing of slightly unripe fruit results in a firmer texture with lower drip loss.[20,21] For example, underripe apricots, cherries, and plums display lower drip loss after freezing and thawing.[21] Similar results have been seen in slightly unripe blackberries.[22]

The fruit variety may also be a factor in freezing performance. Differences in the texture of thawed fruit due to variety differences have been found for strawberries,[23,24] red and black currants,[25,26] and raspberries.[27] However, fruit maturity and grade are probably more important factors than cultivar differences on the texture of thawed fruit.[28,29]

Immediate freezing of fruit after harvest can also improve texture. For example, strawberries frozen within two hours of picking and stored for three months resulted in better texture than those frozen after five hours.[30] In some cases, harvested fruit may be stored for longer times at lower temperatures and in modified atmospheres prior to freezing without affecting textural quality.[20,30]

Fruits are seldom blanched before freezing. Any benefits to be had by blanching are outweighed by the significant cellular damage it causes. Fruits do not have the more extensive fibrous network of vegetables, and therefore become mushy or even disintegrate upon heating. There are some exceptions to the rule, however. For example, polyphenol oxidase can be inactivated in bananas by blanching, without causing unacceptable changes in firmness.[31]

Similarly, blanched breadfruit segments display acceptable texture when thawed and cooked.[32] Some work has also been done on non-thermal blanching of fruits. For example, gas mixtures with sulfur dioxide have been shown to inhibit enzyme activity in sliced and frozen apples.[33]

Many fruits are cut or diced prior to freezing, and have pits or stones removed. As freezing rate is of particular importance to the quality of frozen fruit, pieces with the shortest dimension greater than 2–3 cm are not typically frozen.[34,35,36] Freezing times increase somewhere between linearly with or as the square of the shortest dimension for heat transfer. In addition, much fruit is used for further processing, and fruit cut prior to freezing is often easier to cut and of better quality than fruit cut after freezing and thawing.[37,38] Removal of pits in apricots, plums, and cherries may cause greater drip loss in thawed fruit, but may be necessary for other reasons.[21,30]

Various ingredients may be added to enhance the quality of frozen fruits. Sugar is often used to improve color, texture, and flavor.[39,40] Water is drawn out of the cells by osmosis, leading to a lower freezing point and a decreased fraction of ice at a given frozen storage temperature. An extension of this is osmotic dehydration, in which high concentrations of sugar solutions are used. With this approach, fruit cells may lose up to 40 to 70% of their water. Fruits such as strawberries, apricots, cherries, and pineapples have been subjected to osmotic drying prior to freezing.[41,42] In general, such fruits are sweet, firm, and have lower drip loss.

In some cases, calcium salts may be infused into fruits to enhance firmness. These act by cross-linking the internal pectic substances. Research has indicated that pectinmelthylesterase can be infused into fruit, allowing enhanced action of the calcium.[43]

The formation of extracellular ice concentrates the solutes in the surrounding aqueous phase, which, in turn, drives water from the cells. The higher concentration of intercellular solutes, coupled with the decreased flexibility of the frozen phase, produces cell and cell membrane damage. In addition, cell wall substances, particularly pectins, may be extracted or solubilized from the cell walls, reducing structural rigidity.

Various freezing methods, including air-blast freezing, fluidized-bed freezing, and cryogenic freezing have been used successfully with fruits. Individual quick frozen (IQF) methods are preferred. Contact plate freezing of fruit in blocks is infrequently used in current practice. A significant body of work exists to show that rapid freezing results in the best retention of quality in thawed foods.[36,37,44,45] This is especially important in frozen fruits, which are subject to greater textural changes in the freezing process. Rapid freezing results in smaller ice crystal size, less cell dehydration, and less cell damage.[38,44] Comparative studies on mangoes,[46] strawberries,[47] apples and peaches,[48] and blueberries[49] have shown that rapid freezing rates result in thawed products with the best texture and least drip loss. Cryogenic freezing with liquid nitrogen often results in the best texture. However, prolonged immersion in cryogens may cause "freeze fracturing" at the surface.[50,51] Sprays of liquid nitrogen generally cause less freeze-fracture damage than immersion.[52]

III. MEAT

Many muscle foods are frozen to preserve quality, including red meats (primarily beef and pork), fish and other seafood, and poultry. Properly frozen and stored meat will maintain good texture and flavor for many months. All muscle foods are high protein foods composed of cells containing an orderly sarcomere structure, and with tissues held together by membranes and collagenous material (Figure 61.5). Each requires the proper mix of firmness and tenderness for optimum texture, as well as the retention of moisture for optimum juiciness. Unlike fruits and vegetables, virtually all meats are cooked before consumption with the goal of affecting muscle texture. This occurs as sarcomeric proteins are denatured and solubilized to form soft gel networks, and as collageneous materials melt and reduce muscle toughness.[53] Differences in muscle type, metabolic processes, and production practices exist, dictating variations in freezing practices. In this section we will discuss texture changes during freezing of red meat, with fish, seafood and poultry meats covered in subsequent sections.

A. Texture Attributes

In general, meats have a more challenging texture for consumers than vegetables and fruits. Intact meats should be relatively firm and cohesive, offering a certain amount of bite resistance; however, cooked meat should be tender enough that excessive chewing is not required. Meat toughness is largely dependent upon the properties of the connective tissue proteins.[54] For example, as an animal ages, irreversible cross-links develop in the collagen, causing the meat to become tough.

Muscle tenderness is usually measured by the amount of force required to shear through the meat. In the Warner-Bratzler shear method, cylinders of cooked meat are cut into cylinders and placed in a holder. A knife blade with a triangular opening is passed through the sample, and the maximum force attained before the sample is cut through is used as the measure of tenderness.[4] Irregular shaped pieces, or restructured products, may be tested with a multi-blade apparatus such as the Kramer shear device.

Quality meat should also be juicy and have maximum water holding capacity (WHC). Meats lacking WHC are dry, tough, and yield unsightly losses of liquids during thawing and cooking. Thaw and cook loss can be quantified by weighing before and after thawing or cooking and

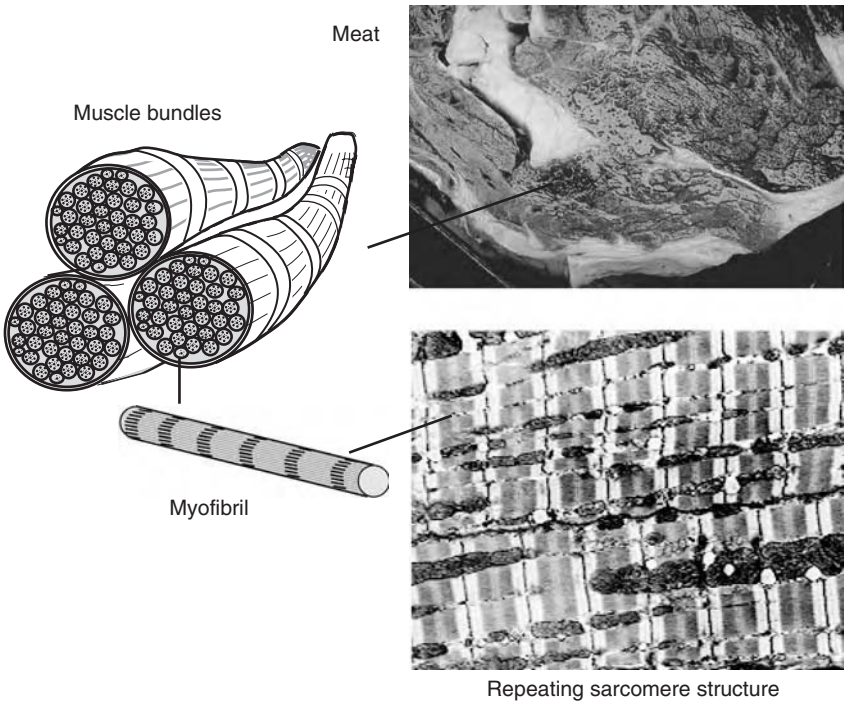

FIGURE 61.5 Muscle foods have a complex hierarchy of structure. In addition to bone and fat, meat is composed of muscle bundles surrounded by a tough connective tissue. Muscle bundles contain many long, parallel myofibrils. Each myofibril, a multinucleated cell, contains a repeating mesh of overlapping proteins, organized into sarcomeres.

calculating the drip loss by the difference in these weights. WHC can also be measured by compressing cooked samples, such as 1 cm cubes, by a given force or deformation, and weighing the amount of liquid expressed onto a filter paper.

B. Processing and Storage

The biochemical state of meat is one of the prime factors determining quality after freezing. After slaughter, most meat must undergo aging and conditioning before being frozen. Much of the meat quality is related to the pH in the muscle. As muscle ages, glycogen in the muscle is converted to lactic acid, which, in turn, reduces muscle pH. The meat is aged in a cold room, so that an ultimate pH will be attained.[55,56,57] An ultimate pH of about 5.6 is attained within a few days, which results in the best meat texture. Typically, the process is speeded up by electrical stimulation of the carcass.[58]

Animals with low glycogen levels may result in meat with higher ultimate pH, which, in turn, causes a dark, firm, and dry (DFD) meat.[59,60,61] In addition, if muscle pH remains high during cold storage, "cold shortening" of the muscle may occur.[62] Such meat is very tough and often unacceptable.[63] Shortening is particularly a problem in meat that has been removed from the bone,[63] but can be minimized with proper cooling.[64] Conversely, if muscle glycolysis occurs too quickly, a pale, soft, exudative (PSE) meat will result,[65] particularly in pork. PSE meat has high drip loss before and after freezing, resulting in colorless, dry meat that lacks juiciness.

As found with most foods, rapid freezing results in optimal texture of the thawed, cooked meat. In some cases, freezing may even increase the tenderness and juiciness of the final product.[66] Slow freezing results in larger ice crystals, greater cell dehydration, more extensive cell damage, and greater protein denaturation.[67,68,69] The movement of cell water to extracellular spaces during slow freezing results in decreased water holding capacity. Such meat is more likely to incur thaw and cook loss, and, consequently, to be less tender and juicy. Freezing times of less than 20 minutes were found to produce the least amount of exudates in frozen or thawed meat.[70] The amount of time that the product spends at temperatures near −3°C may be particularly important, as the freeze-concentration of ions at this temperature produces the greatest protein denaturation.[69]

Air-blast, cryogenic, and contact plate freezing have all been successfully used for meat freezing. Fast freezing rates attained with cryogenic freezing may be advantageous, but such methods are limited to meat pieces of modest volume that can be individually exposed to the cryogenic fluid.

Frozen meat may also incur a drying of the meat surface known as "freezer burn." Although a slight amount of surface drying is useful for limiting microbial growth, excessive drying results in a pale, dry-looking surface with

greater toughness and reduced juiciness. Freezer burn may occur during freezing, and is a particular problem during extended frozen storage. It is enhanced by low humidity and fast moving air, and meat with less surface fat tends to suffer more surface dehydration.[71] Appropriate wrapping can help limit the occurrence of freezer burn.[72,73]

Other textural changes can occur during frozen storage, and storage temperatures below −18°C are recommended to limit these changes. Ice recrystallization is a problem in that it encourages protein denaturation, and subsequent toughening and loss of water holding capacity are all possible. Higher temperatures, and, in particular, repeated temperature fluctuations, are detrimental to texture quality.

Thawing methods can also affect the final texture of the meat. Unfortunately, not many thawing alternatives exist. Rapid thawing, particularly through temperatures near −5° to 0°C, prevents further toughening and loss of WHC.[70] In some cases, thawing and holding the meat may help improve tenderness. By this logic, thawing in a cooler or refrigerator would seem not to be recommended. However, thawing at higher temperatures may result in parts of the product reaching elevated temperatures before all of it has thawed. This would subject the meat to greater microbial spoilage. Thawing by direct immersion in water can be beneficial. In a few instances, meat may be cooked directly from the frozen state, but the possibility for uneven cooking or uncooked centers limits this approach. Research on vacuum, dielectric, infrared, and microwave thawing is ongoing.

IV. FISH AND SEAFOOD

Although fish and seafood are muscle foods, they do have unique characteristics that distinguish the way they are processed for freezing. First, harvesting of particular fish has more seasonal variability, so that freezing becomes necessary to ensure availability throughout the year. In addition, fish are harvested at locations remote from where they are frozen and stored. Fish also have shorter muscle fibers and less connective tissue than land animals, and are expected to be more tender than red meat and poultry. Different metabolic processes also lead to more substantial flavor changes in frozen fish.

A. Texture Attributes

Changes in flavor are perhaps the greatest concern in frozen fish. Fish have different types of muscle fibers, and do not have the large skeletal system and great amounts of connective tissue associated with land animals. Fish meat is generally expected to be firm but tender, and remain juicy and succulent. Some changes in texture do occur in the freezing process, and are often associated with protein denaturation and drying out of the meat.

B. Processing and Storage

Fresh fish are often chilled, placed in ice, or frozen while on board ship. Ice glazes using salts, phosphates, antioxidants, or sodium alginate help prevent surface drying and lipid oxidation during transport.[74] Moisture-proof packaging is important to prevent dehydration, and vacuum-packaging is useful for limiting lipid oxidation.[75] In some on-board operations, fish are individually placed in plastic bags and vacuum-sealed, then frozen in brine freezers.[76]

Like red meats, fish and seafood should be frozen as rapidly as possible. Slow freezing results in cell dessication and damage, resulting in toughness and lack of moisture retention. Fish is especially subject to protein denaturation during freezing and frozen storage.[77,78,79] Freeze concentration of solutes enhances the denaturation of myofibrillar proteins. Some fish, particular cod and deep water fish, contain trimethylamine oxide (TMAO). TMAO breaks down to dimethylamine and formaldehyde in frozen storage, creating protein crosslinks that produce a spongy texture.[74] Such fish release moisture quickly, leaving behind a dry, tough texture.

V. POULTRY

Poultry is another excellent source of protein, and the poultry market has enjoyed substantial growth in the last few decades. Much of the chicken and turkey sold these days has been further processed. Most chicken is sold in cut pieces rather than as whole birds, and often incorporates phosphate or other marinades. Turkey may be sold as whole birds or pieces, and is usually injected with salt and phosphate solution. According to USDA regulations, poultry that is held at temperatures below 0°F must be labeled as “frozen,” while “fresh” poultry is that held at temperatures above 26°F.

A. Texture Attributes

As with other muscle foods, poultry meat is expected to be tender and juicy. Loss of juices during thawing can be a particular problem. Meat from different parts of the bird typically have different textural characteristics. Red meat has more fat, is generally less dry, and has more lubrication when chewed. White meat has more of a tendency to be dry and tough if improperly processed. Simple tests, similar to those used for red meats, are used to assess chicken texture, including Warner-Bratzler shear tests, water holding capacity tests, and analysis of yield, drip loss, and cook loss characteristics.

B. Processing and Storage

As with all products, the quality of the commodity before freezing is perhaps the most important factor in determining

final texture. The USDA provides grades (A, B, C) which may or may not be indicators of good texture in the cooked meat. The age of the bird at slaughter is one determinant of texture.[80] Young "broilers" tend to have more tender meat than older hens. Similarly, young turkey fryer-roasters have more tender meat than older hens and toms.

Rough handling during processing may result in carcass damage, but the effects on texture are not known. In general, poultry must be chilled to 4°C within 4–8 hours to meet USDA specifications. Although used primarily to limit microbial growth, rapid chilling also ensures optimum tenderness. During ice-water chilling, moisture may be absorbed, but must be limited to less than 8% for chicken and 4.3% for turkey. This water, in turn, may increase freezing times and influence final juiciness. In general, freezing immediately following slaughter is not recommended. As with other meats, poultry should be aged to allow development of the ultimate pH, which results in the most tender meat.[80,83] For chicken, the hold period is 6–8 hours, while for turkey it is 12–24 hours.[81] The aging time for poultry is much shorter than that for red meat, and some studies suggest that the pre-freeze hold time is not a major factor affecting quality after frozen storage.[82] Generally, poultry should be frozen to less than −18°C within 72 hours.[80] Although, not strictly freezing, there has been a move towards deep-chilling of poultry to −2 to −3°C, particularly for younger broilers.

A wide variety of further processing operations are performed on poultry, including deboning, chopping, forming, and breading. Poultry must be raw or fully cooked prior to freezing. Operations that slice, cut, or expose more surface area allow for greater drip loss. However, drip loss may be controlled in such products. In particular, marination combined with tumbling or injection has a profound affect on the yield, tenderness, and juiciness of poultry meat.[53] The solubilization of muscle proteins and breakdown of sarcomeric structure result in lower shear values, enhanced swelling of myofibrils, and greater water holding capacity.

Appropriate packaging is critical to maintaining quality during freezing.[83] In addition to preventing oxidation and flavor changes, moisture barriers help prevent dry meat by limiting surface dehydration and "freezer burn". In general, vacuum packaging or gas flushing is used to limit flavor changes, while also preventing overly slow freezing.

As with other muscle foods, rapid freezing provides the best product quality. Excess time spent at sub-freezing temperatures is detrimental, and leads to protein denaturation, resulting in toughening and drip loss. Rapid freezing also helps ensure a light, chalky surface.[84]

Poultry is frozen by a variety of methods, including air-blast, cryogenic, and immersion freezing. As long as the freezing rate is sufficiently rapid, there does not seem to be much effect on meat texture for different freezing processes. Some research has shown that tenderness in chicken breast is not dependent on freezing rate,[85,86] but rapid freezing may decrease the amount of drip loss.[87,88,89] Fried broiler pieces frozen with liquid nitrogen have shown greater tenderness than those frozen in air[90]. It has been recommended that the freezing front progress at rates greater than 2–5 cm per hour.[81] Combination freezing, which is initial freezing with liquid nitrogen or by immersion followed by air-blast freezing at less than −30°C, may be advantageous.

If properly stored, some poultry meat may be kept in frozen storage for up to two years without significant deterioration in quality.[91] Storage temperatures should be below −18°C for optimum quality. Some studies suggest that drip loss may increase during extended frozen storage.[92] As with other frozen foods, large temperature fluctuations are particularly detrimental. The resulting recrystallization and formation of large crystals leads to toughening and drying of the meat.

Pre-processing and freezing seem to be greater factors than thawing method on drip loss and tenderness. Thawing of whole turkeys is especially of interest. However, studies have shown that different thawing procedures have little effect on texture and moisture retention.[93,94]

VI. EGGS

Egg products are used extensively in restaurant and fast-food establishments, and as an ingredient in baked goods and entrees. Much of the eggs are prepared at an egg-breaking facility, then dried, cooked, or pasteurized and frozen. The resulting products are frozen liquid eggs or frozen cooked eggs. Eggs lack the fibrous structure of vegetables and muscle foods, so are not subject to the same deterioration of structure. Although the physical process of freezing is the same, eggs have unique characteristics that determine how they are processed and frozen. The freezing of eggs is governed by USDA regulations.

A. Texture Attributes

In products in which eggs are a minor ingredient, the texture of eggs is not a major issue. However, the functionality of egg proteins in such products is important, as it may determine foam or gel structure and emulsification properties. As a liquid product, the viscosity of thawed, uncooked eggs is characterized by its viscosity, and viscosity is usually a good indicator of the suitability of liquid eggs for further processing. The texture of eggs cooked from frozen liquid eggs should be similar to that of unfrozen eggs. In general, cooked eggs should be firm but tender as well as have adequate cohesion and little run-off. The association of egg white proteins produces a gel network. However, excessive cross-linking may produce an undesirable rubbery texture.

Eggs have clearly distinct phases, the egg yolk and egg albumen, and these have unique chemical compositions.

The albumen is approximately 89% moisture, 10% protein, 0.5% carbohydrate, 0.5% ash, and 0.03% lipid.[95] The yolk contains 48% moisture, 16% protein, 0.8% carbohydrate, 1.1% ash, and 33% lipid. In addition, the functionality of protein and lipid differs in each region. Compositional factors produce differing rheological properties for egg yolk and albumen, while functional properties also dictate the textural characteristics of products made from eggs or egg components.

B. Processing and Storage

Most pre-processing of eggs includes washing, breaking of the shell, and relatively low temperature pasteurization. In addition, yolks may be separated from the whites, then later remixed or sold as separate products. Due to compositional differences in egg yolk and albumen, the effects of freezing are different for each.

Liquid egg white has a relatively high level of solutes, including protein, glucose, and salts. Freeze concentration during freezing produces high solute concentrations and ionic strength. This, in turn, may cause denaturation of the egg white proteins.[96] The concurrent aggregation of denatured protein reduces functionality. However, egg whites are not greatly changed by the freezing process. Cakes and other baked goods made from frozen/thawed egg white have volumes similar to that made from unfrozen egg white.[97] However, gels made directly from frozen/thawed egg white are firmer than those from unfrozen egg white.[98] Viscosity and denaturation depends somewhat on the freezing rate. Rapidly frozen egg white has a viscosity, foam stability, and native protein content closest to that of unfrozen eggs.[99] Lower freezing rates result in greater denaturation, lower viscosity, and less foam stability. In some cases, adjuncts may be added to modify the rheological properties of egg albumen. The addition of 5–10% sucrose helps to prevent loss of protein functionality caused by freezing. Gels made from egg white with added sugar have higher gel strength and elasticity.[100] The addition of 5–10% NaCl, however, reduces gel strength, elasticity and viscosity.

Egg yolk undergoes greater changes in texture during freezing than does egg albumen. The yolk contains granules and lipoprotein micelles that can become unstable and aggregate. Freezing causes thickening and partial gelation of the yolk[101,102] at freezing temperatures below −6°C, with maximum gelation occurring at temperatures below −18°C. The degree of gelation increases during frozen storage. Textural changes occur as freeze concentration of salts and changing pH produces protein denaturation.[103,104,105] Gelation has also been attributed to interactions between granules and low density lipoproteins.[106]

While deterioration of egg yolk is ameliorated by rapid freezing, storage at low temperatures increases the amount of yolk gelation.[106] Gelation is also reduced by the addition of low molecular weight solutes such as sucrose, NaCl, or glycerin.[107,108]

Liquid egg products are generally frozen in cans or pouches in air-blast freezers. Cryogenic freezing with liquid nitrogen or carbon dioxide provides more rapid freezing, but is used mostly for cooked egg products. These are generally flat, thin sandwich eggs or omelets. In addition, there has been some promising research on drum freezing of liquid eggs to form flakes.[96]

VII. DAIRY PRODUCTS

Unlike fruits, vegetables, and muscle food products, most frozen dairy products are eaten while still frozen. Such products include ice cream, sherbet, and frozen yogurt. Products such as milk, cheese or butter are typically refrigerated and consumed within relatively short periods. However, in some cases, dairy products are frozen in order to extend shelf life.

A. Texture Attributes

Desirable texture attributes vary with the type of dairy product. The chemical composition of milk, cheese, yogurt, butter, and frozen desserts are narrowly defined. Processing methods are also usually standardized, so that uniform texture is expected for most dairy products. Liquid milk is typically expected to have a certain viscosity and mouthfeel, be free of aggregated material, and have some cling in the mouth. As protein and carbohydrate content are similar in most milk, the serum viscosity does not vary much. Fat content plays a more important role in viscosity. Milk with more fat has greater viscosity and mouthfeel. The degree of homogenization has some affect on viscosity; however, most homogenization schemes produce particle sizes between 0.25 to 3 μm. Milk with much larger particle sizes would tend to separate. Milk with higher average fat particle size will have higher viscosity. Changes in particle size which occur through aggregation or coalescence will have a profound effect on the fluid rheology.

Cheeses have perhaps the most textural variety. The firmness, cohesiveness, adhesiveness, and chewiness vary with the moisture and fat content, the type of milk used, and the preparation procedure. For example, cream cheese is expected to be soft, smooth, cohesive, and spreadable. Hard cheddar cheese should be firm, less cohesive, and more springy.

Ice cream may also have some textural variety. An ideal ice cream should be cold but not icy, firm but not too hard, have a smooth body and meltdown, and be free of any grittiness. Some textural differences are expected, for example, in soft-serve ice creams or frozen shakes. Some differences in texture may be attributed to price and composition. Premium ice cream has higher fat (14–18%) and

contains lower levels of stabilizers. Shorter shelf life and more stringent distribution channels may be needed to ensure product stability. Other ice creams have at least 10% fat, and may incorporate a variety of stabilizers, sugars, and dextrins. These help prevent changes in ice crystal structure during frozen storage, but may contribute to stickiness or unexpected meltdown characteristics.

B. Processing and Storage

Very little fluid milk is frozen for storage. Concentration of milk prior to freezing increases the efficiency and improves the quality of frozen milk.[109] However, significant textural changes can occur in frozen milk.[109,114] Due to freeze concentration, casein micelles tend to precipitate or coagulate, resulting in increased serum viscosity. In addition, fat separation can be significant, and fat must be redispersed after thawing. The texture of milk has been found to deteriorate in frozen storage.[110] When stored at −10°C, the texture has been found to deteriorate significantly after two months of frozen storage.

There is a greater potential for freezing of high fat cream for use in other products. Cream with more than 25% fat can be frozen with minimal separation. The addition of up to 10% sucrose increases stability. The use of drum freezing to produce flaked cream has been researched.[111] The texture of butter is little affected by freezing, although lipid oxidation may occur at storage temperatures above −20°C.[109] Cheese can also be frozen to good effect, particularly for cheese to be used on pizzas or entrees that are cooked or baked. However, frozen and thawed cheese has a tendency to be more "crumbly" than unfrozen cheese.[112]

Ice cream and dairy desserts comprise the major portion of frozen dairy products. Ice cream is a complex food in which air is incorporated in a composite of ice, unfrozen aqueous solution, and oil in water emulsion. The formulation and processing of ice cream has a profound effect on the final texture. Higher fat content produces a rich, smooth texture and body, with excellent melting properties.[113] During freezing, the protein-stabilized fat membrane can disrupt, producing coalescence with other fat particles. A modest amount of coalescence can increase smoothness and richness. Excessive coalescence is produced when there is too much shear. The resulting ice cream has a "churned" quality reminiscent of butter.

The nonfat solids present in ice cream products include milk proteins, lactose, minerals, and other components. The surface active milk proteins help stabilize the relatively small (0.5–2 μm) fat globules produced during homogenization.[114] The proteins also contribute to serum viscosity, and help stabilize the foam structure produced during initial freezing.[115]

To produce ice cream, homogenized and pasteurized ice cream mix is placed in a swept-surface heat exchanger. Air is incorporated into the product, either by whipping or direct injection of compressed air.[116] as the heat of fusion is removed by the freezer. The amount of air, or overrun, in the product, as well as the air bubble size have an important effect on ice cream texture. Up to half the volume of the frozen ice cream may be air. Increased air provides a lighter, less dense texture. Generally, smaller, more uniform air bubbles produce better texture.[116]

After approximately 50% of the water in the product is frozen, the soft-frozen ice cream is placed in packages, and then stored in hardening freezers. Once the ice cream reaches the consumer, perhaps the most important determinant of texture is temperature. At progressively lower temperatures, more and more water exists as ice, and the ice cream becomes progressively harder. As the temperature is increased towards the freezing point, the ice cream becomes softer.

Several defects in texture may occur during frozen storage. Crystallization of lactose causes a gritty texture known as "sandiness." Lactose often exists as a supersaturated solute in the unfrozen phase. Crystallization is limited by control of diffusion by high viscosity.[117] In this sense, the incorporation of whey protein and hydrocolloid stabilizers may help limit sandiness. Current stabilizers include carrageenan, locust bean gum, gelatin, guar gum, and sodium alginate.[118,119,120]

Iciness is another defect in texture that is manifest during frozen storage. Small ice crystals produce smooth body in ice cream, but, unfortunately, are inherently unstable. Larger crystals produce a texture that lacks smoothness and is overly cold (Figure 61.6). At constant temperature, "Ostwald ripening" causes migration of water from small crystals to form larger ones. Temperature fluctuations are even more detrimental to texture. As the temperature rises, ice melts. As the temperature is lowered, this water refreezes slowly to form relatively large ice crystals. Stabilizers provide some protection against recrystallization and subsequent iciness.[121,122] Presumably, stabilizers limit diffusion of water, preventing continued growth of large crystals in neighboring regions.

VIII. BAKED GOODS

A variety of doughs and baked products are made from cereal-based flours. In the United States, bread is a major staple of the diet. However, the flavor and texture of bread deteriorates quickly, and it would be advantageous to develop processing systems to extend shelf life. Freezing is one technique that has had some success in preserving the quality of dough and bread. Currently, it is more advantageous to freeze dough than to freeze bread. With the advent of in-store baking in supermarkets and fast food restaurants, the accessibility of frozen dough allows for the continuous production of fresh bread.

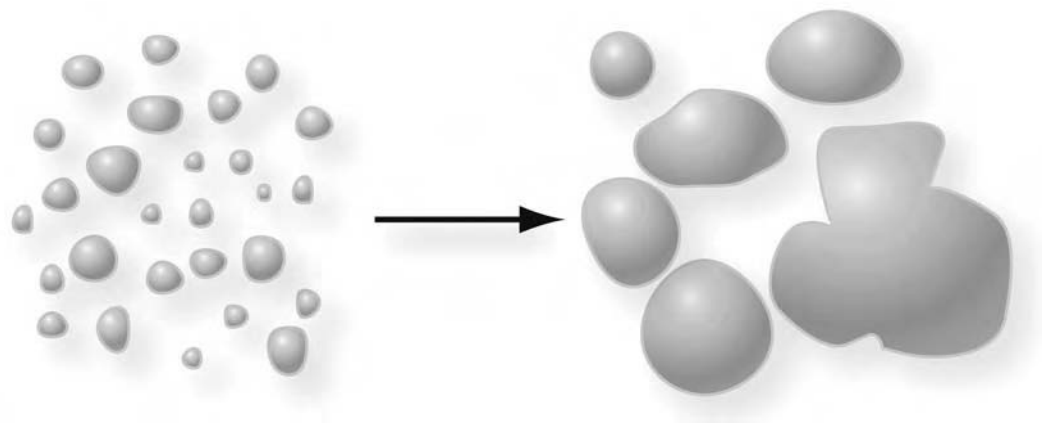

FIGURE 61.6 Over time, and particularly with temperature fluctuations, ice crystals become fewer and larger, and may fuse together.

A. Textural Attributes

Bread has a semi-solid foam structure, which incorporates different levels of air depending on the bread type. Dense, heavy breads have less air cell structure while light, fluffy breads have more air cells. Bread is expected to be relatively soft and easy to shear, but firm enough to resist the forces of spreading. Bread is particularly susceptible to changes in texture due to staling. Linked primarily to the recrystallization of starch, staling causes a firm, tough, dry texture. Firmness is normally measured by the maximum resistance developed when a cylindrical probe compresses a bread sample.

B. Processing and Storage

When freezing dough, the most important consideration is retention of yeast activity. After thawing, the dough should expand as carbon dioxide is formed from yeast fermentation. This provides the finished bread with a lighter, softer, less dense texture. Curiously, slow freezing is the least harmful to yeast activity.[123,124,125] Although slow freezing produces more yeast cell dehydration, it has been proposed that this helps protect the cells from freeze damage.[124] It has also been found that freezing should be done as soon as possible after dough formation.[126,127] Dough systems in which fermentation has commenced prior to freezing display lower yeast activity after thawing. Yeast strains with greater freeze-thaw tolerance have been developed for use with frozen dough.[128] Rapid rise yeast does not withstand freezing as well as slower rising varieties. Adding higher levels of yeast initially may help make up for loss of activity during freezing and frozen storage.[129] Loss of yeast activity increases with time in frozen storage.

Weakening of the gluten network is also a problem during freezing and frozen storage of dough. Weakened dough is less able to retain the gases produced during fermentation. One way to mitigate the effects of dough weakening is to add more protein to the initial mix. French bread made from 12.8% protein, as opposed to 11.1% protein, yielded better dough properties and texture in the finished bread.[130] Protein content may be increased by using a harder wheat or by adding vital wheat gluten. A weaker dough has also been associated with high amylase flour[131] as well as excessive dead yeast cells.[132,126] Using 3–5% less water in the dough mix can also help improve dough quality.

Non-flour ingredients also affect the dough properties. Oxidants can help strengthen the dough.[133,131] Adding higher levels of shortening can help improve the quality of frozen dough.[134] Surface active agents can also improve dough properties and, subsequently, the loaf volume of the baked bread.[135,136]

Doughs meant for freezing are usually prepared at lower temperatures to limit pre-freezing fermentation,[137] with best results achieved with mixing temperatures around 20°C.[138] A compromise freezing rate is needed to freeze the dough, slow enough to maximize yeast activity but fast enough to limit dough weakening. Freezing rates of 0.3 to 1.2°C/min have been recommended.[125] Air-blast and contact freezers have been used successfully for freezing dough.

Baked bread can also be frozen, and studies have shown that freezing can retard staling.[139,140] However, as retrogradation proceeds faster at temperatures between −3°C to 5°C, it is important to cool and freeze the bread rapidly.[141] Cryogenic freezing is an excellent means for freezing bread, but air-blast freezing can also be effective.[142] Avoiding dead spaces in the wrapping helps enhance the rate of freezing.

Moisture loss during frozen storage can result in a dry, firm texture.[143,144] The useful storage life of frozen bread is often limited by the pickup of undesirable flavors. Storage temperatures should be less than −18°C to prevent retrogradation during storage.[144] As with other frozen foods, temperature fluctuations in frozen storage are particularly detrimental to textural quality. Proper thawing of bread is also important to limit staling, firming, and loss

of moisture.[145] Breads rapidly thawed at 40–60% relative humidity display the minimal changes in texture.[146]

IX. CONCLUSIONS

Due to the chemical and structural differences in different food groups, each has unique issues associated with changes in textural quality. With the exception of bread dough, most food groups suffer the least changes in textural quality when frozen at a rapid rate. In addition, storage at low temperatures is preferential, and particular care should be taken to limit temperature fluctuations during frozen storage. Methods of thawing can also affect the texture of foods. However, the variety of thawing regimes is limited, particularly as much thawing is accomplished by consumers.

This chapter has reviewed the effects and processes of freezing major food groups. In addition to these, there are a number of further-processed entrees and heterogeneous foods that are frozen and prepared as convenience meals. Although the issues associated with these foods are too numerous to cover here, it is hoped that this chapter can also be used as a starting guide for understanding such foods.

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62 Chemical and Physical Aspects of Colour in Frozen Muscle-Based Foods

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CONTENTS

I. INTRODUCTION

In the mind of the consumer, one of the most important quality attributes of many foods is colour (1). Colour is correlated with aspects of quality and disagreement may occur between buyer and seller. It is therefore not surprising that the measurement of colour has become an area of much interest and practical importance in recent years (2).

In general, colour properties and their measurement are critical quality control parameters. The quality of food products as perceived by humans is very difficult to describe and quantify as it is usually a function of several food properties (3) including colour, which is affected by most of the technological processes involved in the manufacture of foodstuffs (4). Freezing is one such process and many problems are associated with the maintenance of the colour of frozen foods (1). However, in general, little attention has been given to the effects of freezing and frozen storage on the final colour of muscle-based food (meat, fish, shellfish and their products).

Frozen storage is an important preservation method for muscle-based foods. Freezing, in general, offers advantages in terms of a long storage life and allows better control of production levels. However, freezing and frozen storage can have marked effects on the structural and chemical properties of muscle foods, including changes in the muscle fibres, lipids and proteins, all of which have the potential for significantly influencing final quality attributes (3), especially colour (5). Quality deteriorates during freezing and frozen storage due to the osmotic removal of water, protein denaturation and mechanical damage (6).

The colour of muscle-based foods at the retail level exerts a strong influence on consumer purchasing decisions, and appearance probably is the most important factor in determining retail selection. This is particularly true in the case of meat (7). Food stores assign a relatively large display space to meat in order to attract consumers, frequently using bright lights (8). Most of the frozen muscle-based foods are sold in cool or warm fluorescent illumination (9) on a gondola, where different types of processing, e.g. glazing, and packaging are evident. However, to avoid colour damage especially in beef, the type of light used should be warm fluorescent light, which shows an excellent colour spectrum and has no adverse effect on meat pigments (10).

Some types of product, especially fish and shellfish, are not sufficiently protected from the action of light and oxygen, and may show surface discoloration during frozen storage, for which reason they are frequently packed in vacuum, in gas permeable packages or in modified atmospheres (11).

II. COLOUR MEASUREMENT

The colour of foods can be defined as the interaction of a light, an object, an observer and the surroundings of the food.

Objective colour measurements may refer to include several properties or various ratios or colour difference indices (12). By summarising all the reflected colours (wavelengths) and expressing them as one colour (13). The colour a consumer sees can generally be described in one or two words, which indicate the main colour and its shade. However, colour measurements whether descriptive or specific, must be made as carefully as other measurements (12).

The colour of foods can be studied in two main ways: chemically by analysing the pigments present or physically by measuring the interaction of light. Colour necessarily requires a light source that illuminates an object, which, in turn, modifies the light and reflects (or transmits) it to an observer. The observer senses the reflected light, and the combined factors provide the stimulus that the brain converts into our perception of colour, a property which has three quantitatively definable dimensions: hue, chroma and lightness (12).

Several methods are available for objectively measuring the colour of foods, some of which depend on the extraction of pigments from food products followed by spectrophotometric determination of pigment concentration (14, 15). However, since such pigment extraction methods are time-consuming and tedious, some researchers have sought simpler methods of colour measurement. For example, several methods measure the light reflected from the surface of foods. There are also tabulated coefficients of various objective values which are correlated with panel scores (16). These objective values consist of numerous combinations of percentage reflectance values and tristimulus values such as Hunter Lab, CIE XYZ, Munsell hue, chroma and value or CIELAB. Others researchers have measured reflectance values, which consist of indices and/or differences of reflectance at different wavelengths (17).

A. Fundamentals of the CIE 1976 Lab (CIELAB) Colour Space

Nowadays, colour can be measured by objective methods, with the CIELAB space being the most widely used method because of its degree of international (industrial and research) acceptance (18). It is often used to facilitate the quality control of coloured products, including foods, by measuring colour differences and characterising colour, so that colour quality decisions can be made**.** This colour space has been adopted throughout the world, and many official quality standards for a variety of different products use it, CIELAB replacing other colour spaces such as those of Munsell, Hunter Lab (HLab) or CIE 1931 (12).

CIELAB has the advantage of being more perceptually uniform, and is based on the accepted colour description theory that colours cannot be red and green, or yellow and blue at the same time. Colours are considered as combinations of red and yellow, red and blue, green and yellow, and green and blue. CIELAB is a reasonable descriptor of colour (2) and is also used for measuring and ordering an object's colour.

The CIELAB space is a mathematical transformation of the colorimetric system first published by the CIE (International Commission on Illumination) in 1931 (19). Although the 1931 system proved useful, its practical application was limited as it did not express differences between colours in a uniform perceptual manner. In CIELAB space, however, the numeric differences between colours agree consistently well with visual perceptions (20). CIELAB colour difference equations contain valuable information about the characteristics of human colour vision and provide quantitative metrics corresponding to psychological colour descriptors (21). Their performance in describing visual colour differences is much improved compared with the tristimulus colour space of CIE (19, 22).

CIELAB space was recommended by the CIE in 1976 for use as a colour difference metric (2). CIELAB space was specifically recommended for meat colour evaluation in 1995 (23), before which time the most used colour space was Hunter Lab (HLab), which is still mentioned in some guidelines (12).

Although the studies of this colour space were developed for flat two dimensional objects (21), the CIELAB colour space can be visualised as a three dimensional space, where every colour can be uniquely located. The location of any colour in the space is determined by its colour co-ordinates: L* (lightness), a* (the red/green co-ordinate, with +a* indicating red, and −a* indicating green.), and b* (the yellow/blue co-ordinate, with +b* indicating yellow, and −b* indicating blue). These colour co-ordinates are adimensional (20). The L*, a*, and b* co-ordinate axis define the three dimensional CIELAB colour space. Thus, if the L*, a*, and b* co-ordinates are known, then the colour is not only described, but also located in the space.

Colours in CIELAB can also be described and located using an alternative method, that of specifying their L*, C*, and h values (18). The resulting L*C*h* colour space is also three dimensional, where L* is the same parameter as in CIELAB, while the C* (chroma) and h (hue) are computed from the a* and b* co-ordinates, L*C*h* notation is preferred, since the concept of hue, and chroma agrees well with visual experience (19). Colour can also be located

using the CIEa*b* chromaticity diagram or C* and h. In both cases, L* is usually displayed separately, as a number.

One of the most important things that the CIELAB colour space describes is the colour difference, defined as the distance between the colour locations of any two colours in CIE space. This distance can be expressed as DE* or DE* where $DE^* = (DL^{*2} + Da^{*2} + Db^{*2})^{1/2}$, where DL* is the lightness difference, Da* is the red/green difference and Db* is the yellow/blue difference. It is possible to express DE* as differences in chroma and hue terminology, instead of Da* and Db*, using $DE^* = (DL^{*2} + DC^{*2} + DH^{*2})^{1/2}$, where DC* is the chroma difference, and DH* the metric hue difference (18).

Experimental knowledge of colour-difference perception is incomplete because the CIELAB colour space lacks distance uniformity. Colour difference perception does not depend only on the measured colour tristimulus values of each member of a colour difference pair but also depends on experimental conditions related to the visual environment, sample characteristics, and the way in which the samples are presented. These are called parametric effects and include the influence of such factors as illumination, illuminance, surroundings, viewing mode, sample size, sample separation, sample structure, and size of visual colour difference (18). These effects are very important for colour measurements in muscle foods, and must be considered or standardised when preparing the samples for colour evaluation (12).

B. Colour Measurement in Muscle-Based Foods

Measuring the colour of muscle-based foods involves two basic methods: human visual appreciation (sensory analysis) and instrumental analysis (absorbance and reflectance methods) (12). Objective determination of colour by means of reflectance spectrophotometry is one of the most commonly used methods due to its close correlation with the visual perception of the human eye. In contrast to absorption spectrophotometry, reflectance is measured over the surface of the object, thus making destruction of the object unnecessary and allowing the changes in colour over a period of time to be evaluated (12).

According to how light interacts with muscle-based foods, they can be classified as translucent or opaque, although such foods rarely fall into one category alone. The colour of fresh meat is known to be largely determined by the relative proportions of deoxymyoglobin (DMb), oxymyoglobin (OMb), metmyoglobin (MMb) for fresh meat, and nitrousmyoglobin (NOMb) are specific for cured meat products (24).

Guidelines for colour measurements in muscle-based foods are not complete and only for meat are they well documented. Fish and shellfish do not have specific guidelines for colour measurements, although the guidelines that do exist could be adapted to their specific characteristics. The same basic principles can be used for these types of food when colour is measured.

In the standardisation of colour measurements of fresh meat the CIELAB colour space has been recommended using D_{65} as light source and 10° as standard observer, with the illumination/viewing system as 45/0 or diffuse 8 (d/8) and specular reflectance excluded if within the capabilities of the instrument used (23). In fresh meat, the sampling should be a cross-section taken perpendicular to the long axis of the muscle, and the sample should have a minimum thickness of 1.5 cm (the sample should be opaque or an opaque backing must be used) (12). In measuring the colour of muscle-based food with low myoglobin concentrations the relationship between samples thickness and light transmittance can be checked with white and black backgrounds. The American Meat Science Association (AMSA) recommended that, in the case of frozen meats, the sample surfaces should be as flat as possible, and that a black rubber gasket slightly larger than the aperture should be used to help level uneven frozen surfaces and block stray light. The history of frozen meat (time, temperature, package, etc) when colour is being measured should also be stated, because temperature fluctuations during transportation or storage can affect the colour of muscle-based foods.

III. OPTICAL PROPERTIES OF MUSCLE-BASED FOODS

From a physical point of view, the colour of muscle-based foods, is considered as a surface phenomenon of an opaque solid, where light falling upon it may undergo processes of absorption, reflection or scattering but, where, generally, there is little transmission (12). MacDougall (25) described meat colour as partly determined by light scattering, presumably due to the gaps existing between myofibrils (26).

Light is unable to penetrate a significant distance into the meat without being scattered (27), such penetration and reflection being pH-dependent. Thus, a low pH causes greater scattering and a high pH less (28). In frozen muscle foods (meat and fish) it is generally accepted that pH decreases during storage (29, 30), although some authors found that the pH of some foods can also increase during frozen storage (31).

Swatland (32) describes the optical properties of meat as several levels of organisation. This author describes four levels; 1: sliding filaments and myofibrilar birefringence, 2: myoglobin and protein precipitation in the sarcoplasm, 3: postmortem fiber shrinkage and 4: the release of fluid and the macroscopic surface reflectance properties of cut meat. All of these phenomena can affect the colour of muscle-based foods in different ways.

Muscle proteins in frozen meat may be affected by factors such as ice crystal formation, dehydration, increased

solute concentration, fat hydrolysis and/or oxidation, the presence of atmospheric gases (particularly oxygen), protein oxidation and proteolysis and rigor temperature. During frozen storage total/myofibrillar protein solubility increases, while the solubility of sarcoplasmic proteins is reduced (33). Sarcoplasmic proteins (in which myoglobin is included) are more susceptible to low temperature and frozen storage denaturation than myofibrillar proteins. Pigment also decreased as a result of ice storage (34). Modifications in sarcoplasmic protein fractions during frozen storage occur because endogenous enzymes are released and are not inhibited.

The colour of frozen meats is governed by the freezing rate, which affects light scattering properties. Fast freezing results in small crystal sizes, which scatter more light than the large, slow growing crystals produced during slow freezing. Fast frozen meat is opaque and pale (reflectance of the small ice crystals), and slow frozen meat is traslucent and dark (35, 36). Frozen meats in any circumstances can appear darker than fresh meat due to the concentration of meat pigments during the freezing process (27).

IV. COLOUR PROPERTIES OF MUSCLE-BASED FOODS DURING FREEZING AND FROZEN STORAGE

In general, the quality of frozen muscle-based foods decreases as the storage period increases. Colour, especially, is affected although other important sensorial characteristics including odour and flavour are also affected (37).

The consumer's acceptability of frozen meat products on display are related to the colour of the product, the redder the meat the higher the degree of consumer acceptability. However, the consumer will reject fish and shellfish when they appear dark. Some authors have used objective parameters to evaluate acceptability as the % of reflectance difference between 630 and 580 nm; the a*/b* ratio, Lovibond tintometer red colour units, chroma or metmyoglobin content (%) etc. (38, 39).

Different factors affect colour and its stability in frozen muscle-based foods. Such factors include the particular freezing technique used (individually quick frozen or IQF, plates, glazing, etc.), temperature (-5 to -60°C), time and storage temperature, storage methods, freezing-thawing cycles, type of packaging and type and product characteristics (pigment concentration, polyunsaturated fatty acid concentration, etc.) (4, 33, 40, 41, 42).

During freezing, enzymes and other components are released. The ice crystals formed may injure the cell and release pro-oxidants (iron) (41). For any unprotected muscle-based food during frozen storage, surface dehydration may, in extreme cases, produce an important defect known as "freeze burn" (caused by ice sublimation) which becomes apparent after thawing and affects the appearance and functional properties of the products. This problem is very important for frozen mussels that are sold in "bulk" without any packaging. "Freezer scorch" is another defect that can appear during frozen storage. This shows as small grey and white areas, and is also caused by dehydration (27).

The freezing-thawing process is also very important for the physical and physicochemical properties of muscle-based foods. The denaturation of muscle, structural changes and lipid oxidation induced by the freeze-thaw process affect colour. Lipid oxidation is one of the major problems with frozen muscle-based foods (fish, shellfish and meats). However, the relation between lipid oxidation and pigment oxidation is not fully understood (43).

The primary catalysts of lipid oxidation in skeletal muscles are hemoprotein and iron (44). In general, iron is distributed among five main components: the insoluble fraction, ferritin, haemoglobin, myoglobin and the low molecular weight fraction (45). The two pools of iron in muscle foods, heme and non heme, are altered during frozen storage. Non heme in cod and mackerel muscle increase during frozen storage (46). Since myoglobin and haemoglobin are the pigments, responsible for colour, this property is affected by the freezing process. The iron released from ferritin may act as a lipid oxidation catalyst in muscle (47). During the freezeing-thawing cycle, the distribution of pro-oxidants and oxidation stability affect colour (41). The freezing-thawing process potentially disrupts muscle cells and leads to the deterioration of subcellular organelles, such as mitochondria. Enzymes are released from the mitochondria into the sarcoplasm, while cytochrome c (48, 49) and other heme pigments present in muscle food may also be released and contribute to colour loss. These proteins are soluble and so drip loss in muscle may also can lead to a less acceptable colour.

A. Meat and Meat Products

The frozen storage time of muscle-based foods is related to the characteristics of the product. Meat origin (pork, beef, turkey, etc), type of muscle, pigment concentration, type of pigment, fatty acid profile, antioxidant type and concentration, etc. are related to colour retention and discoloration (50, 51, 52). Also important to colour is the frozen storage time and type of freezing process used.

The effect of packaging on the colour of frozen meat is very important especially if one takes into account the relation of meat colour and oxygen or other gases such as carbon dioxide. If packaging highly permeable to gas is used, oxygen can come into contact with frozen meat and different myoglobin states (OMb, MMb) may be formed. As occurs in fresh meat, MMb is formed from OMb (53). Metmyoglobin plays a pro-oxidant role in fresh and cooked muscle foods, although, in fish, the oxidation

mechanism is still not fully understood. MMb formation increases with the freezing-thawing cycles (41).

Colour parameters (CIELAB) and reflectance ratios vary with the different states of myoglobin, while redness decreases as MMb concentration increases (54). Brownness can be observed in packed frozen stored meats (55). In the case of cured meat products, Sakata and co-workers (56) confirmed that in a model system, nitrousmyoglobin, the main pigment responsible for the colour of this type of product, is stable under frozen storage conditions.

During frozen storage MMb% values gradually increase (4, 55), changing the appearance of muscle-based foods. Farouk & Swan (33) explained this increase by the fact that metmyoglobin-reducing activity decreases during frozen storage and consequently MMB is accumulated. When meat or fish is treated with carbon monoxide during frozen storage MMb% values remain constant (4), while meat stored under carbon dioxide atmosphere is less red than meats stored in other types of packaging.

In general, the colour of salted fresh meat (57) and cured products deteriorates (sodium chloride promotes lipid oxidation), more rapidly in oxygen-permeable packaging than in vacuum packaging (58). Bhoyar and co-workers (31) reported that vacuum-packaged restructured meat products scored higher in colour and other sensorial attributes (flavour, juiciness, texture) and showed greater overall acceptability during frozen storage. Vacuum packaging also reduce the amount of MMb in meat (39).

To prevent colour deterioration, natural antioxidants (alpha-tocopherol, rosemary extract, ascorbic acid, carnosine, etc.) are frequently added to muscle-based foods (30). The use of antioxidants during frozen storage is particularly useful for low fat meat products (59). Industrially, other solutions are sometimes used to avoid this problem, one of them being to dip the product in gelatine solutions to create a coating that improves colour stability during frozen storage (58). Another method is to dip the product in a 3% brine solution before freezing at −45°C, especially in the case of Ascidians (60). In some cases, colour changes during frozen storage are related with lipid oxidation, both of which occur as the same time. This is the case of meats with a low myoglobin concentration, but when the myoglobin concentration is high (e.g. beef), discoloration occurs much earlier than lipid oxidation (50).

When meat is stored in carcass form or as primals, before being cut and packed the exposure of the meat surface to harmful environmental, effects is minimised (61, 62). When large blocks of meat are frozen, colour loss is more pronounced on the exterior than in the interior (55).

Thawed pork chops showed better colour stability after storage when vitamin E had been added to the feed (63)

From a sensorial point of view, the colour shelf life of frozen muscle-based foods depends on species and muscle type (43). The quality characteristics of ovine, for example, begin to deteriorate after 180 days of storage. Other attributes also diminish its acceptability to consumers.

A general rule governing the effect of frozen storage on the colour of muscle-based foods cannot be provided, because the effect changes according to the product in question and storage conditions. For example, the colour of the same sample can vary with the length of storage. For short and long-term frozen storage, different parameters may act as colour indicators. For example, hue may be a better indicator of colour in short-term frozen storage (33), while redness is the best indicator during long-term frozen storage.

Objective colour parameters may behave differently during frozen storage. Farouk & Price (51) reported that lightness decreased in ovine muscles. Heat & Owens, (64) described a similar behaviour for chicken meat, while rabbit and bull meat were seen to be darker after frozen storage (40). In the case of lamb chops brightness increased, and lightness increased in rainbow trout, as a result of the colour oxidation pattern of carotenoids. Decreased redness in meat and meat products is caused by a reduction in OMb and DMb concentrations (55), a phenomenon which has been observed in ground pork (51), low fat ground beef patties (45), rainbow trout and Atlantic salmon (1, 4). Less information is available, concerning yellowness although it is known to depend on the type of product, for example in low fat ground beef patties it decreased, while in rainbow trout and chicken it increased during frozen storage (4, 64, 65).

B. Fish and Fish Products

In the case of fish and shellfish, most are caught at sea and are processed immediately, mainly in frozen form, on board ship, to ensures a high degree of freshness.

Fish and fish products are very prone to discoloration. As mentioned above, lipid oxidation takes places, and changes in meat structure or pigment degradation are common. Minces produced from "white" fish have an unacceptable colour after six months of frozen storage (66), while "blue" fish products need antioxidants to prevent colour changes (30).

To prevent discoloration in tuna fish (the pigments of which are very prone to colour changes) a combined treatment is made. First the meat is frozen in a $CaCl_2$ solution at −30 to −45°C and then the $CaCl_2$ treatment is repeated. The freezing process is intensified by using liquid nitrogen during the first 2–3 h of freezing. Flesh colour changes and general spoilage can be limited by freezing and storing at −35 to −40°C, while lowering the temperature to −50 to −60°C ensures a high degree of freshness retention over several months' storage.

In some types of fish, it is important to preserve skin colour during frozen storage. This can be done by using natural or artificial antioxidants such as tocopherols, ascorbic acid (the most effective skin colour preserver) alone or

with butylated hydroxytoluene (BHT) and sodium erythorbate. These antioxidant, alone or mixed, significantly improved colour retention during the frozen storage of Thornyhead rockfish (*Sebastolobus alascanus*) (67).

Fish and shellfish both contain significant levels of polyunsaturated fatty acids. Benjakul and Bauer (41) postulated that the decrease in heme iron due to heme breakdown in fish muscle was affected by freeze-thaw cycles.

Metmyoglobin plays a pro-oxidant role in fresh and cooked muscle foods. In the case of fish muscle the oxidation mechanism is still not fully understood, although MMb formation tends to increase with the number of freezing-thawing cycles (41).

It is known that MMb reductase is a component of red blood cells and is found in fish muscles (68). Since some blood is retained in the fillets, the residual activity of this enzyme could result in the retardation of colour deterioration (41).

1. Salmonids

It is very important to maintain the colour of farmed Salmonids. This can be largely done by dietary means because colour is affected by dietary carotenoid pigment. The shelf life or frozen storage stability of these farmed fish are affected by the fatty acid (saturated and unsaturated) profiles and natural antioxidant levels present in the flesh (69). Colour studies carried out to date have mainly concerned farmed Salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

The effects of frozen storage are also related with the structural changes that take place during the freezing process, as was mentioned above. However, the changes which take place during frozen storage depend on carotenoid content and stability, characteristics which are mainly related with astaxanthin and canthaxanthin (from natural or synthetic sources) (70).

Salmonid colour and its stability depend on storage time and the packaging system. Sheehan and co-workers (1) reported that canthaxanthin-fed Atlantic Salmon were more sensitive to frozen storage than astaxanthin-fed fish. For the same storage time (3 months) canthaxanthin levels decreased by 59%, visual colour scores and redness values decreasing at the same time, while lightness increased. The same authors reported that no differences in visual colour score or carotenoid content were observed in astaxanthin-fed fish.

Salmonoid flesh is mainly dedicated to producing smoked specialities, while freezing affects colour. When frozen astaxanthin-fed fish is smoked, the astaxanthin content decreases, while the canthaxanthin content of canthaxanthin-fed fish remains unaffected after 3 months. Canthaxanthin-fed fish are better for smoking (if stored for less than 6 weeks) although, when frozen, they lose colour more rapidly than astaxanthin-fed fish. Nevertheless, Sheehan and co-workers, (1) reported that the astaxanthin content depends on the frozen storage time of the raw material, falling from 9.39 (fresh) to 7.99 after 6 weeks and 7.26 mg/kg after 12 weeks.

Packaging plays an important role in preventing the discoloration of Salmonoid flesh and its processed products. Different packaging materials (69) and techniques are used (vacuum packed, modified atmospheres, etc.), and it is uncommon to find Salmonid flesh in light display cabinets without any protection to avoid discolouration. When flesh (fillets) of rainbow trout is vacuum-packed, astaxanthin or canthaxanthin remains stable (maximum 5% loss) for 6 months at −20 or −80°C (52, 70). When this meat was analysed by colour objective methods, no change was observed in redness (a*), even if a thawing/refreezing cycle had taken place. In general, prolonged frozen storage increases lightness (L*), redness (a*) and yellowness (b*), but decreases the hue (h) values.

Light exposure (mainly fluorescent light) has a catalytic effect on lipid deterioration in frozen Salmonoids flesh. Oxygen-impermeable packaging materials inhibit rancidity and increases the length of time the frozen can be exposed to light.

Bjerkeng & Johnsen (69) pointed to the antioxidative effects of astaxanthin. during the frozen storage of rainbow trout fillets (70), while the concentration of dietary vitamin E are also important. The synergistic activity of alpha-tocopherol and rosemary oleoresin during refrigerated/frozen storage increased the retention of carotenoids and reduced lipid oxidation.

C. Shellfish

The frozen storage of shellfish decreases overall quality, particularly in the case of shell-on ascidians and mussels. This phenomenon is caused by the development of ice crystals in the muscle, which discolours after thawing. Choon (71) reported that, the muscle of shrimps (*Penaeus japonicus*) after 60 days of frozen storage began to blacken, while colour sensory scores fell after 7 months of storage (72). The colour of the giant Pacific oyster (*Crassostrea gigas*) becomes unacceptable during frozen storage, a problem caused by freezing temperatures rather than glazing in storage.

Shell-on cold-water shrimps (*Pandalus borealis*) packaged in atmospheric air and exposed to light, during frozen storage, lose colour. In this case the exclusion of O_2 from the pack would improve overall storage stability and extend the shelf life to at least 9 months. Light influences lipid oxidation and colour stability and its exclusion can extend the shelf life by up to 3 months. One of the main problems with shell-on shellfish are shrimp horns because they can break the packaging materials, and so it is not possible to maintain anoxic conditions (11).

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Part F

Fermentation Principles

63 Fermentation

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CONTENTS

I. INTRODUCTION

In this chapter the term fermentation is used to describe the process of intended chemical change in foods catalyzed by microbial enzymes. Prescott and Dunn (1) defined fermentation, in its broad sense, as "a process in which chemical changes are brought about in organic substrates through the action of enzymes elaborated by microorganisms." Steinkraus (2) defined more specifically fermented foods as "food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavours, aromas and textures pleasant and attractive to the human consumer."

A. History

Fermentation is an ancient tradition. In all parts of the world indigenous fermented foods have been prepared and consumed for many centuries. These foods have influenced the settlement sites and cultures of many communities. The first fermented foods eaten were probably fermented fruits, as scarcity of adequate food supplies would require the maximum amount of a crop to be consumed. There is evidence that fermented drinks were prepared many thousands of years ago (3,4). Compromises between starvation and disease from over-ripe/spoilt foods needed to be continually made before the advent of preservation technology. Solutions to the problems of food wastage and food-borne diseases were of major concern even before man had any concept of microbiology. Along with cooking, smoking and sun-drying fermentation is one of the oldest methods for food preservation. The methods established were obtained by trial and error and experiences obtained by many generations with information being passed between generations.

For many centuries cereals and breads have been a basic, major component of the diet of many individuals. Several loaves of bread were found in tombs estimated to be over 3,500 years old in Egypt (5). These are some of the first indications that man had begun to use the process of fermentation to develop new foods and not just to eat naturally fermented fruits, vegetables and grains (6).

B. Classification

A number of methods can be used to classify fermentation processes. They can be segregated on the basis of the microorganisms involved, the biochemical changes that occur or on the commodity type (7). In this chapter the fermentations are grouped in terms of the substrates used e.g. cereal products, fruit and vegetable products, dairy products and meat products (8). Campell-Platt (8) classified foods in 7 classes: (1) beverages; (2) cereal products; (3) dairy products; (4) fish products; (5) fruit and vegetable products; (6) legumes; and (7) meat products. Whereas, Steinkraus (9) classified fermentations according to the type of fermentation, e.g., alcoholic — wines and beers, alkaline — Nigerian dawadawa.

TABLE 63.1
Examples of Common Fermented and Microbial Foods

Food	Principal Ingredient	Key Microorganisms
Wine	Grapes	Yeasts
Beer	Barley	Yeasts
Cider	Apples	Yeasts
Sake	Rice	Moulds
Bread	Wheat	Yeasts
Yoghurt	Milk	LAB
Cheese	Milk	LAB
Buttermilk	Milk	LAB
Kefir	Milk	LAB + yeasts
Vinegar	Grapes	Yeasts followed by *Acetobacter* + *Gluconobacter*
Tempeh	Soyabeans	Moulds
Soy sauce	Soyabeans	Moulds + LAB + yeasts
Pickled cucumbers	Cucumbers	LAB + yeasts
Sauerkraut	Cabbage	LAB
Pickled olives	Olives	LAB + yeasts
Fish pastes	Fish	LAB
Fermented sausages	Meat	LAB + moulds
Microbial biomass/Products	Many including ethanol, molasses, whey, petroleum hydrocarbons	Bacteria, yeasts, moulds
Quorn	Mycoprotein	Mould *Fusarium venenatum*

LAB represents lactic acid bacteria.

C. Benefits of Fermented Foods

In many parts of the world, particularly in developing countries, fermented food products form an essential part of the human diet. Today a large variety of foods are produced either from microorganisms or as a consequence of their biochemical activity. Many of the foods listed in Table 63.1 are amongst the most common and have wide geographical distributions.

TABLE 63.2
Potential Benefits of Fermented Foods

Increased	Decreased
Safety	Toxicity
Revenue from the sale of the transformed food	Cooking time
Nutritional value	Processing costs
Digestibility	
Suitability of food commodities for subsequent processing	
Acceptability	
Sensory properties	
Ease of storage and transportation	
Shelf-life	
Health benefits	

Specific foods were developed simultaneously by many cultures using their available resources for two main reasons:

1. to preserve harvested or slaughtered products, which were abundant at certain times and scarce at others.
2. to improve the sensory properties, e.g., taste and aroma of an abundant produce.

The importance of fermentation processes for food preservation are less important now as new preservation techniques have been developed. However, fermentation is a low energy-requiring process that is relatively efficient at extending the shelf-life of foods, and it remains a very appropriate method for use in developing countries and rural communities that have no access to alternative preservation methods. The simple techniques mean that the procedures can often be carried out in the home (10). Also, a number of studies have shown that consumers regard fermented food products as healthy and natural (11). A range of potential benefits can be gained from fermented foods (Table 63.2).

II. MICROORGANISMS ASSOCIATED WITH FERMENTED FOODS

A. Starter Technology

Fermented foods may be produced with the use of starter microorganisms or by the action of natural fermentative microorganisms present in the raw materials or production environment. The activities of the fermenting organisms are dependent on the intrinsic and extrinsic growth factors. Food fermentations frequently involve a complex succession of microorganisms induced by dynamic environmental conditions. A range of properties constitutes an ideal fermentative microorganism (Table 63.3). Of paramount importance is that the organism must be safe for the consumer to ingest even when in large numbers. A variety of groups of microorganisms are frequently used in fermented foods (Table 63.4).

TABLE 63.3
Factors that Contribute to the Suitability of an Organism for Fermentation

Factors
Safe to consume in high numbers
Produces considerable amounts of the required end-product
Does not breakdown the required metabolised end-product
Not too fastidious
Genetically stable
Competitive

TABLE 63.4
Principal Groups of Fermentative Microorganisms

Microbial Group	Product
Lactic Acid Bacteria (LAB)	Lactic acid
Acetic acid bacteria	Acetic acid
Yeasts	Alcohol and carbon dioxide
Moulds	Enzymes
Micrococcus spp.	Proteases
Bacillus spp.	Amylases

Most substrates used for food fermentations are not sterile, therefore, a knowledge of the composition of the natural, heterogeneous microflora of the food substrate is required. In the past starter cultures were not used, the closest procedure was to use one batch of food to inoculate subsequent batches. However, in many traditional approaches, the advantages of some form of inoculation of new batches is recognized and generally practiced by simple techniques like back-slopping or the repeated use of the same container (12).

Problems may arise from natural fermentations as the composition and selectivity of the natural microflora determine which microorganisms dominate the fermentation and this determines the acceptability of the final product. Consequently, natural fermentations have a degree of unpredictability, which may be unsatisfactory when a process is industrialized. Starter cultures are increasingly used to provide a degree of assurance of quality fermentation and to improve the rate at which fermentation is initiated. Failed, poor quality or unsafe products lead to loss of customers and revenue and are to be avoided at all costs.

Modern biotechnology has moved a long way since ancient times when only empirical food fermentations occurred. Starter cultures are now developed mainly by design rather than by screening (13, 14). The composition of starter cultures is based on knowledge of food-grade microbial genetics (15, 16), metabolism and physiology as well as their interactions with foods (16). The overall aim is to produce reproducible, acceptable, good quality, wholesome, easily digestible, low toxicity, safe products by exploiting the properties of the starter cultures (17).

Innovations in starter technology are being made in many areas including probiotics, increased yields and quality of high revenue products and also for development of new fermentation products.

B. Yeasts

Yeasts are widely distributed in nature, with their natural habitats usually being nutritionally rich and high in carbohydrates. They are regularly associated with plant nectars and fruits (18). Consequently, in fermentations they are frequently used for beverages and foods based on fruit or vegetable substrates. Yeasts are ideal agents for fermentation of food products as they are generally acceptable to consumers and are rarely toxic or pathogenic (19). As a consequence of their value they have been extensively studied, and are well classified (20). Separate classification of yeasts and moulds is well accepted and has proved useful. The oldest and still widely used approach to grouping of yeasts is based on morphological, biochemical and physiological characteristics (21).

More recently, progress in molecular biology has enabled yeasts to be characterised at the genomic level using methods based on polymerase chain reaction (PCR) technology (22, 23). Determination of individual genes coding for 18S and 26S ribosomal RNA has resulted in many changes in the identification and classification of yeasts (24). Also rapid techniques such as API kits are being evaluated for their identification (25).

Although there are about 500 species of yeasts, (20), only a few are commonly used in the production of fermented foods. These are all either ascomycetous yeasts or members of the imperfect genus *Candida* (18). The sexual process of ascomycetous yeasts involves the production of haploid ascospores contained within an ascus (ascocarp).

Sacchaharomyces cerevisiae is the most important and frequently used and there are more than 80 different variants of this species available. All strains ferment glucose and many ferment other plant-associated carbohydrates such as sucrose, maltose and raffinose, although none ferment lactose (18). In general, yeasts do not ferment starch in nature.

Yeasts are used to produce ethanol, CO_2, flavour and aroma. The important reaction of yeasts can be represented by the following equations:

- $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$
- glucose $\rightarrow$ ethyl alcohol and carbon dioxide

In addition to alcohol and carbon dioxide various metabolic products including ethyl acetate, fusel alcohols (pentanol, isopentanol and isobutanol) sulphur compounds and leakage of amino acids and nucleotides can all contribute to the fermentation changes induced by yeasts (19). They contain good concentrations of amino acids such as lysine, tryptophan and threonine and are also rich in B-group vitamins.

The technology for the propagation and storage of starter yeasts has developed. Traditionally yeasts were collected from the sediments of beer making. Compressed yeasts were the most common form of starter cultures, this is a compressed mass of dormant cells that must be stored refrigerated. Other more convenient forms are now available such as instant dried yeast which is small beads of freeze dried yeasts. This is sold vacuum packed and can be stored at room temperature for up to two years. Rehydration in water between 30–40°C is required before use, or gas production may be impaired. Protected active dried yeast is the most convenient, it is similar to instant dried yeast, but can be used without any prior rehydration.

TABLE 63.5
Characteristics Common to Lactic Acid Bacteria

Characteristics
Gram positive
Catalase negative
Oxidase negative
Non-sporeforming
Fermentative anaerobes that are aerotolerant
Produce most of their cellular energy from the fermentation of sugars
Produce lactic acid from hexoses

C. Lactic Acid Bacteria

Lactic acid bacteria are a group of bacteria that predominate in many fermented foods. These bacteria are grouped together based on a set of common properties (Table 63.5) (26).

Lactic acid bacteria are subdivided based on their end products from glucose metabolism. Two distinct groups known as homofermenters and heterofermenters are identified. The homofermenters produce lactic acid as virtually the sole product of glucose fermentation. The heterofermenters produce equimolar amounts of lactate, carbon dioxide, and ethanol/acetate from hexoses. Unlike the homofermenters, the heterofermenters are responsible for the production of other compounds involved with flavour, such as acetaldehyde and diacetyl, and other characteristics. The principal genera of lactic acid bacteria are shown in Table 63.6.

Lactic acid bacteria are generally fastidious on artificial media but they grow readily in most food substrates and lower the pH rapidly to a point where other competing organisms are no longer able to grow. Leuconostocs and lactic streptococci generally lower the pH to about 4.0–4.5 and some of the lactobacilli and pediococci to about 3.5 before inhibiting their own growth (27).

In addition to producing lactic acid, the lactobacilli also have the ability to produce hydrogen peroxide through oxidation of reduced nicotinamide adenine dinucleotide

TABLE 63.6
The Predominant Genera of Lactic Acid Bacteria Used in Food Fermentations

Genus	Cell Shape/Grouping	Homofermenter	Heterofermenter
Lactobacillus	Rods — single or chains	+	+
Lactococcus	Oval cocci — pairs or chains	+	−
Leuconostoc	Oval cocci — pairs or chains	−	+
Pediococcus	Cocci — pairs and tetrads	+	−
Streptococcus	Cocci — pairs and chains	+	−
Weissella	Coccoid/short rods — single, pairs or short chains	−−	+
Enterococcus	Cocci — single, pairs or short chains	+	−

Modified from (18, 26).

(NADH) by flavin nucleotides, which react rapidly with gaseous oxygen (28). Flavoproteins such as glucose oxidase also generate hydrogen peroxide and produce an antibiotic effect against other organisms that might cause food spoilage (27). The lactobacilli themselves are relatively resistant to hydrogen peroxide (29). Accumulation of hydrogen peroxide in some fermented foods has been demonstrated, but its effects are likely to be small.

Lactococcus lactis produces the polypeptide antibiotic nisin that is active against Gram-positive organisms including *L. cremoris* which, in turn, produces an antibiotic "diplococcin" active against Gram-positive organisms including *L. lactis.* Thus, these two organisms compete in the fermentation of milk products while inhibiting growth of other Gram-positive bacteria (30). Carbon dioxide produced by heterofermentative lactobacilli also has a preservative effect in foods resulting partially from its flushing action leading to anaerobiosis if the substrate is properly protected (27).

Lactic acid bacteria perform an essential role in the preservation and production of wholesome foods. Examples of lactic acid fermentations include: a) fermented vegetables such as: sauerkraut, pickled cucumbers, radishes, carrots, olives, b) fermented milks such as: yogurt, kefir, cheeses, c) fermented/leavened breads such as sourdough breads and d) fermented sausages (Table 63.1).

In recent years there has been extensive research into the use of lactic acid bacteria to control pathogenic and spoilage microorganisms and to promote health. Consumers are taking a greater interest in the quality of foods and are creating a demand for chemical free, 'natural health' foods. Lactic acid bacteria can inhibit other microorganisms by many methods (Table 63.7) and several applications are already in common use in the food industry, whilst others are the subject of research to establish their efficacy and safety.

Bacteriocins are bactericidal peptides or proteins that are usually active against species closely related to the producing organism (18). There is interest in the use of LAB bacteriocins as food additives to inhibit food-borne pathogens and to act as preservative as they are produced by food-grade organisms and could therefore be classed as "natural" and hence more acceptable. Nisin is a Class I bacteriocin, a lantibiotic that contains the unusual amino acid lanthionine. Nisin is active against most Gram-positive bacteria including *Listeria* and spore-formers such as *Clostridium botulinum.* It is especially useful for inhibiting the outgrowth of spores from heat-resistant spoilage organisms such as *Bacillus* and *Clostridum* spp. in heat-processed foods. It does not inhibit Gram-negative bacteria, yeasts, or filamentous fungi.

TABLE 63.7
Antimicrobial Factors Used by Lactic Acid Bacteria to Inhibit Competing Organisms

Factors
Competitive exclusion
Bacteriocins (184)-nisin, natamycin, lactococcin, pediococcin
Hydrogen peroxide
Carbon dioxide
Ethanol
Diacetyl
Organic acids
Low pH, by production of lactic acid and minor amounts of acetic and formic acid
Low redox potential
Reduced levels of available nutrients (32)

Nisin is the only bacteriocin being used in the food industry (18). It offers processors a "natural" label as well as extending refrigerated shelf life by 14 to 30 days depending on the product. While most bacteriocins are produced only during exponential growth, nisin is produced in large amounts after cells reach their stationary phase, making it appropriate for foods in which lactic acid bacteria are not expected to grow after processing.

Nisin creates pores in the plasma membrane of vegetative cells allowing leakage of intracellular, cytoplasmic components and causes disruption of the transmembrane potential required to drive active transport. In Gram negative bacteria the outer membrane provides protection (18).

TABLE 63.8
Potential Health Benefits of Lactic Acid Bacteria

Benefits
From foods
Improved nutritional value e.g. production of vitamins or essential amino acids
Reduced toxicity e.g. by degradation of noxious compounds
Increased digestibility and assimilability of nutrients (31)
From colonisation
Control of intestinal infections
Improved digestion of lactose
Inhibition of tumor growth
Lowering of serum cholesterol levels
Immune stimulation (185)

Drouault and Corthier (186).

Some Gram negatives become sensitive when their outer membrane has been damaged. Bacterial spores are particularly sensitive, the most important use of nisin has been to inhibit the outgrowth of spores in heat processed foods, particularly processed cheese, canned foods, but also sometimes clotted cream, dairy desserts and pasteurized soups.

There are several potential health or nutritional benefits possible from some species of lactic acid bacteria, although at present many are controversial (31) and are the subject of research to identify specific roles (31, 32, 33). The benefits may arise from the growth and activity of the lactic acid bacteria during food fermentation or during colonisation of the gastrointestinal tract (Table 63.8).

Selection of species with the properties to perform the required functions e.g. preservation, good fermentation properties or health promotion can be made from wild-type species or by genetic manipulation where strains are engineered to produce the required activities.

D. Bacteriophage

Bacteriophage are viruses that are host specific and multiply inside bacterial cells. Although bacteriophage have no direct role in fermentation as they exist as inert particles on food and only replicate inside susceptible host cells, they cannot be ignored when discussing fermentations. Bacteriophage infection can lead to inadequate fermentation and possible safety risks. They have caused major problems in milk fermentations. Several steps can be taken to reduce the risk of phage infection. These include:

1. Using mixed culture starters (34)
2. Rotation of starter culture species with different phage susceptibility (35)
3. Following rigorous hygiene strategies.
4. Propagating starter cultures in phage inhibitory media (18)
5. Inserting a plasmid which confers phage resistance.
6. Using phage-insensitive bacterial strains.

III. FERMENTED PRODUCTS

A. Fermented Fruits and Vegetables

1. Sauerkraut

Sauerkraut is the product resulting from the natural lactic acid fermentation of shredded fresh cabbage to which salt is added at a concentration of 2.25–2.5%. Figure 63.1 shows the main steps in the manufacture of sauerkraut (36). The fermentation yields lactic acid as the major product. The salt extracts liquid from the vegetable which serves as a substrate for growth of lactic acid bacteria. Anaerobic conditions should be maintained to prevent growth of microorganisms that might spoil the sauerkraut (27). Many researches have shown a sequential involvement for different species of lactic acid bacteria (3, 37, 38, 39). It is now well established that *Leuconostoc mesenteroides* grows first producing lactic acid, acetic acid and CO_2. The pH of the product is lowered quickly, thus limiting the activity of undesirable microorganisms and enzymes that might soften the cabbage shreds. The carbon dioxide flushes out residual oxygen making the fermentation anaerobic which stimulates the growth of many lactic acid bacteria. Then *Lactobacillus brevis* grows continuing the acid production. Finally *Lactobacillus plantarum* grows producing more acid and lowering the pH to below 4.0, allowing the cabbage to be preserved for long periods of time under anaerobic conditions.

The end products formed during the fermentation of the cabbage contain significant amounts of lactic acid and a small amount of acetic acid and propionic acid, and a mixture of gases, CO_2 being the most important. Minor end products also appear. Diacetyl and acetaldehyde were reported by Hrdlicka *et al.* (40) as the primary carbonyls produced during kraut fermentation. According to Lee *et al.* (41), the major amount of the volatiles in sauerkraut is accounted for by acetal, isoamyl alcohol, n-hexanol, ethyl lactate, cis-hex-3-ene-1-ol, and allyl isothiocyanate. The predominant volatiles are cis-hex-3-ene-1-ol, and allyl isothiocyanate define the character of sauerkraut, they do not contribute significantly to its quality. Instead, they believe that the fresh, fruity aroma of compounds such as ethyl butyrate, isoamyl acetate, n-hexyl acetate and mesityl oxide probably are more important in determining the acceptability of sauerkraut (41).

Microbial spoilage of sauerkraut can induce changes in texture, color and flavour. Sauerkraut softening occurs when fermentation takes place at too high a temperature, when the cabbage is exposed to air, too little salt is added; or by faulty fermentation when the lactic acid content

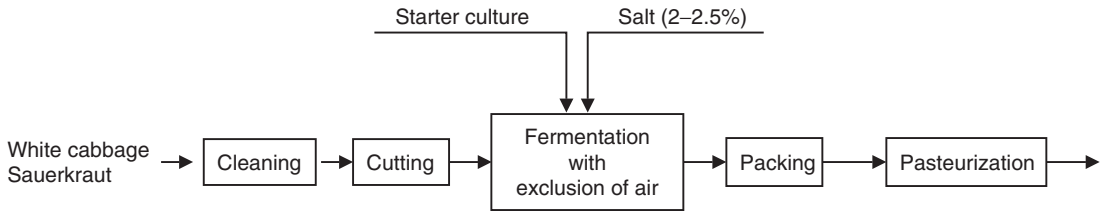


FIGURE 63.1 Flow chart for production of sauerkraut.

remains too low (36). Pink kraut is caused by the surface growth of yeasts mainly of *Torula* spp., especially *T. glutinus* (42). Rotted sauerkraut may be caused by bacteria, molds and/or yeasts.

2. Pickled Cucumbers

Pickled cucumbers are acid fermented in the presence of salt. Cucumbers for pickling are harvested while still immature. The fermentation of cucumbers varies according to the salt concentration used, and two quite different products can be produced, namely high-saltstock (8–10% increasing to 15%) and low-salt dill pickles (3–5% salt containing dill and spices) (43). Usually, the salt solution is poured onto the cucumbers in tanks and then fermentation is allowed to proceed, if necessary, glucose is added to stimulate activity. Fermentation takes place at 18–20°C and yields lactic acid, CO_2, some volatile acids, ethanol and small amounts of various aroma substances (36).

Once the cucumbers have been brined and the tank closed, there is a rapid development of microorganisms in the brine. The normal mixed flora of the cucumbers form the initiating culture. The rapidity of the fermentation is correlated with the concentration of salt in the brine and its temperature. In general, the lower the salt concentration, the more kinds of bacteria will grow at the start, the faster the acid production, and the greater the acidity produced. The lactic acid bacteria chiefly responsible for production of high-salt pickles are initially *Pediococcus cerevisae* followed by the more acid-tolerant *Lactobacillus plantarum* and *Lactobacillus brevis. Leuconostoc mesenteroides* makes little contribution in the high-salt pickles but is active in the low-salt pickles (43). The lactic acid initially formed is later metabolized partly by film yeast or oxidative yeasts that grow on the surface of the brine, thus increasing slightly the original pH value of the fermenting medium (36).

In controlled fermentations the cucumbers are washed and soaked in a chlorinated brine of 25° salinometer. The brine is acidified with acetic acid, buffered with sodium acetate or sodium hydroxide, and inoculated with *L. plantarum* alone or in association with *P. cerevisae* (42, 44). The controlled fermentation reduces economic losses and leads to a more uniform product over a shorter period of time (1).

The two main defects of pickles are bloaters and softening. Pickled cucumbers are often softened due to endogenous or microbial pectolytic enzymes (36). Softening is caused by microorganisms growing inside or on the cucumbers (18). Pectolytic organisms causing pickle softening belong to the genera *Bacillus*, *Fusarium*, *Pencillium*, *Phoma*, *Cladosporium*, *Alternaria*, *Mucor*, *Aspergillus* and others (42). Bloaters are those pickles that float on the brine and are hollow or have large air spaces in the interior. This condition is caused by gas-forming microorganisms, i.e. gaseous fermentation. *Enterobacter* spp., lactobacilli, and pediococci have been implicated as causes of bloaters (42).

3. Olives

Green olives are brined and fermented in a manner similar to cucumbers. Spanish, Greek and Sicilian are the most important types of fermented olives. Before brining, green olives are treated with a 1.25 to 2% lye solution (sodium hydroxide), depending on the type of olives usually at 21 to 25°C for 4 to 7 hours. This treatment is necessary to remove some of the oleuropein, a bitter principal in the olives. Some microorganisms may be destroyed during lye treatment. After treatment, the lye is removed by soaking and washing the olives in fresh water. Excessive washing may induce a loss in some nutrients and a low acidity development due to a high loss of carbohydrates. To overcome these problems inoculation with *Lactobacillus plantarum* may be necessary to neutralize any residual lye. Adding sugar to the brined olives for acid production has also been suggested. Washed olives are then placed in a brine solution, the concentration of which varies from 5 to 15% salt, depending upon the variety and size of olives (3). Ten to 15% is recommended for Manzanillo olives, whereas a 5 to 6.25% is recommended for Sevillano olives. Additional salt is added during fermentation of olives in order to maintain a constant salt concentration, usually at 28° to 30° Salinometer level. The microbiology of the olive lactic acid fermentation is complex with a number of microbial strains being involved. Vaughn *et al.* (45) have divided the normal olive fermentation into three stages. The initial stage is the most important from the standpoint of potential spoilage if the brines are not acidified. Acidification eliminates the original contaminating population of

dangerous Gram-negative and Gram-positive spoilage bacteria and, at the same time provides an optimum pH for activity of the lactic acid bacteria (46). The natural flora of green olives, consisting of a variety of bacteria, yeasts, and molds carries out the fermentation with the lactic acid bacteria becoming prominent during the intermediate stage. *Leuconostoc mesenteroides* and *Pediococcus cerevisiae* are the first lactics to prominate, followed by lactobacilli, mainly *Lactobacillus plantarum* and *Lactobacillus brevis* (47). The fermentation takes as long as 6 to 18 months, and the acidity of the final product varies from 0.18 to 1.27% (48). A level of at least 0.6 to 0.7% acid is needed for proper preservation and flavour of the product.

For the fermentation of black lactic-fermented olives, the ripe, violet to black fruit is washed and directly allowed to undergo spontaneous lactic fermentation in an 8–10% salt solution. No alkali treatment is performed on black olives. Lactobacilli and yeasts are involved, but the yeasts normally dominate. The final product has a pH value of 4.5–4.8 and contains 0.1–0.6% lactic acid (49).

Olives may undergo spoilage owing to the growth of undesirable microorganisms. Zapateria spoilage is characterized by a malodorous fermentation, due apparently to the production of propionic acid by *Propionibacterium* (50). Sloughing spoilage is a defect that includes severe softening, skin rupture and flesh sloughing. It is caused by Gram negative pectinolytic bacteria, especially *Cellulomonas flavigena* (51). Softening of olives is caused by the pink yeasts, *Rhodotorula*, and fermenting pectinolytic yeasts, *Saccharomyces* and *Hansenula* (52, 53).

4. Cocoa Fermentation

Cacao beans grow on the *Theobroma cacao* tree. They can be processed to make cocoa and chocolate. The popularity of cocoa is not its nutritional value but rather its unique flavour, colour and aroma. To obtain these sensory characteristics the beans must be fermented, as roasting unfermented dried cacao does not result in the desired flavour, aroma or colour (54, 55, 56). To induce fermentation the pods are opened following harvesting to reveal the seeds, called beans, covered with pulp. The beans are heaped into piles or placed into boxes on the farm. Fermentation methods vary between different countries and even between different processors (57). The fermentation is natural; therefore, the microbial species present also differ between batches and different geographical locations. Occasionally a more controlled situation is required and starter cultures are used, however, these generally do not compare favorably with the natural fermentations (57).

The pulp is predominantly water with 10–15% sugars and has a low pH, between pH 3.6–4.0 (58). The sterile pulp is inoculated with a variety of microorganisms during handling and storage which perform the fermentation. The beans are fermented for 2–12 days (42, 54). During this time the sticky pulp becomes a turbid broth from which the beans absorb flavours (57, 59). Yeasts and acetic acid bacteria are the most important fermenters of cacao beans.

Fermentation takes place in two stages; firstly the sugars in the acidic pulp are converted to alcohol, which is subsequently oxidised to acetic acid. Yeasts ferment the sugars to ethyl alcohol in the anaerobic piles. *Candida krusei*, *Saccharomyces* spp., *Geotrichium candidum*, *Hansenula anomala* and *Schizosaccharomyces pombe* are amongst the most frequently isolated species (42, 54, 60, 61). In addition the yeasts hydrolyze the pectin that covers the beans (57). Without pectin, the bitter alkaloids may leach out of the bean or be altered by alcohol that can now enter the beans (54). Although yeasts have an important role in producing alcohol, in this fermentation their presence appears even more essential to the development of the final desirable chocolate flavour (42). It has been observed that the endoenzymes released by autolysing yeasts and the levels of reducing sugars and free amino acids have some role in the development of chocolate precursor compounds (42, 62).

As the microorganisms ferment the sugars they produce heat and high temperatures, up to 45–50°C develop (54). As the temperature rises and alcohol accumulates the proportion of yeasts falls rapidly. Lactic acid bacteria such as species of *Lactobacillus*, in particular *L. plantarum*, and *Streptococcus* spp. start to grow, although their numbers and activity is thought be subordinate to that of the acetic acid bacteria (59, 61, 63). In some countries the levels of lactic acid bacteria are higher and their activity is thought to account for the acidity of cocoa from these areas (63).

The pulp is stirred and drained which increases the level of aeration (57). The presence of oxygen and the low pH favor the growth of acetic acid bacteria, including *Acetobacter* spp. (42, 62, 64). The acetic acid makes the bean tegument permeable to the yeast enzymes.

If the fermentation is prolonged microbial growth may start on the beans instead of just the pulp. Off-tastes result when *Bacillus* and filamentous fungi, including *Aspergillus*, *Penicillium*, and *Mucor*, hydrolyze lipids in the beans to produce short-chain fatty acids which cause rancid tastes (54). As the pH approaches pH 7, *Pseudomonas*, *Enterobacter*, or *Escherichia* spp. may grow and produce off-tastes and odors.

When fermentation is complete the beans are sun- or air-dried to reduce the water content to below 7.5%. As they dry moulds including *Geotrichium* grow and may oxidize lactic acid to acetic and succinic acid. The beans are then roasted at 121°C to obtain the characteristic smell and flavour of chocolate. Roasting kills most of these microbes although some species of *Bacillus* may survive (54).

Microorganisms are also used in the production of finished chocolate products. Alpha amylase from *Aspergillus* is used to hydrolyze starch for chocolate syrup and invertase from *Saccharomyces* to hydrolyze sucrose in filling mixtures to make soft-centered chocolates.

Additionally, chocolate is tested for the following specific bacteria: *Staphylococcus aureus*, coliforms, and *Salmonella.* Several outbreaks of salmonellosis have been traced to chocolate that was contaminated after roasting (62, 65).

B. Dairy Products

1. Yogurt

Yogurt is a fermented milk product produced with a yogurt starter culture consisting of *Streptococcus thermophilus* and *L. dulbrueckii* subsp *bulgariecus* in a 1:1 ratio. The symbiotic growth of the two organisms results in lactic acid production at a rate greater than that produced by either when growing alone, as well as the production of various metabolites. Carbonyl compounds have been the subject of intensive research in recent years, because of their possible role in flavour balance or in flavour defects in dairy products (66). Yogurt is prepared using either whole or skim milk, to which approximately 5% by weight of milk solids or condensed milk is usually added. The concentrated milk is heated to 82–93°C for 30–60 min and cooled to around 45°C. The yogurt starter is then added at a level of around 2% by volume and incubated at 45°C for 3–5 h, followed by cooling to 5°C (23). For optimum shelf life at refrigeration temperature the product should have a titratable acidity around 0.85–0.90%. The symbiotic association of the *streptococci* and the *lactobacilli* have been reported by many workers. *Streptococcus thermophilus* produces lactic acid, formic acid, and carbon dioxide. Formic acid stimulates the growth of the *lactobacilli*. The latter exhibit proteolytic activity and liberates some amino acids used by the *streptococci* for their growth. The streptococci tend to be inhibited at pH values of 4.2–4.4, whereas the lactobacilli can tolerate pHs in the 3.5–3.8 range. The lactic acid of yogurt is produced more from the glucose moiety of lactose than the galactose moiety (67).

Substances that contribute to flavour are particularly significant in the preparation of natural yogurt. Various researchers have shown that strains of *Streptococcus thermophilus* and *L. delbrueckii* subsp *bulgaricus* produce different amounts of carbonyl compounds, in particular acetaldehyde, when grown in skimmed cow's milk (68, 69, 70). More acetaldehyde is produced by *L. bulgaricus* when growing in association with *S. thermophilus* (71). Acetaldehyde is the compound that contributes mostly to the typical flavour of yogurt, while acetoin, diacetyl and ehanol are produced in lower concentrations (66). Yogurt flavour continuously changes during manufacture and storage. Flavor changes may vary depending on the cultures, mix formulation, and incubation and storage conditions (72, 73).

The selection of the appropriate starter culture is of great importance in yogurt manufacture. The type of yogurt starter used can change the physical characteristics of the final yogurt product, thus altering both its texture and appearance. For example, "ropy" and the "non ropy" starters are used for the manufacture of "stirred" and "set" types of yogurt respectively. Ropy cultures comprise *Streptococcus salivarius* ssp *thermophilus* and *Lactobacillus* strains capable of producing extracellular polysaccharides during fermentation (74). These starter cultures are used to enhance the viscosity of stirred yogurt.

2. Kefir

Kefir is produced by the fermentation of milk with a mixture of lactic acid bacteria, yeasts and other bacteria. The final product is acidic, slightly alcoholic, liquid to semiliquid and effervescent, and is consumed as a beverage (75, 76).

Kefir is prepared by the use of kefir grains, which contain Lactic acid bacteria and lactose-fermenting yeasts held together by layers of coagulated protein. The yeast flora of kefir varies with its source of production, but mixtures of lactose and non-lactose fermenting species, identified as *Klyveromyces marscianus*, *Candida kefir*, *C. pseudotropicalis*, *Saccharomyces. cerevisae*, *S. exiguus* and *Torulopsis holmii* have been reported (76, 77, 78). The essential bacterial component in the grain is *L. brevis* (79, 80). Kefir fermentation requires a moderate room temperature (17 to 23°C). Acid production is controlled by the bacteria, and the yeast produces alcohol. The final concentration of lactic acid and alcohol may be as high as 1%.

3. Koumiss

Koumiss is similar to kefir except that mare's milk is used, and the alcohol content may reach 2%. The primary fermenting microorganisms are *L. bulgaricus* and Torula yeasts (81). Fermentation produces mainly lactic acid, ethanol, and carbon dioxide. These products give koumiss its effervescence and sour, alcoholic flavour.

4. Acidophilus Milk

Acidophilus milk is produced by the inoculation of a strain of *Lactobacillus acidophilus* into sterile milk. The inoculum of 1–2% is added, followed by holding of the product at 37°C until a smooth curd develops. Acidophilus milk contains from 1.5 to 2.0% acid (as lactic) and no alcohol (81).

5. Cheese

Cheese is commonly made from cow, ewe, goat, or buffalo milk. Less commonly used is milk from mare, reindeer, camel, and yak. The majority of cheeses are made from heat-treated or pasteurized milk. Research groups in many countries have extensively investigated the subject of cheese making, and in-depth information has been

reported, for example, by Kosikowski (82), Scott (83), and Fox *et al*. (84).

Cheese making is initiated by a process of destabilization of casein micelles followed by the formation of a protein network and sequential expression of whey caused by a spontaneous process termed syneresis, which is often complemented by application of external pressure. All modern cheeses, with the exception of whey cheeses, are made using variations of the same basic process: acidification; coagulation into curds and whey; curd drainage/syneresis; shaping of the coagulum; and salting. In case of ripened cheeses, the curd is allowed to ripen under conditions appropriate to the cheese in question. Slight variations of these and the use of different milks combine to generate the huge range of cheeses available today. There are over 400 varieties of cheeses representing fewer than 20 distinct types, and these are grouped or classified according to texture or moisture content, whether ripened or unripened, and if ripened, whether by bacteria or molds (85). Table 63.9 shows the classification of cheeses based on moisture content, with subdivision depending on the milk type and the role of microorganisms in cheese ripening.

In general, the process of manufacture starts with the preparation of milk. Although it is not compulsory, a heat treatment equivalent to pasteurization is usually applied at the start of the processing. The milk is then cooled to the fermentation temperature, which depends on the type of cheese to be manufactured, 29–31°C for Cheddar, Stilton, Gouda, Camembert and Leicester; higher temperatures are employed in the manufacture of high scalded cheeses such as Emmental, Gruyere and Italian cheeses. Milk is inoculated with an appropriate lactic starter. The starter culture produces lactic acid, which, with added rennin, gives rise to curd formation. The starter organisms most used for cheese production are mesophilic starters, strains of *Lactococcus lactis* and its subspecies. Thermophilic starters such as *Lactobacillus helveticus*, *Lb*. Casei, *Lb. Lactis*, *Lb. Delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are used in the production of cheeses where a higher incubation temperature is employed. Propionic bacteria, molds, such as *Penicillium camemberti, P. candidum*, *P. roqueforti*; red- or yellow-smearing cultures, such as *Bacterium linens* are also added, depending on the type of cheese to be manufactured. The time of renneting and the amount added differ with cheese type. Rennet is a preparation from the fourth stomach or abomasom of suckling calves, lambs or goats. Recently, due to world shortage of calf rennet, recombinant or genetically engineered pure chymosin derived from different microorganisms is available on the market, and is currently used by many cheesemakers in different countries. Microbial rennets are produced from fungi such as *Mucor miehei*, *Mucor pusillus*, and *Endothia parasitica*. Genes from chymosin have been cloned into a number of organisms such as the bacterium *E. coli* and yeasts to produce nature-identical chymosin.

TABLE 63.9
Cheese Varieties and Their Classification

Moisture Content	
50–80%	SOFT CHEESES
	Unripened
	Cottage, Quark, Cream, Neufchatel
	Ripened by surface molds, mainly
	Camembert, Brie
	Salt-cured or pickled
	Feta, Domiati
45–50%	SEMI SOFT/SEMI HARD CHEESES
	Ripened principally by internal mould growth
	Roquefort, Stilton, Gorgonzola, Danish blue
	Ripened by bacteria and surface microorganisms
	Limburger, Brick, Trappist, Port du Salut
	Ripened primarily by bacteria
	Bel Paesa, Pasta Filata, Provolone, Brick, Gouda, Edam
<39%	HARD CHEESES
	Without eyes, ripened by bacteria
	Cheddar, Caciocavallo, parmesan, Romano
	With eyes, ripened by bacteria
	Emmental, Gruyère
<34%	VERY HARD CHEESES
	Asiago old, Parmesan, Romano, Grana

After coagulation of the milk, the curd is cut into small cubes for whey expulsion. The curd is further shrunk by heating it and then pressed to expel more whey, followed by salting. Finally the cheese is ripened under conditions appropriate to the cheese in question.

Cheese ripening involves a complex series of chemical and biochemical reactions. Proteolysis and lipolysis are two primary processes in cheese ripening with a variety of chemical, physical, and microbiological changes occurring under controlled environmental conditions (86, 87). Several workers have indicated the importance of free fatty acid levels on the flavours of different types of cheeses (88, 89, 90). Free fatty acids form during ripening and are precursors for methyl ketones, alkanes, lactones, and aliphatic and aromatic esters, all of which are characteristic aroma components in cheese (91). Moreover, short chain fatty acids contribute directly to aroma in many aged cheeses (92, 93, 94). During ripening, a part of the casein is converted by proteolysis into water-soluble nitrogenous compounds, such as peptides and amino acids, which contribute to the flavour and texture (86). Specifically, flavour development has been associated with an increase in total amino acids (95, 96). Particular amino acids, glutamine, methionine, and leucine have been found to influence cheese flavour (96, 97).

According to Fox *et al.* (98) probably five agents are involved in the ripening of cheese: (1) rennet or rennet

substitute (i.e. chymosin, pepsin or microbial proteinases); (2) indigenous milk enzymes, which are particularly important in raw milk cheeses; (3) starter bacteria and their enzymes, which are released after the cells have died and lysed; (4) enzymes from secondary starters (e.g. Propionic acid bacteria, *Brevibacterium linens*, yeasts and molds, such as *Penicillium roqueforti* and *P. candidum*) are of major importance in some varieties; (5) non-starter bacteria, i.e. organisms that either survive pasteurization of the cheese milk or gain access to the pasteurized milk or curd during manufacture; after death these cells lyse and release enzymes.

Although most ripened cheeses are the products of metabolic activities of the lactic acid bacteria, several known cheeses owe their particular character to other related organisms. In the case of Swiss cheese, *Propionibacterium shermanii* is added to the lactic bacteria organisms *Lb. bulgaricus* and *S. thermophilus*. Propionibacteria contribute to the typical flavour and texture of Swiss-type cheese (99). The lipolytic and proteolytic activities of molds play an important role in the maturation of some cheeses. In blue cheese such as Roquefort and Stilton *Penicillium roqueforti* grows throughout the cheese and impart the blue-veined appearance characteristic of this type of cheese. *P. camemberti* is associated with surface-ripened soft cheeses such as Camembert and Brie.

C. Fermented Soybean Products

The use of various bacteria, yeasts, and fungi to make fermented foods from soybeans or a mixture from soybeans and cereals, such as rice or wheat, has been known in the orient for centuries. A variety of fermented soybean foods, including tempeh, sufu, miso, shoyu are produced in the Orient. It is worth noting that fungi are the organisms most frequently used to process soybeans.

1. Soy Sauce

Soy sauce is a dark brown liquid food condiment that is used to add flavour and color to the normally bland Oriental diet including rice, raw fish, beancurd, fermented beans, and boiled vegetables. Figure 63.2 illustrates the different steps for soy sauce manufacture. Soy sauce fermentation is a two stage process; the first is aerobic and the second anaerobic. The first stage, the koji, consists in growing *Aspergillus oryzae* or *A. soyae* on either soybeans or a mixture of soybeans and wheat. The mixture is allowed to stand for 3 days. The mold grows and produces enzymes mainly amylases and proteases that break down, respectively, the carbohydrates and the proteins present in the substrate. This results in the production of large amounts of fermentable sugars, peptides, and amino acids. The second stage, the moromi, consists of adding the fungal-covered material to a 17–19% sodium chloride solution and incubating it for at least 1 year at ambient temperature to give the product a good quality. The high salt concentration ensures the development of flavour enhancing yeasts (100). During the first stage of the fermentation, salt-tolerant homofermentative lactic acid bacteria principally *Pediococcus cerevisae* and *Lactobacillus delbrueckii*, lower the pH to 4.9 (101). One of the most important characteristics of the lactic acid bacteria *P. cerevisae* is the ability to form aroma and flavours essential for soy sauce. In the later stages, salt tolerant yeasts primarily *Saccharomyces rouxii* ferment parts of the sugar to produce ethanol and various aroma compounds (102). Other yeasts such as *Hansenula* and *Torulopsis* may also be involved. Yeasts are also responsible for the production of furfual, an important flavour compound (103). In the final stage of the fermentation, the pH is lowered to about 4.5-4.8. At this stage the fermented mash is filtered and the liquid obtained is soy sauce. The liquid soy sauce is pasteurized at 70–80°C to help remove heat-coagulable material as well as preserve the product.

2. Tempeh

Tempeh is a protein-rich meat substitute produced in Indonesia and Africa by overgrowing soaked, dehulled, partially cooked beans with *Rhizopus oligosporus* or related species such as *R. oryzae* or *R. arrhizus* (27). The general principle of tempeh manufacture starts with the cleaning and overnight soaking of beans for hydratation and to remove the hulls. During this initial soaking acid fermentation lowers the pH to 5.0 or below, which is inhibitory to many organisms but not to the mold. Dehulled beans are cooked in boiling water and spread on a bamboo tray to cool and surface dry. Surface drying inhibits growth of bacteria that might spoil the product (7). They are then inoculated with tempeh from a previous fermentation and wrapped in banana leaves or perforated plastic. The wrapped packages are kept at room temperature for 1 to 2 days during which mold growth occurs and knit the cotyledons into a compact cake — the tempeh. During the fermentation, proteins are partially hydrolyzed, the lipids are hydrolyzed to their constituent fatty acids, stachyose is reduced (decreasing flatulence in the consumer), riboflavin nearly doubles, niacin increases sevenfold and vitamin B-12 is synthesized (7, 27, 104).

D. Fermented Grain/Cereals

1. Beer

Beer is a product of fermentation of barley malt and other grain starch by yeasts. Beer making involves several steps. The first step is to make malt (105). Barley is first allowed to germinate by soaking in water for 5 to 7 days. During this time germinated grains produce amylases and

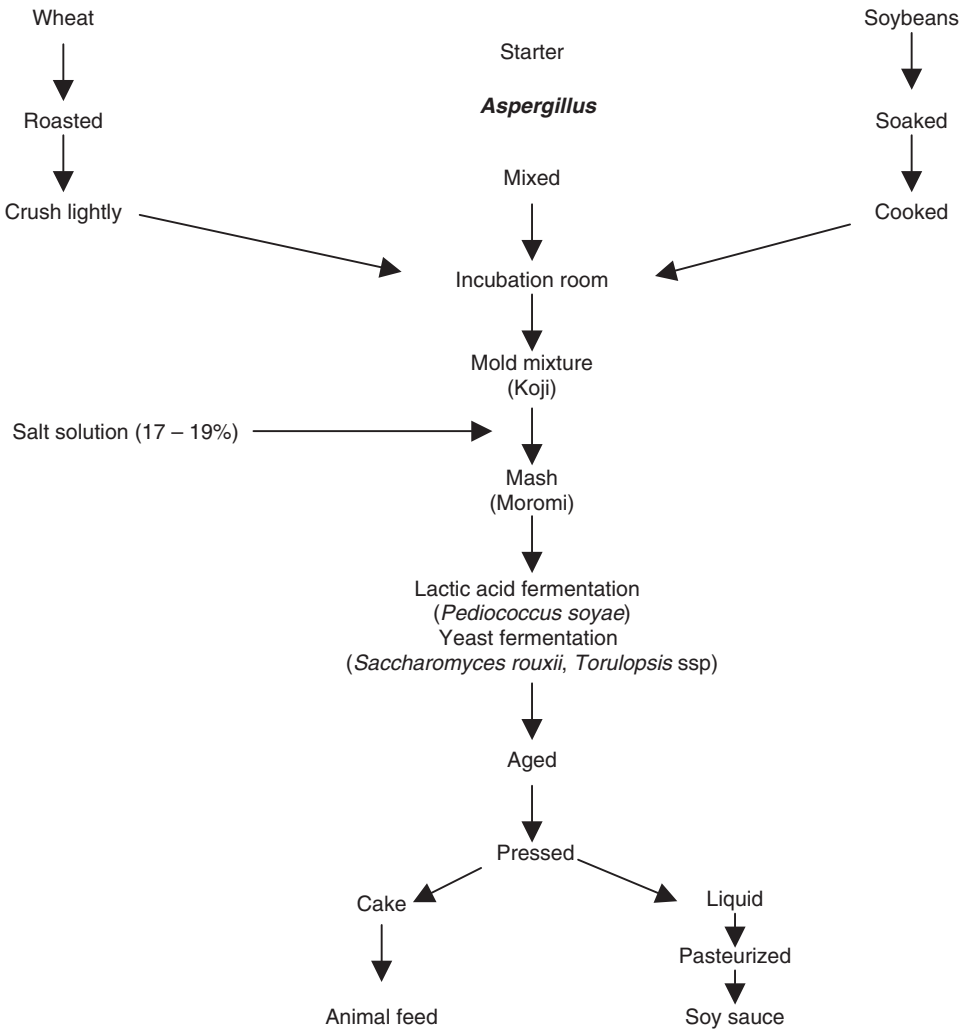


FIGURE 63.2 Flow sheet for the manufacture of soy sauce.

proteases. These two enzymes are essential to the brewing process. Amylases degrade starch to glucose, a sugar needed for the yeast fermentation, and proteases solubilize compounds in the grain and hops important for the quality of beer. After germination, heat is applied to stop the sprouting process and to dry the grain. The dried and crushed malt is suspended in water and mixed with boiled malt adjuncts, such as ground rice and corn. Adjuncts are low in enzyme activity, hence their use may necessitate the addition of microbial preparations with α-amylase and proteinase activities. The addition of amylase is important in order to assure complete hydrolysis of starch.

The mash is then incubated at 65–70°C for a short time to allow the amylase to degrade the starch to glucose. The temperature is raised to 75°C to inactivate the enzymes and then allowed to settle. Insoluble matter sinks to the bottom and serves as a filter as the liquid, called wort, is taken from the container.

Here, hops or hop extracts are then added to the wort. Hops are a very important ingredient in beer production. Their function is to (i) act as a clarifier, since they precipitate the proteins in wort, (ii) change the wort character to give a specific aroma and bitter taste and, (iii) to contribute to the stability of beer because of their antibiotic properties, which act together with ethanol and carbon dioxide. In addition, the protein content of hops enhances the foam-building ability of beer (36). The mixture is then boiled for 1.5–2.5 h to obtain the correct delicate hop flavour (106, 107). Boiling with hops serves several purposes: concentration, sterilization by killing many spoilage microorganisms, further inactivation of enzymes in the mash and solubilization of important compounds in the hops and mash. The color and roasted aroma of the malt are formed during kilning by Maillard reactions. The roasting temperature is important in the development of the color of the beer. The malt is heated to the roasting temperature for 4–5 hours at 80°C for light malt or at 105°C for dark malt (36). Some of these add to the flavour of the beer while others, especially from the hops, have antiseptic qualities and help preserve the beer. The wort is then

separated, cooled and fermented. Water is a key ingredient of beer and its quality has a significant effect upon the final product. The calcium content is important as it can precipitate as calcium phosphate lowering the pH of the wort to around 5.4 which is close to the optimum for some of the malt enzymes (18).

There are two major types of beer separated by their fermentation characteristics; lagers and ales, both of which can be either light or dark in appearance. Lagers are produced using pure cultures of *Saccharomyces carlsbergensis* which is a yeast that flocculates to the bottom. Lager fermentation temperature is generally low, in the range 8–12°C and consequently takes longer than that of ale (18). Most beer produced is of the lager variety. Ale is produced mainly in Great Britain and Continental Europe using *Saccharomyces cerevisiae* which flocculates to the top and is recovered by skimming and reused after each batch. Fermentation temperature is generally warm, 18–25°C. A huge variety of beers exist. Changes in any of the process conditions can result in vastly different beer styles. Beers can be classified by region and original gravity (OG). OG refers to the amount of sugar present prior to pitching (yeast inoculation) and determines the final beer alcohol content.

Fermentation begins by adding the yeast *Sacchromyces cerevisae* to the wort. Ale results from the activities of top-fermenting yeasts, which depress the pH to around 3.8 whereas bottom-fermenting yeasts, *S. carlsbergensis* strains, give rise to lager with pH values of 4.1–4.2. A top fermentation is completed in 5–7 days; a bottom fermentation is completed in 7–12 days (1, 42). Fermentation results in the conversion of glucose to ethanol and CO_2. Other compounds in the wort are also fermented to add to the characteristic flavour of beer.

The fermented wort, called green beer, is aged at 0°C for a period of weeks or months depending on the brewer. At this time the yeast settle to the bottom of the vessel, bitter flavours are mellowed and other compounds are formed that enhance flavour.

The beer is then filtered to remove yeast cells. The beer is finished by addition of CO_2 to a final content of 0.45 to 0.52% CO_2. The pasteurization of beer at 60°C or higher, may be carried out for the purpose of destroying spoilage microorganisms (42).

There are a number of factors that protect beer from the growth of contaminating microorganisms. These include low pH and redox potential, the isohumulones of hops that inhibit Gram-positive bacteria and the alcohol produced by the yeast (44). The usual spoilage agents are acetic acid bacteria, lactic acid bacteria and wild yeasts. The industrial spoilage of beers is commonly referred to as beer infections (42). Four types of spoilage defects have been identified: ropiness, sarsinae sickness, sourness, and turbidity. Ropiness is caused by *Acetobacter*, *Lactobacillus*, *Pediococcus cerevisiae*, and *Gluconobacter oxydans* (108, 109, 110). Ropiness is characterized by an increase in the viscosity of the beer, which pours as an "oily stream" (42). Sarcinae sickness produces a honey like odor as a result of diacetyl production by the spoilage organism *P. cerevisiae* in combination with the normal of odor of beer (42). Sourness in beer is caused by increased levels of acetic acid, as a result of oxidation of ethanol by the action of the acetic acid bacteria *Acetobacter* and *Gluconobacter* (44). *Zymomonas anaerobia* and several other yeasts such as Saccharomyces spp. can cause turbidity and off-odors in beer.

2. Bread

Bread is one of the most common, convenient and inexpensive foods, with leavened forms now the most popular in many countries although flat breads remain very common in India and the Middle East (111). Bread has made a major contribution to the diets of many countries for centuries (111). Bread is basically made from flour or meal by moistening, kneading and baking. Bread has always been made from flour or meal from a number of grains and seeds. However, only flours from wheat and to a certain extent rye are able to produce dough that is able to hold sufficient leavening gases to produce loaves with good volume and a fine, soft cellular structure (5, 112). The characteristics of bread made from wheat flour are directly related to the presence and properties of gluten which is the hydrated protein of wheat. Gluten can form elastic, extensible films within dough which enable it to stretch and retain gas (111, 113, 114). The gas content comes from air incorporated during mixing and CO_2 from yeast fermentation of sugars. Most breadmaking processes involve modifications of the natural properties of the gluten which leads to improved gas retention and a thinner dough.

Although, many microorganisms ferment sugars with the release of CO_2, *Saccharomyces cerevisiae*, or baker's yeast is the best adapted for leavening bakery products (5). A number of advantages are gained by using yeast fermentation for bread making:

- Yeasts produce a delicious flavour and an appealing aroma
- Gas evolution can take place over long periods of time
- Yeast activity helps to ripen and mellow the gluten in the dough enabling it to expand and retain accumulating gases
- The product is viewed as more natural by the consumer
- Yeasts often are easier and more economical to use

Although some yeasts can hydrolyse starch present in flour, *S. cerevisiae* cannot. Amylases naturally present in the flour, or added hydrolyze the starch to maltose making

it available to the action of *S. cerevisiae* enzymes. Sucrose is sometimes added to give the bread a sweat taste. Yeast invertase enzymes convert added sucrose to the monosaccharides glucose and fructose. Glucose is then metabolised by the yeast cells to CO_2 and ethanol. It takes approximately 4 hours for the yeasts to replicate in dough so little reproduction occurs during production of bakery products. Also, the level of ethanol produced is quite low and this evaporates during baking.

Protease enzymes from the yeasts are also active in the dough, they change the soluble flour proteins into simpler forms which can pass through the semipermeable membrane of the yeast for use in replication. There is very little oxygen within the dough so most of the processes take place anaerobically.

Bread is a perishable product with a relatively short shelf-life. Moulds, particularly *Rhizopus stolonifer* and *Nerospora sitophila* are the main spoilage agents due to the low water activity (115, 116) Yeasts have been shown to be responsible for the defect known as "chalky bread" (21). Calcium propionate, up to a level of about 3000 ppm, is permitted as a preservation agent in bread (117). Ascorbic acid is also frequently used for the same purpose. Although, they have been associated with a number of *Bacillus subtilis* outbreaks, baked goods such as bread have an excellent reputation for safety (7), *B. subtilis* is responsible for a defect known as ropey bread where spores, naturally contaminating the flour survive the baking (118, 119), germinate and grow degrading the loaf's internal structure and producing a sticky slime (120). This defect is not always dramatic enough to prevent people eating the bread.

a. Sourdough

Sourdough starters and bread have existed for thousands of years (121); however, the term sourdough originally referred to specific bread making starter cultures that were carried by settlers in the San Francisco area. Prized cultures were handed down from generation to generation. The term is now used for any sour bread.

Sourdough bakery products have remained popular due to their superior sensory qualities and extended shelf life (122, 123). These properties are achieved by combining the metabolic activity of both LAB and yeasts during the sourdough fermentation. In general, the yeasts are responsible for leavening the bread and the bacteria for the souring. However, the bacteria do produce some carbon dioxide from sugars in the flour which helps the bread rise. The sourness of the product depends on many factors including temperature, fermentation time, type of grains, (wheat or rye are most frequently used), amount of water and primarily the strains of yeast and *Lactobacillus* in the starter culture (124). Lactic and acetic acids produced by LAB contribute to the rich complexity of bread flavours. Flavor compounds are formed by the activity of endogenous cereal enzymes, microbial metabolism, and the baking process. LAB and yeasts also form a range of desirable aroma volatiles (125, 126, 127).

Traditionally sourdough fermentations use a natural starter culture of wild yeast and bacteria. The culture is continuously propagated and is frequently maintained by constant back-slopping from one fermentation to the next (128). Several varieties of yeast and *Lactobacillus* have been identified in sourdoughs from around the world. The ratio of yeast to bacteria in San Francisco sourdough cultures is about 1:100. The dominant yeast strain was classified as *Saccharomyces exiguous* called *Torulopsis holmii*s, (129) later reclassified as *Candida milleri*, (postulated to be reclassified as *Candida humilis*). The most common LAB in sourdough fermentations are *Lactobacillus* spp. including *L. sanfranciscensis* (also referred to as *L. sanfrancisco*) (130) *L. reuteri*, *L. brevis* and *L. pontis.*

Fermentation conditions must encourage high levels of metabolic activity by both yeasts and LAB for a stable symbiotic relationship to occur that can exclude other microorganisms. The two organisms do not compete for the same carbon source, degradation of starch by amylases in the dough provides maltose which is utilized by the lactobacilli (131). *Candida milleri* cannot metabolise maltose but catabolises the other sugars present. During the assimilation of maltose by the lactobacilli the activity of maltose phosphorylase releases glucose which stimulates the yeast (132). In turn, the nutritional requirements of the lactobacilli are complex, they require a number of amino acids and fatty acids which may be derived from dead yeast cells (133, 134). *Candida milleri* has a tolerance to the acid environment produced by the lactobacilli (135). The pH of sourdoughs ranges from pH 3.8–4.5 (23).

Mould growth is the most frequent cause of bread spoilage. The LAB produce antimould compounds. *L. sanfranciscensis* has a broad spectrum of inhibitory activity against a range of moulds related to bread spoilage such as *Fusarium*, *Penicillium* and *Aspergillus*. A combination of organic acids including acetic, caproic, formic, propionic, butyric and n-valeric cause the antimould activity with caproic acid playing a major role (136). The lactobacilli also secrete an antimicrobial cycloheximide which kills many organisms in the dough. *Candida milleri* is resistant to cycloheximide.

E. Fermented Grains or Fruits

1. Wine

Wine is a beverage obtained by full or partial alcoholic fermentation of fresh, crushed grapes or grape juice (must), with an aging process. However, it is also produced from the fermentation of berries, fruit, honey, palm juice, rhubarb, and dandelion. The major grape cultivars used to produce wine belong to *Vitis vinifera*, *L.* ssp. *vinifera* (36).

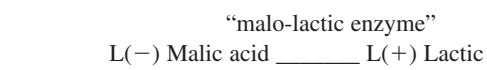
Grapes are either wine-type grapes, for white or red wine making, or table grapes. The cultivars are different in sugar content and aroma. The quality of the grape is influenced by some factors such as climate, soil conditions, and variety of grape (137).

Grape clusters cut from vine stocks are cleaned of rotten and dried berries and then separated from the stems using a roller crusher. The crushed grapes are then subjected to pressing to release juice, the must. The remaining grape skins and seeds (pomace) are loosened or shaken-up and pressed again to provide the second or post extract. The pomace is then removed. In red wine making the must is fermented together with skin in order to extract the red pigments, which are released only during fermentation. The extraction of the red pigments is sometimes facilitated by raising the temperature to 50°C prior to fermentation of the mash, or to 30°C after the main fermentation, followed by a short additional fermentation.

The fresh sweet must is treated with sulfur dioxide to suppress the growth of undesirable microorganisms and to stabilize wine color. Due to health problems caused by the consumption of sulfites, there is a desire to eliminate this additive. Some strains of *S. cerevisiae* can stabilize wine color, apparently by producing sulfite. One strain produced 30 to 80 mg/L (ppm) (138).

The must is then inoculated with a suitable wine yeast strain of *Saccharomyces cerevisiae var. ellipsoides* or *pastorianus*. The must is then fermented for up to 21 days at temperatures 12–14°C for white wines and 20–40°C for red wines (36). Ethanol production could reach 14–18%. For red wine, the must is allowed to ferment until the correct amount of color is extracted from the skin. Following this initial fermentation, the wine is racked to get rid of the sediment. The wine is pumped into barrels, vats, or tanks for aging. The clearing and the development of flavours occur during this stage. Various compounds, such as esters, are formed and contribute to the bouquet and aroma of the wine. In general the wine is removed from vats after 3–9 months and poured into bottles in which aging continues. The duration of aging and stability differ and depend on wine quality (36).

A fermentation called malolactic fermentation can be achieved to reduce the acidity and mellows the wine. Malic and tartaric acids are two of the predominant organic acids in grape must and wine. They account for about 90% of the acidity in grapes (44). The malolactic fermentation can be carried out by many lactic acid bacteria, mainly of the genera, *Lactobacillus*, *Leuconostoc* (*L. oenos*), and *Pediococcus* (*P. cerevisiae*). This fermentation usually follows the alcoholic fermentation and degrades malic acid to lactic acid and CO_2:



The monocarboxylic lactic acid is not as acidic as the dicarboxylic malic acid. Hence, the acidity of the wine is reduced (36).

Not all lactic acid bacteria can tolerate the alcohol and low pH required to convert malic acid to lactic acid. The bacterium *Oenococcus* is an acidophile that can grow in grape must and wine at pH 3.5–3.8, and actually prefers an initial growth pH of 4.8 (139).

Wine can be subjected to some defects. Spoilage is evident by appearance, odor, and flavour changes. Spoilage may be due to molds (140), lactic acid bacteria (141), acetic acid bacteria (142, 143) and chemical reactions (144). Browning of wine is a chemical defect caused by oxidative reactions of phenolic compounds, which in red wines, may result in complete flocculation of the color pigments. Yeast spoilage is induced by species of the genera *Candida*, *Pischia* and *Hansenula*. Other microorganisms are involved in the formation of viscous, moldy and ropy wine flavour defects. Bacterial spoilage may involve acetic acid and lactic acid bacteria. In this case vinegar or lactic acid souring is detectable. The most serious and the most common disease of table wines is referred to as "tourne disease" (42). Tourne disease is caused by a facultative anaerobe or an anaerobe that utilizes sugars and seems to prefer conditions of low alcoholic content. This type of spoilage is characterized by an increased volatile acidity, a silky type of cloudiness, and later in the course of spoilage, a "mousey" odor or taste.

2. Distilled Spirits

Distilled spirits are alcoholic products that result from the distillation of yeast fermentations of grain, grain products, molasses, or fruit and fruit products. Whiskeys, gin, vodka, rum, and liqueurs are examples of distilled spirits. Although the process for producing most products of these types is quite similar to that for beers, the content of alcohol in the final products is considerably higher than for beers.

a. Whiskey

Whiskey is an alcoholic distillate made from fermented mash of grain. Rye whiskey may be made from rye and barley malt. At least 51% of the mash must be rye. For bourbon (corn), the mash must contain more than 51% corn. For example, a typical mash could contain 70% corn, 15% rye and 15% malt. The cereals are first ground, mixed with acidified water, and made into an uniform mash by starch gelatinization. A sour wort is maintained to keep down undesirable organisms, the souring occurring naturally or by the addition of acid. The mash is generally soured by inoculating with a homolactic such as *L. delbrueckii*, which is capable of lowering the pH to around 3.8 in 6–10 h (3). Saccaharification is then accomplished by incorporating 15% kiln-dried malt in a pre-mashing vat and stirring constantly at 56°C. Saccharification proceeds rapidly

through the action of malt diastase enzymes. The enzymes are inactivated by heating the mash to 62°C, followed by cooling to 19–23°C. The cooled mash is then pitched with a suitable strain of *Saccharomyces cerevisiae* for the production of ethanol. When fermentation is completed, the liquid is distilled to obtain higher alcohol levels. Whiskey is aged in white oak barrels. This process increases the solids, esters, acids, fusel oil, aldehydes, furfural and color (1).

b. Rum

Rum is an alcoholic distillate made from the fermented juice of sugar cane, sugar cane syrup, sugar cane molasses or other sugar cane by-products. The alcoholic content is less than 80%. Strains of *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* are used in the fermentation. Phosphorous and a nitrogen source are added to black-strap molasses (12–14% sugar) or a similar carbohydrate material. The pH is adjusted to 4.0–4.7 with H_2SO_4. This is done to speed up the fermentation and produce a light-flavoured rum. The fermentation is usually run at 21°–35°C for 3 to 7 days. The mash is then distilled to increase the alcohol content of the beverage. Rum is normally aged in charred white oak barrels. The flavour, color, and aroma develop during aging due to an increase in esters, organic acids, and solids (1).

3. Vinegar

Vinegar is a clear, sour tasting liquid that may be colourless, may take the colour of the raw substrate or be coloured by the addition of caramel (144). Vinegars are classified according to the raw material from which they are made e.g. grapes are the substrate for wine vinegar (7). Basically, vinegar can be produced from any food that can be fermented by yeasts to ethyl alcohol. Frequently used substrates include fruits, honey, coconut, malt and cereal grains (44). Distilled grain or spirit vinegar is made from an alcoholic solution that has been distilled.

The common types of vinegar in a region often reflect the local alcoholic beverage, e.g., rice vinegar is popular in Japan, wine vinegar in France and malt vinegar in the UK (18, 145). This suggests that vinegar was discovered as a consequence of defects occurring during alcoholic beverage production. In fact the term vinegar comes from the French for "sour wine", vin aigre (18). The extensive history of vinegar is confirmed by reference to it in ancient texts (146).

Vinegar is frequently used as a condiment, but is also an important preservative and flavouring agent in products such as pickled vegetables, sauces and dressings. Higher strength vinegars are frequently used for pickling (7). Some types of vinegar, e.g., komesu, which is made from polished rice, are used as health drinks (145). Increasing consumer interest in health has made any health benefits of traditional vinegar a matter of interest (7, 145, 146).

Vinegar is the product of a two-stage fermentation:

1. Yeasts convert sugars to ethanol anerobically, an alcoholic fermentation**.** The alcoholic vinegar stock is produced using essentially the same processes to those used to make alcoholic beverages. Alcoholic fermentation should be complete before vinegar production occurs since acetic acid and the environmental conditions inhibit further alcohol production.
2. Ethanol is oxidised to acetic (ethanoic acid) aerobically by bacteria of the genera *Acetobacter* and *Gluconobacter*. In a reaction known as acetification, this is a common reaction in the spoilage of alcoholic drinks. Acetification can be represented by the reaction

$$C_2H_5OH + O_2 = CH_3COOH + H_2O$$

The minimum acceptable acetic acid content of vinegar is between 4–6% w/v (40 and 60 g/l) and the pH value should fall between pH 2.0 and pH 3.5 (18, 144).

There are various systems for converting the alcoholic vinegar stock to vinegar. All the methods provide a means of effectively combining alcohol, air, and acetic acid bacteria. Acetic acid bacteria gain energy during the oxidation of ethanol. As members of the genera *Acetobacter* and *Gluconobacter* are strict aerobes techniques for acetification have been developed to maximize the available oxygen supply to the bacteria. Increasing the available oxygen is not only desirable in that it increases the metabolic rate of the bacteria and hence the rate of vinegar production, it is also essential. An interruption in the oxygen supply, even for a short period of time will result in death of the bacteria and failure of the process (18).

Acetobacter are better acid producers and are more commonly used in commercial vinegar production. However, they can oxidise acetic acid to CO_2 and H_2O (over-oxidation) which is not a problem with *Gluconobacter* (146). Ethanol represses over-oxidation. Consequently, to reduce the risk of over oxidation during acidification a residual level of ethanol is always maintained. This is easier to achieve using semi-continuous methods where samples of fresh alcoholic vinegar stock are added into residual amounts of finished vinegar (18, 144, 147).

Three main techniques are used in vinegar production, namely surface culture, the trickling method (quick vinegar process) and submerged fermentation (18, 146, 147). Static, surface fermentation is the oldest method and is still employed in making traditional vinegars. The Orleans method is one of the oldest, static, surface fermentation methods. In the Orleans process a large wooden barrel is laid on its side and partially filled with vinegar stock. Air holes situated just above the level of the stock increase the level of available oxygen Figure 63.3. The inlets to the

barrels are covered with muslin or similar material to prevent insects entering, but to allow free flow of air.

A surface culture of acetic acid bacteria forms at the interface between the acetifying medium and the air, forming a bacterial 'mat' (Figure 63.3). The barrel is left undisturbed until the acidity reaches the required level, then point about two-thirds of the volume of the vinegar is removed through a port near the bottom of the barrel (Figure 63.3). New stock is added through a tube to the bottom of the mix so that the film of bacteria remains undisturbed. The residual volume charges the next batch and the process restarts. It usually takes about 14 days for one acidification cycle (18) but the same barrel may be used continuously for extended time periods. The surface film of acetic acid bacteria has oxygen readily available and consequently is active. Whilst succeeding batches of vinegar will proceed if this mat is broken, they will start and proceed more rapidly if the layer remains intact. The optimum temperature for this conversion is about 29°C.

The advantages of this technique are that it is simple and does not require expensive plant investment, also the quality of the product in terms of both aroma and flavour is excellent. The rather long time required to complete the fermentation makes the Orleans technique less attractive for mass production and only a small proportion of vinegar is now produced by this method.

The "quick vinegar process" or "trickling method" gets its names from the faster rate of vinegar production or from the fact that the alcoholic stock trickles down over the bacteria. This process employs a larger area of bacterial film in conjunction with improved oxygen transfer to the acetifying stock. The acetifying stock is sprayed onto the surface of packing material and slowly trickles down against a counter current of air (Figure 63.4). The packing material is loosely packed and lignocellulosic in nature, e.g., birch, vine, rattan, corn cobs, beachwood shavings. Films of active bacteria develop throughout the packaging material greatly increasing the surface area of bacteria that the stock comes into contact with. Making the acetator larger also increases the distance that the stock travels through the packaging and the time of contact between the bacteria and the vinegar stock. The process is operated semi-continuously; samples are collected at the bottom of the equipment and recirculated until the required acidity is achieved (Figure 63.4). When the vinegar is harvested most of the bacteria are retained in biofilms in the packaging material. The process time is about 4–5 days in a well-controlled system (18, 147).

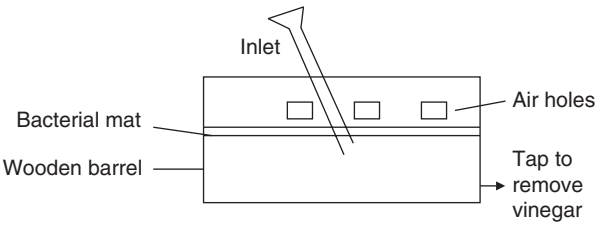


FIGURE 63.3 Schematic diagram of the Orleans process for acetification.

The latest advance in vinegar technology is the use of submerged fermentation, which began around 1952. In this system the entire volume of vinegar stock is oxygenated by tiny bubbles of air that continually sparge through the culture (Figure 63.5). This enables the acetic acid bacteria to grow and metabolise suspended within the volume of the stock. The culture is mixed to ensure homogeneity. Semi-continuous runs are used. The most commercially successful system is the Frings Acetator (18, 144, 146).

Due to the sensitivity of the bacteria, submerged cultures require continuous monitoring. Bacteriophage infection can also result in fermentation failures. The enhanced efficiency of these systems can reduce the time for conversion of alcoholic vinegar stock to vinegar to 24–48 h (18, 144, 146).

Many of the acetobacter species are difficult to culture on conventional media, however they have been extensively studied. Genes have been characterized which encode indispensable components of the acetic acid fermentation, such as alcohol dehydrogenase and aldehyde dehydrogenase. In addition, spheroplast fusion of the *Acetobacter* strains has been applied to improve their properties for use in vinegar production (148, 149).

F. Fermented Proteins

Initially, the main purpose for fermenting meat and fish was to extend the shelf-life of these highly valued, perishable

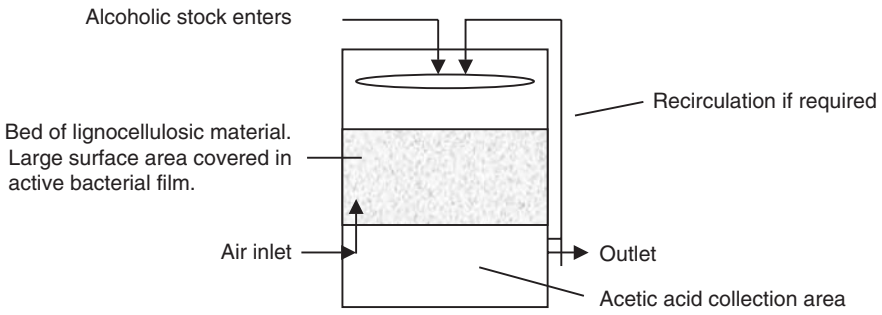


FIGURE 63.4 Schematic diagram of the quick method for acetification.

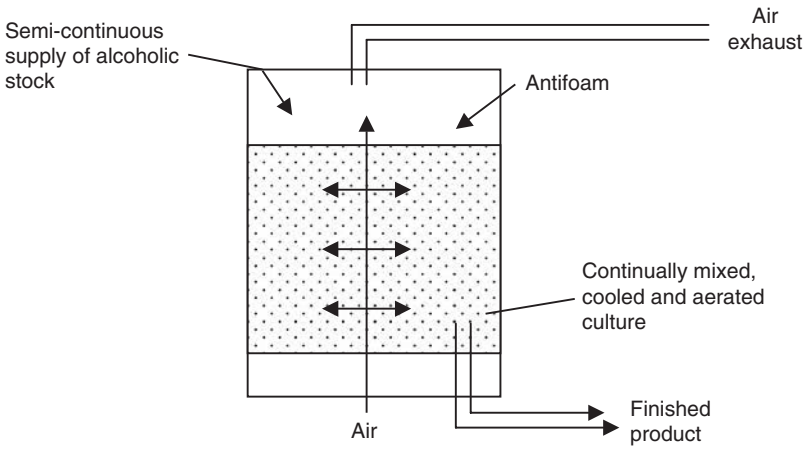


FIGURE 63.5 Schematic diagram of the submerged process for acetification in vinegar production.

foods. Sausages became the most popular method of preserving meat with a host of different recipes and techniques developing around the world. Although many other preservation methods are now available the production of fermented sausages and fermented fish continues, as they are highly valued for their enhanced flavours.

1. Fermented Sausages

The characteristic properties of fermented sausages are partially or completely dependent on the fermentative activity of bacteria. Sausage processing may also include curing, smoking, drying and ageing. The two principle groups of fermented sausages are semi-dry and dry (Table 63.10). The meat used in semi-dry sausages is often finely chopped resulting in spreadable products.

Fermented sausages are prepared by mixing ground meat with various combinations of spices, flavourings, salt, sugar, additives and frequently bacterial cultures. The levels and types of spices and flavouring agents influence the flavour and odour of the final product (150). It is not possible to cover all the types and potential variants of fermented sausages, but the typical ingredients of European sausages are:

Lean meat	55–70%
Fat	25–40%
Curing salts	3%
Fermentable carbohydrates	0.4–2.0%

Additional ingredients include, spices, flavourings, acidulant, colouring etc. (18).

The quality of the meat is critical to both the safety and sensory properties of the sausage. Most production methods do not heat the meat before processing as this leads to defects in the texture of the sausage (18). Also, fermented sausages are frequently eaten without cooking. The hygienic properties of the raw meat influence the production of biogenic amines (tyramine, cadaverine, putrescine; aromatic amines such as histamine, phenylethylamine, or tryptamine; polyamines, e.g. spermine and spermidine) (151) By using appropriate conditions to favor starter development and the use of the raw materials with good hygienic quality it is possible to produce fermented sausages nearly free of biogenic amines (152). Spices can be heavily contaminated with microorganisms, which makes contamination control of spices critical. Sometimes spices are sterilized by radiation.

TABLE 63.10
Properties of Semi-Dry and Dry Sausages

Property	Semi-Dry Sausages	Dry Sausages
Starter culture	Frequently used	Less successful than with semi-dried
Flavour	Tangy, predominantly from fermentation	From fermentation, and biochemical and physical changes during drying and aging
Processing time	Usually <several days	May be up to 90 days
Approx. water content	35–50%	<35%
Examples	Lebanon bologna	Pepperoni, Lola sausages, Genoa
	Mortadella	Salami

The physical characteristics of the meat and fat particles affect drying. The size of the meat particles must encourage efficient release of moisture and the cut edges must not be smeared with fat.

Sausages were traditionally produced in sections of gastrointestinal tract and then regenerated animal hides as they provided the required range of properties (Table 63.11) (18). When the casings are filled air should be excluded as this will lead to discolouration of the meat and reduce the shelf life of the sausage.

The mixed ingredients are allowed to ferment at different temperatures for varying periods of time. Natural fermentations are still used, but more often starter cultures

TABLE 63.11
Properties Required from Sausage Casing Material

Permeable to moisture and smoke
Acceptable to the consumer
Shrink as the meat is dried
Retain the sausage mixture
Form the required sausage shape

are added. The principal components of starter cultures are lactic acid bacteria and nitrate reducing bacteria and the high salt tolerant yeast *Debaryomyces hansenii* (18, 153). *Lactobacillus* spp. and *Pediococcus acidilactici* are primarily responsible for converting sugars into lactic acid thereby lowering the pH of the meat product. Whilst species such as *Micrococcus varians* and *Staphylococcus carnosus* are important for conversion of nitrates to nitrates (18, 154). A major role for nitrates is to control *C. Botulinum*. Lactobacilli with or without micrococci are components of starter cultures used to slow fermentations whereas pediococci with or without micrococci are used for rapid fermentation. *Pediococci* spp. do not occur in fresh meat at high enough levels to be significant in traditional slow fermentations and therefore are only important in meat product fermentation when added in starter cultures.

Following fermentation, the product may be smoked and/or dried under controlled conditions of temperature and relative humidity, further developing the flavour and odour of the final product. Several chemical changes occur during the ripening of dry-fermented sausages including 1) acidification of the sugars by lactic acid bacteria 2) reduction of nitrates and nitrites to nitric oxide by micrococci 3) degradation of the proteins and lipid.

Superficial mold growth can occur on unsmoked sausages which has been shown to significantly improve the sensory properties, including the flavour, of sausages at the end of ripening by altering the levels of amino and free fatty acids, and volatile compounds (155). The fungi should be atoxigenic and have proteolytic, lipolytic and antioxidative activity (155).

Microbial growth and the activity of endogenous meat enzymes are only partially responsible for the development of flavour and odour compounds (90). Lipid autooxidation reactions also have an important fuction in sausage ripening. Research has focused on the breakdown of triglycerides into free fatty acids, diglycerides, and monoglycerides during ripening. Carbonyl compounds probably play a significant role in determining flavour (90). Protein breakdown to peptides and amino acids is involved in microbial and chemical reactions that generate many flavour compounds (90). Also smoking sausages provides distinctive flavours e.g. in salami and hot dogs by accumulation of phenolic componds from the wood smoke (18).

A number of parameters contribute to the final microbial safety of sausages (Table 63.12). The extent of drying

TABLE 63.12
Parameters Contributing to the Microbial Safety of Sausages

Parameter	Comments
Phenolic componds	From smoking, have important antioxidant and antimicrobial properties
Acidity	Approximately 2.5% w/w as lactic acid
Salt	Approximately 3% NaCl and curing salts
pH	4.9–6.0
Various antimicrobial compounds	E.g. hydrogen peroxide
Drying	Reduces the water activity
Spices	Stimulate LAB and inhibit normal spoilage microflora
Low availability of oxygen	Oxygen reduced by growth of LAB

Adams and Moss (18); Adams and Nout (153).

varies; the weight loss in less shelf-stable varieties like Bologna is about 20–30%, and reaches 50% in the more stable salamis (156). The lowered water activity inhibits the proteolytic spoilage Gram negatives and allows the proportion of LAB to increase. LAB growth results in a decrease in pH and available oxygen, these inhibitory factors help control both Gram negative and Gram positive pathogens that are commonly present on the raw meats. When the growth of aerobic spoilage bacteria is inhibited, lactic acid bacteria may become the dominant component of the microbial flora (157). The safety and stability of fermented sausages depends upon fermentation caused by lactic acid bacteria (157).

A combination of antimicrobial hurdles (Table 63.7) in fermented sausages usually provides a shelf-stable, safe product. However, outbreaks of disease following ingestion of fermented sausages caused by *Staphylococcus aureus*, *Salmonella*, and verotoxigenic *E. coli*, and more dramatically, *C. Botulinum*. Salmonellae have been shown to survive during the production of pepperoni. Their persistence was lowest in sausages fermented with a lactic acid starter (158). Although the counts of *Salmonella* and other Enterobacteriaceae have been shown to decline during fermentation and drying we can not rely on this being sufficient to eliminate these organisms, again emphasising the need for high-quality meat and the fermentation process to be controlled (18, 159).

To minimize the risk from outgrowth of *C. botulinum* spores and toxin development in the shelf-stable cured product nitrite/nitrate salts should be added. However, the level should not interfere with the process of fermentation.

2. Fermented Fish

Fermented fish products fall into two major groups, sauces and pastes; they are most popular in Asia. In this region of the world, fish provides a major contribution to the human diet. They are often used as condiments much like salt in

Western cooking and soy sauce in Chinese cooking. They are often the most important flavouring ingredient of the diet. They can be added to soups, curries, salads, rice etc.

Sauces and pastes are primarily made from whole or eviscerated small schooling fish that are found in abundance and that would otherwise have little value for consumption, many of which are anchovies or related species. Larger varieties of fish, such as mackerel and sardines, also make good fish sauce, but because they are relatively more expensive due to their value as a food fish, they are seldom used in the commercial production of fish sauce. Fresh water fish, fish roe and shrimps are also used to a lesser extent.

Fish sauce and paste are considerably different products, but are produced by similar processes. The fish variety, fermentation conditions, cure duration and technique all affect the texture, amino acid content and volatile flavour profile of the finished product. Good-quality fish sauce imparts a distinct aroma and flavour. Products where less extensive autolysis of the fish occurs are classified as fish pastes (18). Some example of fermented fish sauces and pastes are shown in Table 63.13.

To obtain products with a pleasant, fragrant aroma and taste, the fish must be very fresh. Fresh whole fish are rinsed and drained, then mixed with salt usually in an approximate ratio of three parts fish to one part salt. The salted fish are packed into concrete tanks or earthenware jars, and are frequently weighted down to prevent them from floating as liquid accumulates inside the tank as a consequence of autolysis. The tanks are sealed and left undisturbed for at least six months and possibly up to eighteen months. To maintain a relatively high temperature the tanks are either placed in sunshine or buried in the ground.

The amount of salt added is sufficient to produce a water activity value below 0.75 (18) inhibiting most microbiological activity and spoilage, with the possible exception of growth of anaerobic extreme halophiles. The higher the concentration of salt, the slower the production rate of fermented fish products, but the longer the shelf life of the product (153, 160).

During "fermentation" the fish protein undergoes hydrolysis by autolytic enzymes (e.g. gastric juices), which are naturally present, and becomes liquefied. This process releases free amino acids and creates volatile flavour components. At the end of the liquefaction process the liquid is harvested and filtered to remove any sediment. The filtrate is transferred to clean jars and ripened in the sun for a couple of months; this allows the strong fish odors to dissipate. The fish sauce is then ready for use. Good fish sauce is clear without any sediment, brown in color, has a pleasant aroma of the sea, not an overpowering fishy smell, and should not be overly salty. Fish sauce is high in protein (up to 10%), and contains all the essential amino acids. It also provides a rich supply of B vitamins, especially B 12, pantothenic acid, riboflavin and niacin. Other beneficial nutrients include calcium, phosphorous, iodine and iron. Low numbers of streptococci, micrococci, staphylococci and *Bacillus* spp. have been isolated from fish sauces and associated with the development of flavour and aroma (161).

Fermented fish paste is prepared from salted fish with or without flavouring ingredients which is allowed to ferment or ripen. It has a smoother consistency and lower moisture content than sauces. Fat is often added to the final product so that it is spreadable and can be used for example as a sandwich spread.

The carbohydrate content of fish is low, usually less than 1%; therefore, for lactic fermentation of fish to occur an additional source of carbohydrate is required. A range of fish/carbohydrate/salt products are available, although they tend to be less popular than the traditionally made and time-tested fish sauces and pastes. Garlic and rice are often used as carbohydrate sources, and inulin is the fermentable carbohydrate in garlic (162, 163).

The higher the level of supplemented carbohydrate the faster the fermentation. Using this technique the product is often ready after only a few weeks, enabling the production of large quantities of product in a shorter time period. Fermentation of the carbohydrate leads to a product with an acidic flavour. Natural fish sauce production is a lengthy process and substantial investment is necessary for large-scale production. This has resulted in the search for more rapid, cost-effective techniques. These have included adding enzymes or acid to accelerate the fermentation.

The high salt content and consequent reduced water activity of fish sauces and pastes usually guarantees microbial safety of the products however fermented fish products have been implicated in outbreaks of botulism (164, 165). Other potential risks of fermented fish products are disease from products contaminated with *L. monocytogenes* (166) and cancer (167, 168).

TABLE 63.13
Examples of Fish Sauces and Fish Pastes

Country of Origin	Fish Sauce	Fish Paste
Cambodia and Vietnam	*Nuoc-mam*	*Ngapi*
Burma	*Ngapi*	*Ngam-pya-ye*
Philippines	*Patis*	*Bagoog*
Thailand	*Nampla*	*Kapi*
Indonesia	*Ketjap-ikan*	*Bladchan*

IV. OTHER MICROBIAL PROCESSES

A. Treatment of Industrial Wastewaters

The treatment of industrial wastewater should be considered as part of the total effort towards conservation of the quality of the natural environment.

The wastewater to be treated varies depending on the type of the industry. It can include floating matter; sand

and grit; oil and grease; toxic elements; biological pollution; chemical pollution; and thermal pollution.

The treatment process required will depend on the composition of the wastewater and the required effluent quality. The main constituents in the wastewater from food processing are animal or vegetable fat, protein and carbohydrates. These can normally be removed by traditional biological wastewater treatment, but it can sometimes be advantageous or even necessary to take special precautions, such as adjusting the nutrient balance, pH etc. (169).

The choice of method to tackle a specific industrial wastewater problem will be based on experience with similar wastewater, analysis of the actual situation and, if it is unusual process, on testing. Testing can determine whether the wastewater will settle or whether it is biologically degradable (170). In principle four types of treatment can be distinguished:

- Mechanical treatment system
- Biological treatment system
- Physico-chemical treatment system
- Combinations of the above three systems

As this chapter deals with microbiology, we will look only at the biological treatment system. For more detail on all the systems the reader is referred to Droste (171).

1. Biological Wastewater Treatment

This is primarily used to remove dissolved and colloidal organic matter in a wastewater. Some suspended organics will also be metabolized and because of the natural flocculation and settling characteristics of the biomass formed in biological treatment, the biomass along with other suspended matter can be removed in a sedimentation.

2. Aerobic Biological Treatment

In aerobic treatment, microorganisms which need oxygen are used to convert the wastewater compound. In this process, air (oxygen) is supplied to microorganisms that are in contact with the wastewater. The microorganisms metabolize the organic material into carbon dioxide and other end products and new biomass. Several types of aerobic systems are possible for the aerobic biological removal of dissolved and suspended organics. These are the activated sludge, the trickling filters, the lagoons, and the tower technology.

The common activated sludge process is a suspended growth process where the microorganisms are mixed with the wastewater. Air is pumped into a basin to supply oxygen and mixing. Pure oxygen may be supplied as an alternative to air but supplemental mixing is required.

Some processes such as trickling filters use a solid support media to provide surfaces on which bacteria grow and accumulate. In this system wastewater is sprayed over a rock or synthetic media bed. Wastewater is applied on a continuous or an intermittent basis. Bacteria attach and grow on the media. Natural air currents supply oxygen.

A recent development is the use of tower reactors. The microorganisms can be dosed in flocculated form (comparable to activated sludge systems) or attached to grains. The main advantages of theses processes are: saving of space, more efficient use of oxygen, and cheaper off-gas treatment.

Processes may also be designed to oxidize nitrogen or remove nitrogen from the wastewater.

3. Anaerobic Biological Treatment

Anaerobic treatment or anaerobic digestion processes are biological degradation processes in the absence of oxygen. Instead of oxygen other electron-acceptors are used. High-strength wastewaters are amenable to treatment by anaerobic process. Industrial and agricultural wastewaters are often highly concentrated. Anaerobic treatment occurs in enclosed reactors to prevent access of oxygen. Biodegradable substances in wastewater are converted by anaerobic bacteria that are in contact with the wastewater into biomass and methane. Thus, anaerobic treatment is a process which not only removes pollution from wastewater but also generates a by-product, methane gas, which is rich in energy and therefore valuable. As in aerobic biological treatment operations, some processes incorporate solid support media to the microorganisms (fixed film process), whereas others keep the microorganisms in suspension. The biological solids produced in an anaerobic process must be settled in a clarifier that may follow the process or be incorporated into the anaerobic reactor.

B. Microbes as Foods

1. Single Cell Protein

In most fermentation production of biomass is incidental; however, there has been considerable interest in the use of microbial biomass as a food or feed supplement to meet the expanding demands for foods. Algae, bacteria, yeasts and fungi have been considered as protein sources. The food value of the cells is dependent upon their protein and amino acid composition and their lipid, vitamin and nucleic acid content (172). Many attempts have been made to manufacture foods that are protein rich as cheaper alternatives to meat. Microorganisms can grow on waste materials that contain appropriate nutrients (e.g. paper, whey, wood, sugar-refining waste, ethanol and paraffins) (173). The protein-rich cellular microbial mass is commonly known as “single cell protein.” It is often odorless and tasteless and can therefore be used in a wide variety of foods.

2. "Quorn" Mycoprotein

The production of mycoprotein represents an economical means of converting any surplus carbohydrate into foods of much higher nutritional and commercial value. From the many studies into single cell proteins one that has obtained commercial success is Quorn which is mycoprotein, the consumption of which is widespread and increasing (174). Cleverly anticipated consumer demand for healthy, vegetarian, convenience foods led to the development of Quorn. Quorn is the processed cellular mass obtained by continuous, aerobic cultivation of the filamentous fungi *Fusarium venenatum* under steady-state conditions. The taxonomic status of this organism has recently been reevaluated. This organism was previously classified as *Fusarium graminearum* (174, 175, 176).

Before Quorn could be marketed, an evaluation of its safety was required (177). Growth conditions that were not conducive to mycotoxin production were determined. *F. venenatum* is grown at a high rate without any nutritional limitations. The culture is supplied with a nutritionally balanced chemically defined fermentation medium containing easily metabolizable nutrients, including glucose as a carbon source, growth factors and ammonium salts (18, 177). Studies have shown the product to be free from mycotoxins that could have posed a major risk to human health (174).

The biomass removed from the fermenter is rapidly heated by injection of steam to kill the cells. The cellular mass is subsequently harvested by centrifugation. The combination of rapid heating and centrifugation reduces the content of RNA in the cellular mass.

To be acceptable for human consumption the RNA content must be reduced. RNA constitutes about 10% of the mycoprotein (18, 177) which is high when compared to traditional sources of protein, such as meat, fish, or soybeans (178, 179). Ingested RNA is ultimately broken down to purines and pyrimidines, and purines are subsequently converted to uric acid. Elevated levels of uric acid can increase the risk of developing gout and kidney stones in susceptible individuals. The RNA content is reduced to below 2% of the dry weight of the mycoprotein (177). The processed cellular mass is subjected to numerous tests to demonstrate compliance with specifications.

Quorn can be prepared following a variety of recipes. Its texture and "mouth feel" are much like chicken, the mycelium is bound together with egg which does not come unglued in cooking (as do some soy products) and flavoring ingredients are added. The filamentous nature of the cells gives the cellular mass a meat-like texture that makes it suitable for a variety of applications in food including use as a muscle fiber replacer in meat-alternative products, a fat replacer in certain dairy products and as a cereal substitute in products such as breakfast cereals or puffed snacks (177). A range of commercially available products are available based on Quorn, these include meat-free burgers and fillets and prepared meals (e.g., stir-fries, curries, and pasta dishes in which mycoprotein is the central component).

Quorn is low in fat, cholesterol-free, and a good source of protein (about 44%, (18)) and dietary fiber (177). The high fiber content of Quorn derives from the cell wall constituents e.g. chitin and beta-glucans. There are indications that the fiber content may also act as a prebiotic in the lower gut (177).The fat content of mycoprotein typically ranges from 12 to 14% dry weight and is more like vegetable fat than animal fat having a low proportion of saturated fatty acids and a high proportion of mono- and polyunsaturated fatty acids (177).

There was concern that inhalation or ingestion of mycoproteins could lead to adverse reactions in allergenic individuals (180). So far studies have given reassuring, largely negative results although this area is still a matter for concern and study (180, 181). The trouble-free history of Quorn in Europe suggests that the levels of adverse reactions are no higher that for other foods.

C. Microbial Products

1. Microbial Metabolites Used in the Food Industry

Biocatalysis is an important part of fermentation. Research and development of microbial products is becoming increasingly important. Enzymes enable reactions to occur under milder conditions, making them particularly beneficial to the food industry. Some microorganisms do not participate in fermentations, but are used to produce metabolites. Vitamins and amino acids increase the nutrient content, whilst gums and dextrins improve the physical characteristics, and enzymes provide a range of functions including improved flavours (182, 183). E.g., the amino acid, glutamic acid is used in the production of monosodium glutamate, which is a flavour enhancer in many foods (44). Microbial gums are used in the food industry for thickening, emulsion stabilization and rheological modifications. One gum that has wide commercial use is xanthan gum, which is produced by *Xanthomonas* spp. as an extracellular polysaccharide coat surrounding the cell wall (44). Yeasts and bacteria are an excellent source of group B vitamins. Autolysed yeast extract can also be used as a flavouring agent in many foods such as soups, sauces, gravies, snack foods, canned meats (173).

In some cases enzymes can be used more effectively than live microbial cells. When a series of reactions or several types of reactions are desired, such as in wine production, the intact microorganism is the system of choice, but if only one reaction is needed, separated enzymes may be beneficial.

Since microorganisms can be grown in large amounts in controlled conditions they offer an unlimited source of many

TABLE 63.14
Examples of Microbial Enzymes/Metabolites Used in the Food Industry

Enzymes/Metabolites	Commercial Source	Use in the Food Industry
Amylases	*Aspergillus* *Rhizopus* *Bacillus*	• Help prevent the staling of bread. • Important in the colour and texture of bread • Manufacture of syrup for use in confections, • Clarification of fruit juices.
Catalase	*Aspergillus niger Micrococcus lysodeikticus*	• Added to milk for cold sterilisation, • Removes excess hydrogen peroxide.
Cellulase	*Aspergillus, Micrococcus*	• Removes cellulose cloud, clarifies citrus juices • Improves the body of beer • Extraction of flavour compounds (such as essential oils) • Increases the digestibility and nutritive value of plant products.
β galactosidase	Many microbial sources including *Aspergillus niger Aspergillus oryzae.*	• Removal of lactose from milk. • Improves the solubility of dried milk and the consistency of concentrated milk, ice cream bases, and frozen milk. • Lower calorie products. • Sweet whey production for yeast cultivation.
Acidulants	*Aspergillus niger*	• Acidify the environment
Colours — β-Carotene	*Dunaliella salina*	• Food coloring
Thickeners — Glycan	*Saccharomyces cerevisiae*	• Viscosity changes
Vitamins — ascorbic acid	*Acetobactersuboxydans*	• Improve nutritional value
Lipases	Many organisms *Aspergillus niger, Aspergillus oryzae Mucor, Rhizopus, Pseudomonas*	• Develops flavour in cheese. • Treatment of milk fat in the production of ice-cream, butter or margarine.
Pectic enzymes	*Aspergillus niger* *Rhizopus oryzae*	• Clarifying agents. • Improve pressability, color extraction, filtration speed and yield of free run juice. • Removal of the gelatinous coating from coffee beans for fermentations.
Proteases	*Aspergillus oryzae, Bacillus subtillis Streptomyces griseus*	• Chillproofing of beer. • Meat tenderization. • Mellowing of dough in bakery products. • Production of fish protein solubles.
Rennets	*Mucor*	• Cheese making.

Adapted from (44).

enzyme systems and metabolites, only a limited number have wide commercial use. Some examples are shown in Table 63.14.

Microorganisms synthesize enzymes internally, although some may be secreted into the surrounding substrate. Extracellular enzymes are easier to obtain (98). To obtain intracellular enzymes it is necessary to break the cell wall and membrane.

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64 Fermented Products: Role of Starter Cultures in Quality and Flavour Generation

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CONTENTS

I. INTRODUCTION

Various fermented products have an origin of spontaneous fermentation of a raw material, such as milk, meat or vegetables. Initially, the main focus was on preservation of the raw material. Raw milk, for example, will be susceptible to microbial spoilage at room temperature; the milk will rapidly acidify, due to the activity of lactic acid bacteria. Apart from preservation, fermented products also develop distinctive tastes and smells, which are widely appreciated. During the years, many variations developed, partly on purpose and partly spontaneous and in relation to the technology used. Their nature depends very much on the type of raw material used, on the pre-treatment of it, on the temperature (climate) and the conditions of fermentation. This practice is still common in various parts of the world: a part of a spontaneous fermented product is transferred deliberately into a new batch of raw material in order to maintain or even strengthen it. This principle is the basis for controlled preservation, sustainable and safe, with many times an attractive flavour, although the process is not controlled.

Many fermented products contain lactic acid bacteria, but other bacteria, yeasts and moulds may be involved as well. In order to be able to produce products with a more consistent quality, starter cultures for the preparation of fermented food have been developed. These cultures are composed of selected micro-organisms, propagated as defined mixtures of pure cultures or as undefined mixtures of different types of bacteria. The use of starters, on the one hand, has been tremendously positive with respect to the quality of the product, but, on the other hand, it has decreased the diversity of fermented products. Since the food industry is keen to explore new possibilities for enhancing the diversity of its product range, there is a new interest nowadays in searching for potential starter organisms from the pool, which existed at the time of spontaneous fermentations. This chapter gives an overview of various fermented products and the main organisms used with their functionalities. It focuses in particular on dairy fermentations, since these fermentations belong to the best-studied cases and information obtained from these studies can also be applied to other fermentations.

II. VARIOUS FERMENTED FOOD PRODUCTS AND STARTER ORGANISMS USED

The preparation of fermented foods is a practice with a very long history. Already thousands of years ago, for instance as reported in scriptures like the Bible, the making of wine and cheese have been reported. In those times, hardly anything was known about the underlying mechanisms, however, many interesting products evolved. All of them were the result of spontaneous fermentations, essentially resulting in products with an extended shelf life and (often) tasteful characteristics. The extension of the shelf life was obviously a major reason for fermentation practices. Later on, after the discovery of micro-organisms as the causal agents for the fermentation processes, these micro-organisms were isolated and used in order to be able to produce products with a consistent quality. This led to the practice of using industrial starter cultures and gave the manufacturer more control about the technological, safety and consistent quality aspects of their food products.

Many fermented products exist and for each of them cultures with specific characteristics are involved. For instance, in the fermentation of meat, combinations of various bacterial species are used. In almost all cases, lactic acid bacteria are present in these starter cultures, with *Lactobacillus sakei, L. curvatus, L. plantarum, Pediococcus pentasaceus* and *P. acidilactici* as most important species. Also *Micrococcus varians*, *Staphylococcus carnosus* and *S. xylosus* species are used. These micro-organisms are able to grow under low pH (4.5–5.0), low water activities (A_w 0.9–0.95), anaerobic conditions, and in the presence of salt (2–3%). Not only do these organisms cause a decline in pH, but they also contribute in the typical flavour of fermented meat products.

A number of fermented meat products also exhibit a fermentation on the surface, resulting from the growth of yeasts (*Debaromyces hansenii, Candida famata* and *C. utilis*) and moulds (*Penicillium chrysogenum* and *P. nalgiovense*). The surface flora usually adds to the flavour formation through specific lipolytic and proteolytic activities.

For the production of bread and sourdoughs, yeast (*Saccharomyces cerevisiae*) and lactic acid bacteria, e.g., *Lactobacillus sanfransiscensis*, *L. amylovorum*, are employed. The required gas formation in these products is by enlarge due to the sugar fermentation of the yeast cells, although also some heterofermentative lactobacilli can add to this. The fermentation of sourdough is more complex, since it requires the interaction of various micro-organisms. The various flavour components formed in these products, like aldehydes, alcohols, pyrols, etc. are partly the result of biochemical conversions by the starter cultures, but also result from the baking process itself [54, 27].

The fermentation of vegetables, such as olives, cabbage and pickles, belong to the least developed practices. The production methods are still rather simple. Basically lactic acid bacteria like *L. plantarum, Leuconostoc spp, Pediococcus* and *Lactococcus* are used in these productions. The acid formation results in the extended shelf life, and also flavour formation by the micro-organisms adds to the overall quality of these products. For the fermentation of soy, which is a very common and traditional process in the East Asia, various mould species are used like *Rhizopus oligosporus* and *R. oryzae*. These fermentations are often solid state fermentations, for which the raw material is inoculated with micro-organisms which grow at the surface of the raw material, making it essentially an aerobic fermentation process. For some of the products there is an extensive proteolysis, leading to products like soy sauce, whereas in other fermentations (e.g., tempeh) only limited proteolysis occurs [10].

Wine and beer are fermented with various yeast strains (*S. cerevisiae*). In some wines, also *Leuconostoc oenos* is used. In these cases, the main pathway related to preservation is the formation of alcohol from sugar of the grain or the grapes, respectively. Again, like in other fermentation processes, many other reactions occur, which contribute to the final sensory quality of the products and which are in fact even more important for the final quality. In the preparation of the malt, for beer brewing, micro-organisms are also employed in order to lower the pH and to reduce the outgrowth of spoilage organisms, e.g., certain moulds [88, 72].

III. CULTURES USED FOR THE MANUFACTURE OF DAIRY PRODUCTS

All types of lactic acid bacteria as well as other micro-organisms are used for the manufacture of various dairy products. Since dairy fermentations are among the best-studied fermentations, with several new developments, we focus on starter cultures in these fermentations and their characteristics. This section will give examples of the main species used, various characteristics required for the quality of the final products and flavour formation in particular. Many of the described characteristics are also of importance in other fermented products.

A. Lactic Acid Bacteria

When raw milk is left at room temperature for some time, a microflora will develop in which lactic acid bacteria generally dominate. These bacteria acidify the milk and, as a consequence, inhibit the growth of other bacteria. Concomitantly, they give the milk a pleasant flavour. This spontaneous fermentation has been the basis of several fermented dairy products in the history. The discovery of the role of lactic acid bacteria in milk fermentation paved the way for their isolation, characterisation and exploitation. This started more than a century ago and has resulted in the development of starter cultures for the manufacture of fermented dairy products. Both industrial and small-scale manufacture now almost always relies on industrially prepared starters [100].

The lactic acid bacteria used in the dairy fermentations can roughly be divided into two groups on the basis of their growth optimum. Mesophilic lactic acid bacteria have an optimum growth temperature between 20 and 30°C and thermophilic strains with an optimum between 30 and 45°C. Traditional fermented products from sub-tropical countries harbour mainly thermophilic lactic acid bacteria, whereas the products with mesophilic bacteria originate mainly from Western and Northern European countries. Currently, most dairy industries use starter cultures for rapid acidification, because a spontaneous fermentation acidifies the milk too slowly. This method of working has reduced the number of strains present, and is responsible for certain uniformity in dairy products. On the other hand, it has made the production very reliable and efficient. The micro-organisms present in natural niches still form a very interesting potential for product diversification, due to their large biodiversity (see below).

Industrial (dairy) starter cultures can be divided into two groups: undefined and defined starters. The undefined starters are a mixture of an unknown number of lactic acid bacteria types, which are originally derived from an artisanal production practice and further selected for the production of good-quality dairy products. Mesophilic undefined starter cultures are commonly used for the manufacture of Cheddar, Gouda and other cheese types. Their use is based on their consistent performance, especially their well-recognised phage resistance [110, 71].

Defined-strain starters are blends of two of more strains; in the case of Cheddar cheese they are frequently used nowadays in stead of the former undefined mixed-strain starters. Since the risk of phage attacks is greater here than with the use of undefined mixed-strain cultures, cultures with different phage sensitivity profiles are used in rotation [55, 71, 56]. Another well-known example of a defined-strain starter is mixture of thermophilic lactic acid bacteria in the manufacture of yoghurt.

B. Lactococci

It is interesting to consider that the industrial starters for the majority of cheeses are based on a single mesophilic species, namely *Lactococcus lactis*. Strains of this species are employed for the manufacture of various cheese types and, although they exhibit different characteristics, they have several biochemical attributes in common [25, 31]. The most important properties are their ability to produce acid in milk, and to form flavour components.

For the manufacture of Gouda cheese, the undefined mixed-strain starters are composed of acid-forming lactococci, *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, possibly in combination with the citrate-utilising strains *L. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* spp (Fig. 64.1). These starters are thus composed of complex mixtures of strains, forming a bacterial population, which is equipped with the properties suitable for the production of the desired cheese. Since their composition would change depending on the conditions of cultivation [58], their subculturing is minimised and the cultures are preserved by freezing or lyophilisation.

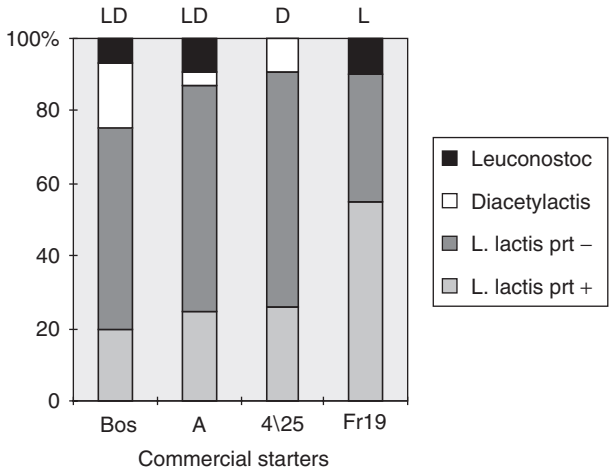

FIGURE 64.1 Composition of some commercial mixed-strain undefined starter cultures used for cheese making.

Also, the manufacture of Cheddar cheese and several other cheese types is entirely dependent on the fermentative activity of lactococci, sometimes in the form of defined-strain starters, consisting of *L. lactis* subsp. *lactis* varieties in case of Cheddar cheese, but frequently still in the form of undefined mixed-strain starters.

Mesophilic lactococci are generally considered to be associated with the milk environment [99], but they can also be isolated from other sources [65]. Lactococci isolated from artisanal manufacture of fermented dairy products without the application of industrially prepared starter cultures and from non-dairy environments are generally referred to as 'wild' lactococci. In an international project funded by the European Community many wild strains of lactic acid bacteria were isolated and partially characterised [21]. Initial studies showed that this pool of lactic acid bacteria contained many *L. lactis* strains, which differ in a number of phenotypic properties from the strains commonly present in industrial starters. Among those characteristics is the ability to form flavour components [122, 4, 5, 6]. This aspect, interesting for diversification in the manufacture of fermented dairy products, will be discussed below.

C. Mesophilic Lactobacilli

Although mesophilic lactobacilli are undoubtedly inhabitants of raw milk and the dairy environment, upon acidification of raw milk they are frequently overgrown by strong acidifiers of the genus *Lactococcus*. However, they do gain access to the cheesemaking process, because they are often found as secondary flora during the ripening of different cheese varieties. This is especially true for raw-milk cheese, but mesophilic lactobacilli are also common in cheese manufactured with modern technologies, using pasteurisation of the milk, defined-strain starters and hygienic processing. The starter is responsible for the acidification during the first stages of cheese manufacture and may reach up to 10^9 colony-forming units (cfu) per gram of cheese. During ripening, however, the number of starter cfu generally decreases rather quickly to lower than $10^7\,g^{-1}$, whereas subsequently the non-starter adventitious lactobacilli grow out and may reach numbers higher than those of the starter culture [103].

The secondary flora in Cheddar cheese have been examined most extensively; they consists mostly of mesophilic lactobacilli and sometimes include pediococci. These bacteria are collectively referred to as non-starter lactic acid bacteria (NSLAB). Isolates from this group belong to the species *Lactobacillus paracasei*, *Lb. plantarum*, *Lb. rhamnosus* and *Lb. curvatus*. The composition of the NSLAB in the cheese varies with the day of manufacture and with the age of the cheese [123, 21]. Adventitious NSLAB have also been reported for Emmental, Comté and other type of cheeses [29, 51, 13].

The NSLAB have the unique ability to grow under the highly selective conditions prevailing in a ripening cheese. Lactose is largely depleted in the first hours of cheese manufacture by the fermentation of the starter bacteria. The pH is between 4.9 and 5.3, the temperature below 13ºC, the moisture content less than 50%, the salt concentration in moisture is 4–6% and oxygen is barely available. All in all, the ripening cheese seems a hostile environment for micro-organisms. Yet, the adventitious lactobacilli manage to grow, obviously with a low rate, but the generally long ripening period allows them enough time to reach considerably high levels of cfu per g of cheese. They apparently consume compounds other than lactose such as lactate, citrate, glycerol, amino sugars, amino acids and perhaps even on material released from lysed starter bacteria. Although the NSLAB, like other lactobacilli, exhibit fastidious nutritional requirements, they clearly find ample opportunities for growth in a ripening cheese. They possess a wide range of hydrolytic enzymes and are able to effect proteolysis and lipolysis [62, 123].

Since NSLAB dominate the microflora of many long-ripened cheeses, they are believed to contribute to the maturation of cheese. The numbers of NSLAB are reported to be higher in Cheddar cheeses made from raw milk than in those from pasteurised milk [78]. Differences in flavour between these cheeses, with a more intense flavour in raw milk cheeses, suggest that the indigenous NSLAB play an important role in flavour development. Indeed, they have been shown to contribute to the formation of small peptides and amino acids, which are the precursors for the flavour components [45].

The observation that the presence of NSLAB in cheese on the one hand leads to a desirable flavour, and on the other hand may induce possible defects or spoilage [115, 114, 60] makes it a delicate choice for the cheese maker to use a certain lactobacillus as adjunct starter. This strain should be selected with care, because only a limited number of the NSLAB present in cheese combine all the required properties with the concomitant lack of imperfections. McSweeney et al [79] were successful in improving the flavour of Cheddar cheese by using strains isolated from raw milk cheese. This improvement was believed to be due to increased formation of amino acids. Cheese made from milk inoculated with strains of *Lb. plantarum* or *Lb. casei* subsp. *pseudoplantarum* received the best gradings [75].

D. Thermophilic Lactic Acid Bacteria

The thermophilic lactic acid bacteria are best known as starters for fermented milks. Several varieties of fermented milks originate from countries in Asia Minor and the Balkans, e.g. Bulgarian yoghurts. These yoghurt products have emerged from spontaneous acidification of raw milk by indigenous organisms, and have now been developed into microbiologically well-controlled industrial processes.

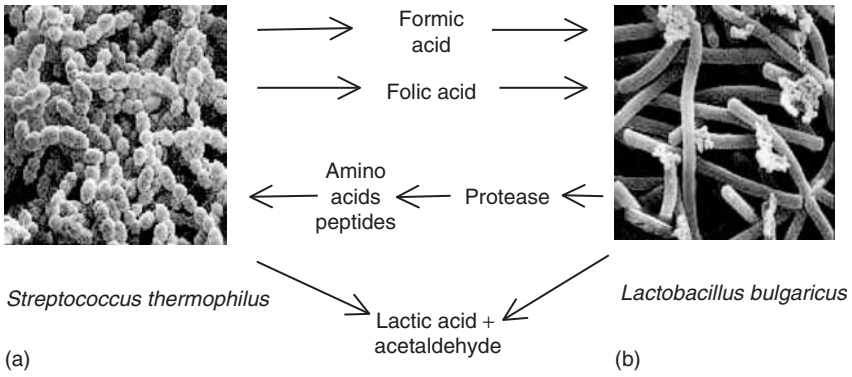

FIGURE 64.2 Proto-co-operation between *Streptrococcus thermophilus* and *Lactobacillus bulgaricus* in a yoghurt culture. This symbiosis results in an increased lactic acid and acetaldehyde (yoghurt flavour) production.

The two most frequently used starter bacteria are classified as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, generally shortened as *Lb. bulgaricus* and *S. thermophilus*, respectively.

Yoghurt is usually made by inoculating an equal mixture of *S. thermophilus* and *Lb. bulgaricus* in milk and incubating it at 37–45°C. *S. thermophilus* grows optimally at these temperatures and hydrolyses lactose via a β-galactosidase. *S. thermophilus* is nutritionally fastidious and requires a complex mixture of amino acids for growth. Since it is proteolytic negative, the coupling with the more proteolytic *Lb. bulgaricus* stimulates its growth in milk. The production of formic acid and carbon dioxide from lactose by *S. thermophilus* on the other hand stimulates the growth of *Lb. bulgaricus* (Fig. 64.2; [35]).

Thermophilic lactic acid bacteria also play an essential role in the manufacture of some cheese types. The starters of Swiss-type and Italian cheeses consist mainly of *S. thermophilus, Lb. helveticus* and *Lb. bulgaricus* [111, 9]. Also in the ripening of Greek hard cheese types made from ewes' and goats' milk, the thermophilic lactic acid bacteria play a dominant role [61]. The specific high cooking temperature used in the manufacture creates their niche in these cheese types. They convert lactose to lactic acid as in all dairy fermentations. This acid plays its usual role as preservative and, for the Swiss-type cheese, is the substrate for the subsequent propionic acid fermentation, leading to large eye formation in the cheeses.

Since thermophilic lactic acid bacteria were found to be very active, they are employed as adjunct cultures in the manufacture of various cheeses. In some cases they are specifically selected to address certain flavour aspects of cheese, e.g. bitterness. Smit et al. [104, 105, 106] reported on thermophilic lactic acid bacteria with high debittering activities, which were found to be very useful in debittering of cheeses made with a mesophilic starter culture which causes bitterness (Fig. 64.3). Various thermophilic adjunct cultures are also employed to enhance the formation of desired flavour notes in a given cheese.

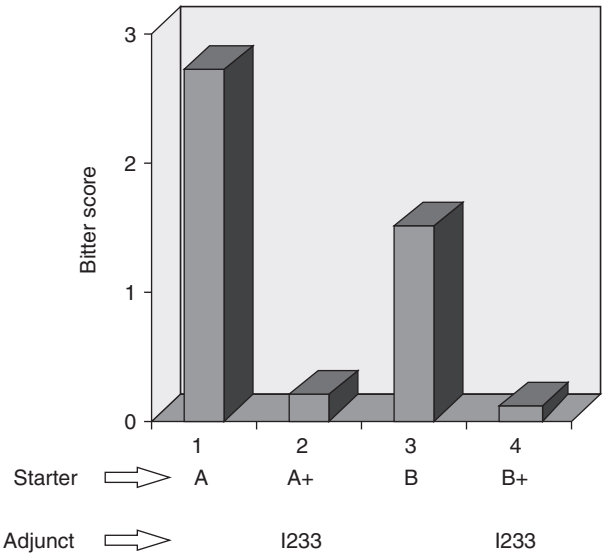

FIGURE 64.3 Effect of addition of the thermophilic adjunct culture I233 on the flavour development of a Gouda cheese after 3 months of ripening. The application of this adjunct culture results in a strong decrease in the bitter score during sensory evaluations. Starters A and B are known for the production of bitter-tasting peptides during cheese ripening.

E. Yeasts

Although yeasts play a minor role in dairy fermentations, several fermented milk products with a natural yeast-containing microflora exist. A distinctive feature of these products is that, in addition to the lactic acid fermentation induced by lactic acid bacteria, a slight alcoholic fermentation due to yeasts takes place [68, 93]. The best known examples of fermentation of milk by a combination of yeasts and lactic acid bacteria are kefir and koumiss, both originating from countries of Eastern Europe and Asia. The predominant species of yeast in kefir and koumiss are *Kluyveromyces marxianus, Candida kefir*, *Saccharomyces cerevisiae* and *Saccharomyces delbrueckii*. The heterogeneity of the

species found can be explained by the different techniques of kefir grain cultivation. Yeasts play an important role in promoting symbiosis among the micro-organisms present, CO_2 formation and development of the characteristic taste and aroma [67].

Yeasts are also involved in the ripening of surface-ripened cheeses. Their primary role is to lower the acidity of the cheese surface, which allows the development of a secondary microbial flora [95, 17]. Positive interactions between yeasts and starter organisms are well documented for semi-soft cheeses, like Limburger and Tilsit [43]. The yeasts metabolise lactate, which causes an increase of the pH, and may secrete factors that promote the growth of *Brevibacterium linens*, which is essential for the ripening of these cheeses [17]. Yeasts are also believed to promote the development of *Penicillium roquefortii* in blue cheeses. They may contribute to the open structure of these cheeses by formation of gas [22]. On the surface of Camembert, the dominating yeast flora inhibits the growth of the spoilage moulds. The most prominent yeasts in cheese are *Debaromyces hansenii, Kluyveromyces marxianus, Kluyveromyces lactis, Yarrowia lipolytica, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* [8; 14].

F. Moulds

Moulds are mainly used in the manufacture of semi-soft cheese varieties together with the lactic acidifiers. Their major role is to enhance the flavour and aroma and to modify the body and the structure of cheese. On the basis of their colour and growth characteristics, they can be divided into white and blue moulds. The former type grows on the outside of the cheese, e.g. Camembert and Brie, and is known as *Penicillium camembertii.* The blue mould is named *Penicillium roquefortii* and grows inside the cheese. Examples of 'blue' cheeses are Roquefort, Blue Stilton, Danish Blue and Gorgonzola [113]. Several varieties of these two *Penicillium* species have been described in the past, but they should all be considered as biotypes. The characteristic feature of mould-ripened cheese is the extensive proteolysis and lipolysis. These biochemical activities ultimately result in the formation of precursors for the typical volatile flavour components of the cheeses. Methyl ketones have a key role in the typical flavour. There is a positive correlation between the free fatty acid level and the amount of methyl ketones formed, and cheeses with limited lipolysis score lower in flavour [52, 53]. Other typical compounds besides methyl ketones are secondary alcohols, esters, aldehydes and lactones. Various compounds arising from the proteolysis and amino acid conversion of course complete the aroma of these cheeses. Clearly, the flavour of mould-ripened cheese is a delicate balance of several compounds, produced by a succession of micro-organisms, which each performs their particular activity. Not only the choice of the *Penicillium* strain is important for the successful production of the soft surface mould cheeses; the selection the concomitant starters is also crucial.

Geotrichum candidum is used as one of the starters for the manufacture of the Finnish fermented milk viili and Camembert cheese. The starter of viili consists of mesophilic lactococci and *Leuconostoc* strains with *G. candidum*. Viili is made from non-homogenised milk and the fungus forms a mouldy layer on the cream. Its growth is generally limited due to the restricted amount of oxygen present in the container with the fermenting mixture [77]. *G. candidum* is one of the contributors to the flora that plays a key role in the ripening of Camembert cheese. It starts to grow on the surface of the rind of Camembert, Pont l'Evêque and Livarot cheeses at the beginning of the ripening process and contributes to typical cheese flavours [57, 81]. Most notably, *G. candidum* is known for its potential to form sulphur-containing flavour components, which contribute to the typical flavour characteristics of Camembert cheeses [30, 11, 108].

G. Other Bacteria

Finally, some other groups of bacteria, which have not been covered above, are nevertheless worth mentioning. The propionic acid bacteria constitute the essential secondary flora in Swiss-type cheese. After the homofermentative lactic acid fermentation, they convert lactate into propionate, acetate and carbon dioxide. The latter is responsible for the characteristic eye formation in these cheeses. Interest in the role of propionic acid bacteria in flavour formation has recently been renewed [48]. Also for this group of bacteria, the raw milk environment appears to be a versatile and interesting source of strain variation [42].

A second group of bacteria, which play a major role in the maturation of surface-ripened cheese, comprises the brevibacteria and other coryneform bacteria. They are obviously present in the smear of these cheeses and are strongly proteolytic, which results in high levels of sulphur-containing volatiles [16, 17]. They also show some lipolytic and esterolytic activity, and produce distinct red-orange pigments [92]. The variation in proteolytic activity, antimicrobial activity and pigment biosynthesis in these bacteria may offer opportunities for the selection of appropriate variants for specific applications in the manufacture of smear-ripened cheeses or even other types of cheeses [15].

IV. STARTER FUNCTIONALITIES

As listed above, various micro-organisms are used as a starter culture for the production of fermented products, e.g., dairy products. Apart from acidification and thus preservation, it is found that these organisms often possess certain characteristics, which offer specific benefits to the consumer. These benefits are now leading in the development of new and improved starter cultures. A number of

specific characteristics will be discussed below. Special attention is paid to the flavour-forming abilities of starter cultures, since flavour perception is the most prominent motivator for the consumer to choose a food product.

A. Bacteriocins

Different lactic acid bacteria are able to produce bacteriocins, proteinaceous substances with bactericidal activity against micro-organisms closely related to the producer strain [63, 64, 26; 89]. Lactic acid bacteria are generally regarded as safe micro-organisms and so are their bacteriocins. Thus these bacteriocins can potentially be used to control the growth of spoilage and pathogenic organisms in food [32]. Bacteriocin-producing lactococcal strains have been used successfully in starter cultures for cheese-making in order to improve the safety and quality of the cheese [73, 76, 28]. For instance, the use of nisin-producing starter cultures was found to effectively reduce the spoilage by *Clostridium tyrobutyricum* strains (Fig 64.4).

Recently it was found that the production of bacteriocins and bacteriocin-like compounds is a property which is very common among strains isolated from natural sources [65, 21, 39, 7]. This phenomenon can possibly be explained by the fact that the ability to produce antimicrobial compounds offers these wild strains the power to withstand the competition of other micro-organisms and thus to survive in their hostile natural environment. The bacteriocin-producing wild lactococcal strains may be useful as starters in cheese making, not only because of their antimicrobial activity but also because of their potential to synthesise interesting flavour compounds (see below). These strains should then be combined with other strains, which are bacteriocin-resistant.

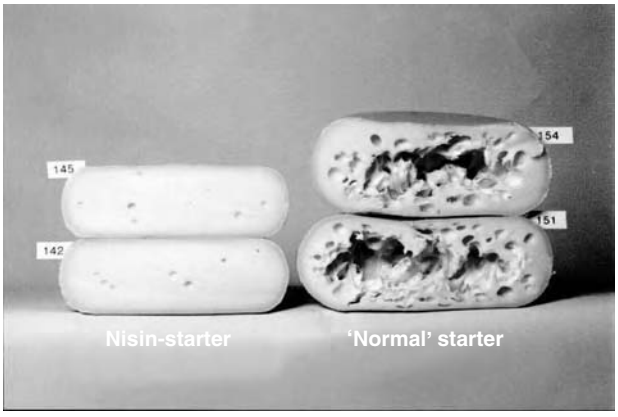

FIGURE 64.4 Effect of a nisin-producing starter culture on the reduction of growth of *Clostridium tyrobutyricum* after 12 weeks of ripening. In the absence of nisin ('normal' starter), this bacterium causes excessive gas formation leading to large holes in the cheese and a strong off-flavour.

B. Vitamin Production

Lactic acid bacteria are able to produce certain vitamins, for in yoghurt a higher concentration of folic acid is found than in milk [44]. *S. thermophilus* is known to produce folic acid during growth in milk [90, 91]. The amount of folic acid found in cow's milk ranges from 20 to 60 µg L^{-1}, whereas its concentration in yoghurt may be increased depending on the strains used for the fermentation and on the storage conditions to values above 200 µg L^{-1}. This level appears also to be dependent on the strain of *Lb. bulgaricus* used, because the latter organism has been shown to use and to degrade folic acid during its growth [124]. It is therefore of utmost importance to select the optimal combination of *S. thermophilus* and *Lb. bulgaricus* strains leading to organoleptically acceptable yoghurt with concomitantly an increased folic acid concentration. The pools of available strains will undoubtedly harbour such strains.

C. Exopolysaccharides

By producing exopolysaccharides, both *S. thermophilus* and *Lb. bulgaricus* contribute to the viscosity and the smooth texture of fermented products like yoghurt. They stabilise the gel and decrease its tendency to synerise [102]. These bacteria produce rather low amounts of exopolysaccharides with a wide variety of chemical structures. Glucose, galactose and rhamnose are its main monomers and the composition might be affected by the fermentation conditions [74, 120]. The chemical structure of the polymer apparently has an effect on the rheological properties, because the use of different starters results in differences in its microscopic structure and viscosity [119]. The underlying mechanism of this effect, however, is not well understood. The emerging knowledge on the genetics of exopolysaccharide biosynthesis *by L. lactis* and *S. thermophilus* strains (Fig. 64.5;

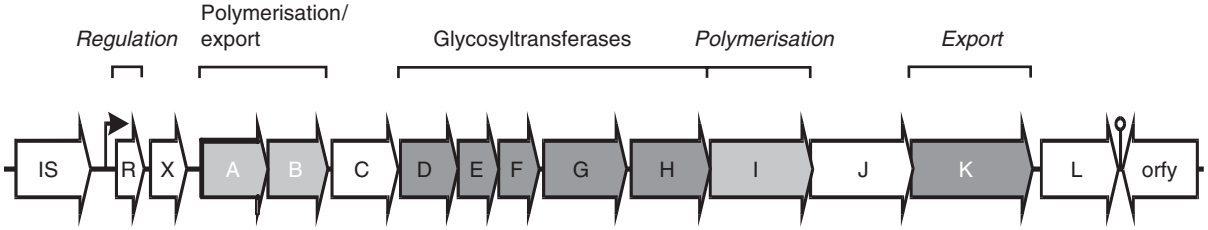

FIGURE 64.5 Genetic map of the genes involved in EPS production in *L. lactis*. (adapted from Van Kranenburg et al. 2000 [117]).

[112, 117]) will offer opportunities to modify the structure of the polymer and to regulate the amount synthesised.

D. Eye Formation

Lactic acid bacteria are able to ferment citrate, which is present in many raw materials used for food fermentations such as fruit, vegetables and milk. Citrate can be fermented by a limited amount of mesophilic lactic acid bacteria [59, 83] for a review). Its degradation results in acetate, diacetyl, acetoin, 2,3-butanediol, and CO_2. Besides the formation of the flavour component diacetyl, which is a crucial flavour attribute in buttermilk, ripened cream butter, sour cream, and quark, the formation of CO_2 is responsible for eye formation in cheese.

In the production of Swiss-type of cheeses, such as Gruyere, Emmental and Maasdammer, propionibacteria are used to achieve the characteristic sweet and nutty flavour notes as well as the large eyes. Various metabolic pathways have been described for the utilisation of lactate as energy source and aspartate as electron acceptor [24]. The eye-formation in Swiss-type of cheese can be controlled by a combination of aspartate-weak propionibacteria in combination with facultative heterofermentative lactobacilli [46].

E. Probiotic Features of Starter Cultures

Probiotics are live microbial food supplements, which benefit the health of consumers by maintaining, or improving their intestinal microbial balance [47]. Species of *Lactobacillus* and *Streptococcus* have traditionally been used in fluid fermented dairy products to promote the human health [98]. These probiotic starters may influence the microbial ecology of the host, lactose intolerance, incidence of diarrhoea, mucosal immune response, levels of cholesterol and cancer. Besides the traditional carrier of probiotics, which is yoghurt, the market share of probiotic drinks is expanding rapidly. The most common probiotic strains are lactobacilli. A number of them are now being successfully commercialised, such as *Lactobacillus rhamnosus* GG [101], *L. casei* Shirota [3] and *L. acidophilus* LA-1 [12]. The scientific basis of these strains in relation to their proposed positive effects on the human health is quite extensive, however most of the evidence is obtained with diseased human populations. Evidence for probiotic claims in healthy populations has still a weak basis.

V. FORMATION OF FLAVOUR COMPOUNDS DURING FERMENTATIONS

Flavour development in fermented products results from a series of (bio)chemical processes in which the starter cultures provide many of the enzymatic activities. Particularly the enzymatic degradation of proteins leads to the formation of key-flavour components, which contribute to the sensory perception of the products [36, 37, 107]. In the following section, the flavour forming enzymes of lactic acid bacteria will be discussed, with dairy products as the main example. On the other hand, it is important to note that very similar activities can be found in other fermented products, such as meat and sourdoughs.

The formation of flavours in fermented dairy products is a complex and, in the case of cheese ripening, rather slow process involving various chemical and biochemical conversions of milk components. Three main pathways can be identified: the conversions of lactose (glycolysis), fat (lipolysis), and caseins (proteolysis) (Fig. 64.6). The enzymes involved in these pathways are predominantly derived from the starter cultures used in these fermentations.

In the case of the lactose fermentation, the main conversion obviously leads to the formation of lactate by LAB, but a fraction of the intermediate pyruvate can alternatively be converted to various flavour compounds such as diacetyl, acetoin, acetaldehyde, or acetic acid, some of which contribute to typical yoghurt flavours.

Lipolysis results in the formation of free fatty acids, which can be precursors of flavour compounds such as methylketones, alcohols, and lactones. Lactic acid bacteria contribute relatively little to lipolysis, but additional cultures, e.g. moulds in the case of surface-ripened cheeses [82] often have high activities in fat conversion. Flavours derived from the conversion of fat are particularly important in soft cheeses like Camembert and Roquefort.

The conversion of caseins is undoubtedly the most important biochemical pathway for flavour formation in hard-type and semi-hard-type cheeses [118]. Degradation of caseins by the activities of rennet enzymes, and the cell-envelope proteinase and peptidases from lactic acid bacteria yields small peptides and free amino acids. A good balance between proteolysis and peptidolysis prevents the formation of bitterness in cheese [104, 105]. Although it is known that peptides can taste bitter and that amino acids can taste sweet, bitter, or broth-like [84], the direct contribution of peptides and amino acids to flavour is probably limited to a basic taste [36]. For specific flavour development,

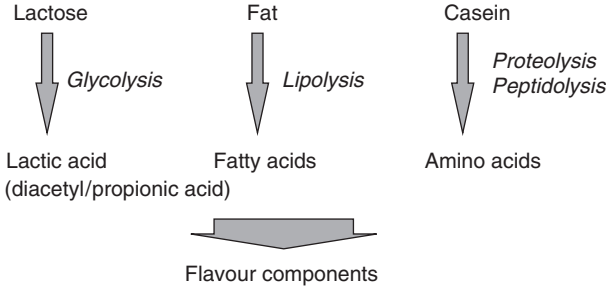

FIGURE 64.6 General conversions of the milk constituents during the ripening of cheese. Three main pathways can be distinguished. The pathway from casein is the most important one, leading to typical cheese flavours.

further conversion of amino acids is required to various alcohols, aldehydes, acids, esters and sulphur compounds. The current knowledge on these pathways is shown in Figure 64.7.

A. Proteolysis Leads to Peptides and Free Amino Acids

Since the concentrations of free amino acids and peptides are very low in milk, the starter cultures depend for growth in milk heavenly on their proteolytic systems. The degradation of milk proteins (caseins) leads to peptides and free amino acids, which can subsequently be taken up by the cells [69, 23]. Proteolysis is initiated by a single cell-wall-bound extracellular proteinase (Prt), which can be either chromosomally or plasmid-encoded. While most dairy LAB strains contain such an extracellular proteinase, several do not and these are mainly dependent on other strains in the starter culture for the production of peptides and amino acids. Such dependency of strains is rather common in starter cultures, and indicates the relevance of knowledge on the population dynamics between strains in order to be able to develop stable starter cultures.

Peptide and amino acid transport systems have been studied extensively in lactococci, but far less is known for other lactic acid bacteria such as mesophilic and thermophilic lactobacilli (see for a review Kunji et al. [69]). Peptide and amino acid uptake occurs via oligopeptide transport systems, di-/tri-peptide transporters, and various amino acid transport systems [66]. Following uptake, the peptides are degraded intracellularly by a variety of peptidases [69, 23]. These peptidases of lactic acid bacteria can be divided into endopeptidases, aminopeptidases, di-/tripeptidases, and proline-specific peptidases. The specialised peptidases in LAB for hydrolysis of Pro-containing peptides have been postulated to be important for the degradation of casein-derived peptides, since these are known to have a high proline content.

The balance between the formation of peptides and their subsequent degradation into free amino acids is very important, since accumulation of peptides might lead to a bitter off-flavour in cheese [109, 121, 104, 105]. Various bitter-tasting peptides have been identified and especially these peptides should be degraded rapidly in order to prevent [109, 121, 70]. Specific cultures have been selected with high bitter-tasting-peptide degrading abilities [104, 105] and such cultures are nowadays frequently used in the preparation of various types of cheese (Fig. 64.3).

The ability of cultures to degrade bitter-tasting peptides was not only found to be dependent on the strain used, but also on the growth conditions. For example, it was found that pH-controlled growth conditions resulted in a higher debittering activity [106]. The mechanism behind these differences was found to be strongly correlated with the sensitivity of the cells to lyse. Meijer et al. [80] showed that the introduction of a transposon in *L. lactis* SK110 not only increased the stability of the cells, but also the bitterness in cheese made with this culture. These results

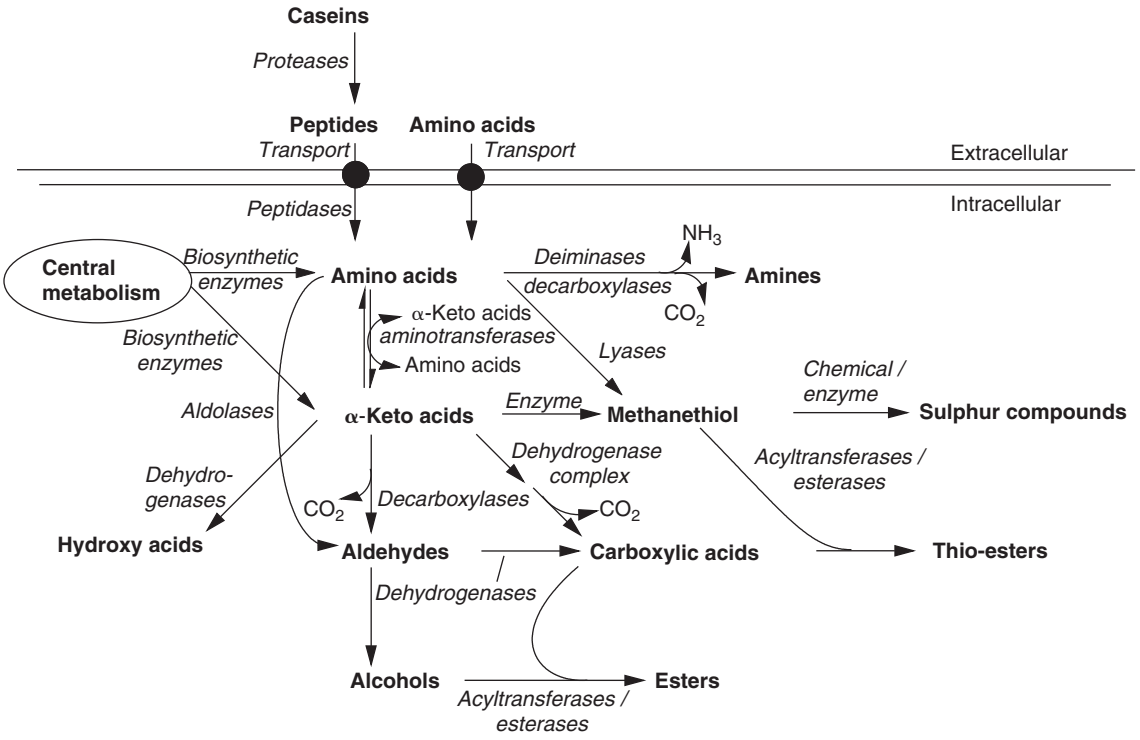

FIGURE 64.7 Pathways from amino acids to flavour compounds (adapted from Van Kranenburg et al. 2002 [118]).

indicate that the cell membrane can be a barrier between the enzymes, located intracellularly, and the peptide substrates, present in the cheese matrix. Apparently, there is not enough active transport anymore with the starter cultures once they are present in the cheese matrix for a certain ripening time.

B. Amino Acid Converting Enzymes (AACEs)

Amino acids are the precursors of various volatile cheese flavour compounds, which have been identified in cheese Engels and Visser, 1996; Engels, 1997]. They can be converted in many different ways by enzymes such as deaminases, decarboxylases, transaminases (aminotransferases), and lyases (Figure 64.7). Transamination of amino acids results in the formation of α-keto acids that can be converted into aldehydes by decarboxylation and, subsequently, into alcohols or carboxylic acids by dehydrogenation. Many of these components are odour-active and contribute to the overall flavour of the dairy product. Moreover, other reactions may occur, e.g. by hydrogenase activity towards α-keto acids resulting in the formation of hydroxyacids, which contribute little to the flavour.

Using biochemical and genetic tools, various flavour-forming routes from amino acids and enzymes involved have recently been identified, mostly in *L. lactis* (see [128, 107 and 118] for a review).

Aromatic amino acids, branched-chain amino acids, and methionine are the most relevant substrates for cheese flavour development. Conversion of aromatic amino acids can result in formation of undesirable flavours, so-called *off-flavours*, such as *p*-cresol, phenylethanol, phenylacetaldehyde, indole, and skatole, which contribute to putrid, faecal or unclean flavours in cheese [23]. Many of these reactions can also occur under cheese conditions and are highly dependent on the strain used [49]. This implies that by target selection of starter bacteria the formation of undesirable flavours can be avoided. A similar strain dependency is also found for enzyme activities, which result in the formation of desired flavour compounds, indicating that a strong potential for starter improvement exists (see below).

Conversion of tryptophan or phenylalanine can also lead to benzaldehyde formation. This compound is found in various hard-type and soft-type cheeses and contributes positively to the overall flavour [37, 82]). In *Lb. plantarum* as well as in other LAB, the formation of benzaldehyde out of phenylalanine is initiated by an aminotransferase reaction followed by a chemical conversion of the intermediate phenylpyruvic acid into benzaldehyde [86, 85]. The latter reaction requires the presence of manganese, for which an efficient uptake system was found [87]. Since this chemical conversion occurs at a high pH and in the presence of oxygen, it is not very likely a main conversion pathway in cheese.

Branched-chain amino acids are precursors of various aroma compounds such as isobutyrate, isovalerate, 3-methylbutanal, 2-methylbutanal and 2-methylpropanal. These compounds are found in various cheese types. Several enzymes that are able to convert these amino acids have been detected in *L. lactis* (see [128, 107 and 118] for a review). The aromatic aminotransferases can convert aromatic amino acids, but also leucine and methionine, while the branched-chain aminotransferases can convert the branched-chain amino acids leucine, isoleucine and valine, but also methionine, cysteine, and phenylalanine.

Volatile sulphur compounds derived from methionine, such as methanethiol, dimethylsulphide, and dimethyltrisulphide, are regarded as essential components in many cheese varieties [116]. In fact, a Gouda cheese-like flavour can be generated by incubation of methionine with cell extracts of *L. lactis* [36]. Conversion of methionine can occur via a aminotransferase-initiated pathway by branched-chain or aromatic aminotransferases, or via an α,γ-elimination of methionine by the lyase activities of cystathionine β-lyase (CBL), cystathionine γ-lyase (CGL), or methionine γ-lyase (MGL) (Figure 64.2) [1, 18, 33, 34, 38, 49, 50, 96, 125, 40, 41, 118].

It was found that the amount of α-keto acids determines the rate of the first step in the conversion of amino acids. Overproduction of the transaminases alone did not lead to a strong increase in amino acid conversion without a simultaneous addition of keto acids as co-substrate [126]. The introduction of a glutamate dehydrogenase gene from *Peptostreptococcus* in *L. lactis* resulted in a similar effect [97]. However, whether this activity also results in a strong increase in the desired flavour components remains to be determined. Ayad et al. [6] found that also the presence of enzymes required for subsequent conversions might be of crucial importance (Fig. 64.8).

Although cystathionine lyases are active under cheese-ripening conditions [1], their activity towards methionine

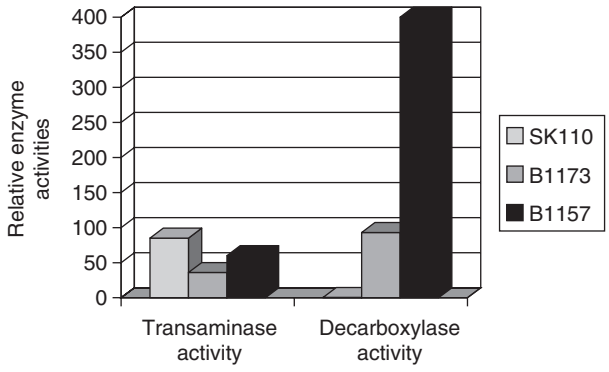

FIGURE 64.8 Variation in enzyme activities between industrial (SK110) and wild *L. lactis* (B1173 and B1157) strains in the two-step conversion of leucine towards 3-methyl aldehyde. The largest difference was found in step 2; the decarboxylation of the ketoacid towards the aldehyde.

could not be detected using ^{13}C nuclear magnetic resonance [49]. With this technique, only the aminotransferase-initiated pathway was observed suggesting that this pathway is most prominent in methionine catabolism to produce methanethiol. On the other hand, strains that overproduce cystathionine β-lyase, where found to be able to degrade methionine, indicating the potential of this enzyme in the production of sulphury flavours [40, 41]. The specificity of CBL [1] is a particular advantage in this respect, since one might expect that only sulphury flavour components would increase in strains with high activity. In case of overproduction of other less-specific enzymes such as transaminases more pathways will be influenced at the same time.

Biosynthesis and degradation of some amino acids are intricately coupled pathways. For instance, the above mentioned cystathionine β-lyase can convert methionine to various volatile flavour compounds, but in bacteria its physiological function is the conversion of cystathionine to homocysteine, which is the penultimate step of methionine biosynthesis [118]. This means that many of the AACEs are in fact involved in the biosynthesis of amino acids. It is well known that biosynthesis of amino acids is highly regulated, and therefore the growth conditions of the starter cultures might affect their flavour forming capacities. For instance, in *L. lactis* the expression of the gene coding for cystathionine β- lyase is strongly influenced by the amounts of methionine and cysteine in the culture medium [40, 41]. High concentrations of these amino acids completely abolish transcription and result in *L. lactis* cells almost deficient of cystathionine β-lyase activity.

Similarly, it is found that the branched-chain aminotransferase is also regulated at the transcriptional level in *L. lactis* [127]. The physiological role of branched-chain aminotransferases in bacterial metabolism is to catalyse the last step in the biosynthesis of branched-chain or aromatic amino acids. Several enzymes can thus been considered as being involved in both biosynthesis and degradation of amino acids, and α-keto acids are intermediates in both directions. These examples illustrate that the choice of culture conditions can strongly influence the flavour-forming capacities of starter cultures like *L. lactis*.

C. Natural Biodiversity

It is already mentioned that various lactic acid bacterial strains differ in amino acid converting abilities and that these activities are in fact linked to the ability to synthesise amino acids. [4, 5] focused on the flavour-forming and the amino acid forming abilities of *Lactococcus* strains isolated from various natural sources, the so-called 'wild lactococci' (Fig. 64.8). These strains originated from dairy and non-dairy environments and they were found to have unique properties, when compared to commercially available starter strains. For instance, many of these strains do not degrade caseins, produce antimicrobial compounds and/or have low acidifying activity. When the amino acids forming capacities of these strains were determined using the single omission technique [20], it was found that these strains had a much larger potential to synthesise amino acids as compared to industrial strains. Lactococci used in dairy fermentations are known for their limited capacity for biosynthesis of amino acids, which explains their complex nutritional requirements; they require several amino acids for growth [2, 94, 19, 4]. For instance, most dairy *Lactococcus* strains need glutamate, valine, methionine, histidine, serine, leucine and isoleucine. Industrial *L. lactis* subsp. *cremoris* strains require even more different amino acids for growth [4]. Wild *L. lactis* subsp. *cremoris* strains generally require two to three amino acids while some *L. lactis* subsp. *lactis* strains only need one amino acid. The absence of some amino acid biosynthetic pathways in dairy lactococci might be a consequence of their adaptation to dairy products, since in milk, the amino acids are readily available from the proteolytic degradation of caseins. Wild strains are not naturally associated with a rich environment such as milk, which makes them more dependent on their own biosynthesis of amino acids compared to industrial strains. The large natural biodiversity, which is found within LAB species, offers good possibilities for flavour diversification. It can obviously also be applied in various other fermented food products.

VI. CONCLUSIONS AND FUTURE PERSPECTIVES

Fermented products bring the consumer a sense of appreciation, which is by enlarge due to the interaction of the right starters cultures, technology and care in manufacturing. The starter cultures play a specific role in this respect and for that reason deserve specific attention. Not only are the starter cultures responsible for the preservation, due to lowering of the pH or increase of ethanol, but they also form various important compounds, most notably flavour components, which contribute directly to the perception of the consumers.

This knowledge makes it necessary to put special emphasis on the research in the area of starter cultures and their functionalities. Especially in view of the natural biodiversity, which still exists in food-grade micro-organisms, it is important to preserve this pool of microbial strains for future applications. The product innovator is challenged to use strains from them in his trials to create the flavour or other attributes the consumer demands.

The variation in microbes and their intrinsic biochemical potential is huge in size. This will make it necessary to have a better understanding of the enzymatic pathways in these starter cultures, in order to be able to select strains with specific desired characteristics. For instance, the rapidly growing knowledge of pathways prevailing in these bacteria which are active in the flavour formation offers

new insights in the required properties of a given starter culture. This should lead to the design of probes to screen collection of microbes (pools of genes) very effectively for the presence of certain desired traits. Moreover, apart from the best-studied examples of starter cultures, e.g. the lactococci, also other micro-organisms receive more attention recently. This will even enlarge the potential possibilities in this field. An interesting time is ahead of us, not only from a scientific point of view, but also from the perspective of manufacturers of fermented products.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Quality and flavor of fermented products," by G. Smit, J. T. M. Wouters and W. C. Meijer. In *Handbook of Food and Beverage Fermentation Technology.* Editors: Y. H. Hui et al. Marcel Dekker, New York, 2004.

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65 Meat Fermentation: Principles and Applications

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CONTENTS

I. INTRODUCTION

Fermented foods can be defined as products whose specific properties are mainly due to the effects of bacterial metabolism during their manufacture. Although meat products are not the most representative examples of fermented foods, it is clear that microbes contribute to both the conserving and flavouring effects of salt added to meat, a practice dating back to the Neolithicum, e.g. anywhere between 5,000 and 10,000 years ago (1). Such contributions are evident when the meat surface flora is evenly distributed by chopping, grinding or comminution of the meat together with the salt. The stuffing of such mixture into a casing produces a « sausage », a product first referred to in writing by Homerus in 900 BC. Centuries BC, a similar product (« lup-cheong ») was already produced in China, adding more sugar however than salt as a preservative. Since these beginnings, variable degrees of mixing and comminution of different « meats » with salt, together with spices, herbs and other plant material have been applied, resulting in a range of products, the stability of which is mainly determined by a combinations, varying in relative importance, of

- acidulation, brought about by lactic acid production and
- lowering of water activity (a_w), brought about by the addition of salt (« curing ») and drying.

Campbell-Platt (2) discusses 36 whole and 56 comminuted fermented meat products, including e.g. an East-African sausage made from fat and bones, a ham like product made by the Greenland Inuits from the eider duck, as well as 33 species of country or raw ham. The contribution of microbial activity in the production of large whole meat products is limited: as the meat interior

is practically free of microbes, the specific characteristics of such products are largely determined by remaining muscle proteolytic and lipolytic activity (3), with a limited contribution of bacterial activity to nitrate reduction, color formation and acceleration of salt introduction by some lactic acid production (4). This introductory discussion will therefore be limited to comminuted products or sausages, subjected to a variable combination of fermentation and drying. The first production of such "fermented sausages" started in Italy in 1730 and spread from there to Germany, Hungary and other countries, including the U.S. and Australia. The basic concept of processing involves comminution of muscle and fat tissue with salt, nitrate and/or nitrite and spices including eventually sugar, starter cultures and other additives such as non meat proteins. After stuffing the mixture into a casing, the resulting sausage is left to ferment and dry. This "ripening" often occurs in two consecutive and separate stages, referred to as fermentation and drying respectively. The lowering of a_w resulting from the presence of salt and drying and the exclusion of O_2 selects for salt tolerant lactic acid bacteria, producing lactic acid from carbohydrates added or present. This lowers pH to final values between 4.5 and 5.5, inducing denaturation of salt solubilised protein to a gel structure that can be sliced. The adequate (fast) reduction of pH and the lowered a_w ensure both product stability and safety. Once these basic requirements are met, the production technology allows for many but imprecise variations, yielding a variety of very different products. A broad distinction can be made between:

- Northern type products (NP): they contain beef and pork and are characterised by relatively short ripening periods, up to about 3 weeks, involving clearly separated fermentation (about 3 days) and drying periods. Rapid acidulation to final pH values just below 5 followed by product dependent weight losses during drying ensure safety and shelf life. Smoking is applied to add specific flavour (taste and aroma).
- Mediterranean or Southern type products (MP) are predominantly pure pork products and production involves longer ripening periods, up to several months. Fermentation occurs at lower temperatures (ca. 20°C vs. ≥25°C), and acidulation to final pH values above 5 is therefore slower and often not clearly separated from drying. Superficial mold growth is often involved, smoke is not applied (with the exception of Hungarian type salami) and shelf life is mainly determined by drying and lowered water activity.

More detailed or official classification of fermented sausages is done in various ways, based on e.g. moisture content, moisture/protein ratio, weight loss, water activity a_w, surface treatment, texture and fat particle size. It has become increasingly clear that the major characteristics of these products are determined by numerous interactions within the trialogue (meat + fat) − (micro-organisms) − (processing technology), whose control presents a considerable challenge to standardization and management of quality. Such lack of control — and thus standardization — is apparent from the between and in batch variability observed in the levels of end products of biochemical changes as well as of volatile flavour compounds, showing variation coefficients between 15 and 35% and exceeding 50% respectively for both NP and MP in a collaborative European project (5, 6). Several aspects of each actor in the trialogue determining variability have been discussed in several recent papers on e.g. meat enzymes (7), the role of bacteria in flavour development (8), the control of bacteriological safety (9), starter cultures (10), overall sausage metabolism (11) and process engineering (12). This paper does not want to add to existing excellent comprehensive literature reviews (4, 11) but to briefly summarize some aspects of the subject, emphasizing the importance of interactions for product quality. The text is inspired mainly by the long term interest of the author's laboratory in an integrated approach to sausage fermentation (13, 14, 15) and the role of muscle enzymes (16).

II. SAUSAGE MANUFACTURE

Table 65.1 lists representative conditions for industrial production of both NP and MP, as studied in a recent collaborative research project (6).

A. Ingredients

1. Meat and Fat

The meat/fat ratio is 2/1 in most industrial sausage mixes, containing 50–70% of frozen and "tempered" (≈−4°C) lean meat, consisting of pork and beef in equal amounts for NP and exclusively pork for the more valued MP. For pork, ham trimmings, shoulders and bellies are used, as well as jowls and throats, in proportions determined by the sausage quality level as reflected in more than twofold price differences (F. Vandendriessche, personal communication, 2002). Pork necks are avoided as well as beef head meat, because of risks of antibiotic and BSE contamination respectively. Only frozen (≤−18°C) pork back and belly fat (excluding less firm sites from the groin and teat line) after removal of rind (lard) and classified according to a visual estimation of fat content (50% vs. 30% respectively) are used as fat source. Pork rind may be added after cooking. Lard and "sausage meat" account for about 10% and 20% of the pig carcass respectively (17) and classic selection criteria for these raw materials are mainly based on bacteriological

TABLE 65.1
Main Production Characteristics for Northern and Mediterranean Types of Fermented Sausages Produced in Belgium (6)

Characteristic	Northern Type	Mediterranean Type
Diameter (mm)	90	60
Weight	1 kg	0.8 kg
Meat species	pork/beef, 1/1	pork only
Lean meat cuts	pork shoulder + beef	shoulder
Fat tissue	Pork back fat	Pork back fat
Meat/fat ratio	2/1	2/1
Starter bacteria (CFU)	$+10^7$/g[b]	$+10^7$/g[c]
Additives (g/kg)[e]		
NaCl	30	30
Nanitrite[f]	180	180
Knitrate[f]	0	0
Saccharose	0	0
Dextrose	7	7
Na-ascorbate	0.9	0.9
Ascorbic acid	0	0
Skimmed milk	0	0
Caseinate	11.5	11.5
Spices[f]	7000[h]	7000[h]
Fungial starters	−	+
Particle size	1–2 mm	1–2 mm
Smoking	+	−
Ripening conditions		
Fermentation		
°C/hrs	20–26/62	5–24/113
pH after 24hrs	5.70	5.80
3 days	4.68	5.20
%rH/hrs	50–90/62	10–90/89
Drying		
°C/% rH	14/78	14/78
Total ripening time	2 weeks	4 weeks

[a] Unspecified *Lactobacillus* [b] 2.10^6 each of *Pediococcus pentosaceous* and *Lactobacillus Sake* as lactic acid bacteria and *Staphylococcus xylosus, Kocuria varians* and *Staphylococcus carnosus* as flavor enhancers. [c] 7.10^5 each of *Pediococcus pentosaceous* and *Lactobacillus Sake* as lactic acid bacteria with 4.10^6 and 2.10^6 of *Staphylococcus xylosus* and *Micrococcus varians* (now properly called *Kocuria varians*) respectively as flavor enhancers. [d] A commercial powder containing a mixture of *Lactobacillus curvatus* and *Kocuria varians* (2.10^{10}/g) was used (50 g/100 kg). [e] per kg meat and fat mince [f] mg/kg [g] oleoresins containing mainly black pepper, with coriander, paptika and cognac (140 mg/kg) and garlic (400 mg/kg) [h] pure black pepper only [i] cracked whole black pepper (700), white pepper (300) and garlic (100).

quality and pH (<5.8) and on oxidation status and unsaturation of fat (<12% of polyunsaturated fatty acids, minimal peroxide vlue) (18,19). Recent developments however report the use of differently specified alternative sources of "meat" and fat as well as the need for more specification for actual meat and fat to improve flavour in the more valued products. Technology for producing fermented sausage from poultry, including ostrich (20), from carp (21) and using olive oil (22) has been proposed. Lyophilized meat can be incorporated to shorten drying time (23), but this may require the lowering of salt addition, unless used after rehydration (24). For the more valued MP, muscle protease and muscle and fat lipase activities are probably important for (spieces dependent) sausage flavour development (25) as described for raw ham (3). Relationships of pig muscle enzyme activities with both carcass and meat quality have been demonstrated (26, 27). Analogous to suggestions made for raw ham production, enzymes and corresponding genes might be considered for specification of raw materials (3) and selection of animals (28) in the production of fermented sausages.

2. Additives

Generally used additives and their ranges of incorporation level include salt (2–4%) containing $NaNO_2$ (80–240 mg/kg aded as curing salt containing 0.4–0.6% $NaNO_2$), glucose (0.5–1%), sodium ascorbate or ascorbic acid (0.5–1%) and spices. The use of nitrite is considered essential because of its antibacterial, color forming, anti-oxidant and flavouring properties. The use of lower nitrite levels, imposed because of health considerations, requires adaptation of technology including e.g. minimal fermentation temperatures and oxygen free chopping (29). For MP part or all of the nitrite is substituted for by KNO_3, although there is little technological and microbiological necessity for the use of nitrate and its reported positive effects on colour and flavour development may in fact be more related the accompanying lower nitrite levels (30, 4). Both the amounts and the nature of carbohydrates have been related to the rate and extent of acidulation (31) but only the use of lactose clearly results in lowering both rate and extent of acidulation (32) whereas its residual presence may improve sensory quality (33). Optimal levels of glucose (dextrose) are reported to be 0.3 and 0.7% for MP and NP respectively (34). Both ascorbate and ascorbic acid are used to improve stability of the red nitrosylated pigment, an effect closely associated with the prevention of lipid oxidation. Both effects may also associated with the presence of anti-oxidants such as BHT and vitamin E in the raw materials, due to their use in animal feeding. However, although protective effects on lipid oxidation and colour stability of supranutritional dosing of animal feeds with γ-tocopherol are clear for beef, and less so, for fresh and cured pork (35), it would seem that it cannot prevent sausage colour deterioration due to incorporation of polyunsaturated fat (36). Ground pepper (0.2–0.3%) is usually present in all types of sausages and especially MP may contain higher levels (1–3%) of other spices such as paprika and garlic that were shown to be effective anti-oxidants, comparable to ascorbate (37). Besides yielding flavor compounds, spices may stimulate lactic acid bacterial activity, e.g., by supplying manganese, and decontamination should be considered (38). Apart

from these "classic" additives, additional additives are sometimes used for NP: phosphates (0.5%) to improve stability vs. oxidation (39), glucono-δ-lactone (GdL, 0.5%) to ascertain fast but chemical acidulation with generally negative effects on flavor development and vegetable proteins (mainly soya isolate) that may also accelerate fermentation (40).

3. Starter Cultures

Starter bacteria were first introduced in the US to ensure rapid fermentation. In Europe, lower fermentation temperatures were and are more common and, certainly in MP, rapid fermentation results in color and flavor defects. In order to avoid the latter, *Micrococci* were introduced as starters by Niïvaara (41). Although excellent fermented sausages can be produced without the addition of starter cultures or re-inoculation with finished sausages, the majority of fermented sausage produced in Europe nowadays makes use of "combined" starter inoculation ($1–2.10^6$/g) as frozen cultures of both *Lactobacilli* and *Micrococcaceae* to ensure rapid acidulation and optimal flavor development respectively (4). Their desired properties have been discussed at length (e.g., 42) and obviously include lag time and rate of lactic acid production for *Lactobacilli* and the resistance of *Micrococcaceae* to an acid environment. Inhibition of the latter by rapid acidulation was indeed found to impair the typical flavor of MP (43). For flavor development lipolytic and proteolytic activities have long been emphasized. It is now realized however that muscle and fat tissue enzymes are by far the more important actors in this respect and more important selection criteria for flavor producing starters such as *S. carnosus* may be their potential for leucine degradation to 3-methyl butanal and for protection of poly-unsaturated fatty acids against oxidation (5, 8). Attention has also been directed recently to biogenic amine (44) and bacteriocin (45) production by starter bacteria. These aspects are considered of less importance, as raw material quality and processing appear to be the main factor controlling amine production (46), and bacteriocin effects are bound to be limited (10). Bacterocin production by *L. sakei* strains, isolated from fermented sausages, may however contribute to safety, without inhibition of *Micrococcaceae* and, thus, of flavor development (46, 47). Besides bacteria, yeasts and fungi are used to a limited extent in the production of MP. *Dabaryomyces hansenii* and *Penicillum nalgiovense* are the major species sold as starters for MP production (19). They are both aerobic organisms and thus situated at the periphery or surface of the sausage, where they oxidize lactic acid and produce ammonia. Their contribution to flavor development is less clear, may involve fatty acid oxidation to methyl-ketones and the strongest argument for the use of fungal starters may be the prevention of growth of mycotoxin-producing fungi (19).

B. Processing

1. Comminution or Chopping

Industrial processing generally uses the cutter, involving a rapidly rotating set of knives ($1–3.10^3$ rpm) producing a batter in a slowly rotating bowl (10–20 rpm) within less than 5 minutes. The relative speeds of rotation of bowl and knives as well the sequence of addition of raw materials and additives determine fat particle size ($1–25\ mm^2$) and are optimized to minimize both, damage to the fat tissue added and increase of the batter temperature ($\leqslant -2°C$). Because air bubbles in the batter and oxygen may interfere with drying and color development respectively, chopping is best carried out under vacuum. The use of blunt rather than sharp knives is to be preferred for good texture (48). For traditional preparation of MP, often characterized by larger particle sizes, a meat grinder rather than a cutter may be used.

2. Stuffing

Traditional methods using nitrate, may still incorporate a "pan curing" phase (49), leaving the batter 24 hrs at low temperatures before stuffing to allow for optimal colour development. In most industrial processes however, vacuum filling devices are used, to immediately stuff the batter into natural or man made collagen or cellulose based casings, permeable to water and air. The size of sausage diameter (e.g., 2–15 cm) is related positively to the relative importance of fermentation (pH) vs. drying (a_w) for stability and a small diameter of collagen casing is required to ensure sufficient oxygen supply for full development of the mould aroma in MP (4, 50).

3. Fermentation and Drying

For industrial production of NP, fermentation and drying are usually carried out in separate rooms. Representative temperature/time/rh combinations for NP and MP respectively are shown in Table 65.1. It is recommended to have air rh values not more than 0.10 points below the associated a_w values of the sausage, to prevent case hardening. When $pH < 5.3$ a steeper rh gradient may be applied. Recommended air speeds are approximately 0.1 m/sec and a back and forth shifting of rh between 80% and 88% during drying is recommended (34). Controlled fermentation and ripening in air conditioned surroundings consumes considerable amounts of energy and alternative methods, involving the use of fresh air, have been proposed, inspired by the traditional methods for MP, adapted to local climatic conditions (51).

4. Smoking

At the end of the fermentation period, NP are subjected to smoke, generated by controlled combustion of wood

(300–600°C) to minimize the production of polycyclic hydrocarbons. Smoke contributes to anti-microbial and anti-oxidant effects, besides generating specific flavor and color components. Smoking is not used in the production of MP, except for Hungarian and Roemanian products, where a light smoking period precedes fermentation (34).

III. THE NATURE AND DYNAMICS OF RIPENING

It has been repeatedly confirmed that safety, stability and sensory quality of fermented sausages are the result of a complex and interacting set of microbiological, physical and chemical changes (4, 11, 13, 14). Within this complexity, most attention has been directed to the role of bacteria in relation to both safety and flavor: a "Web of Science" search for "fermented sausage" in April 2002 shows 194 papers published since 1977 (0.00075% of all documents!), 100 of which were published since 1997, containing 62 dealing exclusively with the behaviour of desirable or undesirable micro-organisms. Safety has been the subject of excellent reviews, describing the "hurdle concept" (34) as well as focussing on the potential of bacterocinogenic lactic acid bacteria (52) and will not be further discussed here. Besides an adequate redox-potential and the presence of nitrite and competitive desirable bacteria, major hurdles ensuring bacteriological safety are considered to be either the rate and extent of acidulation (10) or the extent of drying (lowered a_w) (9) for NP and MP respectively.

A. The Dynamics of Acidulation and Drying

Both the rates and extents of acidulation and drying can be quantified using simple exponential models (13) allowing demonstration of effects of processing factors such as the use of back slopping and starter cultures, sausage diameter, as well as the use of additives such as spices and soy protein (13, 53, 54, 55). The same models allow for the quantitative evaluation of the relations between rates of acidulation and those of drying and texture development (19). Predicted changes of DM content are assumed to be determined by water loss (drying) only, can thus be used to predict weight losses (55), for the calculation of empirical or "effective" diffusion rates (13,56) and, together with data for salt content, for the prediction of a_w values with acceptable precision (57, 58).

B. The Nature and Dynamics of Sausage Metabolism

The carbohydrate, protein and lipid fractions of the sausage are subject to changes, brought about by chemical as well as biochemical changes and the latter are mediated by microbial as wells by muscle and fat tissue enzymes. Multifactorial effects involving raw materials, additives, starter cultures as well as processing determine the relative intensities of these changes, mainly reflected in the rate and extent of acidulation and the development of sensory quality characteristics.

1. Sausage Metabolism and Acidulation

It is now clear that acidulation of the sausage matrix is induced by the interaction of salt solubilized muscle proteins with both lactic acid and ammonia formed during fermentation (33, 59). Molar amounts of lactate and ammonia present (59) as well as produced (54) during sausage ripening have indeed been related to pH and pH changes respectively. Lactate is mainly produced from (added) carbohydrates but may also be formed during microbial fermentation of glycerol, liberated in lipolysis, and, together with ammonia, from fermentation of amino acids. Besides lactic acid, variable amounts of acetic acid are produced, determined by the nature of the bacteria and their metabolism, both affected by the processing conditions. Finally, considerable amounts of oxygen are consumed during "fermentation", therefore better referred to as "metabolism"(60). This overall "sausage metabolism" affects the relationship between the amounts of carbohydrate "fermented" and lactate produced and has been described in a simplified "stoichiometric model" (61, 14), represented in Fig. 65.1.

Such model ignores the complexity of bacterial amino acid metabolism (11), representing it by either deamination to ammonia or decarboxylation to amines. Nevertheless, it has been supported by experimental data, within experimental error (60). Together with simple exponential analytical models, stoichiometry can be used to characterize the respective rates and relative importance of carbohydrate and protein oxidation (respiration) and fermentation, as well as the nature of fermentation (lactate/acetate) (14). In contrast to the kinetic and analytical models, referred to under III.A, models for metabolism are mechanistic, require more analytical data and are therefore better suited for comprehensive rather than predicting purposes (62). They have e.g. been used to illustrate effects of the processing factors mentioned earlier (IIIA) as wells as chopping intensity (63) on the relative importance of respiration and protein fermentation. Table 65.2 shows that in a series of experiments characterizing ripening metabolism of NP, carbohydrate disappearance accounted for between 82 and 94% of pyruvate equivalents metabolized.

An increase of diameter was shown to increase the relative contribution of protein fermentation as well as the accumulation of free amino acids, buffering the increased rate of lactate production. The relative importance of protein (amino acid) fermentation with ammonia production was lowered by addition of active lactic acid bacteria.

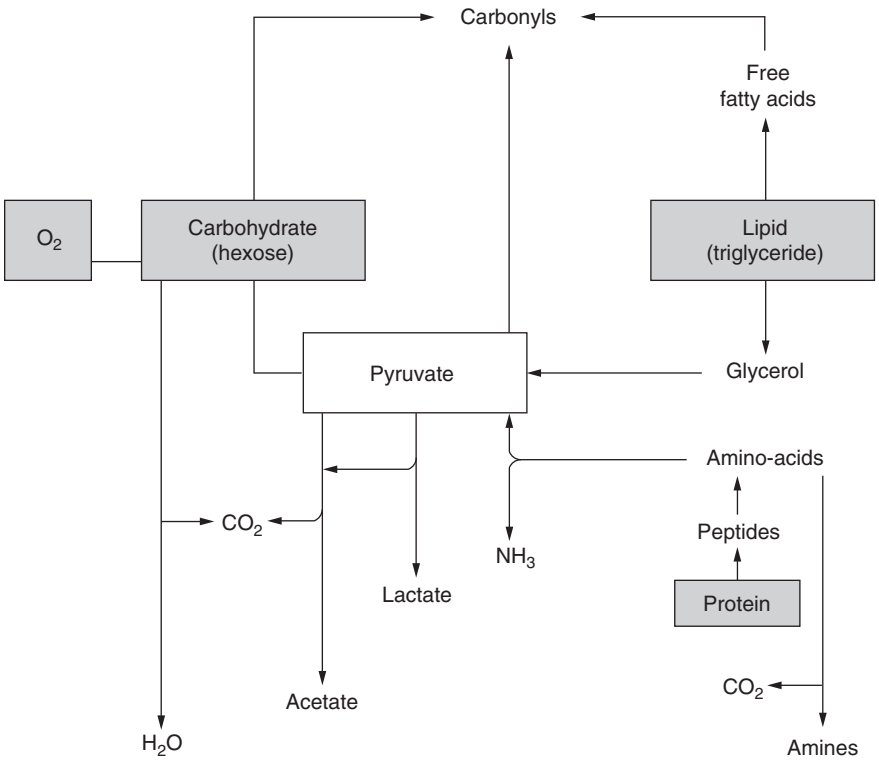

FIGURE 65.1 Reaction scheme underlying the stoichiometric model of sausage metabolism.

TABLE 65.2
Effect of Processing Contitions on the Pattern of Sausage Metabolism During Ripening of NP (21 days): % of Pyruvate Equivalents Derived of Carbohydrate and Protein and Utilised in Fermentation to Lactate and Acetate and in Respiration

						Chopping Time[c] (min)			
Effect of	Added Starters[a]			Sausage Diameter[b]		4.5		7	
Metabolism (%)	None	S1	S2	47 mm	90 mm	(1)	(2)	(1)	(2)
of:									
carbohydrate	82	86	91	93	92	87	89	89	88
protein	18	14	9	7	8	13	11	11	12
to:									
lactate	54	57	86	60	78	73	83	69	77
acetate	16	7	7	7	6	6	10	6	10
respiration	30	35	7	33	16	21	7	25	13

[a] Data from (52): starters used were fermented sausage, e.g. back slopping (S1) or a lyophylized culture of bacteria (S2) [b] Data from (13) [c] Data from (62): results of two experiments are shown involving longer chopping with salt (1) or pork back fat (2) respectively.

When the latter were added during backslopping however or as isolates from fermented sausages, proteolytic activity is apparent, an important effect for flavour, as recently confirmed (47). Inhibitory effects on deamination activities of both rates and extent of acidulation, more outspoken with larger diameters, associated with lower rates of drying have been confirmed in recent work (Demeyer http://fltbwww.rug.ac.be/animalproduction). Protein fermentation seems to be associated with the importance of acetate in the recovery of pyruvate equivalents. The most striking finding however is the large variability in respired substrate: between 7 and 43% of pyruvate equivalents metabolized. These calculated values obviously reflect an enormous variability in oxygen consumption, one that may involve chemical oxidations besides respiration. Factors determining variation are probably related to processing characteristics such as sausage diameter and chopping (Table 65.2), but variability in factors such as

vacuum stuffing, gaseous exchanges during ripening and respiratory activity of bacteria and molds may be more important.

2. Sausage Metabolism and Sensory Quality

Estimates of the nature of sausage metabolism as discussed above, allow better comprehension of the mechanisms determining both rate and extent of acidulation. It is however clear that the generalized pathways used in such estimates cover and/or accompany a set of detailed (bio) chemical reactions and end products affecting the development of sensory quality. Flavor is considered to be the more important sensory characteristic, determining renewed purchase by the consumer, whereas texture and color determine initial purchase and rejection respectively (64).

a. Texture

Texture, measured e.g. as the force necessary to penetrate the sausage surface or interior (sausage slice) under standardised conditions, e.g., "hardness", is determined by two processes, occurring consecutively in NP and simultaneously in MP:

- Gel formation because of acidulation. During chopping, myofibrillar structures are degraded (65) and myofibrillar proteins are solubilized into a sol, e.g. a network of filamentous aggregations of myosine molecules, whose dimensions and formation depend on factors such as pH and NaCl concentration that determine the relative rates of filament formation and aggregation (66). Acidulation induces coagulation, e.g. the conversion of a sol into a gel by intensification of aggregations, associated with the release of water and the formation of a matrix surrounding fat and connective tissue particles. The pH necessary for coagulation increases with increasing salt concentration and is 5.3 for salt concentrations between 2 and 3% (67)
- Drying: after gel formation, hardness is further increased because of loss of water, determined by diffusion limited water transport.

  The rate and extent of pH decline in the sausage, itself a reflection of overall sausage metabolism, determines both processes. During fermentation, muscle cathepsin D is activated by the decrease in pH and degrades sausage myosin (68, 69). It is known that such damage lowers the strength of heat coagulated myosin gels (16) and negative effects on texture of sausage proteolytic activity (70) and of increased myosin degradation because of added proteases (71) have indeed been reported. It is therefore clear that acidulation during fermentation induces two opposing effects on texture development: coagulation of the myosin sol into a gel as well as accelerating proteolytic cleaving of myosin molecules, lowering their contribution to gel strength, and, possibly, to water retention within the gel. Differences in gel structure and its water retention because of different relative rates of acid induced coagulation and proteolysis may explain the positive relationship found between initial rates of acidulation and texture development during drying. The use of PSE pork (72), spices (54), starter organisms (13) and soy protein (40) increase rates of acidulation and drying and thus, of texture development. For obvious reasons, an increase of sausage diameter decreases rate of drying and thus, rate of hardness development (73). Also however the rate of pH decline is lowered because of an increasing contribution of proteolytic processes to metabolism (14, 74).

b. Colour

The stable red colour of fermented sausage is due to nitrosylation and subsequent acid induced denaturation of myoglobin. In MP, formation of the nitrosylating NO occurs after bacterial reduction of added nitrate to nitrite, a process generally attributed to *Micrococceae*. Its inhibition by pH values below 5.2 hampers the use of lactic acid bacterial starters to ensure rapid acidulation. In NP, characterised by rapid acidulation, nitrite is the additive ensuring colour development. However, upon addition it acts as a very reactive oxidant for myoglobin: it is reduced to NO during chopping with an immediate grey discoloration of the batter, due to (nitrosylated) metmyoglobin formation. The rates of both this initial oxidation and the subsequent reduction and denaturation to the red nitrosylated myochromogen during ripening, as well as the stability to subsequent oxidation of the colour formed are determined by a complex set of factors, including e.g. the amounts of nitrite used, the rate of pH drop during fermentation, the use of anti-oxidant additives and the anti-oxidant activities of the meat and starter bacteria used. It would seem that for the Northern ripening process the use of sodium ascorbate (e.g. 600 ppm) with minimal amounts of sodium nitrite (e.g. 150 ppm) is sufficient to obtain an acceptable colour stability also reflected in a low redox potential (30) and minimal lipid oxidation (75). These conditions are promoted by the use of starter organisms with antioxidant activities (catalase, superoxide dismutase and/or nitrate reductase activities in *Micrococcaceae*) also contributing to flavour development (76) and/or low hydrogen peroxide producing activity (lactic acid bacteria). Net peroxide production is low at lower rates of acidulation (4) and minimal oxidation during sausage metabolism because of a low redox

potential may be reflected in its high potential of oxygen removal/ consumption (Torfs & Demeyer, in preparation).

c. Flavor

The simultaneous confrontation of the consumer with texture, taste and smell of the product during chewing creates his impression of flavour. Aroma (smell, odour) determined by volatile compounds, is considered to be the most important component because of the very high sensitivity of the nasal receptors. It is often considered separately from taste, determined by non-volatiles sensed by the receptors predominatly situated on the tongue. One should however be conscious of a "taste-olfaction integration" of senses (77) also apparent from the aroma enhancement due to the glutamate-umami taste. Peptides in fermented sausages may have a similar effect. In this respect it may be significant that fermented sausages were found to be better distinguished by taste than by odour for the descriptor "dry-sausage" (78).

i. Volatile aroma compounds More than 200 chemical compounds have been identified by gas chromatography — mass spectrometry or gas chromatography-olfactometry in volatiles present in the sausage "head space" or isolated by steam distillation (5, 19). Not all compounds in such "spectra" are of sensory relevance and the majority is derived from spices and smoking (NP). A limited group of compounds, thought to be responsible for the specific "fermented sausage" flavour however, consists of:

- Compounds considered to be derived from carbohydrate metabolism represented by acetic, propionic and butyric acids, acetaldehyde, diacetyl and acetoin.
- Compounds considered to be derived from protein metabolism, mainly represented by branched aldehydes and the corresponding acids and alcohols.
- Compounds derived from lipid degradation, mainly represented by methyl ketones, produced by microbial β-oxidation. Chemical autooxidation of unsaturated fatty acids produces a whole range of volatile carbonyl compounds, such as hexanal, contributing to the rancid notes and as such important for the overall flavour.

These compounds have been clearly associated with sensory descriptors such as maturity and salami and their relative importance, as well as that of esters, is increased by the Mediterranean low temperature and long time ripening process with use of *Staphylococci* as starter organisms (79). Higher temperatures and the use of *Pediococci* as lactic acid producing starters promote the production of dairy related volatiles such as diacetyl and accelerate the rate of pH drop, inhibiting *Staphylococci* (5).

ii. Non volatile taste compounds In contrast to MP, an "acid" taste is often sought for in NP and is positively correlated with the contents of D-lactate and acetate (61, 80). The extent of proteolysis, as reflected in the levels of low molecular weight peptides and free amino acids is clearly correlated with sensory analysis (14), specifically in relation to mould growth (10) and it is known that peptides affect taste rather than aroma, as shown for raw ham (81). Preliminar data (15) have associated small peptide (<500 D) and amino acid containing fractions isolated by gel permeation chromatography with sensory descriptors such as salami and bouillon, in analogy with the known importance of such fractions for raw ham flavour (82). Also, the non-protein nitrogen fraction will affect sausage pH (59) and may thus affect liberation of aroma determining acid compounds during chewing (83). ATP metabolites such as IMP and hypoxanthine contribute to taste, whereas free higher fatty acids are generally considered of less importance (64).

The relative importance of the different flavour compounds is determined by interactions between muscle and microbial metabolism as well as chemical reactions. The use of antibiotics and paucibacterial meat incubations has clearly established that initial proteolytic changes mainly involve myosin and actin degradation through the action of cathepsin D like enzymes. The contribution of bacteria in further endo- and, mainly, exoproteolytic changes increases down to ammonia production, the end of the proteolytic chain. Mediterranean, low temperature ripening, lowers rate of pH drop and thus, cathepsin D activity and initial protein degradation, but further proteolysis is not affected. Paucibacterial meat incubations demonstrate free amino acid production by meat enzymes.

In similar experiments, it was clearly demonstrated that endogenous lipases are by far the main responsible enzymes for the liberation of free fatty acids during ripening, with preferential release of poly-unsaturated fatty acids, both because of the more important phopholipase activity on muscle membrane phospholipids and the specificity of fat cell lipases. The importance of lipolysis for lipid oxidation and thus, flavour, remains unclear but a promoting effect is often assumed (15). Our laboratory has participated in studies on the impact of processing and of bacteria on the production of volatiles important for flavor in meat and meat products (6, 15, 84), using standardized methodology and our results suggest that the major contribution of bacteria to dry sausage ripening may be more related to lowering of lipid oxidation than to amino acid fermentation. Anyway, both amino acid fermentation and anti-oxidant characteristics are probably more important selection criteria for flavor enhancing starter bacteria than their lipolytic and proteolytic properties. It is indeed now generally accepted that initial proteolytic and lipolytic changes during dry sausage ripening are brought about by enzymatic activity of the raw materials, rather than from

micro-organisms (10). It is known that such activity in muscle shows considerable variation, related to anatomical location, gender, animal age and post-mortal rate of pH drop (26, 27, 85) The effects of such metabolic variability on flavour development in relation to bacterial activity should be further investigated.

ACKNOWLEDGMENT

The information in this chapter has been derived from "Meat fermentation: principles and applications," by D. Demeyer. Editors: Y. H. Hui et al. In *Handbook of Food and Beverage Fermentation Technology*, Marcel Dekker, New York, 2004.

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66 Fermented Liquid Milk Products

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CONTENTS

I. INTRODUCTION

Fermented milk products have been an important part of the diet for thousands of years. These products are believed to have originated in the Middle East region as a means of extending the shelf life of milk and over time they have spread around the world. They now form an important industry in most countries of the world (1) and consumption in many countries is considerable (2), especially in Europe (Table 66.1). Scandinavian countries and Russia are among the world's largest consumers of fermented milks (1).

In the early years the objective in fermented milks manufacture was primarily that of production of acid (usually lactic) to enable extended storage of milk in the absence of refrigeration. Now, consistency in fermentation and production of selected specific flavors along with a clean acid flavor are the norm. With the proper selection and propagation of cultures it is possible to produce numerous types of cultured products with a wide range of flavor and texture characteristics. In addition, these products offer various functional and nutritional characteristics for ingredient applications and therapeutic value for the maintenance of proper intestinal microbial ecology. The therapeutic properties of fermented milks have been recognized for many years (3). Many of these properties are generally not available in the original milk from which they are produced. Fermented milk products have thus added much to the diversity of the diet. While the commercial technology for the production of these products has advanced significantly

TABLE 66.1
Consumption of Fermented Milks around the World, 1998 (Ref 2)

Country	Per Capita (kg)	Country	Per Capita (kg)
Netherlands	45.0	Czech Republic	10.0
Finland	38.8	Portugal	9.8 (1997)
Sweden	30.0	Hungary	9.4
Denmark	27.3	Poland	7.4
France	26.9	Slovakia	7.4
Iceland	25.3	USA	7.1 (1997)
Germany	25.0	Australia	6.4
Israel	24.8	Argentina	6.0
Norway	19.3	Canada	3.6
Bulgaria	15.6	Ukraine	3.4
Austria	14.7	South Africa	3.1
Spain	14.5	China	0.2

TABLE 66.2
Examples of Fermented Milks (Refs 1, 4)

Name	Characteristics
Acidophilus milk	Medium acid
Bulgarian buttermilk	High acid
Cultured buttermilk	Low acid
Kefir	Acid and alcohol
Koumiss (Kumys)	Acid and alcohol
Lassi	Medium acid (with added salt or sugar)
Langfil	Medium acid, ropy
Mala	Medium acid with added fruits
Viili	Medium acid, mold, ropy

in the realms of cultures, production techniques and packaging, many types of cultured products are routinely prepared on a small scale in the home because of the relative ease of manufacture.

A selection of fermented milks from around the world is listed in Table 66.2. A comprehensive list can be found in the *Encyclopedia of Fermented Fresh Milk Products* (4) and some are described elsewhere (5, 6). The primary focus of this chapter is on the discussion of Acidophilus milk, Kefir, and Koumiss.

II. ACIDOPHILUS MILK

Acidophilus milk is produced mainly by the fermentation of milk with *Lactobacillus acidophilus* and it can have up to 2% lactic acid (1). The manufacture of acidophilus milk is outlined in Figure 66.1. With DNA-DNA homology studies, the acidophilus group of bacteria has been classified in to six major species: *Lactobacillus acidophilus* (A1), *Lactobacillus crispatus* (A2), *Lactobacillus amylovarus* (A3), *Lactobacillus gallinarum* (A4), *Lactobacillus gasseri* (B1), and *Lactobacillus johnsonii* (B2) (7). It is possible therefore, that acidophilus milks manufactured prior to this classification may have actually contained one or more of the above species. Currently, *Lactobacillus acidophilus* and *Lactobacillus johnsonii* are used in the manufacture of fermented milks commercially.

Therapeutic properties of *Lactobacillus acidophilus* have been recognized for long (8) and it is believed that this organism is able to implant in the large intestine and survive (9, 10). Consequently, acidophilus milk is found in health food stores and is used as a therapeutic product. A major characteristic that limits its consumption is poor palatability owing to its extremely high acid content. Furthermore, the sterilization treatment applied to the milk also imparts a strong cooked flavor to the product. Most strains of *Lactobacillus acidophilus* are not able to survive the high acid conditions created by their growth in milk so maintenance of high numbers in products is difficult. Various alternative products have therefore been developed that have better flavor qualities but supposedly have

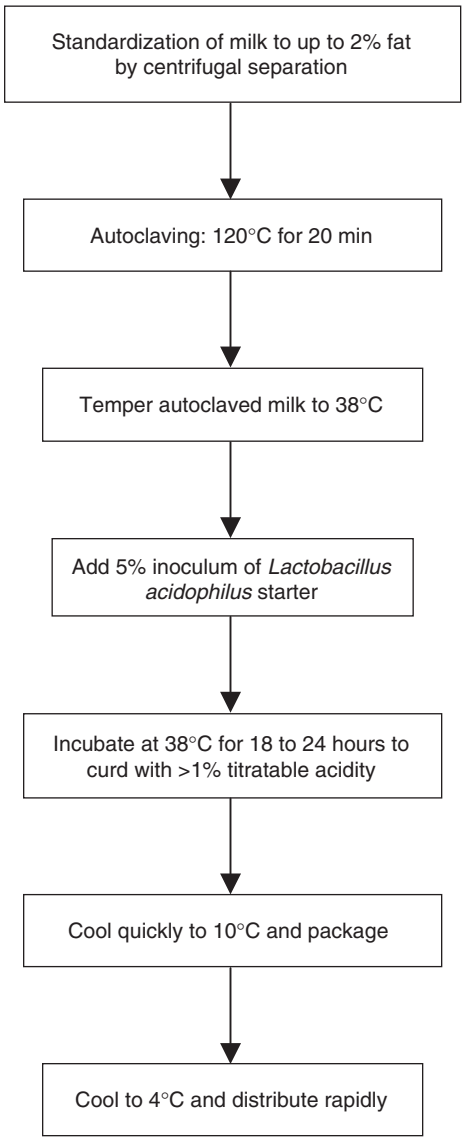

FIGURE 66.1 The manufacture of acidophilus milk.

therapeutic properties similar to acidophilus milk because of the presence of large population of *Lactobacillus acidophilus*. Among these are sweet acidophilus milk (11), also marketed as AB milk in the USA, BRA in Sweden (1), Actimel (Dannon company), which contains *Lactobacillus casei*, and LC1 of the Nestle company in Switzerland. This product contains *Lactobacillus johnsonii*.

As implied by the name, some of these alternative liquid products are not fermented, hence the term "sweet". Early versions of sweet acidophilus milk contained only *Lactobacillus acidophilus* but now also include one or more strains of bifidobacteria. The strain used for such milk generally is *Lactobacillus acidophilus* NCFM, which is a member of the *Lactobacillus acidophilus* A1 group (12). The BRA milk of Sweden has viable bifidobacteria, *Lactobacillus acidophilus* and *Lactobacillus reuteri.*

The latter produces an antibiotic (reuterin) that inhibits the growth of undesirable bacteria in the intestinal tract. In the manufacture of these acidophilus beverages, concentrated cell masses of the above selected bacterial strains are added to pasteurized, cooled, low fat milk, packaged and stored at 4°C until consumed. The cell count of the added bacteria is approximately 5 million per milliliter of milk. There is no fermentation, hence no acid is produced but the consumer ingests large amounts of live lactobacilli and/or bifidobacteria cells that are subsequently implanted in the intestinal tract. The shelf life of such milks is limited to 2 weeks under refrigeration because of the presence of live lactic acid producing bacteria. Extended storage under refrigeration or exposure to higher temperatures during storage will initiate the growth of the added cultures and rapid production of acid, followed by curdling of milk, which would be regarded by consumers as spoilage even though the curdling is caused by lactic acid bacteria.

In Finland a liquid product is manufactured by fermenting demineralized, lactose-hydrolyzed whey concentrates or concentrated whey with *Lactobacillus* strain GG (1). This strain, named after Goldin and Gorbach, the scientists who discovered the organism, is isolated from the human intestinal tract and is believed to possess therapeutic properties especially those pertaining to gastrointestinal disorders. This product is produced with or without any added fruit flavors and has a pleasant acid flavor.

III. KEFIR

Kefir has a long history in Russia, where it originated, but is now manufactured and consumed in several other parts of the world as well (1, 13), though the per capita consumption remains high in Russia at 4 to 5 kilograms. The name kefir is derived from the Turkish term kef or from kefy in the Caucasus region implying pleasant taste (4). Kefir is a viscous pourable liquid, with a smooth, slightly foamy body and whitish color. It is yeasty, acidic, mildly alcoholic, refreshing and slightly effervescent. Kefir is generally made from cows' milk but in the early years it was also made from goats' and sheeps' milk in leather sacks. It perhaps still is in some rural areas. Industrial manufacture of kefir began in Russia in 1930 (14).

Like many other milk products, Kefir ranges in fat content from nonfat to 3.5% but it is in many ways a unique product in that it entails acid and alcohol fermentation. Alcohol fermentation is the result of the addition of yeasts in the form of kefir grains. Because of the multiple fermentation process, the resulting product possesses flavor that is characterized by a balance of lactic acid (0.8%), diacetyl, acetaldehyde, and ethanol (1%). Diacetyl and acetaldehyde in a 3:1 ratio provide optimum flavor. The complex flavor is rounded out by fizz from carbon dioxide that is also produced during fermentation.

Kefir is manufactured using one of two main procedures. In the traditional process, kefir grains are employed for fermentation. Another method involves the use of lyophilized concentrates of cultures. In the latter method, kefir grains are not used.

A. Manufacture of Kefir with Grains

Kefir may be manufactured with grains in one of two ways (1). In the traditional system, Kefir grains are added to cows' milk that has been heated to 85°C for 30 min and cooled to 22°C. Incubation is for 24 hours at 22°C with occasional stirring. The grains are sieved when they rise to the surface due to carbon dioxide production and ripening for alcohol production continues at 10 to 15°C for 24 hours.

In another process a kefir starter is first obtained from the grains for fermentation. Here, freeze dried kefir grains are rehydrated in a sterilized 0.9% sodium chloride solution at 20°C for 5 hours. The grains are then washed with sterile water and added in a 1:30 ratio to skim milk that has been heated to 95°C for 30 min and cooled to 25°C. After incubation for a day the grains are sieved and the process of fermentation in skim milk is repeated two more times. After the final fermentation, the grains are sieved and the skim milk without the grains is then added as the kefir culture at the rate of 5% to milk that has been heated to 85°C for 30 min and adjusted to 22°C. Fermentation ensues for 12 hours (pH 4.5 to 4.6) followed by ripening for 1 to 3 days at 8 to 10°C. During ripening yeast fermentation occurs.

The ratio of kefir grains to milk affects the microflora of the starter. If a relatively large proportion of grains are added to milk (e.g., ratio of 1:10), the proportion of lactococci and yeasts is lower than when the proportion is smaller (1:30 to 1:50) because of the rapid accumulation of lactic acid. The optimum development of all groups of organisms in the grains takes place at a ratio of 1:50 (14).

B. Manufacture of Kefir from Concentrated Culture

Kefir may also be produced with a concentrated culture that is prepared from isolates obtained from kefir grains (1, 15). In the actual manufacturing process, no grains are used, as the organisms have already been isolated as a concentrated culture. This culture consists of 75% homofermentative lactococci, 24% citric acid fermenting lactococci, 0.5% lactobacilli, and 0.1% *Candida kefir* yeasts. A bulk starter made from this concentrated culture is added to heated milk (94°C, 5 min, cooled to 22°C) at the rate of 1%. Incubation is for 18 to 22 hours until pH is 4.5 to 4.6.

C. Kefir Grains

Kefir grains are the most important components of kefir manufacture (Figure 66.2). The concept of kefir grains is

believed to have originated in the accidental discovery of kefir many hundreds of years ago in the Caucasus region. These grains vary in size from a wheat grain to the size of a walnut. They are of whitish to yellowish color and gelatinous and irregular in shape and with a rough surface. The dominant microflora of kefir grains are listed in Table 66.3 and consist of mesophilic, homofermentative and heterofermentative lactococci, heterofermentative lactobacilli, lactose fermenting and lactose non-fermenting yeasts, and acetic acid bacteria. The microbial ecology within the grains depends on the origin and method of cultivation of the grains (4, 16, 17, 18). In one study 120 strains of lactobacilli were isolated from kefir grains. The most predominant was *Lactobacillus kefirogranum* (19). These groups of bacteria when purified do not grow at all or grow very slowly in milk. In fact it has been demonstrated that manufacture of good quality kefir by using isolated bacteria is difficult (20). Thus, the symbiosis within kefir grains is critical in optimal growth for kefir production. Koroleva has described such symbiosis (14), i.e., proteolysis and vitamin production by yeasts and acetic acid bacteria stimulates lactic acid bacteria, which in turn utilize lactose to promote growth of lactose non-fermenting yeasts. The metabolites of growth of lactic acid bacteria also check the growth and alcohol production by yeasts.

FIGURE 66.2 Kefir grains. Courtesy F. V. Kosikowski LLC.

TABLE 66.3
The Microflora of Kefir Grains

	Species
Yeasts	*Candida kefir*
	Candida pseudotropicalis
	Kluyveromyces marxianus subsp. *marxianus*
	Saccharomyces kefir
	Saccharomyces turicensis
	Torula spp.
	Other Yeasts
Bacteria	*Acetobacter aceti*
	Lactobacillus casei
	Lactobacillus helveticus
	Lactobacillus kefir
	Lactobacillus kefirogranum
	Lactobacillus kefironafaciens
	Lactococcus lactis subsp. *lactis*
	Leuconostoc mesenteroides subsp. *dextranicum*
	Leuconostoc mesenteroides subsp. *cremoris*

Newer strains from kefir grains such as *Lactobacillus kefiranofaciens* (21) and *Saccharomyces turicensis* (22) have been isolated. It is conceivable that as new microbiological techniques become available, newer strains will be identified and the microbial ecology of kefir grains will be better understood.

Kefir grains may be contaminated with coliforms, bacilli, micrococci, and mold, which will rapidly spoil the product manufactured with such grains. According to Koroleva, a good quality kefir culture prepared in skim milk should contain 10^8 to 10^9 lactococci, 10^7 to 10^8 leuconostocs, 10^5 thermophilic lactobacilli, 10^2 to 10^3 mesophilic lactobacilli, 10^5 to 10^6 yeasts and 10^5 to 10^6 acetic acid bacteria per milliliter (23).

Kefir grains consist of approximately 24% polysaccharide called kefiran, which consists of glucose and galactose in equal proportion and is not easily attacked by enzymes. Various lactobacilli such as *Lactobacillus kefiranofaciens* (24) and others (25, 26) that are present in the grains produce these polysaccharides. The polysaccharide content of kefir is approximately 0.2 to 0.7% and provides a slightly ropy texture to the final product.

Kefir grains are an excellent example of the coexistence of yeasts and bacteria. When kefir grains are soaked in milk, they swell and turn white and fermentation proceeds. During this process the grains also increase in number, possibly by splitting into smaller ones. Viable microbes from the grains are also shed into the milk during this process. The grains can be propagated and recovered in this manner and used in subsequent kefir production but it has not been possible to synthesize kefir grains.

Newly purchased kefir grains become fully active after two or three propagations (1). Kefir grains when left wet have a limited shelf life. When stored at 4°C they lose activity within 8 to 10 days if not used. Dried grains are active for 12 to 18 months of storage. Kefir grains if properly used and maintained can be reused many times. Grains that are recovered in a strainer should be washed with sterile water and can be stored wet in cold sterile water for reuse within a few days. Excessive washing can alter the microflora of the grains and hence the quality of the final product. They can be dried at room temperature for 36 to 48 hours and stored in a cool and dry place until reused (1) or be maintained in frozen state (−20°C) for extended storage (27).

In place of kefir grains freeze-dried kefir microflora have been suggested for kefir manufacture for uniformity

in quality (15). This was accomplished by cultivating kefir bacteria and yeast isolated from grains under controlled pH and incubation conditions. Bacterial concentrates obtained by centrifugation were freeze-dried with protective agents with survival rates of up to 70%. Including 20% sucrose and starch solutions enhanced survival rates of yeasts during freeze-drying.

D. Application of New Technologies

Kefir has gained popularity in many parts of the world outside its location of origin because of its refreshing taste and potential therapeutic value. The product has therefore continued to evolve and manufacturing procedures have been modified to produce products that meet consumer preferences and also improve the efficiency of manufacture.

Acid and alcohol fermentation can continue in finished kefir during storage because of the relatively high residual lactose content and the presence of yeasts. The result of this is extremely strong and undesirable products. Methods have been developed to control such post-production fermentation. In one such method (28), kefir is manufactured with selected lactic acid bacteria and yeasts. The selected lactic acid bacteria, which include *Streptococcus fecalis, Lactobacillus casei, Lactobacillus lactis, Lactobacillus fermenti,* and *Lactobacillus yogurtii*, are able to hydrolyze galactose and prevent the build up of galactose. Yeasts strains that do not hydrolyze lactose and galactose are used, e.g., *Saccharomyces bisporus, Saccharomyces mellis*, etc. Calculated amounts of sugars fermented by these yeasts such as glucose and fructose are added such that all sugar is utilized by the time the required amount of alcohol has been produced. During storage therefore, there is no more alcohol production.

Kefir with enhanced nutritional value may be produced by the application of ultrafiltration (29). In this process, milk is ultrafiltered to raise the solids content by 0.5 to 4%. This increases the protein content and reduces the lactose content of the milk and hence kefir. After high heat treatment, this milk is fermented to a pH of 4.5 to 4.7 and packaged. This product is especially designed for patients who rely heavily on kefir for therapeutic purposes but do not have other significant sources of protein. It has been recommended that for manufacturing kefir, skim milk should be ultrafiltered to no more than 1.8 fold in order to retain optimum texture quality (30). The application of ultrafiltration to increase the protein content by 0.2 to 0.3% is also useful for improving the consistency of low fat kefir (31).

Kefir products with added value have also been developed such as kefir fortified with a patented colostrum product for enhancing the immune system (32). Kefir products with prebiotics such as fructooligosaccharides (33) and probiotics such as bifidobacteria (34) have also been developed.

IV. KOUMISS (KUMYS)

Koumiss is a frothy beverage with greyish-white color, a refreshing taste and characteristic aroma (1). The name Koumiss is derived from the Kumanes tribe of the central Asian steppes, where it is believed to have originated many hundreds of years ago and was a common drink of the Tartar and Mongol tribes. Traditionally, this product was obtained by fermentation in a vessel made of horse-skin (35) but now it is made on a large scale in some parts of the world from mares' milk. It is an important product of Russia and Mongolia, where horses have been maintained especially for the production of this product. A distinctive feature of koumiss is that it, like kefir, is also produced by acid-alcohol fermentation. Koumiss is regarded as a product of high therapeutic value and a thirst-quenching beverage of high nutritional value in Russia.

Vladimir, Soviet and Russian heavy draft horse breeds are used for koumiss production in Russia and they yield 10 to 20 liters of milk per day (36). Milking occurs every 2 to 3 hours because of a low milk-holding capacity of the udder (37). Mares' milk resembles human milk more than it does cow-milk. On average, it has 1.9% fat, 1.3% casein, 1.2% whey proteins, 6.2% lactose and 0.5% ash (38), which gives it a lower viscosity than cows' milk. There is no visible curd when the milk is set and there is no wheying off. Protein characterization studies of mares' milk have shown that the isoelectric point of casein is 4.2 compared to 4.6 for cows' milk (39).

While Koumiss is traditionally made from mares' milk, limited availability of such milk has led to the development of procedures for its manufacture from cows' milk as well. There is however, a concerted effort in Russia to increase the production of mares' milk for koumiss production via improvements in horse breeding and management techniques (40, 41, 42).

There are several types of Koumiss available depending on the amount of acid and alcohol (Table 66.4). The manufacture of Koumiss therefore requires controlled lactic acid using *Lactobacillus delbrueckii* subsp. *bulgaricus* and alcohol fermentation using yeasts such as *Torula* spp. and others. In addition to acid and alcohol, carbon dioxide is also produced to impart fizziness to the final product.

Various regional products similar to koumiss are also made in central Asia on a relatively small scale. Examples

TABLE 66.4
Types of Koumiss

	Lactic Acid %	Alcohol %
Weak	0.7	1.0
Ordinary	1.1	1.8
Strong	1.8	2.5

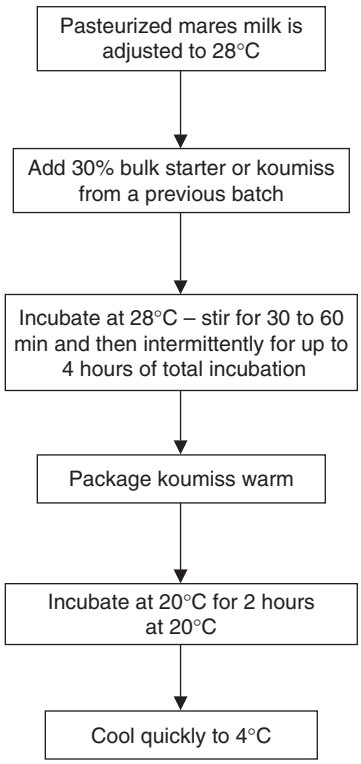

FIGURE 66.3 Manufacture of Koumiss from mares milk.

include Chal, which is manufactured from camels' milk, Kuban and Kurunga (4).

A. Manufacture of Koumiss

1. Mares' Milk

Koumiss can be manufactured using a previous batch as bulk starter or by preparing a bulk culture from pure strains. For preparing a bulk starter, yeast and lactic cultures are propagated separately (1). Two liters of nonfat cows' milk that has been heated to 70°C for 30 min and cooled to 30°C is inoculated with *Torula* spp. yeast. Incubation is for 15 hours at 30°C. A liter of similarly treated milk is inoculated with a lactic culture (*Lactobacillus delbrueckii* subsp. *bulgaricus*) and incubated at 37°C for 7 hours. The two separately fermented milks are mixed with a liter of mares' milk and incubated at 28°C. Additional mares' milk is added until about day four when the acidity is 1.4%. This mixture is then used as bulk starter for the production of koumiss as outlined in Figure 66.3. Aeration during incubation is important to allow the yeasts to grow. The three categories of acid-alcohol in koumiss cited in Table 66.4 can be achieved by adopting short or long ripening periods as follows: 1 day for weak, 2 days for ordinary, and 3 days for strong. Obviously, koumiss should be consumed quickly after production, as acid and alcohol production may continue during storage and the product will become stronger.

2. Cows' Milk

Koumiss that is similar in sensory and therapeutic qualities to mares' milk can be manufactured from cows' milk if appropriate adjustments are made for composition (43). As stated above, mares' milk has a significantly lower protein content and higher lactose content than cows' milk and the proportion of whey proteins is higher relative to casein in mares' milk. In one procedure utilizing cows' milk (1), 2.5% beet sugar is added to skim milk and pasteurized and a 10% culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus,* and *Saccharomyces lactis* is added. The mixture is incubated at 27°C until curd forms and acidity is 0.8%. It is then held at 17°C with aeration via stirring until acidity is 0.9%. Aeration permits the growth of yeasts. The product is then packaged and ripened for a further 2 hours at 17°C before cooling to a storage temperature of 4°C.

In another procedure (23), cows' milk is first standardized as follows: 34.6 parts whole milk, 0.8 parts skim milk and 64.6 parts of cheese whey are mixed. Starter similar to the one described above for cows' milk is added to the mixture at the rate of 20%. Ascorbic acid is also added at the rate of 0.2 grams per kilogram. Standardization lowers the casein content, and raises the whey protein and lactose contents. Ascorbic acid is added as a supplement to match the vitamin C content of mares' milk. The mixture is incubated at 28 to 30°C for 3 to 4 hours under continuous stirring. When the titratable acidity is approximately 0.7%, it is cooled to 16 to 18°C and stirred for 1 to 2 hours. The product is then packaged and ripened at 6 to 8°C for 1 to 3 days.

A Swiss process (44) employs the ultrafiltration of whey prior to standardization of cows' milk to simulate the composition of mares' milk. The lactose in the mix is partly hydrolyzed by beta-galactosidase to improve yeast fermentation. The sensory quality and composition of the resulting koumiss were similar to that made from mares' milk.

3. Starters

In the early years, koumiss from a previous batch was used as starter but purified starters have since become available (14). Such purified starters may be in the form of concentrates and include *Lactobacillus bulgaricus* and *Saccharomyces lactis* (45). The microflora of commercial koumiss is a reflection of the microflora of the starter. Various strains of lactic acid bacteria and yeasts have been isolated from commercial koumiss. One study, for example, isolated 43 strains of lactic acid bacteria and 20 strains of lactose-fermenting yeasts (46) and another isolated 417 yeast cultures from 94 samples of koumiss (47).

TABLE 66.5
Microflora of Koumiss (Refs 14, 46, 47)

	Species
Yeasts	*Candida kefir*
	Candida buensis
	Kluyveromyces marxianus subsp. *lactis*
	Saccharomyces unisporus
	Saccharomyces cartilaginosus
	Other yeasts
Bacteria	*Lactobacillus causasicum*
	Lactobacillus curvata
	Lactobacillus delbrueckii subsp. *bulgaricus*
	Lactobacillus paracasei subsp. *paracasei*
	Lactobacillus paracasei subsp. *tolerans*
	Lactobacillus rhamnosus

Lactose-fermenting as well as lactose non-fermenting yeasts were isolated (Table 66.5).

V. THERAPEUTIC PROPERTIES OF LIQUID FERMENTED MILK PRODUCTS

The medicinal value of liquid fermented milk products has been suggested for hundreds of years and various studies have implied that individuals for whom fermented milks such as acidophilus milk, Bulgarian buttermilk and kefir is a part of the diet as in some parts of Russia, live long lives (1). This value goes beyond the accepted high nutritional quality of unfermented milk. The fermentation process apparently adds therapeutic qualities to milk that are not found in the original milk. These properties are imparted by the cultures used that have the ability to survive product manufacturing and storage procedures and the harsh environment in the gastric system and establish themselves in the intestinal tract. Through their activities in this environment they are able to perform functions that are of benefit to the consumer. Also, some metabolic products of their growth during the fermentation process add therapeutic value to the finished fermented product. For example, a pasteurized Japanese product (Ameal S) is produced by the fermentation of a milk-based medium with *Saccharomyces cereviseae* and *Lactobacillus helveticus.* The fermentation process produces two tripeptides, valine-proline-proline, and isoleucine-proline-proline, which have the ability to reduce blood pressure. In this product live bacteria do not exist and are not important but the products of their growth are (48).

Much has been written about studies on the therapeutic value of fermented milks involving in vitro, animal and human studies. Various benefits have been suggested including the reduction in risk of gastrointestinal illnesses such as diarrhea, lactose digestion, enhancement of the immune function, decreasing *Helicobacter pylori* infection, reduction of cholesterol and certain allergies, anticarcinogenic action, etc (49, 50). As a result of this, commercial fermented milks with health claims have been developed in some countries and in Japan the Ministry for Welfare developed FOSHU (Foods for specified health use) regulations in 1993 for such products (51).

Lactobacilli such as the types used in the manufacture of some liquid fermented milks described above have been linked to various therapeutic properties (12). *Lactobacillus acidophilus, casei* and *reuteri* are believed to control the growth of undesirable microorganisms such as *Escherichia coli* and Salmonella in the intestinal tract (50). A large amount of acid produced by these organisms is one method of such control, as is competitive exclusion, but these bacteria are also able to produce bacteriocins that are antagonistic against undesirable organisms. *Lactobacillus acidophilus* LB (52), *Lactobacillus johnsonii* LA1 and *Lactobacillus casei* YIT9209 (53) produce antimicrobial substances against *Helicobacter pylori*, which is a human gastric pathogen. The antibiotic reuterin produced by *Lactobacillus reuteri* is effective against a wide range of organisms including pathogens (50). Control of undesirable microorganisms that produce carcinogenic metabolites in the intestines may also result in anticarcinogenic effects by limiting the production of such metabolites (50).

Some lactic acid bacteria have been shown to stimulate immune functions by activating macrophages, increasing the levels of cytokines, and increasing immunoglobulin levels (especially those of IgA). Organisms identified in such functions include *Lactobacillus johnsonii* LA1, *Lactobacillus* GG, and *Lactobacillus casei* Shirota, among others (48).

The improvement of lactose digestion, for which *Lactobacillus acidophilus* has particularly been identified, is useful for individuals who are not able to digest lactose. *Lactobacillus acidophilus,* unlike yogurt bacteria, is able to survive and grow in the intestinal tract. Here the increased permeability of the bacterial cells allows for the permeation of lactose and subsequent hydrolysis by beta-galactosidase (50).

A reduction in serum cholesterol has also been linked to certain lactobacilli, including acidophilus, johnsonii and casei (50, 54). This activity is believed to occur by the assimilation of cholesterol or via deconjugation of bile acids in the small intestine and subsequent excretion from the body. Bile acids are precursors for cholesterol; hence their removal from the body helps in reduction of cholesterol.

The therapeutic properties of traditional kefir have been identified as an increase in the excretion of urea and some nitrogen metabolism products (14) and the treatment of atherosclerosis, allergic disease, and gastrointestinal disorders (55). The polysaccharides are able to decrease the cholesterol content of blood and bind toxins. Anti-cancer effects of kefir have also been suggested (14). The inclusion of pre- and probiotics, as stated earlier, and colostrum products has expanded the therapeutic value of kefir.

Koumiss made from mares' milk has been used widely in Russia for many years for the treatment of tuberculosis (56, 57), and chronic enteritis and gastrointestinal disorders (58). The first hospital specializing in koumiss therapy was established in 1858 in Samara, Russia. The intestinal microflora is apparently normalized with koumiss therapy and partly because of the intense yeast fermentation, the vitamin B12 absorption and levels in blood are raised (59). The dose for adults is 1.5 liters per day and 0.4 to 0.8 liters for children (1). Koumiss is readily digested and assimilated because of its lower casein content and a larger proportion of peptones and free fatty acids that are generated during fermentation.

Many of these therapeutic properties of fermented milks are strain specific, i.e., not all strains have all of the above characteristics. In the manufacture of fermented milk, therefore, it is important to use specific strains if health claims are made (60, 61, 62).

ACKNOWLEDGMENT

The information in this chapter has been derived from "Fermented Liquid Milk Products," by V. V. Mistry. Editors: Y. H. Hui et al. IN *Handbook of Food and Beverage Fermentation Technology*, Marcel Dekker, New York, 2004.

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67 Semi-Solid Cultured Dairy Products: Principles and Applications

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CONTENTS

I. SEMI-SOLID CULTURED DAIRY PRODUCTS

A. Introduction

Fermented semi-solid dairy foods have a long history of consumption worldwide. This category of dairy foods that include yogurt, sour cream, fromage frais and many variations continue to show increased sale because of their healthful reputation and convenience. The $2.9 billion yogurt industry saw a 10.5% increase in sales from 1999 to 2000 (1). Within the yogurt category, new product entries are continually appearing in marketplaces that promote probiotic-enhanced benefits. Innovative packaging concepts that target convenience and fun also drive the market surge.

Classification of fermented semi-solid dairy products is difficult because of the wide variety of products that can be manufactured. Traditional dairy products such as

cheese varieties have been classified based on moisture content (2). Although widely used, this system suffers a serious drawback in that dairy products with different characteristics and manufacturing protocols are grouped together. In other attempts products are classified based on similarities that exist, such as starting ingredients, the type of fermentation and rheology (3). However, it is important to define rheology, which is the mathematics of a set of behaviors rather than as a set of materials (4). Rheology of a product is related to the flow of fluids and the deformation of matter. Texture is defined as the tendency to resist flow, and is often used interchangeably with rheology. Typically, texture relates to solid foods and viscosity relates to fluid products. Many foods can exhibit characteristics of both solids and liquids (5). Fluid milk and cream are considered liquids and hard cheeses are classified as solids. Semi-solid fermented dairy foods show an intermediate behavior, with properties of both solids and liquids, and can be defined as viscoelastic (6). As viscoelasticity characteristics are very dependent on the protein network structure formed by casein micelles (6), a great deal of research has focused on processing parameters that optimize a network structure that favors good rheological properties and desired firmness without syneresis.

In this chapter, yogurt, fromage frais and sour cream are considered semi-solid fermented dairy products based on the consensus in the literature. Typical manufacturing protocols for these products require raw milk selection, milk pretreatments, ingredient blending and standardization, heat processing, starter addition and other technological procedures to produce satisfactory products with desired body, texture, flavour and organoleptic and rheological properties. Keeping these aspects in mind, principles and applications of fermented milk manufacture are discussed under two sections, Section I involving selection of raw milk, milk pretreatments, ingredient addition, heat processing, role of lactic starter system, gel formation during fermentation and proactive phage control program. Section II is devoted to probiotics in semi-solid dairy foods covering health issues, probiotic cultures and product development and production of probiotic dairy foods.

B. Selection of Raw Milk

As the old saying goes — there is no substitute for good quality raw milk. This is also true for milk destined for fermented milk manufacture. Quality criteria for raw milk should be based on compositional quality of milk, microbial count, somatic cell count, freedom from inhibitory substances and reception temperature (7). In the U.S., grade A milk is required for fermented milk manufacture. Such milk has a standard plate count of less than 100,000 cfu/ml and a storage temperature not exceeding 7°C (within 2 hr of milking). Somatic cells are indicators of udder health and individual states have set the limits for acceptable somatic cell count (8). Generally somatic cell counts above 750,000/ml are not acceptable. A high somatic cell count is indicative of mastitis conditions; and milk may also be abnormal in composition. Leucocytes in milk may cause inhibition of lactic starters by phagocytosis and abnormal composition can influence other technological parameters affecting quality of resultant fermented milks.

Good quality raw milk is free from all kinds of inhibitory substances including residual antibiotics, traces of detergents, sanitizers or insecticides. Mastitis treatment of cows involves application of various antibiotics, and milk from treated cows may occasionally contain residual antibiotics if it is not withheld for the recommended period. The resulting low-level contamination may be sufficient to inhibit starter microorganisms (9); for example, penicillin at 0.01 IU/ml of milk will inhibit thermophilic starters. Apart from the possibility of partial or complete inhibition of starter bacteria due to antibiotic action, antibiotic testing is also mandatory for consumer safety reasons due to potential allergic reactions in sensitive individuals (10). High levels of residual detergents and sanitizers can inhibit starter bacteria. Under good sanitation practices, the amount of residual sanitizers that might enter into milk from milk contact surfaces is not sufficient to cause culture inhibition except when the sanitizer solution is not completely drained from tanks or trucks (11). Quaternary ammonium compounds (QAC) present a potential problem due to their residual activity, and lactic-acid bacteria are sensitive to low concentrations of QAC. For example, Lactococus strains are inhibited at 10 μg/mL level of QAC (12) and thermophilic cultures are inhibited at 0.5 to 2 μg/mL of QAC (13).

C. Milk Pretreatments

Commonly employed pretreatments in the fermented milk manufacture include filtration, clarification and homogenization.

1. Filtration

Filtration is carried out by pumping milk through specially woven cloth. This results in the removal of extraneous matter. Filtration of the recombined dairy powders is also helpful in the removal of undissolved or scorched particles from base mix. This may be further helpful in minimizing wear and tear to the homogenizer orifice and minimizing the deposition of milk solids on the heat exchanger plates.

2. Clarification

Clarification refers to the process of centrifugal separation carried out in a specially designed separator, called a clarifier. This process removes extraneous matter such as straw, hay and large clumps of bacteria and thereby improves the aesthetic quality of fermented milks. A specially designed centrifuge can be used to carry out bactofugation. This

process can reduce bacterial load by about 80–90%. It is especially effective for removal of spores and leucocytes from milk (~98%).

3. Homogenization

Homogenization is a process of attaining homogeneous emulsions. This is achieved by passing the milk under high pressure through a small orifice; the shearing effect reduces the average diameter of the milk fat globules to less than 2 μm. As a result, globules are less inclined to coalesce into large units and do not rise to form a cream line.

Homogenization is an important processing step in order to improve consistency and viscosity of yogurt due to incorporation of the finely divided fat globules within the coagulum structure. Further, coagulum stability also improves due to adsorption of casein micelles onto fat globules. The increased number of small fat globules tends to enhance the ability of the milk to reflect light, and as a result the fermented milk appears whiter.

Use of unhomogenized milk leads to fat accumulation on top of the packaged product which is disliked by most customers except in products where cream line is specifically required, e.g., crusty yogurt in Greece.

Milk processing for fermented milk manufacture typically employs homogenization pressure in the range of 10–20 MPa at 50–70°C temperature. Homogenization preceding heat processing is preferable to avoid risk of contamination after heat processing.

D. Ingredient Addition and Blending

Optional ingredients can be added to base mix to improve viscosity and to minimize syneresis in fermented milk products. Additionally, with the development of low-fat dairy products, stabilizers are often added to improve mouthfeel and to reduce calories (14).

1. Stabilizers

Stabilizers serve two basic functions when added to semi-solid fermented dairy foods. Good stabilizer systems will have the ability to bind water, react with milk proteins to increase their level of hydration, and stabilize protein molecules into a network that prevents the movement of water. Gelatin and hydrocolloids such as guar gum, pectin, carageenan and pre-gelatinized starch are the most common stabilizers (15). A stabilizer is added as a single component or as a blend. Blends of stabilizers have the benefit of overcoming a limiting property of a single stabilizer. It is important to balance the desired level of stabilizer with undesirable flavor changes the stabilizer may impart. The stabilizer concentration will vary widely depending on the type of system chosen. Processing conditions may need to be considered when choosing the stabilizer system.

2. Sweeteners

Products are typically sweetened by sucrose, honey, high fructose corn syrup (HFCS), and aspartame. Some manufacturers use apple and pear concentrates when a healthier image is desired (15).

3. Fruits, Colorants, and Flavorings

Addition of fruit, colorants and flavorings are also common in fermented semi-solid dairy products and are typically added following fermentation. Typically, heat-treated purees that range from 30–50° Brix are used. Because fruit is commonly added after the dairy product is heat treated, the microbiological quality is critical to the safety and shelf life of the product. In set yogurts, a layer of gelled or pureed fruit is placed into the container prior to addition of cultured milk (14).

4. Other Ingredients

Recently, other less common ingredients are making their way into fermented dairy products. Addition of vitamins and minerals has become a method for increasing nutritional value. Adding calcium to the fermented milk base has been difficult due to solubility and bioavailability issues. Recent patents for methods of increasing calcium in fermented dairy products focus on technology that overcomes the low solubility of calcium salts in milk (16).

5. Prebiotics

Prebiotics are defined as "non-digestible food that beneficially affects the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon" (17). Prebiotics, such as oligosaccharides, are therefore used to maximize the effectiveness of probiotic microorganisms. Based on their chemical structure, some oligosaccharides are resistant to digestive enzymes and are bioavailable to *bifidobacterium* in the large intestine as a carbon and energy source (18). Research suggests that fructo-oligosaccharides, lactose derivatives, galacto-oligosaccharides, and soybean oligosaccharides have commercial significance as prebiotic ingredients (19). Addition of the prebiotic fructo-oligosaccharide and galacto-oligosaccharide in combination with bifidobacterium in yogurt has been shown to increase the intestinal probiotic level by two log cycles. The current trend in yogurts marketed in Australia, Europe and Japan is to contain a prebiotic component (18).

E. Heat Processing

Pasteurization of milk is a legal requirement in the United States for manufacture of fresh cultured products (CFR Title 21, part 133:1995). Heat treatments typically employed for selected fermented milk products are depicted in Table 67.1.

TABLE 67.1
Typical Heat Treatments Employed for Fermented Milk Preparations

Product	Typical Heat Treatment(s)	Reference
Yogurt	90 to 95°C for 2 to 5 min	20
	80 to 85°C for 30 min	
Sour cream	74°C for 30 min	21
	85°C for 25s	
	80°C for 30 min	7
	90°C for 5 min	
Fromage frais	93 for 120s	

The most important impact of heat processing is microbiological. Due to heat treatment, most vegetative bacteria, yeast, molds and pathogens are destroyed ($\geq$5 log cycle reduction is required). This has a strong impact on safety and quality of the resultant product. Surviving spores are unlikely to cause problems, as *bacillus* and *clostridium* endospores do not germinate at the pH of most fermented milks, i.e., pH $<$ 4.5 (20). Heat-stable enzymes are reported to not pose a significant problem in semi-solid fermented milk products (21) with the exception of products like sour cream that may be critical for lipolytic or proteolytic spoilage. With regard to starter culture, reduced competition between surviving flora and starter organisms is beneficial for the growth of the latter. Due to high heat processing employed for milk destined for fermented milk preparation, germicidal activity of milk is destroyed along with natural inhibitors of milk making conditions more suitable for starter growth. Severe heat treatment denatures whey proteins in milk and α-lactalbumin and β-lactoglobulin become attached to casein micelles, especially k-casein. This leads to increased viscosity and enhanced water-binding capacity of coagulum (22).

F. Role of Lactic Starter System

In the limited context of fermented milks discussed in this chapter, starter culture systems can be defined as safe, pure, actively growing, selected lactic acid bacteria that bring about desirable changes to produce a satisfactory fermented product.

Typical starter microflora, inoculation rates, incubation temperature and incubation periods employed in fermented milk manufacture are summarized in Table 67.2. Typical starter flora for yogurt include: *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB). In the United States, the National Yogurt Association recommends the level of at least 10 million live yogurt bacteria per g at the time of consumption if manufacturers wish to display the "Live and Active Cultures" symbol on yogurt packages (23).

The relationship between ST and LB is best described as that of protocooperation and antibiosis (24) as the former is stimulated by free amino acids and peptides produced by LB (25). In turn, LB is stimulated by formic acid, pyruvic acid and carbon dioxide produced by ST at low oxygen concentration (26). As a result, acid production is significantly enhanced in mixed cultures compared to ST and LB grown individually, and yogurt gel tends to set faster. The unique relationship between the two starter organisms also makes yogurt flavour unique compared to other dairy products. Unique flavouring compounds in yogurt include acetaldehyde (optimum flavour and aroma at 23 to 41 ppm), acetone and butanone (contributed from milk), diacetyl, and acetoin (27). Presence of diacetyl (0.5 ppm) contributes to delicate and full flavor and aroma. Acetaldehyde production is mainly attributed to LB. However, in association with ST, acetaldehyde production is considerably enhanced as compared to LB alone (28).

Typical starter flora of sour cream or cultured cream includes: *Lactococcus lactis* ssp. *lactis* (*LL*) *and/or Lactococcus lactis* ssp. *cremoris* (LC)*, Lactococcus lactis* ssp. *lactis biovar. diacetylactis* (LD), *Leuconostoc mesenteroides* ssp. *cremoris* (LeuC). The main sensory attributes of cultured cream are sour taste (lactic acid), buttery aroma (due to diacetyl) and nutty flavor. Starter flora in sour cream exhibit positive (mutually beneficial) association. LD and LC can ferment citrate under aerobic conditions to produce diacetyl. Acetaldehyde in cultured cream is not desirable because it imparts "green" flavor. In such cases inclusion of LeuC in the starter system is advantageous as it scavenges acetaldehyde produced by lactococci. Conversely*, Leuconostocs* that lack proteolytic activity and do not grow well in milk grow better in association with *Lactococci*.

During sour cream manufacture when the citrate level is depleted, diacetyl is reduced to acetoin. Increasing oxygen content in milk may enhance production of diacetyl, however, addition of citrate in milk (0.05 to 0.1%) is a preferable option. Production of cultured cream requires proper selection of bacteria as well as manufacturing conditions to induce the right balance of acid and flavour, because flavor producers are fastidious in their temperature requirements. If the temperature of incubation is maintained much above 22°C, insufficient diacetyl will be produced (29).

Purity and activity checks are very important for optimum performance of the starter system. Purity can be checked by microscopic examination of culture suspension or a catalase test to detect contamination. Starter flora are typically gram-positive, catalase-negative, nonsporulating rod or cocci. An activity test or vitality test can be conducted by monitoring rate of acid development (30). For active cheese culture, acidity development is expected to be at least 0.4% lactic acid after 3.5 hr incubation at 37.8°C with 3% inoculation. For cultured cream and cheese cultures, the activity test can be run at 30°C instead

TABLE 67.2
Typical Starter Microflora, Inoculation Rates, Incubation Temperature, and Incubation Period for Selected Fermented Milk Products

Product	Starter Microflora	Inoculation Rate	Incubation Temperature	Incubation Period
Yogurt	*Streptococcus thermophilus* and	2–3%	40–45°C	2.5–4 hr
	Lactobacillus delbrueckii ssp. *bulgaricus*		30°C	18 hr
Sour cream	*Lactococcus lactis* ssp. *lactis* and or	1–2%	18–21°C	14–20 hr
	Lactococcus lactis ssp. *cremoris*			
	Lactococcus lactis ssp. *lactis bio* var. *diacetylactis*			
	Leuconostoc mesenteroides ssp. *cremoris*			
Fromage frais	*Lactococcus lactis* ssp. *lactis* and or	0.5–3%	27°C	14–16 hr
	Lactococcus lactis ssp. *cremoris*			
	Lactococcus lactis ssp. *lactis bio* var. *diacetylactis*			
	Leuconostoc mesenteroides ssp. *cremoris*			

of 37.8°C. For checking aroma-producing starter bacteria in a mixed culture, a Voges-Proskauer or creatine test can be used. It is also useful to detect gas producing contaminants in a non-gas-forming culture (31).

G. Gel Formation during Fermentation

During the manufacture of semi-solid dairy foods, an irreversible gel is formed due to the destabilization of the casein micelle. The gel can be classified into one of the following groups based on whether it is enzymatic, acid induced, or salt/heat induced (14).

The gel or coagulum formation in fermented semi-solid dairy foods is primarily of the acid type, similar to that of acid-set cheeses. In acid-set cheeses, however, syneresis is desirable, whereas in yogurt or other semi-solid fermented foods it is not desirable.

In studies investigating the acidification of skimmed milk with glucono-δ-lactone at 30°C, the following changes were observed in the casein micelles:

- pH 6.6–5.9, casein micelles were homogeneously distributed with no appearance of change
- pH 5.5–5.2, appearance of micellar disintegration and at pH 5.2 or less, aggregation of casein micelles was observed
- pH 5.2–4.8, casein aggregates contracted and micelles were much larger than the native form
- pH < 4.5, protein network formed with rearrangement and aggregation of casein consisting of micellular chains and clusters with trapped water and milk constituents in the network (32)

H. Proactive Phage-Control Program

Current trend in the fermented milk industry is consolidation of smaller plants in to larger plants and increased production volume. Starter suppliers are now offering ranges of options like direct vat set cultures or frozen concentrated cultures. As a result, the problem of bacteriophage and other microbiological risk factors has been reduced considerably. Nevertheless, preparation of bulk culture cannot be entirely eliminated due to certain practical considerations and in such situations bacteriophage contamination remains a distinct possibility, especially during in-house handling.

In light of this, a proactive phage-control program is necessary. Such a program can be very helpful in maintaining consistent starter performance and thereby in assuring consistent quality of fermented milks.

Many strategies employed in bacteriophage control are summarized below.

- Good whey handling and processing practices. Whey is a principal site where phage multiplication and buildup can occur.
- Adequate heat treatment of bulk culture milk, e.g., 95°C for 30 min to inactivate phages in milk.
- Use of phage-resistant starter cultures or multiple-strain starter or mixed-strain starter used in rotation. Where feasible, direct-vat inoculation with deep-frozen or lyophilized concentrated bulk cultures.
- Use of phage-inhibitory media or phage-resistant media for bulk culture propagation.
- Use of exopolysaccharide-producing strains that are more resistant to phages (33).
- Use of a mechanically protected (e.g., Lewis Jones Alfa Laval) system to maintain asepsis and to avoid contamination of bulk culture.
- Good sanitation practices to ensure freedom from phage build-up on vat surfaces and other potential milk contact surfaces. Chlorine is a better sanitizer compared to iodine or acid sanitizers for phage control.

II. PROBIOTICS IN SEMI-SOLID DAIRY FOODS

A. Introduction

In the early 1900s, Nobel Prize-winning scientist Élie Metchnikoff was the first to propose that acid-producing organisms in fermented dairy foods offered a prolongation of life by preventing fouling in the large intestine (34). From his idea evolved the idea of functional foods as a marketing term in the 1980s. This term is used to describe foods fortified with ingredients capable of producing health benefits. The trend for healthful foods is becoming increasingly popular with consumers because of the awareness that diet can influence health. Probiotic foods are defined as foods containing live microorganisms in significant concentrations that actively enhance the health of consumers by improving the balance of microflora in the gut when ingested (18). This restricts probiotics to products that contain live microorganisms, improve health and well being of humans or animals, and can affect host mucosal surfaces, including the mouth and gastrointestinal tract, and the upper respiratory tract, and the urogenital tract (35). In Japan, a standard for probiotic foods was developed stipulating that a product contain more than 1×10^7 viable *bifidobacteria*/g or mL of product (36). The probiotic must be viable and available at a high enough concentration to effect health benefits (18). These factors are often overlooked or ignored and many of the commercially available probiotic products have shown low populations of probiotics (37).

Fermented dairy products have evolved as the predominant carriers of probiotics in foods. They already have a healthful image that facilitates recommendations of daily consumption, and consumers are aware that fermented foods contain live microorganisms (38). Many of the fermented dairy foods have been optimized for survival of the fermentative microorganisms, which creates important technological advantages for the use of dairy products as probiotic carriers (39).

B. Health Issues

Foods that help prevent disease are becoming increasingly popular with consumers, with 70% of American shoppers believing that certain foods contain components that reduce the risk of diseases and improve long-term health (35). Additionally, 97% of shoppers surveyed in the United Kingdom were willing to change their eating habits to better their health. The benefits of probiotics include inflammatory disease control, the treatment and prevention of allergies, cancer prevention, immune stimulation, and respiratory disease reduction. Many of the suggested benefits of probiotic dairy foods are based on the involvement of the gastrointestinal microflora in resistance to disease. Unfortunately, few well-controlled studies have looked at clearly defined health effects, and many of the reported health benefits are based on unsubstantiated reports. However, the use of probiotics for the treatment of lactose maldigestion, diarrheal disease, lowering of serum cholesterol, and prevention of cancer or formation of carcinogens appear to be well-substantiated (41). Lactose intolerance or maldigestion causing abdominal discomfort affects approximately 70% of the world's population to varying degrees (35). The probiotic bacterium *Lactobacillus acidophilus* has been clinically shown to alleviate the symptoms of lactose intolerance. The recent work in this area indicates that lactose in yogurt, possibly due to lactase-producing bacteria, can be utilized more efficiently than lactose in milk. Additionally, many of the probiotic strains that produce lactase have shown promise in alleviating symptoms of lactose intolerance (40).

Rotavirus is one of the leading causes of gastroenteritis worldwide (41). Gastroenteritis characterized by acute diarrhea and vomiting is a leading cause of death and illness among children, affecting approximately 16.5 million children annually (35). Ample evidence has shown that the probiotic strain *Lactobacillus GG* reduced the duration and severity of rotavirus infection. Oral administration of *Bifidobacterium bifdum* has shown potential for reducing the incidence of diarrhea in infants hospitalized for rotavirus infection (41). Other research suggests that the probiotic bacteria *Bifidobacterium longum* and *Saccharomyces boulardii* prevent antibiotic-associated diarrhea (35).

Much attention has been given to the cholesterol-lowering potential of probiotics. However, the influence of lactic-acid bacteria such as *Lactobacillus acidophilus*, although widely studied, remains controversial (42).

The role of lactic acid bacteria in reducing the incidence of DNA damage and other carcinogenic changes has also been investigated (43). In addition, microflora and composition of the intestinal flora with probiotics might suppress the growth of bacteria that convert procarcinogens to carcinogens. An important index of carcinogenic activity is the activity of enzymes that convert procarcinogens to carcinogens. Several studies have shown an inhibitory effect on these enzyme activities following the consumption of probiotics (41).

Additional research on the prevention or delay of tumor development by probiotic bacteria suggests that they might bind to mutagenic compounds in the intestinal tract. A reduction of mutagens in urinary excretions was found when meals were supplemented with fermented milk containing *Lactobacillus acidophilus*. This suggests that lactobacilli were binding to the mutagenic compounds, thereby reducing their absorption in the intestine. It is inconclusive whether this will lead to a decreased incidence of cancer, but research looks promising (41).

C. Probiotic Cultures

LB and ST are the bacteria traditionally used in yogurt manufacture. While these bacteria have some health benefits, they do not naturally inhabit the gastrointestinal tract.

They do not survive the acidic conditions of the stomach and the bile concentration of the GI tract (18). The probiotic bacteria used currently in commercial products include members of the genera *Lactobacillus (Lb) and Bifidobacterium (B).* The Lactobacillus (Lb) strains that are mainly used in commercial production include: *Lb. acidophilus*, *Lb. johnsonii, Lb. casei, Lb. rhamnosus, Lb. gasseri, Lb. reuteri, Lb. helveticus* and *Lb. plantarum. Bifidoacterium bifidum, B. longum, B. infantis, B. breve* and *B. lactis* are some of the *bifidobacterium* strains that are used commercially (38). Therefore, to be considered a probiotic product, many of these strains are incorporated into the fermented dairy product. Typically, combinations of these strains are used. A product manufactured entirely with probiotic bacteria would require a long incubation time and lowered taste quality. Therefore, it is common practice to manufacture products with both yogurt and probiotic cultures (39).

D. Product Development and Production of Probiotic Dairy Foods

1. Food Interactions and Probiotics

There are several basic requirements for the development of marketable probiotic products. The most basic requirement is that the probiotic bacteria survive manufacturing and storage in sufficient numbers in the product so that they are viable when consumed. Additionally, the probiotic of choice should not have an adverse effect on the sensory properties of the food product. The chemical composition of the food product will also be important in determining the metabolic activity of the probiotic and how successfully it will survive. The amount and type of carbohydrate available, the degree of hydrolysis of milk proteins (amino acid content) and the composition and availability of short-chain fatty acids are essential variables in developing a probiotic semi-solid dairy food.

The interaction between the probiotic organisms and the starter organisms is another important factor to consider in the production of semi-solid fermented dairy foods. Both synergistic and antagonistic effects between organisms exist. This will require the identification of specific strains of probiotics that work in synergy with the starter organisms to achieve efficient acidification and multiplication of organisms during the fermentation process. Antagonistic effects that cause the production of undesirable compounds, such as hydrogen peroxide, benzoic acid and bigenic amines, have been investigated and can be a limiting factor for combinations of starters and probiotics (38).

A critical factor in the development of probiotic dairy foods is the manufacturing procedure. When the probiotic is added following fermentation and before or after cooling, the interactions are kept to a minimum. The metabolic activity of both the starter organisms and the probiotic is drastically reduced at this point and will dramatically reduce interactions. It is critical to maintain cold storage of the product without fluctuations in temperature in order to maintain the quality of the finished product (38).

Another factor of considerable importance is the physiological state of the probiotic. When the probiotic culture is harvested, whether it is in the logarithmic or the stationary phase of its growth and how it is handled following harvesting are critical to it successful addition to a food product. Bacteria from the logarithmic phase have been shown to be much more susceptible to environmental stresses. Environmental conditions that signal to the bacteria the transition from the logarithmic phase to the stationary phase will have an effect on survival during the stationary phase.

2. Production of Semi-Solid Dairy Foods with Probiotic Properties

Because each semi-solid dairy product is manufactured differently, there is considerable variation with respect to starter culture, processing temperatures, ingredients and fermentation time and temperature. All of these factors influence the successful production of fermented dairy products containing probiotics. The methods used to manufacture stirred yogurt and yogurt drinks are best suited to probiotic incorporation following fermentation. Immediately before packaging, the probiotic can be stirred into the product. With set yogurts, the probiotic must be added during fermentation, because stirring in the final stages of manufacturing can destroy the products' consistency (38). The chosen strain must first meet the technological and functional requirements and thus might compromise the full expression of the health properties.

The addition of probiotics to the manufacturing of cottage cheese can also vary. When the probiotic is added with the starter culture during fermentation, a high amount of the probiotic organism will be lost from the coagulum during draining of the whey. It is therefore very difficult to control the concentration of probiotics in the final product. During manufacturing, the whey–coagulum mixture temperature is raised to 50–55°C; this temperature can be destructive to the probiotic organisms. The addition of the probiotics with the cream and spices in the final stages of manufacturing offers the best chance of survival for the probiotic bacteria (38).

III. CONCLUSIONS

Despite considerable advances in scientific understanding of semi-solid cultured dairy products, their manufacture involves fine art and skill. A full understanding of the complex interplay between biological, chemical and technological entities is slowly emerging. Cultured dairy products are different from other dairy products in that they contain deliberately added live lactic acid bacteria with or

without other probiotic bacteria. Therefore, the choice of starter cultures and control of the fermentation process are key parameters in producing quality products. The demand for probiotics-based foods is growing. However, it can be expected that consumer confidence will decrease, unless the dairy industry offers products that have been scientifically proven to exhibit probiotic effects. Thus, additional research on the mechanisms by which probiotic cultures exert their effects on the host is required.

ACKNOWLEDGEMENT

The information in this chapter has been derived from "Semi-solid cultured dairy products: principles and applications," by D. Patel and M. Walker. In Editors: Y. H. Hui et al. *Handbook of Food and Beverage Fermentation Technology*, Marcel Dekker, New York, 2004.

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68 Principles of Production of Cheese

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CONTENTS

Cheese, which can be considered as a nutritious and delicious concentrate of valuable nutrients of milk in a stable form, has been produced since prehistoric times. The basic steps of modern cheese production are the same as empirically based cheese making thousands of years ago. The present scientific knowledge on cheese production, however, has been developed mainly during the last 125 years.

I. BASIC PRINCIPLES OF CHEESEMAKING

A. Concentration

The first step in making cheese is coagulation of milk. Milk can be coagulated by the action of rennet enzyme or by acidification. The coagulum has a natural tendency toward syneresis — contraction and expelling of whey (i.e., water and water-soluble compounds of the milk (Fig. 68.1)). Larger particles such as fat globules and bacteria will be retained in the shrinking coagulum, the cheese curd. The syneresis can be accelerated by cutting up the gel and by heating. The resulting firm curd can be pressed and formed into cheeses of various shapes.

B. Preservation

The fresh curd contains valuable nutrients and can therefore be quickly spoiled by bacteria, yeasts and moulds if not preserved. The main factors contributing to preservation are low moisture content (concentration), acidification by adventitious or added lactic acid bacteria, and salting. Spoilage of the cheese by moulds on the surface can be prevented by drying and cleaning the surface or by exclusion of oxygen by immersion in brine or by coating the cheese with wax or synthetic film material.

C. Ripening

Cheese may be consumed as fresh curd or it may undergo ripening by means of enzymes from the milk, from the rennet and from microorganisms in the cheese or on its surface. During ripening, which may last for months, the organic solids of the cheese are gradually hydrolysed and metabolized, during which time the characteristic flavour, consistency and texture of the cheese is developed.

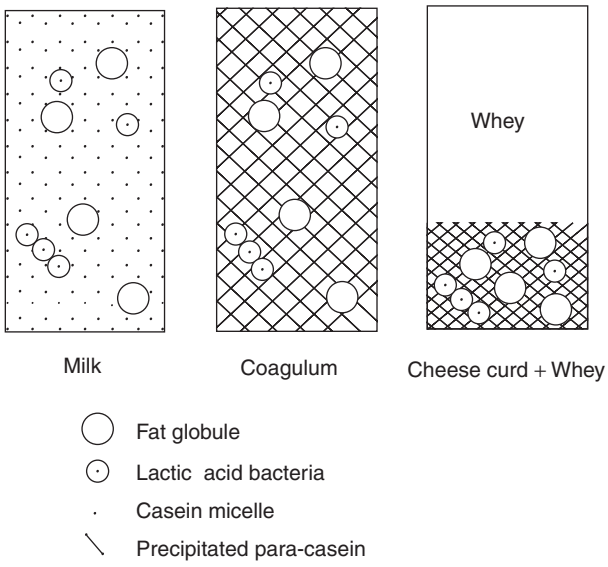

FIGURE 68.1 Outline of the structure of milk, rennet coagulum and cheese curd (1).

In Table 68.1 some of the important types of cheeses are listed and grouped according to method for coagulation of the milk, their firmness and the degree of acidification.

II. YIELD OF CHEESE

The majority of cheeses produced in the world are made from cow's milk. Substantial amounts of cheese, however, are also produced from milk of sheep, goat and buffalo. Many of the milk components vary quantitatively, both between and within species. Estimated mean component figures are shown in Table 68.2.

TABLE 68.1
Cheeses Grouped According to Method of Coagulation, Firmness, and pH Minimum

Coagulation of Milk By	Firmness (Moisture Content on Fat-Free Basis, MFFB)	Acidification, pH of Young Cheese	Examples of Cheese Types
Rennet	Extra hard (<51% MFFB)	5.10	Grana
	Hard (49–56% MFFB)	5.30	Emmentaler
		5.10	Cheddar
	Semihard (54–63% MFFB)	5.20	Gouda, Danbo, Edam, Jarlsberg, Herrgård, Fontina, Svecia, Havarti, Tilsiter
			Heated after acidification: Mozzarella, Kashkaval, Provolone
	Semisoft (61–69% MFFB)	6.10	Heated in whey: Halloumi
		5.20	Heated after acidification: Soft Mozzarella
		5.15	Esrom, Limburger, St.Paulin
		4.70	Blue-veined mould cheeses: Roquefort, Gorgonzola, Stilton, Danablu
	Soft (>67% MFFB)	4.60	White mould cheeses: Camembert, Brie
			Ripened in brine: Feta
			Milk salted: Domiati
Acid	Semisoft (61–69% MFFB)	4.50	Matured sour milk cheese:Gamal ost, Pult ost, Harzer Käse, Olomoucke tvaruzky
	Soft (>67% MFFB)	4.50	Fresh unmatured curd: Homogenous curd: Tvorog, Quarg, Skyr Curd in grains: Cottage cheese
Made from cheese whey:			
Proteins of whey are precipitated by heating			Ricotta, Anari, Whey protein cheese
Solids of whey concentrated by evaporation			Myse ost, Whey cheese

TABLE 68.2
Estimated Mean Figures for Gross Composition of Main Solids of Milk

Species	Fat	Total Protein	Lactose	Citrate	Ash
Cow	3,8	3,3	4,7	0,2	0,7
Buffalo	7,4	3,8	4,8	N.D.	0,8
Goat	5,6	3,8	4,8	N.D.	0,7
Sheep	7,4	5,5	4,8	N.D.	1,0

N.D.: not determined.
Adapted after Edelsten (2)

A. Recovery of Milk Solids and the Yield of Cheese

When milk is coagulated, either by rennet or by acid, the caseins form a three-dimensional network enclosing all the other milk constituents. When the coagulum contracts, water and water soluble constituents will be squeezed out, whereas fat globules and bacteria will be retained in the casein network as outlined in Fig 68.1.

1. Retention of Protein

The caseins, constituting about 75 to 80% of the milk proteins, are the basic structure-forming elements of coagulated milk and cheese curd. "Casein" is defined as those proteins of milk which become non-soluble when milk is acidified to pH of 4.6 (i.e., α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein and γ-casein; see Table 68.3). The caseins in milk are aggregated in micelles 0.01 to 0.3 μm in diameter, and of the milk salts, much of the calcium, phosphate, magnesium and citrate are associated with these micelles. When cheese is made by means of rennet enzymes, coagulation takes place at about pH 6.6. The coagulation is brought about by enzyme-catalysed hydrolysis of a single peptide bond of κ-casein:

κ-casein ----> para-κ-casein + glyco-macro-peptide (GMP).

The glyco-macro-peptide (GMP) constitutes about one-third of the mass of the κ-casein. The para-κ-casein component of the κ-casein remains as a part of the para-casein in the curd. The GMP is soluble and separates with the other soluble compounds (whey proteins, lactose, water-soluble vitamins and the soluble salts) into the whey. Typical figures for the amount of the various groups of milk proteins as percent of total nitrogen, and their distribution on the non-soluble part (in the cheese curd) and the soluble part (in the whey) after precipitation of casein with acid at pH 4.6 or with rennet at pH 6.6, are listed in Table 68.3; 76% is given as a typical value for retention of protein for rennet cheese. Variations in the relative amounts of the various protein fractions can cause actual protein retention values to vary from about 75 to 78% of total protein (N x 6.38).

Heat treatment of the milk can cause denaturation of the whey proteins. Heat-denatured whey proteins precipitate together with the caseins, giving higher protein retention. *Higher* retention of protein can also be achieved by

TABLE 68.3
Typical Values for Distribution of Nitrogen Compounds (Protein Nitrogen and Nonprotein Nitrogen) of Whole Milk in the Nonsoluble Versus the Soluble (Whey) Components after Precipitation of Casein by Acid at pH 4.6 or by Rennet at pH 6.6

Percent of Total Nitrogen				
After Precipitation at pH 4.6			**After Precipitation by Rennet at pH 6.6**	
	Non-soluble		*Non-soluble*	
Fat globule membrane protein (FGMP)[a]	1		1	Fat globule membrane protein (FGMP)
α_{s1}- and α_{s2}-casein	37		37	α_{s1}- and α_{s2}-casein
κ-casein	10	→ para-κ-casein 6.5	6.5	Para-κ-casein
		→ GMP 3.5		
γ-casein[b]	3		3	γ-casein
β-casein[b]	27		27	β-casein
			1.5	Non-soluble proteose-peptone
Total *acid casein* + FGMP	78		76	Total rennet casein + FGMP
	Soluble		*Soluble*	
			3.5	Glyco-macro-peptide (GMP)
Proteose-peptone (pp)[b]	3	→ Non-soluble 1.5[c]		
		→ Soluble 1.5[c]	1.5	Soluble proteose-peptone
Whey proteins	13.5		13.5	Whey proteins
NPN (non-protein-N)	5.5		5.5	NPN (non-protein-N)
Total *acid-whey*-N	22		24	Total *rennet-whey*-N

[a] When cheese is made from whole or part skimmed milk the proteins of the fat globule membrane will be incorporated in the network of the precipitated caseins.

[b] I milk a part of β-casein may be hydrolysed by the action of the milk enzyme plasmin resulting in γ-caseins (the more hydrophobic parts of β-casein) and proteose-peptones (the more hydrophilic parts).

[c] A part of the proteose-peptones, which all are soluble at pH 4.6 , is precipitated with the caseins at pH 6.6. The partition of proteose-peptones on the soluble and the non-soluble parts, after precipitation by rennet at pH 6.6, which in the table is set to 1:1, may be somewhat lower or higher (3, 4).

Source: Data from various sources.

concentrating the protein by ultrafiltration. *Lower* retention of protein may be caused by inflammation of the udder or by growth of psychrotrophic, proteolytic bacteria during cold storage of cheese milk.

2. Retention of Fat and Bacteria

The fat globules with diameters 0.5 to 10 μm will be incorporated into the casein network. Consequently, the retention of fat is high (85 to 95%).

For bacteria the retention is of the same magnitude as for the fat globules.

3. Retention of Lactose

Lactose is dissolved in the water phase of milk and follows the whey out of the curd; hence retention of lactose in curd is low, usually in the range of 3 to 5%, depending on the final moisture content of the cheese and on the amount of water added.

4. Retention of Ash Compounds

Some of the minerals (K^+ Na^+, Cl^-) in milk are dissolved in the aqueous phase and these minerals separate with the whey. Others are present as colloidal particles integrated into the casein micelles (Ca^{2+}, Mg^{2+}, PO_4^{3-}). At the pH of fresh milk, these salts remain in the curd, but as the pH decreases during the syneresis these salts gradually become dissolved.

Some of the phosphate is covalently bound in the casein molecules as serin-phosphate. This phosphate, which accounts for about 2½% of the mass of casein, will be included in the ash in analysis of both milk and cheese, a fact which should be taken into consideration in calculations of cheese yield. The figures found for retention of ash typically are about 35 to 40% for hard and semihard rennet cheese. For Cheddar cheese, with a higher degree of acidification during syneresis, retention of ash is lower — 30 to 35%. For cheeses with acid precipitation of the casein at pH around 4.6 the retention of ash is low — 10 to 15% of total ash.

5. Retention of Citrate

Most of the citrate in milk is in aqueous solution, with 10% linked to the caseins together with calcium in the same way as with inorganic phophate. The retention of citrate therefore depends partly on the whey content of the cheese and

TABLE 68.4
Example of Calculation of Milk Solids in Cheese Curd, Yield of Cheese, and Composition of Cheese, Based on Typical Figures for Composition of Cheese Milk and for Retention of Milk Solids in Cheese Curd

	Milk Solids in Cheese Curd			
	In 1 kg Milk	Retention in Cheese Curd	Gross Calculated for Cheese Curd from 1 kg Milk	Net Calculated for Cheese Curd from 1 kg Milk, with Correction the Part of the Phosphate in the Ash Originating from Serine-PO4
Fat (standardized to give minimum 45% fat in dry matter of cheese)	30.0 g	0.92	27.60 g	27.60 g
Protein	34.0 g	0.76	25.84 g	25.84 g
Lactose (anhydride)	46.0 g	0.035	1.61 g	1.61 g
Ash	7.2 g	0.35	2.52 g	1.87 g
Citrate	1.8 g	0.10	0.18 g	0.18 g
Total solids in fresh cheese curd from 1 kg milk				57.10 g

Yield of cheese:
In addition to the milk solids, the cheese after salting includes extraneous NaCl. If the cheese after salting cheese contains 43% moisture and 57% dry matter, of which 2% NaCl and 55% milk solids, the yield of cheese per kg milk will be:
$57.1 \times 100/55 = 103.8$ g.
Then the composition of the salted cheese can be calculated:

Composition:		%
Fat:	$27.60 \times 100/103.8 =$	26.59
Protein:	$25.84 \times 100/103.8 =$	24.89
Lactose: (converted to lactic acid in cheese)	$1.61 \times 100/103.8 =$	1.55
Ash:	$2.52 \times 100/103.8 =$	2.43
Less the casein-PO_4 included in ash:		−0.62
Citrate:[a]	$0.18 \times 100/103.8 =$	0.17
Total milk solids in cheese		55.0
Plus extraneous NaCl		2.0
Total dry matter in cheese		57.0
Moisture in cheese[b]		43.0

[a] In cheeses like Danbo with citrate fermenting starter bacteria, the citrate is converted within 1 to 2 weeks to volatile compounds such as carbon dioxide, diacetyl and acetic acid.

[b] Under storage and ripening the moisture content of the cheese (and the weight of the cheese) will decrease because of evaporation, unless the cheese is packed in synthetic film or covered with cheese wax.

partly on the acidification during syneresis. For semihard cheeses the retention of citrate is typically around 10%.

B. Calculation of Composition and Yield of Cheese

Table 68.4 shows an example of the calculation of yield of cheese and of composition of cheese. The example is based on typical figures for production of a semihard cheese, e.g., Danbo or Gouda.

III. MILK FOR CHEESE

For production of cheese the milk has to be of good quality.

A. Storage of Milk for Cheese

Unless the cheese is made shortly after milking, the milk should be kept cool until the start of cheese production in order to prevent excessive growth of bacteria. During storage at low temperatures the rennetability may be slightly reduced.

B. Pasteurization

Cheese is often made of raw milk, and it is a common assumption that cheese made of raw milk of good quality can be more rich in flavour. Some pathogenic bacteria, however, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, can survive in cheese for months, and in many countries pasteurization of milk for cheese is mandatory. Pasteurization at 72°C for 15 seconds typically kills about 99% of the bacteria of the raw milk. Bacterial spores, however, will not be killed. About 95% of milk lipase will be inactivated at 72°C for 15 seconds, a fact which may help to explain a part of the assumed richer flavour of raw milk cheese. Most of the whey proteins remain unchanged by pasteurization at 72°C for 15 seconds, but at slightly more intensive heat treatment, the whey

proteins begin to denature. Denatured whey proteins precipitate together with the caseins by the action of rennet, or by acid at pH 4.6, yielding a higher total retention of the proteins. For most cheeses, however, a heating greater than 72°C for 15 seconds is normally not applied, because more intensive treatment entails certain drawbacks: slower renneting of the milk and a weaker coagulum; inactivation of the enzyme xanthine oxidase, which catalyzes the reduction of added nitrate to nitrite; and slower development of cheese flavour and texture during ripening.

C. Homogenization

For some types of cheese, e.g., Danablu and Feta produced by ultrafiltration, homogenization may be applied in order to whiten the cream and increase the water-binding capacity of the cheese curd. Homogenization also increases the lipolysis of the fat, provided active lipase is present.

D. Bactofugation and Microfiltration

These processes are widely used in the production of cheese types liable to damaging fermentation by the spore forming bacteria *Clostridium tyrobutyricum* (e.g., Emmental and Gouda/Danbo cheeses). With *bactofugation* a large part of the bacteria, and more than 90% of the spores, can be removed from the milk. With *microfiltration* more than 99.9% of all bacteria and spores can be removed from the skim-milk part of the milk. The cream of the milk then has to be heat-treated separately, as the fat globules otherwise would block up the filter membranes.

In both processes a part of the bacterial spores follow the cream, adhering to fat globules. In order to produce cheese with a low number of *Clostridium tyrobutyricum* spores, the cream can be pasteurised at about 120°C for a few seconds, prior to mixing cream with skim milk from bactofugation or with permeate from microfiltration. Both the retentate from microfiltration and the bactofugate from bactofugation contain most of the spores from the milk and also a part of the casein, and for utilization of this casein, the retentate/bactofugate can be mixed with the cream before the heat treatment.

E. Ultrafiltration

By ultrafiltration, milk can be separated into a *retentate* in which fat, bacteria, casein micelles and whey proteins are concentrated, and a *permeate* containing water and lactose. Salts linked to the caseins in the micelles (most of the calcium and phosphate and a part of the citrate) will be concentrated in the retentate, whereas other salts will follow the water into the permeate. Ultrafiltration thus resembles the natural filtration process in the curd grains in traditional cheesemaking, in which the casein network acts as a filter, with the exception that the ultrafiltration membranes also retain the whey proteins. Concentrating milk for cheese by ultrafiltration is utilized in large scale for production of UF-Feta cheese, where the retentate, with about 40% total solids after addition of starter culture and rennet, can be *cast* directly into tins. Because the whey proteins are retained in the retentate, the yield of cheese by ultrafiltration is higher than in conventional cheesemaking. By ultrafiltration of a part of the milk, the protein content of the cheese milk can be increased and standardized for conventional cheese production.

F. Standardization of Fat Content

Cheeses are usually classified by percent fat in dry matter (FDM) according to legal standards. The fat content of the cheese milk has to be adjusted according to the percent FDM desired and according to the protein content of the milk.

IV. ADDITIVES TO CHEESEMILK

A. Colouring Agents

The colour of cheese is determined by the carotene colour of the milk fat, which may vary with the seasons and with type of milking animals. Jersey milkfat, for example, is usually high in carotene, while sheep milk fat is low. Where colouring is permitted, the colour of cheese may be adjusted by means of annatto extract or carotene dye.

B. Salt

All cheeses are salted, either by addition of salt to milled curd after acidification and before moulding or by absorption of salt by diffusion after the cheese has been formed. A special case is Domiati cheese, where 8 to 15% salt is added to the milk, yielding coagulum and resulting cheese that is very soft.

C. Spore-Inhibiting Additives

For cheese of Gouda/Danbo type there is a risk of *Clostridium tyrobutyricum* spore growth during ripening, resulting in excessive gas production and the development of an unpleasant smell. The spores can be inhibited by means of nitrate added to the milk (0.1 to 0.2 g KNO_3/L); the nitrate is reduced to nitrite (NO_2^-) which inhibits the spores. This reduction is catalyzed by the milk enzyme xanthin oxidase, which is active in milk heated to about 72°C for 15 seconds but inactive at a slightly higher heat treatment. Other possible additives for inhibition of spores are the enzyme lysozyme (from hen's egg white) and nisin (bacteriocin). The use of spore-inhibiting additives is legally restricted in cheese-producing countries.

D. Starter Cultures and Acidification

For most cheese varieties, starter cultures of lactic-acid bacteria are added to the cheese milk. Where the curd is either

TABLE 68.5
Optimal pH Minima for Various Cheeses

Cheese	pH Minimum
Emmental	5.25
Gouda/Danbo group	5.20–5.25
Tilsiter/Havarti	5.15–5.20
Mozzarella	5.15–5.25
Cheddar	4.95–5.10
Feta, Danablu	4.65–4.7
Camembert	4.60–4.70

not heated or heated to about 40°C, starters with mesophilic *Lactococcus* sp. and *Leuconostoc* sp. are used. If formation of carbon dioxide is undesirable (e.g. for Cheddar cheese, and for Feta cheese destined to be put in tins) mesophilic starters without citrate-fermenting *Leuconostoc* sp. and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (=*Lactococcus diacetylactis*) are utilized. In cheeses scalded at higher temperatures (e.g. Grana, Emmental), lactic-acid fermentation is performed by thermophilic cultures of *Streptococcus thermophilus* and *Lactobacillus helveticus*.

Starter cultures can be local natural starters or laboratory cultures from culture manufacturers either for propagation in the dairy or, as frozen or lyophilized starter concentrates, for direct inoculation in the cheese milk. The amount of starter culture added to the milk is typically about 1% for starter propagated in milk and about 0.01–0.05% for starter concentrates.

Slow acidification due to antibiotics in the milk or the development of bacteriophages can cause serious problems. Bacteriophages will not be destroyed by heat treatment of the milk at about 72°C for 15 seconds, so there will always exist a risk of development of bacteriophages, along with the starter bacteria, in a cheese factory. Counteractive measures include careful cleaning and disinfection with chlorine or disinfectants with peracetic acid (hydrogen peroxide has no effect on bacteriophages), preventing any contamination of milk and curd with whey, propagation of starter culture in highly heated milk in a separate room, the use of starter cultures with good bacteriophage resistance, and if necessary, change of starter culture. Fast development of lactic acid bacteria is of utmost importance for cheese quality. For each type of cheese there is an optimal range for minimum pH; Table 68.5 gives some examples.

The effects of lactic acid bacteria are:

1. *Coagulation and syneresis:* The lactic acid promotes the action of rennet enzyme and the acidification of the curd increases syneresis. In the production of sour milk cheese (Quarg, etc.) the milk is coagulated only by the acid produced by the starter bacteria.
2. *Inhibition of detrimental bacteria:* Lactic acid bacterial growth inhibits harmful bacteria by fermentation of the lactose and by the formation of lactic acid. Some of the lactic acid bacteria also produce bacteriocins which may inhibit other bacteria.
3. *Control of consistency:* The acidification controls the consistency and texture of the cheese. If the pH becomes too low, much of the calcium is dissolved from the casein network and the cheese will be brittle and less coherent. If the acidification is weak, little calcium is dissolved and the cheese mass will be rubbery. For semihard, sliceable cheese, the best consistency is obtained with a fresh cheese pH at about pH 5.2.
4. *Control of enzyme activity:* pH determines the activity of the various enzymes of the cheese during ripening.
5. *Taste:* The lactic acid is important for the fresh acid taste of young cheese.
6. *Low redox potential:* The lactic acid bacteria lowers the redox potential to about −150 mV, yielding cheese that can be kept for a long time without the development of off-flavours owing to oxidation of milk fat.
7. *Formation of proteolytic enzymes:* The lactic-acid bacteria have proteolytic enzymes important for the breakdown of proteins during ripening. Intracellular peptidases, released after autolysis of the cells, are responsible for the formation of free amino acids; starter bacteria may also contribute to conversion of amino acids into various flavour compounds.
8. *Formation of CO_2 and diacetyl:* If the starter contains citric-acid-fermenting bacteria, such as *Leuconostoc* sp. and *Lactococcus diacetylactis*, the citric acid will be converted to acetic acid, diacetyl/acetoin, and CO_2.

E. The Course of the Acidification of Cheese

As described above, acidification is of fundamental importance for the quality of cheese. The velocity of the acidification is also important. A too-rapid drop in pH may cause the cheese to become too sour and too hard (increased syneresis); on the other hand, slow acidification gives the opposite effects, and there will be a risk for growth of detrimental bacteria. Fig. 68.2, left, illustrates the rate of acid development during the first 24 hours for a typical semihard cheese such as Gouda/Danbo, for a blue-veined cheese (Danablu) and for milk, all starting with inoculation with 1% mesophilic starter culture at 30°C. Fig. 68.2, right, shows the rise in pH for both types of cheese during ripening owing to release of ammonia by the breakdown of proteins and by degradation of lactic acid. The faster increase in pH during ripening for Danablu compared to

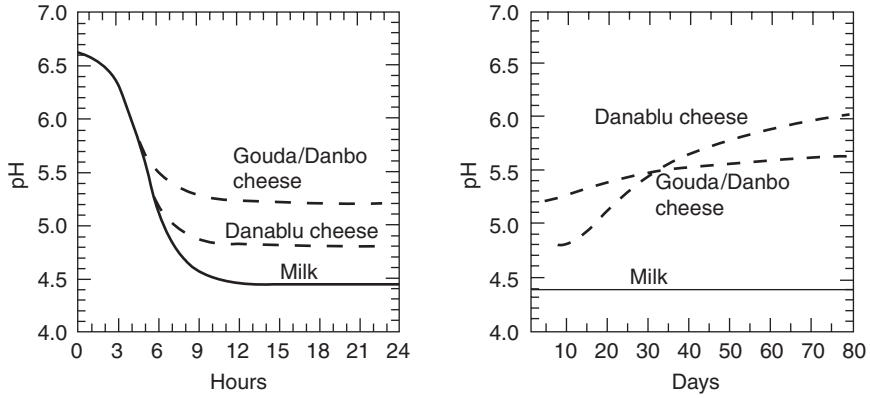

FIGURE 68.2 The course of acidification for a Gouda/Danbo cheese, for a blue-veined cheese (Danablu) and for milk, respectively, during the first 24 hours after inoculation with 1% mesophilic starter culture, all starting at 30°C (left). The graph to the right show pH changes during storage and ripening. The fall in pH during the first 24 hours is caused by the fermentation of lactose into lactic acid by the lactic acid bacteria. The rise during storage is caused by release of amino acids and ammonia by breakdown of proteins and by degradation of lactic acid. The bottom curve applies to milk inoculated with lactococci and which is protected from infection by yeasts and moulds during storage (1).

Gouda/Danbo is a result of very active proteolytic enzymes from the mould and aerobic degradation of lactic acid. In milk acidification stops at about pH 4.4 because the bacteria are completely inhibited by lactic acid at low pH, although there will still be surplus of nonfermented lactose. In cheese the inverse situation prevails: The growth of the bacteria and the decrease in pH end when all of the lactose is used up, not because pH has become too low.

For the Gouda/Danbo cheese the pH levels off at about pH 5.2, while for the blue-veined Danablu cheese pH continues to decrease until a minimum pH at 4.7. This difference is due to a difference in the lactose/buffering capacity ratio. The amount of lactose determines the amount of lactic acid formed, and the amount of buffers (i.e., protein and inorganic phosphate) determines the change in pH for a given amount of acid. In the young blue-veined cheese there is a higher moisture content compared to the Gouda/Danbo cheese, hence a higher content of lactose. At the same time, the blue-veined cheese has a lower content of buffers (protein and inorganic phosphate) than the Gouda/Danbo cheese.

A further important factor which contributes to a higher pH minimum in Gouda/Danbo cheese compared to the blue-veined cheese is that for Gouda/Danbo, water is added to the mixture of whey and cheese grain in order to dilute the whey, thereby reducing the lactose concentration.

However, the pH minimum is not solely a function of the ratio lactose/buffers. As long as the curd grains are dispersed in the whey, lactose can diffuse from the whey into the grains in replacement of that which has been fermented into lactic acid during stirring. (About 90% of the lactic bacteria are concentrated in the curd grains, hence the lactose fermentation mainly takes place here). Consequently, the total decrease in pH may be seen as two phases: Phase one, the decrease of pH in the curd grains until their final separation from the whey, and phase two, the decrease in pH in the cheese curd after it has been finally separated from the whey; in this phase the decrease in pH is determined solely by the lactose/buffers ratio.

An approximate calculation of pH minimum:

$$\text{pH minimum} = \text{pH}_1 - 0.8298 \times (\text{L/B}) \times (\text{pH}_1)^2 + 24.89 \times \text{pH}_1 \times (\text{L/B})^2,$$

where pH_1 is pH at moulding/start of pressing (when cheese grains are finally separated from the whey), L is % lactose hydrate of the pressed cheese, and B is buffering capacity = % protein of the pressed cheese + 19 x % inorganic phosphate (5). For Cheddar cheese the addition of salt to the milled curd may, depending on the concentration of salt, delay or stop the acidification before all lactose has been completely fermented (5). In the model it is assumed that all lactose is converted to lactic acid. Lactose is calculated here as hydrate; therefore, one weight unit of lactose corresponds to one weight unit of lactic acid.

Calculation:

$$\text{L} = \frac{\text{Lm(100-A(100-Tw-h*Pw)/(100-Tm-h*Pm)) *((100-Tc-h*Pc)/100)}}{\text{(100-Tm-h*Pm)-A(100-Tw-h*Pw)/100 + W}},$$

where Lm is % lactose hydrate in milk, Tm and Pm are % total solids and % protein, respectively, in milk, Tw and Pw are % total solids and % protein in whey, and Tc and Pc are % total solids and %protein in cheese (24 h). A is the amount of whey (% of milk by volume) removed during stirring,

W is Water (as % of milk by volume) added to the mixture of whey and curd grains during stirring. h (exclusion factor (2)) is the amount of water bound to protein in such a way that it cannot function as solute for lactose. h can be set to 0.3 g H_2O per g protein (6).

F. Non-Bacterial Acidification

In some countries it is allowed for certain types of cheeses (eg. Quarg, Cottage Cheese and Mozzarella) to acidify the curd by phosphoric, acetic or citric acid, or by gluconodelta-lactone which is slowly converted to gluconic acid.

G. Other Microorganisms

1. *Propionibacteria.* For Emmental cheese and other types with large eyes, cultures of *Propionibacterium* sp. are added in order to initiate fermentation of lactic acid to propionic acid, acetic acid and CO_2, the latter contributing to the formation of the large holes.
2. *Moulds.* For blue-veined mould cheeses, spores of *Penicillium roqueforti* are added to the milk. For white mould cheeses, spores of *Penicillium camemberti* and other *Penicillium* species are added to the milk or onto the surface of the cheese.
3. *Secondary flora of lactic acid bacteria.* In semi-hard cheeses the number of starter bacteria culminates at about 10^9 cells per gram after 1–2 days. Thereafter these bacteria gradually die out and autolyse. During the first month of ripening 99% of the lactococci may die out, and simultaneously a secondary flora of lactobacilli develops and may grow to a number of 10^7–10^8 cells per gram (7). These lactobacilli, mainly belonging to the group of facultatively heterofermentative lactobacilli (e.g., *Lactobacillus casei/paracasei*) develop spontaneously in the cheese and are found only in low numbers, e.g., 1–10 per mL or less, in pasteurized cheese milk; they may originate from the flora in the cheese factory or they may be lactobacilli surviving pasteurization. Pediococci have also been found in the secondary flora. Although the presence of a secondary flora in cheese has been known for more than hundred years, knowledge of their growth and their effects on the cheese is still relatively scarce. There are indications that the secondary flora may have positive effects on the quality of cheese, for example, by inhibition of detrimental bacteria such as heterofermentative lactobacilli (8) and *Clostridium tyrobutyricum* (9) and by the consumption of oxygen diffusing into the cheese (10).

H. Rennet

The primary effect of rennet is coagulation of the milk. Later, rennet enzymes play an important role in the hydrolysis of proteins during ripening. The coagulating activity of *calf rennet*, or *standard rennet*, is due to the enzyme chymosin. Traditionally rennet is made from the fourth stomach of calves, abomasum, which is sliced and extracted in a weak-acid/salt solution. The extract is filtered and the pH adjusted to 5.5. Finally, 15–20% salt and benzoic acid is added. Milk-coagulating enzymes can also be extracted from stomachs of oxen or from other animals, by the fermentation of certain moulds, or by microorganisms into which the gene for chymosin has been cloned.

1. The Enzymatic Coagulation Process

The coagulation takes place in two phases. First a negatively charged part of the κ-casein (1/3 of the κ-casein molecule) is split off by hydrolysis (catalysed by the rennet enzyme) of one peptide bond (no. 105, phenylalanin/no. 106, methionin) in κ-casein. The casein micelles thereby lose a part of the negative charge which otherwise prevents the micelles from coagulating. The casein (paracasein) is now insoluble in the presence of Ca^{2+}. In the second phase of renneting the paracasein micelles aggregate by hydrophobic attraction between hydrophobic amino acid residues in the caseins. With 30 mL standard rennet per 100 L and at 30°C, the first phase takes about 10 minutes and the second phase about 1 minute. It then takes about 20 minutes before the coagulum is sufficiently firm for cutting.

I. Addition of Calcium Chloride

Addition of $CaCl_2$, 5 to 20 g per 100 L, can increase the rate of renneting partly because the addition of $CaCl_2$ produces a slight reduction in pH.

V. PRODUCTION OF CHEESE CURD

After the milk has formed a firm coagulum, the next steps (cutting, stirring and heating/scalding) have the purpose of controlling the syneresis of the curd in order to achieve the appropriate level of moisture.

A. Cutting

The coagulum is cut in grains by means of knives or fine steel wires. The size of the grains influences the syneresis — the finer the cut, the lower the moisture content in the cheese. For soft cheese the grain size is 15 mm or larger, for semi-hard cheese 5 to 10 mm, and for hard cheese 2 to 5 mm.

B. Stirring

After cutting, whey begins to be squeezed out of the grains and the syneresis begins. The mix of whey and grains is

then stirred, at first gently, in order to enhance syneresis. The stirring is continued until the grains have reached the desired firmness. The stirring may take from one to two hours. The main factors for the syneresis are: fat content of milk, size of the grains, pH and temperature during stirring. Lower fat, smaller grains, lower pH and higher temperature increase syneresis.

C. Heating/Scalding

Heating during stirring is usually not applied for soft and semi-soft cheeses; however, for semi-hard cheese heating to 34 to 38°C is typical, for hard/semi-hard cheeses to 37 to 40°C and for hard cheeses 50 to 56°C (scalding). The heating/scalding can be done by heating the mixture of whey and grains by steam in the jacket of the cheese vat or by the addition of hot water to the whey. Addition of water reduces the lactose content of the curd and, consequently, the amount of lactic produced in the pressed cheese. Another effect of heating/scalding is that the lactic-acid bacteria, and their acid production under stirring, will be inhibited because the temperatures applied are above their optimum.

If a higher heating temperature for Gouda/Danbo cheese is used, the starter bacteria will grow more slowly in the cheese grains, and the pH at the end of stirring/moulding will consequently be higher. Although higher pH during stirring will in itself *reduce* syneresis, the higher heating temperature will *increase* syneresis. Experience shows that the net result will be lower moisture content in the cheese. A higher heating temperature will also cause a higher pH minimum of the cheese, because the cheese grains will have a higher pH at the end of stirring/at moulding and because the pressed cheese has a lower moisture content, and consequently, a lower content of lactose. In summary: Higher heating temperatures for a semihard cheese such as Gouda/Danbo type will result in a more firm, less acid cheese.

D. Final Treatment of the Cheese Curd

When the cheese grains have reached the desired firmness they must be separated from the whey. The curd can then be put directly in moulds or it can be given further treatment before final moulding.

On the whole, a few different moulding methods are used for the many varieties of cheese:

- Curd grains are pressed under whey before moulding (round-eyed cheeses).
- Curd grains are pressed under whey before moulding and boiled in whey (Halloumi cheese).
- Grains are separated from the whey before moulding (granular cheeses, soft cheeses).
- Grains are separated from the whey and the curd is left a few hours for continued acidification (cheddaring) and then milled, salted and moulded (Cheddar, Stilton) or milled and then warmed and kneaded before moulding (e.g. Mozzarella).
- After being concentrated by ultrafiltration and added rennet etc., the cheese is cast directly in moulds or in the package (e.g., UF-Feta).
- Sour-milk cheese: After acidification of the milk to about pH 4.6 the whey is separated from the precipitated curd, which can then be packed for consumption (Tvorog, Quarg, Skyr) or formed into small cheeses and ripened (Olmützer quargeln, Harzer käse, Pult ost).
- Grains of acid precipitated coagulum, gently cut and stirred and heated, are separated from the whey and the loose grains are packed for sale (Cottage cheese).

The following describes more in detail some of the above-listed principles for handling the curd.

1. *Round-eyed cheeses:* The curd grains are collected under whey, avoiding mixing with air, lightly pressed and cut in pieces, one for each cheese, placed in moulds, and finally pressed for 1 to 2 h or more. The moulds are equipped with clothes or perforated for drainage of whey and for formation of a closed rind on the cheese. This method results in a closed texture with only microscopic bubbles of air. The closed texture is an important factor for obtaining the desired structure of round-eyed cheeses. The starter cultures used for semi-hard round eyed cheeses, e.g., Gouda/Danbo, contain bacteria which in 1 to 2 weeks can ferment the citric acid, thereby producing CO_2.

   Initially CO_2 is dissolved in the cheese but eventually the combined pressure of CO_2, and that of the N_2 dissolved in the cheese begins to expand the largest of the microscopic air bubbles. If the velocity of the formation of CO_2 is suitable, the gas will expand only a few of the largest air bubbles because of the physical law

$$\text{Pressure in a bubble} = \frac{\text{surface tension}}{\text{radius of the bubble}},$$

   which states that less gas pressure is required for enlarging a large hole than a small one (11). The same applies for large-eyed cheeses, e.g., Emmental and Jarlsberg, where the eyes are expanded by CO_2 produced by propionic-acid bacteria by fermentation of lactic acid.
2. *Halloumi cheese.* After stirring, the grains are pressed under whey, then cut in 300-gram pieces, put into boiling whey and kept at about 90°C

for 30 minutes. After cooling, the Halloumi cheese is stored in brine.

3. *Granular cheeses, soft cheeses*. The whey is drained from the curd grains, which are then scooped into moulds. If the grains are relatively firm, as in semi-hard cheeses, e.g., Tilsiter, air will be mixed in between the grains, which then cannot fuse completely; therefore a large number of tiny air pockets will be incorporated, giving a granular texture. When CO_2 is formed during ripening, the gas enlarges the air pockets, producing irregularly shaped holes.

   In blue-veined cheeses, e.g., Roquefort and Danablu, the air pockets, enlarged by CO_2 from bacteria and yeasts, make up the spaces in which *Penicilium roqueforti* can grow provided atmospheric oxygen is given access via openings in the cheese. If the curd grains are large and soft when put in moulds, as for Camembert and Brie, the texture of the cheese can be without mechanical openings between the grains. Production of CO_2 may produce holes, but most of the CO_2 will diffuse out of small-sized cheeses. Soft cheeses are usually pressed only by their own weight.
4. *Cheddar and "Pasta filata" cheese — Provolone, Mozzarella and Kashkaval*. The curd grains are separated from the whey and the curd is left for a few hours for continued acidification (cheddaring) until a pH value about 5.1 to 5.3. Then the curd is milled into chips.

   For Cheddar, salt is added (2–2½%). Some of the whey, which dissolves about 25% of the added salt, is squeezed out from the chips, while the remaining 75% of the salt is absorbed in the cheese. Finally the curd is hooped, pressed, wrapped in plastic film and packed in cartons and placed in storage for ripening.

   In the production of Provolone, Mozzarella and Kashkaval, the chips of milled, acidified curd are conveyed or shovelled into a container filled with hot water or brine at 75 to 85°C and the curd is kneaded until it is smooth and elastic. The curd then is formed in moulds and cooled.

VI. SALTING OF CHEESE

Salt is important for conservation of cheese and for a balanced, pleasant taste and for consistency. For many cheeses salting also plays a role in reduction of the moisture content. For most semi-hard and semisoft cheeses, 1.5 to 2% NaCl is suitable. For Emmental and other large-eyed cheeses the salt content is lower, 0.8 to 1.4% , in order to facilitate the growth of propionic-acid bacteria. For blue-veined cheese, 3 to 3.5% NaCl is suitable.

The salting can be achieved either by adding salt to the curd before moulding, or by diffusion of salt into the cheese after moulding, by immersion in brine or by dry salting. The lactic-acid bacteria are sensitive to salt and will be severely inhibited if the cheese is salted before acidification has finished.

A. Salting before Moulding

For Cheddar cheese and Stilton, the curd is allowed to continue acidification until about pH 5.2 during the cheddaring process. Then the curd is milled, salted and moulded.

B. Salting after Moulding

For most other cheeses made without a cheddaring process, the curd is put in moulds and pressed shortly after the end of stirring. At that point the pH typically will be about 6.2 to 6.4, and, if more than a small amount of salt is added, the growth of starter bacteria and the acidification will be severely inhibited; the formed cheese therefore has to be salted by absorption of salt from dry salt or from brine.

1. Dry Salting

For dry salting the surface of the cheese is rubbed with dry salt or a salt paste.

2. Brine Salting

For salting in brine, cheeses are immersed in brine (20 to 22% NaCl) at 10 to 15°C. Salt is taken up by the cheese while at the same time some moisture is forced out. The loss of moisture, which is higher than the uptake of salt, gives the cheese a firm rind. The uptake of salt and loss of moisture depends on the geometrical dimensions of the cheese, its moisture content, and its fat content and on the concentration of the brine.

Some examples for salting duration in brine with 20 to 22% salt:

- Grana cheese, high cylinder, 45 kg, 1.7% NaCl: 3 weeks.
- Emmental cheese, flat cylinder, 100 kg, 0.8 to 1% salt: 3 days.
- Semi hard cheeses (Gouda/Danbo etc.), 2 to 12 kg, 1.6 to 2.2% NaCl: 1 to 3 days.
- Soft cheeses (Camembert, Brie etc.), 0.1 to 1 kg, 1.5 to 2.5% NaCl: 0.5 to 3 hours.

The uniform diffusion of the absorbed salt throughout the cheese may take up to several week depending on the weight, moisture content and dimensions of the cheese.

VII. RIPENING OF CHEESE

Although cheeses like cottage cheese and Quarg are consumed fresh, most cheeses require a ripening period (from

a few weeks up to several years) for development of characteristic flavour, consistency and appearance. The changes during ripening are brought about by enzymes and microorganisms. The effects of the enzymes and the microorganisms depend on pH and temperature, on the content of moisture and salt and the surface treatment.

Examples of regimens for storage for ripening:

- Emmental: 2 weeks at 12°C; 3 to 6 weeks at 20 to 24°C; then at 8 to 10°C.
- Cheddar: 4 to 12 months at 6 to 10°C.
- Semihard cheeses, Gouda/Danbo, etc.: 2 to 4 weeks at 12 to 18°C; then at 6 to 10°C.
- Blue-veined cheese: 3 to 5 weeks at 8 to 12°C; 1 to 3 months at 2 to 6°C.
- White mould cheese: 1 to 2 weeks at 12 to 15°C; 1 to 2 weeks at 6 to 10°C; 2 to 6 weeks at 2 to 5°C.

A. Fermentation of Lactose and Citric Acid

The fermentation of lactose to lactic acid by lactic-acid bacteria and the fermentation of citrate by citrate using bacteria in DL-starters are described in the sections above. In cheeses such as Emmental and other large-eyed cheeses lactic acid is further fermented by propionic acid bacteria to propionic acid, acetic acid and CO_2.

B. Proteolysis

The caseins are gradually hydrolysed by rennet proteinase enzymes and by plasmin, an indigenous milk protease, which yields a number of large polypeptides, but which do not influence the flavour of the cheese (some hydrophobic peptides may be astringent or may have a bitter taste, however). These first steps of the proteolysis are important for changing the structure of semi-hard cheeses from a rubber-like to a sliceable consistency.

Some of the peptide bonds in the polypeptides can be further hydrolyzed by enzymes from the lactic-acid bacteria to yield smaller peptides and free amino acids, contributing to the basic taste of cheese. Amino acids may be further converted to smaller molecules — ammonia, organic acids, amines, esters, low molecular sulphur compounds, etc., contributing to the aroma of ripened cheese.

1. Extent and Depth of Proteolysis

The speed of the breakdown of proteins and the amounts of resulting compounds produced during ripening are very different for the various kinds of cheese and depend on a range of factors: Type and amount of rennet, type of starter bacteria, level of moisture, pH of the cheese, salt percentage and storage temperature.

The progression of proteolysis can be monitored by electrophoresis, by HPLC and by separation of the casein and its breakdown products into groups according to their solubility. In the classical ripening analysis the *extent* of proteolysis is defined as the amount of total nitrogen which is soluble at pH 4.4 (12). The *depth* of the ripening is defined as the amount of the nitrogen present as amino acids and low molecular nitrogen compounds. The amount of free amino acids can be estimated by nitrogen determination in the filtrate after precipitation with phosphotungstic acid (13) or by a titration with formaldehyde (14) or by other methods (15).

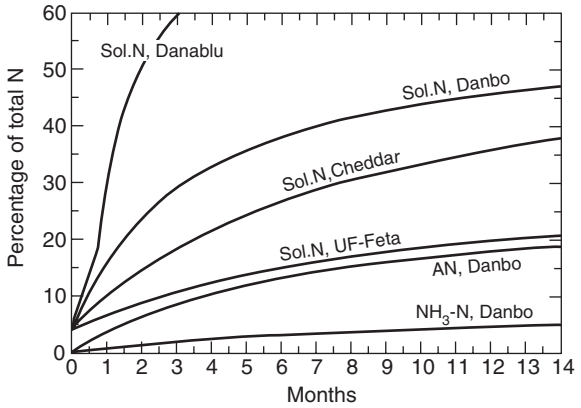

FIGURE 68.3 Typical development of soluble nitrogen at pH 4.4 during ripening as percent of total nitrogen for Danablu cheese, for semihard Danbo cheeses and similar Danish cheeses with moderate smear ripening, for Cheddar cheese and for Feta cheese (made by ultrafiltration). For Danbo and similar Danish cheeses curves are also shown for typical development for amino nitrogen (determined by precipitation with phosphotungstic acid or by formol titration) and for NH_3 nitrogen during ripening. (Based on data from (5) and (17)).

Fig. 68.3 shows curves for soluble nitrogen production during ripening for blue-veined Danablu cheese, for semi-hard Danbo cheese with moderate smear ripening, for Cheddar cheese and for Feta cheese. For the Danbo curves are also given for amino nitrogen (determined by phospho-tungstic acid or by formol titration) and for NH_3 nitrogen. (Based on data from (5) and (16)).

2. Surface-Ripening

Aerobic microorganisms may develop on the surface of cheese. If the surface is moist, bacteria and yeasts may form a red-brownish smear, in which protein and fat is catabolized, producing fatty acids, amino-acid breakdown products such as ammonia, and various low molecular sulphur compounds; these compounds diffuse into the cheese and impart a spicy flavour to the cheese, the intensity of which depends on the growth of the surface flora, the dimensions of the cheese, its moisture content, and the temperature and duration of surface ripening.

3. Feta and Similar Cheeses

Feta and similar cheeses are acidified to a low pH, about 4.4 to 4.6, and kept in brine. The enzymes of rennet retain

some activity; the peptidases of the lactic-acid bacteria, however, are practically inactive at this low pH. Hence proteolysis in Feta is rather limited, and the amounts of free amino acids and ammonia produced are low and there is no increase in pH during ripening.

4. White Mould Cheeses

Like Feta, fresh white mould cheeses are low in pH, about 4.5 to 4.7. The ripening of a Camembert cheese is dependent on the growth of white mould on its surface, which consumes lactic acid and produces ammonia from proteins. The pH of the rind soon rises to pH >7, and a pH gradient is created from the surface to the center (17). The pH increases gradually throughout the cheese, thereby activating peptidases of the lactic acid bacteria and releasing amino acids from the peptides. The ammonia and other metabolic products from the mould diffuse into the cheese and contribute significantly to the flavour.

5. Blue-Veined Mould Cheeses

The pH of fresh blue-veined cheeses is rather low, about 4.6 to 4.8, so a complete ripening process is dependent on the growth of mould, analogous to that described above for white-mould cheeses. *Penicillium roqueforti* is able to grow at rather low oxygen tension, hence it can develop in openings and caves inside the cheese, if there is oxygen access via perforations of the cheese. The moulds in blue-veined cheeses produce highly active protein- (and fat-) degrading enzymes.

C. Lipolysis

By lipase enzymes the triglycerides of milk fat can be hydrolysed into free fatty acids and glycerol. The short-chain fatty acids have a sharp, pungent flavour, which although undesirable in milk and butter, may contribute to a balanced flavour in some ripened cheese because of these fatty acids and other metabolites, such as metyl ketones and esters. In some cheeses, lactones, produced from hydroxy acids of the fat, may also contribute to flavour.

1. Lipolytic Activity in Cheese

a. *Milk lipase.* The indigeneous lipase of milk can produce a significant lipolysis in cheese made from raw milk (Table 68.6). Pasteurization of the milk at 72°C for 15 seconds inactivates about 95% of the milk lipase.
b. *Rennet and starter bacteria.* Standard rennet do not contain lipase and the starter cultures of lactic acid bacteria do not produce enzymes which can liberate fatty acids from milk triglycerides.

TABLE 68.6
Examples of Typical Acid Degree Values for Cheese Fat (16)

Cheese	Acid Degree* (BDI)
Non hydrolyzed milk fat	0.5–0.8
Flavour threshold value for milk	1.2
Semi hard cheese from raw milk, ripened 6 months	3–5
Semi hard cheese, made of pasteurized milk of good quality, ripened 6 months	1–2
Semi hard cheese, made of pasteurized milk with 500,000 of psychrotrophic bacteria per mL before pasteurization, ripened 6 months	3–5
Blue-veined cheese (Mycella) made of raw milk, ripened 3 months	10–20
Blue-veined cheese (Danablu) made of raw homogenized milk, ripened 3 months	30–50

* mL 1 N alkali per 100 g fat

Consequently there will be only slight lipolytic acitivity in cheese made of good quality pasteurized milk.

c. *Added enzyme preparations.* Traditional rennet paste, made by macerating the stomachs and pregastric regions of lambs or kid goats, contains pregastric lipases with high specificity for the liberation of short-chain fatty acids, which in cheese can contribute to a piquant flavour, e.g., Provolone and Feta (18).
d. *Lipases from psychrotrophic bacteria.* In raw milk psychrotrophic, Gram negative bacteria may produce lipases (and proteases). These bacteria are killed by pasteurization, but their enzymes may persist (19). If the milk is high in psychrotrophic bacteria before pasteurization, thermoresistant bacterial lipases can split fat during ripening. Concomitantly an unclean flavour may develop, owing to thermoduric protein degrading enzymes from the psychrotrophic bacteria.
e. *Lipases from surface microflora.* Bacteria and yeasts in the smear on surface-ripened cheeses can hydrolyse fat on the surface and the liberated fatty acids can diffuse into the cheese (20) and contribute to the flavour, in combination with ammonia and other low molecular compounds from degraded proteins of the surface smear.
f. *Mould lipases.* The moulds of blue-veined and white-mould cheeses produce very active lipases. However, in Camembert, for example, the contribution of free fatty acids to the flavour is reduced because of the dissociation of the acids at the high final pH.

2. The Influence of Homogenization on Lipolysis

Homogenization increases the effect of lipase dramatically. If raw milk is homogenized, e.g., for blue-veined cheese, then significant lipolysis, catalyzed by the milk lipase, will occur quickly in the cheese vat. The combined effect of milk lipase and the mould lipases during ripening is also increased by homogenization of the milk (21).

3. Extent of Lipolysis

The hydrolysis of fat can be measured by titration of fat extracted from the cheese, e.g., by boiling with BDI-reagent after adjustment of pH to 4 (21). This method recovers about 70% of the free fatty acid. Table 68.6 give some examples for acid degree of fat from various cheeses.

D. Finishing the Cheese

Hard and semi-hard cheeses after a stable rind has been formed are often given a layer of cheese wax in order to prevent further loss of moisture. For semi-hard cheeses with surface ripening, the smear should be removed after 2 to 3 weeks, and the cheese dried carefully, before waxing can be applied. Mould cheeses, soft cheeses and formed, ripened sour-milk cheeses usually are packed in aluminium foil. Feta-type cheeses and Halloumi, kept in brine in containers, are sold directly from the container or packed in plastic bags with brine.

ACKNOWLEDGMENT

The information in this chapter has been derived from "Principles of production of cheese," by E. W. Nielsen. In Editors: Y. H. Hui et al. *Handbook of Food and Beverage Fermentation Technology*, Marcel Dekker, New York, 2004.

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69 Yeast

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CONTENTS

I. INTRODUCTION

The general public is often confused between baking powder, incorrectly called "chemical yeast," and baker's yeast, which is biological in origin. The availability of active dried yeast, a staple product, only adds to the confusion. The packaging is similar and contains a powder, whilst the baker retails or sells small 42-gram cubes of a semi-moist product, with a plastic appearance.

The craft baker, however, knows that the bread he makes will not be as light, or have the same texture and organoleptic properties as the first product. Bread is a symbolic food about which there is universal agreement, despite the different ways it is made, because a special production method is used: fermentation.

Fermentation is a 5,000-year-old process which was based on the renewal of a previously risen dough: the starter dough (levain). Since earliest antiquity, the manufacture of bread and beer went hand in hand. In the palaces of ancient Egypt, leavens were activated by collecting the foam which rose to the surface of mash tubs. Pliny the Elder, a 1st century Roman naturalist, writes that the bread made in Gaul and on the Iberian peninsula owed its incomparable lightness to the addition of this foam. This method, which was taken up again in the 17th century to make a soft bread and was much talked about, was subsequently widely used (1). When brewers in the 16th century opted for low-fermentation yeast, instead of high-fermentation yeast, it was not at all suitable for bread manufacture. Distiller's yeast filled the gap. It soon opened the door in Europe to a fledgling industry, which expanded, particularly in Austria in 1846 with the Mautner process, then in England in 1886, with continuous aeration of a culture medium. This was the beginning of the biotechnology era, although people were unaware of this at the time. In fact, it was only in the years between 1857 and 1863 that Louis Pasteur demonstrated the role of yeast as the microorganism responsible for fermentation. The yeast industry made a crucial advance between 1910 and 1920 when Sak, a Dane, and Hayduck from Germany developed the continuous sugar-feed process in the presence of oxygen, known as "Zulaufverfahren" or feed-batch process (2).

TABLE 69.1
Yeasts for Different Baking Applications

Applications	Genus	Species
Multipurpose	*Saccharomyces*	*Cerevisiae*
High sugar doughs	*Saccharomyces*	*Rosei*
	Saccharomyces	*Rouxii*
Flavor enhancement	*Saccharomyces*	*Delbrukii*
	Candida	*Lusitaniae*
Sourdough starters	*Saccharomyces*	*Exiguus*
	Torulopsis	*Holmii*
	Candida	*Milleri*

Source: G. W. Sanderson (1985).

Worldwide production of baker's yeast was about 2.8 million tonnes a year in 2003. This is the largest quantity of microorganisms produced, thanks to the enormous technical and scientific progress this industry has been able to exploit and develop. As a result of the innovative processes it uses, the yeast industry has also supplied all the fermentation industries, which are a feature of biotechnology today: production of enzymes, amino acids, vitamins, and substances with a therapeutic use: hormones, antibiotics, vaccines, and more.

II. YEAST: A LIVING ORGANISM MADE UP OF A SINGLE CELL

A. BIOLOGY

1. Its Place in the Living World

The yeasts found in the flora of cereals belong to different genuses: *Pichia, Candida, Saccharomyces*. The latter is the most common comprising the species *cerevisiae* (80%), *exiguus, minor, uvarum, ellipsoïdus, turbidans* (3).

Although the *Saccharomyces* genus is generally used in brewing, wine-making and baking, the *cerevisiae* species does not give the best results in each of these applications. This is why *S. carlsbergensis* (which became *S. uvarum*) is used in the first instance and *S. bayanus* in the second. Within the *cerevisiae* species, some varieties have been improved by genetics, giving rise to new strains which are better suited to their specific applications. Although *Saccharomyces cerevisiae* is used throughout the world in bread-making, there are other groups of yeasts which have very specialized applications in baking (Table 69.1) (4, 5, 6).

Saccharomyces cerevisiae is undoubtedly the most cultivated yeast. All research and development work focuses on it because it has the cell characteristics of higher organisms, simple nutritional requirements, and a beneficial multiplication rate, although it does not multiply quite as fast as bacteria. From an economic point of view, its suitability for industrial production and its wide range of applications make it unique. Strains are generally stable, can be improved, and pose no risk in terms of food safety. Some of the different genuses and species of yeast listed above for special applications cause production problems, such as flocculation or very low yields.

Yeasts as a whole are microorganisms which have a high nutritional value and are completely harmless. However, a few of them are pathogenic. This applies to some species belonging to the *Candida* genus. Yeast producers are very cautious about using some genuses whose taxonomy is uncertain. They can be classified more accurately using very delicate genetic techniques when morphological or biochemical characteristics are not sufficient.

2. The Cell: Organized in a Similar Way to Higher Life Forms

A baker's yeast preparation, through the electron scanning microscope, reveals a multitude of distinctive cells (Fig. 69.1), ovoid in shape. Some of them have buds or scars which they have left. They vary in size from 6 to 8 micrometers. One cm^3 of compressed yeast with a 30% dry matter content contains about 10 billion cells!

Transmission electron microscopy reveals the following ultrastructure (Fig. 69.2), from the outside toward the inside:

- The cell wall: composed of glucans and mannans bound to proteins, it provides physical external protection. It is completely permeable to water, minerals and small organic molecules.

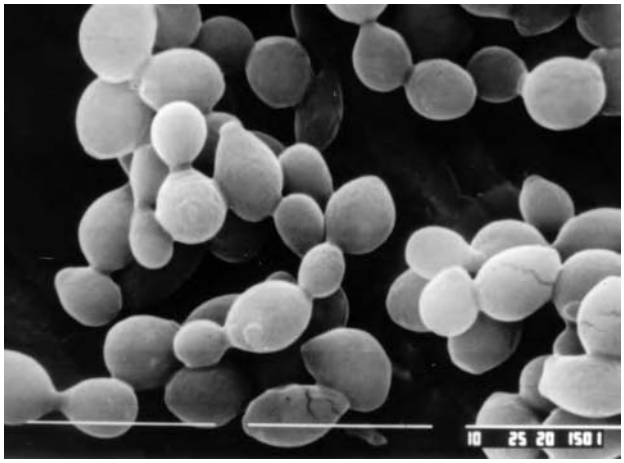

FIGURE 69.1

- The cytoplasmic membrane: made up of glycolipids and glycoproteins, it controls the exchanges between the intracellular and extracellular media. It is characterized by a selective permeability, i.e. it allows water and some solutions to circulate, whilst retaining the large molecules.
- The cytoplasm: a colloidal substance in which a multitude of biochemical reactions take place and which contains organelles in suspension:
  - The nucleus, which contains the chromosomes (carriers of genetic information) which transmit hereditary characters and control the synthesis of proteins.
  - The ribosomes, sites of protein synthesis.
  - The endoplasmic reticulum and Golgi bodies, a network of membranes involved in the secretion of proteins.
  - The mitochondria, energy-producing bodies in the cell in the presence of oxygen.
  - The vacuoles, places where different storage substances are found.

B. Biochemical Composition

The composition of yeast depends on its type and the conditions in which it is stored. Table 69.2 gives average indicative values for samples of fresh European yeast.

- The proteins contribute to a considerable degree of potential metabolic activity as they are made up of a high proportion of enzymes. The protein content is therefore directly related to fermenting power and the ability to produce biomass (Cf. § III.C. et IV.B.).

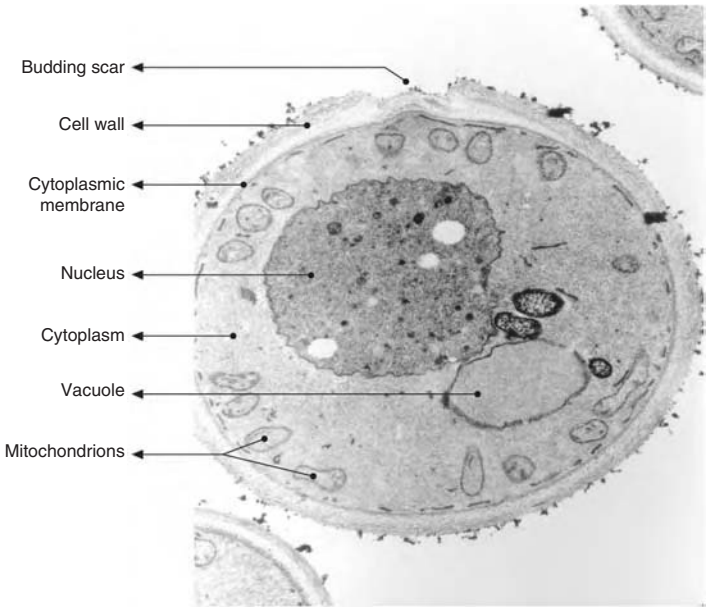

FIGURE 69.2

TABLE 69.2
Average Composition of European Yeasts

Dry Matter * (DM)	30.0 to 33.0 (%)			(%)
Nitrogen/DM	6.5 to 9.30			
Proteins/DM (nitrogen x 6.25)	40.6 to 58.0	of which	Glutathione	0.5 to 1.5
Carbohydrates/DM	35.0 to 45.0	of which	Glycogen	5 to 10
			Trehalose	8 to 20
Cell lipids/DM	4.0 to 6.0	of which	Phospholipids	1 to 2
Minerals/DM	5.0 to 7.5	of which	Potassium	0.8 to 2.0
			Sodium	0.01 to 0.2
			Calcium	0.02 to 0.15
			Magnesium	0.04 to 0.18
			Phosphorus	0.8 to 1.3
			(P_2O_5)	2.0 to 3.0
Vitamins		of which	Thiamin (B1)	0.002 to 0.015
			Riboflavin (B2)	0.002 to 0.008
			Pyridoxine (B6)	0.002 to 0.006
			Niacin (PP)	0.010 to 0.050

- The carbohydrates are mainly:
  - glucans and mannans, wall constituents;
  - glycogen, a storage macromolecule usually found in animal cells, which is used when there is a long-term deficiency of nutrients;
  - trehalose, a disaccharide which is called up preferentially when there is a short-term deficiency. The storage of this sugar is very important whenever the yeast cell has to undergo stress, such as drying, high osmotic pressure or freezing.
- The lipids, particularly lipoproteins and phospholipids, are involved in the make-up of the cytoplasmic membrane and in maintaining its properties in the various processes used for drying active dried yeast.
- The minerals, including phosphorus which is essential as it is involved in the formation of nucleic acids, molecules with a high energy potential (ATP) and membrane phospholipids.

Due to its high number of proteins with the presence of all the essential amino acids in addition to phospholipids, minerals and vitamins, deactivated yeast is regarded as a top quality food supplement.

III. BEHAVIOR OF YEAST DURING BREAD FERMENTATION

A. The Roles of Yeast in Bread-Making

1. Dough Rising

This is the most obvious phenomenon for anyone who is unfamiliar with bread manufacture. Air is incorporated into dough during mixing and the yeast is able to establish respiratory-type metabolism. A few minutes after mixing, all the oxygen which has been introduced is used up by the yeast. Due to the anaerobic conditions, yeast metabolism is geared towards fermentation. The carbon dioxide produced firstly dissolves in the free water in the dough. When it reaches a saturation point, it accumulates in gaseous form, exerting internal pressure on the impermeable gluten network. This network, which is elastic and extensible, enables the dough to rise, whilst the external structure is maintained. Contrary to what is generally thought, the pores in the crumb of bread have nothing to do with the distribution of yeast cells but correspond to the dilatation of the CO_2 they produce. This diffuses into the air bubbles which are incorporated and dispersed in the dough during mixing and various mechanical operations.

2. Acidification

The formation of carbon dioxide and organic acids results in a lowering of pH and an increase in the total titratable acidity (TTA) of the dough during fermentation, in spite of the high buffering capacity of proteins in the flour. This acidification confirms that bread fermentation is working properly. It is often measured (pH and TTA) in routine checks in industrial bakeries using prefermentation processes as water brew, stiff or liquid sponge. (Cf. § II.D.4.).

3. Flavor Production

The alcohol formed, the lowering of pH, and the release of metabolites from secondary fermentation are directly involved in, or act as precursors to, the development of bread taste and flavor (7, 8, 9). Long fermentation times, slightly low dough temperatures and sensible quantities of yeast result in a bread with excellent organoleptic properties. This assumes, of course, that the dough has not been subjected to excessive oxidation which is the result of high-speed mixing and the presence of bean or soya flour.

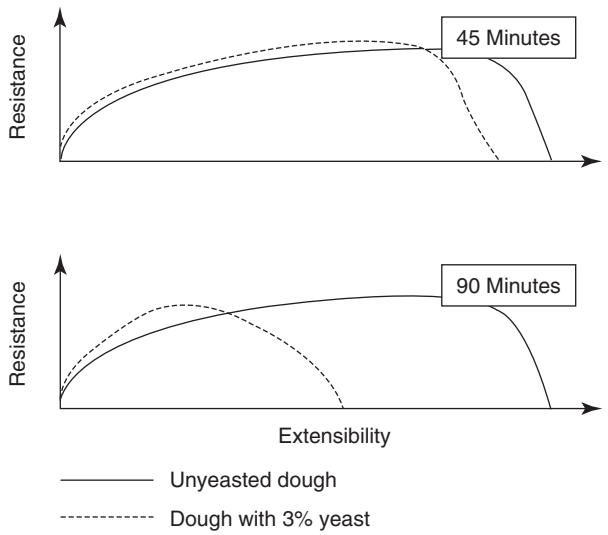

FIGURE 69.3

Particular genuses and species of yeast have metabolisms which result in bread products with very characteristic flavors (Cf. § 1.A.1.).

4. Change in Dough Rheology

Apart from the physical changes dough undergoes during the various operations of mixing, dividing, rolling or moulding, its viscoelastic properties are transformed throughout the fermentation process. This is a very familiar phenomenon to the craft baker, for whom the effects of bread fermentation do not simply mean the inflation of dough (10). The dough strengthening to which bakers refer, and which is occurring during bulk fermentation, is a perfect illustration of this. It involves a reduction in gluten extensibility combined with an increase in its elastic resistance (Fig. 69.3).

The causes are known but it is difficult to find a scientific explanation for the mechanisms because of the complexity of the dough system. It involves:

- Firstly, a purely mechanical effect which is the result of gluten development, namely, its extension and organization into a three-dimensional network under gaseous pressure;
- Secondly, the formation of physicochemical bonds, strengthening the cohesion of the gluten network. The lowering of pH, reactions with the different metabolites produced by secondary fermentation and variations in surface tension between the different dough phases seem to play a part in these phenomena. According to K. Hoseney, the phenomenon is essentially oxidative, involving the production of oxygen peroxide by the yeast (11).

Understanding the notion of dough strength is complicated by certain factors which can cause confusion, particularly in long fermentation processes. After a long first fermentation, or the fermentation of a sponge, the dough softens, which seems to go against this idea of strength. This effect, which is real, is heightened by the undermixing inherent in this type of dough. In fact, it is linked to the gradual hydration of gluten and to the action of enzymes in the flour: the amylolytic activity, which contributes to the release of water previously fixed by the starch, a slight proteolysis affecting the gluten; and possibly the reducing effect of the glutathione excreted by some types of dried yeast.

These changes in no way preclude the strengthening of dough elasticity that takes place in the gluten and is evident during the moulding operations. Too much strength will affect the external and internal characteristics of the finished product, so the baker's skill lies in knowing how to control fermentation processes to fit the characteristics of the flours and the type of mixing used. This skill is somewhat restricted by high-speed mixing methods and the use of dough conditioners. The fact remains that in reality the technology is complex, making it difficult for bakers and technicians or engineers in the world of industry or applied research to communicate. The only way forward is for the latter to "get their hands dirty" if they wish to establish a fruitful dialogue based on mutual trust.

B. Use of Fermentable Substrates: The Enzymes Involved

- Monosaccharides, simple sugars with six carbon atoms (such as glucose, fructose and galactose), are preferentially used by *S. cerevisiae*. Nevertheless, the assimilation of galactose depends on the concentration of glucose, with the latter exerting catabolic repression. It is generally acknowledged that glucose and fructose can penetrate the cell by facilitated diffusion, involving phosphorylation.
- Disaccharides can be assimilated after enzymatic hydrolyses.
  - The sucrose already present in flour or added to the ingredients is converted to glucose and fructose by invertase in the yeast. This reaction takes place in the periplasmic space between the wall and the cytoplasmic membrane. The two hexoses then diffuse into the cytoplasm, where they are metabolized. Invertase acts very quickly, practically doubling the osmotic pressure in the region next to the cell.
  - Maltose, which mainly comes from the conversion of starch by action of the α- and β-amylases in flour, is split into two glucose molecules by maltase, an enzyme in yeast cells. However, the maltose must have previously been carried inside the cell by maltopermease.

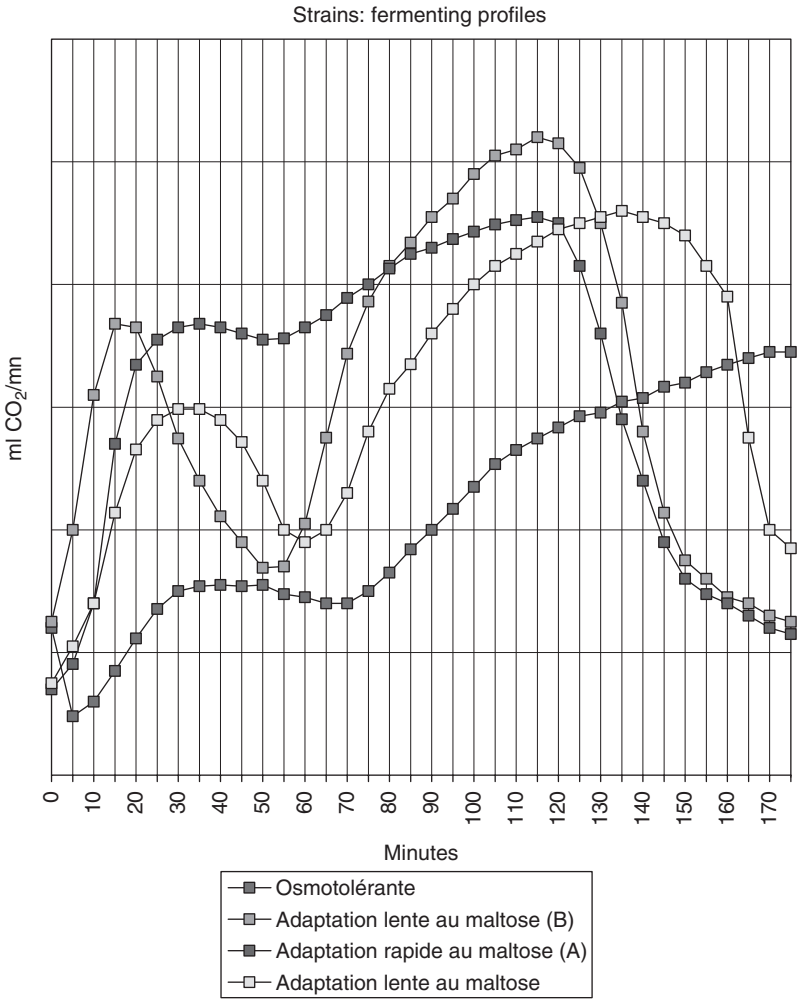

FIGURE 69.4

Not all strains of yeast have the same ability to ferment maltose. Strains used in the U.S. and in Japan have adaptive maltopermease and maltase. The synthesis of these enzymes is catabolically repressed by glucose and induced by maltose. So the cell can only produce the two enzymes which enable it to use the maltose present in the medium after the glucose has been exhausted (13, 14).

In Europe, most industrial baker's yeasts have constituent maltopermease and maltase. These strains were developed in Great Britain in the early 1960s for the Chorleywood Bread Process. They were then used on the continent, where high-speed mixing was catching on. These types of strains were able to rapidly adapt to maltose in bread-making processes with no added sugar, since mechanical dough development replaced first fermentation in straight doughs, or sponge in sponge-and-dough processes. The risk of exhausting the fermentable sugars in the dough was thus eliminated by systematically supplementing flours with malt or α-amylases.

- There are also small quantities of tri- and polysaccharides which are fermentable to varying degrees.

C. Fermentative Profiles

This ability to ferment maltose is easily demonstrated by the rheofermentometer, a device for recording the variations in a yeast's rate of fermentation as a function of time (for example, CO_2 released per min). The dough used must have no sugar added to the flour (so-called "normal" dough).

Depending on the quantity of yeast, its fermentative capacity, and the fermentation temperature, a "depression of adaptation to maltose" varying in size can be seen on the curve. This depression in fermentation speed is linked to the exhaustion of directly fermentable sugars already present. If the yeast contains constituent maltase and maltopermease, the depression is much less pronounced or non-existent, depending on the above-mentioned conditions.

If there is added sugar (sucrose, glucose/fructose syrup), the depression disappears as yeast preferentially uses these substrates (Figs. 69.4 and 69.5).

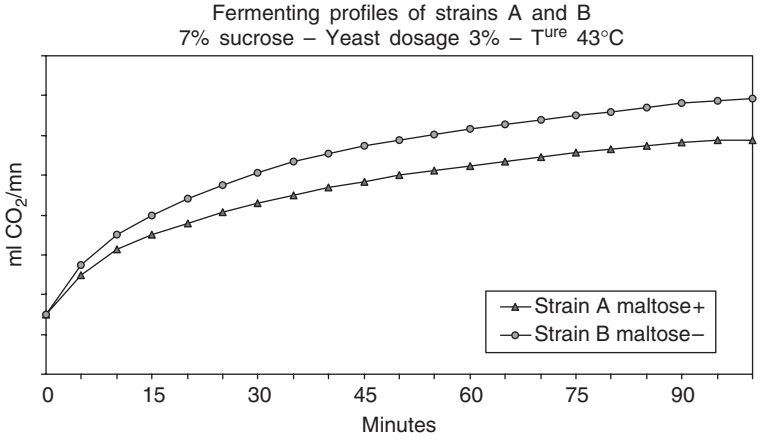

FIGURE 69.5

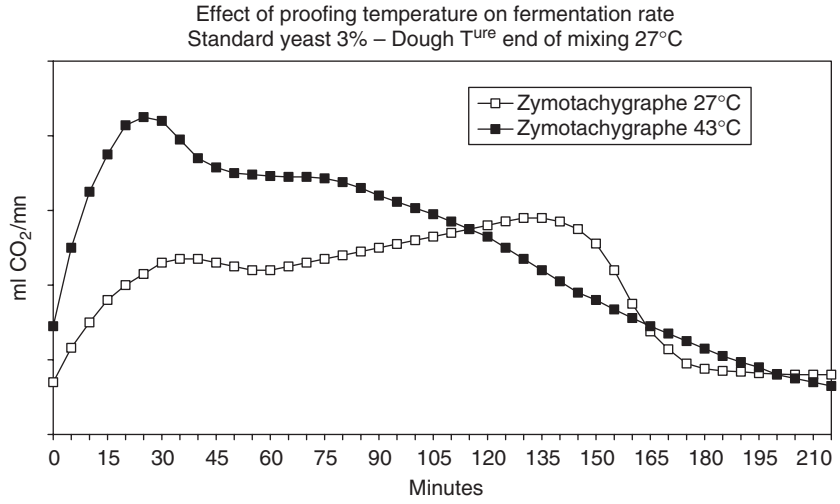

FIGURE 69.6

D. Influence of Various Factors on Fermentative Activity

For a strain with a given biochemical composition, the conditions of the medium affect the rate of fermentation of yeast. The effects must be taken into account in baking, not only for the sake of productivity and economics but also for technological reasons. For example, there are the problems with varying dough density when it is passed through a volumetric divider, or variations in dough strengthening between the start and end of moulding (the larger the dough mass being divided, the greater the difficulty).

Although numerous factors have a bearing on the fermentative activity of yeast, we should remember that the baker judges this activity by the rising of the dough. This is the result of the force exerted by the increase in internal pressure (impermeability plus CO_2 production) and the resistance of the dough to deformation. Extensibility, elastic resistance, permeability, viscosity of dough (cold or warm, soft or firm) are among the many parameters which affect the rate of dough rising, regardless of the activity of the yeast itself. This is clearly demonstrated by observing a dough piece as it rises in a mould, in a fermentation chamber at 43°C and 85% relative humidity. The dough "moves" slowly at first but its volume grows more and more quickly. Over a final fermentation period of one hour, the last five minutes are crucial as, in that time, there is a spectacular increase in the height of dough in the mould. This is related, amongst other things, to a reduction in internal pressure as the dough swells, as can be seen on the alveograph curve.

1. Effect of Temperature

Enzymes activity in the yeast depends on the temperature of the medium. Within the range of 20 to 40°C, an increase in dough temperature of 1°C results in an increase in fermentation speed of 8 to 12% according to the type of yeast (Fig. 69.6). In production conditions, therefore, it is vital to check and control dough temperatures very

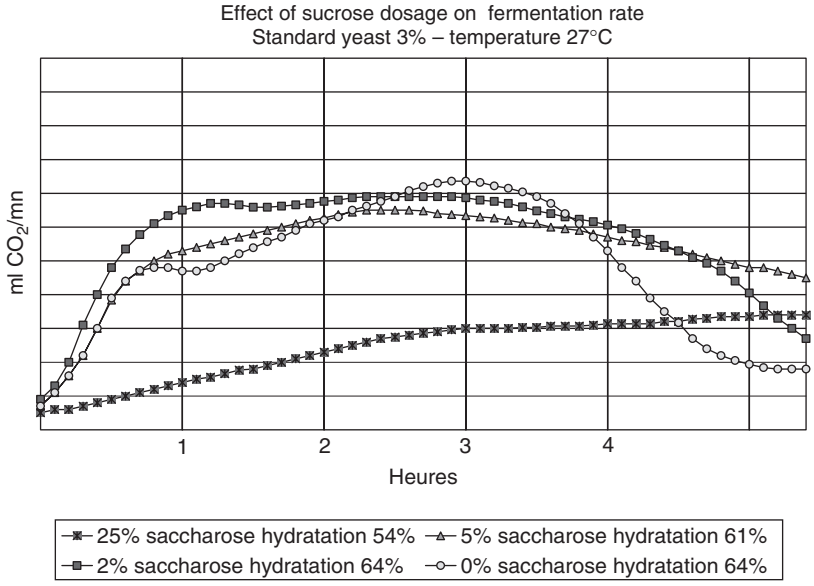

FIGURE 69.7

accurately at the end of mixing, and particularly in the laboratory when comparative studies are being carried out.

This explains the variations of dough and fermentation temperatures required for various applications. Cooler doughs are needed for baguette production (22 to 24°C) in order to restrict dough strength, an excess of which would hinder moulding, and to encourage certain flavors to develop. When working with raw frozen doughs, temperatures of between 18 and 20°C restrict the start-up of yeast activity. At 4°C fermentation is held up. Whilst final fermentation temperatures of 30 to 35°C are used for tinned sandwich bread in France, the general average in the U.S. is 48°C, which increases productivity but at the expense of the overall quality of the finished product. In the United Kingdom, fermentation temperatures of 55°C for soft bun manufacture are quite common, and can be as much as 75°C in the case of industrially produced pizza crusts, all at a relative humidity of 95%. It should be noted that yeast is very quickly killed when the internal dough temperature exceeds 55°C.

2. Effect of Osmotic Pressure

The semi-permeable cytoplasmic membrane of the cell delimits two compartments. When there is a difference in concentration between the medium inside and outside the cell, water gradually moves from the less concentrated medium to the more concentrated one so that equilibrium can be established. This transfer is influenced by a force which exerts a virtual pressure linked to the semi-permeability of the membrane. This movement of water can be prevented by exerting hydrostatic pressure. The difference in pressure between the two media defines the *osmotic pressure*. This is in proportion to the number of dissolved particles per unit of volume. A solution containing 1 mmol/l of sodium chloride (58 mg/l) will release 2 ions per molecule, and the osmotic pressure can be twice that of a glucose solution also containing 1 mmol/l (180 mg/l).

In a dough, osmotic pressure mainly depends on the proportions of sugar and salt used. It can be very high, as much as 35 to 100 atmospheres. This explains the slowdown in the activity of yeast in which metabolism cannot take place normally because water has leaked outside the cell and fewer fermentable sugars can enter.

In bread-making, sugar, often added with fat, is responsible for a reduction in dough consistency. It then becomes necessary to decrease dough hydration, which accentuates the concentration phenomenon in the medium. The baker compensates for the drop in fermentative activity by increasing dough and fermentation temperatures, reducing the quantity of salt and increasing the amount of yeast. The rheofermentometer shows the fermentation rate of yeast with different levels of sucrose, or salt (with or without calcium propionate added in the dough) (Figs. 69.7, 69.8). For a standard French yeast, there is a low level of sugar stimulating fermentation and prolonging it, whilst from 5% of sugar upwards, the rate of fermentation is slowed down and tends to decrease. The rapid accumulation of alcohol, to which the yeast is very sensitive, could be the reason for this. The higher the fermentation temperature, the more obvious this phenomenon is.

There are some bread-making processes where the concentration of sugars is such that the methods described above cannot guarantee that dough will rise within a reasonable time using ordinary yeasts. This has led to the development

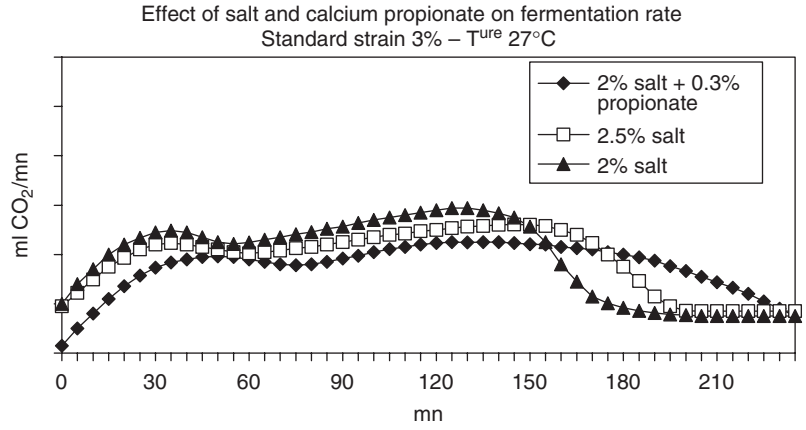

FIGURE 69.8

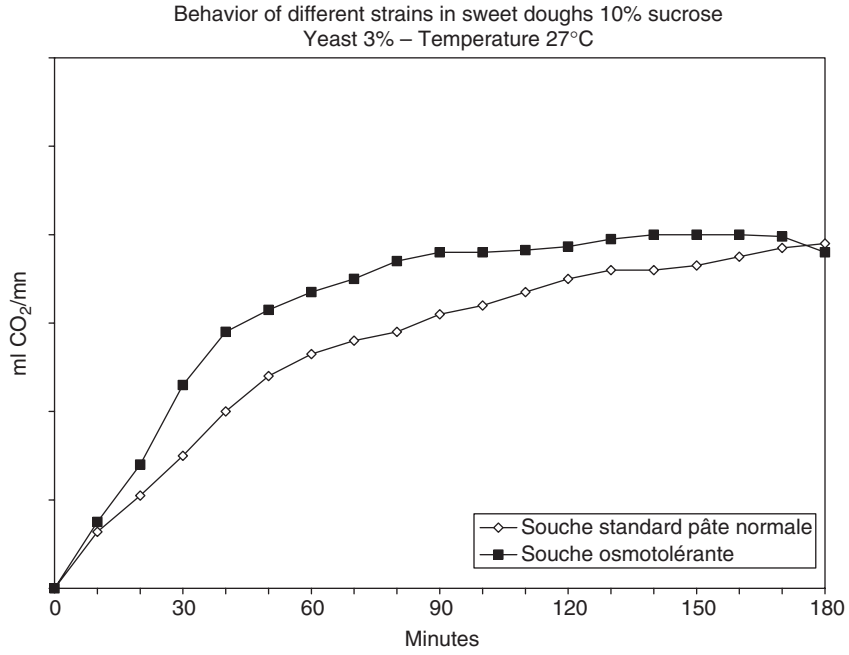

FIGURE 69.9

of osmotolerant strains (Fig. 69.9) which are used in France, (Vendée brioche), the U.S. (Danish pastries, sweet doughs, Hawaiian sweet bread), Indonesia (roti mani), and Japan (kashipan), and where the levels of sugar vary from 20 to 50% based on flour weight.

The osmotolerance of these strains is associated with various characteristics, including:

- a low level of invertase so that sucrose is gradually converted to glucose and fructose, resulting in fewer dissolved molecules at a given moment. Obviously, this ability does not apply to sugars such as glucose/fructose syrups.
- the capacity to synthesize molecules such as glycerol or trehalose, so that the exit of water from a cell is counterbalanced when the concentration of the medium outside the cell is too high.
- the propagation conditions on which, regardless of strain, the osmotolerance of a yeast depends.

3. Effect of Yeast Dosage

At a given temperature, the rate of fermentation depends on the quantity of yeast used (Fig. 69.10). We can see a depression in the curves in the 1st hour because the sugars already present are being used up more quickly.

If no sugar is added, the quantity of maltose resulting from amylolysis is a limiting factor. When the amount of yeast is too high, a flour with low levels of damaged starch

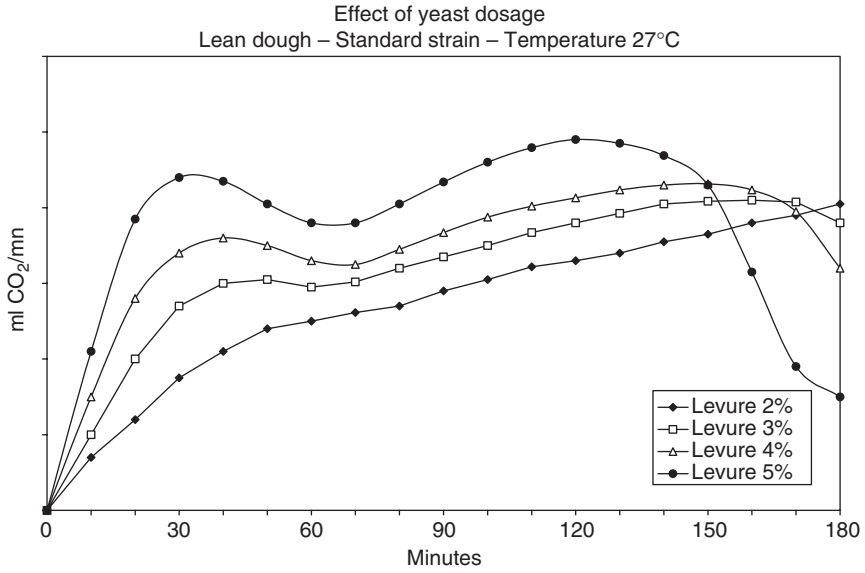

FIGURE 69.10

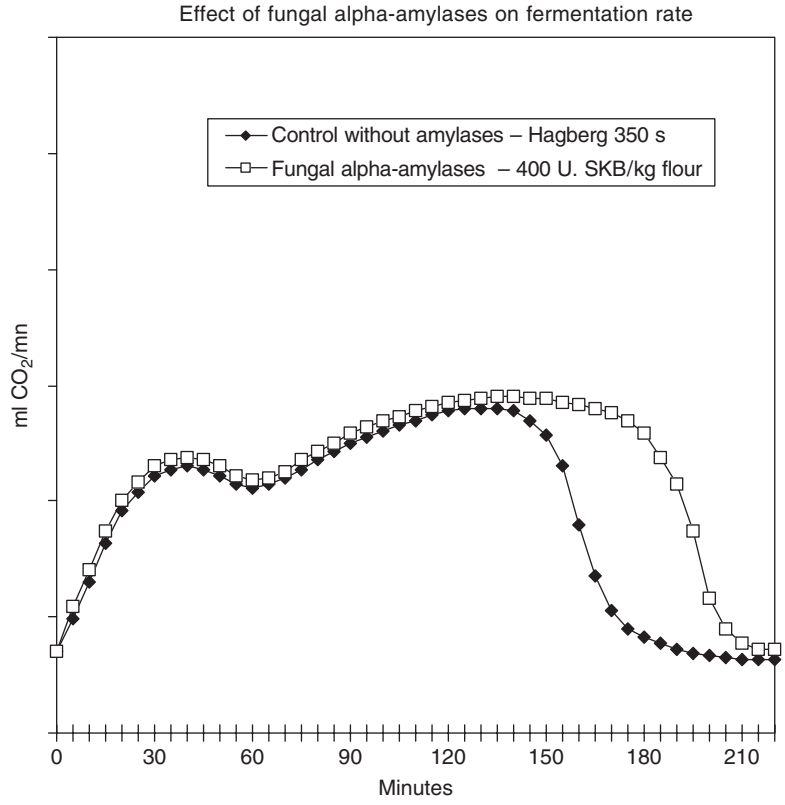

FIGURE 69.11

or α-amylases can be exhausted before proofing is completed. Adding fungal α-amylases at a level of 40,000 SKB units per 100 kg of flour prolongs fermentation by about 1 hour in standard bread-making conditions (Fig. 69.11).

4. Effect of pH

The internal pH of a yeast cell is of the order of 5.6 to 5.8 and varies very little. The enzyme systems involved in the metabolism of *S. cerevisiae* are intracellular as a whole,

TABLE 69.3
Changes in Dough Acidity at Different Stages of Fermentation

	pH	TTA
American Sandwich Bread		
Sponge (70% flour)	4.8	4.6
Dough at start of proofing	5.2	4.0
Dough at end of proofing	5.0	5.3
American Sandwich Bread		
Liquid preferment (20% flour)	4.5	12
Liquid preferment (50% flour)	4.8	10
French Bread — Straight Dough		
Dough at end of mixing	6.1	1
Dough at end of proofing (2 h 1/2 fermentation)	5.8	1.5
French Bread — 30% Sourdough		
Dough at end of mixing	5.3	2.0
Dough at end of proofing (6 h fermentation)	4.2	7.0

which explains why yeast is very tolerant to variations in the pH of a medium, within the range of 2 to 8.

At pH of between 4 and 6, the fermentative activity of yeast is at its optimum. These conditions are found in most bread-making processes with yeast, where the pH at the end of final fermentation is of the order of 5.2 ± 0.2. This pH can be lower when using sourdough starters (cf. chapter II, § III. 1), deactivated sourdoughs or organic acids, particularly for rye bread manufacture. Unlike glucose, the fermentation of maltose is affected when the pH is less than 4.

The actual acidity of a dough cannot be assessed by pH alone due to the high buffering capacity of flour proteins. It is often associated with titratable total acidity which is an arbitrary notion often used in the U.S. (TTA) and Germany (Säuergrad). In the first case, it corresponds to the quantity of 0.1 N sodium hydroxide (4 g/l) needed to neutralize a ground mixture of 15 g of dough in 100 ml of distilled water to pH 6.6. In the second, the pH is 8.5. This method enables plant bakers to control the consistency of their fermentation processes (Table 69.3).

Volatile organic acids and their salts inhibit fermentative activity in their non-dissociated form. A low pH, which shifts the balance of dissociation toward the non-dissociated form, means that the acetic acid or propionates used as anti-mould agents will have a much more detrimental effect. The critical zone falls right inside the range of pH (4 to 5) found at the end of fermentation in most types of bread-making methods (Table 69.4).

5. Inhibiting Ions

In the cases, fortunately rare, where bread fermentation is inhibited, water is often responsible as a carrier of certain ions which are toxic to yeast. Cu^{++} from the copper in old pipes, quaternary ammoniums in antiseptic products from poorly rinsed equipment, and excess Cl^{-} in city supply water have often been blamed.

TABLE 69.4
Dissociation of Acetic and Propionic Acids at Different pH

	Non-Dissociated Fraction (%)					
	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7
Acetic acid	99.8	98.2	84.7	35.7	5.3	0.6
Propionic acid	100	99	88	42	6.7	0.7

IV. INDUSTRIAL PRODUCTION OF YEAST

In 2003, an estimated 2.8 million tonnes of baker's yeast, expressed as compressed yeast, was produced throughout the world. Half was produced on the continent of Europe, with the other half equally divided between the Americas and the rest of the world. Regardless of their physical forms (compressed, dried, etc.), the yeasts produced are not the same in the various regions of the globe as manufacturers have to adapt them to the local bread-making conditions. However, they have one problem in common, ensuring that the fermenting capacity of the product they supply is consistent and stable. This is achieved by controlling the quality of raw materials, production technology and logistics.

A. Raw Materials

The raw materials used must satisfy the nutritional requirements necessary for the growth and multiplication of yeast cells.

Molasses are the substrates of choice both economically and technically. Viscous, highly colored liquids, their composition varies according to the sugar-making process from which they derive, and the quality of the sugar-beet or cane harvest. 77 to 82% of dry matter essentially provides: sucrose as a source of carbon (45 to 55%), minerals, trace elements and vitamins. However, they supply very little nitrogen for protein synthesis (the betaine they contain cannot be assimilated) and not enough minerals such as phosphorus, magnesium and often zinc. The problems they cause are rarely related to any deficiencies but are more likely to be due to the presence of elements which are toxic to yeast. They may be substances which derive from farming methods or sugar-producing processes: fungicides, quaternary ammonium, sulfites, but also an excess of minerals (Na^{++}), short chain fatty acids and a great many trace elements.

In order to homogenize the composition of the nutrient medium and reduce the risks of toxicity, all batches are analyzed and tested before they are mixed (up to 10 or 12 different sources).

After an initial filtration to remove coarse foreign elements, the molasses are diluted and heated to reduce the viscosity. Clarifiers continuously remove the fibers and colloids by centrifugation. The next is flash pasteurization when the molasses are heated to 130°C under pressure for a very short time. This treatment removes vegetative forms of microbial contaminants as well as *Clostridium* and *Bacillus* spores, without caramelizing or degrading the sugars. After they have cooled down on plate exchangers, the molasses are stored in a buffer tank, ready for use.

Cane molasses, which contain a lot of fibers and colloids, are difficult to process but have a very high nutrient content. When they are mixed at a level of 20% with sugar beet molasses, biotin does not need to be added.

Minerals which are added as nutrients (phosphoric acid, ammonium salts, ammoniac) or to control pH (sulfuric acid, soda) comply with food standards, in particular for heavy metal content. The water for diluting the medium is potable and chlorinated to avoid any contamination.

B. Storage and Protection of Strains

Thousands of strains have resulted from research studies but fewer than about ten are used in large-scale industrial production. Manufacturers store them in their own laboratories but also, as a precautionary measure, in public collection centers. With the development of identification methods, the claims of patent applications dealing with the creation and improvement of strains can be described more accurately.

The methods of storing strains vary, for example −80°C on a glycerol medium with subcultures made after 1 to 5 years, or 4°C on a gelose medium and subcultures after 1 to 3 months.

C. Propagation

How yeast cells multiply (wrongly called "fermentation") is of major importance in carrying out the yeast manufacturer's two objectives.

The first relates to quality. A specific strain must satisfy several criteria:

- the best fermentative power in a specific application ("normal", sweet, or acid doughs….), or fermentation kinetics which are most suited to a given bread-making method (sponge and dough, Chorleywood bread process, etc.);
- the nature of the finished product: compressed yeast, rehydratable dry yeast or instant yeast;
- the stability of fermenting power.

The second objective has to do with economics. The manufacturer must achieve the quality dough for the best price. The result is a compromise between:

- yields (% of yeast obtained in proportion to the substrate used);
- productivity (rate of multiplication and optimum use of tank equipment).

It is evident, therefore, that there are many complex — and sometimes contradictory — obstacles. To untangle the knot, engineers and researchers rely on data on the biochemistry and physiology of cells. Although attempts at modeling have been made in order to optimize production, experience and a certain degree of pragmatism are still called for.

Yeast is propagated in sequences during which cells multiply under different conditions. Multiplication has two aims which follow from each other:

- to massively increase the cell population so that a final so-called "commercial" generation can be seeded;
- to multiply the yeast so that its biochemical composition and physiological condition meet the requirements of producer and user.

The number and method of these stages can differ noticeably from manufacturer to manufacturer, but the principle is the same. In each stage, enough yeast is produced to inoculate the next stage (Fig. 69.12). This is carried out using a series of containers, from lab to plant, whose contents must be managed extremely well when the industrial stage is reached.

The third industrial generation (commercial generation) must finish with a maturing phase, the aim of which is to ensure the yeast is stable. It means making the yeast, at the end of the manufacturing process, synthesizing as many stock sugars as possible (trehalose and glycogen) and reducing the rate of budding to very low levels. In order to do this, the yeast is deprived of nitrogen, and is supplied with molasses only, and the temperature is slightly increased above the 32 ± 2°C which is the average temperature during production.

Next comes the separation of yeast cells from the wort. This operation takes place continuously using centrifugal equipment where the yeast is washed and a cream is obtained, i.e., a suspension of cells in water at a dry matter concentration of between 15 and 20%. This figure is equivalent to about 50% of cells, in volume. Above 23% dry matter, the suspension becomes too viscous and cannot be pumped. This cream is cooled down to 4°C in a plate exchanger and kept at this temperature in storage tanks. About 400 tonnes of yeast (compressed yeast equivalent) will be finally collected 10 days after the first test tube was cultured in the laboratory. The principle of yeast manufacture is summarized in Fig. 69.13.

French yeast producers, who are concerned about environmental regulations, have concentrated on investing in methods of pollution control. After the yeast cream has been separated, there is a great deal of effluent which cannot be discharged, as is, into the environment. The most diluted effluent is treated by aerobic or anaerobic

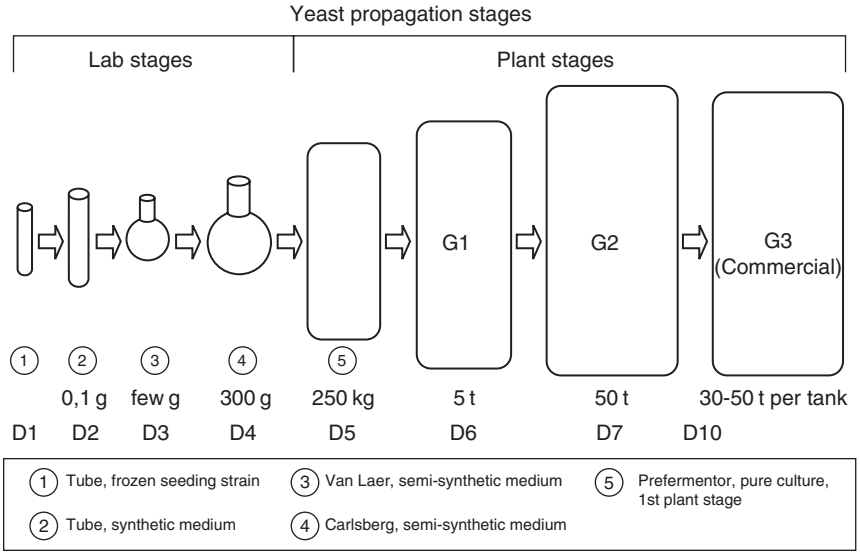

FIGURE 69.12

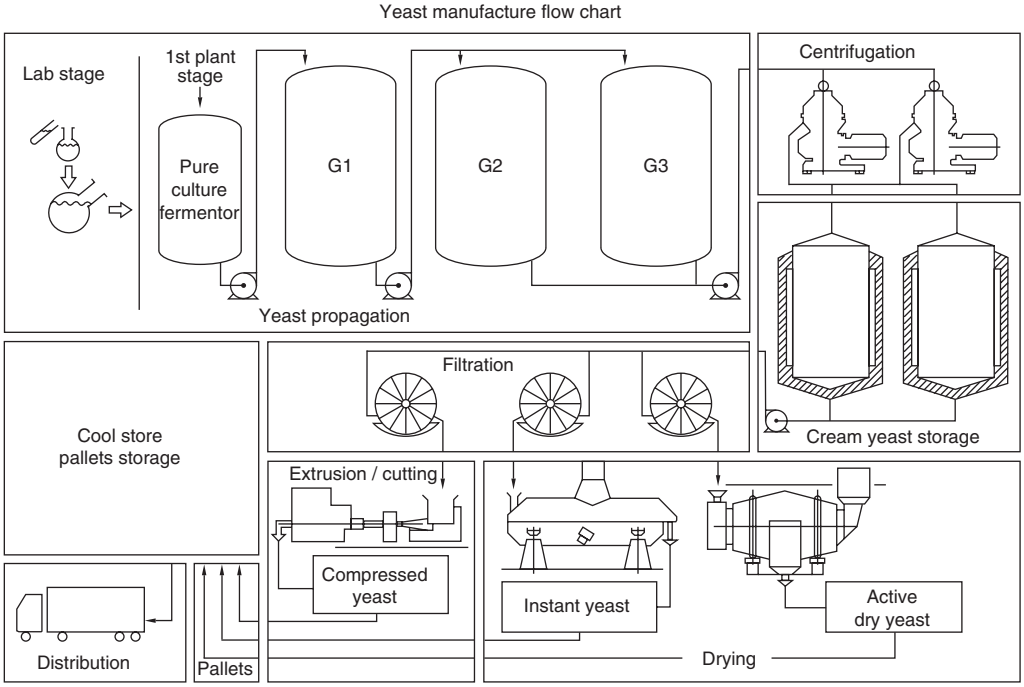

FIGURE 69.13

biological methods. The must from which the yeast has been removed and which still contains non-assimilated organic nitrogen (betaine) and mineral salts rich in potassium sulfate is very highly concentrated by evaporation and inverse osmosis. The vinasse obtained is used as a fertilizer, spread on directly as manure or in the form of potassium extracts after crystallization, decantation and drying. It can also be used in animal feed after treatment to reduce the potassium content. Pollution control treatments have a considerable effect on production costs.

V. COMMERCIAL YEASTS

A. Strains

The strains used in France and in Europe as a whole are of the "rapid adaptation to maltose" type. They were developed in the early 1960s in response to straight bread-making methods which had appeared as a result of high-speed mixing used in bread-making with no added sugar. Because of their poor performance in very sweet doughs (over 15% of sucrose/flour), osmotolerant strains already

TABLE 69.5
Comparison of Commercial Strains and Wide-Range Laboratory Strains

Type of Yeast and Strain	Fermenting Power Lean Dough (Base 100)	Fermenting Power 25% Sweet Dough (Base 100)	Invertase Activity (Ui)*
Commercial — Rapid Adaptation to maltose	100	60	60 to 200
Commercial — Osmotolerant	50	100	5 to 10
Lab — Hybrid wide range	85	105	<5
Lab — Genetic engineering Wide range	90	100	<10

Source: P. Clément, ICC Conference, Paris 1992.

*1 Unit invertase = 1 µmole of reducing sugar released/5min/mg of yeast DM.

TABLE 69.6
Change in the Fermenting Power of Various Types of Yeast in Accelerated Storage Tests

Yeast Quality	DM (%)	Nitrogen/ DM (%)	Fermenting Power Lean Dough (Base 100)	Loss After Storage 7 Days/26°C
"Standard"	≃33	7.0	100	−10%
"Rapid"	≃32	7.3	110	−25%
"Ultra rapid"	≃31	8.2	125	−50%
"High activity"	≃29	9.3	165	Autolysed

used for the large export markets were introduced onto the French market at the end of the 1980s. The difficulty bakeries had in using several qualities of yeast resulted in research work into strains with a wide range of applications (Table 69.5).

B. Biochemical Compositions Adapted for Applications

The need to increase the rate of fermentation, particularly in an industrial environment, is met by yeasts with a high nitrogen — and therefore protein — content. The major drawback of these yeasts is their instability, which is due to higher enzyme activity and a storage sugar content which is proportionally lower. In the case of compressed yeast, as has been mentioned earlier, the increase in protein level calls for a decrease in DM content so that the product maintains its physical cohesion. The stability of its fermentative activity is affected by this, which means that low temperatures must be maintained. The higher the protein level, the more rigorously these low temperatures need to be observed. An increase in temperature accelerates the consumption of trehalose, then glycogen to cover the needs of energy metabolism. When these carbohydrates are exhausted, the yeast attacks its own proteins. This autolysis is responsible for the degradation of yeast activity and its physical appearance (Table 69.6). The nitrogen content of the various types of dried yeast is restricted to the maximum level, which is unlikely to be detrimental to them during drying.

C. Controls on The Finished Product

A yeast, regardless of its form, must comply with several criteria before it can be marketed:

- Physical and organoleptic: consistency and friability in the case of compressed yeast, shape and size of particles in the case of dried yeast, color and smell.
- Biochemical: dry matter, nitrogen and phosphorus composition, stocks carbohydrates.
- Bacteriological: appearance of cells, budding rate; most attention is given to identifying and counting foreign microflora.
- Fermenting power: most of the methods used measure the release of carbon dioxide which is produced over a given time, by fermentation of a medium with known characteristics. Mostly, it is a wheat flour dough whose consistency varies from liquid to very firm. Various levels of sugar can also be used to evaluate the behavior of yeast at increasing osmotic pressure (18).
- Suitability of yeast for storage: the stability of the fermenting power of various types of yeast can be evaluated in storage tests, at temperatures of between 20 and 43°C. There are correlations between the results of these accelerated tests and those obtained in real time, at temperatures found in different markets.
- Bread-making tests: for specific applications, laboratory fermentation tests, although useful, do not supply enough information about the overall yeast activity (cf. § II.A.). Bread-making tests can result in a better understanding of the effect of yeast on dough rheology, on dough behavior during proofing and baking, and finally on its effect on bread properties: color, structure, crumb texture, and flavor. Monitoring the dough in a rheofermentometer at the same time can provide a wealth of information.

D. Commercial Forms and Methods of Use

The different commercial forms of baker's yeast can be discussed in the order in which they appear in the manufacturing process, which corresponds to a continuity in their historical development. And although the 1990s saw a sudden demand for liquid yeast in industry, this was

no revolutionary product unless we are thinking of the mechanical meaning of the term revolution: a return to the point of origin!

1. Liquid Yeast

Until 1825, when compressed yeast was introduced by Tebbenhof, yeast was sold in liquid form (2) . The return to this form correlates with the demand from plant bakeries. In general use in Australia, it is used much less widely in the U.S. and Canada and has its followers in the United Kingdom and Ireland.

- Advantages
  - dosage of the yeast can be automated, as it is for the other raw materials, resulting in improved control and stock management;
  - no handling and no packaging materials to dispose of;
  - even dispersion in the dough during high-speed mixing;
  - yeast activity is standardized: the producer controls fermenting power using a substitution rate, for example 1.5 liters of cream per 1 kg of compressed yeast;
  - stability is noticeably improved compared with other forms, because the cream is cooled immediately on a plate exchanger. This stability is ensured by monitoring the storage temperature (Table 69.7). However, a temperature of less than 4°C must be maintained to prevent any bacteriological contamination.
- Specific points
  - the installation must be specially designed for each user;
  - profitability is guaranteed when the quantity of yeast used at the same site is sufficiently large;
  - hygiene, cleaning and disinfection conditions must be strictly observed.

The installation made entirely of stainless steel consists of two double-walled storage tanks with separate cooling systems, as well as feed systems for the tanks and mixers. The metering systems must be accurate and reliable. The whole unit is supplemented by a cleaning plant and equipment for discharging liquids: soda, antiseptics, rinsing water. The capacity of each tank must allow at least one week's production.

The liquid yeast is stirred in the tank and circulates continuously through the distribution system so that it remains homogeneous and there is no risk of infection. The temperature is strictly controlled and held at between 2 and 4°C. The system must be cleaned and disinfected once a week or whenever the installation is shut down, hence the need for two tanks. The receiving circuits must be cleaned each time there is a delivery. Procedures should be put in place and followed meticulously by operators.

2. Compressed Yeast

This is the most widely used form in industrialized countries, for economic and practical reasons. It comes in the form of compact blocks so that there is limited contact with the oxygen in the air. White in color and very friable in France, it can have a deeper color and a plastic consistency in other countries. The packaging of "waxed paper" or "sulfurized paper and cellophane" restricts gaseous exchanges and controls the migration of moisture, giving a longer shelf life.

TABLE 69.7
Change in the Fermenting Power of Different Quality Liquid Yeasts, Stored at Different Temperatures

Yeast Quality	Length of Storage (Days)	Loss of Fermenting Capacity Lean Dough (%)		Loss of Fermenting Capacity Sweet Dough (%)	
		4°C	12°C	4°C	12°C
"Standard"	7	0	0	0	0
	14	0	0	0	0
"Rapid"	7	0	0	0	0
	14	0	0	0	0
"Ultra-rapid"	7	0	0	0	0
	14	3	4	5	10
"High activity" A	7	0	0	–	–
(Great Britain)*	14	0	4	5	10
"High activity" B	7	0	3	5	10
(Great Britain)*	14	5	30	15	50

* Yeasts made using two different propagation methods. The stability of yeast B can only be achieved by positive temperatures of less than 4°C.

TABLE 69.8
Change in the Properties of Compressed Yeast after Freezing

Properties	t 0	11 Months at −18°C
Dry matter %	32.9	30.5
Nitrogen %/DM	7.2	–
Fermenting power %	100	97
Appearance	White, Friable	White, Very Friable, Normal Smell

The stability of the fermenting power of yeast in its packaging depends on the storage temperature. One month is guaranteed for a "standard" quality French yeast stored at 10°C maximum (ideally 4°C). At 20°C, its activity is stable for two weeks but is reduced by 60% after 1 month; the DM loses 4 points, which corresponds to the consumption of storage sugars and to the product drying out; there is considerable contamination from mould. At 35°C compressed yeast is only stable for 24 hours; after 3 days the loss of activity is equivalent to that found after 1 month at 20°C, and the product becomes brown, soft and sticky.

The performance of "high activity" English yeast is guaranteed for 17 days if the storage temperature is strictly kept at a maximum of 2°C.

No special precautions are necessary when using compressed yeast; it can be easily crumbled into the mixer and there are no repercussions when it is dispersed in cold water. It should not be in contact with salt for too long, although this is not actually harmful in practice, contrary to common ideas.

Compressed yeast tolerates slow freezing very well (<1°C/min) and maintains its initial performance after a year's storage at −18°C (Table 69.8), provided that when it is used, it is thawed at positive temperatures (°C) and is used within 24 hours.

It is worth noting that there is an increase in the friability of yeast due to the evaporation of free water and a loss of dry matter as a result of storage sugar consumption.

3. Crumbled Yeast

This is in the form of relatively fine, free flowing particles which means that it can be either weighed or measured automatically. It is packed in 25-kg, multi-layer polyethylene-lined paper sacks which are sealed to keep the product airtight. Crumbled yeast is very sensitive to oxygen in the air because of its large surface of contact. Respiration which continues in a very active manner after extrusion and during the bagging operation causes the yeast to heat up, making it very difficult to cool it down. However, the carbon dioxide released creates an inert atmosphere which stabilizes it.

The storage temperature must be under 10°C. Under these conditions, the crumbled yeast loses 5% of its initial fermentative power after a week and 10% after a month (losses are much higher in sweet dough).

TABLE 69.9
Change in the Temperature of Crumbled Yeast, Not Refrigerated

Conditions	Time	Temperature (°C)
"Standard" Quality yeast	t 0	8
Ambient temperature: 20°C	0H30	10
Quantity of yeast: 1.5 kg	1H00	14
Thickness of layer: 9 cm	1H30	21
	2H00	26
	–	–
	3H30	41
	4H30	49
	5H00	50
	5H30	47

Opened sacks should be used up within a day, as the product heats up quickly (Table 69.9).

Crumbled yeast is often used by plant bakers as a suspension in water so that metering can be automated. The installation includes a storage tank with identical characteristics to those previously described for liquid yeast. Upstream, a smaller tank with a very vigorous stirring action is used to disperse the yeast in water.

4. Rehydratable Active Dry Yeast

Rehydratable active dry yeast (Fig. 69.13) is packed in air in 125-g or 500-g containers (metal or plastic), 10- or 25-kg sacks, or 25-kg plastic drums. It is packed in 5 to 11 g sachets for the consumer market. It is stored at ambient temperature. This rustic product is very stable, a fact appreciated in parts of the world where the climatic conditions are unfavorable (high temperature and humidity).

When reconstituted, the yeast undergoes a rehydration stage in about 5 times its weight of water, at a temperature between 35 and 42°C, the optimum being 38°C. Rehydration takes 15 minutes, during which time attempts should not be made to disperse the yeast mechanically or it will be damaged. After this resting time, it goes into suspension very easily and forms a cream.

Observing the rehydration temperature is vital as it ensures that the enzymatic mechanisms by which the cell membranes become semipermeable again are functioning properly. If the temperature is too low (<5°C), the membranes do not regain these properties quickly enough and the cells release their contents, resulting in their death. At 20°C, the loss of gassing power is about 30 to 40%.

In view of its dry matter content and fermentative capacity, its rate of substitution compared with "standard" quality French compressed yeast is about 40 to 50%,

i.e., 1 kg of rehydratable dried yeast instead of 2 to 2.5 kg of compressed yeast.

Rehydratable dried yeast releases enough glutathione to change dough consistency and reduce its development time during mixing. Adjustments are therefore necessary; in particular, the quantity of oxidizing agent may need to be increased.

5. Instant Dry Yeast

Instant dry yeast vermicelli-like particles are vacuum packed in 125-g or 500-g sachets, or in packs of 10 kg or more. There are also 7- to 12-g sachets packed with inert gas for home bread-making. The vacuum-packed sachets are hard: They ensure that the product is stable at ambient temperature. (Strict checks after packing eliminate sachets which no longer have this vacuum.) At temperatures of under 20°C, a loss of fermentative activity is apparent during the first month, and then stabilizes; after 1 year it is about 10% ± 2. At 35°C, 1/3 of activity is lost in 1 year. Sachets which have been partially used should be kept in a cold place, carefully closed: the yeast's normal activity will be maintained for at least a week.

Instant dry yeast owes its name to the fact that it does not need to be rehydrated before it is added to flour. It is as easy to use as compressed yeast. In most applications, the fineness and porosity of the particles mean that the yeast can be dispersed quickly and evenly in the dough. However, the use of very high-speed mixers (mixing times of less than 3 min) can pose some dispersion problems.

Nevertheless, when using instant dry yeast, it should not come into direct contact with cold water, ice or the refrigerated walls of mixers. It should just be mixed dry with the flour or sprinkled over the dough during mixing at first speed. In these conditions, the water heats up before it reaches the yeast cells, the membranes of which can regain their biological properties normally. However, it is less sensitive to cold than rehydratable dried yeast (−15% at 20°C/38°C).

The high fermentative power and dry matter content mean that it can be substituted at 33% for compressed yeasts with an equivalent composition. In other words, 1 kg of instant dry yeast can be used instead of 3 kg of compressed yeast. In view of the difference in dry matter content, it is essential to compensate for the difference in weight of water, i.e., 2 kg in the example given.

As a small amount of glutathione may be released, it is advisable to reduce the mixing time slightly or increase the quantity of oxidizing agent in some applications.

Because of the additional energy costs arising from drying and packaging technology, instant dry yeast is a product which cannot compete economically with compressed yeast when the latter is of good quality and the distribution networks are effective.

6. Free-Flowing Frozen Dry Yeast with Intermediate Moisture

In applications such as frozen dough, the results achieved with instant dry yeast are only comparable to those obtained with compressed yeast (CY) when the dough is stored for about two weeks. In fact, the yeast membranes, weakened by drying, cannot withstand being frozen in the dough, then stored, and thawed again without damage. At lower dry matter contents (≃75%) the yeast membranes remain intact. In its physical appearance as a powder, it has the characteristics of CY. It can be packed in air and frozen. It can be stored for long periods and exported to distant regions where there is no compressed yeast, and used in applications where instant dry yeast is unsuitable.

7. Dry Yeast with Reducing Power

- Active
  In the form of small granules packed in air in plastic containers, this yeast is used by pizzerias. Its moderate, consistent fermenting power gives the tolerance during fermentation which is necessary for this type of application. The quantity of glutathione released means that pizzas can be easily moulded, dough extensibility is increased and shrinkage phenomena are reduced.
- Deactivated
  This yeast has no fermenting capacity at all. In dough, the membranes which have been made completely permeable, allow all the cell contents through including glutathione which has a reducing effect on the gluten.

  The quantities used vary from 0.3 to 1%, depending on the flour type and the effects required. The reducing effects work in the following way:
  – by improving the machinability of doughs which are too stiff or very strong;
  – by accelerating dough development during mixing, which requires a 15 to 20% reduction in mixing time. In the absence of lipoxygenase (from bean or soya flour), this property can be exploited to preserve the flavor of bread by limiting the oxidation of the flavor compounds.

Deactivated dried yeast is packed in polyethylene-lined paper sacks. As the product is very hygroscopic, sacks should be closed carefully after use to prevent it becoming lumpy and to ensure it retains all its functions.

VI. CONCLUSIONS

Very little is known about yeast by the people who use it and its true worth is certainly underestimated. Indeed, it is

a product which has become banal in industrial countries, perhaps because the manufacturing process is well controlled and problems are therefore quite rare. Nevertheless, the yeast industry is a high-tech industry in the field of biotechnology where progress has been made due to intensive research and development work. As a result of genetic techniques, strains are being improved all the time. There have been advances in methods of culturing strains as we have gained a better understanding of biology and cell physiology. A good grasp of raw materials and manufacturing methods, intensive automation, and logistical controls are guarantees of product quality in a world increasingly concerned by food safety issues.

ACKNOWLEDGMENTS

To Eric Duquenne for his help with laying out the artwork, and Dorothy Ellerington, for her remarkable translation from French into good English of this unusual topic. The information in this chapter has been derived from "Yeast," Bernard Poitrenaud, In *Handbook of Food and Beverage Fermentation Technology*. Editors: Y. H. Hui et al. Marcel Dekker, New York, 2004.

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70 Pickle Manufacturing in the United States: Quality Assurance and Establishment Inspection

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CONTENTS

I. QUALITY ASSURANCE

A. Introduction

This section is not designed to explain how pickles are manufactured in the United States. Rather, it is designed to show you the critical factors you should look for in assuring the quality of your pickles. The information has been modified from a document issued by the United States Department of Agriculture: *United States Standards for Grades of Pickles*. Consult the original document for complete details.

This section contains 11 tables of reference data, numbered 1 to 11, and they do not appear in the same order as the text.

B. Product Description

Pickles means the product prepared entirely or predominantly from cucumbers (*Cucumis sativus* L). Clean, sound ingredients are used that may or may not have been previously subjected to fermentation and curing in a salt brine. The product is prepared and preserved through natural or controlled fermentation or by direct addition of vinegar to an equilibrated pH of 4.6 or below. The equilibrated pH value must be maintained for the storage life of the product. The product may be further preserved by pasteurization with heat or refrigeration, and may contain other vegetables, nutritive sweeteners, seasonings, flavorings, spices, and other permissible ingredients defined by the U.S. Food and Drug Administration (FDA). The product is packed in commercially suitable containers to assure preservation.

C. Styles of Pickles

1. *Whole style* means the pickles are whole and are relatively uniform in diameter as indicated in a later discussion (Table 70.2).
2. *Whole, mixed sizes style* means the pickles are whole pickles of mixed sizes.
3. *Sliced lengthwise style* means the pickles are cut longitudinally into halves, quarters, or other triangular shapes (spears, strips, or fingers), or otherwise into units with parallel surfaces with or without ends removed.
4. *Sliced crosswise, Crosscut, or Waffle-cut style* means the pickles are cut into slices transversely to the longitudinal axis. The cut surfaces may have flat-parallel or corrugated-parallel surfaces.
5. *Cut style* means the pickles are cut into chunks or pieces that are of various sizes and shapes.
6. *Relish style* means finely cut or finely chopped pickles containing no less than 60 percent of cucumber ingredient and possibly containing other vegetable ingredients (cauliflower, onions, pepper, tomatoes, cabbage, olives, mustard or any other suitable vegetable).

D. Types of Pack

1. Cured Type

The pickles are cured by natural or controlled fermentation in a salt brine solution and may contain the dill herb or extracts thereof. The pickle ingredient may be partially desalted. The pickles may be further processed or preserved by the addition of vinegar and may contain other ingredients (spices, flavorings, firming and preserving agents) that constitute the characteristics of the particular type of pickle. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. The characteristics of the various types of cured pickles are as follows:

1. *Dill pickles (natural or genuine)* are cucumbers that are cured in a brine solution with dill herb and other flavoring agents.
2. *Dill pickles (processed)* are brine-cured pickles that have undergone a freshening process and are packed in a vinegar solution with dill flavoring and other flavoring agents.
3. *Sour pickles* are cured pickles that are packed in a vinegar solution with or without spices.
4. *Sweet pickles* and *mild sweet pickles* are cured pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s).
5. *Sour mixed pickles* are cured pickles that are packed in a vinegar solution. The pickles may be of any style or combination of styles other than relish and may contain other vegetable ingredients as outlined in Table 70.1 or any other suitable vegetable.
6. *Sweet mixed pickles* and *mild sweet mixed pickles* are cured pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s). The pickles may be of any style or combination of styles other than relish and may contain other vegetable ingredients as outlined in Table 70.1 or any other suitable vegetable.
7. *Sour mustard pickles* or sour chow chow pickles are cured pickles of the same styles and ingredients as sour mixed pickles except the pickles are packed in a prepared mustard sauce of proper consistency with or without spices and flavorings.
8. *Sweet mustard pickles* or sweet chow chow pickles are cured pickles of the same styles and ingredients as sweet mixed pickles except the pickles are packed in a sweetened, prepared mustard sauce of proper consistency with or without spices and flavorings.
9. *Sour pickle relish* consists of finely cut or chopped cured pickles that are packed in a vinegar solution. Sour pickle relish may contain other chopped or finely cut vegetable ingredients as listed in Table 70.1, and may contain a stabilizer such as a starch or gum.
10. *Sweet pickle relish* and *mild sweet pickle relish* are finely cut or chopped cured pickles that are packed in a vinegar solution with a suitable nutritive sweetening ingredient(s). Sweet pickle relish and mild sweet pickle relish may contain other chopped or finely cut vegetable ingredients as listed in Table 70.1 and may contain a stabilizer such as a starch or gum.

TABLE 70.1
Proportions of Pickle Ingredients in Certain Types and Styles

Pickle Ingredients and Styles	Cured; Fresh-Pack; and Refrigerated Types (Percent by Weight of Drained Weight of Product)	
	Sour Mixed; Sweet Mixed; and Mild Sweet Mixed; Sour Mustard or Sour Chow Chow; Sweet Mustard or Sweet Chow Chow	Sour Pickle Relish; Sweet Pickle Relish; Dill Relish; Hamburger Relish; Mustard Relish
Cucumbers, and style other than relish	60 to 80%	
Cucumbers, chopped or finely cut	60 to 100%	
Cauliflower pieces	10 to 30%	
Cauliflower, chopped of finely cut		30% maximum (optional)
Onions, whole (maximum diameter of 1-1/4 inches), sliced or cut	5 to 12%	
Onions, chopped or finely cut		12% maximum (optional)
Green tomatoes, whole or pieces	10% maximum (optional)	
Green tomatoes, chopped or finely cut		10% maximum (optional)
Red, green, or yellow peppers, or pimientos, cut, finely cut, or pieces	Optional	Optional
Celery	Optional	Optional
Cabbage	Optional	Optional
Olives	Optional	Optional
Tomato paste	Optional	Required in hamburger relish
Mustard or prepared mustard	Required in chow chow and mustard pickles	Required in mustard relish, optional in hamburger relish

11. *Hamburger relish* consists of relish style pickles and other chopped or finely cut vegetable ingredients as listed in Table 70.1 with tomato product added.
12. *Mustard relish* consists of sweet pickle relish with mustard and other chopped or finely cut vegetable ingredients as listed in Table 70.1.
13. *Dill relish* consists of relish style pickles containing dill flavoring and other chopped or finely cut vegetable ingredients as listed in Table 70.1.

2. Fresh-Pack Type

The pickles are prepared from uncured, unfermented cucumbers and are packed in a vinegar solution with other ingredients to produce the characteristics of the particular type of pack. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. In addition, the pickles are sufficiently processed by heat to assure preservation of the product in hermetically sealed containers. The distinguishing characteristics of the various types of fresh-pack pickles are as follows:

1. *Fresh-pack dill pickles* are pickles that are packed in a vinegar solution with dill flavoring.
2. *Fresh-pack sweetened dill pickles* are pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s) and dill flavoring.
3. *Fresh-pack sweetened dill relish* consists of finely cut or chopped pickles packed in a vinegar solution with suitable nutritive sweetening ingredient(s) and dill flavoring. The relish may contain other finely cut or chopped vegetable ingredients as listed in Table 70.1.
4. *Fresh-pack sweet pickles* and *fresh-pack mild sweet pickles* are pickles that are packed in a vinegar solution with nutritive sweetening ingredient(s).
5. *Fresh-pack sweet pickle relish* and *fresh-pack mild sweet pickle relish* consists of finely cut or chopped pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s). The relish may contain other finely cut or chopped vegetable ingredients as listed in Table 70.1.
6. *Fresh-pack hamburger relish* consists of relish style pickles and other chopped or finely cut vegetable ingredients as listed in Table 70.1 with tomato product added.
7. *Fresh-pack mustard relish* consists of sweet pickle relish with mustard and other chopped or finely cut vegetable ingredients as listed in Table 70.1.

8. *Fresh-pack dill relish* consists of relish style pickles containing dill flavoring and other chopped or finely cut vegetable ingredients as listed in Table 70.1.
9. *Fresh-pack dietetic pickles* are pickles that are packed with or without the addition of sweetening ingredient(s), salt (NaCl), or other suitable ingredient(s) as declared and permitted under FDA regulations.

3. Refrigerated Type

The pickles are prepared from fresh cucumbers and are packed in a vinegar solution with other ingredients to produce the fresh crisp characteristic of refrigerated type. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. They are stored, distributed, and displayed under refrigeration and may or may not contain one or more chemical preservatives. The various types of refrigerated pickles are the same as the types listed for fresh-pack type in earlier discussion with respect to ingredients except that they conform to the requirements for refrigerated type.

E. Sizes of Whole Pickles

Sizes of whole pickles are based on the diameter and the relationship of diameter to the count per gallon. Size designations, applicable counts, and diameters are outlined in Table 70.2 of this subpart. The diameter of a whole cucumber is the shortest diameter at the greatest circumference measured at right angles to the longitudinal axis of the cucumber.

F. Definitions of Terms

For an interpretation of this standard, some definitions of terms are:

Analytical definitions refer to analytical laboratory requirements.

1. *Acid* means total acidity calculated as acetic acid in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC).
2. *Brix value* (Brix) means the percent sugar, by weight, corrected to 20°C (68°F), as determined with a sugar scale, Brix hydrometer or other instrument that gives equivalent results.
3. *Degrees Baumé* means the density of the packing medium determined with a Baumé hydrometer (modulus 145) corrected to 20°C (68°F).
4. *Equalization* means the natural (osmotic) or simulated blending between the soluble solids of the pickle ingredient and the packing medium.

   Natural equalization means equalization brought about after a period of time has elapsed after processing as follows. *Sweetened pickles* are considered to be equalized 15 days or more after processing. If the pickles have been sweetened in a tank prior to packing, the pickles will be considered equalized 15 days after the sweetening process began. *Sour and dill pickles* are considered to be equalized 10 days or more after processing.

   Simulated equalization means a method of simulating equalization by comminuting the finished product in a mechanical blender, filtering the suspended material from the comminuted mixture and making the required tests on the filtrate.
5. *Total chlorides or salt* means the salt content expressed as grams NaCl (sodium chloride) per 100 milliliters packing medium; except that total chlorides in mustard pickles and chow is determined and expressed in grams NaCl per 100 grams of product.

Blemished means any unit that is affected by discoloration, pathological injury, insect injury, or similar causes to the

TABLE 70.2
Sizes of Processed Whole Pickles

Word Designation	Diameter	Approximate Counts In			Glass	Metal
		1 qt	1/2 gal	1 gal	No. 10	No. 12 (1 gal)
Midget	19 mm (.75 in) or less	67 or more	135 or more	270 or more	202 or more	270 or more
Small gherkin	Up to 2.4 cm (.94 in)	33–66	67–134	135–269	101–201	135–269
Large gherkin	Up to 2.7 cm (1.06 in)	16–32	32–36	65–134	48–100	65–134
Small	Over 2.7 cm (1.06 in) but not over 3.5 cm (1.38 in)	10–15	20–31	40–64	30–47	40–64
Medium	Over 3.5 cm (1.38 in) but not over 3.8 cm (1.50 in)	6–9	13–19	26–39	19–29	26–39
Large	Over 3.8 cm (1.50 in) but not over 4.4 cm (1.73 in)	4–5	9–13	18–25	13–18	18–25
Extra large	Over 4.4 cm (1.73 in)	2–3	6–8	12–17	9–12	12–17

extent that the appearance or edibility of the product is adversely affected:

1. Slightly — those blemishes which detract only slightly from the appearance of the unit;
2. Seriously — those blemishes which strongly detract from the appearance or edibility of the unit.

1. Color

1. Good color in cured type means the typical skin color of the pickles ranges from a translucent light green to dark green and is practically free from bleached areas. Not more than 10 percent, by weight, of the pickles may vary markedly from such typical color. In mixed pickles, chow chow pickles, and pickle relish, all of the ingredients possess a practically uniform color typical for the pertinent ingredient. The pickles and other vegetable ingredients are free of off-colors.
2. Good color in fresh-pack and refrigerated types means the typical skin color of the pickles ranges from an opaque yellow-green to green. Not more than 15 percent, by weight, of the pickles may vary markedly from such typical color. In pickle relish, all of the ingredients possess a good uniform color typical for the pertinent ingredient. The pickles and other vegetable ingredients are free of off-colors.
3. Reasonably good color in cured type means the typical skin color of the pickles ranges from light green to dark green and is reasonably free from bleached areas. Not more than 25 percent, by weight, of the pickles may vary markedly from such typical color. In mixed pickles, chow chow pickles, and pickle relish, all of the ingredients possess a reasonably uniform color typical for the pertinent ingredient. The pickles and other vegetable ingredients are free of off-colors.
4. Reasonably good color in fresh-pack and refrigerated types means the typical skin color of the pickles ranges from light yellow-green to green. Not more than 30 percent, by weight, of the pickles may vary markedly from such typical color. In pickle relish, all of the ingredients possess a good, fairly uniform color typical for the pertinent ingredient. The pickles and other vegetable ingredients are free of off-colors.
5. Poor color in all types of pickles means the pickles fail to meet the requirements for good or reasonably good color for the pertinent type.

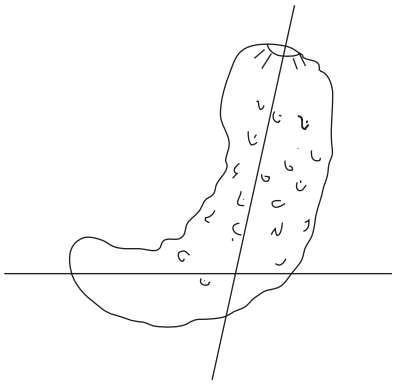

FIGURE 70.1 Crooked pickles (also see the definition of misshapen).

Also see the definition of misshapen.

Crooked pickles mean whole pickles that are curved at an angle greater than 60 degrees, as illustrated by Figure 70.1.

Curved pickles mean whole pickles that are curved at an angle of 35 to 60 degrees when measured as illustrated by Figure 70.2.

Diameter in whole style means the shortest diameter measured transversely to the longitudinal axis at the greatest circumference of the pickle. Diameter in cross-cut style is the shortest diameter of the largest cut surface.

Defect means an imperfection such as curved, misshapen, mechanically damaged, discolored, and other imperfection that affects the appearance or edibility of the product.

End cut means a pickle unit intended for crosscut (sliced crosswise) style that has only one cut surface.

Extraneous Vegetable Material (EVM) means any harmless vegetable material, other than stems, that is not

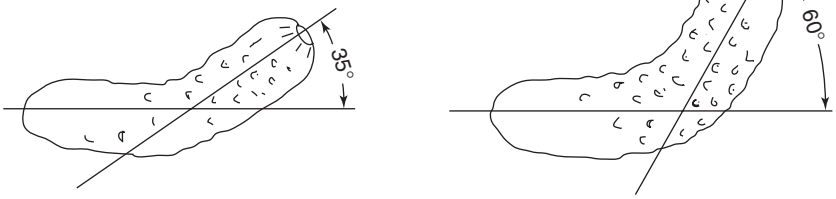

FIGURE 70.2 Curved pickles.

normally part of the pickle ingredient. EVM such as leaves or other vegetable material not associated with proper pickle preparation or packaging is considered a defect if it affects the appearance or edibility of the product either:

Slightly — Practically free of EVM and does not more than slightly affect the appearance or edibility; or

Materially — Reasonably free of EVM and does not more than materially affect the appearance or edibility.

2. Flavor and Odor

1. Good flavor and odor means characteristic flavor and odor (e.g. characteristic dill flavor or the like) typical of properly processed pickles, for the type, that is free from objectionable flavor and odor of any kind.
2. Reasonably good flavor and odor means flavor and odor that may be lacking in characteristic flavor for the type but is free from objectionable flavor and odor.
3. Poor flavor and odor means flavor and odor that fails to meet the requirements for good or reasonably good flavor and odor.

Length in sliced lengthwise style means the longest straight measurement at the approximate longitudinal axis.

Mechanical damage refers to crushed or broken units that affect the appearance of the units. In relish, mechanical damage refers to units that are poorly cut and have a ragged or torn appearance.

Misshapen pickles mean whole pickles that are crooked or otherwise deformed (such as nubbins). Also, see the definition for crooked pickles.

Nubbin is a misshapen pickle that is not cylindrical in form, is short and stubby, or is not well developed.

Texture means the firmness, crispness, and condition of the pickles and any other vegetable ingredient(s) and freedom from large seeds, detached seeds, and tough skins that may be present. The following terms also relate to texture:

1. Hollow centers in whole style, means the pickles, when cut transversely to the longitudinal axis, are missing 1/3 or more of the seed cavity.
2. Soft, shriveled, and slippery units refers to pickles that are wrinkled, not crisp, slick, flabby, or lack firmness.
3. Good texture means the pickle units have been properly processed and possess a texture that is firm and crisp.
4. Reasonably good texture means the pickle units have been properly processed but lack some of the firmness and crispness that is characteristic for the style and type of pack.
5. Poor texture means the pickle units do not meet the requirements for good or reasonably good texture.

3. Uniformity of Size (Relish Style Only)

1. Practically uniform in size means the size of the units may vary moderately in size but not to the extent that the appearance or the eating quality is seriously affected.
2. Poor uniformity of size means the units fail the requirements for practically uniform.

Unit means one whole, half, slice, or piece of pickle as applicable for the style.

Units missing one third or more of the seed cavity in crosscut style means pickles that have lost a substantial portion of the seed cavity such as a crosscut unit missing one third or more of the seed cavity portion.

G. Recommended Fill of Container

The recommended fill of container is not a factor of quality for the purposes of these grades. Each container of pickles should be filled with pickle ingredient, as full as practicable, without impairment of quality. The product and packing medium should occupy not less than 90 percent of the total capacity of the container.

H. Quantity of Pickle Ingredient

The recommended minimum quantity of pickle ingredient is designated as the percentage of the declared volume of product in the container for all items except pickle relish. Minimum quantity of pickle relish is designated as a relationship of the drained weight of the pickle ingredient to the declared volume of the container. The minimum quantities

TABLE 70.3
Recommended Pickle Ingredients

All Styles Except Relish	
Type of Pack	**Minimum Fill (Volume)**
Cured	55 percent
Fresh-Pack	57 percent
Refrigerated	57 percent

TABLE 70.4
Recommended Drained Weight to Container Volume, Relish

Type of Pack	Minimum Fill (Weight/Volume)
Cured:	
Sweet	92 percent
Other than sweet	88 percent
Fresh-Pack:	
Sweet	85 percent
Other than sweet	80 percent

recommended in Tables 70.3 and 70.4 are not factors of quality for the purposes of these grades.

The percent volume of pickle ingredient is determined for all styles, except relish, by one of the following methods in accordance with the procedures prescribed by the USDA:

1. Direct displacement (overflow-can method)
2. Displacement in a graduated cylinder
3. Measurement of pickle liquid
4. Any other method that gives equivalent results and is approved by the USDA

Drained weight/volume. The percent weight/volume (w/v) of relish shown in Table 70.4, is determined as follows: The drained weight of pickle relish of all types is determined by emptying the contents of the container upon a U.S. Standard No. 8 circular sieve of proper diameter containing 8 meshes to the inch (0.0937 inch ±3 percent, square openings) so as to distribute the product evenly, inclining the sieve slightly to facilitate drainage, and allowing to drain for 2 minutes. The drained weight is the weight of the sieve and the pickles less the weight of the dry sieve. A sieve 8 inches in diameter is used for 1 quart and smaller size containers and a sieve 12 inches in diameter is used for containers larger than 1 quart in size.

I. Sample Unit Size

For all styles of pickles and types of pack, the sample unit used in analyzing the quality factors is the entire contents of the container unless otherwise specified in 7 CFR Part.

J. Grades

1. *U.S. Grade A* is the quality of pickles that meets the applicable requirements of Tables 70.5–11 and scores not less than 90 points.
2. *U.S. Grade B* is the quality of pickles that meets the applicable requirements of Tables 70.6–11 and scores not less than 80 points.
3. *Substandard* is the quality of pickles that fails the requirements of U.S. Grade B.

K. Factors of Quality

The grade of pickles is based on the following quality factors:

1. Analytical requirements in Table 70.5
2. Flavor and odor
3. Color
4. Uniformity of size
5. Defects
6. Texture

L. Requirements for Grades

See tables 70.5–11.

II. ESTABLISHMENT INSPECTION

The FDA has issued guidelines for the inspection of a pickles processing plant. Some of the information is provided in this chapter. The quality control officer in

TABLE 70.5
Analytical Requirements [a]

Cured Type Pickles, All Styles	Totally Acidity Expressed as Acetic Acid Grams/100 mL, Unless Otherwise Indicated — Maximum	Total Chlorides Expressed as NaCl Grams/100 mL, Unless Otherwise Indicated — Maximum	Degrees Brix — Minimum	Degrees Baumé — Minimum
Dills (natural, genuine or processed)	1.1	5.0		
Sour, sour mixed, dill pickle relish, sour relish	2.7	5.0		
Sweet whole, sweet mixed, and sweet relish	2.7	3.0	27.0	15.0
Mild sweet, mild sweet mixed, mild sweet relish			20.0	12.0
Sour mustard or sour chow chow	2.7 [b]		3.0 [b]	
Sweet mustard or sweet chow chow	2.7 [b]	3.0 [b]	28.0	15.5
Fresh pack and refrigerated types, all styles				
Dills and sweetened dills	1.1	4.25		
Sweetened dill relish	1.1	4.25		
Sweet and mild sweet relish	1.65	2.75		
Sweet and mild sweet pickles	1.65	2.75		
Dietetic				

[a] All pickle products must have an equilibrated pH of 4.6 or below.
[b] Expressed as "grams/100 grams."

TABLE 70.6
Quality Requirements

	Grade A		Grade B	
Whole Style Pickles	**Maximum (By Count)**	**Score**	**Maximum (By Count)**	**Score**
Flavor and Odor	Good		Reasonably Good [a]	
Color	Good	18–20	Reasonably Good [a]	16–17
Uniformity of Size [b]		18–20		16–17
Diameter variation				
Midget and Gherkin [over 8 mm (.31 in)]	10%		20%	
Small and medium [over 10 mm (.39 in)]	10%		20%	
Large and extra large [over 12 mm (.47 in)]	10%		20%	
Defects	Practically Free	27–30	Reasonably Free [a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Curved pickles	10%		20%	
Misshapen	5%		15%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (.98 in)]	10%		20%	
Extraneous Vegetable Material (EVM)	Practically Free		Reasonably Free [a]	
Texture	Good	27–30	Reasonably Good [a]	24–26
Large seeds, detached seeds, tough skins	5%		10%	
Soft, shriveled, and slippery units	5%		10%	
Hollow centers	15%		25%	
Total Score (minimum)		90 points		80 points

[a] Cannot be graded above U.S. Grade B, regardless of the total score.

[b] Pickles that are Substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

TABLE 70.7
Quality Requirements

	Grade A		Grade B	
Whole Style Pickles, Mixed Sizes	**Maximum (By Count)**	**Score**	**Maximum (By Count)**	**Score**
Flavor and Odor	Good		Reasonably Good [a]	
Color	Good	18–20	Reasonably Good [a]	16–17
Uniformity of Size [b]	18–20	16–17		
Defects	Practically Free	27–30	Reasonably Free [a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Curved pickles	10%		20%	
Misshapen	5%		15%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (.98 in)]	10%		20%	
Extraneous Vegetable Material (EVM)	Practically Free		Reasonably Free [a]	
Texture	Good	27–30	Reasonably Good [a]	24–26
Large seeds, detached seeds, tough skins	5%		10%	
Soft, shriveled, and slippery units	5%		10%	
Hollow centers	15%		25%	
Total Score (minimum) [b]		90 points		80 points

[a] Cannot be graded above U.S. Grade B, regardless of the total score.

[b] Total score is adjusted by dividing the total score by .80 to allow for the absence of the quality factor of uniformity of size in whole mixed sizes style.

TABLE 70.8
Quality Requirements

	Grade A		Grade B	
Sliced Lengthwise Style Pickles	**Maximum (By Count)**	**Score**	**Maximum (By Count)**	**Score**
Flavor and Odor	Good		Reasonably Good [a]	
Color	Good	18–20	Reasonably Good [a]	16–17
Uniformity of Size [b]		18–20		16–17
Length variation [over 2.6 cm (1.02 in)]	10%		20%	
Defects	Practically Free	27–30	Reasonably Free [a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (.98 in)]	10%		20%	
Extraneous Vegetable Material (EVM)	Practically Free		Reasonably Free [a]	
Texture	Good	27–30	Reasonably Good [a]	24–26
Large seeds, detached seeds, tough skins	Practically Free		Reasonably Free [a]	
Soft, shriveled, and slippery units	5%		10%	
Total Score (minimum)		90 points		80 points

[a] Cannot be graded above U.S. Grade B, regardless of the total score.

[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

TABLE 70.9
Quality Requirements

	Grade A		Grade B	
Sliced Crosswise or Crosscut Style Pickles	**Maximum (By Count)**	**Score**	**Maximum (By Count)**	**Score**
Flavor and Odor	Good		Reasonably Good [a]	
Color	Good	18–20	Reasonably Good [a]	
Uniformity of Size [b]		18–20		16–17
Diameter [over 5.4 cm (2.13 in)]	10%		20%	
Defects	Practically Free	27–30	Reasonably Free [a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Mechanical damage	15%		25%	
Broken pieces and end cuts	10%		15%	
Thickness over 10 mm (.38 in)	10%		15%	
Attached stems [over 2.5 cm (.98 in)]	10%		15%	
Units missing 1/3 seed cavity	10%		15%	
Extraneous Vegetable Material (EVM)	Practically Free		Reasonably Free [a]	
Texture	Good	27–30	Reasonably Good [a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically Free		Reasonably Free [a]	
Soft, shriveled, and slippery units	5%		10%	
Total Score (minimum)		90 points		80 points

[a] Cannot be graded above U.S. Grade B, regardless of the total score.

[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

such a plant should use the information to implement its in-plant inspection procedure.

The information is presented in the teacher/student format for ease of reference.

1. Direct special attention to the following areas when inspecting these types of food establishments. If the establishment is producing acidified fresh-pack pickles, determine if the establishment is complying with the requirements of 21 CFR 114, Acidified Foods.
2. Salt Stations and Salt Stock Tanks.
3. Insects which breed in decomposed pickles or other decaying organic matter such as the lesser or little house fly, the latrine fly, the house fly, the rat-tailed maggot, and *drosophila* spp are of major sanitary significance. Examine 25% of the tanks for insect filth.

TABLE 70.10
Quality Requirements

	Grade A		Grade B	
Cut Style Pickles	**Maximum (By Count)**	**Score**	**Maximum (By Count)**	**Score**
Flavor and Odor	Good		Reasonably Good [a]	
Color	Good	18–20	Reasonably Good [a]	16–17
Uniformity of Size [b]		18–20		16–17
Small pieces 5 g or less	5%		10%	
Defects	Practically Free	27–30	Reasonably Free [a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Mechanical damage	10%		15%	
Attached stems [over 2.5 cm (.98 in)]	10%		20%	
Extraneous Vegetable Material (EVM)	Practically Free		Reasonably Free [a]	
Texture	Good	27–30	Reasonably Good [a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically Free		Reasonably Free [a]	
Soft, shriveled, and slippery units	5%		10%	
Total Score (minimum)		90 points		80 points

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

TABLE 70.11
Quality Requirements

	Grade A		Grade B	
Relish	**Maximum (By Weight)**	**Score**	**Maximum (By Weight)**	**Score**
Flavor and Odor	Good		Reasonably Good [a]	
Color	Good	18–20	Reasonably Good [a]	16–17
Uniformity of Size [b]		18–20		16–17
Overall appearance	Good		Reasonably Good [a]	
Defects	Practically Free	27–30	Reasonably Free [a]	24–26
Blemished (slightly and seriously)	15%		25%	
Blemished (seriously)	5%		10%	
Poorly cut	10%		15%	
Loose stems over 3.0 mm (.12 in)	10%		15%	
Extraneous Vegetable Material (EVM)	Practically Free		Reasonably Free [a]	
Texture	Good	27–30	Reasonably Good [a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically Free		Reasonably Free [a]	
Soft, shriveled, and slippery units	5%		10%	
Total Score (minimum)		90 points		80 points

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

4. "Mill run" salt may be used but workers should not walk in the salt.
5. Tanks should be skimmed daily for debris and insects, and the skimmings should be properly disposed of.
6. Newly salted stock ferments: scum growth should be removed regularly and disposed of so that insects are not attracted.

A. Pickle Products

Examination of raw materials used in relish.

1. Obtain the usual composition of relish in percent by weight of cucumbers as well as other ingredients to help appraise the filth load found in the sample.
2. Salt stock used for relish may consist of poor quality pickles, i.e., deformed, bloated, or blemished. However, in the absence of filth, grit, or partly or wholly rotted pickles, there is no objection to their use. Mushy pickles are caused by certain pectin-splitting enzymes during fermentation. Soft pickles may be invaded by bacteria and fungi, but it is

frequently difficult to determine if any mold or bacteria are present by field examination.

3. Examination of cucumber salt stock for relish: When whole pickles or large pieces are used, examine a representative sample of 100 units going to chopper.
4. Segregate and list objectionable pickles as follows:

Class	Number	Percent
– with rot spots over 1/2 in.	_____	_____
– insect-infested or damaged	_____	_____
– mushy or very soft	_____	_____

5. For class 1 pickles, make a further determination of the surface area of the rot spots by size; up to 1 inch; from 1 inch to half of the pickle; and over half of the pickle. Take close-up color photographs of objectionable pickles. Collect exhibits of pickles showing typical rot and insect damage.
6. Laboratory examination of mushy pickles for mold is necessary to establish if they are objectionable. If over 5% of the units are mushy, cut a thin cross-section from each pickle. Place the slices in a quart jar with water and add 20 cc formaldehyde for later examination.
7. When small pieces of salt stock cucumbers, cauliflower, and peppers are used, rot determination by count is impractical. If rotten pieces are observed, collect a separate quart of each pickled vegetable. Preserve the samples with 20 cc formaldehyde. At the same time, collect a sample totaling half a gallon of finished relish.

B. Peppers

1. Check for insect larvae (maggots or larvae of pepper weevil) in fresh and salt stock peppers and figure percent of infestation on a representative sample. Examine any fresh pack peppers in which infested stock was used.
2. If peppers with rot are found, evaluate in the same fashion as for cucumbers.
3. Examine vinegar storage tanks for drosophila infestation and for vinegar eels.
4. Insect filth in sweet stock pickles: insects, particularly drosophila, are attracted to the sweetening tanks, and may be found in the finished sweet pickle products.
5. Sweet brine is frequently circulated within a tank and from one tank to another dispersing insects in the circulating brine. It is sometimes difficult to estimate the number of insects and parts in such circulating brine. Close examination of the inside tank walls may reveal drosophila above the brine level. These are the best indices of infestation in a tank.

When insects are found in a sweetening tank:

1. Determine whether sweet brine in the tank is an intermediate or finishing brine and if it is circulated within the tank or between sweetening tanks.
2. If the finishing brine is used as a packing medium, determine whether it is filtered prior to use and evaluate the filtration step.
3. If sweet stock is held in infested tanks, determine anticipated date of packing.
4. Evaluate tank covers used.
5. List quantitatively, the extent of insect infestation by the collection of representative samples of filth from a definite area, e.g., square feet of the walls of the tank on the sweet stock and in a specified amount of brine from different areas of the tank if the infestation is widespread. If infestation seems to be isolated, collect specimens showing the types of insects.

C. Other Points of Interest

1. Grit in pickles: Excessive grit is frequently found in fresh pack pickles and in midget sweet pickles. Salt stock may occasionally contain excessive grit. If dirty cucumbers are packed, collect in-line and finished product samples.
2. Use of color and preservatives: Green artificial color is sometimes used in relish without label declaration. Ascertain if the color is permitted for use and declared on the label.
3. Sorbic acid may be used in salt stock, to prevent yeast growth, and in finished pickle products, as a preservative. Where sorbic acid is present in the finished product, determine if it is declared on the label.
4. Examination of warehouse stocks: Examine for evidence of spoilage, particularly in fresh pack pickles that may have been inadequately pasteurized.
5. If heavy insect infestation is found, examine 24 jars of the pickle product (other than relish) most likely to contain insects by inverting jars under strong light. Collect jars containing insects as a factory sample.

D. Sample Collection

1. Bulk Salt Stock for Filth

If in barrels, collect a minimum of 12 half-gallon jars of salt with their brine; 2 from each of 6 previously unopened

barrels to make 6 duplicate subs. Collect 1 sub from the top and the other sub from the bottom, if possible. If in tank cars, collect a minimum of twelve 1/2 gallon jars of salt stock and brine. If live flies are observed inside tank during sampling, note and estimate their number.

2. Finished Pickle Product — All Types

Filth and Grit

Quarts and smaller jars	*Minimum to collect*
Up to 100 cases in lot	24 jars
More than 100 cases	48 jars
Gallon jars	*Minimum to collect*
Up to 100 cases	12 jars
More than 100 cases	24 jars

3. Undeclared Color and Chemical Additives

Collect 6 quarts or 12 pints for examination.

ACKNOWLEDGMENT

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Part G

Food and Workers Safety, Food Security

71 U.S. Food Standards and Food Grades

Nanna Cross
Chicago, Illinois

CONTENTS

I. INTRODUCTION

In the United States, three federal agencies have responsibility to make sure that processed foods (excluding meat and poultry) in the market are safe and do not pose any economic fraud. The U.S. Food and Drug Administration (FDA) issues regulations or mandatory requirements to achieve both goals. The U.S. Department of Agriculture (USDA) and U.S. National Marine Fisheries Service (NMFS) issue voluntary guides to achieve the same goals. However, "voluntary" can become "mandatory" under certain circumstances.

When studying information in this chapter, please note the following premises:

1. It is most useful for food companies of all sizes that sell products across state lines.
2. It is most useful for small food companies, especially new ones.
3. Although the information is designed for domestic food companies, it is definitely beneficial for foreign food manufacturers that export food to the United States.
4. Abbreviations used:
   CFR = U.S. Code of Federal Regulations.
   21 CFR 113 = U.S. Code of Federal Regulations, Title 21, Part 113.

II. FDA FOOD STANDARDS

Food standards are a necessity for both consumers and the food industry. They maintain the general quality of a large part of the national food supply and prevent economic fraud. Without standards, different foods could have the same names or the same foods could have different names. Both situations would be confusing and misleading to consumers and create unfair competition.

Section 401 of the Federal Food, Drug, and Cosmetic Act requires that whenever such action will promote honesty and fair dealing in the interest of consumers, regulations shall be promulgated fixing and establishing for any food, under its common or usual name so far as practicable, a reasonable definition and standard of identity, a reasonable standard of quality, and reasonable standards of fill-of-container. However, no definition and standard of identity or standard of quality may be established for fresh or dried fruits, fresh or dried vegetables, or butter, except that definitions and standards of identity may be established for avocados, cantaloupes, citrus fruits, and melons.

Standards of identity define what a given food product is, its name, and the ingredients that must or may be used in the manufacture of the food. Standards of quality are minimum standards only and establish specifications for quality requirements. Fill-of-container standards define

how full the container must be and how this is measured. FDA standards are based on the assumption that the food is properly prepared from clean, sound materials. Standards do not usually relate to such factors as deleterious impurities, filth, and decomposition. There are exceptions. For example, the standards for whole egg and yolk products and for egg white products require these products to be pasteurized or otherwise treated to destroy all viable Salmonella bacteria. Some standards for foods set nutritional requirements, such as those for enriched bread, or nonfat dry milk with added vitamins A and D, etc. A food which is represented as, or purports to be, a food for which a standard of identity has been promulgated must comply with the specifications of the standard in every respect.

The standards that have been promulgated by the FDA are listed below.

A. 21 CFR 131 — Milk and Cream

1. Requirements for Specific Standardized Milk and Cream

21 CFR.110 — Milk.
21 CFR.111 — Acidified milk.
21 CFR.112 — Cultured milk.
21 CFR.115 — Concentrated milk.
21 CFR.120 — Sweetened condensed milk.
21 CFR.125 — Nonfat dry milk.
21 CFR.127 — Nonfat dry milk fortified with vitamins A and D.
21 CFR.130 — Evaporated milk.
21 CFR.147 — Dry whole milk.
21 CFR.149 — Dry cream.
21 CFR.150 — Heavy cream.
21 CFR.155 — Light cream.
21 CFR.157 — Light whipping cream.
21 CFR.160 — Sour cream.
21 CFR.162 — Acidified sour cream.
21 CFR.170 — Eggnog.
21 CFR.180 — Half-and-half.
21 CFR.200 — Yogurt.
21 CFR.203 — Lowfat yogurt.
21 CFR.206 — Nonfat yogurt.

B. 21 CFR 133 — Cheeses and Related Cheese Products

1. Requirements for Specific Standardized Cheese and Related Products

21 CFR 133.102 — Asiago fresh and Asiago soft cheese.
21 CFR 133.103 — Asiago medium cheese.
21 CFR 133.104 — Asiago old cheese.
21 CFR 133.106 — Blue cheese.
21 CFR 133.108 — Brick cheese.
21 CFR 133.109 — Brick cheese for manufacturing.
21 CFR 133.111 — Caciocavallo Sicilian cheese.
21 CFR 133.113 — Cheddar cheese.
21 CFR 133.114 — Cheddar cheese for manufacturing.
21 CFR 133.116 — Low sodium cheddar cheese.
21 CFR 133.118 — Colby cheese.
21 CFR 133.119 — Colby cheese for manufacturing.
21 CFR 133.121 — Low sodium Colby cheese.
21 CFR 133.123 — Cold-pack and club cheese.
21 CFR 133.124 — Cold-pack cheese food.
21 CFR 133.125 — Cold-pack cheese food with fruits, vegetables, or meats.
21 CFR 133.127 — Cook cheese, Koch kaese.
21 CFR 133.128 — Cottage cheese.
21 CFR 133.129 — Dry curd cottage cheese.
21 CFR 133.133 — Cream cheese.
21 CFR 133.134 — Cream cheese with other foods.
21 CFR 133.136 — Washed curd and soaked curd cheese.
21 CFR 133.137 — Washed curd cheese for manufacturing.
21 CFR 133.138 — Edam cheese.
21 CFR 133.140 — Gammelost cheese.
21 CFR 133.141 — Gorgonzola cheese.
21 CFR 133.142 — Gouda cheese.
21 CFR 133.144 — Granular and stirred curd cheese.
21 CFR 133.145 — Granular cheese for manufacturing.
21 CFR 133.146 — Grated cheeses.
21 CFR 133.147 — Grated American cheese food.
21 CFR 133.148 — Hard grating cheeses.
21 CFR 133.149 — Gruyere cheese.
21 CFR 133.150 — Hard cheeses.
21 CFR 133.152 — Limburger cheese.
21 CFR 133.153 — Monterey cheese and Monterey jack cheese.
21 CFR 133.154 — High-moisture jack cheese.
21 CFR 133.155 — Mozzarella cheese and scamorza cheese.
21 CFR 133.156 — Low-moisture mozzarella and scamorza cheese.
21 CFR 133.157 — Part-skim mozzarella and scamorza cheese.
21 CFR 133.158 — Low-moisture part-skim mozzarella and scamorza cheese.
21 CFR 133.160 — Muenster and Munster cheese.
21 CFR 133.161 — Muenster and Munster cheese for manufacturing.
21 CFR 133.162 — Neufchatel cheese.
21 CFR 133.164 — Nuworld cheese.
21 CFR 133.165 — Parmesan and reggiano cheese.
21 CFR 133.167 — Pasteurized blended cheese.
21 CFR 133.168 — Pasteurized blended cheese with fruits, vegetables, or meats.
21 CFR 133.169 — Pasteurized process cheese.
21 CFR 133.170 — Pasteurized process cheese with fruits, vegetables, or meats.
21 CFR 133.171 — Pasteurized process pimento cheese.
21 CFR 133.173 — Pasteurized process cheese food.

21 CFR 133.174 — Pasteurized process cheese food with fruits, vegetables, or meats.
21 CFR 133.175 — Pasteurized cheese spread.
21 CFR 133.176 — Pasteurized cheese spread with fruits, vegetables, or meats.
21 CFR 133.178 — Pasteurized Neufchatel cheese spread with other foods.
21 CFR 133.179 — Pasteurized process cheese spread.
21 CFR 133.180 — Pasteurized process cheese spread with fruits, vegetables, or meats.
21 CFR 133.181 — Provolone cheese.
21 CFR 133.182 — Soft ripened cheeses.
21 CFR 133.183 — Romano cheese.
21 CFR 133.184 — Roquefort cheese, sheep's milk blue-mold, and blue-mold cheese from sheep's milk.
21 CFR 133.185 — Samsoe cheese.
21 CFR 133.186 — Sapsago cheese.
21 CFR 133.187 — Semisoft cheeses.
21 CFR 133.188 — Semisoft part-skim cheeses.
21 CFR 133.189 — Skim milk cheese for manufacturing.
21 CFR 133.190 — Spiced cheeses.
21 CFR 133.191 — Part-skim spiced cheeses.
21 CFR 133.193 — Spiced, flavored standardized cheeses.
21 CFR 133.195 — Swiss and Emmentaler cheese.
21 CFR 133.196 — Swiss cheese for manufacturing.

C. 21 CFR 135 — Frozen Desserts

1. Requirements for Specific Standardized Frozen Desserts

21 CFR.110 — Ice cream and frozen custard.
21 CFR.115 — Goats milk ice cream.
21 CFR.130 — Mellorine.
21 CFR.140 — Sherbet.
21 CFR.160 — Water ices.

D. 21 CFR 136 — Bakery Products

1. Requirements for Specific Standardized Bakery Products

21 CFR.110 — Bread, rolls, and buns.
21 CFR.115 — Enriched bread, rolls, and buns.
21 CFR.130 — Milk bread, rolls, and buns.
21 CFR.160 — Raisin bread, rolls, and buns.
21 CFR.180 — Whole wheat bread, rolls, and buns.

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1. Requirements for Specific Standardized Cereal Flours and Related Products

21 CFR 137.105 — Flour.
21 CFR 137.155 — Bromated flour.
21 CFR 137.160 — Enriched bromated flour.
21 CFR 137.165 — Enriched flour.
21 CFR 137.170 — Instantized flours.
21 CFR 137.175 — Phosphated flour.
21 CFR 137.180 — Self-rising flour.
21 CFR 137.185 — Enriched self-rising flour.
21 CFR 137.190 — Cracked wheat.
21 CFR 137.195 — Crushed wheat.
21 CFR 137.200 — Whole wheat flour.
21 CFR 137.205 — Bromated whole-wheat flour.
21 CFR 137.211 — White corn flour.
21 CFR 137.215 — Yellow corn flour.
21 CFR 137.220 — Durum flour.
21 CFR 137.225 — Whole durum flour.
21 CFR 137.250 — White corn meal.
21 CFR 137.255 — Bolted white corn meal.
21 CFR 137.260 — Enriched corn meal.
21 CFR 137.265 — Degerminated white corn meal.
21 CFR 137.270 — Self-rising white corn meal.
21 CFR 137.275 — Yellow corn meal.
21 CFR 137.280 — Bolted yellow corn meal.
21 CFR 137.285 — Degerminated yellow corn meal.
21 CFR 137.290 — Self-rising yellow cornmeal.
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1. Subpart B — Requirements for Specific Standardized Macaroni and Noodle Products

21 CFR 139.110 — Macaroni products.
21 CFR 139.115 — Enriched macaroni products.
21 CFR 139.117 — Enriched macaroni products with fortified protein.
21 CFR 139.120 — Milk macaroni products.
21 CFR 139.121 — Nonfat milk macaroni products.
21 CFR 139.122 — Enriched nonfat milk macaroni products.
21 CFR 139.125 — Vegetable macaroni products.
21 CFR 139.135 — Enriched vegetable macaroni products.
21 CFR 139.138 — Whole-wheat macaroni products.
21 CFR 139.140 — Wheat and soy macaroni products.
21 CFR 139.150 — Noodle products.
21 CFR 139.155 — Enriched noodle products.
21 CFR 139.160 — Vegetable noodle products.
21 CFR 139.165 — Enriched vegetable noodle products.
21 CFR 139.180 — Wheat and soy noodle products.

G. 21 CFR 145 — Canned Fruits

1. Requirements for Specific Standardized Canned Fruits

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145.115 — Canned apricots.

145.116 — Artificially sweetened canned apricots.
145.120 — Canned berries.
145.125 — Canned cherries.
145.126 — Artificially sweetened canned cherries.
145.130 — Canned figs.
145.131 — Artificially sweetened canned figs.
145.134 — Canned preserved figs.
145.135 — Canned fruit cocktail.
145.136 — Artificially sweetened canned fruit cocktail.
145.140 — Canned seedless grapes.
145.145 — Canned grapefruit.
145.170 — Canned peaches.
145.171 — Artificially sweetened canned peaches.
145.175 — Canned pears.
145.176 — Artificially sweetened canned pears.
145.180 — Canned pineapple.
145.181 — Artificially sweetened canned pineapple.
145.185 — Canned plums.
145.190 — Canned prunes.

H. 21 CFR 146 — Canned Fruit Juices

1. Requirements for Specific Standardized Canned Fruit Juices and Beverages

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146.120 — Frozen concentrate for lemonade.
146.121 — Frozen concentrate for artificially sweetened lemonade.
146.126 — Frozen concentrate for colored lemonade.
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146.135 — Orange juice.
146.137 — Frozen orange juice.
146.140 — Pasteurized orange juice.
146.141 — Canned orange juice.
146.145 — Orange juice from concentrate.
146.146 — Frozen concentrated orange juice.
146.148 — Reduced acid frozen concentrated orange juice.
146.150 — Canned concentrated orange juice.
146.151 — Orange juice for manufacturing.
146.152 — Orange juice with preservative.
146.153 — Concentrated orange juice for manufacturing.
146.154 — Concentrated orange juice with preservative.
146.185 — Pineapple juice.
146.187 — Canned prune juice.

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1. Requirements for Specific Standardized Fruit Butters, Jellies, Preserves, and Related Products

150.110 — Fruit butter.
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J. 21 CFR 152 — Fruit Pies

1. Requirements for Specific Standardized Fruit Pies

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1. Requirements for Specific Standardized Canned Vegetables

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155.130 — Canned corn.
155.131 — Canned field corn.
155.170 — Canned peas.
155.172 — Canned dry peas.
155.190 — Canned tomatoes.
155.191 — Tomato concentrates.
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1. Requirements for Specific Standardized Vegetable Juices

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161.170 — Canned Pacific salmon.
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161.176 — Frozen raw lightly breaded shrimp.
161.190 — Canned tuna.

P. 21 CFR 163 — Cacao Products

1. Requirements for Specific Standardized Cacao Products

163.110 — Cacao nibs.
163.111 — Chocolate liquor.
163.112 — Breakfast cocoa.
163.113 — Cocoa.
163.114 — Lowfat cocoa.
163.117 — Cocoa with dioctyl sodium sulfosuccinate for manufacturing.
163.123 — Sweet chocolate.
163.124 — White chocolate.
163.130 — Milk chocolate.
163.135 — Buttermilk chocolate.
163.140 — Skim milk chocolate.
163.145 — Mixed dairy product chocolates.
163.150 — Sweet cocoa and vegetable fat coating.
163.153 — Sweet chocolate and vegetable fat coating.
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Q. 21 CFR 164 — Tree Nut and Peanut Products

1. Requirements for Specific Standardized Tree Nut and Peanut Products

164.110 — Mixed nuts.
164.120 — Shelled nuts in rigid or semirigid containers.
164.150 — Peanut butter.

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1. Requirements for Specific Standardized Beverages

165.110 — Bottled water.

S. 21 CFR 166 — Margarine

166.40 — Labeling of margarine.
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1. Requirements for Specific Standardized Sweeteners and Table Sirups

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168.111 — Dextrose monohydrate.
168.120 — Glucose syrup.
168.121 — Dried glucose syrup.
168.122 — Lactose.
168.130 — Cane syrup.
168.140 — Maple syrup.
168.160 — Sorghum syrup.
168.180 — Table syrup.

U. 21 CFR 169 — Food Dressings and Flavorings

1. Requirements for Specific Standardized Food Dressings and Flavorings

169.115 — French dressing.
169.140 — Mayonnaise.
169.150 — Salad dressing.
169.175 — Vanilla extract.
169.176 — Concentrated vanilla extract.
169.177 — Vanilla flavoring.
169.178 — Concentrated vanilla flavoring.
169.179 — Vanilla powder.
169.180 — Vanilla-vanillin extract.
169.181 — Vanilla-vanillin flavoring.
169.182 — Vanilla-vanillin powder.

III. USDA STANDARDS FOR GRADES OF PROCESSED FRUITS AND VEGETABLES

Voluntary U.S. grade standards are issued under the authority of the Agricultural Marketing Act of 1946, which provides for the development of official U.S. grades to designate different levels of quality. These grade standards are available for use by producers, suppliers, buyers, and consumers. As in the case of other standards for grades of processed fruits and vegetables, these standards are designed to facilitate orderly marketing by providing a convenient basis for buying and selling, for establishing quality control programs, and for determining loan values.

The standards also serve as a basis for the inspection and grading of commodities by the Federal inspection service, the only activity authorized to approve the designation of U.S. grades as referenced in the standards, as provided under the Agricultural Marketing Act of 1946. This service, available as on-line (in-plant) or lot inspection and grading of all processed fruit and vegetable products, is offered to interested parties, upon application, on a fee-for-service basis. The verification of some specific recommendations, requirements, or tolerances contained in the standards can be accomplished only by the use of on-line inspection procedures. In all instances, a grade can be assigned based on final product factors or characteristics.

In addition to the U.S. grade standards, grading manuals or instructions for inspection of several processed fruits and vegetables are available upon request for a

nominal fee. These manuals or instructions contain detailed interpretations of the grade standards and provide step-by-step procedures for grading the product.

Grade standards are issued by the Department after careful consideration of all data and views submitted, and the Department welcomes suggestions that might aid in improving the standards in future revisions.

Those products with such grade standards are listed below. They are all located in 7 CFR 52.

A. Canned Fruit

Apples
Apple Butter
Apple Juice
Applesauce
Apricots, Regular and Solid Pack
Blackberries and Other Similar Berries
Blueberries
Cherries, RTP
Cherries, Sweet
Cranberry Sauce
Figs, Kadota
Fruit Cocktail
Fruit Jelly
Fruit Preserves (Jams)
Fruits for Salad
Grapefruit
Grapefruit Juice, from Concentrate and Concentrated Grapefruit Juice for Manufacturing
Grapefruit and Orange for Salad
Grapefruit Juice and Orange Juice (Blend)
Grape Juice
Grapes
Lemon Juice
Lemon Juice, Concentrate for Manufacturing.
Orange Juice: from Concentrate, Pasteurized, Concentrated, and Concentrate for Manufacturing
Orange Marmalade
Peaches, Clingstone
Peaches, Freestone
Pears
Pineapple
Pineapple Juice and Pineapple Juice from Concentrate
Plums
Prunes, Dried
Raspberries
Tangerine Juice
Tangerine Juice Concentrate for Manufactuing

B. Canned Vegetables

Asparagus
Beans, Baked, Dried, Pork and
Beans, Green and Wax
Beans
Beets
Carrots
Celery
Chili Sauce
Corn, Cream Style
Corn, Whole Kernel
Hominy
Leafy Greens
Mushrooms
Okra
Okra and Tomatoes and Tomatoes and Okra
Olives, Green
Olives, Ripe
Onions
Peas
Peas and Carrots
Peas, Field and Blackeye
Pickles
Pimientos
Potatoes, White
Pumpkin (Squash)
Sauerkraut
Spinach
Squash (Summer type)
Succotash
Sweet potatoes
Tomatoes
Tomato Catsup
Tomato Juice and Tomato Juice from Concentrate
Tomato Juice, Concentrate
Tomato Paste
Tomato Puree (pulp)
Tomato Sauce

C. Frozen Fruit

Apple Juice, Concentrate
Apricots
Berries (Black, Boysen, etc.)
Blueberries
Cherries (red tart pitted)
Cherries, Sweet
Cranberries
Grape Juice, Concentrate, Sweetened
Grapefruit
Grapefruit Juice
Conc. Grapefruit Juice
Conc. Grapefruit Juice for Manufacturing
Grapefruit Juice and Orange Juice Concentrate, Blended
Lemonade, Concentrate
Limeade, Concentrate
Melon Balls
Orange Juice
Orange Juice from Concentrate

Pasteurized Orange Juice
Concentrate Orange Juice
Concentrate Orange Juice for Manufacturing
Reduced Acid Orange Juice, Concentrate
Peaches
Pineapple Juice
Pineapple Juice from Concentrate
Pineapple
Plums
Raspberries
Strawberries

D. Frozen Vegetables

Asparagus
Beans, Green and Wax Beans, Lima
Beans, Speckled Butter (Lima)
Broccoli
Brussels Sprouts
Carrots
Cauliflower
Corn, Whole Kernel
Corn on the Cob
Leafy Greens (includes Spinach)
Okra
Onion Rings, Breaded
Peas
Peas and Carrots
Peas, Field and Black-eye
Peppers
Sweet Potatoes
French Fried Potatoes
Hash Brown
Rhubarb
Squash (Cooked)
Squash (Summer)
Succotash
Sweet Potatoes
Tomato Juice
Tomato Juice from Concentrate
Turnip Greens with Turnips
Vegetables, Mixed

E. Dried Dehydrated Products

Apples, Dehydrated
Apples, Dried
Apricots, Dehydrated
Apricots, Dried
Dates
Figs
Grapefruit Juice, Dehydrated
Orange Juice, Dehydrated
Peaches, Dehydrated
Pears, Dried
Prunes
Prunes, Dried
Raisins, Processed

F. Sugar Products

Honey, Comb
Honey, Extracted
Maple Syrup
Molasses, Sugarcane
Syrup, Refiners'
Syrup, Sugarcane

G. Miscellaneous Products

Cherries, Sulphured
Olive Oil
Peanut Butter
Potatoes, Peeled
Sauerkraut, Bulk

IV. NMFS STANDARDS FOR GRADES OF FISHERY PRODUCTS

The regulatory agency, the National Marine Fisheries Service (NMFS), has issued some minimal criteria for several seafood and seafood products: what they are, what types and styles are available, and so on. A product grade is established to achieve two objectives: assure product safety and minimize economic fraud. The fishery products with standards for grades include the following.

A. 50 CFR 261 — United States Standards for Grades of Whole or Dressed Fish

Whole or Dressed Fish
Frozen Headless Dressed Whiting

B. 50 CFR 262 — United States Standards for Grades of Fish Steaks

Frozen Halibut Steaks

C. 50 CFR 263 — United States Standards for Grades of Fish Fillets

Fish Fillets
Cod Fillets
Flounder and Sole Fillets
Haddock Fillets
Ocean and Pacific Perch Fillets

D. 50 CFR 264 — United States Standards for Grades of Frozen Fish Blocks and Products Made There from

Frozen Fish Fillet Blocks
Frozen Minced Fish Blocks
Frozen Raw Fish Portions
Frozen Raw Breaded Fish Sticks
Frozen Raw Breaded Fish Portions
Frozen Fried Fish Sticks
Frozen Fried Fish Portions

E. 50 CFR 265 — United States Standards for Grades of Crustacean Shellfish

Fresh and Frozen Shrimp
Frozen Raw Breaded Shrimp

F. 50 CFR 266 — United States Standards for Grades of Molluscan Shellfish

Frozen Raw Scallops
Frozen Raw Breaded Scallops and Frozen Fried Scallops

G. 50 CFR 267 — United States Standards for Grades of North American Freshwater Catfish and their Products

ACKNOWLEDGMENT

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72 Standards for Meat and Poultry in the United States

Nanna Cross
Chicago, Illinois

CONTENTS

I. INTRODUCTION

In the United States, the federal government has total control over the safety of all land muscle foods, i.e., beef, pork, chicken, and many others. This approach is similar to that of major Western countries. The federal agency is called Food Safety Inspection Service (FSIS). FSIS is one arm of the United States Department of Agriculture (USDA). FSIS has the major responsibility of assuring that nation's meat supply and other land muscle foods are save for the public to consume. However, apart from safety, the FSIS has another responsibility, assuring that economic fraud is kept to a minimum. Because meat and many land muscle foods are high-priced items, many dishonest food operations cannot resist the temptation to cheat the consumers when the opportunities arise. Fortunately, most operators are honest businessmen. Still the FSIS has spent an enormous amount of effort to prevent economic fraud.

All information in this chapter has been derived from publication documents issued by the USDA, with legal citation and languages removed. To obtain original documents, please consult the USDA with respect to standards and labeling of products under USDA jurisdiction.

II. DEFINITIONS

For ease of reference, some important terms are defined here.

Red Meat

Required percentages of meat required for red meat products are shown on the basis of *fresh uncooked weight* unless otherwise indicated. Whenever the terms beef, pork, lamb, mutton, or veal are used they indicate the use of skeletal muscle tissue from the named species (9 CFR 301.2).

Poultry

Required percentages for poultry products are based on a *cooked deboned* basis unless otherwise stated. When the standards indicate "poultry," the skin and fat are not to exceed natural proportions per (9 CFR 381.117(d)).

III. ABBREVIATIONS

AMS Agriculture Marketing Service
BHA Butylated Hydroxyanisole (antioxidant)
BHT Butylated Hydroxytolune (antioxidant)
CRDSM Calcium Reduced Dry Skim Milk
FDA Food and Drug Administration
FR French
FSIS Food Safety and Inspection Service
FTC Federal Trade Commission
GRAS Generally Recognized as Safe
HVP Hydrolyzed Vegetable Protein
IMPS Institutional Meat Purchase Specifications
IT Italian
LCPS Labeling and Consumer Protection Staff
MPR Moisture Protein Ratio
MSG Monosodium Glutamate
NAMP National Association of Meat Purveyors
NFDM Nonfat Dry Milk
OPPD Office of Policy Program Development
PDBFT Partially Defatted Beef Fatty Tissue
PDCB Partially Defatted Chopped Beef
PDCP Partially Defatted Chopped Poultry
PDPFT Partially Defatted Pork Fatty Tissue
PER Protein Efficiency Ratio
PFF Protein Fat Free
pH Measure of Acidity
PPM Parts Per Million
SP Spanish
TVP Textured Vegetable Protein
URMIS Uniform Retail Meat Identity Standards
USA United States of America
USDA United States Department of Agriculture
VPP Vegetable Protein Product

IV. STANDARDS, LABELS, AND OTHER INFORMATION

Andouille (FR)

Made with pork and/or pork byproducts stuffed into large intestines. Product can be sold cooked or uncooked. Andouille is a coined name and must be accompanied by a true product name, e.g., "sausage" or "pudding" depending on formulation. If beef is used, it must be shown in the product name, e.g., "Beef Andouille Sausage" or "Beef Andouille Pudding."

Arroz Con Pollo (SP)

The product must contain at least 15% cooked chicken meat. The label must show, the true product name, in English, i.e., "Rice with Chicken," except if the product is distributed solely in Puerto Rico.

Au Gratin Potatoes and Bacon

At least 8% fully cooked bacon (based on 40% yield).

Baby Food

High Meat Dinner — At least 26% meat. High Meat Poultry Dinner — At least 18.75% cooked poultry meat, skin, fat and giblets. Meat and Broth — At least 61% meat. Vegetable with Meat — At least 8% meat. Poultry with Broth — At least 43% cooked poultry meat, skin, and giblets. Poultry and Rice — At least 5% cooked deboned poultry meat.

Note: Wine, mechanically separated species, nitrites, and nitrates are not acceptable in baby and toddler foods.

Baby Food with Fresh Ham or Bacon

Ham or bacon without nitrates or nitrites must be shown in the ingredients statement as ham or bacon (water, salt, sugar, etc., without nitrates or nitrites).

Bacon

The term "bacon" is used to describe the cured belly of a swine carcass. If meat from other portions of the carcass is used, the product name must be qualified to identify the portions, e.g., "Pork Shoulder Bacon." "Certified" refers to products that have been treated for trichinae.

Bacon and Pork Sausage

Product is formulated with a high percentage of bacon (usually bacon ends and pieces) with at least 20% pork.

Bacon Arkansas and Arkansas Style Bacon

Product which is identified as Arkansas Bacon or Arkansas Style Bacon is produced from the pork shoulder blade Boston roast. The pork shoulder blade Boston roast includes the porcine muscle, fat and bone, cut interior of the second or third thoracic vertebrae, and posterior of the atlas joint (first cervical vertebrae), and dorsal of the center of the humerus bone. For Arkansas Bacon, the neck bones and rib bones are removed by cutting close to the underside of those bones. The blade bone (scapula) and the dorsal fat covering, including the skin (clear plate), are removed, leaving no more than one-quarter inch of the fat covering the roast. The meat is then dry cured with salt, sugar, nitrites, and spices, and smoked with natural smoke. The meat may not be injected or soaked in curing brine, nor may any artificial or liquid smoke be applied to the meat. Product that is prepared outside the state of Arkansas but in the manner prescribed may be identified as "Arkansas Style Bacon." The true product name must be shown as "Boneless Cured Pork Shoulder Butt."

Bacon (Canned — Pasteurized)

A shelf stable item, which must have at least 7% brine concentration.

Bacon (Canned, Prefried)

In "Canned Prefried Bacon," e.g., "Bacon Crumbles," the following criteria should be applied:

1. M/SP Index of 0.4 or more. M/SP = Moisture/(Salt × Protein).
2. A Brine Ratio of 9.0 or less. Brine Ratio = Moisture/Salt.
3. A Brine concentration of 10% or more. Brine concentration = Salt/(Moisture + Salt).
4. Maximum 40% yield.

Bacon (Cooked)

Not to yield more than 40% bacon — 60% shrink required. BHA and BHT may be used as antioxidants in precooked bacon at level of 0.01% individually or 0.02% collectively, based on fat content. TBHQ can be used in products as an antioxidant in combination with BHT and BHA; but it can not be used alone except in cooked bacon.

Bacon Dressing for Stuffing

The product must contain at least 8% bacon.

Bacon-Like Products

Bacon-like products, including poultry bacon, labeled with "bacon" in the name must follow the same requirements as those applied to pork bacon. These requirements include, but are not limited to, limits on restricted ingredients and the requirement that the bacon must return to green weight.

Beef bacon is a cured and smoked beef product sliced to simulate regular bacon. It is prepared from various beef cuts and offered with a variety of coined names, including "Breakfast Beef," "Beef Bacon," etc. A common or usual name is required, e.g., "Cured and Smoked Beef Plate," and should be shown contiguous to the coined name.

Poultry bacon products are acceptable and may be designated as (Kind) Bacon. However, a true descriptive name must appear contiguous to (Kind) Bacon without intervening type or design, in letters at least one-half the size of the letters used in the (Kind) Bacon, and in the same style and color and on the same background. An example of an acceptable designation is "Turkey Bacon-Cured Turkey Breast Meat-Chopped and Formed."

The descriptive name can serve alone as the product name.

Bacon Products

The bacon products intended for further cooking before consumption, i.e., slab bacon for deli slicing, can be labeled "certified," "roasted" or "partially cooked" provided the product is cooked to 148°F and the labeling clearly indicated the product is intended to be further cooked before consumption.

Bangers

A sausage-like product prepared with meat and varying amounts of rusk or other cereals. The label must show percentage of rusk (or other cereal) adjacent to product

name in prominent lettering. May be labeled British, Scottish or Irish Style.

Barbecue (BBQ) Products

Barbecue (BBQ) products that are composed of uncured red meat products that are injected, massaged, tumbled, etc., and which are cooked back to or below the weight of the raw meat product (green weight), must use the term "seasoned" or "flavored," in conjunction with the meat product in the product name, e.g., "BBQ Seasoned Pork," or "Sliced Seasoned Beef with Barbecue Sauce."

The labeling for uncured red meat products containing some solutions that are used to make BBQ products (9 CFR 319.312 or 319.80) which are not cooked back to green weight or are not in compliance with the cooking yield must have a containing statement on the label. A containing statement is required in the product name when the cooking yield is not met, e.g., "BBQ Pork Containing up to 15% of a solution." Similarly, a containing statement is required in the product name when the product does not have sufficient quantities of meat minus the solution to meet the minimum meat requirement. However, in limited situations when the minimum meat requirement (minus the solution) is met and when cook yield is compensated for by adding additional meat, the containing statement can either be placed in the product name or attached to the meat component in the ingredients statement, e.g., "Ingredients: Beef Containing up to 25% … sugar, spices."

Red meat components that contain binders and extenders and do not meet one of the barbecue standards (9 CFR 319.80, 319.312) shall be descriptively labeled to include the extender, nomenclature in the product name, e.g., "BBQ Seasoned Beef, Modified Food Starch and Gelatinized Wheat Starch," "Pork and Binder Product with Barbecue Sauce", or "BBQ Cooked Beef and Binder Product" followed by a parenthetical list of all of its ingredients. Bone-in red meat products do not have to comply with Federal meat regulation, 9 CFR 319.312 or 319.80 with regard to cooking yield and must indicate the presence of bones in product name, e.g., "Seasoned Cooked Pork Ribs with Barbecue Sauce" or "Barbecue Beef Ribs."

When bone-in red meat products are injected, massaged, tumbled, etc., and do not return to green weight after cooking, the containing statement shall appear once on the label in (1) the ingredients statement as part of the red meat component (only if there is enough Beef Ribs without solution to meet the requirement for "Beef Ribs and BBQ Sauce"), or (2) in the product name, e.g., "Beef Ribs, containing 10% of a solution and BBQ Sauce."

Barbecue (Infrared Cooked)

The label must indicate heat source, e.g., "infrared cooked," with lettering no less than one-half the size of the largest letter in the word "barbecue."

Barbecue Meat or Poultry "Eastern North Carolina Style"

Acceptable identification for a product that is enhanced in a vinegar-based solution, apple or white. The solution is seasoned with pepper, i.e., black pepper, red pepper, or cayenne pepper. Other ingredients may include salt, sugar, and hot pepper sauce.

Barbecue Sauce with Chicken

The product must contain at least 15% cooked chicken meat. Changing the size of the term "Chicken" does not change the 15% cooked chicken meat requirement.

Barbecue Sauce with Meat

The product must contain at least 35% cooked meat. When the name of the product shows meat in smaller letters, not more than one-half the size of the largest letter in the product name, 25% cooked meat is required.

Beef a La King

The product must contain at least 20% cooked beef.

Beef a La Mode

A product consisting of sliced beef (marinated in wine, cognac, vegetable stock) with carrots, onions, and other ingredients covered with wine sauce. The product must contain at least 50% beef.

Beef Almondine with Vegetables

The product must contain at least 18% cooked meat on the ready-to-serve basis. The product must contain almonds.

Beef and Dumplings with Gravy

The product must contain at least 25% meat and not more than 25% water blanched dry dumplings.

Beef and Gravy

The product contains at least 50% cooked beef.

See: Gravy and Beef

Beef Blood

This is an acceptable ingredient for beef patties provided the product name is qualified, such as "Beef and Blood Patties" or "Beef Patties with Blood."

Beef Blood Glaze

A coating of beef blood is permitted on cured products (e.g., ham, hamette, etc.) if the product name is prominently

qualified to reflect the coating. Nitrite is not permitted in the glaze.

Beef Brisket (Canned)

The minimum brine concentration required is 5.5%.

Beef Burgundy or Bourguignonne

The product must contain at least 50% beef. Product contains beef cubes, mushrooms, onions, and red wine or burgundy gravy. May include other vegetables, e.g., carrots, shallots, tomato paste, or potatoes. Other acceptable names include "Beouf A La Bourguignonne," "Beef Burgundy Style," "Beef Burgundy," and "Burgundy Beef."

Beef Burgundy with Noodles

The product must contain at least 50% beef in the beef burgundy portion. Total product should not contain more than 50% cooked noodles.

Beef Cheek Meat and Beef Head Meat and Pork Cheek Meat and Pork Head Meat (Use and Labeling as an Ingredient in Meat Food Products)

Beef cheek meat and pork cheek meat refers to beef and pork cheeks from which the glandular material has been removed.

Beef head meat and pork head meat refer to muscle tissue remaining on the beef and hog skull after removal of the skin, cheeks, tongue, and lips. The meat normally attached to and considered as part of the tongue trimmings when detached from the tongue trimmings may also be included as beef head meat or pork head meat although it can be labeled as "beef" or "pork."

When beef cheek meat and/or beef head meat are included in boneless beef, its presence must be specifically declared. Examples include: "Boneless Beef — Contains Beef Cheek Meat and Beef Head Meat," "Boneless Beef Head Meat," "Boneless Beef — Ingredients: Beef, Beef Head Meat, Beef Cheek Meat," or "Boneless Beef — 20% Beef Head Meat, 15% Beef Cheek Meat."

Beef cheek meat and/or beef head meat may be used in unlimited quantities and identified as "beef" in meat food products unless restricted by regulatory standards for specific products as indicated in 9 CFR 319.15(a) (Chopped beef, ground beef), 319.15(b) (Hamburger), 319.15(d) (Fabricated steak), 319.81 (Roast Beef parboiled and steam roasted), 319.100 (Corned beef), 319.300 (Chili con carne), 319.301 (Chili con carne with beans), and 319.303 (Corned beef hash).

The presence of pork head meat is not required to be identified on the labeling of boneless pork. However, pork cheek meat and/or pork head meat may be used in unlimited quantities and identified as "pork" in meat food products, unless restricted by regulatory standards as indicated in 9 CFR 319.300 (Chili con carne) and 319.301 (Chili con carne with beans).

Beef Concentrate and Salt

Broth derived from cooking fresh beef containing 3% to 4% solids is centrifuged and evaporated to approximately 60% solids under vacuum. The water fraction is salted to a level of 25.5% of the water weight (100 lbs. concentrated stock at 60% will have 10.2 lbs. of salt added, making a total weight of 110.2 lbs.). There is no need for refrigeration.

Beef Consomme

The standard requires beef as an ingredient and a minimum protein content of at least 3% in the finished product.

"Beef stock" or "beef broth" (or mixture of both) may be used to comprise the beef ingredient. Additional optional ingredients are gelatin, beef extract, tomato puree, hydrolyzed plant protein, and seasoning.

Beef (Dried or Air Dried)

Product name is "Air Dried Beef" or "Dried Beef." MPR 2.04:1. It is usually cured by rub and/or stitch pump followed by cover pickle for 4 to 8 weeks with several overhauls (turned over for the application of additional cure), then placed in smokehouse or drying chambers for 3 to 10 days.

Beef Fibrin

This is a component mixture of beef fibrinogen and beef thrombin plasma protein used to bind pieces of meat or poultry together. It is limited to ten percent.

1. If used from seven percent of ten percent, it must appear in the product name, e.g., "Bacon Wrapped Beef Tenderloin Steak Formed with Beef Fibrinogen and Thrombin." Therefore, the smallest letter in the product name must be at least 1/3 size of the smallest letter in the product name.
2. If used at less than seven percent, it must be a product name qualifier, e.g., "Formed with Beef Fibrinogen and Thrombin." As a product name qualifier, there is no size requirement, however, it must contiguous to the product name and be prominent and conspicuous. Additionally, the terms "Beef Fibrin" or "Fibrin" may be used in the product name as a qualifier and its components identified elsewhere on the principal display panel. In this situation, the terms "Beef Fibrin" or "Fibrin" and its components are linked to each other by means of asterisks.

Acceptable terminology's for the components are "Beef Fibrinogen and Thrombin Plasma Protein," or "Beef Fibrinogen and Thrombin."

Beef Gravy Mix

The product must contain at least 15% dried beef.

Beef Marsala

The product must contain at least 50% beef. Product contains beef cubes, Marsala wine sauce, and usually mushrooms and onions. White wine may be used, but it may not replace Marsala wine.

Beef Oriental or Oriental Beef

The product must contain at least 12% meat and oriental style vegetables and sauce. The label must show true product name, e.g., "Beef Oriental with Vegetables."

Beef Roulade

The product must contain at least 50% cooked meat. Usually a thin strip of flank meat wrapped around vegetables and cooked.

Beef Slices a-La-Pizzaiola

The product must contain at least 50% cooked beef.

Beef Stroganoff

A dish with a creamy sauce prepared with beef cut into narrow strips or cubes and sautéed. Product labeled "Beef Stroganoff" should be prepared with a formula, which includes at least 45% beef, or 30% cooked beef.

1. The product must contain at least 10% sour cream, or
2. 7.5% sour cream, and 5% wine, or
3. 9.5% whole milk, 2% sour cream, and 2½% wine.

Beef Stroganoff with Noodles

Meat and sauce portion must meet the standard for Beef Stroganoff. Total product shall contain no more than 50% cooked noodles.

Beef Sukiyaki

The product must contain at least 30% meat based on total product. Consists of thinly sliced beef and various vegetables cooked in a flavored beef stock. This is not a stew as the vegetables and components are mixed during the cooking process. Vegetables used with this food are celery, bean sprouts, leeks, onions, mushrooms, Chinese cabbage, carrots, spinach, water chestnuts, bamboo shoots, and bean curds.

Beef Tripe Stew

There are two versions of this product. One is of Mexican origin and merchandised in association with the term "Menudo."

Corn is a prominent ingredient in its formula. The standard for an item of this nature requires that it contain not less than 33% beef tripe computed on the basis of the uncooked tripe in relation to total ingredients.

The second product is popular in Puerto Rico. It is referred to as "Mondungo." The product is made with 25% raw beef tripe. The remainder consists principally of potatoes, a squash with pumpkin-like appearance and flavor, and a native vegetable called "Tanier." When the vegetables are not distinguishable, this product can be labeled as "Dominican Style Mondungo."

Beef Wellington

It is made with beef tenderloin that is roasted very rare. It is then spread with a liver pate, covered with pastry, and baked in a hot oven until pastry is brown. The product must contain at least 50% cooked meat and no more than 30% pastry.

Alternatively, mushroom duxelle is an acceptable substitute for liver pate, but a true descriptive product name is required, e.g., "beef tenderloin covered with mushroom duxelle and wrapped with pastry."

Beerwurst, Bierwurst

A cooked smoked sausage. Same requirements as beef salami, with the exception that pork may be used.

Berliner

A cooked smoked sausage usually made from coarsely cut cured pork in large casings. When beef is used, it shall not exceed 50% of the meat block. Pork stomachs or beef tripe not permitted.

Berliner Blood Sausage

A cooked blood sausage containing diced bacon. After cooking it is dried and smoked. Ham fat, snouts, and lips are not permitted.

See: Blood Sausage

Bier Schinken (GR)

The literal translation is "Beer Ham." If product is made of all pork, it may be labeled "Bier Schinken."

Binders in Poultry, Boneless, Raw, or Cooked

Binding agents may be added individually or collectively in amounts not to exceed 3% for cooked poultry products and 2% for raw poultry products based on total finished product. When binders are added in excess of these levels,

the common or usual name of the binder or the generic term "Binders Added" shall be included in a product name qualifier, e.g., "Turkey Breast-Gelatin Added." In all cases, the presence of these ingredients must be shown in the ingredients statement.

This policy is intended to apply to binders which are used in chopped or chunked poultry products that are formed into rolls, loaves, etc., but not to binders added directly into whole muscle by injection, massaging, tumbling, etc., which then act as extenders.

Blockwurst

A semi-dry type sausage. The maximum MPR is 3.7:1.

Blood and Tongue Sausage

Same as blood sausage, except cured and cooked pork or beef tongues are used.

Blood Sausage

A cooked sausage formulated with blood and some meat. Usually contains pork skins and/or pork jowls. May also contain sweet pickled ham fat, snouts, and lips. If the product does not contain meat, it must be labeled as "Blood Pudding."

Boingghetti

This label must show a true product name, "Spaghetti with Chicken Sauce." The product must contain at least 6% cooked chicken meat.

Bone-In Meat with Sauce

Must have at least 50% meat (cooked basis). Product with barbecue sauce must comply with 9 CFR 319.312.

Boneless Breast Trimmings

Boneless breast trimmings (turkey or chicken) are defined as trimmings that are removed from the breast portion only. When a product is formulated with boneless breast trimmings, the amount of skin should be indicated in order to determine that the meat requirement is met for a standardized product and that the product is properly labeled. Trimmings from the ribs may be identified as white turkey or white chicken trimmings, or white turkey or white chicken rib meat (excluding skin).

Breakfast Links or Patties

The names "Breakfast Links" and "Breakfast Patties" can be considered fanciful names, which must be followed by a descriptive product name. Such products are acceptable without compliance with the fresh pork sausage or breakfast sausage standard. If the names "Breakfast Links" or "Breakfast Patties" are used without further qualification, the products must meet either the fresh pork sausage standard or the breakfast sausage standard.

Breakfasts (Containing Meat)

The product must contain at least 15% cooked meat or poultry or meat or poultry food product based on the total net weight of breakfast.

Broth, Beef or Pork

No distinction has been made between "broth" and "stock." They may be used interchangeably as the resulting liquid from simmering meat and/or bones in water with seasonings. Both products have an MPR of 135.1 or a 67.1 MPR for concentrate.

Brotwurst

A cured and cooked sausage that may be smoked.

Brown and Serve Sausage

The standard is based on one of the four options as listed below:

1. Moisture Protein Ratio (MPR) is no more than 3.7:1, fat limited to 35%, and 10% water at formulation.
2. No more than 10% added water at formulation and a yield of no greater than 80%.
3. No more than 8.8% added water at formulation and a yield no greater than 85%.
4. Product must meet fresh sausage standard before cooking. The label must show true product name, e.g., "Brown and Serve Pork Sausage."

Brown and Serve Sausage (Canned)

A cooked sausage, usually without cure, and not more than 8% water. The weight of the sausage at canning shall not exceed weight of fresh uncured meat ingredients plus weight of curing and seasoning ingredients.

Brunswick Stew

The product must contain at least 25% (fresh basis) of at least two kinds of meat, one of which may be poultry. Product must contain corn as one of the vegetables.

See: Poultry Brunswick Stew

Buffalo Style

Buffalo style is acceptable on any product (e.g., poultry parts other than wings, chicken patties, nuggets etc.) that is cooked and coated with a mild or spicy sauce containing, Cayenne red pepper, vinegar, salt and garlic. It is no longer

exclusive to chicken wings only. It would also be acceptable on any product made in Buffalo, NY.

Burgundy Sauce with Beef and Noodles

The product must contain at least 25% cooked beef in the product, with up to 20% cooked noodles. Product must contain enough wine to characterize the sauce.

Burritos

A Mexican style sandwich-like product consisting of a flour tortilla, various fillings, and at least 15% meat or 10% cooked poultry meat. The flour tortilla is rolled and may or may not have tucked ends. Fillings may contain, in addition to meat or poultry meat, such major ingredients as beans, potatoes, cheese, rice, tomatoes, and chilies.

Examples of product names are "BEEF BURRITO," "TURKEY BURRITO," "CHICKEN FAJITA BURRITO," AND "CHILI VERDI WITH BEANS BURRITO." If ingredients, e.g., rice or beans, are declared in the product name, they must appear in the proper order of predominance. Ingredients cannot be mentioned in the product name unless all other ingredients present in amounts equal to or above the declared ingredient are included in the name, e.g., "BEANS, BEEF, TOMATO, ONION, AND RICE BURRITO."

The use of "Red Chili" or "Green Chili" or a similar designation of the chili content in a starburst, flag, or similar display, separated from the product name, is acceptable. If such designations are used as part of the descriptive name, the presence of the chilies must appear in the correct order of predominance, and all other ingredients present in amounts equal to or greater than the chilies must appear in the product name.

A claim or name that identifies the use of shredded meat or shredded poultry meat is permitted. However, if ground meat or ground poultry meat is also used, its presence must also be identified in the claim or name, e.g., "Shredded Beef and Ground Beef Burrito."

"BURRITO" alone, may be used to name the product without a descriptive name. However, the ingredients statement must appear directly beneath "burrito."

Burritos with Sauce or Gravy

Product must contain at least 50% burritos.

Butifarra-Sausage

An uncured sausage. Labeling that features the term "Butifarra" would require an additional product name:

Pork Sausage — for those products that meet the fresh pork sausage standard.

Fresh Sausage — for those products that include byproduct but do not meet the standard for pork sausage.

Sausage — for those products that are incubated or fermented. The term Puerto Rican Style would be applicable if manufactured in Puerto Rico.

Caddies

Caddies or display cards used to display fully labeled product shall not bear an inspection legend and, therefore, can be reused. The caddies or display cards may contain a picture of a product that has a legend on it.

Cajun

Refers to product made in Louisiana.

Cajun Style/Cajun Recipe

Acceptable identification for products containing onion/onion powder/dehydrated onion, garlic/garlic powder/dehydrated garlic, white pepper, red pepper, and black pepper.

Calabrese (IT)

A salami originating in Southern Italy. Usually made entirely of pork seasoned with hot peppers.

Calzone, Calzoni (IT)

Turnover-like product made with dough stuffed with meat or poultry, cheese, and seasonings and baked. It must contain 25% meat or 14% poultry meat. The label must show a true product name, e.g., "Sausage and Cheese Calzone."

Canadian and Canadian Style Bacon

"Canadian Bacon" and "Canadian Style Bacon" are synonymous and should not be considered geographical terms.

The term "Canadian Style Bacon," when featured on the label as a product name or part of a product name (i.e., as a description, etc.), may stand alone without an additional qualifier indicating the true geographical origin of the product.

"Chunked and Formed" and "Water Added" products are permitted, provided proper labeling is applied.

Uncooked and/or unsmoked "Canadian Style Bacon" is also permitted, provided labeling describes the product as uncooked and/or unsmoked.

Product which is identified as "Canadian Style Bacon" is made from a trimmed boneless pork loin. On the shoulder end, the cross section of the longissimus dorsi muscle shall be equal to or larger than the combined cross sectional areas of the splenius and semispinalis capitis muscles. The ham end shall be removed anterior to the ilium. The exposed faces shall be approximately perpendicular with the skin surface. The dorsal and ventral side on each end of the "Canadian Style Bacon" shall not be more

than 1.0 inch different in length. The belly is removed adjacent to the longissimus dorsi muscle. All bones and cartilage shall be removed. The tenderloin and the flesh overlying the blade bone are excluded. The surface fat (and false lean when necessary) shall be trimmed to 0.3 inches thick at any point. The fat on the ventral and dorsal sides is neatly beveled to meet the lean.

Canadian Style Bacon Made with/from Pork Sirloin Hips

The sirloin is obtained by removing a 5- to 7-inch section of the pork loin immediately in front of the hip or pelvic bone. The sirloin hip is obtained by removing the half of the sirloin which comprises the posterior end of the pork loin. The tenderloin is not included and surface fat shall be trimmed to 0.3 inches in thickness.

The labeling for these Canadian Style Bacon products must bear a qualifying statement, adjacent to the product name, clarifying that pork sirloin hips are included or that the product is made entirely from pork sirloin hips, e.g., "Canadian Style Bacon — Includes Pork Sirloin Hips" or "Canadian Style Bacon — Made from Pork Sirloin Hips." The smallest letter in the qualifier should not be less than one-third the size of the largest letter in the product name. The qualifier must be of equal prominence to the product name.

Chunked (or chopped) and formed varieties and substances controlled by the protein fat free (PFF) regulation for cured pork products 9 CFR 319.104 shall be labeled in accordance with applicable guidelines.

Use of this type of product in a secondary product, e.g., a pizza, requires complete identification only in the ingredients statement; the product name of the secondary product need only refer to Canadian Style Bacon, e.g., Canadian Style Bacon Pizza.

Canned Chopped Beef or Pork

Cured product with no more than 3% water in formula.

Canned Meat

"Canned meat with Natural Juices;" is acceptable for product that has been pumped or contains up to 10% of a solution before canning and processing. Processed canned uncured meat products, when water or broth is added to the can may not be called "with natural juices," but the acceptable name would be "with juices."

Cannelloni (IT)

Product must contain at least 10% meat or 7% cooked poultry meat. Cannelloni is an Italian term referring to a product with the same characteristics as "Ravioli" except Cannelloni has a tubular form. The product name should show the type of species, e.g., "Beef Cannelloni."

Cantonese Style Species

Marinated in a solution of soy sauce, cooked and returned weight. In addition, product is mildly seasoned with sugar, salt, wine, and spices.

Capacollo, Cooked (Capicola, Capocolla, Capacola, Capicollo, Cappicola, Capacolo) (IT)

Boneless pork shoulder butts which are cured and then cooked. The curing process may be dry curing, immersion curing, or pump curing. The cured product is coated with spices and paprika before cooking. This product shall always be labeled with "Cooked" as part of the product name. Water added is permitted.

Caramel Coloring

Caramel is considered a natural color. However, when caramel coloring is added to a product, the product name must be qualified to indicate the presence of artificial coloring, e.g., "Cooked Roast Beef-Caramel Coloring Added" or "Artificially Colored." This requirement does not apply to gravies, sauces, and similar products where the use of such coloring is customary. Seasoning mixes containing small quantities of caramel coloring may be used if the caramel coloring does not impart color to the finished product.

Caramel coloring may be used on the surface of raw products, e.g., beef patties, if the name is appropriately qualified. However, caramel coloring may not be added directly to the formulation of a raw product where the caramel coloring becomes an integral part of the total product.

Carbonade (FR)

Product must contain at least 50% meat. It may contain beef, pork, or mutton, and beer or wine. Product is slowly cooked, either by braising or stewing.

Label must show a true product name, e.g., "Beef Carbonade."

Carriers

Substances, as defined by the Food and Drug Administration, that carry flavoring compounds, e.g., essential oils, on their surface, and are not expected to provide a functional effect, e.g., binding and emulsifying, in the finished food product and are considered incidental. Some substances, e.g., maltodextrin and modified food starch, are not carriers but actually diluents or bulking agents, and must be declared in the ingredients statement. Dextrose and/or sugar are commonly used as carriers for spice extracts and resins of spices. The

carrier must be declared in the ingredients statement, except in those cases where a sweetening agent is used separately in formulating the meat or poultry product and the use of the spice mixture will not result in the quantity of the carrier being more than 0.75% of the seasoning mix. When a determination cannot be made from the information on the label application, declaration is required.

Salt, when used as a carrier, will always be declared regardless of amount used.

Casing, Artificial

Frankfurters packaged in retail containers with the artificial casing left on must bear a prominent statement, e.g., "Remove casing before eating," contiguous to the product name on the label.

Casserole

Product must contain at least 25% meat or 18% cooked meat.

Cassoulet (FR)

Product must contain at least 25% meat. A complex stew consisting of dried white beans and a combination of pork, lamb, game, and sausages. The ingredients are cooked, then put into a casserole, usually covered with crumbs, and baked. Label must show true product name, e.g., "Beans and Bacon in Sauce."

Center Slice

When the term "Center Slice" is used on labels for slices of ham from smoked and cooked, smoked, or water cooked hams, product must be sliced from an area of the original ham positioned about 1 inch on each side of a center cut.

Certified

With the exception of the term "Certified Pork" the term "certified" implies that the United States Department of Agriculture (USDA) and the Agriculture Marketing Service (AMS) have officially evaluated a meat product for class, grade, or other quality characteristics. When used under other circumstances, the term should be closely associated with the name of the organization responsible for the "Certification" process (e.g., "XYZ Company's Certified Meat," or "Our Certified Meat").

Cervelat

A cured and cooked sausage, often a semi-dry or dry summer sausage. Hog stomachs, beef tripe and extenders are permitted. There is no MPR (moisture protein ratio) requirement.

Cha Shu Bow (CH)

A steamed bun with a dry roasted pork filling requiring 15% cooked pork. Label must show true product name, e.g., "Steamed Bun with a Pork and Cabbage Filling."

Cheek Meat, Beef

Natural proportions are considered to be 2%.

See: 9 CFR 319.15.

The use of cheek meat is limited to 25% in ground beef, chopped beef and similar type products. If cheek meat exceeds 2% (natural proportions), its presence must be declared.

Cheese

1. When cheese is declared in the ingredients statement of a fabricated product, cheddar cheese must be used in the product's formulation.
2. Swiss, Gruyere: The term "Gruyere" pertains to a cheese that closely resembles "Swiss Cheese" both in its appearance and on analysis, although it has smaller holes than Swiss Cheese. FDA advises that Gruyere Cheese is a suitable substitute for Swiss Cheese and gives the same character to a finished food product, e.g., "Chicken Cordon Bleu."
3. The term Cheese may appear in the product name, e.g., "Ham and Cheese Loaf," provided the common name is declared in the ingredients statement.
4. When a cheese product and meat or poultry food product are packaged together, the product name shown on the label must show the name of each component product.

   For example, if slices of ham and slices of a cheese product are packaged together, the product name should include "Ham" and the name of the cheese product (e.g., Ham and Pasteurized Processed American Cheese). Alternatively, the Pasteurized Processed American Cheese could be parenthetically qualified contiguous to the product name (e.g., "Ham and Cheese (Pasteurized Processed American Cheese"). The name "Ham and Cheese" alone would be acceptable if the cheese used was "Cheddar Cheese."
5. Use of substitute or imitation cheese in products where real cheese is expected (e.g., Cordon Bleu) requires the product name be changed or qualified to indicate the presence of the ersatz cheese. Substitute and imitation cheeses cannot be described as "cheese" in the product name. There is no limitation on the amount of ersatz cheese used.

6. Reduced fat cheeses may be identified on the label as "Reduced Fat Cheese." However, the name of a standardized cheese may not be associated with the phrase "Reduced Fat Cheese" (e.g., Reduced Fat Cheddar Cheese).
7. Cheese is a standardized product. See: 21 CFR 130.10 and 133 for a listing of standardized cheeses.

Cheese (Pasteurized Processed Cheese Food or Spread)

A cheese food product with a standard of identity, but is not considered a cheese. Therefore, it cannot be used in meat food products where cheese is an expected ingredient, e.g., "Cheesefurters" or "Veal Cordon Bleu." It is acceptable in non-specific loaves, etc.

Cheese Products Containing Meat

Homogeneous cheese products, e.g., cheese balls, must contain more than 50% meat to be amenable. When cheese and meat are separate components, the products are amenable with 2% meat.

Cheese Standardized Products

Cheese standardized products that require real cheese, e.g., chicken cordon bleu, must use FDA standardized cheese or those FDA standardized cheeses specified. Use of a substitute, imitation cheese or other non FDA standard cheeses, if permitted, must be declared in the product name, or a suitable qualifier, e.g., chicken cordon bleu made with reduced fat cheese. The 90/10-cheese rule is only applicable to pizza.

Chicharrones (PR)

The Spanish name for fried pork skins. Product must have an English product name, "Fried Pork Skins" except in Puerto Rico.

Chicharrones de Pollo (PR)

An acceptable product name for "Marinated Cut-up Fried Chicken" sold in Puerto Rico. When product is destined for sale only in Puerto Rico, "Chicharrones de Pollo" can be the product name. When destined for sale in other places, "Chicharrones de Pollo" must be explained with true product name.

Chicken, Aloha

"Aloha Chicken" is acceptable as a coined name which must be followed by a true product name, e.g., "Chicken and Sauce with Rice." The standard for the product is 22% cooked poultry meat.

Chicken and Noodles Au Gratin (FR)

Product must contain at least 18% cooked chicken meat.

Chicken Cordon Bleu (FR)

Product must contain not less than:

1. 60% chicken breast meat (sliced). If it is made from any other part of the chicken, then the product name must be qualified to indicate the part used.
2. 5% ham or Canadian Style Bacon.
3. Cheese (either Swiss, Gruyere, Mozzarella, or Pasteurized Processed Swiss).
4. Not more than 30% batter and breading (if used).

Chicken Enchilada Suiza

The product consists of chicken enchiladas with a cream sauce. The sauce used must be made with sour cream, heavy cream, or whipping cream in an amount sufficient to characterize the sauce. The label must show a true product name, e.g., "Chicken Enchilada with Cream Sauce."

Chicken Ova

These can not be used for human consumption without first going to an egg products plant for pasteurization (because of problem with potential Salmonella contamination). Chicken Ova can not use the poultry inspection legend.

Chicken Paprika

Product must contain at least 35% chicken. A Hungarian dish. Sauce must contain either sour or sweet cream and enough paprika to give a pink color.

Chicken Tocino

Acceptable with a true product name such as sliced, marinated, cured chicken thigh meat.

Chicken Wellington

It is made with roasted chicken that is spread with liver pate, covered with pastry, and baked in a hot oven until pastry is brown. The product must contain at least 59% cooked meat and no more than 30% pastry.

Chili

1. "Brick Chili" or "Condensed Chili" requires 80% meat. Cereal is limited to 16%.
2. Chili with reconstitution directions should meet the chili standard when reconstituted.
3. When beef heart meat, cheek meat, or head meat is used in excess of 25% of the meat

block, it must be reflected in the product name, e.g., "Chili with Beef and Beef Heart Meat."
4. When beef appears in the product name, BEEF MAY BE THE ONLY MEAT SOURCE USED. Beef Chili may not contain beef fat or other beef byproducts.
5. "Chili Gravy with Meat" requires at least 40% fresh meat and no more than 8% cereals.
6. Cured meats are not an expected ingredient in chili; when used, they must be shown as part of the product name.
7. The terms "Chili" or "Chili con Carne" may be used interchangeably.
8. Since "con carne" means "with meat," products labeled as chili con carne should include only red meat and not poultry. Products which meet the chili standard and include poultry may be labeled "beef and chicken chili," "beef chili, chicken added," etc., as appropriate. The binder and extender limitation of 8% is based on total formulation.

See: 9 CFR 319.300.

Chili Colorado

Product must meet 9 CFR 319.300 requirements. Chili peppers must be exclusively of the red variety. If a prepared chili powder is used, it must be prepared exclusively from red chili peppers.

The term "Colorado" is used for red more than "Rojo" in Mexico. The term "Rojo" is used more in Spain, Puerto Rico, and Cuba.

Chili-Mac

Product must contain at least 16% meat. The label requires a true product name, e.g., "Bean, Macaroni and Beef in Sauce."

Chili Pie

Chili component of the total product must have at least 40% fresh meat.

Chili Pups

An emulsion stuffed in casing and smoked. Label requires a true product name, e.g., "Chili con Carne and Ground Beans Product." Product must contain at least 60% fresh meat in total formulation.

Chili Relleno

Product must contain at least 12% fresh meat and be coated with a batter and then fried. Sometimes product is called "Chili Pepper Relleno." Relleno means stuffed.

Chili Spaghetti

Product must contain at least 16% meat.

Chili Verde (SP)

Product must meet 9 CFR 319.300 requirements. Chili peppers must be exclusively of the green chili or Verde chili pepper varieties. If a prepared chili powder is used, it must have been prepared exclusively from green chili or Verde chili peppers. Products, e.g., "Chili Verde with Beans" shall comply with 9 CFR 319.301 and the above requirements for "Chili Verde."

Chili with Beans

1. "Brick Chili with Beans" or "Condensed Chili with Beans" requires 50% meat and cereal is limited to 16%.
2. Chili with Beans with reconstitution directions should meet the Chili with Beans standard when reconstituted.
3. When beef heart meat, cheek meat, or head meat is used in excess of 25% of the meat block, it must be reflected in the product name, e.g., "Chili with Beef and Beef Heart Meat with Beans."
4. When beef appears in the product name, beef may be the only meat source used. Beef Chili with Beans may not contain beef fat or other beef byproducts.
5. Cured meats are not an expected ingredient in Chili with Beans; when used, they must be shown as part of product name.
6. "Chili with Beans" formulae usually contain up to 25% of beans in a product. About one-fourth of these beans may be incorporated in the product as ground beans and should be listed in the ingredients statement as ground beans.
7. The terms "Chili with Beans" or "Chili con Carne with Beans" may be used interchangeably.
8. The binder and extender limitation of 8% is based on total formulation.

See: 9 CFR 319.301.

Chimichanga

Product must contain at least 15% meat or 10% poultry meat. A Mexican specialty from the State of Sonora. Like burritos, product is made by wrapping a flour tortilla around a filling; but unlike the burrito, chimichanga is fried until brown and crisp. "Fried Burritos" is acceptable.

Chinese Brand Links

Raw nonspecific sausage-like products. These products are permitted to contain artificial red coloring; however, if pork is used it must be certified. Unlike the term "links,"

"Chinese Brand Links" is considered a coined or fanciful name, and [as a] nonspecific product, it must be accompanied by an ingredients statement. Furthermore, "made in USA" must be contiguous to the word "brand" but cannot intervene between "links" and the ingredients statement.

Chinese Pepper Steak

A Chinese main dish, usually served with rice, must contain at least 30% cooked beef. Beef steak is cut into thin strips, browned in fat or oil, and added to a soy flavored sauce. Vegetables are also added to the sauce. Green pepper strips are always used and other vegetables may be included.

Chinese Style Barbecue Meat

Acceptable identification for a product that is enhanced in a solution with soy sauce, grain alcohol or dry sherry wine, and a sweetener, i.e., sugar or honey. Other ingredients may include garlic or scallions, ginger or ginger juice, sesame or peanut oil. The product may be artificially colored. If artificially colored, a qualifier is needed.

Chinese Style Beef

Product must contain grain alcohol and soy sauce.

Chinese Style Sausage

Product must contain grain alcohol and soy sauce.

Chipped Beef

Beef that is dried, chipped, or sliced and may be cured or smoked. An MPR 2.04:1 is required. It may be chunked, ground, chopped, and formed. If so, the product name must be qualified, e.g., "Chipped Beef, Chunked and Formed."

Acceptable fill:

1. 2 oz. in a 4 fluid oz. glass, or
2. 2 1/2 oz. in a 5 fluid oz. glass, or
3. 5 oz. in a 9-5/8 fluid oz. glass.

Chitterlings

Approved label must identify the species of food animal from which the product is derived. Hog bungs may be labeled "Pork Chitterlings." The purge under normal conditions should not exceed 20% of the net weight of frozen chitterlings.

See: 9 CFR 317.8(b)(30).

Choice Grade, Fancy Grade Poultry

"Choice" or "Fancy" may not be used in conjunction with "Grade" on poultry labels. These terms and others like "Prime" and "Top Quality" on poultry labels indicate only that product is equal to U.S. Grade A.

Chopped Chicken Livers

Total product must contain at least 50% cooked chicken livers. Wheat flour and similar ingredients are acceptable.

Chopped Chicken Livers Combined with other Characterizing Components

Product must contain at least 30% cooked livers, e.g., "Chopped Chicken Livers with Eggs and Onions."

Chopped Ham

A total of 15% shank meat is permitted. This is 3% above the normal proportion of 12% shank meat found in a whole ham.

See: 9 CFR 319.105.

Chop Suey, American

Product must contain at least 25% fresh meat in total formulation. A stew-like dish prepared with beef, pork, or veal. Vegetables include onion and celery. Macaroni, noodles, or rice are usually incorporated in the product, although recipes suggest serving chop suey over one of these.

Chop Suey (Vegetables with Meat)

Product must contain at least 12% fresh meat.

Chorizo (SP)

The product name "Chorizo" can be used for any type of chorizo sausage that is cooked, dry, semi-dry, cured and fresh without further product name qualification. Other requirements for various types of chorizo apply, including the sausage standard. It is seasoned with Spanish pimento and red pepper.

Partially defatted pork fatty tissue is acceptable in Chorizo. Wine is considered a flavoring and need only appear in the ingredients statement. However, the liquid is credited as added water.

Chorizo, Fresh

These products may contain vinegar. The vinegar used must have a strength of no less than 4 grams of acetic acid per 100 cubic centimeters (20°C).

See: 9 CFR 318.7(c)(1).

Chorizo in Lard

Product must contain at least 55% chorizo.

Chorizo in Lard, Canned

Canned chorizos that are packed hot, usually in lard, and are not thermally processed must have a moisture protein ratio of 1.8:1 and a pH of not more than 5.5. An alternative standard is a water activity (Aw) of 0.92.

Chow Mein with Meat

Product must contain at least 12% fresh meat.

Chulent (Cholent)

Product must contain at least 25% fresh meat. A meal-in-one dish of Jewish cuisine made in various ways. The product name can stand without qualification.

Coarse Ground Meat Trimmings

Coarse ground trimmings may be shipped from an establishment without meeting the 30% fat limitation if a specific fat content is declared, e.g., "Coarse Ground Beef Trimmings — 40% fat beef." If the labeling terminology is "Coarse Ground Beef" or "Ground Beef," the 30% fat limitation shall apply.

Colored Casing

Colored casings on meat and poultry products which do not transfer color to the product, but which change and give a false impression of the true color of the products, must be labeled to indicate the presence of the casings. Acceptable terminology includes "Casing Colored" or "Artificially Colored." These phrases must appear contiguous to the product name.

Casings which are the same color as the product and not misleading or deceptive, e.g., a white opaque casing on a summer sausage, do not have to be so labeled. Also, products consisting of whole muscle bundles, e.g., hams, pork butts, etc., packaged in colored wrappings where a cut surface is not visible through the casing are exempt. The color agent must be specifically identified on the label either in the product name qualifier or ingredient statement.

See: 9 CFR 319.15(d).

Composite Ingredients Statement

Processors who use a multi-ingredient product, e.g., pepperoni from various sources, as an ingredient, may identify all the ingredients that may be present from all the various formulations (i.e., a composite ingredients statement). However, the ingredients identified as those that may be present can only be those ingredients that are minor in nature and cannot include ingredients, e.g., the meat component that have a bearing on the overall characteristics or value of the product. The minor ingredients must be identified using one of the following examples of acceptable formats:

1. pepperoni (pork, beef, water, salt, spices, sodium nitrite. May also contain lactic acid starter culture, sugar, and sodium ascorbate).
2. bacon bits (cured with water, salt, dextrose and/or sugar, sodium nitrite).
3. pepperoni, pork, beef, water, sweeteners (contains one or more of the following: sugar, dextrose, fructose, corn syrup), salt, spices, sodium nitrite).

Labeling records must identify all the ingredients of each type of component that is used so the accuracy of the composite ingredients statement can be determined. All labeling for meat and poultry products must either comply with this type of format or, alternatively, accurately list all ingredients used in the product.

Cooked Beef, Equivalency

In lieu of fresh beef, a 70% yield figure is used if no yield information is provided.

Cooked Breakfast Sausage

Antioxidants are permitted when product is formulated on a raw basis (no more than 3% water).

Cooked Red Meat Products Containing Added Substances

Cooked corned beef products and cooked cured pork products not addressed by the cured pork products regulation (9 CFR 319.104), that weigh more than the weight of the fresh uncured article, may be prepared if they are descriptively labeled to indicate the presence and amount of the additional substances. Acceptable product names include: "Cooked Corned Beef and X% Water" or "Cooked Cured Pork and Water Product, X% of Weight is Added Ingredients," and "Cooked Pastrami and Up to 20% of a Solution." The ingredients of the solution may accompany the product name or appear in locations prescribed for ingredient statements. Product name prominence guidelines are found in Policy Memo 087A and Policy Memo 109. If product name qualifiers, such as "X% of Weight is Added Ingredients," are used, the labeling prominence guidelines used for cured pork products as found in 9 CFR 319.104(b) apply.

Uncured red meat products that weigh more than the weight of the fresh article after cooking should be labeled with a qualifying statement indicating the amount of solution remaining after cooking, e.g., "After cooking, contains X% of a seasoning solution of …." The ingredients of the solution may accompany the qualifying statement

or appear in locations prescribed for ingredient statements. The qualifying statement must be one-fourth the size of the largest letter in the product name. If the ingredients of the solution accompany the qualifier, they must appear in print one-eighth the size of the most prominent letter in the product name. Other labeling prominence guidelines are found in Policy Memo 087A.

If cooked, uncured red meat products that contain added solutions/substances prior to cooking are cooked back to or below the weight of the fresh (green weight) article, words, such as "seasoned" and "flavored," are to be used to reflect the addition of the added substances, e.g., "Seasoned Cooked Beef."

For cooked products, the percent added substances for the label statement is determined by subtracting the fresh (green) weight of the article from the weight of the finished cooked product, (i.e., after injecting, marinating, etc., and cooking), dividing by the weight of the finished product, and multiplying by 100. This policy is intended to apply to solutions that impart favorable flavor and other sensory characteristics, but not to solutions containing ingredients used to extend a product, such as isolated soy protein and carrageenan.

Uncooked red meat products containing added substances are addressed in Policy Memo 066C.

Corn Dog or Korn Dog

A coined name which must be accompanied by a true product name, e.g., "Batter Wrapped Franks on a Stick." Product is limited to 65% batter and a minimum of 35% frankfurter.

Corn Dog or Korn Dog (Poultry)

"Corn Dogs" made from poultry cooked sausage, e.g., poultry franks or poultry frankfurters, must show the "kind" of poultry used in conjunction with the coined name "Corn Dogs," e.g., "Chicken (or Turkey) Corn Dogs." The "kind" name should be shown in type size at least one-third the size of the largest letter of the coined name. A descriptive name, e.g., "Batter Wrapped Chicken Frank on a Stick," must accompany the coined name. If the descriptive name is at least one-third the size of the coined name, the "kind" name need not precede the coined name.

Corn Meal Mush with Bacon

Product must contain at least 15% cooked bacon.

Corned Beef and Cabbage

Product must contain at least 25% cooked corned beef.

Corned Beef (Canned, Cooked with Natural Juices)

Canned product labeled "Cooked Corned Beef with Natural Juices," is limited to 10% added solution before cooking. If the added solution is greater than 10%, the label must indicate the total added solution, e.g., "Cooked Corned Beef and Water product-X% of weight is added ingredients."

See: Cooked Corned Beef Products with Added Substances.

Corned Beef, Gray

Gray corned beef is not a cured product but one that contains water, salt, sugar, flavorings, etc. It should be labeled as "Gray Corned Beef," "Gray Corned Beef Rounds," etc. The label must show an ingredients statement rather than a curing statement as shown on other corned beef labels.

Corned Beef with Juices

Uncooked corned beef with juices (or without juices and spices) is unacceptable terminology for corned beef products meeting standards in 9 CFR 319.101 and 319.102 for corned beef brisket and corned beef round (and other cuts). The presence of free flowing juices in a package does not change this policy. The net weight includes free flowing juices.

Cornish Style Pasty

Product must contain at least 25% beef. Product consists of a round or square of piecrust with a filling of chopped beef, potatoes, and onions.

Cotechino (IT)

Pork skin sausage. Meat and meat by-products other than pork skin can be used in this product. It could also be given the name of pork skin sausage in parentheses as a common name. Italian sausage. A variety of cooked sausage.

See: 9 CFR 319.140.

Country

A geographical term that refers to an unincorporated area. To use country, the product must be made in the country.

Country Fried

Refers to a fried product that is usually breaded. It is not considered a geographical term.

Country of Origin

Statement, "Product of …" need only appear beneath the product name on the principal display panel on imported product.

Country Style Chicken

Cut up chicken in which the wishbone is left whole.

Country Style (Farm Style) Sausage

When sausage products are labeled "farm style" or "country style," they must be prepared with natural spices with the exclusion of oleoresins, essential oils, or other spice extractives. Sugar is the sweetening agent for "farm style" or "country style." HVP, MSG, and antioxidants are permitted ingredients. Products so labeled are not necessarily prepared in the country (on the farm) but are expected to have these characteristics.

See: 9 CFR 317.8(b)(2).

Creamed Beef (Chipped or Dried)

Product must contain at least 18% dried beef. It may be produced using a cured beef, or beef product which has been chopped, pressed, or cooked.

Creamed Cheese with Chipped Beef

Product consists of cream cheese, chipped beef, cream and chopped onions. The meat component must be at least 12% of the total formulation.

Creamed Sauce with Meat or Creamed Meat Products (Chipped Beef, Cooked Beef, Sausage, Ham, Franks, Meatballs, etc.)

Product must contain at least 18% meat or meat products (on a cooked basis). The kind of meat product used should be reflected in the product name (e.g., "Creamed Cured Beef, Chopped, Pressed, Cooked").

Creole Style

Term applies to many dishes made with tomatoes, spices, and green peppers. Spices include onion, garlic, bell pepper, white pepper, red pepper, black pepper, parsley, and other Louisiana seasonings, e.g., bay leaf, filét, paprika, or pepper sauce.

Crepe Filling

Must contain at least 40% cooked meat or 20% cooked meat if filling has one other characterizing ingredient, e.g., cheese, and at least 14% cooked meat when the filling has two other characterizing ingredients, e.g., cheese and mushrooms. This is based on the total weight of the filling.

Crepes

Product must contain:

1. At least 20% cooked meat when the filling contains no other major characterizing component.
2. At least 10% cooked meat when the filling contains one other major characterizing component (e.g., cheese).
3. At least 7% cooked meat when the filling contains two or more other major characterizing components (e.g., cheese and mushrooms).

These percentages are based on the total weight of the product.

Croissant

A crescent shaped roll requiring 18% cooked meat. Label must show a true product name, e.g., "Croissant with a ham and cheese sauce filling."

Croquette

Product must contain at least 35% cooked meat, based on total formulation. Beef, ham, etc., must appear as part of the product name.

Curdlan

A substance identified by the common or usual name "curdlan" has been approved for use in foods (see 12/16/1996 Federal Register), and for non-standardized meat products, poultry products, and in Policy Memo 123 and 121B products as a binder/stabilizer/thickener/texturizer.

Cured Meat Products — Labeling of Mechanically Reduced

The traditional names of cured meat products, e.g., bacon, may be used even though mechanical reduction-like chopping or chunking has taken place before the product has acquired the characteristics expected of the product, provided the finished product acquires the characteristics expected. Furthermore, the mechanical reduction must be noted in the product name or in a qualifier to the product name (e.g., chopped bacon or bacon-chopped and formed).

Cured Meat Products — Packed in Brine

Cured meat products, e.g., pork tails, pork snouts, and cured boneless beef brisket, that contain 120–200 PPM nitrite and are packed and sold in brine solution, do not require a handling statement, e.g., "Keep Refrigerated," provided the finished product has at least 10% brine concentration, and the packing medium contains a sufficient quantity of salt to maintain the 10% brine concentration in the product.

Cured Pork Bellies

Such products are assumed to be further processed into bacon. Therefore, cured pork bellies must meet the restricted ingredients requirement for bacon.

Cured Pork

Cured pork products, that contain modified food starch, X% solution ISP, carrageenan or sodium caseinate, that fall into the PFF value of "Ham, Water Added" and the "Ham and Water product X% solution" category, must be labeled with the appropriate PFF Nomenclature, Descriptive Labeling, e.g., "ham, water and binder product," will be used if:

1. Binders are at levels above those permitted by the regulations
2. Binders other than those permitted are used
3. Two or more binders are used in combination or
4. The PFF value of the finished product falls in the "ham" or "ham with natural juice" category which do not permit binders.

Cured Turkey Thigh Meat

A product labeled "cured turkey thigh meat" (without turkey ham in the name) must follow the turkey ham standard. The product "cured turkey thighs" (which includes skin and bone), is not required to meet the standards for turkey ham and cannot be labeled "turkey ham."

Curried Sauce with Meat (Poultry) and Rice Casserole

Product must contain at least 35% cooked meat or poultry meat based on the sauce and meat portion only.

Curry Product

1. Meat Curry — Must contain at least 50% meat (lamb, beef, etc.)
2. Poultry Curry — Must contain at least 35% cooked poultry meat.

Cutlet, Beef

Beef cutlet may be chopped and formed.

Cutlet, Pork

"Pork Cutlet" may consist of pork temple meat, inside masseter muscles, and small pieces of lean from the tip of pork jaws. These are flattened and knitted together in "cutlet" size products by means of "cubing" or "Frenching" machines, or by hand pounding with "cubing hammers." The term "cutlet" relates to thin slices of meat. They can be identified as sliced pork meat product when the designation clearly states the specific part of the carcass from which the meat in the product is derived (e.g., "Pork Loin Cutlets"). All of the terms should be conspicuously displayed on labels.

Cutlet, Poultry

Poultry cutlets may be fabricated as opposed to using whole pieces of poultry meat. However, the term "cutlet" must be properly and distinctly qualified to describe the product, e.g. "Turkey Cutlet from a Turkey Loaf" "Chicken Cutlet from Chicken Roll" "Turkey Cutlet, Chopped and Formed"

Cooked poultry cutlets, which are solid pieces and contain added water, should not be labeled as patties. A solution statement is not needed.

Cutlet Veal

Must be a solid piece of meat from the round; slice thickness may vary. However, combining several thin slices to represent a single cutlet is not permitted.

Dehydrated Meat Calculation Factor

The fresh meat equivalent based on a given amount of dehydrated meat can be found by multiplying the weight of the dehydrated beef by the factor 2.8. This factor was derived as follows: Assuming canners and cutters grade beef was used, the composition of meat would be approximately 12% fat, 18% protein, 69% water, and 1% ash. Then 100 pounds of beef, when dehydrated to 5% moisture, would be 100 less 64 or 36 pounds dehydrated meat. Thus, 100 divided by 36 equals 2.8.

Assuming that the amount of dehydrated beef equivalent of 100 pounds of fresh beef is that quantity containing 18 pounds of protein, then 18 divided by the percentage of protein found by analysis of dehydrated beef would be the amount of dehydrated beef equivalent to 100 pounds of fresh meat.

Dehydrated Poultry Calculation Factor

The moist deboned cooked poultry or poultry meat equivalent based on a given amount of dehydrated poultry or poultry meat which can be found by multiplying the weight of the dehydrated poultry or poultry meat by the factor of 4.0.

Dehydrated Products when Water is Added

Three methods are acceptable for listing dehydrated products. Listing of the ingredients (1) As "water, dehydrated potatoes" or "dehydrated potatoes, water," whichever is the proper order, (2) As "reconstituted potatoes," or (3) As "rehydrated potatoes." If the reference was to meat instead of potatoes, the word beef, pork, or whatever was appropriate would be substituted for the word "potatoes."

Deviled Poultry

Is a semi plastic cured poultry food product made from finely comminuted poultry in natural proportions and containing

condiments. Deviled poultry may contain poultry fat, provided that the total fat content shall not exceed 35% of the finished product and the moisture content shall not exceed that of the fresh unprocessed poultry. When skin is in excess of natural proportions, skin must be included in the product name (e.g. "Deviled (Kind) with (Kind) Skin Added").

Dinner Dog

A coined name — must show true product name, e.g., "A Meat and Soy Protein Concentrate Product."

Dinners and Suppers, Frozen

Frozen products labeled as "dinner" or "supper" must weigh at least 10 ounces and shall contain at least 3 components consisting of the following: meat, poultry, cheese, eggs, vegetables, fruit, potatoes, rice or other cereal-based products (other than bread or rolls). This is not intended to include products like casseroles and stews that have all of the components combined. Sauces and gravies are not considered one of the components. They may also contain other servings of food, e.g., soup, bread or rolls, appetizer, beverage, and dessert, and these components may be included in the minimum 10-ounce net weight requirement. If meat is featured in the product name, e.g., Beef Dinner, the requirement is 25% or 2.5 ounces cooked meat. If a meat food product is featured in the product name, e.g., Beef Burgundy Dinner, then 25% or 2.5 ounces of meat food product is needed. If poultry is featured in the name, e.g., Chicken Dinner, the standard is 18% or 2 ounces cooked deboned poultry meat, whichever is greater. However, if a poultry food product is featured in the product name, e.g., Chicken a La King Dinner, the 25% or 2.5 ounces of poultry food product, whichever is greater, is needed. The meat requirement for products with net weights greater than 10 ounces may be established exclusive of the appetizer, bread, and dessert, provided the remaining components weigh not less than 10 ounces.

The name for dinner and supper products shall consist of or include a listing of each of the dish components in descending order of predominance by weight, for example, Fried Chicken Dinner — Fried Chicken, Mashed Potatoes, Peas and Carrots. Dinner or supper identification may appear on side panels without the complete product name shown, for example, "Fried Chicken Dinner" or "Beef Dinner."

When a dessert is one of the components of a frozen dinner or supper, i.e., a multi-component item, it may appear out of the order of predominance in the product name and appear as the last component in the product name.

Dipped Steaks

Steaks made from a solid piece of meat may be dipped in a solution of water and flavoring. The result in gain shall not be more than 3% above the weight of the untreated product. A prominent statement, like "Dipped in a Solution of contiguous to the product name," shall show.

Dixie Bacon

True product name, e.g., "Pork Jowl Dixie Bacon, Cured and Smoked" shall appear on the label.

Dixie Square

Same as for Dixie Bacon.

Dog Food

See: 9 CFR 355.29.

Dough Conditioner

A generic or class name that cannot stand alone in the ingredients statement. The term "Dough Conditioner" must be followed immediately by the common or usual name of all ingredients present.

Dried Egg White Added

See: **Wheat Gluten Dried Soup Mixes (Meat)**.
Dried meat soups are not amenable. Poultry — See: 9 CFR 381.15.

Dry Aged

Fresh Meat is held (without vacuum packing) for various periods of time (usually 10 days to 6 weeks) under controlled temperatures (34°F to 38°F), humidity, and airflow to avoid spoilage and ensure flavor enhancement, tenderness, and palatability.

There is a difference of opinion regarding the best cooler humidity. Some prefer low humidity of from 70 to 75% so that exposed surfaces of meat remain dry. Others use humidity's up to 85 to 90% in order to purposely develop a mold growth on the outside of the meat and reduce evaporation losses. Ultraviolet light may be used to reduce microbial load in the aging room. The number of days aged does not have to appear on the label when the product is identified as "Dry Aged" (e.g., "Dry Aged Beef").

Dry Cured

Product labeled as "dry cured" shall not be injected with a curing solution or processed by immersion in a curing solution.

Dry Milk Products

Approved dry milk items include whole dry milk, nonfat dry milk, calcium-reduced dried skim milk, dried whey and lactose-reduced dried whey. If nonfat dry milk is reconstituted prior to addition to product, it would be declared on the label as "Reconstituted Skim Milk."

Dry Salt Cured

Dry salt cured product may contain a curing solution injected directly into the tissue but not through the circulatory system before it is covered with a dry curing mixture. It may be momentarily moistened to facilitate initial salt penetration but shall not be immersed in a curing solution.

Dual Weight Requirement for Stuffed Poultry Labels

Poultry products that consist solely of bone-in poultry and stuffing, e.g., a "Stuffed Turkey," shall bear weight statements on the label indicating the total net weight of the product and a statement indicating the minimum weight of the poultry in the product.

When a stuffed poultry product is a component of a dinner or an entree, only the total net weight needs to be shown on the label.

Duck, Salted

This product should reach an internal temperature of 155°F.

Dumplings with Beef

The product must contain at least 18% meat in total formulation.

Dutch Brand Loaf

A nonspecific loaf that must be qualified as "Made in USA."

Easter Nola

Salami that is made with pork that is coarsely chopped and mildly seasoned with black pepper and garlic.

Egg Foo Young with Meat

The product must contain at least 12% meat.

Egg Foo Young with Poultry

The product must contain at least 3% poultry meat.

Egg Roll, Vietnamese Style

The product must contain soy bean noodles or cellophane noodles, and fish sauce or anchovy extract. They are usually rolled in a thin spring roll skin or a dry rice paper skin.

Eggs Benedict

The product must contain at least 18% cured smoked ham. A poached egg on a toasted English Muffin, topped with a slice of ham, and covered with hollandaise sauce.

Eggs, Fresh

For breakfast-type foods the egg portions may be referred to in the product name and the ingredients statement as "Fresh U.S. Grade A Large." The eggs must be received in shells or broken and blended and not in dry or frozen form.

Empanadillas (SP)

A turnover containing 25% fresh meat or poultry (raw basis). The species or kind is part of the product name, e.g., "Beef Empanadillas." The product may vary in size from large to hors d'oeuvre size.

Empanadillas Chorizo

An empanadilla that contains at least 25% fresh chorizo or 17% dry chorizo.

Encapsulation

An encapsulated additive, e.g., salt is an acceptable name. It does not require a sublisting if encapsulated in vegetable oil. If encapsulated in an animal fat, the specific animal fat must be identified in the ingredient statement.

Encapsulated lactic acid starter culture does not need to be sublisted.

Enchilada (SP)

The product must contain at least 15% meat or 10.5% poultry meat. A Mexican type food consisting of a "tortilla" which has been filled with a variety of fillings and then rolled. The species must appear in the product name, e.g., "Beef Enchilada."

Enchilada with Beef Chili Gravy or Enchilada Prepared with Meat and Sauce

The product must contain at least 50% Enchilada.

Enchilada — Sonora Style

The product consists of two or more tortillas stacked "pancake style" with filling spread between each tortilla. Cheese may be mixed into the tortilla dough prior to frying.

Entree (Principal Dish or Main Course)

Product labeled entree should fall into one of the following categories:

1. All meat or meat food product — 100% meat or meat food product.
2. Meat or meat food product and one vegetable; or meat or meat food product and gravy — 50% cooked meat or meat food product.

3. Meat and Vegetable with Gravy — 30% cooked meat portion; meat and gravy portion at least 50%, (e.g., Salisbury Steak with Potatoes and Gravy).
4. Meat or Entree portion of a meal type — 25% cooked meat or meat food product, (e.g., Meat Loaf Dinner would require 25% meat loaf).

Enzyme Treated Product

Product from carcasses of animals injected with papain; liver, heart, tongue, cheek and head meat, trimmings, boneless beef, tenderloin, tails, tripe and cuts of meat not showing an imprint of the roller brand reading, "tenderized with papain," shall be properly identified and kept separate from other product. Kidneys must be segregated and properly labeled.

When such product leaves an official establishment, the immediate container shall bear a label showing, in addition to the other required labeling, a statement like "tenderized with papain" prominently displayed contiguous to the product name.

The establishment will furnish retail dealers handling such product with labels bearing the statement, "tenderized with papain" prominently displayed contiguous to the product name for use by such dealers on consumer packages or on product prepared from carcasses of animals injected with papain. Inspection personnel visiting retail markets should observe the effectiveness of this requirement. When retail outlets do not follow this identification, these facts should be immediately reported to the Food Labeling Division.

Enzyme Trimmings from Ante-Mortem Injected Beef

Beef trimming from this operation may be used in fresh meat products without label declaration.

Enzymes — Proteolytic

A 3% limit permitted pickup on dipped items, e.g., steak and solid pieces of meat. The label must declare the presence of the enzyme, e.g., "Tenderized with Papain." Trimmings from this method may be used in fresh meat products up to 25% of the formula, provided the finished product is immediately frozen and that distribution is limited to institutional use only. The labeling record should state the conditions and means of inspection control. Meat from this method may be used in cooked ground beef products up to 25% of the formula without showing the ingredients of the solution.

See: 9 CFR 317.8(b)(25) 9 CFR 318.7(a)(1) 9 CFR 381.120.

Exotic/Non-Amenable Products — Use of Cure Agents

Only amenable meat/poultry products can contain curing agents (i.e., nitrites etc.), with the exception of ratites (ostrich, rhea, emu) and squab. The prior function of nitrite and nitrate, according to FDA regulations, applies only to those species that were considered "meat" or "poultry" prior to September 1958. Therefore, amenable species that can contain cure agents are identified as the following:

1. Poultry — (domesticated birds) chicken, turkey, duck, geese, and guineas.
2. Meat — cattle, sheep, swine, and goat. Non-amenable products, such as buffalo, reindeer and pheasant, can not contain curing agents; such products are considered to be regulated under FDA regulations. However, if non-amenable products are included in an amenable product, curing agents would be permitted. The curing agents would be calculated based on both the amenable meat/poultry product and non-amenable meat/poultry product. For example, the formula includes 3 lbs. Cooked chicken and 97 lbs. buffalo. The calculation for the curing agents would be based on 100 lbs. of meat. In addition, in those situations where the meat block consists of an amenable product and a nonamenable product (refer to the example), the appropriate inspection legend should represent the amenable product. Therefore, using the example above, the label would have a poultry legend.

   Product derived from exotic/non-amenable species that contain over 3% raw meat (cattle, sheep, swine, goat, horses or other equine) are subject to inspection. The game meat used in these products must be derived from carcasses slaughtered under the Food Safety and Inspection Service. Products made with meat from exotic and non-amenable exotic species with 3% or less of meat or edible portion from cattle, sheep, swine, goat, horses or other equine, or up to 30% fat from these species are non-amenable provided the only reference to meat or meat byproducts on the labeling is in the statement of ingredients and the product name includes the term "flavored with (amenable species)."

   Custom prepared products composed of meat from exotic/nonamenable species and up to 30% animal fat are not amenable. Labeling such products with the term applies (see: 303.1 (a) (2)).

   Products made with meat from game animals with 3% or less of meat or edible portion from cattle, sheep, swine, goat, or up to 30% meat fats provided the only reference to meat or meat

byproducts on the labeling is in the statement of ingredients or referred to as "flavored with."

Custom prepared products composed of meat from game animals and up to 30% animal fat. Labeling "Not For Sale" applies.

See: 303.1 (a)(2)

Buffalo and venison must be federally or state inspected; however, venison may also be produced under the supervision of inspection officials of a country approved to export meat products into the United States. All other meat from exotic/non-amenable species that is used in formulating amenable products must be derived from carcasses slaughtered under the Food Safety and Inspection Service.

Extra and More Than

The terms "extra" or "more (component) than" may be used provided the following guidelines are followed:

1. There is at least a 10% increase in the particular component of interest over the amount that is found in the usual or "regular" formulation.
2. Information must be provided with the label application that compares the product formulation containing the "extra" amount of the component to the regular formulation of the same product to establish that at least a 10% increase in the component has occurred. Therefore, the usual or "regular" component claims at the time of label review must be presented so that the necessary comparison of formulations can be made.
3. In the situation where production of the "regular" product formulation ceases, the "extra" or "more (component) than" product labels would be given a 6-months temporary approval.
4. A comparison to a similar product on the market may be made to support the "extra" or "more/than" type claim, provided suitable market basket data are submitted with the label application that establish the similarity of formulations and show the increased amount of the component over the "usual" amount.

Fabricated Steak

1. Steaks that include large sections or pieces of meat that are molded or shaped to form one large piece and the sliced. A qualifier such as "formed" must be included in the product.
2. Fabricated steaks may contain added solutions if labeled in accordance with Policy Memo 066B.
3. Antioxidants are permitted.
4. When made from simulated fat covering and or marbling, the name must reflect this fact, e.g., artificially marbled-simulated fat covered.

Fajitas

The Spanish translation is "little belts" or strips of meat. Fajitas are strips of seasoned or marinated red meat or poultry meat, which have been cooked. Red Meat Fajitas require labeling in accordance with the current policy memo on added solutions. Fajitas may also be sandwich-like product, requiring 15% strips of cooked meat or poultry meat (excluding the marinade), topped with onions, peppers, and sauce, and rolled in a flour tortilla. Fajita, including the name of the meat or poultry, may stand alone, e.g., "Beef Fajita," "Chicken Fajita."

Farm Style Sausage

See: Country Style (Farm Style) Sausage.

Farmer Sausage Cervelat

Is usually a semi-dry sausage; but may be made in dry form. Usually made of equal parts of pork and beef delicately seasoned without garlic.

Farmer Summer Sausage

This is a special type of sausage made of beef and pork, salt, spices, nitrite or nitrate, and heavily smoked. It is classed as "Cervelat," and no extenders are permitted. It is dry with an MPR of 1.9:1. The word "Farmer" is considered a generic term, and labels can be approved without any qualifying words like "Style" or "Brand." Such labels are not required to bear a statement identifying the place of manufacture. The product must be trichina-treated.

Fiber Products

Fiber products such as bran are acceptable only in non-specific products. Fiber type foods are permitted in meat and poultry products and must be identified by their common or usual name, such as oat bran. However, fiber is not permitted in meat or poultry products, e.g., soy fiber, oat fiber, and wheat fiber. Presently, there is no recognized definition for fiber.

Fillet Style

"Fillet style" must be qualified, e.g., "chunked and formed," if the meat or poultry product is not made from a solid piece of meat or poultry. The term "fillet" is defined as a solid piece of meat or poultry.

Flanken in the Pot

The product must contain at least 25% beef. Product is made from beef plates and may contain such components as Matzo Balls, Noodles, and Vegetables. True product

name, e.g., "Flanken in the Pot with Matzo Balls, Noodles and Vegetables" must be used.

Flavored with

Any product with a standard in Section 9 CFR 319 and 9 CFR 381 of the regulations must meet that standard and may not be designated "flavored with." If a product does not meet the standard as it appears in the Policy Book it can be labeled "Flavored with." "Flavored with" can be anything from over 3% fresh meat or 2% cooked meat to below the standard for the product.

Flavoring

Ingredients, e.g., thiamine hydrochloride, monosodium glutamate, disodium inosinate, disodium guanylate, hydrogenated vegetable oil, and other commonly used materials must be listed separately.

Such ingredients as diacetyl, hexanal, ethyl alcohol, dimethyl sulfide, diallyl sulfide, and furfuryl mercaptan may be declared as artificial flavors or artificial flavorings without naming each.

When spices and/or flavorings are presented on labels coming from foreign countries, the identity of the spices and/or flavorings must be made known.

Foie Gras Products, Duck Liver and/or Goose Liver

Goose liver and duck liver foie gras (fat liver) are obtained exclusively from specially fed and fattened geese and ducks. Products in which foie gras is used are classified into the following three groups based on the minimum duck liver or goose liver foie gras content:

(A) French Product Name	Acceptable English Product Name
Foie Gras d'Oie Entier	Whole Goose Foie Gras
Foie Gras de Canard Entier	Whole Duck Foie Gras

These are products in which goose liver or duck liver foie gras are the only animal tissues present. They may contain added substances, e.g., seasonings and cures and when truffles are featured in the product name, they are required at a minimum 3% level.

(B) French Product Name	Acceptable English Product Name
Foie Gras d'Oie	Goose Foie Gras
Foie Gras de Canard	Duck Foie Gras
Bloc de Foie Gras d'Oie	Block of Goose Foie Gras
Bloc de Foie Gras de Canard	Block of Duck Foie Gras
Parfait de Foie Gras d'Oie	Parfait of Goose Foie Gras
Parfait de Foie Gras de Canard	Parfait of Duck Foie Gras

These products are composed of a minimum 85% goose liver or duck liver foie gras, although "parfaits" may contain mixtures of goose liver and/or duck liver foie gras. These products may also contain a wrapping or stuffing consisting of the lean or fat of pork, veal, or poultry, pork liver, and/or aspic jelly. When these ingredients are used, their presence must be indicated in a product name qualifier. Truffles, when featured in the product name, are required at a minimum 3% level.

(C) French Product Name	Acceptable English Product Name
Pate de Foie d'Oie	Pate of Goose Liver
Pate de Foie de Canard	Pate of Duck Liver
Galantine de Foie d'Oie	Galantine of Goose Liver
Galantine de Foie de Canard	Galantine of Duck Liver
Puree de Foie d'Oie	Puree of Goose Liver
Puree de Foie de Canard	Puree of Duck Liver

These products must contain a minimum of 50% duck liver and/or goose liver foie gras and may also contain a wrapping or stuffing of the lean or fat of pork, veal, or poultry, pork liver, aspic jelly, extenders, and/or binders. When these ingredients are used, their presence must be indicated in a product name qualifier. Truffles, when featured in the product name, are required at a minimum 1% level.

In all groups, an English translation of the term "foie gras" is not required, although all other product name terms must be translated into English. The kinds of poultry liver(s) used must be indicated in the product name. Also, other species and/or binders used must be indicated in a product name qualifier immediately following the product name, while the ingredients statement must follow the product name or qualifier as the case may be.

For Further Processing

Products which require further processing at another federally inspected plant may leave a federally inspected plant under one of the following three conditions:

1. With the name of the finished product qualified by a "For Further Processing" statement (e.g., Turkey Ham For Further Processing); or
2. With a fully descriptive name (e.g., uncooked ham contains up to 30% of a solution).
3. Not acceptable on a label when a product is formulated or processed in a manner contrary to the regulations.

"Fresh," "Not Frozen" and Similar Terms when Labeling Poultry Products

The word "fresh" may *not* be used to describe:

1. Any cured product, e.g., corned beef, smoked cured turkey, or prosciutto.

2. Any canned, hermetically sealed shelf stable, dried, or chemically preserved product.
3. Any raw poultry, poultry part, or any edible portion thereof whose internal temperature has ever been below 26 degrees Fahrenheit.
4. Any injected, basted, marinated poultry, poultry part or any edible portion thereof whose internal temperature has ever been below 26 degrees Fahrenheit.
5. Any other finished processed poultry product (including cooked poultry products) where its temperature has ever been below 26 degrees Fahrenheit, e.g., turkey sausage, chicken meatballs, cooked breaded chicken nuggets, etc.
6. Any uncured red meat product permitted to be treated with a substance that delays discoloration, such as, ascorbic acid, erythorbic acid, or citric acid.
7. Any product treated with an antimicrobial substance or irradiated.
8. The phrase "never frozen" or similar verbiage is not permitted on an unprocessed or processed poultry product where the internal temperature of the product has ever been below 0 degrees Fahrenheit or on any red meat product that has ever been frozen. Further, the phrase "never frozen" or similar verbiage is not permitted on refrigerated secondary products where the meat or poultry component has ever been frozen, e.g., multi-component meals, dinners, etc.

Generally, trademarks, company names, fanciful names, etc., containing the word "fresh" are acceptable, even on products produced in a manner described in one through seven above, provided the term is used in such a manner that it remains clear to the purchaser that the product is not fresh.

Secondary products, e.g., pizza, multi-component meals, dinners, etc., sold in the refrigerated state, i.e., not frozen or previously frozen, may be labeled as "fresh" when the term is used to describe the product as a whole even when made from components processed in a manner described in one through seven above.

This entry cancels Policy Memo 022C dated January 11, 1989, since 022C is out of date.

Fresh Thuringer

Not an acceptable name.

Fried Noodles with Pork

The product must contain at least 12% fresh pork in total formulation.

Fried Products

1. Frying medium need not be shown on the label.
2. Breading is not limited to 30% unless breaded is in the product name.
3. Fried chicken labels do not need to state "fully cooked" or refer to breading because fried denotes fully cooked and breading is expected. Fried poultry products in dinners are limited to 30% breading.

Fried Rice with Meat

The product must contain at least 10% meat; may contain eggs and vegetables.

Fries

1. Beef testicles may be labeled as "Beef Fries." They are not permitted to be used as an ingredient in meat food products.
2. "Fries" is not a required part of the product name, "species mountain oyster."

Fritters

The product must contain at least 35% raw red meat/poultry or red meat/poultry food product in the total formulation depending on the name, i.e., "Beef Fritter" must contain 35% beef and a "Chicken Patty Fritter" must contain 35% chicken patty. Fritters can contain up to 65% batter/breading (coating). If "breaded" is included in the product name, the batter/breading is limited to 30%.

Frizzes

An acceptable name. Similar to pepperoni but not smoked. MPR of 1.6:1.

Galician Sausage

Cured beef and pork is seasoned and stuffed into beef rounds. It is then smoked at a high temperature. Cooling is done in a blast of air which produces a wrinkled appearance which is characteristic of Galician sausage.

Gelatin

Gelatin is a binder/extender and is only permitted in a few meat and poultry products. Examples where gelatin is permitted include:

1. Non-specific products
2. Jellied products, e.g., souse, jellied beef loaf and head cheese
3. As a covering for products such as paté, to bind two pieces of meat together and in products where "gelatin" would be part of the product name

Gelatin is permitted as a thickening agent in menudo (i.e., beef tripe stew). If it is used in red meat paté products, its presence must be indicated by product name qualification.

It is not permitted in products like sausage, luncheon meat, and meat loaves. Gelatin is an acceptable ingredient in souse, jellied beef loaf, headcheese. Canned whole hams require qualifier if gelatin is added.

Gelatin in Poultry Rolls

If gelatin or some other binder comprises more than 3% of the formula, the name of the product must be qualified by wording, e.g., "Gelatin Added."

General Offices

The company's grant of inspection permits the general office address to be used in the signature line for any firm "doing business as."

Genoa or Genoa Salami

Is a dry sausage product with an MPR not in excess of 2.3:1. It is prepared with all pork or with a mixture of pork and a small amount of beef. The meat is given a coarse grind and enclosed in a natural casing. No smoke is used in its preparation.

Geographic and Related Terms (Requirements for the Use on Product Labels)

Any label representation that expresses or implies a particular geographical origin of the product, or any ingredient of the product, shall not be used except when such representation is:

1. A truthful representation of geographical origin, e.g., "Virginia Ham" for a ham produced in the State of Virginia; or
2. A trademark or trade name which:
   a. has been so long and exclusively used by a manufacturer or distributor that it is generally understood by consumers to mean the product of the particular manufacturer or distributor, e.g., "Swiss Chalet;" or
   b. is so arbitrary or fanciful that it is generally understood by the consumer not to suggest geographical origin, e.g., "Moon Sausage;" or
3. A part of the name required or allowed by an applicable Federal law, regulation or standard, e.g., "Frankfurter," "Vienna;" or
4. A name whose market significance is generally understood by consumers to connote a particular class, kind, type or style of product, or preparation rather than to indicate geographical origin of the product, e.g., "Mexican Style Dinner," "Italian Style Pizza." Such terms must be qualified with the word "style" or "type," unless specifically approved by the administrator as a generic term, e.g., "Lebanon Bologna," "Genoa Salami," "Milan Salami."

Any geographical representation that does not meet the aforementioned guidelines should be qualified by the word "brand," provided that the word "brand" is not used in such a way as to be false or misleading. A qualifying statement identifying the place where the product was actually made is required in proximity to the brand name, e.g., "Milwaukee Brand Bacon, Made in Chicago, Illinois." The word "Brand" must be in the same size and style of type as the geographical term. If the product has a foreign brand name, it may be identified as having been made in this country, e.g., "Scandinavian Brand Bacon, Made in U.S.A."

Geographic Terms

1. Country, Ranch, and Farm in Trade, Branch and Fanciful Names: Trade names, brand names, or fanciful names that include the words country, ranch, or farm, e.g., "Country Kitchen," "Ranch House," "Hickory Farms," or "Carabeef Ranch Brand" do not invoke section 9 CFR 317.8 of the regulations regarding the use of the term "Country" or "Farm." However, if the terms are used alone in conjunction with the product name, e.g., "Country Stew," then such products must be prepared in the country or on the ranch or farm and meet any other requirements prescribed.
2. Southern: The term "Southern" is restricted to use only in areas south of the Mason-Dixon Line and east of the Mississippi River as well as Arkansas, Louisiana, and Missouri, which are also considered southern states.

German Potato Salad with Bacon

The product must contain at least 14% cooked bacon in total formulation.

See: Salad–German Potato Salad.

German Sausages with Milk

Whole milk is a permitted ingredient in the following meat food products when the ingredients statement is shown immediately under the name of the product or the milk is shown in a qualifying statement contiguous to the product name: Speckblutwurst, Kalbsbratwurst, Langblutwurst, Blutwurst, Gelbwurst, Zengenwurst, Brand Tongue and Blood Pudding Kalbslebenwurst. (Swiss Liver Sausage, Kalbslebenwurst should be considered on the same basis as Bockwurst (e.g., no limit on water or milk).)

Milk is a characterizing ingredient in German sausages and not an extender. Products which contain milk should be called by their proper names.

Giblet Gravy (Kind)

Requires 7.25% giblets. The product must contain an equal number of livers, hearts, and gizzards.

Giblets and/or Necks Sold with Carcasses

Poultry giblets consist of approximately equal numbers of hearts, gizzards, and livers, as determined on a count basis.

Although often packaged with them, the neck is not a giblet. Rabbit giblets consist of the liver and heart. Giblet packs are expected within the cavities of eviscerated whole birds or eviscerated whole rabbits or when packaged with cut-up whole birds or cut-up whole rabbits, therefore, qualifying the presence of giblets is not required on labeling. However, when giblets are not expected, therefore, a product name qualifier is required, e.g., "Packed with Giblets."

In situations where parts of giblets are missing, a product name qualifier is required, e.g., "Parts of Giblets Missing" or "Parts of Giblets May Be Missing." In situations where the giblets are missing entirely from an eviscerated carcass or a cut-up whole carcass, a product name qualifier is required, e.g., "Packed Without Giblets." In addition, an excess of one of the giblet components can be added to make up for another missing giblet component. In this case, a proper qualifying statement is required, e.g., "Packed with 2 Gizzards, 1 Liver."

A neck, when not attached to the carcass of a whole bird, is also expected to be present within the carcass or packed with the cut-up whole carcass. Accordingly, a product name qualifier is not required to flag the presence of the neck. Rather, when the neck is missing, a product name qualifier is required, e.g., "Without Neck."

See: 9 CFR 381.170 (b) (21) and 9 CFR 354 (m).

Glazes

If a glaze component contributes to the flavor profile of the product, we will allow the ice glaze coating to be counted toward the total net weight of the finished product. However, the coating and coating percentage must be included as part of the descriptive product name in accordance with Policy Memo 044A, e.g., "chicken breast filets with rib meat, coated with a butter garlic flavoring and containing up to 22.5% of a solution of…" or "chicken breast filets with rib meat, coated with a 6.5% butter garlic flavoring and containing up to 16% of a solution of…."

Glycerin

May not be added to any product as such; may be used in proprietary mixes.

Goetta

An oatmeal product similar to scrapple. Goetta is prepared with a formula containing not less than 50% meat and meat byproducts. The cereal component should consist of oats or oat products and just enough water to prevent product from sticking and burning during the preparation process. The term "Old Fashioned" when noted on a label for "Goetta" refers to the round shape.

Goettinger Cervelat

A dry cervelat with no byproducts or binders.

Goose Liver or Goose Liver Sausage

At least 30% cooked goose liver. When pistachio nuts are added, product name must be qualified, i.e., "pistachio nuts added."

Gorditas, Meat/Poultry

The product must contain at least 15% cooked meat/poultry based on the weight of the total product. The "species" or "kind" gorditas name (Beef Gordita, Chicken Gordita) may stand alone. If other characterizing ingredients such as potatoes, rice, beans, etc, are included in the name, they must be reflected in their order of predominance, e.g., Beef and Potato Gorditas, Rice and Chicken Gorditas, as determined by the formula.

Goteborg

A Swedish dry sausage made of coarsely chopped beef and sometimes pork. Mildly seasoned with thyme. It has a somewhat salty flavor and is heavily smoked, usually in long casings and air dried.

Gothaer Cervelat

Originated in Gotha, Germany. Usually made of very lean pork finely chopped and cured.

Goulash

A stew-like product with at least 25% meat or 12% poultry meat. Unless designated "Hungarian," generally means stew, whether veal, pork, beef, turkey, etc., are used. Product may be just meat and gravy or meat and gravy with vegetables served with or without rice, potatoes, or noodles.

Goulash, Hungarian Style

The product must contain paprika and at least 25% meat or 12% poultry meat. May not contain noodles, potatoes or dumplings.

Grade Marks

There are no acceptable grade marks for products imported from foreign countries. Foreign countries do not have a grading service exactly like AMS.

The grading term "good" on poultry is considered puffery and is acceptable.

Red meat grading terms, "prime," "choice," "select," may not be used immediately preceding "kind" poultry, unless the poultry is equivalent to USDA Grade A. For example, "Choice Turkey" or "Select Chicken" must come from Grade A birds.

Gravies

The product must contain at least 25% meat stock or broth, or 6% meat. Mono and diglycerides allowed in amount of 1% in gravies.

Gravy and Beef

The product must contain at least 35% cooked beef (beef same size lettering as gravy). For 25% cooked beef (beef lettering no larger than one-half size gravy).

Gravy and Dressing with Pork or Gravy and Pork with Dressing

The product must contain at least 14% cooked pork.

Gravy and Poultry Salisbury Steak

Not more than 65% gravy and at least 35% poultry salisbury steak.

Gravy and Swiss Steak

The product must contain at least 35% cooked meat.

Gravy and Yankee Pot Roast

The product must contain at least 35% cooked beef. Beef is cooked with or without vegetables.

Greek Sausage

The product must contain orange peel.

Ground Beef

May not contain added fat. Maximum total fat 30%. Cheek meat is permitted up to 25% and must be declared in the ingredients statement. For more than 25%, show as "Ground Beef and Cheek Meat," all the same size.

Beef of skeletal origin, or from the diaphragm or esophagus (weasand) may be used in the preparation of chopped beef, ground beef, or hamburger. Heart meat and tongue meat as organ meats are not acceptable ingredients in chopped beef, ground beef, or hamburger.

Ground Beef Chuck and Round

Product to be labeled "Ground Beef Chuck" or "Ground Beef Round" must comply with the following guidelines:

1. "Ground Beef Chuck" must be derived from all or part of the primal part of the beef carcass commonly referred to as the "Beef Chuck," except as provided for in 3. The product must comply with the fat requirements of 9 CFR 319.15(a).
2. "Ground Beef Round" must be derived from all or part of the primal part of the beef carcass commonly referred to as the "Beef Round," except as provided for in 3. The product must comply with the fat requirements of 9 CFR 319.15(a).
3. Generally, shank meat may be added but may not exceed the natural proportion of the beef carcass, which is considered to average 6%. Higher quantities of shank meat may be used if the shank meat remains attached during the cutting and boning of the boneless chuck or round, or if the processor can demonstrate that a higher percentage is applicable.

Ground Beef — Hamburger and Soy Products

Combinations of ground beef or hamburger and soy products may be descriptively labeled, e.g., "Hamburger and Textured Vegetable Protein Product" or "Ground Beef and Isolated Soy Protein Product" if the combination product is not nutritionally inferior to hamburger or ground beef. If the combination products are nutritionally inferior, they are to be labeled as Imitation Ground Beef (or Imitation Hamburger) or Beef Patty or Beef Patty Mix in accordance with Section 9 CFR 317.2(j)(1) and Section 9 CFR 319.15(c) respectively.

Gum Arabic

May be used up to 2% in breading and batter mixes.

Gum Tragacanth

A carrier and stabilizer in liquid spice extractives not to exceed 0.1% in finished product. Not permitted in sausage products.

Gum-Vegetable

Spice extractive products which employ vegetable gums as emulsifiers have been approved. The addition of vegetable gum is limited to no more than 15% in the seasoning blend emulsion.

Gumbo

A Creole word for okra. It is now recognized as meaning a dish or a soup thickened with okra. To qualify, the dish must have okra as an ingredient. Either the soup or the stew standard would apply, depending on product name ("Chicken Gumbo"). Product identified as "Creole Style — Gumbo" does not contain okra; however, it must contain a roux (flour, milk, or water, etc.) or gumbo file (dried powder from young leaves and leaf buds of sassafras).

Gyros

Products identified with this term must contain at least 65% meat and no more than 12% extenders and binders. Examples include gyro loaf, gyro cone, gyro portions, and gyro slices.

Halal and Zabiah Halal

Products prepared by federally inspected meat packing plants identified with labels bearing references to "Halal" or "Zabiah Halal" must be handled according to Islamic law. The federal meat and poultry inspection program does not certify to the "Halal" or "Zabiah Halal" preparation of products, but rather accepts the statements and markings in this regard offered and applied under the supervision of any Islamic organization. The words "Halal" or "Zabiah Halal" may be used only on the labeling of meat and poultry products prepared under such Islamic authority. The identity of the Islamic organization should be available upon request by agency official. Qualification of the words "Halal" or "Zabiah Halal" by such terms as "style" or "brand" does not negate these requirements.

Ham a La King

Must contain at least 20% ham (cooked basis).

Ham and Bacon Loaf

There is a limit of 3% water in this product.

Ham and Cheese Loaf

Nonspecific loaf. Cheese is chopped into small cubes and combined with finely ground ham.

Ham and Cheese Salad

Product must contain at least 25% ham (cooked basis).
See: Salads.

Ham and Cheese Spread

Product must contain at least 25% ham (cooked basis).

Ham and Dumplings and Sauce or Gravy

Product must contain at least 18% cooked ham.

Ham, Boiled

A fully cooked, boneless product which must be cooked in water and may be processed in a casing or can. The product may be of various shapes and may be partially cooked in boiling water.

Hamcola

Not an acceptable product name; should be accompanied by true product name, i.e., "Boneless Cooked Ham Coated with Spices."

Ham Capacolla, Cooked

Ham that has been cured and then cooked.

Ham Chowder — Condensed

Product must contain at least 10% cooked ham.

Ham, Cooked — Sectioned and Formed

The qualifying phrase "sectioned and formed" is no longer required on boneless ham products, e.g., "ham" and "ham-water added." The addition of small amounts of ground ham added as a binder to such products may be used without declaration. The amount of ground ham that may be used can represent no more than 15% of the weight of the ham ingredients at the time of formulation. Products containing more than 15% ground ham trimmings must be labeled to indicate the presence of the ground ham, e.g., "a portion of ground ham added." Policies regarding the required use of terminology such as "chunked and formed" and "ground and formed" will continue.

Whole hams require a cooking temperature to differentiate the ready-to-eat products from trichinae-treated products. The reason that the temperature is required is to determine the label requirements (e.g., safe handling) and proper serving size.

Ham Croquettes

Product must contain at least 35% cooked ham. If chopped ham is used, the product name must be "Chopped Ham Croquettes."

Ham, Fresh (or Uncured)

Ham that does not contain a cure must be labeled either "Fresh" or, if the ham meets the requirements of 9 CFR 319.2, "Uncured." This also applies to cooked product, and must be labeled cooked product "Cooked Uncured Ham."

Ham Half

"Half Ham" is permitted on labels for semi- boneless ham products which during their processing have had the shank muscles removed. The two halves of the finished product have approximately an equal amount of bone. The term "No Slices Removed" has also been deemed suitable for use with a ham item referred to as "Half Ham."

Ham Omelet

Product must contain at least 18% cooked ham.

Ham/Parma Ham/Prosciutto Di Parma

Ham, when labeled "Parma Ham" and/or "Prosciutto di Parma," would have to be produced in the region of Parma, Italy, in accordance with Italian Law, which defines the denomination of origin, the territorial limits of production, characteristics of the product, and the method of manufacture.

Ham, Quarter, Semi-Boneless (No Slices Removed)

The product consists of a ham prepared as a "Regular Semi-Boneless, Half Ham" which is sectioned again to result in four pieces just about equal not only in weight but also in content of bone.

Ham Roll Sausage

Ham trimmings and ham shank meat are permitted.

Ham Salad

Product must contain at least 35% cooked ham. Chopped ham may be used without it appearing in the product name.

See: Salads.

Ham, Scotch Style

A cured, uncooked, boned, and rolled whole ham either tied or in a casing.

Ham, Shankless

When the term, "shankless" is used in reference to a ham, it indicates that the shank has been removed by a cut through the joint at a right angle to the femur bone. The distal tip of the semitendinous muscle may be severed above its tendinous attachment, leaving an extension approximately 2 inches long. The extension is considered an integral part of the ham's body and is usually folded over the femur's end.

Ham Shortcake

Product must contain at least 25% cooked ham.

Ham, Smithfield

This is an aged, dry cured ham made exclusively in Smithfield, Virginia. The use of the words "brand" or "style," e.g., "Smithfield Brand Ham," "Smithfield Style Ham," does not eliminate this requirement.

Ham Trimmings

Ham trimmings, to be labeled as ham, cannot contain excess shank meat. The fat content will not exceed 35%. It will consist of at least 65% lean meat as determined by chemical analysis.

Ham, Westphalian or Westphalian Style Ham

Ham is cut with bone in, the hip bone cut out, cured in a combination of dry and pickle cure but not a pickle alone. It is smoked in a medium warm (no greater than 100°F.) smokehouse until a shining red brown or chestnut color is acquired. Beechwood may be used and will impart the characteristic Westphalian flavor. Other hard woods are also acceptable. Juniper berries are permitted.

Handling Statements

Acceptable handling statements, in addition to those required in sections 9 CFR 317.2(k) and 9 CFR 381.125, include "Keep Refrigerated — May be Frozen" or "Keep Refrigerated — Can be Frozen."

Handling Statements on Retorted Products

Handling statements may appear on labels for shelf stable product, even though such product does not have to be refrigerated or frozen, and provided the statement will accurately reflect conditions of distribution and sale. These products are to be handled in the plant as shelf stable items including incubation and condition-of-container examinations. Once the product is refrigerated or frozen for shipment, distribution, and display for sale it is to be handled as a refrigerated or frozen item.

The statement "previously handled frozen for your protection, refreeze or keep refrigerated" is now acceptable on poultry products under the usual restriction of use for such statements.

Head Meat

After removal of the cheeks, lips, snout, skin, and tongue from the head, there remains small pockets and areas on the skull to which muscle tissue is attached. This muscle may be removed and used in product and declared on

labeling as beef or pork as the case may be. However, there are a few standardized products in which the regulations limit the amount of this meat that may be used and require that it be specifically declared on the label (e.g., chili, chili with beans, and corned beef hash).

See: Beef Cheek Meat and Beef Head Meat (Use and Labeling as an Ingredient in Meat Food Products).

Headcheese

A jellied product consisting predominantly of pork byproducts and seasoning ingredients. It must contain some product from the head. Extenders like cereal, soy derivatives, nonfat dry milk, etc., are not permitted ingredients of headcheese.

Hearts/Heart Meat

Hearts/heart meat may not be labeled as "beef," "pork," etc. in the ingredients statement. When used in a product, they must be identified by species, e.g., "Beef Hearts." Hearts/Heart Meat, including the heart cap, may be considered meat for calculating the meat to textured vegetable protein ratios.

Heat and Eat Sausage

Not the same as Brown and Serve Sausage. When the "heat and eat" term is used, product must comply with cooked sausage regulations, e.g., limitation of 10% added water and not more than 3½% binder.

Hickory Smoked

"Hickory flavored" ad "hickory taste" are acceptable terms on products that have been smoked with some hickory in the sawdust. They do not need to be smoked with 100% hickory smoke.

High Fructose Corn Syrup (HFCS)

HFCS may be used to flavor meat or poultry products in amounts sufficient for its intended purpose, provided the following conditions are met:

1. HFCS must contain not less than 40% fructose on a solids basis.
2. HFCS must have a dextrose equivalence (D.E.) of not less than 93.
3. HFCS must have a sweetening power greater than or equal to sugar (sucrose).
4. HFCS must be identified on the label as High Fructose Corn Syrup in the ingredients statement, curing statement, etc.

Holstein or Holsteiner

Product is the same as **Farm Style Sausage**, except that it is stuffed into wide casings and heavily smoked, usually in long casings, and air dried. No extenders are permitted.

Honey Claim in Product

A honey claim may be made or implied on a product label if:

1. The product contains at least 3% honey.
2. Honey contains at least 80% solids, U.S. grade C or above.
3. When other sweeteners, (sugar, dextrose, maltose, invert sugar, corn syrup solids, and similar ingredients) are used, the quantity may not exceed one-half that of the honey, e.g.. If 3% honey is used, then no more than 1½% of all other sweeteners may be used.
4. Product to be identified as "Honey Glaze" must contain honey to other sweeteners at a ratio no less than 2:1. If dried honey is used, the ratio is to be no less than 1.6:1.
5. When honey is included in a breading, a honey claim may be made regardless of the quantity of honey used.

Honey Cured or Sugar Cured

"Honey Cured" may be shown on the labeling of a cured product if:

1. the honey used contains at least 80% solids or is U.S. grade C or above;
2. honey is the only sweetening ingredient or when other sweetening ingredients are used in combination with honey, they do not exceed one-half the amount of honey used; and honey barbecue — touch of honey.
3. honey is used in an amount sufficient to flavor and/or affect the appearance of the finished product.

Traditionally, cured products which are labeled to indicate the presence of honey, e.g. Honey ham, must meet the parameters prescribed herein.

"Sugar Cured" may be used on the labeling of a cured product if:

1. the sugar used is cane sugar or beet sugar;
2. sugar is the only sweetening ingredient or when other sweetening ingredients are used in combination with sugar, they do not exceed one-half the amount of sugar used; and
3. sugar is used in an amount sufficient to flavor and/or affect the appearance of the finished product.

"Honey and Sugar Cured" or "Sugar and Honey Cured" may also be used on labeling if:

1. the honey and sugar are of the nature described above;
2. the honey and sugar are the only sweetening agents or when other sweetening ingredients are used in combination with the honey and sugar, they do not individually exceed either the amount of honey or sugar used and collectively do not exceed one-half the total amount of honey and sugar; and
3. the honey and sugar are used in amounts sufficient to flavor and/or affect the appearance of the finished product.

Hors d'oeuvre (Snack)

Product must contain at least 15% cooked meat or 10% bacon (cooked basis). True product name must be shown, e.g., "Puffed Pastry Wrapped Frank."

Hot Dog Chili Sauce with Meat

Product must contain at least 6% meat.

Hot Dog Chili with Meat

Product must contain at least 40% meat. Sausages and bologna rework not permitted.

Hunan Style Seasoned Pork

Acceptable for pork shoulder sliced into one inch pieces and marinated in a solution of soy sauce, garlic, and ginger, cooked and returned to green weight. The product may be flavored with other seasoning ingredients, e.g., star anise and coriander.

Hydrolyzed Beef Stock

A beef stock which has been treated with acid, alkali, or enzymes to digest the protein. The protein molecules are broken down into amino acids, peptides, polypeptides, and peptones. As the digestion is carried out for longer periods of time, more and more of the larger molecules are broken down into amino acids, with free alpha-amino groups. By analyzing these alpha-amino nitrogens one can determine the degree of hydrolysis. 100% hydrolysis would mean that all the nitrogen (protein) is in the form of amino acids. 10% of hydrolysis would mean that only 10% of the nitrogen is in the form of free amino acids, while the rest is still present in polymeric form.

The label should indicate the degree of hydrolysis. This is determined from the ratio of amino nitrogen to total nitrogen.

$$\textit{amino nitrogen} = \%\ \text{hydrolysis total nitrogen}$$

A product labeled 50% Hydrolyzed Beef Stock must, therefore, have 50% of the total nitrogen present as amino nitrogen. Adding % solids is optional. The % solids would not necessarily be the same % as hydrolysis depending on the thickness (consistency) of product.

Hydrolyzed Gelatin

Hydrolyzed gelatin is permitted in frankfurters and similar products (9 CFR 319.180) at levels typically used for flavorings (less than 2%). Hydrolyzed gelatin may currently be used a 2% in 9 CFR 319.180 and like products as a flavoring. If gelatin is used in a product, make sure it is permitted for use in the product. Hydrolyzed gelatin is acceptable in poultry franks 9 CFR 319.180 and like products at levels not to exceed 2% of total formula until otherwise notified.

Hydrolyzed gelatin is recognized as a binder rather than flavoring and may be used at levels of 2% or less in 9 CFR 319.180 type products.

Hydrolyzed Oat Flour

Hydrolyzed oat flour is safe and may be used in non-standardized meat/poultry products as a binder at below typical binder use levels, i.e., 3%. It may be used in low fat hamburger, water, and hydrolyzed oat flour product as per Policy Memo 121.

Hydrolyzed Protein

"Hydrolyzed Protein (milk, egg, soy)" is an acceptable common or usual name provided all components are hydrolyzed. "Hydrolyzed Protein (potato, gelatin)" is an unacceptable ingredient declaration and must be declared as "hydrolyzed potato protein and hydrolyzed gelatin."

Salt is present in hydrolyzed protein and must appear in the sublisting of the hydrolyzed protein if it does not appear elsewhere in the ingredients statement.

Hydroxypropyl Methylcellulose (HPMC)

Emulsifying agent, binder, thickener, and a stabilizer. This is accepted for its emulsifying qualities when prepared as a solution and applied as a dip.

1. Not more than 2% in solution
2. Not more than 4% weight gained in product
3. Not more than .08% hydroxypropyl methylcellulose in finished product
4. Must be identified in the ingredients statement for purpose
5. Approved on individual basis only

Ice-Glazed Breaded Chicken Nuggets

If an ice glaze is applied for the purpose of setting the breading, the term "ice glazed" needs to appear in close contiguous to the product name. The water cannot be included as part of the net weight statement and the transmittal form should indicate this.

Imitation Flavors

Imitation beef flavor, imitation mushroom flavor, flavor base for gravies and similar substances which enhance, fortify, or help to simulate a flavor are usually composed of food additives and, as such, are not "artificial flavors" for labeling purposes. This class of imitation flavors can be composed of such ingredients as flour, fats, oils, salt, hydrolyzed vegetable protein, vegetable gums, thiamine hydrochloride, beta alanine, disodium inosinate, glutamic acid, and a host of other ingredients. These flavorings must be identified on labels by showing each individual ingredient by its common name. Class names, e.g., amino acids are not acceptable. Each specific amino acid must be listed.

Incidental Additives

As defined in the Food and Drug Administration regulations (21 CFR 101.100(a)(3)), incidental additives are substances present in foods at insignificant levels and that do not serve a technical or functional effect in that food. In determining whether a substance is an incidental additive, the following criteria may be applied:

1. Substances that are present in a food as a result of having been present in an ingredient added to the food and have a technical or functional effect on the ingredient but not on the finished food, or
2. Substances that are processing aids, defined as:
   a. substances added during processing but removed before the food is packaged in its finished form, or
   b. substances added during processing but that are converted to constituents normally present in the food, and do not significantly increase the amount of those constituents naturally found in the food, or
   c. substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food.

Ingredient Labeling

1. All ingredients in FDA standardized products, e.g., Cheddar Cheese (Water, salt, cheddar, etc.), and on standardized products, e.g., Soy sauce, Worcestershire sauce, require complete disclosure of all ingredients on the labels of meat and poultry products.
2. Protein hydrolysates must identify the common and usual names and identify the source from which the protein is derived, e.g., "hydrolyzed vegetable protein" would be declared as "hydrolyzed corn protein."
3. FDA certified color additives require the listing of the common or usual names, e.g., FD&C Blue No. 1, Blue 1, or Blue 1 Lake. Color additives not subject to certification may be declared as "artificial color," "artificial color added," or "color added." Alternatively, color additives not subject to certification may be declared as "colored with _____," or "_____ color," with blank space filled in with the name of the color additive listed in 21 CFR 73, e.g., "colored with annatto" or "caramel color."
4. Cured meat products used as ingredients, regardless of their level of use, require complete disclosure of all ingredients in the formulation of meat and poultry products.

Insert Labels for Use at Retail Stores

No inspection legend is permitted on insert labels.

Inspection Legends (Dual)

Products consisting of mixed meat and poultry ingredients shall bear either the official meat inspection legend or poultry legend, depending on which ingredients are present in the greater amounts. If meat or poultry ingredients exist in equal proportions, either official legend may be used. If meat and poultry ingredients exist in exact proportions and both appear in the product name, the official legend must reflect the ingredient appearing first in the product name.

Containers of products intended for sale to household consumers can bear only the official mark of inspection of the product enclosed. Containers of products intended for distribution to other than the retail trade may bear both the official meat inspection legend and the official poultry products inspection legend.

Inspection Mark on Wing Tag

When the inspection mark is shown on a wing tag, either the plant number or the firm's name and address must also appear.

Intestines

Intestines can be prepared as edible product and bear the mark of inspection.

Irish Stew

Product does not require a geographical qualifying statement nor the words, "Style," "Type," or "Brand." Usually it contains lamb or mutton but beef may be used. It must meet the stew standard. Vegetables include onions, carrots, potatoes, and turnips. Dumplings are often used. Beans are not acceptable in "Irish Stew."

Isolated Soy Protein

This food ingredient is similar to soy protein concentrate except that additional extraction has removed more of the non-protein fraction, thereby increasing its protein content. It is prepared by alkaline solubilization of the soy protein and then precipitation of same in an acid bath. It may be powdered, extruded, or spun into fibrils and has a protein content of 90 to 95%. Products of spun fibrils may be referred to as "Textured Soy Protein Isolate," "Isolated Soy Protein Fibers," or "Spun Isolated Soy Protein." The PER of isolated soy protein is about 1.9 and indicates a poorer quality protein than that of soy flour or soy protein concentrate (PER 2.2). When hydrated textured (structured) protein isolate is added to meat food products, the ingredients statement should read "Hydrated Textured (Structured) Isolated Soy Protein."

Italian Sausage

Beef and pork Italian sausage is acceptable. Tomato products and other unexpected ingredients can be added if the product name indicates their presence. Red pepper permitted under 9 CFR 319.145(b)(1).

See: 9 CFR 319.145.

Italian Style

Acceptable term for products containing anise or fennel or Italian type cheese (e.g., Mozzarella, Parmesan, Provolone, Ricotta, Romano) or at least three of the following: basil, garlic, marjoram, olive oil, or oregano. Sausage products must meet the Italian Sausage requirements as per Regulation 9 CFR 319.145.

Italian Style Smoked Sausage

This is a smoked sausage (10% added water) and is not a 9 CFR 319.145 (Italian sausage) product. However, the product must contain Italian style ingredients found in the policy book under Italian style.

Jagwurst

The product is the same as yachtwurst (The Americanized name for the item). It is a cooked sausage made from a fine emulsion with cubes of lean meat rather than fat (as in mortadella).

Jamaican Style

Term may be used to identify meat and poultry food products made with allspice, garlic, onion, red pepper, and thyme. The name of the product must be further qualified with a statement, like "with Jamaican Style Seasoning," e.g., "Jamaican Style Chicken Wings-with Jamaican Style Seasonings." If the product formula contains textured soy product, then the ration rules apply.

Jamaican Style Patties

Product has at least 25% meat enclosed in a crust. The label must show true product name, e.g., "Beef Turnover." If the formula contains textured soy product, then the ratio rules apply.

Jambalaya

Product must contain at least 25% cooked ham and one other meat or seafood must be included. A New Orleans dish involving rice and ham and usually tomatoes (shrimp or other shellfish, other meat or poultry), together with seasonings. Must show true product name, e.g., "Ham and Shrimp Jambalaya."

Jamon

Spanish word for "ham." In the usage of Spanish-speaking people outside Spain, it has come to mean cured pork. "Jamon di Cocinar" is cured pork for cooking as opposed to slicing. When the term "Jamon" appears before the name of a limb, it means the product is cured. With the exception of products available for sale in Puerto Rico, all Spanish product names followed with the English translation.

Examples of acceptable product names are:

Jamon de Paleta — Cured Pork Shoulder
Jamon de Pierna — Cured Pork Leg

Jardiniere (FR)

Must contain at least 50% cooked meat based on total product. It means "in the manner of the gardener." The term applies to dishes made with diced garden vegetables which have been cooked with meat. Jardiniere should be followed by a true product name, e.g., "Beef with Vegetables."

Jerk or Jerk Style

The terms "Jerk" or "Jerk Style" can be used to describe red meat or poultry whole muscle, fabricated products, and other meat poultry food products, that are mixed or placed in a "spicy seasoning." The seasoning usually contains scallion, onion, thyme, allspice (pimento), hot peppers, and usually contains at least one or more of the

following: nutmeg, cinnamon, sugar, brown sugar, garlic, and rice or wine vinegar. The seasoning may be in the form of paste, marinade, sauce, or dry seasoning mixture. The product is mixed or placed in the spicy hot seasoning raw or the product may be grilled, cooked, or smoked. Examples of acceptable product names are, e.g., "Oven Roasted Jerk Chicken," "Jerk Pork Sausage" or "Jerk Style Smoked Beef Sausage."

Jerky

All Jerky products must have a MPR of 0.75:1 or less; "species" or "kind" must be in the name. Products may be cured or uncured, dried, and may be smoked or unsmoked, air or oven dried. A reference to the particular type of drying method is not a labeling requirement.

1. "Beef Jerky" — Produced from a single piece of beef. May also be classified as "Natural Style Beef Jerky" provided this product name is accompanied by the explanatory statement "made from solid pieces of beef" or comparable terminology. When a "Natural" claim (not natural style) is made, the policies as outlined in Policy Memo 055 are to be applied.
2. "Beef Jerky Chunked and Formed" — Produced from chunks which are molded and formed and cut into strips.
3. "Beef Jerky Ground and Formed or Chopped and Formed" — Produced as described, molded and formed and cut into strips.
4. Jerky products that contain over 3½% binders (2% ISP) must reflect the binder in the product name, i.e., "Beef Soy Protein Concentrate jerky, ground and formed. Jerky products that contain binders at levels below 3½% should express the binder in a qualifying statement, e.g., beef jerky — soy protein added.
5. "Species (or Kind) Jerky Sausage." The word "Jerky" can appear on labels for product in which the "species" or "kind" has been processed by chopping or grinding and stuffed into casings under the following conditions only:
   a. The word "Sausage" must appear immediately contiguous to "Jerky" whenever it is shown. "Sausage" must be in type at least one-third as high as "Jerky" in the same color ink and on the same background. The words "stick," "piece" etc. cannot be used as substitutes for "sausage" in the product name. "Sausage" means that the product has been chopped.
   b. The product may be dried at any stage of the process.

Junior Meat Snacks

Product must conform to the sausage standards going into the jar before processing. Limited to 3½% extenders.

Juniper Berries

Juniper berries and twigs are normally thrown on the fire from which dry cured hams are smoked. Juniper berries have been approved in the curing ingredients of Westphalian Ham.

Kabobs

Product consists of chunks of red meat or poultry and vegetables placed on a metal or wooden skewer. "Kabob" may be included in the descriptive name, e.g., "Beef, Mushrooms, and Onion Kabob." A kabob may be cooked or uncooked, but the label must clearly indicate this. This product may contain but does not require vegetables.

Kalbelwurst

Product is similar to Bockwurst with no limit on water or milk.

Katrifitas

A coined word used to describe a type of empanadillas. The product consists of dough containing yucca made to resemble a meat turnover and has a special meat filling. The product must contain at least 25% raw meat (beef) in total formulation. Label must include a true product name in conjunction with "Katrifitas," e.g., "Katrifitas, Beef Turnover made with Yucca Shell," or similar wording.

Kelco-Gel

A thickening agent used in sauces. It contains sodium alginate, calcium carbonate, and disodium phosphate. The amount of disodium phosphate in the finished product is approximately 0.099%. Its use should be judged on an individual basis.

Kidneys from Enzyme Injected Beef

Product may be exported to other countries. They must be labeled "Beef Kidneys, Tendered with Papain — For Export Only."

Kielbasa

A sausage that is cured, cooked, and usually smoked. Kolbassy is Czechoslovakian spelling; other variations include Kielbassy, Kolbasa and Kolbase. Kielbasa is made from coarsely ground pork or coarsely ground pork with added beef or mutton. "Hungarian Style Kolbase" is finely ground product, seasoned and stuffed into casings.

The 70/30 rule can be used, however, pork must always be the predominant meat ingredient. "Beef Kielbasa" is prepared with only beef as the meat ingredient. Byproducts are not permitted ingredients in these sausages.

An uncured (fresh), uncooked variety, with no more than 3% water exists. "Fresh" shall be used in the name when the product is uncured. When fresh Kielbasa is cooked or smoked, then cooked or smoked is required in the product name. The requirements of Policy Memo 110 apply when these perishable, cooked, uncured products are packaged in hermetically sealed containers.

Kippered Beef

A cured dry product similar to beef jerky but not as dry. MPR of 2.03:1 is applied to product.

Kiska; Kisba, Kishka, or Stuffed Derma

Ingredients statement is part of the product name. A meat food product prepared two ways:

1. Prepared with meat byproducts, including beef blood, pork snouts, pork livers, pork cheeks, etc. Packaged in fully labeled retail size packages or individually banded. When beef blood is used, it must be shown as part of product name.
2. Prepared with no more than 30% animal fat, mixed with farinaceous (consisting of or made of flour or meal) materials containing no other meat byproducts and ordinarily stuffed into beef casings and cooked. Product containing 30% or less fat is not considered amenable to the Federal Meat Inspection Act.

Knishes

Product must contain at least 15% cooked meat or poultry or 10% bacon (cooked basis). Same as snack standard. The type of meat or poultry should be identified in a true product name, e.g., "Chicken Knishes."

Konjac Flour

Food ingredient that provides the effects of thickening, gelling, texturizing, and water-binding, e.g., "binder," similar to that of starch vegetable flours, such as potato flour. Konjac flour can be used in meat and poultry products in which starch vegetable flours are permitted, e.g., 3.5% in cooked sausage products such as frankfurters and bologna.

Kosher and Kosher Style

Products prepared by federally inspected meat packing plants identified with labels bearing references to "Kosher" or Rabbinical markings must be handled under Rabbinical supervision. The Federal meat and poultry inspection program does not certify to kosher preparation of products, but rather accepts the statements and markings in this regard offered and applied under the supervision of the Rabbinical authority. The word "Kosher" may be used only on the labeling of meat and poultry products prepared under Rabbinical supervision. The identity of the Rabbinical authority must be made available upon request from agency official. Qualification of the word by such terms as "Style" or "Brand" does not negate the requirement.

Kosher (Product Containers)

Containers must be labeled "Kosher tags attached" when used for hearts, livers, and other products or tissues with attached metal tags indicating kosher inspection.

Krakow

Acceptable name for a cooked sausage similar to "Berliner."

Kreplach

Product must contain at least 20% meat. The type of kreplach should be identified in a true product name, e.g., "Beef Kreplach."

Kubbee

Other acceptable names are: Kubbe, Kibbe, Kabeda, Kilin, Kibbes, Kibby, Kabbo, or Kabe." A product popular in Syria and Lebanon. It must contain at least 25% meat based on total formulation; it must contain soaked cracked wheat and show the true product name, e.g., "Fried Cracked Wheat and Beef Balls," "Baked Stuffed Wheat and Beef Patty." Products may be shaped like a hamburger and fried or shaped into balls and fried.

Kuemmelwurst

An acceptable name. The product is the same as Carawaywurst and is a cooked sausage of the ring variety, with whole caraway seeds. Usual ingredients are beef, pork, salt, caraway, flavorings, and cure.

Kurma

Product must contain at least 50% meat or at least 35% poultry meat.

Labeling, Check-Off Blocks

The use of check-off blocks on immediate containers for identifying products that look alike but are different in composition is not permitted.

Examples of product that may look alike but are different in composition are as follows:

- Ground Beef and Beef Patty Mix
- Partially Defatted Chopped Beef and Partially Defatted Beef Fatty Tissue
- Frankfurters and Frankfurters with Variety Meats
- Finely Ground Chicken and Finely Ground Chicken Meat
- Comminuted Chicken and Comminuted Chicken With Kidney and Sex Glands Removed

However, exceptions to this policy may be granted. Exceptions would require that the establishment operators develop a procedure which the assigned inspector can readily monitor to ensure correct labeling. Such procedures, accompanied by written comments from the assigned inspector and where possible, the circuit supervisor, must be forwarded to the area supervisor for review and approval.

Approved procedures must be attached to the label records accompanying new or modified labels submitted for approval.

Labeling for Substitute Products

If a product fails to comply with a standard only because the meat or poultry content is lower than required and the product has generic identity as a nonmeat product (e.g., pizza, stew, pies), then the product may be designated by the nonmeat terminology in the standardized name (e.g., "Pizza," "Stew," "Pie"), provided the meat/poultry content of the product is conspicuously disclosed contiguous to the product name along with a statement of the amount of meat/poultry in the standardized product. (For example, Pizza contains 5% sausage; Sausage Pizza contains 12% sausage.) Such product may not be nutritionally inferior to the standardized product it resembles. For this purpose, nutritional inferiority is defined, consistent with the requirement of 21 CFR 101.3(e)(4), as any reduction in the content of an essential nutrient that is present at 2% or more of the U.S. RDA per serving of protein or any of the vitamins or minerals for which U.S. RDAs are established. A quality control procedure must be approved for such products by the Processed Products Inspection Division before the label can be used.

If a product is nutritionally inferior to the standardized product it resembles, it must be labeled "imitation" in accordance with 9 CFR 317.2(j) and 9 CFR 381.1(b).

Labeling of Modified Breakfast Sausage, Cooked Sausage, and Fermented Sausage Products Identified by a Nutrient Content Claim

Modified breakfast sausage, cooked sausage, and fermented sausage products are substitute versions of the standardized or traditional products that have been formulated and processed to reduce the fat contents to qualify for use of nutrient content claims, but do not comply with the standard of identity or composition as described in the meat and poultry regulations or the Standards and Labeling Policy Book (Policy Book) because of the use of ingredients used for fat replacement which are precluded or restricted by these standards. The deviation from the standard or the traditional, i.e., "regular product," is conveyed by associating an expressed nutrient content claim for the appropriate reduction in fat content and the standardized or traditional product name, e.g., "Reduced Fat Frankfurter" or "Low Fat Pepperoni." The nutrient content claims that may be used are those related to a reduction in fat contents that are identified in the regulations for meat products in 9 CFR Part 317 and for poultry products in 9 CFR Part 381.

1. Maintaining Product Integrity

The following guidelines must be applied to assure that the modified versions of the subject meat and poultry sausage products do not violate the integrity of the standardized or traditional product for which they purport to be substitutes: (1) the product must be similar in shape, flavor, consistency, and general appearance to the product as prepared according to the regulatory or traditional standard, (2) the meat or poultry used to formulate the modified product must come from the same anatomical location when the standardized term is related to an anatomical region on an animal, e.g., "ham" is expected to be from the hind leg of the hog and cured; thus, "lean smoked ham sausage" would be comprised of meat from the hind leg of a hog that has been smoked and cured, (3) the modified sausage product must result from the same processing procedures as those specified for the subject sausage products described by regulatory or Policy Book standards, (4) there must not be deviations from product safety criteria (e.g., salt content, curing agents, pH, water activity and/or moisture/protein ratio) that are provided in the regulatory or Policy Book standards for sausages, and (5) the modified product must achieve the appropriate reduction in fat content to be eligible to use a nutrient content claim in conjunction with the standardized or traditional product name.

2. Performance Characteristics

In producing modified, substitute versions of sausages, the deviations from ingredient provisions of the regulatory and Policy Book standards should be the minimum necessary to qualify for the nutrient content claim while maintaining the performance characteristics similar to the standardized or traditional product, i.e., similar preparation, cooking, and handling characteristics. If a modified version of the standardized or traditional sausage does not perform in substantially the same way as the standardized

or traditional item, the label must include a prominent statement informing the consumer of such differences. For example, a "low fat frankfurter" that essentially has all of the characteristics of a frankfurter, but cannot be grilled, would indicate "not recommended for grilling." A "reduced fat pepperoni" that displays essentially all the characteristics of pepperoni, but cannot be cooked, would, for example, indicate "not recommended for cooking" or "do not cook."

3. Safe and Suitable Ingredients

A modified, substitute sausage product must be formulated with approved safe and suitable ingredients, e.g., those identified in 9 CFR 318.7(c)(4) and 9 CFR 381.147(f)(4), and those determined to be safe and suitable by the Food Standards and Ingredients Branch, Product Assessment Division. Such ingredients are to be used at the lowest level necessary to achieve the intended effect of reducing fat as compared to the standardized or traditional product. Safe and suitable ingredients are those used to replace fat, improve texture, and prevent syneresis.

An ingredient or component of an ingredient that is specifically required by the regulatory or Policy Book standard for characterizing purposes, e.g., cheese in a cheesefurter, fresh livers in liver sausage, cured ham in a ham sausage, and fennel or anise in an Italian sausage, shall be present in the required amount, if applicable, or otherwise in a significant amount to provide a characterizing identity to the product. Moreover, an ingredient or component of an ingredient that is not permitted by regulations for use in any meat or poultry sausage product, e.g., sodium benzoate, shall not be added to a modified, substitute product.

4. Product Identity

The name of the modified version of the standardized or traditional product that complies with all parts of the policy prescribed herein is the appropriate expressed nutrient content claim for the meat and/or poultry product with a reduction in fat content and the applicable standardized or traditional term, e.g., "Lean Sausage," "97% Fat-Free (or "Low Fat") Kielbasa," "Low-Fat Frankfurter Made with Beef, Pork and Turkey," "Reduced Fat Pepperoni," "Extra Lean Turkey Italian Sausage," and "Lite Genoa Salami." The size and style of type must conform to the nutrition labeling regulations.

5. Ingredients Statement

To assist the consumer in differentiating between the standardized or traditional sausage product and the modified, substitute version, ingredients that are not provided for by regulatory or Policy Book standards, or used in excess of the allowable levels specified, must be appropriately identified with an asterisk in the ingredients statement. The statement(s) defining the asterisk(s), e.g., "*Ingredient(s) not in regular" (fill in name of the standardized or traditional product), or "*Ingredients(s) in excess of amount permitted in regular" (fill in name of the standardized or traditional product), or both as appropriate, must be legible and conspicuous, and shall immediately follow the ingredients statement in the same size and style of type.

Labeling of Meat and Poultry Stick Items

Stick items such as beef jerky, pepperoni sticks, and beef sticks must be labeled (i.e., contain the required label features as outlined in 9 CFR 317 and 9 CFR 381, Subpart N) according to the following guidelines:

1. If sold in *fully labeled* bulk containers, i.e., canisters, caddies, or similar containers, stick items do not have to be fully labeled unless they are individually wrapped. This type of container cannot be reused.
2. If sold in bulk containers, i.e., canisters, caddies, or similar containers that are not *fully labeled*, stick items must be fully labeled. Bulk containers such as these may only be refilled with fully labeled product.
3. If sold in small, fully labeled cartons, boxes, or similar containers (e.g., 3 oz., net weight) that are only intended for retail sale intact, stick items may be individually wrapped and unlabeled.

Labeling of Modified Substitute Versions of Fresh (Species) Sausage, Hamburger, or Ground Beef Products

This policy allows modified versions of fresh (species) sausages, ground beef, or hamburger to contain non-meat or poultry, "fat-replacing ingredients" (e.g., binders such as carrageenan, modified food starch) and to be identified by certain nutrient content claims in accordance with nutrition labeling regulations effective on August 8, 1994, in conjunction with descriptive labeling, e.g., "Lean Pork Sausage with a X% Solution of …," or "Low Fat Ground Beef, Water, and Carrageenan Product."

This policy allows for the use of terms defined in regulations, e.g., "Lean," "Reduced Fat," "Low Fat," etc., to be used to describe fresh (species) sausage, ground beef, or hamburger products with a reduction in fat content resulting from the use of added ingredients (i.e., "fat replacers" such as carrageenan and isolated soy protein). These products must meet the criteria for use of the nutrient content claim associated with the fat reduction. The nutrient content claim may be used in conjunction with the standardized name provided the consumer is informed of the actual components of the product through labeling,

i.e., descriptive product name, ingredients statement, and Nutrition Facts.

Meat products, including those that meet the criteria established for claims, such as "Lean," "Low Fat," "Lower Fat," "Reduced Fat," etc., that combine fresh (species) sausage, ground beef, or hamburger, and other safe and suitable ingredients, for the principal purpose of replacing fat, may be descriptively labeled. Examples of such products are "Lean Ground Beef, Water, and Carrageenan Product," "Low Fat Ground Beef With a X% Solution of …," "Lean Beef Sausage, Water, and Carrageenan Product," or "Reduced Fat Pork Sausage, Water, and Binders Product," provided conditions prescribed in the regulations, viz., 9 CFR 317, for use of the nutrient content claim are satisfied. In contrast, modified versions of fresh (species) sausage, ground beef or hamburger product containing added ingredients that do not qualify for use of a nutrient content claim prescribed in the nutrition labeling regulations must be labeled as Imitation Pork Sausage, Imitation Beef Sausage, Imitation Ground Beef, Imitation Hamburger, Beef Patty or Beef Patty Mix in accordance with 9 CFR Section 317.2(j)(1) and Sections 9 CFR 319.141 (Fresh pork sausage), 319.142 (Fresh beef sausage), and 319.15 (Miscellaneous beef products), respectively.

Descriptively labeled, modified, substitute versions of fresh (species) sausage, ground beef, or hamburger product with a reduction in fat content must comply with the following guidelines:

1. The descriptive name of a modified, substitute product with a reduction in fat content is the applicable nutrient content claim used in conjunction with the appropriate standardized name and fat-replacing ingredients, e.g., "Low Fat Ground Beef, Water and Carrageenan Product," or "Lean Pork Sausage With a X% Solution of Water, Modified Food Starch, Spices, and Salt." Words in the descriptive name may be of a different size, style, color, or type but, in all cases, the words must be prominent, conspicuous, and legible. Moreover, no word in the descriptive name should be printed in letters that are less than one-third the size of the largest letter used in any other word in the descriptive name. The solution statement, when used, is considered to be part of the descriptive product name and must comply with descriptive name sizing requirements.
2. Fat-replacing ingredients (e.g., binders and water) and fat in the finished product may not exceed 30% of the product as formulated for the modified, substitute ground beef, hamburger, or fresh beef sausage product, and no more than 40% of the product formulation for the substitute fresh pork sausage. The fat content must be in accordance with requirements for use of the applicable nutrient content claim.
3. The product includes mandatory nutrition labeling prescribed in the meat inspection regulations, viz., 9 CFR 317.
4. The product is formulated with approved safe and suitable ingredients, e.g., those identified in 9 CFR 318.7(c)(4), and which are determined to be safe and suitable by the Labeling and Consumer Protection Staff, that are used at the lowest level necessary to achieve the intended effect as a fat-replacing ingredient (i.e., binder).

Labeling of Product Names, Fanciful Names, Word Size

Words in product names or fanciful names may be a different size, style, color, or type, but in all cases, the words must be prominent, conspicuous, and legible. Moreover, no word in a product name, i.e., a common or usual name, a standardized name, or a descriptive name should be printed in letters that are less than one-third the size of the largest letter used in any other words of the product name. The same guidelines apply to letters of words in fanciful names that may accompany the product name.

For example, for a product labeled Chili Mac — Beans, Macaroni and Beef in Sauce, "Chili Mac" is the fanciful name and "Beans, Macaroni and Beef in Sauce" is the product name. No letter in "Chili Mac" may be smaller than one-third the size of the largest letter in "Chili Mac." Similarly, no letter in the descriptive name may be smaller than one-third the size of the largest letter in the descriptive name. This policy is not intended to address the relative size of words in fanciful names versus product names. The size of words in qualifying statements, e.g., "Water Added," "Contains up to …," "Smoke Flavoring Added," etc., are not affected by this policy memo.

Labeling of Products Containing Meat with Added Solutions or other Nonmeat Ingredients in Secondary Products

In those situations where meat containing an added solution or other nonmeat ingredients, e.g., Ham-Water Added, Corned Beef and Water Products, Beef — Containing up to 10% of a solution, are used in secondary products in sufficient quantities to meet the minimum meat requirement without including the added solution, or nonmeat ingredients, the product name need not include any reference to the added solution or nonmeat ingredients; e.g., Corned Beef and Cabbage would be an acceptable name for a product if the corned beef portion of the corned beef and water product was present in a sufficient quantity to satisfy the 25% cooked corned beef requirement. The

ingredients statement, however, must include nomenclature as required by the regulations or policy. In this example, the ingredients statement would list "Corned Beef and Water Product — X% of added ingredients are …"

For products in which the added solution ingredient as a whole is used to meet the minimum meat requirement, the product name must include nomenclature required for the component, e.g., Beef (containing up to 10% of a flavoring solution) Burgundy. The ingredients statement must also include the same nomenclature for the meat ingredient.

Labeling of Products which are Artificially Colored

Labels of products which are artificially colored either by artificial colors or natural colors must bear a statement to indicate the presence of the coloring, e.g., "artificially colored" or "colored with annatto." Products whose true color is disguised by packing media, e.g., colored pickling solutions, must also have labels that include a statement that indicates the presence of the color. The statement must appear in a prominent and conspicuous manner contiguous to the product name. When a component within a product is artificially colored, e.g., breading, sauce, and sausage, a qualifying statement is necessary. However, in all cases, the presence of the coloring must appear in the ingredients statement. Whenever FD&C Yellow No. 5 is used, it must be declared in the ingredients statement by FD&C Yellow No. 5 or Yellow 5. Some products, e.g., chorizos and some of the sausages of the longaniza variety, are expected to be characterized by coloring. In these situations, the presence of the coloring need only be indicated in the ingredients statement.

Labeling of Products which Include Packets of other Components

Wording indicating that the product contains, in addition to the meat or poultry product, another component, e.g., a gravy, sauce, or seasoning packet must appear in conjunction with the name of the product in such a manner that it is obvious to the purchaser that he or she is also purchasing that packet along with the meat and/or poultry product. The wording must be shown in print no smaller than one-third the size of the largest letter in the rest of the product name, of such color that will insure it not being overlooked at point of purchase, and positioned contiguous to the rest of the product name, so as not to appear in whole or part on any panel except the main display panel. The net weight individual components may be shown but are not required.

Labeling of Safe Thawing Instructions on Consumer Packages

Thawing instructions which appear on the label of a frozen meat or poultry product must be given in accordance with FSIS' recommendations for safe thawing procedures. These procedures are as follows:

1. Thawing product in the refrigerator.
2. Thawing product in cold water, changing water every 30 minutes until product is thawed.
3. Thawing product in a microwave oven for less than 2 hours. Cook immediately.

Upon request, alternative thawing procedures may be considered. However, scientific evidence which thoroughly establishes the safety of an alternative thawing procedure must be presented with the procedure when it is submitted for review.

Labeling Prominence Guidelines for Cured, Cooked Products with Added Substances that Do Not Return to Green Weight

The cured, cooked products covered by sections 9 CFR 319.100 ("corned beef"), 319.101 ("corned beef brisket"), 319.102 ("corned beef round and other corned beef cuts"), and 319.104(a) ("cured pork products" under PFF) of the Federal meat inspection regulations; and by Policy Memos 057A ("Labeling Turkey Ham Products Containing Added Water") and 084A ("Cooked Corned Beef Products and Cured Pork Products with Added Substances"), whose weights after cooking exceed the weight of the fresh uncured article, shall bear the product name and qualifying statements on the principal display panel using the following guidelines:

1. The product name and the qualifying statements must be prominent and conspicuous.
2. The label will bear the product name on the principal display panel in lettering not *less than* one-third the size of the largest letter in terms commonly associated with the product name, e.g., cooked, boneless, chopped, pressed, smoked, or words which could be a part of the product name, e.g., steak, butt portion, shank portion.
3. The product name will be judged prominent if the lettering is of the same style and color, and on the same color background as that which is used for the terms commonly associated with the product name or words which could be a part of the product name [see guidelines (2)]. If other styles, colors, and/or backgrounds are used, the prominence must be judged equal to those terms and words which could be associated with or part of the product name.
4. The product name must be distinct and separate from other label information. Thus, the product name should not be part of or embedded in

qualifying phrases or descriptions that include a list of added solution ingredients.

Examples of acceptable terminology are "Corned Beef and Water Product" and "Cured Pork and X% of a Solution."

5. The label for the products covered by this policy memo must also bear qualifying statements that conform to established policies on the size of the lettering in these statements in relation to product name (as outlined in Policy Memo 087A, FSIS Directive 7110.2, and Policy Memo 057A).

Labeling Requirements for Pump-Cured Bacon Products Treated with D- or D1-Alpha-Tocopherol in Surface Applications

Pump-cured bacon treated on the surface with d- or d1-alpha-tocopherol must be labeled with a product name qualifier which identifies the substances involved and the method of application. The qualifier must identify both the carrier and active substance in their order of predominance. The specific names, d- or d1-alpha-tocopherol, or the term, Vitamin E, may be used in the name qualifier. Examples of acceptable name qualifiers are "Sprayed with a solution of vegetable oil and Vitamin E" or "Dipped in a solution of corn oil and d-alpha-tocopherol." The name qualifier must be contiguous to the product name and printed in a style as prominent as the product name. The type used for the statement must be at least one-fourth the size of the most prominent letter in the product name, except that the ingredients of the mixture may be in print not less than one-eighth the size of the most prominent letter in the product name. The specific name of the ingredients, d-alpha-tocopherol or dl-alpha-tocopherol, and of the carrier must be listed as such in the ingredients statement or curing statement, as required by 9 CFR 317.2(f)(1).

Lamb Curry

Product must contain at least 50% fresh meat.

Landjaeger Cervelat

A semi-dry sausage that originated in Switzerland. It is about the size of a large frankfurter but pressed flat, smoked and dried giving it a black appearance.

Lard Continuous Process

This nomenclature identifies the commodity produced from clean and sound edible tissues of swine by a low-temperature separation process in which the oil is separated from the fatty tissue by means of a combination of heat and centrifugal force. Labeling records containing the above designation should identify in detail the process and equipment used in producing the commodity.

Lard — Cured Pork Tissue Use

Cured pork trimmings may be rendered to produce lard manufactured in compliance with the lard and leaf lard standard. Rendered bacon is not acceptable in lard.

Lard Refined

This term is applied to open-kettle rendered, prime steam, or dry-rendered lard put through a filter press, with or without bleaching agent.

Lasagna

Sauce is an expected ingredient of lasagna products and its declaration in the product name is optional.

Cheese Lasagna with meat: 12% meat
Lasagna with Meat and Sauce: 12% meat
Lasagna with Meat Sauce: 6% meat in total product
Lasagna with Poultry: 8% poultry meat
Lasagna with Tomato Sauce, Cheese, and Pepperoni: 8% pepperoni
Meat Lasagna: 12% meat
Poultry Lasagna: 8% poultry meat

Lau–Lau

Product must contain at least 25% meat. A Hawaiian dish made with pork and fish, wrapped in taro leaves. Label must have a true product name, e.g., "Pork and Fish Stuffed Taro Leaves."

Lebanon Bologna

A coarse ground, fermented, semi-dry sausage. If the product has a MPR of 3.1:1 or less and a pH of 5.0 or less, no refrigeration is required. It is made with beef. No extenders or hearts are permitted in the product. This is not a 9 CFR 319.180 product.

Legends

Products consisting of mixed meat and poultry ingredients shall bear either the official meat inspection legend or poultry legend, depending on which ingredients are present in the greater amounts. If meat or poultry ingredients exist in equal proportions, either official legend may be used. If meat and poultry ingredients exist in exact proportions and both appear in the product name, the official legend must reflect the ingredient appearing first in the product name.

Lentil Soup with Bacon — German Style

Acceptable name for a lentil soup containing only bacon. The bacon requirement is 4.0% for condensed and 2.0% for ready to eat.

Leona

An acceptable name. A coarse ground cooked sausage.

Lima Beans with Ham or Bacon in Sauce

Product must contain at least 12% ham or bacon.
See: 9 CFR 319.310.

Linguica

A Portuguese type sausage containing pork and excluding other meat and meat byproducts. Usually contains nonfat dry milk and condiments, e.g., vinegar, cinnamon, cumin seed, garlic, red pepper, salt, and sugar. Paprika and cures are acceptable in this product.

Links

This designation falls into four categories:

1. "Links" without further qualification refers to an all pork fresh sausage in links.
2. "Links Sausage" can be used to designate any sausage type formulation usually cured and smoked in links, except for those formulations containing poultry.
3. "Links cereal and nonfat dry milk added" usually formulated with meat and meat byproducts cured and smoked, and approved with the understanding each link is banded with an approved band label.
4. "Links, A pork and textured vegetable protein product" followed immediately by the ingredients statement is acceptable. "Links," "Top's Links," "Joe's Links" are coined names and must be followed immediately by true product name.

Little Smokies

A smoked small variety sausage link made with beef and pork.

Liver and Onions

Product must contain at least 45% liver.

Liver, Chopped

Product must contain at least 50% liver.

Liver, Onions, and Eggs

Product must contain at least 40% liver.

Liver Products

The product name does not have to include the species for multi-ingredient liver products, such as chopped liver, liver pate, and pureed liver. However, the species must be identified in the ingredients statement. For single ingredient liver products, such as sliced beef liver, the species must be identified in the product name. "Kind" of liver must always be identified.

Products with liver in the name (except for products listed) must contain a minimum of 30% liver.

Liver Spread (Streich Leberwurst)

The product name "Liver Spread (Stretch Leberwurst)" is acceptable. Product name must contain at least 30% liver in total formulation.

Liverwurst or "Pate De Foie — Style Liverwurst"

Product must meet liver sausage requirements. (See Regulation 9 CFR 319.182.)

Loaf

A "Loaf" (other than meat loaf) consists of meat in combination with any of a wide range of nonmeat ingredients. These products are not identified with the term "Meat Loaf," "Beef Loaf," or the like but with designations, e.g., "Olive Loaf," "Pickle and Pimiento Loaf," "Honey Loaf," "Luxury Loaf," and others that are descriptive.

Loaf, Canned, Perishable

Canned perishable products in the loaf category must:

1. Meet the perishable requirements. (See 9 CFR 317.2(k).)
2. Show a brine concentration of not less than 3.5% in finished product. Show a brine concentration of not less than 6.0% when the products contain cereal, starch, or other extenders.
3. Be cooked to a minimum internal temperature of at least than 150°F.
4. When extenders are added the product name must be qualified, e.g., "(Name of extender) added."

Lola and Lolita (IT)

Dry sausage products of Italian origin. Consists of mildly seasoned pork and contains garlic. Lolita comes in 14 oz. links, while Lola comes in 2 1/2 lb. links.

London Broil

Name can only be applied to a cooked product. Products including the expression "London Broil" on labels must be prepared with beef flank steak. Uncooked product must be labeled to indicate this, e.g., "Beef Flank Steak for London

Broil." If prepared from another cut, the identity of that cut must accompany the term "London Broil," e.g., "Sirloin Tip London Broil."

Long Island Style or Type

Not acceptable for poultry products.

Longaniza

Longaniza is a fresh sausage product. If it is prepared otherwise, the product name must indicate its nature, e.g., "Cured Longaniza." Paprika is an acceptable ingredient because it is expected.

Longaniza and Puerto Rican Style Longaniza

Longaniza is an acceptable name for Puerto Rican sausage made from pork which may contain beef but does not contain annatto. Added fat is not permitted.

Puerto Rican Style Longaniza is acceptable labeling for sausage made from pork which may contain beef and does contain annatto. Added fat is not permitted, although up to 3% lard may be used as a carrier for annatto. When annatto is used, it should be included in the ingredients statement as "annatto" in accordance with Section 9 CFR 317.2(j)(5) of the meat inspection regulations.

Loukanika

An acceptable name for cooked fresh Greek sausage. It is usually made with lamb and pork, oranges, allspice, whole pepper, and salt.

Lumpia or Loompya

A Philippine style or Filipino style egg roll. There are no special ingredient requirements, it refers to a shape of the egg roll. Lumpia or Loompya are generally longer and thinner than traditional egg rolls.

Luncheon Meat

1. "Luncheon Meat" cannot contain livers, kidneys, blood, detached skin, partially defatted pork or beef tissue, or stomachs.
2. On the label the meat components of "Luncheon Meat" are identified in the ingredients statement as "beef," "pork," "beef tongue meat," "pork tongue meat," "beef heart meat," and "pork heart meat."
3. In the ingredients statement "Beef" and "Pork" means lean meat with overlying fat and the portions of sinew, nerve, and the blood vessels which normally accompany muscle tissue and which are not separated in the process of dressing but not including bone and skin. Up to 10% of the meat portion of the formula can consist of cured and smoked meat trimmings which does not require special declaration in the ingredients statement except included under "pork" and "beef."
4. Heart or heart muscle, tongues, or tongue meat and cheek meat can be included in "Luncheon Meat" under the following restrictions:
   a. Hearts or heart meat or tongues or tongue meat must be declared individually by species in the ingredients statement on the label.
   b. No restriction on the percentage limits of hearts, heart meats, tongues, and tongue meats in the formulation.
   c. The terms "heart meat" and "tongue meat" refer to the muscle tissue remaining after heart caps, glands, nodes, connective tissue, etc. are trimmed away.
5. Water added to "Luncheon Meat" during manufacture cannot exceed 3% by weight of the total ingredients, this is controlled by weighing ingredients and not by analysis. Care must be used to see that water is not added indirectly through the use of undrained hearts and tongues.
6. The only ingredients permitted in "Luncheon Meat" are curing ingredients, sweetening agents, spices, and flavoring. All of these substances must be declared in the ingredients statement by name, except the various "flavorings" and "spices" which need not be named individually. "Spices" refer to natural spices and not to extracts.

Lyoner Wurst

A cooked, smoked, and finely ground sausage originating in Germany. It is usually made with beef, pork, (no chicken) flavoring, cure, and contains green peppercorns.

Lyons Sausage (FR)

A dry sausage made exclusively of pork (four parts finely chopped lean and one or two parts small diced fat) with spices and garlic which is stuffed into large casings, cured and air-dried.

Macaroni and Beef in Sauce

Product must contain at least 12% beef.

Macaroni and Cheese with Ham

Product must contain at least 12% cooked ham.

Macaroni Salad with (Meat or Poultry)

Product must contain at least 12% cooked meat or poultry meat.

Made with … Qualifiers

Need only mention the species or kind in the statement even when only a byproduct of the specific species or kind is used, e.g., pork, chicken and beef hearts in a sausage would carry a qualifier "made with pork, chicken and beef."

Made with 100% Real Cheese

This statement is acceptable on products as long as the cheese components are all 100% real cheese. It is not acceptable if a cheese food product or imitation cheese is included in the formula.

Malic Acid

Malic acid has been used extensively for many years as part of flavoring/seasoning mixtures which are added to components of meat or poultry products. It may be approved as a flavoring agent, and is acceptable as a component in a seasoning mix, e.g., in marinades and sauces, but may not be added alone to a product.

Manicotti (IT)

Product must contain at least 10% fresh meat. An Italian main dish consisting of rectangular-shaped pasta spread with a filling of meat (e.g., sausage, ground beef, or chopped prosciutto) and/or cheeses (e.g., ricotta and mozzarella). The pasta is rolled, edges pressed to seal, and covered with grated parmesan cheese and tomato sauce. A true product name must be shown, e.g., "Beef Manicotti in Sauce."

Margarine Substitutes

Meat food products that are substitutes for margarine because they contain less than 80% fat and/or oil need not be labeled "imitation" if the product has a fully descriptive name and the finished product contains 15,000 international units of Vitamin A per pound.

The descriptive name of the product may include the term "Spread" (or "Spred"), which has been widely adopted as a generic fanciful name for this class of product.

The following guidelines shall be used in selecting the appropriate descriptive product name:

1. "Animal Fat Spread (or Spred)" is an acceptable product name for a product prepared from animal fat as the sole source of fat.
2. "Animal Fat and Vegetable Oil Spread (or Spred)" is an acceptable product name for a product prepared with a combination of animal fat(s) and vegetable oil(s) in which the vegetable oil(s) content is greater than 20% of the total of the fat(s) and oil(s) used but less than 50% of the total.
3. "Animal Fat Spread (or Spred) — Vegetable Oil Added" is an acceptable product name for a product prepared with a combination of animal fat(s) and vegetable oil(s) in which the vegetable oil(s) content is 20% or less of the total of the fat(s) and oil(s) used but greater than 2% of the total.
4. The fanciful name "Spread" (or "Spred") accompanied by a list of all ingredients individually identified by their common or usual name in order of decreasing predominance is acceptable regardless of the nature and amount of fat(s) and/or oil(s) used.

In 1, 2, and 3 above, the descriptive product name may include the % of each fat and/or oil and may include the common or usual name of each fat and/or oil used.

Marengo

Product must contain at least 35% cooked meat or poultry meat. It has chicken or veal in a sauce containing tomatoes, mushrooms, onions, and wine, and label must show true product name, e.g., "Chicken Marengo."

Marinated

To be labeled "marinated," a product must use a marinade that is a mixture in which food is either soaked, massaged, tumbled, or injected in order to improve taste, tenderness, or other sensory attributes, e.g., color or juiciness. Time allotted in a marinade depends on many factors, e.g., thickness and size of the meat and strength of the marinade. Marinade should be that amount necessary to affect the finished product, and limited to 10% pickup in red meat, 8% pickup in boneless poultry and 3% in bone-in poultry.

Marine Oil

Herring oil and other marine species oils found by FDA to be satisfactory may be combined with animal and mixture of animal and vegetable oils processed as meat food products. Labels will bear statements identifying the presence of such substances, e.g., a shortening consisting of 50% herring oil and the remainder equal amounts of animal and vegetable oils would be "Shortening, Prepared with Herring Oil, Animal and Vegetable Oils."

Marking

Labeling may consist of a combination of printing, stenciling, box dyes, etc. for large true containers and for shipping containers. Crayons are unacceptable for applying required labeling features except for figures indicating content quantity. Approval of official marks appearing in newspaper advertisements, billboards, etc. is not necessary; however,

such marks may be reviewed locally before publication. Such markings should conform to the illustrations in the regulations and not be misleading.

"May Contain" Statements

The use of "may contain" or "and/or" labeling may be used in the ingredients statement's sublisting of sliced and/or diced products from various sources.

See: "Composite Ingredients Statement."

Meat and Dressing

Product must contain at least 50% cooked meat.

Meat and Dressing with Gravy

Product must contain at least 30% cooked meat.

Meat Base

A granular, paste-like product which is shelf-stable primarily because of its high salt content (30–40%).

1. Beef Base — 15% beef or 10.5% cooked beef
2. Pork Base — 15% pork or 10.5% cooked pork
3. Ham Base — 18% ham

Meat Broth or Meat Stock

MPR 135:1. Condensed 67:1.

Meat Byproducts

Byproducts must be individually declared by species and specific name in the ingredients statement, e.g., Pork Liver, Beef Tripe, and Beef fat.

Meat Casseroles

Product must contain at least 25% meat or 18% cooked meat.

Meat Curry

Product must contain at least 50% meat.

Meat Flavoring

Meat flavoring — when characteristic meat flavorings such as bacon are added in amounts less than 2% in addition to the required meat component of a product, such meat flavorings need not appear in the product label.

Meat Foldover Mit Dressing

Product must contain at least 50% meat (chopped and formed).

Meat Food Products Containing Poultry Ingredients — Labeling

Meat food products containing poultry ingredients in amounts that exceed 20% of the total livestock and poultry product portion of the meat food product must have product names that indicate the presence of the poultry ingredients, e.g., "Beef and Chicken Chili" or "Chili made with Beef and Chicken."

Meat food products containing poultry ingredients in amounts at 20% or less of the total livestock and poultry product portion of the meat food product must have product names that are qualified to indicate the presence of the poultry ingredients, e.g., "Beef Stew — Turkey Added."

However, meat food products that do not meet specified minimum livestock ingredient requirements because poultry ingredients are replacing any part of the required livestock ingredients must have product names that indicate the presence of the poultry ingredients, e.g., "Beef and Turkey Stew" or "Stew Made with Beef and Turkey."

This policy does not apply to: (1) red meat products that are expected to contain poultry ingredients, e.g., "Brunswick Stew and Potted Meat Food Product" (Section 9 CFR 319.761); (2) cooked sausages identified in Section 9 CFR 319.180 of the meat regulations (Policy Memo 005A); or (3) nonspecific loaves, rolls, logs, etc., e.g., Pickle and Pimento Loaf.

Meat Loaf

Uncooked or cooked pork, beef, veal or lamb, and other ingredients in loaf form, but not canned.

1. Ingredients, e.g., cracker meal, oatmeal, bread crumbs, nonfat dry milk, soy ingredients (untextured), milk, and whole eggs are not required in the product name.
2. Product may contain:
   a. Head meat, cheek meat, heart meat, and tongue meat under label declaration in the ingredients statement only.
   b. Not more than 12% extenders and binders.
   c. Partially defatted chopped beef or pork up to 25% and declared as meat in the ingredients statement.
3. Product must contain at least 65% meat.
4. Onion, tomato juice, water, and other liquid extenders are not directly controlled.

Meat Loaf, Canned (Perishable)

Canned perishable products in the loaf category must:

1. Meet the perishable labeling requirements. See: 9 CFR 317.2(k),

2. Be cured with at least 1 ounce nitrate per 100 pounds of product and 1/2% dextrose or 1% sugar.
3. Have a brine concentration of at least 3.5% in the finished product. Products that contain cereal, starch, or other extenders must have a brine concentration of at least 6.1%.

Meat Loaf, Canned (Sterile Packed)

No head, cheek, heart, or tongue meat permitted. Other requirements are the same as uncanned cured meat loaf. Binders and extenders must be shown in the product name, e.g., "Meat Loaf, cereal added."

Meat Pasty or Pasties

Product must contain at least 25% meat. The label must show the true product name, e.g., "Beef Pasty."

Meat Pie Filling

Product must contain at least 37% meat.

Meat Pies (or Vegetable Meat Pies)

Product must contain 25% meat; meat in gravy may be counted towards meat content.

Meat/Poultry Extended Products

These should always be listed in the ingredients statement of the secondary product by their correct name, e.g., "Beef, water and binder product," unless it is included in the name of the product, e.g., "Chili made with beef and binder product."

Meat Ravioli

Product must contain at least 10% meat in ravioli.

Meat Ravioli in Meat Sauce

Product must contain at least 10% meat in ravioli and at least 50% ravioli in total product, and at least 6% meat in sauce.

Meat Ravioli in Sauce

Product must contain at least 10% meat in the ravioli and at least 50% ravioli in the total product.

Meat Sauce

Product must contain at least 6% ground meat.

Meat Spreads

Product must contain at least 50% meat or 35% cooked meat. When another major component is considered a significant source of protein such as cheese is added the requirement is reduced to 25% cooked meat. Product must show a true product name, e.g., "Sausage and Cheese Spread."

Meat Stick and Cheese Combination Products

The following criteria are used for dry meat stick and cheese combination products that need not bear a "keep refrigerated" handling statement.

1. The dry meat stick portion must have a water activity of less than 0.90, the cheese portion must have a water activity of less than 0.94, and the equilibrium of the water activity of the two components must be no greater than 0.92;
2. the dry meat portion, if fermented, must be fermented by an active fermentation culture (typically to a pH 5.0 or below) and;
3. for products where the meat portion and the cheese portion are packaged together, there must be a heat seal between the dry meat stick and cheese components which separates the meat stick from the cheese stick by at least 4 mm.
4. Dry meat stick and cheese combination products not meeting these criteria must be labeled with a "keep refrigerated" statement in lieu of compelling data that establish safety.

Products not meeting the criteria stated above can be labeled without a "keep refrigerated" statement if a control program ensuring safety and shelf stability is established by the establishment.

Meatballs

Uncooked or cooked pork, beef, veal, and lamb, and other ingredients in a ball form.

1. Product must contain at least 65% meat.
2. Binders and extenders are limited to 12% of the total product. 6.8% of isolated soy protein is considered the equivalent to 12% of the other binders or extenders. The permitted binders and extenders include, but are not limited to, cereal, bread crumbs, cracker meal, soy flour, soy protein concentrate, isolated soy protein, and textured vegetable protein.
3. Cheeks, hearts, and tongues are not allowed, but product may contain head meat, cheek meat, heart meat, and tongue meat when declared in the ingredients statement.

4. Partially defatted chopped (PDC) (species) may be used up to 25% of the meat block. PDC (species) can be identified as (species) in the ingredients statements. [See entry for Partially Defatted Chopped (species).]

Meatballs in Sauce

Requires a 50% minimum of meatballs, by weight in finished product.

Meatball Stew

Meatball stew contains at least 25% meatballs and usually contains vegetables such as potatoes, peas, carrots, etc., and gravy or thick broth resulting from cooking all ingredients together. The meatballs must meet the Meatball Standard.

Meatballs, Swedish Style

Product must contain at least 65% fresh meat. "Swedish Meatballs" or "Swedish Style Meatballs" are small in size and usually contain two or three different varieties of meat, nutmeg and/or allspice, potatoes, and milk. "Swedish Brand Meatballs Made in USA" means any meatball.

Meatballs, Turkey

Product must contain at least 65% raw turkey meat. Skin is permitted in natural proportions of meat used, if skin is in excess of natural proportions, it shall be reflected in the product name.

Mediterranean Style

Acceptable identification for product containing onion or garlic, olive oil and four of any of the following groups:

1. Vegetable or fruit: dried apricot, artichoke, dried date, dried fig, eggplant, tomato, pepper (green or red), squash, lemon or lemon juice, raisin and olives.
2. Legume or nut: fava bean, chick pea, white cannelloni bean, green bean, lentil, almond, pine nut, pistachio.
3. Seasoning: dill, coriander, cinnamon, cumin, fennel, basil, oregano, thyme, saffron, rosemary, parsley, mint, sumac, turmeric.
4. A regional dish as component, e.g., pita bread, yogurt, Italian or Greek type cheese, pasta, couscous or bulgur.

Merguez, Mergues or Merghez Sausage

A hot and spicy fresh sausage originating in North Africa and common in France which contains hot pepper and/or paprika. The meat component must contain beef and may contain lamb or mutton when labeled as "Merguez Sausage." When pork is used as part of meat component, the product is labeled as "Merguez Sausage with Pork." When pork is the only meat ingredient, the product is labeled "Pork Merguez Sausage."

Methyl Cellulose

May be used as an ingredient in formulas for meat and vegetable patties and various poultry products (mainly patties) at a level of 0.15% of the total weight of the product, which includes batter and breading of these products.

The internal technical effect is to extend and to stabilize products as well as to act as a carrier.

See: 421.24(4) and 9 CFR 381.147(f)(4).

Mettwurst

An uncooked cured smoked sausage in which byproducts and extenders are not permitted. Beef heart meat is acceptable. Water is limited to 3% and the fat content shall not exceed 50%.

Mettwurst, Cooked

Mettwurst which is cooked must be labeled "Cooked Mettwurst," and may contain up to 10% water based on the finished product.

Metz Sausage

Cured lean beef and pork and bacon are finely chopped, seasoned, and stuffed into beef middles. It is air-dried for 5 days, then given a cool smoke. It is classed as a semi-dry sausage.

Mexican Style

Acceptable for products that contain at least four of the following: jalapeno peppers, chili peppers, green chilies, cumin, cayenne peppers, red or green peppers, chili powder, jalapeno powder, Monterey Jack cheese, or cheddar cheese. This policy applies to a single food and does not supersede Policy Memo 068.

Mexican Style Dinners

Products like tamales, enchiladas, and tacos must make up 25% of the dinner or entree to qualify as "Mexican Style." The individual product standard must also be met.

Mexican Style Sauces

A garnish (decoration) of cheese in or on the sauce of Mexican style foods does not require the presence of the cheese to be declared in the product name or qualifying statement.

Milan or Milano Salami

A dry sausage with a maximum MPR of 1.9:1. It is an Italian-type salami, except the meat is finely cut. It is made with beef, pork fat, spiced with garlic, and has a distinctive cording.

Mince Meat

Product must contain at least 12% fresh meat or 9% cooked meat. Heart meat may be substituted. In addition to "Mince Meat," the product name should include kinds of meat, e.g., "Mince Meat with Beef" or "Mince Meat with (species) Heart Meat." When 2% or more cooked meat but less than 9% cooked meat is present in the formula, the product is amenable and the name must state that the product is "Mince Meat Flavored With ____."

A product marketed as "Mince Meat" which contains less than 2% cooked meat or contains only beef suet as the ingredient of animal origin, is not considered as a meat food product and is not amenable.

Mixtures

Mixtures of nonfat dry milk (NFDM), calcium reduced dry skim milk (CRDSM), or dried whey, reduced lactose whey, reduced minerals whey, and whey protein concentrate with other substances are not allowed, except in batter and gravy mixes and breaders. Mixtures of cereal, soy preparations and/or sodium caseinate with other substances are permitted to come into the plant for use in batter and gravy mixes, but they must be labeled to show their intended use, e.g., "Patty Mix" or "Gravy Mix." The labels of the mixtures must show the ingredients in order of their predominance.

Mock Drumsticks

An imitation product; nonspecific.

Mock Turtle Soup

Product must contain at least 10% beef and may be made with beef and beef byproducts.

Mofongo

Pork skins and plantain type product with at least 20% pork skins in the total formulation. It must show true product name, e.g., "Pork Skin Filling Wrapped in Plantain."

Moisture Protein Ratio (MPR)

Frizzes 1.6:1 Ukrainian Sausage 2.0:1 Jerky 0.75:1 Kippered Beef 2.03:1 Pepperoni 1.6:1 Dry Salami 1.9:1 Dry Sausage 1.9:1 Genoa Salami 2.3:1 Tropic Cure Pork 3.25:1 Sicilian Salami 2.3:1 Thuringer 3.7:1 Italian Salami 1.9:1 Dried Meat 2.04:1 Roast Beef, Canned 2.25:1 Chipped Beef 2.04:1 Farmer Summer Sausage 1.9:1.

Moisture Protein Ratio (MPR) — PH

Nonrefrigerated or shelf-stable sausages must have an MPR of 3.1:1 or less and a pH of 5.0 or less, unless commercially sterilized. This does not apply to products containing more than 3.5% binders or 2% isolated soy protein.

Mondongo

A mixture of one or more of the following: (a) beef tripe, (b) cattle feet with or without hide on, (c) chitterlings, and (d) beef intestines.

See: Beef Tripe Stew.

Morcella Blood Pudding

Nonspecific. The product is made from pork fat, beef blood and/or pork blood, and may contain meat.

Mortadella

Normally a cooked sausage but can be dry or semi-dry. It is similar to salami and cervelat except that it has large chunks of pork fat. Red sweet peppers up to 4% and pistachio nuts up to 1% are acceptable as long as they are shown in the true product name.

Mortadella (Canned)

Canned items designated "Mortadella" must be labeled with the phrase "Perishable, Keep Under Refrigeration" and must have an MPR of 3.85:1 or less.

Mortadella — Poultry

Poultry Mortadella is a dry, semi-dry, or cooked sausage formulated with poultry. The sausage must contain large chunks of pork fat and may contain extenders and/or binders. Red sweet peppers are permitted up to 4% and pistachio nuts up to 1% and shown as added in the true product name.

If product is canned, the MPR must not exceed 3.85:1, the internal temperature must have reached 160°F and the product labeled "Perishable, Keep Under Refrigeration" or similar wording.

Mortadella without Fat Cubes or Chunks

Product must meet the standard for Mortadella and the label be qualified to indicate the absence of Fat Cubes or Chunks, e.g., "Mortadella without Fat Cubes" or "Mortadella without Fat Chunks."

Mousaka, Moussaka, Musaka (GK)

Must contain at least 25% meat. Mousaka is a casserole containing layers of meat and eggplant made in various

ways throughout the Middle East. A true product name is required, e.g., "Eggplant and Meat Casserole."

Mullicatawny Soup

Product must contain at least 2% cooked poultry meat and enough curry powder and pepper to characterize the product. The label must show a true product name, e.g., "Chicken Mullicatawny Soup."

Mulligan Stew

Product must contain at least 25% fresh meat or meat and poultry. Mulligan stew is a mixture of vegetables and meat combined in a gravy or sauce. The label must have a true product name, e.g., "Chicken and Meat Mulligan Stew."

Mustard Bran

This is not considered a spice and must be declared as "Mustard Bran." It is not acceptable in sausage.

Mustard Flour

It is a spice that is commonly used in sausage products.

Myvacet

(Distilled Acetylated Monoglycerides). Acceptable for use as a coating on sausage casings. Sausages coated with Myvacet shall show, adjacent to the product name, a qualifying statement disclosing the presence of the compound, e.g., "Summer Sausage Coated with a Solution of Distilled Acetylated Monoglycerides."

Nacho Style, Nacho Flavor, and Similar Terms

Acceptable terminology for products possessing the commonly expected flavor characteristics associated with "Nachos," a Mexican hors d'oeurve. The characterizing flavor components generally include, but are not limited to, cheese (Cheddar or Monterey Jack), tomato (tomato solids, tomato powder), spices, or other natural seasonings and flavorings (usually garlic and onion), and chili peppers (mild or hot). Romano and Parmesan cheese are also often present. However, these cheeses may not be used to satisfy the above cheese requirement.

Natural Claims

The term "natural" may be used on labeling for meat products and poultry products, provided the applicant for such labeling demonstrates that:

1. The product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and
2. The product and its ingredients are not more than minimally processed. Minimal processing may include:
   (a) those traditional processes used to make food edible or to preserve it or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or
   (b) those physical processes which do not fundamentally alter the raw product and/or which only separate a whole, intact food into component parts, e.g., grinding meat, separating eggs into albumen and yolk, and pressing fruits to produce juices.

Relatively severe processes, e.g., solvent extraction, acid hydrolysis, and chemical bleaching would clearly be considered more than minimal processing. Thus, the use of a natural flavor or flavoring in compliance with 21 CFR 101.22 which has undergone more than minimal processing would place a product in which it is used outside the scope of these guidelines. However, the presence of an ingredient which has been more than minimally processed would not necessarily preclude the product from being promoted as natural. Exceptions of this type may be granted on a case-by-case basis if it can be demonstrated that the use of such an ingredient would not significantly change the character of the product to the point that it could no longer be considered a natural product. In such cases, the natural claim must be qualified to clearly and conspicuously identify the ingredient, e.g., contains refined sugar.

All products claiming to be natural or a natural food should be accompanied by a brief statement which explains what is meant by the term natural, i.e., that the product is a natural food because it contains no artificial ingredients and is only minimally processed. This statement should appear directly beneath or beside all natural claims or, if elsewhere on the principal display panel, an asterisk should be used to tie the explanation to the claim.

The decision to approve or deny the use of a natural claim may be affected by the specific context in which the claim is made. For example, claims indicating that a product is natural food, e.g., "Natural chili" or "chili — a natural product" would be unacceptable for a product containing beet powder which artificially colors the finished product. However, "all natural ingredients" might be an acceptable claim for such a product.

Natural Smoked Color

Approval can be properly granted to labels with this statement when the products involved are "Smoked" and not artificially colored. The results of the use of artificial smoke materials can, by means of a number of processing operations, result in a color characteristic being acquired by the

frankfurters, bologna, and the like. The term "Natural Smoked Color" can be used to properly identify this point.

Navarin

Navarin is a stew containing lamb or mutton and vegetables and considered a national dish of France. It must meet the meat stew standard of 25% meat. Show true product name, e.g., "Navarin-Lamb Stew."

Negative Labeling

1. Negative labeling is allowed if it is unclear from the product name that the ingredient is not present. For example, the use of the term "no beef" on the label of "turkey pastrami" would further clarify that the product does not contain beef.
2. Negative labeling is allowed if the statement is beneficial for health, religious preference, or other similar reasons. For example, highlighting the absence of salt in a product would be helpful to those persons on sodium-restricted diets.
3. Negative labeling is allowed if the claims are directly linked to the product packaging, as opposed to the product itself. For example, flexible retortable pouches could bear the statement "no preservatives, refrigeration or freezing needed with this new packaging method."
4. Negative labeling is allowed if such claims call attention to the absence of ingredients because they are prohibited in a product by regulation or policy. The statement must clearly and prominently indicate this fact, so as not to mislead or create false impressions. For example, "USDA regulations prohibit the use of preservatives in this product" would be an acceptable statement for ground beef.
5. Negative labeling is allowed to indicate that absence of an ingredient when that ingredient is expected or permitted by regulation or policy. This could also apply to ingredients which are not expected or permitted by regulation or policy if the ingredients could find their way into the product through a component. For example, the use of "no preservatives" on the label of "spaghetti with meat and sauce" (where regulations do not permit the direct addition of preservatives) would be acceptable if the product contained an ingredient, such as cooking oil, which could contain antioxidants but do not.

These guidelines do not preempt the requirements of the nutrition labeling regulation. Therefore, negative claims such as "unsalted" would have to comply with the provisions stated in the nutrition labeling regulations.

Net Quantity of Contents on Combination Packages

The guidelines for stating the net quantity of contents on combination packages containing both liquid and solid products are as follows:

1. The declaration of net quantity of contents for a combination package shall be expressed in terms of fluid measure for individual products that are liquid and in terms of avoirdupois weight for individual products that are solid, semisolid, or viscous, provided the quantity statements for identical packages or units are combined. For example, the fruit drink would be expressed in fluid measure and the meat, cheese, crackers, and cookies would be expressed in the combined avoirdupois weight.
2. The declaration of quantity shall be preceded by one of the following terms, as appropriate: "Net Weight," "Net Wt.," or "Net Contents."

   The net quantity of contents declaration may appear in more than one line. Therefore, both stacked and side-by-side declarations would be considered appropriate.
   - Descriptive terms may be used to identify the liquid and solid components of the package, e.g., entree, meal, or drink; however, such terms shall not include brand names.
   - Connecting words such as "and" or "plus" are permitted to be used as part of the declaration of contents.

   Examples of acceptable net content declarations are as follows:
   - Entree Net Wt. 8 oz., Drink 4 fl. oz. (120 ml)
   - Net Contents: lunch 8 oz. plus fruit drink 4 fl. oz.
   - Net Wt. 8 oz. Drink 4 fl. oz. (120 ml)
   - Net Weight 8 oz. and 4 fl. oz.

Federally inspected meat and poultry products are exempt from the requirements of the Fair Packaging and Labeling Act (FPLA), including the mandatory metric labeling provisions that went into effect February 14, 1994. However, if metric labeling is included voluntarily, such labeling should comply with the FPLA.

The guidelines contained in this policy memo will be subject to the provisions prescribed in 9 CFR 317.2(h) and 9 CFR 381.121 of the Federal regulations.

Net Weight Statement

1. Divider Pak

On a product where two cans are taped together, one of which contains the meat or poultry item and the other a

vegetable, e.g., "Chicken Chow Mein," the meat or poultry label may include the net weight on the 20% panel. The vegetable can bears the true name of the product with the total net weight of the other can and the drained weight of the vegetable can.

2. Double Packing

When a poultry product and a non-poultry product are separately wrapped and placed in a single immediate container bearing the name of both products, the net weight shown on the immediate container may be the total net weight of the two products or the net weight of the poultry product and the non-poultry product separately.

3. Additional Net Weight Information

Nonregulatory information of a net weight nature, e.g., 4–3 oz. packages, accompanying a net weight statement is acceptable and need not adhere to the size and spacing restrictions.

4. Open Net Weights

Open net weights may be presented in pounds and ounces, decimals, decimal fractions, or fractions, e.g., 1½ lbs., 1.6 lbs.

5. Net Weight Requirements

The statement of net quantity of contents is required on all products intended for sale at retail intact. In addition, shipping containers must bear a net quantity of contents statement if product inside is not uniform in weight (i.e., random weight). Piece counts may not be used in lieu of a required net quantity of contents statement on a shipping container but may be used as additional information.

6. Multi-Unit Retail Packages

Fully labeled packages of more than one of the same meat or poultry product packages in an open (i.e., clear) overwrap do not have to include a net weight statement.

See: 9 CFR 317.2(h), and 9 CFR 381.121(b).

Net Weight Statements on Packages with Header Labels

The guidelines for determining the size and location of net weight statements on meat food product packages with header labels are as follows:

1. The entire front of the package is considered the principal display panel of the package and its area is used to determine the size of the net weight statement. Print size specifications for the net weight statement specified by the regulations must be followed.
2. The net weight statement should be placed within the lower 30% area of the header label if no other mandatory labeling features are printed on the rest of the principal display panel of the package. If mandatory features do appear below the header label, the net weight statement must be placed within the lower 30% of the total area containing any mandatory information.

A "Header Label" is a small label applied across the top of a package usually bearing all of the mandatory labeling information. The rest of the package is most often a clear film containing a meat or poultry product, e.g., luncheon meat. This type of packaging is designed to be used on pegboard type displays.

"New" and Similar Terms

Terms like "new," "now," "improved," and similar terms may be used within the following guidelines:

1. The terms may only be used for a period of 6 months from the date of the initial approval, except as noted in 2, 3, and 4 below.
2. Extensions to the 6-month period may be granted if:
   a. Processors can demonstrate that production or distribution delays precluded the use of the approved labeling as scheduled. In such situations, the lost time can be restored.
   b. Processors can demonstrate that labeling inventory needs for the 6-month period were over estimated due to poor sales. The processors must maintain records which indicate the amount and the date the labeling was originally purchased. In this situation, up to an additional 6 months can be granted. No further extension will be considered.
3. In those situations where it is customary to distribute "new" products to various geographical regions, each geographic area may receive a sketch approval for 6 months if the processor can assure adequate controls over the segregation and distribution of the products.
4. In situations where it is customary to test market product in no more than approximately 15% of the intended total marketing area before total distribution begins, labeling for the test market area can receive a sketch approval and also be included in the 6-month sketch approval given to the labeling of the product distributed to the total marketing area. Processors must be able to assure that only 15% of the total market is involved in test marketing.

New England Boiled Dinner

Product must contain at least 25% cooked "Corned Beef."

New Orleans Style

Acceptable for products that contain any five of the following ingredients:

Roux base, rice, onion, green onions garlic, celery, bell peppers, cayenne pepper, white pepper, parsley, or tomato.

The product may contain various protein sources including seafood and game.

Nitrite

Calculations should be based on the total meat block including the muscle tissue, fat and blood (e.g., "Blood Pudding"). If the product is cured, the blood would be included and considered part of the meat.

Non-Amenable Product/Voluntary Inspection

[Examples of non-amenable products are sandwiches containing meat or poultry, clam chowder which has less than 1% bacon for export to Japan, and natural casings for export]. Any non-amenable product can be produced under voluntary inspection when requested [9 CFR 318.13 and Subchapter B, Part 350.3(c)]. However, most FSIS requirements have to be met concerning labeling, i.e., mandatory labeling features, an accurate ingredients statement, handling statement, etc. Safe Handling Instructions are not required even for raw non-amenable products. FDA nutrition labeling rules apply to such products.

Non-Dairy White Sauce or Non-Dairy Sauce

A sauce made with a non-dairy creamer. If this type of a sauce is proposed for use with "Chipped Beef," a suitable name would be "Non-Dairy White Sauce with Chipped Beef" or "Non-Dairy Sauce with Chipped Beef." The reference to "Cream" or any of its derivations should not appear in the product name.

Nonspecific Meat Food Products

Red meat items of this type do not have specific requirements, i.e., they do not possess a standard of identity or composition. Consequently, these products shall be identified by one of two ways: (1) A descriptive name that identifies characterizing components and/or ingredients, or (2) a fanciful or coined name that is accompanied by an ingredients statement. The latter approach should be used when the use of a descriptive name is not practical, e.g., when the descriptive name would read like an ingredients statement.

When a fanciful name or coined name is used, the ingredients statement should appear contiguous to the product name on the principal display panel of an immediate container.

Nonstandardized Cooked Sausage Products Containing Both Livestock and Poultry Ingredients

The labeling of nonstandardized cooked sausage products must comply with 9 CFR 319.180.

Meat food products are those in which more than 50% of the livestock and poultry product portion consists of livestock ingredients. Such cooked sausage products which contain poultry ingredients at more than 15% of the total ingredients (excluding water) must have product names that indicate the species of livestock and kind(s) of poultry ingredients, e.g., "Beef and Turkey Frankfurter" or "Frankfurter Made From Beef and Turkey."

Poultry food products are those in which more than 50% of the livestock and poultry products portion consists of poultry. Livestock ingredients at more than 20% of the total poultry and livestock ingredients must have product names that indicate the kind(s) of poultry and species of livestock ingredients, e.g., "Turkey and Beef Frankfurter" or "Frankfurter Made from Turkey and Beef." Such cooked sausage products which contain livestock ingredients at 20% or less of the total poultry and livestock ingredients must have product names that are appropriately qualified to indicate the inclusion of livestock ingredients, e.g., "Turkey Frankfurter — Pork Added or Turkey Frankfurter" — With Pork." (The product names of cooked sausage products which contain no livestock ingredients designate the kind(s) of poultry ingredients, e.g., "Turkey Frankfurter.") Cooked sausage products containing over 50% meat ingredients would carry the red meat legend while those containing over 50% poultry ingredients would carry the poultry legend.

Noodle Chicken Vegetable Dinner or Noodle Chicken Dinner with Vegetables

(Canned or in glass jars). Product must contain at least 6% cooked chicken.

Nugget Labeling

Nuggets are irregularly shaped, usually bite-sized meat and/or poultry products which are usually breaded and deep fat fried and intended to be used as finger foods. There are a number of different types of nuggets, the labeling for which is:

1. Products made from a solid piece of meat or poultry may use the term "Nugget" as part of the product name without further qualification (e.g., "Chicken Nugget," "Beef Nugget").
2. Products made from chopped and formed meat or poultry may use the term "Nugget" as part of the product name, provided a qualifying statement describing such process is shown

contiguous to the product name (e.g., "Chicken Nugget, Chopped and Formed" or "Beef Nugget, Chopped and Formed").

3. Products made from chopped meat or poultry and containing binders, extenders and/or water may use the term "Nugget" as a fanciful name, provided a descriptive name immediately follows "Species" or "Kind" nugget (e.g., "Breaded Nugget-Shaped Chicken Patties").
4. Products described in (1), (2), and (3) above which are breaded shall be labeled as "breaded" and shall be limited to 30% breading.

Oat Fiber

"Oat fiber," should be identified in the ingredients statement as "isolated oat product." It may be used in non-standardized products and in products, such as, "taco fillings."

Oleomargarine

The Establishment Number may be omitted from the outer container, provided that articles are completely labeled including Establishment Number inside.

See: 9 CFR 317.2(i).

Omelet, Denver or Western Style

Product must contain at least 18% ham with onions and green and/or red peppers.

Omelet, Florentine

Product must contain at least 9% cooked meat and must contain spinach.

Omelets with

Bacon — must contain at least 9% cooked bacon
Chicken Livers — must contain at least 12% cooked liver
Corned Beef Hash — must contain at least 25% corned beef hash
Creamed Beef — must contain at least 25% creamed beef
Ham — must contain at least 18% cooked ham
Sausage — must contain at least 12% dry sausage
Sausage and Cheese (omelet with pepperoni, cheese and sauce) — must contain at least 9% sausage in the total product.

Open Dating

Labels showing further qualifying phrases in addition to the explanatory phrase must submit with the application sufficient documentation to support these additional claims. See [9 CFR 317.8(b)(32) and 9 CFR 381.129(c)]. Some local authorities require that packaged foods heated and sold hot from industrial catering vehicles be dated with the day the foods were placed in the warming units (e.g., Tuesday, Friday, etc.). When assured by the local authorities that the foods are under a rigid local inspection program, the designations may be approved without an explanatory statement as required by the regulations. To date, only the county of Los Angeles, California, has provided this assurance.

The packing date should be shown on immediate or shipping containers of poultry food products as required by regulations [9 CFR 381.126 and 381.129(c)]. When meat or poultry products are packed and held in freezer storage for later repacking, the explanatory phrase on repacked product should be in terms of "sell by" or "use before." However, if a "packed on" phrase is desired, the date shown shall be that of the original packing of the product.

Ostrich and other Ratites (Emu)

Products that do not contain 3% of beef, pork, chicken or turkey, can not contain cure ingredients, i.e., Nitrite, nitrate.

Paella Con Bacalao (SP)

Product must contain at least 35% cooked meat or poultry meat and include seafood and no more than 25% cooked rice. The label must show true product name, e.g., "Beef and Fish with Rice."

Papain

Meat and poultry products that are dipped in a solution containing papain shall show in conjunction with the product name a statement, e.g., "Tenderized with a solution of (list ingredients of solution)." Carcasses of animals treated with papain by ante-mortem injection shall be roller branded "Tendered with Papain." Parts not so marked shall be labeled as "Tendered with Papain."

See: 9 CFR 317.8(b) (25) 9 CFR 381.120

See Enzymes-Proteolytic.

Paprika

Generally, paprika and/or oleoresin of paprika are not permitted in or on fresh red meat products, fresh ground poultry, or fresh poultry sausage. They are permitted under the following conditions:

1. In both red meat and poultry products where such ingredients are acceptable and expected, including Italian Sausage, Salisica, Chorizo, Longaniza, and Hungarian Style products. All requests for additional products should be referred to the Labeling and Consumer Protection Staff to determine their acceptability.

2. On red meat products where their use does not misrepresent the leanness or freshness, e.g., application to a surface layer of fat and not to the muscle tissue. However, the name must be appropriately qualified, e.g., "coated with paprika" or "artificially colored."
3. In or on products where they are expected and the product name discloses this fact, or the product name refers to a component expected to contain the ingredients. Examples include: "Beef with Barbecue Sauce," "Beef — Barbecue Flavor," "Chicken Paprikash," "Chicken with Orange Sauce," or similar type products.
4. In fresh whole muscle poultry products, provided their presence is properly described, e.g., "coated with paprika," or "artificially colored," as appropriate.

Partially Cooked

1. Partially cooked bacon — acceptable nomenclature if shrink requirement for fully cooked bacon is not met must meet requirements for trichinae treatment. Cooking instructions are required.
2. Partially cooked poultry — unacceptable for cooked poultry products.

Partially Defatted (Beef or Pork) Fatty Tissue

These are byproducts produced from fatty trimmings containing less than 12% lean meat. These ingredients may be used in meat products in which byproducts are acceptable. Products include nonspecific loaves, beef patties, frankfurters with byproducts, bologna with variety meats, imitation sausage, potted meat food product, sauces, or gravies. May be used in excess of the amounts of meat necessary to satisfy the standard for only the products listed in the Policy Book. However, in this situation, the PDCB or PDCP must always be declared in the ingredients statement.

See: 9 CFR 319.15(e) 9 CFR 319.29(a).

Partially Defatted Chopped (Beef or Pork) (PDCB, PDCP)

1. Partially Defatted Chopped Beef is not permitted in hamburger, ground or chopped beef. The School Lunch Program requires that when PDCB is used in products like taco mix, which later may be used in preparing other products

The Amount and Labeling of PDCB and PDCP in Food Products

Class	Food Category	Amount	Labeling
I	Beef Patties	No Limit	Beef or Pork, or both
	Imitation Sausage	No Limit	Beef or Pork, or both
	Non Specific Loaf	No Limit	Beef or Pork, or both
	Potted Meat Food Product	No Limit	Beef or Pork, or both
	Patty Mix	No Limit	Always must be declared
	Beef for Roasting	12% of Meat Block	
II	Chinese Eggroll and other Chinese Specialties	Up to 12% of the Meat Block	Beef or Pork
	Chopped Beef Steak	Up to 12% of the Meat Block	Beef or Pork
	Corned Beef Hash	Up to 12% of the Meat Block	Beef or Pork
	Fabricated Steaks	Up to 12% of the Meat Block	Beef or Pork
	Pepper Steak	Up to 12% of the Meat Block	Beef or Pork
	Salisbury Steak	Up to 12% of the Meat Block	Beef or Pork
	Luncheon Meat (nonspecific)	Up to 25% of Meat Block	
	Pizza Meat Topping	Up to 25% of Meat Block	Beef or Pork
	Pizza With Meat	Up to 25% of Meat Block	Beef or Pork
	Cooked Sausage (9 CFR 319.180 (b))	Up to 15% of Meat Block	Always must be declared
	Pepperoni	Up to 15% of Meat Block	Must be declared
III	Chili	Up to 25% of Meat Block or larger	As beef; or pork, if larger must be declared
	Meat Loaf	Up to 25% of Meat Block or larger	As beef; or pork, if larger must be declared
	Meat Balls	Up to 25% of Meat Block or larger	As beef; or pork, if larger must be declared
	Meat Fillings for Tacos, Burritos, Enchiladas, Tamales and other Mexican Foods	Up to 25% of Meat Block or larger	As beef; or pork, if larger must be declared
IV	Corned Beef Hash	Up to 12% of Total Product Formulation	Beef

Note: All percentages as calculated on the basis of the fresh weight of meat content.

(e.g., tacos or patties), the PDCB or PDCP must always be declared in the ingredients statement on the labeling of the taco mix. All Beef or 100% Beef is acceptable as product name.

2. Partially Defatted Chopped may be used in excess of meat necessary to satisfy the standards on only the products listed in the Policy Book. However, in this situation, the PDCP must always be declared in the ingredients statement.

Partially Defatted Cooked (Beef or Pork) Fatty Tissue

This product may be used as an ingredient in: beef patties (cooked and uncooked), potted meat food product, sauces, gravies, imitation sausage, and nonspecific loaves. No limit on quantity is made. It is believed to be self-limiting.

Partially Hydrolyzed Whey Protein

An acceptable ingredient name for a binder.

Pastelles (SP)

Product must contain at least 10% fresh meat. Product is always made with pork in Puerto Rico. The label must show the true product name, e.g., "Pork Pastelles."

Pastellillos (SP)

Puerto Rican Style product containing at least 8% cooked meat. Species is part of the product name. The label must show the true product name, e.g., "Pork Pastellillos."

Pastitsio

(Greek for casserole). Product must contain at least 25% fresh meat or 18% cooked meat. A product containing macaroni, ground beef, tomato paste, wine, white sauce, and Parmesan cheese that may be labeled "Greek Style Pastitsio."

Pastrami

Cooked cured beef with spices, generally made from the plate but other cuts can be used. The product must be smoked or treated with smoke flavoring. "Pastrami, Water Added" is not permitted, although similar products labeled according to Policy Memo 084A are permitted. The term "Unsmoked Cooked Pastrami" must be used when the product is not smoked or does not contain smoke flavoring. Pastrami may or may not be coated with spices. When product is coated, a qualifier is not required.

Pastrami Jerky

Acceptable name for product processed as pastrami prior to meeting the requirements for jerky.

Pastrami, Turkey

A cured turkey product that is cooked. The product must be smoked or treated with smoke flavoring. The term "Unsmoked Cooked Turkey Pastrami" must be used when the product is not smoked or does not contain smoke flavoring. Cured turkey thigh meat is an acceptable name.

Pate De Foie

Product must contain at least 30% liver. Pate means paste; foie means liver.
See: **Foile Gras Products**.

Pattie Fold Over Mit Dressing

Product must contain at least 50% pattie.

Patties

Chopped and shaped and similar terms not required on products labeled patties.

1. Paprika not permitted in fresh meat patties.
2. PDCB or PDCP may be listed as beef or pork, except in patties with mechanically separated (species) product and school lunch labeled products.
3. PDBFT and PDPFT permitted. Must show as such in the ingredients statement.
4. Meat patties, with added fat up to 20% of the meat block, from a source other than that shown in the name, show as added (ex., Veal Patties, Beef Fat Added): over 20% to be part of the product name, e.g., "Veal and Beef Fat Patties."
5. Ground beef patties — no extenders or water added. Hamburger patties — no extenders or water added. Same requirement as hamburger.
6. Pre-broiled beef patties with simulated stripes (patties are deposited on conveyor and pre-broiled). Parallel stripes are applied with a solution of caramel coloring and water through parallel spigots. Product name will identify artificial color marks on the label.
7. Antioxidants are permitted in pork or beef patties both raw and cooked.
8. Beef Patties: If beef byproducts are added which are not permitted by the standard, the list of ingredients must immediately follow the product name. See: 9 CFR 319.15(c).
9. Pork Patties: The standard for beef patties 9 CFR 319.15(c) shall be applied with the exception that the species is pork.

Paupiette (FR)

Thinly sliced pieces of meat stuffed and rolled. Same standard as "Beef Roulade," which is at least 50% cooked meat.

Peanut Flour

Can only be used in nonspecific products that are not subject to moisture controls.

Pectin

Can be used at a maximum use level of 3% in nonstandardized meat and poultry food products. The common and usual name of the ingredient, regardless of its source, is "pectin" (21 CFR 184.1588).

Pepper

The term "pepper:" as used in the Italian sausage regulation refers to the pungent spices, such as: black, white, cayenne, or red pepper. "Paprika" as an optional ingredient is less pungent and is used primarily for its coloring qualities. Bell peppers, chilies, paprika and cayenne or red pepper, are from the capsicum pepper family. These products have specific uses and are recognized by specific names. "Paprika" should not be substituted for "pepper" in a meat or poultry food product.

Pepperoni

A dry sausage prepared from pork or pork and beef. Combinations containing more than 55% beef are called beef and pork pepperoni. Pepperoni made with beef must be called beef pepperoni. Pepperoni must be treated for destruction of possible live trichinae and must have an MPR of 1.6:1 or less. Antioxidants are permitted in pepperoni. The casing, before stuffing, or the finished product, may be dipped in a potassium sorbate solution to retard mold growth. Extenders and binders are not permitted in pepperoni. Hearts, tongues, and other byproducts are not acceptable ingredients.

Pepperoni, Cooked

Cooked pepperoni is not an acceptable product name.

Pepperoni with Poultry

Poultry may be added to pepperoni if properly labeled. If the meat block contains 20% or less poultry, the product is labeled "Pepperoni with Turkey (kind) Added." When poultry over 20% of the meat and poultry block product is labeled "Pork and Turkey (kind) Pepperoni," an MPR of 1.6:1 is applied. If the amount of poultry exceeds that of the meat, the product label reads "Turkey and Pork Pepperoni." This would carry a poultry legend.

Peppers and Cooked Sausage in Sauce

Product must contain at least 20% cooked sausage in total formulation.

Perishable Uncured Meat and Poultry Products in Hermetically Sealed Containers

Establishments seeking approval of label applications for perishable, uncured products which have received a less rigorous heat treatment than traditionally canned product (9 CFR 318 and 381, SUBPARTS G and X, respectively) must submit a sufficiently detailed processing procedure either incorporated on or attached to the FSIS Form 7234-1, APPLICATION FOR APPROVALS OF LABELS, MARKING OR DEVICE. The procedure must include a description of product formulation, method(s) of preparation, cooking and cooling temperatures, type of container, and cooking and handling instructions.

Hermetically sealed containers include glass jars, metal cans, flexible retortable pouches, plastic semi-rigid containers, etc., that are airtight and/or impervious after filling and sealing.

The policy does not apply to raw meat or poultry, cooked or roast beef, cooked poultry rolls and similar products, whole or uncut cured products, or products that are distributed and marketed frozen. However, products containing cured meat or poultry as components in combination with raw vegetables, e.g., pasta salads and other chilled meat/poultry meals or entrees containing raw or partially cooked vegetables, are covered under this policy, provided the above-mentioned procedural attributes are indicative of the manufacturing process.

In addition, an approved partial quality control program (PQCP) is required which must address the critical points in the manufacturing process. As such, the PQCP must contain a detailed description of: ingredient storage controls, product formulation and preparation, container filling and sealing, any heat treatment (times/temperatures) applied, including a description of the equipment used, any other treatments applied, cooling procedures (times/ temperatures), lot identification procedures; finished product storage conditions, inplant quality control procedures, and records maintenance procedures. The PQCP must be forwarded to the Processed Products Inspection Division (PPID) for appropriate review and approval before the product label may be used. Guidelines for development of PQCP's for these products may be obtained from PPID upon request.

Pet Food

1. Certified pet food is manufactured under fee-for-service inspection in a facility approved for the manufacture of animal food. Labeling regulations for certified animal food specify that approval is granted by the labeling staff. However, final approvals are not granted since LCPS no longer grants final approvals. Rather, the company should keep a copy of the final label attached to the sketch approval.

2. Most food for animal consumption produced in a Federal facility is non-certified. It is not an inspected product; therefore, it is inedible product and does not bear any mark of inspection. The product has to be conspicuously labeled to distinguish it from human food. Additionally, the labeling must be in conformance with 21 CFR Part 501, Animal Food Labeling since animal food labeling is also under the jurisdiction of the Food and Drug Administration.

Pfefferwurst (GR)

Product should conform to sausage standard and contain whole peppercorn. Pork livers, pork stock, and beef blood are not acceptable ingredients.

Phosphated Trimmings in Loaves

Trimmings from preparation of pork cuts, cured with approved phosphates besides other curing ingredients, may be used without limitation in loaves other than meat loaves. When such trimmings are used, phosphates may be listed in the ingredients statement using the term "sodium phosphates" or other applicable generic terms.

Phosphates in Dipping Solutions Containing Proteolytic Enzymes

Phosphates have been approved for use as buffering agents in dry mixtures intended for solutions containing proteolytic enzymes. The phosphates should not exceed 0.1% of the "tenderizing" solution if they are to be considered incidental additives.

Picadillo (SP)

Product must contain at least 35% cooked meat. A Mexican style hash usually made with beef, garlic, onions, vinegar, and raisins. The species should be in the product name, e.g., "Beef Picadillo."

Pickled Products, Dry Packed

Products that are pickled and dry packed should be qualified with the name of the pickle as part of the product name, e.g., "Knockwurst Pickled with Vinegar," or "Knockwurst Pickled." The weight of the package shall be the weight of the product less the weight of the pickle that will weep out of the product.

Pie Filling

Product must contain at least 37% meat. Poultry pie filling must contain at least 18.75% cooked poultry meat.

Pies

Product must contain at least 25% meat. Meat in the gravy may be counted. Poultry pies require at least 14% cooked poultry meat.

Pies, English Style–Australian Style

Product must contain at least 25% meat or meat byproduct. Contains gravy and no vegetables with a puff pastry top.

Pimento (SP)

Refers to allspice, but must be specifically named. It is also known as Jamaica pepper.

Pimiento Sausage

Pimientos permitted when declared in product name as "Pimiento Sausage."

Pinkelwurst (GR)

A cooked product that is stuffed in a casing with a diameter of from 1½ to 2 inches and a length of about 10 to 12 inches. It is formulated with beef fat, pork fat, onions, oat groats, water, and sufficient spice to satisfy seasoning requirements.

Piroshki or Pirogi

Product must contain at least 10% cooked meat. A Russian or Jewish dish made of thin rolled dough or pastry that is filled and either steamed, baked, or fried. They resemble small turnovers, pockets, or raviolis.

Pizza

The required meat fill is to be calculated on the stated net weight of the product, not on the formula weight.
(See: 9 CFR 319.600) with

Meat: At least 15% meat
Sausage: At least 12% cooked sausage or 10% dry sausage (e.g., pepperoni)
Poultry: At least 12% cooked poultry meat.
Bacon: At least 9% cooked bacon.
Chili with beans: At least 25% chili with beans.
Meat Pattie Crumble: At least 15% pattie crumbles (fresh) or 12% cooked.

An antioxidant used in pepperoni or sausage need only be reflected in the ingredients statement as "BHA or BHT added to improve stability."

Pizza, Chicago Style

Acceptable labeling for a product which has been manufactured by first placing the cheese on the crust, then following with the meat and then the sauce. Condimental

quantities of a grated cheese may then be placed on the top. The product usually has the deep dish characteristics. The requirements for pizza as designated in 9 CFR 319.600 and various policies must be met.

Pizza, Combination or Deluxe

Product must meet the requirements for pizza as designated in 9 CFR 319.600. In a combination pizza, e.g., "Sausage and Pepperoni Pizza," the component declared last must be at least 25% of its required level in a pizza containing a single meat component.

Pizza Containing Cheese Substitutes

Meat requirements of 9 CFR 319.600 must be met. Labels which contain cheese in a ratio less than one part cheese to nine parts cheese substitute must contain additional qualifying information. Example: Pizza — Sausage, cheese substitute and cheese; Combination Pizza — Sausage — Pepperoni — Imitation Cheese and Cheese.

Pizza Dogs

A nonspecific product.

Pizza, Pan Style

Pizza that is marketed in a pan and contains a thick crust.

Pizza Pups

Product has two crusts, filled with a mixture of pork, tomato puree, and condimental substances. The finished article is approximately 8 inches in length, 2½ inches wide with a thickness of ¾ inches. It is a type of pizza. The label must show a true product name, e.g., "Pork and Sauce Filling in A Crust."

Pizza Roll

This is a nonspecific meat food product. When the name appears on a label, there must be a contiguous statement identifying the major components of the product or a complete ingredient listing. There are two major types of pizza rolls. One is a cooked sausage-like meat food product that contains cheese, usually contains peppers and has no water limitation. The second type consists of a roll-shaped dough enclosure with various fillings. A manufacturer of the latter type of product has asserted trademark protection of the term "pizza roll."

Pizza Sausage

Not an acceptable name. Product must be labeled "Sausage for Pizza."

Pizza, Sicilian Style

A thick crust pizza. The crust is usually 50% or greater of the total pizza product.

Pizza Topping Containing Sausage

The sausage portion of cooked pizza topping is permitted to contain up to 10% water and 3.5% binders (9 CFR 319.140). In addition, the application must indicate the sausage portion within the pizza topping formula. However, the ingredients statement of the cooked pizza topping does not have to list "sausage" in its sublisting. There are no restrictions on the amount of seasonings in the sausage portion.

1. Pizza topping must be cooked
2. In the sausage portion:
   a. Water $<10\%$ of the sausage portion
   b. Binders including TV $<3.5\%$ of the sausage portion
   c. Seasonings are unlimited
3. The ingredients statement of the pizza topping can be arranged in different ways:
   a. Composite e.g., cooked pizza topping [port, water, TVP (…), Seasonings (…)]
   b. Component, e.g., cooked pizza topping [sausage (pork, water, seasonings (…), TVP (…), water (…)].

These parameters do not apply when specific sausage products are governed by other regulations, e.g., Italian Sausage. In these situations, the specific regulation (i.e., Italian Sausage) dictates the requirements, such as, the amount of water permitted and binders are permitted.

Pizza Topping Mix

A nonspecific product, including those products which indicate the type of meat or poultry in the product name (e.g., Chicken and Pork Pizza Topping or Beef Pizza Topping). Antioxidants are permitted, see 9 CFR 318.7(c)(4). Water, extenders, and binders are acceptable.

Pizza, Word Size

When a pizza has a true product name, e.g., "Combination Sausage and Pepperoni Pizza," the true product name must be prominent, conspicuous, and legible, with all words at least one-third the size of the largest letter in any word of the product name. If on the label the manufacturer also elects to display elsewhere the word "Pizza" in exaggerated fashion, the word "Pizza" is not considered in the determination of the size of the letters within the true product name.

PFF (Protein Fat Free) Adjusting for Use

Protein Fat Free (PFF) controlled cured pork products with qualifying statements, e.g., "Ham-Water Added," may be

used in place of PFF controlled cured pork products without qualifying statements, e.g., Ham, to meet the minimum meat requirements of various products. However, the amounts of the PFF controlled cured pork products with qualifying statements used will need to be increased. For example, if a standard requires a certain amount of Ham and a processor wishes to use "Ham-Water Added," a greater amount of the "Ham-Water Added" will be needed to meet the standard. The magnitude of the additional amount is directly related to the relationship between the respective PFF values.

Example: Ham Salad requires 35% Cooked Ham. "Ham Water Added" will be used in the product formula.
Calculation: Multiply the PFF value for Ham (20.5) by the amount of required Ham (35%). Divide this answer by the PFF value of the product being used to formulate the product. (In this example, PFF value for "Ham-Water Added" is 17.0.)
Answer: [(0.35 × 20.5)/17.0] × 100 = 42.21% "Ham-Water Added" needed in the formula.

Example: Ham Pie requires 25% Ham based on green weight. "Ham with Natural Juices" will be used in the product formula.
Calculation: Multiply the PFF value for Ham (20.5) by the amount of required ham (25%). Divide this answer by the PFF value of the product being used to formulate the product.
(In this example, PFF value for "Ham with Natural Juices" is 18.5.)
Answer: [(0.25 × 20.5)/18.5] × 100 = 27.70% "Ham with Natural Juices" needed in the formula.

1. Adjusting for "Ham and Water Product X% of the Weight is Added Ingredients"

Consider a formulated product which is required to contain at least 50% Cooked Ham. If the processor chooses to use a "Ham and Water Product (HWP)" in which 20% of the weight is added ingredients as the source of the Ham in the formulation, this product contains 80% Ham and 20% added ingredients. Clearly, the processor must use more than 50% HWP in the process. Using 50% HWP would result in only 40% Ham in the finished product, i.e., the added ingredients in the HWP represents 25% of the ham content. (If it were a 10 lb. HWP, there would be 8 lbs. of Ham and 2 lbs. of added ingredients (2/8 × 100 = 25%)). Consequently, an additional 25% of HWP is required in the formulation.

The following example may be used to determine the percentage HWP needed to equal Ham:

Ham and Gravy requires 50% Cooked Ham. "Ham and Water Product 20% of Weight is Added Ingredients" will be used in the formulation.

Step 1: Subtract the % added ingredients from 100%. (In this example: 1.00 − 0.20 = 0.80)
Step 2: Determine the amount of Ham needed in the formula. (In this example: 50%)
Step 3: Divide the amount of Ham required. Determined in Step 2 by the answer in Step 1 (In this example: (0.50/0.80 = 0.625))
Step 4: Multiply the answer in Step 3 by 100. Answer for this example is 62.50% "Ham and 20% Water Product" is needed as the equivalent of 50% Ham.

Plantation

The regulations and policies applicable to "Farm" also apply to plantation.

Point of Purchase Materials

Point of purchase materials which refer to specific meat or poultry products are considered labeling under certain circumstances. When printed and/or graphic informational materials (e.g., pamphlets, brochures, posters, etc.) accompany or are applied to products or any of their containers or wrappers at the point of purchase, such materials and the claims that they bear are deemed labeling and they are subject to the provisions of the Federal Meat Inspection Act and the Poultry Products Inspection Act.

Although the Food Labeling Division (FLD) does not exercise its authority to subject point of purchase materials to specific prior approval (materials shipped with the products from the federally inspected establishment are an exception), we do expect point of purchase materials to be in accordance with the Federal regulations and all current labeling policies. Upon request, FLD will review and comment on the point of purchase materials submitted to our office. During the review process, promotional materials will be scrutinized for special claims, particularly those related to nutrition, diet, and animal husbandry practices.

Claims related to nutrition and diet must be made in accordance with all current nutrition labeling regulations. Continuing compliance with stated claims will be assured through periodic sampling, as necessary. Claims are expected to be within the compliance parameters identified in the nutrition labeling regulations.

Animal husbandry claims (e.g., the nonuse of antibiotics or growth stimulants) may be made only for products shipped in containers or wrappers labeled with the same animal production claims.

Polish Sausage

A sausage that is cured, cooked, and usually smoked. Pork and pork byproducts shall comprise at least 50% of the meat and meat byproducts ingredients. To have beef as a predominant ingredient, the product name would be "Beef and Pork Polish Sausage." Green peppers are permitted up to 4% in total formulation.

An uncured (fresh), uncooked variety with no more than 3% water also exists. "Fresh" shall be used in the name when the product is uncured. When Fresh Polish Sausage is cooked or smoked, then the product name is either "Cooked Fresh Polish Sausage" or "Smoked Fresh Polish Sausage." The requirements of Policy Memo 110 apply when these perishable, cooked, uncured products are packaged in hermetically sealed containers.

Polynesian Style Sausage

Product must contain fruit juices, a sweetening agent, and soy sauce.

Polysorbate

Permitted in pickling solutions without declaration.

Pork and Bacon Sausage

Up to 50% bacon permitted provided:

1. Bacon is brought back to green weight before use
2. Product is trichinae treated
3. Product name is "Pork and Bacon Sausage"

The standard for "Pork Sausage and/with Bacon" is 10 to 20% bacon, and for "Pork and Bacon Sausage" is more than 20% but not more than 50% bacon.

Pork and Dressing

Product must contain at least 50% cooked pork.

Pork and Dressing with Gravy

Product must contain at least 30% pork.

Pork Cracklings

Product eligible to be labeled as "Pork Cracklings" must be prepared from fatty tissues from which the skin has been detached. If the skin is not removed from the product before rendering, a descriptive name, e.g., "Pork Cracklings, Fried-Out Pork Fat with Attached Skin," must be used.

Pork Fat

Pork fat shall be declared as such in the ingredients statement. Clear fatbacks and clear shoulder plates must be declared as "Pork Fat." Pork fat may be declared as pork in the ingredients statement if it contains visible lean and it is used in a standardized product which has a fat limitation.

Pork Jowls

Product may be declared as pork if skinned.

See: Pork Skins.

Pork Sausage

Product identified as pork sausage does not include the use of pork cheeks. When such an item is offered as "Whole Hog," tongues, hearts, and cheeks may be used in the natural proportion as found in the hog carcass. "Fresh" shall be used in the name when the product is not cured, cooked and/or smoked.

Pork Skin Residue after Gelatin Extraction

This material consists of back fat skins from which the gelatin has been extracted by means of soaking the skin in acid and subsequent low temperature cooking for the extraction of gelatin. It is not permitted in sausage but may be used in imitation sausage, potted meat food product, loaves (other than meat loaves), and other nonspecific products.

Pork Skins

Not permitted in salami, bologna, frankfurters, Vienna sausage, and braunschweiger. When packed in vinegar pickle, they are not permitted to be artificially colored. When pork skin, either attached to fat and/or muscle tissue or detached from fat and/or muscle tissue, is used to manufacture meat or poultry products, it must be specifically listed in the formulation on the label approval application form and in the ingredients statement on the label, e.g., "Pork Skins," "Unskinned Pork Jowls," "Unskinned Pork Shoulder Trimming," "Unskinned Pork Fat," and "Unskinned Pork Bellies."

"Detached skin" refers to the portion of skin from which most of the underlying fat is removed, e.g., skin from bacon intended for slicing, skin from closely skinned hams, shoulder cuts, fat backs, etc. If removal of skin portions is incidental to removal of a considerable proportion of underlying fat from ham, shoulder, back, etc., preparatory to rendering such fat, portions of skin so removed should not be regarded as detached skin and may be included with fats and rendered into lard. Ham facings are not regarded as detached skin.

Pork Skins, Fried

When prepared from the skin of smoked pork bellies, it may be labeled as "Fried Bacon Skins," "Fried Bacon Rinds," or "Fried Pork Skins." The kind of skin used must be stated on the labeling records when submitted for label approvals.

Pork Spare Ribs, Center Cut

Center cut pork spare ribs refers to pork spare ribs with the loin portion, the brisket (brisket must be removed at a point which is dorsal to the curvature of the costal cartilages), the tail and two ribs from the shoulder removed, this remaining center section may be further portioned or left in one piece.

Pork Spareribs, St. Louis Style

St. Louis Style Spare Ribs are the same as "Pork Spareribs" except that the sternum and the ventral portion of the costal cartilages are removed with the flank portion. This cut is made at a point in which the sternum and costal cartilages are removed dorsal to the curvature of the costal cartilages. If specified by the purchaser, the diaphragm shall be removed.

This anatomical description of the cut must be provided with the information for label approval.

Potato Sausage, Swedish Style or Potato Ring Swedish Style

A cooked or uncooked meat food product with the following requirements:

1. At least 45% meat and no byproducts.
2. Water limited to 3% at formulation.
3. Extenders or binders limited to 3.5% of the finished product, except that 2% of isolated soy protein shall be deemed to be equivalent of 3.5% of any of the other binders or extenders.
4. Contains at least 18% potato.

Poultry

Cuts of poultry that are not identified in 9 CFR 381.168, Table V, may use the maximum amount of poultry skin permitted for that "kind." For example, "turkey" is listed in the table and may contain up to 15% skin. Therefore, a product identified as "white turkey" can be placed in this category for a maximum of 15% skin.

Poultry Standards

Name	**Minimum or Maximum percentage**
Poultry a la King	At least 20% poultry meat
Poultry Barbecue	At least 40% poultry meat
Poultry, Breaded	No more than 30% breading
Poultry, Brunswick Stew	At least 12% poultry meat
Brunswick Stew with Poultry	At least 8% poultry meat
Poultry Burgers	
Poultry Cacciatore	
Poultry Canneloni	
Poultry Chili	
Poultry Chili with Beans	
Poultry Chop Suey	
Chop Suey with Poultry	
Poultry Creole with Rice	
Poultry Chow Mein (w/o noodles)	
Poultry Croquettes	
Poultry, Creamed	
Poultry Dinners	
Poultry Fricassee	
Poultry Fricassee with Wings	
Poultry Gizzards and Gravy	

Name	**Minimum or Maximum percentage**
Poultry Hash	100% meat with skin and fat in natural proportions
Poultry Liver	At least 20% poultry meat or 40% with bone.
Omelet Poultry	At least 7% poultry meat
Meatloaf	At least 28% poultry meat
Poultry Noodle Dinner	At least 17% poultry meat
	At least 4% poultry meat
	At least 2% poultry meat
	At least 35% cooked poultry meat and sauce portion. Not more than 50% cooked rice in total product
	At least 4% poultry meat
	At least 25% poultry meat
	At least 20% poultry meat
	At least 18% poultry meat
	At least 20% poultry meat
	At least 40% poultry wings (cooked basis with bone)
	At least 35% cooked gizzards
	At least 30% poultry meat
	At least 12% cooked poultry liver
	At least 65% raw poultry or 50% poultry meat and at least 15% poultry meat
Poultry Noodle Dinner with Gravy	
Poultry with Noodles or Dumplings	
Noodles or Dumplings with Poultry	
Poultry Paella	
Poultry Parmigiana	
Poultry Pies	
Poultry Ravioli	
Poultry Salad Mix	
Poultry Salad	
Poultry Soup	
Poultry-Flavored Soup	
Poultry Spread	
Poultry Stew	

Name	**Minimum or Maximum percentage**
Poultry Stew with Dumplings	At least 15% poultry meat or 30% poultry meat with bone
Poultry Subgum	At least 6% poultry meat
Poultry Tamales	
Poultry Tetrazzini	

At least 35% poultry meat or 35% poultry meat and other meat, no more than 35% cooked rice, must contain seafood
At least 40% breaded poultry See: Veal Parmigiana
At least 14% poultry meat
At least 2% poultry meat
At least 45% poultry
At least 25% poultry See: Salad, Poultry
At least 2% poultry meat
No minimum requirement (less than 2% poultry meat)
At least 30% poultry
At least 12% poultry meat
Minimum or Maximum Percentage
At least 8.4% poultry meat (Based on 70% of Stew requirement)
At least 12% poultry
At least 6% poultry meat
At least 15% poultry meat
Poultry Turnover
Poultry with Gravy/Sauce
Gravy with Poultry
Poultry with Gravy and Dressing
Poultry with Rice
Poultry Scrapple
Poultry with Vegetables

Poultry, Assorted Pieces

At least 14% poultry meat
At least 35% poultry meat
At least 15% poultry meat
At least 25% poultry meat
At least 15% poultry meat
At least 30% poultry and/or poultry byproducts
At least 15% poultry meat

The product name "Poultry (Kind) Assorted Pieces" is acceptable and does not require the product to be in natural proportions. In addition, the term "piece" is not the same as the term "part," i.e., a piece does not have to be a whole part, e.g., a breast, thigh, or drumstick.

Poultry Bacon

See: Bacon-Like Products.

Poultry Breasts

When poultry breasts with ribs are boned and the resulting product contains portions of the scapula (shoulder) muscles and/or muscle overlying the vertebral ribs, they must be labeled to indicate that fact. Proper names for such products are "Boneless Breast with Rib Meat," "White Chicken Meat or White Turkey Meat," or if the skin is left intact, "White Boneless Chicken or White Boneless Turkey." Product labeled "Boneless Breast" without further qualification may not contain scapula or rib meat.

Poultry Frankfurters (Similar Cooked Sausages)

Products which contain pork fat must be labeled with pork fat added in the product name.

Poultry Grading (Labeling)

Indicates the quality grades of poultry (U.S. grade A, B, or C). The shield design contains the letters "USDA," the U.S. grade of the product, and if not shown elsewhere, the class of poultry. Any letter grade on a consumer package or individual carcass indicates the product was graded by a licensed grader of the Federal or Federal-State grading service, and may not be applied otherwise. Letter grades on bulk packaging or shipping containers only indicate that the product is equal to that particular U.S. Grade.

1. Applying Grademarks to Shipping Containers

All poultry classes and kinds listed in 9 CFR 381.170, except necks, giblets, detached tails, wing tips, skin and stripped backs (below Grade C) are eligible for grading.

In addition, the following poultry parts may be officially graded:

Boneless, Skinless Breast and Thigh Tenderloin or Boneless Breast without Tenderloin
Boneless Breast Quarters
Breast Quarters with Bone in
Boneless Thigh Halves
Wing Portion or Section Breast Halves
Broiler Turkey or Duck Halves
Split Breast Split Fryers
Skinless, bone-in Thighs, Drums and Breasts
Boneless Breast, Thigh Bone-in products marinated in a colorless solution

Poultry cuts other than those identified above may not be eligible for grading; therefore, particular attention should be given to the product name when approving labels for various poultry products which include grade marks (e.g., "Thin Breast Fillets, Thigh Strips").

Grade marks on raw poultry parts processed with solutions that may impart color (e.g., injected with a solution of water, salt, butter) or cooked poultry products must include a statement, e.g., "Prepared from Grade A Poultry." The USDA grader in the plant makes the final determination concerning the necessity of the "Prepared from" statement in situations where it is not apparent at the time of label approval that the added solutions have the ability to impart color to the finished poultry product. Products which may not be grade marked:

Detached Necks
Giblets Packed Separately

Detached Tails Wings, Tips
Stripped Backs
Below C Quality Diced or Shredded Meat

2. Wing Descriptio

The wing is made up of three sections. The section attached to the carcass is the first section. The wing tip is the third section.

3. Grading Backs with Necks

In applying grade standards, when necks are packed with backs following these steps:

1. When backs are graded as provided for in the standards, the name of the product shall read as follows:
   a. Grade A Backs "with necks," or "and necks"
   b. Grade B Backs "with necks," or "and necks"
   c. Grade C Backs "with necks," "Graded backs and necks," or "backs and necks"
2. Necks are to be packed with backs in natural proportions.
3. Necks may or may not be attached to backs. Necks for all officially graded backs are to be free from serious discolorations, feathers, pin feathers, and accumulations of blood and/or excess water.
4. A neck, front, or hind portion of back, when removed from birds which meet the stated quality, may be used to achieve exact weights. Only one of these portions may be used per package. Scraps of backs or necks may not be used.
5. Labels for packages with portions must indicate which portions, e.g., first (1st) portion, 2nd portion, 1st and 2nd portions, 2nd and 3rd portions, etc.

4. Pressure Sensitive Stickers and Tape

1. Inserts or pressure sensitive stickers with the grademark must have plant number.
2. Grademarks on pressure sensitive tape should not be used on consumer packages.
3. Insert with the grademarks are not to be used inside opaque bags.

Poultry Hindquarters

The term "hindquarters" on labels for single cut poultry items is an acceptable alternative to the recognized terminology "Leg Quarter" specified in the regulations. The use of the term "Hindquarters" requires only a specified class of poultry to be considered a true product name, e.g., "Chicken Hindquarters." Either term refers to a poultry thigh and drumstick, with a portion of the back attached.

Poultry Hindsaddles

Poultry hindsaddles are connected poultry leg quarters (the rear of the bird). The product name "Poultry Hindsaddles" may be used alone on the product's label if the product is not intended for retail sale. In contrast, the name "poultry hindsaddles" on the label of a product intended for retail sale must be accompanied by a fully descriptive name (e.g., "Poultry Hindsaddles, Connected Leg Quarters").

Poultry Parts

Specific net weight packages for poultry parts, usually those containing legs or wings, include a single part, e.g., a drumstick or thigh, to make the stated weight. The name on the label must reflect this practice, e.g. "Chicken Legs — Chicken Thigh added to make weight." The single part must be cut at the joint. Wing tips are not permitted as added parts.

Poultry Products

In poultry products where "meat" appears in the product name, e.g., "White Meat Chicken Roll," and "Dark Meat Turkey Loaf," skin and attached fat are permitted in greater than natural proportions. However, the ingredients statement must have the poultry skin or poultry fat listed. When skin and attached fat appear in the ingredients statement, their placement should be in the correct order of predominance and determined by the amount present over the permitted natural proportions.

Poultry Products Containing Meat Ingredients-Labeling

Poultry products containing meat in amounts that exceed 20% of the total meat and poultry product portion of the poultry product must be descriptively labeled to indicate the presence of the meat ingredients, e.g., "Chicken and Beef Stew or Stew made with Chicken and Beef."

Poultry products containing meat ingredients in amounts at 20% or less of the total meat and poultry product portion of the poultry product must have names that are qualified to indicate the presence of the livestock ingredients, e.g., "Chicken Stew-Beef Added."

However, poultry products that do not meet specified minimum poultry ingredient requirements because meat ingredients are replacing any part of the required poultry ingredients must be descriptively labeled to indicate the presence of meat ingredients, e.g., "Turkey and Pork Chop Suey."

Poultry Products with other than Natural Proportions of White and Dark Poultry

Poultry products containing white and dark chicken or turkey of a distinguishable nature and in quantities other

than natural proportions of white to dark meat must bear a qualifying statement identifying the types of poultry meat used in conjunction with the kind of poultry in the product name. The poultry block of white and/dark meat [excluding products labeled as "Mechanically Separated (Kind of Poultry)"] solely determines the usage of the terms "white and dark," "dark and light," "white," "dark," etc. in the product name. Ground poultry (excluding the skin) that bears the terms "white/light," "dark," "breast," "thigh," etc. in the product names is also considered as part of the poultry block for determining the usage of terms "white and dark," "dark and light," "white," "dark," etc. However, products labeled as "Mechanically Separated (Kind)" do not have any bearing on the use of terms "white," "light" or "dark" in the product name since, "Mechanically Separated (Kind)" is as indistinguishable paste-like product that is considered a separated standardized poultry food product ingredient.

Additionally, products with mixture of distinguishable poultry (white or dark) and "Mechanically Separated (Kind)" can not bear claims of "all white," "pure breast," "100% dark," or similar terms. In this situation, the poultry portion of the product contains at least two separated poultry ingredients, or one of which is "Mechanically Separated (Kind)."

See: 9 CFR 381.117(c), Table 1.

Poultry Puffs

Product must contain at least 15% cooked poultry meat. Chicken or Turkey Puffs are classified as hors d'oeuvres and must show a true product name, e.g., "Breaded Chicken and Rice Balls."

Poultry, Raw Solution

Unless addressed by other regulations and policies, water and/or oil based solutions may be added to raw poultry and poultry parts at various levels with an appropriate qualifying statement to the product name.

The statement must include terms adequate to inform the consumer of the amount and manner of the addition and include the common or usual names of the ingredients in their proper order of predominance (e.g., "Injected with up to 12% of a solution of water, salt, and phosphates"). Other similar designations will be considered on their merits. The statement must be contiguous to the product name and printed in a style and color as prominent as the product name. The statement of the manner and amount of addition must be one-fourth the size of the most prominent letter in the product name. The ingredients of the solution can be printed one-eighth the size of the most prominent letter of the product name.

Terms like "Basted," "Marinated," "For Flavoring," and similar terms contemplated within the provisions of Section 9 CFR 381.169 of the poultry products inspection regulation cannot be used if the amount of the solution added is more than needed to baste, marinate, or flavor the product. Bone-in poultry and poultry parts are limited to 3% as prescribed by the regulations. Boneless poultry is limited to 8% to use these terms.

1986 Poultry Roast

May be formulated with up to 10% liquids without a qualifying statement. If more than 10% liquid is used, the name must be qualified with a statement, e.g., "containing up to x% …"

Poultry Salami Products

Poultry sausages prepared to resemble salami and offered to consumers as a salami shall bear product names as follows:

1. "(Kind) Salami" shall be the product name when the moisture-to-protein ratio in the finished product does not exceed 1.9:1. This product resembles a dry salami made from red meats.
2. "Cooked (Kind) Salami" shall be the product name when the product is cooked and the moisture-to-protein ratio is above 1.9:1. This product resembles "cooked salami" made from red meats.

Poultry Sausage

Sausage products made from poultry must be labeled to indicate kind, e.g., "(Chicken) Sausage," "(Turkey) Bologna," etc. Products containing more than one kind of poultry or red meat must declare the added ingredient in the product name, e.g., "Chicken Bologna, Beef Added" and "Turkey Franks, Chicken Hearts Added" per Policy Memo 029 dated September 4, 1981. The basic sausage standards, per meat 9 CFR 319.140, also apply to poultry, except for added water and fat.

Poultry Skin

When determining the amount of poultry skin allowed, refer to 9 CFR 381.168. If the specific part is not identified in this part, use the figure for boneless kind.

Poultry Tenders and Poultry Tenderloins

A "(Kind) Tender" is any strip of breast meat from the kind of poultry designated. A "(Kind) Tenderloin" is the inner pectoral muscle which lies alongside the sternum (breast bone) of the kind indicated.

Poultry Wing Sections (Kind)

Wing Sections is an acceptable designation for a product consisting of equal proportions of the parts of a wing. It may be and is usually used for equal proportions of wing portions and drummettes.

Premier Jus or (Oleo Stock)

The product obtained by rendering at low heat the fresh fat of heart, caul, kidney, and mesentery collected at the time of slaughter of bovine animals. The raw material does not include cutting fats. Premier Jus is not an acceptable name unless accompanied by the term "Oleo Stock."

Pressure-Sensitive Labels

Labels applied to packages shall be of the self-destructive type and must adhere to the packages under all conditions of use.

Pressure-Sensitive Stickers

Pressure sensitive stickers are as a means for manufacturers to use existing labeling material by covering inaccurate and/or misleading labeling information with corrected text or used as a promotional tool, e.g., a starburst encircling sweepstakes terminology. A pressure sensitive sticker must be the type that destroys the underlying label or package if removed, or be self-destructive.

Temporary label approval is not required when the entire label including the pressure sensitive sticker is truthful, not misleading, and the product is not misbranded. Corrected text on the pressure sensitive sticker can cover mandatory or non-mandatory information.

Labeling bearing pressure sensitive stickers falls under the Provisions of the generically approved labeling regulations 9 CFR 317.5 or 9 CFR 381.133, which indicates the conditions for use of final labeling without prior Washington approval. Companies need to create and maintain records of all final labeling, otherwise known as generic approvals.

Consistent with the rules on generic labeling, sketch labeling approval is required for the entire label when pressure sensitive stickers contain special claims [quality, nutrient content, health, negative, geographical origin, other claims (natural, animal production, e.g., "no antibiotics administered," breed claims, etc.), guarantees, foreign language or a change of the nutrition facts serving size].

Primal Parts and Subprimal Meat Cuts

Red meat carcasses, primals, subprimals or cuts can be labeled: 1) as the species of origin, e.g., beef tenderloin bearing the simple product name of "beef," 2) as species without identifying the primal or subprimal when certain terms associated with various sizes are part of the product name, i.e., chop, cutlet, steak, fillet, filet, roast, strips, etc., e.g., "Beef Steak," 3) as species and primal or subprimal cut, e.g., "Veal Shoulder Blade Steak," and 4) as species, coin name (butt, cala, daisy, picnic, etc.) and primal or subprimal cut. The species and coin name are not appropriate as a complete product name since it is missing the primal or subprimal cut, e.g., the phrase "pork picnic" is incomplete without "shoulder."

Recent editions of the "Uniform Retail Meat Identity Standards (URMIS)," published and distributed by the National Livestock and Meat Board, and "The Meat Buyers Guide," published by the National Association of Meat Purveyors, may be used to identify recommended names. These guides have been prepared through extensive review and analysis of the most recent edition of "Institutional Meat Purchase Specifications (IMPS)" and in cooperation with the U.S. Department of Agriculture, Agriculture Marketing Service (AMS) and public and industry associations.

Prime Rib of Beef or Standing Beef Rib Roast for Prime Rib

These products do not have to be derived from USDA prime grade beef.

Principal Display Panel, Alternate

The determination as to whether or not a panel is an alternate principal display panel shall be based on whether or not the panel is likely to be displayed, presented, shown, or examined under customary conditions of sale. If the intent of the panel cannot be determined and demonstrated, and if it has the appearance of a principal display panel, the presence of three or more mandatory labeling features shall serve to characterize the panel as an alternate principal panel. As such, any remaining mandatory features required to be placed on a principal display panel must be also included.

Product Names

1. A product standard should only be applied if the product name is the same as that described by the standard in the regulations or in the Policy Book. For example, the product, "Beef, Cheese and Vegetables in a Crust," would not be required to meet the standard for a "turnover" in the policy book.
2. Products such as "Pizza Pouches" must meet the standard for pizza since they are named "Pizza" even though they are not traditional type pizza. Furthermore, cheese may not be substituted for meat in products named "Pizza."

Product Name Qualifiers

Product name qualifiers have no sizing requirements other than appearing contiguous to the product name and being prominent and conspicuous. Examples of product name qualifiers are "Smoked Flavor Added," "Made in

Sheboygan," and "Colored with Paprika." Examples of phrases that are not product name qualifiers are "Water Added," and "Containing up to X% of a Solution." Such phrases are actually part of the product name and do have particular sizing requirements.

Product Name Qualifiers in Secondary Products

Product name qualifiers, e.g., "binders added," are not required on secondary products with labeling, with the exception of the statement "Calcium Propionate Added to Retard Spoilage of Crust" on pizza labeling. Secondary products are those meal like products that contain a multi-ingredient meat or poultry component, e.g., "Lemon Pepper Seasoned Chicken Breast with Rib Meat, Binders Added in 'Lemon Pepper Chicken Breast with Vegetable Medley.' " The characteristics of the meat or poultry added ingredients, are disclosed in the ingredients statement.

Product of USA

Labeling may bear the phrase "Product of U.S.A." under one of the following conditions:

1. If the country to which the product is exported requires this phrase, and the product is processed in the U.S., or
2. The product is processed in the U.S. (i.e., is of domestic origin). This entry cancels Policy Memo 080 dated April 16, 1985.

Prosciutto

Italian for ham, dry cured. The product name "Prosciutto" is acceptable on labeling to identify a dry-cured ham.

Prosciutto, Cooked

The product name "Cooked Prosciutto" is acceptable on labeling to identify a dry-cured Prosciutto ham that is cooked.

Prosciutto Cotto, Cooked Ham

The product name "Prosciutto Cotto, Cooked Ham" is acceptable on labeling to identify a regular pickle-cured cooked ham. Prosciutto Cotto is the Italian name for cooked ham.

Protective Coverings (Meat) Processed or Prepared Product

Immediate containers, e.g., bags, cardboard cartons, tray packs, and film bags enclosing processed or prepared product can be considered protective coverings and exempt from the marking and labeling requirements if placed in a shipping container which meets all mandatory labeling requirements of an immediate container. This does not exempt the mandatory identification and marking which is specifically required on the immediate container of cooked beef (9 CFR 318.17). In addition, the shipping container must be clearly marked "Packed for Institutional Use" or an equally descriptive statement of intended limited distribution. Unlabeled product may not be removed from shipping containers for further distribution nor displayed or offered for sale.

Unprocessed Meat Cuts — Transparent film bags enclosing individual meat cuts in an unprocessed state can be considered protective coverings and exempt from the marking and labeling requirements if placed in a shipping container which meets all mandatory labeling of an immediate container. These unlabeled meat cuts may only be removed from the shipping container for resale and further distribution to retailers, hotels, restaurants, and similar institutions if the product itself or the film bag bears a clearly legible official mark of inspection and the establishment number.

Protective Coverings (Poultry)

Under provision of the Poultry Products Inspection Act, protective coverings may be exempt from labeling requirements for immediate containers. Under certain circumstances, some protective coverings are considered immediate containers; under different circumstances, they are regarded only as protective product coverings.

When plastic film bags, cardboard cartons, etc., are used for protecting poultry sold for export or to institutions, e.g., hotels, restaurants, and hospitals (where the contents are consumed on the premises), they are exempt from the mandatory labeling of immediate containers, provided the shipping container meets all the labeling requirements for an immediate container. Such product may not be diverted to retail channels and displayed for sale or be sold to household consumers unless they bear all labeling features required for immediate containers.

See: 9 CFR 381.65(p).

Pudding

Nonspecific product.

Pulled Pork

Refers to pork removed from bones by hand or by mechanical means. The meat must retain its natural striated muscle fiber structure, i.e., it can be shredded, chunked, etc., but may not be ground, chopped, or comminuted.

Quality Grade Terms and Subjective Terms on Labels

Terms designated as grades of meat, i.e., prime, choice, select, good, etc., may only be used on red meat which has

been officially graded. However, the Food Labeling Division (FLD) will take no action to rescind currently approved labels which contain the word "select." Labels for new or reformulated products or new product lines will be approved in accordance with the policy for grading terms described above.

Letter grades A, B, C, which are designated grades for poultry, may only be used on poultry (whole birds and parts) that are officially graded and may not be used on red meat. Although poultry grade terms (U.S. grade A, etc.) are not allowed to be used on red meats, the terms prime, choice, and select may be used on poultry (whole birds or parts) that are equivalent to U.S. grade A. The use of a possessive, e.g., XYZ's Prime, does not relieve a company of this requirement. The use of quality grade terms on further processed meat and poultry products will be evaluated on a case-by-case basis to determine if they wrongly imply that the meat or poultry used in these products has been graded.

Terms which are subjective in nature, e.g., but not limited to, fancy, finest, super, supreme, ultimate, premium, greatest, best, old fashioned, homestyle, hotelstyle, deluxe, special, famous, and old time may be used unqualified on labels for meat and/or poultry products. The term "selected" as well as other terms, will be considered individually by the Labeling and Consumer Protection Staff, again to determine if these terms wrongly imply that the meat or poultry has been graded.

Quiche Products

The term "Quiche" does not have to be qualified to indicate it is a custard cheese pie. However, when characterizing ingredients, e.g., bacon, ham, chicken, onion, etc. are used either alone or in combination, the ingredients shall be either clearly identified as part of the product name or prominently displayed elsewhere on the principal display panel (PDP) of the label (e.g., Bacon Quiche, Ham and Onion Quiche, etc.). Similarly, the characterizing ingredients in Quiches bearing fanciful names shall be identified as part of the product name or highlighted elsewhere on the PDP (e.g., Quiche Bercy — made with ham and wine). Since "Quiche Lorraine" is widely recognized, the characterizing ingredients do not have to be identified as a part of the product name or elsewhere on the PDP.

Meat and poultry quiches must contain at least 8% cooked meat or poultry and sufficient cheese so that the combined total at least comprises 18% of the finished product. Quiche Lorraine must contain cooked bacon and/or ham and the only cheeses are Swiss and/or Gruyere.

If other characterizing ingredients (excluding cheese), e.g., onions, peppers, olives, etc., are used in addition to the meat or poultry ingredient in Quiche Lorraine or in any other quiche, the combination of these other characterizing ingredients and the meat or poultry ingredients must comprise at least 8% of the total product, and the cooked meat or poultry portion must be at least 5% of the total product.

Ranch

The regulations and policies applicable to "Farm" also apply to ranch.

Ravioli (Meat)

This product must contain at least 10% meat.

Rehydrated Dehydrated Vegetables

Rehydrated dehydrated vegetables acceptable as name. The specific vegetable must be identified in the ingredient statement.

Relleno De Papa (PR)

This product must contain 8% cooked meat. A Puerto Rican product that must show a true product name, e.g., "Potato Balls with Beef," or "Potato Dough with a Beef Filling."

Rendered Beef Fat Tissue Solids

The solid remains of a fat extraction process from beef that was ground and rendered by a high temperature (180°F) continuous wet rendering system.

See: Beef Gravies.

Rework

Rework is allowed in unlimited quantities when added to a like product. However, if breaded/battered rework is added to similar products, the rework is limited to 2%.

Rice and Meat

The product must contain at least 12% meat.

Roasted

The term "roasted" may be used to describe products that have been subjected to cooking methods that result in a roasted appearance.

Rolls

Six uses exist for the term "Roll" in conjunction with names for meat food products:

1. Items consisting of a solid piece of meat, e.g., "Boned Veal Rib," formed and tied as a roll and usually offered with seasonings.
2. Chopped meat in combination with condiments, also formed and processed. It can be and often is offered in the fresh meat state. Water is not an ordinary or usual ingredient in these two

"Meat Roll" items. If water is an ingredient in these products, then a statement indicating the addition of a solution has taken place must appear contiguous to the product name wherever it appears on the label.

3. "Sausage Rolls" have similar formulas and water limitations to cooked sausage. The finished product may contain up to 10% added water, is in roll shape, and is Cooked, or Smoked and Cured (species) Roll Sausages.
4. Non-descriptive rolls, e.g., "Pizza Roll," "Pickle Roll," "Relish Roll," etc., contain meat with cheese, peppers, pimentos, relishes, and other similar materials. An ingredients statement is required as a part of the product name on the basis of instructions in 9 CFR 317.2(c)(1) and (2), and 317.2(e).
5. Product made from meat and water that has been chunked, ground, chipped, wafer-sliced, etc., and formed into a roll containing a plant protein product or other binder could be labeled as a "Meat, Water, and Textured Vegetable Protein Roll." The same size lettering shall be used for the product name.
6. Product made from meat that has been chunked, ground, chipped, wafer-sliced, hydroflaked, etc., and formed in a roll containing a plant protein product or other binder shall be labeled as "Beef and Textured Vegetable Protein Roll" or "Beef and Soy Protein Concentrate Roll."

Rolls, Poultry

Only natural proportions of skin to the whole carcass or designated part may be used. If skin is in greater than natural proportions, the name must be qualified with the term "Skin Added."

See: 9 CFR 381.159.

Romano Cheese

Label must show "kind" of milk, e.g., (Caprino), "Romano Cheese made with Goat's Milk;" (Pecornia), "Romano Cheese made from Sheep's Milk;" or (Vaccino), "Romano Cheese made from Cow's Milk." The words in parenthesis are not required to be shown.

Rumaki

This product must contain at least 50% chicken livers. An hors d'oeuvre or appetizer. Rumaki is a combination of chicken livers, water chestnuts, and bacon.

Salad — Freeze Dried Ham

Antioxidants have been permitted in Freeze Dried Ham at a level of 0.01%, based on total weight of the ham.

Salad — German Style Potato Salad with Bacon

Requires at least 14% cooked bacon.

Salad Mix, Poultry

Product must contain at least 45% cooked poultry.

Salads

Standards for salads:

Meat salads must include at least 35% cooked meat or meat food product (e.g., corned beef, ham). Ingredients, e.g., "Ham water added" or "Corned Beef and water product" may be used if the formula is adjusted to account for the amount of added substances. Example: if 85% of a meat food product is meat, then 35% required meat divided by 0.85 equals 41% required meat food product in the salad.

Cobb Salad — Contains lettuce and chicken or turkey. The other ingredients that may be found include bacon, hard cooked eggs, tomatoes, Roquefort or other blue cheese or dressing. The product name must include the poultry component(s) and also identify any meat ingredient when present about 2%, e.g., "Bacon and Chicken Breast Cobb Salad."

Caesar Salad — is an acceptable product name and normally contains cheese, meat, or poultry pieces and may contain other vegetables.

Ham and Cheese Salad — Must contain at least 25% cooked ham.

Macaroni with ham or beef — Must contain at least 12% cooked meat.

Poultry Salad — Must contain at least 25% cooked poultry (natural proportions of skin and fat).

Chopped egg and ham salad — Must contain at least 12% ham.

Chopped egg and bacon salad — Must contain at least 12% bacon (9% fully cooked bacon).

Vegetable and/or fruit with poultry — Must contain at least 25% cooked poultry.

Cracker meal, bread crumbs, and similar ingredients may be included in meat or poultry salads up to 2% of the total formula. If more than 2% is used, a product name qualifier is required. Modified food starch and textured vegetable protein cannot be substituted for cracker meal and bread crumbs in salad products.

Salami

A dry sausage that requires an MPR of 1.9:1 or less. Extenders and binders are permitted. It may be cooked to shorten drying period.

Salami, Beef

A cooked, smoked sausage, usually mildly flavored, in a large casing, containing coarsely ground beef. Cereals and extenders are permitted. May contain fat. Product does not have to be labeled cooked.

Salami, Cooked

The product "Salami" must be labeled to include the word "Cooked," regardless of the type and size of its packaging, unless it is one of the following:

1. A salami with a moisture protein ratio of no more than 1:9 to 1
2. "Genoa salami" with a moisture protein ratio of no more than 2.3:1
3. "Sicilian salami" with a moisture protein ratio of no more than 2.3:1
4. Labeled, as…
   a. Kosher Salami
   b. Kosher Beef Salami
   c. Beef Salami
   d. Beer Salami
   e. Salami for Beer. Pork skins are not a permitted ingredient in cooked salami.

Salami, Cotto

A mildly flavored cooked, cured sausage, in a large casing, usually containing coarsely ground beef and pork. The product contains whole or visible pieces of peppercorns. It is cooked in dry heat.

Salami, Hard

A dry sausage with an MPR of 1.9:1. It is made with beef and pork and seasoned with garlic. Less highly flavored but usually more heavily smoked than Italian Salami. It is tied with loops or twine that gives a scalloped appearance.

Salami, Italian

This kind of dry salami is usually prepared in the San Francisco area and is easily distinguished by its covering of a white mold. This salami consists of about 80% finely chopped pork, to which a small amount of pork fat may be added. Nonfat dry milk can comprise $3\frac{1}{2}$% of the finished product. The remainder consists of chopped beef, seasoning, salt, and curing agent. The product should have an MPR not in excess of 1.9:1 to insure the fat content and dryness properties associated with a "dry salami."

Salchichon (SP)

This term, meaning "Large Sausage," This term may only be used for large casing sausage products that are 3 inches in diameter or more. Label must show a true product name.

Salisbury Steak

Finished product must contain at least 65% meat. Fat is limited to 30%. Other requirements are:

1. It is an unbreaded cooked product.
2. The meat block may contain 25% pork, with the remainder beef. Or, the meat block may contain up to 12% partially defatted chopped beef and pork.
3. Extenders are permitted up to 12%. When isolated soy protein is used, 6.8% is the equivalent of 12% of the other extenders. Those extenders include, but are not limited to: cereal, bread crumbs, cracker meal, soy flour, soy protein concentrate, isolated soy protein, and textured vegetable protein.
4. Meat byproducts are not permitted. Beef heart meat is permitted.
5. Permitted liquids include, but are not limited to: water, broth, milk, cream, skim milk and reconstituted skim milk (9 parts water to 1 part NFDM).
6. Product not cooked which conforms to the above may be labeled "Patties for Salisbury."

Salisbury Steak, Turkey

Product must contain at least 55% turkey meat in natural proportions (light and dark) or 65% turkey with skin and fat in natural proportions (skin 10%, turkey meat 55%). Maximum amount of binders and extenders is 12%.

Salpicao

A smoked sausage. The label must show a true product name, e.g., "Smoked Sausage." No more than 3% water can be added at formulation.

Salsiccia (IT)

A fresh pork sausage, highly spiced, in which paprika is permitted. It is a rope style sausage made of finely cut pork trimming.

Salt as a Cure

Dry processed hams, pork shoulders, and bacon are ordinarily cured with mixtures that contain mostly salt along with sugar and nitrates plus nitrites. However, some processors use salt alone in preparing their products. The salt in contact with the meat provides the desired cured color, taste, and necessary product protection.

Salt is an acceptable cure when used singly in the curing and salt equalization of dry processed hams, pork shoulders, and bacon. The cured products must have a 10% brine concentration.

Samosa

This product originated in India, although it is also associated with Pakistan. It resembles a "Meat Turnover" and consists of a spiced vegetable and meat mixture in a dough crust. At least 25% meat is required. Label must show a true product name, e.g., "Beef Turnover."

Samples

Free samples included along with the meat and poultry food products are not to be included in the net weight statement, and the ingredients do not need to be identified in the ingredients statement as long as the ingredients appear on sample package.

Sandalwood

According to FDA regulations, Red Saunders (red sandalwood) is not an acceptable ingredient in meat and poultry products. It is a permitted coloring and flavoring agent in alcoholic beverages only. In contrast, white or yellow sandalwood is acceptable in meat and poultry products as a flavoring agent in an amount that is "sufficient for purpose." White or yellow sandalwood extract may be labeled as "sandalwood extract" or "flavoring."

Sandwich — Closed

Product must contain at least 35% cooked meat and no more than 50% bread. Sandwiches are not amenable to inspection. If inspection is requested for this product, it may be granted under reimbursable Food Inspection Service.

Typical "closed-faced" sandwiches consisting of two slices of bread or the top and bottom sections of a sliced bun that enclose meat or poultry, are not amenable to the Federal meat and poultry inspection laws. Therefore, they are not required to be inspected nor bear the marks of inspection when distributed in interstate commerce.

Sandwich — Open

Must contain at least 50% cooked meat. Sandwiches are amenable only if they are open faced sandwiches. Product must show a true product name, e.g., "Sliced Roast Beef on Bread."

This regulatory policy in no way alters the Department's present policy with respect to caterers who include meat sandwiches in their dinners.

Sandwiches (Meat or Poultry as Components of "Dinner Products")

Dinners containing a sandwich type product, e.g., a frankfurter, hamburger, or sliced poultry meat with a bun, are amenable and subject to inspection.

Santa Fe Style

Acceptable for products that contain chilies with corn or beans and one of the following ingredients: Cheese (jack, cheddar, Mexican Style or fresh goat), bell pepper, onion, garlic, tomatoes, tomatillos, cumin, oregano or cilantro. The beans should be either black, kidney, navy, pink, pinto, red, or white beans or an indigenous variety.

Sarno

A dry smoked sausage that is air dried. The label must show a true product name, e.g., "Smoked Sausage." Coarsely chopped beef, pork, and garlic are not permitted.

Satay

This term refers more to a preparation method than to the nature of a finished product. Satay can be made from chicken, beef, lamb, pork, and other food items, and prepared in two ways:

1. Meat is cut into one inch cubes, then dipped into a spicy sauce, skewered, and roasted over an open fire (similar to "Kebobs" except no vegetables or fruit). Label must show a true product name, e.g., "Beef Cubes on Stick."
2. Meat is cut into one inch cubes, then dipped into a spicy sauce and canned. Label must show a true product name, e.g., "Beef Cubes in Spicy Sauce."

Sauce with Meat or Meat Sauce

Product must contain at least 6% ground meat.

Sauerbrauten (GR)

"Sauerbraten" must contain at least 50% cooked beef. "Gravy with Sauerbraten" must contain at least 35% cooked meat. Sauerbrauten is cooked beef in a vinegar flavored sauce. The beef is marinated in vinegar sauce, then separated from the sauce and partially cooked, and put back in the sauce and cooked completely.

Sauerkraut Balls with Meat

Product must contain at least 30% meat or meat food product.

Sauerkraut with Franks and Juice

Product must contain at least 20% franks.

Sausage Classification

1. Fresh Sausage

Made of fresh, uncured meat, generally cuts of fresh pork, and sometimes beef. Its taste, texture, tenderness, and

color are related to the ratio of fat to lean. Trimmings from primal cuts, e.g., pork, loin, ham, and shoulders are often used. When ice or water is used to facilitate chopping and mixing, it is limited to a maximum of 3% of the total formula. It must be kept under refrigeration and thoroughly cooked before serving. Bratwurst is in this class. Binders and extenders are permitted in fresh sausages except where regulations do not permit the use of such ingredients, i.e., 9 CFR 9 CFR 319.140 (Pork Sausage), 9 CFR 319.142 (Beef Sausage), 9 CFR 319.144 (Whole Hog Sausage), and 9 CFR 319.145 (Italian Sausage).

See: 9 CFR 319 Subpart E.

2. Uncooked Smoked Sausage

Has all the characteristics of fresh sausage except it is smoked, producing a different flavor and color. It must be thoroughly cooked before serving. "Smoked Pork Sausage" is included in this class. If it is a mixture of pork and other meats, regardless of size, it must be treated for trichinae.

See: 9 CFR 319 Subpart F.

3. Cooked Sausages and/or Smoked Sausages

These products are chopped or ground, seasoned, cooked and/or smoked. Added water is limited to 10% of the finished product. Meat byproducts may be used when permitted by standard. Cure is required for particular sausages, e.g., wieners or Polish sausage. These sausages come in various shapes and sizes, e.g., short, thin, long and chub. Cotto salami, liver sausage, and cooked weisswurst are included in this category. Wieners, bologna, knockwurst, etc., are also in this class but are further distinguished by a fat and moisture limitation.

See: 9 CFR 319.180.

4. Dry and Semi-Dry Sausages

Dry sausages may or may not be characterized by a bacterial fermentation. When fermented, the intentional encouragement of a lactic acid bacteria growth is useful as a meat preservative as well as producing the typical tangy flavor.

The meat ingredients, after being mixed with spices and curing materials, are generally held for several days in a curing cooler. Afterward, the meat is stuffed into casings and is started on a carefully controlled air-drying process. Some dry sausage is given a light preliminary smoke, but the key production step is a relatively long, continuous air-drying process.

Principal dry sausage products are salamis and cervelats. Salamis are coarsely cut, cervelats finely cut with few exceptions. They may be smoked, unsmoked, or cooked. Italian and French dry sausage are rarely smoked; other varieties usually are smoked.

Dry sausage requires more production time than other types of sausage and results in a concentrated form of meat. Medium-dry sausage is about 70% of its "green" weight when sold. Less dry and fully dried sausage range from 80% to 60% of original weight at completion.

Semi-dry sausages are usually heated in the smokehouse to fully cook the product and partially dry it. Semi-dry sausages are semi-soft sausages with good keeping qualities due to their lactic acid fermentation.

Although dry and semi-dry sausages originally were produced in the winter for use in the summer and were considered summer sausage, the term "summer sausage" now refers to semi-dry sausages, especially Thuringer Cervelat.

Sausage Containing Cheese

Sausages may contain cheese under the following conditions:

1. If there is a standard for that particular sausage, it must be met as though it contained no cheese.
2. The cheese must characterize the product and appear as part of the product name. Example "Italian Sausage with Cheese."

Sausage — Liquids Added

Sausages containing fluid ingredients that are expected such as fruit and juice and vinegar, are permitted at any level as long as the product is descriptively labeled. The sausage portion of the product, however, must meet any applicable standard. Vinegar is an expected ingredient in chorizos and the name does not have to indicate its presence.

Sausage — Shelf Stable

Dry sausage must have a Moisture Protein Ratio (MPR) of 1.9:1 or less, unless an MPR is cited under Moisture Protein Ratio.

Non-refrigerated, semi-dry, shelf-stable sausage must have an MPR of 3.1:1 or less and a pH of 5.0 or less, unless commercially sterilized or unless an MPR is cited under Moisture Protein Ratio. Alternately, non-refrigerated, semi-dry, shelf-stable sausages are those that:

1. are fermented to a pH of 4.5 or lower (or pH may be as high as 4.6 if combined with product water activity no higher than 0.91),
2. are in an intact form or, if sliced, are vacuum packed,
3. have internal brine concentration no less than 5%,
4. are cured with nitrite or nitrate, and
5. are smoked with wood.

Sausage Rework

This term applies to a fully or partially processed product (excluding uncooked trimmings) re-routed for reasons

other than unwholesomeness or adulteration (i.e., emulsion residue, product breakage, slicing operations, smoked meats, returns, etc.) and intended for inclusion in cooked sausage, loaves, and similar products. Rework may be used provided it does not adulterate the product, violate its standard of composition, change the order of predominance of ingredients, or perceptibly affect the normal characteristics of the product. Rework is subject to the following restrictions:

1. Cooked sausage, meat loaves may be used in similar products without limitation.
2. Except in products covered by section 9 CFR 319.180, pieces of cooked and/or smoked meat may be used without limitation if properly identified in the ingredients statement.
3. Pieces of uncooked, cured pork from primal parts may be used without limitation if properly identified in the ingredients statement.
4. Sausage products in edible collagen casings may be used in similar finely comminuted products without limitation and need not be peeled.
5. Finished cooked sausage in natural casings may be used in similar finely comminuted products without limitation, except sausages in bungs, middles, beef rounds, bladders, or stomachs, which must be stripped of the casings before use. Also, natural casings of any type that break during the stuffing operations should not be included in emulsions.

Sausage Type Products with Fruits and Vegetables

Sausage type products that contain unexpected ingredients that significantly alter the character of the product may be descriptively labeled as (characterizing ingredient) Sausage, e.g., "Cherry Pecan Sausage," "Wild Rice Sausage," or other equally descriptive names, e.g., "Sausage with Wild Rice."

The sausage portion of fresh sausage products must meet any applicable standards, including fat and added water limitations, moisture/protein ratios, and use of binders and extenders prior to the addition of any characterizing ingredient(s). For cooked, smoked, or dry sausages, the finished sausage type product must meet the sausage standard prior to the addition of any characterizing ingredients.

The unexpected ingredient must be present in sufficient quantity or form to characterize the sausage type product in flavor, texture, or other sensory attributes. However, there are no minimum use levels.

This policy applies to products containing unexpected food ingredients, e.g., fruits and vegetables, e.g., cherries, pecans, tomatoes, etc., that change the character of the product by the addition of unique flavor and other sensory characteristics. The policy does not apply to imitation products, i.e., products formulated to resemble in taste, texture, color, etc., the traditional sausage products, but which are nutritionally inferior. Sausages containing cheese are addressed Policy Memo 010, and Potato Sausages are addressed in Policy Memo 011.

Sausage with Sauerkraut in Sauce

Product must contain at least 40% sausage.

Sausage (Species)

(Species) sausages identified in 9 CFR 319.141, 319.142, 319.144, and 319.160 of the meat inspection regulations may be cooked, cured or smoked (or any combination), but must comply with the standards before being processed if the product name is to include "(species) sausage." For example, fresh beef sausage identified in 9 CFR 319.142 which is cured and cooked may be labeled "cured, cooked beef sausage." Prior to this processing, these products could not contain more than the 3% water permitted by the standard.

Cooked cured sausages or smoked cured sausages containing up to 10% added water in the finished product and prepared from one species may be labeled as "cooked cured sausage," "smoked sausage," "cooked cured sausage made with (species)," or "smoked sausage made with (species)."

Semi-dry and dry sausages made from a single species may be labeled "(species) sausage," e.g., "beef sausage."

This policy does not apply to cooked sausages identified in section 9 CFR 319.180 of the meat regulations.

Scalloped Potatoes and Ham

Product must contain at least 20% cooked ham.

Scalloped Potatoes and Sausage

Product must contain at least 20% cooked sausage.

Scalloped Potatoes Flavored with Sausage

Product must contain at least 3% sausage.

Scalloppini

Product must contain at least 35% cooked meat or poultry meat. Thin slices of cooked veal, sometimes beef or poultry, seared or fried. Label must show a true product name, e.g., "Veal Scalloppini" or "Chicken Scalloppini."

Schickenwurst (GR)

The product is made of two parts, one of which is an emulsion prepared from pork and beef cuts. The second component consists of chunks of ham measuring from 2 to 3 inches in size. The two parts are mixed, stuffed into large casings, and smoked while being cooked. The final product appears as a luncheon sausage with large pieces of red

ham meat held together by a light pink binder. The ham sections comprise at least 50% of the product and the item has a distinct smoked flavor. This product is very similar in appearance to the product sold as "Ham Bologna."

Scrambled Eggs with Bacon

Product must contain at least 10% cooked bacon.

Seaweed

The term is not an acceptable ingredient declaration. There are many types of seaweed, some are not as safe.

Select or Higher

This phrase has been extended to retail beef products that have been officially graded prime, choice or select.

Shepherds Pie (with or without Vegetables)

Product must contain at least 25% meat in total formulation. Shepherds Pie is a meat food product consisting of chopped, minced, or cubed beef or lamb, seasoned with gravy or sauce, with or without vegetables, and baked with a covering layer or surrounding border of seasoned mashed potatoes. The label must show a true product name, e.g., "Beef Shepherds Pie."

Shipping Containers

A mark of inspection and a handling statement is required on all shipping containers. Safe Handling Instructions are required with all other required features only when they shipping container is also the immediate or primary container.

See: 9 CFR 316.13.

Shu-Mai

Product must contain at least 10% meat. A Chinese product that resembles a dumpling. It is similar to a meat ravioli. The label must show a true product name, e.g., "Pork Dumpling."

Signature Line

It is not necessary to include the term "General Office" in signature lines on labels used by companies with multiple plant operations. A zip code shall appear following the address.

See: 9 CFR 317.2(g)(1) 9 CFR 381.122.

Sloppy Joe

A coined name that must be qualified by a true product name, e.g., "Barbecue Sauce with Beef." The meat content depends on the name of the product. Heart meat and tongue meat can be used but not to satisfy the minimum meat requirement.

Smoke

For imported Canadian products, e.g., bacon, which are physically smoked during processing, the word "Smoke" is acceptable in the ingredients statement. Although not required or customary, smoke can also appear in the ingredients statement of domestically produced products which are physically smoked. If included in the ingredients statement, smoke should appear as the last item.

Smoke Flavoring

The use of smoke flavoring (natural or artificial) in a component of a meat or poultry food product, e.g., ham in a ham salad, does not require that the product name be qualified to indicate the presence of the smoke flavoring. However, the smoke flavoring must be declared in the ingredients statement on the meat or poultry product labels.

Secondary product — when meat and extender product is produced using a meat product in which smoke flavoring is added, the secondary product name does not have to be qualified with a phrase as "smoke flavoring added."

When smoke flavor (natural or artificial) has been directly added to a product as part of a seasoning mix, the presence of the smoke flavor must be identified in a qualifying statement to the product name, e.g.,

1. "Chicken soup smoked flavor added," and in the ingredients statement.
2. "Beef soup smoke flavor."
3. If a product is simply sprayed with liquid smoke, it must be labeled "smoke flavoring added."

Smoked Products

The guidelines for approving labels for products prepared with natural smoke and/or smoke flavor (natural or artificial) are as follows:

1. Meat or poultry products which have been exposed to smoke generated from burning hardwoods, hardwood sawdust, corn cobs, mesquite, etc., may be labeled as "Smoked" or with terms, e.g., "Naturally Smoked" to indicate that the traditional smoking process is used.
2. Meat or poultry products which have been exposed to natural liquid smoke flavor which has been transformed into a true gaseous state by the application of heat or transformed into vapor by mechanical means, e.g., atomization, may be labeled "Smoked."
3. Meat or poultry products may be labeled "Smoked" if natural liquid smoke flavor is applied by spraying, dipping, liquid flooding,

or similar processes prior to or during heat processing. In such cases, the natural liquid smoke flavoring must be transformed into a true gaseous state by the heat of processing. If a product is simply sprayed with liquid smoke it must be labeled "smoke flavoring added."

4. Meat or poultry products to which smoke flavor (natural or artificial) has been directly applied to the exposed product surface, e.g., massaging or margination, or incorporated into the product by such means as injection, must be labeled to identify the smoke flavor as part of the product name, e.g., "Ham-Natural Smoke Flavor Added," and in the ingredients statement.
5. Meat or poultry products that are smoked, as provided for in (1), (2) and (3) above and also treated with smoke flavor as described in (4), may only be labeled "Smoked" or with terms, e.g., "Naturally Smoked," if it is clearly disclosed that the product is also treated with smoke flavor. The presence of the smoke flavor must be identified as part of the product name, e.g., "Smoked Ham-Smoke Flavoring Added" and in the ingredients statement.
6. Product may be labeled as "hickory smoked" only if the plant provides the inspector with appropriate certification that such sawdust or wood for smoking is 100% hickory.

Smoked Thuringer Links

A cooked smoked sausage made with pork only.

Snacks (Hors d'oeuvres)

Product must contain at least 15% cooked meat or 10% cooked bacon. The label must show a true product name, e.g., "Liver Pate on Toast."

Sodium Alginate

This is added as a binder in "Taquitos." Approval may be given for use at a level of less than 1% with .25% of calcium citrate to stabilize a pizza sauce or pizzas heated in household toasters.

Sodium alginate when used as glue to seal burrito and burrito like products is acceptable, if declared in the ingredients statement, or if a statement such as "sealed with sodium alginate" appears at the end of the ingredients statement.

Sodium Benzoate

Sodium Benzoate is not an acceptable ingredient for meat and poultry products, except in oleomargarine. It is accepted as an incidental additive when it is a part of a product prepared under FDA rules, e.g., sauces, gravies, and similar substances.

Sofrito with Pork

This is a sauce containing 6% smoked pork.

Soppresate (IT)

This is an acceptable name for a dry salami with an MPR of 1.9:1. This is an Italian salami that is lightly flavored with garlic and, generally, hotly seasoned with paprika and black or red peppers. It is smoked to varying degrees depending on regional tastes.

Sorbitol

This is only permitted in 9 CFR 319.180 products, cured pork products, dried beef, kielbasa and products similar to kielbasa. Do not approve when used in other products.

Souffle (Species) or (Kind)

Product must contain at least 18% cooked meat or poultry meat.

Soujouk (TK)

This is a Turkish sausage made from beef which is very dry and highly spiced with an MPR of 2.04:1. The product is usually flattened or resembles a dry salami or ring bologna. The label must show a true product name, e.g., "Dried Beef Sausage."

Soup

1. Soups that declare meat stock in the product name are meat food products and shall contain at least 25% meat stock with an MPR of not less than:
   a. Condensed soup — 67:1
   b. Ready-to-eat — 135:1
   c. Beef Bouillon — 67:1 and at least 50% beef stock
2. Soups made with meat shall contain not less than:
   a. Condensed soup — 4% cooked meat
   b. Ready-to-eat — 2% cooked meat
3. Soups containing smoked meats shall contain not less than:
   a. Condensed soup — 4.0% smoked meat
   b. Ready-to-eat — 2.0% smoked meat
4. Soups made with cooked sausages shall contain at least 4% cooked sausage.

Soup Products

Bean & Ham Shank: When soup is made from ham shanks, they must be shown in the true product name, e.g., "Bean and Ham Shank Soup."

Blood: Product must contain at least 1% blood and be made under inspection.
Chowders: Follow standard for soups.
Consomme: A broth cooked with vegetables and then strained. Must have an MPR of 135:1.
Consomme Instant: Dehydrated — not amenable.
Cream: Condensed cream soups may be made from various creams, whole milk, or dry milk powder. The amount of cream, whole milk, or dry milk powder should provide a minimum of .45% butterfat to the final product. Examples:

1. A cream containing 18% butterfat should make up the product formulation; this provides .45% butterfat to the product formulation.
2. Dry milk powder containing 27% butterfat should make up 1.67% of the product formulation.

Dried Meat Soup Mixes: Not amenable.
Italian Style Minestrone: Soup must contain zucchini. Identify meat in the true product name.
Pepper Pot: Soup must contain at least 20% scalded tripe.
Petite Marmite (FR): A soup made with meat, chicken, and vegetables.
Scotch Broth: Soup must contain at least 3% mutton in a thick mutton broth.
Vegetable: Vegetable soups made with soup stock are not considered amenable.

Souse

This is a nonspecific product that can be made with all pork byproducts. The ingredients statement directly follows the product name.

Southern Fried

Southern fried poultry cuts or patties are breaded and fried. This is not geographical.

Southwestern Style

An acceptable identification for products containing any five (5) of the following types of food ingredients:

Beans (kidney beans, black beans, pinto beans, red or pink beans), corn, chili peppers, bell peppers, cheddar cheese, cilantro, onions or onion powder, cumin, oregano, garlic or garlic powder, paprika, chili powder, either mesquite smoked, or mesquite smoke flavor added.

Soy Protein Products

Whenever soy flour, defatted soy grits, soy protein concentrate, isolated soy protein, and similar products are used as ingredients of meat and poultry products, they must be called by their common or usual name (e.g., soy flour, soy protein isolate, etc.).

Two percent isolated soy protein is equivalent to 3.5% binders.

If these products are textured, then "textured" should also be included in the name. We allow the use of the term "textured vegetable protein" when the textured soy products are mixed with spices, colorings, enrichments, etc., and the ingredients of the textured vegetable protein are listed parenthetically. "Vegetable Protein Product" is an acceptable declaration for a soy product fortified in accordance with Food and Nutrition Service regulations. The ingredients of the VPP must be listed parenthetically.

Spaghetti

Sauce with meatballs Sauce with meat with meatballs with meatballs & sauce with meat and sauce with franks and sauce.

Must contain at least 35% cooked meatballs
Must contain at least 6% meat
Must contain at least 12% meat
Must contain at least 12% franks

Spaghetti Sauce with Meat Stock

This spaghetti sauce consists mainly of tomatoes with seasoning. Product must contain 5% fresh beef and 12.5% concentrated meat stock.

Spaghettios in Cheese Sauce with Ground Beef

Product must contain at least 12% meat.

Spanish Rice with Beef

Product must contain at least 20% cooked beef.

Speckwurst

Product should conform to sausage standard (9 CFR 319.140) without the use of byproducts. Chunks of fat are usually present.

Starch

Starch, wheat starch, and cornstarch are synonymous in meaning. When "Vegetable Starch" is used as a designation, it refers to the starchy materials derived from any vegetable source, e.g., potatoes, peas, etc. Tapioca starch cannot be declared as "starch."

See: Tapioca Starch.

Steak, Chinese Pepper

Product must contain at least 30% cooked steak. A Chinese dish usually served with rice. Beef steak is cut in

thin strips, browned, and added to a sauce. Vegetables are also added to the sauce; green pepper strips are always used, and other vegetables may include celery, onions, scallions, red pepper, bean sprouts, tomatoes, or water chestnuts.

Steak, Country Style

This term is popular in the Southern region of the country. It resembles a "Gravy and Swiss Steak" product. Characteristics of this product are:

1. It is prepared from the steaking portions of beef (usually from the round) and braised.
2. The meat is mechanically "tenderized" and floured prior to browning.
3. The meat is browned by sautéing or oven browning, but not flame browned nor cooked in water.
4. When a true product name is shown as "Gravy and Beef Steak," at least 35% cooked steak must be used.
5. When a true product name is shown as "Beef Steak with Gravy," at least 50% cooked steak must be used.

Steak, Pepper

Product must meet the standard for "Fabricated Steak" in 9 CFR 319.15(d) and contain green and/or red peppers.

Sticks

There are three types of meat or poultry sticks.

1. Meat Sticks, which are an extended "pattie-like" product and are usually breaded. No more than 10% extenders and 30% breading are permitted. When whole egg, tomato, and nonfat dry milk are used, they must appear as added ingredients in the true product name, e.g., "BREADED MEAT STICK — NONFAT DRY MILK ADDED.
2. The infant finger food type of sticks is usually packed in jars. It conforms to the sausage standard and must show a true product name, e.g., "Meat Stick."
3. Nonspecific dry or semi-dry sticks that do not meet the sausage standard must be followed by the ingredients statement. If products meet the sausage standard, they may be identified as "Smoked Sausage."

Stroganoff, Meatball

Product must contain at least 45% cooked meatballs. Sauce portion shall comply with the Stroganoff Sauce standard.

Stroganoff Sauce

The sauce must contain at least 10% sour cream or a combination of at least 7.5% sour cream and 5% wine, or 2% sour cream, 2½% wine, and 9½% whole milk.

Stroganoff Sauce with/and Beef

Product must contain at least 31% beef or 21% cooked beef based on the total weight of the product, with sauce portion complying with the stroganoff sauce standard.

Stroganoff Sauce with/and Meatballs

Product must contain at least 31% cooked meatballs. Sauce portion shall comply with the stroganoff sauce standard.

Stromboli (IT)

Product is not considered a traditional sandwich. Minimum meat requirement is 25% fresh or 18% cooked meat. The label must show a true product name, e.g., "Pepperoni and Cheese Wrapped in Dough."

Stuffed Cabbage with Meat in Sauce

Product must contain at least 12% meat or at least 8% cooked poultry.

Stuffed Peppers with Meat in Sauce

Product must contain at least 12% meat or at least 8% cooked poultry.

Sukiyaki

Product must contain at least 30% beef. Sukiyaki consists of cut up vegetables, e.g., mushrooms, leeks and celery, which are cooked briefly with thin slices of beef and soy sauce.

Sulfiting Agents

The presence of sulfiting agents (sulfur dioxide, sodium sulfite, sodium bisulfite, potassium bisulfite, sodium metabisulfite, and potassium metabisulfite) must be declared on the label if their concentration in the finished meat or poultry food product is 10 PPM or higher. However, some finished meat and poultry food products may be comprised of multiple separable components, e.g., potatoes or apple cobbler in frozen dinner. For these products, if a separable component contains 10 PPM or more sulfiting agents, the sulfiting agents must be declared even though the total product contains less than 10 PPM of sulfiting agents. When sulfiting agents are required to be declared under conditions described above, their declaration shall be according to the following:

1. Sulfiting agents shall be declared by their specific name or as "sulfiting agents."

2. Declaration shall be in the ingredients statement in order of predominance or at the end of the ingredients statement with the statement "This Product Contains Sulfiting Agents" (or specific name(s)).
3. When the total product contains less than 10 PPM, but a separable component contains 10 PPM or more, the sulfiting agent must be declared as part of the component according to (1) and (2) above.

Summer Sausage

Product may be a semi-dry or cooked sausage. Meat byproducts and extenders are permitted.

Swiss Steak

Swiss Steak and Gravy: Contains not less than 50% cooked beef.

Gravy and Swiss Steak: Contains not less than 35% cooked beef.

Product labeled "Swiss Steak" must be floured or dusted before searing, or may have flour added to gravy.

Sweet and Sour Pork, Beef or Poultry

Product requires at least 25% meat or poultry meat, or 18% cooked meat or poultry meat. Product also requires sufficient traditional sweet and sour ingredients (fruit, fruit juices, vinegar, etc.) to impart sweet and sour characteristics.

Szechwan Style

Acceptable identification for any product containing one item from three or the four groups below.

1. Soy sauce
2. Spring onions, scallions or leeks
3. Garlic, ginger, ginger root
4. Chili, Szechwan peppercorn, Chili oil

Taco

Product must contain at least 15% meat.

Taco Filling

Product must contain at least 40% fresh meat. The label must show true product name, e.g., "Taco Filling with Meat," "Beef Taco Filling," or "Taco Meat Filling."

Taco Filling, Kind

Product must contain at least 40% raw poultry meat.

Tags, Tissue Strips, Brands

When tags, tissue strips, brands, etc. are used to apply ingredients statements on sausages and other products in casings or link form, the only additional marking required is the official inspection legend. However, if other features are added, e.g., the product name, all applicable required labeling features are required.

See: 9 CFR 316.10.

Tallow

Acceptable product name for the meat food product consisting of rendered beef fat or mutton fat or both.

Tamales

Product must contain at least 25% meat. Tamales prepared with meats other than beef and/or pork must include them in the product name, e.g., "Chicken Tamale" or "Chicken and Beef Tamale."

See: 9 CFR 319.305.

When inedible wrappings are used, they must be indicated:

a. In the product name, e.g., "Beef Tamale Wrapped in Corn Husk."
b. As a qualifier to the product name, e.g., "remove parchment paper prior to eating," or
c. As information in the preparation instructions, e.g., "remove the inedible covering prior to serving." The wrapper cannot be included as part of the net weight.

Filling — must contain at least 40% beef.

Pie — must contain at least 20% fresh meat. Filling must be at least 40% of the total product.

(kind) — must contain at least 6% poultry meat.

(kind) With sauce or gravy — must contain at least 5% poultry meat.

(species) — must contain at least 25% meat.

(species) With sauce or gravy — must contain at least 20% fresh meat.

If byproducts are used, their presence must be included in the product name.

Tapioca Product

Tapioca flour — can be used as a binder in some products in which "starchy vegetable flour" is permitted, as long as it is declared as tapioca flour.

Tapioca starch — can be used as a binder in some meat and poultry products in which "vegetable starch" is permitted as long as it is declared as "tapioca starch or food starch." Tapioca starch cannot be declared as "starch."

Taquitos

A Mexican dish requiring at least 15% meat. Cooked meat product is cut into strips or shredded and placed in center of tortilla. The tortilla is then rolled around the filling.

Tasajo Salted Beef (SP)

MPR not to exceed 2:1. Product is stitch pumped and cured in salt brine for 72 hours or more after which it is dried with circulated warm air for a period of at least 20 days. If the item is dipped in a tallow mixture, a statement must be shown contiguous to the product name identifying the constituents of the dipping mixture.

Teawurst or Teewurst

A cooked or uncooked product processed with or without curing and cold smoked 2 to 5 days. It is ground or coarsely chopped and is characterized by a soft spreadable texture. Typical meat ingredients include: pork, beef, pork bellies, and bacon. Fresh pork bellies may be used in place of pork fat and bacon.

Tempura

A Japanese dish consisting of shrimp, fish, vegetable, meat, poultry etc., each dipped in an egg batter and deep fried. The label must show true product name, e.g., "Chicken Tempura," "Pork Tempura," etc.

Teriyaki, Meat or Poultry

Cubes or slices of meat or poultry meat which have been marinated in a sauce containing soy sauce, some kind of sweetener, and usually ginger, garlic, or wine. When the marinated product is combined with additional sauce the product name must reflect the sauce; for example, "Beef Teriyaki with Sauce."

See: **Teriyaki Products** when product has not been cooked.

Teriyaki Products

Meat and poultry teriyaki products are not required to be cooked, provided a prominent statement is on the principal display panel informing the consumer that the product is not cooked. Example: "Ready to Cook," "Raw," and "Ready to Bake."

Tetrazzini, Poultry or Beef

Product must contain at least 15% cooked poultry or cooked beef. Made with diced cooked poultry or meat in a rich cream sauce containing sherry. This is added to cooked spaghetti or noodles in a casserole. Usually topped with bread crumbs or grated cheese.

Textured Vegetable Protein (Textured Vegetable Protein Product) for Cooked Meat and/or Poultry Meat

If the cooked meat and/or poultry meat to TVP ratio exceeds 9:1, then the TVP is declared by its common or usual name in the ingredients statement only.

If the cooked meat and/or poultry meat to TVP ratio is less than 9:1 but at least 7:1, the label must contain a qualifying phrase contiguous to the product name, e.g., "Chicken Salad, Textured Vegetable Protein Added."

If the cooked meat and/or poultry meat to TVP ratio is less than 7:1, the TVP must be shown in the product name, e.g., "Chicken and Textured Vegetable Protein Salad."

Textured Vegetable Protein (TVP) Products — Fresh Meat or Poultry Meat Ratios

The following guidelines and labeling requirements have been established regarding use of TVP in products other than patties and pizza toppings.

If the ratio of fresh meat or poultry meat to TVP is greater than or equal to 13:1, the TVP product is not considered to be characterizing or deceptive, e.g., 40% fresh meat: 3% textured soy flour = 13.3:1, and the TVP only needs to be shown in the ingredients statement only.

If the ratio of fresh meat or poultry meat to TVP product is less than 13:1 but greater than or equal to 10:1, the TVP is characterizing and must be shown contiguous to the product name, e.g., "Hot Dog Chili Sauce made with Beef Textured Vegetable Protein added."

Thai Style

Acceptable identification for products containing at least five of the following:

Basil, chilies or chili products, cilantro, coconut or coconut products, coriander, cumin, fish sauce, galangal, garlic, ginger, green onions, jasmine rice, lemon grass, peanuts or peanut products, rice noodles, shallots, or soy sauce.

Thuringer

Usually classed as a "Semi-Dry" sausage with an MPR of 3.7:1. It is usually smoked and complies with the following factors:

1. Pork fat as such may comprise up to 10% of the total ingredients.
2. Heart meat (Beef or Pork) may comprise up to 50% of meat ingredients.
3. Tongue meat (Beef or Pork) may comprise up to 10% of meat ingredients.
4. Cheek meat (Beef or Pork) may comprise up to 50% of eat ingredients.

5. No binders or extenders are allowed.
6. "Cooked Thuringer" can contain up to 10% added water.
7. Acceptable product names for uncooked Thuringer include: "Beef Summer Sausage — Thuringer Cervelat" and "Summer Sausage — Thuringer Cervelat."

Tips

Is the sub-primal of the beef round and is often referred to as the "Sirloin Tip." If the term "Tips" is used for other than from the "Sirloin Tip," it must be qualified as to the specific part of the primal such as "Beef Ribs Tips."

Titanium Dioxide

When Titanium Dioxide is used in poultry salads, a qualifying phrase should appear under the product name stating that the product has been "Artificially Whitened" or "Artificially Lightened."

Tocino

Spanish word for salt pork or bacon. Except in Puerto Rico, must show and use true product name in English, e.g., bacon, salt pork.

Tocino, Poultry

A fanciful name for a tocino product made from poultry. The fanciful name must be followed by a true descriptive product name, e.g., "Chicken Tocino, Sliced, Marinated, Cured Chicken Thigh Meat."

Tocino (Filipino or Philippine Style)

The thinly sliced piece of meat taken from either the hind leg or shoulder portion of the pork carcass. The product is treated with salt, sugar, and nitrite and/or nitrates, with optional ingredients of ascorbic acid, spices, monosodium glutamate, and phosphates. Acceptable color agents are annatto, beet powder, and paprika that must be shown as "artificially colored." A true product name must be shown on the label, e.g., "Sliced Marinated Cured Pork Shoulder Butt, followed by the solution statement."

Tocopherol

May be listed as "Topcopherol (Vitamin E)" on the label but not "Vitamin E (Tocopherol)." Tocopherol and Vitamin E are not synonyms. Also, acceptable in rendered or unrendered fat.

Tomato and Bacon Spread

Product must contain at least 25% cooked bacon.

Tongue Trimmings

Labeling terminology for the various kinds of tongue and cheek trimmings shall be as follows:

1. "(Species) tongue trimmings" shall be used to identify all tissues except cartilage and bone that are obtained by converting long-cut to short-cut tongues. This conversion is done by making a transverse cut anterior to the epiglottis, removing the soft palate and epiglottis, and cutting through the hyoid bone. Approximately $1\frac{1}{2}$ inches of the bone is left with the tongue. "(Species) tongue trimmings" may also be used to identify salivary glands, lymph nodes, and fat from which the muscle tissue has not been removed.
2. "(Species) salivary glands, lymph nodes, and fat (tongue)" must be preceded by the name of the species from which derived. Tongue meat should not include any tissues described in paragraph 2.
3. Trimmings from the tongue itself should be identified as "tongue meat" preceded by the name of the species from which derived. Tongue meat should not include any tissues described in paragraphs 2 and 3 above.
4. Trimmings with fat from tongue is acceptable ingredient in cooked sausage products covered under section 9 CFR 319.180 of the regulations. Lymph nodes and salivary glands are not acceptable ingredients.

Topping — (Species) or (Kind)

Topping is an acceptable product name for a nonspecific product containing the species or kind indicated as well as various other ingredients. The ingredients statement must follow the product name.

See: Pizza Topping Mix.

Tortellini with Meat

Product must contain at least 10% meat.

Tortilla with Meat

Product must contain at least 10% meat. Tortilla is a thin, flat unleavened masa cake which is baked on both sides.

Tostada with Meat

Product must contain at least 15% meat. A tortilla is usually topped with refried beans, meat, cheese, and fresh vegetables.

Touristen Wurst

A semi-dry type of sausage. The MPR must not exceed is 3.7:1.

"Tropic Cure" Pork Products

Pork products when ready for shipment from the official establishment must have a moisture protein ratio not in excess of 3.25:1, and a salt content not less than 6%.

Truffles

Meat food product, e.g., "Liver Pate with Truffles" or "Sandwich Spread with Truffles" would be expected to be prepared with at the least 3% truffles. Labels of product containing less than 3% truffles should indicate the amount of truffle content in the name, e.g., "Liver Pate with 2% truffles." If the name does not feature truffles and they are mentioned only in the list of ingredients, we have no minimum requirement, provided the illustration does not show truffles.

Turkey Braunschweiger

The product name must be shown on the label as "Turkey Liver Sausage." No byproducts other than liver are permitted in the product.

Turkey Chops

Turkey chops are prepared by cutting the frozen breast into slabs with each cut being made perpendicular to the long axis of the keel bone (sternum). The larger slabs are split in half through the center of the sternum, resulting in two individual servings of meat with a piece of bone on one side and a thin layer of skin on the other. The smaller pieces at each end of the breast are left intact as individual servings. The word steak is unsuitable because a turkey steak is boneless by definition.

Turkey Ham Products Containing Added Water

Product otherwise conforming to the standard for turkey ham under section 9 CFR 381.171 of the poultry products inspection regulations but weighing more than the original weight of the turkey thigh meat used prior to curing shall be descriptively labeled as follows:

1. The product name must include in addition to "Turkey Ham," words that specify the amount of the additional substances, e.g., "and % Water," "With % Water Added," or "Turkey Ham and Water Product % of Weight is Added Ingredients." (The ingredients of the added solution may be incorporated into the product name, e.g., "Turkey Ham and Water Product % of Weight is Added Water, Salt, Dextrose, Sodium Phosphate, and Sodium Nitrite.") The blank is filled in with the % determined by subtracting the original weight of the turkey thigh meat from the weight of the cooked finished product. "Turkey Ham and 12% Water" is an example.
2. In retail and non-retail size packaging, the qualifying statements described in 1 above must be shown in lettering that is either not less than three-eighths inch in height or is at least one-third the size of the letters used in the product name and in the same color and style and on the same background as the product name. Full length of the product labeling is not required.
3. The "Turkey Ham" portion of the product name must be qualified with the statement "Cured Turkey Thigh Meat" in the manner described in 9 CFR 381.171(e). This may be effected by using an asterisk as long as there is no type or other designs between the total product name and the qualifying statement. Other means of qualifying "Turkey Ham" will be evaluated based on clarity. Alternatively, the total name as described in 1 and 2 above may be qualified with a statement that includes "Cured Turkey Thigh Meat" and the amount of added water, e.g., "Cured Turkey Thigh Meat and 12% Water." The statement should be presented in the manner described in 9 CFR 381.171(e).
4. The product name shall be further qualified with the statement(s) required by section 9 CFR 381.171(f) and any other statements required in Part 381. A product complying with the standard for Turkey Ham, containing added water, and descriptively labeled as stated above, must be produced under a Partial Quality Control (PQC) program approved by the Processed Products Inspection Division (PPID) prior to the use of the approved label.

Turkey Ham and Water Products Containing Binders

Turkey ham products containing added water and binders must be labeled as "Turkey Ham and Water Products" X% of weight is added ingredients as described in Policy Memo 057a to provide freeze/thaw stability and reduce purge in packages. The binders that are acceptable for use in cured pork product can be used in these turkey ham products. The binders must be used in accordance with 9 CFR, Sections 9 CFR 319.104(d) and 424.21(c). Where several limits are listed, depending upon the cured pork product, the maximum amount permitted in the regulation is acceptable.

Turkey Ham Products Containing Ground Turkey Thigh Meat (Labeling)

Small amounts of ground turkey thigh meat may be added as a binder in turkey ham products as defined in 9 CFR

381.171 without declaration, provided the ground turkey thigh meat is made from trimmings that are removed from the turkey thighs during the boning and trimming process. The amount of ground turkey thigh meat that may be used can represent no more than the amount that was trimmed and in no case more than 15% of the weight of the turkey thigh meat ingredients when formulated. Products containing any ground turkey thigh meat not removed during the boning and trimming processes or products containing more than 15% ground turkey thigh meat must be labeled to indicate the presence of the ground turkey thigh meat, e.g., "a portion of ground turkey thigh meat added." The provision in the regulations (9 CFR 381.171(f)) regarding the required use of terminology, e.g., "Chunked and Formed," "Chopped and Formed," and "Ground and Formed" will continue to be followed.

Turkey Loaf: Cured, Chopped (Canned)

May contain seasonings, cures, and no more than 3% water at formulation. Binders and extenders are not permitted.

Turnovers

Product must contain 25% meat or 14% poultry meat. Similar to pies except the dough is folded. Cheese may be substituted for meat or poultry meat in an amount not to exceed 50% under the conditions outlined below:

1. Cheese must be part of the product name, e.g., "Beef and Cheese Turnover" or "Chicken and Cheese Turnover."
2. Imitation Cheese, substitute cheese, cheese food, and cheese spreads are not acceptable replacements for cheese.

Tzimmes

The true product name is "Beef and Vegetables" (or similar wording) when at least 50% beef is present in the product. "Vegetables with Beef" (or similar wording) is acceptable when at least 35% raw beef is used.

Ukrainian Sausage

A dry sausage made from lean pork and/or veal chunks, containing large amounts of garlic which dominates the flavor. It is cooked and smoked at high temperatures and then air dried. The water activity (Aw) of the finished product shall not exceed 0.92 or a moisture/protein ratio 2.0:1 or less.

Variety Meats in Franks

Cooked sausages with variety meats (byproducts) identified in 9 CFR 319.180(b) must contain not less than 15% red skeletal meat based on total meat block weight. The meat block includes meat, meat by-products, and if applicable, poultry.

Variety Packs — Hors d'oeuvres

Whenever FDA regulated products are included as a part of a variety pack bearing the legend (e.g., seafood hors d'oeuvres included with meat and poultry hors d'oeuvres), the labeling information must still be reviewed to assure accuracy. FDA regulated products that are found mislabeled should be corrected according to the policies of the FDA before the label can be approved.

Veal and Peppers in Sauce

Product must contain at least 30% cooked veal.

Veal Birds

Product is similar to a turnover made with meat and no more than 40% stuffing. Categories of products are as follows:

1. Veal Birds — At least 60% veal
2. Veal Birds Beef Added — At least 60% veal and beef of which 20% may be beef
3. Veal and Beef Birds — At least 60% veal and beef of which up to 50% may be beef
4. Veal Birds (made from patties) — Birds made from patties shall bear a true product name descriptive of patty used, e.g., "Veal Birds made with Veal Patties — Beef Added." The patty portion shall contain 70% meat.

Veal Cordon Bleu (FR)

The standard requires at least:

1. 60% veal;
2. 5% ham, Canadian bacon, or cooked cured pork loin; and
3. Cheese (either Swiss, Gruyere. Mozzarella, or Pasteurized Processed Swiss).

If the product is breaded, it must be shown in the product name. When the product is made with other than solid pieces of meat, "Chopped and Formed" must be shown contiguous to the product name. Beef is not permitted in this product.

Veal that has been injected with water and phosphates and used for Veal Cordon Bleu should be labeled "Veal Roll Cordon Bleu" or other descriptive names as appropriate.

Veal Drumstick, Breaded

May not contain more than 15% water or more than 10% extenders.

Veal Fricassee

Must contain at least 40% meat.

Veal Parmigiana

The following categories of products exist:

1. "Breaded Veal Parmigiana" is the product name for a solid piece of veal that is breaded and topped with cheese and tomato sauce. Breaded cooked veal must represent 40% of the finished product.
2. "Breaded Veal Parmigiana, Chopped and Formed Beef (or Beef Fat) Added" is the product name for chopped veal with up to 20% beef and/or beef fat added that is formed, breaded, and topped with cheese and tomato sauce. The chopped and formed beef added statement is shown one-third the size of "Veal" contiguous to the product name. Breaded cooked patty must represent 40% of the finished product.
3. "Breaded Veal Parmigiana made with Veal Patties, Beef (or Beef Fat) Added" is the product name for a veal patty containing at least 70% fresh meat (in unbreaded patty) of which 20% may be beef or beef fat. The patty is breaded, topped with cheese and tomato sauce. The entire qualifying statement in the product name is to be shown 1/3 size of "Veal" contiguous to product name. The breaded cooked patty represents 40% of the finished product.
4. Breaded Veal and Beef Patty Parmigiana. The patty may be prepared in proportions as governed by 9 CFR 317.2(f)(1)(v) of the regulations; the minimum meat patty requirement is 50%. If the product is breaded, the name must reflect this fact. The cheese component of the product does not have to be shown in the name of the product. A specific kind of cheese is not required, although Romano, Mozzarella, and Parmesan are the usual types used. No specific spelling of the word "Parmigiana" is required. Name applies to a "Cooked Product Assembled, Ready to Heat and Eat."

The labeling of Veal Parmigiana made from a veal patty shall include veal patty in the product name, e.g., "Breaded Veal Parmigiana made with Veal Patties" or "Breaded Veal Patty Parmigiana." The ingredients of the veal patty do not have to be part of the product name.

Veal Patties

Up to 20% beef and/or beef fat of the meat block permitted. Beef and/or beef fat must show in the true product name, e.g., "Veal Patties, beef added" or "Veal Patties, beef fat added." Beef and/or beef fat in excess of 20% of the meat block must show as "Veal and Beef Fat Patties."

Veal Scaloppini

Veal and sauce type product that must contain at least 35% cooked sliced veal.

Vegetable Declaration on Labels

1. The use of the terms onion, garlic, celery, and parsley shall mean fresh, frozen, or canned.
2. Processed onion or garlic must be qualified in a manner, e.g., "dried" or "dehydrated onion" or may be shown as "onion flakes" or "powdered."
3. It is usually not necessary to show vegetables as whole, diced, sliced, granulated, powdered, or pureed; however, whenever the name of the vegetable is necessary to describe a food, then the name of the vegetable should be modified to show the form of its degree of processing.
4. Onion or garlic juice to which water has been added shall be noted, e.g., onion juice with water added.
5. Celery seed may be listed as a spice.
6. Celery salt shall be shown as celery salt.
7. Oil of celery may be listed as a flavoring.

Vegetable Extract

The source must be identified i.e. "soy," "corn" and "beet."

Vegetable Gum

Declare common or usual name of each vegetable gum, e.g., Guar Gum.

Vegetable Pie with

"Species" meat must contain 12% meat on a raw basis. "Kind" poultry must contain 7% cooked poultry.

Vegetable Stew with: Minimum Meat Content

Vienna Sausage — Packed in Beef Broth

Meatballs	12% meat
Meat	12% meat
Meat Sauce or Gravy	6% meat
Sauce and Meat	12% meat
Poultry	6% cooked poultry meat

Product must contain 80% sausage to be in compliance prior to inclusion in can. Broth component to have a MPR

of not more than 135:1. A manufacturer holds trademark rights to the terms "Vienna" and "Vienna Beef."

Vinegar

Product must contain at least 4 grams of acetic acid per 100 cubic centimeters (approximately 4% acetic acid). This strength is referred to as 40 grain vinegar. Cider vinegar, which during the course of manufacture has developed an excess of acetic acid over 4%, may be reduced to a strength not less 4%. Cider vinegar so reduced is not regarded as adulterated but must be labeled as to its nature as "diluted" or "water added" cider vinegar. However, when vinegar of any concentration (not less than 4% acetic acid) is used in a food product, the only labeling requirement is "vinegar." Statements like "diluted" or "water added" are not required.

Vinegar Pickle

Sausage in vinegar pickle is approved with the understanding that sausage is completely covered with pickle and that the pickle has a pH level not higher than 4.5.

Water Base Solutions in Red Meat in Meat Products

Solutions intended to impart flavor (not extend the product) may be added in any amount to uncooked, cured and uncooked, uncured red meat products including those that have been chunked, ground, wafer sliced, etc., and formed/shaped. Whenever an uncooked, cured red meat product is injected, massaged, tumbled, etc., with a flavoring or seasoning solution, the product name must be qualified with a statement indicating that the addition of a solution has taken place, e.g., "Containing 6% of a Solution," "Injected with up to 12% of a Flavoring Solution." The qualifier must appear contiguous to the product name whenever it appears on the label. The ingredients of the solution may accompany the qualifier or appear in locations prescribed for ingredient statements.

For products marinated (i.e., soaked, steeped, massaged, tumbled, or injected in order to improve taste, texture, tenderness, or other sensory attributes, such as color or juiciness) and identified as "marinated," the solution added is limited to 10%. The qualifying statement must include the percentage of solution contained in the product, e.g., "Marinated with up to 8% of a Solution of Water, Salt, and Sugar."

In situations where it has been customary to coat a product by rubbing, spraying, or dipping water mixed with seasonings, flavorings, etc., onto the surface of the meat, the qualifying statement describing this treatment does not have to include the amount and a partial quality control program is not needed. If, however, these components are incorporated into the meat by excessive rubbing, massaging, or tumbling, a qualifying statement indicating the composition and the amount of any solution absorbed is needed as described herein. An approved partial quality control program is also needed.

The addition of an enzyme solution to meat products is limited to 3% of the raw meat product (green weight) by the meat inspection regulations (9 CFR 318.7(c)(4)). If a product is treated with an enzyme solution and a flavoring solution, separately or in one step, both treatments must be separately identified on the label, e.g., "Tenderized with Papain," and "Marinated with up to 7% of a Solution." No particular order is required for these qualifying statements. Combined tenderization/marination solutions are limited to 10% of the raw meat product (green weight).

For all products, the qualifying statement must be at least one-fourth the size of the largest letter in the product name. If the ingredients of the solution accompany the qualifier, they must appear in print at least one-eighth the size of the largest letter in the product name. Product name labeling prominence guidelines are found in Policy Memo 087A.

For uncooked products, the percent added substances for the label statement is determined by subtracting the fresh (green) weight of the article from the weight of the finished (total) product, i.e., after injecting, marinating, etc., dividing by the weight of the fresh article, and multiplying by 100.

In all situations where the percentage of a solution is disclosed, a partial quality control (PQC) program for the addition of solutions must be approved before the label can be used regardless of the amount of solution added.

Since the meat inspection regulations (9 CFR 319.101 and 102) allow uncooked corned beef brisket to contain 20%, and uncooked corned beef round and other cuts to contain 10% of a curing solution above the weight of the fresh, uncured (green weight) product without disclosure, the above labeling scheme does not apply until these levels are exceeded. If these levels are exceeded, the total amount of added solution, not just the level above compliance, must be indicated in the format described for other uncooked, cured products. Similarly, the labeling scheme does not apply to uncooked cured pork trimmings or uncooked cured pork products that are not labeled to indicate the presence of hams, loins, shoulders, butts, picnics, or cured pork made from parts not covered by the cured pork products regulation (9 CFR 319.104) until more than 10% added substance is present.

This policy memo does not apply to uncooked cured pork products covered by the cured pork products regulation. The labeling schemes for indicating the presence of added substances in these products are outlined in the meat inspection regulations (9 CFR 319.104 and 105) and FSIS Directive 7110.2 (Rev. 1). The percentage of the weight of added ingredients is determined as described above.

Water-Declaration

The use of water must be declared in the ingredients statement of all products with the exception of the following:

1. The water added to lactic acid starter culture (.05% or less) for the purpose of rehydration.
2. The water added to products which are freeze-dried or sprayed-dried.

Water in Canned Sausage

Water, not to exceed 8% of the total product weight, may be used in the preparation of precooked pork sausage links intended for canning. The amount of water used is for the purpose of replacing that which is lost during the processing operation that takes place prior to canning. The weight of the sausage at the time of canning shall not exceed the weight of the fresh uncured meat ingredients plus the weight of the curing ingredients and the seasoning ingredients.

Water-Misted and Ice-Glazed Meat and Poultry Products

When meat or poultry products are water-misted or ice-glazed, the net weight of the product may not include the weight of the water or ice. An acknowledgment to this effect must be indicated on the label application form. A prominent and conspicuous statement must appear on the principal display panel adjacent to the product name, describing that the product is protected with a water-mist or ice glaze (e.g., "Product Protected with Ice Glaze").

If the manufacturer can show that a water or ice glaze is sublimed from the unpackaged product during freezing so as not to compromise the integrity of the product's formulation or the standard with which it must comply, the labeling of the product need not bear the statements identified above.

Because the regulatory standard 9 CFR 319.15 precludes the addition of water — hamburger, ground beef and chopped beef patties cannot be ice-glazed and, if there is evidence of an ice-glaze on such patties subsequent to freezing, they must be labeled appropriately to be sold in commerce, e.g., as "beef patties." However, water-misting of formed hamburger, ground beef, or chopped beef patties just prior to freezing individual patties is permitted if (1) the water applied in misting acts as a processing aid to prevent shrinkage of the patties, and (2) the misted water sublimes from the surface of the patties during the freezing process such that the weight of the patty exiting the freezer does not exceed the green weight of the patty just prior to water-misting and freezing.

Weisswurst

An acceptable name for fresh sausage. It is usually made of pork or veal and must be thoroughly cooked before eating. It is of German origin, which means White Sausage-similar to Bratwurst. Weisswurst with milk should be labeled "Kalbsbratwurst." Weisswurst with milk and eggs should be labeled "Bockwurst."

Welsh Rarebit Sauce with Cooked Ham

Product must contain at least 20% cooked ham in the total formulation.

Wheat Gluten

Acceptable for use to bind fresh meat cuts, e.g., boneless loins, boneless legs, and livers together, so that they may be cooked and sliced without falling apart. The amount used should not exceed 2% of the weight of the total product. The product name shall be qualified by the phrase "Wheat Gluten Added."

Wheat gluten is not acceptable for use with chunked and/or chopped specific products as roasts, rolls, and reformed meat cuts.

Acceptable in nonspecific products and home-style meat loaves within the prescribed limits of other extenders and binders.

Whole Hog Sausage

Must contain all primal parts of a hog. Hearts and tongues, in natural proportions, are permitted ingredients in whole hog sausage when declared in the ingredients statement. Other meat byproducts are not permitted in whole hog sausage.

See: 9 CFR 319.144.

Wiener Schnitzel (GR)

A veal cutlet prepared by dipping in egg, flour, and bread crumbs and frying to a golden brown.

Wild Boar

Products prepared from wild boar from feral swine are amenable and subject to the meat inspection regulations.

"Wild Boar" is an acceptable label term for a product, provided the words "Wild Boar" are directly followed by the statement "Meat from Feral Swine." The statement "Meat from Feral Swine" must appear prominently on the principle display panel as described in 9 CFR 317.2(d)(1)(2) and (3). If the statement "Meat from Feral Swine" does not directly follow the term "Wild Boar," then an asterisk may be included with the term "Wild Boar" and the statement "Meat from Feral Swine" should appear prominently elsewhere on the principal display panel. "Wild Boar from Feral Swine," "Wild Boar Meat from Feral Swine," "Wild Boar (byproduct) from Feral Swine," are also acceptable product names.

In order to obtain approval for a product label bearing the name "Wild Boar from Feral Swine," or similar acceptable names, a statement describing and verifying the following physical and environmental characteristics typical of wild boar is required: color patterns, e.g., white stripes or spots, longer bristly haircoat, elongated snout with visible tusks, a "razorback" body shape, and wild boar males which are uncastrated. (We acknowledge both males and females under the term "Wild Boar.") The purchased hogs should be obtained from a nonrestrictive environment which permits foraging for uncultivated feed, natural selection, and breeding and farrowing without confinement. A letter should be submitted with "Wild Boar from Feral Swine" labels describing the environment where such swine live and their method of capture or entrapment. These same criteria would also apply to imported "Wild Boar Meat from Feral Swine" and arrangements should be made through foreign programs for slaughter and export from approved establishments.

In multi-ingredient products, e.g., "Beans in Sauce with Wild Boar," the "Wild Boar" part of the product name must be followed by an asterisk and a statement "(meat or meat byproduct) from Feral Swine" must appear somewhere on the principal display panel. The ingredient wild boar, wild boar meat, or wild boar byproduct must be listed as "Wild Boar [(Meat or meat byproduct) from Feral Swine]" in the ingredients statement in its proper order of predominance.

Wing Sections

First wing section is described as the wing drummette Second wing section is described as the wing portion. Wings Sections is an acceptable wing term for both wing drummette and wing portion when in natural proportions.

With Natural Juices (Poultry)

The term "With Natural Juices" may be used with poultry products to indicate the presence of cooked out juices derived solely from the liquid normally associated with the poultry prior to cooking. If liquids have been added to the poultry prior to cooking, natural can not be used.

Wraps

A ready-to-eat meat/poultry food product that may contain vegetables and seasoning ingredients and is wrapped in a dough based component, e.g., tortilla. The product name must bear the kind or species, e.g., "Ham Wraps." The minimum meat or poultry requirement is 2% cooked meat or 2% cooked poultry meat.

Yearling

The term "yearling" (e.g. yearling beef) may be used to describe an animal of either sex that is too old to be classified as a calf or lamb but less than 2 years of age. The company is required to segregate carcasses and provide product identification to insure that no commingling occurs between qualifying and nonqualifying products.

The terms "Yearling Ovine", "Yearling Mutton" and "Yearling Sheep Meat" are acceptable product names for meat derived from sheep between 1 and 2 years of age. Yearling Lamb is **not** an acceptable name for this product.

ACKNOWLEDGMENT

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73 FDA's GMPs, HACCP, and the Food Code

Peggy Stanfield
Dietetic Resources

CONTENTS

I. INTRODUCTION

Nearly 25 years ago, the United States Food and Drug Administration (FDA) started the approach of using umbrella regulations to help the food industries to produce wholesome food as required by the Federal Food, Drug, Cosmetic Act (the Act). In 1986, the FDA promulgated the first umbrella regulations under the title of Good Manufacturing Practice (GMP) regulations (GMPR). Since then, many aspects of the regulations have been revised (1).

Traditionally, industry and regulators have depended on spot-checks of manufacturing conditions and random sampling of final products to ensure safe food. The Current Good Manufacturing Practice Regulations (CGMPR) forms the basis on which the FDA will inform the food manufacturer about deficiencies in its operations. This approach, however, tends to be reactive, rather than preventive, and can definitely be improved.

For more than 30 years, FDA has been regulating the low acid canned food (LACF) industries with a special set of regulations, many of which are preventive in nature. This action aims at preventing botulism. In the last 30 years, threats from other biological pathogens have increased tremendously. Between 1980 and 1995, FDA has been studying the approach of using Hazard Analysis and Critical Control Points (HACCP). For this approach, FDA uses the LACF regulations as a partial guide. Since 1995, FDA has issued HACCP regulations (HACCPR) (2) for the manufacture or production of several types of food products. These include the processing of seafood and fruit/vegetable juices.

Since 1938, when the Act was first passed by Congress, FDA and State regulatory agencies have worked hard to reach a uniform set of codes for the national regulation of food manufacturing industries and state regulation of retail industries associated with food, e.g., groceries, restaurants, catering, and so on. In 1993, the first document titled *Food Code* was issued jointly by the FDA and state agencies. It has been revised twice since then. This chapter discusses CGMPR, HACCPR, and the *Food Code*.

TABLE 73.1
Contents of the Current Good Manufacturing Practice Regulations (CGMPR)

Section 110.3	Definitions
Section 110.5	Current good manufacturing practice
Section 110.10	Personnel
Section 110.19	Exclusions
Section 110.20	Plant and grounds
Section 110.35	Sanitary operations
Section 110.37	Sanitary facilities and controls
Section 110.40	Equipment and utensils
Section 110.80	Processes and controls
Section 110.93	Warehousing and distribution

II. CURRENT GOOD MANUFACTURING PRACTICE REGULATIONS (CGMPR)

The Current Good Manufacturing Practice Regulations (CGMPR) cover the topics listed in Table 73.1. These regulations are discussed in detail here. Please note that the word "shall" in a legal document means mandatory and is used routinely in FDA regulations published in the U.S. Code of Federal Regulations (CFR). In this chapter, the words "should" and "must" are used to make for smoother reading. However, this in no way diminishes the legal impact of the original regulations.

A. Definitions (Section 110.3)

FDA has provided the following definitions and interpretations for several important terms.

1. *Acid food* or *acidified food* means foods that have an equilibrium pH of 4.6 or below.
2. *Batter* means a semi-fluid substance, usually composed of flour and other ingredients, into which principal components of food are dipped or with which they are coated, or which may be used directly to form bakery foods.
3. *Blanching*, except for tree nuts and peanuts, means a prepackaging heat treatment of foodstuffs for a sufficient time and at a sufficient temperature to partially or completely inactivate the naturally occurring enzymes and to effect other physical or biochemical changes in the food.

4. *Critical control point* means a point in a food process where there is a high probability that improper control may cause a hazard or filth in the final food or decomposition of the final food.
5. *Food* includes raw materials and ingredients.
6. *Food-contact surfaces* are those surfaces that contact human food and those surfaces from which drainage onto the food or onto surfaces that contact the food ordinarily occurs during the normal course of operations. *Food-contact surfaces* include utensils and food-contact surfaces of equipment.
7. *Lot* means the food produced during a period of time indicated by a specific code.
8. *Microorganisms* means yeasts, molds, bacteria, and viruses and includes, but is not limited to, species having public health significance. The term *undesirable microorganisms* includes those microorganisms that are of public health significance, that promote decomposition of food, or that indicate that food is contaminated with filth.
9. *Pest* refers to any objectionable animals or insects including, but not limited to, birds, rodents, flies, and insect larvae.
10. *Plant* means the building or facility used for the manufacturing, packaging, labeling, or holding of human food.
11. *Quality control operation* means a planned and systematic procedure for taking all actions necessary to prevent food from being adulterated.
12. *Rework* means clean, unadulterated food that has been removed from processing for reasons other than insanitary conditions or that has been successfully reconditioned by reprocessing and that is suitable for use as food.
13. *Safe moisture level* is a level of moisture low enough to prevent the growth of undesirable microorganisms in the finished product under the intended conditions of manufacturing, storage, and distribution. The maximum safe moisture level for a food is based on its water activity, a_w. An a_w will be considered safe for a food if adequate data are available that demonstrate that the food at or below the given a_w will not support the growth of undesirable microorganisms.
14. *Sanitize* means to adequately treat food-contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance, and in substantially reducing numbers of other undesirable microorganisms, but without adversely affecting the product or its safety for the consumer.
15. *Water activity* (a_w) is a measure of the free moisture in a food and is the quotient of the water vapor pressure of the substance divided by the vapor pressure of pure water at the same temperature.

B. Personnel (Section 110.10)

Plant management should take all reasonable measures and precautions to ensure compliance with the following regulations.

1. Disease Control

Any person who, by medical examination or supervisory observation, is shown to have an illness, open lesion, including boils, sores, or infected wounds, by which there is a reasonable possibility of food, food-contact surfaces, or food-packaging materials becoming contaminated, should be excluded from any operations which may be expected to result in such contamination until the condition is corrected. Personnel should be instructed to report such health conditions to their supervisors.

2. Cleanliness

All persons working in direct contact with food, food-contact surfaces, and food-packaging materials should conform to hygienic practices while on duty. The methods for maintaining cleanliness include, but are not limited to, the following:

a. Wearing outer garments suitable to the operation to protect against the contamination of food, food-contact surfaces, or food-packaging materials.
b. Maintaining adequate personal cleanliness.
c. Washing hands thoroughly (and sanitizing if necessary to protect against contamination with undesirable microorganisms) in an adequate hand-washing facility before starting work, after each absence from the work station, and at any other time when the hands may have become soiled or contaminated.
d. Removing all unsecured jewelry and other objects that might fall into food, equipment, or containers, and removing hand jewelry that cannot be adequately sanitized during periods in which food is manipulated by hand. If such hand jewelry cannot be removed, it may be covered by material which can be maintained in an intact, clean, and sanitary condition and which effectively protects against their contamination of the food, food-contact surfaces, or food-packaging materials.
e. Maintaining gloves, if they are used in food handling, in an intact, clean, and sanitary condition. The gloves should be of an impermeable material.

f. Wearing, where appropriate, hairnets, headbands, caps, beard covers, or other effective hair restraints.
g. Storing clothing or other personal belongings in areas other than where food is exposed or where equipment or utensils are washed.
h. Confining the following personal practices to areas other than where food may be exposed or where equipment or utensils are washed: eating food, chewing gum, drinking beverages, or using tobacco.
i. Taking any other necessary precautions to protect against contamination of food, food-contact surfaces, or food-packaging materials with microorganisms or foreign substances including, but not limited to, perspiration, hair, cosmetics, tobacco, chemicals, and medicines applied to the skin.

3. Education and Training

Personnel responsible for identifying sanitation failures or food contamination should have a background of education or experience, to provide a level of competency necessary for production of clean and safe food. Food handlers and supervisors should receive appropriate training in proper food handling techniques and food-protection principles and should be informed of the danger of poor personal hygiene and insanitary practices.

4. Supervision

Responsibility for assuring compliance by all personnel with all legal requirements should be clearly assigned to competent supervisory personnel.

C. Plant and Grounds (Section 110.20)

1. Grounds

The grounds surrounding a food plant that are under the control of the plant manager should be kept in a condition that will protect against the contamination of food. The methods for adequate maintenance of grounds include, but are not limited to, the following:

a. Properly storing equipment, removing litter and waste, and cutting weeds or grass within the immediate vicinity of the plant buildings or structures that may constitute an attractant, breeding place or harborage for pests.
b. Maintaining roads, yards, and parking lots so that they do not constitute a source of contamination in areas where food is exposed.
c. Adequately draining areas that may contribute contamination to food by seepage or foot-borne filth, or by providing a breeding place for pests.
d. Operating systems for waste treatment and disposal in an adequate manner so that they do not constitute a source of contamination in areas where food is exposed. If the plant grounds are bordered by grounds not under the operator's control and not maintained in an acceptable manner, steps must be taken to exclude pests, dirt, and filth that may be a source of food contamination. Implement inspection, extermination, or other countermeasures.

2. Plant Construction and Design

Plant buildings and structures should be suitable in size, construction, and design to facilitate maintenance and sanitary operations for food-manufacturing purposes. The plant and facilities should:

a. Provide sufficient space for such placement of equipment and storage of materials as is necessary for the maintenance of sanitary operations and the production of safe food.
b. Take proper precautions to reduce the potential for contamination of food, food-contact surfaces, or food-packaging materials with microorganisms, chemicals, filth, or other extraneous material. The potential for contamination may be reduced by adequate food safety controls and operating practices or effective design, including the separation of operations in which contamination is likely to occur, by one or more of the following means: location, time, partition, air flow, enclosed systems, or other effective means.
c. Taking proper precautions to protect food in outdoor bulk fermentation vessels by any effective means, including:
   – Using protective coverings.
   – Controlling areas over and around the vessels to eliminate harborages for pests.
   – Checking on a regular basis for pests and pest infestation.
   – Skimming the fermentation vessels, as necessary.
d. Be constructed in such a manner that floors, walls, and ceilings may be adequately cleaned and kept clean and kept in good repair; that drip or condensate from fixtures, ducts and pipes does not contaminate food, food-contact surfaces, or food-packaging materials; and that aisles or working spaces are provided between equipment and walls and are adequately unobstructed and of adequate width to permit employees to perform their duties and to protect against contaminating food or food-contact surfaces with clothing or personal contact.

e. Provide adequate lighting in hand-washing areas, dressing and locker rooms, and toilet rooms and in all areas where food is examined, processed, or stored and where equipment or utensils are cleaned; and provide safety-type light bulbs, fixtures, skylights, or other glass suspended over exposed food in any step of preparation or otherwise protect against food contamination in case of glass breakage.
f. Provide adequate ventilation or control equipment to minimize odors and vapors (including steam and noxious fumes) in areas where they may contaminate food; and locate and operate fans and other air-blowing equipment in a manner that minimizes the potential for contaminating food, food-packaging materials, and food-contact surfaces.
g. Provide, where necessary, adequate screening or other protection against pests.

D. Sanitary Operations (Section 110.35)

1. General Maintenance

Buildings, fixtures, and other physical facilities of the plant should be maintained in a sanitary condition and should be kept in repair sufficient to prevent food from becoming adulterated within the meaning of the Act. Cleaning and sanitizing of utensils and equipment should be conducted in a manner that protects against contamination of food, food-contact surfaces, or food-packaging materials.

2. Substances Used in Cleaning and Sanitizing; Storage of Toxic Materials

a. Cleaning compounds and sanitizing agents used in cleaning and sanitizing procedures should be free from undesirable microorganisms and should be safe and adequate under the conditions of use. Compliance with this requirement may be verified by any effective means including purchase of these substances under a supplier's guarantee or certification, or examination of these substances for contamination. Only the following toxic materials may be used or stored in a plant where food is processed or exposed:
   – Those required to maintain clean and sanitary conditions;
   – Those necessary for use in laboratory testing procedures;
   – Those necessary for plant and equipment maintenance and operation; and
   – Those necessary for use in the plant's operations.
b. Toxic cleaning compounds, sanitizing agents, and pesticide chemicals should be identified, held, and stored in a manner that protects against contamination of food, food-contact surfaces, or food-packaging materials.

3. Pest Control

No pests should be allowed in any area of a food plant. Guard or guide dogs may be allowed in some areas of a plant if the presence of the dogs is unlikely to result in contamination of food, food-contact surfaces, or food-packaging materials. Effective measures should be taken to exclude pests from the processing areas and to protect against the contamination of food on the premises by pests. The use of insecticides or rodenticides is permitted only under precautions and restrictions that will protect against the contamination of food, food-contact surfaces, and food-packaging materials.

4. Sanitation of Food-Contact Surfaces

All food-contact surfaces, including utensils and food-contact surfaces of equipment, should be cleaned as frequently as necessary to protect against contamination of food.

a. Food-contact surfaces used for manufacturing or holding low-moisture food should be in a dry, sanitary condition at the time of use. When the surfaces are wet-cleaned, they should, when necessary, be sanitized and thoroughly dried before subsequent use.
b. In wet processing, when cleaning is necessary to protect against the introduction of microorganisms into food, all food-contact surfaces should be cleaned and sanitized before use and after any interruption during which the food-contact surfaces may have become contaminated. Where equipment and utensils are used in a continuous production operation, the utensils and food-contact surfaces of the equipment should be cleaned and sanitized as necessary.
c. Non-food-contact surfaces of equipment used in the operation of food plants should be cleaned as frequently as necessary to protect against contamination of food.
d. Single-service articles (such as utensils intended for one-time use, paper cups, and paper towels) should be stored in appropriate containers and should be handled, dispensed, used, and disposed of in a manner that protects against contamination of food or food-contact surfaces.
e. Sanitizing agents should be adequate and safe under conditions of use. Any facility, procedure, or machine is acceptable for cleaning and

sanitizing equipment and utensils if it is established that the facility, procedure, or machine will routinely render equipment and utensils clean and provide adequate cleaning and sanitizing treatment.

5. Storage and Handling of Cleaned Portable Equipment and Utensils

Cleaned and sanitized portable equipment with food-contact surfaces and utensils should be stored in a location and manner that protects food-contact surfaces from contamination.

E. Sanitary Facilities and Controls (Section 110.37)

Each plant should be equipped with adequate sanitary facilities and accommodations including, but not limited to:

1. Water Supply

The water supply should be sufficient for the operations intended and should be derived from an adequate source. Any water that contacts food or food-contact surfaces should be safe and of adequate sanitary quality. Running water at a suitable temperature, and under pressure as needed, should be provided in all areas where required for the processing of food, for the cleaning of equipment, utensils, and food-packaging materials, or for employee sanitary facilities.

2. Plumbing

Plumbing should be of adequate size and design and adequately installed and maintained to:

a. Carry sufficient quantities of water to required locations throughout the plant.
b. Properly convey sewage and liquid disposable waste from the plant.
c. Avoid constituting a source of contamination to food, water supplies, equipment, or utensils or creating an unsanitary condition.
d. Provide adequate floor drainage in all areas where floors are subject to flooding-type cleaning or where normal operations release or discharge water or other liquid waste on the floor.
e. Provide that there is no backflow from, or cross-connection between, piping systems that discharge wastewater or sewage and piping systems that carry water for food or food manufacturing.

3. Sewage Disposal

Sewage disposal should be made into an adequate sewerage system or disposed of through other adequate means.

4. Toilet Facilities

Each plant should provide its employees with adequate, readily accessible toilet facilities. Compliance with this requirement may be accomplished by:

a. Maintaining the facilities in a sanitary condition.
b. Keeping the facilities in good repair at all times.
c. Providing self-closing doors.
d. Providing doors that do not open into areas where food is exposed to airborne contamination, except where alternate means have been taken to protect against such contamination (such as double doors or positive air-flow systems).

5. Hand-Washing Facilities

Hand-washing facilities should be adequate and convenient and be furnished with running water at a suitable temperature. Compliance with this requirement may be accomplished by providing:

a. Hand-washing and, where appropriate, hand-sanitizing facilities at each location in the plant where good sanitary practices require employees to wash and/or sanitize their hands.
b. Effective hand-cleaning and sanitizing preparations.
c. Sanitary towel service or suitable drying devices.
d. Devices or fixtures, such as water control valves, so designed and constructed to protect against recontamination of clean, sanitized hands.
e. Readily understandable signs directing employees handling unprotected food, unprotected food-packaging materials, of food-contact surfaces to wash and, where appropriate, sanitize their hands before they start work, after each absence from post of duty, and when their hands may have become soiled or contaminated. These signs may be posted in the processing room(s) and in all other areas where employees may handle such food, materials, or surfaces.
f. Refuse receptacles that are constructed and maintained in a manner that protects against contamination of food.

6. Rubbish and Offal Disposal

Rubbish and any offal should be so conveyed, stored, and disposed of as to minimize the development of odor, minimize the potential for the waste becoming an attractant and harborage or breeding place for pests, and protect against contamination of food, food-contact surfaces, water supplies, and ground surfaces.

F. Equipment and Utensils (Section 110.40)

1. All plant equipment and utensils should be so designed and of such material and workmanship as to be adequately cleanable, and should be properly maintained. The design, construction, and use of equipment and utensils should preclude the adulteration of food with lubricants, fuel, metal fragments, contaminated water, or any other contaminants. All equipment should be so installed and maintained as to facilitate the cleaning of the equipment and of all adjacent spaces. Food-contact surfaces should be corrosion-resistant when in contact with food. They should be made of nontoxic materials and designed to withstand the environment of their intended use and the action of food, and, if applicable, cleaning compounds and sanitizing agents. Food-contact surfaces should be maintained to protect food from being contaminated by any source, including unlawful indirect food additives.
2. Seams on food-contact surfaces should be smoothly bonded or maintained so as to minimize accumulation of food particles, dirt, and organic matter and thus minimize the opportunity for growth of microorganisms.
3. Equipment that is in the manufacturing or food-handling area and that does not come into contact with food should be so constructed that it can be kept in a clean condition.
4. Holding, conveying, and manufacturing systems, including gravimetric, pneumatic, closed, and automated systems, should be of a design and construction that enables them to be maintained in an appropriate sanitary condition.
5. Each freezer and cold storage compartment used to store and hold food capable of supporting growth of microorganisms should be fitted with an indicating thermometer, temperature-measuring device, or temperature-recording device so installed as to show the temperature accurately within the compartment, and should be fitted with an automatic control for regulating temperature or with an automatic alarm system to indicate a significant temperature change in a manual operation.
6. Instruments and controls used for measuring, regulating, or recording temperatures, pH, acidity, water activity, or other conditions that control or prevent the growth of undesirable microorganisms in food should be accurate and adequately maintained, and adequate in number for their designated uses.
7. Compressed air or other gases mechanically introduced into food or used to clean food-contact surfaces or equipment should be treated in such a way that food is not contaminated with unlawful indirect food additives.

G. Processes and Controls (Section 110.80)

All operations in the receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing of food should be conducted in accordance with adequate sanitation principles. Appropriate quality control operations should be employed to ensure that food is suitable for human consumption and that food-packaging materials are safe and suitable. Overall sanitation of the plant should be under the supervision of one or more competent individuals assigned responsibility for this function. All reasonable precautions should be taken to ensure that production procedures do not contribute contamination from any source. Chemical, microbial, or extraneous material testing procedures should be used where necessary to identify sanitation failures or possible food contamination. All food that has become contaminated to the extent that it is adulterated within the meaning of the Act should be rejected, or if permissible, treated or processed to eliminate the contamination.

1. Raw Materials and Other Ingredients

a. Raw materials and other ingredients should be inspected and segregated or otherwise handled as necessary to ascertain that they are clean and suitable for processing into food and should be stored under conditions that will protect against contamination and minimize deterioration. Raw materials should be washed or cleaned as necessary to remove soil or other contamination. Water used for washing, rinsing, or conveying food should be safe and of adequate sanitary quality. Water may be reused for washing, rinsing, or conveying food if it does not increase the level of contamination of the food. Containers and carriers of raw materials should be inspected on receipt to ensure that their condition has not contributed to the contamination or deterioration of food.
b. Raw materials and other ingredients should either not contain levels of microorganisms that may produce food poisoning or other disease in humans, or they should be pasteurized or otherwise treated during manufacturing operations so that they no longer contain levels that would cause the product to be adulterated within the meaning of the act. Compliance with this requirement may be verified by any effective means,

including purchasing raw materials and other ingredients under a supplier's guarantee or certification.

c. Raw materials and other ingredients susceptible to contamination with aflatoxin or other natural toxins should comply with current FDA regulations, guidelines, and action levels for poisonous or deleterious substances before these materials or ingredients are incorporated into finished food. Compliance with this requirement may be accomplished by purchasing raw materials and other ingredients under a supplier's guarantee or certification, or may be verified by analyzing these materials and ingredients for aflatoxins and other natural toxins.
d. Raw materials, other ingredients, and rework susceptible to contamination with pests, undesirable microorganisms, or extraneous material should comply with applicable FDA regulations, guidelines, and defect action levels for natural or unavoidable defects if a manufacturer wishes to use the materials in manufacturing food. Compliance with this requirement may be verified by any effective means, including purchasing the materials under a supplier's guarantee or certification, or examination of these materials for contamination.
e. Raw materials, other ingredients, and rework should be held in bulk, or in containers designed and constructed so as to protect against contamination and should be held at such temperature and relative humidity as to prevent the food from becoming adulterated. Material scheduled for rework should be identified as such.
f. Frozen raw materials and other ingredients should be kept frozen. If thawing is required prior to use, it should be done in a manner that prevents the raw materials and other ingredients from becoming adulterated.
g. Liquid or dry raw materials and other ingredients received and stored in bulk form should be held in a manner that protects against contamination.

2. Manufacturing Operations

a. Equipment and utensils and finished food containers should be maintained in an acceptable condition through appropriate cleaning and sanitizing, as necessary. Insofar as necessary, equipment should be taken apart for thorough cleaning.
b. All food manufacturing, including packaging and storage, should be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms, or for the contamination of food. One way to comply with this requirement is careful monitoring of physical factors such as time, temperature, humidity, a_w, pH, pressure, flow rate, and manufacturing operations such as freezing, dehydration, heat processing, acidification, and refrigeration to ensure that mechanical breakdowns, time delays, temperature fluctuations, and other factors do not contribute to the decomposition or contamination of food.
c. Food that can support the rapid growth of undesirable microorganisms, particularly those of public health significance, should be held in a manner that prevents the food from becoming. Compliance with this requirement may be accomplished by any effective means, including:
   – Maintaining refrigerated foods at 45°F (7.2°C) or below as appropriate for the particular food involved.
   – Maintaining frozen foods in a frozen state.
   – Maintaining hot foods at 140°F (60°C) or above.
   – Heat treating acid or acidified foods to destroy mesophilic microorganisms when those foods are to be held in hermetically sealed containers at ambient temperatures.
d. Measures such as sterilizing, irradiating, pasteurizing, freezing, refrigerating, controlling pH or controlling a_w that are taken to destroy or prevent the growth of undesirable microorganisms, particularly those of public health significance, should be adequate under the conditions of manufacture, handling, and distribution to prevent food from being adulterated.
e. Work-in-process should be handled in a manner that protects against contamination.
f. Effective measures should be taken to protect finished food from contamination by raw materials, other ingredients, or refuse. When raw materials, other ingredients, or refuse are unprotected, they should not be handled simultaneously in a receiving, loading, or shipping area if that handling could result in contaminated food. Food transported by conveyor should be protected against contamination as necessary.
g. Equipment, containers, and utensils used to convey, hold, or store raw materials, work-in-process, rework, or food should be constructed, handled, and maintained during manufacturing or storage in a manner that protects against contamination.
h. Effective measures should be taken to protect against the inclusion of metal or other extraneous material in food. Compliance with this

requirement may be accomplished by using sieves, traps, magnets, electronic metal detectors, or other suitable effective means.

i. Food, raw materials, and other ingredients that are adulterated should be disposed of in a manner that protects against the contamination of other food. If the adulterated food is capable of being reconditioned, it should be reconditioned using a method that has been proven to be effective or it should be reexamined and found not to be adulterated before being incorporated into other food.

j. Mechanical manufacturing steps such as washing, peeling, trimming, cutting, sorting and inspecting, mashing, dewatering, cooling, shredding, extruding, drying, whipping, defatting, and forming should be performed so as to protect food against contamination. Compliance with this requirement may be accomplished by providing adequate physical protection of food from contaminants that may drip, drain, or be drawn into the food. Protection may be provided by adequate cleaning and sanitizing of all food-contact surfaces, and by using time and temperature controls at and between each manufacturing step.

k. Heat blanching, when required in the preparation of food, should be effected by heating the food to the required temperature, holding it at this temperature for the required time, and then either rapidly cooling the food or passing it to subsequent manufacturing without delay. Thermophilic growth and contamination in blanchers should be minimized by the use of adequate operating temperatures and by periodic cleaning. Where the blanched food is washed prior to filling, water used should be safe and of adequate sanitary quality.

l. Batters, breading, sauces, gravies, dressings, and other similar preparations should be treated or maintained in such a manner that they are protected against contamination. Compliance with this requirement may be accomplished by any effective means, including one or more of the following:
   - Using ingredients free of contamination.
   - Employing adequate heat processes where applicable.
   - Using adequate time and temperature controls.
   - Providing adequate physical protection of components from contaminants that may drip, drain, or be drawn into them.
   - Cooling to an adequate temperature during manufacturing.
   - Disposing of batters at appropriate intervals to protect against the growth of microorganisms.

m. Filling, assembling, packaging, and other operations should be performed in such a way that the food is protected against contamination. Compliance with this requirement may be accomplished by any effective means, including:
   - Use of a quality control operation in which the critical control points are identified and controlled during manufacturing.
   - Adequate cleaning and sanitizing of all food-contact surfaces and food containers.
   - Using materials for food containers and food-packaging materials that are safe and suitable.
   - Providing physical protection from contamination, particularly airborne contamination.
   - Using sanitary handling procedures.

n. Food such as, but not limited to, dry mixes, nuts, intermediate-moisture food, and dehydrated food, that relies on the control of a_w for preventing the growth of undesirable microorganisms should be processed to and maintained at a safe moisture level. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
   - Monitoring the a_w of food.
   - Controlling the soluble solids/water ratio in finished food.
   - Protecting finished food from moisture pickup, by use of a moisture barrier or by other means, so that the a_w of the food does not increase to an unsafe level.

o. Food, such as, but not limited to, acid and acidified food, that relies principally on the control of pH for preventing the growth of undesirable microorganisms should be monitored and maintained at a pH of 4.6 or below. Compliance with this requirement may be accomplished by any effective means, including employment of one or more of the following practices:
   - Monitoring the pH of raw materials, food-in-process, and finished food.
   - Controlling the amount of acid or acidified food added to low-acid food.

p. When ice is used in contact with food, it should be made from water that is safe and of adequate sanitary quality, and should be used only if it has been manufactured in accordance with current good manufacturing practice.

q. Food-manufacturing areas and equipment used for manufacturing human food should not be used to manufacture nonhuman-food-grade animal feed or inedible products, unless there is no reasonable possibility for the contamination of the human food.

H. Warehousing and Distribution (Section 110.93)

Storage and transportation of finished food should be under conditions that will protect food against physical, chemical, and microbial contamination as well as against deterioration of the food and the container.

I. Natural or Unavoidable Defects in Food for Human Use that Present No Health Hazard (Section 110.110)

1. Some foods, even when produced under current good manufacturing practice, contain natural or unavoidable defects that at low levels are not hazardous to health. FDA establishes maximum levels for these defects in foods produced under current good manufacturing practice and uses these levels in deciding whether to recommend regulatory action.
2. Defect action levels are established for foods whenever it is necessary and feasible to do so. These levels are subject to change upon the development of new technology or the availability of new information.
3. The mixing of a food containing defects above the current defect action level with another lot of food is not permitted and renders the final food adulterated within the meaning of the Act, regardless of the defect level of the final food.
4. A compilation of the current defect action levels for natural or unavoidable defects in food for human use that present no health hazard may be obtained from the FDA for printed or electronic versions.

III. HAZARD ANALYSIS CRITICAL CONTROL POINTS REGULATIONS (HACCPR)

In 1997, FDA adopted a food safety program that was developed nearly 30 years ago for astronauts and is now applying it to seafood, and fruit and vegetable juices. The agency intends to eventually use it for much of the U.S. food supply. The program for the astronauts focuses on preventing hazards that could cause foodborne illnesses by applying science-based controls, from raw material to finished products. FDA's new system will do the same.

Many principles of this new system now called Hazard Analysis and Critical Control Points (HACCP) are already in place in the FDA-regulated low-acid canned food industry. Since 1997, FDA has mandated HACCP for the processing of seafood, fruit juices, and vegetable juices. Also, FDA has incorporated HACCP into its *Food Code*, a document that gives guidance to and serves as model legislation for state and territorial agencies that license and inspect food service establishments, retail food stores, and food vending operations in the United States.

FDA now is considering developing regulations that would establish HACCP as the food safety standard throughout other areas of the food industry, including both domestic and imported food products. HACCP has been endorsed by the National Academy of Sciences, the Codex Alimentarius Commission (an international, standard-setting organization), and the National Advisory Committee on Microbiological Criteria for Foods. Several U.S. food companies already use the system in their manufacturing processes, and it is in use in other countries, including Canada.

A. What is HACCP?

HACCP involves seven principles:

1. Analyze Hazards. Potential hazards associated with a food and measures to control those hazards are identified. The hazard could be biological, such as a microbe; chemical, such as a toxin; or physical, such as ground glass or metal fragments.
2. Identify critical control points. These are points in a food's production — from its raw state through processing and shipping to consumption by the consumer — at which the potential hazard can be controlled or eliminated. Examples are cooking, cooling, packaging, and metal detection.
3. Establish preventive measures with critical limits for each control point. For a cooked food, for example, this might include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbes.
4. Establish procedures to monitor the critical control points. Such procedures might include determining how and by whom cooking time and temperature should be monitored.
5. Establish corrective actions to be taken when monitoring shows that a critical limit has not been met — for example, reprocessing or disposing of food if the minimum cooking temperature is not met.
6. Establish procedures to verify that the system is working properly — for example, testing time-and-temperature recording devices to verify that a cooking unit is working properly.
7. Establish effective record keeping to document the HACCP system. This would include records of hazards and their control methods, the monitoring of safety requirements and action taken to correct potential problems.

Each of these principles must be backed by sound scientific knowledge such as published microbiological studies on time and temperature factors for controlling foodborne pathogens.

B. Need for HACCP

New challenges to the U.S. food supply have prompted FDA to consider adopting a HACCP-based food safety system on a wider basis. One of the most important challenges is the increasing number of new food pathogens. For example, between 1973 and 1988, bacteria not previously recognized as important causes of foodborne illness — such as *Escherichia coli* O157:H7 and *Salmonella enteritidis* — became more widespread. There also is increasing public health concern about chemical contamination of food: for example, the effects of lead in food on the nervous system.

Another important factor is that the size of the food industry and the diversity of products and processes have grown tremendously — in the amount of domestic food manufactured and the number and kinds of foods imported. At the same time, FDA and state and local agencies have the same limited level of resources to ensure food safety. The need for HACCP in the United States, particularly in the seafood industry, is further fueled by the growing trend in international trade for worldwide equivalence of food products and the Codex Alimentarius Commission's adoption of HACCP as the international standard for food safety.

C. Advantages and Plans

HACCP offers a number of advantages over previous systems. Most importantly, HACCP

1. Focuses on identifying and preventing hazards from contaminating food,
2. Is based on sound science,
3. Permits more efficient and effective government oversight, primarily because the record keeping allows investigators to see how well a firm is complying with food safety laws over a period rather than how well it is doing on any given day,
4. Places responsibility for ensuring food safety appropriately on the food manufacturer or distributor,
5. Helps food companies compete more effectively in the world market,
6. Reduces barriers to international trade.

Here are the seven steps used in HACCP plan development.

1. Preliminary Steps
   a. General information
   b. Describe the food
   c. Describe the method of distribution and storage
   d. Identify the intended use and consumer
   e. Develop a flow diagram
2. Hazard Analysis Worksheet
   a. Set up the Hazard Analysis Worksheet
   b. Identify the potential species-related hazards
   c. Identify the potential process-related hazards
   d. Complete the Hazard Analysis Worksheet
   e. Understand the potential hazard
   f. Determine if the potential hazard is significant
   g. Identify the critical control points (CCP)
3. HACCP Plan Form
   a. Complete the HACCP Plan Form
   b. Set the critical limits (CL)
4. Establish Monitoring Procedures
   a. What
   b. How
   c. Frequency
   d. Who
5. Establish Corrective Action Procedures
6. Establish a Record Keeping System
7. Establish Verification Procedures

It is important to remember that apart from HACCPR promulgated for seafood and juices, the implementation of HACCP by other categories of food processing is voluntary. However, the FDA and various types of food processors are working together so that eventually HACCPR will become available for many other food processing systems under FDA jurisdiction. Using the HACCPR for seafood processing as a guide, the following discussion for a HACCP plan applies to all categories of food products being processed in United States.

D. Hazard Analysis

Every processor should conduct a hazard analysis to determine whether there are food safety hazards that are reasonably likely to occur for each kind of product processed by that processor and to identify the preventive measures that the processor can apply to control those hazards. Such food safety hazards can be introduced both within and outside the processing plant environment, including food safety hazards that can occur before, during, and after harvest. A food safety hazard that is reasonably likely to occur is one for which a prudent processor would establish controls because experience, illness data, scientific reports, or other information provide a basis to conclude that there is a reasonable possibility that it will occur in the particular type of product being processed in the absence of those controls.

E. The HACCP Plan

Every processor should have and implement a written HACCP plan whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur. A HACCP plan should be specific to:

1. Each location where products are processed by that processor; and
2. Each kind product processed by the processor.

The plan may group kinds of products together, or group kinds of production methods together, if the food safety hazards, critical control points, critical limits, and procedures required to be identified and performed are identical for all products so grouped or for all production methods so grouped.

1. The Contents of the HACCP Plan

The HACCP plan should, at a minimum:

- List the food safety hazards that are reasonably likely to occur, as identified, and that thus must be controlled for each product. Consideration should be given to whether any food safety hazards are reasonably likely to occur as a result of the following: natural toxins; microbiological contamination; chemical contamination; pesticides; drug residues; decomposition in products where a food safety hazard has been associated with decomposition; parasites, where the processor has knowledge that the parasite-containing product will be consumed without a process sufficient to kill the parasites; unapproved use of direct or indirect food or color additives; and physical hazards;
- List the critical control points for each of the identified food safety hazards, including as appropriate: critical control points designed to control food safety hazards that could be introduced in the processing plant environment; and critical control points designed to control food safety hazards introduced outside the processing plant environment, including food safety hazards that occur before, during, and after harvest;
- List the critical limits that must be met at each of the critical control points;
- List the procedures, and frequency thereof, that will be used to monitor each of the critical control points to ensure compliance with the critical limits;
- Include any corrective action plans that have been developed to be followed in response to deviations from critical limits at critical control points;
- List the verification procedures, and frequency thereof, that the processor will use;
- Provide for a record keeping system that documents the monitoring of the critical control points. The records should contain the actual values and observations obtained during monitoring.

2. Signing and Dating the HACCP Plan

The HACCP plan should be signed and dated:

- Either by the most responsible individual on site at the processing facility or by a higher-level official of the processor. This signature should signify that the HACCP plan has been accepted for implementation by the firm.
- Upon initial acceptance; upon any modification; and upon verification of the plan.

F. Sanitation

Sanitation controls (3) may be included in the HACCP plan. However, to the extent that they are otherwise monitored, they need not be included in the HACCP plan.

G. Implementation

This book is not the proper forum to discuss in detail the implementation of HACCPR. Readers interested in additional information on HACCP should visit the FDA HACCP website http://vm.cfsan.fda.gov/ that lists all the currently available documents on the subject.

IV. FDA FOOD CODE

The FDA *Food Code* (the Code) (4) is an essential reference that guides retail outlets such as restaurants and grocery stores and institutions such as nursing homes on how to prevent foodborne illness. Local, state and federal regulators use the FDA *Food Code* as a model to help develop or update their own food safety rules and to be consistent with national food regulatory policy. Also, many of the over one million retail food establishments apply *Food Code* provisions to their own operations. The *Food Code* is updated every two years, to coincide with the biennial meeting of the Conference for Food Protection. The conference is a group of representatives from regulatory agencies at all levels of government, the food industry, academia, and consumer organizations that works to improve food safety at the retail level (5). A brief discussion of the Code is provided below. Further information, including access to the Code, may be obtained from the Food Safety Training and Education Alliance (www.fstea.org).

The Code establishes definitions; sets standards for management and personnel, food operations, and equipment and facilities; and provides for food establishment plan review, permit issuance, inspection, employee restriction, and permit suspension. The Code discusses the good manufacturing practices for equipment, utensils, linens, water, plumbing, waste, physical facilities, poisonous or toxic materials, compliance and enforcement. The Code also provides guidelines on food establishment inspection, HACCP guidelines, food processing criteria, model forms,

guides, and other aids. A brief introduction to the *Food Code* in this chapter is important for two reasons:

- Firstly, at the end of this book, two chapters cover retail food protection from the perspectives of food sanitation. The *Food Code* forms the backbone of these chapters.
- Secondly, although this guide is designed for retail food protection, more than half of the data included are directly applicable to food processing plants, e.g. equipment design (cleanability), CIP system, detergents and sanitizers, refrigeration and freezing storage parameters, water requirements, precautions against "backflow" (air, valve, etc.), personnel health and hygiene, rest rooms and accessories, pest control, storage of toxic chemicals, inspection forms, inspection procedures, and many more. Some of the data in the present book can be readily traced to the Code.

The Code consists of 8 chapters and 7 annexes. Some of the information found in the Code will be further explored in two chapters at the end of this book. The annex that covers inspection of a food establishment applies equally as well to both retail food protection and to sanitation in food processing. According to the Code, the components of an inspection would usually include the following elements:

- Introduction
- Program planning
- Staff training
- Conducting the inspection
- Inspection documentation
- Inspection report
- Administrative procedures by the state/local authorities
- Temperature measuring devices
- Calibration procedures
- HACCP inspection data form
- Food establishment inspection report
- FDA electronic inspection system
- Establishment scoring.

Details of these items will not be discussed here; some are further explored in various chapters in this book (please consult the index for specific topics). Instead, the next two sections trace the history and practices of food establishment inspection and how basic sanitation controls are slowly evolving into the prerequisites for HACCP plans in both retail food protection *and* food processing plants.

A. Purpose

A principal goal to be achieved by a food establishment inspection is to prevent foodborne disease. Inspection is the primary tool a regulatory agency has for detecting procedures and practices that may be hazardous and for taking actions to correct deficiencies. *Food Code*-based laws and ordinances provide inspectors science-based rules for food safety. The *Food Code* provides regulatory agencies with guidance on planning, scheduling, conducting, and evaluating inspections. It supports programs by providing recommendations for training and equipping the inspection staff, and attempts to enhance the effectiveness of inspections by stressing the importance of communication and information exchange during regulatory visits. Inspections aid the food-service industry in the following ways.

1. Serving as educational sessions on specific Code requirements as they apply to an establishment and its operation;
2. Conveying new food safety information to establishment management and providing an opportunity for management to ask questions about general food safety matters; and
3. Providing a written report to the establishment's permit holder or person in charge so that the responsible person can bring the establishment into conformance with the Code.

B. Current Applications of HACCP

Inspections have been a part of food safety regulatory activities since the earliest days of public health. Traditionally, inspections have focused primarily on sanitation. Each inspection is unique in terms of the establishment's management, personnel, menu, recipes, operations, size, population served, and many other considerations.

Changes to the traditional inspection process were first suggested in the 1970s. The terms "traditional" or "routine" inspection have been used to describe periodic inspections conducted as part of an on-going regulatory scheme. A full range of approaches was tried and many were successful in managing a transition to a new inspection philosophy and format. During the 1980s, many progressive jurisdictions started employing the HACCP approach to refocus their inspections. The term "HACCP approach" inspection is used to describe an inspection using the "Hazard Analysis and Critical Control Point" concept. Food safety is the primary focus of a HACCP approach inspection. One lesson learned was that good communication skills on the part of the person conducting an inspection are essential.

FDA has taught thousands of state and local inspectors the principles and applications of HACCP since the 1980s. The State Training Branch and the FDA Regional Food Specialists have provided two-day to week-long courses on the scientific principles on which HACCP is based, the practical application of these principles including field exercises, and reviews of case studies. State and local jurisdictions have also offered many training opportunities for HACCP.

A recent review of state and local retail food protection agencies shows that HACCP is being applied in the following ways:

1. Formal Studies — Inspector is trained in HACCP and is using the concepts to study food hazards in establishments. These studies actually follow foods from delivery to service and involve the write-up of data obtained (flow charts, cooling curves, etc.).
2. Routine Use — State has personnel trained in HACCP and is using the hazard analysis concepts to more effectively discover hazards during routine inspections.
3. Consultation — HACCP-trained personnel are consulting with industry and assisting them in designing and implementing internal HACCP systems and plans.
4. Alternative Use — Jurisdiction used HACCP to change inspection forms or regulations.
5. Risk-Based — Jurisdiction prioritized inventory of establishments and set inspection frequency using a hazard assessment.
6. Training — Jurisdiction is in the active process of training inspectors in the HACCP concepts.

Personnel of every sort of food establishment should have one or several copies of the *Food Code* readily available for frequent consultation.

V. APPLICATIONS

The sanitary requirements in the CGMPR and the *Food Code* serve as the framework for the chapters in this book. The HACCPR will be touched on when they help to clarify the discussion. Essentially, this book shows how to implement the umbrella regulations provided under the CGMPR. Each chapter handles one aspect of these complicated regulations. Most chapters discuss the regulations applicable to all types of food products being processed. Several chapters concentrate on the sanitary requirements from the perspectives of the processing of a specific category of food.

The appendix of this book reproduces the complete coverage of CGMR in Section 110.

ACKNOWLEDGMENT

The information in this chapter has been derived from *Food Safety Manual*, published and copyrighted by Science Technology System, West Sacramento, California, 2004©. Used with permission.

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74 Filth and Other Foreign Objects in Food: A Review of Analytical Methods and Health Significance

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CONTENTS

I. THE FOOD, DRUG AND COSMETIC ACT

This chapter is based in large part on earlier reviews [1–3] and other contributions to the scientific literature [4, 5], but the text has been amended and expanded to include additional information.

The language of the Federal Food, Drug and Cosmetic Act (FD&C Act) seems rather unequivocal whenever acts of adulteration by filth are mentioned [6–8]. One paragraph, 301(k), prohibits any act done to a food after shipment in interstate commerce that results in the food being adulterated. Another, 402(a)(1), declares "a food shall be

deemed to be adulterated if it bears or contains any poisonous or deleterious substance which may render it injurious to health." Still another, 402(a)(3), states that a food shall be deemed adulterated if it consists in whole or in part of any filthy, putrid, or decomposed substance or if it otherwise is unfit for food. The final example, from paragraph 402(a)(4), states that a food shall be deemed to be adulterated if it has been prepared, packed, or held under unsanitary conditions by which it may have been contaminated with filth, or by which it may have been rendered injurious to health [9].

A. Enforcement and Guidance

In the United States of America, the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) share responsibility for food safety. Federal drinking water standards are set, monitored and enforced by the Environmental Protection Agency. The task of enforcing the FD&C Act has been given to the FDA [10–13]. Scientists and policy managers at the FDA, along with collaborating personnel from other federal agencies and from universities, have endeavored to provide helpful guidance to the food industry by generating explanatory documents designed to spell out in detail just what FDA understands to be the meaning of these basic paragraphs of the FD&C Act [14–19].

B. Codex Alimentarius

Two United Nations agencies, the Food and Agricultural Organization (FAO) and the World Health Organization (WHO), have also issued food safety guidelines under the general title of Codex Alimentarius [20, 21] that are similar in scope and principle to the food safety guidance documents issued by the FDA and the USDA. Moreover, WHO has promulgated a global strategy for food safety [22]. The European Union has established the European Food Safety Authority (ESFA), and some European nations, such as Spain [23], have reorganized their national food safety programs to meet the EFSA guidelines.

II. ANALYTICAL METHODS FOR FILTH

This short review should in no way be construed as a technical guide to the detection of filth in food; it is meant merely to put the practice of filth analysis in its proper place in the overall arena of food safety [24]. Reliable sources of technical information are readily available [25–30].

A. History of Filth Analysis

The current edition of the *Official Methods of Analysis of AOAC International* [25] is the product of nearly 100 years of development of analytical methods. Dr. Harvey W. Wiley, who helped create the USDA-sponsored Association of Official Agricultural Chemists (AOAC) in 1894, directed the publication of the first book of official methods, a manual devoted to the analysis of agricultural fertilizers. Wiley and his group of intrepid food-tasters/testers gave much of their attention to toxic chemical adulterants [31]. However, even in those early days, around the time of the passage of the first federal food purity law in 1906 [32], some effort (apparently minimal) was made to detect filth in food. Since the detection process required some form of analysis of each kind of food, analytical methods were created out of necessity.

Over the years, the subject matter of the official methods book was expanded to include (to mention just a few among many) animal feeds, dairy products, filth, microbiological contaminants, and pesticides. Sponsorship for the Association shifted to the FDA when that arm of government was established in 1927. Because of the ever-broadening scope of analytical work, the organization's name was changed to the Association of Official Analytical Chemists in 1965. AOAC became a fully independent organization in 1979 (but still retained close ties to USDA and FDA). The ever-widening international scope of food analysis called for a more inclusive "Association of Analytical Communities" and a further name change in 1991 to AOAC International [33].

B. The Practice of Food Analysis

The practice of food analysis occurs at many different levels of sophistication. The more informal the analysis, i.e., at the lower end of the sophistication scale, the less expensive it is. The more formal the analysis, i.e., higher on the scale of sophistication, the more expensive it is. This expenditure of money and effort must be justified; in other words, there has to be some compelling reason to carry out the analysis. Generally, the more sophisticated procedures take place in a laboratory setting; the less formal procedures may occur in a wide variety of settings, including the laboratory.

C. Why Analyze Food

The reasons for performing analyses vary widely, but the basic motive of food manufacturers is to present to the consumer food that tastes good, smells good and *is* good. Achieving this maintains or enhances one's reputation, whether individual or corporate. The production and marketing of only good and wholesome food also means that the food establishment is complying with the pertinent federal, state and local laws pertaining to food purity, thus avoiding the unpleasant consequences of violating these laws: fines, prison time, and civil law suits, to mention a few [34–36].

Other reasons for conducting food analyses arise from the law enforcement side of the equation. Voluntary compliance with the laws and with good manufacturing

practices [37, 38] is very beneficial to the consumer, but the only way to know for certain the purity status, as defined by the pertinent laws and regulations, of any given food at any given time is to analyze it. Here, with regard to the Federal FD&C Act [39, 40], at least, analysts are guided by the two major filth provisions in the Act, namely that filth has no place being *in* food or being *around* food.

D. Analytical Varieties

The quick and casual observations by sight and smell that happen whenever a can or package of food is opened in the home kitchen constitute food analysis — informal but often quite telling. Similar things happen in the grocery; cans and packages are usually not opened, unless in connection with a deli operation, but cans and packages are observed for defects, and substandard items of all kinds are culled before being presented to the public. Visual inspection (read "analysis") takes place in the warehousing and distribution sectors of the food chain. Items that look bad or smell bad are discarded.

In the food-manufacturing sector, food analysis is an integral part of the operation's quality assurance protocols. Similar analyses, most informal but some involving technical expertise, happen at the places where raw materials are collected for offer as ingredients in the manufacture of foods. Analyses occur, too, at the many places where food materials are harvested.

Food materials may be visually inspected on the wharf or in the warehouse, or at the granary or flourmill. It is common for samples to be collected in all of these places by FDA and USDA investigators and sent to a government food laboratory for further examination. In the laboratory setting, the whole range of analytical techniques may be employed, from the simplest to the most technically complex.

III. ANALYSIS FOR FILTH, DECOMPOSITION AND FOREIGN MATTER

A. Heavy Filth

Although the word "filth" is in the title of this chapter, it is really just the first word in a triad of contaminants: filth, decomposition, and extraneous materials (or foreign matter) [41]. This last term covers all the others, but in common practice it refers to sand, gravel, paint chips, metal fragments, bits of glass, fruit pits, and a whole host of other hard foreign objects (these kinds of extraneous materials, often collectively called "heavy filth," are discussed below).

Visual inspection procedures and other "macroanalytical" techniques designed to detect hard or sharp foreign objects in raw ingredients or processed foods are discussed in the *Macroanalytical Procedures Manual* [42]. These hard foreign objects can be sieved out of food; or they tend to settle out of the food materials upon agitation. Analytical techniques for glass detection in infant foods packaged in glass have been developed at FDA [43].

B. Decomposition and Quality Evaluations

1. Decomposition

The detection of decomposition is a specialized kind of analysis. The process of decomposition often leads to the production of characteristic odors that a specially trained and experienced analyst can detect by *organoleptic methods* (smell, in this case, but taste is also employed in some cases). Organoleptic methods [44] have been widely used in the past to detect decomposition in seafoods and in the carcasses of meat animals and poultry. With the onset of voluntary or mandatory HACCP (Hazard Analysis and Critical Control Points) programs, organoleptic analyses have become less common.

2. Quality Evaluations

Panels of sensory analysts are often used to evaluate the quality status of dairy products, especially cheeses. The organoleptic method applied here is called *descriptive sensory analysis*. Considerable progress has been made in the development of an "electronic nose" designed to do much of the work of the human evaluation panel. The electronic nose technology has been utilized in a wide variety of applications including evaluating the microbial quality status of milk and of cereal grains, cheese and juice quality, fermentation status of sausage, and meat quality with reference to spoilage [45].

Another line of research is focused on developing an artificial tongue that mimics human taste buds [46]. These sensory elements consist of various conducting polymers embedded in an ultrathin film. Besides the basic taste categories (sweet, sour, etc.), the artificial tongue can detect slight differences among different kinds of bottled water or different batches of wine from the same winery. Toxic chemicals and extremely bitter tastes, that would be very unpleasant or dangerous for human tasters to detect, can be identified by the artificial tongue.

3. Mold as Evidence of Decomposition

Molds often occur on fruits, nuts, berries, seeds, and many other food materials. These defects may be detected by visual examination — techniques that are referred to as macroanalytical methods [42, 47, 48]. On occasion, to get an approximation of the level of moldiness, the product in question is subjected to a special sampling program that is designed to make the sample representative of the entire lot. These macroanalytical techniques are often used when

unprocessed foods are presented to the food manufacturer for use as ingredients.

In other techniques, such as the Howard Mold Count, the sample of food is prepared for analysis in such a way that the mold fragments become visible under light microscopy and that the mold count in the sample accurately reflects the level of mold contamination in the entire lot that was sampled [49].

C. Light Filth

The detection of insect fragments, feather barbules and rat hairs — all properly labeled filth but sometimes referred to as light filth — has been a major focus of food analysts for more than half a century. Basically, these kinds of analytical methods, often referred to as microanalytical methods, involve dispersing an extraction oil (usually mineral oil) into an aqueous or alcoholic mixture of the food sample. Since the bits of insect cuticle, feather barbules and hairs are oleophilic, they tend to rise with the oil to the surface. The food materials, being largely hydrophilic, tend to settle to the bottom. The analyst then simply separates the two polar opposites and examines the mineral oil fraction under the microscope [50].

This oil/water technique, frequently utilized for routine analyses in many laboratories, is the procedure that was most often used in the days when it was necessary to establish (or reexamine) a food defect action level (DAL, discussed below) for a particular product [51]. For example, in one instance in which questions were raised about filth adulterants in canned sardines, canned tuna and canned crabmeat, samples were collected, using a statistically valid sampling protocol, from many localities around the United States [52]. Before the current moratorium on new DAL development [53], other products were sampled and analyzed in a similar manner. These sample collection programs produced such huge quantities of product that it was usually not feasible for a single government laboratory to do all the analytical work. Therefore, contract laboratories in the private sector performed most of the microanalytical work.

D. Soluble Filth

In recent years there has been a trend to go beyond trying to detect merely particulate filth. These efforts have focused on identifying and quantifying the biochemical signatures left behind by food-contaminating animals (birds, bats, rodents, insects, mites) [54–60].

IV. QUALITY ASSURANCE

A. Methods of Quality Assurance

One very important aspect of filth analysis is perhaps little known and little appreciated by the general public. We refer to the great emphasis on quality assurance on the part of the analyst population. For example, when an analyst develops a new analytical technique, that technique does not simply and immediately become a standard method. The technique has to be evaluated by a panel of expert analysts to determine its reliability in the hands of several different analytical technicians. The new technique will most likely get wide exposure by its publication in a scientific periodical such as the *Journal of AOAC International*. If it survives the examination occasioned by the journal publication, the technique may eventually make it into the *Official Methods of Analysis of AOAC International* [25].

B. Analyst Quality Assurance

Most filth analysts are tested periodically to evaluate their analytical skills. Most often, this testing is done under programs sponsored by AOAC International. Test samples are sent out to the analysts and the analytical results derived therefrom are then evaluated. In this way, a high level of consistent expertise is maintained, and any deficiencies in analyst performance can be remedied.

The achievement and maintenance of a high and consistent level of skill comes by way of unending practice and the pursuit of perfection in carrying out analytical procedures. A huge body of literature [see, for example, references 61–65, as well as the other references at the end of this chapter] and a wide array of analytical techniques [25] must be mastered by the analyst.

C. Laboratory Quality Assurance

Two international bodies, the International Standards Organization and the International Electrotechnical Commission, have promulgated, as rule ISO/IEC 17025, a minimum set of standards for analytical laboratories [66]. While remaining fully functional and in compliance with internal standards, all FDA analytical laboratories (along with virtually all commercial and governmental laboratories) are in a state of transition as they strive to fully comply with ISO/IEC 17025.

D. Benefits to the Public

This emphasis on quality assurance directly benefits the general public. When any given food in found to be adulterated, the public can have confidence in that finding and reassurance in knowing that such adulterated food will not reach the dinner table. Similarly, when analysts find no filth in food and it has been certified that all applicable sanitation standard operating procedures (SSOPs) and HACCP protocols have been observed, the public can be confident that these findings reflect the actual state of the food — it is wholesome and safe to consume [67].

V. HEALTH SIGNIFICANCE OF ADULTERATION BY FILTH

A. Filth and the FD&C Act

The federal courts have never insisted that there must always be a cause-and-effect relationship between "filth" and "injurious to health" (see Section I, above). Since 1942, when District Court Judge Chestnut (sometimes spelled Chesnut) read during a trial the definition of *filth* from *Webster's International Dictionary*, the ordinary sense of the word has been used in judicial interpretations of the FD&C Act [68]. Judges and juries have rendered verdicts both for and against the government's allegations of filth in food, but always based on the practical application of this dictionary definition [35].

As used in this chapter, *filth* is defined as insect, bird, rodent, or other objectionable matter, usually of animal origin, found in or associated with food [4, 69]. The more common kinds of filth found during food inspections and analyses are whole insects and mites and fragments thereof; rodent and other mammalian hairs; feathers and feather barbules; and urine, uric acid, and feces (usually from rodents and birds); and molds.

B. Competition for Food

Human foods and animal feeds, as well as the places where these materials are stored, processed and sold, are attractive to a wide variety of opportunistic animals and fungi. Competition between "us" and "them" for nutritive substances is keen and continuous. Pests of the food industry may be counted upon to take full advantage of every opportunity to convert our foods and feeds to their own use. To prevent this, nothing less than constant vigilance is required of the food industry (or the housekeeper in the home setting) [70]. But even under the best of circumstances, it is difficult to consistently provide the consumer with foods and feeds that are completely free from contamination by food pests [69].

C. Indicators of Insanitation: Four Categories

We emphasize again that competition for food is keen. Food pests continually search for opportunities to share our food resources [71]. Commensal pests such as houseflies, cockroaches, ants and mice are considered *opportunistic* pests. They typically exhibit synanthropic behavior, that is, they are closely associated with human foods or animal feeds and with structures built by humans. *Adventive* (*facultative*) pests, such as birds, bats and various insects, sometimes utilize human-built structures for shelter and this behavior (synanthropy) may bring them into contact with foods or food-contact surfaces.

Pantry pests, such as the sawtoothed grain beetle (*Oryzaephilus surinamensis*) or the Indianmeal moth (*Plodia interpunctella*), are categorized as *obligatory* pests because they are largely dependent on human or animal foods for their own food and for shelter. All of the categories of insects noted here may also be parasitized by small wasps or flies, termed *parasitoids*; this is the fourth and final category of pests considered to be indicators of insanitation.

D. Early Ideas and Discoveries

The idea that pests might be vectors of pathogenic microbes was very slow to dawn upon the human mind. The germ theory itself had first to be thoroughly established, and that was another idea that was slow to take hold. But once the germ theory was accepted, people began to ask about the ways that germs make their way from a person who is sick to one who is well.

By the dawn of the twentieth century, it was well known that germs causing certain diseases could be transmitted directly from one person to another. This method of transmission, seemingly so simple and logical, became the overriding theory that was applied, correctly or incorrectly, to a wide variety of diseases. In some cases, the theory worked perfectly; in other cases, it seemed to work well most of the time; and in still other cases, it did not work at all.

E. Transmission Cycles

Classic studies of malaria, yellow fever, and Texas cattle fever firmly established the fact that some disease transmission cycles include an obligatory intermediary, the *vector* [72]. Moreover, the disease agents themselves were identified as belonging to a wide range of living organisms — viruses, bacteria, protists/protozoa, helminths, and fungi. The transmission cycles of these disease agents varied from the very simple to the very complex.

Before the first half of the twentieth century came to a close, a large number of vectorborne diseases had been studied to the point where their mechanisms of transmission were well understood. Two basic cycles of pathogen transmission, biological and mechanical, had been defined. In the simplest form of biological transmission, the pathogen must enter the vector, undergo at least one mandatory phase of multiplication and/or morphologic transformation, and be carried by the vector to a susceptible host. Biological transmission and the role of food pests as direct agents of disease (that is, in the production of toxins and allergens, as the cause of physical injury, or the invasion of the digestive tract by insects and mites) are not discussed is this chapter [see 72 for numerous examples], except for very brief note below regarding biological transmission. In mechanical transmission [5], the pathogen

remains on the external surface of the vector, or in its gut, or in its excretory system. The vector merely transports the pathogen from an infectious source to a susceptible host, to something that a susceptible host eats or drinks, or to a food-contact surface.

F. Mechanical Transmission

The mechanical transmission of foodborne pathogens is another idea that continues to be very slow to take hold. It seems very easy for professional health-care providers to accept the concept that a diarrheic food handler, emerging from a bathroom without washing his or her hands and immediately preparing a shrimp salad, could contaminate food with pathogenic bacteria that might cause illness in susceptible people who eat that food. This is, obviously, the classic *fecal-oral route* of transmission that every clinician and epidemiologist remembers when confronted by a case or an outbreak of foodborne illness. However, when an entomologist suggests that a housefly or a cockroach might pick up pathogens from feces and mechanically transport them to human food (or food-contact surfaces) and that people might become ill from eating that food, the traditional medical professional usually tends to look for another explanation.

1. Nosocomial Infections

A good example of this kind of tunnel-vision approach can be found in the literature on the epidemiology of nosocomial infections in which the proposition that hospital pests might be vectors is rarely considered. In spite of an extant and growing literature on the subject (some examples are given in the references cited in this chapter), the possibility of mechanical transmission by insects is typically excluded whenever environmental factors are discussed in relation to nosocomial infections [73]. Even in those hospitals, such as perhaps one in Calcutta, where one might understandably expect to have flies, ants and cockroaches as frequent visitors, usually no role is given to and no discussion is offered concerning insects as mechanical vectors [74].

In contrast to this mindset of entrenched neglect of the evidence regarding insects as mechanical vectors, infection control specialists in an Israeli hospital were quick to ban the carrying and use of cell phones by patient-care personnel when *Acinetobacter baumannii*, a multidrug-resistant, desiccation-resistant microbe that causes nosocomial infections with mortality rates in the range of 50–69%, was isolated from 12% of the phones carried by care givers [75].

2. The Housefly Menace

Before the twentieth century was even a decade old, Dr. Harvey W. Wiley, that great champion of food purity, had already made plans to study the role of houseflies (*Musca domestica*) as mechanical carriers of bacteria [76]. In the same time period, Dr. L. O. Howard [77], an entomologist, and Dr. Samuel Crumbine [78], a physician and public health administrator (to mention just two activists among many), castigated the housefly as a menace to public health (Figure 74.1) [79]. Scientists have long sought to build the case for the role of vectors in food contamination and foodborne illnesses. Several lines of thought that researchers and reviewers have applied to this question are discussed here, along with examples from the literature.

FIGURE 74.1 "Kill flies!" poster, Chilean Red Cross, circa 1920. Courtesy American National Red Cross Museum, Washington, D.C., and the International Red Cross and Red Crescent Museum, Geneva, Switzerland. Used by permission.

G. Examination of Pests for Microbial Pathogens

A common approach for evaluating the role of pests as vectors in the epidemiology of foodborne illnesses is to capture a pest from its natural environment and then employ microbiological culture methods to find out what microorganisms the pest carries on its body surface and/or in its gut. Hospitals have been a common venue for these pest-screening programs in which ants, cockroaches and other pests are surveyed for nosocomial and community-acquired pathogens [80–87].

Early in the twentieth century, scientists often cultured samples from flies and cockroaches to determine their bacterial associates [88, 89]. Numerous investigators have continued these vector-screening programs right up to the

present day [85, 90–96]. Various suspect vectors have been assayed for pathogenic fungi [97–99], protists/protozoans [100–102], helminths [103–105], bacteria [106, 107], and viruses [108].

1. Flies

The two groups of insects most frequently screened for foodborne pathogens are houseflies and synanthropic cockroaches. Houseflies have been reported to carry foodborne and other human pathogens including *Campylobacter jejuni* [91, 92], *Shigella* spp. [109], *Yersinia enterocolitica* [110], *Salmonella* spp. [111], *Klebsiella* spp. [112], *Aeromonas caviae* [113], *Corynebacterium pseudotuberculosis* [114], *Mycobacterium* spp. [115], *Giardia lamblia* [116], and *Cryptosporidium parvum* [117]. In Japan, houseflies have been shown to carry enterohemorrhagic *Escherichia coli* O157:H7, a pathogen that causes the sometimes fatal "hamburger disease" (hemolytic uremic syndrome and hemorrhagic colitis) [118–120].

Enterobacter sakazakii, an opportunistic pathogen sometimes found in powdered milk, causes neonatal meningitis. A possible environmental reservoir has been identified: *E. sakazakii* was isolated from the gut of larval stable flies (*Stomoxys calcitrans*) [121]. A study of the role of houseflies in the ecology of *E. coli* O157 is currently underway at Kansas State University [122].

2. Cockroaches

German cockroaches (*Blattella germanica*) were reported to carry *Salmonella* spp. in feed mills and poultry hatcheries [123]. American cockroaches (*Periplaneta americana*) have been reported to carry and disseminate *Cryptosporidium parvum*, a pathogenic protist that causes diarrhea [124]. The make up of the microbial population of the American cockroach gut, long the subject of numerous investigations, has been shown to include a variety of microorganisms: bacteria, archaeans, protists (protozoa), and nematodes [125].

H. Fate of Pathogens on and in Pests

Another common technique is to expose (either externally or orally) a "clean" potential vector, such as a fly or a cockroach, to some known pathogen and then determine how long the microbes remain viable in or on the insect, how long the insect sheds viable pathogens to its environment, and how long the pathogens remain viable and infective after being disassociated from the host insect.

Experimental work along these lines was recently carried out with laboratory populations of a free-living soil nematode, *Caenorhabditis elegans* (Rhabditidae) [126]. Colonies of non-pathogenic *E. coli* serve as the standard food medium for these nematodes. The worms, having fed readily on bacterial colonies representing four different bacterial species (all non-pathogenic surrogates of known foodborne disease pathogens), were able to transfer the ingested bacteria to sterile substrates during a three-hour period. In the case of fruits and vegetables that are in contact with soil just before harvest and are promptly consumed without further processing, there is some concern that nematodes could transport fecal pathogens to the food item while it is in or lying on soil.

1. Cockroaches

Pai et al. [100] recovered viable cysts of *Entamoeba histolytica* from the external surfaces and from the guts of cockroaches (*Periplaneta americana*, *Blattella germanica*) that had fed on cyst-laden feces. Bacteria (*Salmonella oranienburg*) fed experimentally to American cockroaches could be isolated from the fecal pellets of the roaches over a period of 140 days [127]. American, German, and Oriental (*Blatta orientalis*) cockroaches, experimentally infected with *Salmonella typhimurium*, disseminated this pathogen for at least four days and contaminated non-infected cockroaches as well as chicken eggs [123]. Bacteria (*Salmonella enteriditis*), fed experimentally to German cockroaches, were recovered from the feces for ten days and from the gut for up to 20 days after the initial exposure [128]. Virulent mycobacteria (*Mycobacterium avium avium*), recovered from cockoach nymphs (*B. orientalis*) ten days after the roaches were orally infected, caused avian tuberculosis in chickens [129].

2. Flies

Root [130] found that cysts of *Entamoeba histolytica*, ingested by flies that were subsequently drowned in water, remained viable for seven days. Houseflies, fed on broth containing pathogenic bacteria (*Yersinia pseudotuberculosis*), retained the microbes internally for about 36 hours and contaminated their environment with viable microbes for about 30 hours after ingestion [131].

Houseflies are now being considered more seriously as potential players in the ecology and transmission of *E. coli* O157:H7 [132]. Sasaki et al. [133] investigated epidemiological significance of housefly defecation and regurgitation in the dissemination of *Escherichia coli* O157:H7 to food. It was shown that this pathogen persisted in the housefly digestive tract for at least four days after the initial exposure and that frequent defecation and regurgitation enhanced the dissemination of *E. coli* O157:H7, especially during the first 24 hours. Kobayashi et al. [134] postulated that houseflies are actually biological vectors of this pathogen because the bacteria increase in numbers while ensconced within the interstices of the spongiform mouthparts of the fly.

Shane et al. [135] demonstrated experimentally that houseflies can effectively transmit *Campylobacter jejuni*,

cause of the most frequently reported foodborne bacterial illness in the Unites States and other developed countries [136].

Zimmerman et al. [137] showed that the pseudorabies virus persisted (but did not replicate) in houseflies for up to 13 hours. Under experimental conditions, houseflies can serve as transport hosts of *Cryptosporidium parvum* oocysts [138]. For up to 11 days after ingesting oocyst-laden feces, the flies regurgitated oocysts and also released them in their own feces [139].

3. Ants

House-infesting and structure-invading ants (Formicidae) have often been investigated for their potential role as mechanical vectors. Several ant species, such as the Argentine ant, *Linepithema humile* (=*Iridomyrmex humilis*), are good candidates as mechanical vectors, but the ants that attract the most attention here are Pharaoh ants, *Monomorium pharaonis*. The frequent occurrence of ants, especially Pharaoh ants, in health-care facilities [80, 140–144], food-manufacturing plants [145], restaurants, and domestic venues [146, 147], has made ants especially suspect.

This level of suspicion is heightened by the fact that representatives of several species and genera of fecal coliforms and human pathogens, such as *Escherichia coli, Streptococcus epidermidis* (=*Micrococcus pyogenes*), *Proteus vulgaris, Pseudomonas aeruginosa, Shigella flexneri, Bordetella bronchiseptica, Klebsiella pneumoniae, Streptococcus* spp., *Staphylococcus* spp., *Salmonella* spp., *Clostridium* spp., *Proteus mirabilis, Serratia marcescens, Listeria monocytogenes* and *Yersinia pestis*, have been recovered from ants [148–152].

Given that these suspect ants have all the needed characteristics to function as mechanical vectors, and the fact that some species are extremely invasive, sometimes even, in hospitals, getting into materials that are presumed to be sterile [153, 154] and foraging on the bodies of patients, further study of their significance in pathogen dissemination may result in enough evidence to elevate this group to a level comparable to that of houseflies and cockroaches as reputed mechanical vectors. Certainly, in the case of Pharaoh ants, their indiscriminant foraging habits, involving such places as floor drains, bedpans, garbage cans, food and food-preparation surfaces, sterile dressings, discarded dressings, surgical incisions, suppurative wounds, and the lips of sick infants, would be sufficient to make them suspect as mechanical vectors.

I. Role of Pest Habits in Mechanical Transmission

A third approach in evaluating the role of pests as vectors is to study the morphology, physiology and behavior of a pest to discover what attributes it possesses that make it especially accommodating to the acquisition, carriage, shedding or deposition of pathogens. Some pests make especially suitable mechanical vectors because they have morphologic features that facilitate carriage of pathogens [155]. Commensal rodents [156], cockroaches [157], ants [158], and flies [159] have habits that often take them to contaminated substrates.

1. Houseflies and Microbes

In a somewhat anthropomorphic, but nevertheless apt, comment on houseflies, West [160] notes that "If the fly were purely and simply a fecal feeder, it would not feel the impulse to spend so much time exploring the human table. This is just what makes the species so dangerous. All day long its restless nature causes it to fly back and forth between the privy and the kitchen, between a wound that is infected and a fresh incision."

Because of their anatomy, physiology and behavior, houseflies are likely the most significant insect vectors of foodborne disease pathogens. In terms of evolutionary ecology, houseflies are very closely associated with microbes; their larvae develop exclusively in decaying organic substrates (manure, compost, garbage, etc.) that harbor large and diverse microbial communities. In fact, housefly larvae cannot develop in substrates free of live microbes [161, 162]. Virtually all environments rich in decomposing organic matter harbor diverse and populous microbial communities and are, therefore, suitable substrates for development of houseflies and other filth flies [163].

2. Larval Nutrition

Housefly larvae are morphologically and physiologically adapted for the uptake and digestion of bacterial cells [164–166]. However, it is not known if the microbial cell mass serves only as a direct source of nutrients or if the microbes also play a role in chemically breaking down the substrate, making it available for larval nutrition [162]. As pupation progresses towards completion, most of the microbes in the gut are destroyed or are left behind in the discarded pupal tissues or puparium. Consequently, newly emerged housefly adults are largely free of bacteria [167, 168]. In any case, newly emerged adult flies start immediately feeding on the substrates in which they developed as larvae. Consequently, their body surfaces and digestive tracts quickly become contaminated by microbes.

Experiments conducted in Israel showed that the numbers of bacteria (*Escherichia coli*) fed to maggots (*Lucilia sericata* — sometimes used in the debridement of wounds) decreased markedly as the bacteria progressed through the alimentary canal [169]. In contrast to these findings, Fobert [170] demonstrated that flies, *Calliphora erythrocephala* (=*C. vicina*) and *Lucilia caesar*, experimentally infected

as larvae with *Salmonella montevideo*, retained the bacteria through metamorphosis to the adult stage. Some bacteria may remain viable in or on the body of the fly after it dies. For example, Fobert [170] showed that viable *Salmonella* spp. could be recovered from flies that had died two months previously.

3. Behavior of Adult Houseflies

Adult houseflies are often attracted to human dwellings (synanthropic behavior) and, once there, seem especially attracted to food and drinks. Adult flies are capable of flying long distances. They move indiscriminately between decaying substrates (where they feed and oviposit) and places where human food and animal feed are prepared and stored [160]. Thus, houseflies have great potential to transport microbes from reservoirs where they present relatively low risk (animal manure, garbage) to places where they pose a great risk (human food and food-contact surfaces) [171].

Another feature that significantly increases the potential of houseflies to disseminate foodborne disease and other human pathogens is their mode of feeding. Their sponge-like mouthparts physically limit ingested foods to only liquids or to finely comminuted solids dispersed in a liquid medium. Houseflies habitually regurgitate a portion of the contents of the foregut onto food substrates that are too large to pass through the interstices of the mouthparts [172]. This results in the deposition of digestive enzymes and bacterial decomposers (both immediately begin to liquefy the substrate or break it up into minute particles), as well as any pathogens that may have been ingested earlier [173]; thus, adult houseflies contaminate the surfaces upon which they feed or otherwise touch with their mouthparts.

4. Other Filth Flies

Other filth flies, including flesh flies (Sarcophagidae) and blowflies (Calliphoridae), also to some small extent synanthropic in behavior, develop in decaying organic matter. However, they usually do not build up to such large populations, as do houseflies. Nevertheless, all filth flies have to be considered as potential carriers of foodborne disease pathogens. For example, the Asian blowfly (*Chrysomya megacephala*) oviposits on human feces (commonly exposed in the Orient) and feeds on confections and fruits in the markets, greatly increasing the risk of contamination with fecal bacteria [174].

J. Correlation of Pest Populations and Disease Incidence

Another line of investigation is largely statistical but still requires intensive field and laboratory work. This involves correlation of pathogen occurrence in a human population (applies also to populations of domestic animals and wildlife) with that same pathogen in the population of a suspected disease vector. Sometimes a marked increase in a probable vector population is followed by an epidemic in the human population [175]. Samples for microbiological analysis must be collected from both people and suspected vectors.

A corollary approach, after an epidemic has been in progress for some time, is to dramatically reduce the suspected vector population by some control measure. If, after allowing enough time for the required incubation periods to play out, the epidemic curve subsequently slopes downward, then the implication follows that there may have been a cause-and-effect relationship between the drop in human cases and the corresponding drop in the vector population.

1. Flies and Outbreaks

In some cases, outbreaks of diarrheal diseases were observed to be closely related to seasonal peaks in filth fly numbers or dense populations of cockroaches. Outbreaks and cases of *Vibrio cholerae*, *V. fluvialis* and *Shigella* spp. in Thailand were associated with marked increases in filth fly populations [176]. Levine and Levine [109], in a review of the literature on the epidemiology of shigellosis, concluded that fly control measures tend to reduce the incidence of shigellosis in human populations. Rates of shigellosis were lower in communities where fly suppression measures were implemented than in communities without fly control programs [177].

Esrey [178] reported that the suppression of the housefly population led to a 40% reduction in frequency of diarrheal diseases among children in certain communities of Africa. West et al. [179] reported that the chance of contracting trachoma (caused by *Chlamydia trachomatis*) was 70% higher in Tanzanian preschool children in areas with high fly populations than in areas with few flies. Hyperendemicity of salmonellosis and shigellosis among infants and young children in Beirut, Lebanon, was linked to high populations of filth flies [180]. Khalil et al. [181] demonstrated that there was a correlation between the reduction of filth fly numbers and a decrease of gastroenteritis cases among infants and young children in Lahore, Pakistan.

Houseflies were postulated to be mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7 after the pathogen was collected from the flies and from patients during an outbreak of colitis in a Japanese nursery school [134]. Quantitative feeding experiments showed that the bacteria, after having apparently proliferated in the mouthparts (especially the labellum) of the fly, passed through the gut of the flies over a period of three days after the initial feeding.

2. Cockroaches and Outbreaks

Cockroaches have been implicated as probable mechanical vectors in numerous reports. The case rate during an

outbreak of infant diarrhea in a Belgian hospital dropped off shortly after the cockroaches (*Blattella germanica*) infesting the ward were exterminated. *Salmonella typhimurium* was isolated from the patients and the cockroaches [182]. An outbreak of infant diarrhea, attributed to *S. bovismorbificans*, in an Australian hospital was traced to defective sanitary practices. The pathogen was isolated from patients and from cockroaches and mice infesting the hospital [183]. *S. typhimurium* was isolated from sparrows infesting the kitchen of a mental hospital where many patients were suffering from gastroenteritis caused by the same strain of *Salmonella* [184]. *Shigella dysenteriae* was isolated from German cockroaches associated with an outbreak of shigellosis in Northern Ireland [185].

Investigators in Poland found that certain species of pathogenic bacteria collected from patients in a provincial hospital were also carried by cockroaches (*Blattella germanica*, *Blatta orientalis*) infesting the hospital [186]. In a similar situation, Indian scientists found an antibiotic-resistant strain of *Klebsiella pneumoniae* in patients and in German roaches in a New Delhi hospital [187].

K. Sampling Pathogens from Foods and Pests

Another technique in the investigation of foodborne illness is to sample suspect foods to see if there is a match between the microorganisms found in the food and those taken from suspected vectors and from people who are ill. In 1935, Staff and Grover [188] did a classic investigation of a foodborne disease outbreak, linking the patients (208, with three fatalities), the food product (a filled pastry), and the vector (*Rattus norvegicus*) to the etiologic agent (*Salmonella enteritidis*).

L. Role of Vectors in Mechanical Transmission

The last and, so far, least productive effort is to try to establish a cause-and-effect relationship between human foodborne disease and the activity of a probable vector [159, 189]. The Staff and Grover [188] investigation was exceptional; too often, the evidence is largely circumstantial. But there is at least one disease in which the route of transmission clearly involves vector contamination (via infective viruses in the urine) of food and drink: Bolivian hemorrhagic fever and the rodent transport host, *Calomys callosus* [190].

Experimental work by Klowden and Greenberg [191] unequivocally demonstrated the importance of mechanical transmission. When the mechanical transmission occurs after the final biocidal step in food processing, consumers may be at risk of ingesting viable organisms, and this may lead to foodborne illness. When bacteria (*Salmonella* sp.) were placed on the bodies of cockroaches that were subsequently killed, the bacteria survived on the dead bodies for as long as 60 days. If the cockroaches had died in a food material that was destined to be eaten without further biocidal treatment, then the consumer would have been at risk of foodborne illness.

M. Role of Pantry Pests in Mechanical Transmission

Left to themselves, the pantry pests — insects and mites that make their home in raw or processed foods and are found most often in homes, granaries, grocery stores and food warehouses — have little opportunity to become contaminated with pathogens. However, the foods in which pantry pests live are also visited by ants, flies, cockroaches, mice, rats, birds and other mobile pests, all well known for their habit of indiscriminately foraging on filthy substrates. Thus the more mobile pests bring pathogens to the home territory of the pantry pests, and they, in turn, inadvertently pick up the pathogens and disseminate them wherever they chance to travel within the food material.

1. Indianmeal Moth Larvae

Larvae of the Indianmeal moth (*Plodia interpunctella*), one of the more common insects found in the stored foods, were collected from various food sources and screened for their extracellular symbionts. Surprisingly, more than 80% of the specimens did not harbor any bacterial cells in the digestive tract that would grow on various artificial culture media [168].

2. Beetles

On the other hand, many other insect pests of stored food, including red flour beetles (*Tribolium castaneum*), lesser mealworms (*Alphitobius diaperinus*) and confused flour beetles (*T. confusum*), collected from various feed mills in the Midwestern United States, carried potentially pathogenic enterococci and aerococci [168]. Immature and adult confused flour beetles and lesser mealworms carried a fungus, *Fusarium roseum* var. *graminearum* (=*Gibberella zeae*), that produces mycotoxin F-2 (zearalenone); this fungus was also recovered from dead beetles [192].

De Las Casas et al. [193] exposed the lesser mealworm to fowl pox virus and Newcastle disease virus. Mealworms carried fowl pox virus for six hours; Newcastle disease virus was not recovered from these insects. Another avian pathogen, infectious bursal disease virus, was carried by lesser mealworms for up to 14 days after the initial exposure [194]. Harein et al. [195] isolated five different species of *Salmonella* and 48 different serotypes of *E. coli* from lesser mealworm beetles collected from poultry farms.

3. Weevils

Artificially infected rice weevils (*Sitophilus oryzae*) retained *Salmonella montevideo* for at least one week [196]. Harein and De Las Casas [197] postulated a potential role for granary weevils (*S. granarius*), collected from stored wheat, in the dissemination of several species of opportunistic pathogens including *Serratia marcescens, Streptococcus* spp., *Micrococcus* spp., and members of the *Klebsiella-Aerobacter* group.

Clearly, many species of common stored-product insect pests have significant potential as transport hosts for various pathogenic microbes; therefore, they should not be considered as merely nuisance or economic pests.

N. New Policies to Meet New Risks

1. Risks: Old and New

Disease-causing foodborne pathogens have been present throughout human history. Over past ages and right up to the present day, transmission from person to person has been effected via the fecal-oral route. The incidence of such infections has always been greater where insanitary conditions — polluted water, contaminated food, and lack of personal hygiene — prevail due to poverty or lack of education. Foodborne diseases, as most of us know from personal experience, are not limited to economically challenged populations. Affluent and well-educated people on occasion acquire foodborne infections in their own homes and in the best of restaurants.

In spite of the denial by many in the medical community, we strongly believe that a small but significant number of transmission incidents are effected through the agency of mechanical transport of pathogens by food-associated, synanthropic pests, most especially filth flies, cockroaches, ants, and rodents. We have supplied here abundant evidence to support our claim. Most people in the health-care and epidemiologic professions seem quite content to ignore the evidence. Policy makers in the food regulatory agencies of several nations have de-emphasized filth testing and terminated filth analyst and entomologist positions in government laboratories. We submit that, based on the following facts, all of which are well accepted and well known, the health of the public is not well served by these just-mentioned presuppositions and trends. There are also applications here to domestic animal and wildlife populations, but we will emphasize human populations.

a. Rapid transportation

Movement of pathogens, including food-associated pathogens, from one geographic area to another is greatly facilitated by rapid modes of transportation, as has been recently evidenced by the episodes of SARS (severe acute respiratory syndrome) in Asia and North America. Mass movements of populations between adjacent geographic regions, due to famine, civil disorders or war, may also facilitate translocation of foodborne disease pathogens, and the insanitary conditions, signaled by large populations of filth flies that often accompany such chaotic population shifts, only serve to facilitate fecal-oral transmission.

The rapid transport of food from one geographic region to another is also a factor in the epidemiology of foodborne diseases. The sanitary standards in the country or region of origin may be set at a far lower level than what would be acceptable in most of the western world. Difficult-to-clean fruits and vegetables that are usually eaten raw (e.g., raspberries, strawberries, cantaloupes) represent the greatest risks. These same foods are also often exposed to contamination by filth flies and other food pests at various stages on the journey from farm to fork: in the production fields, in transport vehicles, in distribution points for the wholesale market, at retail outlets, and in the home environment. Certain specialty food items such as smoked fish and jerky (*carne seca*) are sometimes exposed to filth flies in the early phases of production and thus to potential contamination by foodborne disease pathogens.

b. Immunocompromised people

The efficiency of the immunologic response can be underdeveloped or compromised for many reasons. The very young (with still developing immunologic systems) and the very old (with varying degrees of deterioration of the immunologic response) first come to mind. Then there are the ill for any reason, some of whom may exhibit a diminution of immunologic response in varying degrees, and the traumatized by injury or surgery (especially splenectomy and organ transplants, both involving an immediate and prolonged suppression of the immune response). Immunosuppression is the constant accessory to the radiation treatment and chemotherapy administered to cancer patients. But by far the most common sort of immunosuppression is that occasioned by the effects of the human immunodeficiency virus (HIV), the precursor to AIDS (acquired immune deficiency syndrome).

c. "Super" pathogens

Pathogens may qualify for this hyperbolic descriptor for any of several reasons. Many pathogens have acquired varying levels of resistance to the effects of certain antibiotics. This is important for anyone who contracts an infectious disease, but it is especially important for the immunocompromised. As a general rule, when a therapeutic agent is administered, cure is accomplished by the combined effects of the drug and the patient's immune system working against the pathogen population. The effectiveness of the drug becomes more crucial when the immune system is (for whatever reason) suppressed. Recovery is delayed or sometimes prevented when the pathogen has developed resistance to the antibiotic agent. Certain strains of some very common pathogens, among them some potential

foodborne pathogens, have become resistant, in varying degrees, to many of the antibiotics currently in use. A few examples are VRE (vancomycin-resistant enterococci), MRSA (methicillin-resistant *Staphylococcus aureus*), and strains of *E. coli* resistant to cephalosporin or cephamycin.

The medical journal *Emerging Infectious Diseases* is largely devoted to "new" pathogens or to "new" strains of previously recognized pathogens that often produce unexpected symptoms or exhibit unexpected virulence. Strains of the intestinal commensal, *Escherichia coli*, such as *E. coli* O157:H7, have acquired exceptional virulence, dangerous enough for healthy individuals but especially dangerous for the immunocompromised. This latter population has become especially vulnerable to opportunistic pathogens such as the non-food-associated *Babesia* recently discovered in Europe [198]. Both immunocompromised and immunocompetent populations may be susceptible to rare strains of foodborne disease pathogens, such as *Salmonella kiambu*, that "jump" unpredictably from animal to human populations.

d. Uncommon virulence

We have already stipulated that food pests play no more than an incidental role, as mechanical transport hosts, in the transmission of foodborne disease pathogens. But we submit that this "small role" is taking on greater significance because of the increasing numbers of immunocompromised people, because some of the pathogens are acquiring resistance to antibiotic drugs, and because some foodborne disease pathogens have taken on enhanced virulence, involving smaller and smaller infective doses (in amounts easily within the capabilities of mechanical vectors to deliver) to such a degree that otherwise healthy individuals, as well as the immunocompromised, may become ill.

e. Summary: why food pests are important

There may have been a time when the medical community (perhaps with some excuse) could have been nonchalant about food pests in or around food and the associated risks of mechanical transmission of foodborne disease pathogens, but now we live in a much different world. We have witnessed the emergence of foodborne pathogens possessing new virulence factors dangerous to healthy people and even more so to the immunocompromised.

A recent report, while not bearing directly on the subject matter of this chapter, does serve to underscore several points made here [198]. Careful investigation may reveal previously undiscovered pathogens or may identify microbes that have been known previously to cause only inapparent or self-limiting infections. These newly discovered pathogens can be characterized and differentiated from pathogens previously described by the application of the molecular and genetic typing techniques briefly noted below (Section VII D). These same techniques may be applied to identify and characterize microbes obtained from potential vehicles and vectors of infection, including human and vertebrate carriers, thus making it possible to establish probable chains of infection. This paper [198] also demonstrates that immunocompromised people are prime targets for opportunistic pathogens, microbes that would stand no chance of establishing infections in immunocompetent hosts.

2. Mechanical Transmission (Again)

As has been demonstrated earlier in this chapter, pathogens may be carried about by insect, rodent and bird vectors. The pathogen load borne by such vectors has traditionally (but not necessarily accurately) been thought of as very small in relation to the infective dose, that is, the minimum number of pathogenic units required to generate symptoms in a normal human host. Even if the pathogen load carried by a mechanical vector is quite small, it may be enough to produce illness in an immunocompromised host.

Some of the opportunistic pathogens, such as *Serratia marcescens* or even our ubiquitous commensal, *Escherichia coli*, isolated from various potential mechanical vectors, especially flies, cockroaches and ants, are thought to be innocuous to healthy human beings. However, for the immunocompromised, these "innocuous" bacteria may become serious, even life-threatening, opportunistic pathogens. Further, the pathogen may be one of the newly emergent "super" microbes; in that case, the "small" pathogen load, deposited by a vector, may be sufficient to make even a healthy person ill.

3. Insects and Mites in Food

The idea that eating insects and mites in our food or eating food that has been contaminated by insects and mites is harmless is becoming so pervasive that some food regulatory agencies have simply stopped doing filth inspections and filth analyses. This trend seems inconsistent with the fact of a long and continuing history of numerous successful prosecutions of filth violations in the food industry. However, consumers may still draw some assurance of safety in their food because of the fact that the policy makers still attach great importance to food pests as "indicators of insanitation."

Of course, it is true: Most of us will not suffer any ill effects from ingesting a few dead insects and mites with our food. But the occasional serious illness, such as anaphylaxis associated with ingesting mites [199–202], jars the complacency usually associated with such a benign phenomenon and justifies the establishment of regulatory action criteria [203].

4. FDA Policy on Filth in Food

Scientists and policy makers at FDA have established and defined categories of filth and extraneous matter and have

developed a flow diagram for use in evaluating the significance of these several categories within the context of a food-manufacturing operation [69].

a. Potentially hazardous

There are three categories of contaminants in the flow diagram: potentially hazardous extraneous materials, indicators of insanitation, and aesthetic defects.

A contaminant is designated as *potentially hazardous* if scientific and medical authorities recognize it as such, or if the contaminant is known to have caused injury, or if the contaminant remains in the food even though the usual processing procedures could be expected to remove it.

Hard or sharp foreign objects immediately come to mind here. When a consumer buys unpitted fruit such as cherries or prunes, the consumer is forewarned that pits are present and it is the consumer's responsibility to remove the pits before chewing the fruit. In this case, the pits are normal constituents of the food product and would not be categorized as potentially hazardous. If, however, a consumer buys pitted fruit, and it is labeled as such, any pits or fragments thereof remaining in the product could be considered as potentially hazardous because of the potential for injury or choking. Even in this instance, the consumer must be wary because FDA, in its DALs, may permit very small amounts of pit fragments in specific retail products.

Already noted above is a different, rather uncommon (and very minor, according to some [204]) sort of potentially hazardous filth: allergic reactions to certain mite-infested foods [199–202].

b. Indicators of insanitation

Another category of contaminants is represented by insects, mites, rodents and birds that are themselves not hazardous but are placed in this category because of their potential for serving as mechanical vectors of food-associated pathogens. Quite apart from any proved or suspected demonstration of pathogen carriage by these pests, their mere presence in a food-processing facility or retail food establishment causes them to be categorized as "indicators of insanitation." The presence of these pests in or around food suggests that one or more basic sanitation safeguards in the food facility have failed and that the defect or defects must be corrected.

c. Aesthetic defects

Aesthetic defects, even though generally harmless, are subject to regulatory action if they exceed the DALs. Some examples are field insects or fragments thereof accidentally carried into the food processing operation, bits of soil picked up during harvesting and not adequately removed before processing, and pieces of stems or pods that remain in the food product.

5. A New Enforcement Strategy

In any evaluation of the significance of filth and extraneous materials in food, including hard or sharp foreign objects, FDA has placed the first priority on the question, *is it a health hazard?* If the answer is yes, then HACCP immediately comes into play and corrects the problem. If the answer is no, the next question is, *does the contaminant represent a violation of good manufacturing practice?* If the answer is yes, then adherence to SSOPs [205] is reviewed for adequacy of compliance. If the answer is no, the next question to ask is, *is it an aesthetic defect?* If the answer is yes, then it must be determined whether the applicable DAL has been exceeded. Appropriate regulatory action may be taken if there is a DAL violation. However, even if there has been no technical violation of a DAL (or if, as often happens, no applicable DAL exists), regulatory action may still ensue if other factors point to deficiencies in sanitary operations.

6. End of an Era

The food defect action levels have had a long history of development in the federal food regulatory agencies of the United States [53]. At least since 1973, the DALs have been set on the basis of a large, statistically valid sample of a particular food product. The filth load in each subsample of that product was determined by standardized micro-analytical techniques. All of these individual results were then considered, along with seasonal and geographic variations, before setting a DAL.

During the last quarter of the twentieth century, entomologists, chemists and analytical technicians at the FDA Center for Food Safety and Applied Nutrition in Washington, D.C. (now located in Beltsville, Maryland), and at the various regional laboratories of the FDA, actively engaged in the development of filth detection procedures for a large variety of food products, a variety far greater than the list represented in the DAL handbook [206, 207]. Shifting priorities on the part of the decision-makers within the FDA and the USDA have resulted in the near elimination of research and development on filth detection methods. A very few modest filth methods projects are underway or planned [208, 209], but neither the expertise nor the funds remain within FDA to support the setting of any new DALs.

VI. HARD AND SHARP FOREIGN OBJECTS IN FOOD

A. Consumer Experience

Most consumers are aware of the fact that hard objects that do not belong in food are, nevertheless, sometimes present. This is because most consumers have personally found such objects. Furthermore, most such encounters are

innocuous in nature. Certain foods are so frequently endowed with physical objects (e.g., grit in spinach; bits of shell in clam chowder) that we are surprised if such objects are not found. In other instances, the encounter between the consumer and a physical object in food or drink may result in an injury, the severity of which may vary widely.

B. Literature on Hard Objects in Food

In view of the rather high frequency with which consumers encounter hard and sharp objects in food, it is surprising that the scientific literature on this subject remained remarkably scarce for so many years, the publications by Hyman et al. [210] and Gorham [211] being the first two of several to follow. Since the implementation of mandatory seafood HACCP on 18 December 1997 [212] and the installation of many voluntary HACCP programs in recent years, three general reviews [1, 213, 214] and one practical paper [215] on the subject have been published, and other numerous publications have appeared, especially regulations, guidelines, methods and didactic materials, the latter often quite redundant but useful nevertheless because they are usually tailored to meet the needs of specific audiences in the food industry [14, 16, 213, 216–220]. The USDA Food Safety and Inspection Service (FSIS) has produced a report on a technical conference on foreign material contaminants [221].

Concern for the possible adverse health effects of hard foreign objects in food and drink was evident early in the twentieth century. One might have guessed that Harvey W. Wiley, principal protagonist for the first federal pure food law [30], would have been interested in this subject, and perhaps he was. But in the record of that era, he makes only passing reference to a study of "cocoa shells in cocoa products" in his 1911 report [76]. His concern then was probably with the shells as an "economic adulterant" (in the same sense that added water was an economic adulterant of milk) rather than with the shell fragments as hard foreign objects in cocoa powder.

C. Product Tampering

Excluded from detailed discussion in this review are acts of fraud, sabotage, and product tampering. Hiding a big rock inside a bale of bay leaves to boost the weight would be considered fraud. The deliberate addition of a foreign object to a food or beverage product by an employee of the product's manufacturing company would be considered an act of sabotage. When, in an effort to cause harm to some other person or to extort money from a manufacturer, someone deliberately adds a foreign substance or a physical object such as a needle, a straight pin or bits of glass to a product offered for sale, the act would be considered product tampering [222–224]. The added object does not have to be hard or sharp to constitute product tampering: Spitting a glob of phlegm onto a fast-food hamburger before serving it to a customer would clearly be actionable.

A common type of product tampering, rarely prosecuted under the Federal Anti-Tampering Act [225] or any other statute, occurs when a consumer deliberately puts a piece of glass, a mouse, or a cockroach — to mention just a few of the possibilities — into a food or beverage, and then reports the "find" to the manufacturer, the motive being to get a free quantity of the product or money from the manufacturer.

D. Filth

Also excluded from discussion in this section on hard foreign objects are those physical objects, already noted above, usually categorized as filth [2, 69]: rodent hairs and feces; bird feathers and feces; insects and mites and their fragments; and molds and rots associated with fruits, seeds, nuts, and many other foods. The tiny metal fragments sometimes generated by opening cans with a can opener in the home or restaurant kitchen are also excluded from this present topic.

E. Hard Objects Absent from the Food, Drug and Cosmetic Act

The FD&C Act says nothing about such things as paint chips, ball bearings, or glass shards in food. When such items happen to be found in food or drink by regulatory officials, each incident is evaluated on its own merits and, if deemed actionable under the FD&C Act, it is because the foreign object has rendered the food "unfit" (402[a][3]) for human consumption due to an offensive "mouth-feel" of the adulterated food, or has rendered it "injurious to health" (402[a][4]).

An FDA Compliance Policy Guide [16] provides further regulatory details based on reviews by the FDA Health Hazard Evaluation Board of nearly 200 cases of hard or sharp foreign objects in food. The Board concluded that objects measuring less than 7 mm in length rarely cause physical injury. However, special risk consumers such as infants, the elderly, and the physically or mentally challenged may be unable to cope with objects 2 mm or less in greatest dimension.

F. Defect Action Levels

The DALs [206, 207] give no ground to physical objects that find their way into foods after the agricultural phase of production [53]. Some modest forbearance is shown in the DALs toward some of the fruits with pits (e.g. cherries, dates, olives, prunes), apparently because even the best pitting technology falls short of 100% efficacy. The DALs address these and many other unavoidable defects That the USDA permits small numbers of bone fragments in certain meats [226] is a fact many of us have discovered

to be true as we seek out a dentist to repair the damage. The DALs do not exempt from regulatory action any hard or sharp foreign object if that object has been shown to constitute a hazard for physical injury or choking or if it has been shown to cause the product to be made unfit for food.

G. The Processor's Goal

Reputable food and beverage processors prefer to produce products that are free of foreign objects. Their zeal is driven perhaps in part by the fact that there is little defense against an obvious hard foreign object, especially one that has caused injury to a consumer. No doubt, however, the overriding motive for keeping objects out of foods and beverages is to protect and preserve the company's reputation. Of course, the economic consequences of downtime on the production line and recalls of shipped products probably also play a role in a company's pursuit of zero defects in the product line, whatever that might be. Many food manufacturers use vendor certification programs to ensure that only high quality ingredients enter the food processing plant [227].

H. Quality Assurance Procedures and Devices

Many companies have invested heavily in quality assurance devices and procedures to prevent the occurrence of foreign objects in food or to discover them before the foods leave the factory [228, 229]. Glass, one of the early mass-produced commercial packaging materials, continues to be widely used. Unfortunately, glass packaging readily breaks during the manufacturing of the container and during the washing, filling, capping, labeling, and boxing of it on the production line. The problem of glass shards in foods and beverages has been reviewed in the technical literature over a span of many years beginning early in the twentieth century [1]. Reputable companies that use glass packaging strictly follow the applicable good manufacturing practice (GMP) statements [37] and carefully keep records of problems so that container-manufacturing and food-processing equipment can be fine-tuned to reduce breakage to a minimum.

Virtually all detection systems for foreign objects involve a human observer to some degree, but most production line detection systems also involve specialized machines to supplement visual observations. There are two different lines of approach to the goal of preventing hard foreign objects from getting into food and drink. One involves various screens, shakers, air blasts, filters, magnets, or other devices to remove foreign objects present in the production stream.

The second, which may sometimes be used in concert with the first, involves machines that "look" for foreign objects in product ingredients or in the finished product before or after packaging [230–236]. These machines may be very sophisticated technically and capable of detecting many kinds of foreign objects — glass, metal, bone, rocks, shell fragments (from nuts, mollusks or crustaceans) and other items.

Rigid observance of well-planned protocols for housekeeping and plant maintenance operations, with strict accountability of supplies, tools, and spare parts, serves well as a further safeguard against adulteration of food products with foreign objects [237, 238]. These protocols should include inspections of raw ingredients for hard objects and inspections of processing equipment and food packaging to detect missing parts or container fragments (Figure 74.2). The shielding of all light fixtures (to prevent broken glass lamps from falling onto the food line) is another essential preventive measure.

I. Ingestion and Inhalation of Foreign Objects

Human injury or death associated with the ingestion or inhalation of foreign objects has been reported widely in the medical literature. Many such cases have nothing to do with food. The offending object could be a coin, thumbtack, earring, marble, balloon — in other words, a vast array of small objects [239].

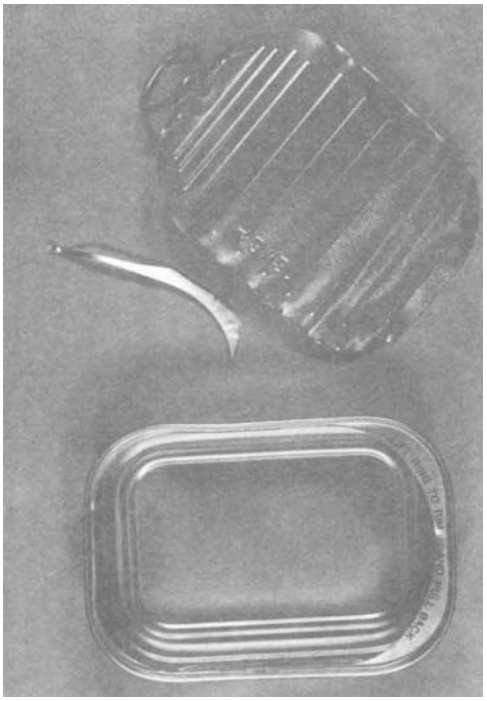

FIGURE 74.2 Metal fragment from a retail can of sardines.

1. Food-Related Items

Many other cases of injury and death have been attributed to various items of food. Hot dogs are notorious offenders, as are grapes, hard candy, nuts, chunks of meat, popcorn, beans, and many other such objects. Liquid foods may cause problems also, especially for infants.

Food regulatory agencies in England, Australia and the United States [240–242] have in recent years issued warnings about a choking hazard from a small, jelly-filled candy (Figure 74.3) made in part of konjac, a flour derived from the tuber of *Amorphophallus konjac* (Araceae) and widely used as a food ingredient in the Orient. The slippery nature of the candy, lubricated by saliva, causes it to be readily shifted, without chewing, into the back of the throat where it may block the airway, sometimes with fatal results. In the United States, a hazardous food product such as konjac candy is subject to regulatory action by both the FDA and the Consumer Product Safety Commission (CPSC) [243].

Another category of objects reported to cause injury or death consists of things that are associated intrinsically with foods: fruit pits and seeds (as in dates, grapes, apples, cherries, limes and other citrus fruits), bones (beef, pork, chicken, fish), shells (nuts, shrimp, clams, oysters), stems (raisins, string beans), pig teeth in sausage, and fish scales in canned tuna. Objects in this category sometimes are considered to be less objectionable than those in the foreign object category because it is predictable that they may occasionally filter through the normal safeguards of good manufacturing practice.

2. Hard and Sharp Foreign Objects in Food

This category consists of foreign objects in foods and beverages. Such objects are described as foreign because they are not intrinsically associated with an edible portion of a food product. Some are obviously associated with harvesting. The small stones or clumps of soil found in dried beans and peas are typical, as are the sand and grit in peanut butter and spinach (Figure 74.4).

Other objects, clearly associated with manufacturing, occur in two cases. The first is associated with the manufacturing of the food container *per se*. Glass, plastic, and metal fragments predominate here. The second occurs during food processing. Glass, plastic, and metal fragments also predominate in this phase. In earlier days, wood splinters were more common in manufactured foods because wood was then more often used in processing equipment.

FIGURE 74.3 Konjac candy (choking hazard). Courtesy of Food Standards Agency, United Kingdom. Used by permission.

FIGURE 74.4 Beans and pebbles from a retail bag of pinto beans.

Today, plastic is frequently seen on the processing lines. Due to normal wear and tear on the equipment, bits of plastic may find their way into foods and beverages.

Inadvertent contamination by foreign objects, such as wood, paper, plastic, etc., may occur during food processing. But most of these unwanted objects are already in the cans and bottles before they leave the container manufacturer. All food processors should have systems for cleaning all containers before the point at which they are filled with the food product.

a. Foreign objects associated with can manufacturing

At can manufacturing plants, small curls of plastic sometimes form on the "fingers" used to move the new cans onto pallets in preparation for shipment. On occasion, the "fingers" rub against the sharp can lip, peeling off thin curls of plastic. Quality-assurance protocols require the palletizer operators to periodically inspect the "fingers" and remove any curls; otherwise, the curls will eventually break off and drop into cans. When it is understood that on any given day, many thousands of cans are fabricated, palletized and readied for shipment, it should not be surprising that a few bits of plastic may escape notice and drop into cans. This is one of the reasons why seemingly clean cans from the can factory must be cleaned and inspected before they are filled by the processor. Glass containers must be similarly cleaned and inspected.

b. Insects associated with can manufacturing

While not bearing directly on the subject of hard foreign objects, it should be noted here that insect contamination may occur during the manufacture of cans. A relatively minor sort of "adulteration" occurs when flying insects, attracted to the lights over the finished cans on the conveyors, accumulator tables and palletizers, fall into the cans.

A far more serious kind of insect "adulteration" problem occurs during warm, humid months when conditions are favorable for the proliferation of minute mycophagous insects called psocids or booklice (Order Psocoptera). The same weather conditions that favor the production of large populations of psocids also favor the growth of fungi on the cellulose fiber (composite) slipsheets or divider boards used to separate tiers of cans in a palletized stack. This abundance of food naturally leads to large numbers of psocids. When the slipsheets are placed over the open cans on the pallet, some of the psocids fall into the cans, creating a potential violation of the FD&C Act, even though these insects have no importance as mechanical vectors.

c. Control of booklice

Two commonly used "control" measures are both uncommonly ineffective. Applying insecticidal aerosols or mists gives only minimal temporary control. The same applies to fumigation of the stacked cans or the slipsheets. In both instances, many of the dead insects remain on the slipsheets and in the cans, rendering them adulterated. Therefore, the cans must be cleaned in order to bring them back into a marketable state. In other words, the end result is the same, so it would seem to be economically prudent to avoid the purchase of ineffective "control" measures.

There is no easy or totally effective way to deal with psocids in this situation. However, the use of plastic slipsheets, instead of the traditional fiberboard slipsheets, goes a long way toward solving the psocid problem. Apparently, plastic slipsheets do not support the fungal growth required for proliferation of the booklice. Although the plastic slipsheets are more expensive than the composite board slipsheets, economy is derived from avoiding expenditures for the virtually useless measures of fumigation and spraying insecticidal mists. The use of plastic slipsheets and the practice of keeping the palletizing area scrupulously clean will minimize both the psocid problem and the incidence of unwanted foreign objects in the cans [244].

d. "Chicken a la plastique"

Plastic shards in a four-ton batch of chicken nuggets prompted a major chicken processor to initiate a recall effort. All but about 2500 pounds of the adulterated chicken were recovered. Children discovered the plastic pieces while eating lunch served at a school. This kind of hard foreign object may cause physical injury or choking; fortunately, no injuries were associated with this incident [245].

The continuing recurrence of recalls due to glass, plastic, metal and other foreign objects in food and drink indicates that, while much progress has been made, much remains to be done toward the end of preventing these unwanted objects from entering the product stream.

3. Review of Case Reports

Case reports or reviews of collections of cases involving foreign objects in the digestive or respiratory tracts typically refer to the more severe, the more unusual, or the fatal cases. The degree of severity ranges from the relative "minor" esophageal tears caused by sharp-edged, solid objects [239, 246], through partial blockages of the esophagus [247] or trachea [248], to complete and fatal obstruction of the airway [249].

A review of the medical literature readily available in the FDA foods library, covering the span of the years 1961–1990, yielded 679 cases of injury or death associated the ingestion of inhalation of foreign objects. Many of these cases (221) had nothing to do with food, but in a relatively large number (437), some kind of food material was involved. In a relatively small number of cases (28), some food-associated, hard foreign object — pit, shell, bone, stem, seed — was the culprit.

In this admittedly incomplete collection of cases from the medical literature, there was no unequivocal report of

injury, illness or obstruction caused by a hard foreign object ingested with food or drink that was not intrinsically associated the food or drink. In other words, ball bearings, wood splinters, conveyor-belt nuts and cleats (staple-like wires used to hold together sections of conveyor belt), glass shards, bits of metal or plastic, and similar extraneous hard foreign objects ingested with food or drink were in no case unequivocally implicated in obstruction of or injury to the digestive or respiratory tracts. There was one report (not included in the above informal survey) of intestinal injury caused by a wood splinter that apparently had been swallowed with food, but the patient could not recall the event [250].

4. The Food and Drug Administration Complaint Reporting System

The situation in the preceding section stands in marked contrast to that reported by Hyman et al. [210]. In a careful analysis of just one 12-month period (October 1988–September 1989), they compiled data on 10,923 complaints about food registered with FDA. Of these complaints, 25% (2726 cases) involved foreign objects in food or drink, and 14% (387 cases) of these involved illness or injury associated with foreign objects ingested in beverages or food. Most of the injuries/illnesses, as might be expected, involved cuts or abrasions in the mouth and throat, damage to teeth or dental prostheses, or gastrointestinal distress. The foreign objects were rank ordered from most to least common: glass, slime or scum, metal, plastic, stones/rocks, crystals/capsules, shell/pits, wood, and paper. Foreign object complaints involving injury or illness were associated most often with soft drinks, followed in descending order by baby foods, bakery products, cocoa/chocolate products, fruits, cereals, vegetables, and seafoods.

J. HACCP, DALs and Hard Foreign Objects

The worldwide food industry is gradually coming under the food safety program now well known as HACCP (Hazard Analysis and Critical Control Points). In the United States, HACCP programs are mandatory for the seafood [212, 251] and juice [252] industries, but many other food processing systems in the United States [253], Canada, and in numerous other countries [254] are protected by voluntary programs. The Canadian Food Inspection Agency plans to implement mandatory HACCP in all federally registered meat and poultry processing plants by mid-2005 [255].

Since HACCP focuses on microbial hazards and their prevention, reduction or elimination, the overall safety of food should be enhanced. In spite of the fact that hard foreign objects have little to do with microbial pathogens, hard foreign objects are nevertheless considered a Critical Control Point. This is because hard foreign objects may be a choking hazard or may cause injury if the consumer attempts to chew or swallow them [1, 213, 214].

The DALs [53, 206, 207] allow very small quantities of fruit pits to be present in food; at these low levels, they are considered to be natural and unavoidable constituents of food. Other hard foreign objects are considered adulterants under the provisions of the FD&C Act if they may cause injury to the mouth and digestive organs [1, 210, 214], if they simply make the food to feel gritty in the mouth, or if they may be a choking hazard for special consumers such as infants and physically challenged individuals of any age who have difficulty chewing and swallowing.

VII. CONCLUSION: HACCP, SSOPs, RESEARCH

A. HACCP

The HACCP program for food safety, when properly implemented and maintained, has the ability to protect everyone from both the traditional foodborne pathogens and parasites and from the newly-emerging ones [253, 256]. Although hard foreign objects are treated as a Critical Control Point, the other adulterants generally referred to as "filth in food" receive very little attention in the HACCP protocols.

B. SSOPs

However, this is not to say that filth is considered to be unimportant. HACCP does not deal specifically with the sources of filth in food because the whole edifice of HACCP is built (and must be built) on a foundation of thorough, persistent and dependable sanitary practices in the food industry, usually referred to as SSOPs (sanitation standard operating procedures) [229]. A substantial part of *preventing pathogens* from getting into our food is *preventing pests* from getting into our food — and this at all stages of production, harvest, storage, manufacture, distribution, retailing, and even to the home kitchen, this latter being the theater where the majority of foodborne infections are staged.

C. Foodborne Diseases

Estimates by the Centers for Disease Control and Prevention of the annual incidence of foodborne diseases in the United States range from 6.5 million to 76 million cases. Annual mortality has been estimated between 5,000 and 9,000 [257, 258]. The rates of foodborne diseases in developing countries are much higher due to poor sanitation, lack of clean water, and inadequate hygiene. With increased international travel, the risk of introduction and spread of pathogens around the world is very high.

After many years of therapeutic and preventive uses of antibiotics, many kinds of bacteria have adapted and become resistant to antibiotics. Multiple uses of antimicrobial agents in medicine, production of food animals, and crop protection have caused an increasing resistance to those agents. In addition, widespread use disinfectants in household products may also contribute to the development of resistance. As effectiveness of existing antimicrobial agents declines, it will be more difficult and expensive to treat infectious diseases and to control epidemics.

D. Research

Within the past few decades, great advances in biology (primarily biochemistry and molecular biology) have offered new and more sensitive approaches for the fields of microbial ecology and epidemiology, as exemplified in the paper by Herwaldt et al. [198]. Techniques based on biochemical analysis and amplification of nucleic acids by the polymerase chain reaction (PCR) technique, followed by genotyping by, for example, restriction fragment length polymorphism (RFLP), pulse field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP), allow for tracking specific pathogens (strains/serotypes/genotypes) in various clinical and environmental habitats. This leads to a better understanding of bacterial and vector ecology and consequently to formulation and implementation of more effective food safety programs.

It is clear that the need for risk assessment and surveillance programs for foodborne and other human and animal diseases, including screening for antibiotic resistance genes and their spread in the environment and an improved understanding of microbial ecology in general, is rapidly growing. Thorough assessment of the significance of mechanical and biological transmission of pathogens by arthropod and other animal vectors must be an integral and essential part of new food safety and foodborne disease prevention programs.

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75 Food Pathogens and Consumer Practices

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CONTENTS

I. INTRODUCTION

In the United States, an estimated 76 million persons, about one in every four, contract foodborne illnesses each year (1). Most cases of foodborne illness are relatively mild, with the affected individuals suffering from a few days of gastrointestinal cramping, headache, vomiting and/or diarrhea. However, some foodborne illness is much more serious, with an estimated 325,000 cases requiring hospitalization and 5000 resulting in death in the United States (1). Persons who are very young, elderly, pregnant (because of risk to the fetus) and those with weakened immune systems are especially susceptible to foodborne illness and the serious consequences that may result.

Consumers can reduce the risk of getting a foodborne illness by following safe food handling guidelines when buying, storing, preparing, cooking and serving food at home. In the United States, consumers tend to believe that food prepared at home is an unlikely source of foodborne illness, although studies indicate that many kitchen environments are contaminated with pathogens, and many consumers are unaware of or do not practice safe food handling methods (2). Many consumers clean their bathrooms more thoroughly than their kitchens, as shown in a bacterial survey of these rooms in 15 households. The kitchen was more heavily contaminated with fecal coliforms and other bacteria than was the bathroom (3). It has been shown that foods consumed in household settings are three times more likely to cause foodborne illness than foods consumed in cafeterias (2).

This chapter discusses causes and sources of common foodborne diseases encountered by consumers, and the

measures that consumers can take to prevent and control foodborne illness.

II. CAUSES OF FOODBORNE ILLNESSES

There are over 200 diseases that are transmitted through food (1), although those who work in the public arena offering food safety advice to consumers concentrate their concern on 10 to 30 of the most significant agents of foodborne disease. Most foodborne illnesses are caused by eating food that contains harmful bacteria, viruses or parasites, or a toxin produced by bacteria growing in the food; metals and prions are other sources of foodborne illness/disease, but will not be discussed here.

The U.S. Centers for Disease Control and Prevention (CDC) uses data from a number of sources to estimate the incidence of foodborne disease in the United States, including the frequency of diarrhea in the general population, and active surveillance for nine pathogens in a defined population of 20 million Americans. However, foodborne illness is underreported for a number of reasons: people who become ill may not seek medical care (and frequently do not if the illness is mild), doctors may not order diagnostic tests or sampling of patients with suspected foodborne illnesses; diagnostic tests may not be measuring the causal organism or detect low numbers of organisms; and diagnosed illnesses may not be reported to public health agencies. Sporadic illnesses, those occurring occasionally or singly, are significantly less reported than outbreak-related cases of foodborne illness, when two or more people come down with the same illness from the same contaminated food. About 81% of the 76 million foodborne illnesses projected by CDC are caused by unidentified or as yet unknown pathogens (1).

A. Viruses

Of the 13.8 million U.S. foodborne illnesses caused by identified pathogens, viruses are responsible for 9.2 million cases, or 67% (1). Viruses are extremely small pathogens that are able to reproduce only within a living host cell; they do not reproduce in foods. Some viruses (such as no rovirus and hepatitis A) can be transmitted by food and water. Other viruses (such as Human Immunodeficiency Virus, the cause of AIDS) are transmitted from person to person, but not through food or water (4). Table 75.1 provides information about the incidence, costs to society, disease severity, food sources, and the control of foodborne illness due to fourteen major foodborne pathogens, including norovirus and hepatitis A.

Norovirus is the most common cause of foodborne illness in the United States (1) because of its low infectious dose, relative resistance to sanitizers and heat, many strains, and the fact that individuals do not acquire long term immunity (5). Fortunately, the illness is usually mild. Norovirus is passed from the feces or vomit of infected persons onto the hands of food handlers or into sewage. Any food subject to human fecal contamination may cause a norovirus infection. Outbreaks are usually associated with food or contaminated water in swimming pools, lakes, or municipal supplies. Inadequately cooked shellfish or salads contaminated by food handlers have been vehicles for norovirus-caused illness. Norovirus was recently responsible for a series of troubling outbreaks of illness aboard cruise ships in the Caribbean; transmission was attributed primarily to person-to-person contact rather than through food (6). The low infective dose means that it is relatively easy for a person who has the virus to spread the disease to other people. The U.S. Food and Drug Administration (FDA) recently advised that hand washing is the best prevention against gastroenteritis (7).

Hepatitis A virus causes about 4000 foodborne illnesses annually in the United States. Similar to norovirus, hepatitis A virus is excreted in feces of infected people, and causes infection in susceptible individuals when contaminated water or foods are ingested. Controlling the spread of hepatitis A can be difficult because infected persons can shed viral particles during the long, 10 to 50 day, incubation period. The greatest danger of spreading the disease to others occurs during the middle of the incubation period, well before the first presentation of symptoms. Hepatitis A is usually mild, but can be one of the more severe foodborne illnesses (8); recovery generally requires 1–2 weeks, but can be much longer. Hepatitis A symptoms include fever, fatigue, nausea, vomiting and abdominal discomfort, followed in several days by jaundice. The infectious dose is thought to be fairly low, 10–100 virus particles (9).

B. Bacteria

Bacterial pathogens are estimated to account for about 4 million of the foodborne illnesses caused by identified pathogens annually in the United States (1). Depending on how the classification is made, there are 19 or so bacterial pathogens that cause nearly all the identified bacterial foodborne illnesses. Bacterial pathogens account for 60% of the hospitalizations attributable to foodborne illness, and 72% of the deaths (1). Bacterial pathogens can be found in virtually any environment, including food, and plants and animals produced for food. They thrive in warm, moist foods that are low in acid. Most disease-causing bacteria grow very slowly in foods at low temperatures, multiply rapidly in mid-range temperatures, and are killed at high temperatures.

Bacterial pathogens cause foodborne illness by three mechanisms: infection, intoxication or toxin-mediated infection; some employ more than one mechanism (10). Some bacterial pathogens produce a literal food poisoning

TABLE 75.1
Fourteen Common Foodborne Pathogens

Pathogen	Estimated Incidence (Foodborne)	Estimated Cost (Millions of Dollars)	Disease Severity	Common Food Sources	Food-Handling Error(s) Associated with Pathogen	Primary Control Factor
Norovirus	9,200,000	8,160	Mild to moderate	Contaminated shellfish and prepared foods handled by infected food handlers	Poor personal hygiene or infected food handlers	Personal hygiene
Shigella spp	90,000	34	Moderate to severe	Prepared foods handled by infected food handlers	Poor personal hygiene or infected food handlers	Personal hygiene
Hepatitis A	4,170	21	Moderate to severe	Contaminated shellfish and prepared foods handled by infected food handlers	Poor personal hygiene or infected food handlers	Personal hygiene
Clostridium perfringens	249,000	46	Mild, self-limiting	Meat, poultry products, and beans	Bacterial spores survive cooking; multiply when food is in danger zone	Keep food at safe temperatures
Staphylococcus aureus	185,000	85	Mild to severe (rarely)	High-protein foods handled frequently during preparation	Food handler contaminates cooked food, *S. aureus* produces toxin while food is in danger zone	Keep food at safe temperatures
Bacillus cereus	27,000	11	Mild, self-limiting	Cooked rice and pasta	Bacterial spores survive cooking; multiply when food is in danger zone	Keep food at safe temperatures
Campylobacter jejuni	1,960,000	1,798	Mild to moderate	Raw milk, poultry, beef, pork, shellfish	Inadequate cooking, fecal/environmental contamination	Adequate cooking/ cross-contamination
Salmonella spp	1,342,000	1,190	Mild to severe	Meat, poultry, raw milk, eggs fresh produce	Inadequate cooking, fecal/environmental contamination	Adequate cooking/ cross-contamination
Toxoplasma gondii	112,500	1,247	Mild to severe	Pork, ground beef, other meats	Inadequate cooking, fecal/environmental contamination	Adequate cooking/ cross-contamination
Yersinia enterocolitica	86,731	77	Mild to moderate	Pork, milk or milk products	Inadequate cooking, fecal/environmental contamination	Adequate cooking/ cross-contamination
Escherichia coli 0157:H7	62,500	205	Moderate to severe	Ground beef, raw milk, lettuce, unpasteurized apple cider	Inadequate cooking, fecal/environmental contamination	Adequate cooking/ cross-contamination
Vibrio spp.	5,218	5	Mild to severe	Raw seafood and shellfish	Inadequate cooking fecal/environmental contamination	Adequate cooking/ cross-contamination
Listeria monocytogenes	2,493	29	Mild to severe	Raw milk, soft cheeses, raw vegetables, raw-meat sausages	Inadequate cooking, post-pasteurization contamination, lengthy refrigeration	Avoid food from unsafe source
Clostridium botulinum	58	1	Severe	Home-canned or fermented vegetables, meat, fish	Inadequate processing time for low-acid food; poor fermentation	Avoid food from unsafe source

Adapted from reference 14.

by producing a toxin as they grow in food. In this case, ingestion of the toxin causes illness (*Staphylococcus aureus* and *Clostridium botulinum* are examples). Most bacterial pathogens cause an infection when ingested, burrowing into the digestive tract lining and colonizing there, increasing in number. Antibiotics are used to control bacterial infection from foodborne illnesses, but some bacterial pathogens are becoming antibiotic-resistant, resulting in illnesses that are not easily treated. Antibiotic-resistant bacteria are increasing in the food supply (11, 12), making prevention of foodborne illnesses increasingly important.

All bacteria exist as vegetative cells, which are relatively easily destroyed by heat, sanitizers and/or other controls. However, a few bacterial pathogens have the ability to form spores (for example *Clostridium botulinum* and *C. perfringens*), a structure that enables the bacteria to withstand harsh environmental conditions, such as heating and the application of sanitizers. This characteristic makes their control more difficult than that of non-spore-forming pathogens. Some bacterial pathogens (for example, *Yersinia enterocolitica* and *Listeria monocytogenes*) are psychotrophs, so refrigeration temperatures are not effective for controlling growth in food (13).

Eleven bacterial pathogens are of particular concern in consumer food safety. The illnesses caused by these pathogens are briefly discussed below in the order of the amount of foodborne disease they cause in the United States. This information is drawn primarily from the Food and Drug Administration's Center for Food Safety and Applied Nutrition's (FDA-CFSAN) *Bad Bug Book* web pages (9); this is a good source for additional information. Table 75.1 lists the estimated incidence, dollar cost to society, and disease severity of these pathogens with organisms grouped by food safety behaviors most useful in controlling each pathogen (14). *Salmonella* (nontyphoidal) and *Campylobacter* are responsible for 43% and 29% of the hospitalizations caused by known bacterial pathogens; *Salmonella* (nontyphoidal) and *Listeria monocytogenes* are responsible for 43% and 38% of the deaths caused by known bacterial pathogens (1).

- *Campylobacter jejuni* causes the most foodborne illness among bacterial pathogens. Its infective dose is small, about 400–500 bacteria may cause illness, although individual susceptibility varies. *Campylobacter* infections are frequently mild, causing diarrhea, which may be watery or sticky. Other campylobacteriosis symptoms include fever, abdominal pain, nausea, headache and muscle pain. The illness usually occurs 2–5 days after ingestion of the contaminated food or water and generally lasts 7–10 days, but relapses occur in about 25% of cases. The illness rarely can progress to further complications including reactive arthritis, hemolytic uremic syndrome, meningitis, recurrent colitis, and Guillain-Barré syndrome.
- Bacteria in the genus *Salmonella* include thousands of serotypes, most of which are pathogenic. Foodborne illness is mainly from the nontyphoidal types. As few as 15–20 bacterial cells may cause the infection, although susceptibility depends upon individual age and health, and on serotype differences. Symptoms of salmonellosis include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. The illness usually occurs 6 to 48 hours after ingestion of the contaminated food or water and lasts 1 to 2 days, but may be prolonged. Complications include post infection reactive arthritis in about 2% of cases.
- *Clostridium perfringens* is a spore-forming organism that frequently causes illness due to temperature abuse of prepared foods, which allows the spores to germinate and grow in a competition free environment. Prefringens poisoning is caused by ingestion of large numbers (greater than 100 million) bacterial cells. When the infection takes hold, toxin produced in the digestive tract is associated with sporulation of the vegetative cells. Perfringens poisoning symptoms include intense abdominal cramps and diarrhea which begin 8–22 hours after consumption of foods containing large numbers of *C. perfringens* bacteria. The illness lasts about 1 day, but residual symptoms may persist for 1 or 2 weeks.
- *Staphylococcus aureus* bacteria occur normally on human skin and in the nose and throat; about 50% of people carry this pathogen. These bacteria can be transmitted to food during handling or preparation. When potentially hazardous foods are held at temperatures where *S. aureus* can grow, any *Staphylococcus* bacteria in the food multiply and produce a heat stable toxin that is not destroyed by cooking. Staphylococcal food poisoning occurs when less than 1 μg of toxin is ingested, a toxin level produced when *S. aureus* populations exceed 100,000 per gram of food. Symptoms of staphylococcal food poisoning occur within 1 to 6 hours, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse

rate may occur. Recovery generally takes two days.

- *Shigella spp.* are highly infectious bacteria that are transmitted by the fecal-oral route. As few as 10 bacterial cells can cause the gastro-intestinal infection. The symptoms of shigellosis occur within 12 to 50 hours of ingesting contaminated food or water and include abdominal pain, cramps, diarrhea, fever, vomiting and blood or mucus in the stools. Complications may include reactive arthritis and hemolytic uremic syndrome. Infants, the elderly, and the infirm are susceptible to the severest symptoms of disease, but all individuals are susceptible to some degree. Shigellosis is a very common malady suffered by individuals with acquired immune deficiency syndrome.
- *Yersinia enterocolitica* is a bacterial infection that causes fever and abdominal pain and often diarrhea and/or vomiting. Yersiniosis is more common in Northern Europe, Scandinavia, and Japan than in the United States. Complications of yersiniosis include reactive arthritis (about 2–3% frequency) and rarely bacteremia (entrance of organisms into the blood stream). Fatalities are rare.
- *Escherichia coli* is a common bacteria, but several strains have the potential to cause foodborne illness, accounting for just over 4% of known bacterial causes of foodborne illness in the United States. *E. coli* 0157:H7 causes the most serious illness, as it is responsible for 3% of total hospitalizations and 3% of total deaths from known pathogens. *E. coli* O157:H7 can cause severe bloody diarrhea. The symptoms begin to occur within one to eight days, and the illness generally lasts 5 to 10 days. About 5% of persons who are infected with *E. coli* 0157:H7 develop serious complications such as kidney failure and blood clots in the brain. Children and the elderly are much more likely to have serious complications from *E. coli* 0157:H7. In young children, *E. coli* 0157:H7 is the leading cause of acute kidney failure.
- *Bacillus cereus* is a spore forming organism that produces two types of toxins when environmental conditions permit its growth in food. These toxins cause two types of symptoms: diarrhea is caused by a large molecular weight protein, while vomiting is caused by a low molecular weight, heat-stable peptide. If the number of *B. cereus* bacteria exceeds one million per gram in a food, toxin is likely to be present. The diarrheal illness, including abdominal cramps, occurs 6–15 hours after consumption of contaminated food and persists for about 24 hours. The type of illness characterized by nausea and vomiting generally occurs more quickly, within 0.5 to 6 hours after consumption of contaminated foods, and lasts less than 24 hours.
- *Vibrio spp.* At least three species of *Vibrio* are involved with foodborne illness: *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholera*. They cause somewhat different illnesses, but all are associated with seafood.
- *Vibrio parahaemolyticus* causes a usually mild to moderate infection with associated diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills. Ingestion of greater than one million organisms causes disease, although the dose may be lowered if stomach acid is buffered by food or antacids. Illness occurs within 4–96 hours when the organism attaches itself to the small intestine and excretes a toxin. Illness usually lasts about 2.5 days.
- The ingestion of *Vibrio vulnificus* can cause serious blood infection in individuals with underlying chronic disease such as diabetes or leukemia, or those taking immunosuppressive drugs. Rapid death occurs in about 50% of cases. For healthy individuals, ingestion of this organism can result in gastroenteritis. The infective dose for gastrointestinal symptoms in healthy individuals is unknown, but for susceptible persons the infective dose is less than 100 total organisms.
- The infection caused by *Vibrio cholera*, which attaches to the small intestine and produces a toxin, creates mild to acute watery diarrhea, abdominal cramps, nausea, vomiting, dehydration, and shock. If there is severe fluid and electrolyte loss, death may occur. Human volunteer feeding studies utilizing healthy individuals have demonstrated that approximately one million organisms must be ingested to cause illness.
- *Listeria monocytogenes* bacteria rarely cause illness (an estimated 2500 foodborne cases per year), but those who get listeriosis are frequently hospitalized and about 20% of cases result in death. Pregnant women, the elderly, and those with compromised immune systems are vulnerable to *Listeria* infection. If a pregnant women gets listeriosis, the infection can be passed to the unborn child and cause death to the fetus. Listeriosis can occur within 1 day or up to 3 weeks of ingesting contaminated food.
- Botulism is a rare, but deadly food poisoning, causing an estimated 58 cases and 4 deaths per

year. This pathogen has the ability to form spores that are very resistant to heat. Ingestion of the spores or vegetative cells is generally harmless (with the exception of infant botulism), but when vegetative cells grow, they produce a toxin that is one of the most deadly agents; a few nanograms is sufficient to cause the death of an adult.

C. Parasites

Parasitic pathogens cause about 3% of known foodborne illnesses in the United States (1). Several types of parasites can be found in food and water, including *Toxoplasma gondii* (the cause of toxoplasmosis), *Trichinella spiralis* (the cause of trichinosis), *Giardia lamblia, Cryptosporidium parvum,* and *Cyclospora cayetanensis*. Parasites do not reproduce in food, and usually require more than one animal species to carry out their life cycle.

- One parasite of note, *Toxoplasma gondii* usually causes a mild, self-limiting infection. About 30% of Americans have likely been infected with *Toxoplasma*. House cats and many other animal species (including animals raised for meat), as well as humans may carry *Toxoplasma*. Pregnant women who become infected with *Toxoplasma gondii* during pregnancy may transmit the disease to their unborn child, resulting in severe illness in the child. Toxoplasmosis has been increasing in frequency due to increases in the numbers of immunocompromised individuals (15). Additional information about *Toxoplasma gondii* is in Table 75.1.

Although causes of foodborne illness like those described above have been recognized for decades, new pathogens that can cause foodborne diseases continue to be identified, and that trend is likely to continue.

III. FOOD SOURCES OF FOODBORNE DISEASE

Almost any food can be a source of foodborne pathogens, but some foods are more likely to be sources, and require more careful handling. In addition, foods which are termed "potentially hazardous" support the rapid growth of bacterial pathogens and must be kept out of the Danger Zone (40° to 140°F). Sometimes these foods are also called "perishable" foods. These foods are usually moist and low in acid and include:

- foods of animal origin such as meat, milk, cheese, poultry, eggs, fish, and seafoods
- foods of plant origin that have been heat treated, including cooked vegetables, beans, and rice
- raw sprouts
- cut melons, peeled carrots, and other peeled vegetables and fruits
- cooked pasta
- tofu and other moist soy protein products
- sauces such as Hollandaise and many other sauces (unless they are high in acid)

The Center for Science in the Public Interest, a consumer activist group that specializes in food issues, has compiled a list of foods that have caused foodborne illness from 1990 to 2001 using outbreak data reported to the CDC (16). The top six food categories linked to food-poisoning outbreaks were multi-ingredient foods, seafood, eggs, produce, beef, and poultry.

Food	Number of Outbreaks	Number of Cases
Multi-ingredient foods, such as salads, pizza, and sandwiches	337	23,142
Eggs and egg-dishes	271	10,827
Produce, including sprouts, lettuce, berries, and cantaloupe	148	9,413
Beef	134	6,089
Seafood	340	5,133
Poultry	79	4,279

Some foods are more likely to be sources of specific pathogens. The foods associated with specific pathogens are discussed below (pathogens arranged in alphabetical order).

- *Bacillus cereus*, which produces two kinds of toxins in foods, has been associated with a wide variety of foods. Meats, milk, vegetables, and cereal dishes are foods that have caused the diarrheal-type illness. The vomiting-type of illness is generally associated with starchy foods, such as rice products, potatoes, and pasta; vegetable sprouts and pasteurized cream have also been implicated (17).
- *Campylobacter jejuni* bacteria are normally found in warm-blooded animals such as cattle, poultry, and pigs. These bacteria may be present in food products that come from these animals, such as raw meat, poultry, eggs, or unpasteurized dairy products. Handling raw poultry and eating undercooked poultry is associated with many sporadic cases of campylobacteriosis (18). A majority of chicken available in the U.S. (63 to 88%) is contaminated with *Campylobacter* (19, 20).

- The bacteria that cause botulism, *Clostridium botulinum*, are indigenous in soil and water and have the ability to form spores. They become dangerous when environmental conditions of low oxygen and low acidity allow them to multiply and produce a toxin. Low-acid foods such as meat, fish, poultry, or vegetables that are improperly canned can provide a growth environment for these bacteria. The toxin also may be produced in low-acid cooked foods left too long at room temperature, such as baked potatoes or potpies. Homemade mixtures of raw garlic stored in oil at room temperature have also caused outbreaks (17).
- *Clostridium perfringens* bacteria are widely distributed in the environment (soil, water, foods) and frequently occur in the intestines of humans and many domestic and feral animals. Spores of this bacterium persist in soil, sediments, and in areas subject to human or animal fecal pollution. Meat, poultry, vegetables, beans, rice, and spices are foods in which *C. perfringens* have been identified. Meat dishes that are prepared one day and served the next day have been the source of a number of perfringens food poisoning outbreaks (17).
- *Escherichia coli* 0157:H7 can be transmitted through food and water. A wide variety of foods, including undercooked ground beef, raw (unpasteurized) milk, uncooked sprouts and unpasteurized juices as well as contaminated drinking water have been associated with *E. coli* 0157:H7 outbreaks. *E. coli* 0157:H7 also can be transmitted from person to person. This is particularly a problem in day care centers where young children are in close contact. If the bacteria are on the hands, they can be spread by touching another person or by touching food that will not be cooked before eating (21).
- *Listeria monocytogenes* has been isolated from a wide variety of foods including milk and dairy products, meat and meat products, vegetables, and seafood. Foods associated with outbreaks have generally been highly processed, capable of supporting the growth of *L. monocytogenes*, had extended shelf life at refrigeration temperature and were ready to eat. *L. monocytogenes* is able to colonize food-processing environments for long periods of time, is tolerant to sodium chloride and sodium nitrate, and can grow on a wide variety of foods at refrigeration temperatures. Thus, food manufacturers must be vigilant to prevent post-processing contamination (22). Pregnant women and others who are at risk of listeriosis are advised to either avoid or reheat paté, smoked seafood, fresh soft cheeses, luncheon meats, hot dogs, smoked fish and deli salads because these foods have been implicated in listeriosis outbreaks (23).
- *Salmonella* are normally found in warm-blooded animals such as cattle, poultry, and pigs, and foods of animal origin cause most of the *Salmonella* infections in humans. Consumers need to be aware that these bacteria may be present in raw meat, poultry, eggs, or unpasteurized dairy products. *Salmonella* outbreaks have also been associated with fresh fruits and vegetables and packaged products (17, 24).
- Since *Staphylococcus aureus* is primarily carried on human skin and in the respiratory tract, foods that require extensive handling during preparation and that are kept at Danger Zone temperatures after preparation are frequently involved in staphylococcal food poisoning. Some of the foods that are often involved in staphylococcal food poisoning include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products (25).
- *Shigella* is principally a disease of the human intestinal tract and is rarely found in animals. These bacteria are found frequently in water that is polluted with human feces. The contamination of foods with *Shigella* occurs via the fecal-oral route when food handlers do not follow proper hand washing practices or when fecally contaminated water is used for irrigation or processing. Salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and poultry have all been involved in outbreaks (26).
- *Yersinia enterocolitica* occurs naturally in animals (pigs, birds, beavers, cats, and dogs) and in the environment (ponds, lakes). This organism has been found in meats (pork, beef, lamb, etc.), oysters, fish, and raw milk, but the exact cause of the food contamination is unknown. Poor sanitation and improper sterilization techniques by food handlers are postulated as contributing to contamination (27). Most cases are associated with pork (17).
- Infections with *Vibrio* spp. have been associated with the consumption of raw, improperly cooked, or cooked, recontaminated fish and shellfish. Improper refrigeration of contaminated seafoods will allow an increase of

pathogen numbers, which increases the possibility of infection. Infection increases during the warmer months of the year (28). Sporadic cases occur along all coasts of the U.S. (17).

IV. CONSUMER PREVENTION AND CONTROL OF FOODBORNE DISEASES

Review of the pathogens of most concern in consumer food safety indicates that the control of these pathogens requires hand washing, kitchen cleanliness, avoidance of cross-contamination and of unsafe foods, heating to appropriate temperatures, and refrigeration. Educating consumers about food safety has coalesced around four or five basic messages. The Partnership for Food Safety Education is a coalition of industry, government and consumer groups formed in 1997 to educate the public about safe food handling and reduce foodborne illness. The coalition developed the Fight BAC!® campaign to promote four steps — Clean, Cook, Chill and Separate — that consumers "can take to fight foodborne bacteria and reduce their risk of foodborne illness" (29). A recent review of the food safety literature identified and categorized common food handling messages, then used a Delphi process with a panel of food safety experts to identify the most important messages (30). They identified five pathogen control factors: 1) Practice personal hygiene, 2) Cook foods adequately, 3) Avoid cross-contamination, 4) Keep foods at safe temperatures and 5) Avoid foods from unsafe sources (1 through 4 are in concert with the Fight BAC!® steps). The pathogens shown in Table 75.1 are grouped according to these control factors needed to prevent them from causing a foodborne illness. The details associated with these messages are presented below.

A. Practice Good Personal Hygiene

The most important food safety message for consumers is adequate and frequent hand washing. Most of the foodborne illnesses that can be prevented by good personal hygiene are those that are transmitted from an infected person's intestines to another person, the fecal-oral route. These include particularly the norovirus and Hepatitis A viruses, and the bacterial pathogen *Shigella*. Consumers need to be reminded about the times to wash hands: always after using the toilet, changing a diaper, or petting an animal, and before handling food or eating food. Pathogens can be transferred to the hands from a variety of sources. Some foodborne pathogens, including norovirus and hepatitis A virus, *E. coli*, and some other types of bacteria, plus some parasites such as *Giardia*, can also be transmitted directly from person to person, often via the hands of an infected person. Animals at home, on the farm, or in a petting zoo are also sources of pathogens, as well as dirt, manure, or a sandbox. *Toxoplasma gondii*, for example, is commonly found in dirt or sand in areas where there are outdoor cats. Consumers should also be encouraged to teach and model good hand washing practices to their children. Regular hand washing can help consumers avoid transferring pathogens from raw food, the environment, or their body onto ready-to-eat foods.

Most consumers know about the need to wash hands, but do not always put knowledge into practice. A survey and observational study conducted for the American Society of Microbiology's (ASM) Clean Hands Campaign found 95 percent of men and women say they wash their hands after using a public restroom, but only 67 percent of people actually do wash before leaving the restroom (31). Hand washing studies also suggest that consumers need to be reminded about the techniques for hand washing that adequately remove pathogens from hands. Use of warm water assists in dissolving foreign materials and encourages longer washing. Soap is a surfactant and is necessary to loosen dirt and pathogens from the skin so they can be rinsed away. In spite of the prevalence of antibacterial soaps available to consumers, they are not necessary and may potentially contribute to the problem of antibacterial resistance (32). A fingernail brush helps remove dirt and feces. Hand washing research suggests that 15 to 20 seconds are necessary for adequate pathogen removal and that thorough drying of hands is necessary to prevent recontamination (33). The use of disposable paper drying towels is preferred, although frequently (daily) changing of a cloth hand towel is acceptable practice in homes.

Consumers who are sick with diarrhea should not prepare food for others. Fecal matter can contain millions of pathogenic viruses or bacteria (5), and the infectious dose is low for many pathogens. People can easily miss a few pathogens when hand washing and unintentionally infect someone else. Personal hygiene messages also need to stress that cuts and burns on hands should be covered with a bandage and disposable plastic gloves during food preparation because infected lesions can be a source of *Staphylococcus aureus.*

Specific food handling messages for consumers that focus on good personal hygiene should include:

- Wash hands after any contact with raw poultry, meat, or seafood.
- Work with clean hands, clean hair, clean fingernails, and wear clean clothing.
- Avoid using hands to mix foods when clean utensils can be used.
- Keep hands away from one's mouth, nose, and hair.
- Cover the nose and mouth with disposable tissues when coughing or sneezing, and wash hands thoroughly afterward.

- Use a clean spoon each time when tasting food while preparing, cooking, or serving.
- Wash hands after smoking.

B. Cook Foods Adequately

Cooking is an essential part of making foods safe to eat since foodborne pathogens are killed by heat. Almost all pathogens are killed when food is heated to 160°F for a few seconds; lower temperatures for longer periods of time also kill pathogens (34). The best way to be certain food has been cooked to the proper temperature is to check it with a food thermometer. However, only a small percentage of consumers use food thermometers when preparing food. For example, 12 to 24% of consumers say they use a meat thermometer (2). Only 3 to 6% use a thermometer to check endpoint temperature of hamburgers (35). Educating and motivating consumers to use instant-read food thermometers more frequently is the focus of USDA's Thermy™ Campaign (36).

Two types of instant-read food thermometers are generally available to consumers: bimetallic coil dial thermometers and thermister digital thermometers. The best kind of thermometer to use is a thermister digital thermometer. The thermister is located in the tip of the probe and is a ceramic semiconductor whose electrical resistance changes with temperature. It can be used in both thick and thin foods. Even though the temperature sensor is in the tip, the tip still must be inserted at least ½-inch deep into the middle of the thickest part of the food. Digital thermometers require about 10 seconds to register the correct temperature. When testing thin or small items for temperature, it is best to insert the thermometer from the side. Instant read dial thermometers use a bimetallic coil to sense temperature; the bimetal coil generally occupies the lower 2 to 3-inches of the thermometer stem, so for these thermometers the entire lower 2 to 3 inches of the stem must be inside the food. For thin foods, it must be inserted sideways. Instant read thermometers, dial and digital, are used to test for doneness near the end of the cooking time; they are not designed to be left in the food during cooking. Instant read thermometers should be tested for accuracy periodically using ice water or boiling water (37).

Consumers need to assume that raw (uncooked or unpasteurized) meats, poultry, fish, shellfish, milk, and eggs contain pathogens, and eating them raw or lightly cooked presents a food safety risk. Many pathogens live naturally in the intestinal tracts of food animals. Surveys of poultry cuts sold in retail food stores indicate that between 1/4 and 3/4 are contaminated with *Salmonella* and/or *Campylobacter* (20). Due to the grinding and mixing process, ground meats such as hamburger, sausage and ground poultry products may be contaminated with pathogens throughout the meat, requiring that ground meat items be cooked throughout to 160°F. Parasites such as *Toxoplasma* or *Trichinella* may be in the muscles of some animals, particularly pigs. Thus, pork also requires thorough cooking to kill parasitic as well as bacterial pathogens. Roasts and steaks from other animals usually only have pathogens on the surface of the meat unless the meat is pierced for tenderizing or pumping. Rare beef steaks and roasts are much less risky than rare pork or undercooked ground meat. Wild game may have a higher level of bacteria than commercially slaughtered meat because of the difficulty of handling game in the field, thus thorough cooking of all game meat is recommended. *Salmonella* sometimes contaminate the inside of the egg as well as the eggshell itself; about 1 egg out of every 20,000 contains *Salmonella* inside the shell (38). Shellfish may pick up bacteria and viruses from contaminated waters (13). Fish may contain parasitic worms. These microorganisms then can infect anyone who eats the shellfish or fish if they are not cooked properly.

Seventy-four to 92 percent of consumers are aware of risks associated with eating rare meats or poultry, raw or lightly cooked fish and shellfish, raw milk, and foods made with raw or lightly cooked eggs. However, 80 to 93% do not know the correct temperatures for safe heating of foods (2). Any foods likely to be contaminated with pathogens should be heated to an internal temperature of 160°F; at this temperature, most pathogens are killed very quickly (34). Safe heating temperatures for foods are shown in Table 75.2. Consumers should be advised to check the temperature with a thermometer to be certain the food is fully cooked.

C. Avoid Cross-Contamination

Caution to prevent the moving of pathogens from one food to another food is an especially critical consideration when dealing with food that is ready-to-eat. Cross-contamination is a relatively common mistake made by home food preparers. A case in point is *Campylobacter jejuni*, a common contaminant of poultry from the grocery store. *Campylobacter* is relatively easy to kill by cooking, and few people eat undercooked poultry. Yet there are about 2 million cases each year of foodborne illness resulting from *Campylobacter*, good indirect evidence that cross-contamination is common. In a United Kingdom study of consumer food handling behavior, it was found that 17% of homemade chicken salads prepared in a model kitchen were positive for *Campylobacter* as a result of cross-contamination during preparation (2).

Although it may be challenging within the confines of some small domestic kitchens, consumers can prevent cross contamination by always keeping cooked and ready-to-eat foods separate from raw foods. Food preparation surfaces need to be cleaned before and after preparing food. After any contact with raw poultry, meat, seafood, or

TABLE 75.2
Endpoint Temperatures to Control Pathogens in Specific Foods

Minimum Interior Temperature	Food Item	Details
160°F	Ground meats (including turkey and chicken)	Using visual signs to determine that ground beef is done is risky because it has been shown that 1 in 4 hamburgers appears brown before reaching 160°F.
	Pork and game meats	These meats may contain parasites. Game meats may contain more pathogens than commercially slaughtered meat due to less controlled slaughter and cutting conditions.
	Poultry	When poultry is cooked to 160°F, the meat may not look and taste 'done', although pathogens will be killed. At 170°–180°F, the flavor, texture, or appearance will be that associated with fully cooked poultry.
	Eggs	Either check the temperature with a thermometer or else cook eggs until both the yolk and white are firm. Visual cues are adequate since pathogens will be killed if the yolk and white are cooked enough to be firm.
	Food mixtures containing eggs or raw meat	Visually difficult to assess doneness; use a thermometer to check temperature.
145°F	Beef steaks and roasts, ham, lamb	Heat exterior sufficiently to kill pathogens. Interior not likely to contain pathogens.
	Fish and shellfish	Visual cues are commonly used to determine if fish and shellfish are thoroughly cooked. Cook fish until it flakes and loses its translucent or raw appearance, and cook shellfish until the shell opens and the flesh is fully cooked. Little research has been done to identify the cooking temperatures required to kill pathogens on fish and shellfish, and the cooking recommendations are based more on quality of the cooked product than on actual scientific data.
165°F	Leftovers, reheating of those to be served hot	Re-heat to kill pathogens that may have survived the first cooking or that were introduced after cooking. However, *Staphylococcus aureus* toxin is not destroyed by heating leftovers.
	Hot dogs, deli meats for some people	Pregnant women, the elderly and those with compromised immune systems should reheat before eating. Because these items have been fully cooked previously, they, like leftovers, should be heated to 165°F.

any other potentially hazardous foods, hands, knives, cutting boards, food preparation surfaces, and sink need to be thoroughly washed. For utensils and hard nonporous surfaces, washing and rinsing should be followed by sanitizing with a dilute chlorine solution and air drying. Mixing 1 tablespoon of bleach (5.25% sodium hypochlorite) to one gallon of water produces an approximately 200 ppm chlorine sanitizing solution (39, 40) that will kill most pathogens. Household bleach is easily available to consumers. For best efficacy, the chlorine solution should be left in contact with the surface for two minutes before rinsing. Storing the dilute bleach solution in a spray bottle can make sanitation more convenient. Dilute bleach solutions do lose strength over time (41) and should be re-made regularly. Kitchen countertops should be washed with soap and water before food preparation if previous use of the surface is not known; for example, if there is a cat in the house that gets on the counters.

Fresh produce should be rinsed thoroughly under running water before preparing or eating. Rinsing does not remove all pathogens, but it reduces the number present by about 90 to 99 percent (42, 43). Pathogens have been isolated from a wide variety of fresh produce, and outbreaks of foodborne illness have been associated with many types of produce, including cantaloupes, lettuce and tomatoes. If contamination is present on the skin of the fruit or vegetable, the pathogens move into the fruit when it is sliced. Removing the skin or rind reduces the risk (44).

During the preparation and storage of raw meat and poultry items it also important to avoid cross-contamination. It is best to have two cutting boards — one for raw meat, fish, and poultry, and another for food that will not be cooked before eating. Colored cutting boards, which allow boards to be more easily restricted for use with one food category, are increasingly available. A hard, nonporous cutting board such as one made of acrylic is easier to clean than a wooden board because the acrylic board can be washed in the dishwasher. In the refrigerator, raw meat and poultry should be placed on a tray or plate to prevent juices from dripping onto other food. The refrigerator "meat drawer" should be used for the storage of either raw or ready-to-eat meats, not both. All family members should be aware of refrigerator storage rules. When moving raw meats to a cooking site (such as grilling outdoors), it is important to remember to bring a clean plate or other clean container for the cooked meat in order to prevent cross contamination.

Kitchen cloths and sponges can be a major source of cross-contamination. Bacteriological surveys of kitchen dishcloths and sponges have shown the presence of many pathogens (45). Dishcloths and sponges need to be laundered frequently. Drying dishcloths in a clothes dryer aids in killing pathogens. Sponges can be washed in the dishwasher. Wet dishcloths and sponges can be sanitized by heating them in a microwave oven for one minute on high (45, 46). They also can be disinfected by soaking in a bleach solution (3 tablespoons of bleach per quart of water) for 5 minutes, then rinsed and dried (40). Paper towels should be used to clean up spilled juices from raw meat or poultry.

D. Keep Foods at Safe Temperatures

Most bacterial pathogens grow most rapidly at temperatures of 60° to 125°F. Controlling bacterial growth in potentially hazardous foods requires temperature control; the old adage "keep hot foods hot" (above 140°F) and "cold foods cold" (below 40°F) is frequently used to reinforce the message. Figure 75.1 summarizes the temperature information needed by consumers to control the growth of bacteria in foods. Temperature control recommendations for consumers differ from those used in foodservice because consumers are unlikely to keep track of the amount of time foods are kept at different temperatures.

Recommendations for consumers center around the temperatures needed for hot holding, the types of foods requiring refrigeration, proper refrigerator temperature and the limitation of time these foods are held at room temperature. Recommendations for consumer refrigerator temperature is 40°F or below (47). Prepared and leftover foods should not be at room temperature more than 2 to 3 hours, including all time involved during preparation, storage, and serving. When shopping, consumers are advised to pick up perishable foods as the last stop in the grocery store, and, especially in hot weather, get them home and into the refrigerator quickly, using an ice chest or cooler for transportation of perishable items when shopping is done more than 30 minutes from home. A recent study found that most (68%) consumers refrigerate or freeze meat within 1 hour of purchase, but 9% did not place meat purchases in cold storage until more than 2 hours (and up to 8 hours) had elapsed (48). Research indicates that about 13% of consumer refrigerators are above 40°F, and most (80%) do not have a thermometer in the refrigerator (49). Brown and colleagues (49) also reported that 78% of people did not know the correct temperature to maintain in home refrigerators. It would aid consumers in maintaining safe food if refrigerators came equipped with built in thermometers that clearly marked the temperatures recommended for cold food storage.

Foods need to be stored to prevent cross-contamination in the refrigerator. It is best to store meat, poultry and fish on a dish or tray. Refrigerators require periodic cleaning. A recent study from Ireland found that foodborne pathogens were commonly present in home refrigerators. *Staphylococcus aureus* was found in 40%, *E. coli* and *Listeria* in 6%, and *Salmonella* in 7% of the home refrigerators, indicating that some refrigerators are unclean and unsafe for food storage (50). Washing refrigerator interior surfaces with a solution of one gallon of water mixed with one-half cup of unscented ammonia is a recommended cleaning method (51).

A common consumer practice is to cool foods at room temperature prior to refrigerating in the mistaken belief that placing hot foods in the refrigerator is harmful to the appliance. Small amounts of warm or hot foods may be put into the refrigerator, but it is advisable to speed the cooling of larger quantities by cooling the container of food in a pan of ice water, stirring and replacing the ice frequently until the food has cooled to about 100°F, then refrigerating the food in shallow containers. Consumers are advised to set a timer for about 30 to 45 minutes as a reminder to check to see if the food is cooled enough to be refrigerated (52).

Planning ahead for proper cooling and reheating of food prepared in large quantities and/or prepared for transporting to other sites, such a picnics, potluck dinners, camping trips and packed lunches, requires special attention. This involves planning the amount of needed refrigerator space (to include good circulation) or ice and cooler space to keep foods at safe temperatures. If perishable foods from picnics or potlucks are not kept adequately chilled or heated during serving, they should be thrown out if in the Danger Zone for 2 to 3 hours.

Frozen perishable foods must be thawed in the refrigerator, under cold running water, or as a part of the cooking process. If food is thawed in a microwave, it should be cooked right after it thaws.

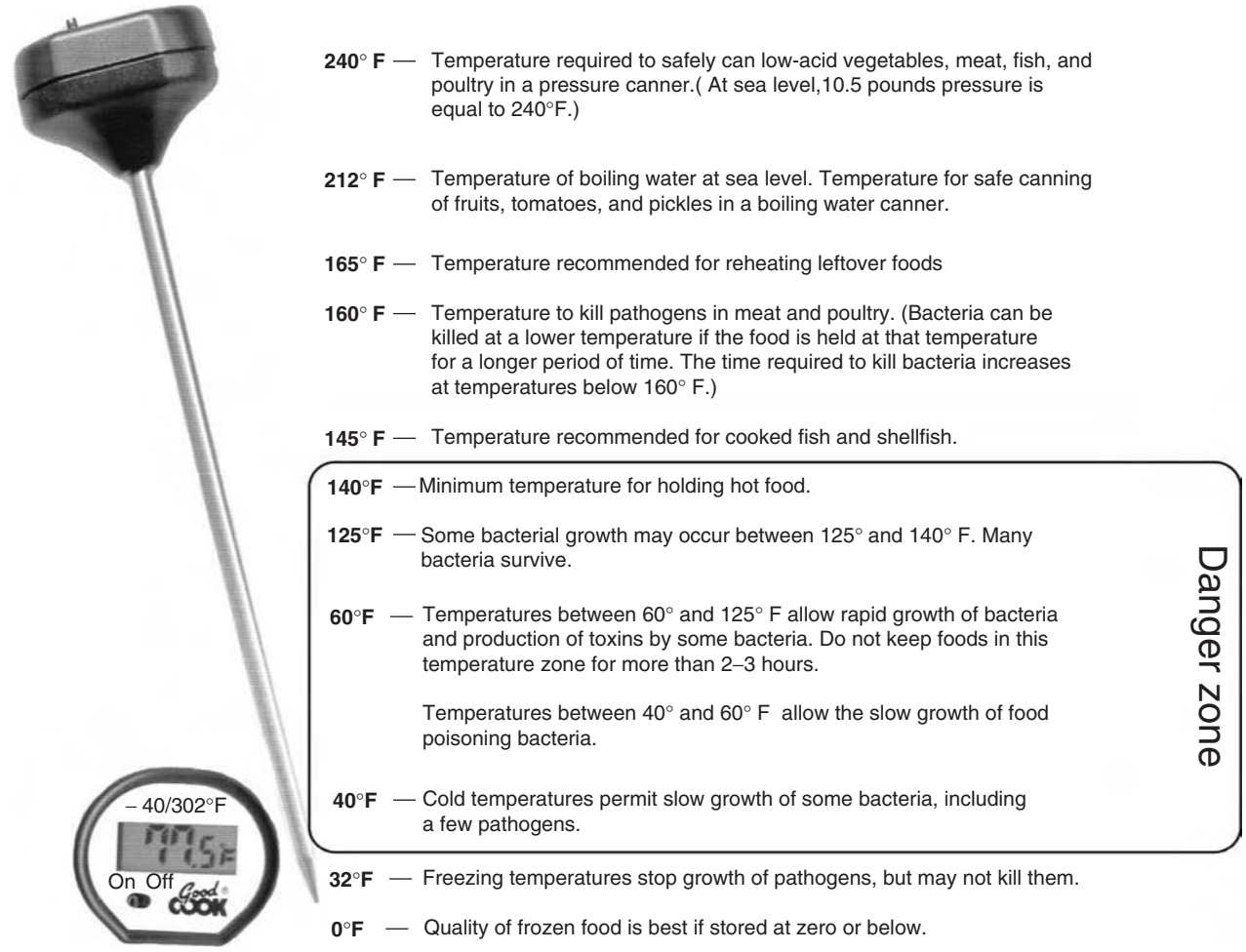

FIGURE 75.1 Safe handling food temperatures.

E. Avoid High-Risk Foods and Water

Some foods have such a high probability of being contaminated with pathogens or toxins that their consumption should be avoided by all individuals. The risk is highest for people susceptible to foodborne illness. Many of these foods are ready-to-eat and have been produced or processed in a way that does not kill pathogens. Foods frequently linked to foodborne illness include:

- Unpasteurized milk and milk products
- Raw or undercooked meats and poultry
- Unpasteurized fruit juice
- Raw sprouts of all types
- Raw seafood and fish
- Raw or undercooked eggs
- Contaminated water

Specific consumer advice for reducing the chance of contracting a foodborne illness includes:

- Drink only pasteurized milk and fruit juices.
- Avoid eating undercooked or raw meat (such as steak tartare).
- Use cheese and yogurt made from pasteurized milk.
- Cook sprouts before eating.
- Cook fish and shellfish before eating them.
- Avoid eating foods containing raw eggs. Use pasteurized eggs or egg products in food containing uncooked eggs.
- Use water from a safe water supply for drinking and for food preparation.
- Obtain shellfish from approved sources such as grocery stores (rather than from an unlicensed roadside vendor) (30).

V. SPECIAL INFORMATION FOR CONSUMERS MORE SUSCEPTIBLE TO FOODBORNE DISEASE

Some people are more susceptible to foodborne disease because of suppressed immune function. These include the very young, elderly, pregnant, people who have diseases such as AIDS, cancer or diabetes, and those whose immune

system is suppressed by other medical conditions. These groups represent 20% of the American population (23).

All food safety guidelines related to hand washing, cooking meat, seafood, poultry and eggs properly, avoiding cross-contamination and keeping foods at safe temperatures are important for control of food illnesses of all people, including those in high-risk groups. In addition to following the guidelines listed above, additional precautions are suggested for the high-risk groups. Pregnant women should be encouraged to avoid ready-to-eat foods known to be sources of *L. monocytogenes*, avoid contact with cat feces, and cook or reheat foods well before eating. Elderly and immune compromised persons should avoid raw or undercooked seafood, raw sprouts, foods that contain raw eggs, and food known to be sources of *L. monocytogenes.* For young children, use of pasteurized milk and fruit juices, avoiding raw sprouts, and washing fruits and vegetables before eating are important food safety behaviors (23).

VI. FOODS THAT CONSUMERS SHOULD HANDLE WITH EXTRA CARE

While all foods should be handled by following the basic rules (practice good personal hygiene, cook foods adequately, avoid cross-contamination, and keep foods at safe temperatures), some foods require extra care or special handling.

- **Eggs and foods containing eggs.** Fresh eggs must be handled carefully, for even eggs with clean, uncracked shells may contain *Salmonella* bacteria. Eggs should be purchased only from a refrigerated case and checked for cleanliness and intact shells. Eggs should be refrigerated promptly in their original carton and not in the rack that may be on the refrigerator door, because the temperature on the door fluctuates widely. Eggs and dishes containing eggs need to be cooked thoroughly, with both the yolk and white cooked to firmness or to 160°F. Dishes containing eggs should be cooked to 160°F as verified by thermometer. Dishes with raw or lightly cooked eggs require the use of pasteurized eggs or a modified recipe (available from the American Egg Board http://aeb.org). Cooked eggs and egg dishes should be reheated or refrigerated within 2–3 hours of their preparation, including hard-cooked eggs used for Easter egg hunts.
- **Thawing meat/fish/poultry.** Recommended thawing of frozen meat, poultry and fish includes thawing in the refrigerator (generally requires 24 hours for each five pounds of product) or submerging the frozen package in a watertight plastic bag under cold water. Since cold water thawing requires about 30 minutes per pound of frozen meat, it should not be used for very large items (10 hours would be needed to thaw a 20 lb. turkey by this method). The risk of bacterial contamination could be high if the water is not changed regularly during thawing (53).

  If meat is microwave-defrosted, it must be cooked promptly after thawing because some areas of the food become warm during microwaving. Raw meat can be thawed at room temperature for short periods of time as long as it is refrigerated or cooked while it is still cold and contains ice crystals. Cooking from the frozen state requires food to be cooked about 1½ times longer than for thawed products of the same size and shape.
- **Ground meats.** Ground meat must be handled with special care because bacteria on the surface are spread throughout the meat during grinding. Ground meats should always be cooked to 160°F, and verified with a thermometer inserted sideways into meat that has been lifted out of the pan on a spatula. Turning ground meat patties frequently assures even distribution (54).
- **Hot dogs and lunch meats.** These products are perishable and should be kept in the refrigerator. Hot dogs should be cooked before eating and luncheon meats should be heated to steaming if they are to be eaten by a person at high risk for listeriosis.
- **Stuffed meat or poultry.** When stuffing meats and poultry, stuffing should be not be tightly packed, to allow heat to penetrate throughout quickly, and these items should be stuffed just before cooking. Stuffing needs to reach a temperature of at least 165°F as verified with a thermometer. Whole, stuffed poultry should not be cooked in a microwave oven because the stuffing may not get hot enough to kill harmful bacteria.
- **Using a microwave oven to cook meat, poultry, or fish.** Cooking in a microwave oven can leave cold spots that present a food safety problem. Using meat that is the same temperature throughout aids in even cooking; if defrosted in the microwave, a rest period of 20 to 30 minutes between defrosting and cooking allows the temperature to equalize. Covering the meat item, cooking at medium power (50%), and rotating food frequently all aid in more even cooking and the elimination of cold spots. A food thermometer should be used to ensure food is cooked to the proper temperature. Letting food

stand covered for 5–10 minutes after cooking also equalizes internal temperatures.

- **Fresh produce.** The U.S. Food and Drug Administration (FDA) has offered specific consumer advice about the purchase and handling of fresh produce: buy produce that is not bruised or damaged; fresh cut produce should be refrigerated or surrounded by ice at the store; perishable produce should be promptly refrigerated; wash produce just before eating using cool tap water and reasonable agitation, and removing bruised or damaged areas (prewashed packaged vegetables labeled "Ready To Eat" do not need to be washed at home; firm produce such as melons and cucumbers should be scrubbed with a clean produce brush) (55). Some outbreaks of *E. coli* O157:H7 have been caused by apple cider; homemade apple cider should be heated to at least 160°F.
- **Honey.** Infants are susceptible to a very rare form of botulism that is caused by ingesting *Clostridium botulinum* bacteria. An infection results when these bacteria grow in the infant's intestinal tract. (In contrast, botulism in adults is caused by eating food contaminated with the toxin that the bacteria produce.) Babies with infant botulism are constipated and have difficulty holding up their heads and sucking. Because honey can contain *C. botulinum*, the U.S. Centers for Disease Control and Prevention state that children who are less than a year old should not be exposed to honey (56). (This includes foods made with honey such as honey graham crackers, muffins and jellies.) Pasteurized honey is not a solution, because the heat treatment that produces the desired crystallization is too mild to kill the spores. While only 5 percent of the nation's infant botulism cases have been traced to honey, protecting children from honey before their first birthdays is one step that parents and caregivers can actively take.
- **Home canned foods.** Low-acid foods (meat, fish, poultry, and vegetables) must be canned in a pressure canner. Boiling-water canners, ovens, steamers without pressure and open kettles do not get the food hot enough to kill the spores of *Clostridium botulinum*, so it is not safe to can vegetables, fish, meat or poultry — or mixtures containing these foods — by any method other than using a pressure canner. Tomatoes with added acid in the form of lemon juice or citric acid, pickled vegetables, and fruits can be canned safely in a boiling water canner because they are more acidic. However, overripe tomatoes or tomatoes from frost-killed vines should not be used for canning since they lose acidity as they mature or if they are frozen. Many Cooperative Extension offices have information and bulletins, and some conduct classes, with specific directions and scientific time and temperature recommendations for home canning and other food preservation methods. Research based information for home canning, the *USDA Complete Guide to Home Canning*, is available online at the National Center for Home Food Preservation (57).

VII. MOTIVATING CONSUMERS TO ADOPT SAFE FOOD HANDLING PROCEDURES

Many consumers are aware that they can reduce their risks of foodborne illness by their own actions regarding hand washing, adequate cooking, avoiding cross-contamination, refrigeration of foods and avoiding risky foods and water. However, they may not have sufficient motivation and knowledge plus the proper equipment to successfully implement all of the recommended behaviors. Some examples of barriers to adopting recommended behaviors and ways to reduce the barriers follow:

- Strong motivation is required to modify habitual behaviors. Change may occur when people participate in programs that make them aware of their actions when they wash their hands or when preparing food in a way that results in cross contamination.
- Knowledge regarding how to implement recommended behaviors may be lacking. To reduce this barrier, food safety education should be more common, including in school curricula, and emphasized in television cooking shows.
- Current recommendations may be different from what the person learned previously. For example, the current recommendation is to cook ground beef to 160°F. Previously, consumers were advised to cook ground beef until it appeared to be cooked and had no traces of pink. Extensive education about the new recommendation is necessary to replace the previous behavior.
- Equipment needed to implement the recommended behavior may not be readily available. For example, if a consumer decides to cook ground beef patties to 160°, they will need an instant-read thermometer, preferably one that has recommended endpoint internal temperatures printed on the sheath. Fortunately, these

types of thermometers are now much more commonly available to consumers.

Educational efforts are needed to increase food handlers' knowledge of correct procedures and motivation to perform recommended behaviors. Equipment should be designed to reduce opportunities to make food handling mistakes. Improvement in behaviors is likely to result in reduction in incidence of foodborne illnesses.

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76 Migratory Chemicals from Food Containers and Preparation Utensils

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CONTENTS

I. INTRODUCTION

Plastics have revolutionized the packaging and transportation of foods, allowing foods to be packaged in light weight materials while offering excellent physical protection against mechanical damage, water vapour transmission and light oxidation. Approximately 70 to 80% of food and ingredients are packaged in polymeric materials (1). However, the use of plastic organic polymers containing functional additives such as plasticizing agents, antioxidants, U.V. light and heat stabilizers or lubricants in the manufacture, packaging and serving of common foodstuffs such as meat, cheese, margarine, bacon, vegetables and beverages may result in these polymer constituents potentially becoming indirect food additives (1, 2). This phenomenon occurs due to the potential for contact and migration or transfer of polymer components from containers or packaging films into foods during processing, packaging and storage. Plastics (e.g. polyethylene, polypropylene, polystyrene etc.) are also extremely prevalent as food service utensils and containers both inside and outside the home (3, 4). Polymer additives can fall into two functional categories: those that modify the physical properties of polymers, namely catalysts, plasticizers, lubricants and colouring agents etc. and those that have a stabilizing or protective effect on polymer degradation (antiaging additives), such as antioxidants, U.V. light and heat protection agents (5, 6). Plasticizers are synthetic organic additives characterized by low molecular weight with low melting points or low volatility with high boiling

points and high miscibility with the parent polymeric matrix (e.g. phthalic acid esters (PAEs)). They are used to improve the plasticity (e.g. flow, workability and flexibility) of polymers such as the vinyl derivatives (e.g. polyvinyl chloride (PVC), polyvinyl acetate (PVA), polyvinyl alcohol (PVAL)) and the polyolefins (e.g. polyethylene (PE), polypropylene (PP)). In this way, plastic formulations can contain up to 60% of plasticizer materials to transform an otherwise rigid polymer into a plastic with improved physical and mechanical properties such as flexibility, durability, and impact resistance (1, 2, 7). However, due to the relatively low molecular weight of residual polymeric monomers and certain additives (i.e. plasticizers and antioxidants), the migration to and absorption of these compounds by both solid and liquid foods is known to occur (8–10). This may result in off-odours or -flavours being imparted to the packaged food (11, 12) resulting in the loss of product and sales. As well, the toxicology of these substances has been the subject of intense investigation in animal models (13–17) and human subjects (18–21). These issues gain relevance due to the prevalence of microwavable convenience foods which are heated in the packaging containers, as well as the increasing interest in irradiating packaged foods for increased microbiological safety against foodborne illnesses (22–24). Gamma-irradiation of packaged foods is known to result in alterations such as crosslinking and scission of polymeric materials and component additives which may be, in turn, adsorbed by the packaged food contained therein. This chapter will discuss these issues as well as examining potential alternatives to the traditional polymeric packaging materials discussed in the recent literature.

II. MIGRATION THEORY

The investigation and mathematical modelling of the diffusion, migration and sorption of materials from food packaging materials into foods or in contrast, from foods into the packaging polymer itself, have been the subject of several recent reviews and reference book compilations (2, 5, 6, 25, 26). Generally, the movement of molecules within a packaging system reflects the motion of mass or energy from one location to another. Mass transport by diffusion will be influenced by the behaviour of the polymer itself (i.e. glassy versus rubbery state) as well as the solubility of the diffusant chemical in the polymeric matrix and the partition coefficient of the diffusing chemical between the two contact media (25). Polymers are classified as either rubbery or glassy depending on whether the temperature of the polymer is above or below the glass transition temperature (T_g) for that polymer, respectively. Above the T_g, polymer chains will be able to move more freely in space versus below the T_g, where chain mobility will cease and the polymer becomes glassy and hard. Rubbery polymers at $T > T_g$, which include polyvinylchloride (PVC, T_g = 80–100°C), polystyrene (PS, T_g = 80–100°C), polyethylene terephthalate (PET, T_g = 67–81°C) and polytetrafluoroethylene (PTFE, T_g = 115–125°C) are able to quickly establish a new equilibrium when a diffusant chemical is absorbed and transported through the rubbery matrix of the polymer (25). Conversely, glassy polymers at $T < T_g$, which comprise the various forms of the olefins PE ($T_g = -30 \pm 15$°C) and PP ($T_g = -17 \pm 5$°C) are unable to rapidly establish a homogeneous environment upon absorption of a diffusant.

In rubbery polymers, diffusion of chemicals obey *Fick's Laws of Diffusion* and can be explained on the basis of the adhesion theory, which refers to the physical interaction of two materials.

Fick's First Law of Diffusion

$$J = -D\frac{\partial C_A}{\partial X}$$

where
J = flux (i.e. moles/sec cm^2)
D = diffusion coefficient (i.e. cm^2/sec)
C = concentration of substance A (i.e. moles/cm^3)
X = distance (i.e. cm)

Fick's Second Law of Diffusion

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial^2 x} \quad 0 < x < l$$

where
C = concentration of substance (liquid or plasticizer)
D = diffusivity in the membrane
x = space coordinate
l = length of the geometric shape in question
t = time

Fick's first law of diffusion describes steady-state diffusion wherein mass transfer from a region of high concentration to a region of low concentration occurs and eventually approaches equilibrium (26, 27). Whereas, unsteady-state diffusion is described by *Fick's second law of diffusion* wherein for a constant diffusivity, the rate of change in the concentration of the substance of interest with time is proportional to the rate at which the concentration gradient changes with distance in a given diffusion direction.

In glassy polymers, on the other hand, diffusion of compounds is more complex, in that diffusion can be:

a. Class I or Fickian, where the rate of diffusion is much less than the relaxation of the diffusant-polymer system; or
b. Class II, where the rate of diffusion is very rapid compared to the relaxation processes of the polymer system; or
c. Anomalous diffusion, where the rates of diffusion and relaxation of the polymer system are comparable to each other (25).

This non-ideal behaviour can be linked to swelling or shrinkage of the food in question, or the packaging polymer. Food components, particularly water, may influence the swelling and plasticity/mobility of the polymer chains leading to non-ideal diffusion (27).

There are two mechanisms by which adhesion plays an important role in the migration of packaging material components. The first is through the adhesion and loss of food constituents (i.e. volatiles) to the packaging material, resulting in a reduction of food sensory qualities and thus, acceptability. The second is through the adhesion of the packaging material to the foodstuff, which may introduce toxicants, unwanted flavours or odour-active substances, again resulting in the potential loss of food quality as well as safety (5, 28). The adhesion phenomena can be explained according to the conditions listed in Table 76.1 (5, 28, 29). The term migration typically refers to the movement of chemical compounds from the packaging material into the food or beverage contained therein. This migration activity would then include the movement of gases, low molecular weight compounds and water vapour from the package into the food (5). This phenomenon is distinct from sorption, which involves the movement and uptake of food constituents, such as flavour and odour components, into packaging materials (28). These processes are dynamic and can occur simultaneously by diffusion (30). At steady state, the flux can be described as a function of the concentration difference of the migrating chemical across the distance of diffusion when referring to *Fick's first law of diffusion* above (31). In general, migration and sorption are similar processes which can ultimately affect food quality. Both phenomena are influenced by the chemical and physical properties of the substrate and the receiving medium.

III. MIGRATION OF PACKAGING MATERIALS

Of primary concern to polymer and food scientists as well as the consumer are the migration and sorption processes which may impact on the organoleptic quality, nutrition and safety of packaged foods. Migration of plastic packaging constituents may result in the introduction of undesirable and/or unsafe substances into the food, thus rendering the food unsuitable for consumption. Similarly, sorption processes may result in nutrient or flavour and odour loss from the packaged food into the packaging material, giving rise to a poor quality product. Thus, sorption phenomena involve packaged foods wherein concern for product organoleptic qualities and nutrient retention are the primary focus. Packaging materials can adsorb flavour components from food systems rendering the food product unacceptable over time (i.e. during the distribution and delivery process) due to "flavour scalping" as in the case of terpenoids in juices (32). In addition, the use of heat treatment or other adhesive phenomena will contribute to weight loss or reduced volume and altered nutrient retention in packaged foods (33).

Migration of packaging materials into food is a function of the chemical composition and concentration of the packaging materials and the solubility of the migrating chemical in the polymer, or its partition coefficient between the polymer and the contact surface of the food. The fat content and more specifically, the fat-releasing properties of contact foods can greatly influence migration of low molecular weight compounds (34). For example, migration of the plasticizer stearyl 3-(3,5-di-*tert*-butyl-4 hydroxyphenyl) propionate into mayonnaise (an oil-in-water emulsion with moderate fat-releasing properties) resulted in a mean concentration of 69 μg/dm^2 compared to 792 μg/dm^2 into a

TABLE 76.1
General Tenets to Adhesion Theory Involving Migration of Food Packaging Materials[1]

Type of Adhesion Interaction	Molecular Interaction	Description of Interaction
Mechanical interlocking	Macro- and microscale	Rugosity of surface increases locking of film components.
Wetting	Liquid dispersion and surface tension	Electrodynamic intermolecular forces create dispersive and attractive forces at surfaces.
Electrostatic	Ionized substrates form electrical double layer	Double layer is positively charged while outer interface is negatively charged. Adhesive forces act by attraction through electrical double layer in films.
Chemical	Covalent bonding between surfaces and polymer components	Contact time and processing dependent.
Diffusion	Polymer and additive migration	Contact time, temperature, molecular weight, polymer type, viscosity and concentration dependent.

[1] References: 5, 28, 29.

test fat (34). Migration efficiency will also be affected by the storage or thermal processing temperature used (i.e. heating the food inside the packaging material, e.g. microwave cooking), as well as the amount of time during which the food is in contact with the package material (3). Thus, the migration of chemicals from packaging materials into foods can be mathematically modelled and predicted (4, 35, 36). Any toxicological hazard associated with the migration of food packaging materials into foods would therefore be a function of the amount of migratory material originating from the polymer which enters the food and the intrinsic toxicity of this material or its metabolites (16, 17, 37).

It is important to recognize that the conditions involved with the use of plastic packaging of foods will not be isothermal throughout the life of the polymer. Moreover, food systems are, by their nature, very complex with aqueous and hydrophobic components often co-existing in one food matrix. In order to study the extent of the migration of polymer components into foods, laboratories use established food simulants (i.e. distilled water, 3% NaCl, 3% $NaHCO_3$, 3% acetic acid, 3% lactic acid, 20% sucrose, 15–50% ethanol and olive oil or iso-octane) as model systems to estimate migration of chemicals into foodstuffs (31, 34–36). Frequently, elevated temperatures are used to simulate accelerated storage conditions and the worst-case scenario. Standard test conditions accepted by regulatory bodies in the United States (US Food, Drug and Cosmetic Act enforced by the Food and Drug Administration (FDA)), Canada (Division 23 of the Food and Drugs Act and Regulations), United Kingdom (Ministry of Fisheries and Foods (MAFF)), and European Union (EU Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE)) include 10 days at 40°C, 2 hr at 70°C and 1 hr at 100°C (10, 34, 38, 39). These data, used in conjunction with toxicological testing, have resulted in the establishment of regulatory allowances for food packaging components migrating into foodstuffs set at a dietary concentration of 0.5 μg/kg in the US by the FDA (36, 40, 41) and maximum total migration limits to be set at 10 mg/dm^2, or less than 60 mg/kg food by the EU (7, 42). Packaging design and construction which incorporate the concept of functional barriers (i.e. laminate films) to limit the migration of noncarcinogenic chemical components to food to a level less than the threshold of 0.5 μg/kg are regarded acceptable in the US (36). Functional barriers represent a concept whereby the specific package design and construction limit the migration of packaging materials into food in amounts regarded below this threshold level (36, 43). This is especially pertinent given the trend to use recycled materials in packaging. Quite often, a contaminated material will be co-extruded with virgin materials acting as a functional barrier against taint migration from the inner layer of recycled polymer material (36). Tripartite polyethylene terephthalate (PET) films made from core material consisting of PET contaminated with toluene and chlorobenzene sandwiched between two virgin PET layers demonstrated significant migration of the contaminants into water, 3% acetic acid and iso-octane. Migration of contaminants into 3% acetic acid (50°C over 10–131 days) from the contaminated PET into 3% acetic acid ranged from 4.1 to 33.3 $\mu g/dm^2$ and <0.1–20.7 $\mu g/dm^2$ without and with barrier layers respectively for toluene and <0.1–17.0 $\mu g/dm^2$ and <0.1–12.3 $\mu g/dm^2$ without and with barrier layers for chlorobenzene (36). Thus, the effects of diffusion of core layer contaminants in plastic films must be considered when using coextruded recycled materials.

IV. CONCERNS WITH MIGRATION OF PACKAGING MATERIALS

The classes of packaging material migrants that fall into the category of food safety concerns include the amine precursors and nitrosamines (44), plasticizers (1, 45) and polymer monomers such as vinyl chloride and styrene (17, 20). Amine precursors and nitrosamine formation have been reported from the interaction of the rubber netting used to package nitrite containing cured meats and from other foods contained in paperboard containers (5, 44, 46). The focus of the following discussion will concentrate on the polyolefin and vinyl derivative packaging materials and additives. The most commonly used plasticizers to improve the working properties of PVC, for example, are the esters of dibasic or tribasic organic acids known as phthalates which comprise as much as 60% of the market (47). Phthalic acid esters are the subject of concern due to the structural similarity of these compounds to the known teratogen thalidomide (phthalidomide) (48). Indeed, di(2-ethylhexyl) phthalate (DEHP) has been demonstrated to have fetotoxic, embryolethal and teratogenic effects in rats at a dose of 1 g/kg/day (14). Finally, vinyl chloride polymers (polyvinylchloride; PVC) are widely used in many types of packaging materials and have also been shown to migrate into foods (8, 48). PVC contains allylic chlorine atoms, which can be released from the polymer upon exposure to light or heat treatment of the packaged food. The primary concern with PVC and the monomer vinyl chloride used in the manufacture of this plastic, is due to animal and human epidemiological data from workers exposed to vinyl chloride indicating that exposure to vinyl chloride is strongly linked to hepatic carcinoma (17, 19, 20, 49).

V. SOURCES OF FOOD PACKAGING MIGRANT CHEMICALS

A. Polymers

Numerous polymers are used in food packaging to store and protect food from deterioration due to chemical or physical damage due to radiant or heat energy, while at

TABLE 76.2
Common Polymers and Migration Chemicals in Food Packaging Materials[1]

Polymer	Example of Use	Migration Chemicals
Polyethylene (PE)	Dairy product and other food containers, water pipes, food wrap films	Acetaldehyde, allyl alcohol, acrolein.
Polyvinylchloride (PVC)	Beverage and water bottles, food wrap films, water pipes	Allylic chlorine, vinyl chloride monomer, plasticizers and stabilizers.
Polypropylene (PP)	Bread packaging, bottles, frozen foods, microwavable foods	Isopropyl alcohol, methyl alcohol, formaldehyde, antioxidants and stabilizers.
Polystyrene (PS)	Microwave meals, cookie bags and syrup containers	Styrene monomers, dimers, trimers, polystyrene.
Polyethylene Terephthalate (PET)	Beverage and water bottles, box liners, boil-in-bag pouches, microwave food trays	Acetaldehyde, cyclic oligomers, ethylene glycol.
Paper and paperboard	Milk and juice cartons	Polychlorinated biphenyls (PCBs), alkyl and aryl aldehydes.
Melamine-Formaldehyde	Tableware	Melamine and formaldehyde residues.
Vulcanized rubber	Rubber nettings for packaged meats, food industry equipment	Polycyclic aromatic hydrocarbons (PAHs) including benzo[a]pyrene, nitrosamines, alkanes, alkenes, acids, antioxidants, plasticizers and sterols.

[1] References: 1, 2, 4, 5, 8, 11, 12, 35, 46, 50.

the same time enabling the consumer to view and evaluate the product. Examples of common polymers used in food packaging materials and migratory components of concern are presented in Table 76.2 and chemical formulae of plastic resin monomers and polymers provided in Tables 76.3 and 76.4, respectively.

B. Vinyl Derivatives

Polyvinyl chloride (PVC) is second only to PE in worldwide production, estimated at approximately 22×10^6 metric tons (2). PVC film is manufactured by the low pressure free radical polymerization of vinyl chloride (Table 76.3) at temperatures ranging from 50–160°C (50). PVC film is a versatile thin cling film (<25 μm or 1 mil thick) which is transparent and has high oxygen permeability but is naturally brittle and therefore requires plasticizing agents, such as the phthalic acid esters (Table 76.5), to be incorporated into the polymer (50). Plasticizer content of PVC polymers can vary from 3.0 to as high as 80% (1). A copolymer of vinyl chloride and vinylidene chloride (Table 76.3), more commonly known by its trade name, Saran™, is a high quality cling-film wrap used commercially and in the home. As plasticizers are not polymerized directly into the polymer matrix, and have high mobility associated with a low molecular weight, migration from the PVC polymer into the food product can occur. Migration is influenced by the fat content, pH and the alcohol content of the foodstuff (48). Vinyl chloride monomer (VCM) is a residue of PVC film manufacture which is removed following the polymerization reaction. However, VC has received considerable attention due to its link to angiosarcoma of the liver (ASL) (17, 19, 20). An Italian study indicated that significant quantities of VC (13 to 83 ppt) could be detected in PVC-bottled drinking water (8). Moreover, the concentration of VC in bottled water was observed to increase linearly with storage time

TABLE 76.3
Monomers and Degradation Products from Food Packaging Polymers

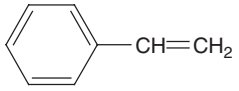
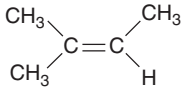
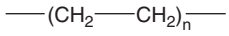
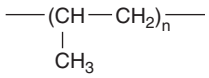
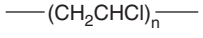
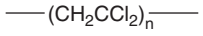
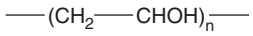
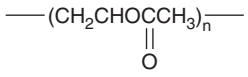
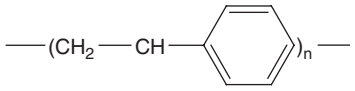
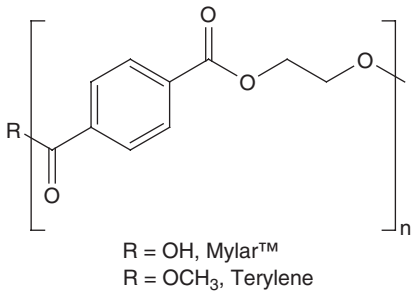
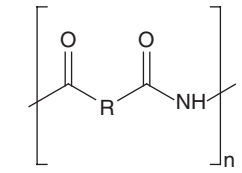
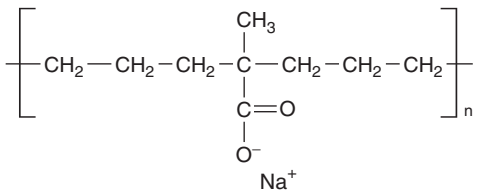
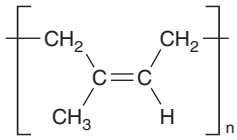
Compound Name	Chemical Structure
Ethylene	$CH_2=CH_2$
Propylene	$H_2C=CHCH_3$
Ethyl acetate	$CH_3COOCH_2CH_3$
Vinyl acetate	$CH_3COOCH=CH_2$
Vinyl chloride	$CH_2=CHCl$
Vinylidene chloride	$CH_2=CHCl$
Styrene	
cis-1,4-isoprene (rubber)	

TABLE 76.4
Common Food Packaging Polymer Materials

Compound Class	Name	Abbreviation	Chemical Structure
Polyolefins	Polyethylene	PE	
	Polypropylene	PP	
Vinyl derivatives	Polyvinylchloride	PVC	
	Polyvinylidene chloride	PVDC	
	Polyvinylalcohol	PVAL	
	Polyvinylacetate	PVA	
	Polystyrene	PS	
Polyesters	Polyethylene terephthalate	PET	
Polyfluorocarbons	Polytetrafluoroethylene	PTFE Teflon™	
Polyamides	Polyamide	PA	
Ionomers	Poly(ethylene-co-methacrylic acid)	Surlyn™	
Rubber	Poly (cis-1,4-isoprene)		

(approx. rate 1 ng/l/day), resulting in the recommendation that storage dates be placed on the labels of PVC-bottled water (8). While migration of VC into vinegar (9.4 mg/L), cooking oil (14.8 mg/kg), butter and margarine (approx. 50 µg/kg) have been reported (48), the greatest risk of health effects from VC is from occupational exposure in manufacturing plants (18, 19). Workers exposed to VC from manufacturing plants have been reported to suffer from primary nonangiosarcoma liver tumours as well as ASL (18, 19, 49). Workplace exposure to VC has also been

reported to result in the dissolution of the distal phalanges in the hands of workers as well as disturbances in the circulation of the extremities (49). Studies with cell culture and rodent animal models have demonstrated that VC is mutagenic and can induce liver, lung and brain carcinogenesis (49). Moreover, mutations to the p53 tumour suppressor gene have been observed in ASL patients in association with serum anti-p53 antibodies (20). Anti-p53 antibodies could also be detected in serum from individuals who were occupationally exposed to VC, leading these workers to suggest that serum anti-p53 antibodies may be a biomarker of individuals at risk for development of ASL.

Another vinyl derivative which is used commonly in food service utensils and dishes is the monomer styrene (Table 76.3) in the manufacture of polystyrene (PS; Table 76.4) foam articles (2, 3, 4, 9, 35, 51). Styrene is classified as a mutagen and is also known to cause irritation of the nose and eyes, drowsiness, weakness and an unsteady gait on overexposure (52, 53). Routes of exposure to styrene monomer include occupational exposure to styrene vapour during the manufacturing process and as a result of migration into food contact articles (53). Concern about styrene exposure is related to its high blood/gas phase solubility ratios whereby styrene easily enters the circulation from the lungs upon inhalation. General purpose and high impact PS are used in numerous food packaging and preparation containers including thermoset cookware, plates, cups, bowls, egg cartons, meat trays and hinged take-out containers. In most cases, foods would only be in contact with the packaging material for a short period of time at relatively mild temperatures (approx. 55°C) in a food service situation or for a longer period at refrigeration temperatures (approx. 4°C) (4). Migration of residual styrene monomer from thermoformed PS foam food contact articles into Crisco™ brand cooking oil has been reported to be proportional to the square root of the time of exposure (4). The diffusion coefficients of styrene migration into cooking oil ranged from 4.5×10^{-11} cm^2/s at 21°C to 3.4×10^{-9} cm^2/s at 65.6°C (4). Migration of styrene into 8% ethanol from egg cartons incubated over 31 days at 4°C was negligible (below the detection limit of 0.01 μg/cm^2) (4). Daily styrene exposure from the diet and inhalation has been estimated at approx. 18.2 to 55.2 μg/person for an annual exposure ranging from 6.7 to 20.2 mg/person according to a recent German survey (54). Others have evaluated the concentration and migration of styrene-7,8-oxide in food contact articles (35). Styrene-7,8-oxide is the oxidation product of styrene and is thought to be formed during the peroxide-initiated polymerization of styrene resin at 200°C. Moreover, styrene is metabolized by hepatic NADPH-cytochrome P_{450}-dependent monooxygenases to epoxides, namely its 7,8 epoxide, which can bind to cellular macromolecules (i.e. proteins, nucleic acids), resulting in cytotoxicity, mutagenicity and ultimately carcinogenicity (53). Thus, it is this styrene derivative which has been hypothesized to be responsible for the toxicity of styrene including renal, hepatic damage as well as pulmonary and cardiac abnormalities (35, 55). Finding the oxide in PS packaging materials but not in the base resin pellets indicates that the epoxides are not formed during the polymerization process but rather are formed during injection moulding or thermoforming of the plastic. However, the oxide decomposed rapidly in aqueous media at 40 and 100°C potentially making exposure levels in foods less of a risk (35).

C. Polyolefins

Polyethylene (PE) is the most wide-spread food packaging material today. Annual world production of PE polymers was 40×10^6 metric tons in the 1990's (2). This hydrocarbon polymer (Table 76.4) can be manufactured with varying amounts of branching within the linear backbone with high-density PE (HDPE) being the least branched and low-density PE (LDPE) containing the most branching (2). The ratio of LDPE to HDPE and linear LDPE (LLDPE) polymers produced was 40:40:20 (LDPE:HDPE:LLDPE) (2). The structural differences between the various forms of PE are associated with the differing uses of these polymers: HDPE has great thermal stability and as such, is used not only for films but also rigid food containers (i.e. yogurt pots) while LDPE is used for bags and cling-films due to its excellent flexibility. The related polymer, polypropylene (PP) has similar characteristics and uses in food packaging as PE. Production of PP polymers is ranked third behind PE and PVC with an annual worldwide production of 21×10^6 metric tons (2). Conditions which favour migration of chemicals from polyolefin packaging materials such as PE include elevated storage temperatures and exposure to oxygen (11, 12). The resultant reaction products (Table 76.2) can migrate into foods and cause wax-like odours that contribute to easily detected off-flavours. Vitamin E has been shown to reduce the migration of off-odour, aldehydes and ketones and hexadec-1-ene into water stored in HDPE bottles, thus reducing the development of off-flavours in stored product (11). Phenolic antioxidants were also demonstrated to reduce the release of off-odours and -tastes from HDPE bottles associated with aldehydes and ketones (low odour thresholds) although a total of 47 volatile components released from bottles could be identified by gas chromatography combined with mass spectrometry (GC/MS) (12).

D. Polyesters

The polyester polyethylene terephthalate (PET) is synthesized by the condensation of ethylene glycol and terephthalic acid and is more commonly known by its trade name Mylar™ in North America (Table 76.4). PET is often used in cola type beverage and water bottles as well as in laminated films to provide excellent strength and abrasion resistance (2). PET and polybutylene terephthalate (PBT)

are noted for the impact and wear resistance of these polymers. PET is also known to be a good barrier to gases, aroma volatiles and lipids, but less so against water vapour transmission (2). Thus, PET is used to bottle edible oils, peanut butter, dressings as well as in cereal box liners (1). In the frozen prepared-food industry, paperboard dishes are laminated with PET or PBT for use in ovens between 200–220°C. Microwavable single-serving dishes are manufactured from heat-formed PET films. Two PET monomers, mono- and di-ethylene glycol (MEG, DEG) have designated specific migration limits (SML) between 15 and 30 mg/kg food. MEG is an hygroscopic poison, more well known as antifreeze, which causes vomiting, drowsiness, coma, respiratory failure, convulsions, renal damage and ultimately death with a lethal dose of approx. 1.4 mL/kg b.wt. or 100 mL (51). DEG is also used in antifreeze solutions and has similar symptoms as MEG upon ingestion. Similar to PE and PVC, PET is also sensitive to thermal and oxidative degradation resulting in the release of acetaldehyde as the principle volatile migratory compound. Acetaldehyde concentrations in mineral water and lemonade have been reported to range between 7.5 and 11 mg/L (1). Acetaldehyde has also been associated with causing colour changes in PET as the packaging material ages. Cyclic oligomers of PET have been reported in various microwavable foods such as french fries, fish sticks, waffles and pizza at levels between 0.012 up to 7.0 g/kg (1).

E. Other Packaging Materials

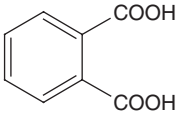
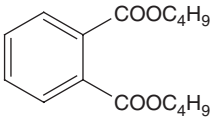
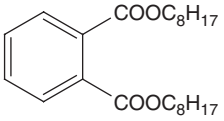
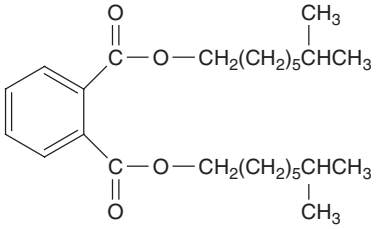
Paper and cardboard are also commonly used as packaging materials. Polychlorinated biphenyls (PCBs) have been reported in foods packaged in paperboard made from recycled paper as a consequence of the recycling process or from the printing ink used in the packaging (48). In Glassine packages, a pine oil hydrogenation product is used in cereal box liners which can contribute to a pine flavour upon migration of chemical constituents (5).

VI. ADDITIVES USED IN POLYMER FORMULATIONS

A. Functionality in Polymers

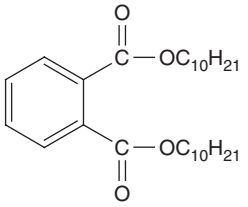
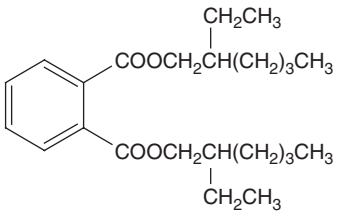
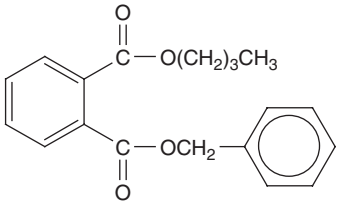
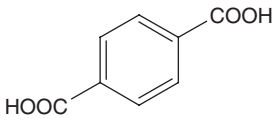
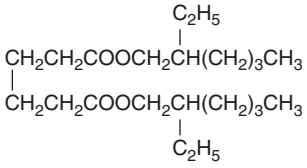
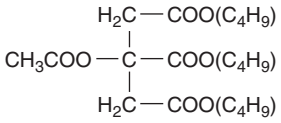
There are in excess of 450 different plasticizers used in polymer formulations, of which approximately 100 are available for commercial use (5). Some common plasticizing agents, in use to decrease the rigidity and brittleness of polyolefin, vinyl derivative and polyester polymers such as PE, PVC, PS and PET, are listed along with chemical formulae in Table 76.5. Important factors in choosing an appropriate plasticizer include low volatility, miscibility with the plastic polymer and avoidance of undesirable properties such as a predisposition to induce colour alterations of the packaging material as well as adverse organoleptic or toxicological properties. Plasticizers are used primarily in

TABLE 76.5
Plasticizer Additives Used in Polymers

Classification	Compound Name	Abbreviation	Chemical Structure
Phthalic acid esters	Phthalic acid		
	Dibutyl phthalate	DBP	
	Dioctyl phthalate	DOP	
	Diisononyl phthalate	DINP	

(*Continued*)

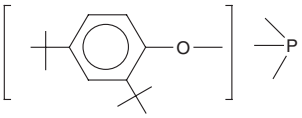
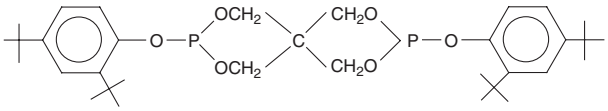
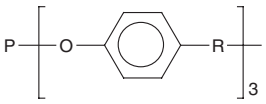
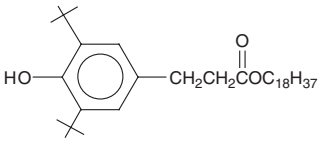
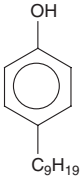
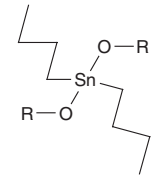
TABLE 76.5
(*Continued*)

Classification	Compound Name	Abbreviation	Chemical Structure
	Diisodecyl phthalate	DIDP	
	Di (2-ethylhexyl)phthalate	DEHP	
	Benzyl butyl phthalate	BBP	
Terephthalic acid esters	Terephthalic acid		
	Di (2-ethylhexyl) terephthalate	DEHT	
Adipic acid esters	Adipic acid		$HOOC(CH_2)_4COOH$
	Dioctyl adipate	DOA	$(CH_2)_4(COOC_8H_{17})_2$
	Di (2-ethylhexyl) adipate	DEHA	
Citrates	Acetyl tributyl citrate	ATBC	
Oils and fatty acids	Epoxidized soybean oil	ESBO	
	Epoxidized linseed (flax) oil	ELO	
	N-butyl stearate		

polymers which are characterized by a glassy or crystalline structure at room temperature (6). The rigid polymer interacts with plasticizer molecules to reduce the brittleness (brittleness temperature, T_b) as well as the glass transition temperature (T_g) allowing an increase in the temperature range of viscoelasticity for the polymer (6). Other routes of exposure to plasticizers from packaging materials include sealants of glass jar lid closures, printing inks on polymer films as well as adhesives and can lacquers (56). Other additives used in polymer manufacture include a variety of antioxidant species, namely phosphites and phenols (Table 76.6) to protect against effects from metal contaminants, photoactive dyes and pigments which may be incorporated into the polymer matrix (57, 58). Polymers are oxidized primarily by two routes: a) long-term heat aging (thermal oxidation), or b) weathering

from U.V. light (photo-oxidation) (57). Polymers are protected from thermal oxidation effects through the incorporation of phenols such as Irganox 1076™ (Table 76.6). The efficiency of the phenol antioxidants can be improved by using these antioxidants in combination with phosphites (e.g. Irgafos 168™, TNPP) which facilitate the decomposition of hydroperoxides (1). Heat stabilizers are necessary in PVC manufacture due to the low thermal

TABLE 76.6
Stabilizers Used in Polymeric Materials

Classification	Compound Name	Abbreviation	Chemical Structure
Antioxidants			
Phosphites			
	Tris(2,4-di-*tert*.butylphenyl) phosphite	Irgafos 168™	
	Bis(2,4-di-*tert*.butylphenyl) pentaerythritol diphosphite	Ultranox 626™ Hostanox PAR24™	
	Tris(nonylphenyl) phosphite	TNPP	 R = C_9H_{19}
Phenols			
	Octadecyl β-(2,6-di-*tert*. butylphenol) propionate	Irganox 1076™	
	4-nonylphenol	NP	
U.V. Stabilizers			
Lead sulfates	Tribasic lead sulfate		$Pb_4O_3(SO_4)H_2O$
Heat and Light Stabilizers			
Organotin compounds	Di-*n*-butyltin compounds		 R = fatty acid chain or other carbon chains.
Lubricants			
Lead stearates	Dibasic lead stearate		$2PbO \cdot Pb(C_{17}H_{35}COO)_2$
	Tribasic lead stearate		$3PbO \cdot Pb(C_{17}H_{35}COO)_2$

stability of these polymers. Thermally aged PVC becomes discoloured due to the formation of conjugated double bonds associated with dehydrochlorination of the polymer during molding or extrusion processes. Heat stabilizers include barium, calcium or zinc fatty acid soaps and various organotin compounds (Table 76.6) (1, 57). Thermo- and light-stabilizers such as tribasic lead sulfate are often used in PVC in combination with organotin stabilizers (1). Lead stearates function as thermo-stabilizers and as a lubricant for PVC and vinyl chloride copolymers, again often in combination with organotin compounds (1).

B. Plasticizers

The commonly used phthalic acid esters (PAEs) are low molecular weight esters that consist of a cyclohexatriene ring (benzene dicarboxylic acid) core esterified to aliphatic substituents (Table 76.5). The PAEs are used in a variety of packaging materials such as PVC, PE, PS, PET, laminated aluminum-PE as well as printed fibreboard and cellophane. DEHP is the most common plasticizer incorporated into polymers with an annual worldwide production of approx. 2.7×10^6 metric tons (38). PAEs, such as DEHP and DOP, are characterized by an affinity for these polymers, low volatility, water resistance and good flexibility of products at low temperatures (6). On the other hand, DBP has a relatively high volatility and is used mainly in cellulose-based varnishes and in adhesives (6). Several other organic acids such as terephthalic acid (an isomer of phthalic acid), adipic and citric acids are the parent compounds of other plasticizers such as DOA, DEHA and ATBC. Other plasticizers include stearyl 3-(3,5-di-*tert*-butyl-4 hydroxyphenyl) propionate used in HDPE, LDPE and PP as well as *n*-butyl stearate which is used in high-impact polystyrene and PVC manufacture (6, 34). Polyepoxide plasticizers, such as ESBO, have a good affinity for the vinylic polymers and are frequently used to plasticize PVC glass jar lid gaskets (6, 56). Frequently, combinations of plasticizers will be used in formulations due to differences in plasticizing, solvation, lubrication and creep properties of finished polymer and plasticizer blends.

VII. FACTORS WHICH INFLUENCE THE MIGRATION KINETICS OF PLASTICIZERS

Over time, plastics containing low molecular weight plasticizers will lose plasticizer due to the evaporation of volatiles or migration of plasticizer during food contact (6). This will result in a change in the physical properties of the polymer (i.e. becoming hard and brittle) accompanied by a decrease in weight due to loss of the plasticizer. The migration of plasticizer from PVC films into foods is thought to follow Fickian behaviour occurring via a two-stage process involving first, the diffusion of plasticizer from the bulk phase within the polymer to the surface followed by the subsequent diffusion of the migrant compound from the polymer into the food. Transfer of the plasticizer to the surface of the film depends on the nature and properties of the film (i.e. functional barrier in a laminate) as well as barrier layer thickness (36). The migration of plasticizer into the food is facilitated by the relatively lower resistance to mass transfer within the food phase compared to that of the polymer. As a result of the differences in diffusion kinetics between the polymer and food source, both the composition (fat content and fat-releasing properties) and phase of the food, as well as the type and concentration of plasticizer used, are important variables in migration kinetics (34, 36). For example, transport of plasticizer by diffusion is expected within an immobile or solid food phase as opposed to convection kinetics if the food is in the liquid phase. In both of these situations, the migrant transportation rate will be reduced as the system approaches an equilibrium. The physico-chemical properties of the plasticizer and food simulant extraction medium will also influence the rate at which equilibrium is established (34, 36). For example, migration of stearyl 3-(3,5-di-*tert*-butyl-4 hydroxyphenyl) propionate from LDPE into distilled water after incubation at 20°C for 60 days resulted in transfer of 2.53 µg plasticizer/dm^2 whereas the transfer into olive oil was 720 µg plasticizer/dm^2 (36). These variables will also be influenced by duration and temperature of exposure of films in contact with foods (34, 36). Moreover, a lower concentration of plasticizer within a polymer will result in ultimately less migration into the foodstuff (47). Diffusion of plasticizers from a polymer will also occur more rapidly when the temperature is significantly above the T_g of the polymer material. However, if the external temperature is approximately equal to the T_g, diffusivity of the plasticizer can decrease by several fold with a change in incubation temperature in food simulant model studies. Bieber and coworkers (34) demonstrated this phenomenon with high impact PS containing *n*-butyl stearate incubated with a test fat resulting in transfer of plasticizer at a rate of 10.2 µg/dm^2 at −20°C compared to 41.7 µg/dm^2 at 20°C after 120 days incubation.

VIII. MIGRATION OF PLASTICIZER FROM POLYMER FILMS

Migration of plasticizers from PVC and PE films, PS as well as PVC-based toys and childcare articles have been studied investigating a variety of foods and food simulants modelling aqueous and fatty food systems as well as saliva extraction for the children's articles (10, 39, 42, 59, 60). Several international studies (Canadian and Danish) confirm that DEHA is present in food-contact films and as a migrant chemical in store-wrapped meat, poultry, fish, cheese and ready-to-eat prepared foods (10, 42, 59, 60).

In the Canadian study, fresh meat, poultry and fish contained up to 14 μg DEHA/g; a noteworthy observation was that smoked salmon filet contained 220 μg/g reflecting its relatively greater surface area exposed to the packaging material (10). Cheeses wrapped in polymer films contained between 2.1–310 μg DEHA/g and mean levels of 2.2 μg DEHP/g. Packaged ready-to-eat foods contained between 7.9–160 μg DEHA/g. Levels of DEHA in store-wrapped cheese were observed to reach a level of 45 mg/kg after only 2 hr at 5°C and then to increase to 150 mg/kg after 10 days of storage (59). Other workers have reported DEHA levels as high as 429 mg/kg in cheeses (60) which means that consumer intakes close to or even exceeding the EU recommended maximum tolerable daily intake (TDI) for DEHA (0.3 mg/kg body weight) are entirely possible given a 50 g portion size for cheese and a 60 kg adult. In fact, a majority of PVC cling film samples (77%) used at the various stages of food distribution (importers, wholesalers and retailers) demonstrated greater rates of overall migration (between 8.3 and 59.4 mg/dm^2) and specifically of DEHA migration into fatty foods as modelled by iso-octane (in place of olive oil) than permissible by the EU limit of overall migration of less than 10 mg/dm^2 (or $<$60 mg/kg food) as well as the specific migration limit (SML) of 3 mg DEHA/dm^2 (or $<$18 mg/kg food) (42). However, when the appropriate 'reduction factors' were applied to these migration data to take into account migration from actual fat-containing foods, overall migration was calculated to decrease to between 6.4 and 13.9 mg/dm^2. Migration of DEHA from PVC films into non-fatty foods as modelled by water was at or below the EU limit of 0.1 mg/dm^2 in this same study. It is noteworthy that while some fresh meats packaged in PVC films have contained 49 to 151 mg DEHA/kg, frozen chicken showed no detectable migration of DEHA (60).

Migration of other plasticizers including DEHP have been reported in beverages (mean 0.065 mg/kg) and foodstuffs (0.29 mg/kg) packaged in glass containers with DEHP-containing cap or lid seals (10). PVC film wrapped cheeses were observed to contain a mean of 2.2 μg DEHP/g. The UK MAFF has established TDI values for various phthalate esters as follows: DEHP 0.05 mg/kg b.wt./day; BBP 0.1 mg/kg b.wt./day; DBP 0.05 mg/kg b.wt./day and DEP 0.2 mg/kg b.wt./day (38). These TDI values relate to the EU total tolerable daily intake (TTDI) for all phthalate esters of 0.3 mg/kg b.wt./day. In an Australian survey, the most predominant plasticizers used in PE, PS, PET, cellophane, printed fibreboard and laminated aluminum-PE packaging materials were DEHP, DBP, BBP and DEHA; the greatest levels of phthalate plasticizers (up to 8160 μg/g film) were observed in printed PE films indicating the importance of printing inks on films in contributing to total phthalate esters in packaging materials (38).

Another source of phthalate ingestion unique to young children are PVC-based toys and childcare articles which may be mouthed for considerable periods of time by young infants (39). In fact, the EU CSTEE has established maximum permissable extracted amounts for several PAEs for a child weighing approx. 8 kg: all values are mg/10 cm^2 of the article mouthed over a 3 hr period, DEHP 0.3; DBP 0.8; DOP 3.0; DINP 1.2; DIDP 2.0 and BBP 1.6 (39). Using a saliva simulant solution of potassium and sodium salts in distilled water, Earls and coworkers (39) reported extractable amounts of PAEs from teethers, bath animals etc. ranging from 0.7–1.6 μg/10 cm^2/min which is less than the CSTEE migration limit of 9 μg/10 cm^2/min for DINP for example. Other plasticizers may enter a child's diet from commerically prepared and bottled baby foods (56). A Swedish study reported that ESBO migration from PVC gaskets in lids into ready cooked baby foods sold in glass jars ranged from $<$1.5–50.8 mg/kg food, with a mean of 11.9 mg/kg and a median value of 7.9 mg/kg (56). The EU CSTEE has proposed a TDI of 1 mg ESBO/kg b.wt. and a maximum total migration of 60 mg ESBO/kg food for adults. When these values are translated for an infant of approx. 8.5 kg, two servings of food containing a mean of 11.9 mg ESBO/kg results in an intake of approx. 0.38 mg/kg b.wt., which is very close to the adult TDI value above (56).

IX. METABOLISM OF PLASTICIZERS

Plasticizers in food are absorbed from the diet over a wide concentration range and can be found in several tissues, with the most concentrated amounts being found in the kidney and liver and metabolites excreted in the urine (61, 62). Studies to estimate dietary intake and excretion of DEHA reported a median intake of 23.7 mg in the United Kingdom (62). Urinary excretion of the metabolite 2-ethylhexanoic acid (EHA) acted as a useful biomarker to assess DEHA intake. Exposure to DEHP occurs not only through environmental factors such as the diet, but also from plastics used in medical settings, namely PVC medical devices such as IV bags and tubing, blood and plasma bags as well as enteral feeding and dialysis equipment (63). Average total daily exposure, excluding occupational exposure, has been estimated at 0.27 mg DEHP in the US. Exposure in the medical setting ranges from 0.01 mg DEHP/kg b.wt. from hemodialysis to a high of 140.0 mg/kg b.wt. from extracorporeal oxygenation of infants (63). There does not appear to be any significant bioaccumulation of phthalates such as DEHP in healthy higher mammals and humans (21, 61, 63). Studies in primates indicate that DEHP is rapidly and extensively metabolized to be excreted in the urine as glucuronide conjugation products of mono(2-ethylhexyl) phthalate (MEHP). DEHP is metabolized to MEHP by intestinal lipases and esterases. Another primary metabolite of DEHP is 2-ethanohexanol (2-EH), which undergoes further hepatic metabolism along with MEHP to secondary metabolites prior to excretion. The alkyl side chains are further oxidized and short chain dialkyl phthalates can be

excreted unchanged, or are completely hydrolyzed to phthalic acid (61). Longer chain DEHP is converted to polar derivatives of the monoester by oxidative metabolism prior to excretion. MEHP metabolism occurs in part by a rate limiting peroxisomal β-oxidative metabolism prior to excretion (21). For both DEHP and MEHP, excretion follows a time and dose-dependent metabolic profile in primates and man. DEHP has been estimated to have a urinary elimination half-life of approx. 6–12 hr (63). Lipoprotein bound DEHP metabolites are efficiently eliminated (e.g. peak elimination is 6 hours after ingestion) as evidenced by the 90% urinary excretion of DEHP and metabolites and 10% fecal excretion (21). Differences in DEHP metabolism between humans and rodents include the fact that rodent gut lipases are more effective in the hydrolysis of DEHP to MEHP compared to man. This results in greater intestinal absorption of MEHP by rodents, and in particular, greater absorption by young rodents compared to adults. These observations become important in the discussion of DEHP toxicity, since MEHP is thought be the active agent involved in this toxicity (63).

X. TOXICITY OF PLASTICIZERS: PHTHALATES

The potential toxicity of phthalate esters is of concern not only from packaging materials but also from the fact that PAEs are noted to leach into the environment (64). A Taiwanese study reported that PAEs such as DOP can be detected in the soil, ground water as well as vegetables grown in these environments (64). The LD_{50} for DEHP in rats has been reported to be 34 g/kg b.wt. (62). Phthalate esters have been shown to have a number of potential deleterious effects in vitro and in vivo (Table 76.7). In young male rats (110 g b.wt.), diets containing 2% (wt/wt) DEHP fed for 3 weeks resulted in decreased body weight gains associated with a decrease in energy retention and cumulative nitrogen balance (65). As mentioned above, PAEs such as DEHP, are the subject of toxicological concern due to the structural similarity of these molecules to the known teratogen thalidomide (phthalidomide) (48). In vitro studies evaluating the potential estrogenicity of various PAEs demonstrated that DBP and BBP weakly competed with 17β-estradiol for estrogen receptor binding and exhibited 36 and 42% activity, respectively, in gene expression assays using MCF-7 breast cancer cells (66). However, in vivo these PAEs did not increase uterine wet weights in the standard uterotrophic assay, nor was there any effect on vaginal epithelial cell cornification (66). Evidence of fetotoxicity, embryolethality and teratogenicity of PAEs have been reported in animal studies with DEHP and other branched PAEs fed at 1000 mg/kg diet to rat dams; no such effects were observed with DEHP fed at 200 or 40 mg/kg diet (14). DEHP at 1000 mg/kg diet, resulted in reduced numbers of live fetuses and 63% malformed fetuses, namely soft tissue malformations and skeletal malformations. Others have reported that rats dosed with 2 g DEHP or DOP/kg b.wt./day resulted in no changes in seminiferous tubular structure or Leydig cell morphology with DOP, but slight cytoplasmic rarefaction or vacuolation of Sertoli cells in seminiferous tubules with DEHP (67). Studies evaluating the developmental toxicity of 7-, 9- and 11-carbon branched PAEs have reported that doses between 500 and 1000 mg/kg b.wt. resulted in increased incidences of supernumerary lumbar ribs in pups and a significant increase in dilated renal pelves (68). Decreases in testicular weight recorded in rodents receiving MEHP at 2% of their diet has been attributed to loss of gonadal zinc. The intravenous administration of 11 mg/kg MEHP daily for 13 days to rabbits has been shown to result in 33% maternal mortality. Enlargement of liver and accompanying hypolipidemia and hepatic peroxisomal proliferation has also been reported in rats administered DEHP (16). Chronic feeding studies of DEHP at dietary intakes of 0.27–0.9 g/kg b.wt. for 2 years has resulted in a significant increase in hepatic tumours. It should be noted, however, that there is a 6,000 fold or more margin between the estimated human intake of PAEs and the dose that produced liver tumours in rodents. Thus, while PAEs have been reported to have teratogenic and fetotoxic effects in animal studies when fed at levels of 1 g DEHP/kg/day, and impaired lipid metabolism accompanied by

TABLE 76.7
Some Detrimental Effects of Phthalate Esters and their Monoester Metabolites[1]

In Vitro **Findings:**
Chromosomal aberrations in mammalian cells;
Base pair mutagenicity with/without S-9 mix (MEHP);
Genotoxicity to *B. subtilis*;
Estrogenic effects via competitive estrogen receptor binding; MCF-7 gene expression;
Inhibition of gap junctional intercellular communication (GJIC) in rat and mouse hepatocytes;
Decreased LH-stimulated secretion of testosterone from rat Leydig cells.
In Vivo **Findings:**
Decreased rat body weight gains and decreased energy and nitrogen balances;
Small or no decrease in rat litter size and pup weight;
Hepatic and pituitary hypertrophy;
Neuromuscular and skeletal deformities, i.e. increased incidence of supernumerary lumbar ribs;
Reproductive tract malformations, decreased testicular weight and sperm counts;
Hypolipidemia;
Hepatic tumorigenesis;
Fetotoxicity, embryolethality and teratogenicity in rats.

[1] References: 13, 14, 16, 62, 65–70.

hepatomegaly at 2% DEHP in the diet, the rapid metabolism and excretion of DEHP in primates and humans suggests that this plasticizer is only a minor health risk (14, 16, 21). Indeed, the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition concluded that phthalates do not represent a significant hazard to healthy humans given the relatively low rates of migration into foods (5). On the other hand, the very young and those undergoing hemodialysis and hemophiliacs with long-term exposure to higher doses of DEHP may be at risk of adverse effects including pulmonary, cardio-toxicity or increased hepatic peroxisomal proliferation (63). The risk for liver cancer in man appears to be relatively low due to the metabolism and excretion of PAE metabolites such as MEHP as well as the absence of inhibition of GJIC in human and hamster hepatocytes in culture compared to rat and mice hepatocytes (69).

XI. EFFECTS OF IONIZING RADIATION ON PACKAGING MATERIALS

Treatment of foods which represent a microbiological health risk using ionizing irradiation to improve the safety of susceptible foods such as chicken (*Salmonella sp*) and ground beef or hamburger (*Escherichia coli* O157:H7) is increasingly recommended given the potential severity of these foodborne diseases (22, 23). Irradiation must also be combined with other safe food handling or preservation techniques (i.e. refrigeration) to prevent cross- or recontamination of the irradiated product. One way to achieve this is to irradiate pre-packaged meat and poultry; however, the irradiation process should alter neither the physico-chemical properties of the packaging film nor result in the transfer of components or residues from the packaging material to contaminate the food in contact with the plastic film (71). Irradiation of plastic films may result in a combination of two basic phenomena: a) chemical cross-linking between polymer structural units ultimately resulting in a potential increase in film tensile strength, or b) fragmentation of polymeric structures resulting in decreased strength and increased permeability of packaging films (72, 73). Polymer additives such as plasticizers and antioxidants (added as stabilizers) will also be affected by the ionizing irradiation of plastic films (72–74). Radiation-induced polymer alterations will be influenced by several factors including the chemical structure and composition (i.e. additives) of the polymer, processing history and the irradiation conditions, namely the dose rate. In addition to scission and crosslinking of polymeric chains, the formation of volatile radiolysis products, which can be influenced by the presence of O_2, occur in irradiated plastics. As reviewed by Buchalla and coworkers (72), under vacuum, the main radiolysis volatiles produced are hydrogen, methane and hydrogen chloride from polymers containing chlorine molecules. In the presence of O_2, the gaseous products will also contain CO_2, CO as well as H_2, CH_4 and other hydrocarbons. The formation of reactive oxygen species as free radicals can result in the oxidation of the polymer, resulting in the formation of peroxide, alcohol and carbonyl compounds (72).

The various classes of polymers are noted to vary in their susceptibility to radiolysis. The most stable polymers include the vinyl derivative PS and the polyester PET, with the polyamides (Nylons) having intermediate stability and the polyolefins (HDPE, LDPE, PP) as the least stable polymers. When exposed to radiation doses or 25 or 50 kGy, PS samples have been reported to contain radiolysis products such as benzaldehyde, acetophenone (30–50 ppm), 1-phenylethanol ($<$10 ppm) and phenol; Nylon samples contained pentanamide (approx. 75 ppm) and caprolactam (75). Exposure of PP to heat, light or ionizing radiation is known to result in the generation of alkyl radicals (PP•) which can subsequently interact with molecular oxygen to form peroxyl radicals (PP-OO•) and ultimately, products containing hydroxyl, carbonyl or carboxyl groups (76). These volatiles can result in adverse flavour changes to irradiated foods packaged in these polymers. The intensity of off-odours is much greater upon irradiation of LDPE compared to HDPE, which in turn is greater than PS and various polyamides and polyesters (77). Moreover, the intensity of off-odours is noted to increase with the availability of oxygen in the atmosphere. Taints from PVC and PS have been reported with doses as low as 2.6 to 3.9 kGy through sensory testing (77). Odors from irradiated LDPE have been described as waxy polyolefin odor in the unirradiated control and plastic, burnt, pungent and waxy in irradiated samples (78). PET samples were described as giving off a slight plastic and a sweet plastic odor in both unirradiated and irradiated samples.

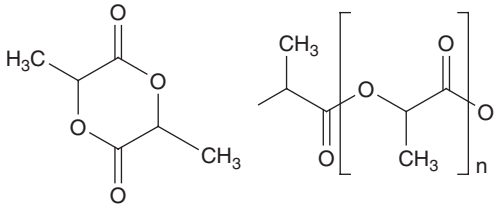
Due to the potential physico-chemical changes to polymers as a result of cross-linking or scission of polymer chains as a result of irradiation, it is necessary to investigate the permeability of irradiated films to O_2, CO_2, water vapour transmission as well as mechanical properties. Recently, Goulas and coworkers (79) reported that irradiation at 5, 10 and 30 kGy had no effect on the O_2, CO_2 permeability or water vapour transmission rates of a variety of polymers including LDPE, HDPE, PS, ethylene vinyl acetate (EVA), bi-axially oriented PP (BOPP) and an Ionomer. The low doses (5 and 10 kGy) had no effect on polymer mechanical properties such as tensile strength, % elongation at break and Young's Modulus. However, 30 kGy of irradiation resulted in decreased tensile strength in HDPE, BOPP and Ionomer and decreased % elongation at break in LDPE and Ionomer (79). There were no changes in Young's Modulus values for all polymers with the high dose irradiation. It is noteworthy that the mechanical properties of neither EVA nor PS were affected by irradiation. The overall migration into food simulants also showed variable results in that migration into distilled

water was not affected by irradiation; at 30 kGy, migration into 3% acetic acid was enhanced and decreased for BOPP and Ionomer, respectively. Similarly, migration into isooctane was increased for BOPP, but decreased for HDPE and Ionomer films (79).

The addition of antioxidants such as BHT is noted to reduce the formation of carboxylic acid derivatives in LDPE films (72). However, phenolic antioxidants such as Irganox 1076™ (Table 76.6), 1010™ and 1330™ and the arylphosphite antioxidant Irgafos 168™ which are frequently added to PVC, PE and PP polymers are susceptible to degradation following gamma irradiation, but also during melt processing and/or storage of polymers such as HDPE (58, 74). For example, low doses of irradiation of approx. 5 kGy to HDPE trays can result in the complete destruction of phosphite antioxidants such as Irgafos 168™ and Ultranox 626™ (Hostanox PAR24™) to their phosphate derivatives (58). Products of Irgafos 168™ decomposition in polymers include 1,3-di-*tert*-butylphenone and 2,4-di-*tert*-butylphenol (78). It is noteworthy that some antioxidant degradation products may become covalently linked to the matrix polymer, thereby decreasing the potential for migration into foodstuffs. This hypothesis has been validated by studies with ^{14}C-labelled packaging antioxidants indicating that following irradiation, the amount of extractable ^{14}C-labelled native antioxidant declined in irradiated polymers. This observation was associated with increases in the non-extractable radioactivity within the polymeric matrix (74). At present, little is known about the toxicity of the radiolysis products from polymers and additives given the variability in migration rates due to potential polymeric entrapment of volatiles from the different polymers into food simulants.

XII. ALTERNATIVE FOOD PACKAGING MATERIALS

Alterations in the formulation of traditional polymers are performed for several reasons including a) improvement of the biodegradability of common polymers such as LDPE once they enter the environment (80) and b) decreasing the bitterness from flavanones and terpenoids in citrus juices through the use of active packaging (enzymes immobilized on cellulose acetate films) (81). Safety concerns about the environment and the food supply with regard to the disposal of packaging materials in the environment and leaching of plasticizers into the soil and ground water (64) have been the impetus behind research into novel food packaging materials. Edible films based on carbohydrate (i.e. starches), lipids (i.e. monoglycerides) and milk protein (i.e. caseinates, whey proteins) have been investigated extensively by food technologists (82–84). However, these films have very high water vapour transmission rates (WVTR) albeit, moderate oxygen permeability, ultimately severely limiting the utility of these biodegradable or edible films in the food industry in any meaningful way (82, 83). Conn and coworkers (85) investigated the use of polylactide (PLA) as a food packaging polymer. Polylactide is a polymer resulting from the polymerization of lactic acid and its dimer lactide as shown below:



Lactide **Polylactide (PLA)**

Lactic acid has a solid history of use as a food ingredient and has had GRAS status in the US since 1984. The main physical characteristic of concern with PLA is the T_g = 60°C which limits the use of this polymer to foods which are not extensively heated. Migrant chemicals from PLA may include lactic acid, lactoyl-lactic acid (the linear dimer of lactic acid), small oligomers of PLA and lactide (85). These chemical species are however expected to be hydrolysed to lactic acid in the aqueous or acidic environment of foodstuffs or ultimately within the gastric contents. Potential applications of PLA include disposable food service items such as dishes and cutlery, packaging for fast-food applications at or below room temperature or at elevated temperatures below 60°C (85).

Other workers have investigated LDPE films which had been formulated to contain from 5 up to 40% rice, potato, wheat or soluble starch to improve the biodegradability of LDPE films when exposed to aerobic and anaerobic bioreactors (80, 86). It is noteworthy that the resultant LDPE/starch blend polymers would consist of 4 separate phases, two each of crystalline and amorphous structure from the two different polymer components. The incorporation of rice and potato starches into LDPE polymers resulted in reductions to film mechanical properties, namely tensile strength and modulus as well as % elongation; however, films retained functionality provided the starch content did not increase past 20% (80). The flexural strength and moduli of the LDPE/starch blend polymers increased with the starch proportion of the films. When evaluating gas permeability and WVTR of the LDPE/starch blends, film permeability to N_2 and CO_2 increased, as did the WVTR, with an increase in polymer starch content. As expected, biodegradability of the LDPE/starch blend polymers was enhanced at rice and potato starch contents greater than 10%, with approx. 50–60% starch removal and some early LDPE degradation observed following bioreactor incubations. Moreover, using soluble versus wheat starch improved the biodegradability of the LDPE/starch blend polymers, in that wheat starch resulted

in up to 55% starch removal, whereas soluble starch resulted in up to 80% starch removal (86). LDPE/starch blend polymers containing up to 20% starch retain mechanical barrier properties, although the susceptibility to high WVTR values and gas permeabilities would be a significant concern for most processed foods.

Active packaging is an emerging technology wherein the packaging interacts with the food inside to improve its quality, shelf-life or safety (81). Citrus juices, particularly grapefruit juice, contain bitter components, namely naringin and limonin, which may be a negative characteristic from an organoleptic perspective. Active packaging, which was comprised of fungal-derived naringinase immobilized to cellulose acetate film, reduced naringin and limonin concentrations in grapefruit juice, and thereby decreased bitterness as evaluated by paired-comparison sensory testing (81). Desorption of enzyme from the film reached only 2% following 4 weeks of storage indicating good stability compared to other acetate films.

XIII. CONCLUSION

In summary, migration of plastic polymer residues (i.e. VC, styrene), plasticizers and contaminants may adversely influence the taste, odour and safety of foods and beverages either stored or heated in specific types of storage containers (i.e. PE, PVC, paperboard etc.). Factors which influence the degree of severity of these effects include: contact surface and time, aqueous or lipophilic nature of the foodstuff, fat-releasing properties of the food, temperature of processing or storage and method of processing (i.e. ionizing radiation). Off-odours and flavours in foods packaged or stored in polymers, or conversely 'flavour-scalping' of food volatiles into packaging materials may reduce the quality and palatability of foods without affecting the health and safety of the consumer. Toxicology of plastics and plasticizers may be associated with occupational exposure as in the case of ASL in those exposed to VC, or consumption of phthalate ester plasticizers in animal studies. Exposure of consumers to the amount of phthalate esters which may migrate into foods is not considered to be a health risk. Food scientists are currently evaluating the utility of such alternate packaging materials such as edible films, polymer/starch blends, polylactide, a polymer of the dimer of lactic acid, lactide, as well as enzymes adsorbed to films as a form of active packaging which can conceivably improve a product as it interacts with its packaging. Limiting factors for the application of these novel packaging films include such problems as high rates of water vapour transmission, high gas permeability as well as glass transition temperatures close to the serving temperature of foods. However, new formulations as well as the use of laminate films may enhance the physicochemical properties of existing films and newer edible or biodegradable films.

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77 Food Plant: Sanitation and Quality Assurance

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CONTENTS

I. PART I: SANITATION

Sanitation in a food processing plant is to assure that the food product the company manufactures is wholesome and safe to eat. This usually means that the food does not contain any potential undesirable substances including:

- Biological toxins
- Chemical toxicants
- Environmental contaminants
- Extraneous substances

To achieve this goal, a food processor with products sold crossing state lines in the United States uses the following approaches:

1. Implementing a basic food plant sanitation program.
2. Compliance with good manufacturing regulations issued by the United States Food and Drug Administration (FDA).
3. Long term plan in developing a Food Hazards Critical Control Points or HACCP program for those food industries that are not mandated to have a current HACCP program.

A. Food Plant Sanitation Program

Most food processors have a sanitation program to make sure that their products are safe. Most programs have the following components, among others:

1. The product and its ingredients
2. Cleaning
3. Housekeeping
4. Personnel hygiene and safety
5. Warehousing
6. Distribution and transportation
7. Sanitation inspections

Let us use the manufacturing of bakery products as an example to study the above factors.

Bakery goods include bread cakes, pies, cookies, rolls, crackers and pastries. Ingredients consisting of flour, baking powder, sugar, salt, yeast, milk, eggs, cream, butter, lard shortening, extracts, jellies, syrups, nuts, artificial coloring, and dried or fresh fruits are blended in a vertical or horizontal mixer after being brought from storage, measured, weighed, sifted, and mixed. After mixing, the dough is raised, divided, formed, and proofed. Fruit or flavored fillings are cooked and poured into dough shells. The final product is then baked in electric or gas-fired ovens, processed, wrapped, and shipped. Loaves of bread are also sliced and wrapped.

B. Raw Ingredients and the Final Product

Sanitation considerations apply to every stage of the processing operation: raw materials, operations.

1. Critical Factors in the Evaluation of Raw Materials

1. Raw materials must come from warehouses that comply with local, county, state and federal requirements for food warehouse sanitation.
2. For certain ingredients such as egg and milk products, their sources, types, etc. should be ascertained. If frozen eggs are used, are they pasteurized and received under a Salmonella-free guarantee? Some food plants require routine testing of critical raw materials for bacterial load including Salmonella and other pathogens.
3. Are raw materials requiring refrigeration (or freezing) or refrigerated (or frozen)?
4. Is there any "blend off," mixing contaminated raw materials with clean raw materials?

2. Critical Factors in Evaluating the Sanitation of Operations

1. Room temperature, bottleneck, and bacterial contamination. During certain stages of an assembly line operation, always check sites where "bottleneck" frequently occurs. Room temperature and period of bottlenecking are related to chances of bacterial contamination.
2. Metal detection. During a production operation, always check metal detection or removal devices to make sure that they are working properly.
3. Time and temperature. Identify stages in the operation where time and temperature are major and/or critical variables. Intense education must assure that any abuses which may allow growth and possible toxin formation of microbial contaminants are strictly forbidden.
4. Equipment design. Be alert for poorly designed conveyors or equipment which might add to bacterial load through product delay or "seeding."

C. Cleaning

Imagine your kitchen. We have to wash the kitchen floor because water, oil, and other cooking ingredients are dropped accidentally or intentionally. Then there are the dishes and pots and pans. They have to be cleaned and put away.

Of course the same problems exist in a bakery processing plant, but on a much larger scale.

Almost all bakery processing plants have a written plan on plant sanitation:

- Is there water on the floor?
- Has all flour dust been removed?
- Are different and clearly identified containers used for salvaged material and returned goods? Any noncompliance may be the cause of contaminating newly produced products
- Is the distance between garbage disposal containers and the station where food ingredients are processed acceptable to avoid any potential contamination?

Those few examples are among hundreds of details that a good sanitation program will carefully identify and establish polity as to who is responsible and what the responsibility is.

Similarly, food processing equipment requires a highly structured sanitation program.

Major components or equipment in the process flow are flour bins, elevator boots, conveyor systems, sifters, dump scale apparatus, production line flouring devices, dough proofers, overhead supports and ledges, and transport vehicles. All have removal inspection ports. Schedule checks to make sure that static accumulation for insect and/or rodent infestations is acceptable. Remove them as frequently as possible.

Most companies require the inspection of equipment prior to production to determine adequacy of cleanup and sanitizing operations.

It is doubtful that a food processing company will survive long if it does not have a comprehensive and workable program to clean the equipment used to manufacture its products. The objective of cleaning any equipment that has been used in food processing is to remove any residue or dirt from the surfaces which may or may not touch any food or ingredient.

Some of the equipment may be subjected to further sanitization and sterilization. Such attempts will be questioned if there is still visible dirt or debris attached to any surface of the equipment.

The wet-cleaning process, used by all food processors, has three components: pre-rinse, cleaning, post-rinse. This can be done manually or by circulation.

- Pre-rinse. This uses water to separate loosely adhered particles (dirt, residue, etc.), using two basic considerations. Perform the cleaning after the production cycle is completed to get ready for the next work day. Predetermined criteria: method for specific surfaces (vessels, components, pipelines), period of rinsing, temperature. For both factors, most food processing plants have established appropriate policy to handle the pre-rinse.
- Clean. Under most circumstances, soaking, scrubbing and more soaking, characterize any cleaning process. The goal is to remove sticky residues or particles from the surface. Of course, cleaning detergents or solutions are used in the soaking and scrubbing. The chemical reactions are the standard: saponification, hydrolysis, emulsification, dispersion, and so on. All chemical reactions are time- and temperature-dependent.
- Post-rinse. This is no difference from rinsing cooking utensils after they have been scrubbed and soaked. This stage removes all detergents/sanitizers used and any particles left behind.

For all three stages, the water used must comply with rigid standards to avoid damage to equipment, corrosion, and status of microbiological presence.

Apart from manual cleaning, we have the Clean In Place or CIP which uses a circulation system of chemical solutions pumped through the equipment "in place." Much food processing equipment is designed to have this built-in feature. Any automatic process has inherent problems that must be dealt with in a manner dictated by circumstances.

The use of a circulatory method in cleaning is dependent on two groups of factors:

1. Substances used in the detergent or cleaning solutions.
2. The variables.

Substances used can include an array of chemicals: caustic soda, acid, etc. Obviously, the concentration of such chemicals is a critical factor. The variables include: contact temperature, contact time, flow rate between surfaces and substances in cleaning solution.

D. HOUSEKEEPING

Again, we can use our home as an example. We keep the inside clean, dusting, vacuuming, sweeping, and so on. We keep the outside of our house clean: garbage, leaves, droppings, peeling paints, and so on. It is of paramount importance that a food processing plant is clean inside and outside.

For internal housekeeping, part of the information has been discussed in the cleaning process we presented earlier. However, we still need to worry about cleaning windows, debris under counters and in the corner of a room, emptying garbage cans, and so on. Most food companies hire regular maintenance crews to do the job. Unfortunately,

the plant manager will still need to develop policy, implement and evaluate procedures.

The environment of a food processing plant has always been a problem: garbage, birds, insects, rodents, and so on. Housekeeping for the immediate vicinity outside a food plant requires close monitoring.

Many professionals consider housekeeping as "nonglamorous" and "menial." However, it is so important that it requires complete attention from management. The reason is simple. Regulatory officials from local, county, and state levels are serious about this aspect of food processing. If the company ships products across state lines, the United States FDA has the authority to issue warning letters about any unacceptable conditions, including sloppy housekeeping.

We will briefly analyze the two components of housekeeping.

1. Housekeeping inside a Food Plant

Every manufacturing company, food or otherwise, has a master schedule of cleaning. Obviously, food particles attract rodents and other undesirable animals and insects and removal is important to the plant operation. A cleaning schedule essentially has the following components:

1. Coverage: rooms, storage areas, toilets, offices, freezers, walls, ceilings, and so on.
2. Frequency: each area requires a different frequency of cleaning, days (1, 2, 3, 4, etc.), weekly, etc.

A cleaning schedule is meaningful only if the methods of cleaning are appropriate and the schedule is enforced or implemented. Also, the frequency of cleaning must be carefully evaluated with the methods of cleaning. This is because, a process of cleaning may increase the dust load in the air which may in turn contaminate other surface areas.

Some areas require frequent cleaning and others don't.

A storage room with infrequent traffic may be cleaned once a week while a storage room with frequent traffic may need to be cleaned once a day.

a. Dust

1. Most dry cleaning methods increase dust in the air, e.g., wiping with a rag, vacuum cleaners, brooms, brushes, pressurized air.
2. Since dust particles are charged electrically, they will adhere to any surfaces that are electrically or electrostatically charged. This results in contamination.
3. Dust contamination is heightened when the environment is moist, including surfaces, resulting in molds. When the molds occur on piping, back of tanks, ducts and cables, corner of ceilings and other places that are obscured from vision, the problem increases.
4. Dust moves from room to room by normal air flow from temperature differences or window and door draft. That is further contamination.
5. Dust dispersion is a risk replacing the risk that has just been removed by cleaning.

The areas to be cleaned should be evaluated with great care.

1. Although most objects, e.g., vats, holding tanks, are raised from the floor with a space for cleaning, it is still difficult to clean this part of the floor because the space is too narrow and hidden from view.
2. Corners always pose a problem for cleaning. Special devices such as suction hose are needed to keep them clean. There are places where insects, rodents, and other undesirable creatures will thrive.
3. Bottoms of most equipment always pose a problem in cleaning. Crawling on one's knees does not always solve the problem. Customized devices may be needed.

Wet-cleaning by hands or machines is acceptable. Modern technology has made available gel, foam, aerosols, and special equipment. However, the water hose is still the method of choice in most food companies. Wet-cleaning must take the following into consideration:

1. All materials that can absorb moisture, such as cardboard boxes, pallets, etc. should be removed.
2. After wet-cleaning, the surfaces must be dried carefully.
3. Proper draining systems should be in place and maintained, clean and free of debris around the openings.

II. PART II: UNITED STATES ENFORCEMENT TOOLS

A. Background Information

The FDA is charged with protecting American consumers by enforcing the Federal Food, Drug, and Cosmetic Act and several related public health laws. What does it do when there is a health risk associated with a food product?

When a problem arises with a product regulated by the FDA, the agency can take a number of actions to protect the public health. Initially, the agency works with the manufacturer to correct the problem voluntarily. If that fails, legal remedies include asking the manufacturer to recall a

product, having federal marshals seize products if a voluntary recall is not done, and detaining imports at the port of entry until problems are corrected. If warranted, the FDA can ask the courts to issue injunctions or prosecute those that deliberately violate the law. When warranted, criminal penalties — including prison sentences — are sought.

However, the FDA is aware that it has legal responsibility to keep the public informed of its regulatory activities. To do so, the FDA uses press releases and fact sheets. The FDA uses this tool before, during, and after an event of health hazard related to a food product. Some of these are briefly described below, emphasizing the sanitation deficiencies of affected food products.

1. Press Releases and Fact Sheets

FDA Talk Papers are prepared by the Press Office to guide FDA personnel in responding with consistency and accuracy to questions from the public on subjects of current interest. Talk Papers are subject to change as more information becomes available.

Below are the regulatory tools used by the FDA.

B. Data on Insanitary Practices

For the FDA to enforce its laws and regulations, it must have specific data regarding the sanitary practices of a food processing plant. The FDA has a number of ways to ascertain or determine if a food product is associated with unsanitary conditions in a food processing plant or if a food processing plant has sanitary deficiencies. They include:

1. Product monitoring
2. Activities based on reports from the public
3. Activities based on reports from other government agencies
4. Establishment inspection reports

1. Product Monitoring

Product monitoring is as old as when modern food processing first started. At present, local, county, state, and federal health authorities conduct market food product sampling and analyses to determine the wholesomeness of food. Such monitoring is restricted by the availability of allocated budget and resources. However, the FDA has the most resources and its monitoring effort produces the most results.

When products are found to be unsanitary (pathogens, rats, insects, glass, metal, etc.) by the FDA, it will implement standard procedures to warn the public, remove such products from the market and a variety of other actions it can do and which will be discussed later in this chapter.

2. Activities Based on Reports from the Public

The FDA has a website and an 800 number for the public to report health hazards including sanitation of food products. Since the establishment of such communication convenience, there is an increasing number of consumers reporting products posing health risks, such as glass in baby food, dead insects in frozen dinners, and so on. Occasionally, so-called whistle blowers, i.e., employees of food companies, inform the FDA of products with contaminants from unsanitary practices. Based on the data provided by the public, the FDA implements standard procedures to handle any potential health hazards related to the products reported.

3. Activities Based on Reports from Other Government Agencies

Health care providers frequently are the source of information that eventually lead to unsanitary practices of food companies. These include physicians, pharmacists, nurses, dentists, public health personnel, and others. Most of these reports involve injury, such as food poisoning, decomposed or spoiled product contents.

These reports become a vital source of leads for the FDA to enforce its laws and regulations.

4. Establishment Inspection Reports

Inspection of a food processing plant by a government authority is the basis on which the government can decide if the food manufactured in the plant is wholesome and poses no economic fraud. The frequency and intensity of the inspection process will depend on resources and budgets, especially for the non-federal agencies. The FDA, as a federal agency, has more authority, resources, and budget.

The framework for inspecting a plant covers the following:

1. Basics
   a. Preparation and references
   b. Inspectional authority
2. Personnel
3. Plants and grounds
4. Raw materials
5. Equipment and utensils
6. Manufacturing process
   a. Ingredient handling
   b. Formulas
   c. Food additives
   d. Color additives
   e. Quality control
   f. Packaging and labeling

After an inspection is completed, the inspector gives the plant management a copy of the report. If there are

sanitation deficiencies, the management will be expected to correct them.

The data collected from this inspectional procedure and other sources discussed earlier become the central operation base on which the FDA will enforce its legal responsibility to make sure that all deficiencies are corrected to reduce any hazard to the health of the consuming public.

The interesting part is the enforcement and compliance of the equation that concerns this chapter. We have seen the manner in which the FDA compiles data on the sanitation of a food product and a food processing plant. We will now proceed to the regulatory activities the FDA uses to assure compliance.

C. Recalls

The *FDA Consumer* magazine has published several articles on the recall of food products in this country. The following information has been compiled in these public documents.

1. Misunderstanding

Recalls are actions taken by a firm to remove a product from the market. Recalls may be conducted on a firm's own initiative, by FDA request, or by FDA order under statutory authority.

The recall of a defective or possibly harmful consumer product often is highly publicized in newspapers and on news broadcasts. This is especially true when a recall involves foods, drugs, cosmetics, medical devices, and other products regulated by FDA.

Despite this publicity, FDA's role in conducting a recall often is misunderstood not only by consumers, but also by the news media, and occasionally even by the regulated industry. The following headlines, which appeared in two major daily newspapers, are good examples of that misunderstanding:

"FDA Orders Peanut Butter Recall," and "FDA Orders 6,500 Cases of Red-Dyed Mints Recalled."

The headlines are wrong in indicating that the Agency can "order" a recall. FDA has no authority under the Federal Food, Drug, and Cosmetic Act to order a recall, although it can request a firm to recall a product.

Most recalls of products regulated by FDA are carried out voluntarily by the manufacturers or distributors of the product. In some instances, a company discovers that one of its products is defective and recalls it entirely on its own. In others, FDA informs a company of findings that one of its products is defective and suggests or requests a recall. Usually, the company will comply; if it does not, then FDA can seek a court order authorizing the Federal Government to seize the product.

This cooperation between FDA and its regulated industries has proven over the years to be the quickest and most reliable method to remove potentially dangerous products from the market. This method has been successful because it is in the interest of FDA, as well as industry, to get unsafe and defective products out of consumer hands as soon as possible.

FDA has guidelines for companies to follow in recalling defective products that fall under the Agency's jurisdiction. These guidelines make clear that FDA expects these firms to take full responsibility for product recalls, including follow-up checks to assure that recalls are successful.

Under the guidelines, companies are expected to notify FDA when recalls are started, to make progress reports to FDA on recalls, and to undertake recalls when asked to do so by the Agency.

The guidelines also call on manufacturers and distributors to develop contingency plans for product recalls that can be put into effect if and when needed. FDA's role under the guidelines is to monitor company recalls and assess the adequacy of a firm's action. After a recall is completed, FDA makes sure that the product is destroyed or suitably reconditioned and investigates why the product was defective.

The FDA has stated the following guidelines several times in its magazine FDA Consumer.

2. Categories

The guidelines categorize all recalls into one of three classes according to the level of hazard involved.

1. Class I recalls are for dangerous or defective products that predictably could cause serious health problems or death.
2. Class II recalls are for products that might cause a temporary health problem, or pose only a slight threat of a serious nature.
3. Class III recalls are for products that are unlikely to cause any adverse health reaction, but that violate FDA regulations.

FDA develops a strategy for each individual recall that sets forth how extensively it will check on a company's performance in recalling the product in question. For a Class I recall, for example, FDA would check to make sure that each defective product has been recalled or reconditioned. In contrast, for a Class III recall the Agency may decide that it only needs to spot check to make sure the product is off the market. Detailed regulations have been promulgated on FDA recalls in the U.S. Code of Federal Regulations.

Even though the firm recalling the product may issue a press release, FDA seeks publicity about a recall only when it believes the public needs to be alerted about a serious hazard. For example, if a canned food product, purchased by a consumer at a retail store, is found by FDA to contain botulinum toxin, an effort would be made to retrieve all the cans in circulation, including those in the hands of consumers. As part of this effort the Agency also

could issue a public warning via the news media to alert as many consumers as possible to the potential hazard.

FDA also issues general information about all new recalls it is monitoring through a weekly publication titled "FDA Enforcement Report."

Before taking a company to court, FDA usually notifies the responsible person of the violation and provides an opportunity to correct the problem. In most situations, a violation results from a mistake by the company rather than from an intentional disregard for the law.

There are several incentives for a company to recall a product, including the moral duty to protect its customers from harm and the desire to avoid private lawsuits if injuries occur. In addition, the alternatives to recall are seizures, injunctions, or criminal actions. These are often accompanied by adverse publicity, which can damage a firm's reputation.

A company recall does not guarantee that FDA will not take a company to court. If a recall is ineffective and the public remains at risk, FDA may seize the defective products or obtain an injunction against the manufacturer or distributor.

The recalling firm is always responsible for conducting the actual recall by contacting its purchasers by telegram, mailgram, or first-class letters with information including:

1. The product being recalled
2. Identifying information such as lot numbers and serial numbers
3. The reason for the recall and any hazard involved
4. Instructions to stop distributing the product and what to do with it.

FDA monitors the recall, assessing the firm's efforts.

3. Initiating a Recall

A firm can recall a product at any time. Firms usually are under no legal obligation to even notify FDA that they are recalling a defective product, but they are encouraged to notify the agency, and most firms seek FDA's guidance. FDA may request a recall of a defective product, but it does so only when agency action is essential to protect the public health.

When a firm undertakes a recall, the FDA district office in the area immediately sends a "24 Hour Alert to Recall Situation" notifying the relevant FDA center (responsible for foods and cosmetics, drugs, devices, biologics, or veterinary medicine) and DEEO of the product, recalling firm, and reason for the recall. FDA also informs state officials of the product problem, but for routine recalls, the state does not become actively involved.

After inspecting the firm and determining whether there have been reports of injuries, illness, or other complaints to either the company or to FDA, the district documents its findings in a recall recommendation ("RR") and sends it to the appropriate center's recall coordinator. The RR contains the results of FDA's investigation, including copies of the product labeling, FDA laboratory worksheets, the firm's relevant quality control records, and, when possible, a product sample to demonstrate the defect and the potential hazard. The RR also contains the firm's proposed recall strategy.

4. The Strategy

FDA reviews the firm's recall strategy (or, in the rare cases of FDA-requested recalls, drafts the strategy), which includes three things: the depth of recall, the extent of public warnings, and effectiveness check levels.

The depth of recall is the distribution chain level at which the recall will be aimed. If a product is not hazardous, a recall aimed only at wholesale purchasers may suffice. For more serious defects, a firm will conduct a recall to the retail level. And if the public health is seriously jeopardized, the recall may be designed to reach the individual consumer, often through a press release.

But most defects don't present a grave danger. Most recalls are not publicized beyond their listing in the weekly Enforcement Report mentioned earlier. This Report lists the product being recalled, the degree of hazard (called "classification"), whether the recall was requested by FDA or initiated by the firm, and the specific action taken by the recalling firm.

A firm is responsible for conducting "effectiveness checks" to verify — by personal visits, by telephone, or with letters — that everyone at the chosen recall depth has been notified and has taken the necessary action. An effectiveness check level of "A" (check of 100 percent of people that should have been notified) through "E" (no effectiveness check) is specified in the recall strategy, based on the seriousness of the product defect.

5. The Health Hazard Evaluation

When the center receives the RR from the district office, it evaluates the health hazard presented by the product and categorizes it as a class I, II, or III. The classification is determined by an ad hoc "Health Hazard Evaluation Committee" made up of FDA scientists chosen for their expertise. Classification is done on a case-by-case basis, considering the potential consequences of a violation.

A class I recall involves a strong likelihood that a product will cause serious adverse health consequences or death. A very small percentage of recalls are class I.

A class II recall is one in which use of the product may cause temporary or medically reversible adverse health consequences, or in which the probability of serious adverse health consequences is remote.

A class III recall involves a product not likely to cause adverse health consequences.

For class I and II, and infrequently for class III, FDA conducts audit checks to ensure that all customers have been notified and are taking appropriate action. The agency does this by personal visits or telephone calls.

A recall is classified as "completed" when all reasonable efforts have been made to remove or correct the product. The district notifies a firm when FDA considers its recall completed.

6. Planning Ahead

FDA recommends that firms maintain plans for emergency situations requiring recalls. Companies can minimize the disruption caused by the discovery of a faulty product if they imprint the date and place of manufacture on their products and keep accurate and complete distribution records.

A "market withdrawal" is a firm's removal or correction of a distributed product that involves no violation of the law by the manufacturer. A product removed from the market due to tampering, without evidence of manufacturing or distribution problems, is one example of a market withdrawal.

A "stock recovery" is another action that may be confused with a recall. A stock recovery is a firm's removal or correction of a product that has not yet been distributed.

Even though the firm recalling the product may issue a press release, FDA seeks publicity about a recall only when it believes the public needs to be alerted about a serious hazard. For example, if a canned food product, purchased by a consumer at a retail store, is found by FDA to contain botulinum toxin, an effort would be made to retrieve all the cans in circulation, including those in the hands of consumers. As part of this effort the Agency also could issue a public warning via the news media to alert as many consumers as possible to the potential hazard.

D. Warning Letters

Under FDA regulations, a prior notice is a letter sent from FDA to regulated companies about regulatory issues. One such notice is the warning letter. If the establishment inspection report includes a list of sanitary deficiencies, the FDA may send a warning letter to the food company to ask for proper correction of such deficiencies.

III. PART III: QUALITY ASSURANCE

Many college graduates in food science, food technology, and food engineering work for food processing plants. Eventually, many of them become operational managers in the company. At this stage, they realize the significance of quality assurance. They are responsible for, not only the quality of the finished product, but also its wholesomeness and safety for public consumption.

The principles and procedures for quality assurance are as applicable and beneficial to small plants as to larger plants. In many cases, quality control systems can be more efficiently administered in small plants because of a simpler organizational structure and more direct communication among employees. The term quality control is not the same as quality assurance. In general "control" refers to one aspect of "assurance." Some professionals equate quality control systems with quality assurance. To avoid this issue and for ease of discussion, we use them (quality assurance, quality control, and quality control systems) interchangeably.

A. Cost versus Benefit

Quality assurance or control is a good management tool. A quality control system specifically tailored to the volume and complexity of a plant operation can be cost-effective.

A properly designed and operated total quality control system will minimize the likelihood of mistakes during processing, give an indication of problems immediately and provide the information quickly to locate and correct the cause of problems. As a result, production delays are reduced, the need for re-processing or re-labeling is lessened, and the possibility of product recall and condemnation is reduced.

B. Product Consistency Improved

Quality control systems provide the information necessary to consistently produce a uniform quality product at a predicted cost. Some processors have questioned whether the cost of implementing a total quality control system would be recovered unless the quality of the plants product had been so poor that the plant suffered reduced sales and a high return of product.

It is true that a plant with a poor product would benefit most. In even the best plants, however, the lack of a quality control system results in a product that is more variable and not as well defined.

With organized controls and objective sampling, the plant has more extensive and precise information about its operation. As a result, management has better control and product quality is stabilized. Records from a quality control system define product quality at the time of shipment, and will aid in dealing with claims of damage or mishandling during shipment.

C. Equipment Costs

Contrary to the impression or idea that quality control systems require highly trained technicians and expensive equipment, a plant quality control system can be fairly simple and inexpensive and still be effective.

The expense of equipment is related to the type and complexity of products and operations and the volume of production. In most cases, a total quality control system in a small plant would require only inexpensive thermometers, calculators, knives, grinders, and existing testing equipment used for traditional inspection and quality assurance.

If necessary, samples may be submitted to commercial laboratories.

Technical skills in food science, mathematics and statistics necessary to establish a quality control system are available from trade associations and professional societies at a reasonable one-time charge. This assistance can be utilized to define defects, defective units, and critical control points and to establish corrective actions for the system. Once the technical details of the system are established, the system can be carried out by plant personnel familiar with the processing operation. It is not necessary to hire a quality control technician.

A critical control point is a point in the food processing cycle where loss of control would result in an unacceptable product. Such points may include the receipt of raw meat just before use, processing and storage operations, and delivery of the product to the customer.

The FDA and USDA have special programs designed to assist in identifying critical control points and setting up a quality control system in a small food processing plant. They will also provide onsite assistance in the startup of the system.

D. Elements of a Total Quality Control System

The first step in developing a plant quality control system is to outline the processes that occur in the plant. An easy way to do this is to visualize the physical layout of the plant operation. The building may be small and consist of only one or two rooms or it may be large and contain many rooms. Make a list of the rooms or areas, and draw a flow diagram of the production process, starting with the incoming or receiving area and ending with the shipping area for finished goods.

For each room or area, list the activities that occur there, making special note of those unusual or important relevant to the process or product. For each, spell out the controls that are imposed-or should be imposed- whether precise or flexible, written or not. Examples would include raw materials examined, ingredients weighed, meters used, scales calibrated, equipment cleaned, bills of lading examined, or trucks checked.

Identify the FDA or USDA inspection regulations that apply to each area of the processing plant, and these should be listed. The good manufacturing regulations promulgated by the FDA are the most appropriate.

For each processing area, designate the person responsible for the controls or inspection — the name of a plant employee or an outside contractor. How often is the control or inspection check to be done? What records are kept? This information can be compiled by a clerical or administrative employee, and the FDA or USDA inspector can assist.

When this exercise has been completed, a rough outline of a total quality control system has been developed. It can be compared, area by area, to descriptions of the elements in the sample system in the appendix of the manual that records the system. If a company does not have such a manual, it is recommended that it start one.

As each element is reviewed, note where controls may be missing. The outline that remains, with missing controls added, is another step closer to a total quality system.

The final step is to convert this outline into a written format, as though it were a set of instructions for plant employees. In reality, it can be the operating manual for the persons responsible for conducting quality control in the plant.

E. General Elements of Total Quality Control

We have just completed a discussion of the general outline of a plant quality control system. Within the system are various elements, determined by the type of operation in the plant.

In this section, the specific operations will be discussed and the elements of a good quality control system will be outlined.

F. Receiving

Examples of controls:

- Examine (and possibly sample) incoming lots
- Verify identification marks
- Check carriers
- Log deliveries

A plant's total quality control system will include written instructions for checking incoming raw materials, such as raw fruit, flour, frozen fish, spices, salt, liquid ingredients, additives and extenders, and for recording the results. These materials must be verified for wholesomeness (free from indications of mishandling, decomposition, infestation), acceptability for intended use, and approval for use.

It may also be desirable at this point — although it is not mandatory — to test for composition (fat, moisture, and so on) to assure proper blending of formulated products. It is preferable to run the most frequent tests on products likely to have the most variation. For example, biological cultures and frozen orange juice need more frequent analysis than frozen dough or dried beef. Sampling plans utilizing statistical quality control procedures are helpful in inspecting incoming lots. These plans are easy to use and may be obtained from several sources, including government booklets.

It is good practice to prepare a suppliers' or buyers' guide outlining the specifications for ingredients, additives and other products bought outside the plant.

The air temperature and product temperature in the receiving area should be checked often enough to assure

that the company's requirements are being met. This would include checks of freezers, doors, door seals, incoming railroad cars, and trucks. The quality control plan should include procedures for taking corrective action in the event a product is contaminated during shipment.

The receiving log should be checked to assure that entries are accurate and up to date and that all requirements regarding incoming products and materials are met. The log will be useful in indicating trends, so problems can be spotted early. The person who checks the log can keep a record of the dates and results of the verifications.

Lots moved from the receiving area to other areas of the plant should be periodically checked to assure that their identity is properly maintained.

In preparing written instructions for the receiving area, identify the various checks to be made, who is to make them, when they are to be made, and how and where the information will be recorded.

G. Manufacturing

Examples of Controls:

- Verifying wholesomeness
- Verifying identification, weight or volume of ingredients
- Verifying ambient temperature
- Handling of rejected ingredients or product

Although ingredients may have been checked earlier for wholesomeness and acceptability, it is a good idea to make another check just prior to actual use in the manufacturing process.

This recheck does not need to be painstaking. It should be ample to assure that unacceptable ingredients are not used, and that ingredients are correctly identified and eligible for use in the product. The frequency of these rechecks can be reduced for small, low-volume plants.

Moreover, a method for controlling the weight of each ingredient is essential in order to assure a uniform and consistent finished product that complies with the company's quality requirements for the products and FDA's good manufacturing regulations for the products.

Maintaining the correct temperature in an area is important to good quality product. Occasional checks should be made during the shift and a record kept of the findings. This will take only a small amount of time and effort on the part of a plant employee, and will identify any situations requiring correction. Inexpensive recording thermometers are useful for maintaining a record of room temperature.

Occasionally, unacceptable ingredients or materials will arrive in the manufacturing area, and procedures should be outlined for these situations. Remember, good management sets realistic and effective controls for dealing with these situations. The procedures that are outlined must be diligently followed.

In cases where a finished product must meet certain requirements, such as fat or moisture limits, consider sampling each lot. Sampling plans may be designed to fit each condition and type of analysis.

For the purpose of verifying formulation or checking wholesomeness, a lot can be each batch during each shift, several batches from the shift, or the shift's entire production. For the purpose of laboratory testing, a lot may consist of one day's production or several days' production of an item, depending on the volume and type of product.

Records of all inspections and tests must be made available to state and federal inspectors and maintained on file.

H. Packaging and Labeling

Examples of Controls:

- Verify label approval
- Verify accuracy of labeling
- Check temperatures
- Finished product sampling

Since this is one of the last steps prior to shipping, it is essential that no regulatory requirement be overlooked.

Checks must be made to assure that all labels have been approved by state and federal regulators and that proper labels are being used. Particular attention should be paid to the new nutrition labeling. It must be verified that illustrations represent the product, net weight and count declarations are accurate, and packaging meets the company's specifications. The temperatures of frozen products, as well as the condition of all containers and cases, should be checked and the findings recorded.

A net weight control program must assure that all lots leaving the plant meet with FDA's requirements for standardized foods as well as other applicable requirements. The sampling rate should be appropriate for the volume, type of product, size of package, and degree of accuracy desired. For instance, cartons of wholesale volumes need less frequent checks than retail packages.

Where applicable, routine systematic sampling, inspection, or analysis of a finished product must be part of the approved total quality control system, especially for a product going to retail outlets.

State and federal regulators in the plant, in regional offices, or in Washington can consult with processors on sampling, including rates, targets, and limits.

I. Shipping

Examples of Controls:

- "First in, first out"
- Record of shipments
- Checking order sizes and temperatures
- Checking containers and carriers

Records of the destination of products shipped from the plant are important to good quality control. In the event recall is necessary, the records will pinpoint the amount and exact location of the product.

The procedure for knowing the destination of each shipment should be explained in the quality control system. The plant may find it beneficial to have some type of container coding and dating system. This would identify the date of processing and packaging for returned goods. Occasional quality control checks should be made to verify the adequacy of the container codes and to verify order sizes, temperatures, (where applicable), and the condition of containers, rail cars, or trucks used for shipping. These controls do not need to be complicated, but adequate to assure effectiveness.

J. General Sanitation

Examples of Controls:

- Rodents and pests
- Product contamination
- Employee hygiene
- Facilities and environmental appearance

A procedure to check the overall sanitation of plant facilities and operations, including outside adjacent areas and storage areas on plant property, should be included in a total quality control system.

In a total quality control system, a designated plant official will make the sanitation inspection and record the findings. If sanitation deficiencies are discovered, a plan for corrective action is necessary. Corrective action might include re-cleaning, tagging a piece of equipment, or closing off an area until a repair is completed.

A frequent systematic sanitation inspection procedure should be used where product contamination is possible, such as packaging failure, moisture dripping, or grease escaping from machinery onto product or surfaces which come into contact with product.

Good employee hygiene should be continuously emphasized through special instruction for new employees and properly maintained, adequate toilet and welfare facilities. Clean work garments in good repair, good personal hygiene practices such as hand-washing, periodic training, and the cleaning of floors and walls in non-production areas are signs of effective sanitation. Plant management will want to use a number of techniques to assure the continued effectiveness of this phase of the quality control system.

K. Employee Training

Examples of Controls:

- New employee orientation
- Refresher training

When new employees begin work at a plant, it is useful to acquaint them with all aspects of the plant. The quality control system should provide for instruction of new employees on the plant's operations and products and on good hygiene practices.

A number of questions concerning hygiene should be addressed in this instruction. What basic things should any new employee know about food handling and cleanliness? Why is cleanliness essential? What are the standards-in other words, what does clean mean? Why are product temperatures important? What is a cooked product? What occurs if something is accidentally soiled? Which chemicals (cleaners, sanitizers, insecticides, food additives) are around? Does the new employee use or have any responsibility for any of these? How does the employee become acquainted with the operation and products? Whom does the employee consult if questions or problems arise?

Make a list of all the items that need to be covered in employee orientation, and indicate generally how and when the orientation will be performed.

Employee training should not end with orientation, but should include an ongoing program to continually remind employees of the importance of good sanitation.

How are employees continually reminded of important functions, such as personal hygiene after a visit to the restroom? Will posting a sign or poster which fades over time communicate the appropriate level of importance? There are many ways of continuing employee training and maintaining sensitivity. Plants may find that occasionally changing methods will help emphasize management's commitment.

A brief description of the methods and time schedule for assuring that employees do not become unconcerned or indifferent is helpful.

L. Completing the Total Quality Control System

When the details of the elements discussed in previous sections are compiled, the result is essentially the plant's "operating manual." It will also serve as the plant's total quality control system.

Upon completion, it should be reviewed. In some cases, a definition or description may be needed for such points as control limits, variability in weights, or number of defects per sample. Also, all critical control points should be covered.

In addition, those sections of the FDA's good manufacturing practice regulations applicable to the operations of the plant must be listed. For each, identify the specific part of the quality control system which is designated to assure compliance.

If one or more full-time quality control personnel are employed at the plant, an organizational chart should be included showing how they fit into the plant's management structure. If there are no full-time quality personnel,

identify who will assume specific responsibilities for quality control and list all other duties of that employee.

When the proposed total quality control system is completed, it is ready to be submitted to the company's management. Let us wish the best of luck to the officer who prepares the plan.

ACKNOWLEDGMENT

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78 Retail Foods Sanitation: Prerequisites to HACCP

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Chicago, Illinois

CONTENTS

I. BACKGROUND

When we talk about retail food protection, we include many categories of business that deal with food e.g., restaurants, cafeterias (schools, prisons, hospitals), public eating places (fairgrounds, events) and so on. As far as health agencies are concerned, delis, grocery stores, etc. are also considered as retail food business. There is no doubt that eventually all large restaurants and cafeterias will be required to implement a HACCP system. Before it can be implemented, most state regulatory agencies want to make sure that all such foodservice establishments have safety programs in place that serve as the foundation or prerequisites to a workable HACCP program. This chapter discusses such fundamental safety practices and how they can eventually be incorporated into a sound HACCP program. The information has been modified from the Food Code.

The discussion for this chapter will be much facilitated by referring to a glossary of terms frequently mentioned and provided by the Food Code as indicated in the next section.

II. GLOSSARY

Some important terms in the glossary are:

Approved source — acceptable to the regulatory authority based on a determination of conformity with principles, practices, and generally recognized standards that protect public health.

Bacteria — living single-cell organisms. Bacteria can be carried by water, wind, insects, plants, animals, and people and survive well on skin and clothes and in human hair. They also thrive in scabs, scars, the mouth, nose, throat, intestines, and room-temperature foods.

CCP — Critical Control Point.

Contamination — the unintended presence in food of potentially harmful substances, including microorganisms, chemicals, and physical objects.

Cross-contamination — the transfer of harmful substances or disease-causing microorganisms to food by hands, food-contact surfaces, sponges, cloth towels and utensils that touch raw food, are not cleaned, and then touch ready-to-eat foods. Cross contamination can also occur when raw food touches or drips onto cooked or ready-to-eat foods.

Corrective action — an activity that is taken by a person whenever a critical limit is not met.

Critical Control Point (CCP) — an operational step or procedure in a process, production method, or recipe, at which control can be applied to prevent, reduce, or eliminate a food safety hazard.

Critical limit — a measurable limit at a CCP that can be monitored to control the identified hazard to a safe level in the food.

Fish — fresh or saltwater finfish, crustaceans and other forms of aquatic life (including alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, if such life is intended for human consumption; includes an edible human food product derived in whole or in part from fish, including fish that have been processed in any manner.

Food — raw, cooked, or processed edible substance, ice, beverage, chewing gum, or ingredient used or intended for use or for sale in whole or in part for human consumption.

Food establishment — an operation at the retail level, i.e., that serves or offers food directly to the consumer and that, in some cases, includes a production, storage, or distributing operation that supplies the direct-to-consumer operation.

Foodborne illness — sickness resulting from acquiring a disease that is carried or transmitted to humans by food containing harmful substances.

Foodborne outbreak — the occurrence of two or more people experiencing the same illness after eating the same food.

HACCP — Hazard Analysis Critical Control Points.

HACCP plan — a written document which is based on the principles of HACCP and which describes the procedures to be followed to ensure the control of a specific process or procedure.

HACCP system — the result of implementing the HACCP principles in an operation that has a foundational, comprehensive, prerequisite program in place. A HACCP system includes the HACCP plan and all SOPs.

Hazard — a biological, physical, or chemical property that may cause a food to be unsafe for human consumption.

Internal temperature — the temperature of the internal portion of a food product.

Meat — the flesh of animals used as food including the dressed flesh of cattle, swine, sheep, or goats and

other edible animals, except fish, poultry, and wild game animals.
Microorganism — a form of life that can be seen only with a microscope, including bacteria, viruses, yeast, and single-celled animals.
Molluscan shellfish — any edible species of raw fresh or frozen oysters, clams, mussels, and scallops or edible portions thereof, except when the scallop product consists only of the shucked adductor muscle.
Monitoring — the act of observing and making measurements to help determine whether critical limits are being met and maintained.
National Shellfish Sanitation Program (NSSP) — the voluntary system by which regulatory authorities for shellfish harvesting waters and shellfish processing and transportation and the shellfish industry implement specified controls to ensure that raw and frozen shellfish are safe for human consumption.
NSSP — National Shellfish Sanitation Program.
Operational step — an activity in a food establishment, such as receiving, storage, preparation, cooking, etc.
Parasite — an organism that grows, feeds, and is sheltered on or in a different organism and contributes to its host.
Pathogen — a microorganism (bacteria, parasites, viruses, or fungi) that is infectious and causes disease.
Personal hygiene — individual cleanliness and habits.
Potentially hazardous food — a food that is natural or synthetic and that requires temperature control because it is capable of supporting 1) the rapid and progressive growth of infectious or toxigenic microorganisms, 2) the growth and toxin production of *Clostridium botulinum*, or 3) in raw shell eggs, the growth of *Salmonella enteritidis*.
Potentially hazardous food — includes foods of animal origin that are raw or heat-treated; foods of plant origin that are heat-treated or consists of raw seed sprouts; cut melons; and garlic and oil mixtures that are not acidified or otherwise modified at a processing plant in a way that results in mixtures that do not support growth of pathogenic microorganisms as described above.
Procedural step — an individual activity in applying this guide to a food establishment's operations.
Process approach — a method of categorizing food operations into one of three modes: 1) Process number one: Food preparation with no cook step wherein ready-to-eat food is stored, prepared, and served; 2) Process number two: Food preparation for same day service wherein food is stored, prepared, cooked, and served; or 3) Process number three: Complex food preparation wherein food is stored, prepared, cooked, cooled, reheated, hot held, and served.
Ready-to-eat food — a food that is in a form that is edible without washing, cooking, or additional preparation by the food establishment or consumer and that is reasonably expected to be consumed in that form and includes potentially hazardous food that has not been cooked; raw, washed, cut fruits and vegetables; whole, raw, fruits and vegetables that are presented for consumption without the need for further washing, such as at a buffet; and other food presented for consumption for which further washing or cooking is not required and from which rinds, peels, husks, or shells have been removed.
Record — a documentation of monitoring observation and verification activities.
Regulatory authority — a federal, state, local, or tribal enforcement body or authorized representative having jurisdiction over the food establishment.
Risk — an estimate of the likely occurrence of a hazard.
SOP — Standard Operating Procedure.
Shellfish — bivalve molluscan shellfish.
Standard operating procedure (SOP) — a written method of controlling a practice in accordance with predetermined specifications to obtain a desired outcome.
Temperature measuring device — a thermometer, thermocouple, thermistor, or other device for measuring the temperature of food, air, or water.
Toxin — a poisonous substance that may be found in food.
Verification — the use of methods, procedures, or tests by supervisors, designated personnel, or regulators to determine if the food safety system based on the HACCP principles is working to control identified hazards or if modifications need to be made.
Virus — a protein-wrapped genetic material which is the smallest and simplest life-form known, such as hepatitis A.

III. PREREQUISITE PROGRAMS

A. Food Code

The provisions of the Food Code provide a foundation on which to develop a food safety system based on the principles of HACCP. Major interventions in the Food Code demonstrate the knowledge of the person-in-charge, regarding employee health, avoiding contact with ready-to-eat food with bare hands, time and temperature control, and the use of a consumer advisory regarding the consumption of raw or undercooked animal foods. These interventions need to be addressed within the overall food safety program which may entail inclusion in SOPs (Standard Operation Procedures) which can be considered as the major frame of reference of sanitation and HACCP.

B. Standard Operation Procedures

1. Introduction

Many provisions of the Food Code address the design of food establishments and equipment as well as acceptable operational practices. Adherence to design criteria and

development of SOPs affect the food preparation environment. Both are considered prerequisite to the development of food safety systems based upon the HACCP principles. SOPs specify practices to address general hygiene and measures to prevent food from becoming contaminated during storage, preparation or service. When SOPs are in place, HACCP can be more effective because it changes the focus from the food preparation facility to the hazards associated with the food and its preparation.

SOPs specific to your operation describe the activities necessary to complete tasks that accomplish compliance with the Food Code, are documented as a written reference, and are used to train the staff who are responsible for the tasks.

Three purposes for establishing SOPs for your operation are to protect your products from contamination from microbial, chemical, and physical hazards; to control microbial growth that can result from temperature abuse; and to ensure procedures are in place for maintaining equipment.

SOP procedures ensure that:

1. Products are purchased from approved suppliers/sources
2. The water in contact with food and food-contact surfaces and used in the manufacture of ice is potable
3. Food-contact surfaces, including utensils are cleaned, sanitized, and maintained in good condition
4. Uncleaned and nonsanitized surfaces of equipment and utensils do not contact raw or cooked ready-to-eat food
5. Raw animal foods do not contaminate raw or cooked ready-to-eat food
6. Toilet facilities are accessible and maintained
7. Handwashing facilities are located in food preparation, food dispensing, warewashing areas, and immediately adjacent to toilet rooms and are equipped with hand cleaning preparations and single-service towels or acceptable hand drying devices
8. An effective pest control system is in place
9. Toxic compounds are properly labeled, stored, and safely used
10. Contaminants such as condensate, lubricants, pesticides, cleaning compounds, sanitizing agents, and additional toxic materials do not contact food, food packaging material, and food-contact surfaces
11. Food, food packaging materials, and food-contact surfaces do not come in contact with, and are not contaminated by physical hazards such as broken glass from light fixtures, jewelry, etc.

2. SOPs to Control Contamination of Food

Procedures must be in place to ensure that proper personnel health and hygienic practices are implemented including:

1. Restricting or excluding workers with certain symptoms such as vomiting or diarrhea
2. Practicing effective handwashing
3. Restricting eating, smoking, and drinking in food preparation areas
4. Using hair restraints
5. Wearing clean clothing
6. Restricting the wearing of jewelry

3. Control Microbial Growth

These procedures ensure that all potentially hazardous food is received and stored at a refrigerated temperature of 41°F (5°C) or below. Note that the Food Code makes some allowances for specific foods that may be received at higher temperatures.

4. Maintain Equipment

These procedures ensure that:

1. Temperature measuring devices (e.g., thermometer or temperature recording device) are calibrated regularly
2. Cooking and hot holding equipment (grills, ovens, steam tables, conveyor cookers, etc.) are routinely checked, calibrated if necessary and are operating to ensure correct product temperature
3. Cooling equipment (refrigerators, rapid chill units, freezers, salad bars, etc.) are routinely checked, calibrated if necessary and are operating to ensure correct product temperature
4. Warewashing equipment is operating according to manufacturer's specifications

C. The Flow of Food

The flow of food, which is the path that food follows from receiving through serving, is important for determining where potentially significant food safety hazards may occur. At each operational step in the flow, active management of food preparation and processes is an essential part of business operations. With a HACCP system, you set up control measures to protect food at each stage in the process.

The illustrations of food processes listed below are not intended to be all inclusive. For instance, quick-service, full-service, and institutional providers are major types of food service operations. Each of these has its own individual food safety processes. These processes are likely to be different from a deli in a retail food store.

Some operations may have all three types of processes or variations of the three. Identifying the flow of food processing specific to your operation is an important part of providing a framework for developing a food safety management system.

D. Food Processing with No Cook Step

1. Receive — Store — Prepare — Hold — Serve

As mentioned in the Introduction, the important feature of this type of process is the absence of a cooking step. Heating foods destroys bacteria, parasites, and viruses, and is often a CCP. But since this particular food flow does not include cooking, there is no step that will eliminate or kill bacteria, parasites, or viruses. An example is tuna salad that is prepared and served cold. Control in this process will focus on preventing:

1. Bacterial growth (e.g., storage under refrigeration)
2. Contamination from employees (e.g., restriction of employees ill with diarrhea, proper handwashing, preventing bare hand contact with ready-to-eat foods, etc.)
3. Cross-contamination from other foods (e.g., raw to ready-to-eat)
4. Cross-contamination from soiled equipment (e.g., cleaning and sanitizing)
5. Obtaining foods from approved sources (e.g., a supplier of raw fish for sushi who adequately freezes fish to control parasites)

You should also think about some other factors.

1. Are there any ingredients or menu items of special concern? (see Food Code)
2. Is this a potentially hazardous food requiring specific temperature controls?
3. How will it be served? Immediately? On a buffet?
4. Does this food have a history of being associated with illnesses?
5. Will this menu item require a great deal of preparation, making preparation time, employee health, and bare hand contact with ready-to-eat food a special concern?
6. How will an employee ill with diarrhea be restricted from working with food?
7. Are you serving food to a population that is known to be highly susceptible to foodborne illness (e.g., residents of health care facilities, persons in child or adult day care facilities, etc.)?

E. Food Preparation for Same Day Service

1. Receive — Store — Prepare — Cook — Hold — Serve

In this process, a food is prepared and served the same day. The food will be cooked and held hot until service, such as chili. Generally, the food will pass through the temperature danger zone only once before it is served to the customer, thus minimizing the opportunity for bacterial growth.

The preparation step may involve several processes, including thawing a frozen food, mixing in other ingredients, or cutting or chopping. It is important to remember that added ingredients may introduce additional contaminants to the food. Cutting or chopping must be done carefully so that cross contamination from cutting boards, utensils, aprons, or hands does not occur. Control points at this operational step include good sanitation and handwashing.

During cooking, food will be subjected to hot temperatures that will kill most harmful bacteria, parasites, and viruses that might be introduced before cooking, making cooking a CCP. It is the operational step where raw animal foods are made safe to eat, and therefore, time and temperature measurement is very important. Temperature of foods during hot holding must be maintained until service so that harmful bacteria do not survive and grow.

F. Complex Processes

1. Receive — Store — Prepare — Cook — Cool — Reheat — Hot Hold — Serve

Failure to adequately control food product temperature is the one factor most commonly associated with foodborne illness. Foods prepared in large volumes or in advance for next day service usually follow an extended process flow. These foods are likely to pass through the temperature danger zone several times. The key in managing the operational steps within the process is to minimize the time foods are at unsafe temperatures.

In some cases, a variety of foods and ingredients that require extensive employee product preparation may be part of the process. A sound food safety management system will incorporate SOPs for personal hygiene and cross contamination prevention throughout the flow of the food.

Before you set up a management system for your operational steps, there are several factors you should consider. Multiple step processes require proper equipment and facilities. Your equipment needs to be designed to handle the volume of food you plan to prepare. For example, if you use a process that requires the cooling of hot food, you must provide equipment that will adequately and efficiently lower the food temperature as quickly as possible. If you find that a recipe is too hard to prepare safely, you may want to consider purchasing pre-prepared items from a reputable source.

IV. GETTING STARTED

The most effective way to get started is to use a team approach to design and implement a plan based on the HACCP principles. A team could be comprised of the owner and the chef or cook. Although managers are responsible for designing the system, implementation involves the efforts and commitment of every employee. Education and training of both management and employees in their respective roles of producing safe foods are important. You may consider working with outside consultants, university extension services, and regulatory authorities to ensure your HACCP system is based on the best available science and will control identified hazards.

V. PROCEDURAL STEP 1: GROUP MENU ITEMS

To get started, review how your menu items flow through your operation, note whether they undergo a cook step for same day serving, receive additional cooling and reheating following a cook step, or have no cook step involved. Refer to the Food Code for organizing your menu items by Process 1, 2, and 3.

Looking at your menu, place each menu item or similar menu items (like "hot soups" or "cold salads") into the appropriate group. You may discover that more than one food process is conducted within your operation. These menu items may pose special hazards that are not always readily apparent. To accomplish the first procedural step in developing your food safety management system, identify the food processes specific to your menu items.

A. Process-Specific Lists

List your menu items that belong to one of the three processes.

Chart 1: Process-Specific Lists

Process #1	Process #2	Process #3
List menu foods:	List menu foods:	List menu foods:
Examples:	Examples:	Examples:
Salad greens	Hamburgers	Soups
Fish for sushi	Soup du jour	Gravies
Fresh vegetables	Hot vegetables	Sauces
Oysters or clams served raw	Entrees for "special of the day"	Large roasts
Tuna salad	Cooked eggs	Chili
Caesar salad dressing		Taco filling
Coleslaw		Egg rolls
Sliced sandwich meats		
Sliced cheese		

Process number 1
Food preparation with no cook step — ready-to-eat food that is stored, prepared, and served.

Process number 2
Food preparation for same day service — food that is stored, prepared, cooked, and served.

Process number 3
Complex food preparation — food that is stored, prepared, cooked, cooled, reheated, hot held, and served.

VI. PROCEDURAL STEP 2: CONDUCT HAZARD ANALYSIS

In developing a food safety system, you need to identify the hazards that exist in the flow of foods in your operation from receiving to serving. Hazards include:

1. Pathogens or toxins present in food when you receive them
2. Pathogens that may be introduced during preparation (e.g., using a raw animal food as one ingredient)
3. Pathogen growth or toxin production during storage, preparation, or holding
4. Pathogens or toxins that survive heating
5. Contaminants (i.e., pathogens, chemicals, physical objects), that are introduced to food by food workers or equipment

Since you have grouped your menu items, including ingredients, into the three processes on Chart 1, you can identify hazards that are associated with each process. You will see that the more complex the process is, the greater are the opportunities for hazards to occur.

In consultation with your regulatory authority, you need to identify the hazards associated with various foods and ingredients, such as:

1. *Salmonella* and *Campylobacter jejuni* in raw poultry
2. *E. Coli* O157:H7 in raw ground beef
3. *Staphylococcus aureus* toxin formation in cooked ham
4. *Bacillus cereus* spore survival and toxin formation in cooked rice
5. *Clostridium perfringens* spore survival and subsequent growth in cooked foods
6. Hazards specific to seafood (see Food Code)

This list is only a brief sample of hazards associated with specific foods. By identifying the hazards, you will be able to determine CCPs and critical limits on the worksheet. Another way of fulfilling the hazard analysis step is to understand the hazards associated with your specific menu items and to adhere to the critical limits established in the Food Code. Those critical limits are based on the anticipated hazards.

A. Food Safety Management Worksheets and Summaries for Operational Steps

Worksheets and summaries are provided to enable you to:

1. Identify those operational steps in the food flow that are specific to your operation
2. Write in your SOPs which are the general procedures that cross all flows and products
3. Reference the CCPs and critical limits pertaining to those process steps
4. Develop monitoring procedures and corrective actions which are customized to fit your operation
5. Consider the type of record keeping you need to document you are controlling significant food safety hazards

HACCP allows the flexibility for you to customize a food safety management system specific to your operations. The worksheets are provided to assist you in developing procedures to:

1. Monitor CCPs
2. Take corrective actions when critical limits are not met
3. Establish a verification procedure
4. Establish a record keeping system

Review the following worksheets and the summary page for each operational step. Determine the ones that are applicable to your operation and make copies of them so you can fill in your groupings of menu items (which you did preliminarily in Procedural Step 1). Then continue to use the forms and complete the information as you work through Procedural Steps 3 through 9.

1. Receiving

At receiving, your main concern is contamination from pathogens and the formation of harmful toxins. Obtaining food from approved sources and at proper temperatures are important purchase specifications for preventing growth and contamination during receiving. Approved sources are suppliers who are regulated and inspected by appropriate regulatory authorities.

Ready-to-eat, potentially hazardous food is a special concern at receiving. Because this food will not be cooked before service, microbial growth could be considered a significant hazard for receiving refrigerated, ready-to-eat-foods. Having SOPs in place to control product temperature is generally adequate to control the hazards present at receiving of these products. Besides checking the product temperature, you will want to check the appearance, odor, color, and condition of the packaging.

Federal regulations require that processors of seafood and seafood products for interstate distribution have a HACCP plan. These establishments are approved sources for seafood, and you may ask your interstate seafood supplier for documentation that the firm has a HACCP plan in place. Processors of seafood and seafood products that are sold or distributed only within a state may or may not be required to have a HACCP plan, depending on the state, local, or tribal regulations.

Special consideration should be given to certain species of finfish and raw molluscan shellfish. Molluscan shellfish (oysters, clams, mussels, and scallops) that are received raw in the shell or shucked must be purchased from suppliers who are listed on the FDA Interstate Certified Shellfish Shippers' List or on a list maintained by your state shellfish control authority. Shellfish received in the shell must bear a tag (or a label for shucked shellfish) which states the date and location of harvest, in addition to other specific information.

Finfish harvested from certain areas may naturally contain a certain toxin that is not readily apparent. This toxin is called ciguatera. Other finfish may develop toxins after harvest if strict temperature control is not maintained. This toxin is called scombrotoxin. Temperature control is important at receiving because this toxin can not be eliminated by cooking. For more information on toxins in reef finfish, histamine formation in certain species, and parasites in raw finfish requiring control, refer to the Food Code.

Operational Step 1: Receiving

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Examples: Salads Sushi	Microbial contamination Bacterial growth Parasites Scombrotoxin Ciguatera or other toxin contamination Chemical contamination	Yes _____ or No _____	Receive at 41°F or below Approved source Seafood HACCP plan Proper chemical storage/use				

(*Continued*)

Operational Step 1: Receiving
(*Continued*)

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 2	Examples: Hamburgers Mahi-mahi	Microbial contamination Bacterial growth Scombrotoxin Ciguatera or other toxin contamination Chemical contamination	Yes _____ or No _____	Receive at 41°F or below Approved source Seafood HACCP plan Proper chemical storage/use				
Process 3	Example: Soups	Microbial contamination Bacterial growth Ciguatera or other toxin contamination Scombrotoxin Chemical contamination	Yes _____ or No _____	Receive at 41°F or below Approved source Seafood HACCP plan Proper chemical storage/use				

2. Storage

When food is in refrigerated storage, your management system should focus on preventing the growth of bacteria that may be present in the product. This is primarily achieved through temperature control. Special attention needs to be given to controlling and monitoring the temperatures of potentially hazardous ready-to-eat foods.

When determining the monitoring frequency of product storage temperature, it is important to make sure that the interval between temperature checks is established to ensure that the hazard is being controlled and time is allowed for an appropriate corrective action. For example, If you are storing potentially hazardous ready-to-eat foods under refrigeration, you may decide to set a critical limit for the refrigeration units to operate at 41°F (5°C) or below. You may also want to set a target, or operating limit, of 40°F (4.4°C) for example, in order to provide a safety cushion that allows you the opportunity to see a trend toward exceeding 41°F (5°C) and to intervene with appropriate corrective actions.

Monitoring procedures for ready-to-eat food ideally include internal product temperature checks. You need to assess whether it is realistic and practical for you to do this, depending on the volume of food you are storing.

You may choose to base your monitoring system on the air temperature of the refrigerated equipment as an SOP. How often you need to monitor the air temperature depends on:

1. Whether the air temperature of the refrigerator accurately reflects the internal product temperature — (remember, your food safety refrigeration temperature must be based on the internal product temperature of the food stored within a refrigeration unit, not the air temperature)
2. The capacity and use of your refrigeration equipment
3. The volume and type of food products stored in your cold storage units
4. The SOPs that support monitoring this process
5. Shift changes and other operational considerations

Standard operating procedures can be developed to control some hazards and assist in implementing a food safety system that minimizes the potential for bacterial growth and contamination. The control of cross contamination can be done by separating raw foods from ready-to-eat products within your operation's refrigeration and storage facilities.

Special consideration should be given to the storage of scombroid fish due to the potential formation of histamine, a chemical hazard. To control histamine formation in scombroid toxin-forming fish, it is recommended that storage be a CCP with the critical limit not to exceed 41°F (5°C), as stated in the Food Code, unless you can show through scientific data that the food safety hazard will not result.

3. Preparation

Of all the operational steps in food processes, preparation has the greatest variety of activities that must be controlled,

Operational Step 2: Storage

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Example: Salads Sushi	Bacterial growth Cross contamination Parasites Chemical contamination	Yes _____ or No _____	Store at 41°F or below Separate raw from ready-to-eat food Freeze fish to be consumed raw @ −4°F for 7 days or −31°F for 15 hours Proper chemical storage/use				
Process 2	Example: Hamburgers Mahi-mahi	Bacterial growth Scombrotoxin Cross contamination Chemical contamination	Yes _____ or No _____	Store at 41°F or below Separate raw from ready-to-eat food Proper chemical storage/use				
Process 3	Example: Soups	Bacterial growth Scombrotoxin Cross contamination Chemical contamination	Yes _____ or No _____	Store at 41°F or below Separate raw from ready-to-eat food Proper chemical storage/use				

monitored, and in some cases documented. It is impossible to include in this model a summary guide that covers the diversity in menus, employee skills, and facility design that impact the preparation of food. The preparation step may involve several processes, including thawing a frozen food, mixing together several ingredients, cutting, chopping, slicing, or breading.

At the preparation step, SOPs can be developed to control some hazards and assist in implementation of a food safety system that minimizes the potential for bacterial growth and contamination from employees and equipment.

Front-line employees will most likely have the greatest need to work with the food. A well-designed personal hygiene program that has been communicated to all employees will minimize the potential for bacterial, parasitic, and viral contamination. Your program must include instructions to your employees as to when and how to wash their hands. Procedures need to be in place that either eliminate employee hand contact with ready-to-eat foods, or implement an alternative personal hygiene program that provides an equivalent level of control of bacterial, parasitic, and viral hazards. It is also very important to identify and restrict ill employees from working with food, especially if they have diarrhea.

Procedures must be in place to prevent cross contamination from utensils and equipment. Designated areas or procedures that separate the preparation of raw foods from ready-to-eat foods minimize the potential for bacterial contamination. Proper cleaning and sanitizing of equipment and work surfaces are an integral SOP to this operational step.

Batch preparation is an important tool for controlling bacterial growth because limiting the amount of food prepared minimizes the time the food is kept at a temperature that allows growth. Planning your preparation ahead assists in minimizing the time food must be out of temperature at this operational step. Batch preparation also breaks the growth cycle of bacteria before they can reach dangerous levels.

When thawing frozen foods, maintaining proper product temperature and managing time are the primary controls for minimizing bacterial growth. Procedures need to be in place to minimize the potential for microbial, chemical, and physical contamination during thawing.

Use of prechilled ingredients to prepare a cold product, such as tuna salad, will assist you in maintaining temperature control for this process.

Special consideration should be given to disallowing bare hand contact in the preparation of ready-to-eat foods. You need to control the introduction of hazards during preparation. How will you accomplish controlling the hazard presented by hand contact with ready-to-eat food? You should review your operation to determine whether this operational step will be controlled as a CCP or an SOP.

4. Cooking

This operational step only applies to those foods that you have listed in Processes #2 and #3. Cooking foods of animal

Operational Step 3: Preparation

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Example: Salads	Bacterial growth Cross contamination Contamination from employees Chemical contamination	Yes _____ or No _____	Store at 41°F or below or use time to control growth Separate raw from ready-to-eat food Restrict ill employees; control bare hand contact Proper chemical storage/use				
Process 2	Examples: Hamburgers Mahi-mahi	Bacterial growth Cross contamination Contamination from employees Chemical contamination	Yes _____ or No _____	Store at 41°F or below or use time to control growth Separate raw from ready-to-eat food Restrict ill employees; control bare hand contact Proper chemical storage/use				
Process 3	Example: Soups	Bacterial growth Cross contamination Contamination from employees Chemical contamination	Yes _____ or No _____	Store at 41°F or below or use time to control growth Separate raw from ready-to-eat food Restrict ill employees; control bare hand contact Proper chemical storage/use				

origin is the most effective operational step in food processes for reducing and eliminating biological contamination. Hot temperatures will kill most harmful bacteria and with relatively few exceptions, such as cooking plant foods, this is a CCP. It is at this step that food will be made safe to eat. Therefore, product temperature and time measurements are very important. If the appropriate product temperature for the required amount of time is not achieved, bacteria, parasites, or viruses may survive in the food.

Critical time and temperature limits vary according to the type of food. Employees should view ensuring proper cooking temperatures as an essential element in producing an acceptable product. A final cooking time and temperature chart for specific foods is included for your review. Simply reference the foods specific to your food establishment and incorporate the appropriate critical time and temperature limits into your management system.

You will need to determine the best system for you to use that will ensure that the proper cooking temperature and time are reached. Checking the internal product temperature is the most desirable monitoring method. However, when large volumes of food are cooked, a temperature check of each individual item may not be practical. For instance, a quick service food service operation may cook several hundred hamburgers during lunch. If checking the temperature of each hamburger is not reasonable for you to do, then you need to routinely verify that the specific process and cooking equipment are capable of attaining a final internal product temperature at all locations in or on the cooking equipment.

Once a specific process has been shown to work for you, the frequency of record keeping may be reduced. In these instances, a record keeping system should be established to provide scheduled product temperature checks to ensure that the process is working.

Special consideration should be given to time and temperature in the cooking of fish and other raw animal foods. To control the pathogens, it is recommended that cooking be a CCP, based upon the critical limits established by the Food Code, unless you can show through scientific data that the food safety hazard will not result.

5. Cooling

This operational step is only used for those foods that you have listed in Process #3. One of the most labor intensive operational steps is rapidly cooling hot foods to control microbial growth. Excessive time for the cooling of potentially hazardous foods has been consistently identified as one of the factors contributing to foodborne illness. Foods

Operational Step 4: Cooking

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Example: Salads Sushi	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply
Process 2	Examples: Hamburgers Mahi-mahi	Bacterial, parasitic, or viral survival or growth	Yes ___ or No ___	Cook to Product Internal Temp Time See Chart 2				
Process 3	Example: Soups	Bacterial, parasitic, or viral survival or growth	Yes ___ or No ___	Same as Process #2				

Chart 2: Food Code Cooking Temperatures and Times

Product	Final Internal Temperature	Time
1a. Poultry Wild Game Animals Stuffed Fish Stuffed Meat Stuffed Pasta Stuffed Poultry Stuffed Ratites or Stuffing containing Fish Meat Poultry or Ratites	1a. 165°F	1a. 15 seconds
1b. Animal foods cooked in a microwave oven	1b. 165°F; food rotated, stirred, covered	1b. Cover and allow to stand for 2 minutes
2a. Pork, ratites, or injected meats	2a. 155°F	2a. 15 seconds
2b. Ground meat, fish, or game animals commercially raised for food	2b. 155°F	2b. 15 seconds
2c. Game Animals under a voluntary inspection program	2c. 155°F	2c. 15 seconds
2d. Raw shell eggs that are NOT prepared for immediate service	2d. 155°F	2d. 15 seconds
3a. Raw shell eggs broken and prepared in response to consumer order and for immediate service	3a. 145°F	3a. 15 seconds
3b. Fish and Meat including Game Animals except as specifically referenced on this chart	3b. 145°F	3b. 15 seconds
4a. Fruit and vegetables cooked for hot holding	4a. 140°F or above	4a. Instantaneous
4b. Ready-to-eat food from a commercially sealed container for hot holding	4b. 140°F or above	4b. Instantaneous
4c. Ready-to-eat food from an intact package (from a food processing plant inspected by the regulatory authority with jurisdiction over the plant) for hot holding	4c. 140°F or above	4c. Instantaneous

(*Continued*)

Chart 2: Food Code Cooking Temperatures and Times (*Continued*)

Product	Final Internal Temperature	Time
5a. Beef Roast/Corned Beef Roasts Preheated Oven Temperatures	5a. LESS THAN 10 lbs. (i) Still dry: 350°F or more (ii) Convection: 325°F or more (iii) High Humidity: 250°F or less MORE THAN 10 lbs (i) Still dry: 250°F or more (ii) Convection: 250°F or more (iii) High Humidity: 250°F or less	
5b. Beef Roast/Corned Beef Roasts Internal Food Temperature for Specified Amount of Time	5b. ACHIEVE ONE OF THE FOLLOWING: (i) 130°F for 121 Minutes (ii) 132°F for 77 Minutes (iii) 134°F for 47 Minutes (iv) 136°F for 32 Minutes (v) 138°F for 19 Minutes (vi) 140°F for 12 Minutes (vii) 142°F for 8 Minutes (viii) 144°F for 5 Minutes (ix) 145°F for 3 Minutes	

Operational Step 5: Cooling

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Examples: Salads Sushi	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply
Process 2	Examples: Hamburgers Mahi-mahi	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply
Process 3	Example: Soups	Bacterial growth Cross contamination Contamination from employees or equipment	Yes ___ or No ___	Cool food from 140°F to 70°F within 2 hours and from 70°F to 41°F within 4 hours Separate raw from ready-to-eat foot Restrict ill employees; control bare hand contact				

that have been cooked and held at improper temperatures provide an excellent environment for the growth of disease causing microorganisms that may have survived the cooking process (spore-formers). Recontamination of a cooked food item by poor employee practices or cross contamination from other food products, utensils and equipment is a concern at this operational step.

Special consideration should be given to large food items, such as roasts, turkeys, thick soups, stews, chili, and large containers of rice or refried beans. These foods take a long time to cool because of their mass and volume. If the hot food container is tightly covered, the cooling rate will be further slowed down. By reducing the volume of the food in an individual container and leaving an opening for heat to escape by keeping the cover loose, the rate of cooling is dramatically increased.

Commercial refrigeration equipment is designed to hold cold food temperatures, not cool large masses of food. Some alternatives for cooling foods include:

1. Using rapid chill refrigeration equipment designed to cool the food to acceptable

temperatures quickly by using increased compressor capacity and high rates of air circulation.
2. Avoiding the need to cool large masses by preparing smaller batches closer to periods of service.
3. Stirring hot food while the food container is within an ice water bath.
4. Redesigning your recipe so that you prepare and cook a smaller or concentrated base and then add enough cold water or ice to make up the volume that you need. This may work for some water-based soups, for example.

Whatever cooling method you choose, you need to verify that the process works. Once again if a specific process has been shown to work for you, the frequency of record keeping may be reduced. A record-keeping system should be established to provide scheduled product temperatures checks to ensure the process is working.

6. Reheating

This operational step applies only to those foods that you listed in Process #3. If food is held at improper temperatures for enough time, pathogens have the opportunity to multiply to dangerous numbers. Proper reheating provides an important control for eliminating these organisms. It is especially effective in reducing contamination from from Staph toxin. Along with personal hygiene, preventing cross contamination through the use of cleaned and sanitized equipment and utensils is an important control measure.

Special consideration should be given to the time and temperature in the reheating of cooked foods. To control the pathogens, it is recommended that reheating be a CCP, based upon the critical limits established by the Food Code, unless you can show through scientific data that the food safety hazard will not result.

7. Holding

All three processes may involve holding. Proper temperature of the food while being held is essential in controlling the growth of harmful bacteria. Cold temperature holding may occur in Processes 1, 2, or 3. Hot temperature holding occurs primarily only in Processes 2 and 3. Where there is a cooking step as a CCP to eliminate pathogens, all but the spore-forming organisms should be killed or inactivated. If cooked food is not held at the proper temperature, the rapid growth of these spore-forming bacteria is a major food safety concern.

When food is held, cooled, and reheated in a food establishment there is an increased risk from contamination caused by personnel, equipment, procedures, or other factors. Harmful bacteria that are introduced into a product that is not held at proper temperature have the opportunity to

Operational Step 6: Reheating

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Examples: Salads Sushi	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply
Process 2	Examples: Hamburgers Mahi-mahi	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply	Does not apply
Process 3	Example: Soups	Bacterial, parasitic or viral survival or growth	Yes _____ or No _____	Reheat to 165°F within 2 hours				

bacterial spore-formers which survived the cooking process and may have multiplied because foods were held at improper temperatures.

Although proper reheating will kill most organisms of concern, it will not eliminate toxins, such as that produced by *Staphylococcus aureus*. If microbial controls and SOPs at previous operational steps have not been followed correctly and Staph toxin has been formed in the food, reheating will not make the food safe.

Incorporating a comprehensive personal hygiene program throughout the process will minimize the risk multiply to large numbers in a short period of time. Once again management of personal hygiene and the prevention of cross contamination impact the safety of the food at this operational step.

Keeping food products at 140°F (60°C) or above during hot holding and keeping food products at or below 41°F (5°C) is effective in preventing microbial growth. As an alternative to temperature control, the Food Code details actions when time alone is used as a control, including a comprehensive monitoring and food marking system to ensure food safety.

Operational Step 7: Holding

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Examples: Salads Sushi	Bacterial, parasitic, or viral introduction, survival, or growth	Yes _____ or No _____	41°F				
Process 2	Examples: Hamburgers Mahi-mahi	Bacterial, parasitic, or viral introduction, survival, or growth	Yes _____ or No _____	140°F or 41°F				
Process 3	Example: Soups	Bacterial, parasitic, or viral introduction, survival, or growth	Yes _____ or No _____	140°F or 41°F				

How often you monitor the temperature of foods during hot holding determines what type of corrective action you are able to take when 140°F (60°C) is not met. If the critical limit is not met, your options for corrective action may include evaluating the time the food is out of temperature to determine the severity of the hazard and based on that information, reheating the food, if appropriate, or discarding it. Monitoring frequency may mean the difference between reheating the food to 165°F (74°C) or discarding it.

When determining the monitoring frequency of cold product temperatures, it is important to make sure that the interval between temperature checks is established to ensure that the hazard is being controlled and time is allowed for an appropriate corrective action. For example, If you are holding potentially hazardous ready-to-eat foods under refrigeration, such as potato salad at a salad bar, you may decide to set a critical limit at 41°F (5°C) or below. You may also want to set a target, or operating limit, of 40°F (4.4°C) for example, in order to provide a safety cushion that allows you the opportunity to see a trend toward exceeding 41°F (5°C) and to intervene with appropriate corrective actions.

Special consideration should be given to the time and temperature in the hot or cold holding of potentially hazardous foods to control pathogens. It is recommended that hot or cold holding be a CCP, based upon the critical limits established by the Food Code, unless you can show through scientific data that the food safety hazard will not result.

8. Setup and Packing

Setup and packing is an operational step used by some retail food establishments including caterers (e.g., restaurant/caterer or interstate conveyance caterer), commissaries, grocery stores (for display cases), schools, nursing homes, hospitals, or services such as delivery of meals to home-bound persons. Setup and packing can be controlled through an SOP and may involve wrapping food

Operational Step 8: Setup and Packing

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Examples: Salads Sushi	Bacterial Growth Microbial contamination from employees	Yes _____ or No _____	41°F No bare hand contact or equivalent alternative				
Process 2	Examples: Hamburgers Mahi-mahi	Bacterial Growth Microbial contamination from employees	Yes _____ or No _____	140°F or 41°F No bare hand contact or equivalent alternative				
Process 3	Example: Soups	Bacterial Growth Microbial contamination from employees	Yes _____ or No _____	140°F or 41°F No bare hand contact or equivalent alternative				

items, assembling these items onto trays, and packing them into a transportation carrier or placing them in a display case. An example would be an airline flight kitchen where food entrees are wrapped, assembled, and placed into portable food carts which are taken to a final holding cooler. Hospital kitchens would be another example where patient trays are assembled and placed into carriers for transportation to nursing stations. Food may be placed into bulk containers for transportation to another site where it is served.

This operational step might not be considered a CCP, but it is a special consideration when setting up your program. This process can be controlled by strict adherence to SOPs to minimize the potential for bacterial contamination and growth, to eliminate bare hand contact with ready-to-eat foods, to ensure proper handwashing, and to ensure food comes into contact with cleaned and sanitized surfaces.

Following final assembly into either individual trays or into bulk containers, the food may be held for immediate service or for transportation to another site for service. This hot holding or cold holding operational step needs to be evaluated in the same manner as other holding operational steps on the worksheet. Temperature control or using time as a control measure during transportation, and holding and serving at a remote site must be evaluated and managed as part of your food safety system.

Special consideration should be given to time/temperature controls and the prevention of cross contamination from equipment and utensils and contamination from employees' hands. This process may be adequately controlled through an SOP; however, holding and transportation should be considered CCPs.

9. Serving

This is the final operational step before the food reaches the customer. When employees work with food and food-contact surfaces, they can easily spread bacteria, parasites, and viruses and contaminate these items. Managing employees' personal hygienic practices is important to controlling these hazards. A management program for employee personal hygiene includes proper handwashing, the appropriate use of gloves and dispensing utensils, and controlling bare hand contact with ready-to-eat foods.

Minimizing the growth of bacteria is also a concern at hot and cold holding customer display areas. Maintaining food products at proper temperature within these display units will control the growth of microorganisms. Refer to the HOLDING worksheet for additional information.

Special consideration needs to be given to minimizing contamination from the customer. Customer self-service displays, such as salad bars, require specific procedures to protect the food from contamination. Some suggestions for protecting food on display include:

1. The use of packaging
2. Counter, service line, or salad bar food guards
3. Display cases
4. Suitable utensils or effective dispensing methods
5. Not mixing an old product with fresh
6. Having employees monitor self-serve stations

Preventing cross contamination from soiled utensils and equipment will minimize the potential for bacterial contamination of ready-to-eat foods.

VII. PROCEDURAL STEP 3: IDENTIFY CCPs AND CRITICAL LIMITS

The CCPs column identifies places in the flow of food where you can have a significant impact in controlling food safety hazards. A measurable critical limit has been identified for each of these CCPs. These critical limits provide the baseline for measuring the effectiveness of your food safety procedures.

For each of your operational steps, within your operation, review the CCPs and critical limits needed to minimize or eliminate significant food safety hazards. Does

Operational Step 9: Serving

Process	Menu Item	Hazard	CCP	Critical Limits	Monitoring	Corrective Actions	Verification	Records
Process 1	Examples: Salads Sushi	Bacterial, parasitic, viral, or physical contamination	Yes _____ or No _____					
Process 2	Examples: Hamburgers Mahi-mahi	Bacterial, parasitic, viral, or physical contamination	Yes _____ or No _____					
Process 3	Example: Soups	Bacterial, parasitic, viral, or physical contamination	Yes _____ or No _____					

your operation currently have control measures in place that are at least equivalent to these critical limits?

On the worksheet, you will need to decide whether the operational step is a CCP or whether the hazard is controlled by your SOPs that address the prerequisite program elements discussed in the Food Code.

In some operational step worksheets, such as the cooking step, the Guide recommends that the step be considered a CCP, because there is no practical alternative to ensure control of the hazard. In other operational steps, you may have a choice as to how you will control the hazard. For example, in the preparation step for ready-to-eat foods, you will identify contamination from employees' hands as a hazard. When controlling that hazard as a CCP, you must also identify the critical limits, establish monitoring and corrective actions, verification procedures, and records. Alternatively, you may choose to control that hazard by instituting an SOP that disallows bare hand contact with ready-to-eat food. You will need to decide the most effective method of controlling the hazard, i.e., as a CCP or through use of an SOP.

VIII. PROCEDURAL STEP 4: MONITOR CRITICAL CONTROL POINTS

Use the worksheet to develop procedures, customized to your operation, for monitoring your CCPs. Consideration should be given to determining answers to the following questions.

1. What critical limit at the CCP are you measuring?
2. How is it monitored?
3. When and how often will the CCP be monitored?
4. Who will be responsible for monitoring it?

Monitoring is observing or measuring specific operational steps in the food process to determine if your critical limits are being met. This activity is essential in making sure your critical food processes are under control. It will identify where a loss of control occurs or if there is a trend toward a loss of control of a critical food process. Needed adjustments will then become obvious.

In your food safety management system, certain processes have been identified as CCPs. What you are going to monitor depends on the critical limits you have established at each CCP. Minimum critical limits for many CCPs have been established by the Food Code. For example, cooking hamburger (which is the CCP) to 155°F (68.3°C) for 15 seconds (which is the critical limit) will kill most harmful bacteria. Therefore, final temperature and time measurements are very important and you need to determine how you will effectively monitor the critical limits for each CCP.

Is monitoring equipment needed to measure a critical limit? The equipment you choose for monitoring must be accurate and routinely calibrated to ensure critical limits are met. For example, a thermocouple with a thin probe might be the most appropriate tool for measuring the final product temperature of hamburger patties.

When deciding how often you need to monitor, make sure that the monitoring interval will be reliable enough to ensure the hazard is being controlled. Your procedure for monitoring should be simple and easy to follow.

Individuals chosen to be responsible for a monitoring activity may be a manager, line-supervisor, or a designated employee. Your monitoring system will only be effective if employees are given the knowledge, skills, and responsibility for serving safe food. Train your employees to carefully follow your procedures, monitor CCPs, and take corrective action if critical limits are not met.

IX. PROCEDURAL STEP 5: DEVELOP CORRECTIVE ACTIONS

Decide what type of corrective action you need to take if a critical limit is not met.

1. What measures do you expect employees to take to correct the problem?
2. Is the corrective action understood by your employees?
3. Can the corrective action be easily implemented?
4. Are different options needed for the appropriate corrective actions, depending on the process and monitoring frequency?
5. How will these corrective actions be documented and communicated to management so the system can be modified to prevent the problem from occurring again?

Whenever a critical limit is not met, a corrective action must be carried out immediately. Corrective actions may be simply continuing to heat food to the required temperature. Other corrective actions may be more complicated, such as rejecting a shipment of raw oysters that does not have the required tags or segregating and holding a product until an evaluation is done.

In the event that a corrective action is taken, you should reassess and modify if necessary your food safety system based upon the HACCP principles. Despite the best system, errors occur during food storage and preparation. A food safety system based upon the HACCP principles is designed to detect errors and correct them before a food safety hazard occurs. It is a benefit to industry and regulators to be able to show that immediate action is taken to ensure that no food product that may be injurious

to health is served to or purchased by a customer. It is important to document all corrective actions in written records.

X. PROCEDURAL STEP 6: CONDUCT ONGOING VERIFICATION

A. Description

Because HACCP is a system to maintain continuous control of food safety practices, implementation of the plan needs to be audited or verified. Verification is usually performed by someone other than the person who is responsible for performing the activities specified in the plan. That person might be a manager, supervisor, designated person, or the regulatory authority.

There is ongoing verification, which is conducted frequently, such as daily, weekly, monthly, etc., by designated employees of the establishment. It is important to note that routine monitoring should not be confused with audit or verification methods or procedures.

There is long-term verification, which is done less frequently. This will be discussed in Procedural Step 8.

Verification is an oversight auditing process to ensure that the HACCP plan and SOPs continue to:

1. Be adequate to control the hazards identified as likely to occur
2. Be consistently followed (i.e., a comparison is made regarding observed, actual practices and procedures with what is written in the plan)

Ongoing verification activities include:

1. Observing the person doing the monitoring: is monitoring being done as planned?
2. Reviewing the monitoring records:
   a. Are records completed accurately?
   b. Do records show that the predetermined frequency of the monitoring is followed?
   c. Was the planned corrective action taken when the person monitoring found and recorded that the critical limit was not met?
   d. Do records of the calibration of monitoring equipment indicate that the equipment was operating properly?

B. Procedures

Procedures may include the following activities:

1. Observe the person conducting the activities at the CCPs and recording information
2. Check monitoring records
3. Check corrective action records
4. Periodically review the total plan
5. Test product in process or finished product
6. Review equipment calibration records
7. Review recording thermometer accuracy (large operations and some processes such as large quantity cook and chill operations or smokers, etc.)

C. Frequency

Verification should occur at a frequency that can ensure the HACCP plan is being followed continuously to:

1. Avoid adulterated/unsafe product getting to the consumer
2. Be able to take corrective action without loss of product
3. Ensure prescribed personnel practices are consistently followed
4. Ensure personnel have the tools for proper personal hygiene and sanitary practices (e.g., handwashing facilities, sanitizing equipment, cleaning supplies, temperature measuring devices, and sufficient gloves, etc.)
5. Follow/comply with the control procedures established
6. Conduct calibrations as needed depending upon the type of equipment (some may be verified daily and others annually)

D. Observations/Documentation — Examples

1. System Verification

a. Receiving

The manager reviews temperature logs of refrigerated products at various intervals such as daily or weekly. An operation may want its HACCP Plan to specify that the manager checks the monitoring records daily if:

1. Receiving constitutes a high volume, or
2. Products include particular items such as fresh tuna, mahi-mahi, mackerel, etc. (scombrotoxin-forming species).

b. Chill step

Weekly, the production manager checks the "chilling log" that is maintained for foods that are either left over or planned for later service. Recorded on the log sheet are the time the food is placed into the cooler, its temperature, the type of container used (depth per SOP), and measurements of the time and temperature involved in cooling the food.

c. Handwashing facilities and practices

Daily, the manager checks the log maintained at the handwashing facilities and corrections made in areas where

ready-to-eat food is prepared. Less frequent checks are made in other areas of the operation.

2. Process Verification

The manager checks daily or weekly, the time/temperature monitoring records at all CCPs (receiving, holding, preparation before cooking for scombrotoxin-forming seafood; cooking time/temp for hamburgers, etc.)

XI. PROCEDURAL STEP 7: KEEP RECORDS

In order to develop the most effective record-keeping system for your operation, determine what documented information will assist you in managing the control of food safety hazards. Some recorded information should already be part of your food safety system, like shellfish tags, and an additional record may not be needed. Your record-keeping system can use existing paperwork, such as delivery invoices, for documenting product temperature. Another method could be maintaining a log to record the temperatures. A record-keeping system can be simple and needs to be designed to meet the needs of the individual establishment. It can be accomplished many different ways that are customized to your operation as long as it provides a system to determine that activities are performed according to the HACCP plan.

Accurate record keeping is an essential part of a successful HACCP program. Records provide documentation that the critical limits at each CCP were met or that appropriate corrective actions were taken when the limits were not met. Records also show that the actions performed were verified.

Involve your employees in the development of your management system. Ask them how they are currently monitoring CCPs. Discuss with them the types of corrective actions they take when a critical limit is not met. Employees are an important source for developing simple and effective record keeping procedures. Managers are responsible for designing the system, but effective day-to-day implementation involves every employee.

The simplest record-keeping system that lends itself to integration into existing-operations is always best. A simple yet effective system is easier to use and communicate to your employees.

Record-keeping systems designed to document a process rather than product information may be more adaptable within a retail food establishment, especially if you frequently change items on your menu. Accurately documenting processes like cooking, cooling, and reheating, identified as CCPs, provides active managerial control of food safety hazards. Consistent process control by management reduces the risk of foodborne illness.

Simple logs for recording refrigeration equipment temperature are perhaps the most common SOP records currently maintained. However, product temperature records are commonly CCP records.

Other records may include:

1. Writing the product temperature on delivery invoices
2. Keeping a log of internal product temperatures of cooked foods
3. Holding shellstock tags for 90 days

Some retail establishments have implemented comprehensive HACCP systems where records are maintained for each CCP. These records may be quality control logs, but they can also constitute CCP records if they are designed to monitor activities that are, in fact, CCPs. The level of sophistication of record keeping is dependent upon the complexity of the food operation. For example, a cook–chill operation for a large institution would require more record keeping than a limited menu, cook–serve operation.

Once a specific process has been shown to work for you, such as an ice bath method for cooling certain foods, the frequency of record keeping may be reduced. In these instances, a record keeping system provides a scheduled check (verification) of the process to ensure that it effectively controls the risk factor. This approach is extremely effective for labor-intensive processes related to:

1. Cooking large volumes of food where a temperature check of each individual item is impractical
2. Implementing a verified process will allow employees to complete the procedure within the course of a scheduled work day
3. Cooling foods or leftovers at the end of the business day
4. Maintaining cold holding temperatures of ready-to-eat potentially hazardous foods in walk-in refrigeration units

XII. PROCEDURAL STEP 8: CONDUCT LONG-TERM VERIFICATION

Once your food safety system is implemented, you will need to confirm that it is effective over time, an activity referred to in this document as long-term verification. You may benefit from both internal (quality control) verifications and external verifications that may involve assistance from the regulatory authority or consultants.

Long-term verification is conducted less frequently (e.g., yearly) than on-going verification. It is a review or audit of the plan to determine if:

1. Any new product/processes/menu items have been added to the menu
2. Suppliers, customers, equipment, or facilities have changed
3. The SOPs are current and implemented

Procedural Step 8: Long-Term Verification

Name of person responsible for long-term verification: _____ Title: _____
Frequency at which the long-term verification is done: _____ _____
Reason, other than frequency, for doing a long-term verification: _____ _____

Date of last long-term verification: _____

The length of time this record is kept on file: _____

1. (a) Has a new product, process, or menu item been added since the last verification?	No ___			
	Yes ___ Go to Question #2	Does this change necessitate a change on the worksheet? No ____ Yes ____		
(b) Has the supplier, customer, equipment, or facility changed since the last verification?	Yes ___ No ___ Go to Question #2			
2. Do the existing worksheets contain accurate and current information?	No ___ >	Worksheet information updated:	Date: _____	Name: _____ _____
	Yes ___ Go to Question #3			
3. Are the existing CCPs correctly identified?	No ___ — >	CCP's updated:	Date: _____	Name: _____
	Yes ___ Go to Question #4			
4. Are the existing critical limits appropriate to control each hazard?	No ___ — >	CL's updated:	Date: _____	Name: _____ _____
	Yes ___ Go to Question #5			
5. Do the existing monitoring procedures ensure that the critical limits are met?	No ___ — >	Monitoring procedures updated:	Date: _____	Name: _____ _____
	Yes ___ Go to Question #6			
6. Do existing corrective actions ensure that no ensure that no injurious food is served or purchased?	No ___	Corrective actions updated:	Date: _____	Name: _____ _____
	Yes ___ Go to Question #7			
7. Do the existing on-going verification procedures ensure that the food safety system is adequate to control hazards and is consistency followed?	No ___	On-going verification procedures updated:	Date: _____	Name: _____ _____
	Yes ___ Go to Question #8			

(*Continued*)

8. Does the existing record keeping system provide adequate documentation that the critical limits are met and corrective actions are taken when needed?	No ___	Record keeping procedures updated:	Date: _____	Name: _____ _____
	Yes ___ Go to Question #9			
9. Are the existing SOPs current and implemented?	No ___	Does this necessitate a change in your plan? If so, start again with number 1.		
	Yes ___			
The long-term verification procedure is now complete. The next long-term verification is due _____. The changes made to the food safety management system were conveyed to the line supervisor or front-line employees on _____.				
	Completed by:	Name _____ Title _____ Date _____		

4. The worksheets are still current
5. The CCPs are still correct, or if new CCPs are needed
6. The critical limits are set realistically and are adequate to control the hazard (e.g., the time needed to cook the turkey to meet the Food Code internal temperature requirement)
7. Monitoring equipment has been calibrated as planned

Long-term verification helps the operator:

1. Ensure the food safety management system is implemented and the HACCP plan is being followed
2. Improve the system and HACCP plan by identifying weaknesses
3. Eliminate unnecessary or ineffective controls
4. Determine if the HACCP plan needs to be modified or updated

ACKNOWLEDGMENT

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REFERENCE

1. Food Code, 2001, DHHS/PHS/FDA, Washington, DC.

79 Seafood Processing and U.S. HACCP QMP

Nanna Cross
Chicago, Illinois

CONTENTS

I. INTRODUCTION

This chapter discusses HACCP (Hazard Analysis Critical Control Point) program management for the seafood industry as recommended by the U.S. National Marine Fisheries Service (NMFS).

HACCP is a nontraditional, noncontinuous inspection technique recommended by the National Academy of Sciences as a more scientific, analytical, and economical approach than that provided by traditional inspection and quality control methods. HACCP, which focuses on problem prevention and problem solving, relies heavily on proper monitoring and record keeping by the industry. One of the primary economic benefits of HACCP is that it provides for reduced destructive sampling of the finished product as compared to the end-product sampling required under traditional inspection systems. The application of HACCP principles to seafood inspection has been adopted by several countries, including Canada, Iceland, and the European Union, and is becoming more broadly recognized by the international community as a mechanism to apply uniform inspection procedures.

Hazard Analysis Critical Control Point (HACCP) is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product. For successful implementation of a HACCP plan, management must be strongly committed to the HACCP concept. A firm commitment to HACCP by top management provides company employees with a sense of the importance of producing safe food. In July 1992, the U.S. National Marine Fisheries Service (NMFS) announced the availability of a new seafood inspection program based on (HACCP) principles. This program is in addition to the Integrated Quality Assurance (IQA) Program that also uses HACCP principles. However, the IQA program, having unique methods for the inspection and grading of products, will continue as an option for applicants to the program. The guidelines for the HACCP Quality Management Program (QMP) have been compiled to inform interested parties that the NMFS is offering an alternative inspection program in addition to what is presently available. Participation in one program over the other is a decision, which must be made by the company's management. Under the Quality Management Program, the company takes on the responsibility of documenting and implementing a quality system. NMFS will then ensure that the quality system in place is adequate to control the critical functions by regular inspections of the system, known as audits. These audits will evaluate the quality system by examining product, processes, and records.

This document includes sections, which explain the specifications or requirements of the QMP program for documenting a quality system that will meet NMFS requirements. The document is also a guide manual for use by interested parties in developing their own quality manual. The HACCP Quality Management Program will allow participants an opportunity to apply their existing quality systems more efficiently, receive the management benefits of producing safe, wholesome, and properly labeled products more consistently and obtain the marketing benefits of using marks associated with the program. In summary, the HACCP-based service is consistent with global activities to harmonize inspection protocols. In addition, NMFS believes that the service will enhance the safety, wholesomeness, economic integrity, and quality of seafood available to consumers, as well as improve seafood industry quality assurance and regulatory oversight.

NMFS policy is to encourage and assist interested parties in the development and implementation of HACCP-based quality management systems to facilitate consistent distribution of safe, wholesome, and properly labeled fishery products of desired uniform quality. The development and implementation of HACCP-quality management systems is optional. However, their use should result in more efficient use of NMFS resources to inspect, grade, and certify fishery products.

NMFS has issued a document "NOAA HACCP Quality Management Program (HACCP QMP). Program Requirements." The latest revision was dated January 1, 2000. This document is designed to provide guidance for the development, implementation, and operation of HACCP-quality management systems, which will meet NMFS approval. All information in this chapter has been modified from this document.

When studying this document, please note the following premises:

1. The data have been obtained from a document issued by the NMFS. Data have been slightly modified for ease of reading. Therefore, for details, users should obtain a copy of the original document from the NMFS.
2. The user is assumed to know the abbreviations.
3. In the U.S., the word "shall" is a legal requirement and is used where applicable.
4. The legal numerical citation is maintained.
5. Although the information in this document is designed for seafood processors in the United States, it can be easily modified to fit foreign producers who may be interested to apply HACCP in a proper manner.

II. DEFINITIONS

1. Auditee: The organization being audited.
2. Auditor: A person qualified to perform audits.

3. Contamination: The occurrence of a contaminant in fish due to microbial pathogens, chemicals, foreign bodies, spoilage, objectionable taints, unwanted or diseased matter, which may compromise fish safety or suitability.
4. Control measure (preventive measure): Action performed to eliminate a hazard or reduce it to an acceptable level. For the purposes of this guide a control measure is also applied to a defect.
5. Control Point: Any step in a process whereby biological, chemical, or physical factors may be controlled.
6. Corrective Actions: An action taken to eliminate the causes of an existing nonconformity, defect, or other undesirable situation in order to prevent recurrence.
7. Critical Control Point (CCP): A point, step, or procedure in a food process at which control can be applied, and a food hazard can as a result be prevented, eliminated, or reduced to acceptable levels.
8. Critical Deficiency: A hazardous deviation from plan requirements such that maintenance of the safety, wholesomeness, and economic integrity is absent; will result in unsafe, unwholesome, or misbranded product.
9. Critical Limit: The maximum or minimum value to which a physical, biological, or chemical parameter must be controlled at a critical control point, or defect action point, to prevent, eliminate, or reduce to an acceptable level the occurrence of the identified food hazard.
10. Decision Tree: A sequence of questions applied to each process step with an identified hazard to identify which process steps are CCPs. For the purpose of this Program this also applies to a Defect Action Point.
11. Decomposition: A persistent and distinct objectionable odor or flavor including texture breakdown caused by the deterioration of fish.
12. Defect: A condition found in a product which fails to meet essential quality, composition and/or labeling provisions of the appropriate product standards or specifications.
13. Defect Action Point (DAP): A point, step or procedure at which control can be applied and a defect can be prevented, eliminated or reduced to acceptable level, or a fraud risk eliminated.
14. Food Safety Hazard: Any biological, chemical, or physical property that may cause a food to be unsafe for human consumption.
15. HACCP Plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety and control of defects which are significant for essential quality, composition, and/or labeling provisions in the segment of the food chain under consideration.
16. Hazard: A chance for, or the risk of, a biological, chemical, physical, or economic property in a food product that could violate established program criteria or cause the consumer distress or illness.
17. Hazard analysis: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
18. High risk products: Seafood that may pose a significant danger to the health of the public when prepared for consumption by conventional or traditional means. For example, ready-to-eat; heat and/or brown and serve products; products which may contain a microbial pathogen, biotoxin, or physical or chemical contaminant which may pose an unacceptable health risk at the time of consumption.
19. Low risk products: Seafood that poses no significant risk to the health of the public when prepared for consumption by conventional or traditional means.
20. Major Deficiency: A significant deviation from plan requirements, such that maintenance of safety, wholesomeness, or economic integrity is inhibited.
21. Minor Deficiency: A failure of the part of the HACCP-based system relative to facility sanitation which is not likely to reduce materially the facility's ability to meet acceptable sanitation requirements.
22. Monitoring Procedures: Scheduled testing and/or observations recorded by the firm to report the findings at each CCP or DAP.
23. NUOCA (Notice of Unusual Occurrence and Corrective Action): The record that outlines the incident and the corresponding corrective action implemented by the facility.
24. Objective Evidence: Information, which can be proved true, based on facts, obtained through observation, measurement, test, or other means.
25. Prerequisite Program: Procedures, including Good Manufacturing Practices that address operational conditions providing the foundation for the HACCP system.
26. Preventive Measure(s) (control measure): Physical, chemical, or other factors that can be used to control an identified food safety hazard. For the purposes of this program, this also applies to a DAP.

27. Process: One or more actions or operations to harvest, produce, store, handle, distribute, or sell a product or group of similar products.
28. Quality: Totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs. The inherent properties of any processed product which determine the relative degree of excellence of such product, and includes the effects of preparation and processing, and may or may not include the effects of packing media, or added ingredients.
29. Quality Audit: A systematic and independent examination to determine whether quality activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives.
30. Record: A document that furnishes objective evidence of activities performed or results achieved.
31. Serious Deficiency: A severe deviation from plan requirements such that maintenance of safety, wholesomeness, and economic integrity is prevented; and, if the situation is allowed to continue, may result in unsafe, unwholesome, or misbranded product.
32. Severity: The seriousness of the effect(s) of a hazard or defect. Specification: A document stating requirements. A detailed document describing the materials, dimensions, and workmanship requirements of a product.
33. Systems Audit: On-site NMFS evaluation of the firm's effectiveness in following the plan after validation.
34. Validation: That element of verification focused on collecting and evaluating scientific and technical information to determine if the Quality Management Plan, when properly implemented, will effectively control the hazards and defects.
35. Verification: Those activities performed by the firm, other than monitoring, that determine the validity of the Quality Management Plan and that the system is operating according to the plan.

III. APPLYING TO ENTER THE PROGRAM

Firms who wish to participate in the Program may apply orally or in writing to the appropriate Regional Inspection Branch of the NMFS. The Regional Inspection Branch will provide the applicant with all necessary materials to inform them of the program and its requirements. This material will also include the requirements and any policies necessary for development and submission of a Quality Management Plan. The firm develops its Quality Management Plan and submits it for review according to the plan review procedures described further in this document. There may be a cost for the application.

IV. EDUCATION AND TRAINING

The success of a HACCP system depends on educating and training management and employees in the importance of their role in producing safe foods. This should also include information concerning the control of food borne hazards related to all stages of the food chain. It is important to recognize that employees must first understand what HACCP and quality management is and then learn the skills necessary to make it function properly. Specific training activities should include working instructions and procedures that outline the tasks of employees monitoring each CCP or DAP. Management must provide adequate time for thorough education and training. Personnel must be given the materials and equipment necessary to perform these tasks. Effective training is an important prerequisite to successful implementation of a HACCP or quality plan.

Each facility must employ a NMFS-certified person knowledgeable in the program's principles to be present during all processing times. The certification must be kept on file and available to NMFS at all times.

NOTE: Retail establishments of significant size do not require the certification of an individual at each store or facility location. However, they must have demonstrated sufficient control of the training of all pertinent individuals and have a sufficient number of management personnel trained and certified in their system to maintain proper control of the concepts and the HACCP plan.

V. PLAN REVIEW AND DESK AUDIT

Each applicant must submit a QMP plan in accordance with this document. At the request of the firm, NMFS will provide consultation toward the development of the HACCP Quality Management Program plan on a fee basis. Plans are submitted to the servicing Regional Inspection Branch for desk review. Reviews of the plan may require requests for changes, clarifications, deletions, etc., from the firm. The servicing region will work with the firm to finalize the development of the QMP Plan. A written review is sent to the firm indicating what changes, if any, are necessary prior to scheduling the site visit. All work of the assigned CSO and the Regional Inspection Branch is performed on a fee basis at established rates.

VI. LABEL REVIEW PROCEDURES AND APPROVAL

All applicable labels must be approved prior to use in accordance with regulations.

The firm should begin following their plan as soon as possible. The firm must adhere to the plan's provisions and keep all records associated with the approved QMP plan for at least five (5) consecutive production days. The firm will contact the Regional Inspection Branch as soon as they believe the approved plan is functioning successfully and when they have records covering the minimum production days. The Regional Inspection Branch will schedule a site visit with the firm. The firm must verify through end-product examination that the process controls result in a product which complies to all regulations and applicable quality standards or specifications. If documentation has not been previously provided, the firm must collect data prior to the site visit, which will be sufficient to demonstrate this relationship. Firms attempting to document this relationship must collect data on not less than 20 percent of their lots using sampling plans comparable in statistical confidence to those in 50 CFR Part 260, with at least one lot representing each product form.

The inspection records must be available to NMFS personnel upon request. Although not required, NMFS recommends that the firm submit end-item verification records with their QMP Plan. This will allow the firm to test their controls, provide plan reviewers more information, and possibly reduce the time and cost of the site visit.

The audit performed on site will determine whether all of the hazards/defects and CCPs/DAPs have been identified, the quality management plan is being followed and monitored by the firm, and is effectively controlling the identified hazards/defects. The site visit will be conducted on a fee basis by a team of personnel assigned based upon the needs of the audit and the expertise available. The number and structure of the team will be determined by the size and complexity of the firm's process and nature of hazards associated with the products covered under the QMP Plan. The audit will include conducting document and record reviews, evaluating sanitation and in process observations and product verification.

All reviews will be performed using accepted auditing practices based on the current standards of ISO 10011. Conducting a combination of statistical reviews of records and finished product sample inspections will complete product verifications. At least one lot for each product form will be verified by inspecting samples of finished product. NMFS inspection personnel may, for cause, sample and verify product in excess of this guideline. Firms will be evaluated using the QMP System Evaluation Criteria. If the firm is determined to be acceptable it will qualify as a participant in the program and may finalize a contract for services with NMFS. If the audit at the firm is favorable, all products under review during the audit, including the previous five (5) production days, are eligible to bear the appropriate official marks or advertising claim.

Note for Vessels: Due to logistical factors, only one NMFS Consumer Safety Officer will perform the site visit. The NMFS Consumer Safety Officer will accompany the vessel, if determined necessary, for an appropriate time period during a fishing season, performing the background checks of critical control points and auditing the plan at one time. The officer may assist the quality assurance/management group on board the vessel in any alterations to make to their QMP Plan to work toward plan approval and a successful audit. Once the QMP plan is approved, the officer is taken off the vessel as soon as is practicable. These procedural accommodations are made in recognition of possible space restrictions and to reduce the numbers of transfers at sea.

VII. QMP PLAN CHANGES

After the QMP plan has been approved, modifications may be made under the following conditions. The firm must notify the servicing Regional Inspection Branch, in writing (Faxes are acceptable), of any modifications in their QMP plan before implementing the changes. However, any changes to address a health or safety issue may be made without prior approval, but must be documented in a corrective action plan. The Regional Inspection Branch must be notified of these immediate changes within one working day.

As the QMP Plan outlines the basic foundation and policies of the firm's quality program, changes to the plan must be approved in advance with Program management. However, the specific work procedures may change as necessary without prior approval, as long as they meet the NMFS Program Quality System Standard found at the end of this document. Prior to signing the contract, it will be determined what of the firm's document requires preapproval.

VIII. SYSTEMS AUDITS

Only with a valid contract and continued demonstrated compliance with all applicable laws and regulations and policies may (1) the firm be eligible to use official marks or other related statements and (2) firm-collected data be used by NMFS towards issuing official certification of the firm's products or facility compliance. After the firm's QMP Plan is approved, NMFS will conduct Systems Audits at a frequency listed below to determine the firm's continued adherence to their QMP Plan.

A. VESSELS

Firms must provide the appropriate NMFS Regional Inspection Branch with their tentative season schedules and off-loading schedules and sites as soon as they are known. Firms must give the servicing Regional Inspection Branch notice prior to each port arrival, providing

sufficient time for auditors to verify and audit the vessel when required. Failure to do so could result in the removal of the vessel from the Program. Vessels will be visited once every other trip, with at least one visit per year. A visit will be composed of a maximum of ten (10) percent of the scheduled fishing days for the trip in question. For example, if a trip is scheduled to last 30 days, the Systems Audit will be performed over approximately three days. Additional days may be necessary if the Consumer Safety Officer has encountered a problem during the audit. Audits may not require the auditor to be on board during fishing, but will require the auditor to be present during offloading.

NOTE: Samples of finished product may be pulled while the NMFS Consumer Safety Officer is on board or at dockside. If samples are pulled while on board, they will be evaluated immediately for compliance.

B. Processing Establishments

NMFS will conduct unannounced Systems Audits to determine the firm's continued adherence to their plan. Facilities will be visited at least once every month.

C. Retail and Food Service Establishments

NMFS will conduct unannounced Systems Audits to determine the firm's continued adherence to their plan. Facilities will be visited at least once every three months.

NOTE: NMFS is interested in providing this program with a minimum possible burden to retail participants. Record keeping should not be so grand as to cause undue hardship on the retailer. Records should be of a precision only to show what products were received by what supplier on a particular day.

D. Procedures for Retail and Food Service Operations with Multiple Outlets and with an Established Quality Assurance Program

Firms which operate a chain of stores may have the stores under the program sampled as outlined in the chart below (provided they have an established approved Quality Assurance System).

In addition, the following criteria apply:

1. All firms will enter the Program at the Tightened level of sampling. After two successive audits at this level, the firm will move to the Normal level of sampling. After two successive audits at the Normal level, the firm will move to the Reduced level of sampling.
2. No stores in the sample may be considered unreliable. If a store in the sample is deemed unreliable (Five Serious deficiencies or One Critical deficiency), the Firm's Quality Assurance System is suspect. NMFS will then perform an audit on the Quality Assurance System of the firm for the next thirty days. This audit will include the sampling of additional stores. During this 30 day period, the stores may continue to use all advertisement claims.
3. If after this audit the Quality Assurance System is deemed to be under control, the firm will be sampled at the Tightened level and the system begins again as described above.
4. If the Quality Assurance System is deemed to not be performing as designed, Regional management and the Quality Team will evaluate the firm's entire program and suggest the necessary changes to continue in the Program. This evaluation could include each store being audited and/or removed from the Program or may result in a permanent or temporary removal of the firm from the Program.
5. During this thirty day period the stores may continue to use all advertisement claims.
6. If the sample of stores does not meet the above requirements, then each store in the chain must be audited on its own until such time as the Quality Assurance System has been re-approved.

E. Tightened Frequency Audit Procedures

A firm at the tightened frequency has demonstrated difficulties in administering their QMP Plan and has rated the facility as unreliable. If a Consumer Safety Officer rates a facility unreliable, he/she will rate the facility and immediately contact his/her Supervisor. The decision to rate a facility unreliable will be made prior to the Consumer Safety Officer performing the exit interview. Once the rating is confirmed, the Chief Quality Officer of the Seafood Inspection Program is to be informed and provided with all documentation, including but not limited to: Final Audit Report, score sheets, supporting documentation, etc. Facilities who are rated unreliable have a period of thirty days to remove the unreliable status. Failure to do so will result in the facility's removal from the NMFS HACCP Quality Management Program, or the EU HACCP Program. A firm who is deemed unreliable may continue to use the mark or other applicable advertising privileges if consent by NMFS is given for daily auditing of the firm. Consent will be on a case by case basis and granted only if NMFS believes the nature of the condition which caused the firm

to become unreliable warrants daily auditing. Daily auditing will be acceptable to NMFS under the following conditions:

1. The firm must submit a corrective action plan to the NMFS Consumer Safety Officer detailing how they will correct the problem (Faxes are acceptable). The corrective action plan must include, at a minimum, detailed descriptions of the following:
   a. A statement of the problem
   b. Identification of the person or persons
   c. Handling the situation
   d. The methods to be used to correct the problem
   e. A schedule which details the time frame to correct the problem
   f. A statement with signatures of top management attesting to their commitment to correct the deficiency The corrective action plan must be written in sufficient detail to provide NMFS with all necessary information for its approval or disapproval.
2. The NMFS Consumer Safety Officer will review the corrective actions identified by the firm and will approve or disapprove the corrective actions and notify his/her Supervisor. Daily auditing will continue until the issue is corrected for a maximum of thirty calendar days.
3. Products may be certified during daily auditing. However, if any condition(s) exists that is considered critical, no product certification will occur until the condition is corrected to the satisfaction of NMFS.
4. At the inspector's discretion, product compliance will be verified by end-item inspection. No products covered by the QMP plan will leave the firm without NMFS approval.
5. Firms deemed unreliable twice in a 12-month period will be removed from the HACCP Quality Management Program or the EU HACCP Program.
6. Firms who have been removed from the HACCP Quality Management Program or the EU HACCP Program may submit a request for reapplication into the program after a period of three calendar months. Application will be accepted by NMFS only if evidence of a change in management philosophy can be provided.
7. Firms who have been removed from the NMFS HACCP Quality Management Program or EU HACCP Program may still be eligible to enter into the traditional Inspection Program.

F. Appeal Procedures

If a facility wishes to appeal this decision, they are to contact, in writing, the Chief Quality Officer in NMFS Seafood Inspection Program headquarters. The facility must provide, in writing, all pertinent information as to why it is believed the rating was determined in error and what the facility expects to be a proper correction. Once the Chief Quality Officer receives all information, he/she will investigate the matter and make a determination. The decision will be communicated to the Regional Inspection Branch and the facility as soon as it is made. A written report will follow.

G. Use of Marks

Participating firms are responsible for using the marks in accordance with the regulations set forth in 50 CFR Part 260 and the Policy and Guidelines for Advertising and Marking Products Inspected by the U.S. Department of Commerce. Facilities who have received official stamping devices must have written procedures in place securing the device and protecting from its abuse.

H. Analytical Testing and Product Verification

The firm must perform periodic end-item verification of product compliance to program requirements. Both the firm and NMFS must agree upon the firm's frequencies of testing and end-item product requirements; however, product samples for analytical testing must be collected and analyzed at least once per year as part of the firm's verification procedures. The level of analytical sampling per lot must also be comparable to that found in the Hazards and Controls Guide of the Food and Drug Administration. Records of all analytical findings will be made available to NMFS inspectors during Systems Audits and at other times as necessary. As part of the product verification discussed below, NMFS will have product tested analytically throughout the year. Six lots will be tested based upon the information found in the FDA Hazards and Controls Guide. Three lots will be tested for any criteria that is considered quality or economic integrity in nature, such as moisture content of scallops. Variation in the described sampling frequency may occur if evidence warrants. However, any changes to the frequency (and their effects) will be discussed with the applicable parties prior to their implementation.

To determine whether the product produced at the firm meets specification and/or U.S. grade standard requirements, NMFS will routinely perform a product audit on up to three (3) lots produced by the firm since the last Systems Audit. This information will be used to guide the auditor in his/her audit of the system. Product audits

will be completed by conducting records reviews and finished product sample inspections. Additional lots may be sampled if the situation warrants. Lots must be defined by the firm in their QMP plan and approved by NMFS.

IX. QMP SYSTEM EVALUATION CRITERIA

A. General Requirements

1. 21 CFR Part 123

a. Hazard analysis not performed

Every processor shall conduct, or have conducted for it, a hazard analysis to determine whether there are food safety hazards that are reasonably likely to occur for each kind of fish and fishery product processed by that processor and to identify the preventive measures that the processor can apply to control those hazards. Such food safety hazards can be introduced both within and outside the processing plant environment, including food safety hazards that can occur before, during, and after harvest. A food safety hazard that is reasonably likely to occur is one for which a prudent processor would establish controls because experience, illness data, scientific reports, or other information provide a basis to conclude that there is a reasonable possibility that it will occur in the particular type of fish or fishery product being processed in the absence of those controls. The hazard and defect analysis is the foundation of the quality plan. If the analysis is not performed, the entire plan and its efficacy is suspect. Firms must provide this analysis to the requesting Consumer Safety Officer in writing. If it is not provided and evidence suggests that it was performed but a written document is not available, a Serious deficiency will only be assessed. Otherwise, a Critical deficiency will be assessed. [Deficiency: Serious/Critical].

b. No written HACCP plan when one is required

Every processor shall have and implement a written HACCP plan whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur. Firms must provide this plan to the requesting Consumer Safety Officer. [Deficiency: Serious].

c. Plan is not location and/or fish species specific

A HACCP plan shall be specific to:

1. Each location where fish and fishery products are processed by that processor; and
2. Each kind of fish and fishery product processed by the processor. The plan may group kinds of fish and fishery products together, or group kinds of production methods together, if the food safety hazards, critical control points, critical limits, and procedures required to be identified and performed in paragraph are identical for all fish and fishery products so grouped or for all production methods so grouped.

[Deficiency: Major]

d. Hazard(s) is not listed in the plan

The HACCP plan shall, at a minimum list the food safety hazards that are reasonably likely to occur and that thus must be controlled for each fish and fishery product. Consideration should be given to whether any food safety hazards are reasonably likely to occur as a result of the following:

1. Natural toxins
2. Microbiological contamination
3. Chemical contamination
4. Pesticides
5. Drug residues
6. Decomposition in scombroid toxin-forming species or in any other species where a food safety hazard has been associated with decomposition
7. Parasites, where the processor has knowledge or has reason to know that the parasite-containing fish or fishery product will be consumed without a process sufficient to kill the parasites, or where the processor represents, labels, or intends for the product to be so consumed
8. Unapproved use of direct or indirect food or
9. Color additives
10. Physical hazards

[Deficiency: Serious]

e. Hazard(s) is not controlled

Firms may not have met the requirements of performing the hazard analysis or writing a required HACCP plan. However, controls may still be in place for the hazards identified by the Consumer Safety Officer. If it is determined that the controls are not in place, a Critical deficiency will be assessed. [Deficiency: Critical]

f. CCPs are not properly identified in the plan

The HACCP plan shall, at a minimum list the critical control points for each of the identified food safety hazards, including as appropriate:

1. Critical control points designed to control food safety hazards that could be introduced in the processing plant environment; and

2. Critical control points designed to control food safety hazards introduced outside the processing plant environment, including food safety hazards that occur before, during, and after harvest.

[Deficiency: Serious]

g. Appropriate critical limit(s) is not listed in the plan

The HACCP plan shall, at a minimum list the critical limits that must be met at each of the critical control points. If evidence is present that the critical limits were improperly identified but those identified were followed, the deficiency will be assessed here. [Deficiency: Serious]

h. Monitoring procedure(s) in the plan is inadequate

The HACCP plan shall, at a minimum, list the procedures, and frequency thereof, that will be used to monitor each of the critical control points to ensure compliance with the critical limits. [Deficiency: Serious]

i. Corrective action listed in plan is not appropriate

Whenever a deviation from a critical limit occurs, a processor shall take corrective action by following a corrective action plan that is appropriate for the particular deviation. [Deficiency: Serious]

j. Verification procedure(s) stated in plan is inadequate

The HACCP plan shall list the verification procedures, and frequency thereof, that the processor will use. Every processor shall verify that the HACCP plan is adequate to control food safety hazards that are reasonably likely to occur, and that the plan is being effectively implemented.

Verification shall include, at a minimum:

1. Reassessment of the HACCP plan. A reassessment of the adequacy of the HACCP plan whenever any changes occur that could affect the hazard analysis or alter the HACCP plan in any way or at least annually. Such changes may include changes in the following: Raw materials or source of raw materials, product formulation, processing methods or systems, finished product distribution systems, or the intended use or consumers of the finished product. The reassessment shall be performed by an individual or individuals who have been trained in accordance with Sec. 123.10 of 21 CFR Part 123. The HACCP plan shall be modified immediately whenever a reassessment reveals that the plan is no longer adequate to fully meet the requirements.
2. Ongoing verification activities. Ongoing verification activities including:
   - A review of any consumer complaints that have been received by the processor to determine whether they relate to the performance of critical control points or reveal the existence of unidentified critical control points
   - The calibration of process-monitoring instruments
   - At the option of the processor, the performing of periodic end-product or in process testing
3. Records review. A review, including signing and dating, by an individual who has been trained in accordance with Sec. 123.10, of the records that document:

   The monitoring of critical control points. The purpose of this review shall be, at a minimum, to ensure that the records are complete and to verify that they document values that are within the critical limits. This review shall occur within 1 week of the day that the records are made. The taking of corrective actions. The purpose of this review shall be, at a minimum, to ensure that the records are complete and to verify that appropriate corrective actions were taken in accordance with Sec. 123.7. This review shall occur within 1 week of the day that the records are made; and the calibrating of any process control instruments used at critical control points and the performing of any periodic end product or in-process testing that is part of the processor's verification activities. The purpose of these reviews shall be, at a minimum, to ensure that the records are complete, and that these activities occurred in accordance with the processor's written procedures. These reviews shall occur within a reasonable time after the records are made.
4. Processors shall immediately follow corrective action procedures whenever any verification procedure, including the review of a consumer complaint, reveals the need to take a corrective action. (See Corrective Action sections listed below.)
5. Reassessment of the hazard analysis. Whenever a processor does not have a HACCP plan because a hazard analysis has revealed no food safety hazards that are reasonably likely to occur, the processor shall reassess the adequacy of that hazard analysis whenever there are any changes that could reasonably affect whether a food safety hazard now exists. Such changes may include, but are not limited to changes in: raw materials or source of raw materials, product formulation, processing methods or

systems, finished product distribution systems, or the intended use or consumers of the finished product. The reassessment shall be performed by an individual or individuals who have been properly trained in accordance with 21 CFR 123.10. (See 2.3.1)
6. Recordkeeping. The calibration of process monitoring instruments, and the performing of any periodic end-product and in-process testing shall be documented in records that are subject to record keeping requirements listed below.

[Deficiency: Serious]

k. Sanitation standard operating procedures not present

Each processor should have and implement a written sanitation standard operating procedure (SSOP) or similar document that is specific to each location where fish and fishery products are produced. The SSOP should specify how the processor would meet those sanitation conditions and practices that are to be monitored. [Deficiency: Serious].

l. Sanitation not monitored

Each processor shall monitor the conditions and practices during processing with sufficient frequency to ensure, at a minimum, conformance with those conditions and practices specified in 21 CFR Part 110 that are both appropriate to the plant and the food being processed and relate to the following:

1. Safety of the water that comes into contact with food or food contact surfaces, or is used in the manufacture of ice
2. Condition and cleanliness of food contact surfaces, including utensils, gloves, and outer garments
3. Prevention of cross-contamination from unsanitary objects to food, food packaging material, and other food contact surfaces, including utensils, gloves, and outer garments, and from raw product to cooked product
4. Maintenance of hand washing, hand sanitizing, and toilet facilities
5. Protection of food, food packaging material, and food contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate, and other chemical, physical, and biological contaminants
6. Proper labeling, storage, and use of toxic compounds
7. Control of employee health conditions that could result in the microbiological contamination of food, food packaging materials, and food contact surfaces
8. Exclusion of pests from the food plant [Deficiency: Serious]

2. Program Requirements

a. Defect action plan is not adequate to control product quality characteristics

Every processor, as applicable, shall have and implement a written Defect Action Plan and a quality defect analysis for products that will either bear an inspection mark or will be advertised as under the NMFS Seafood Inspection Program. Firms must provide this plan to the requesting Consumer Safety Officer. [Deficiency: Critical]

b. Quality manual is inadequate

Every processor, as applicable, shall have and implement a written quality manual which covers each of the elements delineated in the Quality System Requirements. Firms must provide this plan to the requesting Consumer Safety Officer. [Deficiency: Serious]

c. Labels and/or specifications are inadequate

Title 50 of the Code of Federal Regulations (CFR) requires that establishments contracting for fishery product inspection service obtain NMFS approval of labels prior to use on products packed under Federal inspection, regardless of whether or not they bear official inspection or grade marks. Additionally, the "Policy for Advertising Services and Marks" identifies additional labeling and advertising of marks and services that must be approved prior to use. The Regulations Governing Processed Fishery Products require that specifications for all products for which U.S. Standards for Grades are not available be approved by the Secretary of Commerce and that end-product samples, when requested, be evaluated to determine their compliance with approved specifications prior to NMFS inspection and certification of such products. [Deficiency: Serious]

B. Adherence to HACCP-Based Plan

1. Procedures

The procedures outlined in a firm's QMP plan must be followed as written. The plan was approved by NMFS as a whole, not procedure by procedure. Not following a procedure could affect the entire critical control point.

a. Monitoring procedures not followed

Monitoring procedures must be followed to maintain control of the process. If any monitoring procedure has not been followed and a corrective action report is not filed, the firm is not in compliance with this item. [Deficiency: Serious]

b. Critical limits not followed
Self Explanatory. [Deficiency: Critical]

c. Corrective action not taken
Whenever a deviation from a critical limit, sanitation, verification, or quality plan occurs, a processor shall take corrective action. Processors may develop written corrective action plans, which become part of their QMP plans by which they predetermine the corrective actions that they will take whenever there is a deviation from a critical limit. A corrective action plan that is appropriate for a particular deviation is one that describes the steps to be taken and assigns responsibility for taking those steps, to ensure that:

1. No product enters commerce that is either injurious to health, is otherwise adulterated as a result of the deviation, or does not meet Program requirements; and
2. The cause of the deviation is corrected. A firm is provided room for error in their plan through a system of corrective actions.

If an error or problem arises in the conducting of the QMP plan, the firm must file a corrective action report (Notice of Unusual Occurrence and Corrective Action — NUOCA). All other deficiencies may possibly be averted in this checklist if corrective action reports are filed for each problem or situation. Failure to file a corrective action report will be considered a failure to take a corrective action and the firm will then not be in compliance with this item.

When a deviation from the QMP occurs and the processor does not have a corrective action plan that is appropriate for that deviation, the processor shall:

1. Segregate and hold the affected product.
2. Perform or obtain a review to determine the acceptability of the affected product for distribution. The review shall be performed by an individual or individuals who have adequate training or experience to perform such a review.
3. Take corrective action, when necessary, with respect to the affected product to ensure that no product enters commerce that is either injurious to health or is otherwise adulterated as a result of the deviation or does not meet other program requirements.
4. Take corrective action, when necessary, to correct the cause of the deviation.

Perform or obtain timely reassessment by an individual or individuals who have been properly trained to do so, to determine whether the plan needs to be modified to reduce the risk of recurrence of the deviation, and modify the plan as necessary. [Deficiency: Critical]

d. Verification procedures not followed
Verification procedures are those that provide for management to determine the overall effectiveness of the plan. Not following these procedures could ultimately cause the plan to fail or misidentify a hazard, defect, or control procedure. Since failure of these procedures will likely not immediately cause the plan to fail, it is rated at a Serious level. This item should be checked on a trend basis, not based on isolated incidences unless they are of such severity to warrant action. Firms must reassess their hazard and defect analyses when information or other evidence indicates the need and at least yearly. The plan must be signed and dated by a management official responsible for the operation of the facility. The plan must be signed upon implementation and at least once each year. [Deficiency: Serious]

e. Sanitation standard operating procedures not followed
This deficiency will be assessed if it is determined that the firm did not follow their written SSOPs, whether or not specific sanitation deficiencies were observed. [Deficiency: Serious]

f. Defect action plan/quality manual not followed
This deficiency will be assessed if the firm did not follow the policies outlined in their Quality manual or did not follow the procedures listed in their defect action plan. This deficiency will be assessed whether or not it was determined that product was affected. [Deficiency: Serious]

2. Records

a. Inadequate information on records (facility name and location, etc.)
Self Explanatory
Based on the required information stated in 21 CFR Part 123. All records required by this part shall include:

1. The name and location of the processor or importer
2. The date and time of the activity that the record reflects
3. The signature or initials of the person performing the operation
4. Where appropriate, the identity of the product and the production code, if any. Processing and other information shall be entered on records at the time that it is observed [Deficiency: Major]

b. Record data is missing
All records must be kept up-to-date. Entries must be made as they are measured. The records shall contain the actual values and observations obtained during monitoring or

measurement. All time schedules outlined in the QMP plan must be maintained. Examples of non-compliance include: measurement observed to be taken but not entered on record; partial entry of information from monitoring procedures; initials for QA verification not recorded in a timely manner; etc. If record data is missing, a Major deficiency will be assessed.

All labels must be up-to-date. All labels must be kept on file by the firm. If labels are not up-to-date, a Serious deficiency will be assessed. The maintenance of records on computers is acceptable, provided that appropriate controls are implemented to ensure the integrity of the electronic data and signatures. [Deficiency: Major (Serious for Labels)]

c. Records are inaccurate

All entries must be accurate or the record is meaningless. If calculations, time test measured, etc., are not correct, the box for this deficiency should be checked. This deficiency will also be used for the compliance of product leaving the firm. [Deficiency: Serious/Critical]

d. Records are not available for inspection

If the firm for any unreasonable amount of time does not surrender the applicable record for inspector review, they are not in compliance with this item. If portions of a record are not available, the firm is not in compliance with this item. All required records shall be retained at the processing facility or importer's place of business in the United States for at least 1 year after the date they were prepared in the case of refrigerated products and for at least 2 years after the date they were prepared in the case of frozen, preserved, or shelf-stable products. Records that relate to the general adequacy of equipment or processes being used by a processor, including the results of scientific studies and evaluations, shall be retained at the processing facility or the importer's place of business in the United States for at least 2 years after their applicability to the product being produced at the facility.

If the processing facility is closed for a prolonged period between seasonal packs, or if record storage capacity is limited on a processing vessel or at a remote processing site, the records may be transferred to some other reasonably accessible location at the end of the seasonal pack but shall be immediately returned for official review upon demand. [Deficiency: Critical]

e. Documents or records are falsified

This item is self-explanatory. However, intent on the part of the firm or its representatives must be shown. For example, if an item on a record was shown to be corrected with correction fluid or other means of obliteration, the inspector must show that someone with, full knowledge, changed the entry to reflect a value that was not the value measured or observed. Otherwise, this will be considered an inaccurate entry. [Deficiency: Critical]

3. Other Requirements

a. Program trained personnel not available

Hazard analysis, reassessment or modification of HACCP plan, or records review performed by untrained personnel. Each firm must employ a person who has been certified by NMFS for this program. At least one NMFS HACCP-certified person is required to be present during production. In addition, copies of all certified personnel's certificates must on file with the firm. Per 21 CFR part 123, these duties are assigned only to properly trained personnel. For the QMP Program, properly trained will be any person who has passed the NMFS Certification Exam. However, failure of this element will not likely cause an immediate hazard or defect. Therefore, it is rated as a Serious deficiency. Per 21 CFR part 123, these duties are assigned to only properly trained personnel. Failure of this element could lead to an immediate hazard or defect. At a minimum, the following functions shall be performed by an individual who has successfully completed training in the application of HACCP principles to fish and fishery product processing at least equivalent to that received under standardized curriculum recognized as adequate by the U.S. Food and Drug Administration or who is otherwise qualified through job experience to perform these functions. Job experience will qualify an individual to perform these functions if it has provided knowledge at least equivalent to that provided through the standardized curriculum.

Developing a HACCP plan, which could include adapting a model or generic-type HACCP plan, that is appropriate for a specific processor, in order to meet the requirements of Sec. 123.6(b); Reassessing and modifying the HACCP plan in accordance with the corrective action procedures specified in Sec. 123.7(c)(5), the HACCP plan in accordance with the verification activities specified in Sec. 123.8(a)(1), and the hazard analysis in accordance with the verification activities specified in Sec. 123.8(c); and Performing the record review required by Sec. 123.8(a)(3). The trained individual need not be an employee of the processor. [Deficiency: Serious]

b. Modification to QMP plan without approval

Any change in procedures whether they are written or not will be considered non-compliance by the firm for this item. This includes all procedures at critical control points, sanitation procedures, recall procedures verification procedures, and consumer complaint procedures. Exceptions will be allowed for those procedures the firm can justify that were necessary to avert or control a public safety or health situation provided a corrective action report is on file for the incident and a request for plan modification is filed with the servicing NMFS Regional Inspection Branch within a 24-hour period. [Deficiency: Serious]

C. Facility Sanitation

1. Safety of Process Water

Process water must be of very high quality as it directly interfaces or becomes part of the product being manufactured. Therefore, no filth, deleterious chemicals, bacteria, or other contaminants may be present in solution as it will directly affect the safety of the product. Available water must pass potability standards established by federal, state, and local authorities. Water that is supplied to the plant must meet certain minimum standards. However, processing water must also be reasonably protected in the facility. Conditions that allow contamination to occur cannot be allowed. These may include cross-connection of plumbing, backsiphonage, or back flow from a contaminated source to the supply system or open vessels of water.

a. Unsafe or unsanitary water supply

The water supply, including seawater, will be in compliance when by certification or direct testing the supply is found to meet the federal standards set forth by the Environmental Protection Agency. Private supplies shall have testing performed at a minimum of every six (6) months. Certification of municipal or community systems should be secured at a minimum of once per year. [Deficiency: Critical]

b. No protection against backflow, back-siphonage, or other sources of contamination

A facility will be in compliance when all cross connections are eliminated, backflow prevention devices are installed wherever backflow or siphonage may occur, or where other possible forms of contamination may be present. [Deficiency: Serious]

c. Inadequate supply of hot water

Hot water is necessary for many cleaning techniques. In addition, a hot water supply is necessary to provide a comfortable means for employees to wash their hands. If the tap is on and a luke-warm supply of water is present in sufficient quantities for the tasks it will perform in the facility, the plant is in compliance. The supply must also be easily accessible for its proper use. [Deficiency: Minor]

d. Ice not manufactured, handled, or used in a sanitary manner

A facility will be in compliance when potable water is used for manufacturing, when the manufacturing equipment is clean, and the ice only touches impervious surfaces; the ice holding containers are clean and made of appropriate impervious material; handling equipment is clean and appropriate for food contact; and ice is not reused on ready-to-eat product. For facilities receiving ice from an outside supply, a certificate of conformance will be necessary to ensure that the ice being received meets the standards set forth in this document. In addition, potability checks must be made at a minimum of every six (6) months on ice received. [Deficiency: Critical]

2. Food Contact Surfaces

a. Equipment and utensils' design, construction, location, or materials cannot be readily cleaned and sanitized; does not preclude product adulteration or contamination

Any equipment used in the manufacturing or handling of the food product must be designed or constructed so that it can be easily taken apart for regular cleaning and inspection. Failure to do so will cause the facility to be out of compliance. In addition, if the materials used are not of a material suitable for its intended purpose or there is reuse of single-service items, then the facility is also out of compliance. [Deficiency: Major]

b. Equipment, primary packaging materials, and utensils not maintained in proper repair or removed when necessary. (Product-contact surfaces)

All product contact surfaces must be kept in good repair. If the contact surface cannot be repaired, then the piece of equipment or utensil should be removed so as not to allow for its use. Primary packaging materials should be adequately covered when stored or not in use. Failure to provide these conditions will result in non-compliance. [Deficiency: Major (Serious for products at a high risk stage of processing)]

c. Product contact surfaces not cleaned or sanitized before use, after interruptions, or as necessary

Product contact surfaces must be cleaned using proper techniques to remove dirt and debris. Sanitizers must be used before product contacts the surface. Sanitizing without cleaning is insufficient. Any violation will be considered noncompliance. [Deficiency: Serious (Critical for products at a high risk stage of processing)]

d. Processing or food handling personnel do not maintain a high degree of personal cleanliness

All persons, while in food preparation or handling areas shall wear clean outer garments, use clean cloths, and conform to hygienic practices while on duty, to the extent necessary to prevent contamination or adulteration of food. This includes occasional workers or visitors to the area. [Deficiency: Major/Serious]

e. Processing or food handling personnel do not take necessary precautions to prevent adulteration or contamination of food

All persons, while in a food preparation or handling area, shall:

1. Wash their hands thoroughly to prevent contamination by undesirable microorganisms before starting work, after each absence from the work station, and at any other time when the hands may have become soiled or contaminated. After washing, the hands must be sanitized using the company-provided hand dip stations.
2. Remove all insecure jewelry, and when food is being manipulated by hand, remove from hands any jewelry that cannot be adequately sanitized.
3. If gloves are used in food handling, maintain them in an intact, clean, and sanitary condition. Such gloves shall be of an impermeable material except where their usage would be inappropriate or incompatible with the work involved. If gloves are used they will be washed and sanitized at the same frequency as employees' hands as described in number one of this list.
4. Wear hairnets, caps, masks, or other effective hair restraint. Other persons that may incidentally enter the processing areas shall comply with this requirement.
5. Not expectorate; nor store clothing or other personal belongings; not eat food or drink beverages; nor use tobacco in any form in areas where food or food ingredients are exposed, or in areas used for food processing, storage of food ingredients and/or packaging materials, washing of equipment and utensils, or in production areas.
6. Take other necessary precautions to prevent contamination of foods with microorganisms or foreign substances including, but not limited to perspiration, hair, cosmetics, tobacco, chemicals, and medicants. [Deficiency: Serious/Critical]

3. Prevention of Cross Contamination

a. Grounds condition can permit contamination to enter the facility

There shall be no conditions on the grounds such as dusty roads or parking lots, mud puddles, chemical spills, etc., that can cause contamination to be carried into the plant through such means as wind drafts, personnel foot traffic, adherence to personnel clothing, flooding, etc. Design of the facility structure should be such that access is easily obtained to all areas. This is necessary for proper cleaning and sanitizing of floors, walls and ceilings, as well as for visual inspections. [Deficiency: Minor]

b. Facility

Design, layout of materials used cannot be readily cleaned and sanitized; does not preclude product adulteration or contamination

If the rooms (including restrooms and employee break rooms) in the facility are laid out or designed in such a way that they cannot be readily cleaned or sanitized, then the facility is not in compliance. This would include improper materials for walls, ceilings, etc., as well as hard-to-reach rooms or corners even when the equipment is removed from the room. [Deficiency: Major]

Insufficient separation by space or other means allows product to be adulterated or contaminated

There must be sufficient separation between different activities in the processing, packaging and handling of food products. This includes the complete separation of living/sleeping quarters or heavy maintenance areas from food-handling areas. The food product should flow easily from one stage to another and not be allowed to come into contact with non-food surfaces if exposed. In addition, the layout of the facility should not be such that product contamination is likely due to heavy employee traffic through work areas. Retail product displays should be arranged so that there is sufficient separation to assure that no cross-contamination can occur between raw, cooked, and live product. [Deficiency: Serious (Critical for products at a high risk stage of production)]

c. Condition of roof, ceilings, walls, floors, or lighting not maintained; lights not protected

Areas directly affecting product or packaging material

For those areas that will directly affect product or primary packaging materials, (packaging immediately surrounding product), the roof, ceiling, walls, floors, and lighting fixtures must be maintained as designed and lights must be protected. Failure to do so causes the facility to be out of compliance. [Deficiency: Critical]

Other

For areas in the facility other than in section above (Areas directly affecting product or packaging material), the roof, ceilings, walls, floors, or lighting fixtures must also be maintained as designed. This does not include those areas designated as offices and in which food products or primary packaging materials in any stage of production will not be handled or stored. [Deficiency: Minor (Major for products at a high risk stage of production)]

d. Cleaning methods permit adulteration or contamination

Employees must take care to use methods that will not adulterate or contaminate the product. Any cleaning or sanitizing procedures or techniques that may cause the

product to become adulterated or contaminated will caused the facility to be in non-compliance. Examples of non-compliance include but are not limited to inadvertent touching of product or product surfaces with wash water, detergent, sanitizers, etc., during production. [Deficiency: Serious (Critical for products at a high risk stage of production)]

e. Finished product not properly covered or protected

Finished product must be either packaged, covered or protected so as to not permit contamination or adulteration prior to shipment. [Deficiency: Major (Serious for products at a high risk stage of production)]

f. Equipment and utensils not maintained in proper repair or removed when necessary (Non-product contact surfaces)

All non-food contact surfaces should also be maintained in good repair. The facility is in noncompliance when the maintenance of all additional equipment or areas of equipment and utensils not referred to in item 3.2.a above is insufficient and may allow indirect product contamination or adulteration. [Deficiency: Minor (Major for products at a high risk stage of production)]

g. Non-product contact surfaces not cleaned before use

Non-product contact areas must also be cleaned prior to use. However, sanitizing is not required. This includes wall, ceilings, floors, and other room areas as well as equipment. [Deficiency: Major]

4. Handwashing, Hand Sanitizing, and Toilet Facilities

a. Hand washing and hand sanitizing stations not present or conveniently located

Hand washing and hand sanitizing stations must be present and located conveniently and in sufficient numbers to provide employees ease of their use. [Deficiency: Serious (Critical for products at a high risk stage of production)]

b. Improper disposal of Sewage

A facility is in compliance when sewage systems drain properly, are vented to the outside, and are connected to an approved private septic system or a public septic and/or sewerage system. [Deficiency: Critical]

c. Inadequate supplies

The restrooms must provide supplies such as toilet paper, soap, etc., sufficient enough to meet employees' needs. [Deficiency: Major]

d. Insufficient number of functional toilets

The facility must have one operable, in good repair, conveniently accessible toilet per fifteen (15) employees, per gender. For men, urinals may be substituted for toilet bowls, but only to the extent of one-third (1/3) of the total number of bowls required. [Deficiency: Minor]

5. Protection from Adulteration

a. Condensation

Areas directly affecting product or primary packaging material

If any condensation, overhead leaks, or water splash is found in areas in the facility where the condensation has the potential to come in contact with product or primary packaging material, the facility is in non-compliance. [Deficiency: Serious (Critical for products at a high risk stage of production)]

Other

Any areas other than those noted above where food is stored, handled, processed, packaged, or displayed shall be condensation-free. If condensation is noted in these areas, the facility shall be in non-compliance. [Deficiency: Major]

b. Adequate air exchange does not exist

A facility is in compliance when adequate air exchange exists to preclude the development of foul odors.

[Deficiency: Minor (Only for products at a high-risk stage of production)]

6. Proper Labeling, Use, and Storage of Toxic Compounds

Plant chemicals are cleaners, sanitizers, rodenticides, insecticides, machine lubricants, etc. They must be used according to manufacturer's instructions, have proper labeling, and be stored in a safe manner or they may pose a risk of contaminating the food product that the establishment is handling or manufacturing. A facility will be in compliance when the chemicals are used according to manufacturer's instructions and recommendations and stored in an area of limited access away from food handling or manufacturing. All chemicals must be labeled to show the name of the manufacturer, instructions for use, and the appropriate EPA or USDA approval.

a. Chemical(s) improperly used or handled

[Deficiency: Critical]

b. Chemical(s) improperly stored

[Deficiency: Serious]

c. Chemical(s) improperly labeled

[Deficiency: Major]

7. Control of Employee Health Conditions

a. Facility management does not have in effect measures to restrict people with known disease from contaminating the product

No person affected by disease in a communicable form, or while a carrier of such disease, or while affected with boils, sores, infected wounds, or other abnormal sources of microbiological contamination, shall work in a food plant in any capacity in which there is a reasonable possibility of food or food ingredients becoming contaminated by such person. Plant management shall require employees to report illness or injury to supervisors. [Deficiency: Serious]

8. Exclusion of Pests

The presence of rodents, insects, and other animals in the facility must not be allowed because they are sources for the contamination of food with foreign material, filth, and bacteria, etc.

a. Harborage and attractant areas present

The facility and grounds are free of harborage areas. These include but are not limited to: uncut weeds, brush or tall grass; improper storage of unused equipment or materials; presence of litter, waste and refuse; or standing or stagnant water. All garbage and refuse containers are rodent/insect-resistant and outside storage areas are properly constructed. [Deficiency: Major]

b. Pest control measures not effective

Exclusion

Openings to the outside of or within the facility may allow vermin or other pests to enter. Openings and cracks should be screened or otherwise sealed. Screens must be of a mesh not larger than 1/16th of an inch in order to exclude insects. Cracks or holes should be sealed and doors and windows should close tightly (no opening larger than 1/4″) to exclude rodents or other animals. Air curtains and strip curtains must be effective. Air curtains shall comply with National Sanitation Standard Number 37 for Air Curtains for entranceways in food establishments. Strip curtains must run the entire width of the opening with sufficient overlap between flaps (1/2 inch). In addition, every effort should be made to keep birds from areas of the plant where food is transferred or processed. [Deficiency: Major]

Extermination

Birds — Nesting areas must be eliminated. Insects — There should not be a significant number of insects present in the facility. Insect electrocution devices, when used, must be located near the entranceway. Approved insecticides should be used whenever insect populations become noticeable.

Rodents — There should not be evidence of rodent activity. Evidence of rodents includes, but is not limited to: fecal droppings present; urine stains on bags or walls; slide marks along rodent runways; or feeding areas around stored dry goods bags that may be excessive. The facility should have appropriate rodent control measures in place. If not, the facility is not in compliance. [Deficiency: Serious]

c. Inadequate disposal of processing waste

A facility is in compliance with regard to processing wastes when they are placed in proper containers, placed at appropriate locations throughout the plant, and removed frequently. [Deficiency: Serious]

d. Inadequate housekeeping

Any excess clutter in production areas, employee areas, or other areas of the facility will cause the facility to be in non-compliance. This does not include those areas designated as office areas. [Deficiency: Minor]

ACKNOWLEDGMENT

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80 Workers Safety and Types of Food Establishments

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CONTENTS

I. STANDARD INDUSTRIAL CLASSIFICATION (SIC) OF FOOD ESTABLISHMENTS

Food and kindred products may be classified according to the "Standard Industrial Classification Manual" (Occupational Safety and Health Administration). This manual is revised periodically by using supplements. Ever since the introduction of the Internet, the use of this manual has increased tremendously.

In this manual, food and kindred products are placed under Major Group 20. This major group includes establishments manufacturing or processing foods and beverages for human consumption, and certain related products, such as manufactured ice, chewing gum, vegetable and animal fats and oils, and prepared feeds for animals and fowls. Table 80.1 describes the industrial establishments according to categories of food and kindred products, and the group number for each category is also given.

Table 80.2 describes food establishment categories, operations, and product examples.

II. POTENTIAL OCCUPATIONAL HAZARDS IN A FOOD PROCESSING PLANT

The potential hazards associated with the manufacturing process in the nearly 50 subdivisions of food and kindred

TABLE 80.1
Classification of Industrial Establishments According to Categories of Food and Kindred Products

Group Number	Establishments of Manufacturing or Processing
201	Meat products
202	Dairy products
203	Canned and preserved fruits and vegetables
204	Grain mill products
205	Bakery products
206	Sugar and confectionery products
207	Fats and oils
208	Beverages
209*	Miscellaneous food preparations and kindred products

*This group includes canned and cured fish and seafoods, fresh or frozen packaged fish and seafood, roast coffee, manufactured ice, macaroni, spaghetti, vermicelli, and noodles, and food preparations not elsewhere classified**.

**This category includes baking powder, yeast, and other leavening compounds; chocolate and cocoa products except confectionery, made from purchased materials; peanut butter, packaged tea (including instant); ground spices; potato, corn, and other chips; and vinegar and cider. There are others.

TABLE 80.2
Food Establishment Categories, Operations, and Product Examples

Food Establishments (SIC)	Operations Product Examples	Product Examples
Packing houses (201)	Slaughtering	Dressed meat and fowl
	Dressing	Meat products
	Packing	Processed meat
	Processing	
Dairies and creameries (202)	Churning	Fluid milk
	Cheese making	Butter
	Condensing	Cheeses
	Freezing	Evaporated milk
		Ice cream
Canneries and preserving (203)	Canning	Canned fruit and vegetables
	Drying	Dried products
	Pickling	Pickled products
	Freezing	Frozen products
Grain mills (204)	Flour milling	Wheat, corn, and rye flour
	Corn meal	Corn products
	Rice milling	Rice
	Wet corn	
	Milling	Prepared foods
	Prepared foods	
Bakeries (205)	Baking	Bread
	"Dry" baking	Cookies
		Crackers
Sugar refineries and confectioneries (206)	Extracting concentrating, and crystallizing cane and beet sugar	Cane and beet sugars
		Molasses
	Processing confectioneries	Syrup
		Candies
		Chewing gum
Fat and oil (207)	Extracting vegetable oils and animal oils by pressing, heating the solution	Vegetable oils
		Animal and fish
	Hydrogenating	Shortening
		Margarine
		Edible oils
Beverages (208)	Alcoholic	Beer, wine, and spirits
	Brewing	
	Fermenting	
	Distilling	
	Nonalcoholic	Bottled and canned soft drinks
	Extracting	
	Carbonating	Carbonated drinks
	Canning	
Miscellaneous prepared foods (209)	Cooking	Canned and frozen seafoods
	Canning	Roasted coffee
	Drying	Noodles
	Curing	Macaroni
	Roasting	Ice
	Ice making	

products are primarily safety hazards (Table 80.3). The following safety hazards were generally common to all the processes:

1. Extensive manual handling of feed and in-process materials and of finished products
2. Extensive exposure to slippery floors and supports
3. Extensive use of sharp implements such as cutting hand tools, saws and knives
4. Exposures to microorganisms, chemicals, allergens, viruses, molds, and dusts on substances in the feed materials

TABLE 80.3
Food Processes, Safety Hazards and Controls

Controls Process	Occupational Condition	Potential Hazard	Control
Meat processing, SIC 201	Handling live, immobile, and slaughtered animals	Strains, contusions	Mechanization, training
	Cutting and use of sharp tools	Lacerations, loss of body members	Protective clothing, gloves, guards, training
	Wet flooring, platforms, decks	Falls, sprains	Drains, shields
	Steam	Burns, scalds	Shields, reliefs
	Animal borne microorganisms	Brucellosis, dermatitis	Inspection
Dairy processes, SIC 202	Handling churns, homogenizers, plasticizers, evaporators, freezers	Lacerations, contusions, etc., from moving machine parts	Guards, shields, layout, clothing, insulation
	Handling in-process materials, products	Strains and contusions	Mechanization, training
Food preservation processes, SIC 203	Cleaning, cutting, screening, peeling raw fruit and vegetables	Lacerations, bruises, pinches in operating and maintaining the tools and machines	Guards, shields, clothing layout, training
	Blanching, cooking, pasteurizing, curing, freezing products	Burns, scalds, extreme temperatures	Insulation, shields
	Storing, packaging, shipping	Cuts, bruises from packaging machines, sprains	Guards, gloves, shields, mechanization
Grain mill processes, SIC 204	Operating and servicing breaking rolls, sieves, conveying and elevating equipment, man-lifts	Bruises, contusions, pinches, lacerations, falls	Guards, barriers, training
	Handling feed, in-process material products	Body strains	Mechanization, training
	Dust, noise, vibration	Respiratory effects, hearing	Ventilation, insulation
Bakery processes, SIC 205	Mixing, kneading, and forming machinery-conveyors	Injuries from moving parts	Guards, shields, layout
	Baking ovens	Burns, hot working environments	Insulation, clothing, air conditioning
	Handling in-process materials, products	Strains	Mechanization, training
Sugar and confectionery processes, SIC 206	Cleaning, grinding, shredding, and extraction machinery	Lacerations, contusions from moving machine parts	Guards, protective clothing, layout, ventilation, drains, overflows
	Purifiers and chemicals	Lime, sulfur dioxide, chlorine dioxide, formaldehyde	Controls, ventilation
	Concentrators, crystallizers		
	Centrifuges, filters, dryers under operating and maintenance conditions	Burns, spills, leaks	Overflows, drains
		Lacerations, contusions, burns	Controls, maintenance, insulation-sizing
Fat and oil recovery processes, SIC 207	Extracting oil and fat from animal and vegetable processes by steam distillation, mechanical expression, solvent extraction	Burns and scalds from steam and liquor leaks, spills; breaks and leaks from presses; vapors and gases from extractors	Insulation, barriers, layout, controls against overloads, spills, ventilation and monitoring
	Cleaning, grinding, shredding feeds	Machine injuries	Guards, training
	Purification, hydrogenation processing	Chemical effects	Ventilation
Beverages, SIC 208	Handling in-Processing materials	Body straining from lifting	Increasing mechanization, training
	Broken glass	Lacerations	Protective clothing, gloves

5. Seasonal operating schedules, reflecting time of harvesting, that influence safety training effectiveness

Specifically, particularly high rates are associated with the meat processing, food preservation, sugar and confectionery, fat and oil recovery, and beverage processes. In general, average rates are associated with the dairy, grain mill, and bakery processes.

The high injury and illness rates in the meat processing and fat and oil recovery processes appear to result primarily from hazards associated with cutting and hand tools, slippery floor conditions, and batch handling. There is little specific information that explains the elevated rates associated with the food preservation and sugar and confection processes, but the seasonal schedules and temporary, untrained work forces employed to meet harvest requirements are important factors. In the

beet and cane sugar industry, contusions and bruises to hands and feet, especially to maintenance workers, are a frequent cause of injury; scalds from hot water are considered to be another important factor. The foremost potential hazards associated with the beverage processes, particularly the bottled and canned soft drink processes, are body strains and sprains arising from the manual handling of the products.

Outbreaks of diseases of bacterial origin in meat processing facilities appear to be foremost among the reported nonsafety related potential health problems associated with that industry. Reports of brucellosis and skin sepsis in slaughtering and rendering plants, psittacosis in a turkey processing plant, and antibodies to *E. coli* enterotoxin in beef and swine meat-packing workers were encountered. Respiratory illness resulting from exposure to polyvinyl chloride pyrolysis fumes is a potential health hazard for meat wrappers.

Brucellosis is an acute or subacute infectious disease with variable manifestations. It is characterized by attacks of irregular fever, chills, sweating, and pain in muscles and joints, which may last for months. The disease shows remissions and although relapses are frequent, brucellosis does produce substantial immunity to reinfection. Because it can be confused with almost any febrile episode, diagnosis is very difficult unless blood cultures are positive. The *Brucella* species that are classically infective for man are found in dairy cattle (*B. abortus*), hogs (*B. suis*), and sheep and goats (*B. melitensis*). Each of these species may occasionally infect the other animals. Brucellae are distributed throughout the infected animal and may remain viable for 21 days in a refrigerated carcass. The tissues, blood, placenta and fetus, milk, and urine may be infectious. They may survive the curing of ham, but are killed by smoking, cooking, and pasteurization. Brucellae may invade through the eye, nasopharynx, genital tract, and gut, but unbroken skin is resistant. Contact with swine is the probable source of infection.

An outbreak of psittacosis among workers in a turkey processing plant had been reported. Cases occurred in employees working in the kill and pick, evisceration, and packaging departments, and inhalation of infectious sprays of poultry blood and other tissues was considered to be the primary route of infection. The results of the investigation suggested, however, that workers having both frequent contact with turkey tissues and skin injuries were more likely to be infected than other processing workers. Psittacosis is a disease of bacterial origin (*Chlamydia psittaci*), which usually takes the form of a pneumonia accompanied by fever, chills, headaches, body aches, cough, and often splenomegaly. Respiratory tract illness has been reported in meat wrappers exposed to polyvinyl chloride (PVC) pyrolysis fumes while working with hot wire cutting machines. From the data available, it appears that the major emissions from the meat wrapping film are di-2-ethylhexyladipate and hydrogen chloride. Meat wrapping, however, is usually performed in the meat departments of retail super-markets. Respiratory distress has also been reported in some meat cutters following exposure to heat-activated price labels; emissions from heated price labels have recently been found to include phthalate anhydride, 2,5-di-tert-amylquinone, and dicyclohexyl phthalate.

The rates for injury and illness in the industry's processes are considerably above the average for U.S. manufacturing and one of the highest in all manufacturing.

Design features that may reduce employee exposure to hazards basically involve factors that ensure steady and uninterrupted equipment operation, such as sizing, strength, capacity corrosion, and wear-resistance properties. Overloading, spills, breakdowns, and failures are major causes of potentially hazardous exposures. Engineering controls should also provide adequate space for easy and safe access to the equipment by production and maintenance workers, and means for sensitive, reliable, and accurate monitoring of process conditions.

In addition to those basic design controls, engineering controls apply to specific working conditions. Adequate and reliable ventilating, scrubbing, and monitoring systems should be provided to ensure good air in working areas. Vents for storage tanks and closed areas may also be required, as well as comprehensive safety guarding systems for cutting tools and moving machine parts, and proper electrical grounding. Insulating and isolating barriers for excessive temperature, noise, or vibration may be appropriate in certain instances. Maintenance tends to increase the potential for hazardous exposures because of the unusual conditions that may develop, and the special procedures that may be involved.

Table 80.3 describes food processes, safety hazards, and controls.

III. AN EXAMPLE OF WORKERS SAFETY IN A BAKERY ESTABLISHMENT

A. Identification

Industry: Bakery products. Sub-group: Bread, cake and related products; cookies and crackers. Standard Industrial Classification: 2051, 2052.

B. Process Description

Bakery goods include bread, cakes, pies, cookies, rolls, crackers and pastries. Ingredients consisting of flour, baking

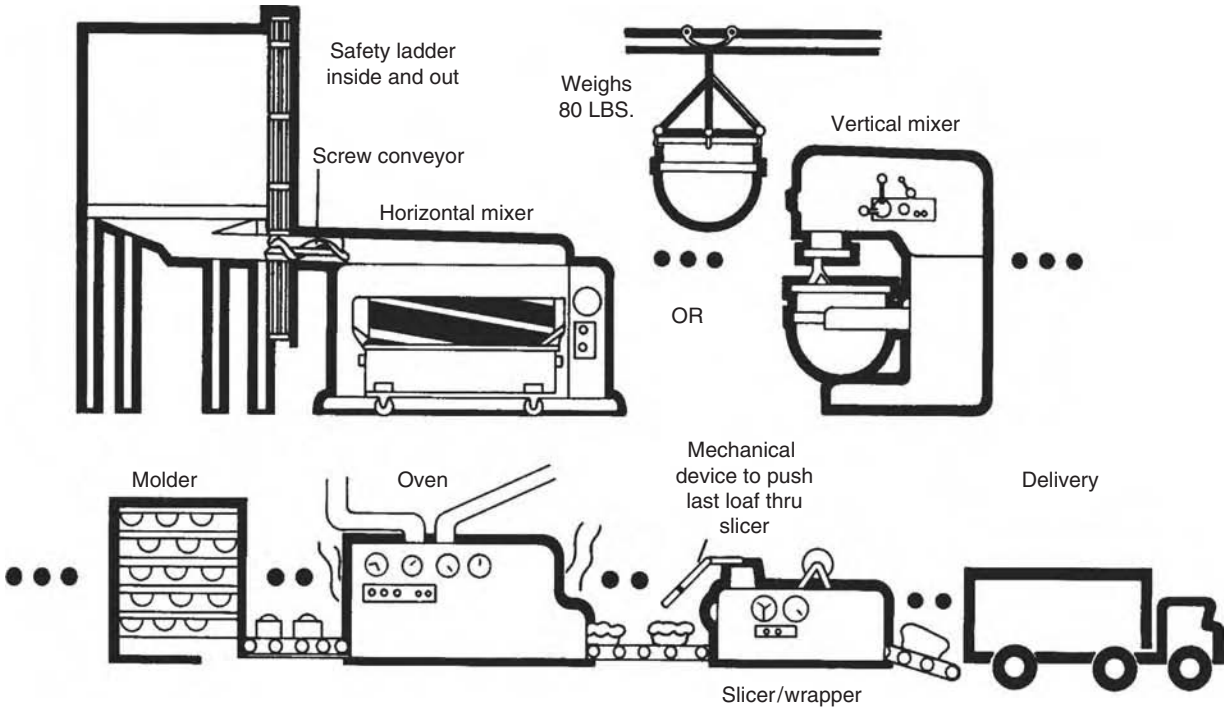

FIGURE 80.1 Process flow for a bakery establishment.

powder, sugar, salt, yeast, milk, eggs, cream, butter, lard, shortening, extracts, jellies, syrups, nuts, artificial coloring, and dried or fresh fruits are blended in a vertical or horizontal mixer after being brought from storage, measured, weighed, sifted, and mixed. After mixing, the dough is raised, divided, formed, and proofed. Fruit or flavored fillings are cooked and poured into dough shells. The final product is then baked in electric or gas-fired ovens, processed, wrapped, and shipped. Loaves of bread are also sliced and wrapped.

Figure 80.1 presents a simple outline of the process flow in this category of food establishments.

C. Injury Type and Sources

In bakery products, most of the injured employees are struck by or struck against some object, fell or slipped or were caught in, under or between objects. These injuries most commonly encountered are dislocations, sprains and strains and often involve machines and working surfaces as sources of injury.

D. Inspection Analysis

When a company officer inspects the bakery establishment for safety concerns, he or she should do the following analysis. The inspection should begin in the receiving and storage areas where bins must be checked for safety ladders of non-splintering material. Any OSHA Class II hazardous locations must have approved electrical fixtures. Mixers should then be checked for interlocks, along with agitator guards, size of openings, and cranes for moving bowls over 80 lbs. Bread rollers must have in-running rollers guarded and the slicing machine must have a device to push the last loaf of bread through and be interlocked. Employees must be checked for personal protective equipment at hot fat kettles. Machines must be grounded and have power transmission and guarded throughout. Any hot water or steam pipe must be guarded, especially in mixing and oven areas. Any conveyor passing over an aisle must have a lower guard to protect employees passing underneath baking machinery. Dividers, dough breaks, biscuit and cracker equipment, sugar and spice pulverizers, cheese and fruit cutters, and dough sheeters shall have guards to protect nip points and points of operation. Aisles must be clear of all tripping and slipping hazards particularly at open fat kettles. High noise areas must be surveyed or referred to an Industrial Hygienist.

E. OSHA Hazards Analysis

Table 80.4 presents the types of hazards, their causes and their occurrence in the bakery processing plant.

TABLE 80.4
OSHA Hazards Analysis

	Activities or Equipment	Location
Major Hazards		
Amputation and mangled limbs from contact with gears, shafts, pulleys, belts, chains and sprockets	Mechanical power transmission apparatus	Throughout plant
Slipping, tripping and falling hazards	Housekeeping	Throughout plant
Amputation and mangled limbs from nip points and sharp blades	Point of operation	Throughout plant
Electrocution from inadequate grounding	Electrical connections	Throughout plant
Burns from hot pipes and hot fat splashes. Inhalation of carbon monoxide	Ovens and open fat kettles	Throughout plant
Other Hazards		
Broken chain links and pulleys causing mixing bowls to fall on employees	Cranes and hoists	Mixing
Back strains and pulled muscles	Lifting	Mixing and baking areas
Explosion or fire	Combustible dusts	Storage

F. Other Pertinent Information

An Industrial Hygienist referral must be made for flour dust, which can cause rhinitis, buccopharyngeal disorders, bronchial asthma and eye diseases. There is a high incidence of pulmonary tuberculosis among bakers.

ACKNOWLEDGMENT

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81 Animal Food (Feed) Product Safety

Nanna Cross
Chicago, Illinois

CONTENTS

I. INTRODUCTION

The safety and economic integrity of animal feeds for domestic animals, e.g., beef cattle and pet foods, are regulated by the U.S. Food and Drug Administration (FDA). This chapter discusses the regulatory guidelines issued by the FDA.

The use of food products is governed by the provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA), and the regulations issued under its authority. These regulations are published in the Code of Federal Regulations (CFR). The FFDCA defines food as "articles used for food or drink for man or other animals…." Therefore, any product that is intended to be used as an animal feed ingredient, to become part of an ingredient or feed, or added to an animal's drinking water is considered a "food" and thus, is subject to regulation. FDA's Center for Food Safety and Applied Nutrition (CFSAN) is responsible for the regulation of

human food products. FDA's Center for Veterinarian Medicine (CVM) is responsible for the regulation of animal food (feed) products.

- Premarket Approval
- Labeling and Claims
- Regulatory Discretion

The FFDCA sets forth requirements for "foods" in the Sections 402 and 403. Failure to meet these requirements can result in a product being deemed adulterated or misbranded. Adulteration includes, among other things, food packaged or held under unsanitary conditions, food or ingredients that are filthy or decomposed, and food that contains any poisonous or deleterious substance. A food may be considered misbranded if its labeling is false or misleading in any way or fails to include required information.

The Dietary Supplement and Health Education Act (DSHEA) of 1994, has affected the way FDA regulates "food for humans," i.e., among other things, it restricts substances from being food additives or drugs if the product meets the definition of a dietary supplement. However, the agency's assessment of the law is that it was not intended and does not apply to animal feed, including pet food. This assessment was published in the Federal Register on April 22, 1996 (61 FR 17706). Thus, products marketed as dietary supplements or "feed supplements" for animals still fall under the FFDCA prior to DSHEA, i.e., they are considered "foods," "food additives," or "new animal drugs" depending on the intended use (see below). The regulatory status of a product is determined by CVM on a case-by-case basis, using specific criteria.

FDA carries out its responsibility for the regulation of animal feed in cooperation with state and local partners through a variety of mechanisms: cooperative agreements, contracts, grants, memoranda of understanding and partnerships. For instance, FDA cooperates with the Association of American Feed Control Officials (AAFCO) and the States for the implementation of uniform policies for regulating the use of animal feed products. This includes the establishment of uniform feed ingredient definitions and proper labeling to assure the safe use of feeds. The ingredient definitions are important because animal feeds and feed ingredients must be correctly and truthfully labeled when they enter commerce. Although FDA has the responsibility for regulating the use of animal feed products, the ultimate responsibility for the production of safe and effective animal feed products lies with the manufacturers and distributors of the products.

A. Premarket Approval

The FFDCA does not require pre-market approval of "food." Foods that animals consume, e.g., grains, hays, etc., are considered safe. Most mineral and vitamins are generally recognized as safe (GRAS) as sources of nutrients; however, some ingredients added to an animal feed must be used in accordance with a food additive regulation (21 CFR 573). Further, a substance that does not become a component of feed but that is used, for example, in preparing an ingredient of the food to give it a different flavor, texture, or other characteristic may be a food additive.

The basis for a food additive regulation is an approved food additive petition. Use of a food ingredient that is neither GRAS nor an approved food additive can cause a "food" to be adulterated, which cannot be legally marketed in the United States. Section 409 of the FFDCA sets forth the statutory requirements for approval of a food additive.

Under Section 409(c)(3) of the FFDCA, FDA is not to approve a food additive petition if a fair evaluation of the data fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe. Only if the petitioner meets this burden can the food additive be approved. Regulations, which apply specifically to food additives in feeds, are published in Title 21, Part 570 of the CFR. Part 571 prescribes the kinds of data that should be submitted by the petitioner and the required format for the petition itself. While the actual content may vary from petition to petition, depending primarily on the composition of the food additive and its intended use, each of the following subject areas should be addressed: a) Human food safety; b) Target animal safety; c) Environmental impact; d) Utility (intended physical, nutritional or other technical effect); e) Manufacturing chemistry; f) Labeling (cautions, warnings, shelf life, directions for use); and g) Proposed regulation.

A list of approved food additives for use in animal feed is found in Part 573 and a partial list of GRAS substances for use in animal feed is found in section 582 of Title 21 of the CFR. Substances affirmed as GRAS for use in animal feeds are listed under 21 CFR 584. Copies of the CFR may be obtained by fax: 202-512-2233 or by mail: Superintendent of Documents, Government Printing Office, Washington, D.C. 20402.

B. Labeling and Claims

A feed label should contain information describing the feed product and any details necessary for the safe and effective use of the feed. The federal regulations concerning the labeling of animal feeds are published in Part 501 of the 21 CFR. The FFDCA defines "labeling" as all labels and other written, printed or graphic matter upon any article or any of its containers or wrappers, or accompanying such articles. The courts have interpreted labeling to include promotional brochures, promotional pamphlets, testimonials, product information sheets, books, etc. Also, promotion of an animal product on the Internet for unapproved drug claims can cause the product to be misbranded under the

FFDCA, if the product label fails to bear adequate directions for the uses promoted on the Internet.

In addition to meeting the federal labeling requirements, animal feed products are also subject to individual state laws. Under many state regulations, the feed label must include the following information:

- Brand Name, if any
- Product Name
- Purpose Statement
- Guaranteed Analysis
- List of Ingredients
- Directions for Use
- Warning or Caution Statements
- Name and Address of Manufacturer
- Quantity Statement

Under the FFDCA, expressed or implied claims that establish the intended use to cure, treat, prevent or mitigate disease, or affect the structure/function of the body in a manner other than food (nutrition, aroma, or taste), identify an intent to offer the product as a "drug." Unless the "drug" product has been shown to be safe and effective for its intended use via approval of a New Animal Drug Application (NADA), it could be subject to regulatory action as an adulterated drug. It is noted that, on a case-by-case basis, CVM has allowed references to "nutritional support" for specific organs or body functions. Further, CVM has incorporated the philosophy of the Nutrition Labeling Education Act (NLEA) in its policies to permit meaningful "health" information on the label of some animal food products. For example, the use of "urinary tract health" and "dental health" claims on cat food diets falls under this policy. Feed manufacturers provide substantiation that the desired statement is truthful and not misleading. CVM review and acceptance of the claim is needed before the claim is utilized on product labeling.

C. Regulatory Discretion

The use of regulatory discretion to allow specific products to be marketed is done to conserve agency, state and industry resources, without jeopardizing human or animal health or subjecting the consumer to fraud. If a problem is found with a product, which is allowed on the market under regulatory discretion, FDA can quickly act to remove it from the marketplace. Decisions concerning the use of regulatory discretion are made on a case-by-case basis by the Center.

II. FEED INGREDIENTS AND ADDITIVES

A. Biotechnology Products

Biotechnology products are a growing proportion of the feed components regulated by the Center for Veterinary Medicine. We anticipate that "new" biotechnology will become an even greater source of products in the future. The spectrum of products being presented to CVM for regulation includes biotechnology products from plants, microbes and animals.

B. Feed Contaminants

There are two classes of feed contaminants. The first is a toxic or deleterious substance that is an inherent, naturally occurring constituent of an animal food and is not the result of environmental, agricultural, industrial or other contaminations. Examples of this class include some of the mycotoxins, such as aflatoxin and fumonisin, the glucosinolates, and the heavy metals, like lead and cadmium. The second class is made up of industrial toxic or deleterious substances, which are not naturally occurring and are increased to abnormal levels in the animal food through mishandling or other intervening acts. Examples of this class are the polychlorinated biphenyls (PCBs) and certain pesticides, like DDT (1,1′-(2,2,2-Trichloroethylidene) bis[4-chlorobenzene]). CVM may prohibit any detectable amount of a contaminant or establish a regulatory limit for the contaminant, taking into account the protection of the public health, the extent to which the presence of the contaminant cannot be avoided, and other ways in which the consumer may be affected by the presence of the contaminant.

C. Feed Ingredients

A feed ingredient is a component part or constituent or any combination/mixture added to and comprising the feed. Feed ingredients might include grains, milling byproducts, added vitamins, minerals, fats/oils, and other nutritional and energy sources. Animal feeds provide a practical outlet for plant and animal byproducts not suitable for human consumption. The Official Publication of the Association of American Feed Control Officials (AAFCO) contains a list of feed ingredients with their definitions. Many of these ingredients are not approved food additives and may not meet the criteria needed to be recognized as GRAS (21 CFR 570.30). Nevertheless, FDA has not objected to the listing of certain ingredients (e.g., those used as sources of nutrients, aroma, or taste) in the AAFCO Official Publication or their marketing in interstate commerce, provided there were no apparent safety concerns about the use or composition of the ingredient.

Federal regulations require ingredients be listed on the product label by their common or usual name in descending order of predominance according to weight (21 CFR 501.4). A common or usual name is one that accurately identifies or describes the basic nature of the ingredient (21 CFR 502.5). FDA has recognized the definitions as they appear in the Official Publication of AAFCO as the common or usual name for animal feed ingredients including pet food

(Compliance Policy Guide 7126.08). There is only one exception to the requirement to list the common or usual name on the label — when the ingredient is part of a collective name. Regulation 21 CFR 501.110 describes the use of collective names. The following are acceptable collective names: animal protein products, forage products, grain products, plant protein products, processed grain byproducts and roughage products. These collective names may be used in the ingredient list for livestock and poultry feeds, but not pet foods.

1. Association of American Feed Control Officials (AAFCO)

AAFCO is composed of state, federal, and international regulatory officials who are responsible for the enforcement of state laws regulating the safe production and labeling of animal feed, including pet food. FDA and AAFCO work together in the area of feed regulation, particularly in the establishment of definitions to describe new feed ingredients. Each year AAFCO publishes its Official Publication which includes a model feed bill for states to adopt in regulating feed products and a list of accepted feed ingredients. Most states have adopted all or part of the model feed bill and allow feed ingredients listed in the publication to be used in their respective territories.

D. Food Additives (Food Additive Petitions)

Any substance intentionally added to an animal feed, including pet food, must be used in accordance with a food additive regulation unless it is generally recognized as safe (GRAS) among qualified experts for its intended use. The basis of a food additive regulation is an approved food additive petition. The food additive petition should include an adequate factual basis to establish that the food additive is safe for its intended use, under the conditions of use specified in the petition. If the petitioner meets this burden of proof, the food additive can be approved for use in animal feed.

There are several types of food additives based on its composition and intended use. A food additive generally provides one or more of the following, i.e., nutrient, aroma/flavor, taste, soluble or insoluble fiber, stabilizer, emulsifier, sequestrant, chemical preservative, anti-oxidant, anti-caking agent, etc.

Section 571 of Part 21 of the Code of Federal Regulations (CFR) prescribes the kinds of data that must be submitted by the petitioner and the format which the food additive petition must follow when sent to FDA. While the actual content may vary from petition to petition, depending primarily on the food additive's composition and intended use, each of the following subject areas must be addressed: human food safety, target animal safety, environmental impact, utility, labeling, proposed regulation, assay methodology, and manufacturing process and controls. Subsequently, when the FDA concludes that the available data for a food additive are sufficient to meet current criteria, the FDA issues a regulation permitting the petitioned use of the additive.

III. PET FOODS

A. General

The FDA regulates that can of cat food, bag of dog food, or box of dog treats or snacks in your pantry. The FDA's regulation of pet food is similar to that for other animal feeds. The Federal Food, Drug, and Cosmetic Act (FFDCA) requires that pet foods, like human foods, be pure and wholesome, safe to eat, produced under sanitary conditions, contain no harmful substances, and be truthfully labeled. In addition, canned pet foods must be processed in conformance with the low acid canned food regulations to ensure the pet food is free of viable microorganisms (see Title 21 Code of Federal Regulations (CFR), Part 113).

There is no requirement that pet food products have premarket approval by the FDA. However, FDA ensures that the ingredients used in pet food are safe and have an appropriate function in the pet food. Many ingredients such as meat, poultry, grains, and their by products are considered safe "foods" and do not require premarket approval. Other substances such as mineral and vitamin sources, colorings, flavorings, and preservatives may be generally recognized as safe (GRAS) or must have approval as food additives. (See Title 21 CFR, Parts 73, 74, 81, 573 and 582.) For more information about pet foods and marketing a pet food, see FDA's Regulation of Pet Food and Information on Marketing A Pet Food Product.

Pet food labeling is regulated at two levels. The FDA regulations require proper identification of the product, net quantity statement, name and place of business of the manufacturer or distributor, and a proper listing of all the ingredients in order from most to least, based on weight. Some states also enforce their own labeling regulations. Many of these regulations are based on a model provided by the Association of American Feed Control Officials (AAFCO). For more information about AAFCO, please visit its website. There are two informational documents on CVM's web site that provide more details about labeling requirements: Interpreting Pet Food Labels and Interpreting Pet Food Labels — Special Use Foods.

FDA also has put into place policies for making health claims on pet food, such as "maintains health of urinary tract," "low magnesium," "reduces plaque and tartar," and "reduces hairballs in cats." Guidance for collecting data to make a urinary tract health claim is available in Guideline 55 on the CVM internet site.

B. BSE (Bovine Spongiform Encephalopathy) and the Safety of Pets

With the exception of cats, no pets (companion animals) are known to be susceptible to the infectious agent that causes BSE (Mad Cow Disease) in cattle. No evidence of BSE has ever been found in dogs, horses, birds, or reptiles.

However, cats are susceptible. Approximately 90 cats in the UK and several cats in other European countries have been diagnosed with the feline version of BSE. Before it was recognized that they were susceptible to the BSE agent, cats were exposed to the infectious agent through commercial cat food and through meat scraps provided by butchers.

Currently in the U.S., rendered products that are prohibited from cattle feed are acceptable for use in pet food. Such products include meat and bone meal, for example. However, FDA believes that the safeguards it has put into place to prevent BSE in the U.S. have protected cats. FDA continues to review these safeguards to be sure they are adequate, especially in light of the BSE case found in Washington State.

Rendered material from the BSE positive cow in Washington State did not pose a risk to cats in the U.S. because none of it was released into distribution. All firms involved with the incident in Washington State were found to be in compliance with the BSE rules.

In addition, when the BSE positive cow was found in Canada in May 2003, the FDA stopped imports of all pet foods made from material derived from mammalian sources, and the pet food manufacturer recalled the food it had manufactured that was thought to contain material from the infected cow.

CVM does not recommend one product over another or offer guidance on individual pet health issues that are normally provided by the pet's veterinarian. Questions regarding your pets' health and/or the specific use of any veterinary drug, pet food, or other product should always be referred to your veterinarian.

C. An Example of a State Regulation for Pet Foods

To illustrate how states regulate pet food, the regulations used by Pennsylvania are presented here. The data have been modified from Pennsylvania Codes, Title 7, Chapter 72, sections 1–13. All legal citations and languages have been removed for easy reading. The original document must be consulted for details.

1. Definitions

Immediate container — The unit, can, box, tin, bag or other receptacle or covering in which a pet food is displayed for sale at retail.

Ingredient statement — A complete listing on the label of the ingredients of which the pet food is composed.

Principal display panel — That part of a label on an immediate container that is most likely to be displayed or examined under normal and customary conditions of display for sale at retail.

2. Label Format

The following information should be shown on the principal display panel:

1. Statement of net content
2. Product name
3. The words "Dog Food," "Cat Food" or similar designations

The following information should be shown either on the principal display panel or elsewhere on the label and should be sufficiently conspicuous as to render it easily read by the average purchaser under ordinary conditions of sale:

1. Guaranteed analysis
2. Ingredients list
3. Listing of artificial color, drugs, and other additives, if any
4. Directions for use, if a limited purpose food
5. Name and address of the manufacturer, packer or distributor

3. Statement of Net Content

The declaration of net content should be made in conformity with the federal regulations.

4. Guaranteed Analysis

The guaranteed analysis should be stated in the following order:

1. Crude protein (minimum)
2. Crude fat (minimum)
3. Crude fiber (maximum)
4. Moisture (maximum)
5. Any additional guarantees

The sliding scale method of expressing a guaranteed analysis, such as "protein 15–18%," is prohibited.

The label of a pet food which is formulated as, and represented to be, a mineral supplement should include a guarantee of all the minerals contained in the ingredient statement.

Pet foods containing 5.0% or more mineral ingredients should include in the guaranteed analysis the minimum and maximum percentage of calcium (Ca) and salt (NaCl) and the minimum percentage of any added phosphorus (P)

and iodine (I). Minerals (except salt NaCl) should be stated in terms of percentage of the element when quantitatively guaranteed.

The label of a pet food which is formulated as, and represented to be, a vitamin supplement should include a guarantee of the minimum content of each vitamin contained in the ingredient statement.

Vitamin guarantees should be stated in units or milligrams per pound or parts per million, with the following exceptions:

1. Vitamin E should be stated in U.S.P. or International units.
2. Vitamin A, other than percursors of vitamin A, should be stated in U.S.P. units.
3. The compounds pyridoxine hydrochloride, choline chloride and thiamine need not be stated in true vitamin units.

Oils and concentrates containing vitamin A or vitamin D or both may be additionally labeled to show vitamin contents in units per gram. The term "d-pantothenic acid" may be used in stating the pantothenic acid guarantee. The vitamin potency of pet foods distributed in container smaller than one pound may be guaranteed in approved units per ounce.

If the label of a pet food does not represent the product to be either a vitamin or a mineral supplement, but does include a table of comparison of a typical analysis of the vitamin, mineral or nutrient content of the product with levels recommended by a recognized animal nutrition authority, such comparison may be stated in the units of measurement used by such recognized authority. The statement, in a table of comparison, of the vitamin, mineral or nutrient content should constitute a guarantee and need not be repeated in the guaranteed analysis.

5. Ingredients Statement

Each ingredient of the pet food should be listed in the ingredient statement in descending order of predominance by weight and names of all ingredients in the ingredient statement should be shown in letters or type of the same size. Any ingredient for which the Association of American Feed Control Officials has established a name and definition should be identified by the name so established. Any ingredient for which no name and definition has been so established should be identified by the common or usual name of the ingredient. Brand or trade names should not be used in the ingredient statement.

The term "dehydrated" should precede the name of any ingredient in the ingredient list that has been artificially dried.

No reference to quality or grade of an ingredient should appear in the ingredient statement of a pet food product label.

6. Brand and Product Names

No flavor designation should be used on a pet food label unless the designated flavor is detectable by a recognized test method, or is one the presence of which provides a characteristic distinguishable by the pet. Any flavor designation on a pet food label must either conform to the name of its source as shown in the ingredient statement or the ingredient statement should show the source of the flavor. Distributors of pet food employing such flavor designation or claims on the labels of the product distributed by them should, upon request, supply verification of the designated or claimed flavor to the Secretary.

The designation "100%" or "All" or words of similar connotation should not be used in the brand or product name of a pet food if it contains more than one ingredient. Water sufficient for processing, required decharacterizing agents, and trace amounts or preservatives and condiments should not be considered ingredients for the purpose of this subsection.

The terms "meat" and "meat byproducts" should be used on a pet food label only if the meat and meat byproducts are from cattle, swine, sheep and goats.

The name of the pet food should not be derived from one or more ingredients of a mixture to the exclusion of other ingredients and may not be one representing any components of a mixture of a pet food product unless all components or ingredients are included in the name except as specified in subsection (a), (e) or

If any ingredient or combination of ingredients is intended to impart a distinctive characteristic to the product which is significant to the purchaser, the name of the ingredient or combination of ingredients may be used as a part of the name of the pet food provided the following conditions exist:

1. The ingredient or combination of ingredients is present in sufficient quantity to impart a distinctive characteristic to the product.
2. It does not constitute a representation that the ingredient or combination of ingredients is present to the exclusion of other ingredients.
3. It is not otherwise false or misleading.

When an ingredient or a combination of ingredients derived from animals poultry or fish constitutes 95% or more of the total weight of all ingredients of a pet food mixture, the name or names of such ingredients may form a part of the product name of the pet food. Where more than one ingredient is part of such product name, then all such ingredient names should be in the same size, style and color printing.

If an ingredient or a combination of ingredients derived from animals, poultry or fish constitutes at least 25% but less than 95% of the total weight of all ingredients of a pet

food mixture, the name or names of such ingredient or ingredients may form a part of the product name of the pet food only if the product name also includes a primary descriptive term, such as "meatballs" or "fishcakes," so that the product name describes the contents of the product in accordance with an established law, custom or usage or so that the product name is not misleading. All such ingredient names and the primary descriptive term should be in the same size, style and color printing.

Contractions or coined names referring to ingredients should not be used in the brand name of a pet food unless it is in compliance with subsection (a), (d), (e), or (f).

7. Balanced and Complete Rations

The label of a pet food should not contain an unqualified representation or claim, directly or indirectly, that the pet food there in contained, or a recommended feeding there of, is or meets the requisites of a complete, perfect, scientific, or balanced ration for dogs or cats unless such product or feeding meets one of the following requisites:

1. It contains ingredients in quantities sufficient to provide the estimated nutrient requirements for all stages of the life of a dog or cat, as the case may be, which have been established by a recognized authority on animal nutrition, such as the Committee on Animal Nutrition of the National Research Council of the National Academy of Sciences.
2. It contains a combination of ingredients which when fed to a normal animal as the only source of nourishment will provide satisfactorily for fertility of male and female gestation and lactation, normal growth from weaning to maturity without supplementary feeding, and will maintain the normal weight of an adult animal whether working or at rest and has had its capabilities in this regard demonstrated by adequate testing.

To the extent that the ingredients of the product provide nutrients in amounts which substantially deviate from those nutrient requirements estimated by a recognized authority on animal nutrition, or in the event that no estimation has been made by a recognized authority on animal nutrition of the requirements of animals for one or more states of these animals lives, the represented capabilities of the product in this regard should have been demonstrated by adequate testing.

8. Limited Purpose Pet Foods

The label of a pet food product which is suitable only for intermittent or supplemental feeding or for some other limited purpose should either bear a clear and conspicuous disclosure to that effect or contain specific feeding directions which clearly state that the product should be used only in conjunction with other foods. (b) Labels for products which are compounded for or which are suitable for only a limited purpose may contain representations that the particular pet food product or a recommended feeding thereof is or meets the requisites of a complete, perfect, scientific, or balanced ration for dogs or cats only in conjunction with a statement of the limited purpose for which the product is intended or suitable, for such as "a complete food for puppies." Such representations and the required qualifications of purpose should be juxtaposed on the same panel and in the same size, style and color printing.

Qualified representations for limited purpose pet food products may appear on labels only if one of the following conditions exist:

1. The pet food contains ingredients in quantities sufficient to satisfy the estimated nutrient requirements established by a recognized authority on animal nutrition, such as the Committee on Animal Nutrition of the National Research Council of the National Academy of Sciences for such limited or qualified purpose.
2. The pet food product contains a combination of ingredients which when fed for such limited purpose will satisfy the nutrient requirements for such limited purpose and has had its capabilities in this regard demonstrated by adequate testing.

9. Moisture Limitation

The maximum moisture in all pet foods should not exceed 78% or the natural moisture content of the constituent ingredients of the product, whichever is greater. Pet foods such as those consisting principally of stew, gravy, sauce, broth or juice which are so labeled, may contain moisture in excess of 78%.

10. Drugs and Additives

An artificial color may be used in a pet food only if it has been shown to be harmless to pets. The permanent or provisional listing of an artificial color by the United States Food and Drug Administration at 21 CFR Parts 8 and 9 as safe for use, together with the conditions, limitations and tolerances, if any, incorporated there in, should be deemed to be satisfactory evidence that the color is, when used, under the regulations, harmless to pets.

Prior to approval of a facility registration for pet foods which contain additives, including drugs, other special purpose additives or non nutritive additives, the distributor may be required to submit evidence to prove the safety and efficacy of the pet food, when used according to

directions furnished on the label. Evidence of the safety and efficacy of a pet food is not required under the following conditions:

The additives used are legal.
The pet food is a drug and approved by the FDA.
The medicated labeling format recommended by Association of American Feed Control Officials should be used.

11. Misrepresentations

A vignette, graphic or pictorial representation of a product on a pet food label should not misrepresent the contents of the package. The use of the word "proven'' in connection with label claims for a pet food is prohibited unless scientific or other empirical evidence establishing the claim is first submitted to the Secretary for review.

No statement may appear upon the label of a pet food which makes false or misleading comparisons between that pet food and another pet food. Personal or commercial endorsements are permitted on pet food labels where said endorsements are factual and not otherwise misleading.

12. Identification of Producer

The label of a pet food should specify the name and address of the manufacturer, packer or distributor of the pet food. The statement of the place of business should include the street address, if any, of the place unless the street address is shown in a current city directory or telephone directory.

If a person manufactures, packages or distributes a pet food in a place other than his principal place of business, the label may state the principal place of business in lieu of the actual place where each package of the pet food was manufactured or packaged or is to be distributed, if the statement is not misleading in any particular.

13. Outer Containers or Wrappers

When a pet food is enclosed in an outer container or wrapper which is intended for retail sale, the required label information should appear on the outside wrapper or container unless all of the required label information is readily legible through apertures or transparencies in the outside container or wrapper.

ACKNOWLEDGMENT

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82 Bioterrorism and Protecting Food Supply in the United States

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Chicago, Illinois

CONTENTS

I. INTRODUCTION

Since the terrorist attack in U.S. soils on September 11, 2001, this country is tightening its security in all fronts. Food and drinking water are considered major weak points susceptible to attack by terrorists. The government considers this as part of bioterrorism. This chapter is not meant to be a comprehensive reference source on the topic. Rather, it is confined to one small aspect of protecting our food supply from enemy attack, preparedness and responses.

For ease of discussion and the lack of a reference standard, the approach to protect our food supply from terrorist attack can be divided into six components listed in the table below, using botulism as an example.

Components	Meaning	U.S. Knowledge Level	Sources of Information
Risks analysis	For example: What food, what beverages, what risks, how significant, etc.?	The best our experts can offer.	Information available from government, industry, and academia. ***Some early data are presented in this chapter.***
Science	The basic scientific information about pathogens, e.g., botulism.	The best our scientists can offer.	Information available from government, industry, and academia.
Delivery (Technology & Engineering)	How to package the pathogen for distribution to cause maximal damage to U.S. population?	The best our technologists and engineers can offer	Information available from government, industry, and academia.
Public health and medicine	How to investigate and treat the public if the attack occurs?	The best our public health officials and physicians can offer	Information available from government, industry, and academia
Threats and strikes	Exactly when, where, how, and so on?	An unknown factor handled by U.S. government?	No one knows at any moment in time.
Preparedness and response	What can be done now?	Preliminary steps have been started	Laws, regulations, guides, etc., issued by responsible U.S. government agencies. ***Some early data are presented in this chapter.***

The next section presents the risk assessment and food terrorism.

II. RISK ASSESSMENT FOR FOOD TERRORISM AND OTHER FOOD SAFETY CONCERNS

A. Introduction

On October 13, 2003, the Food and Drug Administration (FDA) issued the following information about risk assessment for food terrorism.

The events of September 11, 2001, and the subsequent anthrax incidents gave rise to concerns about unconventional terrorist attacks, including the threat of attacks on the U.S. food supply. Those events also heightened international awareness that nations could be targets for biological or chemical terrorism — a threat that had long concerned military and public health officials.

In the aftermath of those incidents, the FDA took steps to improve its ability to prevent, prepare for, and respond to, incidents of food sabotage. Though motivated by the concerns about deliberate contamination, those activities built upon and expanded the agency's continuing efforts to protect consumers from foods that have been unintentionally contaminated (e.g., through processing failures or handling errors).

As part of those activities, FDA assessed the risk to, and vulnerability of, the U.S. food supply to an act of terrorism. However, most of those assessments contain classified information. To promote transparency, FDA prepared this publicly available assessment of the risks to public health of a terrorist attack on the food supply and of serious illness due to inadvertent food contamination.

The Risk Assessment follows the generally accepted framework for risk assessments endorsed by the Codex Alimentarius Commission, the U.S. National Academy of Sciences, and other authoritative bodies. The framework divides risk assessment into four components: (1) hazard identification, (2) hazard characterization (or dose-response assessment), (3) exposure assessment, and (4) risk characterization. Unlike traditional risk assessments, however, which focus on one hazard, this assessment addresses the broad range of hazards available to terrorists intending to sabotage food, as well as hazards that accidentally are introduced into food.

This Risk Assessment uses scientific evidence on food terrorism to the extent that it exists and is available, but balances this disclosure with the need to maintain the integrity of classified information. Thus, this assessment is based solely on unclassified information. In addition, the very nature of "bioterrorism" and the fact that it is a relatively new and evolving threat present challenges in quantitatively evaluating the associated risks. FDA has prepared a qualitative risk assessment that presents various risk scenarios, as well as discusses the quality of information available for, and the uncertainties associated with, the assessment. The agency has determined that this qualitative risk assessment, which discusses prior incidents of food contamination and available unclassified information on prior acts of food sabotage, is appropriate to the circumstances.

B. Risk Assessment

1. Hazard Identification

Even before the September 11 attacks, the U.S. Centers for Disease Control and Prevention (CDC) had developed a strategic plan on biological and chemical terrorism. The CDC plan identified and ranked several foodborne pathogens as critical agents for possible terrorist attacks. Among the high-priority biological agents ("Category A" agents) were *Bacillus anthracis* (anthrax) and *Clostridium botulinum* (botulism), both of which are deadly pathogens and may contaminate food. Most of the foodborne biological agents identified by CDC were classified as "Category B" agents because they are moderately easy to disseminate and cause moderate morbidity and low mortality. The Category B biological agents include *Salmonella* spp., *Shigella dysenteriae, E. coli* O157:H7, and ricin. Notably, several of the pathogens identified by CDC as critical biological agents also are known to pose a significant threat due to unintentional contamination of food.

In addition, the CDC identified certain chemicals as possible agents for a terrorist attack. Those included heavy metals, such as arsenic, lead, and mercury, and pesticides, dioxins, furans, and polychlorinated biphenyls (PCBs), all of which may be used to contaminate food. These toxins also have been introduced inadvertently into foods and linked to human health effects.

The CDC further warned:

> … [P] ublic health agencies must prepare also for the special features a terrorist attack probably would have … Terrorists might use combinations of these agents, attack in more than one location simultaneously, use new agents, or use organisms that are not on the critical list (e.g., common, drug-resistant, or genetically engineered pathogens).

Acts of deliberate food contamination have already occurred in the U.S. In 1984, for example, the members of a religious cult contaminated salad bars with *Salmonella typhimurium* in order to disrupt a local election. This incident caused 751 cases of salmonellosis and resulted in the hospitalization of 45 of the victims. In another incident, in 1996, a disgruntled laboratory worker deliberately infected food to be consumed by co-workers with *Shigella dysenteria* type 2, causing illness in 12 people. Four of the victims had to be hospitalized and five others were treated in hospital emergency rooms. Furthermore, in May 2003, a supermarket employee pleaded guilty to intentionally

poisoning 200 pounds of ground beef with an insecticide containing nicotine. Although the tainted meat was sold in only one store, 111 people, including approximately 40 children, were sickened.

Examples of food sabotage can be drawn from other countries' experiences, as well. In September 2002, nearly 40 people died and more than 200 were hospitalized near Nanjing, China after the owner of a fast-food outlet poisoned a competitor's breakfast foods with rat poison. One year earlier, 120 people in China were sickened when the owners of a noodle factory reportedly laced their food with rat poison. A dozen children in Holland and West Germany were hospitalized in 1978 after citrus fruit from Israel was deliberately contaminated with mercury. Finally, in Canada in 1970, a postgraduate student tainted his roommates' food with the parasite *Ascaris suum*. Four of the victims became seriously ill; two of these suffered acute respiratory failure.

The incidents discussed above illustrate a few of the possible agents for food terrorism. The range of such agents is broad, and their characteristics varied; they may include:

- Biological and chemical agents
- Naturally occurring, antibiotic-resistant, and genetically engineered substances
- Deadly agents and those tending to cause gastrointestinal discomfort
- Highly infectious agents and those that are not communicable
- Substances readily available to any individual and those that are more difficult to acquire
- Agents that must be weaponized and those that are accessible in a useable form

This assessment addresses the risk to public health not only of deliberate contamination of the food supply, but also of hazards that are inadvertently introduced into foods that FDA regulates.

2. Hazard Characterization

The hazard characterization analyzes the magnitude of the risk (i.e., the severity and duration of adverse effects), using reports of foodborne disease caused by unintentional contamination. Such reports are relevant to an analysis of both inadvertently and deliberately introduced hazards, because many of the pathogens that historically have been linked to unintentional food contamination, such as *E. coli* O157:H7 and *Salmonella* spp., were identified by the CDC as "critical" agents for food terrorism. Moreover, the risk to consumers and the public health response to these known pathogens would be comparable, regardless of whether the contamination was deliberate or accidental. Officials responding to an outbreak of foodborne illness probably would not know whether the contamination was accidental or intentional until an investigation was performed to determine the source of the outbreak. Even then, officials might never be able to conclusively determine whether the food was deliberately sabotaged or inadvertently contaminated. For all of these reasons, the risk assessment uses incidents of unintentional food contamination as the basis for a characterization of hazards that could be either deliberately or unintentionally introduced into food.

a. Illnesses and deaths

The World Health Organization (WHO) estimates that microbiologically contaminated food and water cause approximately two million children worldwide to die from diarrhea each year. Even in industrialized countries, WHO estimates that one person in three suffers from a foodborne disease annually. Recognizing that foodborne illnesses "significantly affect people's health and well-being," the World Health Assembly in 2000 adopted a resolution stating that the assembly was "[d]eeply concerned that foodborne illness associated with microbial pathogens, biotoxins and chemical contaminants in food represent a serious threat to the health of millions of people in the world."

In the U.S., the CDC estimates that 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths occur annually due to food that has been inadvertently contaminated by pathogens. Based on current population data, this roughly translates to an estimate that, each year, one out of every four Americans will develop a foodborne illness.

Major outbreaks of foodborne illness occur all too frequently, sometimes affecting hundreds of thousands of people. Among the largest reported outbreaks caused by unintentional biological contamination was an outbreak of *Salmonella typhimurium* infection that sickened approximately 170,000 people in 1985 and was linked to post-pasteurization contamination of milk from a U.S. dairy plant. An outbreak of hepatitis A caused by tainted clams affected nearly 300,000 people in China in 1991 and may be the largest foodborne disease incident in history. In 1994, an outbreak of *Salmonella* Enteritidis infection linked to a contaminated ice cream pre-mix sickened an estimated 224,000 people in 41 states in the U.S. In 1996, about 8,000 children in Japan became ill, and some died, after eating *E. coli* 0157:H7-tainted radish sprouts served in school lunches.

Illnesses from pesticides, mycotoxins, heavy metals, cyanide, and other acutely toxic chemicals also have been reported. In one deadly incident, over 800 people died and a chemical agent present in cooking oil sold in Spain in 1981 injured about 20,000. In 1985, nearly 1,400 people in the U.S. reported becoming ill after eating watermelon grown in soil treated with the pesticide aldicarb. In Iraq in 1971–1972, more than 6,500 people were hospitalized with neurological symptoms and 459 died after eating bread made from mercury-contaminated wheat. Additionally, in the 1960's, more than 200 people in Japan suffered from mercury poisoning after eating highly contaminated fish caught in polluted waters.

In today's global marketplace, the contamination of food in one country can have a significant effect on public health in other parts of the world. In 1989, approximately 25,000 people in 30 states in the U.S. were sickened by *Salmonella* chester in cantaloupes imported from Mexico. In 1996 and 1997, 2,500 people in 21 states in the U.S. and two Canadian provinces developed *Cyclospora* infections after eating tainted Guatemalan raspberries.

If an unintentional contamination of one food, such as clams, can affect 300,000 individuals, a concerted, deliberate attack on food could be devastating, especially if a more dangerous chemical, biological, or radionuclear agent were used. It would be reasonable to assume that a terrorist using the food supply as a vehicle for attack would use an agent that would maximize the number of deaths associated with the contamination. Many of these agents are the same pathogens that have been linked to significant outbreaks of foodborne illness due to unintentional contamination.

b. Economic effects

Deliberate or accidental contamination of food also may have enormous economic implications in the U.S., where one out of every eight Americans is estimated to work in an occupation directly linked to food production. Indeed, food terrorists may have economic disruption as their primary motive. For example, the sabotage of Israeli citrus fruit exports, discussed above, was allegedly intended to damage Israel's economy.

At least three types of economic effects may be generated by an act of food terrorism: direct economic losses attributable to the costs of responding to the act; indirect multiplier effects from compensation paid to affected producers and the losses suffered by affiliated industries, such as suppliers, transporters, distributors, and restaurant chains; and international costs in the form of trade embargoes imposed by trading partners.

Though the costs associated with the food sabotage discussed above are unavailable, reports from unintentional contamination incidents demonstrate the tremendous costs of responding to such events. In 1998, a company in the U.S. recalled nearly 16,000 metric tons of frankfurters and luncheon meats potentially contaminated with *Listeria monocytogenes*, at a total cost of $50 million to $70 million. The company reported spending more than $100 million in the following two years to improve food safety and convince consumers that its products were safe. Indirect costs can be staggering as well. The U.S. Department of Agriculture estimates that foodborne illnesses linked to five pathogens costs the economy $6.9 billion annually. The outbreak from *Salmonella*-contaminated ice cream, discussed above, was estimated to have cost the U.S. economy about $18.1 million in medical care and time lost from work. In addition, costs arise from the disruption of international trade, as was demonstrated when Belgium's dioxin incident caused the recall of food products distributed globally.

c. Social and political implications

The response to the 2001 anthrax incidents showed that limited dissemination of biological agents by simple means could cause considerable disruption and public anxiety, even if only a few cases of illness result. The potential magnitude of the social impact arising from a food terrorism incident may be best illustrated by examining the effects of the Bovine Spongiform Encephalopathy (BSE) (also known as "mad cow disease") crisis in Great Britain in the 1990's. When researchers first discovered BSE in British cattle in 1986, some speculated that BSE could be spread to humans. However, for the next decade British authorities consistently reassured citizens that BSE was only an animal disease. When, in 1996, authorities acknowledged that BSE could be linked to human disease and announced that ten people in the U.K. had been infected or died from a human form of mad cow disease, widespread panic erupted.

Even though the toll of human fatalities — 137 deaths to date — was lower than the death toll from many other diseases, the effects of this public health crisis were widespread and long lasting. Bans on imports of British beef lasted for several years. Furthermore, the public's shattered confidence in government forced the creation of a new food regulatory authority, the Foods Standards Agency. The slaughter of millions of cattle and other BSE control measures, together with depressed markets for British beef, crippled the country's cattle industry. Some key issues from the BSE crisis are unresolved. In addition, BSE has been identified in cattle in both Canada and the U.S.

Fear and anxiety may contribute to reduced confidence in the political system and government, and may result in political destabilization, as was seen in Great Britain during the mad cow crisis.

3. Exposure Assessment

As discussed above, the CDC estimates that unsafe food causes 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year in the U.S. This roughly translates to an estimate that, each year, one in every four Americans will develop an illness due to the accidental food contamination.

Although the CDC has identified certain pathogens as critical agents for food terrorism, it is difficult for FDA to predict with any certainty the likelihood that an act of food terrorism will occur. Indeed, Codex has recognized that uncertainty may be prevalent in the risk assessment process:

Many sources of uncertainty exist in the process of risk assessment of food related hazards to human health. The degree of uncertainty and variability in the available scientific information should be explicitly considered in the risk analysis.

FDA further recognizes that the potential impact of a food terrorism event is influenced not only by the likelihood of occurrence, but also by the specific target and agent selected by a terrorist.

Despite these uncertainties, the WHO has warned that "the malicious contamination of food for terrorist purposes is a real and current threat." The CDC's infectious disease experts similarly have concluded that sabotage of food and water is the easiest means of biological or chemical attack largely because such attacks (albeit on a small scale) have been successful in the past. In addition, the CDC experts explain, the relative centralization of food production in the U.S. and the global distribution of food products give food a "unique susceptibility," and many points of vulnerability to sabotage intended to affect a large number of people exist in the food production and food distribution processes.

The threat to the U.S. food supply is more than theoretical. When U.S. troops entered the caves and safe houses of members of the al Qaeda terrorist network in Afghanistan in the months following the September 11th attacks, they found hundreds of pages of U.S. agricultural documents that had been translated into Arabic. A significant part of the group's training manual was reportedly devoted to agricultural terrorism — specifically, the destruction of crops, livestock, and food processing operations.

Moreover, recent threats of food sabotage from known terrorist groups have been reported. Specifically, the U.S. Central Intelligence Agency stated in January 2003 that it was investigating whether one of al Qaeda's leading experts on chemical and biological warfare was involved in a plot to poison food intended for British troops. The investigation stemmed from the discovery of ricin in a London apartment linked to suspected militants, one of whom worked for a catering company. The suspects were believed to have been in contact with people who worked on at least one British military base.

In early September 2003, the U.S. Federal Bureau of Investigation (FBI) issued a bulletin warning that terrorists might use two naturally occurring toxins, nicotine and solanine, to poison U.S. food or water supplies. The FBI noted that terrorist manuals and documents recovered in Afghanistan refer to the use of these substances as poisons. Citing the supermarket employee that deliberately contaminated ground beef with an insecticide containing nicotine, FBI officials advised: "Such lone offenders, whether al-Qaida [*sic*] sympathizers or domestic criminals, are a concern to FBI because they are so difficult to detect."

The U.S. is not alone in its concern about a food terrorist event. The WHO Secretariat noted last year that several countries have reported heightened states of alert for a biological or chemical attack on air, water, or food. The events of September 11, 2001, and evidence from al Qaeda validate concerns about threat of terrorism against the United States.

4. Risk Characterization

This Risk Assessment addresses a broad range of hazards that may be deliberately or accidentally introduced into the food supply that FDA regulates. Despite the difficulty of developing a quantitative risk assessment of cumulative risk in the present circumstance, FDA notes that the public health impact of unintentionally contaminated food has been documented by the CDC estimate that 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths from foodborne illness occur annually in the U.S. Other journal articles and risk assessments, referenced here, also provide data on risk of illness from the accidental introduction of pathogens into food. For example, the government's quantitative risk assessment for *Salmonella* Enteritidis (SE) in shell eggs and egg products estimated, based on modeling, that an average of 660,000 illnesses occur each year in the U.S. due to SE-contaminated eggs. The predicted average risk is 3.5 SE illnesses per one million egg servings per year.

It is more difficult for FDA to characterize the risk of food terrorism, largely because of uncertainties associated with estimating the likelihood of occurrence — even though documented incidents of sabotage have occurred and recent reports have surfaced of al Qaeda plots to poison food supplies. Traditional risk assessments for acute hazards, particularly those using modeling, often generate estimates of risk on an annual basis, as was done in the SE risk assessment. In the present circumstance, the agency has determined that the magnitude of the risk of food terrorism, and the uncertainty associated with that risk characterization, depend on the temporal basis of the risk estimate. Specifically, if a small increment of time, such as one day, is used to estimate the risk of an act of deliberate contamination, then the likelihood of occurrence would be low. If, however, a larger increment of time, such as the period of one month, is used, then the risk would be greater, and if the agency considers the likelihood of occurrence over the period of one year, then the risk of an act of food terrorism is significantly higher. To ensure that this assessment is useful for planning purposes, FDA has determined that it is appropriate to characterize the risk of occurrence of an act of food sabotage or a significant incident of unintentional food contamination on an annual basis.

The agency has considered, for the purposes of risk characterization, the known exposure to food that has been inadvertently contaminated and the past incidents of deliberate contamination, as well as the evidence that terrorists have targeted our food supply. In light of this information and the uncertainties attendant to characterizing the risk of an act of food terrorism, FDA has concluded that there is a high likelihood, over the course of a year, that a significant number of people will be affected by an act of food terrorism or by an incident of unintentional food contamination that results in serious foodborne illness.

C. Conclusion

The CDC's strategic planning workgroup on biological and chemical terrorism warned, more than a year before the September 11th attacks and the anthrax incidents.

An act of biological or chemical terrorism might range from dissemination of aerosolized anthrax spores to food product contamination, and predicting when and how such an attack might occur is not possible. However, the possibility of biological or chemical terrorism should not be ignored… Preparing the nation to address this threat is a formidable challenge, but the consequences of being unprepared could be devastating.

Though the likelihood of a biological or chemical attack on the U.S. food supply is uncertain, significant scientific evidence documents the risk to public health of food that has been inadvertently contaminated. Notwithstanding the uncertainties described in this risk assessment, and given the broad range of agents that may contaminate the food supply that FDA regulates, the agency concludes that there is a high likelihood, over the course of a year, that a significant number of people will be affected by an act of food terrorism or by an incident of unintentional food contamination that results in serious foodborne illness.

FDA has determined that this qualitative risk assessment, which discusses prior incidents of food contamination and available unclassified information on prior acts of food sabotage, is appropriate to the circumstances.

Using the above information as a premise, there are two types of preparedness and response we can do at this stage: tracing the sources of domestic food supply; and tracing the sources of imported foods from foreign countries. The importance of the information is based on one assumption. When any stage of a food chain is attacked by an enemy, the authority wants an answer to one question. What is the source of the food being attacked, a manufacturing plant, a truck doing the transport, a warehouse, a grocery store? Advance information is vital. So, the FDA has issued regulations and guidelines to achieve two goals: registration of domestic food facilities and registration of foreign manufacturing plants that export foods to the United States.

III. REGISTRATION OF FOOD FACILITIES

A. Background Information

The information is this section is presented under the following premises:

1. At time of writing, some of the data are laws and regulations and some are general guidelines. Their legal status may be different in the foreseeable future.
2. All legal citations and languages have been modified to facilitate reading. Always obtain the original document for details.

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (the Bioterrorism Act) directs the Secretary of Health and Human Services to take steps to protect the public from a threatened or actual terrorist attack on the U.S. food supply. To carry out the provisions of the Bioterrorism Act, FDA published, on October 10, 2003, an interim final regulation, *Registration Of Food Facilities*, which requires domestic and foreign facilities that manufacture/process, pack, or hold food for human or animal consumption in the United States to register with the FDA. In the event of a potential or actual bioterrorism incident or an outbreak of food-borne illness, facility registration information will help FDA to determine the location and source of the event and permit the agency to notify quickly facilities that may be affected.

This new regulation pertains *only* to facilities that manufacture/process, pack, or hold food, as defined in the regulation, for consumption in the U.S. Examples of "food" include:

- Dietary supplements and dietary ingredients
- Infant formula
- Beverages (including alcoholic beverages and bottled water)
- Fruits and vegetables
- Fish and seafood
- Dairy products and shell eggs
- Raw agricultural commodities for use as food or components of food
- Canned and frozen foods
- Bakery goods, snack food, and candy (including chewing gum)
- Live food animals
- Animal feed and pet food

Food contact substances and pesticides are not "food" for purposes of the interim final rule. Thus, a facility that manufactures/processes, packs, or holds a food contact substance or a pesticide is not required to register with FDA.

B. Questions and Answers

Let us look at some questions and answers about the registration of food facilities that are usually made available when a new law is initiated.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance describes the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements

are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

1. Who Must Register?

The owner, operator, or agent in charge of a domestic or foreign facility that manufactures/processes, packs, or holds food for human or animal consumption in the U.S., or an individual authorized by one of them, must have registered that facility with FDA by December 12, 2003. A domestic facility must register whether or not food from the facility enters interstate commerce. A foreign facility must designate a U.S. agent (for example a facility's importer or broker), who must live or maintain a place of business in the U.S. and be physically present in the U.S., for purposes of registration.

2. What Types of Facilities Do Not Need to Register?

- *Private residences of individuals*, even though food may be manufactured/processed, packed, or held there.
- *Non-bottled water drinking water collection and distribution establishments and structures*, such as municipal water systems.
- *Transport vehicles that hold food only in the usual course of their business as carriers*.
- *Farms*, i.e., facilities in one general physical location devoted to the growing and harvesting of crops, the raising of animals (including seafood), or both. Washing, trimming of outer leaves, and cooling of produce are considered part of harvesting. The term "farm" also includes facilities that pack or hold food, provided that all food used in such activities is grown, raised, or consumed on that farm or another farm under the same ownership, and facilities that manufacture/process food, provided that all food used in such activities is consumed on that farm or another farm under the same ownership. A farm-operated roadside stand that sells food directly to consumers as its primary function would be exempt from registration as a retail food establishment.
- *Restaurants*, i.e., facilities that prepare and sell food directly to consumers for immediate consumption, including pet shelters, kennels, and veterinary facilities that provide food directly to animals. Facilities that provide food to interstate conveyances, such as commercial aircraft, or central kitchens that do not prepare and serve food directly to consumers are not restaurants for purposes of the rule.
- *Retail food establishments*, such as groceries, delis, and roadside stands, that sell food directly to consumers as their *primary function*, meaning that annual sales directly to consumers are of greater dollar value than annual sales to other buyers. An establishment that manufactures/processes, packs, or holds food and whose primary function is to sell food directly to consumers, including food that the establishment manufactures/processes, from that establishment is a retail food establishment and is not required to register.
- *Nonprofit food establishments*, which are charitable entities that meet the terms of §501(c)(3) of the Internal Revenue Code and that prepare or serve food directly to the consumer or otherwise provide food or meals for consumption by humans or animals in the U.S. Central food banks, soup kitchens, and nonprofit food delivery services are examples of nonprofit food establishments.
- *Fishing vessels* that harvest and transport fish. Such vessels may engage in practices such as heading, eviscerating, or freezing fish solely to prepare the fish for holding on board the vessel and remain exempt.
- *Facilities regulated exclusively and throughout the entire facility by the U.S. Department of Agriculture*, that is, facilities handling only meat, poultry or egg products.

3. Questions and Answers about "Private Residences"

1. *Q*: If a person has a business in his/her home that involves manufacturing/processing, packing, or holding food, does that person need to register his residence as a food facility?
   A: No. A private residence is not a facility as defined in the Interim Final Rule (21 CFR 1.227(b)(2)) and thus, need not be registered. [CFR = U.S. Code of Federal Regulations].

2. *Q*: If a person is selling food from his or her private residence through the Internet, does that person need to register his residence as a food facility?
   A: No. A private residence is not a facility as defined in the Interim Final Rule (21 CFR 1.227(b)(2)) and thus, need not be registered.

3. *Q*: Is a private residence in which low acid canned food is produced exempt from the regulations for low acid canned food (21 CFR Part 113)?
   A: No. Although such a residence is not required to be registered as a food facility under

21 CFR Part 1, Subpart I, it is not exempt from any other requirements established by any other laws or regulations (21 CFR 1.240).

4. Questions and Answers about "Farms"

4. *Q*: Is a facility that manufactures/processes and sells seed to farmers required to be registered if the seed is intended for cultivation? What if the seed is an ingredient that will be included in animal feed?
   A: FDA requires registration of any facility that manufactures/processes, packs, or holds food for consumption in the U.S. As noted in a response to a comment in the Interim Final Rule (Comment 62), FDA will consider a product as one that will be used for food if the owner, operator, or agent in charge of the facility reasonably believes that the substance is reasonably expected to be directed to a food use. Therefore, if the owner, operator, or agent in charge of the facility in this question reasonably believes that the seed is reasonably expected to be used as an ingredient for animal feed, the seed is considered "food" and thus, the facility is required to be registered. However, if the seed is reasonably expected only to be cultivated, the facility is not required to be registered.

5. *Q*: Is a farm that grows tomatoes and sells them directly to consumers from a roadside stand located on the farm exempt from registration?
   A: Yes. Assuming that the farm on which the tomatoes are grown otherwise satisfies the definition of farm (21 CFR 1.227(b)(3)), it is exempt from registration. If the primary activity of the roadside stand is selling food (including the tomatoes) directly to consumers, it is exempt as a retail food establishment (21 CFR 1.227(b)(11)).

6. *Q*: If a farm located in a foreign country ships food directly to the U.S., is it required to register?
   A: No. Assuming that the farm otherwise satisfies the definition of farm (21 CFR 1.227(b)(3)), the farm is exempt from registration if it ships food directly to the U.S. However, if prior to export to the U.S., food grown on the farm is shipped to a foreign facility that manufactures/processes, packs, or holds the food, the second facility must register unless the food subsequently undergoes further manufacturing/processing of more than a *de minimis* nature at another foreign facility (21 CFR 1.226(a)). The *de minimis* provision is discussed further in question 21 of this guidance and in the preamble to the Interim Final Rule (responses to comment 17, 21, 25, and 26).

7. *Q*: Is a mixed-type facility, such as a farm that grows oranges and processes them into orange juice for sale to a distributor, required to register?
   A: Yes. FDA uses the term "mixed-type facility" in the preamble to the Interim Final Rule (response to Comment 46) to refer to an establishment that engages in both activities that are exempt from registration and activities that require the establishment to be registered. In this example, the farm is required to be registered because its processing activities are not covered by the farm definition (21 CFR 1.227(b)(3)).

8. *Q*: Is applying pesticides on a farm considered a "traditional farming activity" within the scope of the farm definition and exemption? Does this include applying a pesticide, for example, on bananas in the field or in the packing station just prior to packing?
   A: Whether the application of a pesticide to a crop is an activity covered by the farm definition depends upon whether the application is prior to or post-harvest. Section 1.227(b)(3) defines a farm as "a facility in one general location devoted to the growing and harvesting of crops, the raising of animals (including seafood), or both." FDA considers application of pesticides to a crop prior to harvest as an integral part of growing crops. Such application generally does not involve close manipulation of the food being grown because the application is usually directed at the entire plant. Therefore, an establishment devoted to the growing and harvesting of crops that applies a pesticide to its crops prior to harvest is a "farm" within the meaning of the Interim Final Rule. However, post-harvest application is necessarily directed at the food, not the entire plant, and thus, is considered to be manufacturing/processing under §1.227(b)(6). Therefore, a farm that treats a crop against pests post-harvest must register with FDA unless it satisfies the conditions of §1.227(b)(3)(ii).

9. *Q*: Is use of chlorinated water to wash lettuce on a farm considered "processing," necessitating registration of a farm?
   A: If the farm is using water directly from a public or other water supply that is chlorinated for other purposes, FDA will consider this activity "washing" within the meaning of 21 CFR 1.227(b)(3). Accordingly, an establishment using chlorinated water in this manner is a "farm" and is not required to be registered. In addition,

FDA's Good Agricultural Practices guidance document (section 2.2) (http://www.foodsafety.gov/~dms/prodguid.html) notes that chlorine is commonly added to water at 50–200 parts per million (ppm) total chlorine, at a pH of 6.0–7.5, for post harvest treatment of fresh produce, with a contact time of 1–2 minutes. FDA recognizes that chlorination at these levels is the only way many growers and packers can raise the microbiological quality of the water they use to a level that is safe and suitable. Addition of chlorine to water at these levels, therefore, does not constitute "manufacturing/processing" within the meaning of 21 CFR 1.227(b)(3)(ii).

In contrast, if water used as a wash on harvested foods on a farm contains added chlorine above levels of 200 ppm to create a specific wash, FDA considers this activity as "treating" food within the meaning of 21 CFR 1.227(c)(6), which is a manufacturing/processing activity that would require the farm to register, unless it falls under another exemption (e.g., foreign facility exemption).

10. *Q*: Does placing stickers on fruit on a farm amount to "manufacturing/processing" and therefore require registration of the facility in which the application of the stickers occurs?
A: A farm that places stickers on produce grown or consumed on the farm is not required to register as long as the farm otherwise satisfies the definition of farm (21 CFR 1.227(b)(3).) Under §1.227(b)(3)(i), FDA considers on-farm facilities that pack or hold food as meeting the farm definition, if all food used in such packing or holding is grown, raised, or consumed on that farm or another farm under the same ownership. As stated in the response to comment 41 in the Interim Final Rule, FDA considers certain activities to be "packing," such as sorting, grading, wrapping, or boxing harvested food for the sole purpose of transporting this food off the farm. FDA also considers placing stickers on produce grown or consumed on a farm part of "packing."

5. Questions and Answers about "Retail Facilities"

11. *Q*: Does a warehouse club that sells to both consumers and businesses need to be registered?
A: A warehouse club is exempt from registration as a retail food establishment (21 CFR 1.227(b)(11)) if it sells food products directly to consumers as its primary function. A retail food establishment's primary function is to sell food directly to consumers if the annual monetary value of sales of food products directly to consumers exceeds the annual monetary value of sales of food products to all other buyers. Businesses are not considered consumers.

12. *Q*: If a supermarket has a bakery on the premises that bakes bread and sells it to other stores in the same chain, is the supermarket required to be registered?
A: The supermarket is exempt from registration as a retail food establishment (21 CFR 1.227(b)(11)) if its primary function is to sell food products directly to consumers from the supermarket. As noted, an establishment's primary function is to sell food directly to consumers if the annual monetary value of sale of all food products directly to consumers exceeds the annual monetary value of sales of food products to all other buyers.

6. Questions and Answers about "Nonprofit Food Facilities"

13. *Q*: Are exporters of food for charity exempt from the registration requirements?
A: Yes. A facility, including a non-profit facility, is not required to be registered if all food manufactured/processed, packed, or held at the facility is not for consumption in the U.S. (21 CFR 1.225 and 1.227(b)(7)).

7. Questions and Answers about "Facilities Regulated Exclusively, Throughout the Entire Facility, by USDA"

14. *Q*: Are facilities that process deer, elk, and bison required to register with FDA?
A: Yes. These facilities are required to be registered with FDA because they are not regulated exclusively by the United States Department of Agriculture (USDA) (21 CFR 1.226(g)).

8. Questions and Answers about Some "Definitions"

a. Holding

15. *Q*: Are local collecting facilities for grains exempt from the registration requirement?
A: All establishments at which food is manufactured/processed, packed, or held are required to be registered, unless otherwise exempt. FDA understands the term "collecting facilities" to refer to facilities that store or hold food, such as silos or grain elevators. Such a facility must be registered with FDA because food (grain) is held by the facility (21 CFR 1.225; 1.227(b)(5)).

16. *Q*: If a facility receives packaged produce for shipping and holds it in cold storage, is it required to register?
A: Yes. The facility in this example is holding food and therefore, must be registered (21 CFR 1.225; 21 CFR 1.227(b)(5)).

17. *Q*: If finished food products for consumption in the U.S. are held at a third party facility before consolidation for import into the U.S., must this facility be registered?
A: Yes, if the finished products are held at a third party facility prior to export to the U.S., the facility is required to be registered (21 CFR 1.225; 1.227(b)(5)).

18. *Q*: In a lessor–lessee relationship, such as a food-producing business that rents space from a landlord, who is legally obligated to register the facility?
A: Either the lessor or the lessee may register the facility as follows. The Bioterrorism Act and the Registration Interim Final Rule place the duty to register a facility on the owner, operator, or agent-in-charge of the facility. Each of these persons has an independent obligation to comply with the registration requirement, and any one of them may satisfy the obligation for the other two. On the other hand, if a facility is not registered, FDA could proceed with an enforcement action against one or all of the three. A facility is defined as "any establishment, structure, or structures under one ownership at one general physical location, or, in the case of a mobile facility, traveling to multiple locations, that manufactures/processes, packs, or holds food for consumption in the United States." Thus, for a public warehouse, either the owner of the entire warehouse may register the warehouse and satisfy the obligation for all lessees, or an individual lessee, functioning as the operator or agent-in-charge of the portion of the warehouse he/she leases, may register that portion of the facility.

19. *Q*: Post offices and similar facilities owned or operated by express couriers may have packages containing food on their premises as part of the shipment process. Are these types of establishments required to be registered with FDA as food facilities?
A: No. For purposes of the registration Interim Final Rule, post offices and express courier facilities are not required to be registered with FDA as food facilities. The activities of both postal services and express courier services are focused on the transport of goods; their facilities generally serve only as a point of transfer of packages and other freight, including packages containing food. Thus, it is appropriate to view both types of facilities as part of the transportation process. The definition of "facility" in the Interim Final Rule (21 CFR 1.226(b)(2)) does not include transportation vehicles "if they hold food only in the usual course of business as carriers." Although the registration Interim Final Rule does not define "transportation vehicles," the proposed rule on the establishment and maintenance of records (68 FR 25188 at 25238; May 9, 2003) defines "transporter" as "a person who has possession, custody, or control of an article of food — for the sole purpose of transporting the food." FDA believes that it is appropriate to apply this same rationale to exclude from registration facilities that house food only because they are part of the process of transporting it from one location to another. This analysis is also consistent with the definition of "facility" in 21 CFR 1.227(b)(2). Thus, for the purpose of the registration Interim Final Rule, post offices and express courier facilities operating in a manner comparable to post offices that are part of the transportation network and have possession, custody, or control of food for the sole purpose of transporting it are not required to be registered with FDA.

20. *Q*: Truck terminals and freight forwarders may have food on their premises as part of the shipment process. Are these types of establishments required to be registered with FDA as food facilities?
A: No. Truck terminals and other stationary facilities that serve merely to assist transportation vehicles in the process of transporting food are not required to be registered with FDA. The analysis for post offices and similar facilities is also applicable here. Thus, for the purpose of the registration Interim Final Rule, truck terminals and freight forwarders that are part of the transportation network and have possession, custody, or control of food for the sole purpose of facilitating its transport are not required to be registered with FDA.

FDA acknowledges that this response is not completely consistent with certain prior guidance (Response to Comment 36; 68 Fed. Reg. 58894 at 58904; October 10, 2003). The agency has further considered this issue, as well as related ones, resulting in a revision of the earlier guidance.

b. Manufacturing/processing

21. *Q*: Is fumigation (such as of bagged cocoa beans) considered *de minimis* processing?
A: No. The Interim Final Rule states that "treating" food is a manufacturing/processing activity (21 CFR 1.227(b)(6); also see the response to Comment 41 in the rule). Therefore, a foreign facility that performs fumigation of food that is for consumption in the U.S., is required to be registered unless another foreign facility conducts further manufacturing/processing of more than a *de minimis* nature before the food is shipped to the U.S. FDA notes that even if fumigation were considered to be a *de minimis* activity, the facility at which the fumigation occurs would be required to be registered. The Bioterrorism Act *de minimis* provision is relevant to whether a particular foreign facility that manufactures/processes, packs, or holds food prior to the "*de minimis* facility" is required to be registered. The response to comment 17 in the preamble of the Interim Final Rule also discusses fumigation of cocoa beans.

22. *Q*: Is it necessary for a facility housing cotton gins to register if the cotton gins separate cotton from its seeds and hulls and the facility then sells these seeds or hulls to a manufacturer who then further processes the seeds and hulls into feed for sale to livestock operations?
A: FDA notes that the answer to this question depends in part on whether the cotton by-products are "food" as defined in the interim final rule (21 CFR 1.227(b)(4)) and whether the establishment housing the cotton gins is domestic or foreign.

In the preamble to the Interim Final Rule, FDA responded to a comment (Comment 62) regarding facilities that manufacture/process, pack, or hold multi-use substances. (68 Fed. Reg. 58894 at 58910; October 10, 2003.) The agency believes that discussion is relevant to this question. In the Interim Final Rule, the agency stated that "a product is one that will be used for food if the owner, operator, or agent in charge of the facility reasonably believes that the substance in question is reasonably expected to be directed to a food use." In this example, the facility containing the cotton gins is a food facility because the owner, operator, or agent in charge of the facility knows or should know that the cotton by-products are reasonably likely to be used as components of animal feed.

If the cotton gin establishment is located in the U.S., the establishment is required to be registered because it is manufacturing/processing food (components of animal feed), and the facility does not appear to satisfy any exemption from registration. FDA notes that any subsequent facility that processes the cotton seed and hulls into animal feed is also required to be registered.

However, if the cotton gin establishment and the establishment that processes the cotton seed and hulls into animal feed are both located in a foreign country, the cotton gin establishment would not required to be registered because a subsequent foreign facility (the feed manufacturer) conducts further manufacturing/processing of the cotton by-products prior to export to the U.S. The foreign feed manufacturing/processing facility must be registered unless, before the feed is exported to the U.S., the feed undergoes further manufacturing/processing of more than a *de minimis* nature at a third foreign facility (21 CFR 1.226(a)).

c. US agent

23. *Q*: For foreign facilities, may the U.S. agent for the facility also serve as the facility's emergency contact?
A: Yes. The U.S. agent will be considered the emergency contact for a registered foreign facility unless another name is provided in the facility's registration as the emergency contact (21 CFR 1.227(b)(13); 1.233(e)).

24. *Q*: Some U.S. law firms are charging fees to serve as a foreign facility's U.S. agent. Some of these firms have the word "FDA" in their name. Must a foreign facility use one of these firms as its U.S. agent?
A: No. A foreign facility's U.S. agent may be an individual, partnership, corporation, or association; the only requirement for such an agent is that the agent must have a place of business or residence in the U.S. and be physically present in the U.S. For example, a foreign facility may use its U.S. importer as its U.S. agent. FDA does not recommend or endorse any particular firm, organization, persons, or company to serve as a foreign facility's U.S. agent. FDA is not affiliated with any firm offering its services as a U.S. agent.

25. *Q*: May a foreign government official residing in the U.S., such as a representative from the foreign country's embassy, act as a foreign facility's U.S. agent for purposes of food facility registration?
A: In the preamble to the Interim Final Rule (Comment 90), FDA noted that the agency has concerns that acting as a U.S. agent may conflict with the duties of foreign government

representatives. Whether it is proper for a foreign government representative to act as a U.S. agent is a fact-specific inquiry, depending on the title and status of the foreign government representative and the functions that the representative assumes as a U.S. agent. FDA will consider such situations on a case-by-case basis in consultation with the U.S. State Department.

26. *Q*: I am a foreign facility that does business with several different brokers. May I use more than one of these as my U.S. agent?
A: No. The Interim Final Rule requires that each foreign facility have only one U.S. agent for food facility registration purposes. However, having a single U.S. agent for FDA registration purposes does not preclude a facility from having multiple brokers for other business purposes. FDA notes that a foreign facility is not required to conduct all of its business in the U.S. through the U.S. agent designated for purposes of registration. 21 CFR 1.227(b)(13)(iii) and the response to comment 86 in the preamble to the Interim Final Rule further discuss this issue.

27. *Q*: Is the U.S. agent legally liable in the event something goes wrong with food manufactured/processed, packed, or held at the foreign facility for which he serves as U.S. agent?
A: FDA generally does not intend to hold a foreign facility's U.S. agent responsible for violations of the Bioterrorism Act that are committed by the foreign facility. FDA, however, would consider legal action against a U.S. agent where the agent knowingly submits false information to FDA or the U.S. agent and the foreign facility are effectively the same entity. Liability issues between the facility and its U.S. agent must be resolved between the private parties (i.e., the facility and its U.S. agent), most likely through the terms of their contractual relationship.

d. Owner, operator, agent in charge, parent company

28. *Q*: How does FDA define "owner," "operator," and "agent in charge?"
A: The owner, operator, or agent in charge is a person (21 U.S.C. 321(e)) who has an ownership interest in, or management authority of, a facility or a portion of a facility (e.g., a lessee of a part of a public warehouse).

29. *Q*: How does FDA define "parent company?"
A: The term "parent company" is used in 21 CFR 1.232(b) and is intended to have the meaning it has in the corporate context. If a facility is part of a company that is owned by another corporation, then the corporation would be the parent company. For example, if a facility is owned by Company X, and Company X is a subsidiary of Corporation Y, then the owner of the facility is Company X and the parent company is Corporation Y.

9. Other Questions

a. Do all foreign facilities that manufacture/process, pack, or hold food for consumption in the U.S. need to register?

No. If a foreign facility that manufactures/ processes, packs, or holds food sends it to another *foreign* facility for further manufacturing/processing or packaging before the food is exported to the U.S., only the *second* foreign facility is required to register. However, if the second foreign facility performs only a *de minimis* activity, such as putting on a label, *both* facilities would be required to register. Also, any foreign facility that *packs or holds* food after the last foreign manufacturer/processor of the food must register.

b. How often must you register?

Registration is required only once for each food facility. However, required registration information must be updated if it changes.

c. What does the registration number mean?

It means that the owner of the facility has complied with this rule by registering with FDA. Assignment of the number does not convey FDA approval or endorsement of the facility or its products.

d. Is there a fee for registration?

There is no fee for registration or for updates of any registration.

e. Is there a mechanism for registering multiple food facilities at one time?

FDA will accept multiple registrations.

f. What information is required?

Each registration must include the name, address, and phone number for the facility and its parent company (if applicable); the name, address, and phone number of the owner, operator, or agent in charge; all trade names the facility uses; applicable food product categories as identified in FDA's regulation, 21 CFR 170.3; a statement certifying that the information submitted is true and accurate and that the person submitting the registration, if not the owner, operator, or agent in charge, is authorized to submit the registration. A foreign facility must also provide the name, address, and phone number of its U.S. agent. The foreign facility must also provide the emergency contact phone number for its

U.S. agent unless the facility designates another person to serve as the emergency contact. A domestic facility must also provide an emergency contact phone number.

g. Is additional information requested?

FDA is asking for, but not requiring, certain *optional* information on the registration form. The optional information will help us communicate more effectively with facilities that may be the target of an actual or potential terrorist threat or other food-related emergency. For example, some food products are not identified in the list of food categories at 21 CFR 170.3, such as certain dietary supplements, infant formula, and animal feed, but foods in these categories may be the focus of a food-related emergency.

h. Is registration information available to the public?

No. Neither the list of registered facilities, any registration documents submitted under this regulation, nor any information derived from the list or the documents that would reveal the identity or location of a specific registered person is subject to disclosure under the Freedom of Information Act (FOIA).

i. What if the submitted registration information changes?

When a required element of a facility's registration information changes, e.g., change of operator, agent in charge, or U.S. agent, the owner, operator, or agent in charge, or an individual authorized by one of them, must submit an update to the facility's registration within 60 days of the change through the Internet at http://www.access.fda.gov/ or through the paper update process.

j. What if a facility goes out of business?

When a facility goes out of business, its registration must be canceled.

k. What if a new owner acquires an already-registered facility?

The former owner must cancel the facility's registration within 60 days of the change, and the new owner must re-register the facility.

l. What happens if a facility does not register?

Failure of a domestic or foreign facility to register, update required elements, or cancel its registration in accordance with this regulation is a prohibited act under the Federal Food, Drug, and Cosmetic Act. The Federal government can bring a civil action to ask a Federal court to enjoin persons who commit a prohibited act, or it can bring a criminal action in Federal court to prosecute persons who are responsible for the commission of a prohibited act. If a foreign facility is required to register but fails to do so, food from that foreign facility that is offered for import into the U.S. is subject to being held within the port of entry for the article unless otherwise directed by FDA or the Bureau of Customs and Border Protection (CBP). FDA plans to issue enforcement guidance regarding the agency's policies regarding refusals of imported food under section 801(m)(1) or holds of imported food under section 801(l). This guidance document will be available to the public, and FDA will publish a notice of its availability in the Federal Register.

IV. PRIOR NOTICE OF IMPORTED FOODS

A. Introduction

On October 10, 2003, the Food and Drug Administration (FDA) published an interim final rule in the Federal Register requiring submission to FDA of prior notice of food, including food for animals, that is imported or offered for import into the United States (68 FR 58974). The prior notice interim final rule implements section 801(m) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (21 U.S.C. 381(m)) which was added by section 307 of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (the Bioterrorism Act) (Public Law 107–188), which requires that FDA receive prior notice of food imported into the United States. This guidance document provides a list of questions that have frequently been asked about the requirements of the prior notice regulation, and the answers to those questions. This document is being issued to help the food industry and others comply with the legal requirements established by the prior notice interim final rule. We intend to issue additional guidance as new questions arise. FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in Agency guidances means that something is suggested or recommended, but not required.

B. Background

a. What is prior notice?

Prior notice is notification to the FDA that an article of food, including animal feed or pet food, is being imported or offered for import into the United States in advance of the arrival of the article of food at the U.S. border (68 FR 58974; October 10, 2003).

b. Why is prior notice required to be submitted to FDA?

Prior notice is required by new section 801(m) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act)

(21 U.S.C. 801(m)). Section 801(m) requires advance notification to FDA prior to the arrival of food imported or offered for import into the United States.

How can the industry learn how to comply and submit prior notice through the U.S. Bureau of Customs and Border Protection (CBP) Automated Broker Interface of the Automated Commercial System (ABI/ACS) or FDA Prior Notice System Interface (PNSI)?

Since publication of the Prior Notice and Registration interim final rule on October 10, 2003, FDA has conducted extensive domestic and international outreach and education about the new rule. FDA and CBP held a satellite downlink public meeting on October 28, 2003, and a series of public meetings across the U.S. to discuss the prior notice and registration interim final rules. Public meetings are announced on FDA's web site at http://www.fda.gov/. In addition, we have prepared several tools, such as tutorials, instructions, and question-and-answer documents, to help importers and other affected persons to submit prior notice under either through ABI/ACS or PNSI. These are available on FDA's web site at http://www.fda.gov/. CBP continues to issue instructions to ABI/ACS filers and brokers on complying with the prior notice regulation.

c. Where can I get more information about FDA's prior notice regulation?

Information on the prior notice regulation may be found on FDA's web site at http://www.fda.gov/. Many of your questions can be answered by reading the prior notice interim final rule or by reviewing the tutorials, fact sheet, and other materials that are posted on the web site. If your questions are not answered by information on that web site, you can send an email to the following address: http://www.cfsan.fda.gov/~furls/helpf2.html. FDA plans to periodically issue guidance documents to answer those questions that are not directly addressed in the prior notice interim final rule or its preamble. Thus, you will not receive an individual response to your email. You should check our web site at http://www.fda.gov/oc/bioterrorism/bioact.html periodically to obtain a copy of these guidance documents.

d. Where can I get more information about CBP's procedures under the Bioterrorism Act and the prior notice regulation?

For additional information on the U.S. Bureau of Customs and Border Protection's (CBP's) procedures for prior notice, you may want to consult the CBP website at http://www.customs.treas.gov/.

e. Is information about prior notice available in languages other than English?

Yes. Information about prior notice to FDA is available in other languages. Translations available are indicated at the top of the English version of documents located on the Internet at http://www.cfsan.fda.gov/~dms/fsbtact.html

C. Definitions

1. Food

a. For the purposes of the prior notice regulation, what is food?

Food is defined in the prior notice regulation by reference to section 201(f) of the FD&C Act, which defines food as articles used for food or drink for man or other animals, chewing gum, and articles used for components of any such articles (21 U.S.C. 321(f)). However, for purposes of prior notice, the definition of food does not include food contact substances or pesticides (21 CFR 1.276(b)(5)(i)). Examples of food subject to prior notice include: fruits, vegetables, fish, including seafood, dairy products, eggs, raw agricultural commodities for use as food or as components of food, animal feed (including pet food), food and feed ingredients, food and feed additives, dietary supplements and dietary ingredients, infant formula, beverages (including alcoholic beverages and bottled water), live food animals, bakery goods, snack foods, candy, and canned foods.

b. Is a bulk commodity like raw cane sugar "food" that is subject to prior notice?

Yes, if a bulk commodity like raw cane sugar is food under the definition if it is for use as food, including for use as a component of food. Raw agricultural commodities for use as food or as components of food are food for prior notice purposes (21 CFR 1.276(b)(5)(ii)). FDA will consider an article as one that will be used for food if any of the persons involved in importing or offering the product for import (e.g., submitter, transmitter, manufacturer, grower, shipper, importer, owner, or ultimate consignee) reasonably believes that the substance is reasonably expected to be directed to a food use.

c. Are live animals "food" for prior notice purposes?

Live animals are food for purposes of prior notice (21 CFR 1.276(b)(5)(ii)) if any of the persons involved in importing or offering the live animal for import (e.g., the submitter, transmitter, manufacturer, grower, shipper, importer, owner, or ultimate consignee) reasonably believes that the live animal is reasonably expected to be directed to a food use (21 CFR 1.276(b)(5)). Note that live food animals are not excluded from prior notice under section 801(m)(3)(B) of the FD&C Act and 21 CFR 1.277(b)(4) or (5) because live food animals do not fall within the exclusive jurisdiction of USDA under the Federal Meat Inspection Act or Poultry Products Inspection Act.

If USDA's Animal Plant and Health Inspection Service (APHIS) inspects the live animals when they are imported into the U.S., are the live animals "food" for prior notice purposes?

Yes. Live food animals that are subject to border inspections by APHIS are also subject to FDA's prior notice requirements: FDA and APHIS may both have jurisdiction over live animals. Note that the requirement for prior notice to FDA for live food animals does not alter the role of APHIS in, or any APHIS requirements relating to, inspection of live animals imported into the U.S.

d. Are game animals "food" for which prior notice must be given?

Yes, if any person involved in importing the animal reasonably believes the animal is reasonably expected to be directed to a food use, the animal is food for which prior notice is required (21 CFR 1.276(b)(5)). So, for example, elk imported to stock a ranch where the elk are hunted and used for food would be food under the prior notice definition. By contrast, elk imported for repopulating a national park where hunting the elk is not permitted would not be food for which prior notice is required. Note that live food game animals are not excluded from prior notice under section 801(m)(3)(B) of the FD&C Act and 21 CFR 1.277(b)(4) or (5) because neither these live food animals nor the products derived there from, fall within the exclusive jurisdiction of USDA under the Federal Meat Inspection Act or Poultry Products Inspection Act.

e. Are chemicals used to manufacture food additives included in the definition of food for prior notice purposes?

Yes, chemicals that are used for food or drink or are used for components of any such articles are food and are subject to the prior notice rule. However, if the chemicals are used for food contact substances or components of food contact substances or pesticides, prior notice is not required (21 CFR 1.276(b)(5)).

f. What are some examples of food contact substances?

Food packaging materials, empty food packages, ceramic dinnerware, brass drinking vessels, and corn husks to be used as tamale wrappers, are examples of food contact substances. Even though these foods are excluded from prior notice requirements in section 801(m) of the FD&C Act, they are still subject to other provisions of the FD&C Act, including section 801(a), and FDA will still make admissibility decisions about them.

g. Are secondary direct additives, many of which are processing aids, exempt from prior notice as "food contact substances"?

The term "secondary direct food additive" is not a defined term. Under 21 CFR 1.276(b)(5), "food" excludes "food contact substances" and "pesticides." Thus, if the "secondary direct food additive" is a food contact substance or a pesticide, it is not included in the definition of food, for purposes of prior notice, and prior notice is not required.

2. FDA Country of Production

a. What is the FDA Country of Production and how does it differ from CBP's Country of Origin?

For food that is in its natural state, the FDA Country of Production is generally the country where the food was grown or collected, including harvested and readied for shipment to the U.S. Articles of food grown, including harvested or collected and readied for shipment, in U.S. territories are considered to be grown in the U.S. (21 CFR 1.276(b)(4)(i)). However, for wild fish, including seafood, that is caught or harvested outside U.S. waters by a vessel that is not registered in the U.S., the FDA Country of Production is the country in which the vessel is registered. (21 CFR 1.276(b)(4)(i)).

For food that is no longer in its natural state, the FDA Country of Production is generally the country where the food was made or processed. However, if the article is made from wild fish aboard a vessel, the FDA Country of Production is the country in which the vessel is registered. If food that is no longer in its natural state was made in a Territory, the FDA Country of Production is the United States (21 CFR 1.276(b)(4)(ii)).

The FDA Country of Production may be different from the CBP Country of Origin. For example, the CBP Country of Origin for beans that are grown and dried in the U.S., then rehydrated and canned in the Dominican Republic would be the U.S. The FDA Country of Production would be the Dominican Republic. However, for purposes of the prior notice provisions of the FD&C Act, the "article of food" is canned beans, not dried beans. From a food safety standpoint, FDA is most interested in knowing where the article of food was processed and canned. To avoid confusion between FDA's prior notice requirements and CBP requirements, the interim final rule uses the term "FDA Country of Production" instead of the term "originating country" or "country from which the article originates." "FDA Country of Production" is already familiar to customs brokers and self-filers using ABI/ACS interface with OASIS.

3. International Mail

a. Are express carriers, such as Federal Express, considered "international mail"?

The term "international mail" only covers foreign national mail services. Express carriers, such as Federal Express, as well as express consignment operators, or other private delivery services are not considered international mail under the prior notice rule (21 CFR 1.276(b)(7)).

4. Port of Arrival/Port of Entry

a. What is the "port of arrival" and how does it differ from the "port of entry?"

The port of arrival is the water, air, or land port at which the article of food is imported or offered for import into the U.S., i.e., the port where the article of food first arrives in the U.S. (21 CFR 1.276(b)(9)). Port of entry is given the same definition the CBP regulations use, which "refer[s] to any place designated by Executive order ..., by order of the Secretary of the Treasury, or by Act of Congress, at which a Customs officer is authorized to accept entries of merchandise, to collect duties, and enforce the various provision of Customs and navigation laws ..." (19 CFR 101.1).

b. Can the port of arrival differ from the port where entry is made?

Yes. The port of arrival is the port where the articles first arrive in the U.S. A consumption or warehouse entry or foreign trade zone admission documentation may be presented to CBP at a different port than the port of arrival. This port is often referred to as the "port of entry" or "port where entry is made." Note that timeframes for submission of prior notice are tied to the time of arrival in the port of arrival, not arrival in the so-called port of entry.

5. United States

a. Is prior notice required for foods that are imported into Puerto Rico?

Yes. The prior notice interim final rule defines the United States to be the 50 states, the District of Columbia, and the Commonwealth of Puerto Rico, but not the U.S. Territories (21 CFR 1.276(b)(13)). Therefore, prior notice is required for food that comes from outside the U.S. into Puerto Rico, but not for food shipped from Puerto Rico into the 50 states or the District of Columbia.

b. Is prior notice required for foods that are imported into the U.S. Territories?

No. The prior notice interim final rule defines the United States to be the 50 states, the District of Columbia, and the Commonwealth of Puerto Rico, but not the U.S. Territories (21 CFR 1.276(b)(13)). Therefore, prior notice is not required for food shipped into a U.S. Territory. However, prior notice is required for food coming from a U.S. Territory (e.g., Guam, the U.S. Virgin Islands, and the Northern Mariana Islands) into the 50 states, the District of Columbia or the Commonwealth of Puerto Rico.

c. Is prior notice required for shipments originating in another NAFTA country?

Yes. Because the "United States" is defined for purposes of prior notice to be the Customs territory of the United States (21 CFR 1.276(b)(13)), food that is imported into the 50 states, the District of Columbia, or the Commonwealth of Puerto Rico from a country that is a signatory to the North American Free Trade Agreement (other than the United States) is subject to prior notice.

D. Scope

1. General

a. What is the scope of prior notice regulation? What shipments of food imported or offered for import into the United States require prior notice?

If the article that is shipped to the United States is food within the meaning of 21 CFR 1.276(b)(5), then prior notice is generally required, even if the item is intended for further processing, is not intended for consumption in the United States, or is not intended for commercial distribution. Thus, prior notice is required for all food for humans and other animals that is imported or offered for import into the United States for use, storage, or distribution in the United States, including food for gifts and trade and quality assurance/quality control samples, food for transshipment through the United States to another country, food for future export, and food for use in a U.S. Foreign Trade Zone.

b. Are there any exceptions from the prior notice requirements?

Yes. Prior notice is not required for:

- Food for an individual's personal use (i.e., for consumption by the individual, family, or friends, and not for sale or other distribution) when it is carried by or otherwise accompanies the individual when arriving in the United States;
- Food that was made by an individual in his/her personal residence and sent by that individual as a personal gift (i.e., for non-business reasons) to an individual in the United States;
- Food that is imported then exported without leaving the port of arrival until export; and Meat food products, poultry products, and egg products that are subject to the exclusive jurisdiction of the U.S. Department of Agriculture (USDA) at the time of importation (21 CFR 1.277(b)).
- Prior notice also is not required under FDA requirements for food brought into the United States in a diplomatic pouch. (The Vienna Convention on Diplomatic Relations (1961) provides: "The diplomatic bag shall not be opened or detained." Art. 27(3) Any baggage or cargo marked "diplomatic bag" or "diplomatic pouch" is immune from search, including by electronic devices, and thus its contents are not subject to FDA's prior notice requirements.)

b. Are there exceptions from prior notice for any of the following:

- Food items of small value or quantity;
- Food samples for research and development or for testing purposes only and not for consumption; or
- Food samples for test marketing?

There are no exemptions from prior notice requirements for:

- Food based on the size or value of the shipment (68 FR 58993);
- Samples of food (including animal feed) for research and development. (However, if the samples are items that are in such early stages of research and development that they cannot yet be considered food under 21 CFR 1.276(b)(5), they would not be subject to prior notice requirements. An example of such an item is a substance being tested for possible preservative qualities before being tested in any food); or
- Samples of food, including animal feed, for test marketing.

2. Shipments from Farms

a. Is prior notice required for tomatoes from a foreign farm that packs and exports tomatoes to the U.S. since farms don't have to register?

Yes. The requirement for prior notice is not based on whether registration is required. FDA registration requirements (21 CFR 1.266(b)) apply to facilities that manufacture/process, pack, or hold food for consumption in the United States. The prior notice requirements apply to articles of food imported or offered for import into the United States. Although registration of farms is not required, the articles of food grown, harvested, or collected on farms are not exempt form prior notice requirements. Thus, generally, the food that a foreign farm exports to the U.S. is subject to the prior notice requirements (21 CFR 1.277).

3. Shipments for Charities

a. Is there an exemption for food imported for charity?

No. Food intended for charity is not exempt from prior notice. Although the registration interim final rule exempts nonprofit food establishments in which food is prepared for, or served directly to, the consumer from the requirements to register their facilities, (21 CFR 1.266(e)), the prior notice interim final rule does not exempt food imported for use by those nonprofit food establishments. Thus, imported food that is imported for or by a U.S. charity is subject to prior notice (21 CFR 1.277).

4. U.S. Goods Returned

a. Is prior notice required for "reimported" food product that was produced in the U.S., shipped to a foreign country, and then shipped back to the U.S. without further processing?

Yes. FDA has determined that, for the purposes of section 801(m) of the FD&C Act, the phrase "imported or offered for import into the United States" applies to articles of food of U.S. origin that are "reimported" back into the U.S. (68 FR 58990; 21 CFR 1.277). These reimportations are most often referred to as American Goods Returned or U.S. Goods Returned.

5. Food Not for Consumption in the U.S.

a. If the food is not for consumption in the U.S., is prior notice required?

Yes. Prior notice requirements apply even when the food is not for consumption in the United States. You must submit prior notice for food for that is for transshipment, further processing and export, or storage and export. In contrast, the requirement to register facilities applies only to food facilities that manufacture/process, pack, or hold food for consumption in the U.S.

b. Is prior notice required for food transiting the U.S. for exportation to another country, e.g., for a Transportation and Exportation (T&E) entry?

Yes. Prior notice is required for food for transshipment through the U.S. to another country and food for future export (21 CFR 1.277(a)).

6. Foreign Trade Zones

a. Will food being admitted into a Foreign Trade Zone need to have prior notice?

Yes. Food for admission into a Foreign Trade Zone is subject to the requirements of the prior notice regulation (21 CFR 1.277). However, prior notice is not required when the food is withdrawn from the FTZ, either as an export or for use within the United States. However, if the food is withdrawn from the FTZ for consumption entry into the United States, FDA will be notified and make the admissibility decision about the consumption entry at that time.

7. Gifts

a. Does prior notice apply to food sent as gifts from family living outside the U.S.?

If the food was made by an individual in his/her personal residence and sent by that individual as a personal gift (i.e., for non-business reasons) to an individual in the United States, prior notice is not required (21 CFR 1.277(b)(2)). Other food products sent as gifts are subject to the prior

notice requirement (21 CFR 1.277). FDA recognizes that, in these circumstances, the sender who purchased the food as a gift may not have the manufacturer/producer registration number. Thus, the sender can use the name and address of the firm that appears on the label. A registration number need not be provided (21 CFR 1.281(a)(6)).

8. Airline Food

Is aircraft food exempt from prior notice, since any excess is incinerated at the U.S. airport? Is in-flight food, imported in bulk and moved in-bond to U.S. caterers, for use on export flights exempt?

If the aircraft food is consumed on the flight or discarded and is not entered into the U.S. for use, storage, or distribution, it is outside the scope of the regulation and prior notice is not required (21 CFR 1.277(a)). By contrast, prior notice is required for in-flight food that is moved to U.S. caterers for use on export flights (21 CFR 1.277).

9. Personal Baggage

a. I am bringing food from a foreign country in my luggage and for my personal use. Do I need to submit prior notice to FDA?

No. Prior notice is not required for food that is carried by or otherwise accompanies an individual entering the United States (e.g., is in his or her carry-on or checked baggage) when the food is for that individual's personal use (21 CFR 1.277(b)(1)). Personal use means that the food is for consumption by the individual or by the individual's family and friends and is not for sale or other distribution.

10. Food Shipped by Express Carriers or Express Consignment Operators

a. Is an article of food that is shipped by an express carrier or express consignment operators like Fed Ex exempt from prior notice?

No. Imported food transported into the U.S. via express carriers or express consignment operators is not exempt from the requirements of the prior notice regulation. Articles imported via these private delivery services are subject to prior notice, which must be submitted within the timeframe for the applicable mode of transportation (21 CFR 1.279).

11. Food Shipped by International Mail

a. Is food imported into the U.S. by international mail exempt from prior notice?

No. Food sent to the U.S. via international mail is not exempt from the requirements of the prior notice regulation. Articles imported via international mail are subject to prior notice, which must be submitted before the article is sent to the U.S. (21 CFR 1.279(c)).

12. Personal Shipments

a. Is prior notice required for food for personal use that is shipped by the individual while overseas and, therefore, does not accompany the individual when returning to the U.S.?

Yes. Food purchased abroad and sent to the U.S. (i.e., does not accompany the individual when arriving in the U.S.) is subject to prior notice (21 CFR 1.277).

a. I have ordered food for my personal use that is being shipped to me through international mail. Do I need to provide prior notice to FDA?

Yes. Prior notice must be provided. The exception for food for "personal use" applies only when the food is carried by or otherwise accompanies an individual when arriving in the U.S. (21 CFR 1.277(b)(1)). This exception does not apply when the food is shipped to the U.S. Although you or any other person with knowledge of the required information may submit prior notice (21 CFR 1.278), it would make sense for the foreign shipper to provide prior notice because the PN Confirmation Number, which indicates that FDA has received and confirmed the prior notice for review, must accompany an article of food that is sent to the U.S. via international mail (21 CFR 1.279(e)).

b. What if my order is shipped by an express carrier or express consignment operators like Fed Ex?

Prior notice is still required. Food transported to the U.S. via express carriers or express consignment operators is not exempt from the requirements of the prior notice regulation. Articles imported via these private delivery services are subject to prior notice, which must be submitted within the timeframe for the applicable mode of transportation (21 CFR 1.279).

14. Trade Samples

a. Is food that is a trade sample and that I carry with me into the United States exempt from prior notice?

No. The exclusion for good carried by an individual applies when the food is for the individual's personal use when it is carried by or otherwise accompanies the individual when arriving in the United States (21 CFR 1.277(b)(1)). However, trade samples are imported or offered for import to generate sales, which is a commercial, not personal, use.

15. Food Moved with Household Goods

a. Are foods included with my household goods subject to prior notice when I move to the U.S.?

Yes.

16. Food Subject to USDA/FSIS Requirements

a. Is prior notice required for meat, poultry, or eggs that are under the jurisdiction of the U.S. Department of Agriculture (USDA)?

If, at the time the food is imported or offered for import, the food is subject to the exclusive jurisdiction of the USDA's Federal Meat Inspection Act (21 U.S.C. 601 et seq.), the Poultry Products Inspection Act (21 U.S.C. 451 et seq.), or the Egg Products Inspection Act (21 U.S.C. 1031 et seq.), the food is not subject to the requirements of prior notice. (21 CFR 1.277(b) (4)-(b)(6)).

E. Requirements to Submit Prior Notice of Imported Food

1. Submitters and Transmitters

a. Who may submit prior notice to FDA?

Any person with knowledge of the required information may submit prior notice for an article of food. This person is the submitter. The submitter may also use another person to transmit the required information on his or her behalf. The person who transmits the information is the transmitter. The submitter and transmitter may be the same person (21 CFR 1.278).

b. May I submit a prior notice on behalf of another person?

Yes. Note that if you transmit the required information on behalf of a submitter, you are the transmitter.

c. Is it possible for the submitter to have his/her legal residence in the country of origin and for the transmitter to have his/her legal residence in the U.S.?

Yes. There are no geographic restrictions on the location of the submitter or the transmitter (21 CFR 1.278).

2. Deadlines for Prior Notice

a. When must prior notice be submitted?

Prior notice must be submitted and the submission must be confirmed by FDA no more than 5 days before arrival, except for food arriving by international mail, and no less than:

2 hours before arrival, if the food is arriving by land by road;
4 hours before arrival, if the food is arriving by land by rail
4 hours before arrival, if the food is arriving by air; and
8 hours before arrival, if the food is arriving by water (21 CFR 1.279(a) and (b)).

For article of food sent by international mail, prior notice must be submitted and confirmed by FDA before the food is sent (21 CFR 1.279(c)). The Prior Notice (PN) Confirmation Number must appear on the Customs Declaration that accompanies the package (21 CFR 1.279(e)).

If you are carrying an article of food or if it otherwise accompanies you (i.e., the food is in your checked baggage), and the food is not for personal use, you must submit prior notice according to the timeframe established for the mode of transportation you are using. You must receive confirmation from FDA and provide a copy of the confirmation, including the PN Confirmation Number, to CBP or FDA when arriving in the U.S. (21 CFR 1.279(f).

3. Submitting Prior Notice

a. How is prior notice submitted to FDA?

You must submit prior notice to FDA electronically either through the U.S. Bureau of Customs and Border Protection's (CBP's) Automated Broker Interface of the Automated Commercial System (ABI/ACS) or FDA's Prior Notice System Interface.

CBP's ABI/ACS allows prior notice to be submitted to FDA through the existing ABI/ACS interface (21 CFR 1.280(a)(1)); and FDA's Prior Notice System Interface (PNSI) is available through FDA's website at http://www.access.fda.gov/ (21 CFR 1.280(a)(2)).

Both ABI/ACS and the FDA PNSI are available 24 hours a day, 7 days a week for information submission.

b. Do I have to submit prior notice information to both FDA and CBP?

No. Prior notice must be submitted to FDA. If you are an authorized user of CBP's ABI/ACS you may submit prior notice to FDA through the ABI/ACS interface or through FDA's Prior Notice System Interface (PNSI) at http://www.access.fda.gov/. If you are not an authorized user of CBP's ABI/ACS, you may arrange for prior notice submission by an authorized user or submit prior notice through PNSI. Except, however, prior notice for the following, must be made through FDA's PNSI:

Articles of food shipped through international mail;

Transaction types that cannot be transmitted through ABI/ACS; and Articles of food that have been refused admission under section 801(m)(1) of the FD&C Act (21 CFR 1.280(a)(2)).

c. I am an authorized user of CBP's ABI/ACS. Can I use FDA's Prior Notice System Interface (PNSI) to submit prior notice?

Yes. You may submit prior notice through either system.

d. I am shipping food by international mail. How do I provide prior notice?

You provide prior notice to FDA through PNSI (21 CFR 1.280(a)(2)). Prior notice must be submitted and confirmed by FDA before the food is sent (21 CFR 1.279(c)). The Prior Notice (PN) Confirmation Number must appear

on the Customs Declaration that accompanies the package (21 CFR 1.279(e)).

e. What happens if the CBP or FDA system is not working?

If CBP's ABI/ACS is not available or if your broker's or your self-filing system is not working, you must submit prior notice through the FDA Prior Notice System Interface at http://www.access.fda.gov/. (21 CFR 1.280(b)).

If we determine that our Prior Notice System Interface is not working, we will issue notification on our website (http://www.fda.gov/) and on the Prior Notice System Interface, as well as through messages in ABI/ACS. If you do not use ABI/ACS, and the PNSI is down, you must submit prior notice by e-mail or fax to FDA.

If FDA determines that its automated import system (OASIS) is not working, FDA will issue notification on our website (http://www.fda.gov/) and on the Prior Notice System Interface and all prior notices must be submitted to FDA by e-mail or by fax. The e-mail address and fax number, as well as more information on how to submit prior notice if FDA or CBP systems are not working, will be available at http://www.fda.gov/.

f. What happens if my computer system is not functioning or I don't have electricity for a period of time?

If your computer is not functioning or there is no electricity to operate your computer but the Prior Notice System Interface and ABI/ACS are functioning, you must arrange to use a functioning computer to submit the required prior notice.

If I have problems submitting prior notice through the Prior Notice System Interface (PNSI), how can I get help?

Tutorials on use of PNSI are available on FDA's website at http://www.fda.gov/. If you are already familiar with how to use PNSI and you are having problems win an online submission through PNSI, in the U.S call toll-free 1-800-216-7331. From elsewhere, call 301-575-0156. You may send a fax to 301-210-0247. These phone numbers will be staffed on business days from 7 AM until 11 PM U.S. Eastern Time. Requests for assistance also may be emailed to http://www.cfsan.fda.gov/~furls/helpf2.html. For assistance with ABI/ACS transmission, contact your CBP client representative.

g. Can I submit the prior notice to the Prior Notice System Interface in a language other than English?

You must submit all prior notice information in the English language, except that an individual's name, the name of a company, and the name of a street may be submitted in a foreign language. All information, including these items, must be submitted using the Latin (Roman) alphabet (21 CFR 1.280(a)).

h. If you are not comfortable with English, you may choose to use a transmitter to enter the information for you. Can anyone submit prior notice through ACS/ABI?

No. CBP allows submissions through ACS/ABI only by brokers or filers that are recognized by CBP, e.g., licensed brokers and filers. Individuals can contract with a broker to transmit prior notice for them. In this case, the submitter is the person responsible for providing the information, but the broker is the transmitter.

Brokers are licensed private individuals or companies that are regulated by CBP and who aid importers and exporters to move merchandise through CBP. Brokers provide the proper paperwork and payments to CBP for clients and charge a fee for this service. Before brokers apply for a license, they must pass the Customs broker examination. See:http://www.customs.gov/xp/cgov/import/broker_management/brokers.xml

Filers are licensed carriers and self-filers who submit entries on their own behalf. Filers are required to submit a written request to CBP port personnel for a filer code, which is subsequently assigned by CBP headquarters. See: http://www.cbp.gov/ImageCache/cgov/content/import/brokers/broker_5fhandbook_2epdf/v1/broker_5fhandbook.pdf

Individuals who choose not to use a broker or who chose not to become recognized by CBP as a filer can submit their prior notice only through the FDA PN System Interface (PNSI).

i. Do I have to submit prior notice if I do not have to file a consumption entry with CBP?

Yes. The requirement to submit prior notice to FDA is different from the requirement to file a consumption entry with CBP. Some foods arriving in the United States do not require a CBP consumption entry at the time of arrival, such as entries that move under bond (in-bonds) from the port of arrival to an inland port and shipments into a Foreign Trade Zone. However, any article of food imported or offered for import into the United States requires prior notice, unless the food is specifically excluded from the requirement to submit prior notice (21 CFR 1.277).

j. Can I submit any CBP entry or admission for food without prior notice?

No, not if the entry or admission contains food subject to prior notice requirements. You can not submit a CBP import entry or admission if you have not submitted prior notice to FDA for an article of food that requires prior notice, because the Harmonized Tariff Schedule (HTS) codes have been flagged to indicate foods that require or may require prior notice. You must submit prior notice either through the ABI/ACS (along with the CBP entry information) or through the FDA Prior Notice System Interface at http://www.access.fda.gov (21 CFR 1.280(a)).

When you submit prior notice through the FDA Prior Notice System Interface, you will receive a Prior Notice (PN) Confirmation Number (21 CFR 1.279(d)). If you subsequently submit import entry or admission information through ABI/ACS, you must enter the PN Confirmation Number for that submission as an Affirmation of Compliance when the CBP entry or admission is filed. The PN Confirmation Number will allow CBP to confirm that prior notice was submitted to FDA.

k. I cannot or do not want to use CBP's ABI/ACS. What other way can I submit prior notice?

If you cannot or do not want to use ABI/ACS, you must submit prior notice through the FDA Prior Notice System Interface at http://www.access.fda.gov/ (21 CFR 1.280(a)). You will receive a confirmation number when you complete the prior notice through the Prior Notice System Interface (21 CFR 1.279(d)). The PN Confirmation Number must accompany the food when the article arrives in the U.S. (21 CFR 1.279(g)).

l. How do I submit prior notice for foods that are covered by immediate transportation or transportation and exportation entries?

The U.S. Bureau of Customs and Border Protection (CBP) has modified the Automated Broker Interface of the Automated Commercial System (ABI/ACS) interface to allow for submission of prior notice to FDA for IT and T&E entries. Prior notice for such entries also may be made through FDA's Prior Notice System Interface.

m. I regularly ship food to the United States in a truck, under bond, to St. Louis. When and how do I need to file prior notice?

The time frame for submitting prior notice is based on the mode of transportation and the port of arrival, i.e., the port where the food first arrives in the United States. This port may be different from the port where the entry documentation is presented to CBP. Prior notice for food arriving by truck (by land by road) must be confirmed by FDA for review at least 2 hours before the truck arrives at the port where it is crossing the border into the United States (21 CFR 1.279).

If you are entering under bond and want to file your CBP entry in St. Louis, you can file your prior notice either through FDA's Prior Notice System Interface or through CBP's ABI/ACS interface (21 CFR 1.280(a)). If you submit the prior notice through the FDA Prior Notice System Interface, you will receive a Prior Notice Confirmation Number (21 CFR 1.279(d)). This Prior Notice (PN) Confirmation Number must be made available to CBP when entering the United States, and must be entered as an Affirmation of Compliance when you file your CBP entry information (21 CFR 1.279(g)).

n. Is there a filing fee for prior notice?

No. FDA does not charge a fee for filing prior notice or for using FDA's Prior Notice System Interface. However, if you chose to use a broker to file the prior notice through the ABI/ACS interface, the broker may charge a fee for providing that service. The collection of duty by CBP is not affected by FDA's prior notice regulation.

4. Prior Notice Data Elements

a. What information must be included in the prior notice?

The information required for prior notice varies, based on the type of entry, mode of transportation for the entry, and whether the food is in its natural state. You should refer to the interim final rule (21 CFR 1.281) for details on the required information. The preamble to the interim final rule includes a chart that summarizes the information requirements (68 FR 58980). The interim final rule is available on FDA's web site http://www.fda.gov/. Tutorials on the FDA Prior Notice System Interface (PNSI) also are available on FDA's website to help guide you through the process for providing the required information when you submit prior notice through PNSI.

b. How does the information required for prior notice of imported food shipments differ from information submitted to the CBP before December 12, 2003?

Most of the information required by 21 CFR 1.281 also is entry data required by CBP before December 12, 2003. The key differences are the information required about the identity of manufacturer, grower, owner, and submitter and the identification of the country from which the article is shipped.

c. What time do I enter for anticipated time of arrival?

For prior notice, anticipated time of arrival is the local time when the food will arrive at the border (21 CFR 1.281(a)(11)(iii)). For vessels, this would be when the vessel will dock in the port where the shipment is offloaded. For planes, this would be when the plane is scheduled to land. For land vehicles, such as trucks, buses, and trains, this would be when they will cross at the border.

5. Changes to Prior Notice Submissions

a. Do I have to resubmit prior notice if the anticipated time of arrival changes?

No. Prior notice does not need to be resubmitted if the anticipated arrival information changes (21 CFR 1.282(a)(2)(ii)). Although a new prior notice submission is not required, FDA staff may need time to respond to the changes in arrival information.

b. What should I do if information changes after I submit prior notice?

The interim final rule requires that if required information (except estimated quantity, anticipated arrival information, and planned shipment information) changes after FDA has confirmed prior notice for review, the prior notice must be resubmitted (21 CFR 1.282(a)(2)).

If the prior notice was submitted as part of a multi-line ABI/ACS entry, and information about one or more of the products changes, the entry must be deleted and resubmitted. Prior notice for the new product can be submitted as part of a new entry. If you submitted the prior notice via the FDA Prior Notice System Interface, you should cancel the prior notice via the FDA Prior Notice System Interface (21 CFR 1.282(b)).

c. Which changes require me to resubmit prior notice and which changes don't?

Changes in the estimated quantity, anticipated arrival information, or planned shipment do not require resubmission of prior notice after FDA has confirmed your prior notice submission for review (21 CFR 1.282(a)(1)(i)-(iii)). For all other changes, e.g., if the identity of the manufacturer changes, you should cancel the prior notice and you must resubmit prior notice if you still intend to import or offer the food for import into the U.S. (21 CFR 1.282).

6. Changes to Shipments

a. May I add another article of food to an existing prior notice after the prior notice has been submitted to FDA?

No. Each article of food requires a separate prior notice (21 CFR 1.281(a)(5) and 1.281(b)(4)) and receives a unique confirmation number (21 CFR 1.279(d)). However, FDA is allowing prior notices to be grouped in an ABI/ACS entry, or in an "envelope" for the FDA Prior Notice System Interface (PNSI) entries and In-Bonds submitted through ABI/ACS, in order to reduce data entry for transmitters and to simplify CBP review at the border. For submissions through PNSI, no articles of food can be added to an envelope after the Prior Notice Confirmation Number(s) has been received. For submissions through ABI/ACS, no additional lines may be added after the entry is accepted by ACS.

b. What can I do if I want to add another article of food to a shipment after prior notice was submitted to the FDA?

New articles of food cannot be added to an entry, or prior notice envelope, after it has been submitted to FDA (21 CFR 1.282). If a new article of food is being added to a shipment for which prior notice(s) has already been submitted and confirmed, a separate prior notice must be filed for that article under a new entry/envelope (21 CFR 1.281(a) and (b)). The submission time for the new prior notice will be different from that of the rest of the shipment, and this may have an effect on the ability of the shipment to enter the United States (21 CFR 1.279). For example, if you add another food to a truck at 9:00 am, that food is not covered by timely prior notice until 11:00 am. The rest of the food on that same truck may be covered by prior notices submitted at 8:00 am and deemed timely at 10:00 am. It is recommended that the time for arrival be anticipated on the last article of food submitted and confirmed by FDA for review.

c. What can I do if I want to remove an article of food from the information provided for a shipment?

If the prior notice was submitted through ACS, you can delete the prior notice using the existing entry delete procedures. If you submitted the prior notice via the FDA Prior Notice System Interface, you should cancel the prior notice via the Prior Notice System Interface (21 CFR 1.282(b)).

7. PNSI and ACS/ABI Features

a. If I need to revise some information on a prior notice, will some of the fields be filled in by the computer automatically or will I need to start from the beginning?

The answer depends on how the prior notice submission is being made. If submitting through the CPB Automated Broker Interface of the Automated Commercial System (ABI/ACS), the ability to pre-fill or otherwise support submission will depend on the functionality of the software used by the filer. The ABI/ACS interface is concerned with the validity of the information in the submission, not with how it was generated.

If submitting through FDA's Prior Notice System Interface, the answer varies with the status of the submission. The Prior Notice System Interface will allow a transmitter to pre-enter information and save the draft until the time of actual submission. Before the draft is submitted, changes can be easily made to the draft because information previously entered will be pre-filled.

After the prior notice has been submitted to FDA, no changes can be made unless requested by FDA to correct an error found during the review process before a Prior Notice (PN) Confirmation Number issues to the transmitter (21 CFR 1.282).

8. Confirmation

a. Will I receive confirmation that FDA has received the prior notice I submitted?

Yes. FDA will notify the transmitter that the prior notice has been confirmed for review with a reply message that

contains a Prior Notice (PN) Confirmation Number. For prior notice submissions through the CPB ABI/ACS, the PN Confirmation Number together with the "PN received" message will be made available to the transmitter (broker or filer) through the ABI/ACS. For prior notice submissions through the FDA Prior Notice System Interface (PNSI), a PN Confirmation Number will be provided to the transmitter through PNSI as soon as FDA confirms your prior notice for review.

b. Is a copy of the prior notice required to accompany the food?

To ensure that entry proceeds as smoothly as possible, the carrier or individual should consider having a copy of the reply message that contains a PN Confirmation Number in his/her possession upon arrival.

For food carried by or otherwise accompanying an individual that is not for personal use, the individual must provide a copy of the PN confirmation to FDA or CBP. Food covered by prior notice submitted through the FDA Prior Notice System Interface must be accompanied by a copy of the reply message that contains the PN Confirmation Number.

For international mail packages, the confirmation number must accompany the package.

c. Does receipt of a PN Confirmation Number mean that the food will not be examined or sampled?

No. Receipt of a PN Confirmation Number is evidence only that a prior notice has been received for FDA review. Based on review of the prior notice, FDA may determine that an article of food should not be allowed to proceed into the United States without further inspection and sampling at the border. The food may be refused under the prior notice regulation and section 801(m) of the FD&C Act and held if the prior notice is inaccurate or if it is untimely and FDA has not had sufficient time to receive, review, and respond to the prior notice information. In addition, FDA may examine or sample the food for food safety and security concerns and for determining whether the food is subject to refusal under section 801(a) of the FD&C Act.

d. If receipt of the Prior Notice (PN) Confirmation Number does not mean the FDA has determined that timely prior notice was submitted or that the information submitted is accurate, what is the value of the PN Confirmation Number?

The Prior Notice (PN) Confirmation Number is FDA's notice to you that that your prior notice was submitted to and received for review by FDA. It is the signal to you that the time frame for prior notice for the food covered by that prior notice submission has started. In addition, the PN Confirmation Number provides a mechanism for prior notice data, submitted to FDA, to be matched with an entry submitted to CBP. The timeliness of prior notice can not be assessed until the food actually arrives in the United States and, often, the accuracy of the prior notice can not be fully determined until the food is examined upon arrival.

F. Consequences

1. Inadequate Prior Notice

a. What does FDA consider to be inadequate prior notice?

Inadequate prior notice is when:

1. There is no prior notice submitted for an article of food imported or offered for import into the United States; and/or
2. The information submitted in the prior notice is inaccurate; and/or
3. The prior notice is not submitted in concurrence with the required timeframes.

FDA's enforcement policies on inadequate prior notice are being set out in a separate guidance document. This Compliance Policy Guide will be available on FDA's website at http://www.fda.gov/ora/compliance_ref/cpg/default.htm

b. What are some examples of inadequate prior notice?

The following examples of inadequate prior notice are for 5 articles of food (foods A, B, C, D, and E) arriving at the border by truck:

1. Prior notice was submitted for only 4 of the 5 articles of food (foods A, B, C, and D). Inadequate prior notice, e.g., no prior notice, was provided for food E.
2. Prior notice for food A was submitted and confirmed for FDA review with PN Confirmation Number 999. Food B arrives associated with PN Confirmation Number 999. Inadequate prior notice, e.g., inaccurate prior notice, was provided for food B.
3. Prior notice for food A was submitted and confirmed for FDA review at 9:00 am on February 2, 2005. Food A arrives at the port of arrival at 9:30 am on February 2, 2005, but CBP has not received an examination decision response from FDA. Inadequate prior notice, e.g., untimely prior notice, was provided for food A.

FDA's enforcement policies on inadequate prior notice are being set out in a separate guidance document. This

Compliance Policy Guide will be available on FDA's website at http://www.fda.gov/ora/compliance_ref/cpg/default.htm

c. What happens to food that is imported or offered for import without adequate prior notice?

Articles of food arriving with no prior notice, inaccurate prior notice, or untimely prior notice may be refused admission and, other than food arriving by international mail or carried by or otherwise accompanying an individual, if refused, will be handled in one of the following ways:

1. Immediately exported, with CBP concurrence, from the port of arrival; or
2. Held within the port of entry, unless directed by CBP or FDA (21 CFR 1.283(a)(1)(i)-(iii) and (b)).
3. Refused food is considered general order merchandise under section 490(a) of the Tariff Act (19 U.S.C. 1490(a)) and may move only under appropriate custodial bond (21 CFR 1.283(a)(2)). If the refused article is moved, the submitter must notify FDA of the hold location within 24 hours of refusal. The refused food may not be delivered to the importer, owner, or ultimate consignee (21 CFR 1.283(a)(2)(ii)).

For food that is carried by or accompanies an individual arriving in the U.S. and the food is not for personal use, if adequate prior notice is not submitted or if the PN confirmation number cannot be provided to FDA or CBP, the food is subject to refusal. If before leaving the port, the individual does not arrange to have the food held at the port or exported, the article may be destroyed (21 CFR 1.283(b)).

For food arriving by international mail, if prior notice is inadequate or if the PN Confirmation Number is not affixed, the article will be held for FDA inspection and disposition. If refused and there is a return address, the parcel may be returned to sender. If there is no return address or the food in the shipment appears to present a hazard, FDA may dispose of or destroy the parcel at its expense. If FDA does not respond within 72 hours of the CBP hold, CBP may return the parcel back to the sender or, if there is no return address, destroy the parcel, at FDA expense.

FDA's enforcement policies on inadequate prior notice are being set out in a separate guidance document. This Compliance Policy Guide will be available on FDA's website at http://www.fda.gov/ora/compliance_ref/cpg/default.htm.

d. Will my food be held if it arrives earlier than the anticipated time of arrival I submitted?

If arrival occurs before the anticipated arrival time, the food could be refused and held until the prior notice timeframe has elapsed (21 CFR 1.279) or until processing is complete, which ever comes first. However, if the prior notice has been fully processed by FDA, the food will not be refused because the anticipated arrival time has not yet come (21 CFR 1.283(a)(1)(ii)). However, if FDA plans to examine the food, it may be held to allow time for FDA staff to arrive.

e. What does it mean if, after I receive a Prior Notice (PN) Confirmation Number, FDA later refuses the same article of food?

The PN Confirmation Number only confirms that the submission is complete and facially valid. If FDA's review process determines that the prior notice is inaccurate after receipt of the prior notice is confirmed by issuance of the PN Confirmation Number, the article of food is still subject to refusal under 21 CFR 1.283(a)(1)(ii).

f. Does meeting all the requirements of prior notice mean that the article of food will not be held or examined further?

No, the food must meet the requirements of all other applicable regulations as well. If FDA decides to take no prior notice action for an article of food under 21 CFR 1.283 or 1.285(a), this decision has no bearing on whether the article of food is admissible or will be granted admission under other provisions of the FD&C Act or other U.S. laws. Thus, for imported food or food offered for import, FDA will continue its normal investigative and enforcement activities for food safety and security concerns and for determining whether the food is subject to refusal under section 801(a) of the FD&C Act.

g. Who will be notified if FDA determines that a food needs to be held for examination when it arrives at the border?

FDA will communicate the decision to examine articles of food to CBP.

ACKNOWLEDGMENT

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Part H

Functional Food Ingredients

83 Food Additives

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CONTENTS

I. INTRODUCTION

The broadest definition of a food additive is any substance that becomes part of a food product, either directly or indirectly, during some phase of processing, storage, or packaging. The universe of food additives encompasses.

Direct food additives, those that are intentionally added to food for a functional purpose, in controlled amounts, usually at low levels (from parts per million to 1–2%, by weight), and

Indirect or incidental food additives, those entering into food products in small quantities as a result of growing, processing, or packaging.

The difference between food ingredients and additives is mainly in the quantity used in any given formulation. Food ingredients can be consumed alone as food (e.g., sucrose), while food additives are used in small quantities (usually less than 2%) relative to the total food composition but which nonetheless play a large part in the production of desirable and safe food products.

Food additives may be looked upon as minor ingredients incorporated into foods to affect their properties in some desired way. Most commonly, the effects desired relate to color, flavor, texture, nutritive value, or stability in storage. There is no rigorous definition that meets all needs.

The *Codex Alimentarius*, which dominates actions in international circles, considers an additive as an ingredient "not normally consumed as a food by itself and normally used as a typical ingredient." This obviously leaves great latitude for judgment by the committee. The U.S. Food, Drug, and Cosmetic Act has a complex definition of food additives that comes close to any component of food introduced into U.S. commerce after 1957 and it will be addressed in the section dealing with government regulations.

II. FUNCTIONS OF FOOD ADDITIVES

Direct food additives serve several major functions. Many additives, in fact, are multifunctional (Table 83.1). The basic functions of direct food additives include the following:

A. Preservation

Food preservation techniques have advanced in the past 100 years and now include thermal processing, concentration and drying, refrigeration and freezing, modified atmosphere, and irradiation. However, the use of chemical preservatives frequently augments these basic preservation techniques and represents the most economical way for food manufacturers to ensure a reasonable shelf life for their product. Antioxidants and antimicrobial agents perform some of these functions as well.

B. Processing

Food processors are increasingly using food additives to ensure the integrity and appeal of their finished products. Emulsifiers maintain mixtures and improve texture in breads, dressings, and other foods. They are used in ice cream when smoothness is desired, in breads to increase shelf life and volume and to distribute the shortening, and in cake mixes to achieve batter consistency. Stabilizers and thickeners assist in presenting an appealing product with consistent texture. Sorbitol, a humectant and sweetener, is used to retain moisture and enhance flavor. With the removal of sugar from many foods for dietetic reasons, a substitute bulking agent is needed.

C. Appeal and Convenience

The changing eating habits of consumers, partly brought about by the large increase in the number of women who work outside the home, is creating a growing need for convenience foods. In many of these types of foods, it is essential that a variety of additives be used to provide the taste, color, texture, body, and general acceptability that are required. This need for convenience, while maintaining aesthetic appeal and taste, is becoming extremely important. Most food additives such as gums, flavoring agents, colorants, and sweeteners are included by food processors because consumers demand that food look and taste good as well as be easy to serve.

D. Nutrition

There have been tremendous advances in the knowledge of human nutrition, and consumers are increasingly aware of the value of good nutrition. Vitamins, antioxidants, proteins, and minerals are added to foods and beverages as supplements in an attempt to ensure proper nutrition for those who do not eat a well-balanced diet. In addition, additives such as antioxidants are often used to prevent deterioration of natural nutrients during processing. Recently more importance has been attributed to disease prevention through proper nutrition, as well as to increasing performance through sport nutrition products. On the other hand, the desire for good nutrition through a balanced diet may adversely affect consumer demand for some food additives such as fat substitutes.

Adopted from the National Academy of Sciences/National Research Council national survey of food industries, the following terms describe the physical and technical effects of various food additives:

- Anticaking agent or free-flow agent: substance added to finely powdered or crystalline food products to prevent caking, lumping, or agglomeration.
- Antimicrobial agent: substance used to preserve food that prevents the growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors, antimicrobial agents, antimyotic agents, preservatives, and mold preventing agents (indirect additives).

TABLE 83.1
Food Additives and Their Functions

Food Additive	Preservation	Process Improvement	Appeal Modification	Nutrition
Anticaking agents		X	X	
Antioxidants	X		X	X
Colors			X	
Emulsifiers		X	X	X
Enzymes		X	X	
Fat substitutes				X
Flavors			X	
Humectants			X	
Leavening agents		X	X	
pH control agents	X	X		
Preservatives	X			
Processing aids		X		
Sweeteners (sugars only)	X	X	X	
Sweeteners, high intensity				X
Thickeners and stabilizers	X		X	X
Vitamins and minerals				X

- Antioxidant: substance used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation.
- Boiler water additive: substance used in a steam or boiler water system as an anticorrosion agent to prevent scale or to effect steam purity.
- Color or coloring adjunct: substance used to preserve or enhance the color or shading of a food including color fixatives and color-retention agents.
- Curing or pickling agent: substance imparting a unique flavor and/or color to food, usually producing an increase in shelf-life.
- Dough strengthener: substance used to modify starch and gluten, thereby producing more stable dough.
- Drying agent: substance with moisture-absorbing ability used to maintain an environment of low moisture.
- Emulsifier or emulsifier salt: substance which modifies surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.
- Enzyme: used to improve food processing and the quality of finished food.
- Firming agent: substance added to precipitate residual pectin, thus strengthening the supporting tissue and preventing its collapse during processing.
- Flavor enhancer: substance added to supplement, enhance, or modify the taste and/or aroma of a food without imparting a characteristic taste or aroma of its own.
- Flavoring agent or adjuvant: substance added to impart or help impart a taste or aroma in food.
- Flour treating agent: substance added to milled flour to improve its color and/or baking qualities, including bleaching and maturing agents.
- Formulation aid: substance used to promote or to produce a desired physical state or texture in food, including carriers, binders, fillers, plasticizers, film-formers, and tableting aids, etc.
- Freezing or cooling agent: substance that reduces the temperature of food materials through direct contact.
- Fumigant: volatile substance used for controlling insects and pests.
- Humectant: hygroscopic substance incorporated in food to promote retention of moisture.
- Leavening agent: substance used to produce or stimulate production of carbon dioxide in baked goods in order to impart a light texture, including yeast, yeast foods, and calcium salts.
- Lubricant or release agent: substance added to food contact surfaces to prevent ingredients and finished products from sticking to them (direct additives), including release agents, lubricants, surface lubricants, waxes, and antiblocking agents (indirect additives).
- Malting or fermenting aid: substance used to control the rate of nature of the malting or fermenting process, including microbial nutrients and suppressants and excluding acids and alkalis.
- Masticatory substance: substance that is responsible for the long-lasting and pliable property of chewing gum.
- Nonnutritive sweetener: substance having less than 2% of the caloric value of sucrose per equivalent unit of sweetener.

- Nutrient supplement: substance necessary for the body's nutritional and metabolic processes.
- Nutritive sweetener: substance having greater than 2% sucrose per equivalent unit of sweetening capacity.
- Oxidizing or reducing agent: substance which chemically oxidizes or reduces another food ingredient, thereby producing a more stable product.
- pH control agent: substance added to change or maintain active acidity or basicity, including buffers, acids, alkalis, and neutralizing agents.
- Processing aid: substances used as a manufacturing aid to enhance the appeal or utility of a food or component, including clarifiers, clouding agents, catalysts, flocculents, filter aids, crystallization inhibitors, etc.
- Propellant: gas used to supply force to expel a product or to reduce the amount of oxygen in contact with the food in packaging.
- Sequesterant: substance which combines with polyvalent metal ions to form a soluble metal complex to improve the quality and stability of products.
- Solvent or vehicle: substance used to extract or dissolve another substance.
- Stabilizer or thickener: substance used to produce viscous solutions or dispersions, impart body, improve consistency, or stabilize emulsions, including suspending and bodying agents, setting agents, and bulking agents.
- Surface-active agent: substance used to modify surface properties of liquid food components for a variety of effects, other than emulsifiers. Including solubilizing agents, dispersants, detergents, wetting agents, rehydrating enhancers, foaming agents, defoaming agents, etc.
- Surface finishing agents: substance used to increase palatability, preserve gloss, and inhibit discoloration of foods, including glazes, polishes, waxes, and protective coatings.
- Synergist: substance used to act or react with another food ingredient to produce a total effect different from or greater than the sum of the effects produced by the individual ingredients.
- Texturizer: substance which affects the appearance or feel of the food.
- Tracer: substance added as a food constituent (as required by regulation) so that levels of this constituent can be detected after subsequent processing and/or combination with other food materials.
- Washing or surface removal agent: substance used to wash or assist in the removal of unwanted surface layers from plant or animal tissues.

III. FOOD ADDITIVE CATEGORIES

Substances that come under the general definition of direct food additive number in the thousands and include

Inorganic chemicals (e.g., phosphates, sulfiltes, calcium chloride, etc.)

Synthetic organic chemicals (e.g., dyes, benzoates, aroma chemicals, vitamin A, etc.)

Extraction products from and derivatives of natural sources (e.g., pectin, essential oils, vitamin E, etc.)

Fermentation-derived products (e.g., enzymes, citric acid, xanthan gum, etc.)

Most food additives have a long history of use; others are the result of recent research and development to fill particular requirements of modern food processing. Some are common chemicals of industry that are upgraded in terms of purity to allow their use in food.

Major categories of food additives include preservatives, colorants, antioxidants, flavors, thickeners and stabilizers, emulsifiers, acidifiers and buffers, enzymes, and sweeteners. Examples of major products in each category are shown in Table 83.2. Within this same category, products may belong to several chemical classes and offer specialized functionality (e.g., water- and oil-soluble antioxidants that include ascorbic acid and hindered phenols, respectively, and water-soluble azo dyes and water-dispersible carotenoids as food colors). Basic foodstuffs are excluded from the definition, although ingredients added to foods (e.g., high fructose corn syrup, MSG, and protein concentrates) are often included among food additives.

Certain food additives, such as colors, flavors, gums, emulsifiers, and preservatives may find use also in pharmaceutical products and in toiletries and cosmetics (e.g., toothpaste, lipstick, etc). The same Food Chemical Codex (FCC) grade as in food is typically used in these applications, however, the combined value of the additive for these other applications does not exceed 10% of food use.

Indirect food additives have no purposeful function in food and may be divided into the following categories:

- Components of adhesives (e.g., calcium ethyl acetoacetate 1,4-butanediol modified with adipic acid)
- Components of coatings (e.g., acrylate ester copolymer coatings and polyvinyl fluoride resins)
- Components of paper and paperboard (e.g., slimicides, sodium nitrate/urea complex, and alkyl ketone dimers)
- Basic components of single- and repeated-use food contact surfaces (e.g., cellophane, ethylene-acrylic acid copolymers, isobutylene copolymers, and nylon resins)
- Components of articles intended for repeated use (e.g., ultrafiltration membranes and textiles and textile fibers)

TABLE 83.2
Selected Major Food Additives

Thickeners and Stabilizers	Vitamins
Agar	Vitamin A
Alginates	Vitamin A acetate
Carageenan	Vitamin B_1
Carboxymethyl cellulose (CMC)	Thiamin hydrochloride
Casein	Vitamin B_2
Gelatin	Thiamin mononitrate
Gellan gum	Vitamin B_6
Guar gum	Pyridoxine hydrochloride
Gum Arabic	Vitamin B_{12}
Locust bean gum	Cyanocobalamin
Modified starches	Vitamin C
Pectin	Ascorbic acid
Xanthan gum	Vitamin D
	Ergocalciferol, cholecalciferol
	Vitamin K
	Menadione
Sweeteners	**Antioxidants**
Acesulfame-K	Ascorbic acid/sodium ascorbate
Aspartame	Erythorbic acid/sodium erythorbate
Dextrose	BHA (butylated hydroxyquinone)
Lactitol	BHT (butylated hydroxytoluene)
Mannitol	PG (propyl gallate)
Sorbitol	TBHQ (tert-butyl hydroquinone)
Saccharin	Tocopherols
Xylitol	Sulfur dioxide/sulfite salts
Colors	**Preservatives**
Certified food colors	Benzoic acid/benzoates
Dyes	Propionic acid/propionates
Lakes	Parabenes
Noncertified colors	Sorbic acid/sorbates
Caramel	Sulfites
Plant extracts	
Synthetic carotenoids	
Fat replacers	**Emulsifiers**
Partially or nonmetabolizable	Mono- and diglyercides
Sucrose polyester (Olestra)	Lactylated esters
Medium-chain triglycerides	Lecithin
Fat mimetics	Polysorbates
Carbohydrate based products	Propylene glycol esters
Protein based products	Sorbitan esters
Emulsifiers	Sucrose esters
Flavors	**Anticaking agents**
Aroma chemicals	Aluminum calcium silicate
Vanillin	Calcium silicate
Essential oils/natural extracts	Salts of fatty acids (stearates)
Menthol	Silicon dioxide
Flavor compositions	Tricalcium silicate
Strawberry flavor	Yellow prussiate of soda
Enzymes	**pH control agents**
Amylases (alpha-amylase, etc.)	Citric acid
Glucose isomerase	Malic acid
Pectinases	Phosphoric acid/phosphates
Proteases	Sodium citrate
Rennin	Sodium hydroxide

- Compounds controlling growth of microorganisms (e.g., sanitizing solutions)
- Antioxidants and stabilizers (e.g., octyltin stabilizers in vinyl chloride plastics)
- Certain adjuvants and production aids (e.g., animal glue, hydrogenated castor oil, synthetic fatty alcohols, and petrolatum)

In many countries, these materials are defined and regulated as contaminants. In the United States, these materials are food additives under the law. They are commonly classed as indirect food additives, but the FDA handles them in the same way as direct additives. Just as with direct additives, they may be generally recognized as safe (GRAS) substances and thereby escape explicit regulation because that status makes them, in fact, not food additives. In general, however, regulation of these materials is more extensive and more rigorous in the United States than in other counties. As might be expected, packaging materials which have been used for a long time, such as glass, receive less close scrutiny than more newly introduced materials and those materials just being proposed for introduction.

IV. FOOD ADDITIVE SUPPLY INDUSTRY

Food additive suppliers are an important part of the food manufacturing system, supplying products to both commodity processors and food processors (Figure 83.1). Practically every food manufacturing operation depends to some degree on the use of food additives, but the range of additives necessary for the formulation varies (Table 83.3).

Overall, the food additive industry appears to be highly fragmented, consisting of more than 500 companies supplying a variety of chemically and functionally different products that serve a common end-use market — the food industry. However, suppliers tend to be either highly specialized participants in the major product categories (e.g., Novozimes with enzymes, Sensient Colors, Inc. with certified food colors, etc.) or large chemical companies that offer food-grade versions of a few industrial products (e.g., Lonza's emulsifiers, FMC Corp.'s cellulose derivatives). Manufacturers are typically involved in supplying additives in a limited number of product categories (e.g., colors, vitamins, or enzymes) or servicing selected food sectors (e.g., processed meats, dairy-based products, or bakery products). While a company or group or companies may tend to dominate sales in each of the specific categories (e.g., Novozimes with enzymes, or Rhodia with vanillin), no single company or small group of companies dominates the entire food additive industry.

Forty years ago it was relatively easy and lucrative for chemical companies to stumble into the role of food additive supplier and reap profits by upgrading the purity and quality of chemicals originally developed for other industrial

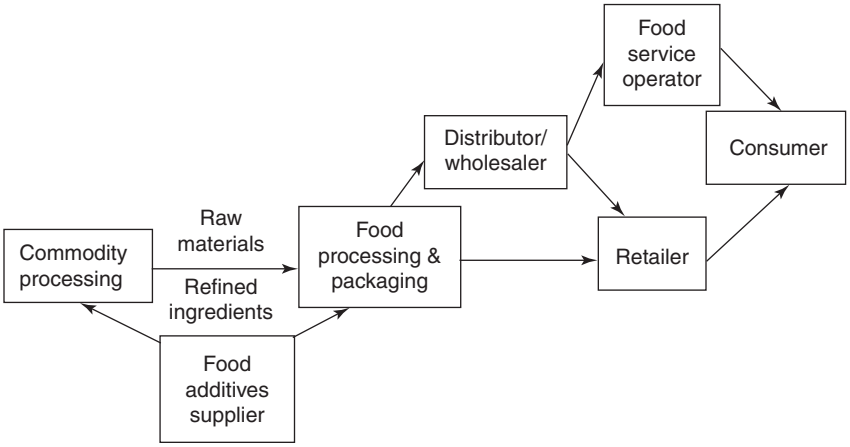


FIGURE 83.1 Integrated view of the U.S. food manufacturing system.

TABLE 83.3
Selected Food Additives and Their Major Uses

Food Additive	Processed/ Cured Meats	Dairy Products	Cheese	Ice Cream	Jams/ Jellies	Pickles/ Sauces	Breakfast Cereals	Cookies/ Crackers
Flavors		X		X		X	X	X
Thickeners/stabilizers	X	X		X	X			
Sweeteners		X		X	X		X	X
Colors	X	X	X	X	X	X	X	
Fat Substitutes		X		X				X
Enzymes		X	X					
Vitamins		X					X	
Antioxidants	X						X	
Preservatives	X		X					
Emulsifiers	X	X		X		X		

Food Additive	Baked Goods	Candy	Chocolate	Chewing Gum	Mayonnaise/ Dressings	Carbonated Beverages	Still Beverages
Flavors	X	X	X	X	X	X	X
Thickeners/stabilizers	X						
Sweeteners	X	X	X	X	X	X	X
Colors	X	X			X	X	X
Fat Substitutes	X		X		X		
Enzymes	X						
Vitamins							
Antioxidants		X		X	X		
Preservatives					X		
Emulsifiers	X		X		X		

Food Additive	Dry Beverage Bases	Tabletop Sweeteners	Snacks/ Chips	Dessert Mixes	Frozen Entrees	Dietetic Products	Fats/ Oils	Essential Oils Margarine
Flavors	X		X	X	X	X		
Thickeners/stabilizers	X		X	X	X	X		
Sweeteners	X	X		X		X		
Colors	X		X	X				
Fat Substitutes			X	X	X	X		X
Enzymes						X		
Antioxidants	X						X	X
Preservatives	X			X	X			X
Emulsifiers	X			X	X	X	X	X

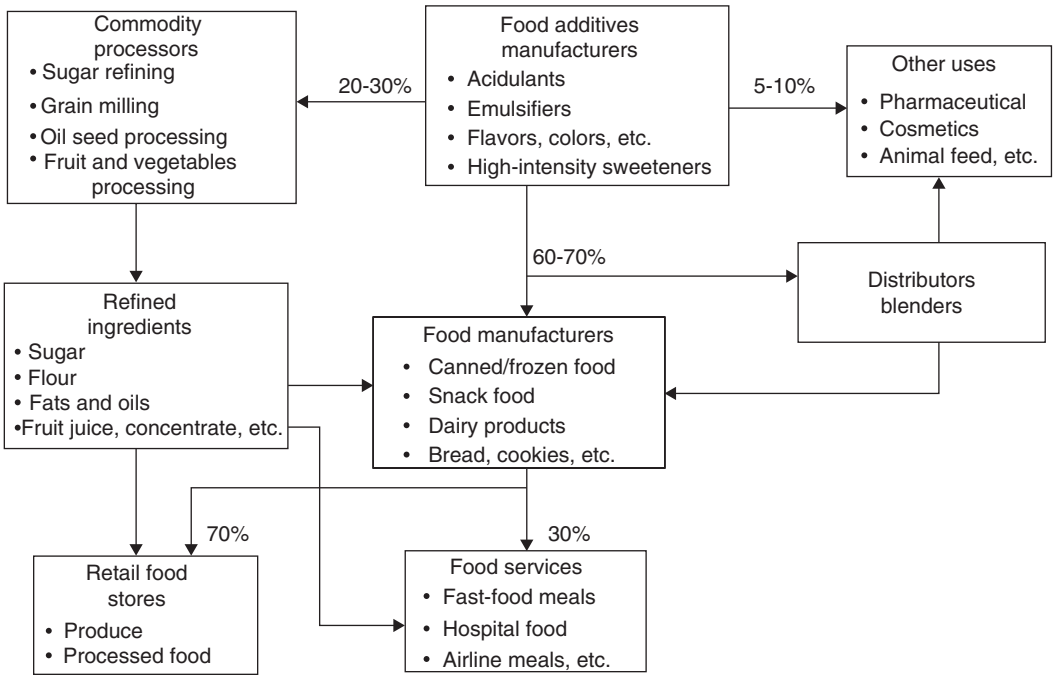


FIGURE 83.2 Food additives, pattern of use in food, and other applications.

markets. Today, however, the long time and high costs associated with gaining regulatory approval (estimated 5–10 years and $15–$40 million) have taken away the incentive to commercialize products from basic research. As a result, food additives represent only a minor portion of large chemical companies' overall business. Most large chemical companies that supply food additives, such as FMC, Monsanto, Lonza, Hoffman-La Roche, Degussa, BASF, Rhoclia, Nutrinova, and Eastman Chemicals, have diversified chemical operations, with perhaps only 5% or less of total sales generated by food additives.

Figure 83.2 depicts the food additive industry structure and the flow of its products. Some 60–70% of food additives are used in the manufacture of food: about 20–30% are used in commodity processing operations such as flour milling, meat packing, oilseed crushing and refining, vegetable packaging, animal feeds, and fruit juice processing; and the remaining 5–10% are used for things such as pharmaceuticals and cosmetics. In addition to basic additive producers, the food additives industry includes companies that specialize in compounding of specialty product mixes, and national and local distributors (Figure 83.2). Specialty compounders formulate mixed products for the food industry such as dairy ingredients, baker's mixes, curing blends, thickener and emulsifier blends, cheese aids, ethnic flavors, total seasoning packages, and spice blends. They are generally very knowledgeable about additive and ingredient properties and are experienced in food technology overall. Compounding companies are often relatively small, sell directly to a food processor, are highly service oriented, and market product lines that have a high level of perceived differentiation. Their formulations offer convenience and enjoy higher gross profit margins than single food additive sales.

Distributors also play an important role in the distribution of food additives. Additive producers typically use distributors to service their smaller accounts or for warehousing and servicing of accounts that geographically the producers cannot cover effectively or economically.

V. RESEARCH AND DEVELOPMENT

Because there are so many dissimilar and unconnected segments of the food additives industry, the participating companies exhibit different approaches to research and development (R&D). Many stress applications research to uncover new niches for existing additives or modifications of currently FDA-approved additives. Some emphasize innovative research or new, high-value products, but these are very few because of the cost and time for basic research, development, regulatory approval, and market acceptance of a new food additive product. For example, NutraSweet's aspartame product took more than 11 years to gain FDA approval; acesulfame-K took 6 years for FDA approval and a total of 21 years since development. The total costs of research, development, and approvals for aspartame were close to $25 million.

Procter & Gamble's Olestra™ was in research and development for 20 years, yet wasn't submitted to the FDA until June 1987. After spending over $200 million

in development costs and waiting for more than 8 years for FDA approval, the fat substitute received approval in January 1996. Approval of the food additive is limited to snacks such as potato chips and tortilla chips. Moreover, it has a special regulatory constraint: Olestra™-containing products require fortification with vitamins A, D, E, and K to compensate for the limited absorption of these fat-soluble vitamins, and the products must be labeled with the statement. "This product contains Olestra. Olestra may cause abdominal cramping and loose stools. Olestra inhibits the absorption of some vitamins and other nutrients. Vitamins A, D, E, and K have been added."

In general, only large, well-financed companies can afford the R&D efforts necessary to bring a new food additive product to market. Small companies simply are unable to deal with the complexity, costs, and required time. Personnel staffing requirements for R&D of food additives vary significantly. Because statistics for the food additives business of most producing companies are not reported separately, only estimates can be made. R&D expenditures as a percentage of sales typically range from 1% or less for products such as preservatives, to 5–6% for more technically oriented products such as fat substitutes and certain natural colors, and 5–10% for flavors.

VI. MANUFACTURING

Manufacturing processes for food additives vary widely in their nature and technological sophistication. Some of the specific processes for the more important food additives are described in later sections of this report. A common characteristic of all food additives manufacturing, however, is that the products must be made to a high degree of purity and under sanitary conditions similar to those of food processing plants. Production equipment must be dedicated to food additive products and cannot be used for other industrial production. Plants producing food additives are subject to periodic inspection by the regulatory agencies. Typically chemical additives made by synthesis (e.g., BHT, saccharin) or by fermentation (e.g., aspartame, microbial enzymes, xanthan and gellan gums) require a high level of capital investment. The former additives have industrial uses and are likely to share their basic production costs with the industrial-grade material; a small portion of the total production is then upgraded and purified to food-grade quality in separate dedicated plant units. Accurate long-term market forecasts for products are essential in order to minimize the risk associated with capital investment decisions in single-purpose plants. Other participants in the food additives business that are not involved in chemical production on a large scale for the extraction and purification of natural products, small-scale synthesis of aromatic chemicals, and for flavor and ingredient compounding have much lower capital requirements.

VII. GOVERNMENT REGULATIONS

A. United States

The application of food additives is highly regulated worldwide, although regulatory philosophy, the approval of specific products, and the level of enforcement differ from country to country. Basic regulations in the United States, Western Europe, and Japan are described below. These three major industrial regions are the largest consumers of food additives. With only 13% of the world's population, these countries account for more than two-thirds of the food additive market.

The U.S. Food and Drug Administration (FDA) is the principal U.S. regulatory body controlling the use of food additives. It does so through the 1958 Food Additives Amendment to the Food, Drug & Cosmetic (FD&C) Act of 1938. The amendment was enacted with the threefold purpose of

1. Protecting public health by requiring proof of safety before a substance can be added to food.
2. Advancing food technology.
3. Improving the food supply by permitting the use of substances in food that are safe at the levels of intended use.

According to the legal definition, food additives that are subject to the amendment include "any substance the intended use of which results or may reasonably be expected to result directly or indirectly in its becoming a component or otherwise affecting the characteristics of any food." This definition includes any substance used in the production, processing, treatment, packaging, transportation, or storage of food.

If a substance is added to a food for a specific purpose it is referred to as a direct additive. For example, the low-calorie sweetener aspartame, which is used in beverages, puddings, yogurt, chewing gum, and other foods, is considered a direct additive.

Indirect food additives are those that become part of the food in trace amounts due to its packaging, storage, or other handling. This class includes all materials that would not usually become part of food if man could completely control food production. In practice, indirect food additives are found in agricultural produce in quantities well within acceptable and legal tolerances. For example, minute amounts of packaging substances may find their way into foods during storage. A variety of chemicals, including plastic monomers, plasticizers, stabilizers, printing ink, and other substances, migrate at extremely low levels into foods. Lead and tin are perhaps the main concerns associated with packaging materials. The storage of acidic foods in inappropriate containers can result in the leaching of toxic heavy metals, such as zinc and copper, into the food. Food packaging manufacturers therefore must prove to

the FDA that all materials coming in contact with food are safe before they are permitted for use in such a manner.

A wide variety of chemicals are used in modern agricultural practice. Residues of these chemicals can linger in raw and processed foods, although federal regulatory agencies evaluate the safety of such chemicals, and regulate and monitor their use on food products. The major categories of agricultural chemicals include insecticides, herbicides, fungicides, fertilizers, and veterinary drugs, including antibiotics.

Industrial and/or environmental pollutants may migrate into foods in small amounts. On rare occasions, hazardous levels of polychlorinated biphenyls (PBCs) and polybrominated biphenyls (PBBs) have been found in foods.

For regulatory purposes, all food additives fall into one of three categories:

1. Generally recognized as safe (GRAS) substances
2. Prior sanctioned substances
3. Regulated direct/indirect additives

GRAS substances (approximately 700 compounds) are a group of additives regarded by qualified experts as "generally recognized as safe." These substances are considered safe because their past extensive use has not shown any harmful effects. Prior sanctioned substances (approximately 1400 compounds) are products that were already in use in foods prior to the 1985 Food Additives Amendment and are therefore considered exempt from the approval process. Some prior sanctioned substances also appear on the GRAS list. This is the grandfather clause of the amendment.

The FDA is involved in an ongoing review of the GRAS and prior sanctioned substance lists to ensure that these substances are tested by means of the latest scientific methods. Likewise, the FDA also reviews substances that are not currently included on the GRAS list to determine whether they should be added.

All other additives are regulated — that is, a specific food additive petition must be filed with the FDA requesting approval for use of the additive in any application not previously approved. A food or color additive petition must provide convincing evidence that the proposed additives perform as intended. Animal studies using large doses of the additive for long periods are often necessary to show that the substance will not cause harmful effects at expected levels of human consumption.

In deciding whether an additive should be approved, the agency considers the composition and properties of the substance, the amount likely to be consumed, its probable long-term effects, and various other safety factors. Absolute safety of any substance can never be proven. Therefore the FDA must determine if the additive is safe under the proposed conditions of use, based on the best scientific knowledge available. In addition, the FDA operates an Adverse Reaction Monitoring System (ARMS) to help serve as an ongoing safety check of all additives. The system monitors and investigates all complaints by individuals or their physicians that are believed to be related to specific foods, food additives, or nutrient supplements.

Color additives for food represent a unique and special category of food additives. They have historically been so considered in legislation and regulation. The current legislation governing the regulation and use of color additives in the United States is the Food, Drug & Cosmetic Act of 1938, as amended by the Color Additive Amendment of 1960. Colors permitted for use in foods are classified either as certified or exempt from certification. Certified colors are man-made, with each batch being tested by the manufacturer and the FDA (certified) to ensure that they meet strict specification for purity.

Color additives that are exempt from certification include pigments derived from natural sources. However, color additives exempt from certification also must meet certain legal criteria for specifications and purity. One of the features of the Color Additive Amendment of 1960 was the equal treatment of synthetic colors and the so-called natural colors in respect to pretesting requirements. Moreover, currently all color additives, certified and uncertified ("natural"), are designated on the label as artificial color.

Flavor substances are regulated somewhat differently, and the rules are less restrictive. However, the use of aroma chemicals as flavor ingredients is regulated under laws that may differ from country to country. Following the lead of the United States, inclusion on a positive list that spells out which chemicals are permitted for food use has become the prevalent legislation for regulating flavor chemicals worldwide. The United States has a list of flavor substances that are deemed GRAS based on the history of use, review of available toxicology, and the opinion of experts. These GRAS lists (through GRAS 20) have been compiled since 1977 by the expert panel of the Flavor Extracts Manufacturers Association of the United States (FEMA). Over the years, more than 1800 materials appeared on FEMA lists. Formed in 1909, FEMA is an industry association that originally started pursuing voluntary self-regulation and later was granted quasi-official status on regulatory matters regarding flavor chemicals by the FDA. The FEMA expert panel was formed in 1960. This independent panel, composed of eminently qualified experts recruited from outside the flavor industry, has expertise in human nutrition, physiology, metabolism, toxicology, and chemical structure-activity relationships. Most industrial countries more or less follow the U.S. system.

Although the FDA has primary jurisdiction over food additives, clearance for use of additives in certain products must be obtained from other government agencies as well. For example, the U.S. Department of Agriculture (USDA) through the Meat Inspection Division (MID) exercises jurisdiction over additives and ingredients for meat and poultry; the Bureau of Alcohol, Tobacco, and

Firearms (BATF) of the U.S. Department of the Treasury controls the ingredients used in alcoholic beverages.

The standards of identity specify in detail what can and cannot be packaged under a given product name. Standards of identity exist for milk, cream, cheese, frozen dessert, bologna products, cereal products, cereal flours, pasta, canned and frozen fruits and vegetables, juices, eggs, fish, nuts, nonalcoholic beverages, margarine, sweeteners, dressings, and flavorings. An approved food additive in the United States may be precluded from use in certain foods characterized by the standards of identity unless the additive is specifically required by or is listed as an optional ingredient in the standards. The standards of identity establish the ingredient composition of a given food, which can then be labeled by its common name. If the manufacturer does not adhere to the standard composition, the food must be labeled "imitation."

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which was issued in 1972 and amended in 1988, covers pesticides used on raw agricultural products. The FDA, however, is responsible for enforcing tolerances for pesticide residues that end up in food products.

In the United States, label disclosure of food additives is mandated with few exceptions. Under FDA, USDA, and BATF regulations, the ingredients of a food or beverage must be stated on the product label in decreasing order of predominance. For many direct additive categories, chemical constituents must be identified by their common names and the purpose for which they were added.

One of the recent regulations involving the food industry, as well as food additive manufacturers, came with the passing of the Nutrition Labeling and Education Act of 1990 (NLEA), which amends the Federal Food, Drug & Cosmetic Act, to make nutrition labeling mandatory for most FDA-regulated foods. The nutrition labeling regulations issued by the FDA and the USDA Food Safety and Inspection Service (FSIS) required compliance by August 8, 1994.

The FDA's nutrition labeling regulations focus on nutrients currently accepted as significantly affecting consumer health. The serving size is the basis for reporting each food's nutrient content. Serving sizes are defined for most foods reflecting the amount people actually eat and are shown in both common household and metric measures. The amount per serving of the following nutrients are required to be included on labels: total calories, calories from total fat, total fat, saturated fat, cholesterol, total carbohydrates, complex carbohydrates, sugars, dietary fiber, protein, sodium, vitamin A, vitamin C, calcium, and iron. Listing other essential vitamins and minerals such as thiamin, riboflavin, and niacin, among other nutrients, is optional. A simplified nutrition label format is allowed for foods containing insignificant amounts of more than half the required nutrients. The minimum label includes total calories, total fat, total carbohydrates, protein, and sodium.

The FDA regulation requires the nutrition content be based on amounts of the product customarily consumed, and expressed in both common household and metric measures (e.g., 1 cup and 240 ml). Serving size reference amounts are based on food consumption survey data on amounts of food commonly consumed per eating occasion by persons 4 years of age and older. Manufacturers must follow the procedures to convert the reference amounts to serving sizes appropriate for their specific products. Any package containing less than two servings is considered a single-serving container.

Nine terms are presently allowed by the FDA to describe a food, including free, low, high, source of, reduced, light/lite, less (or, for calories, fewer), more, and fresh. Claims for cholesterol are tied to levels of saturated fat in the food. Meal-type products are not allowed to use the terms reduced.

Health claims are allowed for only the following nutrient/disease relationships:

- Calcium and osteoporosis
- Sodium and hypertension
- Unsaturated fats, low cholesterol intake, and cardiovascular disease
- Dietary lipids and cancer
- Fiber-containing grain products, fruits and vegetables, and cancer
- Fruits, vegetables, and grain products that contain fiber and risk of coronary heart disease
- Fruits and vegetables and cancer
- Folic acid and neural tube defect
- Sugar alcohols and dental caries
- Psyllium-containing foods and the risk of heart disease (when consumed as part of a diet low in saturated fat and cholesterol
- Soy protein and reduced risk of coronary heart disease

Changing dietary recommendations and labeling requirements impact food additive producers both positively and negatively. Products used for fat-sparing/substitution (e.g., hydrocolloids, starches, other fat substitutes) and low-calorie sweeteners fare well, as food manufacturers strive to lower the caloric and saturated fat content of their products. Natural colors (provided they can be substituted), as well as other natural or seemingly natural products will also be in higher demand so as to provide consumers with a more healthy and nutritious product (or at least one with a more healthy-sounding label).

The Food Additives Amendment also contains what is known as the Delaney clause, which mandates the FDA to ban any food additive found to cause cancer in man or animals, regardless of dose level or intended use. The clause applies not only to new food additives but also to those in use prior to 1958. The Delaney clause is totally inflexible

in that it does not recognize any threshold level below which the additive might not present a health hazard. Thus it has caused a number of problems for the food industry and for food additives. Certain additives (e.g., the sweetener cyclamate, etc.) have been banned after they were found to be potential carcinogens — even though feeding tests in animals at massive dose levels may not bear any correlation to the potential risk to man of chronic ingestion at very low levels. Were it not for a moratorium mandated risk to man of chronic ingestion at very low levels. Were it not for a moratorium mandated by Congress, saccharin would also have been banned in the United States several years ago by the FDA in compliance with current U.S. food laws. Although congressional sentiment has been running for some time in favor of repealing the Delaney clause, to date, attempts to replace it with a more practical and realistic law and have been unsuccessful.

1. Approval Process

A new substance gains approval for food use through the successful submission of a food additive petition that must document the following:

- Safety, including chronic feeding studies in two species of animals.
- Intended use.
- Efficiency data at specific levels in the specified food system.
- Manufacturing details and product specifications.
- Methods for analysis of the substance in food.
- Environmental impact statement.

Quite frequently, this process can be lengthy — up to 10 years in the case of aspartame and Olestra™ — and costly in terms of man-hours and dollars. There is little doubt that every level of the U.S. food additives business is affected by regulations, and operates with a constant awareness of the importance of FDA decisions. Not only is the introduction of a new food additive impossible without FDA approval, but the additives in use are under constant scrutiny by the regulatory agency and remain vulnerable to new unfavorable toxicology findings. While the barring of an additive may create opportunities for suppliers to develop new or substitute materials, the potential market is often too small to create sufficient incentive, and the loss of the ingredient may cause havoc within affected sectors of the food industry. For example, the ban on cyclamates, followed by the close call on saccharin, almost caused the demise of the diet soft-drink industry. The well-recognized need for alternative safe sweeteners undoubtedly was a stimulus for G.D. Searle (now Monsanto's NutraSweet Kelco division) to engage in a 10-year effort to have aspartame cleared for food use.

B. European Union (EU)

Food additives intended for human consumption are regulated by the member states as described in Directive 89/107/EEC of December 21, 1988. The EU food additive law recognizes 106 food additives. Later, several amendments and adaptations of the directive were introduced or proposed including

- A list of additives the use of which is authorized to the exclusion of all others.
- The list of foodstuffs to which these additives may be added, the conditions under which they may be added and, where appropriate, a limit on the purpose of their use.
- The rule on additives used as carrier substances and solvents, including their purity criteria.
- In 1990 the commission proposed a first specific directive relating to sweeteners and food additives other than colors and sweeteners. The Sweetener Directive took effect on July 30, 1994.

Efforts have been toward a uniform registration process so that a registration obtained in one country would be valid in all EU member countries. The new EU food additive law, however, will not prevent individual countries from asking for additional or country-specific requirements for new product registrations. At the EU level, several institutions and groups are involved in the development of food additives law, including the Scientific Committee for Food (SCF), one of the institutions of the European Commission which deals with safety issues, representatives from different national professional organizations, representatives from the food industry, retailers, etc. The Standing Committee on Foodstuffs ensures close cooperation between the commission and the member states.

The EU rules for the evaluation, marketing, and labeling of novel food such as genetically modified foods are also being developed. The new marketing rules would also oblige manufacturers to obtain permission before placing new foods or ingredients on the market, with the exception of products that are substantially equivalent to existing foods. The new rules have still to be cleared by the European Parliament, which has the power to veto under the new co-decision procedure introduced in 1995.

In many countries, additives must be declared in the labeling. Within the EU, some additive groups have been uniformly codified with "E" numbers for the orientation of consumers. Some countries, such as Germany, have gone further, adopting regulations on an acceptable daily intake (ADI) basis that build on the latest toxicological knowledge. Some examples of "E" numbers are presented in Table 83.4.

Under EU food law, any claim that a food has the property of preventing, treating, or curing a human disease

TABLE 83.4
Selected EU Food Additives and Codes

Colorants	
E100	Curcumin
E101	Riboflavin
E102	Tartrazin
E110	Yellow no. 6
E120	Carmin
E150	Caramels
E160	Annatto
E160a	Beta-carotene
E160c	Paprika
E162	Beetroot red (betanin, betanidin)
E163	Enocyanin (grape-skin extract)
Preservatives	
E200	Sorbic acid
E201	Sodium sorbate
E202	Potassium sorbate
E203	Calcium sorbate
E210	Benzoic acid
E211	Sodium benzoate
E212	Potassium benzoate
E281	Sodium propionate
E282	Potassium propionate
E283	Calcium propionate
Antioxidants	
E300	L-ascorbic acid (vitamin C)
E307	Synthetic alpha-tocopherol (vitamin E)
E311	Propyl gallate (PG)
E320	Butylhydroxyanisole (BHA)
Thickeners and stabilizers	
E401	Sodium alginate
E415	Xanthan
E420	Sorbitol
E440a	Pectin
Emulsifiers	
E322	Lecithin
E471	Mono- and diglycerides of fatty acids
E475	Polyglycerol esters of fatty acids
E481	Sodium steroyl-2 lactilate

or condition, or any implication of such properties, is prohibited. This aspect of the law has been strictly enforced in all member states of the EU. As early as 1980, the European Commission recognized that the area of food claims required harmonization and circulated the first proposal for a directive. By the end of 1998, this approach had not succeeded.

Recently the introduction of genetically modified (GM) corn and soy into Europe has caused considerable activity within governments and consumer organizations. European Parliament and Council Regulation no. 258/97 on novel foods and novel food ingredients requires prior approval of foods and food ingredients containing or consisting of a GM organism, and food and food ingredients produced from, but not containing GM organisms. More recently, Council Regulation 1139/98 came into force, requiring that any product containing GM soy or corn, or derivatives of GM soy or corn containing protein or DNA, must be labeled with the statement "produced from genetically modified soy" or "produced from genetically modified maize." However, refined oils or lecithin that are very unlikely to contain GM protein or DNA are exempt from such labeling statement requirements.

A further labeling change that came into force in February 2000 is the quantitative declaration of ingredients (QUID). This applies to foods and beverages with more than one ingredient, with very few exceptions. The quantity of ingredients, expressed as a percentage of the food or drink, must appear in or immediately next to the name of the food or in the list of ingredients next to the ingredient concerned.

C. JAPAN

In Japan, the Food Chemistry Division of the Ministry of Health and Welfare (MHW) has jurisdiction over food additives through the Food Sanitation Law. It was in 1948 that the term "food additive" appeared in the law (in the Food Sanitation Law) and a positive list of food additives was created in Japan. It was the first positive list created in the world, and it did not distinguish between synthetic or natural additives. Several amendments were adopted later. Amendments to the regulations, as well as additions or deletions to *Kohetisho* (the Japanese Codex of Food Additives), were mostly influenced by two major objectives: protection of food sanitation and customer safety, and harmonization with international regulatory requirements.

In the Food Sanitation Law, the term "additive" means anything added to, mixed into, permeating, or otherwise put in or upon food for the purpose of processing or preserving it. Most discussions on regulating food additives in Japan have been related to defining what food additives should be under legal restriction and on labeling requirements. Very often in these discussions, differentiating "synthetic" and "natural" food additives had been at issue. In Japan, those two generally used terms have often misled customers into a blind belief in natural food additives. However, regulatory bodies, as well as the food additive industry, no longer distinguish additives with these terms. The latest amendment of the law (May 24, 1995) includes deletion of the term "chemically synthesized substances." Thus "natural" food additives are regulated under the amended law (being enacted from May 24, 1996), unless they are listed as "existing food additives." The MHW then disclosed the list of "existing food additives" on August 10, 1995.

Under the amended Japanese Food Sanitation Law (1995), substances that are permitted for use as food additives fall into four categories listed below:

- Substances that are generally recognized as food (about 70 substances).
- Natural-based flavors (about 580 substances).

- Natural-based substances that are recognized as safe to human health on the basis of actual results for use as food additives (about 490 substances).
- Synthetic substances that are recognized as safe to human health on the basis of the results of safety evaluations (about 350 substances).

The amended law requires that any new substances, regardless of whether they are natural-based or synthetic, be verified to be safe to human health through safety evaluations and then approved by MHW before being used as food additives. Also, it is required, that the type of substance and the intended purpose of addition be labeled on the surface of food containers or packaging.

However, in general, data requirements for natural additives are still not as strict as those for chemical substances. Natural food additives reported to the MHW are listed in a table separate from the conventional positive list for chemical food additives. There are about 1200 items in the natural additive list, while the conventional list contained 349 compounds as of 1992.

VIII. TRENDS AND ISSUES

While there are many differences in food tastes and preferences among consumers, the major trends driving the food additives industry appear to be very similar:

Concern for health, nutrition, naturalness, quality.
Food safety, free of contaminants.
Desire for convenience.
Price-value relationship.

Growing awareness of the connection between diet and diseases such as cancer and heart disease has caused consumers to reexamine their diets and lifestyles and seek healthier alternatives. Consumer desire for healthier, more nutritious foods favors natural additives and ingredients (and those that are perceived as natural), as well as those that reduce calories, sodium, cholesterol, and the overall fat in foods. Fortification with the "right level" of vitamins, amino acids, and trace minerals is important, and additives that sound natural (e.g., gelatin, pectin, vitamins, etc.) versus chemical (e.g., potassium benzoate, butylated hydroxyanisole, etc.) have a more favorable consumer image.

The shift away from commodity to more processed, higher-value food products favors an increased use of additives in processing. Additives that are perceived favorably by consumers as healthy or natural foods are likely to grow faster. Finally, demands are high for fat replacers, high-intensity sweeteners, low-calorie bulking agents, certain gums, freeze/thaw stabilizers, and natural flavors.

Sales of ingredient and additive blends will dominate in the future. The synergistic effects that enhance the functionality of these materials, while reducing the quantity needed, will play an ever-more significant role in formulated foods. Information on these blends will be scarce, because they will be developed in house by food additive suppliers and food manufacturers wishing to maintain confidentiality in order to optimize exclusive commercial benefit. Other issues affecting the growth and broadening of the food additives industry include increasing government regulatory activity; increasing R&D and legal expenses; and the great length of time needed to perfect, gain approval for, and market a new food additive product.

In addition to traditional processed food products, a variety of health-related products known as "functional foods" and "nutraceuticals" have appeared on the market. Functional foods are food products that improve performance or provide a health benefit beyond meeting the basic nutritional needs of humans. Although functional foods are consumed for their taste, aroma, or nutritional value, they are also consumed by health conscious adults for their perceived benefits in preventing the onset of degenerative diseases such as arthritis, cancer, or heart disease.

Nutraceuticals are specific vitamins, minerals, amino acids, herbs and other botanicals, or constituent parts thereof that are taken in oral form to promote natural ways of preventing or treating various degenerative disease conditions. Nutraceuticals differ from functional foods in that they are only consumed for their health benefits rather than for taste, aroma, or nutritive value. In the United States nutraceuticals will have to overcome regulatory constrained before they can gain a large market. In contrast, in Japan and several countries in Europe the concept of nutraceuticals is well established, both in terms of regulations and consumer acceptance.

The safety of the food supply continues to receive a great deal of attention from the press, the public, and the governments. In 1993, an outbreak of food poisoning in the United States, eventually traced to undercooked beef, caused fundamental changes in regulatory policies and demonstrated to food processors the need for increased caution against food pathogens. The recent European outbreaks of bovine spongiform encephalopathy (BSE), known as "mad cow disease," have created more serious and worldwide concerns about cattle-derived food products, including some dietary supplements (e.g., gelatin capsules) and personal care products.

Fast-paced lifestyles will continue to drive the demand for savory, high-quality convenience foods. Microwaveable and shelf-stable products that are tasty and healthy require additives such as specialized flavors, colors, and stabilizers to enhance/maintain quality and will result in continuing growth of the market for these additives.

The concept of value-added products is also of great interest to food processors as foods with added value, or at least perceived added value (e.g., low-fat, low-calorie, vitamin fortified, more convenient form/package, perceived prevention against particular diseases), garner higher margins.

Therefore consumption of additives that can aid in adding value to processed foods will continue to increase.

Following trends in the United States, the European market shows increased interest in ethnic foods and vegetarianism. The motivation in both cases is to promote health. Also, more products are being introduced which are aimed at very specific groups. These include children, teenagers, women, and in particular the growing population of elderly persons.

IX. DESCRIPTION OF MAJOR FOOD ADDITIVES

Direct food additives comprise more than 30 types. With about 3000 food additives, including more than 1800 flavoring substances currently approved for use in the United States (and more petitioning for approval), it would be difficult in a chapter such as this to discuss each and every substance. Ten major food additives were selected for discussion in this chapter.

A. Sweeteners

Sweeteners are used in formulated foods to impart sweetness and to perform several other functions. They render certain foods palatable and mask bitterness; add flavor, body, bulk, and texture; change the freezing point and control crystallization; control viscosity, which contributes to body and texture; and prevent spoilage. Certain sweeteners act as preservatives by binding moisture in food that is required by detrimental microorganisms. Alternatively, some sweeteners can serve as food for fermenting organisms that produce acids that preserve the food, thus extending shelf life by retaining moisture. These auxiliary functions must be kept in mind when considering applications for artificial sweeteners.

Sweeteners can be classified in a variety of ways:

- Nutritive or nonnutritive. Materials either are metabolized and provide calories, or are not metabolized and thus are noncaloric.
- Natural or synthetic. Commercial products that are modifications of a natural product, for example, honey or crystalline fructose, are considered natural.
- Regular or low-calorie/high-intensity. Although two sweeteners may have the same number of calories per gram, one may be considered low-calorie or high-intensity if less material is used for equivalent sweetness.
- As foods. For example, fruit juice concentrates can impart substantial sweetness.

Sweetness is measured via sensory methods by taste panels. It is a subjective perception influenced by a multitude of variables including the temperature of the food being tasted, pH, other flavors and ingredients in the food, physical characteristics of the food sweetener, concentration, rate of sweetness development, and permanence of sweetness and flavor. Also, results can vary depending on the foods consumed prior to testing (even several hours before testing), the flavors to which the taster is accustomed, tasting experience of the panelist, time of day, and the physical surroundings in the test room.

Sucrose, commonly known as table sugar (or refined sugar), is the standard against which all sweeteners are measured in terms of quality of taste and taste profile. It is consumed in the greatest volume of all sweeteners. Sucrose, high-fructose corn syrup (HFCS), and other natural sweeteners (e.g., molasses, honey, maple syrup, and lactose) are food commodities and are not considered as food additives, therefore they will not be covered here. The discussion that follows is limited to the polyol alternative sweeteners and the high-intensity sweeteners.

1. Polyols

Polyols (sugar alcohols or polyalcohols) are chemically reduced carbohydrates. These compounds are important sugar substitutes that are utilized where their different sensory, special dietary, and functional properties make them desirable. Also polyols are utilized in low-calorie food formulations. The sweetness of polyols relative to sucrose and their caloric values are shown in Table 83.5. Moreover, because polyols are absorbed more slowly from the digestive tract than is sucrose, they are useful in certain special diets. When consumed in large quantities (in excess of 25–50 g/day), however, they have a laxative effect, apparently because of the comparatively slow intestinal absorption. In the EU countries, if a food product contains more than 10% by weight of a polyol, a warning statement must be added to the label stating that excessive consumption may induce a laxative effect.

In the United States food products sweetened with polyols and containing no sucrose can be labeled as "sugarless," "sugar free," or "no sugar" but must also bear the

TABLE 83.5
Relative Sweetness and Calorie Value of Polyols

	Relative Sweetness	Caloric Value
Polyol	(sucrose = 100)	(U.S. allowance; kcal/g)
Erythritol	60–70	0.2
Hydrogenated starch Hydrolysates	25–50	3.0
Isomalt	45–65	3.0
Lactitol	40	2.0
Maltitol	90	3.0
Mannitol	70	1.6
Sorbitol	50–70	2.6
Xylitol	100	2.4

statement "Not a reduced calorie food," "Not a low calorie food," or "Useful only for not promoting tooth decay."

Sorbitol occurs naturally many edible fruits and berries including pears, apples, cherries, prunes, and peaches. Its nontoxic nature has long been recognized. In 1974 the FDA included sorbitol as one of the first four chemicals in its revised list of GRAS substances.

Sorbitol is only 70% as sweet as sucrose. However, it has many functional properties desirable in a sweetener, such as bulking agent ability, high viscosity (contributing to body and texture), hygroscopicity (resulting in its humectant as well as its softening nature), cool taste, sequestering ability, and crystallization modification (retardation). Because sorbitol can be digested without insulin and is also noncarinogenic, it is used as a sugar substitute in diabetic and sugarless foods and candies.

In general, sorbitol is used in foods to aid retention of product quality during aging, or to provide texture or other product characteristics to the formulation. In its major applications — sugarless chewing gum, candies, and mints — liquid sorbitol is used primarily as a bulking agent and not for its sweetness. Sorbitol's noncariogenic nature and the fact that it does not promote tooth decay may account for its wide use in these applications.

Mannitol is only about 70% as sweet as sucrose and is also noncariogenic. Because of its nonhygroscopic nature, mannitol is used as a dusting powder and anticaking agent, besides its special dietary food application. The highest demand for mannitol is in sugarless chewing gum and sugar-free chocolates. However, mannitol has a more serious laxative effect than sorbitol and a warning label is required when consumption is likely to exceed 20 g/day.

Xylitol is a five-carbon polyol with sweetness similar to sucrose. It is found in small amounts in a variety of fruits and vegetables, and is formed as a normal intermediate in the human body during glucose metabolism.

Xylitol has good solubility, blends well with foods, and has a lower melting point than sucrose, an advantage in the manufacture of confectionery products. There is also evidence that xylitol is not only noncariogenic but reduces tooth decay when used as a replacement for sucrose. It is mainly used in compressed candies, chewing gum, and over-the-counter pharmaceutical products. Xylitol is expensive, therefore it is usually used in small amounts in combination with other sweeteners. In a blend with aspartame, the two compounds have an excellent synergistic effect. Also, xylitol is blended with other polyols to minimize undesirable properties, such as hygroscopicity or the laxative effect of sorbitol, or to improve the solubility of mannitol.

Lactitol monohydrate, a sugar alcohol, has physicochemical properties different from those of sugars. It has a sweetness value approximately one-third of that of sucrose and is therefore suitable where bulking with low sweetness is required. To increase the sweetness it can be blended with high-intensity sweeteners. It is derived from milk sugar and used as a sweetener in Japan, Israel, and Switzerland. In the United States a self-affirmation GRAS statement petition has been submitted to the FDA for its use in chocolate, confections, and baked goods.

Erythritol is about 70% as sweet as sucrose. Like other polyols, it does not promote tooth decay and is safe for diabetics. However, it is distinctive for its caloric content (the lowest of the polyols — 0.2 calories/gram) and its high digestive tolerance. Studies have shown that, due to its small molecular size and structure, more than 90% of ingested erythritol is absorbed and excreted unchanged through the kidneys within 24 hours, so that the laxative side effects sometimes associated with polyol consumption are unlikely. As a result, foods containing substantial amounts of erythritol are very unlikely to cause gaseous and laxation side effects. A recent clinical study showed that daily consumption of 1 gram per kilogram of body weight is well tolerated by adults and compared to sucrose-containing foods.

Maltitol, approximately 0.9 times as sweet as sucrose with similar sweetness and body, has application in products such as chewing gum, dry bakery mixes and chocolate. It is said to behave similarly to sucrose in chocolate making. Some believe that maltitol has the best flavor of the polyol family and it has been particularly successful as an alternative to mannitol in the manufacture of sugar-free chocolates. Also because of its heat stability and good handling properties, it is suitable for use in sugar-free baked goods. Maltitol is also increasingly being used in dairy products.

Hydrogenated Starch Hydrolysates (HSH), also known as maltitol syrup and hydrogenated glucose syrup. Hydrogenated starch hydrolysates are a mixture of sorbitol, maltitol and hydrogenated oligosaccharides. Depending on the sorbitol and maltitol content, the sweetness of HSH can vary from 0.25 to 0.5 times that of sucrose. HSH serve a number of functional roles, including use as bulk sweeteners, viscosity or bodying agents, humectants, crystallization modifiers, cryoprotectants and rehydration aids. They also can serve as sugar-free carriers for flavors, colors and enzymes. HSH have been used by the food industry for many years, especially in confectionery products.

Isomalt, approximately 0.45–0.65 times as sweet as sugar. Isomalt can be used in candies, gums, ice creams, jams and jellies, fillings and frostings, beverages and baked products. As a sweetener/bulking agent, it has no off-flavors and works well in combination with other sweeteners. Isomalt is made from sucrose and looks much like table sugar. It lacks the "cooling" effect characteristic of some other polyols. It is white, crystalline and odorless, and is more stable chemically and enzymatically than sucrose.

2. High-Intensity Sweeteners

High-intensity sweeteners, once used mainly for dietetic purposes, are now used as food additives in a wide variety

TABLE 83.6
Regulatory Status and Sweetness Relative to Sugar[a]

	Sweetness (Sucrose = 1)	U.S.	Canada	European Community	Japan
Cyclamate, Na salt	30	P	A	A	N
Aspartame	200	A	A	A	A
Acesulfame K	200	A	A	A	P
Saccharin	300	A	N[e]	A	A
Sucralose[b]	600	A	A	A	N
Thaumatin (talin)	3000	A	A	A	A
Alitame[c]	2000	P	P	P	P
Neohesperidin DC	2000	N	N	A	N
Stevioside	300	N	N	N	A
Glycyrrhizin	300	N[d]	N	N	A
Neotame	7000–13000	A	N	N	N

[a] A = approved; P = petition filed; N = not approved.

[b] Sucralose is approved in Australia, Russia, Brazil, New Zealand, Quasar, Romania, and Mexico.

[c] Alitame is approved in Australia, New Zealand, People's Republican of China, Indonesia, Colombia, and Mexico.

[d] Glycyrrhizin is approved as a flavoring, but not as a sweetener in the United States.

[e] Saccharin in Canada is limited for use in personal care products and pharmaceutical, but it is banned in foods and beverages.

of products. They are termed high-intensity because they are many times sweeter than sucrose. But because of their very low use levels, high-intensity sweeteners cannot perform other key auxiliary functions in food and often must be used in conjunction with other additives such as low-calorie bulking agents. High-intensity sweeteners are also used in pharmaceuticals, cosmetics, animal feed, and biocides. The regulatory status and sweetness relative to sugar of high-intensity sweeteners are shown in Table 83.6.

Aspartame was approved in the United States in 1981 for use in prepared foods, dry beverage mixes, and as a tabletop sweetener, and in 1983 for use in liquid soft drinks. It gained instant popularity and has become the sweetener in virtually all diet soft drinks in the United States. Aspartame has impacted not just the dietetic soft drink market but also many other sweetener markets. Its success has encouraged R&D, and FDA approval is currently being sought for its use in baked products, since aspartame can now be made heat-stable through an encapsulation process.

Aspartame first appeared in the U.S. diet soft drink market in combination with saccharin (30% aspartame and 70% saccharin). Presently about 98–99% of canned or bottled diet sodas contain 100% aspartame. However, aspartame may be replaced in many products because in 1998 other high-intensity sweeteners were approved for beverages.

Aspartame can be used in many diverse applications. It is approved for use in more than 100 countries worldwide, and more than 5000 products contain aspartame. In the United States, FDA approval is being given to more and more applications. In 1981 it was approved for use in prepared foods, dry beverage mixes, and as a tabletop sweetener and for carbonated liquid products in 1983. More recently, in 1993, FDA approval was extended to many other products, and the list of approved products now includes the following categories:

- Nonalcoholic beverages and ready-to-serve nonrefrigerated, pasteurized, aseptically packaged fruit juice beverages, including sport drinks
- Frozen desserts (dairy and nondairy)
- Refrigerated, flavored milk beverages
- Fruit and wine beverages containing less than 7% alcohol
- Yogurt-type products in which aspartame is added after pasteurization and culturing
- Refrigerated, ready-to-serve gelatin desserts
- Confectionaries (hard and soft candies)
- Baked goods, including baking mixes
- Low-alcohol beer (containing less than 3% alcohol)

Aspartame is about 200 times sweeter than sucrose. Unlike many other low-calorie sweeteners, aspartame is digested by the body to amino acids, which are metabolized normally. However, because of its intense sweetness, the amounts ingested are small enough that aspartame is generally considered noncaloric. Aspartame has a sugar-like taste, and enhances some flavors.

Before aspartame was approved by the FDA, it underwent the most rigorous review the agency ever gave a food additive. The process took approximately 10 years to complete. In early 1984, aspartame's safety in beverages was again brought into question by researchers at the University of Arizona and the Community Nutrition Institute. The FDA, however, rejected a request for further hearings, saying it was satisfied that aspartame is safe in soft drinks. More recently, some research reports show that artificial sweeteners have had no effect on lowering weight levels and suggest that artificial sweeteners may actually increase appetite and thirst. To date, these findings have not appeared to affect the American consumer's perceived benefit of low-calorie sweeteners. A few cases relating aspartame consumption to severe medical reactions have been reported in medical journals. About 4000 consumer health complaints of headaches and other reactions have been received by the FDA, allegedly due to the consumption of aspartame. The clinical validity and resultant outcome of these claims are not known at the present time.

Saccharin was discovered in 1879 and has been used as a food additive since the early 1900s. Saccharin is approximately 300 times as sweet as sucrose. Because it is acidic

and not very soluble in water, it is used primarily as its sodium salt. Saccharin combines well with other sweeteners and has an excellent shelf life. Its main disadvantages are a bitter, metallic aftertaste and concern over its safety. Saccharin is the most widely used nonnutritive sweetener worldwide and is the least expensive on a sweetness basis.

The FDA took saccharin off the GRAS list in the early 1970s as a result of a study suggesting is caused cancer in rats. A ban on saccharin used in the United States was proposed by the FDA but was stayed by Congress in 1977 because of the ensuing public uproar fueled by the fact that there was then no noncaloric sweetener to replace it. However, saccharin has now been cleared of the possibility of causing bladder cancer by a number of studies. It is banned in Canada.

Saccharin has been used primarily in soft drinks, but also as a tabletop sweetener and in a wide range of other beverages and foods. A drop in the demand for saccharin for use in soft drinks occurred in early 1985 after Coca-Cola and Pepsi-Cola substituted a major portion of their saccharin use with aspartame. However, it is still used in other products in the rapidly growing dietetic soft drink market.

In July 1988, the FDA approved the use of Hoechst AG's (now Nutrinova) acesulfame K (Sunette™) for use in chewing gum, dry beverage mixes, instant coffee and tea, gelatins, puddings, and nondairy creamers. In 1998, the FDA approved its use in nonalcoholic beverages. Acesulfame K has a rapidly perceptible sweet taste 200 times that of sucrose. It has a good shelf life and is relatively stable across temperature and pH ranges associated with the preparation and processing of foods. Baked goods, candies, and dry mixes are believed to be the most viable markets for this low-calorie sweetener. A limitation is an unusual taste detected at levels required for adequate sweetness, which will no doubt prevent its widespread use in diet soft drinks. No toxicity problems have been reported in a multitude of studies to date.

Sucralose is the only low-calorie sweetener made from sugar. Since 1991 it has been authorized for use in foods and beverages in more than 30 countries worldwide, including the USA, Canada, EU, Mexico, Brazil, Australia, New Zealand, Argentina, Lebanon, Russia, and Romania, and it received FDA approval in April 1998. Developed by Tate & Lyle (UK), sucralose is a chloroderivative of sucrose, 600 times sweeter than sucrose, made by altering the sucrose molecule. Unlike sugar, sucralose is not converted into energy by the body, and therefore contains no calories. In addition, sucralose does not promote tooth decay, and is stable in a wide range of pH and thermal process conditions. Its uses include soft drinks, dairy products, baked and extruded products, puddings, breakfast cereals, jams and jellies, canned fruit, and chewing gum.

Neotame, formed through a modification of aspartame's dipeptide base, is approximately 7,000 to 13,000 times sweeter than sugar. It is a free-flowing, water soluble, white crystalline powder that is heat stable and can be used as a tabletop sweetener as well as in cooking applications. Examples of uses for which neotame has been approved include baked goods, nonalcoholic beverages (including soft drinks), chewing gum, confections and frostings, frozen desserts, gelatins and puddings, jams and jellies, processed fruits and fruit juices, toppings and syrups. In determining the safety of neotame, FDA reviewed data from more than 113 animal and human studies.

Other high-potency sweeteners not approved for use in the United States but used elsewhere include the following compounds:

Cyclamate is 30 times sweeter than sucrose. It has a sugarlike taste, a good shelf life, and a synergistic effect when combined with saccharin or aspartame. Cyclamate was introduced as a food sweetener in the 1950s, but was banned in 1970 because of its suspected carcinogenic potential. Since then, Abbott Laboratories, the developer and main producer of cyclamate, has undertaken further studies and submitted petitions to the FDA that demonstrate its safety. In June 1985, the National Academy of Sciences concluded that cyclamate was not a carcinogen. The FDA, however, has not reapproved use of the sweetener. Cyclamate use is currently permitted in more than 40 countries, including Canada and the EU (excluding the United Kingdom). Cyclamate is used as a tabletop sweetener, in beverages, and in low-calorie foods, particularly in combination with saccharin. The use of cyclamate with saccharin gives a better taste to beverages than saccharin alone. Thus saccharin producers would welcome reintroduction of cyclamate in view of competition from aspartame.

Developed by Pfizer in 1979 (prior to selling its food business), alitame is a dipeptide made of two amino acids, L-aspartic and D-alanine. It is 2000 times as sweet as sugar, with the same taste as sugar; thus its use level would be 25–400 ppm. Composition and use patents had been issued in 32 countries. The U.S. patent expired in 2000. Alitame is approved in Australia, New Zealand, the People's Republic of China, Indonesia, and Mexico for use in food, beverage, and tabletop applications. Approval is still pending in the United States, Japan, Canada, and the EU. Potential market applications for alitame include bakery products, snack foods, candies and confectionaries, ice cream, and frozen dairy products. A reported advantage of alitame over aspartame is lower loss during cooking and heating, since it is heat-stable.

Thaumatin, a mixture of sweet-tasting proteins from the seeds of *Thaumato coccusceus daniellii*, a West African fruit, is about 2000–2500 times sweeter than sucrose. Its taste develops slowly and leaves a licorice aftertaste. Thaumatin acts synergistically with saccharin, acesulfame K, and stevioside. Potential applications include beverages and desserts; it cannot be used in baked products. Thaumatin is generally recognized in the United States as

safe for chewing gum, and the supplier. Tate & Lyle, is seeking GRAS extensions for other foods. Thaumatin has been permitted in Japan as a natural food additive since 1979. Although it is approved for use as a sweetener in the United Kingdom and Australia, it is used primarily as a flavor enhancer.

Stevia rebaudiana, a plant native to South America, is the source of the stevia extract, which is a natural sweetener. Stevia can be used in food products that require baking or cooking because of its stability in high temperatures. This product is approved for use as a sweetener in Japan, but is not approved for use in the United States. There is currently a proposal in Brazil that the sweetener be included in any sugar-free soft drink in that country. Brazil is believed to be the third-largest soft drink market in the world, after the United States and Mexico.

Dihydrochalcones (DHCs) are derived from bioflavonoids of citrus fruits and are 300–2000 times sweeter than sucrose. They leave a licorice aftertaste and give a delayed perception of sweetness. Currently DHC that is 2000 times sweeter than sucrose is produced from bitter Seville oranges by hydrogenation of natural neohesperidin (the main flavone of some oranges). In low concentration in combination with other sweeteners, it has potential uses in chewing gum, candies, some fruit juices, mouthwash, toothpaste, and pharmaceuticals. It is approved for use in Spain, the Netherlands, Germany, Belgium, and Zimbabwe.

Several other high-potency plant constituents (in addition to stevia and thaumatin) that have been considered as food sweeteners include monellin from the African "serendipity berry"; glycyrrhizin, also discussed as a flavor enhancer and extracted from the licorice root; and hernandulcin, an oil extracted from a Mexican plant. Such sweeteners could potentially be used in addition to or as substitutes for synthetic sweeteners that are now used to sweeten low-calorie or dietetic foods and beverages.

B. Thickeners and Stabilizers

Thickeners and stabilizers (also called hydrocolloids, gums, or water-soluble polymers) provide a number of useful effects in food products. The technical base for these effects results from the ability of these materials to modify the physical properties of water. Most food and beverage products largely consist of water. Water-soluble materials function as rheology modifiers, affecting the flow and feel (mouth) of food and beverage products; act as suspension agents for food products containing particulate matter; stabilize oil/water mixtures; act as binders in dry and semidry food products; and create both hard and soft gels in food products that require this physical form.

During the 1990s, fat replacement (discussed in detail in a later section) became a major application for modified starches and gums as these additives provide unique texturizing, bulking, and emulsifying properties. Moreover, natural gums have been preposed as good sources of dietary fiber. Thickeners and stabilizers are generally used in very small amounts in most food products (e.g., 0.15% in jam, 0.35% in ice cream, and 1–2% in salad dressings). Table 83.7 indicates the primary functions of many food thickeners.

Two principal classes of these materials are recognized: natural materials obtained from plants and animals, and semisynthetic materials that are manufactured by chemical derivatization of natural organic materials, generally based on a polysaccharide on microbial fermentation-based substances. A third class known as "synthetic polymers," obtained from petroleum or natural gas precursors, is not used as a food additive. Figure 83.3 shows the sources and the various hydrocolloids used by the food processing industries.

Unmodified or natural corn starch, produced by the wet milling of field corn, supplies the majority of thickening material for the American food and beverage market. Other natural starches of significance include potato, tapioca, arrowroot, and sago starches. Unmodified cornstarch, commonly called pearl starch, is used in the food processing industry in the preparation of sauces, gravies, and fillings. It is the choice thickening ingredient in many formulated food products because of its modest price. It is modestly priced in comparison to modified starch, but even more so compared with additives such as cellulose ethers, guar, xanthan, and alginates. Major use categories for unmodified starch include

- Meat gravies
- Cooked puddings
- Cream-style sauces
- Pie fillings
- Barbecue sauces
- Salad dressings
- Baked good fillings

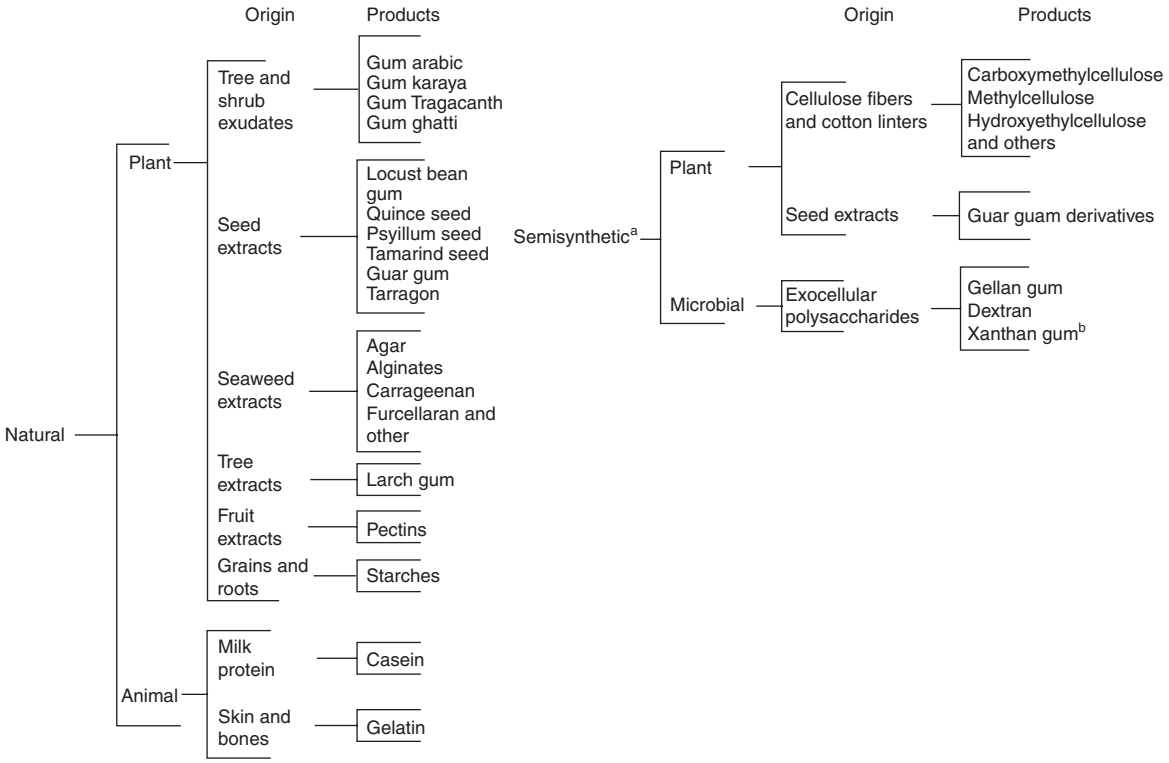
Modified starches used in food products or food processing have been modified to

Extend the bodying or gelling effect of normal starches
Improve resistance to acid or heat degradability and to low temperature and freeze/thaw (eliminating aggregation)
Improve texture
Modify gelling tendencies as desired
Increase viscosities at high temperature without gelling on cooling
Provide instant solubility and gelling in cold water

Modified or derivatized starches are generally designed for more selective food applications than unmodified starch.

TABLE 83.7
Major Food Thickeners and Stabilizers and Their Functions

	Thickening	Emulsion Stabilization	Suspending Properties	Gelation	Crystallization Control	Water Binding	Mouth Feel	Foam Stabilization	Flavor Fixation	Protective Film Forming	Synergistic Effect	Fat Substitution
Unmodified starches	X	X	X	X								
Modified starches	X	X	X	X		X	X					X
Casein						X		X				
Gelatin						X		X		X		
Carboxymethylcellulose	X	X			X	X	X		X	X		X
Methylcellulose	X	X		X				X		X	X	
Guar gum	X	X				X					X	
Alginates	X	X	X	X		X	X	X		X	X	
Xanthan gum	X	X	X		X		X					X
Pectin	X			X			X			X		X
Locust bean gum	X	X										
Gum arabic		X	X		X			X		X		
Carageenan	X	X	X	X	X	X	X				X	X
Agar				X								



[a] Natural-based, but highly derivatized and commercially significant products.

[b] Although xanthan gum is sourced from a natural product (grain), it is classified as a semisynthetic polymer because it is manufactured by fermentation in a chemical plant. Some industry sources disagree with this classification.

FIGURE 83.3 Natural and semisynthetic hydrocolloids used as food additives.

Modified starches are used in a wide variety of products, including baby foods, purees, candy (e.g., bonbons and butter creams), jellies, cake mixes, dough, various soup powders and liquids, instant noodles, puddings, pie fillings, batter mixes, sauces, salad dressings, dairy desserts, snack foods, and canned foods. In meat products such as sausage, ham, and luncheon meat, modified starches serve as a binder as well as thickener. Recently, modified starches have been used as fat substitutes in margarine-like spreads, salad dressings, cookies, and baked products.

Casein is a protein occurring naturally in, and obtained from, milk; it is the main ingredient in cheese. Casein is marketed as sodium, calcium, potassium, or magnesium caseinate and is used in confections, puddings, bakery fillings and frostings, coffee whiteners, and whipped toppings. In 1970 only five protein products were available from milk. Since then, numerous advances have been made used in the methods to isolate and modify proteins. As a result, most suppliers now offer multitudes of specialty casein products.

Gelatin is obtained from pork skin and bones (type A), or beef skin and bones (type B). Type A is mostly used for confectionery products and type B for dairy applications. Gelatin is about 97% protein, but it has no beneficial value to human nutrition. Food applications for gelatin includes dairy products such as yogurts, confectionary products such as gummy animal chewables, meats such as canned hams, and gelatin desserts. Gelatin is hygroscopic, capable of absorbing up to 10 times its weight in water. Under refrigeration it forms a thermally reversible gel of high strength.

Gelatin seems to exhibit little synergy with other thickeners and stabilizers. Therefore it appears to be of little benefit to blend gelatin with other gums to produce custom formulations.

Carboxymethylcellulose (CMC) is the primary cellulose ether consumed in food and beverage applications, mainly in pet foods, frozen dairy products, beverages, bakers' goods, dry drink mixes, syrups, glazes, icings, and toppings. The current search for microwave-compatible food additives makes CMC a candidate for this rapidly growing formulated food market. CMC, a nonnutritive substance, is also popular in diet food formulations requiring thickeners and stabilizers. Methylcellulose (MC) and hydroxypropylcellulose (HPC) are also used in specialized food and beverage applications, but their relatively high market prices preclude them from large volume applications.

Guar gum is the galactomannan derived from the endosperm of guar seeds (*Cyamoposis tetragonolobus*)

grown in India and Pakistan since ancient times. It is one of the most economical and widely used gums, with extensive use in a variety of food applications since the 1950s. In spite of several attempts by major domestic guar concerns to encourage domestic production of guar in Texas and other arid agricultural areas of the American Southwest, most of the guar gum consumed in the United States is derived from imported degermed guar beans (splits), mostly from India. Because of changing supplies due to weather and harvest conditions, as well as the demand for guar gum for industrial applications, the price of guar gum tends to shift dramatically. However, guar is expected to remain one of the most cost-effective thickeners on the market. Major uses for guar gum include ice cream, dessert toppings (e.g., Cool Whip®), frozen and refrigerated prepared meals, cheese, imitation bakery jellies and dry-mix bakery formulations, fruit drinks, soups, gravy and sauce mixes, water-based frozen desserts, salad dressings, and instant hot cereal.

Alginates are extracted from different types of seaweeds, mainly from brown seaweed, *Macrocytis pyrifera* and *Laminaria sp.* The alginates include the various salts of alginic acid and propylene glycol alginate (PGA). Sodium alginate is used primarily as a binder in frozen desserts, reconstituted onion rings, crab and shrimp analogs, instant pudding mixes, fabricated puddings, sauces and gravies (particularly those containing milk or requiring the low "weep" property of alginates), and re-formed meats.

Propylene glycol alginate (PGA) is used in the United States as a foam stabilizer in beers and ales. In addition, PGA is used by major food manufacturers in salad dressing formulations. The nonsodium light metal salts of alginic acid are used as sodium alginate alternatives in low-sodium and dietary food specialties.

Xanthan gum, a fermentation product, is used in salad dressings, relishes, syrups, sauces, bakery fillings, prepared puddings, glazes and toppings, processed cheese products, dry cake and beverage mixes, and fruit and carbonated beverages. A significant use is in dairy products, where it prevents the separation of the contained whey from the rest of the food product. Xanthan does not exhibit any reactivity with milk proteins and therefore is often used in combination with other hydrocolloids, particularly carrageenan. Moreover, the stability of xanthan gum to acid and high salt content makes it very useful for many types of foods.

Gellan gum is the latest hydrocolloid approved for food use, produced with a fermentation process like that used for the fermentation of xanthan gum by the organism *Auromonas elodea.* The FDA approved gellan gum for use in icings, frostings, bakery fillings, and low-solids jams and jellies and confections. It is also approved for food use in Japan. Gellan gum can be used at levels substantially below those required by conventional hydrocolloids. There are two forms of gellan gum. The first is a high-acetyl gum, which is partly acetylated and provides thermoreversible gels. The second is a low-acetyl gum forming a firmer and more brittle gel.

Pectin is a fruit extract from the peel of citrus fruits and apple pomace. The main commercial types used in foods are pectin itself and potassium pectinate, sodium pectinate, and amidated pectin. Commercial products include high ester [degree of esterification (DE) of 50] or low ester (DE of less than 50) pectins. Traditional food uses of pectins are in jellies and jams. Newer applications include gummy candies and fruit-flavored juices and carbonated drinks. In gummy candies and jellies, it is replacing starch for improvement of fruity flavor. In fruit-flavored drinks, it stabilizes the constituents and makes the product more appealing. A constraint on the supply of pectin is the approval required by the EPA to start up a new plant. Because of the high costs of compliance to dispose of the large volume of waste generated during pectin production, the last North American pectin production plant was relocated from Florida to Mexico.

Locust bean gum is obtained from the carob tree. The major source of locust bean gum is the Mediterranean countries. The size and quality of the crop is directed related to climatic conditions, producing periodic shortages of supply and great fluctuations of price. Chemically locust bean gum is similar to guar gum. Anionic, cationic, and hydroxyalkyl derivatives are also produced commercially. Locust bean gum swells in cold water, but heating is necessary for maximum solubility. Locust bean gum is widely used in frozen dairy products, in conjunction with guar gum and carrageenan, and is used for preventing syneresis in cream cheese. In addition, locust bean gum is used in many nonemulsified sauces and dressings as a thickener, in prepared meals, and in bakery products as a moisture retention aid. Much of the locust bean gum is supplied in a blended form to the dairy industry.

Gum arabic is obtained from various trees of the genus *Acacia*, primarily from *A. senegal.* It is highly soluble in water (up to 50%), and its solutions are of relatively low viscosity. Other advantages of gum arabic as a food additive are its nontoxicity and lack of odor, color, and taste. These properties are especially useful in systems requiring emulsifying properties, such as high solid suspensions. It is used as an emulsifier in beverages for citrus oil and flavors, a foam stabilizer in beer, as a crystallization retarder and emulsifier in confectionaries, and as a stabilizer in dairy and bakery products. Since the source of supply has sometimes been unreliable because of political and social events in the Middle East, many U.S. users have turned to substitutes, including starch derivatives.

Carrageenan is extracted from Irish moss (*Chondrus* and *Gigartina* species) that is harvested off the Atlantic shores of New England, the Canadian Maritime Provinces, and several European countries. Carrageenan is readily soluble in water to form an inelastic gel and is commonly used with other gums. Its most unique property is a high

degree of reactivity with certain proteins, such as casein. The largest application of carrageenan in food use is in dairy products (e.g., frozen desserts, flavored milk powder, nondairy creamers). For example, coca can be suspended in milk with the use of about 0.025% carrageenan.

One of the most significant recent developments for carrageenan suppliers has been its widespread use in poultry applications for moisture retention. The product serves to retain moisture before and during cooking and allows the poultry to be pumped with large amounts of water.

In 1990, the FDA decided to allow an unrefined seaweed extract known as Philippine Natural Grade (PNG) to be sold under the carrageenan heading. Traditionally, refined carrageenan is made in a 10-step process in which carrageenan is extracted from the seaweed and then filtered to remove the cell walls, or cellulose, the other substances from the seaweed. PNG carrageenan is prepared in a five-step process that extracts the unwanted substances from the seaweed, leaving both carrageenan and about 10–14% cellulose, as opposed to the less than 1% found in traditionally refined product. PNG is currently being used in the meat, cheese, and pharmaceutical markets.

Agar (also called agar-agar) is obtained from various red-purple seaweeds of the class *Rhodophycae*. Agar is used primarily in baked foods (icings, toppings, meringues) and in confectionary products. Because agar is the most expensive of the seaweed extracts, there have been efforts to substitute with other gums such as carrageenan.

A number of other thickening agents are used by the food industry, but the represent a very minor portion of the food additives market. Most are higher priced, in erratic supply, and face increasing competition from the principally used thickeners. Such other thickening and stabilizing agents and their principal uses include

- Ghatti gum. Obtained from India and Sri Lanka; no other functional properties are known than thickening and emulsion stabilization.
- Tragacanth gum. Obtained from the Middle East and used in salad dressings and sauces.
- Karaya gum. Obtained from India and used for extreme thickening to pastelike gels.

C. Colors

Colors are additives used to improve the overall appearance of foods and influence the perception of texture and taste. Products are derived from either natural origins or produced synthetically. In the United States, colors are divided into two types: certified (FD&C) and natural (exempt from certification) colors. Food colors are listed in the *Code of Federal Regulations* (CFR) Title 21 parts 70–82. If an additive is not specifically included in these sections, it may not be used for coloring food, drug, or cosmetic products that will be sold in the United States.

There is some confusion about the term "natural" colors. The definition of "natural" varies between the United States, Europe, and Japan. This section will concentrate on the U.S. regulations, with occasional reference to others. In the United States, from a regulatory point of view, there is no definition for natural colors, only "certified dyes" and "color additives exempt from certification." Certified colors are synthetic materials whose purity is checked by the FDA. Colors, obtained from animal, plant, or mineral origins are not certified because they often contain complex mixtures of many components. The exact composition of natural color varies from plant variety to plant variety, from region to region, and from season to season. Users depend on the integrity of their suppliers to ensure product quality.

The certification process concerns only batch purity, it does not guarantee the safety of the color molecule. There is no inherent reason why certified colors are either more safe or less safe than natural colors (colors exempt from certification). In order to market their products, U.S. producers must submit product samples from each batch of material and pay a certification fee. The materials are analyzed in an FDA laboratory to ensure that they meet specific purity specifications. In other parts of the world, only self-certification exists, except in Japan where certification of synthetic colors has been required since 1994. Certified food colors, both primary and blends, are produced in a variety of forms including powder, liquid, granules, plating blends, nonflashing blends, pastes, and dispersion; the least expensive form is powder.

A number of formerly certified FD&C colors have been banned under the provisions of the Delaney clause of the Food, Drug & Cosmetic Act, either because they were found to be carcinogenic or because there was no assurance that they could be made free from carcinogenic impurities. These actions have steadily reduced the number of certified dye colors available to the U.S. food industry from more than 22 in 1950 to 7 in 2003. (In addition FD&C citrus red no. 2, is permitted for coloring the skins of oranges that are not intended or used for processing, but it has not been produced in the United States in recent years; and orange B may be used for coloring the casings or surfaces of frankfurters and sausages.) Certified food colorants can be divided into dyes and lakes. Dyes are chemical compounds that exhibit their coloring power or tinctorial strength when dissolved in a solvent. Lakes are insoluble colored materials that color by dispersion. Table 83.8 shows the physical properties of these seven certified food colorants.

Color regulations specify a legal minimum of 85% pure dye for primary colors, but most dye lots contain from 90–93% pure dye. Certified dyes fall into several chemical classes: azo-dyes (yellow no. 5, yellow no. 6, red no. 40, citrus red no. 2), triphenylmethane dyes (blue no. 1, green no. 3), xanthine type (red no. 3), and sulfonated indigo (blue no. 2).

TABLE 83.8
Physical and Chemical Properties of Certified U.S. Food Colorants

FD&C Name	Common Name	Light	Stability to Oxidation	pH Change	Compatibility with Food Components	Tinctorial Strength	Hue	Water Solubility
Red no. 3	Erythrosine	Fair	Fair	Poor	Poor	Very good	Blue	9
Red no. 40	Allura red AC	Very good	Fair	Good	Very good	Very good	Yellow	25
Yellow no. 6	Sunset yellow FCF	Fair	Fair	Good	Good	Good	Red	19
Yellow no. 5	Tartrazine	Good	Fair	Good	Good	Good	Lemon yellow	20
Blue no. 1	Brilliant blue FCF	Fair	Poor	Good (unstable in alkali)	Good	Excellent	Green-blue	20
Blue no. 2	Sodium indigo disulfonates	Very poor	Poor	Poor	Very poor	Poor	Deep blue	1.6
Green no. 3	Fast green FCF	Fair	Poor	Good	Good	Excellent	Blue	20

FD&C dyes are also used in the production of lakes, which are pigments prepared by combining a certified dye with an insoluble alumina hydrate substratum. Lakes are both water and oil insoluble and impart color through dispersion in food. Thus they are suitable for coloring foods that cannot tolerate water and products in which the presence of water is undesirable. Examples include bakery products (icings, fillings, cake and doughnut mixes), confections, dairy products (hard fat coatings for ice cream novelties, wax coatings for cheese, yogurt with fruit syrups), dry pet foods, dry beverage bases, and dessert powders. The FD&C lakes do not have a legally specified minimum dye content; manufacturers use formulations of from 11% (standard) to 42% pure dye (concentrated).

Noncertified colors can be from either natural origins (primary sources), such as vegetables and fruits, or produced synthetically. Traditional markets for noncertified food colors include lipid-based, high-fat food systems such as butter, margarine, shortening, popcorn oil, processed cheeses and spreads, salad dressing, and snack foods. Water-soluble forms are also available and are used in beverages, baked goods, confections, and dairy products. Food color additives exempt from certification, their colors and sources are listed in Table 83.9 and described in more detail below.

Annatto extract (Bixin, Norbixin, etc.) is an extract of a seed from a shrub called *Bixa orellana L.* that grow in South America, East Africa, and the Caribbean. Oil- and water-soluble forms exist depending on the method of extraction. Annatto extracts exhibit various yellow shades, and are commonly used in cheddar cheese and bakery products, often in combination with turmeric or paprika oleoresin.

Beet juice/powder (betanin, beet-root red, etc.) is a water-soluble color found as the predominant pigment in red beets (*Beta vulgaris*). Several forms exist, including dried ground beets, or dehydrated beet powder; beet juice concentrate, the liquid obtained by concentrating the expressed juice from mature beets; and beet powder, made by spray drying beet juice concentrate onto a carrier of maltodextrin.

TABLE 83.9
Food Color Additives Exempt from Certification[a]

Additive	Color	Source
Annatto extract	Yellow	Vegetable
Beet juice	Red	Vegetable
Dehydrated beets	Purple	Vegetable
Canthaxanthin[b]	Red	Synthetic
Caramel	Brown	Semi-synthetic
Apocarotenal[c]	Orange	Synthetic
Beta-carotene	Yellow	Synthetic
Carrot oil	Yellow	Vegetable
Cochineal extract (carmine)	Red	Insect
Corn endosperm oil[d]	Yellow	Vegetable
Dried algae meal[d]	Yellow	Plant
Ferrous gluconate[e]	Black	Synthetic
Ferrous lactatee	Black	Synthetic
Fruit juice (grape and cranberry)	Red	Fruit
Grape skin extract (enocianina)[f]	Red	Fruit
Paprika	Red	Vegetable
Paprika oleoresin	Red	Vegetable
Riboflavin	Yellow	Synthetic
Saffron	Yellow	Vegetable
Titanium dioxide[g]	White	Synthetic
Turmeric	Yellow	Vegetable
Turmeric oleoresin	Yellow	Vegetable
Vegetable juice	Red	Beet and red cabbage juice

[a] Under the Code of Federal Regulations, Title 21. No color additive may be used in foods for which standards of identity have been promulgated under Section 401 of the Federal Food, Drug & Cosmetic Act, unless the use of added color is authorized by such standards.
[b] May not exceed 66 mg/kg of solid, or pint of liquid, food.
[c] May not exceed 33 mg/kg of solid, or pint of liquid, food.
[d] To enhance yellow color of chicken skin and egg yolk.
[e] To color ripe olive.
[f] Used only in beverages.
[g] May not exceed 1% by weight of the food.

Canthaxanthin (Roxanthin) is a synthetically prepared carotenoid that is commercially available as a water-dispersible powder. It exhibits reddish orange to dull violet shades.

Caramel (burnt sugar) color results from the controlled heat treatment of food-grade carbohydrates. Often catalysts are added to drive the reaction to the desired color end point. Caramel colors exhibit a colloidal charge and a variety of shades from yellow brown to reddish brown, and is available in powder and liquid forms. Caramel has a very large market in cola beverages. It is also used in bakery products and confectionaries.

Apocarotenal (beta-apo-8′-carotenal) is a red-orange synthetically prepared carotenoid that is oil soluble. The pigment is found in oranges and tangerines and is commonly used in products such as cheese spreads and snack foods. In the United States, a usage restriction of 15 mg/lb of semisolid or solid food exists.

Commercial products of natural beta-carotene exist from several sources, including the alga *Dunaliella salina* and palm oil. Beta-carotene can be also synthesized. It is oil soluble and exhibits a characteristic butter to egg-yolk shade. It is commonly used in baked goods, beverages, and confections.

Cochineal extract, or carmine, the lake pigment of cochineal extract, is an extract of a female cochineal insect *Dactylopius coccus*, or *Coccus cacti.* It is a stable colorant used since antiquity. At pH 4 and below it is orange, at pH 4–6 it is magenta-red color, and at pH greater than 6, it has a blue-red shade. The insect is commonly cultivated in Peru, Ecuador, and the Canary Islands. Approximately 70,000 insects are required to produce one lb of 50% carminic acid lake. It is commonly used in beverages, sausage products, aperitifs, and confections. Cochineal extract is not kosher.

Fruit juices that typically contain carotenoid- or anthocyanin-type pigments are often used in concentrated or single-strength forms as coloring agents. In the United States, fruit juices must be expressed from mature varieties of edible fruits or a water infusion of the dried fruit. Fruit juices that are used for coloring include cranberry, cherry, raspberry, elderberry, grape, orange, and tomato.

Grape skin extract (enocianina) is obtained by an aqueous extraction of fresh, deseeded marc remaining after grapes have been pressed to produce grape juice or wine. It contains the common components of grape juice but not in the same proportion. During the steeping process, sulfur dioxide is added, and most of the extracted sugars are fermented to alcohol. The extract is concentrated by vacuum evaporation, during which practically all of the alcohols are removed. A small amount of sulfur dioxide may be present. In the United States, grape skin extract is permitted only for use in coloring beverages.

Paprika is the ground form of sweet red peppers (*Capsicum annum*). Paprika oleoresin is a solvent extract of the coloring principles of sweet red peppers. Extraction of the peppers is carried out with several permitted solvents, including hexane, ethylene dichloride, and various alcohols. Oil is commonly added to the extract to standardize the strength, with typical designations in American Spice Trade Association (ASTA) units and color value units (CVU). Paprika oleoresins are oil soluble, reddish orange shades. Typical applications include coloring salad dressings, snack foods, cheese product, baked goods, breading, and crackers.

Riboflavin, a bright yellow color, is also referred to as lactoflavin and vitamin B_2. It is a naturally occurring yellow pigment isolated from milk, and it can also be synthesized. It has limited solubility, a bitter taste, and is light sensitive, therefore it has limited use.

Saffron is the dried stigmas or extract or *Crocus sativus.* The predominant pigments are crocin and crocetin. Saffron is limited in its application due to its very high cost; approximately 165,000 blossoms are required to produce 1 kg of colorant. Saffron is commonly used as a spice and colorant in rice products. Its bright lemon-yellow color is also used in applications such as soups, baked goods, and certain dairy products.

Titanium dioxide is a white pigment that is reacted product from a mineral oxide called ilmenite, a type of iron ore. The crystal form, anatase, is the form of choice as a colorant for food. In the United States, purity of 99% or greater is required. Titanium oxide is the only white pigment currently permitted as a color additive in the United States. It is often used to opacify systems such as low-fat/no-fat salad dressings and dairy products, pet foods, baked goods, sugar-coated candies, and other confections. It colors by dispersion, as it is not water or oil soluble.

Turmeric and turmeric oleoresin is a bright yellow pigment from the rhizome *Curcuma longa*, which is grown predominantly in India. The principle coloring agent is curcumin. The oleoresin form is extracted by solvents, such as alcohol and acetone. It is available with or without flavor components.

Some vegetable juices, typically in a concentrated form, are used as coloring agents. In the United States, vegetable juices must meet the criteria of the federal regulation, which describes juice expressed from mature varieties of edible vegetables. An example of a commercially available vegetable juice colorant is red cabbage juice, which contains anthocyanins. Most other vegetable juice concentrates contain chlorophyll pigments and are often not of sufficient color concentration nor stable enough to be used commercially. In addition, the flavor impact is often undesirable.

Eight synthetic dyes are permitted for use in the EU countries. Red no. 4 (Ponceau 4R) was banned in the US in 1970, but it is still approved for food use in the EU countries. At the same time Red no. 40 (Allura Red AC) the most widely used red color in the United States has not been approved yet in the EU countries. Twelve water-soluble dyes and eight of their lake colors are allowed in Japan for foods. Compared to American consumers, European and Japanese consumers are more prone to demand that their food products contain natural colorants.

There is a growing body of evidence that many natural colorants perform additional functions. They also act

as vitamins, antioxidants, and antimicrobial and antiviral agents. Natural colorants also may have anticancer properties and can be used to treat vascular disease and improve night vision. This information was discovered in recent years.

D. Fat Replacers

Although fats are essential for a healthy diet, excessive consumption of fat has been related to health problems. Ft replacers are those ingredients that can help to reduce a food's fat and calorie levels while maintaining some of the desirable qualities fat brings to food, such as "mouth feel," texture, and flavor. Fat replacers can be carbohydrate, protein, or fat based.

Three alternative approaches are being pursued in this area:

- Fat substitutes. There are partially or fully nonmetabolizable compounds that possess fatlike properties and can replace fats on a one-for-one basis. Most fat substitutes are synthetic compounds that possess fatlike properties and can replace fat in a broad range of applications.
- Fat mimetics. These are nonfat ingredients that mimic the mouth feel and other functional properties provided by fat, but have fewer calories than fat. In recent years, numerous approaches have been undertaken to partially replace or to eliminate fats in food by using FDA-approved traditional nonfat food ingredients such as novel carbohydrates and gums, as well as other innovative ingredients, including microparticulated milk and egg proteins, and modified oat fibers. These products are capable of duplicating many of the functional properties of fats, such as lubricity, tenderization, opacity, flavor release, slipperiness, melt, and plasticity. These products cannot substitute for fats on a one-to-one replacement basis. Moreover, these ingredients are suitable as fat replacements only in foods that do not require extensive heat processing (e.g., salad dressings, frozen desserts, margarine-like spreads, etc.)
- Emulsifiers are fat- or fatty acid-derived compounds that have the ability to modify the surface properties of solids or liquids and possess many of the properties of a fat or an oil. The caloric value of most emulsifiers is similar to that of triglycerides. However, depending on the degree of esterification and polymerization, some emulsifiers such as polyglycerol esters may have a lower calorie content. Polyglycerol esters contribute only 6 kcal/g. Typically 2% fat in a formula can be replaced with 1% emulsifier with no loss of functionality. However, due to regulatory constraints and flavor considerations, emulsifiers are usually used at 1% concentration or less in formulated foods.

1. Low and Noncaloric Lipids

Olestra (brand name Olean™), developed by Procter & Gamble Co. (P&G), was approved by the FDA in January 1996 for use in preparing potato chips, tortilla chips, and other savory snacks. Olestra is a sucrose polyester made from sucrose backbone and six to eight fatty acids. The number and type of fatty acids vary depending on the performance characteristics desired. The fatty acids are derived from vegetable oils found in soy, corn, and cottonseed oils. Olestra molecules are much larger than those of ordinary fats, so the body's digestive enzymes cannot break it down. Thus Olestra is neither digested nor absorbed, passing straight through the body. It is noncaloric and nonsweet.

Olestra was discovered about 25 years ago. Its submission to the FDA was withdrawn numerous times as the information on it was refined. At the time of its approval, more than 300 volumes covering more than 100 laboratory studies on seven species and 98 clinical investigations involving 2500 humans comprised the body of knowledge on this compound. P&G spent more than $200 million for the development and regulatory approval process of olestra.

There are some concerns about olestra because it blocks the absorption of fat-soluble vitamins consumed with it. Therefore the FDA requires that fat-soluble vitamins A, D, E, and K be added to foods made with olestra. Also it is reported to cause abdominal discomfort and may act as a laxative in some cases. Therefore products with olestra have to carry a warning label that these effects are possible. Approval of olestra brought a range of responses from scientists and consumer advocates who disapprove of its use, as well as endorsements by groups such as the American Dietetic Association, which identifies olestra as "one more choice for consumers in the war against fat."

Medium-chain triglycerides (MCT) are esters of fractional coconut oil fatty acids. These compounds provide 8.3 kcal/g, only slightly less than conventional fats. However, recent physiological studies suggest that MCTs are burned readily for energy and have little tendency to be incorporated into tissue lipids that are not deposited as fat. MCTs are GRAS compounds, have been used in medical and infant feeding products for more than 30 years, but have not been used in consumer food products because of their high cost. More recently, their use has been expanded into sport/nutrition foods.

2. Fat Mimetics

a. Carbohydrate-based substitutes

Nearly 40 different products based on starch have been recommended for fat replacement. Some of these exist as

products with other uses, although several have been developed specifically as fat mimetics. Most of the materials in this category are used to form a gel containing modified starch and water. The gel is then substituted for fat in the formula on an equal-weight basis. Starch-based fat mimetics have many different properties depending on the parent starch and on the degree of cross-linking, substitution, and acid modification. In many instances, two or more starches must be used together to give the desired effect, or they can be combined with other polymers and emulsifiers.

Maltodextrins are products of the acid hydrolysis of starch, and act as bulking agents, giving the mouth-feel qualities of fat. One of the first on the market, in 1984, was N-Oil, a hydrolyzed tapioca maltodextrin. The substance forms a thermoreversible gel in aqueous foods, and therefore creates the mouth feel of fat, N-oil and several similar products are used in frozen desserts, salad dressings, margarine-type spreads, dips, baked products, and snacks. Several other starch-based maltodextrin fat replacers are available.

One of the most widely used fat mimetics is Avicel™, a cellulose derivative used in frozen desserts, salad dressings, and baked goods. Methocel, a food gum, is made of cellulose ethers for use in bakery products, fried foods, and salad dressings.

Pectin is a gum that forms a gel. It has a large water-holding capacity and therefore helps to overcome some of the dry impression of fat-free foods. A gum is a soluble fiber, so it must be counted as a carbohydrate (4 kcal/g) in the calculation of calories for labeling purposes. In 1991 Splenda, a specialty pectin, was introduced as a fat replacer. Another gum fat replacer, carrageenan, that was used in low-fat hamburger, failed to achieve wide consumer acceptance.

Other hydrocolloids and gums are frequently promoted as fat-sparing agents. Xanthan, gelatin, carrageenan, algin, guar konjak, locust bean gums, etc., can be utilized as well for their fat-sparing function.

Polydextrose — "Litesse™" and "Veri-Lo™" — is recommended for replacement of fats. Polydextrose is a water-soluble, reduced-calorie polymer is dextrose that contains small amounts of sorbitol and citric acid. It provides 1 cal/g as it is only partially metabolized by the human body.

Oatrim, developed in the USDA laboratory in Peoria, Illinois, is an amylodextrin with 5% β-glucan extracted from oat flour. It is used as a fat replacer in baked products.

b. Protein-based substitutes

Lita is based on zein, a microencapsulated protein from corn. Like other protein-based fat replacers, it contributes less than 2 kcal/g. It is used in frozen desserts, whipped toppings, and mayonnaise.

c. Emulsifiers

Emulsifiers are fat-based substances that are used with water to replace all or part of the shortening content in cake mixes, cookies, icings, and vegetable-dairy substitute. Most emulsifiers provide the same calories as fat, but less is used, resulting in fat and calorie reductions. Many emulsifiers simply play a "fat-sparing" role. However, polyglycerol esters may have a lower caloric content than triglycerides, depending on the degree of esterification and polymerization.

The commonly used food emulsifiers that have applications as fat replacers include lecithin, mono- and diglycerides, and derivatives such as acetylated, succinylated, and diacetyl tartaric esters of distilled monoglycerides, polyglycerol esters, polysorbates, and sucrose esters.

More information on specific compounds can be found in the emulsifier section of this chapter.

While fat replacement is still very important to consumers in the United States, over the last years the interest in low- and non-fat products has begun to weaken. Even the advent of olestra in snack-food products has not provided the solution. Still, the fastest growth for fat substitute-containing products is expected to be in the United States, where diets have historically been higher in fat and sugar and consumers appear to have more problems with obesity/weight control and associated diseases. In Europe, consumer preference for low-fat, low-calorie foods perceived as natural, rather than artificial, has tended to limit acceptance of fat substitutes. On the regulatory side, fat substitutes face more daunting approval challenges in Europe than do other types of food additives. In Japan, and other Asian countries the market for fat substitutes is currently very small.

E. Enzymes

Enzymes are catalysts used during food processing to make chemical changes to the food. They are biological catalysts that make possible or greatly speed up chemical reactions by combining with the reacting chemicals, bringing them into the proper configuration for the reaction to take place. They are not affected by the reaction. All enzymes are proteins and become inactive at temperatures greater than about 40°C or in unfavorable conditions of acidity or alkalinity. Some of the specific functions food enzymes perform include:

- Speed up reaction
- Reduce viscosity
- Improve extractions
- Carry out bioconversions
- Enhance separations
- Develop functionality
- Create/intensify flavor
- Synthesize chemicals

Food enzymes are usually classified into the following categories:

- Carbohydrates and amylases are commercially the most important subgroup, hydrolyzing 1,4-glycosidic bonds in carbohydrates
- Proteases, hydrolyze peptide bonds in proteins
- Lipases, split hydrocarbons from lipids
- Pectic enzymes and cellulases, hydrolyze plant cell wall material
- Specialty enzymes

These enzyme categories can be divided further into 15–20 subgroups. The traditional roles of enzymes in the food industry have been in the processing of bakery goods, alcoholic beverages, and starch conversion. But interest is now focused on newer and more varied applications, such as hydrolysis of lactose, the preparation of modified fats and oils, the processing of fruit juices, and other processes where newer enzymes are being identified.

Today many food processes utilize enzymes. Food-grade enzymes encompass a wide variety of commercial products that are employed in the production, conversion, and modification of foods because of their highly efficiency and selective catalytic functions. Table 83.10 lists many of the major food enzymes and gives some applications in foods and food processing. The largest application of enzymes in the food industry is the use of alphaamylase, glucoamylase, and glucose isomerase for starch conversion and production of high fructose corn syrup.

Rennin, a protease enzyme used in cheese making, is also of significant value, followed by a host of other enzymes, including pectinases, invertase, lactase, and maltase (used for the modification of starches and sugars), catalase, pepsin, glucose oxidase (an antioxidant for canned foods), and bromelin, ficin, and papain (plant proteases used for tenderizing meat and producing easily digestible foods). Enzymes are highly specific and can act only on a single class of chemicals, such as proteins, carbohydrates, or fats. These same enzymes are also used in nonfood applications such as pharmaceuticals, textiles, detergents, and waste treatment.

Enzymes are produced from animal tissues (e.g., pancreatin, tripsin, lipase), plant tissues (e.g., ficin, bromelin), and most frequently by microorganisms (e.g., pectic, starch enzymes). Microbial production from a variety of species of molds, yeasts, and bacteria is increasingly becoming the predominant source of enzymes.

Application of genetic engineering to the development of enzymes has already made a significant impact. The first food additive produced by genetic engineering was chymosin, "Chy-Max,™" a microbial rennet that has been approved by regulatory agencies in the United States, Canada, the United Kingdom, Australia, Italy, and several other countries. Advantages of the bioengineering product are increased yields, relative ease of manufacture, lower price, and the ability to label the product as kosher.

TABLE 83.10
Applications for Enzymes in the Food Industry

Food Processing	Enzyme	Application
Alcohol production	Amylases	Starch liquefaction
Analytical testing	Cellulase, pectinase, amylases	Testing for dietary fibers, sugars
Baking	Fungal proteases	Dough conditioning, flour bleaching, malting, and antistaling
Brewing	Microbial proteases, papain, pectinase	Low-calorie beer, chill proofing, barley, alternative adjunct liquefaction, and saccharification
Confections	Invertase	Cream candy centers
Coffee processing	Pectinases, cellulase	Removal of burnt flavor in UHT (ultra-heat-treated) milk Separation of bean, viscosity control of extracts
Dairy	Rennins, lactase, lipase	Cheese making, accelerated cheese ripening, natural cheese flavor concentrations, whey utilization, lactase intolerance
Fats and oils	Lipase, phospholipase	Coca-butter substitutes, flavor-ester synthesis
Flavors	Protease, lipase	Synthesis of savory flavors, natural flavor esters
Fruits and vegetables	Cellulase	Breakdown of cellulose structure
Fruit juice and wine	Pectinases	Mash treatment, depectinization, starch/araban haze removal, citrus pulp wash viscosity reduction, natural cloud production
Protein	Bromelin, papain, pepsin, pancreatin	Rendering, soy milk production, egg white replacement emulsifier production, functional hydrolysates
Sugar processing	Amylases, cellulase	Removal of undesirable starches and polysaccharides in the processing of cane sugar
Starch conversion	Glucose isomerase	High fructose corn syrup, maltose, and dextrin syrups
Waste treatment	Proteases, cellulases, lipase	Breakdown of cellulose, lignins, oil residues and other solid waste material
Other	Proteases	Meat tenderizing, coffee soluble-extract viscosity reduction

Approximately, 60% of food enzymes used globally are now sold as recombinant proteins.

F. Vitamins

Vitamins are nutritive substances required for normal growth and maintenance of life. They play an essential role in regulating metabolism, converting fat and carbohydrates into energy, and forming tissues and bones. Vitamins can be used as functional ingredients in foods. Vitamin E (tocopherol) and vitamin C (ascorbic acid) protect foods by serving as antioxidants to inhibit the destructive effects of oxygen. This helps protect the nutritive value, flavor, and color of food products. In addition, ascorbic acid enhances the baking quality of breads, increases the clarity of wine and beer, and aids color development and inhibition of nitrosamine formation in cured meat products. Beta-carotene and beta-apo-8′-carotenal are vitamin A precursors, which are brightly pigmented and may be added to foods such as margarine and cheese to enhance their appearance. The roles of these substances outside their nutritional functions are discussed elsewhere in this chapter (see Antioxidants, Preservatives, and Color).

Thirteen vitamins are recognized as essential for human health, and deficiency diseases occur if any one is lacking. Because the human body cannot synthesize most vitamins, they must be added to the diet. Most vitamins are currently consumed as pharmaceutical preparations or over-the-counter vitamin supplements. Some, like vitamins B, C, D, and E are added directly to food products. Ready-to-eat breakfast cereals are a good example of fortification. Because the primary use of these cereals is as a complete breakfast entrée, they are commonly formulated to provide 25% or more of the daily value (% DV) per serving of the 10–12 important vitamins and minerals common to cereals. Another important example is the fortification of fruit drinks with vitamin C. Other foods that typically have added vitamins include margarine, infant formula, meat replacements, and breakfast bars (Table 83.11).

Vitamins are added to processed foods for several reasons:

- To restore vital nutrients lost during processing — this is important with dried milk, dehydrated vegetables, canned foods, and refined and processed foods.
- To standardize nutrient levels in foods when these fluctuate because of seasonal variations, soil differences, and methods of preparation.
- To fortify fabricated foods that are low in nutrients and promoted as substitutes for traditional products; this includes complete breakfasts, breakfast drinks, meat extenders, and imitation products such as eggs, milk, cheese, and ice cream.

TABLE 83.11
Fortified Food Groups

Food	Vitamin	Use Level	Remarks
Milk	Vitamin D	420 IU/1	Optional, but generally added
Beverages (noncarbonated)	Vitamin C	15–100% of U.S. RDI per serving	Optional; also added as an antioxidant
Cereals	Most essential vitamins	25–100% of U.S. RDI per serving	Optional; added to 90% of cold cereals
Flour	Thiamin, Riboflavin, Niacin	8–15% of U.S. RDI per 2 oz. serving	Mandatory
Margarine	Vitamin A	33,100 IU/kg	Optional, but generally added
Miscellaneous foods (e.g., instant breakfast, energy bars, etc.)	Most essential vitamins		Added to position food as complete meal replacement

- To fortify a major staple, such as bread, with a nutrient known to be in short supply.
- For the preparation of functional foods (nutraceuticals) containing vitamins that are shown to be useful in preventing chronic diseases.

Vitamins are typically divided into two groups: fat soluble and water soluble. Fat-soluble vitamins are usually measured in international units (IUs) and consist of vitamins A, D, E, and K. The water-soluble group, usually measured in units of weight, consists of vitamin C (ascorbic acid) as well as the B vitamins. Humans need eight nutritive B-complex vitamins: niacin, riboflavin, panthotenic acid, pyridoxine, folic acid, thiamin, biotin, and vitamin B_{12}. Table 83.12 outlines synonyms for and the most commonly marketed forms of the major vitamins consumed in the United States as food additives.

In 1993 the FDA introduced reference daily intakes (RDI) [formerly recommended daily allowance (RDA)] for the labeling of foods and vitamin supplements. The RDI was designed to spell out the nutritional requirements of an average American. Those with greater than average needs (young woman, the elderly, and cigarette smokers, for example) are responsible for knowing their additional requirements and supplementing their diet, RDI values for vitamins established by FDA regulations are listed in Table 83.12.

Vitamin A is generally added to margarine and milk. Much of the vitamin A content of milk is obtained by feeding cows supplements of the vitamin. In addition, vitamin A is frequently added to instant breakfast foods, granola bars, and quick preparation or energy bar food products to better position those foods as complete meal replacements.

TABLE 83.12
Vitamins Consumed as Food Additives and U.S. RDI Values

Vitamin	Principal Synonyms	Major Market Form	U.S. RDI Value
Vitamin A			5000 IU
A_1	Retinol	Vitamin A acetate Vitamin A palmitate	
A_2	Dehydroretinol		
Vitamin B			
Niacin	Vitamin B_3	Nicotinic acid	20 mg
Thiamin	Vitamin B_1	Thiamin hydrochloride	1.5 mg
Riboflavin	Vitamin B_2	Riboflavin	1.7 mg
Pantothenic acid	Vitamin B_5	Calcium pantothenate	10 mg
Pyridoxine	Vitamin B_6	Pyridoxine hydrochloride	2 mg
Cyanocobalamin	Vitamin B_{12}		6 µg
Folic acid	Vitamin B_c	Folate	0.4 mg
Biotin			0.3 mg
Vitamin C	Ascorbic acid	Ascorbic acid Sodium ascorbate Calcium ascorbate	60 mg
Vitamin D			400 IU
D_2	Ergocalciferol		
D_3	Cholecalciferol		
Vitamin E	Tocopherols	DL-alpha tocopherol acetate D-alpha tocopherol D-alpha tocopheryl acid succinate	30 IU
Vitamin K			
K_1	Phytonadione	Phylloquinone	65 µg
K_3	Menadione		

Most natural vitamin A is derived from fish oils or carotenoid pigments found in chlorophyll-containing plants. These carotenoid pigments are the source of several provitamins, of which alpha- and beta-carotene are the most important. Important commercial forms include beta-carotene, retinol, retinol acetate, and retinol palmitate. Practically all the vitamin A used today is obtained by synthesis from the chemical intermediate, beta-ionone.

Thiamin (vitamin B_1) is found in all plants, but cereal grains, milk, legumes, nuts, eggs, and pork contain large amounts. Thiamin is essential for the proper functioning of the central nervous system. Important commercial products include thiamin hydrochloride and thiamin mononitrate. Thiamin is obtained synthetically by several different routes, including linking chlorate-thylpyrimidine with 4-methyl-5-(hydroxy-ethyl)thiazole. Another method is the conversion of 4-amino-5-cyano-pyrimidine into a thioformylaminomethyl derivative via catalytic hydrogenation and reaction with sodium dithioformate.

Riboflavin (vitamin B_2) occurs in plant and animal cells. Important dietary sources include organ meats, yeast, and dairy products. Riboflavin is produced synthetically from D-ribose and fermentation processes.

Pantothenic acid (vitamin B_3) occurs in all animals and plants and in some microorganisms. Natural sources of pantothenic acid include liver, eggs, broccoli, cauliflower, tomatoes, and molasses. Commercially available forms include the liquid D-pantothenyl alcohol (panthenol), as well as calcium D-pantothenate and racemic calcium pantothenate. It is produced commercially by condensation of D-pantolactone with beta-alanine.

Niacin (vitamin B_4) is a generic term that includes both niacin (nicotinic acid) and niacinamide (nicotinamide). Poultry, meats, and fish are the most important sources of niacin. Both niacin and niacinamide are important commercial forms. Niacin is produced synthetically by the oxidation of quinoline, or 2-methyl-5-ethyl-pyridine. Niacinamide is produced by amidation of niacin.

Pyridoxine (vitamin B_6) refers to naturally occurring pyridine derivatives that have vitamin B_6 activity. Most forms of the vitamin occur in plant and animals, but chemical synthesis is a far more efficient and economical method of production than natural isolation. Pyridoxine hydrochloride is produced by the condensation of ethoxyacetylacetone with cyanoacetamide.

Cyanocobalamin (vitamin B_{12}) is found in dairy and meat products. Cyanocobalamin and hydroxocobalamin are the important commercial forms, produced by fermentation using either *Streptomycetes griseus* or *S. aureofaciens.* Vitamin B_{12} is essential for bone marrow cells, the nervous system, and the gastrointestinal tract, as well as for normal blood function.

Folic acid, a member of the vitamin B complex, is a yellow-orange crystalline powder found in brewer's yeast, wheat, nuts, legumes, and liver tissues. Folic acid and the calcium salt of folic acid can be obtained synthetically by a number of routes from triaminohydroxypyridine and para-aminobenzoylglutamic acid. Folic acid functions as a coenzyme in the synthesis of nucleic acid, purine-pyrimidine metabolism, and other systems.

Recently folic acid gained importance because of its role in reducing the chances of neural tube birth defects and its role of controlling homocysteine, a risk factor in atherosclerosis. Medical studies indicate that folic acid and pyridoxine (vitamin B_6) can reduce high levels of homocysteine, an amino acid, in the blood. A high level of blood homocysteine was found to be an independent factor from cholesterol leading to increased risk of heart attack and stroke. Therefore folic acid and possibly vitamin B_6 use in nutraceuticals and other fortified food products (e.g., breakfast cereals, cereal bars, calorie control and fitness food products, etc.) will increase substantially in the next few years.

Vitamin C (ascorbic acid) is the most important vitamins used as a food additive in terms of volume. Primary applications include fruit juices, still beverages, juice-added

sodas, and dry cocktail or beverage powder mixes. Ascorbic acid is also used as a food preservative. Fruit juice makers, in particular, are applying the vitamin to preserve and protect against color change in fruit ingredients. By doing so, they can also promote the high vitamin content of juices.

Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are produced synthetically by the irradiation of the provitamins ergosterol and 7-dehydrocholesterol, respectively. Vitamin D_3 can also be isolated from fish liver oils. Although vitamins D_2 and D_3 are both important commercial products, most of the vitamin D is synthesized by the photochemical conversion of 7-dehydrocholesterol to D_3.

Vitamin E is found widely throughout nature, but the main dietary sources include vegetable fats and oils, dairy products and meat, eggs, cereals, nuts, and leafy green and yellow vegetables. The role of tocopherol as an antioxidant that can reverse the damaging effects of free radicals and therefore prevent certain chronic diseases has received increasingly wide attention in recent years. Naturally occurring vitamin E can be obtained from vegetable oil sources by distillation, and recently two major vegetable oil processors initiated the commercial production of natural tocopherols. However, large quantities of tocopherols are synthetically derived.

Vitamin K, found in green leafy vegetables, tomatoes, cauliflower, egg yolks, soybean oil, and liver, is essential for the formation of prothrombin and other blood-clotting factors in the liver. Menadione and its sodium bisulfite and diphosphoric acid ester derivatives are the most common commercial forms of the vitamin K group of compounds. Menadione (vitamin K_3) is produced synthetically by treating 2-methylnaphthalene with chromic acid in the presence of sulfuric acid.

G. Antioxidants

Antioxidants are food additives that retard atmospheric oxidation and its degrading effects, thus extending the shelf life of foods. Examples of food oxidative degradation include products that contain fats and oils in which the oxidation would produce objectionable rancid odors and flavors, some of which might even be harmful. Antioxidants are also used to scavenge oxygen and prevent color, flavor, and nutrient deterioration of cut or bruised fruits and vegetables.

Recently, definitive studies have shown and been widely publicized in the news media that antioxidant nutrients such as ascorbic acid (vitamin C) and tocopherols (vitamin E) can protect against harmful cell damage and thus prevent certain human diseases. Foods formulated with antioxidants and other vitamins are now recommended to prevent and cure cancer, cardiovascular diseases, and cataracts. The same antioxidants that are used to prevent oxidative deterioration of food may be used in functional foods (nutraceuticals, designer foods, etc.) to create products that prevent or cure certain chronic diseases. In this section, however, only the food preservation function of antioxidants will be discussed.

To improve the performance of antioxidants, two other types of food additives, sequestrants (e.g., EDTA, citric acid) and synergists (e.g., mixtures of antioxidants and lecithin), are frequently used with them. Antioxidants may also be present in food packaging as indirect food additives, but such use is not covered in this chapter.

Food antioxidants can be divided into water-soluble and oil-soluble compounds and also classified as natural or synthetic, as shown in Table 83.13. The most frequently used natural antioxidants are ascorbic acid (vitamin C), its stereo isomer erythorbic acid, and their sodium salts, plus the mixed delta and gamma tocopherols. While ascorbic acid finds its major use as a nutritive supplement or in pharmaceutical preparations, smaller amounts are intentionally used for antioxidant purposes. Erythorbic acid (isoascorbic acid) is virtually devoid of vitamin C activity (only 5% that of ascorbic acid). Citric acid and tartaric acid are also natural antioxidants (and antioxidant synergists), but are predominantly added to foods as acidulants.

Synthetic antioxidants used as direct food antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallate (PG). These antioxidants are effective in very low concentrations (0.01% or less in animal fact) and not only retard rancidity but also protect the nutritional value of the food by minimizing the breakdown of vitamins and essential fatty acids. At one time, safety

TABLE 83.13
Food Antioxidants and Their Manufacturing Processes

Antioxidant Compound	Manufactured By
Oil-soluble products	
Butylated hydroxyanisole (BHA)*	Synthesis
Butylated hydroxytoluene (BHT)*	Synthesis
Tert-butyl-hydroquinone (TBHQ)*	Synthesis
Propyl gallate (PG)*	Synthesis
Tocopherols*	Extraction or synthesis
Thiodipropionic acid	Synthesis
Dilauryl thiodipropionate	Synthesis
Ascorbyl palmitate	Synthesis
Ethoxyquin	Synthesis
Water-soluble products	
Ascorbic acid*	Fermentation or synthesis
Sodium ascorbate*	Fermentation or synthesis
Erythorbic acid*	Fermentation or synthesis
Sodium erythorbate*	Fermentation or synthesis
Glucose oxidase/catalase enzymes	Fermentation
Gum guaiac	Extraction
Sulfites*	Synthesis
Rosemary extract	Extraction

* Major products.

questions were raised about several synthetic antioxidants. These have largely been resolved, although the impact on the market for synthetic antioxidants still exists.

The major applications of antioxidants in foods are listed in Table 83.14. The fats and oils industry and the snack/fast food/convenience food industries are the major users of food antioxidants.

While the growth of fat-containing foods is declining because of consumer concerns related to the adverse effects of fat and high caloric intake to health, the increasing preference for "healthier" unsaturated fats will increase demands for oil-soluble antioxidants because these fats require more protection against rancidity.

Butylated Hydroxyanisole (BHA) was introduced commercially in foods in 1948. The major applications for BHA are in frying fats and oils, salad oils, and shortenings. BHA can be used in blends with BHT, TBHQ, or PG to optimize performance. For example, blends of BHA and propyl PG are used as stabilizers in edible lard and tallow.

BHA is approved by the FDA as a GRAS substance, but the use is limited to a maximum 0.02% of the total fat and oil content of the product. Past developments have had a detrimental effect on the demand for BHA. In 1982, the Japanese government reported that, according to feeding studies done in Japan with BHA at 2% of the entire diet, BHA was found to be carcinogenic. Consequently BHA would not be allowed in food products sold in Japan after July 1982. Various governments, including the United States, Canada, and the United Kingdom, requested a delay in the implementation date until further studies could be done. The date was then deferred to February 1, 1983, and the ban was never implemented. Subsequently the World Health Organization's Food and Agriculture Organization studies showed that BHA dosage levels would have to be high (e.g., about 2% of the oil or fat content of the food) before any carcinogenic effects would become apparent. (The normal BHA content level is 200 ppm of the fat or oil content of the food). However, because of Japan's announcement of its initial study, BHA was removed from some of the food and food packaging sold in the United States and Japan. The findings of the Japanese study relative to BHA were surprising since several other studies conducted worldwide had found BHA to be noncarcinogenic. The original Japanese researcher has now agreed that BHA is not carcinogenic; however, irreparable damage to BHA as a food antioxidant has occurred, and the product's unhealthy image is unlikely to be reversed in the future.

Butylated hydroxytoluene (BHT) was approved for use as a food antioxidant in 1954. BHT is often used in blends with BHA or BHA/PG in vegetable oils and in edible animal fats to take advantage of the synergism obtained. Although BHT was never removed from the FDA's GRAS list, demand for BHT as a direct food additive declined throughout the 1980s. That proposal, as well as the general trend toward the use of all-natural ingredients in foods, has negatively impacted BHT use in foods.

Producers of both BHA and BHT have petitions filed with the FDA to recognize the existence of "prior sanctions" for the use of the chemicals as food antioxidants at levels not to exceed 0.02%. Such recognition would eliminate the necessity of classifying the chemicals as food additives.

Tertiary-butyl hydroquinone (TBHQ) is related to BHA and has good heat stability. It was first introduced for food applications in 1972. TBHQ shows exceptional ability in protecting unsaturated vegetable oils and animal fats from rancidity. One of its largest applications is in soybean oils. Although mostly used by itself, TBHQ can be used in combination with BHT and BHA. TBHQ is often used as a replacement product for PG.

Propyl gallate (PG) has been used as a food antioxidant since the 1950s. Its current primary use is more as a synergist in combination with BHA and BHT. The active part gallic acid, can be extracted from natural sources and can be synthesized. Propyl gallate is effective in vegetable oils as well as animal fats, but it is not heat stable, even at cooking temperatures. Total consumption of PG is very small because of its relatively high price and competition from TBHQ.

Ascorbic acid (vitamin C) and sodium ascorbate are widely used as natural antioxidants and vitamin supplements. As an antioxidant, ascorbic acid is used primarily in prepared foods (canned fruits and vegetables, juice drinks, applesauce, potatoes) and in processed meats (sausages). Manufacturers use it if for its protective function in soft drinks, for example, but declare it as "added vitamin C." Ascorbic acid is insoluble in fats and oils, and its almitoyl

TABLE 83.14
Food Applications for Antioxidants

Oil-Soluble Antioxidant Applications (to Retard the Onset of Rancidity)	Water-Soluble Antioxidant Applications (to Prevent Oxidative Deterioration of Color, Flavor, and Nutrients)
Edible fats	Fresh fruits and vegetables
Vegetable oils	Dried fruits
Nuts	Fruit juices and drinks
Shortenings	Frozen fruits and vegetables
Margarine	Frozen entrees
Salad oils	Confectionery products
Fast food frying oils	Flavoring compositions
Bakery products	Frozen potatoes
Processed meat	Meat and poultry spreads
Cheeses	Processed cheese
Processed chicken	Thermally processed fruits in nonmetallic containers
Canned meat and poultry	
Pancake/cake mixes	
Breakfast cereals	
Dehydrated potatoes	
Chewing gums	

ester is synthesized to impart some lipid solubility. Used alone, the ester is not very effective in fats and oils, and it is normally used in combination with tocopherol.

Erythorbic acid (iso-ascorbic acid) and sodium erythorbate are used primarily as antioxidants in cured meats (e.g., bacon, sausages) and by salad bars as an oxygen scavenger. They are also used in frozen fruits, vegetable fats and oils, and frozen fish and seafood. Erythorbic acid (and its salts) has benefited significantly from the FDA's ban on the use of sulfites for fresh or uncooked vegetables in salads. Approximately 80% of the total U.S. consumption is estimated to be in the form of sodium erythorbate. The greatest use is in cured meat to minimize the formation of nitrosamines during the curing or cooking process. USDA regulations governing the maximum level of nitrite permitted for curing bacon require the use of 500 ppm of ascorbates or erythorbates. Other food uses of erythorbates are in fresh cut meat, frozen fruits and vegetables, and raw fresh cut vegetables. In Europe, erythorbic acid use has been permitted since 1995.

Tocopherols are the fastest growing antioxidants used in the United States. Because the United States trades heavily with Japan, where synthetic antioxidants are banned, U.S. food exporters are reformulating products using natural antioxidants. Mixed tocopherols appear to be the product of choice.

Although the isomeric forms (alpha, gamma, and delta) of tocopherol show antioxidant activity, the 80% gamma/20% delta mixture of natural tocopherols has the best antioxidant activity. Mixed natural tocopherol products can be used to protect a variety of food products, including dehydrated and processed vegetables, pasta and noodles, animal fats, salad dressings and oils, snacks, meats, and baked foods.

Residues from vegetable refining contain a small but significant level of tocopherols. Using techniques such as molecular distillation, these can be concentrated to give a brown oily product with good antioxidant properties. The composition varies with the origin (type of vegetable oil), and both gamma-rich and delta-rich versions are used.

Sulfites serve multiple functions in foods: (1) inhibition of enzymatic and nonenzymatic browning, and (2) control of microbial growth. For years, sulfur dioxide and sulfite salts have been widely used to help preserve the color of dried fruits and vegetables. Sulfites are used in wine making and the wet milling of corn to prevent the browning of fresh produce with the use of sulfites. But because of the allergic reactions of some consumers (especially asthmatics) to sulfites, regulations were issued and alternatives sought. In July 1986, six sulfiting agents — sulfur dioxide, sodium sulfite, sodium bisulfite, potassium bisulfite, sodium metabisulfite, and potassium metabisulfite — were banned by the FDA for use in raw vegetables and fruits on salad bars. In July 1987, the FDA ruled that all packaging foods containing 10 ppm or more of sulfur dioxide equivalents must disclose on the label that sulfiting agents are present. In 1990, the use of sulfites on fresh potatoes was banned.

Treatment of fruits and vegetables with sulfites is the most effective means available today to control browning. However, because sulfites have been banned in certain food categories and their regulatory status in other categories is in question, alternative treatments to retard enzymatic browning and other oxidative reactions have been investigated. To date, however, alternatives to sulfites are not equivalent to sulfites in their effectiveness, cost, or functionality.

The promising antioxidant/preservative alternatives generally contain ascorbic acid or erythorbic acid in combination with one or more adjuncts, such as citric acid or some other acidulant, a calcium salt, a phosphate, sodium chloride, cysteine, or a preservative such as potassium benzoate or sorbate. The ascorbic acid derivatives, ascorbic acid 2-phosphate and ascorbic acid-6-fatty acid esters, are also reportedly effective. Another suggested substitute (which functions in water but not with fats and oils) is the sequesterant and chelating agent ethylenediaminetetraacetic acid (EDTA), which has been widely used in processed potatoes, salad dressings, sauces, and beverages.

Cyclodextrin is another sulfite alternative that can be used to prevent browning. Finding a good substitute for sulfites, however, has not yet been realized. This is because sulfites not only act as antioxidants to prevent browning, but also perform preservative functions in preventing unwanted microbial spoilage. The above chemicals are ineffective against microbes.

Ethoxyquin is included in the FDA regulation but limited to specific applications only. It is cleared for retarding oxidation of carotene, xanthophylls, and vitamins A and E in animal feed and fish food, and as an aid in preventing the development of organic peroxides in canned pet food.

Gum guaiac is an approved antioxidant for natural flavoring substances and other natural substances used in conjunction with flavors. It is also approved for addition to animal feed and food-packaging materials.

H. Preservatives

Preservatives (antimicrobial agents) are capable of retarding or preventing the growth of microorganisms such as yeast, bacteria, molds, or fungi and subsequent spoilage of food. The principal mechanisms are reduced water availability and increased acidity. Sometimes these additives also preserve other important food characteristics, such as flavor, color, texture, and nutritional value. Important food preservatives used include sorbic acid and its potassium salt, calcium and sodium propionates, sodium and potassium benzoates, and parabens.

Sulfur dioxide and sulfites are also used extensively for controlling undesirable microorganisms in soft drinks, juices, wine, beer, and other products. Salt, organic acids,

TABLE 83.15
Major Uses of Preservatives by Use Sector

Preservative	Application
Sorbates	Mold and yeast inhibition in processed cheese and spreads, other low-acid foods, and dried fruits. Effective in the acidic pH range up to pH 6.5
Benzoates	Antibacterial use in beverages, fruit juice, pickles. Effective in pH range 2.5–4.0
Propionates	Mold and rope inhibitors in bread and baked goods
Parabens	Antibacterial for use in low-acid foods (pH greater than 5.0) such as meat and poultry products

sugar, alcohol, spices, essential oils, and herbs also inhibit the growth of microorganisms, but usually their primary function is different when added to food.

Chemical preservatives play a very important role in the food industry, from manufacture through distribution to the ultimate consumer. The choice of a preservative takes into consideration the product to be preserved, the type of spoilage organism endemic to it, the pH of the product, the shelf life, and the ease of application. No one preservative can be used in every product to control all organisms, and therefore combinations are often used. In certain foods, specific preservatives have very little competition. In the concentrations used in practice, none of the preservatives discussed here is lethal to microorganisms in foods. Rather, their action is inhibitory. Major uses for preservatives by food industry sector are listed in Table 83.15.

In general, increased demand by consumers for lightly processed, lightly prepared foods (as people tend to do less cooking at home but at the same time are looking for products that are fresh, such as prepared salads) has stimulated use of antimicrobial preservatives over the past several years. At the same time, however, media and consumer reaction to chemical preservatives has stymied or limited the growth of several preservatives in favor of "all natural" and "no preservatives added" food products. However, significant displacement of traditional preservatives with naturally derived new products is not expected in the near future.

Important areas for preservatives are in fruit beverages and convenience foods. For example, low fat/low calorie salad dressings require a preservative, while the traditional high oil-containing products had lower water activity and therefore an acceptable shelf life without chemical preservatives.

Potassium sorbate and sorbic acid are used as preservatives in a great variety of foods and can be used as direct additives, as sprays or dip baths, and as coatings on wrapping materials, inhibiting yeasts, molds, and bacteria. Potassium sorbate is used where high water solubility is desired. Because sorbates have no effect on the microorganisms that produce lactic acid, they can be utilized to prevent yeast and mold spoilage of foods, such as most cultured dairy products and pickles, without interfering with the desired bacterial cure. Potassium sorbate solutions may also be used for spray and dip bath applications on cheese, dried fruits, smoked fish, and similar products.

The effectiveness of potassium sorbate is based on its ability to depress fatty acid metabolism in the microorganisms. Use of sorbic acid is limited because of its low solubility in water. Therefore potassium sorbate is the primary form used in foods. It is effective against microbes at pH 6.5 or less. As the pH decreases, the antimicrobial activity of this preservative increases. On an equal weight basis, potassium sorbate has 74% of the activity of sorbic acid.

Sorbic acid and potassium sorbate are GRAS additives. Normal use levels are in the range of 0.05–0.01%. Sorbates are used in cheeses, baked goods, spreads, margarine, dried fruits, jams, and jellies.

Because of its corrosive nature, propionic acid, a liquid, is rarely used in the food industry. Its sodium and calcium salts are used in its place, yielding the free acid within the food at low pH. They are highly effective mold inhibitors, but have essentially no effect against yeast. They have negligible activity against bacteria, except for their effectiveness against the rope-causing *Bacillus mesentericus.*

Propionic acid occurs naturally in Swiss cheeses at levels as high as 1%. Its calcium salt, and to a lesser extent its sodium salt, have been used for more than 30 years as an inhibitor of mold growth in bread. The main market for propionate salts is in bakery products, chiefly because these salts do not inhibit yeast action (they also have almost no activity against bacteria).

The propionates have GRAS status for use in foods and have no upper limits imposed except for breads, rolls, and cheese, which come under the Standards of Identity. They can be used up to 0.3% in cheese products and to 0.32% by weight of the flour in white bread and rolls.

Benzoic acid is one of the oldest chemical preservatives used in foods, having been described as a preservative in the 1800s. It has been used in foods since the early 1900s. Benzoic acid occurs naturally in some fruits and spices, such as cranberries, prunes, cinnamon, and cloves. Sodium or potassium benzoates are most effective in the pH range of 2.5–4.0. Benzoates have activity against yeasts, molds, and bacteria. However, benzoates are not recommended for bacterial control because their antimicrobial activity is poor above pH 4, where bacteria are the greatest problem. As benzoates are very efficient in controlling yeasts, they cannot be used in dough or in other yeast-raised bakery products.

The most important uses for benzoates are in fruit juices and carbonated beverages, jams and jellies, and condiments. In carbonated drinks, 0.03–0.05% is used; in noncarbonated drinks, up to 0.1% is used. Benzoates are also used for fats and oils, gravies, frostings, puddings, and gelatins. Potassium benzoate became in many of the above food products. It is also useful in margarine, potato salad,

fresh fruit cocktails, and pickles. Although the amount of sodium added with the benzoate salt is nutritionally insignificant, the potassium salt was developed specifically for use in reduced or low-sodium food products to avoid sodium declaration on the label.

The potassium or sodium salts of benzoic acid are more soluble in water than is benzoic acid and consequently are preferred for use in many food products. They do not destroy yeasts or molds but instead retard further growth of organisms already present, provided the degree of contamination is not too high. Benzoic acid and benzoates are GRAS substances and are permitted for use in foods up to a maximum of 0.1% concentration.

Parabens are esters of para-hydroxybenzoic acid. A combination of methyl and propyl esters and sodium benzoate is most often used, but the ethyl and butyl esters also have utility. Parabens are the only phenols approved for microbiological preservation of foods. Parabens are effective against molds and yeasts and are relatively ineffective against bacteria, especially the gram-negative bacteria. Their antimicrobial activity extends up to pH 7.0, making the parabens the only antimicrobial agents effective at higher pH values.

The methyl and propyl parabens are GRAS ingredients, but their use is limited to 0.1% (combined). *n*-Heptyl paraben is permitted in beer at a minimum concentration of 12 ppm. Parabens are used in baked goods, beverages, fruits, jams and jellies, and olives and pickles, but not in dairy products. Because parabens are the most expensive of the available preservatives and have some technical problems associated with their use in foods, use by the food industry remains limited.

About 10 years ago, sodium nitrite and sodium nitrate were used in curing bacon and other meats to prevent the growth of bacteria that cause botulism. The nitrate and nitrite were linked to the formation of nitrosamines in the meat, which were considered carcinogenic in experimental animals. Though the public outcry has largely subsided and nitrites continue to be used in smaller amounts, the continued use of these preservatives probably stems from the absence of suitable alternatives. Sodium ascorbate and sodium erythorbate are effective catalysts in the curing process, and the addition of one of these antioxidants to bacon makes it possible to reduce the quantity of sodium nitrite used.

Most chemical preservatives in use today have specialized uses and established niche markets in the food industry. A great amount of interchangeability does not exist because of specific inhibitory actions toward bacteria, molds, or yeasts. Blends of antioxidants and preservatives (some natural and some synthetic chemicals) can provide multiple functions for multiple food products. One such combination of ingredients is a blend of erythorbic acid, citric acid, and potassium sorbate as an antioxidant and antimicrobial substitute for sulfites on fresh vegetables.

I. EMULSIFIERS

Emulsifiers are additives that allow normally immiscible liquids, such as oil and water, to form a stable mixture. They are widely used in foods in order to achieve the texture, taste, appearance, fat reduction, and shelf life desired in foods. Bread and bakery products is the largest food segment utilizing emulsifiers. In this application, they soften the bread and strengthen the dough by distributing the fat within the product so less fat (shortening) needs to be added. Emulsifiers are utilized as fat-sparing agents in salad dressings and bakery and dairy products. Visible fats and oils routinely need emulsifiers for food-product processing, appearance, maintenance of shelf life, texture, and taste uniformity. They are also included in low-fat formulations (e.g., frozen desserts, bakery products), often more so than in formulations with normal fat levels. In addition, food emulsifiers are widely used in convenience, snack, and microwaveable food products. The multiple applications and functions of food emulsifiers are shown in Table 83.16, and several of the more prominent food uses of emulsifiers are shown in the following listing:

Breads
Frozen desserts
Icings
Cream fillings
Chocolate milk
Whipped toppings
Coffee creamers
Instant breakfasts
Infant formula
Dessert mixes
Rolls
Cake mixes
Fresh cakes
Donuts
Cereals
Food coatings
Instant potatoes
Pastas
Snack foods
Ice cream
Dips
Shortenings
Margarine and diet spreads
Peanut butter
Candy
Caramels
Chewing gum base
Chocolate
Toffees

The most common and commercially important emulsifiers are monoglycerides and diglycerides of fatty acids and their esters (e.g., glyceryl monostearate), lactylated esters (e.g., sodium stearoyl lactylate), propylene glycol mono- and diesters (e.g., propylene glycol monostearate), lecithin, sorbitan esters (e.g., sorbitan monostearate), and polysorbates (e.g., polyoxyethylene 80 sorbitan monolaurate). With the exception of lecithin, few emulsifiers are used as a single additive. Most food emulsifiers are used as blends of emulsifiers, water, fats, and other classes of food additives such as gums. These products are formulated for specific applications (or specific customers) so that the combination provides both enhanced performance and ease of use. Emulsifiers are regulated as food additives in most countries. The FDA classifies lecithin, monoglycerides and diglycerides, diacetyl tartaric acid ester (DATEM), and triethyl citrate as GRAS substances.

TABLE 83.16
Functions of Emulsifiers

Function	Typical Application	Process
Emulsifying	Margarine, creamy salad dressing, coffee whiteners, frozen desserts	Disperses small droplets of immiscible substances
Antistaling	Most baked goods	Complexing action on starch reduces firming of crumbs
Modifying texture	Bread, cakes, macaroni	Complexing action on starch reduces clumping, improves consistency and uniformity
Wetting	Coffee whiteners, drink mixes, instant breakfasts	Reduces interfacial tension between liquid and solid
Solubilizing	Color and flavor systems	Improves solubility
Crystal modification	Shortenings, margarine, peanut butter	Modifies mode and rate of crystal formation
Preventing agglomeration	Ice cream, frozen desserts	Controls coagulation of fat particles
Foaming	Whipped toppings, icings, cakes, convenience desserts, ice cream	Controls dispersion of a gas in a liquid
Defoaming	Processing of syrups, yeast	Breaks emulsions
Reducing tackiness	Candies, chewing gums	Assures texture
Fat sprang	Baked products, frozen desserts, whipped toppings, margarine, spreads, imitation sour cream	Reduces size of fat globules, resulting a wider dispersion and reduced fat levels
Improving palatability	Icings, confectionary coatings	Improves mouth feel

The other emulsifiers have specific regulations that permit their use in specific products at set levels.

Monoglycerides and diglycerides are used in the largest amounts (more than 50% of the total volume for emulsifiers), mainly because of their low cost. Important applications are in the preparation of shortenings, in bread and other bakery products, and in ice cream.

Lecithin. Commercial lecithin is usually a by-product from the refining of crude soybean oil. The term lecithin describes a complex mixture of phospholipids, triglycerides, fatty acids, and other components that occur naturally in soybean oil. The major phospholipids of lecithin are phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidic acid (PA). The unique structure of these phospholipids and other minor constituents gives lecithin its emulsification properties. Lecithin is often modified to improve its effectiveness as an emulsifier. The common modified lecithins that are commercially available are the hydroxylated, acetylated, and enzyme-modified lecithins. The result is more hydrophilic, water-dispersible lecithin with enhanced oil-in-water emulsion properties. The primary applications for lecithin are in baked products, dairy blends, baby foods, nutritional drinks, margarines, chocolates, chewing gums, and confectionaries.

Polysorbates are a group of emulsifiers that contain sorbitans, various types and amounts of fatty acids, and polyoxyethylene chains. Heating sorbitol with stearic acid in the presence of a catalyst cyclizes sorbitol and forms an ester to produce sorbitan monostearate and tristearate. Other sorbitan esters of importance are monooleate and tristearate. Any of the three esters may be reacted with ethylene oxide to give polyoxyethylene derivatives, which are much more hydrophilic than sorbitan esters. The monostearate derivative is known as polysorbate 60, the tristearate is polysorbate 65, and the monooleate is polysorbate 80. Polysorbates and sorbitol esters are used chiefly in ice cream, imitation dairy products, and in baking applications.

Polyglycerol esters contain polymerized glycerol and various types and amounts of fatty acids. The polyglycerol portion is synthesized by heating glycerol in the presence of an alkaline catalyst. The polyglycerol backbone is then esterified either by direct reaction with a fatty acid or by interesterification with triglyceride fat.

Sucrose esters are manufactured by adding fatty acids to a sucrose molecule. Sucrose has eight free hydroxyl groups that are potential sites for esterification with fatty acids. Derivatives containing one to three fatty acid esters are emulsifiers and are approved for food use.

There are a large number of other emulsifiers used in the food industry, but their volumes are negligible. Examples include lactylated esters, used in direct baking (not the shortening) and in imitation dairy products, and propylene glycol esters, used in various prepared mixes, shortening, and baking.

J. Flavors

Flavors consist of concentrated preparations, with or without solvents and carriers, used to impart a specific taste to food. Flavor ingredients are the largest single group of direct food additives utilized by the food industry. They also represent the highest value among the food additives segments. Flavoring substances are classified as

- Natural flavoring substance — obtained by physical separation, enzymatic processes, or microbial processes from vegetable or animal sources, either in the raw state or after processing (including drying, torrefaction, and fermentation).

- Nature-identical flavoring substance — obtained by synthesis or isolated by chemical processes and chemically identical to substances naturally present in the vegetable or animal sources (this classification is used in Europe but not allowed in the United States).
- Artificial flavoring substance — obtained by chemical synthesis and not found in nature.
- Flavoring preparation — products other than natural substances, whether concentrated or not, with flavoring properties, obtained by physical separation or enzymatic or microbial processes from material of vegetable or animal origin, either in the raw state or after processing (including drying, torrefaction, and fermentation).
- Process flavorings — products obtained by heating to a temperature not exceeding 180°C for a period not exceeding 15 minutes using a mixture of ingredients, not necessarily having flavoring properties themselves, of which at least one contains nitrogen (amino) and another is a reducing sugar.
- Smoke flavorings — smoke extracts used in traditional foodstuff smoking processes.
- Flavor enhancers — some amino acids and nucleotides, as well as sodium salts (such as monosodium glutamate, sodium inositate, and sodium guanylate), have only a weak taste by themselves but have the power to considerably enhance the taste sensation caused by other ingredients in savory flavors.

The flavor industry is not a single homogeneous entity, but a composite of closely interrelated and somewhat overlapping sectors including essential oils and natural extracts, aroma chemicals, and compounded flavors. The first two sectors provide the raw materials used for compounding flavors.

Essential oils are usually defined as the volatile aromatic material obtained from botanical or animal sources by the process of distillation, expression, solvent extraction, or maceration. The most common physical process used for removal of essential oils is steam or water distillation.

The term "extract" refers to a material that has been removed from a plant by a solvent, after which the solvent is evaporated to concentrate the oil. Absolutes, which are alcohol-soluble liquids, and concentrates, which are usually waxy solids, are both extracts.

Oleoresins are thick, viscous products obtained by extraction of plant material with a nonaqueous solvent (e.g., hydrocarbon) that is subsequently removed. Extracts of vanilla beans and other fruit extracts are the most important product examples of this class.

Essential oils and natural extracts represent complex aroma mixtures containing hundreds of chemical constituents. They may be used for imparting scent or aroma to consumer products or may be used as raw materials for compounding flavor and fragrance compositions, or they may be the source of isolated aroma chemicals, also used in compounding. Essential oils can be classified into three chemical groups: straight hydrocarbons, oxygenated compounds, and benzene derivatives.

Aroma chemicals comprise organic compounds with a defined chemical structure that are isolated from microbial fermentation, plant or animal sources, or produced by organic synthesis. Isolation consists of the physical removal of the flavor compound of interest from a natural source that contains it (e.g., L-menthol isolated from cornmint oil). Isolates may be further chemically modified.

Aroma chemical used to compound flavors are of two types: (1) isolates, which have been physically removed from natural sources that contain them and which may be further chemically modified; and (2) synthetic aroma chemicals that duplicate the structure and aroma characteristics of their counterparts found in nature. Synthetic aroma chemicals that duplicate the structure and aroma characteristics of their counterparts found in nature are known as "nature identical." Those that are not know to occur in nature but display an aroma reminiscent of known natural products with unrelated chemical structure are defined as "artificial." However, the legal definition of natural and artificial varies, depending on each country's legislation. Aroma chemicals are used as raw materials for flavor compositions. While technical merits are not at issue, naturally occurring aroma chemicals may enjoy preferential status for their use in certain countries because of labeling regulations.

More than 80% of aroma chemicals in use contain only carbon, hydrogen, and oxygen in their structure, the large majority being esters, ketones, aldehydes, and alcohols. A few contain nitrogen (nitro and nitrile groups, pyrazines) and/or sulfur (mercaptane, thiazoles). About 4% of the chemicals are unsaturated hydrocarbons, primarily with cyclic and acyclic terpene structures (e.g., limonene, pinenes, etc.). Most of the aroma chemicals are oil-soluble, water-insoluble liquids.

Aroma chemicals of commerce can be broadly classified according to their chemical structure and are grouped into three categories as follows:

1. Benzenoids (including naphtalenoids): chemicals containing a benzene or naphthalene ring, including alcohols, acids, esters, aldehydes, ketones, phenols, phenol esters, and lactones.
2. Terpene and terpenoids: chemicals with (or closely related to) characteristic terpene structures, both acyclic and cyclic, having two or more isoprene (C_5H_8) moieties and oxygenated derivatives of the terpene hydrocarbons, including alcohols, aldehydes, ketones, and esters.

TABLE 83.17
Commercial Flavor Compositions

Type of Flavor	Classification	Manufacturing Process	Raw Materials	Product Form
Compounded flavors	Natural or synthetic	Blending, mixing	Essential oil, natural extracts, fruit juice concentrates, aroma chemical	Liquid, spray-dried, encapsulated
Natural extracts	Natural	Extraction, enzymatic treatment	Food substrates (e.g., plants, fish, meat, etc.)	Liquid, paste
Reaction flavors (thermally processed)	Natural	Heating/pressure cooking	Amino acids and sugars, hydrolyzed proteins	Paste, powder
Enzymatically modified flavors	Natural	Enzymatic/microbial reaction	Food substrates (e.g., cheese)	Paste, powder

3. Other aroma chemicals: includes aliphatic, alicyclic, and heterocyclic compounds and esters of lower fatty acids.

Of the thousands of aroma chemicals included in compounded flavors, the following compounds are used in very large quantities: 3-phenetyl alcohol and esters, vanillin, ethyl vanillin, esters of lower fatty acids, benzyl acetate, alpha-hexyl-cinnamaldehyde, 1-menthol (synthetic), geraniol/nerol and esters, and anethol.

The universally applicable definition of flavor compositions is that of mixtures of aromatic materials that are added to foods and beverages in order to improve palatability. Flavor compositions consist of complex mixtures of various aromatic materials from few to 100 or more constituents. Compounded flavors may contain aroma chemicals, natural extracts, essential oils, solvents, and in some cases other functional additives (e.g., antioxidants, acidulants, emulsifiers, etc.). Certain raw materials that can be used directly as flavors without compounding (e.g., vanilla, peppermint) and those products with a taste of their own, such as sweeteners, acidulants, and salts, are not included in the above definition.

Flavors serve all sectors of the food processing industry, including carbonated and still beverages, processed foods, confectionary, and dairy foods, and are added to foods and beverages for the following reasons:

- To create a totally new taste
- To enhance, extend, round out, or increase the potency of flavors already present
- To supplement or replace flavors to compensate for losses during processing
- To simulate other more expensive flavors or replace unavailable flavors
- To mask less desirable flavors — to cover harsh or undesirable tastes naturally present in some foods

The types of flavor compositions, their manufacturing process and the starting materials for manufacturing them, and their common product form are summarized in Table 83.17.

X. ADVERSE EFFECTS OF FOOD ADDITIVES

The practice of adding chemicals (e.g., salt, spices, herbs, vinegar, and smoke) to food dates back many centuries. In recent years, however, the ubiquitous presence of chemical additives in processed foods has attracted much attention and public concern over the long-term safety of additives to man. Although the safety issue is far from subsiding, there is scientific consensus that food additives are indispensable in the production, processing, and marketing of many food products. Moreover, the judicious use of chemical additives — typically in the range of a few parts per million (ppm) to less than 1% by weight of the finished food — contributes to the abundance, variety, stability, microbiological safety, flavor, and appearance of food. While food additives offer a major contribution to the palatability and appeal of a wide variety of foods, their level of use is relatively insignificant in the total human diet. For the most part, the permitted food additives are safe, highly effective, and have been in continuous use for a long time.

There is much discussion about whether a food additive or food product is natural or synthetic. The fact is that this classification, in many instances, has become somewhat arbitrary. Many food additives synthesized in chemical laboratories are also naturally occurring in normal food. Monosodium glutamate, a flavor-enhancing food additive, is the sodium salt of glutamic acid, an amino acid found in many foods such as mushrooms and tomatoes and metabolized by the human body using the same biochemical pathways of digestion. Synthetic vitamin C (ascorbic acid) and its isomer, erythorbic acid, are the same chemicals that are found in citrus fruits. Similarly, citric acid, which is today produced commercially by enzymatic fermentation of sugars, is the same chemically as the naturally occurring chemical that has been found to make lemons and limes tart.

Much of the worldwide public concern about the use of food additives relates to fears about safety and has generated some sort of regulatory structure in every major country, as well as in international bodies, to monitor this aspect of the field. There is a Joint Experts Committee on Food

Additives, set up by the Food and Agriculture Organization and the World Health Organization, to consider the safety of additives and set specifications and limits for them. These limits take the form of an acceptable daily intake (ADI). The Codex Committee on Food Additives is required to follow the safety guidelines of the Joint Express Committee. Its safety criteria are generally not very different from those used in the United States, although they are not codified. In the United States, criteria for food additives are stated in 21 CFR §170.22, "Safety factors considered," and 21 CFR §170.20, "General principles for evaluating the safety of food additives." The key sentence, which also runs through the decisions in other countries, says, "A food additive for use by man will not be granted a tolerance that will exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals."

It should be remembered, however, that in the United States, these criteria apply only to substances that are legally food or color additives and, by interpreting regulation, to those substances that are GRAS on the basis of "scientific procedures." For those substances that are GRAS because of "experience based on common use in food," there are no rules. Decisions of safety depend on the knowledge and judgment of the "experts qualified by scientific training and experience to evaluate its safety."

One other aspect of the safety question deserves discussion. In the United States only, there is a special provision, known as the Delaney clause, that says "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal." This means that an additive is not to be permitted at any level, no matter how low, if it induces cancer at any level, no matter how high.

The risks posed to the consumer by the food supply are rated in decreasing order of severity as follows:

1. Microbiological hazards (food poisoning from bacteria or bacterial toxins salmonellosis, botulism, etc.).
2. Nutritional hazards (excessive consumption of sodium, saturated fat, etc.).
3. Environmental pollutants (mercury in fish, lead from car exhaust, etc.).
4. Natural toxicants (mushroom poisoning, solanine in potatoes and other solanaceous plants, shellfish toxins, etc.).

TABLE 83.18
Food Additives Prohibited from Use in Human Food

Food Additive	21 CFR Section	Date of Ruling	Functionality
Calamus and its derivatives	189.110	May 9, 1968	Flavoring compound
Cinnamyl anthranilate	189.113	Oct 23, 1985	Flavoring compound
Cobaltous salts and its derivatives	189.120	Aug. 12, 1966	Foam stabilizer
Coumarin	189.130	March 5, 1954	Flavoring compound
Cyclamate and its derivatives	189.135	Oct. 21, 1969	High-intensity sweetener
Diethylpyrocarbonate (DEPC)	189.140	Aug. 2, 1972	Ferment inhibitor in beverages
Dulcin	189.145	Jan. 19, 1950	High-intensity sweetener
Monochloroacetic acid[a]	189.155	Dec. 29, 1941	Preservative in beverages
Nordihydroguaiarectic acid (NDGA)	189.165	Apr. 11, 1968	Antioxidant
P-4000 (5-nitro-2-n-propoxyaniline)	189.175	Jan. 19, 1950	High-intensity sweetener
Safrole	189.180	Dec. 3, 1960	Flavoring compound
Thiourea (thiocarbamide)	189.190		Antimycotic preservative
Chlorofluorocarbon	189.191	Mar. 17, 1978	Propellant
Flectol H (1,2-dihydro-2,2,4-tri-methylquinoline	189.220	Mar. 15, 1977	Food packaging adhesive
Lead solders	189.240	June 27, 1995	Can solder
Mercaptoimidazoline and 2-mercaptoimidazoline	189.250	Nov. 30, 1969	Packaging material
4,4′-methylenebis (2-chloroanaline)	189.280	Dec. 2, 1969	Packaging adhesive and polyurethane resin
Hydrogenated 4,4′-isopropylidene-diphenolphosphite ester resins	189.300	Feb. 17, 1989	Antioxidant and stabilizer in vinyl chloride resins
Tin-coated lead foil capsules for wine bottles	189.301	Feb. 8, 1996	Capsule for wine cork

[a] Permitted in food package adhesives with an accepted migration level up to 10 ppb under §175.105.

5. Pesticide residues (maximum residue levels are enforced in the United States but may exceed federal limits in imported produce).
6. Food additives (documented cases of poisoning due to food additives are rare and were due to noncompliance with federal regulations).

Although the risk to human health from food additives ranks the lowest among food hazards, some potential risks from food additives to exist.

A. Food Additives Banned from Use

In the United States, the FDA prohibited the use of a number of chemicals in foods for human consumption because they either present a risk to public health or have not been shown to be safe by adequate scientific data. Table 83.18 lists the food additives that are presently prohibited from addition to food. Use of any of these substances causes the food to be in violation of FDA regulations.

In the years since 1970, food colors, especially the synthetic dyes, have received tremendous publicity — nearly all of it bad. Color additives for food represent a unique and special category of food additives. They have historically been so considered in legislation and regulation. The current legislation governing the regulation and use of color additives in the United States is the Food, Drug & Cosmetic Act of 1938, as amended by the Color Additives Amendment of 1960. This amendment allowed for the provisional or temporary listing of food colorants, pending completion of scientific studies determining the suitability of these colorants for permanent listing. Pharmacological testing of synthetic "certified" colors was initiated in 1957. Many of the synthetic colors that had been approved for use at some time in the past have been removed from the approved list as a result of new toxicological test results. This has steadily reduced the number of certified dye colors available to the U.S. food industry from more than 22 in 1950 to 7 in 2003. Table 83.19 provides a history of the status of synthetic colorants in the United States. Another eight dyes are permitted in the EU, but are not permitted in foods in the United States.

The EU works on a positive list system using EU Directive no. 95/2/EC, which is the general directive on food additives (other than colors and sweeteners that are covered in separate directives). This law recognizes 106 food additives. If the additive is mentioned in the doctrine then it is allowed, if not it is forbidden. However, the directive includes a list of substances that cannot be used in flavorings (Table 83.20).

TABLE 83.19
Chronological History of Certified Food Colors in the United States

Year Listed for Food Additive	Name of Certified Food Color	Year Delisted
1970	Red no. 1	1961
1907	Red no. 2	1976
1907	Red no. 3	*
1907	Orange no. 1	1956
1907	Yellow no. 1	1959
1907	Green no. 2	1966
1907	Blue no. 2	*
1916	Yellow no. 5	*
1918	Yellow no. 3	1959
1918	Yellow no. 4	1959
1922	Green no. 1	1966
1927	Green no. 3	*
1929	Red no. 4	1976
1929	Yellow no. 6	*
1929	Blue no. 1	*
1939	Yellow no. 2	1969
1929	Orange no. 2	1956
1939	Red no. 32	1956
1950	Violet no. 1	1973
1959	Citrus red no. 2	**
1966	Orange B	**
1971	Red no. 40	*

* Currently permitted.

** Currently permitted with certain restrictions.

B. Industrial Chemicals

Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) are toxic industrial chemicals. Because of their widespread, uncontrolled industrial applications, they have become a persistent and ubiquitous contaminant in the environment. As a result, certain foods, principally those of animal and marine origin, contain PBCs and PBBs as environmental contaminants.

PCBs are transmitted to the food portion (meat, milk, and eggs) of food-producing animals ingesting PCB-contaminated animal feed. In addition, a significant percentage of paper food-packaging materials contain PCBs, which may migrate to the packaged food. Therefore temporary tolerances for residues of PCBs as unavoidable contaminants are established by the FDA (21 CFR §109.15 and §109.30). The temporary tolerances for residues of PCBs are as follows:

- 1.5 ppm in milk (fat basis)
- 1.5 ppm in manufactured dairy products (fat basis)
- 3 ppm in poultry (fat basis)
- 0.3 ppm in eggs
- 0.3 ppm in finished animal feed for food-producing animals
- 2 ppm in animal-feed components of animal origin, including fish meal and other by-products of marine origin and in finished animal feed

TABLE 83.20
European Union List of Food Additives Prohibited for Use in Flavorings (Directive 95/2/EC)

EU Number	Compound
E230	Biphenyl, diphenyl
E231	Orthophenyl phenol
E232	Sodium orthophenyl phenol
E233	Thiabendazole
E234	Nisin
E235	Natamycin
E239	Hexamethylene tetramine
E242	Dimethyl dicarbonate
E249	Potassium nitrite
E250	Sodium nitrite
E251	Sodium nitrate
E252	Potassium nitrate
E280	Propionic acid
E281	Sodium proprionate
E282	Calcium proprionate
E282	Potassium proprionate
E284	Sodium tetraborate (Borax)
E311	Octyl gallate
E312	Dodecyl gallate
E315	Erythorbic acid
E316	Sodium erythorbate
E579	Ferrous gluconate
E620	Glutamic acid
E621	Monosodium glutamate
E622	Monopotassium glutamate
E623	Calcium diglutamate
E624	Monoammonium glutamate
E625	Magnesium diglutamate
E626	Guanylic acid
E627	Disodium guanylate
E628	Dipotassium guanylate
E629	Calcium guanylate
E630	Inosinic acid
E631	Disodium inosinate
E632	Dipotassium inosinate
E633	Calcium inosinate
E634	Calcium 5′-ribonucleotides
E635	Disodium 5′-ribonucleotides
E912	Montan acid esters
E914	Oxidized polyethylene wax
E927b	Caramide
E950	Acesulfame K
E957	Thaumatin
E1105	Lysozyme

concentrates, supplements, and premixes intended for food-producing animals
- 2 ppm in fish and shellfish (edible portion)
- 0.3 ppm in infant and junior foods
- 10 ppm in paper food-packaging material intended for use with human food or finished animal feed

TABLE 83.21
Most Frequent Causes of Food Allergy

Cereals containing gluten (wheat, rye, barley, oats)
Crustacea and products
Eggs and egg products
Fish and fish products
Peanuts and products
Milk and milk products
Tree nuts and nut products
Soybeans and products

C. Food Allergies and other Adverse Reactions to Food Additives

Food allergies and other food sensitivities are individualistic adverse reactions to foods. These food-related illnesses are individualistic because they affect only a few people in the populations; most consumers can eat the same food with no ill effects. Many different types of reactions are involved in these individualistic adverse reactions to foods. Adverse food reactions can include IgE (immunoglobulin E) and non-IgE-mediated primary immunological sensitivities, non-immunological food intolerances, and secondary sensitivities. While these various types of reactions are often considered collectively as food allergies, true food allergies represent only a fraction of the individualistic adverse reactions to foods.

True food allergies are abnormal responses of the immune system to components of certain foods. The components of food that elicit these abnormal immune responses are typically naturally occurring proteins in foods, although some may be polysaccharides (Table 83.21).

Certain foods can elicit adverse reactions that resemble true food allergies. These foods contain elevated levels of histamine, one of the principal mediators of allergic reactions in the body. When large doses of histamine are ingested with foods, the body's protective mechanism can be overwhelmed resulting in histamine poisoning.

Food intolerances are abnormal reactions to foods or food components that do not involve the immune system. Lactose intolerance is an example of a metabolic food disorder resulting from a defect in the metabolism of a food component. Favism is an example of a metabolic disorder resulting from food-born substances that interfere with normal metabolic processes. Sulfite-induced asthma is an example of an idiosyncratic reaction that occurs among certain consumers, although the mechanism remains unknown.

Although food allergy rarely constitutes a serious, life-threatening concern, it may result in chronic illness. As complete avoidance of the incriminated food is the best defense against adverse reactions, information is of foremost importance. Consumers with various types of food allergies and intolerances must alter their lifestyles on a continuing basis to avoid the offending food or food ingredient.

Symptoms. The most common manifestations of food-allergic reactions are gastrointestinal, dermal, and respiratory, and they represent interaction of released mediators with receptors on those tissues. Gastrointestinal symptoms are common, especially in infants and young children. These include nausea, vomiting, cramping, and diarrhea. Skin reactions can include itching and swelling of the lips, tongue, gums, oral mucosa and pharynx. A topic dermatitis, a chronic inflammatory skin disease, is characterized by dry, easily irritated, intensely pruritic skin. Urticaria (hives) and dermatitis are the most common skin manifestations, and can be a general or localized nature.

The reactions are very individualistic and diverse; initially some individuals may experience immediate contact reactions on their lip and tongue, while others do not experience a reaction until the offending food has moved farther down the gastrointestinal tract. Respiratory symptoms can include upper airway distress, produced as a consequence of bronchoconstriction, with resulting swelling and mucus production. Asthma has been associated with allergies to certain foods. Anaphylactic shock, the most severe manifestation of a food allergic reaction, is a rare, acute, and potentially fatal response.

Despite the multitude of additives used in foods, only a small number have been associated with adverse reactions. Table 83.22 provides a list of the food additives that have been associated with adverse reactions.

Burning of sulfur-containing coal has been used for centuries to preserve food. In addition, sulfite salts (sodium and potassium sulfite, bisulfite, or metabisulfite) are used as a sanitizing agent for fermentation containers and are added to a wide variety of food products, including dried fruits and vegetables, wine, shrimp and other seafood, and citrus beverages. Because of complaints about severe allergic reactions from asthmatic consumers, in 1986 the FDA banned the use of sulfites in fresh cut fruits and vegetables and sulfites must be listed on the label if a food product contains in excess of 10 ppm sulfite. The FDA estimates that about 1% of the U.S. population may be sulfite sensitive. However, among the asthmatic patient population, the sensitivity to sulfites is more prevalent, ranging from 2% to 5%.

The antimicrobial food preservatives benzoate and paraben are believed to cause adverse reactions, such as asthmatic reactions in some individuals. Benzoates occur naturally in certain berries and are used in beverages, and their use is limited to 0.1% concentration. Parabens are effective antioxidants in low-acid products. However, they are primarily used in cosmetic and pharmaceutical products and rarely in food. They have been implicated as a cause of eczematous or contact dermatitis reactions.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been linked to adverse reactions in a small number of individuals. These antioxidants are frequently used in fats and oils and in cereal products to retard rancidity.

TABLE 83.22
Food Additives Suspected of Causing Adverse Reactions

Antioxidants
Sulfur dioxide
Sodium sulfite
Sodium bisulfite
Sodium metabisulfite
Potassium bisulfite
Potassium metabisulfite
Preservatives
Sodium benzoate
Potassium benzoate
Parahydroxy benzoic acid
Methyl-paraben
Ethyl-paraben
Butyl-paraben
Antioxidants
Butylated hydroxyanisole (BHA)
Butylated hydroxytoluene (BHT)
Sodium nitrate
Potassium nitrate
Sodium nitrite
Potassium nitrite
High-intensity sweetener
Aspartame (aspartic acid and methylphenylalanine)
Flavor enhancer
Monosodium glutamate (MSG)
Certified food colors (dyes)
Tartrazine (FD&C yellow no. 5)
Sunset yellow (FD&C yellow no. 6)
Brilliant blue (FD&C blue no. 1)
Indigotin (FD&C blue no. 2)
Erythrosine (FD&C red no. 3)
Ponceau (Red no. 4, permitted in Europe and Japan)
Amaranth (Red no. 5, permitted in Europe)

Aspartame (L-aspartyl-L-phenylalanine methyl ester), a widely used artificial sweetener, is a dipeptide. Humans metabolize it. Aspartame has been cited as the most frequently complained-about product. Soft drinks have been mentioned most often as the aspartame-containing product, and headaches are the common reaction reported. In the United States, the FDA requires that aspartame-containing products include the following label declaration: "Phenylketonurics: contains phenylalinine." Also, in the EU countries, the label declaration "contains a source of phenylaniline" is required.

Monosodium glutamate (MSG) is used as a food additive because of its flavor-enhancing properties. The most commonly reported adverse reaction associated with MSG consumption is Chinese restaurant syndrome. Symptoms of the Chinese restaurant syndrome include nausea, headache,

TABLE 83.23
Selected Food Additives Derived from Allergenic Food Staples

Milk protein derivatives
Casein
Caseinates
Lactose
Lactitol
Whey
Egg protein derivatives
Albumin
Globulin
Livetin
Lysozyme
Ovalbumin
Soybean derivatives
Hydrolyzed soy protein
Hydrolyzed vegetable protein
Natural flavoring
Meat flavoring (natural)
Lecithin
Soy protein
Soy concentrate
Soy isolates
Wheat derivatives
Gluten
Vital gluten
Starch
Vital gluten
Vegetable gum
Vital gluten
Corn derivatives
Caramel coloring
Corn sweetener
Citric acid
Dextrin
Dextran
Erythritol
Food starch
Gellan gum
Lactic acid
Maltodextrine
Mannitol
Modified food starch, vegetable gum
Sorbitol
Xanthan gum

sweating, thirst, facial flushing, and abdominal pain. These symptoms typically occur 15–30 minutes after consuming food containing a large amount of MSG. A Chinese food meal may contain from 5 to 10 g of MSG.

Among the coloring agents used in the food industry, tartrazine (FD&C yellow no. 5) has most often been implicated as a cause of allergies, especially urticaria and asthma. Respiratory problems subsequent to tartrazine ingestion have been reported by several sources. Tartrazine produces a bright yellow color and it is used in a variety of beverages, baked products, confectionaries, dessert mixes, etc. Tartrazine is also used to produce other food colors such as green, maroon, and rust. Lack of yellow color, therefore, is not a guarantee of tartrazine's absence. Thus the FDA requires that FD&C yellow no. 5 be specifically stated by name on food ingredients labels. Food colorings other than tartrazine (listed in Table 83.23) have also been implicated as causing adverse reactions in some individuals.

D. Food Additives Derived from Allergenic Foods

A few foods are responsible for the majority of allergic reactions. In adults, these foods include nuts, peanuts, fish, and shellfish. In children, the main culprits include eggs, milk, peanuts, soy, wheat, and fish. Elimination of these foods from the allergic individual's diet is essential. However, many food additives are derived from these basic food items, and the allergen compound may be carried over even into highly refined derivatives. Recognition of the presence of such potentially allergenic compounds is sometimes difficult.

In Table 83.23, selected food additives derived from allergenic natural food sources are listed. Recognition of these additives is crucial to avoid potential health hazards to consumers sensitive to certain foods.

The industry must provide these consumers with the information necessary for them to practice such avoidance effectively. Ingredient labeling statements are the key to implementation of safe and effective avoidance diet. FDA regulation exempts specific substance declaration of flavors, spices and colorings. However, the agency strongly encourages specific declaration of an allergenic ingredient if present among these exempt ingredients (e.g. FD&C Yellow No. 5). Also, manufacturers must be aware that certain processing practices such as the use of shared equipment can result in undeclared residues of allergenic foods existing in other products. These situations can be hazardous for allergic consumers especially if larger quantities of the allergenic foods are present on an undeclared basis.

ACKNOWLEDGMENT

This chapter is a revised and updated version of Chapter 15, Food Additives, L.P. Somogyi, pp. 447–516. In *Foodborne Disease Handbook*, Volume 4: *Seafood and Environmental Toxins*, edited by Y.H. Hui, D. Kitts, and P.S. Stanfield, Marcel Dekker, Inc., New York, 2001.

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84 Food Processing Biofilms and Antimicrobial Agents

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CONTENTS

I. INTRODUCTION

While more than two decades have passed since the term "biofilm" was coined, the effect of surface contamination by attached bacteria has long been known in the food processing industry. It has only been over the past 10 years, during which time the production of food, and especially meat products, has come under increasing pressure to improve sanitation and reduce the incidence of foodborne pathogens, that biofilms have been targeted as a major concern to food processing operations. Most food processing operations now use HACCP to closely monitor and record critical food processing steps to ensure that high standards of plant and employee hygiene are maintained, and that the potential for health hazards in the final product is minimized. However, biofilms continue to pose a serious problem in many facilities despite efforts to control the situation. The solution to this problem in part lies with improved education about what biofilms are and how they form, as well as proven and novel control strategies for their removal and killing.

Most foods are a source of pioneer bacterial flora, and as such represent a major source of contamination and recontamination in food production facilities. Cleaned or

new surfaces in a plant rapidly become fouled with spoilage and/or pathogenic bacteria soon after processing operations begin, and if unchecked, a surface fouling, or biofilm, problem may result. Once established, biofilms are difficult to dislodge and completely kill, features evidenced by their rapid regrowth following typical cleaning and sanitization operations. Much is already known of the ecology and nature of biofilms despite the fact that specific studies haven't always been performed in food processing environments; hence, extrapolation of this knowledge to food systems is possible in many instances. For example, it is known that biofilms are different, in terms of physiology, gene expression and behaviour, from pure cultures grown in planktonic culture (the traditional approach to the study of bacteria). Biofilm bacteria express different genes, up- or down-regulate production of various proteins, undergo physiological changes that impact their ability to survive imposed stress factors such as antimicrobial agents, and become integrated within complex assemblages of different microbial species rather than just a single species. The condition of these sessile bacteria is very far removed from that experienced by a pure culture of bacterial cells suspended in liquid growth medium.

No universally effective control strategy or antimicrobial agent presently exists for biofilms; hence, research for developing and testing new approaches for biofilm control is ongoing. The most common strategy for controlling biofilms involves the application of antimicrobial agents to vulnerable surfaces, typically in conjunction with other agents as well as surface sanitation techniques. Development of new chemical agents and/or techniques, especially in light of new revelations of the distinct physiological and molecular status of biofilm bacteria, is an obvious goal of those researchers in the discipline. This review is intended to provide key foundational information describing biofilms, as well as biofilm control measures currently in use or under development that are relevant to food processing.

II. BIOFILMS DEFINED

Other than Antonie van Leeuwenhoek, who used a handmade single lens microscope to view microbial populations from his teeth back in the late 1600s, the first individual credited with speculating that significant populations of attached bacteria existed was Zobell, who in 1943 examined surfaces associated with oligotrophic aquatic systems (1). It is now known that virtually any surface over which liquid flows, including contact lenses, ship hulls, dairy equipment, teeth, water distribution systems, petroleum pipelines, rocks in streams, heat exchangers and all varieties of biomedical implants and transcutaneous devices are all prone to colonization by biofilm bacteria over time. While biofilms may be either beneficial or detrimental, the detrimental effects are best characterized.

Exactly what is a biofilm tends to vary in the scientific literature; however, they are consistently described as bacterial cells attached to a surface and enmeshed in an extracellular exopolysaccharide (EPS) matrix. A more descriptive explanation of how these elements are arranged is useful, however, if one is to appreciate the complexity and organization exhibited by most microbial biofilms. Thus, biofilms are the result of a variety of different bacteria and their EPS material forming a highly-hydrated matrix positioned at a solid liquid interface, which will eventually develop to include extraneous particulate matter, proteins, nucleic acids, lipids, enzymes, etc. The structural arrangement of cells and polymer has been described as "architectural," due to the high degree of apparent organization in three-dimensional space; given time, embedded microcolonies and cellular aggregates become separated by channels, voids or tunnels. A number of review articles have addressed the topic of biofilm architecture and its importance to biofilm nutritive and metabolic processes (2–4).

III. THE FORMATION OF BIOFILMS

Biofilm development is a complex process that includes the following "phases"; i) molecular film adsorption, ii) bacterial attachment, iii) biofilm growth, spread or maturation, and iv) biofilm dispersion or cell shedding. It is useful to view the events governing biofilm formation in terms of a particular time frame involved (e.g., either relatively short or very long), as these events may then be considered in light of the sanitation and operation of a typical food processing operation.

A. Early Events

When biofilm research intensified during the 1980's, much effort was focused on bacterial attachment, as cells must first attach to surfaces before they form a biofilm. The adsorption of a molecular film from the liquid phase is significant in that any clean surface placed in an impure liquid will rapidly accumulate a molecular film. This process starts immediately following the immersion of a surface in liquid, and where there is an abundance of complex organic materials and exudates such as in a meat processing plant, the accumulated film can be significant. Molecular films rapidly modify the chemistry of the surface to reflect that of the solution, and consequently impact surface physicochemical properties. For example, surface charges and zeta potentials, contact angles, and surface tensions can be expected to vary as a function of the properties of the liquid as well as that of the once-clean substratum (5–9). The adsorption of this film has been documented to have measurable effects on bacterial attachment. Meadows (10) demonstrated that sorption of casein and gelatin enhanced attachment processes whereas Fletcher (11) determined that precoating polystyrene

surfaces with albumin, gelatin, fibrinogen, and pepsin all inhibited the attachment of a marine Pseudomonad. Chemotactic bacteria have also been shown to respond to the presence of organic molecules, providing an explanation for the directed movement and preferential positioning of many bacterial species on solid substrata (12–15).

While sanitizing protocols in food processing settings are unable to eliminate all surface organic and inorganic molecules (or even all bacteria for that matter), they should be effective enough to prevent the surface from acting as a major source of nutrients for bacteria. As soon as the next production shift begins, a new film will accumulate fairly rapidly; thus, the daily challenge is to minimize the amount of surface soil remaining following cleaning along with any bacteria that may have become established or grown during the preceding shift, thereby preventing the uncontrolled growth of attached microorganisms for any longer than the time required for a single shift.

The actual attachment of bacteria involves close cell-surface interactions at solid liquid interfaces. Non-motile bacteria initially contact attachment surfaces via gravitational settling, convection, or Brownian motion/diffusion (16, 17). In contrast, motile organisms migrate either chemotactically or randomly to surface attachment sites. The process of adhesion has been described by Marshall et al. (18) to involve two phases, a theory that still sees extensive use. The first, or reversible attachment, phase is a non-specific process involving the association of cells near ($\geqslant$50 nm), but not in actual contact with, the substratum. Adhesion is reversible as only weak bonds (Van der Waal's forces, hydrogen bonding, hydrophobic interactions, or electrostatic interactions) secure the bacterium to the surface; thus, any significant shear force will remove the cells. At these distances, the microbial and solid surfaces are too far apart for molecular recognition of most surface features. Once the cell is positioned 10 to 20 nm from a solid surface, interplay between attractive Van der Waal's and repulsive electrostatic forces (while most bacteria and surfaces carry a net-negative charge; non-uniform, oppositely charged, ion clusters exist on the cell surface) now occurs. For attachment to occur, attractive forces must exceed repulsive forces. The presence of pili and/or flagella (pili are ~4–7 nm in diameter and up to 1000 nm in length) that penetrate the repulsive barrier from long range both enable bacterial cells to become stably attached. Overall, reversible attachment is rapid and may occur within a fraction of a second following a bacteria-surface contact.

Other factors also affect attachment of bacteria. For example, surface composition (e.g., plastic, metal, or wood), surface condition (e.g., whether it is new or has scratches or pits), and surface charge and hydrophobicity all contribute to microbial attachment success. Other factors like solution ionic strength, liquid shear forces and viscosity, and temperature have been shown to play roles of varying importance (5, 19). Lastly, bacteria-specific parameters, such as the type of organism, growth phase and prior growth conditions, flagellum and motility factors, and specific cell–cell coaggregation events all affect attachment success (20–24). These factors not only impact initial attachment success of bacteria but also the tenacity of long-term attachment by various organisms — conditions that result in stronger adhesion translate into increased difficulty of bacterial removal during plant sanitation. While perhaps self-evident, the condition of the surface is especially important, not only because it may contribute to cellular adhesion, but because surface imperfections like scratches and corrosion provide refuge for biofilms and subsequently shield cells from the effects of antimicrobial agents (25). Work on bacterial attachment continues today, reflect the fact that this phenomenon is both complex and pervasive.

B. Late(r) Events

Once reversibly attached, stronger cell-surface interactions may cause the cell to become permanently, or irreversibly, attached (the second phase in Marshall's (18) two stage attachment description; see above). The key to this process is the production of extracellular polysaccharides (EPS), a substance that bridges the bacterial cell surface and the solid substratum. Bacterial EPS are high molecular weight polymers that are highly hydrated (26, 27); thus, food processing surfaces with a biofilm contamination problem often have a "slimy" feel. Biofilm EPS constituents includes, in addition to polysaccharides, globular glycoproteins, amino acids and sugars, nucleic acids, lipids, phosphates, organic acids and ions. While EPS does not form strong bonds with the substratum, it does form a large number of weak bonds that collectively immobilize bacterial cells within the biofilm and more importantly, secure the biofilm to the substratum.

Specific EPS moieties also play important adhesive roles. For example, glycoproteins contain positive charges that ionically cross-bridge the EPS and substratum. Other EPS related factors that influence bacterial adhesion include EPS age, complexation of EPS with other molecules, chemical makeup of the EPS, and the types of organisms present. Biofilm EPS, like the EPS produced by planktonic bacteria, vary greatly in terms of their physical and chemical properties and it has been reported that EPS from different bacterial species actually function synergistically to strengthen overall biofilm adhesion (26). Another effect of an aging cell-EPS matrix is a reduction in diffusional transport, a factor tending to make biofilms more substrate- and oxygen-limiting. This alters the physiological status of the cells trapped within, and embedded cells typically have decreased rates of growth and metabolism. The role of EPS is greater than that of simple adhesion, and biofilm EPS is widely thought to participate in nutrient capture and uptake, maintaining an optimal biofilm microenvironment,

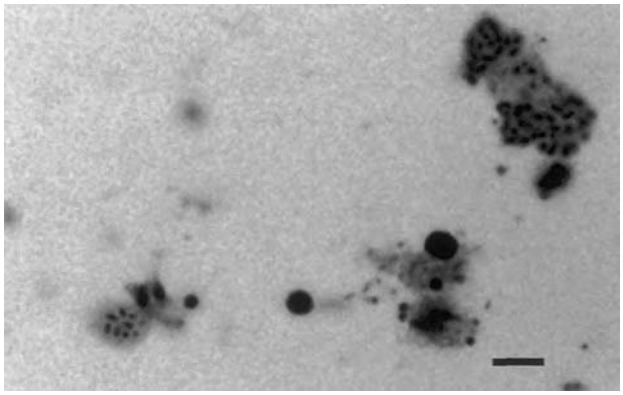

FIGURE 84.1 Confocal laser microscope image of negatively stained (0.1% fluorescein solution) mixed species biofilms showing microcolonies surrounded by dense EPS material. Note that the EPS material tends to exclude the low molecular weight fluorescein (289 MW) in regions immediately adjacent to the cells. Scale bar = 10 μm.

and protecting biofilm inhabitants from predators, desiccation and antimicrobial agents (27, 28). The contribution of EPS in the protection of food processing biofilms from antimicrobial agents is discussed further below (see section on *Biofilm resistance to sanitizing and antimicrobial agents*). Figure 84.1 shows bacterial microcolonies enmeshed in dense EPS material.

Factors like flagella, pili and adhesins have also been shown, using adaptation-minus mutants, to play important roles during adhesion and subsequent biofilm formation. The primary function of flagella in biofilm formation is assumed to be in transport and in initial cell-surface interactions (see above). However, the presence of a functional flagellum has also been shown to act as a adhesion factor during the colonization of potato and wheat surfaces by *Pseudomonas* species (29, 30), whereas pili-associated adhesins are known to be important during the colonization of epithelial surfaces of mammals (31–34).

C. Microcolonies, Maturation and Architecture

The colonization of surfaces by bacteria may be based on either solitary or colonial strategies (3). Single cell strategies are employed by budding bacteria like *Rhizobium* or *Caulobacter* spp. These cells attach to surfaces either in a polar or non-polar manner and then release their progeny into the aqueous phase (35). Budding organisms most commonly exist in oligotrophic waters systems or are associated with plant roots and are typically slow growing and non-pathogenic; hence, they are of limited importance to food processing. Of greater significance are colonial cells, of which *Pseudomonas aeruginosa* or *P. fluorescens* provide examples. Soon after these cells attach to surfaces, they proliferate and develop into microcolonies. The formation of microcolonies are considered to represent primitive behavioural patterns, and follow a defined (often species-specific) developmental pathways or colonization strategy (3). These organisms attach as single cells, multiply on the surface into packed, or dense, microcolonies, and then release progeny as single cells to recolonize new surfaces (19). The majority of food processing pathogens colonize surfaces in this manner.

Microcolony formation patterns considered "behavioural" have been shown to vary with the physical, chemical and biological environment; thus, flow velocity and substratum type, nutrient status and Eh, and presence of competing or communal organisms affect microcolony developmental kinetics and appearance. Wolfaardt et al. (36) and Moller et al. (37) showed that by changing to a labile carbon source from a recalcitrant carbon source resulted in a different biofilm appearance. While biofilms are the dominant mode of growth in many environments, specific examples exist of bacterial adaptation to both the planktonic and biofilm modes of growth. *Vibrio parahaemolyticus* clearly shows this trait; a short, polarly flagellated variant is adapted for migration within low viscosity environments such as the open ocean, whereas an elongated, laterally flagellated variant is suited for colonization of high viscosity environments (e.g., mucus layers of fish) (38). Switching between these two phenotypes ensures the organism is poised for success in either system. Based on other adaptations like motility and chemotaxis, it is clear that the free swimming or suspended versions of bacteria are key parts of their life cycle. For more information on the bacterial surface colonization behaviour, readers are referred to a review by Lawrence et al. (1996).

Prior to the advent of confocal scanning laser microscopy (CSLM), biofilm models were based on electron microscopic observations and thus described as homogeneous layers of cells on surfaces. After the first examination of native, fully-hydrated biofilms using CSLM (39) it was revealed that biofilms exhibited considerable organization and variability of cells and their extracellular products in three-dimensional space. The organization exhibited by many pure cultured and mixed species biofilms in large part contribute to the description of biofilms as having a "primitive circulatory system." A number of biofilm models have subsequently been described, including mushroom structures interspersed by a network of channels, mounds, aggregates, clusters, channels, void spaces, holes, bridges, etc. (40, 41). The variables controlling architecture are equally diverse, and include the organism or organisms involved, nutrient status, predation, flow velocity and substratum type and condition. Figure 84.2 shows a series of optical thin sections of a positively-stained *Salmonella enteritidis* biofilm where voids and cell aggregates (or mounds) may clearly be seen.

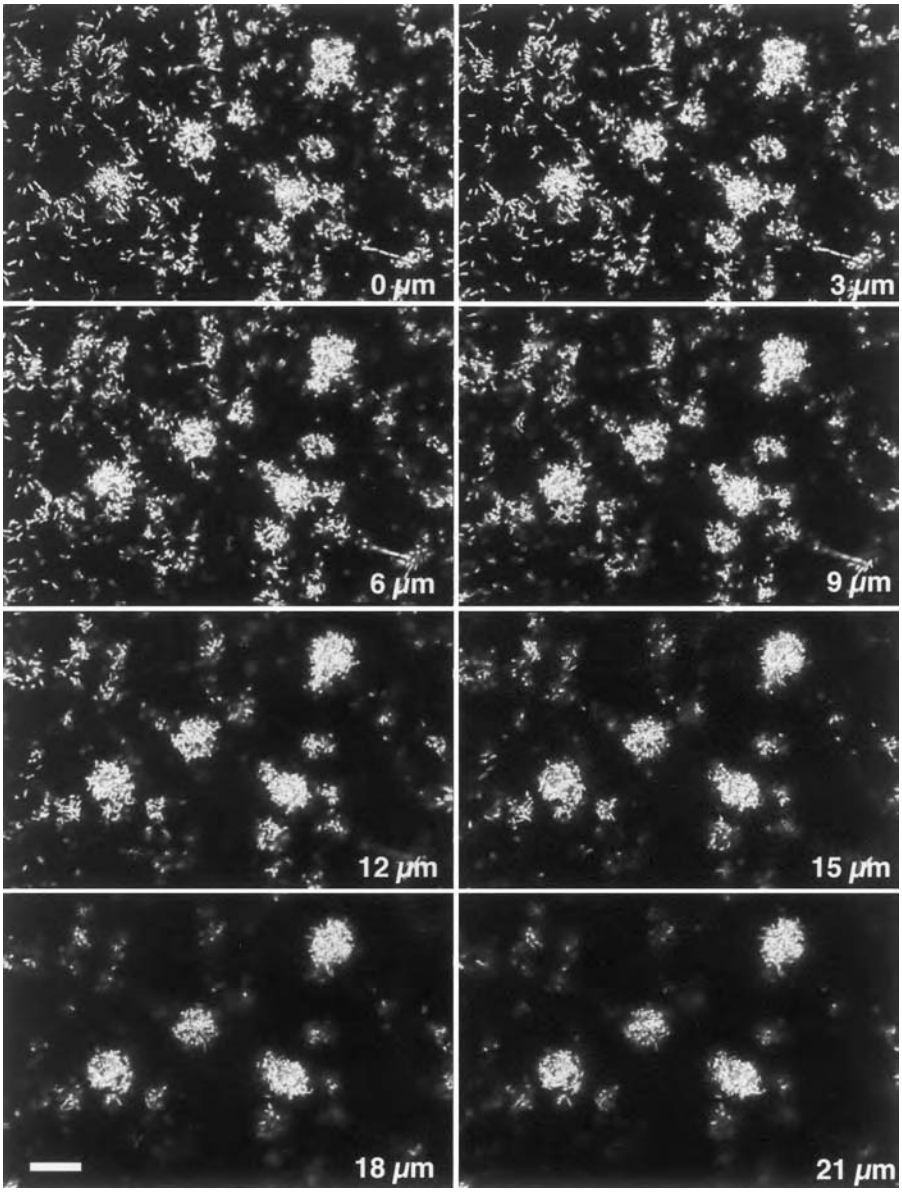

FIGURE 84.2 Confocal laser microscope images showing cell aggregates and channels in a *Salmonella enterica* serovar Enteritidis biofilm grown in a model flow cell system. Optical thin sections from the 0, 3, 6, 9, 12, 15, 18 and 21 μm section depths are shown. Cells were positively stained using Syto 9. Scale bar (lower left) = 20 μm.

D. Biofilm Maturation

Biofilm bacteria are quite different from their planktonic counterparts, as judged by gene expression, cellular physiology and increased resistance to antibiotics and antimicrobial agents (40, 42, 43). These differences involve not only the proliferation of attached cells into microcolonies and biofilm along with the production of EPS, but also the establishment of a biofilm structure (architecture; as described above) along with the creation of distinctive physical and chemical zones, or microenvironments (44).

The biofilm microenvironment is physically and chemically distinct from the macroenvironment and is the consequence of metabolic activity of microorganisms themselves (consumption of O_2, production of CO_2, acid and end product formation) in conjunction with the physical characteristics of the cells and EPS polymer matrix. Biofilm microenvironments tend to be diffusion-dependent, and thus gradients of all chemical parameters may be encountered. For example, *in situ* CSLM work with molecular probes has directly confirmed the existence of zones of hindered diffusion, gradients of pH, dense polymer and varied cell metabolic activity. The depths of actively growing heterotrophic biofilm such as *P. aeruginosa* rapidly become oxygen limiting, with the highest O_2 concentrations located near the biofilm-liquid interface, and the most

oxygen depleted regions located at the biofilm-substratum interface (the attachment surface) (40). As the exchange of nutrients and end products into and out of the biofilm are diffusion-dependent, the velocity of the overflowing liquid also impacts nutrient exchange.

The consequences of the formation of the chemical microenvironment are two-fold: 1) establishment of a spatial gradient of growth conditions for biofilm organisms, altering their rates of growth and ability to carry out metabolic reactions, and 2) creation of a gradient of growth conditions for different organisms with different growth requirements. It is widely believed that the formation of the microenvironment is a key factor in resistance of biofilm bacteria to antimicrobial agents (see discussion below). The integration of pathogens within a non-pathogenic biofilm is obviously of the greatest concern with respect to food processing biofilms. Thus, as biofilms "mature," the capacity to harbor pathogenic organisms, like *E. coli* and *Listeria monocytogenes*, may increase (45). More research is needed for a better understanding of how different microbial species become integrated into complex communities.

Recently, gene and protein expression patterns have demonstrated genetic and physiological differences between biofilms and planktonic cells, as well as between biofilms at different developmental stages. Brözel and coworkers monitored global protein expression patterns in attached *P. aeruginosa* cells and observed as many as 11 proteins whose levels became altered during various stages of attachment (46). Similar studies revealed fairly high levels of gene expression existed for certain proteins (35% of total) of *E. coli* following transition from the planktonic to biofilm growth state (47). More recently, Sauer et al. (43, 48) reported the regulation of as many as 30 genes and 40 gene products became altered in *P. putida* within 6 h following attachment, and using direct microscopic observation were also able to characterize five stages of biofilm development in *P. aeruginosa*. The varied physical and chemical conditions experienced in the biofilm microenvironment would also result in differences in the expression of genes and production of proteins.

In *P. aeruginosa*, changes in gene expression following bacterial attachment include the surface-induced gene activation of *algC*, a gene involved in lipopolysaccharides core biosynthesis, as well as the biosynthesis of the exopolysaccharide alginate (49, 50). Others have linked the down-regulation of flagellar synthesis with the alginate up-regulation (51). As cells adjust to an immobile life on a surface, they lose their flagella and increase the production of EPS.

A seemingly important step in the maturation of biofilms is the formation of characteristic biofilm architecture. While the advent of CSLM was a pivotal tool in revealing the complexity of the different biofilm architectures, until recently it wasn't clear how the development of these structures was regulated. The observation that a mutant of *P. aeruginosa* unable to synthesize the quorum-sensing molecule acylhomoserine lactone (acyl-HSL) developed a radically altered biofilm architecture, suggested that architecture was indeed regulated. As published in the journal Science, a *lasI* mutant (defective for production of acyl-HSLs) formed a biofilm without the typical well-spaced microcolonies (attaining heights of over 100 μm) and lacked resistance to SDS treatment typically seen in the wild-type strain (52). This study suggested that cell-cell communication was essential for *P. aeruginosa* to establish structured biofilms. However, more recent work using similar *P. aeruginosa* AHL mutants have found contradictory results (53, 54). The role of quorum sensing in the development of biofilm architecture is therefore unclear for the time being. This continues to be an enticing research area with potential for benefits in the area of biofilm control. One interesting study approach would use furanone AHL analogs, produced by *Delisea pulchra* (a macroalga that naturally resists biofilm formation), to disrupt or block AHL communication, and hence biofilm formation.

Overall, post-attachment events are considered by some to culminate in the "maturation" of the biofilms into a thick layer of organized cell material interspersed by channels within a matrix of EPS material. While it is useful in theory to envision biofilms developing sequentially (e.g., molecular film, attachment phases, microcolony development, biofilm formation, and biofilm sloughing and progeny dissemination), and there is evidence to indicate that this indeed is the way things occur for *P. aeruginosa* (43), these models are based on sterile laboratory systems that are "inoculated" after which biofilm formation is monitored over time. In real-world situations, it is altogether more likely that many phases (e.g., attachment, detachment, shedding/sloughing, microcolony formation, etc.) of biofilm formation will be occurring concurrently, and would involve different bacterial species.

IV. BIOFILM DETECTION

Detection of biofilm contamination depends on rinsing or swabbing surfaces, followed by dilution and plating of the samples on agar media. While some modifications to the sampling methods exist (e.g., spray gun samplers, sonication-assisted removal of cells), these are essentially the same culture-based techniques. Owing to the complexity and variable geometry of surfaces and equipments (e.g., pipelines) within a processing plant it may be desirable to perform "in line" surface sampling. The Modified Robbin's Device (MRD, Tyler Research, Edmonton, Canada) permits the removal of plugs that mount flush with a surface or a fluid delivery pipeline. These plugs or coupons may be removed at defined intervals, with the biofilm stained and

observed directly, and/or disrupted and enumerated using culture based methods. One obvious advantage of culture-based methods is that various selective and differential growth media are readily available. Their main shortcoming is the time to obtain a result, since most organisms require 24 or more hours of cultivation. As in other disciplines, molecular methods like polymerase chain reaction (PCT) and real-time PCT can be used to identify and estimate the number of specific pathogens that may be plaguing a facility. Use of ATP-sensitive luciferase assay kits can also provide a measure of biofilm buildup. ATP-bioluminescence is a non-culture based commercially-available method that relies on the ATP-dependent production of light from the luciferin-luciferase system. Surfaces are sampled with sterile cotton swabs and are then reacted with kit reagents. After reaction, light generation is read in a hand-held luminometer. ATP bioluminescence may provide an index of plant surface hygiene that can then be used to estimate microbial contamination. Since it is a rapid method it can be used on-site providing results in as little as 5–10 minutes. However, it is important to note that non-microbial sources of ATP cannot be differentiated from those of microbial origin, thus ATP associated with meat exudates can affect the readings. It is important to remember that the biofilm sampling methods described above are to be performed in addition to routine plant cleaning and sanitation, and not as a diagnostic step to prescribe cleaning. Lastly, there are numerous model systems and approaches for the study of biofilms. Many of these are highly applicable to the food processing situation, and thus many techniques and methods are directly transferable. Reviews describing these culture systems are available (55, 56).

V. PREVENTION AND REMOVAL OF BIOFILMS IN FOOD PROCESSING FACILITIES

Microbial attachment and biofilm formation may be beneficial (vinegar production, enzyme production); however, it is far more common for biofilm formation to be detrimental to food production. Fouling of food processing equipment and foods by spoilage or pathogenic bacterial biofilms is the main concern, however damage to metal surfaces (pitting and corrosion) and breakdown of plastics are other possible outcomes. Biofilms also contribute to decreased heat transfer, lost sensor sensitivity, and plugging of filters. Low pressure, large diameter pipes do not always fill and thus are prone to biofilms and are hard to clean without disassembly. Pathogens known to form biofilms include *Salmonella*, *Klebsiella*, *Pseudomonas*, *Campylobacter*, enterohaemorrhagic *E. coli* and *Listeria*. These organisms are of special significance where ready-to-eat and minimally-processed meat products are produced as they aren't controlled in a terminal processing step.

Those areas of food processing plants where biofilms become problematic are often non-food contact surfaces with high humidity or moisture levels. Machine shrouds, overhead structures, floor drains and walls are examples. These surfaces do not directly contact foods; however, indirect transfer of biofilm bacteria may occur as a consequence of moisture dripping onto food contact surfaces below, worker activity, and especially during cleaning where aerosolized bacteria in water droplets may be propelled many meters during pressure washing.

Other areas where even the best efforts may fail to control biofilms include conveyor surfaces, brine injectors, belts, gaskets, damaged steel cutting surfaces, floor drains, elbows or dead-ends in pipelines, pumps, valves, and food or liquid storage tanks. Injectors for brining or tenderizing meats have been identified as a location for recurrent *L. monocytogenes* biofilms. Czechowski (57) reported finding biofilms in dairies and breweries on bends in pipes, rubber seals, conveyor belts, waste water pipes, floors, etc. Teflon® and Buna-n® seals were determined to be excellent sites for biofilm formation and when cracked provide reservoirs for microbes which are difficult to inactivate or kill during cleaning. *Listeria monocytogenes* persists on the rubber fingers of poultry pluckers and the trolleys that carry the carcasses after cleaning (58), as does *S. aureus* (59). Notermans and co-workers (60) have observed biofilms in poultry slaughterhouses by scanning electron microscopy. They found that after several weeks of operation the whole surface of the rubber plucker finger was pitted, trapping microorganisms in places like surface scratches and metal welds so that microorganisms become protected from routine cleaning and disinfection. Such biofilms represent a recurrent source of contamination to foods coming in contact with them, potentially with health consequences.

Because of the sensitive nature of certain foods, the occurrence of food processing biofilms has the potential to impact both food shelf-life and safety. During the processing of meats and minimally processed meat products, persistent biofilms may cause significant reductions in product quality and consumer safety. Most of the problem associated with biofilms is associated with the inability or failure to adequately clean the affected surface; hence, the age and design of the facility, as well as the design of the equipment, play important roles in providing cleaning access to vulnerable sites in the processing stream. Routine inspections of potential sites for biofilm formation, as well as scheduled replacement of problem surfaces (gaskets and cutting boards) will assist in limiting problems of this nature.

VI. BIOFILM RESISTANCE TO SANITIZING AND ANTIMICROBIAL AGENTS

The fact that biofilm bacteria have greater resistance to antimicrobial agents than planktonic cells has received

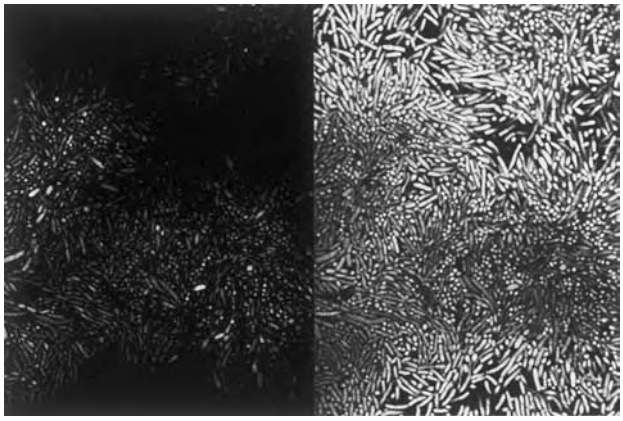

FIGURE 84.3 Dual-channel confocal laser microscope image showing *E. coli* biofilm bacteria after treatment with 0.625% trisodium phosphate (TSP) and staining with the BacLight™ Live/Dead Viability probe. Cells in the right hand panel have been killed by the treatment and thus fluoresced in the red wavelengths. Cells in the left hand panel have not been killed and thus fluoresced green. It is suggested that incomplete penetration of the antimicrobial agent, in combination with interaction of the agent with cells and EPS material, resulted in a reduction of TSP efficacy.

considerable attention over recent years. Specific reports detailing this phenomenon are regularly published. For example, Williams et al. (61) reported that cells of *S. aureus* biofilms on silicon surfaces were 10 times more resistant to vancomycin than were their planktonic counterparts. Ceri and coworkers (62) reported that *P. aeruginosa* required as much as 1,024 μg of imipenem and *E. coli* required a much as 512 μg of ampicillin as the minimal biofilm eradication concentrations compared to just 1 and 2 μg, respectively, for their planktonic counterparts. A similar increase in resistance was reported against ceftazidime and doxycycline in both *P. pseudomallei* and *Streptococcus sanguis* biofilms, respectively (63, 64). Investigators have also observed increases in resistance of biofilms and attached microorganism on food contact surfaces to various sanitizing agents (65–69). Frank and Kofi (66) found that attached microcolonies of *L. monocytogenes* were resistant to prolonged treatment (~20 min) with the anionic acid sanitizer, benzalkonium chloride, whereas cell suspensions were killed by a 30 sec exposure to the same concentration of sanitizer. Results of work conducted by Mustapha and Liewen (70) and Wirtanen and Matti-la-Sadholm (71) suggest that the age of a biofilm affects the resistance of microorganisms to sanitizers. Sodium hypochlorite and quaternary ammonium compounds were effective against a 24 h biofilm of *L. monocytogenes* on food contact surfaces. Biofilms of *P. fluorescens*, *L. monocytogenes*, and *Bacillus subtilis* challenged with 0.1% and 1.0% chlorine at 25°C were shown to become more resistant with biofilm age. Figure 84.3 shows a confocal laser microscope dual channel image where *E. coli* biofilm bacteria have been treated with trisodium phosphate (a foodgrade antimicrobial) but surviving cells exist at the base of the biofilm.

VII. MECHANISMS OF BIOFILM RESISTANCE TO ANTIMICROBIAL AGENTS

At least three mechanisms have been proposed to account for the increased resistance of biofilm bacteria to antimicrobial agents: (i) delayed penetration of the antimicrobial agent, (ii) altered growth rate of biofilm organisms, and (iii) physiological changes due to the biofilm mode of growth.

Bacterial cells enmeshed in an EPS matrix present a diffusion barrier for these molecules by influencing either the rate of transport of the molecule (hindered diffusion) to cells in the biofilm interior or the reaction of the antimicrobial material with the matrix material. Suci and coworkers (72) demonstrated the delayed penetration of ciprofloxacin into *P. aeruginosa* biofilms; what normally required 40 s for a sterile surface required 21 min for a biofilm-fouled surface. DuGuid and coworkers (73) examined *Staphylococcus epidermidis* susceptibility to tobramycin and concluded that the organization of cells within biofilms could in part explain the resistance of this organism to this antimicrobial agent. Studies examining antimicrobial penetration and interaction with the EPS of biofilms have revealed reduced efficacy due to poor diffusion of the antimicrobial agent through the biofilm matrix. Hatch and Schiller showed that a 2.0% suspension of alginate, a key EPS component, from *P. aeruginosa* inhibited diffusion of gentamicin and tobramycin, and this effect was reversed by using the enzyme alginate lyase (74). Souli and Giamarellou (75) demonstrated that *S. epidermidis* slime hindered the antimicrobial efficacy of *B. subtilis* against a large number of agents.

The rate of cell growth has also been shown to influence the resistance of biofilms to antimicrobial agents. Biofilm-associated cells grow significantly more slowly than planktonic cells in logarithmic growth (the common target of previous antibiotic susceptibility tests). Many antibiotics depend on inhibiting a key component of cell biosynthesis (e.g., cells walls, protein synthesis, DNA replication), and thus tend to have a greater effect on actively growing cells. Using a method of cell culture designed to determine the effect of growth rate apart from other biofilm processes, Evans et al. (76) found that the slowest growing cells of *E. coli* were most resistant to cetrimide. Eng et al. (77) controlled the growth rate of bacteria through nutrient limitation and demonstrated that only fluoroquinolone antibiotics produced bactericidal effects against stationary-phase gram-negative organisms. However, increasing the

growth rate of target biofilm cells was followed by an increase in antimicrobial efficacy. Anwar and coworkers (78) also demonstrated age-related differences in the response of *S. aureus* biofilms to antimicrobial therapy; 4-day-old biofilms exposed to tobramycin and/or cephalexin caused a rapid decrease in the numbers of viable cells, whereas biofilms developed over a 13-day period demonstrated marked resistance to either drug or a combination of both. Korber et al. (79) provided indirect evidence of a gradient of cell growth rates through *P. fluorescens* biofilms, using the antimicrobial agent fleroxicin (which inhibits DNA gyrase and causes growing cells to elongate) and confocal scanning laser microscopy. Cells nearest the biofilm-liquid interface elongated significantly more than those cells near the biofilm-substratum interface and were more susceptible to fleroxacin.

Other physiological factors may also play a role in biofilm resistance. For example, Gram-negative bacteria respond to nutrient limitations and other stresses by synthesizing sigma factors. In *E. coli*, those sigma factors that are under the control of the *rpoS* regulon regulate the transcription of genes whose products mitigate the effects of stress. By studying *E. coli* biofilms formed by strains with or without *rpoS* genes, Adam and McLean (80) found that the *rpoS*$^+$ *E. coli* biofilms were more dense and had higher number of viable cells. Since *rpoS* is activated during slow growth of this organism, it appears that conditions that elicit the slowing of bacterial growth, such as nutrient limitation or build-up of toxic metabolites, favor the formation of biofilms. Nutrient limitation and increase in toxic metabolite concentrations might be particularly acute within the depths of established biofilms, and therefore slow growth and cell physiology are likely linked. Tresse et al. (81) found that agar-entrapped *E. coli* cells were more resistant to an aminoglycoside as oxygen tensions were decreased. They suggested that the effect was due to lowered uptake of the antibiotic by the oxygen-starved cells. It is also thought that at least some of the cells in a biofilm adopt a distinct and protected biofilm phenotype. This phenotype is not a response to nutrient limitation, but is thought to be a biologically programmed response to growth on a surface. This phenotype is likely the consequence of the expression and translation of unique genes and proteins (see earlier discussion on biofilm maturation). Our group has identified a number of differentially expressed proteins found in *Campylobacter jejuni* and *Salmonella enterica* that are likely to play roles in this regard (82, 83).

Thus, multiple factors likely play additive or combinatorial roles in the resistance of biofilms to antimicrobial compounds. It follows that a key element of any surface sanitation program is to first use methods and compounds sufficient to dislodge cells and their EPS from the food processing surfaces, as physical removal of biofilm material is not dependent on their killing *per se*. This will, in turn, enhance the efficacy of any antimicrobial treatment that is then applied. For more details on the resistance of biofilms to antimicrobial agents, the reader is directed to the following review articles (4, 84).

VIII. CONTROL OF FOODBORNE BACTERIA

Bacteria may become associated with foods because of their presence in the raw materials or their introduction during processing. Control of foodborne bacteria is essential to hazard free and acceptable food for consumption. In the absence of appropriate controls, bacteria present in foods will change the quality or devalue the product and in extreme cases cause spoilage and become serious source of hazard to the consumers.

A. Foodgrade Surface Agents/Novel Agents

Food surfaces are the prime areas of exposure to air, processing instrument, and human and animal contact. Surfaces in general and food surfaces in particular, pose exceptional challenge because they may be uneven and contain cracks (both macro- and nano-sizes) and be inaccessible to removal of microorganisms. Additionally, many foods are prepared in contact with various surfaces (tables, cutting knives, handlers, etc.). Due care in the sanitation of the processing environment, instruments and personnel become critical issues in the manufacture of bacteria free foods. A number of chemical treatments allow for cleaning or disinfecting foods undergoing processing or the surfaces with which they come in contact. The types of agents used for this purpose vary.

Surface active agents such as wetting agents or surfactants are non-polar, long chain organic molecules with a polar head. They aid cleaning by reducing water surface tension and their ability to be suspended in fats, including bacterial membranes and cause disruptions. Other agents such as organic alkalies, inorganic and organic acids, at critical concentrations are bactericidal. Other options include the use in combination of e.g., sucrose laurate (SL) ethylenediaminetetraacetate (E), and butylated hydroxyl anisole (B) (SLEB) as an effective antimicrobial agent against both gram-negative (aerobes) and gram-positive (facultative anaerobes) foodborne bacteria (85).

B. Trisodium Phosphate

Trisodium phosphate (TSP) is an orthophosphate salt of phosphoric acid. It has a minimum of 41.5% P_2O_5 and a pH of approximately 11.8 in a 1% solution (86). Uses of TSP include as a food additive, dietary supplement, paint remover, and detergent or industrial cleaner. The beneficial effects of phosphates on food products include: (i) water binding, (ii) retardation of oxidative rancidity,

(iii) emulsification, and (iv) color development and stabilization (87). The ability of phosphates to enhance microbial safety and stability of certain food has been discussed by Wagner (87).

The AvGard™ process is an USDA-approved process where TSP solution is applied to reduce the incidence of Salmonella contamination during poultry processing (88). The AvGard™ process was the first commercial process for which a claim of successfully reducing Salmonellas (2 to 5 log decrease) on processed carcasses can be made (86). The process has been patented for poultry (86), specifically covering solutions of trialkali metal phosphate in concentration of 4% (Wt./Vol.) or greater with the pH values of 11.5. TSP in solution results in an alkaline pH which contributes to its antimicrobial action. Exposure of *Salmonella enterica* serovar Enteritidis ATCC 4931 to different concentrations of TSP resulted in membrane damage and leakage of cellular contents leading to cell death in a concentration dependent manner (89). The antimicrobial action of TSP treatment has been reported to successfully reduce numbers in *E. coli*, *Campylobacter jejuni* and *L. monocytogenes* (90). The treatment with TSP is as effective as alkali treatment without the adverse effects on the meat or skin associated with inorganic alkali treatments (90). The process was also found to have little or no effect on the taste, texture, and appearance of poultry (91). The phosphate residue can therefore be left on the poultry surfaces to provide reduced bacterial activity and improved shelf life (86). Efforts to extend the efficacy of TSP, using combinatorial or sequential approaches (i.e., EDTA and TSP) have been performed. Korber et al. (92) examined a process termed plasmolysis-deplasmolysis, where cells were plasmolyzed in the absence of the antimicobial agent using high salt solutions, and then deplasmolyzed in the presence of TSP. This approach did reduce the TSP concentrations required against a panel of pathogen and spoilage organisms in model systems, but failed to universally enhance TSP efficacy on meat tissues.

C. Biological Antibacterial Products

There are several naturally synthesized antibacterial products that are efficient, and often effective means for the control of foodborne bacteria. The choice for the type of agents may depend on several factors including cost, availability, compatibility and safety for human consumption. Certain plant synthesized products, e.g. tannins (93) in addition to having antimicrobial properties carry other properties such as antinutritional factors and antiinfective agents. Tannins are water-soluble polyphenols found in many plant foods. Tea polyphenols and many tannin components were suggested to be anticarcinogenic. The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins and tannic and propyl gallate are inhibitory to foodborne bacteria and off-flavor-producing microorganisms. Their antimicrobial properties seemed to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. Tannic acid is used in food processing to increase the shelf life of certain foods, such as catfish fillets. Tannis also play a role in detaching attached bacteria from surfaces, and thus act by both removing bacteria and by killing the bacteria.

D. Lactic Acid Bacteria Derived Peptides

Nisin is an antibacterial peptide (35 kDa) produced by the dairy fermentation bacterium *Lactococcus lactis* subsp. *lactis*. It exhibits a broad spectrum of inhibitory activity against gram-positive microorganisms such as *L. monocytogenes*, *S. aureus* and *Clostridium botulinum* (94). Although nisin was once thought to inhibit only gram-positive bacteria, researchers have found that the spectrum of nisin activity can be extended to gram-negative bacteria, including a variety of pathogens. Stevens et al. (95) reported that when gram-negative cells were treated with a combination of 20 mM EDTA and 50 μg/ml of nisin in a complex buffer and incubated at 37°C for 60 min, populations of *Salmonella* sp., *Enterobacter aerogenes*, *Shigella flexineri*, *Citrobacter freundii*, and *E. coli* O157:H7 were reduced by 3 to 6 log. In this process, EDTA is thought to chelate magnesium ions from the lipopolysaccharide (LPS) layer of the outer membrane of gram-negative bacteria. This causes the outer cell surface to be more permeable, thereby allowing the bacteriocin access to the cytoplasmic membrane where nisin-mediated inactivation occurs (95). Compounds such as 1% sodium hexametaphosphate or 500 mM lactate, as well as EDTA (50 mM) or citrate (100 mM), have been reported to enhance nisin activity against gram-negative pathogens *in vitro* (96). Synergistic effects between nisin and thymol on antimicrobial activities in *L. monocytogenes* has also been shown to be useful in certain traditional fermented dairy and cultured-milks. There is an additional need for methods to substantially reduce or inhibit food-borne and biofilm bacteria using food-grade compounds. These methods would be of considerable interest to the food industry.

E. Lactoferrin

Lactoferrin is an iron-binding glycoprotein present in milk and many exocrine secretions that bathe the mucosal surface. Lactoferrin contains 703 amino acids and has a molecular weight of 80 kDa. Lactoferrin co-ordinately binds to metal ions and occurs in divergent biological milieu including saliva, tears, seminal fluids, mucins, and the secondary granules of neutrophils. Lactoferrin is

considered a multifunctional or multi-tasking protein. It appears to play several biological roles. Owing to its iron-binding properties, lactoferrin is thought to play a role in iron uptake by the intestinal mucosa of the suckling neonate. Lactoferrin also appears to have antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immunomodulatory activities. A number of laboratories have identified lactoferrin as a broad spectrum antimicrobial and reported a variety of inhibitory mechanisms on both Gram-positive and Gram-negative bacteria (97). Lactoferrin occurs naturally as iron saturated, iron free and immobilized/activated forms. The iron free and immobilized forms of lactoferrin have the highest antibacterial ability. However, the current processes used to isolate lactoferrin from cow's milk alter the protein structure so that the majority of lactoferrin that is extracted is in the less effective, iron saturated state. "Activated" lactoferrin is a term used to describe a unique combination of natural ingredients that mimic the optimum environment necessary for lactoferrin to have maximum antimicrobial activity. Activation biases lactoferrin to its iron free and immobilized forms, in effect returning lactoferrin to its most natural and functional state. Activated lactoferrin has been approved by the FDA and USDA for use on meat to prevent contamination by a number of foodborne pathogens, such as *E. coli* O157:H7. Activated lactoferrin is sprayed onto a beef carcass as one of the final steps on a multiple-intervention system. Activated lactoferrin reacts with pathogenic bacteria that are already attached to the meat surface and binds with them causing their detachment. Once the activated lactoferrin has been applied, it is followed by a final rinse, allowing for the detached bacteria to be washed away safely. Activated lactoferrin protects meat from pathogenic bacteria in three ways: 1) detachment of adherent pathogens, 2) prevention of pathogen attachment by rendering bacterial attachment structures non-reactive, and 3) inhibition of pathogen growth through the binding of iron required by bacteria for growth (98). Sing et al. (99) showed that lactoferrin can reduce bacterial colonization and biofilm development in a dose-dependent manner. This suggests that lactoferrin could be used as an antimicrobial agent to maintain hygiene on food processing equipments. Exploring the potential of such methods is clearly of interest to those striving to maintain and improve processing plant hygiene and sanitation.

F. Antibacterial Plant Peptides

Antibacterial plant peptides are short peptides that are produced by plants to defend themselves through restricting attack or invasion of bacteria. Several hundred peptide antibiotics are produced by a broad range of plant species as defense molecules. Plant antimicrobial peptides are approximately 50 amino acids in length and contain cysteine residues that allow for the formation of disulfide bonds, which contribute to their high stability (100–102).

TABLE 84.1
Known *In Vitro* Antibacterial Activity of Different Plant Antimicrobial Peptides. IC50 Equals the Concentration Required for 50% Growth Inhibition of the Test Organism

	Amount of AMP Needed for 50% Growth Inhibition	
Test Bacteria	***M. jalapa* (AMP2)**	***I. balsamina* (Ib-AMP4)**
Bacillus megaterium	2	—
Bacillus subtilis	—	20
Clavibacter michiganensis	—	<10
Erwinia amylovora	—	>100
Erwinia carotovora	>500	—
Escherichia coli	>500	>500
Micrococcus luteus	—	5
Proteus vulgaris	—	>500
Pseudomonas solanancearum	—	>100
Sarcina lutea	50	—
Staphylococcus aureus	—	20
Streptomyces faecalis	—	5
Xanthomonas campestris	—	6
Xanthomonas oryzae	—	15

Plant-based antimicrobial peptides are classified into three groups, antifungal peptides, factors against microbial toxins (e.g., tablotoxin), and those determining the need for synthesis of other natural defense products. The genetic expression of plant antimicrobial peptides can be adaptive or constitutive. The constitutive ones are found in herbs and spices, e.g. phenolics, essential oils (allicin) and various thiocyanates. The most abundant source of these products appears to be the seeds; however, they have been isolated and purified from the seeds, kernels and roots. Table 84.1 shows the antibacterial potency of these proteins *in vitro.* Biotechnology should not only help in our understanding of the mechanisms of action, but also in application of these molecules in the production and processing of foods. A large number of AMPs have not yet been evaluated against organisms of concern to the food processing sector, thus there is potential for these compounds as antimicrobial agents.

G. Surface Antimicrobial Agents

Newer types of food-packaging material, which uses combinations of food-packaging materials (plastic and rubber articles, paper-based materials, textile fibrils, and a low-density polyethylene film with triclosan and chitosans as a choice antimicrobial substance is being used to control microbial surface contamination of foods. This strategy can be used for vacuum packaged meat, fish, poultry or

cheese. Many classes of antimicrobial compounds have been evaluated in both synthetic polymer and edible film states, including organic acids and their salts, enzymes, bacteriocins, and miscellaneous compounds such as triclosan, chitosan, silver zeolites, and fungicides. In the adoption of antibacterial agents in wrapping material, special consideration is needed as the presence of fatty acids or adipose tissues may diminish the antimicrobial activity of certain compounds on meat surfaces. Chitin and chitosan matrices are known for their adsorptive capacities, however their potential as antimicrobial coatings has only recently been considered. While these results are preliminary, edible chitosan coatings (e.g., hotdogs or deli-products) may offer some protection against food-borne pathogens like *L. monocytogenes*. At this time, antimicrobial packaging materials cannot legally be used in the EU. The state of this technology for its particular commodities and use of different types of antimicrobial concepts is under research and development (103, 104).

H. Surface Sanitizing for Biofilm Control

The general approach for cleaning food processing surfaces should be inherently the same. First to be addressed is the problem of surface soil or fouling. Following an initial rinse, surface fouling is best addressed by physical cleaning or pressure washing using either an acidic or alkali based detergent — acidic detergents dissolve fats and mineral buildup, whereas alkali based detergents dissolve microbial polymeric substances. This wash step must be followed by a rinse which will remove any residual detergent that might interfere with subsequent sanitizing steps. The surface is then treated with a sanitizing agent, which typically involves use of chlorine-based or peroxyacetic acid (POAA) treatments. The POAA treatment may be left on as peroxide and acetic acid are food grade additives, whereas chlorinated compounds must be rinsed off — this is important not only because of food flavor effects, but also because strong alkaline compounds tend to corrode even food grade stainless steels. All the rinsing steps should use potable water. The incorporation of novel antimicrobial agents such as surface-incorporated polychlorinated compounds (triclosan) is a somewhat different approach — it would tend to prevent initial colonization and growth by biofilm forming bacteria. However, both advocates of this type of antimicrobial approach insist that this doesn't eliminate the need for proper sanitation and/or hygiene, but rather must be complimentary. Such considerations are best made during the planning stage of processing plant construction; the appropriate type of material or equipment design should be selected to prevent microbial adhesion and facilitate cleaning. In cases where an existing facility is of concern, a suitable starting point involves a microbiological audit in order to determine where biofilms are likely to occur followed by strict HACCP implementation.

IX. CONCLUSION

Microorganisms have been observed to adhere and aggregate on surfaces, grow into microcolonies, form complex 3-dimenisonal structures and communal relationships, resulting in complex biofilm. Our knowledge of biofilm comes from studies of nutrient limited environments or simulations of these environments. However, very little understanding regarding the kind of biofilm found in the food industry is available because of a lack of direct observation of biofilms in these facilities or research using appropriate model systems. Foodborne pathogens and spoilage organisms can form biofilm on many food contact and environmental surfaces and can coexist with other environmental flora leading to contamination of food that come in contact with them. This challenges the plant hygiene as well as safety of the food product and can lead to significant loss to business and public health. Biofilms on food processing surfaces are difficult to control due to the problem associated with cleaning complex food processing equipment, and also because biofilms are quite resistant to antimicrobial agents in general. Thus, biofilm control relies on the implementation of effective cleaning and sanitation procedures employed in processing facilities specifically designed for effective and thorough cleaning.

Currently, there is an increased interest in methods to monitor biofilm development on surfaces, as well as to develop surfaces with functional antimicrobial coatings to inhibit bacterial colonization. There is also a seemingly prosperous research direction involving the design and engineering of novel antimicrobial agents. As much of this research is ongoing, there is no universal measure to control biofilms. At present, education and vigilance are the best tools for ensuring food safety and plant hygiene.

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85 Antioxidants

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CONTENTS

I. INTRODUCTION

A. General

This review covers the chemistry, in particular the mode of action, the analysis and the evaluation of the efficacy of antioxidants. It briefly touches on synergism in antioxidant mixtures and the legal implications of addition of antioxidants to foodstuffs. At the outset it should be noted that the medical and economic aspects are scarcely mentioned as it falls outside the expertise of the authors.

The word antioxidant is not found in English dictionaries of the first half of the last century. One of the earliest definitions of an antioxidant appears in the 1966 edition of the *Random House Dictionary of the English Language* that reads "Any of a group of substances that inhibit the deterioration of rubber, gasoline, soaps, etc." It is noteworthy that at that time no mention was made of the deterioration of foodstuffs although the prevention of this now occupies the energy and ingenuity of many scientists. The *Cambridge Encyclopaedia* of 1990, however, gives the following definition: "Substances which slow down the oxidation of others, often by being oxidized themselves. The term is usually applied to additives in foods and plastics" clearly illustrating the shift in emphasis from rubber, gasoline, and soaps to foods and plastics.

B. Chemistry of Action

Upon exposure to atmospheric oxygen, lipids (fats and oils) are slowly oxidized to peroxides. This reaction is of immense practical importance as it leads to the spoilage of foodstuffs and deterioration of tissues in living organisms (ageing) (1,2). Even freeze-dried foods can be spoilt by peroxides formed during cold storage. These peroxides are the reaction products of free radicals and atmospheric oxygen. Free radicals are uncharged, highly reactive chemical entities with a lone electron that are initially formed by the loss of a hydrogen atom from a lipid fatty acid moiety induced by ultraviolet light or heat. Once generated, these reactive free radicals propagate themselves by a theoretically endless chain reaction involving oxygen unless halted by an antioxidant. Antioxidants act sacrificially by donating a hydrogen atom to the reactive free radical and regenerating the original molecule. In the process, the chain reaction is terminated and the antioxidant itself becomes a free radical. The difference, however, is that this free radical is unreactive as antioxidants possess structures capable of delocalizing the lone electron, thus rendering it unreactive, and no further damage to the lipid or tissue is done. This is illustrated in the following simplified reaction scheme:

1. Initiation
   $RH \longrightarrow R^{\bullet} + H^{\bullet}$
2. Propagation
   $R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet}$
   $RO_2^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$
3. Termination
   $\left.\begin{array}{l} R^{\bullet} + R^{\bullet} \\ RO_2^{\bullet} + RO_2^{\bullet} \\ RO_2^{\bullet} + R^{\bullet} \end{array}\right\}$ products

In the propagation step ROOH is a hydroperoxide and $\dot{R}\ddot{y}$ is either another reactive free radical as in the initiation step, or an unreactive free radical if RH is the antioxidant. The primary reaction products, hydroperoxides ROOH and peroxides ROOR then decompose further into secondary products such as aldehydes, ketones, alcohols and carboxylic acids. These secondary oxidation products are responsible for the unpleasant taste and odours of spoilt foods, variously described as "rancid," "tallowy" or "fishy." But it is not only the oxidation of the fat itself which is important; other vulnerable components in the foods such as vitamins, pigments and even proteins are liable to be destroyed as a consequence of lipid oxidation. Vitamins and pigments are normally minor components of foods but they are important in determining its appearance, palatability and nutritional value thus damage to them can easily make the food unacceptable. This deterioration is not confined to foods of high fat content, it can also occur in cereals that contain relatively little fat, vegetables such as unblanched frozen peas, and potato chips. This is because the flavour threshold of the aldehydes and ketones that are formed is often less than 1 part per million (ppm), sometimes as low as 1 part per billion (ppb). Such traces, that can wreak havoc to the economy of food companies, are readily detectable by the human senses of taste and smell (3). It must also be realized that research on antioxidants is never straightforward: antioxidants are anomalies in that they do not follow rules and nothing is ever predictable, but it is precisely this unpredictability which constitutes the challenge and attraction of this field of research. Much has been learned over the years by empirical methods of trial and error and we are now able to protect valuable foodstuffs much more effectively than half a century ago.

C. Chemical Structures

The question may now be asked: "What are the structures of those compounds, small amounts of which are capable of stabilizing foodstuffs?" Several structures are known and occasionally new structures are discovered, as for instance those incorporating the indole and indoline chromophores (4). However, because of the very stringent safety requirements of the United States Food and Drug Administration (FDA), it is doubtful whether these novel antioxidants will eventually be approved for the use in foodstuffs. Most antioxidants both synthetic and natural possess an aromatic ring.

D. Synthetic Antioxidants

Structures such as hydroquinone [1,4-dihydroxybenzene; **1**], *t*-butylhydroquinone [TBHQ; 1,4-dihydroxy-2-*t*-butylbenzene; **2**], butylated hydroxytoluene [BHT; 3,5-di-*t*-butyl-4-hydroxytoluene; **3**], butylated hydroxyanisole [BHA; a 9:1 mixture of 3-*t*-butyl-4-hydroxyanisole and 2-*t*-butyl-4-hydroxyanisole; **4**] and ethoxyquin [EQ; 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline; **5**] (Figure 85.1) are examples of synthetic antioxidants. These antioxidants do not occur naturally and they act in very small doses, typically ppm. Since the parent compound, hydroquinone, is a suspected carcinogen it is not permitted as an antioxidant in food. However it is used as an antioxidant in rubber and in the preparation of methyl esters of highly unsaturated lipids (5,6). TBHQ [**2**] and BHT [**3**] are pure chemical compounds, properly characterized with sharp melting points of 128–129° and 69–70°C respectively. Food grade BHA [**4**] is normally a 9:1 mixture of the 3- and 2-isomers and shows a melting range of 48–55°C. The ratio in which they are produced from 4-hydroxyanisole and isobutene is 9:1 and it appears uneconomical to separate the two. The bulky *t*-butyl groups greatly enhance the antioxidant character of these compounds by inhibiting other molecules from approaching

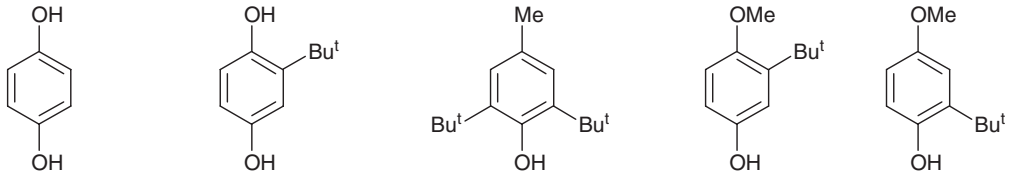


FIGURE 85.1 Synthetic antioxidants.

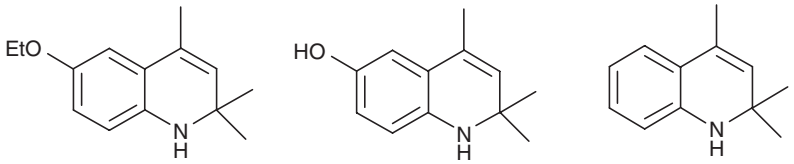


FIGURE 85.2 Ethoxyquin and two analogues.

the active site. Other examples of unreactive hindered free radicals are known, for example, the triphenylmethyl radical (7) and 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) of melting point 36–40°C that can even be purified by sublimation and is listed in various commercial catalogues. TBHQ is the latest and also the best phenolic antioxidant for oils and is often used for prolonging the shelf life of potato crisps (8). However, it is a poor antioxidant for fish meal (A.J. de Koning, unpublished work). BHT, prepared from 4-hydroxytoluene and isobutene, was originally an antioxidant in the petroleum industry and as a result suffers from a slight stigma. In spite of this it is very effective in protecting animal fats and is used often in combination with BHA, resulting in an antioxidant mixture that shows synergism (see below) (8). BHA, on the other hand has no stigma and is normally the antioxidant of choice in the food industry, where it finds extensive use in oils for deep-fat frying. Both BHT and BHA are volatile in steam, which is a disadvantage when using them at high temperatures. These phenolic antioxidants have degradation products that act as powerful antioxidants in their own right, some possessing greater efficacy than the parent compound. Japanese workers in particular have extensively investigated these products (9,10,11). This, however, was found to depend on the substrate used for testing the degradation products.

EQ [**5**], an antioxidant used in spice blends, as a postharvest dip for apples and to prevent spontaneous combustion in fish meal, also has a benzene ring and its structure is reminiscent of vitamin E [**9**]. Its antioxidant activity centres on the NH grouping which donates its hydrogen atom forming an unreactive free radical stabilized by delocalization in the benzene ring assisted by the ethoxy group in position 6.

TABLE 85.1
Efficacy Values of a Number of Antioxidants Relative to Ethoxyquin in Fish Meal and Fish Oil

	Efficacy Value (%)	
Antioxidant	**Meal**	**Oil**
Ethoxyquin (**5**)	100	100
Butylated hydroxytoluene (**3**)	65	75
Hydroxyquin (**6**)	74	350
Hydroquin (**7**)	101	52
Methoxyquin (**11**)	105	100
Propoxyquin	n.d.*	100
i-Propoxyquin	n.d.	100

* n.d. means not determined.

Analogues of EQ have been prepared and their efficacies compared to EQ (12,13,14). It was found that the efficacies in oil and meal were, for unknown reasons, often markedly different. One analogue, hydroxyquin [1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline; [**6**] (Figure 85.2), where a hydroxyl replaces the ethoxy group, was 3.5 times as effective in fish oil as EQ, while in fish meal it had only three quarters of its activity. On the other hand, hydroquin [1,2-dihydro-2,2,4-trimethylquinoline; **7**] the simplest analogue, was equally effective as EQ in meal but in oil it showed only half its efficacy. BHT only showed about 75% and 65% of the efficacy of EQ in oil and meal respectively. This is recorded in Table 85.1. Oxidation of EQ gives rise to two products that have been detected in aged fish meals originally treated with EQ. These products, a dimeric oxidative coupling product and a quinolone, both showed antioxidant activity; the dimer

having about 30% and the quinolone about 75% of the activity of EQ (15).

E. Natural Antioxidants

The naturally occurring antioxidants, vitamin C or ascorbic acid [**8**] and vitamin E or tocopherol [**9**] (Figure 85.3), are generally preferred by the public due to negative perceptions surrounding the use of synthetic chemicals in foodstuffs. The efficacy of these natural antioxidants is, however, sometimes far less than that of their synthetic counterparts. For example, 2% vitamin E gave much less protection of the polyunsaturated fatty acids [PUFA] in dietary fish oil capsules than 100 ppm of synthetic dodecyl gallate (16). This observation seems to contradict the results of Burton and Traber who claim that vitamin E is superior to phenolic antioxidants (17). However, this only seems to be a contradiction as Burton and Traber refer to the *in vivo* efficacy of vitamin E whereas in the protection of fish oil capsules the *in vitro* efficacy was measured.

Vitamin C [**8**] is a pure chemical compound with a sharp melting point of 190–192°C, and it is water-soluble in contrast to the above mentioned oil-soluble antioxidants. It is abundantly present in citrus fruits and a variety of vegetables. It has no aromatic benzene ring to delocalize a lone electron, however careful scrutiny of its structure shows two enolic hydroxyl groups (which are responsible for its acidity), similar to a phenolic hydroxyl group in TBHQ, BHT and BHA. Removal of a hydrogen atom from an enolic hydroxyl group creates a free radical which can be stabilized by delocalization, albeit not as extensively as in a benzene ring. Vitamin C is used to prevent browning in fruits and vegetables, to preserve the aroma and colour of fruits and to improve the shelf-life of beer. This form of spoilage is due to oxidation of phenolic substances under the catalytic influence of traces of copper. It can therefore also act as a chelating agent and enhance the action of other antioxidants (18,19). In living organisms vitamin C acts as a back-up of vitamin E which it regenerates after this has been used up by oxidation (20). To render vitamin C more soluble in oil the semi-synthetic derivative, ascorbyl palmitate has been made and this is frequently used to stabilize oils and fats. For instance 0.01% ascorbyl palmitate has been shown to be more effective than BHT or BHA in stabilizing vegetable oils (21).

Vitamin E or tocopherol [**9**] has four different isomers α, β, γ and δ. These isomers have different antioxidant activities, the α isomer being the most potent and also the most naturally abundant. Foods of both animal and vegetable origin contain tocopherols, but it is mainly in vegetable foods, in particular seeds, that substantial amounts occur. Seed oils are a rich source of tocopherols, with the α isomer occurring at a level of approximately 600 ppm in refined sunflower seed oil, whereas the δ isomer is often not detectable (22). Large amounts (between 30 and 70%) of the tocopherols are lost during refining of these seed oils, especially in the vacuum steam deodorizing process (23). The distillate thus becomes a rich source of tocopherols but extensive purification is required to isolate them (24). In the body vitamin E is present in the lipid bilayers of biological membranes where it plays a structural role. It used to be "the vitamin in search of a disease" but it is now known as a powerful *in vivo* antioxidant to prevent the oxidation of unsaturated lipids to peroxides (25). Peroxides can cause extensive damage to cells when insufficient vitamin E is present. Increasing the polyunsaturated fatty acid (PUFA) content of a diet, which is frequently suggested by medical practitioners, should ideally be accompanied by a simultaneous increase in vitamin E and at least 0.6 mg of vitamin E per g of linoleic acid is recommended (26). Vegetable oils having high concentrations of linoleic and linolenic acid fortuitously also have sufficiently high levels of vitamin E to give adequate protection.

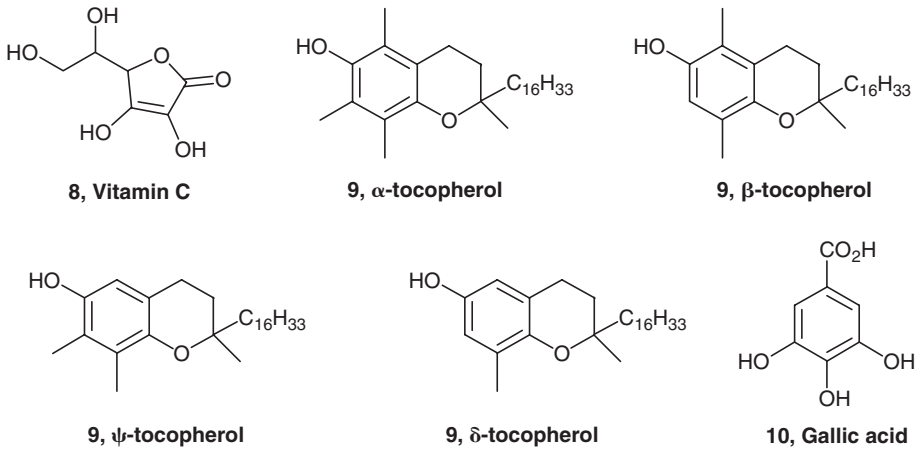


FIGURE 85.3 Natural antioxidants.

However, consuming fish oil capsules as a dietary supplement with high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) without an additional dosage of vitamin E might lead to cell damage. It is recommended therefore that for each gram of DHA in a fish oil dietary capsule at least 1.8 mg of vitamin E should be added (27).

Propyl, octyl and dodecyl gallate are esters of 3,4,5-trihydroxybenzoic acid [**10**] or gallic acid (Figure 85.3), a naturally occurring substance abundantly present in tannins; for instance tea leaves contain about 10% gallic acid derivatives. Gallic acid itself acts as an antioxidant, but it is not used as such because of its insolubility in oils and fats. The esters do not occur naturally and must be prepared by esterification of gallic acid with the appropriate alcohol. They are therefore neither synthetic nor natural but hybrids, similar to ascorbyl palmitate. No bulky *t*-butyl groups are present in these gallates, nevertheless they act as powerful antioxidants for the protection of fats, oils and margarine in snack foods, for instance (8).

II. ANALYSIS OF ANTIOXIDANTS

A. General

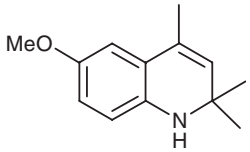
Food laboratories mostly have their own in-house methods for the analysis of antioxidants. These normally originate from procedures of the American Oil Chemists' Society (AOCS), the Association of Official Analytical Chemists (AOAC), the International Union of Pure and Applied Chemistry (IUPAC), or the British Pharmacopoeia. It should be emphasized, however, that procedures of these professional societies are not necessarily the best and most convenient for a particular analysis in a laboratory. A literature search can sometimes unearth a more suitable procedure, or a novel method may even be devised by an enterprising scientist. A few selected procedures will be briefly discussed, mainly concentrating on the principles involved.

B. TBHQ, BHT, BHA, and Gallates

The phenolic antioxidants and the gallates are usually determined by High Performance Liquid Chromatography (HPLC). The food sample is extracted with hexane and the antioxidant in the hexane is quantitatively extracted into multiple volumes of acetonitrile, which is immiscible with hexane. The acetonitrile is concentrated, an aliquot injected into the chromatograph and the antioxidants eluted with a mixture of acetonitrile and aqueous acetic acid. A standard mixture of antioxidants is treated in the same manner, and each one quantified by UV detection at 280 nm. Excellent separation of the antioxidants can be achieved by selecting the correct HPLC column and eluting solvent (28). However, the accuracy of this analysis could be much improved and time and energy saved if a suitable internal standard were adopted. Replacing the *t*-butyl group with an *i*-propyl group in the three phenolic antioxidants seems to provide an ideal internal standard and it is suggested that suppliers of fine chemicals prepare these analogues with the prediction that they will find a ready market. Recently, a convenient and rapid gas chromatographic method for the determination of TBHQ, BHT and BHA in fats and oils was developed in Taiwan. The sample is simply dissolved in diethyl ether, the internal standard 8-hydroxyquinoline (oxine) is added and the mixture injected into a gas chromatograph fitted with a capillary column. As the fats and oils are injected together with the antioxidants the capillary column will undoubtedly need frequent replacement (29). Earlier determinations of BHT and BHA were based on the volatility of these phenolic antioxidants in superheated steam. After separation from the non-volatile food residues with superheated steam, the antioxidant in the distillate is determined spectrophotometrically at 515 nm using the ferric chloride-2,2-dipyridyl reagent for BHT and at 620 nm using the 2,6-dichloroquinonechloroimide reagent for BHA. This method is still the official IUPAC procedure (30).

C. Ethoxyquin

Ethoxyquin (EQ) can be determined in foodstuffs and fish meals by gas chromatography making use of the internal standard methoxyquin (MQ; 1,2-dihydro-6-methoxy-2,2,4-trimethylquinoline [**11**]) (Figure 85.4) (15). An accurate amount of MQ is added to the weighed sample, which is then treated with hexane and filtered. The hexane extract is subsequently washed with 1M HCl, separating the EQ and MQ into the aqueous phase, as EQ and MQ are organic bases and thus soluble in aqueous HCl. The HCl solution is made alkaline and the EQ and MQ extracted back into hexane. This solution, after concentration, is injected into the gas chromatograph. Gas chromatography on almost any column perfectly separates both compounds and EQ is quantified by comparing peak areas. Earlier methods separated EQ from other components by liquid chromatography over a column of alumina. The EQ is eluted with a mixture of hexane: diethyl ether (9:1, by vol.), while the progress of the elution is followed with an UV lamp, as EQ is fluorescent, and the EQ is collected in a volumetric flask. The absorbance of the solution is read at 362 nm and using its molar absorptivity of



11, MQ

FIGURE 85.4 Internal standard in EQ analysis.

3254 moles^{-1} l.cm^{-1} the EQ content of the sample is calculated (14,15). Other methods make use of the fluorescing characteristic of EQ and minute residual quantities (of the order of 0.05 ppm) of EQ in chicken tissues and eggs were determined in this manner (31).

D. Vitamin C

Vitamin C is a mild reducing agent and can be determined simply and inexpensively in, for instance, fruit juices by titration in strong aqueous acid (>3M HCl) with the oxidizing agent potassium iodate using starch and a small amount of chloroform as indicator. Initially iodate is reduced to iodine by the vitamin C, but when this is consumed, oxidation of iodine to ICl takes place. The end point of the reaction is signalled by complete disappearance of the iodine shown by decolorization of the purple starch-iodine colour of the chloroform phase (32). A more recent method relies on the fluorescence of vitamin C on reaction with *o*-phenylenediamine (1,2-diaminobenzene). The fluorescence is read on a fluorometer and standard solutions of vitamin C are used to quantify the results (33). This sophisticated analysis yields results for the vitamin C content of fruit juices comparable to the simple titration procedure just mentioned.

A gas chromatographic procedure making use of the volatility of the tetra-*O*-trimethylsilyl ether derivative of vitamin C using octadecane as internal standard has also been described (34). This method does not elaborate on the difficulty of identifying vitamin C amongst the multiplicity of carbohydrate peaks present when determining it in food stuffs. A similar gas chromatographic method also determines vitamin C in food products by extraction in 95% ethanol followed by precipitation with lead acetate, addition of the internal standard glutaric acid, and conversion into its tetra-*O*-trimethylsilylether. This publication shows a chromatogram illustrating the multitude of peaks accompanying the vitamin C and glutaric acid signals (35).

E. Vitamin E or Tocopherol

A vast amount of work has been done on the analysis of tocopherols, as shown in a recent review (36). The IUPAC, the AOAC and the AOCS have published their own methods (37,38,39), all of which rely on the fact that tocopherols end up in the non-saponifiable fraction of the substance to be analysed. For example in the IUPAC method (37), tocopherols are determined in the non-saponifiable material by capillary gas chromatography of their respective trimethylsilyl ethers at a temperature of 250°C. The internal standard squalane (a high molecular weight hydrocarbon) is used to quantify them. However, it must be realized that squalane, which is not an isomer of vitamin E, is only added at the final stage of the analysis. This means that all chemical manipulations prior to the chromatographic separation must be carried out quantitatively. As with the phenolic antioxidants, if a suitable isomer of vitamin E were to become available (for instance, the ethyl analogue of tocopherol) a great deal of time and energy could be saved by using it as an internal standard. An alternative IUPAC procedure determines vitamin E in the non-saponifiable material by thin layer chromatography on silica gel. The spots, after visualization under UV light, are cut out, extracted into ethanol and the tocopherols determined colorimetrically at 520 nm with the ferric chloride-2,2-dipyridyl reagent (37). Other methods combine HPLC separation with fluorescence detection of vitamin E (40,41).

III. EVALUATION OF THE EFFICACY OF AN ANTIOXIDANT

A. General

The efficacy of an antioxidant depends, for as yet unknown reasons, not only on the antioxidant but also on the substrate that it is meant to protect. For instance, the efficacies of analogues of EQ are totally different in fish oil and fish meal (14). The selection of an antioxidant should ideally be based on its efficacy in a realistic situation using subjective criteria such as taste and smell. Unfortunately, time constraints often necessitate accelerated tests at higher than storage temperatures, which then serve as a guide. There are many methods for assessing the efficacy of an antioxidant and the Journal of the American Oil Chemists' Society has numerous papers dealing with ancient and modern methods. Marco, for example, lists eight different methods and then proceeds to add another one (42). A few selected procedures will be discussed in some detail.

B. Procedures for Evaluating Antioxidants

A frequently used method for determining the efficacy of oil-soluble antioxidants is the Active Oxygen Method (AOM) (43). In this method air is blown simultaneously into several oil samples held in glass tubes in a water bath maintained at a constant temperature, typically 50°C. The air flow is kept constant at 5 ml/second. Oxidation of the oil is followed by determining peroxide values of the oil at predetermined intervals, i.e. every 4 hours. A control oil together with a number of other oils containing the antioxidants to be tested is included in the run. The oil is normally a refined vegetable or animal oil, free of natural antioxidants. The efficacy of an antioxidant is obtained by determining the time taken for the antioxidant-treated oil to show a rapid increase in peroxide value compared to the control. This time is known as the induction time. The efficacy of the antioxidant is expressed as a difference (or a ratio) of the induction time of the control and the antioxidant-treated oil. There are a number of variables in this test which can be altered to suit almost any requirement, such

as the temperature of the water bath, the rate of air flow, and the level at which the antioxidant is tested. It is an accelerated test and often criticised for not being realistic. The test has the characteristics of a three-stage oxidation process, firstly, an induction period during which the peroxide value remains low and increases slowly over time, this is followed by a period in which the peroxide value increases sharply and finally a period in which the peroxide value decreases. It is, of course, only necessary to complete the first and second stages to obtain the efficacy of the antioxidant. Fully automated instruments have been marketed under the name Rancimat, rendering antioxidant assessment a routine matter. These Rancimats use either peroxide values or titration of volatile fatty acids formed during oxidation, to measure the efficacy of an antioxidant. A more realistic procedure for testing antioxidants in fish meal has recently been described (44). This test measures the remaining polyunsaturated fatty acids (PUFA) in the extracted meal lipids over a one-year storage period at 25°C. The efficacies of the different antioxidants are assessed by periodically determining the amount of PUFA in the residual meal lipids and calculating their average daily decrease. A control fish meal is included in the test. Lipid extractions are done at approximately bi-monthly intervals over a total period of about 12 months. At the end of the experiment, regression lines and correlation coefficients are calculated using at least 6 data points. Several analogues of EQ have been tested by this procedure. It was found that efficacies of the antioxidants in fish meal were entirely different from those in fish oil. Some comparisons are shown in Table 85.1. A rather interesting and rapid method for evaluating antioxidants is described by scientists from Monsanto. It involves the preservation of the yellow colour of β-carotene in a mineral oil film at 75°C. The preservation of the β-carotene colour, compared to a control, serves as an index of the efficacy of the antioxidant (45). A total of forty quinoline derivatives, including both ethoxyquin (**5**) and hydroquin, (**7**) were tested by this procedure. However, it failed to recognize hydroquin (**7**) as a potentially viable alternative to ethoxyquin (**5**) for the protection of the PUFA in fish meal (44,45), illustrating the importance of the substrate when testing antioxidants.

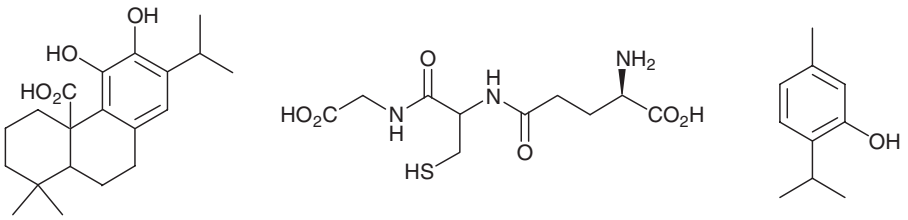
IV. SYNERGISM

The dictionary defines synergism as "the joint action of agents, such that the total effect is greater than the sum of the two effects taken independently," in other words "one gets something for nothing." Mixtures of antioxidants sometimes show synergism and have a higher efficacy than expected from the individual components. A 1:1 combination of BHT and BHA shows this and is very effective in stabilizing animal fat (8). However, it is prudent to carefully investigate every claim of a superior antioxidant mixture on the grounds of synergy, as frequently an expensive antioxidant is merely diluted with a cheaper one. For instance, no synergy was found between EQ and the less expensive BHT when tested with the active oxygen method in Marinol (a bleached and refined fish oil) (46), and neither was the proprietary mixture Oxiquin Super (EQ mixed with BHT and soyabean lecithin) found to be superior to EQ (47).

A curious fact emerges from the study of synergism. Since mixtures of antioxidants display synergism, a random occurrence, it follows logically that other mixtures will display antagonism, i.e. the joint action being less than the sum of the two effects thus offsetting the mysterious gains of synergism and, as it were, "balancing the books." One comes across it in the medical field where the public is warned that certain drugs are antagonistic towards each other. However, it appears that cases of antagonism remain unreported in the food world (no doubt due to the expense involved in reporting it). This is unfortunate, as it inhibits scientists from trying to unravel the as yet unexplained mystery of synergism, where seemingly the laws of thermodynamics are broken: "in synergism one gets something for nothing." Examples of chelation (sequestering or complexing) of metal ions are sometimes wrongly classified under the heading of synergism. Small amounts of metal ions such as copper and iron (as low as 0.02 ppm) often cause off-odours in milk and butter. These off-odours are due to the formation of minute amounts of complex ketones and aldehydes from the milk lipids under the catalytic influence of metal ions. Milk and milk products are particularly vulnerable as milk is deficient in natural antioxidants and therefore easily develops a "soapy," "fishy," or "metallic" taste. Complexing agents such as citric acid or ethylenediaminetetra-acetic acid (EDTA) are used to combat this type of spoilage (48,49,50,51). Another method to protect milk products is to mix them with cocoa butter, which has sufficient vitamin E to stabilize the butter fat (52). Instances of phospholipids acting as both chelating and solubilizing agents are reported and often an antioxidant mixture will contain phospholipids for this very purpose. The chelating action of the phospholipids seems to be confined to the phosphatidylethanolamine fraction while its solubilizing property is a characteristic of the whole phospholipid (53).

V. ANTIOXIDANT CONCOCTIONS

Modern consumers frequently shun synthetic antioxidants and demand natural ones. This had led to the proliferation of plant extracts claiming to have antioxidant properties similar or superior to synthetic ones. The chief difference between the natural antioxidants mentioned earlier and these concoctions is that the latter are not properly defined and are therefore not reproducible. If a plant extract shows antioxidant properties, the active ingredient should be isolated and characterized. This has been done for rosemary extracts



12, Carnosic acid **13, Glutathione** **14**

FIGURE 85.5 Antioxidants present in rosemary (**12**), vegetables (**13**) and eucalyptus leaves (**14**).

with the identification of carnosic acid (**12**) (54,55) and for broccoli, parsley and spinach with glutathione (**13**), a tripeptide, as the active ingredient (56). Similarly, thymol (3-hydroxy-4-*i*-propyltoluene) (**14**) has been identified as the main antioxidant in the volatile components of eucalyptus leaves (57) (Figure 85.5). In other cases, however, this identification is sadly lacking. A brief glance at recent titles in food journals will confirm this, and a cursory look on the Internet identified more than 482,000 publications dealing with these concoctions. It is the proliferation of these publications, which is the real danger. It is impossible to keep up with these claims, therefore those with real potential are in danger of being drowned in a flood of unverifiable claims.

VI. ANTIOXIDANTS AND THE LAW

Government regulations concerning the use of food antioxidants differ from country to country and are liable to be modified continually. Travelling through this maze of regulations is a lawyer's job, not a scientist's, although the scientist cannot escape the legal implications of work carried out in the laboratory. Antioxidants to be used in the food industry all aspire to receive GRAS (Generally Recognised As Safe) status. This is the brainchild of the U.S. FDA, and it has given GRAS designation to antioxidants in use prior to January 1, 1958, based on common knowledge accumulated through years of usage. Clearly it was not the intention of the FDA to ban all food antioxidants until they were shown to be safe, as this would have virtually halted the production of many foodstuffs. New antioxidants, however, must now undergo years of rigorous testing to ensure freedom from harmful effects. The European Union also evaluates antioxidants and, if approved, grants them *E numbers*, which must appear on food labels. Many of these *E number* antioxidants are the naturally occurring substances such as vitamins C [**8**] and E [**9**], which are often prepared using a synthetic route; others are not naturally occurring such as BHT [**3**] and BHA [**4**] (58). It appears that the practice of granting *E numbers* is gaining ground and is set to become the international standard.

A vast amount of work has been done to establish the toxicity of antioxidants. The results are surprising, as it was found that in large doses antioxidants, including BHT and BHA, might be the cause of cancer in animals. Evidently a number of antioxidants become pro-oxidants when used in large concentrations; even vitamins C and E are potentially toxic at high doses (59,60). The regulatory authorities, however, decided to permit the use of BHT [**3**], BHA [**4**] and the gallates [**10**] whilst keeping the situation under review as new research results become available (59). Since little is known of the long term toxicity of TBHQ [**2**], the latest and also the best phenolic antioxidant for oils and fats, it has been banned as antioxidant in foods in a large number of countries, including the U.S. (59). The European Union has followed suit and has not assigned an *E number* to it (58). Predictably, neither EQ (**5**) nor any of its analogues have received either GRAS or *E number* status. In spite of this, it is allowed in foodstuffs in many countries up to a certain maximum level differing from country to country. For instance, in Belgium, Denmark, Greece, Italy, Luxembourg, the Netherlands, and Spain it is not allowed in any food for human consumption. Sweden and the U.S., however, allow it at levels of as high as 100 ppm in spice blends and spice extracts, while in the United Kingdom only 3 ppm is allowed as a pesticide on apples and pears (14). This is just one example illustrating the complexity of the regulations governing food antioxidants with which some scientists are forced to familiarize themselves.

VII. CONCLUDING REMARKS

In our opinion, the cost of introducing new synthetic antioxidants still outweighs any potential benefits due to the stringent testing criteria that have to be met. This is in all probability the main reason for the phenomenal surge in research in the field of natural antioxidants and their mixtures. So far the results of these natural antioxidants have fallen dismally short of expectations, as they are unable to compete in efficacy and price with synthetic ones. However, as antioxidants are compounds that continue to surprise it is probably prudent to remain optimistic and say "in antioxidant research nothing is ever predictable."

On the other hand, in the medical arena great strides have been taken in the elucidation of the protection afforded by antioxidants *in vivo*, and this seems to be the area of research yielding the largest dividends in the future.

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86 Synthetic Colorants

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CONTENTS

I. INTRODUCTION

Color is one of the most important quality attributes of foods. No matter how nutritious, flavorful, or well textured, a food is unlikely to be eaten unless it has the right color. Color affects the overall judgments on the worth of food from both aesthetic and safety point of view. It plays an important role in taste thresholds, flavor identification, food preference, pleasantness, acceptability and, ultimately, food choice (10, 15, 28, 32, 47).

Specific colors of foods are often associated with freshness or ripeness. Redness of raw meat is associated with freshness, while brownish-red meat as not fresh. A green apple, tomato, or peach may be judged as unripe, although some apples are green when ripe (32, 44).

Colors also influence flavor perception. The consumer expects red drinks to be strawberry, raspberry, or cherry flavored, yellow to be lemon, and green to be lime flavored. The impact of color on sweetness perception has also been demonstrated (6, 40, 44).

Color in foods is due to naturally occurring pigments, which are normal constituents of cells. Many pigments are, unfortunately, unstable during processing and storage. Depending on the pigment, stability is affected by factors such as light, oxygen, heavy metals, oxidizing or reducing agents, temperature, water activity, and pH (6, 30, 44).

Colorants are added to foods to help preserve the identity or character by which foods are recognized; to intensify the natural color of food; to correct for natural variations in color; to ensure uniformity of color from batch to batch due to natural variations in color intensity; to restore the original appearance of the food when natural colors have been altered during processing and storage; and to enhance colors naturally occurring in foods in less intensity than the consumer would expect. They are also used to give an attractive appearance to certain colorless foods, such as imitation foods, and to help protect flavor and light-sensitive vitamins during storage via sunscreen effect. But it has also been used for adulteration purposes — to disguise food of poor quality, to mask decay, to redye food, to mask effects of aging or to simulate a higher biological value (4, 14, 32, 40).

II. HISTORY

The practice of coloring foods dates back to very early times. It is known from Pliny, that wine was colored through the use of smoke or aloe as far back as 200–300 BC. Pliny also noted the practice of adding seawater to wine to increase the color's brightness. As early as 1396, an edict was issued in Paris that forbade the coloring of

butter (5, 40, 46). The increasing use of colorants and other adulterants led Frederick Accum to publish in 1820 a treatise on adulteration of food and culinary poisons, in which he described, among other practices, the coloring of hedge leaves with verdigris to resemble green tea and the artificial greening of pickles and candies with copper salts (40).

Until the middle of the nineteenth century, the only coloring matters available were derived from natural sources of animal, vegetable and mineral origin (10, 46). The first synthetic organic colorant, mauve, was discovered in 1856 by Sir William Henry Perkin from the coal-tar derived methylanaline. This synthetic purple dye sparked not only the birth of the modern organic chemical industry, but also the quest for synthetic colorants (40). Soon after that, new and different colorants were available in a wide range of shades, higher tinctorial value and stability, more uniform, and more permanent than natural organic dyes and, in the opinion of some, were safer for use in foods than many of the mineral colorants in use at that time. These colorants were applied to foods immediately (5, 10, 27).

Accum's book initiated a flurry of investigations and reports on food adulterations and a number of treatise were published to prompt state government increasingly into legislating the safety of the food supply (40). The United States first legalized the use of synthetic organic dyes in foods by the act of Congress that authorized the addition of coloring matter to butter in 1886. The second came ten years later when Congress officially recognized coloring matter as a legitimate constituent of cheese. By 1900, Americans were eating a wide variety of artificially colored products, including ketchup, jellies, cordials, butter, cheese, ice cream, candy, sausage, noodles and wine. The rapid growth in the use of color additives was prompted not just by the availability of these new colorants but also by the numerous changes that were taking place in food technology. The development of food substitutes or imitation products such as margarine for butter, jellied glucose for jam, soft drinks for fruit juices, etc., created needs for colorants. Furthermore, the increased use of food preservatives, refrigeration, canning and large-scale food processing, all of which tended to alter the natural color of food products, demanded new approaches to restore the normal appearance of food products (27).

The abundant use of color additives was soon recognized as a threat to the public's health. Of particular concern was the fact that substances known to be poisonous were often incorporated into food and the colorants were frequently used to hide poor quality, to add bulk and weight to certain products and to pass off imitation foods as real. Another concern, besides the misuses of colorants, was that little or no control was exercised over the purity of the colorants added to foods. Towards the end of the 1800s, concern began over some of the dangerous and deceptive ways colorants were being used in foods and so a number of legal steps were taken. In the early 1900s, several European countries wrote laws that either outlawed the use of certain colorants in foods, or limited the colorants that could be used. At about the same time, state and municipal governments in the United States moved to control the use of colors in foods, too. American food manufacturers also moved to stop misuse of colorants in food. A circular was issued on 1899 by the executive committee of the National Confectioners' Association that enumerated 21 coal-tar colorants which were considered harmful and therefore unfit for adding to foods (10, 27).

Eventually, it became obvious that the individual efforts of industry and state and local governments were not enough to restrain the misuse of colorants in foods, and that some form of national government control was needed if the public's health was to be protected. The beginning of this control in the United States was probably 1862, when the Congress established the Department of Agriculture (USDA) and its Division of Chemistry, later named the Bureau of Chemistry (27, 40).

The Food and Drug Act of 1906 banned the addition of poisonous colors to confectionery products and prohibited the addition of colorants to foods for the purpose of concealing inferiority. That same year, Dr. Bernhard C. Hesse was hired to determine which colorants could be safely added to food and which restrictions should be placed on their use. Hesse began his work by examining the chemistry and the physiology of 695 colorants then known to be in use throughout the world for foods and by reviewing the laws of numerous countries that controlled their use. After much study, Hesse and the Bureau concluded that coal-tar dyes should not be used indiscriminately. They also concluded that only specific colorants that were proven to be safe should be permitted in foods, and that each batch of colorant intended for use in food should be tested and certified as pure before allowing its use (27, 31).

Based on these principles and taking into consideration recommendations of the National Confectioners' Association, the current needs of industry and suggestions by the German and American dye manufacturers, seven colorants, among them, amaranth, ponceau 3R, orange I, erythrosine, naphthol yellow S, light green SF yellowish and indigo disulfo acid sodium salt were permitted through the Food Inspection Decisions (FID 76, July 13, 1907). Furthermore, procedures for certification of these colorants were established (FID 77, September 25, 1907) and made it clear that each batch of dye had to be certified (27, 40).

Soon after Hesse published the list of permitted colorants, pleas began to expand it. In response to the appeals, testing began in 1915 and soudam I, butter yellow OB, and yellow AB were added to the list (FID 175, April 23, 1918). However, soudam I and butter yellow were delisted (FID 180, June 7, 1919) for causing severe contact dermatitis in workers handling the colorants (31). During the 1920s, the number of colorants on the list continued to grow, with the addition of guinea green B (FID 184, July 10, 1922),

fast green FCF (FID 207, April 9, 1927) and brilliant blue FCF, ponceau SX and sunset yellow (1929) (27).

The Food, Drug and Cosmetic Act of 1938, resulted in a number of important changes for color additives. It made certification mandatory for three specific certified categories: colors suitable for foods, drugs, and cosmetics (FD&C), colors suitable for drugs and cosmetics (D&C) and colors suitable for externally applied drugs and cosmetics (Ext. D&C). It made it clear that certification had to be on a batch-wise basis at the expenses of the industry. It also allowed manufacturers to add harmless diluents to colorants. In 1939, Food and Drug Administration (FDA) added orange SS, dipotassium salt of naphthol yellow S and oil red XO to the list and proposed a new systematic method for naming certified colorants — FD&C colorants (27, 40).

In January of 1950, FD&C violet No. 1 was added to the list. In the same year, three incidents involving excessively applied colorants in popcorn and candies prompted FDA to begin a more rigorous toxicological testing program (31). FDA ordered a complete retesting of all FD&C colorants, including two-year chronic oral toxicity experiments. While all the testing was going on, another event was taking place that would have significant effects on color additives — the hearings of the House Select Committee to investigate the use of chemicals in food products, whose chairman was James Delaney. These focused on the numerous chemicals being added to foods, including colorants which raised the public's concern regarding the safety of the food supply. In 1953, results of the new round of animal testing indicated that FD&C orange No. 1 and FD&C red No. 32 could cause adverse effects (18, 27). As a consequence, FD&C orange Nos. 1 and 2 and FD&C red No. 32 were delisted in 1958 and FD&C yellow Nos. 1, 2, 3 and 4 were delisted in 1959. In this same year, the Department of Health, Education, and Welfare submitted the Color Additives Amendment, which was approved in 1960. This amendment allowed for the continued use of existing color additives pending the completion of investigations to ascertain their suitability for listing as permanent colorants, and authorized the Secretary of Health, Education, and Welfare to establish limits of use for colorants, thus eliminating the harmless-per-se interpretation formerly employed. A special provision, commonly known as the Delaney Clause, specifically directed the Secretary not to list a color additive for any use if that colorant could be shown to induce cancer in humans or animals. Another feature of the Color Additives Amendments gave the FDA jurisdiction over all color additives by eliminating any distinction under the law between coal-tar colors and any other color additives, regardless of their source or method of manufacture. The Amendments also addressed the issue of the need to certify colorants such as beta-carotene by empowering the Secretary to decide which colors must be certified and which could be exempt from certification (27).

III. DEFINITIONS AND CLASSIFICATION

Color refers to the human perception of colored materials. Foods have color because of their ability to reflect different quantities of energy at wavelengths which stimulate the retina in the eye. The energy range to which the eye is sensitive is referred to as visible light. Depending on the individual's sensitivity, it encompasses wavelengths of approximately 380 to 770 nm, which is a portion of the electromagnetic spectrum. The perception of color results from differences in absorption of radiant energy at several wavelengths. If the shorter wavelengths of 400–500 nm are reflected to a greater extent than other wavelengths, the color is described as blue. Maximum reflection in the medium wavelength range results in green or yellow color, and maximum reflection at longer wavelengths 600−700 nm indicates red objects (8, 27).

According to the 1938 Act, a color additive is defined as "a dye, pigment or other substance made or derived from a vegetable, animal, mineral or other source and that, when added or applied to a food, is capable of imparting a color thereto" (15, 40). Even though colorants have been classified as natural or synthetic, the FDA does not recognize any category of colorant as being natural, as the addition of any colorant to food (natural source or nature-identical) results in an artificially colored product (32). Naturally occurring colorants or pigments are described in another chapter. Synthetic colorants or dyes are attractive to the food industry because they are superior to natural colorants in tinctorial power, consistence of strength, range and brilliance of shade, hue, stability and ease of application (2, 8). Synthetic colors provide a larger spectrum of colors. They also have lower prices and higher availability (32, 35).

IV. CHEMICAL AND PHYSICAL PROPERTIES

The synthetic food colors are categorized in different ways. The E or EEC references are the numbers allotted in the European Economic Community Directive of 23/10/62 to the coloring matters listed in Annex I of that Directive. The FD&C No. is a systematic method for naming certified colorants permitted in the United States. The CI Food Color Numbers are the Color Indexes established by the Society of Dyers and Colourists (United Kingdom) and the Society of Dyers Colorists of the United States (8).

Synthetic food colorants are also classified according to their chemical structures into the classes: azo, triarylmethane, xanthene, quinoline and indigoid compounds (39, 44). The chemical structures of some synthetic food dyes are shown in Figures 86.1 and 86.2.

The azo colorants comprise the largest group. They are characterized by the presence of the azo group (-N=N-) in association with one or more aromatic systems. There may be one or more azo groups present in the molecule. They are synthesized by the coupling of a diazotized

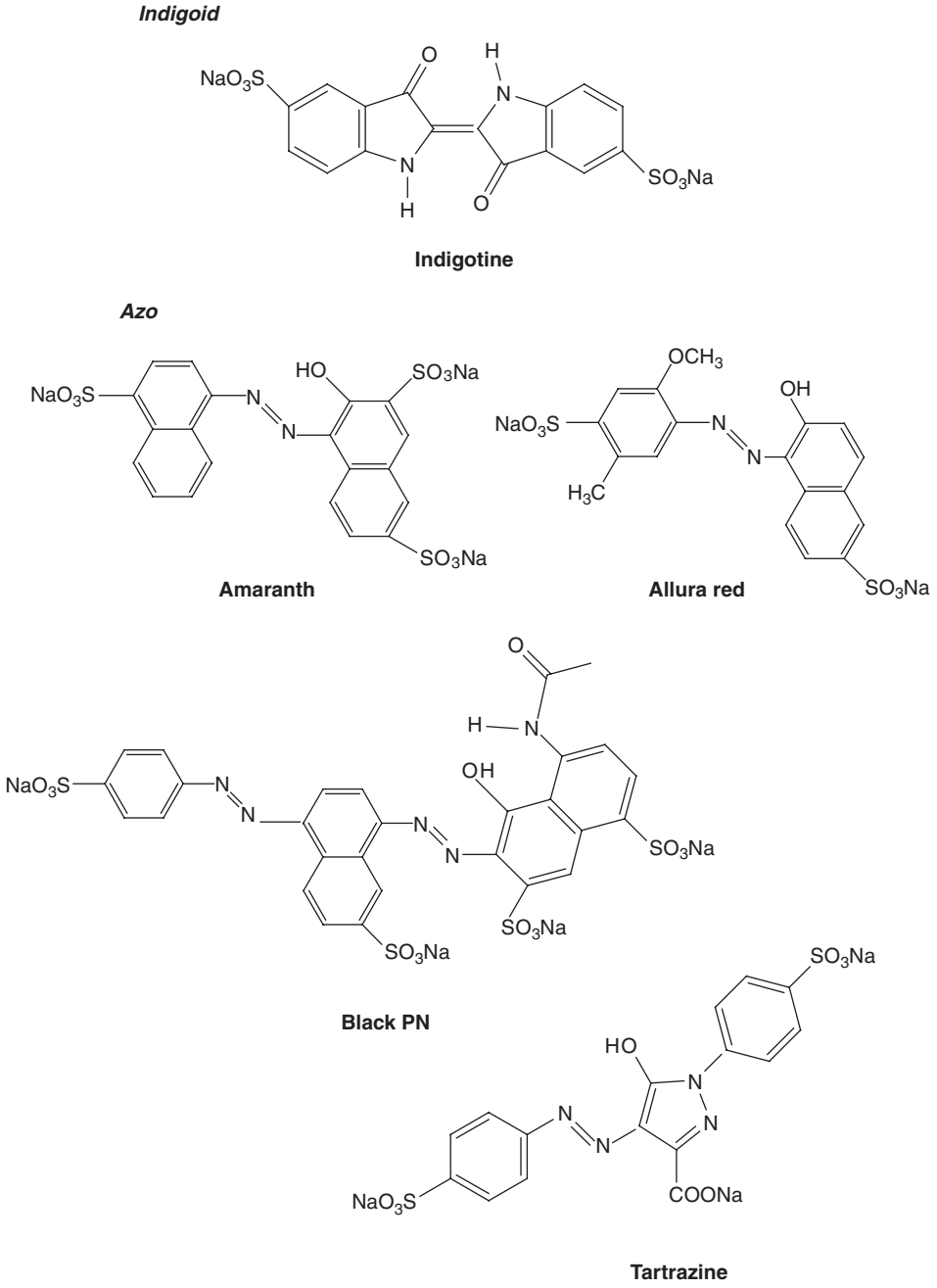

FIGURE 86.1 Chemical structures of azo (amaranth, allura red, black PN, and tartrazine) and indigoid (indigotine) colorants.

primary aromatic amine to a component capable of coupling, usually a naphthol. The soluble azo dyes can contain one or more sulfonic acid groups. Their degree of solubility is determined by the number of sulfonic groups present and their position in the molecule. The azo dyes give rise to colors in the yellow, orange, red, blue, violet, brown and black range. Amaranth, allura red, black PN and tartrazine (Figure 86.1) belong in this class (8, 27, 44).

There are colorants of the indigoid type, including indigotine (Figure 86.1), which is the water-soluble disodium sulfonate derivative of indigo, one of the oldest known and most extensively utilized natural pigments. The pigment is derived from various species of the indigo plant found in India. It is made by sulfonating indigo, yielding 5,5′-indigotine disulfonate. The color, which results from the resonance hybrid of structures, is a deep blue, compared to the greenish-blue of brilliant blue FCF. It has the lowest water solubility of any of the FD&C colorants and it is highly susceptible to oxidation by ultraviolet light and fades rapidly (8, 27, 44).

The triarylmethane or triphenylmethane colorants consist of three aromatic rings attached to a central carbon

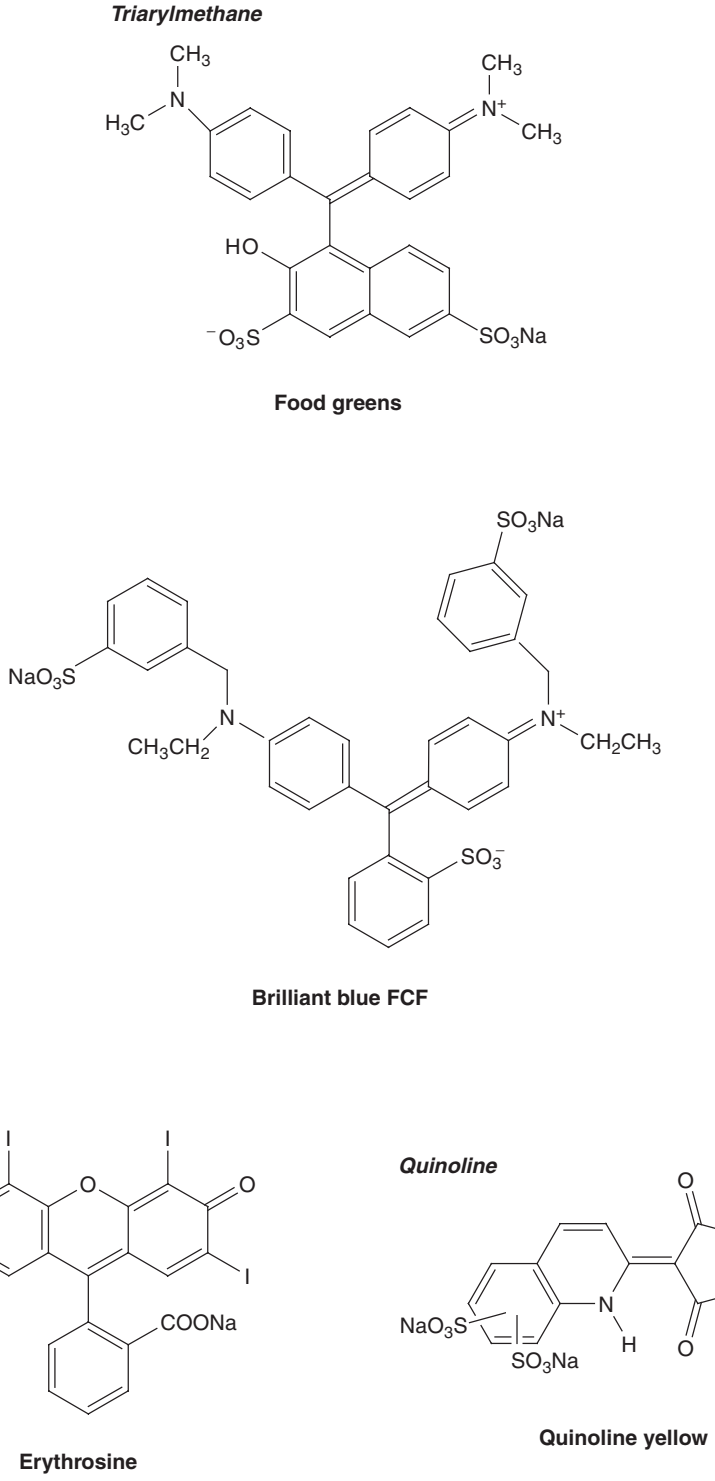

FIGURE 86.2 Chemical structures of triarylmethane (Food green S and brilliant blue FCF), xanthene (erythrosine) and quinoline (quinoline yellow) colorants.

atom (Figure 86.2). They are water soluble, anionic, sulfonated compounds. They are distinguished by their brilliance of color and high tinctorial strength, but they have poor light-fastness properties. They are characteristically bright green or blue. Alkali decolorization of a triphenylmethane colorant involves formation of a colorless carbinol base. The ortho-substituted sulfonic acid group sterically hinders access of the hydroxyl ion to the central

TABLE 86.1
Generic Names, International Numbering System, Food, Drug and Cosmetic Number, Color Index Constitution Number, Chemical Abstract Service Register Number, Chemical Classes, and Names of some Synthetic Food Colorants

Synthetic Colorants	INS, EEC[a]	FD&C No.[b]	CI[c]	CAS Reg[d]	Chemical Class[e]	Chemical Name
Carmoisine, azorubine, D&C red No. 10	122		14720		A	Disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)-1-naphthalene sulfonate
Ponceau 4R, brilliant scarlet 4R, new coccine	124		16255		A	Trisodium-2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalene 1,3-disulfonate
Amaranth, bordeaux S, CI acid red 27	123	red 2	16185		A	Trisodium 3-hydroxy-4-(4-sulfonato-1-naphthylazo)-2,7-naphthalene disulfonate
Erythrosine, CI Food red 14	127	red 3	45430	16423-68-0	X	Disodium 2′,4′,5′,7′tetraiodo 3′,6′-dioxidospiro-[isobenzo-furan-1(3H), 9′-[9H]xanthen]-3-one hydrate
Allura red AC, CI Food red 17	129	red 40	16035	25956-17-6	A	Disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalene sulfonate
Sunset yellow S or FCF, grelborange, CI Food yellow 3	110	yellow 6	15985	2783-94-0	A	Disodium 6-hydroxy-5-(4-sulfonatophenylazo]-2-naphthalene sulfonate
Tartrazine, CI Food yellow 4	102	yellow 5	19140	1934-21-0	A	Trisodium 5-dihydro-1-(4-sulfonatophenyl)-4-(4-sulfonato phenylazo)-H-pyrazole-3-carboxylate
Quinoline yellow, CI Food yellow 3	104	(D&C yellow 10)	47005	8004-92-0	Q	Disodium 2-(1,3-dioxo-2-indanyl)-6,8-quinolinesulfates
Indigotine, indigo carmine, CI Food blue 1	132	blue 2	73015	860-22-0	I	Disodium 3-3′-dioxo-2,2′-bi-indolylidene-5-5′disulfonate
Brilliant blue FCF, CI Food blue 2	133	blue 1	42090	2650-48-2	TAM	Disodium [4-(N-ethyl-3-sulfonato benzylamino)phenyl]-[4-(N-ethyl-3-sulfonato benzyl-imino)cyclohexa-2,5-dienylidene]toluene-2-sulfonate
Patent blue V, CI Food blue 5	131		42051		TAM	2-[(4-diethylaminophenyl)(4-diethylimino-2,5-cyclohexadien-1-ylidene)methyl]-4-hydroxy-1,5-benzenedisulfonate
Fast green FCF, CI Food green 3	143	green 3	42053	2353-45-9	TAM	Disodium 3-[N-ethyl-N-[4-[[4-[N-ethyl-N-(3-sulfonato benzyl)-amino]phenyl](4-hydroxy-2-sulfonatophenyl)-methylene]-2,5-cyclohexa-dien-1-ylidene]-ammoniomethyl]-benzene sulfonate
Chocolate brown HT	156		20285		A	Disodium-4,4′-(2,4-dihydroxy-5-hydroxymethyl-1,3-phenylene bisazo)di(naphthalene-1-sulfonate)
Brilliant black N, black PN	151		28440		A	Tetrasodium-4-acetamido-5-hydroxy-6-[7-sulfonato-4-(4-sulfonatophenylazo)-1-naphthylazo]naphthalene-1,7-disulfonate

Source: Refs. 14, 22 and 27.

[a] INS — International numbering system or European Economic Community number.

[b] Food, Drug & Cosmetic color Number.

[c] CI — Color index.

[d] Chemical Abstracts Service Register Number.

[e] Chemical classes: A — Azo, I — indigoid; Q — quinoline; TAM — triarylmethane, X — xanthene.

carbon atom, thus preventing formation of the carbinol base. Substitution of a sulfonic group for a hydroxyl group in these colorants improve the resistance to alkali and also the stability to light (8, 44).

In the xanthene group, the chromophoric system is the xanthene or dibenzo-1,4-pyran heterocyclic ring with amino or hydroxyl groups in the meta position with respect to the oxygen bridge. The xanthenes are usually water-soluble. Erythrosine (Figure 86.2) is insoluble in acids and it is quite stable to alkali. The chromophoric group imparts a brilliant red shade to erythrosine. It also exhibits fluorescence (8, 27, 44).

The quinolines are derived from quinaldine by condensation with phthalic anhydride. The chromophoric system is the quinophthalone or 2-(2-quinolyl)-1,3-indandione heterocyclic ring. Bright greenish yellow shades with poor light fastness are characteristic of the group. Quinoline yellow (Figure 86.2) is the only dye in this group of importance for use in food coloration (8, 14, 39, 44).

The generic and chemical names, the chemical classes, and color identification according to the International Numbering System (INS) or European Economic Community (EEC or E number), United States Food, Drug and Cosmetic Act, Color Index Constitution Number, and the Chemical Abstract Service Register Number of some synthetic colorants are shown in Table 86.1. The chemical formulas, molecular and milliequivalent weights, color shade, maximum absorbance wavelength, and extinction coefficients of some synthetic food colorants are indicated on Table 86.2.

It is the bond conjugation within the organic molecule which is responsible for color. Delocalization of the π-bonding electrons lowers their excitation energies, allowing them to absorb light. Extensive conjugation, or the presence of electron donor and acceptor groups within the molecule, serves to shift the absorption of light to the lower energies (longer wavelengths) comprising the visible spectrum. The tinctorial strength is an intrinsic property of the dye's chemical structure, i.e., the extinction coefficient. The coloring power is thus best manipulated through optimization of the physical form of the dye used and the carrying vehicle (40).

The chemical and physical properties of some colorants, as summarized in Tables 86.3 to 86.6, confer advantages and disadvantages, and thus fulfill a specific application need (8, 9, 32, 43). However, the chemical and physical properties desired in a food coloring material are not easy to achieve. Furthermore, many factors can contribute to the instability of colorants.

Water solubility is conferred by the presence of at least one salt-forming moiety (-SO_3H, -CO_2H). These dyes are usually isolated as their sodium salts. They have colored anions and are known as anionic or acid dyes. Dyes containing basic groups, such as -NH_2, -$NHCH_3$ and -$N(CH_3)_2$ form water soluble salts with acids. These are the cationic or basic dyes, and the colored ion is positively charged. Some dye molecules have both acidic and basic groups, giving rise to an internal salt or zwitterion structure. Indigotine, in particular, has many possible zwitterionic structures besides that shown in Figure 86.1 (39).

TABLE 86.2
Generic Names, Chemical Formulas, Molecular and Milliequivalent Weights, Color Shade, Maximum Absorbance Wavelength, and Extinction Coefficients of some Synthetic Food Colorants

Synthetic Colorants	Chemical Formula	Molecular Weight	Milliequivalent Weight	Color Shade	λ max[c] (nm)	$E^{1\%}_{1\,cm}$ (water)
Carmoisine	$C_{20}H_{12}O_7N_2S_2Na_2$	502.44	0.1256	Red	515	545
Ponceau 4R	$C_{20}H_{11}O_{10}N_2S_3Na_3$	604.48	0.1511	Strawberry red	505	431
Amaranth	$C_{20}H_{11}O_{10}N_2S_3Na_3$	604.49	0.1511	Magenta red	523	438
Erythrosine	$C_{20}H_6O_5I_4Na_2$	879.92		Bright pink/red	526	1154
Allura red AC	$C_{18}H_{14}O_8N_2S_2Na_2$	496.42	0.1241	Orange/red	502	556
Sunset yellow FCF	$C_{16}H_{10}O_7N_2S_2Na_2$	452.37	0.1131	Orange	480	551
Tartrazine	$C_{16}H_9O_9N_4S_2Na_3$	534.39	0.1336	Lemon yellow	426	527
Quinoline yellow	$C_{18}H_9O_8NS_2Na_2$	477.38		Lemon yellow	414	800
Indigotine	$C_{16}H_8O_8N_2S_2Na_2$	466.37	0.2332	Royal, deep blue	610	489
Brilliant blue FCF	$C_{37}H_{34}O_9N_2S_3Na_2$	792.85	0.3964	Turquoise, greenish blue	629	1637
Patent blue V	$C_{27}H_{31}O_6N_2S_2Na_2$	566.66	0.2899	Turquoise blue	635	2000
Fast green FCF	$C_{37}H_{34}O_{10}N_2S_3Na_2$	808.85	0.4045	Sea/bluish green	625	1560
Chocolate brown HT	$C_{27}H_{18}O_9N_4S_2Na_2$	652.57	0.0816	Chocolate brown	462	367
Brilliant black N	$C_{28}H_{17}O_{14}N_2S_4Na_5$	867.69	0.1085	Violet black	568	553

Source: Refs. 10, 14 and 45.

[a] INS — International numbering system or European Economic Community number.

[b] Food, Drug & Cosmetic color Number.

[c] Maximum absorbance at neutral media.

TABLE 86.3
Solubility of Synthetic Food Colorants in Water, Alcohol, Glycerin, and Propylene Glycol

Synthetic Colorants	Solubility (g/100 mL)												
	Water			Alcohol		50% Alcohol		Glycerin		50% Glycerin		Propylene Glycol	
	2°C	25°C	60°C	25°C	60°C	25°C	60°C	25°C	60°C	25°C	60°C	25°C	60°C
Carmoisine		8.0				(Slight)							
Ponceau 4R		30.0				(Slight)							
Amaranth		7.2				(Slight)		18.0				1.0	
Erythrosine	9.0	9.0	17.0		0.01	1.0	1.0	20.0	20.0	16.0	16.0	20.0	20.0
Allura red	18.0	22.0	26.0	0.001	0.05	1.3	5.5	3.0	8.0	12.0	14.0	1.5	1.5
Sunset yellow S	19.0	19.0	20.0		0.001	3.0	4.0	20.0	20.0	20.0	20.0	2.2	2.2
Tartrazine	3.8	20.0	20.0		0.001	4.0	8.4	18.0	18.0	20.0	20.0	7.0	7.0
Indigotine	0.8	1.6	2.2		0.008	0.30	0.35	1.0	1.0	1.0	1.5	0.1	0.1
Brilliant blue	20.0	20.0	20.0	0.15	0.15	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Fast green	20.0	20.0	20.0	0.01	0.01	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Chocolate brown		20.0				(Insoluble)							
Brilliant black		5				(Slight)							

Source: Refs. 5, 8, 14 and 27.

TABLE 86.4
Tinctorial Strength, Stability to Light, Oxidation, Heat and pH and Compatibility with Food Constituents of Synthetic Food Colorants

Synthetic Colorants	Tinctorial Strength	Stability to			Stability to pH				Compatibility with Food Constituents
		Light	Oxidation	Heat	3.0	5.0	7.0	8.0	
Carmoisine		Very good		Good		Good	Good		
Ponceau 4R		Good		Very good		Good	Poor		
Amaranth	Good	Very good		Good		Good	Fair		Poor
Erythrosine	Very good	Fair	Fair	Very good	Insoluble	Insoluble	Very good	Very good	Good
Allura red	Very good	Very good	Fair	Very good	Very good	Very good	Very good	Very good	Very good
Sunset yellow S	Good	Moderate	Fair	Very good	Very good	Very good	Very good	Very good	Moderate
Tartrazine	Good	Very good	Fair	Very good	Very good	Very good	Very good	Very good	Moderate
Indigotine	Poor	Poor	Poor	Poor	Poor	Poor	Poor	Very poor	Very poor
Brilliant blue	Excellent	Good	Poor	Very good	Fair	Good	Good	Good	Moderate
Fast green	Excellent	Fair	Poor		Fair	Good	Good	Fair (bluer)	Moderate
Chocolate brown		Very good		Very good		Good	Good		
Brilliant black		Excellent		Fair		Fair	Good		

Source: Refs. 8, 14 and 27.

TABLE 86.5
Stability of Synthetic Food Colorants in the Presence of Different Acids and Alkalis

Synthetic Colorants	Stability to 10% Acids				Stability to 10% Alkalis			
	Citric	Acetic	Malic	Tartaric	$NaHCO_3$	Na_2CO_3	NH_4OH	NaOH
Erythrosine	Insoluble	Insoluble	Insoluble	Insoluble	Very good	Fair	Fair	Very poor
Allura red	Very good	Very good	Very good	Very good	Good (bluer)	Fair (bluer)	Fair (bluer)	Poor (much bluer)
Sunset yellow S	Very good	Very good	Very good	Very good	Very good	Very good	Very good	Good
Tartrazine	Very good	Very good	Very good	Very good	Very good	Very good	Very good	Poor
Indigotine	Very poor	Very poor	Very poor	Very poor	Very poor	Very poor	Very poor	Poor (yellower)
Brilliant blue	Very good	Very good	Very good	Very good	Fair	Very poor	Poor	Very poor
Fast green	Very good	Very good	Fair	Fair	Very good	Poor (bluer)	Poor (bluer)	Very poor

Source: Refs. 8 and 27.

Azo and triarylmethane dyes are susceptible to discoloration or precipitation during processing and storage in the presence of reducing agents (monosaccharides, aldehydes, ketones, and ascorbic acid), heavy metals, metal packaging, exposure to light, excessive heat, or exposure to acid or alkali (Tables 86.4 and 86.5). Azo dyes give rise to colored subsidiary products and to the colorless hydrazo forms or sometimes to the primary amine.

TABLE 86.6
Stability of Synthetic Food Colorants in the Presence of Different Sugars and Food Additives

	Stability to 10% Sugars			Stability to Food Additives			
Synthetic Colorants	Cerelose	Dextrose	Sucrose	1% Sodium Benzoate	1% Ascorbic Acid	SO_2 25 ppm	SO_2 250 ppm
Erythrosine	Very good	Very good	Very good	Very good	Insoluble	Insoluble	Insoluble
Allura red		Very good	Very good	Very good	Very good	Very good	Very good
Sunset yellow S	Very good	Very good	Very good	Very good	Poor	Poor	Poor
Tartrazine	Very good	Very good	Very good	Very good	Poor	Poor	Poor
Indigotine	Poor	Poor	Good	Good	Poor	Very poor	Very poor
Brilliant blue	Very good	Very good	Very good	Very good	Good	Very good	Good
Fast green	Very good	Very good	Very good	Very good	Good	Very good	Very good

Source: Refs. 8 and 27.

Azo dyes are also subject to SO_2 decolorization through HSO_3 addition to the nitrogens, resulting in the colorless hydroazo sulfonic acids. Triarylmethane dyes are reduced to the colorless leuco base (11, 40, 44).

Free trace metals, including zinc, tin, aluminum, iron, and copper are known to cause fading of some additives (27). Of most concern are iron and copper. The presence of calcium and magnesium can also result in the formation of insoluble salts and precipitates (44). Azo dyes in particular are troublesome in that they often react with food cans at a rate proportional to their concentration, causing corrosion of the container and a corresponding loss in the food's dye content. Some colorants lack stability in retorted protein foods, whereas others are affected by peroxides (27).

The stability of a colorant toward light, either by itself or in solution, is not necessarily the same as its stability toward light in a product. Various ingredients, including aldehydic flavors, reducing sugars, and oils, are known to enhance the effects of light on some colorants, whereas, others prove to be more light stable in a product than alone. Several methods are used to minimize the effects of light on colorants in products, including packaging in light-proof packing, the incorporation of ultraviolet absorbers into the products, the use of color lakes, and the careful selection of the other ingredients used in the product. In general, the most stable colorants to light are tartrazine, carmoisine, amaranth, allura red, chocolate brown HT and brilliant blue FCF. Erythrosine and indigotine are the least stable (8, 27).

The pH value must also be considered when choosing a colorant, since not everyone of them can be used at all pH values. Indigotine fades rapidly in acid or alkaline media, while erythrosine precipitates from acid solution, whereas fast green FCF turns blue under alkaline conditions. Other colors exhibit important pH related changes in their properties, including shifts in shade, variations in shelf life, changes in solubility, and loss of tinctorial strength (8, 27).

The stability of synthetic food colors towards the conditions prevailing in food processing depends upon a number of factors. These include the medium in which the color is used, the concentration of the color and of the various food additives used and the temperature and time of exposure. Changes that occur as a result of these factors vary for each color used so that all of the components of a blend of food colors will not fade at the same rate. The resulting shade may, therefore, be entirely different from the original blend (8).

Temperatures used in cooking can be detrimental to food colors. The most stable of the commonly used synthetic colorants to average boiling and baking temperatures of 100 and 200°C, respectively are ponceau 4R, allura red, sunset yellow, tartrazine, chocolate brown HT and brilliant blue FCF. Indigotine is not stable to processing heat. At very high temperatures some carbonization may occur which will cause color loss or change of shade. All colors should be added at the lowest possible temperature, and as late as possible in the food manufacturing process, when little further heating will take place (8).

The synthetic colorants are stable to the levels of benzoic acid generally used as a preservative in foodstuffs, except patent blue V, quinoline yellow and indigotine. Erythrosine is precipitated from the solution in the presence of benzoic acid. The addition of sulphur dioxide as a preservative in fruit based-products causes color loss in patent blue and indigotine (1, 8).

Vitamins and antioxidants are added to a wide range of processed foods and vitamin C is frequently used to perform both functions. Sunset yellow, tartrazine, fast green FCF, and brilliant blue FCF are moderately stable to levels up to 160 ppm, while amaranth is stable in the presence of 70 ppm of ascorbic acid (1, 8).

When subjected to higher processing temperatures, particularly at above atmospheric pressure while retorting, color loss can occur as a result of reaction with proteins. This applies particularly in fish and meat processing but may also occur in processed milk products. In view of the varying protein contents of each product, it is important to carry out small-scale tests on each type of raw material to be used. Amaranth is the most susceptible food color to this type of action, while erythrosine is the most stable (8).

V. COLORANTS USE IN FOODS

Colors are added to foods to make them recognizable and pleasing to the consumer. What consumers want in the color of a food depends on a variety of factors, including cultural background, geographic, and sociological aspects of the population, past experiences, desire for color coordination, esthetic appeal, local customs, among others (6, 27).

The reason for coloring any particular food depends on a number of factors. The major use is in products containing little or no color of their own. These include many liquid and powdered beverages, gelatin desserts, candies, ice creams, sherbets, icings, jams, jellies, and snack foods. Without the addition of colors to some of these products, all flavors of the product would be colorless, unidentifiable and probably unappealing to the consumer (27).

The process used to prepare foods results in the formation of a color which depends largely on the time, temperature, pH, air exposure and other parameters experienced during processing. Therefore it is necessary to supplement the color of the product to ensure its uniformity from batch to batch. Foods that fall into this category include certain beers, blended whiskies, brown sugars, table syrups, toasted cereals, and backed goods. The storage of foods can also be a problem because natural pigments often deteriorate with time due to exposure to light, heat, air and moisture or because of interactions of the components of the products with each other or with packaging material. The color of maraschino cherries, for example, changes so easily with storage that they are bleached and colored artificially (27).

The problems of the citrus fruit industries are typical of those encountered with products produced in different areas of the country or at different times of the year. Most varieties of Florida oranges tend to be green, suggesting unripeness, even though they contain the proper ratio of solids to acid for fully nutritious, ripened fruit. Therefore, there is a need to color these oranges to make them comparable in appearance and thus of commercial acceptability as naturally colored fruit from other areas of the country. The percentage of the total crop colored varies from year to year and depends largely on the weather. However its use is not permitted for oranges intended for processing (27).

The problems associated with dairy products are more complex. Approximately 90% of the yellow color in milk is due to the presence of beta-carotene, a fat-soluble carotenoid extracted from feed by cows. Summer milk is more yellow than winter milk. This is largely due to seasonal feeding practices in which cows grazing on lush pastures consume much higher levels of carotenoids than do cows barn-fed on hay and grain in the fall and winter. Furthermore, various breeds of cows and even individual animals differ in the efficiency to extract beta-carotene from feed and in the degree to which they convert it into colorless vitamin A. The differences in the color of milk are more obvious in products made from milk fat. Thus, unless standardized though the addition of yellow colorant, products like butter and cheese can show a wide variation in shade and in many cases appear unsatisfactory to the consumer. It is also frequently necessary to use various amounts of blue or green colorants when making gorgonzola, provolone, blue and other cheeses to neutralize the yellow of the curd used to prepare them (27).

Other products whose natural color varies enough to make standardization desirable include the shells of certain kinds of nuts, the skins of red and sweet potatoes and ripe olives (27, 40).

Ink used by inspectors to stamp the grade or quality on meat must, by law, be made from food-grade colors. Dyes used in packaging materials that come in direct contact with a food must also be food-grade or, if not, it must be established that no part of the colorant used migrates into the food product (27).

There are numerous restrictions on the use of color additives. They cannot, for example, be employed to deceive the public by adding weight or bulk to a product or by hiding quality. In addition, special permission is needed to use colorants or products containing them in foods for which standards of identity say so. Other restrictions pertaining to the areas of use and the quantities of colorants allowed in products are specified in regulations for particular additives. Citrus red No. 2, for example can only be used to color the skins of oranges not intended for processing (27).

The amount of color additive allowed in a product depends on both the colorant and the food being colored. When numerical limits for use of food colorants are not specified, the amount allowed is controlled by "good manufacturing practices," e.g., one cannot use more colorant in a product than it is needed to achieve the desired effect. Today, the excessive use of colorants is rarely a problem since manufacturers are not likely to waste costly additives and, at the same time, run the risk of making their products appear unnatural (27).

VI. FORMS OF COLORANTS AVAILABLE

Food colors are available as straight colors, referred to as primaries. They are also available as blends of two or more straight colors, with or without diluents, referred as blends, mixtures, secondaries or secondary blends. The blends are standardized in strength by mixing the colors with approved diluents such as sucrose, salt, caramel, flour, etc. Mixtures of just one straight color with one or more diluents are also provided on special order for color users. However, mixtures must be made only from previously certified straight colors (23).

Typical forms of certified primary food colors are powder, ultra-fine powder, plating grade powder, granules,

dry blends, wet-dry blends, solutions, pastes and dispersions. The latter three preserved forms are used less frequently because of the added costs incurred in their preparation (23).

Secondary blends are the forms used for creating a wide variety of shades ready for immediate use in food preparations, such as grapes, raspberry, lime and various chocolates. The processing of secondary blends is dictated by the needs of the consumer. Many blends can be combinations of all dry ingredients. Some blends, especially those containing blue or green, must be combined in a wet state, then redried in order to prevent flashing at the time the consumer combines the product with water or milk. The dried products are ground, blended and standardized in strength within carefully controlled limits as required by the product specifications (23).

In manufacturing any colorant, the major difficulty is to meet the specifications of purity given for certification. However, the color manufacturing industry not only meets these purity specifications, but most manufacturers exceed them. The pure colorant content of a typical certified colorant is 86–96%. Variation of 2–3% in total colorant content of a colorant is of little practical significance since such variation has no significant effect on the ultimate color of a product. The moisture content of the colorant powder is between 4 and 5% and the salt (ash) content is approximately 5%. The high ash content comes from the salt used to crystallize (salt out) the colorant (44).

Where water-insoluble colorants are desired, the lakes are useful as insoluble pigments. Lakes were first approved for use in 1959. They are a special kind of color additive prepared by precipitating a straight color as aluminum salts on a substratum of alumina hydrate. To make a lake, the alumina substrate is first prepared by adding sodium carbonate or sodium hydroxide to a solution of aluminum sulfate. Next, a solution of colorant is added to the resulting slurry, the aluminum chloride is added to convert the colorant to an aluminum salt, which adsorbs onto the surface of the alumina. The slurry is filtered, and the cake is washed, dried, and ground to an appropriate fineness, typically 0.1 to 40 μm (8, 23, 27).

Lakes are available with pure dye contents ranging from less than 1% to more than 40% and with moisture levels of 6–25%. Typical use levels are 0.1 to 0.3%. Griding or mixing a lake in oil or fat achieves a result which, for some applications, is superior to coloring the product with a water-soluble dye. Lakes are marketed individually or mixed with other lakes or approved diluents, or dispersed in various edible vehicles such as hydrogenated vegetable oil, coconut oil, propylene glycol, glycerin, or sucrose syrup, or dispersed in other approved media that make the mixtures appropriate for printing food packagings (10, 23, 27).

Although lakes are insoluble in water, some bleeding or leaching may be observed in hot water, in strongly acid (pH <3.5) and alkaline (pH >9.0) conditions, or in solvents in which the unlaked dye is soluble. Lakes often show amphoteric properties with both acid and alkali tending to solubilize the inorganic substrate releasing free colorant (23, 27).

Properties of lakes that enhance their usefulness include opacity, ability to be incorporated into products in the dry state, relative insolubility, and superior light, chemical and thermal stability (40). Such properties have made possible the more effective and more efficient preparation of candy and tablet coatings and often eliminate the need to remove moisture from dry products before coloring them. Lakes have also made possible the coloring of certain products that, because of their nature, method of preparation, or method of storage, cannot be colored with ordinary color additives (23, 27). Lakes have proven particularly valuable for coloring water-repelling foods such as fats, gums, waxes, and oils, and for coloring food packaging materials including lacquers, containers, plastic films and inks from which soluble dyes would be quickly leached out (10, 27).

Unlike dyes that color objects through their adsorption or attachment from solution to the material being colored, lakes impart color by dispersing them in the medium to be colored. As a consequence of this pigment-like character, both the shade and the tinctorial strength of lakes are highly dependent on the conditions used in their manufacture as well as their physical properties, including their particle size and crystal structure (27, 40).

Lakes are considerably more expensive than the water-soluble colorants, however, there are several specialty uses where their application is practical (23). Some specific products in which lakes are used include icings, fondant coatings, sandwich cookie fillings, cake, doughnut mixes, decorative sugar crystals, coated and compression tablets, hard candy, candy wafers, chewing gums, wax coatings for cheese, yogurts, dry beverage bases, dessert powders, snack foods, spice mixes, printing inks, plastic films, decorative coatings, can linings, meat trays, produce containers, and margarine tubs (27).

When selecting a colorant for food use, several requirements must be attended. First of all, it should posses a desirable hue range and should have a high tinctorial strength. The tinctorial strength or coloring power of a colorant determines the amount and thus the cost of the colorant that must be added to a product to achieve a particular effect. A colorant's tinctorial strength is an inherent property of its chemical structure and cannot be changed, although maximum use can be made of it by selecting the physical form, vehicle, and conditions under which it is used (27).

The colorant must not impart any offensive property to the product. Offensive properties that can be transferred to a product include taste and odor, which can originate from the colorant itself or from trace impurities (27).

The colorant must be stable in the presence of light, oxidation and reduction, pH change, and microbial attack. Furthermore, it must be nonreactive with the products and the containers in which it is used. Tables 86.4 to 86.6 describe the performance of some colorants. An even more serious problem can result from the instability of a colorant, whether it is inherent or caused by reaction with a product or a product's container. Generally, color additives have shown excellent stability when stored in the dry state. For example, most food colors show little degradation after storage periods of 15 years or more. However, the stability of a colorant stored neat is no guarantee of its stability in a product. Consequently, use tests must still be performed and on an individual product/colorant basis (27).

The dye must also be easy to apply to products and should be highly soluble (Table 86.3) in water and in other inexpensive acceptable polar food grade solvents (e.g., alcohol, propylene glycol); it might need to be soluble in edible fats and oils, and if it is not soluble, it should be easily dispersed (27).

The colorant should also be inexpensive. The cost per pound of colorants is affected by the cost of the raw materials, equipment, and labor needed to produce it, as well as the supply and demand of the colorant. To these expenses, the additional cost needed to ensure the ultrahigh purity required must be added as well as the cost of certification. These factors make certified colors more expensive than typical technical dyestuffs. The saving feature is that in most cases, relatively little colorant is needed to achieve the desired depth of shade in a product, and, thus, the cost of the colorant adds relatively little to the cost of the finished product (27).

Although all of these factors should be considered when selecting the colorant for food use, the safety criteria, e.g. its safety for humans and the levels allowed are of extreme importance and should be fully satisfied, while the other factors are seldom completely met.

VII. TOXICOLOGICAL ASPECTS

The safety of food colorants has been a matter of concern. Most synthetic colors have been extensively tested in conventional toxicity studies. However, divergent views have often been expressed on the significance of the same toxicity data (11). Most of the questions have been associated with the azo dyes with respect to hypersensitive reactions. Some individuals show allergic reactions, such as urticaria (hives), asthma, and rhinitis, after ingestion of tartrazine (15, 17, 26, 29, 37). Tartrazine was observed to provoke bronchospasms in asthmatics and aspirin-intolerant persons. Newer studies indicated that the incidence of tartrazine hypersensitivity was rare (fewer than 1 out of 10,000 people being susceptible) and that tartrazine responses in asthmatic or aspirin-sensitive were exceptional and idiosyncratic (20). However, a double-blind, placebo-controlled study linked tartrazine ingestion with irritability, restlessness, and sleep disturbance in some children (38). Therefore, the use of tartrazine in a food product should be declared on the label (44).

Carcinogenic potential has been confirmed for some colorants, such as, ponceau 3R, butter yellow, methyl red, soudam R brown, soudam 7B red, orange SS and crisoidine (11, 41). Studies by FDA in 1972, evidenced that amaranth is embryophatic to rats. Allura red was observed to cause no significant and consistent adverse effect. However, there is concern about the presence of impurities, such as *p*-cresidine, which has been proven to be carcinogenic (36). However, recent studies have indicated that tartrazine, indigotine and erythrosine are potent inhibitors of skin tumor promotion in mice treated with 7,12-dimethylbenz[*a*]anthracene and with 12-*O*-tetradecanoylphorbol-13-acetate (24).

Erythrosine has been regarded as probable adventitious source of iodine, and toxicological studies confirmed its carcinogenic action on thyroid of male rats (11, 34, 41, 44). Although erythrosine is permanently listed, its long term toxicity is questionable. The lake of this colorant is no longer permitted for use in foods because of toxicological concerns (44).

The most publicized and controversial food color is amaranth. It had been used in the US since 1908. However, two Russian studies alleged that it was both carcinogenic and embryotoxic. While FDA discounted the charges of carcinogenicity, it was concerned about the teratogenic and reproduction effects. After several studies, ADI was reduced to 0–0.5 mg/kg body weight. The US prohibited the use of amaranth, however Canada, after studies of their own, observed that amaranth was not organ specific and allowed its use in foods together with the European Community, Japan, among others (19, 41). Amaranth has been recently delisted in Canada (7).

Many azo colors are not stable under certain conditions. Therefore, they undergo fading giving rise to colorless and colored subsidiary products whose toxic properties are not known. Indigotine is unstable in aqueous solution giving isatin-5-sulphonic acid and 5-sulphoanthranilic acid which are less clearly defined toxicologically than the parent molecule (11).

The International Life Sciences Institute, Nutrition Foundation's Catalog of Food Colors reports on the regulatory status of colorants in use throughout the world and on the safety assessment data, including international expert committee evaluations (21). The Joint Expert Committee on Food Additives Safety (JECFA), FAO, WHO, also reports on the regulatory status of colorants in use throughout the world and on safety assessment data (11, 41). JECFA also evaluates and provides updated information (22) on acceptable daily intakes (ADI) of colorant in mg per kg of body weight, making it accessible as it is evaluated (Table 86.7).

TABLE 86.7
Acceptable Daily Intake of Some Synthetic Food Colors

Food Color	ADI (mg/kg Body Weight) (Last Evaluation)
Carmoisine	0–4 (1983)
Ponceau 4R	0–4 (1983)
Amaranth	0–0.5 (1984)
Erythrosine	0–0.1 (1990)
Allura red	0–7 (1981)
Sunset yellow	0–2.5 (1982)
Tartrazine	0–7.5 (1964)
Quinoline yellow	0–10 (1984)
Indigotine	0–5 (1974)
Brilliant blue FCF	0–12.5 (1969)
Patent blue V	not allocated (1982)
Fast green FCF	0–25 (1986)
Chocolate brown HT	0.25 (1979)
Brilliant black PN	0–1 (1981)

ADI = Acceptable daily intake in mg/kg body weight.
Source: Refs. 11, 22 and 41.

TABLE 86.8
Some Synthetic Food Colorants Permitted in Different Countries

Food Color	Brazil	Canada	EEC	Japan	USA
Carmoisine	+	+	+	−	−
Ponceau 4R	+	−	+	−	−
Amaranth	+	−	+	−	−
Erythrosine	+	+	+	+	+
Allura red	+	+	−	−	+
Sunset yellow FCF	+	+	+	+	+
Tartrazine	+	+	+	+	+
Quinoline yellow	−	−	+	−	−
Indigotine	+	+	+	+	+
Brilliant blue FCF	+	+	+	+	+
Patent blue	+	−	+	−	−
Brilliant green BS	−	−	+	−	−
Fast green FCF	+	+	−	+	+
Chocolate brown HT	−	−	−	−	−
Brilliant black PN	−	−	+	−	−
Acid red	−	−	−	+	−
Ponceau SX	−	−	−	−	+

Source: Refs. 3, 7, 10, 33 and 44.

According to JECFA (22), it is important to constantly gather information on the levels of synthetic colorants in foods to assess where the dietary intake stands compared to the ADI. Therefore, each country, and even regions within a country, should verify periodically the dietary intake of colorants, and additives in general, to make sure that intake does not exceed the ADI. In 1992, Toledo et al. (42) investigated the potential weekly intake of synthetic colorants by 3–14 years-old children from Barão Geraldo, Brazil. Results indicated that male children, especially from lower classes were most exposed to artificial colors. Comparison of estimated potential intakes with the ADI showed that consumption of amaranth, sunset yellow, indigotine and tartrazine represented ca. 24, 3, 0.05 and 0.4% of the actual ADI values, respectively.

VIII. LEGISLATION

Legislation on the use of colors in foods arose from the need to protect the consumer from harm and from fraudulent practices. The regulations promulgated by various countries world-wide differ in detail rather than in concept, as all have the same ultimate goal. Today, the food colors permitted in most countries are allowed only when a need has been demonstrated and their safety assured. Before these colors are authorized for food use, they have been subjected to rigorous toxicological testing and numerous debates between government officials, scientists, and representatives from the industry and consumer associations. Furthermore, the colors have been manufactured to exacting standards resulting in a high degree of purity (33).

Previously, several different colorants were used in foods. There was a great deal of research into the safety of food colors in the past years. Even thought results of those studies were often ambiguous and open to interpretation, there has been a decrease on the number of synthetic colorants permitted in many countries. However, differences in interpretation accepted by the various countries have led to the situation in which a color can be considered safe and acceptable in one country, but prohibited in another (12, 33).

Today, the types of colorants allowed varies greatly among countries, which reflects the different opinions about their toxicity (Table 86.8). However, there is a trend toward using fewer synthetic colorants. It is hoped that the trend to international standardization of food colorants will gain momentum (12, 44).

In the United States seven synthetic colorants are permitted for general food use, while in Canada and Japan, eight and nine colorants, respectively, are allowed (7). In Brazil and Mercosur (common trade among Brazil, Uruguay, Paraguay and Argentine), eleven synthetic colorants are allowed for food use (3). The European Economic Community allows much more than that (seventeen). Norway, however, prohibits the use of any synthetic colorant in the manufacture of foods. Lake pigments and dispersions are also permitted (10, 14).

In order to prevent indiscriminate use, many countries limit the types, uses and amounts of colorants permitted by groups or specific foods (11, 25). Since different countries allow the use of specific food colorants, it is possible that foodstuffs may be imported into a country, which forbids the coloring agent present in the product. Therefore,

the importer country legislation must be known in order to comply with regulations (4, 13, 16).

Colors used in the food products, like other additives, must be declared in the ingredients list, either the name of the color or the number, E number for the European Union and the FD&C number for the U.S. (10). The amount of colorants added to any food today is small. Furthermore, it is self-limiting due to good manufacturing practices and also by the fact that the certified colors in use today possess high tinctorial strength, thus only an extremely small amount is needed to color a product properly (4, 13, 16).

IX. CONCLUSIONS

Throughout time there was a significant reduction in the availability of colorants (23). This reduction resulted from several reasons, among them, the advances in the science of toxicology which enabled the access of more subtle effects; the excessively high use levels on the part of a few uninformed users; the unwarranted conclusion sometimes drawn from less than the best toxicology work (FD&C red no. 4); toxicology studies using a test substance probably not meeting purity standards (FD&C violet no. 1); political pressure (FD&C red no. 2); and lack of market demand (FD&C green no. 1 and green no. 2).

Although today's list is much shorter than in some other times, the safety in use of these products rests on more firm grounds. This is due to the fact that these synthetic organic colors have been more thoroughly tested than almost all other food additives.

Many advances in the development of food colorants have been made over the last 25 years, particularly in terms of harmonized legislation and advances in processing and formulation technology. However, there is still room for future development, specially on customized blends and technical support as well as improved handling properties.

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87 Biosynthesis of Natural Aroma Compounds

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CONTENTS

I. INTRODUCTION

In recent years, the consumers' demand for natural food flavor has increased continuously. This trend can be attributed to increasing health- and nutrition-conscious lifestyles. As a consequence, the demand for natural ingredients has risen from 10% of the food company requests to 80% within the last decade. The comparison of the costs for natural flavor compounds and their synthetic counterparts show that the chemically produced compounds are by a factor of 100–400 cheaper than the natural ones (1).

Three flavor categories exist in Europe: Natural, nature-identical and artificial flavors. In the US, natural flavors are defined as: essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit juice, vegetable or vegetable juice, edible yeast, herb, bud, bark, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products or fermentation products thereof whose significant function in food is flavoring rather than nutrition (2). The definition of naturals in Europe is almost identical to the US guidelines. Materials (mixture or single substances) are called natural if they are obtained exclusively by physical, microbiological, or enzymatic processes from material of vegetable or animal origin, either in the raw state or after processing for human consumption by traditional food preparation process (including drying, roasting, and fermentation) (3). Flavors and fragrances that can be labeled "natural" both in the United States and Europe have to be produced from natural sources by physical,

enzymatic or microbiological processes (US Food and Drug Administration 2001). Biotechnologically produced flavors are also covered by the term natural. The major advantage of biotechnologically produced products is attainment of the natural status and the ability to make such a claim on the product label and the ingredient list. Duplicating plant secondary metabolism in microbial systems ("fermentative processes") leads to aroma compounds that are classified as natural by the European and US food legislation. Aroma chemicals, such as vanillin, benzaldehyde (bitter almond, cherry) and 4-decanolide (fruity-fatty) are marketed on a scale of several thousand tons per year. Their production by single-step biotransformations, bioconversions and de novo synthesis using microorganisms, plant cell or isolated enzymes has been studied intensively (4).

Metabolic engineering strategies that involve inactivation of undesired genes and/or over-expression of existing or novel ones have been used to create rerouting of the metabolic fluxes by changing the energy metabolism, or the concentration of metabolic intermediate, or of existing or completely new end-products (5). Three sources for the biotechnological production of flavors are plants, enzymes, and microorganisms. Plants cells used for the production of flavors can either be manipulated by conventional plant breeding methods such as intraspecific crossing, hybridization and nonspecific mutagenesis by chemicals or irradiation, or by novel plant breeding methods such as tissue culture techniques, protoplast fusion techniques and recombinant DNA techniques. Microorganisms have the advantage that they can use relatively inexpensive substrates such as carbohydrates and amino acids to form complex flavor mixtures. Screening for highly productive strains and genetic engineering is necessary to obtain reasonable amounts. The use of enzymes on industrial scale is now a common practice. The enzyme transformation produces extremely pure products. However, of the 1500 chemicals that are used by the US flavor industry only 20 has been produced commercially by fermentation routes (6).

II. AMINO ACID-DERIVED FLAVORS

A. 2-Phenylethanol

2-Phenylethanol (2-PE) is an important flavor and fragrance compound with a rose-like odor. Most of the world's annual production of several thousand tons is synthesized by chemical means but, due to increasing demand for natural flavors, alternative production methods are being sought. Characterized by a delicate fragrance of rose petals, 2-phenylethnol is the main commercial alcohol following ethyl alcohol. It is the most used fragrance in the food, cosmetic, and perfume industries. In addition to presenting interesting sensory characteristics which influence the quality of alcoholic beverage or fermented food, it is also widely used to modify certain flavor compositions of food stuffs such as fruit formulas, ice cream, candy, pudding, and chewing gum (7). 2-PE occurs naturally in the essential oils of many flowers and plants, such as hyacinths, jasmine, narcissi, and lilies. In most cases, concentrations are too low to justify extraction. One exception is rose oil, which can contain up to 60% 2-PE. Both 2-PE and its ester, especially phenylethyl acetate (GRAS 2857), are valuable flavor and fragrance compounds (8).

Like many other higher alcohols, 2-PE is a metabolite of microbial fermentation, thus synthesis by microorganisms is an obvious option. The commonly accepted route for 2-PE formation is by conversion of phenylanine through deamination, decarboxylation and reduction, first described by Ehrlich and consequently named after him. It is assumed that for industrial purposes, only yeast strains improved by conventional screening, selection, and mutagenesis rather than by genetic engineering have been used so far (9). Various organisms including *Cladosporium cladosporioides, Kluyveromyces lactis, Saccharomyces cerevisiae, Hansenrula anomala*, and *Kluyveromyces marxianus* have been reported to be capable of producing 2-phenylethnol. 2-Phenylethanol and 2-phenylethylacetate are obtained in substantial amounts when *Hansenula anomala* CBS 110 and *Kloeckera saturnus* CBS 5761 are grown with L-phenylalanine as sole nitrogen source. While in the first microorganism the alcohol predominates, in the second the acetate is formed almost exclusively (10). Production of 2-phenylethnol increases almost in parallel with the growth of *Pichia fermentans* during the initial stage of fermentation (11).

B. Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most important aromatic flavor compounds used in foods, beverages, perfumes, and pharmaceuticals and is produced on a scale of more than 10,000 tons per year by the industry through chemical synthesis. Vanillin is the major component of vanilla flavor extracted from the fermented pods of *Vanilla* orchids. Alternative biotechnology-based approaches for the production are based on bioconversion of lignin, phenolic stilbenes, isoeugenol, ferulic acid, or aromatic amino acids, and on de novo biosynthesis, applying fungi, bacteria, plant cells, or genetically engineered microorganisms (12).

Phenylalanine is known as the starting point of flavonoid, coumarine, stilbene, and ferulic acid biosynthesis in plant. The shikimate pathway was discovered as the biosynthetic route to the aromatic amino acids phenylalanine, tyrosine, and tryptophan. This pathway has been found only in microorganisms and plants. All phenylpropanoids are derived from cinnamic acid, which is formed from phenylalanine by the action of phenylalanine ammonia lyase (PAL, EC 4.3.1.5) (13). The enzyme phenylalanine

ammonia lyase (PAL) catalyzes the first metabolic step from primary metabolism into phenylpropanoid metabolism, which is the deamination of phenylalanine to produce cinnamic acid (14).

One of the most intensively studied biotransformations to produce natural vanillin is the conversion of ferulic acid. Ferulic acid is an extremely abundant cinnamic acid derivative found in the cell walls of woods, grasses, and corn hulls (15). A novel CoA-dependent, non-β-oxidative pathway for ferulic acid cleavage was identified in *Pseudomonas fluorescens* (16). Ferulic acid is activated to the CoA thioester, catalyzed by feruloyl-CoA synthetase (FCS, EC 6.2.1.34). The gene *fcs* of *Pseudomonas* encoding this enzyme has been cloned and expressed in *Escherichia coli* (17). Feruloyl-CoA is subsequently hydrated and non-oxidatively cleaved to vanillin and acetyl-CoA. Both reactions are catalyzed by one distinct enzyme, designated as enoyl-CoA hydratase/aldolase (EC 4.2.1.17). This function was confirmed with the purified enzyme and by cloning and expressing the corresponding genes of *Pseudomonas* in *E. coli*. Cloning of *fcs* of *Pseudomonas* in *E. coli* resulted in a recombinant strain that was able to convert ferulic acid to vanillin at a rate of 0.022 mole per min per ml (18).

C. Benzaldehyde

In quantity, benzaldehyde is the second most important flavor molecule after vanillin. Natural benzaldehyde is produced by microbial degradation of natural phenylalanine. This process is added by a plentiful, cheap supply of natural L-phenylalanine, which has become available as an intermediate of the synthesis of aspartame (19). L-phenylalanine was almost completely converted to the flavor compounds benzaldehyde and 3-phenylpropanol following two different degradation pathways of submerged cultured *Ischnoderma benzoinum* (20). The oxidative degradation pathway to benzaldehyde was also found in bacteria and subsequently patented (21).

D. 4-Vinylguaiacol

Vinylphenols (4-vinylphenol and 4-vinylguaiacol) are natural constituents of wine and can play a role in wine aroma. 4-Vinylguaiacol (PVG) is an important aroma in soy sauce. However, 4-Vinylguaiacol (PVG), a major off-flavor in citrus products, was detected in stored model solutions of orange juice containing ferulic acid, and its amount increased with time and temperature (22). These volatile phenols have a distinct flavor that is evaluated as smoky, pharmaceutic, clove, or leather, and impart a phenolic characteristic to wine. High levels of 4-vinylphenol and 4-vinylguaiacol were detected in wines made from grape juice initially treated with some enzyme preparations. Two enzyme activities, which operate successively, are responsible for this formation. First, the cinnamoyl esterase (feruloyl esterase, ferulic acid esterase, hydroxycinnamoyl esterase, EC 3.1.1.73) activity from enzyme preparation liberates cinnamic acids from their corresponding tartaric acid esters. Second, cinnamic acids are transformed into 4-vinylphenol and 4-vinylguaiacol by decarboxylase activity provided by the yeasts (23).

Bacillus pumilus PS213 isolated from bovine ruminal fluid was able to transform ferulic acid and *p*-coumaric acid to 4-vinylguaiacol and 4-vinylphenol, respectively, by nonoxidative decarboxylation. The enzyme responsible for this activity has been purified and characterized. The purified enzyme shows a single band of 23 kDa; the molecular mass calculated by size exclusion chromatography is 45 kDa. Enzyme activity is optimal at 37 degrees C and pH 5.5 (24). Seven strains of *Lactobacillus* isolated from malt whisky fermentations and representing *Lactobacillus brevis*, *L. crispatus*, *L. fermentum*, *L. hilgardii*, *L. paracasei*, *L. pentosus*, and *L. plantarum* contained genes for hydroxycinnamic acid (*p*-coumaric acid) decarboxylase. With the exception of *L. hilgardii*, these bacteria decarboxylated *p*-coumaric acid and/or ferulic acid, with the production of 4-vinylphenol and/or 4-vinylguaiacol, respectively. It seems likely that the combined activities of bacteria and yeast decarboxylate *p*-coumaric acid and then reduce 4-vinylphenol to 4-ethylphenol more effectively than either microorganism alone in pure cultures (25).

E. 2-Acetyl-1-Pyrroline

2-acetyl-1-pyrroline has been reported as a potent flavor component of an aromatic rice (26). 2-acetyl-1-pyrroline was synthesized naturally in Pandan (*Pandanus amaryllifolius* Roxb.) leaves (27), bread flowers (*Vallaris glabra* Ktze) (28) and pearl millet (*Pennisetum americanum*) (29). In addition to plant materials, microorganisms, especially, *Bacillus cereus* (30), and bakers' yeast (31) biosynthesizes 2-acetyl-1-pyrroline as well.

Proline apparently plays an important role in formation of 2-acetyl-1-pyrroline. Increases in concentration of 2-acetyl-1-pyrroline occurred when proline, ornithine, and glutamate were added to the medium for seeding and callus of rice. Results of tracer experiment using ^{15}N-proline, ^{15}N-glycine, proline-1-^{15}C indicated that the nitrogen source of 2-acetyl-1-pyrroline was proline. 2-acetyl-1-pyrroline was formed in the aromatic rice at temperature below that of thermal generation in bread baking, and formed in the aerial part of aromatic rice from proline as the nitrogen precursor (32).

Previous investigations concluded that this trait of rice underwent monogenic inheritance, whereas others stated that two or three recessive or dominant genes controlled the construction of the trait (33). Lorieux et al. (34) reviewed the genetics of aromatic fragrance and concluded that a single recessive gene was responsible for the production

of fragrant rice plants. This single recessive fragrance gene (*fgr*) was linked to the RFLP clone RG28 on chromosome 8, at a genetic distance of 4.5 cM. They confirmed the close linkage between RG28 and *fgr* (5.8 cM) and also identified two quantitative trait loci for fragrance, one on chromosome 4 and the other on chromosome 12. Fragrance can be detected by tasting the associated flavor in individual seeds or assessing the aroma of leaf tissue or grains after either heating in water or reacting with solutions of KOH or I 2-KI. PCR-based molecular marker for the fragrance gene detection in rice (*Oryza sativa*. L.) has been developed (35). The genomic DNA clone RG28, linked to the major fragrance gene of rice (*fg*r), was assessed for polymorphism in order to produce a PCR-based marker for fragrance. A small mono-nucleotide repeat, that was polymorphic between a pair of fragrant and non-fragrant cultivars, was identified and developed into a co-dominant PCR-based marker. The polymorphism-information-content determinations for three microsatellite markers, that have been genetically mapped near RG28, are also presented. These PCR-based markers were used to distinguish fragrance-producing alleles from non-fragrance-producing alleles at the *fgr* locus.

The arginine deiminase or dihydrolase (ADI, EC 3.5.3.6) pathway for arginine degradation is comprised of three enzymes: ADI (EC 3.5.3.6), which degrade arginine into citrulline and ammonia; ornithine transcarbamoylase (OTC, EC 2.1.3.3), which cleaves citrulline into carbamoyl phosphate and ornithine; and carbamate kinase (CK, EC 2.7.2.2), which produces ATP, ammonia, and carbon dioxide through dephosphorylation of carbamoyl phosphate. A fourth membrane transport protein, which catalyzes an electroneutral exchange between arginine and ornithine, is also fundamental for this pathway. Sequence analysis of the genetic organization of the ADI pathway has been studied largely either with gram-negative bacteria or with gram-positive bacteria such as *Clostridium perfringens*, *Bacillus licheniformis*, *Streptococcus sanguis*, *Lactobacillus sakei*, and *Oenococcus oeni*. High similarities among species and revealed that genes are clustered to form the operon arcABCTD and encode ADI(*arcA*), OTC(*arcB*), CK(*arcC*), and the membrane transport protein (*arcD*)(36). Oxygen, carbohydrate metabolism, arginine concentration, pH, repression by glucose, and intracellular ATP, NADH, or other intermediate metabolites seemed to be signals for ADI expression, with an influence which depended on bacterial species (37).

The cytoplasmic extracts of 70 strains of the most frequently isolated sourdough lactic acid bacteria were screened initially for arginine deiminase (ADI), ornithine transcarbamoylase (OTC) (EC 2.1.3.3), and carbamate kinase (CK) (EC 2.7.2.2) activities, which comprise the ADI (or arginine dihydrolase) pathway. Only obligately heterofermentative strains such as *Lactobacillus sanfranciscensis* CB1; *Lactobacillus brevis* AM1, AM8, and 10A; *Lactobacillus hilgardii* 51B; and *Lactobacillus fructivorans* DD3 and DA106 showed all three enzyme activities. The three enzymes were purified from this microorganism to homogeneity by several chromatographic steps. ADI, OTC, and CK had apparent molecular masses of ca. 46, 39, and 37 kDa, respectively, and the pIs were in the range of 5.07 to 5.2. The OTCs, CKs, and especially ADIs were well adapted to pH (acidic, pH 3.5 to 4.5) and temperature (30 to 37°C) conditions which are usually found during sourdough fermentation. ADI, OTC, and CK of *Lactobacillus sakei. L. sanfranciscensis* CB1 expressed the ADI pathway either on MAM broth containing 17 mM arginine or during sourdough fermentation with 1 to 43 mM added arginine. Cell survival during storage at 7°C, and tolerance to acid environmental stress and favored the production of ornithine, which is an important precursor of crust aroma compound, 2-acetyl-1-pyrroline (38).

F. Pyrazine

Alkylpyrazines are heterocyclic, nitrogen containing molecules found in wide variety of foods. They are responsible for different flavors, according to the nature of the alkyl substituents. Many products were formed with a heterocycle production such as pyrazines and methylpyrazines, and dimethylethyloxazoles. These various compounds present odors of corn-like, pungent, nut, popcorn, roasted hazelnut, toasted, roasted, and ripe fruits (39).

The first evidence that microorganisms were able to synthesize pyrazines were provided by Kosuge et al. (40) who showed that tetramethylpyrazine could be produced by *Bacillus subtilis.* 2,5-Dimethylpyrazine (2,5-DMP) and tetramethylpyrazine (TTMP) were produced using *Bacillus subtilis* grown on soybeans. 2,5-DMP was obtained using soybeans enriched with threonine, giving metabolite after 6 days. TTMP production involved addition of acetoin to soybeans, and was recovered after 14 days. These results demonstrated the suitability of solid-state cultivation for production of high-added-value compounds (41). Optimization studies demonstrated that solid-state cultivation of *Bacillus subtilis* IFO 3013 involving massive enrichment of the medium with L-threonine and acetoin could be used for production of high-added-value compounds (42).

G. Methyl-Branched Aldehydes and Carboxy Acids

Enzymatic degradation of amino acids plays an important role in the development of cheese flavor. In particular, branched chain amino acids (BCAAs) are precursors of cheesy aroma compounds, such as isovalerate (precursor, leucine) and isobutyrate (valine), and aromatic amino acids are precursors of floral or phenolic aroma compounds, such as phenylacetate and phenylacetaldehyde (phenylalanine),

indole (tryptophane) and phenol (tyrosine) (43). Branched chain amino acid degradation seems to follow chain reaction that commences by transamination producing a keto acid, subsequent non-oxidative decarboxylation into aldehydes which is then oxidized or reduced to acids or alcohols. Amino acid catabolism of valine, leucine, isoleucine, phenylalanine and methionine may also lead to the corresponding aldehydes and acids (44).

Staphylococcus carnosus and *S. xylosus* are important starter cultures for production of essential flavor compounds, such as aldehydes, alcohols, esters, and carboxy acids in dried fermented sausages. Many of these compounds are derived from degradation of amino acids and provide the product with its unique taste and aroma. Catabolism of the methyl-branched amino acids, leucine, isoleucine, and valine, leads to production of methyl-branched aldehydes, 3-methylbutanal, 2-methylbutanal and 2-methylpropanal, respectively. Leucine catabolism is of particular interest, since key sausage aroma compounds like 3-methylbutanal and 3-methylbutanoic acid are derived from catabolism of leucine.

The genetic and physiological evidence of the role of the *Iiv*E enzyme in the degradation of BCAAs in *S. carnosus* has been studied intensively (45). The first step in the catabolism is most likely a transamination reaction catalyzed by BCAA aminotransferase (*Iiv*E proteins). The *Iiv*E enzyme in *S. carnosus* was reported to be the only enzyme that catalyzed the deamination of leucine, isoleucine, and valine. Aminotransferases catalyze the transfer of an alpha-amino group from ArAAs and BCAAs to an alpha-keto acid receptor using pyridoxal 5'-phosphate as a coenzyme, seem to be responsible for deamination of these amino acids (46). The *Iiv*E gene from *S. carnosus* was characterized. An *Iiv*E deletion mutant by gene replacement was constructed. The mutant strain provides the direct evidence of the critical role of aminotransferases in biosynthesis of methyl-branched aldehydes and carboxy acids.

III. CARBOHYDRATE-DERIVED FLAVORS

A. 4-Hydroxy—2,5-Dimethyl-3(2H)-Furanone (HDMF)

Among flavor compounds exhibiting caramel-like odors, HDMF is of special interest because of its relatively low flavor thresholds (47). The odorant was reported for the first time in pineapples and strawberries (48). 4-Hydroxy—2,5-dimethyl-3(2H)-furanone (HDMF) also called furaneol is a carbohydrate-derived natural flavor. HDMF has also been detected in several heat-processed foods, e.g. beef broth (49). HDMF has also been isolated from shoyu (shoyu is the Japanese name for soy sauce) (50).

An aldolase catalyzed reaction was involved in the formation of 4-Hydroxy—2,5-dimethyl-3(2H)-furanone (51). The formation of a homolog of HDMF, HEMF and concluded that D-sedoheptulose 7-phosphate is the precursor of HEMF.A. This study demonstrates that HDMF is secondary metabolite of *Zygosaccharomyces rouxii* that is produced when the medium is supplemented with six-carbon ketoses. Aerobic conditions are more favorable to its production than anaerobic conditions. Two of the tested compounds, 6-deoxyketose and fructose 1,6-bisphosphate, can act as precursors or inducers of the biosynthesis. These findings demonstrate that HDMF is produced by *Z. rouxii* grown aerobically with D-fructose 1,6-bisphosphate (10%) as precursor (52).

Biosynthesis route of HEMF was claimed to be through the pentose-phosphate cycle by *Z. rouxii* (53). Several biotechnological routes have been proposed for the production of natural furaneol. One approach uses the aldolase reaction to form dihydroxyacetone that reacts with lactaldehyde in the presence of aldolase to 6-deoxyfructose-1-phosphate. The equilibrium can be shifted in favor of the product by the addition of triosephosphate isomerase (EC 5.3.1.1). Acid hydrolysis yields 6-deoxyfructose and during heating with base furaneol is formed. The second approach was 6-deoxy-L-sorbose, an isomer of 6-deoxyfructose for the production of furaneol. In this case the deoxysugar is generated by the action of transketolase (EC 2.2.1.1) for 4-deoxy-L-threose and hydroxypyruvate. Hydroxypyruvate is formed from L-serine by serine glyoxylate aminotransferase (serine glyoxylate transaminase, EC 2.6.1.45). Hydroxypyruvate is also the starting material for 4-deoxy-L-erythrulose catalyzed by transketolase (EC 2.2.1.1). The 4-deoxy-L-threose is generated by a microbial isomerization from 4-deoxy-L-erythrulose.

B. Diacetyl

Diacetyl is considered to be one of the most important compounds contributing to the final flavor and aroma in a range of fresh fermented dairy products, such as butter, buttermilk, culture cream, and quark. Diacetyl is formed during the fermentation of milk by the gram-positive microaerophilic bacterium *Lactococcus lactis* subsp. *lactis biovar diacetylactis* and *Leuconoccus* spp. Based on its aroma value, efficient diacetyl (or its precursor alpha-acetolactate) production from lactose rather than from citrate by *Lactococcus lactis* has been one of the targets of various metabolic engineering strategies. *Lactococcus lactis* has been developed as a cell factory for high-level diacetyl production (54).

Metabolic engineering strategies that involve inactivation of undesired genes and/or over-expression of existing or novel ones have been used to create rerouting of the metabolic fluxes by changing the energy metabolism or the concentrations of metabolic intermediates or of existing or completely new end-products (55).

Diacetyl is a by-product of fermentation by many microorganisms. It is produced by oxidative decarboxylation of the metabolic intermediate alpha-acetolactate. One molecule of alpha-acetolactate is produced from two molecules of pyruvate by the condensing enzyme, alpha-acetolactate synthase (EC 2.2.1.6) (56). Specific *Lactococcus lactis* strains isolated from dairy cultures that produce large amounts of alpha-acetolactate from citric acid were shown to lack the alpha-acetolactate decarboxylase enzyme (57). New selection methods and deletion of alpha-acetolactate decarboxylase encoding *ald*B gene by genetic engineering have made these mutants more ready available.

Recent studies of pyruvate and citrate metabolism in *Lactococcus lactis* and *Leuconoccus* spp demonstrate that diacetyl is formed by an oxidative decarboxylation of alpha-acetolactate. A possible method for improving the diacetyl level would be to redirect and adjust the metabolic activities involved in the formation of alpha-acetolactate (58). An innovative approach is to increase the amount of enzyme involved in conversion of pyruvate to alpha-acetolactate. Two enzymes are known in *Lactococcus lactis* that catalyze this reaction: alpha-acetolactate synthase (EC 2.2.1.6, ALS-encoded by the *als* gene) and alpha-acetohydroxy acid synthase (IL VBN-encoded by the *ilv*BN gene). A 100-fold increase in ALS enzyme level was achieved by cloning the *als* gene on a multi-copy plasmid, resulting in a 40% rerouting efficiency of the pyruvate pool towards acetoin under aerobic conditions. This is due to the endogenous alpha-acetolactate decarboxylase (EC 4.1.1.5, ALDB-encoded by the *ald*B gene) activity that efficiently converts alpha-acetolactate to acetoin was inactivated. An elegant approach to obtain ALDB deficient natural *Lactococcus lactis* variants has recently been developed (59). A strain in which all three mutations are combined (LDH- and ALDB-deficiency and ALS or IL VBN overproduction) could be an effective diacetyl producer. The introduction of an NADH oxidase encoding gene in an alpha-acetolactate decarboxylase deficient strain (*aldB* mutant) led to the efficient re-routing of the pyruvate metabolism towards alpha-acetolactate and diacetyl (60). Carroll et al. (61) suggested that manipulation of the enzyme alpha-acetolactate synthase from *Leuconostoc lactis* would also yield strains with increased diacetyl production.

IV. LIPID-DERIVED FLAVORS

A. Hexenals

Leave aldehyde (E)-2-hexenal and (Z)-3-hexenal are responsible for the green flavors and aromas of fruits and vegetables. The natural compounds derived from plant tissue that have been disrupted in some fashion are tried to replace the chemically synthetic compounds. In general, the unsaturated fatty acids linoleic and linolenic acids are degraded via a lipoxygenase (EC 1.13.11.13)-catalyzed formation of hydroperoxides and a subsequent cleavage by a lyase to form aliphatic C6-compound such as (Z)-3-hexenal, which may further isomerized to (E)-2-hexenal. Much research has already been conducted on lipoxygenases especially those from soybean. They catalyze the addition of molecular oxygen to the molecule at carbon 13 (62). Lipoxygenases are essential components of the oxylipin pathway, converting unsaturated fatty acids into (Z)-3-hexenol and (E)-2-hexenal. Lipoxygenases have also been detected in microorganism and plant lipoxygenases have been expressed in host organism. They are available for the biotechnological production of the 13-hydroperoxide. The other decisive enzyme is the hydroperoxide-lyase. Recently, the construction of recombinant yeast cells containing the hydroperoxide lyase gene from banana fruit (*Musa* sp.) has been published (63). Researchers from Givaudan succeeded in the isolation and transfer of the lyase gene from banana to yeasts. The yeast produces hexenals from fatty acids.

B. Lactones

The naturally occurring, organoleptically important lactones generally have gamma or delta-lactone structure, and are straight-chained. Lactone flavor substances play an important role in the overall aroma presentation of many foods and beverages. Lactones are important flavor substances for pineapple, apricots, strawberry, raspberry, mango, papaya, passion fruit, peach and plum. At present, lactones are made fairly expensively via chemical synthesis from keto acids. On the other hand, microbially produced lactones have the advantage of being optically pure and natural. Application focuses mainly on the production of added-value products such as gamma and delta-lactone (from US$6000/kg to US$1200/kg) (64). The gamma-and delta-lactone of less 12 carbons constitutes a group of compounds of great interest to the flavor industry. Gamma-lactone is one of the most produced lactones. Its production through biotechnology results in a natural lactone. The annual market for gamma-lactone has been growing in recent years and was estimated in 1997 at about 10 t (65).

Usually, the precursor used for the production of a lactone is a hydroxy fatty acid. Depending upon the position of the hydroxy group on the aliphatic chain, this acid could lead to the formation of a gamma or delta-lactone. Lactones can be formed either by de novo synthesis, by beta-oxidation from ricin-oleic acid, free fatty acids or hydroxy acids. The 9-10-epoxyoctadecanoic acid formed by epoxidation from oleic acid was proposed as the precursor for dodecano-4-lactone in strawberry fruits. Subsequent beta-oxidation and cyclization leads to the formation of the lactone. Gamma-lactone is generally obtained by biotransformation of a long-chain hydroxy fatty acid precursor by yeast cells. Ricin-oleic acid is a convenient substrate since this hydroxy acid represents almost 90% of

hydrolyzed castor oil (66). 4-Decanolide is an important component in a number of fruits and can be obtained from coriolic acid, which is the major fatty acid present in the seed oil of *Coriana nepalensis* and in rice lipids. Delta-octalactone can be produced by *Saccharomyces cerevisiae* from 3,11-dihydroxymyristic acid, which is present in Jalap resin. After a bioprocess was established, the price for natural 4-decanolide decreased from US$20000/kg to $1200/kg (67).

The general strategy used for the production of lactone by microorganisms involving the bioconversion of a hydroxy fatty acid. The main pathway by which a microorganism catabolizes a fatty acid is the beta-oxidation system. Free fatty acids are converted to coenzyme A (CoA) esters before being catabolized. Beta-oxidation is a four-reaction sequence resulting in a two-carbon chain-shortening catalyzed in peroxisomes by the following activities: acyl-CoA oxidase (EC 1.3.3.6), 2-enoyl-CoA hydratase (EC 4.2.1.17), 3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) and 3-ketoacyl-CoA thiolase (EC 2.3.1.16). The commonly accepted pathway from ricin-oleyl-CoA to gama-decalactone was proposed to follow the four beta-oxidation cycles, yielding 4-hydroxy-decanoly-CoA, which is then cyclised to gamma-decalactone (68).

Yarrowia lipolytica is one of the yeast species that are able to produce gamma-decalactone from ricin-oleic acid or its methyl ester. *Y. lipolytica* was also reported in patents dealing with the production of some other lactone aroma compounds: gamma-dodecalactone, gamma-nonalactone, unsaturated lactones and delta-lactones (69). Because there are few natural sources of cheap fatty acid precursors other than castor oil, processes have been developed for the biotechnological production of hydroxy fatty acids, such as the microbial production of 10-hydroxystearic acid by *Nocardia cholesterolicum* for the synthesis of gamma-dodecalactone (70). Recently, solid-state fermentation with economical and practical advantages had been used as a model for the production of volatile flavor compounds. These include high productivity, low capital investment, reduced energy requirement, low wastewater output, improved product recovery and elimination of foam products. The production of 6-pentyl-alpha-pyrone, a compound with a strong coconut-like aroma, by *Trichoderma harzianum* in solid state cultivation was developed (71).

C. Esters

The distinctive flavor of wine, brandy, and other grape-derived alcoholic beverages is affected by many compounds, including esters produced during alcoholic fermentation. The characteristic fruity odors of the fermentation bouquet are primarily due to a mixture of hexyl acetate, ethyl caproate (apple-like aroma), iso-amyl acetate (banana-like aroma), ethyl caprylate (apple-like aroma), and 2-phenylethyl acetate (fruity, flowery flavor with a honey note) (72).

1. Acetate Esters

The synthesis of acetate esters by the wine yeast *Saccharomyces cerevisiae* during fermentation is ascribed to at least three acetyltransferase activities, namely, alcohol acetyltransferase (AAT, EC 2.3.1.84), ethanol acetyltransferase (EC 2.3.1.84), and iso-amyl acetyltransferase (AAT) (EC 2.3.1.84) (73).

Esters are produced by fermenting yeast cells in an enzyme-catalyzed intracellular reaction. In the volatile ester production by *S. cerevisiae* alcohol acetyltransferases, the respective genes Atf1p, Atf2p and Lg-Atf1p were either deleted or over-expressed in a laboratory strain and a commercial brewing strain. Analysis of the fermentation products confirmed that the expression levels of *ATF1* and *ATF2* greatly affect the production of ethyl acetate and isoamyl acetate. Atf1p and Atf2p are also responsible for the formation of a broad range of less volatile esters, such as propyl acetate, isobutyl acetate, pentyl acetate, hexyl acetate, heptyl acetate, octyl acetate, and phenyl ethyl acetate. Atf1p and Atf2p are responsible for the total cellular isoamyl alcohol acetyltransferase activity.

Interestingly, over-expression of different alleles of *ATF1* and *ATF2* led to different ester production rates, indicating that differences in the aroma profiles of yeast strains may be partially due to mutations in their *ATF* genes. The over-expression of acetyltransferase genes such as *ATF1* could profoundly affect the flavor profiles of wines and distillates deficient in aroma, thereby paving the way for the production of products maintaining a fruitier character for longer periods after bottling (74). The *ATF1* gene, located on chromosome XV, was cloned from a widely used commercial wine yeast strain of *S. cerevisiae*, and placed under the control of the constitutive yeast phosphoglycerate kinase gene (*PGK1*) promotor and terminator. The level of ethyl acetate, iso-amyl acetate, and 2-phenylethyl acetate increased 3- to 10-fold, 3.8- to 12-fold, and 2- to 10-fold, respectively, depending on the fermentation temperature, cultivar, and yeast strain used (75). The drastic increases in the levels of ethyl acetate and iso-amyl acetate had a pronounced effect on the aroma of the fermented and distilled products. Some of other esters whose concentrations were also increased as a result of the over-expression of *ATF1* include hexyl acetate, ethyl caproate, and 2-phenylethyl acetate, presenting a flowery and fruity aroma. Oda et al. (76) developed a system based on the double coupling of glucose metabolism for the production of acetyl-CoA and microbial esterification of alcohol by using a nutrient agar plate as hydrophilic carrier and decane as a hydrophobic organic solvent. This system can be used to produce acetate ester of alcohol without adding any acetyl donors.

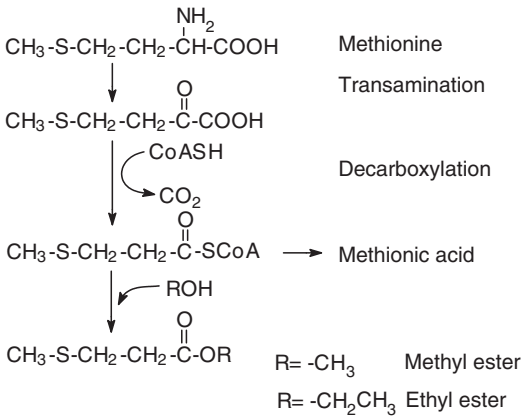

FIGURE 87.1 Proposed biosynthesis pathway for methyl and ethyl esters.

2. Methyl and Ethyl Esters

Methyl and ethyl esters and interesting thioesters, methylmethylthiopropanoate, and ethyl 3-methylthiopropanoate could be synthesized following a pathway shown in Figure 87.1. Amino acid may undergo a transamination to form a keto acid, subsequent decarboxylation leads to the formation of a CoA ester. The release of the CoA moiety results in the generation of an acid following the similar pathway proposed by Dirinck et al. (77). Incorporation of methanol and ethanol to the CoA ester may form methyl and ethyl esters, respectively.

The fate of amino acids in relation to aroma biogenesis has been studied in strawberries using the in vitro growth approach. Incubations of strawberries with L-isoleucine gave rise to an increase of fourteen compounds in this fruit aroma, either not detected previously or constituents of strawberry aroma. Strawberry feeding with L-isoleucine resulted in a 7-fold increase in the sum of 2-methylbutanoate esters, and a double production of 2-methylbutyl esters compared to those of control fruits. Around 94% of the ester increase corresponded to 2-methylbutanoates, with ethyl 2-methylbutanoate being the most representative compound (92%). On the other hand, among the 2-methylbutyl esters, comprising around 6% of total aroma volatiles increase, 2-methylbutyl acetate was the major compound (95%) arising from L-isoleucine strawberry feeding. The role of enzymatic activities within the amino acid metabolic pathway in strawberry fruits is discussed (78).

In *Lactococcus lactis*, which is widely used as a starter in the cheese industry, the first step of aromatic and branched-chain amino acid degradation is a transamination that is catalyzed by two major aminotransferases. In lactococci, transamination is the first step of the enzymatic conversion of aromatic and branched-chain amino acids to aroma compounds. The major aromatic aminotransferase (AraT) of a *Lactococcus lactis* subsp. *cremoris* strain has been purified and biochemically characterized. The corresponding gene was characterized , and evaluated for the role of AraT in the biosynthesis of amino acids and in the conversion of amino acids to aroma compounds. It is also highly involved in methionine and leucine conversion (79).

Pseudomonas fragi strain CRDA 037 produced a fruity aroma when grown in skim milk-, whey and whey permeate-based culture media. Addition of C(3)-C(7) fatty acids to the culture stimulated the production of the corresponding fatty acid esters from 12 to 1570 times compared to unsupplemented media. Lipase is responsible for the biosynthesis of these fatty esters. Lipases are generally defined as glycerol ester hydrolases (e.g., triacylglycerol lipase EC 3.1.1.4) hydrolyzing tri-, di- and mono-glycerides present at an oil-water interface. They can also synthesize and transfer esters. As water is a product of ester synthesis reactions, organic solvents with a very low water activity are generally used and the water formed during the reaction must be extracted (80). Synthesis of esters had been observed in the presence of the lipase from *Candida parapsilosis* (81). The enzyme was inactive in anhydrous systems, however its activity increased with increasing water concentration in the liquid phase or water activity of the system. In the case of very high Aw values, the enzyme preparation contained a large amount of free water, and diffusion of hydrophobic substrates was limited, causing a decrease of the reaction rate (82).

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88 Natural Flavors

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CONTENTS

I. INTRODUCTION

Flavor not only is a major quality attribute to foods, but it also plays a critical role in consumer acceptability. There are many ways to classify flavors. An easier and more realistic way for flavor classification is based on its mode of formation, either biosynthesized naturally from known raw materials or formed artificially by processing imposed on natural or artificial starting materials. Therefore, the terms "natural flavors" and "synthetic flavors" are used. According to the Code of Federal Regulations, the Title 21, Section 101.22 (a)(3) [1] published by the U.S. Government, the term "natural flavor" or "natural flavoring" is defined as:

> *the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional. Natural flavors include the natural essence or extractives obtained from plants listed in Secs. 182.10, 182.20, 182.40, and 182.50 and part 184 of this chapter, and the substances listed in Sec. 172.510 of this chapter…*

Thus, on one hand, "natural flavors" include those volatile compounds derived from nuts, meats, and seafood that possess natural, mild or weak aromas unless further processed thermally; on the other hand, the term also comprises chemical components produced by enzymatic and/or fermentation processes. The latter could be savory flavors or flavor modifiers such as cheese and soy sauce. Since the processed "natural flavors" will be discussed in other chapters, this chapter will focus on those derived from selected natural fruits, vegetables, herbs and spices based on the preference of the authors, to illustrate the chemical basis of key flavors responsible for the natural materials. Detailed discussion on a single character-impact compound for a specific raw material will not always be attempted since many flavor profiles from the natural source are attributed to a mixture of aromatic compounds rather than a single compound though it might play an important role in flavor contribution in certain cases. Besides, it is not the objective of this chapter to cover many biosynthetic pathways of the natural flavors in depth. However, examples will be given, where appropriate and when the information is available, to illustrate the mechanisms, characteristics and biofunctions of some of the important flavors. The aim of this chapter is to present an outline of the most important aspects of the current knowledge and the recent progress in natural flavors, rather than to present an in-depth review on one subject. Nevertheless, interested readers can find more detailed information for the topics in excellent references, reviews and books [2,3,4,5,6,7] listed at the end of this chapter.

II. FRUIT FLAVORS

Fruits are widely accepted by consumers because of their floral, pleasant, and elegant aromas, as well as their association with the firm idea of benefits to health [8]. Natural flavors in ripened fruits usually exist in complicated and delicate ratios of hundreds of volatile compounds composed of short chain alcohols, aldehydes, ketones, esters, acids, lactones, terpenic compounds, etc. The flavors are formed gradually during ripening and usually reach their richest profiles when fruits become mature [9]. At that time, the fullest aromas in fruits are often correlated well with the sugar content, acidity, and colors [9]. When fruits become overripe, their colors turn unappealing, their sugars grow syrupy, and their flavors might lose the attractive freshness. Nevertheless, successful commercial blending of various fresh fruit juices together to make desirable beverages, such as the fruit-flavored teas, lemonades, and yogurts, have created many well-known products being the myth of many companies. As a result, enthusiasm to better understand the components in fruit flavors increases. Although many characteristic aromas in fruits have been identified so far, many more aromas in exotic fruits are still a mystery due to their low concentrations in the fruits.

This section will focus on some character-impact and quantitatively important flavor components in selected fruits to provide a basis to those determined readers who are interested in formulating fruity-flavored products and in performing quality control during fruit processing and storage of the fruits.

A. APPLE

Apples are the fruits of the plant *Malcus sylvestris* Mill., and one of the most common edible fruits accepted by consumers all over the world. In most varieties, apples are spherical in shape and in different sizes, with skin colors from green to red. Apples are generally either freshly consumed or made into apple juice, or cider after fermentation. The volatile flavor constituents of apples have been studied over half a century and have been reviewed by Dimick and Hoskin [10]. Common flavor components in apples

TABLE 88.1
Important Flavor Compounds Found in Apples

Ethyl acetate	Hexyl acetate	6-methyl-5-hepten-2-one
Propyl acetate	Acetylaldehyde	Ethanol
2-Methylpropyl acetate	Propanal	1-Propanol
Ethyl butyrate	Butanal	1-Butanol
Propyl propionate	2-Methylbutanal	2-Methyl-l-butanol
Ethyl 2-methylbutanoate	Hexanal	β-damascenone
Butyl acetate	Trans-2-hexenal	γ-octalactone
2-Methylbutyl acetate	Octanal	4-methoxylallybenzene
Ethyl pentanoate	Acetone	
Butyl proprionate	1-Phenyl ethanone	

Source: From Refs. 4, 7, 11, and 12.

are short-chain esters, alcohols, lactones and aldehydes (Table 88.1). For example, 6-methyl-5-hepten-2-one and γ-octalactone were often detected. Other compounds claimed to be important contributors to the aroma of apples include: ethyl 2-methylbutanoate, n-hexanal, *t*-2-hexenal, 4-methoxyallylbenzene, etc. Though β-damascenone in apples is in very low concentration, it is likely the most potent aroma compound due to its low threshold value of 2 ppt in water [13]. For instance, β-damascenone has been measured to contribute 32% of the total aroma potency in thermally processed apple juice [14]. However, similar to many other fruits, apple aroma does not simply rely on the presence of a limited number of character-impact components but a more complex mixture of the aforementioned chemicals in a delicate ratio.

B. Banana

Banana is a collective term for the fruits of several *Musa* species which are grown throughout the tropics. Commercially, it is picked at its green, unripened stage and consumed at its yellow, ripened stage for easy digestion and absorption of its rich nutrients [15]. Banana is easily subject to physical damages during transportation and distribution, also it is very sensitive to storage conditions and readily turns brown after enzymatic reactions. Nevertheless, banana is one of the most favorite fruits by many consumers all over the world because of its soft, smooth texture and distinctive, mild but delectable aromas released during consumption. Though majority of the banana is freshly consumed as a table fruit, some others are consumed as a snack food, e.g. banana chips.

Analysis of the aroma compounds in banana has been carried out for the past 40 years. With the aid of modern sensitive, high resolution state-of-the-art instruments such as gas chromatography coupled with mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR), many flavor components in bananas have been identified, which were discussed by several researchers [7,15,16,17,18], and summarized and published by TNO-CIVO [4]. Major compounds of banana fruits are acetates, butanoates, and/or 3-methylbutyl esters. Other principle or characteristic flavor components isolated from bananas include pentan-2-one, 3-methyl-1-butanol, 2-methyl-1-butanol, and esters of pentan-2-ol, hexan-2-ol, hept-4(*Z*)-en-2-ol, oct-4(*Z*)-en-2-ol, etc. Shiota [17] also reported some uncommon esters in bananas, such as alkenyl esters and alkan-2-yl esters. They possessed green, sweet, fruity, banana-like or pineapple-like flavors which might also be important contributors to the overall banana aroma. Interested readers may consult the article [17] that listed more than 150 aromas and provided some odor descriptions to the flavor-impact esters.

C. Citrus Fruits

The citrus family contains many popular table fruits with significant commercial values [19]. It has been extensively studied not only for its nutritional benefits but for its distinguished aromatic fruity flavor sensation. Traditionally, citrus flavorings, either in the form of oil phase (essential oils) or of water phase (concentrated fruit juices), are used to extend, enhance or substitute for fresh fruit juices in beverages or other finished food products [19]. With the continued demand and growth of natural flavorings, research in the citrus fruits has been receiving more attention. However, when taking into account of the enormous economic importance of citrus fruits, the published information on its flavorings is still sparse, let alone the confidential restrictions imposed by the beverage companies. Therefore, it might be helpful to summarize some of the previous works found in the literature on citrus flavorings in order to facilitate the readers to get an access to some of the data available. The natural flavors in the orange, tangerine, grapefruit, lemon and lime are discussed in the subsequent sections.

1. Orange

Among the essential oils from the commercially important citrus species, orange oil has been analyzed the most extensively. Shaw and Coleman in 1974 [20] determined 17 components in the cold-pressed orange oil, in which *d*-limonene was found to be the most predominant chemical accounting for an amount of more than 94% in the oil. Beside limonene, other identified chemicals were pinene, myrcene, linalool, citronellal, decanal, neral, geranial, perillaldehyde, dodecanal, β-elemene, β-caryophyllene, β-copaene, β-farnesene, valencene, β-sinensal, and α-sinensal. Subsequent investigations on the chemical composition of the orange oil were reviewed by Shaw [21], who summarized and reported that there were 109 volatile constituents that had been identified. Since the peel oil is inevitably dropped in the fruit juice during the expressing, it is not surprising that many orange oil components have also been found in the fruit essence though there is a remarkable difference in flavor profiles between the

TABLE 88.2
Important Flavors Found in Citrus Fruits

Compounds	Orange	Grapefruit	Lemon	Lime	Tangerine
Δ-3-Carene	X		X	X	X
Camphene		X	X	X	X
Carvone	X	X	X		X
Caryophyllene	X	X	X	X	X
Citronellal	X	X	X	X	X
Citronellol	X	X	X	X	X
Copaene	X	X			
Elemene	X	X		X	X
Farnesene	X		X	X	
Eugenol			X		
Geranial	X	X	X	X	X
Humulene		X	X	X	X
Limonene	X	X	X	X	X
Linalool	X	X	X	X	X
Myrcene	X	X	X		X
Menthone			X		
Neral	X	X	X	X	X
Nerol	X	X	X	X	X
Nootkatone	X	X	X	X	X
Ocimene	X	X	X		
Perillaldehyde	X		X	X	X
Phellandrene	X	X	X	X	X
Sabinene	X	X	X		X
Terpinene	X	X	X	X	X
Terpinen-4-ol	X	X	X	X	X
Terpinolene	X		X	X	X
Thujene	X	X	X	X	X
Trans-carveol	X		X	X	
Valencene	X	X	X		
Vanillin	X	X	X	X	
α,β-Pinene	X	X	X	X	X
α,β-Sinesal	X	X			X
α,β-Terpineol	X	X	X	X	X
β-Bisabolene			X	X	
γ-Cadinene	X				
1-*p*-Methene-8-thiol		X			

Source: From Refs. 4, 22, 23 and 25.

essential oil and the essence [22]. Other than the major chemical classes (Table 88.2) such as terpenes and terpenoids, alcohols, esters, lactones, and carbonyls, trace amounts of *N*-containing compounds were also identified in the oil [24,25]. Among them, alkyl and phenylpyridines were among the most potent flavor constituents. For example, 3-hexylpyridine found in the oil was described as having a fatty, citrus, orange note and has a threshold value of 0.28 ppb [24]. Therefore, these naturally occurring pyridines are also considered as the major aroma contributors. Recent development in the search of novel chemicals in the citrus essential oils has resulted in the identification of some oxygen-containing heterocyclic compounds [26] and vanillin [27]. However, their roles in the oil are still uncertain.

2. Grapefruit, Lemon, Lime, and Tangerine

Grapefruit oils have been studied by utilizing many of the same analytical methods used for orange essential oils. Many of the same aromas in orange oils were also found in grapefruit oils (Table 88.2). In the grapefruit oil, both nootkatone [28] and 1-*p*-methene-8-thiol [29] were considered to be the major flavor contributors providing synergistic grapefruit odor. Interestingly, the magic facet of nootkatone is not only limited to its aromatic character, but to its wide-spectrum efficacy against insects, such as the *Formosan* subterranean termites, cockroaches, fire ants, and ticks [30]. For 1-*p*-methene-8-thiol, it is one of a few *S*-containing compounds that play important roles in the citrus flavors.

Lemon oil usually has a higher market value per unit than orange and grapefruit oils, and is nearly as equally valuable as lime oil [21]. However, the demand for lemon juice, although significant and popular for its fresh, acidic taste commonly used in soft drinks, is not as high as for orange juice. Similar to the chemical composition of orange oils, lemon oil contains a large quantity of terpene hydrocarbons and oxygenated terpenic compounds [21, 31] that include limonene, β-pinene, γ-terpinene, α-pinene, myrcene, sabinene, citral, linalool, citronellal, neral, geranial, α-terpineol, etc. In addition, esters, short chain acids, ketones and aldehydes have also been found. Like lemon juice, lime juice is very acidic but has a pleasant flavor and cooling taste, which makes it suitable for the production of a refreshing beverage. Lime oils are commercially produced in much higher quantity by distillation method than cold-pressed method since the former product possesses a stronger harsh, lime-type flavor. Tangerine, or mandarin essence was analyzed by Moshonas and Shaw [32] who identified 34 volatile compounds that were categorized into alcohols, aldehydes, ester, ethers, ketones and limonene. Like orange and grapefruit oils, tangerine oils contain a higher percentage of limonene. Other investigations have led to the identification of additional volatile flavor components in the tangerine. These compounds in the tangerine oils and in other citrus oils are collectively shown in Table 88.2.

D. Mango

Mango (*Mangifera indica* L.), one of the earliest cultivated fruits grown in India with a history of more than 5000 years, is one of the most popular tropic fruits in southeast Asia [33]. Mangoes vary considerably in size, shape, and flavor, as well as color from green to yellow. The fruit is usually picked at its unripened stage when it is still raw and firm. Within a week of storage at room temperature, it gradually ripens and is ready for consumption. But the ripening process slows down if the fruit is refrigerated. The reason that mango is so welcomed by consumers is particularly attributed to its strong and desirable

aromas. Though different mango varieties are distinguished by the amount and type of volatile compounds present, similar chemical groups such as esters, lactones, furanones and certain terpenes are commonly found in them. The flavor components of mangoes have been investigated by various researchers [34,35,36,37] and the following constituents were reported: terpenic compounds including Δ-3-carene, α- and β-pinene, β-myrcene, limonene, *cis*- and *trans*-ocimene, terpinene, α-copaene, β-caryophyllene, α-guaiene, α-humulene, *alto*-aromadendrene, gurujunene, δ-cadinene, β-selinene, β-phellandrene, α-terpinolene, β-cubebene, linalool, α-terpineol, etc.; esters with isobutyl acetate, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, hexyl butanoate, etc.; and alcohols, aldehydes ketones and other miscellaneous compounds such as acetophenone, benzaldehyde, dimethylstyrene, benzothiazole, ionone, as well as 2(3H)-furanones. The presence of furaneol (see I.2.F Pineapple), a characteristic pineapple-like aroma that imparts a herbal, sweet, fruity aroma and is commonly found in pineapples and several other fruits, might be responsible for the major flavor attribute in mangoes that contain pineapple-like aroma [35].

E. Peach

Peaches (*Prunus persica* L.), one of the most popular fruits due to their various textures and flavors, are associated with many selectable commercial varieties in the fruit market. Peaches are usually consumed fresh or processed into canned food because they are easily subject to physical wounding and enzymatic browning that will significantly deteriorate the quality of products (i.e., texture and flavor) and shorten their shelf-life for sale. Since peach flavor is a critical factor influencing the product value, many investigations [38,39,40,41] have been conducted on the topic and have resulted in the identification of about 70 volatile compounds. Lactones are considered as a group of important flavor contributors in peach aroma. Generally, C-5 to C-12 γ-lactones have low threshold values and bear the characteristic peach aroma. Among them, γ-decalactone is an important flavor compound. In nature, it exists as two enantiomers (R and S) at a ratio of 9 to 1. As a result, γ-decalactone has been used as an index to detect adulteration in food products since the synthetic one is in racemic form [42]. The status of δ-lactones is uncertain due to their low concentrations in peaches. Other major compounds identified in peaches comprise hexanal, (E)-2-hexenal, benzaldehyde, linalool, esters and other aldehydes [41,43,44].

F. Pineapple

Pineapple (*Ananas cosomus* (L.) *Merr.*) is a herbaceous, perennial plant of the bromeliad family with large, pointed leaves. It might be one of the most beautiful fruits in the world with many hexagonal sections arranged spirally and embedded in the juicy pulp. It is cultivated in the tropical regions of the world, with plantation centers in Hawaii, Florida, and Puerto Rico in the United States. Though pineapple is frequently consumed as a dessert fruit together with other fresh fruits, a large quantity of them are processed into canned or juiced products, and both are excellent food flavoring ingredients used in the preparation of marinated and baked goods.

The taste and flavor profiles of pineapples change markedly with seasons from sweeter in summer to more aromatic in winter. With the aid of instrumental analysis such as GC-MS, Takeoka et al. [45,46] separated and identified in pineapples more than 180 compounds, among which the following constituents were considered as the important flavor contributors: 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), methyl-2-methylbutanoate, ethyl-2-methylbutanoate, ethyl acetate, ethyl hexanoate, ethyl butanoate, ethyl-2-methylpropanoate, methyl hexanoate, and methyl butanoate. Some compounds have a very high odor unit value (an odor intensity value) indicative of their importance in contributing to the overall flavor of pineapples. For instance, furaneol [47] has an odor threshold value of 0.03 ppb, its odor unit value in pineapple has been measured as high as approximately 2.5×10^5. Besides, many esters listed above largely possess pineapple or apple aromas. Ethyl 2-methylbutanoate, a character-impact flavor found in apples, was measured at a threshold value of 0.006 ppb that made it the second largest odor contributor (after furaneol) to pineapple aroma in the pineapple essence [46]. Other than those flavor compounds discussed, terpenes and terpenoids were also detected in pineapples, though their odor contributions were less significant. Meanwhile, it is worth noting that the enantiomeric ratio of numerous chiral components, such as 3-and 5-hydroxy esters, 3-, 4-, and 5-acetoxy esters, γ- and δ-lactones, were found to remain nearly unchanged during the ripening process in pineapples [42,48]. Such stability in the ratios of chiral constituents at different physiological stages is therefore very useful for the investigation of the intended adulterations in fruit products.

III. VEGETABLE FLAVORS

Vegetable flavors continue to be of interest commercially and scientifically for a variety of reasons that rely heavily on health concerns [49,50]. Contemporary dietary recommendations encourage consumption of more fresh vegetables not only to satisfy the gustatory feelings, but to be good for the health. Whether the marketed vegetables can be successfully acceptable to consumers is largely influenced by their inherent flavor sensation delivered after processing and storage. Therefore, characterization of the important vegetable flavor components and illustration of their formation mechanisms are important and will provide

a solid foundation for their quality and desirability evaluation. This is possible by taking advantage of the current modern analytical techniques.

A. *Allium* Species

Interest in the flavor components of plants in the genus *Allium*, e.g. onion and garlic, has led to the isolation and identification of the strong, penetrating, and lachrymatory sulfur-containing chemicals. Today the general features of the biosynthesis of the flavor constituents, the enzymatic conversion to primary products, the rearrangements, decomposition and interaction to produce the secondary products responsible for those irritating aromas have been generally understood and agreed. Not only did the *S*-containing compounds in the *Allium* species contain strong lachrymatory sensation, but they were suggested to have many positive health claims [51,52,53,54]. However, a discussion on the health benefits of these plants is beyond the scope of this section. The subsequent sections will be confined to illustrate the flavor biogenesis and flavor profiles in the genus.

1. Onion

Onion (*Allium cepa* L.) flavors usually do not "break out" unless the tissue is damaged releasing the embedded enzyme alliinase which reacts with the precursor compounds. The enzyme alliinase is a pyridoxal 5'-phosphate dependent α, β-eliminating lyase that hydrolyzes the flavor precursor S-(1-propenyl)-L-cysteine sulfoxide to a hypothetical sulfenic acid intermediate along with ammonia and pyruvate [29,55]. The sulfenic acid is very unstable and readily undergoes further rearrangements to produce the thiopropanl S-oxide, which releases spicy, lachrymatory odor, a typical flavor of cut onions. Besides, part of the unstable sulfenic acid decomposes to form a wide range of *S*-containing compounds. Investigations in the chemical composition of fresh onions and onion oils [56,57,58,59] have identified the following chemical classes: thiosulfinates, thiosulfonates, monosulfides, disulfides, trisulfides, tetrasulfides, thiophene derivatives, thiols, ketones, aldehydes, and other miscellaneous compounds (Table 88.3). The abundant disulfides and other polysulfides were suggested to be formed by disproportionation of the unstable thiosulfinates. Sensory evaluation and threshold determinations (Table 88.4) of the aforementioned chemicals revealed that thiosulfonates with four or more carbon atoms displayed powerful and distinct odor of freshly cut and/or cooked onions, while the propyl- and propenyl-containing di- and trisulfides possessed the flavor of cooked onions. Also, dimethylthiophenes were shown to have distinctive fried or fresh onion flavors. 3-Mercapto-2-methylpentan-1-ol was recently identified [63] as another powerful onion aroma with an odor threshold value at 0.15 ppb in 5% saltwater.

TABLE 88.3
Important *S*-containing Aromas in Onion and Garlic

Dimethyl sulfide	Hydrogen sulfide	2,5-Dimethylthiophene
Allyl propyl sulfide	Methyl propenyl sulfide	3,4-Dimethylthiophene
Dipropenyl sulfide	Methyl propenyl trisulfide	Allylthiol
Tetrasulfide	Isopropyl propyl trisulfide	Propanethiol
Propenyl propyl sulfide	Methyl propyl trisulfide	Propyl methanethiosulfonate
Allyl methyl disulfide	Allyl methyl trisulfide	Propyl propanethiosulfonate
Isopropyl propyl disulfide	Diisopropyl trisulfide	Methyl methanethiosulfonate
Methyl propyl disulfide	Propenyl propyl trisulfide	Allyl isothiocyanate
Propenyl propyl disulfide	Allyl propyl trisulfide	
Diallyl disulfide	Diallyl trisulfide	
Allyl propenyl disulfide	Dipropyl trisulfide	
Dipropyl disulfide	Dimethyl trisulfide	
Dipropenyl disulfide	Dimethyl tetrasulfide	
Allyl propyl disulfide	3,4-Dimethyl-2, 5-dihydrothiophen-2-one	
Methyl propenyl disulfide	Methanethiol	

Source: From Refs. 4, 56, 60, 61 and 66.

TABLE 88.4
Threshold Values of Some *S*-Containing Compounds in *Allium* Species

Compounds	ppb in Water	Description
3,4-Dimethylthiophene	1.3	Fresh onion
2-Methanethiophene	3	Heated onion, sulfury
Dipropyl disulfide	3.2	Cocked onion
Methyl propenyl disulfide	6.3	Cocked onion
Propenyl propyl disulfide	2.2	Cocked onion
Propyl methanethiosulfonate	1.7	Fresh onion
Methanethiol	2.0	Sulfurous
Dimethyl disulfide	3.0–12	Onion, Cabbage
Dimethyl trisulfide	10	Alliaceous, Meaty
Allyl isothiocyanate	375	Strong, Pungent

Source: From Refs. 56, 60, 61 and 62.

2. Garlic

Unlike the flavor precursor in onion, the primary precursor in garlic (*Allium sativum* L.) is S-(2-propenyl)-L-cysteine sulfoxide. One of its products is the volatile chemical diallyl thiosulfinate (allicin) which has a characteristic garlic flavor [64]. With further degradation, additional important components such as methyl allyl and

diallyl disulfide, thiosulfonate, and trace quantities of allyl trisulfide are formed as part of the overall garlic flavor profile (Table 88.3) [65,66,67]. In recent years, garlic with its inherent bioactive components has attracted much attention for its nutritional and clinical values [54,68]. Like other medicinal herbs and spices such as tea, ginger, ginseng, ginkgo, etc., garlic has been recognized as a major functional food or "nutraceutical" that helps to reduce the occurrence of cardiovascular disease and cancer [54,68].

B. Tomato

Tomatoes (*Lycopersicon esculentum* Mill.) are no doubt one of the most popular and favorite vegetables because of its colors, flavors and many claimed healthy benefits. The study of the chemical composition of tomatoes, particularly its characteristic flavors, has been the subject of much research over many years [69,70,71,72,73,74]. Many different and important aspects of tomatoes have been reviewed by Petro-Turza [75]. In particular, the author reported more than 300 volatile compounds in tomatoes. The volatile components which contribute to the flavor of tomatoes are (*Z*)-3-hexenal, 3-methylbutanal, β-ionone, 1-penten-3-one, hexanal, (*Z*)-3-hexenol, (*E*)-2-hexenal, 2- and 3-methylbutanol, 2-(2-methylpropyl)-thiazole, eugenol, 6-methyl-5-hepten-2-one, geranylacetone, 2-phenylethanol, dimethyl sulfide, β-damascenone, methional, 1-nitro-2-phenylethane and 3-methylbutyric acid [69,70,76]. Amino acids, fatty acids and carotenoids serve as their major precursors [72,77]. Furaneol, a character-impact flavor in pineapple, was also suggested to be an important flavor contributor to tomato in subsequent investigations [73,76]. Some investigators believed the tomato flavors were significantly influenced by its rich sugar and acid contents [7,78]. Besides, investigation in flavor precursors in tomatoes, particularly the glycosides, has received considerable attention like that in other fruits [79,80]. 2-Phenylethanol and benzyl alcohol were found to be the major components bound as glycosides in tomatoes, and other glycosidically bound chemicals existing in small quantities include monoterpene alcohols and C13 norisoprenoids [78].

IV. HERBS, SPICES, AND ESSENTIAL OILS

In the olden days, herbs, spices and silk were once objects of relentless exploration by various countries and a symbol of wealth. Currently, as defined in the Title 21, Section 101.22 (a)(2) in the Code of Federal Regulations published by the U.S. Government, the term "spices" is given the meaning of

> *any aromatic vegetable substance in the whole, broken, or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed. Spices include the spices listed in Sec. 182.10 and part 184 of this chapter, such as the following: Allspice, Anise, Basil, Bay leaves, Caraway seed, Cardamon, Celery seed, Chervil, Cinnamon, Cloves, Coriander, Cumin seed, Dill seed, Fennel seed, Fenugreek, Ginger, Horseradish, Mace, Marjoram, Mustard flour, Nutmeg, Oregano, Paprika, Parsley, Pepper, black; Pepper, white; Pepper, red; Rosemary, Saffron, Sage, Savory, Star aniseed, Tarragon, Thyme, Turmeric, Paprika, turmeric, and saffron or other spices…*

while "herbs" are traditionally defined for those botanical materials with aromatic leaves, flowers, stems, and in many cases roots, which have been used in history as medicines or considered to be with health benefits, though some of them have not been specifically defined or approved by the US FDA [81]. In close connection with herbs and spices, essential oils extracted from spices and herbs have found ready use in the food and pharmaceutical industries for their various properties [6,27]. Essential oils are generally obtained by either steam/water distillation, expression extraction, or solvent extraction. Modern extraction of essential oils utilizes supercritical fluid (especially the liquid carbon dioxide) extraction (SFE) method by taking advantage of both its environmental-friendly property and extraordinary extraction ability [82].

A. Tea

Plant tea (*Camellia sinensis* L.) has a long history in China with more than 5000 years. According to legend, it was discovered in the year 2737 BC by a Chinese emperor when some tea leaves accidentally blew into a pot of boiling water. In 800 A.D., Lu Yu wrote the first definitive book on tea, *The Bible of Tea*, that significantly promoted the plantation and consumption of tea. Since then tea culture has spread throughout the Chinese history, as coffee has in the Western countries, reaching into every aspect of the society. Tea was imported to the continental Europe from China in the early 1600s by Dutch traders. In the late 1700s, tea shared a part of historical events when a demonstration against taxation by the English known as the "Boston Tea Party" occurred. This event was one of the fuses for the revolution and founding of the United States of America. Today, tea has become one of the most widely consumed beverages in the world. Its popularity depends largely upon its fragrant, pleasant aromas combined with its medicinal effects that revive, refresh and relax the human bodies and minds before and after their daily work [83]. Depending on the processing procedures, tea leaves are classified into black tea, oolong tea, and green tea after full, semi and without fermentation, respectively. Fermentation by natural enzymes in tea leaves causes many changes in the taste, flavor profile and color

of the end tea products. The chemical constituents of teas have been investigated by many researchers [84,85,86, 87,88,89,90,91], and more than 600 volatile compounds have been identified [91,92,93,94]. Although some character-impact flavors and flavor precursors were identified, there is no uniform flavor profile for various types of teas [86,92] or even with the same type of tea. For example, by using GC-O with the aid of AEDA method, Kumazawa and Masuda [87] found the following most potent flavors in the Japanese green tea: 4-methoxy-2-methyl-2-butanethiol (meaty), (*Z*)-1,5-octadien-3-one (metallic), 4-mercapto-4-methyl-2-pentanone (meaty), (*E,E*)-2,4-decadienal (fatty), β-damascone (fruity), (*Z*)-methyl jasmonate (floral), and indole (animal-like), while Guth and Grosch [94] reported (*Z*)-1,5-octadien-3-one, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, and 3-methylnonone-2,4-dione were the most important odorants in the Chinese green tea. Such variation in the potency of compounds in green teas reflected the difference in chemical compositions that might be caused by the differences in raw materials and in processing methods. In contrast, investigation by Kumazawa and Masuda [88] on black teas showed that 3-methylbutanal, methional (potato-like), β-damascenone (sweet), dimethyl trisulfide (putrid), and 2-methoxy-4-vinylphenol (clove-like) had the highest flavor dilution (FD) values, and aliphatic alcohols and terpenic alcohols were found to be the main aromas of black and oolong teas.

B. Licorice

Native to both Asia and the Mediterranean region, licorice (or liquorice) (*Glycyrrhiza glabra* L.) belongs to the family Leguminosae and is a well-known plant in China called "Gancao," which literally means "sweet grass." Licorice root is one of the oldest and most frequently used folk medicine in China for its diverse therapeutic functions. In the first Chinese medicinal book "Shen Nong Ben Cao Jing," licorice was claimed to possess some special healthy benefits such as "prevention from diseases" that have been confirmed by the modern medical studies due to the presence of some inherent bioactive chemicals, such as the chemical classes of alkaloids, polysaccharides, polyphenols, flavonoids, terpenoids, etc. These compounds have been demonstrated to possess either cancer chemopreventive or detoxification capabilities [95]. Licorice extract is currently widely used in the food and pharmaceutical industries as a flavoring and sweetening agent because of its distinguished flavors. In particularly, it contains glycyrrhizin, a sweetener, that is about 50 times as sweet as sucrose [96]. Licorice extract is typically used in tobaccos, chewing gums, candies, toothpastes, and beverages. Despite its well-known intrinsic sweetness, there is limited information of the volatile flavor components of licorice. Frattini et al. [97] reported that the most abundant components in heated licorice essential oil were acetol, propionic acid, 2-acetylpyrrole, 2-acetylfuran and furfuryl alcohol, whereas the furan derivatives was the largest chemical class due to the pyrolysis and condensation reactions among sugars. Further investigation by Fenwick et al. [98] revealed the following volatiles might be additional important flavor contributors: estragole, eugenol, indole, cumic aldehyde and nonalactone.

C. Angelica

Angelica with the scientific name of *Angelica archangelica* (L.), also known as *Archangelica officinaiis* Hoffm., is unique amongst the family of Umbelliferae for its pervading, strongly aromatic odor in musky, earthy flavors extracted from its root or seed. Its bitter-sweet, warmly pungent but pleasant flavor profile makes it different from others of the same family members like anise, caraway, fennel, and parsley. The major components in angelica root oil (seed oil has similar profile) were identified by Taskinen and Nykanen [99] with the following quantities: α-pinene (24.0%), limonene (13.2%), Δ-3-carene (10.1%), β-phellandrene (10.1%), para-cymene (9.8%), α-phellandrene and myrcene (7.6%), trans-ocimene (2.68%), α-copaene (1.91%), camphene (1.3%), α-muurolene (1.2%) and cis-ocimene (1.2%). Other terpenic compounds are less than 1% each.

D. Anise

Anise is native to Egypt and the Mediterranean countries, then its cultivation extends to the central Europe, Russia, India, China, etc. This dainty, white-flowered Umbelliferous annual plant yields 1.5–6% of colorless to pale yellowish aniseed oil. This syrupy oil is characterized by its main component, *cis/trans*-anethole, which is in approximately 90 percent of the whole oil that is readily solidified due to the crystallization of anethole when cooled down below 19°C. The other major components of aniseed oil, reported by Lawrence [100,101], include methyl chavicol (1.02%), *trans*-ocimene + acetoanisole (0.94%), anisaldehyde (0.91%), and safrole (0.58%). Other terpenes and terpenoids are in trace amounts.

E. Basil

Sweet basil (*Ocimum basilicum* L.) oil is extracted from the dried, broken leaves and floral parts of the labiate, herbaceous plant which belongs to the Family *Labiatae*. Typical India sweet basil leaves give off a strong aromatic scent very much like that from cloves. In France, sweet basil is also a common culinary flavoring ingredient. There are more than one hundred varieties of basil from which the essential oils are extracted. Their differences in flavor profiles have been discussed by Lawrence [102] and Reineccius [6]. The following components were commonly found in and considered as major constituents among

different sweet basils, though their quantities might vary significantly. These compounds are: 1,8-cineole, ocimene, camphor, linalool, methyl chavicol, geraniol, methyl eugenol, methyl-cinnammate, and eugenol.

F. Caraway

Caraway (*Carum Carvi* L.) is a biennial Umbelliferous plant that is mainly distributed throughout the northern and central European regions. The caraway oil is commonly used as a food flavoring in cookery, confectionery and liqueurs. The major constituents of its oils summarized by Lawrence [103] are listed below: limonene (49.8%), carvone (47.1%), *trans*-dihydrocarvone (0.6%), *cis*-dihydrocarvone (0.2%), *trans*-carveol (0.4%), *cis*-carveol (0.2%), and other terpenic compounds in less than 0.1% or in trace amounts.

G. Cinnamon

Cinnamon trees are typically grown in the hot tropical rain forests. Two of the most important species [104] used to prepare commercial cinnamon oils are: Ceylon cinnamon (*Cinnamomum zeylanicum* Nees.) and Cassia-cinnamon (*C. cassia* L.). The former is indigenous to and mainly cultivated in Sri Lanka and China, while the latter is grown in southern China, Burma, and Vietnam. Commercial cinnamon oils are extracted by steam-distillation from scabrous bark of the plant after drying. Though oils from both sources give slightly different sensory characters, both of them are characterized by sweetly aromatic, warmly pungent flavors and burning effect. The main components in Ceylon cinnamon oil [105] include cinnamic aldehyde, eugenol, linalool, 1,8-cineole, α-terpineol, caryophyllene, α-pinene, α-humulene, etc., whereas the China cassia-cinnamon oil has cinnamic aldehyde, cinnamyl acetate, cinnamic acid, benzaldehyde, salicylaldehyde, courmarin, etc. Comparison between the constituents of the two cinnamon oils is listed in Table 88.5.

TABLE 88.5
Comparison of the Major Chemical Constituents between the Ceylon Cinnamon Oil and the China Cassia-Cinnamon Oil

Celylon-Cinnamon	China Cassia-Cinnamon
Cinnamic aldehyde	Cinnamic aldehyde
Cinnamylacetate	Methoxy cinnamaldehyde
Eugenol	Cinnamylacetate
Caryophyllene	Benzaldehyde
Linalool	Ethyl cinnamate
1,8-Cineole	Salicylaldehyde
α-Terpineol	Coumarin
Terpinen-4-ol	

Source: From Refs. 6, 23, and 105.

H. Clove

Clove (*Eugenia caryophyllata* Thumb.) oils are commercially classified into oils of clove bud, clove leaf and clove stem. They are an important source of the chemical eugenol which is usually used as a quality index for the product. Generally, clove bud oil gives out more fruity and pleasant flavors than its counterparts, leaf oil and stem oil. The chemical profile of the clove bud oil as investigated by Deyama and Horiguchi [106] was found to contain: eugenol (80.9%), eugenyl acetate (7.3%), caryophyllene (9.12%), α- and β-humulene (1.7%), and α-ylangene (0.4%).

I. Coriander

Coriander, an annual Umbelliferous plant (*Coriandrum sativum* L.) originated from the East, is now cultivated in many temperate countries. Coriander fruit contains about 1% of pale yellowish to colorless volatile oil, and its odor could be fruity or warmly spicy. In British Pharmacopoeia, it is described as a functional agent to disguise unpleasant medicines. Taskinen and Nykanen [107] identified the following major components in the Coriander oil: linalool (65.0%), γ-terpinene (10.1%), camphor (5.0%), α-pinene (6.5%), *p*-cymene (3.7%), geranyl acetate (2.6%), limonene (1.7%), geraniol (1.7%) and other chemicals in minor or trace amounts. Since the chemical linalool is dominant in quantity in the oil, it is usually recommended as a flavoring quality index for the recovered essential oil.

J. Fennel

Fennel is a perennial Umbelliferous herb (*Foeniculum vulgare* Mill.) that grows wild in most temperate European countries, but is generally considered indigenous to the Mediterranean regions. Fennel is well known for its succulent, edible shoots and aromatic leaves which are an excellent flavoring ingredient for culinary use. Fennel oils are generally pleasant in flavor, though they differ in some degrees with varieties and sources. There are two principle varieties of fennel: bitter and sweet. Bitter fennel is a synonym for the most common variety. Its principle constituents are anethole (approx. 50%) and fenchone (>10%). Anethole is also a major component in anise oil (see section 1.4.4 Anise). Fenchone is a colorless

TABLE 88.6
Important Flavors in Fennel

α-Thujene	α-Terpinene	p-Cymene	Δ-3-Carene
β-Fenchene	Limonene	Camphor	Myrcene
α-Pinene	l,8-Cineole	Terpinen-4-ol	α-Phellandrene
α-Fenchene	cis-β-ocimene	Methyl chavicol	Terpinolene
Camphene	β-Phellandrene	Linalool	Anisaldehyde
β-Pinene	γ-Terpinene	Fenchone	cis-Anethole
Sabinene	trans-β-Ocimene	α-Terpineol	trans-Anethole

Source: From Refs. 6, 108, and 109.

TABLE 88.7
Major Components in Ginger Oil

Hydrocarbons		Oxygenated Compounds	
α-Thujene	*trans*-β-Farnesene	*trans*-2-Hexenal	Elemol
α-Pinene	Selina-4, 11-diene	Nonanal	*cis*-sesquisabinene hydrate
Camphene	Selina-3, Il-diene	Decanal	Nerolidol
β-Pinene	δ-Amorphene	Undecanal	β-Bisabolol
Sabinene	Zonarene	Citronellal	Zingiberenol
δ-3-Carene	α-Cadinene	Myrtenal	10-α-cadinol
Myrcene	γ-Cadinene	Phellandral	α-Eudesmol
α-Phellandrene	δ-Cadinene	Neral	β-Eudesmol
α-Terpinene	γ-Bisabolene	Geranial	*trans*-β-sesquiphellandrol
Limonene	β-Bisabolene	α-Terpineol	Cubebol
β-Phellandrene	Zingiberene	Citronellol	2-Heptyl acetate
γ-Terpinene	β-Sesquiphellandrene	Geraniol	α-Fenchyl acetate
cis-Ocimene	Germacrene B	1,4-Cineole	Bornyl acetate
Copaene	β-Curcumene	1,8-Cineole	Citronellyl acetate
Sesquithujene	ar-Curcwnene	Linalool	α-Terpinyl acetate
β-Ylangene	Calamenene	Isopulegol	Methyl heptenone
β-Elemene		Caryophyllene oxide	2-Hexanone
γ-Elemene		Terpinolene epoxide	2-Heptanone
trans-α-Bergamotene			2-Nonanone
Caryophyllene			2-Heptanol

Source: From Refs. 6, 23, 111, 112, and 113.

liquid possessing a pungent, camphoraceous flavor, and accounts for the undesirable bitterness of the oil. Sweet fennel is also known as French or Roman fennel that is derived from *Foeniculum vulgare* var. *dulce* Mill. Since it has much less anethole and no fenchone, its oil possesses a milder and sweeter taste. The constituents of fennel oils are shown in Table 88.6 [108,109].

K. Ginger

Commercially traded ginger (*Zingiber officinale* Rosc.) is a herbaceous perennial monocotyledon plant that belongs to the *Zingiberaceae* family. The *Zingiber* genus is estimated to consist of about 80–90 species. Ginger is native to southeast Asia and has been cultivated in countries such as India and China for over 3,000 years. Today, it is widely cultivated in almost all tropical and subtropical countries for its end use either as a fresh root vegetable or as a dry culinary spicy ingredient. In some Asiatic countries, ginger also serves as a folk medicine to cure diarrhea and to prevent catching cold [90,110]. The dry rhizome of ginger yields about 1 to 4% of a light yellowish essential oil which is characterized by its distinctive and strong pungent smell. However, considerable differences exist in sensory properties of ginger oils extracted from different sources [111]. Representative chemical composition of the ginger oil prepared by the steam distillation includes the following major components: α-pinene, camphene, β-pinene, sabinene, Δ-3-carene, myrcene, β-phellandrene, limonene, tricyclene, zingiberene, 1,8-cineole, borneol, zingiberol, methyl heptanone, citral, n-decylaldehyde, linalool, geraniol, farnesene, curcumene, and bisabolene. More comprehensive information on ginger oil constituents (Table 88.7) has been contributed by various research teams [112,113] and summarized in a review by Lawrence [111] who reported that the ginger oil was mainly composed of about 83.0% hydrocarbons, 10% oxygenated compounds and other unidentified compounds determined by using a combination of techniques, e.g., distillation, column chromatography, GC, NMR, IR, and MS. Among the aforementioned volatile chemicals, Bednarczyk and Kramer [114] used statistical chemometrics to correlate the following compounds in ginger oil to the overall faintly lemon-like, earthy, woody characters of the ginger aroma. These compounds are α-terpineol, neral, geraniol, β-sesquiphellandrene, ar-curcumene, nerolidol, and β-sesquiphellandrol. Nevertheless, the pungency of ginger is attributed to some nonvolatile analogues which include gingerols, shogaols, paradols and zingerone (Figure 88.1) [110,115]. Connell noted that some of the ginger homologues would undergo a retro-aldol reaction into zingerone and aliphatic aldehydes if heated, and some of them would be dehydrated to shogaol under alkaline conditions. These degradations and changes would increase the pungency in the products.

L. Mustard

In general, mustard is a herbaceous perennial plant that can be found wildly throughout Europe, India, North African

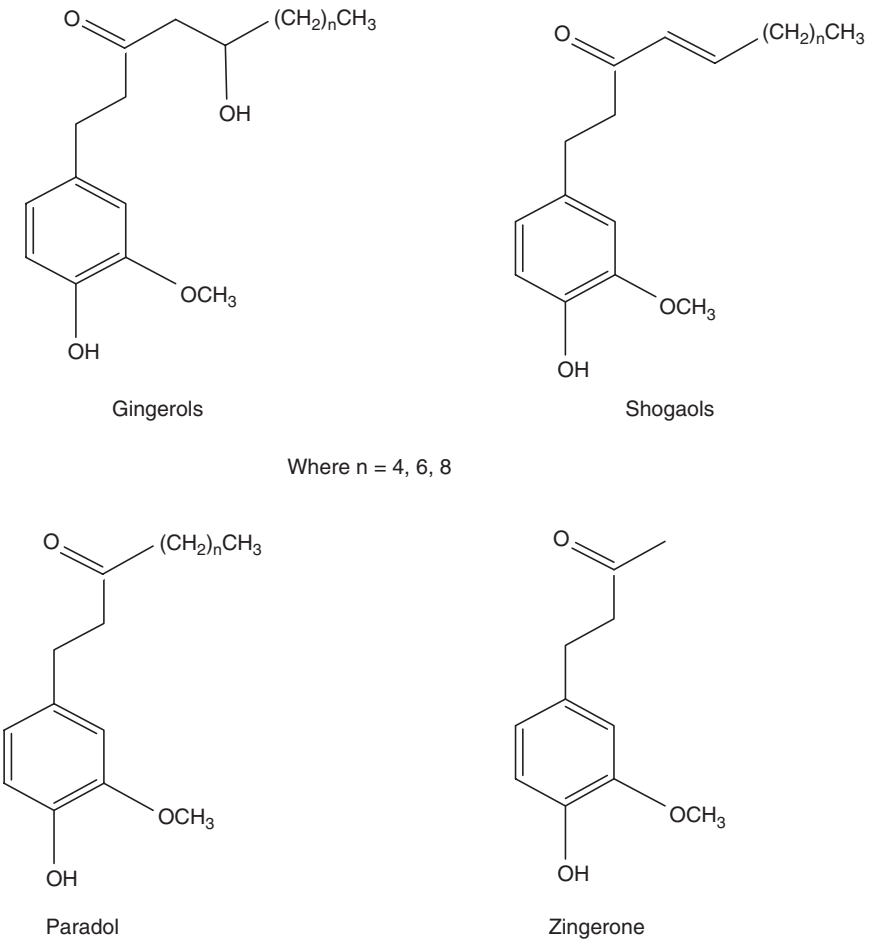

FIGURE 88.1 Key components responsible for ginger pungent aroma.

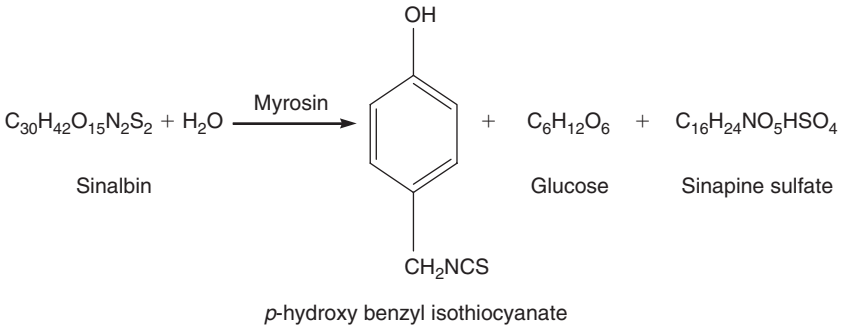

FIGURE 88.2 Proposed enzymatic scheme for the formation of *p*-hydroxy benzyl isothiocyanate in mustard.

and American continent. Under the family Cruciferae, the White mustard (*Brassica alba* Boiss. or *Sinapis alba* L.) and the Black mustard (*Barssica nigra* L.) are known to be associated with other common vegetables such as cabbage, cauliflower, turnip, radish, horseradish, watercress, etc. due to their pungent aromas attributed to the presence of some sulfur-containing compounds. The compound responsible for the characteristic pungent flavor in white mustard is *p*-hydroxyl benzyl iso-thiocyanate which is a decomposed product of the glycoside precursor, sinalbin. The reaction takes place in the presence of both the enzyme myrosin and water (Figure 88.2). By contrast, the pungency of black mustard is caused by the presence of allyl isothiocyanate which is formed from another glycoside sinigrin under the same enzymatic reaction as in white mustard. Generally, mustard oil is extracted by boiling alcohols instead of the steam distillation method. Since mustard oil is dominated in quantity by isothiocyanate that

TABLE 88.8
Major Components in Parsley Leaf Oil

α-Pinene	Dimethylsulphide	α-Thujene	Camphene
β-Pinene	δ-3-Carene	Apiole	α-Phellandrene
Limonene	2-Pentylfuran	γ-Terpinene	β-Elemene
p-Mentha-1, 8-triene	α-p-Dimenthylstyrene	Benzaldeyde	β-Ocimene
Phenylacetaldehyde	α-Terpineol	Dimethylbenzofuran	Copaene
p-Methylacetopheneone	β-Sesquiphellandrene	β-Bisabolene	γ-Cadinene
α-Elemene	Myristicin	*cis*-β-Ocimene	Elemicin
Sabinene	Caryophyllene	α-Cubebene	β-Phellandrene
α-Terpinene	Myrcene	Terpinolene	γ-Elemene

Source: From Refs. 6, 116, 117, and 118.

TABLE 88.9
Major Components in Pepper Oil

α-Pinene	α-Thujene	Myrcene	1,8-Cineole
Sabinene	δ-3-Carene	β-Phellandrene	Linalool
α-Terpinene	Limonene	Citronellal	p-Cymene
γ-Terpinene	trans-Sabinene hydrate	Phellandral	α-Bisabolene
Terpinen-4-ol	α-Terpineol	*cis*-α-Bergamotene	trans-β-Farnesene
Terpinolene	Copaene	Humulene	ar- Curcumene
trans-a-Bergamotene	Caryophyllene	*cis*-γ-Bisabolene	γ-Eudesmol
β-Bisabolene	δ-cadinene	Elemol	*cis*-β-Bergamontene
Piperitone	Cubebol	δ-Elemene	Zingiberene
α-Bisabolol	α-Cubebene	α-Muurolene	Borneol
trans-β-Bergamontene	Ledene	Myrtenol	α-Selinene
Nerol	Geraniol	Methyl citronellate	Terpinen-4-yl acetate
trans-Carveol	*trans*-Pinocarveol	α-Terpinyl acetate	*cis*-Dihydrocarvone
Calamenene	Methyl geranate	Isopinocamphone	α-Humulene
Camphor	Pinocamphone	β-Elemene	*trans*-p-Menth-2-en-l-ol
Carvotanacetone	β-Cubebene	*cis*-Sabinene hydrate	Methyl carvacrol
β-Selinene	Sesquisabinene	trans-Anethole	Terpinolene epoxide
Myrtenal	Benzaldehyde	Dehydro-l, 8-cineole	cis-Nerolidol
2-Undecanone pinol	Methylheptenone	Caryophyllene oxide	β-Bisabolol
Carvone	*trans*-Limonene epoxide	Viridiflorol	
Cubenol	epi-Cubenol	β-Pinene	
Muurolol	Camphene	α-Phellandrene	

Source: From Refs. 6, 121, and 122.

may cause intensive irritating sensation and lachrymatory effects, considerable precautions should be taken before handling the material.

M. Parsley

Fresh green parsley (*Petroselinum ctispum* Mill.) is popular and welcomed for its pleasant aroma. It is often used in culinary preparations as a flavouring material to spice up sauces, soups, stuffings, and even vegetables or salads. The composition of parsley leaf oil was studied by several researchers [116,117,118], and is summarized and presented in Table 88.8. Among the flavors listed in the table, MacLeod et al. [118] noted that β-phellandrene, terpinolene, α-*p*-dimethyl styrene, *p*-mentha-1,3,8-triene, myristicin and apiole were the character-impact components in parsley leaf.

N. Pepper

The black and white peppers are derived from the same plant (*Piper nigrum* L.) of family Piperaceae, but prepared under different procedures. The plant is now widely cultivated in the tropical regions, especially in the southern Asian countries such as India, Malaysia, Indonesia, Cambodia, Sri Lanka, and China. Since pepper powders are one of the most popular food flavorings in our everyday life, intensive research has been carried out over the years to characterize their chemical profiles [119,120]. Early studies revealed that the volatile compounds contributed to the pepper flavor, and the nonvolatile components, mostly piperine, resulted in the pungent taste. Delicate balance between these two chemical groups determines the quality of peppers and distinguishes peppers that come from different sources. For example, Indian peppers are generally

more aromatic, whereas the counterparts from Malaysia and Indonesia are more pungent. Further investigation [121] in the essential oil of pepper has led to the identification of more characteristic flavors which are listed in Table 88.9. Along with the characterization of the oil, the principle pungency of pepper was found to be attributed, in most part, to the alkaloid piperine, which was less pungent than capsaicin, but stronger than gingerol [122].

O. Peppermint and Spearmint

Peppermint (*Mentha piperita* L.) and spearmint (*M. viridis* L.) are two major species of mint in the family Labiatae. Both of them can thrive best in an environment with a fairly warm climate, favorable humidity and nutritious soil. Peppermint might be one of the world's most popular flavoring materials. Peppermint is used in a wide range of applications in candies, chewing gums, and medicinal supplements, whereas spearmint, in many ways resembling peppermint, is mainly used for culinary products. Their essential oils with special flavors are so important that a lot of research [123,124,125] has been conducted in an attempt to better understand their chemical profiles, which are presented in Table 88.10. As noted in the table, menthol and menthone in the peppermint oil are the two major quantitative components that account for approximately 70% of the amount in the oil. In particular, menthol provides the cooling sensation. Other quantitatively important volatiles include menthyl acetate, 1,8-cineole, menthofuran, limonene, iso-menthone, pulegone, octan-3-ol, oct-1-en-3-ol, and mint lactone. Since the pulegone is suspected to be toxic, peppermint oil is used with provisional limits for pulegone levels ranging from 25 ppm in food to 350 ppm in mint confectionery [23]. In spearmint oil, carvone is the predominant volatile compound that presents a sweet, spearmint odor, followed by other terpenic compounds and esters, such as limonene, myrcene, 1,8-cineole, carvyl acetate, dihydrocarvyl acetate, dihydrocarveol, *cis*-jasmone, etc. (Table 88.10).

TABLE 88.10
Important Flavors in Peppermint and Spearmint Oils

Peppermint	Spearmint
Menthol	Carvone
Menthone	Limonene
Menthyl acetate	Myrcene
1,8-Cineole	1,8-Cineole
Menthofuran	Carvyl acetate
Terpinolene	Dihydrocarvyl acetate
Limonene	Dihydrocarveol
Iso-menthone	3-Octanol
3-Octanol	*cis*-Jasmone
Oct-1-en-3-ol	
Mint lactone	

Source: From Refs. 6 and 23.

TABLE 88.11
Major Components in Tea Tree Oil

Terpine-4-ol	aromadendrene	α-Terpinene
γ-Terpinene	δ-Cadinene	1imonine
Terpino1ene	α-Thujene	Sabinene
α-Terpineol	Phellandrene	α-Pinene
1,8-Cineole	Viridiflorene	myrcene

Source: From Refs. 129 and 130.

P. Rosemary

Rosemary (*Rosmarinus officinalis* L.) (family Laminaceae) is a small evergreen shrub with small needle-like leaves. It is cultivated in nearly all countries around the Mediterranean Sea, as well as England and the US. Rosemary leaves contain about 1–2% of the essential oil, which is colorless to pale yellowish and gives off sweetly aromatic flavors typical of camphoraceous, herbaceous and bitter odor. No doubt rosemary is one of the most popular spices in many Western countries, especially Italy and France where rosemary is often used as a flavoring ingredient in their cookery. In Italian cuisine, mutton is hardly ever cooked without rosemary, and broiled poultry wrapped in rosemary twigs is also very popular. Rosemary is also well known for its presence of natural antioxidants, which contribute to the retention of a good red color in processed meats [126]. Rosemary oil is typically composed of the following major components: α-pinene, 1,8-cineole, camphor, camphene, β-pinene, borneol, myrcene, bornyl acetate, and α-terpineol.

Q. Tea Tree

The essential oil of *Melaleuca alternifolia*, commonly known as Australia tea tree oil, is obtained by steam distillation of a small perennial shrub species which is cultivated in the northern New South Wales, Australia. With recent recognition of its strong and wide-spectrum antibacterial and antifungal properties [127,128], tea tree oil has gained widespread acceptance and has been used in a wide range of consumer products from pharmaceuticals to cosmetics. The essential oil has a characteristic strong greenish aroma and its principle component is terpinen-4-ol [129], which is now known to possess some bioactive functions mentioned above. Other major constituents of this oil include monoterpenes or monoterpenoids, such as γ-terpinene, α-terpinene, 1,8-cineole, and α-terpineol. Using the simultaneous steam distillation and solvent extraction (SDE) method, more volatile chemicals have been identified [130]. These chemicals are presented in Table 88.11 and its chromatographic profile is shown in Figure 88.3.

R. Vetiver

Vetiver grass (*Vetiveria zizanoides*, L.), a fast growing perennial plant originated from India, belongs to the same

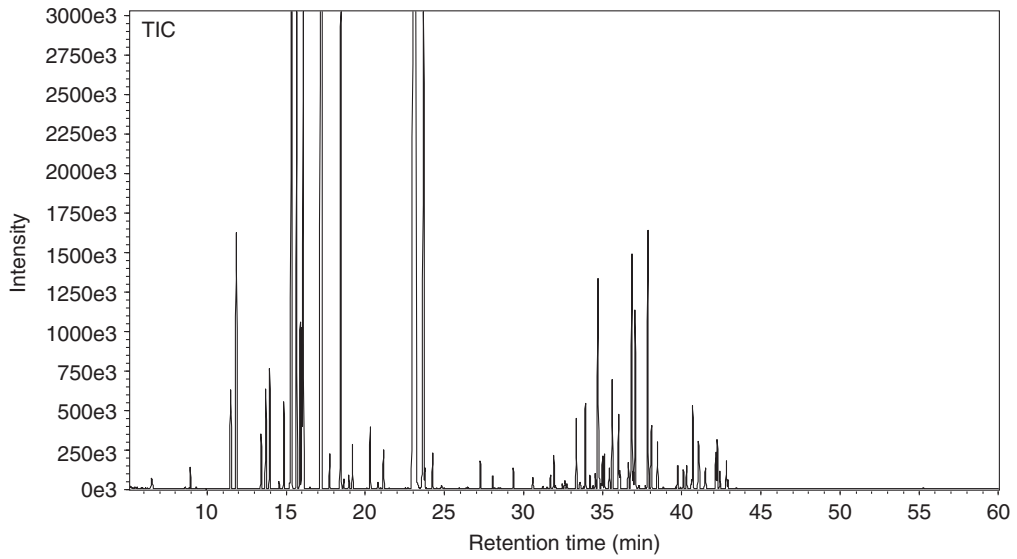

FIGURE 88.3 Total ion chromatogram of tea tree oil (TTO) using Shimadzu GC-17A/QP5000 GC-MS system with DB-5 column (60 m × 0.25 mm × 0.25 μm). The oven temperature was programmed from 60°C to 240°C at 3°C/min and held at 240°C for 10 min.

grass family as maize, sorghum, sugarcane, and lemongrass. So far twelve known species of vetiver grass and many hundreds of different cultivars have been found in the tropical and the semi-tropical countries such as southern Africa countries, India, Thailand, Malaysia, Indonesia, China, Central and South America, the Caribbean, and the United States. The driving force for the popularity in cultivating this plant is attributed to the commercial profits gained by the essential oil industries from vetiver oil, which contains a strong, penetrating, fragrant odor with versatile applications, not limited to be used as food flavoring or medicinal additive but also used a as cosmetic. Another recent principle application of the vetiver oil is its use as a strong repellent and toxic efficacy against the *Formosan* subterranean termite (*Coptotermes formosanus*) discovered by Henderson and co-workers [30].

Vetiver oil is extracted by distillation from vetiver roots in yield of 1–1.5%. The perfumery industry describes it as "heavy, woody, earthy" in sensory character. Investigation of the chemical composition of the vetiver oil, which was thought to possess one of the most complex flavor profiles in essential oils, has been conducted by several research groups during the last four decades [131,132,133,134,135]. Though vetiver oils from different sources do not have a uniform flavor profile, the following chemicals are generally found and considered as the major components of the oils: zizanol, khusimol, bicycle-vetivenol, β-vetivone, α-vetivone (syn. isonootkatone), zizanal, khusimon, β-vetivenene, α- and β-bisabolol, nootkatone, and β-eudesmol, which are shown in Table 88.12. More detail on the composition of the vetiver oil can be found in the literature as noted.

TABLE 88.12
Major Components in Vetiver Oil

α-Vetivone	α-Ylangene
β-Vetivone	β-Cedrene
Khusimone	α-Cubebene
Zizanal	Cadinene
Epizizanal	Vetivenene
Nootkatone	Zizaene
Zizanyl acetate	Elemol
Khusimol	Eudesmol
Zizanol	Bicyclo-vetivenol
α,β-bisabolol	

Source: From Refs. 30, 131, 132, 134 and 135.

V. SUMMARY

A comprehensive review to cover all natural flavors is not possible facing more exotic aromas identified and more biosynthetic pathways illustrated. What has been possible to achieve is to select some materials typically bearing characteristic flavor profiles to enable the readers to gain an overview and other more important aspects of the natural flavors. This chapter has therefore examined some fruits, vegetables, herbs, and spices, as well as their extracts, which are excellent sources of natural flavors that have been extensively used in food industries and in our lives. Finally, this chapter has outlined the main forms of aromatic flavors in plants, and hopefully can provide food chemists, flavorists, and culinary artists who are involved with natural flavors the basic information to approach to the complexity of the natural flavor in a more systematic way.

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89 Spices and Herbs

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CONTENTS

I. INTRODUCTION

A. Use of Spices in History and Various Cultures

Spices and herbs have been used in various cultures for centuries in food preparation for better flavor, appearance, and consumer acceptance and, to a lesser extent, for therapeutic purposes. They were and still are important in commerce among different countries, and also led to the discovery of the New World. With the current cultural exchange activities among countries nowadays, spices, herbs, and seasonings such as Indian curry, used by one ethnic group, are common in many countries, and have been adapted into their cultures with modifications, such as in Thailand and Japan. In the U.S., spices, herbs, and seasonings not common in the past have emerged as important flavoring agents in the food industry. Flavoring of food products by ethnic grouping such as Italian, Mexican, or Chinese in the past has expanded into Mediterranean, Latin American, Carribean, and Asian. Many technical books and review articles on spices, herbs, and seasonings have been published in the past several decades. This chapter attempts to present to readers, especially in the food science and technology area, a concise summary on spices, herbs, and seasonings. Readers are encouraged to consult books published in this area such as those listed in references at the end of this chapter (1–17).

B. Definitions by Various Agencies and Groups (6–21)

There is no clear distinction between spices and herbs and their definitions. Even in the same country, such as the U.S., various definitions are available, even within the federal government. In addition, each country has its own definition(s). However, for international trade, commonly used names and scientific names are used to distinguish the identity of a spice, and international standards are available such as the ISO (International Organization for Standardization) (http://www.iso.ch). Generally speaking, spices are derived from a variety of plant parts, e.g., the bark (cinnamon), buds (cloves), flowers (saffron), fruit (allspice, chilies), roots, (ginger, licorice), or seeds (caraway, mustard). They are obtained from plants that normally flourish in semitropical and tropical climates where the sun's heat is said to influence the strength and pungency of the spice. Spices are highly aromatic due to their high content of essential oils.

In contrast, herbs are the leaves and stems of soft-stemmed plants of which the main stem dies down to the ground at the end of the growing season. Herbaceous plants usually grow in temperate climates and may be annuals, biennials, or perennials. However, herbaceous plants are not the exclusive source of the substances we consider culinary herbs. For example, woody- or semiwoody-stemmed plants give rise to rosemary, sage, and thyme; and a large, woody-trunk evergreen tree, the laurel, is the source of bay leaves. An additional point of interest is that some herbaceous plants such as coriander and fennel are sources of herbs (the leaves), spices (the seeds), and vegetables (the bulb of fennel). Herbs are further distinguished from spices by their low content of essential oils, and are used to produce delicate or subtle flavors in contrast to the aromatic flavors imparted by spices.

The American Spice Trade Association (ASTA) defines spices in very broad terms dried plant products used primarily to season food. This definition encompasses all types of products found on the supermarket spice shelf, such as the spices considered "true spices" (e.g., pepper, cinnamon, nutmeg), as well as herbs (e.g., basil, marjoram), aromatic seeds (e.g., sesame, poppy, cardamom), blends (e.g., pumpkin pie spice), and dehydrated vegetable seasonings (onions, garlic, celery, sweet pepper).

Similarly, the U.S. Food and Drug Administration (FDA) has a broad definition for spices — with one important exception: it excludes dehydrated vegetables such as onions, garlic powder, and celery powder from the spice list. According to their definition, a spice is "any aromatic vegetable substance in the whole, broken, or ground form that is used primarily to season food rather than to contribute nutrients." This definition also requires spices to be true to the name and unmodified so that no volatile oil or other flavoring principle has been removed. FDA regulations state that spices may be labeled as "spices;" however, color-contributing spices — paprika, turmeric, and saffron — must be declared as "spice and coloring" or by their common names. Essential oils, oleoresins, and other natural plant extractives containing flavor constituents may be declared as "natural flavor." Dehydrated vegetables must be declared by their usual or common names.

The USDA defines the term spice as any aromatic vegetable substance in the whole, broken, or ground form, with the exception of onions, garlic, and celery, whose primary function in food is seasoning rather than nutritional and from which no portion of any volatile oil or other flavoring principle has been removed.

Spices and herbs are the basis of several spice blends such as chili powder, curry powder, poultry seasoning, Chinese five spices, and pumpkin pie spice. These blends, which are considered seasonings by consumers, are different from the industrial product called seasonings. Industrial seasonings contain one or more spices or spice extractives in addition to a number of other dissimilar ingredients such as acidulants, salts, sugars, monoglutamate, and ribonucleotides. Prepared by a specialized process called compounding, seasonings are used to enhance the flavor of food and improve its acceptance to consumers. They are added during the processing or manufacture of food and so are distinguished from condiments (e.g., mustard, catsup) which are also spice- or spice extractive-containing compounds but are added to the food after it is served. Industrial seasonings are widely used in meat products (e.g., bologna, frankfurthers, sausage), soups (e.g., French onion soup seasoning and mix), dry gravy mixes, instant sauces, and salad dressings.

II. THE VARIOUS SPICES AND HERBS AND THEIR BASIC INFORMATION

It is important to have some basic understanding about spices, as different names may be used for one particular spice in the same or different countries. It is not the intention of this chapter to list all the names for one spice. Interested readers should consult the references listed in the earlier section. (1 to 15).

Common and scientific names, plant family, botanical classification, and parts used in selected spices are presented in Table 89.1. Place of origin and current major sources of some common spices are presented in Table 89.2. The product forms and appearances of some common spices are presented in Table 89.3.

III. USES OF COMMON SPICES AND HERBS

As mentioned earlier, spices and herbs are used for various purposes. Table 89.4 summaries some of the basic

TABLE 89.1
Scientific Name, Plant Family, Botanical Classification, and Parts Used in Some Common Spices

Spice	Scientific Name	Plant Family	Growth Characteristics	Plant Part
Allspice	*Pimenta doica*	Myrtle	Evergreen	Berries
Anise seed	*Pimpineela anisum*	Parsley	Annual	Seeds
Basil	*Ochimum basilicum*	Mint	Annual	Leaves
Bay leaf	*Laurus nobilis*	Laurel	Evergreen	Leaves
Cassia	*Cinnamonum cassia*	Laurel	Evergreen	Inner bark
Caraway seed	*Carum carvi*	Parsley	Annual or biannual	Seeds
Cardamon	*Elettraia cardamomum*	Ginger	Perennial	Seeds
Celery seed	*Apium graveolens*	Parsley	Biannial or annual	Fruits
Chervil	*Anthriscus cerefolium*	Parsley	Annual	Leaves
Chives	*Appium schoenoprasum*	Onion	Perennial	Leaves
Cinnamon	*Cinnamomum zelancium*	Laurel	Evergreen	Inner bark
Clove	*Syzgium aromaticum*	Myrtle	Evergreen	Unopened flower buds
Coriander	*Coriandrum sativum*	Parsley	Annual	Fruits
Cumin seed	*Cumium cyminum*	Parsley	Annual	Ripe fruits
Dill seed	*Anethum graveciens*	Parsley	Annual	Seeds
Dill weed	*Anethum graveciens*	Parsley	Annual	Leaves
Fennel seed	*Foeniculum vulgare*	Parsley	Perennial	Seeds
Fenugreek	*Trigonella foenum-gracecum*	Bean	Annual	Fruit
Garlic, dehydrated	*Allium sativum*	Onion	Perennial	Bulbs
Ginger, dehydrated	*Zingiber officinale,* Roscoe	Ginger	Perennial	Rhizomes
Lemongrass	*Cymbopogon citratus*	Grass	Perennial	Stem and leaves
Lovage	*Laristicum efficuale*	Parsley	Annual	Stem and leaves
Mace	*Myristica fragans*	Nutmeg	Evergreen	Aril of seed
Marjoram, sweet	*Origanum majorana*	Mint	Perennial	Leaves
Mint, Peppermint (Black mint)	*Mentha piperita* variety *vulgaris*	Mint	Perennial	Leaves
Mint, Spearmint	*Mentha spicata*	Mint	Perennial	Leaves
Mustard seed, black	*Brassica hira* or *B. alba*	Cabbage	Annual	Seeds
Mustard seed, brown	*Brassica juncea*	Cabbage	Annual	Seeds
Mustard, white	*Brassica nigra*	Cabbage	Annual	Seeds
Nutmeg	*Myristica fragans*	Nutmeg	Evergreen	Seeds
Onion, dehydrated	*Allium cepa*	Onion	Biannial	Bulbs
Oregano	*Origanum spp.*	Mint	Perennial	Leaves
Paprika (pepper red)	*Capsicum annum*	Nightshade	Annual or perennial	Fruit pericarp
Parsley	*Petrosalinum crispum*	Parsley	Biannial	Leaves
Pepper, black	*Piper nigum*	Pepper	Perennial	Unripe berries or peppercorns
Pepper, chili	*Capsicum minimum*	Pepper	Annual or Perennial	Fruit
Pepper, red (Capsicum)	*Capsicum fructescens*	Nightshade	Perennial	Fruit pods
Pepper, white	*Piper nigum*	Pepper	Perennial	Skinless, ripe berries
Poppy seed	*Papaver somniferum*	Poppy	Annual, biannial, or perennial	Seeds
Rosemary	*Rosemarinus officinalis*	Mint	Perennial	Leaves
Saffron	*Crocus sativus*	Iris	Perennial	Stigma
Sage	*Salvia officinalis*	Mint	Perennial	Leaves
Savory, (sweet summer)	*Satureja indicum*	Mint	Annual	Leaves
Sesame seed	*Sesamum indicum*	Unicorn	Annual	Seeds
Star anise, Chinese	*Illicum vercum*	Illiciaceae	Perenial	Fruit
Tarragon	*Artemisia dracunculus*	Sunflower	Perennial	Flowering tops and leaves
Thyme	*Thymus vulgaricus*	Mint	Perennial	Leaves and flowering tops
Turmeric	*Curcuma longa*	Ginger	Perennial	Rhizomes
Vanilla	*Vanilla fragrans, V. planilfolia, or V. tahitensis*	Orchid	Perennial	Fruits

Source: References 1–14.

TABLE 89.2
Places of Origin and Current Major Sources of Some Common Spices

Spice	Places of Origin	Current Major Sources
Allspice	Western hemisphere	Carribean and Central America
Anise seed	Middle East	Turkey, Egypt, Syria, Spain, China, Mexico
Basil	Middle East	Egypt, U.S., France, Hungary, Bulgaria, India
Bay leaf	Mediterrian countries	Greece, Turkey
Caraway seed	Europe and Western Asia	Canada, The Netherlands, Egypt, Poland, Denmark
Cardamom	Southern India and Sri Lanka (Ceylon) Guatemala	Guatemala, India, Sri Lanka
Celery seed	Eurasia	India, China, France
Chervil	South Europe or Caucasus region	France, U.S.
Chives	Unknown, maybe Central Asia	Freshly grown
Cinnamon	China	China, Indonesia, Vietnam
Clove	Madagascar, Zanzibar, Pemba	Madagascar, Indonesia, Brazil, Zanzibar, Sri Lanka
Coriander	S. Europe, Asia Minor, S.W. Russia	Canada, Mexico, Morroco, Roumania, Argentina
Cumin seed	Egypt and Mediterrian region	Middle Eastern countries, India, Pakistan, Switzerland
Dill, seed	Mediterrain region	Canada, India, Indonesia
Dill weed	Mediterrain region	Canada, India, Indonesia
Fennel seed	Europe	Egypt, India, Turkey, Lebanon, China
Fenugreek (Foenugreek)	Southern Europe and parts of Asia	Southern Europe and parts of Asia
Garlic	Central Asia	U.S., China
Ginger dehydrated	China	India, Jamaica, Nigeria, China
Lemon grass	India	Tropical Asia
Lovage	Central Asia	South Europe
Mace	Moluccas Islands and East Indian Archipelago	East Indies, Indonesia, West Indies
Marjoram, sweet	Mediterrian region and Western Asia	Egypt, France
Mint, Peppermint	Central or Southern Europe	Egypt, Bulgaria, Yugoslavia
Mint, Spearmint	Central or Southern Europe	Egypt, Bulgaria, Yugoslavia
Mustard seed	Middle East	Canada, U.S., UK, Denmark
Nutmeg	Moluccas Islands and East Indian Archipelago	East Indies, Indonesia, West Indies
Onion	West or Central Asia	U.S.
Oregano	Mediterrian region	Turkey, Mexico, Greece
Paprika	Western hemisphere	U.S., Spain, Hungary, Morocco
Parsley	Mediterrian region	U.S., Israel, Hungary
Pepper, black	Malabar Coast of Southern India	Indonesia, India, Brazil, Malaysia
Pepper, red	Western hemisphere	Africa, China, India, Pakistan, Mexico
Pepper, white	Malabar Coast of Southern India	Indonesia, India, Brazil, Malaysia
Poppy seed	West Asia	Australia, Netherlands, Turkey, Rumania
Rosemary	Mediterrian region	Yugoslavia, Portugal, Spain, France
Saffron	Mediterrian region	Spain
Sage	Mediterrian region	Albania, Croatia, Germany, Italy, Macedonia, Turkey
Star anise	China	China
Savory, sweet (summer)	Southern Europe and Mediterrian region	Yugoslavia, France
Sesame seed	Tropical Africa	Mexica, Central America
Tarragon	Southern Russia, Asia	U.S., France, Yugoslavia
Thyme	Mediterrian region	Spain, France
Turmeric	Southern and Southeastern Asia	India, Jamaica, Haiti, Peru
Vanilla	Mexico, certain tropical South American countries	Bourbon Islands, Indonesia, Mexico, Tonga, Tahiti, Moorea

Source: References 1–14.

TABLE 89.3
Product Forms and Product Appearance of Some Common Spices

Spice	Product Forms (Dried)	Product Appearance (Dried)
Allspice	Whole and ground	Dark reddish-brown pea-sized fruit
Anise seed	Whole and ground	Greyish brown, oval-shaped and about 3/16 inches long
Basil	Bits of leaves and ground	Green leaves (bits)
Bay leaf	Whole and ground	Deep green leaves up to 3 inches long
Caraway seed	Whole only	Brown, hard seeds of 3/16 inches long
Cardamom	Whole pods (bleached and green), decorticated (seed alone), and ground	Irregularly round pod with seeds about 3/32 inches long
Celery seed	Light brown seeds up to 1/16 inches long	Whole, ground, and ground mixed with table salt (celery salt)
Celery flakes	Flakes, granulated, powdered	Light green dehydrated celery leaves
Chervil	Whole and ground	Small and smooth leaves
Chives	Whole only	Bright green, cross-cut section of leaves (Freeze-dried)
Cinnamon	Whole and ground	Brown bark
Clove	Whole and ground	Brown nail-shaped, 1/2 to 3/4 inches in length
Coriander	Whole and ground	Tan to light brown, globular with verticle ridges up to 3/16 inches in diameter
Cumin seed	Whole and ground	Yellow-brown seeds of 1/8 to 1/4 inches long
Dill, seed	Whole and ground	Light brown, oval shaped, up to 1/16 inches in length
Dill weed	Whole (bits of leaves)	Green leaves
Fennel seed	Whole and ground	Green, oblong-oval, straight or slightly curved, size varies up to 5/16 inches
Fenugreek seeds	Whole	Brownish yellow seeds
Garlic, dehydrated	Large sliced, chopped, minced, ground, granulated, powder; also powder or granulated with table salt (garlic salt)	Off white
Ginger dehydrated	Whole, cracked or ground	Even light biff hands or fingers
Mace	Whole or ground	Yellow-orange aril or skin
Marjoram, sweet	Whole (bits of leaves), and ground	Grey-green leaves
Mint, Peppermint	Flakes (broken dried leaves)	Pale green
Mint, Spearmint	Flakes (broken dried leaves)	Pale green
Mustard seed, black	Whole, mustard flour and ground	Black seeds
Mustard seed, brown	Whole, mustard flour and ground	Brown seeds
Mustard, white	Whole, mustard flour and ground	White seeds
Nutmeg	Whole, ground	Greyish-brown, oval fruit size up to 1 1/4 inches in length
Onion, dehydrated	Sliced, chopped, diced, minced, ground, granulated, powdered, toasted (all sizes), powder or granulated onion with table salt (onion salt)	Off white
Oregano	Whole (bits of leaves) and ground	Light green with size about 1/8 inches long
Paprika	Powder	Bright rich red stemless pods
Parsley	Flakes of dried leaves	Green leaves
Pepper, black	Whole, ground, cracked, decorticated	Black seeds
Pepper, red	Whole and ground	Various sized red-colored pods
Pepper, white	Whole and ground	White seeds
Poppy seed	Whole	Bluish grey to black, kidney-shaped seeds about 3/64 inches long
Rosemary	Whole and ground	Brownish-green needles less than 1 inch in length
Saffron	Whole and ground	Orange-yellow stigma
Sage	Whole, cut, rubbed and ground	Silver-grey leaves
Savory, sweet (summer)	Whole (bits of leaves) and ground	Brown-green leaves less than 3/8 inched in length
Sesame seed	Whole (unhulled and hulled)	White, oval-shaped, shiny seeds about 1/8 inch long
Tarragon	Whole (bits of leaves) and ground	Dark green, narrow with small round tips, about 5 cm long
Thyme	Whole (bits of leaves) and ground	Brownish-green leaves seldom exceed 1/4 inch long
Turmeric	Ground	Dark yellow
Vanilla	Whole beans, powder, extract	Dark brown

Source: References 1–14.

TABLE 89.4
Basic Uses of Selected Spices in Food Preparation

Basic Use	Selected Spices
Flavoring	
Sweet	Cardamon, anise, star anise, fennel, allspice, cinnamon
Sour	Tamarind
Bitter	Fenugreek, mace, clove, thyme, bay leaf, oregano, celery
Spicy	Clove, cumin, coriander, ginger
Hot	Chili pepper, mustard, black pepper, white pepper, wasabi
Pungent	Pungent, wasabi, ginger, garlic, onion
Fruity	Fennel, coriander, root, tamarind, star anise
Floral aromatic	Lemongrass, sweet basil, turmeric, ginger, black pepper
Woody	Cassia, cardamom, clove
Piney	Rosemary, thyme, bayleaf
Cooling	Peppermint, basil, anise, fennel
Earthy	Saffron, turmeric, black cumin
Herbaceous	Parsley, rosemary, tarragon, sage, dillweed
Sulfury	Onion, garlic, chives
Nutty	Sesame, poppy seeds, mustard seeds
Texture	Garlic, mustard, onion, sesame seed, shallot
Masking/ Deodoring	Garlic, clove, rosemary, onion, bayleaf, thyme, sage, coriander, caraway, oregano
Colorant	
Water-soluble	Saffron, annatto
Oil-soluble	Paprika, turmeric, parsley, basil

Source: Refereences 1–14, 18.

uses of selected spices and herbs in food preparations. Spices and herbs are used mainly for flavoring purposes, and to a less extent in coloring foods and providing texture to the final product. Occasionally, spices are used to mask or deodorize food products as the odor of some spices are fairly strong and definitely can serve these purposes.

Spices and herbs are used for special purposes, and there is not one spice or herb that can be used in all food preparations. Consumers learned long ago that certain spices and herbs are more suitable for some food groups than others. Table 89.5 presents the flavor characteristics of some common spices and their applications. Table 89.6 gives some typical components in some common spice mixtures or blends. It should be noted that the percentage of each spice in a spice mixture or blend is proprietary to the spice producer except in special cases such as the US military standard that specifies some minimum content for each spice. Readers interested in this information should consult the US Military Specification EE-S-631J and Reference 9 for general proportions of some spice mixtures and blends.

Consumers in various cultures learned long time ago that selected spice and/or herb combinations from spices available in their regions provide special flavor characteristics. This consumer preference has developed into typical flavors of various cultures. Table 89.7 gives a comparison of basic sensory characteristics of some common spices

TABLE 89.5
Flavor Characteristics and Commercial Applications of Some Common Spices

Spice	Flavor Characteristics	Commerical Applications
Allspice (Pimento)	Warm, sweet and slightly peppery, reminiscent of clove	In almost all meats, sausages, pickles and sauces
Anise seed	Taste and aroma similar to licorice	In fish, shellfish, baked goods
Basil	Mild, sweet, and slightly pungent	In potato dishes, soups, processed meats, tomato sauce
Bay leaf	Warm, spicy, and somewhat bitter	In pickles, soups, fish and vegetable dishes
Caraway seed	Warm, slightly sweet with a slightly sharp after-tone	In cheese products, confectionery, meat stews and pork dishes
Cardamom	Bitter-sweet, aromatic with slight lemony back note	Primarily in curries, pickles and sauces
Cassia	Sweet spicy and distinctve	In spiced confectionery, curries, tomato sauce and to a limitd extent in meat dishes
Celery seed	Powerfully warm with bitter after-flavor	In clam, potato, or other creamy soups and some cheese dressings
Chervil	Sweet and aromatic	In soups, salad and fish dish
Chives	Similar to onion, but less dominant but more subtle	In soups, vegetable stews, egg- or yogurt- based sauces
Cinnamon	Sweet spicy and distinctive	In spiced confectionery, apple products, wines, pickles, and beef and game casseroles. Can mask other flavors if used in excess
Clove	Very aromatic, warm and astringent	In hams, tongue and pork products, pickled and preserved fruits and vegetables, apple dishes, pudding mixes and cream soup
Coriander	Mild and pleasantly aromatic	In pickles, pea soup, cakes and biscuits, various meat stuffings and apple products
Cumin seed	Salty sweet, similar but coarser than that of caraway, bitter after taste	Basic ingredient of curries, cream cheese, processed meats, pickled cabbage and certain fish products
Dill, fruit and seed	Penetrating sharply aromatic flavor similar to that of carraway but with fresher lemony note	In pickles, sauces, confectonery, soups and chicken dishes
Dill weed	Fresh sweet spicy having a slight anise-like back-note	In pickles and sauces
Fennel seed	Strong and penetrating but fragnant	In sweet pickles, fish dishes, confectionery, and as a sauce to pork dishes

(*Continued*)

TABLE 89.5
(*Continued*)

Spice	Flavor Characteristics	Commerical Applications
Fenugreek	Intensely sweet, spicy and caramel-like	In chutneys, pickles and sauces
Garlic, dehydrated	Strong and characteristic	With care in most types of product
Ginger dehydrated	Aromatic, hot, and biting	In biscuits, spice cakes, preserved fruits, chutneys, most meat and seafod products, pickles, and wines
Lemon grass	Fresh and lemon like	In meat dishes
Lovage	Strong nad characteristic, reminiscent of celery	In meat dishes, soups, and sauces
Mace	Finer than but similar to that of nutmeg	In pickles, fruit cakes, gingerbread, processed meat loaf, sausages, and mincemeat
Marjoram, sweet	Distinctive, delicately sweet and spicy with a slightly bitter back note	In most meat dishes, soups, and stuffings
Mint, Peppermint	Quite characteristic, sweet with a cool aftertaste	In canned and frozen peas, soups, lamb, mutton, and veal products, and mint sauce
Mint, Spearmint	Quite characteristic, sweet with a cool aftertaste	In canned and frozen peas, soups, lamb, mutton, and veal products, and mint sauce
Mushroom, Boletus	Characteristic and slightly meaty	In soups and certain meat dishes
Mustard seed, black	No fragrance, but exhibit a pungent taste after chewing	Pungency destroyed during cooking
Mustard seed, brown	No fragrance, but exhibit a pungent taste after chewing	Pungency destroyed during cooking
Mustard, white	No fragrance, but exhibit a pungent taste after chewing	Used as a paste for its pungency
Nutmeg	Sweet and aromatic, adding a certain richness to spice mixes	In spiced confectionery, biscuits, and many meat products
Onion, dehydrated	Characteristic and penetrating	In many dishes requiring an onion flavor
Oregano	Strong, distinctive with bitter after flavor	In spaghetti products, canned pork dishes, cheese spreads, soups, and sauces
Paprika (red pepper)	Piquant and moderately pungent	In all foods to give added zest
Paprika, Rosen	Characteristic and stronger than normal Paprika with a definite pungency	In many specialty meat products
Parsley	Mild and agreeable	In soups, fish and poultry products, and many meat dishes
Pepper	Penetrating, strong, and characteristic	In almost all dishes a small quantity improves the overall flavor
Pepper, Chile	Intense pungent	Used with discretion in any product requiring heat
Pepper, Red	Hot and pungent	Used with discretion in any product requiring heat
Poppy seed	Nutty and pleasant	In baked products and confectionery
Rosemary	Distinctive, delicate and sweet, with a piney slightly bitter aftertaste	In lamb and certain other meat products, cheese sauces, stuffings, and soups
Saffron	Distinctive flavor	Used mainly to color food golden yellow
Sage, Dalmatian	Strongly aromatic, warm with slightly bitter aftertaste	With care in all pork dishes, fish and poultry stuffings, cream soups, cheese, and sauces
Sage, English	A milder and much pleasanter flavor than that of Dalmatian sage, lacks the objectionable “thujone” note	With care in all pork dishes, fish and poultry stuffings, cream soups, cheese, and sauces
Savory, sweet (summer)	Strongly aromatic	With vegetables, legumes, and in mixtures for sausages, pates, or pickles
Sesame seed	Unroasted seeds have mild flavor, but oil from roasted seeds gives strong distinctive flavor	Can be used in seasoning almost any kind of food
Star anise	Warm, sweet, aromatic	In meat dishes, also as a component for Chinese five spices
Tarragon	Distinctive sweetly aromatic with a bitter undertone	In poultry and fish sauces, pickles, and spiced vinegar, and in small quantities in certain meat products
Thyme	Penetratingly warm and pungent, the aroma is long lasting	In tomato products, stuffings, fish dishes, soups, and many other meat dishes
Turmeric	Warmly aromatic with musky undertones	In pickles, relishes, prepared dressings, and to color a wide range of products, in particular curries
Vanilla	Rich, mellow perfumed, tobacco-like aroma	Chocolate, coffee, pudding, ice cream, in dishes such as those made with veal and lobster

Source: References 1–21.

TABLE 89.6
Components in Some Common Spice Mixtures or Blends

Name of Mixture (Blend)	Components
Apple pie spice	Cinnamon, cloves, nutmeg or mace, all spice, ginger
Barbecue spice	Chili, peppers, cumin, garlic, cloves, paprika, salt, sugar
BBQ seasoning in chips	*Main:* Onion, garlic, chili pepper, paprika
	Optional: Celery, cumin, cloves, all spice, cinnamon, black pepper, red pepper
Bologna or weiner seasoning	Pimento, nutmeg, black pepper, clove leaf or stem, capsicum, paprika, coriander, cassia, mustard, onion, garlic
Chicken-flavored gravy	Turmeric, celery seed, black pepper, onion, garlic, other spices
Chili powder	Chili pepper, cumin, seed, oregano, garlic, salt
Five spices, Chinese	Black pepper, star anise or anise, fennel, clove, cinnamon
Cinnamon spice	Cinnamon, sugar
Crab boil or shrimp spice	Peppercorns, bay leaves, red peppers, mustard seed, ginger, and other spices
Curry powder (dry masalas)	*Mandatory*: Coriander, cumin, fenugreek, turmeric (yellow coloring)
	Optional: Cardamom, cinnamon, cloves, nutmeg, onion, garlic, poppy seeds, sesame seeds, basil, bay leaves, dill seeds, hot chilies, saffron, black pepper, kiffer lime
Curry paste (wet masalas)	*Main ingredients*: Same as curry powder
	Additional: Coconut milk, yogurt, clarified butter (ghee), mang, cilantro, and tamarind accents
Ham	Cassia, clove stem or leaf, pimento, capsicum, celery, bay leaf, garlic
Herb seasoning	Milder-flavored herbs such as marjoram, oregano, basil, chevil, etc., salt
Hot and spicy seasoning	Chili pepper, red pepper, black pepper, onion, garlic, paprika, capsicum
Italian sausage seasoning	Anise, fennel, paprika, black pepper, red pepper
Italian seasoning	Oregano, basil, red pepper, rosemary, garlic (optional)
Japanese seven spices	Perilla, basil, sesame, poppy seed, hemp, red pepper, Japanese pepper
Marinara-type tomato sauce	Garlic, onion, oregano, basil, parsley
Mixed pickling spice	Mustard seeds, bay leaves, black and white peppercorns, dill seeds, red peppers, ginger, cinnamon, mace, all spice, coriander seeds, etc.
Pork sausage seasoning	Sage, black pepper, red pepper, nutmeg
Poultry seasoning	Sage, thyme, marjoram, savory, rosemary (optional)
Pumpkin pie spice	Cinnamon, nutmeg, cloves, ginger
Ranch tortilla chips	Onion, garlic, parsley, paprika
Roast beef or corned beef rub	Corriander, onion, garlic, celery, oregano, basil, black pepper, paprika
Seafood seasoning	Similar to crab boil and shrimp seasoning, plus salt
Seasoned or flavored salts	Salts with different dehydrated vegetables and spices, e.g., celery salt (celery and salt), garlic salt (garlic and salt), onion salt (onion and salt)
Smoked sausage seasoning	Garlic powder, black pepper, coriander, pimento

Source: References 2, 6–9, 11, 14–15, 18–21.

with cultural differences. It should be noted that the flavor characteristics described in one culture may not be the same in another culture. Table 89.8 shows some ethnic usage of spices and herbs; and each culture has its own characteristic spice(s). Usually, spices and herbs not available in a region are not used commonly there.

In 1993, Nakamasa (22) in Japan proposed the "patterning theory" to describe the relationship of spices to cooking ingredients and techniques. This "patterning theory" of spice use considers the suitability of a spice and certain raw materials to be the result of a "synthesis" that occurs in the mouth. A preference for a specific spice is determined by individual judgement, and the suitability of any spice and any other ingredients is based on individual preference. Flavor preference is evaluated using the senses of taste and smell. If a spice and an ingredient tasted together are well received, it follows that these two can be combined in cooking. This "patterning theory" can be considered as a scientific description of the relationship of spice combinations and consumer patterns in various cultures. Readers interested in this topic should consult References 18 and 23.

Consumers prefer certain spices because these spices contain certain compounds typical of their flavor characteristics, mainly in their essential oils. Principal constituents in common spices are presented in Table 89.9. It should be noted that spices from the same family usually contain some similar flavor compounds.

In the past 20 years, researchers recognized that people sensitive to sodium should reduce their intake of salt (sodium chloride) in order to reduce the risk of hypertension. However, reduction in the use of common salt in most cases makes food bland. It is suggested that these

TABLE 89.7
Comparison of Basic Sensory Characteristics of Some Common Spices

Spice	Basic Sensory Characteristics	Indian	Chinese
Allspice	Sweet	Not commonly used	Not commonly used
Anise seed	Sweet cooling	Anise	Not commonly used
Basil	Floral aromatic	Not commonly used	Pungent
Bay leaf	Bitter	Bitter	Not commonly used
Cassia	Woody, sweet	Not commonly used	Pungent, sweet
Caraway seed	Warm and slightly sweet	Not commonly used	Slightly pungent
Cardamon	Sweet	Sweet	Not commonly used
Celery seed	Bitter	Not commonly used	Not commonly used
Chervil	Floral aromatic	Not commonly used	Not commonly used
Chives	Sulfury	Not commonly used	Not commonly used
Cinnamon	Woody, sweet	Bitter	Pungent, sweet
Clove	Bitter	Bitter	Pungent
Coriander	Spicy	Sweet	Pungent
Cumin seed	Spicy	Sweet	Pungent
Dill, seed	Herbaceous	Not commonly used	Pungent
Fennel seed	Sweet	Sweet	Pungent
Fenugreek	Bitter	Bitter/stringent	Not commonly used
Garlic, dehydrated	Pungent, spicy	Spicy/pungent	Pungent
Ginger, dehydrated	Spicy	Spicy/pungent	Pungent
Lemongrass	Floral aromatic	Not commonly used	Not commonly used
Licorice	Sweet	Astringent	Sweet
Mace	Bitter	Not commonly used	Not commonly used
Marjoram, sweet	Sweet	Not commonly used	Pungent
Mint, Peppermint (black mint)	Cooling	Not commonly used	Pungent
Mint, Spearmint	*Mentha spicata*	Not commonly used	Pungent, sweet
Mustard seed	Hot, pungent, nutty	Spicy/pungent	Not commonly used
Mustard powder, white	Pungent	Not commonly used	Warm
Nutmeg	Bitter	Not commonly used	Pungent
Onion, dehydrated	Pungent	Spicy/pungent	Not commonly used
Oregano (wild marjoram)	Bitter	Not commonly used	Not commonly used
Paprika (Pepper red)	Hot	Not commonly used	Not commonly used
Parsley	Herbaceous	Not commonly used	Not commonly used
Pepper, black	Hot	Spicy/pungent	Pungent
Pepper, Chili	Hot	Spicy/pungent	Pungent
Pepper, red (Capsicum)	Hot	Not commonly used	Not commonly used
Pepper, White	Hot	Not commonly used	Not commonly used
Poppy seed	Nutty	Not commonly used	Not commonly used
Rosemary	Piney	Not commonly used	Pungent
Saffron	Earthy	Not commonly used	Sweet
Sage	Herbaceous	Not commonly used	Not commonly used
Savory, sweet (summer)	Fruity	Not commonly used	Not commonly used
Sesame seed	Nutty	Not commonly used	Not commonly used
Star anise, Chinese	Fruity	Not commonly used	Pungent, sweet
Tamarind	Sweet–Sour	Sour	Not commonly used
Tarragon	Herbaceous	Not commonly used	Not commonly used
Thyme	Bitter	Not commonly used	Not commonly used
Turmeric	Earthy	Bitter	Not commonly used

Source: References 1–15, 18, 22.

consumers can consider the addition of spice(s) and/or herb(s) in their foods to increase their acceptance. Some spice companies provide free information to consumers about low sodium spice combinations. For example, McCormick/Schilling Company recommends the combination of several spices for a few food groups in their Low Sodium Spice and Herb Chart. Table 89.10 shows examples of three low sodium herb blends.

TABLE 89.8
Some Ethnic Usage of Spices and Herbs

Culture	Commonly Used Spices and Herbs
Chinese	Anise, cinnamon, clove, fennel seed, garlic, onion, red pepper, star anise
French	Black pepper, fine herbs, marjoram, rosemary, shallot, tarragon, thyme
German	Caraway seeds, dill seeds, ginger, nutmeg, onion, paprika, rosemary, white pepper
Greek	Bay leaves, black peppper, cinnamon, fennel seed, garlic, onion, oregano, dill, basil, thyme, fennel seed
Hungarian	Caraway seeds, cinnnamon, dill seeds, garlic, onion, paprika, poppy seeds, white pepper
Indian (Eastern)	Black pepper, coriander, cumin seed, curry, ginger, red pepper, tumeric, cardamom, fenugreek, celery, cinnamon, clove, nutmeg
Indonesian	Caraway seeds, cinnamon, clove, curry, garlic, ginger, nutmeg, red pepper
Italian	Basil, fennel seeds, garlic, marjoram, onion, oregano, red pepper, sage, thyme, anise, rosemary, black pepper
Jamaican	All spice, garlic, red pepper, red pepper, thyme, onion, oregano
Mexican	Chili pepper, cinnamon, coriander, garlic, onion, oregano, sesame seeds, cumin seeds, cilantro, red pepper, bay leaves, all spices, cloves, anise
Morracan	Anise, cinnamon, coriander, cumin seeds, mint, red pepper, saffron
Southwestern U.S.	Garlic, onion, chili pepper, oregano, cilantro, cumin, cinnamon, clove
Spanish	Bay leaves, cumin seeds, garlic, onion, paprika, parsley, saffron, sweet pepper, thyme, basil, hot pepper, innamon, nutmeg
Spanish Carribean (Puerto Rican, Cuban)	Garlic, cumin, oregano, bay leaves, onion, cloves, nutmeg, ginger, thyme, cilantro, cayenne, cinnamon, black pepper, turmeric, paprika
Thai	Curry paste (red and green), coriander, cilantro, coriander root, garlic, ginger, lemon grass, basil, mint, black, white, and red peppers, cinnamon, cloves, cardamom, kiffer lime, garlanga
Vietnamese	Chilies, garlic, lemon grass, cilantro, basil, ginger, turmeric, red, white, and black pepper, curry powder, five spice powder, cloves, star anise, galangal

Source: References 6–9, 15, 18, 20–21.

TABLE 89.9
Principal Constituents in Some Common Spices

Spice	% Essential Oil (Range)	Principal Constituents in Essential Oil	Significant Constituents in Oleoresin
Allspice	3.3 to 4.5	Eugenol (60–75%), eugenol methyl ether, cineole, phellandrene, caryophylene	None
Anise seed	1.5 to 3.5	Anethole (major), anisaldehyde, anisketone, methyl chavicol	None
Basil	0.1 to 1.0	Methyl chavicol (estragole), linalool, cineols	None
Bay leaf	1.5 to 2.5	Cineole	None
Caraway seed	1.5 to 3.5	Carvone (major), limonene, dihydrocarvone, dihydrocarvone, dihydrocarveol, acetaldehyde methyl alcohol, furfural, and diacetyl	None
Cardamom	2 to 10	Cineole, alpha-terpinyl acetate, liminone, linalyl linalool, borneol, alpha-terpineol, alpha-pinene, limonene, myracene	None
Celery seed	1.5 to 3	*d*-Limonene, *beta*-selinene	None
Chervil	0.3 to 0.9	Methylcharviol (estragol), hendecane (undecane)	None
Chives	??	Dipropyl disulfide, methyl pentyl disulfide, penthanethiol, pentyl-hydrodisulfide, *cis/trans*-trans-3,5,-diethyl-1,2,4 trithiolane	None
Cinnamon	1.5 to 3.0	Eugenol (clovelike flavor), cinnamic aldehyde (cinnamonlike flavor)	None
Clove	Up to 20%	Eugenol, eugenol acetate, beta-caryophyllene	None
Coriander	0.1 to 1.5	*d*-Linalool (coriandrol), Pinenes, terpinenes, geranol, vorneal, decylaldehyde	None
Cumin seed	2 to 5	Cumaldehyde (cumic aldehyde), dihydrocumaldehyde, ciminyl alcohol, *dl*-pinene, *p*-cumene, diterpene	None
Dill seed	2 to 5	Carvone, *d*-limonene, phellandrene, eugenol, vanillin	None

(*Continued*)

TABLE 89.9
(*Continued*)

Spice	% Essential Oil (Range)	Principal Constituents in Essential Oil	Significant Constituents in Oleoresin
Dill weed	0.3 to 1.5	Carvone, methyl 2-methylbutanoate, alpha-phyllandrene, dill ether, myristicin	None
Fennel seed	1 to 3	Anethole, *d*-fenchone, *d*-alpha-pinene, *d*-alpha-phlladrene, dipentene, methyl chavicol, feniculun, anisaldehyde, anisic acid	None
Fenugreek	Trace	None	Trigonelline, choline
Ginger	??	Gingerols (pungency)	Zingerone, shogoals
Ginger dehydrated	1.5 to 3.0	(−)-alpha — zingiberene, (−)-*beta*-bisabolene, (+)-ar-curcumene (ginger flavor), farnesene, *beta*-sequiphellandrene (ginger flavor), alpha-terpineol (lemony flavor), citral (lemony flavor)	None
Lemon grass	0.2 to 0.5	Citral, nerol, limonene, β-caryphyllene	None
Mace	15 to 25	*Alpha*-pienene, *Beta*-pinene, sabinene, myristicin (main flavor)	None
Marjoram, sweet	0.7 to 3	Terpen-4-ol, *alpha*-terpineol	None
Mint, Peppermint (dried leaves)	2.5	Menthol, menthone and their methyl esters, pulegone, piperitone, methofurane, jasmone	None
Mint, Spearmint	2.5	Carvone, liminene, dihydrocarvone, methone, pulegone, 1,8-cineol, β-pinene	None
Mustard seed, black	0.6 (av.)	Allyisothiocyanate	None
Mustard seed, yellow	None	None	Sinabin mustard oil (pungent)
Nutmeg	6 to 15	*Alpha*-pienene, *Beta*-pinene, sabinene, myristicin (main flavor)	None
Onion, dehydrated	Trace	Ethyl and propyl disulfides, vinyl sulfide	None
Oregano (wild marjarom)	ca. 2	Thymol, carvacol	None
Paprika	None	None	Capsanthin, carotene, capsorubin (pigments)
Parsley	0.05	Myristian, limonene, 1,3,8-*p*-methatriene	None
Pepper, black	0.6 to 2.6	*alpha*-pinene, *beta*-pinene, 1-*alpha*-pellandrene, *beta*-caryophyllene, limonene, sabine-delta-3-carene pungency: piperine, piperettine, peperyline, piperolein A and B, piperanine	None
Pepper, red		Capsaicin, Dihydrocapsaicin	None
Pepper, white	1.0 to 3.0	*alpha*-pinene, *beta*-pinene, 1-*alpha*-pellandrene, *beta*-caryophyllene, limonene, sabine-delta-3-carene pungency: piperine, piperettine, peperyline, piperolein A and B, piperanine	None
Poppy seed	N/A	2-pentylfuran	None
Rosemary	0.5 to 2.0	*Alpha*-pinene, *d*-pinene, camphene	None
Saffron	<1	None	Crocin (coloring agent) picrocrocin (bitter flavor)
Sage	1.5 to 2.5	Thujone, borneol, cineole	None
Savory, sweet (summer)	<1	Carvacrol, thymol, *p*-cymene	None
Sesame seed	N/A	Pyrazines (in toasted seeds)	Sesamin, sesamolin
Star anise	5 to 8	Anethol	None
Tarragon	0.2 to 1.5	Methyl chavicol (estragole), anethole (anise or licorcelike tone), ocimene, phellandrene, *p*-methoxy-cinnamaldhyde	None
Thyme	0.8 to 2.0	Thymol, *p*-cymene, *d*-linalool	None
Turmeric	1.5 to 6	Turmerone, *ar*-turmerone (dehydroturmerone)	Curcumin, desmethoxy-curcumin, bis-desmethoxy-curcumin (pigments)
Vanilla	2 to 2.5	Vanillin	None

Source: References 4–9, 11, 14, 16, 20.

TABLE 89.10
Examples of Low Sodium Herb Blends

Blend	Usage	Spices Used	Quantity Ratio
A	For salads or salt shakers	Thyme leaves	2 teaspoons
		Basil leaves	2 teaspoons
		Ground savory	2 teaspoons
		Marjoram leaves	1 tablespoon
		Rubbed sage	2 teaspoons
B	For soups, stews, poultry, or pot roast	Thyme leaves	1 tablespoon
		Rubbed sage	1 teaspoon
		Majoram leaves	2 tablespoons
		Rosemary leaves	1 tablespoon
C	For beef, cooked vegetables and added seasoning at table	Celery seed	1 teaspoon
		Majoram leaves	1 tablespoon
		Thyme leaves	1 tablespoon
		Basil leaves	1 tablespoon

Source: McCormick/Schilling Company's Low Sodium Spice and Herbs Chart.

IV. SPICE QUALITY STANDARDS AND SPECIFICATIONS

It is generally accepted that spice quality differs year to year, and from country to country. In addition, spices are produced in various countries in the world and then exported to spice-consuming countries. Also, the same spice may be produced in different countries (24–26). It is therefore deemed necessary to have unified spice standards or specifications for exporting and importing countries in order to make international trade easier. However, there are no unified standards or specifications worldwide for spices. Each country has its own importing or domestic spice standards or specifications to meet its own needs. The quality standards most used are those of the ASTA and the U.S. federal specifications: spices, ground and whole, and spice blends. U.S.-FDA also has an import procedure for spices. Readers interested in this topic should visit FDA's website (http://www.*fda.gov/ora/import/oasis/home*) for details. ISO also has its own established standards. However, exporting nations usually have their own exporting spice standards or specifications to guarantee that their products meet certain quality standards, which in turn usually follow the quality standards or specifications of the importing countries. Although differences from country to country may exist, most spice standards or specifications of importing nations emphasize the following:

- Cleanliness of the spice products (placing limits on the extraneous matters, see section below on US-FA Defective Action Limits)
- Microbial content
- Extraneous matters (such as rodent hair, insect fragments, and mammalian excreta)
- Specific chemical components or sensory potency for selected spices
- Chemical residues (such as pesticides, sterilant gas residues, heavy metal residues, solvent residues, and mycotoxins)
- Moisture content
- Volatile oil content

A. Extraneous Matters

Many spices are produced in Third World countries where production and handling practices differ from those that are used in developed or important countries. These differences resulted in the presence of extraneous matters that are undesirable but sometimes unavoidable. Even spices produced in developed countries may have unavoidable problems. The ASTA published its own "Cleanliness Specifications for Spice, Seeds, and Herbs, Revised Edition, 1999" which can be purchased for reference. U.S.-FDA has established its own standards for cleanliness of spices (such as limits on rodent hair, insect fragments, mammalian excreta, and mold) that are included in its document on "Defective Action Levels (DALs)." Table 89.11 is a summary of DALs for spices.

Spice processors also remove extraneous matters such as metallic pieces, stones, mud balls, other plant materials and foreign matter through their processing lines which may include magnets, sifters, air tables, destoners, sir separators, indent separators, and spiral separators. This extraneous matter should be excluded in the final spice products through processing.

Presence of undesirable bacteria and live insects in spices has been a problem in the spice trade for many decades. Various procedures such as gas fumigation, irradiation, steam treatment, and carbon dioxide/nitrogen treatment are available to overcome the presence of undesirable bacteria and insects. Table 89.12 presents a comparison of various procedures available in reducing bacteria in spices, and their advantages and disadvantages. It should be noted that individual countries have their own regulations on which procedure can be used. For example, irradiation can be used in the U.S., but is not permitted in Japan and many European countries. Live insects can easily be eliminated by carbon dioxide or fumigation treatment. However, care and proper storage precautions must be taken to avoid re-infestation of processed products and growth and development of mold and yeast.

B. Specific Tests for Quality Comparison of Selected Spices

Spices are complex biological materials, and their compositions vary according to production environment. Comparison of quality of spices still relies mainly on sensory evaluation and their volatile content. Even though

TABLE 89.11
Defective Action Levels of Spices

Spice	Defective Action Level (Average)	Direct Source	Significance
Allspice, ground	Insect filth: 30 or more insect fragments per 10 gm	Insect fragment: Pre/post harvest and processing insect infestation	Aesthetic
	Rodent filth: 1 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Allspice, whole	Mold: 5% or more berries by weight are moldy	Mold: Preharvest and/or post harvest infection	Potential health hazard — mold may contain mycotoxin producing fungi
Bay (Laurel) leaves	Mold: 5% or more pieces by weight are moldy	Mold: preharvest infection	Aesthetic
	Insect filth: 5% or more pieces by weight are insect-infested	Insect infestation: preharvest and/or post harvest and/or processing insect infestation	
	Mammalian excreta: 1 mg or more of mammalian excreta per lb after processing	Mammalian excreta: post harvest and/or processing animal contamination	
Capsicum pods	Insect filth and/or mold: more than 3% of pods weight are insect-infested and/or moldy	Insect infestation: preharvested and/or post harvest insect infestation	Aesthetic
	Mammalian excreta: more than 1 mg of mammalian excreta per lb	Mold — preharvest and/or post harvest infection	Potential health hazard — mold may contain mycotoxin producing fungi
		Mammalian excreta: post harvest and/or processing animal contamination	
Capsicum, ground (excluding paprika)	Mold: mold count is more than 20%	Mold: preharvest and/or post harvest mold infection	Aesthetic
	Insect filth: more than 50 insect fragments per 25 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Potential health hazard — mold may contain mycotoxin producing fungi
	Rodent filth: more than 6 rodent hairs per 25 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Cassia (or) cinnamon bark, whole	Mold: 5% or more pieces by weight are moldy	Mold: preharvest infection	Aesthetic
	Insect filth: 5% or more pieces by weight are insect-infested	Insect infestation: preharvest and/or post harvest and/or processing insect infestation	
	Mammalian excreta: 1 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
Cinnamon, ground	Insect filth: 400 or more insect fragments per 50 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 11 or more rodent hairs per 50 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Cloves	Stem: 5% or more stems by weight	Stem: Harvest	Aesthetic, economic adulteration
Condimental seeds other than fennel seeds and sesame seeds	Mammalian excreta: 3 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	Aesthetic
Cumin seed	Sand and grit: 9.5% or more ash and/or 1.5% or more acid insoluble ash	Sand and grit: harvest contamination	Aesthetic

(*Continued*)

TABLE 89.11
(*Continued*)

Spice	Defective Action Level (Average)	Direct Source	Significance
Curry powder	Insect filth: 100 or more insect fragments per 25 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 4 or more rodent hairs per 25 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Fennel seed	Insects: 20% or more subsamples containing insects	Insects: pre-harvest and/or post harvest insect infestation	Aesthetic
	Mammalian excreta: 3 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
Ginger, whole	Insect filth and/or mold: 3% or more by weight are insect-infested and/or moldy	Insect infestation: preharvest and/or post harvest and/or processing	Aesthetic
		Mold: post harvest and/or processing infection	Potential health hazard — mold may contain mycotoxin producing fungi
	Mammalian excreta: 3 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
Mace	Insect filth and/or mold: 3% or more by weight are insect-infested and/or moldy	Insect infestation: preharvest and/or post harvest and/or processing	Aesthetic
	Mammalian excreta: 3 mg or more of mammalian excreta per lb	Mold: post harvest and/or processing infection	
		Mammalian excreta: post harvest and/or processing animal contamination	
	Foreign matter: 1.5% or more of foreign matter through a 20-mesh sieve	Foreign matter: post harvest contamination	
Marjorum, whole plant, unprocessed	Insect filth and/or mold: 3% or more by weight are insect-infested and/or moldy	Insect infestation: preharvest and/or post harvest and/or processing	Aesthetic
	Mammalian excreta: 1 mg or more of mammalian excreta per lb	Mold: post harvest and/or processing infection	
		Mammalian excreta: post harvest and/or processing animal contamination	
Marjoram, ground	Insect filth: 1175 or more insect fragments per 10 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 8 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Marjoram, unground	Insect filth: 250 or more insect fragments per 10 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 2 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Nutmeg, whole	Insect filth and/or mold: 10% or more by weight are insect-infested and/or moldy	Insect infestation: preharvest and/or post harvest and/or processing	Aesthetic
		Mold: preharvest and/or post harvest infection	Potential health hazard — mold may contain mycotoxin producing fungi
Nutmeg, ground	Insect filth: 100 or more insect fragments per 10 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 1 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	

Oregano, whole plant, unprocessed	Insect filth and/or mold weight: 5% or more insect infested and/or moldy pieces by weight	Insect infestation: preharvest and/or post harvest and/or processing Mold: post harvest and/or processing infection	Aesthetic
	Mammalian excreta: 1 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
Oregano, ground	Insect filth: 1250 or more insect fragments per 10 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 5 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Oregano, crushed	Insect filth: 300 or more insect fragments per 10 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 2 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Paprika, ground	Mold: mold count is more than 20% Insect filth: more than 75 insect fragments per 25 gm	Mold: preharvest and/or post harvest infection Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: more than 11 rodent hairs per 25 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Pepper, whole (black or white)	Insect filth and/or insect-mold: 1% or more pieces by weight are infested and/or moldy	Insect infestation: preharvest and/or post harvest and/or processing Mold: preharvest and/or post harvest infection	Aesthetic Potential health hazard — mammalian encreta contain Salmonella
	Mammalian excreta: 1 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
	Foreign matter: 1% or more of pickings and siftings by weight	Foreign matter: post harvest contamination	
Pepper, ground	Insect filth: more than 475 insect fragments per 50 gm	Insect fragments: pre-harvest and/or post harvest and/or processing insect infestation	Aesthetic
	Rodent filth: 2 or more rodent hairs per 50 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Sage, whole plant, unprocessed	Insect filth: 5% or more pieces by weight are infested	Insect infestation: preharvest and/or post harvest and/or processing	Aesthetic
	Mammalian excreta: 1 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
Sage, ground	Insect filth: 200 or more insect fragments per 10 gm	Insect infestation: preharvest and/or post harvest and/or processing	Aesthetic
	Rodent filth: 9 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Sesame seeds	Insect filth: 5% or more seeds by weight are insect-infested or damaged Mold: 5% or more seeds by weight are decomposed	Insect infested: preharvest and/or post harvest and/or processing infestaion Mold: preharvest infection	Aesthetic

(*Continued*)

TABLE 89.11
(*Continued*)

Spice	Defective Action Level (Average)	Direct Source	Significance
	Mammalian excreta: 5 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
	Foreign matter: 0.5% or more of foreign matter by weight	Foreign matter: post processing and/or processing contamination	
Spices, leafy, other than bay leaves	Insect filth and/or mold: 10% or more by weight are insect-infested and/or moldy	Insect infestation: preharvest and/or post harvest and/or processing Mold: preharvest and/or post harvest infection	Aesthetic
	Mammalian excreta: 1 mg or more of mammalian excreta per lb	Mammalian excreta: post harvest and/or processing animal contamination	
Thyme, whole plant, unprocessed	Insect filth: 5% or more seeds by weight are insect-infested and/or moldy	Insect infested: preharvest and/or post harvest and/or processing infestaion	Aesthetic
	Mammalian excreta: 1 mg or more of mammalian excreta per lb after processing	Mammalian excreta: post harvest and/or processing animal contamination	
Thyme, ground	Insect filth: 925 or more insect fragments per 10 gm	Insect fragments: preharvest and/or post harvest and/or processing infestation	Aesthetic
	Rodent filth: 2 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	
Thyme, unground, processed	Insect filth: 325 or more insect fragments per 10 gm	Insect fragments: preharvest and/or post harvest and/or processing infestation	Aesthetic
	Rodent filth: 2 or more rodent hairs per 10 gm	Rodent hair: Post harvest and/or processing contamination with animal hair or excreta	

Source: US-FDA-Food Defect Action Levels, Revised 1998.

TABLE 89.12
Methods to Reduce Bacteria in Spices

Method	Procedure	Advantages/Disadvantages
Ethylene oxide	Product introduced to chamber Replacement of air by pulling vacuum Heating of chamber contents to 110–120 F Introduction of moisture Introduction of ethylene oxide Holding of spice under ethylene oxide for specific period of time relative to bulk density and bacterial reduction level desired (12–18 hr) Expellation of ethylene oxide and flushing air prior to returning to ambient pressure Removal of product to quarantine area to ensure residual ethyl oxide gas level	Proven method for whole spices and seeds Safety of using ethylene oxide is a concern Long treatment time Banned in some countries
Irradiation	Product loaded to carrier and travels on conveyor through a series of doors and locked into cell area containing cobalt 60 Product exposed to high and low levels of gamma radiation for specific time (5 to 15 hr) in the cells to reduce bacterial load relative to initial bacterial load and bacterial reduction level desired Product returned to its place of origin through a series of doors and locks	Cost effective Overall effectiveness Can process packed products Consumer concerns on irradiated products Not universally accepted
Dry steam	Product dropped into chamber containing shaking bed through airlock Product exposed to dry steam between 108 to 125°C briefly Products cooled immediately with continuous filtered airflow Product is dropped through airlock into a second closed chamber to continue the cooling process with ambient air without development of condensation, dust is filtered during the entire process	Highly effective More expensive than irradiation process, but less expensive than etheylene oxide and wet steam process Absence of residual fumigant
Wet steam	Product dropped into chamber containing shaking bed through airlock Exposure of saturated steam as required to inactivate microbes Termination of steam treatment followed by drying process to adjust the moisture content back to original	Absence of residual fumigant Loss of volatile oils Loss of color in some spices Development of unaccepted flavor

Source: References 6–11, 14, 18.

proximate compositions of common spices are available (25), the other measurable chemical components are usually not used in the comparison of quality except for a few spices that have been agreed upon (Table 89.13). It should be noted that the heat level of red pepper (Scoville test) is basically a sensory test even though chemical procedures are used in the preparation of the samples. Even though ASTA has recommended the use of the HPLC (High Performance Liquid Chromatography) method, there is doubt in the U. S. spice industry on its application to replace the Scoville test.

C. Chemical Residues

A few chemicals may be used in the disinfestation of insects and elimination of bacteria, yeast and/or fungi associated with spices. It is unavoidable that these spices have to be examined for these chemical residues. Application of these chemical treatments in one nation may not be permitted in another country. In addition, agricultural chemicals used in the production of spices have to be monitored especially when these chemicals are acceptable in one country but may not be acceptable in another country. Chemical treatment of spices and the use agricultural chemicals have caused complications in international spice trade. The presence of heavy metals in spices is mostly related to production environment, and heavy metals in spices have to be monitored as importing countries usually have defined limits. Readers interested in this topic should consult Reference 8 for further information.

D. Moisture Content

Moisture content in spices not only affects the weights of the spices, but also is related to the water activity. Water activity is one of the primary controlling factors on the growth and development of microorganisms. Microorganisms can grow during the handling, transportation, and storage if the moisture content is not properly controlled. Spice processors usually control the moisture content of their products carefully so that microorganisms and even insects will not grow. Readers should consult references listed in the earlier sections of this chapter for further information.

E. Volatile Oil

Volatile oils are essential in most of spices. Percentage of volatile oil differs from spice to spice (Table 89.9), and is used as one specification for spices. Volatile oils are also

TABLE 89.13
Specific Tests on Quality Comparison for Selected Spices

Spice	Test Conducted	Methodology
Allspice	Total acid	Titration
	Acid-soluble ash	Ashing, extraction, and gravimetry
	Water-soluble ash	Ashing, extraction, and gravimetry
Capsicum	Scoville heat units	Sensory, High Performance Liquid Chromatography
Capsium oleoresin	Capsaicin	High Performance Liquid Chromatography
Nutmeg, mace	Phenol	High Performance Liquid Chromatography
Paprika	Carotenoids	Spectrophotometry
Paprika, defatted	Sample structure	Microscopy, solvent extraction
Parsley	Green color (Chlorophylls)	Sensory
Pepper (black)	Piperine	Spectrophotometry
Saffron	Crocin	Hydrolysis and spectrophotometry
Turmeric oleoresin	Curcumin	Spectrophotometry
Residual solvent tolerance	Various solvents	Gas chromatography

Source: References 6–11, 14, 18.

used as one of the basic components in the manufacture of soluble spices (see below). The equivalencies of some common spices are listed in Table 89.14.

V. PRINCIPLE FORMS OF PROCESSED SPICES

Spices come in a variety of forms for adding the "bite," "pungency," or "character" so desired in food. They are available whole, ground, or as extractives — e.g., essential oils and oleoresins, and their derivatives. Essential oils are the aromatic, volatile compounds present in most spices, and provide the characteristic flavor and aroma of the spice. They are recovered from plant materials primarily by steam distillation, although some are cold expressed, dry distilled, or vacuum distilled. Oleoresins consists of essential oils, organically soluble resins, and other non-volatile principles — such as heat components, fixatives, natural antioxidants, and pigments. Solvents typically used for oleoresin extraction include ethyl acetate, ethanol, and ethylene chloride. Supercritical extraction using carbon dioxide also is used by some companies. Figure 89.1 shows a schematic diagram on the preparation of spice essential oils and oleoresins (16–17).

Spice extractives are further formulated to produce secondary products such as essences, emulsions, liquid-soluble spices, dry-soluble spices, encapsulated spices, heat-resistant spices, and fat-based spices.

TABLE 89.14
Some Common Essential Oils Available, and Their Equivalencies (Per 100 lb of Spice/Herb)

Spice	Oil Produced From	Equivalency
Allspice	Berries	2.5 lb
Basil	Flowering tops	0.8 lb
Bay (Laurel) leaf	Leaves	1 lb
Caraway seed	Seeds	2.5 lb
Cardamom	Seeds	3 lb
Clove	Flowering buds	15 lb
Coriander	Seeds	0.75 lb
Cumin seed	Seeds	3 lb
Fennel seed	Seeds	3 lb
Nutmeg	Seeds	5 to 15 lb
Oregano	Flowering plant	13 oz
Pimenta leaf	Leaves	0.5 to 1 lb
Rosemary	Flowering tops	11 oz
Sage or Salmatian	Leaves	1.25 lb
Thyme	Flowering plant	2 lb

Source: References 6–9, 11, 14, 16, 20.

- Essences are extractives prepared by macerating the ground spice with 70% ethanol. "Top notes" may be provided by the addition of essential oils or oleoresins (Figure 89.2).
- A spice emulsion is liquid seasoning prepared by emulsification of essential oils and/or oleoresin with gum arabic or other emulsifiers.
- A heat-resistant spice made from oleoresins and/or essential oils is encapsulated with a water-insoluble coating, which extends the product's shelf life and renders it suitable for baking applications.
- A fat-based spice is made from essential oils and/or oleoresins blended with a liquid edible oil or hydrogenated fat, and sold either in bulk or as a spray-cooled, fat-encapsulated spice. Examples of applications include high fat products such as mayonnaise and cream soups. Each spice form is prepared by a different method, and has unique properties that make it more suitable for one particular application over another.
- Water-soluble (dry-soluble) spice is made by dispersion of a blend of volatile oil and oleoresin in sugar, salt, corn syrup solids, gums or modified corn starch.

All these spice extractives have the advantages of constant quality, stability them, and easy handling. They can also be formulated to have certain equivalencies to make user-friendly. Figure 89.2 shows a schematic diagram of the preparation of some of these spice extractives (6–9, 16–17). Tables 89.14 and 89.15 shows the product appearance and spice equivalent (replacement strength) of some common spice volatile oils and oleoresins, respectively. Products

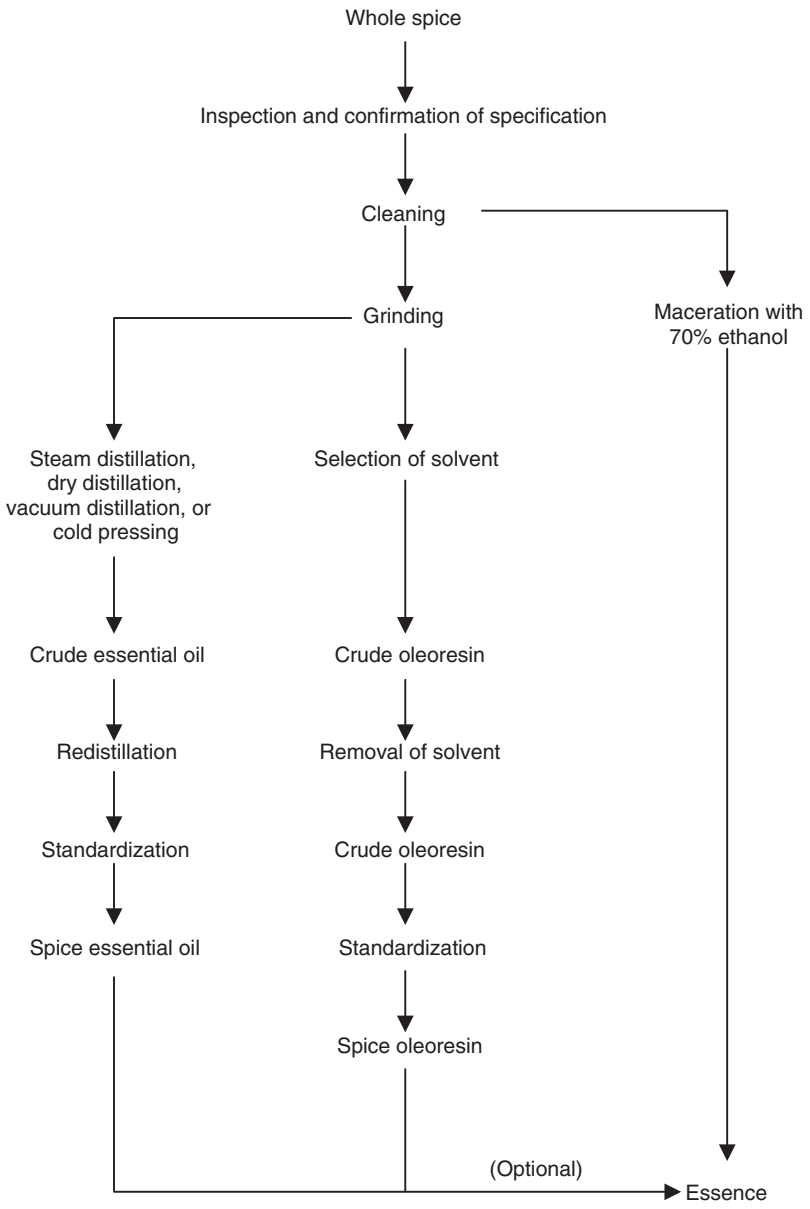

FIGURE 89.1 Schematic processing steps in the production of spice essential oils and oleoresin.

from various spice processors are not the same and have their own spice equivalents due to methods used for oleoresin extraction, and source of raw materials. Table 89.16 compares the advantages and disadvantages of different forms of spices and their derivatives.

VI. BIOACTIVITY IN SPICES AND HERBS

A. Natural Occurring Toxicants in Selected Spices

It has been known for at least several decades that some spices are toxic even when consumed in small quantities. Some spices are now proven to be either estrogenic, carcinogenic, hallucinogenic, goitrogenic, antithyroidic, lacinmatic, and/or mutagenic to humans, test animals or on Ames test (27–36). Table 89.17 presents examples of these toxicities of some common spices. However, readers should not be scared away from using small amount of spices in their food preparations. No reported case of harmful or unhealthy effects exists from consuming spices in quantities commonly applied in food preparation. Anything consumed in excessive amounts is bound to have undesirable effects.

B. Antioxidative Effects of Spices

In recent years, interest in antioxidants has increased because of their possible beneficial health effects. Many common food items have been observed for their

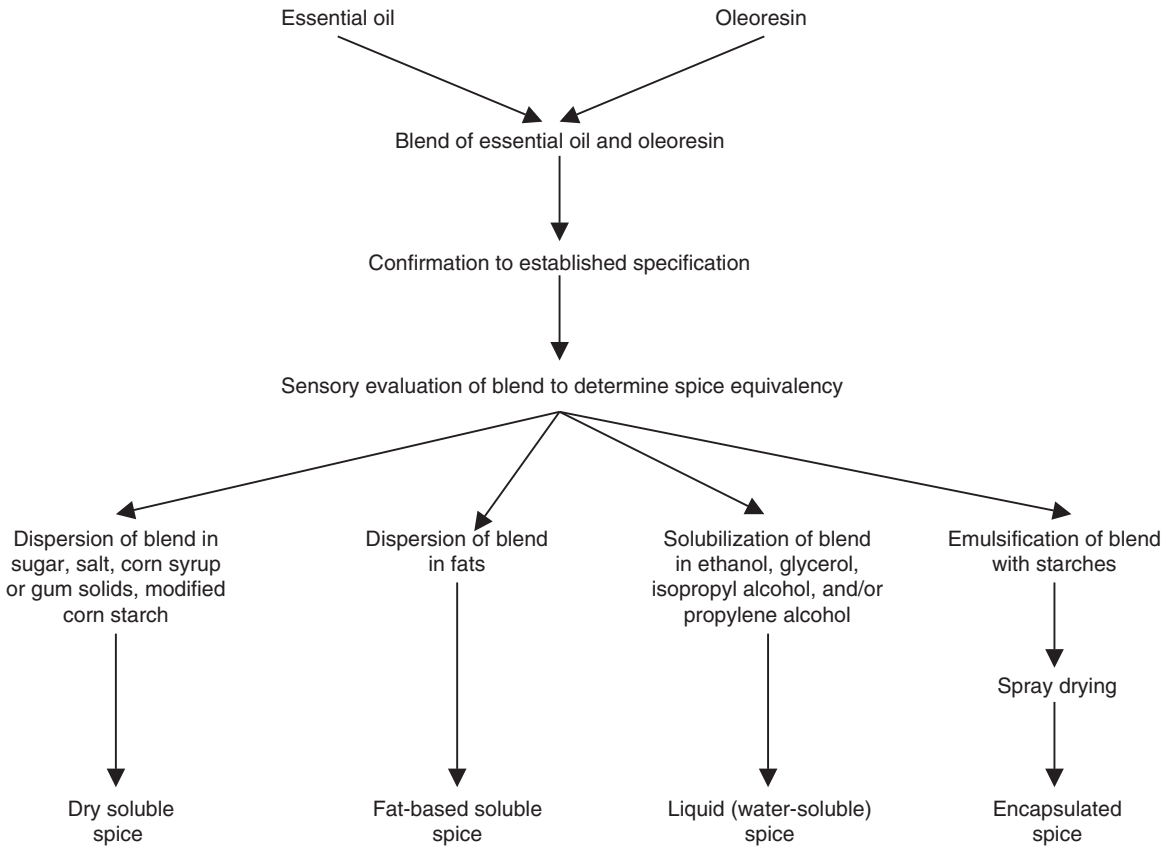

FIGURE 89.2 Schematic diagram on production of various soluble spices.

TABLE 89.15
Product Appearance and Spice Equivalent (Replacement Strength) of Common Spice Oleoresins

Spice	Product Appearance	Spice Equivalent or Replacement Strength
Allspice	Reddish brown to green liquid	1 lb to 28–30 lb
Basil	Dark green semi-solid	1 lb to 0.5–1 lb
Bay leaf	Dark green semi-solid	1 to 5 lb
Caraway seed	Greenish yellow to light green liquid	1 lb to 5 lb
Cardamom	Green to dark green liquid	1 lb to 4 lb
Celery seed	Green to light green liquid	1 lb to 3–7 lb
Cinnamon	Reddish or dark brown liquid or Yellowish green liquid	1 lb to 40 lb
Clove	Reddish brown or yellowish green liquid	1 to 20 lb
Coriander	Greenish brown or brownish yellow liquid	1 to 15 lb
Cumin seed	Brownish to yellowish-green liquid	1 to 5 lb
Dill seed	Green or light amber liquid	1 to 4–6 lb
Fennel seed	Brownish green liquid	1 to 5–6 lb
Garlic, dehydrated	Brownish-tan or brownish viscous liquid	1 to 2 lb (fresh) 1 to 8 lb (dehydrated)
Ginger, dehydrated	Brown to dark brown liquid or semi-solid	1 to 28 lb
Mace	Amber or brownish- or reddish-orange liquid	1 to 10–20 lb
Marjoram, sweet	Dark green viscous liquid	1 to 7–9 lb
Nutmeg	Yellow-orange or pale yellow viscous liquid	1 to 6–10 lb
Onion, dehydrated	Brown to brownish-tan liquid	1 to 0.5 lb (fresh) 1 to 1 lb (dehydrated)

(*Continued*)

TABLE 89.15
(*Continued*)

Spice	Product Appearance	Spice Equivalent or Replacement Strength
Oreganum	Dark-brown to brownish green viscous liquid	1 to 3–5 lb
Paprika	Dark red liquid	1 to 15 lb
Parsley	Deep green semi-viscous liquid	1 to 1/3 lb (fresh) 1 to 3 lb (dry)
Pepper, black	Thick liquid	1 lb to 20–25 lb
Pepper, red	Oily orange, reddish or brown liquid	1 lb to 5–7 lb
Rosemary	Thick green to greenish brown semi-solid	1 to 4–7 lb
Sage	Brownish green heavy liquid	1 to 6–9 lb
Thyme	Dark green to brown viscous semi-solid	1 to 4 lb
Turmeric	Reddish brown to dark brown liquid	1 to 15–20 lb
Vanilla	Dark brown viscous semi-solid	1 to 10 lb

Source: References 6–9, 11, 14, 16–17.

TABLE 89.16
Comparison of Various Forms of Spices

Forms	Advantages	Disadvantages
Whole/ground spice	Slow release of flavor at high temperature processing	Inconsistent aroma, taste, and color
	Easy to weigh and handle	Greater volume and space during storage
	No labeling problem	Presence of foreign matter and undesirable organisms
		Possibility of contamination during storage
Essential oil		
Liquid	Uniform and standardized flavor	Incomplete flavoring agents
	Undiluted essential oil highly concentrated	Lack of natural antioxidants in spice
	Sterile and free of extraneous matter	Undiluted essential oils difficult to handle
	Space saving	
	Stable under good storage condition	
	Emulsifiable with other liquid solutions	
Encapsulated oil	Uniform and standardized flavor	Incomplete flavoring agents
	Sterile and free of extraneous matter	Loss of volatiles during encapsulation
	Space saving	Very expensive
	Ease of handling and dispersing in food system	Not suitable for liquid
	Stable under good storage condition	
	Can be formulated to be water-soluble	
Oleoresin		
Undiluted	Uniform and standardized flavor	Very viscous and hard to handle
	Highly concentrated	Possible presence of undesirable flavor components
	Hygienic and free of extraneous matter and organisms	Possible presence of undesirable organic solvent
	Presence of natural antioxidants	
	More complete flavor than essential oil	
	Long shelf life	
	Space saving	
Encapsulated oleoresins	Same as encapsulated oil	Same as encapsulated oil
Essential oil + oleoresin	Uniform and standardized flavor	Very expensive
	Benefits of combined flavor from essential oil and oleoresin	Loss of volatiles during encapsulation
		Not suitable for liquid
	Hygienic and free of extraneous matter and organisms	Possible presence of undesirable flavor components
	Ease of handling and dispersing in food system	Possible presence of undesirable organic solvent
	Stable under good storage condition	
	Can be formulated to be water-soluble	

(*Continued*)

TABLE 89.16
(*Continued*)

Forms	Advantages	Disadvantages
	Space saving	
	Can be formulated to be water-soluble	
Dry soluble spice	Uniform and standardized flavor	
	Hygienic and free of extraneous matter and organisms	Carrier such as salt and sugar must be considered in formulation of food products
	Ease of handling and dispersing in food system	
	Stable under good storage condition	Not as stable as encapsulated products
	Can be formulated to be water-soluble	Possible presence of undesirable flavor components
	Space saving	Possible presence of undesirable organic solvent
	Price more stable than raw spice	
Liquid spice	Uniform and standardized flavor	Expensive
	Sterile and free of extraneous matter	Possible presence of undesirable flavor components
	Space saving	Possible presence of undesirable organic solvent
	Stable under good storage condition	Nature of carrier must be considered
	Emulsifiable with other liquid solutions	

Source: References 6–9, 11, 14, 16–21.

TABLE 89.17
Natural Occurring Toxicants in Selected Spices

Spice	Toxicant or Toxic Activity
Anise volatile oil	Anethole (estrogenic activity)
Basil oil	Estragole (carcinogenic activity)
Black pepper	Safrole (carcinogenic activity)
	Piperine (carcinogenic activity)
Black pepper + nitrate	*n*-Nitrosopiperidine (carcinogenic activity)
Fennel volatile oil	Anethole (estrogenic activity)
Mustard, brown	Sinalbin then allylisothiocyante (lacinmater, mutagenic in Ames test,carcinogenic in rats)
Mustard, white	Glucosinolates (goitrogenic and antithyroid activities)
	Sinalbin then allylisothiocyante (lacinmater, mutagenic in Ames test, carcinogenic in rats)
Parsley	Evidence of estrogenic activity
Paprika + nitrate	*N*-Nitrosopyrrolidine (carcinogenic activity)
Nutmeg	Myristicin (hallucinogen)
Sage	Evidence of estrogenic activity
Tarragon oil	Estragole (carcinogenic activity)
Turmeric	Evidence of carcinogenic activity

Source: References 6, 7, 9, 14, 27–33.

TABLE 89.18
Antioxidative Effect of Spices

Medium	Antioxidative Effectiveness	Spice
Lard	Remarkably effective	Rosemary, sage
	Effective	Oregano, thyme, nutmeg, mace, turmeric
Oil-in-water emulsion	Strongest	Clove
	Strong	Turmeric, allspice, rosemary, ginger, cassia, cinnamon, oregano, savory, sage
	Some	Aniseed, basil, cardamom, marjoram, black pepper, white pepper

Source: References 6, 9, 14, 23, 34–37.

antioxidative effect(s), and spices are no exception. In fact, spices have been looked at for their antioxidative effect at least two decades ago for possible applications in food processing. A notable example is rosemary. This discovery was first patented and now is available as a food antioxidant (9, 6, 14, 18, 34–37). Table 89.18 shows examples of antioxidative activity of some common spices in lipid and oil-in-water emulsions.

C. Some Common Foodborne Bacterial Pathogens Inhibited by Selected Spices

Synthetic food preservatives have not enjoyed a good reputation over the years. With the trend of using more natural ingredients in formulations targeted for special groups of consumers, spices have been looked at as possible alternatives, and there are some successful reports. About 150 scientific reports have been published in this area between 1970 to 2000, and it is not the intention of this chapter to go into the details. Instead, a summary of inhibitory effect of spices [either in the form of the spice itself, its extractive or its effective compound(s)] on some common foodborne pathogens is presented in Table 89.19. (38–94). Garlic and cloves proved to be most effective in inhibiting these foodborne pathogens. It is not surprising that garlic is one of the most effective spices, as garlic has long been suspected of providing beneficial health effects. Even the slaves building the great pyramids used garlic and reported health benefits. With this better understanding of the inhibitory effects of various spices, it is possible that combinations may be developed to serve this purpose in certain food preparations. In fact, attempts are being made to achieve this goal (Daniel Y.C. Fung, personal communication, 2003). Research works on inhibitory effects on yeast and fungi are also available (95–106).

TABLE 89.19
Some Common Foodborne Bacterial Pathogens Inhibited by Selected Spices

Spice	*Staphylococcus aureus*	*Clostridium botulinum*	*Clostridium perfrigens*	*Bacillus cereus*	*Listeria monocytogenes*	*Salmonella enterica*	*Shigella dysenteriae*	*Escherichia coli*	*Vibrio parahaemolyticus*	*Yersinia enterocolitica*	*Camphobacter jejuni*
Allspice		X			X			X			
Bay leaves	X	X						X	X	X	
Cinnamon	X			X	X			X		X	
Cloves	X	X	X	X	X	X		X		X	
Cumin	X	X		X	X	X					
Dill	X										
Fennel	X			X		X					
Garlic	X		X	X		X		X	X	X	X
Lemongrass	X			X		X		X			
Mint								X		X	
Onion	X						X	X			
Oregano	X	X						X			
Rosemary	X	X		X	X				X		
Sage	X	X			X				X		
Tarragon	X							X			
Thyme	X			X				X	X	X	

Source: References 38–94.

TABLE 89.20
Prospective Beneficial Medical Effects of Some Common Spices

Spice	Scientific Name	Prospective Beneficial Medical Effects
Allspice	*Pimenta dioica*	High blood pressure, infection, rheumatism
Anise seed	*Pimpineela anisum*	Not known
Basil	*Ochimum basilicum*	Not known
Bay leaf	*Laurus nobilis*	Cancer, infection
Cassia	*Cinnamonum cassia*	Cancer, cold/flu, dyspepsia
Caraway seed	*Carum carvi*	Cancer, cold/flu, hepatosis
Cardamom	*Elettraia cardamomum*	Cold/flu, hepatosis
Celery seed	*Apium graveolens*	High blood pressure, rheumatism
Chervil	*Anthriscus cerefolium*	Not known
Chives	*Appium schoenoprasum*	Not known
Cinnamon	*Cinnamomum zelancium*	Dyspepsia, infection
Clove	*Syzgium aromaticum*	Bronchosis, toothache
Coriander	*Coriandrum sativum*	Not known
Cumin seed	*Cumium cyminum*	Not known
Dill seed	*Anethum graveciens*	Not known
Dill weed	*Anethum graveciens*	Not known
Fennel seed	*Foeniculum vulgare*	Not known
Fenugreek	*Trigonella foenumgraecum*	Dermatosis, diabetes, high cholesterol
Garlic, dehydrated	*Allium sativum*	Cardiopathy, infection
Ginger, dehydrated	*Zingiber officinale, Roscoe*	Cold/flu, dyspepsia
Lemongrass	*Cymbogon citratus*	Cold/flu, pain
Mace	*Myristica fragans*	Cancer, infection
Marjoram, sweet	*Origanum majorana*	Not known
Mint, Peppermint (black mint)	*Mentha piperita* variety *vulgaris*	Not known
Mint, Spearmint	*Mentha spicata*	Not known
Mustard seed, black	*Brassica hira* or *B. alba*	Not known
Mustard seed, brown	*Brassica juncea*	Not known
Mustard, white	*Brassica nigra*	Not known
Nutmeg	*Myristica fragans*	Cancer, infection
Onion, dehydrated	*Allium cepa*	Colds, diabetes
Oregano	*Origanum spp.*	Not known
Paprika	*Capsicum annum*	Cardiopathy, cold/flu, rheumatism
Parsley	*Petrosalinum crispum*	Not known
Pepper, black	*Piper nigum*	Dermatosis, high cholesterol
Pepper, red (Capsicum)	*Capsicum fructescens*	Cardiopathy, cold/flu, rheumatism
Pepper, white	*Piper nigum*	Dermatosis, high cholesterol
Poppy seed	*Papaver somniferum*	Not known
Rosemary	*Rosemarinus officinalis*	Not known
Saffron	*Crocus sativus*	Cancer, infection
Sage	*Salvia officinalis*	Not known
Savory, sweet (summer)	*Satureja indicum*	Not known
Sesame seed	*Sesamum indicum*	Cancer, dermatosis
Star anise, Chinese	*Illicum verum*	Bronchitis, dyspepsia, fungus
Tarragon	*Artemisia dracunculus*	Not known
Thyme	*Thymus vulgaricus*	Not known
Turmeric	*Curcuma longa*	Cancer, dyspepsia, infection, antiinflamatory
Vanilla	*Vanilla fragrans, V. planilfolia,* or *V. tahitensis*	Cancer, cramp, infection

Source: References 107–112.

D. Prospective Beneficial Medical Effects of Some Common Spices

It was common practice in ancient cultures such as Greek, Egyptian, Indian, and Chinese to use spices and herbs to treat patients with various problems. It should be understood that in these cultures, the lists of spices and herbs are much more extensive than what is presented in this chapter. However, this method of treating patients is still practiced to some extent in China and India. With the modern

TABLE 89.21
Use of Spices as Food Cures in Traditional Chinese Medicine (TCM)

Spice	Food Cures According to Traditional Chinese Medicine (TMC)
Caraway seeed	Stomach ache, abdominal pain, hernia, lumbago
Cinnamon bark	Cold limbs, abdominal pain, diarrhea, hot sensations in the upper region with cold sensations in the lower region
Cinnamon twig (stick)	Pain in the back and shoulder, chest pain, menopause
Clove	Vomiting, hiccuping, upset stomach, diarrhea, abdomnal pain, hernia
Coriander (Chinese parsley)	Indigestion, measles prior to rash
Dill seed	Abdominal pain, poor appetite, shortage of milk secretion after childbirth
Fennel seed	Hernia, cold pain in lower abdomen, lumbago, stomachache, vomiting, dry and wet beriberi
Garlic	Common cold, abdominal pain, edema, diarrhea, dysentery, whooping cough
Ginger, dried	Commn cold, abdominal pain, vomiting, and diarrhea, cold limbs, rheumatism
Ginger, fresh	Common cold, vomiting, cough, asthma, diarrhea
Licorice	Abdominal pain, poor appetite, fatigue, fever, cough, palpitation, convulsions, sore throat, digestive ulcers, drug poisoning, food poisoning
Marjoram	Common colds, fever, vomiting, diarrhea, jaundice, malnutrition in children, skin rash
Nutmeg	Abdominal swelling and pain, diarrhea, vomiting, indigestion
Pepper, black and white	Common cold, adbominal pain, upset stomach, vomiting of clear water diarrhea, food poisoning
Pepper, red	Abdominal pain, vomiting diarrhea
Peppermint	Common cold, headache, sore throat, indigestion, cores, toothache, skin eruptions
Rosemary	Headache
Saffron	Congested chest, vomiting of blood, suppression of menstruation, abdominal pain after childbirth due to blood coagulation, injuries from falls
Spearmint	Common cold, cough, headache, abdominal pain, menstrual pain
Star anise, Chinese	Hernia, abdominal pain, lumbago, beriberi, vomiting
Sweet basil	Headache in common cold, diarrhea, indigestion, stomachache, irregular menstruation
Thyme	Whopping cough, acute bronchitis, laryngitis

Source: Reference 22.

methodology in studying therapeutic effects of chemicals or natural products, there are indications that some spices do have certain therapeutic effects. Table 89.20 lists some examples, and Table 89.21 lists some claims in Traditional Chinese Medicine (TMC) on therapeutic effects of some common spices and herbs. Readers interested in this area should consult the references cited for these two tables and also References 22, 107–112 listed at the end of this chapter. With the increasing interest of Complimentary and Alternative Medicine (CAM), it is expected that research reports and books in this area will be more abundant in the immediate future. However, consumers must be very careful in the use of dietary supplements that include spices and herbs in ground or concentrated forms to treat ailments, as responses differ among people. Consumers are advised to consult physicians or professionals who have had training in this area before taking these products to treat ailments.

ACKNOWLEDGMENT

We would like to thank Dr. Daniel Y. C. Fung, Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas for his generosity in sharing his database on spices.

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90 Eggs as Nutritional and Functional Food Ingredients

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CONTENTS

I. INTRODUCTION

Eggs have long been recognized as an important source of nutrients, providing all of the proteins, lipids, vitamins, minerals, and growth factors for the developing embryo, as well as defense factors against bacterial and viral invasion.

More recently, however, eggs have been recognized as a source of biologically active substances, with significant therapeutic potential. Some pharmacologically significant egg components, for example, have been shown to possess antibacterial, antiviral, and anticancer action, suggesting potential medical, nutraceutical, and food-fortification applications.

Some of these egg components have been the subject of intense study and are currently produced and used on an industrial scale, including egg white lysozyme and avidin. As well, a thorough understanding of the egg and its components has allowed for the manipulation of egg contents, to further enhance their nutritional and therapeutic potential. Examples include the feeding of hens special diets to produce eggs with increased levels of ω-3 polyunsaturated fatty acids, which have demonstrated numerous health benefits, and the immunization of hens to produce specific egg yolk immunoglobulins, capable of preventing and treating bacterial and viral infections.

Here we describe in detail the biological activities of egg components, and review their numerous applications in the food and pharmaceutical industries.

II. STRUCTURE AND CHEMICAL COMPOSITION OF HEN EGGS

A. Structure of Hen Eggs

The hen egg is composed of three main parts: the shell and its membrane, the albumen (egg white), and the yolk (1, 2).

The egg shell is an inflexible, mineralized structure which gives the egg its shape (1). It is covered with fine pores, each approximately 10 to 30 μm in diameter, which allow for the exchange of atmospheric gases and water vapor (1, 2). These pores are in turn covered by a cuticle layer, which permits the exchange of gases, and protects the egg from moisture and invasion of microorganisms (2). On the inside of the shell is an inner and outer membrane, with a mesh-like structure for obstructing invading microorganisms (2, 3).

The egg albumen consists of thin and thick albumen, and a chalaziferous layer. The thick albumen is sandwiched between an inner and outer layer of thin albumen (2). The thick albumen, with increased viscosity due to a higher concentration of ovomucin, covers the inner thin albumen and chalaziferous layer, keeping the egg yolk in the center of the egg (2). The chalaziferous layer is a fibrous layer which covers the yolk, and is twisted at both ends of the yolk membrane, stretching into the thick albumen and suspending the yolk in the center of the egg (2).

The egg yolk, surrounded by the vitelline membrane, is composed of yellow yolk, and at the center, white yolk, which originates from the white follicle which matures in the ovary. The yellow yolk contains layers of alternate light and deep yellow yolk, and it is here that the embryo develops (1, 2).

B. Biosynthesis of Egg Components

The formation of an egg involves the conversion of nutrients in the feed into egg constituents through several intricate and highly coordinated steps (4).

The reproductive system of the hen consists of the ovary and oviduct (4). The ovary is the site of assembly of the yolk, each yolk beginning as a cell formed in the embryo. As the hen matures, the yolk cells are transformed into oocytes. A mature ovary contains many oocytes, and at least 600–700 of them will become mature yolk. Each oocyte becomes a follicle after being covered with a granular layer. The follicles in the ovary are surrounded by the hen's veins (4). Yolk constituents are synthesized in the liver in response to hormonal stimulation, some as protein precursors which are later enzymatically modified, and are transported to the follicular walls in the blood. The follicle undergoes several changes, including a separation of the cells of the granular layer, in order to facilitate the transfer of material to the yolk, which occurs via receptor-mediated endocytosis, producing yolk granules (5). The follicle undergoes a rapid development during which most of the yolk is deposited 6–10 days prior to ovulation.

When sufficient yolk has accumulated, the follicle then enters the oviduct. As it travels through the oviduct the vitelline membrane of the yolk and chalazal layer of the albumen are added, followed by the secretion of the albumen (2, 6). The proteins of the albumen are synthesized in the oviduct, also in response to hormonal stimulation, with ovalbumin and the other major proteins being synthesized by the tubular gland cells, and avidin being synthesized in the goblet cells (6). Following albumen deposition, the shell membranes are immediately added.

The egg is then held in the uterus, where several glands secrete fluid with a high concentration of calcium ion, which is transported from the blood, onto the inner surface of the uterus. The mechanism by which the calcium reaches the shell is unclear, but the eggshell structure is formed by assembling a crystalline-like calcium structure on the newly formed shell membranes (2, 4).

C. Chemical Composition of Eggs

1. General Composition

In general, an egg is composed of 9–11% eggshell (shell and shell membrane), 60–63% egg white, and 28–29% egg

yolk. The main components are 12% lipids, 12% proteins, and around 75% water, carbohydrates and minerals (1, 7).

Proteins are distributed throughout the egg, but most of them are present in the egg yolk (44%) and egg white (50%), the remaining 6% in the eggshell and eggshell membrane (7). Very small amounts of free amino acids have also been reported, presumably for immediate use by the developing embryo (1).

Egg lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins, and include phosphorus, nitrogen, and sugar-containing lipids. A very small amount of lipid has also been reported in the egg shell (1, 7).

Carbohydrates are a minor component of eggs, with 40% of the carbohydrate content being present in the egg yolk, and they are present as free and conjugated forms which are attached to proteins and lipids. Glucose is found in the egg white, and mannose and galactose are present as complex carbohydrates attached to proteins (7).

Numerous minerals have also been found in eggs, most of them in the egg shell (7).

2. Egg Shell and Membrane

The egg shell is made up of a matrix consisting of interwoven protein fibers and spherical masses, and interstitial calcite or calcium carbonate crystals (8). Approximately 95% of the egg shell is minerals, around 98% of this being calcium, along with 0.9% magnesium, and 0.9% phosphorus (7, 8).

The egg shell is covered with a cuticle layer which is composed of about 90% protein, with a high content of glycine, glutamic acid, lysine, cystine, and tyrosine, a small amount of carbohydrate, including galactosamine, glucosamine, galactose, fucose, glucose, and sialic acids, as well as a very small amount of lipid (7, 8).

The egg shell membranes are made up of protein fibers, with a high content of arginine, glutamic acid, methionine, histidine, cystine, and proline; they are considered keratins, due to a lack of hydroxyproline and presence of high concentrations of sulfur-containing amino acids (8).

3. Egg White

The egg white, or albumen, makes up about 60% of the total egg weight (7). Water and protein are the major constituents of albumen, accounting for about 88% and 11%, respectively (7, 8). The albumen proteins include ovalbumin, which is the major protein, followed by ovotransferrin and ovomucoid. Other egg white proteins include ovomucin, which is responsible for the viscosity of the albumen, lysozyme, avidin, cystatin, and ovoinhibitor (7).

Carbohydrates, minerals, and lipids are minor albumen components. The carbohydrates exist both in free form and bound as glycoprotein. Most of the free form carbohydrate is glucose; however, small amounts of fructose, mannose, arabinose, xylose, and ribose have also been detected (7). The major inorganic compounds found in the albumen are sulfur, potassium, sodium, and chlorine, as well as phosphorus, calcium, magnesium, and traces of iron. Lipid is found in only trace amounts (7).

4. Egg Yolk

Egg yolk is a homogeneously emulsified fluid. The major constituents of the solid matter of yolk are proteins and lipids, present mainly in the form of lipoproteins, which can be separated by high speed centrifugation into a sedimented fraction called the granules, and a clear fluid supernatant called plasma (7).

The granules are composed of 70% α- and β-lipovitellins (the high density lipoprotein fraction), 16% phosvitin, and 12% low-density lipoproteins (9). The plasma is divided into the low-density lipoprotein fraction (87%) and the water soluble fraction (WSF) (13%), which contains the livetins, which are lipid-free globular proteins, including γ-livetin, also referred to as immunoglobulin Y (8).

The egg yolk lipids include triglycerides, phospholipids, cholesterol, cerebrosides or glycolipids, and some other minor lipids (7).

Egg yolk also contains minerals, of which phosphorous is the most abundant. More than half of the phosphorus is contained in phospholipids. The content of carbohydrates in the yolk is approximately 1%, most of it as oligosaccharides bound to protein; that which is free is in the form of glucose. Finally, egg yolk contains pigments, primarily carotenes and riboflavin, which are the source of the colour of the yolk (7).

III. BIOLOGICAL ACTIVITIES OF EGG COMPONENTS

A. Egg Shell

1. Egg Shell Calcium and Matrix Proteins

Chicken eggshell is a highly specialized mineralized structure which consists of approximately 95% calcium carbonate by weight, and of the remaining material, 3.5% is an organic matrix consisting mainly of glycoprotein and proteoglycans (10, 11). Currently, eggshells are disposed of in many ways, and are generally considered a waste product. Only recently has significant research been conducted into the possibility of the value-added utilization of eggshells as a food ingredient (12, 13). Eggshell calcium has been proposed for pharmaceutical applications for calcium deficiency therapies in humans, and in animals for bone mineralization and growth (14). Eggshell powder was shown to have antirachitic effects in rats (15). *In vitro*, eggshell powder stimulated the growth of chick embryo

cartilage cells (16), and in humans the use of the eggshell powder resulted in decreased pain and increased bone mineral density (17). In piglets, the apparent absorbability of calcium from eggshell powder was found to be at least as good as that from purified $CaCO_3$ (17). However, the factors resulting in increased bone mineral density have not been discovered.

The eggshell consists of various organic matrix components. Numerous proteins which are present in various mineralized tissues and thought to be involved in the mineralization process possess calcium binding properties (18). Some eggshell matrix proteins also possess calcium-binding properties (19). Cortivo et al (20) separated a low molecular weight acidic protein with calcium affinity; this property was associated with a high content of aspartic and glutamic acid and a high content of sulphate groups. Similarly, ovotransferrin (78 000 Da), ovalbumin (45 000 Da) and others (36 000 Da) express calcium affinity (19, 21). A recent study demonstrated that a 21 000 Da protein present in soluble eggshell matrix proteins plays an important role in increasing calcium transport across intestinal epithelial cells *in vitro*, using Caco-2 cell monolayers (22). Eggshell calcium is proposed as an excellent calcium supplement to increase bone mineral density in individuals with osteoporosis (23).

2. Egg Shell Membrane

Eggshell membrane is composed of collagen-like proteins (collagen type I and V) (24), in a ratio of 100 of type I to 1 of type V. Coarse fibers (2.5 μm in diameter) contain more type I collagen, while type V collagen predominates in the fine fibers (0.6 μm in diameter), which are largely located in the inner membrane (25).

Eggshell membrane protein hydrolysate has been prepared by alkaline treatment, and the peptides derived from the membrane were shown to stimulate human skin fibroblasts *in vitro* (13). The membrane proteins (peptides) are currently utilized as a cosmetic ingredient for their emollient properties (26). Eggshell membranes also contain antimicrobial substances. Lysozyme activity was reported in the outer and inner membranes (27) and an anti-bacterial enzyme, β-N-acetylglucosaminidase, was found in the outer membrane (28).

B. Egg White

Various biological activities are associated with the egg white, and are summarized in Table 90.1.

1. Ovomucin

Hens' egg white ovomucin is a macromolecular and heavily glycosylated glycoprotein, consisting of a peptide-rich α-subunit and a carbohydrate-rich β-subunit (29). Ovomucin serves physical functions such as maintaining the structure and viscosity of the egg white albumen (30), thus serving to prevent the spread of microorganisms (31), and possessing good foaming and emulsifying properties (32). However it has also demonstrated several biological applications.

Ovomucin has been shown to possess virus hemagglutination inhibition activity, attributed primarily to its carbohydrate-rich β-subunit (33). Antiviral activity against newcastle disease virus, bovine rotavirus, and human influenza virus has been demonstrated *in vitro* (30, 33–36). It has also been demonstrated that ovomucin fragments, produced by treatment with pronase, showed increased solubility, as compared to its native form, while still retaining virus-binding activity (30, 36).

Pronase-prepared glycopeptides of ovomucin have also demonstrated anti-tumor effects in a double grafted tumor system in mice (37), suggested to be related to the anti-angiogenic activity of ovomucin, inhibiting tumor growth (38).

Ovomucin peptides may also act as immunomodulators, showing macrophage-stimulating activity *in vitro* (39). Finally, ovomucin was found to inhibit cholesterol uptake *in vitro* by Caco-2 cells, and reduce serum cholesterol in rats, displaying hypocholesterolemic action (40).

2. Ovotransferrin

Belonging to the transferrin family, a group of iron-binding proteins which are widely distributed in various biological fluids, ovotransferrin is a monomeric glycoprotein consisting of a single polypeptide chain of 686 amino acids, which has the capacity to reversibly bind two iron ions per molecule (41). Its suggested function is as an iron scavenger, preventing availability of iron to microorganisms, and as an iron delivery agent (42).

Ovotransferrin has been suggested as a natural food antimicrobial, and its antibacterial activity has been shown against a wide spectrum of bacteria, including *Pseudomonas* spp., *Escherichia coli*, *Streptococcus mutans* (43), *Staphylococcus aureus*, *Bacillus cereus* (42) and *Salmonella enteritidis* (44). Similar results have been obtained with immobilized ovotransferrin (43, 45) and iron-saturated ovotransferrin (46), indicating that its antibacterial activity may not be due to its iron binding alone, but also due to interaction between the protein and the bacterial cells. A 92-amino acid ovotransferrin antimicrobial peptide, OTAP-92, was found to be capable of killing Gram-negative bacteria by crossing the bacterial outer membrane by self-promoted uptake, and damaging the cytoplasmic membrane (47). The OTAP-92 peptide shares sequence similarity with a group of peptides referred to as defensins, which kill bacteria by forming a pore or ion channel in the cytoplasmic membrane (47). It has also been shown that ovotransferrin possesses both antiviral activity, against Marek's disease virus in chicken embryo fibroblasts (48), as well as antifungal activity, against species of *Candida* (46).

TABLE 90.1
Biological Properties of Egg white Proteins

Protein	Properties	Reference
Ovomucin	Antiviral activity	30, 33–35
	Antiviral activity of ovomucin-derived fragments	36
	Anti-tumor activity	37, 38
	Immunomodulating activity	39
	Hypocholesterolemic activity	40
Ovotransferrin	Antibacterial activity	41, 43–45
	Antimicrobial activity of ovotransferrin-derived peptide, OTAP-92	47
	Antiviral activity	48
	Antifungal activity	46
	Immunomodulating activity	49–51
	Enhancement of activity of certain antibiotics	52
Ovalbumin	Anti-mutagenic and anti-carcinogenic activity	55
	Immunomodulating activity	56–58
	Antioxidant activity	59, 60
	Anti-hypertensive activity of ovalbumin-derived peptides ovokinin and ovokinin (2-7)	61-63
	Angiotensin 1 converting enzyme (ACE) inhibitory activity of ovalbumin-derived peptides	64
	Immunomodulating activity of ovalbumin-derived peptides	65–68
Ovomucoid	Serine proteinase inhibitor	69, 71, 72, 76
	Drug delivery to small intestine	73, 75
	Biospecific ligand to lectins in gastrointestinal tract	74
	Immunomodulating activity	77
Lysozyme	Antibacterial activity	81, 83–85, 88, 89
	Enhanced antimicrobial activity when coupled to hydrophobic carrier or phenolic aldehyde	90, 91
	Antimicrobial action of lysozyme-derived peptides	92-95
	Application as a food preservative	81, 83, 84, 96–99
	Protection against periodontis-causing bacteria when added to oral health care products	100, 101
	Antiviral activity	81, 100, 102
	Anti-inflammatory activity	100
	Immunomodulating and immune-stimulating activity	100, 102, 103
	Anti-tumor activity	104–107
Avidin	Antimicrobial activity	111, 112
	Pre-targeting and drug delivery of anti-cancer drugs	114–118
	Application in adoptive immunotherapy	119
	Drug delivery to brain	120
Cystatin	Cysteine proteinase inhibitor	8
	Antibacterial activity	125, 129, 130
	Inhibition of growth of *P. gingivalis* by cystatin-derived peptides	131
	Immunomodulating activity	134–137
	Inhibition of tumor invasion	140
Ovomacroglobulin	Serine, cysteine, thiol, and metallo proteinase inhibitor	142, 143, 149–152
	Antibacterial activity	143–146,
	Enhancement of wound healing	147, 148
Ovoinhibitor	Serine proteinase inhibitor	155–157
	Antiviral activity	158
	Potential anti-inflammatory and anti-mutagenic activity	159
	Protein purification applications	161, 162

Ovotransferrin has been described as an acute phase protein in chickens, with serum levels increasing during inflammation and infections. It has been suggested that acute phase proteins may facilitate the biological activities of phagocytic cells and help produce factors that lead to the restoration of homeostasis (49). Xie et al. (49) demonstrated that ovotransferrin can act as an immunomodulator by modulating macrophage and heterophil functions in chickens. Further immunomodulating effects of ovotransferrin have also been shown, including the

inhibition of proliferation of mouse spleen lymphocytes (50) and the enhanced phagocytic response of peripheral blood mononuclear cells and polymorphonuclear cells in dogs (51).

Finally, when administered with Syn 2190, an inhibitor of AmpC lactamases, lactoferrin increased the activity of various antibiotics against beta-lactamase-producing bacteria (52).

3. Ovalbumin

Ovalbumin constitutes over half of the total egg white proteins (31). It is a monomeric phosphoglycoprotein, with a molecular weight of 45 000 Da, and has been used extensively as a model for studying the structure-function relationships of proteins (31, 53). Functionally, ovalbumin is important for the gelling, foaming, and emulsifying properties of egg white (54); however, its biological role in the egg remains unknown. It has been suggested that ovalbumin may serve as a source of amino acids for the developing embryo (31). Although ovalbumin shows no proteinase inhibitory activity, it shows structural homology with a family of serine proteinase inhibitors, called serpins (31).

The anti-mutagenicity of ovalbumin toward N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a well known and highly reactive model nitrosamine, has been examined (55). Vis et al. (55) found that ovalbumin, especially when heat-denatured, was strongly anti-mutagenic against MNNG, suggesting a role for ovalbumin as an anti-mutagen and anti-carcinogen.

Ovalbumin may also possess some immunomodulatory activity, as it was found to induce the release of tumor necrosis factor (TNF) alpha in a dose-dependant manner *in vitro*, when modified with dicarbonyl methylglyoxyl (MGO) (56), and immunogenic ovalbumin peptides have been used to enhance immune responses for cancer immunotherapy (57, 58).

Ovalbumin was found to possess a strong antioxidant activity against linolenic acid and docosahexaenoic acid (DHA) (59). Three antioxidant peptides with sequence of Ala-His-Lys, Val-His-His and Val-His-His-Ala-Asn-Glu-Asn derived from ovalbumin hydrolysis were identified and it has been suggested that metal chelation plays a major role in the antioxidant activity (60).

A vasorelaxing peptide (ovokinin; OA 358–365) was isolated from the peptic digestion of ovalbumin (61), and was found to be a weak bradykinin B1 agonist peptide. Ovokinin(2–7), a peptide produced by the chymotryptic digestion of ovalbumin, corresponding to OA 359–364, was also found to possess vasorelaxing effects (62). Following oral administration of ovokinin(2–7) at a dose of 10 mg/kg, the systolic blood pressure in a spontaneously hypertensive rat was significantly lowered (62). The anti-hypertensive activity of ovokinin(2–7) was enhanced by replacement of the C-terminal Phe residue with Trp (63). Two angiotensin I converting enzyme (ACE)-inhibitory peptides were also identified in ovalbumin by peptic (OA 183–184) and tryptic (OA 200–218) digestions (64). Furthermore, phagocytic activity of macrophages was increased by the addition of OA 77–84 and OA 126–134 peptides, derived by peptic and chymotryptic digestions of ovalbumin, respectively (65).

Immunoactive peptides produced by *Saccharomyces cerevisiae* fermentation of egg white were prepared and the administration of these peptides enhanced non-specific resistance in mice (66) and calves (67) infected with *Escherichia coli*. These peptides also exhibited high phagocytic activity in rainbow trout (68). However, their active components have not yet been identified.

4. Ovomucoid

Ovomucoid is a glycoprotein, with a molecular weight of 28 000 Da, comprised of 186 amino acids arranged into three domains, which are cross-linked by intradomain disulfide bonds (69). It is relatively resistant to treatment with heat or digestive enzymes, and it is this stability that has led to its being one of the dominant egg white allergens (70). Ovomucoid is also one of several egg white proteinase inhibitors, belonging to the group of serine proteinase inhibitors, namely inhibiting trypsin (69, 71). The inhibitory activity of ovomucoid toward trypsin and chymotrypsin was recently shown to be enhanced by site-directed mutagenesis, replacing amino acids around the reactive site of ovomucoid domain 3 (72).

The incorporation of ovomucoid into polymeric microparticles, to overcome the degradation of protein drugs by proteolytic enzymes, has been examined. Agarwal et al. (73) found that when ovomucoid was included, the stability of insulin in polymethacrylate based microparticulates was increased significantly. Inclusion of ovomucoid also resulted in targeting of drugs to the blood, by acting as a biospecific ligand to lectins on the walls of the gastrointestinal tract (74). The presence of ovomucoid was found to enhance insulin flux across rat jejunum (75), suggesting the use of ovomucoid to enhance the oral delivery of insulin. Using a rat model of experimental pancreatitis, intravenous ovomucoid was found to decrease the trypsin-like activity to the level of intact rats, and reduce the primary pancreas destruction (76).

Synthetic ovomucoid peptides have also demonstrated immunomodulating activity, inducing T-cell secretion of cytokines interleukin-(IL) 4, IL-10, IL-13, interferon-(IFN) gamma, and IL-6 (77).

Because of its allergenic nature, ovomucoid has limited biological and medical applications. It has however been used as a ligand in HPLC columns for the affinity and chiral separation of compounds of pharmaceutical importance such as trypsin, pentazocine and bupropion hydrochloride enantiomers (78, 79).

5. Lysozyme

Lysozyme is a ubiquitous enzyme, present in almost all secreted body fluids and tissues of humans, as well as plants, which plays an important role in the natural defense mechanism (80). The most plentiful source, however, is hens' egg white, containing around 0.3–0.4 g of lysozyme per egg (81).

Lysozyme acts as a mucopeptide N-acetylmuramyl hydrolase, exerting bacteriolytic activity by hydrolyzing the β(1–4) linkage between N-acetylmuraminic acid and N-acetylglucosamine of peptidoglycan, which is the structural component of bacterial cell walls (82). Gram-positive bacteria are more susceptible to lysozyme, as the cell wall contains around 90% peptidoglycan. Gram-negative bacteria, on the other hand, contain only 5–10% peptidoglycan, and this peptidoglycan lies beneath the outer membrane of the cell envelope (81). The lipopolysaccharide layer of the outer membrane acts as a barrier against macromolecules and hydrophobic compounds, making it more resistant to the action of lysozyme (81).

Lysozyme is ideal as a natural food preservative, as it is endogenous to many foods, specific to bacterial cell walls, and harmless to humans (81). It has been found to be most effective against *Bacillus stearothermophilus*, *Clostridium tyrobutyricum*, *Clostridium thermosaccharolyticum*, *Clostridium sporogenes* and *Bacillus* spp. (81). However, this spectrum can be broadened by the use of lysozyme in conjunction with other compounds, such as nisin and EDTA, to include *Enterococcus faecalis*, *Weissella viridescens* (83), *Brochothrix thermosphacta*, *Lactobaccilus sakei*, *Leuconostoc mesenteroides*, *Listeria monocytogenes* (84), and *Carnobacterium* sp. (85).

The physical treatment of lysozyme, including heat denaturation (86, 87) has been found to increase its antimicrobial activities against Gram-negative bacteria, as does high pressure treatment (88, 89). Other strategies have also been adopted to increase its activity against Gram-negative bacteria, including equipping the enzyme with a hydrophobic carrier to enable it to penetrate and disrupt the bacterial membrane (90, 91), and coupling it with a safe phenolic aldehyde with lethal activity toward the bacterial membrane (91). Enzymatic treatment, to expose amino acids 98–112 of lysozyme, which have been found to exert antimicrobial action (92, 93), as well as synthetic peptides corresponding to the C-terminal of lysozyme (94), have also been examined. Recently, Pellegrini et al. (95) demonstrated that polypeptides derived from lysozyme were capable not only of damaging the outer membrane of *Escherichia coli*, but could also inhibit DNA and RNA synthesis.

The use of lysozyme has been proposed for numerous antimicrobial applications in food, including inhibiting *C. tyrobutyricum* and *L. monocytogenes* in cheese and dairy products (81), controlling lactic acid bacteria in beer and wine (96–98), preventing *Edwardsiella tarda* infection in fish (99), inhibiting spoilage bacteria in meats (83–85), and for the bacterial decontamination of vegetables (81). Lysozyme has also been added to oral health care products, such as toothpaste, mouthwash and chewing gum to protect against periodontis-causing bacteria and to prevent infections in the oral mucosa (100, 101).

Lysozyme has been shown to exert antiviral activity, reportedly associated with its charge, rather than its lytic ability (81). Oral and topical applications of lysozyme were found to be effective in preventing and controlling several viral skin infections, including herpes simplex and chicken pox (100), as well as acting as exerting anti-inflammatory action (100). It has also been shown, when combined with immunotherapy, to be effective in improving chronic sinusitis (102), and to normalize humoral and cellular responses in patients with chronic bronchitis (100).

Finally, lysozyme has also been shown to act as an immune-modulating and immune-stimulating agent, enhancing immunoglobulin production, and regulating and restoring the immune responses in immune-depressed patients undergoing anti-cancer treatments (100, 103), and as an anticancer agent, the inhibitory action of lysozyme being demonstrated in a number of experimental tumors (104–107).

6. Avidin

Chicken avidin is a tetrameric glycoprotein, with an extremely high affinity for the water soluble vitamin biotin (108). The unique feature of this binding is the strength and specificity of the formation of the avidin-biotin complex, formed when avidin binds four molecules of biotin, the resulting affinity constant being the highest known for a protein and an organic ligand (109).

Due to the lack of interaction between the carboxy-containing side chain of biotin with avidin, biotin lends itself to chemical modification and conjugation to a wide variety of biologically active molecules, without altering its binding to avidin (109). This has led to numerous biochemical and diagnostic applications, including affinity chromatography, enzyme immunoassays, microscopy, and biosensors (110).

It has been suggested that avidin possesses antimicrobial properties, and it has been found to inhibit the growth of biotin-requiring bacteria and yeasts (108, 111). The antibacterial activity of avidin has also been attributed to its ability to bind to various Gram-negative and Gram-positive bacteria, including *Escherichia coli* K-12, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermis*, in a dose-dependent manner, and independent of the saturation of the biotin-binding sites of the bacteria (112).

The strong binding between avidin and biotin was recently used as a model system in mice to examine the

use of proteins with high affinity binding sites to establish an absorptive barrier in the gastrointestinal tract to prevent the uptake of low molecular weight chemicals (113).

Avidin has also been found to be useful in medical applications, to localize and image cancer cells and to pre-target drugs to tumors. Because of its tight biotin binding and signal amplification due to the tetrameric structure of avidin, it leads to the accumulation of higher effective doses and increased persistence of biotinylated anti-cancer drugs, as compared to other immunotherapeutic procedures (114). Tumor pre-targeting with avidin has also been found to be effective in increasing the uptake of tumor necrosis factor (TNF) alpha conjugated to biotin *in vitro*, improving the anti-tumor activity of TNF (115–117). Yao et al. (118) found that radiolabelled avidin also bound to lectins expressed on the surface of tumor cells, and localized highly and rapidly in various types of tumors in mice, reducing radioactivity accumulation in other organs. These results suggest that avidin may be a promising vehicle for the delivery of radioisotopes, drugs, toxins or therapeutic genes to tumors (118).

Avidin was also found to be essential for the activity of adoptively transferred T-cells at tumor sites (119), and the utilization of avidin in drug delivery through the blood-brain barrier has also been demonstrated, allowing delivery of therapeutics to the brain (120).

Several chemically modified forms of avidin have also been developed to improve their pharmacokinetics and biodistribution, while reducing immunogenicity (121–123).

7. Cystatin

A member of a "superfamily" of cystatins, egg white cystatin belongs to the Type 2 cystatins, which have about 115 amino acids and two disulphide bonds, but no carbohydrate (124). Egg cystatin inhibits most cysteine proteinases, including ficin, papain, and cathepsins B, C, H and L, and is found in the egg white at levels of around 60–80 μg/mL (8). Low contents of cystatins in natural resources may limit their applications (125). However, genetic modification and expression of cystatin has been carried out, not only providing a source for increased quantities of cystatin, but also resulting in the production of recombinant cystatin with enhanced proteinase inhibitory activity (126), and increased stability to heat (127) and freezing (128).

Egg white cystatin has been shown to possess antibacterial activity, preventing the growth of group A streptococcus (129), *Salmonella typhimurium* (125), and the periodontis-causing *Porphyromonas gingivalis* (130). Blankenvoorde et al. (131) found that cystatin-derived peptides were also capable of inhibiting the growth of *P. gingivalis*.

It has been suggested that cystatins may not only have a general function of protecting cells against uncontrolled activities of their own proteinases, but may also protect against viral proteinases responsible for viral infection (8). Similar cysteine protease inhibitors have shown antiviral activity against herpes simplex virus, poliovirus (129, 132), and human rotavirus (133).

Cystatins may also have medical applications, resulting in less intensive side effects than other synthetic protease inhibitors currently used in medical treatments (125). A relationship between cystatins, cytokines, and the immune response has been suggested. It has been observed that cystatin induced the synthesis of various cytokines (134), resulting in an up regulation of nitric oxide release *in vitro* using mouse peritoneal macrophages (135, 136), as well as *in vivo*, greatly reducing parasite numbers in a mouse model of visceral leishmaniasis (137).

Increased levels of cysteine proteases, and the concomitant decrease of cystatin, has been observed in various cancers (138, 139), and cystatin has been shown to inhibit tumor invasion in ras-transformed breast epithelial cells (140), suggesting a role for chicken cytstatin in cancer therapy.

The use of chicken cystatin has also been recently suggested for food preservation applications, including its use to inhibit autolysis or gel softening in seafood (141).

8. Ovomacroglobulin (Ovostatin)

Ovomacroglobulin, also referred to as ovostatin, is a glycoprotein composed of four subunits, each with a molecular weight of 175 000 Da, joined in pairs by disulfide bonds (142). It has demonstrated broad-spectrum inhibitory activity against various types of proteases, including serine proteases, cysteine proteases, thiol proteases, and metalloproteases (142, 143).

The antimicrobial effects of ovomacroglobulin against *Serratia marcescens* and *Pseudomonas aeruginosa*, due to its proteinase inhibitory action, have been studied extensively both *in vitro* (143–146). *In vivo*, it was found to reduce corneal destruction in an experimental keratitis model in rabbits, as well as to accelerate wound healing (146, 147). Ovomacroglobulin was also found to enhance periodontal wound healing in rats, by accelerating fibroblast growth, collagen deposition, and capillary formation in tissue (148).

The proteinase inhibitory effects of ovomacroglobulin have also demonstrated a number of other biological effects, including the suppression of septicemia by *P. aeruginosa* and *Vibrio vulnificus* due to the inhibition of kinin generating proteases (149, 150), the *in vitro* inhibition of the inflammatory proteinase medullasin (151), and the suppression of metalloproteinases and enhanced vascular permeability in skin tissues, which play a role in tumor metastasis (152).

9. Ovoinhibitor

Another proteinase inhibitor in egg white, ovoinhibitor is a glycoprotein composed of a single 447-amino acid

polypeptide (153, 154) with a reported molecular weight between 46 000 and 49 000 Da (153, 155, 156). Like ovomucoid, ovoinhibitor is a serine proteinase inhibitor, inhibiting enzymes such as trypsin, chymotrypsin, and elastase, as well as various bacterial and fungal proteinases (155–157).

Ovoinhibitor has been found to prevent the development of rotavirus induced gastroenteritis in a mouse model of rotavirus infection (158), and to inhibit the formation of active oxygen species by human polymorphonuclear leukocytes, which are associated with inflammatory diseases, mutagenicity and carcinogenicity (159). It has also been used to study models of autoimmune arthritis in mice (160) as well as for biochemical applications such as the removal of contaminating proteinases from protein solutions, and the purification of serine proteinases (161, 162).

Proteinases are involved in the regulation of a number of biological processes, and have been implicated as contributors in several diseases, including viral diseases such as HIV (163), and Alzheimer's disease (164). Proteinase inhibitors, therefore, such as those from egg white, have significant potential for the treatment and prevention of proteinases-mediated diseases.

C. Egg Yolk

Several biological activities are associated with egg yolk components, and are summarized in Table 90.2.

1. Immunoglobulin (Ig) Y

Immunoglobulin (Ig) Y is the functional equivalent of IgG, the major serum antibody in mammals (165). It is transferred to the developing embryo, to give acquired immunity to the chick (166, 167). The yolk then may contain from 5 to 25 mg/mL of IgY (168, 168). Specific IgY can be produced by immunization of chickens with the desired protein, and then conveniently purified from the egg yolk (165, 170). The use of egg yolk antibodies presents many advantages over those produced in mammals, including being less invasive and less stressful on the animal, being more cost-efficient, and resulting in a higher yield of antibody per animal (165, 169). As well, yolk antibodies do not activate the mammalian complement system or interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract (165).

While IgY has been applied for numerous immunodiagnostic and immunoaffinity purification purposes,

TABLE 90.2
Biological Properties of Egg Yolk Components

Component	Properties	Reference
Immunoglobulin Y	Antibacterial activity	177–191, 194, 195
	Antiviral activity	172–176, 192, 193
	Reduction of dental caries	196–199
	Anti-venom applications	200, 201
	Anti-inflammatory action	203
	Cancer targeting and drug delivery of anti-tumor drugs	204
Phosvitin	Enhancement of calcium binding by phosvitin-derived peptides	212, 213
	Antioxidant activity	214
LDL	Immunomodulating activity	217, 218
	Cryoprotectant	219
Sialic acid	Receptor functions	223
	Anti-inflammatory activity	224–227
Sialyloligosaccharides	Antiviral activity	232
	Antibacterial activity	233
Yolk lipids	Antioxidant activity	220, 234
	Important for maintenance of cell membranes	234, 237
	Drug carrier	220
	Treatment of Smith-Lemli-Opitz syndrome	239
Phospholipids	Drug delivery to tumors and brain (via liposomes)	242–245, 246
	Reduction of necrotizing enterocolitis in infants	247
	Reduction of serum cholesterol	248, 249
	Increase in acetylcholine concentrations, and improvement in memory retention and brain function	250–252
	Antiviral activity	253
	Source of DHA and AA, which possess anti-tumor, anti-thrombic, anti-inflammatory, anti-hypertensive, vasodilatory, and hypolipidemic effects	255, 266–274

considerable research has focused on the use of IgY for immunotherapeutic applications, especially for the oral passive immunization of immunocompromized individuals against various bacteria and viruses. IgY has been produced against several bacteria and viruses, and has been shown to bind to and inhibit the infection and disease symptoms, *in vitro* and *in vivo*, of gastrointestinal pathogens such as human and bovine rotavirus (171–175), bovine coronavirus (176), *Escherichia coli* (177–183), *Salmonella* spp. (184–188), *Yersinia ruckeri* (189), *Edwardsiella tarda* (190), *Helicobacter pylori* (191), porcine epidemic diarrhea virus (192), infectious bursal disease virus (193), *Staphylococcus aureus* (194, 195) and *Pseudomonas aeruginosa* (194).

Egg antibodies against *Streptococcus mutans* have been shown to prevent adhesion of the bacteria *in vitro* and *in vivo*, and reduced dental caries development in an animal model (196–199). Chicken anti-venom IgY has been produced, for treatment of snake and spider bites, and was found to have a higher bioactivity than anti-venom raised in horses (200, 201). IgY also has a lower likelihood of producing significant clinical side effects, such as serum sickness and anaphylactic shock, which can occur upon administration of mammalian serum proteins (200, 202).

The use of IgY has also been examined to replace the anti-inflammatory drugs used to treat Crohn's disease and ulcerative colitis. Worledge et al. (203) reported that anti-TNF antibodies produced in chickens were capable of effectively treating acute and chronic phases of colitis in rats, and were also found to neutralize human TNF *in vitro*, indicating its possible use for the treatment of inflammatory bowel disease in humans.

Finally, IgY has been suggested for use in targeting cancer cells, to act as a carrier for anti-tumor drugs (204).

2. Phosvitin

Phosvitin is a highly phosphorylated protein with a molecular weight of 35 000 Da, containing 10% phosphorus and 6.5% carbohydrates (205). It contains 123 serine residues, which account for 57.5% of the total amino acid residues (206). Ninety-five percent of the iron in eggs is present in the yolk and is bound to phosvitin (207); however, its bioavailability is very low (208). This is due to the strong binding of phosvitin, or its phosphopeptide derivatives, with Fe^{3+}, and the formation of phosvitin-iron complexes which promote the precipitation of iron in the small intestine. Thus, phosvitin is presumed to be responsible for the poor bioavailability of egg yolk iron (209–211). However, Jiang and Mine (212, 213) produced functional phosphopeptides, with a molecular weight of 1000–3000 Da, derived from tryptic hydrolysis following partial alkaline dephosphorylation. These peptides exhibited enhanced calcium-binding capacity and inhibited the formation of insoluble calcium phosphates. These results suggest that novel functional phosvitin phosphopeptides may have potential applications as nutraceuticals.

Phosvitin also demonstrated a capacity to inhibit iron catalysis of phospholipid oxidations (214). Thus, phosvitin could be useful in foods as a natural antioxidant.

3. Lipoproteins (Low Density Lipoproteins, LDL)

The low density lipoproteins (LDL) fraction of yolk plasma is composed of 89% lipid and 11% protein (215). The lipid content of LDL is 70% triacylglycerol, 4% cholesterol, and 26% phospholipids (216).

The proliferative activity of LDL has been demonstrated using human histocytic lymphoma cells (U-937), human monocytic leukemia cells (THP-1) and U-937-derived macrophage-like cells (U-M) (217). LDL was also shown to enhance the production of IgM in human-human hybridomas (218).

LDL is widely used as a cryoprotectant for bull semen and it has shown better efficiency than commercial extenders (219).

4. Sialic Acid

Sialic acid is a general term for derivatives of neuraminic acid which have an acyl group on the amino nitrogen. The most widely distributed sialic acid in nature is N-acetylneuraminic acid (Neu5Ac) (220). Egg yolk has been examined as a source of sialic acid, and was found to be an excellent source for the large scale preparation of Neu5Ac (221, 222).

Sialic acids possess many biological functions, including acting as receptors for microorganisms, toxins, and hormones, and masking receptors and immunological recognition sites of molecules and cells (223).

Sialic acid may act as an anti-inflammatory agent. Cells carrying a carbohydrate ligand called sialyl-Lewis X were found to bind to endothelial leukocyte adhesion molecule-1 (ELAM-1), which mediates the adhesion of circulating leukocytes to the vascular endothelium during inflammation (224, 225). The anti-inflammatory effects of sialyl-Lewis X were also shown *in vivo*, reducing lung injury in rats (226) and reducing tissue injury in rabbit ears, caused by a temporary increase in blood supply (227).

Finally, a sialic acid analogue, zanamivir, has demonstrated potent antiviral effects, and was found to reduce the symptoms of influenza infections in humans, by inhibiting influenza A and B virus neuraminidases, enzymes essential for the release of virus from infected cells (228).

5. Sialyloligosaccharides

Several sialyloligosaccharides in chalaza, egg yolk membrane and delipidated egg yolk have been isolated by acid hydrolysis or protease digestion (229, 230). These

sialyloligosaccharides are likely to be naturally present as glycoproteins or glycopeptides (230). A sialylglycopeptide, Lys-Val-Ala-Asn-Lys-Thr, the Asn of which was modified with the disialylglycan moiety, was isolated from egg yolk (231). Sialylglycoconjugates, sialylgangliosides, sialyloligosaccharides and sialylglycoproteins have been reported to play various important roles in animal and human tissue cells.

The oligosaccharide-enriched fraction of sialyloligosaccharide was found to significantly inhibit rotavirus infection both *in vitro* and *in vivo*, with the sialic acid moiety of the oligosaccharide playing an important role in the inhibition (232). Sialyloligosaccharides also inhibited *Salmonella* infection not by activating macrophages, but by inhibiting the entry of bacteria through the gut (233).

6. Yolk Lipids

Dry egg yolk contains approximately 60% lipids (234), and of this around 65% is triglyceride, 28% is phospholipid, and 5% is cholesterol (8). The fatty acid composition of the lipid fraction of egg yolk varies, and is influenced by the type of fat in the hen's diet (8). Egg yolk lipids have found numerous applications in the food, cosmetic, pharmaceutical and nutraceutical industries.

Egg yolk lipids are natural surfactants, and have been applied in the food industry as emulsifying, wetting, and dispersing agents, releasing agents, sealants and lubricants (234). Food products containing unsaturated fatty acids are often susceptible to oxidation. To prevent this antioxidants are commonly added; however the safety of synthetic antioxidants is often questioned (220, 235). Yolk lipids have been shown to possess antioxidant activity in fish oil, vegetable oil, and animal oil, both alone and in conjunction with primary antioxidants (234–236). Egg yolk lipids have also been used in the cosmetic industry, due to their non-toxic nature, as they replenish lipid deficiency in the skin and closely resemble skin lipids (234).

Rabinowich et al. (237) noted that the administration to elderly individuals of an egg yolk-derived lipid mixture, formulated for *in vivo* rectification of rigidified cell membranes in an attempt to restore proper physiological function, resulted in an increase in lymphocyte responsiveness, and suggested that immune functions may be modulated by the dietary manipulation of lipids.

A well established parenteral fat emulsion derived from egg yolk lipids is often used as a carrier of fat soluble drugs (220).

Cholesterol is an important component in cell membranes and is needed for the growth of infants. As well, it is a precursor of bile acids, sex hormones and cortex hormones (234). The supplementation of infant formulas with egg yolk lipids has been suggested to more closely resemble the mother's milk; it has been found that while providing essential nutrients, the yolk lipids did not result in an increase in plasma cholesterol, indicating that it could safely be included in the infant diet (238). Egg yolk cholesterol has also been suggested for use in the treatment of Smith-Lemli-Opitz syndrome, a condition in which the activity of 7-dehydrocholesterol delta(7)-reductase, the final enzyme in cholesterol biosynthesis, is reduced resulting in reduced plasma and tissue concentrations and an accumulation of cholesterol precursors. Patients receiving supplemental dietary cholesterol from egg yolk exhibited a significant increase in plasma cholesterol levels, and a decrease in plasma levels of cholesterol precursors which may be toxic (239).

7. Phospholipids

Phospholipids (PLs) are lipids which contain phosphate and have a glycerol-phosphate backbone (234). About 31% of the egg yolk lipids are PLs (234), of which phosphatidylcholine (PC) makes up around 73% of the total PLs, followed by phosphatidylethanolamine (PE 15%), lysophosphatidylcholine (LPC 5.8%), and sphingomyelin (SM 2.5%). The remaining 3.7% is made up of lysophosphatidylethanolamine (LPE 2.1%), plasmalogen (0.9%) and inositol phosholipid (IP) (240).

Phospholipids are amphiphilic molecules, having both polar and non-polar groups, therefore most PLs will spontaneously form a bilayer when exposed to an aqueous environment (234), making them ideal for studying membranes and preparing liposomes (aqueous compartments enclosed by a lipid bilayer), the latter of which the main constituent is PC (220). Phospholipids from soy beans are typically used to prepare liposomes, but egg yolk PLs present several advantages, including high entrapment efficiency, stability, and low cost (241). Egg PC-containing liposomes have been shown to be effective for drug delivery to tumors (242–254), and for drug delivery to the brain (246), reducing the toxicity and side effects, as well as prolonging the concentration compared to the free drug by itself.

Feeding infants with formula containing egg PLs was found to reduce the incidence of necrotizing enterocolitis, suggesting that one or more of the compounds of egg PLs may enhance the immature intestinal functions of infants (247).

Egg yolk PLs were also shown to decrease serum cholesterol levels in rats (248), with PC in particular reducing the intestinal absorption of cholesterol (249). As well, PLs have been found to improve memory retention and increase concentrations of acetylcholine, a neurotransmitter which decreases in concentration in cases of Alzheimer's disease (250, 251). A diet including egg PC was also found to enhance "maze-learning" ability and brain functions in old mice (252).

It has been suggested that egg PLs may exert a degree of antiviral activity by interfering with the viral envelope formation, and by protecting or restoring transmembrane

signaling functions in host cells (253). Furthermore, bacterial or viral infections may also be reduced by an activation of immune cells by egg PLs, as immune cells grown *in vitro* require exogenous fatty acids, which could be provided by egg PLs (253).

The more recent focus has been on the specific PL components namely arachidonic acid (AA), docosahexaenoic acid (DHA), and choline, whose metabolic products may play a role in membrane integrity, modulation of the membrane, and activation of immune cells (253). Egg yolk PC is a significant source of choline, which is an important nutrient in brain development, liver function, and cancer prevention (254). DHA and AA are important in the maintenance of normal neural functions, and have been shown to promote effects that are hypolipidemic, antithrombic, vasodilatory, and anti-inflammatory (255). DHA and AA will be discussed in more detail later.

IV. MODIFICATION OF YOLK LIPIDS FOR HEALTH BENEFITS

It has already been demonstrated that eggs possess a number of compounds beneficial for human health. However, the laying hens' diet can be manipulated to modify the resulting egg yolk lipids, and further enhance the nutritional benefits of the eggs.

Considerable focus has been placed on the enrichment of eggs with long chain omega (ω-3 polyunsaturated fatty acids (PUFAs), the ω referring to the position of the first double bond counted from the terminal methyl carbon of the fatty acid (255). The health benefits of ω-3 fatty acids are significant, suggested for the prevention and treatment of hypertension, arthritis, autoimmune disorders, as well as inhibiting certain cancers, and being essential for fetal brain and visual development (255).

It is widely recognized that the Western diet is deficient in ω-3 PUFAs, which include linolenic acid (LNA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), and which can generally be obtained through the consumption of fish (256). Consequently, the ratio of ω-6 fatty acids, which include linoleic acid (LA) and arachidonic acid (AA) and come from sources such as vegetable oils, to ω-3 fatty acids is approximately 20–30:1, rather than the desired ratio of 1–2:1 (257).

Researchers have been actively pursuing the design of a commercially viable ω-3 fatty acid-rich egg for several years (258). A number of dietary supplements have been shown to increase the ω-3 PUFA levels in hens' eggs, each producing a different ω-3 fatty acid distribution. These include flax seed, canola seed, fish oils, marine algae, linseed oil, vegetable oils and oil mixtures (256, 259, 260). This research has resulted in the production of so called "designer eggs," capable of supplying around 600 mg of total ω-3 fatty acids, which is approximately equivalent to a 100 g serving of fish (261). The feeding of conjugated linoleic acid (CLA) to hens' has also been suggested as a method to manipulate the fatty acid profile of egg yolk (262, 263).

Because ω-3 PUFAs are highly susceptible to peroxidation, the addition of vitamin E to hens' diets, along with the ω-3 PUFAs, has been examined, to control the production of cytotoxic aldehydic lipid peroxidation products during production and storage of the eggs (264). Cherian et al. (265) also described the addition of palm oil to hens' diet to increase the levels of antioxidants (tocopherol, tocotrienol, carotene, and retinol) in the egg yolk.

Lewis et al. (260) found that feeding individuals four ω-3 PUFA-enriched eggs a day for four weeks did not significantly increase plasma cholesterol and low-density lipoprotein (LDL) levels. Rather, plasma triglycerides and blood platelet aggregation decreased, and it was suggested that the inclusion of ω-3 PUFAs may influence LDL particle size, resulting in a less artherogenic particle.

The health benefits of ω-3 PUFAs have been well documented. Dietary ω-3 PUFAs have been shown to exert antihypertensive effects and decrease blood pressure in hypertensive rats, as well as altering the fatty acid composition of plasma triacylglycerols, cholesterol esters and red blood cell total lipids (266–268). DHA has demonstrated anti-tumor activity against various cancers, inhibiting the growth of and inducing apoptosis in Caco-2 colon cancer cells (269, 270), inhibiting carcinogenesis and reducing tumor size and number in a multi-organ carcinogenesis model in rats (271), as well as increasing the toxicity of anti-tumor drugs in tumor cells. The results indicate that DHA may have the potential to selectively target anti-tumor drugs to the tumor cells while at the same time reducing toxicity towards host cells (272). It has also been suggested that ω-3 PUFAs possess immunomodulatory activity, and their consumption may be useful for the management of inflammatory and autoimmune diseases (273, 274). Finally, ω-3 PUFAs, and DHA in particular, have demonstrated a key role in infant health, increasing visual acuity and cognitive development (275, 276), and improving PUFA profile in infants with cholestasis (277).

Therefore, ω-3 PUFA-enriched eggs may help to increase the intake of ω-3 fatty acids in the general population, leading to a restoration of the ω-6 to ω-3 fatty acid ratio, and resulting in increased health benefits of egg consumption.

V. SUMMARY

Egg is the largest biological cell known which originates from one cell division and is composed of various important chemical substances that form the basis of life. Therefore, the avian egg is considered to be a storehouse of nutrients

such as proteins, lipids, enzymes and various biologically active substances including growth promoting factors as well as defence factors against bacterial and viral invasion. In the last decade, numerous extensive studies characterizing biophysiological functions of egg components and seeking novel biologically active substances in the hen eggs have been conducted. By compiling a review of these studies into a single focussed work, this review provides evidence that hen eggs contain various biologically active substances with specific benefits for human health and would be ideal sources for medical, cosmetic, nutraceutical and food-fortification applications. Our base of knowledge is dynamic and rapidly expanding with new information continually appearing.

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91 Enzymes as Functional Ingredients

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CONTENTS

I. INTRODUCTION

This chapter will attempt to cover the importance of enzymes in the biotransformation of a number of food products. The existence of enzymes has been known for well over a century. In 1835 the Swedish chemist Jon Jakob Berzelius found the catalytic action of enzyme. The first enzyme obtained in pure form was in 1926, by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work earned him the 1947 Nobel Prize (1).

Although a reaction may have a large negative Free Energy ($-\Delta G$) value, it does not necessarily proceed at a measurable velocity. For example the oxidation of glucose to CO_2 and H_2O has a ΔG value of -163 kJ/mole. Yet, crystalline glucose or a sterile solution of glucose does not spontaneously oxidize in the presence of air. The rate of a reaction depends on the number of molecules that attain a certain minimum "activation energy." The activation energy is a barrier that must be overcome for the reaction to take place spontaneously.

The activation energy for a chemical reaction could be supplied by adding heat to the system to raise the temperature. However, living biological systems can only exist within narrow temperature limits and have no mechanism to specifically add heat to certain reactions and not to others. Enzymes are catalysts that increase the rate of specific chemical reactions in biological systems by lowering the activation energy required. All known enzymes are proteins. They have molecular weights ranging from 10,000 to 2,000,000, but most fall in the range 15,000 to 60,000. Many enzymes require the presence of other compounds called cofactors before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the holoenzyme (1).

$$\text{Apoenzyme} + \text{Cofactor} = \text{Holoenzyme}$$

The cofactor could be:

1. A coenzyme — a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part.
2. A prosthetic group — an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.
3. A metal-ion-activator — these include K^+, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++}, and Mo^{+++}.

Specificity of enzymes makes them important as diagnostic and research tools. A few enzymes exhibit absolute specificity. Other enzymes are specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

- Absolute specificity — the enzyme will catalyze only one reaction.
- Group specificity — the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- Linkage specificity — the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- Stereochemical specificity — the enzyme will act on a particular steric or optical isomer.

Though enzymes exhibit great degrees of specificity, cofactors serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor, e.g. alcohol dehydrogenase, malate dehydrogenase, and lactate dehydrogenase reactions. Enzymes are used in the food industry as processing aids or to improve or maintain the quality of various food products. Use of enzymes further provide the possibilities for novel product and process developments.

The world market for sale of industrial enzymes has more than doubled in the last six years and is projected to more than triple in 2005 what it was in 1992 (Table 91.1).

II. ENZYME CLASSIFICATION

There are six classes of enzymes and they are denoted by an EC number according to the Enzyme Commission. The

Table 91.1
Industrial Enzymes: Worldwide Market Forecast, 1997–2002 ($Million)

Market Sector	1997	1998	2002	% Average Annual Growth Rate 1997–2002
Food and animal feed	705.0	729.7	833.1	3.5
Detergents/cleaners	475.2	498.0	600.9	4.8
Textiles, leather, and fur	161.0	164.2	182.7	2.0
Pulp and paper	97.6	104.3	136.0	6.9
Chemicals manufacture	59.2	60.8	67.6	2.7
Total	1,498.0	1,557.0	1,820.3	4.0

Source: Business Communications Company, Inc. (May 11, 1998)

first digit is the class (general reaction type — see below). The second digit is the bond involved. The third digit defines the bond more closely and a fourth, the number of enzymes identified to date performing the function (over 90 in the case of alpha-amylases).

Six classes of enzymes published in *Enzyme Nomenclature* 1992 (2) are shown below:

1. Oxidoreductases (EC 1…) — Catalyse oxidation–reduction reactions. Act on a number of groups, each group has a number. For example: CH-OH is 1.1; Aldehyde is 1.2; peroxide is 1.11; superoxide radical is 1.15, etc. Examples: dehydrogenases, oxidases, hydroxylases.
2. Transferase (EC 2…) — Catalyse functional group transfers. Transfer the following groups. For e.g. one carbon is 2.1; aldehydes or ketones 2.2; acyl 2.3; glycosyl 2.4; etc. Examples: kinases, aminotransferases.
3. Hydrolases (EC 3…) — Catalyse hydrolysis reactions. Hydrolyse the following bonds. For e.g. ester 3.1; glycosidic 3.2; ether 3.3; peptide 3.4 etc. Examples: trypsin, carboxypeptidase, amylase, maltase.
4. Lyases (EC 4…) — Catalyse elimination or addition of groups to form double bonds. Lyse the following bonds. For example, C-C 4.1; C-O 4.2; C-N 4.3; C-S 4.4; C-halide 4.5; P-O 4.6; other 4.99. Examples: synthases, decarboxylases, fumarase, aldolase.
5. Isomerases (EC 5...) — Catalyse reactions that alter the structure but not the composition (optical, geometric or structural isomers). Isomerise a given molecule. For example, racemases and epimerases EC 5.1; cis-transe isomersase EC 5.2; intra oxidoreductase EC 5.3; etc. Example: gluose-6-phosphate isomerase.
6. Ligases (EC 6…) — Catalyse coupling of two compounds along with hydrolysis of a phosphoanhydried bond. Act on compounds forming the following bonds. For eg. C-O 6.1; C-S 6.2; C-N 6.3; C-C 6.4; P-ester 6.5. Example: synthetases, DNA Ligase.

III. PRODUCTION OF ENZYMES

Enzymes were originally extracted in very impure forms from animal and plant sources. Today, however, industrial production is usually via a bacterial, fungal, or yeast fermentation. Such fermentations are usually carried out using well tried and tested species — notably the fungi *Aspergillus niger*, *A. oryzae*; bacteria *Bacillus subtilis*, *Streptomyces griseus*; or yeasts *Saccharomyces cerevisiae*, *Kluyveromyces fragilis* (3). These species have the advantage that regulatory authorities worldwide recognize them as safe.

A major problem with many enzymes is that the output from fermentation is not a single pure enzyme of well defined concentration. Much work has been concentrated on achieving reproducible products. There are a number of companies in the western world producing enzymes commercially, of which 10–12 are major players.

A. Applications

Classic enzyme modulated reactions in food processing include:

- Starch hydrolysis using amylases (brewing, ethanol and glucose production, baking)
- Other carbohydrases such as beta-glucanase and pectinase in wine processing, beer, and juice clarification
- Proteinases both have a role in viscosity reduction (endoproteases) and Maillard reaction flavouring (exoproteases)
- Lipases and proteases are critical to the development of cheese flavours — especially enzyme modified cheese

B. Legislation

Enzymes in the EU are almost always treated as processing aids, rather than components of the food matrix. In these circumstances, they do not currently need to be declared on a label.

In recent years, the issue of genetically modified (GM) foods has caused concern to food manufacturers. Without considering the scientific logic of the issue, the current legislative climate in the EU requires that labeling identifies the possibility that GM ingredients are, or may be, present. In this context, it is likely that the processing aid exception may be reviewed and this could have a significant effect in slowing the development of novel and more efficient enzyme systems.

IV. USE OF ENZYMES IN FOODS

A. Use of Enzymes in Juice Manufacture

Plant tissue contains vacuoles, which contain all the water-soluble components and their precursors such as sugars, acids, salts etc. The vacuole is enclosed in a semi-permeable membrane system consisting of lipoproteins that allows transport of only the water molecules.

This is how osmotic pressure is created — presses the membranes against the cytoplasm and cell wall — gives turgor pressure, which keeps the fruits and vegetables firm and fresh.

The cell walls are also permeable. They are rigid structures consisting of pectin, cellulose, and hemicellulose. Pectin is the main constituent of middle lamella, which glues the cells together (Figure 91.1).

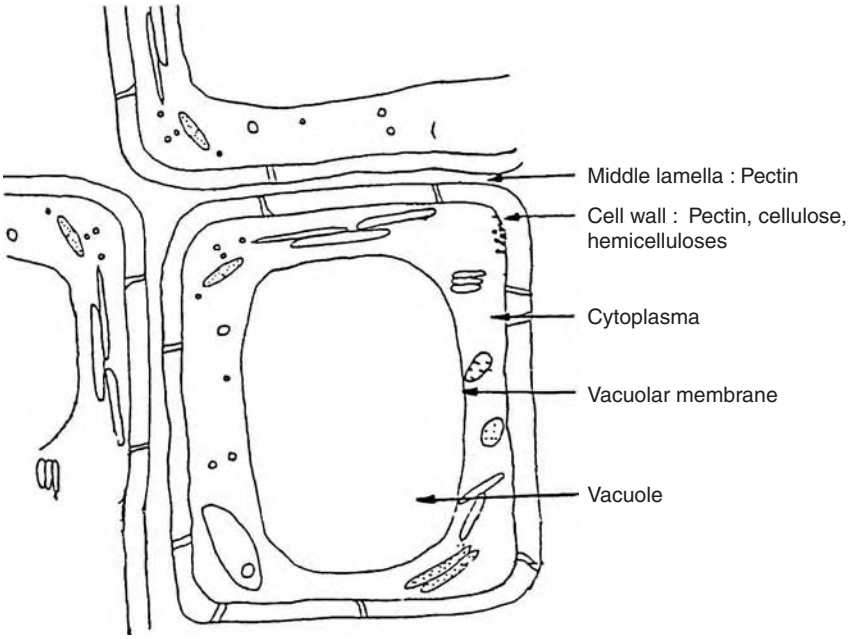

Figure 91.1 Schematic diagram of a parenchymal cell.

Enzymes are located in mitochondria found in the cytoplasm. Juice is really the cell sap, which can only be obtained by destroying the membrane. This can be done by mechanical cell disruption, or exposing the vacuoles when the restraining cell walls are enzymatically removed, or by denaturing the membrane in diffusion processes with hot water or alcohol.

1. Pressing

The traditional way of making fruit juice is by pressing. It is essentially a process of cell disruption with mechanical separation. Different techniques are used for clear and cloudy juices. Cell disruption means the compartmentalization of the tissue is destroyed. As a consequence many chemical, biochemical, and physical changes may occur.

Pectin is important in juicing, because a fraction of the pectin is found in the soluble form in the cell wall. It becomes dissolved in the liquid phase during grinding and pressing. The pectin in the juice can cause the following changes:

1. Increase in viscosity
2. Stabilize the cloud particles
3. Cause gelling of concentrates
4. Cause formation of flocks during storage

Clarification treatments therefore include enzymatic degradation of pectin using commercial fungal enzymes with strong pectolytic activity. A schematic diagram of a pectin molecule and the points of attack of the pectolytic activities (4, 5) are illustrated in Figure 91.2. Pectin is a chain of 1-4 linked galacturonic acids, part of which is esterified with methanol.

Pectin Lyases depolymerise highly esterified pectin by splitting glycosidic linkages next to methylesterified carboxyl groups through a β-elimination process. Another depolymerisation pathway is by the combination of pectin esterase (PE), also known as Pectin methyl esterase (PME) and polygalacturonase (PG).

PE splits off methanol from highly esterified pectin, transforming it into low ester pectin, which is hydrolyzed by PG attacking glycosidic linkages next to a free carboxyl group. PE and PG are also found as endogenous enzymes.

Pectate Lyase (PAL) also attacks glycosidic links next to a free carboxyl group. Thus PE also prepares the substrate for this enzyme.

PAL is a bacterial enzyme and is not found in fruits and vegetables or enzyme extracts from fungal preparations. It has a high pH optimum and is unsuitable for fruit processing.

2. Clarification of Juice

For clarification of juice, only the pectolytic activities are necessary. Raw pressed juice is a viscous liquid with a persistent cloud of cell wall fragments and complexes of such fragments with cytoplasmic protein.

Addition of pectinase lowers the viscosity and causes the cloud particles to aggregate and sediment, and can be easily centrifuged off (see Figure 91.3). This mechanism was first proposed by Yamasaki in 1964 (6) and elucidated in another paper by him and his colleagues in 1967 (7).

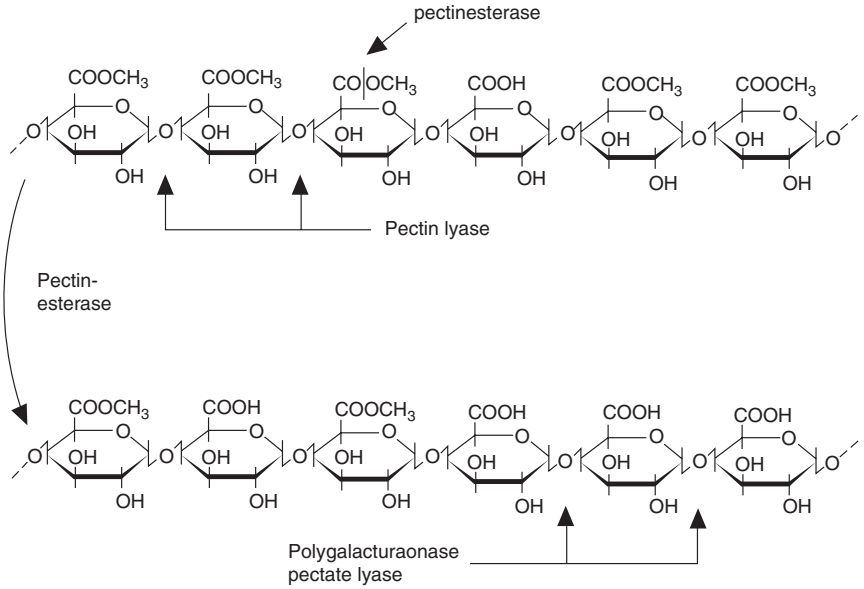

Figure 91.2 Schematic diagram of a pectin molecule and the points of attack of the different pectinase enzymes.

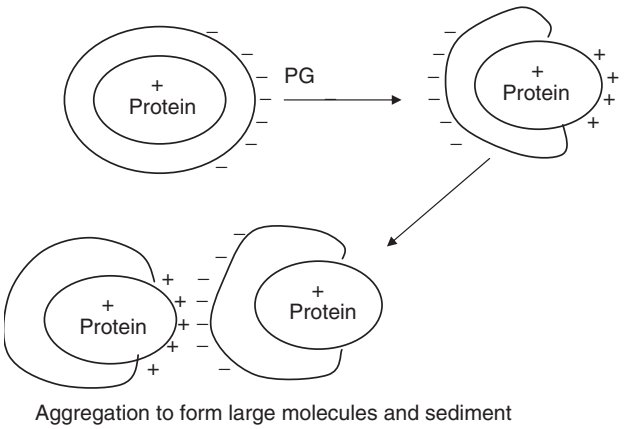

Figure 91.3 Schematic diagram of the principle of juice clarification.

The cloud particles have a protein nucleus with a positive surface charge, coated by negatively charged pectin molecules. Partial degradation of these pectin molecules by PG enzymes results in the aggregation of oppositely charged particles.

The reduction in viscosity of raw juice is also brought about by the action of pectinase enzymes (PG and PE). This same effect is also brought about by pectin lyase (PL), which is found in small amounts in commercial enzyme preparations. Pectin degradation is also important in the manufacture of high Brix concentrates to avoid gelling and the development of haze.

Clarification also includes starch degradation by amylase in cases where starch is present and has had the chance to gelatinize. Haze formation occurs in apple and pear juices that have been sparkling clear, after concentration and storage. Analysis of the haze has shown that most of it due to arabinans with α–(1-5) main chain and α–(1-3) side chain. These can be hydrolyzed by a combination of exo- and endo-arabinases (4).

3. Pulp Enzyming

Certain fruits do not press well after storage or if they are over-ripe. e.g. apples, plums, nectarines, etc. develop a mealy texture — does not give rise to good juice extraction. This is due to large fractions of pectin that have been solubilised, so that the viscous juice adheres to the pulp. Use of pressing aids such as cellulose fiber or rice hulls can improve this situation.

Pectolytic enzymes can also be used to for juicing of soft fruits. Breakdown of pectin releases thin free-flow juice, so that at high pressures a thin juice can be extracted. Prevention of the inactivation of added enzymes by polyphenols is an important aspect of the process.

In the case of apples, endogenous Polyphenol oxidase (PPO) is encouraged to oxidize the phenols by aeration and polymerize. The polymerized phenols are unable to combine with the added enzymes. An alternative is to add PVPP to bind the phenols.

Enzyme preparations that work well with juice clarification are also suitable for enzyme treatment of pulp. In the case of apples, any combination of enzymes that de-polymerize highly esterified pectin can be successfully used (8).

A better release of anthocyanins of fruits into the juice is also achieved by cell wall destruction by pectinase enzymes. This is also a distinct advantage in red-wine making.

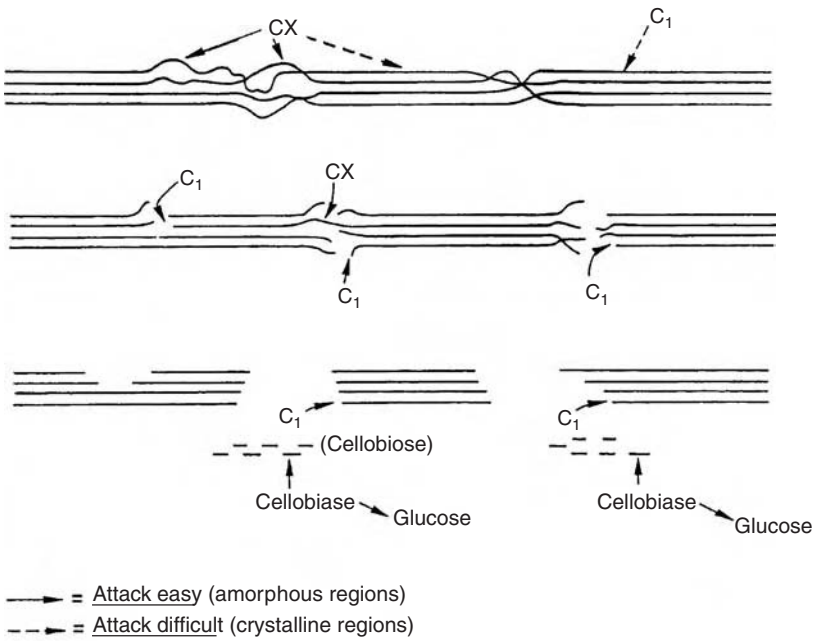

Figure 91.4 Schematic diagram of the points of attack of the endo- and exocellulose enzyme on the cellulose molecule.

4. Liquefaction

Commercial preparations of cellulase obtained from *Trichoderma* species became available in the 1970s. Degradation of crystalline cellulose requires a particular set of enzymes. With respect to hydrolysis, cellulases can be divided into endo- and exo-acting enzymes. The presence of exo-β-glucanase (C1 cellulase or cellibiohydrolase) is typical of cellulase preparations which are able to degrade highly ordered cellulose to form cellobiose (4).

Degradation of crystalline cellulose is believed to occur at the surface of the cellulose fibril by endo-β-glucanase, followed by exo-β-glucanase. Cellibiose, a competitive inhibitor of cellulases, is hydrolyzed by cellobiase to glucose.

A combination of cellulase and pectinase act synergistically, to decrease the viscosity dramatically (9). The low viscosity values reached corresponds to complete liquefaction shown by the disappearance of cell walls under a microscope. Enzyme liquefied papaya and cucumber are almost clear, apples and peaches are cloudy, carrots are pulpy. This depends on the accessibility of the cell compounds to the enzymes.

The exo cellulase (C1 cellulase) works in cooperation with endo-β-glucanase (CX-cellulase) and a β-glucosidase also known as cellobiase (Figure 91.4). Cellobiose, the disaccharide obtained by partial hydrolysis of cellulose, consists of two D-glucopyranoses joined by a 1,4′,-beta-glycoside bond (Figure 91.5). Both maltose and cellobiose are reducing sugars because the anomeric carbons on the right-hand sugar are part of a hemiacetal.

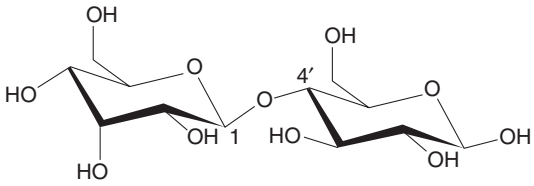

Figure 91.5 Cellobiose, a 1,4′-beta-glycoside [4-O-(beta-D-Glucopyranosyl)-beta-Dglucopyranose].

Liquefaction products can be clarified further by the usual techniques. The action of enzymes in these products can be followed by comparing the amount of galacturonic acid and neutral sugars found in alcohol insoluble solids (AIS) compared to those of the untreated (control) products. It has been found that the pectinase activity alone releases about 75% of the pectic material. Cellulose alone had very little activity on pectin and solubilized only 22% cellulose. No glucose was released from cellulose. Combined pectinase and cellulase released 80% of the polysaccharides. This is the synergistic effect noticed in pulp viscosity experiments. The breakdown products increase the soluble solids content in the juices. Thus high yields of juice and solids are obtained.

Liquefaction enzymes are important in the manufacture of fruit and vegetable juices, which yield no juices on pressing. For example mango, guava, banana, durian, etc. No presses have been developed for extracting juices from such products. Therefore there is good potential for using enzyme liquefaction technique to manufacture juices from such tropical fruits.

One has to be carefully with the choice of selecting commercial enzymes used for enzyme liquefaction. Most commercial enzymes have a number of side activities other than the major activities. Thus some of the fruity esters may be hydrolyzed and the finished product may give rise to bland/reduced flavor intensities (4, 9).

Enzyme liquefied juices may have an increase in titratable acidity and may have an enhanced the acid taste. Free methanol from Pectinesterase is also present from about 60–450 mg/L of the juice. The presence of these high levels of MeOH is of concern to consumers as MeOH is regarded as a poison.

The lethal dose is highly variable, ranging from less than 10 mL to more than 500 mL of MeOH, which is well within the amounts found in highly enzyme treated products.

- Although methanol itself is relatively innocuous, it is metabolized to the toxic byproducts formaldehyde and formic acid, which are responsible for its ocular and CNS toxicity.
- Because of the relatively slow conversion of methanol to its toxic metabolites, there is frequently a delay between the time of ingestion and the development of toxic signs and symptoms. This delay typically ranges from 12–24 hours.
- Toxic signs and symptoms include:
  - Ocular: blurred vision, scintillations, decreased visual acuity, scotomata, unreactive pupils, papilledema, partial or complete blindness. Blindness may be permanent in survivors.
  - CNS: drunkenness, stupor, coma, seizures, meningeal signs, cerebral edema, basal ganglia infarction.
  - GI: nausea, vomiting, epigastric pain, gastritis, GI hemorrhage, pancreatitis.
  - Respiratory: faint odor of methanol or formaldehyde on breath, respiratory failure.
- Severe degrees of poisoning may be associated with profound metabolic acidosis, circulatory shock, and death.

In the modern fruit juice industry, volatiles are stripped off from the juice and are traded separately. Therefore it is possible to control the MeOH content in the finished product. Enzyme treated juices can undergo more rapid browning reactions compared to the traditional juices, due to the presence of reducing sugars.

Enzymatically extracted apple juice is yellowish in color because of the yellow flavanol glucosides (quercitin).

The comparison of volatiles of apple juice obtained by pressing and by liquefaction. One notable fact is the reduction in esters, reduction of aldehydes to alcohols and disproportionately high increase of C6 aldehydes in the enzyme treated juices. The high C6 aldehyde content is explained by the increase in endogenous lipoxygenase activity which results in increase in C6 aldehyde. Hence precautions should be taken to minimize these changes.

5. Cloudy Juices

Cloudy juices are becoming popular in the market place. It is most important to pasteurize the freshly pressed juice immediately to inactivate endogenous enzymes. The pasteurized juice should be passed through screens or centrifuged to remove large particles, which would sediment on standing. Short enzyming of pulp is also described as a factor which would improve the cloudiness and cloud stability (4, 8).

B. Enzyme Applications in Other Products of Plant Origin

1. Vanillin from Vanilla Beans

When the Spanish conquistadores were in Mexico in 1520, one of their officers observed that the emperor Montezuma was drinking a beverage consisting of powdered cocoa bean and ground corn, flavored with ground black vanilla pods and honey. This was the first introduction of vanilla and chocolate to the world.

Today vanilla is one of the most expensive natural flavors in the food, pharmaceutical, and perfumery industry. World production of vanilla beans is in excess of 1800 tons (worth nearly $1.5 billion).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a crystalline phenolic aldehyde and is the most abundant aromatic compound in vanilla bean. However, in its natural state it is found as a glucoside, glucovanillin, which is not aromatic. During the drying and curing process the natural beta glucosidase found in the bean slowly converts the glucovanillin to vanillin and the aroma is developed (10, 11). The conversion of glucovanillin to vanillin by Beta-glucosidase is shown in Figure 91.6.

2. Removal of Cyanogens from Cassava and Other Foods

A number of food products are known to contain cyanogenic glucosides which may give rise to cyanide toxicity after ingestion due to enzymatic reactions in the stomach. Table 91.2 lists some of the most prominent cyanogenic glucosides found in common foods. Cassava is a well known example of a staple food containing cyanogenic glucosides. Lactic fermentation is a common processing technique used for cassava in Asia and Africa

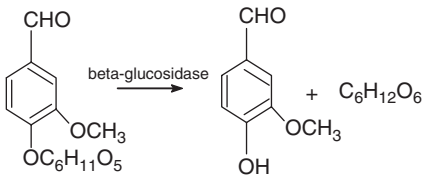

Figure 91.6 Conversion of gluco-vanillin to vanillin.

Table 91.2
Food Sources of Cyanogenic Glycosides and Amount of hydrogen cyanide (HCN) Produced

Plant	mg/100g HCN	Glucoside
Bitter almond	250	Amygdalin
Cassava root	53	Linamarin
Whole sorghum	250	Dhurrin
Lima beans	10–312	Linamarin

to eliminate these toxic factors. Fermentation is carried out on the grated roots by soaking the roots. Knowledge of the mechanisms of cyanogen reduction during lactic fermentation is important to optimize the reaction.

Microorganisms play little or no role in cyanogen reduction during the fermentation of grated cassava. Although a high proportion of the microorganisms present have the ability to hydrolyze linamarin, 90% of the initial linamarin content is hydrolyzed within 3 hours of grating (12). This demonstrates that grating is the key step in cyanogenic glucoside hydrolysis bringing endogenous linamarase into contact with linamarin.

In soaked roots, microbial growth is essential for efficient cyanogen reduction. Although there is some reduction in cyanogens in the absence of microbial growth, efficient cyanogen reduction only occurs when microbial growth takes place and the roots soften. The mechanisms of cyanogen reduction are more complex for fermented roots than for grated roots, but leaching of cyanogens from the softened roots plays a significant role. It has been demonstrated that after root softening, approximately one third of the initial linamarin of the roots was present in the soaking water. The enzymatic reactions involved in the reduction of cyanogens from linamarin is shown in Figure 91.7.

Because of the above reaction involving beta-glucosidase and hydroxynitrile lyase at relatively low temperatures, cyanide can be removed by simple grinding and drying slowly at low temperature. Slow boiling in water in an open pan is also useful in reducing the linamarin content.

3. Amygdalin

Amygdalin is a natural substance found in a variety of plants. Common sources of amygdalin include seeds of apples, pears, black cherry, almond, cherry, plum, peach, and apricot. Amygdalin is a cause of concern, because once ingested

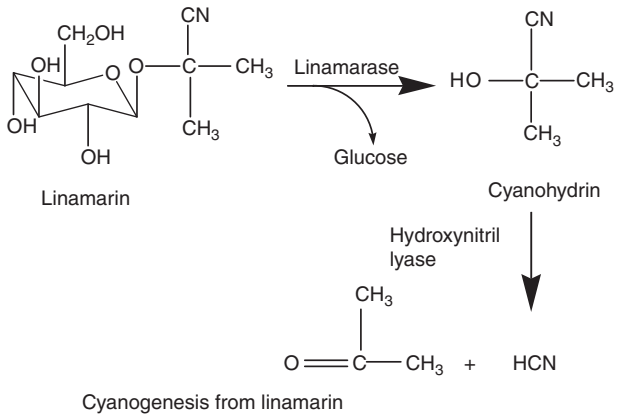

Figure 91.7 Removal of cyanogens from Linamarin in cassava.

it is metabolized to the deadly poison hydrogen cyanide (HCN). Cyanide causes toxic effects by binding to the terminal enzyme in the electron transport chain of the mitochondria, essentially halting ATP generation and oxygen utilization. In small doses, the body can detoxify cyanide. However, if untreated, large doses of cyanide are fatal.

Despite amygdalin's potentially deadly effects, in the 1970s and early 1980s, amygdalin was proposed as an anticancer drug (Laetrile) capable of killing cancerous cells. However, the dangers and ineffectiveness of laetrile were soon uncovered (13). The American Cancer Society has since dubbed Laetrile as a "toxic drug that is not effective as a cancer treatment."

Amygdalin is converted to HCN and benzaldehyde by the reaction shown in Figure 91.8. The above reaction may be used to manufacture the important natural flavour compound benzaldehyde (almond flavour).

4. Removal of Beany Flavour

During soya processing, volatile degradation compounds such as hexanal, pentanal, etc. are produced, which give a "beany" flavour to products, because of the action of lipoxygenase on unsaturated fatty acids such as linoleic and linolenic acids (14). These defects can be overcome by the enzymatic oxidation of the aldehydes to the corresponding carboxalic acids by the use of aldehyde dehydrogenase as follows.

$$\text{n-hexanal} + NAD^+ \xrightarrow{\text{Aldehyde dehydrogenase}} \text{caproic acid} + NADH + H^+ \quad (91.1)$$

5. Removal of Bitter Taste in Grapefruit and Other Citrus Juices

Some citrus juices, especially grapefruit, contain naringin, a dihydro-calcone with a bitter taste. The causes of the bitter taste that leads many shoppers to reject grapefruit juice

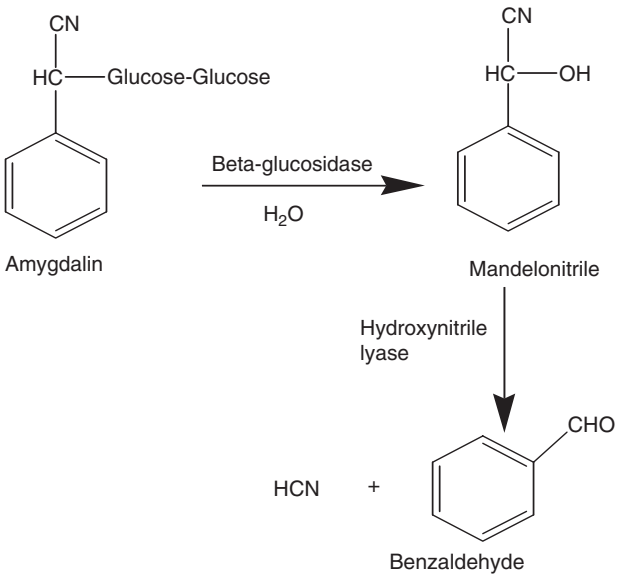

Figure 91.8 Conversion of amygdalin to benzaldehyde.

are two compounds, glycosidic flavanone naringin and triterpenoid lactone limonin (15). Naringin is the bitter component found in most fresh citrus fruit, and therefore, in freshly processed citrus juices. Limonin forms when the fresh fruit juice is pasteurized. Fruit acids give an undesirable bitterness to the packaged juice. Naringin bitterness can be reduced by the application of naringinase (16). Naringinin is non-bitter.

$$\text{Naringin} \longrightarrow \text{naringinin} + \text{rhamnose} + \text{glucose} \qquad (91.2)$$

A thin cellulose–acetate film layer on the inside of the packaging is used in place of the normal polymer lining. This layer of film contains an enzyme naringinase (which contains alpha-rhamnosidase and beta-glucosidase); when the juice comes into contact with the food-safe film, its bitter taste is reduced due to the activity of the enzymes (17).

6. α and β-Amylases

For starch hydrolysis, α and β-amylases are normally used. Bacterial amylases have a high temperature tolerance and hence they are of commercial value. α-amylase added to the wort in the beer manufacture accelerates starch degradation. These enzymes are also used in the baking industry.

7. Starch Saccharification

Acid hydrolysis of starch has had widespread use in the past. It is now largely replaced by enzymatic processes, because the acid hydrolysis requires the use of corrosion resistant materials, gives rise to high colour and ash content (after neutralisation), needs more energy for heating, and is relatively difficult to control.

Of the two components of starch, amylopectin presents the great challenge to hydrolytic enzyme systems. This is due to the residues involved in 1,6-glycosidic branch points which constitute about 4–6% of the glucose present. Most hydrolytic enzymes are specific for 1,4-glucosidic links yet the 1,6-glucosidic links must also be cleaved for complete hydrolysis of amylopectin to glucose (18). Some of the most impressive recent exercises in the development of new enzymes have concerned debranching enzymes.

It is necessary to hydrolyse starch in a wide variety of processes, which may be condensed into two basic classes:

1. Processes in which the starch hydrolysate is to be used by microbes or man.
2. Processes in which it is necessary to eliminate starch.

In the former processes, such as glucose syrup production, starch is usually the major component of reaction mixtures, whereas in the latter processes, such as the processing of sugar cane juice, small amounts of starch, which contaminate non-starchy materials, are removed. Enzymes of various types are used in these processes. Although starches from diverse plants may be utilised, corn is the world's most abundant source and provides most of the substrate used in the preparation of starch hydrolysates.

There are three stages in the conversion of starch (see Figure 91.9):

1. Gelatinisation, involving the dissolution of the starch granules to form a viscous suspension
2. Liquefaction, involving the partial hydrolysis of the starch, with concomitant loss in viscosity
3. Saccharification, involving the production of glucose and maltose by further hydrolysis

Gelatinisation is achieved by heating starch with water, and it occurs necessarily and naturally when starchy foods are cooked. Gelatinised starch is readily liquefied by partial hydrolysis with enzymes or acids and saccharified by further acidic or enzymic hydrolysis.

The starch and glucose syrup industry uses the expression dextrose equivalent, or DE, similar in definition to the DH units of proteolysis, to describe its products, where:

$$DE = 100 \times \left(\frac{\text{Number of glycosidic bonds cleaved}}{\text{Initial number of glycosidic bonds present}} \right) \qquad (91.3)$$

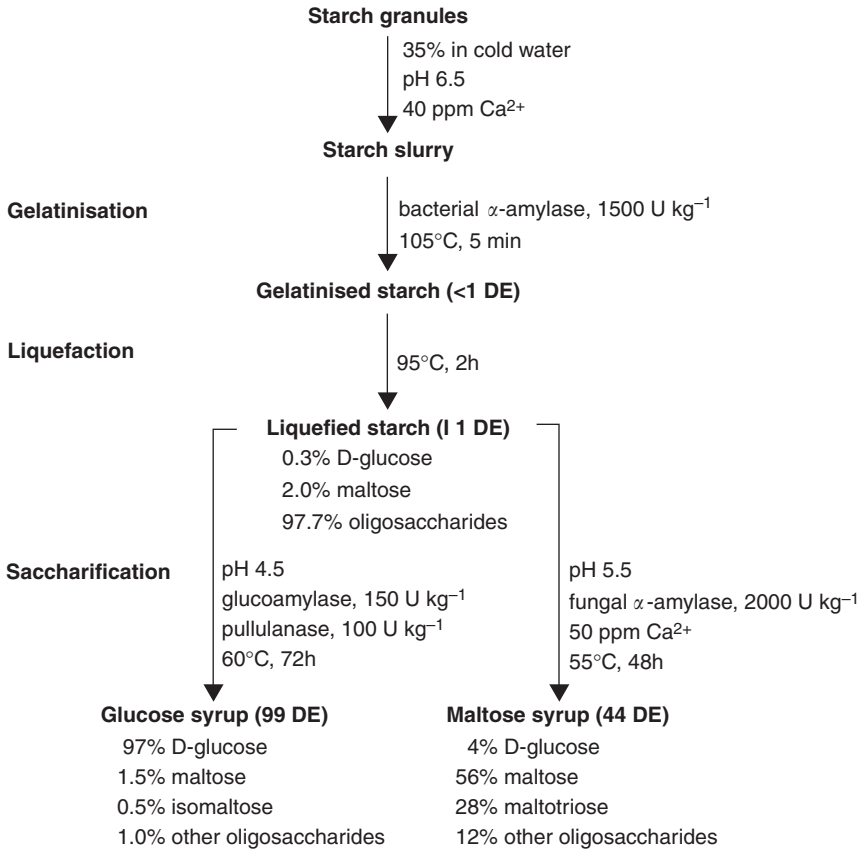

Figure 91.9 Schematic diagram of the use of enzymes in starch saccharification.

In practice, this is usually determined analytically by use of the closely related, but not identical, expression:

$$DE = 100 \times \left(\frac{\text{Reducing sugar, expressed as glucose}}{\text{Total carbohydrate}} \right) \qquad (91.4)$$

Thus, DE represents the percentage hydrolysis of the glycosidic linkages present. Pure glucose has a DE of 100, pure maltose has a DE of about 50 and starch has a DE of effectively zero. During starch hydrolysis, DE indicates the extent to which the starch has been cleaved. Acid hydrolysis of starch has long been used to produce "glucose syrups" and even crystalline glucose (dextrose monohydrate). Very considerable amounts of 42 DE syrups are produced using acid and are used in many applications in confectionery. Further hydrolysis using acid is not satisfactory because of undesirably coloured and flavoured breakdown products. Acid hydrolysis appears to be a totally random process which is not influenced by the presence of α-1,6-glucosidic linkages.

The nomenclature of the enzymes used commercially for starch hydrolysis is somewhat confusing and the EC numbers sometimes lump together enzymes with subtly different activities. For example, α-amylase may be subclassified as liquefying or saccharifying amylases, but even this classification is inadequate to encompass all the enzymes that are used in commercial starch hydrolysis. One reason for the confusion in the nomenclature is the use of the anomeric form of the released reducing group in the product rather than that of the bond being hydrolysed; the products of bacterial and fungal α-amylases are in the α-configuration and the products of β-amylases are in the β-configuration, although all these enzymes cleave between α-1,4-linked glucose residues.

The α-amylases (1,4-α-D-glucan glucanohydrolases) are endohydrolases, which cleave 1,4-α-D-glucosidic bonds and can bypass but cannot hydrolyse 1,6-α-D-glucosidic branch-points (19). Commercial enzymes used for the industrial hydrolysis of starch are produced by *Bacillus amyloliquefaciens* and by *B. licheniformis* (supplied by Novo Enzymes as Termamyl). They differ principally in their tolerance of high temperatures, Termamyl retaining more activity at up to 110°C, in the presence of starch, than the *B. amyloliquefaciens* α-amylase (20). The maximum DE obtainable using bacterial α -amylases is around 40 but prolonged treatment leads to the formation of maltulose (4-α-D-glucopyranosyl-D-fructose), which is resistant to hydrolysis by glucoamylase and

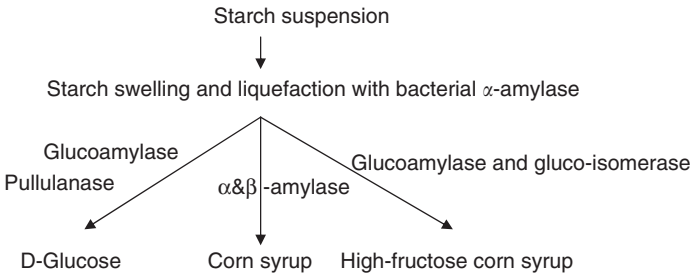

Figure 91.10 Summary of starch conversion to glucose syrup, corn syrup, and high-fructose syrup.

α-amylases. DE values of 8–12 are used in most commercial processes where further saccharification is to occur. The principal requirement for liquefaction to this extent is to reduce the viscosity of the gelatinised starch to ease subsequent processing.

Various manufacturers use different approaches to starch liquefaction using α-amylases, but the principles are the same. Granular starch is slurried at 30–40% (w/w) with cold water, at pH 6.0–6.5, containing 20–80 ppm Ca^{2+} (which stabilises and activates the enzyme) and the enzyme is added (via a metering pump). The α-amylase is usually supplied at high activities so that the enzyme dose is 0.5–0.6 kg tonne^{-1} (about 1500 U kg^{-1} dry matter) of starch. When Termamyl is used, the slurry of starch plus enzyme is pumped continuously through a jet cooker, which is heated to 105°C using live steam. Gelatinisation occurs very rapidly and the enzymic activity, combined with the significant shear forces, begins the hydrolysis. The residence time in the jet cooker is very brief. The partly gelatinised starch is passed into a series of holding tubes maintained at 100–105°C and held for 5 min to complete the gelatinisation process. Hydrolysis to the required DE is completed in holding tanks at 90–100°C for 1 to 2 h. These tanks contain baffles to discourage backmixing. Similar processes may be used with *B. amyloliquefaciens* α-amylase but the maximum temperature of 95°C must not be exceeded. This has the drawback that a final "cooking" stage must be introduced when the required DE has been attained in order to gelatinise the recalcitrant starch grains present in some types of starch which would otherwise cause cloudiness in solutions of the final product.

The liquefied starch is usually saccharified but comparatively small amounts are spray-dried for sale as "maltodextrins" to the food industry mainly for use as bulking agents and in baby food. In this case, residual enzymic activity may be destroyed by lowering the pH towards the end of the heating period.

Fungal α-amylase also finds use in the baking industry. It often needs to be added to bread-making flours to promote adequate gas production and starch modification during fermentation. This has become necessary since the introduction of combine harvesters. They reduce the time between cutting and threshing of the wheat, which previously was sufficient to allow a limited sprouting so increasing the amounts of endogenous enzymes. The fungal enzymes are used rather than those from bacteria as their action is easier to control due to their relative heat lability, denaturing rapidly during baking.

Corn syrup, high-fructose corn syrup, and glucose can be obtained from starch by enzymatic process as shown in Figure 91.10.

8. Interesterification of Fats

There is no opportunity or established need to build up polymers or oligomers using lipases or esterases, yet it is possible and commercially advantageous to use these enzymes as transferases in transesterifications (carboxyl group exchange between esters) (21), acidolyses (carboxyl group exchange between esters and carboxylic acids), and alcoholyses (alcohol exchange between esters and alcohols). Certain triglycerides, cocoa butter being the outstanding example, have high value because of their physical properties and comparative rarity. There is a commercial pull, therefore, to fund routes to the production of high value triglycerides from more plentiful, cheaper raw materials. This may be done using lipases (e.g. from *Rhizopus* or *Mucor Miehei*) acting as transacylases.

This acidolysis reaction may be used for increasing the value of rapeseed oil by exchanging linoleic acid for linolenic acid residues and increasing the value of palm oil and sunflower oil by increasing their content of oleic acid residues.

Cocoa butter fat is a high value product, because TAGs are high in stearates, which give a melting point of 37°C. It is used in confectionery, because of its sharp melting point between room temperature and body temperature; chocolate literally melts in the mouth. This is due to the fairly small variation in the structure of the constituent triglycerides; 80% have palmitic acid or stearic acid in the 1 and 3 positions with oleic acid in the central 2 position. For the production of cocoa butter substitute from palm oil, a process, which increases the value of the product three-fold, the acidolysis utilises stearic acid

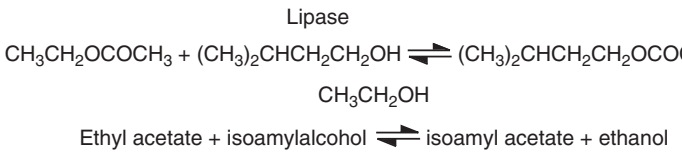

Figure 91.11 Interesterification of ethyl acetate with isoamylalcohol to form isoamyl acetate.

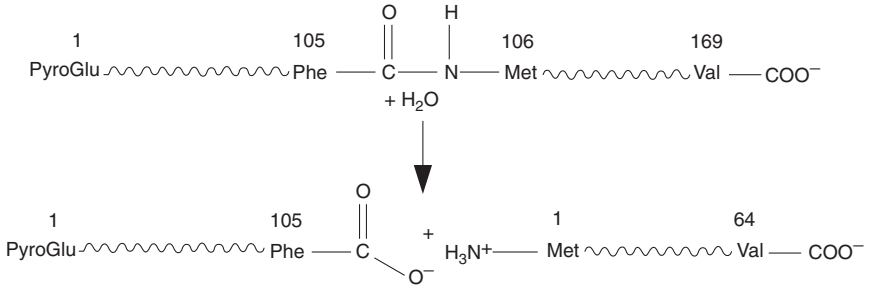

Figure 91.12 Action of chymosin on casein.

(i.e. R_4 is $C_{17}H_{35}$) in hexane containing just sufficient water (~10%) to activate the lipase (22).

Palm oil TAGs are high in palmitate and they give a melting point of 23°C. Therefore, it is an oil at room temperature and is a low value product. Conversion of palm oil into cocoa-butter fat substitute can be achieved by interesterification and is now a commercial process.

The products may be recovered by recrystallisation from aqueous acetone. Such reactions may also be used for the resolution of racemic mixtures of carboxylic acids or alcohols, the lipase generally being specific for only one out of a pair of optical isomers.

The secret of success has been the selection of lipases with the correct specificity and the selection of reaction conditions that favour transacylation rather than hydrolysis. Because the hydrolytic activity of industrial lipases is 10–15 times the transacylation activity, it is advantageous to minimise the water content of the reaction system and use the aqueous/organic biphasic systems described earlier. An example of lipase being used in an alcoholysis reaction is the biphasic production of isoamyl acetate, a natural aroma (Figure 91.11).

C. Use of Proteases and Other Enzymes in the Food Industry

1. Milk

Certain proteases have been used in food processing for centuries and any record of the discovery of their activity has been lost in the mists of time. Rennet (mainly chymosin), obtained from the fourth stomach (abomasum) of unweaned calves has been used traditionally in the production of cheese. Similarly, papain from the leaves and unripe fruit of the pawpaw (Carica papaya) has been used to tenderise meats. These ancient discoveries have led to the development of various food applications for a wide range of available proteases from many sources, usually microbial. Proteases may be used at various pH values, and they may be highly specific in their choice of cleavable peptide links or quite non-specific. Proteolysis generally increases the solubility of proteins at their isoelectric points.

The action of rennet in cheese making is an example of the hydrolysis of a specific peptide linkage, between phenylalanine and methionine residues (-Phe_{105}-Met_{106}-) in the κ-casein protein present in milk (23, 24). The κ-casein acts by stabilising the colloidal nature of the milk, its hydrophobic N-terminal region associating with the lipophilic regions of the otherwise insoluble α- and β-casein molecules, whilst its negatively charged C-terminal region associates with the water and prevents the casein micelles from growing too large. Hydrolysis of the labile peptide linkage between these two domains, resulting in the release of a hydrophilic glycosylated and phosphorylated oligopeptide (caseino macropeptide) and the hydrophobic para-κ-casein, removes this protective effect, allowing coagulation of the milk to form curds, which are then compressed and turned into cheese (Figure 91.12). The coagulation process depends upon the presence of Ca^{2+} and is very temperature dependent ($Q_{10} = 11$) and so can be controlled easily. Calf rennet, consisting of mainly chymosin with a small but variable proportion of pepsin, is a relatively expensive enzyme and various attempts have been made to find cheaper alternatives from microbial sources. These have ultimately proved to be successful and microbial rennets are used for about 70% of US cheese and 33% of cheese production world-wide.

The major problem that had to be overcome in the development of the microbial rennets was temperature lability (25, 26). Chymosin is a relatively unstable enzyme and once it has done its major job, little activity remains. However, the enzyme from *Mucor miehei* retains activity during the maturation stages of cheese-making and produces bitter off-flavours (27). Treatment of the enzyme with oxidising agents (e.g. H_2O_2, peracids), which convert methionine residues to their sulfoxides, reduces its thermostability by about 10°C and renders it more comparable with calf rennet. This is a rare example of enzyme technology being used to destabilise an enzyme. Attempts have been made to clone chymosin into *Escherichia coli* and *Saccharomyces cerevisiae* but, so far, the enzyme has been secreted in an active form only from the latter.

The development of unwanted bitterness in ripening cheese is an example of the role of proteases in flavour production in foodstuffs. It has been found that peptides with terminal acidic amino acid residues give "meaty," appetising flavours akin to that of monosodium glutamate. Non-terminal hydrophobic amino acid residues in medium-sized oligopeptides give bitter flavours, the bitterness being less intense with smaller peptides and disappearing altogether with larger peptides. Application of this knowledge allows the tailoring of the flavour of protein hydrolysates. The presence of proteases during the ripening of cheeses is not totally undesirable and a protease from *Bacillus amyloliquefaciens* may be used to promote flavour production in cheddar cheese. Lipases from *Mucor miehei* or *Aspergillus niger* are sometimes used to give stronger flavours in Italian cheeses by a modest lipolysis, increasing the amount of free butyric acid. They are added to the milk (30 U l^{-1}) before the addition of the rennet.

2. Lactase in Dairy Industry

Lactose is present at concentrations of about 4.7% (w/v) in milk and the whey (supernatant) left after the coagulation stage of cheese-making. Its presence in milk makes it unsuitable for the majority of the world's adult population, particularly in those areas which have traditionally not had a dairy industry. Real lactose tolerance is confined mainly to peoples whose origins lie in Northern Europe or the Indian subcontinent and is due to "lactase persistence;" the young of all mammals clearly are able to digest milk but in most cases this ability reduces after weaning. Of the Thai, Chinese, and Black American populations, 97%, 90%, and 73% respectively, are reported to be lactose intolerant, whereas 84% and 96% of the U.S. White and Swedish populations, respectively, are tolerant. Additionally, and only very rarely, some individuals suffer from inborn metabolic lactose intolerance or lactase deficiency, both of which may be noticed at birth. The need for low-lactose milk is particularly important in food-aid programmes as severe tissue dehydration, diarrhoea, and even death may result from feeding lactose-containing milk to lactose-intolerant children and adults suffering from protein-calorie malnutrition. In all these cases, hydrolysis of the lactose to glucose and galactose would prevent the severe digestive problems.

Another problem presented by lactose is its low solubility resulting in crystal formation at concentrations above 11% (w/v) (4°C). This prevents the use of concentrated whey syrups in many food processes as they have an unpleasant sandy texture and are readily prone to microbiological spoilage. Adding to this problem, the disposal of such waste whey is expensive due to its high biological oxygen demand. These problems may be overcome by hydrolysis of the lactose in whey; the product being about four times as sweet, much more soluble and capable of forming concentrated, microbiologically secure, syrups [70% (w/v)].

Lactose may be hydrolysed by lactase, a β-galactosidase. Commercially, it may be prepared from the dairy yeast *Kluyveromyces fragilis* (*K. marxianus* var. *marxianus*), with a pH optimum (pH 6.5–7.0) suitable for the treatment of milk (28), or from the fungi *Aspergillus oryzae* or *A. niger* (29), with pH optima (pH 4.5–6.0 and 3.0–4.0, respectively) more suited to whey hydrolysis. These enzymes are subject to varying degrees of product inhibition by galactose (30). In addition, at high lactose and galactose concentrations, lactase shows significant transferase ability and produces β-1,6-linked galactosyl oligosaccharides.

Lactases are now used in the production of ice cream and sweetened flavoured and condensed milks. When added to milk or liquid whey (2000 U kg^{-1}) and left for about a day at 5°C about 50% of the lactose is hydrolysed, giving a sweeter product, which will not crystallise if condensed or frozen. This method enables otherwise-wasted whey to replace some or all of the skim milk powder used in traditional ice cream recipes. It also improves the "scoopability" and creaminess of the product. Smaller amounts of lactase may be added to long-life sterilised milk to produce a relatively inexpensive lactose-reduced product (e.g. 20 U kg^{-1}, 20°C, 1 month of storage). Generally, however, lactase usage has not reached its full potential, as present enzymes are relatively expensive and can only be used at low temperatures.

3. Protease in Meat

The action of endogenous proteases in meat after slaughter is complex but "hanging" meat allows flavour to develop, in addition to tenderising it (31). The evidence indicates that the cysteine proteases, in particular the calpains, are responsible for post-mortem proteolysis and tenderization of meat (32).

Meat tenderisation by the endogenous proteases in the muscle after slaughter is a complex process, which varies

with the nutritional, physiological, and even psychological (i.e. frightened or not) state of the animal at the time of slaughter. Meat of older animals remains tough but can be tenderised by injecting inactive papain into the jugular vein of the live animals shortly before slaughter. Injection of the active enzyme would rapidly kill the animal in an unacceptably painful manner, so the inactive oxidised disulfide form of the enzyme is used. On slaughter, the resultant reducing conditions cause free thiols to accumulate in the muscle, activating the papain and so tenderising the meat. This is a very effective process, as only 2–5 ppm of the inactive enzyme needs to be injected. Recently, however, it has found disfavour as it destroys the animal's heart, liver, and kidneys that otherwise could be sold and, being reasonably heat stable, its action is difficult to control and persists into the cooking process.

Proteases are used to recover protein from parts of animals (and fish) would otherwise go to waste after butchering. About 5% of the meat can be removed mechanically from bone. To recover this, bones are mashed incubated at 60°C with neutral or alkaline proteases for up to 4 h. The meat slurry produced is used in canned meats and soups.

When proteases are used to depolymerise proteins, usually non-specifically, the extent of hydrolysis (degree of hydrolysis) is described in DH units where:

$$DH = 100 \times \left(\frac{\text{Number of peptide} - \text{bonds cleaved}}{\text{initial number of peptide bonds present}} \right) \quad (91.5)$$

Commercially, using enzymes such as subtilisin, DH values of up to 30 are produced using protein preparations of 8–12% (w/w). The enzymes are formulated so that the value of the enzyme: substrate ratio used is 2–4% (w/w). At the high pH needed for effective use of subtilisin, protons are released during the proteolysis and must be neutralised:

$$\mathrm{H_2N\text{-}aa\text{-}aa\text{-}aa\text{-}aa\text{-}aa\text{-}COO^-} \xrightarrow{\text{subtilisin (pH 8.5)}} \mathrm{H_2N\text{-}aa\text{-}aa\text{-}aa\text{-}COO^-} + \mathrm{H_2N\text{-}aa\text{-}aa\text{-}COO^-} + \mathrm{H^+} \quad (91.6)$$

where, aa is an amino acid residue.

4. Proteases on Soy Proteins

Correctly applied proteolysis of inexpensive materials such as soya protein can increase the range and value of their usage, as indeed occurs naturally in the production of soy sauce. Partial hydrolysis of soya protein, to around 3.5 DH greatly increases its "whipping expansion," further hydrolysis to around 6 DH improves its emulsifying capacity. If their flavours are correct, soya protein hydrolysates may be added to cured meats. Hydrolysed proteins may develop properties that contribute to the elusive, but valuable, phenomenon of "mouth feel" in soft drinks.

5. Bakery Products

Proteases are also used in the baking industry. Where appropriate, dough may be prepared more quickly if its gluten is partially hydrolysed. A heat-labile fungal protease is used so that it is inactivated early in the subsequent baking. Weak-gluten flour is required for biscuits in order that the dough can be spread thinly and retain decorative impressions. In the past this has been obtained from European domestic wheat but this is being replaced by high-gluten varieties of wheat. The gluten in the flour derived from these must be extensively degraded if such flour is to be used efficiently for making biscuits or for preventing shrinkage of commercial pie pastry away from their aluminium dishes.

D. Glucoseoxidase in Food Industry

Glucose oxidase is a highly specific enzyme for D-glucose, obtained from the fungi *Aspergillus niger* and *Penicillium*, which catalyses the oxidation of β-glucose to glucono-1, 5-lactone, and releasing hydrogen peroxide (Figure 91.13).

It finds uses in the removal of either glucose or oxygen from foodstuffs in order to improve their storage capability. Hydrogen peroxide is an effective bacteriocide and may be removed, after use, by treatment with catalase (derived from the same fungal fermentations as the glucose oxidase) which converts it to water and molecular oxygen:

$$2\mathrm{H_2O_2} \xrightarrow{\text{catalase}} 2\mathrm{H_2O} + \mathrm{O_2} \quad (91.7)$$

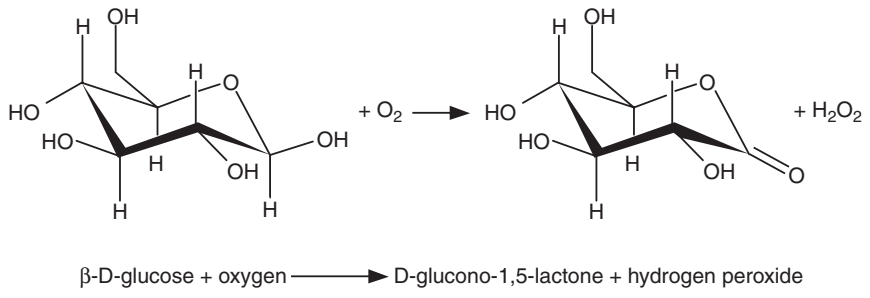

Figure 91.13 Oxidation of glucose to gluconolactone.

For most large-scale applications the two enzymic activities are not separated. Glucose oxidase and catalase may be used together when net hydrogen peroxide production is to be avoided.

A major application of the glucose oxidase/catalase system is in the removal of glucose from egg-white before drying for use in the baking industry. A mixture of the enzymes is used ($165\,U\,kg^{-1}$) together with additional hydrogen peroxide (about 0.1% (w/w) to ensure that sufficient molecular oxygen is available, by catalase action, to oxidise the glucose. Other uses are in the removal of oxygen from the head-space above bottled and canned drinks and reducing non-enzymic browning in wines and mayonnaises.

E. Enzymes in Brewery Industry

In brewing, barley malt supplies the major proportion of the enzyme needed for saccharification prior to fermentation. Often other starch containing material (adjuncts) are used to increase the fermentable sugar and reduce the relative costs of the fermentation. Although malt enzyme may also be used to hydrolyse these adjuncts, for maximum economic return extra enzymes are added to achieve their rapid saccharification. It is not necessary nor desirable to saccharify the starch totally, as non-fermentable dextrins are needed to give the drink "body" and stabilise its foam "head." For this reason the saccharification process is stopped, by boiling the "wort," after about 75% of the starch has been converted into fermentable sugar (33).

The enzymes used in brewing are needed for saccharification of starch (bacterial and fungal α-amylases), breakdown of barley β-1,4- and β-1,3- linked glucan (β-glucanase), and hydrolysis of protein (neutral protease) to increase the fermentation rate later. Cellulases are also occasionally used, particularly where wheat is used as adjunct. Due to the extreme heat stability of the α-amylase from *B. amyloliquefaciens*, where this is used, the wort must be boiled for a much longer period (e.g. 30 min) to inactivate it prior to fermentation. Papain is used in the later post-fermentation stages of beer-making to prevent the occurrence of protein-tannin "chill-haze" otherwise formed on cooling the beer.

Recently, "light" beers, of lower calorific content, have become more popular. These require a higher degree of saccharification at lower starch concentrations to reduce the alcohol and total solids contents of the beer. This may be achieved by the use of glucoamylase and fungal α-amylase during the fermentation.

1. Malting

Malting serves the purpose of converting insoluble starch to soluble starch, reducing complex proteins, generating nutrients for yeast development, and development of enzymes. The three main steps of the malting process are steeping, germination, and kilning (33).

Generally, barley variety (six-row) that has a higher enzyme content, more protein, less starch, and a thicker husk than "two-row" barley is used for malting. Malting barley has higher level of diastatic enzymes (α and β amylases).

Steeping begins by mixing the barley kernels with water to raise the moisture level and activate the metabolic processes of the dormant kernel.

During malting, enzymes break down the cell structure of the endosperm, release nutrients necessary for yeast growth, and make the starch available for enzyme degradation during mashing. Modification of the endosperm correlates with growth of the acrospire. As the acrospire grows, chemical changes are triggered that result in the production of numerous enzymes. Their function is to break down the complex starches and proteins of the grain (33).

2. Biochemical Changes at Mashing

The mashing process is done over a period of time at various temperatures to activate the enzymes responsible for acidulation and reduction in proteins and carbohydrates.

Although there are numerous enzymes present in the mash, only three groups and their respective processes are paramount to biochemical transformations in beer manufacture (33). These enzymes are

1. Phytases (acidifying)
2. Proteolytic enzymes (protein-degrading)
3. Carbohydrase enzymes (starch-degrading)

The acid rest is responsible for reducing the initial mash pH for traditional decoction mashing of lager beers. In recent years, because of the use of well-modified malts, the general trend has been to simplify and shorten the lager mash by eliminating the acid rest in mashing.

The protein rest is responsible for reducing the overall length of high-molecular-weight proteins, which cause foam instability and haze, to low-molecular-weight proteins in the mash. The enzymes proteinase and peptidase are two main enzymes of this group.

By far the most important change brought about in mashing is the conversion of starch molecules into fermentable sugars and unfermentable dextrins. The principal enzymes responsible for starch conversion are alpha and beta-amylase. Alpha-amylase very rapidly reduces insoluble and soluble starch by splitting starch molecules into many shorter chains polysaccharide fractions — dextrins and maltotriose that can be attacked by beta-amylase (33).

Given a long enough "rest," the alpha-amylase can dismantle all the dextrins to maltose, glucose, and small, branched "limit dextrins." However, starch conversion is more effective by the faster-acting beta-amylase.

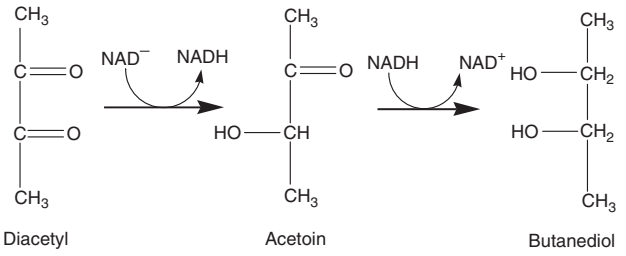

Figure 91.14 Reduction of diacetyl to acetoin and butanediol.

Beta-amylase is more selective than alpha-amylase since it breaks off two sugars at a time from the starch chain.

A number of factors lead to diacetyl formation, but only one reliable method can reduce diacetyl levels: enzymatic reduction by yeast using butanediol dehydrogenase in the presence of NADH (Figure 91.14). Acetoin, the intermediate product, has a rather unpleasant, musty taste, but because it has a flavor threshold of 3.0 mg/L its effect is not nearly as damaging to beer flavors as an equivalent amount of diacetyl. The final product, butanediol, is neutral as far as beer flavor is concerned.

F. Soy Sauce Fermentation

Soy sauce is a very important condiment in most cooking in the Orient as well as in the west. Its great antiquity is illustrated by the fact that it is mentioned in the book of Chau Lai, one of the 13 classics of Confucius, written before 1000 B.C.

The basic process of soy sauce manufacture is a two-stage fermentation process. In the first stage of the fermentation, the soybeans or a mixture of equal parts of beans and wheat are inoculated with *Aspergillus oryzae* (34). The mould grows rapidly on this substrate and after three days the first stage of the fermentation is complete. The mould covered material known as Koji is then broken up and immersed in 18% brine solution. The introduction of pure cultures in soybean fermentations has not only improved process efficiency, but has also improved product quality and consistency. New and better microbial strains introduced into the process include the fungi *A. oryzae* (35) and *A. sojae* for koji production (36).

Because of the activities of the enzymes produced by the moulds, considerable breakdown of proteins and polysaccharides present in the substrate takes place. Thus substantial amounts of fermentable sugars, peptides, and amino acids are released into the brine.

The Koji preparation utilizes principally *Aspergillus oryzae*. This mould releases a variety of enzymes to its surrounding, such as acid, neutral and alkaline proteases, peptidases, amylases, cellulases, lipases, and invertases.

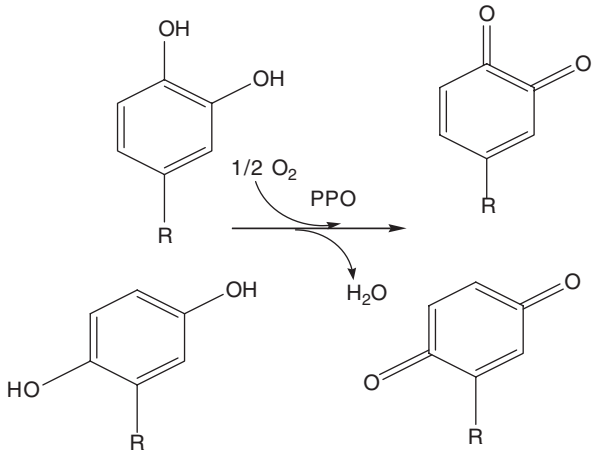

Figure 91.15 The oxidation of phenolic compounds in the presence of PPO in tea fermentation.

G. Tea Fermentation

In the case of tea leaves, fermentation is the process during which the polyphenols in the tea leaf are oxidized in the presence of polyphenoloxidase enzymes and subsequently condensed to form colored compounds contributing to the quality attributes of black tea. Fermentation starts immediately after cell rupture and proceeds through the enzymatic reactions shown in Figure 91.15.

Formation of the black pigments, viz theaflavins, thearubigins etc., during enzymatic fermentation of different components of tea leaves followed their PPO activity (37).

H. Enzymes in Health and Wellbeing

Celiac Sprue (a.k.a. celiac disease, coeliac disease, or gluten intolerance) is a hereditary disorder characterized by a sensitivity to the toxic effects of gluten in the diet, leading to abnormal small-intestinal structure, malabsorption, and intolerance to gluten, a component of nutritionally important proteins found in common dietary food grains such as wheat, rye, and barley (38).

The disease commonly presents in early childhood with severe symptoms including chronic diarrhea, abdominal distension, and failure to thrive. The general condition of these children is severely impaired. In many patients, symptoms may not develop until later in life when the disease presents with fatigue, diarrhea, and weight loss due to malabsorption of nutrients and vitamins, anemia, and neurological symptoms (38).

This is a lifelong disease, and if untreated, patients have a substantially enhanced risk for the development of complications such as infertility, osteoporosis, intestinal cancer, and lymphoma. There is no therapeutic option available to Celiac Sprue patients, the only treatment being a lifelong adherence to a strict gluten-free diet.

Recently it was discovered that a relatively short fragment of the gluten protein is exceptionally toxic to Celiac Sprue patients. This gluten fragment is unusually resistant to breakdown by digestive enzymes in the intestine, where it remains intact to have a destructive effect on the intestinal lining. Using this information, the researchers identified a bacterial enzyme (a peptidase) that can rapidly degrade this peptide and other related toxic fragments from gluten (39). Thus it may be possible to cure certain diseases by the use of enzymes in our diet.

V. CONCLUSION

The enzymes used as functional ingredients in food processing and manufacture of food ingredients are numerous. Because enzymes can lower the activation energy of a particular reaction under different conditions of temperature, pH, and prosthetic groups, they are ideal catalysts for biological systems. In the preceding pages, it was demonstrated how various biotransformations could be effected to produce desirable end-products with the mediation of enzymes. Thus basic knowledge about the structure and composition of various plant and animal tissues and enzyme reaction kinetics would lead to interesting novel developments, such as the enzymatic peeling of citrus products, yams, tubers, removal of skin from animal carcasses, and so on. Such applications would give rise to low wastage of raw materials and lead to production efficiencies and environmentally friendly processes. Fermentation, which utilizes microorganisms and hence enzymes, is used extensively to produce a large number of natural food ingredients and will continue to play a strong part in the future, especially in the biotechnology area.

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92 Composition and Structure-Function Relationships in Gums

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CONTENTS

I. INTRODUCTION

In the food industry the term "gum" refers to polysaccharides, which are widely distributed in nature and possess a broad range of functional properties. Particularly important examples of their functionality are the capacity to alter the flow characteristics of fluids and to interact in the hydrated state with other dispersed or dissolved molecular species, which they may bind, chelate, complex, emulsify, encapsulate, flocculate, stabilize, or suspend (1). As a result, they perform either by themselves or in association with lipids, proteins, or other molecules vital physicochemical functions, by providing structural support and energy reserve, and by mediating various biological processes such as cellular recognition and growth. This broad range of functional properties, frequently unique in their nature, is increasingly exploited by the food industry and since the 1970s has lead to a flurry of activity in the

published literature (2,3). Normally, a review of the subject provides an outline of gums and discusses within each chapter the properties and uses of individual cases (4). The approach of the present compendium will emphasize the understanding of why gums behave in different ways. Groupings of primary structure and conformational characteristics of the most popular gums should rationalize their functional properties and indicate to the reader the best material for a particular end-use application.

II. THE CHEMISTRY AND CONFORMATION OF MONOSACCHARIDES

The functionality of gums is derived by the chemical characteristics of the individual residues and the conformation of the linkages between them (5). In almost all gums of industrial importance, the repeat unit is based on a six-membered (pyranose) ring structure. Figure 92.1 shows the two stable chair conformations of the pyranose ring ($^4C_1$ and $^1C_4$) in which all bonds are fully staggered (6). In the chair conformations, groups attached by bonds that project vertically from the ring are sterically crowded whereas substituents that point outwards have much more space. The former type of bond is called axial whereas the latter is called equatorial. According to the steric arrangement at C(5), hexose sugars are classified into two categories: the D-series where the bulky hydroxymethyl group is equatorial in the $^4C_1$ ring form and the mirror image L-series where the hydroxymethyl group is equatorial in $^1C_4$. In both series, monosaccharides are named according to the position of hydroxyl-group bonds on carbons 2, 3 and 4 of the pyranose ring. For example, the stable ring form of glucose has all the hydroxyl groups equatorial, whereas in mannose and galactose O(2) and O(4) are respectively axial. Axial linkage on carbon 1 of the monosaccharide ring is denoted as α and equatorial as β.

III. ORDERED CONFORMATIONS OF GUMS WITH LINEAR CHAINS

The linkage between adjacent monosaccharides in carbohydrate chains is known as the glycosidic bond, and involves the hemiacetal OH group at carbon 1 on one residue and one of the alcohol groups of the next residue, with formal elimination of water. There are three significant classes of chain bonding pattern (7):

- Polysaccharides linked di-equatorially at positions 1 and 4 of the pyranose ring adopt **flat ribbon** structures upon gel formation.
- Where linkage is again at carbons 1 and 4, but through axial bonds at each position, the chains adopt a highly **buckled ribbon** structure.
- Finally, where the bonds to and from the ring are no longer parallel (e.g. 1,3 linkages or 1,4 axial-equatorial), this introduces a systematic change in chain direction at each residue, which gives rise to **helical structures** in the ordered state.

Those ordered conformations are the origin of the wide range of structural properties of industrial gums, as it will be demonstrated below.

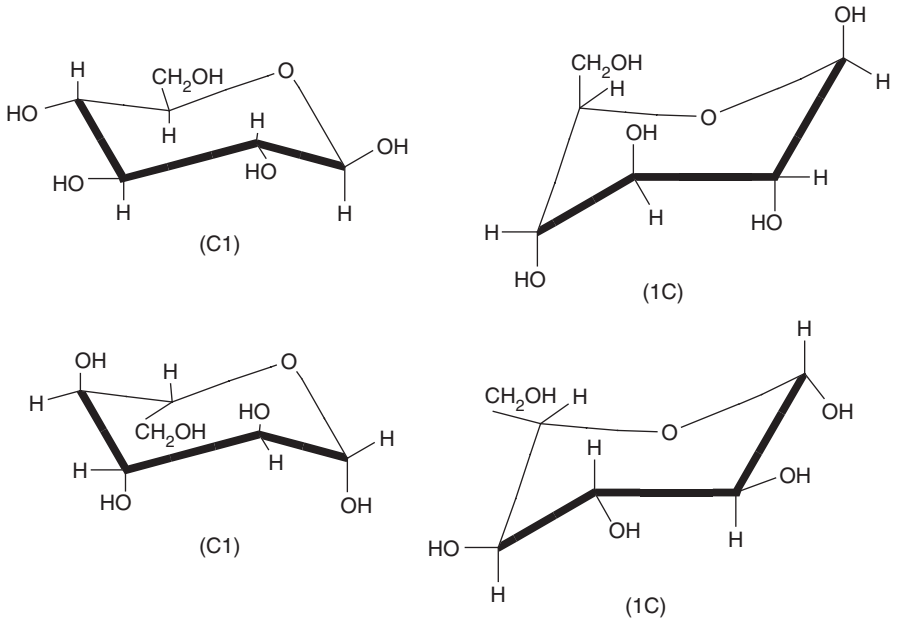

FIGURE 92.1 Pyranose chair conformations illustrated for β- and α-glucose (from reference 6, with permission).

A. Helical Structures

Three different modes of helix formation have been suggested. They are based on the principle that an empty space along the axis of a hollow helix would not be expected to be stable in a natural situation. Depending on the state of extension of helix the three models are (8,9):

i) A least extended helix that uses a molecule of suitable diameter to fill the void along its axis (inclusion complex).
ii) A more extended helix that forms a close-packed structure, by turning round two or three chains, in an embrace that can fill the void of the each other.
iii) An even more extended helix where the chains pack by nesting together without twisting round each other.

Tertiary structures in the form of hollow helix conformation have been characterized in the solid state for starch and a number of marine red algal polysaccharides (10). It is not within the scope of this treatise to discuss the properties and applications of starch and its derivatives but in the context of the hollow helix, there is now evidence that the linear component of starch, amylose forms a double helix in the ordered state. This can be converted by crystallisation to a less extended form (V-amylose) in which the chains include suitable small molecules, such as in the familiar complex with iodine.

Any hollow-helix gum can form these inclusion complexes provided it is hydrophobic because the D-glucopyranose units are adjusted in a way that arranges the axial C-H bonds on carbons 3 and 5 as well as one of the H atoms on each carbon 6 inwards, giving hydrophobic character to the vacant space (11). Therefore the bonding which holds the enclosed molecule serves as a source of helix stability. Stability is enhanced further by hydrogen bonds between oxygen 2 and 3 of successive residues and between oxygen 2 and 6 of glucose units which are separated by several units in the chain and are therefore adjacent on the helix surface.

1. Carrageenans

Among the carbohydrate sequences with more than one type of sugar unit and/or more than one type of linkage, the family of carrageenans is a prime example (12). Carrageenans are extracted from marine red algae (seaweed). In the helix-forming members, the basic repeating unit contains 1,3-linked β-D-galactose-4-sulphate and 1,4-linked 3,6-anhydro-α-D or L-galactose, with various degrees of 2-sulphation of the latter, but no 2-sulphation in kappa carrageenan (Figure 92.2). The anhydride bridge has the effect of holding the sugar ring in the normally unfavoured chair conformation with C(6) axial, which facilitates the

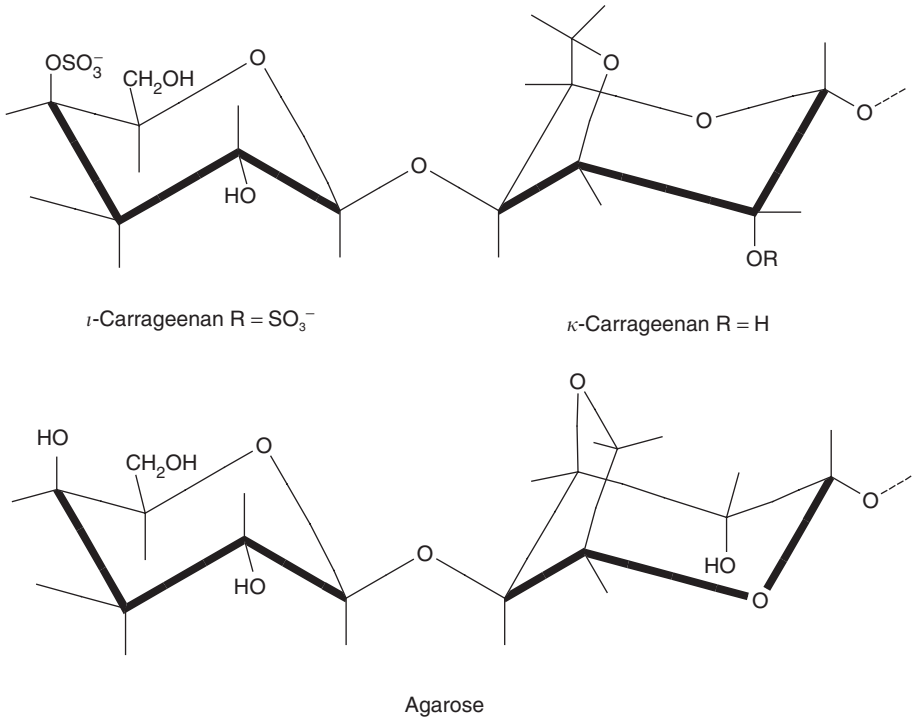

FIGURE 92.2 Idealized disaccharide repeating units of carrageenan and agar polysaccharides (from references 12 and 19, with permission).

formation of a coaxial double helix. However, complete ordering and precipitation is avoided because a proportion of the 1,4-linked residues in natural carrageenans occur as unbridged D-galactose sulphate or disulphate. These residues adopt the normal chair conformation and act as 'kinks,' which limit the extent of helix formation and thus solubilise the network (13).

Sigmoidal changes in optical rotation verified the disorder-to-order transformation of kappa carrageenan chains during cooling. X-ray diffraction studies and model building taking into account the angles of glycosidic bonds, the closeness of adjacent atoms and the space available to the partner chain, argued for the formation of a double helical structure with three disaccharides per turn of helix (14). This is further stabilised with hydrogen bonding, perpendicular to the helix axis, between the OH groups at positions 6 and 2 of the galactose rings of the two strands. Helices come together to form aggregates, which constitute the structural unit of the kappa carrageenan network (15). K^+ are the main stabilizing counterions of the aggregates and according to the "domain model," they form an array between adjacent helices in the gel (Figure 92.3).

Iota carrageenan is the most highly sulphated member of the helix-forming family. Its double helix structure is three-fold, parallel and right-handed with the sulphate groups protruding away from the helix area. As in kappa carrageenan, the double helix is further stabilized by hydrogen bonds between O(2) and O(6) of D-galactose residues on different strands. Because of the high negative charge of iota carrageenan, helix-helix aggregation and thus gelation occurs only in the presence of cations which can suppress electrostatic repulsion between the participating chains. The dependence of the gel strength on cation type follows the series (16):

$$Ca^{2+} > Cs^+ > Rb^+ \sim K^+ \gg Na^+ > Li^+$$

Aggregation stabilizes the ordered conformation to temperatures above those at which it would normally form, giving thermal hysteresis between gel formation and melting. Lambda carrageenan has a high ester sulphate content (three sulphate groups per disaccharide repeat unit) and it does not gel (17).

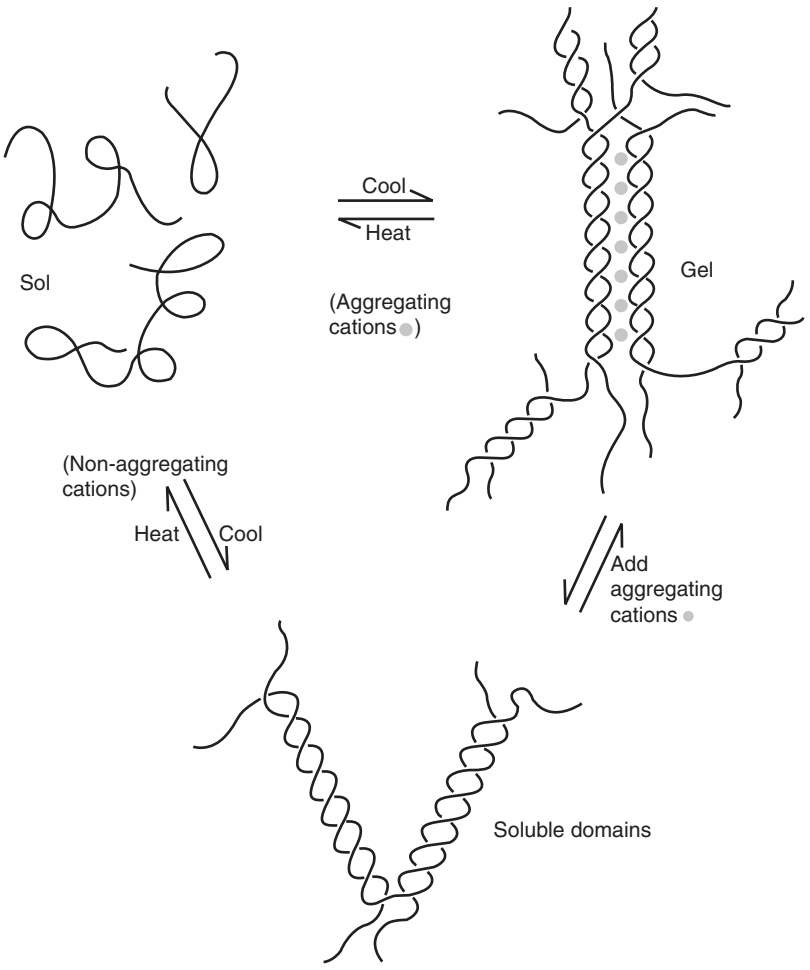

FIGURE 92.3 Domain model of carrageenan gelation (from reference 15, with permission).

Carrageenans have a wide range of food applications functioning as gelling agents, stabilizers or viscosity enhancers (18). Chocolate or evaporated milk is stabilized against sedimentation of cocoa particles or creaming of the fat globules by addition of the gum between 0.02 and 0.05% in the formulation. In gelled water deserts and low sugar preserves carrageenans (0.5 to 1%), often in mixture with galactomannans, form thermally stable gels with gelatin-like textural properties. In meat, fish and bakery products, the polysaccharide (0.2 to 2%) is used to maintain the moisture content in cooked hams and rolls, increase the binding properties of canned products and aspics, and gel or glaze tart or pie fillings.

2. Agarose

Agarose is a structural gum occurring in different species of red seaweed (*Rhodophyceae*). As illustrated in Figure 92.2, its primary structure comprises an alternating repeating sequence of 1,3-linked β-D-galactopyranose and 1,4-linked (3→6)-anhydrogalactopyranose (A and B units, respectively). The 3,6-anhydro units are in the L form which is reflected in left-handed helix geometry. Furthermore, the anhydride bridge may be absent in a proportion of the 4-linked residues, which changes the geometry of the sugar ring to a form that is sterically incompatible with incorporation in the ordered double helix (19). The presence of this "kinking" residue is essential for the development of gel networks, as it terminates the ordered junction zone association and allows each chain to participate in more than one intermolecular junction zones.

At high temperature, agarose exists in solution as disordered, random coils, but upon cooling it undergoes a co-operative conformational transition to a rigid, ordered form (20). It is generally accepted that agarose gels are cross-linked by ordered aggregates of double helices. The double helix appears to have an interior cavity with a diameter of 0.45 nm (measured at the narrowest points between atomic centers). Oxygen atoms, namely O(2) of galactose and O(5) of 3,6-anhydrogalactose which are engaged in hydrogen bonds can be found in the interior of the helix. According to this, it was suggested that the agarose cavity is occupied by water molecules, which participate in a hydrogen bond that contributes to the stability of the double helix (21). This comes in agreement with the proposal that water is the single solvent in which agarose forms gels and that it is necessary part in the aggregation of the helices and the formation of the junction zones (22). The agarose network consists of long, flexible chains connecting junction zones, which are somewhat crystalline, and the other regions are amorphous.

Agarose is insoluble in cold water but soluble in hot. Its solutions are heat-stable with essentially constant viscosity in neutral pH and it gives gels even at concentrations of 0.1% or lower. Agarose gels exhibit thermal hysteresis and the melting temperature is dependent on the concentration used (23). The gel finds a wide range of non-food applications especially as a moulage material capable of reproducing fine details with great accuracy thus being used in dentistry, plastic surgery, criminology, etc. (24). In U.S., it is mostly used in microbiology at liquid media concentrations of 0.007 to 0.08%. Even at such low concentrations, agarose is able to prevent the entry of oxygen into liquid media, making the cultivation of anaerobes feasible in air-exposed broths. Agarose is also used in a wide range of food products to improve texture and stability (yoghurts, cream cheeses), as an antitackiness or antistaling ingredient (cookies, cakes, pie fillings, meringues), and in vegetarian and health food products (cereals, meat/fish substitutes).

B. Flat Ribbons

The monomer units of this group of polysaccharides are diequatorially linked through positions 1 and 4, diagonally opposite each other across the pyranose ring. Cellulose and chitin provide a simple illustration of the way in which intermolecular associations can arise.

1. Cellulose

Cellulose is a 1,4-linked β-D-glucan that occurs in almost all higher plants as extended ribbons of 2-fold chain geometry. The ribbon-like chains lie side by side in sheets, which are laid on top of each other in a way that staggers the ribbons. The chain packing is parallel, i.e. the chain termini at each end of the bundle are all of one type. The chain geometry is stabilized by hydrogen bonding of each successive residue to its nearest neighbours through O(3) and O(5). This compact and tightly bonded structure is characterized by high strength, fibrous character, insolubility and inertness, providing strength and support in plant tissues (25).

Food applications utilize cellulose derivatives like the carboxymethylcellulose (CMC). Modification of the cellulose is achieved by introducing a controlled amount of sodium carboxymethyl groups onto the molecule. This holds the chains apart thus imparting water solubility. Therefore, CMC is able to increase viscosity, prevent syneresis and stabilize suspensions (26). Other cellulose derivatives include methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC). Upon heating, these form gels from a hydrophobically crosslinked network in regions of dense substitution. Despite the presence of hydrophobic character, unsubstituted or sparingly substituted regions of cellulosic structure impart enough hydrophilicity for the molecule to be highly water-soluble at ambient temperatures (27). Thus, MC and HPMC possess unique hydration-dehydration characteristics acting

as emulsifiers and adhesion improvers of the batter or coating in a food product. Finally, microcrystalline cellulose (MCC) is used in dairy and nondairy frozen desserts including ice cream, ice milk and soft-serve to control growth of ice crystals under adverse heat shock conditions, and in novelty products to improve extrusion characteristics and foam control (28).

2. Chitin

Chitin is the structural polysaccharide of insects and crustaceans. The structure of chitin is identical to that of cellulose except that the hydroxyl group on each C(2) is replaced by an acetyl group (-$NHCOCH_3$). Therefore the ordered conformation is closely similar (extended ribbons of two-fold symmetry) and the way in which the chains pack side by side in a strongly hydrogen-bonded crystalline manner is also very similar (29). However, both parallel and anti-parallel sheets can occur in nature. Native chitin, like cellulose, is totally insoluble in water and most other solvents.

Commercial chitin is available at various degrees of acetylation (DA). At high degrees of acetylation, the polymer is only soluble in a few solvents, which limit its applications. Partial deacetylation, however, drops the DA below 50% making the polymer soluble in aqueous acidic conditions. This is known as chitosan, which can be considered as a copolymer containing (1→4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose residues (30). Chitosan in aqueous solutions of dilute acids exhibits a polyelectrolyte character owing to the presence of protonated amino groups, which have an intrinsic pK_0 value of 6. Thus, the physicochemical properties of solutions of chitosan are expected to be governed by factors such as pH, ionic strength, degree of acetylation and temperature. It is documented that the charge density along the chitosan macromolecule increases with decreasing degree of acetylation. This means that chain flexibility can be manipulated in response to DA (31).

In addition, the hydrophobicity of the chitosan chain influences the steady shear viscosity and dynamic mechanical properties of solutions and gels. The hydrophobic character can be intensified by chemical modification on the free amino groups or the hydroxyl groups present along the main chain by grafting, for example, alkylated side chains. Numerous food and non-food applications for chitin are foreseen and some are actively being developed (32). Currently, chitosan is used in animal feeds in amounts up to 0.1%. Its chelating/binding properties are explored in structuring of pelleted fish foods, the separation of heavy metals, recovery of proteinaceous materials and in pharmaceuticals. The polysaccharide forms good quality films with potential use in dye, textile, photographic and paper sheet applications.

C. Buckled Ribbons

The 1,4-diaxial linkage arrangement, in which the effective residue is changed from the almost linear form described above to 'Z' shaped, gives rise to buckled ribbon structures. Well-characterised examples of this type of structure occurs in the alginates from brown seaweed and pectin.

1. Alginates

These are 1,4-linked linear polymers of β-D-mannuronate and α-L-guluronate, containing homopolymeric sequences of both types and heteropolymeric regions in which residues occur in various arrangements (33). The latter can range from almost statistically random to almost regularly alternating, depending on the botanical source and state of maturation of the plant. Pure polymannuronic acid, having 1,4-diequatorial linkage geometry, adopts a flat ribbon-like two-fold conformation (as in cellulose and chitin), which converts in the salt form to a similarly extended three-fold ribbon (Figure 92.4). The corresponding x-ray fibre diffraction structure for polyguluronate, by contrast, is a highly buckled two-fold zig-zag, irrespective of charge or counterion (34).

Investigation of molecular models of polyguluronate in the invariant two-fold structure identified by x-ray shows cavities of the size required to accommodate a calcium ion, and with oxygen atoms well placed for cation chelation. It has therefore been proposed that the interchain junctions in alginate gels involve polyguluronate sequences locked in a two-fold conformation, with arrays of site-bound cations sandwiched between them like eggs in an egg box (35). The "egg-box model" rationalizes a well-known property of alginates, which is of major importance for both their biological function and industrial applications. This is the formation of strong, rigid gels with divalent cations, usually Ca^{2+} (Figure 92.5). Gel strength increases with increasing content of polyguluronate and calcium ion activity is solutions of guluronate oligomers shows a sharp decrease above a critical sequence length of about twenty residues. This indicates the onset of co-operative binding behaviour, where the binding of each ion facilitates the binding of the next. Such effects are not seen for the corresponding mannuronate sequences, which remain soluble in the presence of Ca^{2+}, and show only simple polyelectrolyte binding behaviour, indicating that they must act as solubilising interconnecting regions in alginate gels (36).

Commercial applications for alginates in foods/non-foods necessitate the formation of a continuous network rather than a precipitate. Thus it is essential to control the affinity of polyguluronates for Ca^{2+} by ensuring that the ions are introduced in a controlled way that allows intermolecular junctions to form slowly enough. Diffusion setting, internal setting, and a third alternative method when

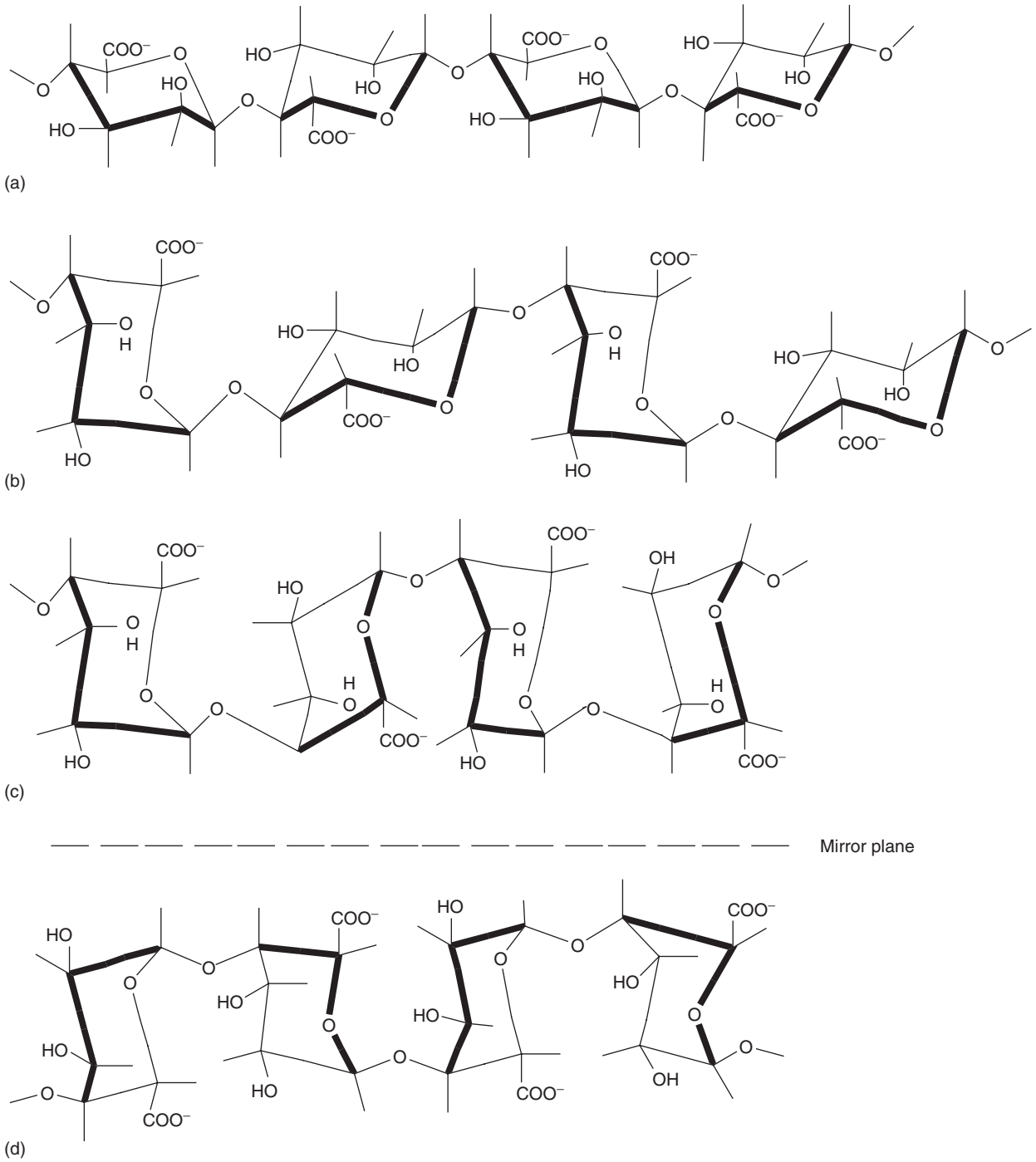

FIGURE 92.4 Comparison of backbone geometry in (a) alginate poly-D-mannuronate sequences, (b) alginate heteropolymeric sequences, (c) alginate poly-L-guluronate sequences and (d) poly-D-galacturonate from pectin (from references 34 and 42, with permission).

setting occurs on cooling in the presence of sequestrants are the recommended recipes to prepare alginate gels (37). Through the years, alginate found its way to a widespread range of products. Alginate setting is used in the production of pimiento strips, onion rings, structured food and fruits, and other thin gelled coating applications. Alginate forms heterotypic interactions with low methoxy pectin due to the mirror-image conformation of the chains of the two gums, which allows applications in low pH jellies, jams and fruit-filled gels (38). Thick pastes, instead of gels, can be

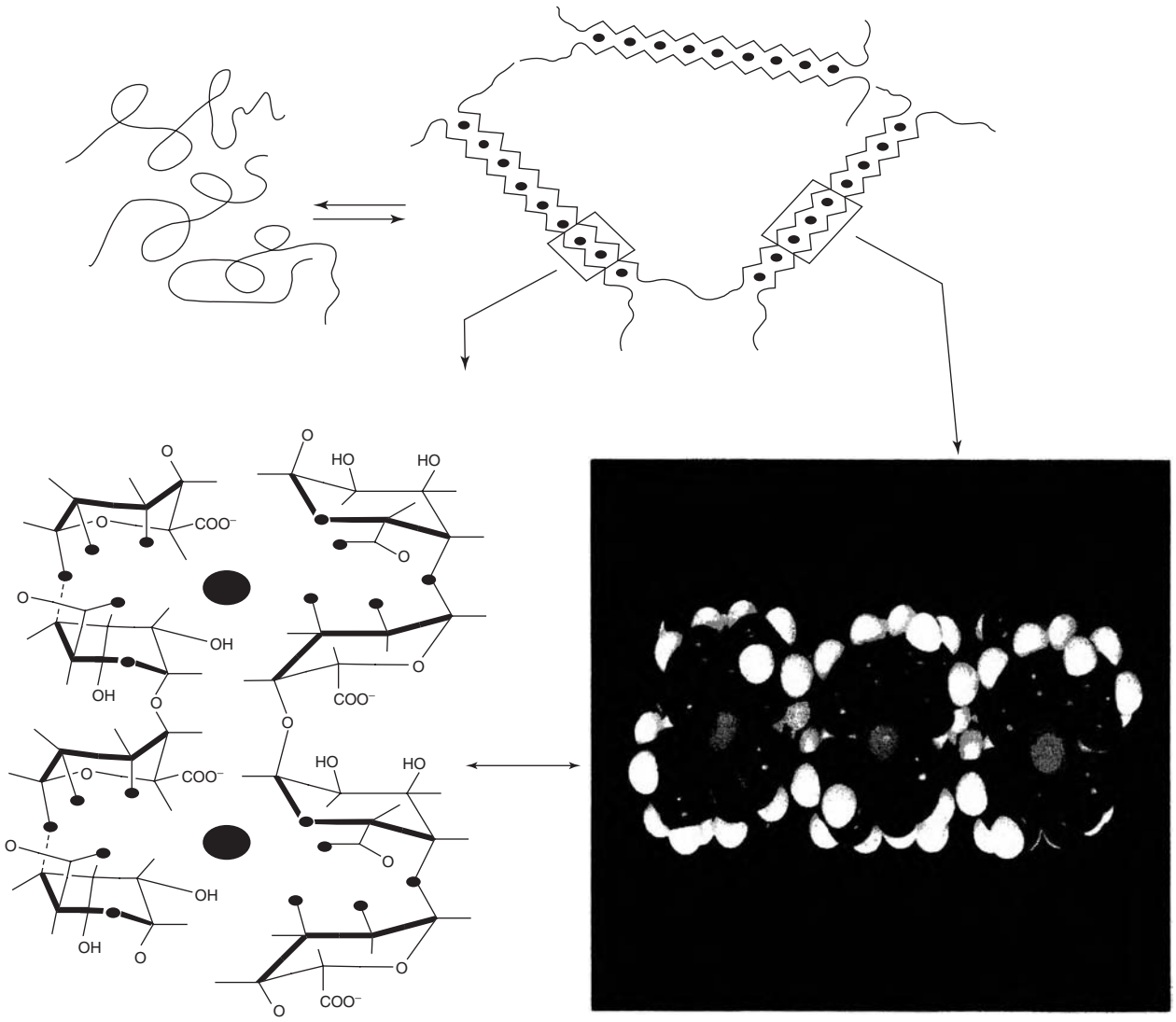

FIGURE 92.5 "Egg-box" binding of Ca^{2+} in gelation of alginate and pectin (top). Oxygen atoms • involved in cation chelation (bottom left). Space filling molecular model showing buckled chain contour (bottom right) (from references 35 and 45, with permission).

achieved with the sodium or potassium salt and the propylene glycol partial ester of the polysaccharide. These are utilized in salad dressings and fruit drinks containing pulp.

2. Pectin

Pectin is a complex, structural polysaccharide present in all plant tissues and its name comes from the Greek word "πηχτos," which means "to thicken." Commercially accepted pectins are produced from either apple pomace or citrus peel formed as residues from fruit juice, citrus oil and cider industries (39). Other sources of pectins are sugar beet residues (after the extraction of sucrose) and sunflower (from production of edible oil). Commercially, pectin is extracted by treating the raw material with hot dilute mineral acid at ≈ pH 2 and filtering. Apple pectin is treated with carbon to remove colour. The clarified extract is concentrated under vacuum and then mixed with alcohol (isopropanol, methanol) to precipitate the gum in the form of a stringy gelatinous mass. The pure powder is pressed, washed, dried and ground. Since pectin is extracted from a variety of raw materials, production of a consistent product is normally achieved by blending of a number of production batches and diluting them with sugar or dextrose to a standard performance (40).

This process yields pectin with a degree of esterification of approximately 70%, which is called "rapid set" pectin. To produce other types, some of the ester groups must be hydrolysed. This is mainly carried out by the action of acid, either before or during a prolonged extraction, in the concentrated liquid, or in the alcoholic slurry before separation and drying. The procedure results in a range of slower

setting high methoxyl and calcium reactive low methoxyl pectins. Alternatively, alkaline can be used, but since pectin readily degrades at neutral or alkaline pH by a β-elimination reaction, this must be carried out at low temperature. The use of ammonia for alkaline hydrolysis leads to a conversion of some of the ester groups into amide groups, producing amidated pectins, which are usually low methoxyl (41).

Compositional analysis produces the following primary structure: (1) "Smooth" regions made up of a backbone of linear (1→4)-linked α-D-galacturonic acid (pectic acid in Figure 92.4) that may be partially methyl esterified and (2) "hairy" regions in which the galacturonic acid residues are interspersed with (1→2) linked α-L-rhamnopyranosyl residues (42). Attached to the latter are oligosaccharide side chains, containing mainly D-galactopyranose and L-arabinofuranose residues. Sidechains degrade during extraction with acid and, for practical purposes, the pectin molecule may be considered as an unbranched chain of 200 to 1000 galacturonic acid units. According to the degree of esterification (DE), pectin is classified into high methoxy pectin (HM) for DE >50%, low methoxy pectin (LM) with DE <50%, amidated low methoxy pectin and pectic acid for DE <10% (43).

The rate of gelation and the gelation temperature of high methoxy pectin on cooling from the pre-gel solution increase with increasing DE for a given level of sugar (usually above 60% in the formulation). The purpose of reducing pH in preparation of HM gels is to suppress electrostatic repulsion further, by partial conversion of COO^- to COOH. Thus gelation of high methoxy pectins requires a subtle balance of hydrophobic and hydrophilic interactions involving formation of aggregated helices supported by hydrogen bonds and grouping of methyl ester groups within a cage of water molecules (44). In contrast with HM, the gelation in low methoxy pectin takes place by ionic linkages *via* calcium bridges between two carboxyl groups belonging to two different chains in close contact. This mechanism of gelation ("egg-box") is congruent with that of alginates and it has been described in Figure 92.5 (45).

Apple pomace gives a heavier, more viscous gel, commonly used in bakery filings, whereas citrus peel has a lighter colour and consistency, which makes it suitable for confectionery jellies. Thus high methoxy pectin is used as a gelling agent in the formulation of high sugar jams, marmalades, jellies and confectioneries (65 to 85% solids). Low methoxy pectin is suitable for the production of low-sugar jams at levels of solids between 30 and 55% (46). Pectin has a good stability at acidic conditions relevant to food produce even when they are prepared hot and as a result it is popular in the making of fruit sauces or fruit preparations in the form of sheared gel particles for yoghurts (47). The usual concentrations for the above products are between 0.3 and 0.8% pectin but higher amounts of the polysaccharide are needed for bakery glazing and jam fillings (0.7–1.3%), where the requirement is for gel films that maintain their integrity at baking temperatures.

IV. EFFECT OF BRANCHING

There are two distinct groups of branched polysaccharides: plant and bacteria. They differ in the distribution pattern of the branches along the backbone, which is normally irregular for the former group but often regular for the latter. Irregular branching, by interfering with ordered intermolecular association and packing, usually provides a drive to enhance solubility in plant polysaccharides where it occurs. Solubility is further increased if the branches (or other irregularly-distributed substituents) are charged. Regularly-spaced sidechains in bacterial polysaccharides can have a similar solubilising effect. In some cases, however, they can have a quite different role, by modifying or restricting the conformational options of the polymer backbone and participating in the formation of ordered structures with different backbone geometry from that adopted when sidechains are absent (48). Illustrative examples of the effects of both types of branching are given in the following section.

A. The Galactomannans

Galactomannans are energy reserve carbohydrates found in the endosperms of some legume seeds. They consist of a linear backbone of 1,4-linked β-D-mannose units, some of which carry a single α-D-galactopyranosyl sidechain at C(6). Commercially available galactomannans include locust bean (carob), tara and guar gum and their typical mannose/galactose ratios are 3.5, 3.0 and 1.6, respectively (49). Early investigations on the "fine structures" of these galactomannans led to the conclusion that they have a block-like distribution of sidechains. Later work was interpreted as showing that guar gum has a uniform structure, with galactose residues attached to alternate mannose residues, so that in the preferred two-fold conformation of the polymer backbone all the sidechains would be located on one side of the main chain. The same workers also suggested a block structure for carob gum. However, more recent results obtained by intelligent use of rigorously-purified enzymes in combination with computer modeling indicate a more irregular distribution pattern of galactose residues along the mannose backbone (50).

The difference in pattern of substitution of galactomannans reflects on their ease of solubilisation. The overall composition is also an important determinant of hydration. In general, their solubility increases with increasing galactose content. Thus guar gum hydrates rapidly in either hot or cold water, complete dissolution of carob gum required heating to elevated temperatures, and their parent mannan is totally insoluble in water (51). On dissolving, the galactomannans adopt an extended, disordered conformation,

giving solutions of high viscosity at low polymer concentration. In the solid state the backbone adopts an ordered, two-fold conformation with the individual chains packing together into flat sheets.

Galactomannans serve the function of economical and natural thickeners in the food industry (52). Their primary function is to bind water and minimize the growth of ice crystals in confectioneries, pie fillings and chocolate-based frozen desserts. As mentioned earlier, they are combined with gelling carrageenans and agarose to increase the pliability and reduce the syneresis of gels used as gelatin mimetics and with carboxymethylcellulose to stabilise ice creams. A wide range of dips, sauces, salad dressings, mayonnaise, soups and toppings utilize galactomannans to provide desirable viscosity and stability at high temperatures and relatively acidic conditions (53). Locust bean gum, in particular, is used as an efficient binding agent thus contributing to a homogenous texture in sausages made of comminuted meat and canned petfoods with gravy.

B. Xanthan

The bacterial polysaccharide xanthan is an example of a true periodic branched repeating sequence. It is produced during fermentation by the organism *Xanthomonas campestris*. Its primary structure consists of a cellulose backbone, substituted on O(3) of alternate glucose residues by charged trisaccharide sidechains of β-D-Man*p*-1,4-β-D-GlcA*p*-1,2-α-D-Man*p* to give a branched pentasaccharide repeating unit (54). Substantial sample-to-sample variations are observed in the degree of acetylation at O(6) of mannose residues adjacent to the polymer backbone, and in the content of pyruvate ketal 4,6-linked to terminal mannose units. X-ray diffraction studies on oriented xanthan fibres can accommodate equally two trial models. One is an extended five-fold single helix stabilized by hydrogen bonding of sidechains along the polymer backbone and the other is a coaxially packed double helix where the individual chains have the same conformation as the single helix model (55).

It is documented that the disorder-to-order transition of xanthan obeys first order kinetics, with little change in molecular weight until conformational ordering is virtually complete, arguing for an intramolecular single helix rather than an intermolecular double helix. A subsequent approximate doubling of molecular weight observed at lower temperature may then be attributed to lateral association of ordered chain sequences. This proposal offers a likely interpretation of the tenuous gel-like behaviour of xanthan solutions, which is utilized technologically in emulsion stabilization and particle suspension (56). The associations indicated by light scattering provide a mechanism for the development of a network comprising intermolecular associations. The network structure, however, breaks down readily under stress, allowing the solution to flow freely. This unusual combination of properties falls into the category of "weak gels."

The weak-gel properties of xanthan are used widely in the food industry. Salad dressings, mayonnaise and ketchup may contain xanthan up to 0.3% in order to impart a functional texture (57). Thus the foodstuffs are readily pumped in the production line, flow easily from the bottle during use and restructure well "at rest" on a salad, piece of pizza, a toast or a burger. Xanthan's ability to disperse and then dissolve in cold water or to retain its stability and viscosity in acidic formulations finds applications in beverages, sauces and gravies. Its interaction with galactomannans create paste-like heterotypic associations (58), which are used in the stabilization and creaming of processed soft cheeses, and to impart smooth texture and improved organoleptic properties in frozen confectionery (ice cream, milk shakes, etc.).

C. Gellan, Welan and Rhamsan

The structurally-related extracellular polysaccharides gellan, welan and rhamsan share the same four-sugar backbone repeating unit. Gellan is unbranched, whereas welan and rhamsan display comb-like branching. Gellan is biosynthesized by the organism *Sphingomonas elodea* with an L-glyceryl substituent at O(2) of each 3-linked glucose unit of the polymer backbone and an acetyl group at O(6) of the same residue; although the latter substituent may be present in substoichiometric proportion (59). Both groups are often removed by alkaline hydrolysis in preparation of gellan for commercial use. In the case of welan, each 4-linked D-glucose residue carries a 1,3-linked single sugar sidechain of either α-L-rhamnose or α-L-mannose, in the approximate ratio of 2:1. In rhamsan the sidechain is a disaccharide (β-D-Glc*p*-1,6-α-D-Glc*p*-) attached at C(6) of the 1,3-linked glucose residue (the same point of attachment as the acetyl substituents in gellan).

The effect of chain branching on backbone conformation and thus on the physical properties of the polymers, is profound (60). The major industrial use of gellan gum is as a gelling agent with physical properties ranging from weak and elastic in the native polymer to stiff and brittle in the fully deacylated form. The strength and thermal stability of the gels formed by the deacylated polymer is strongly influenced by the type and concentration of cations present (61). For monovalent cations, gel strength increases in the order:

$$Me_4N^+ < Li^+ < Na^+ < K^+ < Cs^+ < H^+$$

whereas for divalent cations, which can form gels at far lower salt concentrations, the order is:

$$Mg^{2+} \sim Ca^{2+} \sim Sr^{2+} \sim Ba^{2+} < Zn^{2+} < Cu^{2+} < Pb^{2+}$$

The ordered structure of gellan involves two left-handed, three fold helical chains organized in a parallel fashion in an intertwined duplex. Gel formation is believed to occur by cation-mediated helix-helix aggregation. The sensitivity of gellan to acyl content and salt composition of the gelation media permits use as a thermosetting, cold-setting, thermo-reversible or thermo-irreversible gelling agent. Additionally, it is highly resistant to enzymic breakdown and can withstand autoclaving, allowing it to be used as a substitute for agarose in microbiology or for plant tissue cultivation. Attempts have been made to find a use for gellan in a wide range of food systems requiring good gelling properties (62). The gum is popular in the making of "halal" table jellies and high sugar confectioneries, which are gelatin and cholesterol free. It may also find use in pie fillings, dairy products and petfoods.

Welan and rhamsan, by contrast, do not gel but give viscous aqueous solutions, which are very stable to temperature, pH and salt, and have characteristic "weak gel" properties similar to those of xanthan (63). Like xanthan, welan was developed initially for commercial application in oil recovery (hence the name). Calorimetric and chiroptical studies showed no evidence of a conformational transition for welan or rhamsan at any accessible temperature. Thus the molecules are either "random coils" which do not order under hydrated conditions, or they are locked in a stable, ordered structure which is very resistant to denaturation. Recent evidence from NMR linewidth, optical rotation, differential scanning calorimetry and response of intrinsic viscosity to changes in ionic strength favours the latter proposal (64). A likely interpretation is that in both of these materials the sidechains pack along the polymer backbone, enhancing the stability of the ordered structure but preventing the helix-helix aggregation required for gel formation.

D. Gum Arabic

Gum arabic is an exudate obtained from the bark of the *Acacia tree* in the form of a very viscous liquid. It is transported all over the world from Sudan, Senegal and other African countries where the main tree species is *Acacia senegal*. Clarified grade is required for food and pharmaceutical applications, which is obtained by filtering, centrifuging, freeze drying and grinding to fine powder (65). Even in this form, the gum is a complex composite of polysaccharide chains covalently linked with proteins. The backbone and side chains of the polysaccharide consist mainly of β-D-galactopyranosyl molecules, which have attached α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl units. The main appeal of gum arabic in the food industry is its ability to stabilize oil-in-water emulsions, for example, citrus oil emulsion concentrates. In addition, foam and cloud producing ability is utilised extensively in beers and non-alcoholic beverages (66). Another major application of gum arabic is in confectionery products where it prevents sugar crystallization, enhances product homogeneity and provides adhesion for film coatings and nuts (67).

V. PRINCIPLES OF GUM PROPERTIES IN SOLUTIONS AND GELS

The advent of microcomputing in recent years has allowed the rapid development of rheological techniques, with computer driven rheometers becoming commonplace in the laboratory or the research and development department of food companies. These are now established as the most productive line of attack for the development of function-structure-texture relationships in food products (68). The aim of this section is to give a basic introduction to rheological approaches sufficient for the practical examination of gum based sample systems, interpretation of results and to provide enough background information for the accounts of food application types described in this chapter.

A. Dilute Solutions

A particularly convenient and useful experimental parameter in studies of dilute solutions is the intrinsic viscosity [η], a measure of the volume occupied by the individual polymer molecules in isolation, which is directly related to molecular weight (M) [69]. This is widely used in routine characterisation of polymer batches (Mark-Houwink equation):

$$[\eta] = KM^{\alpha} \qquad [92.1]$$

where K and α are constants whose values depend on the shape of the polysaccharide, the solvent used and the temperature of measurement. Typical values of α for flexible coils are 0.5–0.8, and for rigid rods 1.5–1.8 (70).

Intrinsic viscosity is defined by the standard equations:

$$\eta_{rel} = \eta/\eta_s \qquad [92.2]$$

$$\eta_{sp} = (\eta - \eta_s)/\eta_s = \eta_{rel} - 1 \qquad [92.3]$$

$$[\eta] = \lim_{c \to 0} (\eta_{sp}/c) \qquad [92.4]$$

where η and η_s denote the viscosities of the solution and solvent, respectively, and η_{rel} and η_{sp} are, respectively the dimensionless parameters of relative viscosity and specific viscosity. Experimental values of η_{sp} for extrapolation to intrinsic viscosity at infinite dilution (Equation [92.3]) should be in the range 0.2 to 1.0 (71).

Treating now molecules as particles widely separated, the Einstein relationship for laminar flow can be derived (72):

$$\eta = \eta_s(1 + k_1\phi) \qquad [92.5]$$

where ϕ is the phase volume of the disperse phase and k_1 takes the value of 2.5 for spheres. From Equations [92.3] and [92.5], η_{sp} can be expressed as a function of phase volume:

$$\eta_{sp} = k_1\phi \qquad [92.6]$$

Combination of Equations [92.4] and [92.6] for infinite dilution gives the identity:

$$k_1\phi = \eta_{sp} = [\eta]c \qquad [92.7]$$

At higher concentrations, where the increase in viscosity is not directly proportional to the mass of the disperse phase, the Einstein relation is extended by including higher order terms in Equation [92.6]:

$$\eta_{sp} = k_1\phi + k_2\phi^2 + k_3\phi^3 + k_4\phi^4 + \cdots \qquad [92.8]$$

Experimentally, a linear intrinsic viscosity-concentration relationship is observed for specific viscosities up to about 1 and its numerical form is obtained by keeping terms up to quadratic in Equation [92.8] and substituting ϕ from Equation [92.7]:

$$\eta_{sp}/c = [\eta] + k'[\eta]^2c \qquad [92.9]$$

where $k' = k_2/k_1^2$. This is the Huggins equation and the extrapolation to give intrinsic viscosity is obtained from a plot of η_{sp}/c *vs* c. Alternative extrapolations are given by the equation of Kraemer and the single point method (73). In practice, these extrapolations may not be strictly linear, but using all of them, intrinsic viscosities can be well bracketed.

B. Effect of Entanglement

Moving now from the case of very dilute solutions, where the intention was to acquire information about the volume occupied by individual molecules, up to the real range of practical viscosity behaviour ($\eta_{sp} > 1$), the Huggins and the other associated extrapolations become irrelevant because higher order powers such as those in Equation [92.8] start to be significant. With further increase in concentration, viscosity begins to show appreciable dependence on shear rate (74). At low shear rates, viscosity remains constant at a fixed, maximum value (the "zero shear" viscosity, η_0), but at higher values "shear thinning" is observed (Figure 92.6). Taking the maximum "zero shear" value, it has been observed empirically that for a wide range of gums with flexible coils the log of specific viscosity varies approximately linearly with the log of concentration over the viscosity range $1 < \eta_{sp} < 10$, with a slope of about 1.4 (75).

At higher values of η_{sp}, however, the concentration dependence changes dramatically to a slope of about 3.3,

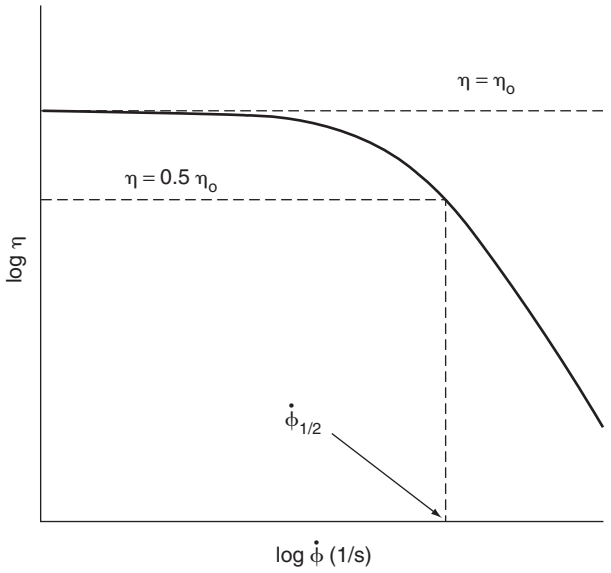

FIGURE 92.6 Shear-rate dependence of viscosity for a typical concentrated gum solution (from reference 75, with permission).

because a point is reached where the individual coils start to entangle ($c = c^*$). The relationship between absolute concentration and the onset of entanglement can be seen from equation [92.7]. According to this equation, increasing concentration leads to a stage where the volume fraction occupied by the coils becomes equal to the total volume ($\phi = 1$). The degree of space-occupancy by the polymer coils is characterized by the right-handside of Equation [92.7] ($c[\eta]$), which is called the "coil overlap parameter" (76). This has been found to have a value of about 4, at the onset of entanglement, regardless of polymer primary structure and molecular weight (Figure 92.7). The zero shear specific viscosity at this critical concentration (c^*) is invariably close to 10 (77).

The spectacular change in concentration-dependence of solution viscosity during the transition from a dilute solution of independently moving coils to an entangled network can be rationalised as follows. At concentrations below the onset of coil overlap and entanglement ($c < c^*$), the main effect of the polysaccharide coils is to perturb the flow of the solvent by tumbling around and setting up "countercurrents," with mutual interference of countercurrents from adjacent chains giving a somewhat more than proportional increase in viscosity with increasing concentration (78). At concentrations above c^*, however, where flow requires chains to move through the entangled network of neighbouring coils, the restriction of mobility increases steeply with increasing network density, giving rise to the higher concentration-dependence of viscosity.

Shear thinning behaviour in polysaccharide solutions can be similarly rationalised in terms of polymer entanglement At concentrations below c^*, shear thinning is minimal (typically less than 30% over several decades of shear rate), and can be attributed to elongation of individual coils in the direction of flow at high enough shear rate. The

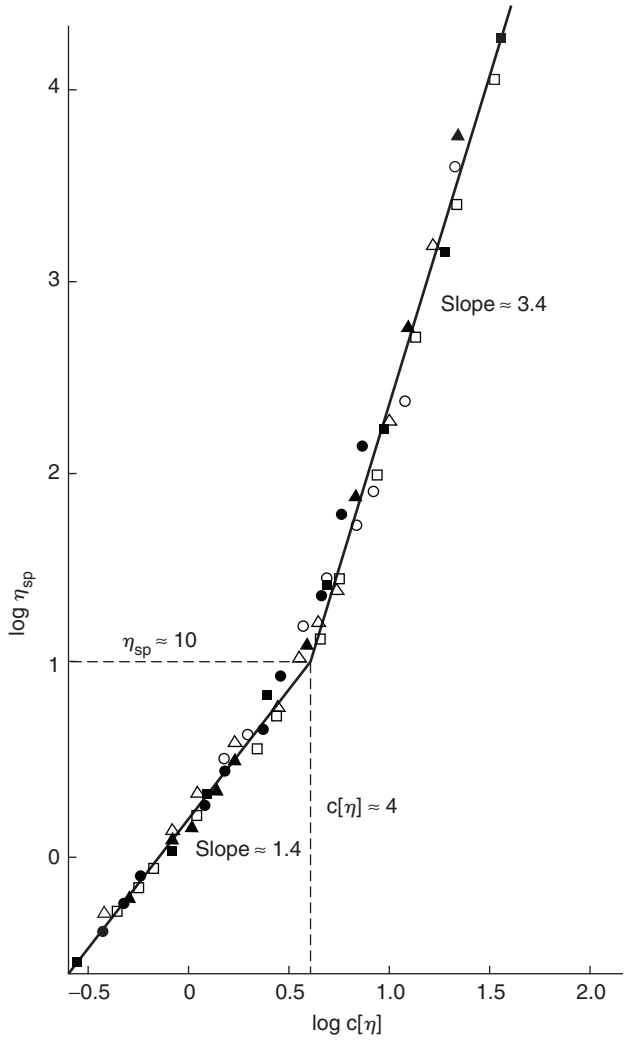

FIGURE 92.7 The variation of "zero-shear" specific viscosity with degree of space-occupancy for linear polysaccharides in solution (from reference 76, with permission).

"Newtonian plateau" observed for entangled coils ($c > c^*$) at low shear rates corresponds to a dynamic equilibrium between forced disentanglement (to allow the solution to flow), and re-entanglement with new partners (79). At higher values of shear rate, where the pace of disentanglement exceeds the pace at which new entanglements can form, the overall crosslink-density of the network is reduced, with consequent reduction in viscosity (often by several orders of magnitude).

C. Viscoelasticity

So far the discussion has been confined to the liquid-like behaviour of gum systems, measured under "large deformation" conditions, i.e. steady shear. Gum networks, including entangled "flexible coils," however, also show a substantial element of solid-like response (80). The degree of solid-like and liquid-like character can be quantified by the technique of mechanical spectroscopy in which the resistance of the sample to a very small oscillatory deformation is resolved into the storage (G') and loss (G'') moduli (68). The oscillatory analogue of shear rate is the frequency of oscillation (ω) and the ratio of the unresolved "complex modulus" ($G^* = (G'^2 + G''^2)^{1/2}$) to the applied frequency gives the "dynamic viscosity," η^* (81).

Polysaccharide systems can give three characteristic types of small deformation response (Figure 92.8). Polymer solutions with concentrations lower than c^* yield the first general type of spectrum (82). There is little variation of dynamic viscosity with frequency and G'' dominates over the G', although with increasing frequency G' approaches G'' due to storage of energy by contortion of the chains into strained conformations. Concentrated gum solutions provide the second type of mechanical behaviour (83). At lower frequencies the principal response is rearrangement of the network to accommodate the strain, and G'' predominates (as in the case of dilute solutions). At high frequencies, where interchain entanglements do not have sufficient time to come apart within the period of one oscillation, $G' > G''$ showing little change with frequency (solid-like response). The dynamic viscosity is independent of frequency initially, but decreases steeply on reaching higher values of ω.

The last general category of spectrum is the permanent network (e.g. true gum gels) that show properties approaching those of elastic solids, hence G' predominates over G'' at all frequencies with neither showing any appreciable frequency-dependence. Dynamic viscosity decreases steeply with increasing frequency, and the slope of log η^* vs log ω approaches the value of -1 expected when G' and G'' are constant (84). An unusual combination of liquid-like and gel-like properties is the case of weak gels, with xanthan the best documented example mentioned above. Xanthan solutions show quite different shear-thinning behaviour from the generalized shear rate dependence observed for flexible polysaccharide coils and, when measured under small deformation conditions, G' is greater than G'' with little frequency-dependence in either modulus, behaviour typical of a gel rather than a normal solution (85). Additionally, the small-deformation dynamic viscosity is greater than the large-deformation steady shear viscosity, in contrast to flexible coil polysaccharides where both types of measurement give the same numerical values. This implies a weak, gel-like structure, which can survive a low amplitude oscillation, but is disrupted by continuous shear (86).

VI. MIXED GELS

Two gelling gums in the same system can create three general types of network structure, as shown in Figure 92.9 (87):

a) Interpenetrating networks
b) Coupled networks and
c) Phase-separated networks

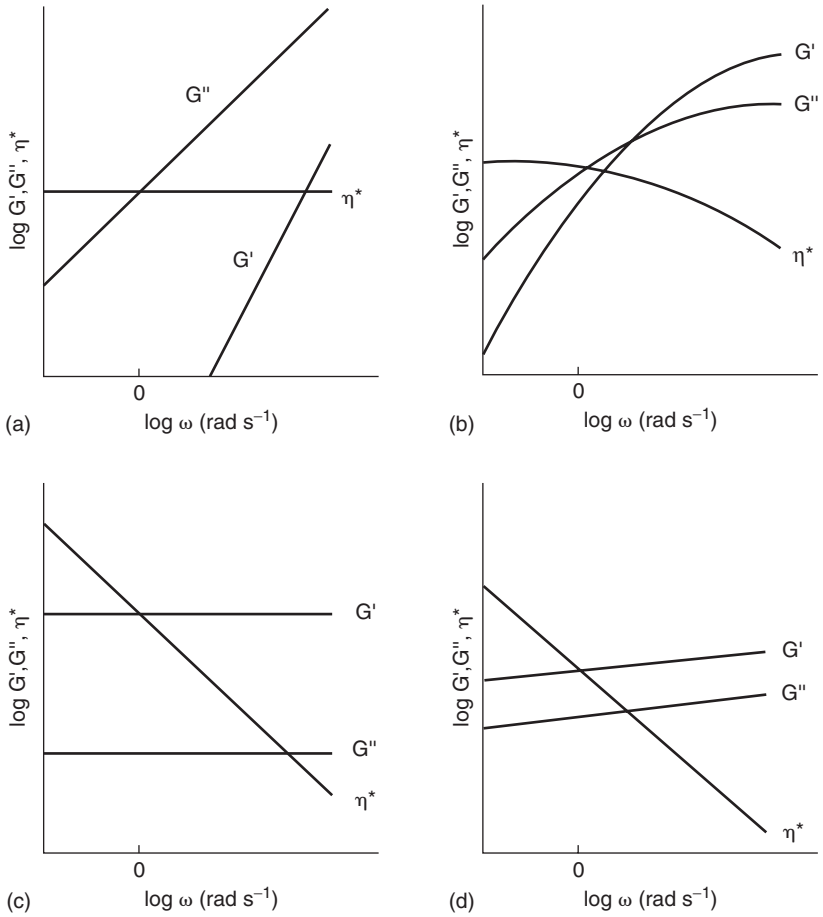

FIGURE 92.8 The four principal categories of mechanical spectra: (a) Dilute solution, (b) entangled solution, (c) strong gel and (d) "weak gel" (from reference 82, with permission).

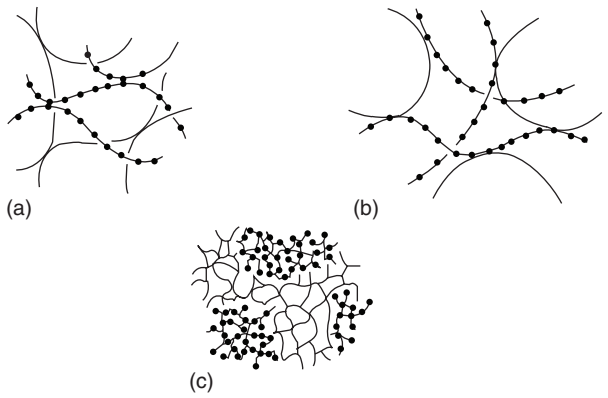

FIGURE 92.9 The three possible network topologies for binary gelling systems: a) interpenetrating, b) coupled and c) phase-separated networks (from reference 87, with permission).

A. Interpenetrating Networks

These represent the simplest situation, rarely encountered in mixed gum gels, where the two components gel separately forming two independent network structures. Both networks span the entire system, interpenetrating one another, but interaction is solely topological (88). One way of ensuring bicontinuity is to introduce a second polymer into the pores of a pre-existing network, and then to alter conditions in such a way that this second species forms its own network without disruption of the original gel. The problem of slow diffusion of a polymer solution into a gel can be tackled by using small globular proteins as the diffusing species. An alternative would be to prepare a xerogel of a gum and then swell this gel in the protein solution. A final requirement is the thermal irreversibility of the pre-existing gum network in order to allow for the heat-set process of the protein.

B. Coupled Networks

This kind of interaction involves a direct association between two different polymers to form a single network. Three different types of intermolecular binding may then arise:

i) Covalent linkages
ii) Ionic interactions
iii) Co-operative junctions

Chemical cross-linking between different chains offers a direct way of forming a gel network. The main characteristic of a system held by covalent bonds is its thermostability. One reasonably well-understood interaction of this type involves the formation of amide bonds between the propylene glycol esters of alginate and uncharged amino groups of gelatin (89). Direct interactions can also occur between biopolymers of opposite net charge, by formation of an insoluble coacervate. Complex coacervation between, for example, gum arabic and gelatin has extensive practical use in microencapsulation. Ionic attraction may also be involved in the highly specific interaction of κ-carrageenan with κ-casein, to give a weak gel network of practical importance in, for example, preventing sedimentation of cocoa particles in chocolate milk desserts (90).

Finally, it has been suggested that in some systems the interactions of unlike polysaccharides may involve formation of specific co-operative junction zones analogous to those in single-component gel systems, such as carrageenans, but with the participating chains being heterotypic rather than homotypic. The mixed gels formed between alginate and pectin under acid conditions are believed to involve junctions of this type (91). As mentioned earlier, another example of co-operative synergism is between certain galactomannans (notably carob and tara gum) and certain helix-forming polysaccharides (agarose, xanthan, κ-carrageenan). In general, the phenomenon is believed to involve unsubstituted regions of the mannan backbone associating with the ordered conformation of the second polymer to create mixed-junction zones.

C. Phase-Separated Networks

When favourable interactions (such as those in polyanion-polycation systems) are absent, thermodynamic incompatibility between chains of dissimilar polysaccharides tends to cause each to exclude the other from its polymer domain, so that the effective concentration of both is raised. This is true even when the energies of interaction between the chains involved are small (disordered chain segments) in comparison with the much stronger interactions of ordered sequences in gum gels (92).

At low concentrations, thermodynamic incompatibility can promote conformational ordering within a single phase, which, for gelling systems can increase the rate of network formation (93). At higher concentrations, the system may separate into two discrete liquid phases. Generally, phase separation in protein-polysaccharide-water systems occurs only when the total concentration of the macromolecular components exceeds 4%, although there are variations from system to system (94). In the case of carboxyl-containing or sulphated polysaccharides, ionic strength and pH play an important role. For example, proteins and carboxyl-containing polysaccharides phase separate at pH values above the isoelectric point (at any ionic strength) or when the pH is equal to or less than the protein isoelectric point but the ionic strength is greater than ≈ 0.25 (95). Phase separation phenomena are manipulated in the food industry to develop novel textures, a notable example of which is low fat spreads, processed soft cheeses, low protein yoghurts and chocolate mousses. In these products, starch and its hydrolysates, pectin, alginates, gelatin and sodium caseinate play an important role (96).

VII. GUMS IN A HIGH SUGAR ENVIRONMENT

As described earlier, gums comprise an important class of confectionery products. These are based on a mixture of sucrose and glucose syrups and typically contain 10–25% moisture in the final product. A hydrocolloid is incorporated to control texture. Gummy sweets are made by preparing first the 'liquor,' which is a mixture with all the ingredients at 30% moisture content (97). Liquors are deposited hot (e.g. 70°C) into dry-powdered starch moulds and the excess moisture is extracted by stoving the sweets in these moulds for the required period of time at about 50°C.

Gelatin which for almost a century has been produced on an industrial scale is the most frequently used structuring agent in confectionery products, but is increasingly falling 'out of fashion' with consumers and producers alike (98). Reasons for the change in attitudes include the need to circumvent diet and health problems or perceptions such as the BSE scare, vegetarianism and religious dietary laws (e.g. Muslim and Hindu). Furthermore, high sugar/gelatin confectioneries tend to become sticky during handling, or when stored in warehouses at high ambient temperatures resulting in partial structural collapse, welding and crystallization (caking) of the product. For these reasons, there is an incentive to understand the behavior of polysaccharides in high sugar environments since this class of biopolymers may provide an alternative to gelatin.

Recent studies have mapped out the structural properties of gums in the presence of increasing levels of sugar (99). Small deformation dynamic oscillation described in the 'Viscoelasticity Section' of this chapter was used to identify the structural characteristics of the gums from the rubbery plateau (elastic consistency) through the transition region to the glassy state (brittle texture). In contrast to the collapse of the firmness of polysaccharide gels (agarose, gellan gum, κ-carrageenan) at intermediate levels of co-solute, gelatin forms reinforced networks between 40 and 70% sugar in the mixture (Figure 92.10). The drop in polysaccharide network strength is accompanied by a decline in the enthalpy of the coil-to-helix transition, whereas the transition enthalpy is more pronounced in gelatin gels in accordance with their strengthening (100). Tangible evidence of the molecular transformations was obtained using electron microscopy, with polysaccharides

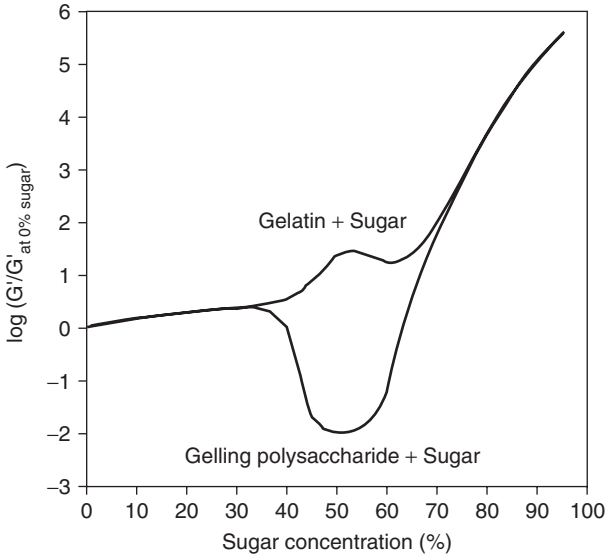

FIGURE 92.10 Variation of normalized shear modulus ($G'/G'_{at\ 0\%\ sugar}$) as a function of sugar concentration for polysaccharide/sugar and gelatin/sugar mixtures (from reference 100, with permission).

disaggregating and dissolving in the saturated sugar environment. Gelatin, on the other hand, is visualized in an aggregated form thus producing a phase-separated topology with sugar. It is speculated that this behaviour may have implications and lie behind the difficulties encountered in the commercial replacement of gelatin with small additions of gelling gums in high sugar confectionery products (101).

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93 Heat Induced Aggregation, Gelation and Phase Separation of the Globular Protein β-Lactoglobulin

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CONTENTS

I. INTRODUCTION

Generally, heat induced denaturation of globular proteins leads to aggregation of the proteins and above a characteristic concentration (C_g) eventually a gel is formed [1–3]. The aggregation process, the structure and the visual aspect of the gels depend on external conditions such as the pH, the ionic strength (C_s), the protein concentration (C) and the temperature (T). At a pH far from the isoelectric point (pI) and at low ionic strength the gels are generally transparent and electron microscopy shows that linear aggregates are formed. At high ionic strength or close to pI the gels are turbid and more densely branched aggregates are observed.

Among the most common globular proteins such as serum albumin (BSA), ovalbumin (OA) and lysozyme, β-lactoglobulin (β-lg) is the most intensively studied. β-lg is the major component of whey and has molar mass 18.4 Kg/mol and radius 2 nm [4–6]. At pH2 β-lg has a purely positively charge of +21, while at pH7 it has a net negative charge of −7, but contains both positively and negatively charged groups. The isoelectric point pI equals 5.2.

Apart from being a good model, β-lg is widely used in food industry and it is important to understand the heat induced aggregation process either because one wants to avoid it, e.g. in sterilization of milk, or because one wants to exploit it for building new textures for specific applications.

The purpose of this chapter is to present some of the major features of heat induced aggregation and gelation of β-lg based mainly on the research carried out in our laboratory over the last decade [7–22]. Experimental and technical details of the results shown in this chapter can be found in the original papers where the reader can also find references to the relevant literature. One aspect that has received relatively little attention until recently, is the influence of phase separation on the gel structure. We will address that issue here through a study of mixed systems of β-lg and κ-carragheenan (κ-car) [18–21]. The latter is a polysaccharide issued from blue algae and is fully

compatible with native β-lg under the conditions of our investigation. However, κ-car and β-lg aggregates are, under some specific conditions, incompatible, which leads to (micro) phase separation with important consequences for the gel structure. We will argue that the effect of micro phase separation may play an important role in the elaboration of new textures with new properties.

II. NATIVE PROTEIN CONSUMPTION

β-lg aggregates are stable to cooling and dilution and can be characterized in dilute solutions at room temperature [7, 14]. Figure 93.1 shows an example of the size distribution of β-lg after different heating times obtained by size exclusion chromatography (SEC). The chromatographs show a clear separation between a narrow peak corresponding to residual native proteins and a broad peak that corresponds to the aggregates. This observation implies that the minimum size of the aggregates contains already many monomers and that no or very few stable oligomers are formed. This separation is also observed in-situ in dynamic light scattering experiments both at pH7 and pH2 [7, 12].

With increasing heating time the aggregates grow in size and the size distribution broadens, while the fraction (F) of native proteins decreases. Unfortunately, the resolution of SEC is rather limited and already relatively small aggregates are fully excluded and no longer resolved. The evolution of F with heating time of β-lg at pH7 and 0.1 M NaCl salt is shown in Figure 93.2 for three temperatures. The rate with which the protein aggregates is strongly temperature dependent, but the shape of the time dependence is the same at each temperature. This is illustrated in Figure 93.3 where the data are plotted as a function of the heating time normalized by the time needed to aggregate half of the protein (t_h). The aggregation rate has an Arrhenius temperature dependence with a large activation energy

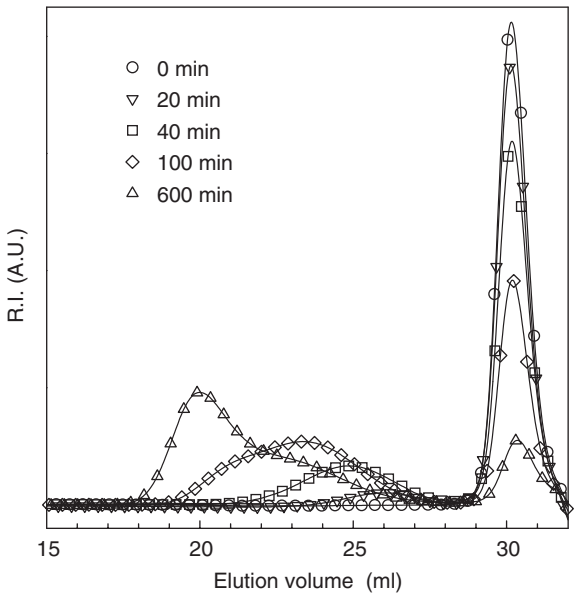

FIGURE 93.1 Chromatograms of β-lg solutions at pH7, 0.1 M salt and C = 18.6 g/L after different heating times at 60°C.

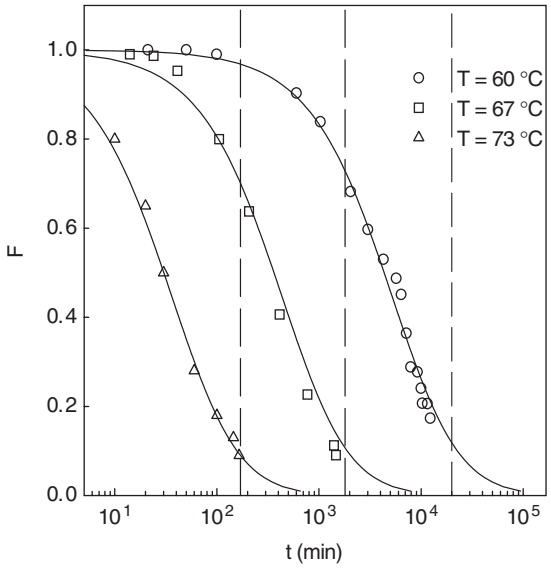

FIGURE 93.2 Heating time dependence of the unaggregated β-lg fraction (F) at pH7, 0.1 M salt and C = 18.6 g/L at different temperatures indicated in the Figure. The dashed lines indicate the gel time defined as the time when we first observed a fraction of non-soluble protein. The continuous lines represent a fit with a reaction order 1.5.

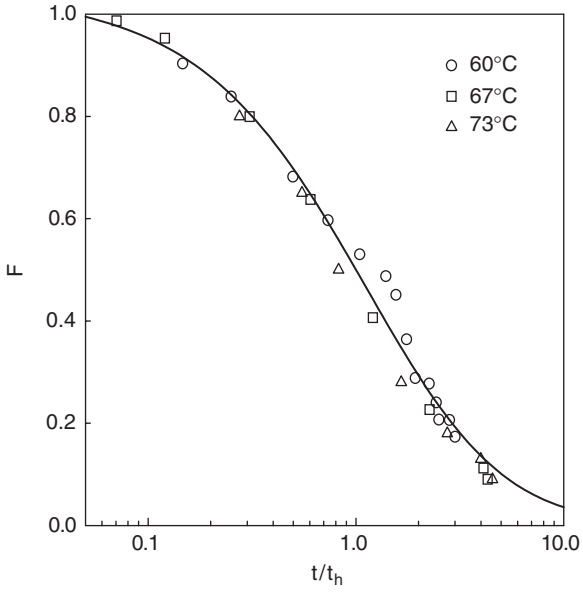

FIGURE 93.3 Same data as in Figure 2 plotted as a function of the heating time normalized by the time needed to consume half of the native proteins t_h. The continuous line represents a fit with a reaction order 1.5.

(250–400 KJ/mol) that depends weakly on the concentration and the ionic strength [13]. The temperature dependence of the aggregation rate is most likely controlled by the temperature dependence of the protein denaturation, which has a large activation energy because it is a co-operative process. For most of the situations that we have investigated the temperature has only an effect on the rate of aggregation and there is no indication for a critical gelation temperature other than the constraint that the gelation will take too long at lower temperatures [13].

The influence of the protein concentration on the aggregation process is more complicated. In general the rate of native protein consumption decreases with decreasing concentration [13, 23]. It has been investigated in detail for β-lg at pH7 and at 0.1 M NaCl ionic strength, for which it is shown that the aggregation rate can be characterized by an apparent order of 1.5 [13]. But, unexpectedly, at low protein concentrations not all proteins aggregate and the fraction of unaggregated β-lg (F) decays to a plateau value, F_p. Figure 93.4 shows examples of the dependence of F on heating time at 80°C for two ionic strengths at pH7. We do not claim that protein depletion stops entirely at F_p, but clearly it decreases relatively rapidly to a given value after which the decrease becomes logarithmically slow. For all ionic strengths, F_p increases with decreasing protein concentration. A systematic study of the concentration dependence of F_p over a range of ionic strengths shows that the proteins only aggregate above a critical association concentration, CAC, which decreases with increasing ionic strength, see Figure 93.5.

At pH2, as at pH7, the rate at which the protein is involved in the formation of aggregates increases with the temperature and the initial protein concentration. The ionic strength has also a strong influence on the decrease of the fraction of unaggregated β-lg (F) as shown in Figure 93.6. After long heating time, the fraction of unaggregated

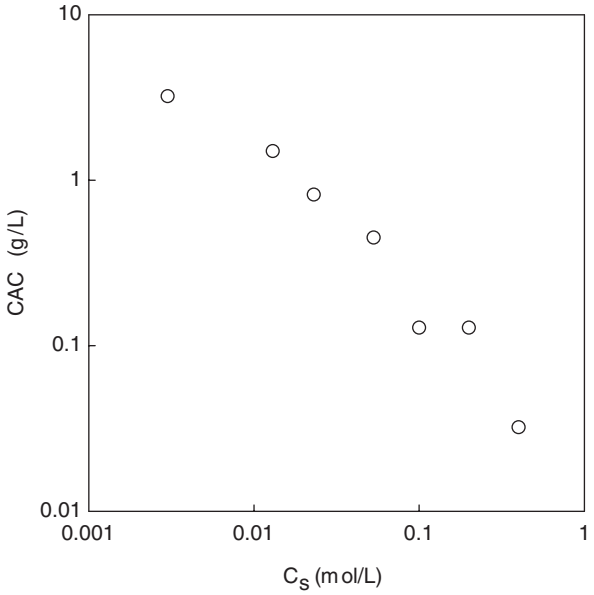

FIGURE 93.5 Ionic strength dependence of the critical association concentration (CAC) of β-lg solutions at pH7 heated 24 h at 80°C.

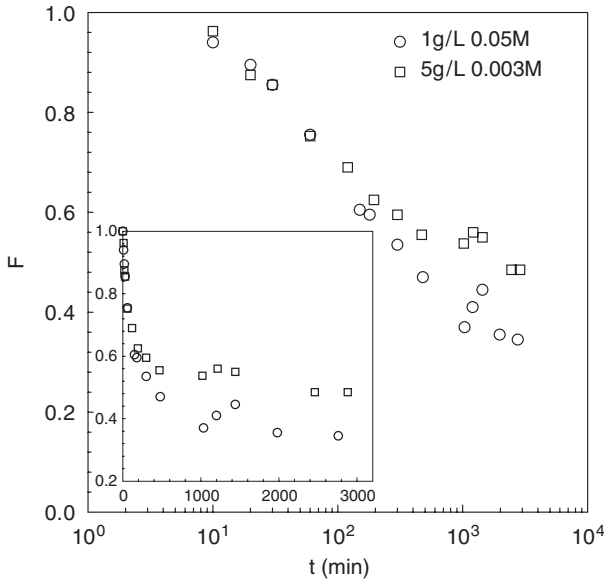

FIGURE 93.4 Time evolution of the fraction of unaggregated β-lg (F) at pH7 during heating at 80°C at different ionic strengths and concentrations indicated in the Figure. The insert shows the same data with linear time scale.

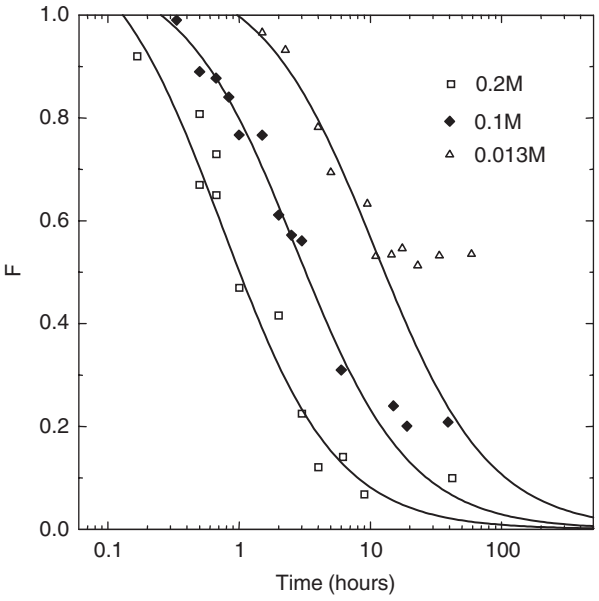

FIGURE 93.6 Time evolution of the fraction of unaggregated β-lg (F) at pH2 during heating at 80°C, C = 5 g/L for different ionic strengths indicated in the Figure. The continuous lines represent a fit with a fractional order 1.5.

protein stabilizes at a value that increases with decreasing ionic strength as was found at pH7.

III. AGGREGATE GROWTH AND GELATION KINETICS

Static light scattering (SLS) has proven to be a powerful technique to characterize the growth of the aggregates [11]. Figure 93.7 shows the scattering wave vector (q) dependence of the intensity scattered by the aggregates formed after different heating times at pH7 and 0.1 M NaCl salt. The solutions were highly diluted so that interaction may be neglected. The scattering intensity (I_r) is normalized by that of β-lg monomers at the same concentration, so that the value of I_r extrapolated to q = 0 corresponds to the weight average aggregation number (m_w), according to the relation $I_{rel}(q) = m_w.S(q)$ where S(q) is the structure factor of the aggregates [24, 25]. In Figure 93.7, clearly m_w increases with increasing heating time, while the q-dependence becomes more important because the average size of the aggregates increases The z-average radius of gyration of the aggregates (R_{gz}) can be obtained from the initial q-dependence of S(q) through the relation $S(q) = (1 + q^2R_{gz}^2/3)^{-1}$ for $qR_{gz} \ll 1$. At larger heating times this initial q-dependence can no longer be reached in the q-range accessible in the experiment. Very large aggregates show a power law q-dependence, $I_{rel}(q) \propto q^{-df}$, which means that the aggregates have a self similar structure with fractal dimension d_f [11, 24]. After dividing $I_r(q)$ by m_w and plotting the result, the so called structure factor S(q) [24, 25], as a function of $q \cdot R_{gz}$, we obtain a master curve, see Figure 93.8, which confirms that aggregates are self similar [11, 24]. We will discuss the structure of the aggregates in more detail in the section IV.

In the following part, we will examine the influence of the temperature, concentration and ionic strength on the kinetics of aggregation and gelation.

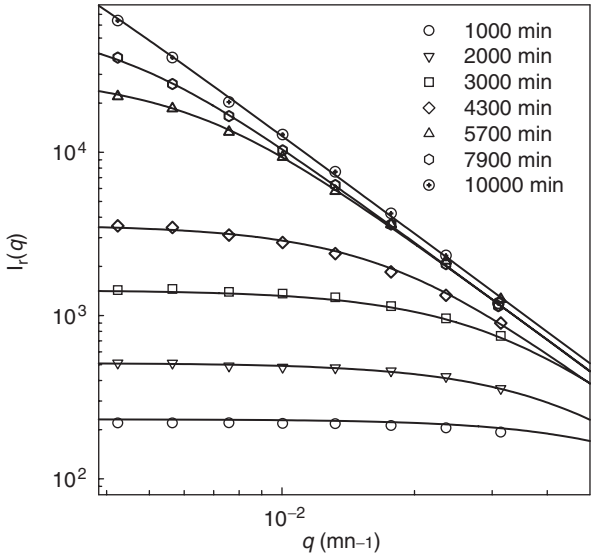

FIGURE 93.7 Scattering wave vector (q) dependence of the relative excess intensity of light scattered (I_r) by dilute solutions of β-lg aggregates formed after different heating times at 60°C, pH7, 0.1 M salt and C = 18.6 g/L. The heating times in minutes are indicated in the Figure. The solid lines represent fits to the equation $I_{rel}(q) = m_w (1 + q^2R_{gz}^2/3)^{-1}$. The straight line through the data at t = 10000 min. has slope −2.

A. Temperature Dependence

Figures 93.9 and 93.10 show, respectively, the effect of heating temperature on the aggregate growth and on the evolution of G″ and G′ at 0.1 Hz at pH7 and 0.1 M salt. It is clear that the gelation rate increases strongly with increasing temperature. However, there is not a significant effect of the temperature on the shapes of $m_w(t)$, G′(t) and G″(t). This is shown in Figures 93.11 and 93.12 where we have superimposed the data by time shifts. Master curves of two other protein concentrations are also shown in Figure 93.12.

In Figure 93.11 we have compared the evolution of m_w, with that of the loss (G″) and the storage (G′) shear moduli. The shear modulus was measured at 0.1 Hz in the linear regime (10% deformation) using a couette geometry [16]. We have defined here the gel time (t_g) as the time when we first observe an insoluble gel fraction after dilution indicated by the dashed line in Figure 93.11. Alternatively, one can define t_g as the time when m_w diverges or when G′ and G″ follow the same power law frequency dependence in the law frequency domain [26].

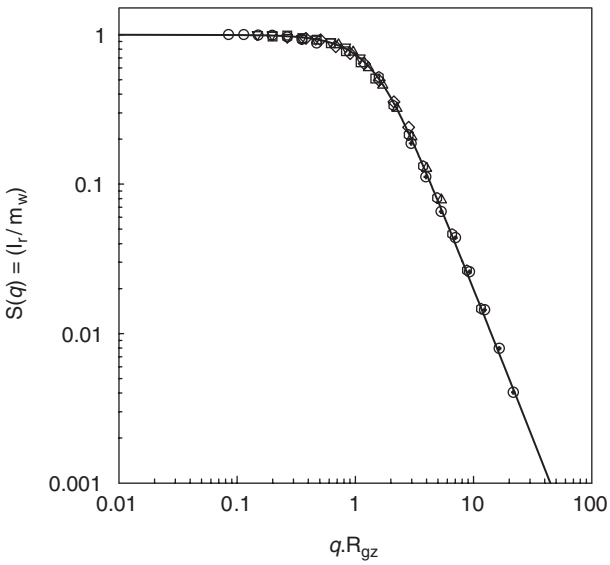

FIGURE 93.8 Same data as in Figure 7 with I_r normalized by the weight average aggregation number (S(q)) and q by the z-average radius of gyration R_{gz}. The solid line represents the theoretical structure factor $S(q) = (1 + q^2R_{gz}^2/3)^{-1}$.

These definitions give similar values for t_g, but they are less convenient for systematic investigation. Taking the observation of an insoluble gel fraction as t_g leads to an overestimation of t_g by at most 20%.

The temperature dependence of the gel time (t_g) is characterized by the same activation energy as that of the protein denaturation. This means that the temperature dependence of the gelation rate is controlled by the process of protein denaturation [13]. Above the gel time G′ increases rapidly at first and later continues to increase slowly over the whole duration of the measurement.

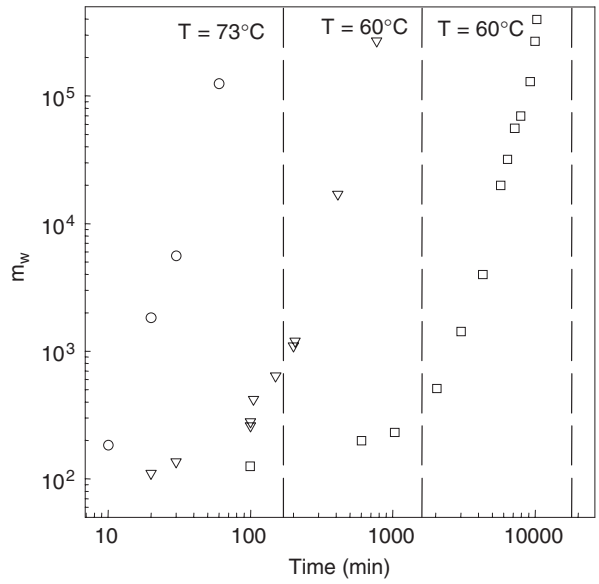

FIGURE 93.9 Time dependence of the weight average aggregation number (m_w) at different temperatures. Symbols are the same as in Figure 2. The dashed lines indicate the time (t_g) when we first observed a fraction of non-soluble protein.

B. Concentration Dependence

In order to form a gel the aggregates have to grow sufficiently large to fill up the whole space. If aggregates have

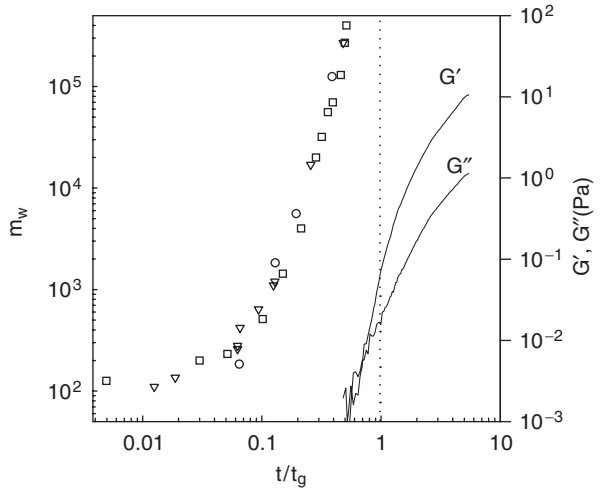

FIGURE 93.11 Same data as in Figure 9 normalized by the gel time and time evolution of the loss (G″) and storage (G′) shear modulus at 70°C (pH7, 0.1 M NaCl salt and C = 18.6 g/L). The dotted line indicates the time (t_g) when we first observed a fraction of non-soluble protein.

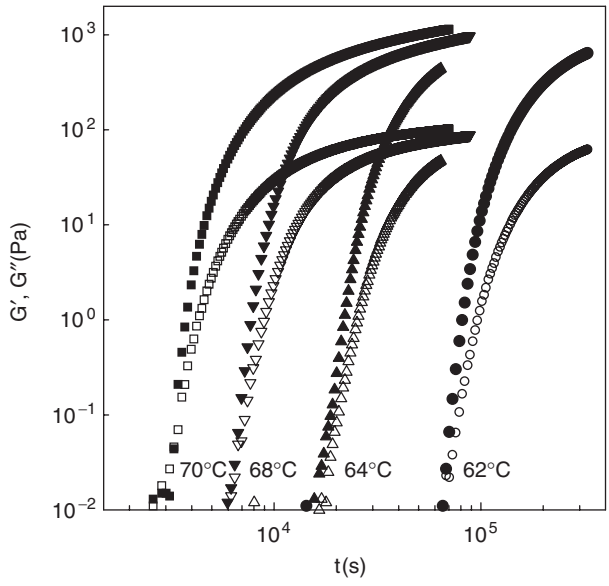

FIGURE 93.10 Evolution of the loss (open symbols) and storage (closed symbols) modulus at 0.1 Hz as a function of heating time for β-lg solutions (C = 5.0 g/L, 0.1 M NaCl, pH7) at different temperatures indicated in the Figure.

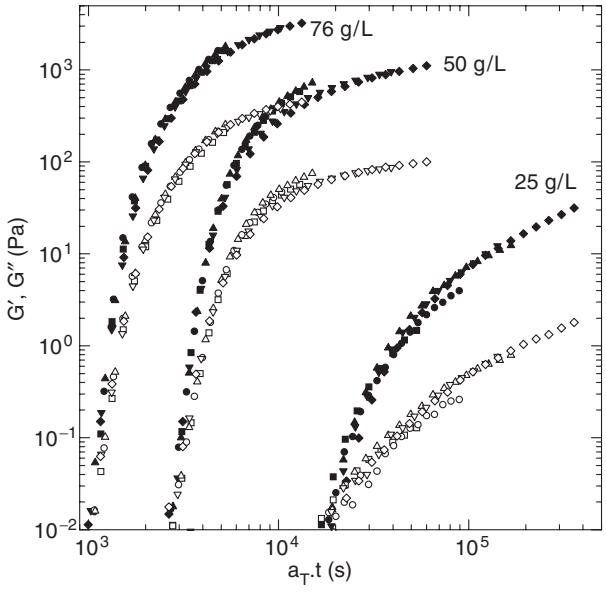

FIGURE 93.12 Master curves of the evolution of G′ and G′′ at reference temperature 70°C constructed by superposition of data obtained at different heating between 61 and 72°C for different β-lg concentrations indicated in the Figure.

a fractal structure there is no lower concentration limit for space filling and gelation to occur, as has been demonstrated by computer simulations [27]. Of course, as the concentration decreases the gel becomes less dense and weaker so that in practice there is always a lower concentration limit below which the gel modulus is no longer measurable. For globular proteins, however, a lower concentration limit for gelation (C_g) is observed for a different reason. This is illustrated in Figure 93.13, where we plotted the growth of the aggregates with heating time at different concentrations for β-lg at pH7 and 0.1 M CH_3COONH_4. Above a critical concentration $C_g = 7$ g/L we observe a clear divergence of the weight average aggregation number m_w after which a gel is formed. Below this concentration $C < C_g$, the growth of the aggregates stagnates when most native proteins have aggregated [13, 17]. The size of the aggregates, at which their growth stagnates, increases with the protein concentration. C_g depends strongly on the ionic strength, but also on the type of salt, see below.

In Figure 93.14 we show the evolution of G′ at 80°C for a range of concentrations at pH7 and 0.1 M NaCl. The gel time increases with decreasing concentration and diverges as $C \to C_g$. As expected, at a given temperature both the rate of gelation and the value of the modulus at the measured longest time increase with increasing concentration.

C. Ionic Strength Dependence

Figure 93.15 shows how m_w varies with the protein concentration at different ionic strengths between $3 \cdot 10^{-3}$ M and 0.4 M. The data were obtained for β-lg solutions at pH7 that were heated for 24 h at 80°C. After this prolonged heat treatment the growth of the aggregates has stagnated. For ionic strength up to $2 \cdot 10^{-2}$ M we observe a plateau at low concentrations. At these concentrations, only small so-called primary aggregates are formed. There appears to be a small increase of the mass of the primary aggregates with increasing ionic strength. The association of the primary aggregates into larger self-similar aggregates leads to an increase of m_w with increasing concentration and a divergence at C_g. The increase occurs at higher concentrations with decreasing ionic strength. For $C > C_g$ we observe the formation of a homogeneous gel if $C_s < 0.2$ M.

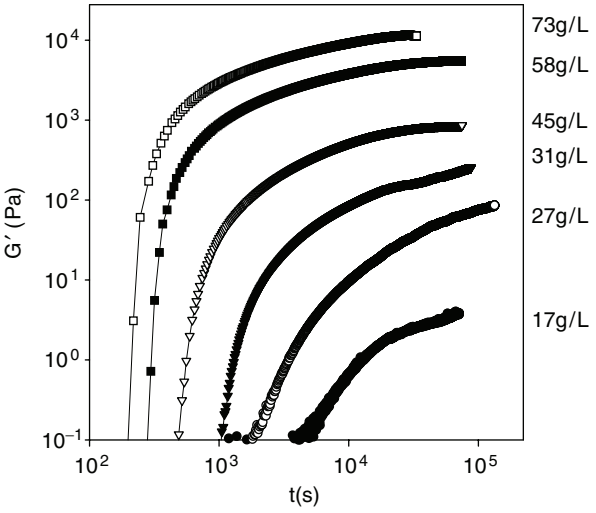

FIGURE 93.14 Evolution of the storage shear modulus at 0.1 Hz with heating time at 80°C for β-lg solutions at different concentrations indicated in the Figure (pH7 and 0.1 M NaCl).

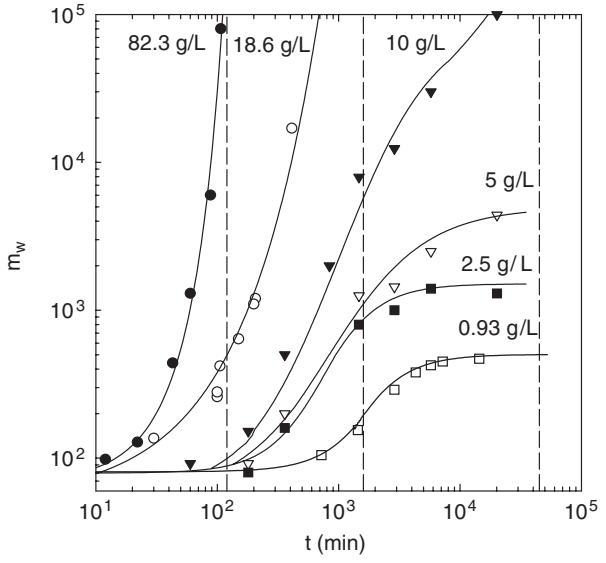

FIGURE 93.13 Growth of the weight average aggregation number (m_w) of β-lg aggregates formed after heating at 67°C for different concentrations indicated in the Figure (pH7, 0.1 M CH_3COONH_4). The vertical line indicates the gel point.

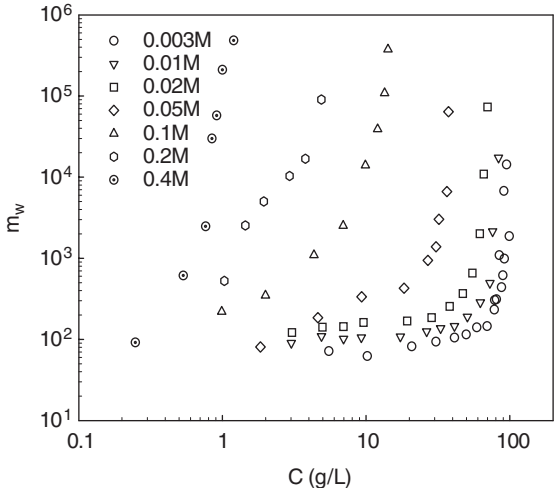

FIGURE 93.15 Concentration dependence of the weight average aggregation number (m_w) of β-lg aggregates formed at pH7 after heating 24 h. at 80°C at different ionic strengths indicated in the Figure.

However, at higher ionic strengths we observe a precipitation of protein flocs between C_g and about 10 g/L. We interpret this precipitation as the formation of a gel that is not sufficiently strong to resist gravity. Figure 93.16 shows the dependence of C_g on the ionic strength. C_g increases strongly with decreasing ionic strength, but the relative variation weakens below 0.02 M. We speculate that the latter effect is caused by the contribution of counterions that becomes important at very low ionic strength where C_g is large. C_g also depends on the type of added salt since at 0.1 M CH_3COONH_4 we observed $C_g = 7$ g/L while at 0.1 M NaCl we found $C_g = 15$ g/L. We note that in the presence of even a small amount of $CaCl_2$ (10^{-3} M) C_g is less that 1 g/L. C_g increases as well if the pH is further from the isoelectric point [28].

At pH2, as at pH7, the growth of m_w is faster when the temperature and the concentration are higher. A remarkable feature is that although the rate of protein depletion is low at low ionic strength, some long fibrilar aggregates are observed even if a vast majority of the proteins is unaggregated, see section IV.

IV. STRUCTURES OF β-lg AGGREGATES AND MECHANISMS OF AGGREGATION

We will discuss now the structure of aggregates formed at pH2 and pH7 and for each pH at low and high ionic strength. These 4 different conditions lead to 4 different situations that are in our opinion typical for a wide range of situations encountered in heat induced aggregation of globular proteins.

At pH7 and 0.1 M salt, as seen in Figure 93.8, the static structure factor S(q) obtained from scattering techniques presents for $q \cdot R_{gz} \gg 1$ a power law behavior $S(q) \propto q^{-df}$ which means that aggregates are self similar with a fractal dimension $d_f = 2$. Independent information about the structure of the aggregates can be obtained from Figure 93.17 where m_w is plotted versus R_{gz}. For fractal objects $m_w = aR_{gz}^{df}$, where the prefactor a depends on the local structure of the aggregates and their size distribution [11, 24]. The value of d_f is consistent with the measured static structure factors and confirms independently $d_f = 2$. The lower limits of R_{gz} and m_w are given by that of the primary aggregates.

At low ionic strength 3×10^{-3} M and low concentrations (see Figure 93.15), the growth leads only to the formation of primary aggregates which have a weight average aggregation number of about 100 and a hydrodynamic radius of about 15 nm. Figure 93.18 shows the structure factors of the aggregates at pH7 at low and high ionic strength by a combination of SLS and Small Angle Neutron Scattering (SANS). SANS measurements were done in D_2O in which the primary aggregates are a bit smaller. The large scale structure of the aggregates formed at 0.1 M is again characterized by a fractal dimension $d_f = 2$. The internal cut-off for the fractal regime corresponds to a weight average aggregation number of about 100, which confirms that the larger aggregates are formed by random association of the primary aggregates. Figure 93.19 shows the combination of Small Angle Light Scattering (SALS), SLS and SANS data for dilute solutions of very large aggregates at pH7 0.1 M. $I_r(q)$ has a power law dependence on q in the range $2 \times 10^{-4} < q < 2 \times 10^{-1}$ nm^{-1} which implies that the aggregates have a fractal structure

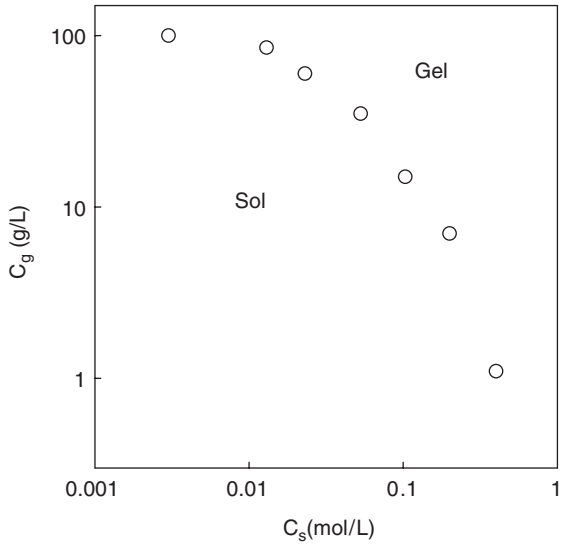

FIGURE 93.16 Ionic strength dependence of the critical gelation concentration for β-lg solutions at pH7 heated 24 h at 80°C.

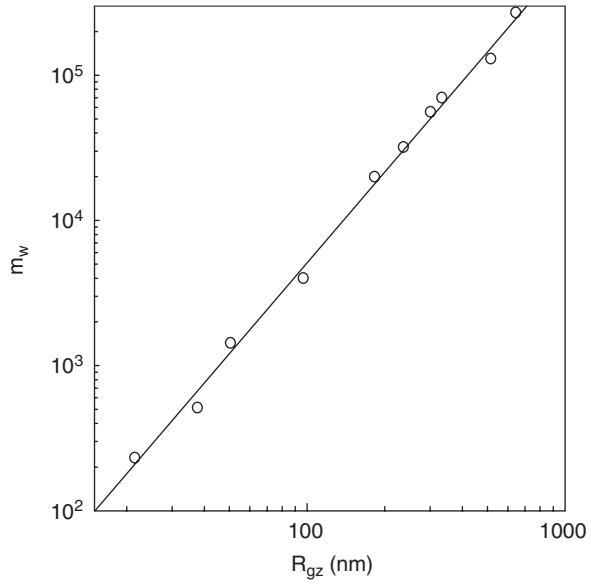

FIGURE 93.17 Weight average aggregation number (m_w) of β-lg aggregates formed after different heating times at 60°C, pH7, 0.1 M salt and C = 18.6 g/L as a function their z-average radius of gyration.

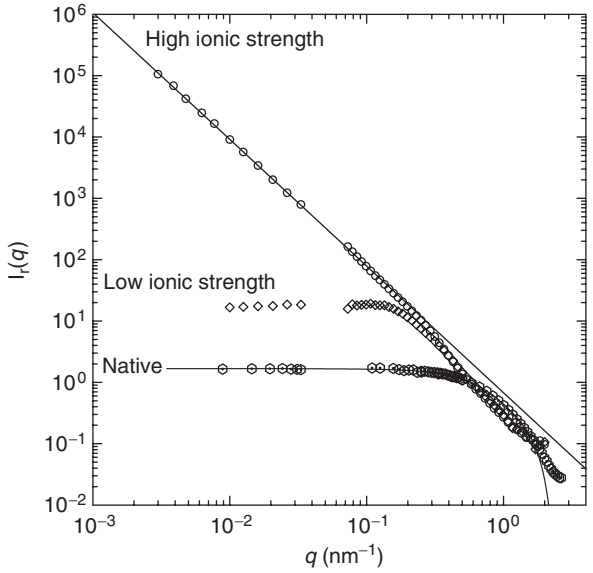

FIGURE 93.18 Scattering wave vector (q) dependence of the relative excess intensity of light (I_r) scattered by dilute solutions of β-lg aggregates formed after heating 24 h at 80°C, pH7 and two ionic strengths 0.1 M (o) and 0.003 M (◇). The straight line through the data at 0.1 M (o) has slope −2. For comparison we also show the results for native protein.

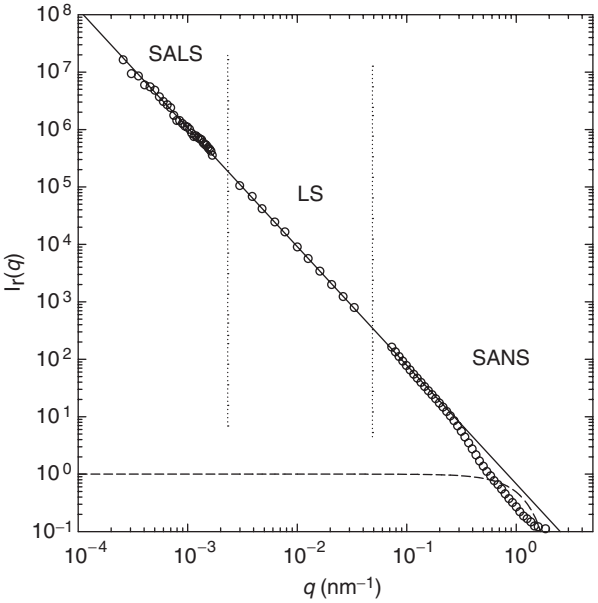

FIGURE 93.19 Scattering wave vector (q) dependence of the relative intensity (I_r) scattered by dilute solutions of native β-lg (dotted line) and very large β-lg aggregates formed pH7, 0.1 M (o). The straight line through the data has slope −2.

over at least three orders of magnitude up to several micrometers. The weight average aggregation number is much bigger than 10^7 protein monomers.

The structure of the aggregates may also be investigated at larger scales using microscopy techniques. Figure 93.20 shows micrographs of β-lg aggregates obtained using Cryo-Transmission Electron Microscopy (Cryo-TEM). At pH 7 small elongated aggregates are formed at low ionic strength (3×10^{-3} M), while at high ionic strength (0.1 M) we observe larger aggregates that appear to be formed by random association of the small aggregates. At low ionic strength the primary aggregates are inhibited from further association, probably due to electrostatic repulsion. However, at high protein concentrations we do observe further association partially caused by the presence of protein counterions that increases the ionic strength. The structure of the aggregates does not depend on the heating temperature nor on the protein concentration although higher concentrations are needed to form large aggregates at lower ionic strength, see Figure 93.16.

The large scale fractal structure is little influenced by the ionic strength, but the local structure varies with the ionic strength [17]. The aggregates become significantly denser for ionic strengths higher than 0.05 M. The origin of this local densification is probably the stronger branching of the aggregates at higher ionic strength. From Cryo-TEM and X-ray scattering experiments it appears that at low ionic strength the primary aggregates associate mainly head to tail, while at high ionic strength side ways association leading to branching becomes more common [29].

The aggregation at pH 2 is different. At pH2 thin linear aggregates are formed which are very rigid at low ionic strength (10^{-2} M) and flexible at high ionic strength (0.2 M), see Figure 93.20. In Figure 93.21 we compare the structure factor of large aggregates formed at pH 2 at different ionic strengths. The amount of residual non-aggregated proteins is negligible for these samples. Over some q-range $I_r \propto q^{-1}$, which is characteristic for a rigid rod-like structure. The q-range over which $I_r \propto q^{-1}$ decreases with increasing ionic strength and is no longer visible at 0.2 M. The fit of the data with the structure factor of large worm-like chains leads to estimate the persistence lengths as 38, 300 and 600 nm at ionic strengths 0.1 M, 0.03 M and 0.013 M, respectively. The value at 0.013 M should be considered as a minimum value, as the deviation from $I_r \propto q^{-1}$ is small. Notice that at high ionic strength the values of d_f happen to be the same for the aggregates formed at pH2 and pH7 even though it is obvious from Figure 93.22 that their local structure is very different. The elementary unit of the fractal structure at pH7 is the primary aggregate while at pH2 it is the persistence length of the strands.

The hydrodynamic radius (R_h) and the degree of flexibility of the aggregates can be probed by dynamic light scattering (DLS). The q-dependence of the apparent diffusion coefficient (D) was measured for highly diluted aggregates formed at pH7 for different ionic strengths. For rigid spherical particles D does not depend on q even for $qR_h > 1$. For rigid fractal aggregates D increases somewhat with increasing q due to the influence of rotational diffusion. Finally, for flexible particles one expects $D \propto q$

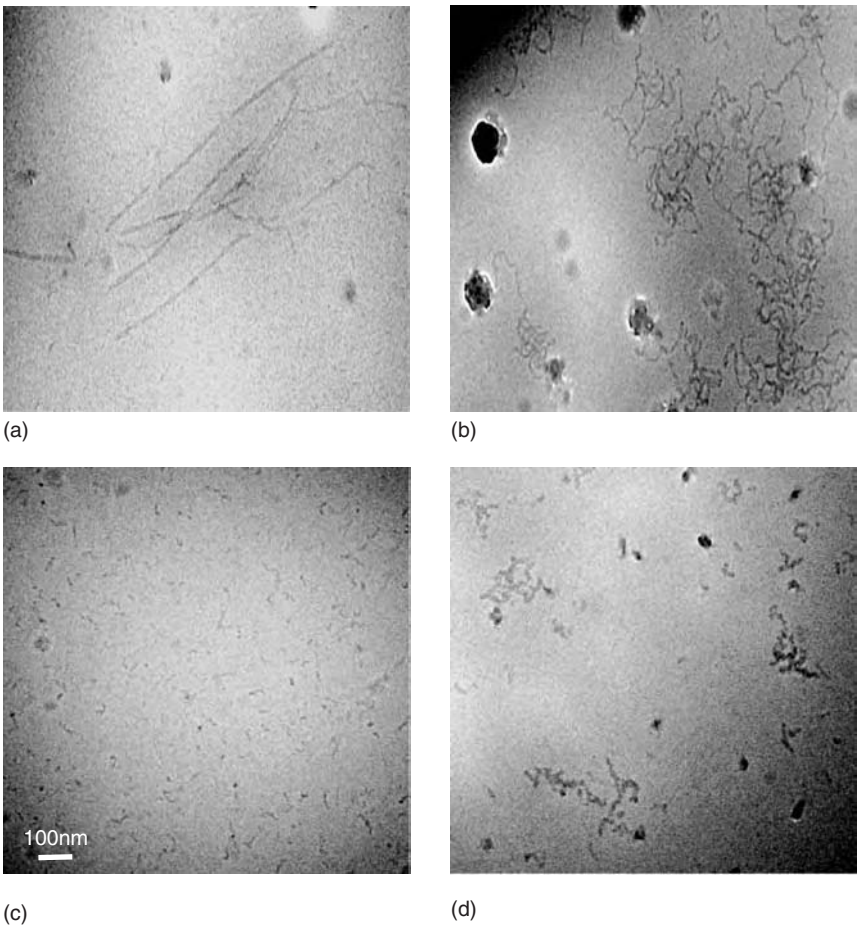

FIGURE 93.20 Cryo-TEM micrographs of β-lg aggregates formed upon heating 24 h at 80°C at pH2, 0.01 M (a); pH2, 0.2 M (b); pH7, 0.003 M (c); pH7, 0.1 M (d).

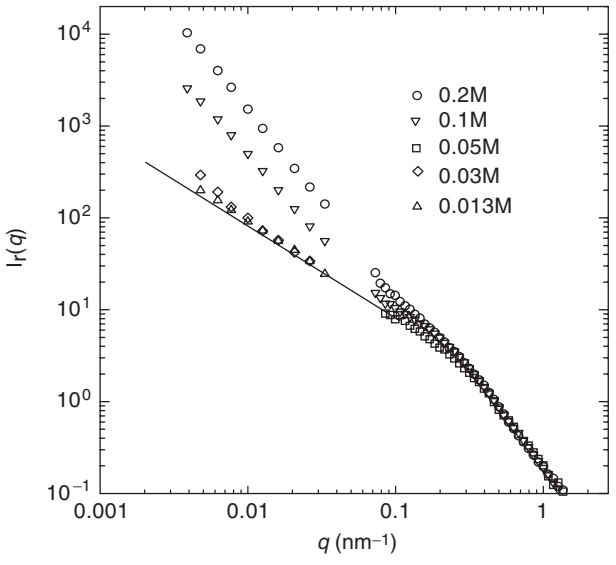

FIGURE 93.21 Scattering wave vector (q) dependence of the relative intensity scattered by dilute solutions of large β-lg aggregates formed after heating at 80°C, pH2 and various ionic strengths indicated in the Figure. The straight line has a slope −1.

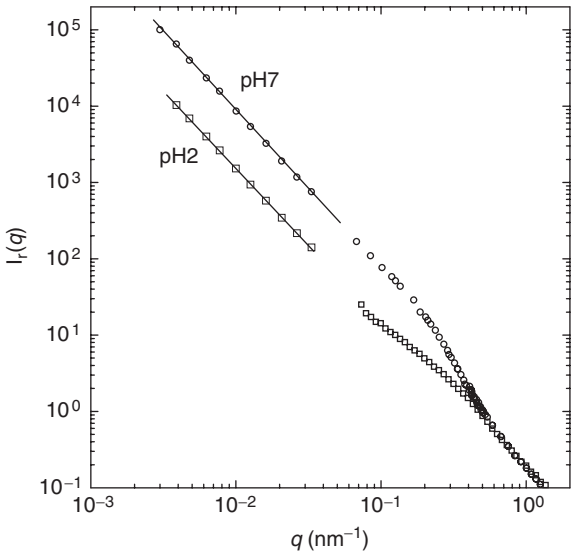

FIGURE 93.22 Comparison of the q-dependence of I_r at pH7 and pH2 at 0.2 M.

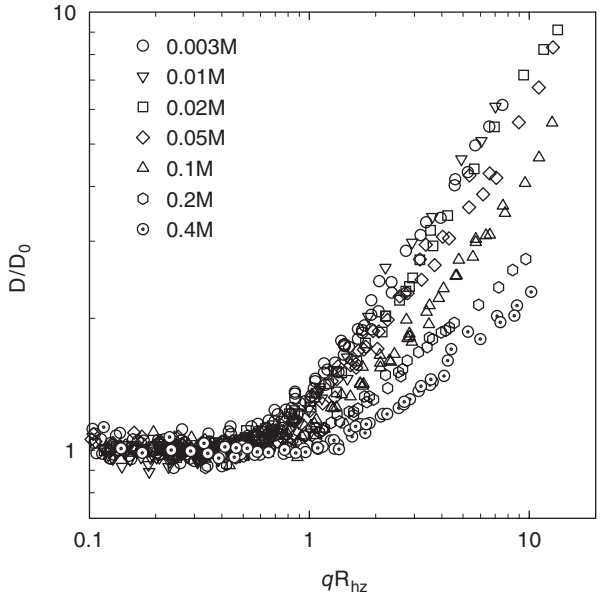

FIGURE 93.23 Dependence of D/D_0 on $q.R_{hz}$ for highly diluted β-lg aggregates formed at ph7 for different ionic strengths. The master curves were constructed by superposition of the data obtained from aggregates that were formed by heating protein solutions for 24 h at 80°C at different protein concentrations.

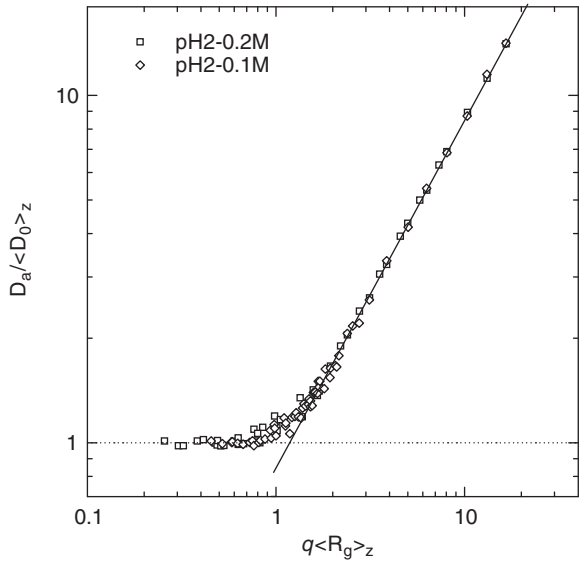

FIGURE 93.24 Dependence of D/D_0 on $q \cdot R_{hz}$ for highly diluted β-lg aggregates formed at pH2 0.1 M and 0.2 M. The master curves were constructed by superposition of the data obtained from aggregates that were formed by heating protein solutions at 80°C during various times.

for $qR_h \gg 1$. If D_0 denotes the extrapolated value of D at $q \to 0$, master curves can be obtained by plotting the values of D/D_0 obtained at different protein concentrations as a function of qR_{hz} [7, 14]. Figure 93.23 shows master curves obtained at different ionic strengths. In all cases D increases with increasing q for $qR_{hz} > 1$, but the increase is more important at lower ionic strength implying that the aggregates formed at lower ionic strength are more flexible. The lower flexibility at higher ionic strength is probably caused by the increasing degree of branching of the aggregates. The full flexibility which leads to $D \propto q$ is only reached at large length scales, i.e. for $qR_{hz} > 10$ [14, 17].

Figure 93.24 shows the dynamic structure factor of diluted aggregates formed after different heating times at 80°C at pH2 at 0.1 M and 0.2 M salt. For $qR_{gz} \gg 1$, the linear q dependence of the apparent diffusion coefficient shows that the aggregates formed at high ionic strength are fully flexible. At lower ionic strength a weaker q-dependence of D was observed because the aggregates are more rigid.

The aggregation of β-lg occurs in first instance by a unidirectional growth. This first step of the aggregation leads to particles of a small and rather well defined size at pH7, but at pH2 it leads to large linear aggregates. One may speculate that the aggregation is initially a process of nucleation and growth. This is especially clear at pH2 and low ionic strength, where immediately very large rod-like aggregates are formed even if only a tiny fraction of the native proteins has been consumed. But also at all other conditions investigated we never observed the formation of oligomers. At present we do not know why the initial aggregation of β-lg stops at a relatively small size at pH7. The formation of well defined primary aggregates was not observed for ovalbumin at similar conditions [30].

The second step of the aggregation, which is clearly distinguished at pH7, involves the entirely different process of random association of the primary aggregates. It is difficult to judge from the micrographs shown in Figure 93.20 to what extent this further association also takes place at pH2. Some further association at pH2 seems implied by the fact that gels are also formed at pH2. With the increase of the ionic strength, the aggregates are more flexible and some branching appears. Figures 93.25 and 93.26 show schematic drawings of the aggregation processes at pH7 and pH2 respectively.

V. STRUCTURE AND RHEOLOGICAL PROPERTIES OF THE GELS

The structure of globular protein gels is traditionally described in terms of finely stranded and particulate gels, but mixed structure are also observed [31]. β-lg at pH2 leads to finely stranded gels, while β-lg at pH7 and high ionic strength leads to particulate gels. Clearly, the fine strands seen in the gels at pH2 are simply more or less associated linear aggregates. On the other hand micrographs of so-called particulate protein gels typically show dense domains with a characteristic size that varies with the ionic strength [32], the pH [23] and the heating conditions [33].

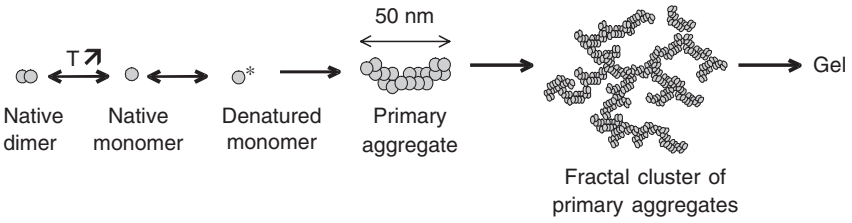

FIGURE 93.25 Schematic representation of the gel formation of heated β-lg at pH7 and 0.1 M salt.

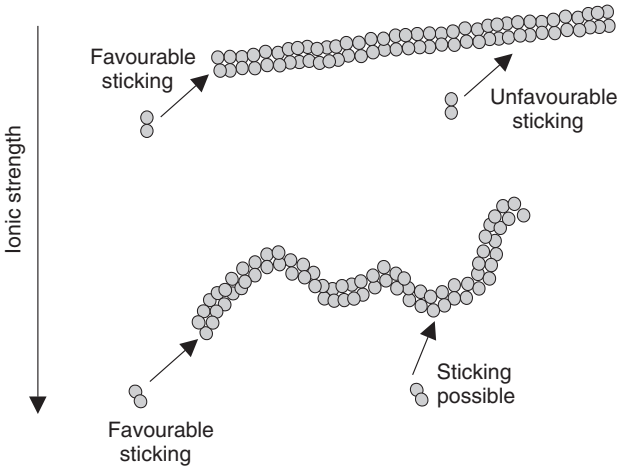

FIGURE 93.26 Schematic representation of the gel formation of heated β-lg at pH2 at low and high ionic strengths.

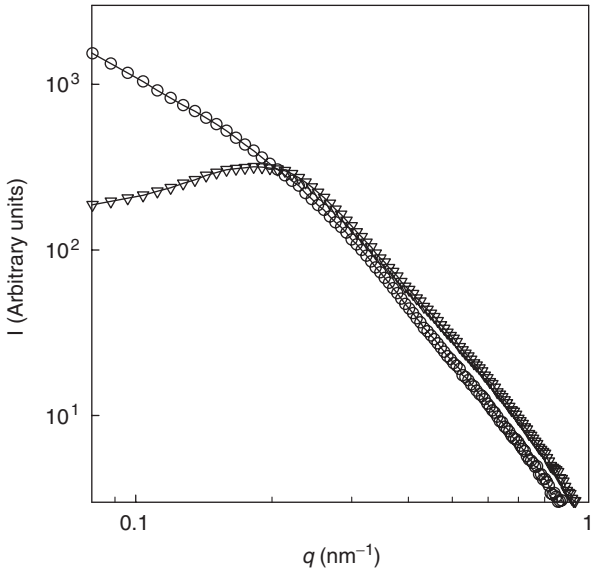

FIGURE 93.27 X-ray scattering wave vector (q) dependence of the intensity scattered by β-lg gels formed after heating 20 h at 80°C β-lg solutions at 60 g/L, pH7 and two ionic strengths 0.1 M (squares) and 0.01 M NaCl (triangles).

The dense domains result from micro phase separation, which is observed very clearly in mixed systems.

The structure factor of heat-set protein gels at small length scales has been studied using neutron and X-ray scattering [29, 34, 35]. They show for transparent gels a maximum caused by electrostatic interactions. Transparent gels are homogeneous at length scales larger than a few tens of nanometers and thus show no q-dependence over the q-range covered by light scattering. When the ionic strength is increased the interaction peak disappears and instead an increase of the scattering intensity is observed at low scattering wave vectors (q), see Figure 93.27. So far structure factors of turbid gels could not be studied at the smaller q-values covered by light scattering because multiple scattering influences the results. However, it has recently been shown that the structure factor of turbid globular protein gels can be determined using cross-correlation dynamic light scattering [16, 36].

This new experimental technique has been used to study the relationship between the rheological properties of globular protein gels and their structure in the specific case of β-lg at pH7 and 0.1 M salt.

A. Gel Structure

As we have already mentioned, the structure of heated globular protein solutions and gels is determined by the interplay between the growth of the aggregates and the electrostatic interaction between the aggregates. This interplay leads to a maximum turbidity and heterogeneity at some protein concentration. Figure 93.28 shows the turbidity of a series of β-lg solutions at pH7 and 0.1 M NaCl that were heated at 80°C during 24 h. The turbidity increases due to the increase of the concentration and the aggregate size up to $C_{max} \approx 30$ g/L, then decreases at higher concentrations because repulsive electrostatic interactions start to dominate. The repulsive electrostatic interactions are screened by added salt. Therefore they increase with decreasing ionic strength. As a consequence the turbidity at a given concentration decreases with decreasing ionic strength, see Figure 93.28. There is no direct correlation between C_{max} and the critical gelation concentration C_g. The weaker is the electrostatic interaction the smaller is C_g compared to C_{max}.

Figure 93.29 shows the q-dependence of I_r of a series of β-lg solutions at pH7 0.1 M salt that were heated at 80°C during 24 h (the fraction of unaggregated protein is about 5% over the whole range of concentrations

investigated). By analogy with the highly diluted system, we define an apparent aggregation number (m_a) which is the value of I_r extrapolated to $q = 0$ and an apparent radius R_a. After dividing $I_r(q)$ by m_a and plotting the result as a function of $q \cdot R_a$, we obtain the structure factor of the samples, see Figure 93.30. The solid line through the data represents the structure factor of the diluted aggregates (replacing R_{gz} by R_a). These two structure factors

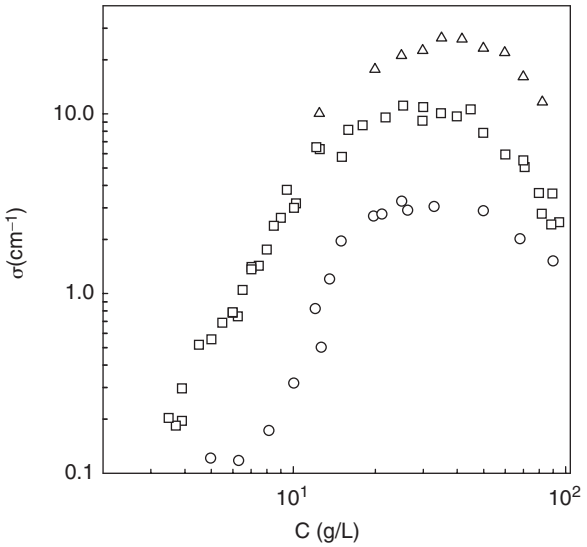

FIGURE 93.28 Concentration dependence of the measured turbidity of β-lg solutions at pH7 after heating 24 h at 80°C for three ionic strengths 0.1 M (circles), 0.15 M (squares) and 0.2 M (triangles).

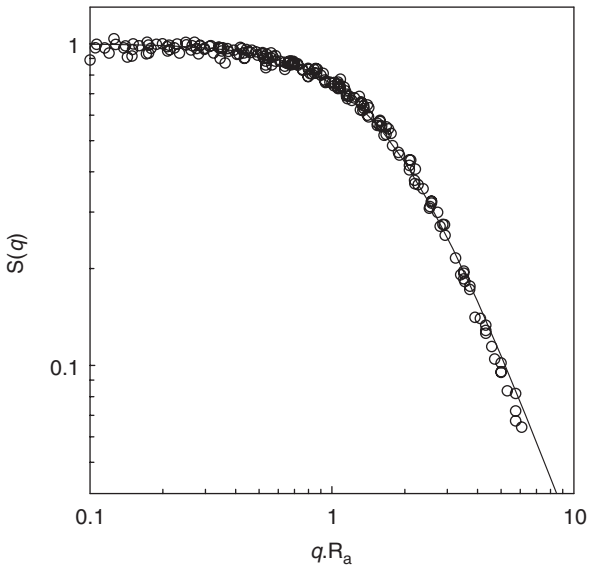

FIGURE 93.30 Structure factor of β-lg solutions at different concentrations after heating 24 h at 80°C (pH7 and 0.1 M NaCl). The solid line represents $S(q) = (1 + q^2R_a^2/3)^{-1}$.

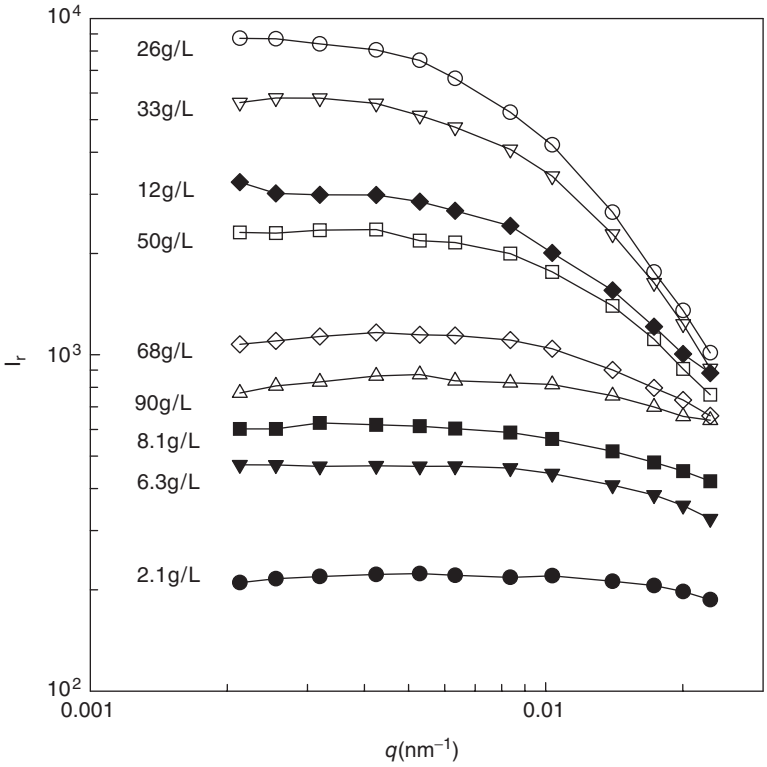

FIGURE 93.29 Wave vector dependence of I_r for β-lg solutions at different concentrations indicated in the Figure after heating 24 h at 80°C (pH7, 0.1 M NaCl). Filled symbols represent data for $C < C_g$ and open symbols represent data for $C > C_g$.

are almost the same. This is consistent with the Figure 93.31 that compares the dependence of m_a on R_a with the dependence of m_w on R_{gz} for the diluted aggregates. The solid line in Figure 93.32 has slope 2 implying that $d_f = 2$.

The concentration dependence of R_a and R_{gz} is shown in Figure 93.32. For very dilute solutions interactions between the aggregates are small and $R_a \approx R_{gz}$. With increasing concentration the aggregates become bigger and R_{gz} diverges at C_g. However, R_a remains finite due to excluded volume interaction that increases with increasing concentration. Above $C \approx 20$ g/L interaction dominates and R_a decreases approximately linearly with C: $R_a \propto C^{-1.1}$ [16]. The system may be described as a collection of randomly close packed blobs with radius R_a and apparent aggregation number m_a if $R_a \propto C^{1/(d_f - 3)}$ and $m_a \propto R_a^{d_f}$. These relations are consistent with the experimental results for $C > 20$ g/L with $d_f = 2.0 \pm 0.1$.

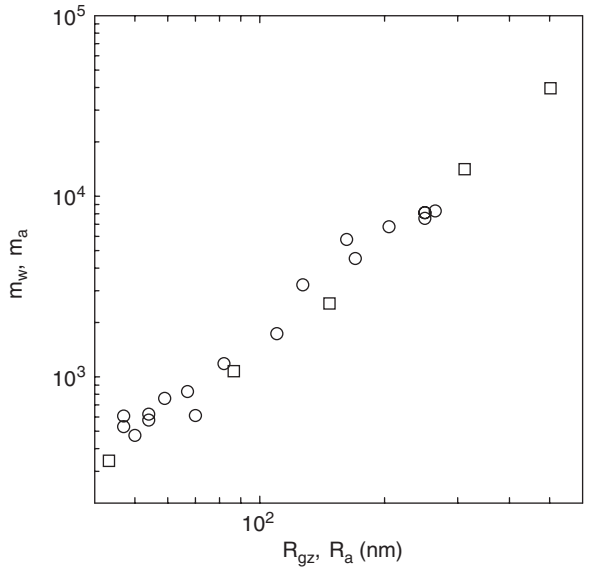

FIGURE 93.31 Comparison of the dependence of m_a on R_a (circles) with that of m_w on R_{gz} (squares). The solid line represents $m_a = 3 \times 10^3 R_a^2$.

B. Shear Modulus

Figure 93.14 shows the evolution of the storage shear modulus (G′) at 0.1 Hz for different concentrations at pH7 and 0.1 M NaCl during heating at 80°C. Above the gel time G′ increases rapidly at first and later continues to increase slowly over the whole duration of the measurement. The gel time increases with decreasing concentration and diverges as $C \to C_g$. As G′ is almost independent of the frequency in the range 0.01–10 Hz and $G' \gg G''$ we consider G′ at 0.1 Hz as the elastic modulus G_0. The concentration dependence of G′ after 24 h heating is shown in Figure 93.33. After 24 h heating at 80°C almost all the native proteins have aggregated, so that the sol fraction is negligible except close to C_g. For $C > 20$ g/L the concentration dependence can be approximated by a power law dependence: $G' \propto C^{4.5}$. At lower concentrations, G′ decreases strongly and the sol-gel transition occurs approximately at $C_g = 15$ g/L. Renard et al. [34] measured the concentration dependence of G′ for β-lg after heating at 80°C for 1 h under the same conditions and observed a stronger concentration dependence ($G' \propto C^{6.1}$) in their tested concentration range 25–60 g/L. Verheul and Roefs [37] found

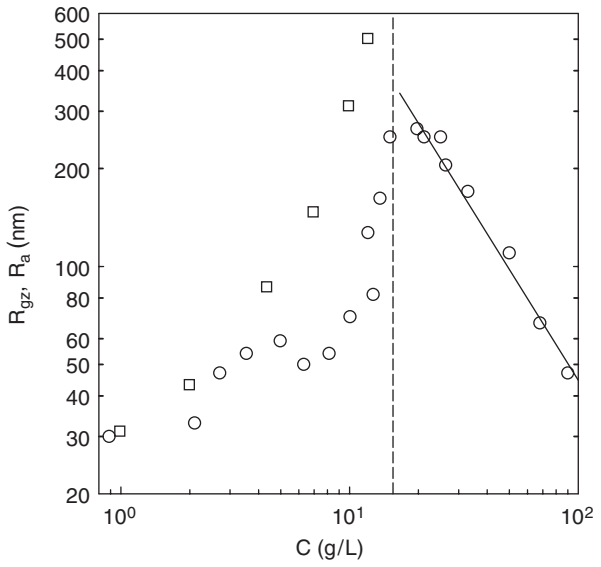

FIGURE 93.32 Comparison of the concentration dependence of R_a (circles) and R_{gz} (squares). The dashed line indicates the gel concentration, C_g, and the solid line has slope −1.1.

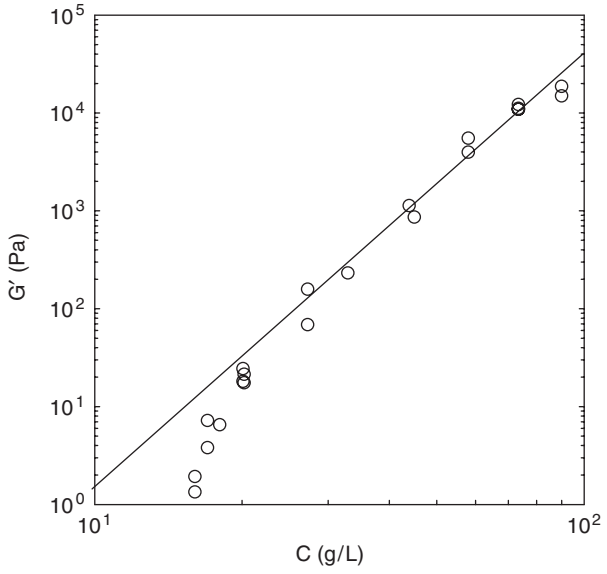

FIGURE 93.33 Concentration dependence of the storage shear modulus at 0.1 Hz of β-lg solutions after heating 24 h at 80°C (pH7, 0.1 M NaCl). The solid line has slope 4.5.

$G' \propto C^{4.5}$ in the range 35–80 g/L for a whey protein isolate with 70% β-lg after heating at 68.5°C for 20 h. Obviously, the concentration dependence of G′ depends on the heating time (and temperature), because G′ evolves differently at different concentrations especially close to C_g. E.g. we also found a stronger concentration dependence of G′ after heating for 1 h at 80°C. Therefore one should be cautious when comparing data obtained with different heat treatments.

Light scattering shows that at C_g the aggregates are already strongly interpenetrated. The sol-gel transition may therefore be described as a bond percolation process of close packed blobs. Whereas light scattering is sensitive to the structure of the whole system, the elastic modulus is determined by the structure of the gel fraction. Percolation of blobs would explain the initial rapid increase of the modulus above t_g for a given concentration (see Figure 93.10) and above C_g after prolonged heating. For $C > 20$ g/L, where C has a power law dependence, all the blobs can be considered as connected and the structural unit of the gel may be identified with R_a.

According to the model of fractal gels [38], $G_0 \propto C^n$ with $n = (3 + d_b)/(3 - d_f)$ where d_b is the fractal dimension of the elastic backbone that is necessarily between unity and d_f. The experimental values $n = 4.5$ and $d_f = 2$ leads to $d_b \approx 1.5$. After cooling to 20°C the gel modulus increases by a factor 2–3, but the concentration dependence is the same as already reported by Renard et al. [34]. After 20°C the elastic modulus is larger. The temperature dependence of the elastic modulus is probably caused by a temperature dependence of the bending constant of the individual bonds.

The frequency dependence of the shear modulus was determined by performing frequency sweeps of G′ and G″ during the gelation process [22]. The values of G′ and G″ at equal times were obtained by interpolation. The values of G′ at all heating times can be superimposed by vertical and horizontal shifts which implies that the system is self-similar at different stages of the gelation process. Master curves obtained in this way are shown in Figure 93.34 for 4 concentrations. These master curves show that the frequency dependence of the shear modulus of fractal globular protein gels is self-similar at different concentrations and heating times. G′ has a close to linear frequency dependence at high frequencies which crosses over to a very weak frequency dependence at low frequencies. The high frequency dependence can be understood in terms of internal relaxation of the fractal blobs that form the gel. The low frequency decay indicates that the relaxation on the larger length scales is not fully elastic.

We conclude that the particular heat-set protein gels that we studied here (pH7, 0.1 M) can be successfully described as ensembles of close packed fractal blobs with an elastic energy dominated by their bending energy [16]. However, this description is only valid if the concentration and heating times are sufficiently high so that the sol fraction

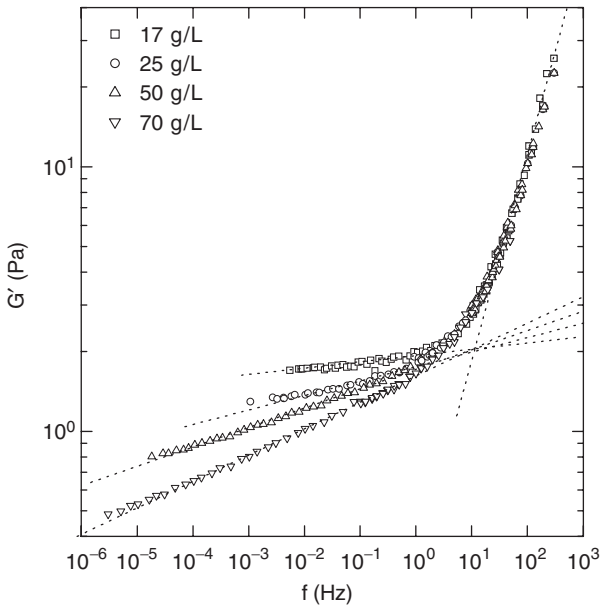

FIGURE 93.34 Master curves of the frequency dependence of G′ obtained from superposition of the data obtained at different heating times for different β-lg concentrations (0.1 M NaCl, pH7).

can be ignored and if the interaction between the protein are short range and repulsive. Long range electrostatic interaction decreases R_a as was observed at lower ionic strengths and can even lead to a peak in the static structure factor. In addition, if electrostatic interactions are important generally linear aggregates are formed that are only weakly branched. On the other hand close to the isoelectric point or at very high ionic strength attractive interactions may lead to the formation of heterogeneous gels containing dense micro-domains. Therefore the fractal gel model is valid for heat-set protein gels only over a limited range of concentrations, pH and ionic strength.

VI. AGGREGATION INDUCED PHASE SEPARATION

As we have already mentioned, aggregation can induce a phase separation if the ionic strength is high enough. Another way to induce phase separation is to add a polysaccharide. We have investigated the aggregation and gelation of β-lg at pH7 in the presence of κ-car [18–21]. At the conditions used in our investigation the two biopolymers are both negatively charged and are compatible. Figure 93.35 compares micrographs of gels with and without κ-car. Clearly the presence of κ-car leads to much more heterogeneous gels containing domains rich in β-lg with the polysaccharide situated between these domains. Under certain conditions of high κ-car concentrations and slow aggregation, we observe a macroscopic phase

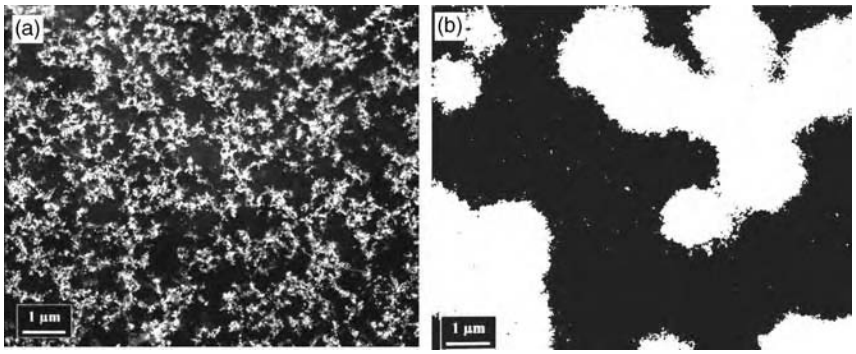

FIGURE 93.35 TEM micrographs of β-lg gels formed after 2 hours at 78°C pH7, 0.1 M NaCl, with 60 g/L β-lg (a) and 60 g/L β-lg + 4 g/L κ-Car (b). Protein domains are white.

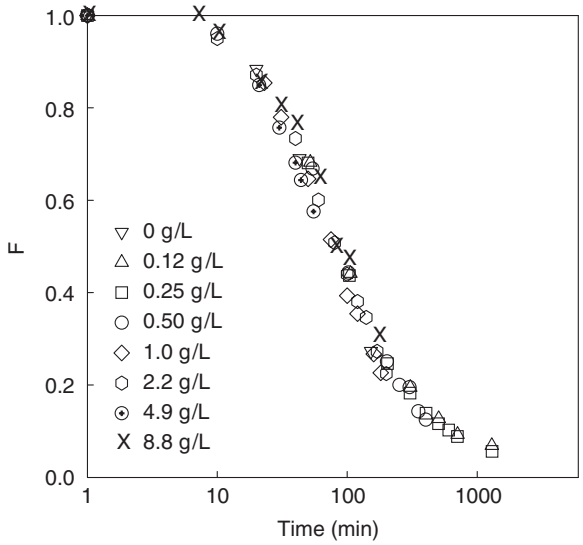

FIGURE 93.36 Fraction of unaggregated β-lg as a function of heating time at 70°C in a solution of 20 g/L β-lg, pH7, 0.1 M NaCl, and various concentrations of κ-car indicated in the figure.

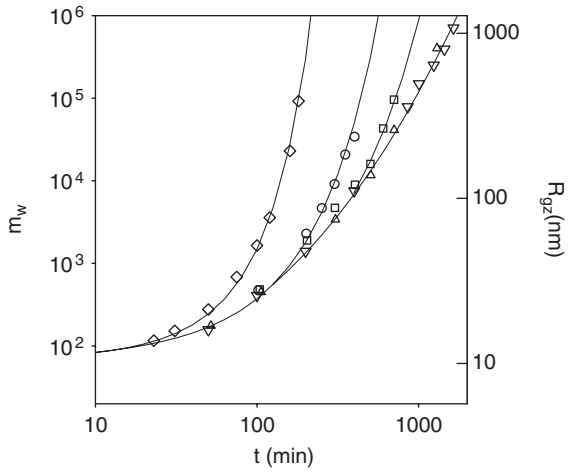

FIGURE 93.37 Time dependence of m_w and R_{gz} for β-lg aggregates formed in a solution of 20 g/L β-lg, pH7, 0.1 M NaCl, and various concentrations of κ-car heated at 70°C. Symbols are as in Figure 93.36.

separation with a gel-like sediment containing most of the protein and a clear supernatant, which contains most of the polysaccharide.

Figure 93.36 shows the fraction of unaggregated proteins as a function of heating time at 70°C for a solution containing 20 g/L β-lg and varying amounts of κ-car between 0 and 8 g/L. Within the experimental error there is no influence of κ-car on the rate of protein consumption, i.e. on the rate of the first aggregation step [21]. It was also shown that the structure of the aggregates is not modified by the presence of κ-car [21].

However, κ-car accelerates the second step of the aggregation process, i.e. the growth of the aggregates, as is shown in Figure 93.37. Comparing the growth at different κ-car concentrations, we find that initially the growth is the same as in the absence of κ-car. But once the β-lg aggregates have reached a certain size the growth rate increases and a gel is formed rapidly. The influence of κ-car becomes significant for $C > 0.2$ g/L, which is somewhat smaller than the overlap concentration of κ-car (0.5 g/L). The size of the β-lg aggregates where κ-car influences the growth rate decreases rapidly with increasing κ-car concentration between 0.25 and 1.0 g/L, although the correlation length of κ-car varies little in this range (16–26 nm) [39].

If we use higher κ-car concentrations we observe very rapid formation of large particles together with small aggregates and the residual fraction of native proteins. These systems either develop into a gel or segregate into a turbid bottom phase and a transparent top phase. Whether these systems gel or segregate, depends not only on the concentrations of β-lg and κ-car but also on the temperature [21].

We have investigated the structure of the gels on a larger scale using confocal microscopy. Within the resolution of the confocal microscope, the β-lg gels formed in the presence of no, or a small amount of κ-car, appear homogeneous. However, in the presence of more κ-car

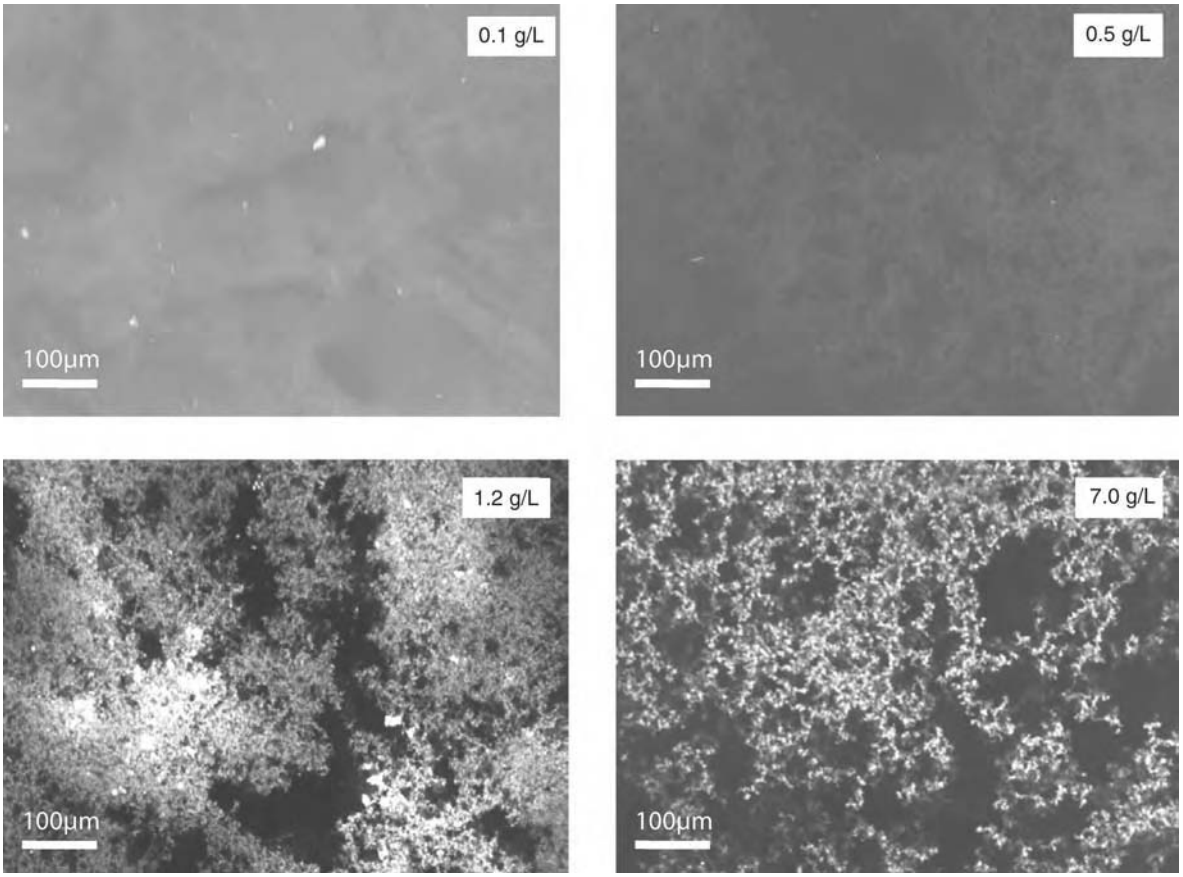

FIGURE 93.38 Micrographs obtained from confocal laser scanning microscopy of solutions of 20 g/l β-lg, pH7, 0.1 M NaCl, and various concentrations of κ-car indicated in the figure after heating 25 h at 70°C. Protein domains are white.

one observes a micro phase separation leading to the formation of β-lg rich domains that appear to cluster [21], see Figure 93.38.

As shown in Figure 93.39, the presence of κ-car has a profound influence on the mechanical properties of the gels [18]. Though the gels form more quickly with increasing κ-car concentration, the coarsening of the gel structure caused by the microphase separation weakens the gel. The gel modulus as a function of κ-car concentration goes through a maximum, and, as mentioned above, in some cases we do not even observe gel formation, but rather macroscopic segregation.

In order to understand better the competition between aggregation and phase separation, we have mixed β-lg aggregates and κ-car at room temperature [20]. After mixing, one observes by optical microscopy the formation of spherical micro-domains rich in protein aggregates, see Figure 93.40. The micro-domains slowly precipitate and form a viscous sediment. The micro-domains contain the larger aggregates while the residual native proteins and smaller aggregates remain in solution together with the polysaccharide. As can be seen in Figure 93.40 the

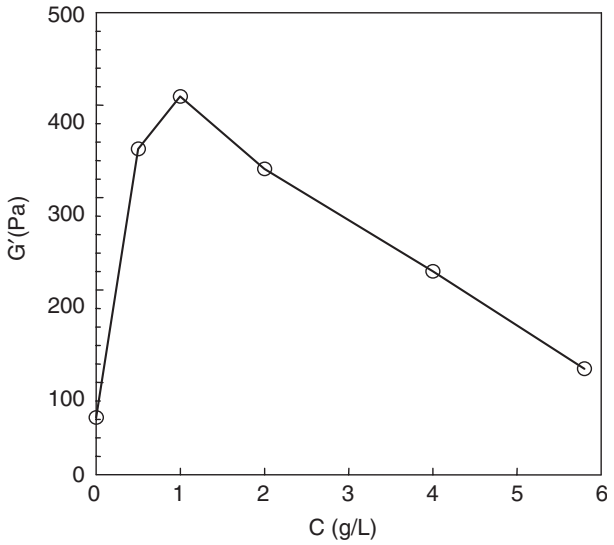

FIGURE 93.39 Dependence of the storage shear modulus G′ at 1 Hz on the κ-car concentrations after heating a solution of 50 g/L β-lg, pH7, 0.1 M for 2 h at 75°C.

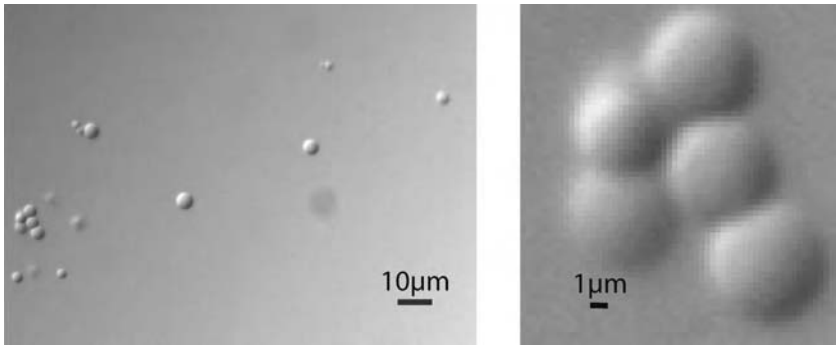

FIGURE 93.40 Micrographs of protein rich micro-domains obtained with optical microscopy for a mixture of 5.7 g/L β-lg aggregates with R_{gz} = 20 nm and 9.2 g/L κ-car.

micro-domains have a tendency to stick together, but the sediment can be easily redispersed in the form of small clusters of micro-domains. If we increase the κ-car concentration, smaller and smaller aggregates will phase separate and become part of the micro-domains.

The formation of the micro-domains is due to decomposition into two liquid phases. If the system is diluted soon after the micro-domains are formed, we recover the initial distribution of aggregates. However, the longer we wait the slower becomes dissolution of the micro-domains after dilution. If we wait for more than a few days the micro-domains can no longer be dissolved by dilution. The reason for the progressive stabilization of the micro-domains is very slow association of the aggregates that are concentrated in the micro-domains. We have observed such slow association at room temperature in more concentrated solutions of β-lg aggregates even without phase separation.

The size of the micro-domains depends somewhat on the concentration of κ-car and β-lg, but is close to that of the dense domains seen in the gels of the heated mixed systems, (compare Figures 93.35 and 93.40). We propose that the dense protein domains in the mixed gel are due to micro phase separation of the growing aggregates. The micro-domains formed in the heated mixtures cannot precipitate because they are trapped in the gel. The structure of the gels formed by the heated mixtures is controlled by the balance between aggregation and phase separation. As long as the aggregates are small enough to be compatible with κ-car, the growth and the structure of the aggregates are not influenced by the presence of the polysaccharide. However, as soon as they become larger they will start to phase separate. The extent of the phase separation and thus the heterogeneity of the gels depend on the rate of phase separation compared to the rate of aggregation. The latter is strongly temperature dependent and can be slowed down by using a lower heating temperature. If the aggregation rate is sufficiently slow the micro-domains can precipitate and we observe a macroscopic phase separation.

The influence of micro-phase separation is particularly clear for the mixed system discussed here. However, as we have said, gel structures very similar to that of the mixed system are observed in pure β-lg gels at high ionic strength or at pH close to the isoelectric point. In these situations the gel structure is also influenced by micro-phase separation.

VII. CONCLUSION

Heat-induced denaturation of β-lactoglobulin leads either to the formation of large linear aggregates or to small primary aggregates. In a second step the primary aggregates may associate further to form self-similar clusters. At low concentrations the growth of the aggregates stagnates, while above a given concentration C_g the aggregates fill up the whole space and form a gel. Under conditions of high ionic strength, pH close to the isoelectric point or added polysaccharide very large aggregates will phase separate, which leads to the presence of protein rich micro domains. In such situations the morphology of the protein gels is determined by the competition between phase separation on the one hand and aggregation and gelation on the other.

The knowledge of these structures is essential for understanding the rheological properties of protein gels as we have seen in the specific case of the system at pH7 and 0.1 M salt where the elasticity of the gels can be explained by the so-called fractal gel model. However, further work is needed in order to elucidate the rheological properties of globular protein gels over the whole range of pH and ionic strength. This work is a necessary step for the development of new routes for texturing globular proteins with new properties.

ACKNOWLEDGMENTS

The work reviewed here has been done in collaboration with the following people: Pierre Aymard, Karine Baussay, Lazhar Benyahia, Jean Pierre Busnel, Isabelle Capron, Allan Clark, Philippe Croguennoc, Jean Christophe Gimel, Christel Le Bon, and Matthieu Pouzot.

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94 Emulsions and Emulsifiers

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CONTENTS

An emulsion is a colloidal dispersion of liquid droplets in another liquid phase. Often this colloidal dispersion is complex because the dispersed phase is partially solidified or because the continuous phase contains crystalline material. All emulsions have in common that they are (thermodynamically) unstable.

Emulsifiers are substances that lower interfacial tension between two non-miscible liquids with different polarity. Emulsifier molecules consist of lipophilic and hydrophilic parts [8]. When an emulsifier is added to a mixture of water and oil, the emulsifier is arranged on the interface, anchoring its hydrophilic part into water and its lipophilic part into oil. On the interface surface of water and air and of oil and air, the hydrophilic part and the lipophilic part are adsorbed and arranged around the interface [11]. The hydrophilicity and lipophilicity are different among emulsifiers, and the balance between the two is called the hydrophilic-lipophilic balance — HLB value [9]. The HLB number is a parameter that has been assigned to each surfactant molecule, and has a range of 0 to 20. A higher number represents a more hydrophilic molecule. Emulsifiers can form two types of emulsions. Emulsifiers with prevalent hydrophilic part form emulsion type oil in water (O/W) and emulsifiers with prevalent hydrophobic component form an emulsion (W/O)[19]. Characteristic behaviours of emulsifiers depending on HLB are summarized in Table 94.1.

Because the molecule of emulsifier has hydrophilic and lipophilic part, its solution does not become a simple aqueous solution but a colloidal one, of which properties vary greatly depending on their concentration. In an extremely-diluted solution, there is no special change, but the emulsifier gathers on the interface and the surface tension is reduced as an increase of its concentration. As further increase of the concentration, a uniform monomolecular layer, is made on the surface and the surface tension drops to the minimum. A further increase of the concentration causes micelle formation. Micelles formation occurs when the excess molecules, in which the lipophilic groups are positioned face to face gather and there is no change in the surface tension. The concentration to start micelle formation is called critical micelle concentration (CMC) and the properties of the solution change greatly with a change of this concentration [20]. Similar changes are developed on the interface of oil and water, when the interfacial tension reaches the CMC point. When the concentration exceeds CMC, spherical micelles appear at first and disperse into water [6].

Emulsions are thermodynamic unstable systems, and their tendency is to reach equilibrium state by linking the dispersed share. This effect is called coalescence [17]. Substances that increase emulsion stability are stabilizers or subsidiary emulsifier agents. Among the principle food

TABLE 94.1
Characteristic Behaviour of Emulsifiers Depending on HLB

Characteristic Behaviours Related to Water	HLB	Ratio Hydrophilic Part	Ratio Lipophilic Part	Functions	
Not dispersing	0	0	100		
				Anti-foaming agent	
Slightly dispersing	2	10	90		
	4	20	80		W/O emulsification
	6	30	70		
				Wetting agent	
Milky dispersion	8	40	60		O/W emulsification
Stable milky dispersion	10	50	50		
Transparent dispersion	12	60	40		
				Cleaning agent	
Colloidal solution	14	70	30		
	16	80	20	Solubilizing agent	
	18	90	10		
	20	100	0		

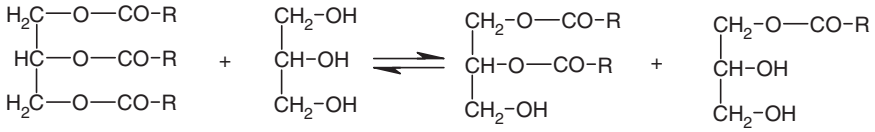

FIGURE 94.1 Transesterification scheme.

stabilisers belong alginates, cellulose derivate, gelatine, agar, pectin, starch and other jelly-forming substances.

It is obvious that temperature is an important factor in relation to the emulsion-forming characteristics of a surface-active agent. An emulsifier that tends to be preferentially soluble in water at relatively low temperatures may become preferentially soluble in oil at higher temperatures at which hydrophobic interactions become stronger. Determination of the temperature at which this inversion occurs provides a useful basis for emulsifier selection. A strong positive correlation has been observed between the phase-inversion temperature (PIT) of emulsifiers and emulsion stability [5].

I. FOOD EMULSIFIERS

Food emulsifiers can be divided into two main groups that include natural and synthetic ones. The first group represents a not very large number of substances present in natural plant or animal sources where it is possible to separate them. Synthetic emulsifiers use natural substances (fats, sucrose) or their components (glycerol, fatty acids) treated with chemicals. To set up synthetic emulsifiers we can use the substances that are not present in natural sources (1,2-propylenglycol, polyglycerol, succinic acid). At present, food industry makes intense use of about one-tenth of available synthetic emulsifiers, with an emphasis on emulsifiers based on monoacylglycerols and their derivatives.

II. MONOACYLGLYCEROL EMULSIFIERS

Fats and oils are an excellent source for many amphiphilic molecules since they are inexpensive, easy to extract and handle [16]. Chemical reactions and/or enzymatic processes are often carried out on certain fats and/or oils, to obtain molecules with hydrophobic and hydrophylic groups attached to each other. One of the most common emulsifiers for water-in-oil emulsions is monoacylglycerol of fatty acids. Hydrogenated oils or natural fats give mixtures of mono and diesters of fatty acids when they are transesterified with glycerol. Mechanism of transesterification is illustrated in Fig. 94.1.

The reaction by itself can be realized most often at temperatures 200–240°C in the presence of 0.1% alkaline catalyst (CaO, KOH and others) under intensive stirring. Transesterification is finished at 230°C in one hour. The reaction mixture contains mainly mono a diacylglycerols. Acyl in monoacylglycerol can occupy border (alpha) or central glycerol position (beta form). This conventional process, even with widespread use, presents two main disadvantages. One is the difficulty in obtaining pure compounds and secondly the use of high temperatures which leads to flavour and colour impurities. When the molecule of interest is

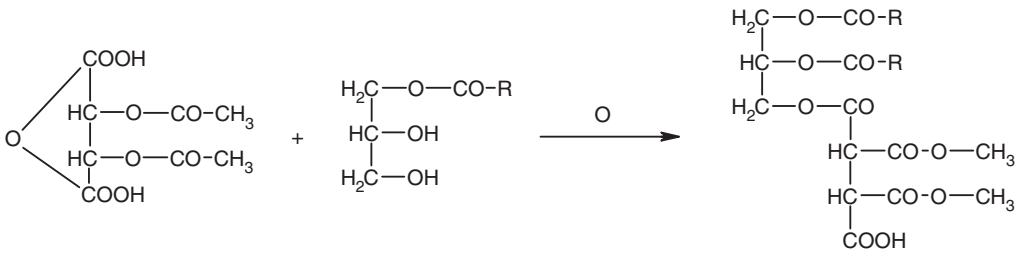

FIGURE 94.2 Reaction of monoacylglycerol ester with diacetyltartaric anhydride.

monoavylglycerol and when high purity is needed, a very interesting alternative route is the use of lipase-catalyzed esterification, taking advantage of the high specificity of the catalyst and of the mild conditions used [4].

From the mixture of acylglycerols a relatively pure >92% alpha monoacylglycerol ester can be prepared. Distillation takes place in a evaporator at 200–210°C and pressure 0.8–4 Pa. Melting point of these distillates moves about 65°C and therefore they are very hard. Because of good handling and application, they are pulverized by spraying molten material in a cold air stream into crystallization vats.

The phase behaviour of monoacylglycerol-water systems is critical for optimum functionality of monoacylglycerols in aqueous systems. With pure monoacylglycerols, the lamellar-type liquid crystal dominates for esters of the 12:0 and 16:0 fatty acids, hexagonal II or cubic type liquid crystals are usually produced from fatty acid esters with longer chains. When the water content is low, unsaturated monoacylglycerols yield lamellar-type liquid crystals at room temperature. By increasing the water content to approximately 20%, a viscous isotropic phase forms that transforms into a hexagonal II phase at temperatures above 70°C. If the water content is increased above 40% the viscous isotropic phase will separate as gelatinous lumps, making uniform distribution very difficult [7].

Distilled monoacylglycerols are widely used, especially in the baking industry because they improve the quality of pastries.

III. DERIVATIVES OF MONOACYLGLYCEROLS

The hydrophilic nature of a monoester can be increased by increasing the number of free hydroxyl groups in the alcoholic moeity of the molecule. Polyglycerol esters with a wide range of HLB values are thus produced by esterification of fatty acids with polyglycerols. Polyglycerol chains containing up to 30 glycerol units can be prepared by polymerisation of glycerol. On the other hand the hydrophobic character of monoacylglycerols can be enhanced by the addition of various organic acid radicals yielding esters of monoacylglycerols with hydroxycarboxylic acids.

IV. ESTERS OF MONOACYLGLYCEROLS WITH DIACETYLTARTARIC ACID (DATEM)

The diacetyltartaric and fatty acid esters of glycerol consist of glycerol esters of mono- and diacetyltartaric acid and fatty acids of food fats. It can be manufactured either by the interaction of diacetyl-tartaric anhydride and monoacylglycerols of fatty acids in the presence of acetic acid, or by the interaction of acetic anhydride and monoacylglycerol of fatty acids in the presence of tartaric acid [15] (Fig. 94.2). Because of inter- and intramolecular exchange of acyl groups, the two methods of production result in essentially the same components. The distribution depends on the relative proportions of the basic raw materials, on temperature and on reaction time. Diacetyltartaric and fatty acid esters of glycerol may contain small amounts of free glycerol, free fatty acids and free tartaric and acetic acids. They may be further evaluated according to the acid value, total tartaric acid content, free acetic acid content, saponification value, iodine value, free fatty acid content and the solidification point of the free fatty acids.

Esters of monoacylglycerols and diacetyltartaric acid form stable O/W emulsion. They interact with flour proteins and raise its quality [14]. Biochemical studies suggest that diacetyltartaric and fatty acid esters of glycerol are hydrolysed in the gastrointestinal tract to yield monoglycerols and acetylated tartaric acid. As monoglycerols are natural dietary constituents, they would be subjected to natural digestion and absorption processes. Diacetyltartaric acid is not a natural constituent of the diet, and there is evidence that it may be further hydrolysed to yield acetic and tartaric acids. The studies reviewed previously indicated little toxicity after administration of a single oral dose of diacetyltartaric and fatty acid esters of glycerol [21].

V. ESTERS OF MONOACYLGLYCEROLS WITH SUCCINIC ACID

They are produced in reaction of distilled monoacylglycerols with succinic acid anhydride in molar proportion 1:1. The product is mixture of partial and total ester.

The diacetyl tartaric acid esters, as well as the succinic acid esters, form lamellar liquid crystals that have

limited swelling capacity in water. However, as is true of distilled monoacylglycerols, their capacity to imbibe water can be increased drastically by the addition of NaOH. Malic acid esters form cubic mesomorphic phases with water contents of up to 20% and hexagonal II phases at higher temperatures and water concentrations. Succinic acid esters do not form mesomorphic phases with water, but they do exhibit mesomorphism [18].

These esters are used in baking industry as an emulsifier to improve rheological properties of dough, enlarging its volume, giving light and uniform porosity and elongating its freshness.

VI. ESTERS OF MONOACYLGLYCEROLS WITH CITRIC ACID

The esters may be produced by the esterification of glycerol with citric acid and edible fatty acids or by reaction of a mixture of monoglycerols of edible fatty acids with citric acid.

Reaction is carried out at 105–150°C during 1.5 hour. The product (Fig. 94.3) is a white to ivory-coloured, oily to waxy material of a consistency determined by the fatty acids. Esters are typically dispersible in hot water, insoluble in cold water and soluble in edible oils and fats.

This product is widely used within the food industry, e.g. as an emulsifier, stabiliser, anti-spattering agent, flour improver, protein-binding agent and as a synergist for antioxidants [2].

VII. ESTERS OF MONOACYLGLYCEROLS WITH LACTIC ACID

These esters, also called lactoglycerols are prepared by esterification of monoacylglycerols with lactic acid in molar proportion about 1:1 at temperatures above 100°C and by lowered press (Fig. 94.4). A less used way of production consists in straight esterification of about equal volumes of higher fatty acid, lactic acid and glycerol.

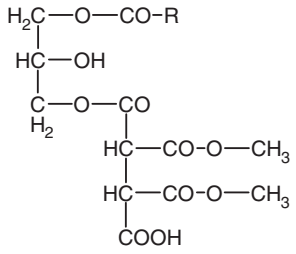

FIGURE 94.3 Ester of monoacylglycerol with citric acid.

The product is a light yellow to amber-coloured, oily to waxy material with a consistency determined by the fatty acids and the proportion of lactic acid. Lactoglycerols are dispersible in hot water, indispersible in cold water and soluble in edible oils and fats. Lactoglycerols are good emulsifiers for emulsion W/O and they crystallize in *alpha* form and moreover in this form they stabilize monoacylglycerols. They are used alone or in combination with 2-stearoyllactates for ice cream and cream-cakes preparation. Lactic acid esters of monoglycerols are used to improve aeration and foam stability as well as texture and volume. Applications include topping powders, non-dairy creams, fine bakery wares, shortening or chocolate compounds.

VIII. ESTERS OF MONOACYLGLYCEROLS WITH ACETIC ACID

They are prepared by esterification of distilled monoacylglycerols with acetic acid anhydride with cooling at 110 to 140°C for about 1 hour. After the reaction has finished, the mixture is thoroughly rinsed with water to remove free acetic acid. The product is a colourless to ivory, oily to waxy material with a consistency determined by the fatty acids and the proportion of acetic acid. Esters of monoacylglycerols with acetic acid are typically insoluble in cold and hot water and dispersible to soluble in edible oils and fats. These compounds have excellent aerating and foam stabilising properties. They are also used as lubricants and release agents.

Acetic esters prepared from total hydrogenated fat give products transparency and are characterized by elasticity and flexibility. These products can be used as an edible coat for some food [1] or in topping powders, chewing gum base or cakes. Emulsifier ability of acetoglycerols is insignificant. Acetic acid esters of monoglycerols of fatty acids have been evaluated by the Joint FAO/WHO Expert Committee.

IX. PROPYLENGLYCOL ESTERS

The product is produced either by the direct esterification of 1,2 propylene glycol with fatty acids or by transesterification

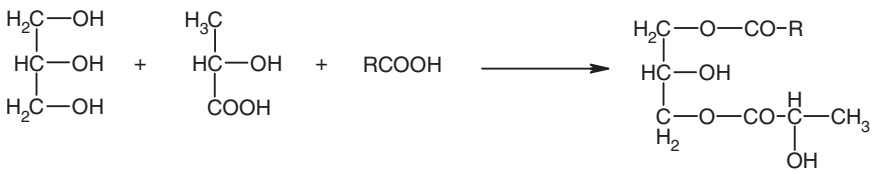

FIGURE 94.4 Synthesis of lactoglycerols.

of 1,2 propylene glycol with fats and oils. The process may be followed by molecular distillation or other separation techniques to separate the monoesters. Propylenglycol ester is white to yellowish-white. The consistency is determined by the fatty acids. The product is dispersible in hot water, indispersible in cold water and soluble in edible oils and fats. Ester imparts excellent aerating and foam stabilising properties. Propane-1,2-diol esters of fatty acids are permitted in fine bakery wares, fat emulsions for baking purposes, milk and cream analogues, beverage whiteners, edible ices, sugar confectionery, desserts, whipped dessert toppings other than cream, dietetic foods intended for special medical purposes or dietetic formulae for weight control [10].

X. POLYGLYCEROL ESTERS

This type of emulsifiers is produced by condensation of glycerol and by partial esterification of polyglycerol with higher fatty acids. Glycerol condensation gives rise to a mixture of linear and cyclic polyglycerols with various molecular weights and their composition depends on condensation temperature and duration. In the group of polyglycerol emulsifiers belong partial glycerol esters (n = 3−10). Higher polyglycerol monoesters are water-soluble or dispersible substances [12] (Fig. 94.5).

Polyglycerol esters are widely used in foods as aerating agents, crystal modifiers, starch-complexing agents, dough conditioners, humectants, defoaming agents and anti-spattering agents. These esters are permitted in fine bakery wares, emulsified liqueurs, egg products, beverage whiteners, chewing gum, milk and cream analogues or dietary food supplements. Polyglycerol esters are often used in mixture with sorbitan esters or their polyoxyethylen-derivates to improve the quality of bakery products.

XI. SORBITAN ESTERS

Sorbitan fatty acids esters (Fig. 94.6) are usually mixed esters of fatty acids with sorbitol anhydride or sorbitan. Sorbitol is dehydrated first to form hexitans and hexides, which are then esterified with fatty acids. The resulting products are known commercially as "Spans."

These agents tend to promote W/O emulsions. Compounds that are more hydrophilic can be produced by reacting sorbitan esters with ethylene oxide. Polyoxyethylene chains add to the hydroxyl groups through ether linkages. The resulting polyoxyethylene sorbitan fatty acid esters are commercially known as "Tweens." In general, these compounds form hexagonal I liquid crystals in water and they can solubilise small quantities of triacylglycerols. With larger amounts of triacylglycerols, transformation to a lamellar-type liquid crystal takes place. The ability of an emulsifier to solubilise nonpolar lipids is important to the formation of phase equilibrium at the emulsion interface [13].

Within the EU sorbitan monopalmitate is permitted in fine bakery wares, toppings and coatings for fine bakery wares, milk and cream analogues, or liquid tea concentrates and liquid fruit and herbal infusion concentrates or edible ices.

XII. SACCHAROSE ESTERS

The products may be prepared from sucrose and the methyl and ethyl esters of food fatty acids or by extraction from sucroglycerols [3]. Depending on the food legislation applicable the following organic solvents are used in their production: dimethylsulphoxide, dimethylformamide, ethyl acetate, propane-2-ol, 2-methyl-1-propanol, propylene glycol, methyl ethyl ketone or isobutanol.

The structural formula of saccharose ester is shown in Fig. 94.7.

Products based on saturated fatty acids are white to slightly greyish powders. Derivatives based on unsaturated fatty acids are yellowish, pasty to waxy substances. The products are sparingly soluble in water and soluble in ethanol. They are insoluble in vegetable oils. The more fatty acids are linked to saccharose, the higher the fat solubility and the lower the swelling ability in water. Sucrose esters of fatty acids are used, for instance, as aerating and starch-complexing agents. They are also used to improve fat dispersion and stability.

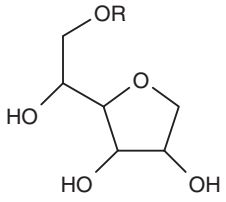

FIGURE 94.6 Sorbitan ester.

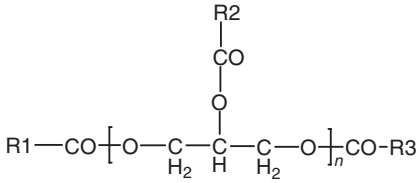

FIGURE 94.5 Polyglycerol ester.

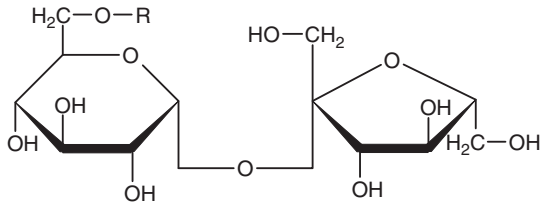

FIGURE 94.7 Saccharose ester.

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95 Phytates

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CONTENTS

I. INTRODUCTION

Phytate discovery is believed to have its beginnings in 1855–1856 when Hartwig first isolated small particles, nonstarch grains, from several plant seeds. He suggested that these small particles to be a source of reserve nutrients for seed germination and plant growth (1). Subsequent studies [1872–1896] on the chemical composition of the particles by Pfeffer, Palladin, Schulze, and Winterstein established the "inosite-phosphoric acid" nature of the particles. Extensive studies that ensued over the next two decades resulted in several suggestions for the structure of the molecule. Anderson (2) finally determined the precise structure of phytic acid [Figure 95.1]. The currently accepted name of phytic acid is *myo*-inositol 1, 2, 3, 4, 5, 6 hexakis dihydrogen phosphate or *myo*-inositol hexakisphosphate. Salts of phytic acid are referred to as "phytate(s)." In seeds, K^+, Na^+, Ca^{++}, Zn^{++}, Fe^{+++}, Cu^+ and Mg^+ are the most frequently encountered cations in naturally occurring seed phytates. In this chapter we will use the term phytate to describe both acid and salt forms. A detailed account of the historical background of phytic acid and deduction of its chemical structure has been described earlier (3–5). Although much of the early work centered around naturally occurring seed and grain phytic acid and phytates, presence of phytic acid in avian erythrocytes (6), several vertebrates (7), domestic fowl (8), as well as soil (9, 10) has been reported. For a more detailed account of occurrence of phytates, please refer to Reddy (5).

A continued and explosive growth in phytate literature over the last twenty years alone is an excellent indication of not only the complexity of reactivity of the molecule but also the diversity of chemical/biochemical reactions it may participate. During the period of 1920–1950 much of the scientific efforts were directed in developing methods to analyze the phytate content of various foods and food products and understanding the chemical behavior of the molecule under a variety of environmental conditions of pH, buffers, temperatures, and presence of other chemical compounds, notably proteins, tannins, and minerals. It is through these early studies that it became apparent that phytate has a strong affinity towards certain minerals (such as Zn^{2+}, Cu^{2+}, Mg^{2+}, and Fe^{2+}) that leads to formation of metals chelates (both soluble and insoluble). In certain instances, formation of such mineral chelates may lead to reduced bioavailability. It was therefore not surprising that during this period early studies on the role of phytate in human nutrition also began to appear (11–14). As methodologies in biochemistry, analytical chemistry, and nutrition continued to make significant progress, sophisticated research tools became available to a larger number of scientists around the world and significant advances in

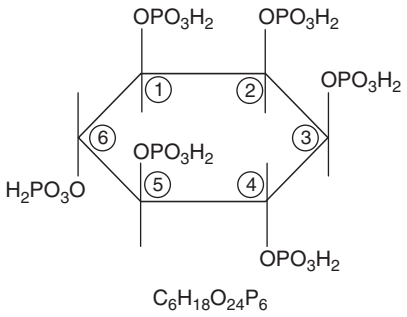

FIGURE 95.1 Phytic acid structure as proposed by Anderson [1914] (2).

molecular understanding of phytate followed over the next several decades (1950–1980). In particular, the structural complexity of the molecule as well as the chemical ramifications of the structural diversity became increasingly evident. During these years, significant progress was also made in unraveling the biochemical synthesis of the molecule in plant seeds (15). From a nutritional point of view, elaborate and extensive studies on role of phytate on nutrient bioavailability also began to appear at regular and increasing frequency during the last few decades (notably 1970–1990). These studies have significantly improved our understanding of phytate in human and animal nutrition, especially with respect to influence of phytate on mineral absorption and utilization. During these decades it also became apparent that phytate interaction with food components is much more complex than originally anticipated.

More recently, phytate is receiving increased attention for its possible importance in human health. With the realization that inositol 3 phosphate (IP3) acting as a second messenger molecule *in vivo* (16) scientific interest in understanding the role of phytate degradation products (IP6-IP1) has increased considerably. In addition, several initial *in vitro* studies also suggest that phytate (and phytate related compounds) may have chemical and biological properties that may be relevant to human health.

Since extensive and very large body of phytate literature is available to readers through various resources (particularly the electronic resources and electronic library databases) as well as some of the recent books on the subject matter, the primary focus of this chapter will not be on providing exhaustive data compilation. Instead, an attempt will be made to summarize important new developments in selected areas and outline a few suggestions for future research.

II. CHEMICAL AND BIOLOGICAL PROPERTIES

A. Chemical Properties

A topic of scientific contention over half a century, modern analytical chemistry techniques (x-ray crystallography and ^{31}P nuclear magnetic resonance in particular) finally solved the problem of determining the chemical structure of phytic acid (Figure 95.1) originally proposed by Anderson in 1914 (2). The accepted name for the molecule is *Myo*-Inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate or *Myo*-Inositol hexakisphosphate. Phytic acid salts are generally referred to as "phytate(s)." Details of recommended name and pertinent references for the IUPAC/IUB nomenclature can be found at the IUPAC website (17). Phytic acid is a small molecule ($C_6H_{18}O_{24}P_6$; MW = 660.08) with six phosphate groups. With the exception of the only one phosphate group on carbon 2 (which is axial to the plane of the inositol ring), the remaining five phosphate groups are in equatorial position with respect to the inositol ring. It is important to mention here that presence of diphosphorylated inositol phosphates is well documented in the literature (18, 19) however presence of triphosphates at a single carbon (or multiple carbons) is not yet demonstrated.

Phytate is synthesized from D-glucose 1-P. The first committed step in this synthetic pathway is the conversion of D-Glucose 1-P to 1L-*myo*-inositol 1-P [also known as Ins(3)P_1 or 1D-*myo*-inositol 3-P] by 1L-*myo*-inositol-1-P-synthase. Ins(3)P_1 synthase (E.C. 5.5.1.4) catalyses the conversion of hexose 6-P to Ins(3)P_1. A magnesium-dependent monophosphatase (E.C. 3.1.3.25) effects the removal of phosphate to release free *myo*-inositol. Free *myo*-inositol (a ubiquitous sugar alcohol), is an important intermediate in a host of biochemical pathways (and therefore its regulation is of interest to many), also serves as the building block for phytic acid synthesis. A detailed account of phytate synthesis can be found in the chapter by Loewus (15).

The phytate molecule has 12 dissociable protons. Of these twelve, six are strongly dissociated with a p*K* value of about 1.8, two are weakly dissociated [p*K* of about 6.3] and the remaining four are very feebly acidic [p*K* value of about 9.7] (20). More recent studies on phytic acid interactions with rare-earth ions [Ce^{3+}, Pr^{3+}, Sm^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}] further indicate that eight protons in phytic acid are highly acidic, two weakly acidic, and two very weakly acidic [titrable in the pH range of 1.2–4.9, 5.0–8.15, and 8.3–11.0, respectively] (21). Consequently, the molecule is chemically very reactive. It can participate in reactions that are electrostatic in nature. The degree of reactivity and the affinity towards the molecule reacting with phytate is dependent on several factors that include:

1. The p*K* of the reactive phytate proton(s).
2. The reaction environmental factors such as pH, ionic strength, temperature, viscosity of the medium, presence or absence of ionic polymers, etc.
3. Stability and reactivity of molecules interacting with phytate.
4. Duration of reaction.
5. Solubility of products resulting from interaction of phytate(s) with component(s) of interest.
6. Whether or not phytate is being degraded during the interaction(s). For example, if phytate is being degraded to lower phosphates, each of the lower phosphates in turn may influence the reactivity of the intact phytate molecule by potentially competing with phytate and also through independent binding with the reactive molecule (the one that is interacting with phytate).

In food, minerals (di- and tri-valent metal ions), proteins, and certain carbohydrates are the likely components

that may react with phytate. The strength of the interaction will obviously depend on a host of parameters. When phytate interacts with such food component(s) the resulting complex(s) may be soluble or insoluble. Depending on the type of complex and its solubility properties, bioavailability of the interacting component(s) may be adversely influenced. If the interacting food component is an essential nutrient, such as an essential mineral, influence of phytate on the bioavailability of that nutrient may be of nutritional concern. Mineral phytate interactions are also of interest for a variety of other reasons that include potential use of phytate as:

1. An antioxidant in foods [such as meats] (22).
2. As an inhibitor of plaque formation in tooth paste (23).
3. Intermediate in production of special unleaded fuel (24).
4. Antirust agent on metal surfaces (25).

Because phytate can form complexes with several minerals, notably the divalent minerals such as Fe^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Sn^{2+}, Co^{2+}, Ni^{2+}, and several others, any chemical, biochemical, or organic reactions that depend on such minerals can therefore be potentially influenced. Depending on the stability and solubility of mineral phytate complexes under a variety of pH, temperature, as well as other environmental factors, several possibilities may be explored. For example, divalent metal ions are involved in catalysis of numerous oxidation and reduction reactions [chemical and biochemical]. Polyphenol oxidases [PPOs] are a group of oxidative enzymes that naturally occur in many fruits and vegetables. PPOs cause enzymatic browning that may be desirable [e.g. in black tea and chocolate manufacture] or undesirable [as in case of cut apples or bananas]. The PPOs partly depend on Cu^{2+} for catalysis. It may be useful to find out whether one can apply acceptable levels of phytate to fruits/vegetables to prevent such undesirable browning. Another example is that of loss of chlorophyll Mg^{2+} during fruits/vegetables processing that results in development of undesirable off-color [dull green pheophorbide formation]. Using phytate to stabilize the chlorophyll Mg^{2+} may be useful in stabilizing green color of several fruits/vegetables. Use of phytate in controlling such reactions remains largely unexplored. Similarly, several non-food uses of phytates also need to be explored to utilize this naturally occurring, abundant compound. It should be mentioned here that the number of studies examining the phytate-mineral complexes are rather limited. Most studies on phytate-mineral complexation are typically restricted to specific experimental conditions such as selected pH, temperature, reactant ratio(s), buffer conditions, and type of resultant salts. Such studies are often limited in scope partly due to number of minerals that can interact with phytate, nature of complexes formed, analytical tools available, environmental influences on the complexation can change significantly depending on the mineral involved in complex formation, and time/resource limitations. It is however important to recognize that targeted, detailed studies are needed to improve our basic understanding of these complex interactions. With the exception of very few studies (26), even less is known about the mineral interactions with lower inositol phosphates [such as inositol penta-, tetra-, tri-, di-, and mono- phosphates]. With the increasing realization and understanding of possible biological role(s) of lower inositol phosphates, notably the triphosphate, it is important to investigate the mineral interactions with lower inositol phosphates as improved understanding of such interactions may help us define the biological role(s) of inositol phosphates more precisely.

B. Biological Properties

The recognition of ubiquitous presence of phytic acid in animal kingdom launched a search for physiological function(s) of phytate in earnest. Of particular interest was the possible involvement of phytate in various cellular regulatory processes with particular reference to health and disease. With the publication of possible role of IP3 in cellular calcium signaling by Berridge and Irvine (16), it became increasingly apparent that inositol phosphates, other than inositol hexakis phosphate, may have important cellular functions *in vivo*. A recent review (18) provides an in-depth overview of role of inositol hexakisphosphate [and some of the lower phosphates] in cellular regulation. Reported functions of phytate [and lower phosphates] are summarized in Table 95.1. Some of the difficulties in interpreting published literature on biological role(s) of phytate [and lower phosphates] include:

1. *In vivo* and *in vitro* studies do not always correlate. For this reason it is important to evaluate the results of *in vitro* investigations with caution.
2. *In vivo* studies from one animal species cannot be directly applied to another species or humans.
3. Purity and chemical structure(s) of inositol phosphate(s) and/or intermediates used in studies.
4. Rigor of controls and experimental variables in a study. For example, were experiments done under conditions relevant to physiological conditions? Was placebo effect accounted for in *in vivo* experiments? Was the placebo relevant and proper? Were the cell line(s) used relevant? What criteria were used to select specific cell line(s) for a particular experiment? Was physiologically appropriate ionic strength employed and was it relevant?
5. Were any attempts made to separate the effect(s) of individual, specific phosphates or were the results representing collective effect?

6. In metal binding experiments, were the concentrations of minerals [mineral salts] used in the experiments physiologically relevant? Were there any attempts to separate the mineral-mineral interactions? How does the presence of specific, relevant protein(s) influence such interactions?

Obviously, not all of the above listed concerns are applicable to all the experiments. However, these and other such concerns, when applicable, need to be addressed in relevant experimental designs.

Despite the inherent difficulties and limitations to accurately define the *in vivo* role of inositol phosphates in cellular regulation, based on the available data, it is apparent that inositol phosphates do have important roles in cellular signaling processes, notably in calcium regulation. Since calcium is a mediator of several important biological processes, it is evident that increased number of investigations designed to understand the role of inositol phosphates in cellular regulation will continue. Indeed the explosive growth of number of papers published in just last ten years supports this notion.

TABLE 95.1
Reported Biological Functions of Phytate (and Lower Phosphates)

Reported Function	Mechanism/Specific Role	Phytates	References
ANIMAL CELLS			
Cellular			
Calcium mobilization	Second messenger in signalling	IP3, IP(s)	27
Receptor regulation	Agonist-induced receptor desensitization	IP5, IP6	28
Vesicle trafficking	Binding to vesicle adaptor proteins AP-2, AP-180, arrestins, synaptotagmin	IP4-IP6	29, 30, 31
Activation of protein kinase C (PKC)	Calcium-dependent enzymes	IP(s)	32
Inhibition of protein phosphatase activities	Serine/threonine protein phosphatases	IP6	33
Nuclear			
DNA repair	Activation of enzymes (DNA dependant protein kinase)	IP3-IP6	34, 35
mRNA export	Regulate conformation of nuclear pore complex, faciliate removal of an export inhibitor	IP6	36, 37
Neural			
Related neuropathological disorders			
Down's syndrome, hypernatremia, stroke	Volume regulation (physiologically important osmolyte)	Inositol	38
Bipolar disorder, neural tube defects, diabetic peripheral neuropathy, ataxia talengiectasia, Alzheimer's disease	—	Inositol	38
Neurotransmitter release	Blocks synaptic transmission by preventing vesicular fusion, vesicle trafficking	IP4-IP6	39, 40
Renal			
Renal lithiasis, kidney stone prevention	Prevent crystallization of cacium salts (oxalate and phosphate)	IP6	41
Immune			
Natural Killer cells	Colon carcinogenesis	IP6	42
Neutrophils	Priming action on stimulated respiratory burst in human neutrophils	IP6	43, 44
Iron/Antioxidant	Chelation of iron, iron transport, prevent formation of free radicals, protection from vitamin E and selenium deficiency, cardiovascular effects, carcinogenesis	IP3-IP6	45, 46, 47
Antidiabetic			
Hypoglycemic effects	Inhibition of starch digestion/absorption, colon carcinogenesis	IP6	48
Hypolipidemic effect	Decrease sucrose induced increases in total hepatic lipids, serum triacylglycerols and phospholipids	IP6	49
Cardiovascular			
Hypercholesterolemic	Alteration of Zinc/Copper ratio	IP6	50
Protection from ischemic and reperfusion injury	Iron chelation, reduce free radical formation and lipid peroxidative damage	IP6	51
Prevent aortic calcification/hypertension	Calcium chelation	IP6	52
Anticancer	Various mechanisms described in this table	IP6, IP(s)	53
PLANT CELLS			
Stomatal pore closing	Mobilization of endomembrane calcium store	IP6	54

III. RESEARCH NEEDS

The ability of inositol hexakis phosphate [and lower salts] to interact with minerals, proteins, fiber, and other positively charged entities are at the heart of their chemical and biological reactivity. The inherent negative charge on the molecule at neutral pH is the driving force behind such interactions. Although sporadic attempts have been made to thermodynamically define and characterize phytate-mineral interactions, there is a clear inadequacy of in-depth understanding of chemical reactivity of inositol phosphates and minerals and other positively charged species. Sound understanding of chemical reactivity of the molecule before embarking on defining the biological implications of reaction(s) is not only desirable but also imperative for several reasons. Perhaps the most compelling reason to precisely define and understand the thermodynamics of such reactions is that such information should enable the researchers to eliminate numerous reactions that may be feasible to occur in an *in vitro* experiment but not feasible in an *in vivo* situation simply based on unfavorable energy of reaction, thus narrowing down the number of experiments to test a hypothesis. In order to derive such data base, ground state(s) of phytate [and lower phosphates] must be defined with respect to free energy associated with such molecules under defined environmental conditions such as the temperature, pH, ionic strength, etc. Since many divalent minerals such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}, Sn^{2+}, Co^{2+}, Zn^{2+}, and others are important in *in vivo* catalytic interactions, improved and thorough investigations of interactions between these minerals and phytate would be of importance.

Phytate interactions with trivalent minerals such as Al^{3+}, Fe^{3+}, Cr^{3+}, and others are much less understood when compared to the interactions of the divalent metal ions. Since many of the trivalent [and higher valency] minerals are important in metallurgical operations, material developments, and material applications, improved understanding of reactions between trivalent (and higher) minerals and phytate is needed.

Any experiments designed to evaluate physiological function(s) of phytate or its lower salts must be done under conditions relevant to *applicable* physiology. Often many studies report findings of experiments done under conditions not physiologically relevant thus severely limiting the utility of the resulting data.

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96 Sorbates

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CONTENTS

I. INTRODUCTION

Preservatives are chemical compounds that prevent or inhibit the activity and growth of microorganisms after adding to foods. The main aim is to prolong the shelf life of foods, by protecting them against food deterioration caused by microorganisms. Various kinds of microorganisms bring about food spoilage and these microorganisms are able to vegetate at different conditions of pH, temperature, water activity, relative humidity, presence or absence of special growth factors. One of the most effective chemical preservatives is sorbic acid (SA) and its sodium, potassium, and calcium salts which are collectively known as "sorbates."

II. DEFINITION

SA is an organic, straight-chain, *trans-trans* unsaturated monocarboxylic fatty acid that is metabolized in the same way as other food fatty acids such as corn oil's linoleic and margarine's oleic acid (1). The carboxyl group of SA is highly reactive and can form various salts and esters. The conjugated double bonds are also reactive and can be influential in its antimicrobial activity and the quality and safety of food products (2). Physicochemical properties as well as other relevant data relating to sorbates are shown in Table 96.1.

TABLE 96.1
Physico-Chemical Properties of Sorbates

Properties	Sorbic Acid	Potassium Sorbate	Sodium Sorbate	Calcium Sorbate
Synonyms	Acetic acid (2-butenylidene); Acetic acid crotylidene; Hexadienic acid; Hexadienoic acid; E,E-2,4-Hexadienoic acid; (E,E)-2,4-Hexadienoic acid; Hexa-2,4-dienoic acid; 2,4-Hexadienoic acid; 2,4-Hexadienoic acid, (E,E)-; 2,4-(E,E)-Sorbic acid; *trans-trans*-2,4-Hexadienoic acid; 1,3-pentadiene-l-carboxylic acid; 2-Propenylacrylic acid; 2-Propenyl-acrylic acid; *alpha-trans-gamma-trans-Sorbic* acid; *trans, trans-Sorbic* acid; Sorbistat; Unistat; Tristat;	BB powder; 2,4-Hexadienoic acid potassium salt; 2,4-Hexadienoic acid, potassium salt (9CI); 2,4-Hexadienoic acid, (E,E)-, potassium salt; 2,4-Hexadienoic acid, (E,E′)-, potassium salt; 2,4-Hexadienoic acid, potassium salt; Potassium 2,4-hexadienoate; Potassium (E,E)-sorbate; Sorbic acid potassium salt; Sorbic acid, potassium salt; Sorbistat; Sorbistat-K; Sorbistat-potassium; Unistat K;	2,4-Hexadienoic acid, sodium salt; 2,4-Hexadienoic acid, sodium salt, (E,E); Sodium (E,E)-hexa-2,4-dienoate; Sorbic acid, sodium salt	2,4-Hexadienoic acid calcium salt; Calcium dihexa-2,4-dienoate; (E,E)-Calcium 2,4-hexadienoate; Calcium sorbate; 2,4-Hexadienoic acid, calcium salt, (E,E)- (9CI); Sorbic acid, calcium salt
Summary formula	$C_6H_8O_2$	$C_6H_7O_2K$	$C_6H_7O_2Na$	$C_{12}H_{14}O_4Ca$
Molecular formula	CH_3-CH=CH-CH=CH-COOH	CH_3-CH=CH-CH=CH-COOK	CH_3-CH=CH-CH=CH-COONa	(CH_3-CH=CH-CH=CH-COO)$_2$Ca
State of matter	solid	solid	solid	solid
Colour	Colourless needles or white powder	White crystals, white powder	White fluffy powder	White fine crystalline powder
Molecular weight [g.mol^{-1}]	112.14	150.23	134.12	
Melting point [°C]	134.5	270 (decomposes, emits toxic fumes of K_2O)	(decomposes, emits toxic fumes of Na_2O)	decomposes, emits acrid smoke and irritating fumes at about 400°C
Boiling point [°C]	228 (decomposes to acridic smoke, irritating fumes)			
pK_a	4.75			
Flash point [°F]	260			
Solubility at 25°C (g/L)	1.6 (water) 0.7 (10% saline) 130 (ethanol) 5–10 (edible oil)	582 (water) 65 (alcohol)	320 (water)	
Density		1.363		
LD_{50} in rate, oral [mg.kg^{-1}]	7 360	4 920	7 160	
ADI [mg.kg^{-1}]	0–2.5	0–2.5	0–2.5	0–2.5
INS	200	202	201	203
EEC	200	202	201	203
CAS	110-44-1	590-00-1	7757-81-5	7492-55-9 (other 90550-09-7)
EINECS	203-768-7	246-376-1		
Regulatory	FDA 21CFR §133,146.115, 146.152, 146.154,150.141, 150.161, 166.110, 181.22. 181.23, 182.3089. GRAS; USDA 9CFR §318.7; BATF 27CFR §240.1051; USA CIR approved. EPA reg.; JSCI approved 0.5% max.; Europe listed 0.6% max. Japan approved with limitations; Europe listed; UK approved	FDA 21CFR §133, 150.141. 150.161.166.110,182.90, 182.3640, GRAS; USDA 9CFR §318.7; BATF 27CFR §240.1051; GRAS (FEMA), CIR approved; Japan approved with limitations; JSCI approved 0.5% max.; Europe listed 0.8% max.	FDA 21CFR §182.90.182.3795. GRAS; USDA 9CFR §318.7; Europe listed	FDA 21CFR §182.3225, GRAS; USDA 9 CFR § 318.7 (not allowed in cooked sausage)

From ref. 4, 190–192.

III. HISTORY

SA as naturally occurring compound was isolated for the first time by A. W. van Hoffman, a German chemist in 1859 in London, who analyzed oil to be obtained from unripe rowanberries (1), where it occurs as the lactone, parasorbic acid (190). The compound was named after the Latin name of mountain ash (*Sorbus aucupartia L.*), which is the parent tree of the rowanberries. The structure of SA was elucidated during 1870–1890, and synthesized for the first time by O. Doebner in 1900 (2,3). In 1945, a U.S. patent was awarded to C. M. Gooding, who found out that unsaturated fatty acids with a double bond in the α-position (crotonic acid and its homologues) behaved as fungistatic agents in foods, or on food wrappers, respectively (4). In 1953, sorbates were approved as food preservatives.

IV. SYNTHESIS AND MANUFACTURE

SA was synthesized in 1900 by Doebner by condensation of crotonaldehyde and malonic acid

$$CH_3\text{-}CH{=}CH\text{-}CH{=}O + HOOC\text{-}CH_2\text{-}COOH \longrightarrow CH_3\text{-}CH{=}CH\text{-}CH{=}CH\text{-}COOH + CO_2 + H_2O$$

Commercial synthesis of SA involves the reaction of ketene $CH_2{=}CH{=}O$ with crotonaldehyde $CH_3\text{-}CH{=}CH\text{-}CH{=}O$. Ketene is produced from acetic acid or acetone by thermal decomposition in a tubular furnace. Crotonaldehyde is formed as a secondary product from acetaldehyde condensation. Methods of industrial production use a catalyst reaction to produce a polyester of molecular weight 1,000–5,000 of the form by reacting the ketene and crotonaldehyde in an excess of aliphatic, alicyclic, aromatic hydrocarbons, or their derivatives as solvents. The polyester is decomposed in either an acid media or by thermal decomposition. The more stable *trans, trans* structure can be obtained in preference to *cis* isomers by solubility or with the use of catalysts. Further purification using recrystallization and carbon treatment is carried out to meet food-grade specifications. The SA is sold as dried powder. The water-soluble potassium, sodium or calcium salts are produced by neutralizing the SA with potassium, sodium or calcium hydroxide, respectively, and by drying the pelletized product or by directly spray granulating the potassium/sodium/calcium sorbate solution. Various modifications and improvements to this process are being practiced at the industrial scale by Hoechst in Germany, Daicel, Ueno, Chisso, and Nippon Gohsei in Japan, Cheminova in Denmark, and Tennessee Eastman in the USA (1).

V. MODES OF ACTION

Sorbates can inhibit yeasts, molds, Gram-positive and Gram-negative and catalase-negative, aerobic and anaerobic, and mesophilic and psychrotropic microorganisms as well as spoilage and pathogenic bacteria. However, come bacterial strains are not inhibited, some may even metabolize the sorbates. Usually, sorbates in foods under the level of 0.3% inhibit microbial activity, at higher level may cause the death. Sorbate inhibits cell growth and multiplication as well as germination and out growth of spore-forming bacteria (2). Several studies have indicated that sorbates inhibit bacterial spore germination (5–9). Inhibition has involved various species of bacteria in laboratory culture media and in foods and has been influenced by species, strains, pH, and sorbate concentration (2). Sorbates can act as a competitive and reversible inhibitor of amino acid-induced germination (6). But, it was also reported that sorbates inhibited spores triggered to germinate or after germinant binding (9). As also postulated, sorbates probably inhibit a postgerminant binding step in the process of germination, and it was thus concluded that it inhibits spore commitment to germination, not triggering. Inhibition of the not well-defined connecting reactions of spore germination may be taking place through inhibition of sporeolytic enzymes involved in germination or through the interaction of sorbates with spore membranes and increase in their fluidity (8,9). Several mechanisms of inhibition of metabolic function by sorbate have been proposed, and it may be possible that several of them may be functional under various conditions, including types and species of microorganisms, type of substrate, environmental conditions, and type of food processing. Under certain conditions sorbates have changed the morphology and appearance of microbial cells (10,11). Such changes have been observed in yeast cells as dense phosphoprotein granules, irregular nuclei, increased numbers and variable sizes of mitochondria, and vacuoles. Cells of *C. botulinum* were long, with bulbous formation and defective division (7,12,13). *C. sporogenes* cells treated by sorbates were usually filamentous and nonseptate, but with distorted shapes characterized by numerous bends and bulges. Septation, when present, resulted in minicells, and the inner cell wall appeared to be thickened; the outer cell wall was absent in many areas (11). Treatment of *C. putrefaciens* with sorbate at pH 7.0 increased cell hydrophobicity and cell wall lysis upon exposure to lysozyme, which could be overcome to some extent by addition of magnesium ions. There was also evidence of other membrane damage in sorbates treated cells (10). Although the significance of such alterations is unknown, they may be due to incorporation of sorbate into specific cell structures and alteration of biosynthetic processes in the cell (14). Other proposed mechanisms of inhibition of microbial growth by sorbates include alterations in the morphology, integrity, and function of cell membranes and inhibition of transport functions and metabolic activity (2). Death of microorganisms exposed to high concentrations of preservatives, such as sorbates, has been attributed to generation of holes in the

cell membrane. Sorbates have decreased the assimilation of carbon from several substrates, including glucose, acetate, succinate, pyruvate, lactate, oxaloacetate, α-ketoglutarate, ethanol, and acetaldehyde (2,15). Inhibition of cell metabolism by sorbates in these studies may have been due to inhibition of enzymes, nutrient uptake, or various transport systems. Sorbates inhibit the activity of several enzyme systems, which may lead to disruption of vital processes involved in transport functions, cell metabolism, growth, and replication. Enzymes inhibited by sorbates include alcohol dehydrogenase, fumarase, enolase, aspartase, catalase, malate dehydrogenase, α-ketoglutarate dehydrogenase, ficin and succinic dehydrogenase. However, some reports have also indicated no inhibition of enzymatic activity by sorbates (56), when aldolase, enolase and pyruvate decarboxylase of *S. cerevisiae* were not inhibited by SA. Therefore it was assumed that SA affected the yeast cell mainly by influencing its cell membrane and its permeability in its undissociated form after the adsorption on surface cells. Inhibition of sulfhydryl enzymes by sorbates has been attributed to binding with sulfhydryl groups and decreasing the number of such active sites on the enzyme. Inhibition of yeast alcohol dehydrogenase has been attributed to formation of a covalent bond with the sulfydryl or ZnOH group of the enzyme and the α- and/or β-carbon of sorbates. It was also proposed that sorbates inhibit the sulfhydryl enzymes through the formation of a thiohexenoic acid derivative. Inhibition of catalase by sorbates was attributed to the formation of sorbyl peroxides through the autoxidation of SA. These peroxides then would inactivate catalase (2). Another postulation has indicated that sorbate may act competitively with acetate at the site of acetyl coenzyme A formation (15). Binding and inhibition of coenzyme A should result in inhibition of oxygen uptake and microbial growth. Lipophilic acid food preservatives, such as sorbates, may interfere with substrate and electron transport mechanisms. Inhibition of substrate transport into the cell by uncoupling it from the electron transport system results in cell starvation (16,17). SA has inhibited the uptake of glucose (18) and amino acids 19), as well as the electron transport system (2). Inhibition of nutrient uptake may be due to neutralization of the proton-motive force needed for substrate uptake, inhibition of the electron transport system, inhibition of synthesis or depletion of ATP, inhibition of transport enzymes, and inhibition of metabolic energy utilization by the amino acid transport systems (2). Since inhibition of microbial growth by sorbates has been associated with decreased levels of ATP (15), a proposed mechanism of ATP depletion includes hydrolysis of ATP by the primary sodium/hydrogen pump in an attempt to maintain ion balance in the cell (20). A proposed mechanism of inhibition of microbial growth by sorbates is based on the theory that lipophilic, weak acid preservatives starve the cells of compounds that are transported actively only by the proton-motive force (PMF) that exists across the cell membranes (21–24). According to this theory, the weak acids, such as SA, reduce the intracellular pH and neutralize the transport-driving PMF. In general, however, more than one mechanism may be involved in inhibition of various microorganisms under different conditions.

VI. EFFECT TO MICROORGANISMS

Microbial inhibition is variable and depends on species, strains composition of substrate, pH, water activity, additives present, processing treatment, temperature of storage, composition of atmosphere, type of packaging and concentration of sorbates. Variations or resistance to inhibition by sorbates may lead to failures in preservation and defective food products (2). In general, various species and strains of microorganisms exhibit different sensitivities to inhibition by sorbates. Early studies indicated that sorbates could be used as a selective agent for catalase-negative lactic acid-producing bacteria and clostridia because exhibited highly inhibition against catalase-positive organisms. Overall, however, the inhibitory action against lactic bacteria by sorbate is less than that against yeasts. Bacterium that appears to be more resistant to inhibition by sorbate than other spore formers is *Sporolactobacillus* (25). Growth of *Gluconobacter oxydans* in the presence of sublethal concentrations of SA before determination of the minimal inhibitory concentration (MIC) resulted in a substantial increase in the MIC within 1 h (26). Varying sensitivities of bacterial species and strains to sorbates may lead to shifts in the microbial population during storage of foods (9,27,28,29,30,31,32,33). Under certain conditions some species and strains of yeasts and molds are also resistant to inhibition by sorbates. Yeast strains resistant to sorbates belong to the genera *Zygosaccharomyces*, *Saccharomyces*, *Torulopsis*, *Brettanomyces*, *Candida*, and *Triganopsis* (34–41). Resistance of yeasts to sorbates depends on species and strains, sorbate concentration/content, pH, inoculum level, storage temperature, and previous exposure of the organism to low levels of sorbate. When the yeast cells have been previously adapted to sorbate in media containing glucose or sucrose, subsequent exposure of the cells shows little effect of solute type on sorbate resistance (42). Resistance of osmotolerant yeasts to inhibition by sorbate was acquired by reconditioning the yeast to sorbate (40). One proposed mechanism of resistance of osmotolerant yeasts has involved an inducible, energy-quiring system that transports the preservative out of the cell (34). Other proposed mechanisms of yeast resistance to sorbates at reduced water activities have been related to yeast cell shrinkage and decreases in membrane are size, retarding the flow of sorbates into the cell (38), or protection of enzyme systems from inhibition by sorbates through production of compatible solutes, such as polyols (40).

VII. METABOLIC DEGRADATION

At normal conditions of alimentation, sorbates are completely metabolized and fully oxidized to carbon dioxide and water like other fatty acids. It means that sorbates yields 6.6 kcal/g, of which 50% is biologically utilizable. Mechanism of degradation in metabolic pathways of organisms has been studied extensively. Sorbates can be metabolized as fatty acids through β-oxidation by animals and certain microorganisms. When sorbate levels are high, there is also evidence of ω-oxidation (44). At special conditions, some mold strains can grow and metabolize sorbates. The decomposition of sorbates by molds has been detected in cheeses and fruit products. Mold strains of the genus *Penicillium* isolated from cheeses treated with sorbates were able to grow and metabolize high (0.18–1.20%) sorbate levels (44, 45). It should be noted that 0.1% sorbates are usually sufficient to inhibit sensitive molds (46). It seems that selection may occur in sorbate-treated cheeses for certain molds tolerant to the compound (47). One of products of sorbate metabolism by molds include 1,3-pentadiene, which is volatile and has an odor like kerosene, plastic paint, or hydrocarbon and is formed through a decarboxylation reaction (48, 49). Other strains of molds that may degrade sorbates belong to the genera *Aspergillus, Fusarium, Mucor,* and *Geotrichum* (2). It appears, however, that there is no apparent relationship between sorbates resistance and the toxigenic properties of molds (50). In general, although many molds are sensitive to inhibition by sorbates, certain strains are resistant and can utilize them, as a carbon source. Degradation of sorbates by molds depends on species and strains, prior exposure to subinhibitory levels of sorbate, level of inoculum, amount of sorbate present, and type of substrate. Some bacterial strains may also degrade sorbates under appropriate conditions. This metabolism is mostly associated with lactic acid-producing bacterial strains present as high inocula in sublethal concentrations of sorbates (46, 51, 52). Degradation of sorbates by lactic acid bacteria has been associated with geranium-type off-odors in wines and fermented vegetables, caused by ethyl sorbate, 4-hexenoic acid, l-ethoxyhexa-2,4-diene, and 2-ethoxyhexa-3,5-diene (53, 54). In general, a geranium-like odor is usually associated with wines treated with sorbates and contaminated with high microbial loads. Yeasts *Zygosaccharomyces rouxii* and *Debaryomyces hansenii* are able to decarboxylate SA to produce 1,3-pentadiene as major compound of petroleum off-odor (72).

VIII. PHYSICOCHEMICAL DEGRADATION

Autoxidation of aqueous SA solutions increased in the presence of citric acid and further increased in the presence of Fe^{3+} ions and lowered in the presence of Cu^{2+} salts unless ascorbic acid was also added. In the absence of metal ions, ascorbic acid had no effect on autoxidation of SA (57). Solutions of SA are also unstable in the presence of SO_2 and light. At pH 2.1 in the presence of excess SO_2, SA was totally degraded in 8 days. SA is also unstable in solutions of dilute H_2SO_4. Two volatile products from the degradation in SO_2 solution and in dilute H_2SO_4 were identified as β-angelica lactone and 2-methyl-5-acetylfuran (58). SA degraded in aqueous glycerol solutions at pH 4.0 over the water activity range 0.71–1.00 and the temperature range 40–60°C by first-order reaction kinetics and to conformed to the Arrhenius equation. Activation energy values obtained were 5.8 kcal mol^{-1} and 7.8 kcal mol^{-1} for systems at a_w 0.80 with and without added Co^{2+} ions, respectively. The rate of SA degradation increased with decreasing a_w i.e. increasing glycerol concentration. The presence of added Co^{2+} decreased the rate of SA breakdown at any particular a_w or temperature. Browning of sorbate solutions during storage was markedly inhibited by Co^{2+} ions (59). Acetaldehyde and β-carboxylacrolein were identified as the major degradation products of SA in the presence of glycine and $CuSO_4$. Crotonaldehyde and acetone were also detected, but in very small amounts. Studies of the role of carbonyl compounds in SA-mediated browning revealed that, in the presence of amino acids, β-carboxylacrolein polymerizes rapidly to brown pigments. In the presence of Cu^{2+} brown pigments were not formed (60). SA degraded in model systems composed of sugar solutions at pH 3.5 and 4.5 and its destruction was described by first-order kinetics. Nonenzymic browning reactions, which occurred in systems containing lysine, greatly influenced the kinetics of SA destruction (61). Aspartame reduced sorbate destruction in aqueous system without sugars as well as in systems containing glucose, but in the presence of sucrose, it enhanced degradation (96). Loss of SA in stored peaches preserved by combined factors followed first order reaction kinetics, when rate constants and activation energy were almost identical to previously reported data (62). NaCl showed a pronounced stabilizing effect on degradation of SA in aqueous solutions to which glycerol and citric acid had been added to adjust a_w to 0.91 and pH between 5 to 6 (63). SA in aqueous solutions underwent to autoxidation, forming malonaldehyde and other carbonyls. Oxidative degradation followed first-order reaction kinetics, and the rate of reaction decreased with a rise in pH. Amino acids except histidine and arginine increased while NaCl, KCl, sucrose, and metal ions (Cu^{2+}; Fe^{2+}; Mn^{2+}) decreased the rate of degradation. In an other study, NaCl enhanced SA degradation when the destruction seemed to be related to the NaCl and concentration rate of SA degradation increased significantly with increasing temperature (68). Acetic acid, glycerol, and the majority of other salts enhanced the rate of degradation (64). In liquid model systems containing water-glycerol-glucose, water-glycerol-sucrose, sucrose and 30 sucrose

glucose mixtures, SA degraded during storage forming brown pigments. Addition of amino acids (glycine or lysine) increased rate of browning development while Cu^{2+} reduced SA degradation and browning development. In all systems, addition of amino acids decreased SA degradation but increased browning development. Rate of SA-induced browning was considerably higher than that caused by sugar-amino acid interactions (65). In an other study it confirmed the accelerate role of SA in the glucose-glycine browning reaction (66). The kinetics of brown pigment formation in a water-glycerol-sorbate-glycine model system at pH 4 was studied as a function of a_w (0.55–0.90) in the interval of 40–60°C. The rate of browning was found to follow zero-order kinetics and to increased with decreasing a_w. Activation energy values ranged from 15.5 to 22.2 kcal/mol, and were higher at the lower a_w levels. Glycerol was found to participate actively in the formation of brown pigments by reaction with sorbates or glycine; the rates also followed zero-order kinetics obeying the Arrhenius equation (67). Important is effects of additives (propyl gallate, sodium nitrite, sucrose, ascorbic acid, glycerol, glycine and polyphosphates) on stability of sorbates in aqueous model systems of a_w 0.91 and pH 5.0. Rate constants of SA destruction could be described by 1st order kinetics and non-enzymatic browning reactions exhibited zero order kinetics. Both were greatly influenced by SA destruction, when the correlation between the natural logarithm of sorbates retention and the increase in absorbance at 420 nm was found in all systems. Addition of glycine increased sorbate destruction and browning development. To the opposite, SA catalysed Maillard reaction between glycine and glucose heated to 100°C, while no loss of the SA was observed during the experiment (83). Polyphosphates and propyl gallate showed an important protective effect against SA degradation and also against non-enzymatic browning. Ascorbic acid showed a dual effect: in the absence of sodium nitrite, it exhibited a pro-oxidant effect; in the presence of nitrite, a protective effect was apparent. Sucrose did not influence the rate of SA degradation but enhanced non-enzymatic browning. The effect of sodium nitrite on sorbate destruction was diverse: it accelerated destruction in systems containing NaCl, glycerol and glycine, diminished the destruction rate when polyphosphates were also present, and had no effect in the presence of sucrose and/or sucrose and polyphosphates. Conversely, in the presence of ascorbic acid, sodium nitrite exerted a protective effect (69). EDTA enhanced sorbate degradation as a result of Fe scavenging from the glass and polypropylene packaging materials through EDTA complexation. It was proposed that SA autoxidation is catalyzed by EDTA-Fe^{2+} complexes, accompanying with production of carbonyls followingly involved in non-enzymatic browning (70). To lower sorbate destruction, the use of glass instead of PET containers, and a mixture of citric and acetic acids, ascorbic acid and EDTA was proposed (97). SA was highly susceptible to radiolytic degradation in aqueous systems exposed to gamma-irradiation. Rate of degradation decreased with rise in pH. Sugars, hydrocolloids except pectin, citric acid, lactic acid, malic acid, arginine and threonine, catalyzed degradation while oxalic acid, maleic acid, Cu^{2+}, nitrite, nitrate and phthalate had protective effects. SA was more stable in alcohols and vegetable oils than in aqueous solutions. In wheat flour radiolytic degradation of SA was less at lower moisture. SA was more stable in chapattis than in dough. Gelatinization and addition of oil into dough reduced degradation of SA (71).

IX. INTERACTION AND FORMATION OF ADDUCTS

SA and methyl ester of SA react with sodium nitrite to form ethylnitrolic acid (ENA) after heating at 90°C for 1 h (73,74). As found, ENA could also be formed at lower temperature of 60°C and pH 3.5–4.2 after 30 min when the formation of an other mutagenic compound 1,4-dinitro-2-methylpyrrole (DNMP) was observed. Formation of both mutagenic compounds was inhibited by ascorbic acid and cysteine (75, 79), and the loss of mutagenicity could be reached by reduction of the C-nitro group to the C-amino group in DNMP (76) by conversion to 1-nitro-2-methyl-4-aminopyrrole (77). Nitrite reacted optimally with SA at pH 3.5, but the reaction did not take place at pH 6. Most mutagenic products formed were C-nitro and C-nitroso compounds, and the conjugated dienoic structure for mutagen formation was essential (78). Thiols (mercaptoethanol, cysteine or an alkylthiol) reacted slowly with SA during heating for several days at 80°C and pH 3.7–7.8 in a bimolecular reaction via nucleophilic attack by thiolate anion at position 5 of the SA molecule, in the case of amines, cyclization to form substituted dihydropyridones may follow (80, 85). The nucleophilic attack by alkylthiols led to formation of diadducts, while reaction with mercaptoethanol, esters of 2-mercaptoacetic acid and cysteine ethyl ester gave the 2,5-addition product. Products of monoaddition of thiols to SA had a double bond in position 3 (81). Cysteine added to the conjugated diene in position 5 formed the substituted 3-hexenoic acid (87). Reaction between SA and thiols could be accelerated by some cationic surfactants i.e. dodecyltrimethylammonium bromide as well as nonionic surfactants also behaved as catalysts (82). Even thought, bovine serum albumin could also behave as catalyst (84). The formation of the reaction intermediate in bovine serum albumin-SA-mercaptoethanol mixtures involved the binding of equimolar amounts of the reactants at up to some 90 sites on the BSA molecule. 3-hexenoic acid reduced the rate-enhancing effect of BSA due to competitive binding between 3-hexenoic acid and SA

to the reaction intermediate (88). SA-thiol irreversible adducts were formed in a dough made from cereal flours (89). Reaction of SA with sulfite species (S^{4+}) was affected in a presence of O_2, and involved a pH-dependent oxidative mechanism leaded to loss of S^{4+}. In the absence of O_2, a much slower nucleophilic addition of sulphite ions to undissociated SA took place (86). SA and ethyl sorbate reacted with various amines (methylamine, ethylamine, butylamine and benzylamine) in model systems. At ambient temperature, the reaction of sorbates with amines resulted in formation of linear mono adducts, while cyclic adducts were formed from double addition in temperature interval of 50–80°C mainly in the presence of O_2 which significantly accelerated adduct formation reaction (90,91). Potasium sorbate reacted with methylamine and butylamine and adducts were identified as cyclic dihydropyridone esters, formed by a double addition reaction (92). Under conditions typical of food processing (50–80°C), the cyclic derivatives resulting from a double addition reaction between SA and various amines (including methylamine, ethylamine, propylamine, butylamine and benzylamine) were formed and analysed. Major formed compounds were N-methyl-6-methyl-3,6-dihydro-2-pyridone, N-ethyl-6-methyl-3,6-dihydro-2-pyridone, N-propyl-6-methyl-3,6-dihydro-2-pyridone, N-butyl-6-methyl-3,6-dihydro-2-pyridone, N-benzyl-6-methyl-5,6-dihydro-2-pyridone and minor compounds were N-methyl-6-methyl-5,6-dihydro-2-pyridone, N-ethyl-6-methyl-5,6-dihydro-2-pyridone, N-propyl-6-methyl-5,6-dihydro-2-pyridone, N-butyl-6-methyl-5,6-dihydro-2-pyridone, N-benzyl-6-methyl-5,6-dihydro-2-pyridone. Mutagenesis studies involving the Ames test using *S. typhimurium* (strains TA98 and TA100), and genotoxicity studies with HeLa cells and plasmid DNA, showed that none of the compounds studied presented either mutagenic or genotoxic activities (93,94). Mutagenicity and DNA damage by products formed during oxidation of sorbates by H_2O_2, produced by reaction of ascorbate with Fe-EDTA, ferric citrate, ferrous gluconate, ferric pyrophosphate or ferrous sulphate, were assessed *in vitro* using the Ames test and a rec-assay, respectively. Products from a reaction carried out in the absence of O_2 did not exhibit mutagenicity, which confirmed that the mutagenic effects resulted from an oxidation reaction (95).

X. TOXICOLOGY

Sorbates are considered for the least harmful antimicrobial preservatives, even at levels exceeding those normally used in foods. The LD_{50} for sorbates in rats ranges from 7.4 to 10.5 g/kg body weight. Rats fed SA at 10% in feed for 40 days had no ill effects. When the feeding period was increased to 120 days, the growth rate and liver weight increased. SA at 5% in the diet of rats did not affect health after 1 000 days (except for two tumors in 100 rats). When rats were given SA (10 mg/100 mL) or potassium sorbate (0.3%) in drinking water or 0.1% levels in the diet, no tumors were observed after 100 weeks. The growth of tumors was not seen when mice were fed 40 mg SA per kilogram body weight. Repeated subcutaneous administration of SA in peanut oil or water in rats produced sarcomas at the site of injection. Sarcomas produced locally in this manner were not considered a valid index of carcinogenicity. Weak mutagenic activity was detected in the ether extract of bacon-curing brines containing potassium sorbate. Retesting the sample indicated that the mutagenic activity was due to a compound in the ether to be used as the extraction solvent. (136). Also other studies did not reveal carcinogenic effects of sorbates (99,100). When SA is used for cosmetic and pharmaceutical products, it may irritate the mucous membranes, and in highly sensitive individuals it may cause skin irritation (98). An allergic-type response has been reported for SA in a study, when panelists tasted bacon treated with sorbate-nitrite mixture (101).

XI. REGULATORY STATUS

The application of sorbates is limited by national legislation of individual countries to prevent to the use them in excessive amounts due to their possible toxicity. In most countries of the world, food legislation has given procedures for application of sorbates in various food products. This legislation identifies approved sorbates, establishes permitted concentrations, and includes labeling requirements. From an international point of view, the Joint Expert Committee for Food Additives (JECFA) deals periodically with evaluation of a real situation and adopts maximum limits for food additives including sorbates (see Table 96.2) on the basis of all available scientific data. Such information provided by JEFCA is used by the Joint FAO/WHO organ — Codex Alimentarius Commission in implementing Joint FAO/WHO Standards Programme, which has been established to elaborate international standards for foods to protect the health of consumers, ensure fair practices in food trade, and facilitate international trade. To identify individual food additives, so called "General Standard for Food Additives" embraces the list of preservatives permitted for application in food industry together with their International Numbering System (INS) numbers (102). Similarly, European Union has adopted the same numbering system of permitted food additives, the only difference is replacing "INS" by "E" letter on labels (103). In the United States, sorbates are generally recognized as safe (GRAS), and this status has been reaffirmed again April 1st 2001 by a select committee of the U.S. Food and Drug Administration, which regulates all antimicrobials used in or on processed food under FFDCA §409, as food additives. According to the Code of Federal Regulations, when a food preservative is used in a food product, its common name should be listed on

TABLE 96.2
Maximum Limits of Sorbates for Food Products Adopted by Codex Alimentarius Commission

Food Cat. No.	Food Category	Max. Level [mg/kg]	Food Cat. No.	Food Category	Max. Level [mg/kg]
01.1.1	Milk and buttermilk	1 000	01.1.2	Dairy-based drinks, flavored and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	300
01.2.1	Fermented milks (plain)	300	01.2.2	Renneted milk	1 000
01.3.2	Beverage whiteners	200	01.6	Cheese	3 000
01.7	Dairy-based desserts (e.g., ice cream, ice milk, pudding, fruit or flavoured yoghurt)	1 000	02.1.1	Butter oil, anhydrous milkfat, ghee	1 000
02.2.1	Emulsions containing at least 80% fat	1 000	02.2.2	Emulsions containing less than 80% fat (e.g., minarine)	2 000
02.3	Fat emulsions other than food category 02.2, including mixed and/or flavoured products based on fat emulsions	1 000	02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7	1 000
03.0	Edible ices, including sherbet and sorbet	1 000	04.1.1	Fresh fruit	1 000
04.1.2.1	Frozen fruit	1 000	04.1.2.2	Dried fruit	2 000
04.1.2.3	Fruit in vinegar, oil, or brine	2 000	04.1.2.4	Canned or bottled (pasteurized) fruit	1 000
04.1.2.5	Jams, jellies and marmelades	1 500	04.1.2.6	Fruit-based spreads (e.g., chutney) excluding products of food category 04.1.2.5	1 000
04.1.2.7	Candied fruit	1 000	04.1.2.8	Fruit preparations, including pulp, purees, fruit toppings and coconut milk	1 500
04.1.2.9	Fruit-based desserts, including fruit-flavoured water-based desserts	1 000	04.1.2.10	Fermented fruit products	1 000
04.1.2.11	Fruit fillings for pastries	1 000	04.1.2.12	Cooked or fried fruit	1 200
04.2.2.3	Vegetables and seaweeds in vinegar, oil, brine, or soy sauce	2 000	04.2.2.4	Canned or bottled (pasteurized) or retort pouch vegetables	1 000
04.2.2.5	Vegetable, and nut and seed purees and spreads (e.g., peanut butter)	2 000	04.2.2.6	Vegetable, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables, soybean curd) other than food category 04.2.2.5	2 000
04.2.2.7	Fermented vegetable products	1 000	04.2.2.8	Cooked or fried vegetables and seaweeds	2 000
05.1.1	Cocoa mixes (powders and syrups)	1 500	05.1.2	Cocoa-based spreads, including fillings	1 500
05.1.3	Cocoa and chocolate products (e.g., milk chocolate bar, chocolate flakes, white chocolate) other than food categories 05.1.1, 05.1.2 and 05.1.4	1 000	05.1.4	Imitation chocolate, chocolate substitue products	1 500
05.2	Confectionery including hard and soft candy, nougat, etc. other than food categories 05.1, 05.3 and 05.4	2 000	05.3	Chewing gum	1 500
05.4	Decorations (e.g., for fine bakery wares), toppings (non-fruit) and sweet sauces	1 500	06.2	Flours and starches	1 000
06.4.2	Pre-cooked or dried pastas and noodles and like products	2 000	06.5	Cereal and starch based desserts (e.g., rice pudding, tapioca pudding)	1 000
06.6	Batters (e.g., for fish or poultry breading)	2 000	07.0	Bakery wares	2 000
08.2	Processed meat, poultry, and game products in whole pieces or cuts	2 000	08.3	Processed comminuted meat, poultry, and game products	2 000
08.4	Edible casings (e.g., sausage casings)	GMP*	09.2.1	Frozen fish, fish fillets, and fish products, including mollusks, crustaceans, and echinoderms	2 000
09.2.2	Frozen battered fish, fish fillets, and fish products, including mollusks, crustaceans, and echinoderms	2 000	09.2.3	Frozen minced and creamed fish products, including mollusks, crustaceans, and echinoderms	2 000
09.2.4.1	Cooked fish and fish products	2 000	09.2.4.2	Cooked mollusks, crustaceans, and echinoderms	2 000
09.2.5	Smoked, dried, fermented, and/or salted fish and fish products, including mollusks, crustaceans, and echinoderms	2 000	09.3	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms	2 000
10.2.1	Liquid egg products	5 000	10.2.2	Frozen egg products	1 000

(*Continued*)

TABLE 96.2
(*Continued*)

Food Cat. No.	Food Category	Max. Level [mg/kg]	Food Cat. No.	Food Category	Max. Level [mg/kg]
10.2.3	Dried and/or heat coagulated egg products	1 000	10.4	Egg-based desserts (e.g., custard)	1 000
11.1	White and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose; sugar solutions and syrups, also (partially) inverted sugars, incl. molasses, treacle, and sugar toppings	1 000	11.2	Other sugars and syrups (e.g., brown sugar, maple syrup)	1 000
11.4	Table-top sweeteners, including those containing high-intensity sweeteners	1 000	12.2	Herbs, spices, seasonings (including salt substitutes), and condiments (e.g., seasoning for instant noodles)	1 000
12.3	Vinegars	1 000	12.4	Mustards	1 500
12.5	Soups and broths	1 000	12.6	Sauces and like products	2 000
12.7	Salads (e.g., macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut based-spreads of food categories 04.2.2.5 and 05.1.2	1 500	13.3	Dietetic foods intended for special medical purposes, including those for infants and young children	1 500
13.4	Dietetic formulae for slimming purposes and weight reduction	1 500	13.5	Dietetic foods (e.g., supplementary foods for dietary use) excluding products of food categories 13.1–13.4	2 000
13.6	Food supplements	2 000	14.1.1.2	Table waters and soda waters	200
14.1.2.1	Canned or bottled (pasteurized) fruit juice	2 100	14.1.2.2	Canned or bottled (pasteurized) vegetable juice	1 000
14.1.2.3	Concentrate (liquid or solid) for fruit juice	1 000	14.1.2.4	Concentrate (liquid or solid) for vegetable juice	1 000
14.1.3.1	Canned or bottled (pasteurized) fruit nectar	2 000	14.1.3.2	Canned or bottled (pasteurized) vegetable nectar	1 000
14.1.3.3	Concentrate (liquid or solid) for fruit nectar	1 000	14.1.3.4	Concentrate (liquid or solid) for vegetable nectar	1 000
14.1.4.1	Carbonated drinks	1 000	14.1.4.2	Non-carbonated, including punches and ades	1 000
14.1.4.3	Concentrates (liquid or solid) for drinks	1 500	14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	1 000
14.2.1	Beer and malt beverages	500	14.2.2	Cider and perry	1 000
14.2.3	Wines	2 000	14.2.4	Fruit wine	1 000
14.2.5	Mead	1 000	14.2.6	Spirituous beverages	600
15.1	Snacks — potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	1 000	15.2	Processed nuts, including covered nuts and nut mixtures (with e.g., dried fruit)	1 000
16.0	Composite foods (e.g., casseroles, meat pies, mincemeat) — foods that could not be placed in categories 01–15				1 000

GMP* — good manufacturing practice.

the product label and its function should be indicated by an explanatory description (e.g., "to maintain freshness," "to extend shelf life," or "as a preservative"). The use of sorbates may be requested for any food product that allows preservatives (2).

XII. ANTIMICROBIAL ACTIVITY

Sorbates have broad spectrum activity against yeast and molds, but are less active against bacteria. Food-related yeasts inhibited by sorbates include species of *Brettanomyces, Byssochlamys, Candida, Cryptococcus, Debaryomyces, Endomycopsis, Hansenula, Oospora, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Torulaspora, Candida,* and *Zygosaccharomyces*. Food-related mold species inhibited by sorbates belong to the genera *Alternaria, Ascochyto, Aspergillus, Botrytis, Cephalosporium, Fusanum, Geotrichum, Gliocladium, Helminthosporium, Humicola, Mucor, Penicillium, Phoma, Pullularia (Auerobasidium), Sporotrichum,* and *Trichoierma* (2). Bacteria sensitive to sorbate embraces genera *Salmonella, Clostridium, Yersinia, Staphylococcus, Bacillus, Lactobacillus, Escherichia, Proteus, Streptococcus* (98). Optimum effectiveness of sorbates extends from lower values to pH ≈ 6.5 (4), but and the antimicrobial activity of SA is greatest when the compound is in the undissociated state. Because of pKa value, the antimicrobial activity is greatest as the pH is rather lower and below the value 6.0 (98). The effect of pH on inhibition activity of SA was determined for *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans*. As observed, the inhibitory action of undissociated acid was 10–600x

greater than that of dissociated acid (22). The inhibitory effect of sorbates may be lethal as well as static. Conidia of *A. parasiticus* lost viability in the presence of sorbate (20). Differential effect on growth and patulin production by strains of *P. patulum* and *P. roqueforti* isolated from cheese was observed, when potassium sorbate delayed initiation of growth, prevented spore germination, and decreased the rate of growth of *P. patulum* in potato dextrose broth, while *P. roqueforti* was affected less (104). Similar results were observed with *A. parasiticus* and *A. flavus* in yeast extract sucrose broth (105). Potassium sorbate reduced or prevented the production of the patulin by *P. patulum* and aflatoxin B_1 by *A. parasiticus* and *A. flavus* (104,105). Potassium sorbate has been found to inhibit markedly the growth and patulin production of *P. expansum* and *B. nivea* in grape and apple juice (106,107,108,109). On the other hand, sorbate delayed only mold growth, but did not inhibit biosynthesis of aflatoxin by *A. parasiticus*. The ability to synthesize aflatoxin was greater in the early stages of growth and then decreased as mold growth progressed (110). Potassium sorbate at the concentration of 0.2% (pH 5.5) or 0.05% (pH 4.5) completely inhibited *A. parasiticus* growth and toxin production for 3 days in a glucose-yeast extract salts medium. However, growth and toxin production were nearly normal by day 7 of incubation (111). *A. flavus* grown on yeast extract-sucrose medium produced higher amounts of aflatoxin B_1 in the presence of 0.025% SA than without it. Addition of 0.05–0.0125% SA stimulated T-2 toxin production of *F. acuminatum* cultures grown on maize meal and highest amounts of the mycotoxin were detected in 14-day-old cultures containing 0.025% SA. It was assumed that certain amounts of SA near the minimum inhibitory concentration reduced the activity of the tricarboxylic acid cycle. This may leaded to an accumulation of acetyl coenzyme A, which is an essential intermediate in the biosynthesis of aflatoxin B_1 and T-2 toxin (152). Potassium sorbate was more effective against injured spores of *A. parasiticus* and an intensity of inhibition depended on the concentration of sorbate and pH (112). Sorbate has been found to inhibit the growth of *Salmonella, C. botulinum,* and *S. aureus* in cooked, uncured sausage (113), of *S. aureus* in bacon (114), of *P. fluorescens* and *P. putrefaciens* in trypticase soy broth (115,116), of *V. parahemolyticus* in crab meat and flounder homogenates (117), of *S. aureus* (118), microbiological growth in cooked turkey products (119), *C. botulinum* toxin production in commercially prepared bacon (120). Sorbates also inhibited *S. typhimurium* in skim and evaporated milk (121). Potassium sorbate at 0.5% inhibited growth and histamine production by *P. morgani* and *K. pneumoniae* strains in a trypticase soy broth fortified with histidine (122). Potassium sorbate inhibited anaerobic growth of *S. aureus* more than growth under aerobic conditions in a model sausage system and inhibition was strengthened after lactic acid addition (123). While combinations of sorbate and NaCl were more effective in inhibition of *S. typhimurium* than sorbate alone at pH 6.3 (124), NaCl did not appear to enhance effectiveness of sorbate on growth of *S. aureus* MF-31 in tryptic soy broth at same pH (125). Microorganisms isolated from seafood showed differential sensitivity to sorbate (126). Potassium sorbate inhibition of *B. cereus* T spore germination induced by L-alanine and L-α-NH_2-n-butyric acid was shown to be competitive in nature. It was also a competitive inhibitor of L-alanine- and L-cysteine-induced germination of *C. botulinum* 62A spores (127). Reports in the 1950s concluded that sorbates had either stimulatory or no effect on clostridia, but later studies concluded that sorbates have acted as an anticlostridial agent in cured meat products (5,14,29). The contradictory results of the studies were most likely due to the fact that in the 1950s, media with pH values approaching 7.0 were used. This was optimal for growth of clostridia but not optimal for the activity of sorbates, since only about 0.6% of SA is in the undissociated form at pH 7.0 (98). Potassium sorbate, sufficient to give an undissociated SA concentration of 250 mg/liter in culture medium at pH 5.5–7.0, retarded the growth of proteolytic strains of *C. botulinum* from spores and vegetative cells (29,30). Sorbate has been shown to prevent spores of *C. botulinum* from germinating and forming toxin in poultry frankfurters, ham, emulsions as well as beef, pork, and soy protein frankfurter emulsions, bacon, and canned comminuted pork (120,128, 129,130,131). *C. botulinum* toxin production was significantly decreased in a model cured meat system adding 3.5% NaCl, 0.26% potassium sorbate at pH less than 6.0, and low storage temperatures (132). A systematic study to determine the concentration of potassium sorbate necessary to inhibit a single *C. botulinum* cell in culture medium showed that 0.1% at pH 5.0 would reduce the probability of growth of *C. botulinum* at 30°C in 14 days to 1 in 10^8. It was also concluded that 0.2% SA alone in meat products at pH 6.5 would not significantly inhibit *C. botulinum* vegetative cells (133). The effect of sorbate on thermal inactivation and recovery of injured microorganisms is variable among species and strains. Low concentrations of sorbic and fumaric acids in the heating medium had little effect on the heat resistance of *E. herbariorum*, an aerophilic mold involved in the spoilage of grape preserves (138). The antimicrobial activity of sorbate is usually enhanced under vacuum or modified gas atmosphere storage conditions, including meat (32,139,140) and fish products (141,142,143). Combinations of carbon dioxide and sorbate have also been reported as effective inhibitors of microbial growth of *S. enteriditis* (144), *S. aureus* on tryptic soy agar (145) as well as on fresh poultry to be dipped into sorbate pollution (146). Synergistic antimicrobial

activity was optimal at pH 5.5 at 1.5% sorbate solution and 100% CO_2 treatment. Although food acids may reduce the water solubility of sorbate, they can enhance its antimicrobial activity by increasing the concentration of undissociated SA. In addition, the specific anion itself may contribute antimicrobial activity (129). Specific effects, however, vary with substrates, microorganisms, and types of acids. Combinations of sorbate with antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary butyl hydroxyquinone, and propyl gallate, had increased antimicrobial activity compared to individual components (147,155). Variations existed, however, with types of microorganisms, antioxidants, and substrates. These combinations offer the advantage of simultaneous inhibition of microbial growth and development of rancidity. A few studies have also indicated increased antimicrobial effects when sorbate was combined with various phosphates (148). Combinations of sorbate with benzoate or propionate may be used to expand the range of microorganisms inhibited with reduced concentrations of each preservative (149). In addition, mixtures of sorbate with various antibiotics have demonstrated increased antimicrobial activity (150,151). Fungicidal activity of SA against *S. cerevisiae* was enhanced 64-fold in combination with half-minimum fungicidal concentration of polygodial, a bicyclic sesquiterpene dialdehyde isolated from the sprout of *P. hydropiper*. This synergistic activity of presumably came from its ability to inhibit the plasma membrane H^+-ATPase (154). Different effect of SA was observed on *P. membranifaciens.* At 25°C, SA completely inhibited yeast growth, but at temperature greater than 43 degree C appeared to protect the yeast from thermal death (156). The antilisterial effects of a sorbate-nisin combination were assessed *in vitro* and on beef at refrigeration temperature. Treatment with the sorbate-nisin combination did not significantly affect pathogenicity of the *L. monocytogenes* cocktail recovered from vacuum- or CO_2-packages after storage, in contrast to the in vitro study, where pathogenicity was clearly attenuated. The reason for this difference was unknown (157).

XIII. RESISTANCE

Potassium sorbate (0.1%) added to scallops (pH 6.3–6.5) resulted in more rapid *C. botulinum* toxin formation than in controls. It was postulated that this might be due to inhibition of microflora competitive to C. *botulinum* (134). Resistance to sorbate was observed at *P. roqueforti*, which was able to decarbolise SA and produce 1,3-pentadiene (135). Lowering the a_w enhanced the resistance of *S. rouxii* to increasing concentrations of sorbate (38.) *S. rouxii* cells, preconditioned in 0.1% sorbate showed an increased resistance to sorbate when inoculated into the growth medium and the chocolate syrup *S. rouxii* cells preconditioned in 60% sucrose/0.1% sorbate were more sensitive to sorbate than cells preconditioned in 0% sucrose/0.1% sorbate (40). Effect of sorbate on growth of *Z. rouxii* was evaluated at reduced a_w, using sucrose, glucose and polyethylene glycol (PEG). Sorbate-adapted and unadapted cells were grown aerobically at pH 5.0 in media adjusted to a_w 0.92 by addition of solutes. Sorbate resistance by unadapted cells increased in the order of glucose less than PEG less than sucrose. Solute type had little effect on sorbate resistance by sorbate-adapted cells in media containing glucose and sucrose (137). Certain strains of microorganisms were not only resistant to inhibition by sorbate, but also increased levels of microbial contamination. Thus, sorbates should be used to preserve foods processed using good manufacturing practices, not as a substitute for sanitation and hygienic practices (2).

XIV. APPLICATION

Sorbates are some of the most widely used food preservatives in the world and they are being applied in human foods of all types, animal feeds, pharmaceuticals, cosmetic products, packaging materials, and technical preparations that come in contact with foods or the human body. As food preservatives, sorbates have found wide application in various foods, especially as yeast and mold inhibitors. Major groups of foods that may be preserved with sorbates include dairy products, bakery items, fruit and vegetable products, edible fat emulsion products, certain meat and fish products, and sugar and confectionery items. Application methods for sorbates include direct addition in the formulation, spraying or immersing the food material in a solution, dusting with a powder, or addition in a coating or packaging material. However, release of sorbates from plastic films is limited, so, the antimicrobial effectiveness is low (194). Selection of the most appropriate method depends on processing procedures, type of food, objectives to be accomplished, equipment available, and convenience (2).

XV. ANALYSIS

Analytical determination and occurrence of sorbates in foods is permanently actual and frequently published/reviewed (159,160,161). There are a lot of procedures and techniques for determination of sorbates in foods. If necessary, sorbates are isolated from the matrix prior to analysis. Two main procedures of isolation methods are generally used as follows:

1. Steam distillation, followed by an extraction of SA from the aqueous distillate with organic solvents

TABLE 96.3
Sample Treatment and HPLC Conditions Used for Determination of Sorbates

Sample, Sample Preparation	Stationary Phase	Mobile Phase	Detector	Ref.
Extraction of margarine, washing with diethyl ether, extraction with NaOH and H_2SO_4	μ-Bonda Pak C_{18}	Phosphate buffer-methanol (20:80) (v/v); phosphate buffer-methanol (40:60) (v/v)	UV at 235 nm	166
Filtration of orange juice	Shodex RP pak DS-(150 × 6 mm) Polystyrene-divinyl benzene resin RP-18 guard column (40 × 3.4 mm)	0.05 M KH_2PO_4 (pH 2.65)-acetonitrile (60:40) (v/v); flow rate 1.0 mL/min	UV 230 nm	160 161 167
Coffee drink steam- distilled, cleanup SPE technique	Nucleosil 5 C_{18} (150 × 3.4 mm), Lichrosorb RP-18 (150 × 4.3 mm); Develpsil ODS 5 μm	Acetonitrile-water-0.2 M phosphate buffer, pH 3.6 (7:12:1) + CTA	UV at 235 nm	168
Fatty samples cleaned on Extrelut column, elution with dichloromethane-diethyl ether (4:1)	LiChrosorb Si 60 (150 × 4.6 mm) μ-BondaPak C_{18} 10 μm (300 × 4.6 mm)	Heptane-diisopropyl ethyl-glacial acetic acid (88:12:0.1), (90:10:0.05), 1.8 mL/min; water-acetonitrile-H_3PO_4 (90:10:0.5), flow rate 0.8 mL/min	UV at 230 nm	160 161 167
Beverages, jams; filtration, Degassing	PhaseSep C_{18}, Spherisorb ODS 1 (250 × 4.6 mm) 5 μm	Methanol-phosphate buffer (8:92) (v/v) pH 6.7 using H_3PO_4 flow rate 1.0 mL/min	UV at 227 nm	169
Fruit juice; filtration	μ-BondaPak CN	2% Acetic acid-methanol (95:5) (v/v)	UV at 254 nm	170
Citrus juices filtration	μ-Bondapak CN	2% Acetic acid-methanol (19:1) (v/v)	UV at 240 nm	171
Citrus juices; filtration	Partisil 10 SAX	0.225 M KH_2PO_4 1.0 mL/min	UV 254 nm	172
Cheese; steam distillation	LiChrosorb RP-18 (250 × 4 mm)	Methanol-0.02 M phosphate buffer (30:70) (v/v) + tetrabutylammonia 1.0 mL/min	UV 235 nm	173
Beverages, tomato sauce; extraction	μ-BondaPak C_{18}	Methanol-acetic acid-water (20:5:75) (v/v/v) (35:5:60) (v/v/v)	UV 254 nm	174
Sweet commercial wines; synthetic wines diluted with 0.01 N H_2SO_4, filtered 0.45 μm	AMINEX HPX 87-H (300 × 7.9 mm); 65°C	0.01 N H_2SO_4:CH_3CN (75:25)	UV 258 nm	175
Fruit juices, sodas, soy sauce, ketchup, peanut butter cream, cheese, other foods liquid samples: dilution acetonitrile/ammonium acetate solid samples: blending with the same solution	reversed phase C_{18} column	Acetonitrile/ammonium acetate	255 nm	176
Wines direct injection	Hydrogen sulfonated divinyl benzene-styrene copolymer	Isocratic elution 0,01 N H_2SO_4:CH_3CN (75:25)	UV 258 nm	177
Quince jams extraction 0,1 M orthophosphoric acid or steam distillation or via Amberlite XAD 2 after mixturing of sample with 0,05 M HCl	C_{18} column	Acetate buffer (pH 4,4) — methanol (65:35)	DAD	178
Fishery products ion-exchange chromatography	Nucleosil 100-5 C_{18}	Gradient elution: A: 0,02 M H_3PO_4/ acetonitrile (93+7, v/v) B: 0,02 M K_2HPO_4 with 5% H_3PO_4, pH 6,7: acetonitrile (80:20, v/v)	DAD,	179

2. Direct extraction using diethyl ether and petroleum ether, chloroform, benzene, acidified methanol or their mixtures as the most suitable organic solvents

XVI. COLOR DETERMINATION

Determination is based on oxidation of SA by $K_2Cr_2O_7$ in acidic medium to form a malonic dialdehyde, which

TABLE 96.4
Sample Treatment and GC Conditions for Determination of Sorbates

Sample, Sample Preparation	Stationary Phase	Carrier Gas	Detector	Ref.
Marmalades, mustard, mayonnaise, Fish products, alcoholic beverages; extraction and derivatization	Packed column, 3% SE-30 on Aeropak(3 m)	Nitrogen, 30 mL/min	FID	180
Cheese; distillation	Packed column with Porapak QS (1 m × 3 mm)	Nitrogen, 40 mL/min	FID	173
Rennet, extraction with diethyl ether	Packed column Chromosorb W AW DMSC,15% EGA (2 m × 2 mm)	Nitrogen, 30 mL/min	FID	181
Mustard, mayonnaise, fish products, juices	Glass capilary column coated with OV-73 (24 m × 0.22 mm)	Nitrogen	FID	182
Soft drinks, skim yogurts, jams, sauces	Fused-silica columns (15 m × 0.53 mm i.d.) packed with 5% diphenyl-95% dimethylsiloxane or 50% diphenyl-50% dimethylsiloxane	Nitrogen	FID	183
Margarine, oil, fresh cheese, mayonnaise	Fused-silica column (30 m × 0.32 mm i.d.) coated with 5% diphenyl-95% dimethylpolysiloxane, HP-5)	Helium	FID	184
Rye bread, extraction with diethyl ether-phosphoric acid	Fused-silica column WCOT (25 m × 0.53 mm i.d.), FFAP-CB coating, 1.0 μm thickness	Nitrogen	FID	185

reacts with thiobarbituric acid to give a red coloration, intensity of which is measured (162).

XVII. TRADITIONAL DETERMINATIONS

The principle of the titration method is based on the iodometric determination by following reactions

$$2KBrO_3 + 3CH_3\text{-}CH{=}CH\text{-}CH{=}CH\text{-}COOH + 10KBr + 6H_2SO_4 \longrightarrow 3CH_3\text{-}(CHBr\text{-}CHBr)_2\text{-}COOH + 6K_2SO_4 + 6H_2O$$

$$KBrO_3 + 6KI + 3H_2SO_4 \longrightarrow KBr + 3K_2SO_4 + 3I_2 + 3H_2O$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

A direct spectrofotometric determination of SA in clarified liquid media is based on a linear dependence of absorbance on SA concentration in a liquid media at the maximum absorption wavelength of 262 nm by Lambert-Beer's law (163).

XVIII. THIN-LAYER CHROMATOGRAPHY

SA may be determined directly in alkaline extracts (164). Separation on a silica gel layer is effective with mobile–phase n-hexane–diethylether–formic acid mixture. Samples are usually developed on by ascending or descending techniques in n-butanol–ammonia–water–ethanol, or n-butanol–ethanol–ammonia mixture, respectively. For detection, $K_2Cr_2O_7/H_2SO_4$ mixture is applied first, then sprayed with saturated thiobarbituric acid solution. After further drying, a pink spot on a white background appears (165).

XIX. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Sample treatment is usually limited only to filtration (removal of solid particles) and sonication (removal of gases, mainly CO_2 from carbonated drinks). The detection limit can be enhanced by measuring the absorption maximum of SA at 259 nm. When solid samples are analyzed, SA is extracted from a matrix by the following procedures:

1. Direct extraction of an acidified sample by organic solvent
2. Solid-phase extraction
3. Extraction as an ion pair
4. Steam distillation

Fatty samples such as margarine are dissolved in non-polar solvent (diethyl ether, hexane, petroleum ether), and extracted twice with NaOH in a separating funnel. The basic aqueous extracts are acidified with H_2SO_4 and diluted to the appropriate volume with methanol. Sample preparation as well as HPLC conditions are summarized in Table 96.3.

XX. GAS CHROMATOGRAPHY

Also, gas chromatography is a simple and reliable method for the determination of SA directly or after derivatisation

TABLE 96.5
Sample Treatment and Determination of Sorbates by Capillary Electrophoresis

Sample	Separation Conditions	Detection Wavelength	Ref.
Fruit juices, cordials, soft drinks, wine, jams, dips, cheese slices	50 mM SDS, 20 mM disodium hydrogen phosphate (pH 9.2)	230 nm	186
Soy sauce, dilution with ethanol-water Plum preserves, sonication and extraction	Running buffer α/β-cyclodextrin in borax-NaOH buffer, fused capillary column 50 μm × 47 cm, electric potential of separation 20 kV	diode array	187
Soft drinks, soy sauces, bean curd	25 mM sodium phosphate (pH 10.0)	248 nm	188

without any interference from coextracts using FID or MS detectors. Sample preparation as well as GC conditions are summarized in Table 96.4.

XXI. CAPILLARY ELECTROPHORESIS

CE is a highly effective analytical technique, applied for sorbates determination over the recent decade. It possesses the advantages of high separation efficiency, excellent resolution and short analysis time. Also, it consumes less solvent and sample than HPLC. Moreover, CE methods employing cyclodextrins as modifiers can satisfactorily separate enantiomers and chiral drugs (193). Some data regarding separation conditions are summarized in Table 96.5.

XXII. ENZYMATIC DETERMINATION

The method is based on spectroscopic measurement of sorbyl coenzyme A (sorbyl CoA) at 300 nm. SA is converted to sorbyl CoA with acyl CoA synthetase in the presence of coenzyme A and ATP. The reaction is made quantitative by the irreversible hydrolysis of pyrophosphate, formed during the acylation reaction, to phosphate in the presence of inorganic pyrophosphatase Absorbance measured at 300 nm is specific for sorbyl CoA and is proportional to the SA concentration in the sample solution (189).

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97 Artificial Sweeteners: An Overview

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CONTENTS

I. INTRODUCTION

Although sweeteners can be grouped in a number of different ways, the grouping "nutritive" and "non-nutritive" acknowledges a difference in the amount of energy provided by sweeteners. (1) Nutritive sweeteners provide a sweet taste and a source of energy; non-nutritive sweeteners are sweet without providing energy. Nutritive sweeteners include sugar sweeteners (e.g., refined sugars, high fructose corn syrup, crystalline fructose, glucose, dextrose, corn sweeteners, honey, lactose, maltose, various syrups, invert sugars, concentrated fruit juice) and reduced-energy polyols or sugar alcohols (e.g., sorbitol, mannitol, xylitol, isomalt, and hydrogenated starch hydrolysates).

The claim that nutritive sweeteners have caused an increase in chronic disease (e.g., obesity, cardiovascular disease, diabetes, dental caries, behavioral disorders) is not substantiated but many consumers want the taste of sweetness without added energy. (2) The food industry has responded to this demand by producing a number of energy-reduced or non-nutritive sweeteners. Although some of the non-nutritive sweeteners are natural (e.g. Thaumatin) consumers refer to them as artificial components resulting from chemical or enzymatic production processes.

Non-nutritive sweeteners (e.g., saccharin, aspartame, acesulfame-K, cyclamate, neohesperidin, etc.) offer no energy, and, as they are to be used in small amounts, can also be referred to as *high-intensity* sweeteners. Both polyols and non-nutritive sweeteners can replace sugar sweeteners and are therefore termed sugar substitutes, sugar replacers or alternative sweeteners. Some of those non-nutritive sweeteners are artificial (e.g., aspartame and acesulfame) others are extracted from natural raw material (e.g. Stevioside, Thaumatin, etc.). (3) With five sweeteners already in use on the European continent, several new sweeteners, including aspartame-acesulfame salt and sucralose are pending approval, and the derivates of aspartame, alitame, and neotame are being reviewed by the U.S. Food and Drug Administration (FDA).

II. SWEETNESS PERCEPTION AND MEASUREMENT

The theory for sweetness perception was developed by Shallenberger and Acree, describing sweet taste as a result of an interaction between a proton donor and a proton acceptor on the sweet molecule, with a distance of approximately 0.3 nm, and a complimentary system located in the membrane of taste receptor cells. (4) This initial step in taste reception takes place at the apical end of the taste receptor cells that are situated in taste buds in the papillae of tongue, palate, and throat. Each bud is composed of 50–100 cells. Sweetness response depends on the accession of the sweetener molecule to a receptor site of the plasma membrane of a receptor cell in the mouth. Chemically a complexation reaction resulting in conformational changes stands at the base of a change in electrical potential of the cell membrane. The sweet taste sensation is caused by the potential change of the membrane, resulting in a release of neurotransmitters. Stereochemical fitting influences the intensity of the sweet taste. (4,5,6)

Extensive studies on three well-known commercial sweeteners (saccharin, acesulfame-K, and sodium cyclamate) resulted in a good understanding of the mechanism responsible for the sweet taste. The sweetness of these molecules has been rationalised on the basis of molecular models of their multiple interactions with the protein receptor in which the hydrophobic attractions are critical for both sweetness and intensity.

Substitution of the crucial bounding positions of the sweetener molecule resulted in a loss of sweet taste. (7,8) Recent studies identified the T1R2–T1R3 receptor responsible for sweet taste (9,10,11).

Artificial sweeteners have low molecular weight in contrast to some sweet tasting proteins (thaumatin) which are large molecules. Temussi (12) proposes that those proteins also do attach to a binding site on the T1R2–T1R3 receptor and probably stabilize the active form of the receptor, resulting in sweet taste. One can expect that future

TABLE 97.1
Sweetness Intensity of Sweetners Compared to Sucrose

Sweetener	Intensity
Sucrose	1
Fructose	1.73
Invert sugar	1.30
Glucose	0.74
Maltose	0.32
Lactose	0.16
Saccharin	550
Aspartame	200
Acesulfame K	200
Cyclamate	35
Neohesperidin dihydrocalcone	330
Thaumatin	3500
Alitame	2500
Neotame	2000
Stevioside	160
Rebaudioside A	250
Sucralose	600

studies will result in a better understanding of molecular mechanisms underlying taste transduction.

Sweetness is commonly measured by comparison to reference solutions of sucrose. Sucrose is the standard to which all sweeteners are compared. Humans can recognize sweetness in about 1 or 2% sucrose solution. Coffee is typically sweetened to about the level of 5% sucrose. A level of 15% sucrose is very sweet and starts to feel syrupy. In between these concentrations, taste panellists usually are trained to quantify sweetness using 2–15% sucrose solutions as references. (13) The evaluation of sweeteners results in intensity factors relatively versus sucrose which is given the intensity factor 1 (Table 97.1) These factors can vary depending on the concentration of the sweetener and the system they are used in. The presence of other substances as salt, starch, and proteins will influence the intensity. Some of the sweeteners have an extremely bitter aftertaste which limits their use in food systems.

III. SUGAR ALCOHOLS: OVERVIEW

Two types of sweeteners, including sugar alcohols and no-calorie or non-nutritive sweeteners, are used to replace sugars in foods.

Sugar alcohols or polyols are bulk sweeteners, also called extensive sweeteners, which provide sweet taste without a bitter aftertaste. They introduce less calories in comparison with sucrose and may be useful to people with diabetes.

They are suitable for a variety of products reduced in calories, sugar, or fat and some of them have been used for almost half a century.

The energetic value of polyols is difficult to measure and varies from product to product. The European Union (EU) has provided a Nutritional Labeling Directive stating that all polyols have a caloric value of 2.4 kcal/g or 10 kJ/g, which is 50% compared to sucrose. The FDA accepts this value and so, as in the U.S. a product qualified as "reduced calorie" should have a reduction in calories of at least 25% and products referred to as "light" must have a one-third reduction, polyols can be used in formulation of "reduced calorie" products.

Another benefit of the use of polyols is that they are non-cariogenic because they are resistant to metabolism by oral bacteria which break down sugars to release organic acids that may lead to cavities or erode tooth enamel. (1)

When sugar alcohols are used, they are slowly absorbed so the increase in blood glucose level and insuline response associated with the ingestion of glucose is significantly reduced.

A. SORBITOL

Sorbitol or glucitol has first been discovered in 1872. It occurs naturally in a wide range of fruits and berries. Today it is commercially produced by the catalytic hydrogenation of glucose at high temperature and pressure, and is available in both liquid and crystalline form.

Sorbitol is used as a humectant in many products for protection against loss of moisture content. The moisture-stabilizing and textural properties of sorbitol are used in the production of confectionery, baked goods, and chocolate, where products tend to become dry or harden. Its moisture-stabilizing action protects these products from drying and maintains their initial freshness during storage.

Sorbitol is very stable and chemically unreactive. It can withstand high temperatures and does not participate in Maillard reactions, which are responsible for the browning of food products containing reducing sugars.

Sorbitol has been affirmed Generally Recognised As Safe (GRAS) by the FDA and is approved for use by the EU and numerous countries around the world, including Australia, Canada, Japan.

The Joint Expert Committee on Food Additives (JEFCA) has established an acceptable daily intake (ADI) for sorbitol of "not specified." In 1985 the Scientific Committee for Food (SCF) of the EU concluded that sorbitol is acceptable for use without limit. When one may expect that daily consumption of a food would result in the ingestion of 50 grams of sorbitol, the label should carry the warning: "*Excess consumption may have a laxative effect*."

B. XYLITOL

Xylitol is a polyol used in foods since the 1960s. It was discovered in 1891. It is a white crystalline powder with a

sweet taste, and is odourless. It occurs naturally in many fruits and vegetables and is even produced by the human body. It is commercially produced from plants such as birch and other hard wood trees and fibrous vegetation. Xylitol can be synthesized by hydrogenation of Xylose. Xylitol can also be produced by microbial transformation reactions, such as from D-glucose by yeast or from D-glucose by yeast and bacteria. For example Xylitol can be produced from D-xylose using the commercial immobilized D-xylose isomerase of *Bacillus coagulans* or immobilized cells of *Mycobacterium smegmatis*.

Xylitol has the same sweetness and bulk of sucrose with one-third fewer calories.

One of the most important advantages of xylitol is his effect in reducing tooth decay rates.

Xylitol is approved for use in foods (chewing gum, hard candy), pharmaceuticals (cough syrup, chewable multivitamins) and oral health products (toothpastes and mouthwashes) in more than 35 countries.

C. Lactitol

Lactitol, discovered in 1920, was first used in foods in the 1980s. Lactitol has a sweet taste that closely resembles the taste profile of sucrose. It has only 40% of sucrose's sweetening power. This mild sweetness makes it an ideal bulk sweetener to partner with low-calorie sweeteners such as acesulfame K, aspartame, and saccharin.

Lactitol is produced by reduction of the glucose part of the disaccharide lactose. Unlike the metabolism of lactose, lactitol is not hydrolysed by lactase. It is neither hydrolysed nor absorbed in the small intestine. Lactitol is metabolised by the bacteria in the large intestine, where it is converted into biomass, organic acids, carbon dioxide, and a small amount of hydrogen. The organic acids are further metabolised, resulting in an energy contribution of 2 kcal or approximately 8.4 kJ per gram.

Internationally it is approved as safe for use in many countries, including the EU, Canada, Japan, Israel, and Switzerland. Due to its GRAS status production, distribution of foods containing lactitol is allowed in the US.

Due to its stability at high temperatures, its solubility similar to glucose, and similar taste to sucrose, lactitol can be used in a variety of low-calorie, low-fat, and/or sugar-free foods such as ice cream, chocolate, candies, baked goods, and chewing gums.

D. Maltitol

Maltitol is made by the hydrogenation of maltose, which is obtained from starch. Like other polyols it does not brown or caramelise as sugars do. Maltitol has a sweet taste similar to sucrose. It is about 90% as sweet as sucrose, with the supplementary advantages of being non-cariogenic and reduced in calories.

Although maltitol is often used to replace sugars in the manufacture of sugar-free foods, it may also be used to replace fat, as it gives creamy texture to food.

IV. NON-NUTRITIVE SWEETENERS

A. Aspartame

Aspartame, currently market leader among artificial sweeteners, was discovered accidently in 1965 by James Schlatter.

Aspartame was approved for use as a tabletop sweetener and in powdered mixes in 1981 by the FDA. Since then, a number of people have questioned the studies that led to this decision. However, repeated studies done on aspartame show that it is harmless to people in the amount that it is normally ingested. In 1996, it was approved for use in all foods and beverages, including products such as syrups, salad dressings, and certain snack foods where prior approval had not yet been obtained. Products that may contain aspartame include low-calorie beverages, sugar-free gelatins, puddings, frozen desserts, and cereals as well as tabletop sweeteners (13).

1. Chemical and Physical Properties

Amino acids are relatively tasteless, nonetheless they contribute to the flavour of foods where they show flavour enhancing properties.

Glycine, for example, has a refreshing sweetish flavour when used as an additive in vinegar, pickles, and mayonnaise. Beyond its attenuation of the sweet taste, it lends some sweetness to the aroma. For same reason, D- and L-Alanine are used in the Far East. For the most part, dipeptides and oligopeptides have a bitter flavour. For this the sweet taste of aspartame was very remarkable (14).

Aspartame (E 951) is a methylester of two amino acids, aspartic acid and phenylalanine. N-L-alpha-Aspartyl-L-phenylalanine 1-methyl ester (Figure 97.1), or aspartame, is 150–200 times sweeter than sucrose. Its molecular weight is 294.31 g/mol and its melting point is 249°C. It is a white crystal solid which has a modest solubility in aqueous solution, but because of its high sweetening capability there is no need to dissolve large amounts (14). It mixes well with other solids.

Aspartame has a sweet taste comparable to saccharose. As aspartame is a dipeptide ester, it can be metabolised and has a nutritive factor of 17 kJ/g. Because of his high sweetening intensity it is only used in small amounts in comparison to bulk sweeteners, so calorie intake is reduced.

It is heat unstable so it can not be added previous to cooking or baking process.

Encapsulation of aspartame is used in chewing gum. Through encapsulation the product can be protected from

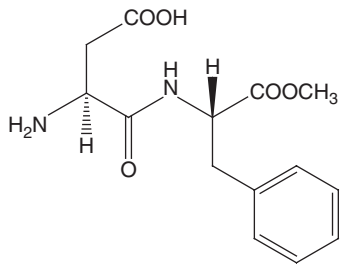

FIGURE 97.1 Aspartame.

moisture, heat, or other extreme conditions, thus enhancing stability and maintaining viability. It also can induce the slow release of flavour (15).

The major degradation product at higher temperatures is dioxopiperazine. The dioxopiperazine is also formed during storage. These two compounds have been satisfactorily assessed in a large number of studies in humans, and an ADI has been established for each of them: 40 mg/kg body weight for aspartame and 7.5 mg/kg body weight for dioxopiperazine.

2. Metabolism

The metabolites of aspartame in humans are compounds generally occurring in food. They are also produced by the endogenous cellular metabolism.

Aspartame is a minor source of phenylalanine, aspartic acid, and methanol compared with the standard dietary intake of these substances. Therefore, one can suppose they cannot be the source of the toxic neurological effects attributed to aspartame. The only problem with aspartame is in the case of phenylketonuric patients, due to inability to metabolise phenylalanine.

3. Synthesis

The synthesis of aspartame can be carried out via enzymatic or chemical methods.

Phenylalanine and aspartic acid are both chiral, which means that they have two isomers. If the incorrect enantiomers are used, the aspartame molecule will not have sweetening capacity due to the fact that it will not fit the binding site of the "sweetness" receptors on the tongue.

In the synthesis of aspartame, the starting materials are a racemic mixture of phenylalanine and aspartic acid. Only the L enantiomer of phenylalanine is used, which is separated from the racemate by reacting it with acetic anhydride and sodium hydroxide. Treatment of L-phenylalanine with methanol and hydrochloric acid esterifies the $-CO_2H$ group, and this ester is then reacted with aspartic acid to give the final product (16).

The chemical synthesis of aspartame requires protection of both the β-carboxyl and the α-amino groups of the L-aspartic acid. Even then, it produces aspartame in low yield and at high cost. If the β-carboxyl group is not protected, a cost saving is achieved but about 30% of the β-isomer is formed and has, subsequently, to be removed. When thermolysin is used to catalyse aspartame production the regio-specificity of the enzyme eliminates the need to protect this β-carboxyl group but the α-amino group must still be protected to prevent the synthesis of poly-L-aspartic acid. More economical racemic amino acids can also be used, as only the desired isomer of aspartame will be formed (17).

4. Applications

Aspartame's most important use is as tabletop sweetener, and it is commercialised under various brands (Canderel™, Equal™, NutraSweet™). Because of its numerous advantages such as low calorie, cost saving, no aftertaste, tooth-friendly, etc., aspartame is used in the manufacture of many sugar-free, energy-reduced, diet or light products, such as beverages (carbonated and still soft drinks, dilutables, fruit-juice drinks, and fruit syrups), dry mixes (hot and cold chocolate and beverage mixes and instant desserts), dairy products, confectionery, and pharmaceuticals (tablets, sugar-free syrups, etc.).

5. Safety and Legislation

Prior to marketing, the safety of the high-intensity sweetener aspartame for its intended uses as a sweetener and flavor enhancer was demonstrated by the results of over 100 scientific studies in animals and humans. In the postmarketing period, the safety of aspartame was further evaluated. The results of the extensive intake evaluation in the United States, which was done over an 8-year period, and the results of studies done in other countries demonstrated intakes which were well below the acceptable daily intakes set by the FDA and regulatory bodies in other countries, as well as the Joint FAO/WHO Expert Committee on Food Additives. Scientific evidence confirms that, even in amounts many times what people typically consume, aspartame is safe for its intended uses as a sweetener and flavour enhancer (18).

B. Saccharin

The first artificial sweetener discovered was Saccharin. It was accidentally found in 1879 by Fahlberg. The name "saccharin" results from the Latin word *saccharum*, which means sugar. Saccharin was used as a sweetener only sporadically until World War I. During and after the war the consumption of saccharin increased, as did the number of products that used saccharin. World War II led to another significant increase in the saccharin use and a proliferation of products that used the sweetener.

When, in 1960, tests began to suggest that saccharin caused bladder cancer in lab rats, the FDA moved to limit

its use. Its use was banned in Canada in 1977. The FDA considered banning it in 1977 based on this animal research. Later, more research on saccharin's safety was carried out. The FDA withdrew the ban in 1991, safety studies carried on. While numerous studies since 1977 have clearly shown that saccharin does not cause cancer in humans in the doses that people take it in, in the U.S. labels on products with saccharin must still carry a statement that says saccharin has caused cancer in laboratory animals. Currently saccharin is used in more than 90 countries, including the EU.

Saccharin is about 300 times sweeter than table sugar. It is used in several brands of tabletop sweeteners and in canned foods and beverages and low-calories soft drinks.

1. Chemical and Physical Properties

Saccharin (E954), discovered in 1879, is the oldest non-nutritive sugar which has been used in foodstuffs since 1884. Saccharin has many desirable properties that make it a valuable food ingredient. It is extremely sweet and counts no calories.

The ionised form of saccharin is about 300–500 times as sweet as sucrose. Its sweetness depends upon concentration and the type of food it is used in.

Saccharin is the chemical compound 1,2-benzisothiazol-3-one-1,1-dioxide (Figure 97.2). Solid saccharin is a white crystalline, odourless powder. It has poor water solubility with a maximum of 40 g/L at 100°C. The most important form is the sodium salt, which has excellent solubility (19). It is heat stable but has an unpleasant metallic, bitter after-taste. Therefore it is combined with cyclamate.

2. Metabolism

Saccharin is not metabolised in the human body.

3. Synthesis

Saccharin is mostly produced by the Remsen-Fahlberg process, a reaction of toluene and chlorosulfonic acid to a mixture of toluene sulfochlorides. After reaction with ammonia, followed by oxidation, saccharin is formed (14).

4. Safety and Legislation

Because of some concerns raised in the late 1970s, saccharin was at the end of 1972 no longer regarded as GRAS. The use of saccharin was only permitted in certain "special dietary foods" as mentioned, soft drinks, dietary products, and candy. The declaration "this product contains … mg saccharin per ounce." Later, a cancer warning label was required in food stores on shelves containing these food products. In January 1997, on advice of the Calorie Control Council, the FDA agreed to disclaim a supplementary warning system for products containing saccharin (20). In 2000, after more than 20 years of scientific studies, saccharin was given green light for application without a warning label.

In June 1995 the SCF of the European Commission set the ADI for saccharin on 5 mg/kg body weight.

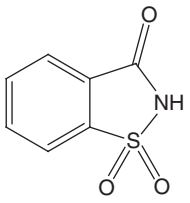

FIGURE 97.2 Saccharin.

5. Benefits and Application

Saccharin provides no calories, can be used in foods for diabetics and has low cost. It is highly stable and shows very good shelf life. Of all sweeteners saccharin has the widest range of applications. It shows some synergetic effect when combined with other sweeteners, resulting in a sweetness which exceeds the sum of both individual additives (21).

It is used in soft drinks, tabletop sweeteners, baked goods, jams, chewing gum, canned fruit, candy, etc. It is also used in cosmetic products, vitamin supplements, and pharmaceuticals.

C. Cyclamate

Cyclamate was developed in 1937 by Abbott Laboratories in the U.S. It has been in broad use in many countries since the 1950s.

Cyclamate is one of the most thoroughly tested food additives. In 1969 cyclamate was banned in the U.K. and U.S. for nearly 30 years. This ban was based on the results of a single study, showing that very high doses of cyclamate caused bladder tumors in rats. These results have not been reproduced in further studies and it was proven that bladder tumors in rats are not relevant for human health.

The FDA concluded in 1984, after reviewing all the scientific evidence available, that there is little data to implicate cyclamate as a carcinogen at any organ or tissue. This was also confirmed by the U.S. Academy of Sciences in 1985. Similar conclusions were reached by the JECFA, the SCF, and the U.K. committee on Toxicity of Chemicals in Foods. (22) As a result, cyclamate was included in the European Directive 94/35/EC (23) on low-calorie sweeteners for use in foodstuffs. In the U.K. it was not permitted until 1 January 1996. It is approved for use in over 55 countries worldwide and has undergone stringent testing 1995 with a review by the European SCF. In July 2002, the SCF reevaluated the sweetener and although the epidemiological data revealed no indications of harmful effects of cyclamates on humans, the committee decided to lower

the ADI for this substance from 11 to 7 mg/kg bodyweight. New scientific evidence has shown the conversion rate of cyclamate in the body to be higher than was previously thought. So the committee proposed reducing the use of cyclamate by banning its use in certain food categories like chewing gums and breath freshening microsweets, and by reducing it in soft drinks (from 400 mg/l to 350 mg/l) to assure that the intake of cyclamates stays below the revised ADI (23,24).

1. Chemical and Physical Properties

The most important cyclamate sweetener is sodium cyclamate. It has been in broad use in many countries since the 1950s.

Cyclamate (E 952) in fact is the name for cyclamic acid or cyclohexylsulphamic acid (Figure 97.3) and its sodium or calcium salts. Its sweetness is approximately 30–50 in comparison with sucrose. Due to its very low sweetening ability and because of the low allowable usage level, it is not possible to use cyclamate on its own to produce an acceptable sweet taste as wished, for example, in soft drinks. So, cyclamate is always used in association with another low calorie sweetener, usually saccharin in a 10:1 mix.

Cyclamate is a white crystalline powder with a melting point of 170°C. It is water soluble up to concentrations of about 150–200 g per litter. It is relatively heat-stable. Cyclamates can add heat stability to formulations with aspartame. It has good shelf life. In high concentrations cyclamate has an unpleasant aftertaste but at low concentrations it has some bitterness-masking ability.

When combined with acesulfame and aspartame it appears to have some synergistic qualities, resulting in a higher sweetness of the sweetener mix than the sum of the individual non-nutritive sweeteners. Cyclamate can serve as an excellent complement to other low-calorie sweeteners which are available and, particularly because of its unique synergistic sweetening properties, makes possible a wider variety of good-tasting low-calorie products.

2. Synthesis

The production of Sodium and Calcium Cyclamate includes the reaction of cyclohexylamine with sulfamic acid, salt determination, isolation, filtration, re-crystallization, drying and selection of particle size desired by the customer.

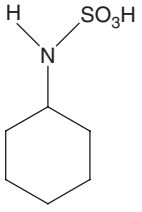

FIGURE 97.3 Cyclamate.

The reaction is carried out under high pressure and elevated temperature (25).

3. Metabolism

Cyclamate is not metabolized in the body, so it is considered as non-nutritive.

The metabolic conversion of cyclamate to cyclohexylamin (CHA) has been extensively studied in over 1000 human subjects. Research showed an extreme individual variability and urinary excretion of cyclohexylamin. The urinary excretion of cyclohexylamin was found to be extremely variable from individual to individual and fluctuated greatly from day to day. Approximately 30% of the test population convert cyclamate to cyclohexylamin, but the majority of these convert less than 0.1% to approximate 8% of the ingested cyclamate. A minor part converted up to 60% of the ingested cyclamate to CHA (26,27,28).

Neither cyclamic acid nor its salts are metabolised in the human body. In humans transcyclohexan-1,2-diol is found as a metabolite of cyclohexylamine which could probably be synthesized from cyclamate due to some *Enterococcus* species in the intestine.

4. Benefits and Applications

Calcium cyclamate is less sweet than sodium cyclamate. The primary use of cyclamate is as a non-caloric sweetener in combination with other sweeteners. Cyclamates can also be used as flavouring agents, such as to mask the taste of unpalatable drugs. Cyclamates have use in a variety of applications: as tabletop sweeteners, in tablet, powder, or liquid form, in beverages, fruit juice drinks, processed fruits, chewing gum and confectionery, salad dressings, gelatin desserts, jellies, jams, and toppings

Due to their good heat stability, cyclamates can be used in baked goods, jams, jellies and candies, and canned fruit. The main application of cyclamates is in blends with saccharin in a 10:1 ratio.

5. Safety and Legislation

Cyclamate is approved for use in over 55 countries worldwide and has undergone stringent testing as recently as 1995 with a review by the European SCF. In the opinion expressed in March 2000, the SCF established a new ADI of 0–7 mg/kg bodyweight for cyclamic acid and its sodium and calcium salts, expressed as cyclamic acid. More recent intake data submitted in Denmark suggest that the intake of cyclamates in children with a body weight of 15 kg (3 years old) could easily exceed the ADI. As a consequence the commission of the European Communities presented in July 2002 a proposal for a directive amending directive 94/35/EC on sweeteners for use in foodstuffs (16). In this proposal the maximum usable dose

of "400 mg/l" is replaced by "350 mg/l" for the category "water-based flavoured drinks, energy reduced or with no added sugar" furthermore, some food categories and maximum usable doses are deleted (confectionery, chewing gum, breath freshening micro-sweets, edible ices). Some commissions within the European Parliament suggest an even lower maximum dose of 250 mg/l and propose that the allowable dose for milk-based drinks should also be reduced.

D. Acesulfame-K

A third artificial sweetener, acesulfame potassium, was discovered in 1967 by Hoechst AG. It was approved for use in the U.S. by the FDA in 1992 for gums and dry foods, and it was finally approved for liquid use in 1998. As soon as it was approved, it was used in a new soft drink (Pepsi One).

1. Chemical and Physical Properties

Acesulfame-K is the potassium salt of 6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide (Figure 97.4). Its properties are very similar to those of saccharin but it has a better flavour with a much reduced bitter aftertaste. It is about 200 times sweeter than sugar and is often used in soft drinks combined with aspartame. It is soluble in water, resulting in a neutral solution. At 225°C it starts decomposing. In a pH range of 3–7 it is virtually stable. It has a very good stability to baking, UHT, and microwave heating.

Acesulfame-K is not metabolised in the body and is in this way excreted by the kidneys.

2. Benefits and Application

It has a nice sweet taste with only slight aftertaste at high concentrations above normal use levels. It has good shelf life and is very stable to processing. It shows some synergistic effects and has the capacity to enhance and intensify flavours.

Acesulfame-K can be used in all areas where sweeteners can be used to enhance taste or to reduce calories by replacing sugar: beverages, dairy products, jams, and baked goods.

3. Safety and Legislation

Like all other low-calorie sweeteners, acesulfame-K has been tested in numerous studies to determine its safety. It is not metabolised by the body but is excreted unchanged and does not affect the normal functions of the human body.

The World Health Organisation's Joint FAO/WHO Expert Committee on Food Additives have set an ADI for acesulfame-K of 15 mg per kg of body weight per day. In addition at European level, in March 2000, the European Commission's SCF confirmed its ADI of 9 mg/kg.

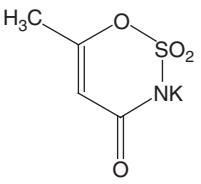

FIGURE 97.4 Acesulfame-K.

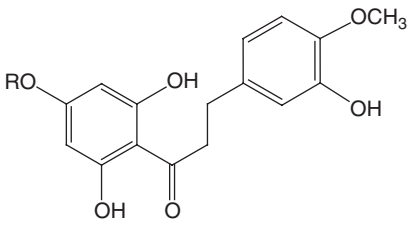

FIGURE 97.5 Neohespiridin DC.

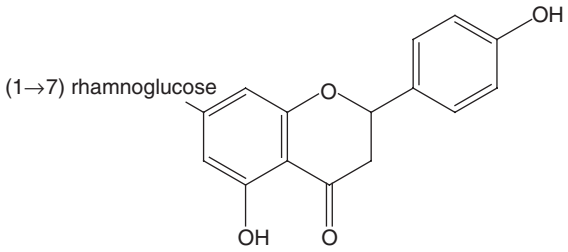

FIGURE 97.6 Hesperidin.

E. Neohesperidin DC

Neohesperidin dicalcone (Figure 97.5) has been known for some time. It is sweet with a specific aftertaste and is always used in combination with other (intensive) sweeteners. It is also used as flavour enhancer. Its produced by hydrogenation of hesperidin (Figure 97.6), a flavonoid occurring in bitter oranges. Neohesperidin is not absorbed by the human body, but it can be metabolised by the intestinal flora.

1. Chemical and Physical Properties

Neohesperidin DC is stable in aqueous solutions and shows a good shelf life at normal storage temperatures and pH 1–7. It is heat stable and can therefore be used in processed food.

Neohesperidin DC is an intensive sweetener and has the capability to intensify the sweetness of intensive and bulk sweeteners when used in combination (31).

Table 97.2 shows the experimental values for maximum synergy of mixtures with Neohesperidin for aqueous systems. This result implicates that the dose of any other sweetener used in combination with Neohesperidin can be lowered. Although Neohesperidin is more expensive, the

TABLE 97.2
Sweetening Synergy of Neohesperidin DC in Combination with Intense Sweeteners and Sugar Alcohols

Sweetener	Sweetening Synergy (%)
Aspartame	64
Acesulfame-K	83
Saccharin	81
Cyclamate	42
Lactitol	60
Xylitol	86
Isomalt	60

use of combinations with other sweeteners saves ingredient cost. As an example, for use in chewing gum a concentration of 3000 ppm of aspartame may be replaced by a mixture of 1500 ppm aspartame in combination with 190 ppm Neohesperidin, resulting in equal sweetness.

Not only is sweetness intensity changed, but there is also an influence on the quality of the taste, resulting, for example, in a less bitter aftertaste when combined with saccharin or acesulfame-K, even at low concentrations. Neohesperidin DC can still improve the overall flavour profile of foods (32).

2. Benefits and Applications

In fruit drinks, carbonated drinks, still drinks, powdered drink concentrates, syrups, sport drinks, dark sweet beer etc. Neohesperidin DC can extend shelf life by masking the off flavour resulting from the decomposition of unstable flavour components. It is used as sweetener or as flavour enhancer, the latter in a much lower concentration level (ex. 5 ppm versus 30 ppm in beverage). Neohesperidin DC enhances creaminess and fruit flavours in yoghurts and other dairy products. Furthermore, it can be used in chewing gum, confectionery, and pharmaceuticals. Neohesperidine DC improves the organoleptic properties and palatability of animal feed and pet foods.

F. SUCRALOSE

The discovery by Hough and Khan of the enhanced sweetness of the chlorodeoxy-derivatives of sucrose raised the problem of the explanation of the enhancement of sweetness after chlorinating (33). Wiet and Miller suggested that chlorine substitution is merely enhancing the intrinsic sensory qualities of sucrose. Other chemical modifications of sugars generally lead to bitter derivatives. Depending on the position of the chloro-substituent and the resulting hydrophilic/hydrophobic balance of the molecule, the derivatives of sucrose may be found to be intensely sweet, sweet, bitter-sweet, or even extremely, bitter (34).

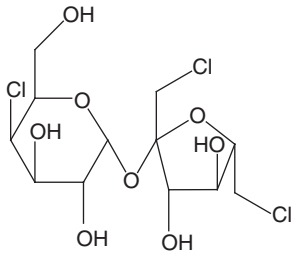

FIGURE 97.7 Sucralose.

1. Chemical and Physical Properties

Sucralose (trichlorogalactosucrose or 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-β-D-galactopyranoside) (Figure 97.7) is the only non-nutritive sweetener based on sucrose which was selectively chlorinated. It has been developed by Tate and Lyle and McNeil Speciality Products in the United States.

Its sweetness is 400–800 times the sweetness of sucrose, depending upon the concentration of sucrose which is being matched. For water the more the concentration of sucralose increases the lower the response.

Its flavour profile indicates that it is very similar to sugar, with less aftertaste often associated with intense sweeteners. This sweetener is relatively heat stable in cooking and baking. The instability of three intense sweeteners, aspartame, acesulfame-K, and sucralose, has been evaluated. They were added as a single product in lime-lemon flavoured carbonated beverage which was stored for 60 days at 4°, 27°, and 37°C. At the end of 60 days storage the loss of aspartame was maximum (29.5%), acesulfame-K decreased with 6.1%, while the loss of sucralose was minimum (1.9%). Thus sucralose was more stable than the other sweeteners (35).

At high temperature in aqueous solution it is hydrolysed to a limited extent producing 4-chloro-4-deoxy-galactose and 1,6-dichloro-1,6-dideoxy-fructose. It is stable during long term storage. It blends with sugar, which enables the production of products with reduced sugar level. It is very soluble in water.

2. Metabolism

The glycosidic link between the two rings is resistant to hydrolysis by acid or enzymes, so it is not metabolised. Sucralose provides no energy and there are no known derivates. It is not well absorbed and is excreted in the urine essentially.

3. Safety and Legislation

Sucralose was approved in the U.S. in April 1998 as a tabletop sweetener and for use in a number of desserts, confections, and non-alcoholic beverages. FDA concluded from a review of more than 110 studies in human beings and animals that this sweetener did not pose

carcinogenic, reproductive, or neurologic risk to human beings. Towards the end of 2000 Tate and Lyle applied for approval of sucralose on an EU-wide basis. The SCF has assessed the data on the safety of sucralose and issued its opinion in September 2000. This concluded that "sucralose is acceptable as a sweetener for general food use and a full Acceptable Daily Intake (ADI) of max 15 mg/kg body weight can be established" The SCF proposed an amendment to the EC Sweeteners Directive, including the addition of sucralose to the list of approved sweeteners. The ADI set for sucralose by the joint FAO/WHO Expert Committee on Food Additives made the same statement in 1990. In the U.K. the Committee on Toxicity in Food, Consumer Products, and the Environment (COT) which advises the U.K. government on food safety issues relevant to the use of additives has endorsed the SCF opinion on sucralose and concluded no further separate safety assessment was needed.

TABLE 97.3
Maximum Usable Dose Proposed for Sucralose in Some Foodstuff Categories

EC No	Name	Foodstuff	Maximum Usable Dose
955	Sucralose	Non-alcoholic drinks	300 mg/l
		Desserts and similar products	400 mg/l
		Confectionery with no added sugar	1000 mg/l
		Breath-freshening micro-sweets, energy reduced or with no added sugar	2400 mg/l
		Chewing gum with no added sugar	3000 mg/l
		Energy reduced jams, jellies, marmalades	400 mg/l
		Fine bakery products, energy reduced or with no added sugar	700 mg/l

In July 2002 the commission of the European Community proposed a Directive amending Directive 94/35/EC on sweeteners for use in foodstuffs with as major amendment the authorisation of two new sweeteners; sucralose and the salt of aspartame-acesulfame.

Table 97.3 gives some examples of foodstuffs with maximum usable dose as listed in the annex of the directive.

4. Benefits and Applications

Intense sweeteners have benefits for diabetics and for people who wish to reduce their calorie intake by reducing the sugar intake. If a new sweetener is introduced and asks for approval some additional benefits should be available. These benefits result from the taste profile and the stability characteristics of sucralose. Its heat stability will enable the food industry to produce a wider range of calorie reduced products and a commercialised tabletop sweetener can be used in home cooking and baking process.

G. Aspartame-Acesulfame Salt

The salt of aspartame and acesulfame (6-methyl-1,2,3-oxathiazine-4(3h)-one-2,2-dioxide salt of l-phenylalanyl-2-methyl-l-alpha-aspartic acid) is, as the name already indicates, a salt of two already authorized sweeteners (E 950 and E 951). It is manufactured from these two substances by replacing the potassium ion of acesulfame K by aspartame. For the purposes of ingredient listing, the product would be known as "aspartame – acesulfame salt." As the salt is not yet approved for use in the EU it does not have an "E" number. The SCF regarded, after assessment of the safety data, the use of the salt of aspartame and acesulfame acceptable as an additive considering that it is in fact an alternative for two already authorized additives, the potential exposure equals that of an equivalent blend of aspartame and acesulfame K, and no additional safety considerations were made (24). The

TABLE 97.4
Maximum Usable Dose for Aspartame-Acesulfame Salt and its Constituents Equivalent in Some Foodstuff Categories

			Maximum Usable Dose		
EC No	Name	Foodstuff	Salt	Aspartame Eq.	Acesulfame K Eq.
E 962	Aspartame-Acesulfame Salt	Non-alcoholic drinks	796 mg/kg	512	350
		Desserts and similar products	796 mg/kg	512	350
		Confectionery with no added sugar	1137 mg/kg	731	500
		Breath-freshening micro-sweets, energy reduced or with no added sugar	5683 mg/kg	3656	2500
		Chewing gum with no added sugar	4546 mg/kg	2925	2000
		Energy reduced jams, jellies, marmalades	1554 mg/kg	1000	350
		Fine bakery products, energy reduced or with no added sugar	2273 mg/kg	1462	1000

use of the salt is therefore proposed for food categories where both aspartame and acesulfame are used. Aspartame and acesulfame moieties are covered by the ADIs established previously for aspartame (0–40 mg/kg body weight) and acesulfame potassium (0–15 mg/kg body weight). The maximum usable doses for aspartame shall, however, not be exceeded by the use in combination with the salt.

1. Physical Properties

The benefits mentioned by the manufacturer and listed in annex of the Directive are the following.

The potassium in acesulfame K is eliminated when the salt is made, as is the moisture contained in aspartame. Therefore, the salt represents a more concentrated source of sweetness without the functionless presence of potassium and water.

It dissolves in water significantly more rapidly than an equivalent blend of aspartame and acesulfame-K. Instant powder products such as desserts, toppings, and powder beverage mixes perform better during reconstitution by the consumer. Similarly, tabletop sweeteners dissolve faster, especially in cold beverages.

It is more stable in certain applications than aspartame alone. Therefore, it can be used in applications where aspartame has either required encapsulation for protection or has been substituted by sweeteners of inferior taste such as saccharin. The consumer benefits from products of an improved taste and prolonged shelf life.

2. Benefits and Applications

It improves the sweetness of chewing gum and gives the gum a very long-lasting quality without recourse to encapsulation of the sweetener, for example with polymer coatings. The consumer experiences an improved product quality at potentially lower costs.

It disperses easily in previously difficult applications, such as sugar-free hard candy and sugar-free chocolate, where a blend of sweeteners is difficult to employ.

In instant powder products, the salt provides a guaranteed balanced sweetness throughout the dissolution process where a blend of the two individual sweeteners does not, since acesulfame K dissolves faster than aspartame.

It is not hygroscopic and thus much easier to store and requires less stringent packaging. This benefits products that are stored in containers that are repeatedly opened and closed allowing contact with atmospheric moisture.

It has superior powder-flow properties and thus avoids inconsistencies in powdered products in which the consumer may previously have experienced variations in taste quality and/or sweetness level.

H. Neotame

Neotame is a derivative of the dipeptide composed of the amino acids aspartic acid and phenylalanine. It is 7,000–13,000 times as sweet as sucrose and 30–60 times as sweet as aspartame. It produces no aftertaste. It can used alone or in blends with other high intensity sweeteners or bulk sweeteners (36).

The stability of Neotame is dependent on pH, moisture and temperature (37). It is stable under dry conditions and has comparable stability to aspartame in aqueous food systems and is more stable in neutral pH conditions. When stored under conditions relevant to commercial use (25°C and 60% RH) no loss of Neotame occurred.

The major metabolic pathway is hydrolysis of the methyl ester resulting in de-esterified neotame and methanol. The peptide is not further metabolised because of blockage of the peptidase responsible for the break down of the dipeptide. It is fully eliminated by the body through urine and feces and does not accumulate.

Because it is not metabolised, no phenylalanine is formed and no special labelling for individuals with phenylketonuria (see aspartame) is required.

Extended research has been conducted on Neotame to establish its safety as a general sweetener. Neotame has been approved for general use in Australia and New

TABLE 97.5
Maximum Application Dose of Intense Sweetener in Foodstuffs (mg/kg)

Product Category	Acesulfame	Aspartame	Cyclamate	Saccharin	Thaumatin	NHDC
Soft drinks	350	600	350	80–100		30–50
Desserts	350	1000	250	100		50
Candy	500	1000		500	50	100
Chewing gum	2000	5500		1200	50	400
Cider, beer	350	600		80		10–20
Ice cream	800	800		100	50	50
Jam	1000	1000	1000	200		50
Sauces, mustard	350	350		160–320		50
Food supplements	350–500	600–2000	400–500	80–500		50–100
Sweet and sours	200	000		160		30
Breakfast cereals	1200	1000		100		50

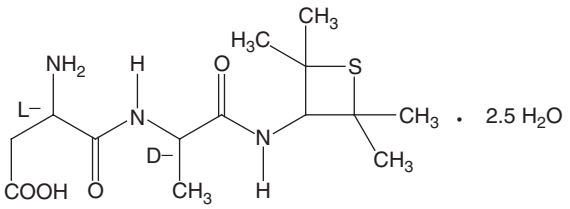

FIGURE 97.8 Alitame.

Zealand. In July 2002 the FDA approved the use of Neotame as a general purpose sweetener. In Europe it is used in Poland, Czech Republic and Roumania (www.neotame.com).

It can be used in foods and beverages and as a table-top sweetener. It can also be used in cooking and baking.

I. Alitame

Alitame (l-a-aspartyl-N-(2,2,4,4-tetramethyl-3-thi-etanyl)-d-alaninamide) is a dipeptide of the complex series l-a-aspartyl-d-alaninamide. It was synthesized in 1979 by Pfizer laboratory researchers and possesses an intense sweet taste, resembling sugar, due to the terminal amide (2,2,4,4-tetramethyl-thietanyl-amine or TTA) (38).

A request of authorization for use was presented to the FDA (U.S.) in 1986 and other countries. It is classified as a non-nutritive sweetener that is approximately 2000 times sweeter than sucrose (39).

The structure of the alitame molecule (Figure 97.8) resembles the well-known artificial sweetener aspartame (N-l-a-aspartyl-l-phenylalanine 1-methyl ester) in the amino acid moiety of aspartic acid. The l-phenylalanine was replaced by d-alanine and the methoxy group of the ester part was replaced by the TTA group in alitame. Alitame owes its increased stability to the terminal amide TTA, forming a second peptide link in the molecule, in comparison with aspartame's methyl ester group (39).

Alitame starts decomposing at 136–137°C. Its solubility is good. Its stability is superior to aspartame at all pH levels.

Alitame is not recognized in the U.S. or Europe. In Australia, New Zealand, Mexico, and China it is used in foods and beverages.

V. NATURAL SWEETENERS

A. Thaumatin

Thaumatin was originally isolated drom fruits of *Thaumatococcus danielli* (Benth), an African plant named in 1839 after Dr. W.F. Daniell. The Talin Food Group markets it under the trade name Talin. It has a sweetness of 1600–2700 times that of a 8–10% solution of surose. The discovery, chemistry, chemical modifications, safety, and practical applications of Thaumatins have been described in several articles (12,40,41,42). Research has been done to reproduce Thaumatin by DNA recombinant technology, but this resulted in a protein with difference in structure and no sweet taste (43).

1. Chemical and Physical Properties

Thaumatin is known as E 957, as a polypeptide with 207 amino acids and a molecular weight for Thaumatin I of 22209 and for Thaumatin II of 22293. The N content is 16%.

Its a creamy coloured powder without smell and with an intense sweet taste. It has a good solubility in aqueous solutions. Energetic value, as for any other protein, is 17 kJ/gram.

Either heat denaturation or cleavage of the disulfide bridges of Thaumatin result in loss of sweetness, implicating that the tertiary structure of the protein is important to sweetness reception.

2. Applications

Applications are few because of a low stability. In the European Community it is used in confectionery, chewing gum, and cacao-based confectionery, ice cream, and tooth paste with a maximum addition of 50 mg/kg. Another important application is the use in animal feed to reduce the bad taste of some raw material used in the formulation.

B. Stevioside and Rebaudioside

Stevia rebaudiana (Bertoni) is one of the 154 members of the genus Stevia. It is a member of the Compositae family with small, white flowers. It is a plant native to the region of Northeastern Paraguay. Stevioside, a diterpene glycoside isolated from the leaves of *Stevia rebaudiana B.*, was first described by Bridel and Laveille in 1993 (44).

The four major glycosides present in the plant tissue are (w/w): stevioside 5–10%; rebaudioside A 2–4% and rebaudioside C 1–2%; and dulcoside A 0.5–1%. During plant breeding studies Brandle noticed high concentrations of rebaudioside C (14.4%) in an *S. rebaudiana* line and a new sweet steviol glycoside F (2.7%) (45,46). They are synthesized in the plant using initially the same pathways as gibberellic acid, a plant hormone. The purpose of the sweet compounds in the plant is not yet determined. Hypothesis exists on their role are as repellent for insects or as a pool for the synthesis of gibberellic acid.

Stevioside is a glycoside with a glycosil and a sophorosyl residue attached to the aglycone steviol. It is a white, crystalline, odourless powder. It ranges in sweetness from 40 to 250 times sweeter than sucrose. Rebaudioside seems to have the best sensory properties.

Good stability has been reported for storage in solution with water at 30°C for 30 days and during long-term storage in carbonated phosphoric acidified beverages (48). Some

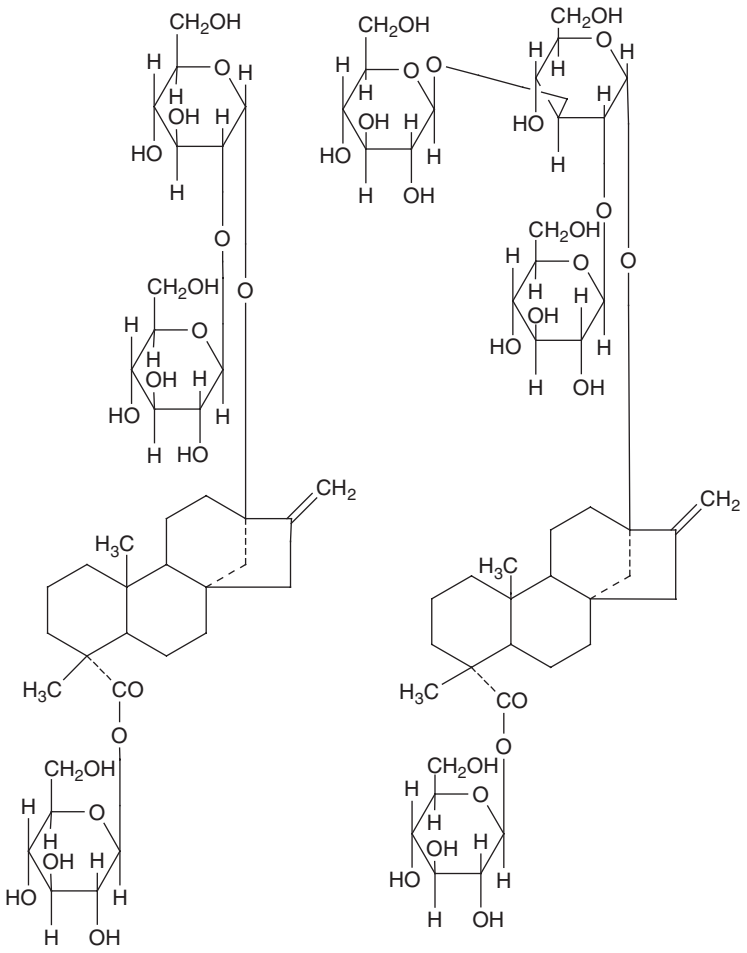

FIGURE 97.9 Stevioside and Rebaudioside.

loss was found for storage at low pH and after heating. Steviol glycosides are heat- and pH-stable, non-fermentable, and do not result in brown colouring effect during the baking process. Therefore they may have a wide range of applications in food products.

Studies carried out mainly in Asian countries have demonstrated no significant toxic activities of stevioside. Stevia sweeteners are in use in Japan, Brazil, and China. In some countries of the European continent (Czech Republic) it is now being used as a food additive. Stevioside is not approved for use in the EU.

VI. METHODS FOR ANALYSIS

The use of sweeteners is, in similarity with all other food additives, regulated and specified by law in most countries. Therefore analytical control of legal limits is essential.

For determination of food additives over the last decades, chromatographic techniques have been applied with large success. HPLC is well suited for the quantitative determination of a wide range of constituents and is well suited for the quantitative determination of complete groups of closely related compounds.

The noncaloric sweeteners do not form a uniform chemical group. Nevertheless simultaneous determination of some sweeteners is possible. In the case of soft drinks the determination is simplified due to the absence of an extraction procedure. For juices, desserts, and other applications an additional extraction step has to be performed. An overview on analysis of noncaloric sweeteners by HPLC is given by Flak and Pilsbacher (52).

Saccharin can be determined with reversed-phase HPLC method and absorbance detection, simultaneous with other sweeteners, namely acesulfame-K, aspartame, etc. (53).

Liquid chromatography with different detection techniques has been evaluated for determination of cyclamate, but the most common method is gas chromatography with flame ionisation detection of the pre-column derivatisation product Cyclohexylamine (18).

An HPLC isocratic method with pre-column derivatization, because of the absence of a chromophore for UV detection, and UV detection for the quantification of

cyclamate and cyclohexylamine in urine samples is described. The method requires very little sample preparation. Free cyclohexylamine is analysed in a first run and subsequently cyclamate is analysed as cyclohexylamine, after the simple process of oxidation of the sample by means of hydrogen peroxide. Cycloheptylamine is used as internal standard. Trinitrobenzenesulfonic acid (TNBS) appears to be a good reagent for the pre-column derivatisation (30)

Acesulfame-K determination with reversed-phase liquid chromatography is reported by Grosspietch and Hachenberg in 1980 and by Veerabhadrarao et al. in 1987 with UV absorption detection respectively at 237 nm and 254 nm (52). Also ion chromatographic methods for the simultaneous determination of saccharin, aspartame, acesulfame-K, and even cyclamate in combination with other food additives were reported (54,55). This method has been successfully applied to the analysis of soft drinks and various food products, and average recoveries varied from 85% to 104%. In 1999 Qu et al. used ion chromatography and amperometric detection to determine aspartame in a mixture of acesulfame and saccharin, without interference of these sweeteners (56).

Vanek et al. described the determination of stevioside in plant material based on water extraction and HPLC isocratic elution (57). Farhadi et al. developed a capillary column gas chromatographic method for the measurement of urinary sucralose used as a probe in an *in vivo* study of intestinal permeability. Concentration levels of 200 ppm were successfully reported (58).

VII. SUGAR REPLACEABILITY

The food industry uses sugar not only for its sweetening function. Sugar also serves some technological purposes and therefore in some way does not seem to be completely replaceable.

Sugar helps to preserve food by lowering the water activity, a parameter which is correlated with the microbiological stability of the product (50).

Sugar increases boiling point and reduces freezing point, acts as a bulking agent, and serves as a flavour-enhancer, important properties in food production and conservation.

Therefore sugar replacement is determined by the technical suitability of a sweetener.

One of the largest applications of intense sweetener addition is the production of soft drinks. In these products sugar can be replaced for the full 100% of its amount. Since sugar can represent up to half of the raw material costs of a regular soft drink and the volume loss is simply compensated by a larger amount of water, significant cost savings can be made. The replacement of sugar by intense sweeteners is estimated to result in a reduction of the input cost up to 50%.

Another advantage is the synergetic effect of a combination of sweeteners. This allows for lower dosage levels and a decrease in the cost of soft drink manufacturing. The first commercial sweetener blend was saccharin and cyclamate. Today blends of aspartame/saccharin, saccharin/sorbitol and others are frequently used in soft drinks, candies, and chewing gum.

The technical substitutability of sugar in food and drink products are estimated to be 100% for soft drinks, ice cream, yoghurt, frozen confectionery, canned fruit, and pickles; 10% for sugar confectionery, and 5% for biscuits, chocolate, and jams.

The sweetening power of each intense low-calorie sweetener varies depending upon the type of product for which it is to be used and the level of its usage.

Therefore each soft drink company decides which low-calorie sweetener to use only after it has considered the type of product and the availability and cost of ingredients in the marketplace at that time.

In addition, owing to technological developments, manufacturers have found that blending different low-calorie sweeteners, sometimes with sugar, can lead to a better product taste profile. For this reason, many producers now use a blend of low-calorie sweeteners rather than one single low-calorie sweetener in their drinks (51).

VIII. ECONOMICS

The European internal market is evolving into one single market without trade barriers. The Sweeteners Directive of the EU is in place and is implemented by the national laws of member states. Low-calorie sweeteners are being used in more products than ever before. The use of intense sweeteners is no longer restricted to the use as a sugar replacer in conventional food products, there are a lot of new products based on the use of sweeteners, such as extra strong breath fresheners. These are products for which there is no high-calorie "normal" version. Cosmetic chewing gum, and newer better tasting food products aimed at the fitness, wellness, and sports markets are showing strong growth. As the sales of diet and light products are growing more slowly than in the past, the use of low-calorie sweeteners in combinations with each other, with nutritive sweeteners, and even as flavour enhancers, is increasing.

The soft drinks sector is the largest consumer of artificial sweeteners and will maintain its position. Because of the cost benefits to food and drink manufacturers, the usage of high intensity sweeteners is continuing to rise (52).

A sugar replacement of about 25% of the total sugar consumption is technically feasible. The International Sugar Organization reported that, with an annual growth high intensity sweeteners could grab 10% of the global sweetener market by 2005 (53). Each year an average amount of 15,000 tonnes of intensive sweeteners are used

worldwide. They replace about 13 million tonnes of white sugar and have contributed to the buildup of annual stocks of around 60 million tonnes of refined sugar (Financial Post, 11/08/2000). In 2001 tabletop sweeteners grew by more than 10%, while diet soft drinks outpaced regular soft drinks with a growth of 0.7% in comparison with 0.5%. Most diet soft drinks are sweetened with aspartame, or a blend of aspartame and saccharin. The global market of artificial sweeteners was estimated in 2000 as €2.5 billion.

Aspartame has the largest share of the market, currently around 90%, and is likely to dominate the market during this decade, with its share expected to fall to only around 70% by 2009. Acesulfame-K and sucralose are expected to share a similar slice of most of the remaining 30% in the years to come.

IX. LEGISLATION REGULATION AND FOOD LABELLING

In the European community all sweeteners, natural or artificial, reduced or low-calorie, have to be approved for use. The European Directive on sweeteners in food 94/35/EC, as amended by directive 96/35/EC, regulates the use of acesulfame K, aspartame, cyclamate, neohesperidine, saccharin, and thaumatin. The amending directive was adopted in 1996 and implemented by 15 member states by June 1998.

In June 2002 an amendment proposes the use of sucralose and aspartame-acesulfame-K salt.

Denmark suggested that cyclamate intake for children can be higher than the ADI. As stated in the directive on food additives 89/107/EC, all additives should be continuously supervised to guarantee their safety and to amend the directive, if necessary, the Comission proposes to reduce the ADI for cyclamate, to reduce the maximum level of use in some food products, and to prohibit the use in some products as chewing gum, ice cream, etc.

The use of sweeteners is prohibited in any foods for infants and young children, and this prohibition now includes foods for infants not in good health. Foods for infants include foods specially prepared for infants (under the age of 12 months) generally known as "baby foods" and young children (under the age of 3 years), healthy or not.

On the label one should find correct information on additives used.

The Directive on Sweeteners defines:

- "*With no added sugar*": without any added mono- or disaccharides or any other foodstuff used for its sweetening properties
- "*Energy reduced*": with an energy value reduced by at least 30% compared with the original foodstuff or a similar hypothetical one with same characteristics but with use of nutritive sweeteners (glucose, fructose, sucrose)

Furthermore, Article 5 of the directive mentions the sales description of tabletop sweeteners must include the term "…-based tabletop sweetener" using the name of the sweetener(s) used in its composition.

When *polyols* or sugar alcohols are used the label should bear the warning "excessive consumption may induce laxative effects." For the purposes of these regulations, polyols are considered to be sorbitol syrup [E420 (i) and (ii)], mannitol (E421), isomalt (E953), maltitol and maltitol syrup [E956 (i) and (ii)], lactitol (E966) and xylitol (E967).

In the case of *aspartame*, the phrase "contains a source of phenylalanine" is to be used.

The directive includes a list with all sweeteners allowed in foodstuffs. Acceptable ADIs are also set for each low-calorie sweetener. These indicate levels for safe consumption of low-calorie sweeteners every day over a lifetime, and have a built-in safety factor of 100 times.

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Part I

Nutrition and Health

98 Chinese Edible Botanicals: Types, Efficacy and Safety

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CONTENTS

I. INTRODUCTION

China, the most populous country in the world, has a territorial area of 9,600,000 km^2. Situated in the Eastern Hemisphere and also including tropical, subtropical, temperate, and frigid zones, it has complex topography, landscape, and climates. As a result of these, China forms a great biodiversity. For higher seed plants, there are a total of 30,586 species recorded on Earth, which are divided into 343 families and 3155 genera. Of these, about

243 genera containing 527 species are found only in China (1).

Many higher plants in China have found various usages such as staple foods (e.g., rice, wheat, maize, etc.), vegetables (e.g., carrot, cucumber, cabbage, etc.), medicines (e.g., ginseng, liquorice, angelica, etc.), forage (e.g., clover, fescue, timothy, etc.), flavors (e.g., perilla, Japanese mint, basil, etc.), pigments (e.g., gardenia, fish storkbill, garden balsam, etc.) and sweeteners (e.g., sugar cane, sugar beet, etc.). These plants have been domesticated and cultivated by Chinese for thousands of years. In addition, there are many wild plants that have been utilized by Chinese as staple foods, food additives, vegetables, and medicines, which were the main source of foods of ancient people (2). In China, there are more than 1,800 edible seed plant species that are rich in proteins, vitamins, dietary fibre and minerals, and are also tasty (2). At the same time, these plants are more disease- and pest-resistant than cultivated crops. Therefore, wild edible plants are considered important gene pools for crop improvement.

In this chapter, some seed plants utilized by Chinese as staple foods, food additives, vegetables, and medicines are introduced. Edible wild plant species and their types, efficacy, and safety will also be discussed in detail. The map of China is shown in Figure 98.1.

II. TYPES OF EDIBLE PLANTS

According to plant parts (or organs) and components, edible plants can be divided into several groups such as edible flowers, edible fruits, edible young stems and leaves, edible roots and tubes, edible pigments, and edible flavors.

A. Edible Flowers

In Europe and North America, the main edible flower plants are *Brassica oleracea* var. *botrytis* and *Brassica oleracea*

FIGURE 98.1 Map of the People's Republic of China.

var. *italica*. In the Middle East, flowers of pumpkin and orange are used for making jams and syrups. In Asian countries, many plant flowers are eaten directly, because these countries have had a flower-eating culture for centuries. Flowers of plants such as *Dendranthema grandiflorum* in Japan, *Rhododendron* spp. and *Caragana arborescens* in Korea, *Musa errans* var. *botoan*, *Sesbania grandiflora*, *Moringa oleifera*, *Nymphaea pubescens*, *Bombax ceiba*, *Amomum maximum*, *Telosma cordata*, *Curcuma sessilis*, *Syzygium malaccense*, *Syzygium samarangense* and *Psophocarpus tetragonolobus* in Southeast Asia are widely consumed as foods by local people.

Eating flowers also has a long history in China (3, 4). In ancient books called "Shi-Jin," "Shan-Hai-Jin" and "Shen-Nong-Ben-Cao-Jin," the flowers of *Chrysanthemum morifolium*, *Hibiscus syriacus*, *Hemerocallis fulva*, *Lonicera japonica*, *Sophora japonica*, *Prunus mume*, *Carthamus tinctorius* were recorded to be edible (4). These flowers, however, are eaten mainly among national minorities of China. Some of the edible flowers in China are listed in Table 98.1 (2–5).

B. Edible Fruits

China possesses a rich resource of wild edible fruits that are the origins of many cultivated fruit varieties. There are more than 30 fruit species such as apple, pear and orange that are widely cultivated in China. The number of wild edible fruit species in China is more than 300 (4–8). More wild edible fruit species are distributed in Tianshan Mountains of Xinjiang (7) and Xishuangbannan of Yunnan (4) than those in any other regions of the country. There are 84 forma specialis of wild apple (*Malus sieversii*), 44 forma specialis of wild apricot (*Armeniaca valgaris*), 21 forma specialis of wild plum (*Prunus divaricata*), and 14 forma specialis of wild Persia walnut (*Juglans regia*) distributed in the region of Tianshan Mountains of Xinjiang at the northwestern part of China (7). These wild fruit species form natural forests in Xinjiang. They possess strong resistance to diseases and cold and salt stresses, and thus are considered the gene bank for breeding fruit resistance, and are also good stock materials in horticulture.

TABLE 98.1
Plants in China with Edible Flowers

Scientific Name	Family Name	Distribution Area	Collection Time
Alpinia kwangsiensis	Zingiberaceae	Southern China	Mar. to Apr.
Bauhinia variegata var. *candida*	Leguminosae	Yunan, Guangxi, Fujian, Taiwan	Feb. to Apr.
Bombax ceiba	Bombacaceae	Yunan, Guizhou, Guangxi	Jan. to Mar.
Cassia fistula	Leguminosae	Yunnan	Mar. to Jul.
Cassia siamea	Leguminosae	Guangdong, Yunnan, Hainan, Taiwan, Sichuan	Jul. to Sep.
Clerodendrum japonicum	Verbenaceae	Southern China	Apr. to Aug.
Curcuma longa	Zingiberaceae	Southern China	Jul. to Aug.
Dendranthema indicum	Compositae	Whole country	Autumn
Etingera elatior	Zingiberaceae	Tropical and subtropical area	Whole year
Globba racemosa	Zingiberaceae	Southern China	Jun. to Sep.
Hemerocallis nana	Liliaceae	Whole country	Spring, autumn
Hibiscus mutabilis	Malvaceae	Southern China	Aug. to Oct.
Hibiscus sabdariffa	Malvaceae	Fujian, Guangdong	May to Oct.
Hibiscus syriacus	Malvaceae	Yunnan, Guizhou, Guangxi	Jan. to May
Hemerocallis fulva	Liliaceae	Whole country	Aug. to Oct.
Jasminum sambac	Oleaceae	Whole country	Apr. to Dec.
Mayodendron igneum	Bignoniaceae	Yunnan	Feb. to Apr.
Musa acuminata	Musaceae	Southern China	Whole year
Musella lasiocarpa	Musaceae	Southwestern China	Whole year
Nymphaea lotus var. *prbescens*	Nymphaeaceae	Tropical and subtropical area	Whole year
Perotis indica	Gramineae	Whole country	May to Jun.
Plumeria rubra	Apocynaceae	Southeast Asia	May to Jun.
Pseuderanthemum polyanthum	Acanthaceae	Yunnan	Jan. to Apr.
Punica granatum	Punicaceae	Tropical and subtropical area	May to Aug.
Saraca indica	Leguminosae	Yunnan	Feb. to May
Sesbania grandiflora	Leguminosae	Southeast Asia	Whole year
Sophora japonica	Leguminosae	Whole country	Apr. to May
Thunbergia grandiflora	Acanthaceae	Guangdong, Yunnan, Guangxi	Dec. to May
Woodfordia fruticosa	Lythraceae	Yuannan, Guangxi	Jan. to Apr.

Source: Data from Ref. 2–5.

TABLE 98.2
Some Wild Edible Sarcocarp Fruit Species in China

Scientific Name	Family Name	Distribution Area	Products
Actinidia callosa var. *henryi*	Actinidiaceae	Whole country	Jam, syrup, canned food, eaten directly
Actinidia chinensis	Actinidiaceae	Whole country	Jam, syrup, canned food, edible directly
Actinidia kolomikta	Actinidiaceae	Whole country	Jam, syrup, canned food, eaten directly
Akebia quinata	Lardizabalaceae	Southern China	Ratafee
Akebia trifoliate	Lardizabalaceae	Southern China	Ratafee
Amalocalyx yunnanensis	Apocynaceae	Yunnan	Vegetable
Amomum maximum	Zingiberaceae	Guangdong, Guangxi, Yunnan, Tibet	Vegetable
Armeniaca valgaris	Rosaceae	Xinjiang	Syrup, canned food, jam
Broussonetia papyrifera	Moraceae	Southern China	Ratafee
Canarium album	Bursteraceae	Guangdong, Gangxi, Fujian, Taiwan, Hainan, Yunnan	Jam
Cajanus cajan	Leguminosae	Yunnan	Vegetable
Carica papaya	Caricaceae	Tropical area	Vegetable, preserved fruit
Celtis sinensis	Ulmaceae	Southern China	Jam
Choerospondias axillaris	Anacardiaceae	Southern China	Syrup, ratafee, jam
Crataegus cuneata	Rosaceae	Henan, Guangdong, Guangxi, Fujian	Canned food, jam, syrup, preserved fruit
Crataegus hupehensis	Rosaceae	Henan, Jiangxi, Jiangsu	Canned food, jam, syrup, preserved fruit
Cyphomandra betacea	Solanaceae	Tropical area	Vegetable
Dendrobenthamia angustata	Cornaceae	Southern China	Jam, ratafee
Dendrobenthamia capitata	Cornaceae	Southern China	Jam, ratafee
Dendrobenthamia japonica	Cornaceae	Southern China	Jam, ratafee
Diospyros kaki	Ebenaceae	Whole country	Eaten directly, preserved fruit
Diospyros lotus	Ebenaceae	Whole country	Eaten directly, preserved fruit
Diospyros rhombifolia	Ebenaceae	Fujian, Jiangsu, Hunan	Eaten directly, preserved fruit
Dolichandrone stipulata	Bignoniaceae	Southern China	Vegetable
Eriobotrya cavaleriei	Rosaceae	Southern China	Canned food, syrup
Eriobotya japonica	Rosaceae	Gansu, Shaanxi, Henan	Canned food, syrup
Euryale ferox	Nymphaeaceae	Whole country	Jul. to Oct.
Ficus carica	Moraceae	Whole country	Canned food, preserved fruit, drink
Ficus henryi	Moraceae	Southern China	Canned food, preserved fruit, drink
Ficus pumila	Moraceae	Southern China	Canned food, preserved fruit, drink
Ficus samentosa	Moraceae	Southern China	Canned food, preserved fruit, drink
Ficus tikoua	Moraceae	Southern China	Canned food, preserved fruit, drink
Fragaria gracilis	Rosaceae	Tibet, Shaanxi, Gansu	Eaten directly, jam, canned food, syrup, ratafee
Fragaria orientalis	Rosaceae	Northern China	Eaten directly, jam, canned food, syrup, ratafee
Hippophae rhamnoides	Elaeagnaceae	Western China	Syrup, jam
Holboellia coriacea	Lardizabalaceae	Western China	Ratafee
Holboellia grandiflora	Lardizabalaceae	Southern China	Ratafee
Hovenia acerba	Rhamnaceae	Southern China	Ratafee, sugar
Hovenia dulcis	Rhamnaceae	Southern China	Ratafee, sugar
Hovenia trichocarpa	Rhamnaceae	Southern China	Ratafee, sugar
Kadsura heteroclita	Schisandraceae	Southern China	Preserved fruit, jam, canned food, syrup
Kadsura longipedunculata	Schisandraceae	Southern China	Preserved fruit, jam, canned food, syrup
Livistona saribus	Palmae	Guangdong, Hainan, Yunnan	Vegetable
Lycium chinense	Solanaceae	Whole country	Jam, syrup
Maclura cochinchinensis	Moraceae	Southern China	Ratafee
Malus asiatica	Rosaceae	Whole country	Jam, canned food, syrup, preserved fruit
Malus hupehensis	Rosaceae	Southern China	Ratafee
Malus sieversii	Rosaceae	Xinjiang	Ratafee, jam, canned food
Morus alba	Moraceae	Whole country	Jam, syrup, ratafee
Morus australis	Moraceae	Whole country	Jam, syrup, ratafee
Myrica rubra	Myricaceae	Southern China	Syrup, canned food, ratafee
Oroxylum indicum	Bignoniaceae	Southwestern China	Vegetable
Physalis alkekengi var. *franchetii*	Solanaceae	Southwestern China	Jam, syrup
Podophyllum emodi var. *chinense*	Berberidaceae	Tibet, Yunan, Sichuan	Jam, syrup

(*Continued*)

TABLE 98.2
(*Continued*)

Scientific Name	Family Name	Distribution Area	Products
Prunus davidiana	Rosaceae	Western China	Canned food, jam, syrup, ratafee, preserved fruit
Prunus divaricata	Rosaceae	Xinjiang	Jam, syrup, ratafee
Prunus domestica	Rosaceae	Xinjiang	Jam, syrup, ratafee
Prunus gandulosa	Rosaceae	Southern China	Canned food, preserved fruit
Prunus japonica	Rosaceae	Whole country	Canned food, preserved fruit
Prunus tomentosa	Rosaceae	Whole country	Syrup, preserved fruit
Pyracantha angustifolia	Rosaceae	Tibet, Yunnan, Sichuan	Jam, ratafee
Pyracantha atalantioides	Rosaceae	Southern China	Jam, ratafee
Pyrus betulaefolia	Rosaceae	Whole China	Canned food, ratafee, syrup
Pyrus calleryana	Rosaceae	Whole China	Canned food, ratafee, syrup
Pyrus pyrifolia	Rosaceae	Southern China	Canned food, ratafee, syrup
Pyrus serrulata	Rosaceae	Southern China	Canned food, ratafee, syrup
Ribes alpestre	Saxifragaceae	Western China	Jam, syrup
Ribes meyeri	Saxifragaceae	Xinjiang	Jam, syrup
Ribes nigrum	Saxifragaceae	Xinjiang	Jam, syrup
Rosa cymosa	Rosaceae	Southern China	Ratafee, syrup
Rosa omeiensis	Rosaceae	Southern China	Ratafee, syrup
Rosa laevigata	Rosaceae	Southern China	Drink, ratafee
Rosa roxburghii	Rosaceae	Yunnan, Guizhou, Sichuan, Guangzhou	Ratafee, confiture, syrup, canned food
Rubus amabilia	Rosaceae	Shaanxi, Gansu, Sichuan, Jiangxi	Canned food, Jam, ratafee
Rubus amphidasya	Rosaceae	Anhui, Zhejiang, Sichuan, Hunan, Fujian	Canned food, Jam, ratafee
Rubus biflorus	Rosaceae	Shaaxin, Gansu, Sichuan, Yunnan, Tibet, Hubei	Canned food, Jam, Ratafee
Schisandra glaucescens	Schisandraceae	Sichuan, Hunan	Preserved fruit, canned food, syrup, jam
Schisandra incarnata	Schisandraceae	Hubei	Preserved fruit, canned food, syrup, jam
Schisandra propinqua	Schisandraceae	Southern China	Preserved fruit, jam, canned food, syrup
Sinofranchetia chinensis	Lardizabalaceae	Shaaxi, Guansu, Southern China	Syrup, ratafee
Solanum coagulans	Solanaceae	Tropical and subtropical area	Vegetable
Solanam indicum	Solanaceae	Sichuan, Guizhou, Yunnan, Guangdong	Vegetable
Solanum nigrum	Solanaceae	Whole country	Jam
Solanum torvum	Solanaceae	Yunnan, Guangdong, Guangxi, Taiwan	Vegetable
Sorbus alnifolia	Rosaceae	Whole China	Ratafee
Sorbus caloneura	Rosaceae	Southern China	Ratafee
Sorbus keissleri	Rosaceae	Southern China	Ratafee
Spondias pinnata	Anacardiaceae	Guangdong, Yunnan	Jam
Stauntonia duclouxii	Lardizabalaceae	Southern China	Jam, ratafee
Trichosanthes villosa	Cucurbitaceae	Southeast Asia	Vegetable
Vaccinium bracteratum	Ericaceae	Southern China	Jam, ratafee
Vaccinium iteophyllum	Ericaceae	Southern China	Ratafee
Vitis adstricta	Vitaceae	Southern China	Wine, currant, syrup
Vitis davidii	Vitaceae	Southern China	Wine, currant, syrup
Vitis ficifolia	Vitaceae	Southern China	Wine, currant, syrup
Vitis flexuosa	Vitaceae	Southern China	Wine, currant, syrup
Ziziphus jujuba var. *spinosa*	Rhamnaceae	Northern China	Syrup, ratafee, jam

Source: Data from Ref. 4–9.

Apart from their edibility, these wild fruits can be processed into all kinds of food products such as jam, syrup, and ratafee. These wild fruits can be divided into sarcocarp fruits and dry fruits, which are tabulated in Tables 98.2 and 98.3 (4–9), respectively.

C. Edible Young Stems and Leaves

The number of plants with edible young stems and leaves may account for up to 60–70% of the total edible plants in China (4, 5, 9). They are usually collected in their growth stages especially in springtime. These edible young stems and leaves can be divided into two groups, herbaceous and woody, which are shown respectively in Tables 98.4 and 98.5 (4, 5, 9).

D. Edible Roots and Tubers

Many plant roots and tubers can be eaten because they contain mainly starches together with other nutrient

TABLE 98.3
Some Wild Edible Dry Fruit Species in China

Scientific Name	Family Name	Distribution Area	Products
Carya cathayensis	Juglandaceae	Southern China	Canned food, drink
Castanea henryi	Fagaceae	Southern China	Starch, canned food, jam
Castanea seguinii	Fagaceae	Shanxi, Shaanxi, Southern China	Starch, canned food, jam
Castanopsis eyrei	Fagaceae	Southern China	Starch, canned food, jam
Castanopsis fargesii	Fagaceae	Southern China	Starch, canned food, jam
Castanopsis jucunda	Fagaceae	Southern China	Starch, canned food, jam
Castanopsis sclerphylla	Fagaceae	Southern China	Starch, canned food, jam
Castanopsis tibetana	Fagaceae	Southern China	Starch, canned food, jam
Coix lacryma	Gramineae	Whole country	Drink
Corylus chinensis	Corylaceae	Yunnan, Sichuan	Starch
Corylus fargesii	Corylaceae	Guizhou, Sichuan, Yunnan, Shaaxi	Starch
Cyclobalanopsis glauca	Fagaceae	Southern China	Starch, canned food, jam
Euryale ferox	Nymphaeaceae	Whole country	Canned food
Fagus longipetiolata	Fagaceae	Southern China	Starch, canned food, jam
Ginkgo biloba	Ginkgoaceae	Southern China, Western China	Canned food, drink
Hodgsonia macrocarpa var. *capniocarpa*	Cucurbitaceae	Yunnan, Tibet, Guangxi	Edible seeds
Juglans cathayensis	Juglandaceae	Southern China	Canned food, drink
Juglans regia	Juglandaceae	Western China	Canned food, drink
Litocarpus cleistocarpus	Fagaceae	Southern China	Starch, canned food, jam
Litocarpus glaber	Fagaceae	Southern China	Starch, canned food, jam
Nelumbo nucifera	Nymphaeaceae	Whole country	Canned food
Pinus armandii	Pinaceae	Southern China	Drink, vegetable
Pinus bungeana	Pinaceae	Southern China	Drink, vegetable
Pinus dabeshanensis	Pinaceae	Southern China	Drink, vegetable
Quercus acutissima	Fagaceae	Liaoning, Hebei	Starch, canned food, jam
Quercus baronii	Fagaceae	Western China	Starch, canned food, jam
Quercus phillyraeoides	Fagaceae	Southern China	Starch, canned food, jam
Quercus spinosa	Fagaceae	Shaanxi, Gansu, Sichuan, Yunnan	Starch, canned food, jam
Trapa bispinosa	Trapaceae	Whole country	Canned food, jam, ratafee
Trapa incisa	Trapaceae	Whole country	Canned food, jam, ratafee
Trapa litwinowii	Trapaceae	Southern China	Canned food, jam, ratafee
Trapa maximowiczii	Trapaceae	Southern China	Canned food, jam, ratafee
Trapa potaninii	Trapaceae	Whole country	Canned food, jam, ratafee
Trapa pseudoincisa	Trapaceae	Southern China	Canned food, jam, ratafee
Zizania caduciflora	Gramineae	Whole country	Vegetable

Source: Data from Ref. 4–9.

components. The roots of sweet potato (*Ipomoea batatas*), wayaka yambean (*Pachyrhizus erosus*), yam (*Dioscorea* spp.), and kudzuvine (*Pueraria* spp.) are the most common edible plant roots. The tubers of potato (*Solanum tuberosum*) and giantarum (*Amorphophallus* spp.) are usually consumed as either staple food or vegetables. Some of these plants have been domesticated and cultivated, whereas many of the plants with edible roots and tubers are still grown in the wild (2). Some examples of the wild edible roots and tubers are shown in Table 98.6 (2, 4, 5, 9).

E. Edible Plant Pigments

There are two groups of edible pigments: chemically synthesized colorings and natural colorings derived from living organisms. Natural plant colorings are believed to be safer than the synthetic ones. Some synthetic pigments are suspected of causing hyperactivity and learning difficulties in children, and causing other diseases such as cancers. Pigments of plant origins are not only safer, but also more environmentally friendly. Hence, with increasing suspicion that certain synthetic food pigments may be harmful, natural food colorings are increasing in popularity (10).

The plant kingdom possesses an abundant supply of plant pigments of which four main groups are suitable for coloring foods: 1) anthocyanines are intensely colored, water-soluble, orange, red, violet, or blue flavonoid pigments commonly found in flowers, fruits, and vegetables. For stability, they are usually kept in a rather acid medium; 2) betalains are a small group of red or yellow pigments sensitive to pH, heat, and light; the most common one is betanin from *Beta vulgaris* (beetroot); 3) carotenoids are red, orange, or yellow in color, and are sensitive to oxidation, hence their usefulness in the food industry necessitates

TABLE 98.4
Wild Edible Herbaceous Plants in China with Young Stems and Leaves

Scientific Name	Family Name	Distribution Area	Collection Time
Achyranthes bidentata	Amaranthaceae	Northern China	Summer
Adenophora stricta	Campanulaceae	Whole country	Summer
Agastache rugosa	Labiatae	Whole country	Summer, autumn
Agrimonia pilosa	Rosaceae	Whole country	Spring
Allium hookeri	Liliaceae	Southern China	Spring, summer
Allium macrostemon	Liliaceae	Whole country	Spring, summer
Aloe vera var. *chinensis*	Liliaceae	Whole country	Whole year
Alpinia nigra	Zingiberaceae	Yunnan	Whole year
Alternanthera sessilis	Amaranthaceae	Southern China	Whole year
Amaranthus lividus	Amaranthaceae	Whole country	Summer, autumn
Amaranthus spinosus	Amaranthaceae	Southern China	Whole year
Amaranthus viridis	Amaranthaceae	Whole country	Whole year
Basella rubra	Basellaceae	Whole country	Spring, summer
Begonia crassirostris	Begoniaceae	Tropical and subtropical area	Whole year
Bidens pilosa	Compositae	Tropical and subtropical area	Whole year
Brasenia schreberi	Nymphaeaceae	Southern China	Spring, autumn
Calystegia hederacea	Convolvulaceae	Northern, western China	Summer
Capsella bursa-pastoris	Cruciferae	Whole country	May to Oct.
Carduus crispus	Compositae	Whole country	Summer, autumn
Celosia argentea	Amaranthaceae	Whole country	Whole year
Cephalonoplos segetum	Compositae	Whole country	Summer, autumn
Centella asiatica	Umbelliferae	Southern China	Whole year
Chenopodium album	Chenopodiaceae	Whole country	Whole year
Cissus luzoniensis	Vitaceae	Hainan, Yunnan	Whole year
Colocasia fallax	Araceae	Yunnan	Whole year
Coccinia grandis	Cucurbitaceae	Tropical and subtropical area	Whole year
Colocasia tonoimo	Araceae	Southern China	Summer, autumn
Commelina communis	Commelinaceae	Gansu, Yunnan	Whole year
Costus speciosus	Zingiberaceae	Southern China	Summer, autumn
Crassocephalum crepidioides	Compositae	Tropical and subtropical area	Whole year
Cryptotaenia japonica	Umbelliferae	Southern China	Spring
Cymbopogon citratus	Gramineae	Tropical area	Whole year
Dendranthema indicum	Compositae	Whole country	Summer, autumn
Dichondra repens	Convolvulaceae	Tropical and subtropical area	Whole year
Eichhornia crassipes	Pontederiaceae	Whole country	Summer, autumn
Eleusine indica	Gramineae	Temperate area	Summer, autumn
Elsholtzia kachinensis	Labiatae	Southern China	Whole year
Enydra fluctuans	Compositae	Southern China	Whole year
Eryngium foetidum	Umbelliferae	Southern China	Whole year
Fagopyrum dibotrys	Polygonaceae	Whole country	Whole year
Gnaphalium affine	Compositae	Whole country	Mar. to Aug.
Gymnopetalum chinense	Cucurbitaceae	Guangdong, Guangxi, Guizhou, Yunnan	Mar. to Oct.
Gymnostemma pentaphyllum	Cucurbitaceae	Shaanxi, Southern China	Whole year
Gynura crepidioides	Compositae	Southern China	Jan. to Sep.
Hemerocallis minor	Liliaceae	Northern China	Summer
Hemerocallis nana	Liliaceae	Whole country	Spring
Houttuynia cordata	Sauruaceae	Southern China	Whole year
Hydrocotyle sibthorpioides	Umbelliferae	Southern China	Whole year
Impatiens balansae	Balsaminaceae	Yunnan	Whole year
Kalimeris indica	Compositae	Whole country	Mar. to Apr.
Kochia scoparia	Chenopodiaceae	Southern China	Summer
Kummerowia striata	Leguminosae	Southern China	May to Jun.
Lactuca indica	Compositae	Northwestern China, southern China	Summer, autumn
Lasia spinosa	Araceae	Tropical area	Whole year
Leonurus heterophyllus	Compositae	Whole country	Summer, autumn

(*Continued*)

TABLE 98.4
(*Continued*)

Scientific Name	Family Name	Distribution Area	Collection Time
Limnophila rugosa	Scrophulariaceae	Southern China	Mar. to Oct.
Lycium chinense	Solanaceae	Whole country	Mar. to Oct.
Lycopus lucidus	Labiatae	Whole country	Summer
Malva verticillata	Malvaceae	Whole country	Summer
Marsilea quadrifolia	Marsileaceae	Southern China	Whole year
Medicago hispida	Leguminosae	Southern China	Spring
Medicago sativa	Leguminosae	Northern China	Spring, autumn
Mentha haplocalyx	Labiatae	Whole country	Whole year
Momordica cochinchinensis	Cucurbitaceae	Southern China	Whole year
Monochoria hastata	Pontederiaceae	Southern China	Whole year
Monochoria vaginalis	Pontederiaceae	Whole country	Summer, autumn
Nasturtium officinale	Cruciferae	Southern China	Summer, autumn
Nymphoides peltatum	Menyanthaceae	Northern China	Spring
Ocimum basilicum var. *pilosum*	Labiatae	Southern China	Summer, autumn
Oenanthe javanica	Umbelliferae	Whole China	Whole year
Opuntia ficus-indica	Cactaceae	Southern China	Whole year
Oxalis corniculata	Oxalidaceae	Southern China	Whole year
Passiflora edulis	Passifloraceae	Tropical and subtropical area	Whole year
Parabaena sagittata	Menispermaceae	Southwestern China	Feb. to Nov.
Patrinia scabiosaefolia	Valerianaceae	Whole country	Summer, autumn
Patrinia villosa	Valerianaceae	Northern China	Summer, autumn
Perilla frutescens	Labiatae	Whole country	Apr. to Oct.
Phyllanthus urinaria	Euphorbiaceae	Southern China	Summer
Piper flaviflorum	Piperaceae	Yunnan	Whole year
Piper sarmentosum	Piperaceae	Southern China	Whole year
Plantago asiatica	Plantaginaceae	Whole country	Summer, autumn
Polygonatum odoratum	Liliaceae	Northern China	Mar. to May
Polygonum aviculare	Polygonaceae	Whole country	Summer
Polygonum hydropiper	Polygonaceae	Whole country	Summer
Polygonum lapathifolium	Polygonaceae	Whole country	Summer, autumn
Polygonum multiflorum	Polygonaceae	Southern China	Summer
Portulaca oleracea	Portulacaceae	Whole country	Apr. to May
Potentilla chinensis	Rosaceae	Whole country	Summer
Pratia nummularia	Lobeliaceae	Tropical and subtropical area	Whole year
Prunella vulgaris	Labiatae	Whole China	Spring
Rehmannia glutinosa	Scrophulariaceae	Whole country	Spring, summer
Rorippa dubia	Cruciferae	Shaanxi, Gansu	Whole year
Rorippa indica	Cruciferae	Southern China	Summer, autumn
Rumex trisetifer	Polygonaceae	Southern China	Feb. to May
Salsola collina	Chenopodiaceae	Whole country	Summer
Sanicula chinensis	Umberlliferae	Northern China	Summer
Scoparia dulcis	Scrophulariaceae	Southern China	Whole year
Smilax china	Smilacaceae	Whole China	Summer, autumn
Solanum nigrum var. *photeinocarpum*	Solanaceae	Southern China	Whole year
Solanum spirale	Solanaceae	Southern China	Whole year
Sonchus oleraceus	Compositae	Whole country	Whole year
Sphenoclea zeylanica	Sphenocleaceae	Southern China	May to Aug.
Tacca chantrieri	Taccaceae	Southern China	Whole year
Thladiantha calcarata	Cucurbitaceae	Southern China	May to Sep.
Tussilago farfara	Compositae	Whole country	Summer
Vicia pseudo-orobus	Leguminosae	Northern China	Summer
Vicia sativa	Leguminosae	Yunnan	Spring
Vicia unijuga	Leguminosae	Whole country	Summer
Zingiber fragile	Zingiberaceae	Yunnan	Apr. to May
Zingiber orbiculatum	Zingiberaceae	Yunnan	Mar. to Jun.
Zingiber zerumbet	Zingiberaceae	Southern China	Apr. to Jun.

Source: Data from Ref. 4, 5, and 9.

TABLE 98.5
Wild Edible Woody Plants in China with Young Stems and Leaves

Scientific Name	Family Name	Distribution Area	Collection Time
Acacia concinna	Leguminosae	Tropical and subtropical areas	Whole year
Acacia pennata	Leguminosae	Southern China	Mar. to Oct.
Acanthopanax trifoliatus	Araliaceae	Southern China	Whole year
Aralia armata	Araliaceae	Southern China	Whole year
Ardisia solanacea	Myrsinaceae	Yunnan, Guangxi	Mar. to May
Arenga pinnata	Palmae	Yunnan, Guangxi	Whole year
Arytera litoralis	Sapindaceae	Guangdong, Guangxi	Mar. to May
Bischofolia javanica	Euphorbiaceae	Shaanxi, Southern China	Summer, autumn
Camellia sinensis var. *assamica*	Theaceae	Southern China	Summer
Caryota ochlandra	Palmae	Tropical and subtropical areas	Whole year
Caryota urens	Palmae	Yunnan	Whole year
Celastrus paniculatus	Celastraceae	Southern China	Mar. to Jun.
Cinnamomum tenuipilum	Lauraceae	Yunnan	Whole year
Citrus maxima	Rutaceae	Southern China	Whole year
Clerodendrum colebrookianum	Verbenaceae	Southern China	Whole year
Crateva unilocularis	Capparidaceae	Guangdong, Guangxi	Apr. to Nov.
Cycas pectinata	Cycadaceae	Yunnan	Summer
Dendrocalamus giganteus	Gramineae	Yunnan	Jul. to Sep.
Dendrocalamus hamiltonii	Gramineae	Yunnan	Jul. to Aug.
Dendrocalamus latiflorus	Gramineae	Southern China	Summer
Doellingeria scaber	Compositae	Northern China	Summer
Dregea volubilis	Asclepiadaceae	Southern China	Mar. to Jun.
Ecdysanthera rosea	Apocynaceae	Southern China	Whole year
Elaeis guineensis	Palmae	Yunnan, Guangdong	Whole year
Embelia ribes	Myrsinaceae	Southern China	Whole year
Erythropalum scandens	Erythropalaceae	Southern China	Mar. to Oct.
Euphorbia antiquorum	Euphorbiaceae	Southern China	Whole year
Ficus auriculata	Moraceae	Southern China	Whole year
Ficus callosa	Moraceae	Southern China	Mar. to May
Ficus oligodon	Moraceae	Southern China	Whole year
Ficus racemosa	Moraceae	Yunnan, Guizhou, Guangxi	Mar. to May
Ficus vasculosa	Moraceae	Southern China	Mar. to May
Hyptianthera bracteata	Rubiaceae	Yunnan	Summer, autumn
Lysimachia clethroides	Primulaceae	Northern China	Summer, autumn
Picrasma chinensis	Simaroubaceae	Guangxi, Yunnan, Tibet	Jan. to Feb.
Pleioblastus amarus	Gramineae	Yunnan	Feb. to Apr.
Rhus chinensis	Anacardiaceae	Whole country	Sep. to Nov.
Rosa multiflora	Rosaceae	Southern China	Summer
Sauropus androgynus	Euphorbiaceae	Tropical and subtropical areas	Whole year
Scorzonera albicaulis	Compositae	Northern Chian	Summer, autumn
Stellaria saxatilis	Caryophyllaceae	Hennan, Hubei	Summer
Tamarindus indica	Caesalpiniaceae	Tropical and subtropical areas	Mar. to May
Toona sinensis	Meliaceae	Whole country	Summer, autumn
Trevesia palmata	Araliaceae	Guizhou, Guangxi, Yunnan	Whole year
Ulmus pumila	Ulmaceae	Northern China	Apr. to Jun.
Zanthoxylum planispinum	Rutaceae	Southern China	Whole year

Source: Data from Ref. 4, 5, and 9.

limiting their exposure to air; and 4) chlorophyll pigments are green, and are sensitive to acidity and light.

Some Chinese plants containing edible pigments are shown in Table 98.7 (4, 5, 10). Even for pigments with the same color, their chemical structures may be quite different. For example, most red pigments from plants belong to anthocyanin, but the red pigment shikonin from *Lithospermum erythrorhizone* is naphthoquinone. Up to now, the chemical compositions of most plant pigments are still not known.

F. Edible Plant Spices

Some Chinese botanicals have been used as spices. Plant spices are the main source of food additives (5). Some cultivated and wild edible spices-producing plants in

TABLE 98.6
Plants in China with Edible Roots and Tubers

Scientific Name	Family Name	Distribution Area	Roots or Tubers
Allium macrostemon	Liliaceae	Whole country	Bulbs (stem)
Amorphophallus yunnanensis	Araceae	Yunnan, Guizhou	Corm (stem)
Arctium lappa	Compositae	Whole country	Roots
Asparagus cochinchinensis	Liliaceae	Southern China	Roots
Daucus carota	Umbelliferae	Southern China	Roots
Dioscorea alata	Dioscoreaceae	Tropical area	Rhizomes (stem)
Lilium brownii	Liliaceae	Southern China	Bulbs (stem)
Lilium concolor	Liliaceae	Northern China	Bulbs (stem)
Lilium duchartrei	Liliaceae	Western China	Bulbs (stem)
Lilium fargesii	Liliaceae	Yunnan, Sichuan	Bulbs (stem)
Lilium henryi	Liliaceae	Guizhou, Hunan	Bulbs (stem)
Lycopus lucidus	Labiatae	Whole country	Rhizomes (stem)
Manihot esculenta	Euphorbiaceae	Tropical area	Roots
Nelumbo nucifera	Nymphaeaceae	Whole country	Rhizomes (stem)
Ophiopogon japonicus	Liliaceae	Southern China	Roots
Phragmites communis	Gramineae	Whole country	Rhizomes (stem)
Platycodon grandiflorus	Campanulaceae	Whole country	Roots
Polygonatum cirrhifolium	Liliaceae	Western China	Rhizomes (stem)
Polygonatum cyrtonema	Liliaceae	Southern China	Rhizomes (stem)
Polygonatum odoratum	Liliaceae	Whole Country	Rhizomes (stem)
Polygonatum sibiricum	Liliaceae	Whole country	Rhizomes (stem)
Potentilla anserina	Rosaceae	Northern China	Roots
Potentilla discolor	Rosaceae	Whole country	Roots
Pueraria lobata	Papilionaceae	Whole country	Roots
Sagittaria sagittifolia	Alismataceae	Whole country	Corm (stem)
Smilax glabra	Liliaceae	Whole country	Rhizomes (stem)
Stachys sieboldii	Labiatae	Southern China	Tubes (stem)
Stachys adulterine	Labiatae	Southern China	Tubes (stem)
Swertia pseudochinensis	Gentianaceae	Northern China	Roots

Source: Data from Ref. 2, 4, 5, and 9.

TABLE 98.7
Plants in China with Edible Pigments

Scientific Name	Family Name	Distribution Area	Pigment Color
Acanthopanax senticosus	Araliaceae	Northern China	Red
Beta valgaris var. *rubra*	Chenopodiaceae	Northern China	Red
Capsicum frutescens	Solanaceae	Whole country	Red
Carthamus tinctorius	Compositae	Whole country	Yellow, red
Caragana intermedia	Leguminosae	Northern China	Red
Celosia cristata	Amaranthaceae	Whole country	Red
Centaurea cyanus	Compositae	Whole country	Red
Cerasus humilis	Rosaceae	Northern China	Red
Citrullus lanatus	Cucurbitaceae	Whole country	Yellow
Coreopsis lanceolata	Compositae	Southern China	Yellow
Consolida ajacis	Ranunculaceae	Southern China	Red
Crataegus cuneata	Rosaceae	Northern China	Red
Cudrania tricuspidata	Moraceae	Whole country	Yellow
Curcuma longa	Zingiberaceae	Whole country	Yellow
Fragaria orientalis	Rosaceae	Northern China	Red
Gardenia jasminoides	Rubiaceae	Whole country	Yellow, blue
Glycine soja	Leguminosae	Northern China	Black, red
Lilium pumilum	Liliaceae	Northern China	Red
Lithospermum erythrorhizon	Boraginaceae	Northern China	Red
Lonicera caerulea var. *edulis*	Caprifoliaceae	Northern China	Red

(*Continued*)

TABLE 98.7
(*Continued*)

Scientific Name	Family Name	Distribution Area	Pigment Color
Lycopersicon esculentum	Solanaceae	Whole country	Red
Malus asiatica	Rosaceae	Northern China	Red
Malva sinensis	Malvaceae	Whole country	Red
Paeonia lactiflora	Ranunculaceae	Whole country	Red
Pelargonium hortorum	Geraniaceae	Whole country	Red
Rosa rugosa	Rosaceae	Whole country	Red, yellow
Rubus crataegifolius	Rosaceae	Northern China	Red
Rubus idaeus	Rosaceae	Northern China	Red
Salix subfragilis	Salicaceae	Northern China	Yellow
Solanum nigrum	Solanaceae	Whole country	Red
Spinacia oleracea	Chenopidiaceae	Whole country	Green
Vaccinium vitis-idaea	Ericaceae	Northern China	Red
Vitis amurensis	Vitaceae	Northern China	Red

Source: Data from 4, 5, and 10.

TABLE 98.8
Edible Spice-Producing Plants in China

Scientific Name	Family Name	Parts Used	Cultivation Area
Agastache rugosa	Labiatae	Leaves	Northern China
Allium fistulosum var. *viviparum*	Liliaceae	Leaves	Northern China
Allium porrum	Liliaceae	Leaves	Whole country
Allium schoenopasum	Liliaceae	Leaves	Southern China
Anethum graveolens	Umbelliferae	Aerial parts	Southern China
Apium graveolens var. *rapaceum*	Umbelliferae	Roots	Northern China
Aralia cordata	Araliaceae	Whole plant	Whole country
Armoracia rusticana	Cruciferae	Roots	Eastern China
Borago officinalis	Boraginaceae	Whole plant	Whole country
Brassica alba	Cruciferae	Seeds	Whole country
Caryopteris mongholica	Verbenaceae	Leaves, flowers	Northern China
Chamaemelum nobile	Compositae	Flowers	Southern China
Chrysanthemum morifolium	Compositae	Flowers, leaves	Whole country
Cnaphalium affine	Compositae	Whole plant	Whole country
Coriandrum sativum	Umbelliferae	Aerial parts	Whole country
Cuminum cyminum	Umbelliferae	Fruits	Xinjiang Province
Dasiphora fruticosa	Rosaceae	Flowers, leaves	Whole country
Daucus carota	Umbelliferae	Fruits	Whole country
Diospyros kaki	Ebenaceae	Leaves	Northern China
Elaeagnus angustifolia	Elaeagnaceae	Flowers	Northern China
Eulrema wasabi	Cruciferae	Rhizomes	Whole country
Foeniculum vulgare	Umbelliferae	Aerial parts	Whole country
Foeniculum vulgare var. *dulce*	Umbelliferae	Aerial parts	Whole country
Houttuynia cordata	Saururaceae	Whole plant	Southern China
Lepidium sativum	Cruciferae	Aerial parts	Imported from Iran
Lindera rubronervia	Lauraceae	Leaves, pericarp	Whole country
Lycopus lucidus	Labiatae	Roots	Western China
Melilotus officinalis	Leguminosae	Whole plant	Northern China
Melissa officinalis	Labiatae	Aerial parts	Whole country
Mentha haplocalyx	Labiatae	Leaves	Whole country
Mentha spicata	Labiatae	Leaves	Whole country
Michelia bodinieri	Magnoliaceae	Leaves, flowers	Northern China

(*Continued*)

TABLE 98.8
(*Continued*)

Scientific Name	Family Name	Parts Used	Cultivation Area
Nasturtium officinale	Cruciferae	Aerial parts	Whole country
Ocimum basilicum	Labiatae	Leaves and stems	Southern China
Oenanthe decumbens	Umbelliferae	Aerial parts	Northern China
Origanum marjorana	Labiatae	Aerial parts	Southern China
Paeonia suffruticosa	Ranunculaceae	Roots, flowers	Northern China
Perilla frutescens	Labiatae	Leaves	Whole country
Petasites japonica	Compositae	Aerial parts	Northern China
Petroselinum crispum	Umbelliferae	Whole plant	Southern China
Philadelphus pekinensis	Saxifragaceae	Flowers	Shanxi, Hebei, Liaonin
Portulaca oleracea	Portulacaceae	Whole plant	Northern China
Prunus padus	Rosaceae	Flowers	Northern China
Rosa banksiae	Rosaceae	Flowers	Northern China
Rosa chinensis	Rosaceae	Flowers, leaves	Whole country
Rosa omeiensis	Rosaceae	Flowers	Shaanxi, Gansu
Rosa rugosa	Rosaceae	Flowers	Whole country
Rosa sertata	Rosaceae	Flowers	Gansu, Shaanxi
Rosmarinus officinalis	Labiatae	Aerial parts	Whole country
Rumex acetosa	Polygonaceae	Whole plant	Northern China
Ruta graveolens	Rutaceae	Aerial parts	Southern China
Salvia officinalis	Labiatae	Aerial parts	Whole country
Syringa oblata	Oleaceae	Flowers	Northern China
Thymus citriodorus	Labiatae	Flowers	Northwestern China
Thymus vulgaris	Labiatae	Flowers	Northwestern China
Tropaeolum majus	Tropaeolaceae	Whole plant	Southern China
Vaccaria pyramidata	Caryophyllaceae	Seeds	Northern China
Zanthoxylum piasezkii	Rutaceae	Fruits	Northwestern China
Zanthoxylum schinifolium	Rutaceae	Fruits	Northern China
Zanthoxylun simulans	Rutaceae	Fruits, leaves	Hebei, Henan
Ziziphus jujuba var. *inermis*	Rhamnaceae	Fruits	Northern China

Source: Data from Ref. 4 and 5.

China are listed in Table 98.8 (4, 5). Among them, some originated from Europe, South America, or Southeast Asia. Spices are widely used in the food industry, e.g., meat products, biscuit, beverages, and salad dressing. Plant spices were the most important component in ancient culture communion between Eastern and Western countries. Some plant spices originated from China and other Asian countries, such as cinnamon (*Cinnamomum zeylanicum*), cassia (*Cassia cinnamon*), cardamon (*Elettaria cardamomum*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*), were exported and were widely used in European and Arabian countries centuries ago.

III. EFFICACY OF EDIBLE PLANTS

Edible plants with rich nutrient contents and medicinal efficacy have been recognized and utilized by the Chinese for thousands of years. These plants are considered either functional foods or medicines. In addition, some edible plants also have other functions such as serving as industrial materials and forages, and are being used for ecological protection. The nutritional and medicinal functions of some edible plants are described below.

A. Nutritional Functions

1. Definition of Nutrients

Nutrient components of a food may embrace protein, carbohydrate, fat, dietary fiber, cholesterol, carotene, retinal, thiamin, riboflavin, niacin, vitamin C, vitamin E, and a variety of mineral elements such as calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), and other trace elements which are essential to human and animals to grow and develop (11). For example, the element magnesium participates in energy transformation in human cells, promotes myocardium activity, promotes the dissolution of fiber protein, inhibits fibrin ferment formation, and prevents arteriosclerosis, high blood pressure, or heart diseases. Calcium is an important cation for maintaining blood moisture balance, acid-alkali balance, and osmotic pressure. Iron produces protoferriheme to

prevent iron-sufficient anemia. The relationship of zinc with impediment to taste sensation has also attracted great attention. In addition, selenium shows functions to retard oxidation and in the regulation of immunity. Selenium combines with other metals in the body and forms certain kinds of unsteady metal–selenium–protein complexes which are helpful in eliminating toxicants from the body.

2. Main Nutrients in Edible Wild Plants

Wild edible plants often have superior nutritional qualities, whether eaten raw or cooked. They contain rich dietary fiber, vitamins, minerals, and other nutritious components (11) which are beneficial to humans and animals. Many edible wild plants are considered functional foods. The contents of organic compounds in some wild edible plants are shown in Table 98.9 (11), while the contents of mineral components are shown in Table 98.10 (11).

B. Medicinal Functions

In China, many edible plants are also used as herbal medicines, which are a part of traditional Chinese medicines (TCM). Chinese medicines have a long history in Eastern cultures, especially in China, where foods and medicines are considered to be equally important in preventing and curing diseases.

Since foods and Chinese medicines are believed to share a common origin in the Chinese tradition, it is not easy to distinguish the two. Difficulty arises in classifying a product as a functional food or as a medicine. To solve this problem, based on the safety profile of Chinese medicines,

TABLE 98.9
Contents of Organic Compounds in Wild Edible Plants (Per 100 g Fresh Weight)

Species (Family)	Protein (g)	Fat (g)	CHO (g)	Dietary Fiber (g)	Carotene (μg)	Thiamin (μg)	Riboflavin (μg)
Allium macrostemon (Liliaceae)	1.0	0.4	7.7	2.2	680	0.03	0.12
Artemisia selengensis (Compositae)	3.7	0.7	9.0	0	0	ND	ND
Cephalanoplos segetum (Compositae)	4.5	0.4	5.9	1.8	5990	0.04	0.33
Corchorus capsularis (Tiliaceae)	4.7	0.3	5.8	1.2	ND	0.13	0.55
Ecballium elaterium (Cucurbitaceae)	ND	ND	0.3	0.3	180	ND	ND
Houttuynia cordata (Saururaceae)	ND	ND	0.3	0.3	3450	ND	ND
Kalimeris indica (Compositae)	2.4	0.4	4.6	1.6	2040	0.06	0.13
Kalopanax septemlobus (Araliaceae)	ND	ND	8.1	8.1	0	ND	ND
Kochia scoparia (Chenopodiaceae)	5.2	0.8	10.4	2.2	5720	0.15	0.31
Lepidium apetalum (Cruciferae)	ND	ND	1.6	1.6	3930	ND	ND
Lycopus lucidus (Labiatae)	4.3	0.7	13.7	4.7	6330	0.04	0.25
Medicago falcate (Leguminosae)	3.9	1.0	10.9	2.1	2640	0.10	0.73
Ocimum basilicum var. *pilosum* (Labiatae)	3.8	ND	4.6	3.9	2460	ND	ND
Origanum vulgare (Labiatae)	ND	ND	0.4	0.4	4110	ND	ND
Platycodon grandiflorus (Campanulaceae)	ND	ND	2.9	2.9	0	ND	ND
Premna microphylla (Verbenaceae)	ND	ND	7.8	7.8	0	ND	ND
Rabdosia forrestii (Labiatae)	ND	ND	6.9	6.9	4270	ND	ND
Rumex acetosa (Polygonaceae)	ND	ND	1.0	1.0	20	ND	ND
Taraxacum mongolicum (Compositae)	4.8	1.1	7.0	2.1	7350	0.03	0.39
Thymus mongolicus (Labiatae)	ND	ND	0.2	0.2	3510	ND	ND
Toona sinensis (Meliaceae)	1.7	0.4	10.9	1.8	700	0.07	0.12
Ulmus pumila (Ulmaceae)	4.8	0.4	7.6	4.3	730	0.04	0.12

Source: Adapted from Ref. 11.
ND: not determined; Tr: trace amount.

TABLE 98.10
Mineral Contents of Wild Edible Plants (mg Per 100 g Fresh Weight)

Species (Family)	Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn
Artemisia selengensis (Compositae)	17	8	40	1.0	2	0.5	0.20	0.05	0.02
Allium macrostemon (Liliaceae)	89	38	231	17.2	13	1.2	0.50	0.03	0.26
Cephalanoplos segetum (Compositae)	252	40	253	0.2	36	2.3	0.24	0.36	0.20
Corchorus capsularis (Tiliaceae)	208	83	559	8.0	64	4.8	ND	ND	ND
Ecballium elaterium (Cucurbitaceae)	74	35	203	2.9	22	2.3	0.28	0.33	0.12
Houttuynia cordata (Saururaceae)	123	38	718	2.6	71	9.8	0.99	0.55	1.71
Kalimeris indica (Compositae)	67	38	285	15.2	14	2.4	0.87	0.13	0.44
Kalopanax septemlobus (Araliaceae)	495	125	1641	0	109	6.5	1.36	0.65	6.13
Kochia scoparia (Chenopodiaceae)	281	66	702	62.4	118	6.5	0.52	0.25	0.42
Lepidium apetalum (Cruciferae)	188	124	335	120.8	42	4.7	0.90	0.06	0.36
Lycopus lucidus (Labiatae)	297	62	416	ND	25	4.4	0.93	0.43	0.26
Medicago falcate (Leguminosae)	713	78	497	5.8	61	9.7	2.01	ND	0.79
Ocimum basilicum var. *pilosum* (Labiatae)	285	65	576	5.7	106	4.4	0.52	0.91	0.68
Origanum vulgare (Labiatae)	218	51	442	16.2	66	10.7	0.89	0.90	0.60
Platycodon grandiflorus (Campanulaceae)	46	53	24	16.7	27	3.6	0.40	0.10	0.21
Premna microphylla (Verbenaceae)	126	97	444	0	38	6.2	1.36	0.22	12.68
Rabdosia forrestii (Labiatae)	356	106	670	8.4	229	10.9	1.27	1.39	0.85
Rumex acetosa (Polygonaceae)	28	21	228	0	26	0.6	0.15	0.04	0.12
Taraxacum mongolicum (Compositae)	216	93	327	76.0	54	4.0	0.35	0.44	0.58
Thymus mongolicus (Labiatae)	218	42	470	36.4	104	27.9	0.72	1.08	0.94
Toona sinensis (Meliaceae)	96	147	172	4.6	36	3.9	2.25	0.09	0.35
Ulmus pumila (Ulmaceae)	62	104	134	0.7	47	7.9	3.27	0.24	0.78

Source: Adapted from Ref. 11.
ND: not determined.

the Ministry of Public Health of the People's Republic of China has formally recognized 97 plant species as both functional foods and Chinese medicines which are shown in Table 98.11 (12–14). The plants listed in Table 98.11 can be consumed either as food or as medicine, and they are almost non-toxic. The plants shown in Table 98.12 (12–14) can be used as functional ingredients to be incorporated into health food. They are also considered safe, provided the amount consumed is not higher than the dosage set in Chinese pharmacopoeia (14).

In the past decades, the consumption of botanicals and nutritional supplements has increased dramatically. The most popular Chinese botanicals in western countries are astragalus (*Astragalus membranaceus*), angelica (*Angelica sinensis*), ginger (*Zingiber officinale*), foti (*Polygonum multiflorum*), jujube (*Ziziphus jujuba*), licorice (*Glycyrrhiza uralensis*), and Asian ginseng (*Panax ginseng*). In recent years, more and more Chinese medicinal herbs and edible plants have been exported to other countries, especially in Europe and North America. Many wild edible plants that have evident pharmacological activities according to modern pharmacology are summarized in Table 98.13 (15–21).

C. Some Medicinal Plants in China

1. Tartary Buckwheat

Buckwheat, which belongs to the family Polygonaceae, has two species, common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*F. tataricum*). Common buckwheat is widely cultivated in many countries over the world, while tartary buckwheat has only been cropped and used in the Loess Plateau of northern China and Yungui Plateau of southwestern China.

a. Nutrient contents

Comparison of nutrients between tartary buckwheat and other cereals is shown in Table 98.14 (22). It is clear that tartary buckwheat flour has higher protein and fat contents than those of wheat or rice. It should be noted that tartary buckwheat has rutin (vitamin P) which is absent in crops from the grass family. Besides, the levels of minerals in tartary buckwheat are higher than those in other crops. The contents of protein, fat, vitamin B2, and vitamin P in tartary buckwheat are higher than those in common buckwheat.

b. Medicinal functions

Tartary buckwheat has significant healing effects on hyperlipidemia, hypoglycemia and diabetes mellitus, as well as gastric disease. In China, tartary buckwheat has been developed into various health foods, drinks, and other products such as powder, health-care paste, custard, dried noodle, jelly, biscuit, cake, vinegar, wine, tea, and soft drinks.

2. Walnut

Although walnut (*Juglans* spp.) of the family Juglandaceae is distributed in many countries of the world, the major

TABLE 98.11
Plants as Both Functional Foods and Medicines Approved by the Ministry of Public Health of China in 2002

Scientific Name	Chinese Name (In Pin Yin)	Family Name	Parts Used
Agastache rugosa	Huoxiang	Labiatae	Dried herb
Allium chinensis	Xiebai	Liliaceae	Dried bulb
Allium macrostemon	Xiebai	Liliaceae	Dried bulb
Alpinia officinarum	Gaoliangjiang	Zingiberaceae	Dried rhizome
Amomum villosum	Sharen	Zingiberaceae	Dried ripe fruit
Amomum longiligulare	Sharen	Zingiberaceae	Dried ripe fruit
Angelica dahurica	Baizhi	Umbelliferae	Dried root
Brassica juncea	Jiezi	Cruciferae	Ripe seed
Canarium album	Qingguo	Bruseraceae	Dried ripe fruit
Canavalia gladiata	Daodou	Leguminosae	Dried ripe seed
Cannabis sativa	Huomaren	Moraceae	Dried ripe fruit
Cassia obtusifolia	Juemingzi	Leguminosae	Dried ripe seed
Cassia tora	Juemingzi	Leguminosae	Dried ripe seed
Chaenomeles speciosa	Mugua	Rosaceae	Dried nearly ripe fruit
Chrysanthemum morifolium	Juhua	Compositae	Dried capitulum
Cichorium glandulosum	Juju	Compositae	Aerial part
Cichorium intybus	Juju	Compositae	Aerial part
Cinnamomum cassia	Rougui	Lauraceae	Dried stem bark
Cirsium setosum	Jiercai	Compositae	Dried aerial part
Citrus aurantium	Suancheng	Rutaceae	Dried fruit
Citrus medica	Xiangyuan	Rutaceae	Dried ripe fruit
Citrus reticulata	Chenpi	Rutaceae	Dried pericarp of the ripe fruit
Citrus wilsonii	Xiangyuan	Rutaceae	Dried ripe fruit
Coix lacrymajobi var. *mayuen*	Yiyiren	Gramineae	Dried ripe kernel
Crataegus pinnatifida	Shanzha	Rosaceae	Dried ripe fruit
Dimocarpus longan	Longyan	Sapindaceae	Aril
Dioscorea opposita	Shanyao	Dioscoreaceae	Dried rhizome
Eugenia caryophyllata	Dingxiang	Myrtaceae	Dried flower bud
Euryale ferox	Qianshi	Nymphaeaceae	Dried kernel of ripe seed
Foeniculum vulgare	Xiaohuixiang	Umbelliferae	Dried ripe fruit
Fritillaria cirrhosa	Beimu	Liliaceae	Dried bulb
Fritillaria delavayi	Beimu	Liliaceae	Dried bulb
Fritillaria thunbergii	Beimu	Liliaceae	Dried bulb
Fritillaria unibracteata	Beimu	Liliaceae	Dried bulb
Fritillaria ussuriensis	Beimu	Liliaceae	Dried bulb
Gardenia jasminoides	Zhizi	Rubiaceae	Dried ripe fruit
Gastrodia elata	Tianma	Orchidaceae	Dried tuber
Ginkgo biloba	Yinxing	Ginkgoaceae	Dried ripe seed
Glycyrrhiza glabra	Gancao	Leguminosae	Dried root and rhizome
Glycyrrhiza inflata	Gancao	Leguminosae	Dried root and rhizome
Glycyrrhiza uralensis	Gancao	Leguminosae	Dried root and rhizome
Hippophae rhamnoides	Shaji	Elaeagnaceae	Dried ripe fruit
Houttuynia cordata	Yuxingcao	Saururaceae	Dried aerial part
Hovenia dulcis	Guaizao	Rhamnaceae	Dried fruit
Illcium verum	Bajiaohuixiang	Magnoliaceae	Dried ripe fruit
Imperata cylindrica var. *major*	Baimao	Gramineae	Fresh rhizome
Lilium brownii var. *viridulum*	Baihe	Liliaceae	Dried fleshy scale leaf
Lilium lancifolium	Baihe	Liliaceae	Dried fleshy scale leaf
Lilium pumilum	Baihe	Liliaceae	Dried fleshy scale leaf
Lonicera confusa	Jinyinhua	Caprifoliaceae	Dried flower bud or opening flower
Lonicera dasystyla	Jinyinhua	Caprifoliaceae	Dried flower bud or opening flower
Lonicera hypoglauca	Jinyinhua	Caprifoliaceae	Dried flower bud or opening flower
Lonicera japonica	Jinyinhua	Caprifoliaceae	Dried flower bud or opening flower
Lophatherum gracile	Danzhuye	Gramineae	Dried stem and leaf
Lycium barbarum	Gouqizi	Solanaceae	Dried ripe fruit

(*Continued*)

TABLE 98.11
(*Continued*)

Scientific Name	Chinese Name (In Pin Yin)	Family Name	Parts Used
Mentha haplocalyx	Bohe	Labiatae	Dried aerial part
Momordica grosvenori	Luohanguo	Cucurbitaceae	Dried fruit
Morus alba	Sang	Moraceae	Dried leaf and fruit-spike
Mosla chinensis	Xiangru	Labiatae	Dried aerial part
Myristica fragrans	Roudoukou	Myristicaceae	Dried kernel
Nelumbo nucifera	Lianzi	Nymphaeaceae	Dried leaf and ripe seed
Perilla frutescens	Zisu	Labiatae	Dried leaf and ripe fruit
Phaseolus angularis	Chixiaodou	Leguminosae	Dried ripe seed
Phaseolus calcaratus	Chixiaodou	Leguminosae	Dried ripe seed
Phragmites communis	Lugen	Gramineae	Fresh rhizome
Phyllanthus emblica	Yuganzi	Euphorbiaceae	Dried ripe fruit
Piper nigrum	Heihujiao	Piperaceae	Dried and nearly ripe or ripe fruit
Platycodon grandiflorum	Jiegeng	Campanulaceae	Dried root
Pogostemon cablin	Huoxiang	Labiatae	Dried herb
Polygonatum cyrtonema	Huangjing	Liliaceace	Dried rhizome
Polygonatum kingianum	Huangjing	Liliaceace	Dried rhizome
Polygonatum odoratum	Huangjing	Liliaceae	Dried rhizome
Polygonatum sibiricum	Huangjing	Liliaceace	Dried rhizome
Portulaca oleracea	Machixian	Portulacaceae	Dried aerial part
Prunus armeniaca var. *ansu*	Xingren	Rosaceae	Dried ripe seed
Prunus humilis	Yuliren	Rosaceae	Dried ripe seed
Prunus mandshurica	Xingren	Rosaceae	Dried ripe seed
Prunus pedunculata	Yuliren	Rosaceae	Dried ripe seed
Prunus sibirica	Xingren	Rosaceae	Dried ripe seed
Pueraria lobata	Gegen	Leguminosae	Dried root
Pueraria thomsonii	Gegen	Leguminosae	Dried root
Raphanus sativus	Laifuzi	Cruciferae	Dried ripe seed
Rubus chingii	Fupenzi	Rosaceae	Dried fruit
Sesamum indicum	Heizhima	Pedaliaceae	Dried ripe seed
Sinapis alba	Jiezi	Cruciferae	Ripe seed
Sophora japonica	Huai	Leguminosae	Dried flower bud and dry flower
Sterculia lychnophora	Pandahai	Sterculiacaeae	Dried ripe seed
Taraxacum mongolicum	Pugongying	Compositae	Dried herb
Taraxacum sinicum	Pugongying	Compositae	Dried herb
Ziziphus jujuba	Zao	Rhamnaceae	Dried ripe fruit and seed

Source: Data from Ref. 12–14.

production of walnut is in China. It is distributed all over China except the cold zone in northern China, and the middle and downstream areas of the Yangtze River. The kernel of walnut has higher nutritional and medicinal values. It has been served as food and raw materials in the food industry for a long time (23).

a. Nutrient contents

The protein content of walnut is about 15% of the whole kernel on a dry weight basis. Walnut contains more than 18 amino acids, with phenylalanine, valine, tryptophan, methionine, threonine, lysine, leucine, and isoleucine as the main ones. The fat from walnut kernel has four main types of fatty acids which form tripalmitin with sugar alcohol and glycerin. The walnut lipid is considered a nutrient product. Besides, there are some soluble compounds such as vitamin C, B1, B2, E, and P, and many different minerals including Ca, Mg, Fe, Mn, and Zn in the walnut kernel.

b. Medicinal functions

Walnut has various health functions for people of all ages and for special groups of people. It is believed that the fetus will develop well if pregnant women consume walnut often during their gestational periods. Children eating walnut often may help their growth and the development of intelligence. In addition, eating walnut may lead to longevity suggesting that walnut is also beneficial for the elderly. Walnut has been shown to possess beneficial effects in preventing and curing cardiovascular diseases. One of the reasons is that walnut contains a large quantity of linoleic acid in its kernel which may help soften blood vessels, prevent cholesterol synthesis, and excrete cholesterols out of the body.

TABLE 98.12
Plants as Ingredients of Functional Food Approved by the Ministry of Public Health of China in 2002

Scientific Name	Chinese Name (In Pin Yin)	Family Name	Parts Used
Acanthopanax gracilistylus	Wujiapi	Araliaceae	Dried root bark
Acanthopanax senticosus	Ciwujia	Araliaceae	Dried root and rhizome or stem
Achyrantes bidentata	Niuxi	Amaranthaceae	Dried root
Alisma orientalis	Zexie	Alismataceae	Dried tuber
Allium tuberosum	Jiucai	Liliaceae	Dried ripe seed
Aloe barbadensis	Luhui	Liliaceae	Dried leaf juice
Aloe ferox	Luhui	Liliaceae	Dried leaf juice
Amomun compactum	Baidoukou	Zingiberaceae	Dried fruit
Amomun kravanh	Baidoukou	Zingiberaceae	Dried fruit
Anemarrhena asphodeloides	Zhimu	Liliaceae	Dried rhizome
Angelica sinensis	Danggui	Umbelliferae	Dried root
Apocynum venetum	Luobuma	Apocynaceae	Dried leaf
Arctium lappa	Niubanggen	Compositae	Dried ripe fruit and root
Asparagus cochinchinensis	Tianmendong	Liliaceae	Dried root tuber
Astragalus complanatus	Shayuanzi	Leguminosae	Dried ripe seed
Astragalus membranaceus	Huangqi	Leguminosae	Dried root
Atractylodes macrocephala	Baishu	Compositae	Rhizome
Atractylodes chinensis	Cangshu	Compositae	Dried rhizome
Atractylodes lancea	Cangshu	Compositae	Dried rhizome
Aucklandia lappa	Muxiang	Compositae	Dried root
Bambusa tuldoides	Zhuru	Gramineae	Dried middle shavings of stem
Bletilla striata	Baiji	Orchidaceae	Dried tuber
Cassia acutifolia	Jueming	Leguminosae	Dried leaflet
Cassia angustifolia	Fanxie	Leguminosae	Dried leaflet
Carthamus tinctorius	Honghua	Compostiae	Dried flower
Centella asiatica	Jixuecao	Umbelliferae	Dried herb
Cimicifuga dahurica	Shengma	Ranunculaceae	Dried rhizome
Cimicifuga foetida	Shengma	Ranunculaceae	Dried rhizome
Cimicifuga heracleifolia	Shengma	Ranunculaceae	Dried rhizome
Cirsium japonicum	Daji	Compositae	Dried aerial part or root
Citrus aurantium	Zhi	Rutaceae	Dried fruit
Citrus reticulata	Qingpi	Rutaceae	Dried pericarp of the young or immature fruits
Citrus sinensis	Cheng	Rutaceae	Dried young fruit
Codonopsis pilosula	Dangshen	Campanulaceae	Dried root
Codonopsis tangshen	Dangshen	Campanulaceae	Dried root
Cornus officinalis	Shanzhuyu	Cornaceae	Dried ripe sarcocarp
Cuscuta chinensis	Tusizi	Convolvulaceae	Dried ripe seed
Cyathula officinalis	Chuanniuxi	Amaranthaceae	Dried root
Cyperus rotundus	Xiangfu	Cyperaceae	Dried rhizome
Dendrobium candidum	Shihu	Orchidaceae	Fresh or dried stem
Epimedium brevicornum	Yinyanghuo	Berberidaceae	Dried aerial part
Epimedium koreanum	Yinyanghuo	Berberidaceae	Dried aerial part
Epimedium pubescens	Yinyanghuo	Berberidaceae	Dried aerial part
Epimedium sagittatum	Yinyanghuo	Berberidaceae	Dried aerial part
Epimedium wushanense	Yinyanghuo	Berberidaceae	Dried aerial part
Equisetum hiemale	Muzei	Equisetaceae	Dried aerial part
Eucommia ulmoides	Duzhong	Eucommiaceae	Dried stem bark, leaf
Eupatorium fortunei	Peilan	Compositae	Dried aerial part
Evodia rutaecarpa	Wuzhuyu	Rutaceae	Dried and nearly ripe fruit
Fagopyrum dibotrys	Jinqiaomai	Polygonaceae	Dried rhizome
Fritillaria hupehensis	Hubeibeimu	Liliaceae	Dried bulb
Ginkgo biloba	Yinxing	Ginkgoaceae	Dried Leaf
Glehnia littoralis	Beishashen	Umbelliferae	Dried root
Gynostemma pentaphyllum	Jiaogulan	Cucurbitaceae	Dried herb

(*Continued*)

TABLE 98.12
(*Continued*)

Scientific Name	Chinese Name (In Pin Yin)	Family Name	Parts Used
Ilex cornuta	Kudingcha	Aquifoliaceae	Dried leaf
Ilex latifolia	Kudingcha	Aquifoliaceae	Dried leaf
Leonurus japonicus	Yimucao	Labiatae	Dried aerial part
Ligusticum chuanxiong	Chuanxiong	Umbelliferae	Dried rhizome
Lycium barbarum	Digupi	Solanaceae	Dried root bark
Lycium chinense	Digupi	Solanaceae	Dried root bark
Lycopus lucidus var. *hirtus*	Zelan	Labiatae	Dried aerial part
Magnolia officinalis	Houpo	Magnoliaceae	Dried stem bark, root bark, branch bark, dried flower bud
Morinda officinalis	Bajitian	Rubiaceae	Dried root
Morus alba	Sang	Moraceae	Dried root bark, young branch
Ophiopogon japonicus	Maimendong	Liliaceae	Dried root tuber
Paeonia lactiflora	Shaoyao	Ranunculaceae	Dried root
Paeonia suffruticosa	Shaoyao	Ranunculaceae	Dried root bark
Paeonia veitchii	Shaoyao	Ranunculaceae	Dried root
Panax ginseng	Renshen	Araliaceae	Dried leaf, root and fruit
Panax notoginseng	Sanqi	Araliaceae	Dried root
Panax quinquefolium	Xiyangshen	Araliaceae	Dried root
Piper longum	Bibo	Piperaceae	Dried and nearly ripe or ripe fruitNDspike
Plantago asiatica	Cheqian	Plantaginaceae	Dried herb, ripe seed
Plantago depressa	Cheqian	Plantaginaceae	Dried herb, ripe seed
Platycladus orientalis	Baiziren	Cupressaceae	Dried ripe kernel, twig and leaf
Polygala sibirica	Yuanzhi	Polygalaceae	Dried root
Polygala tenuifolia	Yuanzhi	Polygalaceae	Dried root
Polygonum multiflorum	Heshouwu	Polygonaceae	Dried root tuber, lianoid stem
Pseudostellaria heterophylla	Taizishen	Caryophyllaceae	Dried root tuber
Psoralea corylifolia	Buguzhi	Leguminosae	Dried ripe fruit
Rehmannia glutinosa	Dihuang	Scrophulariaceae	Fresh or dried root tuber
Rheum officinale	Dahuang	Polygonaceae	Prapared dried root and rhizome
Rheum palmatum	Dahuang	Polygonaceae	Prapared dried root and rhizome
Rheum tanguticum	Dahuang	Polygonaceae	Prapared dried root and rhizome
Rhodiola crenulata	Hongjingtian	Crassulaceae	Dried herb
Rhodiola fassigisa	Hongjingtian	Crassulaceae	Dried herb
Rhodiola sachalinensis	Hongjingtian	Crassulaceae	Dried herb
Rosa davurica	Cimeiguo	Rosaceae	Fruit
Rosa laevigata	Jinyingzi	Rosaceae	Dried ripe fruit
Rosa rugosa	Meigui	Rosaceae	Dried flower bud, and fruit
Rubia cordifolia	Qiancao	Rubiaceae	Dried root and rhizome
Salvia miltiorrhiza	Danshen	Labiatae	Dried root and rhizome
Schisandra chinensis	Wuweizi	Magnoliaceae	Dried ripe fruit
Scrophularia ningpoensis	Xuanshen	Scrophulariaceae	Dried root
Similax glabra	Tufuling	Liliaceae	Dried rhizome
Sinocalamus beecheyanus var. *pubescens*	Zhuru	Gramineae	Dried middle shavings of stem
Sophora japonica	Huaishi	Leguminosae	Dried fruit
Tamarindus indica	Suanjiao	Leguminosae	Dried fruit
Terminalia chebula	Hezi	Combretaceae	Dried ripe fruit
Trigonella foenumNDgraecum	Hulouba	Leguminosae	Seed
Trubulus terrestris	Jili	Zygophyllaceae	Dried ripe fruit
Typha angustifolia	Puhuang	Typhaceae	Dried pollen
Typha orientalis	Puhuang	Typhaceae	Dried pollen
Vaccinium vitisNDidaea	Yueju	Ericaceae	Dried fruit

Source: Data from Ref. 12–14.

TABLE 98.13
Pharmacological Activities of some Wild Plants in China

Pharmacological Activities	Plant Species	Family Name
Cardiovascular System		
1. Herbs with multiple actions	*Acanthopanax gracilistylus*	Araliaceae
	Acanthopanax senticosus	Araliaceae
	Codonopsis pilosula	Campanulaceae
	Gynostemma pentaphyllum	Cucurbitaceae
	Panax ginseng	Araliaceae
	Panax quinquefolium	Araliaceae
2. Cardiac herbs	*Alpinia oxyphylla*	Zingiberaceae
	Apocynum venetum	Apocynaceae
	Lycopus lucidus var. *hirtus*	Labiatae
	Polygonatum kingianum	Liliaceae
	Polygonatum odoratum	Liliaceae
3. Antihypertensive herbs	*Chrysanthemum indicum*	Compositae
	Equisetum hiemale	Equisetaceae
	Eucommia ulmoides	Eucommiaceae
	Morus alba	Moraceae
	Raphanus sativus	Cruciferae
	Trubulus terrestis	Zygophyllaceae
4. Antianginal herbs	*Cassia angustifolia*	Leguminosae
	Chrysanthemum morifolium	Compositae
	Ginkgo biloba	Ginkgoaceae
	Ophiopogon japonicus	Liliaceae
	Panax notoginseng	Araliaceae
	Pueraria lobata	Leguminosae
	Rehmannia glutinosa	Scrophulariaceae
	Salvia miltiorrhiza	Labiatae
	Ziziphus jujuba	Rhamnaceae
5. Antihypercholesterolemic herbs	*Alisma orientalis*	Alismataceae
	Allium chinensis	Liliaceae
	Allium macrostemon	Liliaceae
	Cassia obtusifolia	Leguminosae
	Crataegus pinnatifida	Rosaceae
	Curcuma longa	Zingiberaceae
	Hippophae rhamnoides	Elaeagnaceae
	Polygonum multiflorum	Polygonaceae
	Trigonella foenumNDgraecum	Leguminosae
6. Antishock herbs	*Citrus aurantium*	Rutaceae
	Citrus sinensis	Rutaceae
Nervous System		
1. Anesthetics and muscle-relaxing herbs	*Rosa davurica*	Rosaceae
	Rubia cordifolia	Rubiaceae
2. Sedatives and hypnotic herbs	*Astragalus complanatus*	Leguminosae
	Canavalia gladiata	Leguminosae
	Platycladus orientalis	Cupressaceae
	Ziziphus jujuba var. *spinosa*	Rhamnaceae
3. Anticonvulsive herbs	*Gastrodia elata*	Ochidaceae
	Piper nigrum	Piperaceae
4. Antipyretic herbs	*Angelica dahurica*	Umbelliferae
	Arctium lappa	Compositae
	Canarium album	Bruseraceae
	Centella asiatica	Umbelliferae
	Cimicifuga heracleifolia	Ranunculaceae
	Dendrobium chrysanthum	Ochidaceae
	Dendrobium loddigesii	Ochidaceae
	Lophatherum gracile	Gramineae

(*Continued*)

TABLE 98.13
(*Continued*)

Pharmacological Activities	Plant Species	Family Name
	Nelumbo nucifera	Nymphaeaceae
	Perilla frutescens	Labiatae
	Phragmites communis	Gramineae
	Phyllanthus emblica	Euphorbiaceae
	Sterculia lychnophora	Sterculiaceae
	Zingiber officinale	Zingiberaceae
5. Antirheumatic herbs	*Cichorium glandulosum*	Compositae
	Cichorium intybus	Compositae
	Eupatorium fortunei	Compositae
Alimentary System		
1. Stomachic and "wind"-dispelling herbs	*Alpinia officinarum*	Zingiberaceae
	Amomum vellosum	Zingiberaceae
	Aucklandia lappa	Compositae
	Citrus reticulata	Rutaceae
	Foeniculum vulgare	Umbelliferae
	Glycine max	Leguminosae
	Illcium verum	Illiciaceae
	Magnolia officinalis	Magnoliaceae
	Mentha haplocalyx	Labiatae
2. Herbs promoting digestion	*Amomun compactum*	Zingiberaceae
	Amomun kravanh	Zingiberaceae
	Citrus aurantium	Rutaceae
	Citrus reticulata	Rutaceae
	Eugenia caryophyllata	Myrtaceae
	Hordeum vulgare	Gramneae
	Piper longum	Piperaceae
	Pseudostellaria heterophylla	Caryophyllaceae
	Tamarindus indica	Caesalpiniaceae
3. Laxative herbs	*Cannabis sativa*	Cannabidaceae
	Cassia acutifolia	Leguminosae
	Cassia angustifolia	Leguminosae
	Cirsium japonicum	Compositae
	Prunus humilis	Rosaceae
	Prunus japonica	Rosaceae
	Prunus pedunculata	Rosaceae
	Prunus persica	Rosaceae
	Rheum palmatum	Polygonaceae
	Rheum tanguticum	Polygonaceae
4. Antidiarrheal herbs	*Agastache rugosa*	Labiatae
	Atractylodes macrocephala	Compositae
	Cinnamomum cassia	Lauraceae
	Euryale ferox	Nymphaeaceae
	Myristica fragrans	Myristicaceae
	Pogostemon cablin	Labiatae
	Terminalia chebula	Compositae
5. Emetic and antiemetic herbs	*Cirsium setosum*	Compositae
	Cyathula officinalis	Amaranthaceae
6. Choleretic and antihepatitis herbs	*Gardenia jasminoides*	Rubiaceae
	Schisandra chinensis	Schisandraceae
7. Tonics and supporting herbs	*Cuscuta chinensis*	Convolvulaceae
	Drynaria fortunei	Compositae
	Lilium lancifolium	Liliaceae
	Lycium barbarum	Solanaceae
	Morinda officinalis	Rubiaceae
	Paeonia lactiflora	Ranunculaceae

(*Continued*)

TABLE 98.13
(*Continued*)

Pharmacological Activities	Plant Species	Family Name
	Psoralea corylifolia	Leguminosae
	Rosa laevigata	Rosaceae
	Rubus chingii	Rosaceae
	Sesamum indicum	Pedaliaceae
Respiratory System		
1. Antitussives	*Allium tuberosum*	Liliaceae
	Fritillaria cirrhosa	Liliaceae
	Fritillaria hupehensis	Liliaceae
	Fritillaria thunbergii	Liliaceae
	Fritillaria ussuriensis	Liliaceae
	Fritillaria unibracteata	Liliaceae
	Laminaria japonica	Laminariaceae
	Phyllanths emblica	Euphorbiaceae
	Prunus armeniaca var. *ansu*	Rosaceae
	Prunus mandshurica	Rosaceae
	Prunus sibirica	Rosaceae
2. Expectorants	*Brassica juncea*	Cruciferae
	Citrus medica	Rutaceae
	Citrus wilsonii	Rutaceae
	Momordica grosvenori	Cucurbitaceae
	Platycodon grandiflorum	Campanulaceae
	Polygala sibirica	Polygalaceae
	Polygala tenuifolia	Polygalaceae
	Sinapis alba	Cruciferae
Genitourinary System		
1. Diuretic herbs	*Cornus officinalis*	Cornaceae
	Imperata cylindrica var. *major*	Gramineae
	Morus alba	Moraceae
	Plantago asiatica	Plantaginaceae
	Plantago depressa	Plantaginaceae
	Vaccinium vitisNDidaea	Vacciniaceae
2. Herbs affecting the uterus	*Achyrantes bidentata*	Amaranthaceae
	Angelica sinensis	Umbelliferae
	Cyathula officinalis	Amaranthaceae
	Cyperus rotundus	Cyperaceae
	Leonurus japonicus	Labiatae
	Rosa rugosa	Rosaceae
Hematopoietic System		
1. Herbs promoting blood formation	*Morus alba*	Moraceae
	Ligustrum lucidum	Oleaceae
2. Immunoenhancing and immunosuppressing herbs	*Dioscorea opposita*	Dioscoreaceae
	Dimocarpus longan	Sapindaceae
	Glehnia littoralis	Umbelliferae
3. Hemostasic and antistasic herbs	*Bletilla striata*	Crchidaceae
	Eclipata prostrata	Compositae
	Imperata cylindrica var. *major*	Gramineae
	Platycladus orientalis	Cupressaceae
	Rhodiola sachalinensis	Crassulaceae
	Sophora japonica	Leguminosae
Endocrine System		
1. Herbs affecting the thyroid gland	*Arctium lappa*	Compositae
2. Herbs affecting the adrenal cortex	*Glycyrrhiza uralensis*	Leguminosae
	Glycyrrhiaza inflata	Leguminosae
	Panax ginseng	Araliaceae

(*Continued*)

TABLE 98.13
(*Continued*)

Pharmacological Activities	Plant Species	Family Name
3. Antidiabetic herbs	*Alisma orientalis*	Alismataceae
	Anemarrhena asphodeloides	Liliaceae
	Atractylodes lancea	Compositae
	Lycium chinense	Solanaceae
	Morus alba	Moraceae
	Panax ginseng	Araliaceae
	Rehmannia glutinosa	Scrophulariaceae
	Scrophularia ningpoensis	Scrophulariaceae
Chemotherapy		
1. Antibacterial, antiviral, and antifungal herbs	*Aloe barbadensis*	Liliaceae
	Aloe ferox	Liliaceae
	Astragalus membranaceus	Leguminosae
	Bambusa tuldoides	Gramineae
	Chaenomeles speciosa	Rosaceae
	Dolichos lablab	Leguminosae
	Fagopyrum dibotrys	Polygonaceae
	Houttuynia cordata	Saururaceae
	Ilex latifolia	Aquifoliaceae
	Ligustrum lucidum	Oleaceae
	Lonicera japonica	Caprifoliaceae
	Morus alba	Moraceae
	Paeonia fuffruticosa	Ranunculaceae
	Perilla frutescens	Labiatae
	Phaseolus calcaratus	Leguminosae
	Phaseolus angularis	Leguminosae
	Portulaca oleracea	Portulacaceae
	Similax glabra	Liliaceae
	Taraxacum mongolicum	Compositae
2. Anthelmintics herbs	*Torreya grandis*	Taxaceae
	Zanthoxylum bungeanum	Rutaceae
	Zanthoxylum schinifolium	Rutaceae
3. Anticancer herbs	*Coix lacrymajobi* var. *maNDyuen*	Dioscoreaceae
	Asparagus cochinchinensis	Lilaceae

Source: Data from Ref. 15–21.

Walnut as a traditional medicine has been used for more than one thousand years in China. Its medicinal functions were reported in many medicinal literatures in ancient China, such as in the Tang Dynasty, Song Dynasty, and Ming Dynasty. The following are some folk remedies using walnut kernel: 1) 15 g of walnut kernels is chewed carefully and swallowed slowly, three times each day to cure hyperchlorhydria, gastritis, and duodenal ulcer; 2) 10–15 g of walnut kernels is roasted with proper brown sugar to be charcoal, decocted in water, and drunk to cure diarrhea; 3) walnuts are pounded with 100 g black sesame and 100 g mulberry leaves to make pills, 7.5 g per pill, two pills each day, one pill each time to cure vertigo, sleeplessness, and poor memory.

3. Yam

Yam (*Dioscorea* spp.) is one of the earliest sources of foods integrated into traditional folklore and ceremonial occasions. It has served as a main sustenance of people living in the tropical and subtropical countries (24). In China, eating the tubers and roots of yam has a long history (25).

a. Nutrient contents

The major components of yam tubers and roots are water and carbohydrates. Starch, the main carbohydrate, consists of amylose and amylopectin in the starch granules. Sugars, proteins, vitamins, minerals, and some polyphenolic compounds as well as certain steroids are also present in the tuber of yam. The major mineral elements in the tuber include calcium, iron, phosphorus, potassium, manganese, zinc, and magnesium (26).

b. Medicinal functions

Yam is a good source of calories for diabetic patients. The main bioactive compounds in the tubers and roots of some yam species such as *Dioscorea zingiberensis* are steroids. One of the bioactive compounds, diosgenin, is widely

TABLE 98.14
Nutritional Compositions of Tartary Buckwheat Flour and Other Cereal Flours

Nutrients	Tartary Buckwheat	Common Buckwheat	Wheat	Rice
Ash (%)	15	13	12	13
Crude protein (%)	10.5	6.5	9.9	7.8
Crude fat (%)	2.15	1.37	1.8	1.3
Starch (%)	73.11	76.59	71.6	76.6
Dietary fiber (%)	6.2	1.01	0.6	0.4
Vitamin B1 (mg/100 g)	0.18	0.08	0.46	0.11
Vitamin B2 (mg/100 g)	5.0	0.12	0.06	0.02
Vitamin PP (mg/100 g)	2.55	2.7	2.5	1.4
Vitamin P (mg/100 g)	3.05	0.21	0	0
K (mg/g)	4.2	2.9	1.95	1.72
Na (mg/g)	ND	ND	0.018	0.017
Ca (mg/g)	0.16	0.3	0.38	0.017
Mg (mg/g)	2.2	1.4	0.51	0.63
Fe (mg/g)	0.086	0.14	0.042	0.024
Cu (μg/g)	4.59	4.0	4.0	2.2
Mn (μg/g)	11.70	10.3	25.0	23.4
Zn (μg/g)	18.50	17.0	22.8	17.2
Se (μg/g)	0.43	ND	ND	ND

Source: Adapted from Ref. 22.

used to produce corticosteroids (e.g., cortisone), sex hormones, fertility control compounds, anabolic agents, and cardicatonic glycosides.

4. Angelica

Angelica (*Angelica sinensis*), called *danggui* in Chinese, belongs to the family Umbelliferae. It is one of the most popular traditional medicinal herbs in China. This herb is mainly distributed in Gansu and Shaanxi, the northwestern part of China. The roots are dried and sliced, and used unprepared or stir-baked with wine (27).

a. Nutrient contents

There are many kinds of nutrients in the roots of angelica. One gram of dry root contains approximately 4,025 μg potassium, 520 μg calcium, 96 μg sodium, 698 μg magnesium, 11 μg manganese, 35 μg copper, 68 μg zinc, 90 μg iron and 1 μg cadmium (28). Other nutrient components in angelica include polysaccharides, terpenoids, amino acids, vitamin B_{12} and vitamin A (29).

b. Medicinal functions

The main pharmacological functions of angelica are promotion of blood circulation, proliferation of the blood cells, relieving pain, and moistening the bowels (30, 31). Angelica has been used by patients with the following symptoms: 1) blood deficiency syndrome characterized by sallow complexion, pale lips and tongue, dizziness, palpitation, or pale nails; 2) irregular menstruation, amenorrhea, and menorrhalgia in case of blood deficiency; 3) various kinds of pains due to blood stasis and rheumatic arthralgia; 4) score, carbuncle, or pyogenic skin infections; and 5) constipation due to blood deficiency and dryness of the intestines.

Better pharmacological results may be achieved by the combination of angelica with other medicinal herbs such as *Astragalus* spp., *Rehmannia* spp., and peony (*Paeonia lactiflora*) (31).

5. Sanchi-Ginseng

Sanchi-ginseng (*Panax notoginseng*) is a well-known Chinese medicine belonging to the family Araliaceae. It is mainly distributed in the Yunnan province in the southwestern part of China. Sanchi-ginseng is similar to Asian ginseng (*Panax ginseng*) in many medicinal effects. Its wild resource has become scarcer because of the increasing demand on the market. It has been cultivated by ethnic groups in Wenshan area of Yunnan and served as a useful plant a long time ago. Dai people in Yunnan traditionally cook it with chicken or pork. A wealth of experience of sanchi-ginseng cultivation has been accumulated over the year by the ethnic groups, and no one knows exactly when and how they began its domestication (32).

a. Nutrient contents

There are many compunds in sanchi-ginseng such as saponins (ginsenosides), amino acids, polysaccharides, essential oils, phytosterol, inorganic ions, etc. (33). One of the main compounds of nutritional and pharmaceutical importance is saponin. Therefore, research has been focused on the separation and structural determination of saponin and its derivatives. A number of dammarane types of triterpenoid saponins are contained as the main constituents

in the plant. Sapogenin panaxadiol and panaxatriol have been obtained by hydrolysis of saponins. Some characteristic saponins such as notoginsenosides R1 and R2 appear only in sanchi-ginseng (34).

There are more than 16 amino acids existing in the roots of sanchi-ginseng. A special amino acid, β-N-oxalo-L-α,β-diaminopropionic acid, which was named as dencichine, has shown clear hemostatic activity. The average content of dencichine reaches 0.87% in the roots. Hence, sanchi-ginseng has been used as a hemostatic product in clinical practice. In addition, more than 79 essential oils have been identified in sanchi-ginseng. There are also some bioactive polysaccharides or sanchinins, which can stimulate immunological functions. Besides, some phytosterols such as carotin, stigmasterol, sitosterol, and some trace elements such as calcium, phosphorus, and iron are found in sanchi-ginseng (33, 35).

b. Medicinal functions

Dai and Zhuang people in Yunnan have used sanchi-ginseng as a hemostat for external application for many years. There are many pharmacological actions of sanchi-ginseng. It has clear hemostatic activity and has been shown to increase the number of red blood cells. It also has antiarrhythmic effects, and may lower the blood pressure transiently. The extracts of sanchi-ginseng, saponins, have many beneficial effects on the metabolism of proteins, sugars, RNA, cholesterols, and lipids in experiments using animal models (36). The non-toxicity of sanchi-ginseng to animals makes it possible for wide applications in clinical practices.

Sanchi-ginseng has been used to treat gastric, intestinal, and cranial cavity gynaecological haemorrhages. It is also the most important component of the famous Chinese medicine "Yunnan-Baiyao" which has shown strong hemostatic, hemotonic, and roborant activities. Recently, the roots of sanchi-ginseng have been used to treat coronary heart diseases, hepatitis, high cholesterol, and high blood lipid. Sanchi-ginseng is being developed into a modern medicine based on its traditional use (37).

IV. SAFETY OF EDIBLE PLANTS

Plant foods may contain, in addition to the many well-known major and minor nutrients, many kinds of naturally occurring toxic compounds which are commonly termed "nature's pesticides" because they are often toxic to predators, such as insects and animals including human. Fortunately, plant foods also contain natural chemicals that may counteract the adverse effects of many natural and synthetic toxins. While much work on these "antitoxins" or "chemopreventives" is still needed, the data available thus far are very encouraging, and some plant foods can actually reduce the incidence of certain types of cancer.

The terms "poisonous" and "non-poisonous" are two relative concepts. It is important for us to recognize plants, collect plant parts in a certain period, process materials with appropriate methods, and cook them correctly. Since it is practically impossible to avoid all plant-derived toxins in a normal diet, the best way to minimize potential hazards would be to eat a wide variety of plant foods, but not too much of any one item.

As poisonous plants grow in wild areas with edible-plants, some reference books are helpful to provide basic knowledge in distinguishing edible plants from the poisonous counterparts (38–41). The compositions of poisonous components in certain plants have been recently summarized (42). In addition, pesticide residues in edible plants are also of great concern to consumers (43).

A. Identification of Edible Plants

There are several groups of plants that are either entirely nontoxic or mostly nontoxic with certain qualifications (39, 40). It is certainly the best way to precede learning each plant that is to be consumed with a study of edible wild plants. Nevertheless, studying the edible plant families should not be considered a shortcut approach, since it still necessitates careful observation of all the floral characteristics. For example, there are small differences between the plants poisonous celery (*Cicuta virosa*) and edible water celery (*Oenanthe javanica*) in terms of their morphological characteristics. It is thus necessary to make sure that a given plant has been clearly identified as a member of certain families before sampling any part of it. Some examples of relatively easily recognized plant families that are nontoxic and primarily edible are shown in Table 98.15 (38–41).

B. Natural Toxins and Chemopreventives in Plants

1. Natural Toxins in Plant Foods

a. Canavanine

Canavanine is produced in some legumes such as edible alfalfa (*Medicago sativa*). It is an analog of arginine, and can substitute for this amino acid in cellular proteins, thereby compromising their functions. Canavanine can inhibit the enzyme nitric oxide synthetase (44) and induce heat-shock proteins in human cells *in vitro* (45).

b. Cyanogenic glycosides

Cyanogenic glycosides are cyanide-containing compounds naturally present in seed of apples, apricots, cherries, peaches, and pears, and also in almonds, sorghum, lima beans, cassava, corn, yams, chickpeas, and kirsch (46). It is well known that cyanide is one of the most acutely toxic chemicals. It binds to and inactivates heme enzymes, in particularly mitochondrial cytochrome oxidase, resulting in an acute, life-threatening anoxia. Cases of acute human poisoning from cyanide released from certain varieties of

TABLE 98.15
Plant Families with Nontoxic and Primarily Edible Species

Family Name	Edible Species	Edibility
Amaranthaceae	*Achyranthus* spp.	The young leaves can be eaten raw or cooked. The older leaves should be cooked since they become bitter with age.
Chenopodiaceae	*Beta vulgaris Chenopodium album*	The leaves can be eaten raw in salads. Older greens may need cooking to render them less bitter and more palatable. Seeds of most members can be harvested, winnowed, and used as a flour or flour extender.
Compositae	*Bedens pilosa* *Lactuca* spp. *Sonchus* spp. *Taraxacum officinale*	When young, many of these herbs can be included in salads, or used as the main salad ingredient. They quickly become bitter as they get older and are then best as cooked greens.
Cruciferae	*Brassica* spp. *Lepidium* spp.	Mustard leaves add a spicy flavor to salads and make a good steamed vegetable or spinach-type dish. The flowers, unopened flower buds, and many of the tender fruits can also be added to salads or cooked foods.
Fagaceae	*Castanea henryi* *Castanopsis* spp. *Fagus* spp. *Quercus* spp.	The nuts are eaten. For some nuts such as acorns, leaching out the bitter tannic acid before use is essential. In some cases, the bark and leaves may be used for medicinal purposes.
Juglandaceae	*Juglans regia*	The nuts of all can be eaten, though in many cases, a rock or hammer is required to break the thick, hard shell.
Labiatae	*Mentha* spp. *Salvia* spp.	Some of these plants are more aromatic than others. Many of the leaves are used for making beverages or medicinal teas. The seeds of many can be harvested and used in bread products, ground into flour, or used in tea.
Malvaceae	*Malva* spp. *Hibiscus* spp.	The leaves of all these plants can be eaten raw in salads or cooked like spinach. The roots of some are tender enough to be used. The flowers and fruits are edible raw or cooked.
Portulacaceae	*Portulaca oleracea*	The entire ground plant can usually be eaten raw. It may need to be steamed sometimes for improved palatability. The seeds can also be harvested and eaten.
Rosaceae	*Fragaria* spp. *Prunus* spp. *Rosa* spp.	Many leaves of this group can be eaten, some raw, some when cooked. The petals can be eaten as well as the fruits, if fleshy and palatable. The leaves and seeds of this group may contain cyanide. There is rarely a problem consuming them in small to moderate amounts, but poisonings have occurred when eaten in large amounts.

Source: Adapted from Ref. 38–41.

lima beans, cassava, and bitter almonds are a regular occurrence (47).

c. *Allyl isothiocyanates*

Allyl isothiocyanates are a group of major naturally occurring compounds that confer a pungent flavor to foods, such as mustard and horseradish, where it is present at about 50 to 100 μg/g. It is also present at much lower levels in cruciferous vegetables such as broccoli and cabbage, and in cassava and other tropical staple foods. Isothiocyanates occur in cruciferous vegetables as glucosinolate conjugates that are hydrolyzed when the plant releases enzymes during chewing (48, 49). Isothiocyanates are toxic goitogens that inhibit the binding of iodine in the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Normal dietary exposures to isothiocyanate-containing foods release milligram amounts of isothiocyanates (43).

d. *Pyrrolizidine alkaloids*

Pyrrolizidine alkaloids (PAs) are common plant toxins produced by over 200 species of flowering plants. PA-containing plants pose significant health hazards to people who consume some kinds of herbal teas and traditional folk remedies and those who eat grain-based foods contaminated with PA-containing plant parts (50).

e. *Acetylcholinesterase inhibitors in potatoes*

Members of the family Solanaceae contain a variety of toxic glycoalkaloids. Potatoes (*Solanum tuberosum*) are an important food staple in many parts of the world and, under certain conditions, produce a variety of glycoalkaloids. Potatoes that have been damaged, exposed to light (green), or sprouted contain the glycoalkaloids α-solanine and α-chaconine. Like physostigmine, solanine and chaconine are highly potent inhibitors of the enzyme acetylcholinesterase. Larger amounts of solanine and chaconine are contained in the green parts of potatoes (51).

f. *Tannins*

Tannins occur in nearly every plant-derived food. They have the ability to bind to and/or precipitate proteins. The ability of tannins to bind to proteins is of great toxicological and nutritional concern. Tannins also bind strongly to metals, such as iron, copper, and zinc, and consequently reduce the gastrointestinal absorption of these metals (52).

g. Caffeic acid and chlorogenic acid

Caffeic acid and its quinic acid conjugate chlorogenic acid occur in an extremely wide range of fruits and vegetables. Caffeic acid inhibits 5-lipoxygenase, a key enzyme in the biosynthesis of various eicosanoids, such as leukotrienes and thromboxanes. These eicosanoids are mediators of a wide variety of physiological and disease status, and are involved in immunoregulation, asthma, inflammation, and platelet aggregation (53, 54).

2. Natural Chemopreventives in Plant Foods

a. Isothiocyanates

Isothiocyanates are a large group of natural plant compounds that exhibit promising anticancer properties. Sulforaphane is a recently discovered powerful chemoprotective often found in broccoli and other cruciferous vegetables (55).

b. Indole 3-carbinol

Indole 3-carbinol (I-3-C), also present in cruciferous vegetables, is another promising chemopreventive (43). I-3-C may inhibit carcinogenesis caused by a number of chemicals in rodents and rainbow trout, most likely by multiple mechanisms. It is thought that I-3-C and its derivatives, produced under acid conditions in the stomach, are most likely to be the bioactive compounds.

c. Polyphenols

Many foods are a rich source of chemopreventive polyphenolics, which are a type of plant tannin. Strawberries, blackberries, cranberries, walnuts, and pecans are particularly good sources of the polyphenol compound ellagic acid that is the hydrolysis product of ellagitannins. Ellagic acid has been shown in numerous studies to be a versatile inhibitor of tumors at a number of sites induced by several compounds (56).

V. CONCLUSIONS AND FUTURE PROSPECTS

Many wild edible plants have been eaten as vegetables for a long time in China. More information on these plants should be collected and carefully studied. While recording information on each food plant, it is necessary to know how it is prepared, cooked or eaten raw, consumed as staple or vegetable, what spices and other ingredients may be added in the preparation, eaten regularly, in scarcity or at particular occasions, etc. Ethnobotanical knowledge could be helpful to understanding what Chinese botanicals are edible and beneficial to health (3, 8, 57).

It is also known that many kinds of edible ferns and fungi are nutritious and widely distributed in China, which are not covered in the present chapter. Further readings on these edible ferns and fungi are thus recommended (58–61).

Wild edible plants are receiving more and more attention due to their multiple beneficial functions. As a result, wild edible plants have been in great demand in recent years. Some Chinese edible plants have been exported to other countries (e.g., Japan, South Korea, Europe, and North America). Domestication and cultivation of these wild edible plants have become a new trend. Some species have also been imported from other countries such as *Opuntia ficus-indica* from Mexico (62), *Eutrema wasabi* from Japan (63, 64) and are now cultivated in China. It is very important for the sustainable development of wild edible plant resources so as to maintain their economic and health contributions to mankind.

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99 Vegetable and Plant Parts as Legal Dietary Supplements

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CONTENTS

I. INTRODUCTION

There is an increasing number of epidemiological studies that support the health benefits of increasing vegetable consumption (1–3). These benefits are generally associated with the typical intake of fresh and cooked vegetables. In addition, there is much interest in potential health benefits from other vegetable components such as fresh and dried culinary herbs, as well as medicinal herbs in the form of dietary supplements (4,5).

This chapter describes many of the known and potential healthful characteristics of typical vegetables consumed as foods or as non-food vegetable components. Also, potential contraindications are identified for the use of various

medicinal plants. In addition, regulations related to the use of vegetable components as dietary supplements are discussed along with the labeling and advertising of these products.

II. BENEFITS AND POTENTIAL RISKS OF VEGETABLES AS DIETARY SUPPLEMENTS

A. Benefits and Risks

With the numerous health-promoting aspects of vegetables and their components, the growing interest in incorporating these into dietary supplements is not surprising. Many of the purported health benefits of commonly consumed vegetables are presumed to be beneficial in dietary supplements that use vegetable products and their components as ingredients (see Chapter 2)(6).

Medicinal herbs of botanical origin extend the use of vegetables into the medical realm. Many plant products have been used in the development of purified drugs with many of the known health benefits claimed by various cultures from traditional use (7,8).

Along with the many benefits, associated health risks related to herbs are quite complicated. Because of the genetic complexities of humans, the variety of potential health/disease conditions, and the addition of an almost limitless number of potential herb and pharmaceutical drug combinations, it is nearly impossible to predict the complete safety of any dietary supplements. And although there is a long history of traditional use for many botanical components of dietary supplements, this is not always the case with new or processed herb products.

1. Culinary Vegetables in Supplement Form

Many popular claims of efficacy and safety are based on beliefs derived from reports of traditional use and ethnobotanical literature. However, when a botanical product is processed into the form of a dietary supplement and used by thousands of people from multiple racial groups for a great variety of health problems, complications are very possible. Manufacturers of herbal dietary supplements can reduce their risk by consulting reliable resources related to the ingredients to be used in a product.

Frequently, assumptions are made that the health benefits of vegetables (as indicated by epidemiological studies) are due to specific compounds in vegetables such as carotenoids, isoflavones, etc. As a major carotenoid in many vegetables, β-carotene has often been considered to be a major beneficial compound. This prompted studies of β-carotene supplements that resulted in the unexpected result of increased incidence of lung cancer in smokers who received the β-carotene supplements (9).

Similarly, traditional use of herbal plant materials does not always translate to similar efficacy and safety when the herb is processed into the form of powders and pills for use as dietary supplements. A highly controversial situation recently developed when the German Federal Institute for Drugs and Medical Devices (BfArM) canceled all registrations for medicinal products containing kava (*Piper methysticum*) due to reports of liver damage. In light of centuries of traditional use of kava or kava-kava in South Pacific cultures, this was unexpected by the supplement industry (10).

2. Functions, Contraindications, Side Effects, and Dangerous Interactions

Major references on botanicals can be contradictory on some issues. It is the authors = opinion that the most reliable science-based references available are *The Complete German Commission E Monographs* (Commission E) published in 1998 and *Herbal Medicine: Expanded Commission E Monographs* (Expanded Commission E) published in 2000 (11, 12). In order for a botanical to be marketed in Germany as an over-the-counter product, approval by the German Commission is required. In addition, these evaluations frequently agree with approvals by both the World Health Organization (WHO) (13) and the European Scientific Cooperative on Phytotherapy (ESCOP)(14).

Commission E includes 380 monographs on various herbs and herbal preparations (11). Expanded Commission E focuses on about 100 of the herbs commonly sold in the United States (12). Each monograph is expanded from the original Commission E monograph to include updated information on botany, history, composition, safety, efficacy, and therapeutic use. Also, extensive references are included that are not included in Commission E. Although many would imply that these references are too limited or outdated, the authors believe that it is important to use a conservative approach both in evaluating function claims based on science and dietary supplement safety.

Table 99.1 provides a list of factors that can affect both the functionality of the botanical and the toxicity of the botanical. The uncontrollable nature of these factors makes standardization of active compounds somewhat of a challenge.

Table 99.2 provides a list of common English names for vegetables and herbs with their botanical (Latin) and pharmacopeial names. Since different species of herbs can sometimes have identical English names, verification of the botanical name for an herb is essential. In addition, various parts of a given plant may be used for very different effects and consequently are not interchangeable. To avoid confusion, the pharmacopeial names identify the plant name and part or type of preparation. For example, the pharmacopeial names basilici herba, basilici folium, and basilici aetheroleum refer to the whole above ground herb, the

TABLE 99.1
Factors that Can Affect the Functionality and Toxicity of Botanical Dietary Supplements

Conditions Affecting Active Compound Concentrations

1. Growing Conditions (soil type and nutrition, climate, weather, and geographical conditions)
2. Processing Effects on Botanical Compounds (powders, extracts, tinctures, dried plant part)
3. Pharmaceutical Quality of Supplement
4. Standardization of Active Ingredients Concentration

Conditions Affecting Botanical Safety for Humans

1. Dosage (per body size, age, gender, health)
2. Mode of Administration (pill, powder, gel, liquid)
3. Health Status of Individual (immune system status, as well as free of illness or injury or already in a disease state)
4. Reproductive State of Individual (pregnancy, lactation)
5. Children and Elderly
6. Intake of Potentially Conflicting Substances (pharmacological drugs, over-the-counter drugs, nutrient dietary supplements, and other botanical dietary products)
7. Duration of Dosage

leaf, and the oil of the herb respectively. See Commission E for a more complete discussion of this nomenclature.

Table 99.2 also identifies the Commission E approval status and provides quick reference to various positive and negative properties that each plant part may have. The table information is derived from three key sources (11, 12, 15) with supplemental information from other respected sources (13, 16). Table 99.2 often reflects that a botanical can have both a positive and a negative effect on the same physiological system depending on dosage, duration of use, or other factors listed in Table 99.1. Contradictory information can be readily identified by comparing stated uses for an entry and the contraindications and side effects given.

Contraindications are conditions with which an herb or other botanical should not be used. Examples of the types of contraindications identified in *Commission E* for various herbs include allergies, children and infants, diabetes, gallstones, HIV, renal inflammation or disease, etc.

Side effects are potential adverse reactions that have been reported for an herb. Similar to purified drugs, the occurrence of an adverse reaction to a particular herb (side effect) may be rare and should not be considered to be an inevitable result of using an herb or herbal product.

Reproduction, lactation, and the effect of botanicals on children are listed in the table only when there has been a known effect. However, it should be cautioned that these populations are at greater risk from potential toxicity side effects of botanicals.

3. Adverse Events Monitoring

The U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Special Nutritionals has established a Special Nutritionals Adverse Event Monitoring System (SN/AEMS). This system obtains and records information from a wide variety of sources with the purpose of identifying emerging public health problems related to marketed products. Since an adverse event associated with a product may or may not have been caused by the product, the system is designed to identify patterns of adverse and unanticipated or unintended safety problems that may call for public health action (17).

B. Additional Sources of Reliable Information

Although there seem to be more sources available that rely on information about traditional use of herbs than on science-based data, there are additional science-based sources available for the public (18–21).

It has become obvious that herbal dietary supplement information on Internet web sites must be used with caution. In July 2002, the Federal Trade Commission (FTC) sent a press release warning consumers about greater than 280 web sites making questionable health claims. This evaluation was made by a 19 member international Internet health network of consumer protection law enforcement agencies led by the Australian Competition and Consumer Commission and can be found at http://www.ftc.gov/opa/2002/07/biopulse2.htm.

Because the area of dietary supplements is changing so rapidly, valid websites may be the best way to stay current. Table 99.3 provides websites of particular interest for dietary supplements.

III. DIETARY SUPPLEMENT HEALTH AND EDUCATION ACT OF 1994

In October 1994, the Dietary Supplement Health and Education Act (DSHEA) was enacted [Public Law 103–417]. This legislation was developed with the intention to provide consumers with the option to include dietary supplements in their overall health choices to decrease both disease and health-care expenses (22).

Rather than view dietary supplements as drugs, DSHEA regulates dietary supplements more like foods. Dietary supplement labels are required on every supplement with a number of similarities to the nutrition facts panel found on foods. In both cases, the manufacturers must ensure that the label information is truthful and not misleading (23).

There are four key features of DSHEA, besides labeling, which include:

1. Legally defines the term dietary supplement.
2. Places the premarket burden of safety on the manufacturers of dietary supplements.
3. Identifies constraints for written information relating to dietary supplement sales requiring that it be scientifically valid and not misleading.

TABLE 99.2
Examples of Edible Botanicals and their Pharmacological Actions when Consumed as a Dietary Supplement

Names			Comm E Approval Status	Primary Sources	Known Functions	Known Contra-Indications	Known Side Effects	Known Interactions
English	Scientific	Pharmacopeial						
Allspice	*Pimento officialis*			3		Ca/G/(Rp/Rpc)	CNS/Np1/G	—
Anise	*Pimpinela anisum*	*Anisi fructus*	*a-1*	1/3	G1/R	Da/Rp	D/R/G/Np/E/Rh3	n
Artichoke leaf (GLobe)	*Cynara scolymus*	*Cynarae folium*	*a-1;a-2*	2	G1	Da/L2	—	—
Asparagus Root	*Asparagus officinalis*	*Asparagi rhizoma*	*a-1;a-2*	2	U1/U2	U2	D	
Basil Herb	*Ocimum basilicum*	*Basilici herba*	*u-1*	1/3		E1/Rpc	E/L1CA	—
Bitter melon; Balsam pear	*Momordica charantia L.*			3		Rpc/Da	L1/G1/NP1	—
Black Pepper	*Piper nigrum*			3		Rpc/Da/L1	G/R/Rpc	—
Borage Flower	*Borago officinalis*	*Boraginis flos*	*u-1*	1/3		(Rpc)	L1	
Borage Herb	*Borago officinalis*	*Boraginis herba*	*u-1*	1/3		(Rpc)	L1	
Burdock Root	*Arctium lappa*	*Bardanae radix*	*u-1*	1/3		E1/CV	E2/CV	—
Burdock Root	*Arctium minus*	*Bardanae radix*	*u-1*	1/3		E1/CV	E2/CV	—
Burdock Root	*Arctium tomentosum*	*Bardanae radix*	*u-1*	1/3		E1/CV	E2/CV	—
Caraway Oil	*Carum carvi*	*Carvi aetheroleum*	*a-1*	1/3	G	Da	D/G3	
Caraway Seed	*Carum carvi*	*Carvi fructus*	*a-1*	1/3	G	Da	D/G3	
Cardamom seed	*Elettaria cardamomum*	*Cardamomi fructus*	*a-1*	1/3	G1	L2/G1/(Rpc)	L2/Da	
Cayenne	*Capsicum spp.*	*Capsici fructus*	*a-2*	2/3	Rh1	(Rpc)	D	—
Celery	*Apium graveolens*	*Apium graveolens*		3		Rp/(Rpc)/U1	CNS/Rp/D/Da/	
Celery Herb	*Apium graveolens*	*Apii herba*	*u-1*	1/3		Rp/(Rpc)/U1/Da	CNS/Rp/D/Da/	
Celery Root	*Apium graveolens*	*Apii radix*	*u-1*	1/3		Rp/(Rpc)/U1/Da	CNS/Rp/D/Da/	
Celery Seed	*Apium graveolens*	*Apii fructus*	*u-1*	1/3		Rp/(Rpc)/U1/Da	CNS/Rp/D/Da/	
Chamomile, German	*Chamomilla recutita*	*Matricariae flos*	*u-1;a-2*	2	G	D		h
Chamomile, German	*Matricaria chamomilla*	*Matricariae flos*	*u-1;a-2*	2	G	D		h
Chamomile, Roman	*Chamaemelum nobile*	*Chamomillae romanae flos*	*u-1*	1/3		Rp/R/Da	R/Da	d/h
Chamomile, Roman	*Anthemis nobilis*	*Chamomillae romanae flos*	*u-1*	1/3		Rp/R/Da	R/Da	d/h
Chicory	*Cichorium intybus*	*Cichorium intybus*	*a-1*	1/3		Rpc/CV/Da/L2	D	
Cinnamon	*Cinnamomum verum*	*Cinnamomi ceylanici cortex*		3		(Rpc)	Cv/CNS/G/R	
Cinnamon Bark, Chinese	*Cinnamomum aromaticum*	*Cinnamomi cassiae cortex*	*a-1;a-2*	2	Np1/G	D/Rpc	D	
Cinnamon Bark, Chinese	*Cinnamomum cassia*	*Cinnamomi cassiae cortex*	*a-1;a-2*	2	Np1/G	D/Rpc	D	
Cinnamon Flower	*Cinnamomum aromaticum*	*Cinnamomi flos*	*u-1*	1			D/G	
Cinnamon Flower	*Cinnamomum cassia*	*Cinnamomi flos*	*u-1*	1			D/G	
Cloves	*Eugenia caryophyllus*	*Caryophylli flos*	*a-1*	1/3	G	(Rpc)	G/R/D	
Cloves	*Syzgium aromaticum*	*Caryophylli flos*	*a-1*	1/3	G	(Rpc)	G/R/D	
Cloves	*Jambosa caryophyllus*	*Caryophylli flos*	*a-1*	1/3	G	(Rpc)	G/R/D	
Coffee	*Coffea canephora*	*Coffeae carbo*	*a-1*	1/3	G3/G	Rp/Rp1/CV	CV/Np/G/U/Rh1	l/d
Coffee	*Coffea liberica*	*Coffeae carbo*	*a-1*	1/3	G3/G	Rp/Rp1/CV	CV/Np/G/U/Rh1	l/d
Coffee	*Coffea arabica*	*Coffeae carbo*	*a-1*	1/3	G3/G	Rp/Rp1/CV	CV/Np/G/U/Rh1	l/d

Cola Nut	*Cola nitida/Cola spp.*	*Colae semen*	*a-1;a-2*	2	Np2	G/(Rpc)/CV/NP	Np3	d/f/h
Comfrey Herb	*Symphytum officinale*	*Symphyti herba*	*a-1*	1/3	D	(Rpc)/L1	L1/G/G1	
Comfrey Leaf	*Symphytum officinale*	*Symphyti folium*	*a-1*	1/3	D	(Rpc)/L1	L1/G/G1	
Comfrey Root	*Symphytum officinale*	*Symphyti radix*	*a-1*	1/3	D	(Rpc)/L1	L1/G/G1	
Coriander Seed	*Coriandrum sativum*	*Coriandri fructus*	*a-2*	2/3	Np1/G	(Rpc)	G/L1/Da	—
Cranberry	*Vaccinium macrocarpon*	*Vaccinium fructus*		3	U	U1	G3/Da	
Dandelion Herb	*Taraxacum officinale*	*Taraxaci herba*	*a-1;a-2*	2/3	G	Rp/E1/U1/G	G/Da	—
Dandelion Root w/Herb	*Taraxacum officinale*	*Taraxaci radix cum herba*	*a-1;a-2*	2/3	G	Rp/E1/U1/G/L2	G/Da	—
Dill Seed	*Anethum gravelons*	*Anethi fructus*	*a-1*	1		(Rpc)/U1	U1/D	
Fennel Oil	*Foeniculum vulgare*	*Foeniculi aetheroleum*	*a-1;a-2*	2	G/R	(Rpc)	CNS/D/R/Ca/G	—
Fennel Seed	*Foeniculum vulgare*	*Foeniculi fructus*	*a-1;a-2*	2	G/R	(Rpc)	CNS/D/R/Ca/G	—
Fenugreek Seed	*Trigonella foenum-graecum*	*Foenugraeci semen*	*a-1;a-2*	2/3	Np1	Rp/D	D/CV/E	—
Flaxseed	*Linum usitatissimum*	*Lini semen*	*a-1;a-2*	2/3	G	(Rpc)/G2/U1	G/D/Np2/CNS/R	d, f
Garlic	*Allium sativum*	*Allii sativi bulbus*	*a-1;a-2*	2/3	CV/H	Rpc/G/D	CNS/G/E2/D	d, h
Ginger Root	*Zingiber officinale*	*Zingiberis rhizoma*	*a-1;a-2*	2/3	G/H/I/E	L2/(Rp)	G1/D	—
Green Tea	*Camellia sinensis*	*Camellia folium*		3	I	U2/G1/CV	CV/CNS/G/D	d, f, h
Horseradish	*Armoracia rusticana*	*Armoraciae rusticanae radix*	*a-1;a-2*	2/3	R/G/Rh1	G/U2/Rpc/E2	G/D	
Kava Kava	*Piper methysticum*	*Piperis methystici rhizoma*	*a-1;a-2*	2/3		(Rpc)/NP	L1/Ca/G/H/R/D	d, f
Kelp	*Laminaria lyperborea*	*Laminariae stipites*	*u-1*	1		Rp/E2/D	H/Rp/CV/G/Da	—
Lemon Balm	*Melissa officinalis*	*Melissae folium*	*a-1;a-2*	2	Np3/G	(Rpc)/E2/D	G1/Np1	
Lemon Grass	*Cymbopogon citratus*	*Cymbopoginis citrati herba*	*u-1*	1/3	I		R	
Licorice Root	*Glycyrrhiza glabra*	*Liquiritiae radix*	*a-1;a-2*	2/3	R/G	L1/CV/U2/(Rpc)	CV/CNS/G1/D/	d, l
Lovage Root	*Levisticum officinale*	*Levistici radix*	*a-1*	1/3	U	U2/CV/H/(Rpc)	G/D	—
Marjoram Herb	*Origanum majorana*	*Majoranae herba*	*u-1*	1/3		(Rpc)	G1/G3/Np1/D	
Marjoram Oil	*Origanum majorana*	*Majoranae aetheroleum*	*u-1*	1/3		(Rpc)	G1/G3/Np1/D	
Mint/Peppermint Oil	*Mentha arvensis*	*Menthae arvensis aetheroleum*	*a-1;a-2*	2/3	G/L2/R/Rh	L/G1/D/(Rpc)	G1/G3/D/Da	
Nutmeg	*Myristica fragrans*	*Myristica frangrans*	*u-1*	1/3		Rp/(Rpc)/Np	Rp/Np/G/D	—
Onion	*Allium cepa*	*Allii cepae bulbus*	*a-1;a-2*	2	Np1/CV			
Oregano	*Origanum vulgare*	*Origani vulgaris herba*	*u-1*	1		(Rpc)/D	R/G/D/	
Parsley Herb and Root	*Petroselinum crispum*	*Petroselini herba/radix*	*a-1;a-2*	2/3	U1/U2	L1/U2/CV/' (Rpc)	CV/G/L1/U2/R/D	—
Peppermint Leaf	*Mentha x piperita*	*Menthae piperitae folium*	*a-1;a-2*	2/3	G/L	L/G1/D/(Rpc)	G1/G3/D/Da	
Peppermint Oil	*Mentha x piperita*	*Menthae piperitae aetheroleum*	*a-1;a-2*	2/3	G/L2/R	L/G1/D/(Rpc)	G1/G3/D/Da	
Plantain	*Plantago lanceolata*	*Plantaginis lanceolatae herba*	*a-1;a-2*	2/3	R	(Rpc)/G2/D	G/Da/	—
Psyllium Seed Husk, Blonde	*Plantago isphagula; P. ovata*	*Plantaginis ovatae testa*	*a-1;a-2*	2	G2/G3	G1/E1	Da	—

(*Continued*)

TABLE 99.2
(*Continued*)

Names			Comm E Approval Status	Primary Sources	Known Functions	Known Contra-Indications	Known Side Effects	Known Interactions
English	**Scientific**	**Pharmacopeial**						
Psyllium Seed, Black	*Plantago psyllium; P. afra*	*Psyllii semen*	*a-1;a-2*	2	G/G2	G1	Da	—
Psyllium Seed, Blonde	*Plantago ovata; P. isphaguta*	*Plantaginis ovatae semen*	*a-1;a-2*	2	G/G2/G3	G1/E1	Da	—
Pumpkin Seed	*Curcurbita pepo*	*Cucurbitae peponis semen*	*a-1;a-2*	2/3	U	(Rpc)/D	U1/CV/G1/D	—
Radish root	*Raphanus sativus*	*Raphani sativi radix*	*a-1*	1	G1/R	L2		
Rhubarb root	*Rheum palmatun; R. officinale*	*Rhei radix*	*a-1*	1	G2	G/(Rpc)	G/U/CV/G3	
Rosemary leaf	*Rosmarinus officianlis*	*Rosmarini folium*	*a-1;a-2*	2	G1	Rp		
Saffron	*Crocus sativa*	*Croci stigma*	*u-1*	1			Rp/G3/Np/O/D	
Sage leaf	*Salvia officinalis*	*Salviae folium*	*a-1;a-2*	2/3	G1	Rp/(Rpc)/E1/CNS	CNS/Rh3/G1/D	
Spinach leaf	*Spinacia oleracea*	*Spinaciae folium*	*u-1*	1		NSI		
Thyme	*Thymus vulgaris*	*Thymi herba*	*a-1;a-2*	2	R	Rp		
Turmeric root	*Curcuma longa*	*Curcumae longae rhizoma*	*a-1;a-2*	2/3	G1	L/G1/(Prc)	G1/Np1/D	—
Watercress	*Nasturtium officinale*	*Nasturtii herba*	*a-1;a-2*	2*	R	G/U1/Rp-c	G	

Commission E approval Status: a = approval, u = unapproval, (#) refers to primary source.
Primary Sources Used: 1 = Commission E (Ref. 11); 2 = Commission E, Expanded (Ref. 12); 3 = Mosby's Handbook of Herbs and Natural Supplements (Ref. 15).
Known Functions: presence of a code for a system or function indicates that reasonable evidence exists for potential benefit.
Known Contraindications: presence of a code for a system or function indicates that reasonable evidence exists for potential negative effects.
Known Interactions: presence of a code indicates that evidence exists for potential negative interactions (d = drug; f = food; h = herb; n = nutrient; l = laboratory tests).
( ) = parenthesis around any of the following initials indicates precautionary steps necessary until information indicates otherwise.
CV = Cardiovascular System.
Ca = Cancer.
CNS = Central Nervous System problems.
D = Dermatitis; Da = Allergic reaction including anaphylactic shock.
E = Endocrine Functions; E1 = Diabetes; E2 = thyroid.
G = Gastrointestinal Tract; G1 = Upper GI functions; G2 = constipation; G3 = diarrhea.
H = hematology/lymphatic.
I = Immune system.
L = Liver + Gall Bladder; L1 = liver; L2 = gall bladder.
NP = Neural/Psychological; NP1 = appetite; NP2 = fatigue; NP3 = normal sleep.
NSI = Not Sufficient information to make claims.
O = Ophthalmologic.
R = Respiratory System.
Rh = Muscle/Joint System; Rh1 = muscle; Rh2 = joint; Rh3 = convulsions.
Rp = Pregnancy and lactation; Rpc = Pregnancy, lactation, and children.
U = Urinary tract and kidney; U1 = Bladder; electrolyte balance; U2 = Kidney function.

TABLE 99.3
Internet Resources for Information on Medicinal and Culinary Herbs

AGRICOLA (Agricultural online Access) National Agricultural Library
Provides access to a large bibliographic database of information on herbs.
http://www.nal.usda.gov/

AMERICAN BOTANICAL COUNCIL
A comprehensive and current source of information on herbs; membership required for full access.
http://www.herbalgram.org/

AMERICAN HERBAL PHARMACOPOEIA AND THERAPEUTIC COMPENDIUM
Good source of authoritative monographs on botanical; new monographs are published on a regular basis.
http://herbal-ahp.org/

AMERICAN HERBAL PRODUCTS ASSOCIATION
A national trade association that promotes responsible commerce of products that contain herbs.
http://www.ahpa.org/

AMERICAN HERBALISTS GUILD
A professional organization promoting standards of competency in herbal medicine.
http://www.americanherbalistsguild.com/

AMERICAN SOCIETY OF PHARMACOGNOSY
A professional scientific organization promoting the science of pharmacognosy.
http://www.phcog.org/

BOTANICAL MEDICINE INFORMATION RESOURCES
Website of the Rosenthal Center for Complementary and Alternative Medicine; provides many links to journals, mailing lists, and regulatory information.
http://www.rosenthal.hs.columbia.edu/Botanicals.html

BRITISH HERBAL MEDICINE ASSOCIATION
Professional society founded to advance the science and practice of herbal medicine in the United Kingdom.
http://info.ex.ac.uk/phytonet/bhma.html

CONSUMER LAB.COM
Independent organization that tests herbs and health & nutrition products for purity and accuracy in labeling.
http://www.consumerlab.com/

DIETARY SUPPLEMENT EDUCATION ALLIANCE
www.supplementinfo.org

FDA CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
Location for industry guidance on labeling.
www.cfsan.fda.gov/~dms/supplmnt.html

HERB RESEARCH FOUNDATION
A nonprofit research and education organization that strives to improve world health through the informed use of herbs.
http://www.herbs.org/

HERBAL ABSTRACT PAGE
Provides hundreds of Medline and other abstracts dealing with herbal and traditional Chinese medical therapies.
http://www.vet-task-force.com/Medline4.htm

HERBAL MEDICINE FROM MEDLINEplus
Provides links to the latest news on herbs and other practical sources of information.
http://www.nlm.nih.gov/medlineplus/herbalmedicine.html

INTERNATIONAL BIBLIOGRAPHIC INFORMATION ON DIETARY SUPPLEMENTS
IBIDS is a database of published, international, scientific literature on dietary supplements, including vitamins, minerals, and botanical produced by the Office of Dietary Supplements (ODS) at the National Institutes of Health. It presently contains 676,453 unique scientific citations and abstracts (8/02)

JOURNAL OF NATURAL PRODUCTS
Full text access to the journal of the American Society of Pharmacognocy
http://pubs.acs.org./journals/jnprdf/index.html

MEDLINE PLUS HEALTH INFORMATION HERBAL MEDICINE PAGE
http://www.nlm.nih.gov/medlineplus/herbalmedicine.html

NATIONAL CENTER FOR COMPLEMENTARY AND ALTERNATIVE MEDICINE (NCCAM)
www.nccam.nih.gov

(*Continued*)

TABLE 99.3
(*Continued*)

OFFICE OF DIETARY SUPPLEMENTS (National Institute of Health)
ODS supports research and disseminates research results in the area of dietary supplements.
http://ods.od.nih.gov/index.aspx
ROCKY MOUNTAIN HERBAL INSTITUTE
Offers continuing education courses and other resources on Chinese herbal sciences for medical and health professionals.
http://www.rmhiherbal.org/
WHO MONOGRAPHS OF MEDICINAL PLANTS
Provides online access to a variety of resources on medicinal plants.
http://www.who.int/medicines/library/trm/medicinalplants/medplantsdocs.shtml
U.S. PARMACOPEIA Dietary Supplement Verification Program (DSVP)
http://www.usp-dsvp.org/

4. Creates the Office of Dietary Supplements (ODS) in the National Institutes of Health (NIH) to coordinate dietary supplement research within NIH.

It is also the responsibility of ODS to advise other Federal agencies regarding dietary supplements.

A. Dietary Supplement Terminology

FDA traditionally considered dietary supplements to be composed only of essential nutrients including vitamins, minerals, and proteins. In 1990 the Nutrition Labeling and Education Act identified herbs and similar nutritional substances as dietary supplements. In 1994, DSHEA legally defined dietary supplement to include non-nutrients such as herbs, fish oils, psyllium, enzymes, glandulars, and mixtures of these (22).

The short formal definition of a "Dietary Supplement" is a product (other than tobacco) that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients (23):

- A vitamin
- A mineral
- An herb or other botanical
- An amino acid
- A dietary substance for use by man to supplement the diet by increasing the total daily intake. This might include enzymes or tissues from organs or glands.
- A concentrate, metabolite, constituent, extract, or combinations of these ingredients

and

- Is intended for ingestion in pill, capsule, tablet, or liquid form
- Is not represented for use as a conventional food or as the sole item of a meal or diet
- Is labeled as a "dietary supplement"

The expanded definition can be found at web site for the Office of Dietary Supplements National Institutes of Health at http://ods.od.nih.gov/health.aspx.

Botanical ingredients include all plant-derived materials whether fresh, preserved, or dried full plants, plant parts, plant species mixtures, plant extracts, and compounds found in such materials. Items that are commonly termed herbs or herbal products, regardless of whether they meet the dictionary definition of herb or that are comprised of parts, extracts, or preparations of woody plants are included as botanical ingredients. Botanicals also include fungi and algae. Herbs are considered to be flowering plants whose stems above ground do not become woody (24).

Any dietary ingredient that was not marketed in the United States in a dietary supplement prior to October 15, 1994 is considered a "new dietary ingredient" (25). If a "new dietary ingredient" is to be marketed in a dietary supplement, the following conditions must be met:

1. The substance is first considered to be a "dietary ingredient" (26).
   A dietary ingredient is defined as a vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients.
2. The product qualifies for the definition of dietary supplement (see DSHEA terminology above) and is not presently nor has it been authorized for investigation as an approved drug, certified antibiotic, or licensed biologic (26).

Also a dietary supplement containing a "new dietary ingredient" shall be deemed adulterated unless it meets at least one of two requirements:

1. The dietary supplement contains only dietary ingredients which have been present in the food supply as an article used for food in a form in which the food has not been chemically altered.
2. There is a history of use or other evidence of safety establishing that the dietary ingredient when used under the conditions recommended

> or suggested in the labeling of the dietary supplement will reasonably be expected to be safe and, at least 75 days before being introduced or delivered for introduction into interstate commerce, the manufacturer or distributor of the dietary ingredient or dietary supplement provides the FDA with information, including any citation to published articles, which is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such dietary ingredient will reasonably be expected to be safe (25).

B. DSHEA Responsibilities

1. Manufacturers

The greatest difference between the regulation of food products and dietary supplements is that the manufacturer is responsible for ensuring that their product is safe prior to marketing. Under DSHEA's guidelines, manufacturers also are responsible for ensuring that product information is both truthful and is not misleading.

Manufacturers must also follow FDA's Current Good Manufacturing Practices in manufacturing, packing, or holding dietary supplements (27).

The DSHEA amends the adulteration provisions of the FD&C Act. Under DSHEA a dietary supplement is adulterated if it or one of its ingredients presents "a significant or unreasonable risk of illness or injury" when used as directed on the label, or under normal conditions of use (if there are no directions). A dietary supplement that contains a new dietary ingredient (i.e., an ingredient not marketed for dietary supplement use in the U.S. prior to October 15, 1994) may be adulterated when there is inadequate information to provide reasonable assurance that the ingredient will not present a significant or unreasonable risk of illness or injury. The Secretary of HHS may also declare that a dietary supplement or dietary ingredient poses an imminent hazard to public health or safety. However, like any other foods, it is a manufacturer's responsibility to ensure that its products are safe and properly labeled prior to marketing.

2. Food and Drug Administration

Under DSHEA, FDA's responsibility for dietary supplements begins after a product reaches the market. This responsibility includes evaluating product information on labels, package inserts, and accompanying literature which may make a product unsafe. FDA recently expanded their evaluations to include product information on company websites.

DSHEA also grants FDA the authority to establish Good Manufacturing Practices (GMPs) for dietary supplements that are similar to those used for food products. These GMPs should ensure the safety of dietary supplements throughout formulation, manufacturing, packaging, storage, and shipping.

3. Federal Trade Commission

The Federal Trade Commission (FTC) bears the responsibility of regulating false advertising claims for both food and dietary supplements. Under DSHEA, the FTC is the lead agency to assure that information within and on dietary supplement packages is both truthful and not misleading. This responsibility carries over to accompanying literature as well.

C. Constraints on Written Product Information

There are multiple levels of dietary supplement information available to the consumer, with a significant amount based primarily on traditional uses and "word-of-mouth" testimonials. Prior to passage of DSHEA, information linked to supplement sales was not always based on science, however DSHEA now requires that written information relating to the sale of dietary supplements be scientifically valid and not be misleading to the consumer. This includes constraints on product labeling described below in Section IV-D "Claims."

D. Office of Dietary Supplements

DSHEA's intention is for the Office of Dietary Supplements (ODS) to be the coordinating body for dietary supplement research within NIH. This responsibility includes: 1) promoting scientific research on supplements related to their potential for decreasing the incidence of chronic diseases; 2) becoming a worldwide reservoir for dietary supplement scientific data, and 3) being a scientific advisor to other federal agencies including Human Health Services and FDA.

IV. LABELING OF DIETARY SUPPLEMENTS

A. General Labeling Information

As with food products, there is a set of information that must be supplied on the product label. This includes a descriptive name of the product that clearly identifies the product as a "dietary supplement" on the front label (e.g. Vitamin E Dietary Supplement). Also required is the name and place of business of the manufacturer, packer, or distributor; a complete list of ingredients; and the net contents of the product. To access current information on basic and specific dietary supplement regulations, see Industry Information and Regulations at the U.S. Food & Drug Administration Center for Food Safety & Applied Nutrition Dietary Supplements web site [http://www.cfsan.fda.gov/~dms/ds–ind.html].

All dietary supplements also require a "Supplement Facts" panel and not "Nutrition Facts" even if the dietary supplement is a nutrient. Below the panel, a statement of ingredients should list all dietary ingredients not already identified in the "Supplement Facts" panel (e.g., rose hips as the source of vitamin C). And the ingredient statement should include other food ingredients (e.g., water, oil, and sugar), and technical additives or processing aids (e.g. colors, flavors, gelatin, preservatives, and stabilizers). See http://www.cfsan.fda.gov/~lrd/fr97923a.html.

In addition, it is also required that ingredient statements clearly indicate which part of the plant is used including that in extracts, oils and any other form.

B. Supplement Facts Panel

The "Supplement Facts" must include the quantity for each dietary ingredient per serving. The listing may include the source of a dietary ingredient (e.g., "300 mg. calcium from calcium gluconate"). If an ingredient is listed in the "Supplement Facts" panel, it is not required to also be listed in the statement of ingredients.

For dietary supplements containing significant amounts of nutrients that have recommendations for daily consumption (Daily Values), ingredient lists must list nutrients in the order that FDA has established for labeling (28). See Table 99.4.

For supplements containing botanicals, including proprietary blends, the total quantity of all dietary ingredients in the blend (excluding inert ingredients) must be listed. Also for products containing herbal and/or botanical ingredients, the label must indicate the part of the plant from which the ingredient is derived (e.g. root, leaf, bark).

DSHEA indicates that when an official compendium exists for a dietary supplement, then the supplement must meet the specifications of the compendium or the product can be considered to be misbranded. Three such official compendia include the U. S. Pharmacopeia, the Homeopathic Pharmacopeia of the United States, and the National Formulary.

TABLE 99.4
Reference Nutrient Values for Dietary Supplements

Mandatory			Voluntary		
Nutrient	**Daily Values**		**Nutrient**	**Daily Values**	
Total Fat*	65 g	DRV**	Vitamin D	400 IU	RDI
Saturated fatty acids	20 g	DRV	Vitamin E	30 IU	RDI
Cholesterol	300 mg	DRV	Vitamin K	80 mcg	RDI
Sodium	2400 mg	DRV	Thiamin	1.5 mg	RDI
Potassium	3500 mg	DRV	Riboflavin	1.7 mg	RDI
Total carbohydrate	300 g	DRV	Niacin	20 mg	RDI
Fiber	25 g	DRV	Vitamin B6	2.0 mg	RDI
Protein	50 g	DRV	Folate	400 mcg	RDI
			Vitamin B12	6.0 mcg	RDI
Vitamin A	5000 IU	RDI***	Biotin	300 mcg	RDI
Vitamin C	60 mg	RDI	Pantothenic acid	10 mg	RDI
Calcium	1000 mg	RDI	Phosphorus	1000 mg	RDI
Iron	18 mg	RDI	Iodine	150 mcg	RDI
			Magnesium	400 mg	RDI
			Zinc	15 mg	RDI
g = grams			Selenium	70 mcg	RDI
mg = milligrams			Copper	2.0 mg	RDI
mcg = micrograms			Manganese	2.0 mg	RDI
IU = International Units			Chromium	120 mcg	RDI
			Molybdenum	75 mcg	RDI
			Chloride	3400 mg	RDI

* Based on diets containing about 2,000 calories a day for adults and children over 4 only.

** Daily Reference Values (DRVs).

*** Reference Daily Intake (RDI).

Nutrients in this table are listed in the order in which they are required to appear on a label. The second column of nutrients follows directly after the first column (21 CFR 101.9(c)).

This list includes only those nutrients for which a Daily Reference Value (DRV) has been established in 21 CFR 101.9(c);(9) or a Reference Daily Intake (RDI) in 21 CFR 101.9(c);(8);(iv) [http://www.cfsan.fda.gov/~dms/flg-7a.html]. Revision. Jan 30, 1998

C. Claims

1. Types of Claims

Based on DSHEA, dietary supplement label claims may only be described in relation to: 1) classical nutrient deficiency diseases; 2) the "well-being" achieved by consuming the dietary ingredient or 3) "structure/function" of the dietary supplement on the body. In the case of deficiency diseases, the prevalence of the disease in the United States must be disclosed on the supplement label. A guide for industry on dietary supplement claims can be found http://www.cfsan.fda.gov/~dms/sclmguid.html (29).

As important as the above stipulations, DSHEA mandates that no supplement can claim directly nor can it be implied that the use of a dietary supplement will assist to diagnose, prevent, mitigate, treat, or cure a specific disease (unless approved under the new drug provisions of the FD&C Act). In other words, it is not permissible to imply that a dietary supplement may detoxify the liver nor can a dietary supplement prevent osteoporosis (29, 30). Numerous examples of permitted and prohibited claims can be found at the MLMLAW site [http://www.mlmlaw.com/index.html].

2. Disease Criteria

Disease is defined as "… damage to an organ, part, structure, or system of the body such that it does not function properly (e.g., cardiovascular disease), or a state of health leading to such dysfunctioning (e.g., hypertension); except that diseases resulting from essential nutrient deficiencies (e.g., scurvy, pellagra) are not included in this definition" (30).

Listed below is a summary of ten major criteria that determine if a statement is considered to be a disease claim (30) and additional examples of permissible and non-permissible claims can be found at http://www.mlmlaw.com/saleswatch (31).

A claim is not permissible if it:

1. Claims to have an effect on a disease or class of diseases
2. Claims to have an effect on characteristic symptoms of disease
3. Claims to have an effect on a condition associated with a natural state such as pregnancy
4. Implies to have an effect on a disease because of product name, formulation, or graphics
5. Implies an effect by belonging to a class of products that are intended to diagnose, mitigate, treat, cure, or prevent a disease
6. Portrays a product as promoting health when in reality the function is disease therapy
7. Assists a therapy or drug intended to diagnose, mitigate, treat, cure, or prevent a disease
8. Relates to preventing a disease or to a vector of disease
9. Implies that the product can treat, prevent, or mitigate adverse events associated with a disease therapy
10. Suggests an effect on a disease or diseases

For FDA authorized health claims related to specific nutrients in vegetables (6, 32), a dietary supplement may make the appropriate claim only if the product meets all criteria to bear the claim.

3. Qualified Health Claims

One exception to the above limitations on health claims is the use of FDA approved "qualified health claims." When the FDA finds that significant scientific agreement does not exist for a health claim, but more evidence is in support of the nutrient/disease relationship than against it, a qualified health claim may be appropriate. The FDA reviews proposed qualified health claims on a case by case basis. An example of an approved qualified health claim for selenium is: "Selenium may reduce the risk of certain cancers. Some scientific evidence suggests that consumption of selenium may reduce the risk of certain forms of cancer. However, FDA has determined that this evidence is limited and not conclusive." To use this or other approved qualified health claims, specific criteria must be met. For additional information, see http://www.cfsan.fda.gov/~dms/ds-ltr35.html.

4. Disclaimer and Warnings

To make any claim on dietary supplements, a manufacturer must verify that the statements are truthful and are not misleading. Supplement product labels with a claim must also bear the phrase, "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease."

Unlike health claims, structure/function statements do not need to be approved by FDA before a manufacturer markets the supplement product bearing the statement. However, the FDA should be notified within 30 days after a product that bears the claim is first marketed (29, 33).

5. Point of Purchase Supporting Literature

To assist the consumer in choosing dietary supplements, DSHEA allows retail outlets to provide "third-party" materials (e.g. book chapters, scientific abstracts) related to any health-related benefits of dietary supplements. These materials may not contain false or misleading information and cannot promote a specific supplement brand or have product promotional literature attached (23).

6. Internet Claims

FDA recently indicated that commercial web sites for a product must comply with the same regulations established for product labels and point of purchase supporting literature. Therapeutic claims that indicate a product is intended for use in the cure, mitigation, treatment, or prevention of disease can force FDA to establish the product as a drug, placing the product under much more stringent regulation (35).

For more information on dietary supplement labeling and claims that can be made, see the Report of the Commission on Dietary Supplement Labels (36).

V. OTHER CONSIDERATIONS

Staying current in the area of dietary supplements will be a challenge for anyone. To prevent wasting valuable time and resources, staying abreast with FDA responses on safety issues will be crucial. For updated warning and safety information on dietary supplements see http://www.cfsan.fda.gov/~dms/ds-warn.html (37).

VI. CONCLUSION

Both the potential benefits and risks associated with the use of vegetable and plant parts in dietary supplements can be compelling. These botanical components range from rather basic and harmless culinary vegetables to powerful herbal extractions that can cause death with inappropriate use. Between these extremes, there is a large grey area. As the studies of this grey area continue, surprises related to both benefits and risks are to be expected.

Developers and manufacturers of dietary supplements need to be aware of the regulatory requirements involved in production and marketing of supplements as well as the rapidly growing body of research literature. Like the products' potential effects on people, the financial benefits and risks to manufacturers can be great. The risks can be extremely great for an uninformed manufacturer. Use of the resources reviewed in this chapter can certainly help to reduce the risks.

ACKNOWLEDGMENT

The information in this chapter has been derived from "Vegetable and Plant Parts as Legal Dietary Supplements," by J. Dobbs and C. A. Titchenal. In *Handbook of Vegetable Preservation and Processing*, editors: Y. H. Hui et al., Marcel Dekker, 2004.

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100 U.S. Regulations for Advertising Dietary Supplements, Health and Diet Products and Services

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CONTENTS

I. INTRODUCTION

The array of dietary supplements — vitamins and minerals, amino acids, enzymes, herbs, animal extracts and others — has grown tremendously over the years. The number of products — and the variety of uses for which they are promoted — have increased significantly in the last few years. Scientific research on the associations between supplements and health is accumulating rapidly. Although the benefits of some of these products have been

documented, the advantages of others are unproven. Officials at the Federal Trade Commission (FTC) and the Food and Drug Administration (FDA) say health fraud promoters often target people who are overweight or have serious conditions for which there are no cures, including multiple sclerosis, diabetes, Alzheimer's disease, cancer, HIV and AIDS, and arthritis.

The role of the FTC, which enforces laws outlawing "unfair or deceptive acts or practices," is to ensure that consumers get accurate information about dietary supplements so that they can make informed decisions about these products. The FTC and the FDA work together under a long-standing liaison agreement governing the division of responsibilities between the two agencies. As applied to dietary supplements, the FDA has primary responsibility for claims on product labeling, including packaging, inserts, and other promotional materials distributed at the point of sale. The FTC has primary responsibility for claims in advertising, including print and broadcast ads, infomercials, catalogs, and similar direct marketing materials. Marketing on the Internet is subject to regulation in the same fashion as promotions through any other media. Because of their shared jurisdiction, the two agencies work closely to ensure that their enforcement efforts are consistent to the fullest extent feasible. In 1994, the Dietary Supplements Health and Education Act (DSHEA) significantly changed the FDA's role in regulating supplement labeling. Although DSHEA does not directly apply to advertising, it has generated many questions about the FTC's approach to dietary supplement advertising. The answer to these questions is that advertising for any product — including dietary supplements — must be truthful, not misleading, and substantiated. Given the dramatic increase in the volume and variety of dietary supplement advertising in recent years, FTC staff is issuing this guide to clarify how long-standing FTC policies and enforcement practices relate to dietary supplement advertising.

The FTC's approach to supplement advertising is best illustrated by its Enforcement Policy Statement on Food Advertising (Food Policy Statement). Although the Food Policy Statement does not specifically refer to supplements, the principles underlying the FTC's regulation of health claims in food advertising are relevant to the agency's approach to health claims in supplement advertising. In general, the FTC gives great deference to an FDA determination of whether there is adequate support for a health claim. Furthermore, the FTC and the FDA will generally arrive at the same conclusion when evaluating unqualified health claims. As the Food Policy Statement notes, however, there may be certain limited instances when a carefully qualified health claim in advertising may be permissible under FTC law, in circumstances where it has not been authorized for labeling. However, supplement marketers are cautioned that the FTC will require both strong scientific support and careful presentation for such claims. Supplement marketers should ensure that anyone involved in promoting products is familiar with basic FTC advertising principles. The FTC has taken action not just against supplement manufacturers, but also, in appropriate circumstances, against ad agencies, distributors, retailers, catalog companies, infomercial producers and others involved in deceptive promotions.

Therefore, all parties who participate directly or indirectly in the marketing of dietary supplements have an obligation to make sure that claims are presented truthfully and to check the adequacy of the support behind those claims.

II. APPLICATION OF FTC LAW TO DIETARY SUPPLEMENT ADVERTISING

The FTC's truth-in-advertising law can be boiled down to two common-sense propositions: 1) advertising must be truthful and not misleading, and 2) before disseminating an ad, advertisers must have adequate substantiation for all objective product claims. A deceptive ad is one that contains a misrepresentation or omission that is likely to mislead consumers acting reasonably under the circumstances to their detriment. The FTC's substantiation standard is a flexible one that depends on many factors. When evaluating claims about the efficacy and safety of foods, dietary supplements, and drugs, the FTC has typically applied a substantiation standard of competent and reliable scientific evidence. To determine whether an ad complies with FTC law, it is first necessary to identify all express and implied claims that the ad conveys to consumers. Once the claims are identified, the scientific evidence is assessed to determine whether there is adequate support for those claims. The following sections describe this two-step process with examples illustrating how principles of ad interpretation and substantiation apply in the context of dietary supplement advertising. The examples have been simplified to illustrate one or two specific points. Therefore, advertisers should use these examples as general guidance only.

III. IDENTIFYING CLAIMS AND INTERPRETING AD MEANING

A. Identifying Express and Implied Claims

The first step in evaluating the truthfulness and accuracy of advertising is to identify all express and implied claims an ad conveys to consumers. Advertisers must make sure that whatever they say expressly in an ad is accurate. Often, however, an ad conveys other claims beyond those expressly stated. Under FTC law, an advertiser is equally responsible for the accuracy of claims suggested or implied by the ad. Advertisers cannot suggest claims that they could not make directly. When identifying claims, advertisers should not focus just on individual phrases or statements, but rather should consider the ad as a whole, assessing the "net

impression" conveyed by all elements of the ad, including the text, product name, and depictions. When an ad lends itself to more than one reasonable interpretation, the advertiser is responsible for substantiating each interpretation. Copy tests, or other evidence of how consumers actually interpret an ad, can be valuable. In many cases, however, the implications of the ad are clear enough to determine the existence of the claim by examining the ad alone, without extrinsic evidence.

Example 1

An advertisement claims that "university studies prove" that a mineral supplement can improve athletic performance. The advertiser has expressly stated the level of support for the claimed benefit and is therefore responsible for having "university studies" that document the advertised benefit. Furthermore, the implied reference to scientific evidence likely conveys to consumers the implied claim that the studies are methodologically sound.

Example 2

An advertisement for a vitamin supplement claims that 90% of cardiologists regularly take the product. In addition to the literal claim about the percentage of cardiologists who use the product, the ad likely conveys an implied claim that the product offers some benefit for the heart. Therefore, the advertiser must have adequate support for both representations. Depending on how it is phrased, or the context in which it is presented, a statement about a product's effect on a normal "structure or function" of the body may also convey to consumers an implied claim that the product is beneficial for the treatment of a disease. If elements of the ad imply that the product also provides a disease benefit, the advertiser must be able to substantiate the implied disease claim even if the ad contains no express reference to disease.

Example 3

An ad for an herbal supplement makes the claim that the product boosts the immune system to help maintain a healthy nose and throat during the winter season. The ad features the product name "Cold Away" and includes images of people sneezing and coughing. The various elements of the ad — the product name, the depictions of cold sufferers, and the reference to nose and throat health during the winter season — likely convey to consumers that the product helps prevent colds. Therefore, the advertiser must be able to substantiate that claim. Even without the product name and images, the reference to nose and throat health during the winter season may still convey a cold prevention claim.

Example 4

An ad for a dietary supplement called "Arthricure" claims that the product maintains joint health and mobility into old age. The "before" picture shows an elderly women using a walker. The "after" picture shows her dancing with her husband. The images and product name likely convey implied claims that the product is effective in the treatment of the symptoms of arthritis, and may also imply that the product can cure or mitigate the disease. The advertiser must be able to substantiate these implied claims.

B. When to Disclose Qualifying Information

An advertisement can also be deceptive because of what it fails to say. Section 15 of the FTC Act requires advertisers to disclose information if it is material in light of representations made or suggested by the ad, or material considering how consumers would customarily use the product. Thus, if an ad would be misleading without certain qualifying information, that information must be disclosed. For example, advertisers should disclose information relevant to the limited applicability of an advertised benefit. Similarly, advertising that makes either an express or implied safety representation should include information about any significant safety risks. Even in the absence of affirmative safety representations, advertisers may need to inform consumers of significant safety concerns relating to the use of their product.

Example 5

An advertisement for a multi-vitamin/mineral supplement claims that the product can eliminate a specific mineral deficiency that results in feelings of fatigue. In fact, less than 2% of the general population to which the ad is targeted suffers from this deficiency. The advertiser should disclose this fact so that consumers will understand that only the small percentage of people who suffer from the actual mineral deficiency are likely to experience any reduction in fatigue from using the product.

Example 6

An advertiser for a weight loss supplement cites a placebo-controlled, double-blind clinical study as demonstrating that the product resulted in an average weight loss of fifteen pounds over an eight-week period. The weight loss for the test group is, in fact, significantly greater than for the control subjects. However, both the control and test subjects engaged in regular exercise and followed a restricted-calorie diet as part of the study regimen. The advertisement should make clear that users of the supplement must follow the same diet and exercise regimen to achieve the claimed weight loss results.

Example 7

An advertiser claims that its herbal product is a natural pain reliever "without the side effects of over-the-counter pain relievers." However, there is substantial evidence that the product can cause nausea in some consumers when taken regularly. Because of the reference to the side effects of other pain relievers, consumers would likely understand

this ad to mean that the herbal product posed no significant adverse effects. Therefore, the advertiser should disclose information about the adverse effects of the herbal product.

Example 8

An herbal weight loss product contains an ingredient which, when consumed daily over an extended period, can result in a significant increase in blood pressure. Even in the absence of any representation about the product's safety, the advertiser should disclose this potentially serious risk.

C. Clear and Prominent Disclosure

When the disclosure of qualifying information is necessary to prevent an ad from being deceptive, that information should be presented clearly and prominently so that it is actually noticed and understood by consumers. A fine-print disclosure at the bottom of a print ad, a disclaimer buried in a body of text, a brief video superscript in a television ad, or a disclaimer that is easily missed on an Internet web site, are not likely to be adequate. To ensure that disclosures are effective, marketers should use clear language, avoid small type, place any qualifying information close to the claim being qualified, and avoid making inconsistent statements or distracting elements that could undercut or contradict the disclosure. Because consumers are likely to be confused by ads that include inconsistent or contradictory information, disclosures need to be both direct and unambiguous to be effective.

Example 9

A marketer promotes a supplement as a weight loss aid. There is adequate substantiation to indicate that the product can contribute to weight loss when used in conjunction with a diet and exercise regimen. The banner headline claims "LOSE 5 POUNDS IN 10 DAYS," the ad copy discusses how easy it is to lose weight by simply taking the product 3 times a day, and the ad includes dramatic before-and-after pictures. A fine print disclosure at the bottom of the ad, "Restricted calorie diet and regular exercise required," would not be sufficiently prominent to qualify the banner headline and the overall impression that the product alone will cause weight loss. The ad should be revised to remove any implication that the weight loss can be achieved by use of the product alone. This revision, combined with a prominent indication of the need for diet and exercise, may be sufficient to qualify the claim. However, if the research does not show that the product contributes anything to the weight loss effect caused by diet and exercise, it would be deceptive, even with a disclosure, to promote the product for weight loss. Qualifying information should be sufficiently simple and clear that consumers not only notice it, but also understand its significance. This can be a particular challenge when explaining complicated scientific concepts to a general audience, for example, if an advertiser wants to promote the effect of a supplement where there is an emerging body of science supporting that effect, but the evidence is insufficient to substantiate an unqualified claim. The advertiser should make sure consumers understand both the extent of scientific support and the existence of any significant contrary evidence. Vague qualifying terms — for example, that the product "may" have the claimed benefit or "helps" achieve the claimed benefit — are unlikely to be adequate. Furthermore, advertisers should not make qualified claims where the studies they rely on are contrary to a stronger body of evidence. In such instance, even a qualified claim could mislead consumers.

Example 10

A company has results from two studies suggesting that the main ingredient in its supplement helps to maintain healthy cholesterol levels. There are, however, significant limitations to each of the studies and a better controlled study is necessary to confirm whether the effect is genuine. The company makes a claim in advertising that "scientific studies show that our product may be effective in reducing cholesterol." The use of the word "may" is not likely to be a sufficient disclaimer to convey the limitations of the science. A disclosure that clearly describes the limitations of the research, in language consumers can easily understand, and states directly and unambiguously that additional research is necessary to confirm the preliminary results is more likely to be effective. As discussed in the following section on substantiating claims, the extent to which studies support an unqualified claim will depend largely on what experts in the relevant field would consider to be adequate support.

IV. SUBSTANTIATING CLAIMS

A. Overview

In addition to conveying product claims clearly and accurately, marketers need to verify that there is adequate support for their claims. Under FTC law, before disseminating an ad, advertisers must have a reasonable basis for all express and implied product claims. What constitutes a reasonable basis depends greatly on what claims are being made, how they are presented in the context of the entire ad, and how they are qualified. The FTC's standard for evaluating substantiation is sufficiently flexible to ensure that consumers have access to information about emerging areas of science. At the same time, it is sufficiently rigorous to ensure that consumers can have confidence in the accuracy of information presented in advertising. A number of factors determine the appropriate amount and type of substantiation, including:

1. *The Type of Product.* Generally, products related to consumer health or safety require a relatively high level of substantiation.
2. *The Type of Claim.* Claims that are difficult for consumers to assess on their own are held to a

more exacting standard. Examples include health claims that may be subject to a placebo effect or technical claims that consumers cannot readily verify for themselves.

3. *The Benefits of a Truthful Claim, and The Cost/Feasibility of Developing Substantiation for the Claim.* These factors are often weighed together to ensure that valuable product information is not withheld from consumers because the cost of developing substantiation is prohibitive. This does not mean, however, that an advertiser can make any claim it wishes without substantiation, simply because the cost of research is too high.
4. *The Consequences of a False Claim.* This includes physical injury, for example, if a consumer relies on an unsubstantiated claim about the therapeutic benefit of a product and foregoes a proven treatment. Economic injury is also considered.
5. *The Amount of Substantiation that Experts in the Field Believe is Reasonable.* In making this determination, the FTC gives great weight to accepted norms in the relevant fields of research and consults with experts from a wide variety of disciplines, including those with experience in botanicals and traditional medicines. Where there is an existing standard for substantiation developed by a government agency or other authoritative body, the FTC accords great deference to that standard. The FTC typically requires claims about the efficacy or safety of dietary supplements to be supported with "competent and reliable scientific evidence," defined in FTC cases as "tests, analyses, research, studies, or other evidence based on the expertise of professionals in the relevant area, that have been conducted and evaluated in an objective manner by persons qualified to do so, using procedures generally accepted in the profession to yield accurate and reliable results." This is the same standard the FTC applies to any industry making health-related claims. There is no fixed formula for the number or type of studies required or for more specific parameters like sample size and study duration. There are, however, a number of considerations to guide an advertiser in assessing the adequacy of the scientific support for a specific advertising claim.

B. Ads that Refer to a Specific Level of Support

If an advertiser asserts that it has a certain level of support for an advertised claim, it must be able to demonstrate that the assertion is accurate. Therefore, as a starting point, advertisers must have the level of support that they claim, expressly or by implication, to have.

Example 11

An ad for a supplement includes the statement "Scientists Now Agree!" in discussing the product's benefit. This statement likely conveys to consumers that the state of science supporting the benefit has reached the level of scientific consensus. Unless the advertiser possesses this level of evidence, the claim is not substantiated.

Example 12

An advertiser claims that its product has been "studied for years abroad" and is now the "subject of U.S. government-sponsored research." In addition to the explicit claim that the product has been studied, such phrases likely convey to consumers an implied claim that there exists a substantial body of competently-conducted scientific research supporting the efficacy of the product. The advertiser would be responsible for substantiating both claims.

C. The Amount and Type of Evidence

When no specific claim about the level of support is made, the evidence needed depends on the nature of the claim. A guiding principle for determining the amount and type of evidence that will be sufficient is what experts in the relevant area of study would generally consider to be adequate. The FTC will consider all forms of competent and reliable scientific research when evaluating substantiation. As a general rule, well-controlled human clinical studies are the most reliable form of evidence. Results obtained in animal and in vitro studies will also be examined, particularly where they are widely considered to be acceptable substitutes for human research or where human research is infeasible.

Although there is no requirement that a dietary supplement claim be supported by any specific number of studies, the replication of research results in an independently conducted study adds to the weight of the evidence. In most situations, the quality of studies will be more important than quantity. When a clinical trial is not possible (e.g., in the case of a relationship between a nutrient and a condition that may take decades to develop), epidemiologic evidence may be an acceptable substitute for clinical data, especially when supported by other evidence, such as research explaining the biological mechanism underlying the claimed effect. Anecdotal evidence about the individual experience of consumers is not sufficient to substantiate claims about the effects of a supplement. Even if those experiences are genuine, they may be attributable to a placebo effect or other factors unrelated to the supplement. Individual experiences are not a substitute for scientific research.

Example 13

An advertiser relies on animal and in vitro studies to support a claim that its vitamin supplement is more easily absorbed into the bloodstream than other forms of the vitamin. However, the animal research uses a species of animal that, unlike humans, is able to synthesize the vitamin, and the in vitro study uses a different formulation with a higher concentration of the compound than the product being marketed. In addition, human research is feasible and relatively inexpensive to conduct in light of the potential sales of the product and is the type of research generally accepted in this particular field of study. The substantiation is likely to be inadequate in this case, both because there are significant methodological problems and because, in this particular instance, human research is both feasible and the accepted approach in the field.

Example 14

A company wants to advertise its supplement as helpful in maintaining good vision into old age. There have been two long-term, large-scale epidemiologic studies showing a strong association between life-long high consumption of the principal ingredient in the supplement and better vision in those over 70. Experts have also discovered a plausible biological mechanism that might explain the effect. A clinical intervention trial would be very difficult and costly to conduct. Assuming that experts in the field generally consider epidemiological evidence to be adequate to support the potential for a protective effect, and assuming the absence of any stronger body of contrary evidence, a claim that is qualified to accurately convey the nature and extent of the evidence would be permitted.

Example 15

An advertisement for a supplement claims that the product will cause dramatic improvements in memory and describes the experiences of 10 people who obtained these results. The descriptions of these anecdotal experiences are truthful, but the advertiser has no scientific substantiation for the effect of its product on memory and cannot explain why the product might produce such results. The individual experiences are not adequate to substantiate the claim without confirming scientific research.

D. The Quality of the Evidence

In addition to the amount and type of evidence, the FTC will also examine the internal validity of each piece of evidence. Where the claim is one that would require scientific support, the research should be conducted in a competent and reliable manner to yield meaningful results. The design, implementation, and results of each piece of research are important to assessing the adequacy of the substantiation. There is no set protocol for how to conduct research that will be acceptable under the FTC substantiation doctrine. There are, however, some principles generally accepted in the scientific community to enhance the validity of test results. For example, a study that is carefully controlled, with blinding of subjects and researchers, is likely to yield more reliable results. A study of longer duration can provide better evidence that the claimed effect will persist and resolve potential safety questions.

Other aspects of the research results — such as evidence of a dose-response relationship (i.e., the larger the dose, the greater the effect) or a recognized biological or chemical mechanism to explain the effect — are examples of factors that add weight to the findings. Statistical significance of findings is also important. A study that fails to show a statistically significant difference between test and control group may indicate that the measured effects are merely the result of placebo effect or chance. The results should also translate into a meaningful benefit for consumers. Some results that are statistically significant may still be so small that they would mean only a trivial effect on consumer health. The nature and quality of the written report of the research are also important. Research cannot be evaluated accurately on the basis of an abstract or an informal summary. In contrast, although the FTC does not require that studies be published and will consider unpublished, proprietary research, the publication of a peer reviewed study in a reputable journal indicates that the research has received some measure of scrutiny.

At the same time, advertisers should not rely simply on the fact that research is published as proof of the efficacy of a supplement. Research may yield results that are of sufficient interest to the scientific community to warrant publication, but publication does not necessarily mean that such research is conclusive evidence of a substance's effect. The FTC considers studies conducted in foreign countries as long as the design and implementation of the study are scientifically sound.

Example 16

An advertiser conducts a literature search and finds several abstracts summarizing research about the association between a nutrient and the ability to perform better on memory tests. The advertiser relies on these summaries to support a claim that its supplement, which contains the same nutrient, aids memory. However, without looking carefully at the specifics of the study design, implementation, and results, there is no way for an advertiser to ascertain whether the research substantiates the product claims. (For example, did the research use a comparable formulation of the ingredient? Was the study adequately controlled? Did the study yield results that are statistically significant?) The advertiser should carefully review the underlying science, with the assistance of an expert if necessary, before drafting advertising claims.

Example 17

An advertiser makes an unqualified claim about the anticlotting effect of a supplement that contains a compound

extracted from fruit. There are three studies supporting the effect and no contrary evidence. One study consists of subjects tested over a one-week period, with no control group. The second study is well-controlled, of longer duration, but shows only a slight effect that is not statistically significant. The third study administers the compound through injection and shows a significant anti-clotting effect, but there is some question whether the compound would be absorbed into the bloodstream if administered orally. Because the studies all have significant limitations, it would be difficult to draft even a carefully qualified claim that would adequately convey to consumers the limited nature of the evidence. The advertiser should not base a claim on these studies.

Example 18

The marketer of an herbal supplement claims that its product promotes healthy vision and is approved in Germany for this purpose. The product has been used extensively in Europe for years and has obtained approval by the German governmental authorities, through their monograph process, for use to improve vision in healthy people. The company has two abstracts of German trials that were the basis of the German monograph, showing that the ingredient significantly improved the vision of healthy individuals in the test group over the placebo group. Animal trials done by the company suggest a plausible mechanism to explain the effect. Although approval of the supplement under the German monograph suggests that the supplement is effective, advertisers should still examine the underlying research to confirm that it is relevant to the advertiser's product (for example, that the dosage and formulation are comparable) and to evaluate whether the studies are scientifically sound. Advertisers should also examine any other research that exists, either supporting or contradicting the monograph, especially if it is not possible to identify and review the research on which the monograph is based.

E. The Totality of the Evidence

Studies cannot be evaluated in isolation. The surrounding context of the scientific evidence is just as important as the internal validity of individual studies. Advertisers should consider all relevant research relating to the claimed benefit of their supplement and should not focus only on research that supports the effect, while discounting research that does not. Ideally, the studies relied on by an advertiser would be largely consistent with the surrounding body of evidence. Wide variation in outcomes of studies and inconsistent or conflicting results will raise serious questions about the adequacy of an advertiser's substantiation. Where there are inconsistencies in the evidence, it is important to examine whether there is a plausible explanation for those inconsistencies. In some instances, for example, the differences in results are attributable to differences in dosage, the form of administration (e.g., oral or intravenous), the population tested, or other aspects of study methodology. Advertisers should assess how relevant each piece of research is to the specific claim they wish to make, and also consider the relative strengths and weaknesses of each.

If a number of studies of different quality have been conducted on a specific topic, advertisers should look first to the results of the studies with more reliable methodologies. The surrounding body of evidence will have a significant impact both on what type, amount and quality of evidence is required to substantiate a claim and on how that claim is presented — that is, how carefully the claim is qualified to reflect accurately the strength of the evidence. If a stronger body of surrounding evidence runs contrary to a claimed effect, even a qualified claim is likely to be deceptive.

Example 19

An advertiser wishes to make the claim that a supplement product will substantially reduce body fat. The advertiser has two controlled, double-blind studies showing a modest but statistically significant loss of fat at the end of a six-week period. However, there is an equally well-controlled, blinded 12-week study showing no statistically significant difference between test and control groups. Assuming other aspects of methodology are similar, the studies taken together suggest that, if the product has any effect on body fat, it would be very small. Given the totality of the evidence on the subject, the claim is likely to be unsubstantiated.

Example 20

Advertisements for a fiber supplement make the claim that the product is "proven" to aid weight loss. Although the company has two published, peer-reviewed studies showing a relationship between fiber and weight loss, neither of these studies used the same proportions of soluble and insoluble fiber or the same total amount of fiber as the supplement product. There are numerous controlled, published human clinical studies, however, using the amount and type of fiber in the supplement product, that provide evidence that the product would not result in measurable weight loss. The totality of the evidence does not support the "proven" claim and, given the stronger body of contrary evidence, even a qualified claim is likely to be deceptive.

Example 21

An advertiser runs an ad in a magazine for retired people, claiming that its supplement product has been found effective in improving joint flexibility. The company sponsored a 6-week study of its supplement, involving 50 subjects over the age of 65, to test the product's effect on improving flexibility. The study was double-blinded and placebo controlled and has been accepted for publication in a leading medical journal. The study showed dramatic, statistically significant increases in joint flexibility compared to placebo, based on objective measurements. In addition, several large trials have been conducted by European

researchers using a similar formulation and dose of the active ingredient in the supplement. These trials also found statistically significant results. The advertiser reviewed the underlying European research and confirmed that it meets accepted research standards. The evidence as a whole likely substantiates the claim.

F. The Relevance of the Evidence to the Specific Claim

A common problem in substantiation of advertising claims is that an advertiser has valid studies, but the studies do not support the claim made in the ad. Advertisers should make sure that the research on which they rely is not just internally valid, but also relevant to the specific product being promoted and to the specific benefit being advertised. Therefore, advertisers should ask questions such as: How does the dosage and formulation of the advertised product compare to what was used in the study? Does the advertised product contain additional ingredients that might alter the effect of the ingredient in the study? Is the advertised product administered in the same manner as the ingredient used in the study? Does the study population reflect the characteristics and lifestyle of the population targeted by the ad? If there are significant discrepancies between the research conditions and the real life use being promoted, advertisers need to evaluate whether it is appropriate to extrapolate from the research to the claimed effect. In drafting ad copy, the advertiser should take care to make sure that the claims match the underlying support. Claims that do not match the science, no matter how sound that science is, are likely to be unsubstantiated. Advertising should not exaggerate the extent, nature, or permanence of the effects achieved in a study, and should not suggest greater scientific certainty than actually exists. Although emerging science can sometimes be the basis for a carefully qualified claim, advertisers must make consumers aware of any significant limitations or inconsistencies in the scientific literature.

Example 22

An ad for a supplement claims that a particular nutrient helps maintain healthy cholesterol levels. There is a substantial body of epidemiologic evidence suggesting that foods high in that nutrient are associated with lower cholesterol levels. There is no science, however, demonstrating a relationship between the specific nutrient and cholesterol, although it would be feasible to conduct such a study. If there is a basis for believing that the health effect may be attributable to other components of the food, or to a combination of various components, a claim about the cholesterol maintenance benefits of the supplement product is likely not substantiated by this evidence.

Example 23

A number of well-controlled clinical studies have been conducted to suggest that a mineral supplement can improve mental alertness and memory in subjects with significantly impaired blood circulation to the brain. A claim suggesting that the supplement will improve memory or mental alertness in healthy adults may not be adequately substantiated by this evidence. Advertisers should not rely on research based on a specific test population for claims targeted at the general population without first considering whether it is scientifically sound to make such extrapolations.

Example 24

An advertiser wants to make claims that its combination herbal product helps increase alertness and energy safely and naturally. The product contains two herbs known to have a central nervous system stimulant effect. The advertiser compiles competent and reliable scientific research demonstrating that each of the herbs, individually, is safe and causes no significant side effects in the recommended dose. This evidence may be inadequate to substantiate an unqualified safety claim. Where there is reason to suspect that the combination of multiple ingredients might result in interactions that would alter the effect or safety of the individual ingredients, studies showing the effect of the individual ingredients may be insufficient to substantiate the safety of the multiple ingredient product. In this example, the combination of two herbs with similar stimulant properties could produce a stronger cumulative stimulant effect that might present safety hazards. A better approach would be to investigate the safety of the specific combination of ingredients contained in the product.

Example 25

Several clinical trials have been done on a specific botanical extract showing consistently that the extract is effective for supporting the immune system. The studied extract is a complex combination of many constituents and the active constituents that may produce the benefit are still unknown. An advertiser wishes to cite this research in its advertising, as proof that its product will support the immune system. The advertiser's product is made using a different extraction method of the same botanical. An analysis of the extract reveals that it has a significantly different chemical profile from the studied extract. The advertiser should not rely on these clinical trials alone as substantiation because the difference in extracts may result in significant differences in the two products' efficacy.

V. OTHER ISSUES RELATING TO DIETARY SUPPLEMENT ADVERTISING

In addition to the basic principles of ad meaning and substantiation discussed above, a number of other issues commonly arise in the context of dietary supplement advertising. The following sections provide guidance on some of these issues including: the use of consumer or expert endorsements in ads; advertising claims based on traditional uses of supplements; use of the DSHEA disclaimer in advertising; and the application to advertising of the DSHEA

exemption for certain categories of publications, commonly referred to as "third party literature."

A. Claims Based on Consumer Testimonials or Expert Endorsements

An overall principle is that advertisers should not make claims either through consumer or expert endorsements that would be deceptive or could not be substantiated if made directly. It is not enough that a testimonial represents the honest opinion of the endorser. Under FTC law, advertisers must also have appropriate scientific evidence to back up the underlying claim. Consumer testimonials raise additional concerns about which advertisers need to be aware. Ads that include consumer testimonials about the efficacy or safety of a supplement product should be backed by adequate substantiation that the testimonial experience is representative of what consumers will generally achieve when using the product. As discussed earlier, anecdotal evidence of a product's effect, based solely on the experiences of individual consumers, is generally insufficient to substantiate a claim. Further, if the advertiser's substantiation does not demonstrate that the results are representative, then a clear and conspicuous disclaimer is necessary. The advertiser should either state what the generally expected results would be or indicate that the consumer should not expect to experience the attested results. Vague disclaimers like "results may vary" are likely to be insufficient.

Example 26

An advertisement for a weight loss supplement features a before-and-after photograph of a woman and quotes her as saying that she lost 20 pounds in 8 weeks while using the supplement. An asterisk next to the quotation references a disclaimer in fine print at the bottom of the ad that reads, "Results may vary." The experience of the woman is accurately represented, but the separate, competent research demonstrating the efficacy of the supplement showed an average weight loss of only 6 pounds in 8 weeks. Therefore, the disclosure does not adequately convey to consumers that they would likely see much less dramatic results. The placement and size of the disclaimer is also insufficiently prominent to qualify the claim effectively. One approach to adequate qualification of this testimonial would be to include a disclaimer immediately adjacent to the quote, in equal print size that says, "These results are not typical. Average weight loss achieved in clinical study was 6 pounds." When an advertiser uses an expert endorser, it should make sure that the endorser has appropriate qualifications to be represented as an expert and has conducted an examination or testing of the product that would be generally recognized in the field as sufficient to support the endorsement. In addition, whenever an expert or consumer endorser is used, the advertiser should disclose any material connection between the endorser and the advertiser of the product. A material connection is one that would affect the weight or credibility of the endorsement, or put another way, a personal, financial, or similar connection that consumers would not reasonably expect.

Example 27

An infomercial for a dietary supplement features an expert referred to as a "Doctor" and a "leading clinician in joint health" discussing the effect of a supplement product on the maintenance of healthy joints. The expert is not licensed to practice medicine, but has a graduate degree and is a trained physical therapist, running a sports clinic. The expert has not conducted any review of the scientific literature on the active component of the supplement. In return for appearing in the infomercial, she is given a paid position as an officer the company. The ad is likely to be deceptive for several reasons. First, her qualifications as an expert have been overstated and she has not conducted sufficient examination of the product to support the endorsement. In addition, her connection to the company is one that consumers might not expect and may affect the weight and credibility of her endorsement. Even if she is adequately qualified and has conducted an adequate review of the product, her position as an officer of the company should be clearly disclosed.

Example 28

A best-selling book about the benefits of a supplement product includes a footnote mentioning the most effective brand of the supplement, by name. The manufacturer of the brand cited in the book has an exclusive promotional agreement with the author and has paid him to reference the product by name. The manufacturer's ad touts the fact that its product is the only brand recommended in this best-selling book. The ad is deceptive since it suggests a neutral endorsement when, in fact, the author has been paid by the manufacturer to promote the product.

B. Claims Based on Traditional Uses

Claims based on historical or traditional use should be substantiated by confirming scientific evidence, or should be presented in such a way that consumers understand that the sole basis for the claim is a history of use of the product for a particular purpose. A number of supplements, particularly botanical products, have a long history of use as traditional medicines in the United States or in other countries to treat certain conditions or symptoms. Several European countries have a separate regulatory approach to these traditional medicines, allowing manufacturers to make certain limited claims about their traditional use for treating certain health conditions. Some countries also require accompanying disclosures about the fact that the product has not been scientifically established to be effective, as well as disclosures about potential adverse effects. At this time there is no separate regulatory process for approval of claims for these traditional medicine products under DSHEA and FDA labeling rules. In assessing claims based

on traditional use, the FTC will look closely at consumer perceptions and specifically at whether consumers expect such claims to be backed by supporting scientific evidence. Advertising claims based solely on traditional use should be presented carefully to avoid the implication that the product has been scientifically evaluated for efficacy. The degree of qualification necessary to communicate the absence of scientific substantiation for a traditional use claim will depend in large part on consumer understanding of this category of products. As consumer awareness of and experience with "traditional use" supplements evolve, the extent and type of qualification necessary is also likely to change. There are some situations, however, where traditional use evidence alone will be inadequate to substantiate a claim, even if that claim is carefully qualified to convey the limited nature of the support. In determining the level of substantiation necessary to substantiate a claim, the FTC assesses, among other things, the consequences of a false claim. Claims that, if unfounded, could present a substantial risk of injury to consumer health or safety will be held to a higher level of scientific proof. For that reason, an advertiser should not suggest, either directly or indirectly, that a supplement product will provide a disease benefit unless there is competent and reliable scientific evidence to substantiate that benefit. The FTC will closely scrutinize the scientific support for such claims, particularly where the claim could lead consumers to forego other treatments that have been validated by scientific evidence, or to self-medicate for potentially serious conditions without medical supervision. The advertiser should also make sure that it can document the extent and manner of historical use and be careful not to overstate such use. As part of this inquiry, the advertiser should make sure that the product it is marketing is consistent with the product as traditionally administered. If there are significant differences between the traditional use product and the marketed product, in the form of administration, the formulation of ingredients, or the dose, a "traditional use" claim may not be appropriate.

Example 29

The advertiser of an herbal supplement makes the claim, "Ancient folklore remedy used for centuries by Native Americans to aid digestion." The statement about traditional use is accurate and the supplement product is consistent with the formulation of the product as traditionally used. However, if, in the context of the ad, this statement suggests that there is scientific evidence demonstrating that the product is effective for aiding digestion, the advertiser would need to include a clear and prominent disclaimer about the absence of such evidence.

Example 30

A supplement manufacturer wants to market an herbal product that has been used in the same formulation in China as a tonic for improving mental functions. The manufacturer prepares the product in a manner consistent with Chinese preparation methods. The ad claims, "Traditional Chinese Medicine — Used for Thousands of Years to Bring Mental Clarity and Improve Memory." The ad also contains language that clearly conveys that the efficacy of the product has not been confirmed by research, and that traditional use does not establish that the product will achieve the claimed results. The ad is likely to adequately convey the limited nature of support for the claim.

Example 31

A supplement manufacturer markets a capsule containing a concentrated extract of a botanical product that has been used in its raw form in China to brew teas for increasing energy. The advertisement clearly conveys that the energy benefit is based on traditional use and has not been confirmed by scientific research. The ad may still be deceptive, however, because the concentrated extract is not consistent with the traditional use of the botanical in raw form to brew teas and may produce a significantly different effect.

Example 32

A supplement ad claims that a supplement liquid mineral solution has been a popular American folk remedy since early pioneer days for shrinking tumors. The ad is likely to convey to consumers that the product is an effective treatment for cancer. There is no scientific support for this disease benefit. Because of the potential risks to consumers of taking a product that may or may not be effective to treat such a serious health condition, possibly without medical supervision, the advertiser should not make the claim.

C. Use of the DSHEA Disclaimer in Advertising

Under DSHEA, all statements of nutritional support for dietary supplements must be accompanied by a two part disclaimer on the product label: that the statement has not been evaluated by FDA and that the product is not intended to "diagnose, treat, cure or prevent any disease." Although DSHEA does not apply to advertising, there are situations where such a disclosure is desirable in advertising as well as in labeling to prevent consumers from being misled about the nature of the product and the extent to which its efficacy and safety have been reviewed by regulatory authorities. For example, a disclosure may be necessary if the text or images in the ad lead consumers to believe that the product has undergone the kind of review for safety and efficacy that the FDA conducts on new drugs and has been found to be beneficial for the treatment of disease. Failure to correct those misperceptions may render the advertising deceptive. At the same time, the inclusion of a DSHEA disclaimer or similar disclosure will not cure an otherwise deceptive ad, particularly where the deception

concerns claims about the disease benefits of a product. In making references to DSHEA and FDA review, advertisers should also be careful not to mischaracterize the extent to which a product or claim has been reviewed or approved by the FDA. Compliance with the notification and disclaimer provisions of DSHEA does not constitute authorization of a claim by FDA and advertisers should not imply that FDA has specifically approved any claim on that basis.

Example 33

A company markets a supplement for "maintaining joint flexibility." The product packaging is similar in color and design to a nonprescription drug used to treat joint pain associated with arthritis and the product name is similar to the drug counterpart. The ad includes statements urging consumers to "ask their pharmacist" and "accept no generic substitute." The various elements of the ad may lead consumers to believe that the supplement is, in fact, an approved drug, or may give consumers more general expectations that the product has been subjected to similar government review for safety and efficacy. A clear and prominent disclaimer may be necessary to indicate that the product has not been evaluated by FDA and is not an approved drug product.

Example 34

An advertisement for an herbal supplement includes strong, unqualified claims that the product will effectively treat or prevent diabetes, heart disease, and various circulatory ailments. The advertiser does not have adequate substantiation for this claim, but includes the DSHEA disclaimer prominently in the ad. In face of the strong contradictory message in the ad, the inclusion of the DSHEA disclaimer is not likely to negate the explicit disease claims made in the ad, and will not cure the fact that the claims are not substantiated.

Example 35

A dietary supplement advertisement makes a number of claims about the benefits of its product for supporting various body functions. The ad also includes the statement, "Complies with FDA notification procedures of the Dietary Supplement Health and Education Act." This statement may suggest to consumers that FDA has authorized the claims made in the ad or that it has reviewed the support for the claims and found the product to be effective. Because there is no review and authorization process for such claims under DSHEA, this would be deceptive.

D. Third Party Literature

Dietary supplement advertisers should be aware that the use of newspaper articles, abstracts of scientific studies, or other "third party literature" to promote a particular brand or product can have an impact on how consumers interpret an advertisement and on what claims the advertiser will be responsible for substantiating. For purposes of dietary supplement labeling, Section 5 of DSHEA provides an exemption from labeling requirements for scientific journal articles, books and other publications used in the sale of dietary supplements, provided these materials are reprinted in their entirety, are not false or misleading, do not promote a specific brand or manufacturer, are presented with other materials to create a balanced view of the scientific information, and are physically separate from the supplements being sold.

The FTC will generally follow an approach consistent with the labeling approach when evaluating the use of such publications in other contexts, such as advertising. Although the FTC does not regulate the content or accuracy of statements made in independently written and published books, articles, or other non-commercial literature, FTC law does prohibit the deceptive use of such materials in marketing products. The determination of whether the materials will be subject to FTC jurisdiction turns largely on whether the materials have been created or are being used by an advertiser specifically for the purpose of promoting its product. As a practical matter, publications and other materials that comply with the elements of the DSHEA provision, particularly with the requirement that such materials be truthful, not misleading and balanced, are also likely to comply with FTC advertising law.

Example 36

An author publishes a book on the curative properties of an herb. The book title is "The Miracle Cancer Cure." The book does not endorse or otherwise mention any particular supplement brand. The author/publisher does not sell the herbal supplement and does not have any material connection to any marketers of the herb. As non-commercial speech, the book itself would not be subject to the FTC's jurisdiction over advertising. However, if a marketer of the herb referred to the book in advertising materials (for instance, by quoting the title and using excerpts to describe the anti-cancer benefits of its product), such references would likely be considered advertising. The advertiser would be responsible for substantiating any claims about the advertiser's product that are conveyed by these references.

VI. GOVERNMENT'S ROLE IN THE WEIGHT LOSS MARKETPLACE

The FDA regulates the labeling of prescription and over-the-counter weight loss drugs and approves new drugs. While, the FTC and the state attorneys general combat misleading advertising claims for weight loss products and programs. The FDA and the FTC share statutory authority over the promotion and advertising of drugs and supplements. The FDA has primary jurisdiction over prescription drug advertising by manufacturers or on behalf of manufacturers as well as jurisdiction over drug labeling.

The FTC regulates the advertising of weight loss products and programs, including dietary supplements for weight loss under Section 5 of the Federal Trade Commission Act. The FTC has filed more than 150 cases against such products and programs since 1927, and 75 of these have been filed since 1990. Most of these cases were filed administratively, but in nearly a dozen of them, the agency sued directly in federal district court to obtain injunctions and monetary redress.

Issues that the FTC addressed in the program cases of the early 1990s included misleading use of consumer testimonials, deceptive claims about costs and credentials of program staff, misleading comparisons of programs, and failure to substantiate success claims for weight loss or weight loss maintenance. Consent orders entered in those cases include remedies that require substantiation for weight loss or weight maintenance claims, disclosures triggered by maintenance claims ("For many dieters, weight loss is temporary"), disclosure of total costs, and prohibitions against misrepresenting staff credentials. As a result of monitoring compliance with the consent orders, the agency is seeing in general that companies are avoiding broad-based maintenance claims, and where maintenance claims are made, they are narrowly focused on discrete subgroups of dieters. Also, companies appear to be making the order-required disclaimers that are intended to limit the applicability of testimonials. The agency continues to work with companies to assure that such disclaimers are featured in a clear and prominent manner.

The offices of the state attorneys general have played a prominent role in pursuing advertisers and promoters of fraudulent pills, nostrums and devices — the bottom-feeders of the weight-loss industry — as well as more mainstream companies. States review diet ads under both their false and deceptive practices statutes as well as state food and drug laws. In addition, some states, have statutes that apply to advertising by medical professionals and prohibit false advertising and advertising for unsubstantiated treatments.

The following summaries represent disclosure models, either in place or recommended, for providing information to prospective clients/patients of weight loss programs. Some states have adopted standards for information disclosure. Additionally, the FTC (through triggered disclosures in individual case-generated consent orders) has defined information consumers should know about weight loss methods in order to understand certain efficacy and success claims. Finally, the Center for Science in the Public Interest, in its petition for rulemaking filed before the FTC, has set out a detailed disclosure model.

Most models require affirmative disclosures of costs, health risks and staff credentials untriggered by specific claims or the need for consumers' first to request the information. The exceptions is the New York City Consumer Bill of Rights where, except for an affirmatively disclosed warning about the risk of rapid weight loss, the consumer is informed of his/her right to receive certain information upon request. The FTC orders require outcome disclosures only when triggered by express or implied success or efficacy claims. The Connecticut statute is based in part upon the FTC model, requiring triggered outcome disclosures but also requiring that the diet program contract affirmatively disclose information about costs, staffing and program duration. The only in-place model that calls for any sort of untriggered outcome data disclosure is Michigan's that requires that consumers receive, through informed consent, information about the likely long term success of the program.

VII. VOLUNTARY GUIDELINES FOR PROVIDERS OF WEIGHT LOSS PRODUCTS OR SERVICES

A. Definitions

The *Partnership for Healthy Weight Management* is a coalition composed of representatives from science, academia, the health care professions, government, commercial enterprises, and organizations promoting the public interest. The Partnership encourages weight loss providers to adopt the *Partnership's Voluntary Disclosure Guidelines for Providers of Weight Loss Products and Programs* and does not endorse any particular product or program for weight loss or weight management.

"Providers" includes any individual or organization involved in providing weight loss services or products to the public, including, but not limited to, physicians, clinical psychologists, dieticians, nutritionists, and commercial programs, as well as any one else selling products or publications designed to cause weight loss or result in weight maintenance.

Providers of a weight loss product should note that use of statements about the risks associated with overweight and obesity, the risks associated with the product, or about outcomes may render the product adulterated, misbranded, or unapproved under the Federal Food, Drug, and Cosmetic Act and relevant regulations promulgated by the Food and Drug Administration.

B. Format and Distribution

These *Voluntary Guidelines* represent a consensus of voluntary consumer disclosure practices reached by a panel of weight management companies, weight loss professionals, and consumer protection groups. They are not binding, do not represent legal standards or interpretation of any legal requirements, and are not sponsored or issued by any government agency. Providers should only make these disclosures to the extent that they are permissible under applicable state and federal law. In order to assure compliance, these guidelines should be placed in the program/procedures

policy manual or such other written guidance provided to program staff.

Providers that subscribe to these *Voluntary Guidelines* may be flexible in tailoring the Guidelines to the structure and needs of their own programs. Different programs will comply in different ways with various portions of the Guidelines. However, providers should not represent, directly or indirectly, in advertising or otherwise, that they subscribe to or comply with the Voluntary Guidelines unless they make all disclosures that are applicable to their particular program or product. The examples set forth in the *Voluntary Guidelines* are provided for the purpose of illustration, and are not intended to represent a required or preferred form or format. Different providers and types of providers may utilize varying formats for disclosure.

Providers following these guidelines should make all disclosures clearly and prominently. Providers that obligate purchasers to make payments in the future or that collect non refundable payments in advance for products or services to be consumed or provided in the future, should make all disclosures in a single document that is given to all prospective clients/patients on their first visit to the center and prior to purchase. Providers that charge for products and services as they are used by the purchaser — "pay-as-you-go programs" — or that collect refundable payments in advance of delivery of the services, should also include all disclosures covered by these guidelines in a single document except that cost information may be provided either as part of the document or on clear and prominent postings at each center. Providers who post notices of costs should do so in a manner that renders them consistently viewable by consumers during their visit and permits consumers to notice and read the contents upon first entering the area occupied by the provider's customer representatives or recruiters.

Providers should instruct their staff to encourage prospective clients/patients to read all disclosures prior to enrolling in the program. Services actually provided should not be inconsistent with the content of these disclosures. (For example, a provider should not offer advice of a medical nature if the disclosures state that medical advice is not provided.) In addition, written or oral representations contained in advertising or any other materials or presentations should not be inconsistent with or contradict these disclosures.

C. Information Content

Providers of weight management services should, at a minimum, voluntarily provide to prospective patients/clients the following information:

1. Staff Qualifications and Central Components of the Program

This includes a description of the program content and goals and pertinent information about the weight management training, experience, certification and education of the customer service personnel where the service, including distribution of products, is being provided, and which is appropriate to the program. The disclosure should include wording that encourages prospective patients/clients to ask additional questions about the qualifications of the provider and should not be deceptive or misleading.

Example 1

Our Staff at the "Weight Away Diet Center" program consists of diet, exercise, and behavioral modification. Program leaders who have successfully used the Weight Away program to lose weight monitor your weekly progress. Staff is required to have completed Weight Away program as well as a six-week in-house staff training program covering nutrition, weight-loss dynamics, customer service, and presentation. One or more Weight Away counselors will see you on an individual or group basis at each meeting. Our staff does not offer medical or psychological counseling. Please feel free to ask for more details about any particular leader's training or experience.

Example 2

Our Staff at the "Community Hospital Obesity Clinic" provides a medically supervised weight loss program for patients with severe disorders related to obesity or whose obesity places them at risk of developing such disorders. The program consists of optional low or very low-calorie diet plans, exercise, and lifestyle education. During this program, patients following a very-low-calorie diet will receive a protein supplement diet formula to substitute for regular meals and a multi-vitamin supplement. Our staff is comprised of one physician who is board-certified in endocrinology, two registered nurses (RNs), three registered dietitians (RDs), one masters level exercise physiologist and one clinical psychologist (Ph.D.) Usually, patients will visit with the dietitians and exercise physiologist. Other professional staff are available for consultation if professional intervention is indicated. Prospective patients are encouraged to ask about staff experience and training and how much time various staff members spend with individual patients.

2. Risks Associated with Overweight and Obesity, and the Benefits to be Derived from Modest Weight Loss

For example, obesity and overweight are associated with increased risk of heart disease, diabetes, some forms of cancer, gall bladder disease, osteoarthritis, stroke, and sleep apnea, among other illnesses, and moderate amounts of weight loss (five to ten percent of total body weight) can reduce many of the risks. While most providers will likely want to include information about the risks of obesity and the benefits of weight loss, any such disclosure is optional, and the failure to provide such a statement will not be considered inconsistent with the guidelines.

3. Risks Associated with the Provider's Product or Program

This includes for programs, the risks associated with any drugs, devices, dietary supplements, or exercise plans that are provided in the course of the program or treatment. In addition to program/product-specific risks, the information provided should indicate that:

1. Consultation with a medical professional is advisable for people who are under treatment for specific medical conditions or taking prescribed medications.
2. Unless medically indicated, weight loss after the first two or three weeks of dieting should not exceed a rate of three pounds or approximately one and one-half percent of body weight per week. More rapid weight loss may cause an increased risk of developing gallbladder disease, a risk which is believed to be higher than the risk of developing gallbladder disease as a result of staying overweight/obese. People who are considered medically appropriate for more rapid weight loss should have their progress monitored by a physician.
3. Very-low-calorie diets (<800 kcal per day) are designed to promote rapid weight loss in people whose obesity has resulted in, or has put them at medical risk of, developing serious health complications. Rapid weight loss may also be associated with some medical problems. This program provides medical supervision to minimize risks associated with rapid weight loss.
4. People undergoing weight loss can experience physical changes in the body (dizziness, interruptions in the menstrual cycle, hair loss, for example) that may indicate more serious conditions. People noticing such changes should be advised to talk immediately to their primary care physician.

Example 3

For providers whose programs are designed to produce weight loss at a rate of approximately two pounds per week.

What You Need To Know About the Safety of the "Healthy Weight Loss Clinic" Diet.

This diet has been designed to promote weight loss of no more than two pounds -or one percent of total body weight — a week. Medical authorities recommend that losing weight at such a rate reduces risk of health problems that have been associated with more rapid weight loss (greater than three pounds per week). Some people may lose weight at a slightly higher rate. However, we will monitor your progress and modify your diet if your rate of weight loss after the first two or three weeks exceeds a rate of three pounds — or one and one-half percent of body weight — a week. Children and adolescents, pregnant or breast feeding women, and people with significant health problems such as bulimia, heart disease, kidney disease, diabetes or psychiatric disorder, should not begin this program without written authorization by their primary care provider. People under treatment for other conditions or taking medications prescribed by their health care provider should tell their providers that they have begun this diet because, in some cases, adjustments to medications or modifications to the weight loss program may be appropriate.

Weight loss can produce physical changes in the body such as interruptions in the menstrual cycle, temporary hair loss, and dizziness. Such changes may indicate more serious health complications. Report any such changes that you notice to your primary care provider.

Remember, people who are overweight or obese are at increased risk of developing heart disease, diabetes, some forms of cancer, gall bladder disease, osteoarthritis and sleep apnea. Losing even small amounts of weight (five to ten percent of body weight), may reduce these risks. The side effects and complications that some people may experience while losing weight by following a healthy eating plan and exercise program are usually minor compared to the risks of overweight and obesity.

4. Information about Program Costs

This includes (1) total program costs, including all fixed costs (administrative fees, entry fees, renewal fees, as appropriate), (2) periodic costs such as weekly attendance fees or mandatory food purchases (expressed for food purchases at the option of the provider as either average approximate costs or a high/low range of costs per scheduled payment unit or per week), (3) optional costs (such as fees charged for re-entering the program or for any optional maintenance program), and (4) discretionary costs (medical tests, for example). Providers should also identify, clearly and prominently, any non-refundable costs. If practicable, providers should disclose total approximate program costs averaged across all dieters. A single, non refundable entry fee or registration fee that does not exceed 150 percent of the fee charged for a single visit or session in an otherwise pay-as-you-go program does not remove the program from pay-as-you-go status.

In the case of telephone sales, inasmuch as providers cannot give written disclosures to prospective patients/clients until they visit a center, sales should not be considered final until the prospective patient/client has received the disclosures and had a chance to read them. Alternatively, providers may include with the disclosures, a clear and conspicuous notice of their right to rescind such a sale within at least five days.

Example 4

"BYE-BYE BMI Weight Loss Centers" Cost Schedule:
Mandatory Charges:
One-Time Entry Fee $xx.xx
Each Weekly Meeting (attended) $xx.xx
Average (approximate) cost of food per week $xx.xx
Nutritional Supplements (30 day supply) $xx.xx
Discretionary Additional Charges (if Center deems necessary)
Blood tests, Physician Exam, EKG $xx.xx
Optional Additional Charges (if client chooses) $xx.xx
Re-entry fee (after absence of at least __ consecutive visits) $xx.xx
Optional Maintenance Program $xx.xx
ALL COSTS NON-REFUNDABLE

5. Outcome Information

Consumers of weight loss products and services are entitled to receive outcome information that would allow people to make informed choices among weight loss products and services.

These guidelines encourage but do not mandate outcome disclosures in terms of weight loss and weight maintenance. Weight loss programs differ, and at the current time, there is no consensus on what the content and the format of such disclosures should be. Research is lacking as to what consumers need and how they interpret or use such information. The Partnership for Healthy Weight Providers are encouraged to collect data, e.g., how much weight consumers of a particular product or program have lost and how long they kept off all or part of their weight loss, and disclose weight loss and maintenance information to prospective clients/patients before they enroll. Providers subscribing to these guidelines should include within the document containing the other disclosures the statement, "Most people who lose weight are likely to find it difficult to keep the weight off. They can improve their chances by adopting a lifelong commitment that includes:

1. Increased frequent and regular physical activity of at least moderate intensity, and
2. Healthy eating in accordance with the Dietary Guidelines for Americans, emphasizing a reduction in total calories, a lowered fat consumption, and an increase in vegetables, fruits and whole grains," and
3. Information about the health benefits of modest amounts of weight loss (5–10 percent of body weight, 10–20 pounds).

Example 5

For providers that make specific disclosures.

Patients under Dr. Doe's weight loss treatment lost, on average, 17.5 pounds, and after 18 months, they kept off 55 percent of their weight loss. This measurement includes all patients who remained in active weight loss for at least three weeks. Most people who lose weight are likely to find it difficult to keep the weight off. They can improve their chances by adopting a lifelong commitment that includes:

1. Increased frequent and regular physical activity of at least moderate intensity, and
2. Healthy eating in accordance with the Dietary Guidelines for Americans, emphasizing a reduction in total calories, a lowered fat consumption, and an increase in vegetables, fruits and whole grains.
3. Moreover, maintaining a modest amount of weight loss over time has been shown to have health benefits.

Example 6

For providers that choose not to make specific disclosures.

Most people who lose weight are likely to find it difficult to keep the weight off. They can improve their chances by adopting a lifelong commitment that includes:

1. Increased frequent and regular physical activity of at least moderate intensity, and
2. Healthy eating in accordance with the Dietary Guidelines for Americans, emphasizing a reduction in total calories, a lowered fat consumption, and an increase in vegetables, fruits and whole grains.
3. Moreover, even weight loss of as little as ten percent of body weight, if maintained over time, has been shown to be beneficial.

VIII. REPRESENTATIVE DIETARY SUPPLEMENT ADVERTISING CASES 1984 — JULY 15, 2003

Michael S. Levey; Gary Ballen; Bentley Myers International Co.; Publisher's Data Services, Inc.; and Nutritional Life, Inc.; Civ. Action No. CV-03-4670 GAF (AJWx) (C.D. Cal.) (Filed 6/30/03) Complaint for Civil Penalties, Injunctive and Other Relief. Alleged false or unsubstantiated efficacy and safety claims for weight loss products containing ephedra, "Zymax" and "MillinexES," or St. John's wort, "Serotril," and unsubstantiated arthritis cure and other claims for a dietary supplement product containing glusosamine and chondroitin, "CartazyneDS." Alleged violation by Michael S. Levey of 1993 FTC order in C-3459.

Health Laboratories of North America, et al., Civ. Action No. 03 1457, (D.D.C.) (Filed 07/01/03) Complaint and Stipulated Final Judgment and Order for a Permanent Injunction and Monetary Relief. Alleged false and unsubstantiated weight loss claims for "Berry Trim Plus" dietary supplement products containing Hydroxycitric Acid or "HCA" and ephedrine alkaloids from Ma Huang and unsubstantiated safety claims for "Berry Trim Plus." Order requires payment of $195,000 in consumer redress.

Seasilver USA, Inc.; Americaloe, Inc.; Bela Berkes; Jason Berkes; Brett Rademacher, individually and dba

Netmark International and Netmark Pro; and David R. Friedman, Civ. Action No. CV-S-03-0676-RLH-LRL, Complaint for Injunctive and Other Equitable Relief (filed June 12, 2003) (D. Nev.) Alleged false and unsubstantiated disease treatment and cure claims and weight loss claims for "Seasilver" liquid supplement. Inventories of Seasilver seized by the Food and Drug Administration.

Unither Pharma, Inc., and United Therapeutics Corp., File No. 022 3036, (June 12, 2003) (Consent Agreement subject to final approval of the Commission following public comment period) Alleged deceptive claims that "HeartBar," a food bar containing the amino acid L-Arginine, is effective against cardiovascular diseases.

Kevin Trudeau; Robert Barefoot, Shop America (USA), LLC, and Deonna Enterprises, Inc. Civ. Action No. 03 C 904 (June 9, 2003) (N.D. Ill.) (Complaint for Permanent Injunction and Other Equitable Relief) Alleged false and unsubstantiated claims that Coral Calcium Supreme can treat or cure cancer and other diseases, such as multiple sclerosis and heart disease, and is superior to other calcium supplements.

A. Glenn Braswell; JOL Management Co.; G.B. Data Systems, Inc., Gero Vita International, Inc., Theraceuticals, Inc.; and Ron Tepper; Civ. Action No. CV 03-3700 DT (PJWx) (C.D. Cal.) (filed May 27, 2003) (Complaint for Permanent Injunction and Other Equitable Relief) Alleged false and unsubstantiated advertising claims for numerous dietary supplements marketed under the Gero Vita and Theraceuticals brand names.

ValueVision International, Inc., Civ. Action No. 03-2890 District of Minnesota (entered April 17, 2003) (Consent Decree and $215,000 Civil Penalty) (Agreement settles charges that company violated a previous 2001 FTC order [C-4022] by making unsubstantiated health claims for Physician's RX, a dietary supplement containing a variety of vitamins, minerals, and antioxidants. In addition to paying the civil penalty, ValueVision will be enjoined from violating the FTC order.)

Snore Formula, Inc., et al., File No. 022 3247 (April 15, 2003) (Consent agreement pending final approval following public comment period) Alleged unsubstantiated claims that "Dr. Harris' Original Snore Formula" herbal supplement tablets were effective in preventing sleep apnea in adults and children and significantly reduced snoring. Alleged failure to disclose material information, specifically, the importance of seeing a physician for people with symptoms of sleep apnea because the condition may be fatal.

Rexall Sundown, Inc., Civ. Action No. 00-7016-CIV-Martinez, (S.D. Fla.) Stipulated Final Order for Permanent Injunction and Settlement of Claims for Monetary Relief (March 31, 2003) Alleged false and unsubstantiated claims that its dietary supplement, Cellasene™, would reduce or eliminate cellulite.

Slim Down Solution, LLC, et al., and Maderia Management, Inc., et al., Civ. Action No. 03-80051-CIV-PAINE (S.D. Fla.), Complaint for Permanent Injunction and Other Equitable Relief and Stipulated Order for Preliminary Injunction (01/21/03) Alleged false and unsubstantiated claims for a purported weight-loss product containing D-glucosamine.

Dr. Clark Research Association, et al., *dba Dr. Clark Zentrum*, Civ. Action No. l:03CV0054, (N.D.Ohio) Complaint for Permanent Injunction and Other Equitable Relief (filed 01/08/03) Alleged unsubstantiated AIDS, terminal cancer, and other disease cure claims for dietary supplements and devices.

Mark Nutritionals, Inc., Harry Siskind, and Edward G. D'Alessandro, Jr., Civ. Action No. SA02CA1151EP, U.S. District Court, Western District of Texas, San Antonio Division, (Complaint for Permanent Injunction and Other Equitable Relief and Stipulated Order for Preliminary Injunction Between Mark Nutritionals, Inc., and FTC filed December 5, 2002) (Stipulated Order for Preliminary Injunction with asset freezes entered against individual respondents on December 23, 2002) Alleged false and unsubstantiated claims for "Body Solutions Evening Weight Loss Formula" in English and Spanish radio ads and website.

David L. Walker, dba DLW Consulting, Inc., File No. X020041, Civ. Action No. C02-5169 RJB, (filed October 28, 2002) U.S. District Court for the Western District of Washington at Seattle, Stipulated Final Judgment and Order, (Complaint alleging unsubstantiated claims on web site and in seminars regarding the efficacy of Walker's purported cancer cure, the "CWAT — Treatment: BioResonance Therapy and Molecular Enhancer." was filed in March, 2002.)

No. 9068-8425 Quebec, Inc. aba Bio Lab, Cellu-Fight, and Quick Slim, and Jean-Francois Brochu, Civ. Action No. 1:02:CV-1128, U.S. District Court, Northern District of NY (September 3, 2002) (Complaint for Permanent Injunction and Other Equitable Relief) Alleged false weight-loss and cellulite-treatment claims by Canadian company marketing dietary supplements to U.S. consumers using mainstream U.S. media.

Interstate Bakeries Corp., File No. 012 3182 (March 6, 2002) Consent Agreement subject to final FTC approval following public comment period. Alleged unsubstantiated claims that its Wonder Bread containing added calcium could improve children's brain function and memory.

Campbell Mithun LLC (Advertising agency for Interstate Bakeries Corp. [IBC]), File No. 012 3182 (March 6, 2002) Consent Agreement subject to final FTC approval following public comment period. Alleged unsubstantiated claims in advertisements developed for IBC that Wonder Bread containing added calcium could improve children's brain function and memory.

Kris A. Pletschke, dba Raw Health, File No. C-4040, (February 22, 2002) Consent Order. (Alleged unsubstantiated claims on "rawhealth.net" web site that its colloidal

silver product could treat or cure 650 different diseases, eliminate all pathogens in the body, and was medically proven to kill every destructive bacterial, viral, or fungal organism in the body, including anthrax, Ebola, and flesheating bacteria. Consumer refunds must be offered under terms of the order.)

TechnoBrands, Inc., et al., File No. 992 3034 (February 19, 2002) Consent Agreement subject to final FTC approval following public comment period. Alleged false and unsubstantiated claims concerning weight-loss products, pain-relief magnets, air cleaners, and hairgrowth stimulants. ($200,000 in consumer redress required by the Consent Agreement) In a separate action, filed in federal district court, TechnoBrands agreed to pay more than $200,000 in consumer redress for its role in the deceptive telemarketing of a buyer's club service after completing the sale of its own products. (Stipulated Final Order for Permanent Injunction and Settlement of Claims for Monetary Relief, Civ. Action No.: 3:02-CV-86, Eastern District of Virginia, Richmond.)

Natural Organics, Inc., et al., D. 9294 (September 6, 2001) (Consent Order) (Alleged unsubstantiated claims that company's dietary supplement, "Pedi-Active A.D.D.," treats or mitigates Attention Deficit Hyperactivity Disorder and improves attention span and scholastic performance of children who have difficulty focusing on their schoolwork.)

Liverite Products, Inc., et al., Civil Action No.: SA 01-778 AHS (ANx) (August 20, 2001) (Complaint and proposed Stipulated Final Order filed in U.S. District Court for the Central District of California, Southern Division) (Alleged unsubstantiated claims on Internet, radio, and print ads about the ability of "Liverite" dietary supplement products to treat or prevent a wide range of liver diseases or disorders, including cirrhosis and hepatitis ($60,000 in redress required).

Panda Herbal International, Inc., et al., File No. C-4018 (Consent Order) (July 30, 2001) (Alleged unsubstantiated efficacy claims for "Herbal Outlook," a dietary supplement containing St. John's Wort, and for "HerbVeil 8," a topical ointment; alleged false claim that Herbal Outlook has no known contraindications or drug interactions. Order requires Panda to pay full refunds upon request to consumers who purchased HerbalVeil 8 during a specified time period.)

ForMor, Inc., et al., File No. C-4021 (Consent Order) (July 30, 2001) (Alleged false and unsubstantiated safety and efficacy claims for dietary supplement products containing St. John's Wort, colloidal silver, and shark cartilage. Order requires ForMor to pay refunds upon request to consumers who purchased colloidal silver and "Ultimate II Shark Cartilage Concentrate" during a specified time period.)

MaxCell BioScience, Inc., et al., doing business as Oasis Wellness Network, File No. C-4017 (Consent Order) (July 30, 2001) (Alleged false and unsubstantiated anti-aging claims for "Longevity Signal Formula," a dietary supplement product containing the hormone DHEA, and for an at-home urine test, "Anabolic/Catabolic Index™²," to gauge overall health and youthfulness. Order requires payment of $150,000 in consumer redress.)

Robert C. and Lisa M. Spencer, dba Aaron Company, File No. C-4019 (Consent Order) (July 30, 2001) (Alleged false and unsubstantiated safety and efficacy claims for dietary supplement products, Colloidal Silver, and Chitosan with Vitamin C; and unsubstantiated claims that "Ultimate Energizer," a product containing ephedra (ma huang), is safe and has no side effects. Order requires warning labels on products containing ephedra sold by respondents in the future).

Western Botanicals, Inc., et al., Civ. Action No. CIV.S-01-1332 DFL GGH, (July 13, 2001) (Complaint and Stipulated Final Order) (Alleged unsubstantiated claims that dietary supplement products containing comfrey were beneficial in the treatment of a variety of serious diseases and health conditions and false claims that the products were safe. A judgment of $50,800 was suspended, but could be reinstated if the court finds defendants made misrepresentations or omissions on their financial statements.)

Christopher Enterprises, Inc., et al., Civ. Action No. 2:01 CV-0505 ST, District of Utah, Central Division (Stipulated Final Order for Permanent Injunction and Settlement of Claims for Monetary Relief filed November 29, 2001) The order would prohibit the defendants from marketing any comfrey product for ingestion, for use as a suppository, or for external use on open wounds, unless they have evidence that the product is free of pyrrolizidine alkaloids and that it is safe. The defendants would also be required to place a warning disclosure in any ad, promotional material or product label for any confrey products intended for topical use. The specific claims challenged in the complaint would also be prohibited by the order. (Complaint for Permanent Injunction and Other Equitable Relief and Stipulated Order for Preliminary Injunction filed July 3, 2001) (Alleged false safety claims and unsubstantiated efficacy claims for products containing the herbal ingredient comfrey.)

Streamline International, Inc., et al., Civil Action No. 01-6885-CIV-Ferguson (Complaint filed in U.S. District Court for the Southern District of Florida) (June 20, 2001) (Alleged false and deceptive earnings claims by operators of fraudulent business opportunity pyramid marketing scheme purportedly distributing a line of dietary supplements and health-care products. Complaint also alleges false and misleading claims that the company sells only dietary supplement products containing ingredients that appear on the FDA's list of ingredients generally recognized as safe. In fact, a number of products contain the herbal ingredient comfrey, which is not on that list and is known to pose a significant risk to humans, including liver damage, when used internally or externally on open wounds.

Western Dietary Products Co., et al., Civ. Action No. CO1-0818R, (U.S. District Court for the Western District of Washington, in Seattle) (Stipulated Final Judgment and Order filed with the court on December 26, 2001) (Complaint filed June 4, 2001, alleged unsubstantiated claims that their products, "Black Walnut Tincture," "Wormwood Tincture," and "Cloves Tincture," and an electrical unit called the "Zapper," were effective in the treatment and cure of cancer, Alzheimer's, diabetes, arthritis, and HIV/AIDS; and that their herbal products would make surgery and chemotherapy unnecessary for persons with cancer.)

Weider Nutrition International, Inc. (File No. C-3983) (Consent Order) (November 15, 2000) (Alleged unsubstantiated efficacy and safety claims in ads for weight loss dietary supplement products called "PhenCal" and "PhenCal 106." $400,000 in consumer redress required by the consent order.)

Lane Labs-USA, et al., (Civ. Action No. CV-00-3174 (WGB), U.S. District Court, District of New Jersey) (Stipulated Final Order for Permanent Injunction as to Cartilage Consultants, Inc., and I. William Lane entered July 6, 2000) (Stipulated Final Order for Permanent Injunction and Settlement of Claims for Monetary Relief as to Lane Labs-USA, Inc., and Andrew J. Lane) (Entered by the court on September 26, 2000) ($1 Million judgement: $550,000 for redress or disgorgement; and the remaining $450,000 to be used to pay for shark cartilage and a placebo in a clinical study of shark cartilage sponsored by the National Cancer Institute and Lane Labs.) (Alleged unsubstantiated claims about the efficacy of "BeneFin," a shark cartilage product, and "SkinAnswer," a skin cream, in the prevention, treatment and cure of cancer. Lane Labs was the manufacturer and distributor of these products.)

Cartilage Consultants, Inc., et al., (Civ. Action No. CV-00-3174 (WGB), U.S. District Court, District of New Jersey) (Entered by the court on July 6, 2000) (Stipulated Final Order for Permanent Injunction) (Complaint alleged that working in conjunction with Lane Labs-USA, Cartilage Consultants, Inc., supplied consumers with information on how to use BeneFin and SkinAnswer to treat cancer)

Michael D. Miller, dba Natural Heritage Enterprises, File No. C-3941 (May 16, 2000) (Consent Order and $17,500 in consumer redress) (Alleged unsubstantiated claims on Internet sites that Essiac Tea is effective in curing a number of diseases, such as cancer, diabetes, AIDS/HIV and feline leukemia.)

CMO Distribution Centers of America, File No. C-3942 (May 16, 2000) (Consent order; company must offer refunds to consumers who purchased the product for personal use or that of their families.) (Alleged unsubstantiated claims that CMO™ (cetylmyristoleate) capsules would regulate and normalize the immune system, cure arthritis and reverse the effects of the disease, and be effective in treating other conditions such as asthma and cancer. Complaint also alleged that certain claims about scientific studies were false.)

EHP Products, File No. C-3940 (May 16, 2000) (Consent order; company must offer refunds to consumers who purchased the product for personal use or that of their families.) (Alleged unsubstantiated efficacy claims for its CMO product, Myristin®, including claims that the product provides long term relief from arthritis symptoms and may prevent rheumatoid arthritis and osteoarthritis, and misrepresentations that scientific studies or the issuance of patents prove the effectiveness of Myristin®.)

J & R Research, Inc., File No. C-3961 (July 19, 2000) (Consent Order) (Alleged unsubstantiated efficacy claims for the product Pycnogenol, marketed and sold by the company for the mitigation or cure of the effects of Attention Deficit Disorder (ADD) or Attention Deficit Hyperactivity Disorder (ADHD).

Rose Creek Health Products, Inc., File No. CS-99-0063-EFS, (May 4, 2000) (Consent decree issued in settlement of Complaint for Permanent Injunction and Other Equitable Relief filed March 1999 in U.S. District Court for the Eastern District of Washington; $375,000 in consumer redress.) (False and unsubstantiated health claims in advertisements for a nutritional supplement called "Vitamin O")

Enforma Natural Products, Inc., et al., Civ. Action No.: 04376JSL (CWx), U.S. District Court, Central District of California, (filed April 25, 2000) Complaint and Stipulated Final Order filed with the court; $10 million in consumer redress ordered. (False and unsubstantiated weight loss claims alleged for "Fat Trapper" and "Exercise in a Bottle" containing the dietary supplements chitosan and pyruvate, respectively)

The Quigley Corp., (File No. C-3926) (February 10, 2000) (Consent order) (Allegedly unsubstantiated claims by Quigley that Cold-Eezer and Cold-Eeze brand zinc lozenges that it manufactures can prevent colds and alleviate allergy symptoms and that Kids-Eeze Bubble-Gum can reduce the severity of cold symptoms in children.)

AST Nutritional Concepts and Research, Inc., *et al*., (Civ. No. 99-WY-2197, U.S. District Court for the District of Colorado) (May 4, 2000) (Permanent Injunction) (Alleged unsubstantiated safety claims made for purported body-building supplements that contain androstenedione, "androgen," and other steroid hormones, and in some cases, stimulants.

Met-RX USA, Inc., et al., (Civ. No. SACV99-1407 DOC (ANX), U.S. District Court for the Central District of California) (Nov. 24, 1999) (Stipulated Final Order For Permanent Injunction and Other Equitable Relief) Unsubstantiated safety claims made for purported body-building supplements that contain androstenedione, "androgen," and other steriod hormones, and in some cases, stimulants.

Arthritis Pain Care Center (APCC), et al., File No. C-3896 (September 7, 1999) (Consent order) (unsubstantiated health benefit claims regarding dietary supplements and false claims regarding scientific studies)

SlimAmerica, Inc., FTC v., No. 97-6072-Civ-Ferguson (S.D. Fla. June 30,1999) (Final Judgment for Permanent Injunction and Damages; $8,374,586 in redress) (Deceptive claims that "Super-Formula" diet product can "blast" up to 49 pounds off user in only 29 days, "obliterate" 5 inches from waistlines, and "zap" 3 inches from thighs, without dieting or exercising)

Bogdana Corp., File No. C-3820 (July 28, 1998) (consent order) (Cholestaway calcium carbonate supplement to lower cholesterol, blood pressure, cause weight loss, treat heart disease;

Western Direct Marketing Group, Inc., C-3821 (July 28, 1998) (consent order) (ad agency for Bogdana Corp.)

Herbal Worldwide Holdings, Inc., File No. C-3827 (September 16, 1998) (consent order) (unsubstantiated weight loss claims for "Fattache," a purported dietary product, in advertisements run on Spanish-language television stations)

TrendMark International, Inc., File No. C-3829 (September 23, 1998) (consent order) (unsubstantiated claims for "THIN-THIN" diet program, consisting of Neuro-Thin weight loss supplement made primarily of amino acids and Lipo-Thin weight loss product made of "chitin," a natural fiber made from the ground-up hydrolyzed exoskeletons of shellfish)

Venegas Inc., C-3781 (Jan. 23, 1998) (consent) (health claims for dietary supplement Alen, including delaying aging process, eliminating anemia, controlling addiction to excess fats and sweets, and helping diabetics to naturally produce insulin – Spanish language)

Mega Systems International, Inc., File No. C-3811 (June 8, 1998) (consent order and $500,000 in consumer redress) (Eden's Secret Nature's Purifying Product to clean and purify body's blood supply and to cure PMS and other illnesses, and to cause significant weight loss, advertised in infomercials) AND Civ. Action No. 98C 8009 (Dec. 15, 1998) (Complaint for Injunctive and Other Relief filed in U.S. District Court for failure to pay $500,000 in consumer redress) (Jan. 4, 2002) (Commission authorized filing of a stipulated judgment in a bankruptcy court action to resolve the Complaint for Injunctive and Other Relief. Under terms of the settlement, the FTC will receive $30,000 now and share in any distribution of assets at the conclusion of the bankruptcy case.

Metagenics, Inc., D. 9267 (Oct. 23, 1997) (consent) (OTC calcium supplement to restore lost bone, eliminate pain)

Global World Media Corp., C-3772 (Oct. 9, 1997) (consent) (explicit safety and no side effects claims for Ecstacy supplement advertised to produce a natural "high," in media with large youth audiences, and without disclosing health and safety risks)

Kave Elahie/M.E.K. International, C-3770 (Sept. 19, 1997) (consent) (NutraTrim Bio-Active Cellulite Control Cream and Weight Loss Tablets with aminophylline — Spanish language)

Rogerio Monteiro and Eliana Crema (Leeka Products), C-3767 (Sept. 12, 1997) (consent) (Super Formula Reductora supplement with chromium picolinate for weight control by regulating metabolism, reducing appetite, burning or dissolving fat — Spanish language)

Efficient Labs, Inc., C-3768 (Sept. 12, 1997) (consent) (Venoflash dietary supplement with vitamins and plant derivatives to remove dangerous clogs in circulatory system and treat symptoms of varicose veins and hemorrhoids — Spanish language)

Nu Skin International, Inc., United States v., No. 2:97-CV-0626G (D. Utah Aug. 6, 1997) (stipulated permanent injunction and $1.5 million civil penalty for violation of 1994 order) (fat loss, muscle maintenance, and other claims for dietary supplements, Metabotrim, OverDrive, GlycoBar, Appeal Lite, and Breakbar, containing chromium picolinate and L-carnitine)

Nutrition 21, C-3758 (Jul. 11, 1997) (consent) (weight loss and health benefit claims for chromium picolinate)

Mountain Springs L.L.C., FTC v., 97-4649 SVW (C.D.Cal. Jun. 25, 1997) (stipulated permanent injunction) (Manaxx Una de Gato (cat's claw) for treatment or prevention of various diseases by strengthening the immune system; effectively treats inflammation; reduces the abnormal irritation of body tissue; cures acne; and is void of any toxic effects — Spanish language)

Amerfit, Inc., C-3747 (Jun. 16, 1997) (consent and $100,000 in disgorgement) (Fat Burners System for weight loss, with tablet and, drink)

Interactive Medical Technologies, Ltd./Effective Health, Inc., C-3751; William Pelzer, Jr., C-3750; and William E. Shell, M.D., C-3749 (Jun. 16, 1997) (consents and $55,000 in consumer redress) (Lipotrol cellulose-bile weight loss product and SeQuester)

Dean Distributors, Inc., C-3755 (Jun. 16, 1997) (consent) (Food for Life Weight Management System and Cambridge Diet low calorie and very low calorie weight loss programs, with formula drinks)

Abbott Laboratories, C-3745 (May 30, 1997) (consent) (many doctors recommend Ensure nutritional beverages as a meal supplement and replacement for healthy adults)

Universal Merchants, Inc., C-3707 (Jan. 23, 1997) (consent) (weight loss and health benefit claims for chromium picolinate)

Zygon International, Inc., C-3686 (Sept. 24,1996) (consent and up to $195,000 in consumer redress for all products) (SuperBrain Nutrient Program to improve intelligence and memory, and to enhance the intelligence

of the children of pregnant women; Fat Burner pills for weight loss; and Day and Night Eyes pills to improve day vision and night blindness, among others)

Home Shopping Network, Inc., D. 9272 (Sept. 26, 1996) (consent) (health-related claims for three vitamin sprays, including prevention of the common cold and reduction of the risk of infectious diseases; and efficacy claims for a stop-smoking spray, including elimination of the anxiety and weight gain associated with quitting smoking — advertised on Home Shopping Network)

Third Option Laboratories, Inc., C-3628 (Nov. 29, 1995) (consent and $480,000 in consumer redress) (Jogging in a Jug apple juice, grape juice, and vinegar beverage for heart disease, arthritis, dysentery, constipation, lowering serum cholesterol and triglycerides, cancer, leukemia, viral disease, blood sugar levels, and other ailments)

National Dietary Research, Inc., D. 9263 (Nov. 7, 1995) (consent and $100,000 in disgorgement) (Food Source 1 weight loss product and Vancol 5000 cholesterol reducing product)

Live-Lee Productions, Inc./Ruta Lee, C-3620 (Oct. 10, 1995) (consent) (health related claims made by television personality Ruta Lee on Home Shopping Network for three vitamin sprays and a stop-smoking spray) (See Home Shopping Network)

Body Wise International, Inc., C-3617 (Sept. 25, 1995) (consent) (dietary supplements for weight loss and cholesterol reduction; failure to disclose that healthcare professionals and others who gave testimonials for the products had a financial interest in promoting them)

Nature's Bounty, Inc., C-3593 (Jul. 21, 1995) (consent and $250,000 in consumer redress) (health related claims for 26 nutrient supplements, including Sleeper's Diet for weight loss during sleep; L-Arginine and L-Ornithine to increase muscle mass while decreasing body fat; L-Cysteine to promote hair growth; Octacosanol to improve reaction time, reduce cholesterol levels, and strengthen muscles; New Zealand Green Lipped Mussel Extract to prevent arthritis and relieve its symptoms; Eye-Vites to reduce the risk of developing cataracts; and Ginsana to improve physical endurance and mental alertness)

Taleigh Corp. (*Choice Diet Products, Inc.*), 119 F.T.C. 835 (1995) (consent agreement, with the requirement that the owner post a $300,000 performance bond to be used for consumer redress should he engage in deceptive practices when marketing weight loss or stop-smoking products in the future) (FormulaTrim 3000, MegaLoss 1000, and MiracleTrim pills for weight loss without diet or exercise, and without nervous jitters, insomnia, or any other side effects; deceptive consumer endorsements and failure to disclose that certain consumer endorsers had been compensated)

Bee-Sweet, Inc., 119 F.T.C. 57 (1995) (consent) (bee pollen products for anemia, back pain, allergies, arthritis, fatigue, and other ailments)

RN Nutrition, 119 F.T.C. 25 (1995) (consent) (BoneRestore calcium product to build bone better than estrogen, slow or stop bone loss, prevent and heal osteoporosis, and restore lost bone)

Schering Corp., 118 F.T.C. 1030 (1994) (consent) (weight loss claims for Fibre Trim supplement, as well as claims that the product provides the health benefits associated with a fiber-rich diet)

Redhead, FTC v., No. 93-1232-JO (D. Ore. Sept. 9, 1994) (stipulated permanent injunction against defendants Demlow, Danek, Lifeline, Inc.) (Imuno-Plex algae-based supplement to cure or treat HIV disease, AIDS, and AIDS related complex)

General Nutrition, Inc., United States v., No. 94-686 (W.D. Pa. Apr. 28, 1994) (permanent injunction and $2.4 million civil penalty for violation of 1970 and 1989 orders) (Protabalase ME, Cybergenics Phase I, Hot Stuff, and others for faster muscle development, improvement of endurance, strength, and stamina; New Zealand Green Lipped Mussel Extract to cure, treat, prevent, or reduce the risk of developing any disease; Quick Shot Energy Pak and other vitamins to prevent, relieve, or treat listlessness; Fat Burners and other weight control and appetite suppressant products)

Nature's Cleanser, Inc., 116 F.T.C. 718 (1993) (consent) (Lady's Comfort herbal remedy to relieve menstrual pain, discomfort in menopause; and Nature's Cleanser herbal remedy for weight loss and weight control)

Nu-Day Enterprises, Inc., 115 F.T.C. 479 (1992) (consent and $30,000 in consumer redress) (Nu-Day diet program with Nu-Day Meal Replacement Formula, and Nu-Day Herbulk, a dietary fiber supplement, for weight loss without exercising; failure to disclose that the infomercial was a commercial, not an independent consumer news program)

Amerdream Corp., FTC v., No. 91-0505 PHX RCB (D. Ariz. Nov. 5, 1991) (permanent injunctions and $50,000 in consumer redress) (Ultimate Solution Diet Program and Night-Trim Diet Tablets for significant weight loss and for reducing cholesterol and blood pressure levels)

International White Cross, Inc., FTC v., No. C-91-0377-TEH (N.D. Cal. Oct. 21, 1991) (stipulated permanent injunction) (Immune Plus nutritional supplement to cure patients with AIDS or AIDS-related complex or help them go into remission, eliminate or reduce AIDS or ARC symptoms, and cause an individual who had tested HIV-positive to become HIV-negative)

Miles, Inc., 114 F.T.C. 31 (1991) (consent) (One-A-Day multiple vitamins to renew essential minerals lost due to strenuous exercise and stress; and to protect lungs against adverse effects of air pollution)

Allied International Corp. (Fat Magnet), FTC v., No. 90-0120 CBM (Kx) (C.D. Cal. Nov. 14, 1990) (stipulated permanent injunction) (Fat Magnet diet pills for weight loss without dieting or exercise)

American Life Nutrition, Inc., 113 F.T.C. 906 (1990) (consent) (bee pollen, fish oil, royal jelly, vitamin, oyster

shell, and calcium food supplements to prevent or treat diseases such as breast cancer, heart disease, diabetes, rubella, arthritis, colds, tuberculosis, asthma, kidney disease, high blood pressure, cerebral apoplexy, low sex drive, among others)

TV Inc., 113 F.T.C. 677 (1990) (consent) (bee-pollen products to prevent, alleviate, or cure allergy symptoms, the aging process, impotence and sexual dysfunction; to promote weight loss; and to relieve pain; failure to disclose that the infomercial was a commercial, not an independent and objective television program)

Nature's Way Products, Inc., 113 F.T.C. 293 (1990) (consent agreement and $30,000 to National Institutes of Health for research in candidiasis) (Cantrol capsules to control yeast infections; Cantrol's diagnostic yeast test can demonstrate that a person is likely to have a yeast infection)

General Nutrition, Inc., 111 F.T.C. 387 (1989) (consent agreement and $600,000 in nutrition, obesity, and physical fitness research) (dietary supplement for reducing the risk of some forms of cancer; six food supplements, including L-Arginine and L-Ornithine, to promote weight loss and muscle growth or retard aging)

Kingsbridge Media & Marketing, Inc., FTC v., No. CIV-88-0003 PHX EHC (D. Ariz. Jun. 8, 1988) (stipulated permanent injunction and $1.1 million in consumer redress) (Dream Away weight reduction pills for weight loss while sleeping and without dieting or exercising)

Great Earth International, Inc., 110 F.T.C. 188 (1988) (consent) (Tri-Amino Plus P.M., L-Ornithine, and L-Arginine, for weight reduction while sleeping, muscle building, protection against mental and physical fatigue, promotion of healing, and strengthening of the immune system)

Viobin Corp., 108 F.T.C. 385 (1986) (consent) (wheat germ oil to improve physical fitness or performance)

Intra-Medic Formulations, Inc., FTC v., No. 85-2819-Civ-Nesbitt (S.D. Fla. Feb. 26, 1986) (permanent injunction) (weight control and baldness-cure products)

Weider Health and Fitness, Inc., 106 F.T.C. 584 (1985) (consent and $400,000 in consumer redress) (Anabolic Mega-Pak and Dynamic Life Essence nutritional supplements for rapid and substantial muscular development and strength)

P. Leiner Nutritional Products Corp., 105 F.T.C. 291 (1985) (consent) (Octacol 4 wheat germ oil capsules to improve endurance, stamina, vigor, and overall athletic performance or physical fitness)

PharmTech Research, Inc., FTC v., 576 F. Supp. 294 (D.D.C. 1983) (preliminary injunction), 103 F.T.C. 448 (1984) (consent) (Daily Greens dehydrated vegetable tablet for reducing the risk of some forms of cancer; claimed support from National Academy of Sciences' report on diet and cancer).

IX. CONCLUSION

Marketers of dietary supplements should be familiar with the requirements under both DSHEA and the FTC Act that labeling and advertising claims be truthful, not misleading and substantiated. The FTC approach generally requires that claims be backed by sound, scientific evidence, but also provides flexibility in the precise amount and type of support necessary. This flexibility allows advertisers to provide truthful information to consumers about the benefits of supplement products, and at the same time, preserves consumer confidence by curbing unsubstantiated, false, and misleading claims. To ensure compliance with FTC law, supplement advertisers should follow two important steps: 1) careful drafting of advertising claims with particular attention to how claims are qualified and what express and implied messages are actually conveyed to consumers, and 2) careful review of the support for a claim to make sure it is scientifically sound, adequate in the context of the surrounding body of evidence, and relevant to the specific product and claim advertised. The FTC works for the consumer to prevent fraudulent, deceptive and unfair business practices in the marketplace and to provide information to help consumers spot, stop, and avoid them.

101 Food Allergy: A Synopsis

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CONTENTS

I. INTRODUCTION

Adverse reactions to foods may be classified as those that are induced by biologically active microbial toxins (e.g., shiga toxin, vomitoxin, etc.) contained in the foods and those that are triggered by specific food constituents by nontoxic mechanisms [1–3]. Some of the nontoxic adverse reactions to foods are mediated by the immune system while others are not. Food induced adverse reactions mediated by the immune system are generally grouped as food induced hypersensitivity reactions.

Whereas the primary role of the immune system is host-defense, some of the activation pathways of the immune responses often results in significant host-damage. Such immune-mediated tissue damages were originally classified by Coombs and Gell as hypersensitivity reactions [4, 5]. The basis underlying this classification, including relevant examples, is summarized in Table 101.1. Accordingly, hypersensitivity reactions mediated by IgE antibodies are classified as classical allergic reactions (or immediate hypersensitivity reaction or Type-I hypersensitivity reaction). Airways allergies and most food allergies belong to this group. Antibodies of other isotypes (IgG, IgA) mediate Type-II or Type-III HS reactions, and contribution of these HS reactions to food induced adverse reactions is unclear. In contrast, those HS reactions that are mediated by specific immune cells are classified as Type-IV HS reactions (or delayed or cell-mediated hypersensitivity reactions). A classical example for the cell mediated adverse reaction to food is gluten enteropathy (or coeliac disease) [6].

Although IgE is associated with many food allergies, IgE independent mechanisms, many of which are unclear at present, appear to underlie other types of food allergies [7–9]. To complicate the matter further, some food allergies may involve mixed type of immune mechanisms involving both IgE antibodies and immune cells. One such condition often seen in infants and children is food-induced atopic dermatitis [7–9]. This disease often begins in infancy. Earliest lesions are erythematous, weepy patches on the cheeks with subsequent extension to other parts of the body including the face, neck, wrists, hands, and abdomen. Similar to food allergies, recent evidence also suggests that other atopic diseases (e.g., atopic rhinitis, dermatitis, and asthma) may also be classified as IgE dependent or independent [10]. There is controversial evidence that conditions such as irritable bowel syndrome and migraines may also be

TABLE 101.1
Coombs and Gell Classification of Hypersensitivity Reactions

	Type I	Type II	Type III	Type IV
Other names	Immediate hypersensitivity Classical allergic reaction Anaphylactic reaction	Cytotoxic reaction	Immune complex reaction Arthus reaction	Delayed hypersensitivity Contact "allergic" reaction
Major immune component involved	IgE antibody	IgG antibody	IgG, IgA antibody	Antigen specific T-lymphocyte
Antigen	Allergens (usually soluble proteins)	Transfused blood cells Altered host cells	Auto antigens or Soluble antigens	Soluble antigen or cell associated antigens
Major tissue damaging mechanism	Mast cell Basophil Eosinophil	Complement mediated cytolysis Phagocytosis	Immune complex deposition Phagocytosis	T-lymphocytes Monocytes and macrophages
Examples	Classical allergies Allergic asthma Most food and drug allergies	Transfusion reactions Some drug hypersensitivity	Lupus IgA nephropathy	Celiac disease (or gluten enteropathy), Nickel "allergy"

References: 4, 5, 9, 6.

TABLE 101.2
Immune System Dependent Food Induced Adverse Reactions

Type of Adverse Reaction	Onset of Symptoms in Sensitized Host When Exposed to Food	Implicated Mechanism	Examples
True food allergy including anaphylaxis (immediate hypersensitivity reaction)	Minutes (most reactions occur within 1 h; most reported severe reactions occur within 1–3 h)	IgE antibody mediated	Peanut allergy Chicken egg allergy Cow's milk allergy Food induced anaphylaxis
Delayed hypersensitivity reaction (non-IgE mediated reactions)	24–48 h	Cell mediated (e.g., T lymphocytes)	Gluten enteropathy (or Celiac disease) Food induced pulmonary hemosiderosis (or Heiner's syndrome) Contact dermatitis Dermatitis herpetiformis Dietery (or food) protein induced enterocolitis Dietery (or food) protein induced proctitis
Mixed hypersensitivity reactions	Variable	IgE plus cell mediated	Food induced atopic dermatitis Allergic eosinophilic gastritis Allergic eosinophilic gastroenteritis Allergic eosinophilic esophagitis
Uncharacterized/controversial food-induced reactions	Unknown	Unknown	Migraine headaches Irritable bowel syndrome

References: 3, 6–9, 11, 12, 19, 41.

related to food [11, 12]. The various types of immune system-dependent food induced adverse reactions with implicated mechanism and relevant examples are summarized in Table 101.2.

Food intolerance is a condition that is often mistaken for food allergy. As opposed to food allergy, food intolerance is not mediated by the immune system. For instance, lactose deficiency leads to milk intolerance [9, 13, 14]. Similar to lactose intolerance, alcohol intolerance is also a consequence of a precise metabolic defect rather than being immune system-mediated [15] (Table 101.3). Furthermore, there are allergy-like food induced adverse reactions that occur due to the formation of histamine or histamine-like substances in certain foods during storage [16]. For instance, histidine can undergo chemical conversion to histamine in presence of histidine decarboxylase produced by certain bacteria [17]. Consequently, consumption of food containing histamine and other pharmacologically active amines can result in allergy-like reactions [2, 3] (Table 101.3). Scombroid fish poisoning is a classic example of this category [18]. As will be discussed later, histamine is one of the major mediators of allergy symptoms. Therefore these conditions that are immune system-independent should be distinguished from actual food allergies as well.

TABLE 101.3
Immune System Independent Adverse Reactions to Foods

Type of Adverse Reaction	Mechanism	Examples
Allergy-like reactions	Preformed histamine in food causes allergy like symptoms (e.g., itching, swelling or hives, diarrhea etc.)	Fermented aged cheese, Scombroid fish poisoning
Food intolerance	Lactase deficiency Aldehyde dehydrogenase deficiency	Lactose (milk) intolerance Alcohol intolerance

References: 13–16, 18, 19.

II. PREVALENCE AND SIGNIFICANCE OF FOOD ALLERGY

Current prevalence of food allergies that are relatively common among many western countries has been estimated to be ~2% among adults and ~7% among children [19, 20]. As seen for several other allergic diseases, some recent reports suggest that incidence of some food allergies may be increasing, particularly among children [19, 21]. For instance, a recent study conducted in the Isle of Wight suggested that sensitization to peanut had increased between 1989 and 1994 to 1996 and that there was a strong but statistically nonsignificant trend for increase in reported peanut allergy [22]. Thus, more data is warranted to test this hypothesis and to examine whether this may be true for all types of food allergies in addition to that of peanut allergy.

Notably, there are an estimated 29,000 emergency room visits and 150–200 deaths per year in the U.S. due to food-induced systemic anaphylaxis — a generalized hypersensitivity response involving multiple organ systems including circulatory system, that can be fatal within minutes to hours after the onset of symptoms if untreated [19]. Because most of the victims are children, food allergy is a major public health problem facing day care centers, pre-schools, and schools [23–26]. Furthermore, food allergies also pose a serious challenge to food industries (i.e., food producers, retailers, and restaurants), as well as regulatory agencies (FDA, EPA) and service providers (such as the airline industry) [20, 27–30]. Thus, these incurable chronic diseases exert a significant negative impact on the economy as well as society at large.

III. ALLERGENIC FOODS vs. FOOD ALLERGENS

Any food can cause allergic reaction in a sensitized individual (i.e., someone who has produced IgE antibodies to the food in question). About an estimated over 100 different types of foods have been documented to trigger an allergic reaction in sensitized subjects [31]. According to the Food and Agriculture Organization (FAO) and the Food Allergy Task Force of the International Life Sciences Institute (ILSI), Europe, 90% of food allergies are caused by only eight food types: Chicken egg, cow's milk, peanut, soybean, wheat, tree-nuts (almond, hazelnut etc.), fish, and shellfish [8, 31–33]. Consequently, these food types have been regarded by the food regulatory agencies as "big eight" or "red flag" allergenic foods [27, 34]. These food allergies appear to be not restricted to any geographical region in particular but rather are widespread in the world [8, 31–33] (Table 101.4). In contrast, some other food allergies appear to be relatively common in certain countries. For instance, rice allergy — a rare form of allergy in the U.S., is relatively common in Japan, where rice is a staple food [35, 36] (Table 101.4). Allergy to sesame, often expressed as systemic anaphylaxis similar to peanut allergy, has been reported to be more common in Israel [37, 38]. A recent recommendation by the ILSI expert panel states that sesame meets the necessary requirements of a major food allergen and therefore may need to be included in this group of major allergenic foods [39]. It has been suggested that the geographical variation in the prevalence of food

TABLE 101.4
Commonly Allergenic Food Types and the Prevalence of Food Allergies

Food Type	Geographic Prevalence	Usually Outgrown
Red-Flag or Big Eight Food Types		
Cow's milk	Global*	Yes
Chicken egg	Global	Yes
Wheat	Global	Yes
Soy	Global	Yes
Peanut	Global	No**
Fish (salmon, cod, less often tuna)	Global	No
Shellfish (crustaceans: shrimps, crayfish, crabs, lobsters)	Global	No
Tree nut (almond, hazelnut/ filberts, walnut, pecans, pistachios, cashews, pine nuts, macadamia etc.)	Global	No
Other Allergenic Foods		
Celery	Mostly in Europe	UN
Buckwheat	Southeast Asia	UN
Rice	Japan	UN
Sesame	Israel	UN

* Global, meaning many countries but not necessarily all countries.
** Some peanut-allergic individuals outgrow peanut allergy over time (47, 67).
UN, Unknown.
References: 7–9, 19, 31, 34, 40–42, 45, 56.

allergies may very well be related to the exposure levels of an allergenic food in the local population [35, 36, 40–42].

A food that triggers an allergic reaction in a sensitized host is regarded as an allergenic food. Notably, not all constituents of an allergenic food are necessarily food allergens. Whereas most proteins in chicken egg are allergenic, only three proteins of peanut are known to be allergenic for most peanut allergic people [32, 33]. Classical allergens are typically proteins or glycoproteins that elicit production of specific IgE antibodies and physically and specifically bind to them. Although this definition of allergen was originally developed for aeroallergens, it has been widely used in the literature for food allergens as well [33].

Following are some of the salient features associated with many food allergens. Thus many food allergens are (i) proteins (usually glycoproteins); (ii) heat resistant (for instance, roasting does not abolish IgE binding ability of peanuts); (iii) acid stable (pH $\leqslant$2.0); (iv) resistant to pepsin digestion; (v) water soluble; and (vi) present in a given food at $\geqslant$1% level of total protein content [7, 32, 43]. Although these characters appear to associate with many food allergens, they do not absolutely predict the allergenic potential of a given protein *per se* [7, 8, 43].

IV. FOOD ALLERGY: CLASSIFICATION

Clinically food allergic reactions are expressed within minutes of exposure with a variety of symptoms. These include urticaria (skin rashes), hives (or raised rashes), angioedema, rhinoconjunctivits, vomiting, diarrhea, airway allergy symptoms (such as asthma, runny and itchy nose) or systemic anaphylaxis and shock. Accordingly, based on the target organ involvement, food allergies have been classified as (i) gastrointestinal food allergy; (ii) dermatological form of food allergy; (iii) respiratory form of food allergy; and (iv) food-induced systemic anaphylaxis involving multiple organ systems including the cardiovascular system [2, 9]. Some food allergies manifest themselves following exercise immediately after eating, presumably due to increased absorption and distribution of allergens via enhanced circulation [19, 44]. Notably, foods such as peanut, tree-nuts, fish, and shellfish are more often associated with systemic anaphylactic reactions than other food types [8, 19, 20].

Clinically food allergies are also expressed in two distinguishable forms: (i) transient food allergies (that are commonly outgrown); and (ii) persistent food allergies (that are rarely outgrown). Allergies to chicken egg, cow's milk, soy, and wheat typically start during childhood and are outgrown before adulthood [9, 24, 45, 46]. In contrast, peanut, tree nut, fish, and shellfish allergies although may start early in life but tend to remain during adult hood and are rarely outgrown [9, 24, 45, 46]. Consequently, these types of food allergies are overrepresented among adults. Notably, a recent study reported that a small but significant number of peanut allergic children ($\sim$20%) may outgrow their clinical disease over time [47].

Antigen/allergen specificity is one of the hallmarks of immune responses. Generally IgE antibodies produced against a given allergen are specific in nature (i.e, they generally do not physically bind to most other unrelated proteins). Consequently, if a given person is allergic to peanut alone, his peanut-specific IgE antibodies do not usually bind to other food types and therefore this individual is not expected to exhibit allergic reaction to foods other than peanut.

In some cases, there are cross-reacting IgE antibodies that bind to more than one allergenic protein due to similarities in the primary and quaternary structure (i.e., three-dimensional structure) shared by these proteins [48]. For instance, many patients allergic to hazel pollen or birch pollen exhibit pollen-specific IgE antibodies that also physically bind to hazelnut proteins (i.e., cross reacting antibody) [49–56]. The presence of such cross-reacting IgE antibodies is associated with hazelnut allergy in some patients [57]. Similarly some grass or ragweed pollen-allergic patients exhibit oral allergy symptoms (such as tingling, burning sensation in the mouth, etc.) when they consume fresh fruits and vegetables [58–61]. This has been proposed to be due to cross-reacting IgE antibodies elicited following exposure to grass or ragweed pollen allergens that also bind to proteins from certain fruits and vegetables [62]. Immune cross-reactivity between latex and many food types has also been described [63]. Food allergies caused by such cross-reacting antibodies have been classified as Type-2 food allergies [9]. Some of the clinical syndromes attributed to immune cross-reactivity are summarized in Table 101.5.

V. MECHANISMS OF FOOD ALLERGY: IMMUNE RESPONSE MODEL

As opposed to food allergy, immune mechanisms underlying other types of allergies such as allergic rhinitis and allergic asthma are very well established [46, 64–67]. Allergies in general are regarded as complex heterogeneous genetic disorders involving interaction between multiple genetic susceptibility genes and environmental factors [68–70]. Similar genetic susceptibility is thought to underlie food allergies as well [64, 66, 67, 71]. However, environmental exposure to allergen is essential for initiation of an allergic immune response as well as expression and maintenance of the clinical disease [65, 68].

Although food allergies are immune system-mediated, specific mechanisms underlying individual types of food allergies are unclear at present [9, 64]. One currently proposed mechanism for peanut allergy is illustrated in Figure 101.1. According to this model, exposure to peanut leads to peanut antigen presentation by professional antigen-presenting cells of the immune system to peanut-specific T helper (Th) cells. This results in the formation of Th2

TABLE 101.5
Type-2 Food Allergy: Food Allergies Attributed to Immune Cross-Reactivity to Aero Allergens

Type of Food Allergy	Implicated Foods	Aero-Allergens that Exhibit Immune Cross-Reactivity
Celery–mugwort–spice syndrome	Celery, carrot, apple, hazelnut, potato, kiwi fruit	Birch tree; pollen allergens from birch tree and mugwort
Birch pollen–hazelnut allergy syndrome	Hazelnut	Pollen allergens from hazel, birch, and alder
Latex–food allergy syndrome	Avocado, banana, chestnut, kiwi, raw potato, tomato, stone fruits (e.g., peach, cherry), hazelnuts, melons, celery, carrot, apple, pear, papaya, and almond	Latex
Oral allergy syndrome	Honeydew melon, banana, cantalope, watermelon	Pollen allergens from ragweed, grass, and birch tree

References: 7, 48, 51, 56–58, 62.

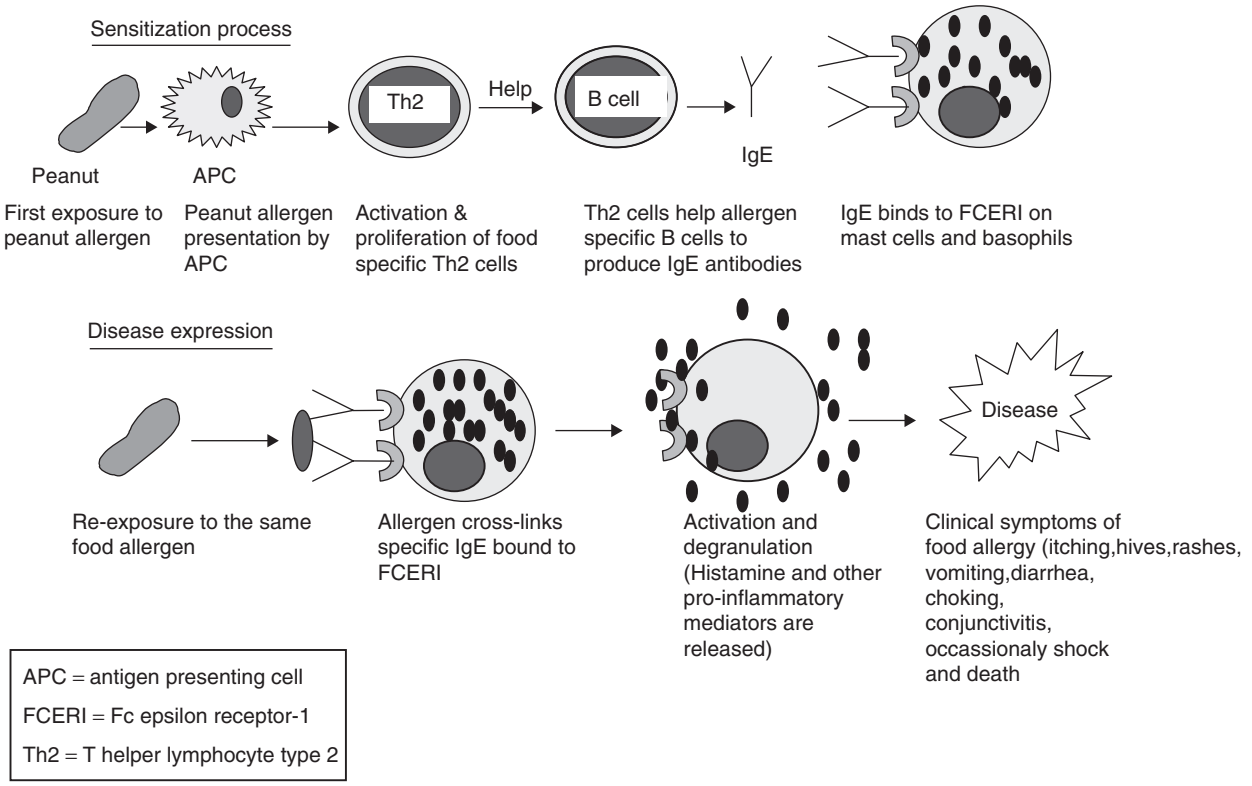

FIGURE 101.1 Mechanism of peanut allergy: immune response model. Exposure of a genetically susceptible individual to peanuts results in presentation of peanut allergens by antigen presenting cells (APC) to T helper (Th) lymphocytes. A consequence of this is the proliferation and activation of peanut specific Th-2 lymphocytes that help peanut-specific B cells to produce peanut-specific IgE antibodies. IgE antibodies bind to the high affinity IgE receptor present on cells such as mast cells that are rich in histamine and other proinflammatory mediator containing granules. Subsequent exposure to the same allergen (peanut in this case) results in cross-linking of surface IgE receptors on mast cells results in cellular activation, degranulation. Host response to histamine and other mediators results in the expression of clinical symptoms of food allergy within minutes of exposure to allergens.

lymphocytes that help peanut-specific B-lymphocytes to produce peanut-specific IgE antibodies [46, 66, 67]. These events are thought to occur when a susceptible individual comes in contact with the peanut for the first time. Once formed, most IgE antibodies in the system bind to high affinity IgE receptor (Fc epsilon receptor I, FCERI) on mast cells and basophils through their Fc portion [8, 72]. These immune cells are equipped with granules containing histamine and other pro-inflammatory mediators [72, 73]. Once these events occur, the individual is regarded as being sensitized to the relevant food. Subsequent encounter with the same food allergen (peanut allergen in this case) results in cross-linking of cell surface-bound IgE molecules by peanut allergens leading to activation and degranulation of mast

cells and basophils. The release of inflammatory mediators results in expression of clinical symptoms of disease. In extreme cases, this reaction could be widespread, involving multiple organs (referred to as systemic anaphylaxis and shock) that is often fatal if untreated [19].

In contrast to peanut allergy (and some other IgE mediated food allergies), mechanisms underlying many IgE independent food allergies are less clear. Food protein-induced enterocolitis syndrome (FPIES) is a group of conditions particularly common among infants and children [1, 74–76]. Commonly implicated foods in these diseases are cow's milk and soy. A recent study suggests that cereals, vegetables, and poultry meats, typically regarded as of low allergenic potential, must be considered in the evaluation of FPIES, particularly in infants previously diagnosed with FPIES to cow's milk or soy, and as an initial cause in patients who have been exclusively breastfed [76]. Notably food-specific IgE antibodies are not usually detectable in the circulation of these patients. Specific mechanisms of disease are not clear, although many appear to involve delayed hypersensitivity reactions. Furthermore, the disease-eliciting food components also remain to be characterized.

One well-characterized non-IgE mediated food-induced disease is gluten enteropathy (or celiac disease). It afflicts children as well adults and is particularly prevalent among Caucasians (1 in 200 individuals) [6]. Gluten is a component of many cereals such as wheat, rye, and barley. Wheat gluten in particular consists of a complex mixture of many gliadins and glutenin polypeptides. Gliadins are monomers and glutenins are polymers. There is clear evidence that celiac disease patients have T cells infiltrating the intestinal mucosa that react to deamidated gluten peptides [6]. Thus, gluten-specific T lymphocytes appear to be involved in the pathogenesis of this disease [6, 77]. However, there is evidence suggesting that the disease mechanism may have an autoimmune component as well. Thus, celiac disease patients on a gluten-containing diet produce IgA and IgG autoantibodies specific to tissue transglutaminase (TG)-2 [78]. The TG2 is an enzyme that deamidates gluten peptides so that T cells can recognize it in complex with the host human leukocyte antigen class II gene products [6, 79].

VI. FOOD ALLERGY: PREVENTION, DIAGNOSIS, AND THERAPY

Currently there is neither a cure nor an effective preventive method such as a vaccine available for food allergy. Strict avoidance of contact with the food allergen is the only recommended preventive method at present [8, 9]. In addition, following are some of the strategies especially for decreasing risk of atopy (i.e., tendency to develop an IgE antibody response) in infancy: (i) prolonged exclusive breast-feeding; (ii) maternal avoidance of commonly allergenic foods so as to prevent transfer of these proteins via breast milk to infants; (iii) delayed introduction of solid foods for the first 6 months of life; and (iv) use of hydrolyzed infant formulas (commonly referred to as hypoallergenic foods) [3, 24, 41, 74]. For food allergy in particular, novel prophylactic approaches are under experimental stages (for peanut allergy) in mouse models. Some of these are summarized in Table 101.6.

Food allergies are typically diagnosed with clinical history in combination with one or more of the following diagnostic tests. (i) Skin prick test: a small amount of allergen is placed on the surface of the skin and a quick prick is done to enable the allergen to cross the skin barrier and reach the underlying mast cells. If an individual has IgE antibodies specific for the allergen bound to the mast cells, it leads to IgE cross-linking, mediator release, and a wheal (swelling) and flare (redness) reaction at the site of allergen prick within 20–30 minutes. A saline solution is used as a negative control and a histamine solution as a positive control. Comparison of wheal size is made to determine the diagnosis. (ii) Double-blind placebo-controlled oral food challenge (DBPCFC) test: this is the gold standard test for food allergy diagnosis. However, the risk of severe reactions such as anaphylaxis makes this a difficult (and potentially very dangerous) choice for individuals with such a history. (iii) Food-specific IgE antibody measurement: immunoassay-based tests (such as radio allergosorbent test, or RAST) are available to determine the level of food-specific IgE antibody levels in the peripheral blood [7]. Recent studies suggest that determination of peanut allergen epitope recognition by IgE antibodies provides an additional tool to diagnose symptomatic peanut allergy, especially in children with peanut-specific IgE below diagnostic decision levels [80]. Furthermore, quantification of food-specific IgE was reported to be a useful test for diagnosing symptomatic allergy to egg, milk, peanut, and fish in the pediatric population and could eliminate the need to perform double-blind, placebo-controlled food challenges in a significant number of children [81]. Given the high rate of false positive reactions for skin testing and the problems with DBPCFC, this might prove useful clinically.

Following are the currently used therapeutic approaches for allergic diseases in general including food allergies. (i) Antihistamines are widely used for most allergic reactions. Antihistamines block histamine binding to H1 receptor and prevent cellular response to histamine. (ii) Corticosteroids are general anti-inflammatory agents that can be used locally or systemically. (iii) Epinephrine injections (intramuscular) are recommended for severe food-induced reactions such as systemic anaphylaxis. Epinephrine is the only life-saving medicine available to treat them. It can save life provided it is administered early in the reaction [3, 82]. It works as follows: constricts blood vessels and restores blood pressure, dilates bronchi and prevents choking, and increases heart action. Notably, as opposed to epinephrine, antihistamines have no demonstrated life-saving properties. (iv) Allergy shots: a technique

TABLE 101.6
Experimental Prophylactic and Therapeutic Approaches for Peanut Allergy

Method	Host System	Route of Administration	Results	References
Prophylactic Approaches				
Gene therapy (allergen-encoding plasmid DNA + chitosan nanoparticles)	Mouse	Oral	Decreased SPT Decreased IgE, plasma histamine, vascular leakage, decreased anaphylaxis	112
Recombinant IL-12 (in liposome)	Mouse	Oral	Decreased specific IgE, Increased IFN-g; Prevented and reversed anaphylactic symptoms Decreased histamine release	113
Therapeutic Approaches				
Humanized IgG1 monoclonal anti-IgE antibody therapy	Human	Subcutaneous injection	Increased the threshold of sensitivity to peanut on DBPCOFC from 178 mg to 2805 mg per person	88
Recombinant IL-12 (in liposome)	Mouse	Oral	Decreased specific IgE, Increased IFN-g Prevented and reversed anaphylactic symptoms Decreased histamine release	113
Chinese herbal formula (FAHF-1)	Mouse	Oral	Decreased specific IgE, IL-4, IL-5, and IL-13 Complete blocked anaphylactic symptoms Decreased histamine release and mast cell degranulation	114
Ara h1-3 plus heat-killed *Escherichia coli*	Mouse	Subcutaneous	Persistent protective effects	116
Ara h1-3 plus heat-killed *Listeria monocytogenes*	Mouse	Subcutaneous	Decreased frequency and severity of anaphylaxis Decreased specific IgE, IL-5 and IL-13 Decreased histamine release Bronchial constriction	115

called Preventive Allergy Treatment (PAT) (also called desensitization) has been used clinically for treating airway allergies. However, they are not recommended for food allergies due to serious risk of systemic anaphylaxis [3, 7, 82].

A number of experimental therapeutic approaches are currently being evaluated for allergies in general [83–86]. These may also be relevant for food allergies. They include the following. (i) Using allergen derived peptides that activate only T cells but fail to bind IgE antibodies and induce tolerance. (ii) Inhibition of IL-4, IL-5, IL-13 responses so that IgE synthesis can be inhibited or reduced. (iii) Use of cytokines such as IFN-gamma, IFN-alpha, IL-10, IL-12, and TGF-beta that can inhibit IgE antibody synthesis. (iv) Use of antibodies to IgE so that IgE can be removed from the system; a recent clinical study suggested that use of anti-IgE antibodies as a therapeutic method may be of some benefit in peanut-allergic subjects by increasing the threshold of the peanut dose required to elicit symptoms [87, 88]. (iv) Use of chemokine receptor (CCR3) antagonists to inhibit allergic inflammation by interfering with immune cell recruitment (i.e., chemotaxis) to sites of tissue damage. (v) Use of anti-leukotriene to block late-phase responses that contribute to chronic inflammation as in allergic asthma [84, 85].

Some recently reported experimental therapeutic approaches with interesting results in mouse models for peanut allergy are summarized in Table 101.6.

VII. FOOD ALLERGY AND FOOD INDUSTRY

The primary focus of the food industry has been on IgE-mediated food allergies. This is mainly because most potentially fatal food allergies are IgE mediated. In this regard, the food industry faces several challenges as listed Box 101.1. According to the United States Food and Drug Agency (US FDA), presence of "big eight" allergenic foods in a product needs to be identified on the label [27]. Absence of such information on the label is the reason for large number of class I food recalls in the U.S. [27].

In view of the current regulations, the food industry needs to ensure that their product is free from known eight commonly allergenic foods if they are not identified on the food label. Because many processing units share machinery, there is a real chance of cross-contamination between food products. One way to address this problem is by strictly following the recommended Good Manufacturing Practices (GMP) for food allergens as described in detail earlier [89]. Some of these in principles include (i) segregating allergenic

Box 101.1

FOOD ALLERGY: SOME OF THE MAJOR CHALLENGES FACED BY THE FOOD INDUSTRY

Consumers at risk of potentially fatal complications

Product recalls

Compensation to victims, law suits, business future at stake

Inadvertent contamination of food (hidden allergen)

Labeling issue

Is there a threshold for allergenic foods?

How to determine whether exotic (or nontraditional) food is/is not allergenic?

Genetically modified food: will they be allergenic?

Does food processing increase allergenicity?

foods from non-allergenic foods in the production lines; (ii) scheduling allergenic foods processing at the end of the day after non-allergenic food processing, followed by adequate cleaning; and (iii) frequent testing of the production lines for contamination of allergenic foods using appropriate methods. Availability of commercial rapid immunoassay (ELISA) screening kits to detect allergenic foods in field samples has helped this process enormously.

Most commercial immunoassays for allergenic foods typically have a detection sensitivity of $\geqslant$10 ppm. However, whether such a detection level is sufficient to ensure safety for consumers is not entirely clear. According to the US FDA, there are no recommended or suggested threshold or cutoff levels for allergenic food contamination of food products. There are two major reasons for this stand: (i) clinical symptoms of allergy can be elicited by "very small" quantities of food exposure in sensitized individuals, and (ii) there are no scientifically validated and well accepted threshold levels for common allergenic foods in different populations.

There have been, however, efforts to determine the threshold levels for various allergenic foods. For example, there are reports that 100 µg total peanut protein or 6 µg of the major peanut allergen (*Ara h2*) are threshold doses in patients by DBPCFC test [90, 91]. Another report suggested that the lowest observed reactive threshold was at 250 µg of peanut protein (equivalent to 1 mg whole peanut). Similar efforts have been recently reviewed for other foods as well [92]. These data suggest existence of dose thresholds and that they are unlikely to be a single dose for all allergenic food types for all age groups.

A number of concerns have been raised on the potential of food processing in increasing allergenicity of proteins and/or creating "neo-allergens" in processed foods [93–95]. There is some evidence that, as opposed to boiling or frying, dry roasting of peanuts increases its allergencity [96]. Other processing methods such as Maillard reaction (a chemical reaction between sugar and proteins) have also been proposed to increase IgE binding property of both allergenic and non-allergenic protein (lectin) in peanuts [97, 98]. In contrast, other processing methods such as protease treatment of soybeans and boiling of milk have been shown to reduce allergenicity of these foods [99–101]. Thus, more work is needed to further study the specific impact of different processing methods on allergenicity of proteins in different food types.

VIII. PREDICTING ALLERGENICITY OF NOVEL FOODS

Exposure to allergens is a critical step in the sensitization process as well as for allergy disease expression. Consequently, it is critical that new allergenic foods are not introduced into the food chain due to application of biotechnology in genetic modification of foods [28, 34]. Thus another significant problem faced by the food industry is to ensure that novel foods are tested for potential allergenicity before being introduced into the food chain (Box 101.1). Novel foods could be exotic/nontraditional foods or genetically engineered food proteins.

As alluded to earlier, food allergens in general are proteins in nature. Since genetically engineered foods express a new protein, potential allergenicity of this new protein is an important issue [8, 43, 92, 102]. This is especially critical if the new gene comes from sources such as peanuts, which are known to be lethally allergenic. In addition to foods, allergens can come from other sources such as insects (house dust mites, cockroaches), pollen from grass, trees, or weeds, and helminth parasites (such as round worms). In the U.S., the FDA requires labeling for a food that contains a new protein derived from known allergens or if a given food contains any of the eight "red flag" allergenic foods [27].

The difficult issue is the assessment of allergenic potential of novel proteins without any allergenic history available, particularly because the characteristics of an allergenic protein that would predictably distinguish it from non-allergenic proteins are largely unknown at present [8, 43, 92, 102]. This is an area of tremendous interest for the regulatory agencies (US FDA, EPA), federal health agencies (NIH), as well as food industries that are developing a variety of genetically engineered foods. The recent episode of StarLink™ corn vs. allergy/asthma has increased awareness and concern among public, regulatory agencies and the food industry on this issue, although the currently available investigative data on whether StarLink™ corn is indeed allergenic is inconclusive [103] (Box 101.2).

There have been extensive efforts to develop and validate methods to predict allergenicity of novel foods. Some of the currently pursued methods are summarized in Box 101.3. Limitations for some of the methods are listed

Box 101.2

StarLink™ Corn: Is This Genetically Modified Food Allergenic in Humans?

1996: Aventis Corporation (Bridgewater Crossing, NJ) produced this genetically modified corn; no product made after 2000

It contained Cry 9c, a pesticidal protein from *Bacillus thuringiensis* (Bt); Cry 9c is thermostable compared to other *Bt* proteins; therefore potential allergencity could not be ruled out based on available scientific knowledge at that time

1998: US EPA approved this corn only for animal feed September 2000: reports confirmed that StarLink had contaminated the human food supply (e.g., DNA was detectable in taco shells)

2001: US FDA, US EPA and USDA Food safety and inspection service asked National Food Processors Association to collect data on consumption, consumer contacts, allergy health contacts, and allergy related product recalls that might be potentially related to StarLink

2001: US Centers for Disease Control conducted analyses of samples from suspected cases; found no evidence for IgE antibody reactivity to Cry 9c in any of the serum samples; however, the test was limited because it used a recombinant Cry 9c protein produced in bacteria rather than the protein that is expressed in plants;

allergenic potential of this genetically modified remains inconclusive

in Box 101.3. It has been reported that results from methods such as stability to digestion do not necessarily correlate with allergenicity, and that such correlations are not absolute [104]. Predicting allergenicity of novel proteins based on primary amino acid and/or nucleotide sequence homology is also challenging, especially for discontinous or nonlinear epitopes [43, 105]. A recommendation from an FAO/WHO expert panel suggests that animal studies will contribute important information on the allergenicity of foods derived from biotechnology [34]. Readers are referred to excellent articles that discuss these issues in detail [43, 106].

There has been a recent effort to test the hypothesis that commonly allergenic foods are relatively more immunogenic than rarely allergenic or non-allergenic foods in mice using a multiple food sensitization protocol [107]. Mice were immunized with protein extracts from commonly allergenic foods (chicken egg, peanut, almond, filbert/hazelnut, walnut, soy, and wheat) or rarely/non-allergenic foods (coffee, sweet potato, carrot, white potato, cherry, lettuce, and spinach) plus alum as an adjuvant. Immunogenicity was measured as a function of the ability of each food type to elicit food-specific IgG1 antibody responses. The hierarchy of immunogenicity in this model of multiple food sensitization was found be: almond = filbert > spinach ≥ peanut ≥ sweet potato > cherry > lettuce > walnut > chicken egg > carrot ≥ white potato > wheat = coffee = soy. Thus there was no evidence supporting the hypothesis that allergenic foods are more immunogenic relative to non-allergenic foods. These data also demonstrated for the first time that (i) food types vary widely in relative immunogenicity in this mice strain; and (ii) relative immunogenicity in this model does not distinguish common vs. rare/non-allergenic food types. Consequently, the results also caution that evaluating immunogenicity of novel foods in mouse using the multiple sensitization method is unlikely to provide useful information on their potential allergenicity among humans.

Box 101.3

Methods for Assessment of Allergenic Potential of Food

Stability to pepsin digestion (pH 2.0)

Heat stability (90°C, 5 minutes)

Primary amino acid and/or nucleotide sequence homologies (6 consecutive amino acid match; or 35% identity)

Allergen skin prick testing and binding to IgE antibody (in exposed population)

Double-blind placebo-controlled oral food challenge test (in exposed population)*

Testing in food allergy animal models

* Ethical concerns have been raised on using this method in humans

A. Assessment of Allergenic Potential of Genetically Engineered Food: Decision Tree Approach

Food allergy is an international problem of critical significance. Consequently, there have been extensive international efforts in developing decision trees (i.e., flow charts) with the objective of helping predict the allergenic potential of novel proteins. The International Life Science Institute/International Food Biotechnology Consortium developed a decision tree approach for examining allergenic potential of genetically engineered (GE) foods in 1996 [31]. Subsequently a number of criticisms were made against this decision tree. To address some of these concerns, the FAO/WHO convened a special expert panel in 2001. The

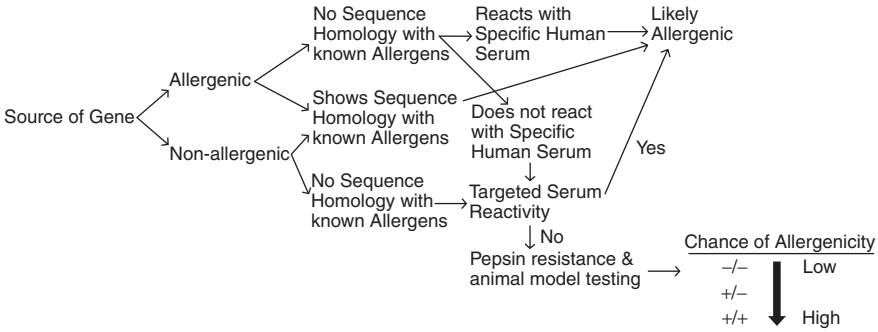

FIGURE 101.2 WHO/FAO Decision tree for assessment of allergenic potential of foods derived from biotechnology. The figure illustrates the principles of the decision tree approach developed by the FAO/WHO expert committee. (For more details, see <ftp://ftp.fao.org/es/esn/food/allergygm.pdf>.)

revised decision tree includes additional methods such as the use of animal models to assess allergenicity [34]. Principles underlying the latest FAO/WHO decision tree for assessment of allergenicity of novel foods is shown in Figure 101.2.

IX. CHALLENGES AND FUTURE DIRECTION

Since only a few (eight) foods account for a vast majority (~90%) of food allergies, one simplistic possibility to deal with food allergy is to eliminate one or more allergenic foods from the food chain. However, this appears to be an impractical solution because of the economic stature of the peanut business in the U.S. Peanut production in the U.S. is ~2 million tons per year (~10% of global peanut production). This translates to consumption of 2.9–3.6 kg per person per year. In addition to this economic importance, peanuts and other nuts offer nutrient (e.g., protein content) and health benefits (e.g., use of almonds in reducing risk of coronary heart disease [108, 109] and the use of nut and peanut butter in lowering risk of type 2 diabetes in women [110]. Thus it would be unwise to consider eliminating one or more of these food types from the food chain.

Alternative strategies are therefore necessary to deal with food allergy. Several areas of current research needs are (i) to develop improved methods to detect the presence of "hidden" allergens in food, (ii) to develop non-allergenic foods (e.g., non-allergenic soybeans and peanuts by producing them without the allergenic proteins; there is some recent success in this effort [111], (iii) to develop and validate methods to accurately predict allergenic potential of novel foods, and (iv) to develop effective preventive and/or therapeutic methods for food allergies.

X. CONCLUSIONS

Food allergies are a large and complex group of immune system-mediated diseases with little understood molecular mechanisms, although some have begun to be uncovered. Extensive research efforts are under way not only to understand the disease process, but also to study the potential utility of novel therapeutic and prophylactic methods such as gene therapy [112–116]. Since these diseases can be potentially fatal, they pose a serious challenge to consumers, food industries, and food regulatory agencies. Assessment of allergenic potential of genetically modified foods is another area that has received international attention. This has resulted in the availability of an FAO/WHO-proposed "decision tree" approach for assessment of allergenicity of novel foods. Clearly, coming years would test the utility of such approaches for predicting allergenic potential of genetically modified foods in humans before they are introduced in to the food chain. With advances in basic and clinical aspects of allergy and immunology and improved biotechnology, there is enormous hope and potential for effective prevention and management of food allergies in the twenty-first century.

ACKNOWLEDGMENTS

This work was supported by funding from the Michigan State University (CANR, MAES, CVM, and MSU Foundation) and the U.S. Environmental Protection Agency.

Author would like to thank Dr. Kent HayGlass (University of Manitoba, Canada) and Dr. Wilfried Karmaus (Michigan State University) for a critical review of the manuscript, and Dr. Lalitha Navuluri, Mr. Caleb Kelly, and Ms. Jankhana Subhashchandra (Michigan State University) for assistance with manuscript preparation.

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Taylor & Francis
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A CRC title, part of the Taylor & Francis imprint, a member of the
Taylor & Francis Group, the academic division of T&F Informa plc.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-9847-9 (Set)
International Standard Book Number-10: 1-57444-551-0 (Vol 1)
International Standard Book Number-10: 0-8493-9848-7 (Vol 2)
International Standard Book Number-10: 1-57444-552-9 (Vol 3)
International Standard Book Number-10: 0-8493-9849-5 (Vol 4)
International Standard Book Number-13: 978-0-8493-9847-6 (Set)
International Standard Book Number-13: 978-1-57444-551-0 (Vol 1)
International Standard Book Number-13: 978-0-8493-9848-3 (Vol 2)
International Standard Book Number-13: 978-1-57444-552-7 (Vol 3)
International Standard Book Number-13: 978-0-8493-9849-0 (Vol 4)
Library of Congress Card Number 2005050551

Library of Congress Cataloging-in-Publication Data

Handbook of food science, technology, and engineering / edited by Y.H. Hui.
p. cm. -- (Food science and technology ; 148)
Includes bibliographical references and index.
ISBN 1-57444-551-0 (v. 1 : alk. paper) -- ISBN 0-8493-9848-7 (v. 2 : alk. paper) -- ISBN 1-57444-552-9 (v. 3 : alk. paper) -- ISBN 0-8493-9849-5 (v. 4 : alk. paper)
1. Food industry and trade--Handbooks, manuals, etc. 2. Food--Analysis--Handbooks, manuals, etc. 3. Food--Composition--Handbooks, manuals, etc. I. Hui, Y. H. (Yiu H.) II. Food science and technology (Taylor & Francis) ; 148.

TP370.4.H38 2005
664--dc22 2005050551

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Preface for Volumes 3 and 4

In the last 30 years, progress in food science, food technology, and food engineering has advanced exponentially. As usual, information dissemination for this progress is expressed in many media, both printed and electronic. Books are available for almost every specialty area within these three disciplines, numbering in the hundreds.

Collective works on the disciplines are also available, though in smaller number. Examples are encyclopedia (food science, food engineering, food packaging) and handbooks (nutrition, food processing, food technology). Since handbooks on these topics are limited, this four-volume treatise is released by Taylor & Francis to fill this gap. The title of these four volumes is *Handbook of Food Science, Technology, and Engineering* with individual volume titles as follows:

- Volume 1: Food Science: Properties and Products
- Volume 2: Food Science: Ingredients, Health, and Safety
- Volume 3: Food Engineering and Food Processing
- Volume 4: Food Technology and Food Processing

This preface introduces Volumes 3 and 4. Each volume contains approximately 1000 printed pages of scientific and technical information. Volume 3 contains 46 chapters and Volume 4 contains 54 chapters. Volume 3 presents the following categories of topics, with the number of chapters indicated:

- Units of food processing, 1
- Food drying, 4
- Thermal processing, 5
- Freezing, 6
- New technology, 12
- Packaging, 6
- Ingredients technology, 5
- Modeling, 4
- Waste management, 3

Volume 4 presents the following topics, with the number of chapters indicated:

- Food categories, 28
- Food fermentation, 8
- Food microbiology, 6
- Water technology, 4
- Food safety and security, 8

Each topic in the two volumes deals with selected subject matters in the discipline. A brief discussion of the coverage for each volume is described below.

In Volume 3, most topics are related to food or chemical engineering. An introduction covers the units of operation in food engineering and food processing. Food drying discusses the basic principles of food dehydration and freeze drying. Two examples are provided: drying tropic fruits and drying pears.

The thermal processing of food covers basic engineering principles. Four examples are provided: heat transfer, temperature-time relationship, modeling, and deep-fat frying. Food freezing is discussed in relationship to: principles and food components, frozen storage, and frozen dough. Two examples are given: microwave and frozen foods and the quality and shelf-life of frozen meat.

The new engineering technology in food processing covers: minimal processed food, modified atmosphere packaging, ohmic and inductive heating, ultra sound, ultra light, aseptic packaging, irradiation, microwave, pulsed electric field, nanotechnology, sensors, and genetic engineering. Food packaging will be discussed from the following

perspectives: plastics, paper, new development, and edible films and coatings. Two special examples include: packaging of frozen foods and thermal processing of packaged foods. Modeling in food engineering is explored in several fronts: kinetics, experimental design, and model building.

Two other topics covered in Volume 3 are: ingredient technology and waste management in food processing. Five areas in ingredient technology are: spices and seasonings, sweet flavors application, emulsion, gums, and pectin. Waste management in food processing covers: water waste, poultry waste, and meat waste.

Volume 4 covers major topics in the application of technology in food processing. The first topic concentrates on the different categories of food products including, but not limited to, bakery products, chocolate confectionery, mozzarella cheeses, processed cheese, yogurt, eggs, frozen dessert, edible fats and oils, hydrogenation of fats, Asian noodles, pasta, seafood processing, fish paste, surimi, caviar, roe, thermal processing of meat, frozen meat processing equipment, dry-cured ham, poultry carcass, canned poultry ham, poultry nugget and pâté, snacks, extrusion, coatings, vegetables horticulture and processing, tofu, beer, Chinese wine, wine biotechnology, and whiskey.

There are two important topics related to food microbiology in food processing. One is fermented food which covers: basic principles, starter culture, and products manufacture. Five examples are covered: sour cream, cheese, meat, Mexican chili, and sourdough. The second topic is analytical methodology in pathogens detection. The topics covered are: conventional techniques, immunology, genetic engineering, and methods for detecting viruses, parasites, and mold.

The role of water in food and beverage processing is a frequently neglected topic. Water technology is discussed in relation to: sources, properties, purification, distribution, chemistry, analysis, and beverage plant sanitation. The last section of the book relates to food safety and food security: contaminants, personal hygiene, cleaning a processing plant, food plant equipment, frozen food plant sanitation, oil and fat plant sanitation, United States guidances for food security, and safety and security guidelines for the transportation and distribution of meat, poultry, and egg products.

When studying the information, please note two important considerations:

- Although major topics in the discipline are included, there is no claim that the coverage is comprehensive.
- Although the scientific information is applicable worldwide, a small number of topics with legal implications are especially pertinent in the United States.

These two texts are the result of the combined effort of more than 150 professionals from industry, government, and academia. They are from more than 15 countries with diverse expertise and background in the discipline of food science. These experts were led by an international editorial team of 12 members from 7 countries. All these individuals, authors and editors, are responsible for assembling 2000 printed pages of scientific information of immense complication and complexity. In sum, the end product is unique, both in depth and breadth, and will serve as an essential reference on food technology and food engineering for professionals in government, industry, and academia.

The editorial team thanks all the contributors for sharing their experience in their fields of expertise. They are the people who make this book possible. We hope you enjoy and benefit from the fruits of their labor.

We know how hard it is to develop the content of a book. However, we believe that the production of a professional book of this nature is even more difficult. We thank the editorial and production teams at Taylor & Francis for their time, effort, advice, and expertise. You are the best judge of the quality of this book.

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Part J

Food Processing

102 Units of Operations

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I. BACKGROUND

Food processing technology to date has made it possible for the U.S. to lead the way in providing the most nutritious, affordable, and safe food supply (Table 102.1). Despite these achievements, new challenges and issues continue to emerge and must be addressed to ensure that our food supply continues in this manner. For example, as the demand for convenient, nutritious, and healthful food products for both targeted groups and the general public continues to grow, of particular interest is the design and optimization of integrative processes that could be used to lead to effective food preservations and productions with enhanced food quality and improved safety (1). Moreover, the food industry is challenged to produce new products with longer shelf life that also possess quality attributes that are more reminiscent of the fresh or native state of a given food. Therefore, the need for improved processing steps continues to grow with consumers' demands for increased convenience and improved quality at an affordable cost.

Food processing, which involves a wide variety of industrial processes with a correspondingly large variety of products, is at a crossroads where proper application of technologies is able to develop and produce palatable products, retain nutritious food constituents, prevent microbial contamination, and ensure proper handling throughout the storage and distribution chain, while operation and energy costs remain a constant concern of all intervenients in the chain. This issue has now become global, as both functional and ethnic foods keep gaining popularity worldwide because of their nutritional values and variety in flavor and taste (2–4). To take on the challenges accompanying these opportunities, it is vital for food scientists to have a clear understanding of the operation of processing units that are crucial to achieving proper preservation of foods.

TABLE 102.1
Methods for Food Preservation

- **(1) Temperature Control**
  - (1) Cold storage (milk, meat, fruits, and vegetables)
  - (2) Frozen storage (poultry, fish, ice cream, juices)
  - (3) Controlled atmosphere (transport of fruit)
- **(2) Heat Processing**
  - (1) Blanching (vegetables and fruits)
  - (2) Pasteurization (milk and fruit juice)
  - (3) Sterilization
    - (i) Canning (batch process for vegetable, meat, fish)
    - (ii) Ultra-High-Temperature-Short time/Aseptic packaging
    - (iii) Radiation preservation (still experimental)
- **(3) Water Removal**
  - (1) Concentration (tomato paste, puree)
  - (2) Vacuum evaporation (milk, orange- and tomato juice)
- **(4) Drying and Dehydration**
  - (1) Solar and wind drying (fish, fruit, fruit slices)
  - (2) Oven drying (cereals, potato chips)
  - (3) Roller drying: low cost process (animal feed)
  - (4) Freeze drying: high cost (instant coffee and tea)
  - (5) Spray drying (large scale processing of dry milk and instant coffee)
- **(5) Microbial Preservation**
  - (1) Yeast fermentation/alcohol production (beer and wine)
  - (2) Lactic acid fermentation (yogurt, cheese, pickles)
  - (3) Complex fermentations (coffee, tea and cocoa)
- **(6) Chemical Preservation**
  - (1) Addition of sugar (jams and jellies)
  - (2) Addition of salt (salted meat or fish)
  - (3) Addition of acids (pickles, soft drinks)
  - (4) Addition of preservatives (soft drinks, wine)
  - (5) Smoking (fish, meat, cheese)
- **(7) Packaging**
  - (1) Protection against contamination
  - (2) Protection against deterioration
    - (i) Loss of moisture (meat, vegetables, cheese)
    - (ii) Gain of moisture (cereals, potato chips)
    - (iii) Loss of aroma (coffee, tea, cocoa)
    - (iv) Oxygen barrier (frying oil, butter)
    - (v) Odor barrier (cheese, fish, some fruits)

II. MATERIALS HANDLING

Correct and efficient materials handling is the key to savings in storage and operating space, better stock control, improved product quality, reduced processing time, lower costs of production, and less wastage of materials and operator time. It also could significantly improve working conditions, while reducing risk of accidents. A food processing worker has to be familiar with the principles of materials handling and control and automation of equipment.

A. General Considerations

Apple in 1977 (5) pointed out that industrial materials handling is concerned with the five elements: Movement—Time—Place—Quantity—Space. Over the last few decades materials handling technique has improved rapidly. A set of rules summarized by Brennan et al. in 1990 (6) that provide guides to action in planning, selection, and operation remain generally applicable in today's industry. Equipment employed for materials handling could be categorized into five main groups, including conveyors, elevators, cranes and hoists, trucks, and pneumatic equipment. It is important to follow the First-In-First-Out (FIFO) principle, while each processing company has to define its own materials handling needs and specify the requirements in its Good Manufacturing Practice (GMP).

B. Shape and Size Sorting

Shape sorting of foods such as potatoes and cucumbers is important in assessing their suitability for mechanized processing (e.g., peeling, blanching, pitting, and coring) as well as their retail value. Size sorting (sieving or screening) is

the separation of solids into two or more fractions on the basis of differences in size. In size sorting, the rate of separation is closely related to the nature of the sieve material, the shape and size distribution of the particles, the amplitude and frequency of shaking, and the effectiveness of methods used to prevent sieve blockage. Drum screen, with its capacity increasing with their speed of rotation up to a critical point, is commonly used for sorting small particulate foods such as peas or beans that have sufficient mechanical strength to withstand the tumbling action inside the screen. Sorted foods are necessary in processes in which uniformity of heat transfer is critical (e.g., sterilization and pasteurization) and they give better control over the weights filled into standard sale containers.

C. Weight and Density Sorting

Used for more valuable foods such as eggs, cut meats, fish fillets, and some tropical fruits, weight sorting is considered more accurate than other methods, since the weight of a food unit is proportional to the cube of its characteristic dimension. Recent developments in weight sorting machines involve the use of electro-mechanical and hydrostatic transducers to sense weight differences and computer-controlled devices to direct the weighed units into collection chutes. Automated weighing systems commonly comprise a load cell, which uses strain gauges bonded to a short metal beam rigidly clamped at one end, linked to a microprocessor linked to recording and control equipment. Density or buoyancy sorting, which exploits differences in hydrodynamic behavior, may be used to separate bruised and sound fruit such as apples, while peas of different maturity (size and density) could be separated in brines of different density.

D. Photometric and Color Sorting

Photometric sorting embraces most of the electromagnetic spectrum, ranging from gamma- and X-rays through ultraviolet, visible light, infra-red, microwaves, and radio frequencies to measurements of resistance at main frequency (6). The reflectance and transmittance characteristics of foods are important indicators of their processing suitability. Reflectance properties could be used to indicate product quality such as raw material maturity (e.g., the color of fruit, vegetables, meat, etc.); the presence of surface defects (e.g., worm-holed cereals or bruised fruits); and the extent of processing (e.g., of biscuits, bread, and potato chips). Transmittance measurements of foods are used to determine their internal properties such as ripeness or core defects in fruits, extraneous matter inclusions, and blood spots in eggs.

Color sorting equipment controlled by microprocessors has been used to sort small particulate foods automatically at very high rates. By manipulating the angle, shape, and lining material of the chute, the velocity of the pieces passing a photo-detector could be precisely controlled. Photo-detectors, which measure the reflected color of each piece and compare it with pre-set standards, send amplified signals to the microprocessor, which then communicates with a short blast of compressed air to separate defective foods or whenever a pre-selected color is used to distinguish between different colored foods that are to be processed separately.

III. CLEANING

Cleaning is the unit operation in which contaminating materials are removed from the food and separated at the earliest opportunity in a food process to leave the surface of the food in a suitable condition for further processing. With adequate cleaning in place, damage to subsequent processing equipment could be prevented, loss of time and money in processing contaminants could be reduced, and the safety hazards as well as loss of bulk products due to microbial growth during storage could also be minimized. A variety of cleaning methods have been adopted due to the wide variety of contaminant encountered in raw food materials and the low tolerances permitted for these contaminants (6).

A. Dry Cleaning

Dry cleaning methods such as screening, brushing, aspiration, abrasion, and magnetic separation have the advantages of relative cheapness and convenience while the cleaned surface is left in a dry condition. Used to remove contaminants of different size from that of the raw material, screens come in different forms, such as rotary drum screens (also referred to as trammels, centrifugal screens or reels) and flat-bed screens. Generally, rotary drum screens have good capacity and are relatively inexpensive to install, maintain, and operate. Flat-bed screens are excellent for cleaning fine materials such as flour and ground spices, since they are not easily blinded. Screening effectiveness is a function of the shape-regularity of the working substance.

Abrasion between food particles or between the food and moving parts of cleaning machinery, such as trommels, tumblers, vibrators, abrasive discs, and rotating brushes, is commonly used to loosen and to remove adhering contaminants. Aspiration cleaning is widely used in removing debris differing in buoyancy from the desired material. Typical applications include combine-harvesters, pea-viners, and bean-harvesters, in which the material to be cleaned is fed into a stream of air at controlled velocity when separation into two or more streams (e.g., light, middle, and heavy) is effected. Magnetic cleaning involves cascading the contaminated product stream over one or more magnets (both permanent magnets and electromagnets are used) that are usually located in the conveyor

trunking. In dry cleaning, recontamination remains a concern unless considerable care is exercised to minimize the spread of dust. Careful control of dust is important in the food industry in general (e.g., in milling, high-speed conveying, etc.) but especially so in dry cleaning where dusty conditions are most likely present in conjunction with stones and tramp metal that generate sparks. Therefore, dust-proofing of equipment, dust extraction, and rigorous housekeeping must be seriously exercised.

B. Wet Cleaning

For raw materials such as vegetables or fruits that are usually covered with soil, mud, and sand, a careful cleaning is needed before processing, whereas a second wash must be done after peeling and/or cutting (7). Washing after peeling and cutting removes microbes and tissue fluid, thus reducing microbial growth and enzymatic oxidation during storage. Washing in flowing or air-bubbling water is preferable to dipping into still water, and the washing water used must be good and its temperature at preferably below 5°C (8). To reduce microbial numbers and to retard enzymatic activity, which improves the product shelf life, preservatives such as chlorine can be used in washing water. The effectiveness of chlorine can be enhanced by using a combination of low pH, high temperature, pure water, and correct contact time (9). Alternatives to chlorine include chlorine dioxide, per acetic acid ozone, trisodium phosphate, and hydrogen peroxide (10).

Soaking, which is the simplest method of wet cleaning, is often used as a preliminary stage in the cleaning of root vegetables and other foods that are heavily contaminated. The efficiency of soaking could be leveraged if warm water is used; if water is moved relative to the product by means of caged propeller-stirrers built into the tank; or if the product is moved relative to the water either using slow-moving paddles or by feeding the raw material into a horizontal perforated drum that rotates and partially submerged in the soak tank. Chlorination is commonly used in soak tank water to decrease its bacterial loadings; however, its high chemical oxygen demand (COD) and blackening on foods such as potatoes have to be taken into consideration. The most widely used wet cleaning method is spray washing, whose efficiency is dependent on the water pressure employed, the volume of water used, the water temperature, the distance of the food from the spray-jet, the time of exposure of the food to the sprays, and the number of spray-jets used. While a small volume of water at high pressure is the most effective combination, damage may be caused to ripe soft fruits and to delicate vegetables. Flotation washing, which depends on a difference in buoyancy between the desired and undesired parts of the food to be cleaned, has been used by fluming the fruit into a tank and collecting the overflow of sound fruit to remove bruised or rotten fruit that sink in water.

In the food industry, cleaning methods are generally used in combination. Many cleaning machines involve several stages combined as a single unit. A typical example or a combination procedure is the cleaning process used for producing cleaned wheat for milling into flour. In addition, accompanying the cleaned product is the excess water that coats product surface. Occasionally it is necessary to resort to drying procedures. For example, with washed cereals or with wet-cleaning fruits that is to be stored or is to be sold as a finished foodstuff.

IV. SEPARATION

Separation is a major operation in food processing, where different components of a product are separated based on certain physicochemical principles (11).

A. Sedimentation

Sedimentation uses gravitational forces to separate particulate material from fluid streams. The particles are usually solid, but they can be small liquid droplets, and the fluid can be either a liquid or a gas. Sedimentation is very often used in the food industry for separating dirt and debris from incoming raw material, crystals from their mother liquor and dust or product particles from air streams. In sedimentation, particles are falling from rest under the force of gravity. Therefore in sedimentation the familiar form of Stokes' Law exists:

$$v_m = D^2 g(\rho_p - \rho_f)/18\mu \qquad (102.1)$$

Note that Equation (102.1) is not dimensionless and so consistent units must be employed throughout. For example, in the SI system D would be m, g in m s^{-2}, ρ in kg m^{-3} and μ in N s m^{-2}, and then v_m would be in m s^{-1}. Particle diameters are usually very small and are often measured in microns (micro-meters) = 10^{-6} m with the symbol μm.

Stokes' Law applies only in streamline flow and strictly only to spherical particles. In the case of spheres the criterion for streamline flow is that Reynolds number (Re) = 2, and many practical cases occur in the region of streamline flow, or at least where streamline flow is a reasonable approximation. Where higher values of the Reynolds number are encountered, more detailed references should be sought (12). In addition, Stokes' Law applies only to cases in which settling is free, that is where the motion of one particle is unaffected by the motion of other particles. Where particles are in concentrated suspensions, an appreciable upward motion of the fluid accompanies the motion of particles downward. So the particles interfere with the flow patterns around one another as they fall. Stokes' Law predicts velocities proportional to the square of the particle diameters. In concentrated suspensions, it is found that all particles appear to settle at a uniform velocity once a

sufficiently high level of concentration has been reached. Where the size range of the particles is not much greater than 10:1, all the particles tend to settle at the same rate. This rate lies between the rates that would be expected from Stokes' Law for the largest and for the smallest particles. In practical cases, in which Stokes' Law or simple extensions of it cannot be applied, the only satisfactory method of obtaining settling rates is by experiment.

B. Centrifugation

The separation by sedimentation of two immiscible liquids, or of a liquid and a solid, depends on the effects of gravity on the components. Sometimes this separation may be very slow because the specific gravities of the components may not be very different, or because of forces holding the components in association, for example as occur in emulsions. Much greater forces can be obtained by introducing centrifugal action, in a centrifuge. Gravity still acts and the net force is a combination of the centrifugal force with gravity as in the cyclone. Because in most industrial centrifuges, the centrifugal forces imposed are so much greater than gravity, the effects of gravity can usually be neglected in the analysis of the separation. The centrifugal force on a particle that is constrained to rotate in a circular path is given by

$$F_c = mr\omega^2 \qquad (102.2)$$

where F_c is the centrifugal force acting on the particle to maintain it in the circular path, r is the radius of the path, m is the mass of the particle, and ω is the angular velocity of the particle. Or, since $\omega = v/r$, where v is the tangential velocity of the particle

$$F_c = (mv^2)/r \qquad (102.3)$$

Rotational speeds are normally expressed in revolutions per minute ($\omega = 2\pi N/60$), so that Equation (102.2) can also be written as

$$F_c = mr(2\pi N/60)^2 = 0.011\, mrN^2 \qquad (102.4)$$

where N is the rotational speed in revolutions per minute. If this is compared with the force of gravity (F_g) on the particle, which is $F_g = m_g$, it can be seen that the centrifugal acceleration, equal to $0.011\, rN^2$, has replaced the gravitational acceleration, equal to g. The centrifugal force is often expressed for comparative purposes as so many "g."

The centrifugal force depends upon the radius and speed of rotation and upon the mass of the particle. If the radius and the speed of rotation are fixed, then the controlling factor is the weight of the particle so that the heavier the particle the greater is the centrifugal force acting on it. Consequently, if two liquids, one of which is twice as dense as the other, are placed in a bowl and the bowl is rotated about a vertical axis at high speed, the centrifugal force per unit volume will be twice as great for the heavier liquid as for the lighter. The heavy liquid will therefore move to occupy the annulus at the periphery of the bowl and it will displace the lighter liquid towards the centre. This is the principle of the centrifugal liquid separator. The simplest form of centrifuge consists of a bowl spinning about a vertical axis. Liquids, or liquids and solids, are introduced into this and under centrifugal force the heavier liquid or particles pass to the outermost regions of the bowl, whilst the lighter components move towards the centre. If the feed is all liquid, then suitable collection pipes can be arranged to allow separation of the heavier and the lighter components. Various arrangements are used to accomplish this collection effectively and with a minimum of disturbance to the flow pattern in the machine.

Whereas liquid phases can easily be removed from a centrifuge, solids present much more of a problem. In liquid/solid separation, stationary ploughs cannot be used as these create too much disturbance of the flow pattern on which the centrifuge depends for its separation. One method of handling solids is to provide nozzles on the circumference of the centrifuge bowl. These nozzles may be opened at intervals to discharge accumulated solids together with some of the heavy liquid. Alternatively, the nozzles may be open continuously relying on their size and position to discharge the solids with as little as possible of the heavier liquid. These machines thus separate the feed into three streams, light liquid, heavy liquid and solids, the solids carrying with them some of the heavy liquid as well (Table 102.2).

C. Crystallization

Crystallization is an example of a separation process in which mass is transferred from a liquid solution, whose composition is generally mixed, to a pure solid crystal. Soluble components are removed from solution by adjusting the conditions so that the solution becomes supersaturated and excess solute crystallizes out in a pure form. This is generally accomplished by lowering the temperature, or by concentration of the solution, in each case to form a supersaturated solution from which crystallization can occur. The equilibrium is established between the crystals and the surrounding solution, the mother liquor. The manufacture of sucrose, from sugar cane or sugar beet, is an important example of crystallization in food technology. Crystallization is also used in the manufacture of other sugars, such as glucose and lactose, in the manufacture of food additives, such as salt, and in the processing of foodstuffs, such as ice cream. In the manufacture of sucrose from cane, water is added and the sugar is pressed out from the residual cane as a solution. This solution is

TABLE 102.2
Applications for Centrifugal Equipment in the Food Industry

Type	Application
Tubular-bowl centrifuges	• Edible oil refining in degumming, neutralisation and washing stages • Clarification of fruit juices, cider and sugar syrup
Disc-bowl centrifuges	• Dairy industry for cream separation • Edible oil refining for separation of soapstocks and washings • Clarification of vegetable, citrus oils and fruit juices
Decanting centrifuges	• Recovering animal and vegetable protein • Harvesting single cell protein • Separating coffee, tea and cocoa slurries • Desludging fish oils • Separating fish blood water and fish press liquor • Fat separation from comminuted meat • Meat deboning • Olive oil extraction • Dewaterin trub in brewing • Clarifying liquor and recovering tartrates in winemaking
Filtering centrifugals	• Sugar refining for recovering, washing and drying sugar crystals • Juice separation from comminuted fruits and vegetables • Vegetable protein recovery • Starch separation from potato slurry • Freeze concentration operations

purified and then concentrated to allow the sucrose to crystallize out from the solution. Once crystallization is concluded, equilibrium is set up between the crystals of pure solute and the residual mother liquor, the balance being determined by the solubility (concentration) and the temperature. The driving force making the crystals grow is the concentration excess (supersaturation) of the solution above the equilibrium (saturation) level. The resistances to growth are the resistance to mass transfer within the solution and the energy needed at the crystal surface for incoming molecules to orient themselves to the crystal lattice.

Crystallizers can be divided into two types: crystallizers and evaporators. A crystallizer may be a simple open tank or vat in which the solution loses heat to its surroundings. The solution cools slowly so that large crystals are generally produced. To increase the rate of cooling, agitation and cooling coils or jackets are introduced and these crystallizers can be made continuous. The simplest is an open horizontal trough with a spiral scraper. The trough is water jacketed so that its temperature can be controlled. During crystallization, the crystals are grown from solutions with concentrations higher than the saturation level in the solubility curves. Above the supersaturation line, crystals form spontaneously and rapidly, without external initiating action. This is called spontaneous nucleation. In the area of concentrations between the saturation and the supersaturation curves, the metastable region, the rate of initiation of crystallization is slow; aggregates of molecules form but then disperse again and they will not grow unless seed crystals are added. Seed crystals are small crystals, generally of the solute, which then grow by deposition on them of further solute from the solution. This growth continues until the solution concentration falls to the saturation line. Below the saturation curve there is no crystal growth, crystals instead dissolve.

When a solution is cooled to produce a supersaturated solution and hence to cause crystallization, the heat that must be removed is the sum of the sensible heat necessary to cool the solution and the heat of crystallization. When using evaporation to achieve the supersaturation, the heat of vaporization must also be taken into account. Because few heats of crystallization are available, it is usual to take the heat of crystallization as equal to the heat of solution to form a saturated solution. Theoretically, it is equal to the heat of solution plus the heat of dilution, but the latter is small and can be ignored. For most food materials, the heat of crystallization is positive, i.e. heat is given out during crystallization. Note that heat of crystallization is the opposite of heat of solution. If a material takes in heat, then the heat of crystallization is positive. Heat balances can be calculated for crystallization (12).

D. Solid-Liquid Extraction

Solid-liquid extraction is a basic operation to separate one or more components contained in a solid phase by a liquid phase or solvent. Liquids have a capacity to dissolve solids up to an extent, which is determined by the solubility of the particular solid material in that liquid. Solubility is a function of temperature and, in most cases, solubility increases with rising temperature. A solubility curve can then be drawn to show this relationship, based on the equilibrium concentration in solution measured in convenient units, for example g kg^{-1}, as a function of temperature (12). The first stage in an extraction process is generally mechanical grinding, in which the raw material is shredded, ground or pressed into suitably small pieces or flakes to give a large contact area for the extraction.

Important applications of the solid-liquid extraction in the food industry include: extraction of animal and vegetable oils and lipids, washing precipitates, obtaining extracts from animal or vegetable materials, obtaining sugar, and manufacturing tea and instant coffee (13). For example in sugar-cane processing and in the extraction of vegetable

oils, a substantial proportion of the desired products can be removed directly by expression at this stage and then the remaining solids are passed to the extraction plant. Fluid solvents are easy to pump and so overflows are often easier to handle than underflows and sometimes the solids may be left and solvent from successive stages brought to them. In the same way and for the same reasons as with counter flow heat exchangers, a counter current (or counter flow) extraction system provides the maximum mean driving force, the log mean concentration difference in this case, contrasting with the log mean temperature difference in the heat exchanger. This ensures that the equipment is used efficiently. In some other extractors, the solids are placed in a vertical bucket conveyor and moved up through a tower down which a stream of solvent flows. Other forms of conveyor may also be used, such as screws or metal bands, to move the solids against the solvent flow. Sometimes centrifugal forces are used for conveying, or for separating after contacting.

E. Electrodialysis and Ion Exchange

Electrodialysis (ED) is defined as the transport of ions through membranes as a result of an electric driving force. The membranes used have anion and cation exchange functions respectively, which gives the ED process a capability to reduce the mineral content of a process liquid, for example seawater or whey. A major limiting factor for using ED in dairy processing is the cost for the replacement of membranes, spacers, and electrodes, which constitutes 35–40% of the total running costs in the plant. The need for replacement is caused by the fouling of the membranes, which in turn is caused by the precipitation of low-soluble calcium phosphate on the cation membrane surfaces and the deposition of protein on the anion exchange membrane surfaces, which is the main factor reducing the life length of the anion membrane. Although frequent high-pH cleaning removes most of the deposits, disassembly of the stack for manual cleaning is recommended with two- to four-week intervals. On the other hand, the processing cost of ED depends very much on the demineralization rate. Increasing the capacity in steps from 50% to 75% to 90% doubles the processing cost per step. This means that it is four times as expensive per kilo of product solids to demineralize to 90% than to 50% and this is due to the reduction in plant capacity when working with 90% demineralization.

In contrast to ED, ion exchange (IE) is a fixed-bed technique involving the use of resins, which have a discrete capacity for the absorption of minerals. When this capacity is used up, the absorbed minerals have to be removed from the resin and the resin regenerated before it can be used again. IE resins are macromolecular porous plastic materials, formed into beads with diameters in the range of 0.3 to 1.2 mm for technical applications. Chemically they act as insoluble acids or bases which, when converted into salts, remain insoluble. The main characteristic of IE resins is their capacity to exchange the mobile ions they contain for ions of the same charge sign, contained in the solution to be treated. Demineralization using IE has long been an established process for water treatment but has, during the last 15 years, also obtained interest for de-ashing of whey, which is not a uniform product as to composition. Whey from an acid casein/cheese curd has a pH of 4.3 to 4.6. The pH of sweet whey is 6.3 to 6.6. The main difference between these two types of whey, apart from the acidifying medium, is the high level of calcium phosphate of the acid whey. Thus, whey can consequently be characterized as a liquid with a high salt load which, as a consequence, results in short cycles when IE is applied. This in turn results in high costs for regeneration chemicals, if they are not recovered.

F. Reverse Osmosis and Ultrafiltration

Reverse osmosis (RO) and ultrafiltration (UF) are both unit operations in which water and some solutes in a solution are selectively removed through a semi-permeable membrane. The main advantages of membrane concentration over concentration by evaporation are: (1) the food is not heated, and there is therefore negligible loss of nutritional or eating quality and particularly less loss of volatiles, (2) in contrast with boiling, membrane concentration doses not involve a change in phase and therefore uses energy more efficiently, and (3) lower labor and operating costs: simple installation, operation with a single control valve, and no requirement for steam boilers (14).

The two processes are similar in that the driving force for transport across the membrane is the pressure applied to the feed liquid. Specifically, RO is concerned mainly with solutions containing smaller molecules such as simple sugars, salts, monosaccharides, and aroma compounds at higher molar concentrations, which exert higher osmotic pressures. To overcome these osmotic pressures, high external pressures have to be exerted, up to the order of 100 atmospheres (4000–8000 kPa). Movement of molecules through RO membranes is by diffusion and not by liquid flow. The molecules dissolve at one face of the membrane are transported through the membrane and then removed from the other face. The flow rate of liquid (the flux) is determined by the solubility and diffusivity of the molecules in the membrane material, and by the difference between the osmotic pressure of the liquid and the applied pressure. Limitations to increased flow rates arise in this case from the mechanical weaknesses of the membrane and from concentration of solutes which causes substantial osmotic "back" pressure. Applications in the food industry are in separating water from solutions such as fruit juices.

UF membranes, on the other hand, have a higher porosity and retain only large molecules (for example proteins or colloids) that have a low osmotic pressure (15). Smaller

solutes are transported across the membrane with the water. UF therefore operates at lower pressures (50–2000 kPa). The main requirement of an UF membrane is the ability to form and retain a "microporous" structure during manufacture and during operation under thermal and mechanical stress. The material and methods used for the manufacture of the microporous structure are the most important factors in determining the properties of the membrane. Rigid or glassy polymers, which are thicker than RO membranes (0.1–0.5 μm), are commonly used. For food applications, membranes should be capable of being cleaned and sanitized. Suitable materials include polysulphones, polyamides, poly(vinyl chloride), polystyrene, polycarbonates, polyethers, and rigid cellulose esters.

The equipment for these membrane separation processes consists of the necessary pumps, flow systems and membranes. In the case of UF, the membranes are set up in a wide variety of geometrical arrangements, mostly tubular but sometimes in plates, which can be mounted similarly to a filter press or plate heat exchanger. Flow rates are kept high over the surfaces and recirculation of the fluid on the high pressure, or retentate, side is often used; the fluid passing through, called the permeate, is usually collected in suitable troughs or tanks at atmospheric pressure. In the case of RO, the high pressures dictate mechanical strength, and stacks of flat disc membranes can be used one above the other. Another system uses very small diameter (around 0.04 mm) hollow filaments on plastic supports; the diameters are small to provide strength but preclude many food solutions because of this very small size. The main flow in reverse osmosis is the permeate. The systems can be designed either as continuous or as batch operations. One limitation to extended operation arises from the need to control growth of bacteria. After a time bacterial concentrations in the system, for example in the gel at the surface of the ultrafiltration membranes, can grow so high that cleaning must be provided. This can be difficult as many of the membranes are not very robust either to mechanical disturbance or to the extremes of pH which could give quicker and better cleaning.

V. DISINTEGRATION

Disintegration is a common unit operation in food processing. The usual concerns when cutting agricultural raw materials include sanitation and the hazard of metal contamination. It is the point stones or other objects could be introduced to damage processing equipment. Prescreening, inspection, and cleaning are important, as well as downstream metal detection.

A. Peeling, Cutting, and Shredding

It is generally agreeable that peeling should be as gentle as possible. However, on an industrial scale, peeling of fruits or vegetables such as carrots, apples or potatoes is normally accomplished mechanically (e.g., rotating carborundum drums), chemically or in high-pressure steam peelers (16). Since adverse effects of carborundum, steam peeling, or caustic acid are known to enhance the possibilities of microbial growth and enzymatic changes, it is a common practice in the industry to combine carborundum (a first stage of rough peeling) and knife peeling (a second stage of finer peeling). Successful enzymatic peeling was also reported in the case of oranges (17). From a microbiological and sensory point of view, many studies show that the cutting and shredding of fruits and vegetables must be performed with knives or blades as sharp as possible and made from stainless steel. Vibration of a slicing machine should be minimized in order not to impair the quality of the sliced surfaces, while mats and blades used in slicing should be disinfected with a 1% hypochlorite solution (18).

B. Size Reduction of Solid Foods

Grinding and cutting reduce the size of solid materials by mechanical action, dividing them into smaller particles. The most extensive application of grinding in the food industry is in the milling of grains to make flour, but it is used in many other processes, such as in the grinding of corn for manufacture of corn starch, the grinding of sugar, and the milling of dried foods (12). Size-reduction processes may be divided into those that produce random particles or pieces and those that produce pieces that are more uniform (19). A random process produces a size distribution. In some cases, the extremes—the smallest or the largest—may be undesirable, while in others the mix can be used completely. If only one portion of the size distribution is sought, there must be a separation or fractionation process. If smaller particles are desired, the larger ones may be screened out and recycled. Very fine particles are often discarded as waste but in some cases may be treated to create larger particles. An example of this situation is sugar grinding followed by granulation to create a size distribution suitable for tableting or compacting.

When a uniform particle is crushed, after the first crushing the size of the particles produced will vary a great deal from relatively coarse to fine and even to dust. As the grinding continues, the coarser particles will be further reduced but there will be less change in the size of the fine particles. Careful analysis has shown that there tends to be a certain size that increases in its relative proportions in the mixture and which soon becomes the predominant size fraction. For example, wheat after first crushing gives a wide range of particle sizes in the coarse flour, but after further grinding the predominant fraction soon becomes that passing a 250 μm sieve and being retained on a 125 μm sieve. This fraction tends to build up, however long the grinding continues, so long as the same type of machinery, rolls in this case, is employed.

VI. PUMPING

Many raw materials for foods and many finished foods are in the form of fluids. These fluids have to be transported and processed in the factory. The transfer is usually accomplished by pumping the materials through pipeline systems. Thus, food technologists must be familiar with the principles that govern the flow of fluids and with the machinery and equipment that is used to handle fluids. In addition, there is an increasing tendency to handle powdered and granular materials in a form in which they behave as fluids. Fluidization has been developed because of the relative simplicity of fluid handling compared with the handling of solids.

A. Control of Fluid Flow

Viscosity is the property of a fluid that gives rise to forces that resist the relative movement of adjacent layers in the fluid. Viscous forces are of the same character as shear forces in solids and they arise from forces that exist between the molecules. In many instances in practice non-Newtonian characteristics are important, and they become obvious when materials that it is thought ought to pump quite easily just do not. They get stuck in the pipes, or overload the pumps, or need specially designed fittings before they can be moved. Sometimes it is sufficient just to be aware of the general classes of behavior of such materials. In other cases it may be necessary to determine experimentally the rheological properties (Newtonian or non-Newtonian) of the material so that equipment and processes can be adequately designed (20).

B. Pipeline and Pump Selection

The ease and cost of pumping are largely dependent on the design of the piping system. The liquid flow rate, vertical lift, and increase in downstream pressure to be overcome are normally fixed by the processes. However, energy losses due to friction and shock losses could be minimized by careful selection of components fabricated. In order to minimize energy losses in a piping system, it is recommended that the total length of the pipe-run is as short as possible; the same pipe diameter is maintained throughout the length of the pipe-run whenever possible; the correct type of fitting for a specific duty is used; and the number of pipe-fittings is kept to a minimum. In addition, a suitable pump matched to suit the system must be selected.

The pump is the heart of the fluid transfer process. Upon choosing a pump, factors such as the volumetric flow rate of liquid to be transferred, the total system head against which the liquid is to be pumped, the fluid properties (e.g., consistency, density, temperature, shear deformation, and lubricating properties), the type of prime mover (electric, steam, or compressed air), and hygienic requirements need to be considered. Pumps are commonly classified as either (1) positive displacement pumps (reciprocating and rotary types) or (2) centrifugal pumps. All positive displacement pumps operate by trapping a certain volume of fluid within the body or the pump and forcing it from the inlet side to the outlet at higher pressure. Centrifugal pumps, the most widely used liquid transfer pump in the general process industries, are also widely used in the food industry because they are relatively cheap and simple. A centrifugal pump consists of an impeller rotating in a casing having inlet and exit ports. Fluid normally enters axially into the eye of the impeller that rotates at a constant speed. The fluid is accelerated and thrown from the tips of the impeller blades at high velocity, passing into an increasing area for flow where the velocity energy is converted into pressure energy. The fluid leaving the pump flows through the pipe system by virtue of the pressure or head imparted by the pump.

VII. MIXING

Mixing is the dispersing of components, one throughout the other. It occurs in innumerable instances in the food industry and is probably the most commonly encountered of all process operations. Unfortunately, it is also one of the least understood. There are, however, some aspects of mixing which can be measured and which can be of help in the planning and designing of mixing operations. The production of fully mixed small volumes, which can be taken or sampled for measurement, is what mixing is all about. Sample compositions move from the initial state to the mixed state, and measurements of mixing must reflect this.

A. Particle Mixing

If particles are to be mixed, starting out from segregated groups and ending up with the components randomly distributed, the expected variances of the sample compositions from the mean sample composition can be calculated. The degree of mixing that is achieved depends on:

- the relative particle size, shape and density of each component,
- the efficiency of a particular mixer for those components,
- the tendency of the materials to aggregate, and
- the moisture content, surface characteristics, and flow characteristics of each components.

The mixing of particles varying substantially in size or in density presents special problems, as there will be gravitational forces acting in the mixer which will tend to segregate the particles into size and density ranges. In such a case, initial mixing in a mixer may then be followed by a measure of (slow gravitational) un-mixing and so the time of mixing may be quite critical.

Mixing is simplest when the quantities that are to be mixed are roughly in the same proportions. In cases where very small quantities of one component have to be blended uniformly into much larger quantities of other components, the mixing is best split into stages, keeping the proportions not too far different in each stage. For example, if it were required to add a component such that its final proportions in relatively small fractions of the product are 50 parts per million, it would be almost hopeless to attempt to mix this in a single stage. A possible method might be to use four mixing stages, starting with the added component in the first of these at about 30:1. In planning the mixing process it would be wise to take analyses through each stage of mixing, but once mixing times had been established it should only be necessary to make check analyses on the final product.

B. Liquid Mixing

The component velocities induced in liquids by a mixer include: (1) a radial velocity which acts in a direction perpendicular to the mixer shaft; (2) a longitudinal velocity (parallel to the mixer shaft); and (3) a rotational velocity (tangential to the mixer shaft). To mix low-viscosity liquids adequately, turbulence must be induced throughout the bulk of the liquid to entrain slow-moving parts within faster moving parts. A vortex should be avoided because adjoining layers of circulating liquid travel at a similar speed and entrainment does not take place. The liquids simply rotate around the mixer. In high-viscosity liquids, pastes, or dough, a different action is needed. Mixing occurs by kneading the material against the vessel wall or into other material, folding unmixed food into the mixed part, and shearing to stretch the material. Efficient mixing is achieved by creating and recombining fresh surfaces in the food as often as possible. However, because the material does not easily flow, it is necessary either to move the mixer blades throughout the vessel or to move the food to the mixer blades.

The rate of mixing is characterized by a mixing index (M). The mixing rate constant (K) depends on the characteristics of both the mixer and the liquids. The effect of the mixer characteristics on K is given by:

$$K \propto \frac{D^3 N}{D_t^2 z} \tag{102.5}$$

where D is the agitator diameter (m), N is the agitator speed (s^{-1}), D_t is the vessel diameter (m), and z is the height of liquid (m). The rheology of the liquid also is crucial to the overall mixing efficiency and as discussed in Section VI.A.

C. Emulsification and Homogenization

Emulsification may be defined as that operation in which two normally immiscible liquids are intimately mixed, one liquid (the discontinuous, dispersed or internal phase) becoming dispersed in the form of small droplets or globules in the other (continuous, dispersing or external phase). In most emulsions the two liquids involved are water and oil. The water phase may consist of solutions of salts, sugars or other organic and colloidal materials (hydrophilic materials). The oil phase may consist of oils, hydrocarbons, waxes, resins, and other substances which behave like oil (hydrophobic materials). In order to form a stable emulsion a third substance, known as an emulsifying agent or an emulsifier, needs to be included. The emulsion formed tends to exhibit most of the properties of the liquid that forms the external phase. Thus two emulsions of similar composition can have quite different characteristics depending on whether the oil or water is the external phase. The factors which influence the type of emulsion formed include the type of emulsifying agent used, the relative proportions of the phases, and the method of preparation.

The essential feature of an emulsion is the small size of the dispersed phase droplets. This can be achieved by imposing very high shearing stresses upon the liquid that is to be dispersed and the shearing forces break the material into the multitude of fine particles. Homogenization is the term used to describe the operation in which the desired reduction in the size of the droplets of the internal phase is brought about by forcing the crude emulsion through a narrow opening at high velocity. The size of the dispersed phase droplets is commonly of the order 1–10 μm diameter. Below 0.1 μm droplet diameter, the dispersion is often spoken of as colloidal. Coalescence of the dispersed phase droplets is hindered by increased viscosity in the continuous liquid phase. The nearer the densities of the components are to each other, the less will be the separating effect of gravitational forces. Stokes' Law gives a qualitative indication of the physical factors that influence the stability of an emulsion. This is because the relative flow of the particles under gravitational forces may break the emulsion, so stability is enhanced by small settling velocities.

VIII. HEAT EXCHANGING

In a heat exchanger, heat energy is transferred from one body or fluid stream to another. In the design of heat-exchange equipment, heat-transfer equations are applied to calculate this transfer of energy so as to carry it out efficiently and under controlled conditions (12). The equipment goes under many names, such as boilers, pasteurizers, jacketed pans, freezers, air heaters, cookers, ovens, and so on. The range is too great to list completely. Heat exchangers are found widely scattered throughout the food process industry.

A. Continuous-Flow Heat Exchanger

It is convenient to use heat exchangers in which one or both of the materials that are exchanging heat are fluids,

flowing continuously through the equipment and acquiring or giving up heat in passing. One of the fluids is usually passed through pipes or tubes, and the other fluid stream is passed round or across these. At any point in the equipment, the local temperature differences and the heat-transfer coefficients control the rate of heat exchange. The fluids can flow in the same direction through the equipment, this is called parallel flow; they can flow in opposite directions, called counter flow; they can flow at right angles to each other, called cross flow. Various combinations of these directions of flow can occur in different parts of the exchanger. Most actual heat exchangers of this type have a mixed flow pattern, but it is often possible to treat them from the point of view of the predominant flow pattern.

The rate of heat transfer can be calculated using the heat transfer coefficient, the total area, and the log mean temperature difference. This same result can be shown to hold for parallel flow and counter flow heat exchangers in which both fluids change their temperatures. In parallel flow, at the entry to the heat exchanger, there is the maximum temperature difference between the coldest and the hottest stream, but at the exit the two streams can only approach each other's temperature. In a counter flow exchanger, leaving streams can approach the temperatures of the entering stream of the other component and so counter flow exchangers are often preferred. A popular heat exchanger for fluids of low viscosity, such as milk, is the plate heat exchanger, where heating and cooling fluids flow through alternate tortuous passages between vertical plates. The plates are clamped together, separated by spacing gaskets, and the heating and cooling fluids are arranged so that they flow between alternate plates. Suitable gaskets and channels control the flow and allow parallel or counter current flow in any desired number of passes. A substantial advantage of this type of heat exchanger is that it offers a large transfer surface that is readily accessible for cleaning. The banks of plates are arranged so that they may be taken apart easily. Overall heat transfer coefficients are of 2400–6000 $J\,m^{-2}\,s^{-1}\,°C^{-1}$.

In some food processes, quick heating is required in the pan, for example, in the boiling of jam. In this case, a helical coil may be fitted inside the pan and steam admitted to the coil. This can give greater heat transfer rates than jacketed pans, because there can be a greater heat transfer surface and also the heat transfer coefficients are higher for coils than for the pan walls. One type of heat exchanger, which finds considerable use in the food processing industry particularly for products of higher viscosity, consists of a jacketed cylinder with an internal cylinder concentric to the first and fitted with scraper blades. The blades rotate, causing the fluid to flow through the annular space between the cylinders with the outer heat transfer surface constantly scraped. Coefficients of heat transfer vary with speeds of rotation but they are of the order of 900–4000 $J\,m^{-2}\,s^{-1}\,°C^{-1}$. These machines are used in the freezing of ice cream and in the cooling of fats during margarine manufacture.

IX. EVAPORATION

Evaporation is the concentration of a solution by boiling off solvent and has received major applications in the food industry. The basic factors that affect the rate of evaporation are the rate at which heat can be transferred to the liquid, the quantity of heat required to evaporate each kg of water, the maximum allowable temperature of the liquid, the pressure at which the evaporation takes place, and the changes that may occur in the foodstuff during the course of the evaporation process. Considered as a piece of process plant, the evaporator has two principal functions, to exchange heat and to separate the vapor that is formed from the liquid. Important practical considerations in evaporators include: (1) the maximum allowable temperature, which may be substantially below 100°C; (2) promotion of circulation of the liquid across the heat-transfer surfaces, to attain reasonably high heat transfer coefficients and to prevent any local overheating; (3) viscosity of the fluid which will often increase substantially as the concentration of the dissolved materials increases; and (4) tendency to foam which makes separation of liquid and vapor difficult.

A. Evaporator Configuration

Evaporation systems may be single-stage or multiple-stage (also called "effect") with 2, 3, or more evaporator or vacuum units. The typical evaporator is made up of three functional sections: the heat exchanger, the evaporating section, where the liquid boils and evaporates, and the separator in which the vapor leaves the liquid and passes off to the condenser or to other equipment. In many evaporators, all three sections are contained in a single vertical cylinder. In the centre of the cylinder there is a steam-heating section, with pipes passing through it in which the evaporating liquors rise. At the top of the cylinder, there are baffles, which allow the vapors to escape but check liquid droplets that may accompany the vapors from the liquid surface. In the heat exchanger section, steam condenses in the jacket and the liquid being evaporated boils on the inside of the tubes and in the space above the upper tube plate. The resistance to heat flow is imposed by the steam and liquid film coefficients and by the material of the tube walls. The circulation of the liquid greatly affects evaporation rates, but circulation rates and patterns are very difficult to predict in any detail. Values of overall heat transfer coefficients that have been reported for evaporators are of the order of 1800–5000 $J\,m^{-2}\,s^{-1}\,°C^{-1}$ for the evaporation of distilled water in a vertical-tube evaporator using condensing steam. However, with dissolved solids in increasing quantities as evaporation proceeds leading to increased viscosity and poorer circulation, heat

transfer coefficients in practice may be much lower than this.

As evaporation proceeds, the remaining liquors become more concentrated and because of this the boiling temperatures rise. The rise in the temperature of boiling reduces the available temperature drop, assuming no change in the heat source. And so the total rate of heat transfer will drop accordingly. Also, with increasing solute concentration, the viscosity of the liquid will increase, often quite substantially, and this affects circulation and the heat-transfer coefficients leading again to lower rates of boiling. Yet another complication is that measured, overall, heat transfer coefficients have been found to vary with the actual temperature drop, so that the design of an evaporator on theoretical grounds is inevitably subject to wide margins of uncertainty. For the evaporation of liquids that are adversely affected by high temperatures, it may be necessary to reduce the temperature of boiling by operating under reduced pressure. When the vapor pressure of the liquid reaches the pressure of its surroundings, the liquid boils. The reduced pressures required to boil the liquor at lower temperatures are obtained by mechanical, or steam jet ejector, vacuum pumps, combined with condensers for the vapors from the evaporator. Mechanical vacuum pumps are generally cheaper in running costs but more expensive in terms of capital than are steam jet ejectors. The condensed liquid can either be pumped from the system or discharged through a tall barometric column in which a static column of liquid balances the atmospheric pressure. Vacuum pumps are left to deal with the non-condensables, which are much less in volume but still have to be discharged to the atmosphere.

B. Heat Transfer and Liquid Boiling Point

Boiling is evaporation that occurs throughout a liquid, as a contrast to a surface phenomenon. Heat transfer in evaporators is governed by the equations for heat transfer to boiling liquids and by the convection and conduction equations. The heat must be provided from a source (e.g., condensing steam in most cases) at a suitable temperature. The steam comes either directly from a boiler or from a previous stage of evaporation in another evaporator. Major objections to other forms of heating, such as direct firing or electric resistance heaters, arise because of the need to avoid local high temperatures and because of the high costs in the case of electricity. In some cases the temperatures of condensing steam may be too high for the product and hot water may be used. Low-pressure steam can also be used but the large volumes create design problems.

While lower evaporation temperatures reduce heat damage to the products, in evaporators that are working under reduced pressure, a condenser to remove the bulk of the volume of the vapors by condensing them to a liquid often precedes the vacuum pump. Condensers for the vapor may be either surface or jet condensers. Surface condensers provide sufficient heat transfer surface, pipes for example, through which the condensing vapor transfers latent heat of vaporization to cooling water circulating through the pipes. In a jet condenser, the vapors are mixed with a stream of condenser water sufficient in quantity to transfer latent heat from the vapors.

X. DRYING

Drying is one of the oldest methods of preserving food. Primitive societies practiced the drying of meat and fish in the sun long before recorded history. Today the drying of foods is still an extremely important food preservation operation. The distinguishing features between drying and concentration are the final level of water and nature of the product (11). Dried foods have water content sufficiently low to give solid characteristics, thus can be stored for long periods without deterioration occurring. The principal reasons for this are that the microorganisms which cause food spoilage and decay are unable to grow and multiply in the absence of sufficient water and many of the enzymes which promote undesired changes in the chemical composition of the food cannot function without water. Preservation is the principal reason for drying, but drying can also occur in conjunction with other processing. For example in the baking of bread, application of heat expands gases, changes the structure of the protein and starch and dries the loaf. Losses of moisture may also occur when they are not desired, for example during curing of cheese and in the fresh or frozen storage of meat, and in innumerable other moist food products during holding in air.

A. Moisture in Foods and Dehydration Principles

Drying of foods implies the removal of water from the foodstuff. In drying, water molecules must make their way through the food product to the surface in contact with drying air. Once at the surface, water molecules are transferred into the air based on the difference in vapor pressure between the air and the surface. When the vapor pressure in the air reaches the same value as the vapor pressure of water at the surface of the food, drying ceases. Understanding the behavior of water is important to reach effective water removal and to obtain a high-quality product. In most cases, drying is accomplished by vaporizing the water that is contained in the food, and to do this the latent heat of vaporization must be supplied. There are, thus, two important process-controlling factors that enter into the unit operation of drying: (1) transfer of heat to provide the necessary latent heat of vaporization, and (2) movement of water or water vapor through the food material and then away from it to effect separation of water from foodstuff.

Water activity (a_w), often defined as the ratio of the vapor pressure of water measured at the food surface (p_w) to the saturation vapor pressure of pure water at the same temperature (p_w^o), is commonly used by food technologists to describe how water interacts in food products. However, since this definition is only true for ideal solution at thermodynamic equilibrium, water activity based on vapor pressure measurements is only an approximation. Recently, use of the state diagram to depict conditions of water in foods has been employed, and its use has improved our knowledge of drying technology (21). It is important to note that, as a food product dries, both moisture content and water activity change. At any given relative humidity of air used for drying, there is an equilibrium water content with the product. However, it has been found that the nature of this relationship depends on whether the food product is being dried or allowed to pick up moisture from the air. In general, water that is removed during processing of foods cannot be replaced in exactly the same manner to yield a product identical to the original material.

B. Drying Methods

There are many factors that influence the rate of drying. These are related to either: (1) the process conditions present during drying, as determined by dryer type and operating conditions (e.g., temperature, air velocity, relative humidity, and pressure), or (2) the nature of the food product placed into the dryer (e.g., surface area, constituent orientation, cellular structure, and type and concentration of solutes). Drying processes based on the type of heating medium used fall into three categories: (a) Air and contact drying under atmospheric pressure. In air and contact drying, heat is transferred through the foodstuff either from heated air or from heated surfaces. The water vapor is removed with the air. (b) Vacuum drying. In vacuum drying, advantage is taken of the fact that evaporation of water occurs more readily at lower pressures than at higher ones. Heat transfer in vacuum drying is generally by conduction, sometimes by radiation. (c) Freeze drying. In freeze drying, the water vapor is sublimed off frozen food. The food structure is better maintained under these conditions. Suitable temperatures and pressures must be established in the dryer to ensure that sublimation occurs.

Osmotic dehydration is already considered a valuable tool in minimal processing of food. It is the partial removal of water by direct contact of plant or animal tissue with a suitable hypertonic solution, i.e., highly concentrated solutions of sugars, salts, sugar/salt mixtures, etc. It can be applied either as an autonomous process or as a processing step in alternative processing schemes leading to a variety of end products. During osmotic dehydration two major countercurrent flows take place simultaneously. Under the chemical potential gradients existing across product-medium interface, water flows from the product into the osmotic medium, while osmotic solute is transferred from the medium into the product. A third transfer process, leaching of product solutes (sugars, acids, minerals, vitamins) into the medium, although quantitatively negligible, is recognized as affecting the sensorial, nutritional, and functional characteristics of the final product.

Most of the work done so far refers to osmotic processing of fruits and vegetables with emphasis on the manipulation of process parameters to control product dehydration or impregnation. Recent recognition of direct formulation possibilities has opened a whole new world of potential applications in conventional and new processes for plant and animal products. The osmotic treatment is now viewed as a complex solid-liquid system that has to be analyzed and modeled as a basic unit operation. In this context, fundamental findings such as properties and behavior of biological materials, mass transfer mechanisms, and process modeling, will be directly applicable to every solid/liquid process. Since transfer mechanisms have to be studied at cell level, newly acquired knowledge on physical, physicochemical, and biological properties of biomaterials has to be taken into account. Extensive industrial applications are awaiting a successful address of fundamental issues, such as adequate process modeling, microbiological process validation, and efficient, environmentally friendly osmotic solution management.

XI. COMBINED OPERATIONS

A. Enzymatic Time-Temperature Integrators

Thermal processing, including blanching, pasteurization, and sterilization, has been and still is one of the most widely used physical methods of food preservation. In the context of food preservation, the quantitative measurement of the impact of a thermal process in terms of food safety is of utmost importance in process design, optimization, evaluation, and control. Consumer demands for higher-quality convenience products and the striving of food companies for energy savings and better process control have resulted in the optimization of existing and the development and application of new heating technologies, such as continuous processing in rotary retorts, aspectic processing, ohmic and microwave heating, and combined processes. However, the commonly used process evaluation techniques, including *in situ* and the physical-mathematical approach, have serious limitations with regard to their applicability in these technologies.

A TTI can be defined as "a small measuring device that shows a time-temperature dependent, easily, accurately, and precisely measurable irreversible changes that mimics the change of a target attribute undergoing the same variable temperature exposure." The development of enzymatic time-temperature integrators (TTIs) allows fast, easy, and

correct quantification of the thermal process impact in terms of food safety without the need for detailed knowledge of the actual temperature history of the product. The experimental work involves extensive kinetic studies of α-amylase at different environmental conditions under steady state and non-steady state conditions. After careful isothermal calibration and validation under variable temperature conditions, several TTIs are successfully applied as wireless device to (1) determine the influence of process and/or product parameters on the spatial distribution of process-lethality in a particulate model food system and to (2) evaluate the lethality distribution and hence determine the coldest zone in a retort. Moreover, TTIs are sufficiently sensitive to distinguish between in-pack spatial variations of the lethal impact, indicating that TTIs can be used in monitoring critical control points (i.e., determination of the in-pack coldest point or evaluation of the coldest zone in a retort) as part of a hazard analysis critical control points (HACCP) program.

B. Computational Fluid Dynamics for Thermal Process Optimization

In many thermal food processes, several product units are processed simultaneously, in batch or continuously. To ensure a uniform quality and microbial inactivation in all units, the heat transfer must be as uniform as possible throughout the heating equipment. However, optimization of the design of the processing unit is a difficult task, because of the interaction of many physical phenomena on a relatively small scale in a complex geometry. Thus, analytical solutions exist only in a small number of simplified cases. The most common approach is to use simplified equations, which result from a combination of approximations and require considerable experimental input. At this scale of detail, experiments become very time-consuming and costly.

With the increasing computational power and memory capacity of computers and the development of efficient numerical algorithms, the numerical solution of the governing fluid flow, mass, and energy equation has become possible. This approach of using computers for solving fluid flow problems is known as computational fluid dynamics (CFD). For example, CFD has been applied to thermal food processing in a forced convection oven. CFD was applied to solve the boundary layer near the surface of complexly shaped food products. Although CFD cannot be used as a stand-alone solution but requires experimental inputs to adjust the turbulence models and also careful validation of the results, it has the advantage of ease of use once a reliable model has been established. Then CFD produces quick answers to complex questions and gives details of all the variables involved. The availability of commercial codes with easy-to-use interfaces and flexible pre- and post-processing facilities has cleared the path for the extensive use of CFD in many applications.

C. Minimal Processing with Electric Heating Methods

Three major types of electric heating processes are available: ohmic heating, high frequency heating, and microwave heating. Thermal treatments are known to produce undesirable changes such as loss of vitamins and minerals, formation of thermal reaction components of biopolymers, and, in minimal processing term, loss of fresh appearance, flavor, and texture of food. The electric heating methods directly transfer the energy from the electromagnetic source to the food, without heating up heat transfer surfaces, etc., in the heat processing equipment. This direct energy transfer is of major advantage, as it gives excellent opportunities for high-energy utilization. The ohmic system has been installed for pasteurization and sterilization of a number of food products with resulting excellent quality. The largest application in the food industry for high frequency heating is in the finish drying or post-baking of biscuits and other cereal products. The interest in high frequency defrosting has increased again in the last number of years. Industrial applications of microwave heating, on the other hand, are found for most of the heat treatment operation in the food processing industries.

In electric resistance (ohmic) heating, the food itself acts as a conductor of electricity. The food may also be immersed in a conducting liquid, normally a weak salt solution of similar conductivity to the food. Heating is accomplished according to Ohm's law, where the conductivity, or the inverse, the resistivity, of the food will determine the current that will flow between the ground and the electrode. High frequency heating is done in the MHz region of the electromagnetic spectrum. Foods are heated by transmitting electromagnetic energy through the food placed between an electrode and the ground. Microwaves used in the food industry for heating are mainly due to the dielectric heating mechanism up to moderated temperatures. Polar molecules, the dominant water, of the food is an important factor for the microwave heating performance of foods, especially the penetration ability of the microwave in foods is limited. An important requirement on microwave equipment and microwave energy application in the food industry is the ability to properly control the heating uniformity. A number of new applications in the microwave heating area have been reported recently in patent literature, often involving the use of the unique heating properties of microwaves with higher energy fluxes and direct heating of the interior. However, electric heating equipment for the food industry has to be designed and operated according to international and national safety standards. The levels of allowable leakage vary over the frequency range according to these standards.

D. High Pressure/Temperature

Non-thermal processes are currently receiving considerable attention from consumers as well as from producers

and researchers. Processes that are under evaluation or development include high hydrostatic pressure treatment, the utilization of high electric field pulses, high-intensity light pulses, the application of supercritical carbon dioxide, or the use of magnetic fields. In addition, treatments with biopolymers or with natural antimicrobials are being applied or attempted. Also various combinations of the above mentioned unit operations with thermal processes are being evaluated. To date, the development of high hydrostatic pressure treatment has come close to commercialization. Processing opportunities integrating high-pressure treatment as a processing step can involve preservation as well as modification or separation processes and can lead to product development opportunities. Therefore, it will most likely be the first nonthermal process with applications in oriental foods.

Key process advantages of high pressure application to food systems are the independence of size and geometry of the samples during processing, possibilities for low temperature treatment, and the availability of a waste-free, environmentally friendly technology. Opportunities for effective and relevant utilization of the potential of high hydrostatic pressure center around preservation processes, product modifications, and processes based on phase transitions or membrane permeabilization. A vast amount of empirical information is available regarding the effects of high hydrostatic pressure on a wide range of vegetative microbial cells. Bottlenecks such as the baroresistance of microorganisms within environments of low water activity could be overcome by combinations with mild heat or by pretreatment with ultrasound. However, challenges exist while adapting the technology for specific products. Scientific challenges are the lack of kinetic data, little understanding of mechanisms involved in high pressure effects on food systems, limited knowledge regarding the role of food constituents, and storage-related changes of pressure-treated products. Technical challenges of commercial application of high-pressure technology include material handling, process optimization, sanitation, cleaning, and disinfections as well as package design. Engineering aspects to be dealt with are process inhomogeneities such as heat transfer issues and temperature distribution within pressure vessels. In addition, work on pathogenic microorganisms is still scarce in the published literature and needs continued attention.

XII. SUMMARY

Using new technological solutions, new packaging materials and technologies, or combinations of different (mild) treatments, a variety of products much closer to fresh and wholesome products is increasingly reaching the market. High-pressure treatment is another very promising technology, of a more severe nature, but that has proven successful in preserving quality attributes. However, it must be borne in mind that the development of new technologies is being made while facing a very conventional industry sector, where less technological ability means that optimization of conventional technologies will have greater potential impact, in the short term. In addition, it is equally important to promote the integration of knowledge drawn from different disciplines required to design quality-efficient food processes for the modern food industry and consumer markets.

Worldwide, the food industry is facing relatively stagnant markets in terms of quality—the most important markets for most food companies still are in the part of the world where population is not growing. Economic success is greatly linked to the ability to pay off investments at attractive rates and is basically associated with growth scenarios. Therefore, the interest in market segments involving higher value-added products and with great growth potential is evident. The changes in lifestyle, consumption habits and patterns, and in the diet-health perception are the basic contours of such opportunities. Hence, the focus of researchers and industrialists on technologies and processing approaches and methodologies that can efficiently lead to adequate responses to the newer market demands. While it is likely that the bulk of food production may differ slightly from what it is today for many years, the greater interest for development lies in paths that may lead us to a completely new way of understanding and producing food products, even if market niches are at the moment comparatively small.

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Part K

Food Drying

103 Dehydrated Vegetables: Principles and Applications

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CONTENTS

I. INTRODUCTION

Natural or artificial dehydration is the most traditional method of vegetable preservation. Nature provides man with preserved vegetables, fruits and herbs in the form of sun-dried products. Dehydration accomplishes preservation of such foods in two major ways: (1) it removes the water necessary for growth of microorganisms and for enzymatic activity and (2) by removing water, it increases the osmotic pressure by concentration of salts, sugars and

acids, creating a chemical environment unfavourable for the growth of many microorganisms.

According to the standard definition, dehydration is rather general water removal process, independently of the type of mechanism and driving forces, such as air moisture content or osmotic pressure differences, etc. Drying is rather air-involved process with driving force of the air humidity difference. However, both of the names are used exchangeable.

The largest end-uses for dried vegetables are seasonings, ingredients of soups and ready meals, sauces, gravies and many other food additives. To fulfill the role of the healthy ingredient of our menu, vegetables we consume have to be of high nutritional value, without any microbial and chemical contaminations (pathogenic bacteria, mycotoxins, pesticides, heavy metals, etc.). The same conditions apply to the dried vegetables with some additional requirements such as a high rehydration rate and extended shelf life. Vegetables dried by a proper method and in appropriate process condition, should preserve the maximum nutritional value and quality of a fresh product. Thus, the selection of the drying method and drying conditions should be mainly based on the required quality of the final product. Additionally, for the massive production scale, the energy consumption, and environmental impact of the greenhouse gases production, are the aspects of some special consideration.

General principles of drying are well described in many resources such as "Handbook of Industrial Drying" [1] edited by Mujumdar (1995), "Drying: Principles, Applications and Design" [2] by Strumillo and Kudra (1986), "Industrial Drying of Foods" [3] edited by Baker (1997), "Dehydration of Foods" [4] by Barbosa-Canovas and Vega-Mercado (1996), "Advanced Drying Technologies" [5] by Kudra and Mujumdar (2001), etc. Thus, in this chapter the only specific drying characteristics for vegetable and vegetable products will be considered and for the basic knowledge of the dehydration process the reader is asked to refer to the above handbooks or other widely available food engineering books or book chapters. Also, drying of potato and potato products will be excluded from this chapter as a separate chapter for this important commodity is presented.

II. PRINCIPLES OF VEGETABLE DEHYDRATION

Freshly harvested vegetables are relatively very perishable and further processing, such as dehydration, freezing, canning and fermentation, should be performed almost immediately after the harvest to prevent or reduce quality deterioration. Most vegetables are dehydrated to very low moisture levels (2 to 3%) for increased stability [6, 7].

A key parameter in the food dehydration is a water activity (a_w), defined as the ratio of water vapor pressure (p) of the food system to the vapor pressure of pure water (p_0) at the same temperature.

$$a_w = p/p_0 \tag{103.1}$$

The microbial stability of dehydrated foods, or more precisely, of foods stabilized by a reduced water activity, results from interruption of vital processes essential to microbial growth or spore germination which is mediated by a depressed availability of water in the food. The number and types of microorganisms, which may be associated with foods, is extremely large and may not remain constant during the life of a food. These microorganisms may originate from the raw material or from contamination (by people, animals and insects, water or air, contact surfaces, etc.). Every microorganism has an optimum and a minimum water activity for growth. A reduction of a_w below optimum delays spore germination and decreases the growth rate, and reduction of a_w below the minimum, presented in Table 103.1, totally inhibits the growth or spore germination. However, the reduction of water activity is not sufficient to prevent the growth of all microorganisms. During air drying the increased temperature of the food could affect the living forms of the microorganisms but not the spores of such the species as *Bacillus* and *Clostridium*. For example, dried onion may contain a heavy load of *Clostridium perfringens* spores, which can cause food poisoning when used in a poultry stuffing [8], while *Shigella* is one of the most common gram-negative food pathogen for almost all vegetables and fruits.

TABLE 103.1
Minimum Water Activity for the Grow of Some Microorganisms in Food

Group of Microorganisms	Examples	Minimum Water Activity
Normal bacteria, viruses	*Clostridium botulinum, Salmonella, Bacillus, Pseudomonas, Escherichia, Lactobacillus, Vibrio*	0.91
Normal yeasts	*Candida, Torulopsis*, most *Saccharomyces,* (Also *Staphylococcus aureus*)	0.88 0.80–0.87
Normal molds	Mycotoxigenic penicillia	0.80
Halophilic bacteria	Most halophilic bacteria	0.75
Some molds	Mycotoxigenic aspergilli	0.75
Xerophilic molds	*Aspergillus chevalieri, A. candidus, Wallemia sebi* (Also yeast *S. bisporus*)	0.65
Osmophilic yeasts	*Saccharomyces rouxi* (Also molds: *A. echinulatus,* and *Monoascus bisporus*)	0.60

Source: [8, 9].

It should be noted that the drying process does not necessarily destroy the food toxins occurring as contaminants prior to or during drying. In the case of the food intoxicated by toxins produced by some food poisoning bacteria (*Clostridium botulinum, Staphylococcus aureus*, *Bacillus cereus*) this food should be excluded from the drying process and consumption.

Other typical microorganisms as viruses, protozoa, algae and prions do not grow on food so the only aspects of importance are their pathogenicity or toxigenicity and their resistance to thermal drying procedures as they can be transferred to a human body and develop some diseases [8]. Almost all of these microorganisms are more heat-sensitive than the average vegetative bacterium.

The effect of water activity on microbial growth is influenced by many other factors, such as temperature, pH, nutrients availability, preservatives and other components of food, oxygen supply, etc. With the same water content in the food, several different water activity values can be achieved by changing the food composition. Table 103.2 presents some examples of maximum water activity values, which are permissible for certain types of dehydrated foods. Practical relation between water activity and water content in the food is given by so-called food stability map, introduced first by Labuza [11]. At present, the measurement of water activity is easy with the use of several types of commercially available single or multi-channel instruments; however, moisture content (wet or dry basis) still remains the most practical criterion in the food drying technology. The water activity of fresh vegetables falls in the range 0.97–0.99. Dried vegetables with the moisture content of 14–24% have water activity of 0.7–0.77. The water activity of most dehydrated foods is below the minimum water activity values of food pathogens, which are inhibited at a water activity of 0.9 (only *Staphylococcus aureus* is capable to growing at a_w values down to 0.85). Most other microorganisms such as food spoilage type bacteria and fungi, which can grow at lower water activities, multiply very slowly in this low a_w condition or require a special environment for the growth. In case of fungi (yeasts and molds) they tend to grow more slowly than bacteria and they are often out-competed in many types of food spoilage, unless bacterial growth is limited, e.g., by drying below their minimum water activity, presence of preservatives, etc. In general, yeasts and molds tend to be more resistant than bacteria to harsh environmental conditions (low a_w) and so cause spoilage under such conditions. The nature of this type of spoilage may be similar to that caused by bacteria, except that the growth may be much more pronounced. As some molds may produce toxins (especially mycotoxins) that can result in a variety of acute or chronic toxicity syndromes in man and animals. The foods that may be affected by molds associated with mycotoxins production are, for example, grains, nuts, figs, cocoa, coffee, etc. Production of mycotoxins is generally associated with poor handling practices during harvesting, drying or storage of these foods.

TABLE 103.2
Maximum Permissible Water Activity for Some Dry Vegetables, Fruits and Additives (20°C)

Dry Product	Maximum a_w
Potato flakes	0.11
Dry soup mix	0.60
Peas	0.25–0.45
Beans	0.12–0.80
Sucrose	0.85
Fructose	0.63
Dextrose	0.83
Maltose	0.92
Sorbitol	0.55–0.65
Honey	0.75
Soluble coffee	0.45

Source: [8–10].

A dehydrated product remains stable only as long as it is protected from water, air, sunlight and contaminants. For small packages of dehydrated vegetables, the use of an in-package desiccant (to bring the moisture content of the storage environment to 1% or lower) permits the storage for six months or more at room temperatures without significant losses of vitamins. Some useful information on proper storage condition of dried foods is presented by Labuza [11].

Extending the shelf life of foods is not the only objective of the drying process, however, it is the most important. Other rationales for controlled vegetable drying are [12]: to reduce the product seasonality, to improve transportability and reduce the costs of transport, to improve storage capability and to reduce nutritional fluctuations.

These advantages of vegetables as well as fruits and herbs drying are associated with some negative changes occurring during drying [13, 14], as for example, some "heat damage" of heat-sensitive constituents like vitamins, enzymes, etc., browning, shrinkage, "case hardening," irreversible loss of ability to rehydration, loss of volatile constituents, and changes in moisture distribution within the product. Generally, these negative aspects of drying are at least in the same order of magnitude as those encountered in other preservation methods (canning, aseptic processing or freezing), and drying of vegetables remains a popular method of food preservation.

III. PRACTICAL VEGETABLE DRYING TECHNIQUES

Drying of the agricultural products could be natural, based on a natural action of sun and wind (field-sun drying), artificial (air drying, contact drying, osmotic dehydration, etc.)

or mixed (solar-assisted air/contact drying) which use both natural and artificial energy sources, as for example, in a hybrid solar dryer [15]. All these drying methods are playing an important role in vegetables, spices and herbs dehydration. The heat necessary for the drying is transferred from the energy source to the material by means of conduction, convection, internal generation (microwaves, radio-frequency), surface radiation (sun, light) or by combination of all these. According to the latest trends, in many existing conventional drying systems, solar, wind and other natural energy is increasingly being considered to provide part or the whole energy required for the drying process.

Figure 103.1 presents a general scheme of vegetables, herbs or spices drying. As the variety of these products is extremely large, in individual cases, some of the processing steps may be omitted or others added. In this chapter, only the drying operation, which is the main part of the full processing scheme, will be considered.

A. Sun and Solar Drying

Field sun drying, used for large tonnages of vegetables or fruits, can be very inexpensive in areas of adequate climatic conditions and additionally (but not necessarily, as for example in California) where labor costs are low. No costs are incurred to heat or to circulate the air. Additionally, the solar energy is natural, abundant and environment friendly. Typically, fruits, herbs, spices and to a lesser extent vegetables are spread out in the sun and wind on the field, special mats, concretes, etc. The radiant energy of the sun provides the heat to evaporate the water while the wind helps to move

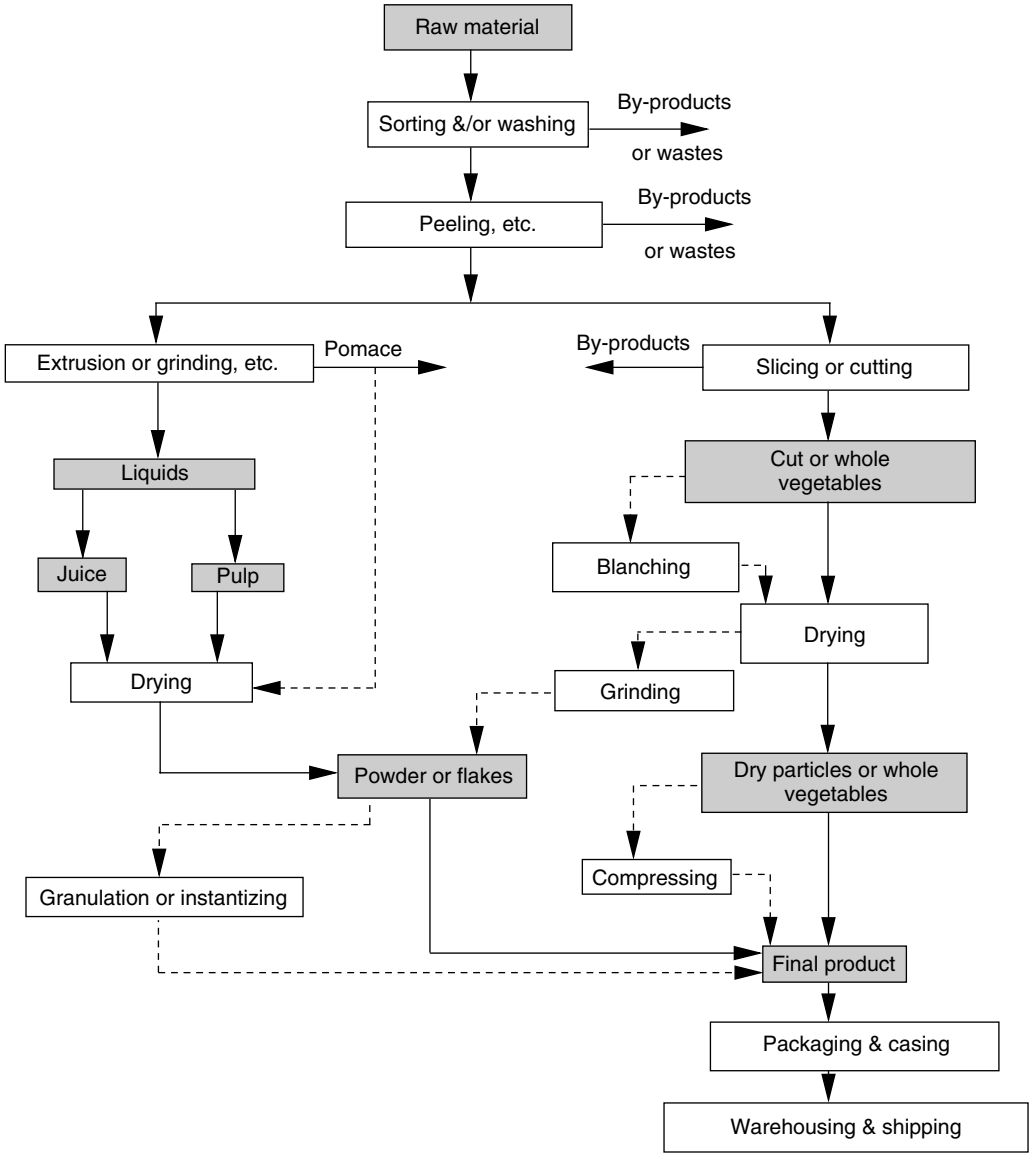

FIGURE 103.1 A general scheme for vegetable drying.

the moisture and accelerates the process. This type of drying performs well in warm and dry conditions on the field or other locations (like shadows for light-sensitive products like herbs, spices, etc.). At night and during the rainy periods, this type of drying cannot be used. The temperature of the food during sun drying is usually 5–15°C above ambient temperature [16]. Sun drying techniques are based mainly on experience and tradition. Thickness of the wet material layer, drying time and material handling before, during and after drying are the conditions normally standardized based on practice and can differ from one location to another. Some very approximate prediction of the sun drying performance could be based on the energy balance from available data on the distribution of solar energy radiation throughout the world, absorbency/reflectance characteristic of the wet and the dry material [16], local climatic conditions such as ambient air temperature, speed and humidity, rainfall frequency and intensity [17]. For example, the thermal efficiency of sun drying of Colombian coffee beans, calculated as a ratio of heat necessary to remove the moisture to the total solar radiation heat, was reported to be in the range, 13.7–22.6% [18].

The time of sun drying depends on the product characteristics and drying conditions and typically ranges from 3 to 4 days, but can be longer [16]. During this time, the product has to be protected from the rain, insects, birds, animals, etc. Several practical methods have been developed to reduce the length time of drying. One of the most important, especially for larger production scale, is the application of different product pretreatment before sun drying. Several pretreatment methods and materials are used for this purpose, for example, steaming, immersion in boiling water or sugar and/or salt solutions or the use of ethyl-, methyl- or sodium oleate, etc.

Another way to reduce sun drying time is to use solar energy concentrators with or without natural or forced airflow inside the dryer. This technique is normally called solar drying. More detailed description of solar drying principles, equipment and conditions may be found in selected publications [19, 20, 21, etc.]. Some practical values of the efficiency of solar drying collectors are given by Hall [22]. Examples of solar dryers developments for vegetables, herbs and spices are detailed below.

1. Solar Natural Dryers (Cabinet, Tent, Greenhouse, etc.)

Solar cabinet is the simplest type of solar natural dryer and very popular in many locations. It is dedicated rather to a small production (family) scale. The wet product is placed in an enclosure and the solar heat, generated through a conversion of solar radiation into low-grade heat, accelerates the evaporation of moisture from the product. The airflow inside the cabinet is driven by natural convection. The dryer throughput and the drying time are estimated from practice (Table 103.3), however considerable effort has been made to model solar collector performance [21]. The thermal efficiencies of solar drying were reported to be from 11 to 13% [24] with the highest value at the beginning of the drying run. These are not the general values for all solar drying applications and an individual level of this parameter should be estimated in each specific drying process.

The quality of the dried product is the most important parameter in the drying technology. Patil [25] compared the quality of peppers after sun drying, polyethylene solar drying and solar cabinet drying. He found that the solar cabinet drying offers some advantages over direct sun drying in terms of better quality and faster rate of drying. Jayaraman et al. [26] compared ascorbic acid retention in cauliflower, cabbage and bitter gourd dried by three different methods: (1) directly under the sun, (2) under the sun in a black polyethylene tent, and (3) in a drying cabinet with three solar collectors. They reported (Table 103.4) that the direct exposure of these products to the sun decreased the quality factors like vitamin retention. The use of solar energy in an indirect way could improve the quality of the final product. In some cases, this was not practical because of large costs of solar collectors. Less expensive solar tents or covers are often used for bigger production scale. Also solar greenhouses, terrace or room dryers are very practical for the larger productions as they are simple in construction and relatively low in investment

TABLE 103.3
Solar Cabinet Drying Throughput*

Product	Amount of Fresh Matter Dried Per Unit Time**	Maximum Allowable Temperature (°C)
Garlic	2.6 kg/per 2 days	60
Grapes	5.7 kg/per 4 days	88
Okra	3.0 kg/per 2 days	66
Onions	3.0 kg/per 2 days	71
Apricots	4.0 kg/per 2 days	66

* *Source*: [23] (with permission).
** Cabinet dimensions: 1.93 × 0.6 m; location: Syria; approximate cost of the dryer: US$ 21.3 (in 1980).

TABLE 103.4
Ascorbic Acid Retention (%) in Some Sun/Solar Dried Vegetables

Drying Mode	Cauliflower*	Cabbage*	Bitter* Gourd	Potato**
Direct sun	2.9	0.005	2.5	45.5
Polyethylene black tent	18.1	24.4	20.2	–
Solar cabinet (with 3 collectors)	27.1	32.9	49.9	61.3***

Sources: * [26], ** [27].
*** Osmosed + Solar (From Reference 27).

costs. Figure 103.2 presents two examples of low-cost solar dryers for relatively larger production scale.

2. Dryers with Solar Collectors and Natural or Artificial Airflow

Some vegetable, fruit and spice dryers utilize both direct and indirect solar radiation. In these dryers, radiant energy from the sun falls directly onto the product being dried. In addition, an air-preheater (solar collector) is also used to raise the drying air temperature. The circulation of air in the solar pre-heater is either free convection or using a fan. In both situations, this air stream accelerates significantly the drying rate. Figure 103.3 presents two examples of very simple and inexpensive dryers of this type. According to published data [21, 23], a family-scale dryer (Figure 103.3a) works well for drying mushrooms, apricots, apples, etc., usually in one day. Similarly, adding a wind-driven fan for more intensive airflow (Figure 103.3b), a higher throughput of the dryer can be achieved. The fan provides air circulation in the dryer and pulls the air up through the product. Dampers can be installed in the stack to control airflow rate, thus also controlling the amount of heat build-up. Several other modifications of this concept are in use for drying purposes in tropical and hot-climate areas. Further extension of the dryer throughput can be achieved by applying forced convection airflow. Another solution in this type of installation is the use of very long metal air-preheater pipes [28] or large surface solar collectors [29]. The flow of air is then artificial with the use of powerful fans. In one construction design [28], the length of the solar air-preheating pipe was about 7 miles. The heating rate of this preheater was reported to be sufficient for proper operation of a solar-assisted sonic dryer for several fruits and vegetables.

3. Solar-Assisted Artificial Dryers

For commercial applications, where a large tonnage of product needs to be dried, some source of auxiliary energy is needed to initiate forced air movement and/or to provide

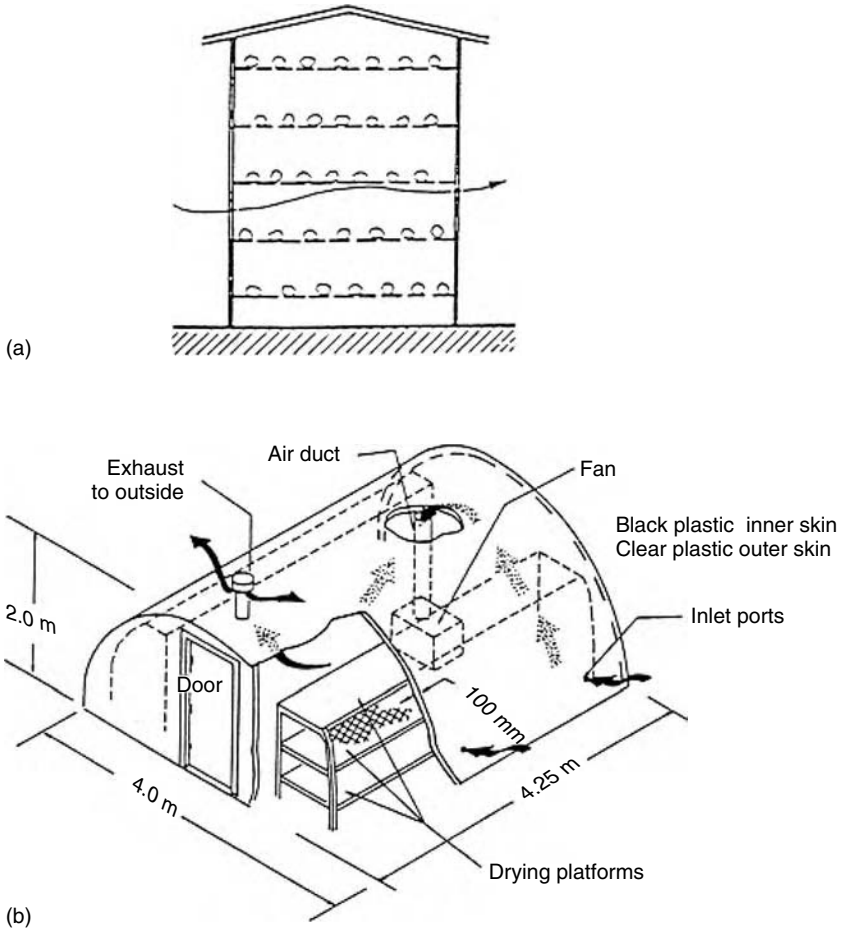

FIGURE 103.2 Examples of large-scale solar dryers: (a) Australian type dryer, and (b) plastic-tent solar dryer. (From References 19 and 21).

the supplemental energy. In this scenario, the solar drying equipment is part of a whole drying system and generally assists operation of a typical air dryer, supplying it some amount of heat. Construction of solar-assisted artificial drying system could be simple or combined with other elements, such as a source of stored heat, heat pumps, etc. In the simplest system the solar energy is directly used to provide a part of the heat required for moisture evaporation in a conventional air dryer. The drying of the food is normally intensive during sunshine hours, while at night or low radiation periods an auxiliary heat source supplements the heat energy. Several conventional types of dryers could be combined with solar energy collectors. For example, Smith et al. [30] describe commercial convective air-drying installation for the final dehydration of potato cubes from 11% down to 4% moisture (wet basis). This system utilizes solar double-glazed collectors and an auxiliary gas burner to heat the drying air. The solar collectors operate at about 40°C above the ambient temperature. In another example, considerable reduction of the fuel-gas was reported by West Growers and Packers [27], when a tunnel dryer was modified for utilization of solar radiation and by using exhaust air to preheat inlet air to the dryer. Another technique that has not been fully explored is the use of a

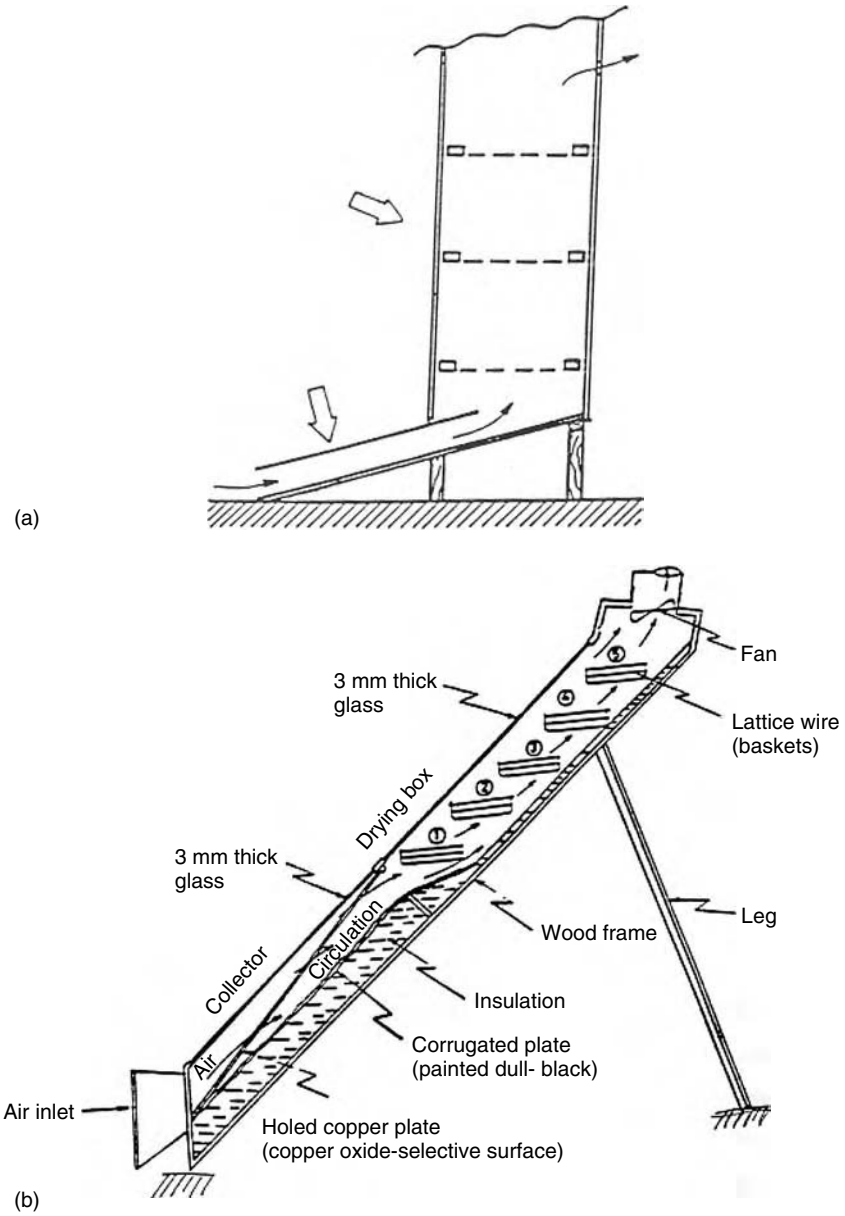

FIGURE 103.3 Low-cost fruit and vegetable solar dryers. (Reproduced with permission of Brace Research Institute, McGill University, Montreal, Canada).

desiccant to remove moisture from the drying agent (used for dehydration of heat sensitive foods), and then solar energy to regenerate the desiccant.

To reduce the effect of periodicity of solar radiation several physical heat storage systems have been developed for drying applications. Water-type and rock-bed type heat storage are the most typical [20].

Proposed schemes of solar energy application should integrate into other drying systems. More generally, solar energy should be applied as much as possible in energy consuming processes such as drying. Active research and development of various methods and components of solar drying systems have been taking place internationally, especially for the reduction of an investment cost associated with solar collectors and adequate control systems [20, 31].

B. Hot Air Dryers

Artificial hot air drying of vegetables and spices covers a very wide range of methods and installations of different production scales. The main mechanism of drying in this case is moisture migration from inside the wet material to the surface and then evaporation to the surrounding air. The drying rate is a function of several parameters such as temperature, humidity and velocity of drying air, moisture content of the moist and dry product, rheological characteristics, specific surface and geometrical form of the initial product, etc. Some general types constructions of the hot air dryers suitable for drying of vegetables and spices will be presented below.

1. Tray, Cabinet, Kiln and Bin Dryers

A tray or cabinet dryer is the simplest type of drying equipment. The operation of the dryer is periodical and the moist product is subjected to unsteady-state process. The dryer allows processing of different products, from liquid slurries to solid piece-form materials. A typical cabinet dryer consists of an insulated chamber into which trayloads of prepared food are placed. A fan pushes or pulls air through a heater and then either horizontally between the trays or vertically through them. By means of a damper system, part of the air may be recycled and part discharged to the atmosphere. Such dryers vary in size from very small units, containing one or few trays to very large units used singly or in groups with a throughput of fresh material up to 20 tons per day. The material to be dried is placed in relatively thin layers (1 to 6 cm thick). Good control of drying conditions is possible. A main disadvantage of these dryers is the large amount of manual work needed to operate the dryer and the relatively low intensity of the drying. However, a wide variety of materials can be dried in these dryers. Because of its versatility, their use is widespread in the food industry. An example of modeling of this type of drying process is given in reference [32].

Bin-type finishing dryers are sometimes used for the final drying and equilibration of some dried vegetables, although often with large modern conveyor dryers they are no longer needed. They are essentially large vertical cylindrical bins with perforated bottoms through which a constant stream of warm air is blown [33].

2. Tunnel Dryers

Tunnel dryers possess all advantages of tray dryers. In addition, they have a semi-continuous operation. This type of dryer is very popular for drying vegetables and fruits. As compared to a tray dryer, the investment costs are higher. A typical dryer may be more than 20 m long with a square or rectangular cross-section of approximately 2 m × 2 m. The wet material is loaded in trays that are stacked on trolleys (Figure 103.4). The trolleys are introduced periodically into one end of the tunnel (the "wet" end), advance through the tunnel and removed at the other end (the "dry" end). A typical tunnel dryer can be operated in co-current or counter-current flow of air and trolleys. Two-stage dryers are also used featuring a short co-current stage followed by longer counter-current stage. In cross-flow designs the drying air moves at right angles to the path of the trays of food. Basic modeling principles for this type of drying are similar to that of a cabinet dryer. The drying times are similar to those in cabinet tray drying while the air flow-rate is linked to the total number of trays. The dimensions of the tunnel are calculated based on the necessary drying throughput, necessary drying time and the dimensions and capacity of a single trolley. More information on the design, construction

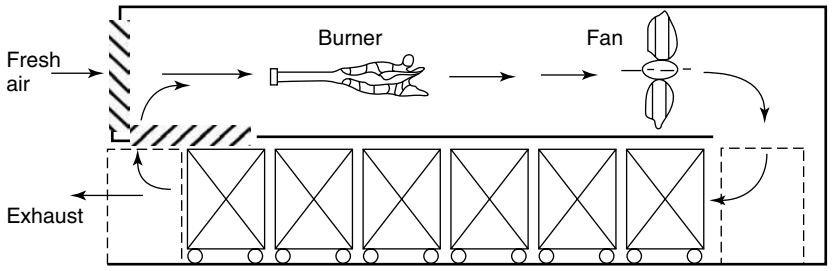

FIGURE 103.4 Simple co-current tunnel dryer with partial air recirculation.

details and operation of this type drier can be found elsewhere [13, 16, 34, etc.].

3. Conveyor-Type Dryers

Fully continuous drying operation is achieved in conveyor, belt or band dryers. This type of dryer is also very popular in vegetable processing industry. The wet product formed or placed in a bed of different thickness is carried through a tunnel on perforated (mesh, slotted or louvered) conveyors. Heated air is directed up or down through the conveyor and the layer of the product; usually up in the early drying stages and down toward the dry product exit, or directed across the material surface for products in thin layers on a non-perforated band. Some models consist of two or more conveyors in a series. Also, either co-current or counter-current configurations can be used. Such dryers are limited to foods that form a porous bed (cut, granulated or naturally particulate foods). For vegetable drying, multiple conveyors (up to 5), one above the other (Figure 103.5) can be used. The wet product is introduced onto the top conveyor and progress downwards from one conveyor to the next. Air circulation is usually a combination of cross-flow and through-flow. A final drying step for some vegetables is provided often in this type of dryer. Gradually, the conveyor dryers are replacing drying trays in tunnels for vegetable pieces such as carrots, onions and potatoes [22]. Infrared, microwave or radio frequency energy is sometimes additionally supplied to the product conveyed through the dryer. The drying unit may operate under vacuum or atmospheric conditions.

4. Fluidized-Bed Dryers

Particles vegetables (whole or diced) may be dried in fluidized bed dryers or several of its modifications: vibro-fluidized, pulsed fluidized or spouted bed dryers [35, 36, etc.]. The main advantage of this type of drying is its short time as a result of high intensity heat and mass transfer achieved due to enhanced air turbulence in fluidized bed. Batch or continuous systems are typical for the drying of food particles in range of 10 to 20 mm. Peas, beans, diced carrots, onions and potatoes are typical vegetables dried in this type of driers. If the particle size of the food covers a wide range, uniform fluidization of the product may be difficult. In such cases, mechanically or pneumatically (pulsed fluidization) induced vibration may overcome the problem. The frequency of the vibration is usually in the range 5–25 Hz with amplitude of few millimeters [36]. This type of drier is often used as a second stage powder dryer or as a granulator after, for example, spray drying in a first stage.

Another modification of the fluidized bed dryer, a spouted bed dryer, may be used in some practical applications, as for example for paprika breaks, carrot cubes, mushrooms and tomato seeds drying [37]. Spouted bed technique could be also used for vegetable or fruit concentrate or paste drying, when it is spread into a bed of inert particles (Teflon, alumina, etc.) circulating as a fountain inside a drying chamber. Pulsed fluidized bed drier, adopting regular pulsations of the drying air (Figure 103.6) as well as other fluidized bed modifications, swirl-fluidizer and centrifugal fluidized bed dryer [35], are still in a development stage. However, they could offer a promising

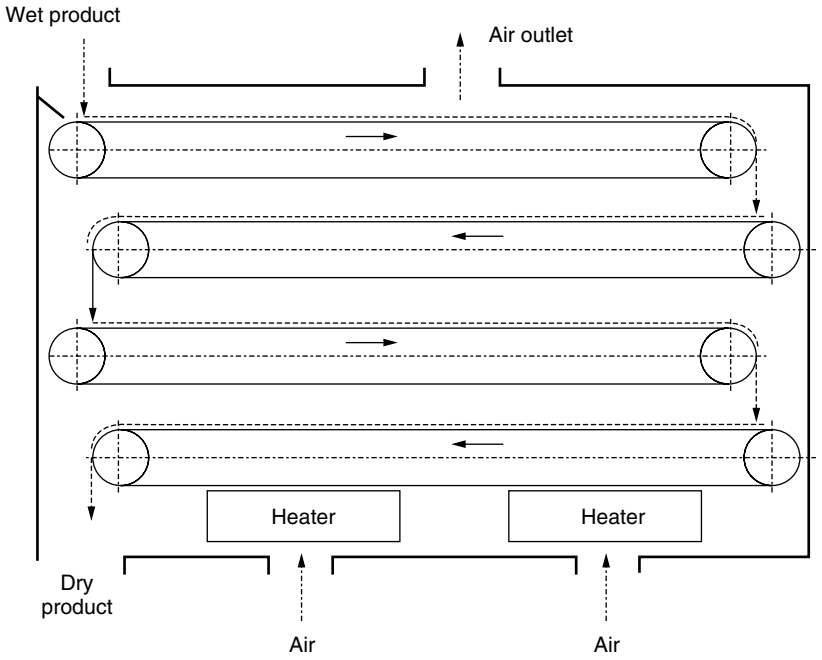

FIGURE 103.5 Diagram of multiple-conveyor dryer.

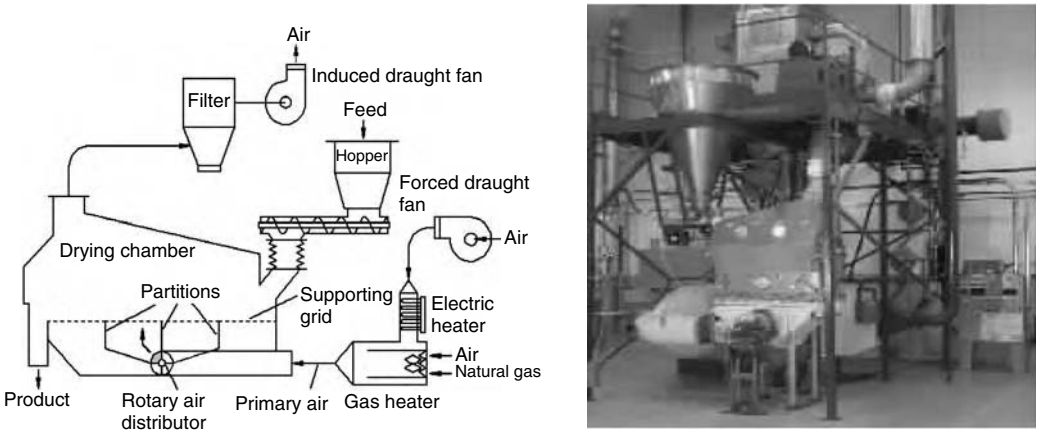

FIGURE 103.6 Schematic diagram and picture of the pilot-scale pulsed fluidized bed dryer (Courtesy of CANMET, Varennes, Canada).

technology for wide range of diced or flaked vegetables: bell pepper, beet, carrot, cabbage, onion and mushrooms [5, 38]. Much more details on fluidized bed drying technique can be found elsewhere [1, 2, 22, 35, etc.].

5. Spray Dryers

Some vegetable powders are produced from juices, concentrates or pulps using a spray drying technique. Dry powders can be directly used as important constituents of dry soups, mixtures or additives. Principles of spray drying are well documented in the literature [39, 40, etc.]. However, its application to vegetable or fruit pastes poses several problems. For example, stickiness, caking and hygroscopicity of the final products must be overcome. Drying of tomato pulp is a typical example of a difficult process as the dried tomato powder tends to become soft and sticky while still warm. The drying is achieved by spraying of the slurry into an air stream at a temperature of 138–150°C and either introducing cold dry air into the outlet end of the dryer or to cool down the dryer walls to 38–50°C [1, 9]. A wide range of vegetable powders can be dried, agglomerated and instantized in spray drying units, specially equipped with internal static fluidized bed, integral filter or external vibro-fluidizer [41].

A typical spray drying unit consists of large volume drying chamber, feed atomizer, hot air supply and dry product recovery/dust collection systems. Three consecutive steps of the process can be distinguished: (1) atomization of the feed, (2) spray-air mixing and moisture evaporation, and (3) separation of the dry product from the exit air.

Atomization is the most critical operation in the spray drying operation. Three general types of atomizers are available with many other individual developments. The most commonly used are rotary wheel atomizers and single-fluid pressure nozzle atomizers. Pneumatic two-fluid (compressed air/liquid feed) nozzles are used rarely in very special applications (slurries, past-like materials, etc.). Co-current, counter-current or mixed flow of the droplets and drying air is typical in spray drying chambers. The geometry of the drying chambers depends mainly on the type of atomizer used. It is generally short and wide (conical type) for rotary wheel atomizers and long and narrow (tall type) for nozzle atomizers. The dry product is collected at the bottom of the drying chamber and/or in cyclones, bag filters or electrostatic precipitators. The final wet separation of the powder from the exhaust air is provided in wet scrubbers, wet cyclones or irrigated fans [39, 40]. Some spray drying systems, equipped with special atomizing devices, are used for drying paste-like materials such as vegetable concentrates.

6. Other Hot Air Dryers

Pneumatic conveying dryers are generally used for finish drying of powders and granulate of vegetable and fruit products. It is extensively used for making potato granules. As the material entering the dryer must be conveyable in an air stream, the incoming material usually is predried in another way (spray, fluidized, spouted bed dryers, etc.) to a moisture level below 40% (w.b.).

Also, rotary dryers may be sometimes used for drying of particulate solid foods. In these dryers, the wet solids (particles, crystals, etc.) enter one end of a rotating drum, move down the drum length cascading from peripherally mounted lifting flights, and exit at the other end suitably dried. The drying medium is either hot air or combustion gases that flow co-currently or counter-currently to the direction of the solids through the drum length. The size of these dryers varies from approximately 0.3 m in diameter by 2 m length to 5 m diameter by 90 m length. Many details of these last techniques are given in the literature [13, 22, 42, etc.].

C. Contact Dryers

Hot surface of the contact dryer is the source of heat necessary to evaporation of moisture from the vegetable or

fruit concentrates, pulps, pastes or slurries. This type of drying is more economical than for example spray drying but the dry product has usually different characteristics than that from a spray dryer. A typical example of the contact dryer is a drum dryer with a single, double or twin drums. The single-drum dryer comprises only one roll. A double-drum dryer comprises two rolls, which rotate toward each other at the top. This gives the possibility to adjust the feed thickness layer by changing the space between them. The twin-drum dryer is similar in appearance to the double-drum dryer, but is quite different in its operation. The two drums occupy the same general position as in a double-drum dryer but rotate away each other at the top and are not spaced closely together. The vegetable pulp is fed onto the metal surface of the drums by mean of various types of feeders. Doctor blades remove the dry product. Depending on the material properties, the product is removed in the form of powders, flakes, or webs. The drum system can be entirely enclosed in a hood for vacuum operation or for supplying with the necessary amount of drying air. Thus, organic solvents or volatile can be recovered in this process. A typical example of single-drum drying operation is the drying of mashed potato. Double-drum or twin-drum dryers are in commercial use for drying tomato and pumpkin purees, mashed white potatoes, etc.

Special types of contact dryers — plate dryers with rotating radial arms are dedicated to drying of solid particulate materials [38]. All contact drying units may operate under vacuum, at atmospheric pressure or in gas-tight chambers.

D. Foam Drying

Two different foam-drying methods are of practical importance: foam mat and foam spray drying. Foam-mat dried vegetable or fruit powders have less heat-induced changes in color and flavor as compared with conventional spray-dried or drum-dried products. A product with density less than that in a conventional drier is obtained. The product density is about equal to the density of instantized or agglomerated powder.

Stable gas-liquid foam is the primary condition for a successful foam drying. Glycerol monostearate, solubilized soya protein and propylene glycol monostearate are the typical additives for the fruit and vegetable foam formulation from juice or pulp. Foam mat drying involves drying a thin layer (0.1–0.5 mm) of the stabilized foam in air at 65–70°C within few minutes only, as the foam structure decreases drying time to about 1/3. The foam is spread on perforated floor craters as the airstream is forced through the bed. A continuous belt tray dryer as well as slightly modified spray dryer [22] can also be used to this process. Good quality tomato powder can be produced using this technique. Optimal initial concentration of feed solids is in the range of 30% for tomato pulp. The cost of such a drying process may be higher than spray or drum drying, although lower than vacuum and freeze-drying. More technical details on foam-mat drying are given in van Arsdel et al. [13].

E. Explosion Puffing

Overheating of a wet food product in a pressurized chamber, called gun, followed by a quick release of the pressure to atmospheric condition is the technique, called explosion puffing, which allows to produce a good quality, porous dry material. The amount of water contained typically in fresh vegetables and fruits, is usually too large to evaporate entirely during the explosion puffing process. An initial pre-drying of the fresh product is therefore necessary when using this technology. Table 103.5 presents some examples of practical values of this initial moisture content for the explosion puffing of selected vegetables and fruits along with some other necessary processing conditions [1, 13]. The average moisture content of vegetables and fruits in order to start the process should be brought down to 15–35%. Such partly dried food pieces are put into a puffing gun. The gun is sealed and pressurized by heating directly (superheated steam) or indirectly, through the walls (gas flame, electric heater, etc.). As the pressure and temperature inside the chamber reach a certain level (Table 103.5), the door of the gun is suddenly opened and explosion of the moisture from the food occurs. The advantages of this technique include shorter drying time (which may be lower by a factor of two or three as compared with conventional air drying), good reconstitution characteristics because of the porous structure, and good organoleptic properties. A final-stage drying is sometimes necessary after the explosion puffing.

A simple batch explosion-puffing gun is essentially a cylindrical pressure chamber with quick-release lid, and is heated internally or externally. A more sophisticated continuous explosion puffing system also has been developed which allows up to 44% reduction in steam consumption as compared with conventional dehydration [43].

F. Vacuum and Freeze-Drying

To intensify moisture removal and lower the drying temperature to protect heat sensitive food components, vacuum drying technique is employed. Some vegetables as well as herbs and spices are often dried using this technique. Typical batch or continuous drying equipment is used with some modifications to sustain vacuum created by a pump connected through a moisture condenser.

A special case of a vacuum drying, i.e., freeze-drying, is very important in food drying operations. Removal of the moisture is a result of sublimation, without of a phase change from solid (ice crystals) to liquid. Figure 103.7 presents a phase diagram of the water with an indication of

TABLE 103.5
Explosion-Puffing Processing Conditions of Some Vegetables and Fruits

Commodity	Moisture Content Before Puffing (% wb)	Steam Pressure (kPa)	Temp. of Process (°C)	Dwell Time (s)	Rehydration Time (s)
Beets	20–26	276	163	120	5
Carrots	25	275	149	49	5
Celery	25	275	149	39	5
Mushrooms	20	193	121	39	5
Onions	15	414	154	30	5
Peppers	19	207	149	45	2
Potatoes	25	414	176	60	5
Apples	15	117	121	35	5
Blueberries	18	138	204	39	4
Cranberries	17–26	138	163	64	3
Pears	18	228	154	60	5
Pineapples	18	83	166	60	1
Strawberries	25	90	177	–	3

Source: [1, 13].

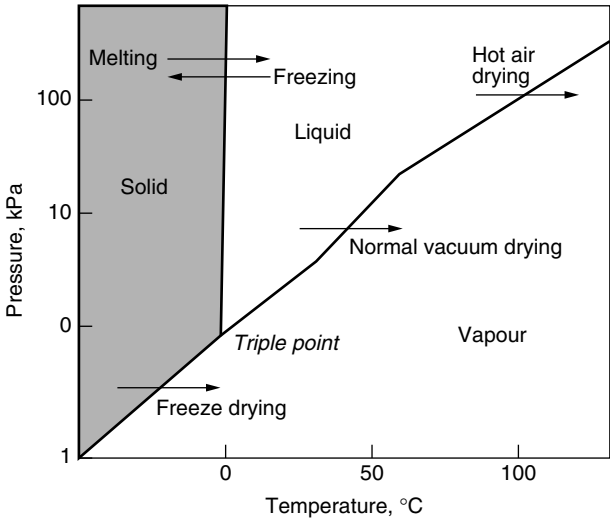

FIGURE 103.7 Phase diagram of water in application to drying process.

conditions for normal pressure air drying, vacuum drying, and freeze-drying processes. The advantage of freeze-drying over other methods of drying is the superior quality of the product obtained. Little or no shrinkage occurs. The dry product will have a porous structure and a color almost as fresh as with the raw material. The only disadvantage of this process is the high equipment and operational costs — the highest amongst all typical drying methods. This explains why the freeze-drying is only used for more expensive products such as coffee extracts, instant dry soups, powders of exotic fruits, spices and herbs. Examples of vegetable freeze-dried products are: tomato, vegetable juices and flavors, asparagus, beans, cabbage, cauliflower, celery, mushrooms, onions, peas, parsley, chives and many herbs.

FIGURE 103.8 Picture of pilot-scale freeze dryer.

There are two main stages in the freeze-drying process: (1) freezing of the food, when most of the water is converted into ice, and (2) sublimation, when the bulk or all of the ice is transferred into vapor under very low pressure or high vacuum, and this vapor is removed from the dryer. In some cases, additional final drying, in the same or other equipment, is necessary. Cabinet or tunnel batch type dryers are typically used with pressures in the range 13.5–270 Pa. Commercial high performance freeze-drying cabinets are fitted with heated shelves on which trays of frozen food are placed (Figure 103.8). Necessary to sublimation heat is transferred from the heated shelves by conduction while microwave or infrared radiation heat is sometimes additionally supplied [44]. In a tunnel freeze dryer the food is placed on specially designed trays, which are assembled on tracks. The tracks enter the tunnel through a vacuum lock. In the main tunnel, the trays move between stationary heated plates and exit through another lock at the dry end. Fully continuous freeze dryers are developed for some specific applications. Particulate

solids are conveyed by a screw, belt or vibrating plates through vacuum chambers. Spray [10] and fluidized-bed atmospheric freeze dryers [45] are examples of modifications of the typical freeze-drying process. All details on the freeze drying technique can be found in several handbooks, manuals, prospects, etc. [1, 44, 46].

G. Radiative and Acoustic Drying

Microwave drying is becoming steadily more popular in drying technology. However, research is needed, especially for bigger production scale. Drying of potato chips, peas, onions, beans and vegetable juice concentrates are examples of such applications [10].

Infrared drying is used for thin layer drying of some foods and especially spices [10]. These products are conveyed beneath infrared radiators on belts or vibrating plates. Some research work was done by Karuri [47] for infrared drying of potatoes and carrots.

Acoustic drying with waves of 12–19 kHz and intensities between 128 and 132 dB was applied to drying of green rice in a fluidized bed at 20 and 40°C. Successful combination of solar and acoustic energy for drying of different agricultural products was also reported [28].

H. Osmotic Dehydration

An example of minimal processing dehydration method of foods, especially vegetables, fruits and meats, is osmotic dehydration. The process is based on a tendency to reach equilibrium between osmotic pressure inside the biological cells (vegetable, meat, fruit, etc.) and the surrounding osmotic solution, which has an increased osmotic pressure by high concentration of soluble osmotic agent. Molecular diffusion of water through semi-permeable cell membrane takes place and the product will loose its water. The osmotic dehydration process is stopped when the osmotic pressure inside the tissue reaches osmotic pressure of the surrounding syrup. Theoretically, molecules of the solute, being large in size, do not migrate en mass from the osmotic syrup into the product. Practically, however, some migration (infusion) of the solute occurs. A net mass loss is therefore a difference between water removal and solids gain. Osmotic dehydration can provide the reduction of the water activity to some extent, which is limited mainly by the water activity of the osmotic syrup itself. For example, typical osmotic syrup, such as a sucrose solution of 60°Brix, has a water activity of about 0.85. This means that food osmotically dehydrated in the above solution would be equilibrated to a water activity of 0.85 and therefore is not necessarily safe from a microbial point of view, and a second stage of drying is often necessary. This second stage could be a typical hot air drying, solar drying as well as vacuum, freeze, microwave or any other type of drying. Several two-step drying processes, with osmotic dehydration in the first step, have been developed for some vegetables and fruits. For example, high quality dry carrot and potato products have been produced while dehydrated celery has not given satisfactory results [48].

FIGURE 103.9 Pilot-scale continuous osmotic dehydration unit (Courtesy of Food Research and Development Centre, Saint-Hyacinthe, Canada).

Typical batch-type osmotic dehydration equipment consists of a mixer tank for the syrup with heating elements to maintain temperature around 50°C; however, higher or lower temperatures are possible depending on the type of dehydrated product. Continuous osmotic dehydration units have also been developed. Figure 103.9 shows a pilot scale continuous osmotic dehydration unit applied to carrot as well as other fruits and vegetables. More detailed data on practical and theoretical aspects of fruit and vegetable osmotic dehydration can be found elsewhere [1, 15, 49, 50, etc.]. Osmotic infusion of some additives as for example caffeine, vitamins, etc., prior to regular drying, is a technique used for fortified new dry foods [51].

IV. DRYING OF SELECTED VEGETABLES

A. Carrot

Carrots can be crosscut or crinkle-cut as rings and dried as such. Starch coating may be used to extend the shelf life of the dry product because oxidation of carotene together with off-flavor and odor can be a significant problem during storage. Typically, steam-blanched and sulfited or steam-blanched and starched dices are spread onto trays at a loading of about 6 kg/m^2. Carrots are then dried in the tunnel or on continuous single or multi-conveyor driers to

a final moisture content of about 4% (w.b.). Hot air temperature in such a typical counter flow tunnel dryer is 71°C and about 7 h of drying is necessary to achieve an optimal moisture content of 8% (w.b.) in the product. Additional bin drying at 60°C through 7 h gives a final product moisture content of 4% (w.b.). A relatively fast drying (50 min) of diced carrot in continuous belt-through dryers was reported [13]. Freeze drying (temperature –33°C and absolute pressure 0.2 mmHg) as well as explosion puffing, are other drying techniques employed for carrot on a limited scale. Low-oxygen packages (vacuum, nitrogen or carbon dioxide atmosphere) with O_2-levels lower than 2% are necessary to prevent carotenoid deterioration during storage.

Sethi and Anand [52] prepared intermediate moisture (IMM) carrot slices using an osmotic solution containing sugar, glycerol, water, acid and preservatives. The processed product has good color, flavor and texture. The slices treated with 500 ppm of SO_2 and 0.45% potassium sorbate remained free from microbial spoilage at 39.2% moisture level (water activity 0.85). At low temperatures (1–3°C), the ready-to-serve product remained acceptable for 6 months in a glass container with a beta-carotene retention of 40%. More details of carrot drying technology can be found elsewhere [13, 53, etc.].

B. Beets

Peeled beets are sliced, diced or strip-cut to specific size. Steam blanching for about 6 min is used in many cases, but not always. Sulfiting of the raw beets is not practical. Tunnel and conveyor dryers of different configurations are typically used. Relatively high drying air temperatures (95–100°C) may be used in the first drying stage, however, a lower temperature of air (71°C) is desirable in the second stage. Final moisture content of beets in such a dryer is 11% (w.b.) and normally an additional drying to 5% (w.b.) in stationary bins is necessary.

Explosion puffing of dices is commercially used for a good quality dry product. Air pre-dried diced beets of 45% moisture (w.b.) are processed in a puffing gun. They are heated to the pressure of 0.3 MPa in steam and then the pressure is quickly released to the atmospheric level. The exploded product, which has lost about 5% moisture, is then finish-dried on trays, continuous belt, etc. The porous structure of the final product results in much lower dehydration time than conventional drying techniques.

C. Onion and Garlic

Dried root vegetables, onion, garlic, potato, etc. have found extensive use in food manufacturing both in dry product formulation and as well as ingredients for wet soups, baby foods and ready or canned meals [6, 13].

a. Onion

Onions are generally dehydrated and pickled. The cultivars with 15–20% total solids content (dry matter) are preferred for dehydration. Onions used for processing should have high pungency, since the dehydrated product is primarily used as a flavoring agent and some pungency is lost during the drying process. Southport White Globe and White Creole are suitable cultivars for dehydration. Sliced, flaked or kibbled onions are mostly dried on continuous belt conveyors or tunnels to a target moisture content of 5% (w.b.) or less. Onions are normally not blanched to avoid the flavor loss that would otherwise occur during this process. When dried in tunnel-type dryers, the sliced onions are automatically spread on wooden trays with a unit load of about 5 kg/m^2. The trays are automatically stacked on cars and conveyed through a typical two-stage tunnel dryer. In the first (co-current) stage, hot air of temperature 70–88°C is normally used and in the second (countercurrent) it is 55–60°C. After 10–15 h the dry material has 5–7% moisture, which may then be dried, in bins to the final moisture less than 5%.

A more practical equipment for onion drying is a multistage continuous belt-conveyor. A stainless-steel perforated belt is normally used with hot air flowing through the belt and a bed of onion slices of 10–15 cm in thickness. The temperature of the drying air is gradually reduced from about 80° to 55°C as the sliced onions move through the dryer. The average drying time is 6 h with a final moisture content of 6% (w.b.). An additional drying (12–30 h) in stationary bins using partially dehumidified air of 43.3–50°C gives the final product of 4% moisture or less. The product may be passed between revolving metal rolls to flake and screened to remove fines (small broken pieces).

A small proportion of dry onion is produced by freeze-drying. Sun-drying of sliced onions is carried out in some areas [6].

For commercial use, sliced, chopped, minced, granulated, or powdered dry onion is produced. High hygroscopic dry onion products require special low air-humidity atmosphere in the final processing stages such as screening, grinding and packaging. A variety of moisture-resistant containers, such as plastic or aluminum bags, jars, clipboard boxes and metal cans, are used for the retail market.

b. Garlic

In the North America the bulk of the garlic production is dehydrated. Garlic is a basic flavoring for multitude of dishes ranging from vegetable soups and sauces, meats, salads, tomato products, spaghetti, sausages, pickles, etc. Fast food restaurants and steak houses invariably offer garlic bread, garlic butter and salt. All other forms and types of dry garlic products, as for onion, are commercially available.

Drying technology of garlic is almost the same as that of onion [6, 13]. The garlic bulbs are broken into individual

cloves, washed, sliced, and then dried up to 8 or 6.5% (w.b.). Compared to the onion, garlic has a considerably lower initial moisture content. The garlic is dried for a short time in an unheated air current to separate the cloves. They are then passed between large rubber rolls, taking care that the cloves are not crushed and the clumps of garlic are broken up into individual cloves. After separating pieces of paper husks, cloves are graded into several sizes. Pieces of skin and other light debris are also separated by floating the cloves in water one to two times. The cloves are separated from water by dewatering screens. They are next sliced by special revolving, very sharp knives and spread automatically on trays or on perforated stainless steel belt of a continuous dryer. Two-stage dehydration is employed in tray-and-tunnel dehydration. The sliced garlic is dried to about 8% moisture, the drying being completed in bins at 43.3 to 48.9°C to less than 6.5% moisture to produce all forms of dried product.

D. Other Vegetables

Many other vegetables are being dried. However, it is to a much less extent than what was described above. Generally, similar drying methods and conditions can be used for most vegetables. Gentle drying conditions are recommended as vegetable tissues are very heat sensitive.

a. Green beans

Green beans are dried on the trays of tunnel dryers or in belt dryers, either while still frozen or in a thawed state. A two-step tunnel drying is recommended; first at 90°C and then at 62°C. A final moisture content of 5% (w.b.) is typically a target value.

b. Cabbage

Cabbage, typically, is cored, trimmed, washed and shredded by kraut cutters before blanching and sulfiting. The pre-treated material is dried in a two-stage tunnel dryer with an inlet air temperature of about 80°C in the first (parallel air flow) stage, and 60°C in the second (counter-current air flow) stage. A finish drying step from the moisture content of about 7 to 4% (w.b.) is provided in stationary bin dryers, supplied with an air at 50°C. A strong compression of the dry product before packing was a standard procedure for cabbage packed in cans during World War II, but this procedure is not in use anymore.

c. Sweet potato

The sweet potatoes are peeled before dehydration in abrasive peelers, steam, or lye-peeled in 15 to 20% sodium hydroxide lye solution. Peeled sweet potatoes are then washed thoroughly, trimmed, sliced or cut into strips or diced. After thorough steam-blanching for about 7 min, they are dehydrated on trays at about 71°C to a moisture content of less than 7%. The tray load is about 5–8 kg/m^2. A cultivar, Goldrush, is recommended for a freshly dehydrated product of highest quality [6].

d. Mushrooms

They are processed into more durable products in various ways. They can be air-dried, freeze-dried, canned or frozen. The processed mushrooms are prepared as buttons, whole or sliced. Being very perishable, mushrooms should be processed as soon as they are harvested, within a period of few hours. More expensive mushroom varieties are freeze-dried while less expensive air-dried. For use in dehydrated soups and specialty products they are mainly freeze-dried (6).

A lot of practical information of other vegetable dehydration procedures can be find in specialized literature and recent publications [1, 6, 13, 54, 55, etc.].

V. SPICES

The quality of dry spices, culinary herbs and spice blends is strongly affected by the drying method and drying conditions that are applied. The main attribute of spices is the improvement of food flavor and taste by some characteristic components, especially volatiles. These components are very heat-sensitive and they should be preserved (to prevent evaporation or decomposition) to the maximum level during all processing steps. The drying process involves some thermal treatment, so the drying method and the conditions should be very delicate and individually chosen for each type of spice or herb. Low temperature natural or artificial drying is the common practice.

The measurement of moisture content in spices presents some unusual problems. Moisture is usually measured in food products by measuring the weight loss of a sample during a drying test at elevated (around 100°C) temperature or under the vacuum. The volatile oils, typically existing in spices, are also lost during drying and this weight loss would be measured as moisture. To resolve this problem, the trade has adopted a co-distillation method for most spices. In this test, the spice is covered with toluene and the toluene brought to its boiling temperature. The moisture in the spice co-distills with the toluene and as the toluene is condensed, the moisture separates from the toluene and is measured. Karl Fisher titration could be also employed for some spices while for other spice products (paprika and other capsicum, etc.) a standard drying oven test is sufficient. Vacuum drying techniques are also frequently used to evaporate moisture at lower temperatures.

The final moisture level in dry spices is of practical importance, as for all other perishable foods. It should be low enough to prevent microbiological growth but not too low to overcome some unfavorable changes in an over-dry tissue such as color losses and increased hardness. An

average of 10% moisture content (w.b.) is desirable for most spices. Detailed data on several typical dry spice chemical and physical specifications and applications are presented by Charalambous [56], Tainter and Grenis [57], Richard [58], Farrell [59], Rosengarten [60] and Purgelove et al. [61].

Sun and field drying of spices and herbs is the oldest and still the most typical drying method for the majority of these products. In many cases direct exposure to the sun can create discoloration of the spice or herb tissue so shadow-open-air or cabinet drying is applied. Larger scale drying of spices and herbs is performed as for drying of typical fruits and vegetables (e.g., tunnel and conveyor dryers). Multi-step processing, with hot air temperatures decreasing with the advance of drying (for example, 110°C initially, then 60°C and 40°C at the end of drying) is very typical. More details of preparation, drying, characteristics and application of the spices can be found in other references [56–61].

VI. CONCLUDING REMARKS

Vegetables as well as fruits, spices and herbs play an extremely important role in human nutrition. Additionally, the recognition that the intake of nutrients, minerals, and antioxidants (vitamin C, E, etc.) may have a protective role against illnesses and tiredness will mean that vegetables and fruits — a potent source of relevant nutrients, minerals and vitamins — will be in demand. However, these have to be preserved in a form that protects these constituents. It is likely that the market for fresh, frozen and properly dried vegetables and fruits will outgrow other forms of processing in the short term. For drying technology, this means that only the methods giving an appropriate quality of the final products may have good practical perspectives. Research should be dedicated to the development of proper drying processes. An improvement of existing as well as the introduction of new drying techniques are equally important aspects of the drying research. For spices, it is well known that they have not only the taste and flavor effect on the food, but some antioxidant and microorganism inhibition activity too. Proper drying techniques have to be applied to each individual product to ensure the highest quality in the product. Energy and environmental aspects of this process should also be considered.

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104 Dehydration of Tropical Fruits

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CONTENTS

I. INTRODUCTION

Tropical fruits are defined as those fruits that grow well in the tropical region (between the Tropic of Cancer in the north and the Tropic of Capricorn in the south), the region where the temperature and the length of the day varies little throughout the year and frosting scarcely occurs. The fruits are rich sources of vitamins, especially A and C, minerals, carbohydrates and flavour for the people of the tropical regions and beyond. Although most tropical fruits are cultivated for fresh consumption, a few of them including avocado, banana, carambola, coconut, guava, kiwi, lychee, mango, papaya, passion fruit and pineapple undergo significant processing before consumption (1). However, they are highly perishable and in the absence of adequate modern handling, transportation and storage facilities in these regions, there is considerable loss due to spoilage which is aggravated by high ambient temperatures

(22–32°C) and humidity and prevailing unhygienic handling practises (2). Among the several process technologies, the major ones being canning, freezing and dehydration, which have been employed on an industrial scale to preserve fruits, dehydration is especially suited for developing countries with poorly established low-temperature and thermal facilities. Drying brings about substantial reduction in weight and volume, minimising packing, storage and transportation costs, and storability of the dried product under ambient temperatures, features that are especially important for developing countries (3).

Fruits may be dried in one of the following forms (4): as whole forms (e.g., various berries) using sun drying, solar cabinet drying and hot air drying; as sliced, chopped or particulate forms (e.g., banana, mango, papaya, kiwi) using hot air drying (with conveyors or trays), freeze drying, and vacuum drying; and as pastes or suspensions using spray-, drum-, and solar drying.

The aim of this chapter is to present an overview of techniques and applications developed for the drying of tropical fruits and their products. The current trends in drying, involving combination drying methods are presented in as far as tropical fruits are concerned. The reader is referred to the literature cited for further details about the methods.

II. METHODS OF DRYING TROPICAL FRUITS

A. Sun and Solar Drying

The traditional open-air sun drying of agricultural crops has been practised since the beginning of civilisation and is by far the most widely practised agricultural processing operation in the developing countries (5). In sun drying, the product is spread on floors or raised platforms and exposed to the direct sun radiation and natural air currents. Occasional stirring of the product is done to expose most of it to the sun and thus speed up the removal of saturated air from the product (6).

The long history of sun drying has not been without drawbacks, which limit its use for large scale production. The drawbacks include: difficulty in controlling the drying rate, the requirement of more land and labour for spreading the product, infestation by insects and microbial contamination (7). Some of these drawbacks however, can be addressed by using solar drying, since this gives improved quality products and has significantly less drying time than open-air sun drying (8).

Solar dryers are identified according to their method of collecting solar energy and transferring it to the product as indirect, direct and mixed-type (also known as convective, radiative and radiative-convective dryers respectively) (9). Solar dryers that use air as the main transport of heat to the drying product may be either free- or forced- convection type.

FIGURE 104.1 Direct solar dryer at NARO-National Banana Research Programme, Kawanda, Uganda.

In the direct solar dryer, the product absorbs direct sun radiation, which passes through a transparent cover or side panels. Evaporation takes place principally from the top surface of the product, which also conducts some of the absorbed heat to the interior of the product. A direct solar dryer is shown in Figure 104.1.

Indirect dryers have a separate compartment, the collector, in which air from the ambient passes through and is heated before passing through the drying chamber. The hot air performs two functions; it supplies the necessary heat to evaporate moisture from the product as well as carry away the evaporated moisture.

The mixed-mode type of dryer combines the features of both the direct and indirect type of dryers (10). The product is therefore dried by a combination of both direct radiation with conduction of heat into the top product and convection of hot air from the collector entering the bottom of the drying bed. Mixed-mode solar dryers give higher drying rates compared with other types of solar dryers (11–13). A mixed-mode solar dryer is shown in Figure 104.2.

Although the use of solar drying has advantages such as free, non-polluting and renewable energy source, the periodic nature of solar energy requires an auxiliary source of energy from fuel or electricity for use during periods of no or low radiation (8). Bolin and Salunkhe (14) have reviewed solar drying methods with and without auxiliary energy source. Further, part of the energy gained during radiation periods can be stored and used later during no or low radiation periods.

Pablo (15) studied the comparative performance of a polyethylene solar dryer with sun drying for drying banana, jackfruit, mango and papaya and found that the polyethylene dryer produced fruits with a better colour and texture than sun drying. Furthermore, the solar dryer reduced the drying time by 25–40%. Similarly, Kalra and Bhardwaj (16) dried mango slices and leather in two solar

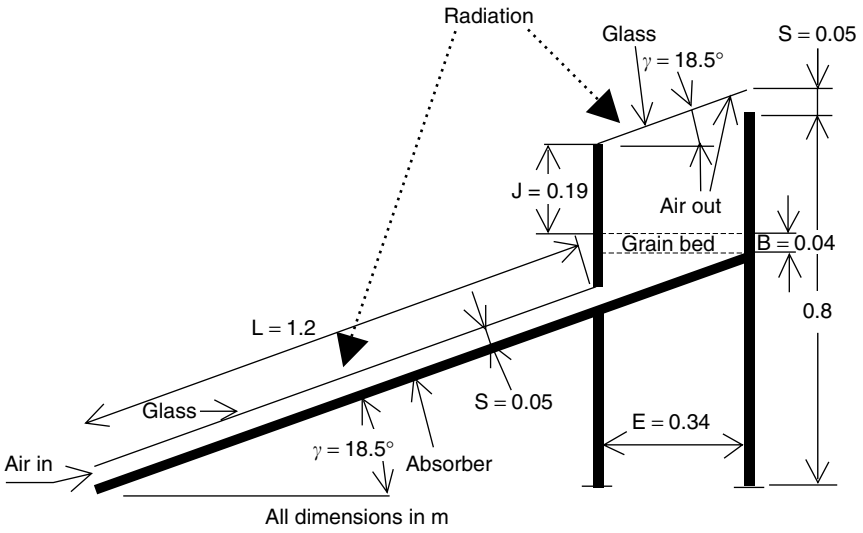

FIGURE 104.2 Schematic representation of mixed mode solar dryer (source ref. 10).

dryers, a direct type and an indirect type, which were suitable for small scale industries and for use in rural areas, and compared their performance to open-air drying. The direct dryer attained temperatures of 10–20°C while the indirect one attained 20–25°C above ambient temperature. The direct dryer reduced the moisture content to 7% in 8 hours while the indirect type reduced it to 6% in 7 hours compared to a reduction to 8% in 9 hours for open-air drying.

Coleman (17) dried mango slices in two small scale solar dryers, one using natural convection and the other using forced air circulation and found that the drying rate was higher with the forced circulation dryer. Higher drying rates for forced convection dryers compared to natural convection have also been reported by Wilhelms and Mullins (18) for drying apples, and Trim and Ko (19) for drying capsicum peppers.

In a study (20) to determine the effect of SO_2 on the quality of solar dried mango, mango slices were soaked in various concentrations of sodium bisulphite followed by solar drying and hot air drying. It was found that fruits prepared for solar drying required greater concentration of sodium bisulphite than those prepared for hot air drying. Also, the SO_2 treated solar dried fruits had better flavour than the hot air dried ones.

Other studies on solar and sun drying of tropical fruits related to modelling, quality of the dry product, production of snacks, fruit leathers and bars, and performance of dryers are given in Table 104.1 in references (21) to (38).

TABLE 104.1
References on Solar and Sun Drying Studies of Some Tropical Fruits Related to Modelling, Quality, Production and Performance

	Banana	Mango	Papaya
Modelling and drying characteristics	(21), (22)		(23)
Quality and storage (nutritional significance, consumer acceptance and reconstitution)	(24), (25), (26)	(27), (28), (29)	(30)
Production process (snacks, leather, beverage, powder, dry fruits)	(31), (32)	(33), (34), (35), (36)	(37)
Comparison of sun and solar drying performance	(38)		

B. Hot Air Drying

In convective drying systems, the drying process takes place in an enclosed chamber where hot air is passed over the product which results in simultaneous heat and mass transfer between the air and the drying product. Energy is supplied from the air stream to provide the heat for evaporation of moisture from the product and at the same time, the evaporated moisture is carried away from the product by the air stream (3). The most common of these dryers used for fruits are cabinet, kiln, belt and tunnel dryers (4).

A typical cycle of air drying comprises three stages, namely: the equilibrium stage in which the food is heated to the drying temperature, the constant rate period in which water is evaporated from the saturated surface of the food at a rate proportional to the moisture content and the falling rate stage starting after the critical moisture content is reached, in which moisture is held internally within the food and the moisture has to overcome a resistance to flow through the porous food material to the surface (39). Factors to be considered when drying heat

sensitive solids are that during the constant rate period, the solid surface temperature becomes equal to the wet-bulb temperature corresponding to the air temperature and humidity at the same location and, during the falling rate period, the temperature of the solid approaches the dry-bulb temperature of the air (40).

Fruit leather, one of the traditional fruit products that has evoked renewed interest in recent years because of its convenience, ease of preparation, shelf stability, simple packaging requirements and nutrition, is prepared by air drying (commonly tray drying) of fruit puree in the form of sheets (41). In improved and standardized methods for mango leather production reported by Rao and Roy (42), the puree is spread evenly at a thickness of 1 cm on a metal tray that has been smeared with glycerine. The puree is then heated at 50–80°C for 2–20 hours until a leathery product with 15–20% moisture content is obtained.

Jayaraman (2) used a cabinet dryer to dry fruit bars with a soft gel like structure which were suitable for direct eating, from the pulp of several tropical fruits. To the pulp was added sugar and pectin before cabinet drying in three phases as follows: initially at 80°C for 1 hour, then at 70°C for 2–3 hours and finally at 65°C for 5–6 hours.

Other studies on hot air drying of tropical fruits related to modelling and drying kinetics, quality and storage of dry products, production of dry fruit products and performance of dryers are given in Table 104.2 in references (43) to (69).

C. Drum Drying

Drum dryers are generally used to produce powdered and flaked ingredients widely used in bakery products, beverages, cereals and dairy products. In this system of drying, the product to be dried, usually fluid, slurry, or paste like form, or in suspension or solution if it is solids, is spread in a thin layer on the surface of an internally heated revolving drum with the dryer comprising either a single or double drum and the drying operation typically continuous (70). Although the drying rate is affected by the thickness of the film applied to the drum surface, the speed of rotation and the temperature of the drum, drum dryers have high drying rates and use heat economically (71). Figure 104.3 shows a double drum dryer.

Mao (72) studied drum drying of banana puree, with special reference to factors influencing the quality of the dried product. Drum rotation rate and drum temperature influenced the colour, moisture content and flavour of the dried product, but had no effect on texture. Drum speeds greater than 2 revolutions per minute and drum temperature less than 142°C gave the best results. Drum clearance influenced the texture and moisture content of the product. Monostearin and lecithin emulsifiers when added to the puree gave excellent film formation on the drum, excellent rehydration properties, and excellent texture of the rehydrated puree, but brittle and powdery dried flakes. Egg yolk gave excellent texture of both the dried and the reconstituted product.

A double drum dryer was used by Brekke *et al.* (73) to produce mango flakes and powder to a moisture content of less than 3% under the operating conditions of steam pressure of 4.2 kg/cm^2, drum spacing of 0.254 mm and residence time of 25 s.

Kitson and MacGregor (74) found that with drum drying, fruit purees with a relatively high fibre content (e.g., banana, guava, papaya) could be successfully dried without additives while low fibre ones required the addition of fibre (e.g., less than 1% low methoxyl pectin) to aid sheet formation at the doctor blade.

Travaglini, Carvalho, Ruiz and Shirose (75) dried mango puree using a convectional drum dryer and found the process to be suitable for the production of mango

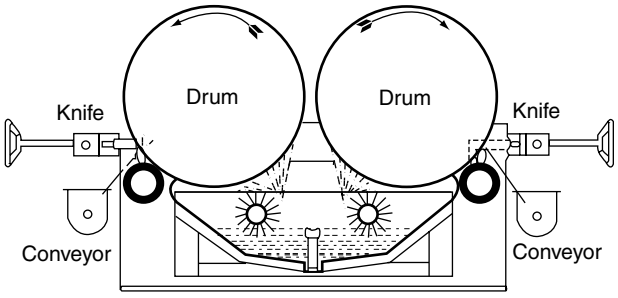

FIGURE 104.3 Double drum dryer (source ref. 70).

TABLE 104.2
References on Hot Air Drying Studies of Some Tropical Fruits Related to Modelling, Quality, Production and Performance

	Avocado	Banana	Mango	Papaya	Pineapple
Modelling and drying characteristics	(44)	(46), (48), (52), (54)	(48), (56), (58)	(62), (63), (66)	(68), (69)
Quality and storage (nutritional significance, consumer acceptance and reconstitution)	(43)	(45), (47), (49), (50) (51), (53), (55)			
Production process (snacks, leather, beverage powder, dry fruits)			(57), (60), (61)	(64), (65), (67)	(67)
Comparison of drying performance			(59)		

flakes. Under operating conditions of steam pressure of 60 psig, drum spacing of 0.06 inches and residence time of 20 s, improved quality flakes were obtained with ascorbic acid and beta-carotene retention of approximately 90%. Furthermore, an addition of 1% glycerol monostearate (MSG) and 4% corn starch to the puree improved the drying process.

D. Spray Drying

Spray drying process is used to transform feed from fluid form into droplets and then into dried particles by continuously spraying it into a heated drying medium. With this method, high drying air temperatures can be applied, resulting in high evaporation rates which maintain a low droplet temperature, and very short drying time compared to other drying processes, thus making it suitable for drying very heat sensitive materials (76).

A two-stage process, consisting of a combined spray-drying and through-flow bed stage was used to produce pineapple solids with less than 2% moisture content (77). The fruit juice is atomized under high pressure as it enters the spray-drying chamber. The atomized particles are mixed with heated air and fall onto a mesh-type conveyor belt where the partially dried particles form a porous mat through which the drying air from the chamber passes. The combination process enables dry crystalline sugars to be obtained on a continuous basis from sucrose, dextrose and lactose syrups.

King (78) added milk or soy milk to an equal volume of banana products diluted with various amounts of water and spray dried at different inlet temperatures. Drying rate and productivity both increased with increasing inlet temperature. At the same inlet temperature, the best drying rate and productivity were with 3x water dilution for banana-milk (BM) samples and with 2x and 1x water dilution respectively, for banana-soy milk (BSM) samples. Hygroscopicity and bulk density of the powdered product decreased with increase of drying air temperature, and the higher the drying rate the lower was the bulk density. The powdered products were light yellow in colour, and had good flavour and taste.

Parameters for the production of instant mango juice powder were studied by Khalil and Sial (79). Mango juice of 23°Brix, with or without addition of 0.25 or 0.5% sodium alginate or glyceryl monostearate, was spray dried with drying outlet temperature of 70–80 or 80–90°C, and voltages of 200 or 250 (which influenced atomization speed). Colour, flavour and taste were good only in the samples without additives.

A study (80) in which dehydrated fruits and fruit powders were prepared by osmotic drying, foam-mat drying and spray drying, and used in suitable combinations along with puffed cereals in the formulation of recipes for compressed ready-to-eat fruited cereals is reported. The products from banana, mango, pineapple and tomato were highly acceptable. The formulations packaged in an inner wrap of cellophane and an outer pouch of paper-aluminium foil-polyethylene laminate were acceptable at more than 6 months at 37°C and for 1 year storage under ambient conditions (20–30°C) except those with osmotic dried fruits wherein hardening of the fruits due to moisture transfer was noticed beyond 6 months under ambient conditions. Microbiological levels in the ingredients and formulations were within safe limits for direct consumption.

E. Osmotic Dehydration

Osmotic dehydration is a process involving partial removal of water from cellular materials like fruits and vegetables by immersing these materials in concentrated solutions of soluble solutes such as sucrose for fruits, and sodium chloride, for vegetables (81, 82). Figure 104.4 shows a flow chart of the osmotic dehydration process.

In this process, a chemical potential of the solute is created and this causes mass transfer of the solvent from a dilute to a concentrated solution through a semi-permeable membrane in order to equalize the potential (83). The nature of the cellular structure of fruits and vegetables is to act as non-perfect semi-permeable membranes resulting in not only transfer of water and small solutes from the tissue into the solution but also a smaller counter transfer of solutes from the solution into the tissue cells (84–86).

The rate at which osmotic dehydration takes place depends on the concentration and temperature of the osmotic solution, the nature and molecular weight of the osmotic solution, the product geometry and size, the product structure (e.g., porosity), the ratio of the mass of the product to that of the osmotic solution, the rate of agitation of the osmotic solution, and pressure (high pressure, ambient or vacuum) (87–91).

Heng, Guilbert and Cuq (92) studied the effect of temperature, concentration and nature of dehydration solution and preliminary calcium adjunction or blanching during osmotic dehydration of papaya cubes. They found that

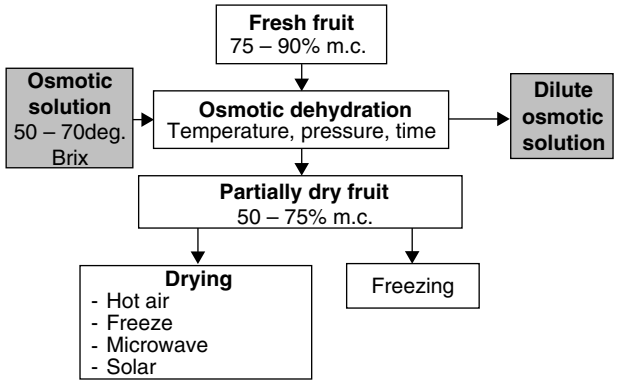

FIGURE 104.4 Flow chart of osmotic dehydration process.

increasing the temperature (from 30 to 70°C), increasing the concentration of the dehydration solutions (45 to 72°Brix), addition of calcium (0.05 M), no preliminary blanching and/or substitution of sucrose with a low DE glucose syrup, all increase water loss without increasing sugar penetration. Similarly, Waliszewski, Texon, Salgado, and Garcia (93) studied the effects of temperature (50, 60 and 70°C), sucrose concentration (50, 60 and 70°Brix) and pH (6, 7 and 8) on the mass transfer during osmotic dehydration of banana chips. They found that temperature and osmotic solution concentration favour faster water loss yielding also higher water loss/sugar gain ratio. Furthermore, water loss and sugar gain took place in parallel and the rate of water loss was always higher than the rate of solute gain. Panagiotou, Karathanos, and Maroulis (94) investigated mass transfer phenomena during osmotic dehydration of banana and other fruits in glucose and sucrose osmotic solution. They found that low molecular weight solute (glucose) leads to higher water loss and solids uptake than high molecular weight solute (sucrose) of osmodehydrated fruits under the same solution concentration.

The osmotic dehydration process may occur at low temperatures such as at ambient and does not involve a phase change thereby minimizing heat damage to the quality attributes like flavour, colour and texture of the product (95). Other advantages of osmotic dehydration include elimination of chemical pre-treatment, efficiency in energy use, reduction in packaging and distribution costs and, product stability in terms of quality and nutrient retention during storage (96, 97).

The osmotic dehydration process does not result in a product that can be considered shelf-stable and further drying by other methods such as air, freeze, or vacuum drying is done to make the product shelf-stable, or the process can be a pre-treatment for canning, freezing and minimal processing operations (98).

Other studies on effects of osmotic dehydration process variables on colour, mass transfer, storage of the dried product and rehydration, and modelling the osmotic dehydration process of some tropical fruits are given in Table 104.3 in references (99) to (133).

F. Freeze Drying

In freeze drying, the substance to be dried is usually frozen and the water is removed from the substance as a vapour by sublimation from the frozen material in a vacuum chamber (134). Although freeze drying is considered as the reference process for the high quality of dehydrated products, the process entails relatively high costs, as much for investment (vacuum technology) as running costs, and these are the reasons why its use in food is limited to high value added products (135).

G. Emerging Technologies

1. Microwave Drying

Microwave heating of foods is accomplished by the absorption of microwave energy by rotation of dipolar water molecules and translation of ionic components of food. When a microwave field that rapidly changes direction is imposed on the dipolar water molecule, the dipole tries to align itself with the field direction. Some response time is required for the water molecule to overcome the inertia and the intermolecular forces in the water, resulting in energy loss to the random thermal motion of the water and giving a temperature rise. The thermal energy developed in the material is related to the frequency of the electric field, the electric field strength in the material and to the dielectric loss factor of the material (136).

Garcia, Leal and Rolz (137) carried out drying tests for ripe and green banana slices and for a foam made from ripe banana puree in (i) a household microwave oven and (ii) a laboratory forced draft warm air oven. They found that the drying time in (i) was 17–20 times lower than that in (ii) and the drying energy efficiency in (i) was about 30%.

However, for initial drying operations, in particular with very high moisture products, conventional drying is

TABLE 104.3
Studies on Effects of Process Variables on Colour, Mass Transfer, Storage of the Dried Product and Rehydration and Osmotic Dehydration of Some Tropical Fruits

	Effect of Process Variables On				
	Colour	Mass Transfer	Storage	Rehydration	Modelling Osmotic Dehydration
Banana	(99), (100), (101), (102)		(117)	(123)	(116), (124), (125)
Coconut		(103)			(126), (127)
Guava			(118), (119), (120)		
Mango		(104), (105), (106), (107), (108)	(121), (122)		(128)
Papaya		(109), (110), (108)			(129), (130)
Pineapple		(111), (112), (113), (114), (115), (116)			(131), (132), (133)

more economic and efficient which makes microwave drying more suitable for finish drying operations (138).

It is therefore usual to combine microwave drying with hot air drying because this usually improves the efficiency and economics of the drying process, with the hot air being relatively efficient at removing free water at or near the surface and the unique pumping action of microwave drying providing an efficient way of removing internal free water as well as bound water (139).

2. Heat Pump Drying

There has been great interest in recent years to look at heat pump drying system as a substitute for freeze drying where, low temperature drying and well-controlled drying conditions are required to enhance the quality of products (140). The advantages of a heat pump dryer are: higher energy efficiency, better product quality, wide range of drying conditions and excellent control of drying environment. Its limitations are: use of CFCs in the refrigerant which pollute the environment, need for regular maintenance, increased capital costs, limited drying temperature and need for process control and design.

Prasertsan and Saensaby (141) dried bananas in an experimental heat pump drier (HPD). The moisture extraction rates (MER) and the specific moisture extraction rates (SMER) of banana drying decreased rapidly with the drying time but the compressor power was relatively constant. Banana drying yielded the highest average MER of 2.710 kg/h when the drying load was highest. The corresponding SMER was 0.540 kg/kWh. Economic analyses of the HPD compared to an electrically heated hot air dryer and a direct-fired dryer revealed that the HPD had the lowest operating cost.

Moisture and ascorbic acid (vitamin C) contents of batch-dried skinned, peeled and sliced guava were measured under several cyclic temperature profiles of the drying air maintained at constant humidity (8.9 g/kg dry air) using a two-stage heat pump dryer (142). Results on ascorbic acid degradation in guava during drying followed first-order kinetics for both isothermal and non-isothermal drying air conditions. Cyclic air-temperature drying yielded higher retention of ascorbic acid.

Chua, Hawlader, Chou and Ho (143) studied the effects of different time-temperature profiles on the drying time and colour change of bananas and guavas, using a tunnel heat pump dryer capable of providing less than or equal to 14.6 kW of cooling capacity. Samples of banana and guava were dried in batches in a 2-stage heat pump drier. Effects of starting temperature (20–35°C) of a selected profile and cycle time (60–120 minutes) on drying kinetics and product quality were studied. Results indicated that by employing a step change in drying air temperature with the appropriate starting temperature and cycle time, it was possible to reduce markedly food drying time required to reach the desired moisture content with improved product colour.

III. COMBINED METHODS OF DRYING TROPICAL FRUITS

A. Osmotic and Sun/Solar Drying

The combination of osmotic dehydration and sun/solar drying has potential for widespread application in drying tropical fruits with good quality and at reduced costs. Firstly, there is a growing and urgent need for simple inexpensive processes to preserve fruits and vegetables in developing countries in tropical regions to minimise losses due to spoilage, and intermediate moisture food technology involving soaking fruits/vegetables in sugar solution, is appropriate for such regions (2). Secondly, the availability of solar energy and the operational marketing and economy reasons offer a good opportunity for using solar drying in the developing countries (8).

Levi, Gagel and Juven (144) studied the effects of short heat (boiling temperature) treatments, consisting of 'blanching' in concentrated sucrose (70%) solution (I), or plain water (II), or osmotic dehydration in cold (room temperature) (III), or hot (boiling) sucrose (70%) solution (IV), and a combination of (III) following (I) or (II), on drying behaviour of papaya, dehydrated to the intermediate moisture (IM) range. Significant increases were observed in dry matter content of I, III, IV, or III following I or II, with the obvious increase in expected production yields and probable reduction of heat energy needed for drying. The drying behaviour of papaya as a raw material for IM products, during and after the above treatments, as well as following hot-air (cabinet) or direct-sun drying, was studied. Drying time needed for cabinet or solar drying following osmotic treatments was considerably shortened, and therefore a significant saving in heat energy could be expected. The optimal treatments to achieve a considerable short drying time, without reducing the quality of IM papaya, seemed to be syrup 'dipping,' or a combination of water or syrup blanching and cold osmotic dehydration. The results are shown in Figure 104.5.

In another study (145), papaya slices were pre-treated as follows: (i) no treatment, (ii) syrup blanch (5 minutes boiling at 70°Brix), (iii) 5 minutes boiling water blanch, (iv) 90 minutes boiling syrup (70% sugar), (v) cold syrup immersion for 24 hours, and (vi) combined (iii)/(v) followed by treatment with/without 5000–10 000 ppm potassium sorbate solution (10–15 min contact time in dip) then dehydration by cabinet or sun drying to about 70% dry matter. Samples were analysed for total soluble solids, acidity, pH, ascorbic acid (AA), colour, pectin esterase activity, total carotenoids, water activity, and microbial quality (total aerobic counts, viable count of 'sugar tolerant' micro organisms). The highest ascorbic acid retention (66–91%)

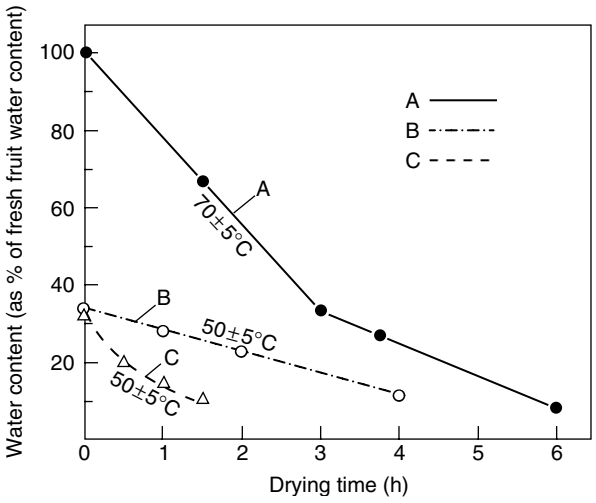

FIGURE 104.5 Drying behaviour of cabinet dried papaya (A — direct drying; B — after 75 min in boiling sugar (70%) syrup; C — after 22 hr in cold syrup) (source ref. 144).

TABLE 104.4
Effect of Pre-Treatments and Drying Method on the Ascorbic Acid (mg/100 g Product) Content of Intermediate Moisture Papaya Slices (Adapted From (145))

Treatment No.	Pre-Treatment	Drying Method	
		Cabinet	Sun
I	None	441	193
II	Syrup blanch (5 min boiling 70% sugar)	263	156
III	Boiling water blanch 5 min	269	134
IV	Boiling syrup (90 min; 70% sugar)	68	–
V	Cold syrup (70% sugar)	161	144
VI	Combined III and V	74	73

was observed in papaya slices directly dried in a cabinet, followed by those that were water blanched + cabinet dried (58–73%) (see Table 104.4). Significantly lower retention of ascorbic acid was observed in sun dried samples. Most products maintained their typical orange colour and their carotenoid content. Total carotenoids content of sun dried slices was lower than that of cabinet dried samples. The relationship between moisture content and water activity value suggests that intermediate moisture papaya products with a moisture content of 28% will be relatively stable microbiologically. They concluded that intermediate moisture papaya products are a promising means of utilizing surplus papayas in developing countries, due to their attractive colour, flavour and other quality characteristics.

Design and construction of a solar osmotic dryer to test the utilization of solar energy in a two step osmovac dehydration of papaya is reported (146). The samples dried by solar osmotic procedure had higher drying rates and sucrose uptake than those dried without solar. Furthermore, drying rates obtained with solar vacuum drying were twice those of vacuum drying without solar.

Uzuegba and Ukeka (147) investigated the combination of osmotic and cabinet or sun drying. Fully-ripe banana, mango and pawpaw slices were cabinet dried or sun dried to 15% moisture, with or without predrying by holding them in saturated sugar solutions (21 kg sugar/10 l water) containing 0 or 1% potassium metabisulphite (PMBS) for 24 hours. Moisture and ascorbic acid concentration of the slices were determined before and after drying and after 12 months of storage in sealed cellophane film at 28–30°C; flavour was evaluated by 8 judges after storage. The best treatment for ascorbic acid preservation and flavour retention appeared to be osmotic dehydration in 1% PMBS solution followed by cabinet drying.

B. Osmotic and Hot Air Drying

Osmotic dehydration has been combined with conventional methods of drying such as hot air drying with the aim of producing better quality shelf-stable products than would be obtained with convectional methods alone. Jackson and Mohamed (148) osmotically dried some mangoes and bananas followed by air drying and found that osmotic dehydration of fruit reduces heat damage to colour and flavour, and loss of fresh fruit flavour, and also prevents enzymatic browning of the fruit and that the process could be carried out with very simple equipment. However, treatment of papaya and guava was not successful.

Rahman and Lamb (149) analysed the air drying behaviour of fresh (non-osmosed) and osmosed pineapple. Pineapple slices of 6.5 mm thickness were osmodried in sucrose solution of 40–60% (m/v) for 5 hours after which they were dried in air at 10% relative humidity, 4 m/s air velocity and 60°C temperature. Non-osmosed samples gave the highest drying rate while osmosed samples at 60% sugar solution and 60°C gave the lowest drying rate. The lower drying rate was due to solute uptake, which resulted in higher internal mass transfer resistance.

In an experiment to determine the effect of initial water activity of mango slices on air drying behaviour at 50, 60 and 70°C with osmotic dehydration pre-treatment, Welti, Palou, Lopez-Malo and Balseira (150) found that the time needed to reach a moisture content of 0.7 g water/g dry solid was 50–75% lower for osmotically dehydrated mango slices than for fresh ones.

Osmotically dehydrated banana slabs were air dried in a cabinet type tray drier to near equilibrium conditions at fixed temperatures from 40 to 80°C and at a constant air speed of 0.62 m/s (151). The drying air temperature was found to significantly enhance the drying rate and the drying constant except at 80°C when the rates fell, possibly due to case hardening of the slabs. The drying rate was also

improved by reducing the slab thickness, but increasing the air speed to 1 m/s did not have any profound effect.

Osmotic dehydration can be applied as a pre-treatment for fruit or fruit pieces, whereby the fruits are soaked in a sugar syrup of 75°Brix at 60–70°C for 1 hour to remove some of the water before further drying or freezing (152). The resultant fruit retains most of the fresh fruit flavour. Enzymatic browning is reduced, the colour is improved without using SO_2, and the loss of fresh fruit flavour is reduced because of the sugar layer surrounding the fruit or fruit pieces. For example, banana slices dehydrated in 70°Brix syrup for 10 hours and then dried in a vacuum-shelf dryer at 10 mm Hg pressure at 70°C for 6 hours resulted in dry slices that were porous, crisp and had retained very nearly their original size and shape.

A combined osmotic drying and air drying process for bananas is described (153). Banana slices (10 mm thickness) were soaked in 2 successive sucrose baths with concentrations of 35 and 70°Brix and both at pH of 3.5. The first bath contained 1% $CaCl_2$ to enhance firmness and this solution was initially heated at 85°C and then cooled slowly to room temperature over 24 hours. A fruit to solution mass ratio of 1:6 was used in the first soaking process. The second soaking in the solution at 70°Brix was conducted at room temperature using a fruit to solution ratio of 1:4. This second treatment served to increase the sugar content of the banana slices, extend the degree of water loss, and maintain good texture and shape in the product. The combined osmotic drying process resulted in a weight reduction of 60% and a solute gain of 2%. Slices were then subjected to air drying at 40°C and relative humidity of 60% for 72 hours. The final dried product had a moisture content of 16%, an average water activity of 0.54 at 25°C and a highly acceptable colour without any brown pigmentation.

C. Osmotic and Microwave Drying

Information on combined osmotic and microwave drying of tropical fruits is not available. However, a study (154) on the combination of osmotic and microwave drying of blueberries gave a faster drying rate and better quality product compared to air drying. In another study (155), the effect of combined osmotic and microwave dehydration of apple on texture, microstructure and rehydration characteristics was done. Osmotic dehydration of apple was found to be a beneficial pre-treatment since it shortened the time of final air-drying in combination with microwaves. It produced dehydrated samples of improved appearance, softer rehydrated texture and lower rehydration capacity compared to the products obtained without osmotic pre-treatment. Rehydration in yoghurt and water gave different results depending on the pre-treatment, implying that the choice of the rehydration medium was important when aiming at restoring the original texture.

D. Sun/Solar and Hot Air Drying

Dehydration of tropical fruits by solar energy followed by hot air drying has not been reported. However, studies (156) conducted in a radiation/convection composite solar dryer for peaches gave high rate of drying during the initial stages of drying which could be considered in relation to possible use of a solar system to produce an intermediate moisture product, which may then be further dried in a conventional dryer.

E. Microwave and Sun/Solar Drying

The feasibility of improving the sun-drying process of grapes by microwave drying was investigated (157). Sultana seedless grapes, dipped in alkali solution (2.5% K_2CO_3 plus 0.5% olive oil, to increase water permeability of the grape skin), were pre-treated in a domestic microwave oven and then dried by direct solar radiation. Microwave pre-treatment reduced the moisture content by 10–20%. The microwave-treated grapes dried nearly two times faster than the controls. Blanching in boiling water had the same effect on the drying rate as microwaves. Colour and appearance of the treated grapes were comparable to the commercial products. It was concluded that microwave pre-treated grapes have an improved colour after drying and this was due to a reduction in enzymatic browning, as enzymes were partially inactivated by the absorption of microwave energy.

F. Microwave and Vacuum Drying

A vacuum microwave oven was used for drying pineapple and passion fruit juice and banana paste (158). In order to produce a stable foam with small diameter bubbles suitable for drying at 45°C and 6–8 torr, raw materials were thickened by addition of sucrose and malto-dextrins to 59°Brix. The percentage retention of total volatiles was 57 for pasteurized, 68 for fresh pineapple juice and 82 for passion fruit juice, compared to 33 for freeze dried and 11 for spray dried passion fruit juice.

The combination of microwave and vacuum drying of banana slices was investigated by Drouzas and Schubert (159). The slices were put in a glass vessel in the interior of a domestic microwave oven and vacuum conditions applied by means of a vacuum pump. The Magnetron power was kept constant for a period of 10 s, followed by a pause of 20 s, and this procedure was repeated several times. Compared to freeze drying, no substantial differences in the samples were observed as far as colour, taste, aroma and shape were concerned. The microwave dried sample absorbed twice as much moisture as that obtained by conventional drying during rehydration. They concluded that microwave dried fruits were more appropriate for use in bakery or related products (cereals, snacks, etc.).

A vacuum microwave dehydration process is described for drying fresh mango and pineapple fruit pieces at low temperature (160). The resulting product, which cannot be achieved using conventional air drying techniques, has a 'fresh,' uncooked flavour and a unique crunchy texture with little or no associated shrinkage.

G. Air and Microwave-Vacuum Drying

Comparative studies (161) were conducted on 5 combined methods for drying of banana slices (25 mm diameter, 6 mm thick): air drying/microwave drying; osmotic drying/air drying; vacuum impregnation/osmotic drying/air drying; osmotic drying/air drying/microwave drying; and vacuum impregnation/osmotic drying/air drying/microwave drying. These combined drying techniques were compared with air drying method as a control. The fastest drying was achieved by the combination of air drying/microwave drying. Combined processes that include vacuum impregnation and/or osmotic drying are slower than conventional air drying, but may improve yield and give dried banana products with improved stability and/or sensory properties.

In a study (162) to characterize the behaviour of 16 volatile compounds of banana during a combination of air drying (AD) and vacuum microwave drying (VMD) of banana chips, samples were subjected to AD to remove 60, 70, 80 or 90% of moisture (wet basis) and then subjected to VMD to achieve a final moisture content of 3% (dry basis). The slices were also dehydrated using only AD, VMD or freeze drying (FD) for comparison. It was found that samples that underwent more VMD had significantly lower levels of volatile compounds, which was attributed to the decreased formation of an impermeable solute layer on the surface of the chips. The optimum process of 90% AD/10% VMD yielded crisper banana chips with markedly higher volatile levels and sensory ratings than the AD ones.

IV. QUALITY OF DRIED TROPICAL FRUITS

Lewicki (163) has reviewed the effects of pre-drying treatment, drying and rehydration on plant tissue properties. The properties that are affected by drying are shrinkage, porosity, biopolymer structure and biochemical activity, and those affected by rehydration are water absorption and leaching of solutes.

A. Texture

The viscoelastic behaviour of dehydrated banana during rehydration was examined under uniaxial compression tests by Krokida, Kiranoudis and Maroulis (164). The samples were dehydrated with 4 different drying methods: conventional, vacuum, freeze and osmotic drying after which they were rehydrated in an air dryer at 50°C and 80% air humidity. It was concluded that dehydrated products do not keep their viscoelastic behaviour after rehydration due to structural damages that occur during drying. Specifically, freeze dried materials loose their elasticity and become more viscous but osmotic pretreament seems to help freeze dried materials to keep their elastic nature, probably due to solids gain, and that air and vacuum dried materials keep their viscoelastic characteristics during rehydration close to those of dried materials. In another study (165), air dried banana samples pretreated by osmotic dehydration, exhibited a viscous rather than elastic behaviour which indicated that the infusion of sugars causes plasticity of the structure of the fruit. Krokida and Maroulis (166) further dried banana samples using 5 different methods (conventional, vacuum, freeze, microwave and osmotic drying) then rehydrated them in an air dryer at 50°C and 80% RH. Then they examined the samples for structural properties (true density, apparent density, porosity and specific volume) during rehydration to moisture contents ranging from 0.01 to 3.5 kg/kg (dry basis). The samples were found to have not recovered the properties due to structural damage that happened during drying and the hysteresis phenomenon that happened during rehydration.

Osmotic dehydration of papaya pieces followed by fast drying in a cryogenic freezer using liquid N_2 (−63°C for 10 minutes) was found to preserve fruit texture better than slow freezing rate in an air blast freezer (−28°C for 1.5 hours) (167).

B. Colour

Effects of sucrose concentration (50–70°Brix), pH (6–8) and temperature (50–70°C) on colour changes in raw papaya slices exposed to 4 hours osmotic dehydration were evaluated (168). It was found that the 3 optimal treatments for changing greenish colour of raw papaya to yellow-orange colour were 60°Brix/60°C with pH 7 or 8, and 70°Brix/60°C with pH 7. Similarly, the effect of chitin on colour during osmotic dehydration of banana slices was studied (169). Banana slices of 0.6 cm thick were placed in sealed jars with 6x their weight of a sucrose solution for up to 4 hours. Chitin was added to syrups at 0.01, 0.05 or 0.1%, and the following conditions of drying were applied: 50°Brix, 70°C and pH 6; 60°Brix, 50°C and pH 6; 60°Brix, 70°C and pH 7; 60°Brix, 60°C and pH 8; and 70°Brix, 50°C and pH 8.3. Slices were removed from each batch every 30 min and analysed for colour values and polyphenol oxidase activity in inner and outer sections. Chitin at 0.01% reduced the tendency of colour L* values to decrease (darkening) during drying (in both inner and outer sections of slices), without affecting the natural drop in polyphenol oxidase activity, or the chroma, hue and total colour difference changes. These changes in L* value were independent of osmotic drying

conditions. In another experiment (170), osmotic drying of banana slices was carried out under 5 sets of conditions (50–70°Brix, 50–70°C, pH 6–8) to determine the effect of EDTA on colour. EDTA was added to the syrup solution at 5 concentrations of 0.01, 0.05, 0.1, 0.5 or 1% w/w. EDTA did not affect polyphenol activity decrease or chroma, hue and total colour difference changes. However, EDTA affected L value decrease in the inner and outer part of the banana slice, independent of osmotic dehydration conditions. It is recommended that EDTA is used during osmotic drying of some fruit that undergo undesirable darkening.

Effect of sucrose concentration (50–70°Brix), pH (6–8) and temperature (50–70°C) on colour changes in raw papaya slices during up to 4 hours of osmotic dehydration followed by air drying was examined (171). The slices were air dried at 50–70°C with air velocity of 0.5–1.5 m/s. Sucrose concentration and pH had no significant effect upon colour changes in the final product. During this combined drying of papaya slices, chroma exhibited a tendency to increase and hue to decrease, indicating that fruit maturity before processing is very important.

The effect of drying conditions on colour changes of banana and other fruits during conventional and vacuum drying were investigated by Krokida, Tsami and Maroulis (172). The Hunter colour scale parameters redness, yellowness and lightness were used to estimate colour changes during vacuum and conventional drying at 50, 70 and 90°C and air humidity of 15, 30 and 40%. The air temperature affected redness and yellowness, but not lightness (see Figure 104.6). Furthermore, the rate of colour deterioration

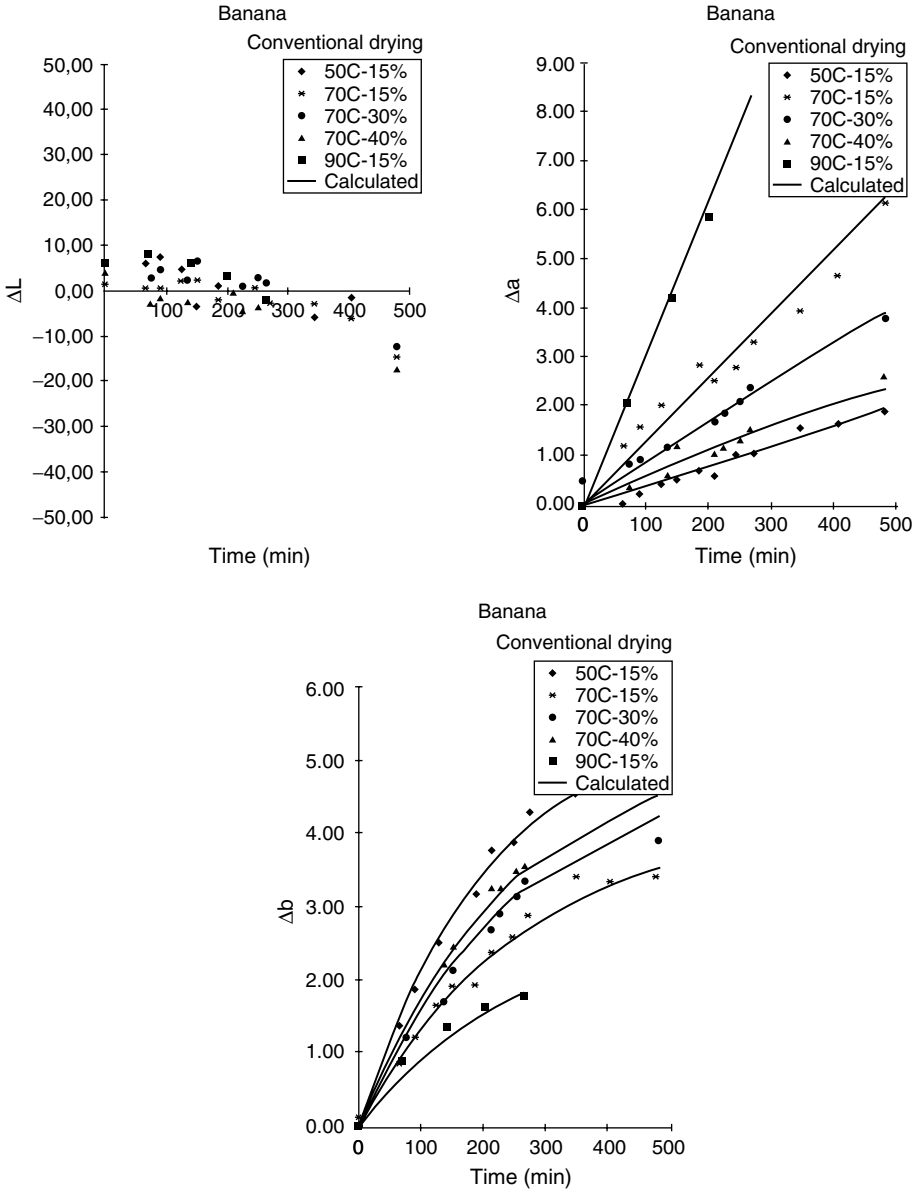

FIGURE 104.6 Changes in lightness ΔL, redness Δa and yellowness Δb (source ref. 172).

was found to increase as temperature increased and humidity decreased, for both drying methods and all the samples examined.

Effects of cyclical variations of air temperature on drying kinetics and colour changes during the drying of banana and guava pieces were investigated (173). Drying was performed in a two-stage heat pump drier capable of precise control of air humidity. Air temperature variations tested were a cosine profile, a reversed cosine profile and 3 different square wave profiles with peak-to-valley variations from 20 to 40°C. Cycle time was approximately 60 minutes and the drying time was approximately 300 minutes. The use of the different temperature profiles allowed individual colour parameters and the overall colour changes in the food products to be controlled. Banana pieces (high in sugar) favoured a sinusoidal temperature wave profile with a starting temperature of 30°C. Overall, results showed that with appropriate choice of temperature-time variations, it is possible to reduce the overall colour change of these products significantly while maintaining high drying rates.

Drying characteristics, colour and rehydration of banana samples dried by hot air, microwave and combined hot air/microwave (microwave finish) treatments were investigated (174). It was found that drying of banana slices took place in the falling rate drying period with all the methods; constant drying rate period was not observed under any studied conditions. Hot air drying took the longest time to dry samples, but when microwave finish treatment was used, drying time was reduced by approximately 64%. The drying rate increased sharply with microwave oven power output. Microwave finish drying had little effect on colour and rehydration capacity of dried banana samples; whereas the colour scores were poorer and rehydration values lower in samples subjected to hot air or microwave drying alone.

Osmotic dehydration as a pre-treatment to air drying has been found to suppress browning during drying of banana (175). In another study, the feasibility of minimizing colour degradation and drying times during osmotic dehydration and subsequent continuous convective air drying with or without intermittent infrared treatment (IR) of pineapple was done by Tan, Chua, Mujumdar and Chou (176). Samples were osmotically dehydrated by soaking in 30, 50 or 70°Brix sugar solutions for 7 hours. Colour changes in pineapple during osmosis were similar irrespective of sugar concentration; all samples turned darker. Osmotically pre-treated pineapple samples retained their colour during air drying, whereas untreated samples browned markedly. However, higher concentration of osmotic solutions affected the magnitude of any colour change whereas lower concentration stabilized colour. Introducing intermittent IR heating increased the initial drying rate by 4–5 times for osmotically treated pineapple, but it also reduced colour degradation compared to continuous heating. Osmotic pre-treatment resulted in a shift in sorption isotherms for both products. They concluded that with the appropriate choice of IR intermittency as well as osmotic pre-treatment, it is possible to reduce overall colour change whilst maintaining high drying rates.

Gujral and Khanna (177) studied the effects of addition of soy protein concentrate, skim milk powder and sucrose at different levels on the drying rate, colour, texture and sensory properties of mango leather. Soy protein concentrate, skim milk powder and sucrose were added to mango pulp at 0, 4.5 and 9%. Control samples were dried at 60°C and the time required for the mango leather to reach 10% moisture content was 7.6 hours. Drying rate was lowered by the addition of all the ingredients tested, with soy protein concentrate having the most pronounced effect. Extensibility and energy required for rupture were markedly reduced with all three ingredients. Sucrose improved the colour of mango leather and samples containing 4.5% skim milk powder and 4.5% sucrose each exhibited the highest acceptability scores.

C. Rehydration

The keeping quality and rehydration characteristics of mango pieces treated with $CaCl_2$, were studied during storage in brine and during sun drying (178). The quality of pickles prepared from the treated dry mango pieces was also studied. Raw mango pieces treated with $CaCl_2$-brine kept well for 1 year and exhibited maximum retention of texture and colour. Pickles prepared from the treated mango also had good texture and colour and no microbial contamination was found. The $CaCl_2$-treated pieces maintained their shape and appearance during sun drying, whereas the control pieces shrivelled and became discoloured. The quality of the treated dried pieces was maintained during storage for 14 months in polyethylene bags. The best rehydration was achieved with a ratio of 1:4 between mango pieces and rehydration medium. Incorporation of $CaCl_2$ into this medium improved the texture of pickles prepared from rehydrated $CaCl_2$-treated pieces but not that of pickles prepared from control pieces.

Candelaria and Raymundo (179) used three different drying methods (convection, vacuum and vacuum puff drying) to process banana and mango and compared the drying efficiency and quality of the dried products. Vacuum puff drying produced the most acceptable product with a significantly shorter drying time (6 hours regardless of raw material) than that obtained with convection drying (17–20 hours) or vacuum drying (10–12 hours). Rehydration ratio and rehydration rates were also significantly higher for products subjected to vacuum puff drying. In further studies (180), optimum conditions for predrying treatments and vacuum dehydration of banana and mango to give products that would rehydrate rapidly were determined. Diced ripe fruit was heated at different pressure and temperature combinations in a vacuum oven. The best

puffing results were obtained at 40 kPa/110°C. Heating times required to reach maximum tissue temperature were 6 minutes for banana and 8 minutes for mango. Optimum dehydration conditions were 6–7 hours at 45–50°C under 70–80 kPa. Rehydration ratios for vacuum-puffed banana and mango were 8.2 and 8.9, with corresponding rehydration rates of 1.8 and 1.7 g/min respectively.

V. CONCLUSION

Dehydration of tropical fruits is a practical alternative to freezing and refrigeration in reducing the wastage of fruits during gluts as well as making them available in a variety of forms during off-season periods. Consumers' demand for quality products and the need to satisfy export requirements of tropical fruits from developing countries will continue to drive development of new drying methods with improved performance.

A lot of research into improved drying methods aimed at producing fruit products with reduced loss of quality, have been done during recent years. Of particular interest to the developing countries in the tropical regions is combination drying methods, which have the potential to address not only quality but also other fundamental problems like long periods of transporting fresh fruit from rural areas to factories in the towns due to poor road infrastructure in most cases. A technique like osmotic dehydration is simple to perform at the farm level in rural areas and by the farmers as the method does not require sophisticated equipment. The semi-processed fruit can then be transported, at a reduced cost and in a more shelf-stable form, to central processing places in the towns for further processing by solar, convection hot air or microwave drying.

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105 Drying Pears

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CONTENTS

I. INTRODUCTION

The fruits that have been traditionally dried are essentially grapes, figs, plums, apricots and peaches, but some other species have recently gained increased importance, like apples, mango, pawpaw, pineapple, banana and pears. The production of dried pears has some relevance in countries like Australia, South Africa, Chile, Argentina and Portugal (1).

It is essential to study the phenomena taking place during drying in order to optimise the process and make it a profitable and competitive production method, offering the consumer products of unquestionable quality. The study of the drying kinetics is therefore essential for the development of reliable process models for such unit operation (2, 3).

In the case of fruits, and of pears in particular, the sugar concentrations are relatively high (60 to 75% dry basis) and increase very significantly as the water evaporation proceeds, offering an additional resistance to the movement of moisture throughout the fruit. Thus, it becomes important to study the influence of this property in the moisture diffusion coefficient along the drying process (4).

Success in food and storing strongly depends on the isothermal relationship between the water content of the food material being processed and its water activity. Therefore, the knowledge of such relations, often represented in the form of sorption isotherms, both at the drying as well as at the storage temperatures, is of unquestionable importance for the food industry (5).

The factors that influence the end of the drying process are the high concentration of sugar and low moisture content as well as the energetic optimisation of the process. The desirable amount of water present at the end of the drying varies according to the type of fruit, and the smaller the fruit the less shall be its final moisture content. However, the choice of the final moisture content must not only have in mind the stability of the fruit but also the final physical and chemical properties that characterize its quality (4).

The dehydration of foods generally involves a series of interdependent unit operations like blanching, pasteurisation or pre-concentration, all of which contribute to the overall quality of the final product, and to improve the efficiency of the process. In ideal dehydration of food products the process of water removal should be reversible, however this is not true due to the inevitable loss of nutritional and functional attributes, which depends on the type and extension of dehydration and the sensitivity of the specific food components (6).

II. SHRINKAGE CHARACTERISTICS

Recently, much attention has been paid to the quality of the food products, which is strongly influenced by the processing conditions. In particular, during drying the water present evaporates in a rather important extension, originating phenomena such as shrinkage, that influences significantly the food physical structure and properties, like texture, contributing for its final quality standards (7, 8).

When studying the physical properties of foods, and particularly fruits, is very important to know with some accuracy the estimations for the size, volume, density and superficial area. Some methods based on the longitudinal and transversal sections of the material were developed for the determination of shape and size. The experimental measurements usually are made with photographic cameras, and include a millimetre scale which is photographed with the object. Other more sophisticated methods have also been developed, based on computer aided image processing. Volume and density are normally determined by liquid displacement, due to the irregularity of the products (9, 10).

In most cases, the shape can be approximated to known geometric forms and its volume and superficial area are thus calculated using the appropriate equations. It is possible to quantify some parameters associated to shape, like sphericity ($= d_e/d_c$, with d_e the diameter of a sphere with the same volume as the object and d_c the diameter of the smallest circumscribing sphere or usually the longest diameter of the object) and roundness ($= A_p/A_c$, where A_p is the largest projected area of object in natural rest position and A_c is the area of the smallest circumscribing circle) (9, 11).

The shrinkage of porous materials during drying is very dependent on the internal vapour pressure and the quality of these products depends on the shrinking behaviour. On the other hand, shrinking of biological products such as foods during drying occurs simultaneously with the water evaporation process, affecting the moisture diffusion and water removal rate, as well as the apparent density. Therefore, knowing the shrinkage behaviour assumes an important role on understanding and modelling drying processes and controlling the characteristics of the food. Within literature various models have been proposed to describe shrinkage of foods in drying, and it has been shown that either volumetric shrinkage or dimensional shrinkage have a strong dependency on the moisture content (12).

The shape of the pears has been observed to correspond to a combination of known geometric forms, after which is possible to estimate their volume and superficial area, assuming that during the drying process the shape does not change. Based on the pear geometry defined in Figure 105.1, the theoretical expressions for volume and superficial area are given by:

$$\text{Volume} = \tfrac{1}{2}\ \text{volume of sphere} + \text{volume of cone} \quad (105.1)$$

$$V = \tfrac{1}{2}\left(\tfrac{4}{3}\pi r^3\right) + \tfrac{1}{3}\ \pi r^2 h = \tfrac{\pi}{3}\ r^2\,(2r + h) \quad (105.2)$$

and,

$$\text{Superficial area} = \tfrac{1}{2}\ \text{area of sphere} + \text{area of cone} - \text{area of base of cone} \quad (105.3)$$

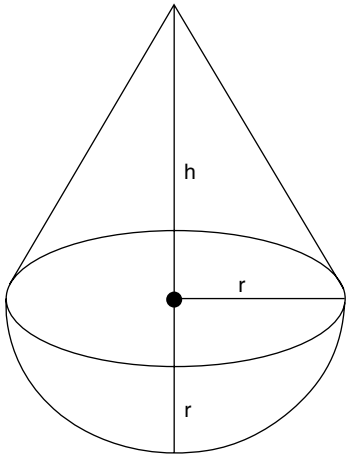

FIGURE 105.1 Approximation of the shape of the pears to known geometries.

$$A_s = \tfrac{1}{2}(4\ \pi r^2) + \pi r(\sqrt{r^2+h^2} + r) - \pi r^2 = \pi r(2r + \sqrt{r^2+h^2}) \quad (105.4)$$

where r is the radius of the semi-sphere and h the height of the cone.

To evaluate the evolution of the pear dimensions during the process of drying, the pears (*pyrus communis*, variety "D. Joaquina") were photographed over a millimetre scale paper, and the dimensions read directly from the scale. The water content was determined with a Halogen Moisture Analyser (Mettler Toledo HG53) and density was determined by liquid displacement, using picnometry (two different solvents were used in the analysis and the results obtained were analogous). The volume of the pears was calculated using two different methodologies: (a) known the mean values of the dimensions, calculate volume from the theoretical formula deduced (Equation (105.2)); (b) directly measure the volume by liquid displacement (the solvents used were water and toluene).

Figures 105.2 and 105.3 show the evolution of the pear dimensions and superficial area as the process of drying proceeds and the corresponding moisture content diminishes. All the three variables obey an exponential raise law described by Equations (105.5) to (105.7), and the quality of the fitting is relatively good as shown by the values of R^2.

$$r = 0.0129 + 0.0268\,(1 - \exp(-0.1281\,W)) \quad R^2 = 0.923 \quad (105.5)$$

$$h = 0.0339 + 0.0180\,(1 - \exp(-0.3598\,W)) \quad R^2 = 0.951 \quad (105.6)$$

$$A_s = 0.0017 + 0.0057\,(1 - \exp(-0.3081\,W)) \quad R^2 = 0.987 \quad (105.7)$$

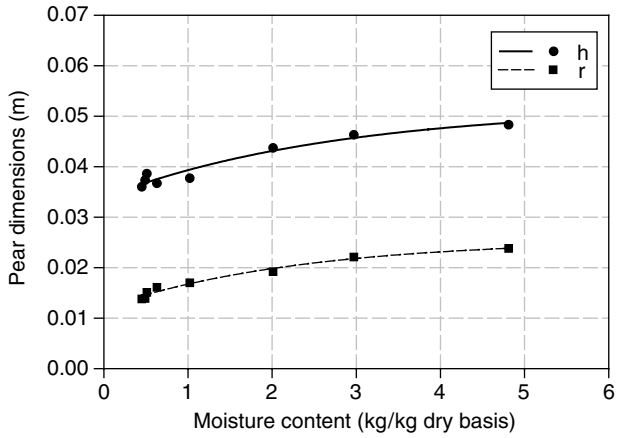

FIGURE 105.2 Evolution of pear dimensions during drying.

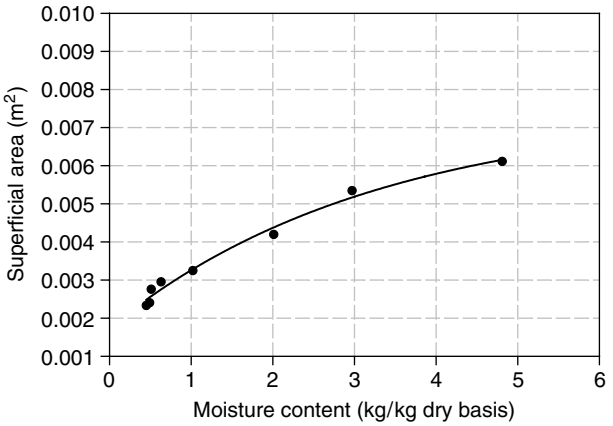

FIGURE 105.3 Variation of superficial area with moisture content.

where r is the radius and h the height of the pears (m), A_s is the superficial area (m^2) and W is the dry basis moisture content.

In Figure 105.4 the variation of the relative density of the pears, dr, with moisture content is presented, and the function that represents this variation is an exponential growth of the form:

$$d_r = 0.2247 \exp(-2.7168\,W) + 1.1527 \exp(-0.0255\,W) \quad R^2 = 0.898 \qquad (105.8)$$

where d_r is dimensionless.

In Figure 105.5 the values obtained for volume by using the two different methodologies described earlier are plotted against moisture content, and a linear relationship between these two variables is very clear, as the value of R^2 highlights. The linear function of Equation (105.9) was obtained by regression using the two sets of data plotted in the graph of Figure 105.5.

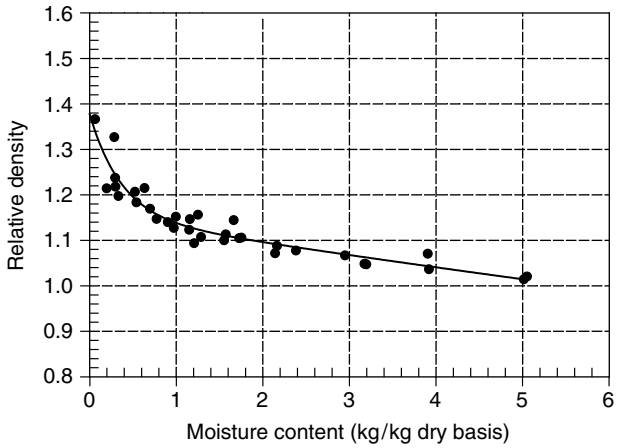

FIGURE 105.4 Variation of density with moisture content.

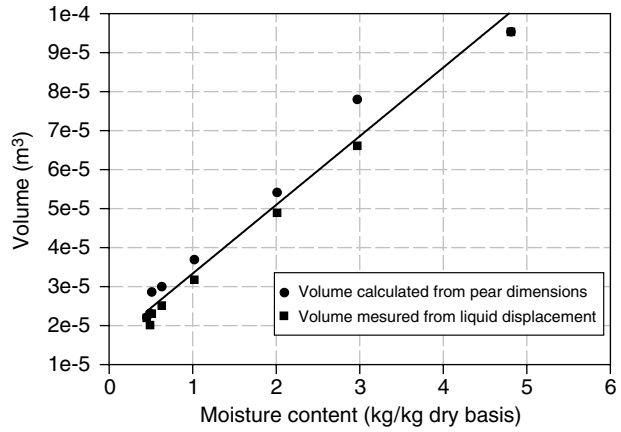

FIGURE 105.5 Variation of volume with moisture content.

$$V = 1.5711\text{e-}5 + 1.7617\text{e-}5\,W \quad R^2 = 0.972 \qquad (105.9)$$

where V is the volume (m^3).

To express the shrinking behaviour during drying, a bulk shrinkage coefficient was defined as the ratio of the sample volume at any stage to the initial volume ($S_b = V/V_0$). This was determined following two different methodologies: (a) S_b was calculated as the ratio of V/V_0 with V given by Equation (105.9), thus obtaining the relation expressed in Equation (105.10), which is in some accordance with the model suggested by Kilpatrick in literature ($S_b = (0.8 + W)/(0.8 + W_0)$) (12, 13); (b) S_b was determined by linear regression applied to the observed values of V/V_0 when plotted against the dimensionless moisture content (W/W_0), as shown in Figure 105.6, thus obtaining the model in Equation (105.11).

$$S_b = \frac{0.89 + W}{0.89 + W_0} \qquad (105.10)$$

$$S_b = 0.1694 + 0.8651\,\frac{W}{W_0} \quad R^2 = 0.990 \qquad (105.11)$$

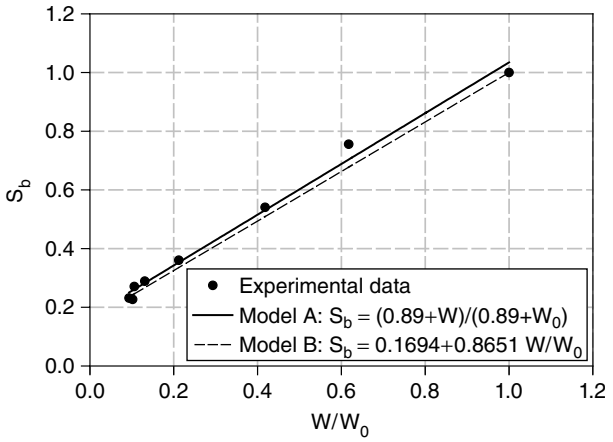

FIGURE 105.6 Evolution of shrinkage coefficient along the drying process.

The linear relation between S_b and W/W_0 is well reported in literature, and Equation (105.11) is quite similar to the one presented by Raghavan and Silveira (14) for the microwave drying of strawberries at 0.2 W/g power level ($S_b = 0.0741 + 0.8121\ (W/W_0)$).

III. SORPTION ISOTHERMS OF PEARS

Equilibrium moisture data provided by sorption isotherms is very important for the determination of optimal storage conditions as well as the prediction of thermodynamic equilibrium models. In drying they are of great importance to properly choose the end-point of the process, corresponding to the optimum residual moisture content of the final product. In fact, a high residual moisture content obtained with a short drying time leads to reduced stability, whereas drying the material to a moisture content below its optimum value leads to energy wasting with no apparent benefit. Such isotherms also give information on how strongly the water is bound to the material and thus are essential for the prediction of the drying and rehydration rates (15, 16).

At the end of the drying process, the moisture content of the food becomes stationary, reaching an equilibrium moisture content with the surrounding atmosphere. The moisture sorption isotherms are obtained when the equilibrium moisture content is plotted against the corresponding water activity of the food product, over a certain range of values and for a constant temperature. Brunauer *et al.* (17) classified adsorption isotherms in terms of the van-der Walls adsorption of gases into five different types, that are illustrated in Figure (105.7). Types I and II have been assigned special names, Langmuir and Sigmoid respectively, but no special names have been attached to the others. For most foods sorption isotherms are nonlinear, usually sigmoidal, as type II in Figure (105.7), however, for foods rich in soluble components such as sugars, the type that best seems to describe the sorption behaviour is type III (6, 16).

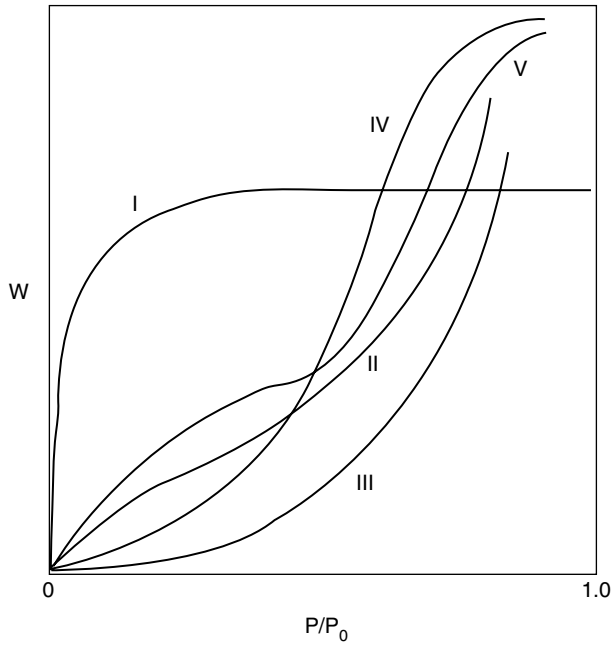

FIGURE 105.7 The different types of adsorption isotherms (adapted from Brunauer *et al.* (17)).

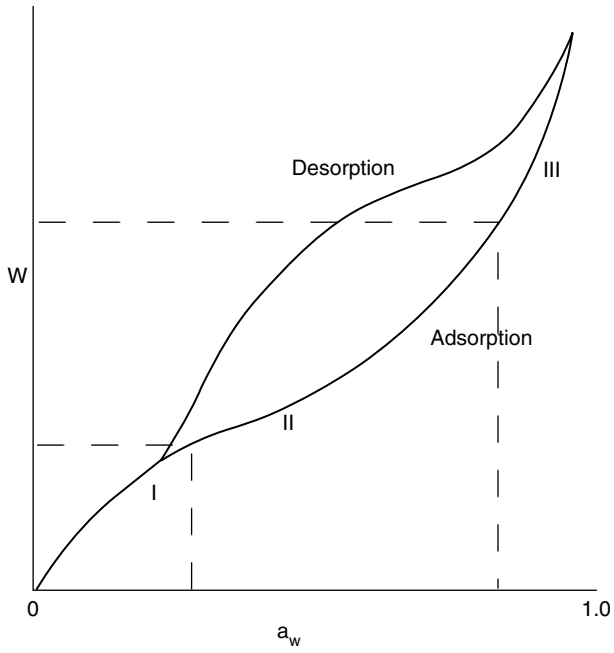

FIGURE 105.8 Sorption isotherm with adsorption and desorption curves (adapted from Fellows (39)).

Another behaviour commonly observed is that different paths are followed dependent upon the direction from which equilibrium is approached, resulting in a hysteresis (see Figure 105.8). The desorption isotherm lies above the adsorption isotherm and therefore more moisture is retained when the approach to equilibrium is made from a wetter state than from a drier one, at a given relative

humidity. In fact, in the original form of the food, the polar sites are almost completely occupied by adsorbed water molecules, whereas upon drying with shrinkage effect, the water holding capacity of the structure is diminished. Therefore, taking into consideration that rehydration is never as complete as the original wet condition of the food, for a specific relative humidity the material holds a higher moisture content along the desorption process as compared to the adsorption one (9).

In Figure 105.8 the isotherm is divided into three different regions, according to the type of water present: (I) bound water or monolayer region, corresponding to absorption of up to a monomolecular layer of water; (II) intermediate water or multiplayer region, corresponding to adsorption of additional layers of water over the monolayer; (III) free water or capillary absorption region, referring to the water held in the pores of the material (9).

Many different models can be found in literature to describe the sorption of water on capillary materials (about 77 (6)), most of them represented by semi-empirical equations with a variable number of parameters. Because water is associated with the food matrix in different ways for different activity regions, is well expected that one sorption model may not be enough to represent the sorption behaviour over the entire water activity range. The quality of fitting of a determined sorption model to the experimental data does not necessarily reveal the nature of the sorption process.

In Table 105.1 some of the more commonly used sorption isotherm models are presented.

In the different models, W_e is the equilibrium moisture content (dry basis), W_m is the monolayer moisture content (dry basis), a_w is the water activity, R is the gas constant ($R = 8{,}31451\ J{\cdot}mol^{-1}{\cdot}K^{-1}$) and T is absolute temperature. In Table 105.1 some models have an explicit temperature dependency (Chung-Pfost, Halsey, Henderson) while some others have parameters that are temperature dependent (Chen, BET, GAB). In the Iglesias-Chirife and Oswin models, the temperature dependency is not considered. The Iglesias-Chirife model, corresponding to a type III curve (see Figure 105.7), proved to be quite adequate to describe the sorption behaviour of many sugar-rich foods, such as most fruits, where the monolayer is completed at a very low moisture content and the dissolution of sugars takes place (6).

The adsorption is an exothermic process while desorption is endothermic. Generally, higher temperatures result in a reduction of adsorbed molecules and therefore, adsorption decreases with increasing temperature, although this dependency is usually small. However, this behaviour is not universal as temperature affects many different phenomena and an increase in temperature can in fact increase the rates of adsorption, hydrolysis and recrystallization processes. Food rich in soluble solids, such as sugars, seem to exhibit antithetical temperature effects for higher values of a_w due to their increase solubility in water (6).

The sorption behaviour of pears was investigated for constant drying temperatures of 20, 25 and 30°C, and the experimental data obtained with the measurement of the moisture content (with a Mettler Toledo, HG53 Halogen moisture analyser) and the corresponding water activity (with a Rotronic Hygrometer) allowed the estimation of the parameters in the different models considered.

The estimation of the parameters in all models was done using an Orthogonal Distance Regression algorithm

TABLE 105.1
Different Models Found in Literature to Describe Sorption Isotherms

Model	Equation	Parameters	References
BET (Brunauer-Emmett-Teller)	$\frac{a_w}{W_e(1-a_w)} = \frac{1}{W_m C} + \frac{C-1}{W_m C} a_w$	W_m, C(T)	(17)
Chen	$a_w = \exp[-A \exp(-B W_e)]$	A(T), B(T)	(18)
Chung-Pfost	$a_w = \exp\left(-\frac{A}{RT} \exp(-CW_e)\right)$	A, C	(19)
GAB (Guggenheim-Andersen-deBoer)	$W_e = \frac{W_m C K a_w}{(1-K a_w)(1-K a_w + C K a_w)}$	W_m, C(T), K(T)	(20, 21, 22)
Halsey	$a_w = \exp\left(-\frac{A}{R T (W_e/W_m)^b}\right)$	W_m, A, b	(23)
Henderson	$1 - a_w = \exp(-CTW_e^b)$	C, b	(24)
Iglesias-Chirife	$\ln[W + (W^2 + W_{0.5})^{1/2}] = A a_w + B$	A, B	(25)
Oswin	$W_e = C\left(\frac{a_w}{1-a_w}\right)^b$	C, b	(26)

TABLE 105.2
Results of the Parameter Estimation for the Non-Explicit Temperature Dependent Models

		Temperature		
Model	**Estimation**	**20°C**	**25°C**	**30°C**
BET	W_m	0.1770 (± 0.0182)	0.1819 (± 0.0215)	0.1887 (± 0.0189)
	C	0.3440 (± 0.0757)	0.3434 (± 0.0884)	0.4653 (± 0.1004)
	Σe^2	1.0892e-2	7.2870e-3	3.9646e-2
	SD	1.8168e-2	1.6741e-2	3.7881e-2
Chen	A	0.4555 (± 0.0251)	0.4433 (± 0.0293)	0.8030 (± 0.0450)
	B	1.0850 (± 0.0931)	1.0942 (± 0.1225)	1.7641 (± 0.1450)
	Σe^2	1.5263e-2	1.0465e-2	6.8704e-2
	SD	2.1839e-2	2.0460e-2	3.7069e-2
GAB	W_m	0.1668 (± 0.0502)	0.2226 (± 0.0994)	0.2372 (± 0.0857)
	C	0.3736 (± 0.1819)	0.2578 (± 0.1596)	0.3379 (± 0.1580)
	K	1.0034 (± 0.0143)	0.9906 (± 0.0192)	0.9880 (± 0.0197)
	Σe^2	7.7148e-3	5.2532e-3	3.7887e-2
	SD	1.5527e-2	1.4496e-2	2.7527e-2
Iglesias-Chirife	A	9.6110 (± 0.5629)	9.9273 (± 0.6010)	10.2884 (± 0.3662)
	B	−7.3664 (± 0.4824)	−7.6327 (± 0.5082)	−7.8718 (± 0.3009)
	Σe^2	3.8286e-2	2.3638e-2	2.2919e-1
	SD	3.4062e-2	3.0152e-2	6.7036e-2
Oswin	C	0.0960 (± 0.0067)	0.0995 (± 0.0080)	0.1228 (± 0.0079)
	b	1.1941 (± 0.0490)	1.1912 (± 0.0579)	1.1441 (± 0.0468)
	Σe^2	1.0792e-2	7.5617e-3	4.0323e-2
	SD	1.8084e-2	1.7054e-2	2.8119e-2

(ODR), where the unknown errors are taken into account in both dependent and independent variables. The software package used to compute the parameters was ODRPACK, developed by the Center for Computing and Applied Mathematics of the National Institute of Standards and Technology (USA) (27). The estimation of the parameters was done using the explicit ODR algorithm with the derivatives approximated by central finite differences.

In Table 105.2 the values of the parameters estimated and the corresponding standard deviation are presented for the models that do not have an explicit dependency on temperature (Chen, BET, GAB, Iglesias-Chirife, Oswin) as well as the statistical information (sum of square of errors, Σe^2, and residual standard deviation, SD). In these cases, the experimental data in the form of sets (W, a_w) was treated separately for the three different temperatures studied, and the number of observations for the temperature of 20°C was 42 sets, for the temperature of 25°C was 40 and for the temperature of 30°C was 56.

In Table 105.3 the values of the parameters estimated with the corresponding standard deviation are presented for the models that have an explicit dependency on temperature (Chung-Pfost; Halsey; Henderson) as well as the statistical information. In these cases, the experimental data in the form of sets (W, a_w, T) was treated all together, and the number of observations was 138.

In Figures 105.9 and 105.10 the sorptions isotherms for the temperature of 30°C, for example, are represented using the 8 different models considered, along with the experimental data. From Figure 105.9 is possible to observe the similarity between the BET and GAB models, that seem to be quite good at predicting the sorption behaviour of the pears for this temperature. The Chen and Chung-Pfost models are also quite similar but apparently are not so adequate to this particular case. In Figure 105.10 all the models are more or less equivalent, although the Iglesias-Chirife model shows a less good approximation to the experimental data.

In Figure 105.11 the isotherms are represented for different temperatures (20, 50 and 80°C) using one of the

TABLE 105.3
Results of the Parameter Estimation for the Temperature Dependent Models

Model	**Parameters Estimation**	
Chung-Pfost	A	1717.61 (± 81.02)
	C	1.7633 (± 0.1117)
	Σe^2	2.9499e-1
	SD	5.0869e-2
Halsey	W_m	0.1281 (± 0.0522)
	A	1638.17 (± 51.25)
	b	0.7679 (± 0.2193)
	Σe^2	7.0618e-2
	SD	2.4999e-2
Henderson	C	0.0067 (± 0.0001)
	b	0.4158 (± 0.0129)
	Σe^2	9.2985e-2
	SD	2.8560e-2

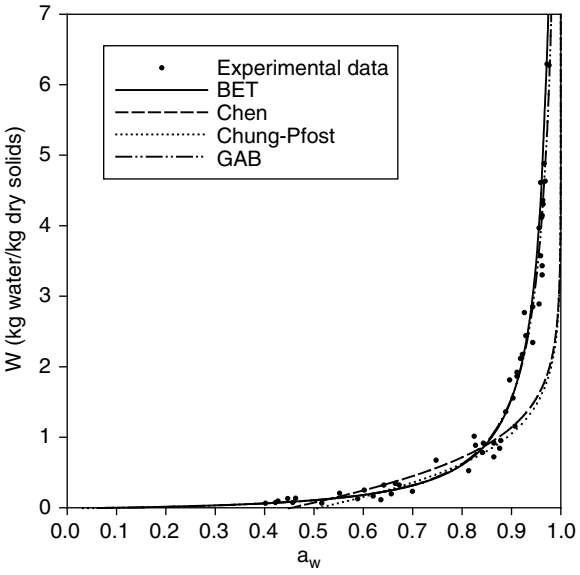

FIGURE 105.9 Representation of sorption isotherms of pears for 30°C with BET, Chen, Chung-Pfost and GAB models.

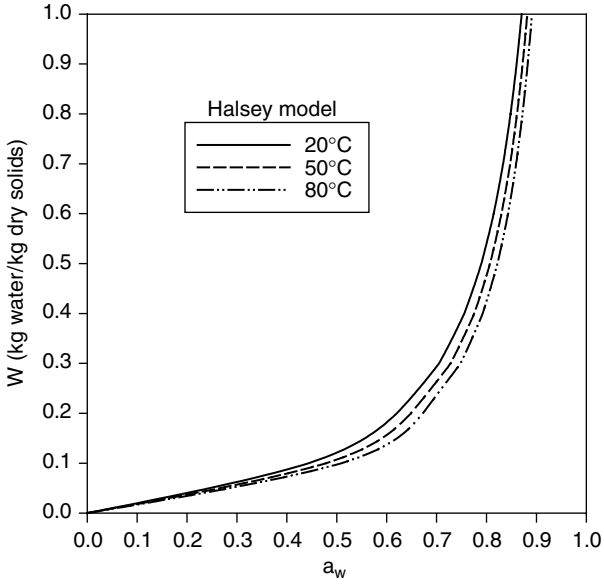

FIGURE 105.11 Influence of temperature on sorption isotherms of pears as shown by the Halsey model.

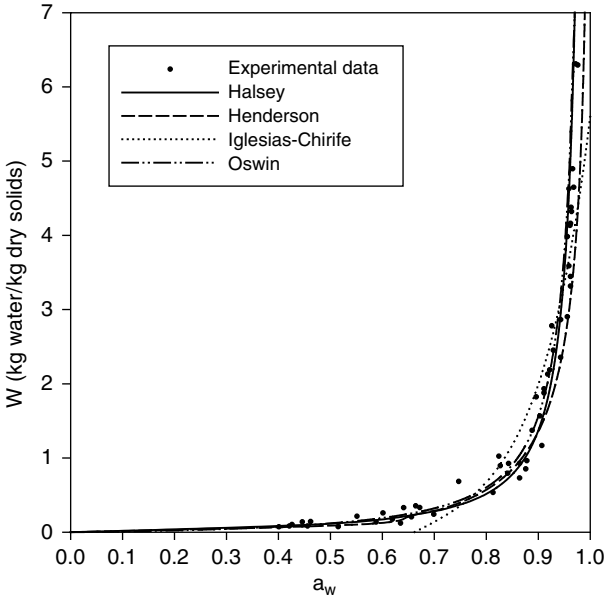

FIGURE 105.10 Representation of sorption isotherms of pears for 30°C with Halsey, Henderson, Iglesias-Chirife and Oswin models.

temperature dependent models, the Halsey model, and is possible to observe that at constant moisture content an increase in temperature enhances water activity. This behaviour has been reported to most solid fruits (2, 28).

IV. DRYING KINETICS

Dehydration consists in the penetration of heat into the food product and the removal of moisture by evaporation into an unsaturated gas phase. This process involves heat, mass and momentum transfer, which are quite difficult to predict with accuracy. In foods such as fruits, with a capillary-porous structure, the food matrix has interstitial spaces, capillaries and cavities filled with gas, and many different mechanisms of mass transfer can be considered, and may act in different combinations. These mechanisms include liquid diffusion due to concentration gradients, liquid transport due to capillary forces, vapour diffusion due to partial vapour pressure gradients, liquid or vapour transport due to the difference in total pressure caused by external pressure and temperature, evaporation and condensation effects, surface diffusion, and liquid transport due to gravity (6, 29).

The most important when developing process models is unquestionably the determination of the drying kinetics, which accounts for the mechanisms of moisture removal and the influence of certain variables during the process (2). In air drying processes two drying stages are usually present: an initial constant rate period corresponding to pure water evaporation and a falling rate period where the moisture transfer is essentially limited by internal resistances.

Figures 105.12 and 105.13 illustrate the different ways of representing the drying behaviour of foods, and show respectively a moisture content versus time plot and the drying rate versus time and moisture content plots.

It is common procedure to interconvert between the three different types of drying curve, as usually the type of data recorded from experiments (moisture-time) is not in the best form to fit mathematically. In fact, the Krischer rate-moisture curve, which is obtained from the

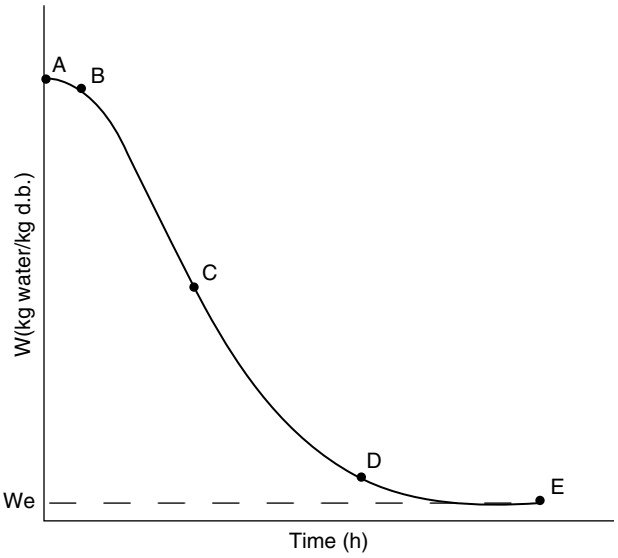

FIGURE 105.12 Drying curve, showing the variation of moisture content with drying time (adapted from Rizvi (6)).

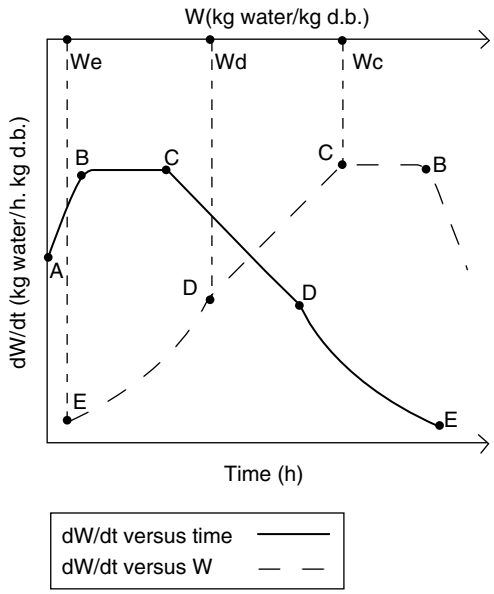

FIGURE 105.13 Drying rate curves, showing the rates of moisture removal as a function of time and moisture content (adapted from Rizvi (6)).

batch drying curve and its differentiation (the drying rate curve), is actually the most suitable form of treating the drying data in terms of the characteristic curve scaling method (30).

In Figures 105.12 and 105.13 some important points, *A* to *E*, are marked in the curves, allowing the visualisation of the different stages of the drying process. The induction stage, that goes from *A* to *B*, corresponds to the initial unsteady-state heating period, and in most cases represents an insignificant portion of the total drying cycle. The unhindered drying or constant rate period is represented by *BC*, and during this phase the drying surface is saturated with water as the rate of migration of water from the inside to the surface equals the rate of evaporation at the surface. The factors that influence the drying rate during this period are the surface area, the temperature and moisture gradients between the surface and the surrounding air and the heat and mass transfer coefficients. In food systems where moisture movement is controlled by capillary and gravity forces the constant rate period is significant and its extension is determined by the food structure and the mechanisms that govern the internal liquid movement. As drying proceeds, a critical moisture content is reached (Wc, corresponding to point *C*) where the rate of migration of water from the interior to the surface is reduced to a degree that no longer is sufficient to saturate the entire surface, and this begins to dry. The critical moisture content usually increases with increasing thickness and drying rate. After point *C* the surface temperature starts to increase and the hindered drying or falling rate period starts. This generally involves two different stages: the first falling rate period, *CD*, and the second falling rate period, *DE*. After point C the rate of moisture migration to the surface is less than the rate of evaporation, originating a continually drier surface. This proceeds until finally all the evaporation from the interior of the food is completed, in point *D*. In the second falling rate period, the path for heat and mass transfer becomes longer and more tortuous as the moisture content diminishes, and the limiting moisture content is reached where the vapour pressure of the food equals the partial vapour pressure of the drying air, thus ending the drying process. This is known as the equilibrium moisture content, We (6, 31).

In typical industrial applications, the kinetic models developed are mostly empirical rather than being mechanistic, due to the complexity of the phenomena involved. However, some empirical kinetic models include parameters of phenomenological nature related to the most appropriate driving force, which can lead to rather good prediction of drying kinetic although they do not have a physical meaning (2).

To determine the drying rate curves, the pears were peeled and left in convective dryers at constant temperature. The temperatures investigated were 30, 40 and 50°C, and every 24 hours two samples were collected from each dryer to analyse their moisture content (with a Mettler Toledo HG53 Moisture Analyser). The water activity was also analysed with a Rotronic Hygrometer. All experiments were carried out until the moisture content of the pears reached about 20%, thus obtaining pears with optimum physical properties like texture, consistency and elasticity, as well as chemical composition that influences their taste and preservation capacity (4).

The batch drying curves (dry basis water content plotted against drying time) are shown in Figures 105.14 and

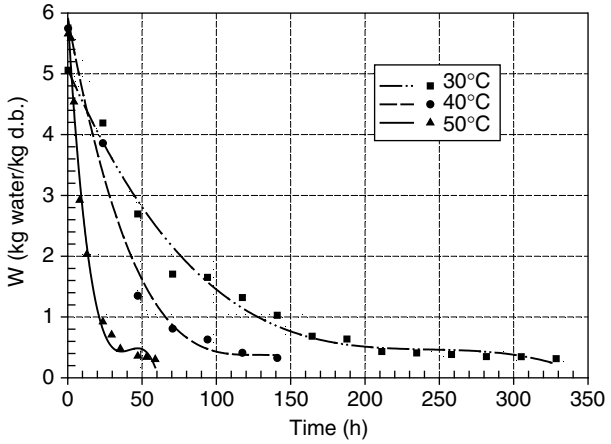

FIGURE 105.14 Drying curves for 30, 40 and 50°C with cubic fit.

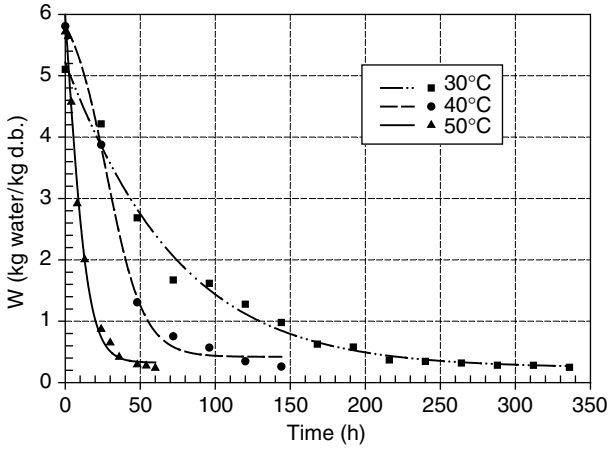

FIGURE 105.15 Drying curves for 30, 40 and 50°C with sigmoidal fit.

TABLE 105.4
Results of the Fitting to the Batch Drying Curves

Parameter	30°C	40°C	50°C
Cubic Fit			
y0	5.1131	5.9685	5.9353
a	−0.0559	−0.1306	−0.4069
b	0.0002	0.0010	0.0098
c	0.0000	0.0000	−0.0001
R	0.9925	0.9912	0.9946
Sigmoidal Fit			
y_0	0.2390	0.4199	0.3254
x_0	−87.9603	27.6879	0.7858
a	25.2585	5.9720	10.7263
b	−62.6247	−12.3753	−7.2140
R	0.9941	0.9985	0.9961

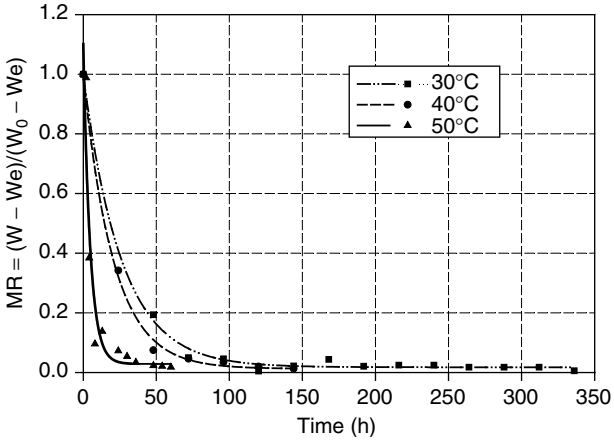

FIGURE 105.16 Moisture ratio curves for 30, 40 and 50°C with exponential fit.

105.15. It is possible to verify that all curves follow the general pattern of a drying curve, and that for higher temperatures the changes in the water content are sharper, because the evaporation process is highly accelerated.

The data was fitted to cubic polynomials as suggested by Kemp *et al.* (30) (Equation 105.12) and also to sigmoidal functions (Equation 105.13):

$$W = y_0 + at + bt^2 + ct^3 \qquad (105.12)$$

$$W = y_0 + \frac{a}{1 + \exp[(x_0 - t)/b]} \qquad (105.13)$$

where W is the dry basis moisture content and t is the drying time in hours. The fitting was done with the software Sigma Plot, version 5.0 (SPSS, Inc.) and the resulting values for the parameters as well as the correlation coefficients are presented in Table 105.4.

From Table 105.4 is possible to verify that the correlation coefficients are higher for the sigmoidal fit, and the best quality of this fitting is quite visible in Figure 105.14 as the cubic fit introduces some degree of instability to the final stage of the drying curve.

The plots of moisture ratio (defined as $MR = (W - W_e)/(W_0 - W_e)$ versus time were also produced, to fit the data to a first order kinetics of the form (32):

$$MR = y_0 + a \exp(-bt) \qquad (105.14)$$

where t is expressed in hours.

The variation of the moisture ratio with the drying time is presented in Figure 105.16 for the three temperatures studied, and the effect of increasing temperature on accelerating the drying process is quite visible. The fitting of the plots MR-time was done with Sigma Plot and the results

TABLE 105.5
Results of the Fitting to the Moisture Ratio Curves

Parameter	30°C	40°C	50°C
y_0	0.0177	0.0127	0.0287
a	0.9834	0.9901	1.0724
b	0.0383	0.0484	0.2111
R	0.9985	0.9989	0.9616

are presented in Table 105.5. The correlation coefficient (R) is also presented to show the quality of the fitting.

V. MOISTURE DIFFUSIVITY PREDICTION

Moisture diffusivity is an unquestionably important transport property necessary for correct modelling and understanding of the food drying processes. The determination of a variable diffusion coefficient is quite complex, not only because of the experimental difficulties that arise, but also due to the mathematical solution of the diffusion equation.

The drying of pears is critically dependent of the temperature as well as of the water and sugar concentrations inside the fruit along drying. In fact, as the drying proceeds, some migration of the sugars occurs with the water that flows from the inside to the surface of the pears. That, combined with the shrinking of the fruit, originates an increase on the sugar concentration close to the surface, offering an extra resistance to the water diffusion (4).

The drying of pears, as the majority of the food products, is complex and the coupled heat and mass transfer phenomena have to be taken into account. The physical characteristics often change significantly along the drying process and despite the complexity of the moisture transfer process, it has been reported by many researchers that Fick's law seems to be fairly good to predict the moisture distribution inside many food materials during drying (33, 34).

For the transient diffusion, assuming a uniform initial distribution of moisture content and a uniform concentration at the surface for $t > 0$, the solution of Fick's Law can be approximated by (35):

$$(W-W_e)/(W_0-W_e) = (8/\pi^2)^3 \exp[-D_e\, t\, (\pi^2/4)\, (1/L_x^2+1/L_y^2+1/L_z^2)] \quad (105.15)$$

where D_e is effective diffusivity, W is the dry basis moisture content at time t, W_e is the equilibrium moisture content, W_0 is the initial moisture content and L_x, L_y, and L_z represent one half the size of the sample in the three spatial dimensions. Thus, the values of D_e can be obtained from the slope of a semi-log plot of the moisture ratio $((W - W_e)/(W_0 - W_e))$ versus time.

The evolution of the pear dimensions along drying was studied by measuring the values of the pear radius and height as the moisture content diminished, and the values for L_x, L_y and L_z were thus calculated from the following equations:

$$L_x = L_y = 0.0129 + 0.0268 * (1 - \exp(-0.1281 * W)) \quad R^2 = 0.923 \quad (105.16)$$

$$L_z = 0.0168 + 0.0099 * (1 - \exp(-0.3285 * W)) \quad R^2 = 0.945 \quad (105.17)$$

that were obtained by adjusting the experimental data with Sigma Plot 5.0 (SPSS, Inc.), and where L_x and L_y are both equal to the pear radius and L_z is the height, expressed in meters.

The temperature and moisture content dependency of the effective diffusivity is represented by a modified Arrhenius relationship of the form (36, 37):

$$D_e = D_0(1 + B\, W) \exp[-E/(RT)] \quad (105.18)$$

where D_0, B and E are parameters to estimate, T is absolute temperature, W the dry basis moisture content and R the gas constant ($R = 8.31451$ J/mol·K). In the above equation E represents the activation energy for moisture diffusion (J/mol).

From the experimental data obtained for W[1] and from the relations that express the evolution of the pear dimensions, different values for diffusivity were estimated for different temperatures (between 30°C and 50°C) and for different dry basis moisture contents (between 0.22 and 4.36). These data were adjusted to the model in Equation (105.18) in the form of triplets (D_e,W,T), and a orthogonal regression algorithm was used, particularly the explicit ODR with the derivatives approximated by central finite differences, and the results of the estimation are presented in Table 105.6. The values of diffusivity obtained with this model vary from 1.174×10^{-10} to 1.909×10^{-9} m^2/s, and the activation energy ($E = 44.234$ KJ/mol) stands in the range of values reported for other food products (15–95 kJ/mol) (6, 35, 36).

As it was stated earlier, this particular drying problem is strongly affected by the sugar concentration, and therefore a modification is suggested to the model of Equation (105.19), to introduce a component that accounts for the dependency of the water diffusivity in relation to the sugar concentration present, S (kg of sugar/kg dry solids):

$$D_e = D_0\,(1+ B\, W)/(1+ C\, S) \exp[-E/R\, T] \quad (105.19)$$

The determination of the parameters of Equation (105.19) was based on the experimental data obtained for

[1] W was measured with a halogen moisture analyser, Mettler Toledo HG53.

TABLE 105.6
Parameter Estimation for the Diffusivity Models

	Optimal Estimate	
Parameters	**Model of Equation (105.18)**	**Model of Equation (105.19)**
D_0	4.2140×10^{-3}	4.0558×10^{-4}
B	7.7634×10^{-1}	2.8082×10^{-1}
C	—	8.5860×10^{-1}
E	4.4234×10^{4}	3.4180×10^{4}
Statistic Information		
N° of observations	19	58
$\Sigma\ \delta^2$ (W, T)/$\Sigma\ \delta^2$(W, S, T)	2.0929×10^{-36}	4.5341×10^{-36}
$\Sigma\ \varepsilon^2(D_e)$	5.6820×10^{-18}	2.4887×10^{-17}
Sum of square errors	5.6820×10^{-18}	2.4887×10^{-17}
Residual standard deviation	5.3301×10^{-10}	6.4948×10^{-10}

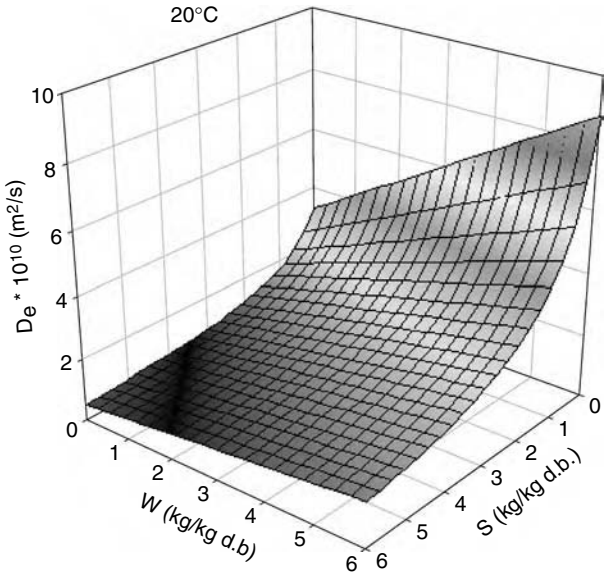

FIGURE 105.17 Variation of diffusivity with moisture and sugar concentrations for 20°C.

the model of Equation (105.18), and also from some data published by Kil Jin Park *et al.* (38). The range of temperatures used was 40–60°C, the moisture concentration varied from 0.10 to 4.00 and the sugar concentration varied from 0.44 to 3.50. The data in the form of sets (D_e,W, S, T) was adjusted to the model using the same ODR algorithm, and the results of the parameter estimation are presented in Table 105.6. With this model the values of diffusivity vary from 4.294×10^{-10} to $1.390 \times 10^{-9}\,m^2/s$, and the activation energy is 34.180 KJ/mol.

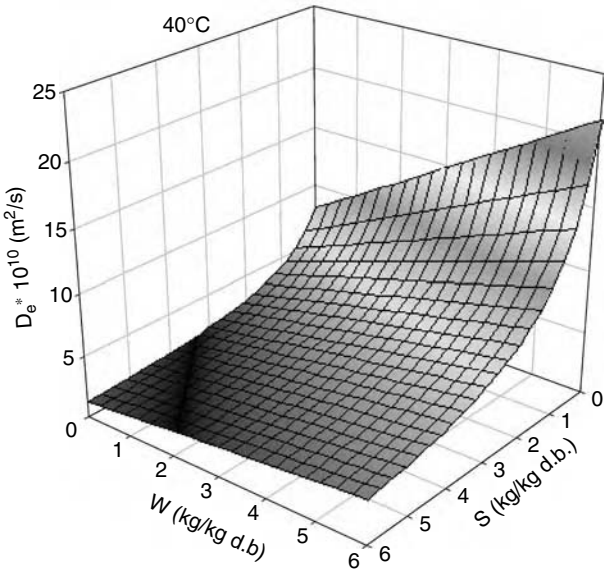

FIGURE 105.18 Variation of diffusivity with moisture and sugar concentrations for 40°C.

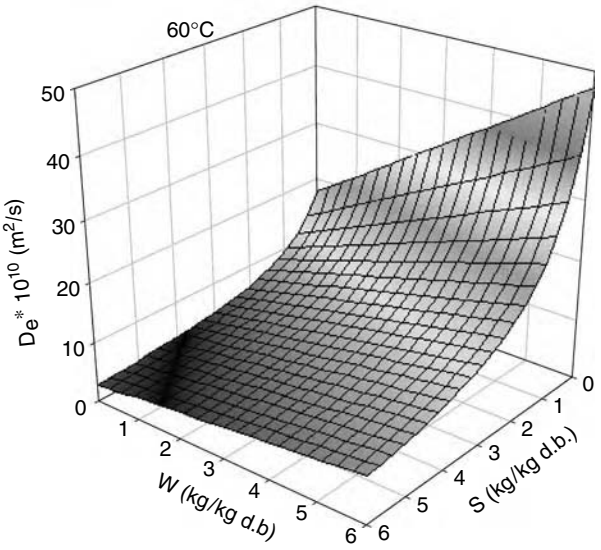

FIGURE 105.19 Variation of diffusivity with moisture and sugar concentrations for 60°C.

Figures 105.17 to 105.19 show the variation of diffusivity with the concentrations of water and sugar, for the temperatures of 20, 40 and 60°C.

DEDICATION AND ACKNOWLEDGMENT

To the memory of Professor José Almiro Castro.

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106 Principles and Applications of Freeze-Concentration and Freeze-Drying

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CONTENTS

In freeze-concentration and freeze-drying processes water is first frozen in the material. Ice is removed by mechanical means during freeze-concentration leaving a concentrated liquid, while ice is removed by sublimation in freeze-drying yielding a dried material. The removal of water by these methods would yield high quality products, but in both processes it is a very expensive operation due to the high consumption of energy. Knowledge of the theoretical principles behind these processes is necessary for minimization of detrimental changes, operating strategies, or optimization purposes. Thus, the fundamental aspects of freeze-concentration and freeze-drying are presented in this chapter with special emphasis on food preservation processes.

I. FREEZE-CONCENTRATION

A. Introduction

Freeze-concentration is the term used to describe the solute redistribution in an aqueous solution with an initial relatively low concentration by the partial freezing of water and subsequent separation of the resulting ice (1, 2). Freeze-concentration is based on the freezing temperature-concentration diagram (Figure 106.1) (3). Freeze-concentration has been applied to preconcentration for analytical purposes (4), desalinitation, concentration of juices (5), concentration of dairy products (6) and waste water treatment (7).

It is necessary to briefly review the physicochemical changes that occur during a freezing process before relating them to the freezing of foods. The phase diagram (Figure 106.1) allows identifying different phase boundaries in a mixture. It consists of the freezing curve (AB), solubility curve (CE), eutectic point (E), glass transition curve (DFG),

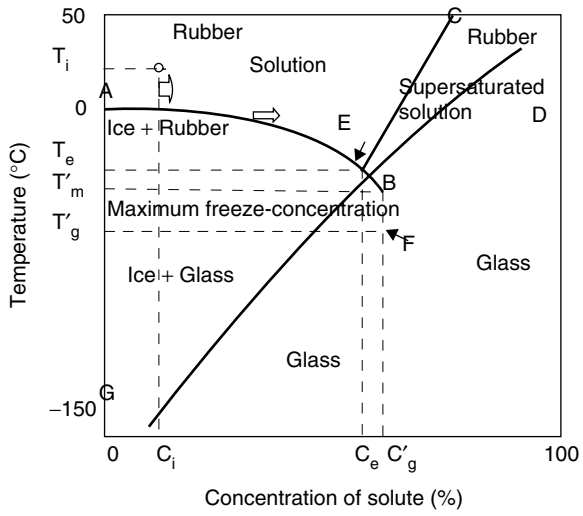

FIGURE 106.1 Typical solid-liquid state diagram for a food system.

and conditions of maximal freeze-concentration. The freezing curve corresponds to solution-ice crystals equilibrium. Along this curve, as water is removed as ice, the concentration of solute increases during the freeze-concentration process. The solubility curve represents equilibrium between the solution and supersaturated solution in a rubbery state. The freezing and solubility curves intersect at the eutectic point E (C_e, T_e), which is defined as the lowest temperature at which a saturated solution (liquid phase) can exist in equilibrium with ice crystals (solid phase). The water content at point E is the unfreezeable water. Below T_e only ice crystals embedded in a solute-water glass exist. The point F (C'_g, T'_g) lower than point B (C'_g, T'_m) represents a characteristic transition in the state diagram. The glass transition curve (DFG) represents the glass-rubber transition of the solute-water mixture, and the type and concentration of the solute and the temperature define it. Above the DFG curve, solutions are in an unstable rubbery or liquid state, below the DFG curve, solutions transform into the glassy state (amorphous solid). The maximum freeze concentration (maximum ice formation) only occurs in the region above T'_g but below the equilibrium ice melting temperature of ice (T'_m) (8, 9). The liquid solute-water mixture is the maximum freeze concentrated and has become glassy. The glass transition temperature of this unfrozen glassy mixture is designated T'_g, and C'_g is the solid content of this glass (3, 8–11). Figure 106.1 also shows the aqueous solution with initial concentration and temperature C_i and T_i undergoing freeze-concentration.

B. Freeze-Concentration System

A typical freeze-concentration system (Figure 106.2) consists of three fundamental components: (a) a crystallizer or freezer, (b) an ice-liquid separator, a melter-condenser, and (c) a refrigeration unit.

In the freeze-concentration system, a solution is usually first chilled to a pre-freezing temperature in a cooler (Figure 106.2), then the solution enters the crystallizer where part of the water crystallizes. Cooling causes ice crystal growth and an increase in solute concentration. The resulting mixture of ice crystals and concentrated solution is pumped through a separator where crystals are separated and the concentrated solution is drained off. Ice crystals are removed and melted by hot refrigerant gas. The final products are cold water and concentrated solution, which flow separately (1, 12, 13).

1. Crystallizers

The heat of crystallization can be taken out directly or indirectly. In direct-contact crystallizers the original solution is allowed to get in contact with the refrigerant, and heat is withdrawn by vacuum evaporation of part of the water, usually at pressures below 3 mm Hg, and by evaporation

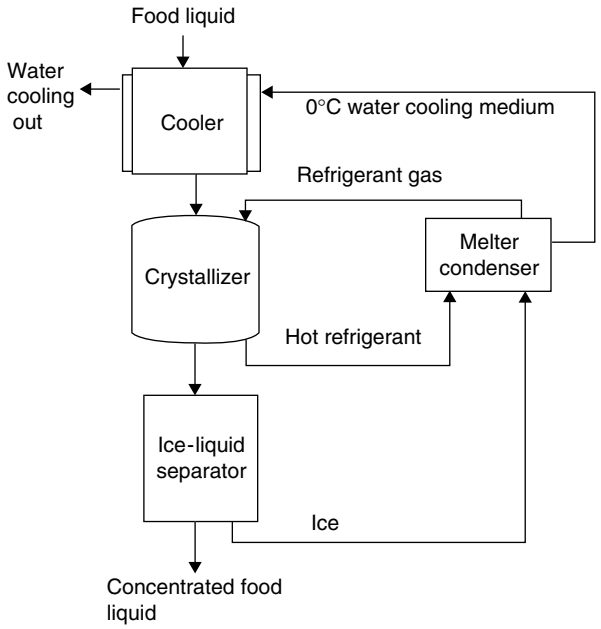

FIGURE 106.2 Schematic diagram for the freeze-concentration process of foods.

of the refrigerant. The refrigerants (CO_2, C_1–C_3 hydrocarbons) form ice-like gas hydrates, which sequester water at temperatures above 0°C. A disadvantage of this method is that part of the aromas will be lost during the evaporation. Direct heat removal is applied in seawater desalinization, but is not suitable for liquid foods, due to the aroma losses and deterioration of the product by the refrigerant. In crystallizers with indirect heat removal, the refrigerant (R22 or ammonia) is separated from diluted solution by a metal wall. So, crystallization takes place on chilled surfaces, from which ice crystals are removed by a scraper. This kind of process has been used commercially for orange juice and coffee concentration (1, 12, 13).

2. Separators of Ice-Concentrated Solution

The separation of ice crystals from concentrated solutions can be performed by use of presses, centrifuges, and washing columns, operating either in batch or continuous mode (12).

Hydraulic and screw presses are used for pressing ice-concentrated slurries to form an ice cake. Pressures around 100 kg/cm^2 are needed to avoid occlusion of solids in the cake, which is the limiting factor of this method. Since the presses are completely closed, aroma loss is negligible (1, 14).

Ice and concentrated solutions may be separated by centrifugation at about 1000 G. Centrifugation must be conducted under inert atmospheres to reduce oxidation and aroma loss. Solute losses may occur if concentrated solution remains adhered to the crystals surface, but washing of the cake with water minimizes such losses. This washing stage is a more efficient centrifugation operation than pressing (1, 2, 14). Application of progressive freeze-concentration (PFC) is used to improve the traditional freeze-concentration process. With PFC, only a single ice crystal is formed as a sheet on a cooled surface immersed in the liquid, thus, the separation of ice crystal from the mother solution is much easier compared with the suspension crystallization method (15).

In washing columns, the ice-solution mixture is introduced at the bottom of the tower and the solution is drained off. The crystals move toward the top of the column in countercurrent to the wash liquid, which is obtained by melting part (5–3%) of the washed crystals leaving the column. In this process the loss of dissolved solids with the ice is less than 0.01% and aroma losses are negligible. Washing columns are preferred in freeze-concentration of low viscosity liquids such as beer and wine (1, 12, 13).

C. Influence of Process Parameters

Crystallization is the main step in freeze-concentration, therefore, it is very important to obtain large and symmetrical crystals. Large crystals can be more easily separated from the concentrated solution. Large crystals also reduce the loss of solutes due to occlusion and adherence to the small crystals (1, 3). During crystallization two kinetic processes take place: the formation of nuclei and the growth of crystals. Nucleation is the association of molecules (at some degree of subcooling) into a small particle which serves as a site for crystal growth. Once nucleus is formed, crystal growth is simply the enlargement of that nucleus. Nucleation and growth of crystals are dependent on solute concentration, bulk supercooling, residence time of the crystals in the crystallizer, freezing rate, molecular diffusion coefficient of water, and heat transfer conditions. These factors should be carefully controlled to regulate crystal formation (2, 16).

1. Solute Concentration

In general an increase in solute concentration produces an increase in nucleation, and a decrease in the growth velocity of the ice crystals and in the mean diameter of the crystal. At critical concentration, solutes may solidify along with ice and are difficult to separate. Practical maximum concentrations for freeze-concentration are between 45–55% range (1, 14, 16).

2. Bulk Supercooling

Supercooling is the driving force responsible for the creation of crystal nuclei and its growth. The nucleation rate is proportional to the square of the bulk supercooling. At high bulk supercooling values the nucleation rate decrease due to the inhibition of molecular mobility.

Crystal growth exhibits a first order dependence of the bulk supercooling (1, 14, 16).

3. Residence Time of the Crystals in the Crystallizer

At constant bulk supercooling and solute concentration the crystal size is proportional to the crystal residence time. At short residence time the crystals produced are very small (1, 16).

4. Freezing Rate

A high freezing rate results in a strong local supercooling near the heat-removing interface, thus leading to high nucleation rates and to small crystals. A decrease in freezing rate results in large, uniform crystals with small surface area (1, 16).

5. Molecular Diffusion Coefficient of Water

A decrease in the value of the molecular diffusion coefficient of water results in a decrease in diameter of the crystals (1).

6. Heat Transfer Conditions

The growth rate of ice crystals increases greatly as the rate of heat removal is increased until some very low sample temperature at which mass transfer difficulties (at high viscosity) causing the growth rate to decline. Very large uniform crystals require large exchange surface at relatively high temperatures (1, 2, 14).

7. Viscosity of the Liquid

Viscosity increases markedly as concentration increases, ice crystals grow very slowly at high viscosity, and large crystals become difficult to separate. The maximum concentration obtainable in freeze-concentration depends on the liquid viscosity. Generally, concentration can be carried out to the point where the slurry becomes too viscous to be pumped. For essentially all liquids this viscosity limit is encountered before eutectic point formation occurs (Figure 106.1). The viscosity of cold concentrated liquid and ice is very high and agitation, which is necessary for proper crystal growth, becomes more difficult (9, 16).

In all ice separators, capacity is inversely proportional to the viscosity of the concentrate and directly proportional to the square of the mean diameter of the crystals as expressed by the equation:

$$Q = \frac{\Delta P\, g\, d_e^2}{0.2\,\mu l} * \frac{\varepsilon^3}{(1-\varepsilon)^2} \qquad (106.1)$$

where Q is the draining rate from the crystal bed (cm^3/cm^2s); ΔP is the pressure difference exerted over the bed by compression, centrifugal or pressure drop of the filtrate (kg/cm^2); d_e is the diameter of the crystals (cm); μ is the viscosity of liquid (poise); l is the thickness of the bed (cm); g is the gravity acceleration (cm/s^2); and ε is the volume fraction in the bed filled by the liquid phase (1).

II. FREEZE-DRYING

A. Introduction

Freeze-drying, or lyophilization, is the process of removing water from a product by sublimation and desorption (17). Sublimation is the transformation of ice directly into a gas without passing through a liquid phase. Sublimation occurs when the vapor pressure and the temperature of the ice surface are below those of the triple point (4.58 mm Hg, 0°C), as shown in the pressure-temperature phase diagram of pure water (Figure 106.3) (18). This technique is a widespread dehydration process in which the quality of final products is very high in comparison with that of products dehydrated using other techniques due the prevention of heat damage. Freeze-dried products have high structural rigidity, high re-hydration capacity, low density, and retain the initial raw material properties such as appearance, shape, taste, and flavor. This process is generally used for the dehydration of products of high added value and sensitive to heat treatments, produced by pharmaceutical, biotechnological and food industries.

The phase diagram of Figure 106.3 is separated by lines into three regions, which represent the solid, liquid, and gaseous states of water in a closed system. The points along the separating lines represent the combinations of temperature and pressure at which two states are in equilibrium: liquid-gas equilibrium (DB line), liquid-solid equilibrium (DA line), and solid-gas equilibrium (DC

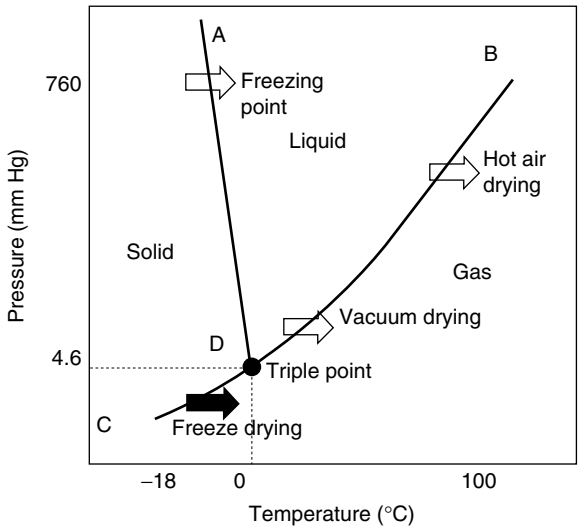

FIGURE 106.3 Pressure-temperature phase diagram of pure water.

line), which is of main concern in freeze-drying. Point D represents the only combination of temperature and pressure at which all three states of water are simultaneously in equilibrium, and it is called the triple point (4, 18).

Freeze-drying can also be conducted at moderated pressures and even at atmospheric pressure. The principle of this process is to produce a vapor pressure difference as large as possible by blowing dry air over the frozen material. In practice, the process is very long because of the low mass and energy transfer rates, but problems related to the application of vacuum do not exist, resulting in an important reduction of operation costs (19, 20).

Compared to air drying processes, which remove water in a single stage, freeze-drying is an expensive process since it takes large operation times and consumes large amounts of energy. Energy is required to freeze the product, heat the frozen product to sublimate ice, condense water vapor, and maintain the vacuum pressure in the system (21, 22). Despite of many advantages, freeze-drying has always been recognized as the most expensive process for manufacturing a dehydrated product (23).

B. Basic Components of a Freeze-Dryer

The typical freeze dryer consists of a drying chamber, a condenser, a vacuum pump, and a heat source (Figure 106.4).

The drying chamber, in which the sample is placed and heating/cooling take place, must be vacuum tight and with temperature controlled shelves. The condenser must have sufficient condensing surface and cooling capacity to collect water vapor released by the product. As vapors contact the condensing surface, they give up their heat energy and turn into ice crystals that will be removed from the system. A condenser temperature of −65°C is typical for most commercial freeze dryers. The vacuum pump removes non-condensable gases to achieve high vacuum levels (below 4 mm Hg) in the chamber and condenser. The heating source provides the latent heat of sublimation, and its temperature may vary from −30 to 150°C (19, 24).

Nowadays different variants of freeze-drying are studied like microwave-freeze-drying and fluidized atmospheric freeze-drier. Neverthless the first has technical problems in food industry and in the second drying times are higher and quality of product is not good due to the use of atmospheric pressure (23). Microwave can speed up the conventional freeze-drying because microwave energy augments the convection heating and drying rates can be increased by as much as an order of magnitude (25). The cost of freeze-drying process depends on the type of raw material, products, packaging, capacity of the plant, duration of the cycle, etc. Freeze-drying not be regarded as a prohibitively expensive preservation process if it gives a reasonable added value to the product (23). Freeze-drying is a method which can be used only for high value vegetables like mushrooms and capsicum or used for products that can be sold at a premium or can withstand only a small amount of sensory deterioration (25, 26).

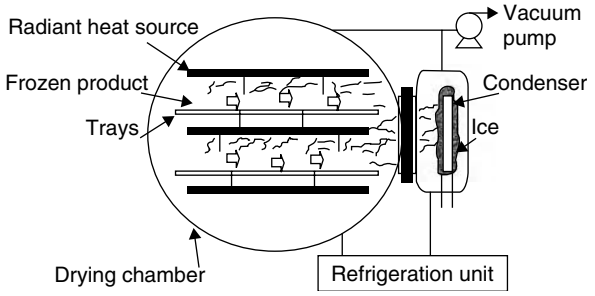

FIGURE 106.4 Simple schematic representation of a freeze dryer system.

C. Freeze-Drying Stages

Freeze-drying involves three essential stages: initial freezing, primary drying, and secondary drying. The objective of the freezing stage is to freeze the mobile water of the product. The product must be cooled to a temperature below its eutectic point, which is the temperature and composition combination that produces the lowest point at which a product will freeze. Freezing has an important influence on the shape, size, and distribution of the ice crystals and thus, on the final structure of the freeze-dried product. In the primary drying, the frozen product is heated under vacuum conditions to remove frozen water by sublimation, while the frozen product is held below the eutectic temperature. During the primary drying, approximately 90% of the total water in the product, mainly all the free water and some of the bound water, is removed by sublimation (27, 28). In the secondary drying, bound water (unfrozen) is removed by desorption from the dried layer of the product, achieving a product that should contain less than 1–3% residual water. This final stage is performed by increasing the temperature and by reducing the partial pressure of water vapor in the dryer (18, 28).

The secondary drying stage requires 30 to 50% of the time needed for primary drying due to the lower pressure of the remaining bound water than free water at the same temperature, yielding a slow process. Freeze-drying is complete when all the free and bound water has been removed, resulting in a residual moisture level that ensures desired structural integrity and stability of the product (18, 22).

D. Heat and Mass Transfer in Freeze-Drying

During the freeze-drying operation, a coupled heat and mass transfer process occurs within the product: energy is transported to the sublimation zone and water vapor is generated. In contrast with mass transfer, which always flows through the dry layer, heat transfer can take place by

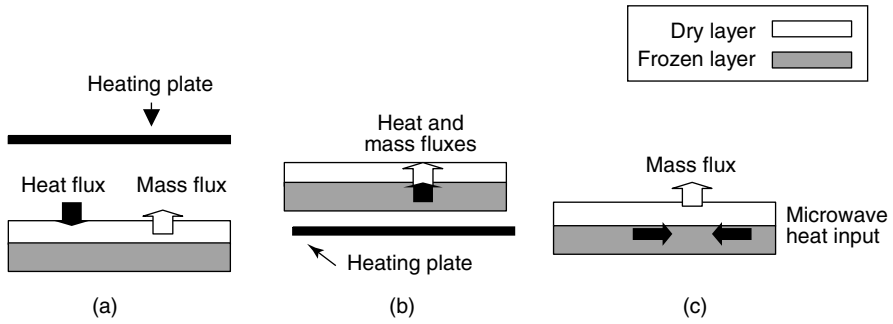

FIGURE 106.5 Basic types of freeze-drying.

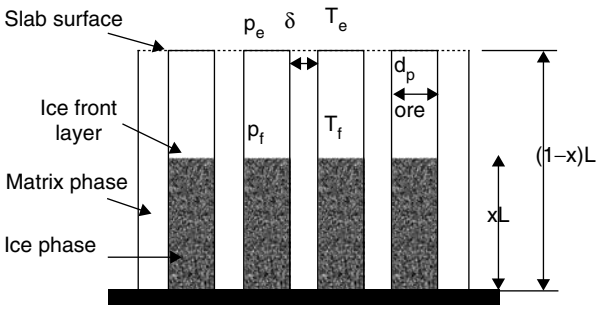

FIGURE 106.6 Schematic representation of freeze-drying of a slab.

conduction through the dry layer (Figure 106.5a) or through the frozen layer (Figure 106.5b), and by heat generation within the frozen layer by microwaves (Figure 106.5c) (12, 20). Microwaves are used as a heat source for drying because they are able to penetrate deeply into product, giving a more effective and uniform heating (29, 30).

Figure 106.6 illustrates a frozen food sample in the form of a slab, with a frozen and a dried porous layer, undergoing one-dimensional freeze-drying (18, 31). The interface between the dried and the frozen layers is referred to as the sublimation or ice front, and it is assumed to move at a uniform rate. The vapor flows through the pores and channels.

In case heat is supplied through the dry layer, the heat flux to the ice front is given by

$$q = k_d \frac{(T_e - T_f)}{(1 - x)L} \tag{106.2}$$

where q is heat flux (J/m^2 s), k_d is the thermal conductivity of dry layer (W/m K), T_e is the temperature at slab surface (°C), T_f is the temperature of sublimation front (°C), L is the thickness of slab (m), and x is the relative height of ice front.

If heat is transferred through the frozen layer,

$$q = k_f \frac{(T_p - T_f)}{xL} \tag{106.3}$$

where k_f is the thermal conductivity of frozen layer (W/m K) and T_p is the heating plate temperature (°C).

If vapor flows in the pores mainly by Knudsen diffusion, the collisions with the dry walls are numerous compared with collisions between water molecules, then the rate of ice sublimation (N_W, kg/s m^2) is given by

$$N_w = \varepsilon D_K \frac{M_w}{RT} \frac{p_f - p_e}{(1 - x)L} \tag{106.4}$$

where ε is the volume fraction of ice, D_K is the Knudsen diffusion coefficient of water vapor (m^2/s), M_w is the molecular mass of water (kg/mol), R is the gas constant (J/mol K), T is the absolute temperature (K), p_f is the vapor pressure at the ice front, and p_e is the vapor pressure at the slab surface (Pa).

The Knudsen diffusion coefficient is related to pore diameter (d_p) and temperature by

$$D_k = \frac{2}{3} d_p \left(\frac{2RT}{\pi M_w} \right)^{0.5} \tag{106.5}$$

At the ice front, T_f represents the sublimation temperature, and by assuming it to be in equilibrium, it is related to vapor pressure (p_w) by the Clausius-Clapeyron equation,

$$\ln(p_w) = 28.9 - \frac{6138}{T_f} \tag{106.6}$$

From a mass balance for water vapor around the drying slab:

$$N_w = \varepsilon \rho_{ice} \frac{d(xL)}{dt} \tag{106.7}$$

where ρ_{ice} is the density of ice (kg/m^3).

Assuming that all supplied heat is used for sublimation of ice, the enthalpy balance gives:

$$q = \Delta H_s N_w \tag{106.8}$$

where ΔH_s is the latent heat of sublimation (J/kg).

For temperature differences not too large, the Clausius-Clapeyron equation can be linearized and the above equations can be solved analytically. The following expressions can be derived for the total drying time

$$t = \frac{\alpha L^2}{D_k}(1 + \beta D_k) \quad (106.9)$$

in which

$$\alpha = \frac{1}{2}\rho_{ice}\frac{RT_f}{M_w}\frac{1}{(p_f - p_e)} \quad (106.10)$$

If heat transfer takes place through the dry layer, then $\beta = 0$; and if heat transfer occurs through the frozen layer,

$$\beta = \varepsilon\frac{M_w^2 \Delta H_s^2 p_f}{R^2 T_f^3 k_{ice}} \quad (106.11)$$

where k_{ice} is the thermal conductivity of ice (W/m K).

Several mathematical equations describing mass and energy transfer have been developed for modeling the freeze-drying process. Such models account for the removal of frozen water only (sublimation model) or the removal of frozen and bound water (sorption sublimation model), examine the methods of supplying heat and the diffusion mechanisms, describe steady or non-steady state processes, or analyze both transfers under various processing conditions. Some models have been found to describe accurately experimental drying rates and freezing times. However, a major problem in the application of some models is the requirement of reliable data on thermal and mass transport properties of food materials such as diffusivity within the porous medium, Knudsen diffusion, water vapor concentration in the dry layer, porosity, effective thermal conductivity, permeability, etc. (21, 27, 29, 32).

E. Influence of Parameters

A number of operation variables influence the performance of the freeze-drying process and the characteristics of the final product.

1. Freezing

The freezing rate has an important influence on the ice configuration and thus on the final structure of the freeze-dried product. Slow freezing rates allow the growth of large ice crystals leading to larger pores, higher mass flow, and thus to shorter freeze drying time (18, 31).

2. Heat Flux

Heat flux that reaches the product is an important factor to reduce drying rate. However, if the drying proceeds too rapidly (high heat flux), the product may melt, collapse, or can be blown out of the container (24). This may cause degradation of the product, and will change the physical characteristics of the dried material. Excessive heat may cause the dry cake to char or shrink. Heating rate can be optimized during operation modifying conveniently product temperatures in the dried zone and at the sublimation front (21, 33).

3. Chamber Pressure

The most important operation variable in the freeze-drying process is chamber pressure. Pressure has a combined effect as controlling mean of sublimation temperature and for modifying the transport parameters that influence the kinetics of vapor removing. At a given temperature, a decrease of the pressure in the drying chamber reduces the vapor pressure at the product's external surface (p_e), thus the driving force ($p_f - p_e$) for drying is enlarged and the total drying time is reduced. Nevertheless, at low pressures, sublimation rate may be limited by the transport of water vapor through the product, if the transport of water vapor falls in the free molecular flow regime (29, 33, 34).

Chamber pressure affects the transport properties, thermal conductivity and water vapor diffusivity. Thermal conductivity of the dry layer is higher at higher chamber pressures, within the range of freeze-drying operation, resulting in high heat transfer rates from the surface to the ice front. Water vapor diffusivity through the dry layer is, however, less at higher chamber pressures, producing low mass transfer rates. So, when pressure is low (low sublimation temperature) freeze-drying is often a heat-controlled process, but at relatively high pressures, freeze-drying becomes a mass-controlled process. In most situations the drying rate is limited by the rate of heat transfer through the dry layer (33–35).

4. Temperature

Aroma diffusivities are very similar to that of water when water content is still high, therefore, maintaining low temperatures during primary drying will reduce aroma losses. The melting point of products has a significant effect on the selection of operation pressures since this is a fundamental factor for the sublimation temperature. Normally, vacuum must be kept so high that no melting occurs in the product during the process, and a true freeze-drying or sublimation takes place. If the temperature of ice in the condenser is higher than product's temperature, water vapor will tend to move toward the product, and drying will stop (34, 35).

When freeze-drying temperature is high enough, the product cake suffers a drastic loss of its structure and is said to have suffered collapse. This phenomenon occurs when the solid matrix can no longer support its own weight, leading to drastic structural changes shown as a marked decreased volume, increase in stickiness of dry powders, loss of porosity, etc. (31). Collapse affects aroma retention, caking and stickiness, rehydration capacity, and final moisture of the product (36). A collapse temperature (T_c) is related to the glass transition temperature (T_g), which in turn depends on temperature and moisture content (Figure 106.1). At temperatures higher than T_g, the viscosity of the amorphous matrix decreases drastically, this decrease being a function of (T−Tg). As the viscosity decreases to a level that facilitates deformation, the matrix can flow, and structural collapse can

occur. A critical viscosity in the range of 10^5–10^8 Pas has been reported to observe collapse (28, 37).

III. CONCLUSIONS AND RECOMMENDATIONS

Despite the reduced use at industrial level of freeze-concentration and freeze-drying processes within food area, both are important to obtain high quality products. Deep knowledge of the thermodynamic fundamentals of phase changes of water in foods and of the effect of the variables on the processes' effectiveness and cost, can open new opportunities for the application of both processes to obtain high quality preserved foods. In the same way and with the development of new ideas about minimal processing and longer storage life for food products, industrial applications of freeze-concentration and freeze-drying could be increased in the near future.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Principles of freeze-concentration and freeze-drying," by J. Welti-Chanes, D. Bermúdez, A. Valdez-Fragoso, H. Mújica-Paz and S. M. Alzamora, IN *Handbook of frozen foods*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.

This work received the support of the Fundacion Produce Puebla, A. C. Mexico.

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Part L

Thermal Processing

107 Thermal Processing of Foods: Principles and Applications

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CONTENTS

I. INTRODUCTION

A. Purpose and Goals

A variety of "non-traditional" processing technologies, such as high-pressure processing or pulsed electric fields, are gaining significant attention in the food industry; however, the application of heat (i.e., thermal processing) remains the dominant means for processing most manufactured food products. Given the practical constraints of cost, throughput, and traditional practice, thermal processing will likely continue as the dominant means for processing value-added food products. Therefore, it is important for practicing food engineers to understand the fundamental principles that underlie the various processing operations that employ heat to achieve the desired purposes.

Thermal processing systems for food products are designed to achieve at least one, but usually several, of the following goals: (i) To destroy undesirable microorganisms that either cause spoilage of the product or are human pathogens, (ii) To inactivate native enzyme systems within the product, which would otherwise affect product quality, and (iii) To impart some desirable quality characteristics to the product, including flavor, color, and textural attributes. For example, heat is the means by which the toxin-producing *Clostridium botulinum* is inactivated in

canned foods. In that same canned product, heat inactivates pectin methylesterase, an enzyme that softens many vegetable products by attacking pectin in the cell wall. In terms of positive quality factors, heat helps create the desired crust and crumb features during baking of bread. In the case of ready-to-eat meat products, heat is used both to inactivate pathogenic bacteria (e.g., *Salmonella*) and to simultaneously create a desirable texture via protein denaturation during cooking. Table 107.1 lists several other examples of how heat plays a positive (+) and negative (−) role in achieving various processing goals.

Often, the best opportunities for improving these types of thermal process occur when operational experience melds with a sound understanding of the fundamental principles behind the process of interest. Operational experience is a valuable asset in food processing; however, without an understanding of the basic principles of heat and mass transfer, attempts at process improvement can result in a haphazard series of hit-and-miss adjustments to a given process. For example, an oven operator might observe that an increase in air velocity in an air impingement oven increases product temperature and therefore enables an increase in product throughput; however, that same operator might not recognize that this increase in velocity also increases mass transfer (via convection), which might have an undesirable effect on product quality, yield, and/or safety. In fact, Deming (1), the "father" of many of the principles embodied in total quality management and continuous quality improvement paradigms, noted that experience alone cannot ensure continuous process improvement. Deming calls for a "system of profound knowledge," in which one must first seek to understand the fundamental characteristics/properties/behaviors of a process or system before attempting to improve that process. In the case of thermal processing of food products, the primary foundation of that "profound knowledge" is comprised of the principles of heat and mass transfer. Only when these fundamental principles are well understood can the food engineer help ensure that any changes in process design or operation are directed toward improvements in the process outcome, whether that is in product safety, quality, or yield.

Therefore the overall goal of this chapter is to outline the general principles of heat and mass transfer that are essential background for a wide range of specific thermal operations that are detailed in subsequent chapters. This introduction is focused on principles most relevant to thermal processing of foods, and therefore cannot cover every aspect of heat and mass transfer. For comprehensive treatment of heat and mass transfer principles, the reader is directed to any of several good monographs or textbooks on the subject (2–5).

TABLE 107.1
Examples of How Heat Affects Various Thermal Processing Targets, Either as a Positive (+) or Negative (−) Influence

	Processing Target		
Product	**Microbial Target**	**Enzyme**	**Quality Factor**
Canned green beans	(+)*C. botulinum* spores	(+)Pectin methylesterase	(−)texture (−)vitamin content
Baked goods			(+)crust/crumb texture (+)color
Pasteurized milk	(+)*Coxelliae burnettii* (+)spoilage organisms		
Ready-to-eat meat products	(+)*Salmonella* (+)*E. coli* O157:H7 (+)*Listeria monocytogenes*		(+)flavor (+)texture (+)color

B. Categorizing Products

Before outlining the underlying principles of thermal processing, it is necessary to define the domain of interest and application. There are a variety of schemes for categorizing food processing operations. With respect to heat and mass transfer, there are two different categorizations that are important — one based on the product and one based on how the product is exposed to the environment within the operation of interest.

In terms of the physical state of the food product, almost every product is actually comprised of matter in a mixture of phases: solid, liquid, and gas. For example, baked bread is a solid matrix containing a gaseous phase in the crumb. At −5°C, a raw meat product is comprised of an aqueous solution, solid water (ice), and insoluble solids (i.e., fats and certain proteins). The physicochemical complexity of such food products can make a detailed, rigorous analysis of heat and mass transfer during processing a very challenging task. Therefore, it is common to describe these products in a simplified manner, in order to make the heat and mass transfer analysis feasible and practical. Therefore, we can presume to place all food products into three distinct categories, according to physical state: (1) *fluid foods* (e.g., milk, ketchup, applesauce), (2) *solid foods* (e.g., meat patties, breakfast cereals, dried pasta), and (3) *mixed phase foods* (e.g., soups with particulates, canned beans in water). The third category (mixed phase) will be reserved to describe those products that have *macroscopically* distinct phases (i.e., "chunks and pieces"), rather than the cellular level mixture of phases that is common to many food ingredients and products.

It is also important to categorize food products according to how they are exposed to the processing environment. The two categories are: (1) *packaged products* and

(2) *unpackaged products*. Although the distinction is simple, the impact on thermal processing is significant. An impervious package (e.g., can, jar, bag) containing a product has the critically important effect of essentially stopping mass transfer in and out of the product during processing. Of course, some types of packaging, such as plastics, have various degrees of permeability to gases, with oxygen and water vapor being the most important. However, because these permeabilities are relatively low, the time scale for gas movement through the packaging is relevant to product transportation and storage (days to months), rather than to thermal processing operations, which are generally relatively short events (on the order of minutes). Thermal processing of packaged products is therefore primarily a *heat transfer* operation.

In contrast, unpackaged products are exposed to the heating medium, such as air, water, or oil. Consequently, thermal processing of unpackaged products is *both* a heat transfer and mass transfer operation. This adds an additional degree of complexity to the analysis of such operations, as heat transfer and mass transfer are rarely independent processes. For example, mass transfer can create a heat transfer effect when water diffusing to the surface of a product evaporates and creates a cooling effect on the product surface, due to the latent heat of vaporization. Also, oil diffusing into a product in an immersion fryer will carry heat energy into the product. Heat transfer can also create a mass transfer effect when, for example, the heating of a product changes the solid-vapor equilibrium for water in the product and in the surrounding air, and thereby affects drying operations.

Given the limitless combinations of product state, packaging, and processing operations, it would be impractical to describe and analyze heat and mass transfer for every application. Therefore, this chapter focuses on the fundamental principles of heat and mass transfer that underlie every thermal process. After the basic principles are covered, a variety of product/process applications will be examined to illustrate how heat and mass transfer principles are relevant to process design and operation.

II. HEAT TRANSFER PRINCIPLES

A. General Concepts

If a system is in thermal equilibrium, then the temperature is the same at every point in the system. In this state, there is no driving force for heat to flow. If the system is not in thermal equilibrium, then a temperature differential becomes the driving force for heat energy to flow through the system. As is the case in most energy transport phenomena, it can be stated, in the most general terms, that:

$$\text{energy flux} = \frac{\text{driving force}}{\text{resistance}} \qquad (107.1)$$

In heat transfer, the driving force is related to the temperature differential mentioned above, and the resistance is a property of the system.

With respect to the thermal processing of foods, the "system" might be the food product itself, some element of the processing equipment, or the walls surrounding the processing facility. In all of these cases, a temperature differential will cause heat to flow. There are three basic mechanisms by which this occurs: *conduction, convection,* and *radiation*. The subsequent sections describe these mechanisms, outline rate laws for each, and illustrate simple analysis methods applicable to thermal processing of foods.

B. Conduction

1. Fourier's Law

Temperature is a measure of the mean kinetic molecular energy of matter. As molecules with higher energy collide with or interact with nearby molecules of lower energy, energy is transferred from the higher energy molecule to the lower energy molecule. This molecule-to-molecule interaction is the basis of heat conduction.

If we consider a simplified slab of solid material that has a higher temperature (T_1) on one face than on the other (T_2), the molecule-to-molecule energy transfer described above will result in a net heat flow (q) from the hotter to colder surface (Figure 107.1). This conduction heat transfer is described by *Fourier's Law*:

$$\frac{q}{A} = -k\frac{dT}{dx} \qquad (107.2)$$

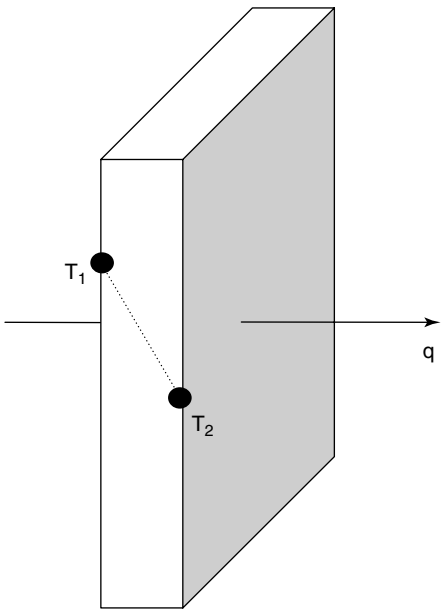

FIGURE 107.1 One-dimensional heat conduction through a slab, from higher temperature to lower temperature.

Where q is the heat flow due to conduction (Watts or Btu/h), A is the cross-sectional area of the slab, q/A is the heat flux (W/m^2), dT/dx is the temperature gradient across the slab, $(T_2 - T_1)/\Delta x$, and k is the thermal conductivity of the material (e.g., W/mK).

In order to illustrate the general principle of heat transfer stated earlier (Equation 107.1), we can rearrange Equation 107.2 to show that:

$$\frac{q}{A} = \frac{\Delta T}{\Delta x/k} \Rightarrow flux = \frac{\text{driving force}}{\text{resistance}} \qquad (107.3)$$

Therefore, in conduction, the driving force for energy transfer is the temperature differential (ΔT) across a finite distance, and the resistance is determined by the thickness (Δx) and thermal conductivity (k) of the material. This resistance term (Δx/k) is commonly referred to as the "R value" for building and construction materials. The concept of thermal resistance in food processing will be further illustrated and applied in subsequent sections.

Within the resistance term, thermal conductivity is an intrinsic property of a given material. From Equation 107.2, it can be seen that a material with a large thermal conductivity conducts heat well, whereas a material with a lower thermal conductivity conducts heat relatively poorly. Table 107.2 lists values of k for a variety of relevant materials. Obviously, metals have a thermal conductivity several orders of magnitude above those of food materials. In fact, the relatively low thermal conductivity of most food materials presents the food engineer with the special challenge of designing thermal processes that optimally match equipment capacities with product characteristics and the particular processing goals (e.g., heating time).

Additionally, there is a wide range of k values among food materials, which depends on chemical composition, physical structure, and temperature. In particular, k is 2–4 times higher for frozen foods than for the same foods in the unfrozen state. Additionally, k increases with moisture content of the food material.

Acquiring accurate k values (and other thermophysical properties) can be one of the most significant challenges in conducting heat transfer analyses of thermal processes for foods. There are essentially three choices for getting k values: (i) finding appropriate values in published sources, such as research articles or handbooks (6), (ii) using predictive equations, usually based on composition and temperature (6,7), or (iii) making experimental measurements with the product of interest, using a thermal conductivity probe (8). The choice of method depends on the application and required accuracy. Using published values is convenient, and suitable estimates can typically be found this way for preliminary or rough analyses involving relatively common, unprocessed food products (e.g., raw meat or vegetables). However, it is difficult to find published values for most processed food products. In contrast, general predictive equations are relatively straight forward, and account for the composition and temperature of the product of interest (assuming these are known); however, most of these general equations were developed for liquid or semi-liquid foods, so any degree of physical structure (e.g., porosity) within a food product can create appreciable errors from the equation-predicted values of k (9). Lastly, thermal conductivity probes can be constructed or purchased commercially, and allow for direct measurement of k for the product of interest. The downside is that the cost and time required for high quality measurements are quite high, and therefore must be weighed against the goal of the analysis and the importance of accuracy.

TABLE 107.2
Typical Thermal Conductivities of Various Example Materials

Material	k (W/mK)
Aluminum	200
Stainless steel	16
Plywood	0.12
Expanded polyurethane	0.02
Chicken breast (unfrozen)	0.41
Chicken breast (frozen)	1.50
Butter	0.20

2. Governing Equation

Fourier's Law (Equation 107.2) describes heat flux at an instant in time and a point in space. If Fourier's Law and the first law of thermodynamics (i.e., conservation of energy) are applied to a control volume, a general governing equation can be derived to describe one-dimensional heat transfer in a system, under any condition:

$$\underbrace{\rho c_p \frac{\partial T}{\partial t}}_{storage} + \underbrace{\rho c_p \frac{\partial}{\partial x}(uT)}_{bulk\ flow} = \underbrace{\frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right)}_{conduction} + \underbrace{Q}_{generation} \qquad (107.4)$$

Where ρ is the material density, c_p is the material specific heat, u is the bulk movement/velocity of the material through the control volume, and Q is a heat generation term (W/m^3).

Each of the four terms in Equation 107.4 represent a different aspect of thermal analysis. Therefore, this general equation is the starting point for all subsequent analyses of conduction heat transfer in food materials. However, solution of the complete equation is rarely needed, because one or more of the terms can usually be neglected for a given analysis. The *storage term* represents the net change in thermal energy in the control volume over time. For a steady-state problem (discussed in the next section), temperature

varies with location but not time; therefore, the storage term can be ignored for that case. The *bulk flow term* represents energy moving in or out of the control volume due to mass flow. This is relevant to problems with fluid foods or materials; however, this term can generally be ignored for analysis of heat flow in solid materials. The *conduction term* describes the change in conductive flux across the control volume, and can rarely be ignored in food processing applications (see next section). Lastly, the *heat generation term* represents heat that might result from biological activity (e.g., respiration of microorganisms) in a food material or from exothermic chemical reactions occurring during processing. In most cases, heat from biological activity is more relevant to food storage applications than to thermal processing applications, where the thermal energy applied by the system (e.g., ovens, retorts, fryers) is orders of magnitude greater than energy resulting from biological activity within the food product. The next few sections detail the general solution methods for each of these specific cases, as relevant to thermal processing of foods.

3. Steady-State Conduction

If the temperatures in a system are constant, but unequal (e.g., Figure 107.1), then the flow of heat is constant, and this case is called *steady-state* conduction. Given that the purpose of thermal processing operations is to change the temperature of the food product, steady-state analysis is not directly relevant to heat transfer in food products, and therefore will not be addressed in detail. Such analysis would, however, be relevant if analyzing heat loss or gain through the walls of a piece of equipment, where the exterior and interior surfaces would be at different, but relatively constant, temperatures. In that case, the steady-state heat flux through the equipment wall could be estimated by:

$$\frac{q}{A} = \frac{\Delta T}{\Sigma R} \tag{107.5}$$

Where ΣR is the sum of the R-values for each "layer" comprising the wall of the equipment (e.g., a layer of insulation material sandwiched between two layers of stainless steel sheet).

4. Unsteady-State Conduction

Most thermal processing applications entail unsteady-state conduction of heat through the food material. In these cases, the goal of heat transfer analysis is generally to estimate the product temperature at a given time or to estimate the time required to achieve a target product temperature. For a rigorous analysis of any such process, Equation 107.4 (or the relevant 2-D or 3-D version of the governing equation) needs to be solved, given the boundary conditions for that analysis. For irregular shapes or non-homogeneous materials, this analysis typically requires numerical solutions methods, such as finite element methods or finite difference methods. However, in some applications (e.g., with a solid, packaged product), it is feasible to conduct a simplified analysis to estimate the desired quantities and/or to evaluate the effects of various product or process parameters (e.g., product thickness) on the process outcome. The next few sections briefly present several of these methods.

In all of these cases, the boundary conditions around the product are necessary to solve for the product temperature or processing time. There are three possible boundary conditions:

Type 1: Fixed surface temperature

$$T = T_{surface} = \text{constant} \tag{107.6}$$

Type 2: Fixed surface heat flux

$$\frac{q_{surface}}{A} = -k\frac{dT}{dx}\bigg|_{surface} = \text{constant} \tag{107.7}$$

Type 3: Convection at the surface

$$-k\frac{dT}{dx}\bigg|_{surface} = h(T_{surface} - T_{\infty}) \tag{107.8}$$

Where T_{∞} is the bulk temperature of a fluid (air, water, or oil) surrounding the product, and h is the convective heat transfer coefficient (W/m^2K). This parameter is a function of the fluid properties, velocity, and product/flow geometry, and will be discussed in more detail in a subsequent section. In analyzing a thermal process, values of h are generally: (i) provided by an equipment manufacturer, (ii) estimated by predictive correlations (see Section II.4.c), or (iii) experimentally determined. In the third boundary condition, note that $T_{surface}$ is not a constant, but instead varies with time. If h is large enough, then $T_{surface}$ approaches T_{∞}, and thereby approximates the first type of boundary condition, which simplifies several of the solutions presented in the following sections.

a. Conduction with negligible internal resistance

As previously mentioned, in rare cases, the conduction term in Equation 107.4 can be neglected in the analysis, which leads to a simplified solution to the conduction problem. For a solid product, this condition can be tested via a dimensionless number called the Biot number (Bi). The Biot number reflects the ratio of the internal to external resistance to heat flow:

$$Bi = \frac{\text{internal (conductive) resistance}}{\text{external (convective) resistance}} = \frac{\frac{L}{kA}}{\frac{1}{hA}} \tag{107.9}$$

Where L is a characteristic dimension of the product, calculated as the ratio of volume V to surface area A. Therefore, Equation 107.9 can be rewritten as:

$$Bi = \frac{h\left(\frac{V}{A}\right)}{k} \tag{107.10}$$

For cases where the internal resistance is more than an order of magnitude less than the external resistance (Bi < 0.1), then the following simplified solution of Equation 107.4, known as the *lumped parameter method*, can be used with solution errors typically less than 5%:

$$\frac{T - T_\infty}{T_i - T_\infty} = e^{\left[\left(\frac{-hA}{mc_p}\right)t\right]} \tag{107.11}$$

Where A, m, and c_p are the surface area, mass, and specific heat of the product, respectively, T_i is the initial temperature, t is time, and T is the product temperature at that time. This solution is typically valid only for very conductive materials, such as metals. However, the Bi < 0.1 condition can also be satisfied for very low airflows around an object, which corresponds to higher external resistances (i.e., less effective convective heat transfer at the surface).

Example

An apple (diameter = 8.5 cm; assuming a sphere) is being cooled with low velocity air. The thermal conductivity of the apple is 0.42 W/mK. How low must the air velocity be to satisfy the Bi < 0.1 criterion and thereby make Equation 107.11 a valid solution method?

$$\text{Want } Bi = \frac{h\left(\frac{V}{A}\right)}{k} < 0.1$$

$$\frac{h\left(\frac{\frac{4}{3}\pi r^3}{4\pi r^2}\right)}{k} = \frac{h\left(\frac{r}{3}\right)}{k} < 0.1$$

$$\therefore h < \frac{(0.1)(k)}{\left(\frac{r}{3}\right)} = \frac{(0.1)(0.42\ \text{W/mK})}{\left(\frac{0.0425\ \text{m}}{3}\right)}$$

$$h < 3.0\ \text{W/m}^2\text{K}$$

Although this is a very small value of h, it is a feasible number at *very* low airflow (~5 cm/s for the given geometry), which is on the order of natural convection conditions. Therefore, in such a case, the lumped parameter method (Equation 107.11) could be used to solve for the product temperature. However, this would be very unusual for any thermal processing applications.

b. Conduction for very thick materials or very short times

This special case is typically referred to as the solution for a *semi-infinite geometry*. In this case, the object/body is so thick, relative to the processing time of interest, that it can be assumed to have only one surface, and to extend to an infinite depth below that surface. This assumption is valid if the following is true:

$$L \geqslant 4\sqrt{\alpha t} \tag{107.12}$$

Where L is the thickness of the object (half-thickness if the object is symmetrically exposed to the boundary condition), α is the thermal diffusivity of the object ($\alpha = k/\rho c_p$), and t is the time of interest. If Equation 107.12 is true for any given case, then the following solutions can be applied for each of the three boundary condition types (2), where x is the distance below the surface:

$$\text{Type 1:} \quad \frac{T - T_\infty}{T_i - T_\infty} = erf\left[\frac{x}{2\sqrt{\alpha t}}\right] \tag{107.13}$$

$$\text{Type 2:} \quad T - T_i = \left(\frac{2}{k}\right)\left(\frac{q_{surface}}{A}\right)\left(\frac{\alpha t}{\pi}\right)^{1/2} \exp\left(\frac{-x^2}{4\alpha t}\right) - \left(\frac{q_{surface}x}{kA}\right)\left[1 - erf\left(\frac{x}{2\sqrt{\alpha t}}\right)\right] \tag{107.14}$$

$$\text{Type 3:} \quad \frac{T - T_\infty}{T_i - T_\infty} = erf\left[\frac{x}{2\sqrt{\alpha t}}\right] + \exp\left(\frac{hx}{k} + \frac{h^2\alpha t}{k^2}\right) \times \left[1 - erf\left(\frac{x}{2\sqrt{\alpha t}} + \frac{h\sqrt{\alpha t}}{k}\right)\right] \tag{107.15}$$

Where the error function is defined as:

$$erf(\phi) = \frac{2}{\sqrt{\pi}} \int_0^\phi e^{-\phi^2}\, d\phi \tag{107.16}$$

for which tabular values can be found in most heat transfer books (2,3,4) or engineering handbooks.

Clearly no food product is infinite in thickness. Therefore, this solution method becomes relevant for very short process times. In these cases, the goal might be to evaluate the surface temperature, or the temperature just under the surface, given a short-time heating process.

Example

A ham steak (2.5 cm thick) is packaged after slicing and subjected to a post-process, surface pasteurization treatment

in a saturated steam environment. The package film is 1 mm thick and has the same thermal diffusivity as the ham (1.05×10^{-7} m²/s). The ham is initially at 7°C, and it is exposed to the condensing steam (100°C) for 30 seconds. The condensing condition results in a very high effective h (and therefore a very low external resistance), so that we will assume that $T_{surface} \sim T_\infty = 100°C$. We want to determine the product temperature at the surface between the ham and the package (i.e., 1 mm depth) after 30 s.

To determine whether the semi-infinite solution is valid for this case, we test the condition from Equation 107.12:

$$4\sqrt{\alpha t} = 4\sqrt{(1.05 \times 10^{-7}\,\text{m}^2/\text{s})(30\text{s})} = 0.007\,\text{m}$$

which is less than the half-thickness of the ham slice (0.0125 m). This test implies that for the given conditions and time, the center temperature of the ham slice does not change appreciably. Therefore, given an assumption of a type 1 boundary condition, Equation 107.13 can be used to solve for T(x = 0.001 m; t = 30 s):

$$\frac{T - 100°C}{7 - 100°C} = erf\left[\frac{0.001\,\text{m}}{2\sqrt{(1.05 \times 10^{-7}\,\text{m}^2/\text{s})(30\text{s})}}\right]$$

$$\frac{T - 100°C}{7 - 100°C} = erf[0.281] = 0.3085$$

(where *erf*[0.281] was taken from table values (2))

$\therefore$ T(x = 0.001 m; t = 30 s) = 71.3°C

It should be noted that the above solution method requires a uniform initial temperature within the product. For this case, however, the solution for a semi-infinite solid can be a relatively useful solution technique to estimate short-time/surface heating processes.

c. *Conduction with non-negligible internal resistance and long times*

When the test criteria for the lumped parameter method and the semi-infinite method are not valid, Equation 107.4 can be solved as an infinite series solution (2), where, for the case of an infinite slab (of thickness 2L, with type 1 boundary condition):

$$\frac{T - T_{surface}}{T_i - T_{surface}} = \sum_{n=0}^{\infty} \frac{4(-1)^n}{(2n+1)\pi} \cos\left[\frac{(2n+1)\pi x}{2L}\right] \times e^{-\alpha\left[\frac{(2n+1)\pi}{2L}\right]^2 t} \quad (107.17)$$

Fortunately, graphical representations of Equation 107.17 (truncated) are often employed for the simplified case of 1-D conduction in an infinite slab, an infinite cylinder, or a sphere, given uniform initial temperature, constant boundary conditions, no internal heat generation, and constant thermal properties (2–4). A portion of such a graph is shown in Figure 107.2 to illustrate how these graphs are used. Whereas the solution for a semi-infinite solid was valid for very short times, the graphical solutions (known as the Heisler charts) are valid for longer times, because they represent the truncated (single term) version of Equation 107.17. Therefore, in order to use the charts, the following condition must be true:

$$Fo = \frac{\alpha t}{L^2} > 0.2 \quad (107.18)$$

where Fo is the Fourier number, which is a dimensionless time parameter, and L is the half thickness of a symmetrical, infinite slab. For the cases of infinite cylinders or spheres, L is the radius.

The Heisler charts involve four dimensionless parameters, as follows:

$$m = \frac{k}{hL} \text{ (ratio of external to internal resistance)} \quad (107.19)$$

$$n = \frac{x}{L} \text{ (n = 0 for center and 1 for surface)} \quad (107.20)$$

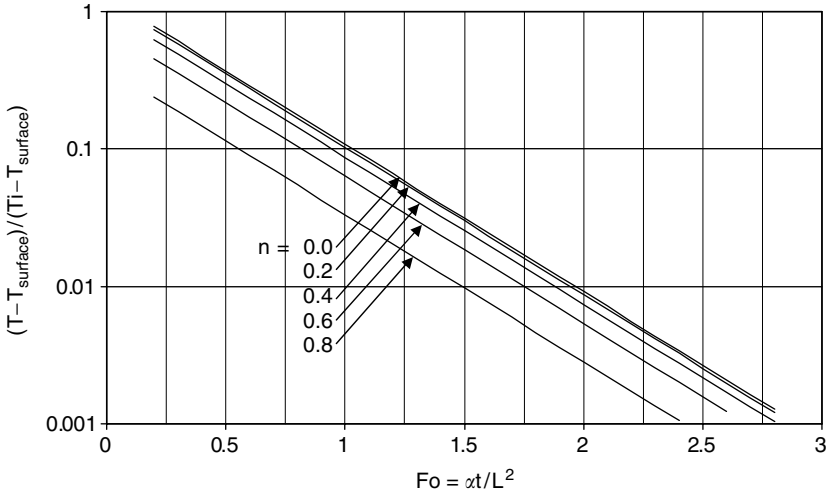

FIGURE 107.2 Example of Heisler-type chart (truncated series) solution for unsteady-state conduction in an infinite slab, for the special case of m ~ 0.

$$Fo = \frac{\alpha t}{L^2} \tag{107.21}$$

$$\frac{\Theta}{\Theta_0} = \frac{T - T_\infty}{T_i - T_\infty} \tag{107.22}$$

where T_i is the initial temperature, T_∞ is the medium (air, water, or oil) temperature, x is the distance from the center line of symmetry in the slab, and the thermal properties (k, α) are those of the product (not the heating medium). In applying graphical solutions, the user can solve for any of the above four parameters, given the other three. For example, one can solve for the product center temperature at a specific time, if the process conditions (h, T_∞, t) and product properties (k, α, R) are known. Conversely, one could estimate the time required to achieve a certain T_{center} if the product properties and process conditions (h, T_∞) were known. As an aside, in many thermal processing operations, which involve very high convective coefficients, m becomes sufficiently small (<0.1) to be assumed to be ~0 when using the graphs.

Example

Consider the packaged ham steak from the previous example (2.5 cm thick), which is now to be fully cooked in flowing hot water. The initial product temperature is 5°C. The thermal conductivity of the product is 0.41 W/mK, and the thermal diffusivity is 1.05×10^{-7} m²/s (assumed to be constant). The water temperature is 95°C, and the convective coefficient (h) is 430 W/m²K. We want to know the center temperature of the product after 8 minutes (480 s) of cooking.

First, assuming that the diameter of the slice is large enough to treat it as an infinite slab (i.e., d >> L), we test whether the graphical solution method is valid by testing whether Fo > 0.2:

$$Fo = \frac{\alpha t}{L^2} = \frac{(1.05 \times 10^{-7}\,\text{m}^2/\text{s})(480\,\text{s})}{\left(\frac{0.025\,\text{m}}{2}\right)^2} = 0.322$$

which indicates that the truncated series (graphical) solution method is acceptable. Now, we calculate the other two dimensionless parameters:

$$m = \frac{0.41\,\text{W/mK}}{(430\,\text{W/m}^2\text{K})(0.0125\,\text{m})} = 0.076 \sim 0$$

$$n = \frac{0}{0.0125\,\text{m}} = 0$$

Now, given Fo = 0.322, m ~ 0, and n = 0, we can estimate (from Figure 107.2) that:

$$\frac{\Theta}{\Theta_0} \approx 0.6 = \frac{T - T_\infty}{T_i - T_\infty} = \frac{T - 95°\text{C}}{5 - 95°\text{C}}$$

$$\therefore T(x = 0;\, t = 480\,\text{s}) = 41°\text{C}$$

It is important to reiterate that the graphical solutions are only valid for uniform initial temperature, constant boundary conditions, and for cases where significant mass transfer (e.g., evaporation) is not affecting the energy balance at the product surface.

d. Multi-dimensional conduction

The graphical solution introduced above for infinite slabs, infinite cylinders, or spheres can also be used to estimate the temperature in a 2-D or 3-D object. For example, a typical cylindrical metal can of food (diameter D and height H) is not sufficiently long to be treated as an infinite cylinder. At any point within the product, the temperature is obviously affected by heat flow through both the side and the ends of the can. Therefore, both dimensions must be considered in the analysis. In order to accomplish via the graphical solutions, the can is treated as the intersection of an infinite cylinder (diameter D) and an infinite slab (thickness H), so that

$$\frac{T(r, z, t) - T_\infty}{T_i - T_\infty} = \left[\frac{T(r, t) - T_\infty}{T_i - T_\infty}\right]_{\text{infinite cylinder}} \times \left[\frac{T(z, t) - T_\infty}{T_i - T_\infty}\right]_{\text{infinite slab}} \tag{107.23}$$

where the dimensionless temperature terms for the infinite cylinder and infinite slab are estimated independently, using the appropriate Heisler charts. The same principle would hold for a rectangular solid, which would be treated as the intersection of three infinite slabs.

C. Convection

In the previous section, the solution of various heat conduction problems required an h value to describe the convective boundary condition, and these coefficients were given as known values. Convective heat transfer coefficients vary widely, depending on fluid properties, flow conditions, and flow geometry (Table 107.3). However, actually determining h values for a thermal processing operation can be a significant challenge, depending on the complexity of the product and process. This section briefly describes the principles behind the convective heat transfer coefficient, general concepts for predicting h, and a basic technique for experimentally determining h.

TABLE 107.3
Typical Values for Convective Heat Transfer Coefficients

Flow Type	h (W/m²K)
Natural air	10
Forced air (fan)	30
Air impingement (dry)	100+
Moving water	~1,000

1. Newton's Law of Cooling and Boundary Layer Concepts

Newton's Law of Cooling describes heat flux between a solid surface and a fluid passing over the surface (Figure 107.3), so that:

$$\frac{q}{A} = h(T_{surface} - T_{\infty}) \tag{107.24}$$

where h is the convective heat transfer coefficient (W/m^2K). As we did for conduction, we can rearrange Equation 107.24 to yield:

$$\frac{q}{A} = \frac{T_{surface} - T_{\infty}}{1/h} \Rightarrow flux = \frac{\text{driving force}}{\text{resistance}} \tag{107.25}$$

Therefore, the driving force for convective heat transfer is the difference between the bulk fluid temperature (T_∞) and the surface temperature, and the resistance is 1/h.

Physically, the convective resistance to heat flow results from the formation of a *boundary layer* near the surface past, which the fluid is flowing (Figure 107.3). Assuming that $T_{fluid,surface}$ equals $T_{solid,surface}$ and a no-slip condition for the fluid at the surface (i.e., $v_{surface} = 0$), then profiles for temperature and velocity are found near the surface (Figure 107.3). The boundary layer thicknesses (δ_T and δ_v) are defined as the point where:

$$\frac{T_s - T_{\delta}}{T_s - T_{\infty}} = 0.99 \text{ (for the temperature boundary layer)} \tag{107.26}$$

$$v_{\delta} = 0.99 v_{\infty} \text{ (for the velocity boundary layer)} \tag{107.27}$$

Equating conductive flux through the boundary layer with convective flux at the surface yields correlations that relate h to the fluid properties, flow conditions, and flow geometry. These correlations are different for forced and natural convection conditions, and they include several dimensionless parameters, as follows:

$$\text{Nusselt Number} = Nu = \frac{hL}{k_{fluid}} \tag{107.28}$$

$$\text{Reynolds Number} = \text{Re} = \frac{v_{\infty}\rho_{fluid}L}{\mu_{fluid}} \tag{107.29}$$

$$\text{Prandtl Number} = \text{Pr} = \frac{\mu_{\text{fluid}}/\rho_{fluid}}{\alpha_{fluid}} \tag{107.30}$$

$$\text{Grashof Number} = \text{Gr} = \frac{\beta_{fluid}\, g\, \rho_{fluid}^2 L^3 \Delta T}{\mu_{fluid}^2} \tag{107.31}$$

$$\text{Rayleigh Number} = \text{Ra} = \text{Gr} \times \text{Pr} \tag{107.32}$$

where μ_{fluid} is the fluid viscosity, β_{fluid} is the coefficient of thermal expansion of the fluid (=1/T for an ideal gas), and g is the gravitation constant.

2. Forced Convection

a. *Predictive correlations*

When a fluid is forced over a surface, the correlations to predict h follow the general form:

$$Nu = f(\text{Re}, \text{Pr}) \tag{107.33}$$

where each geometric case yields a different correlation. These have been determined for various common geometries, such as the following for flow over a flat plate:

$$\text{Local: } \text{Nu}_x = 0.332\ \text{Re}_x^{1/2}\ \text{Pr}^{1/3} \tag{107.34}$$

$$\text{Average: } \text{Nu}_L = 0.664\ \text{Re}_L^{1/2}\ \text{Pr}^{1/3} \tag{107.35}$$

$$\text{where Re} < 200{,}000$$

or for the case of forced flow over a spherical object:

$$Nu_D = 2 + (0.4\ \text{Re}_D^{1/2} + 0.06\ \text{Re}_D^{2/3})\ \text{Pr}^{0.4} \tag{107.36}$$

$$\text{where } 3.5 < \text{Re}_D < 7.6 \times 10^4 \text{ and } 0.71 < \text{Pr} < 380$$

Additionally, the general form of Equation 107.33 has been used to develop a variety of product specific correlations (6),

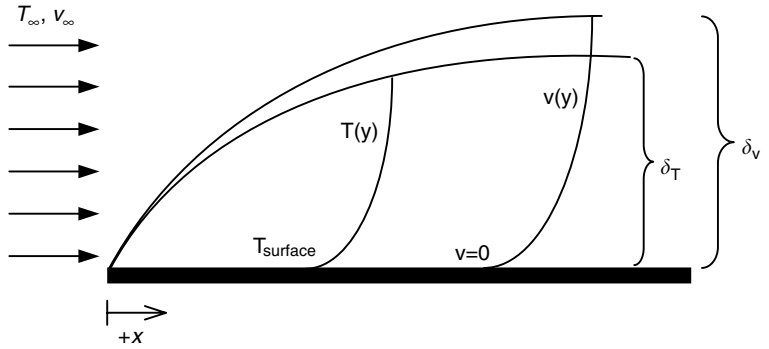

FIGURE 107.3 Temperature and velocity profiles in the boundary layer for laminar flow over a flat plate.

such as the following for airflow over an egg (10), which was developed for an equivalent diameter of 3.4 cm and air velocity from 2 to 8 m/s:

$$Nu = 0.46\ \mathrm{Re}^{0.56} \tag{107.37}$$

Example

Calculate the convective heat transfer coefficient for hot air (220°C) blowing over a pea (T_i = 14°C; 0.85 cm diameter) at a velocity of 3.5 m/s.

In calculating the relevant dimensionless parameters, the fluid properties need to be determined, generally from table values. Because fluid properties can be quite temperature dependent, they should be evaluated at an average temperature called the film temperature: $T_{film} = (T_\infty + T_{surface})/2$. So, for the case of the pea, at the start of the process, $T_{film} = (220 + 14°C)/2 = 117°C$. Therefore, from tables, the following property values were found: $\rho = 0.9050\ kg/m^3$; $\mu = 2.2447 \times 10^{-5}$ kg·m/s; $k_f = 0.03295$ W/mK; $\alpha = 3.5942 \times 10^{-5}\ m^2/s$; and Pr = 0.690. Now,

$$\mathrm{Re} = \frac{\rho v D}{\mu} = \frac{(0.9050\ kg/m^3)(3.5\ m/s)(0.0085\ m)}{2.2447 \times 10^{-5}\ kg \cdot m/s}$$

$$= 1{,}199$$

which is within the acceptable range for Equation 107.36. Although the Pr value (0.690) is slightly below the range for Equation 107.36, we will go ahead and use that equation (lacking any better option and assuming the slight deviation to have a very minor effect on the correlation validity).

$$Nu_D = \frac{hD}{k_{fluid}} = \frac{h(0.0085\ m)}{0.03295\ W/mK}$$

$$= 2 + (0.4(1{,}199)^{1/2} + 0.06(1{,}199)^{2/3})0.690^{0.4}$$

$$\therefore h = 76.7\ W/m^2K$$

Obviously, many real factors, such as the surface characteristics of the food product, would affect the accuracy of these Nu correlations when predicting h values for forced convection. However, the correlations are useful for generating order of magnitude estimates and for estimating the effects that would result from changing product or process parameters (e.g., increasing fluid velocity).

b. Experimental determination

For irregular product or flow geometries, the basic Nu correlations will not work; however, it is reasonably simple to conduct experiments to determine h for a given case (assuming that the processing system/equipment is available). The first step is to create a metal replica of the food product of interest. With aluminum, almost any food product geometry is sufficiently small to meet the $Bi < 0.1$ criterion and thereby make the lumped parameter method (Equation 107.11) an appropriate solution. Therefore, Equation 107.11 can be rewritten as:

$$\ln\left(\frac{T - T_\infty}{T_i - T_\infty}\right) = \left(\frac{-hA}{mc_p}\right)t \tag{107.38}$$

Now, a temperature sensor should be placed in the center of the model (metal) food product, and temperature data should be collected as the metal model is exposed to the processing environment, as if it were being processed like the food product of interest. Given known properties of the metal and known fluid temperature, a linear regression of the time-temperature data (in the form of Equation 107.38) will yield an estimate for h. Depending on the process, one should be cautious that the resulting data indicate a relatively constant h, and that the data are not strongly influenced by mass transfer (e.g., via evaporation of condensation at the surface). Otherwise, the regression will not yield a true h value for the process. Continuously varying boundary conditions create a special challenge for analysis of convective coefficients, which is beyond the scope of this chapter; however, one needs to be aware if this case is encountered.

Natural convection

Even when a fluid is not being mechanically forced past a food product, it is possible for convection to occur. This phenomenon, known as natural convection, is driven by buoyancy differences in the boundary layer near a product surface that is at a different temperature than the surrounding fluid. Analogous to the Nu = f(Re, Pr) correlations for forced convection, correlations for natural convection are typically of the form:

$$Nu = f\,(Ra, \mathrm{Pr}) \tag{107.39}$$

Obviously, h values for natural convection are much lower than those for forced convection, and thermal processes for food products are rarely designed for natural convection conditions. Therefore, correlations for these cases are not presented here, but can be found in most heat transfer texts (2–4).

D. Radiation

In contrast to conduction and convection, radiation heat transfer does not require a transfer medium or directly physical contact. In radiation heat transfer, thermal energy is transferred via electromagnetic waves. Every object emits thermal energy in this way, with hotter objects emitting more energy, as described by the Stefan-Bolzmann Law:

$$\frac{q}{A} = \varepsilon\sigma T^4 \tag{107.40}$$

where ε is the emissivity of the object, T is the absolute temperature (K) of the object, and σ is the Stefan-Bolzmann constant ($5.676 \times 10^{-8}\ W/m^2K^4$).

The emissivity of a material is a function of its composition and surface characteristics, and can range from 0 to 1. For an ideal "black body," which is a perfect emitter, $\varepsilon = 1$. For a perfect reflector, $\varepsilon = 0$. Most food materials (and most biological materials, in general) have relatively high emissivities, in the range of 0.90 to 0.95.

While Equation 107.40 describes the *total* thermal energy radiated by an object, it is generally of more practical interest to evaluate the *net* radiative flux between two objects. For the most general case, with object 1 and object 2:

$$q_{1-2} = \frac{\sigma(T_1^4 - T_2^4)}{\frac{1-\varepsilon_1}{\varepsilon_1 A_1} + \frac{1}{A_1 F_{1-2}} + \frac{1-\varepsilon_2}{\varepsilon_2 A_{12}}} \qquad (107.41)$$

where F_{1-2} is a dimensionless view factor that quantifies (0 to 1) the fraction of total radiation emitted that is exchanged between two objects, given their geometry and orientation.

For the special case when one of the two objects (object 1) is completely enclosed inside the other (Figure 107.4), Equation 107.41 can be simplified to yield:

$$\frac{q_{1-2}}{A_1} = \varepsilon_1 \sigma (T_1^4 - T_2^4) \qquad (107.42)$$

where A_1, ε_1, and T_1 are the properties of the enclosed object. Keeping with our general concept of driving forces and resistances, it can be seen in Equation 107.42 that the driving force for radiation heat transfer is the difference between T^4 values, and the resistance is $1/\varepsilon$, a property of the product.

Example

An infrared/radiative tunnel oven is being proposed to brown the surface of a meat roast before it is cooked in a moist-air oven. The initial temperature of the roast is 6°C (~279 K), and it has an emissivity of ~0.93. The surface temperature inside the oven is 815°C (~1,088 K). What is the initial radiative flux to the surface of the roast when it enters the oven?

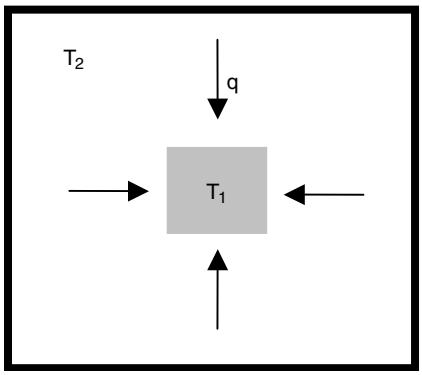

FIGURE 107.4 Radiative flux when one object (1) is completely enclosed by a much larger surface (2).

Using Equation 107.42:

$$\frac{q_{1-2}}{A_1} = (0.93)(5.676 \times 10^{-8}\,\mathrm{W/m^2K^4})(279^4 - 1{,}088^4\,\mathrm{K^4})$$
$$= -73.6\,\mathrm{kW/m^2}\ \text{(The negative sign reflects energy into the roast.)}$$

III. MASS TRANSFER PRINCIPLES

A. General Concepts

As was the case with heat transfer, mass transfer occurs when a system is not equilibrium, so that an analogous generalized transport equation for mass transfer can be stated as:

$$\text{mass flux} = \frac{\text{driving force}}{\text{resistance}} \qquad (107.43)$$

where the mass flux describes the movement of a single species (e.g., water or oil) through a system (e.g., a food product). The distinction between heat transfer analyses and mass transfer analyses is primarily related to the characterization of equilibrium state. In heat transfer, equilibrium is characterized by equal temperature throughout the system. However, in mass transfer, equilibrium is not necessarily characterized by equal concentration throughout the system.

Rather, specific relationships define the equilibrium states at gas-liquid, gas-solid, and liquid-solid interfaces. For a gas-liquid interface, the equilibrium state is defined by *Henry's Law*:

$$p_A = Hx_A \qquad (107.44)$$

where p_A is the partial pressure of component A in the gas, x_A is the molar concentration of A in the liquid, and H is Henry's constant, which is specific for a given liquid, gas, and temperature. For a gas-solid interface, the equilibrium state is defined by *equilibrium (sorption) isotherms* that relate (for example) the moisture content of a food material to the humidity in the surrounding air. These isotherms are product specific and very dependent on product composition. Therefore, they are typically determined experimentally for each food product. Lastly, at liquid-solid interfaces, equilibrium isotherms also describe the relationship between the concentration of A in the liquid and in the solid. In small ranges of concentration, this relationship is often characterized for a given case by a *partition coefficient* (K^*), where:

$$K^* = \frac{C_{A,solid}}{C_{A,liquid}} \qquad (107.45)$$

The next sections will show that the mass transfer rate laws are directly analogous to those for heat transfer. However, it is important to note that accurate mass transfer analyses are highly dependent on good information regarding the equilibrium relationships. Although in-depth analysis of these relationships are beyond the scope of this chapter, the food engineer generally is left to seek out product specific, empirical data for the particular application being analyzes.

B. Diffusion

Transport of a species (e.g., water) through a real food product can result from the combined effect of several mechanisms, including capillary forces, gravitational forces, osmotic potential, and molecular diffusion, making a detailed analysis of a complete mass transfer problem fairly complex for most food products. Transport of a liquid through a porous medium is often described by *Darcy's Law*:

$$n = -K\frac{\partial H}{\partial x} \tag{107.46}$$

where n is the volumetric flux (m^3/m^2s), K is the hydraulic conductivity (m/s), and H is the hydraulic potential (m), which is the sum of the various potential differences driving flow. Many food products are, in fact, porous media, with several of the above flow potentials present. However, it is fairly difficult to isolate and independently describe each of these potential terms.

In contrast, concentration-driven flow of one species (e.g., water) through another (e.g., fruit to be dried) can be described by *Fick's Law*:

$$j_A = -D_{AB}\frac{dc_A}{dx} \tag{107.47}$$

where j_A is the diffusive flux (kg/m^2s) of species A, D_{AB} (m^2/s) is the mass diffusivity of A in B (e.g., water in fruit), and c_A (kg/m^3) is the concentration of A in B. Unlike thermal properties (e.g., k), which are relatively easy to find in the literature, to predict, or to measure, mass diffusivity is experimentally more difficult to measure and therefore far more scarce in the literature. Therefore, it is often necessary to make use of estimates based on data found in the literature for similar food products.

C. Convection Mass Transfer

Convection mass transfer at a surface can be described via an analogy to Newton's Law of Cooling, so that:

$$\frac{j_A}{A} = h_m(c_{A,fluid,surface} - c_{A,fluild,\infty}) \tag{107.48}$$

As with heat transfer, h_m can be predicted by correlations analogous to the Nu = f(Re, Pr) relationships. In fact, the same correlations can be used simply by substituting two of the dimensionless numbers. The Sherwood number (Sh) replaces the Nusselt number, and the Schmidt number (Sc) replaces the Prandtl number, where:

$$Sh = \frac{h_m L}{D_{AB}} \tag{107.49}$$

$$Sc = \frac{\mu_{fluid}/\rho_{fluid}}{D_{AB}} \tag{107.50}$$

where D_{AB} is now the diffusivity of A in the fluid (not the solid).

It is also important to point out that $c_{A,fluid,surface}$ in Equation 107.48 is the concentration of A in the fluid at the surface, rather than the concentration of A in the solid at the surface. As mentioned earlier, $c_{A,fluid,surface}$ and $c_{A,solid,surface}$ are two distinct quantities, which are directly related via equilibrium (sorption) isotherms.

D. Condensation and Evaporation

Condensation and evaporation are often treated as distinct mechanisms of heat transfer; however, both are actually mass transfer phenomena that can create a significant heat transfer effect. In the case of moist air, condensation to a food product surface occurs when $c_{A,air,\infty} > c_{A,air,surface}$, which also corresponds to the state when $T_{air,dewpoint} > T_{product,surface}$. In fact, in condensing conditions, $c_{A,air,surface}$ can be assumed to be the saturation vapor concentration in the air at the surface temperature. Evaporation is simply the opposite phenomenon, which occurs when the converse of the above inequalities are true.

Condensation can be a particularly important mechanism in thermal processing, as condensing steam can transfer significant energy to a product surface. When a condensing-convective boundary condition occurs, the heat flux at the surface becomes:

$$\frac{q}{A} = \underbrace{h(T_{surface} - T_\infty)}_{\text{convection}} + \underbrace{\lambda_v h_m(c_{air,surface} - c_{air,\infty})}_{\text{condensation}} \tag{107.51}$$

where λ_v is the latent heat of vaporization (2,070 kJ/kg for water). Along the course of a dynamic thermal processing operation (e.g., in a continuous, moist-air oven), the driving forces (ΔT and Δc) would vary, while the resistances (1/h and $1/h_m$) would typically remain relatively constant. From the perspective of fundamental principles, this is an important point to consider when designing systems that appropriately match product objectives with process capabilities.

IV. APPLICATION CONCEPTS

The purpose of this section is to illustrate how the various principles introduced in this chapter can be used to evaluate the relative importance of heat transfer mechanisms on thermal processes or to evaluate the impact of changing one or more product/process parameters.

Example

In a dry air impingement oven, with a very large convection coefficient (~110 W/m^2K), which mechanism (convection or conduction) presents the largest resistance to increasing the product core temperature at the beginning stages of baking a cookie (0.8 cm thick, with an estimated k of ~0.39 W/mK)?

The conductive (internal) resistance is calculated as L/k, where L is the half-thickness (assuming symmetrical boundary conditions on the top and bottom). Therefore L/k = (0.004 m)/(0.32 W/mK) = 0.012 Km^2/W. In comparison, the convective (external) resistance is 1/h = 1/(110 W/m^2K) = 0.009 Km^2/W. (Recall that the Biot number, Bi, is a dimensionless measure of the ratio of these resistances.) Although the conductive resistance is slightly larger in this case, the two resistances are of the same order of magnitude. From an equipment or process design perspective, this is a fairly reasonable place to be. If the internal resistance were significantly larger than the external resistance, then we are probably putting too much energy (i.e., via fan size) into achieving a very high convection coefficient, when the internal resistance is the rate-limiting factor. Conversely, if the external resistance were much larger than the internal resistance, then we may have under-engineered our processing system, assuming that rapid heating is a desirable outcome. Even without conducting a complete analysis of the heat transfer in this process, this example illustrates that the basic principles of heat transfer can be used to evaluate various aspects of the process and product design.

Example

In the impingement oven mentioned above, can radiation be ignored in the heat transfer analysis, if the oven air temperature is 205°C (~478 K), the product surface temperature is 10°C (~283 K), and the product emissivity is 0.9?

For the convection component:

$$q_{conv}/A = h(T_\infty - T_{surface}) = (110\ W/m^2K)(205 - 10°C) = 21{,}450\ W/m^2$$

For the radiation component, assuming that the walls in the oven are the same temperature as the air:

$$q_{rad}/A = \varepsilon\sigma[(T_\infty)^4 - (T_{surface})^4] = (0.9)(5.676 \times 10^{-8}\ W/m^2K^4)(478^4 - 283^4\ K^4) = 2{,}339\ W/m^2$$

The convection component is approximately 10 times greater than the radiation component, so that the radiation component can probably be neglected for most simple analyses of this process. However, keep in mind that both of these components of the flux will decrease as the process continues, thereby affecting their relative importance to the process outcome.

Example

In a moist-air, convection oven, which contributes more energy to the product, convection or condensation?

Consider the following set of conditions, which were taken as a "snapshot" from actual experimental trials in a commercial-scale oven: $T_\infty = 127°C$; $c_\infty = 0.45\ kg/m^3$ (corresponds to 83% moisture by volume in the air); $T_{surface} = 30°C$; $c_{surface} = 0.0296\ kg/m^3$ (based on the saturation vapor concentration at $T_{surface}$); $h = 100\ W/m^2K$; and $h_m = 120$ mm/s. Now, from Equation 107.51, we can calculate the instantaneous convective and condensing fluxes:

For the convection component:

$$q_{conv}/A = h(T_\infty - T_{surface}) = (100\ W/m^2K)(127 - 30°C) = 9{,}700\ W/m^2$$

For the condensing component:

$$q_{condensing}/A = \lambda_v h_m(c_\infty - c_{surface}) = (2{,}070 \times 10^3\ J/kg)(0.120\ m/s) \times (0.45 - 0.0296\ kg/m^3) = 104{,}427\ W/m^2$$

Clearly, at the instant in time presented, condensation is responsible for more than 90% of the heat flux into the product. However, as the product temperature rises, eventually $c_{surface}$ will approach and exceed c_∞, thereby eliminating condensation and potentially causing evaporation, depending on the product.

Example

How does doubling the air velocity affect the heating time for any thermal process? Does it cut it in half?

The principles of convection and unsteady-state conduction have shown that the heat transfer mechanisms are non-linear, so that a one-to-one relationship should not be expected between process parameters and product outcomes. In this case, the Nu = f(Re, Pr) correlations show that doubling of the fluid velocity will double Re, but will result in something less than doubling of h, given Re^n, where $n < 1$. Additionally, depending on the processing goal, and therefore the solution method used, the relationship between h and processing time is also non-linear. However, for any specific case, the basic principles introduced in this chapter could be used to evaluate the relative impact that any process or product change would have on the outcome.

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108 Heat Transfer

Lorna Zach
System Solutions for the Food Industry

CONTENTS

DEFINITION OF VARIABLES

A = cross-sectional area (m^2)
a = variable power
b = variable power

$$\text{Bi} = \text{Biot number} = \frac{internal\_resistance}{external\_resistance} = \frac{hL}{k} = \frac{UL}{k} \text{ (dimensionless)}$$

C = a constant

Cp = heat capacity (kJ/kg°C)

D = diameter of the pipe (m),

D = (in section 4 on unsteady-state heat transfer), D refers to the critical dimension of a geometry, as defined in the text. This is in general useage.

D_h = hydraulic diameter (m)

E = electromagnetic energy

F_{1-2} = shape factor for radiation absorption

Fo = Fourier number

$$= \frac{\text{rate of heat conduction across D in volume } D^3}{\text{rate of heat storage in volume } D^3}$$

$$= \frac{\alpha t}{D^2} \text{(dimensionless)}$$

f = friction factor

h = the convective heat transfer coefficient (W/m²°C)

k = thermal conductivity (W/m°C)

l = characteristic length

L = length of the cylinder (m)

Nu = Nusselt number

$$= \frac{diffusivity\_by\_convection}{diffusivity\_by\_conduction}$$

$$= \frac{hl}{k} \text{(dimensionless)}$$

Pr = Prandtl Number

$$= \frac{diffusivity\_of\_momentum}{diffusivity\_of\_energy}$$

$$= \frac{C_p \mu}{k} \text{(dimensionless)}$$

q_r = rate of heat or energy flow in radial direction (W) or (kJ/s)

q_x = rate of heat or energy flow in x direction (W) or (kJ/s)

q_{1-2} = heat radiated by surface 1 to surface 2

R_t = total thermal resistance

R_{mi} = resistance of the metal on the inside

R_{ins} = resistance of the insulation

R_{mo} = resistance of the metal on the outside

Re = Reynolds number

$$= \frac{inertial\_forces}{viscous\_forces} = \frac{Dv\rho}{\mu} \text{(dimensionless)}$$

r_o = outside radius (m)

r_i = inside radius (m)

T = Temperature in °C or °K

T_1 at x_1 = temperature at point x_1 (°C or °K)

T_2 at x_2 = temperature at point x_2 (°C or °K)

T_i = temperature on inside surface (°C or °K)

T_o = temperature on outside surface (°C or °K)

T_s = temperature of the solid surface (°C or °K)

T_∞ = temperature of the bulk fluid (°C or °K)

ΔT_{lm} = log mean temperature difference (°C or °K)

U = overall heat transfer coefficient (W/m²°C).

v = fluid velocity in m/s

w = mass flow rate (kg/s)

x = lineal distance (m)

Symbols

α = thermal diffusivity units of m²/s

Δ = difference

ε = represents the emissivity

ρ = density (kg/m³)

δ = thickness of the boundary layer

σ = Stephan-Boltzman constant = 5.669 $(10)^{-8}$ W/m²K⁴

ζ_{1-2} = accounts for both the shape factor and the emissivity of both surfaces 1 and 2

μ = fluid viscosity (Pa · s)

μ_b = bulk fluid viscosity

μ_w = viscosity of the fluid at the hot wall

Subscripts

CF = cool fluid

HF = hot fluid

Heat transfer refers to the rate or speed at which the temperature changes with time and across a distance. Heat transfer applies to processes of cooking, pasteurization, blanching, commercial sterilization, baking, and evaporation, to name a few. In this chapter heat processing equipment and mathematical models of various types of heat transfer are presented. Because this short chapter covers an extensive area, the author recommends several excellent textbooks, which give a detailed coverage of specific areas.

Several mathematical expressions will be given without derivations. For additional background material the reader is referred to the following texts by Holman (1), Bird *et al*. (2), and Chapman (3). In addition, specific examples of heat transfer theory, as it applies to the food processing industry, can be found in some excellent general food engineering texts, Singh and Heldman (4), and Hallström *et al.* (5). These texts also give the reader thermal property data for a variety of foods and some good examples of the various principles. Finally, Perry's chemical engineer's handbooks (6, 7) have a great deal of general thermal property data and design factors

for heat exchangers, as well as good references for difficult-to-find information.

This chapter will broadly cover heat transfer, as it applies to common food processing operations. First, the various heat processing equipment for food products will be addressed. Section II covers thermal properties of food that are necessary for heat transfer estimations, with references to tabulated thermal property data. Three major types of heat transfer will be discussed and methods for estimating each type will be reviewed in section three. This includes methods to estimate heat conduction in a solid, as well as methods to estimate convection in fluids, and radiative heat transfer from hot surfaces. Also a brief section on simple heat exchanger design is included. Finally, two more areas will be addressed, steady-state and unsteady-state heat transfer.

I. HEAT PROCESSING OF FOOD PRODUCTS

Heat is used to process food in a variety of forms: liquids and slurries, semi-solids, and solids. Sometimes the form changes with heating, for example, water evaporates, fat melts, starches gelatinize, a thin fluid may change into a viscous one with different heating characteristics. The process and the equipment chosen depend, among other things, on the physical properties of the food both before and after heating. This section will cover descriptions of methods of heat processing and equipment used.

A. Liquids and Semi-Solids

Liquids, slurries, and semi-solids are most efficiently heat processed in continuous heat exchangers, although stirred, jacketed kettles are still used in smaller operations. Types of heat exchangers include plate, tubular (also called shell-in-tube), and scraped-surface heat exchangers. Swept-surface, jacketed kettles are similar, theoretically, to scraped-surface heat exchangers. These heat exchangers keep the heating medium (for example, hot water or steam) separate from the food and are sometimes referred to as indirect heat exchangers. Heat exchangers that allow direct contact of the heating fluid, usually steam, with the food product are in a separate category from the above. The one type of heat exchanger that will be discussed in this category is termed a steam injection heat exchanger. For further, more detailed information on heat exchanger design and useage conditions, see the following general texts: Hallström *et al*. (5), Singh and Heldman (4), or find information directly from heat exchanger manufacturers on the Internet.

1. Heating Medium

The heating medium is usually treated water or pressurized, treated steam. However, significant energy savings can be realized if heat regeneration can be used. In this case, the already-heated product is cooled by preheating the incoming product stream. This will be discussed further in the plate heat exchanger section and is addressed well in Singh and Heldman (4).

As a heating medium, pressurized steam provides the most efficient heat transfer due to its high temperature and release of latent heat of condensation as it changes state. However, especially in viscous fluid applications, hot water is often a more popular heating medium than steam. This is because it provides for finer temperature control at the heating metal surface. Poor temperature control can lead to under-processed or over-processed product, as well as product deposition (fouling), or sometimes burn-on, which can decrease heat transfer. The disadvantage of hot water is that heating rates are not as efficient as steam.

2. Fouling

No matter what heating medium is used with indirect heat exchangers, deposition, also referred to as surface fouling, will decrease the heat transfer rate from the heating medium to the product. For example, during pasteurization, milk proteins will deposit onto the hot metal surfaces, and as the deposition increases with time, the heat transfer rate will decrease. This will increase the time needed for heat penetration. Over a long period of time, the flow rate will be constricted and the pressure drop will increase. Eventually the process is stopped and the fouled surfaces are cleaned.

The fouling on the food product side should be minimized because time required for cleaning is lost processing time. For dairy products that require ultra-high temperature processing, the operational time of a heat exchanger may be limited to 3 or 4 hours before the heat exchanger surfaces must be cleaned, see Petermeier *et al*. (8).

Fouling also occurs on the heating medium surface side. The fouling on the heating medium side is minimized by treatment of steam and hot water with anti-fouling chemicals, such as treatment for water hardness and also by addition of corrosion inhibitors. This surface should be checked at least yearly.

3. Plate, Tubular, Shell-in-Tube, and Scraped-Surface Heat Exchangers

a. Plate heat exchangers

These units consist of a series of parallel, closely spaced stainless-steel rectangular plates pressed together in a frame, see Figure 108.1. Gaskets made of natural or synthetic rubber, which can be chosen to fit the specific needs of the fluids to be processed, are used to seal the plates together to prevent leakage. These gaskets are also used around the ports for inflow and outflow and this prevents intermixing of the heating fluid and the product stream. The flow through the plates can be arranged for co-current flow (also called parallel flow, where the heating stream and the

FIGURE 108.1 The gasketed, sanitary plate heat exchanger is specially designed for pasteurizing and general heating or cooling of beverages, dairy products, brewery products and other viscous products. (Photo courtesy of Alfa Laval, Inc.)

product stream flow in the same direction) or countercurrent flow, (where the respective streams flow in opposite directions). The plates are corrugated to induce turbulence at low lineal flow rates in the flowing liquid and therefore to increase the heat transfer rate for particular viscosities. Corrugation also increases heat transfer by preventing channeling across the plates. A variety of corrugation patterns is available for these purposes. The food product flows over the plate heat transfer area in a thin film, therefore the retention time is designed to be short. This may reduce thermal damage to the food product, see Kim *et al.* (9).

Plate heat exchangers are designed for low viscosity fluids (Cherry-Burrel Inc. suggest viscosities less than 5 Pa · s) with no particulates, or very small particles (less than 3 mm), depending on the corrugate pattern pressed into the heat exchanger plate. Larger particles can get caught between the plate contact points and burn on, making it difficult to remove using automatic cleaning procedures.

These units provide fast, efficient heat exchange. The plates can be configured for energy savings by using a flow pattern that will take advantage of waste heat by heat regeneration. In the example of pasteurization of juice or milk, the heat given up for cooling can be used to preheat the product for significant energy savings. Plate heat exchangers also have the advantage that they can be easily disassembled and re-assembled for cleaning or inspection. This provides for a sanitary design and is particularly important with heating fluids that cause surface fouling, such as UHT milk. Although, disassembly and re-assembly

FIGURE 108.2 ViscoLine™ sanitary corrugated tubular heat exchangers are designed for food products with a viscosity range between that of Plate-and-Frame Heat Exchangers and that of Scraped-Surface Heat Exchangers such as juices, tomato sauces, puddings, icings, soups and high-acid foods, the production of hot water with steam and heating of CIP solutions with steam. (Photo courtesy of Alfa Laval, Inc.)

is a comparatively fast and efficient process compared with other heat exchangers, this time should be minimized. Also, they offer excellent temperature control for comparably low capital investment and their capacity can easily be increased by adding more plates to the frame.

b. Tubular heat exchangers

Tubular exchangers are a simple, less expensive type of heat exchanger. This heat exchanger consists of a pipe located concentrically inside another pipe and the two streams flow in the annular space and the inner pipe, respectively (see Figure 108.2). These two streams may flow in the same direction (co-current flow) or in opposite directions (counter-current flow). Shell-and-tube heat exchangers are a variation of the tubular unit, where one of the streams flows inside many small tubes, while the other stream is pumped over the tubes through the shell. Baffles are often present in the shell making the flow pattern over the tubes to be more similar to cross-flow. This allows high rates of heat transfer.

Tubular and shell-and-tube heat exchangers are preferred for their low capital cost and also the smaller heat exchanger surface required per unit length of tube, although they are less commonly used in food contact. Examples include preheating and then cooling juices to below ambient temperatures, prior to freezing.

Cleaning such a heat exchanger is similar to cleaning pipes in a manufacturing facility, the automatic CIP (clean-in-place) system is used. Especially with this unit, it is important to avoid heating situations where fouling on the food surface can occur. Compared to the plate heat exchanger, cleaning is time consuming and it is difficult to disassemble by hand to examine for problems such as build up of bio-films or plugging of some of the small tubes.

In contrast to usual uses of this heat exchanger, Satyanarayana *et al.* (10) found a simple triple-tube heat exchanger, to be useful for short runs of UHT milk production for rural, tropical co-operatives. Its low investment cost and high heat transfer per unit length was thought to be better suited to small-scale operations. The triple-tube unit was used to produce UHT milk, using steam in the

innermost and outermost tubes, with the product flowing in the annular space between the two heating fluids. Although the investment costs are attractive, it is apparent that the application will require further examination, especially given an application where milk-side fouling rates are high.

c. *Scraped-surface heat exchanger*

Scraped-surface heat exchangers are good for heat processing viscous fluids and slurries, including fluids that may have high surface deposition. Although these units have higher capital costs than the previous units discussed, they are chosen because previously discussed units would not address the special needs of the food product. See Figure 108.3. This heat exchanger consists of a cylindrical tube with a rotator shaft mounted in the center. Scraper blades are mounted on the center rotator shaft so that they are pushed against the inside of the cylindrical surface when the shaft is rotated. Food material is pumped into the annular space between the heated wall and the rotator shaft. The general public may identify this type of heat exchanger as an ice cream freezer, because originally, it was developed for this purpose. The rotating blades on the center shaft were meant to physically scrape frozen cream particles off the chilled surface of the freezer, resulting in smaller-sized ice crystals and a smoother-textured ice cream. These units continue to be used in the ice cream making industry, but in this chapter, the designs for heating purposes are described.

FIGURE 108.3 The Contherm™ Scraped-Surface Heat Exchanger is particularly suitable for viscous and sticky products including those that contain particulate matter such as fruit or vegetable pieces. (Photo courtesy of Alfa Laval, Inc.)

The scraping-action of the rotator blade against the wall results from centrifugal force of the shaft rotation. In modern units, the blades may not actually contact the inside of the wall, although this depends on the design of the blades and the material of construction. Some blades are made from stainless steel and are meant to physically scrape the processed food from the heated wall, while others are made of fiberglass and are designed to more gently (but more efficiently) process a viscous or particulate-laden fluid by creating turbulence at the fluid boundary with the heated surface. In this way the thickness of the fluid boundary at the wall is decreased, resulting in higher efficiency heat transfer.

In general, scraped-surface heat exchangers increase the fluid turbulence at the tubular wall. However, the rotator blades will not prevent fouling, they will only minimize it. The rotation speed of the blade-mounted shaft varies between 150 and 500 rpm. The higher rotational speed allows for better heat transfer, especially at high viscosities, and the mechanical action provides a whipping action that may impart desirable attributes for some products. However, the mechanical action may also cause mashing of particulates and, in some cases, may significantly reduce product viscosity of some starch-based sauces.

The heat transfer in the scraped-surface heat exchanger is controlled by three major steps.

1. The rate at which the heat is conducted from the heating medium to the metal wall and the rate of conduction through the metal wall.
2. The rate of heat transfer (conductive) from the metal wall into the thin product, surface-boundary layer.
3. The rate at which the thin product layer is removed by turbulence or direct scraping and then mixed in to the bulk fluid.

The rotation speed controls radial mixing, and the overall product flow rate controls axial mixing and some radial mixing. Both flow mechanisms influence the rate at which the thin boundary layer at the wall is mixed into the bulk fluid. Often there may be one optimum rotator speed for heat transfer and a different optimum rotator speed for product qualities such as viscosity, and particulate integrity.

It is important to note that a certain amount of axial back-mixing may occur over the length of the heat transfer cylinder. This can contribute to a lower temperature driving force and therefore, poorer heat transfer. Harrod (11) reviewed both the flow mixing mechanisms as well

as the heat transfer in scraped-surface heat exchangers. Results from experimental studies are summarized as well as theoretical and empirical correlations. It is important to remember that many of the theoretical correlations developed over-predict the heat transfer in these units because of the assumption of complete equalization of temperature in the liquid film at the surface.

4. Steam Injection Heat Exchangers

Steam injection or so-called direct contact heat exchangers are specialized units that may be higher in capital cost than other heat exchangers on a per liter processed basis. One of the major reasons for this is that high quality, food-grade steam is needed for direct contact with the product in the injection process. This special steam must be available as a plant utility or the unit must make its own. This special food-grade steam is termed culinary steam because any steam additives, such as anti-corrosives or anti-foulants must be food-grade chemicals. The advantage of this direct steam injection is that processors may take advantage of the latent heat of condensing steam. This allows for large amounts of heat to be transferred directly to the product. Although the viscosity of the product determines the application, this type of heat process works best when the liquid or slurry is applied through a spreader in a chamber as a thin sheet or in droplets. The process is very efficient, but care must be taken in the application so that over-heating does not occur. This type of heat exchanger has been used in cooking or sterilizing a variety of products including puddings, concentrated soups, and milk.

B. Solids

Heat processing of solids includes such operations as blanching vegetables and fruit in water or steam, frying potato chips or chicken, retort canning, cooking items such as steaks and hamburgers for process-prepared frozen meals, baking, and surface cleaning (steam or hot water) of fruits and vegetables for fresh market, as well as surface cleaning of meat carcasses, or seed for sprouting.

Surface cleaning of fresh fruits and vegetables for the fresh produce market has become more important as more virulent strains of pathogenic microorganisms evolve (12, 13). Surface cleaning of fresh fruits and vegetables with steam or hot water prior to cutting may be one weapon in the arsenal to prevent pathogenic food-borne contamination (see Figure 108.4). Similar high-temperature, short time surface heat treatments are being investigated for sanitizing fresh food items from alfalfa sprout seeds to beef carcasses.

Steam blanching is generally carried out on a stainless steel mesh belt in a steam tunnel for a prescribed amount of time to deactivate enzymes, remove tissue air for further packing, and preserve color. Because the blanch end-point is determined by the needs of the specific fruit or vegetable (that is, the temperature at which appropriate enzymes are activated or deactivated), heat treatment is usually determined for each specific instance in the laboratory. Factored into this treatment is the largest piece size, as well as the needs of the process.

FIGURE 108.4 The Thermosafe™ process uses steam to reduce the microbial load on the outside surface of cleaned, fresh fruits and vegetables by at least 5 logs. (Photo courtesy of Biosteam Technologies, Inc.)

Fluidized beds are used for large volume, continuous heat processing situations. They would commonly be used in the freezing of, for example, green peas, but they are also used in such large-scale operations as roasting coffee beans. They provide uniformity of product and good process control. However, when particles are large (in some cases large might be >5 mm) or unevenly shaped, unstable fluidization patterns (slugging or sticking) can occur which result in poor process control.

The retort canning process is an example of unsteady-state heat transfer. Heat transfer is greater if the can is rolling in a retort rather than stationary. In either case, the center can temperature must be tested to meet time-temperature regulations. If the can contents are liquid with solid pieces, such as green beans or consumé, the faster heat transfer mechanism of convection will predominate inside the can. If, however, the contents are very thick and almost solid, such as thick, chunky beef stew, the slower heat transfer mechanism of conduction will predominate. Therefore, attaining the objective of commercial sterilization in the canning retort will be achieved faster in the can with liquid contents.

II. THERMAL PROPERTIES OF FOOD

The rate of heat transfer depends not just on the equipment or process being used but also on the properties of the food being heated. Estimating the heating rate of pure substances such as water or oil is a relatively easy exercise compared to estimating the heating rate of chili or of a steak. Not only is chili a compound mixture with a non-Newtonian viscosity, but it is also heterogeneous, and many of the solid particles in it are non-isotropic.

Furthermore, on heating, the many components respond to latent heats of fusion and evaporation at a variety of temperatures, depending on composition (14, 15). A change of state can complicate an otherwise straightforward estimate of temperature profile in a substance. Not only does steak have some of the same change of state issues as chili, but also moisture and fat vary with each slice, as does the fiber orientation. This makes estimation of heat transfer properties and rates in food materials more challenging to estimate, but also more fascinating.

Specific heat, thermal conductivity, and thermal diffusivity are important parameters that are discussed in this section, however, other properties impact thermal estimates and are not covered here. These material properties include density, enthalpy, latent heat, dielectric properties, and viscosity. These are discussed in detail in previous sections of this reference volume as well as in Hallström *et al.* (5).

A. Specific Heat

Specific heat of a food is a measure of the amount of energy required to raise the temperature of a unit mass of the food by one degree of temperature. The property is generally represented by the symbol, "Cp," and the units for this value are kiloJoules per kilogram per degree of absolute temperature difference, kJ/(kg°K) or, in this case, equivalently, kJ/(kg°C). Specific heat, also called the mass heat capacity, has been determined experimentally by several methods including the method of mixtures, method of the guard plate, and using a differential scanning calorimeter (16).

Specific heat is a material property that is relatively constant with changing temperature but that varies with food composition (for example, moisture), and that varies with change of state. Therefore, the specific heat of ice at −2°C is 2.09 kJ/kg°C and steam at 110°C and atmospheric pressure is 2.01 kJ/kg°C, while the specific heat of water at room temperature is 4.18 kJ/kg°C (see Table 108.1). Furthermore, the specific heat of a raw food compared to the specific heat after a cooking process may also be markedly different because the composition of the food may have changed. For example, when meat cooks the fat melts and may flow away, the moisture evaporates, and the proteins denature.

TABLE 108.1
Thermal Properties of Food Constituents at Approximately 20°C

Component	Spec. Heat (kJ/kg°C)	Thermal Conductivity (W/m°C)
Water	4.18	0.60
Carbohydrate	1.42*	0.58*
Protein	1.55*	0.20*
Fat	1.67*	0.18*
Air	0.96	0.025
Ice	2.09 (at −2°C)	~2.4 (below zero °C)**
Inorganic minerals	~0.84*	
Metals	0.05–1.0	50–400

* Hallström *et al.* (5).
** Singh and Heldman (4).

The specific heat of a food depends on the composition of the food. For example, the specific heat of fat is 1.67 kJ/kg°C, while the specific heat of water is 4.18 kJ/kg°C. See Table 108.1. A number of correlations to predict specific heat have been developed by Siebel (17) for high moisture foods above the freezing point, and by Charm (18), and Choi and Okos (16) for liquid foods, based on food composition. The food composition includes moisture and fat, the two most critical parameters, as well as protein, fiber, carbohydrate, and ash. Food composition values can be found in Agriculture Handbook No. 8 (19) and selected values may be found in Singh and Heldman (4).

B. Thermal Conductivity

Thermal conductivity is the rate at which heat will be conducted through a unit thickness of material if a unit temperature gradient exists across that thickness. This property is generally represented in mathematical formulae as the symbol, k, and the units for this value are Joules per second per meter per degree of temperature gradient, (J/sm°C), or equivalently, Watts per meter per degree of temperature gradient, (W/m°C). The conduction heat transfer equation relating this constant to the rate of heat conduction is equation 108.2 in Section III. The relative thermal conductivities for a variety of food constituents and materials are shown in Table 108.1. Note that metals, which are good conductors of electricity, are also good conductors of thermal energy.

Similar to specific heat, the thermal conductivity varies with food composition but, unlike the property of specific heat, it varies more with temperature; see Singh and Heldman (4) for graphs of these properties as a function of temperature. Thermal conductivity in heterogeneous, non-isotropic food materials, particularly fibrous foods such as meat, also varies with the heat flow direction in relation to the orientation of the fibers. Tabulated values that have been determined experimentally are available in Singh and Heldman (4) and Sweat (20, 21). In

addition, both these references have regression equations to estimate this constant based on food composition.

C. Thermal Diffusivity

Thermal diffusivity, α, is a property of a material incorporating previously defined properties of thermal conductivity, k, density, ρ, and the heat capacity, Cp. It is represented by the symbol, "α," and has the units of m^2/s. It is defined as follows:

$$\alpha = \frac{k}{\rho C_p} \tag{108.1}$$

and is more often encountered in complex solutions to steady-state and unsteady-state problems. This material property constant was developed prior to computational analysis of differential equations, as a way to conceptualize and solve complex thermal relationships. For example, thermal diffusivity is linked intimately to both density, and moisture during the process of bread baking. See Zanoni *et al*. (22) for an example of the estimation of this continually changing property for the process of baking bread. This is a fascinating example because density and moisture both change appreciably during the baking process. Therefore heat capacity, thermal conductivity, and of course, thermal diffusivity change as well. In addition, because of crust formation, those changes are not homogeneous.

III. STEADY-STATE HEAT TRANSFER

Three types of heat transfer will be discussed in this section: conduction, convection and radiation in the steady-state case. Heat is transferred conductively in a solid when there is only molecular vibrational movement from the cold end to the hot end. Heat is transferred convectively from a solid to a liquid (or vice versa) when, for example, a cool liquid flows over a hot pipe surface. Finally, heat is transferred radiatively when, for example, meat is baked in an oven (neglecting the heat transferred convectively from the moving air).

Furthermore, these types of heat transfer will be discussed in the idealized case, that of steady-state. Steady-state conditions are defined such that the energy going into a system or an object is equivalent to the energy going out of the system or object. This implies that time has no influence on the temperature distribution within the system or object. An example of steady-state heat transfer is taken from Section I of this chapter, the plate heat exchanger that might be used to pasteurize milk. If we look at a short section of the heat exchanger at the entrance, where the temperature of the hot water going into the heat exchanger is constant, at 98°C, and the temperature of the milk coming into the heat exchanger is constant, at 4°C, then the heat transfer through the metal wall separating the two streams is at steady state. The temperature of the metal between the two surfaces will vary with thickness of the metal wall, but, as long as the system is at steady-state, it will not vary with time.

However, in real life, the system may be at steady-state for only brief periods of time. For example, during the start-up phase the equipment must heat up. Also, the hot water will vary around a set point, perhaps between 95°C and 99°C, and the incoming milk will vary with the bulk temperature of the milk coming in, for example, if a fresh batch of milk from a tanker truck, at 10°C, were added to a steady-state system where the initial bulk milk temperature was 4°C. When the temperature of the incoming hot water is changing or the temperature of the incoming milk is changing, the temperature profile through the metal wall changes with time as well as location.

A. Conductive Heat Transfer

In conductive heat transfer there is no physical movement of the material. It is the common mode of heat transfer in heating of solid media. Conduction is the mode of heat transfer in which the transfer of energy takes place at the molecular level. As a molecule gains thermal energy it vibrates in its own location. As the level of thermal energy increases, so does the amplitude of the vibration. These vibrations are then transmitted from one molecule to another.

1. Fourier's Law

Conductive heat transfer takes place in a solid body when a temperature gradient exists, that is, heat will flow from the high temperature region to the low temperature region. This heat flux, q/A (or energy per unit area), is proportional to the temperature gradient. The constant of proportionality required to make the proportion an equation is k, the thermal conductivity. If the heat, q, is flowing perpendicularly through a cross-sectional area, A, and T is the temperature and x is the lineal distance through the cross-sectional area that the heat traverses, then the heat flux is defined mathematically as:

$$\frac{q_x}{A} = -k\frac{dT}{dx} \tag{108.2}$$

Equation 108.2 is known as Fourier's law for heat conduction. The negative sign in equation 108.2 indicates that the heat will flow from the higher temperature to the lower temperature, dT/dx representing a negative gradient. If there were no negative sign, then the negative differential would result in a negative heat flux.

Fourier's law can be solved for many scenarios including rectangular, cylindrical, and spherical geometries and both steady and unsteady-state. It can even be solved for heat transfer situations where there is heat generation, such as cool storage for respiring fruits or vegetables.

a. Fourier's Law for rectangular co-ordinates

If one assumes steady-state conductive flux in a rectangular slab of cross-sectional area, A, and the temperature is

T_1, at x_1 and T_2 at x_2, then, integrating equation 108.2 for those boundary conditions, the solution is:

$$\frac{q_x}{A} = -\frac{k(T_1 - T_2)}{(x_1 - x_2)} \tag{108.3}$$

Equation 108.3 refers to Figure 108.5 where the heat energy is shown flowing from the hot side of a rectangle through the thickness of the rectangle, to the cool side. This rectangle could represent a cross-section of the insulated stainless-steel wall of a hot water vegetable blancher.

In this heat transfer estimate we will neglect the resistance due to the inside and outside layers of metal skin of the blancher. We will also assume that the system is at steady-state. This means that the hot water inside the blancher is boiling at 100°C and the heat flux is constant, flowing through the wall so that heat is given off to the outside surface as fast as it is supplied by conduction through the wall. When the blancher is started at the beginning of the day, the metal walls will have to warm up; this is the unsteady-state time period, which we will not evaluate here. For this example the following conditions hold: wall thickness = 0.075 m, inside blancher temperature, T_1 = 100°C, ambient outside temperature, T_2 = 27°C, thermal conductivity of insulating material is approximately 0.0360 W/m°C for insulation.

Therefore,

$$\frac{q_x}{A} = -\frac{0.036(W/m°C)[100°C - 27°C]}{[0\,m - 0.075\,m]} \tag{108.4}$$

and the estimated heat flux through the insulated blancher wall is equivalent to 35.04 W/m^2. If the sides and the top of the blancher walls were the same thickness and had uniform inside temperatures and uniform outside temperatures and if the total area was 48 m^2, then the total conductive heat flux would be 1685 W. Conversely, if the heat flux could be measured, this equation may also be used to predict the temperature profile through the wall of the blancher. In this case, that temperature profile would be linear.

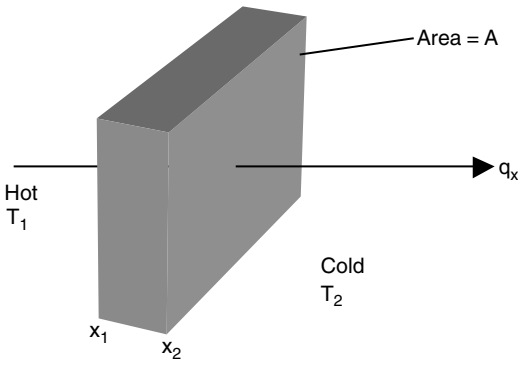

FIGURE 108.5 Heat conduction through a section of wall. Heat flows from the hot side of a section of wall at temperature T_1 to the cold side at temperature T_2. The thickness of the wall is equivalent to the difference between x_1 and x_2.

b. Fourier's Law for other geometries

Solutions to Fourier's Law for other geometries such as the cylindrical case for cans, pipes, and tubular heat exchangers (or even baking bread) and the spherical case for roasts are available in the basic texts noted at the beginning of the chapter. The solution to Fourier's equation in radial coordinates for a hollow tube is:

$$q_r = \frac{2\pi Lk(T_i - T_o)}{\ln\left(\frac{r_o}{r_i}\right)} \tag{108.5}$$

Where the boundary conditions are $T = T_i$ at $r = r_i$ and $T = T_o$ at $r = r_o$ where "i" refers to the inside and "o" refers to the outside. Also, L, refers to the length of the cylinder, r_o refers to the outside radius, r_i refers to the inside radius, so that the heat flux area is defined by $2\pi rL$, and in the process of integration becomes a logarithmic term.

2. Heat Conduction in Multi-Layered Systems

In order to discuss conduction in multi-layered systems, we will go back to the example in Section III.A.1, regarding one dimensional heat conduction in the rectangular coordinates of the insulated walls of a vegetable blancher. In this example, for reasons of simplicity, we neglected heat conduction in the metal layer on the inside of the blancher, next to the hot water and vegetables and we neglected heat conduction in the metal layer on the outside of the blancher next to the ambient air. In order to estimate the amount of heat conduction through several materials of different thicknesses and of different conductivities (and sometimes at different temperatures) one must first estimate the total resistance from the composite materials. Firstly, we must assume that the materials are in complete and full contact with no gaps. Estimating this total resis-tance to heat transfer is similar in theory to estimating the total resistance of several resistors in a series circuit. Therefore, Singh and Heldman (4) show the more detailed derivation from Ohm's law and from equation 108.3:

$$q_x = \frac{T_2 - T_1}{R_t} \quad \text{where } R_t = \left(\frac{x_2 - x_1}{kA}\right) \tag{108.6}$$

such that R_t represents the thermal resistance. Thermal resistance is additive, so that the heat conduction, q_x, through the composite blancher wall would be written:

$$q_x = \frac{T_2 - T_1}{R_{mi} + R_{ins} + R_{mo}} \tag{108.7}$$

Where R_{mi} is the resistance of the metal on the inside of the blancher, R_{ins} is the resistance of the layer of insulation, and R_{mo} is the resistance of the metal on the outside:

$$R_{mi} = \frac{\Delta x_{mi}}{k_{mi}A} \quad \text{and} \quad R_i = \frac{\Delta x_{ins}}{k_{ins}A} \quad \text{and} \quad R_{mo} = \frac{\Delta x_{mo}}{k_{mo}A} \tag{108.8}$$

The x_{mi} refers to the lineal distance, in the cross section that the heat flows through the inside metal skin, the insulation, and so on. Therefore, the heat loss for one square meter of a blancher wall, where the metal on the inside and outside surface is stainless steel (k = 19 W/m°C) and assuming metal thickness of 0.005 m:

$$q_x = \frac{[100°C - 27°C]}{\frac{0.005\,m}{(19W/m°C)(1m^2)} + \frac{0.075\,m}{0.036(W/m°C)(1m^2)} + \frac{0.005\,m}{(19W/m°C)(1\,m^2)}} \tag{108.9}$$

$$q_x = \frac{73°C}{(0.00026 + 2.083 + 0.00026)°C/W}$$

$$= \frac{73}{2.08352} = 35.037\,W \tag{108.10}$$

Therefore the conductive heat flux is 35.037 W for one square meter of surface area. This compares closely to equation 108.4 where the heat flux was estimated at 35.040 W/m^2. In this case, the initial assumption to neglect the resistance of the stainless steel skin was justified.

B. Convective Heat Transfer

1. General Theory

Convective heat transfer occurs when a fluid, such as a liquid or a gas, flows over a solid, such as pipe wall, or a solid food item. Heat will be transferred between the solid surface and the liquid when there is a temperature difference between the two. The rate of heat transferred convectively is not only dependent on the temperature gradient, but also on the properties of the fluid and the fluid flow characteristics. The resistance to heat transfer between the solid and the fluid is thought to be localized in a boundary layer within the fluid, between the flowing fluid and the solid surface. This concept was hypothesized for an ideal system by Prandtl (3), but has been widely used in studying many types of non-ideal systems.

2. Boundary Layer Theory

The boundary layer concept for the rate of convective heat transfer, q, through an area, A, may be written as

$$\frac{q}{A} = \frac{k}{\delta}(T_s - T_\infty) \tag{108.11}$$

where k is the thermal conductivity, δ is the thickness of the boundary layer, T_s is the temperature of the solid surface (and hence also the temperature of the infinitesimally thin layer of fluid on the surface), and T_∞ is the temperature of the bulk fluid, far away from the hot surface. The thickness of the boundary layer is not readily measurable and so the coefficient k/δ is expressed as "h," the convective heat transfer coefficient (also referred to as the surface heat transfer coefficient). This coefficient is expressed in units of W/m^2°C and has been measured by a number of researchers for a variety of conditions. Some approximate ranges of values are shown in Table 108.2. The higher the heat transfer coefficient, h, the faster the heat transfer.

There are two types of convective heat transfer: 1) forced convection, where the fluid is pumped or blown over the hot surface, and 2) natural (or free) convection, where the fluid rises from the surface due to localized heating and the resulting density differences induced by the temperature gradients. Natural convection is slower and therefore is less often used in food processing and will not be addressed in this chapter; see Hallström *et al.* (5) or any other basic heat transfer text.

The flow rate of the fluid affects the rate of heat transferred and therefore, a slow flow rate (characterized by the laminar flow regime) or fast flow rate (characterized by the turbulent flow regime) will affect the thickness of the boundary layer and also how fast the moving fluid can remove heat from the hot surface.

An example of convective heat transfer that is easy to visualize is how air (the fluid) will cool the surface of a hot spoonful of soup (the "solid" in this example). The heat from the liquid surface and the vapor will naturally rise in a curling fashion from the hot surface of the soup into the cooler ambient air. The air at the soup/air interface is much warmer than the air several inches above and therefore, the warmer air will be more buoyant than the cooler air and so it will rise. The liquid in the spoonful of soup will cool by natural convection.

However, if we blow gently across the surface of the hot liquid, the warm air interface directly above the hot liquid will be disturbed and cooler air will be forced to flow past

TABLE 108.2
Ranges of Values of the Convective Heat Transfer Coefficient

Fluid and Mechanism	h, Convective Heat Transfer Coef. (W/m^2°K)
Air – Natural convection	5–50
Air – Forced convection	25–500
Water – Natural convection*	20–100*
Water – Forced convection	250–15,000
Boiling water	2,500–25,000
Condensing water vapor	5,000–100,000

* Singh and Heldman (4).
Source: Holman (1).

the hot liquid. In this way, the air boundary-layer will become thinner when we blow on the liquid and the spoon of hot liquid will cool more quickly through forced convection than if it is allowed to cool via natural convection.

Convective heating is the dominant heat transfer mechanism in heating of liquids, such as milk and juice pasteurization, and often may be important in the heating of solids, such as frying and blanching. See Martens *et al.* (23) for an example of convective heat transfer to a broccoli stem in a blancher, then conductive heat transfer inside the stem. A mathematical model was used and correlated with experimental results so that the blanch time and temperature could be estimated for appropriate enzyme inactivation. Convective heating mechanisms are also used for modeling the drying of food pieces (or drops of milk) when a current of dry air is blown across the surface (spray drying).

3. Estimating the Convective Heat Transfer Coefficient

Estimation of the convective heat transfer coefficient involves an understanding of the rate and quality of fluid flow over the hot surface. Neglecting entrance effects of the fluid and unsteady state heating of the surface, let us look directly at the steady-state case.

The convective heat transfer coefficient is dependent on properties of the fluid, such as the thermal conductivity, density, heat capacity, and viscosity, as well as the temperature difference, the velocity of flow, and the geometrical shape of the solid giving up the heat. Researchers have developed a variety of correlations, based on these parameters, to predict the convective heat transfer coefficient for a specific situation. The correlations are both theoretical and empirical, see Bird *et al*. (2) and Perry *et al*. (6). The parameters are generally grouped for analysis into dimensionless numbers.

a. A word about dimensional analysis

Dimensional analysis is also referred to in terms of dimensionless numbers. Long before CFD (computational fluid dynamics), equations of momentum were used to theoretically model situations were a fluid flowed over a hot surface. The so-called Equations of Change in Bird *et al*. (2) were partial differential equations used to represent the mechanisms of all possible operations within a fluid. Occasionally these equations were able to be simplified enough so that they could be solved analytically. But few interesting problems could be simplified enough to be solved analytically. Finally, when computers came into more common usage, it became easier to solve the more complex equations using numerical techniques.

However, before it became routine to numerically solve these partial differential equations, engineers found alternative ways to solve practical problems using empirical methods based on theory. Certain groups of key variables and constants seemed to appear again and again in the solutions and equation set-ups. Therefore, these resourceful engineers concluded that any theoretical analytical solution must somehow incorporate these key variables and constants. Hence, such dimensionless groups for heat transfer as the Reynolds number, the Prandtl number and the Nusselt number were born. These dimensionless groups are essentially ratios of critical quantities of fluid and heat flow, which effect the boundary layer for the fluid and the boundary layer for the thermal transfer. Notice that the groups are dimensionless. That is, the units of meters and watts and so on, cancel each other out. This made it easier to find mistakes when long, complex manual calculations were made.

$$\text{Reynolds Number: Re} = \frac{inertial\_forces}{viscous\_forces} = \frac{Dv\rho}{\mu} \tag{108.12}$$

$$\text{Prandtl Number: Pr} = \frac{diffusivity\_of\_momentum}{diffusivity\_of\_energy} = \frac{C_p\mu}{k} \tag{108.13}$$

$$\text{Nusselt Number: } Nu = \frac{diffusivity\_by\_convection}{diffusivity\_by\_conduction} = \frac{hl}{k} \tag{108.14}$$

In the Reynolds number, D is a critical dimension, usually the diameter of the pipe in meters, v is the fluid velocity in m/s, ρ is the density of the fluid in kg/m^3, and μ is the fluid viscosity in Pa · s.

In the Prandtl number, Cp is the heat capacity of the fluid in kJ/kg°C, μ is the fluid viscosity in Pa · s, and k, is the thermal conductivity, in W/m°C.

The Nusselt number, in effect, represents the boundary layer heat transfer coefficient, h, in dimensionless form in units of W/m^2°C. This number also uses two other values, "l," a characteristic length in meters (such as the pipe diameter if the heat transfer is occurring inside a pipe), and, k, the fluid thermal conductivity in W/m°C.

The relationships that use these dimensionless groups are empirical, experimentally based correlations developed for specific ranges of fluid flow rate and geometry (among other variables that will not be discussed here). The relations are not the elegant, analytically derived theoretical equations, similar to the Fourier equation, but they are useful in practice and they really work; indeed, commercial heat exchanger design is based on them. Although a great deal of experimental data must be generated in order to predict film coefficients for the variety of flow rates, geometries, viscosities, etc., the dimensionless groups greatly simplify the organization of the data.

In order to estimate a heat transfer coefficient, h, dimensionless numbers are used in the following way.

The experimenter is looking for a relationship that is in the form of the following expression:

$$Nu = C\,\mathrm{Re}^{a}\,\mathrm{Pr}^{b} \qquad (108.15)$$

Where "C" is a constant, and "a" and "b" are powers for the Reynolds number and the Prandtl numbers. The data from a specified range of flow rates and geometries is plotted on log-log paper with the Nusselt number plotted as a function of the Reynolds number for a variety of Prandtl numbers. If the data fall into relatively straight lines, the coefficients are determined for the best fit and hence the constant "C" and powers "a" and "b" are determined.

b. *Estimation of the convective heat transfer coefficient using empirical correlations*

In previous sections and in basic textbooks in fluid mechanics, the velocity profile is estimated and modeled for a variety of channels, pipes, vortices in stirred vessels, and flow around, for example, cylindrical objects. Understanding the velocity profile is critical for estimation of the convective heat transfer coefficient.

The velocity profile

A velocity profile develops in the fluid when it flows over a surface because of the viscous properties of the fluid material. Similarly, a boundary layer develops in the flowing fluid and the thickness of the boundary layer will depend on the viscosity of the fluid (and whether the viscosity is a function of temperature, and shear force), as well as the flow rate, or in effect, the flow regime of the moving fluid.

In the case of fluid flow in a pipe at the lower flow rates, the velocity profile of the fluid will be parabolic so that the highest velocities will be found in the center of the pipe. This regime of fluid flow is referred to as the laminar regime and this regime exists for $\mathrm{Re} < 2100$ for pipe flow.

As fluid velocities increase, the flow enters the so-called transition region where flow patterns are too variable to predict and the charts in Perry and Chilton (7) should be consulted. Even so, the uncertainty of estimation in this regime is greater than the other two regimes. Reynolds numbers for this regime are between 2100 and 10,000 for flow in pipes.

The regime for the highest liquid flow velocities is the turbulent regime, (see charts for flow in pipes in Perry and Chilton (7)). In the turbulent regime, the thin film next to the hot surface is thinner than in the laminar regime, but the velocity profile is no longer parabolic in a pipe. Instead, the fluid above the surface film is filled with eddies and swirls and the velocity profile in this regime is considered to be relatively flat. Good mixing occurs in flows of high turbulence.

Approach to determine the convective heat transfer coefficient, "h"

An experimental approach was described in Section III.B.3.a to determine the coefficients for an empirical correlation for finding the convective heat transfer coefficient, "h." But we may not want to do an experiment every time we need to estimate this number. We would like to look up values in tables and estimate these coefficients using similar scenarios. Therefore, correlations already developed and published may be used. Care must be taken to use this published experimental work judiciously, for the specific conditions, ranges of flow, and assumptions used in the referenced heat transfer work. The following guidelines can be used to check the similarity of correlations to specific needs.

a) Identify the geometry of the flow situation and the characteristic dimension.
   The shape and dimension of the solid surface in contact with the fluid must be identified. Flow in pipes is the classic example, but flow in non-circular ducts, such as plate heat exchangers is also common. The characteristic dimension required for the various dimensionless numbers is the diameter or the equivalent diameter. For flow in pipes this is the pipe diameter, D, but for a non-circular duct, this is referred to as the hydraulic diameter, D_h and is defined:

$$D_h = \frac{4(\mathit{flow\_area})}{(\mathit{wetted\_perimeter})} \qquad (108.16)$$

   Flow around immersed objects has, in the past, concentrated on flow around single spheres or cylinders oriented in a specific way to the flow. However, these models simulate many food processing situations poorly. Multiple pieces vary in orientation to the flow, for example, roasting coffee beans in hot air, blanching broccoli stems, or aseptic processing of green peas. In such situations where the suspension of particles is 15 to 30% of a flow, Chakrabandhu and Singh (24) suggest use of the packed bed flow geometry, where the hydraulic radius of the duct is the important geometry. They found this better represented the characteristic dimension.

   Ghisalberti and Kondjoyan (25) investigated forced air-flow around a variety of geometries including cylinders, truncated cones, and square-sectioned bars. The characteristic dimension was the height to diameter ratio for these objects, depending on flow direction. They found that the fluid flow properties were more significant predictors of the heat transfer coefficient than the geometries for the forms studied in the turbulent regime. Because heat processing situations vary considerably, this important characteristic dimension should be checked with the heat exchanger manufacturer,

the published literature, or in Perry and Chilton (7) for the exact situation.

b) Identify the fluid and determine property data. The fluid may be water, air, or a liquid food product. The bulk fluid temperature (far from the heating surface) or the average temperature between the inlet and outlet should be the temperature at which to obtain the physical and thermal properties of the fluid. Examples are density, viscosity, and thermal conductivity. If these properties vary substantially with temperature between the inlet and outlet temperatures, then two possibilities exist: 1) an overall convective heat transfer coefficient may be calculated based on average properties, or 2) if, for example, the linear average does a poor job of representing the exponential increase of density and viscosity with temperature, then calculate the convective heat transfer coefficient for several short sections of the exchanger.

If the fluid is non-Newtonian, as is common in fluid food product applications, the viscosity must be handled in a slightly different manner and special empirical correlations are available to treat this special case, see Heldman and Singh (26) or Bird *et al*. (2).

In some cases, the ratio of the bulk fluid viscosity to the viscosity of the fluid at the hot wall (μ_b/μ_w), is important and the viscosities should be evaluated at these appropriate temperatures.

c) Calculate the Reynolds number. Using the characteristic dimension, the fluid properties and the fluid velocity, calculate the Reynolds number. The Reynolds number is necessary to determine the flow regime (laminar, transition, or turbulent). This is critical to selection of the appropriate empirical correlation.

d) Select the appropriate empirical correlation. Use the calculations from guidelines a) to c) to choose a correlation that will fit the scenario of the heat process. Calculate the Nusselt number and then finally the convective heat transfer coefficient.

Examples of empirical relations for simple cases three flow regimes

Laminar Regime

For Newtonian fluid flow, Reynolds numbers less than 2100, and flow in a horizontal pipe of diameter, D, and length, L, and for conditions where

$$(\mathrm{Re})(\mathrm{Pr})\left(\frac{D}{L}\right) \geq 100, \text{ then} \qquad (108.17)$$

$$Nu = 1.86\left(\mathrm{Re}\,\mathrm{Pr}\frac{D}{L}\right)^{0.33}\left(\frac{\mu_b}{\mu_w}\right)^{0.14} \qquad (108.18)$$

Therefore, the convective heat transfer coefficient between the hot pipe wall and the Newtonian fluid in the pipe is equivalent to h. This is found by solving the Nusselt number so that

$$h = \frac{Nuk}{D} \qquad (108.19)$$

where k is the thermal conductivity of the fluid in the pipe.

Transition Regime

For Newtonian fluid flow in a pipe at Reynolds numbers greater than 2100 and less than 10,000:

$$Nu = \frac{(f/8)(\mathrm{Re}-1000)\mathrm{Pr}}{1+12.7(f/8)^{0.5}(\mathrm{Pr}^{0.67}-1)} \qquad (108.20)$$

Where, similar to the laminar correlation for pipes, the fluid properties are evaluated at the average fluid temperature. The variable, f, is the friction factor and is estimated for smooth pipes in charts in Perry and Chilton (7) or by using the following correlation:

$$f = \frac{1}{(0.790\ln\mathrm{Re}-1.64)^2} \qquad (108.21)$$

Turbulent Regime

The following correlation for the convective heat transfer coefficient is used for Reynolds numbers greater than 10,000 in pipes for Newtonian fluids:

$$Nu = 0.023\mathrm{Re}^{0.8}\,\mathrm{Pr}^{0.33}\left(\frac{\mu_b}{\mu_w}\right)^{0.14} \qquad (108.22)$$

Kim *et al.* (9) found good correlation of experimental values with the following expression for the convective heat transfer coefficient of orange juice in turbulent flow conditions in a specific plate heat exchanger:

$$Nu = 1.12(10)^{-5}\,\mathrm{Re}^{1.39}\,\mathrm{Pr}^{1.63} \qquad (108.23)$$

This holds true for Re between 156 and 567 and Pr between 41 and 98.

4. The Overall Heat Transfer Coefficient

In common heating situations convective and conductive heat transfer occur at the same time. For example, in the plate heat exchanger, the hot water from the plant boiler transfers its heat to the plate metal wall by means of convection, then the heat conducts through the metal wall, and finally the metal wall heats the food product by means of convection. The method to combine these series thermal resistances is similar to that used previously in Section III.A.2, combining resistances in series in heat

conduction. Therefore the total resistance R_t is equivalent to:

$$R_t = R_{HW} + R_M + R_P = \frac{1}{UA} = \frac{1}{h_{HW}A} + \frac{\Delta x}{k_M A} + \frac{1}{h_P A} \quad (108.24)$$

Where R_{HW} is the resistance due to convective heat transfer from the hot water to the metal plate wall in this example, R_M is the resistance due to the conductive heat transfer through the metal wall, and R_P is the resistance due to convective transfer from the hot metal to the product.

Summing the resistances for rectangular coordinates, where areas are equivalent, yields:

$$q = \frac{(T_{hw} - T_{hotm})}{\frac{1}{h_{HW}A}} + \frac{(T_{hotm} - T_{prodm})}{\frac{\Delta x}{k_M A}} + \frac{(T_{prodm} - T_p)}{\frac{1}{h_P A}} \quad (108.25)$$

In this equation, h_{HW} is the convective heat transfer coefficient on the hot water side of the plate, and T_{HW} and T_{hotm} are the temperatures of the hot water and hot metal respectively. In addition, k_m is the thermal conductivity of the metal, Δx is the thickness of the metal, and T_{hotm} and T_{prodm} is the temperature driving force (the temperature of the water and product sides of the metal, respectively). In addition, h_p is the convective heat transfer coefficient on the product side of the plate and T_{prodm} and T_p provide the temperature boundary layer gradient, the temperatures of the metal on the product side, and the temperature of the bulk product, respectively.

The two convective heat transfer coefficients and the conduction term are often combined into an overall heat transfer coefficient, noted as "U," as in equation 108.24. This is a more useful term because the temperature driving force for the overall coefficient, that is, the bulk hot water and the bulk product temperature, is easier to measure than the film temperatures at the metal wall. Usually the manufacturer will suggest a range of overall heat transfer coefficients, U, based on their own experimentation with the heat exchanger and for the conditions and the product in the range of flow rates that are expected to be used. Therefore, equation 108.25 has been rewritten using the overall heat transfer coefficient and the overall temperature gradient for the specific heat flux area:

$$q = U_{PlateHE} A_{plateHE} (T_{HW} - T_p) \quad (108.26)$$

This example used rectangular coordinates, where area is a constant, but cylindrical coordinates, used with pipes, tubular, and swept-surface heat exchangers, are significantly different because the radius, hence the area of each heat transfer surface, varies with each thickness considered, hence:

$$q = U_i A_i (T_{HW} - T_p) \quad (108.27)$$

Where U_i and A_i are the overall heat transfer coefficient and areas based on the inside area of the tube and T_{HW} is the outside temperature of the bulk heating medium and T_P is the inside temperature of the bulk heated product. The definition of the term U_iA_i, which incorporates the three thermal resistances, based on each area is:

$$\frac{1}{U_i A_i} = \frac{1}{h_i A_i} + \frac{\ln\left(\frac{r_o}{r_i}\right)}{2\pi L k} + \frac{1}{h_o A_o} \quad (108.28)$$

The subscript "i" refers to the dimensions of the inside of the pipe, while the dimension "o" refers to the outside dimensions of the pipe. An example of the estimation of the overall heat transfer coefficient and the resulting heat loss is an insulated hot water pipe, see Heldman and Singh (26).

5. Design of a Tubular Heat Exchanger

In Section I of this chapter a variety of heat exchange equipment was discussed. An objective of this chapter was to determine the capacity or the size of the process unit needed to provide the necessary heat based on the rate at which the heat can be transferred. This section will address the calculations necessary to design a simple tubular heat exchanger to meet those needs.

In order to determine the required capacity of a heat exchanger for a given application, the needed heat transfer area must be estimated. The following assumptions will be used:

a) Heat transfer is under steady-state conditions.
b) The overall heat transfer coefficient is constant over the length of the pipe.
c) There is no longitudinal or axial conduction of heat in the metal pipe.
d) The heat exchanger is well insulated so that heat is only transferred between the two liquid streams in the exchanger. There is negligible loss to the surroundings.

First, the change in the heat energy, q, of a liquid stream, flowing at a mass flow rate of "w" kg/sec is estimated by the change in the temperature from T_1 to T_2:

$$q = wC_p(T_1 - T_2) \quad (108.29)$$

where Cp is the heat capacity (kJ/kg°C) of the liquid stream. In this example, T_1 is the inlet temperature and T_2 is the outlet temperature.

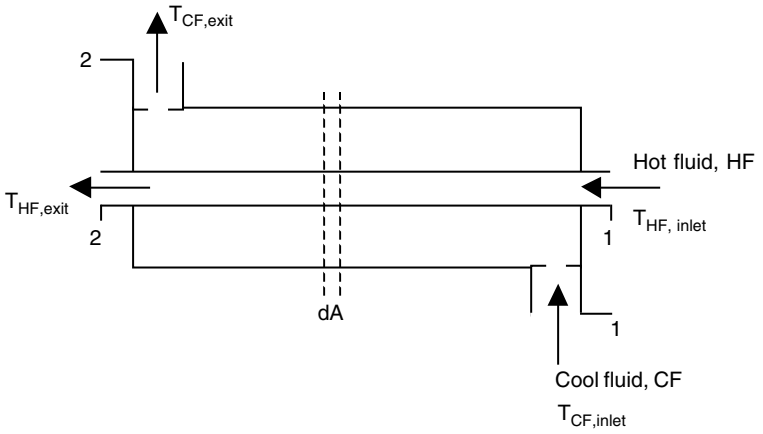

FIGURE 108.6 Tubular heat exchanger showing co-flow of hot and cold liquids for purposes of heat exchanger design.

Figure 108.6 shows an example tubular heat exchanger. In this example the hot fluid could be either water or product and will be noted as HF. It enters the inner tube of the heat exchanger at point 1 and exits at point 2. Its temperature decreases from $T_{HF,inlet}$ to $T_{HF,\ exit}$. Similarly the second fluid, CF, is the cool fluid that enters the annulus, between the inner tube and the outer tube, at point 1 and exits at point 2. Its temperature increases from $T_{CF,inlet}$ to $T_{CF,exit}$. Because there is perfect insulation around the exchanger and because the unit is operating at steady-state conditions, all the heat given up by the hot fluid must be taken up by the cold fluid. Therefore, an energy balance for the rate of heat transferred between the two fluids is:

$$\begin{aligned} q &= w_{HF}Cp_{HF}(T_{HF,inlet} - T_{HF,exit}) \\ &= w_{CF}Cp_{CF}(T_{CF,exit} - T_{CF,inlet}) \end{aligned} \quad (108.30)$$

Where w_{HF} and w_{CF} are mass flow rates of the hot and cold fluids, respectively, in units of kg/s, and Cp_{HF} and Cp_{CF} are the heat capacities of the hot and cold fluids, respectively, in units of kJ/kg°C. This equation determines the needed rate of heat transfer, or heat flux, q, in units of kJ/s or W for the heat process to be successful. This equation is the place to include any phase change information, such as, for example, the heat of condensation for steam, or the heat of fusion for melting fat. However, phase changes will not be considered in this example.

The next step is to determine the heat transfer perpendicular to the direction of flow. The estimation of the convective transfer coefficients and conduction through the metal wall are used here. A simple differential equation can be used to describe the situation. For a thin slice of heat exchanger, dA, perpendicular to the stream flow, the amount of heat, dq, from the hot fluid to the cold fluid will be estimated. The quantity, ΔT, is the difference in temperature between the hot and cold streams for every step, dA, from inlet to exit. Therefore,

$$dq = U\Delta T dA \quad (108.31)$$

where U is the overall heat transfer coefficient for every step, dA, from inlet to exit. In this example, one of the assumptions was that the overall heat transfer coefficient, U, would be constant over the length of the heat exchanger. The term, ΔT, is not assumed to be constant over the length of the heat exchanger. In fact, we already know that this term at point 1 ($T_{HF,inlet} - T_{CF,inlet}$) is different from the term at point 2 ($T_{HF,exit} - T_{CF,exit}$).

In order to easily solve equation 108.31 the term, ΔT, must be constant. In addition, we do not know the temperature at every point along the fluid flow stream, so we must use something similar to the average value, a value, preferably, that is easily measured. Using an arithmetic average temperature would imply that the temperatures are linear along the fluid flow stream and this is not true. Therefore, another method will be used to develop ΔT.

If we were to slice through the tubular exchanger perpendicular to the stream flow we would have two concentric rings. A small differential ring element, using the energy balance for the hot stream would yield:

$$dq = -w_{HF}Cp_{HF}dT_{HF} \quad (108.32)$$

and similarly for the cold stream. Solving for dT_{HF} and dT_{CF}:

$$dT_{HF} = -\frac{dq}{w_{HF}Cp_{HF}} \quad \text{and} \quad dT_{CF} = \frac{dq}{w_{CF}Cp_{CF}} \quad (108.33)$$

Then, subtracting these two equations:

$$dT_{HF} - dT_{CF} = -dq\left(\frac{1}{w_{HF}Cp_{HF}} + \frac{1}{w_{CF}Cp_{CF}}\right) \quad (108.34)$$

Using equation 108.34 above, to substitute into equation 108.31:

$$\frac{d(T_{HF}-T_{CF})}{(T_{HF}-T_{CF})}=-U\left(\frac{1}{w_{HF}Cp_{HF}}+\frac{1}{w_{CF}Cp_{CF}}\right)dA \tag{108.35}$$

Then integrating equation 108.35 from points 1 to 2:

$$\ln\frac{(T_{HF,exit}-T_{CF,exit})}{(T_{HF,inlet}-T_{CF,inlet})}=-UA\left(\frac{1}{w_{HF}Cp_{HF}}+\frac{1}{w_{CF}Cp_{CF}}\right) \tag{108.36}$$

At point 1, $T_{HF,inlet} - T_{CF,inlet} = \Delta T_1$ and at point 2, $T_{HF,exit} - T_{CF,exit} = \Delta T_2$. Substituting these expressions into equation 108.36 and then substituting equation 108.30 into equation 108.36 gives

$$\ln\left(\frac{\Delta T_2}{\Delta T_1}\right)=-\frac{UA}{q}\left[\left(T_{HF,inlet}-T_{HF,exit}\right)-\left(T_{CF,exit}-T_{CF,exit}\right)\right] \tag{108.37}$$

Substituting in the difference equations at points 1 and 2 and rearranging gives the equation for determining heat exchanger design, area and overall resistance:

$$q = UA\frac{\Delta T_2-\Delta T_1}{\ln\frac{\Delta T_2}{\Delta T_1}} \tag{108.38}$$

which is also seen in the form:

$$q = UA(\Delta T_{lm}), \tag{108.39}$$

where ΔT_{lm} is the log mean temperature difference. This equation is commonly used to calculate unknown exit temperatures, as well as necessary area for heat exchange purposes. Equation 108.39 was developed for a simplistic example of a tubular heat exchanger, but this type of exchanger is less common in the food industry. Consult the general references of Hallström *et al*. (5) and Perry *et al*. (6) for specific help in this area or check the Internet.

C. Radiative Heat Transfer

Radiative heat transfer is very different from conduction and convection. Radiative heat transfer has an electromagnetic mechanism, which allows energy to be transported at the speed of light through regions of space that are a complete vacuum. In comparison, for heat conduction to occur there must be a temperature differential at neighboring points in a material. For heat convection to occur there must be a fluid that is free to move and transport energy with it. Radiative heat transfer depends on the emissivity and the absorptivity of the radiating surface as well as the receiving surface, and the rate of heat transferred is also a function of the temperature difference between the surfaces as well as the shape of the object.

Too often the radiative heat transfer in an operation is neglected because it is considered too difficult to estimate or assumed to be negligible in comparison to the other forms of heat transfer. Radiative heat loss from the uninsulated hot surface of a boiler or kettle can be substantial, as can the heat load on the roof of a structure on a clear, sunny day. In addition, radiative heat transfer in a cooking operation, especially one such as grilling or baking in a continuous indirect gas-fired oven is significant; see Broyart and Trystam (15). Utilization of solar radiative energy is important in a variety of operations including drying, and water and air heating. Furthermore, infrared ovens are gaining in popularity because of three basic characteristics: high heat transfer capacity, heat penetration directly into the product, and fast regulation response; see Hallstrom *et al*. (5), Shilton *et al*. (14), and Sheridan and Shilton (27).

1. Radiative Flux

The rate of radiative energy transport between two fully absorptive bodies in a vacuum is proportional to the difference of the fourth powers of their absolute temperatures. The energy radiated from a surface is also dependent on the surface characteristics of the body that is emitting the radiation. A so-called "black body" has a perfect emissivity, that is, emissivity is equivalent to 1. The symbol, ε, for emissivity, represents the degree to which the surface is similar to a black body. The heat flux emitted or radiated from a surface is expressed with the following equation:

$$\frac{q}{A} = \sigma\varepsilon T_A^4 \tag{108.40}$$

In this equation, q/A represents the heat flux in W/m^2, σ, represents the Stephan-Boltzman constant, equal to 5.669 $(10)^{-8}$ $W/m^{2\circ}K^4$, ε, represents the emissivity, and T_A is the temperature of the emitting body in the absolute temperature scale, Kelvin. At a temperature of 0° Kelvin the emission of radiation stops.

The wavelength, in the electromagnetic spectrum, gets smaller as the temperature increases, for example, the radiation emitted by the sun has a much smaller wavelength than the radiation emitted by the element in an electric oven. The infrared part of the electromagnetic spectrum covers the wavelengths between visible light (including solar) and radiowaves (0.6–1000 μm). Infrared energy is naturally emitted by all objects as a result of the thermal vibrations of the molecules and this increases as the temperature is raised. For more discussion of wavelength in radiative heat transfer, as well as the general theory, see Watson and Harper (28).

2. Radiative Heat Transfer Between Two Objects

In order to estimate how much heat is received from the mechanism of radiation, one must consider both the amount

of radiation emitted as well as the incoming radiation. The difference between these two streams is the net exchange and this must always be in the direction of higher to lower temperature.

When electromagnetic energy, E, of a certain wavelength falls on matter, part of it will be absorbed, E_A, to cause heating, part will be reflected, E_R, and part will be transmitted, E_T.

$$E = E_A + E_R + E_T \tag{108.41}$$

In addition, in an isothermal system, Kirchoff's law states that the emissivity of a body surface is equal to its absorptivity for the same wavelength. Emissivity is a property of the material of a surface and tables of emissivities for various material surfaces are available in many heat transfer references including Watson and Harper (28), as well as Singh and Heldman (4). In general, emissivities (and absorptivities) of common surfaces are close to the maximum, so-called, blackbody value of 1, although polished (and even unpolished) metal surfaces have low emissivities. See Table 108.3 for selected examples of emissivities for two temperatures and wavelengths.

The heat transferred by radiation between two surfaces is dependent on the emissivity as well as the absorptivity of the radiating surface. The expression used to describe and estimate the heat, q_{1-2}, radiated by surface 1 and fully absorbed by surface 2, is as follows:

$$q_{1-2} = A\sigma(\varepsilon_1 T_{A1}^4 - E_{A(1-2)} T_{A2}^4) \tag{108.42}$$

where A is the area of the emitting surface (assuming full absorption), σ represents the Stephan-Boltzman constant, ε_1 and T_{A1} represent the emissivity and temperature, respectively, of surface 1, and $E_{A(1-2)}$ represents the absorptivity of surface 2 for radiation emitted from surface 1, at the surface 2 temperature of T_{A2}. It is important to point out that the caveat in equation 108.42 is the assumption that *all* the heat radiated by surface 1 is absorbed by surface 2. This is generally a poor assumption because the geometry as well as the angle of the receiving surface determines how much radiation is absorbed. So in order to account for the variety of shapes, including disks, squares, and rectangles at various incident angles to each other, a shape factor, F_{1-2}, is introduced:

$$q_{1-2} = \sigma F_{1-2} A_1 (T_{A1}^4 - T_{A2}^4) \tag{108.43}$$

This shape factor represents the fraction of the total radiation leaving the surface A_1 that is intercepted by the surface A_2. Singh and Heldman (4) and also Bird *et al*. (2) have some figures of shape factors (also termed view factors) for various dimension ratios. More comprehensive view factors may be found in Hottel (29).

An alternative to using a shape factor is to use Lambert's law, derived in Bird *et al*. (2), for the incidence at angle θ from the normal to the surface. The example illustrating this is the radiant heat flux entering the earth's atmosphere from the sun, also termed the solar constant. This radiant energy is important in solar energy utilization, in particular, and solar food drying operations. Bird *et al*. (2) solve the radiant heat transfer equation using black body assumptions for the sun and the receiving surface on earth and estimate the radiant heat flux to be 1355 W/m^2.

The shape factor in equation 108.43, however, assumes perfect emissivity and absorptivity, i.e., blackbodies and avoids derivations using Lambert's law. In order to account for both shape and emissivity the expression becomes:

$$q_{1-2} = \sigma \zeta_{1-2} A_1 (T_{A1}^4 - T_{A2}^4) \tag{108.44}$$

where ζ_{1-2} accounts for both the shape factor as well as the emissivity of both surfaces 1 and 2 and is defined as such:

$$\zeta_{1-2} = \frac{1}{\dfrac{1}{F_{1-2}} + \left(\dfrac{1}{\varepsilon_1} - 1\right) + \dfrac{A_1}{A_2}\left(\dfrac{1}{\varepsilon_2} - 1\right)} \tag{108.45}$$

Therefore, equations 108.44 and 108.45 may be used to compute the net radiant heat transfer between two gray bodies, each at their own uniform temperature. For further discussion of this useful mode of heat processing see Chapman (3). Hallström *et al*. (5) give some interesting examples of this type of heating in industrial use.

TABLE 108.3
Emissivities of Selected Materials for Specified Wavelengths and Temperatures

Material	9.3 μm Wavelth. and 38°C	0.6 μm Wavelth. and Solar
Polished aluminum	0.04	~0.3
Anodized aluminum	0.94	
Lampblack paint	0.96	0.97
White paint (ZnO)	0.95	0.18

Source: Singh and Heldman (4).

IV. UNSTEADY-STATE HEAT TRANSFER

Unsteady-state heat transfer conditions mean the temperature changes not only with location, but also with time. Steady-state conditions, in comparison, mean the temperature varies only with location. In spite of the fact that transient operating conditions are common, and steady-state conditions are uncommon, steady-state is more often an assumption made in a mathematical analysis because it is simpler than unsteady-state.

However, during the unsteady-state period many important reactions take place. Furthermore, in some operations the unsteady-state period is the major component of

the process. This is especially the case with heating cans for commercial sterilization or cooking, cooling, or freezing solid foods. We cannot use steady-state conditions because the time frame of interest is when the temperature is changing with time and, in the case of canning, microbes are being killed.

A. Use of the Fourier Relationship

In order to mathematically model unsteady-state conductive heating in one dimension, we will use equation 108.2, Fourier's law, and a basic energy equation to result in equation 108.46. For a detailed derivation see Myers (30), Holman (1), Bird *et al.* (2), or Chapman (3).

$$\frac{\partial T}{\partial t} = \frac{k}{\rho C_p}\left(\frac{\partial^2 T}{\partial x^2}\right) \tag{108.46}$$

Therefore, the time evolution of temperature is a function of the combination of material properties, k/ρCp (also defined as the thermal diffusivity, α, from equation 108.1), and the second derivative of the temperature as it varies with distance in the x direction. (This partial differential equation is also the diffusion equation.)

Equation 108.46 has been solved analytically for simplified geometrical shapes (see Myers (30)) such as the sphere (possibly a roast), an infinite cylinder (perhaps a can), and an infinite slab (possibly cooking a hamburger or a box of spinach to be plate-frozen). Take care when using these simplified geometries for real situations that end effects are not significant. For most interesting problems, analytical solutions are not available and advanced computational techniques are generally required to solve the equation.

For reasons similar to those discussed in the dimensional analysis, Section III.B.3.a, food processors found alternative ways to solve practical unsteady-state problems using empirical methods based on theory. In the development of these empirical methods certain groups of key variables and constants seemed to appear again and again in the solutions and equation set-ups. In unsteady-state heat transfer these groups include the Nusselt number, Nu, thermal diffusivity, α, the Fourier number, Fo, and the Biot number, Bi. The Nusselt number and the thermal diffusivity were defined previously and the Biot and Fourier numbers will be discussed below.

B. External and Internal Resistance to Heat Transfer

If an object, say a pineapple at ambient temperature, were placed in a retort of pressurized steam, the pineapple temperature would start to increase, as a function of material properties, time, and of the steam temperature. The temperature profile from the outside surface to the center or pineapple core would change for each second that the whole pineapple was in the retort until the temperature within the pineapple reached equilibrium with the retort (something that might not be desirable). The heat flowing into the pineapple will encounter two major resistances: 1) the convective resistance outside the pineapple of the steam flowing across the surface of the pineapple, and 2) the conductive resistance inside the pineapple of the heat moving from the pineapple surface toward the core.

The Biot number is another dimensionless number and is the ratio of the internal resistance to heat transfer in the solid pineapple to the external resistance to heat transfer from the fluid to the pineapple surface:

$$Bi = \frac{internal\_resistance}{external\_resistance} = \frac{L/k}{1/h} = \frac{hL}{k} = \frac{UL}{k} \tag{108.47}$$

In this equation, L is the characteristic dimension of the object (the diameter for a cylinder or the thickness for a slab), h (or U) defines the surface conductance, and k is the internal conductance of the object.

For Biot numbers greater than about 40, there is generally negligible surface resistance to heat transfer, that is, the "h" value is much greater than "k" value. An example of this might be a metal can of salmon in a steam retort where there is very efficient heat transfer from the flowing, and condensing steam to the metal and also fast heat transfer across the metal can surface, but relatively slow heat transfer from the salmon touching the can through to the center of the can. The heat transfer inside the can is almost 100% conduction in meat, which has a relatively small conductivity.

For Biot numbers less than about 0.1, there is a negligible internal resistance to heat transfer, that is, the "k" value is much greater than the "h" value. Examples of this might occur in the heating of a block of solid metal where the k value, thermal conductivity, is relatively high, 2 to 3 orders of magnitude (see Table 108.1), compared to foods. A more realistic example of this case for the food industry might be a well-stirred liquid in a hot water–jacketed kettle, where the temperature profile is uniform across the container due to the agitation. No temperature gradient with location occurs because the heat transfer is immediate to the bulk liquid.

Between Biot numbers of 0.1 and 40 there is a finite internal and external resistance to heat transfer.

C. Finite Internal and External Resistance to Heat Transfer

The empirical solution to equation 108.46 when the Biot number is between 0.1 and 40 has been simplified to temperature-time charts for the three main geometrical shapes. See time-temperature charts in (4) and (28). In these charts,

a dimensionless unaccomplished temperature is plotted as a function of the thermal diffusivity for several Fourier numbers. The dimensionless unaccomplished temperature is defined as $(T_o - T)/(T_o - T_i)$, where T_o is the outside temperature (also referred to as the temperature of the surrounding medium), T_i is the initial temperature, and T is the variable temperature changing with time. This temperature ratio indicates the change of temperature with time compared to the maximum possible temperature difference. It is not initially obvious that the dimensionless unaccomplished temperature change should be useful for both infinite and finite objects of a slab, as well as a cylinder. Myers (30) provides convincing mathematical derivations.

The dimensionless Fourier number, Fo, is the ratio of the rate of heat conduction across D in volume D^3 (in Watts per degree C) to the rate of heat storage in volume D^3 (in Watts per degree C) and is defined by:

$$Fo = \frac{k\left(\frac{1}{D}\right)D^2}{\rho C_p \frac{D^3}{t}} = \frac{\alpha t}{D^2} \qquad (108.48)$$

where D is a characteristic dimension that might be the shortest distance from the heated surface to the center of the object. For the purposes of this relationship, the characteristic dimension, D, refers to half of the thickness of an infinite slab, the radius of an infinite cylinder and the radius of a sphere. Heldman and Singh (26) suggest another way to think about this parameter: Fo is a measure of the rate of heat conduction per unit of heat storage for a given volume element. Therefore, a larger Fo number indicates a deeper heat penetration into the solid in a given time period.

D. Use of the Temperature-Time Charts for Unsteady-State Heat Transfer in Finite Objects

In order to use the temperature-time charts for estimation of heat transfer rates in appropriately shaped geometries the following steps may be used:

1. Calculate the Fo number using the radius of the cylinder (or half of the height of a slab, or the radius of a sphere) as the characteristic dimension, D.
2. Calculate the Bi number using the radius of the cylinder (or half of the height of a slab, or the radius of a sphere) as the characteristic dimension, D.
3. Use the appropriate temperature-time chart to estimate the unaccomplished temperature ratio, above. From this ratio the center temperature may be calculated providing the initial temperature, T_i and the outside temperature T_o are known.

Be aware that the assumptions within this method are for infinite geometric shapes so that end effects can be quite significant. For example, if the height of a can is smaller than its diameter, then it might be appropriate to treat the short cylinder as a slab in the initial analysis.

For further discussion of unsteady-state heat transfer and use of Schmidt plot, Gurney-Lurie charts and thermal processing examples (1, 4, 5, 30, 31).

ACKNOWLEDGMENT

Credit is given to Alfa Laval, Inc. (Figures 108.1, 108.2, 108.3) and Biosteam, Inc. (Figure 108.4).

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109 Heat Processing: Temperature–Time Combinations

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CONTENTS

I. INTRODUCTION

The principles of heat processing discussed in the chapters in the heat processing section, and in particular those that refer to the heat resistance of the microorganisms and the mechanisms and rates of heat transfer, are among the absolutely necessary information in establishing a heat treatment for a particular product. No matter if one is interested in establishing a process for steam sterilization or thermal pasteurization, or is aiming in enzyme inactivation through a water blanching procedure, or is simply heating the product as an intermediate step during a particular process, the basic principles are the same. Thus, the concepts that will be presented in this chapter, although they will be, most of the time, focused and referred to thermal processes used for commercial sterilization, they are of general applicability, independently of the severity or the goals of a particular heat treatment.

Products with different characteristics will, most probably, require processing at different *temperature–time combinations* in order to accomplish the objectives set in a particular process. Any change in product or process characteristics requires reevaluation of the necessary time–temperature treatment. It is somewhat intuitive that what could be achieved by heating the product at a given process temperature for a given process time, it can be also achieved by heating the product at a higher temperature for less time. Quantifying this idea will be the task of this chapter.

By introducing more than one objective during a heat treatment, it is usually possible to optimize the process.

Product safety coupled with quality is the basis for proper thermal process design. The required theoretical background and the mathematical procedures, with example calculations, used in designing and evaluating a thermal process will be presented in the remainder of this chapter.

II. BASIC PRINCIPLES

The extent of thermal destruction of a heat labile substance during thermal processing is a function of the sensitivity (or the "thermal resistance") of the particular substance to the various temperatures at which it is exposed and the time that the substance remains at each one of these temperatures during the process. Substances with different thermal resistances will be affected differently, even if they are processed under identical time–temperature conditions. Thus, the severity of a thermal process relates to a particular substance or food attribute and it can be assessed by measuring or evaluating the degree of destruction of that substance or attribute after the process (*e.g.,* remaining microbial spores per can).

The heat labile nature of the microorganisms and the other agents of spoilage makes preservation of foods by heat treatment possible. However, the same destructive effect of a heat treatment on quality factors requires precise and accurate design and control of a thermal process. Differences in thermal sensitivities between the various undesirable and quality agents [1, 2] are in fact what make a thermal process effective.

To facilitate our presentation, in the remainder of this chapter, we will assume (unless otherwise explicitly stated) that the target (undesirable) heat labile substances that we refer to are heat resistant microbial spores. With this note, and before attempting to fully quantify the ideas behind the design of a thermal process, let us introduce and define the term *equivalent processes*. Two thermal processes are considered equivalent only if they produce the same destructive effect on a microbial population, that is, if the number of spores surviving after each process is the same, providing that the two processes are applied to the same type and initial load of spores with all conditions, except the time-temperature exposure of the spores, being the same for the two processes [3].

So, let us assume that by holding a sample of spore suspension at a lethal constant temperature, T_1, for a time period, t_1, we reduce its initial number of spores by 90%. The same 90% reduction can be presumably achieved by heating the sample at a different, higher temperature, T_2, for a shorter time period, t_2. One must very precisely estimate (by experimentation or calculation) this new, t_2, processing time in order to achieve exactly the desired amount of 90% destruction. Obviously, one might choose any appropriate temperature and estimate the required, for 90% destruction, processing time, or, in a similar way, find equivalent processes for any given degree of microbial destruction. If the temperature of a food sample going through a thermal treatment is not constant throughout the process (the usual scenario), there still exists an equivalent process at any desired constant reference temperature.

With the above introductory remarks, we can now approach the subject in quantitative terms. Assuming that thermal destruction of microbial spores follows first order kinetics [4], then the rate of spore destruction (*e.g.,* concentration change, C, over time) during processing at a constant temperature T, is given by Equation (109.1).

$$-\frac{dC}{dt} = k_T C \tag{109.1}$$

The subscript T in the reaction rate constant, k_T, denotes its temperature dependence. Integration of Equation (109.1) between the beginning, t_a, of the process (where spore concentration is C_a) and the end, t_b, of the process (where spore concentration is C_b) gives:

$$\int_{C_a}^{C_b} -\frac{dC}{C} = \int_{t_a}^{t_b} k_T\, dt \equiv f(C_a,C_b) \tag{109.2}$$

or

$$\ln(C_a) - \ln(C_b) = \int_{t_a}^{t_b} k_T\, dt \tag{109.3}$$

The type of the $f(C_a,C_b)$ function defined by Equation (109.2) is determined by the kinetics the thermal destruction obeys. For first order reactions, comparison of Equations (109.2) and (109.3) reveals:

$$f(C_a,C_b) = \ln(C_a) - \ln(C_b) \tag{109.4}$$

Different reaction kinetics give rise to different types of $f(C_a,C_b)$ functions. Thus, for zero and for nth order reactions ($n \neq 1$) the $f(C_a,C_b)$ functions (also referred to as quality functions when the change — destruction — of a quality attribute is examined) are given by Equations (109.5) and (109.6) respectively [5].

$$f(C_a,C_b) = C_a - C_b \tag{109.5}$$

$$f(C_a,C_b) = \frac{1}{n-1}(C_b^{1-n} - C_a^{1-n}) \tag{109.6}$$

As it can be inferred from the right hand side of Equation (109.2), the value of $f(C_a,C_b)$ depends upon the time temperature process conditions (beyond the destruction kinetics). Note that k_T, is a function of temperature while time explicitly appears in Equation (109.2). Furthermore, from the definition of equivalent processes, it is inferred that the $f(C_a,C_b)$ value must be the same for two processes to be equivalent. We must clarify here that it is not necessary that the values of the initial and final concentrations of two processes be the same, in order for the values of the $f(C_a,C_b)$ function of the two processes to be

equal. For example, for inactivation following first order kinetics, the value of $\ln(C_a) - \ln(C_b)$ or $\ln(C_a/C_b)$ or finally the value of C_a/C_b, that is, the ratio of the initial over the final concentration must be equal for the values of the $f(C_a,C_b)$ to be equal, and, therefore, for the two processes to be equivalent. Thus, a process with $C_a = 10^9$ spores/can and $C_b = 10^3$ spores/can is equivalent to a process with $C_a = 10^6$ spores/can and $C_b = 1$ spore/can under the earlier mentioned assumptions.

A thermal process at time varying temperature conditions resulting in an $f(C_a,C_b)$ value, as it is calculated from the right hand side of Equation (109.2) for a particular heat induced change, has an equivalent process at any constant, reference, temperature T_{ref}. The required process time at T_{ref}, in order for the two processes to be equivalent, can be estimated by evaluating the last integral in Equation (109.2). This, for the process at constant temperature ($k_T = k_{T_{ref}}$ = constant, and for t_1 being the beginning and t_2 the end of the process) gives:

$$f(C_a,C_b) = k_{T_{ref}}\,(t_2 - t_1) \tag{109.7}$$

where $(t_2 - t_1)$ is the total (required) processing time at the constant reference temperature. Note that we assumed that processing at the reference temperature starts at t_1 and ends at t_2 to explicitly indicate that processing time at T_{ref}, *i.e.*, $t_2 - t_1$, does not have to be equal to the processing time $t_b - t_a$ of the process with the variable temperature. It is customary to denote that time with $F_{T_{ref}}$ (the index T_{ref} in the "F" symbol denotes the reference temperature used in calculating the required time) that is,

$$F_{T_{ref}} = t_2 - t_1 \tag{109.8}$$

and through Equations (109.2), (109.7) and (109.8), we obtain

$$F_{T_{ref}} = \frac{1}{k_{T_{ref}}} \int_{t_a}^{t_b} k_T\,dt \tag{109.9}$$

With the presentation so far we arrived to the fundamental concept in thermal process design and evaluation, the *F value*. The F value of a process is the equivalent processing time of a hypothetical thermal process at a constant, reference, temperature that produces the same effect (in terms of spore destruction) as the actual thermal process. Note that the mathematical definition of the F value through Equation (109.9) is independent of the order of the thermal destruction kinetics.

The reaction rate constant depends upon a variety of factors, but in the context of the present issue we will only focus upon the temperature dependence of the k_T value. Using the Arrhenius equation to express the effect of temperature on k_T, that is, in one representation [6]

$$k_T = k_{T_{ref}}\,10^{-\frac{E_a}{\ln(10)\cdot R_g}\frac{(T_{ref}-T)}{T_{ref}T}} \tag{109.10}$$

Equation (109.9) reduces to:

$$F_{T_{ref}} = \int_{t_a}^{t_b} 10^{-\frac{E_a}{\ln(10)\cdot R_g}\frac{(T_{ref}-T)}{T_{ref}T}}\,dt \tag{109.11}$$

Note that the parameters T ant T_{ref} in Equations (109.10) and (109.11) are representing absolute temperatures (*i.e.,* expressed in K).

In the classical thermobacteriological approach [7–9] but also in the majority of the recent thermal processing literature, the D_T and z values are used in place of the k_T and the E_a (or better E_a/R_g) parameters. The D_T value (*decimal reduction time*) is defined as the time, at a constant temperature T, required to reduce by 90% the initial spore load. The same definition applies for any heat labile substance. Traditionally, D_T is estimated from a semilogarithmic plot of the number of (surviving) spores, N, *vs* processing time, t, as they are experimentally measured during treatment at constant temperature T. A "straight line" fit of the experimental data (according to the first order destruction kinetics) can be drawn (*Thermal Death Rate Curve* or *Survivor Curve* of Figure 109.1) and the D_T value can be measured directly on the graph, as the time required for the straight line to traverse a logarithmic cycle (a logarithmic cycle corresponds to 90% reduction).

Alternatively, the D_T value can be calculated from the slope of the *Thermal Death Rate Curve*. The slope of the straight line, the *Thermal Death Rate Curve* (Figure 109.1), is given as:

$$\text{slope} = \frac{d(\log(N))}{dt} = \frac{\log(N_2) - \log(N_1)}{t_2 - t_1} \tag{109.12}$$

(for 1 and 2 representing any two points on the straight line). For $N_2 = 0.1 \cdot N_1$ we are referring to 90% destruction, where, by definition, $t_2 - t_1 = D_T$. Under these conditions Equation (109.12) reduces to:

$$\text{slope} = \frac{\log(0.1N_1) - \log(N_1)}{D_T} \quad \text{or} \quad D_T = -\frac{1}{\text{slope}} \tag{109.13}$$

Thus, a statistical linear least square regression can be applied to the experimental log(N) *vs* t data, and D_T can be calculated from the slope of the line through Equation (109.13).

Referring to isothermal conditions, Equation (109.1) suggests a linear relationship, with slope equal to $-k_T$, between ln(C) (or equivalently ln(N)) and t. Going from natural to base 10 logarithms, the following relationship is obtained between D_T and k_T:

$$D_T = \frac{\ln(10)}{k_T} \tag{109.14}$$

As already said, the activation energy, through the Arrhenius equation, is used to express the effect of temperature on k_T. In a similar way, in thermobacteriological

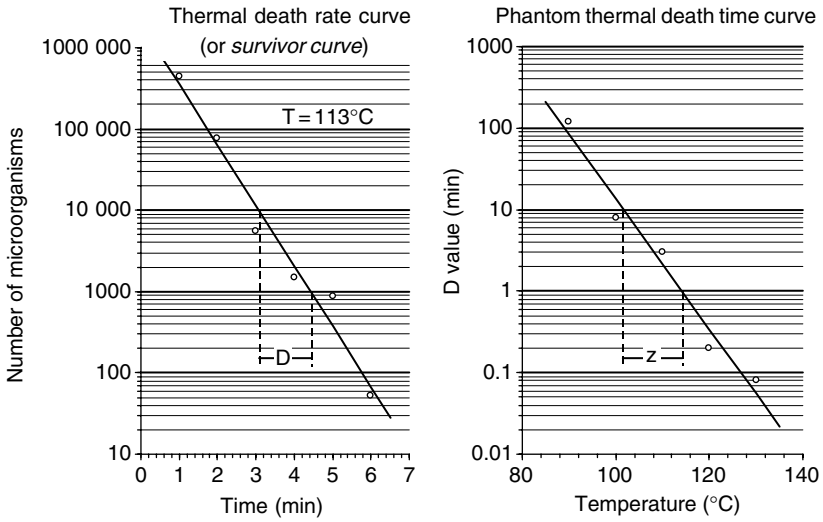

FIGURE 109.1 Graphical determination of the thermal inactivation kinetic parameters D and z through the thermal death rate and the phantom thermal death time curves, respectively.

terms, the z value is used to express, through Equation (109.15), the effect of temperature on the decimal reduction time, D_T.

$$D_T = D_{T_{ref}} 10^{\frac{T_{ref}-T}{z}} \qquad (109.15)$$

The z value is defined as the temperature difference required to achieve a decimal change of the D_T value. In converting z values from one system of units to another, we must remember that z represents a temperature difference. Thus a z value of 10°C is equivalent to a z of 10 K or a z of 18°F. Equation (109.15) is an empirical equation resulted from the observed linear relationship between $\log(D_T)$ and T values. In an analogous way as for the D_T value, the z value can be measured directly on $\log(D_T)$ *vs* T plots (*Phantom Thermal Death Time Curve*, Figure 109.1), as the temperature interval required for the straight line to traverse a logarithmic cycle. Obviously, estimation of the z value requires kinetic (destruction) experiments at different temperatures.

Comparison between Equations (109.10), (109.14) and (109.15) allows for a relation between E_a and z to be determined:

$$z = \frac{\ln(10) \cdot R_g \cdot T_{ref} \cdot T}{E_a} \qquad (109.16)$$

Note that in Equation (109.16) the temperatures T and T_{ref} are representing absolute temperatures (*i.e.,* expressed in K) while the z value is either in °C or K. Furthermore note that although both E_a and z are assumed independent of temperature (it is inherent in the nature of Equations (109.10) and (109.15)) Equation (109.16) suggests the opposite. The contradictory nature of the two models (Equations (109.10) and (109.15)) is given [10]. However, as long as we are not extrapolating beyond the experimental temperature range used for collecting thermal destruction data, both models are considered satisfactory for microbial destruction calculations [11–13]. At this point we should mention that although deviations of microbial thermal destruction from first order kinetics do exist, the "straight-line semilogarithmic model" presented so far (Figure 109.1) is considered as an appropriate engineering tool for thermal process design [14].

As mentioned earlier, the classical thermobacteriological analysis is still followed in the thermal processing literature and industry [2]. From the equations presented so far following the k_T, E_a approach, by appropriate substitutions (through Equations (109.14) and (109.16)), one can obtain the fundamental equations that govern thermal processing in the D, z notation. Nevertheless, for better comprehension, we will, at the risk of being redundant, in brief derive them based on the new nomenclature. Thus, from Equations (109.12) and (109.13) we obtain:

$$\frac{d(\log(N))}{dt} \quad \text{or} \quad \frac{d(\log(C))}{dt} = -\frac{1}{D_T} \qquad (109.17)$$

Upon integration, between t_a (where spore concentration is C_a) and t_b (where spore concentration is C_b) Equation (109.17) gives

$$\int_{C_a}^{C_b} d\log(C) = \int_{t_a}^{t_b} -\frac{1}{D_T} dt$$

$$\Rightarrow \quad \log(C_a) - \log(C_b) = \int_{t_a}^{t_b} \frac{1}{D_T} dt \qquad (109.18)$$

For equivalent processing at constant temperature, T_{ref}, ($D_T = D_{T_{ref}}$ = constant, and for $t_a = t_1$, $t_b = t_2$, where by definition $F_{T_{ref}} = t_2 - t_1$) integration of Equation (109.17) gives:

$$\log(C_a) - \log(C_b) = \frac{t_2 - t_1}{D_{T_{ref}}}$$

$$\Rightarrow \quad \log(C_a) - \log(C_b) = \frac{F_{T_{ref}}}{D_{T_{ref}}} \qquad (109.19)$$

Comparison between Equations (109.18) and (109.19) results to (note the analogy between Equations (109.9) and (109.20)):

$$F_{T_{ref}} = D_{T_{ref}} \int_{t_a}^{t_b} \frac{1}{D_T}\, dt \qquad (109.20)$$

Finally, for variable T, substitution of D_T from Equation (109.15) into Equation (109.20) gives:

$$F_{T_{ref}} = D_{T_{ref}} \int_{t_a}^{t_b} \frac{1}{D_{T_{ref}}\, 10^{\frac{T_{ref}-T}{z}}}\, dt$$

$$\Rightarrow \quad F_{T_{ref}} = \int_{t_a}^{t_b} 10^{\frac{T-T_{ref}}{z}}\, dt \qquad (109.21)$$

By putting together Equations (109.19) and (109.21) we have:

$$F^z_{T_{ref}} = D_{T_{ref}} (\log(C_a) - \log(C_b)) = \int_{t_a}^{t_b} 10^{\frac{T-T_{ref}}{z}}\, dt \quad (109.22)$$

Note that a superscript z is introduced in the notation of the F value. This is to indicate that, as suggested by the right hand side of Equation (109.22), heat induced changes to attributes with different z values correspond to different F values, even if they occur during the same time–temperature conditions. (The symbol F_o is uniquely used for a reference temperature of 250°F and a z value of 18°F.) Equation (109.22) represents the *fundamental equation* in thermal processing. It uniquely indicates the methodologies to be used for thermal process design and evaluation.

There are a few points that must be noted before going further. While different thermal processes can be compared based on their corresponding F values, the accomplishment of the objectives of a process (*e.g.,* commercial sterilization) can be assessed by comparing the F value of the process (which we will term $F_{process}$) with an F value corresponding to a process known to meet our objectives (which we will term $F_{required}$). Given the relationship between concentration and F value (left hand side of Equation (109.22)) the $F_{required}$ value can be defined as the time, at a constant temperature, required to destroy a given percentage of microorganisms whose thermal resistance is characterized by a particular z value.

Furthermore, unless the temperature of the sample is uniform during processing, something that is not the usual case (see the chapter on heat transfer), Equation (109.22) can be used to calculate the F value of a process at a single "point" in the sample where temperatures or concentrations are known throughout the thermal process (termed *point F value*). The objectives of a process have been met throughout the whole product, if they have been met at the *critical point* of the product, that is, the point that receives the least effects, in terms of spore destruction, of the heat treatment (the F value at the critical point is noted as F_c). Integration of the effect of the heat treatment for each of the volume elements of the sample must be performed if equivalent processes in terms of remaining concentration for the total volume of the food sample have to be defined. This (second) integration enables calculation of *integrated F values* (F_s). The significance in calculating F_s values is obvious when we are interesting in quality retention calculations. In microbial destruction calculations, according to Hayakawa [15] the two procedures (F_c *vs* F_s calculations) produce similar results. A further discussion on this matter is given by Stoforos [16]. The relative simplicity in calculating point F values compared to integrated ones gives an advantage to the former procedures, and most of the discussion in the remainder of this chapter will be devoted to them.

The integrated time-temperature effect of a thermal process on a heat labile substance can be evaluated using *in situ*, time-temperature integrators (TTI), or physical-mathematical procedures [17]. The *in situ* measurements as well as the use of TTIs involve direct monitoring of the concentration, before and after processing, of either the heat labile substance of interest itself (*in situ* method) or of another heat labile substance, chosen so as to mimic the original substance as far as its response to thermal treatments is concerned (TTI approach). F value calculations associated with these two methods involve use of the left hand side of Equation (109.22). The basic requirement in using TTIs in thermal process calculations is that the activation energies, or the z values, of the TTI and the substance of interest should be the same [5, 17]. When this is not attainable, the use of multi-component TTIs (with each component having its own activation energy or z value), and correlation of the responses of these multi-TTI systems with the thermal degradation of the substance of interest has been proposed as an alternative [17–20].

Physical-mathematical procedures rely on temperature data, of the product undergoing a thermal process, which together with appropriate thermal destruction kinetics can be used to evaluate the lethal effects of the process. F values by these physical-mathematical procedures are calculated through the evaluation of the integral in Equation (109.22). While the *in situ* or the TTI approach can be the only choice in several cases where time temperature data are not available (*e.g.*, during aseptic processing of liquid/particulate foods) due to inherent difficulties in working with these two procedures [21] physical-mathematical methods are considered to be the most appropriate procedures when product temperature data can become available [22].

III. MATHEMATICAL PROCEDURES FOR THERMAL PROCESS CALCULATIONS

There is a large number of publications dealing with the issue of thermal process calculations through mathematical methods. The core of all these procedures is the right hand side of Equation (109.22). Methodologies have been reported for calculation of point F values from discrete (usually experimental) product temperatures (General

Methods) or from time-temperature curves obtained through appropriate models (Formula Methods). Critical reviews of these methodologies have been presented in the literature [3, 15, 23]. Procedures for evaluating the effects of a thermal process through calculation of integrated F values are also available and have been reviewed [15, 23, 24].

The objectives of any methodology on thermal process calculations consist of:

1. Process evaluation (first type of problem): This refers to the evaluation of the F value of a given process (that is, calculation of $F_{process}$ value). Information on the process (in particular processing time and processing (retort) temperature, etc.) and on the product is available. This is a rather straightforward step.

2. Process design (second type of problem): This step involves the estimation of the proper processing time in order to achieve a particular required F value. A variety of parameters needed for the calculations (*e.g.,* retort temperature, initial product temperature, cooling medium temperature, speed of rotation (if any), can size, headspace volume, etc.) can become available. Obviously this step needs some "predictive" work. Before answering this question we must notice that a cooling cycle always follows the heating cycle in a thermal process in order to control and cease the destructive effects of the elevated temperatures. So, in the design problem we are in fact interested in calculating the time needed to end the heating cycle, *i.e.,* the steam-off time. (The exact duration of the cooling cycle, provided that it is long enough to allow for the food temperature to fall below lethal temperatures, is not important as far as microbial thermal destruction is concerned.) In terms of mathematical representation of this step, dividing the thermal process into the two cycles, Equation (109.22) can be written as:

$$F^{z}_{T_{ref}} = \int_0^{t_g} 10^{(T-T_{ref})/z}\, dt_h + \int_0^{t_{c_b}} 10^{(T-T_{ref})/z}\, dt_c \qquad (109.23)$$

where the first integral is associated with the heating and the second integral with the cooling cycle of the thermal process. What we are in fact looking for, is the value of t_g (steam-off time) — upper limit of first integral — in order for the whole process to result in a given $F_{required}$ value.

Equation (109.23) gives the desired F *vs* t_g relationship. The ultimate objective of all thermal process calculation procedures is to express the F value as an explicit function of the total heating time (and vice versa). An analytical solution of Equation (109.23) can be obtained for only a few product temperature profiles. Given the complexity of product temperature profiles encountered, in most practical cases, during thermal processing of foods, the methodologies developed for thermal process calculation rely on numerical or graphical procedures and/or make use of appropriate graphs and tables. In the remainder of this chapter we will mainly present two of the methodologies used for thermal process calculations: the General Method and Ball's Formula Method.

A. General Method

The first ever scientific procedure for thermal process calculations was the General Method introduced by Bigelow and his coworkers at the National Canners Association over 80 years ago [25]. In approaching the first type of problem, that is, calculation of the F value of a given process, the method, in its original application, calculates the integral appearing in Equation (109.22) graphically (that is why it is also referred to as the *Graphical Method*) from discrete data (usually experimental product temperature values at the critical point *vs* time). It does not make any assumptions about process or product characteristics (termed for this *General Method*) and it calculates an exact (depending on the accuracy of the graphical integration) $F_{process}$ value.

Several complementary works were introduced to make the General Method less laborious, consisting of the use of a special coordinate paper [26–29], or of some numerical integration scheme, such as trapezoidal rule [30], Simpson's rule [31], and Gaussian integration [32]. In fact, a numerical integration scheme is nowadays incorporated to any method that uses a computerized model for product temperature predictions.

In attacking the second type of problem, that is, estimation of the required processing time in order to achieve a particular $F_{required}$ value, any method, including the General Method, has to make some reasonable assumptions in extrapolating time–temperature data to different conditions. The problem, in its simplest form, can be stated as: Given experimental time–temperature data for a particular product during a specific heat treatment (*i.e.*, with specified retort temperature during both heating and cooling cycles of the process, given initial product temperature and steam-off time, specific product characteristics and container type and size, etc.), determine the steam-off time required to achieve a particular $F_{required}$ value for all conditions being the same with those used to obtain the experimental time–temperature curve. General Method approaches this problem using a "geometrical similarity," that is, by assuming that the shape of the integrant in

Equation (109.22) *vs* time curve, after steam-off time, is geometrically the same irrespectively of the total heating time. This is an approximation. With the exception for the case where the product temperature had approached equilibrium with the heating medium temperature, the shape of the lethal rate curve during the cooling phase is a function of the difference between the product and the heating medium temperature at the steam-off time. In the next paragraphs, we will illustrate the application of the General Method in process calculations by the following example.

1. Example Problem

Experimental time–temperature data during processing of canned fish in a still retort at 240°F are given in Table 109.1. Determine the steam-off time for a required F_o value of 6 min.

2. General Method Approach

In one of its applications, General Method uses the lethality of process, that is, the ratio of $F_{process}/F_{required}$ as the design variable.

TABLE 109.1
Heat Penetration Data

Product:	Diced fish in sauce
Can size:	308 × 113
Process:	Still retort at 240°F
Retort come up time:	10 min
Cooling water temperature:	78°F

Time (Min)	Can Center Temperature (°F)	Time (Min)	End of Heating, Start of Cooling Cycle*
0	77.4	57	237.5
3	86	60	238
6	112	End of heating,	
9	132	start of cooling cycle.*	
12	151	63	238.5
15	170.5	66	239
18	185	69	239
21	194	72	238
24	202	75	237
27	211	78	236.5
30	217	81	225.5
33	223	84	200
36	226	87	181.5
39	229	90	166
42	232	93	149
45	233	96	136
48	234.5	99	125.5
51	236.5	102	116
54	237	105	109

*Steam is turned off after 60 min of heating. Therefore, the 61st minute is the end of the first minute of cooling.

$$\text{lethality} = \frac{F^z_{T_{ref}}\big|_{process}}{F^z_{T_{ref}}\big|_{required}} \tag{109.24}$$

For a process that meets our objectives, $F_{process}$ must be equal to $F_{required}$, or, in other words, lethality must be unity. That is,

$$\text{lethality} = 1 \tag{109.25}$$

Using the right hand side of Equation (109.22) we obtain:

$$\text{lethality} = \frac{1}{F^z_{T_{ref}}\big|_{required}} \int_{t_a}^{t_b} 10^{\frac{T-T_{ref}}{z}}\, dt \tag{109.26}$$

or, by defining TDT (thermal death time) as

$$TDT = F_{required}\, 10^{\frac{T_{ref}-T}{z}} \tag{109.27}$$

Equation (109.26) can be written as

$$\text{lethality} = \int_{t_a}^{t_b} \frac{1}{TDT}\, dt \tag{109.28}$$

Note that the TDT (thermal death time) is the equivalent to the required $F_{T_{ref}}$ time at a constant temperature T (one can check this by evaluating the integral in Equation (109.22) for constant temperature T and letting $TDT = t_b - t_a$) and that the variable 1/TDT is called *lethal rate*.

Based on Equation (109.27) and the experimental data from Table 109.1, the lethal rate (1/TDT) *vs* time curve is plotted in Figure 109.2. The area under this curve (corrected appropriately for the time and the lethal rate scales used) gives the lethality of the process. In the particular example, the area under the original (steam-off time = 60 min) lethal rate curve is equal to 663.7 small (dxdy) squares. This area multiplied by the time scale and the lethal rate scale used is converted to process lethality. Specifically, for the example (Figure 109.2) we have:

$$\text{lethality} = 663.7 dxdy \cdot \frac{1\text{min}}{dx} \cdot \frac{0.010\ \text{min}^{-1}}{5dy}$$

$$\Rightarrow \quad \textbf{lethality = 1.33} \tag{109.29}$$

(From the above value one can calculate, through Equation (109.24), the F value of the process, as: $F_{process} = 1.33 \cdot 6 \Rightarrow F_{process} = 8$ min. If one seeks the $F_{process}$ value without knowing the value of $F_{required}$ — or is not interested in steam-off time calculation — one can assume an $F_{required}$ value of unity. In this case, the area under the lethal curve will give directly the $F_{process}$ value.)

The area under the curve that corresponds to unit lethality (our goal) is called Unit Sterilization Area (USA) and is calculated based on the particular time and lethal rate scales used in constructing the lethal rate curve. That is,

$$USA = \frac{1}{\text{time scale}} \cdot \frac{1}{\text{lethal rate scale}} \tag{109.30}$$

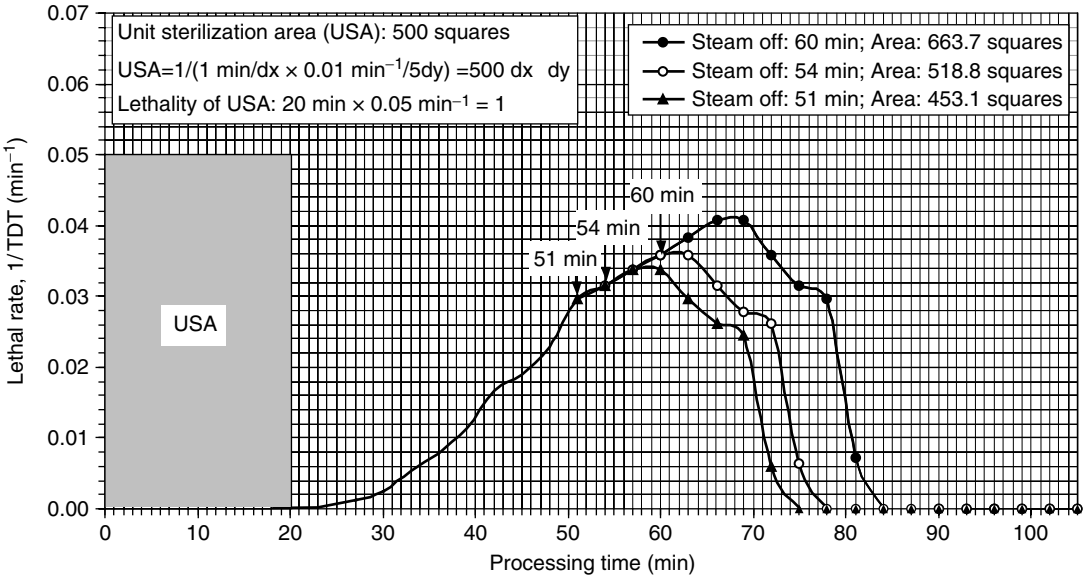

FIGURE 109.2 Graphical determination of process lethality, by trial and error, through the evaluation of the area under the lethal rate curve.

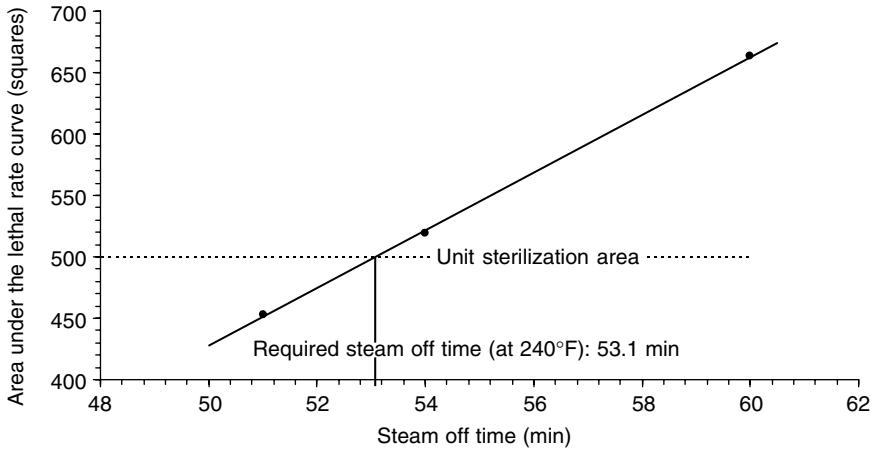

FIGURE 109.3 Graphical interpolation between trials for steam-off time determination.

and for our example,

$$\text{USA} = \frac{1}{\frac{1\,\text{min}}{\text{dx}}} \cdot \frac{1}{\frac{0.010\,\text{min}^{-1}}{5\,\text{dy}}} \Rightarrow \mathbf{USA = 500\,dx\,dy} \tag{109.31}$$

A surface with area equal to USA (*i.e.*, of 500 dx dy squares) is pictured in Figure 109.2 corresponding to a total processing time of 20 min at a temperature resulting to a lethal rate (1/TDT) value of 0.050 min^{-1} (one can calculate from Equation (109.27) that temperature to be equal to 240.59°F).

Thus, so far we calculated the lethality of the original process (data from Table 109.1, steam-off time at 60 min). Proceeding with our goal in finding the steam-off time that gives the unit lethality, we will assume (following a trial and error procedure) a steam-off time of 54 min (based on the early calculations, it should be less than 60 min). We do not know what the product temperature profile will be when the steam will be turned off at 54 min (the only data we have are the ones for 60 min steam-off time), but we will assume, as the General Method dictates, that the new lethal rate curve will follow the shape of the initial lethal rate curve after steam-off time. Such a curve is presented in Figure 109.2. The area under the new curve can be estimated as being equal to 518.8 small (dxdy) squares. This slightly exceeds our target value of 500 dxdy squares.

Repeating the procedure for a new steam-off time of 51 min we find an area under the new lethal rate curve of 453.1 dx dy squares. By interpolation between the three values calculated so far, we obtain the steam-off time corresponding to the Unit Sterilization Area of 500 squares, that is (Figure 109.3), 53.1 min.

The problem we solved, as stated, was in its simplest form. Going to different processing conditions, including, for example, changes in retort temperature, calculations by the General Method become more elaborated. To account for variations in retort and initial temperatures beyond the experimentally obtained values, the use of the dimensionless temperature differences, in the form of $(T_{RT} - T)/(T_{RT} - T_{IT})$, as a function of time only, based on pure conduction or ideal, perfect mixing, models (and for constant T_{RT} and uniform T_{IT}), was suggested [26]. Based on the analytical solution to the heat conduction equation, a procedure to further convert heat penetration data from one can size to another, during the heating cycle only, for conduction heating products, was also suggested [33]. Recently, Simpson *et al.* [34] extended the idea of dimensionless temperature ratios to include linear changing retort temperature profiles, and expanded the applicability of General Method to alternative processing conditions, different than those used in collecting the experimental heat penetration data.

Ending the presentation on General Method, we should mention that the graphical approach followed here was basically chosen for illustration purposes in an attempt to underline some of the basic principles involved. In the reality, the calculations shown here can be done (and were done) in a computer environment following the General Method principles. In the past, area estimations required by the method were done by counting squares, approximating the area with regular geometrical figures of known areas, use of planimeters, weight comparisons (with known areas), etc. This made the method laborious and difficult to use. Formula methods originated, at least in part, in an attempt to make calculations simpler.

B. Ball's Formula Method

Ball, in 1923 [35], introduced a new method for thermal process calculations, named Formula Method from the approach that he used in attacking the problem. He used an equation, a formula, to describe the temperature as a function of time at the critical point of a can, and then he substituted the equation into Equation (109.22) (or better into Equation (109.23)) and performed the integration.

Ball [35] introduced, basically, two empirical parameters, the f and j values, to describe the time-temperature curves of any product during thermal processing. Ball's basic assumption (and simplification) was that any heating or cooling curve when appropriately plotted (as shown in Figure 109.4 and Figure 109.5, respectively) after an initial lag was asymptote to one (*straight-line curve*) or more (*broken-line curve*) straight lines. Therefore, after that initial lag, the curve could be approximated by its asymptote. So, for a straight-line heating curve, the following equation has been suggested:

$$T = T_{RT} - j_h(T_{RT} - T_{IT})10^{-t_h/f_h} \qquad (109.32)$$

Both the f_h and j_h values can be determined from the "heating curve," an "inverted," semi-logarithmic plot of the difference between retort and product temperature (at the critical point) *vs* time, as illustrated in Figure 109.4 (for the data presented in Table 109.1). The j_h value, a dimensionless correction factor, is defined as

$$j_h = \frac{T_{RT} - T_A}{T_{RT} - T_{IT}} \qquad (109.33)$$

for T_A being an extrapolated pseudo-initial product temperature at the beginning of heating defined as the intercept, with the temperature axis at time zero, of the straight line that describes the late, straight, portion of the experimental heating curve plotted as shown in Figure 109.4.

The f_h value is related to the slope of the heating curve. It is defined as time required for the difference between the medium and the product temperature to change by a factor of 10, that is, in relation to Figure 109.4, as the time needed for the straight line heating curve to traverse through a logarithmic cycle.

With these definitions, it becomes clear that Equation (109.32) is the "Exponential Equation" curve shown in Figure 109.4, that is, the straight line that fits the portion of the experimental data after an initial lag period. Although Equation (109.32) approximates the experimental heating curve only after some initial time lag, Ball used this equation for the entire heating curve, reasoning that the thermal destruction taking place at the beginning of a thermal process is negligible since, under common commercial practices, the temperature of the product has not yet reached the lethal temperature range.

Nevertheless, during the initial cooling phase, the product experiences lethal temperatures, and an accurate description of the product temperature during this stage is necessary. Thus, Ball used a hyperbola (Equation (109.34)) to describe the initial part of the cooling phase for $0 \leq t_c \leq f_c \log(j_c/0.657)$,

$$T = T_g + 0.3(T_g - T_{CW}) \left[1 - \sqrt{1 + \left(\frac{1}{0.5275\log(j_c/0.657)}\right)^2 \left(\frac{t_c}{f_c}\right)^2}\right] \qquad (109.34)$$

and another, exponential equation — as for the heating cycle — for the final, the "straight-line," portion of the cooling curve, *i.e.*, for $f_c\log(j_c/0.657) \leq t_c$,

$$T = T_{CW} + j_c(T_g - T_{CW})10^{-t_c/f_c} \qquad (109.35)$$

Note that cooling time t_c counts from the beginning of the cooling cycle ($t_c = 0$ at steam-off) and the f_c and j_c parameters are determined from the cooling curve

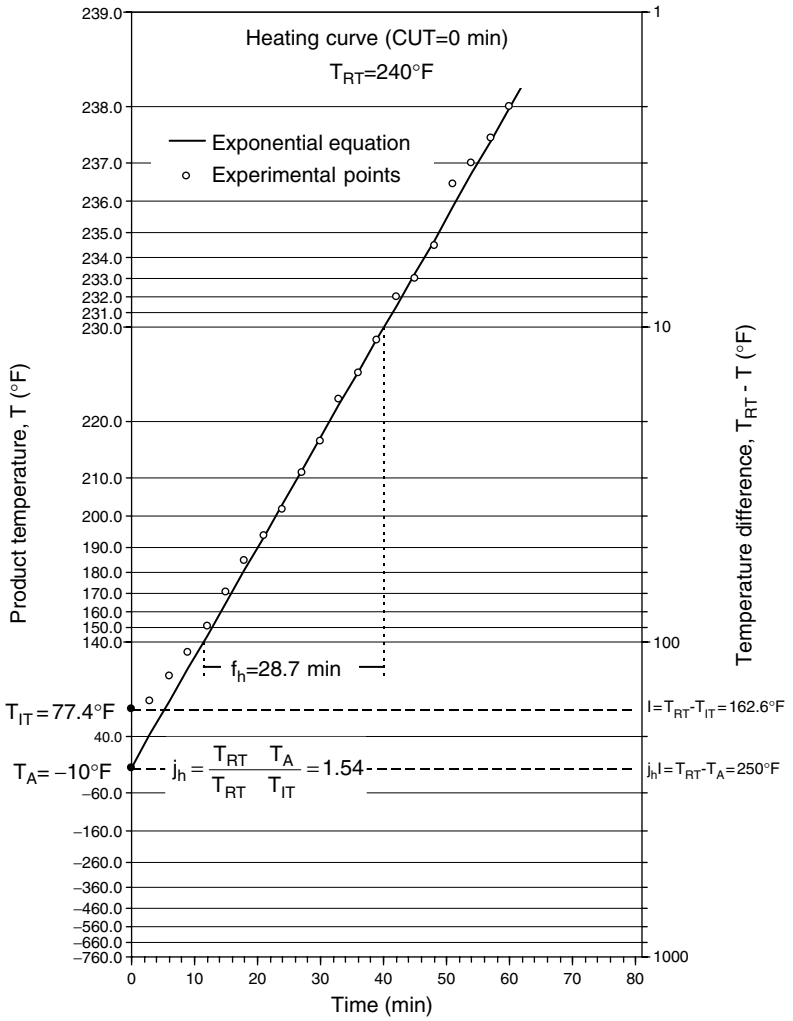

FIGURE 109.4 Experimental *straight-line* heating curve (CUT = 0 min).

(Figure 109.5) in the same way as the f_h and j_h values are determined from the Heating Curve. In particular, j_c is defined as:

$$j_c = \frac{T_B - T_{CW}}{T_h - T_{CW}} \tag{109.36}$$

Before substituting Equations (109.32), (109.34) and (109.35) into Equation (109.23) and proceeding in obtaining a relationship between F and t_g, Ball, based on experimental observations and conservative assumptions, fixed the j_c value at 1.41 and he further considered $f_c = f_h$. After this, Ball proceeded with the substitution into Equation (109.23) and thereafter he solved Equation (109.25) (seeking unity lethality) for the lethality given by Equation (109.24). He ended up with an equation [36] that could not be integrated analytically. So, he graphically evaluated the resulting integrals and he presented the results of the integration in tabular or graphical forms. This step involved some additional (usually minor) assumptions (*e.g.*, that there is no microbial destruction after product center temperature falls 80°F below heating medium temperature).

Ball's final results correlated, graphically, the f_h/U values *vs* log(g) having only the z and the m + g values as parameters. The parameter U is the F value at retort temperature, T_{RT}, that is,

$$U = F^z_{T_{ref}} F_i \tag{109.37}$$

for

$$F_i = 10^{\frac{T_{ref} - T_{RT}}{z}} \tag{109.38}$$

U was used as the depended variable (instead of the $F_{T_{ref}}$ value). The parameter "g" is the difference between retort and product temperature (at the critical point) at steam-off time and was used as the independent variable. Steam-off time, B (in Ball's notation), was related to g

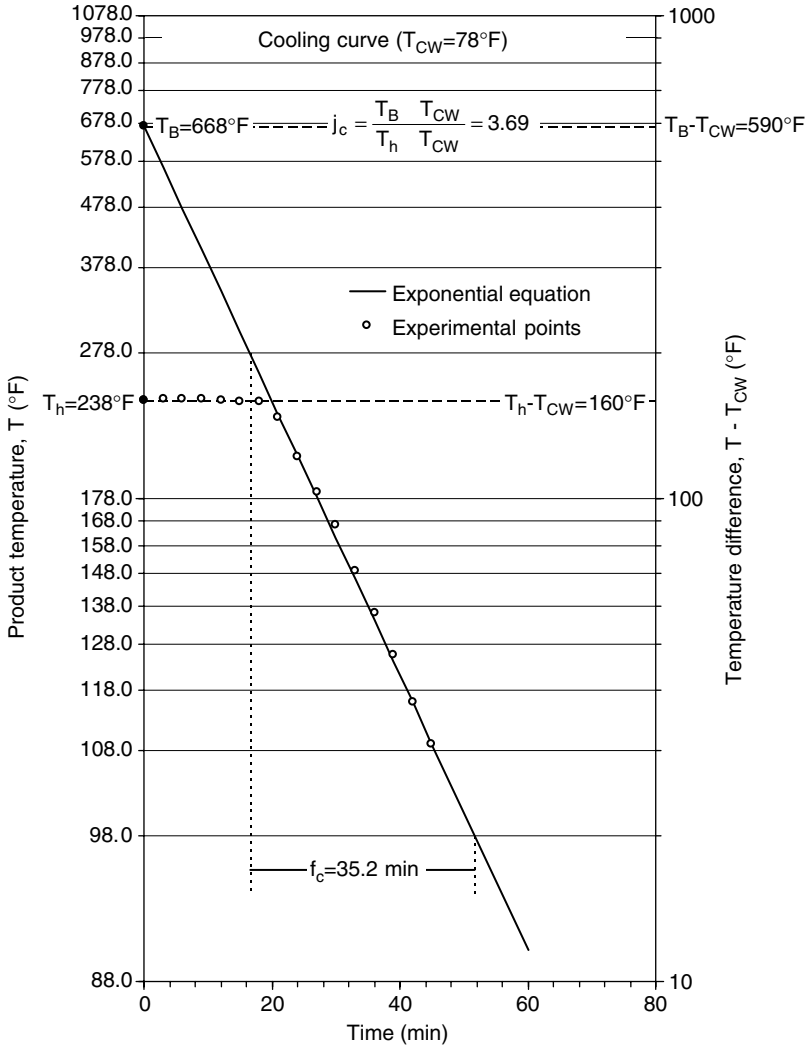

FIGURE 109.5 Experimental cooling curve.

through the following *formula* (originated from Equation (109.32)):

$$B = f_h(\log(j_h I) - \log(g)) \tag{109.39}$$

for

$$I = T_{RT} - T_{IT} \tag{109.40}$$

The parameter "m" was defined as the difference between product temperature at steam-off and retort temperature during cooling, resulting in the following expression for m + g:

$$m + g = T_{RT} - T_{CW} \tag{109.41}$$

In expanding the range of applicability of his Formula Method, Ball further addressed some issues concerning thermal process calculations. By providing additional tables (and working equations) he allowed calculations for *broken-line* (heating) curves, that is, heating curves that could not be adequately described by a single straight line but needed a set of straight lines (for practical purposes two lines), each one with different slope, to be described. In the same context, corrections for the case where $f_c \neq f_h$ were suggested. Finally, Ball accounted for the contribution of the time period needed for the retort to achieve the constant operating temperature during the heating phase, the coming-up time (CUT). For this, based on experimental observations, he concluded that a process at constant retort temperature of duration equal to 42% of the CUT was equivalent to the process at the variable retort temperature profile during the CUT. So he suggested to correct the heating time, by shifting the zero heating time axis by 0.58 · CUT and apply his method, as for a process with CUT = 0, based on this *corrected "zero" axis*.

In brief, the use of Ball's method for determining the F value of a given process involves the following steps:

1. Determination of the f_h and j_h values from the experimental heating curve
2. Calculation of log(g) from Equation (109.39) and the experimental steam-off time, B
3. Finding the f_h/U value, for the log(g) value calculated in step 2, from Ball's graphs [7, 8, 35, etc.]
4. Calculation of F value (from the f_h/U value found in step 3) through the following equation (originated from Equation (109.37) (the definition of U):

$$F^{z}_{T_{ref}} = \frac{f_h}{(f_h/U)F_i} \qquad (109.42)$$

The second type of problem, that is, estimation of the required processing (steam-off) time in order to achieve a particular $F_{required}$ value follows the same steps (in reverse order) and will be illustrated for the problem solved earlier with the General Method, that is, for the data given in Table 109.1.

1. Ball's Formula Method in Calculating Process Time for the Example Data of Table 109.1

Since the data refer to a process at a still retort with 10 min of come up time, the heating curve is replotted on Figure 109.6 to illustrate the correction for the zero time that must be made. From Figure 109.6 we can estimate the heat penetration parameters f_h and j_h as: $f_h = 28.7$ min and $j_h = 0.96$.

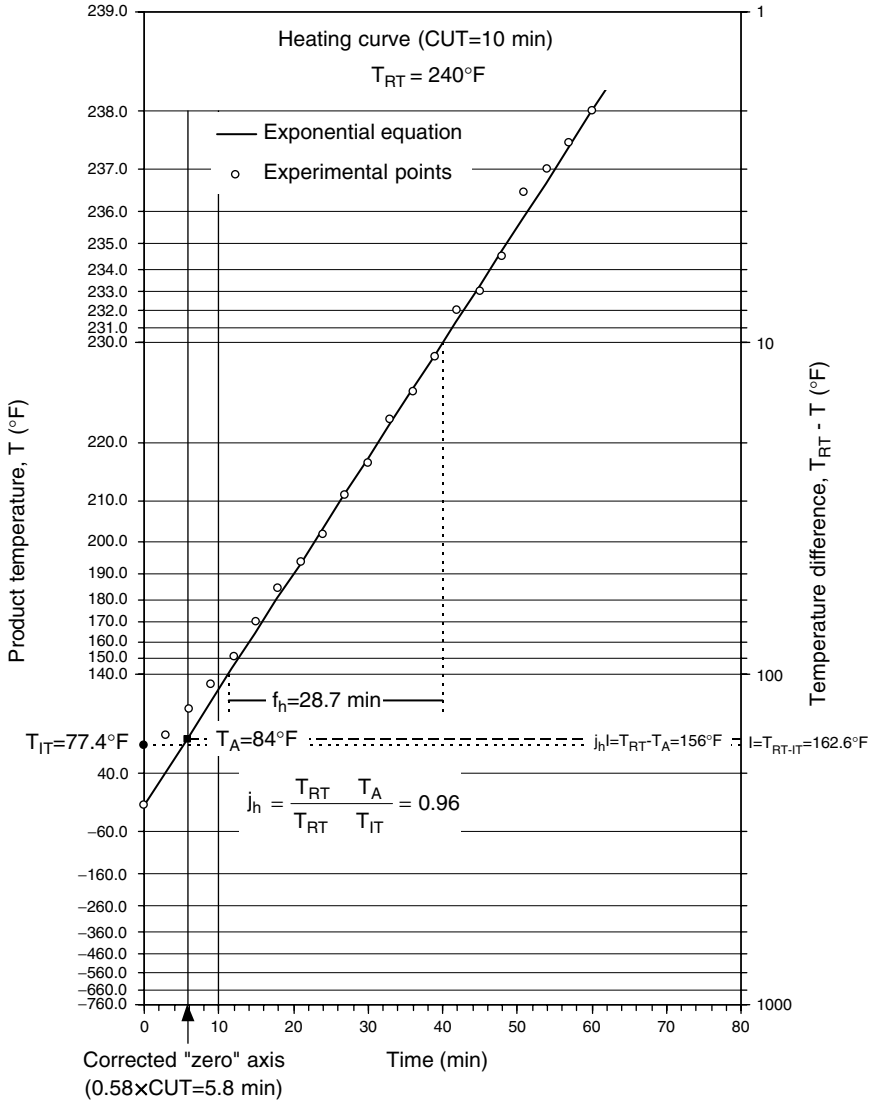

FIGURE 109.6 Experimental *straight-line* heating curve (CUT = 10 min).

For

$$F_i = 10^{\frac{T_{ref}-T_{RT}}{z}} \Rightarrow F_i = 10^{\frac{250-240}{18}} \Rightarrow F_i = 3.5938$$

and Equation (109.37) for U, the value of the parameter f_h/U becomes:

$$f_h/U = \frac{f_h}{F^z_{T_{ref}} \cdot F_i} \Rightarrow f_h/U = \frac{28.7}{6 \cdot 3.5938}$$

$$\Rightarrow f_h/U = 1.33$$

From the f_h/U *vs* log(g) diagrams [8], and specifically for $m + g = 160°F$ (the exact value being: $m + g = T_{RT} - T_{CW}$, *i.e.,* $m + g = 240 - 78 = 162°F$), $z = 18°F$ and for $f_h/U = 1.33$, we find the value of the log(g) equal to: $\log(g) = 0.0$. Hence, for $I = T_{RT} - T_{IT}$ (Equation (109.40)), that is, $I = 240 - 77.4 \Rightarrow I = 162.6°F$ from Equation (109.39), we have: $B = 28.7 \cdot (\log(0.96 \cdot 162.6) - 0.0) \Rightarrow B = 62.95$ min.

Therefore, the time from steam-on to steam-off, t_g, is

$$t_g = B + 0.58 \cdot CUT = 62.95 + 0.58 \cdot 10$$
$$\Rightarrow \mathbf{t_g = 68.8\,min}$$

Comparing this time with the corresponding time found using the General Method, *i.e.*, the 53.1 min found earlier, we conclude that in this particular example, Ball's method overestimates processing time (assuming the General Method's answer as the basis) by about 30%. This must be mainly due to the value of j_c. Ball fixed that value to 1.41. However, for our data (Figure 109.5) this value was equal to 3.69. Ball's assumption about a smaller j_c value means faster cooling than the actual, thus Ball's Formula Method has to allow for longer heating time. The extensive lag period during the cooling cycle observed for the experimental data used can be due to the retort cool down period, that is the time needed for the retort to reach, after steam-off, the cooling temperature (T_{CW}). This is not taken into consideration by any of the thermal process calculation procedures (it can be viewed though as an additional safety factor).

The importance of the j_c value was recognised by Stumbo and his co-workers who in a series of publications [37–39] developed and published [9] a new set of tables for thermal process calculations. The procedure in calculating lethality or process time, through this new set of tables, was identical as for Ball's original formula method. Nevertheless, Stumbo included j_c as a parameter (ranged in his tables from 0.40 to 2.00) and he presented values for a broader range as far as the z value is concerned. In Ball's graphs the z value lied between 10°F and 24°F, while Stumbo employed z values from 8°F to 200°F. While z values in the range of Ball's graphs are sufficient for microbial inactivation, higher z values characterize quality degradation. Aiming in quality retention calculations Stumbo developed a procedure for integrated F values calculations. Although further discussion on this matter is beyond the scope of this chapter, we must notice that quality retention calculations based on mass average values constitute the basis for process optimization.

Several researchers, following Ball's example, used the empirical heat penetration parameters f and j in different formulas to describe the temperature inside the product during thermal processing [40–46]. Although empirical in nature, the use of these parameters resulted in simple, flexible, and of wide applicability, independently of the mode of heat transfer involved, methodologies for thermal process evaluation and design. Theoretical values of these parameters for conduction heating foods under various conditions, as well as for perfectly mixed, forced convection heating foods were presented [7, 47–49]. Factors, used to convert f values from one can size to another (for metal cans) or between different types of containers (*e.g.,* between glass jars and metal cans) for pure conduction or perfectly mixed heating products, have been also reported [35, 50, 51]. So, it is possible, to use Ball's Formula Method to design processes for conditions different from those used in obtaining the experimental data (for example, for different retort temperatures and can sizes).

IV. CONCLUDING REMARKS

This chapter focused on the basic principles involved in establishing the necessary time–temperature combination for an appropriate thermal process. Two of the basic mathematical procedures used for thermal process calculations, namely the General Method and Ball's Formula Method were presented in sufficient detail. Both methods have contributed to the success of the canning industry. Representative example calculations, with the above methods, for establishing the necessary processing time for commercial sterilization were given.

It should be noted that in our entire example calculations temperatures were in degrees Fahrenheit and time units in minutes. This is because literature in thermal process calculations, including working graphs and tables, is traditionally and invariably using these particular units.

The analysis in this chapter referred to "in container" processing. However, the principles presented are of general applicability. Caution, though, must be taken with regards to the assumptions involved in the development of each method. Specific critical factors associated with particular processes must be considered. For example, residence time distribution during aseptic processing could make calculations rather complex and should be properly addressed.

V. NOMENCLATURE

A. Latin Letters

B steam-off time (measured from *corrected zero*), s (unless otherwise explicitly stated)

C concentration of a heat labile substance, number of microorganisms/ml, spores per container, g/ml, or any other appropriate unit

CUT duration of retort come-up time, s (unless otherwise explicitly stated)

D_T (noted also as D) *decimal reduction time* or *death rate constant*—time at a constant temperature required to reduce by 90% the initial spore load (or, in general, time required for 90% reduction of a heat labile substance), s (unless otherwise explicitly stated)

dx the unit length of the x axis scale of a lethal rate curve

dy the unit length of the y axis scale of a lethal rate curve

E_a activation energy, J/mol

F_T^Z (or simply F) time at a constant temperature, T, required to destroy a given percentage of microorganisms whose thermal resistance is characterized by z, *or*, the equivalent processing time of a hypothetical thermal process at a constant temperature that produces the same effect (in terms of spore destruction) as the actual thermal process, s (unless otherwise explicitly stated)

F_c critical point F value

F_i factor defined by Equation (109.38), which when multiplied by $F_{T_{ref}}$ gives the F value at the retort temperature, dimensionless

F_o a reference F value for $T_{ref} = 250°F$ and $z = 18°F$

F_s integrated F value

$f(C_a, C_b)$ function of initial and final concentrations and the destruction kinetics, variable units

f time required for the difference between the medium and the product temperature to change by a factor of 10, s (unless otherwise explicitly stated)

g difference between retort and product temperature (at the critical point) at steam-off time, °C (unless otherwise explicitly stated)

I difference between retort and initial product temperature, °C (unless otherwise explicitly stated)

j a correction factor defined by Equations (109.33) and (109.36) for the heating and cooling curve, respectively, based on the intercept, with the temperature axis at time zero, of the straight line that describes the late, straight, portion of the experimental heating or cooling curve plotted in a semi-logarithmic temperature difference scale as shown in Figures 109.4 and 109.5, respectively, dimensionless

k_T reaction rate constant, s^{-1} for first order reactions

lethality ratio of $F_{process}$ over $F_{required}$, dimensionless

m difference between product temperature at steam-off and retort temperature during cooling, °C (unless otherwise explicitly stated)

N spore load, number of spores

n apparent reaction order, dimensionless

R_g universal gas constant, 8.314 J/(mol · K)

T (product) temperature, °C (unless otherwise explicitly stated)

T_A extrapolated pseudo-initial product temperature at the beginning of heating defined as the intercept, with the temperature axis at time zero, of the straight line that describes the late, straight, portion of the experimental heating curve plotted as shown in Figure 109.4, °C (unless otherwise explicitly stated)

T_B extrapolated pseudo-initial product temperature at the beginning of cooling defined as the intercept, with the temperature axis at zero cooling time, of the straight line that describes the late, straight, portion of the experimental cooling curve plotted as shown in Figure 109.5, °C (unless otherwise explicitly stated)

T_h product temperature at the beginning of the cooling cycle, °C (unless otherwise explicitly stated)

t time, s (unless otherwise explicitly stated)

TDT (thermal death time) time at a constant temperature, T, required to destroy a given percentage of microorganisms, s (unless otherwise explicitly stated)

U the F value at T_{RT}, defined by Equation (109.37)

USA (Unit Sterilization Area) area under the lethal rate curve that corresponds to lethality of unity, units of area, depending on the graph scales

z temperature difference required to achieve a decimal change of the D_T value, °C (unless otherwise explicitly stated)

B. Subscripts

1, 2 refers to a particular condition

a initial condition

b final condition

CW (water) cooling medium

c cooling phase

g condition at steam-off time

h heating phase

IT initial condition (for product temperature only)

process referring to process values

RT (retort) heating medium

ref reference value

required referring to required values

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110 Modeling of Thermal Processing of Foods

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CONTENTS

I. INTRODUCTION

Thermal treatments of foods can be dated back to prehistoric times when cavemen accidentally discovered the wonder of fire (and heat): the sensory quality of roasted animal carcasses was superior to the raw meats, and later the healthful effect of cooked foods was also appreciated. Frenchman Nicholas Appert, is credited as the one who started the canning industry even though he did not understand why canning techniques he invented prevented food spoilage. It took the genius of Louis Pasteur, a half century later, to discover the relationship between thermal processing techniques on a scientific basis. Thermal processes in modern-day food industry are more or less designed to destroy undesirable microorganisms and enzymes that are either harmful to human health or destructive to food quality during storage. Thermal processing of foods in food industry is generally referred to heating of foods packaged in hermetically sealed containers and pasteurization of liquid foods (may contain some particulates). It is carried out either continuously or batchwise in a consecutive process of heating, holding and cooling. Eating quality (which includes sensory quality) of foods is not always improved through thermal processing. Loss of flavors and nutrients, and changes of textures and colors are recognized as the commonest complaints about thermally processed foods. As a result of this recognition, new thermal processes such as high-heat-short-time (HTST) and ultrahigh-temperature (UHT) have been implemented in thermal processing of some foods, and non-thermal processes (electrical pulse field, high-pressure, ultraviolet light, and ultrasound) have also been developed and tested for certain foods for possible replacement of conventional heat processing (1). Aseptic processing and packaging further reduce heat damage to processed foods.

Thermal processes are generally not designed to sterilize foods completely (or bacteriologically). Rather, they are constructed on the objective of destroying microbial pathogens, parasites and deleterious enzymes present in foods, and of minimizing damage of heat on nutritional and sensory profiles in foods by optimizing temperature and heating time based on models of thermal processing. Even in the heat treatment of canned foods, the objective of the sterilization process is to produce a condition of "commercial sterilization" in canned foods. Some thermophilic or heat-loving microorganism spores could survive the thermal treatment of a retort process, but these microorganisms are not pathogenic or poisonous and therefore of no significance regarding public health (2).

In order to determine the extent of thermal processing for a particular food matrix in a particular environment, several fundamental questions have to be answered:

1. The type of microbial flora associated with all raw materials that are to be treated.
2. The pH value of the food.
3. The reaction kinetics that are related to microbial inactivation, enzyme inactivation, chemical change, and physical change subject to heat treatment.
4. The heating temperature and time combination required to inactivate the most heat resistant pathogens and spoilage microorganisms in a particular food.
5. Heat-penetration characteristics in a particular food including the packaging materials.
6. The thermal, surface, and physical properties of the food and the container (for packaged foods in hermetically sealed containers).
7. Heat transfers that produce the optimal conditions (based on thermal death curves and a sufficient margin of safety) that are required by (4) and (5).

The rate at which microorganisms are killed at high temperature is, of course, a very important study critical to thermal processing of foods. Different microorganisms have inherently different resistances to temperature, in that vegetative cells and yeast are generally susceptible while endospores are much more resistant, with virus between these two extremes. The medium that surrounds the microorganisms also has an extremely large influence, especially pH, water activity, concentration, and type of food components (3). The importance of pH is its link to the death of vegetative cells or the ability of spores to germinate and grow. The pH classification is of importance to the canning industry since *Clostridium botulinum*, a highly heat resistant and deadly bacterium, will not grow in foods at pH 4.6 or below. The pH 4.6 is the demarcation line between low-acid foods and acid foods (2).

The type of food to be thermally treated will have associated microorganisms with a high thermal resistance. These microorganisms must be inactivated to ensure the safety of consumers. For example, in canning operations, it is *Clostridium botulinum* that should be targeted to eliminate to the extent that it will not pose a health threat to consumers. Often, the other microorganisms that co-exist with the targeted microorganism are also destroyed, along with deleterious enzymes in the food. In pasteurization of liquid foods or solid-liquid mixtures, the thermal treatment is mild and does not destroy the entire populations of the targeted microorganisms due to the concern with sensory quality of the processed food. It is imperative that pasteurized foods must be refrigerated after thermal processing and preserved with packaging or/and chemical preservatives.

The death of microorganisms at constant elevated temperature follows a first-order reaction kinetic, i.e.,

$$\frac{dN}{dt} = -kN \tag{110.1}$$

which can be integrated to give

$$\mathrm{N/N_0} = 10^{-\mathrm{t/D}} \tag{110.2}$$

Where D is the decimal reduction time, which is defined as the time necessary for a 90% reduction in the microbial population. N_0 is the initial microbial population and k is the rate constant.

Bigelow (4) showed a linear relationship between the logarithm of the decimal reduction time (also called D-value) for spores and their temperatures. Mathematically, Bigelow's model can be expressed as

$$\frac{D_1}{D_2} = 10^{(T_2-T_1)/z} \tag{110.3}$$

where z is a constant over the temperature range for a particular microorganism. This so-called z-value is defined as the increase in temperature necessary to cause a 90% reduction in the decimal reduction time D. Another model, with similar fashion, defines a Q_{10} value as the ratio of decimal reduction time values at a temperature interval of 10°C, i.e.,

$$Q_{10} = \frac{D_T}{D_{T+10}} = 10^{10/z} \tag{110.4}$$

The most commonly used criterion for commercial sterilization is the F value (5). It is defined as

$$\mathrm{F} = \int_0^\infty \mathrm{L}\ \mathrm{dt} = \int_0^\infty 10^{(\mathrm{T}-\mathrm{T_{ref}})/z}\ \mathrm{dt} \tag{110.5}$$

where L is the lethality and T_{ref} is a reference temperature. The reference temperature should always be close to the range of temperatures normally used in that process (3). For commercial thermal processes, the determined F value is always larger than F_0 (reference thermal death time, defined as the time for a given reduction in population of a microbial spore with a z value of 10°C at 121°C).

Once the information with respect to thermal resistance characteristics of the test microorganism (z and F_0) is known, the temperature history of the product going through the process should be obtained before the thermal processing schedule is formulated. This leads to the development of a heat penetration curve. For detailed information regarding heat penetration curves of packaged

foods and F-values related to processing packaged foods, the reader is referred to the chapter titled, "Thermal Processing of Packaged Foods" by Chung, Papadakis and Yam in this handbook.

II. STEADY STATE HEAT CONDUCTION: FOURIER'S FIRST LAW

Thermal processing involves heat transfer between heating sources and foods. Heat transfer is the movement of thermal energy from one point to another driven by a difference in temperature. When heat transfers between adjacent materials due to temperature difference, the heat transfer mode in this case is called heat conduction. Fourier's law of heat conduction prescribes the mechanism of heat conduction. Fourier's first law of heat conduction can be loosely interpreted as follows, the rate of heat flow between point A of temperature T_1 and point B of temperature T_2 under *steady state conditions* over a distance of y is proportional to the area perpendicular to heat flow, and $(T_1 - T_2)$. The constant of proportionality is termed thermal conductivity of the medium between points A and B, k. It can be mathematically expressed as

$$Q = k\,A\,\frac{T_1 - T_2}{y} \quad \text{or} \quad q = \frac{Q}{A} = -k\frac{\Delta T}{y} \tag{110.6}$$

where q is the heat flux (unit: kJ/m^2 s). However, strictly speaking, a one-dimensional form of Fourier's first law of heat conduction should be

$$q = -k\frac{dT}{dy} \tag{110.7}$$

This equation often serves as the definition of k (6). If the temperature varies in all directions, Fourier's first law of heat conduction in a Cartesian coordinate system would be expressed as

$$q_y = -k_y\frac{\partial T}{\partial y}, \quad q_x = -k_x\frac{\partial T}{\partial x}, \quad q_z = -k_z\frac{\partial T}{\partial z} \tag{110.8}$$

In an isotropic media of heat transport, $k_x = k_y = k_z = k$. For a cylindrical coordinate system (r, θ, z), Fourier's first law of heat conduction may be expressed as

$$q_z = -k\,\frac{\partial T}{\partial z}, q_r = -k\frac{\partial T}{\partial r}, q_z = -k\frac{1}{r}\frac{\partial T}{\partial \theta} \tag{110.9}$$

While in a spherical coordinates (r, θ, φ), the equations would look like

$$q_\phi = -k\frac{1}{r\sin\theta}\,\frac{\partial T}{\partial \phi}, \quad q_r = -k\frac{\partial T}{\partial r}, \quad q_z = -k\frac{1}{r}\frac{\partial T}{\partial \theta} \tag{110.10}$$

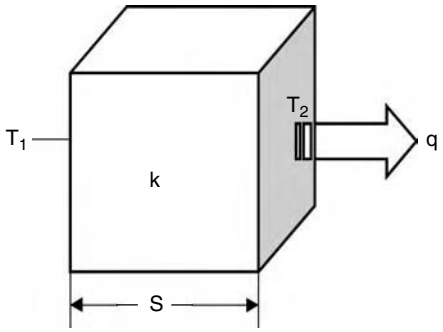

FIGURE 110.1 Schematic diagram of conductive heat transfer in a rectangular slab under steady state conditions.

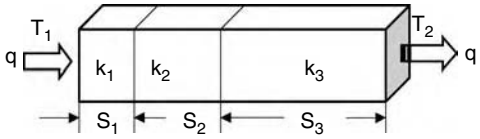

FIGURE 110.2 Schematic diagram of conductive heat transfer in a multilayered rectangular composite slab under steady state conditions.

The heat conduction across a slab under steady state conditions in a Cartesian coordinate system is expressed as (see Figure 110.1)

$$q = k\frac{T_1 - T_2}{S} \tag{110.11}$$

where S is the distance in the direction of heat conduction. The term, k/y, in Equation (110.6) is actually a heat transfer coefficient for heat conduction; the temperature difference $(T_1 - T_2)$ is the driving force. Analogous to an electrical circuit or mass transfer from the bulk of one phase across the interface into the other phase, the reciprocal of the heat transfer coefficient would be the heat conduction resistance. For the case of steady-state conductive heat transfer through a composite system made of several materials of different thermal conductivities and thickness as shown in Figure 110.2, the following resistance-in-series relationship should hold:

$$\frac{S}{k} = \frac{S_1}{k_1} + \frac{S_2}{k_2} + \frac{S_3}{k_3} + \cdots + \frac{S_n}{k_n} \tag{110.12}$$

or

$$R = R_1 + R_2 + R_3 + \cdots + R_n \tag{110.13}$$

where R signifies "resistance." The heat flux through a multiply-layer system (e.g., packaged food or composite wall of a cold room) may be expressed as

$$q = (T_1 - T_2)/R \tag{110.14}$$

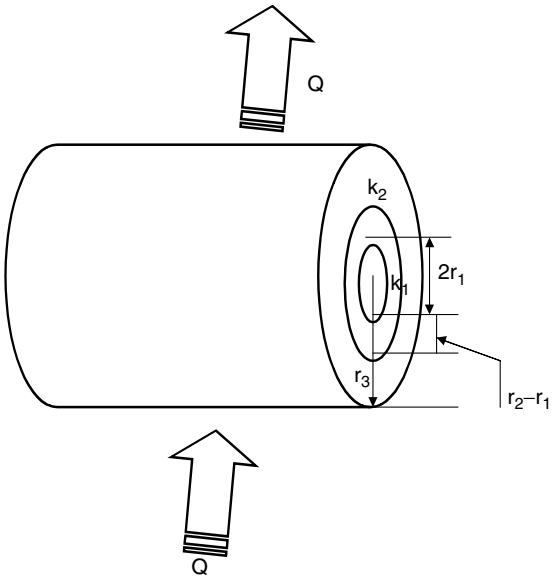

FIGURE 110.3 Schematic diagram of conductive heat transfer in a multilayered composite cylindrical object under steady state conditions.

For composite cylindrical tubes (Figure 110.3) made of multiple layers of different materials, the conductive heat transfer rate (not flux) in the radial direction under steady state conditions can be expressed as

$$Q = (T_1 - T_2)/R \tag{110.15}$$

Where

$$R = R_1 + R_2 + R_3 + \cdots + R_n = \frac{\ln(r_2/r_1)}{2\pi L k_1} + \frac{\ln(r_3/r_2)}{2\pi L k_2} + \frac{\ln(r_4/r_3)}{2\pi L k_3} + \cdots + \frac{\ln(r_{n+1}/r_n)}{2\pi L k_n} \tag{110.16}$$

Heat conduction in a multilayered sphere with spherical hollow core (Figure 110.4) can be written as

$$Q = (T_1 - T_2)/R \tag{110.17}$$

Where

$$R = R_1 + R_2 + R_3 + \cdots + R_n = \frac{r_2 - r_1}{4\pi k r_1 r_2} + \frac{r_3 - r_2}{4\pi k r_3 r_2} + \frac{r_4 - r_3}{4\pi k r_3 r_4} + \cdots + \frac{r_{n+1} - r_n}{4\pi k r_n r_{n+1}} \tag{110.18}$$

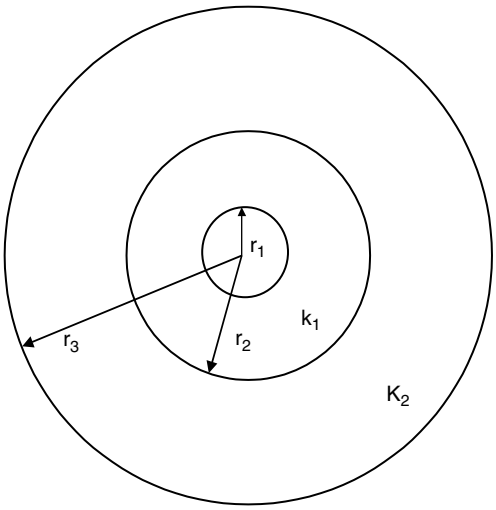

FIGURE 110.4 Schematic diagram of conductive heat transfer in a multilayered composite sphere under steady state conditions.

The values of heat conductivities commonly encountered vary considerably in magnitude. For example, thermal conductivities of metals range from 50 to 400 W/(m°C); alloys 10–120 W/(m°C); water 0.597 W/(m°C) at 20°C; air 0.0251 W/(m°C) at 20°C; insulating materials 0.035–0.173 W/(m°C) (Singh and Heldman, 2001). Many high-moisture food products have thermal conductivities close to that of water and the thermal conductivity values of dried, porous foods are highly affected by the presence of air with its low value (similar to the case of insulating materials). Several publications (7–9) provide tabulated thermal conductivity values of a number of common foods. Alternatively, empirical correlations of thermal conductivities may also be used to estimate the values in heat transfer process calculations where temperature is not constant and/or the values of the temperature entry cannot be found in the tables. Sweat (10) proposed a predictive equation of thermal conductivity for fruits and vegetables (little or no proteins) with moisture content exceeding 60%:

$$k = 0.148 + 0.493X_w \tag{110.19}$$

where X_w is water content expressed as a mass fraction. This equation is able to predict thermal conductivity values within ±15% of experimental data. It is not suitable for foods with low density or/and void spaces such as apples since the values of thermal conductivity of these types of foods would be underestimated using the equation. The reader is referred to the published experimental data on these foods (7–9). Sweat (11) also proposed a predictive equation for meats and fishes

TABLE 110.1
Coefficients of k_i and ρ_i in Equation (110.22) (Ref. 13)

Thermal Conductivity (W/m K)	Density (kg/m^3)
$k_w = 0.57109 + 0.0017625T - 6.7306 \times 10^{-6} T^2$	$\rho_w = 997.18 + 0.0031439T - 0.0037574T^2$
$k_{ic} = 2.2196 - 0.0062489T + 1.0154 \times 10^{-4} T^2$	$\rho_{ic} = 916.89 - 0.13071T$
$k_p = 0.1788 + 0.0011958T - 2.7178 \times 10^{-6} T^2$	$\rho_p = 1329.9 - 0.51814T$
$k_f = 0.1807 - 0.0027604T - 1.7749 \times 10^{-7} T^2$	$\rho_f = 925.59 - 0.41757T$
$k_c = 0.2014 + 0.0013874T - 4.3312 \times 10^{-6} T^2$	$\rho_c = 1599.1 - 0.31046T$
$k_{fi} = 0.18331 + 0.0012497T - 3.1683 \times 10^{-6} T^2$	$\rho_{fi} = 1311.5 - 0.36589T$
$k_a = 0.3296 + 0.001401T - 2.9069 \times 10^{-6} T^2$	$\rho_a = 2423.8 - 0.28063T$

(no carbohydrates) with moisture content ranging from 60–80% between 0–60°C:

$$k = 0.08 + 0.52X_w \tag{110.20}$$

Equations (110.19) and (110.20) ignore the factor of composition of food solids in estimating thermal conductivities, thus limiting their usefulness to high moisture content foods where food solids have much lower values of thermal conductivities. The compositions of most foods in food industry are more complex and therefore an empirical equation of predicting thermal conductivities that accounts for food composition is desirable. Sweat (12) developed the following equation for calculating thermal conductivities of foods at 25°C:

$$k = 0.25X_c + 0.155X_p + 0.16X_f + 0.135X_a + 0.58X_w \tag{110.21}$$

where X is the mass fraction and subscripts c, p, f, a, and w stand for carbohydrate, protein, fat, ash, and water, respectively. The coefficients in Equation (110.21) are the values of the thermal conductivities for carbohydrate, protein, fat, ash, and water. The thermal conductivity of pure water at 25°C is actually 0.606 W/(m°C). The value of 0.58 W/(m°C) betrays the fact that not all water in a food matrix is free water. Notice Equation (110.21) does not include a term for contribution from the thermal conductivity of fiber.

The equations proposed by Sweat (10–12) do not address the issue of change in thermal conductivity due to temperature change. Choi and Okos (13) have postulated the following expression for liquid foods based on the food composition and temperature:

$$k = \sum_{i=1}^{n} k_i Y_i \tag{110.22}$$

The volume fraction Y_i of each component is calculated from the mass fraction, X_i, and the individual densities, ρ_i:

$$Y_i = \frac{X_i/\rho_i}{\sum_{i=1}^{n} (X_i/\rho_i)} \tag{110.23}$$

TABLE 110.2
Temperature Functions of c_{pi} in Equation (110.28) (Ref. 13)

Major Components	Temperature Functions (kJ/kg °C)
Fat	$c_p = 1.9842 + 1.4733 \times 10^{-3}T - 4.8008 \times 10^{-6}T^2$
Carbohydrate	$c_p = 1.5488 + 1.9625 \times 10^{-3}T - 5.9399 \times 10^{-6}T^2$
Fiber	$c_p = 1.8459 + 1.8306 \times 10^{-3}T - 4.6509 \times 10^{-6}T^2$
Ash	$c_p = 1.0926 + 1.8896 \times 10^{-3}T - 3.6817 \times 10^{-6}T^2$
Protein	$c_p = 2.0082 + 1.2089 \times 10^{-3}T - 1.3129 \times 10^{-6}T^2$

where X_i is the mass fraction and ρ_i is the density of *i*th component.

The thermal conductivities in W/(m °C) and individual densities in kg/m^3 of water, ice, protein, fat, carbohydrate, fiber, and ash are computed as functions of temperature in °C and with first letter of each component name as subscript, shown in Tables 110.1 and 110.2.

While thermal conductivity, the rate of heat conduction through a unit cross-sectional area when a unit temperature difference is maintained over a unit distance, describes how fast heat can penetrate through foods and packaging materials, it does not directly give out information on thermal energy requirement for heating certain amounts of foods to prescribed temperature levels. For that aspect of thermal calculations and analyses, a thermodynamic property, specific heat, defined as a measure of energy required by a unit mass of food to raise a unit degree temperature without a change in state, is more practical. Specific heat values of foods can be experimentally obtained through several methods (13). They can also be estimated by several correlations. Dickerson (14) developed the following equation for calculating specific heat of high-moisture content foods in which the influence of the food composition and temperature on c_p is not strong is:

$$c_p = 1.675 + 0.025X_w \tag{110.24}$$

where X_w is the water content expressed as water mass fraction. If the food composition has to be taken into

account for calculations of specific heat, the expression from Charm (15) is used in the following form:

$$c_p = 2.093X_f + 1.256X_s + 4.187X_w \qquad (110.25)$$

The X is the mass fraction of each component. The subscripts, f, s, and w, represent fat, non-fat solids, and water respectively. The coefficients of Equation (110.25) are actually the specific heat values for fat, solids, and water at 70°C. Heldman and Singh (16) expanded the approach used by Charm (15) to include specific heat values of more basic components into the following equation:

$$c_p = 1.424X_c + 1.549X_p + 1.675X_f + 0.837X_a + 4.187X_w \qquad (110.26)$$

where new subscripts, c and a, stand for carbohydrate and ash, respectively. Notice the specific heat value for solid fat is 1.675 kJ/kg°C while the specific heat value for liquid fat is 2.093 kJ/kg°C.

Further expanding Equation (110.26) leads to the following expression:

$$c_p = 1.424X_c + 1.549X_p + 1.675X_f + 0.837X_a + 1.9776X_{fi} + 4.187X_w \qquad (110.27)$$

where X_{fi} is the mass fraction of fiber in the food.

If the temperature dependence of specific heat values is needed, the comprehensive mode proposed by Choi and Okos (13) should be used. Their model is expressed as follows:

$$c_p = \sum_{i=1}^{n} c_{pi} X_i \qquad (110.28)$$

where X_i is the mass fraction of the *i*th component, n is total number of components in a food, and c_{pi} is the specific heat of the *i*th component.

III. CONVECTIVE HEAT TRANSFER

Convective heat transfer is the result of bulk fluid movement in which a temperature gradient exists between the solid surface and surrounding fluid. In any convective heat transfer situation, heat conduction between the bulk fluid and the solid does exist simultaneously but the magnitude of heat conduction is far less than that of convective heat transfer. There is no reason to analyze heat conduction in a convective heat transfer situation. Instead, a heat conduction effect that is associated with convective heat transfer is automatically included in the convective heat transfer analysis in the form of part of a lumped heat transfer parameter called heat transfer coefficient.

It is widely believed that convective heat transfer resistance comes from a thermal boundary layer near the solid surface. The thickness of this "film" epitomizes the resistance to heat transfer as is shown in the equation based on Newton's law of cooling (or heating) as follows:

$$q = \frac{k}{\delta}(T_s - T) = h(T_s - T) \qquad (110.29)$$

where δ is the thickness of the boundary layer or film and T_s is the temperature of the surrounding fluid. Since the thickness of the film is not measurable, the use of the heat transfer coefficient is the commonly preferred method. The heat transfer coefficient, h, however, is not a material property. It depends on physical properties of fluid and solid as well as the hydrodynamic situation in the region where heat transfer takes place. In general, boiling liquids and condensing vapors have very high values of h and gases are poor heat transfer fluids. Steams are often the choice of heating media and the presence of air will reduce the effectiveness of heat transfer of steams (17). Although analytical or numerical treatment of continuity equation, equation of motion and energy equation under steady state, constant density conditions to get temperature profile in the thermal boundary layer (as well as velocity profile in the hydrodynamic boundary layer) is theoretically possible, it is generally neither recommended nor needed. Some classic literature provides some case studies of very simple convective heat transfer situations in the boundary layer (6, 19). Most convective heat transfer calculations involving food thermal processing are carried out using an empirical approach that is based on experimental data in the form of dimensionless number correlations.

There are two modes of convective heat transfer depending on the fluid flow characteristics. Natural (or free) convective heat transfer is due to fluid motion caused by difference in density resulting from temperature difference. If the fluid flow is caused by artificial means such as agitation, pumping, or blowing, the convective heat transfer is named as forced convective heat transfer.

A. Dimensionless Quantities

Nusselt number (N_{Nu}) = hd_c/k = ratio of external convective heat transfer to internal heat conduction.

Prandtl number (N_{Pr}) = $\mu c_p/k$ = ratio of kinematic viscosity to thermal diffusivity.

Reynolds number (N_{Re}) = $\rho uD/\mu$ = ratio of inertial force to viscous force, where D is diameter and u is velocity.

Grashof number (N_{Gr}) = $\frac{(d_c^3 \rho^2 g \beta \Delta T)}{\mu^2}$, where d_c is characteristic length, β is the coefficient of volumetric expansion; ΔT is temperature difference between a solid surface and the surrounding fluid.

Graetz number (N_{Gz}) = $0.25\pi[N_{Re}N_{Pr}(d_c/L)] = \frac{\dot{m}c_p}{kL}$.

B. Dimensionless Number Correlations

Equations for calculating heat transfer coefficients usually take the form of

$$N_{Nu} = f\left(N_{\mathrm{Re}}, N_{\mathrm{Pr}}, \left(\frac{L}{d_c}\right), N_{Gr}, \left(\frac{\mu}{\mu_w}\right)\right) \qquad (110.30)$$

The Grashof number is associated with natural convection and (L/d_c) is related to laminar flow. When calculating these dimensionless numbers, the thermophysical properties of fluids at the arithmetic mean temperature at the entrance and the exit are used. The viscosity of the fluid at the wall (μ_w) is included in the equations to account for the heating process since it affects convective heat transfer. The correlations for forced convective heat transfer can be expressed as a general equation:

$$N_{Nu} = \alpha(N_{\mathrm{Re}})^{\beta}(N_{\mathrm{Pr}})^{\gamma}\left[\frac{L}{d_c}\right]^{\delta} \qquad (110.31)$$

where α, β, γ, and δ are constants from correlation analysis of experimental data and d_c is characteristic diameter (19).

For Newtonian fluids in laminar-flow inside circular pipes, the following equation is used:

$$N_{Nu} = 1.86\left(N_{\mathrm{Re}}N_{\mathrm{Pr}}\frac{d}{L}\right)^{1/3}\left(\frac{\mu}{\mu_w}\right)^{0.14} \qquad (110.32)$$

The characteristic diameter, d_c is the inside diameter of the pipe, d. For fluids flowing outside of but parallel to the pipes, substitute d_e for d in the Reynolds number calculation. The equivalent diameter is defined as

$$d_e = 4\left(\frac{\text{flow area}}{\text{heated perimeter}}\right) \qquad (110.33)$$

This equation can also be used to calculate d_c for noncircular ducts.

For Newtonian fluids in turbulent-flow inside circular pipes and $N_{Re} > 10^5$, $0.7 < N_{Pr} < 700$, and $L/d > 60$, the correlation of N_{Nu} (known as Sieder-Tate equation) is

$$N_{Nu} = 0.023(N_{\mathrm{Re}})^{0.8}(N_{\mathrm{Pr}})^{1/3}\left(\frac{\mu}{\mu_w}\right)^{0.14} \qquad (110.34)$$

For Newtonian fluids flowing around a sphere, the following equation is used:

$$N_{Nu} = 2 + 0.60(N_{\mathrm{Re}})^{0.5}(N_{\mathrm{Pr}})^{1/3} \qquad (110.35)$$

This correlation is useful in predicting convective heat transfer to or from droplets or bubbles. Another correlation that has been proven useful is

$$N_{Nu} = 2 + (0.4(N_{\mathrm{Re}})^{0.5} + 0.06N_{\mathrm{Re}}^{2/3})(N_{\mathrm{Pr}})^{0.4}\left(\frac{\mu}{\mu_w}\right)^{1/4} \qquad (110.36)$$

where the physical properties appearing in N_{Nu} and N_{Re}, and N_{Pr} are evaluated at the approaching stream temperature (20). This correlation is recommended for $3.5 < N_{Re} < 7.6 \times 10^4$, $0.71 < N_{Pr} < 380$, and $1.0 < \mu/\mu_w < 3.2$.

For Newtonian fluids flowing along a flat plate with a sharp edge, oriented parallel to the flow, the local Nusselt number correlation in the laminar region near the leading edge, the following equation is used (18):

$$N_{Nu} = 2\sqrt{\frac{37}{1260}}\,(N_{\mathrm{Re}\,x})^{0.5}(N_{\mathrm{Pr}})^{1/3} \qquad (110.37)$$

in which $N_{Re\,x}$ is the Reynolds number at the point that has a distance of x from the leading edge.

For Newtonian fluids flowing around irregular shaped objects

$$N_{Nu} - N_{Nu,0} = 0.60(N_{\mathrm{Re}})^{0.5}(N_{\mathrm{Pr}})^{1/3} \qquad (110.38)$$

in which $N_{Nu,0}$ is the mean Nusselt number at zero Reynolds number.

The Nusselt correlations for natural convection is more complicated than those for forced convection. In general, these correlations can be expressed as

$$N_{Nu} = \alpha(N_{Gr}N_{Pr})^{\beta} \qquad (110.39)$$

where α and β can be evaluated using the table provided by Singh (8). Alternatively, simplified equations for natural convection to air or water in several geometrical shapes and arrangements is formulated by Toledo (19) based on the data from McAdams (21). A similar set of simplified

equations can also be found in Özisik's (22) book. They all have the following form:

$$h = C(\Delta T)^{0.25} \quad \text{or} \quad h = C(\Delta T/L)^{0.25} \qquad (110.40)$$

for laminar flows, and

$$h = C(\Delta T)^{1/3} \quad \text{or} \quad h = C(\Delta T/L)^{1/3} \qquad (110.41)$$

for turbulent flows where L is replaced by d for cylinders.

More heat transfer coefficient correlations can be found in several books (6, 19). For correlations for non-Newtonian fluids, the textbooks by Heldman and Singh (16) and Toledo (19) are recommended.

C. Overall Heat Transfer Resistance

In most heat transfer processes, particularly those involving indirect heating devices such as heat exchangers, there will be more than one resistance to heat transfer. For a simple heat exchanger, there are two convective heat transfers: one inside and one outside, and one conduction: heat transfer across the walls of the tubes in the heat exchanger. Often in situations like that, an overall heat-transfer coefficient, U, is used and can be calculated as:

$$\frac{1}{U} = \frac{1}{h_1} + \frac{1}{h_2} + \frac{S}{K} \qquad (110.42)$$

IV. UNSTEADY STATE HEAT TRANSFER: FOURIER'S SECOND LAW

When heat transfer across a solid is not uniform, i.e., there is a rate of change in temperature with time; this condition is called unsteady state heat transfer. For example, in sterilization processes, temperature histories of heating and cooling are vital to process operators determining the lethal effect of the thermal processing on microbial populations. Fourier's second law of heat transfer guides unsteady state heat transfer and is mathematically expressed as

$$\frac{\partial T}{\partial t} = \frac{k}{\rho c_p}\left[\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right] = \alpha\left[\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right] \qquad (110.43)$$

Equation (110.43) is derived in a Cartesian coordinate system. For cylindrical objects with temperature symmetry in the circumferential direction, Fourier's second law of heat transfer takes the following form:

$$\frac{\partial T}{\partial t} = \alpha\left[\frac{\partial^2 T}{\partial r^2} + \frac{\partial^2 T}{r\partial r^2} + \frac{\partial^2 T}{\partial z^2}\right] \qquad (110.44)$$

For sphere with symmetrical temperature profile, the governing equation becomes

$$\frac{\partial T}{\partial t} = \alpha\left[\frac{\partial^2 (rT)}{r\partial r^2} + \frac{1}{r^2 \sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\,\frac{\partial T}{\partial\theta}\right)\right] \qquad (110.45)$$

In order to solve Equations (110.43), (110.44), and (110.45) to obtain temperature histories of foods, it is important to know the relative importance of heat transfer at the surface and the interior of an object submerging in a heating environment. In many unsteady state heating processes, the food often initially has a uniform low temperature. Upon immersing in the heating fluid, the heat transfer to the food object from the fluid will experience two types of resistances: convective heat resistance and conductive resistance. The ratio of these two resistances, called Biot number, is defined as

$$N_{Bi} = \frac{\text{internal conductive resistance within } \textit{the} \text{ body}}{\text{external convective resistance at the surface}} = \frac{hd_c}{k} \qquad (110.46)$$

For the case of negligible internal resistance to heat transfer ($N_{Bi} < 0.1$) where the shape and size of food object are irrelevant, the temperature history is expressed as

$$\frac{T - T_m}{T_i - T_m} = \exp\left(\frac{-hA}{\rho c_p V}\right)t = \exp(-N_{Bi}N_{Fo}) \qquad (110.47)$$

where T_m is the temperature of the heating fluid. T_i is the initial temperature of the food object. N_{Fo} is Fourier number, a dimensionless number that is equal to $\alpha t/d_c^2$.

For the case of negligible internal resistance to heat transfer ($N_{Bi} > 40$) where the shape and size of food object are relevant, the temperature history of an infinite slab is expressed as

$$\frac{T - T_m}{T_i - T_m} = \sum_{n=1}^{\infty}\frac{2}{C_n}(-1)^{n+1}\cos\frac{C_n x}{d_c}\exp\left(-C_n^2\frac{\alpha t}{d_c^2}\right) \qquad (110.48)$$

where x is the variable distance from the central axis, d_c is the half-thickness of the slab, and C_n is $(2n - 1)\pi/2$. Note that Equation (110.48) is obtained by solving Equation (110.43) for an infinite slab (one-dimensional conduction through the thickness of the slab).

In cylindrical coordinates,

$$\frac{T - T_m}{T_i - T_m} = \sum_{n=1}^{\infty}\frac{2}{C_n J_1(C_n)}J_0\left(\frac{C_n r}{d_c}\right)\exp\left(-C_n^2\frac{\alpha t}{d_c^2}\right) \qquad (110.49)$$

where r is the variable distance from the central axis and d_c is the radius of a cylinder.

$$J_0(C_n) = 0 \tag{110.50}$$

Note that Equation (110.49) is obtained by solving Equation (110.44) for an infinite cylinder (one-dimensional conduction in the radial direction).

In spherical coordinates,

$$\frac{T - T_m}{T_i - T_m} = \sum_{n=1}^{\infty} 2(-1)^{n+1} \frac{d_c}{rC_n} \sin\frac{C_n x}{d_c} \exp\left(-C_n^2 \frac{\alpha t}{d_c^2}\right) \tag{110.51}$$

where d_c is the radius of a sphere and r is the variable distance from the central axis. $C_n = n\pi$.

Note that Equation (110.52) is obtained by solving Equation (110.45) for a sphere (one-dimensional conduction in the radial direction).

For the case of finite internal and external resistances to heat transfer ($0.1 < N_{Bi} < 40$) where the shape and size of food object are relevant, the temperature history of an infinite slab is expressed as

$$\frac{T - T_m}{T_i - T_m} = \sum_{n=1}^{\infty} \frac{2\sin C_n}{C_n + \sin C_n \cos C_n} \cos\frac{C_n x}{d_c} \times \exp\left(-C_n^2 \frac{\alpha t}{d_c^2}\right) \tag{110.52}$$

where d_c is the half-thickness of an infinite slab and x is the variable distance from the central axis.

$$C_n \tan C_n = hd_c/k \tag{110.53}$$

In cylindrical coordinates,

$$\frac{T - T_m}{T_i - T_m} = \sum_{n=1}^{\infty} \frac{2}{C_n J_1(C_n)} J_0\left(\frac{C_n r}{d_c}\right) \exp\left(-C_n^2 \frac{\alpha t}{d_c^2}\right) \tag{110.54}$$

This expression is identical to Equation (110.49).

In spherical coordinates,

$$\frac{T - T_m}{T_i - T_m} = \sum_{n=1}^{\infty} \frac{2\sin C_n - C_n \cos C_n}{C_n - \sin C_n \cos C_n} \frac{d_c}{rC_n} \times \sin\frac{C_n x}{d_c} \exp\left(-C_n^2 \frac{\alpha t}{d_c^2}\right) \tag{110.55}$$

where

$$C_n \cot C_n = 1 - hd_c/kT \tag{110.56}$$

The analytical solutions to the governing equations in Cartesian, cylindrical, and spherical coordinate systems are not difficult to use to obtain temperature histories in this age of super fast computer hardware and sophisticated software. For example, a programmed spreadsheet (such as MS Excel®) will crunch these numbers easily. However, for someone who is uncomfortable with using computer programs, there are simple temperature-time charts available (8, 9, 23, 24). These charts are semi-log plots of temperature distribution vs. Fourier number. The charts in Singh and Heldman (9) cover a wide range of Fourier numbers; however, the transient temperatures in the charts are those in the geometrical centers of infinite slab, infinite cylinder, and sphere. Although the other charts mentioned above cover much narrower range of Fourier numbers, the temperatures in those charts are not limited to the geometrical centers. The general procedure for using these charts is very simple. First one calculates Fourier number and Biot number, then locate these numbers on the chart that is designated for the shape of an object similar to one you are interested in (Fourier number is on the abscissas and Biot number is the slope of the straight lines in the chart). Finally, one finds the corresponding temperature distribution on the coordinate axis.

V. THERMAL PROCESSES

Thermal processes vary greatly in their severity ranging from mild treatment of pasteurization to more severe such as retort processes involving foods packaged in hermetically sealed containers. Commercial sterilization processes can be categorized into two basic modes: conventional canning in retorts and aseptic canning where foods and containers are sterilized separately and assembled later in a sterile environment. A wide range of food products, ranging from less viscous fluids to high-viscosity fluids, can be heat treated. The presence of particles (up to 25 mm in diameter) poses a challenging problem for engineers involved in multiphase heat transfer, as it becomes necessary to at least heat both liquid and solids adequately and, if possible, equally. Alternative technologies, such as ohmic heating and microwave heating may help alleviate some of the problems associated with heating of solid-liquid mixture (25–29). The presence of air in either of phases becomes a problem in thermal processing as it escapes from the fluids when temperature increases. Also air is a very poor heating fluid.

A. RETORT

Retort is a closed vessel or other equipment that is used for thermal sterilization of packaged foods. The heating medium is usually pure saturated steam. Canned foods are not the only type of foods that can be treated to commercial sterility. Retort pouches are also widely processed in retorts.

The type of retort equipment includes discontinuous (batch), non-agitating (still), and vertical or horizontal retorts.

Many factors contributing to the conditions necessary to produce commercially sterile packaged foods, include [1] pH, [2] the storage conditions after thermal treatment and expected shelf-life, [3] the heat resistance of the targeted microorganism(s), [4] the heat transfer characteristics of the food, its container, and the heating medium, and [5] the initial load of microorganisms. For packaged low-acid foods, it is *C. botulinum* spores that cause the greatest concern and should be the target for destruction. The modes of heat transfer in thermal processing of foods in retorts could be heat conduction, convective heat transfer, or both, depending on the viscosity of foods in the package and the retort equipment (still or agitating), although conductive heat transfer is probably the predominating heat transfer mode. For low-viscosity foods such as juices, natural convection could arise due to temperature differences in different locations of the package. Many retorts can rotate and therefore forced convection is induced in the package. In the rotating retort, the headspace of the package (such as a can) becomes an important factor to consider when one evaluates the combined effect of natural convection and forced convection. It is common to insert the thermocouple probe in the location that is not the geometrical center of the package but slightly below the center in the retort for measurement of the heat penetration curve since this location is believed to be the coldest spot in the package for low-viscosity foods. The drawback with the thermocouple probe approach is that the probe will disturb the flow patterns, which, in turn, distort the measurement of temperature distribution in the package. Various alternatives were considered and tested. Among them are temperature time integrators (TTI). TTI is designed to replace thermocouple probes and to validate overall process of thermal processing of foods, especially for those containing particulates. A marker is an alternative temperature probe used in TTI, be it a microorganism, or a spore, or an enzyme, or a chemical or a physical marker whose degradation kinetics are first-order, which is stable and has been accurately measured. Markers may be intrinsic (already present in the food) or extrinsic (added to the food) and are immobilized in solid particulates (30). The advent of nanotechnology could revolutionize the TTI approach with chemical markers. Computer simulations of food sterilization in packaged foods are also thriving as the computing power goes up and the cost of computing declines. Many papers have been published in this field (31–35). Commercial computational fluid dynamics (CFD) software package such as FLUENT and PHOENICS is a useful tool available to food engineers and technologists to conduct complex computer simulations (36, 37). The latest approach to predicting temperature histories in retorts is the use of artificial neural network (ANN) coupled with genetic algorithms (GA) based on seminal works by Goldberg (38) and Holland (39). Ramaswamy group of McGill University in Canada has several publications in this area (40).

B. Continuous Heat Processing

Conventional continuous thermal processing of liquid or liquid-particulate foods is intended to pasteurize the foods while minimizes the thermal damage to food quality. Steam or hot water is the common heating medium and the thermal energy is delivered to foods through two means: direct steam heating and indirect heating with steam or hot water using one of several types of heat exchangers.

With direct steam heating, the food is heated to sterilization temperature by mixing it with steam. In the process, some of steam may condense and release latent heat of vaporization. Direct steam heating of foods is fast but the processed product is dilute. The extra water sometimes has to be removed. Regardless the way of direct heating is carried out, either the food is injected to steam or steam is injected to the food; the mathematical modeling is not easy. A thermodynamics approach may be used in calculating energy requirement.

Indirect heating involves using plate or tubular or scraped surface heat exchangers. Each of these types of heat exchangers has their own advantages and disadvantages. Although designs of heat exchangers are many, in principle the only significant difference between them is the direction of flows of the two fluids. In general, there are two designs of heat exchangers commonly used in food industry: cross flow and parallel flow. Parallel flow can be subdivided into concurrent flow and counter-current flow exchangers. Under comparable conditions, more heat is transferred in counter-current exchangers than in

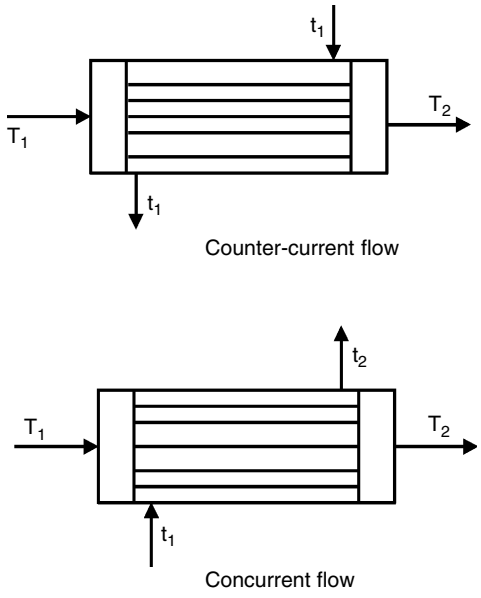

FIGURE 110.5 Schematic diagram of parallel heat exchangers.

concurrent exchangers. Figure 110.5 illustrates the flow arrangement for parallel flow exchangers. There are situations where cross flow exchangers may transfer more heat than counter-current heat exchangers. Parallel flow heat exchangers carry two fluids: one hot and one cold. Heat is transferred to the cool fluid primarily through forced convection.

The rate of heat transfer between the two fluids is

$$Q = UA(\Delta T)_{lm} \tag{110.57}$$

where

$$(\Delta T)_{lm} = \frac{(\Delta T)_2 - (\Delta T)_1}{\ln(\Delta T)_2 - \ln(\Delta T)_1} \tag{110.58}$$

where (ΔT) is the temperature difference at each end of the exchanger and $(\Delta T)_{lm}$ is called the log mean temperature difference.

Scraped surface heat exchangers are often used in heating high-viscous foods or foods containing large particulates (often non-Newtonian fluids). Singh (8) provided the following Nusselt number equation based on Higbie's penetration theory for calculating convective heat transfer coefficient:

$$N_{Nu} = 2\pi^{-0.5}(N_{Re}\,N_{Pr}\,n)^{1/2} \tag{110.59}$$

where n is the fluid behavior index for non-Newtonian fluids.

Plate heat exchangers are commonly used in heating applications in the dairy and food beverage industry. It consists of a series of plates clamped together on a frame. Channels are formed between the plates. The food product and heat transfer medium flow through alternate channels. Because of the narrow channels, plate heat exchangers can be designed to give a high level of induced turbulence in the flowing streams of the food and heating medium. This arrangement along with the small channel size between the plates gives high heat transfer coefficients (3). The plate heat exchangers are compact for any given heating loads. The downside of the design is the narrow channels preclude the flow of high-viscous fluids and liquids containing particulates.

Tubular heat exchangers can be divided into two groups: concentric tubes and shell-and-tube exchangers. Concentric tube type of heat exchangers consists of one pipe inside another. The walls of the inner pipe form the heat transfer surface. Shell-and-tube heat exchangers consist of a bundle of tubes enclosed by a shell. The arrangement allows for one tube pass or multiple passes for the food. In a one-pass arrangement, the product stream enters at one end and exits at the opposite end. In a multiple-pass arrangement, the product stream may flow back and forth through different tubes with each pass before finally exiting the heat exchanger. Tubular heat exchangers can do the jobs that plate heat exchangers cannot. Relatively large tubes of tubular heat exchangers can handle highly viscous fluids or solid-liquid mixtures with ease. But the heat transfer area per unit volume of tubular heat exchangers is smaller than that of the plate exchangers and therefore the heat transfer rate is slower.

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111 The Engineering Aspects of Deep-Fat Frying

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CONTENTS

I. INTRODUCTION

Deep-fat frying is one of the oldest processes of food preparation. For decades, consumers have desired deep-fat fried products because of their unique flavor-texture combination, ranging from potato chips, French fries, doughnuts, extruded snacks, fish sticks, and the traditional fried chicken products.

Frying is the process of cooking foods using oil as the heating medium. In deep-fat frying, the food is immersed in the oil, which should be enough to cover the food by at least 2 cm. The objective of deep-fat frying is to seal the food by immersing it in hot oil so that all the flavors and juices are retained in a crisp crust.

A. Deep-Fat Frying Systems

The processes used to fry food products can be divided into two broad categories: (1) those that are static and smaller (whose capacity can range from 8 L–28 L of oil), classified as batch fryers used in the catering restaurants; and (2) those that fry large amounts of products in a moving bed, used in the food industry, classified as continuous fryers (having a throughput that varies from 250 kg product hr^{-1} – 25,000 kg product hr^{-1}). These fryers can operate at atmospheric and vacuum and high pressure conditions.

Deep-fat frying of foods is usually performed at high temperature (around 190°C) under atmospheric pressure. Excessive darkening or scorching of the product may occur even before the product is completely cooked. In addition, some of the oil decomposition products have been implicated in producing adverse health effects when fried oils degraded with continued use (Taylor et al., 1983).

High-pressure is another way of producing deep-fat products. Pressure fryers were developed primarily to be used in the food service industry for chicken frying. The time required to fry chicken parts is faster in a pressure fryer than in atmospheric (open) fryers and the food is more moist and uniform in color and appearance. The process increases the boiling points of frying oil and moisture in the food, thus increasing the rate of heat transfer to the

interior of the food and reducing the frying time. The result is a moist and flavorful food product. Pressure batch fryers can be electric or gas fired and are available in many sizes. Oil capacity can range from 11 L to 25 L and food capacity from 5.6 kg to 10.9 kg. The operating temperature of pressure fryers can vary from 160°C to 177°C and the frying time from 7 to 10 min. Two pressure ranges are used, some fryers employ 9–14 psi and others 28–32 psi (Moreira et al., 1999). Some disadvantages of pressure fryers are that the frying oil will degrade faster because steam from the food is retained within the fryers thus increasing buildup of free fatty acids. The oil must be filtered more frequently because bread and crumbs can burn or oxidize causing off flavors (Lawson, 1985).

In vacuum frying operations, food is heated under reduced pressure (<60 Torr) in a closed system. In these systems, the boiling point of the oil and the moisture in the foods are lowered. Some of the advantages include: (1) water in the product can be rapidly removed; (2) natural color and flavors of the product can be preserved due to the low temperature and oxygen content during the process; and (3) vacuum frying has less adverse effects on oil quality (Shyu et al., 1998). In addition, under vacuum, food products can be fried at much lower temperature (around 110°C) and yield products with lower oil content (e.g., potato chips) and same texture and less darkness than those fried at traditional atmospheric fryers (170–190°C) (Garayo and Moreira, 2002).

Continuous vacuum frying was a concept developed by Florigo (H&H Industry Systems B.V., The Netherlands) in the early 1970s to produce high quality French fries. Due to the improvement in quality of the raw materials and blanching techniques, the use of vacuum fryers almost died out with the exception of one or two production companies who still insist in producing a non-blanched product. Today, the Florigo automatic continuous vacuum fryers are used mainly to produce fruit-chips and very delicate snack products.

French fries processed in a vacuum fryer can achieve the necessary degree of dehydration without excessive darkening or scorching of the product. With the natural preoccupation with lowering the fat contents of diets, vacuum frying can be an efficient way of reducing the oil content in fried snacks. In addition, vacuum fryer can be an alternative to produce dehydrated fruits and vegetables with high quality in a short time.

II. FRYING OIL CHARACTERISTICS

During deep-frying, fats and oils are repeatedly used at elevated temperatures in the presence of atmospheric oxygen and receive maximum oxidative and thermal abuse. Heating in the presence of air causes partial conversion of fats and oils to volatile chain-scission products, nonvolatile oxidized derivatives, and dimeric, polymeric, or cyclic substances. By affecting the properties of the heat transfer medium (cooking oil), deep-fat frying in turn affects the quality of the fried food. Chemical and physical changes in the oil can prolong the process time, increase the total amount of oil in the product, induce toxicity, and lower food nutritional value.

The term *fat* is used to refer to animal-origin fat while *oil* is used for plant-origin fat. Fat is one of the body's basic nutrients, providing energy by furnishing calories. It is composed primarily of triglycerides, which are three fatty acid molecules joined by a *glycerol* backbone (Stockwell, 1988). There are several fatty acids, but only about eleven are common.

Glycerol can combine with these fatty acids through esterification to form mono-, di-, and *triglycerides.* The characteristics of a particular fat or oil and therefore its frying properties are dictated by the actual fatty acids that are present in the individual triglyceride molecules. Some of these component fatty acids are longer or shorter in chain length than others.

The various fatty acids are placed in groups to reflect their functionality in products (and in the human body). If a fatty acid is composed of a chain of carbon atoms with no double bonds, it is said to be *saturated*. These fatty acids are generally solid at room temperature. It is possible for the double bonds to occur in different places. In the case of fatty acid with two double bonds, they can be sited close to each other or far apart. The closer they are together, the more unstable the molecule will be. The position of the double bonds seems to be more important than the number of them. A molecule with two double bonds close to each other is likely to be more unstable than one with three positioned well apart from each other (Stockwell, 1988).

From these descriptions, it should be clear that even with only a few types of fatty acids, a larger number of different triglycerides are possible. With eleven distinct fatty acids that occur commonly in nature, over 1300 different triglycerides can be formed.

Unsaturated fats are derived primarily from plants and are liquid (in the form of an oil) at room temperature. Generally speaking, oils are composed (in varying percentages) of both monounsaturated and polyunsaturated fats. In general, the more double bonds in a fatty acid and the longer its carbon chain length, the lower the temperature at which an oil remains liquid. Also, the more double bonds, the more unstable the oil is and the more likely it is to undergo various forms of degradation (Stockwell, 1988). Thus, there is a chemical basis for the trade off between stability (polyunsaturated oils are less stable) and health benefits (polyunsaturated oils are considered to be more healthy).

The three most widely used oils that are high in monounsaturates are olive oil, canola oil, and peanut oil. Polyunsaturated fats are also considered relatively healthy and include the following, ranked in order, most to least, of polyunsaturates: safflower oil, soybean oil, corn oil,

and sesame oil (Stockwell, 1988). Soybean, safflower, sunflower, and canola oils are always partially hydrogenated before being used for frying to increase their stability. Cottonseed, corn, peanut, and olive oils are used as a stable source of polyunsaturated fatty acids because of their low linolenic acid content (Pavel, 1985). Other oils such as jojoba oil have also been tested for frying (Clarke and Yermanos, 1980; Saguy et al., 1996).

III. PRODUCT CHANGES DURING DEEP-FAT FRYING

Fried products absorb oil during frying and as they cool which contributes to a high fat and high calorie product (Moreira et al., 1999). Oil content in fried foods has been related to initial moisture content (Gamble et al., 1987; Moreira et al., 1995), pre-frying treatment (Gamble et al., 1987; Moreira et al., 1997), structural changes during baking (Lee, 1991; McDonough et al., 1993; Rock-Dudley, 1993), and cooling time (Sun and Moreira, 1994). Bulk density decreases, and porosity and oil uptake increase with frying time during frying of tortilla chips (Kawas and Moreira, 2000).

A. Shrinkage, Porosity, and Texture Changes

In general, fried products will shrink during frying, becoming more porous and crispy after frying.

Tortilla chips, for example, decrease in diameter by about 9%, expand in thickness by 10%, and puff by 100% by the end of frying. The chips become more porous by the end of frying due to the decrease in bulk density caused by the water loss during the process. As frying time increases, the amount of large pores increases filling out the entire chips' structure in a normal distribution. Tortilla chips become harder up to 30 s of frying, and then crunchy until the end of frying (60 s). The chips' crunchiness increases during frying (Kawas and Moreira, 2000).

B. Degree of Starch Gelatinization and Oil Absorption

The degree of starch gelatinization (and therefore the structure) of the product prior frying can affect the mechanism of oil absorption during the process. Starch goes through a series of transformations when heated. Gelatinization is typically defined as a physicochemical phenomenon of swelling of starch granules as they imbibe water. It occurs as the starch system is heated above a gelatinization temperature. Gelatinization is both a physical and a chemical process. The physical process happens when the granules hydrate and swell, and leach of amylose and amylopectin molecules into the solution. The chemical process is performed by water breaking molecular bonds and replacing them with water-polysaccharide hydrogen bonds.

Experiments performed with tortillas prepared with different levels of starch gelatinization before frying showed that the higher the amount of starch gelatinized the lower the oil content after frying (Kawas and Moreira, 2001).

The structure of the chips formed during frying also play an important role in the mechanism of oil absorption (Kawas and Moreira, 2001). Tortillas chips that show higher number of small pores uniformly distributed all over absorb more oil during frying. Little diameter shrinkage and no puffiness contribute to the chips higher oil content, which in turn results in low porosity. These chips tend to be crunchy.

Tortilla chips that produce a structure during frying with few large pores distributed all over the surface tend to shrink and puff significantly resulting in higher oil content at the surface and low at the core. These chips are also less crunchy and very hard.

IV. FACTORS THAT AFFECT OIL ABSORPTION

Several factors affect oil absorption in fried foods, including process conditions (temperature and residence time), initial moisture content of product, raw material composition, slice thickness, pre-frying treatment, degree of starch gelatinization prior to frying, and oil quality.

A. Oil Temperature Effect

During frying, the rapid drying is critical for ensuring the desirable quality of the final product. However, the loss of water results in a substantial absorption of oil by the product. Higher oil temperatures lead to a faster crust formation thus favoring the conditions for oil absorption. As the moisture is reduced with frying time, the ratio of oil content to the amount of water removed becomes independent of the oil temperature indicating that the oil content is not directly related to the oil temperature but to the remaining moisture present in the product.

B. Specific Gravity

Potatoes with high specific gravity ($>$1.10) and dry matter ($>$24.0%) produce chips with lower oil content (Lulai and Orr, 1979). French fries made from high dry matter content raw potatoes (around 24.0%) contain 9% less oil content than those made from potatoes containing lower dry matter content (around 19.5%). Thickness of potato slices is another factor that affects oil uptake in chips. The thicker the potato slice, and thus the smaller surface area relative to the chip volume, the lower will be the chip's volume oil content (Gamble and Rice, 1987).

C. Pre-Frying Treatment

Oil uptake varies with the product pretreatment before frying. In producing French fries, blanching alone does not

reduce significantly oil absorption in these products. Blanching makes the fries' color more uniform, reduces oil uptake by gelatinizing the surface starch, shortens frying time, and improves the fries' texture.

The amount of water in the raw material before frying can affect the oil content of the fried product. Removing some of the water prior to frying can help reduce oil uptake after frying. However, depending on the drying process used, the final product will develop a pore structure that will affect how the absorbed oil will be distributed after frying. Microwave dried potato slices can produce chips with lower oil content than chips that are only fried. This drying process provides chips with heterogeneous moisture distribution structure and with large oil-free zones. In hot-air dried chips, the structure of the fried product shows oil more uniformly distributed around the surface than the fried only chips (which have most of the oil concentrated at the edges). Hot-air dried chips also have less oil content than the microwaved potato chips. On the other hand, freeze-drying will produce oily potato chips after frying. Freeze-dried potato slices have shown no major change in the product's structure during freeze-drying, thus resulting in an even distribution of oil all over its surface after frying (Gamble and Rice, 1988).

Baking is used in the manufacturing of tortilla chips, for example. The objective of baking is to reduce the raw tortilla moisture content from about 55% wet basis (w.b.) to around 40%–38% w.b., to strengthen the product structure and to develop its flavor. During baking, the raw tortilla is exposed to high temperatures (320–420°C) resulting in severe starch gelatinization (>40%) (Gomez et al., 1992). Baking time can produce tortillas with different moisture contents and consequently different degree of starch gelatinization before frying.

D. Particle Size Distribution

Another important factor in oil uptake in fried products is the level of particle size distribution in the dry mass flour. Tortilla chips prepared from finely ground masa showed excessive puffing and pillowing, resulting higher oil absorption and lower porosity; tortilla chips made from coarse particles presented the lowest oil content and no puffing; and intermediate masa produced tortilla chips with final oil content closer to the control sample. The function of the coarse particles is to produce fissures in the product that allow water to escape during frying and reduce oil absorption, and to reduce the extent pillowing during baking and frying (Gomez at al., 1987).

E. Degree of Starch Gelatinized

The degree of starch gelatinization in the product before frying can significantly affect oil absorption after frying. Tortillas containing a high degree of starch gelatinized (80%) before frying yielded chips with lower oil content (14% w.b.) than tortilla chips made with tortillas containing 45% degree of starch gelatinized (23% w.b.) and those made with tortillas having 5% degree of starch gelatinized (35% w.b.). The degree of starch gelatinization affects the oil distribution. The higher the degree starch gelatinized the more oil will be at the chip's surface than at the core. Starch gelatinization and subsequent swelling of the starch granules inhibits oil uptake in these products (Kawas and Moreira, 2001).

F. Oil Quality

Used or poor quality frying oil will make the product look oilier although the total oil content will be the same as those fried in fresh oil. Most of the oil absorbed by chips fried in degraded oil is concentrated on the surface. The higher viscosity of the degraded oil could cause the oil to adhere to the product's surfaces thus making it more difficult for the oil to be drained off from the chip's surface during cooling (Tseng et al., 1996).

V. HEAT TRANSFER AND THE MECHANISM OF OIL ABSORPTION

Heat transfer in deep-fat frying of foods is coupled to the transport of mass. Heat is transferred from the hot oil to the product surface by convection and from the surface to the center of the chip by conduction. The liquid water moves from the inside of the chips to the evaporation zone, leaving the surface as vapor. The simultaneous aspect of both heat and mass transfer in addition to the fact that the physical properties of the material vary with changing temperature and moisture content make theoretical treatment more complicated. Many models have been developed to predict the frying process, and the reader is referred to Moreira et al. (1999) and Yamsaengsung and Moreira (2002a,b) for detail descriptions of these models.

Moreira et al. (1997) showed that, during frying, only a small amount of oil is absorbed (20 percent of the total final oil content) during the first 0–5 s and remains constant until the end of the process (60 s). It was shown that for tortilla chips (Moreira et al., 1995) starch gelatinizes, moisture evaporates, the crust develops, and the capillary pores expand during frying. During the post-frying period, the interfacial tension between oil and gas increases as temperature decreases resulting in an increasing capillary pressure which causes the surface oil to imbibe into the porous chip, thus increasing its internal oil content.

Therefore, the frying process of potato or tortilla chips, for example, can be divided into three distinct stages:

1. **Pre-Frying**: This stage is characterized by the raw material conditions before frying. Oil content is zero at this stage. The product physical

properties of importance are the initial moisture content, initial porosity, and density of each component of the product (solid, water, air, oil).

2. **Frying**: The product's properties change during this stage. Moisture content is reduced, the physical properties such as thermal conductivity, specific heat, and bulk density decrease, porosity increases as a result of water evaporation, and the product's thickness increases as a result of puffing. Only 20 percent of the total oil is absorbed during this stage.
3. **Post-Frying (Cooling)**: This period starts immediately after frying, when the product is removed from the fryer. The temperature inside the chip decreases until it reaches the ambient (or cooling) temperature. The water content is constant, and the physical properties, with exception of the bulk density, remain essentially the same. Most of the oil is absorbed during this period.

The most important oil absorption process conditions are product initial moisture content and cooling air temperature. Surface tension and pore radius are also important but their effects on final oil content of fried products are minor.

In conclusion, we can say that deep-fat frying is a complex process that involves heat and mass transfer mechanisms and a variety of physical and chemical changes in both food and frying oil. Understanding the mechanism of oil uptake is crucial to product quality control since oil content is one of the most important quality factors of fried foods in deep-fat frying processes.

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Part M

Freezing

112 Principles of Food Refrigeration and Freezing

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CONTENTS

I. INTRODUCTION

Developments in frozen storage in the 18th century established the international food market. Further developments in temperature control for chilled products have led to the rapid expansion of the "fresh" food market. Refrigeration is the most common form of food preservation and has considerable further potential. The International Institute of Refrigeration (IIR) estimates that 240MT of food is refrigerated throughout the world. They believe that at least a further 1800MT of food would benefit from refrigeration in its production and distribution.

Refrigeration stops or reduces the rate at which changes occur in food. These changes can be biochemical (growth of micro-organisms), physiological (e.g. ripening, senescence and respiration), chemical and enzymatic reactions (e.g. browning reactions, lipid oxidation and pigment degradation) and physical (such as moisture loss). Effective refrigeration produces safe food with a long high quality shelf life.

A. Safety and Shelf Life

The American Food and Drug Administration (FDA) reckons — 'conservatively,' according to one official — that there may be anywhere between 24 and 81 million cases and 10,000 'needless deaths' from food poisoning in the US every year. In England and Wales reported cases of food poisoning have increased nearly five-fold over the last decade. In addition to the human cost the economic loss in terms of working days and medical treatment in the US is between $5 billion and $6 billion. A study of one outbreak of *Salmonella enteritidis* in the UK calculated that it cost the country between £224 and £321 million. Even a superficial examination of the problem reveals that temperature control is the prime factor that determines the safe distribution life of most foods.

Temperature is one of the major factors affecting microbiological growth. Microbiological growth is described in terms of the lag phase and the generation time. When a microorganism is introduced to a particular environment there is a time (the lag phase) in which no increase in numbers is apparent followed by a period when growth occurs. The generation time is a measure of the rate of growth in the latter stage. Microorganisms have an optimum growth temperature at which a particular strain grows most rapidly, i.e. the lag phase and generation time are both at a minimum. They also have a maximum growth temperature above which growth no longer occurs. Above this temperature, one or more of the enzymes essential for growth are inactivated and the cell is considered to be heat-injured. However, in general, unless the temperature is raised to a point substantially above the maximum growth temperature then the injury is not lethal and growth will recommence as the temperature is reduced. Attaining temperatures substantially above the maximum growth temperature are therefore critical during cooking and re-heating operations.

Of most concern during storage, distribution and retail display of food is a third temperature, the minimum growth temperature for a microorganism. As the temperature of an organism is reduced below that for optimum growth then the lag phase and generation time both increase. The minimum growth temperature can be considered to be the highest temperature at which either of the growth criteria, i.e. lag phase and generation time, becomes infinitely long. The minimum growth temperature is not only a function of the particular organism but also the type of food or growth media that is used for the incubation. Although some pathogens can grow at 0°C, or even slightly lower (Table 112.1), from a practical point of view the risks to food safety are considerably reduced if food is maintained below 5°C.

TABLE 112.1
Minimum Growth Temperatures for Pathogens Associated with Red Meats

	Minimum Temperature (°C)
Campylobacter spp.	30
Clostridia perfringens	12
Pathogenic *Escherichia coli* strains	7
Salmonella spp.	5
Listeria monocytogenes	0
Yersinia enterocolitica	−2

Source: (25).

Food may also become microbiologically unacceptable as a result of the growth of spoilage microorganisms. Their growth can produce unacceptable changes in the sensory quality of many foods and their rate of growth is also very temperature dependent. The development of off odours is usually the first sign of putrefaction and in meat it occurs when bacterial levels reach approximately $10^7\,cm^{-2}$ of surface area (1). When bacterial levels have increased a further 10 fold slime begins to appear on the surface and meat received in this condition is usually condemned out of hand. At 0°C beef with average initial contamination levels can be kept for at least 15 days before any off odours can be detected. Every 5°C rise in the storage temperature above 0°C will approximately half the storage time that can be achieved. So from a spoilage point of view, and the underlying economic consequences of extended storage/distribution life, temperature is again a very important factor in food production.

B. Quality Aspects

Microbial safety and spoilage are not the only aspects of food quality that are temperature dependent. The rate of loss of vitamins from fruit and vegetables during storage also depends upon the storage temperature. It is of interest to note that it is not always a case of the lower the better, especially with citrus and tropical fruits. The optimum temperature for oranges is approximately 12°C with the rate of loss increasing at temperatures warmer or colder than this value.

Some foods exhibit particular quality advantages as a result of rapid cooling. In meat the pH starts to fall immediately after slaughter and protein denaturation begins. The result of this denaturation is a pink proteinaceous fluid, commonly called 'drip,' often seen in prepackaged joints. The rate of denaturation is directly related to temperature and it therefore follows that the faster the chilling rate the less the drip. Investigations using pork and beef muscles have shown that rapid rates of chilling can halve the amount

of drip loss (2). Fish passing through rigor mortis above 17°C are to a great extent unusable because the fillets shrink and become tough (3). A relatively short delay of an hour or two before chilling can demonstrably reduce shelf life.

However, chilling has serious effects on the texture of meat if it is carried out rapidly when the meat is still in the pre-rigor condition, that is, before the meat pH has fallen below about 6.2 (4). A phenomenon known as cold shortening occurs, which results in the production of very tough meat after cooking. As 'rules of thumb' cooling to temperatures not below 10°C in 10 hours for beef and lamb (5) and in 5 hours for pork (6) can avoid cold shortening.

The rate of sugar loss (sweetness) in freshly harvested sweet corn is very temperature dependent. After 20 h at 30°C almost 60% of the sweetness is lost compared with 16% at 10°C and less than 4% at 0°C. Prompt cooling is clearly required if this vegetable is to retain its desirable sweetness. Similarly, the ripening of fruit can be controlled by rapid cooling, the rate of ripening declining as temperature is reduced and ceasing below about 4°C (7).

For several fruits and vegetables, exposure to temperatures below a critical limit, but above the initial freezing temperature, may result in chilling injury. Typical symptoms of chilling injury are internal or external browning, superficial spots, failure to ripen, development of off-flavour and off-taste, etc. It is primarily fruits and vegetables from the tropical and subtropical zones that are susceptible to chilling injury; several Mediterranean products are also susceptible (7).

The extent of damage depends on the temperature, duration of exposure and the sensitivity of the fruit and vegetable. Some commodities have high sensitivity, while others have moderate or low sensitivity. For each commodity the critical temperature depends on species and/or variety. In some cases, unripe fruits are more sensitive than ripe fruits.

A final, but important, quality and economic advantage of temperature control is a reduction in weight loss, which results in a higher yield of saleable material. Meat for example, has a high water content and the rate of evaporation depends on the vapour pressure at the surface. Vapour pressure increases with temperature and thus any reduction in the surface temperature will reduce the rate of evaporation. The use of very rapid chilling systems for pork carcasses has been shown to reduce weight by at least 1% when compared with conventional systems (8).

II. REFRIGERATION COLD CHAIN

The complete refrigeration cold chain for a food will contain many of the following unit operations: prechilling preparation, primary or secondary chilling, freezing, thawing, tempering, storage, transportation and display. Storage, transport and display are temperature maintenance operations. Chilling and freezing produce substantial decreases in the mean temperature of the product. Thawing and tempering produce substantial increases. Whilst during the preparatory treatment there can be a range of temperature responses from a large gain to a small decrease in the temperature of the foodstuff.

The initial and final temperatures, and the maximum and minimum desirable rates of temperature reduction during chilling, depend on the food being processed. In all cases the final product temperature must be above the initial freezing point of the food, but in a number of products biological factors dictate higher storage temperatures.

The same fundamental modes of heat transfer govern processes that change and those that maintain food temperatures throughout the cold chain.

A. Fundamental of Heat Transfer

The principle factors, which control the rate of heat and mass transfer during cooling, are independent of the foodstuff. Heat can be lost from the surface of a body by four basic mechanisms: radiation, conduction, evaporation or convection.

To determine the rate of heat transfer (Q_r) by radiation from food the following approximation may be applied:

$$Q_r = \varepsilon A \sigma (T_s^4 - T_e^4), \tag{112.1}$$

where ε is the emissivity and A is the surface area of the food, σ the Stefan-Boltzman constant and T_s and T_e the temperature of the surface and the enclosure respectively. To achieve substantial rates of heat loss by radiation large temperature differences between the product and the enclosure are required. Such differences are not normally present during food cooling operations except in the initial chilling of bakery products.

The rate of one dimensional heat transfer (Q_{cd}) by conduction is given by:

$$Q_{cd} = kA\frac{\partial T}{\partial x}, \tag{112.2}$$

where k is the thermal conductivity of the medium through which the heat is passing and $\partial T/\partial x$ the temperature gradient.

Physical contact between the product and a cooler surface is required to extract heat by conduction. The irregular shape of most foodstuffs precludes this mechanism in many applications. Plate coolers are routinely used to freeze fish and offal, which have soft pliable surfaces. Belt freezers are used for steaks, fish fillets and other thin products. However, the rate at which heat can be conducted away from the surface is not the sole criterion that governs the time taken to cool a product. Heat must also be conducted from within the product to its surface before it can be removed. Most foodstuffs are poor

conductors of heat and this imposes a severe limitation on attainable chilling times for either large individual items or small items cooled in bulk.

The rate of mass transfer (M) from the surface of an unwrapped food is described by the equation:

$$M = mA(P_s a_w - P_m), \quad (112.3)$$

where m is the mass transfer coefficient, A the area, P_s the saturated vapour pressure at the surface, a_w the water activity and P_m the vapour pressure above the food surface. Q_e, the heat loss due to evaporation, can be obtained by multiplying M by the appropriate latent heat. The heat lost in the evaporation of water from the surface of the product is a minor component of the total heat loss for most foodstuffs. In spray cooling systems, the rate of heat removal is enhanced by evaporation from the surface. However, the main advantage of spray cooling is a reduction in weight loss. In a number of specific cases i.e. vacuum cooling, evaporation is the primary cooling agent.

Most food chilling systems rely on convection as the principal means of heat removal. The rate of one dimensional heat transfer (Q_c) by convection is given by:

$$Q_c = h_c A(T_s - T_a), \quad (112.4)$$

where h_c is the convection or film heat transfer coefficient and T_a the temperature of the cooling medium. The most common media are air or water, although salt solutions, sugar brines and other refrigerants have been used. Each combination of product and cooling system can be characterized by a specific surface heat transfer coefficient. The value of the coefficient depends on the shape and surface roughness of the foodstuff and to a much greater degree on the thermophysical properties and velocity of the medium. Values range from 7 for still air to over $500\,W\,m^{-2}\,K^{-1}$ for agitated water.

B. Primary Chilling

1. Chilling Methods for Solid Foodstuffs

a. *Moving air*

This is the most widely used method as it is economical, hygienic and relatively non-corrosive to equipment. Systems range from the most basic in which a fan blows the cooled air around an insulated room, to products on conveyors passing through blast chilling tunnels or spirals. Low rates of heat transfer are attained in air cooling systems, but this is not important if conduction within the product is the rate-controlling factor. A major disadvantage is excessive dehydration from the surface of any unwrapped product, whilst the need to avoid surface freezing limits the lowest air temperature that can be used in a single stage system. In practice, air distribution is a major, often overlooked, problem.

A batch system where warm food is placed in refrigerated rooms is the most common method of chilling. Individual items such as carcasses, tuna or bunches of bananas are hung from rails, smaller products are placed on racks or pallets, and bulk fruits and vegetables in large bins.

Conveying products overcomes problems of uneven air distribution since each item is subjected to the same velocity/time profile. In the simplest continuous systems the food is suspended from an overhead conveyer and moved through a refrigerated room. This process is often used in air chilling of poultry or in the prechilling of pork carcasses. Some small cooked products are continuously chilled on racks of trays, which are pulled or pushed through a chilling tunnel using a simple mechanical system. In more sophisticated plants the racks are conveyed through a chilling tunnel in which the refrigeration capacity and air conditions can be varied throughout the length of the tunnel.

In larger operations, it is more satisfactory to convey the cooked products through a linear tunnel or spiral chiller. Linear tunnels are far simpler constructions than spirals but require more space.

b. *Hydrocooling/immersion cooling*

Hydrocooling is probably the least expensive method of achieving rapid cooling in small products. The product is immersed in, or sprayed with, cool water, either at ambient or near 0°C. Practical systems vary from simple unstirred tanks to plants where the product is conveyed through agitated tanks or under banks of sprays. Such systems are typically used for celery, asparagus, peas, sweet corn, carrots, peaches etc.

Immersion chilling also has application with larger products. Most poultry to be frozen is initially cooled by immersion in chilled water or an ice/water mixture. The procedure is very fast and the birds gain weight during the process. The maximum weight gain is controlled by legislation.

An alternative system to air or immersion is spray chilling. Practical spray chilling systems use a combination of air and sprays for the initial part of the chilling period and then air only for the rest of the chilling cycle. The sprays of cold water at 2–3°C are not applied continuously but in short bursts. The main advantage claimed for these systems is reduced weight loss.

c. *Plate cooling*

With thinner materials a plate cooling system has the potential to halve the cooling time required in an air blast system. Continuous horizontal and rotary plate freezing systems have been produced commercially and could be modified to operate at higher temperatures. They tend to be expensive, especially if automatic loading and unloading is required, but have low running costs. Designs have been produced for continuous belt coolers similar to those used for belt freezing.

d. Ice or ice/water chilling

Chilling with crushed ice or an ice/water mixture is simple, effective and commonly used for fish cooling. The individual fish are packed in boxes between layers of crushed ice, which extract heat from the fish and consequently melt. The temperature of the 'coolant' remains at a constant 0°C until all the ice has turned into water. The process is labour intensive although automatic filling systems have been developed. Cabbage and root crops are also cooled with crushed ice.

e. Vacuum cooling

Solid products having a large surface area to volume ratio and an ability to release internal water are amenable to vacuum cooling. The products are placed in a vacuum chamber and the resultant evaporative cooling removes heat from the food. In general terms, a 5°C reduction in product temperature is achieved for every 1% of water that is evaporated. Pre-wetting is commonly applied to facilitate cooling without loss of weight.

Vacuum cooling is rapid and economical to operate because of low labour costs, but the capital cost of the large vacuum vessels is high. Large amounts of lettuce, celery, cauliflower, green peas and sweet corn are vacuum cooled.

f. Cryogenic cooling

Avoiding surface freezing of the product is the main problem in using liquid nitrogen or solid carbon dioxide for chilling. Continuous chilling systems using liquid nitrogen either immerse the product in the liquid, spray the nitrogen onto the surface or vaporize the nitrogen in a forced draught and pass it over the surface of the foodstuff.

Direct spraying of liquid nitrogen onto a food product, whilst it is conveyed through an insulated tunnel, is the most common method. Surface freezing is still a problem but an extra refrigeration effect is obtained by precooling the food with the cold gas produced by the vaporization.

2. Chilling Methods for Liquid Foodstuffs

The majority of liquid foodstuffs require cooling after a heat processing operation such as cooking, pasteurisation or sterilization. Milk is cooled at the point of collection to maintain its quality; unpasteurized fruit juices are cooled immediately after production, whilst fermented beverages are often cooled during primary and secondary fermentation, and before storage.

In simple or small scale processes containers of hot liquids are allowed to cool in ambient air or placed inside chill rooms. Other cooling systems for liquid foods rely on direct expansion refrigeration, the use of a secondary refrigerant, which is passed through or around the foodstuff, vacuum cooling, or a combination of liquid and vacuum.

Batch coolers for liquid foods range in capacity from 100 to 10,000 litres with the foodstuff contained in a stainless steel vessel. The cooling medium may circulate through the jacket of the vessel, through a coil immersed in the liquid, or both. Most vessels are provided with agitators to improve convective heat transfer and stop temperature stratification. One common method used to decrease cooling times in a closed vessel is to apply a vacuum to produce evaporative cooling.

Continuous cooling used to be achieved in falling film or surface coolers in which the hot liquid was pumped over the top of a horizontal bank of refrigerated coils and flowed down over the cooled surfaces. Totally enclosed coolers have now replaced these systems. Double pipe coolers have also been employed in specialized applications but have a limited heat transfer surface. Multiplate coolers are extensively used for liquid foods. They have the highest available heat transfer surface, lowest material requirements, maximum efficiency, are very flexible in operation and easy to clean. In certain applications, such as beer and wine cooling, multitube coolers that have a much higher resistance to pressure and can use primary refrigerants have advantages over multiplate coolers.

Scraped surface heat exchangers can have advantages in the cooling of very viscous liquid foods and where surface fouling is a potential problem. Currently there is interest in the use of pumpable ice in the form of an ice 'pig' to clean heat exchangers and pipeline systems for liquid foods.

C. Freezing

The main aim of freezing is to extend the storage life of the raw material or food product beyond that achieved at temperatures above the initial freezing point of the material. In a number of cases freezing is used to produce a product i.e. ice cream, sorbet, ice-lollies, etc. that cannot be produced in any other way.

1. Freezing Rate

The need for fast freezing to maximise the quality of frozen food is increasingly open to argument. The clear message from a recent international food refrigeration conference (9) was that 'fast freezing does not equate to high eating quality.' Fast freezing may well be the most economical practical process and it is very likely to reduce weight loss from unwrapped food. However, papers from food researchers and refrigeration equipment manufacturers failed to show any eating quality advantages of fast freezing. These papers covered meat, fish, fruit and vegetable products. Fast freezing produced more smaller ice crystals than the slower processes. However, the differences in crystal make up did not translate to effects that could be detected by taste panels or qualitative sensory measurements.

For example with hamburgers the effects of three different freezing methods — spiral freezing, cryogenic freezing (liquid nitrogen) and impingement freezing — have

been compared. The parameters studied were appearance, dehydration during freezing, cooking losses, meat-structure by microscopically analysis and sensory properties by sensory analysis.

The times required to freeze a 10 mm thick 80 g hamburger from +4°C to −18°C ranged from under 3 to over 22 minutes. Weight loss in the slow process was over three times that in the fast. Ice crystals were significantly larger in hamburgers frozen in 22 compared with 3 minutes. However, no significant difference could be seen in cooking losses. Sensory analysis revealed no difference in eating quality between the three freezing methods, even after 2 months storage.

Freezing always tends to decrease water-holding capacity and hence increase drip. When meat is frozen quickly the water, released by the fibrils as the meat has gone into rigor, and that which is still held are frozen simultaneously. Consequently, there is no change in their relative positions or amounts. At slower freezing rates, however, the water balance is altered, the extra-cellular water freezing first. As freezing continues, the existing ice crystals grow at the expense of water from the intra-fibrillar space. This can result in salt crystallization and pH changes that potentially cause protein denaturation. It has been well documented for frozen fish, which is much more susceptible to freezing damage than meat.

A number of scientific investigations have defined the effect of freezing rate on drip production in meat (10). These state that the optimal conditions for freezing portioned meat are those that achieve freezing rates between 2 and 5 cm h^{-1} to −7°C. 'Slow freezing' up to 0.39 cm.h^{-1} resulted in decreased solubility of myofibrillar proteins, increase in weight loss during freezing, thawing and cooking, lower water binding capacity and tougher cooked meat. 'Very quickly frozen' meat (>4.9 cm h^{-1}) had a somewhat lower solubility of myofibrillar proteins, lower water binding capacity and somewhat tougher and drier meat. In the studies samples were thawed after storage times of 2 to 3 days at −20°C so the relationship between freezing rates and storage life was not investigated.

The results on freezing rate are scientifically very interesting however, in industrial practice most meat is air frozen in the form of large individual pieces or cartons of smaller portions. In commercial situations, freezing rates of 0.5 cm h^{-1} in the deeper sections would be considered 'fast' and there would be considerable variation in freezing time within the meat. In laboratory studies samples are often much smaller than most commercial products i.e. 77.6 g in one study (11). Even with such small samples there was no significant difference in drip after 48 hours between cryogenic freezing at −90°C and a walk in freezer operating at −21°C.

Rapid freezing of products such as poultry joints results in very small reflective ice crystal at the surface. These give the product a very light appearance that is very desirable in some markets. Poor temperature control during storage will cause the small crystals to grow, merge and loose their lightness.

2. Freezing Systems

Many of the chilling systems already described (air, immersion, plate and cryogenic) can be used to freeze food when operated at a lower temperature. Since freezing is the object not something to avoid then with most foods there is no lower limit to the temperature that can be used in a freezing system. In practice the temperature used is based on availability of equipment, capital and running costs, throughput achieved and with unwrapped products weight loss.

Few mechanical refrigeration systems operate at temperatures below −45°C. Most food is frozen in air blast systems, operating typically at −35°C and an air speed of 3 m s^{-1}. Cryogenic systems using liquid nitrogen can produce temperatures down to −170°C and those using carbon dioxide −78°C.

D. Chilled Storage

Theoretically, there are clear differences between the environmental conditions required for chilling, which is a heat removal/temperature reduction process, and storage where the aim is to maintain temperature. However, in many air based systems chilling and storage take place in the same chamber and even where two separate facilities are used, in many cases not all the required heat is removed in the chilling phase. With some fruits and vegetables the rate of respiration during storage is sufficient to require heat removal if product quality is to be maintained.

1. Bulk Storage Rooms

Most unwrapped meat and poultry and all types of wrapped foods are stored in large air circulated rooms. To minimize weight loss, air movement around the unwrapped product should be the minimum required to maintain a constant temperature. With wrapped products low air velocities are also desirable to minimize energy consumption. However, many storage rooms are designed and constructed with little regard to air distribution and the maintenance of low localised velocities over products. Horizontal throw refrigeration coils are often mounted in the free space above the racks or rails of product and no attempt is made to distribute the air around the products. Using air socks it is claimed that an even air distribution can be maintained with localised velocities not exceeding 0.2 m s^{-1}.

2. Controlled Atmosphere Storage Rooms

Controlled atmosphere storage rooms were developed for specialized fruit stores, especially those for apples. Interest

is growing in the application of this technique to other commodities including meat and fish. In addition to the normal temperature control plant these stores also include special gas-tight seals to maintain an atmosphere which is normally lower in oxygen and higher in nitrogen and carbon dioxide than air. Additional plant is required to control the CO_2 concentration, generate nitrogen and consume oxygen.

There is growing interest in the use of controlled atmosphere retail packs to extend the chilled storage and display life of meat and meat products. Since the packs insulate the products efficient precooling before packaging is important.

E. Frozen Storage

If the temperature of the food is reduced to below −10°C within a few hours and maintained below that temperature then microorganisms, bacteria, moulds or yeasts will not grow. Microbially, the food will always remain as safe to eat as it was before freezing, irrespective of the length of frozen storage. Some microbiologists consider −5°C to be a practical maximum temperature to prevent the growth of microorganisms on food.

The textural quality of the food will change with time and conditions of storage. More importantly, the flavor will deteriorate with time. Ultimately these changes will result in an unacceptable product.

1. Textural Changes

Most of the textural changes that occur due to freezing and frozen storage only become apparent when the food is thawed. A few changes can be observed while the food is still frozen. The most noticeable of these are 'freezer burn' and in package frosting.

a. Freezer burn

Moisture is lost from the surface of a food when the vapour pressure at its surface is higher than that in the surrounding environment. Freezing considerably reduces the vapour pressure at the surface of a food but it is still likely to be higher than that in a frozen storage room or display cabinet. Ice from the exposed surface of unwrapped frozen food will sublimate directly into the surrounding environment. The rate of the subsequent desiccation will depend mainly on the rate of air movement over the surface and its humidity. Extreme desiccation will result in a layer of spongy, dry dark material developing on the surface. This is known as 'freezer burn.'

b. In package frosting

Tight packaging in heavyweight, moisture-resistant wrap will prevent freezer burn. Loose packaging will reduce freezer burn but can allow another problem 'in package frosting' to develop. In package frosting occurs when moisture from the surface of the food freezes on the inner surface of the package. Its main cause is poor temperature control in the store or cabinet.

c. Changes apparent on thawing

However, most of the textural changes caused by freezing are not noticeable until thawing. During the freezing process as the water turns to ice, it expands and the ice crystals formed cause the cell walls to rupture. Consequently, the texture of the product will be much softer when the product thaws.

These textural changes are more noticeable in fruits and vegetables that have a higher water content and especially those that are eaten raw. For example, when a frozen tomato is thawed, it turns into mush and liquid. This explains why celery, lettuce and tomatoes are not usually frozen. It is also the reason that frozen fruit are best served before they have completely thawed. In the partially thawed state, the effect of freezing on the fruit tissue is less noticeable.

In the case of vegetables the normal blanch treatment has a marked softening effect but further changes during freezing or frozen storage are comparatively small. However, if the vegetable is frozen in the raw state or is not correctly blanched then the enzymic action on pectic substances may lead to unacceptable changes in texture.

Textural changes due to freezing are not as apparent in products which are cooked before eating because cooking also softens cell walls. These changes are less noticeable in high starch vegetables, such as peas, corn and lima beans.

Consumers expect to purchase frozen meat at a lower unit price than a similar chilled product. This price differential is due to a belief that the quality of frozen meat is inferior to that of chilled. There is little in the published scientific literature to substantiate this view. Some publications show that freezing, frozen storage and thawing produce beneficial effects on the textural properties of pork loins. The taste panel used found the meat slightly tenderer with improved juiciness and an easier breakdown of particles. This was evidenced by the samples being less cohesive, easier to chew and releasing more moisture during mastication.

Meat frozen quickly after slaughter before the rigor process is complete and thawed rapidly contracts vigorously and exudes much drip. The resulting meat is very tough and this toughness does not disappear with extended cooking. The phenomenon is called 'thaw rigor' or 'thaw shortening' and can be avoided by holding meat prior to freezing. A long thawing process, where the meat remains in the −5 to −2°C range for a long period will alleviate the effect. A similar phenomenon is observed in fish filleted pre-rigor and frozen individually.

2. Storage Life

Three factors during storage — 1. storage temperature, 2. degree of fluctuation in the storage temperature and 3.

type of wrapping/packaging in which the food is stored — are commonly believed to have the main influences on frozen storage life.

Storage temperature has a marked effect on the behaviour of ice crystals that could be detrimental to the ultimate quality of the food. It has been demonstrated that frozen tissue stored for 180 days at −20°C has small ice crystal formations of irregular shape in the extracellular spaces. Storage for the same period at −3°C results in the development of large rounded ice formations with a concomitant compression of the muscle fibres. It is thus desirable that frozen meat should be stored at a sufficiently low temperature to prevent growth of ice crystals in the extracellular spaces.

Generally fluctuating temperatures in storage are considered to be detrimental to the product. Statements have been made that "temperature fluctuations produced during storage and transport of frozen food lead to activation of the recrystallization processes, causing enlargement of the ice crystals and diminishing advantages obtained in quick freezing of products." However it has been reported that repeated freeze-thaw cycles do not cause any essential change in the muscle ultrastructure and that several freeze-thaw cycles during a product's life cause only small quality damage (in terms of juice loss) or possibly no damage at all (12). A slight but significant improvement has been found in samples that have been frozen and unfrozen several times when tested by a taste panel.

If the temperature cycles were so severe that the surface temperature of the food rose above 3°C during the process then this could create a potential microbiological hazard.

Minor temperature fluctuations in a stored product are generally considered to be unimportant, especially if they are below −18°C and are only of the magnitude of 1 to 2°C. Well packed products and those that are tightly packed in palletized cartons are also less likely to show quality loss. When ground pork and beef were placed under fluctuating conditions of between −18°C and −23°C for a 6 month period the well packed samples showed no deterioration. However poorly packed samples were severely affected by the temperature swings.

The proteins of fish differ from those of meat in their higher susceptibility to damage in frozen storage. Frozen storage results in an increasing tendency to lose moisture on thawing and an increased firmness leading to toughness and dryness on cooking. These changes are the result of denaturation and cross linking reactions predominantly of the myofibrillar proteins.

3. Drip

Drip loss in frozen storage has been shown to increase with storage time and increase in storage temperature (13). After approximately 42 and 63 days drip from beef stored at −10 or −15°C had reached 80% and 90% respectively of its maximum. At −25°C, it required over 120 days to reach the 80% value.

Drip loss during thawing from ground beef patties was also found to increase with the length of time the patties had been in frozen storage (14). For higher fat content samples, drip loss increased from 1.8% in fresh samples to 12.5% after 20 weeks in storage. Higher drip losses in thawing were obtained from samples stored at −12.2°C than lower temperatures. However, there was no difference between storage temperatures of −23.3 and −34.4°C.

4. Flavour Changes

No marked changes in the flavour of fruit occur on freezing except after an extended process at elevated temperatures. Changes in flavour constituents of frozen fruit are generated during prolonged storage and increase as storage temperature is increased. The first significant change in stored frozen fruits is a loss of fresh aroma. After this some off flavours may develop that are dependent on the type of fruit. It is known that some fruits are more stable than others but the exact reason is not known.

Enzymes in fruits and vegetables are slowed down, but not destroyed during freezing. If not inactivated, these enzymes can cause colour and flavour changes as well as loss of nutrients.

Blanching inactivates enzymes in vegetables. Blanching is the exposure of the vegetable to boiling water or steam for a brief period of time. The vegetable must then be rapidly cooled in ice water to prevent cooking. Blanching is essential for top quality frozen vegetables.

Another type of chemical change that can take place in frozen products is the development of rancid off flavours. This can occur when fat, such as in meat, is exposed to air over a period.

Species has an influence on rancidity development. Investigations have been carried out on rancidity development in cooked and uncooked ground meat patties made from chicken, beef and pork. Initially TBA values in uncooked patties were higher in beef than in those made from either pork or chicken (Figure 112.1). Levels in chicken patties did not change appreciably during 150 d storage at −20°C. After 150 days TBA values in beef were approximately 7 times higher than those in chicken. Values in pork were 4 to 5.5 times as high as those in chicken. Cooked chicken patties had higher lipid oxidation potential than cooked beef or pork patties.

Other studies found a temperature and species interaction for pork and beef. In experiments the practical storage life (PSL) and high quality life (HQL) of pork were superior to beef at −5°C but at −10 and −20°C the storage life of beef was much longer. In other trials where more than one species was compared storage times were not fully presented or direct comparisons were not possible.

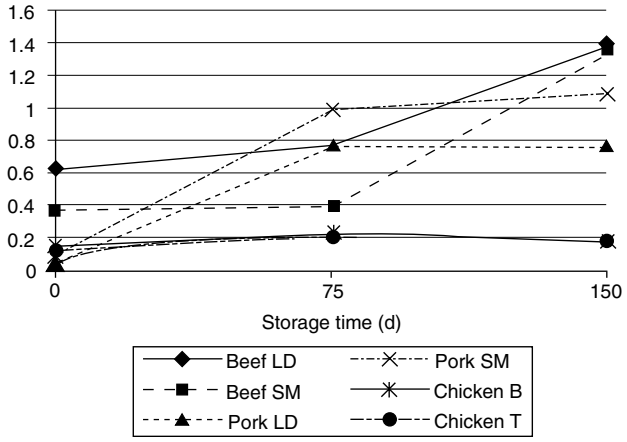

FIGURE 112.1 TBA values during storage at −20°C.

It seems fair to conclude that most work points towards a difference in frozen storage life between species. However, we currently lack the understanding to use data on the frozen storage life of one species under a given condition to predict the likely storage life of other species at that condition.

Animal to animal variation is believed to cause wide variations in storage times. Investigations in New Zealand found that animal to animal variation can lead to storage life differences as great as 50% in lamb, but does not give any definite reason for this variation. Two trials were carried out where lamb was stored at −5°C. In the first trial the lamb was judged rancid after 20 weeks and in a duplicate trial using identical temperature, processing and packaging conditions the lamb was found to store for 40 weeks. The only variation that could be determined was that different animals were used in the two trials.

Processing of meat prior to freezing generally results in a lengthened storage time. It has been found that the amount of TBA-reactive substances, in pre-rigor cooked boar, did not change during 3 months storage at −20°C. Heating prior to freezing can result in a 50% longer PSL for sausages and is thought to be due mainly to an inactivation of some enzymes present. Alternatively, it could be due to the inactivation of the catalytic activity of haemoglobin. However the heating process could be critical since muscles cooked to higher temperatures are most susceptible to oxidative changes during storage. Investigations have also found that pressure-cooking chicken breast at 0.36 kg cm^{-2} produced higher TBA values than cooking at atmospheric pressure.

Smoking is generally advantageous due to the antioxidant properties of the smoke. It has been suggested that wood smoke may not only aid frozen storage due to its antioxidant properties, but that the smoke may also mask off flavors. Smoked broilers were found to store well for over a year without serious quality change. Smoked ham was also found to store well for one year at −18°C without loss of flavour or weight.

Studies on chicken breast and salmon showed that fast and slow freezing, at undefined rates, had no effect on the rate of development of lipid oxidation during storage. Slightly higher levels were found in the fast frozen muscle. In studies on buffalo meat plate frozen meat was scored higher for colour, texture and overall quality than blast frozen samples. Tests were carried out after 3 months storage at −15°C and freezing times, for 500 to 600 g pieces, from 4 to −30°C were 280 and 140 minutes respectively for the plate and blast freezing operations. After 1 month storage TBA values in plate frozen cuts were significantly higher than in those blast frozen but the difference had disappeared after 3 months.

Few would disagree that storage temperature influences frozen storage life and that in general the lower the temperature the longer the PSL. However, few papers have been located where data are presented from experiments on the PSL of food at different storage temperatures. Many of those that have been located are on products that do not meet the lower temperature longer storage rule (normal stability).

It has been shown that rancidity in bacon is increased by higher salt content and that the rates of chemical reactions are accelerated as the temperature is lowered when packed in permeable wrap. Studies have shown that free fatty acids accumulate more readily in bacon stored at −30°C than bacon stored at −12°C. The highest rate of free fatty acid accumulation in bacon was found to be at −62°C. A similar trend was found in trials with liver paste. Cured pork products are known to have an abnormal temperature profile between −5°C and −60°C and store less well between −30°C and −40°C.

Pork chops, Vienna sausage and pork sausages have been found to have straight line quality loss data at lower temperatures but were non linear at higher temperatures. Their storage trials were carried out at −40, −30, −24, −18 and −12°C. Ground veal and pork were also found to have cubic curves at all temperatures.

Improved aroma scores have been found to be only moderately related to lower freezing temperatures, but were not related to flavour. Aroma scores for minced beef improved during 6–12 month storage at −12.2, −17.8 or −23.3°C, although a slight increase in rancidity also occurred. Temperature abuse of −6.7°C for 48 hours caused rancidity on the outside of the samples but did not affect the inner portion. After 24 weeks storage at −12, −18 or −23°C no differences due to storage temperature were found in ground beef patties containing mechanically recovered meat. Ground beef patties stored at −7°C were found to be tougher than those stored at −18 or −23°C. Severe quality deterioration was found in patties stored for 6 months at −7°C. Little difference was found between storage temperatures of −18 or −23°C over an 18-month period.

Consumer panels are often not very sensitive to quality changes. When a consumer panel was used, it could not tell the difference between samples of lamb stored at −5 and −35°C. A trained taste panel could differentiate between the two temperatures and scored the samples stored at −5°C as being rancid.

In recent years energy conservation requirements have caused an increased interest in the possibility of using more efficient storage temperatures than have been used to date. Researchers have questioned the wisdom of storage below −20°C and have asked whether there is any real economic advantage in very low temperature preservation. There is a growing realization that storage lives of several foods can be less dependent on temperature than previously thought, and since research has shown that meat and poultry often produce non-linear time-temperature curves there is probably an optimum storage temperature for a particular product. Improved packing and preservation of products can also increase storage life and may allow higher storage temperatures to be used. It has been suggested that foods should be split into high, medium and low stability categories, with −12°C for products with high stability and −28°C for products with low stability. Some believe that based on storage at −18°C, low stability meats such as mechanically recovered meat should be stored for 8 months or less. Medium stability meats such as pork and processed meats should be stored for between 8 and 15 months. High stability meats, which include all meat and poultry except pork, could be stored for more than 15 months.

F. Thawing and Tempering

Thawing has received much less attention in the literature than either chilling or freezing. In commercial practice there are relatively few controlled thawing systems.

Frozen meat, as supplied to the industry, ranges in size and shape from complete hindquarters of beef to small breasts of lamb, although the majority of the material is 'boned-out' and packed in boxes approximately 15 cm thick weighing between 20 and 40 kg. Frozen fish ranges from large plate frozen slabs to individually quick frozen (IQF) prawns in bags or boxes. Thawing is usually regarded as complete when the centre of the block or joint has reached 0°C, the minimum temperature at which the meat or fish can be boned or cut by hand. Lower temperatures (e.g. −5 to −2°C) are acceptable for product that is destined for mechanical chopping, but such product is 'tempered' rather than thawed. The two processes should not be confused because tempering only constitutes the initial phase of a complete thawing process.

Thawing is often considered as simply the reversal of the freezing process. However, inherent in thawing is a major problem that does not occur in the freezing operation. The majority of the bacteria that cause spoilage or food poisoning are found on the surfaces of food. During the freezing operation, surface temperatures are reduced rapidly and bacterial multiplication is severely limited, with bacteria becoming completely dormant below −10°C. In the thawing operation these same surface areas are the first to rise in temperature and bacterial multiplication can recommence. On large objects subjected to long uncontrolled thawing cycles, surface spoilage can occur before the centre regions have fully thawed.

Most systems supply heat to the surface and then rely on conduction to transfer that heat into the centre of the meat. A few use electromagnetic radiation to generate heat within the meat. In selecting a thawing system for industrial use a balance must be struck between thawing time, appearance and bacteriological condition of product, processing problems such as effluent disposal and the capital and operating costs of the respective systems. Of these factors, thawing time is the principal criterion that governs selection of the system. Appearance, bacteriological condition and weight loss are important if the material is to be sold in the thawed condition but are less so if it is for processing.

G. Secondary Chilling

Products are normally chilled immediately after slaughter or harvest. Most of the subsequent operations in the cold chain are designed to maintain the temperature of the food. Cooking is a very common operation in the production of many products and operators appreciate the importance of rapidly cooling the cooked product. However, any handling such as cutting, mixing or tumbling will add heat to the food and increase its temperature. A secondary cooling operation is always required with chilled products to reduce their temperature to approaching 0°C and maintain their storage life.

The aim of any cooking process is to ensure the destruction of vegetative stages of any pathogenic microorganisms. However, there is always the possibility that the cooking process will not kill some microorganisms that produce spores or that the food can become recontaminated. Therefore, microbiologists recommend that the temperature of the meat, for example, should be rapidly reduced, especially from approximately 60 and 5°C, to prevent multiplication of existing or contaminating bacteria. Rapid cooling is also desirable with cooked products to maintain quality by eliminating the overcooking that occurs during slow cooling.

There are specific cooling recommendations for cook-chill and cook-freeze catering systems. However, even with thin products these are difficult to achieve without surface freezing. Cooling large hams and other cooked meat joints is inherently a much slower process and studies have shown that companies often have very poor cooling systems.

The methods available to cool meat joints, pies and other cooked products have been described in detail (15). A review of the use of vacuum cooling in the food industry has been published (16).

The majority of plants rely on air blast cooling systems for the chilling of pre-cooked meat products. In batch systems the products, packs or trays of cooked material are placed directly on racks in the chiller or on trolleys that can be wheeled into the chiller when fully loaded. Continuous systems range from trolleys pulled through tunnels to conveyorized spiral or tunnel air blast chillers.

Some meals and products are chilled using cryogenic tunnels, however care must be taken to avoid surface freezing. Imperviously packed products can be chilled by immersion in cooled water or other suitable liquid. With some cooked products such as large hams in moulds and sausages, chlorinated water sprays can be used in the initial stages of cooling. Increasingly pie fillings are pressure-cooked and vacuum cooled. With many products an initial cooling stage using ambient air can often substantially reduce the cooling load in the cooling system.

H. Transportation

Developments in temperature controlled transportation systems for products have been one of the main factors leading to the rapid expansion of the chilled food market. The sea transportation of chilled meat from Australasia to European and other distant markets, and road transportation of chilled products throughout Europe and the Middle East, is now common practice. Air freighting was initially used for high value perishable products such as strawberries, asparagus and live lobsters. It is now increasingly used to provide consumers with a year round supply of locally out of season products such as green beans, mange tout and green on carrots.

1. Overland Transport

Overland transportation systems range from 12 m refrigerated containers for long distance road or rail movement of bulk chilled product to small uninsulated vans supplying food to local retail outlets. The majority of current road transport vehicles for chilled foods are refrigerated using either mechanical eutectic plates or liquid nitrogen cooling systems. Irrespective of the type of refrigeration equipment used, the product will not be maintained at its desired temperature during transportation unless it is surrounded by air or surfaces at or below that temperature. This is usually achieved by a system that circulates moving air, either forced or by gravity, around the load. Inadequate air distribution is probably the principle cause of product deterioration and loss of shelf life during transport. Conventional forced air units usually discharge air over the stacked or suspended products either directly from the evaporator or through ducts towards the rear cargo doors. Because air takes the path of least resistance it circulates through the channels that have the largest cross sectional area. These tend to be around rather than through the product. If products have been cooled to the correct temperature before loading and do not generate heat then they only have to be isolated from external heat ingress. Many trucks are now being constructed with an inner skin that forms a return air duct along the sidewalls and floor, with the refrigerated air being supplied via a ceiling duct.

2. Sea Transport

Recent developments in temperature control, packaging and controlled atmospheres has increased substantially the range of foods that can be transported around the world in a chilled condition. Control of the oxygen and carbon dioxide levels in shipboard containers have allowed fruits and vegetables such as apples, pears, avocado pears, melons, mangoes, nectarines, blueberries and asparagus to be shipped from Australia and New Zealand to markets in the USA, Europe, Middle East and Japan.

International Standard Organisation (ISO) containers for food transport are 6 or 12 m long, hold up to 26 tonnes of product and can be 'insulated' or 'refrigerated.' The refrigerated containers incorporate insulation and have refrigeration units built into their structure. Insulated containers either utilize plug type refrigeration units or may be connected directly to an air-handling system in a ship's hold or at the docks. Close temperature control is most easily achieved in containers that are placed in insulated holds and connected to the ship's refrigeration system.

3. Air Transport

Although air freighting of foods offers a rapid method of serving distant markets, there are many problems because the product is unprotected by refrigeration for much of its journey. Up to 80% of the total journey time is spent on the tarmac or transport to and from the airport. Perishable cargo is usually carried in standard containers, sometimes with an insulating lining and/or dry ice but is often unprotected on aircraft pallets.

I. Retail Display

The retail display of chilled food is probably still the weakest link in the cold chain. In the UK, the Food Hygiene (Amendment) Regulations 1990 required food retailers to maintain the temperature of certain chilled foods below 5 and others 8°C during storage, transport and display. This legislation, which contained severe financial penalties for temperature infringements, produced a marked improvement in the design, construction and operation of retail display cabinets. However, the leg-

TABLE 112.2
Temperatures Measured in 8 Surveys of Domestic Refrigerators in Homes

Reference	Country	Number of Samples	Measurement	T_{min}	T_{mean}	T_{max}	% > x°C
(17)	Northern Ireland	150	Thermometer (3 levels: T, M, B)	0.8	6.5	12.6	71% > 5°C
(18)	U.K.	252	Data logger (3 levels: T, M, B)	0.9	6.0	11.4	23% > 7°C
(19)	France	102	Thermometer (3 levels: T, M, B)			14	70% > 6°C
(20)	The Netherlands	125	Thermometer				70% > 5°C
(21)	New Zealand	50	Thermometer (2 levels: T, B)	0	4.9	11	60% > 4°C
(22)	Greece	136	Thermometer				50% > 9°C
(23)	France	119	Data logger (3 levels: T, M, B)	0.9	6.6	11.4	80% > 5°C
(24)	Northern Ireland	30	Data logger (1 level M)	−5	4.5	13.0	53% > 5°C

islation has since been relaxed and there is currently less emphasis on the maintenance of food temperatures.

The required retail display life and consequent environmental conditions for wrapped chilled products differ from those for unwrapped products. The desired display life for wrapped meat, fish, vegetables and processed foods ranges from a few days to many weeks and is primarily limited by microbiological considerations. Retailers of unwrapped fish, meat and delicatessen products normally require a display life of one working day.

Display cabinets for delicatessen products are available with gravity or forced convection coils and the glass fronts may be nearly vertical or angled up to 20°. In the gravity cabinet cooled air from the raised rear mounted evaporator coil descends into the display well by natural convection and the warm air rises back to the evaporator. In the forced circulation cabinets air is drawn through an evaporator coil by a fan and then ducted into the rear of the display. It then returns to the coil after passing directly over the products, or forming an air curtain, via a slot in the front of the cabinet and a duct under the display shelf.

Although the same cabinets can be used for wrapped foods, most are sold from multi-deck cabinets with single or twin air curtain systems. It is important that the front edges of the cabinet shelves do not project through the air curtain since the refrigerated air will then be diverted out of the cabinet. On the other hand, if narrow shelves are used the curtain may collapse and ambient air can be drawn into the display well. External factors such as the store ambient temperature, the position of the cabinet and poor pretreatment and placement of products substantially affect cabinet performance. Computational fluid dynamics (CFD) is increasingly being used as a valuable tool to improve air distribution and temperature control in retail display cabinets and retail stores themselves.

J. Domestic Handling

It is well known that unprotected chilled food will warm up during transportation to the home. Survey results showed that consumers took on average 43 minutes to bring meat, fish or dairy items home from the shops and place them in a refrigerator (10). Although insulated bags and boxes are widely sold only a small percentage (12.7%) used them to transport some of their food home. The vast majority (87.3%) of people did not use any means of protecting food from temperature gains during transportation. Some of the meat product temperatures in samples placed in a car boot rose to around 30°C during the one hour car journey whilst most of the samples placed in a insulated box cooled during the car journey except for a few at the top of the box which remained at their initial temperature. The temperature of frozen products (starting at −25°C) placed in both the cold box and ambient rose during the 1-hour journey. Temperatures of chickens and meat pies placed in ambient reached temperatures approaching 10°C. Frozen meat products, in the cold box kept below −10°C for the period of the journey.

In the past decade there have been at least eight surveys of temperatures in domestic refrigerators. The results are very similar (Table 112.2) with overall mean temperatures ranging from 4.5 to 6.6°C and maximum temperatures from 11 to 14°C. These results are very worrying since they imply that the average temperature of at least 50% of domestic refrigerators is above 4.5°C. When we look at the percentage of temperatures measured that were above set points the results are even more worrying. In the last French study 80% of the temperatures were above 5°C and in the Greek work 50% above 9°C. In Jackson's ongoing investigations only 17% operated below and 20% operated above 5°C for the entire week of monitoring.

III. CONCLUSIONS

Refrigeration has a key role in maintaining the safety and eating quality of most foods. However, it is critical that foods are cooled to the optimum temperature after harvest and processing and that temperature is maintained throughout the cold chain.

Due to the introduction of freezing in the early part of the 20th century meat, fish and some vegetables became readily available year round. Now many 'fresh' chilled products are available at all times of the year due to developments in the chill chain especially in refrigerated storage and transportation. Refrigeration is continuing to develop and an increasing range of raw materials and completely finished refrigerated meals are appearing on the market. Due to demographic changes in the developed world these trends are predicted to continue in the future.

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113 Microwavable Frozen Food or Meals

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CONTENTS

I. INTRODUCTION

The microwave oven was first developed during the 1950s and became a popular household appliance when its penetration level soared during the 1980s—currently, more than 90% of the US households own at least one microwave oven. The major driving forces for the microwave oven are changing lifestyles and development of microwavable food products.

The changing lifestyles in recent years (two income families, single parents, school-age children home alone) are increasingly putting a premium on convenience and quick preparation of food. The microwave oven captures this opportunity by providing a convenient means for the consumer to cook or reheat food quickly and easily. At the same time, the food industry has also developed new products or reformulated existing products for the microwave oven. Microwavable food products are now ubiquitous in the supermarket. In particular, microwavable frozen meals are a major category since they provide a convenient solution to people who do not have time to prepare their own meals. The packaging industry has also contributed to developing new technologies and packages that are compatible to microwave heating. The consumer can quickly microwave a frozen meal in a container and enjoy the meal from the same container.

While microwavable food products have made significant inroads in the past two decades, there still remain many challenges for the developer and manufacturer. Besides convenience, other factors such as taste and texture are also important to the consumer. Quite often, the consumer perceives food heated by the microwave oven do not taste as good as that heated by the conventional oven. This is because the heating mechanisms of food in the microwave oven and conventional oven are indeed quite different. To develop successful microwavable food products, the developer must have a good understanding of the microwave heating of food, as well as careful considerations of the package design and consumer expectation.

II. BASICS OF MICROWAVE HEATING OF FOOD

Microwave heating of food is a complex process that requires a good understanding of several relevant disciplines: electromagnetism, food engineering, food chemistry, food packaging, and food microbiology. It is beyond the scope of this chapter to provide detailed descriptions of the many aspects of this complex process. Instead, this chapter is aimed at acquainting the reader with the basic working knowledge most relevant to the microwave heating of frozen foods. More general information can be found from references in the literature (1–3).

A. Microwaves

Microwaves are short electromagnetic waves located in the portion of electromagnetic wave spectrum between radio waves and visible light. The energy is delivered in the form of propagating sine waves with an electric field and a magnetic field orthogonal to each other. Microwaves are relatively harmless to human because they are a form of non-ionizing radiation, unlike the much more powerful ionizing radiation (such as x-ray or gamma ray) that can damage the cells of living tissue. Microwaves are used in daily applications such as cooking, radar detection, telecommunications, and so on.

Most microwave ovens for food applications operate at two frequencies. The household microwave oven operates at 2450 MHz (2.45×10^9 cycles per second), and the industrial microwave oven operates at 915 MHz (9.15×10^8 cycles per seconds). The wavelengths associated with those frequencies are 0.122 and 0.382 m, respectively, when the microwaves are assumed to travel at the speed of light (3×10^8 m/sec). Microwaves travel at approximately the speed of light in air, but they travel at a lower speed inside a food material. The relationship between frequency and wavelength is expressed by the equation, $v = f\lambda$, where v is velocity (m/sec), f is frequency (Hz), and λ is wavelength (m) of electromagnetic wave.

There are three possible modes of interaction when microwaves impinge upon a material: absorption of microwaves by the material, reflection of microwaves by the material, and transmission of microwaves through the material. The material may be a food or a packaging material. The food must absorb a portion of the microwave energy in order for heating to occur. Most foods do not reflect microwaves, and thus all the remaining unabsorbed microwave energy is transmitted. Some packaging materials, such as susceptors, absorb microwave energy and become hot. Metals, such as aluminum foils, reflect microwaves. Paper, plastics, and glass are transparent to microwaves. To optimize the microwave heating of food, it is necessary to consider the reflection, absorption, and transmission of microwaves by the food and the package.

In the microwave oven, microwaves are generated by an electronic vacuum tube known as magnetron. The microwaves then travel through a hollow metal tube called "waveguide" to the oven cavity. To improve the heating uniformity, the microwave oven is often equipped with a stirrer or a turntable. The stirrer is a fanlike set of spinning metal blades used to scatter the microwaves and disperses them evenly within the oven. The turntable rotates the food during the microwave process. The history, features, standardization, and safety matters relating to the microwave oven are discussed by Decareau (1).

B. Microwave/Heat Conversion

Microwave energy is not heat energy. In order for microwaves to heat food, they must first be converted to heat. There are two mechanisms by which this energy conversion can occur: dipole rotation and ionic polarization. The two mechanisms are quite similar, except the first involves mobile dipoles while the second involves mobile ions. Both dipoles and ions interact only with the electric field, not the magnetic field.

Figure 113.1(a) illustrates the dipole rotation mechanism of a polar molecule. In the presence of an electrical field, the polar molecule behaves like a microscopic magnet, which attempts to align with the field by rotating around its axis. As the polarity of the electric field changes, the direction of rotation also changes. The molecule thus absorbs microwave energy by rotating back and forth billions of times at the frequency of microwaves. Since the molecule is often bound to other molecules, the rotating action also causes it to rub against those other molecules. The rubbing action disrupts the bonds between the molecules, which in turn causes friction and heat dissipation.

Water molecule is the most abundant polar molecule in food. The water molecules in liquid water are quite mobile, and they readily absorb microwave energy and dissipate it as heat through dipolar rotation. In contrast, the water molecules in ice are much less mobile due to the confined crystal structure, and they do not absorb microwave

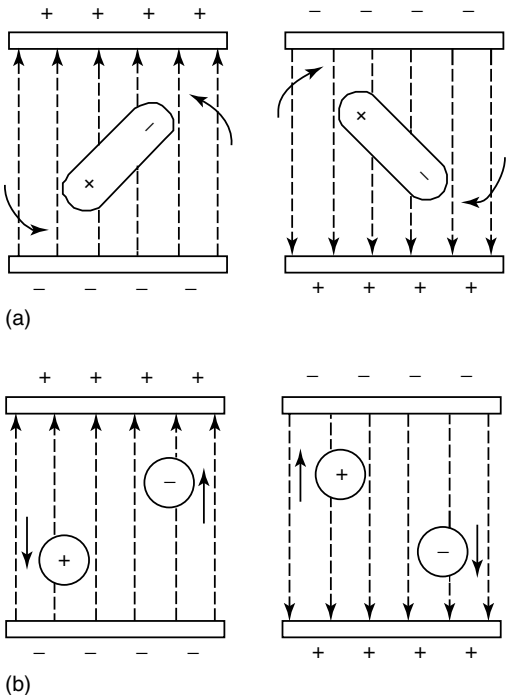

FIGURE 113.1 Microwave/heat conversion mechanisms. The dashed lines denote the alternating electric field at the frequency of microwaves. (a) A dipole rotates back and forth. At high frequencies (such as 2450 MHz), there is not sufficient time for the dipole to rotate 180°, and thus the actual rotation angle is much smaller. (b) A positive ion and a negative ion oscillate in an alternating electric field.

well. The distribution of moisture and the state of water (liquid water or ice) are often two critical factors that determine the behavior of microwave heating of foods.

Figure 113.1(b) illustrates the ionic polarization of a positive ion and a negative ion in solution. In the presence of an electric field, the ions move in the direction of the field. As the polarity of the electric field changes, the ions move in the opposition direction. The ions absorb microwave energy by oscillating themselves at the frequency of microwaves. The oscillating action in turn causes heat dissipation through friction. The common ions in food are those from salts such as sodium chloride. Since ions are less abundant than water molecules in most foods, ionic polarization often plays a less important role than dipole rotation.

C. Dielectric Properties

While dipole rotation and ionic polarization provide a qualitative understanding of the microwave/heat conversion mechanisms, the dielectric properties provide a quantitative characterization of the interactions between microwave electromagnetic energy and food. The dielectric properties, along with thermal and other physical properties, determine the heating behavior of the food in the microwave oven.

TABLE 113.1
Dielectric Constant (ε′), Dielectric Loss Factor (ε″), and Penetration Depth (D_p) of Various Foods at 2450 MHz

	ε′	ε″	D_p (cm)
Ice (−12°C)	3.2	0.0029	1203
Water (1.5°C)	80.5	25.0	0.71
Water (25°C)	78	12.48	1.38
Water (75°C)	60.5	39.93	0.40
0.1 M NaCl (25°C)	75.5	18.1	0.94
Fat and Oil (average)	2.5	0.15	20.6
Raw Beef (−15°C)	5.0	0.75	5.83
Raw Beef (25°C)	40	12	1.04
Roast Beef (23°C)	28	5.6	1.85
Boiled Potatoes (−15°C)	4.5	0.9	4.6
Boiled Potatoes (23°C)	38	11.4	1.07
Boiled Spinach (−15°C)	13	6.5	1.11
Boiled Spinach (23°C)	34	27.2	0.45
Polyethylene	2.3	0.003	986
Paper	2.7	0.15	21.4
Metal	∞	0	0
Free Space	1	0	∞

An important dielectric property is dielectric loss factor (ε″), which indicates the ability of the food to dissipate electrical energy. The term "loss" refers to the loss of energy in the form of heat by the food. It is useful to remember that a material with a high ε″ value (also known as a lossy material) heats well, while a material with a low ε″ value heats poorly in the microwave oven. The dielectric loss factor is related to two other dielectric properties by the equation:

$$\tan \delta = \varepsilon''/\varepsilon' \qquad (113.1)$$

where tan δ is loss tangent and ε′ is dielectric constant.

The dielectric properties (ε′ and ε″) are functions of frequency, temperature, moisture content, and salt content. Values of dielectric properties for foods and other materials can be found in the literature (4–6). Examples of ε″ and ε′ values at 2450 MHz are shown in Table 113.1. Although the literature values can be used as guidelines, actual measurements are often required because of the variability of composition of the materials.

The dielectric properties provide a quick indication of how well a material heats in the microwave oven. For example, the ε″ value of water (12.48) at 25°C is several orders of magnitude higher than that of ice (0.0029) at −12°C. This means water heats far better than ice in the microwave oven. Ice is almost transparent to microwaves because its molecules are tightly bound and do not rotate easily through the mechanism of dipolar rotation. The dramatic increase in ε″ value is also observed, when ice changes to water, during the thawing of frozen foods

including beef, potato, and spinach (Table 113.1). Plastics and paper have low ε'' values because they are almost transparent to microwaves.

D. Penetration Depth

The speed of microwave heating is due to the deep penetration of microwaves into the food, and the dielectric properties can be used to determine the extent of penetration. When microwaves strike a food surface, they arrive with some initial power level. As microwaves penetrate the food, their power is attenuated since some of their energy is absorbed by the food. The term penetration depth (D_p) is defined as the depth at which the microwave power level is reduced to 36.8% (or 1/e) of its initial value, which can be estimated using the equation:

$$D_p = \frac{\lambda_o}{2\pi\sqrt{2\varepsilon'}}\left[\left(1 + \tan^2\delta\right)^{1/2} - 1\right]^{-1/2} \quad (113.2)$$

where λ_o is wavelength in free space. At 2450 MHz, $\lambda_o = 12.24$ cm

$$D_p = 1.38\left\{\varepsilon'\left[\left(1 + \tan^2\delta\right)^{1/2} - 1\right]\right\}^{-1/2} \quad (113.3)$$

where D_p is in cm. The penetration depth is a "visual term" that describes how well a food absorbs microwaves: the shorter is the penetration depth, the more the food absorbs microwaves.

The meaning of penetration depth is further illustrated in Figure 113.2. At the first D_p, 36.8% of the initial power remains, while 63.2% of the power is absorbed. At the second D_p, $(0.368)^2 = 13.5\%$ remains and 86.5% is absorbed. At the third D_p, $(0.368)^3 = 5.0\%$ remains and 95% is absorbed. The penetration depth depends on the composition of material, the frequency of microwaves, and temperature.

Typical values of D_p for various materials at 2450 MHz are also shown in Table 113.1. As mentioned earlier, liquid water absorbs microwaves far better than ice. The D_p of water at 25°C is 1.38 cm, but D_p of ice at −12°C is 1203 cm! Frozen foods have longer penetration depths than unfrozen foods. For example, the D_p values for frozen beef and unfrozen beef are 5.83 cm and 1.04 cm, respectively.

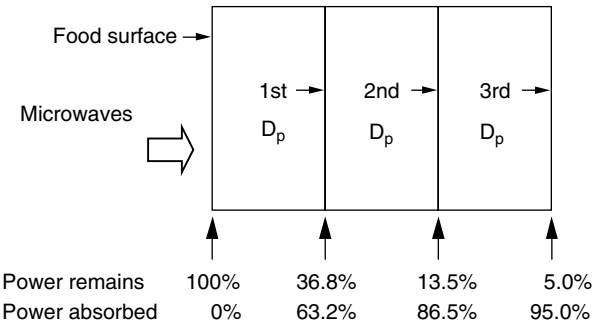

FIGURE 113.2 Power levels at various penetration depths.

E. Mathematical Equations and Models

Besides Equation (113.2), other simple equations can also provide researchers and food product developers a better understanding of the microwave heating process. For example, the microwave power absorption can be estimated using the equation:

$$P = k\,\varepsilon''\,f\,E^2 \quad (113.4)$$

where P is power absorption (watts/cm^3), k is constant (5.56×10^{-13} farads/cm), ε'' is dielectric loss factor of food (dimensionless), f is frequency of microwaves (Hz), and E is electric field strength of microwave (volts/cm). The power absorption is directly proportional to the dielectric loss factor, indicating that a lossy material (which has high ε'' value) is also a good absorber of microwave energy.

The rate of temperature increase of the food can be estimated using the equation:

$$\frac{dT}{dt} = \frac{k\,\varepsilon''\,f\,E^2}{\rho\,C_p} \quad (113.5)$$

where T is average temperature of food (°C), t is time (sec), ρ is density of food (g cm^{-3}), and C_p is specific heat of food (J g^{-1} °C^{-1}). Equation (113.5) is an energy balance equation, which assumes the microwave energy absorbed is balanced by the heat gain of the food. Note that this is a relatively simple equation, and it does not consider the fact that temperature is not evenly distributed within the food.

Mathematical models based on heat-and-mass transfer principles are also available to provide more sophisticated information during the microwave heating of food (7,8). A typical model consists of a set of partial differential equations with the proper initial and boundary conditions. The models can be used to predict the temperature and moisture distribution histories of foods during microwave heating. To use the models, values for dielectric properties, thermal properties, density, electrical field strength, and product dimensions are required. Models for frozen food are more complicated than those for unfrozen food, because the microwave heating behavior changes greatly from the frozen state to unfrozen state.

The models can simulate what-if scenarios and thus can help to minimize the number of experiments and shorten the product development time. However, the models are limited mostly to the predictions of temperature and moisture content, and they do not deal with other important factors such as taste and texture. Most models are also limited to foods that are homogenous and have regular shapes.

III. CHALLENGES IN MICROWAVE HEATING OF FROZEN FOOD

While microwave heating offers the benefits of speed cooking and convenience, it also presents many technical challenges to the food scientist or technologist. Those challenges arise from the need to deal with the many variables relating to the food, package, and microwave oven. For the food, there are variables of food composition, shape, size, specific heat, density, dielectric properties, and thermal conductivity. For the package, there are variables of shape, size, and properties of packaging material. For the microwave oven, there are variables relating to the design of the oven. A related and more important challenge is to solve the problems of the consumer. From the consumer's point of view, the most noticeable problems are those associated with nonuniform heating, lack of browning and crisping, and variation in microwave ovens.

A. Nonuniform Heating

Nonuniform heating is a major problem associated with microwave heating. The problem is especially noticeable for frozen food. It is not uncommon for a frozen food heated in a microwave oven to boil around the edges while the center remains frozen. The problem is caused by the differences in microwave energy absorption of liquid water and ice.

In frozen foods, the water molecules on the surface are relatively free to move compared to the water molecules inside the food. When a frozen food is microwaved, heating begins at the surface where the water molecules are more ready to absorb microwave energy. This causes the adjacent ice crystals to melt and the surface temperature to rise, while the inside temperature is still little affected. As more liquid water is available, the heating of the surface becomes more rapid. This can lead to "runaway heating," in which heating is excessive at the surface while the inside is still frozen. To minimize runaway heating during thawing, microwave energy should be delivered at a slow rate, which allows more time for heat to conduct from the surface to the inside.

Irregular shape of the food can also cause nonuniform heating. The thin parts tend to overcook, while the thick parts tend to undercook. This situation also occurs in conventional cooking but is less pronounced because the cooking is slower. Another cause of non-uniform heating is that different foods have different dielectric and thermal properties. When a microwave meal consists of two or more items, it is possible that the items heat at different rates. For example, when microwave heating a frozen meal consisting of meat and vegetable, the vegetable often becomes overheated and dried out before the meat reaches the serving temperature.

B. Lack of Browning and Crisping

Another problem is that, unlike the conventional oven, the microwave oven is not able to produce foods that are brown and crisp. This is because the heating mechanisms of the conventional oven and the microwave oven are quite different.

In the conventional oven, the food is heated by hot air in the oven, and if the heating element is not shielded, the food is also heated by radiated heat. Heating is concentrated on the food surface by means of heat convection and radiation. The inside of the food is also heated, at a slow rate, by means of heat conduction. The heating causes the moisture on the food surface to evaporate rapidly, and later, browning and crisping to occur. Although the moisture inside the food tends to migrate to the surface, the rate is not sufficiently fast to prevent browning and crisping. As a result, the food surface becomes brown and crispy while its inside remains moist and soft.

In the microwave oven, there is no hot air, and heating is mostly due to the interaction between microwaves and water. Microwave heating is not concentrated on the food surface, but it is distributed within the food depending on the penetration depth. The heating on the food surface is no longer sufficiently intense to cause browning and crisping. Unless the food is microwaved for a long time to remove all or most of the water in the food (which is not desirable because the food quality may no longer be acceptable), browning and crisping either do not occur at all or are inadequate.

Browning formulations have been developed for various meat and dough products (1). Commercial steak sauces, barbecue sauces, soy sauces, and the like are brushed on meat before microwave heating. Reusable browning dishes are also available for browning food surfaces in the microwave oven. Most of the commercial browning dishes are made of glass-ceramic substrate with tin oxide coating on the underside. The packaging industry has also developed a disposable browning and crisping material, known as susceptor, discussed later in this chapter.

C. Variation in Microwave Ovens

Yet another problem is the large variation of performance in different microwave ovens. Microwave ovens are available in different powers, oven cavity sizes, with or without a turntable, with or without a stirrer (to distribute microwaves more evenly in the oven). Consequently, different microwave ovens may produce greatly different results, even if the same cooking instructions are used. To accommodate the differences, the food manufacturer can only place vague microwave heating instructions on their packages. For example, a package may contain vague instructions such as "heat between 4 to 8 minutes, depending on the microwave oven."

D. Meeting the Challenges

There is no easy solution to deal with the complex process of microwave heating. In developing a microwavable food product, the scientist or technologist has to rely on the somewhat useful but incomplete scientific knowledge described in the previous sections, as well as trial-and-error or empirical methods.

From the discipline point of view, there are three approaches to deal with the challenges. The first is the food chemist's approach, in which food ingredients are modified and browning formulations are added to make the food more microwavable. The second is the packaging engineer's approach, in which the package is modified to enhance the performance of microwave heating. The third is the microwave engineer's approach, in which new and useful features are added to the microwave oven. Ideally, these approaches should be integrated into a system to deliver the highest quality of microwavable foods to the consumer.

Many microwavable food products have failed in the past because of lack of performance or high cost. Good technical and marketing tools are essential for developing better tasting microwavable products, without increasing the cost or decreasing the effectiveness of cooking. Although the food manufacturer and the packaging supplier have been working together to develop microwavable products, there has been relatively little collaboration between them and the oven manufacturer. There is a need to have all parties (including also the academia) to work more closely together to bring about innovations that can deliver better microwavable products to the consumer.

IV. MICROWAVABLE PACKAGING

The primary functions of the package are to contain, protect, and sell the product. A general discussion on the packaging of frozen foods is presented in Chapter 133. If the package is used to hold the food during microwave heating (as is the case for many microwavable frozen meals), the interactions between microwave and the package must also be considered. Since the package can transmit, reflect, or absorb microwaves, it can also greatly influence the microwave heating behavior. The package may act "passively" by simply transmitting microwaves. The package may also act "actively" by reflecting and absorbing microwaves in a manner that the power distribution of microwaves and the surface temperature of package are modified. To optimize microwave heating, it is necessary to properly balance these three microwave/package interactions (transmission, reflection, and absorption) to optimize the heating of the food. Microwavable packaging materials may be classified into microwave transparent materials, microwave reflective materials, and microwave absorbent materials.

A. Microwave Transparent Materials

A microwavable package must be wholly or partly transparent to microwaves. The most common microwave transparent materials are paper and plastics. Although glass is also transparent to microwaves, it is seldom used to package frozen food.

Plastic-coated paperboard trays are popular for microwavable frozen meals, mainly because of their low cost. The trays combine the rigidity of the paperboard and the chemical resistance of the plastic. The inside of the trays is either extrusion coated with a resin or adhesive laminated with a plastic film. For microwave-only applications, plastics such as low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polypropylene (PP) are used. For dual oven applications (i.e., usable in both microwave and conventional ovens), polyethylene terephthalate (PET) is used because of its high temperature stability (up to about 200°C).

Molded pulp trays are another common paper product. The containers are dual ovenable and can be molded into several compartments. They are stronger and can carry more load than the paperboard containers.

Thermoformed plastic trays are also common heating containers for microwavable frozen foods. LDPE trays are suitable for light microwave heating because the trays tend to distort at temperatures as low as 75°C. PP trays have a distortion temperature of about 110°C. Homopolymer PP trays are brittle at low temperatures and can crack during distribution and handling at freezer temperatures. Copolymer PP trays have somewhat improved low-temperature durability. Crystallized PET (CPET) trays are the most widely used plastic trays for microwavable frozen meals. The CPET trays are functional in the temperature range from −40 to 220°C. Thus the trays can withstand not only the low temperatures encountered in distribution and handling, but also the temperatures in conventional oven (i.e., the trays are dual ovenable).

B. Microwave Reflective Materials

Aluminum foil, aluminum/plastic laminate, and aluminum/plastic/paperboard laminate are the most common microwave reflective materials. Since these materials do not allow the transmission of microwave, they are also known as microwave shielding materials.

Aluminum is often used to selectively shield microwaves from certain areas of a food (Figure 113.3). For example, a multi-component meal may consist of food items that heat at different rates in the microwave oven. The more microwave sensitive food item(s) can be shielded so that the entire meal can be heated more evenly.

Aluminum is also used as an electromagnetic field modifier to redirect microwave energy in a manner to optimize the heating performance (10). Aluminum can intensify the microwave energy locally or redirect it to places

FIGURE 113.3 Trays with selectively shielded areas. (Courtesy of Graphic Packaging Inc.)

FIGURE 113.4 Metallized film susceptors. (Courtesy of Graphic Packaging Inc.)

in the package that otherwise would receive relatively little direct microwave exposure. This approach has been used to redirect microwave energy from the edges to the center for frozen food products such as lasagna.

When aluminum foils are used in the microwave oven, precautions are necessary to prevent arcing, which can occur between foil packages and the oven walls, between two packages, across tears, wrinkles, and so on. Arcing can be prevented by following several simple design rules (11). For example, any foil components should be receded from the edge of the package to avoid arcing with the oven walls. In additional to following those rules, it is also necessary to thoroughly test the package/product to ensure that the package is safe to use.

C. Microwave Absorbent Materials

Microwave absorbent materials used for food packaging are commonly known as susceptors. The major purpose of susceptors is to generate surface heating to mimic the browning and crisping ability of the conventional oven. Although many types of susceptors have been invented (12), the only commercially available type is the metallized film susceptor (Figure 113.4). This type of susceptor consists of a metallized polyethylene terephthalate film laminated to a thin paperboard. The metal layer is a very thin (less than 100 angstroms), discontinuous layer of aluminum, which is responsible for generating localized resistance heating when exposed to microwaves. The heating can cause the susceptor to reach surface temperatures over 200°C within seconds.

Susceptors have been used for products such as frozen pizza, frozen French fries, frozen waffles, frozen hot pies, and popcorn. Susceptors are available in the forms of flat pads, sleeves, and pouches. The flat pads are suitable for products (such as pizza) that require heating only on one surface. The sleeves and pouches are suitable for heating on multiple surfaces (9). Susceptors are also available in various patterns, in which portions of the metallized layer are deactivated (10). The patterns are designed to provide more control of heating. A company uses a printed checkerboard pattern to generate various levels of heating based on the size of the check.

There is a public concern of migration of mobile compounds from the susceptor to the food, because the susceptor can reach high temperature. The FDA has issued voluntary guidelines regarding the safe use of the susceptor for the packaging company to follow.

V. ADVANCED OVENS

Although the oven manufacturer has made many improvements to the microwave oven, especially during the last two decades, it has also encountered the challenges described earlier. Recently, the oven manufacturer has responded to those challenges by introducing more advanced ovens, such as the high-speed ovens and intelligent ovens described below. These advanced ovens are superior to the microwave oven, and they can provide better heating for frozen food products.

A. High-Speed Ovens

High-speed ovens are essentially multimode ovens equipped with advanced technologies. The move towards using multimode heating instead of single-mode heating to deliver higher speed and food quality is a technically sound one, because any single-mode heating (microwaves or convective heat) has too many inherent limitations. The current commercial high-speed ovens differ from one another in terms of hardware and software.

In hardware, the major difference is in the heating sources: microwaves, convective heat, hot-air impingement, and halogen light. Although it is possible to use more than two heating sources, most ovens are limited to only two heating modes because of the considerations in cost and

power consumption. The high-speed ovens typically use microwaves to provide speed cooking, along with another heating source for browning and crisping. Major oven manufacturers have marketed high-speed ovens using microwave/light and microwave/convective heat. A manufacturer has also developed a vending machine that heats frozen meals using microwave/jet impingement.

In software, the major difference among the ovens is in the ways of controlling the cooking process. Various cooking algorithms have been developed to control the heating sources and heating time. In the future, software development will likely incorporate more food science and technology (to develop cooking instructions, provide nutritional information, etc.), better user interface technology, and the use of fuzzy logic.

B. Intelligent Ovens

In recent years, researchers have also developed the so-called "intelligent ovens," which use information technology to overcome some of the limitations of the microwave oven and enhance the cooking experience of the consumer. An example of the intelligent oven is shown in Figure 113.5.

The intelligent oven is a multimode oven (e.g., microwave/convective heat oven) equipped with a bar code scanner and a microprocessor (13). The oven is connected to an input/output device (such as a touch screen) and the Internet. Below are some advantages of the oven:

1. As mentioned earlier, the heating instructions on food packages are intentionally vague to accommodate the many types of microwave ovens in the market. The intelligent oven can overcome this problem. By scanning the bar code on the food package, its microprocessor is able to generate the precise heating instructions that match the food and the oven.
2. The scanning eliminates the need of entering the heating instructions manually. This is particular convenient when the instructions involve complicated multiple heating sequence, especially for multimode ovens. This feature is also helpful for visual impaired consumers.
3. The Internet connection allows the access of information relating to nutrition, product recall, allergenic ingredients, and so on.

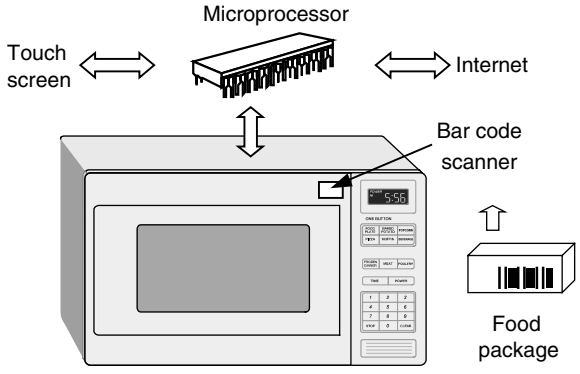

FIGURE 113.5 An intelligent oven.

VI. SUMMARY

While microwavable frozen food and meals have become an integral part of our lifestyle, improvements are still needed to continue to justify their place in the freezer case. Although microwavable frozen products can provide the consumer with convenience, they often fail to impress the consumer with taste and texture. There are many technical and economical challenges for developing new and improved microwavable frozen products. To meet those challenges, the industry (food manufacturer, packaging supplier, and oven manufacturer) and the academia should work more closely together to innovate and develop better microwavable food products that are more tasty, healthy, and convenient to use.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Microwave frozen foods and meals," by K. L. Yam and C. C. Lai. In: *Handbook of frozen foods*, editors: Y. H. Hui et al., Marcel Dekker, NY, 2004.

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114 Frozen Food: Components and Chemical Reactions

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CONTENTS

I. INTRODUCTION

One of the greatest challenges for food technologists is to maintain the quality of food products for an extended period. A decrease in temperature generally decreases the rate of chemical reactions that are responsible for the deterioration in food quality over time; therefore freezing is frequently used to extend the shelf life of food products. However, freezing is not a perfect method of preservation because even at low temperatures deterioration of quality may still occur. The formation of ice may result in textural changes and disruption of cell compartments that cause the release of chemically reactive components. Furthermore, the removal of water during ice formation concentrates the solutes in an unfrozen matrix, which can affect reaction conditions, such as pH and ionic strength. Therefore, in order to extend the shelf life of frozen food products it is crucial to understand the chemical reactions and their kinetics that occur in food components that can lead to quality deterioration.

The effect of freezing on the food components is diverse and some components are affected more than others. For example protein can be irreversibly denatured by freezing whereas carbohydrates are generally more stable. This chapter focuses on chemical and biochemical reactions that affect the quality of frozen food systems. These reactions and specific examples in food are summarized in Table 114.1. Methods for reducing the rate of deterioration are also discussed in this chapter.

II. CHEMICAL AND BIOCHEMICAL REACTIONS IN FROZEN FOOD

A. Protein

Protein may undergo changes during freezing and frozen storage, primarily because of denaturation. Denaturation can be defined as a loss of functionality caused by changes in the protein structure due to the disruption of chemical bonds and by secondary interactions with other constituents [1]. Alterations of the secondary and higher structures of proteins affect the spatial arrangement of the polypeptide chain with respect to other neighboring polypeptide chains [1, 2]. Structural and spatial alterations can cause a range of textural and functional changes, such as the development of toughness, loss of protein solubility, loss of emulsifying capacity and loss of water holding capacity [3–5]. The myofibrillar proteins (mainly myosin and actomyosin) are

TABLE 114.1
Chemical Reactions of Food Components that Affect Food Quality

Food Components	Mechanism of Degradation	Effect on Food Quality	Studies in Food
Protein	Denaturation	Degradation of texture and functional properties	• Toughening and functional changes, particularly loss of protein solubility, in fish [4, 12, 14, 19] • Loss of protein solubility, emulsifying capacity and water holding capacity of meat for processing [3]
Lipid	Hydrolysis	Release of FFA — known to contribute to unpleasant flavors	• Short chain FFA giving rancid odor in dairy product [88] • Medium chain FFA caused a "sweaty" flavor in mutton [89] • Toughening of muscles in frozen Indian oil sardine [8]
	Oxidation • Autoxidation • Enzymatic oxidation	Unstable hydroperoxides break down to reactive compounds and interact with other components to produce off-flavors, discoloration and toughening of muscle protein	• Off-flavors shelled oysters [35] • Detection of rancid flavor in silver pomfret [90]
Carbohydrates	Hydrolysis	Increases the amount of smaller molecular weight components — leads to lower melting temperatures	• Sucrose hydrolysis [47]
		Change of texture	• Firmness of ice cream decreased as hydrolysis progressed [46]
Color Pigments			
(a) Chlorophyll	Pheophytinization	Green chlorophyll forms olive-brown pheophytin in the presence of acid or heat	• Greenness in Brussels sprouts decreased [80] • Stability of green colour in kiwifruit [54, 55] • Frozen blanched spinach had a higher amount of pheophytin than fresh [91]
(b) Anthocyanin	Enzymatic reaction	Glucosidase hydrolyses glycosidic linkages and produces sugars and agylcone compounds	• Loss of anthocyanin in raspberry in the late cultivar was more severe than the early cultivars [59]
	Structure of anthocyanin depends on pH value	Depending on the pH of the food, different forms of anthocyanin exist, usually from red to blue as pH increases	• Red color hue of sour cherry weakens during frozen storage [92]
(c) Carotenoids	Oxidation	The loss of pigments causes fading of color and loss of nutritive value	• Loss of carotenoid in salmon [40]
Flavor Compounds	Enzymatic degradation	Change of flavor profile	• Change in the composition of aromatic compounds of strawberries [67–69] • Change in aroma profile of frozen green peas [70]
	Lipid oxidation	Produces off-flavors	• Green and fatty off-flavor notes in frozen trout due to breakdown products of unsaturated FFA [74]
	Leaching of components during the blanching process	Weakens and changes the sensory perception	• Decrease of organic acids in green beans and Padron peppers [72] • Change in volatile composition of guava [76]
	Effect of heat		• Changes in concentration of odorants during heat treatment [69]
Micronutrients			
(a) Vitamins	Oxidation	Loss of nutritional value because of the loss of vitamins	• Loss of AA and vitamin B6 due to blanching in french fries [81] • Oxidation of AA in peas, lima beans, corn and green beans [82]
(b) Minerals	Generally stable		• Unchanged mineral composition in artichokes, green beans and peas after freezing [87]
	Loss mainly through leaching		• Mineral content of boiled fresh vegetables was not different from frozen vegetables [78]

considered to be the most susceptible to denaturation, while sarcoplasmic proteins have been reported to be more stable [6]. For example, in fresh-water whitefish stored for 16 weeks at −10°C the actomyosin solubility decreased by 50% but the sarcoplasmic proteins were unaffected [4].

In addition to the physical and chemical changes associated with ice formation, loss of protein stability may also be caused by interactions of protein with other components and by enzyme activity. These factors have been reviewed by several authors as causes for denaturation in fish proteins [1, 6, 7]. Lipid oxidation has been shown to be highly correlated with protein denaturation during freezing. For example, the oxidation and hydrolysis of lipids in sardines at −20°C was inversely related to protein solubility and other functional changes [8]. The addition of malonaldehyde, a commonly occurring product of lipid oxidation, to trout myosin solutions during storage at −4°C accelerated protein denaturation [9]. On the other hand, actomyosin in fatty fish, such as halibut and rosefish, is more stable than the actomyosin in lean fish, such as cod and haddock, after one year of frozen storage at −23°C [10]. The actomyosin from fatty fish had little or no denaturation, whereas that from lean fish was almost completely denatured.

Enzymes, particularly trimethylamine oxide demethylase (TMAO demethylase), have been linked to protein denaturation. TMAO demethylase hydrolyses trimethylamine oxide, which is naturally present in some fish and shellfish, into dimethylamine (DMA) and formaldehyde [7]. Formaldehyde is reported to form covalent cross-links with protein and therefore accelerating denaturation [11]. Myofibrillar patterns of hake muscle soaked in formaldehyde had a higher amount of high molecular weight aggregates caused by covalent cross-linking of structural proteins [12]. The effect of the addition of formaldehyde on actomyosin denaturation during frozen storage has also been studied in hake, pork and chicken [13]. The stability of the actomyosin in the presence of formaldehyde differed according to species, with chicken being the most stable to formaldehyde.

Cross-linking of polypeptide chains due to disulfide bond formation may also contribute to protein denaturation in frozen fish, although it may be a secondary factor [14–16]. In minced halibut stored at −10°C, insolubilisation of the myofibrillar proteins was reduced when a disulfide bond reducing agent, such as β-mercaptoethanol, was added [14]. Disulfide bond formation has also been implicated as a factor contributing to the loss of water holding capacity in haddock during frozen storage [12].

Various processing factors may influence protein denaturation during frozen storage. The factors include handling and processing prior to freezing, temperature of storage and the rate of freezing. Mincing has been shown to promote toughening in frozen fish, for example minced hake accumulated formaldehyde at a faster rate than fillets [11]. Depending on the species, aging fish on ice prior to freezing may be advantageous to quality [15]. Aging red hake on ice before processing and frozen storage was shown to produce a more acceptable product than other treatments, such as the removal of dark muscle or dipping in sodium tripolyphosphate or erythorbate [17].

The kinetics of protein denaturation is influenced by temperature. At temperatures just below the freezing point, denaturation is very rapid. For example, the maximum rate of protein denaturation in rabbit and trout myosin solutions was reported to be at approximately −10°C [9]. Lowering the storage temperature generally reduces the degree of protein denaturation [12, 18, 19]. In whiting fillets stored at −20°C and lower, losses in protein extractability, calcium ATPase activity and gel forming ability were reduced [19]. However, there was no significant improvement in the quality of the whiting fillets when stored lower than −20°C. Similar results were observed in halibut [5]. The differences in the development of toughness and off-flavors were insufficient to justify lowering the temperature below −20°C.

Protein stability is also affected by freezing rate. In general, faster freezing rates result in less damage because smaller ice crystals are formed. However, fluctuating temperatures during frozen storage, due to overloading, power failures and equipment breakdowns, can lead to growth of ice crystals, which could counteract the benefit of using fast initial freezing rates [20]. Storage temperature is therefore believed to be a more critical factor than freezing rate.

B. Lipids

Lipids can degrade in frozen systems by means of two well known chemical processes: hydrolysis and oxidation [21]. These processes lead to undesirable changes in the nutritional and sensory quality of foods, such as the production of rancid flavors and discoloration. The hydrolysis of lipids, also known as lipolysis, results in the release of free fatty acids, which can alter the flavor of frozen products. The rate of hydrolysis depends on storage temperature and time, and the type of food product [22–24]. In frozen foods, lipid hydrolysis is generally catalyzed by enzymes. Both phospholipase A and lipases from muscle tissues are responsible for the hydrolysis of phospholipids and neutral lipids in frozen fish [24].

Lipid oxidation is a free radical reaction involving three major steps: initiation, propagation and termination. Oxidation is frequently initiated by a spontaneous reaction with oxygen (autoxidation) or by an enzymatic reaction [25]. Hydroperoxides are the initial products of lipid oxidation. These compounds are unstable and subsequently enter into numerous complex reactions that involve further degradation and interaction with other food components, causing off-flavors, discoloration, loss of nutrients and toughening of protein [8, 21, 26]. The rate of reaction increases with degree of lipid unsaturation, therefore lipid oxidation is particularly a problem in high fat fish [27].

Lipid degradation can be reduced in frozen foods by lowering of the storage temperature, excluding oxygen, adding antioxidants and supplementation of antioxidants in the diet of animals. The effect of lowering the storage temperature from −10°C to −30°C on lipid oxidation and hydrolysis was studied in cod and haddock [28]. At −30°C lipid degradation in both species was reduced to the same extent but at −10°C the haddock was more susceptible to lipid degradation than the cod. Since oxygen is a key component in lipid oxidation, limiting its presence by modified atmosphere packaging can be beneficial to the shelf-life of food, as shown in frozen pork [29], chicken and turkey [30, 31], shrimp [32] and pulverised niboshi (boiled and dried sardines) [33]. The quality of processed fish fingers stored at −20°C was shown to be influenced by the thickness of the packaging and the composition of the packaging material. The control, which was kept without packaging, was discarded after four weeks of storage due to rancid odors, a tough texture and undesirable color changes. Fish fingers packed in 62.5 μm thick low-density polyethylene/high-density polyethylene (LD/HDPE) remained in a satisfactory condition for 28 weeks. The same product packaged with 100 μm thick low-density polyethylene/Nylon-Primacore (LDPE/NY/PC) was stable for 32 weeks [34].

Adding antioxidants, such as butylated hydroxytoluene (BHT) and natural vitamin E reduced lipid oxidation in shelled oysters during storage at −20°C [35]. Frozen rock oysters glazed with 1% sodium ascorbate alone or with 1% monosodium glutamate was efficient in extending their shelf life [36]. Similar results were observed in frozen processed turkey meat treated with antioxidants (a mixture of BHT, propyl gallate and citric acid) where the degree of lipid oxidation was reduced [37]. In addition, supplementing the diet of farm animals with vitamin E was shown to reduce lipid oxidation in meat [38, 39]. Antioxidants may also occur naturally in food. The naturally occurring color pigment astaxanthin has been shown to reduce rancidity in farmed trout [40].

Other factors influencing lipid degradation include handling prior to freezing and the rate of freezing. Precooking of meat prior to freezing can increase lipid degradation during frozen storage, because the denatured heme proteins and ferritin release free iron that catalyse oxidation [41]. It was shown that rancid flavors and thiobarbituric acid (TBA) values increased in precooked chicken meat during two to four months of frozen storage [42]. Rapid freezing of chicken meat in liquid nitrogen produced a product with a lower degree of rancidity than air-blast freezing [42]. Similar results have been observed in pork sausage [43].

C. Carbohydrates

Carbohydrates are susceptible to hydrolysis during frozen storage, as observed in frozen papaya [44]. Starch hydrolysis can be extensive in frozen green beans even when stored at −18°C; almost all of the starch was enzymatically hydrolyzed into reducing sugars, leading to an increase in sweetness and softening [45]. Sugar hydrolysis increases the number of moles of solutes in the food matrix, thus depressing the freezing temperature. This leads to a reduction in the amount of ice in the product, which may alter certain physical properties, for example the firmness of ice cream was inversely related to the degree of hydrolysis [46]. In a model system, the rate of sucrose hydrolysis, by enzymes and under acidic conditions, was investigated at low temperatures [47]. The results indicated that even at temperatures as low as −22°C, hydrolysis still takes place.

D. Color Pigments

The color of raw red meat tissues is mainly attributed to myoglobin. Carotenoid compounds, including astaxanthin, canthaxanthin and β-carotene, contribute to the color of some marine products and poultry [48]. Most of the color in fruit and vegetables is provided by chlorophylls (green leafy vegetables), carotenoids (orange, yellow and red fruits and vegetables) and anthocyanins (majority of berries and red to purplish vegetables).

The stability of color pigments during frozen storage is affected by treatment prior to processing, processing and storage conditions (light, oxygen, heavy metals, temperature, water activity, pH, oxidizing and reducing agents). Furthermore, oxidoreductase enzymes, such as lipoxygenase, catalase, peroxidase, polyphenol oxidase and lipases can cause bleaching of pigments and browning discoloration in fruits and vegetables [49, 50]. Other chemical reactions within the food, particularly lipid oxidation, can also lead to changes in color.

Carotenoids are relatively stable during frozen storage. Sweet potato [51], tomato pulp [52], green beans and broccoli [53] have shown minimal loss of β-carotene during the freezing process. The stability of meat colors, metmyoglobin in meat and carotenoids in fish and poultry, is generally decreased by lipid oxidation [26].

Chlorophyll and anthocyanins are considered to be less stable than carotenoids and can degrade through a variety of mechanisms. In the presence of heat and acid, chlorophyll changes from green to an olive-brown colored pheophytin when the magnesium atom in chlorophyll is displaced by two hydrogen ions [47]. Cultivar type and maturity have been shown to influence the stability of chlorophyll in kiwifruit during frozen storage [54, 55]. This may be because changes in total acidity and pH cause pheophytinization of chlorophyll [54, 55]. However, it was pointed out that at low temperature storage (<-18°C), color of green beans was stable and was probably because of the formation of metal-chlorophyll compounds, which is different from change in color attributing to pheophytisation when stored at high storage temperatures [56]. It was observed in some studies that there was no perceptible

change in greenness of parsley during storage at −20°C for three years [57].

Anthocyanins are relatively unstable during frozen storage and are greatly affected by pH, organic acid content, sugar concentration, enzyme reactions, initial anthocyanin composition and anthocyanin content. Anthocyanins are more stable under acidic conditions [48]. In blackberries, maturity rather than cultivar was found to be more important for preventing blackberries from turning red during storage because the change in color was correlated with lower soluble solids, total anthocyanins and higher titratible acidity [58]. Glycosidases and polyphenolases are two classes of enzymes that promote loss of anthocyanin color intensity [48]. Glycosidases hydrolyze cyanidin 3-glucoside thus contributing to color loss in raspberries [59].

The choice of processing method can affect the stability of color pigments during frozen storage. Light and oxygen have been shown to accelerate color loss in tomato sauce [52], possibly by solubilisation, isomerization and degradation of lycopene [60]. The lycopene content of tomato sauces prepared in an open kettle decreased faster during frozen storage than samples prepared in a tubular pasteurizer [60]. If the tomato product is carefully processed in the absence of oxygen, it can exhibit excellent lycopene stability throughout the normal frozen storage shelf life [61]. The effect of still air freezing versus air-blast freezing on the color of pineapple slices was evaluated [62]. The results showed that the freezing rate did not have any affect on the subsequent frozen storage stability of the pineapple color.

Various methods, such as blanching, modified storage atmosphere, and addition of antioxidants, have been used to enhance the stability of the color pigments in frozen foods. The main purpose of blanching prior to freezing is to inactivate enzymes that degrade color pigments. For example, lipoxygenase is known to degrade β-carotene and lutein [53]. However, blanching can also lead to color losses. Heating can cause the development of an undesirable olive-green color in green vegetables because of chlorophyll degradation [53]. Therefore, minimization of blanching time is generally recommended [49]. Modified atmospheres can be used to reduce color changes. For example, boiled shrimp flushed with nitrogen and sealed in a bag with low oxygen transmission reduced color fading and improved the overall quality of the shrimp [32]. Furthermore, flushing precooked crabmeat with carbon dioxide was shown to prevent blue discoloration [63].

Color can also be stabilized using chemical additives such as antioxidants, applied either as a dip or added directly to the food product or even to the diet of the animal. Natural antioxidants, including ground tomato seeds and spices (rosemary and marjoram) have proved successful for color retention when added to tomato sauce to stabilize carotenoid pigments during frozen storage [52]. Frozen avocado purees can be prevented from browning by adding sodium bisulfite and ascorbate prior to freezing [26]. Browning in frozen crabmeat was reduced by a citric acid dip but no significant reduction in the browning was observed with dips of sodium nitrite, ascorbic acid, sodium acid pyrophosphate, and brine with sodium citrate or tripolyphosphate [63]. Sulfite can inhibit melonosis (blackspot) on crustaceans [64]. However, if it is used in excess (2% for ten minutes), it can cause decomposition of TMAO, thus initiating a chain of lipid and protein degradation reactions [64].

Dietary vitamin E supplementation for Holstein steers increased the color stability of the meat during storage at −20°C [65]. The color and carotenoid content salmon fed with diets supplemented with astaxanthin was stable during frozen storage for up to 12 weeks at −20°C, but salmon fed with cathaxanthin had significant loss in color scores and carotenoid content [66]. In contrast, smoking of the fish significantly reduced the carotenoid content of astaxanthin-fed salmon, but did not cause any decrease in color in canthaxanthin-fed Atlantic salmon during frozen storage [66].

E. Flavor Compounds

Food flavors are composed of volatile aroma compounds and taste components, such as organic acids and sugars. The effect of freezing and frozen storage on flavor compounds in food is variable with flavor changes being affected mainly by enzymatic activities and lipid oxidation. Freezing and thawing processes have been shown to change and weaken the flavor profile of fresh strawberries [67–69] and peas [70], but minimal changes were observed in the aroma volatile composition of raspberries studied under similar conditions [59]. The flavor of fruit and vegetables may also be modified by the decrease in organic acids as observed in frozen kiwifruits, pineapples and blanched green beans [55, 71, 72]. In seafood, the development of off flavors has been associated with the enzymatic degradation of TMAO that produces trimethylamine and dimethylamine [73]. Another type of off-flavor in fish is caused by autoxidation of long chain ω-3-unsaturated fatty acids, which produce carbonyl compounds characterized by an "oxidized fish oil" aroma [73, 74].

Blanching of vegetables prior to freezing can be used to inactivate the enzymes that cause changes to the flavor. The flavor of carrot, cauliflower and beans during frozen storage has been stabilized by blanching [78]. However, complete inactivation of enzymes may not be necessary as it was shown that 5% residual peroxidase activity did not affect the quality of these blanched vegetables during storage [75]. In contrast, the flavors of unblanched leek, onion and swede did not change appreciably during frozen storage and were preferred to blanched products. The disadvantages of blanching with regard to flavor preservation are the loss of flavor compounds through leaching [72] and creation of a different flavor profile through heating [76]. Therefore, it is important to identify and specifically target enzymes such

as lipoxygenases, lipases, lyases and proteases for adequate blanching, rather than to utilize excessive blanching regimes for peroxidase destruction, which is commonly used as an indicator enzyme for vegetables [49]. Foods, such as white truffle, that are unsuitable for blanching, are susceptible to deterioration. These, along with the loss of other volatile compounds were responsible for the typical truffle flavor [77].

Pasteurization is another heat pretreatment frequently used for frozen foods. Changes in the volatile composition of a pasteurized guava puree product were investigated during four months of storage at −10°C and −20°C [76]. Pasteurized guava puree had increased levels of aldehydes and hydrocarbons and decreased levels of esters compared to unpasteurized guava puree. There was only a slight decrease in the concentration of alcohols and hydrocarbons in pasteurized guava puree stored at −20°C.

F. Vitamins

Freezing is considered as one of the best processing methods for preserving nutrients in food. In a comparison of different storage methods, the nutrient content of frozen beans, sweet corn and peas was similar to fresh vegetables that had been cooked by boiling. Frozen vegetables were higher in vitamin C, riboflavin and thiamin content than canned vegetables [78]. Processing foods prior to freezing can result in vitamin losses through oxidation and leaching, with heat being a secondary factor [79]. Generally, vitamin C has been used as a nutritional and quality marker for monitoring the effect of manufacturing processes on nutrient losses, therefore it has been the focus of many studies in food [81]. Vitamin C usually refers to the sum of both ascorbic acid and dehydroascorbic acid, since both are active forms of the vitamin [79], although studies have shown that ascorbic acid has been shown to have faster degradation rate than dyhydroascorbic acid [45]. Beta-carotene is also commonly used to monitor the effect of processing on nutrient losses.

Blanching of foods prior to freezing can contribute to vitamin loss. The loss of 47% ascorbic acid and 10% vitamin B6 in commercially processed french fries during freezing was attributed mainly to the blanching process; the decrease caused by other unit operations was insignificant [81]. Steam blanching is generally better for maintaining vitamin activity than hot water blanching. Large-size cut french fries had better vitamin retention than small-size french fries because leaching was reduced. The effect of alternate freezing and thawing of peas, lima beans, corn and green beans increased the destruction of ascorbic acid. This may have been due to an increase in the concentration of ascorbic acid and oxidative catalysts in the fluid of the tissue matrix [82].

The use of blanching to preserve nutrients depends largely on the type of food. Red peppers and kiwifruit, usually frozen without blanching, showed no significant loss of ascorbic acid during freezing [83]. In contrast, the ascorbic acid content of unblanched okra and beans decreased by about 50% during freezing, while after blanching there was no significant change in the ascorbic acid content. The maximum decrease in ascorbic acid content in this study was observed at −1°C to −8°C [83]. Another study showed that even though blanching reduced the vitamin C content of beans by 28%, it reduced further losses during twelve months of frozen storage [84]. Unblanched beans and peppers lost more than 97% of their vitamin C content within one month of freezing, strongly indicating the need for blanching of vegetables before frozen storage in order to preserve nutrients. Similar results were obtained for broccoli and green beans [85]. Vitamin C could be the shelf life limiting factor, where it was shown that beyond the 45th day of storage, even when the sensory parameters are well retained, the nutritional parameter were at diminished levels [45].

G. Minerals

Minerals in food matrices may be present in many different forms such as chemical compounds, molecular complexes and even as free ions. Minerals present in any form can dramatically affect the color, texture, flavor and stability of foods [86]. Minerals cannot be easily destroyed by exposure to heat, light, oxidizing agents or extremes in pH, but they can be lost through leaching or by physical separation of components. Even so, there were no changes observed in six mineral elements (Ca, Cu, Mg, Mn, Ni and Zn) between fresh and frozen artichokes, green beans and peas [87]. Furthermore, the mineral content of boiled fresh vegetables was shown to be similar to boiled frozen vegetables [78].

III. CRYOPRESERVATION OF FROZEN FOOD

In the previous section different techniques were discussed for reducing the rate of chemical reactions in foods, such as lowering the temperature, blanching and modified atmosphere packaging. In conjunction with these techniques, further reductions in the rate of these reactions can be achieved by cryopreservatives, which can be natural components of the food or added as a pre-freezing treatment. Cryopreservatives can be classified as cryostabilisers or cryoprotective agents. Cryostabilisers are added to increase the viscosity of the frozen system while cryoprotective additives are used to change or stabilize the structure of components that are susceptible to undesirable changes caused by freezing. The variety of frozen products may require special “cocktails” of cryopreservatives to be developed for each individual product [93].

The underlying principle of cryostabilization is based on increasing the viscosity of the frozen system, which

controls the diffusion of solutes and therefore the rate of quality deterioration [94, 95]. When food is frozen, ice is formed and the solutes are concentrated in an unfrozen matrix. At the temperature of maximum freeze-concentration, where no more ice is formed, the unfrozen matrix undergoes a transition from viscous rubbery to a highly viscous glassy state. This temperature is called the glass transition temperature (Tg′) [96]. The extremely high viscosity associated with the glassy state limits the mobility of solutes and therefore, reduces the rate of diffusion-controlled reactions.

Various types of reactions respond differently to this change in viscosity. Simple diffusion-controlled reactions, which have low activation energies (8 to 25 kJ/mol), are especially sensitive to changes in the viscosity [97, 98]. The rate of lipolysis, lipid oxidation and diffusion of ^{14}C-fructose were studied in mackerel mince at temperatures between −5°C and −20°C [99]. The results indicated that the diffusion of ^{14}C-fructose and the production of thiobarbituric acid–related substances (TBARS) in mackerel mince decreased dramatically when the temperature was just below the Tg′ of mackerel. Similar results were observed in reformulated kumara (sweet potato) frozen paste products [100]. However, the rate of lipolysis and accumulation of peroxides were only moderately influenced when stored below the Tg′ of mackerel. Other studies have shown that ice recrystallization is reduced at temperatures below the Tg′ of the unfrozen matrix [95, 101].

The rate of diffusion-controlled reactions has been studied in frozen systems by adding maltodextrins with high Tg′ values (Tg′ ranged from −10°C to −18°C) and carboxymethylcellulose (CMC, Tg′ of −12°C) [102]. It was shown that in frozen systems containing maltodextrins the diffusion-controlled reaction rates were reduced at temperatures below their corresponding Tg′, but frozen systems containing CMC were not reduced. These results showed that even though the adding of selective compounds with a high Tg′ may reduce the rate of diffusion controlled reactions but may not always be true for all.

Typical cryostabilisers are high MW compounds, such as starch and starch hydrolysates, which elevate the Tg′ of the product [94, 95]. In general, low molecular weight components have a lower Tg′ than high molecular weight components [95]. The glass transition temperature is also affected by structural variation, such as polymer branching or linking of monomers [95]. For instance, the Tg′ for various disaccharides with a MW of 342 g/mol can differ by up to 7 K [95]. Table 114.2 summarizes the Tg′ and dextrose equivalent (DE) values for starch hydrolysates.

Cryoprotective agents are widely used to protect protein from denaturation [93, 104]. The additives interact with the surrounding water of the protein and create a preferential hydration of the protein and thus protects functional groups in protein from dehydration [104, 105]. The structure of cryoprotective compounds is diverse. Several small sugars, polyols, amino acids, nucleotides, salts and even surfactants have been shown to stabilize protein at sub-ambient temperature [93]. Researchers have carried out extensive studies to evaluate the effect of different cryoprotective agents and freezing conditions on the preservation of gamates of different origin [106–108]. Such studies could be considered in the future for freezing preservation of food products.

TABLE 114.2
Tg′ and DE Values for Sugars and Polysaccharides [103]

Solute	Tg′ (°C)	Dextrose Equivalent (DE)
Glucose	−43	100
Maltose	−31	53
Star Dri 42F[a]	−27	42
Maltotriose	−24	35
Maltoheptose	−13	16
Star Dri 20[a]	−13	22
Star Dri 5[a]	−10	5

[a] Star Dri 5–42 corresponds to the dextrose equivalent, that is, the higher the number the smaller the average molecular weight.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Frozen food: components and chemical reactions," by M.H. Lim, J.E. McFetridge and J. Liesebach. In *Handbook of frozen foods*, Editors: Y. H. Hui et al., 2004, Marcel Dekker, NY.

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115 Frozen Meat: Quality and Shelf Life

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CONTENTS

I. INTRODUCTION

The main objective of freezing is to prolong shelf life of foods. On this basis, freezing is considered an excellent means of maintaining acceptable quality in red meats for months and even years.

Currently consumers prefer chilled to frozen red meat, probably because of a desire on the part of the purchaser to assess several meat quality attributes, such as ratio of bone to muscle, presence of fat, and the like (49). Such an assessment is difficult when meat is frozen. Despite this, freezing and frozen storage of red meat are gradually increasing. Frozen storage is regarded as a useful technological aid because it makes possible the supply of large amounts of meat for catering and supermarkets, extends the storage life of meat when supply exceeds demand, and represents a flexible tool for sellers.

The quality of red meat brings together sensory (i.e., texture, flavor and color), microbial, nutritional and functional characteristics. In most cases, freezing does not modify quality in any considerable way. However, frozen meat may present defects in quality and naturally can suffer deterioration in quality during storage. Colour, flavour and texture defects, together with moisture losses, are the principal factors to which due regard must be paid.

Colour and flavour can become altered during storage, with lipid and myoglobin oxidation being the main adverse changes affecting the eating quality of frozen red meats.

With respect to texture, there are two circumstances having detrimental effects on the quality of frozen meat, both related to a significant decrease of temperature in prerigor meats. These are cold shortening and thaw rigor, which lead to toughness. In order to avoid these processes the advice has generally been to freeze post-rigor, instead of pre-rigor, meat. Nevertheless, pre-rigor freezing is also possible, provided that preventive measures are brought into play, such as electrical stimulation and tempering during thawing.

In addition, frozen meat production implies some profit losses related to the reductions in weight resulting from freezing. Generally, in comparison with rapid freezing, slow freezing produces greater damage to the muscle structure, resulting in a decrease in myofibrillar protein solubility and water holding capacity (WHC) and an increase in weight losses on defrosting or thawing (44).

The principal factors affecting frozen meat quality include the intrinsic quality of the meat, chilling and freezing conditions, and protection and storage conditions throughout the cold chain (17, 20, 38) (Table 115.1). These

TABLE 115.1
Main Considerations Influencing Frozen Red Meat Quality

Pre-Freezing:	species, genetic, livestock, feeding — lipids, vitamin E –, transport and slaughtering, postmorten processes — electric stimulation, chilling, hot deboning, freezing pre or post-rigor, ageing –, quality of raw materials including microbial status, type of muscle, processing of meat — cutting, cooking, packaging.
Freezing:	freezing rate, freezing technology.
Frozen Storage:	temperature, time, temperature fluctuations.
Thawing:	rate, technology, tempering or cooking of frozen meat.
Culinary Elaboration:	technology, temperature, time.

Adapted from Genot (17).

factors are not independent of one other. The effects on the eating quality of red meats of ageing time of meat before freezing, the freezing rate and the cooking method used, and also on thawing and cooking losses, showed a large number of significant interactions (28).

In relation to the effect of storage temperature on the quality of frozen foods, glass transition temperature under conditions of maximal freeze concentration (with a slow-frozen sample), Tg', is a potential means of assessing the stability of foods that deteriorate as a result of diffusion-limited events. In the case of beef, apparent Tg' values were approximately $-12°C$ (6). This temperature seems also to be the lower limit for microbial growth in meat.

In this chapter, the topics reviewed are the structure, moisture losses, microbiology, organoleptic properties, nutritional value and shelf life of frozen red meat.

II. STRUCTURAL DAMAGE IN FROZEN MEAT (EFFECTS OF FREEZING RATE AND FREEZER TEMPERATURE)

Muscle cells are flexible and elongated, share their alignment, have minimal air spaces and are separated by a matrix rich in glycoproteins. Although muscle cells are difficult to rupture by freezing and thawing, freezing and frozen storage can produce marked effects on the structural properties of red meat tissues, with cell-cell separation being the most evident structural change.

The freezing point in lean red meats is approximately $-1.5°C$, depending on meat composition. After this point, as temperature decreases a higher proportion of water turns into ice crystals. At temperatures below $-7°C$ more than 80% of the water is frozen. Water outside the muscle fibres freezes first, thus intracellular water tends to be drawn out of the fibres by osmotic effects, increasing the intracellular concentration of solutes. The consequences of this cryoconcentration (i.e., changes in osmotic pressure, pH, ionic force, viscosity, *Aw*) trigger the denaturing of and damage to muscle (i.e., myofibrillar and sarcoplasmic) protein, which progress with longer time and lower temperature of frozen storage (23). These changes in the functional properties of proteins may be responsible for changes in texture, colour and flavour. Finally, as solutes reach saturation point two outcomes are possible: the formation of eutectics or of a supersaturated metastable rubbery phase (glass transition). According to Brake and Fennema (6) the formation of eutectics during the freezing of meat is uncommon.

Freezing rate has an important influence on crystal size, location (intra- or extra-cellular) and morphology. Slow freezing rates (i.e., a rate of advance of the frozen front below the range 0.1–0.2 centimetres per hour) in matured meats produce large and, according to Grujic et al. (21), for the most part extracellular crystals, which damage muscle proteins and cell membranes, causing considerable distortions in tissue microstructure. Thus, the WHC of the meat decreases and the exudate produced during thawing increases. At intermediate freezing rates (from 0.2 to 1.0 cm/h) medium-sized ice crystals are formed both inside and outside cells. In this case cellular damage and drip losses are also high. By contrast, fast freezing rates (1.0 to 10.0 cm/h) bring about the formation of numerous small crystals, mainly intracellular, uniformly distributed. In this case, there is a minimum of physical harm to cells and of chemical changes to proteins (15). However ultra-fast freezing (in excess of 10.0 cm/h) may cause freeze-cracking in joints of frozen meat, due to sudden changes of volume. The full effects of fast and slow freezing on the eating quality of frozen meat are still unclear. In general, it is considered that freezing rate has only limited effects (17).

As storage times get longer ice crystals undergo gradual growth (39). Temperature fluctuation is considered a major stimulator of crystal growth through inducing recrystallization. Crystal growth is associated with a decrease in the WHC of meat protein, an increase in tissue damage, and a subsequent increase in drip losses from thawing meat.

In order to reduce structural damage during freezing, a number of cryoprotectants have been used in meat, including aspartate, cystine and β-alanine, sugars, dicarboxylic acids, phosphates and starch hydrolysis products. Unfortunately, the use of cryoprotectants has sometimes been associated with a sweet taste or lipid oxidation defects in meat. Nonetheless, these substances are used in cut, minced and restructured meat, and some of them have even been incorporated into meat by injection in live animals before slaughtering (14). Hence, a topic of great research interest is the application of antifreeze glycoproteins found naturally in cold-water fish or similar substances, incorporated in meat by brining or injection. These substances have the ability to make crystals smaller in size, to inhibit recrystallization and so to reduce drip loss.

III. MOISTURE LOSSES

During freezing, storage and thawing, meat loses water by evaporation, sublimation and exudation, respectively; moisture is also lost during cooking. Moisture losses are of great monetary importance. Although moisture losses make meat less attractive, they do not significantly influence its eating quality after cooking, except in the case of very large losses, which could affect juiciness and tenderness (17).

Evaporation losses depend on freezing conditions, as relative humidity (RH) and temperature, and meat characteristics, as the size and area-to-volume ratio of pieces, fat covering, and the presence of skin or packaging. Moisture losses by evaporation during freezing of non-packed carcasses or joints normally amount to between 0.5 and 1.2% of the total weight (17).

Sublimation during storage is another important cause of moisture loss. Greater weight losses occur when there is a higher air speed, more surface of lean exposed, longer storage or a higher temperature. Monthly losses of 0.15% to 0.7% were found in traditional stockinette-wrapped meat stored at −30°C and −10°C, respectively (9). In general, moisture loss increases when temperature fluctuates, that is, when it rises and falls by more than 2°C. In the extreme case, a severe local loss of water through sublimation may cause freezer burn.

The exudate formed during thawing (purge or drip loss) generally accounts for between 1% and 5% weight of meat pieces (17). Drip loss has a negative financial impact on processors, makes the meat visually unattractive, and also involves loss of soluble nutrients. Amounts of drip loss from frozen meat are determined by two main factors: the volume of the exudate generated during defrosting and its migration speed.

The former depends on the freezing and thawing conditions and features intrinsic to the meat itself (4, 33, 41), the water-binding capacity of proteins being involved in both. The latter depends on the size and shape of the pieces of meat, the myofibrillar orientation and geometry, the presence of large blood vessels and the intensity of physical cellular or macroscopic damage (freeze-cracks) produced during freezing, that is, with fast freeze rates.

As previously mentioned, drip loss has been associated with cellular damage caused by large crystals and extracellular crystal presence, these crystals being formed at slow and intermediate freezing rates. Furthermore, the water from the extracellular crystals formed seems not to be reabsorbed on thawing, thus producing drip loss. Hence, intermediate and slow freezing rates (those in which the time taken for the temperature to fall from −1°C to −7°C exceeds 10 minutes) show greater drip loss than faster rates (4). Within this range, these authors found a peak in drip losses in defrosting at a rate of 15–20 min. In the light of the effect of freeze rates on drip loss, several authors recommend freezing rates in the range 2.0 to 5.0 cm/h (17, 21).

When industrial freezing methods based on thermal gradient are used, large volume carcasses and joints undergo different freezing rates depending on depth, so that the thermal centre shows larger and more numerous extracellular ice crystals, which would lead to a poorer quality product (34, 47). New methods like high-pressure-assisted freezing (for example at a pressure of 200 MPa) would be necessary to overcome these difficulties (39). Pressure brings the freezing point of water down to a minimum of −22°C at 207.5 MPa (8). Thus, meat would be cooled to −20°C under pressure, so included water remains in its liquid state, and then pressure would be released, so that an instantaneous and homogeneous micro-crystallization would occur.

In addition, high and fluctuating temperatures and long storage favour large crystal formation and hence increased drip loss. Drip losses in vacuum packed pork, beef and veal are reported as 1.2%, 4.2% and 2.2%, respectively, after four months of storage (17). However when storage was 16 months the losses were 4.7% for pork and more than 8% for beef and veal. In other work, it has been reported that drip losses from fast-frozen and stored meat increase with storage time, reaching the same level as the drip losses observed from meat frozen at a slow rate but not stored for any length of time (42).

Packaging also shows an influence on drip loss, vacuum and oxygen-impermeable packaging being the most effective. For instance, the volume of exudate from cut pork kept frozen for 13 weeks at −17°C was between 2.5% and 5.0%, in accordance with the packaging characteristics (7).

Moreover, thawing time seems to be correlated with extracellular water re-absorption, which is a slow process. This might be the reason that faster thawing rates (defined as involving a time of under 50 min. for the temperature to rise from −5°C to −1°C) produced more drip loss in frozen beef (19). In contrast, other authors found fast thawing rates resulted in smaller drip losses (41, 42). The most recent suggestion has been that slow thawing causes more structural damage through recrystallization (2) and more protein denaturation. These authors recognized that fast freeze rates were more suited to fast thaw rates and vice versa.

Several intrinsic factors have been noted as affecting drip loss: species, type of muscle, pH, which has an inverse correlation with drip loss (33), pre-rigor and post-rigor freezing (the latter presenting more exudate in the case that thaw rigor is avoided) or ageing time (matured meat gives less drip loss).

To sum up, under industrial freezing conditions, storage time, temperature fluctuations and the intrinsic characteristics of the meat, rather than freezing rate, seem to have the biggest effects on drip loss.

Finally, cooking loss from frozen meat depend principally on the processing of meat before freezing, especially rigor onset temperature (10), and on the cooking method,

particularly the cooking temperature. Although cooking loss is accepted as being higher when freezing rates are slow (see review of Genot), the effect of freezing rate on cooking loss seems to be slight (13).

IV. MICROBIAL EFFECTS (HYGIENE)

Low freezer temperatures (i.e., below −18°C) bring about a practically total inhibition of the cellular metabolism in animal tissues and substantially decrease the rate of almost all chemical reactions. Microbial development in meat virtually comes to a stop when a temperature of −12°C is reached (33), this being the lowest point reported as allowing growth of moulds on meat. In this way, mould spoilage may be the most substantial problem affecting frozen carcasses that have been subjected to temperature abuse.

In general, a large proportion of the microbiota of a foodstuff will be killed or sublethally damaged by freezing (owing to thermal shock, ice formation, dehydration or solute concentration). In fact, a decrease in aerobic spoilage organisms has commonly been observed in frozen meat (25). Freezing process and animal species involved influence the microbial counts. Faster freezing rates have been demonstrated to have a less injurious effect than slower ones, and in addition, the longer the storage time, the more serious the damage.

Among the various organisms, gram-positive bacteria are generally more resistant to freezing than gram-negative, cocci more than bacilli and yeast and moulds more than bacteria. Bacterial and fungal spores and toxins of *Clostridium botulinum* and *Staphylococcus aureus* are highly resistant to freezing (18). In any case, pathogenic micro-organisms are commonly isolated from thawed frozen meats.

Furthermore, freezing has a sanitizing effect on frozen red meat. Freezing can be used to destroy larvae of *Taenia* spp. and *Trichinella spiralis*. These organisms are killed after 1 to 3 weeks at −18°C or after an ultra-rapid freezing at −29°C (48).

Nonetheless, defrosted meat seems to spoil faster than chilled meat because of the damage to tissues and because the exudate formed enhances microbial growth (17). Thus, the thawing process represents a fundamental phase with respect to the microbiological quality of thawed meat. In this way, increases in bacterial numbers become unacceptable in the air thawing of beef and pork carcasses or quarters if temperatures in excess of 10°C are maintained (20). Also, the time for which meat surfaces are at temperatures in excess of 1°C must be kept short if pathogen development is to be prevented. In this respect, tempering meat pieces during thawing at temperatures between −5°C and −2°C, and processing the meat (undertaking operations such as cutting, mincing and the like) at these temperatures may be really advisable. Apart from thawing, microbial counts in frozen meats depend on the initial level of microbial contamination and contamination during freezing and storage.

Microbiological status of good-quality meat (a satisfactory standard has aerobic plate counts at 25°C from the surface of frozen prime joints of beef under 10^5 colony forming units per square centimetre (cfu cm^2) and coliform counts under 10^3 (26); and in frozen minced beef under 10^5 and under 10^2, respectively (25) is largely due to hygiene in dressing and butchery of the carcasses, short time and low temperature of chilled storage, high freezing rate, proper packaging and so forth.

V. ORGANOLEPTIC PROPERTIES OF FROZEN RED MEAT

A. Texture (Cold Shortening and Thaw Rigor)

In frozen red meat, changes in the main attributes of meat texture, namely tenderness and juiciness, depend principally on changes in myofibrillar and stroma proteins (rigor shortening, denaturation, crosslinking, linking between proteins and free fatty acids or lipid auto-oxidation products and similar). These changes affect the functional properties of proteins (WHC, solubility and enzyme activity) throughout freezing and thawing processes. The severest effects appear at low freezing rate and long-time-high-temperature storage (46, 51). However, the mechanisms of protein denaturation and other changes in frozen muscles are still not fully understood, and their real effects on meat eating quality have not been totally established (37).

The freezing process for post-mortem meat in industrial practice seems to have only a slight effect on tenderness and juiciness. These attributes are more dependent upon the intrinsic factors of meat, specifically maturation before freezing, which improves tenderness. On this point, it has been found (50) that beef frozen after 14 days of ageing was the tenderest.

In any case, there have been several instances in which meat frozen then thawed was found tenderer than chilled meat (30, 50). This may represent an advantage for beef and lamb eating quality. The tenderizing effect of freezing might be explained by tissue damage due to ice formation (16) or an improved ageing process in red meats resulting from a decrease in calpastatin activity (52).

On the other hand, pre-rigor freezing could effectively produce a detrimental effect on meat tenderness, if cold shortening and/or thaw rigor took place. Thaw rigor may account for 40% of myofibrillar shortening (33), which induces toughness. These effects are not frequent in entire carcasses of pork and beef, especially if they are covered by a layer of fat and the freezing rate is not too fast (33), for rigor occurring before the decrease in temperature becomes critical. However, in lamb carcasses and hot-deboned joints

both processes can occur. In these cases, lower freezing rates, i.e., attaining −1°C within 5 hours post-mortem (34), are responsible for cold shortening and higher ones for thaw rigor, which is the case of meat frozen before the onset of rigor mortis.

Nonetheless, cold shortening and thaw rigor can be prevented. Post-mortem electrical stimulation, which accelerates the glycolytic process and hence the onset of rigor mortis, has been used to, and can effectively, prevent cold shortening and also thaw rigor (16, 33). For instance, electrical stimulation of lamb carcasses prevented cold shortening at freezing rates such as those needed to attain −4°C within 12 hours post-mortem (17).

Besides, if meat is frozen before rigor onset, tempering the meat at temperatures between −2°C and −5°C for approximately three days during thawing can prevent thaw rigor. At these temperatures the ATPase activity is enough to trigger rigor in the frozen state, thus preventing shortening. ATPase activity has been observed even at −12°C (33). Another way of preventing thaw rigor consists of adding salt, ca. 1.5–1.8%, to meat pieces (5). Salt inhibits glycolysis and rigor mortis onset, thus the levels of ATP, pH, ionic force and WHC of meat remain high during the freezing and thawing processes.

To conclude, provided that the measures to prevent cold shortening and thaw rigor are brought into play, prerigor frozen meat can be better than post-rigor, that is, prerigor frozen meat has been reported to preserve to a great extent the functional properties of proteins and produce less drip loss during thawing and cooking (11, 53).

B. Flavour and Colour (Lipid Oxidation and Discoloration)

Raw meat has a weak or "flat" flavour, but contains flavour precursors from which more than a thousand volatile substances are generated during cooking by a complex sequence of chemical reactions. It appears that a freezing-thawing process applied to beef loin (50), or to red meats in general, has no significant effect on flavour.

However, during frozen storage the flavour of meat can be somewhat altered. Firstly, flavour seems to decrease and lose balance but does not become unpleasant. In this regard, Lawrie (33) pointed out that there is a gradual loss of the most volatile compounds even at temperatures below freezing. Also, flavour and flavour precursors which are soluble substances (reducing sugars, amino acids, peptides and nucleotides such as IMP and GMP) may be lost or degraded during freezing and, above all, during thawing. Equally, freezing-related protein changes can account for differences in their ability to bind taste and volatile compounds, thus modifying flavour perception.

Finally, after longer storage times unacceptable "off" flavours may be detected, normally rancid flavours, which are produced by numerous volatile compounds coming from lipid oxidation. This process can be avoided in full only if oxygen is completely eliminated and storage temperature is extremely low, i.e., under −60°C (31). Furthermore, flavour may be altered by interactions between several oxidation products and different flavour compounds, or hydrophilic peptides (27). Apart from flavour alteration, lipid oxidation may affect texture and nutritional properties of frozen meat. Lipid oxidation is definitely one of the principal limiting factors for frozen meat shelf life.

Theoretically, factors favouring or inducing meat lipid oxidation are numerous (traces of transition metals, sodium chloride, light, heat, protons, active oxygen species, damage to muscle structures, and enzymes). These enzymes (lipases, lipo-oxigenases and so forth) may remain active at sub-zero temperatures (12, 24). On the contrary, muscle possesses endogenous antioxidant capacity (involving α-tocopherol, carotenoids, ubiquinone, glutathione, carnosine, anserine, ascorbic acid and the enzymes with antioxidant activities, such as glutathione peroxydase, superoxyde dismutase and catalase). Storage of meat may result in a decrease in the activity of the antioxidant enzymes (35).

During freezer storage, when the balance of the factors favouring oxidation and of the antioxidant capacity of muscle foods tilts towards lipid oxidation, the latter is no longer controlled. In these circumstances lipid oxidation begins, first in the highly unsaturated phospholipid fraction in cellular biomembranes, thanks to its amphiphylic nature and proximity to the aqueous medium (20). In addition, triglycerides and cholesterol can be oxidised.

On the other hand, visual attributes such as red bright colour, fat colour, fat content (including perceived marbling and external fat cover) and general appearance serve as the first or only consumer indicators of the quality of chilled, and where possible frozen, red meat quality at the time of purchase.

Browning of meat during frozen storage and thawing is a consequence of myoglobin oxidation, which turns the pigment to metamyoglobin. If the percentage of metamyoglobin on the meat surface reaches a certain point (ca. 40% of total myoglobin), purchasers will reject meat. Accumulation of metamyoglobin depends on the relative rates of oxymyoglobin auto-oxidation and enzymatic or non-enzymatic reduction of metamyoglobin to oxymyoglobin. The rate of auto-oxidation of myoglobin is strongly dependent on temperature and exposure to light. Unlike chilling, where thermal auto-oxidation is predominant, under freezer storage conditions, light-induced processes become increasingly important (3, 36). Oxygen concentration is another important factor for colour stability. According to Lanari et al. (32), vacuum packaging and packaging material with incorporated light adsorber increase frozen red meat shelf life. Furthermore a blooming time (some 6 to 48 hours) in oxygen before freezing is advisable.

TABLE 115.2
Times of Storage (Months) at Which Lipid Rancidity or Browning Are Manifested in Lean Meat

	Storage Temperature			
	−8°C	−15°C	−22°C	−30°C
Beef	3	6	12	—
Pork without skin and fat cover	—	3	6	12

Adapted from Lawrie (33).

Colour instability together with lipid oxidation definitely constitute the principal limitations on frozen meat shelf life (Table 115.2).

Brown discoloration (metamyoglobin formation) and lipid oxidation in frozen red meats seem to be related (1, 40). Thus, Lanari et al. (32) and Guidera et al. (22) claimed that frozen meat colour stability and susceptibility to rancidity indicated the oxidation status of meat. Both processes demonstrate similarities in their progression in meat and meat products. Experimental results support the view that lipid oxidation contributes to oxymyoglobin oxidation and meat discoloration (43). However, in frozen meats, colour losses generally occurred much earlier than lipid oxidation (1, 3), these authors stating that pigment oxidation might be an initiator of lipid oxidation. In fact, the exact mechanism for a possible coupling between pigment oxidation and lipid oxidation is not well understood (20).

The susceptibility of specific frozen red meats to lipid oxidation and discoloration is influenced by intrinsic and extrinsic factors, which are shown in Table 115.3 (17).

Frozen meat colour at the surface may also be affected by excessive dehydration during storage, appearing as greyish-brown leathery spots: freezer burn, which leaves the meat dry and stringy. Moreover, dehydrated surface conditions enhance protein denaturation, lipid and colour oxidation. Freezer burn is not necessarily correlated with moisture loss in a simple manner (45). It tends to happen when meat surfaces are initially wet and freezing is very fast, when the lean meat surface is not protected, air speed in storage chambers is fast, and there are temperature fluctuations. In order to prevent freezer burn the recommendation is to prevent air from reaching the surface of the meat, mainly by using vapour-proof packaging materials.

Other defects in colour and/or aspect of frozen meats are yellow coloration of fat through lipolysis, deformations and freeze-cracks arising from sudden changes of volume during freezing, and mould development caused by temperature abuse.

Finally, meat may show different colours according to the size of ice crystals. When the freezing rate at the surface of the meat is high, crystals are smaller and scatter more light than larger ones, and then meat is more opaque and its colour lighter. In the case of red meats, fast freezing at very low temperatures may yield too pale a colour, thus reducing quality.

VI. NUTRITIONAL VALUE OF FROZEN RED MEAT

Red meats are considered as an important source of high quality food protein, minerals and B vitamins. Although freezing implies some losses in those compounds (specifically a loss of water-soluble nutrients in the exudate during thawing), the losses generally lack nutritional impact. Freezing can preserve the nutritional value of meat better than most other preservation methods. Vitamin loses are variable depending on the process. In general, the decrease in B vitamin content of frozen meats should be less than 25% for vitamin B1, 15% for B2, 10% for niacin and 20–50% for B6 or pyridoxine (17).

VII. SHELF LIFE OF RED FROZEN MEAT (CONCLUSIONS)

In terms of quality, freezing is one of the best technologies for preserving red meats. The main limitations on frozen red meat shelf life are the changes in sensorial attributes, specifically colour and flavour, due to pigment and lipid oxidation, respectively. Sublimation and drip loss during freezing and thawing may mean a considerable financial loss, however their influence on eating quality seems in reality to be slight (17).

In spite of the extensive literature on the shelf life of frozen meat, experimental data to support that body of theory seem to be scarce and rather old, based on freezing conditions that nowadays are old-fashioned. Furthermore, there is a lack of agreement among data on the storage life of industrial frozen meat (20). Nonetheless, general rules have been established. In this respect, it has been stated that the shelf life of red meat normally ranges from 8 months to 3 years (20), and likewise that beef and lamb can be stored at −18°C for at least 6–12 months and pork for 6 months. Moreover, frozen meat shelf-life data, defined as the practical storage life (PSL) recommended by the International Institute of Refrigeration (IIR), are shown in Table 115.4. In general, PSL seems to be two to five times high quality life (HQL) (20).

Quality problems in frozen meats subjected to extended storage are substantially reduced following some technological indications (specialist packaging, low (−18°C) and constant storage temperature). Packing/wrapping has a large direct effect on storage life. It can be effective in reducing oxidation, as packaging material can constitute a light and/or oxygen barrier, and dehydration. Fluctuating temperatures during storage are considered to be detrimental to the product.

Although appropriate freezing technology conditions appear to be more important than intrinsic meat quality in extending shelf life, it is nevertheless true that the best way to improve frozen storage life of red meats consists in an integrated package of measures including production, muscle to meat conversion and freezing technology.

TABLE 115.3
Intrinsic and Extrinsic Factors Influencing Lipid Oxidation and Discoloration in Frozen Meats

Factor	Several Significant Effects	Main Causes
	Intrinsic	
Species differences[a]	Pork is more sensitive than beef.	Pork has more PUFA[b] than beef.
Animal to animal variation: genetic, season, physiology		Not fully understood.
Feeding	The more the PUFA — or linoleic acid contents — in the diet, the more unstability, especially in monogastric. Higher content of antioxidant or prooxidant compounds in the diet represent an increase or decrease in stability, especially vitamin E.[c]	Meat composition: vitamins E and C, β-carotenes, carotenoids, prooxidants such as Cu and Fe, oxidative status of lipids in the feed.
Cuts of meat and muscle type	Red muscles are more unstable than white ones.	Oxidative pattern and composition differences, i.e., pH, % PUFA, antioxidant dipeptides, etc.
	Extrinsic	
Transport and slaughtering conditions	Stressed lambs carcasses with high pH were unstable to rancidity (23).	Variations in pH.
Chilling conditions, time and ageing	Increasing the time in chilling reduces frozen storage life. Hot-deboned frozen meat is more stable (47).	
	Processing of Meat	
Cutting and muscle disintegration,[d] structure damage	It favours the deterioration reactions contributing to reduce shelf life.	Enzyme/substrate contact is facilitated.
Salting	Salt contributes to unstability in frozen red meats, especially with light exposure (1).	Amplifies Fe reactivity in autooxidation, modifies enzymatic activity (35), may be a source of traces of prooxidant compounds.
Pre-cooking[e]	On the one hand, pre-cooking increases the propensity of meat to undergo oxidation during freezer storage. On the other hand, cooking may help to increase shelf life.	Cooking produces a structural change and accounts for liberation of prooxidant compounds.
Antioxidants addition in meat (mainly minced or restructured)	Tri-polyphosphates (TPF), fenolics — i.e. BHT, TBHQ, BHA, propyl gallate–, α-tocopherol, spices — i.e. rosemary, marjoram, cumin, ginger–, carnosine at 0.5–1.5% (w/w).	Antioxidant activity.
Packing/wrapping	Proper packaging[e] increased stability. This stability may represent the same as a decrease in 10°C temperature of storage — from −10 to −20°C (17).	Oxygen and light barriers.
	The Freezing Process	
Freezing rate	Freezing rates have not shown a significant effect.	
Storage temperature	The lower the temperature the higher the stability.	Oxidation reactions are temperature dependent.
Temperature fluctuations[f]	Oxidation is favoured.	
Thawing conditions	Lipid oxidation may suppose a problem in long thawing processes at sub-zero temperatures (33).[g]	

[a] Lipid oxidation has been an important problem in pork as well as comminuted lamb and beef (23, 46).

[b] Unstability of frozen meat to lipid oxidation depends mainly on poly-unsaturated fat acids (PUFA) content.

[c] Supplemented vitamin E in diet — usually α-tocopherol — at supranutritional level, i.e., >100 mg/kg feed during 50 days before slaughtering, accounts for a significant increase of vitamin E content in muscle, reaching levels of 3.5–7 μg/g. At this amount vitamin E appears to delay oxidation processes during frozen storage. This protective effect against oxidation of vitamin E might be reinforced with added antioxidants and vacuum packaging in order to eliminate practically the risk of oxidation of meat. Furthermore additional amounts of vitamin E in diet were responsible for decreased drip loss in pork.

[d] Mechanically deboned meat is especially unstable.

[e] The thumb rule to achieve a long shelf life of cooked-frozen meat consists on oxygen and lights barrier-packaging materials, i.e., aluminium foil, polivinidene chloride and storage below −18°C.

[f] Temperature fluctuations cause the rate of several changes to increase or decrease, especially in the vicinity of the freezing point, the glass transition point, and other phase transition temperatures.

[g] Thawing must be fast enough to delay oxidation reactions but slow enough to permit water re-absorption.

TABLE 115.4
Practical Storage Life Expressed as Months

Product	−12°C	−18°C	−24°C
Beef carcasses	8	15	24
Beef steaks/cuts	8	18 (max. 12)	24
Ground beef	6	10 (max. 10)	15
Veal carcass	6	12	15
Veal steaks/cuts	6	12	15
Lamb carcasses	18	24	>24
Lamb steaks	12	18 (max. 10)	24
Pork carcasses	6	10	15
Pork steaks/cuts	6	10 (max. 6)	15

Adapted of Gray (20).

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Frozen meat: quality and shelf life," by M.L. Pérez-Chabela and J. Mateo-Oyague. In: *Handbook of frozen foods*, editors: Y. H. Hui et al., Marcel Dekker, NY, 2004.

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116 Principles of Frozen Storage

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CONTENTS

The use of freezing for food preservation has rapidly developed; the fact that products, mainly meat and fish, could be stored for considerable periods and served, after thawing, as fresh products, is at the origin of its development as one of most common methods for food preservation.

Freezing implies two linked processes: a lowering of temperature and a phase change of water from liquid to solid. Both processes tend to reduce rates of physical and chemical changes and might be expected to enhance the shelf life of the products.

At the same time, quality degradation during storage remains the most common problem for manufacturers and for bringing food freezing to the fullest possible extent. So it is vital to understand well the essentials of the technology. Freezing seldom does not improve the quality of food products; the raw material quality is of primordial importance, and this quality must be preserved during processing and storage. There is no single universal rule governing frozen food preservation; just as with optimal freezing rates, which vary from product to product, the storage time depends not only on the temperature but also on the type of product and packaging. Most physical and chemical reactions are slowed with the decrease in temperature, but they are not stopped at common storage temperatures. The product deterioration during cold storage is a slow, continuous, cumulative, and irreversible process.

The physical properties of food products change dramatically depending on water availability and tempera-

ture. The major assumption relating to quality, and thus shelf life, is that stability is maintained in the glassy state. In a glass, the diffusion of solutes and degradation reactions should be strongly limited, and long-time stability during storage expected. The shelf life of frozen food products should be largely controlled by the physical state of the freeze-concentrated fraction produced by the ice separation. It is desirable to better know how this physical state changes with temperature.

I. PHYSICAL STATE AS A FUNCTION OF TEMPERATURE

A. Physical Changes during the Freezing Process

The freezing of pure water takes place at 0°C (freezing point); this temperature is steady as long as the liquid and solid phases coexist because the chemical potentials of two phases are equal. The freezing of food products is more complex, due to the presence of their soluble and dispersed components. The water begins to freeze at lower temperature than 0°C (freezing point depression); the ice formation increases the solute concentration, reducing the chemical potential of water, and therefore requires a decrease in temperature which depends on the product composition.

For practical purposes, the freezing process is considered complete when most of the water at the center of the food product has been converted into ice. At −15°C, more than 80% of total water is transformed into ice [1]. The system is segregated into a crystalline phase of pure water and an amorphous domain, which contains solutes and residual water. As the temperature decreases, the viscosity of the interstitial fluid increases rapidly as a result of both concentration increase and temperature decrease. When the viscosity reaches 10^{11}–10^{12} Pa.s, a solidification (vitrification) occurs, and the concentrated phase surrounding the ice crystals becomes a glass. The temperature at which this transition appears is called Tg′, the glass transition temperature of the maximally freeze-concentrated system [2]. The freezing of water is stopped at this temperature; the water still unfrozen at Tg′ is often called "unfreezable" water.

Since the water content of foods is often close to 80–90%, its behavior during the freezing process can be considered as that of an aqueous solution. The proposed model is often a sucrose solution, which could be representative of the freezing behavior of a wide range of solutions when no solute crystallizes during cooling. That can easily be checked on fruits, fruit juices, vegetables like tomatoes, and ice creams which show very similar thermal changes. The equilibrium temperatures and concentrations vary with the nature and composition of the product. If cooling is slow enough compared to the kinetics of ice formation, the concentration in the liquid phase depends only on the temperature for a given product and is independent of its initial water content. As a consequence, Tg′ is also independent of the initial water content.

B. Determination of Tg′

Differential scanning calorimetry (DSC) is the technique most often used to measure the glass transition temperature; this transition appears as a typical heat capacity jump on the DSC traces and can be characterized by different temperatures (Figure 116.1). In the presence of ice, the glass transition, which is visible just before the ice-melting peak on the heating scans, appears more complex, presenting a larger heat capacity change than predicted from the solid content in macromolecule frozen solutions [3]. For frozen solutions of small solutes (sugars, polyols), the thermograms show a two-step endotherm (Figure 116.2). These features appear to be very similar to those observed with synthetic polymers and are interpreted as a glass transition with associated enthalpy relaxation [4,5]. There is still no agreement on how the DSC data should be interpreted. The

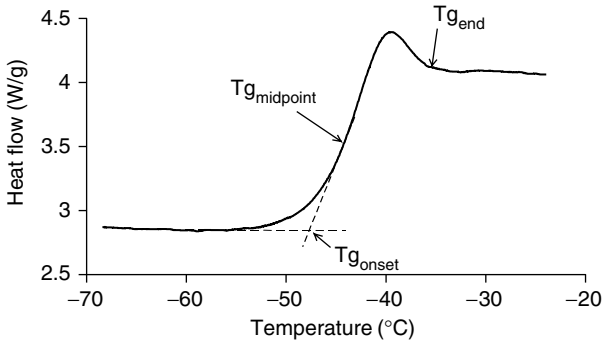

FIGURE 116.1 DSC thermogram of a 80% sucrose solution (cooling and heating rates: 10°C/min).

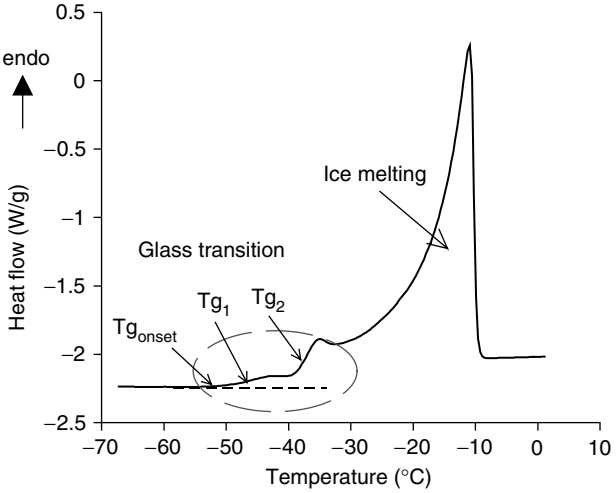

FIGURE 116.2 DSC thermogram of a frozen sucrose solution (50% w/w) (cooling and heating rates: 10°C/min).

reported Tg' value, according to some authors, is the temperature of the midpoint or the onset of the first transition; for others, it is the midpoint of the second step. They are called Tg_{onset}, Tg_1, and Tg_2 respectively, on Figure 116.2. This explains the variations in published Tg' values [6].

Because of the ambiguity of the Tg' determination from simple DSC traces, it was suggested to use simplified state diagrams of solute-water blends, which represent their different physical states in the low-temperature range. The two curves, corresponding, respectively, to the equilibrium liquid/solid or ice melting curve (*Tm* curve), and the kinetically controlled glass transition curve (*Tg* curve), are drawn. The diagram representing the various states of the sucrose-water system as a function of concentration and temperature is appropriate to illustrate the demonstration (Figure 116.3). The curve *Tm* represents the temperature at which ice begins to separate as a function of initial concentration, or the concentration of the freeze-concentrated phase as a function of temperature. For all points on this curve, the freeze-concentrated phase is in equilibrium with ice; its partial water vapor pressure is equal to that of ice at the same temperature. For soluble solutes, the temperature of the glass transition depends on the water content; it decreases when the water content increases. The intersection of the two curves could provide a better estimation of Tg' [7]. The freezing of water is stopped at this temperature; the concentration of the maximally freeze-concentrated phase is called Cg'.

There are obvious problems with the accuracy of the *Tm* and *Tg* curves. The experimental *Tm* values as obtained by DSC for the most concentrated solutions are considered as more or less reliable; an extrapolation of the *Tm* curve can be subjective; therefore the curve represented in Figure 116.3 is derived from excess properties and solid-liquid equilibrium using a UNIQUAC model [8]. For the *Tg* curve, the experimental data taken into account can be the temperature of the beginning Tg_{onset}, or the middle of the transition $Tg_{mid\text{-}point}$ (value most often used) or even of the end of the transition (see Figure 116.1 for sucrose solution). The cooling/heating rates contribute to some changes in *Tg* due to the time dependence of the glass transition feature. The DSC values of Tg_{onset} and Tg_1 of frozen sucrose solutions, in Figure 116.2, were found to be close to the intersection of the *Tm* curve with the Tg_{onset} and Tg_{mp} curves in Figure 116.3. The Tg'-Cg' coordinates vary from −45°C, 82.2% to −41°C, 81.2% [8] depending on the *Tg* value taken into account. Using the method of optimal annealing temperature, Abblet and coauthors have obtained very similar data in considering the mid-point: −40°C, 82% for Tg'-Cg' [9].

The liquid-glass conversion must be considered to occur in a temperature range that is characteristic of the fraction forming the glassy matrix. The coordinates Tg'-Cg' can be far away from the eutectic point predicted by equilibrium thermodynamics; for example, with sucrose-water binary *Te*, *Ce* are −14°C, 64%, respectively.

Thermomechanical spectroscopy (DMTA) studies of frozen sucrose solutions show that the change in mechanical properties, obtained with low sollicatations, becomes perceptible at Tg_{onset} as seen by DSC. A maximum in the loss modulus (E″ or G″) occurs at a temperature between Tg_1 and Tg_2 [10]. Apart from its scientific interest, the sucrose-water phase diagram (Figure 116.3) could be considered as representative for a wide range of products containing low molecular weight solutes; very similar diagrams are reported for some fruits [11].

C. Storage Conditions and Shelf Life of Frozen Foods

The choice of the storage temperature is of considerable practical importance in the frozen food industries. It is

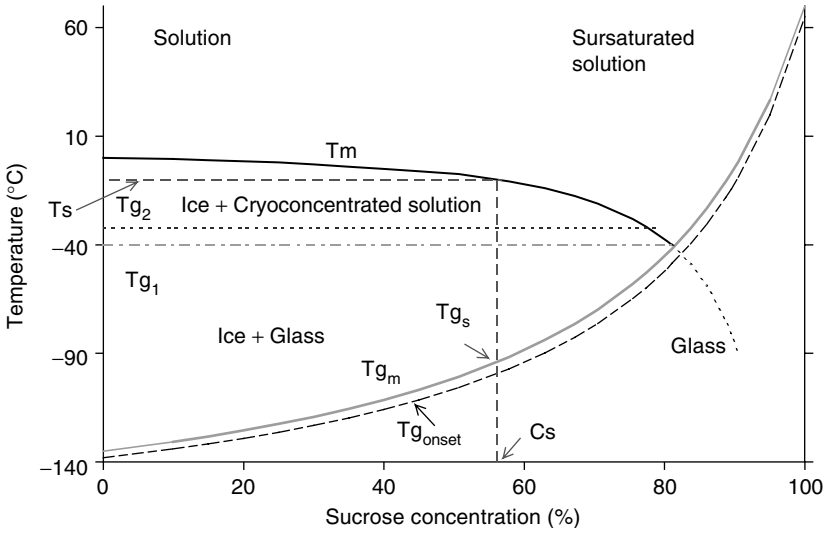

FIGURE 116.3 State diagram of sucrose-water binary, adapted from (8).

the major variable which can affect stability; a product retaining a good quality for months at −20°C can lose it in a few days at −10°C. Moreover, it is well demonstrated that frozen foods stored at fluctuating temperatures have not the same shelf life as products stored at constant temperatures [12–14]. The weakest links of the frozen food chain are handling time, temporary storage, and transport temperatures. The last link of the chain, the retail shops, is often critical. Frozen products delivered to the retail outlets are not always placed in refrigeration immediately; the problem includes temperature increases during defrosting. In Europe, where food stores are not open 24 h per day as in the United States, using night covers and programming the defrosting during the night may help to minimize the temperature fluctuations.

In order to ensure product quality, temperature control is necessary throughout the cold chain, and the required temperature must be maintained from production to consumption. European and international regulations concerning the storage temperature of frozen foods set −18°C as the highest temperature during storage and distribution, although many studies have shown that slow degradation of the product quality may occur even at this temperature. This temperature must be constant and maintained at all points in the product (therefore at the surface); possible brief upward fluctuations of 3°C maximum are admitted during transport, as well as a tolerance of 3°C during local distribution and in retail display cabinets [15,16]. To maintain an optimal quality, the products are generally held at temperatures colder than this in primary cold stores (−25, −30°C).

There is a clear effect of temperature on storage life, with lower temperatures resulting in extended shelf life; but −18°C corresponds to an optimum between the financial costs and the shelf life of frozen foods. As it is the same storage temperature for all frozen products, the marketing shelf life varies from 6 months to 2 years (Table 116.1) [1].

At this temperature frozen foods are neither fully frozen, nor inert, the Tg′ of most products being below −18°C. Table 116.2 shows the Tg' of some food products or more exactly the temperatures corresponding either to the second step of the transition (called Tg_2 on Figure 116. 2) or to the midpoint for a single transition. The Tg' data for solutions of numerous simple ingredients (sugars, polysaccharides, proteins) can be found in an article by Levine and Slade [25]. It must be emphasized that these temperatures, and consequently the glassy state, is readily attained by a very limited number of frozen food products at temperatures commonly employed in the distribution chains. That means that the freeze-concentrated phase is more or less fluid, and solute mobility may be significant. Frozen storage usually continues for long periods, so products undergo deterioration, which can be as important as those due to the freezing and thawing processes. The underlying reason for necessary extensive studies about change of quality during storage is that the causes of deterioration are not known enough to be able to propose efficient predictive models.

TABLE 116.1
Practical Storage Life (PSL) in Months at Several Temperatures

Product	−12°C	−18°C	−24°C
Beef steaks/cuts	8	18	24
Ground beef	6	10	15
Pork steaks/cuts	6	10	15
Fatty fish (glazed)	3	5	>9
Lean fish	4	9	>12
Butter lactic unsalted	15	18	20
Butter, lactic salted	8	12	14

Source: Adapted from IIR-IIF (1).

TABLE 116.2
Tg_2 Values for Frozen Food Products

	Tg_2 (°C)	Reference
Lemon juice	−38	Maltini (17)
Orange juice	−34	Moreira (18)
Raspberry juice	−31 to −35	Moreira (18)
Strawberry	−43	Sa-Sereno (11)
Strawberry juice	−45	Torregiani (19)
Apple	−37	Guegov (20)
	−71	Sa (21)
Tomato	−21	Guegov (20)
Carrot	−32	Guegov (20)
Egg white	−38	Simatos (22)
Egg yolk	−32	Simatos (22)
Beef muscle	−85	Simatos (22)
	∼−70	Sator (23)
Tuna	−70	Inoue (24)
Commercial ice creams	−27.5 to −40*	Levine (25)
	−34*	Blond (26)
Model ice creams	−25 to −43*	Hagiwara (27)

*Depending on the recipe, particularly of the used sugars.

II. CHANGES IN FROZEN FOODS DURING STORAGE

The main factors affecting frozen food quality during storage can be divided into two categories: processing and compositional factors. The quality factors associated with processing parameters are mostly related to the ice phase. Defects associated with compositional factors are related to chemical reactions and affect flavor, texture, appearance, color, and nutritional properties.

A. Physical Changes

1. Water Migration

The main physical change that occurs during storage of frozen foods results from water migration. Solid/liquid and

especially liquid/crystal transformations can occur with temperature fluctuations, but important water motions can take place without temperature changes. There is always, inside a package or a product, some difference of water vapor pressure due to a temperature gradient or a surface energy difference. As water molecules are not completely immobilized by low temperatures [28], a significant redistribution can be observed in frozen products because of the duration usually involved for the storage. The water migration causes either water content changes or changes in ice crystal size called recrystallization. Water migration is highly temperature dependent, and it occurs at any storage temperature, although good conditions (e.g., low temperature, no temperature fluctuations) minimize its importance.

a. Migration with change in water content

Water migration can correspond either to a water loss through surface dehydration or to only a water location change for products with heterogeneous water contents.

The surface dehydration adds to dehydration suffered during the freezing process, which can vary from almost zero for packaged products and cryogenic freezing to 3–4% for naked products and poorly designed freezers [29]. These moisture losses have the same origin, i.e., a temperature difference between the product and the surrounding atmosphere, resulting in a difference of water pressure that produces a water molecular flux from the surface of the frozen product to a colder area. Ice sublimation progresses during storage, and it is more pronounced when the storage temperature is higher. A common example is observed with frozen fruits and vegetables that are not protected by transparent films in close contact with them. The films cool faster than the surface of the product, setting up a water pressure gradient; thus a small amount of vapor water migrates from the product to the inside surface of the package. When the freezer temperature increases, the process is reversed and the water vapor condenses on the product surface. The dehydration must be considered irreversible, reabsorption of water in the product being not possible; this removed water crystallizes and remains inside the package as frost. As this cycle is repeated, the buildup of frost in the package becomes noticeable, this effect being amplified by the frequency and magnitude of temperature fluctuations; more than 2°C fluctuations promote an important sublimation of ice. The consequences are more or less important depending on the products, as the water losses can reduce the weight of the product, thereby reducing the market value for large pieces of meat sold before transformation; overall, the dehydrated surface of a product reduces its appeal to the consumer. Meat appears darker when frozen, since oxygen cannot produce the bright red color of oxymyoglobin (its "natural" color returns on thawing). A surface dehydration on this product leads to the important defect known as freezer burn, particularly for meat carcasses, cuts, and poultry, stored without an adequate packaging. Dehydrated surfaces come in grayish patches, because the disappearance of ice crystals by sublimation forms small cavities on the surface that appear grayish because of the light scattering. Dehydration of unprotected fish can lead to irreversible quality loss when the fish surface becomes dry with a "woody" texture. This dehydration also contributes to increase the rate of rancidity and discoloration. The dehydrated surfaces of vegetables may also appear clearer before thawing, but color changes for these products are predominantly caused by oxidative reactions.

The dehydration effects almost completely disappear on thawing and cooking, except if the product has been stored in bad conditions and dehydration was too severe. But the appearance in the frozen state needs to be taken more into consideration, because it is decisive for the consumer considering whether to buy the product or not. Moreover, the melting of frost during thawing causes an unpleasant appearance when a wet layer appears on the product surface.

Today these defects are more rarely observed, because efficient packaging prevents products from dehydration. Each product to be stored in a freezer over an extended period should be wrapped. Moisture-impermeable films offer considerable protection against moisture loss, but products must be packed with a minimum head space to reduce frost; vacuum packing, which ensures maximum contact of film to product, suppresses all frost and also limits the thermal gradient. Its use is limited because it is more expensive and unsuitable for delicate products. Surface dehydration can also be limited by coating with a continuous film of ice or glaze. Glazing consists in the application of a potable water spray immediately after freezing, a thin layer of ice covering each piece (fish, shellfish, fruit, vegetable) as protective coating; the water amount is normally in the range of 5 to 10%. Ice glaze is considered to be a cheap means of protection for frozen fish. The glaze is also used for giving some mechanical protection to the delicate frozen florets of cauliflower and broccoli. The formulated coatings, which are added-value processing for fish and meat, are also a protection against dehydration.

More difficult to suppress is the water migration in frozen foods containing regions with large water content differences, generally prepared frozen foods that are a combination of several dissimilar components. There is no particular problem if each component contains ice crystals: the water activity is the same in the whole food. But when a part of the food has a low water content, and consequently contains no ice, there is a high water pressure gradient between the "dry" part and the ice of the "frozen" part, irrespective of temperature. The resulting water migration produces moisture redistribution. These local water content changes can be important, and detrimental, for the product quality. They are found in frozen pies, fancy cakes, and desserts. Water migrates from the filling to the crust

of frozen pies and pizzas or from ice cream to the wafer, and the crispy components become moist and soggy. In frozen bread, if a thermal gradient adds to the difference in moisture, water migrates from the center to the region just under the crust and then freezes to form an icy area, which produces trust separation from the crumb at thawing.

As the water vapor pressure is always higher in the part containing ice, edible barriers with low water permeability must be developed, based on hydrophobic substances such as lipids and waxes, to stop or at least slow down the water transfer. The top of the baked trust of pies or pizzas may be sprayed with shortening to keep the sauce moisture from soaking into the crust. Cocoa butter or chocolate coatings are widely used in the ice cream industry for protecting wafer.

b. Migration without water loss: recrystallization

The quality objectives of the frozen food industry are to set a homogeneous ice crystal distribution and to preserve it during storage. The initial freezing process, particularly when occurring rapidly, yields a highly dispersed crystal phase. This initial distribution promotes metastability: small crystals are thermodynamically less stable than large ones, since they have a higher surface/volume ratio and therefore a higher excess surface free energy. The size and consequently ice crystals number must change.

It is not sufficient to produce products with a specified structure through control of the nucleation and crystallization rates. During the storage not only the size and number of ice crystals change but also their shape and orientation. This process is known as recrystallization; it corresponds to water migration as a result of local water motions allowing molecular diffusion from one ice crystal to another, more often without change in ice content. Mazur [30] and then Fennema [31] described several mechanisms of recrystallization; they include migratory, accretive, isomass, and irruptive recrystallizations. Melting and refreezing (regelation) due to temperature fluctuations is also defined as a recrystallization process, which causes the most significant and rapid changes in ice crystal distribution. Except for irruptive crystallization, the mean ice content is not altered by these different mechanisms.

i. Migratory recrystallization

Migratory recrystallization, which is referred to as "grain growth," is also known as the Ostwald ripening. It corresponds to the tendency for the large crystals to grow at the expense of small crystals.

The equilibrium shape of a crystal is the one that minimizes its surface free energy (at constant temperature and volume of crystal); the free energy of a crystal is at a minimum when its structure is perfect and its size infinite. A difference in stability brings about the size difference at constant temperature. The thermodynamic basis is the Kelvin equation, which states that the chemical potential of a curved surface differs from that of a plane one, and relates the vapor pressure over a spherical solid surface to the radius of curvature (r) [30]:

$$\mu_2 - \mu_1 = RT \log p_2/p_1 = 2V\,\sigma\,[1/r_2 - 1/r_1] \quad (116.1)$$

where μ_2 and μ_1 are the chemical potential of the two crystals, p_2 and p_1 their respective vapor pressures, r_2 and r_1 their mean radii, V is the molar volume of ice, and σ the interfacial tension. The relationship between pressure and melting point (Clausius-Clapeyron equation) allows us to calculate the crystal melting point (*Tm*) as a function of its curvature radius. The undercooling due to the curvature radius decreases as the sphere size increases. For a given storage temperature there is one crystal size that is in equilibrium: a solution with small crystals could also be regarded as being undercooled as compared to the one with large crystals. For pure water, *Tm* varies from −0.004 to −3.99°C when the crystal radius decreases from 10 to 10^{-2} μm [32]. Different values can be found in the literature, indeed some parameters are difficult to estimate, particularly the interfacial tension.

ii. Accretion

Accretion, also termed sintering, is the joining together of two crystals or more to form one larger crystal. A neck is formed between two adjacent crystals, and this neck grows until the original crystals are indistinguishable. The chemical potential, and consequently the vapor pressure, of a spherical surface is greater than that of a concave surface (*r* is negative). Reducing the surface concavity can lessen this difference in chemical potential. The vapor pressure difference is always the driving force that explains the neck construction. Kingery (cited by Mazur [30]) found that this growth was strongly dependent on temperature: in pure water the neck growth rate decreased from 3.3 to 0.033 $\mu m \cdot s^{-1}$ when the temperature decreased from −2 to −25°C. When the ice volume is important, this mechanism is certainly dominant, the ice crystal contacts making migration easier. Sutton and coworkers [33] showed that the recrystallization rate was dependent on the ice volume, and the accretion rate decreased as ice phase volume decreased.

iii. Isomass recrystallization

Ice crystals generally tend to become more spherical through isomass rounding during storage even at constant temperature [34]. The driving force is also the water pressure gradient; the Kelvin equation can be used considering different local curvature radii due to the irregular surface. Experimental control has been made in model solutions [35]. In actual foods, the ice crystals are so closely packed that isomass crystallization may not be clearly identified, as water molecules may easily diffuse between different crystals; however, the end results, are identical: all crystals become smoother. It must be emphasized that the

transport processes by which a crystal can change its shape at constant volume may be very slow by comparison with those involved in its growth. Theoretically, recrystallization could occur at a significant rate only for crystals with diameters less than about 2 μm [31]. In frozen foods, the average crystal size is generally much greater than 2 μm, and temperature fluctuations enhance recrystallization.

iv. Melt-refreeze recrystallization

Melt-refreeze recrystallization is observed during temperature oscillations. When the temperature increases, ice crystals partly melt and become smaller; they grow again when the temperature decreases. The smaller ones, which have the highest free energy and the lowest melting point, melt more, grow less, and so gradually decrease in size over time. They can completely disappear; as there is no formation of new nuclei, water refreezes on the surface of remaining crystals, and the total number of crystals decreases and their mean size increases. Fluctuating temperatures stimulate crystal growth; small temperature fluctuations are common during the long life of frozen products, particularly during distribution, but also because of the cyclic nature of refrigeration systems and the need for defrosting. Melt-refreeze recrystallization occurs in combination with other growth modes; most often it is not possible to distinguish which mechanism is the most important. The duration of temperature cycles can also be important for allowing migration of water molecules from one crystal to another; if the temperature fluctuations are fast relative to the diffusion rate of water and/or solute molecules, the crystal changes can be slowed down.

v. Irruptive recrystallization

Additional ice formation may occur during rewarming; this is referred to as irruptive recrystallization or devitrification. These terms could be discussed because (a) they assume that the medium had already been frozen and in fact they are generally used for systems cooled without or with incomplete crystallization and (b) they should imply that a vitreous state was obtained. Cooling may result in products that are not in equilibrium with respect to the freezing point (*Tm* curve). The amount of ice is smaller than predicted; the unfrozen phase is diluted compared to the theoretical equilibrium state. This situation is possible only when ultrarapid freezing can be employed or for products with high viscosity and/or relatively low water content. This state is stable only at temperature below the corresponding glass transition temperature (*Tg*). Above, the product crossing the temperature range where crystal nucleation and growth rates are maximum [36] the equilibrium state is obtained by a sudden appearance of a crystallized state.

Most foods, however, contain large amounts of water, and the freezing rates used in industrial processes are sufficiently low to produce a cryoconcentrated phase in thermodynamic equilibrium. This feature could be found in the high pressure assisted freezing process; after pressure release, the nucleation, in the undercooled product, is instantaneous and the crystallization rate is very fast [37].

c. Consequences of crystal growth

In many frozen food products, large crystals have an important adverse effect on the texture. In ice cream and frozen dairy desserts, a coarse texture results from an increase in the mean size of ice crystals, which become perceptible by consumers. For delicate plant tissues (strawberries) a disruption of cell walls is often observed, which produces a loss of turgidity because of a decrease in the water retention capacity. Meat and fish are a little less sensitive to ice crystal size changes, but crystal growth might enhance shrinkage of muscle fibers and even disrupt the cellular structure, resulting in a greater chance of oxidative catalysts coming into contact with reactive components. In view of the importance of ice crystals in modifying the texture of frozen foods, it is not surprising that there have been considerable efforts to control ice crystal size, and scientific investigations to bring to light the parameters that play on the different recrystallization modes. The higher the storage temperature, the faster is the crystal growth. The influence of the storage temperature is well documented: for example, the mean ice crystal diameter, which was of 10 μm in beef muscle frozen at −40°C, becomes equal to 40 μm after a 150 h storage at −5°C [38]. This crystal growth leads to greater tissue disruption, protein denaturation, and exudate production [39].

Crystal size has considerable importance in ice cream manufacture; ice cream is the only frozen product consumed in the frozen state, and large ice crystals make the product appear colder and sandy. Polysaccharides were reported as efficient stabilizers in several ice cream studies; they alter both initial crystal size and recrystallization rate [40]. In a study on the cryoconcentration process, Smith and Schwartzberg [41] observed that the presence of gelatin resulted in a decrease in ripening rates. The reported effects of polysaccharides on the slowing down of recrystallization are real; but the mechanisms by which they exert such effects are not well understood. Among the potential mechanisms that have been suggested are an effect on viscosity of the cryoconcentrated phase [10], and the polymer adsorption on ice crystals [42]. If the formation of a weak gel slows down the initial crystallization rate [43,44] by a hindering mechanism, the water diffusion that allows the recrystallization is not slowed down by the existence of a network [45].

There is no evidence to support the view that polysaccharides could affect the amount of ice formed, which is in agreement with the thermodynamic properties of solutions. They can even enhance the process of nucleation by the introduction of specks (outside impurities) [46].

2. Solute Crystallization

Many solutes are supersaturated in the unfrozen phase, particularly sugars. After a while, they may crystallize, as

is the case of lactose in ice cream. Even if the lactose crystal size is smaller than that of ice crystals they give a sandy texture because they melt more slowly than ice crystals in the mouth. This effect is limited by polysaccharide addition, but an important lactoprotein content increases its risk if lactose proportion becomes large. Sugars may also crystallize on the surface of high sugar fruit spreads during frozen storage, giving the appearance of mold colonies on the surface. The same effect can be observed with icing or glazes of frozen desserts; the soft texture of these latter must be preserved by a modification in formulation: replacement of sucrose by invert sugar and addition of stabilizers such as polysaccharides. The formulations used must have the greatest degree of tolerance to temperature abuse. While a certain amount of temperature fluctuation is unavoidable, efforts should be made to minimize it.

In most cases, the texture quality of frozen food products is better preserved when their temperature is maintained as low as practicable during storage. The temperatures used to monitor recrystallization rates are generally high compared to the actual storage temperatures. At −18 to −20°C, the recommended temperatures, crystal growth is much slowed, and the main problem remains the temperature oscillations that induce the melt refreeze process. This leads to adopting lower storage temperatures (around −30°C for ice creams) for long-term storage of frozen products awaiting distribution.

B. Chemical Changes

An abundance of chemical and biochemical reactions may take place in animal and plant tissues, even in dead cells. If during the freezing process most reactions are stopped, some may be only reduced. As a frozen product is typically available after several months in storage, some changes can be observed thus far, but they are minimal if optimal conditions of storage are respected, i.e., low and stable storage temperature. Chemical reactions are slowed down by the temperature effect, but small solutes retain some mobility in the unfrozen phase. Because of freeze-concentration, reactants are in close proximity, and reactions rates may increase. It has been shown that freeze-concentration is likely to increase reaction rates over the subzero temperatures, the temperature range depending on substrate composition [47,48]. At lower temperatures, reactions may become diffusion-controlled and the rates decrease as the maximal freeze-concentration, i.e., Tg', is approached.

Evaluation of the chemical changes during frozen storage is a difficult task: (a) because of the duration of the experiments; at −18°C, they have to spread over one or two years; (b) the difficulty for dissociating storage influence from other process effects: blanching, freezing, and thawing; and (c) because of the diverse nature of the sensitive compounds, the chemical heterogeneity should require experiments for each vitamin, lipid, and protein.

Apart from the general problem of analysis methods, there are many possible sources of variations in product quality: for animals, breed, age, diet, preslaughter treatment; for plants, cultivar, maturity, harvesting practice; and recipes for prepared foods. The complexity, variability, and diversity of interactions between different ingredients, and thus the physical laws that control their reactions, are unknown. This may also result from incomplete destruction of enzymes during blanching or cooking, or to oxidations permitted by packaging materials permeable to oxygen.

The analyses done at different temperatures allow the determination of the reactions Q_{10}, i.e., the ratio of deterioration rates at two temperatures separated by a 10°C interval; it indicates how much longer the product quality will be retained when the temperature is decreased by 10°C. The Q_{10} values can be high for frozen products, varying from 2 to 30, while for temperatures above 0°C they are generally between 2 and 3.5.

This complexity can be illustrated by a comparison of chlorophyll degradation in frozen green beans either blanched or unblanched [49]. The rate of color degradation was faster in blanched than in unblanched green beans at −6°C, while the opposite was observed at −18°C. This study well demonstrated that comparison at only one temperature can be misleading; this can be explained by the difference in Q_{10} values, respectively equal to 7.8 and 2.8 at the studied temperature range (Figure 116.4). The degradation ways are different: in unblanched green beans, the degradation is associated with the peroxidation of some fatty acids by lipoxygenase, which gives uncolored substances; in blanched green beans, the bright green chlorophyll is converted to olive

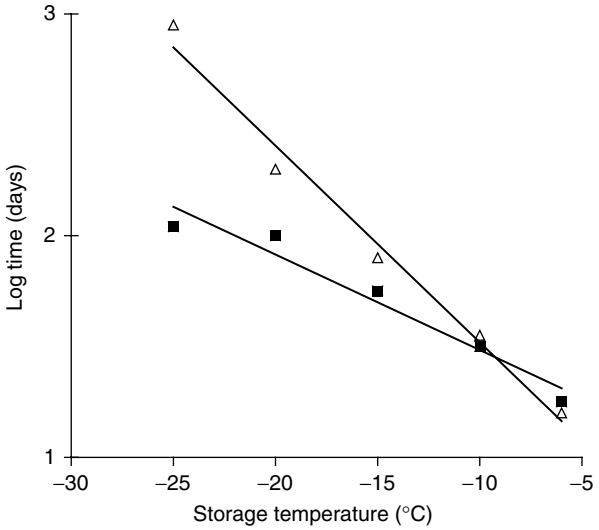

FIGURE 116.4 Days of storage required for a 10% decrease in chlorophyll of blanched (Δ: r = −0.99; Q_{10} = 7.8) and unblanched (▪ r = −0.97; Q_{10} = 2.8) frozen green beans (var. Koba) as a function of temperature, redrawn from Ref. (49).

green pheophytins, independently of enzymes and oxygen presence.

The difficulty of analysis and of interpretation is also demonstrated by the review by James and Evans [50] on the storage life of beef, pork, and lamb at different temperatures. Experimental data from many publications show there is agreement that storage temperature influences frozen state life, and that in general the lower the temperature the longer the shelf life. But considerable scatter between results at any one temperature is observed. Moreover, few papers present data at different storage temperatures, and many experiments are realized at too high a temperature compared to storage common practice.

Different types of chemical changes are particularly troublesome: oxidative processes and protein degradation, denaturation/aggregation, result in texture, flavor, and color degradations, and vitamins losses, i.e., decreases in sensory and nutritional qualities.

1. Oxidative Deterioration

Lipid oxidation corresponds to the most important chemical reactions associated with quality change during frozen storage. In foods, lipids can be oxidized by both enzymatic and nonenzymatic mechanisms. It is generally agreed that autoxidation, that is, the reaction with molecular oxygen, is the main reaction involved in oxidative deterioration of lipids. The major pathway for oxidation involves a self-catalytic free radical mechanism, which can be shown as a three-step simplified scheme: (a) initiation in which free radicals R° are formed from unsaturated lipid molecules RH by their interaction with oxygen in the presence of catalysts; (b) propagation in which the free radicals continue to be generated, and (c) termination when an antioxidant, an oxygen or free radical scavenger, reacts with free radicals. Lipases and phospholipases are enzymes, which hydrolyze lipids, the release of fatty acids can lead to rancid flavor. Lipoxygenases have also proved to be active in frozen foods and to cause changes in lipid constituents. This does not only hold for animal tissues, rich in lipids, but also for plant products. Lipoxygenase is mainly responsible for the deterioration of flavor occurring in frozen unblanched vegetables.

Lipid oxidation most commonly occurs in fatty meats and is considered as the main cause of frozen shelf life reduction compared to lean meat species. The importance of lipid oxidation varies with the quantity and the nature of the lipids; highly unsaturated lipids are less stable than saturated ones. Polyunsaturated fatty acids are autoxidized in the presence of oxygen to hydroperoxides that decompose into volatile compounds, forming flavor and aroma compounds characteristic of rancid foods. Fatty acids formed during autoxidation may produce indirect effects on textural degradation by promoting protein denaturation [51].

Color degradation is also related to oxidation during storage. For example, the discoloration of tuna is due to the oxidation of myoglobin to metamyoglobin; for salmon, the pink color, given by carotenoids, can disappear by oxidation after a long storage.

For animal tissues, the stability studies focus on lipid oxidation. Clearly, composition has an appreciable effect on the storage stability of frozen products. Fish products are the best example, their shelf life being related to their fat content: fatty fishes have a shelf life of 4–6 months at −18°C, whereas the lean ones can be preserved 7–12 months [52]. Pork meat fat contains a higher proportion of unsaturated fatty acids than other meat fats such as beef, lamb, and chicken; consequently pork has a shorter storage life (Table 116.1). Antioxidants are regularly used to stabilize the flavor of composed foods; they delay the development of rancidity by interfering with the initial step of the free radical reactions or by interrupting propagation of the free radical chain [53]. Antioxidants are of little effect in intact muscle; they are more effective when mixed with ground meats; their efficiency appears better when used as nutritional supplements. Also, the very undesirable effect of salt in flesh products is well noticed [54].

For vegetables and fruits, color changes are predominantly caused by oxidative reactions. Enzymatic browning is due to polyphenoloxidase promoting the direct reaction of polyphenolic substances with air oxygen. This problem can be overcome by blanching, but fruits generally are not blanched, because this process gives them a cooked flavor and a soft texture. Color stability is improved by the presence of ascorbic acid or the addition of citric acid, which preserve the phenolic substances in a reduced colorless state. Sugar or syrups added to fruits before freezing also help to limit browning by slowing down the enzymes' action and by protecting against oxygen. The stability studies focus on vitamin changes over time. Vitamin C (ascorbic acid) is the vitamin most readily associated with vegetables; it is also the most labile vitamin. It is oxidized by oxygen, the phenomenon being enhanced by active oxidizes (ascorbases, catecholoxidases) and metallic elements (Fe, Cu). Vitamin C content varies considerably between different vegetables, but also in a vegetable, depending upon variety, maturity, and agronomy. The level of vitamin C is not an indicator per se, but this latter is generally used as the most appropriate "marker" for monitoring quality changes during processes. Favell [55] compared the vitamin C content in some commercially quick- frozen vegetables stored for up to 12 months with the same fresh vegetables at various stages of distribution. For frozen products the major loss was attributed to pretreatments; changes, observed after storage at −18°C for 12 months, were minimal, and the frozen products presented the best nutritional qualities among products usually available to the consumer. This report confirms that for all frozen vegetables, nutritional quality is equal to and even

better than that of fresh vegetables bought in supermarkets; frozen vegetables are also "fresher" than those preserved by other thermal processes.

2. Protein Denaturation

The conformation of protein derives from its secondary and tertiary structure. As a result, every treatment of proteins with concentrated saline solutions, organic solvent, heat, and cold may modify this conformation. Denaturation is an elaborated phenomenon during which new conformations appear. The effects of protein denaturation are numerous: decreased solubility, altered water binding capacity, loss of biological activity, particularly enzymatic, and increased susceptibility to attack by proteases due to the unmasking of peptide bonds in unfolded structures. The sensitivity of a protein to denaturation is related to the readiness with which the denaturing agent breaks the stabilizing interactions. Since the latter vary according to the protein, the resulting effect of the same treatment is variable. Freezing concentrates solutes, including salts and small organic molecules. Changes in ionic strength and possibly pH [56] in the remaining unfrozen aqueous phase ensue, leading to the denaturation of proteins. Aggregation and precipitation may occur. The toughening and poor water retention observed in some species of fish, the precipitation of casein micelles in milk, and the gelation of egg yolk lipoproteins result from a decrease in protein-water hydrogen bonding and an increase in protein-protein interactions in the frozen product. The myofibrillar proteins, which aggregate during frozen storage, are probably linked by secondary interactions and disulfide bonds. As these aggregates tend to grow in number and size, the proteins lose more or less of their water-binding capacity.

The most popular tests to determine the denaturation during storage of frozen meat or fish, for example, are the determination of the loss in solubility or extractability of proteins and the measurement of the water-retention properties of the muscle system. The use of cryoprotectants in cured and processed meats can stabilize the functional properties of myofibrillar proteins; for example, addition of polyhydroxy compounds such as sorbitol and sugar in comminuted fish flesh allows one to preserve the gel-forming ability essential to obtain a surimi pasta.

Even at the low temperatures used for storage, most enzyme systems are still active. Quality deterioration is most commonly observed in meat and fish products because they are raw materials, whereas vegetables are generally subject to blanching, which destroys most enzymes, before freezing. However, the high concentration of the unfrozen phase increases the possibility of contacts between residual enzymes and substrates. Since some proteins also act as enzymes, attempts have been made to assess denaturation from the angle of enzyme inactivation. The loss of protein activity is more reflected by enzymatic activities than by extractability or solubility. It is possible that enzymatic reactions are accelerated during freezing because of the cofactors' concentration and because of membrane disruption, which allows easier enzyme-substrate interaction than in intact cells.

C. Microbial Processes

Frozen food products and particularly fish products are far from sterile and cannot be considered as microbial safe products. Many microorganisms are not destroyed by the freezing process and may survive even if they remain inactive during storage.

The effects of frozen storage on microorganisms are variable, depending upon the organism considered. The lower limit for bacterial growth in food is about −10°C and for yeasts it is about 5°C lower. However, it is not possible to store food for long periods at temperatures slightly below the growth limits of microorganisms. Foods thus stored show noticeable signs of deterioration that should be also due to the activity of microorganism enzymes, adding to that of the product specific enzymes. Whereas a heat treatment used before freezing decreases the number of surviving microorganisms, the enzymes secreted into the food, such as lipases and proteases, are much less affected by heat.

At −18°C no growth of microorganisms will occur. The reduction in number owing to storage is possible but not of practical importance. Therefore the quality of raw products and a good hygiene during the production are most important.

In the practical application of freezing technology, microbial processes are essentially limited to the effects of microbial enzymes; frozen products with a maximum shelf life can only be produced when raw materials of initially outstanding quality are used [57].

III. STABILITY AND SHELF LIFE

A. Which Model for Stability Studies in Frozen Foods?

The principal factors affecting the quality of frozen foods during their storage are (a) storage temperature and time, (b) nature and quality of the product at the time of freezing, (c) preparation and freezing processes, and (d) packaging.

The first two are referred to as TTT factors (time-temperature tolerance). They have been widely analyzed during the 1950s and 1960s and have allowed important progress in frozen food quality [58]. The two following decades have considered the PPP factors (product, process, packaging) [54]; it has been clearly proved that frozen product quality is directly related to the quality of the raw material used and to the optimization of preparation processes (heat treatment); moreover good packaging may often more than double their storage life.

Today the studies' objectives are to elaborate predictive models based on a more accurate knowledge of physical state and molecular mobility in the frozen product as a function of the storage temperature. Levine and Slade [2] have promoted the idea that the transition of a liquid to a glassy state for the maximally freeze-concentrated fraction, and consequently the temperature at which this glass transition takes place (Tg'), was the threshold of instability, and that the kinetics above this temperature were controlled by the difference between the storage temperature and Tg'.

It is essential, however, to define a shelf life for each frozen food product put on the market. Product shelf life can be estimated by (a) objective measurements of properties or characteristic changes related to food quality, *e.g.*, the ascorbic acid content of frozen fruits, and (b) shelf life failure criteria based on some noticeable quality difference between experimental and control samples.

Around 1960, it was suggested that in regulations pertaining to the keeping quality of a frozen product one should use the concept of time first to *just noticeable difference* (JND). In IIR-IIF publications [1] this is referred to as a product's *high quality life* (HQL). HQL is defined as the elapsed time between the freezing of a high-quality product and the storage time when 70% of an experienced taste panel is able to distinguish, by triangle tests, the product from the control stored at a very low temperature ($<-40°C$) [58]. Sensory quality can also be estimated using an hedonic scale method. But obviously consumers are not as sensitive to a small change as is a taste panel, and an acceptability time has been introduced that corresponds to the concept of *practical storage life* (PSL). It corresponds to the consensus of opinions about the useful "storage life" to be reasonably expected for the product.

There are many occasions for frozen products to suffer heat shock abuse during distribution and to reach an unacceptable level of quality. Since there is no way of identifying the conditions under which frozen products will be handled once they leave the manufacturer's control, it is problematic to predict an exact shelf life for any frozen product. A practical approach can be based on the profile of received consumer complaints, combined with the evaluation of products purchased at or near the sell by date. The level of complaints involving chosen quality attributes in most aged products would support a decision to extend or reduce the sell by date.

Thus the shelf life of a product essentially becomes a measure of the suitability (or lack of suitability) of a product for consumption.

No precise relationship has been demonstrated between HQL and PSL, the time gap measured between the two varying with the product. The TTT relationships are deduced from experimental studies, and the data can only be compared so long as the PPP factors are the same. Moreover, the TTT relationships are not mathematical functions. The books by Van Arsdel and coauthors [59], Jul [54], and IIR-IIF [1] reported numerous results of storage investigations on the keeping quality of frozen foods and PSL and HQL data.

B. Glass Transition and Stability Prediction

1. Vitreous State in Frozen Materials

As described before, all physical, chemical, and biochemical changes occurring during the storage of frozen foods are strongly affected by temperature, and also by water availability; the temperature effect is associated with the increasing concentration of the liquid phase as the temperature is lowered. Comprehensive procedures have been searched for accurately predicting stability. Temperature is of little value; the shelf life of different products is not the same at a given temperature. Water activity (Aw) is also not effective as a predicting parameter. The freeze-concentrated phase is in equilibrium with ice, so its partial water vapor pressure is equal to that of ice at the same temperature. Thus for all frozen foods the same water activity would be expected at the same temperature; the water activity in frozen foods only depends on temperature (Figure 116.5). Levine and Slade [2] proposed that the changes in amorphous concentrated systems should be controlled by kinetic rather than thermodynamic constraints. Consequently, the viscosity and other relaxation properties would be appropriate to predict the rate of reactions when molecular mobility is reduced.

When temperature decreases, diffusion-controlled processes slow down with the increase in viscosity due to the combined effects of concentration and temperature. The shelf life of frozen food products should be largely controlled by the physical state of their freeze-concentrated fraction. In the glassy state, i.e., below Tg', chemical reactions should be strongly limited, and a long time stability expected. In practice, foods are rarely maintained at storage temperatures below Tg'; as described before, they become

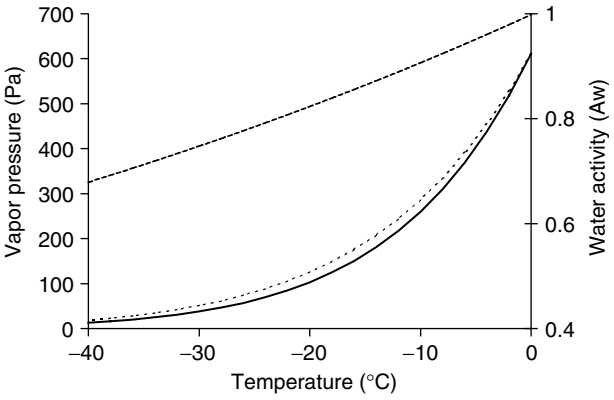

FIGURE 116.5 Water activity (Aw) of ice as a function of temperature; vapor pressure of ice: full line, of liquid water: dotted line, Aw: dashed line.

vulnerable to crystal ripening and chemical changes. Then, the properties and behavior of the unfrozen phase appear to be the key factor for understanding and improving the stability of frozen foods and their shelf life.

The glass-liquid transition is characterized by a rather abrupt change in many physical properties of the material. The main observable phenomenon is a change in the mechanical properties: the glassy brittle solid is changed into a "rubber" in the case of a polymeric material, or in a viscous liquid the viscosity of which strongly decreases as the temperature increases. The most important implication of the glass transition concept in frozen foods, and foods in general, is that translational motions of molecules, especially larger ones, do not occur in the glassy state during a practical time frame. Storage at a temperature above this characteristic temperature range would result in pronounced changes in mobility of solutes and an increase of degradation rates. There should be two possibilities for improving the stability of a frozen product: to lower the storage temperature below its Tg' and to modify this latter by a recipe change, a possibility for manufactured foods.

However, experimental data that analyze quantitatively the relationship between the stability of actual frozen foods and the glass transition are very scarce, and the few tests [27,60,61] are not always conclusive. This scarcity cannot be attributed to a lack of interest in this parameter but rather to the credibility of data as found in the literature. The published Tg' values for sucrose frozen solutions, as collected by Sahagian and Goff [6], vary from −32 to −46°C, and even their designation is different: Tg', $T'm$, Tg_2, and also Tg_{r2}; for fish the values vary from −70 [24] to −13°C [62]. There is general agreement on the concept, but it is necessary to clarify the important discrepancies as regards its practice. The question is not so much what is the true value of Tg' but more about the molecular mobility changes in that temperature range. Two aspects must be considered: (a) to have reliable information on the glass transition temperature of the maximally freeze-concentrated phase (Tg'), and (b) to know in which conditions Tg' is the reference temperature.

2. Influence of Composition on Tg'

For a series of homologous polymers it is well known that the temperature of the glass transition is increasing with the molecular weight; the same is shown to be true for sugars [63]. Other molecular properties such as branching [64] and cross-linking [65], play a lesser role. For a mixture, the glass transition temperature can be predicted from the characteristics of each component by means of expressions such as the semitheoretical Couchman and Karasz expression [66]:

$$\ln \mathrm{Tg} = \frac{\Sigma_1^n(\mathrm{C_i}\Delta \mathrm{Cp_i} \ln \mathrm{Tg_i})}{\Sigma_1^n(\mathrm{C_i}\Delta \mathrm{Cp_i})} \qquad (116.2)$$

where C_i is the mass fraction, Tg_i the glass transition temperature, and ΔCp_i the increment in heat capacity at Tg_i for each component.

However, given the uncertainties in the Tg_i and Cp_i values for biopolymers, and the poor agreement with experimental data when one component is water, the more empirical Gordon-Taylor equation is preferred [67]:

$$\mathrm{Tg} = \frac{\mathrm{C_s Tg_s} + \mathrm{kC_w Tg_w}}{\mathrm{C_s} + \mathrm{kC_w}} \qquad (116.3)$$

where C_S and C_w, are the mass fractions of solid and water, Tg_s and Tg_w their Tg values, and k a fitting parameter.

The thermodynamic properties of solutions depend on the size and nature of the solute; thus composition changes modify both the Tm and Tg curves, and consequently the Tg' value. Tg' will be influenced mainly by the molecular weight (MW) of the solute and to a lesser extent by its conformation and structure; for example, Tg′ is around −40°C for sucrose solutions and −46°C for glucose solutions.

The presence of high MW solutes could increase the Tg' and could be expected to improve stability. Hydrocolloids (polysaccharides or gelatin) must be added at a rather high concentration to a solution of low MW solutes, to have a significant impact on Tg'. In fact, they essentially alter the second step (Tg_2) of the transition; Tg_{onset} and Tg_l do not increase (Figure 116.6) [68]. This may indicate that the temperature at which some mobility appears is not raised upon the addition of the polymer. At the opposite, low MW solutes have a plasticizing effect and thus lower the Tg' of multicomponent samples. The addition of mineral salts largely decreases Tg'. For frozen saline sucrose solution containing 26% sucrose + 6% NaCI, Tg' decreases to −75°C [69]; with $CaC1_2$ in the same ratio, Tg' equals −90°C [66]. The Tg' of fish at −70°C can be explained by the presence of salts in the flesh composition [24]; this low-temperature transition is confirmed by

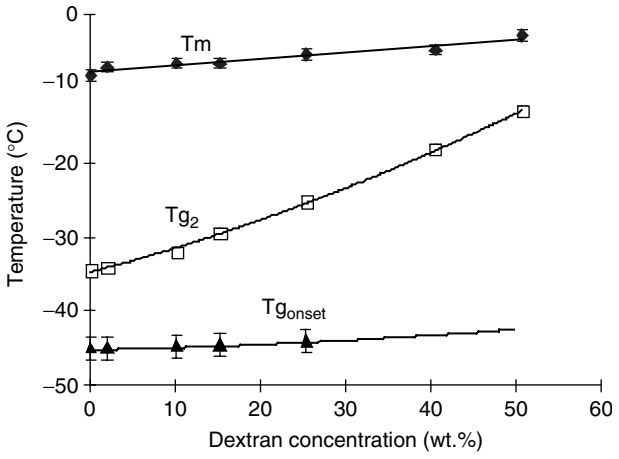

FIGURE 116.6 DSC transition temperatures as function of composition for frozen sucrose dextran solutions (50% w/w).

mechanical measurements [70]. Chang and Randall [71] showed that if for frozen pure protein solutions the Tg' is high (−10, −12°C), it decreases with the addition of small solutes (sugars, polyols). It is a common observation that in mixed materials, the glass transition range broadens; for instance, with a mixture of salts, the transition may spread over a range of nearly 30°C [72]. The presence of ice may promote this broadening, amplifying the Tg_2 feature supposed to be due to enthalpy relaxation associated with the beginning of ice melting. Low Tg' values are not always reported for actual frozen food products (meat, fish) because experimental works may not extend to a low enough temperature range for observing the beginning of the glass transition feature.

The addition of hydrocolloids greatly increases the viscoelastic modulus of the freeze-concentrated phase just above Tg′. Mechanical measurements show a tendency toward the development of a rubbery plateau (Figure 116.7); this effect increases in the order of increasing shear thinning behavior of polysaccharides [10]. These properties are certainly more representative of the macroscopic properties than of the molecular ones. They depend, moreover, on the behavior of both coexisting phases: the ice crystals and the freeze-concentrated phase. In the latter, the freeze-concentrated polymers are above their critical concentration for entanglement.

3. Molecular Mobility Around the Glass Transition and Consequence for Stability

The stability of frozen foods strongly depends upon the storage temperature. This temperature dependence is due to the dramatic decrease in viscosity at $T > Tg'$. It was tempting to explain the drastic effect of temperature by Williams, Landel, and Ferry kinetics [73], the WLF equation [Equation (116.4)] specifying a much greater temperature dependence of molecular viscosity and viscosity-dependent properties than the Arrhenius equation [Equation (116.5)].

$$\log(\eta_T/\eta_{Tg}) = C_{1g}(T - Tg)/(C_{2g} + (T - Tg)) \quad (116.4)$$

$$\eta_T = \eta_0 \exp(-Ea/RT) \quad (116.5)$$

where η_T and η_{Tg} are viscosity at T and Tg, respectively, Ea activation energy, R gas constant, η_0, C_{1g} and C_{2g} are phenomenological coefficients.

Note that the evolution of molecular mobility above the glass transition temperature may be different depending on the material, as shown by the strong/fragile classification of Angell and coworkers [74,75]. Strong liquids are those with Arrhenius or near-Arrhenius behavior above glass transition, in contrast, fragile liquids show an important increase in mobility with increasing temperature.

The major complication of the WLF theory as applied to frozen systems is that the composition of the unfrozen fraction changes as the temperature increases owing to ice melting [60]. Thus dilution of the unfrozen phase results in an even greater decrease in mobility than predicted by the WLF equation where Tg' is taken as the glass transition temperature (Figure 116.8).

In a glass, long range motions are restricted; motions (reorientation of small groups of atoms) are mainly local. The temperature dependence of dynamic properties is generally considered to obey the Arrhenius law [79]. In maximally frozen systems, ice seems to promote structural relaxations in the glass over a broad temperature range below Tg', which may explain the complex features that are observed with frozen sugar solutions by DSC analysis; a

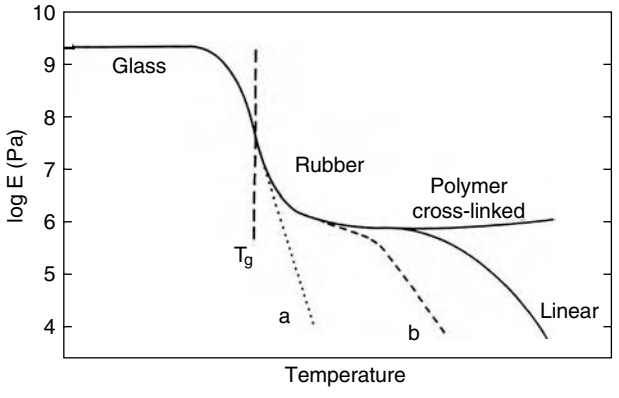

FIGURE 116.7 Theoretical thermomechanical spectra (storage modulus E′) versus temperature for different molecular weight components in the glass transition range (a = frozen sugar solution, b = frozen sucrose + polymer solution).

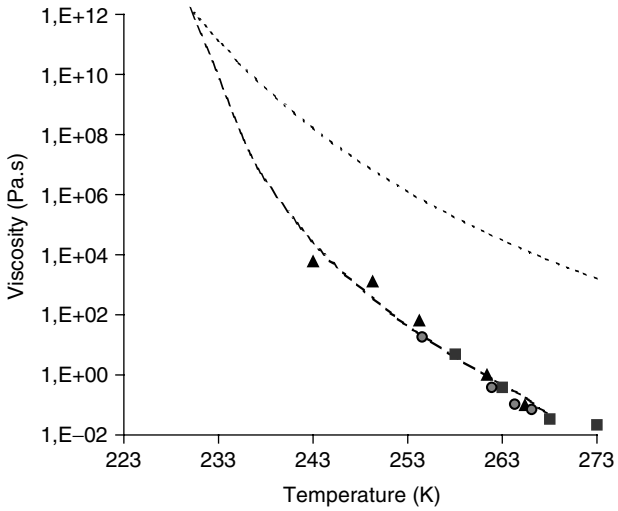

FIGURE 116.8 Viscosity of freeze-concentrated sucrose solutions as function of temperature. Symbols are experimental values: ■ (76), ▲ (77), ● (78) and dashed line represents the WLF prediction of viscosity, dotted line the viscosity predicted for a 82% sucrose solution (maximum cryoconcentration admitted as constant).

slowing down of enthalpy relaxation processes is induced by polymer addition [10].

C. Storage Temperature (*Ts*) and Stability

For products stored at a temperature $Ts < Tg'$, long-term stability may be expected. Translational molecular motions of glass-forming molecules are effectively inhibited; the diffusion-controlled processes cannot be observed on a practical time scale. By contrast, in the product stored at *Ts* between Tg' and *Tm,* the mobility of reactive solutes is higher, resulting in losses of nutrients and quality.

The relevance of Tg' as a reference temperature, for predicting the rate of physical and chemical changes during storage in the frozen state, presents no clear bases, although it has often been mentioned. Different experimental data, essentially the rate of ice crystal growth and some enzymatic reactions, have been analyzed with WLF kinetic equation. Sutton and coworkers [80] showed that ice crystallization rates in fructose solutions increased exponentially with increasing temperatures and followed WLF rather than Arrhenius kinetics, but the authors stressed the point that the range and the number of tested temperatures were too small for exact determination of the best model of temperature dependence. The possibility of inhibiting the recrystallization in ice creams by raising Tg' through the addition of high MW compounds was explored in many studies. There was a general trend where the recrystallization rate increased with increasing $(Ts - Tg')$, but the data did not fit WLF kinetics well [25]. For these models, the reference temperature was taken as constant (Tg'), although the composition of the amorphous phase was variable in the tested temperature range. From a practical point of view, the high MW solutes used as stabilizers in ice creams are added at too low levels (0.1–0.3%) for having significant impact on Tg' of ice cream mix [27], but they certainly slow down the ice recrystallization.

The main mechanism of the beneficial effect of the stabilizer addition to ice cream texture should be related to the perception in the mouth [81].

Manzocco and coworkers studied polyphenoloxidase and peroxidase activity in media with marked differences in viscosity at equal subfreezing temperatures; the rate values for the catalyzed reactions were within the same order of magnitude, in spite of the large differences in viscosity; and Arrhenius kinetics gave a fit at least as good as WLF kinetics over the temperature range of −30 to −5°C [82]. Biliaderis and coworkers [83] presented similar results for the temperature dependence of the oxidation rate of ascorbic acid in the presence of starch hydrolyzates.

More generally, the temperature dependence of many deterioration processes is much too weak to be correlated with the drastic decrease in viscosity above Tg' resulting from ice melting associated with the WLF effect when the latter is calculated using a constant Tg' [84].

For the glass transition concept to be relevant for predicting the rate of diffusion-controlled reactions, several requirements have to be met: diffusion and viscosity should be linked according to the Stokes-Einstein relation, and viscosity should have a WLF temperature dependence; for frozen products, an appropriate glass transition temperature has to be considered, taking into account the varying concentrations in the prevailing storage conditions.

Appropriate WLF parameters C_{1g} and C_{2g} have to be used [85], and *Tg* values much lower than Tg' have to be considered. For frozen food stored above Tg' (Table 116.2), the freeze-concentrated phase is more diluted than at Tg'; this latter parameter cannot be used as a reference temperature [86]. For example, in ice creams, the Tg' value is close to −34°C; if the storage temperature is −20°C (*Ts*), the freeze-concentrated fraction is liquid. Because its state diagram can be compared with that of sucrose-water binary, the reference *Tg* to consider in the WLF equation to predict the influence of temperature can be estimated at around −75°C, i.e., the temperature at which the liquid phase would turn into a glass if it could be cooled without further crystallization. The mobility and the resulting stability in the matrix would be related to the difference between *Ts* and *Tgs* and not between *Ts* and Tg' (see Figure 116.3).

Figure 116.9 shows that a good correlation can be found between the experimental and the calculated data for the rate constant of the hydrolysis of disodium *p*-nitrophenol catalyzed by alkaline phosphatase in sucrose solutions in the [+20, −24°C] temperature interval [76]. The prediction assumes that the reaction rate is viscosity dependent; in the studied temperature range, translational

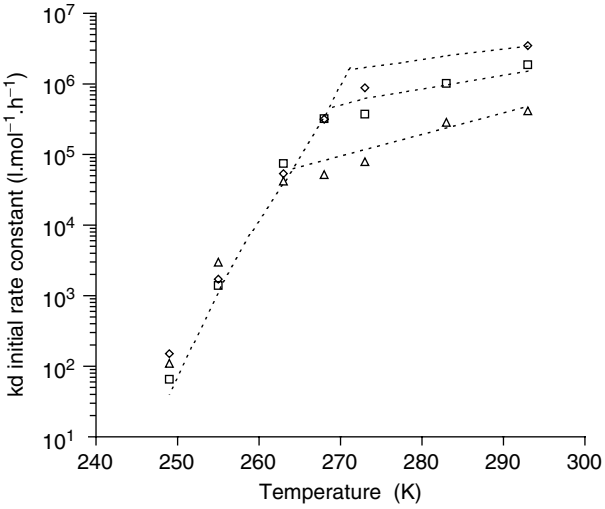

FIGURE 116.9 Initial reaction rate constant kd of alkaline phosphatase as a function of temperature in sucrose solutions (◇ 30% sucrose, □ 40%, Δ 57.5%). Dotted lines: k_d predicted from the measured viscosity (samples without ice) or from the predicted viscosity for the freeze-concentrated phases. (From Ref. 76.)

diffusion *(D)* of reactants and viscosity *(η)* were found to be linked by the Stokes-Einstein relation [87]:

$$D = KT/6\pi r\eta \quad (116.6)$$

where r = hydrodynamic radius of the diffusing molecule.

The prediction is based on the measured and calculated viscosity; the WLF equation was used to predict the medium viscosity taking as temperature reference the glass transition temperature *Tgs* corresponding to the concentration at the storage temperature *Ts* (Figure 116.3).

Even if reaction rates are viscosity dependent, this parameter is not the only one that should be taken into account to predict the complex changes during low temperature storage [82]. A simple relationship: stability = $f(Ts - Tgs)$, cannot be expected; the kinetics of physical and chemical processes taking place in frozen products stored above Tg' may be controlled by many factors depending or not on the concentration changes: solute concentration, pH, ionic strength. In structured products cell membranes limiting the solute diffusion, aggregation, and conformational change of protein or enzyme with temperature must also be taken into account. Sometimes enzyme activity is better preserved in model solutions than in the food product [88].

The discrepancy between theory and experiment can also be explained by the difficulty of knowing if, in the temperature range considered, the reactions are diffusion controlled or still activation-controlled or even in a transition region where activation and diffusion constraints coexist. A more important point is that the molecular diffusion of some components might be influenced by temperature and water content in a way very different from macroscopic properties. When the size of diffusing molecules is very small compared with the matrix molecules, the macroscopic viscosity may not reflect the "local" viscosity and be the parameter that controls diffusion.

Despite the limitation of the glass transition concept with regard to quantitative prediction of reaction kinetics, the Tg' value of a food product is of practical significance because it should provide a guideline to the industry in the formulation of manufactured foods.

A good stability being related to the possibility of maintaining the product below Tg', the formulation change could be a possible improvement. Attention must be given to the presence of small solutes, which contribute to provide some mobility at low temperatures. For example, the experiments of Wang and Jane [89] show that the addition of sugar increases the starch degradation, even for storage temperatures below the apparent Tg'.

It should also be remembered that storage in the glassy state does not guarantee long-term stability, as this state appears to have only a weak direct impact on the diffusivity of small molecules such as oxygen and water. The relatively high mobility of oxygen in glassy matrixes is often responsible for the oxidation in frozen foods stored below their Tg', and thus for their limited shelf life.

Tg' has been proposed and remains as the temperature of critical importance. This parameter represents a interesting limit to characterize the product stability, but it cannot be reliably used as a reference temperature for evolution kinetics above Tg'. The relevant temperature is then the glass transition temperature *Tgs* of the analyzed medium at the storage temperature.

IV. CONDITIONS OF BETTER FROZEN STORAGE

How long can a food be maintained in a state of satisfactory quality during frozen storage depends on the type of food, how it is processed and packaged before freezing, and on the storage temperature and how it is handled during the storage time.

Exposure to high temperature and/or fluctuations of storage temperature produces cumulative adverse effects on the quality of stored foods, which are the primary cause of damage to food marketed through retail channels. The rate of quality deterioration increases with increase in storage temperature, and such changes (reactions) are irreversible. If microorganisms will not grow in frozen foods, chemical and physical changes occur at rates that are of commercial significance during insufficiently controlled storage. Violations of recommended storage conditions should be detected and corrections instituted.

Many investigations have been made to control temperature rise in the logistic frozen food chain; these studies show large variations of product temperature even after a short exposition to ambient conditions. The temperature of the most exposed packaged product in a pallet can exceed −15°C at core for an ambient temperature of 21°C over a 25 min period; the surface temperature was 3 to 5°C higher than the core temperature. Today mathematical models are more precise and allow us to calculate the consequence of this temperature rise; it should be possible to specify what the initial temperature of the product must be maintained at −18°C throughout the distribution chain [90].

Time-temperature indicators (TTI) applied to cases or pallets could be of great help in this regard; but they are very little used yet.

A. Time-Temperature Indicators

Considerable research has been conducted to develop and design reliable systems to monitor temperature abuses. There exist different indicator kinds: temperature indicators showing only exposure above a reference temperature and temperature-time indicators taking into account the cumulative effects of time and temperature above a reference temperature; the latter would be preferred because

both temperature and time are important for quality control of frozen foods during storage and transportation.

Various types of time-temperature indicators have been developed that are based on mechanical, chemical, microbial, or enzymatic irreversible reactions for visualization of the degree of food quality change [91–93]. The problem is that for accurately monitoring changes in frozen food quality the TTI activation energy should be closely matched to that of the food quality factor. To meet such a requirement is a difficult but possible challenge [94]; moreover, an important limit is the cost of such indicators, which should be placed on the outside of each package.

Temperature management requires monitoring devices; today, many recording thermometers are available and must be used to monitor distribution conditions.

Samples must also be protected against light for preserving the color stability. Exposure to the levels of light found in some retail frozen food display areas can cause appreciable color changes within days. Products kept in dark or opaque packaging may be expected to retain color longer than those exposed to light.

Realizing the importance of creating quality reserve for the consumers, most large food industries in Europe are demanding much lower temperatures in the first links of the chain. Two main challenges for the years to come will be to improve consumer education for proper handling of frozen foods throughout the whole frozen chain and to find simple and inexpensive ways for control and checking.

V. CONCLUSION

Appropriate freezing and thawing processes and optimized temperature stability during storage preserve the quality and characteristics of fresh foods in the frozen product to a degree that is scarcely reached by any other preservation method. Since the glassy state is only readily attained by a very limited number of frozen food products, product deterioration in frozen storage is a continuous irreversible process. Physical and biochemical activities continue at −18°C, but the reaction rates at this temperature have only a limited practical significance during the commercial life.

It must be borne in mind that raw material characteristics are the most important factors to final frozen food quality. No process improves the intrinsic qualities of a food product, but a most efficient way to preserve them over time always exists. The challenge of the freezing process is to reduce the rate of physicochemical deterioration through improved storage, particularly its temperature stability and packaging material and also through the quality of the raw material.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Principles of frozen storage," by G. Blond, D. Champion and M. Le Meste. In *Handbook of frozen foods*, Editors: Y. H. Hui et al., 2004, Marcel Dekker, NY.

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117 Freezing Bread Dough

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CONTENTS

Some modifications involving several constituents of bread formulation take place during preparation and baking of bread from frozen dough. This work analyzes the effect of freezing on the several ingredients, regardless of the processes and formulations used.

I. FLOURS

As in any baking process, flour quality is essential for obtaining a good product. In this case, the freezing process, transportation, possible temperature variations and thawing are all factors demanding flours of better quality than those used for traditional baking.

In breadmaking, the proteins play a key role. After water addition, a cohesive dough is formed which is structured by gluten. Gluten proteins associated with lipids are responsible for dough cohesive and viscoelastic properties. These properties make the dough capable of retaining the gases produced by yeast action, leading, after baking, to a spongy product bearing elastic crumb. Processes that affect the proteins will also affect the quality obtained.

In the USA, flours recommended for breadmaking should have a protein content between 12 and 14% (1), though classical baking is carried out with flours containing 11% of protein (2). European recommendations suggest flours with 12.5% of protein with about 30% of wet gluten (3). Nevertheless, the quality of these proteins might be as important as the amount shown by Inoue and Bushuk (4). These authors studied the effect of freezing on baking performance with bread made of selected flours. These flours were quite similar in protein content (between 13.7 and 14.4%) but quite different in quality. Extensigraph results (maxima resistance and extension) showed that dough strength and loaf volume were decreasing after freezing and thawing and during frozen storage. Some experiments were realized with constant yeast activity (same level as in non-frozen doughs) and showed that loss of dough strength on freezing, thawing and during frozen storage was the main reason for the decline in bread loaf volume. Inoue and Bushuk (4) showed that protein content, in the range covered, appeared to be less important than protein quality. Doughs made from extremely strong flours resulted in firmer crumb texture (5).

During frozen dough processing, dough weakening occurs which, together with yeast damage, are the main causes of the shortcomings of this methodology. This is evidenced by the production of bread loaves with lower volume, by the increase in fermentation times, and by alterations in textural properties (4,6–9).

The decrease of dough strength during freezing and the freezing-thawing cycles has been attributed to several factors. Ice crystals formation in non-fermented doughs stored for 24 weeks was found to cause rupture of the gluten network as observed in previous works by scanning electron microscopy (SEM) (10).

Freezing process causes yeast death and the release of reducing substances; these effects were investigated. Some authors (11–12) have proposed that owing to the reducing nature of these substances (mainly glutathione), disulfide bridges could be broken, thus leading to dough weakening. However, it has been suggested in other works that structural changes induced in frozen and thawed doughs are unrelated to the release of reducing substances (8,13–14).

It was observed that, during dough storage at −18°C, glutenin aggregates of molecular weight 129,100 and 88,700 experience depolymerization, which becomes more noticeable for longer storage times (15). This confirms that long-term frozen storage of doughs causes gluten depolymerization.

The release of reducing substances from dead yeasts added to the rupture of gluten network caused by ice crystals may explain the decrease of strength in frozen doughs, the loss of CO_2 retention capacity and the corresponding volume loss in bread loaves prepared with this technology.

Perron et al. (16) have not found a clear correlation between the quality of breads obtained from frozen dough and protein content. However, it is known that the shortcoming of quality in this type of product is more noticeable when weak flours are used. In the USA it was reported that vital gluten supplementation improves product quality, lessening the difficulties mentioned above (9,15).

The effect of freeze damage on the crumb grain was studied using Scanning Electron Microscopy (SEM) (18–19). Figure 117.1 clearly show the structural damage caused on dough ultra structure by frozen storage.

Starch is another important component of flour. This storage polysaccharide participates in breadmaking process by absorbing water. For this reason, it is recommended for breadmaking from frozen dough that the flour used does not have more than 7% damaged starch (2), since excessively high levels of damaged starch increase the water absorption capacity of flours, creating problems during dough handling and fermentation (19).

From the technological point of view, the role of starch is more important in bread firming. Despite the nature of physico-chemical modifications that explain bread hardening that are still widely discussed, a key role is assigned to starch in all hypotheses.

The mechanism causing bread firming has been studied for many years; its elucidation will allow the appropriate selection of methods to lessen this process. At first, the popular belief was that bread hardens with time only because of the moisture loss, but Boussinggault in 1852 (20) stored hermetically packaged bread and also observed firming (21).

A century later it was postulated that starch was responsible for hardening (22). Later, several works contributed with elements to relate bread firming with starch retrogradation (23–27). However, other results were in conflict with the hypothesis explaining bread firming as a consequence of amylopectin retrogradation: (i) the rate of bread hardening is linear up to day 5 while amylopectin retrogradation rate increases linearly up to day 3 (28). (ii) Breads with low moisture content harden faster while starch retrogradation rate keeps unaltered (29); (iii) breads added with α-amylase as additive harden more slowly but they show increased crystallinity studied by X-ray (30).

Upon these results Martin et al. (31) have proposed a bread firming mechanism based on the increase in interactions between starch molecules and gluten proteins. However, later works have shown that gluten addition in model systems did not affect firming rate (32–33).

León et al. (27) have developed a new technique aimed for studying the changes occurring during bread storage, which is based on direct observation of the transformations in progress. To this end, bread dough is baked in a differential scanning calorimeter capsule, following the temperature profile at the crumb center, to test samples by new calorimetric runs after different storage times. By applying this methodology to frozen dough, greater retrogradation rates were found in samples kept frozen for more than 30 days compared with control samples.

By deepening this research, it was found in frozen samples stored for 60 days and then baked in the DSC capsules that amylopectin retrogradation was faster than in fresh doughs. When stored both at 4 and 20°C, though in the former temperature, the effect of freezing was more noticeable (Table 117.1) (34).

II. YEASTS

Freezing induces stress to microorganisms. Five major factors might affect the cell during freezing (35): (i) low temperature, (ii) extracellular ice formation, (iii) intracellular ice formation, (iv) concentration of the extracellular solute and (v) concentration of intracellular ice. (iv) and (v) can be due to ice formation (intra or extra cellular) or to water/solute diffusion through the cell membrane. Factors (i) and (ii) cannot cause *per se* cell damage (35). Most microorganisms can support cell dehydration. Factors (iii) and (iv) are most likely to be the major phenomena involved in cell damage. Intracellular freezing is very difficult to achieve in most conventional industrial

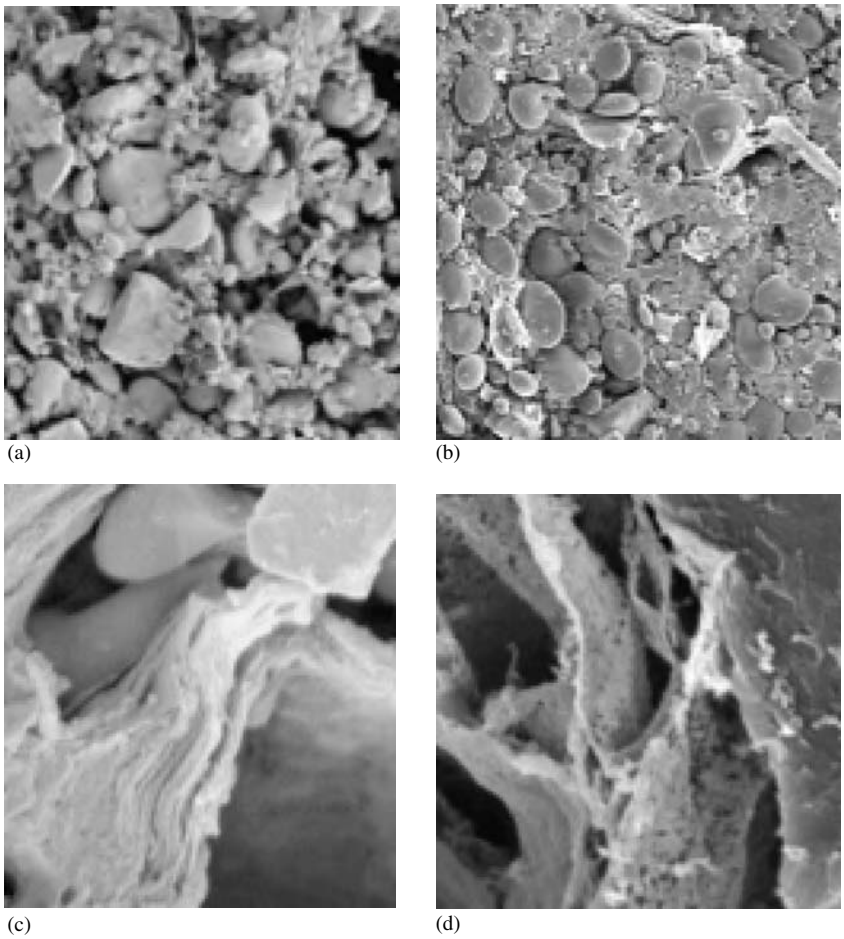

FIGURE 117.1 Scanning electron microscopy of non-frozen (a and c) and frozen (b and d) dough samples.

TABLE 117.1
Effect of Storage at Different Temperatures on Amylopectin Retrogradation

	Stored at 4°C		Stored at 20°C	
Storage Time (Days)	Bread ΔH_R	Bread Obtained from Frozen Doughs ΔH_R	Bread ΔH_R	Bread Obtained from Frozen Doughs ΔH_R
0	−0.07 ± 0.01	−0.07 ± 0.01	−0.07 ± 0.01	−0.07 ± 0.01
24	−1.13 ± 0.06	−1.92 ± 0.13	−0.94 ± 0.09	−1.11 ± 0.19
48	−1.76 ± 0.59	−2.89 ± 0.07	−1.30 ± 0.06	−1.32 ± 0.18
96	−2.22 ± 0.29	−3.44 ± 0.17	−1.82 ± 0.15	−1.92 ± 0.07
144	−3.01 ± 0.06	−3.46 ± 0.04	−2.03 ± 0.09	−2.19 ± 0.0
168	−3.12 ± 0.24	−3.68 ± 0.03	−2.18 ± 0.06	−2.16 ± 0.21

freezing processes. Freezing rate in excess of 10°C/min to 100°C/min can produce intracellular ice formation for yeast and bacteria, respectively (35). The lower the size of the cell, the higher the freezing rate should be; this is due to the cell volume and to the thermodynamics associated to ice crystallization. It has been demonstrated that a critical ice nuclei radius exists below which the nuclei is not stable and cannot exist or grow (36). Conventional freezing process as the one used to freeze bread dough has limited possibilities in term of freezing rate. Yeast is a quite resistant microorganism but the activity of yeast is affected by freezing.

Higher yeast proportions are thus recommended in frozen dough formulations to compensate for the activity loss which is due to freezing *per se* but also during the storage periods, leading to lower gas production capacity (37). This is provoked by the changes induced during freezing, which can cause yeast death. The cells exposed

TABLE 117.2
Dead Yeasts Percentage during Freezing Dough and Yeasts for Several Storage Times

	%Dead Yeasts			
Time (days)	0	40	60	90
Frozen dough	2.7	7.5	20.2	27.7
Frozen yeast	1.4	1.7	2.5	5.2

to temperatures below 0°C are damaged in different ways depending on the temperatures reached and cooling rate. Besides, higher yeast content formulations produced bread with a higher specific volume; whereas during storage, the specific volume decreased, probably because the ice crystallization caused damage to the bread structure (38).

Although freezing point of the cytoplasmic content of cells is about −1°C, it can remain unfrozen even in the presence of ice crystals in the external medium (39). The higher water vapor pressure in the cell interior compared with that of the external ice causes water loss. This dehydration equilibrates vapor pressures at both sides of the membrane, leading to solutes concentration in the cytoplasm (40).

It has been suggested that the optimum cooling rate for destroying as few yeast cells as possible is 7°C/min. For rates below the optimum, the proportion of deaths increase by 'solution effect' (increase in concentration) while rates above recommended values increase the damage by intracellular ice formation (40). Besides, ice crystals recrystallization leads to cells death by causing damage inside the cell and on the plasmatic membrane.

The solution effect is explained by four stages occurring during freezing: a) water is transferred towards the ice, b) solutes become concentrated, c) cell volume decreases, d) some solutes precipitate (41).

The results of Mazur (40) were obtained with cell suspension and cannot be directly applied to the case of frozen dough. When incorporated in the bread dough yeast does not behave the same as yeast alone. The relationship between freezing and yeast activity becomes more complex. The proportion of dead cells caused by freezing is higher in yeast incorporated to the dough than in freezing yeasts (37,42) (Table 117.2).

This phenomenon can be explained by considering that active cells (as those found in the dough) have their plasmatic membranes thinner than in dormant cells, and therefore less resistant to damage. Besides, molecules produced by fermentation such as ethanol, acetic acid and lactic acid concentrate in the unfrozen region of the aqueous phase. This concentrated solution of organic substances can produce autolysis of yeast cells, as it is known that active cells are more sensitive to this autolytic action (12).

The decrease in yeast yield exceeds cell death since CO_2 production losses of 13.2% are found in ready frozen dough; this loss increases to 37.7% at 45 days and 52.4% at 60 days of frozen storage. Therefore, freezing causes yeast death and impairs CO_2 production capacity of surviving yeast. By adding frozen yeast extract to bread formulation, it was observed that the longer the yeast remained in frozen state before extraction, the lower the specific volume of bread obtained with that extract (42).

The dissimilar sensitivity of yeasts from different origins is related to lipid composition, mainly the sterols/phospholipids ratio, which affects plasmatic membrane fluidity (43). In recent years work is being carried out to obtain yeast strains of improved resistance to freezing, in order to use them in breadmaking via frozen dough (44–49).

Other substances were used to protect yeasts, such as trehalose (50–51). This carbohydrate is known to be an efficient protective agent to keep membrane integrity and intracellular structure in a wide range of physiological and room conditions (52). Meric et al. (51) showed that a 5% minimum trehalose content was necessary to achieve a significant improvement of yeast resistance to freezing. No real benefit was observed above this limit. For this reason, in new yeast strains, efforts are directed to make trehalose synthesis more active and to lessen the effect of catabolic routes (41).

III. OXIDIZING AGENTS

The role of these oxidizing agents is essential in breadmaking because they increase gluten strength and allow breads of higher volume to be obtained. In breadmaking via frozen dough, oxidant addition is particularly necessary due to protein matrix weakening caused by the mechanical action of ice and by the effect of reducing substances released by yeasts.

In the USA, flour for breadmaking via frozen dough was usually added with 45 ppm potassium bromide combined with 100 ppm ascorbic acid (1). After potassium bromide prohibition as breadmaking additive in almost all the world, azodicarbonamide was proposed as a substitute, to be used in combination with ascorbic acid and enzymes such as lipoxygenase, or else enzymatic complexes contained in active soy flours.

The comparative analysis of potassium bromide and ascorbic acid actions showed that both improve product quality and that the quality of bread added with ascorbic acid is higher than that added with potassium bromide (53).

Azodicarbonamide is a 'quick action' oxidant, very sensitive to glutathione and to other substances released on yeast death (54). Thus, it is best used, according to recommendations, when combined with benzoyl peroxide (55). Concerning fermentation time, Dubois and Blockolsky (6) showed that potassium bromide, used in low concentrations shortens fermentation time compared to that obtained with ascorbic acid.

If potassium bromide is used in high concentrations, it is found that from 20 weeks of frozen storage on,

TABLE 117.3
Principal Additives Used in the Formulation of Frozen Dough

Additive	Effect	Reference
Gluten 2%	Increase dough strength	Wang and Ponte, 1994 (57)
SSL 0.5%	Decrease the freezing effect on bread volume	Abd El-Hady et al. 1999 (53)
DATEM 0.6%	Increase bread volume	Sahlstrom et al. 1999 (56)
Mix of sucrose, fatty acid ester, DATEM, fatty acid monoglycerides and sugar	Avoid the harmful effects of freezing on the quality of baking products	Nakamura et al. 1996 (61)
Mix of gums, tensioactive agents and forming film proteins	Avoid the harmful effects of freezing on the quality of baking products	Larson et al. 1983 (62)
Mix of gluten, emulsifier and polymeric substances	Inhibit the deterioration of dough eliminating the damage produced by big ice crystals	Yamaguchi and Watanabe 1987 (63)
Hidroxy propyl methyl cellulose (HPMC)	Reduce the hardness and increase the specific volume of bread	Barcenas et al. 2004 (64)
Enzymes that produce maltotrioses and glucose oxidase and/or hemicelulase	Prevent volume reduction due to the freezing	Tanaka et al. 1997 (65)
Glycerol 0.75–1.0%	Improve the structure of the crumb	Dubois and Blockolski 1986 (6)
Ethanol-water 5–20%	Decrease the water melting point and to diminish the thawing time previous to fermentation	Lidstrom and Slade 1987 (66)
Sucroglycerides	Protect the yeast against the harmful effect of freezing on baking products	Le Duff 1987 (67–68)
Lecithin 0.2–0.3%	Flour strength	Grandvoinnet et al. 1986 (69)
Alfa-amylase 0.05–0.1%	Produce fermentable carbohydrates	Larsen 1991 (70)
Skim milk-whey protein 2.2%	Provide humidity in baking products, avoiding the use of gluten that alters the organoleptic properties	Seneau 1989 (71)
Carboxy methyl cellulose (CMC), bean gum, gum arabic	Improve the dough and bread characteristics	Sharadanant and Khan 2003 (72–73)
Guar gum 0.5%, DATEM 0.5%	Improve volume and texture of bread	Ribotta et al. 2004 (18)

fermentation times become longer than those obtained with ascorbic acid, potassium iodide and, specially, than that found when using azodicarbonamide (54).

IV. ADDITIVES

Emulsifiers are increasingly used in those bakery products including fat in its formulation, since they improve dough stability and retention capacity of the gases produced by fermentation, improve loaf volume and crumb freshness with longer shelf-life.

For frozen doughs, effective additives have been proposed to improve product quality such as SSL and DATEM (8). By adding SSL, freezing-induced quality losses can be alleviated (53). DATEM addition significantly increased volume, shape ratio and bread quality (56).

Glycerol used at 0.75–1.5% (on flour basis) causes little effect on the volume obtained via frozen doughs, though crumb structure is improved. In turn, xanthan gum addition does not improve volume and reduces crumb quality (6).

In order to have a flour bearing the required 'strength,' a gluten additivation percentage of 2% has been proposed as the optimum level to improve bread quality, gas retention capacity and fermentation time (57). Apart from improving protein quality, a cryoprotectant effect on yeast is obtained since, on increasing glass transition temperature, the free water proportion is decreased (58). Other additives capable of reducing, at least partially, the shortcomings caused by dough freezing have been described. Among them, we find egg yolk and sugar esters (59), and addition of vegetal fats to form water/oil emulsions at different proportions (60).

A summary of the principal additives and their effects on the characteristics of frozen bread are shown in Table 117.3.

V. CONCLUSIONS

Over frozen dough processing, dough weakening occur which, together with yeast damage, are the main causes of the shortcomings brought about by this technology. The increase in fermentation times, the production of bread

loaves with lower volume and alterations in textural properties are detected.

The success in the expanding use of frozen dough for the production of bread and bakery products depends of the resolution of these problems.

The fact that the freezing process affects the viability of yeast cells and the CO_2 and the reducing substance production by yeast opens an important technological and research field. In this sense, the obtainment of new strains of yeasts and the searching of additives and variables of process that help the cryoresistance of the microorganisms are essential.

Another important phenomenon that deserves attention is the protein depolymerization during the frozen storage, which may partially reduce using different additives.

In the production of bread from frozen dough complex formulations must be used for reducing the harmful effects of freezing. These formulations include lipids, high percentages of yeast and oxidizing agents, as well as mixes of different oxidizing agents, surfactants or emulsifiers that improve the bread matrix, gluten, soy flour, sugars and enzymes. For this reason the freezing process is more profitable for the bakery products with a high added value.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Effect of freezing on dough ingredients," by M.C. Añon, A. Le Bail, and A.E. Leon. In: *Handbook of frozen foods*, editors: Y. H. Hui et al., Marcel Dekker NY, 2004.

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Part N

New Technology

118 Minimal Processing: Fundamentals and Applications

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CONTENTS

I. INTRODUCTION

Consumer's wants, needs and desires have driven the greatest advances and the most significant changes in food processing in the last two decades (Exhibit 118.1). Consumers are increasingly aware of the health benefits and risks associated with consumption of food. At a time when they are requiring less extreme treatments (less heat and chill damage), with lower levels of salts, fats, acids and sugars and/or the complete or the partial removal of chemically synthesized additives, there is also an increased demand for convenience foods with fresh attributes and long shelf-lives.

Food trends towards fresh image on one side and convenience on the other side often conflict. In most cases the fresh quality is negatively affected by the processing procedure. The most crucial current and future challenge is to retain the natural functional properties and the sensory and nutritional quality with the appropriate shelf-life and safety.

To meet these expectations, the food industry and the researchers have developed in the last years a range of strategies and minimal processing (MP) techniques for food preservation that allow better retention of product

Exhibit 118.1

CONSUMER REQUIREMENTS AND DEMANDS

High quality standards with acceptable price levels
High safety standards
Convenience of distribution and preparation
Nutritional concepts and healthy appeal to a broad age group
Satisfaction of cultural and ethnic local habits
Suitable to modern lifestyles
Variety of choice

Adapted from Jiménez-Laguna et al. (1).

flavour, texture, colour and nutrient content than comparable conventional treatments. The MP concept includes a series of products and processes that may be grouped in diverse food categories such as: minimally processed, with invisible processing, carefully processed, partially processed, and high moisture shelf-stable (2). These terms are not exactly equivalent but reflect the great diversity of alternatives for high quality products.

In this chapter, the fundamentals of the main minimal techniques (already used industrially or still in development or testing) are considered, a number of recent achievements are highlighted and some safety and quality considerations for a successful design of minimally processed products are presented.

II. DEFINITION OF MINIMAL PROCESSING

The MP concept has evolved, giving a wider approach than those terms used earlier by Rolle and Chism (3), Shewfelt (4), Huxsoll and Bolin (5), Wiley (6), and Ohlsson (7) (2). According to Rolle and Chism, the manipulation and basic preparation of foods, as well as life permanence in the biological tissue, are the elements that distinguish the minimal processing. Shewfelt added to the definition the possible use of low level irradiation and individual packaging as preservation factors that allow safety and high quality. Examples of minimally processed products presented by Shewfelt included vegetable stick snacks, packaged tossed salads, chilled peach halves, peeled and cored whole pineapple, shelled fresh legumes, microwaveable fresh vegetable trays, gourmet chilled dinners, among others. The use of refrigeration appears as a fundamental preservation factor for this type of foods. Huxsoll and Bolin included those foods whose tissues could be not alive, but freshness should be kept as an important objective of preservation. These authors also expressed that minimally processed foods (MPF) are not necessarily for direct consumption, but can be considered as preserved foods that maintain their freshness characteristics and can be later transformed into processed products using conventional techniques.

Wiley presented a very important alternative to the use of refrigeration alone: the modification and control of atmospheres in food packages as other preservation factor to be employed during the MPF elaboration. Ohlsson mentioned, as an important element, the necessity that this processing should be enough and adequate to keep the product quality during the storage period that goes from preservation to consumption. Manvell (8), more recently, situated MP methods within the broader objective of food processing to extend the shelf-life of foods. He defined that MP must be a safe balance between desired and adverse effects of preservation processes and accordingly, a minimal process is "the least possible treatment" to achieve a purpose. Thus, he considered as minimal processes available traditional techniques that have been improved to obtain safe and high quality products (i.e., aseptic packaging, ohmic heating, radio frequency heating, microfiltration, etc) as well as techniques based on emerging preserving factors (i.e., high pressure, ultrasound, pulsed electric field, etc.). In the same trend, Ohlsson (9) stated that developments in thermal technologies are considered "minimal" when they minimized quality losses in foods compared to conventional thermal techniques. Lastly, and in partial agreement with these terms, Snyder (10), taking into account that there was no regulatory definition for MPF, gave an operating definition of them as "foods in which the biological, chemical, and physical hazards are at tolerable level." He divided them into three groups according to the type and severity of treatment: those foods that need no cook intervention (i.e., raw foods, nuts, grains and eggs); foods with a mild disinfection or reduction by 10^{-2} to 10^{-5} of pathogenic substances, chemicals and microorganisms by blanching, washing without or with chemicals, etc. (i.e., cut fresh fruit and vegetables, berries); and pasteurized foods with a 10^{-5} to 10^{-7} reduction of pathogens by applying irradiation, heat, pulsed light, ultraviolet light, high pressure, etc. (i.e., *sous-vide* products, pasteurized and refrigerated products, tomato sauces and salad dressings with preservatives), and also foods that are formulated to be safe by fermentation, drying, salting or acidification.

The expansion of MP concept has been reflected in new, renewed, and improved products and processes formulated and designed to produce a greater diversity of MPF. The reviews and/or books of Ahvenainen et al. (11–13), Singh and Oliveira (14), Wiley (15), Oliveira and Oliveira (16), Alzamora et al. (17) and Ohlsson and Bengtsson (18) on minimal processing could be used as further reference on the subject.

III. USE OF MULTITARGET PRESERVATION IN THE DESIGN OF MINIMALLY PROCESSED FOODS

Homeostasis or internal media stability (composition and volume of fluids) is vital for survival and growth of microorganisms. Preservation procedures are effective when they overcome, temporally or permanently, the various homeostatic reactions that microorganisms have evolved in order to resist stresses (19). Table 118.1 shows, as mode of example, the mechanisms of action of some environmental stresses in foods or during processing and the microbial homeostatic reactions produced by their application.

Homeostatic mechanisms that vegetative cells have evolved in order to survive extreme environmental stresses are energy dependent and allow microorganisms to keep functioning. In contrast, homeostasis in spores is passive, acting to keep the central protoplast in a constant low-water-level environment, this being the prime reason for the extreme metabolic inertness or dormancy and resistance

TABLE 118.1
Modes of Action of Selected Preserving Factors and Homeostatic Response in Microorganisms (19–22)

Stress Factor	Principal Effects	Homeostatic Mechanisms
Vegetative Cells		
Low water activity	Loss of water and consequent reduction or prevention of metabolism.	Accumulation by synthesis or active transport of compatible cytoplasmic solutes to balance external osmolality avoiding excessive loss of water from cell and of membrane turgor, changes in membrane lipids.
Low pH		
Strong acids	Denaturation of enzymes on cell surface and lowering of cytoplasmic pH due to proton permeation through membrane in response to the increased pH gradient.	Control of the activity of ion transport systems that facilitate proton entry to maintain the intracellular pH within a narrowed range.
Lipophilic weak acids	Lowering of citoplasmic pH due to undissociated acid passage through the membrane; specific effects of acid anion on metabolism; extensive protein denaturation and DNA damage in some organisms.	Increase of ATPase activity, synthesis of new proteins and synthesis of positively charged aminophospholipids that impart to the membrane surface a net positive charge that acts as a barrier to protons, etc.
Low temperature	Reduction or inhibition of growth rate; alteration of metabolic activity and substrate uptake (i.e., permeases unable to combine with substrates due to changes in the molecular architecture of lipid bilayer of the cytoplasmic membrane and arrest of active transport of solute across membrane due to insufficient energy in mesophilic organisms).	Depending on the organisms involved, alterations in fatty acid and phospholipid composition of membrane so as to obtain lipids with a lowered-gel-to-liquid-crystalline state transition temperature maintaining the membrane fluidity; increased enzyme synthesis in psychrophiles to compensate for reduced enzyme activity.
Thermal treatment	Single- and double-strand breaks in DNA, inactivation of specific enzymes, RNA degradation, leakiness of membranes and heat-induced damage of the various chemiosmotic and transport functions of the cell.	Repair of DNA; synthesis of "heat-shock" proteins leading to a rise in heat resistance when long, slow heating is involved.
Ionizing radiation	Damage to DNA	Development of enzymic mechanisms for repairing ionizing radiation–induced lesions in DNA.
Nisin	Pore formation in cytoplasmic membrane, leakage of ATP from target cells.	Synthesis of nisinase. Changes in the bacterial membrane structure to decrease membrane fluidity.
Spores		
Thermal treatment	Depurination and depyrimidation of spore DNA; enzyme attack on apurinic DNA after germination; destruction of the activity of the enzyme(s) on the germination pathway.	Resistance/dormancy mechanisms comprise low core water content by some osmotic and/or pressure-generating function of the cortex and "mineralization" and immobilization of ions in the core of the spore.

of these cells. It seems likely that the structural arrangement of the cortex of the spore is responsible for the dehydration of the protoplast (23, 24).

In foods preserved by combined methods (so called "hurdle technology" or "multitarget preservation"), the active homeostasis of vegetative microorganisms and the passive refractory homeostasis of spores are disturbed by a combination of gentle antimicrobial factors at a number of sites or in a cooperative manner (19, 25, 26). For example, for vegetative cells (where homeostasis is energy dependent), the goal is to reduce the availability of energy (removing O_2, limiting nutrients and reducing the temperature) and/or to increase the demand for energy (reducing water activity (a_w), reducing pH and adding membrane active compounds). Placing a number of sublethal stresses (i.e., preserving factors) on a microbial cell potentially results in metabolic exhaustion and death. For spores (where homeostasis is non-energetic and depends on the structures of the organism), the goal is to damage key structures (by chemical, enzymic, or physical attack on coats, cortex, etc.) or to release spores from dormancy (initiating germination with natural germinants or with false triggers, or applying high pressures).

Antimicrobial preservation of foods by combined methods should be considered not only as interference of the homeostasis by additive or synergistic hurdles on the same microorganism, but also as the selective application of preservation factors that may be effective against a specific organism or group of organisms, while not against others.

In the last fifteen years, the popularity of the hurdle concept has dramatically increased and numerous publications have now indicated its potential for the development of minimally processed foods.

As it will be discussed, combined technology can be applied in the design of the preservation system of MP

foods in many ways: (i) at various stages of the food distribution chain, in storage, in processing and/or in packaging as a "back-up" measure in existing MP products with short shelf-life, in order to diminish the risks and/or increase their shelf-life (26); (ii) as an important tool for improving quality of long shelf-life products without sacrificing their microbiological stability; or (iii) as a new preservation procedure deliberately intended for obtaining novel MP foods.

IV. GENERAL OVERVIEW OF MP TECHNIQUES

According to previous definitions, MP focuses on preservation while keeping food quality attributes, following the principle "as little as possible, but much as necessary" (8, 27). As said before, this concept involves a wide variety of processes with a correspondingly large variety of products. Three major initiatives are currently being purposed by the research community and the industry to make MPF with improved quality (28, 29):

1. Optimization of traditional preservation methods to enhance sensorial, nutritional and microbiological quality of foods, yield and energy efficiency.
2. Development of mild processes by novel combinations of traditional physical and chemical preserving factors, each one applied at low intensity, to obtain products with quality attributes reminiscent of the fresh or native state of a given food but with longer shelf-life.
3. Development of new techniques to obtain novel foods with fresh quality attributes by using combinations of emerging preservation factors or combinations of emerging factors with traditional ones, all of them applied at low doses.

These approaches to obtain foods products of higher perceived quality face different limitations and possibilities for the design, optimization and experimental assessment as well as validation of process conditions. Table 118.2 lists selected examples for each approach.

TABLE 118.2
Selected Existing and Novel MP Technologies

Enhanced Traditional Techniques
Aseptic packaging of thermally processed foods (tube in tube heat exchangers, etc.)
Semi-aseptic processes (high heat infusion, vacuum steam + vacuum cooling, etc.)
Ohmic heating
Radiofrequency heating
Microwave heating
Inductive electrical heating
Infrared heating
Microfiltration
Osmotic dehydration
Vacuum dehydration
Microwave and dielectric drying
Cryogenic freezing
Freezing in dynamic dispersion medium
New Mild Techniques Based on Novel Combinations of Traditional Preserving Factors
Sous vide and cook-chilled processing
Modified/controlled atmosphere packaging (MAP/CAP)
Active packaging techniques
"Ready-to-eat foods"
New Techniques Based on Combinations of Emerging Preserving Factors
Emerging Factors:
- High hydrostatic pressure
- Ionizing radiation
- High electric field pulses
- Ultraviolet light
- Light pulses
- Natural antimicrobials
- Ultrasound
- Biopreservation

A. Improved Traditional Technologies

The first category refers to the many advances in invention and improvements of equipment and techniques connected to traditional methods of food preservation — canning, aseptic packaging, dehydration, a_w reduction, freezing, distribution, storage, analytical devices, new packaging materials, etc. over the past 30 years. Before that, the traditional food industry was, in general, technically unsophisticated compared to several other industries. These above engineering advances, which have had broad economic and quality product impact, have been also accompanied by replacing empirical fashion for establishing processing parameters and formulations with sound knowledge and skills, many times taken over from different science branches (i.e., biomaterial and colloid sciences) and adopted to foods. Attention has been particularly focused on research that aims at maximising the product quality, since mechanisms of microbial inactivation/inhibition under these improved techniques are in general the same as under the conventional processes.

These significant developments were accompanied by: 1) the application of statistical quality control to all phases of food operations; 2) the growing safety regulations and recommendations of governments and international organisms (FDA, USDA, FAO, WHO, Codex Alimentarius, etc.); 3) the adoption of automatic inspection devices and many computer-driven process controllers; and 4) the advances in mathematical modelling of the phenomena. In the following, some of them are commented.

Thermal processing affects the quality of foods significantly (loss of vitamins and minerals, loss of fresh appearance, flavour and texture, etc.). Quality optimization greatly depends on the ability to process foods under the high temperature–short time (HTST) concept, since activation energies for inactivation of microorganisms are higher (and hence the rate is more temperature-dependent) than those for many undesirable quality changes. These HTST processes are currently done for packaged liquid foods and liquids with particles, but for large solid products the food in contact with the hot surfaces will be overheated during the time needed to heat the coldest spot of the food. Suitable industrial systems to overcome this problem are based on heating unpackaged foods followed by aseptic packaging, or on the use of new heating technologies, such as the so called "volumetric electric heating" (30).

Electric heating is done by applying electromagnetic energy to increase food temperature. These methods directly heat the whole volume of the food, without heating up heat transfer surfaces and requiring less time to come up to the desired temperature. According to the frequency of the electromagnetic spectrum, we can distinguish three types of processes:

- electric resistance/ohmic heating (50/60 Hz) (application in the food industry: pasteurization and sterilization of fruit juices, with or without particulates; blanching of vegetables; cooking of foods in catering sector and in preparing low-acid-ready-to-eat meals);
- high frequency/radio frequency heating (10–60 MHz) (application in the food industry: post-baking of biscuits and cereals; drying, bulk defrosting of meat and fish);
- microwave heating (1–3 GHz) (application in the food industry: baking bread, cakes and pastry; tempering of foods; drying, pasteurization of pumpable foods).

In ohmic heating, the food itself acts as a conductor between the ground and a charged electrode, or it can be immersed in a conducting liquid. The resistivity of the food will determine the current that will flow between the ground and the electrode (voltages up to 5000 V) according to Ohm's law. Main advantages of ohmic heating are: 1) the food is heated by internal heat generation and so does not exhibits large temperature gradients during process; 2) particulates in liquid-particulate mixtures achieve higher temperatures over liquids; 3) high energy efficiency (≅90%); and 4) reduced maintenance cost and ease of process control.

Microwave and radio frequency heating generate heat in the material by primarily two mechanisms: dielectric and ionic (31). In dielectric heating, water molecules, due to their dipolar characteristic, try to follow the electric field associated with electromagnetic radiation, oscillating at the very high frequencies and producing heat. In ionic mechanism, the oscillatory migration of ions under the influence of the oscillating electric field results in heating. Microwave and radio frequency heating may be relatively more uniform than conventional treatment but heating uniformity is hard to predict. The product may be turned on or off instantly, and can be pasteurized after being packaged.

For all these electric processes, the thermal effect is presumably the principal lethal mechanism and the most heat-resistant pathogens of concern as well as their thermal inactivation kinetics are already well known. The influence on chemical constituents is also the same than in conventional heating methods. So, time-temperature history at the coldest location will determine the safety of the process. But the application of electric heating in the food industry is limited at present by a number of reasons: 1) for ohmic heating, the lack of quantification of the heat generation rate and the electrical field distribution as affected by the electrical heterogeneity of particles and the different conductivity between solid and liquid phases; scarcity of data about critical factors affecting heating, such as residence time, loading levels, etc., and the difficulty in localization of hot/cold spots (30); 2) for microwaves, the high energy costs, the non-uniformity of heating and the inability to ensure sterilization of the entire package (31); and 3) for radiofrequency heating, equipment and operating costs are higher than for conventional or ohmic heating techniques (30).

In the freezing area, the attention had been aimed at maximising the product quality during storage. Frozen foods are not stable and deteriorate at commercially significant rates during frozen storage due to chemical and physical changes. The improvement of quality had been focused with an integrated approach:

- firstly, by selecting adequate processing conditions, product characteristics and formulation for conventional freezing processes;
- in second place; by considering new techniques;
- on the other hand, by application of the fundamentals of bio-materials sciences to the formulation of frozen products with cryoprotectants and other additives for improving their keeping quality. Important advances had been made in the understanding of glass transition and of crystallization/recrystalization phenomena in the frozen matrix (32).

B. Techniques Based on Novel Combinations of Traditional Preserving Factors

An important sector of the MP foods market is formed by chilled foods, e.g., foods that rely either exclusively or

primarily on cold storage for preservation. These foods may contain entirely raw foods; low-risk raw and uncooked ingredients ("prepared chilled" or "ready-to-eat-foods," typical shelf-life ≅10–14 days) such as salad or cheese components; entirely cooked or baked ingredients ("cooked ready-to-eat-foods," typical shelf-life ⩾ two weeks) or they can be in-pack pasteurized and stored for extended periods ("ready-to-eat products for extended durability" or REPFED, typical shelf-life >40 days) (28, 33, 34).

Major concerns over the microbiological safety of chilled foods have been extensively detailed in the literature (33–35). The principal situation that affects safety is improper refrigeration (accidental due to mechanical failure or intentional to save energy costs) during manufacture, distribution, retail sale and at home. Chilled foods are not free from abuse. Refrigeration temperatures are not standardized. In the real world conditions, during distribution and retail presentation and storage of perishable foods, temperatures often cycle between low and high temperatures (36). In addition, refrigerated foods can be stored by the consumer for a longer time than intended because sensory quality is still good. The main concern is that spoilage bacteria are inhibited sufficiently to allow toxin formation, toxigenesis preceding organoleptic spoilage.

Although modified atmosphere packaging (MAP) extends shelf-life and visual appearance of food, its use continues to be questionable because of the possible growth of anaerobic and psychrotrophic pathogens. Microbial safety is affected in three ways: (i) the stimulatory effect of CO_2 on spore germination; (ii) the inhibition of the spoilage microorganisms, which are indicators of incipient spoilage; and (iii) the potential for temperature abuse (37).

Storage at chilled temperatures cannot prevent all pathogenic growth, but can inhibit the growth of some types of microorganisms and decrease the growth rate of others (Table 118.3). Therefore, the shelf-life of chilled foods depends on a balance between the minimum temperature for growth, the growth rate at chilled temperatures and the storage time and temperatures. As temperature control is one of the key issues with regard to chilled foods to control the survival or growth of any microorganisms that remain in the food or that enter food after processing, and there are many opportunities for temperature abuse to occur, authorities in different countries are increasingly suggesting the use of others factors in combination with refrigeration.

For instance, many possible additional hurdles have been proposed for being used in combination with *sous vide* process (i.e., cooking a food product inside a hermetically sealed vacuum package, rapid chilling and chilled storage of end product) (38). As these products usually have a pH > 4, $a_w > 0.93$ and contain no preservatives, abuse temperatures can lead to germination and outgrowth of spore-forming pathogenic (i.e., *Clostridium botulinum*) as well as to repairing thermally injured cells. Thus, as soon as the food is purchased by the consumer, it is outside of any of the legislative requirements. The UK Advisory Committee on the Microbiological Safety of Food (1992) has recommended, in addition to chill storage, the following preservation factors to be used singly or in combination: 1) heat treatment at 90°C for 10 minutes or equivalent process; 2) a pH < 5.0 throughout all components of complex foods; 3) a minimum salt concentration of 3.5% in the aqueous phase of all components; 4) an $a_w < 0.97$ throughout all components of complex foods; and 5) a combination of heat and preservative factors that have been shown to prevent growth of and production of toxin by psychrotrophic *C. botulinum*. Biopreservation, the use of sodium lactate, a combination of a slight reduction in a_w and pH and irradiation have also been proposed by many authors as promising potential "back up" hurdles when temperature is out of control (38).

TABLE 118.3
Pathogenic Microorganisms of Concern in Chilled Foods and their Minimum Growth Temperature, pH and a_w (Adapted from Alzamora (38))

Critical Hazard	Microorganisms	T (°C)	pH	a_w
Infectious pathogens	*Salmonella* species	5.1	3.8–4.0	0.92–0.95
	Listeria monocytogenes	−0.1	4.4	0.90–0.92
	Vibrio parahaemolyticus	5	4.8	0.94–0.96
	Aeromonas hydrophila	0	4.0	0.94
	Campylobacter jejuni and *coli*	25–30	5.3	0.985
	Escherichia coli	4–7.1	4.4	0.935–0.95
Toxigenic pathogens	*Staphylococcus aureus*	7.7	4.0	0.86
Toxigenic spore-forming pathogens	*Clostridium botulinum* type E and non proteolytic strains B & F	3.3	5.0	0.96
	Bacillus cereus	<4	4.4–4.9	0.90–0.93
	Clostridium perfringens	5	5.5	0.93–0.95
	Clostridium botulinum (proteolytic strains)	10–12	4.6	0.94

C. Techniques Based on Combinations with Emerging Preserving Factors

There is a wide range of modern preserving factors, developed in the last few years and decades, that cause physical inactivation of microorganisms at ambient or sublethal temperatures (i.e., high hydrostatic pressure, pulsed electric fields, ultrasound, pulsed light, ultraviolet light, etc.), avoiding the deleterious effects that severe heating has on flavour, texture and nutritional quality of foods. Table 118.4 lists some new physical food preservation techniques, the mechanisms of action on the microorganisms and the factors of the technique itself that influence the efficacy of the preservation. Some of these processes are still under

TABLE 118.4
Main Emerging "Non-Thermal" Preserving Factors (27, 31, 39)

Factor	Mechanism of Inactivation	Critical Factors	Potential Application/ Products on the Market
High Pressure Processing (HPP)			
Subjecting liquid and solid foods, with/without packaging, to 100–800 MPa, below 0°C to 100°C, from seconds to about 20 min, instantaneously and uniformly throughout food, independent of size, shape and food composition	Membrane damage, protein denaturation, leakage of cell contents	Temperature, pressure magnitude, rate of compression and decompression, and holding time at pressure, time to achieve treatment pressure, composition, pH and a_w of the food, product initial temperature; and critical factors of other constraints in combination	Jam, jellies, fruit juices, oysters, guacamole, ham, fruit yogurts, dairy-based fruit smoothie, processed meat products, pressure-treated ready-to-eat rice, sauces (in use since 1990)
Pulsed Electric Field (PEF)			
Pulses of high voltage (≅ 20–80 kV/cm) to foods between two electrodes, in the form of exponentially decaying, square wave, bipolar or oscillatory pulses at ambient, sub-ambient or slightly above ambient temperature, for less than 15 seconds	Electrical breakdown and electroporation of cell membranes	Electric field intensity, pulse width, treatment time and temperature, and pulse waveshapes, pH, ionic compounds, conductivity and medium ionic strength, and critical factors of other constraints in combination	Only two industrial-scale PEF systems recently available. Fluid foods (fruit juices, liquid, eggs, milk, sauces, beverages)
Ultraviolet Light			
Radiation from the ultraviolet region of the electromagnetic spectrum (UVC 200–280 nm), at least 400 J/m^2	DNA mutations induced by UV light absorption	Transmissivity of the material, homogeneity of the flow pattern and the radiation field, UV wavelength, thickness of the radiation path through the food (geometric configuration of the system); product composition, solids content, and starches	Pasteurization of apple and clear juices. Surface and air decontamination
Pulse Light			
Few flashes applied in a fraction of a second of intense pulses of broad spectrum "white light" (ultraviolet to the near infrared region)	Chemical modifications and cleavage of the DNA and destruction of cellular components by the high peak power and the broad-spectrum of the flash	Light characteristics (wavelength, intensity, duration and number of pulses); packaging and type, transparency and color of food are considered to be critical process parameters	Sterilization or reduction of microbial load on surfaces and transparent products
Ultrasound			
Energy generated by sound waves of 20 kHz or more	Disruption of cellular structure and functional components and cell lysis attributed to cavitation	Power and amplitude of ultrasonic waves, exposure time, volume and composition of the food to be processed, temperature of treatment and the critical factors of procedures used in combination	No commercial food products. Limited to product modification and process efficiency improvements (enhancement of mass and heat transfer, degassing of liquids, cleaning of surfaces)

development but have commercial potential. Others have completed development and are waiting to be licensed or transferred to industry. Others are commercially applied.

The usefulness of many of these techniques is limited due to the following aspects. Most of these emerging factors are effective in inactivating vegetative cells of most microorganisms but spores are far more tolerant, and very high treatment intensities are required to achieve spore destruction in low acid foods. These big doses may significantly affect the sensory and/or functional properties of foods. So, these alternative factors for inactivating microorganisms are analogous to thermal pasteurization and their potential use for the sterilization of low acid foods is not yet possible.

The scheduled process for traditional thermal processes is based on the knowledge of the thermal inactivation kinetics of the most heat-resistant pathogen of concern for each specific product and of the heat transfer rate. The validity of the established process is often confirmed using an inoculated test pack study (under actual plant conditions) (31). As the mechanism of microbial inactivation for electric heating is basically the same as under conventional thermal treatment, the indicator microorganisms to validate the processes are the same used for conventional heating. But alternative non-thermal techniques involve different mechanism for microbial destruction and the organisms of concern are not the same, and neither are the inactivation kinetics that follow the exponential relationship that is usually the basis of the thermal process design. The most resistant pathogens and the corresponding surrogate organisms for alternative preservation processes are yet to be clearly defined. Moreover, limited information is available about the mechanisms of inactivation of microorganisms by alternative factors and survivor plots commonly exhibit a shoulder and/or a tail (44). A highly resistant subpopulation remains usually viable over a long period of treatment time, causing a substantial decrease in the efficacy of the processes.

Antimicrobial systems naturally present in plants (e.g., phenolics, essential oils, phytoalexins), animals (e.g., lisozyme, lactoperoxidase system, lactoferrin, chitosan), or microorganisms (e.g., nisin, pediocin and other bacteriocins, pimaricin and others antibiotics) have potential, in the context of "natural preservatives," to replace or reduce reliance on synthetic food preservatives (28, 40, 41). Because of interactions with food components (proteins, lipids, aldehydes and many macromolecules), natural antimicrobial concentrations required for microbial inhibition in real foods are considerable higher than in laboratory media and frequently above tolerable thresholds, limiting their industrial application (42).

However, a multifactorial preserving approach can minimize sensory changes in foods with natural antimicrobials, or enhance inactivation of highly resistant microbial subpopulations by alternative non-thermal factors, so that lower factor intensities can be used (42). Combining emerging factors with conventional preserving ones or with other novel techniques has been explored in the last years with promising results (42–45). Some successful additive or synergistic combinations are the following:

- High hydrostatic pressure and lowered pH: this combination prevents microbial growth and the germination of spores that can survive HHP treatment at acidic pH.
- High hydrostatic pressure and antimicrobial agents: sublethal injured cells by HHP become more susceptible to antimicrobials.
- Pulsed electric fields and heat: high temperatures increase the fluidity and the thickness of membranes, increasing the lethality of PEF treatment.
- Pulsed electric fields and antimicrobials: antimicrobials that act on the cell membrane may increase the susceptibility of membranes to dielectric breakdown and/or PEF may facilitate the access of antimicrobials that cross the membrane and acts in the cytoplasm.
- Ultrasound and natural antimicrobials (i.e., cinnamon): the lipophilic antimicrobial could accumulate in the lipid bilayer of the membrane, sensitizing this structure to the action of ultrasonic waves.

V. STRATEGIES TOWARDS MICROBIAL SAFETY OF MINIMALLY PROCESSED FOODS

The primary concern of the approach to the management of food from raw material to a consumed item is safety. Many MPF are good media for growth of microorganisms and represent a potential health risk. Because of the reduced microbiological safety margins, work is now being focused not only on the application of strict hygiene and good manufacturing practices, and implementation of hazard analysis and critical control points principles, but also on the design of the preservation system with additional hurdles so that an imperfect processing and/or packaging, distribution and storage can still guarantee safe products.

An analysis of the voluminous literature on the subject serves to highlight various important points to take into account to design effective MP techniques.

A. Formulation of the Combined MP Preservation System

– Most work has been focused on studying the response to the stress factors of key microorganisms in laboratory media and in model system, and few studies have been undertaken using actual foods. Moreover, when actual foods were

assayed, in most cases the native flora had been previously inactivated. The ecology of pathogens and non pathogens in foods is very complex and their interactions are difficult to predict.
- In many cases, intensity of the hurdles is detrimental to the food quality (e.g., flavour) and should be lowered, keeping in mind that hurdles levels must not only consider safety, but also desired total quality.
- The success of the hurdle technology used to design and/or to increase the safety of MP foods can be compromised in many ways. Two phenomena that appear to be significantly affecting the efficiency of the combined approach are next addressed.

1. Adaptation of Microorganisms to Sublethal Stresses

Spore-forming and non-spore-forming microorganisms sometimes react or adapt to mild stress factors by developing some mechanisms to repair the damages and to become even more resistant, surviving more severe homologous or heterologous stresses (46). Much of the research on the so-called "global stress response" has only been focused on the heat stress response and on acid adaptation, with the physiological bases involved not yet being fully understood. For example, bacteria that have been exposed to mildly acidic conditions acquire the ability to survive not only normally lethal pH values but also other stresses that microorganisms may meet in foods (47).

After adaptation to mild stresses, cells behave differently from unadapted ones and can grow at values outside the traditionally known ranges of temperature, water activity and pH determined under nearly optimal conditions (47). Therefore, microbiological challenge testing to assess the risk of food poisoning or to establish MP product stability needs careful design, and stressed known or potential pathogens would be also selected to validate the process.

Another point to be considered is that in certain instances, subinhibitory levels of additives, preservatives or chemicals may stimulate mold growth and/or toxin accumulation (28). The use of additives or preservatives at subinhibitory levels can not only stimulate toxin production but also eliminate other indigenous microflora that might be competitive with the growth of the mold favouring mold growth and toxin production (28).

2. Interactions between Stress Factors and Food Matrix

The intensity of the hurdles may change along product storage. Alternatively, the initial intensity of the hurdle may be less than the level applied. It is well known that sorbates, commonly used as preservatives in many food products, can be destroyed through an autoxidation mechanism, its destruction affecting food product safety (28).

Many natural antimicrobials (e.g., bacteriocins, some phenolics and essential oils) are less effective inhibitors in food matrix than *in vitro* (41). For instance, vanillin activity against *L. monocytogenes* in fluid milk decreases with increasing fat concentration. The preservatives probably bind to the fat, which thereby reduces their availability to inhibit microorganisms. The presence of di- and trivalent ions (such as Mg^{2+}, Ca^{2+}) has been reported to reduce the efficiency of nisin against Gram-positive bacteria due to inhibition of electrostatic interactions between positive charges on the bacteriocins and the negatively charged headgroups of the phospholipid molecules of the cytoplasmic membrane (21).

These and other phenomena that can reduce the intensity of the barriers are summarized as follows: binding to food components such as proteins and fats; chemical degradation; inactivation and/or biological destabilization by other ingredients or components; pH and temperature effects on hurdles stability and activity; physical losses by mass transport from the food to the environment; poor solubility and uneven distribution in the food (21, 48).

The interaction of antimicrobial factors can be additive, synergistic or antagonistic. It is not easy to anticipate the type of the interaction resulting of the combination of many factors. Moreover, the characteristics of the interaction can change along storage time or with minimal alterations in the food formulation (42).

Based on the facts cited above, much remains to be done regarding the contribution of the hurdles approach to minimal food preservation. In addition, Gould et al. (49, 50) pointed out that, although much progress on the physiology of the most important target microorganisms relevant to factors affecting growth and survival has been made, this has not yet been sufficiently exploited in the control of food poisoning microflora in a practical way of commercial interest.

B. Design of Minimally Processed Foods and Predictive Microbiology

Until recently, combined factors techniques were designed through observations of cardinal parameters for growth or decay as well as trial and error. In the last 20 years, a number of models to mathematically describe the growth or inactivation/decline of microorganisms under specific environmental conditions have been developed. Within this frame of risks, hazards and consumer trends, predictive microbiology emerges as a powerful tool to quickly explore the microbiological impact of varying conditions within MPF formulation, processing and/or distribution and retail conditions.

Information provided by the models can be used advantageously in the design of combined technologies for obtaining MPF in many ways (51):

- Although most of these MPF are preserved by more than one hurdle, there is a lack of quantitative data available to allow predicting the adequate and necessary levels of each hurdle. Thus, if we can predict with accuracy the growth kinetics (lag phase duration, growth rate), or the interface between microbial growth and no growth, or the decay kinetics for an identified target microorganism under several combinations of factors, the selection of such factors can be made on sound basis, and the selected hurdles can be kept at their minimum doses.
- Modelling has application in products where the perceived "naturalness" prevents the use of large intensities of chemical preservation and/or solutes and/or acids or/and heating or/and other non-thermal factors. Sensory selection of hurdles and their levels may be done between several "safe" equivalent combinations of interactive effects determined by the models.
- While much relevant information is available in the scientific literature concerning factors/interaction of factors that influence microbial activities in foods, it is seldom usable in formulating combined techniques. Many times, information involves only data from traditional challenge testing in particular food conditions, microbial presence or absence tests, or growth or no growth determination. These isolated results do not allow us to compare quantitatively what happens in a food system when the levels of the independent preservation variables are changed. Neither can the sensitivity of the key microorganisms to the different factors be inferred.
- Rather than inactivating the microorganisms, many MP techniques inhibit their growth through the manipulation of various growth-controlling factors. Thus, microbiological safety and stability margins are being reduced, and improved control in manufacture and distribution are required to ensure the recommended shelf lives. Temperature abuse, emergent pathogens, induced tolerance of microorganisms to stress factors and food matrix-stress factor interactions have caused concern about the safety of some of these nonsterile products. In this type of product, end-product testing is useless, and mathematical modelling can help developing qualitative and quantitative information to describe microbial behaviour, allowing a better control and prediction of the shelf-life.

VI. STRATEGIES TOWARDS SENSORY QUALITY OPTIMIZATION OF MINIMALLY PROCESSED FOODS

The concept of hurdle technology has been usually used to improve the microbial stability and safety of foods but the preservation of other food attributes is of additional concern. The combined technology concept refers not only to microbiological stability, but also to total quality of foods, comprising the application of preservation factors to inhibit or delay physicochemical and biochemical reaction deleterious to colour, texture, flavour and nutritive value of foods (22). Somehow, the application of combined processes for the enhancement of sensory or nutritional properties is not still sufficiently studied (51, 52).

Next, some examples of the application of combined techniques for enhancing quality retention are addressed.

A key quality problem in minimally processed vegetables and fruits, such as peeled and sliced apple and potato, is enzymatic browning by polyphenol oxidase (PPO). The prevention of browning reactions in this kind of product has been traditionally studied, but the effect of browning inhibitors on the sensory quality has not been examined. Sensory attributes such as appearance, flavour, odour and texture are also important factors affecting consumer acceptability and must be taken into account when a browning prevention method is selected (53). Enzymatic browning requires four different components: oxygen, an enzyme, copper and a substrate. At least one component must be removed from the system to inhibit browning (52). Historically, enzymatic browning has been controlled by heat inactivation of the enzyme or by the application of sulphites. In addition to the adverse health effects on asthmatics, sulphites may produce tissue softening and off-flavours. Some potential alternatives to sulphites include: 1) ascorbic acid (AA), that reduces quinones back to phenolic compounds before they can undergo further reaction to form pigments, chelates copper and acts as a competitive inhibitor of PPO; 2) citric acid (CA), which acts as chelating agent and acidulant; 3) 4-hexilresorcinol (HR), that interacts with PPO, avoiding its participation in the browning reaction; 4) EDTA, that acts as complexing agent; 5) sulphydril-containing amino acids like cisteine, which prevent brown pigment formation by reacting with quinine intermediates; and 6) MAP, which reduces the oxygen concentration in the atmosphere surrounding a product (52). Hurdle approaches for inhibiting browning can be formulated by using some of these alternatives in combination. For instance, AA or AA derivatives, like AA-2-phosphate and AA-2-triphosphate, have been used in combination with CA, Ca_2Cl, phosphoric acid and sodium acid pyrophosphate (SAPP) in potatoes (54, 55); with HR in apples (56) and with CA in potatoes (57). Digestion with hot ascorbic acid/citric acid solutions also improved the shelf-life of pre-peeled potatoes (55). However, high

concentrations of ascorbic acid (0.75%) may impose an unpleasant taste on fruits (58). Apple pieces treated with 0.01% HR, 0.5 & AA and 0.2% Ca_2Cl and packed under partial vacuum could be stored at 0.5% for 50 days with acceptable colour (58). A natural combined system is pineapple juice, which has been found to be an effective browning inhibitor in fresh as well as in dried apples (59). Pineapple juice compounds which may have potential as PPO inhibitors include organic acids, proteins, amino acids, certain metals and sulfhydryl compounds. Dipping potatoes into solutions of L-cisteine and CA followed by packing and flushing with N_2 also allowed to obtain an extended shelf-life (60).

Texture is another quality determinant in fruits and vegetables. Calcium has widely been reported to play an important role in preserving structural integrity and mechanical strength of cell walls (61, 62). Depending on the impregnation method and on the pretreatment (blanching), it has been successfully used as a texture enhancer when added in infusion solutions as calcium lactate in minimally processed kiwi, strawberry and melon (63). However, applied as calcium chloride during osmotic adjustment of a_w and pH in apple preserved in a similar way, it was not able to prevent softening, probably due to the high calcium concentration employed and solubilization of pectin substances (64). Calcium (as 1% $CaCl_2$) and ascorbic acid dips have also been employed as firming agents to extend postharvest shelf life in sliced pears and strawberry stored in controlled atmosphere (65). The combined effects of heat treatment (20, 40, 60°C) and calcium dips (2–5% $CaCl_2$) on the firmness of fresh-cut cantaloupe melons was studied by Luna-Guzmán et al. (66). A dip in 2.5% $CaCl_2$ solution at 60°C for 1 min not only resulted in a great improvement in firmness but the fruit seemed to hold firmness over 10 days of storage samples (Figure 118.1). This effect in calcium and heat treated melon was attributed to PE activation, allowing more calcium complexing following PE-activated and middle lamella, and/or to a membrane or turgor pressure effect.

The impact of processing and the vegetable stress reactions that often take place during storage may be minimized by refrigeration and suitable modified atmosphere packaging. MAP fresh produce requires that a narrow range of concentrations be maintained in a package, typically 2–5% O_2, 2–5% CO_2 and the rest nitrogen (67). This is achieved by means of a dynamic interaction between the respiring produce, the gas permeability of the packaging film and environmental conditions such as storage temperature. High O_2 MAP treatment has been found to be particularly effective at inhibiting enzymatic browning, preventing anaerobic fermentation reactions and inhibiting aerobic and anaerobic microbial growth. It is hypothesized that high O_2 levels may cause substrate inhibition of PPO or alternatively high levels of colourless quinones subsequently formed may cause feedback production of PPO (53). When

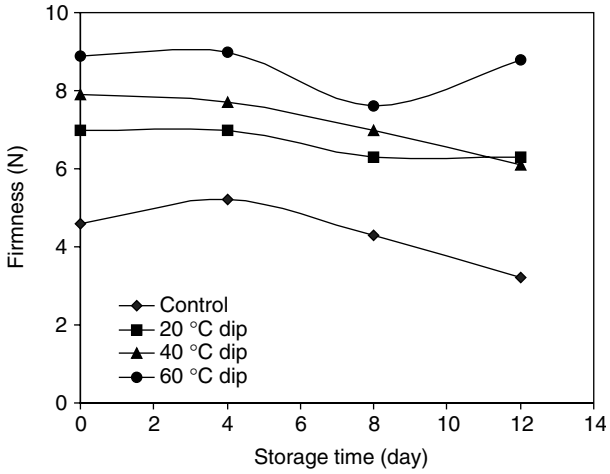

FIGURE 118.1 Firmness of fresh-cut cantaloupe melon during storage at 5°C and 95% RH for 12 days after being dipped in 2.5% calcium chloride solution for 1 min at different temperatures. Adapted from Luna-Guzmán et al. (66).

the packaging material is not sufficiently permeable and the produce has high respiration, microholes of defined sizes and a defined quantity can be made in the material in order to avoid anaerobiosis (68). Barry-Ryan et al. (69) showed by using different films and storage temperatures (3 and 8°C) that deterioration in shredded carrots (slime production, loss of firmness and the development of off-odours) occurred more rapidly with the depletion of O_2 than by the rise in CO_2 and that a film with similar permeability to O_2 and CO_2 was the most suitable for the storage. Packaged perforation significantly improved, for example, the shelf-life of grated carrots (52), but it was not needed in shredded Iceberg lettuce dipped in betaine solutions (70). According to Gil et al. (71), the colour preservation (anthocyanin content) of pomegranate seeds was similar after storage at 1°C in perforated polypropylene (air atmosphere) or in unperforated polypropylene (MAP). The potential benefits of controlled atmospheres and heat treatment to maintain high quality and to extend shelf-life of green onions were reported by Hong et al. (72). Atmospheres of 0.1–0.2% O_2 or 0.1–0.2% O_2 containing 7.5–9% CO_2 were the conditions that best maintained the visual appearance in both intact and cut onions, and in combination with a pre-storage treatment (55°C for 2 min), provided a shelf-life of more than 2 weeks at 5°C. This combination of factors did not reduce the soluble sugar and thiosulfinate concentration of produce. A controlled atmosphere (air + 15% CO_2) at 5°C greatly reduced development of macroscopic decay, translucency and off-odours in pieces of honeydew melons (73). Controlled atmospheres reduced colour changes, but not firmness loss; soluble solids concentration was maintained during storage in air or controlled atmospheres. Edible coatings are an alternative packaging method for extending the post-harvest storage of minimally processed

fruit and vegetables, because they have the potential to reduce moisture loss, restrict oxygen entrance, lower respiration, retard ethylene production, seal in flavour volatiles and carry additives (such as antioxidants) that retard discoloration and microbial growth (52).

The control of nutritive value (i.e., vitamin, sugar, amino acid, fat and fibre content) of minimally processed produce is an area that needs more research. The steps often involved during processing and storage conditions usually have an effect on nutrients and these changes must be taken into account. Important components of vegetables include vitamins, particularly those which act as antioxidants, such as ascorbic acid and β-carotene. Loss of these nutrients is one of the main limiting factors of nutritional quality (15). Recently, the importance of changes of the total antioxidant activity as an indicator of overall quality of any processed product has also been highlighted (74, 75). Barry-Ryan and O'Beirne (76) analyzed the effects of slicing method, packaging atmosphere and storage temperature on ascorbic acid retention and quality of shredded lettuce. Modified atmosphere packaging, nitrogen flushing, manual tearing or slicing and low temperatures were effective postharvest storage treatments for conserving ascorbic acid and visual quality. Kaur and Kapoor (75) studied the effect of different dip treatments and storage period on the antioxidant activity and ascorbic acid and carotenoids contents of Indian cabbage. Incorporation of ascorbic and citric acids in dip water improved the overall appearance and retained the maximum antioxidant activity, ascorbic acid and total carotenoids (Figure 118.2).

Emerging non-thermal factors, such as ultrasound, electric pulses, light pulses and high pressure are promising procedures, from the point of view of both shelf-life and product modification. The impact of cell membrane permeabilizing features of non-thermal processes to introduce modifications in plant foods, such as the retention and availability of nutritionally relevant metabolites from plant materials, as well as the improvement of mass transfer of functional ingredients during drying processes, was highlighted by Rastogi et al. (77) and Knorr (78). Pulse electric fields have been applied successfully prior to osmotic dehydration to enhance mass transfer of water and sugar in carrots (79), and pineapple (80). Ade-Omowaye

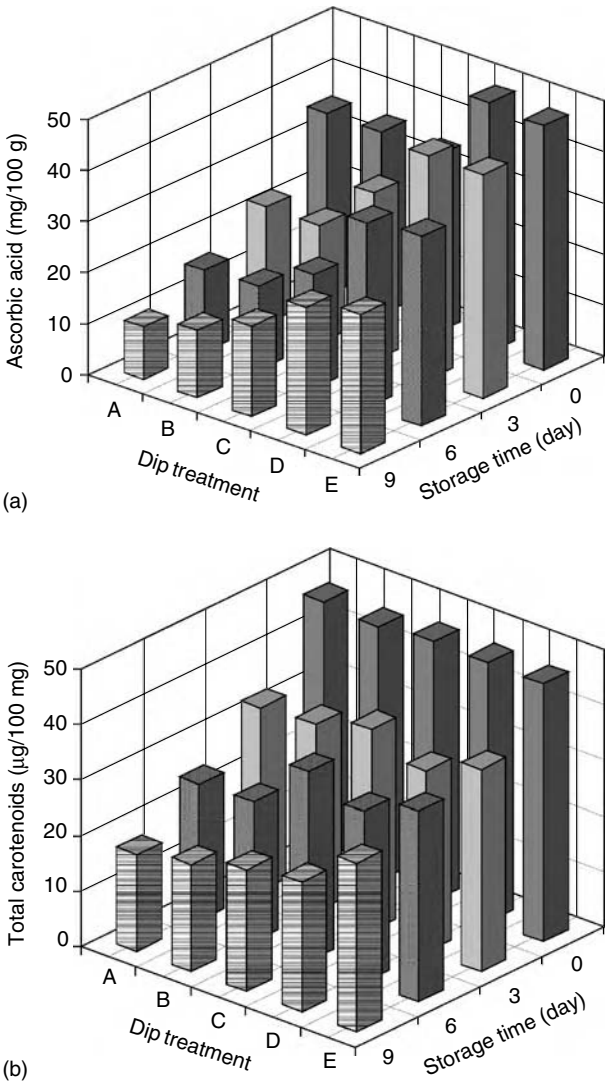

FIGURE 118.2 Effect of different dip treatments (A = water at 4°C, B = chlorinated water at 4°C, C = chlorinated water + citric acid (10 g/l) at 4°C, D = chlorinated water + ascorbic acid (10 g/l) at 4°C, E = chlorinated water + citric acid (10 g/l) + ascorbic acid (10 g/l) at 4°C) on: a) ascorbic acid content, and b) carotenoids content of minimally processed cabbage (var. Golden Acre) throughout the storage time. Adapted from Kaur and Kapoor (75).

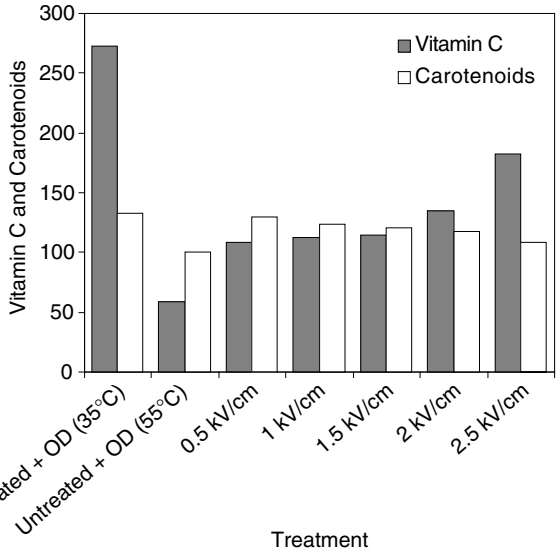

FIGURE 118.3 Vitamin C and carotenoids contents of dried osmosed bell pepper treated at different temperatures (35°C and 55°C) and high intensity electric field pulses at different field strengths (E = 0.5–2.5 kV/cm). Adapted from Ade-Omowaye et al. (81).

et al. (81) evaluated the influence of high intensity electric pulses field at varying field strengths (E = 0.5–2.5 kV/cm), osmotic agent (sucrose or sodium chloride) and moderate thermal treatment (25–55°C) on selected quality parameters (vitamin C and carotenoids) of bell peppers. Increasing temperature resulted in water loss from 32% to 48% and increasing field strength resulted in water loss from 36% to 50% of initial moisture content. But combination of high intensity electric pulses (2.5 kV/cm) with subsequent osmotic dehydration at 35°C gave products higher in vitamin C and carotenoids than osmotic treatment alone at 55°C, suggesting high intensity electric pulses as an alternative to thermal processing to improve mass transfer (Figure 118.3). A combination of high pressure treatment, in conjunction with subsequent freezing prior to fluidized bed drying of green beans, carrots and potatoes appeared to improve mass transfer and enhance product quality regarding to texture and colour of rehydrated product (Figure 118.4) (82). Simal et al. (83) also reported the applicability of sonication to increase water loss and solute gain during osmotic dehydration of apple cubes.

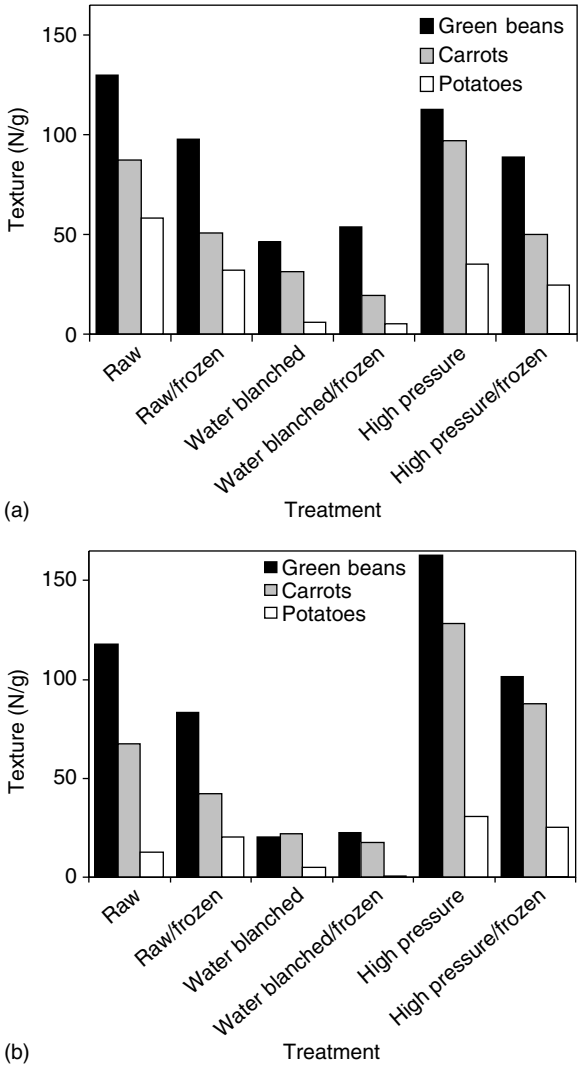

FIGURE 118.4 Texture measurements of green beans, carrots and potatoes: a) prior fluidized bed drying, and b) after rehydration. Adapted from Estiaghi et al. (82).

VII. FINAL REMARKS

Minimally processed foods are becoming an important component of the food supply. The older methods of food preservation continue to be used extensively and technological advances to enhance their efficiency and effectiveness are being developed at a high rate. The adoption of newer techniques for industrial application, in spite of the enormous advancement in technological and fundamental knowledge made in the last decade, is slow. More research is needed. Most minimal processing technologies need to be considered as combined techniques. So, it is imperative that reactions that are responsible of microbial, sensory, nutritional, physical and chemical quality be understood and quantified as a function of the hurdles involved in the combined preservation system. In addition, food safety and quality aspects have to be integrated along the entire food chain, from "farm to table." The production of MPF should include: scrutinizing materials entering the food chain; inhibiting microbial, biochemical and physicochemical deleterious reactions (i.e., refrigerated storage and distribution); and decreasing or eliminating the microbial load by processing and preventing post-processing contamination as well as decreasing quality changes. That is, the safety and quality of food should be based on controlling all steps in the food production process.

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119 An Interactive Design of MA-Packaging for Fresh Produce

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CONTENTS

A. NOMENCLATURE

A = Area, m^2
A_F = Area of film, m^2
A_H = Total area of holes, m^2
D_{CO_2} = Diffusivity of CO_2 in air, $m^2.sec^{-1}$
D_{O_2} = Diffusivity of O_2 in air, $m^2.sec^{-1}$
D_P = Diameter of perforation, m
e = Thickness of film, m
E_{CO_2} = Activation energy of permeability for CO_2, $kJ.mol^{-1}$
E_{O_2} = Activation energy of permeability for O_2, $kJ.mol^{-1}$
H_T = Height of package, m
I_F = Intercept of film line, atm
I_N = Intercept of a line perpendicular to film line, atm
L_P = Length of perforation, m
L_T = Length of package, m

M = Weight of commodity, kg
m_M = Molar mass
N_H = Number of holes
N_{ap} = Number of perforations
$P^*_{CO_2}$ = Permeability pre-exponential factor for CO_2, $ml.mil.m^{-2}.hr^{-1}.atm^{-1}$
$P^*_{O_2}$ = Permeability pre-exponential factor for O_2, $ml.mil.m^{-2}.hr^{-1}.atm^{-1}$
P_{CO_2} = Permeability for CO_2, $ml.mil.m^{-2}.day^{-1}.atm^{-1}$
P_{O_2} = Permeability for O_2, $ml.mil.m^{-2}.day^{-1}.atm^{-1}$
P_T = Total pressure, Pa
R_{CO_2} = Rate of CO_2 production rate, $ml.kg^{-1}.h^{-1}$
R_{O_2} = Rate of O_2 consumption rate, $ml.kg^{-1}.h^{-1}$
$R^A_{CO_2}$ = Rate of CO_2 production at the $y^{min}_{O_2}$ and $y^{min}_{CO_2}$, $ml.kg^{-1}.h^{-1}$
$R^A_{O_2}$ = Rate of O_2 consumption at the $y^{min}_{O_2}$ and $y^{min}_{CO_2}$, $ml.kg^{-1}.h^{-1}$
$R^B_{CO_2}$ = Rate of CO_2 production at the $y^{max}_{O_2}$ and $y^{max}_{CO_2}$, $ml.kg^{-1}.h^{-1}$
$R^B_{O_2}$ = Rate of O_2 consumption at the $y^{max}_{O_2}$ and $y^{max}_{CO_2}$, $ml.kg^{-1}.h^{-1}$
$R^{best}_{CO_2}$ = Rate of CO_2 production at the intersection point, $ml.kg^{-1}.h^{-1}$
$R^{best}_{O_2}$ = Rate of O_2 consumption at the intersection point, $ml.kg^{-1}.h^{-1}$
$R^{mean}_{CO_2}$ = Rate of CO_2 production at average of optimum window, $ml.kg^{-1}.h^{-1}$
$R^{mean}_{O_2}$ = Rate of O_2 consumption at average of optimum window, $ml.kg^{-1}.h^{-1}$
R = Gas constant ($0.008314\,kJ.mol^{-1}.K^{-1}$)
R_H = Radius of hole, m
RQ_A = Respiratory quotient at the $y^{min}_{O_2}$ and $y^{min}_{CO_2}$
RQ_B = Respiratory quotient at the $y^{max}_{O_2}$ and $y^{max}_{CO_2}$
RQ_{mean} = Respiratory quotient at average of optimum window
S_F = Slope of film line
S_N = Slope of a line perpendicular to film line
T = Storage temperature, °C
t_{O_2} = Equilibrium time, sec
$t^{eq}_{CO_2}$ = Equilibrium time for CO_2, sec
$t^{eq}_{O_2}$ = Equilibrium time for O_2, sec
y_{CO_2} = CO_2 concentration, atm
y_{O_2} = O_2 concentration, atm
$y^{best}_{CO_2}$ = CO_2 concentration at the intersection point, atm
$y^{best}_{O_2}$ = O_2 concentration at the intersection point, atm
$y^{eq}_{CO_2}$ = Equilibrium CO_2 concentration, atm
$y^{eq}_{O_2}$ = Equilibrium O_2 concentration, atm
$y^{e}_{CO_2}$ = External CO_2 concentration, atm
$y^{e}_{O_2}$ = External O_2 concentration, atm
$y^{i}_{CO_2}$ = Initial CO_2 concentration, atm
$y^{i}_{O_2}$ = Initial O_2 concentration, atm
$y^{min}_{CO_2}$ = Minimum CO_2 of optimum window, atm
$y^{min}_{O_2}$ = Minimum O_2 of optimum window, atm
$y^{max}_{CO_2}$ = Maximum CO_2 of optimum window, atm
$y^{min}_{O_2}$ = Maximum O_2 of optimum window, atm
$y^{mean}_{CO_2}$ = Average of optimum CO_2 range, atm
$y^{mean}_{O_2}$ = Average of optimum O_2 range, atm
V_f = Free volume of package, m^3
V_t = Total volume of package, m^3
W_B = Bottom width of package, m
W_T = Top width of package, m
β = Permeability coefficient, fraction
β_{max} = Maximum β value, fraction
β_{mean} = Average β value, fraction
β_{min} = Minimum β value, fraction
ε = Porosity of perforation, fraction
ρ = Density of produce, $kg.m^{-3}$
Γ = Tortuosity factor of perforation, fraction

B. Symbols

C_2H_4 = Ethylene
CA = Controlled atmosphere
CO_2 = Carbon Dioxide
EMA = Equilibrium Modified Atmosphere
MA = Modified Atmosphere
MAP = Modified Atmosphere Packaging
O_2 = Oxygen
RQ = Respiratory Quotient
a = constant
b = constant
c = constant

I. INTRODUCTION

There has been an increasing interest in using polymeric films for packaging of fresh and fresh-cut horticultural commodities to provide several benefits such as protection against physical injuries, improved sanitation, control of light conditions, and more importantly modification of atmospheric composition. There is a wealth of published information on Modified Atmosphere Packaging (MAP), yet no systematic theoretical study has been conducted to establish which commercially available plastic films would be most suitable for MAP of a particular produce. Such analysis could provide an initial screening of polymeric films, point out potential limitations, and help minimise the number of experimental trials.

The objective of MAP design is to define conditions that will create the atmosphere best suited for the extended storage of a given produce and to minimise the period of time to achieve this atmosphere. A MAP system not properly designed may be ineffective or even shorten the storage life of a product. If the desired atmosphere is not established rapidly, the package has no benefit. For example, high perishable products may deteriorate before the recommended atmosphere is attained. If O_2 and/or CO_2 levels are not within the recommended ranges of O_2 and CO_2 concentrations the product may experience serious alterations and its storage life is shortened. It may

even induce anaerobiosis, with the possible growth of pathogens and concomitant effects on product safety.

The success of MAP depends largely on the selected package material and thickness, as well as on the package surface area and the free volume inside the package. These depend on the respiration rate of the product, weight to be packed and temperature of the distribution chain. Design criteria for modified atmosphere packaging of respiring products have been widely reported in literature. Yet, their industrial application is limited, owing to the numerical complexity of the design equations, the lack of relevant data and the non-availability of user-friendly simulator tools.

Simulation of a MAP system is the most appropriate method to allow a correct MAP design and consequently obtain a successful commercial product. The "pack and pray" procedure may have economic and safety hazard consequences and the "trial and error" approach is an extremely time consuming procedure. The Department of Process Engineering, University College, Cork, Ireland has developed user-friendly software, called PACKinMAP, for designing MAP for fresh produce. PACKinMAP can be used as a tool to ensure the proper equilibrium modified atmosphere for each packaged fresh whole/fresh cut product. To reach this optimal atmosphere inside the package, PACKinMAP simulates O_2 and CO_2 concentrations for different combinations of films, area, and weight, avoiding time-consuming experiments to determine the best film to be used in packaging each type of product. The resulting PACKinMAP solution takes into account factors such as respiration rate, temperature, packaging film and its area, produce weight, package geometry and its volume.

II. DESIGN METHODOLOGY

Figure 119.1 schematically shows respiring produce stored in a package comprised of a plastic film. The simplest concept is to let the plastic film serve as the regulator of O_2 flow into the package and the flow of CO_2 out. Assuming that there is no gas stratification inside the package and that the total pressure is constant, the differential

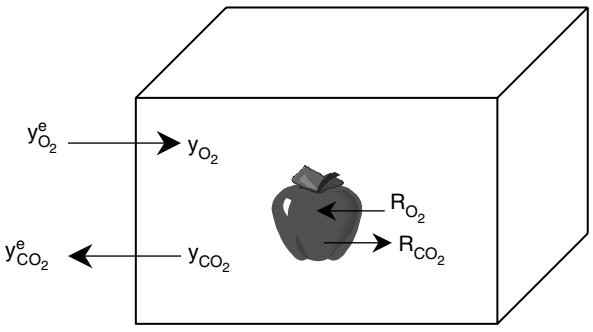

FIGURE 119.1 Gas exchange mechanism in MA package containing fresh produce.

equations of mass balance for O_2 and CO_2 in MAP containing a respiring product are (balance with N_2):

$$V_f \times \frac{d(y_{O_2})}{dt} = \frac{P_{O_2}}{e} \times A \times (y^e_{O_2} - y_{O_2}) - R_{O_2} \times M \quad (119.1)$$

$$V_f \times \frac{d(y_{CO_2})}{dt} = \frac{P_{CO_2}}{e} \times A \times (y^e_{CO_2} - y_{CO_2}) + R_{CO_2} \times M \quad (119.2)$$

Equations 119.1 and 119.2 are first-order linear differential equations, which are useful for describing the unsteady state behaviour of MAP system during the process of passive modification. When the accumulated term is zero, Equations 119.1 and 119.2 are reduced to the steady state as:

$$y^e_{O_2} = y_{O_2} + \frac{R_{O_2} \times e \times M}{P_{O_2} \times A} \quad (119.3)$$

$$y^e_{CO_2} = y_{CO_2} - \frac{R_{CO_2} \times e \times M}{P_{CO_2} \times A} \quad (119.4)$$

The above equations describe the dynamic equilibrium behaviour of the MAP system, when the CO_2 evolution rate equals the efflux rate of CO_2 through the package and the O_2 consumption rate equals the influx rate of O_2 through the package. In most commercial package situations, steady state or dynamic equilibrium is approached within two days. For long storage of the product, the dynamic equilibrium behaviour is more important than the unsteady state behaviour. To use Equations 119.3 and 119.4 as design equations, it is necessary to keep track of how many independent or design variables are available. As per Table 119.1 there are a total of 11 variables: R_{O_2}, R_{CO_2} and M are associated with the product; P_{O_2}, P_{CO_2}, e and A are associated with the package; $y^e_{O_2}$, y_{O_2}, $y^e_{CO_2}$ and y_{CO_2} are associated with the environment. Once the product and the temperature are selected, six out of the 11 variables are already decided: R_{O_2} and R_{CO_2} from the respiration rate model as reported in the literature; y_{O_2} and y_{CO_2} are assumed to be the optimum O_2 and CO_2 concentrations and $y^e_{O_2}$ and $y^e_{CO_2}$ are constant, i.e., 0.21 atm and 0.0003 atm, respectively. With six variables fixed and two equations to satisfy, there are only $(11-6-2) = 3$ design variables. That is, only

TABLE 119.1
Variables Used in MAP Design

Input variables	Surrounding-related	$y^e_{O_2}$, $y^e_{CO_2}$
	Package-related	A, e
	Commodity-related	M
Calculated variables	Package-related	P_{O_2}, P_{CO_2}
	Commodity-related	R_{O_2}, R_{CO_2}
Response variables	System-related	y_{O_2}, y_{CO_2}

three out of the remaining five variables (M, e, A, P_{O_2} and P_{CO_2}) can be specified arbitrarily. Combining Equations 119.3 and 119.4 we get:

$$\frac{y^e_{CO_2} - y_{CO_2}}{y^e_{O_2} - y_{O_2}} = -\frac{RQ}{\beta} \qquad (119.5)$$

Where, RQ is the respiratory quotient and β is the permeability ratio, defined as R_{CO_2}/R_{O_2} and P_{CO_2}/P_{O_2}, respectively.

III. COMMODITY RELATED ASPECTS

For any packaging design, the product characteristics such as its weight, respiration rate, density and the optimum conditions for the best shelf life are needed for the mathematical calculations. Such data is widely available in literature. For the present software a database is prepared which includes all these parameters. This database is flexible as users can change it as per their requirements.

A. Respiration Rate

Control of respiration is an important effect of atmosphere modification on post harvest life of fruits and vegetables (1). High respiration rates are associated with rapid deterioration of the product. Design of a MAP system that will prolong the storage or shelf life of fresh product requires the mathematical equation for predicting the respiration rate at the various influencing factors. Thus, the respiration rate model is central to the design of MAP for fresh fruits and vegetables.

B. Mathematical Modelling of Respiration Rate

There are a number of limitations to the development of predictive models. Potentially large experimental errors and time consuming experiments for the determination of respiration rates for MAP design as well as the complex nature of the process are limitations to the development of predictive models. Thus, a constant respiration rate is sometimes considered in MAP modelling reported in the literature (2). However, this approach can only be accepted as a simplified model, as, in fact, MAP relies on the ability to control the respiration rate by changing the atmospheric composition. Hence, the design should take into consideration not only steady-state conditions, but also the dynamic process, because if the product is exposed for a long time to unsuitable gas composition before reaching the adequate atmosphere, the package may have no benefit. Moreover, the success of MAP greatly depends on the accuracy of the predictive respiration rate models. Table 119.2 presents the factors for conversion of the different units used in the published works to the International System (SI) of units.

The fruits and vegetables listed in Table 119.3 have been selected based on the availability of respiration rate models in the published literature. These models are function of O_2, CO_2, temperature and time or combinations thereof. All the models were modified to yield uniform units of respiration rate, i.e., $m^3.kg^{-1}.s^{-1}$. The respiration rate of each selected fruit and vegetable is calculated at 5°C with air and is shown in Table 119.3.

TABLE 119.2
Conversion Factors to SI Units

Units to be Converted	Conversion Factor	Units Obtained
$mg.kg^{-1}.h^{-1}$	$2.778 \times 10^{-7}/m_M$	$mol.kg^{-1}.s^{-1}$
$ml.kg^{-1}.h^{-1}$	$3.341 \times 10^{-11} \times \frac{p_T}{R \times T}$	$mol.kg^{-1}.s^{-1}$
$mol.kg^{-1}.h^{-1}$	2.778×10^{-4}	$mol.kg^{-1}.s^{-1}$
%	$p_T \times 10^{-2}$	Pa
$mol.kg^{-1}$	$p_T \times m_M/1000$	Pa
atm	101325	Pa

Source: Banks et al. (3).

C. Commodities and their Optimum Conditions

The best atmosphere to extend product shelf life varies from product to product and depends on temperature. Recommended concentrations also depend on the quality parameter under evaluation. Criteria include lowering of C_2H_4 production or action, lowering of the number and extent of physiological disorders, retention of quality and better colour, flavour or texture. Normally the most important one is selected, that is, the one that limits shelf life. Usually, the recommendations for CA storage are also applied to MA. However, optimum MA concentrations may be more severe (lower O_2 and higher CO_2 concentrations) than long CA conditions because the product is submitted to the atmosphere over a much shorter period of time.

The first commercial recommendations for the CA storage of apples were formulated over 80 years ago by Kidd and West in England (34). CA/MA requirements and recommendations on the optimum atmospheric conditions for selected whole and cut vegetables and fruits are periodically presented in the International Controlled Atmosphere Research Conference. The recommendations are updated over the years when research introduces new data. The range of gas composition that best extends product shelf life is often called the "window" of recommended atmosphere. However, the extension of product shelf life by use of low O_2 and high CO_2 is not convenient for all products. Some vegetables, such as asparagus and okra, do not benefit from low O_2 levels. There are great differences in tolerance to CO_2 levels. While most fruits may tolerate up to 5% CO_2, some fruits may show CO_2 injury at this level and others may even tolerate 15–20% CO_2. The recommended atmospheres for fruits and vegetables are shown in Figures 119.2 through 119.4.

TABLE 119.3
Respiration Rate of Fruits and Vegetables Predicted by Models Presented in the Literature

Commodity	Reference	Respiration Rate* ml of O_2/kg hr	Respiration Rate* ml of CO_2/kg hr	RQ	Commodity	Reference	Respiration Rate* ml of O_2/kg hr	Respiration Rate* ml of CO_2/kg hr	RQ
Apple:					French beans	18	115.77	107.67	0.93
var. Elstar	4	17.68	15.21	0.86	Garlic	13	31.53	23.46	0.74
var. Red delicious	5	16.97	17.10	1.01	**Onion:**				
var. Golden delicious	4	12.51	10.76	0.86	Cut Onion Cepa	13	11.11	5.78	0.52
var. NY 640 slices	6	29.07	37.79	1.30	Green Onion Fistulosum	13	50.92	40.97	0.80
var. Delicious	7	8.42	10.31	1.23					
var. Red delicious slices	8	8.91	5.56	0.62	Green Onion Wakeyi	13	51.38	47.80	0.93
Asparagus	9	41.76	36.33	0.87	Litchi	22	13.90	11.60	0.83
Banana	10	14.14	15.01	1.06	**Mango:**				
Bell pepper	11	12.28	11.15	0.91	var. Nam dok mai	23	22.10	17.53	0.79
Blueberry:					Mango slices	24	787.86	1005.95	1.28
var. Blueray	12	259.08	204.73	0.79	Whole Mango	25	19.28	17.74	0.92
var. Coville	12	365.62	290.16	0.79	Mixed lettuce	18	6.71	6.24	0.93
var. Duke	12	16.69	14.21	0.85	Mungbean	18	59.93	55.74	0.93
var. Jersey	12	211.50	167.12	0.79	Mushroom	9	118.36	106.34	0.90
var. Michigan	13	3.83	3.90	1.02	Nopal	26	34.90	35.30	1.01
var. Sharpblue	14	3.14	3.21	1.02	Peeled garlic	13	33.43	24.65	0.74
Cabbage	15	112.56	90.05	0.80	Pepper	13	19.38	12.00	0.62
Carrot	13	26.05	14.58	0.56	Raspberry	27	43.46	48.63	1.12
Cauliflower	16	9.80	13.88	1.42	Red pepper	25	15.15	14.09	0.93
Cherry:					Rutabaga cut	28	10.04	9.21	0.92
var. Burlat	17	36.66	33.73	0.92	Rutabaga whole	28	4.59	4.27	0.93
var. Sunburst	17	13.77	12.66	0.92	Shredded lettuce	15	48.97	39.18	0.80
var. Sweatheart	17	48.32	38.66	0.80	Snap bean	29	92.15	83.85	0.91
Chicory	4	56.63	52.67	0.93	Soybean	13	50.52	14.22	0.28
Chicory var. Belgium	18	19.98	18.58	0.93	Strawberry	30	21.62	18.11	0.84
Chopped lettuce	19	7.13	6.63	0.93	Strawberry var. Elsanta	31	20.46	18.73	0.92
Coleslaw	20	11.98	13.07	1.09	**Tomato:**				
Cucumber	13	24.39	16.52	0.68	Tomato	32	36.01	18.30	0.51
Cucumber Belgium	18	6.90	6.42	0.93	Green	32	15.13	14.07	0.93
Cut bell pepper	18	7.09	6.59	0.93	Heinz	33	18.67	17.36	0.93
Cut broccoli	13	225.62	200.82	0.89	Pink	32	15.79	14.68	0.93
Cut Galega kale	21	101.29	94.20	0.93	Red	32	13.80	12.84	0.93
Cut pepper	13	2.21	3.68	1.66	Whole broccoli	4	120.77	113.53	0.94
					Whole chicory	18	47.51	44.18	0.93

* Temperature = 5°C, O_2 = 21% and CO_2 = 0.03%.

D. Calculation of Limiting β Values

Selection of a film having optimal β is necessary to achieve the optimum atmosphere in the package. Use of a polymeric film with a high β (e.g., >4–6) results in an equilibrium atmosphere that is low in CO_2, and films with low β (e.g., <2) tend to accumulate high levels of CO_2 without regard to absolute permeability rates. Equation 119.5 implies that, for a product with a given RQ, a given packaging can only yield a limited combination of O_2 and CO_2 concentrations, which is controlled by the relative transfer rate of CO_2 to O_2. Thus, the selection of packaging material basically relies on the choice of a material with a permeability ratio that will allow it to reach the desired atmosphere composition at steady state. This requires computation of the extreme β values adequate for the chosen commodity and can be calculated by solving Equation 119.5 at the maximum and minimum values of O_2 and CO_2 concentration as:

$$\beta_{min} = RQ_B \left(\frac{y_{O_2}^{e} - y_{O_2}^{max}}{y_{CO_2}^{max} - y_{CO_2}^{e}} \right) \tag{119.6}$$

$$\beta_{max} = RQ_A \left(\frac{y_{O_2}^{e} - y_{O_2}^{min}}{y_{CO_2}^{min} - y_{CO_2}^{e}} \right) \tag{119.7}$$

$$\beta_{mean} = \frac{\beta_{min} + \beta_{max}}{2} \tag{119.8}$$

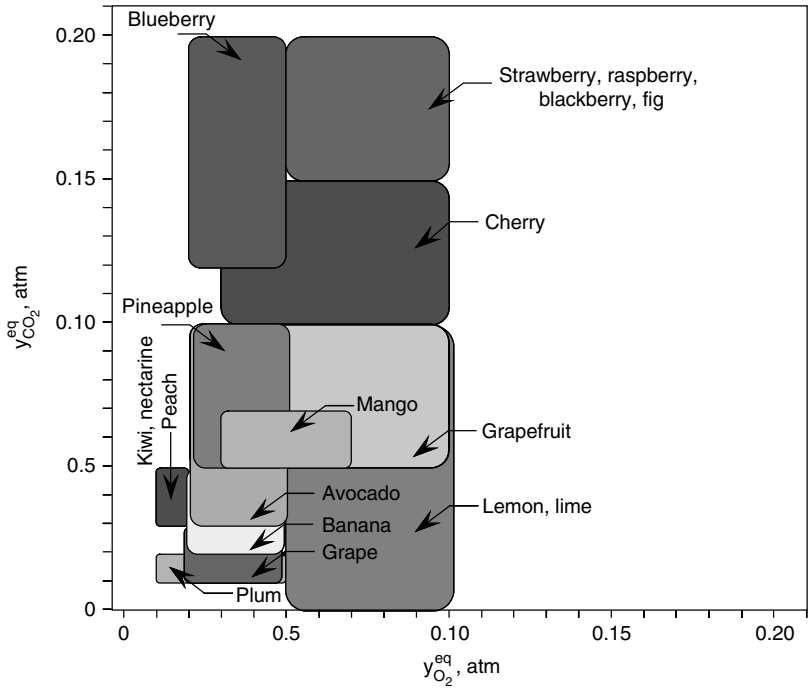

FIGURE 119.2 Recommended atmospheres for fresh fruits (boxes show the recommended atmosphere for each product).

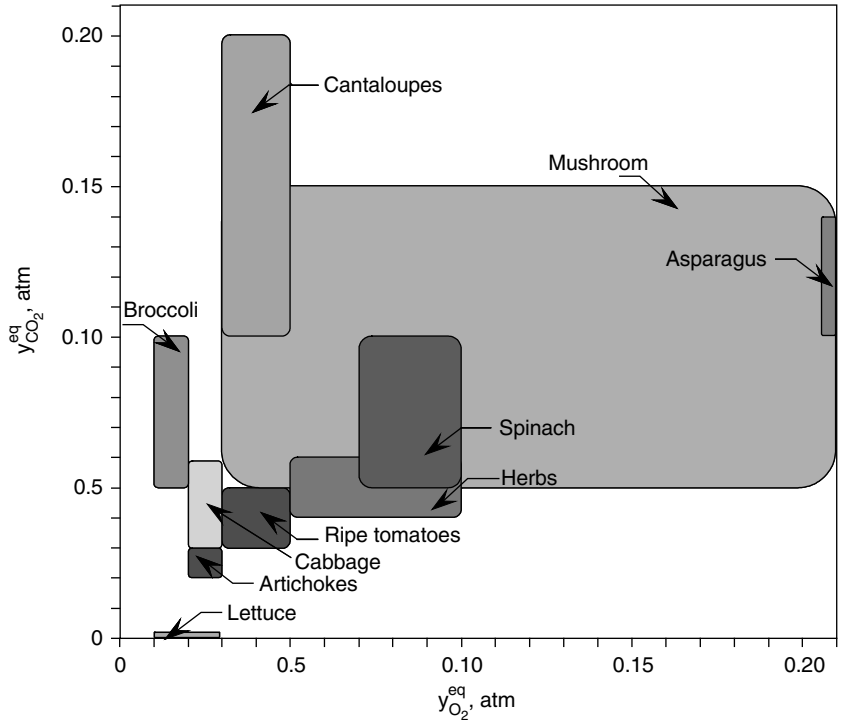

FIGURE 119.3 Recommended atmospheres for fresh vegetables (boxes show the recommended atmosphere for each product).

The Respiratory Quotients (RQs) are calculated at the bottom-left (A) and top-right (B) corners of the "Optimum Window" of recommend gas composition, i.e., for lowest O_2 and CO_2 concentrations and for highest O_2 and CO_2 concentration. These calculations are done for the temperature specified by the user. The model is selected based on the commodity chosen. In order to set the limit of RQ by Equations 119.6 and 119.7, the

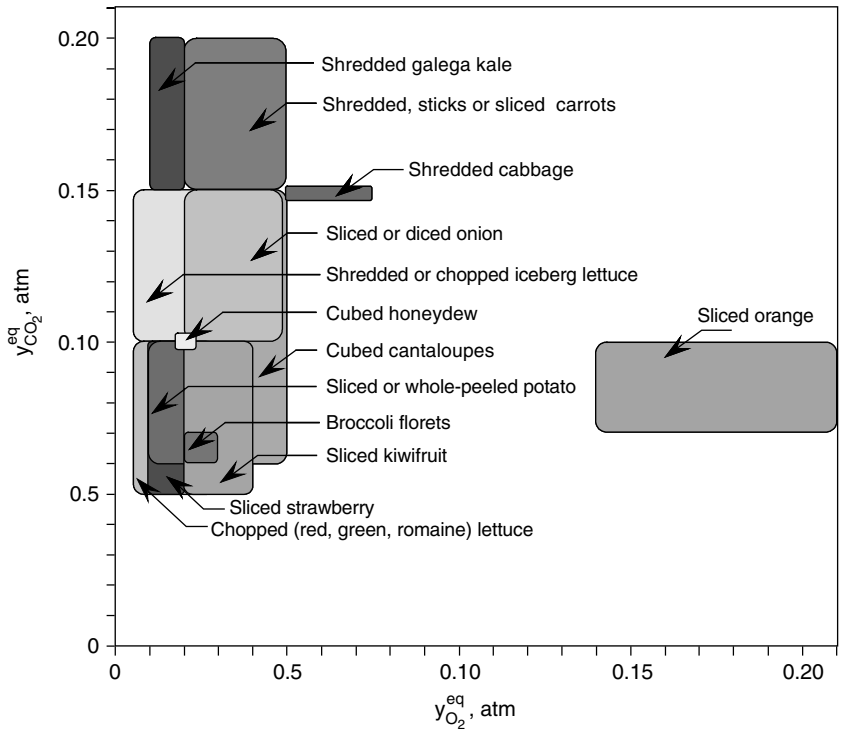

FIGURE 119.4 Recommended atmospheres for minimally processed produce (boxes show the recommended atmosphere for each product).

program calculates the respiration rate at the bottom-left and top-right corners of the "Optimum Window" as shown below.

$$R^{A}_{O_2} = \text{model}(y^{min}_{O_2}, y^{min}_{CO_2}, T) \quad (119.9)$$

$$R^{A}_{CO_2} = \text{model}(y^{min}_{O_2}, y^{min}_{CO_2}, T) \quad (119.10)$$

$$R^{B}_{O_2} = \text{model}(y^{max}_{O_2}, y^{max}_{CO_2}, T) \quad (119.11)$$

$$R^{B}_{CO_2} = \text{model}(y^{max}_{O_2}, y^{max}_{CO_2}, T) \quad (119.12)$$

$$RQ_B = \frac{R^{B}_{CO_2}}{R^{B}_{O_2}} \quad (119.13)$$

$$RQ_A = \frac{R^{A}_{CO_2}}{R^{A}_{O_2}} \quad (119.14)$$

IV. PACKAGING SYSTEM

Since there are many varieties of produce, a wide range of permeabilities is required. High permeabilities are needed for rapidly respiring produce, low permeabilities for slowly respiring produce. The required permeability may be achieved by three different types of packaging systems:

i. Polymeric films without perforations;
ii. Macroperforated polymeric films;
iii. Perforation-mediated packaging systems.

The details of each system are shown in Figure 119.5. Non-perforated polymeric films yield low O_2 and low CO_2 concentrations because the CO_2 permeability of these materials is generally 3 to 6 times that of O_2 permeability. These materials are suitable for less CO_2 tolerant commodities such as mango, banana, grapes and apples. Perforated films have higher permeability rates, but the ratio of CO_2 and O_2 permeability is much lower, approaching one. Such films are, therefore, of great interest for commodities tolerating simultaneously low O_2 and high CO_2 levels such as fresh-cut products, strawberry and mushroom. Perforation-mediated packaging is a system where tubes, which may be packed with an inert filling, are inserted in an otherwise airtight package. This system is also adequate for products requiring high CO_2/low O_2 concentrations and minimises water accumulation inside the package; because the package is rigid, it is suitable for bulk products and for products sensitive to mechanical damage.

V. MAP USING POLYMERIC FILMS

Permeable polymeric films are the most popular among available barriers to create modified atmospheres. These materials present different properties depending on the chemical structure, production process and additives.

A. Available Polymeric Films

The permeability coefficient of most commercially available films may be obtained from the company data.

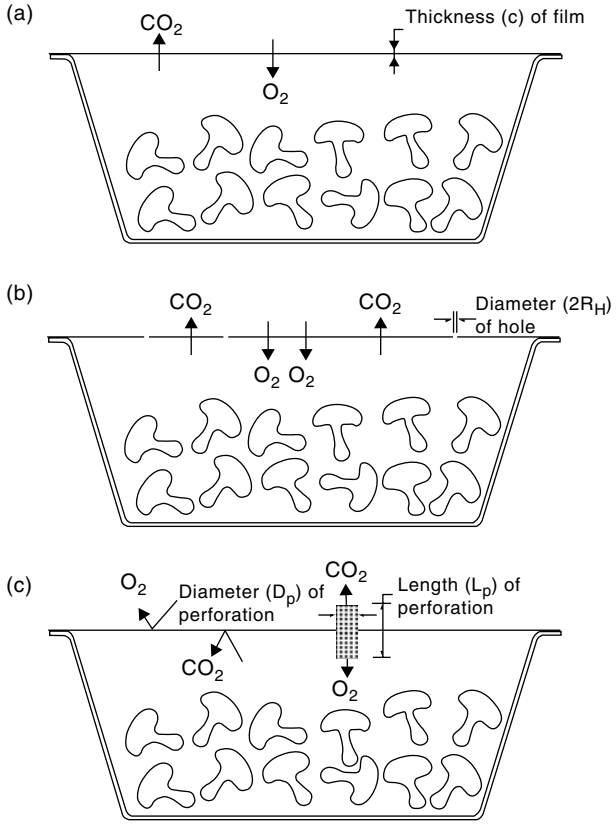

FIGURE 119.5 Schematic representation of three packaging systems (a) MAP with polymeric film (b) MAP with macroperforated polymeric film (c) perforation-mediated MAP.

However, these values were determined at some fixed temperature, usually in the ambient temperature range, 20–25°C. Most packaging films used for fresh fruits and vegetables are exposed to much lower temperatures. Moreover, the permeability for O_2 and CO_2 of a packaging film is temperature dependent; hence, data on the film permeability must be converted to the exact storage temperature of the produce. For this purpose the following Arrhenius model can be used:

$$P_{O_2} = P^*_{O_2} \exp\left[\frac{-E_{O_2}}{R \times T}\right] \quad (119.15)$$

$$P_{CO_2} = P^*_{CO_2} \exp\left[\frac{-E_{CO_2}}{R \times T}\right] \quad (119.16)$$

As per the above equations, permeability increases with temperature. If this increase were in the same magnitude as the increase in respiration due to temperature, then the net effect of temperature would be negligible, as far as the gas composition inside the package is concerned. In most cases however, the rates of increase of permeability and respiration due to temperature are not the same. Usually, the respiration rate tends to increase faster than the permeability. As the respiration rate is increased, less O_2 is available, and that, in turn, causes anaerobic respiration of packed products. This indicates that temperature, O_2 and CO_2 are the prime parameters to be considered while calculating film permeability and produce respiration rate. Using the pre-exponential factor and activation energy as specified in Table 119.4, P_{O_2} and P_{CO_2} are calculated at 5°C and are shown in Figure 119.6. The film permeability of each film is calculated as per the given values of activation energy and pre-exponential factor and accordingly the functions are written in Matlab (Version 13.1, The Mathworks Inc., Natick, US) programming language. Three parameters, i.e., A_F, e and T, are required to solve these functions giving the values of P_{O_2} and P_{CO_2}.

$$P_{O_2} = \text{film}\ (A_F, e, T) \quad (119.17)$$

$$P_{CO_2} = \text{film}\ (A_F, e, T) \quad (119.18)$$

A fortunate situation occurs when the desired permeability requirements are met by one or more existing commercial films. If this is the case, a good chance exists for a successful design. Unfortunately, this is not often the case because the choices of suitable polymeric films are rather limited. The problem can be appreciated by examining Figure 119.6, which reveals that the β values for most films fall within a rather narrow range between 3 and 6; however, Figures 119.2 to 119.4 show that many fruits and vegetables require β values outside this narrow range. It should be noted that there is a great deal of variation in measured and published permeability values due to variation in film thickness and its structure and measurement methods. Hence, it is advised to verify the gas permeability values of the selected film, if precision is required. In addition, it is necessary to study the feasibility of the package containing produce before actually putting them in the market.

B. Film Selection

The polymeric material with β within the set interval of β_{min} and β_{max} is considered as the best one for the product under study. The film selection steps are described in Figure 119.7. Matlab programming language was used to solve these steps. If the number of selected films is more than one, then the film having β value close to the β_{mean} would be selected. If there is an option left, a film having minimum equilibrium time is selected. Then this selected film is used to calculate equilibrium modified atmosphere parameters and gas kinetics. The rest of the films are displayed as the alternative optimal films for the given commodity.

C. Calculation of the Equilibrium Modified Atmosphere (EMA)

The program calculates the equilibrium gas composition, commodity weight, film area and equilibrium time that is

TABLE 119.4
Pre-exponential Factor and Activation Energy of Different Films

	$ml.mil.m^{-2}hr^{-1}atm^{-1}$		$kJ.mol^{-1}$	
Polymeric Film	$P^*_{O_2}$	$P^*_{CO_2}$	E_{O_2}	E_{CO_2}
Butyl rubber[a]	9.83×10^9	1.02×10^{10}	44.7	41.4
Cast polypropylene[b]	5.30×10^1	1.51×10^2	-	-
Cellulose acetate (Lumarith)[a]	3.45×10^5	1.25×10^8	20.9	29.7
Ceramic-filled LDPE[b]	1.18×10^9	1.48×10^8	36.8	28.4
Ceramic filled polystyrene[b]	2.59×10^8	4.17×10^7	34.5	26.2
Ethyl cellulose (Plasticied, Ethocel)[a]	2.23×10^6	4.81×10^4	16.7	5.90
Ethylene vinyl acetate[b]	1.34×10^{11}	6.33×10^9	48.4	37.0
High density polyethylene[b]	6.04×10^6	3.38×10^6	35.1	30.1
Low density polyethylene[b]	1.11×10^2	3.71×10^2	30.2	31.1
Linear low density polyethylene[b]	2.57×10^2	1.00×10^3	-	-
Methyl rubber[a]	4.47×10^{10}	1.34×10^{11}	47.3	46.8
Microporous film[b]	9.56×10^9	1.83×10^8	13.0	3.70
Natural rubber[a]	7.96×10^8	4.33×10^8	31.43	25.52
Neoprene[a]	7.97×10^9	4.69×10^9	41.5	35.5
Nylon laminated multilayer film[b]	8.11×10^9	9.52×10^9	52.6	50.0
Oriented polypropylene[b]	3.40×10^1	1.05×10^2	-	-
Polybutadiene styrene[a]	4.25×10^8	2.06×10^8	30.5	23.8
Polyethylene (Irradiated)[a]	2.71×10^9	2.65×10^9	39.7	35.9
Polyvinyl chloride (VF)[a]	1.21×10^9	9.50×10^8	41.4	33.1
Polyvinyl chloride (RMF)[a]	2.61×10^{10}	1.34×10^7	36.9	27.6
Polyvinyl chloride (AF)[a]	5.70×10^8	2.69×10^7	40.5	30.6
Polyvinyl chloride (VA)[a]	1.19×10^{10}	5.10×10^9	40.6	34.3
Polybutadiene[a]	3.37×10^8	1.04×10^8	29.7	21.8
Polyethylene terephthalate[b]	1.53×10^5	3.56×10^5	26.8	25.9
Rubber hydrochloride[a]	6.77×10^7	2.25×10^8	35.1	36.0
Silicone rubber[b]	3.93×10^5	7.13×10^4	8.4	-
Saran[a]	1.61×10^{11}	2.25×10^9	66.5	51.5

[a] Exama (35).
[b] Yam (36).

achievable by the selected film(s) as described in the following steps.

It calculates the average of O_2 and CO_2 composition in the recommended range and corresponding respiration rates:

$$y_{O_2}^{mean} = \left(\frac{y_{O_2}^{max} + y_{O_2}^{min}}{2} \right) \quad (119.19)$$

$$y_{CO_2}^{mean} = \left(\frac{y_{CO_2}^{max} + y_{CO_2}^{min}}{2} \right) \quad (119.20)$$

$$R_{O_2}^{mean} = \text{model}\ (y_{O_2}^{mean}, y_{CO_2}^{mean}, T) \quad (119.21)$$

$$R_{CO_2}^{mean} = \text{model}\ (y_{O_2}^{mean}, y_{CO_2}^{mean}, T) \quad (119.22)$$

$$RQ_{mean} = \frac{R_{CO_2}^{mean}}{R_{O_2}^{mean}} \quad (119.23)$$

i. It calculates the slope (S_F) of the line corresponding to the selected film(s):

$$S_F = \frac{-RQ_{mean}}{\beta} \quad (119.24)$$

ii. It calculates the slope (S_N) of the line that passes thorough the average gas composition and is perpendicular to the line corresponding to the film selected, i.e., the line that passes through 0.0003 atm CO_2 and 0.21 atm O_2 and has a slope equal to S_F as shown in Figure 119.8.

$$S_N = \frac{\beta}{RQ_{mean}} \quad (119.25)$$

iii. It finds the intercept of the line corresponding to the film:

$$I_F = y_{CO_2}^e + \frac{RQ_{mean} \times y_{O_2}^e}{\beta} \quad (119.26)$$

iv. It finds the intercept of the line perpendicular to the line of the film:

$$I_N = y_{CO_2}^{mean} - \frac{\beta}{RQ_{mean}} \times y_{O_2}^{mean} \quad (119.27)$$

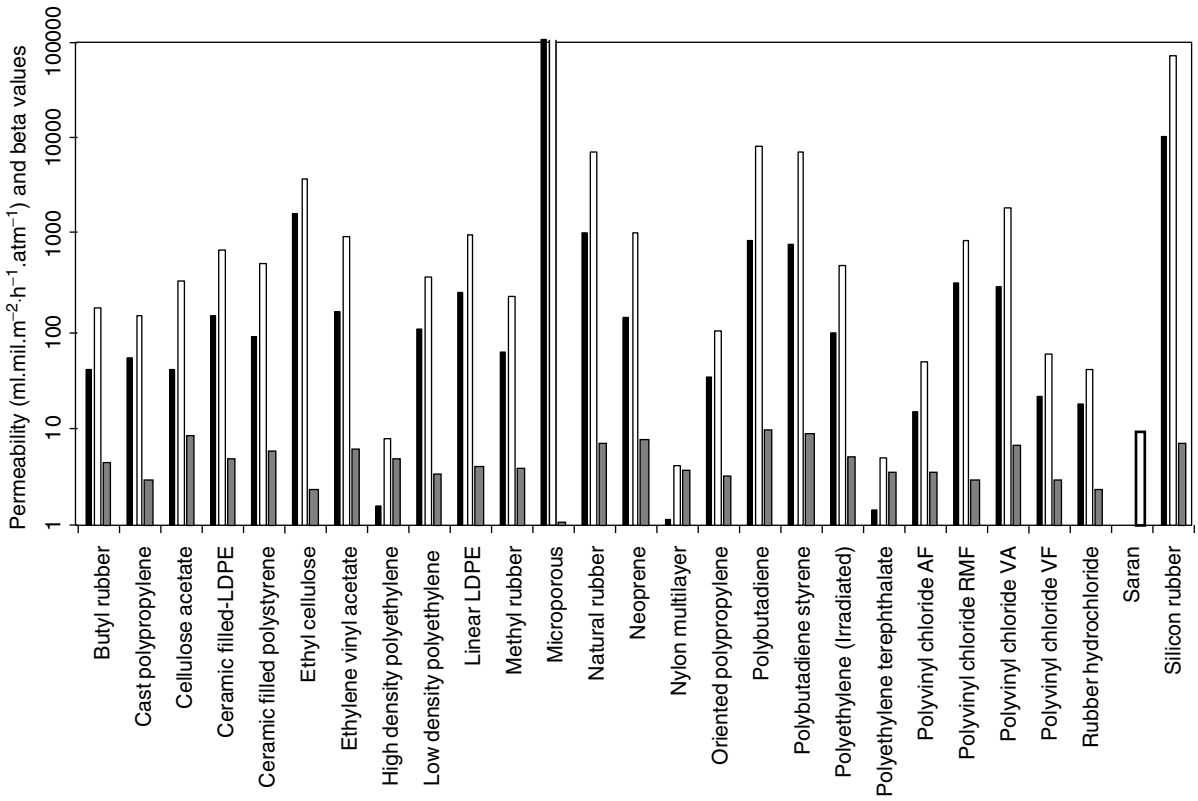

FIGURE 119.6 Permeability and β values of different polymeric films at 5°C (solid square: P_{O_2}; empty square: P_{CO_2}; lined square: β).

v. It finds the co-ordinates of a point (equilibrium gas composition) where the two lines intersect:

$$y_{O_2}^{best} = \frac{I_F - I_N}{S_N - S_F} \tag{119.28}$$

$$y_{CO_2}^{best} = \frac{RQ_{mean}}{\beta}(y_{O_2}^{e} - y_{O_2}^{best}) + y_{CO_2}^{e} \tag{119.29}$$

$$R_{O_2}^{best} = \text{model}(y_{O_2}^{best}, y_{CO_2}^{best}, T) \tag{119.30}$$

$$R_{CO_2}^{best} = \text{model}(y_{O_2}^{best}, y_{CO_2}^{best}, T) \tag{119.31}$$

vi. It calculates the area of film and weight of commodity by combining Equations 119.1 and 119.2 at equilibrium. We get Equation 119.32 from this which we can use to predict the film area required to achieve the desired gas composition at given weight of commodity or predict the weight of commodity to achieve the desired gas composition at given film area.

$$A_F = \left[\frac{M(R_{CO_2}^{best} + R_{O_2}^{best})}{\frac{P_{O_2}}{e}(y_{O_2}^{e} - y_{O_2}^{best}) - \frac{P_{CO_2}}{e}(y_{CO_2}^{e} - y_{CO_2}^{best})}\right] \tag{119.32}$$

vii. Approximate volume of the package can be calculated by multiplying the produce volume by the factor 1.8 to account for typical headspace found in retail packages (35). By using this volume, the dimensions of the package are decided. Finally, these dimensions are used to calculate the actual total volume of the package. The free volume inside the package containing produce is calculated using Equation 119.34.

$$V_t = W_B \times H_T \times L_T + \frac{(W_T - W_B) \times H_T \times L_T}{2} \tag{119.33}$$

$$V_f = V_t - \frac{M}{\rho} \tag{119.34}$$

viii. In order to know if the atmosphere inside the package may achieve the gas concentrations desired to preserve the produce in a reasonable period of time, a simplified model can be developed in order to simulate the process. This simplified model considers a constant respiration rate, a constant temperature and uniform concentrations inside the package. With these assumptions, Equations 119.1 and 119.2 can be integrated, yielding the history of concentrations

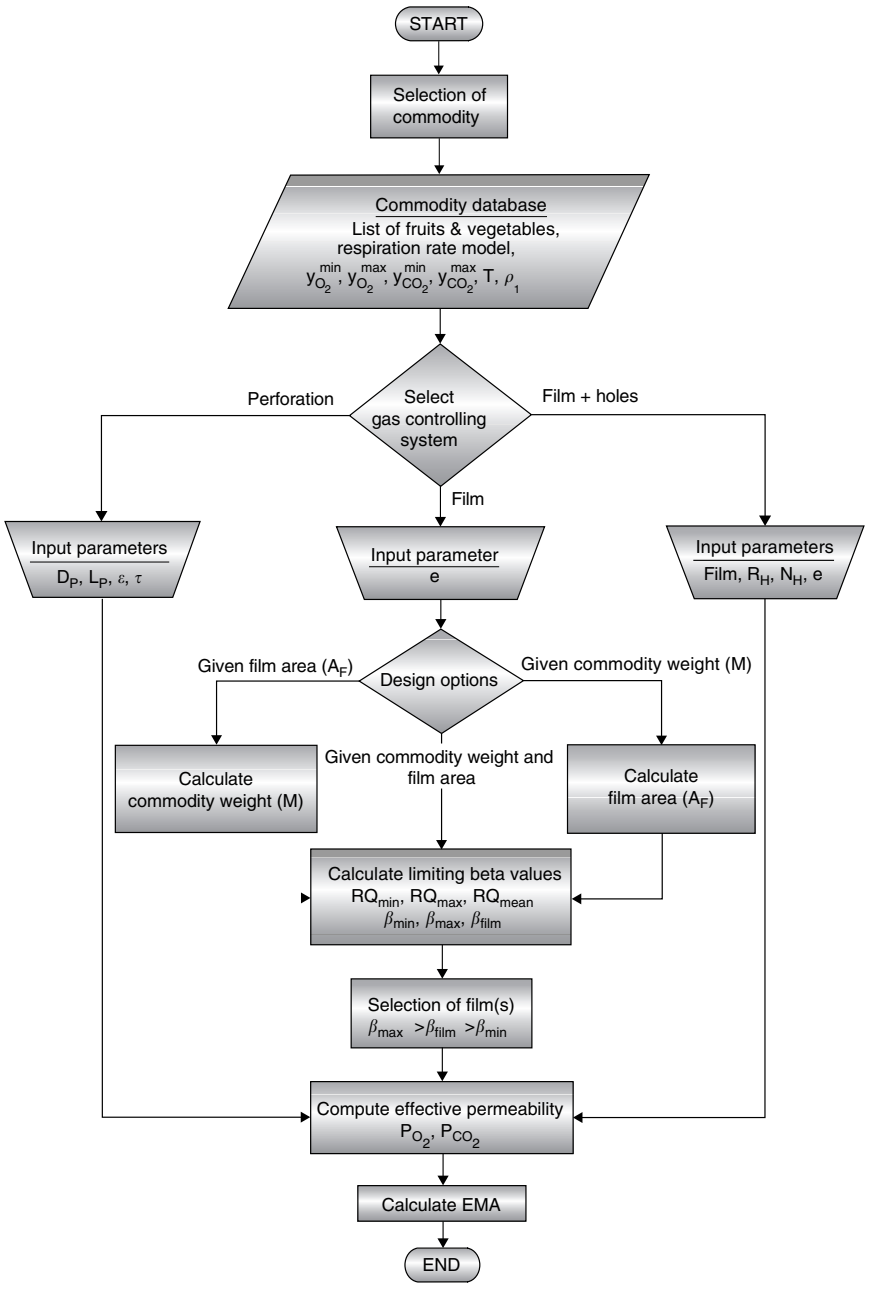

FIGURE 119.7 Description of steps involved in MAP design.

of both gases in the package from which the equilibrium time can be calculated as follows:

$$t_{O_2}^{eq} = \frac{-V_f \times e}{24 \times 3600 \times P_{O_2} \times A} \times \ln \left| \frac{\left(y_{O_2}^{eq} - y_{O_2}^{e}\right) + \dfrac{R_{O_2} \times M \times e}{P_{O_2} \times A}}{\left(y_{O_2}^{i} - y_{O_2}^{e}\right) + \dfrac{R_{O_2} \times M \times e}{P_{O_2} \times A}} \right| \quad (119.35)$$

$$t_{CO_2}^{eq} = \frac{-V_f \times e}{24 \times 3600 \times P_{CO_2} \times A} \times \ln \left| \frac{\left(y_{CO_2}^{i} - y_{CO_2}^{e}\right) + \dfrac{R_{CO_2} \times M \times e}{P_{CO_2} \times A}}{\left(y_{CO_2}^{eq} - y_{CO_2}^{e}\right) + \dfrac{R_{CO_2} \times M \times e}{P_{CO_2} \times A}} \right| \quad (119.36)$$

Equations 119.35 and 119.36 show that the variables that affect the time required to reach the

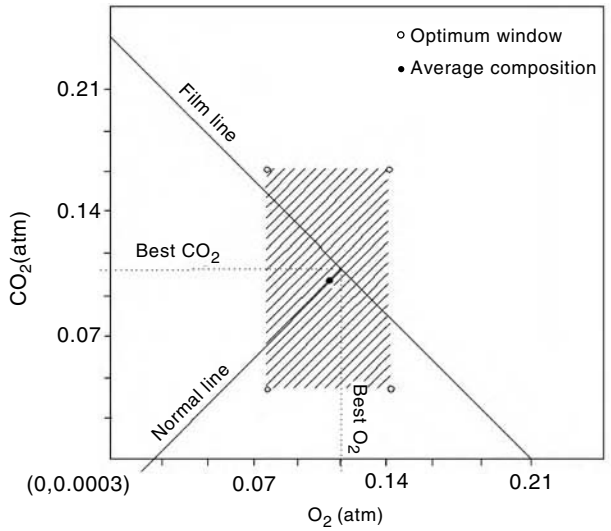

FIGURE 119.8 Determination of equilibrium gas composition to be achieved by the selected film.

equilibrium are O_2 and CO_2 permeability of film, free volume inside the package, the concentrations of O_2 and CO_2 at the beginning and at equilibrium and the product weight, A and e. The highest time of t_{O_2} and t_{CO_2} is the one considered for simulation of gas composition inside the package. Usually the initial concentrations are those in atmospheric air and the concentrations at equilibrium are the ones for optimal preservation of the produce. But in passive MAP the gas composition changes with time due to which respiration rate also changes and achieves constant rate at the once equilibrium gas composition reaches. Hence, it is necessary to consider the effect of change in gas composition on the respiration rate and thereby on the equilibrium time.

ix. The last step consists of the calculation of the changes in package gas composition with time. The program uses an optimisation routine named fsolve to solve the two ordinary differential Equations 119.1 and 119.2 for O_2 and CO_2 concentrations, respectively. The program calculates the O_2 and CO_2 concentrations at every minute. The respiration rate is re-calculated at new gas composition, i.e., composition after one minute and is used to calculate the next O_2 and CO_2. In this way gas composition is predicted until the equilibrium time t^{eq} ($=t^{eq}_{O_2}$ or $t^{eq}_{CO_2}$, whichever is higher) is reached.

VI. PERFORATION-MEDIATED MAP

A major challenge is to develop films that have greater permeability and have a wider range of β values than existing types. Films of enhanced permeability are necessary for packaging high respiration rate products and for preventing the development of anaerobiosis. A wider range of β values, especially those below 3, is necessary to better match the respiration behaviour of many products. The use of either perforation systems or microporous films is a possible solution to meet these two requirements. These systems and films have permeabilities many orders of magnitude higher than those of non-perforated polymeric films, as well as β values between 0.8 and 1. The use of perforations in MAP has been recently reported by Fonseca et al. (36), Silva (37) and Emond (38). The gas transfer coefficients through perforations can be described by Equations 119.37 and 119.38 characteristics (36). This model has a functional form similar to the one that would be expected by dimensionless analysis and has the advantage of having less number of parameters than those suggested by Emond (38) and Silva (37). Γ is the tortuosity of the path through which the pores pass and is equal to 1.14. The other parameters are shown in Table 119.5.

$$P_{O_2} = N_P \times \frac{\varepsilon}{\Gamma^2} \times a \times D_p^b \times L_p^c \qquad (119.37)$$

$$P_{CO_2} = N_P \times \beta \times \frac{\varepsilon}{\Gamma^2} \times a \times D_p^b \times L_p^c \qquad (119.38)$$

VII. MACROSCOPIC HOLES IN COMBINATION WITH POLYMERIC FILM

Since β value of polymeric films is much larger than the respiratory quotient, RQ, there will be more CO_2 leaving the package than O_2 entering. Nitrogen cannot make up the volumetric difference, so the package free volume tends to shrink during storage. This volume shrinkage can be eliminated by introducing a very small hole that allows a convective influx of air to balance the total pressure inside the bag with the air outside. This, of course, alters the composition of the gas in the package relative to what it would have been in the absence of the hole. For some products, it is necessary to have a lower CO_2/O_2 permeability ratio than available polymer choices permit in

TABLE 119.5
Parameters of the Mathematical Model Shown in Equations 119.37 and 119.38

Parameter	Value
a	6.42×10^{-6}
b	1.45
c	−0.598
Γ	1.14
D_{O_2}	$16.4 \times 10^{-6}\,m^2.sec^{-1}$
D_{CO_2}	$20.6 \times 10^{-6}\,m^2.sec^{-1}$

order to achieve the CO_2/O_2 composition for optimal extension of shelf life. This can be solved by making the area of the holes large enough to provide non-selective permeation in parallel with the film; thus reducing the net CO_2/O_2 permeability ratio.

The macroscopic perforation in the polymeric film represents an alternate route for gas transport, which is in parallel to the barrier formed by the plastic material. The total flow through polymeric film having N_H number of holes is

$$\text{Total permeation} = \left\{\begin{matrix}\text{Permeation}\\ \text{through film}\end{matrix}\right\} + \left\{\begin{matrix}\text{Permeation through}\\ \text{one hole} \times N_H\end{matrix}\right\} \quad (119.39)$$

The macroscopic perforations in polymeric films have diameters of the order of 10^{-4} m or greater, whereas the mean-free path of gas molecules at atmospheric pressure is much less, being about $(1 \text{ or } 2) \times 10^{-7}$ m (25). Therefore, transport through the perforation may be treated as macroscopic diffusion in a cylindrical pathway filled with air. If the distance between perforations is much greater than their radius, the diffusive pass length becomes the length of cylindrical pore plus the radius of the hole. Diffusive flux in this case obeys Fick's law:

$$P_{O_2} = \left[P_{O_2}^{film} + \frac{\pi R_H^2 \times D_{O_2}}{(e + R_H)} \times N_H\right] \quad (119.40)$$

$$P_{CO_2} = \left[P_{CO_2}^{film} + \frac{\pi R_H^2 \times D_{CO_2}}{(e + R_H)} \times N_H\right] \quad (119.41)$$

VIII. DEVELOPMENT OF SOFTWARE

The graphical user interface of the software was built in Matlab programming language. The same tool was used to solve the design steps as explained in Figure 119.7.

IX. CASE STUDY

A case study is presented to illustrate the use of the software to design MAP for whole mango. In this case study, the design procedure is explained for two kinds of package: permeable polymeric film and microscopic perforation (hole) with permeable polymeric film. A tray type package as shown in Figure 119.5 is selected for the present case study. The product and package characteristics are shown in Table 119.6.

For the selected commodity and at given optimum conditions β_{min} and β_{max} were found to be 1.71 and 3.84, respectively. As in Figure 119.9, the thick rectangle indicates the limits, inside which lines must run so that the corresponding films are eligible for selection. As can been seen, there are 11 films selected from the database of 27 films. These films will modify the package atmosphere leading to the optimum level for the selected commodity provided that the area of film is kept as shown in Table 119.7. The equilibrium modified atmosphere parameters such as $y_{O_2}^{eq}$, $y_{CO_2}^{eq}$, A_F and t^{eq} are calculated and are shown in Table 119.7. All the 11 films were found to give the optimum atmosphere, but the area of film required is different for all of them. Moreover, the film area required to achieve equilibrium is different from the possible area

TABLE 119.6
Product and Package Characteristics for the Case Study

Product Characteristics	
Product selected	Mango
Varity/cultivar	Nam dok mai
Product weight (M), kg	1.0
Respiration rate model[a]	MMU[c]
True density (ρ), $kg.m^{-3}$	1044
Optimum MA conditions[b]	
O_2 minimum ($y_{O_2}^{min}$), atm	0.03
O_2 maximum ($y_{O_2}^{max}$), atm	0.07
CO_2 minimum ($y_{CO_2}^{min}$), atm	0.05
CO_2 maximum ($y_{CO_2}^{max}$), atm	0.08
Temperature (T), °C	10
Package Characteristics	
Top Width (W_T), m	0.16
Bottom width (W_B), m	0.12
Length (L_T), m	0.14
Height (H_T), m	0.09
Total volume (V_t), m^3	1.792×10^{-3}
Free volume (V_f), m^3	0.834×10^{-3}
Film area (A_F^{Tray}), m^2	0.0224

[a] Charoenchaitawornchit et al. (23).
[b] Kader (39).
[c] Michaelis-Menten type equation with uncompetitive inhibition of CO_2.

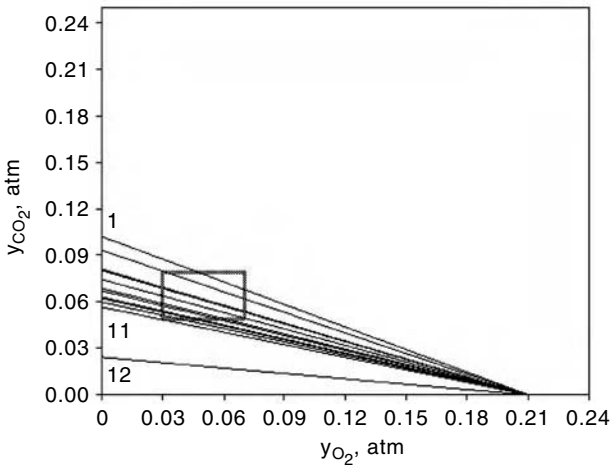

FIGURE 119.9 Optimum window and lines established from the β relation of selected films for mango (the number given to each line corresponds to the serial number of films in Table 119.7).

TABLE 119.7
List of Selected Polymeric Films for Mango

Sr. No.	Selected Polymeric Films*	Film β	A_F, m^2	Equilibrium Parameters of MAP $y_{O_2}^{eq}$, atm	$y_{CO_2}^{eq}$, atm	t^{eq}, day
1	Ethyl cellulose	2.09	0.021	0.055	0.076	3.47
2	Polyvinyl chloride (RMF)	2.64	0.093	0.049	0.062	3.97
3	Low density polyethylene	3.33	0.363	0.045	0.050	2.84
4	Methyl rubber	3.71	0.458	0.045	0.045	2.55
5	Cast polypropylene	2.85	0.745	0.047	0.058	3.46
6	Oriented polypropylene	3.09	1.170	0.046	0.054	3.10
7	Polyvinyl chloride (VF)	2.64	1.350	0.049	0.062	3.96
8	Rubber hydrochloride	2.27	1.670	0.052	0.070	4.64
9	Polyvinyl chloride (AF)	3.13	1.970	0.046	0.053	3.05
10	Polyethylene terephthalate	3.39	22.20	0.045	0.049	2.79
11	Nylon multilayer	3.53	23.70	0.045	0.047	2.67
12	Polybutadiene ¢	9.40	0.022	0.065	0.037	3.33

* Thickness = 1 mil; ¢ Not suitable for mango.

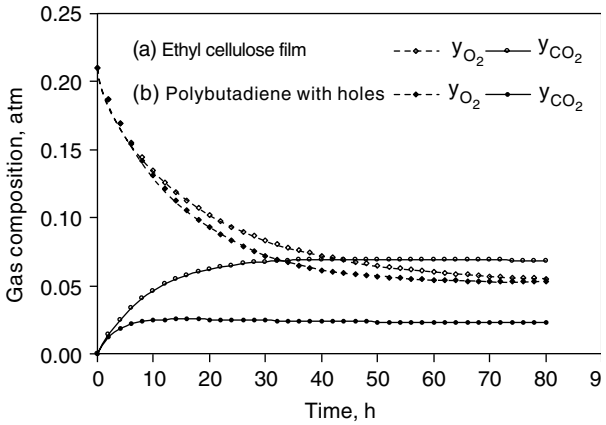

FIGURE 119.10 Steady-state establishment of O_2 and CO_2 in MAP with (a) Ethyl cellulose film having thickness = 1 mil, area = 0.0146 m^2; (b) Polybutadiene film having thickness = 1 mil, area = 0.0224 m^2, number of holes = 2, diameter of each hole = 0.0001 m.

($A_F^{Tray} = 0.16 \times 0.14 = 0.0224\ m^2$) for the selected tray type of package. For the present case, ethyl cellulose film is selected because it presents the minimum difference between A_F^{Tray} and A_F. If the area of any other chosen film is kept 0.0224 m^2, the package will not achieve the optimum levels. The change in O_2 and CO_2 in MAP with ethyl cellulose film is shown in Figure 119.10 (a).

The rest of the films are not suitable due to their high β values. Nevertheless, these films could be used for MAP of mango after making certain number of holes to increase the gas transfer across the film (Figure 119.10(b)). Table 119.8 shows the results of microperforations with polymeric films having β value more than β_{max}. It is necessary to decrease the β value of the film in order to produce the atmosphere within the optimum range of mango, by making tiny holes in polymeric film. The number of holes and diameter depends on the permeability and area of the film as shown in Table 119.8.

Although the polymeric films are the most useful packaging material, they are unable to produce the optimum atmosphere for the high respiration and transpiration rates and higher CO_2 tolerance commodities. The perforation-mediated package is one such alternative, where the regulation of the gas exchange is achieved by single or multiple perforations or tubes that perforate an otherwise impermeable covering. This perforation-mediated MAP has special interest in fresh-cut products, e.g., cut Galega kale (21). Table 119.9 shows the design output for the perforation-mediated MAP for cut Galega kale at 20 and 5°C. The resultant steady state establishment of O_2 and CO_2 in perforation-mediated MAP is shown in Figure 119.11.

TABLE 119.8
EMA Parameters Achieved by Microperforated Polymeric Film

Polymeric Film*	Film β	A_F, m^2	D_H, m	N_H	Net β	Equilibrium Parameters of MAP $y_{O_2}^{eq}$, atm	$y_{CO_2}^{eq}$, atm	t^{eq}, day
Butyl rubber	4.31	0.0224	0.0001	14	0.84	0.141	0.075	1.0
Cellulose acetate	8.14	0.0224	0.0001	13	0.88	0.137	0.076	1.0
Ceramic-filled LDPE	4.73	0.0224	0.0001	14	0.94	0.140	0.067	1.0
Ceramic filled polystyrene	5.79	0.0224	0.0001	14	0.90	0.140	0.070	1.0
Ethylene vinyl acetate	5.93	0.0224	0.0001	14	0.96	0.139	0.066	0.9
Linear LDPE	3.90	0.0224	0.0001	14	0.95	0.140	0.066	0.9
Natural rubber	6.93	0.0224	0.0001	03	4.05	0.059	0.035	2.4
Neoprene	7.50	0.0224	0.0001	14	1.04	0.138	0.062	1.0
Polybutadiene	9.40	0.0224	0.0001	03	4.97	0.052	0.030	2.0
Polybutadiene styrene	8.72	0.0224	0.0001	03	4.52	0.050	0.034	2.0
Polyethylene (Irradiated)	5.04	0.0224	0.0001	14	0.91	0.140	0.070	1.0
Polyvinyl chloride (VA)	6.49	0.0224	0.0001	14	1.20	0.136	0.054	1.0
High density polyethylene	4.85	0.0224	0.0001	09	0.80	0.147	0.072	2.0

* Thickness of film = 1 mil.

TABLE 119.9
Design Output of Perforation-Mediated MAP System for Cut Galega Kale

Temperature, °C	D_P, m	L_P, m	N_P	ε	Equilibrium Parameters of MAP $y^{eq}_{O_2}$, atm	$y^{eq}_{CO_2}$, atm	t^{eq}, day
20	0.015	0.019	1	0.43	0.041	0.194	0.5
5	0.009	0.015	1	0.43	0.022	0.200	1.2

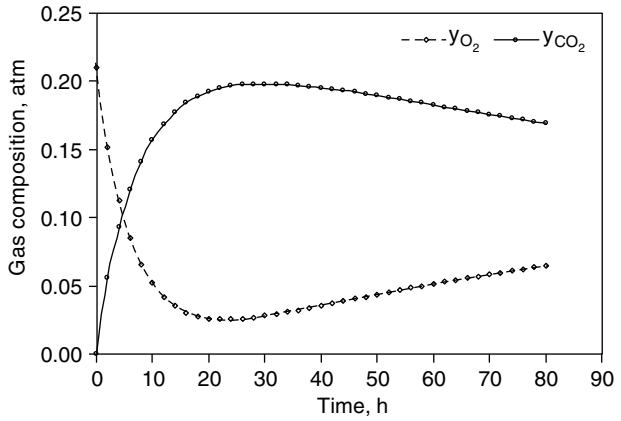

FIGURE 119.11 Steady state establishment of O_2 and CO_2 in perforation-mediated MAP at 5°C, diameter of perforation = 0.009 m, length = 0.015 m, Np = 1, ε = 0.43, weight of cut Galega kale = 0.4 kg.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from the Irish Government, under National Plan 2000–2006. Dr. Luís M. Cunha acknowledges financial support from *Fundação para a Ciência e a Tecnologia* (FCT) through "Financiamento Plurianual — POCTI."

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120 Ohmic and Inductive Heating

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CONTENTS

I. OVERVIEW

Ohmic and inductive heating are alternative methods of heating to conventional heating techniques. Ohmic heating, also known as Joule heating, electric resistance heating, direct electric resistance heating, electro heating, and electro conductive heating, is a process in which alternating electric current is passed through food material; heat is internally generated within the material due to its resistance to the applied electrical current. Inductive heating is a technique that uses electric coils to generate oscillating electric fields that send currents through the food.

Ohmic heating is not a new technology; it was used as a commercial process in the early 20th century for the pasteurization of milk (1). However, the "Electropure Process" was discontinued between the late 1930s and 1960s, ostensibly due to the prohibitive cost of electricity. Interest in ohmic heating was rekindled in the 1980s, when investigators were searching for viable methods to effectively sterilize liquid-large particle mixtures, a scenario for which aseptic processing alone was unsatisfactory. Very little information exists on inductive heating because its development in food processing is so recent.

The purpose of this chapter is to present general information regarding ohmic heating, and to identify areas of study which will add to the knowledge base of this subject. This chapter is separated into several sections: (1) general information on ohmic heating, (2) modeling of

ohmic heating processes, (3) novel uses of ohmic heating, and (4) future research directions.

II. GENERAL INFORMATION ON OHMIC HEATING

A. Advantages

Ohmic heating exhibits several advantages with respect to conventional food processing technologies, as follows:

- Particulate foods up to 1 in^3 are suitable for ohmic heating; the flow of a liquid-particle mixture approaches plug flow when the solids content is considerable (20–70%).
- Liquid-particle mixtures can heat uniformly under some circumstances (for example, if liquids and particles possess similar electrical conductivities, or if properties such as solids concentration, viscosity, conductivity, specific heat, and flow rate are manipulated appropriately).
- Temperatures sufficient for UHT processing can be rapidly achieved.
- There are no hot surfaces for heat transfer, resulting in a low risk of product damage from burning or overprocessing.
- Energy conversion efficiencies are very high.
- Relatively low capital cost.

B. Applications

Ohmic heating can be applied to a wide variety of foods, including liquids, solids, and fluid-solid mixtures. Ohmic heating is being used commercially to produce liquid egg product in the United States. It is also being used in the United Kingdom and Japan for the processing of whole fruits such as strawberries. Additionally, ohmic heating has been successfully applied to a wide variety of foods in the laboratory, including fruits and vegetables, juices, sauces, stews, meats, seafood, pasta, and soups.

Widespread commercial adoption of ohmic heating in the United States is dependent on regulatory approval by the FDA, a scenario that requires full understanding of the ohmic heating process with regard to heat transfer (temperature distributions), mass transfer (concentration distributions, which are influenced by electricity), momentum transfer (fluid flow), and kinetic phenomena (thermal and possibly electrothermal death kinetics, and nutrient degradation).

Larkin and Spinak (2) examined safety considerations for ohmically heated, aseptically processing, multiphase low acid food products, and discussed the need for providing information on equipment design, product specification, process design, and process validation for regulators. Full knowledge of these areas is critical to ensure that the food product receives adequate thermal treatment. Significant research strides toward widespread commercial use have been made, though more work remains to be done.

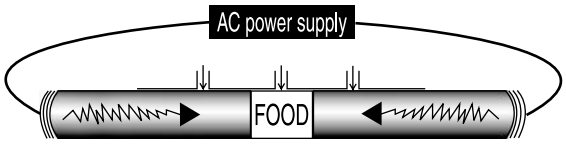

FIGURE 120.1 Graphic of a static ohmic heating apparatus. (Courtesy of Barbara Corns, LSU Ag Center Communications.)

FIGURE 120.2 APV ohmic heater used for industrial applications. (Courtesy of Dr. Sudhir Sastry, Ohio State University.)

C. Design

Ohmic heating devices consist of electrodes, a power source, and a means of confining the food sample (for example, a tube or vessel). Appropriate instrumentation, safety features, and connections to other process unit operations (pumps, heat exchangers, holding tubes, etc.) may also be important. Ohmic heaters can be static (batch) or continuous. Figure 120.1 contains a graphic of a static ohmic heating apparatus, while Figures 120.2 and 120.3 represent an early[1] continuous ohmic heater used by APV Baker, a U.K. company using ohmic heating for food processing, and a more recent continuous ohmic heating system developed by S. Sastry, respectively.

Important design considerations include electrode configuration (current flows across product flow path or parallel to product flow path), the distance between electrodes, electrolysis (metal dissolution of electrodes, particularly at low frequencies), heater geometry, frequency of alternating

[1] 1980s and 1990s.

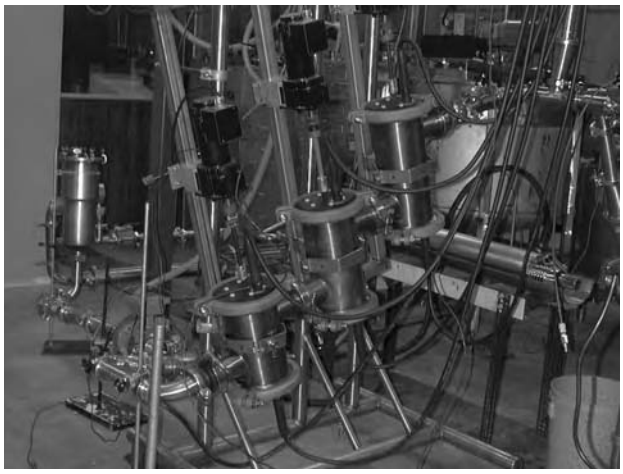

FIGURE 120.3 Ohmic heater developed by Dr. Sudhir Sastry. (Courtsey of Dr. Sudhir Sastry, Ohio State University.)

current, power requirements, current density, applied voltage, and product velocity and velocity profile. Additional factors regarding the food system used in an ohmic heater include the type of product and its properties, especially electrical conductivity and heating rate; others include percent solids, acidity, product viscosity, specific heat, and density, and solid particle size, shape, and orientation to the electric field. Substantial literature has been devoted to these topics; see, for example, references (3–10).

Coated electrodes can minimize or eliminate electrolytic reactions; temperature measurement remains an area of concern, as many measurement methods influence the electric field during ohmic heating. Some success has been seen with thermocouples that are coated with material such as Teflon, however, non-invasive temperature measurements that do not interfere with the electric field remain a challenge, particularly with regard to temperature measurement inside particles.

D. Cost

Investigators (11) conducted an economic engineering analysis of ohmic food processing for low and high acid foods. They found that ohmic heating is an economically viable technology for processing low acid foods. Though ohmic heating was found to be more costly than conventional methods for processing high acid foods, the authors believed that ohmic heating was still viable in these cases because of its potential to produce superior product quality.

III. PARAMETERS OF IMPORTANCE IN OHMIC HEATING

A. Product Properties

The most important parameter of interest in ohmic heating is the electrical conductivity of the food and/or food mixture. Substantial research was conducted on this property in the early 1990s because of the importance of electrical conductivity with regard to heat transfer rate and temperature distribution.

The electrical conductivity is determined using the following Equation (120.1):

$$\sigma = \frac{L}{AR}$$

where
σ = specific electrical conductivity (Siemens/m)
A = area of cross section of the sample (m^2)
L = length of the sample (m)
R = resistance of the sample (ohm)

General[2] findings of numerous electrical conductivity studies are as follows:

- The electrical conductivity is a function of food components; ionic components (salt), acid, and moisture mobility increase electrical conductivity, while fats, lipids, and alcohol decrease it.

Electrical conductivity is linearly correlated with temperature when the electrical field is sufficiently high (at least 60 V/cm). Non-linearities (sigmoidal curves) are observed with lower electrical field strength (3, 12).

- Electrical conductivity increases as temperature and applied voltage increases, and decreases as solids content increases.
- Lowering the frequency of alternating current during ohmic heating increases the electrical conductivity.
- The waveform can influence the electrical conductivity; though alternating current is usually delivered in sine waves, sawtooth waves increased the electrical conductivity in some cases, while square waves decreased it (12).
- Electrical conductivity increases by heating cycle; preheated samples showed increased electrical conductivity as opposed to raw samples when both were subsequently subjected to ohmic heating (13).

The electrical conductivity of solids and liquids during ohmic heating of multiphase mixtures is also critically important. In an ideal situation, liquid and solid phases possess essentially equal electrical conductivities, and would thus (generally) heat at the same rate. When there are differences in the electrical conductivity between

[2] These findings are true in general; some exceptions may exist depending on the situation.

a fluid and solid particles, the particles heat faster than the fluid when their conductivities are lower than the fluid. Also, solid particulates heat more slowly than a fluid when the electrical conductivity of the solid is higher than that of the fluid. Fluid motion (convective heat transfer) is also an important consideration when there are electrical conductivity differences between fluids and particles.

Other product properties that may affect temperature distribution include the density and specific heat of the food product. When solid particles and a fluid medium have similar electrical conductivities, the component with the lower heat capacity will tend to heat faster. High densities and specific heats are conducive to slower heating. Fluid viscosity also influences ohmic heating; higher viscosity fluids tend to result in faster ohmic heating than lower viscosity fluids.

B. Texture Analysis

Sensory evaluation is critically important to any viable food process. Numerous publications have cited the superior product quality that can be obtained through decreased process time, though few published studies specifically quantify sensory and texture issues. Six stew formulations sterilized using ohmic heating before and after three years of storage were analyzed; the color, appearance, flavor, texture, and overall food quality ratings were excellent, "indicating that ohmic heating technology has the potential to provide shelf-stable foods closely equivalent to those prepared from scratch" (14).

C. Gelatinization

Starch gelatinization is an important parameter in food processing, and can be either advantageous or disadvantageous depending on the desired product formulation. The electrical conductivity of a food product is influenced significantly by starch gelatinization (13). These investigators found that electrical conductivity decreased with the degree of gelatinization, and suggest that ohmic heating can be used in the development of a sensor to detect starch gelatinization.

Ohmic heating was used to maximize the gel functionality of a seafood product (15). The ohmic heating process was superior to the conventional heating process due to rapid heating that deactivated enzymes, which in turn enabled strong gel formation.

IV. MODELING OF OHMIC HEATING PROCESSES

A. Basic Equations

Considerable effort has been expended to model the heat transfer mechanisms and microbial death kinetics involved during ohmic heating. Models are of interest in the analysis and design of ohmic heating processes to provide information about the temperature distribution throughout the process, especially "the cold spot," and to provide accurate predictions of the minimum lethal processing time. Complexities in modeling heat transfer processes during ohmic heating arise when the liquid and particle possess different electrical conductivities, and because electrical conductivity is a (sometimes non-linear) function of temperature and frequency of alternating current. Basic equations regarding ohmic heating are included below.

The temperature distribution in a fluid during ohmic heating is based on an energy balance as follows:

$$\rho C_p v_z \frac{\partial T_f}{\partial z} = \nabla \cdot (k_f \nabla T_f) - n_p A_p h_{fp} (T_f - T_{ps}) + \dot{u}_f$$

where
ρ = density
C_p = specific heat
T = temperature
z = distance
v_z = fluid velocity
f = fluid
p = particle
ps = particle surface
k = thermal conductivity
n_p = number of particles
A = surface area of particles
h_{fp} = fluid to particle heat transfer coefficient
$\dot{u}_f$ = internal energy generation rate of the fluid

The temperature distribution in a particle during ohmic heating can be predicted with the conduction heat transfer equation with internal energy generation:

$$\nabla \cdot (k \nabla T) + \dot{u} = \rho C_p \frac{\partial T}{\partial t}$$

where k = thermal conductivity

The internal energy generation is:

$$\dot{u} = |\nabla V|^2 \sigma$$

where
V = voltage
σ = electrical conductivity
$\dot{u}$ = energy generation rate per unit volume

The voltage field is determined by solving:

$$\nabla \cdot (\sigma \nabla V) = 0$$

Numerous models have been developed based on numerical solution of these equations with appropriate boundary conditions and assumptions, and also from

dimensional groupings. Though these models have contributed significantly to the understanding of heat transfer in ohmic heating, none have completely described the ohmic heating process to date.

The voltage field (Laplace) equation for a single solid particle in a static heater has been solved (16). Numerical solutions and experimental simulations to more complex ohmic heating situations have been developed (17–22) using magnetic resonance imaging to rapidly map the temperature of food particles during ohmic heating.

B. Microbial Death Kinetics

In terms of microbial death kinetics, considerable attention has been paid to the following question: does electricity result in microbial death, or is microbial death due only to heat treatment? The challenge in modeling microbial death kinetics is precise matching of time-temperature histories between ohmic processes and conventional processes. The FDA has published a comprehensive review of microbial death kinetics data regarding ohmic heating (23).

Initial studies in this area showed mixed results, though the experimental details were judged insufficient to draw meaningful conclusions (1). Researchers compared death kinetics of yeast cells under time-temperature histories as identical as possible and found no difference between conventional and ohmic heating (24).

More recent work in this area has indicated that decimal reduction times of *Bacillus subtilis* spores were significantly reduced when using ohmic heating at identical temperatures (25). These investigators also used a two step treatment process involving ohmic heating, followed by holding, followed by heat treatment, which accelerated microbial death kinetics; they hypothesized that electroporation may positively influence microbial death kinetics.

The inactivation of yeast cells in phosphate buffer by low-amperage direct current electrical treatment and conventional heating at isothermal temperatures was examined (26). These researchers concluded that a synergistic effect of temperature and electrolysis was observed when the temperature became lethal for yeast.

Further research regarding microbial death kinetics, survivor counts subsequent to treatment, and the influence of electricity on cell death kinetics are necessary to address regulatory issues. At the present time, assuming that microbial death is only a function of temperature (heat) results in an appropriately conservative design assumption.

C. Vitamin Degradation Kinetics

Limited information exists regarding product degradation kinetics during ohmic heating. Researchers measured vitamin C degradation in orange juice during ohmic and conventional heating under nearly identical time-temperature histories, and concluded that electricity did not influence vitamin C degradation kinetics (27). This study was conducted at one electrical field strength (E = 23.9 V/cm). Others found that the ascorbic acid degradation rate in buffer solution during ohmic heating was a function of power, temperature, NaCl concentration, and products of electrolysis (10). Further research in this area could include the influence of electrical field strength, endpoint temperature, and frequency of alternating current on the degradation of food components during ohmic heating. The characterization of electrolysis is also a critical need in this area.

V. NOVEL USES OF OHMIC HEATING

A. Background

Early research on ohmic heating was conducted on heat transfer and sterilization of liquid-particle mixtures. In executing such studies, investigators observed unanticipated phenomena. For example, ohmically heating beetroot resulted in enhanced diffusion of betanin from the beetroot tissue when compared to beetroot tissue heated conventionally (28). These investigators hypothesized that the enhanced mass transfer could be due to electroosmosis.

Investigators expanded on the aforementioned work and found that diffusion of beet dye from beetroot into a carrier solution from was enhanced as much as 40% during heating from 20°C to 80°C, and that the concentration of diffused dye was proportional to particle surface area, and a linear function of electric field strength (29). Other researchers ohmically heated Japanese white radish and found that the ohmic heating rate was influenced by frequency; as the frequency of alternating current decreased, the heating rate increased (30). These investigators used H-NMR analysis and hypothesized that at low frequency (50 Hz), rapid heating is due to electroporation of radish tissue membrane, which resulted in a decrease of electrical impedance. Subsequent studies (9, 31) have concluded that electroporation is the most likely mechanism for enhanced mass transfer effects during ohmic heating.

Electroporation is defined as the formation of holes in a cell membrane resulting from local pressure of ions, which cannot initially permeate the cell membrane, but are forced against it by the electric field (32). The relatively low alternating frequencies employed during ohmic heating enable this charge build up to occur on the cell wall, resulting in the formation of pores. This also suggests that the lower the frequency of ohmic heating, the more pronounced the mass transfer effect; this concept has been demonstrated in the literature (31, 33–34). It was found that direct current resulted in less mass transfer enhancement than low AC frequency ohmic heating (at 15 V/cm, 250 Hz $<$ DC $<$ 50 Hz). Kulshrestha and

Sastry postulated that a monopolar electric charge (DC) is not as effective as a bipolar electric charge at creating stress on the cell membrane, thus yielding less of an effect than low frequency alternating current (31).

Electrically heating foods influences their mass transfer properties. This phenomenon has important implications for food processing operations that involve mass transfer. In 2001, the FDA reported that "A large number of potential future applications exist for ohmic heating, including its use in blanching, evaporation, dehydration, fermentation, and extraction" (23). In this section, we will report on some of the research regarding these novel uses.

B. Blanching

Because blanching requires large volumes of water during processing, and often requires dicing vegetables, studies to increase the efficiency of blanching using ohmic heating are important. Wigerstrom (35) found that electric fields enhanced moisture loss during the blanching of potato slices. Mizrahi (36) determined that ohmic heating was an effective method for blanching because the rapid, uniform heating exhibited by ohmic heating eliminated the need for dicing vegetables. The quick process time and reduction in surface area (no dicing) reduced solute losses by an order of magnitude during blanching. Sensoy, Sastry, and Beelman (37) found that using ohmic heating during the blanching of mushrooms resulted in the shrinking of mushrooms at a lower temperature and with less water use as compared to conventional blanching. Lakkakula, Lima, and Walker (34) showed significant lipase deactivation in rice bran during ohmic heating, with and without a corresponding temperature increase. Taken collectively, these studies show that ohmic heating can increase process efficiency in blanching.

C. Evaporation

Wang and Chu (38) studied the effect of ohmic heating on the vacuum evaporation of orange juice, and found that the evaporation rate could be increased as much as three times using ohmic heating, and resulted in enhanced product quality. The authors conclude that ohmic heating has potential as a fast evaporation method and recommend further development in this area.

D. Dehydration

Ohmic heating has also been used to enhance the drying rate of vegetable tissue. Wang and Sastry (39) showed that ohmically treating sweet potato prior to dehydration accelerated the hot-air drying rate significantly compared to raw, conventionally treated, and microwaved samples. Lima and Sastry (33) found that the lower the frequency of alternating current used in ohmic heating, the faster the hot-air drying rate. Maximum drying benefits were seen when drying to initial or intermediate moisture contents. Zhong and Lima (40) showed that ohmic pretreatment accelerated the vacuum drying rate of sweet potato as much as 24%; these investigators also demonstrated that minimal ohmic treatment (electrical field strength of 50 V/cm and an endpoint temperature of 40°C) resulted in the maximal or near maximal acceleration of drying rate. These investigators suggested that because ohmic heating enhances drying rates and enhances extraction yields, the process could be ideal for the recovery of high value, heat labile components from biological materials using unit operations such as supercritical fluid extraction.

E. Fermentation

Cho, Sastry, and Yousef (41) found that mild electrical treatment significantly decreased the lag time of *Lactobacillus acidophilus*, possibly due to electroporation, which could enhance the transport of substrates across the cell membrane. These investigators also found that electricity applied later in the microbial growth cycle proved detrimental, possibly due to the enhanced transport of inhibitory substances across the cell membrane.

F. Extraction

Ohmic heating has been used to enhance the extraction of components from foods. Katrokha, Matvienko, Vorona, Kupchik, and Zaets (42) used an electric field to extract sugar from sugar beets. Kim and Pyun (43) extracted soymilk from soybeans. Lima and Sastry (33) and Wang and Sastry (44) found that ohmically heating apple tissue prior to mechanical juice extraction significantly increased apple juice yields with respect to non-treated apple tissue, and that the lower the frequency of alternating current, the greater the extraction yield.

Several studies have examined the diffusion of beet dye from beetroot. In addition to the pioneering work mentioned above, Lima, Heskitt, and Sastry (45) found that the diffusion enhancement beet dye due to ohmic heating was especially pronounced at lower temperatures (42°C vs. 58°C and 72°C), and could be related to the difference in electrical conductivity of beet tissue between conventional and ohmic cases at the same temperature. Kulshrestha and Sastry (46) showed that significant leaching of beet dye occurs with temperature increases of 1–2°C in ohmic heating. Lakkakula, Lima, and Walker (34) used ohmic heating to significantly increase the extraction of rice bran oil from rice bran (with moisture addition), especially at low (1 Hz) frequency.

G. Summary of Novel Processes

There exists a strong potential to enhance mass transfer operations using ohmic heating, particularly because mild ohmic treatment has been shown to significantly increase

dehydration and extraction efficiencies. Future work in these areas includes establishing a more complete body of knowledge regarding the mechanisms for mass transfer effects, and process design to establish industrial processes that take advantage of this technology.

VI. FUTURE RESEARCH DIRECTIONS

Though there has been a proliferation of published research on ohmic heating during the past fifteen years, there exist many opportunities for contributing to the body of knowledge regarding ohmic and inductive heating.

In terms of inductive heating, so little information exists that substantial contributions can be made in all areas of research, including equipment design and instrumentation, process characterization, properties of importance in inductive heating, and modeling process transport phenomena and kinetics (microbial death and food degradation).

In terms of ohmic heating, areas of future work include the following:

- Developing temperature measurement methods that are (preferably) non-invasive and that do not interfere with the electrical field for the internal monitoring of solid particles during ohmic heating.
- Developing models that correlate process parameters and process design with properties of the product (physical, electrical, chemical, biological, microbial) in order to standardize ohmic heating design and analysis, and to accurately quantify changes in process or product.
- Determining the influence of temperature and electrical field on the degradation kinetics of key pathogenic microorganisms.
- Developing the knowledge necessary to quantify the effects of electrical field on mass transfer properties in order to optimize promising applications of ohmic heating, including drying, extraction, blanching, fermentation, evaporation, and gelatinization.
- Quantifying electrolytic effects during ohmic heating, particularly the minimization of electrolysis at low frequencies, where several novel process options exist.

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121 Power Ultrasound

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CONTENTS

I. INTRODUCTION

Sound waves are mechanical vibrations that travel through solids in the form of transverse waves, and through liquids and gases in the form of longitudinal waves. Ultrasound refers to sound waves having a frequency higher than the range audible to humans. The lowest ultrasonic frequency is commonly taken as 20 kHz (1 Hertz = 1 cycle per second). The upper limit of ultrasound frequencies is not clearly defined but is usually taken to be 5 MHz for gases and 500 MHz for liquids and solids (1). Applications of ultrasound can be divided broadly into two categories: low- and high-power ultrasound (Figure 121.1).

The first category involves low amplitude sound waves and is also referred to as "low intensity," "diagnostic," or "high frequency" ultrasound. Low power ultrasound uses very high frequencies of 2 MHz to 20 MHz with low sound intensities of 100 mW/cm^2 to 1 W/cm^2. It measures the velocity and attenuation of the wave in a medium and utilizes such information in medical imaging (e.g., scanning an unborn fetus), chemical analysis, food quality assessment, and non-destructive testing (e.g., regular crack testing for aircraft structures). A low-power ultrasound measurement system is composed of a transducer, a signal generator, a digitizer, and a measurement cell. Possible applications of low-power ultrasound in food

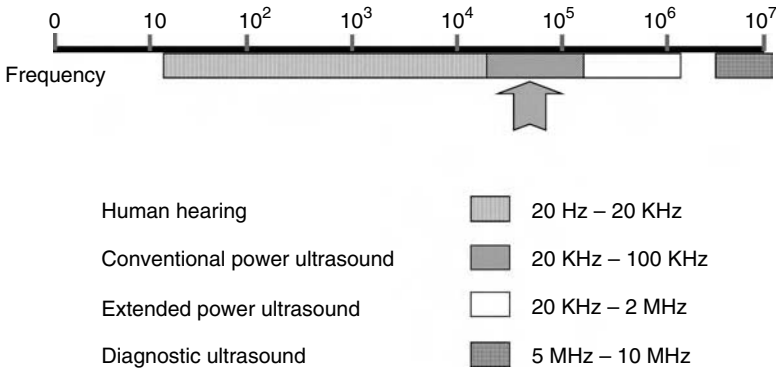

FIGURE 121.1 Sound wave spectrum (from ref. (1)).

processing and quality determination include measurement of temperature and flowrate, determination of composition, determination of particle size, determination of creaming and sedimentation profiles, monitoring of phase transitions, study of gelation, ultrasonic imaging, fouling detection, and the study of molecular properties.

The second category in often known as "power ultrasound" or "high intensity ultrasound." Power ultrasound uses lower frequencies (typically 20 kHz to 100 kHz) and produces sound intensities of 10 to 1,000 W/cm^2 with amplitudes ranging from about 5 to 50 microns. It finds applications in food processing operations such as emulsion generation, dispersion of aggregated materials, drying, inactivation of microbes and enzymes, heat and mass transfer enhancement, biological components separation, and modification and control of crystallization process (2).

The purpose of this chapter is to provide an insight into the general principles of power ultrasound, as well as to review recent progress in the research and development of power ultrasound and its applications in the food processing industry. Readers who are interested in low-power ultrasound applications can refer to the comprehensive review articles of McClements (3, 4) for detailed information.

II. GENERATION OF ULTRASOUND AND ULTRASOUND SYSTEMS

A. Ultrasound Generation

Ultrasound is generated via an ultrasonic transducer — a device by which mechanical or electrical energy can be converted into sound energy. There are two fundamental transducer designs used for power ultrasonic applications today, magnetostrictive and piezoelectric, both powered by electricity.

1. Magnetostrictive Transducers

Magnetostrictive transducers consist of a large number of nickel plates or laminations arranged in parallel with one edge of each laminate attached to the bottom of a process tank or other surfaces to be vibrated. A coil of wire is placed around the magnetostrictive material. When a flow of electrical current is supplied through the coil of wire, a magnetic field is created. This magnetic field causes the magnetostrictive material to contract or elongate, thereby introducing a sound wave into a sonicating fluid.

2. Piezoelectric Transducers

The heart of a piezoelectric transducer is a single or double thick disc of piezoelectric ceramic material, such as barium titanate, lead metaniobate, or lead zirconate titanate (PZT), sandwiched between electrodes that provide the attachment points for electrical contact. The ceramic assembly is compressed between metal blocks (one aluminum and one steel) to a known compression with a high strength bolt. When a voltage is applied across the ceramic through the electrodes, the ceramic expands or contracts, depending on polarity, due to changes in its lattice structure. This physical displacement causes a sound wave to propagate into a treatment solution.

3. Comparison between Magnetostrictive and Piezoelectric Transducers

Piezoelectric transducers utilize the piezoelectric property of a material to convert electrical energy directly into mechanical energy. Magnetostrictive transducers utilize the magnetostrictive property of a material to convert the energy in a magnetic field into mechanical energy. Both types of transducers have advantages and disadvantages. A comparison between two types of transducers is given in Table 121.1.

B. Power Ultrasound Systems

A typical ultrasonic system comprises three essential parts (2): a generator that converts electricity into required high frequency alternating current, a transducer that converts the high-frequency alternating current into

TABLE 121.1
A Comparison between Piezoelectric Transducers and Magnetostrictive Transducers

Transducer	Frequency Range (kHz)	Noise	Reliability	Sweeping	Aging	Energy Efficiency
Piezoelectric	Wide range	Less noisy	Improving reliability	Not easy	Noticeable	>70%
Magnetostrictive	18–30	Noisy	Reliable	Easy	NA	35–40%

mechanical vibrations, and a delivery system to convey the vibration into a food system, such as the tank of the ultrasonic bath and the horn of the ultrasonic probe system.

1. Conventional Power Ultrasound Systems (1)

In practice, ultrasound can be introduced into a food system to perform various applications in two ways: direct and indirect contacting. With the direct contacting method, a food is in direct contact with an ultrasonic element, which can be a thin metal blade in the case of a liquid whistle apparatus or a sonic horn for probe type designs. Transducer arrays can also be arranged with sonic horns attached to them inserting into a treatment chamber or flow cell of different geometry to facilitate various sonication treatments. The advantage of this arrangement is that ultrasound can be directly transmitted into a food system with less energy loss. It can be used to design a high surface power density (W/cm^2) system or a high volumetric acoustic energy (W/cm^3) system by controlling the treatment chamber volume. The disadvantages may include difficulty of temperature control, and the possibility of generating free radicals on the contacting surfaces when surface power density is high. Another direct contacting design is a sonic vibrating bar developed by a Canadian company (5). The metallic bar is driven by three magnets and vibrates at the audible frequencies. It can operate at power as high as 75 kW and is effective as a mixer and grinder.

In an indirect contacting system, the ultrasound transducer is usually mounted onto a large surface to perform sonication treatments. The widely used ultrasonic baths are a good example of this design. In this category, ultrasound has to be transferred through a wall to reach the food system under treatment and thereby both the ultrasound power intensity (W/cm^2) and the volumetric acoustic energy density (W/cm^3) are low. It also has the problems of poor temperature control and difficulty in quantifying power delivered into the food system during sonication. Systems developed based on this design are widely used owing to their simplicity and ease of operation. The most successful application of the indirect contacting system is surface cleaning of jewelry and gun parts. In recent years, various food surface decontamination applications have been explored.

2. Variable Frequency Systems

Due to the ultrasound generation mechanism, traditional ultrasound systems are inherently single frequency units. In recent years, the concept of using more than one transducer each with different frequencies to enhance cavitation has been explored. Researchers have demonstrated several-fold increases in cavitation activities in lab testing in a dual or multi-frequency ultrasound system (6). Ultrasound units using this concept have to use more than one transducer, each with its own generator. This will complicate the design and operation, and will increase the cost of the unit. A new concept, the multi-frequency, multimode, modulated (MMM) technology was recently developed to facilitate variable frequency sonication applications. In a MMM unit (Figure 121.2), the ultrasonic power supply is able to produce variable frequency-sweeping oscillations around a central operating frequency, and has an amplitude-modulated output signal (where the frequency of amplitude modulation follows sub-harmonic low frequency vibrating modes of the mechanical system). The MMM technology can utilize the coupled vibrating modes in a mechanical system by applying advanced digital signal processing to create driving wave forms that synchronously excite many vibrating modes (harmonics and sub-harmonics) of an acoustic load. It will help to produce uniform distribution of high-intensity acoustical activity to make the entire available vibrating domain acoustically active while eliminating the creation of potentially harmful and problematic stationary and standing wave structures (7).

C. Cavitation

Most power ultrasound applications are based on the activity of cavitation, which refers to the formation, growth, and implosion of gas- or vapor-filled cavities in liquids when large acoustic pressure differences are applied. When sound waves travel through a liquid in the form of longitudinal waves comprising a series of compression and rarefaction portions, negative pressures are generated at the rarefaction portions. It is believed that nucleation is initiated at sites where the tensile strength of the liquid is dramatically lowered. One generally accepted nucleation mechanism states that gas entrapped in small-angle crevices, when subjected to negative

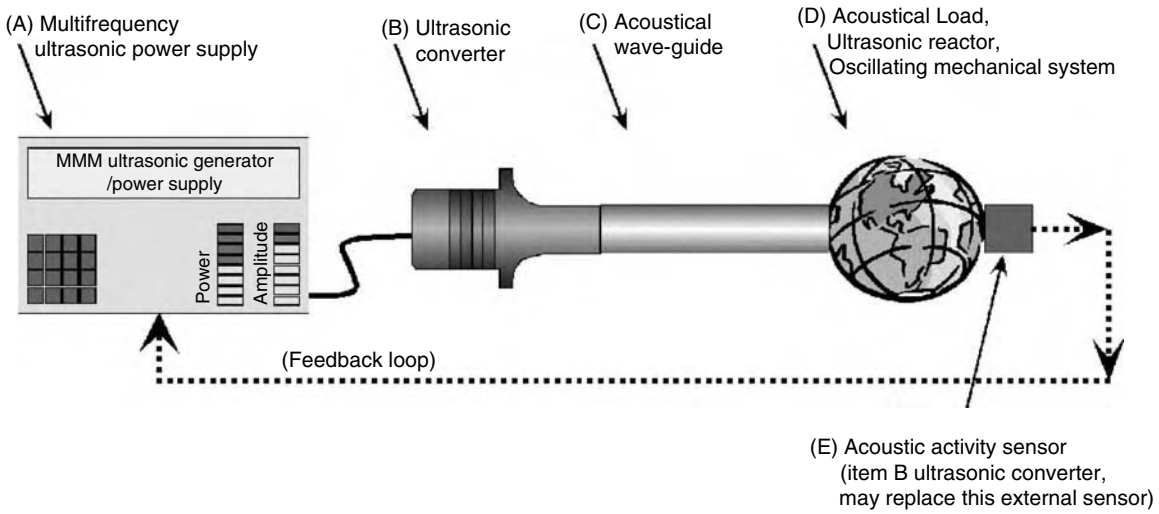

FIGURE 121.2 A multifrequency, multimode, modulated (MMM) ultrasound system (courtesy of MP Interconsulting).

acoustic pressure, undergoes expansion and forms small free bubbles (8). The behavior of the bubbles in a sonicating liquid determines the cavitation dynamics. Transient cavitation bubbles are generated when sound intensity is greater than 10 W/cm^2. These bubbles, with effective residence time of $<$100 nano-seconds, will experience large expansion in size in a few acoustic cycles and terminate in a violent collapse. The collapse of transient micro-bubbles can create extreme physical conditions, such as temperatures and pressures as high as 5,000 K and 1,000 atmospheres (8). It is believed that the localized high temperatures and pressures are responsible for most of the sonochemical and bactericidal effects. The release of the high pressure results in the formation of shock waves that often provide mechanical cleavage of large biopolymers. Stable cavitation is produced at fairly low sound intensities (1–3 W/cm^2). Stable bubbles have a much longer residence time so that the mass transfer of gas into them will result in growth of bubble sizes. As bubble sizes increase, stable bubbles can transform into transient bubbles and undergo collapse. They can also float to the surface and be expelled. Stable bubbles oscillating in resonance with the applied acoustic field can generate intense local strains in the bubbles' vicinity, which are the cause of many of the disruptive mechanical effects of sound (1).

There are several parameters affecting cavitation activity in a sonicating liquid. Increasing frequency decreases the intensity of cavitation because, at high frequencies, the rarefaction cycle is too small to permit a bubble to grow to a size sufficient to cause implosion. Since the prerequisite for micro-bubble formation in a liquid is that the negative pressure must overcome the cohesive forces, it is more difficult to generate cavitation in viscous and high surface tension liquids. Cavitation intensity increases with temperature when the acoustic power density is a constant. Increased vapor pressure makes it easier to surpass the crushing force, the difference between the hydrostatic pressure and the acoustic pressure, to generate cavitation. The effect of applied external pressure is two-fold. Higher external pressure will result in a higher cavitation threshold, as well as an increase in the intensity of bubble collapse.

III. SELECTED POWER ULTRASOUND APPLICATIONS

A. EMULSIFICATION

Emulsification is one of the earliest applications of power ultrasound. When a bubble collapses in the vicinity of the phase boundary of two immiscible fluids, the resulting shock wave can provide a very efficient mixing of layers (9, 10). Stable emulsions generated with ultrasound have been used in the textile, cosmetic, pharmaceutical, and food industries. The emulsions obtained by mechanical oscillations at ultrasound frequencies are known to have a number of advantages, including stable emulsions even without the addition of surfactant, and narrow mean droplet size distribution compared to other methods (11). Mongenot et al. (11) reported that encapsulations spray-dried from ultrasound-generated emulsions of maltodextrin better retained cheese aroma. In milk homogenization tests with ultrasound, shorter fermentation time of yogurt was found. Ultrasound treatment also altered the physico-chemical properties of the milk (12).

The mechanical device used to acoustically generate emulsification can be a sonicator with a piezoelectric or magnetostrictive transducer, or a "liquid whistle" that is

widely used for homogenization and emulsification applications in the manufacture of fruit juices, tomato ketchup, and mayonnaise (9).

B. Cutting

Ultrasonic knives made of titanium have been used in slicing or slitting of different food products. An ultrasonic cutting machine consists of a specially designed horn, used as a knife, driven by an ultrasound transducer, usually through a booster, and a precision positioning mechanism. The reciprocating vibration of the blades at ultrasonic frequency greatly reduces friction between the knife and the product, which ensures straight and clean cuts and results in products with uniform size, shape, and density. Ultrasonic knives can be used to cut sticky and brittle products. Nuts, raisins, and other hard fruits are cut cleanly without plowing or displacement, and peanuts are cut with minimum waste. Production costs and downtime associated with conventional cutting methods are minimized. With greatly reduced friction, there is minimal knife abrasion and blades stay sharper longer, which reduces annual maintenance costs. An ultrasonic cutting unit can be easily incorporated into an existing production line. It can also be a complete custom-built combined ultrasonic slitting and guillotine cutting station.

C. Inactivation of Microorganisms

The bactericidal effect of ultrasonic waves has long been observed (13). At that time, the effect was attributed to the compression that ultrasound would generate in a liquid. However, the relatively low inactivation rate of sound waves compared with other methods prevented ultrasound from being used as a food preservation method. In the early years, the low inactivation capacity of ultrasound was related to the low power density used.

A few studies have examined the use of ultrasound in conjunction with other preservation methods for the destruction of microorganisms. Neppiras and Hughes (14) reported that static pressure helped to increase the inactivation capacity of sound waves. Burgos et al. (15) showed that the heat resistance of bacteria spores decreased under sonication. Ultrasound has been combined with ozone (16) and H_2O_2 (17) to inactivate bacteria and spores and a synergistic effect was observed. When combining heat with power ultrasound (20 kHz) (thermosonication), the microbial inactivation rate was greater than the addition of the inactivating effect of heat to that of ultrasound when acting independently (18). Pagán et al. (19,20) and Mañas et al. (21) documented in their studies inactivation of *L. monocytogenes* with ultrasonic waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures and reported a significant increase in inactivation due to pressurization.

Several theories have been proposed to describe the inactivation mechanism of ultrasound. When ultrasonic waves pass through a liquid, bubbles or cavities can be formed if the amplitude of the waves is high enough. This phenomenon is known as cavitation. Cavitation can affect a biological system by virtue of a highly localized temperature rise and mechanical stress (22), which cause double-strand DNA breaks, enzyme inactivation, and damage to liposomes. Application of ultrasound to a liquid also leads to the formation of OH^- and H^+ species and hydrogen peroxide (23). These species also have important bactericidal properties. When ultrasound is combined with heat and pressure, the synergistic effect was attributed to the disruption of the bacterial spore cortex, which resulted in protoplast rehydration and loss of heat resistance (24). In the case of sonication assisted by elevated pressures, the increase in inactivation rate was probably due to an increase in bubble implosion intensity, as postulated by Pagán et al. (19). For certain microorganisms, such as *L. monocytogenes*, *S. enteridis*, and *A. hydrophila,* only additive effects were observed under the conditions tested by Pagán et al. (19).

Ultrasound has also been tested for its efficacy on surface decontamination of poultry (25) and fresh produce (26), as well as on removal of biofilm (27). The use of ultrasound in microbial inactivation for a food system is still in the stage of laboratory testing. No commercial applications of food microbial reduction have been documented.

D. Enzyme Activity Control

Ultrasonic energy has been used either to increase or inhibit enzyme activity, depending upon the ultrasound intensity. At low ultrasound intensities, Ishimori et al. (28) achieved a two-fold increase in α-chymotrypsin activity in a casein substrate. Enzyme activity inhibition at high ultrasound intensity levels has long been observed. Inactivating enzymes with ultrasound, however, usually requires long treatment times and the presence of oxygen (29). To increase the effectiveness of ultrasound treatment, different combined processes have been used to inactivate food enzymes. The most recent development includes treatments using a combination of heat, low pressure and ultrasound to increase the inactivation rate (manothermosonication). Lopez et al. (30) studied tomato pectinmethylesterase (PME) and polygalacturonase (PG) inactivation kinetics using manothermosonication (MTS) in a buffer and reported D values of 0.85 min for PME, and 1.46 and 0.24 min for PG1 and PG2, respectively. Vercet et al. (31) applied MTS to inactivate enzymes in a tomato paste at 200 MPa and 70°C for 1 min. They found that PME residual activity in treated samples was undetectable and PG residual activity was 38%. As observed with microorganisms, enzyme inactivation by a combination of heat and ultrasound under pressure exhibited a synergistic effect. The enzyme inactivation efficacy of

heat is increased by a factor that is dependent on the nature of the enzyme and the working conditions (29). In laboratory tests, MTS has been proven to be an effective means to inhibit enzyme activity. An advantage of using MTS is that both microbial inactivation and enzyme activity inhibition can be achieved in one processing step.

E. Modification of Biopolymers

Jackson et al. (32) used ultrasound to dissolve corn and sorghum starch granules after heating. They reported that ultrasonic vibrations disrupted swollen granules, thereby releasing amylose and amylopectin from the granules, which resulted in an increase in water solubility of the starch. Chung et al. (33) used power ultrasound to treat mung bean, potato, and rice starches with heating followed by ultrasound and reported that the average degree of polymerization did not change after sonication. They postulated that the changes in starch properties were induced by the disruption of swollen granules rather than the breakage of glucosidic linkages. In a study to examine the effect of ultrasound treatment on starch at different pH (2.0, 4.5, 7.0, 9.5, and 12.0), Zhang et al. (34) found that starches treated with power ultrasound had lower peak viscosity (PV) and higher pasting stability than did native starch. Ultrasound treated starches exhibited a decrease in the gelatinization enthalpy (ΔH) and an increase in the gelatinization onset (T_o). Ultrasound also significantly increased the in vitro digestibility of starches in the earlier stage.

F. Separation of Bio-Polymers and Bio-Components

Studies utilizing the mechanical action of sonication have been performed in recent years to realize various bioseparation operations. Mason (35) reported a study using ultrasound to treat rice grains in which surface erosion and particle size reduction resulted in shorter cooking and gel times. In a study on rice starch isolation, Wang et al. (36) found that high-intensity ultrasound treatment resulted in a high starch recovery but a slightly higher residual protein content. Zhang et al. (37) tested power ultrasound as a means to recover starch from degermed corn flour and hominy feed, which are reasonably high in starch and may be a source of non-sulfate treated starch. They used five treatments to produce starch from degermed corn flour and hominy feed slurries (10% solid): control, ultrasound only, ultrasound followed by fine grinding, fine grinding followed by ultrasound, and fine grinding only. The total starch recovery data are listed in Table 121.2. The starch yield from the corn flour was 37.1% for the control, and those by ultrasound treatments were 65.5 to 67.0%, a 28.4 to 29.9% increase compared to the control. Similarly, starch yields from the hominy feed by ultrasound treatments were 45.4 to 45.8%, a 16.1 to 16.5% increase compared to the control (Table 121.2). Comparing with the total starch contents in the two products, ultrasound treatments recovered 97.3 to 99.5% starch from the degermed corn flour and 97.8 to 98.9% from the hominy feed. Obviously, ultrasound treatment is a very effective method to recover starch from low value degermed corn flour and hominy feed.

TABLE 121.2
Starch Yields from Corn Flour and Hominy Feed Treated with Different Methods

Product	Treatment	Fractions (%)			Starch Recovery (%)
		Starch	Gluten	Fiber	
Corn flour	Ultrasound	66.8	15.7	16.7	99.1
	Ultrasound-Grind*	65.5	16.3	17.5	97.2
	Grind-Ultrasound	67.0	15.9	15.8	99.4
	Grind	34.7	8.4	55.2	51.5
	Control	37.1	5.9	56.0	55.0
Hominy feed	Ultrasound	45.6	22.5	29.0	98.3
	Ultrasound-Grind	45.4	26.7	24.5	97.8
	Grind-Ultrasound	45.8	27.5	23.1	98.7
	Grind	34.6	21.2	39.9	74.6
	Control	29.3	12.3	54.6	63.1

*Grind = fine grounding.

Power ultrasound was also used to increase starch yield in a novel corn processing method, the quick-germ/quick-fiber process (38). In the experiments, yellow dent corn soaked in deionized water at 52°C for 24 hrs without addition of SO_2 was wet-milled using a 100-g laboratory procedure with some modifications. Ultrasound treatments were performed at different process steps: first grind followed by ultrasound, ultrasound followed by second grind, second grind followed by ultrasound, fine fiber slurry treatment with ultrasound, and milling only (no ultrasound). A conventional wet milling treatment was used for comparison. Starch yield resulting from no ultrasound treatment was 61.7%, ultrasound treatments were 66.9–68.7%, and conventional wet milling was 68.9%. The characteristics of the starches produced with ultrasound treatments are similar to that from a conventional wet milling method as shown by color measurements and RVA curves.

G. Case Study — Power Ultrasound Enhanced Corn Pericarp Separation (39, 40)

Corn pericarp is a main source of dietary fiber. Refined corn pericarp has at least 92% dietary fiber, which places it among the most concentrated sources of edible fiber. It can be used as a supplement in dietary beverages, extruded breakfast cereals and snack foods, and breads and other bakery products. Currently, there are no rapid and effective pericarp separation methods available. It is imperative for

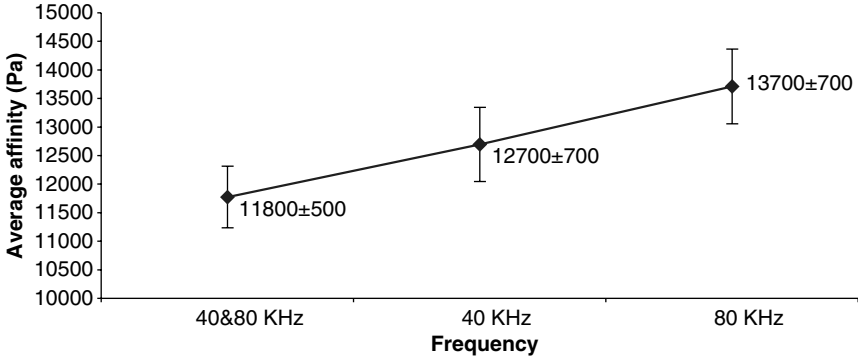

FIGURE 121.3 Pericarp affinity at three sonication frequencies. The samples were sonicated for 1 min at room temperature using the Zenith sonicator with a rating power of 925 W.

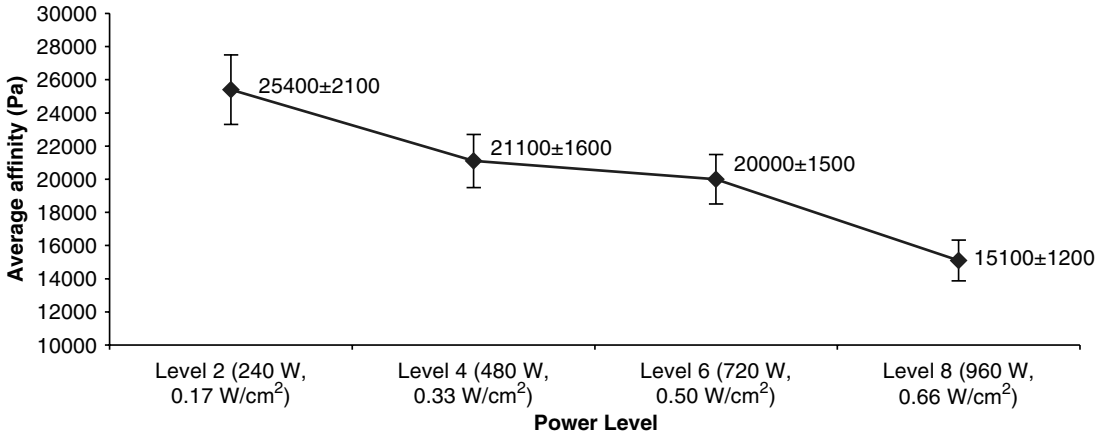

FIGURE 121.4 Pericarp affinity at four ultrasonic power levels. The samples were sonicated for 1 min at room temperature using a VWR sonicator at 40 kHz. The wattage for power levels of 2, 4, 6, and 8 was 240, 480, 720, and 960 W, respectively, and the corresponding power intensities were 0.17, 0.33, 0.50 and 0.66 W/cm^2, respectively.

such methods to be developed to facilitate the lab use for transgenetic research of corn as well as for industrial applications of the corn pericarp and other components.

Yellow dent corn at about 13% moisture content was treated with two ultrasonic baths at different frequencies, treatment times, temperatures, and power levels. Corn sample (50 g) was sealed in a plastic bag containing 200 ml of water and placed in the ultrasonic baths for sonication tests. After sonication, the pericarp can be easily separated by mechanical friction or abrasion. To quantify the separation effect of ultrasound, the affinity of the pericarp (Pa) was measured with an Instron Testing Machine (Instron Corporation, Canton, MA).

Figure 121.3 shows the average pericarp affinity for each of the three frequency conditions tested in the experiments: 40 kHz, 80 kHz, and a combination of 40 and 80 kHz. From Figure 121.3 it can be seen that pericarp sonicated at 40 kHz had a lower affinity (i.e., was easier to separate) than that sonicated at 80 kHz. Compared to the pericarp sonicated at 40 kHz or 80 kHz alone, the pericarp sonicated with a combination of 40 & 80 kHz resulted in the lowest pericarp affinity. Figure 121.4 shows the average pericarp affinity at four power levels. One can see that the pericarp affinity showed a decreasing trend with the power level. The power level had a significant effect ($\alpha = 0.05$) on the pericarp affinity. However, changes in sonication duration and sonication temperature did not show a marked effect on pericarp affinity.

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122 Ultraviolet Light

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CONTENTS

I. THE ULTRAVIOLET PORTION OF THE ELECTROMAGNETIC SPECTRUM

Ultraviolet light forms part of the electromagnetic spectrum. The ultraviolet wavelength range is from about 10 to 400 nm, placing it between X rays and the visible part of the spectrum (Figure 122.1).

Ultraviolet is frequently referred to as 'non-ionising' radiation, however the shortest ultraviolet wavelengths do bring about some ionisation. The ultraviolet portion of the spectrum has been sub-divided on a more or less arbitrary basis primarily for convenience. The term 'vacuum ultraviolet' is reserved for wavelengths below 200 nm, because in this region ultraviolet is strongly attenuated by air. It is usual to refer to the region between 200 and 300 nm as 'far ultraviolet' and that between 300 and 400 nm as 'near ultraviolet.' Alternative sub-divisions are often quoted in the scientific literature: thus UV-C is used for wavelengths in the range 100 to 280 nm, UV-B for 280 to 315 nm and UV-A for 315 to 400 nm. In what follows here, the abbreviation 'UV' will be used to denote UV-C.

II. THE EFFECT OF UV ON MICROORGANISMS

The fate of a microbial cell, or a population of such cells, following exposure to UV will depend on a number of factors. The range of wavelengths used to irradiate the cells will be one such factor. All UV sources used for commercial and industrial disinfection are polychromatic but the spectral range emitted will depend on the type of source used.

The most directly lethal wavelengths will be those that are maximally absorbed by the bases of DNA. The precise wavelength for maximum lethal effect varies from species to species because the DNA composition of species differs and because each DNA base has its individual peak absorptivity. Giese and Darby (2) compared the lethality of two UV wavelengths (280 and 301 nm) using a variety of bacteria and a bacteriophage (Table 122.1). There were greater differences in UV susceptibility between species at the highest wavelength (301 nm) but as the lethal effect of wavelengths in this part of the UV spectrum is low, the

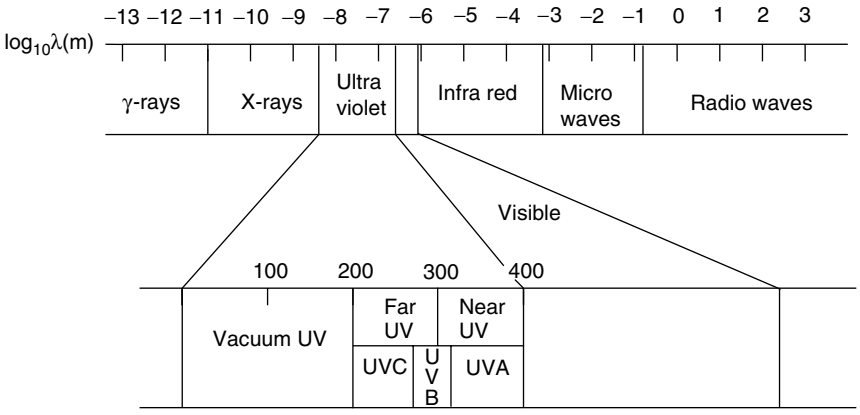

FIGURE 122.1 The electromagnetic spectrum.

TABLE 122.1
Germicidal Efficiencies at 280 and 301 nm

Micro-organism	Mean Germicidal Efficiency* at 280 nm	Mean Germicidal Efficiency* at 301 nm
Citrobacter diversus	0.894	0.025
Citrobacter freundii	0.834	0.017
Klebsiella pneumoniae	0.787	0.043
Bacteriophage ϕX-174	0.899	0.054

* Relative to 254 nm.

Data from (2).

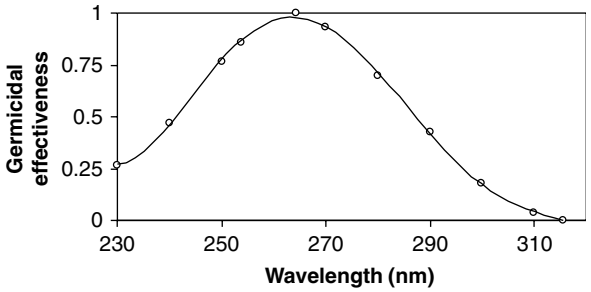

FIGURE 122.2 Relative effectiveness (with reference to 254 nm) of wavelengths of the UV spectrum. Data from ref. (3).

differences are of no real significance and for all practical purposes it appears justifiable to use data that is species-independent (see Figure 122.2). This figure shows that wavelengths in the vicinity of 265 nm are the most effective at inactivating microbial cells.

As the UV penetrates the microbial cell it will be attenuated by the various cellular structures and components. Attenuation effects are wavelength-specific. For example, the cell membrane may absorb 25% of the UV light at 280 nm but only 10% of the UV light at 254 nm, thereby decreasing the germicidal efficiency at 280 nm (2). Some organisms have evolved strategies for surviving irradiation by synthesising various UV-screening compounds (4). However, the majority of these compounds provide protection against UV-A and UV-B. A recent comparison of the sensitivity of a pigmented and a non-pigmented mutant of *Rhodobacter sphaeroides* to short wave UV showed no significant difference between the two cultures (5).

Although UV can bring about changes to a number of cellular components, the most significant reactions in determining cell survival are those that occur between UV and the nucleic acids. If the retroviruses, which contain only RNA, are omitted from further discussion, it becomes possible to concentrate solely on DNA. The interaction between UV and DNA will result in the formation of so-called 'photoproducts.' The most important of these are pyrimidine (i.e., thymine and cytosine) dimers. These are formed between two pyrimidine bases adjacent to one another on the same strand of DNA. Thymine dimers tend to predominate because thymine has a greater absorbance than cytosine in the germicidal wavelength range (6). Thymine (T) occurs in DNA in equal amounts with adenine (A) and it might be thought that species that contained a high proportion of A + T in their DNA might be particularly sensitive to UV, but this is not borne out by existing experimental evidence (5). Another type of photoproduct, 'pyrimidine adducts' are also formed between adjacent pyrimidine bases but at reduced rates of formation compared to dimers. At sufficiently high UV doses DNA-protein cross-links are formed, whilst at higher doses still, DNA strand breakages may be induced. A unique photoproduct — the so-called 'spore photoproduct,' another dipyrimidine — has been found in bacterial spores.

Nearly all living cells possess the ability to reverse the damage caused to their DNA by UV by using one or more repair mechanisms of which there are three principal types. In photoenzymic repair, dipyrimidine dimers are

enzymically monomerized in the presence of light. The second type of repair process, excision-resynthesis repair, involves removing sections of damaged DNA and resynthesising them using the intact strand as template. Whilst in postreplication repair, undamaged sections of DNA are replicated and combined in such a manner that an intact double stranded molecule, identical with the original, is formed.

The physiological state of cells is another factor that determines survival. It has been shown that microorganisms harvested at different stages of growth show differences in susceptibility to UV (7). Moreover, the physical state of cells can also have an effect on cell survival: when cell aggregates are irradiated, the cells on the outside can effectively shield those towards the centre of aggregates. This phenomenon manifests itself as a 'tail' in the so-called 'dose–response curve' for that species of organism. The dose response curve is simply a plot of the reduction in cell viability against dose.

It has generally been accepted that the effect of UV exposure on living cells is solely determined by the dose absorbed. The UV dose is defined as the product of the exposure time and UV intensity (or 'fluence rate'). In other words, a short, high intensity exposure is equivalent to a protracted low intensity one. This is the Bunsen-Roscoe reciprocity law. However, it has been known for some time that experimental data exists which casts doubt on the veracity of this law. Sommer *et al.* (8) compared survival data for three different strains of *E. coli* irradiated at constant doses achieved by varying the UV intensity and time of exposure. In all cases the most lethal effects were achieved at the highest intensities. These findings can be interpreted in mechanistic terms: irradiation of microbial cells will lead to the formation of DNA lesions as explained above, however this process is counteracted by the cells' repair capabilities. As the UV intensity is increased the rate of lesion formation will eventually exceed the capacity of the repair systems. A similar argument has been used to explain the presence of 'shoulders' on the UV dose–response curves of certain microbial species.

The most commonly used method of assessing cell viability following UV irradiation is to plate the cells out onto an agar medium. However, recent work suggests that there may be risks in using this type of assay to definitively define viability. Physical and chemical stresses applied to microbial cells can induce them to enter, what has been termed a 'viable but non-culturable' (VBNC) state. Of particular relevance is work that shows that cells of *E. coli* 'killed' by UV were capable of performing a number of metabolic functions 48 hours after being irradiated. These functions included expression of esterase activity, uptake of glucose and cell elongation (9). In the context of food processing knowledge of whether VBNC cells retain their pathogenicity could be of crucial importance. The evidence accumulated to date is that cells of *Salmonella typhimurium* do not retain their pathogenicity (10) whereas *E. coli* cells do (11).

III. UV DOSE LETHALITY

Compilations of data have been published which show the UV doses necessary to bring about specific reductions in the populations of a variety of microbial species (3). These data are often given in the form of the doses necessary to reduce the population size by one tenth (the 'decimal reduction dose,' D_{10}) or by 1/e, i.e., 37% (D_{37}). The very existence of this data seems to have perpetuated the notion that a single value of D_{10} can be ascribed to a particular species in much the same way as can the density of a solution of sugar of known concentration. Closer comparisons of data published for individual species reveal some quite large discrepancies. To quote just one example for the radioresistant bacterium *Deinococcus radiodurans*, estimates for the D_{37} dose in kJ/m^2 include 0.40 ± 0.13 (12), 0.34 ± 0.05 (5) and 0.55 to 0.60 (13). Moreover, it is generally assumed that taxonomically closely related species have similar UV susceptibilities, whereas some quite large variations have been shown to exist (14). Decimal reduction doses, in common with all other measures of the effects UV on microbial populations, are subject to influence by all of the factors discussed above, i.e., the physiological and physical state of the cells as well as irradiation conditions.

Van Gerwen *et al.* (15) adopted an interesting approach to the treatment of D_{10} doses for ionising radiation which might profitably be applied to existing UV data. These workers analysed over 500 estimates of D_{10} doses from the literature. After eliminating data clusters for unusually tolerant species such as *D. radiodurans* as well as data for particularly sensitive and highly resistant spores, they were able to specify an average D_{10} value for bacterial spores as well as one for vegetative cells. Until such an analysis becomes available of the UV data, the existing D_{10} compilations should be viewed upon as simply providing general guidance.

IV. INACTIVATION OF MICROORGANISMS ON FOODS

Downes is credited in 1886 with discovering that the ultraviolet portion of the solar emission is lethal towards microorganisms (16). The first artificial UV source was patented in 1903 and soon after this UV sources were being used to disinfect water. The first recorded use of an artificial UV source for food disinfection was in 1906, for milk (17). Subsequent applications of UV in the early stages of its development as a method of disinfection are covered in the reviews of Moldovan (18) and Proctor and Goldblith (19).

Decimal reduction doses (D_{10}) are typically obtained by irradiating dilute suspensions microorganisms in water or buffer. The difficulties in interpreting data obtained under these relatively well-defined conditions, as discussed above, are greatly compounded when such data is required for the surfaces of foods.

There are a number of considerations that will influence the nature of the data obtained. Firstly, researchers have a choice of either working with the microflora naturally present on a particular food, or of artificially culturing organisms of interest and then applying them to the food in some way. Whilst the former approach might hold obvious attractions, it presents certain experimental difficulties. Samples of foods, even those taken from a single source, may harbour both different numbers, and different species of microorganisms, a problem made worse if more than a single source is used. This makes replication and statistical evaluation of results difficult. In addition to this, it has been accepted for some time that not all microbial species present on environmental samples — this includes foods — can be cultured under laboratory conditions. Whilst such difficulties may be greatly reduced by working with artificially cultured microorganisms, other complications arise. The association of microorganisms with foods is complex and can take more than one form. Microbial cells coming into contact with the food may adsorb to the food and then go on to multiply at its surface. This may simply lead to greater cell assemblages, however certain species may produce polysaccharides that serve not only to attach the cells more strongly to the food, but which can also protect them from external stresses. Moreover, the physiology of cells embedded in polysaccharide matrices, or 'biofilms,' is known to differ from those of planktonic cells (20). In addition, recent work has shown that certain elements of the natural microflora may offer protection against pathogens (22). In many cases the true state of the association between a particular food and its natural microflora is unknown, and the methods chosen by researchers to apply microorganisms to food surfaces may result in highly artificial associations. Often there are no alternatives to such methods. However, if these inherent experimental limitations are taken into account then the existing experimental data available for a number of different foods can provide a useful guide to assessing whether UV treatment of a particular food is feasible.

Table 122.2 shows a compilation of recent data. The range of foods is quite diverse as are both the targeted microbial species and the dosages applied.

Relatively few evaluations have been made of the effect of UV on the organoleptic characteristics of the foods. Such considerations are far from trivial and deleterious changes could lead to rejection by consumers despite the fact that treatment may have produced a safer product.

When treating solid materials, such as foods, with UV, it is characteristic of the surfaces that are of primary importance, as UV radiation will not penetrate very far beyond the surface layers of the material. On this basis, these surface properties can be expected to influence strongly the chances of microorganisms surviving irradiation. Evidence of the importance of macroscopic surface features is provided by work done on the irradiation of fish fillets by Huang and Toledo (33). These researchers found that UV was more effective in inactivating microorganisms on the surfaces of smooth-fleshed fish, such as mackerel, than on rough-fleshed fish such as mullet. This was because the surface of the mullet provided ridges around which microorganisms were shielded from incident UV light. Microscopic surface characteristics and irregularities may similarly be expected to affect microbial survival: surfaces which might appear smooth to the human eye, may at scales comparable to the dimensions of microorganisms (i.e., of the order of 1 to 10 μm) resemble a topography as rugged as that of the Himalayan foothills.

One approach to account for surface topography and surface–organism interactions is to extend the concept of the decimal reduction dose (D_{10}). A comparison of the D_{10} value for a particular microorganism in conditions where the nature of the medium surrounding the organism does not unduly influence microbial survival with that obtained on the surface of a food can provide some sort of measure of surface-related protective effects. When in dilute aqueous suspensions, microorganisms are essentially fully exposed to incident UV but, presumably for reasons of convenience, many workers have opted instead to irradiate colonies on the surface of agar to obtain 'basal' D_{10} values. Relatively few published studies contain sufficient data to enable this to be done but the data available is presented in Table 122.3.

Implicit in this approach is the assumption that the inactivation data is approximately linear in form. This is the case for most of the data shown in the table, but there are instances where the data deviates significantly from linearity. A case in point is the data of Wong *et al.* (35) for both *E. coli* and *Salmonella senftenberg* on the surface of agar (i.e., the basal D_{10}). In order to obtain D_{10} values under these conditions these workers effectively imposed linearity on their data to enable comparisons to be made with data for the surface of pork which was linear.

Gardner and Shama (40) suggested an alternative approach to the use of decimal reduction factors. Working with model materials (cellulose filter papers), they proposed that the surfaces of foods could be considered as constituting a discrete number of zones each of which contained a certain fraction of the microbial surface population. Each zone was typified by a so-called 'exposure factor.' This quantified the degree of attenuation to incident UV. Thus factors near 1.0 indicated that the zone offered little attenuation, whilst zones with low factors offered high levels of attenuation, and therefore high UV doses were required in order to inactivate the organisms

TABLE 122.2
UV Disinfection of Foods

Food	Microbial Flora	UV Dose kJ/m^2	No. of Log Reductions	Adverse Effects	Other Comments	Reference
Beef (slices)	Cultivated psychrophilic *Pseudomonas* sp. *Thamnidium* sp. *Candida scotii*	77.0	Results highly variable: 0.1 to 2.2 for a dose of 77 kJ/m^2 for *Pseudomonas* sp.	None evaluated.	Effects obtained by continuous long exposure (c. 3.5 days) at low UV intensities. UV irradiation resulted in increased growth lags and decreased growth rates for *Penicillium* sp., *Pseudomonas* sp. and *Thamnidium* sp. but not for *C. scotii*.	21
Beef (muscle and adipose tissue)	Natural microflora	Not measured.	Not stated.	Consumer desirability ratings based on colour were measured. Best results were obtained for samples wrapped immediately after irradiation.	Type of UV source not stated but peak emissivity was at 366 nm with some contribution at 253.7 nm. Shelf life increased by 1.5 to 2 days.	23
Beef	Cultivated *Pseudomonas* spp. *Micrococcus* spp. *Staphylococcus* spp.	0.66	2.0 for the mixed bacterial culture.	No significant differences in odour or appearance between irradiated samples and controls.		24
Bread (Baguette)	Natural microflora	0.14–0.54	Not stated.	None evaluated.	Shelf life extended by approx. 9 days.	25
Chicken (whole)	Natural microflora	0.1	Not stated.	No adverse effect on organoleptic quality.	Although an immediate effect on surface contamination of treated, as opposed to untreated, chickens was observed, the shelf life was not significantly increased.	26
Chicken (halves)	Cultivated *S. typhimurium* and natural psychrotrophic microflora	0.8	0.5 (*circa*) for *S. typhimurium* no significant effect on psychrotrophs.	No significant effects on colour or rancidity.		27
Chicken (skin)	Cultivated *S. typhimurium*	0.16–0.97	80.5% reduction (mean value for all doses).	None evaluated.		28
Chicken (breasts)	Cultivated *S. typhimurium, L. monocytogenes, E. coli* O157:H7	0.015	0.48 for *L. monocytogenes;* 1.02 for *S. typhimurium;* 1.28 for *E. coli*. For chicken with the skin left on.		Number of log reductions for all three bacterial species were decreased when the chicken was treated with the skin left on.	29
Chocolate	Cultivated salmonellae	1.7	5.3 for *S. eastbourne* in a 0.1 mm film of chocolate. No reduction in a 0.5 mm film.	None evaluated.	Photoreactivation was not observed.	30
Cider	Cultivated *E. coli* O157:H7	0.1–0.6	3.81 (mean reduction for all treatments).	None evaluated.	Treatment was affected by the original background microflora. Unable to consistently achieve the 5 log reduction required for the product by the regulatory authorities.	31

(*Continued*)

TABLE 122.2 (*Continued*)

Food	Microbial Flora	UV Dose kJ/m^2	No. of Log Reductions	Adverse Effects	Other Comments	Reference
Cider	Harvested *Cryptosporidium parvum* oocysts	0.14	5	None evaluated.	Oocyst viability was determined using mice.	32
Fish (fillets)	Natural microflora	3.0–75	2.5 for mackerel 0.3 for mullet For low pressure source at a dose of 3.0 kJ/m^2. 2.7 for mackerel 0.4 for mullet For high intensity source at a dose of 60 kJ/m^2	None evaluated.	Both low pressure and high intensity sources used. Poor effect on mullet attributed to the rough surface of the fish. Best results obtained by combining UV irradiation with chlorinated water wash. Shelf life extended by at least 7 days for mackerel.	33
Maple syrup	Natural microflora	Not stated.	Not determined.	The treated sap retained its flavour and colour.	Maple sap was stored in tanks and continuously irradiated. Under these conditions the bacterial count was maintained below 4.0×10^5 per ml for 11 days.	34
Pork (skin and pork muscle)	Cultivated *E. coli* *S. senftenberg*	1.92	3.8 on skin 4.6 on muscle for *S. senftenberg*. 1.6 on skin 1.5 on muscle for *E. coli*.	None evaluated.	D values At 1000 uW/cm^2 *S. senftenberg*: Skin 490 s Muscle 1064 s *E. coli*: Skin 592 s Muscle 1205 s	35
Sausages	Cultivated *E. coli* *B. subtilis*	2	5 for *E. coli* 4 for *B. subtitlis.*	None evaluated.	High intensity source. Sterilisation was not as effective at the ends of the sausages as towards the centre due to shadowing effects.	36
Shell Eggs	Natural microflora	Up to 39	2 at a dose of 10 kJ/m^2. 3 at a dose of 30 kJ/m^2.	None evaluated.	Type of source not stated. Eggs were rotated during treatment.	37
Shell Eggs	Cultivated *S. enteritidis*	Up to 0.5	1.1 at a dose of 0.08 kJ/m^2. 2.5–4.0 at a dose of 0.5 kJ/m^2.	None evaluated.		38
Strawberries	*Botrytis cinerea*	0.25 and 1.0	Not explicitly stated.	Extensive tests performed. At a dose of 2.5 kJ/m^2 fruit had a higher anthocyanin content and were firmer than controls. Suggestion of damage to fruit at the higher dose (10 kJ/m^2) on the basis of electrical conductivity measurements.	Shelf life extended by 4–5 days.	39

TABLE 122.3
Decimal Reduction Doses (D_{10}) for Various Foods

Food	Bacteria	D_{10} Values kJ/m²			UV Intensity W/m²	Reference
Chicken	*Listeria monocytogenes*	Peptone water 0.079	Chicken (with skin) 1.948	Chicken (without skin) 1.911	5	
	Salmonella typhimurium	Peptone water 0.13	Chicken (with skin) 0.888	Chicken (without skin) 2.439	5	29
	E. coli O157:H7	Peptone water 0.061[a]	Chicken (with skin) 0.677	Chicken (without skin) 1.06	5	
Chocolate	*Salmonella eastbourne*	Peptone water 0.18[a]	Chocolate (0.1 mm thickness) 0.797	Chocolate (0.3 mm thickness) 16.28	19	30
Pork	*E. coli*	Agar 1.77	Pork skin 5.92	Pork muscle 12.05	10	35
	Salmonella senftenberg	Agar 0.21	Pork skin 4.90	Pork muscle 10.64	10	

[a] Estimate based on only two data points.

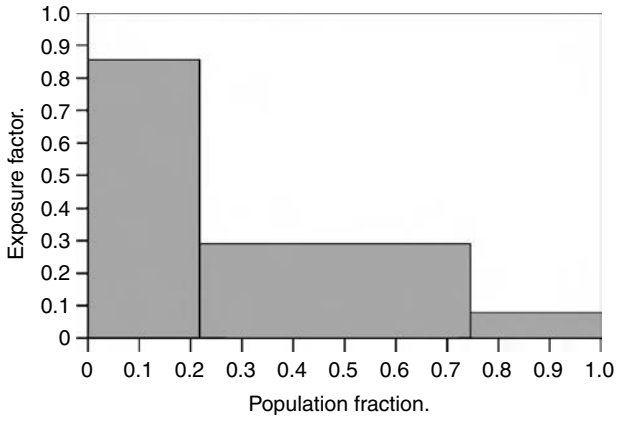

FIGURE 122.3 Zone model for UV inactivation of microorganisms on a solid surface (40).

present. Figure 122.3 shows a typical food surface simulation. In this instance the surface of the food comprises three zones, containing 22, 53 and 25% of the microbial population and with exposure factors of, respectively, 0.85, 0.29 and 0.08.

An extension of this approach to actual food surfaces might go some way to providing data that would enable the UV doses necessary to bring about given reductions in the viability of microbial populations to be predicted.

V. COMBINED TREATMENTS INCORPORATING UV

A. UV AND HYDROGEN PEROXIDE

The germicidal effects of UV can be enhanced by hydrogen peroxide. Hydrogen peroxide possesses antimicrobial properties in its own right, but when combined with UV produces synergistic disinfection effects. This has been demonstrated both for bacterial spores (41) and for vegetative bacteria (42).

TABLE 122.4
Enhancement of UV Disinfection by Hydrogen Peroxide

Medium	Ratio of First Order Inactivation Rate Constants (UV + H_2O_2[a]/UV)	Reference
Coarse cellulose filter paper	3.2	43
Fine cellulose filter paper	5.3	43
Distilled water	4.8	44

[a] H_2O_2 concentration 1% (w/v).

Hydrogen peroxide undergoes photolysis in the presence of UV to yield hydroxy radicals (OH°):

$$H_2O_2 \rightarrow 2OH^\circ$$

These short-lived radicals are highly reactive and will attack microorganisms indiscriminately. The germicidal effect of hydrogen peroxide in aqueous solutions increases with concentration. However, as hydrogen peroxide absorbs UV quite strongly, concentrated solutions of hydrogen peroxide will attenuate incident UV resulting in reduced activity against microorganisms. Therefore, an optimum concentration exists at which the synergistic germicidal effect is at its greatest. This concentration has been shown to be approximately 1% (w/v) for *Bacillus subtilis* spores either in suspension (41) or on solid surfaces (43). Irradiation of hydrogen peroxide solutions at this concentration results in enhanced rates of spore inactivation compared to UV alone as Table 122.4 shows.

Perhaps the most successful commercial application of the combined UV–hydrogen peroxide treatment has

been in the decontamination of beverage cartons (45). Although hydrogen peroxide treatment is currently being evaluated for decontaminating a wide variety of foods, it is somewhat surprising that not more attention has been paid to the combined treatment, particularly as UV can reduce residual hydrogen peroxide levels to those considered acceptable.

B. UV and Ozone

The classification of ozone by the Food and Drugs Administration in the USA as being generally recognised as safe (GRAS) has led to growing interest in its use as a germicide in the food industry (46). In common with hydrogen peroxide, ozone is known to exhibit synergistic disinfection effects under conditions of UV irradiation. The mechanism by which free radicals and other oxidising species are liberated during UV irradiation are quite complex but well established (47).

Despite the advantages that ozone residuals are short-lived, very little work has been published on the combination of ozone and UV of direct relevance to foods. One early study (22) did show that a synergistic inhibitory effect against two species of moulds, (*Thamnidium* sp. and *Penicillium* sp.) was obtained when beef slices were irradiated with UV in the presence of gaseous ozone.

C. Other

An interesting combination involves the use of long wave UV with a class of compounds known as the furanocoumarins. These tricylcic compounds exert their lethality by intercalation within the double helical structure of DNA and cross-linking with DNA bases. One particular attraction in the context of food processing is that furanocoumarins occur naturally in a number of food plant species including lemons, celery and parsley. Bintsis *et al*. (48) showed that the combination of UV and relatively high concentrations of furanocoumarins inactivated both pathogenic bacteria such as *E. coli* O157:H7 and *Listeria monocytogenes*, and the natural brine microflora. Further work in this field, possibly in relation to minimal processing, might yield more positive outcomes.

The anatase crystalline form of titanium dioxide when irradiated by UV light in the presence of water generates free hydroxy radicals. This phenomenon has been widely exploited in environmental applications and some of these are of interest in food processing. One such application is the destruction of ethylene by UV-irradiated titanium dioxide (49, 50). Ethylene is known as the 'ripening hormone' and its accumulation in storage depots of certain horticultural products, i.e., fruit, vegetables and flowers, leads to considerable losses through accelerated senescence resulting in unmarketable produce.

Immobilized titanium dioxide was recently shown to be active in treating the water used in bean sprout cultivation (51).

VI. UV HORMESIS

The uses of UV described so far have related to its direct effects against microorganisms. However, there seems to be a growing interest in the treatment of fruit and vegetables with UV in order to bring about indirect effects in plant tissues that confer resistance to a variety of fungal pathogens.

To date this interest appears to be restricted to that of the research community with little evidence of its adoption by growers or processors. To some extent this is due to the fact that the magnitude of the effects obtained is due to the particular cultivar used or the precise conditions under which it was grown. For example, it is known that there are seasonal variations in susceptibilities of fruits to stresses (52) and such factors would need to be taken into account in commercialising any process based on irradiation with UV. However, as the changes that UV induces in plant materials become better defined and understood, irradiation of agricultural produce might well provide a viable method of reducing postharvest losses in the future.

It has been claimed that postharvest losses of agricultural produce are significant in developed countries, but that in developing countries they can be catastrophic. A number of measures are applied in developed countries to reduce such losses. Principal amongst these is postharvest storage at low temperature. Fewer losses are sustained if in addition to cold storage fungicides are used. These are typically applied to the crops some short while before they are harvested. Interest in the potential use of UV stems from pressures from the regulatory authorities to reduce the dependence on chemical fungicides as primary disease control agents. To these concerns must be added those of consumers who are increasingly demanding food free from chemical additives and produced by methods that cause minimal environmental impact.

The application of UV to the treatment of agricultural produce is termed 'hormesis.' Hormesis may be defined as a beneficial plant response resulting from the application of a low dose of a so-called 'stressor.' A variety of physical treatments, including UV irradiation, may serve as stressors. Although the phenomenon of hormesis was described in the 1940s, it is really in the last 20 years that it has excited interest as a practical method of reducing postharvest losses of crops (53).

Irradiation of fruits and vegetables with UV will result in at least some direct inactivation of microorganisms present at the surface, but hormesis is quite distinct from purely surface disinfection and may even be considered as additive to it. The evidence for hormetic effects is that fungi deliberately inoculated into fruit some distance from

the surface are inhibited following low level irradiation (54). Moreover, hormetic effects have been shown to be reversible by illuminating the plant tissue with visible light following UV irradiation. This would seem to implicate genetic involvement in the response of the plant to UV. Indeed, the isolation of a gene in grapefruit that is activated in response to UV and produces an isoflavone reductase-like protein has recently been announced (55). In addition, it seems that there is a delay after irradiation before the maximum protective effect is achieved; this delay can be as long as 96 hours (56). Table 122.5 reveals that hormesis can be induced in a number of different crops.

The only exception appears to be cactus pears where at best UV treatment resulted in no positive benefits (58). The optimal doses for achieving hormetic effects range from 0.12 to 9.0 kJ/m^2, this is a much narrower range of doses than those used in irradiating foods for obtaining germicidal effects (see Table 122.3). For certain crops the nature of at least one 'hormetin,' the substance produced in response to the application of UV, has been identified. However, it seems likely that the formation of a number of quite different classes of compounds is probably elicited by hormesis. In many cases the exact identity of these compounds have yet to be described. Typically researchers have confined themselves to assaying only one or two previously identified compounds. The enzyme phenylalanine ammonia-lyase (PAL) has been found in a number of different fruits including sweet potato (54). This enzyme is associated with lignin biosynthesis which is a common plant response to fungal attack. There is mounting evidence to suggest that phytoalexins are produced following irradiation. Phytoalexins are low molecular weight compounds that are produced by plants in response to microbial infection or physical damage. The phytoalexin scoparone has been detected in a number of citrus fruits (52, 62), whilst 6-methoxymellein has been found in carrots (59) and resveratrol in grapes (72).

The effects of irradiation with low doses are not restricted to the inhibition of fungal pathogens. A number of workers have shown that ripening of fruit can also be delayed (70). Premature ripening, as may occur for example in storage, is also the cause of postharvest losses as over-ripe produce is not marketable.

There is compelling evidence that even more subtle effects than hormetic ones may be at work and that if these are better understood even greater protection may be given to agricultural produce without resorting to fungicides. The natural epiphytic (i.e., surface-associated) microflora found on certain fruits may inhibit certain fungal pathogens but is itself not affected by low UV doses. This has been demonstrated for the epiphytic yeast *Debaromyces hansenii*, which survives UV doses on the surfaces of peaches which would normally inactivate it on artificial surfaces. This strongly implicates one or more control factors which are imposed by the fruit. *D. hansenii* has been shown to be inhibitory towards the soft rot fungus *Monilinia fructicola* without forming antibiotics. A synergistic protection of peaches against this particular fungus has been demonstrated by the application of the yeast with low UV doses (21).

Of related application to the subject discussed above is the possibility of producing functional foods through UV treatment. Mau *et al*. (73) showed that the vitamin D_2 content of a number of different mushroom species could be increased by irradiating them with UV-B. Cantos *et al*. (74) proposed subjecting grapes to UV-C pulses to increase their resveratrol content.

VII. DELETERIOUS EFFECTS OF UV ON FOODS

Most of the data available on the damaging effects of UV on foods relates to the work done on applying hermetic UV doses to fruits and vegetables. These effects are summarised in Table 122.4. In general, UV doses much above the levels that bring about beneficial hermetic effects, approximately 0.12 to 9.0 kJ/m^2, may result in surface discoloration, accelerated senescence or sprouting. Relatively few adverse effects following UV irradiation were reported in the range of foods listed in Table 122.2, this was mainly because such evaluations were often outside the scope of the work reported. In fact, very few studies have specifically focussed on this aspect of the UV treatment of foods. The extended irradiation of cold liver oil resulted in the formation of toxic aldehydes (75). Studies with artificial food colouring showed that prolonged UV irradiation led to the formation of breakdown products that were DNA damaging (76). However, the UV doses necessary to bring about these effects were not specified.

VIII. UV TECHNOLOGY

A. UV Sources

Despite being published 20 years ago, a very useful source of fundamental information on UV sources remains that of Phillips (1). UV sources are in the main gas discharge sources containing xenon and mercury or xenon and argon. The low vapour pressure mercury source is perhaps the most commonly used method of achieving disinfection. It operates optimally at a temperature of approximately 40°C and is often referred to as 'monochromatic' but actually 90% of its output is emitted at 253.7 nm which is fortuitously close to the maximum absorptivity of DNA. Increasing use is being made of medium and high pressure UV sources in water disinfection and this trend may extend to the food industry. These are not as efficient as the low pressure sources in emitting in the germicidal range but their higher power ratings

TABLE 122.5
UV Hormesis of Horticultural Produce

Crop (Variety)	Targeted Pathogen	UV Dose Range Investigated kJ m^{-2}	Optimal UV Dose kJ m^{-2}	Additional Details	Reference
Apple (Red Delicious)	*Penicillium expansum*	7.5	Not determined	Treated fruit stored at 24°C for 14 days. The earliest application of UV treatment (96 hours) before inoculating with *P. expansum* provided the best defence against disease. Combining UV irradiation with other disease prevention measures, harpin, chitosan and yeast antagonists *Candida saitoana* and *C. oleophilia* offered no advantages.	56
Cabbage seeds (Acc 16 Hybrid)	*Xanthomonas campestris*	1.3–7.5	3.6	Irradiation performed on cabbage seeds which were stored for up to 8 months. Improvements in quality and growth response observed at optimum dose. Disease resistance of treated seeds decreased with storage time.	57
Cactus Pear (Gialla)	Not specified	0.75	Not determined	UV treatment did not reduce the incidence of decay. Skin damage observed following irradiation.	58
Carrot (Caropak)	*Botrytis cinerea*	0.11–0.88	0.44–0.88	Both fresh and aged carrots were studied. Aged carrots had been stored at 1°C for 4 months. After irradiation carrots were stored at 1°C for 25 days. Only carrots that had been surface-wounded responded to UV treatment at 1°C but intact roots responded to treatment at 20°C. Treatment of fresh carrots gave higher resistance to storage rots. Exposure to UV induced 6-methoxymellein production.	59
Grape (Italia)	*Botrytis cinerea*	0.125–4.0	0.125–0.5	Irradiated grapes were stored at either 3°or 21°C. Grapes irradiated 24–48 hours before inoculating with *B. cinerea* showed a lower disease incidence than those inoculated immediately before irradiation. Doses above 1.0 kJ m^{-2} resulted in skin discolouration. Treatment within the optimum range did not significantly reduce the numbers of yeasts antagonistic towards pathogenic moulds.	60
Grapefruit (Star Ruby)	*Penicillium digitatum*	0.5–3.0	0.5	Quality and disease resistance determined after storage at 7°C for 4 weeks followed by 1 week at 20°C. Scoparone and scopoletin levels were increased at all UV doses. Rind browning and tissue necrosis occurred at UV doses >1.5 kJ m^{-2}.	61
Kumquat (Nagami)	*Penicillium digitatum*	0.2–15	1.5	Scoparone levels increased following irradiation at all UV exposures. After 2 weeks of storage at 17°C UV-treated fruit showed signs of damage, however at lower temperatures UV damage was practically absent even at the highest dose used.	62
Lemon (Eureka)	*Penicillium digitatum*	0–15	5	Irradiated fruit was stored in the dark at 17°C. UV was only effective in suppressing decay in fruit that had been irradiated at least 24 h before inoculation with *P. digitatum*. Increased levels of scoparone were found in irradiated fruits.	63
Mango (Tommys Atkins)	Not specified	4.9 and 9.9	4.9	Quality and disease resistance determined after storage at 5°C for 14 days followed by 7 days at 20°C. Treatment at 4.9 kJ m^{-2} resulted in improved appearance and texture of fruit. Irradiation induced spermidine and putrescine. The higher dose induced senescence.	64
Onion (Walla Walla)	*Aspergillus* spp., *Penicillium* spp., *Erwinia* spp.	0.44–19.10	7.33	Quality and disease resistance determined after storage at 20–25°C for four weeks. UV exposure was generally better than gamma or electron beam irradiation at	65

(*Continued*)

TABLE 122.5 (*Continued*)

Crop (Variety)	Targeted Pathogen	UV Dose Range Investigated kJ m^{-2}	Optimal UV Dose kJ m^{-2}	Additional Details	Reference
				reducing the incidence of disease and improvement in marketability and storage life. UV irradiation did not affect texture or flavour. Some UV doses induced sprouting.	
Orange (Biondo Comune, Washington Navel, Tarocco, Valencia Late)	Not specified	0.5–3.0	Not determined	Quality and disease resistance determined after storage at 7°C for 4 weeks followed by 1 week at 20°C. Peel quality was affected in all cultivars with the exception of Valencia L. Percentage of damaged fruit at the higher dosages decreased as the season progressed. UV irradiation at 0.5 kJ m^{-2} was effective in reducing decay development. The higher dose of 1.5 kJ m^{-2} was more effective but only in early harvested fruit. Concentrations of scoparone and scopoletin increased in all varieties with increasing dose.	52
Oranges (Shamouti, Valencia)	*Penicillium digitatum*	0.2–15	9.0	After 2 weeks of storage at 17°C UV-treated fruit showed signs of damage, however at lower temperatures UV damage was practically absent even at the highest dose used. Scoparone levels increased following irradiation at all UV exposures.	62
Peach (Elberta)	*Monilinia fructicola*	0.84–40	7.5	Exposure to UV delayed ripening, suppressed ethylene production and increased phenylalanine ammonia-lyase activity. Doses of 40 kJ m^{-2} increased susceptibility to brown rot. Irradiation resulted in increased numbers of the antagonist yeast *Debaryomyces hansenii* on the surface of the fruit.	66
Pepper (Bell Boy, Delphin)	Natural infections and *Botrytis cinerea*	0.22–2.20	0.88 for *Botrytis cinerea*	Fruit were stored at either 13° or 20°C following irradiation. All doses tested provided protection against natural infection. UV provided protection against *B. cinerea* only when artificial inoculation occurred after irradiation but not before. Two successive exposures at 0.44 kJ m^{-2} were equivalent to a single exposure at 0.88 kJ m^{-2}.	67
Potato (Superior)	*Fusarium solani* *Erwinia carotovora*	7.5–15	Not determined	Potatoes stored at 8°C for 3 months. Disease suppression increased with UV dose. Doses higher than 15 kJ m^{-2} associated with induction of sprouting and were not investigated.	68
Sweet Potato (Jewel)	*Fusarium solani*	1.3–20	3.6	Tubers stored for up to 8 weeks at 25−27°C. All UV exposures resulted in increased phenylalanine ammonia-lyase activity.	54
Tomato (Tuskegee 80–130, Floradade, Better Boy)	*Alternaria alternata* *Botrytis cinerea* *Rhizopus stolonifer*	1.3–40	3.6–7.5	UV doses of 3.6 and 4.8 kJ m^{-2} delayed ripening whilst doses of 40 kJ m^{-2} resulted in skin discolourization.	69
Tomato (Capello)	-	3.7–24.4	3.7	Study aimed at delaying senescence only. Treated fruit were stored at 16°C for 35 days. High UV doses caused abnormal browning of the surface of fruits. Treatment with doses of 3.7 kJ m^{-2} delayed ripening for 7 days. This correlated with increased amounts of putrescine in the fruits.	70
Zucchini Squash (Tigress)	Not specified	0.49–9.86	Not determined	Fruit sliced prior to irradiation and stored at 5° and 10°C for up to 18 days. Doses above 4.9 kJ m^{-2} retarded microbial growth. Treatment at the higher doses resulted in slight discolouration for fruit stored at 10° but not 5°C.	71

mean that greater overall outputs can be obtained. 'Doping' with e.g., metal halides can modify the spectral output of these sources.

Recent advances have led to the development of pulsed UV sources in which the duration of the pulse varies from nano- to milli-seconds. Comparisons with continuous i.e., conventional sources have shown that pulsed sources are more effective at decontaminating surfaces (77, 78). Similarly tunable excimer lasers have been used to disinfect food packaging (79). Plasmas, generated in vacuum or at atmospheric conditions also have potential for use in surface disinfection (80).

B. UV Irradiation Equipment

The history of treating water precedes that of food disinfection and it is therefore not surprising therefore that most innovations relate to the treatment of liquids and water at that. Novel thin film devices have been proposed for disinfecting liquids, such as cider and syrups, that have low UV transmittance (81) but such systems have low liquid throughputs. Solids are generally conveyed on belts past UV sources. These may be mounted on the walls of enclosures to create 'UV tunnels.' Shama *et al*. (82) proposed the hydraulic conveying of particulate matter over UV sources. Some innovative designs have been reviewed in relation to UV curing of inks on curved surfaces which could well have applications to food treatment (83).

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123 Aseptic Processing: Basic Principles and Advantages

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CONTENTS

I. INTRODUCTION

A. Components of an Aseptic Processing System

Aseptic processing consists of pumping, deaeration, heating of the product (also referred to as "pre-sterilization"), passage of the product through a holding tube wherein the product attains the required temperature for the required amount of time (achievement of commercial sterility), cooling of the product, possible holding of the product in an aseptic surge tank, and subsequent packaging of the product in a pre-sterilized container under aseptic conditions. This process results in a high quality shelf-stable product in a hermetically sealed container.

Pumping of the product at a constant rate (also referred to as "timing") is very important to ensure that all parts of the product receive uniform and the required amount of heat treatment. The most common type of pump used for this purpose is the piston type positive displacement pump wherein slippage (back flow of the product) is minimal. This type of pump delivers the product at a constant rate even if the pressure against which it is pumping fluctuates (for example, when fouling in the heat exchanger increases the pressure or when the opening and closing of valves causes changes in pressure). For certain fluid products, homogenization of the product may be required and this is achieved by using a homogenizer, which can also serve as the "timing" device.

Deaeration of the product removes excess air in the product. It is accomplished in a vessel maintained at a certain degree of vacuum by means of a vacuum pump. The product is fed into the vessel at 55–70°C through a nozzle at the center of the vessel. Vacuum is controlled to obtain a product flash of about 5°C. An internal spiral

condenser condenses vapors and other condensable gases. The deaerated product is discharged through the bottom and pumped to the heating section. This serves the purposes of increasing the rate of heat transfer during heating and cooling, maintaining the specific volume of the product in the holding tube (to avoid reduction in holding time due to expansion of product), maintaining a constant fill rate and prevention of foaming during packaging, and minimizing oxidation reactions within the product during storage. The deaerator is placed before pre-heating in cases where loss of volatiles at elevated temperatures is of concern and after pre-heating in other instances since it is easier to remove air at higher temperatures due to its expansion.

The high temperatures required for aseptic processing (125–145°C) are achieved by direct or indirect contact heat exchangers (usually with steam as the heating medium) and a back pressure valve (placed after the cooling unit) that maintains high enough pressure to prevent boiling (or flashing) of the product. The required back pressure for the process can be achieved by using a piston-type air actuated valve, diaphragm valve or pressurized tanks. Heating of the product can be accomplished by direct (steam injection of infusion) or indirect (tubular, shell and tube, plate, scraped surface, microwave, radio frequency, or ohmic) means. The choice of the type of heating and cooling equipment depends on a variety of factors such as type of product (acid or low-acid, viscous or non-viscous, fluid or particulate, heat sensitive or heat stable), potential for fouling, ease of cleaning, and as always the cost of the heat exchanger. An additional useful characteristic of the back pressure valve is that it also provides a dampening effect against the fluctuating or pulsating action of pumps used in aseptic processing.

The holding tube is an important part of the aseptic processing system since this is where the product receives the required time-temperature treatment in order to render the product commercially sterile. The holding tube is always inclined upwards (requirement from regulatory agency) with a vertical rise of at least ¼″ per foot length of the tube. This vertical rise ensures that the product completely fills the holding tube (with no air pockets) and that the product returns to the supply tank when the pump is shut off (thereby minimizing contamination issues).

An aseptic surge tank provides the means for product to be continuously processed even if the packaging system is not operational due to any malfunction. It can also be used to package the sterilized product while the processing section is being re-sterilized.

1. Historical Perspective

Historically, the first aseptic packaging of food (milk in metal cans) was done by Nielsen in Denmark in 1913. In 1917, Dunkley of the U.S. sterilized cans and lids by saturated steam and filled pre-sterilized product in it. In 1923, aseptically packaged milk from S. Africa reached a trade fair in London in perfect condition. The work of Olin Ball and the American Can Research Department laid the foundation of aseptic processing in the U.S. as early as 1927 when the HCF (heat, cool, fill) process was developed. This was followed by the Avoset process in 1942 (steam injection of the product coupled with retort or hot air sterilization of packages such as cans and bottles) and the Dole-Martin aseptic process in 1948 (product sterilization in a tubular heat exchanger, metal container sterilization using superheated steam at temperatures as high as 450°F since dry heat requires higher temperature than wet heat, followed by aseptic filling and sealing of cooled product in a superheated steam environment). The early 1960s was marked with the advent of a form-fill-seal package — tetrahedron package. The late 1960s saw the advent of the Tetra Brick aseptic processing machine and the late 1970s saw the advent of the Combibloc (blank carton) aseptic system. Soon, aseptic filling in drums and bag-in-box fillers were established. One of the major landmarks in the history of aseptic processing is the approval of use of hydrogen peroxide for the sterilization of packaging surfaces by the FDA in 1981. In recent years, a major break-through for the aseptic processing industry was in 1997 when Tetra Pak received a no-objection letter from the FDA for aseptic processing of low-acid foods containing large particulates.

2. Advantages and Disadvantages

Better product quality (nutrients, flavor, color, texture), less energy consumption, fewer operators, less space requirements, eliminating the need for refrigeration, easy adaptability to automation, use of any size package, use of flexible packages, and cheaper packaging costs are some of the advantages of aseptic processing over the conventional canning process. It also does not have the problem of texture changes associated with frozen products and increased permeability of packaging material such as ethylenevinylalcohol (to oxygen) due to the high temperature as in retortable pouches. Some of the reasons for the relatively low number of aseptically processed products include slower filler speeds and higher overall cost. Aseptic processing also requires better quality control of raw products, better trained personnel, and better control of process variables and equipments. It is also subjected to stringent and extensive validation procedures. Some of the disadvantages of aseptic processing include increased shear rates, degradation of some vitamins (some vitamins are stable at pasteurization temperatures but not at sterilization temperatures), separation of solids and fats, precipitation of salts, and change in flavor or texture of the product (steam injection followed by flash cooling may eliminate off-flavors) relative to what consumers are accustomed to.

Though aseptic processing could potentially result in better product quality, one has to keep in mind that several chemical changes take place during temperatures encountered during aseptic processing which do not occur under normal processing conditions. Some of these chemical changes include age gelation, browning reactions, oxidation reactions, and changes in pigments and have been described in detail in references (1, 2).

Due to some of the stringent regulatory requirements of aseptic processing, many processors adopt an aseptic process, but package it in non-aseptic containers. This results in products that are called "extended shelf-life products." Such processes are easier to adopt, require less monitoring (since the resulting product-package combination does not need to be sterile), and are easier to file with regulatory agencies. One such process involves ultra-pasteurization of milk wherein extended shelf-life can be obtained.

Notwithstanding the problems associated in producing aseptically processed foods, several companies have adopted this technology. Some of the products that are aseptically processed include fruit juices, milk, condensed milk, coffee creamers, puddings, soups, butter, gravies, and jelly. Some of the companies that deal with aseptic processing and packaging equipment are International Paper, Tetra Pak, Combibloc, Elopak, Cherry Burrell (tubular: Unitherm; plate: Thermaflex; SSHE: Thermutator; steam injection: Aseptic direct steam incorporation), Alfa Laval (Plate: Steritherm; SSHE: Contherm; steam followed by SSHE: Viscotherm; steam injection: VTIS — Vacu-therm instant sterilizer; corrugated tube: Spiraflo), ASTEC, VRC, APV (Plate: Juicematic; Plate for low-acid: Super ultramatic; steam injection: Uperizer), FranRica, Benco, Scholle, Bosch, and Metal Box.

B. Important Facets of Aseptic Processing

Some of the important facets of aseptic processing include fluid mechanics (residence time and residence time distribution of the fluid elements and particles in the product), kinetics (of microbial destruction, enzymatic inactivation, and nutrient destruction), and heat transfer (transfer of heat from the heating medium to the liquid and particulate portions of the product and accumulation of F_0 or F-value at 121.1°C and z = 10°C, where z is the temperature change required for an order of magnitude change in the decimal reduction time, D).

1. Fluid Mechanics

The Food and Drug Administration (FDA) only credits heat treatment experienced in the holding tube, which makes its design critical. The velocity profile of the fluid in the holding tube is affected by the degree of its deviation from the behavior of a Newtonian fluid. The degree of deviation is characterized by the flow behavior index, n', for Ostwald-de-Waale fluids. For a Newtonian fluid ($n' = 1$) flowing under laminar conditions in a straight tube of circular cross-section, the maximum velocity occurs at the center of the holding tube and its magnitude is twice the average velocity of the fluid. For pseudoplastic fluids ($n' < 1$), differences between the maximum and average velocity becomes smaller as n' decreases. In other words, the velocity profile becomes flatter. For the extreme case ($n' = 0$), the plug flow profile is attained. However, for most cases ($n' > 0$), the maximum velocity occurs at the axis of the tube, which means that the minimum residence time corresponds to the residence time of particles located along the center-line of the tube. Consequently, these particles receive the least amount of heat treatment. Thus, the holding tube length required to achieve the required F_0 value (time-temperature effect) can be calculated based on the knowledge of this minimum residence time, but this will result in an over-processed product. This is where the residence time distribution (RTD) of the particles comes into the picture. To understand RTD, we begin with the following equation, which describes the velocity profile for flow of a Newtonian fluid under laminar conditions in a pipe of circular cross-section:

$$u = 2\,\bar{u}\,[1 - (r^2/R^2)] \qquad (123.1)$$

Where u is fluid velocity in m/s, $\bar{u}$ is average fluid velocity in m/s, r is the radial distance from the center of the tube in m, and R is the radius of the tube in m.

Thus, it can be seen that different fluid elements (at different radial locations) spend different amounts of time in the tube. For instance, a fluid element traveling at the center of the tube will travel twice as fast as the average fluid element. The distribution of times spent by various fluid elements within the tube is referred to as the residence time distribution (RTD) of the fluid elements. Similarly, when different particles are flowing through the tube, they spend different times in the tube, and the distribution of these times is the RTD of the particles. The RTD of the particles depends a great deal on the RTD of the fluid. It also depends on flow rate and viscosity of the carrier medium, and also the size, density, and concentration of particles. Analysis of particle RTD is relatively simple when there is only one type of particle in a system. However, when different types of particles (especially particles of different densities) are present in a product, the flow behavior is quite different from the situation when they are each present as the only particle type in suspension. For instance, in a mixture of two types of particles, denser particles (which traveled slowly at the bottom of the tube when present alone) could be sped up by foreign particles due to collisions, and in turn, the foreign particles could get slowed down. Thus, an analysis has to be performed for each combination of particle types present in a system and direct inferences cannot be made from RTD of each particle type separately.

The existence of a RTD for the particles results in some particles receiving more heat treatment than others in the holding tube. From a safety standpoint, the fastest particle is what is of concern and the holding tube length is based on the fastest particle residence time. Thus, it can be seen that if the particle RTD is narrow, the quality of the product would be high since the difference between the fastest and slowest particle residence time is not very high. The wider the RTD of the particles in the holding tube section, the more non-uniform the process. One of the techniques that can be used to narrow the RTD of the fluid and particles is the use of helical tubes.

When a non-Newtonian (power-law) fluid flows through a straight tube under laminar flow conditions, the velocity profile is given by the following equation.

Non-Newtonian fluids:

$$u = \frac{3n' + 1}{n' + 1}\,\bar{u}\left[1 - \left(\frac{r}{R}\right)^{\frac{n'+1}{n'}}\right] \qquad (123.2)$$

Thus, for a pseudoplastic fluid ($n' < 1$), the maximum fluid velocity is given by:

$$u_{max} = \frac{3n' + 1}{n' + 1}\,\bar{u} \qquad (123.3)$$

Hence it can be seen that the maximum velocity in the case of a pseudoplastic fluid is less than twice the average fluid velocity. Thus, the RTD of the fluid is narrower for a pseudoplastic fluid in comparison with that for a Newtonian fluid. Hence, the RTD of particles is also narrower when the carrier medium is a pseudoplastic fluid. Further details of RTD of fluid elements and particles have been presented by (3).

The average fluid velocity can be calculated once the volumetric flow rate of the product is known. To determine the distribution of fluid residence times, salt injections, dye tracers, and fine particles are used. Magnetic resonance imaging can also be used under certain circumstances to obtain a fluid flow profile. Fluid flow profiles, though important, are usually not the target, since the species of concern are the slow-heating particles. Particle residence times, residence time distributions, and velocities can be determined by using a stop-watch, digital image analysis, LASER-Doppler velocimetry, and also with the aid of magnetically tagged particles.

2. Kinetics

Sufficient heat has to be applied to a food product to inactivate microorganisms that cause food spoilage and food poisoning, and inactivate enzymes. However, the color, texture, flavor, and nutrients within the food must not be destroyed to an unacceptable level. This is where optimization of the process comes into play. Several combinations of time and temperature could be used to destroy the microorganisms of concern. Out of these, the combination that results in the least nutrient destruction would be the desired combination. In order to arrive at this combination, a thorough understanding of the kinetics of microbial and nutrient destruction is essential.

The heat resistance of microorganisms is affected by several factors. Some of these factors include water activity, pH, lipids and oily materials, dielectric constant, ionic species, ionic strength, oxygen level, organic acids, and antibiotics. Methods to measure heat resistance in the temperature range of 60–135°C are the end-point method (a number of replicate containers with a known number of spores are heated successively for longer periods of times until no survivors are obtained by culturing each container) and the multiple-point method (a batch of spores is heated continuously and samples are withdrawn at selected intervals, followed by determination of the number of survivors).

The most general equation representing the kinetics of microbial inactivation, enzymatic inactivation, nutrient destruction, or other chemical reactions is

$$-\frac{dc}{dt} = k_n c^n \qquad (123.4)$$

with c being the concentration of the reacting species at time t, k_n being the specific reaction rate, and n being the order of the reaction. To determine the order of the reaction, the logarithm of the equation is taken on both sides and a graph is plotted between $\ln(-dc/dt)$ versus $\ln(c)$. The intercept of the graph is $\ln(k_n)$ and the slope is the rate of the reaction.

$$\text{Zero order reaction } (n = 0): -\frac{dc}{dt} = k_0 \qquad (123.5)$$

$$\text{Integrating, we get: } c_0 - c = k_0 t \qquad (123.6)$$

Caramelization of sugar and degradation of vitamin C in model meat systems are examples of reactions that fall under this category.

$$\text{First order reaction } (n = 1): -\frac{dc}{dt} = k_1 c \qquad (123.7)$$

$$\text{Integrating, we get: } \ln(c/c_0) = -k_1 t$$

$$\text{This can also be written as: } c/c_0 = e^{-kt} \qquad (123.8)$$

Most reactions, including microbial inactivation, enzymatic inactivation, and nutrient destruction, fall under this category.

$$\text{Second order reaction } (n = 2): -\frac{dc}{dt} = k_2 c^2 \qquad (123.9)$$

$$\text{Integrating, we get: } 1/c - 1/c_0 = k_2 t \qquad (123.10)$$

Destruction of thiamin in milk falls under this category.

Other orders of reaction (fractional): Reactions involving the color ($n = 1.31 \pm 0.18$), texture (1.13 ± 0.20) of peas and the texture (0.36 ± 0.17), and appearance (0.44 ± 0.16) of beans fall under this category.

The more common way of representing the rate of microbial destruction is through the use of decimal reduction time (D). The relationship between the rate of a reaction and decimal reduction time is

$$N/N_0 = e^{-kt} \tag{123.11}$$

$$\log_{10}(N/N_0) = -kt/2.303$$

$$\text{with } D = 2.303/k \tag{123.12}$$

if k is represented in s^{-1} and D in s.

Thus, $N/N_0 = 10^{-t/D}$ where N_0 and N are the initial and final number of microorganisms, respectively, and t is time.

The D value determined at a reference temperature (T_{ref} or T_r) is denoted by D_{ref} or D_r.

The ratio of D_{ref} to D is referred to, as the lethal rate.

The effect of temperature on rate of reaction can be described by one of the following two models:

1. Thermal death time (TDT) method (D-z model): TDT is the time required for total destruction of a microbial population or the time required for destruction of microorganisms to an acceptable level. The plot of D (logarithmic scale) versus T is referred to as the "phantom" TDT curve (or the thermal resistance curve). The slope of this curve is "$-1/z$."

$$\log(D_1/D_2) = (T_2 - T_1)/z \tag{123.13}$$

   The points on this curve correspond to the combination of time and temperature that results in 90% reduction in microbial count. A similar curve with either 100% reduction in microbial count (based on experiments) or acceptable levels of reduction in microbial counts is referred to as the TDT curve and the time on the curve at a given temperature is the TDT at that temperature. Note that the slope of this curve is also "$-1/z$."
2. Arrhenius kinetics method (k-E model): The basic equation is as given below.

$$k = A\, e^{-E/R_gT} \tag{123.14}$$

   where A is a pre-exponential factor, collision number, frequency factor (s^{-1}), E is the activation energy (J/kg-mol), and R_g is the universal gas constant (= 8314 J/kg-mol-K).

Writing the above equation for two different temperatures, yields:

$$\frac{k}{k_{ref}} = e^{-\frac{E}{R_g}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \tag{123.15}$$

The activation energy is the minimum energy which molecules must have for the reaction to occur and the exponential term "e^{-E/R_gT}" is the fraction of molecules that collectively have the minimum energy.

The above equation can be simplified to:

$$E = 2.303\, R_g(T)(T_{ref})/z \tag{123.16}$$

For unimolecular reactions A varies from 10^{14} to $10^{20}\,s^{-1}$, for bimolecular reactions it varies from 10^4 to $10^{11}\,s^{-1}$, and intermediate values for chain reactions. For heat resistant bacterial spores, A is extremely large, of the order of 10^{30}–$10^{60}\,s^{-1}$ and E can be up to 500 kJ/mol. For *Clostridium botulinum* spores, $A = 2 \times 10^{60}\,s^{-1}$ and E = 310.11 kJ/mol-K for the range of temperatures from 100°C to 150°C (4).

It is assumed that A and E are independent of temperature, but this may not be the case always. There are several approaches available to correct this assumption or approximation — absolute reaction rate theory and quotient indicator method are two of them.

Once the time-temperature profile within a product is determined, the degree of lethal treatment delivered is determined by determining the F-value of the process. The F-value of a

$$F = \int_0^t 10^{\frac{T - T_{ref}}{z}}\, dt \tag{123.17}$$

process is the time (in mins) at a reference temperature that would produce the same degree of microbial destruction as in the process under consideration. It is computed as follows.

For a constant temperature process, the above equation reduces to

$$F = 10^{\frac{T - T_{ref}}{z}}\, \Delta t \tag{123.18}$$

In aseptic processing, the reference temperature is usually chosen as 121.1°C (250°F) and the z value chosen is 10°C (or 18°F). The F-value computed with these values of T_{ref} and z is referred to as the F_0 value of the process.

The use of a time-temperature integrator (TTI) as an alternative to temperature measurement or microbiological testing for process evaluation (determination of F-value) is becoming popular. A TTI can be an enzyme such as amylase or peroxidase that denatures (an unwinding of the structure) as it is heated. If the reaction kinetics of the

temperature-induced denaturation match those of the microbial death kinetics for the target species, it is possible to use such TTIs as non-biological markers of a process.

3. Heat Transfer

Heat is transferred from the heating medium (steam or hot water) to the product in the heat exchanger. For liquid foods, the rate of increase of product temperature depends on the overall heat transfer coefficient between the heating medium and the product. For particulate foods, there is an additional factor — heat from the liquid portion of the product has to be transferred to the particulates. This is affected by the convective heat transfer coefficient between the particles and the fluid and also thermal conduction within the particulates (governed by the thermal diffusivity of the particles). Efforts have been geared towards decreasing fouling (deposition of the food material on the heat exchanger) and increasing the rate of heat transfer between the heating medium and the product by not only minimizing fouling but also by new designs of heat exchangers and the change of processing parameters.

During aseptic processing, the FDA does not credit lethality accumulation (accumulation of F-value) within a product in the cooling section. This is because particulates could possibly break up in the cooling section and thus, due to their smaller size, cool rapidly, thereby not accumulating the required F-value. Also, due to the uncertainties in the temperature distribution within the product in the heat exchanger, lethality credit is not given in this section. Lethality credit in the heat exchanger could possibly be included if the time-temperature history within the heat exchanger could be determined or modeled conservatively.

Some of the techniques used to determine the temperature distribution within a product include the use of thermocouples, resistance temperature detectors, data tracers, infrared imaging, thermochromic dyes, magnetic resonance imaging, thermoluminescent markers, and magnetic particles. The non-invasive techniques among these are of more interest in aseptic processing. However, each of those techniques has their limitations in determining the center temperature of particles under unobstructed flow conditions. Thus, researchers are focusing on developing reliable techniques that can be used to non-invasively determine the internal temperature of flowing particles.

Further details of the heat transfer aspects of aseptic processing have been described in detail by (5). This includes discussion of convective heat transfer coefficient, steam quality, dimensionless numbers governing heat transfer, natural (free) and forced convection, transient heat transfer within particles, hydrodynamic and thermal entrance lengths, heat transfer coefficient in straight tubes, heat transfer coefficient in helical tubes, heating media and equipment, co-current and counter-current heat exchangers, governing heat transfer equations and energy balance, fouling and enhancement of heat transfer, and techniques to estimate the temperature history of a product.

C. Processing Details

Some of the important processing and packaging details to be considered during aseptic processing and packaging of liquid and particulate foods are discussed in this section.

1. Issues to be Dealt with for Liquid and Particulate Foods

In aseptic processing, if we ensure that the slowest heating point (critical point) within the product is sufficiently processed, the entire product will be sufficiently processed. The critical point for a fluid product is generally at the center of the product. There are exceptions to this — for example, for flow in a helical tube, the fastest fluid element is located away from the center of the tube (towards the wall of the tube). The critical point with a particulate product is usually the center of the particle that receives the least heat treatment (critical particle). The critical particle in a system containing only one type and size of particle is the fastest particle in the holding tube. In a multi-particle product, the critical particle is the slowest heating particle, which is not necessarily the fastest particle since slower particles may potentially have a lower thermal diffusivity than the fastest particle. Mathematical modeling (with conservative assumptions) of the process would be one way to narrow down the choice of the critical particle.

2. Product Heating and Cooling

Heating of the product is the first step towards delivering the required F-value to the product. For fluid products, a plate, tubular, scraped surface, steam injection/infusion, and volumetric heating mechanisms (ohmic, microwave, and radio frequency) can be used. It has been shown that the use of higher temperatures for shorter times is the generally preferred technique from the standpoint of nutritive value of the product. Thus, techniques to rapidly heat products are being sought.

Product cooling is the final heat exchange operation prior to filling into aseptic packaging containers. Since the product temperatures achieved in aseptic processing are typically higher than in other thermal sterilization methods, and no creditable microbial lethality is accumulated in the cooling section (as opposed to the canning where creditable lethality can also be accumulated during the cooling stage), there is a need for rapid reduction of product temperature during cooling in order to minimize the negative effects of high temperature on various product quality characteristics such as nutrient, color, flavor, and texture retention. This reduction of temperature can be

performed, using appropriate coolant fluids, in the same types of heat exchangers listed for product heating (plate, tubular, and scraped surface heat exchangers), with the exception of steam injector equipment and volumetric heating equipment. For products where water is added through direct steam injection, flash or vacuum cooling can be employed to remove the added water as well as to effect very rapid cooling by boiling off the water under reduced pressure. The removal of the sensitive heat of evaporation causes the product material to cool to ambient or final filling very rapidly, typically within seconds.

Indirect vacuum cooling equipment employs evaporating environment of cooling fluid to surround the flow-through tubes of process material to effect rapid indirect, vacuum-driven cooling of product.

3. Sterilization of Equipment

Sterilization of the processing, packaging, and the airflow system prior to processing are of utmost importance. This is what is referred to as pre-sterilization. Pre-sterilization of the air system is done by high efficiency particulate arresting (HEPA) filtering or incinerated air. For equipment, it is accomplished by steam, hydrogen peroxide, or other disinfectant solutions. For filling lines, pre-sterilization is done with steam or water at high pressure. The recommended heating effect for pre-sterilization (using hot water) of the processing equipment for low-acid foods is the equivalent of 250°F for 30 minutes. The corresponding combination for acid or acidified products is 220°F for 30 minutes. This often involves acidification of the water (to below a pH of 3.5 for acid products) used for sterilization. Pre-sterilization of an aseptic surge tank is usually done by saturated steam and not hot water due to the large volume associated with the surge tank.

Once the product is processed, the system has to undergo a clean in place (CIP) operation. The CIP cycle for low-acid foods involves the use of hot water, alkali, hot water, acid, and hot water sequentially. The CIP cycle for high-acid foods is hot water, alkali, and hot water sequentially. A detailed description of fouling, cleaning, and disinfection has been presented by (6).

Sterilization of the food contact surface of packaging material is the next point of consideration. For non-sterile acidic products ($pH < 4.5$), a 4D process is required. For sterile, neutral, low acid products ($pH > 4.5$), a 6D process is required. However, if there is possibility that *C. botulinum* is able to grow in the product, then a full 12D process is required. It has been suggested that only 3% of the total number of microorganisms on the package surface are spores. An upper value of 1,000 microorganisms per m^2 (30 spores per m^2) has been assumed for plastic films and paperboard laminates on reels, and 3,000 microorganisms per m^2 (90 spores per m^2) for prefabricated cups.

Some of the techniques used for sterilization are listed below.

a. Radiation

UV-C Radiation (250–280 nm)

Optimum effectiveness at 253.7 nm; applicable only to smooth, even surfaces.

Infrared Radiation

Applicable only to smooth, even surfaces (Al lids coated with plastic laquer).

Ionizing Radiation

Co-60 or Cs-139; 25 kGy (2.5 Mrad); 100 keV of electron beam (empty sealed containers such as bag-in-box).

b. Heat

Saturated Steam

165°C and 600 kPa for 1.4 s (cups) and 1.8 s (lids); disadvantages include need for high pressure, removal of air (to promote heat transfer), and possible dilution of product as steam condenses.

Superheated Steam

220–226°C for 36–45 s.

Hot Air

315°C (surface temperature reaches 145°C for ~3 min); suitable only for acidic products.

Hot Air and Steam

Hot air is blown through a nozzle in such a way that the base and walls are uniformly heated; used for cups and lids made of PP which is thermally stable up to 160°C.

Extrusion

During extrusion of plastic granules prior to blow molding of plastic containers, temperatures of 180–230°C are reached for up to 3 min. However, because the temperature distribution inside the extruder is not uniform and the residence time of the plastic granules varies considerably, it is not possible to guarantee that all particles will achieve the minimum sterility. It has been suggested that extrusion results in a 3–4D process. Thus, aseptic filling into extruded containers should be used only for acidic products. For low-acid products, a hydrogen peroxide treatment is usually done.

c. Chemical Treatment

Hydrogen Peroxide

Dipping, spraying, rinsing processes, or combined with UV-C or heat at least 80°C and 30% concentration is required; residual peroxide should be less than 100 ppb at time of filling and must decrease to 1 ppb within 24 hours. Since it is hard to detect peroxide in foods, containers filled with water are run through the machine initially.

Peracetic Acid

Produced by oxidation of acetic acid by hydrogen peroxide; effective even at 20°C (1% solution will eliminate

7–8 logs of the most resistant spores in 5 min at 20°C; maximum usable temperature is 40°C).

Ethylene Oxide

It is a toxic gas and can penetrate porous materials; thus it is used for pre-sterilization of paperboard-based packaging materials (particularly preformed carton blanks which are to be assembled in an aseptic filler).

Verification of sterilization is done by inoculation of the surface of the web, cup, or lid stock with the proper concentration of the test organism and allowing this to dry. The system is then run as in a commercial run and the finished containers are filled with an appropriate growth medium and observed for growth. Two of the most important factors affecting the success of the tests are the choice of the indicator organisms and the physical state of the microorganisms used. The indicator organisms used are: *B. stearothermophillus* strain 1518 (for superheated steam, peroxide + steam, and extrusion), *B. polymyxa* PSO (for dry heat), *B. subtilis* strain A (for peroxide + UV), *C. sporogenes* PA 3679 (for ethylene oxide), and *B. pumilus* (for gamma radiation).

4. Heat Exchange Equipment and their Suppliers

The various types of heat exchangers used in heating and/or cooling products include plate, tubular, scraped surface, microwave, radio frequency, and ohmic heating devices. Each of these devices has their advantages, disadvantages, and range of applicability. Some of the systems that handle the liquid and solid portions of a particulate food separately are the Jupiter system, rotaholder, and the fluidized bed system (7).

a. Plate Heat Exchanger

Numerous companies produce plate heat exchangers. Some of the well-known major suppliers of integrated aseptic processing lines including plate heat exchangers are Tetra Pak Inc (Vernon Hills, IL, USA), Alfa Laval, Inc. (Glen Allen, VA, USA), FMC FoodTech (FranRica, Madera, CA, USA), Waukesha Cherry-Burrell (Delawan, WI, USA), Invensys/APV (New York, NY, USA), and Stork Food and Dairy Systems (Gainesville, GA, USA).

b. Tubular Heat Exchanger

In addition to companies listed above, there are several companies producing Tube in tube and tube in shell heat exchangers. They are Feldmeier Equipment Inc. (Syracuse, NY, USA) and Rossi & Catelli (Parma, Italy).

c. Helical Heat Exchanger

VRC Co. Inc. (Cedar Rapids, IA) and GEA-AG (Bochum, Deutschland).

d. Steam Injection and Steam Infusion Unit

Most of the major heat exchanger producer companies listed under plate heat exchanger also offer steam injection direct heaters in various configurations.

e. Scraped Surface Heat Exchanger (SSHE)

Major suppliers of scraped surface heat exchanger equipment are the same equipment companies providing the integrated aseptic processing systems.

f. Continuous Flow Volumetric Heaters

Radio Frequency Heater

Radio Frequency Co., Inc. Millis, MA.

Continuous Flow Ohmic/Electro Heater

Invensys/APV (New York, NY, USA) and Raztek (Sunnyvale, CA, USA).

Continuous Flow Microwave Heaters

Industrial Microwave Systems (Morrisville, NC, USA), Keam Holdem Associates (Auckland, NZ), and Armfield Limited (Ringwood, England).

D. Packaging Details

1. Types of Packaging Systems

Modern aseptic packaging units for foods cover an extensive range of materials, shapes, and sizes. For individual and family-size packages, traditional paperboard laminate package types are still the dominant form, with reclosable lids and pouring spouts introduced more recently. Some of the well known companies providing the packaging equipment and materials are Tetra, SIG Combibloc and Elopak. These types of packages — thermoformed polymer cups and pots with peel-off laminate layer tops and, depending on package size, re-closable lids are also well known and on the market for a considerable time — the packaging units and materials are provided by Robert Bosch Corporation. Individual serving size bottles with re-closable snap-on or screw-on lids made of high density polyethylene, polyethyleneterepthalate, or other polymers have also been introduced to the market by companies like Sidel, Krones AG, and Stork. Production of fluid products and beverages aseptically packaged into conventional packages such as glass bottles and aluminum cans has also been increasing during the last several years.

Multi-layer laminate bags and pouches in forms from single serving mini-pouches, stand-up pouches and bags fitted with various pouring and dispensing closures and spouts are also on the market. Small 2–3 oz packages to 1–3 gallon bag and bag-in-box package types for institutional use to 50–60 gallon bags in boxes for industrial ingredient and raw material use are provided by Liqui-Box, Rapak, Astepo, Scholle, etc.

Aseptic packaging systems are generally classified into the following categories.

a. Can Systems

Pioneered by Martin in the late 1940s, the first system was commissioned by Dole Corp. (CA) in 1950 for soups. It uses superheated steam at 225°C for up to 40 s to sterilize can and ends, with temperature not to exceed 232°C since

tin flow underneath the enamel can occur, resulting in blister formation during seaming of the lid (the lining compound is still at ~220°C and is plastic; thus, the seamed can should be transported in vertical position for at least 15 s after seaming to allow compound to settle and hermetically seal the can). For composite cans consisting of a spirally wound body made from laminations of foil, plastics, and paper with metal ends, hot air at 143°C for 3 min is used to sterilize the packaging materials as steam would cause swelling of the paper layers. The Dole, Serac, and Remy systems are examples of systems that fall under this category.

b. Bottle Systems

Glass

Saturated steam or dry heat (when dry heat is used, extended cooling by sterile air is required to minimize thermal shock when cool product is filled in it; no commercial unit, yet).

Plastic

Non-sterile bottles: After blowing, the plastic bottles are conveyed into a sterile chamber which is kept at a slight over-pressure of sterile air. The bottles are inverted and sprayed inside with hydrogen peroxide and passed through a hot air tunnel to evaporate the residual peroxide. The bottles are rinsed with sterile water and then filled. A chemically sterilized, heat sealable closure such as a plastic film or cap is then applied.

Sterile Blown Bottles

Bottles are extruded, blown with sterile air, and sealed under conditions that ensure internal sterility of the container. The sealed bottles are introduced into a sterile chamber (maintained at a slight positive pressure) where the outside surfaces are sterilized by hydrogen peroxide sprays. The closed top of the bottle is cut away, the neck trimmed, the bottle filled, and a foil cap or heat sealable sterile closure applied.

Single Station Blowing, Filling, and Sealing

This is a complex system. The separate operations of parison extrusion, blow molding, bottle filling, and sealing all take place in sequence in a single mold. Sterility of the inside surface of the container is ensured by the high temperature (164 to 234°C) of the plastic material during extrusion of the parison, and the use of sterile air for blowing. After filling, the tube projecting from the bottle mold is vacuum-formed or sealed with jaws into a cap which closes the bottle. No special arrangements to ensure sterility are required since the filling and sealing are carried out within the closed mold.

c. Sachet and Pouch Systems

Form-Fill-Seal Systems

A vertical form-fill-seal machine operates in a sterile chamber. The packaging material is passed through hydrogen peroxide and then drained and dried. The Asepak, Impaco, DuPont, Prodo-Pak, and Thimmonier systems fall under this category.

Layflat Tubing

This system uses a blown film polymer in the form of a layflat tubing so that only a transverse seal is required to form the bag. It is assumed that the inside of the tubing is sterile due to the temperature achieved during the extrusion process. The tubing is fed from the reel into a sterile chamber in which an over-pressure of air is maintained. The sachets are sealed at the bottom, cut, and moved into a filling station. After filling, they are sealed at the top and leave the chamber through a water seal.

d. Cup Systems

Preformed Plastic Cups

The cups are fed onto a conveyor which is inside a sterile tunnel supplied with sterile air. The cups are sprayed with hydrogen peroxide and after about 3 s, the solution is removed with compressed hot air at ~400°C with the inside surface of the cups reaching ~70°C which completes the surface sterilization and reduces the peroxide residue to acceptable levels. The cups are then filled and sealed with aluminum foil (sterilized by peroxide with residue removed by heat) with a thin coating of a thermoplastic to provide heat sealability. The Metal Box, Gasti, Crosscheck, Hamba, Ampack, and Remy systems fall under this category.

Form-Fill-Seal Cups

The plastic material (usually polystyrene) in the form of a web is fed from a roll into a thermoformer. Sterilization of the web is done prior to forming using a hydrogen peroxide bath. It then passes through a tunnel where it is heated to 130–150°C to prepare it for thermoforming. Mechanical force and compressed air is used to form the container in a water-cooled mold below the web.

e. Carton Systems

Form-Fill-Seal Cartons

The packaging material is supplied in rolls which have been printed and creased (for ease in the forming process). A polyethylene (PE) strip is sealed to one edge and the packaging material sterilized using a wetting system or a deep bath system. The sterilized packaging material is fed into a machine where it is formed into a tube and closed at the longitudinal seal by a heat sealing element. In this process, the PE which was added prior to sterilization is heat sealed across the inner surface of the longitudinal seal to provide protection of the aluminum and paperboard layers from the product which could corrode or swell the layers if such a strip were absent. Product is then filled into the tube and a transverse seal made below the level of product, thus ensuring that the package is completely filled. The Tetra-Pak and International Paper systems fall under this category.

Prefabricated Cartons

In this method, prefabricated carton blanks are used, the cartons being die-cut, creased, and the longitudinal seam

completed at the factory of origin. The cartons are delivered to the processors in lay-flat form, ready to be finally shaped in the filler and the top and bottom seams formed and bonded. Stacks of blanks are loaded into a magazine from which they are individually removed by suction pads, opened up into a rectangle, and placed on a mandrel. PE at the bottom of the carton is softened by hot air. The bottom is then folded by transverse and longitudinal folders and sealed. The top is then pre-folded. All of this takes place in a non-sterile zone. The inside surface is then sterilized by peroxide in a sterile zone (over-pressure of sterile air). The carton is then filled, closed, and heat sealed.

f. Bulk Packaging Systems

Metal Drum

Two major systems are in use and both use a 55 gallon metal drum constructed from steel with an electrolytically coated tin lining outside. The ends are double-seamed onto the body of the drum during manufacture and filling takes place through a threaded hole in which a cap is swaged after filling. The Scholle, FranRica, and Cherry-Burrell systems fall under this category.

Bag-in-Box

In this system, the product is filled into a plastic bag which when full is put into an outer container such as a drum or a paperboard box. For large containers, filling occurs after the bag is placed in the box. The Scholle, FranRica, Liquibox, and ELPO systems fall under this category.

Further details of aseptic packaging systems are described in reference (8). In addition, details of some of the "FDA-accepted" low-acid packaging systems have been presented in reference (9).

2. Testing of Package Integrity

Testing of the integrity of aseptic packages is usually done by one of the following methods.

a. Destructive Methods

Teardown

The flaps of the package are unfolded and pressure applied to the package to check the tightness of the transverse seals. The quality of the transverse and longitudinal seals is determined by carefully pulling apart the seals — if the seal is good, the PE layers will be removed and the aluminum foil laid bare in the sealing zone.

Electrolytic Test

This test is based on the principle that a tight plastic container is an electrical insulator. By introducing an electric potential across a brine-filled package which is partially immersed in a brine solution, the existence of holes in the package can be determined. Positive tests are generally followed by a dye test for confirmation.

Dye Test

After rinsing with water and drying, a solution of 0.5% Rhodamine B in isopropanol is applied to the critical areas of the package including the longitudinal and transverse seals. The carton is then allowed to develop for 5 minutes and dried in a warm cabinet overnight. The flaps of the package are unfolded and the dye coated paper removed and examined for ink penetration. Any sign of the pink ink indicates the presence of holes in the PE layers.

b. Nondestructive Methods

These include visual inspection, computer-aided video inspection, and automatic profile scanning.

c. Biotest Methods

The package is filled with a nutrient broth, sealed, and placed in contact with a medium infected with a test organism. After contact for a certain period of time, the package is placed in an incubator and microbial growth is assessed after an appropriate period of time.

E. Issues Related to Process Validation

Thermal process validation involves three stages: process establishment, lethality assurance, and record keeping. Process establishment involves considerations related to product formulation and properties, initial temperature, container size and shape, location of thermocouple, critical point, container stacking, retort controls, and steam and water controls. Lethality assurance involves comparison of the actual lethality delivered to that based on the scheduled process. Record keeping involves maintenance of full records of the process history of all production runs which contain the details of all critical factors related to the scheduled process.

In aseptic processing, biological validation tests are performed at various stages of the process — just after start-up, during the middle of the run, and just before shut-down. These tests account for variations during the process and also for factors such as fouling. The validation tests are conducted at different temperatures to document a positive/negative result at the end of the process. This will aid in determining the minimum allowable process temperature that will result in a safe process. Microbiological validation tests are done using PA 3679 inoculated within alginate particles. Care should be taken to ensure that the spores do not leach out into the fluid. If the target for the process was a 5D process, and an initial load of 10^5 spores per particle is used, a final count of <1 would indicate a safe process. The decimal reduction time of the organisms used is determined by means of thermal death time studies. Based on all of these tests, a process is designed and finally verification of the established process has to be conducted. During this process of verification, comparisons are made between actual temperatures and lethalities to the predicted temperatures and lethalities in

order to ensure that the mathematical model developed (details of mathematical models have been provided by (3)) results in a conservative prediction of process lethality.

Once verification is successful, all the process and system parameters are noted down and care should be taken to ensure that these parameters remain within an acceptable range. Some of the parameters include hydration time, mixing/batching time, temperatures at various locations, product flow rate, back pressure, and product properties. The final step in commercialization of the product involves process filing with the FDA using form 2541c. A comprehensive overview of the procedures and processes involved in process filing for a product such as the one discussed above has been given in a report prepared based on the workshops organized by the Center for Advanced Processing and Packaging Studies (CAPPS) and the National Center for Food Safety and Technology (NCFST) — (10) and the detailed discussion of the form has been presented in reference (3).

At pH values below 4.6, processes are aimed at controlling the survival and growth of spore-forming organisms such as *B. coagulans*, *B. polymyxa*, *B. macerans*, and butyric anaerobes such as *C. butyricum* and *C. pasteurianum*, but not *C. botulinum*. An $F_0 = 0.7$ min is generally regarded as adequate for this. NFPA suggests $F^{8.3}_{93.3} = 10$ min when pH is between 4.3 and 4.5; $F^{8.3}_{93.3} = 5$ min when pH is between 4.0 and 4.3. Below a pH of 3.7, processors are concerned with the control of non-sporing bacteria, yeasts, and molds. They can be generally controlled by heat processes with temperatures below 100°C.

Aseptic process validation requirements differ depending on the country of marketing and distribution and product type being marketed. In North American countries, requirements and procedures are regulated by government regulatory agencies like the Food and Drug Administration in the U.S. In most other countries, validation procedures and requirements are typically defined and implemented by the food producers. In either case, the producers need to design and implement a treatment that imparts the degree of sterility to the least treated food segment sufficient to inactivate all microorganisms of public health significance and sufficiently reduce the risk of product spoilage by the more resistant spoilage-related microorganisms.

Unlike in European countries (11, 12) where regulations are based on spoilage tests, the FDA requires microbiological tests to prove the safety of a process with sufficient latitude for variability in process conditions. In the U.S., different regulatory agencies and rules apply to different products. For example, UHT milk processing is covered under title 21 (parts 108, 113, 114) of the code of federal regulations (CFR). The process should also adhere to the pasteurized milk ordinance (PMO). When meat is involved, the regulations are imposed by the USDA. In addition to these regulations, certain states have state regulations imposed on certain processes. During the past few years, HACCP has gained tremendous importance and its implementation has been extended by the FDA to various products after its initial application to certain acidified and low-acid canned foods. The details of the requirements of a HACCP program are described in detailed in 21 CFR 113, 114. In addition, details of the evaluation of a HACCP program for a multiphase food product aseptically produced has been presented in reference (3).

Shelf-stable low-acid food products constitute a special case since the conditions of storage and chemical composition are conducive to the growth and toxin formation by various strains of *Clostridium botulinum*, microorganisms capable of producing one of the most potent toxic substances known, the causing agent of potentially fatal botulism poisoning. Processes for treating these types of products need to be designed and validated to consistently deliver a 12D reduction of spores of the most resistant proteolytic strains of *C. botulinum*. For homogeneous materials like beverages, dairy products, purees, homogenates, and clear and smooth soups, procedures and methodology to establish and validate these types of processes are well known and established.

General FDA Requirements for Establishment of Registration, Thermal Process Filing, and Good Manufacturing Practices for Low-Acid Canned Foods and Acidified Foods are covered in 21 CFR 108, 21 CFR 110, 21 CFR 113, and 21 CFR 114. These and other listed regulations and forms are also accessible through contact with FDA directly or from their web site.

Aseptically processed low-acid particulate products present a formidable challenge to the processor in terms of the ability to design, document, and validate a process that will deliver adequate treatment to the fastest moving, slowest heating particle within a continuously processed system. Three decades of intensive research and development by numerous researchers and engineers have been invested in the development of knowledge methodology and a technology database to perform these documentation and validation studies to meet the regulatory agency requirements. Typical thermal process design for aseptically processed liquid foods, aseptically processed fluid homogeneous foods, and aseptically processed low-acid heterogeneous foods containing discrete particulates have been presented in reference (9).

A series of industry-university-government workshops on aseptic processing of multiphase foods in 1995 and 1996, sponsored by the National Center for Food Safety and Technology in Chicago and the Center for Advanced Processing and Packaging Studies at North Carolina State University in Raleigh, resulted in a publication of the "Case study for condensed cream of potato soup from the aseptic processing of multi-phase foods workshop" (10).

As a result of these workshops, criteria for demonstrating a safe process for aseptic particle-containing foods were established (13). Within one year, Tetra Pak Inc., in

conjunction with the NFPA, developed the necessary data required for a low-acid canned foods filing for cream of potato soup, which was accepted by the FDA in May 1997. After this first successful filing, no further filings are known that were submitted to FDA and no aseptically processed and packaged low-acid products are known to be on the North American market.

This illustrates the still-prevailing hurdle of regulatory requirements and the lack of appropriate validation techniques as one of the major remaining obstacles to wider commercialization of multiphase aseptic products and aseptically processed foods in general.

As a result, there is an abundance and wide variety of fluid and homogeneous semi-fluid aseptically processed products on the market, but low-acid products containing particles, in spite of the obvious advantages of aseptic processing technologies to their quality and distribution, remain limited world-wide and absent on the North American market. Further development of technologically more sophisticated, but easier to implement validation methods and tools is expected to reduce this last remaining obstacle to aseptic technology penetration and implementation into a wider range of processed food products and biomaterials.

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124 Food Irradiation Using Electron-Beam Accelerators

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CONTENTS

I. INTRODUCTION

Food irradiation involves exposing the food (packaged or in bulk) to controlled amounts of ionizing radiation to achieve certain desirable objectives. The technology has recently been identified by the Food and Drug Administration (FDA) and World Health Organization (WHO) as having significant strategic importance for the future of food safety worldwide.

Food irradiation has been around for over 60 years and can offer a number of potential benefits including inactivation of microorganisms, inhibition of many enzymatic processes (such as those that cause sprouting and ripening), in addition to the fact that it can be used as an alternative to chemical treatment for disinfestations. The recent progress in the development of electron beam accelerators, together with the increased number of illnesses associated with produce-related foodborne disease outbreaks in the last years, provide the incentive for the development of an efficient technique to ensure hygienic quality of food products, especially those to be consumed raw or undercooked, to protect consumer health.

It is estimated that up to 81 million people a year are infected by foodborne illness, and of that number, 10,000 die (1). With an estimated 25% of all food production lost after harvesting to insects, bacteria, molds, and premature germination and the potential for continued rise in food poisoning incidence, irradiation could be used more widely to improve the quality and safety of the food supply in the future.

II. SOURCE OF IONIZING RADIATION

The type of radiation used in processing materials is limited to high energy *gamma-rays*, *X-rays,* and *accelerated electrons*. These radiations are also referred to as *ionizing radiations* because their energy is high enough to dislodge electrons from atoms and molecules and to convert them to electrically charged particles called ions. Gamma-rays and X-rays, like radiowaves, microwaves, ultraviolet, and visible light rays, form part of the electromagnetic spectrum, occurring in the short wave length, high energy region of the spectrum. Their properties and effects on materials are

the same, but their origins are different. In most cases, X-rays with varying energies are generated by machines. Gamma-rays with specific energies come from the spontaneous disintegration of radionuclides. Only certain radiation sources can be used in food irradiation. These are the radionuclides cobalt-60 (^{60}Co) or caesium-137 (^{137}Cs); X-ray machines having a maximum energy of 5 MeV, or electron machines having a maximum energy of 10 MeV. Energies from these radiation sources are too low to induce radioactivity in food.

As shown in Figure 124.1, gamma-rays and X-rays radiation can penetrate distances of a meter or more into the product, depending on the product density, whereas electrons, even with energy as high as 10 MeV, can penetrate only several centimeters.

A. Cobalt-60

Naturally occurring and man-made radionuclides, also called radioactive isotopes or radioisotopes, are unstable, and emit radiation as they spontaneously disintegrate, or decay, to a stable state. The Becquerel (Bq) is the unit of radioactivity and equals one disintegration per second.

The radionuclide used almost exclusively for the irradiation of food by gamma rays is ^{60}Co. Until 1993, food irradiation in the United States occurred exclusively from the use of ^{60}Co (2). It is produced by neutron bombardment, in a nuclear reactor, of the metal ^{59}Co, and then doubly encapsulated in stainless steel "pencils" to prevent any leakage during its use in a radiation plant. ^{60}Co has a half-life of 5.3 years.

The emitted gamma rays are photons with very short wavelengths, similar to ultraviolet light and microwaves but with much higher energies. Because gamma radiation does not elicit neutrons (i.e., the subatomic particles that can make substances radioactive), irradiated foods and their packaging are not made radioactive (3). Since 1960, the worldwide use of ^{60}Co has resulted in the accumulation of a vast and successful record of safety and reliability (4).

One economic factor related to ^{60}Co is the fact that its supply is limited and is practically a monopoly of Nordion Inc. (Ontario, Canada). Prices have increased and a sufficient future supply is questionable. A significant increase in demand would require the development of increased nuclear reactor capabilities.

In a typical gamma radiation facility, the radioactive material (^{137}Cs or ^{60}Co) is placed at the top of an elevator that can be moved up for use or down under water when not in use. Materials that need to be irradiated are placed around the radioactive material at a suitable distance for

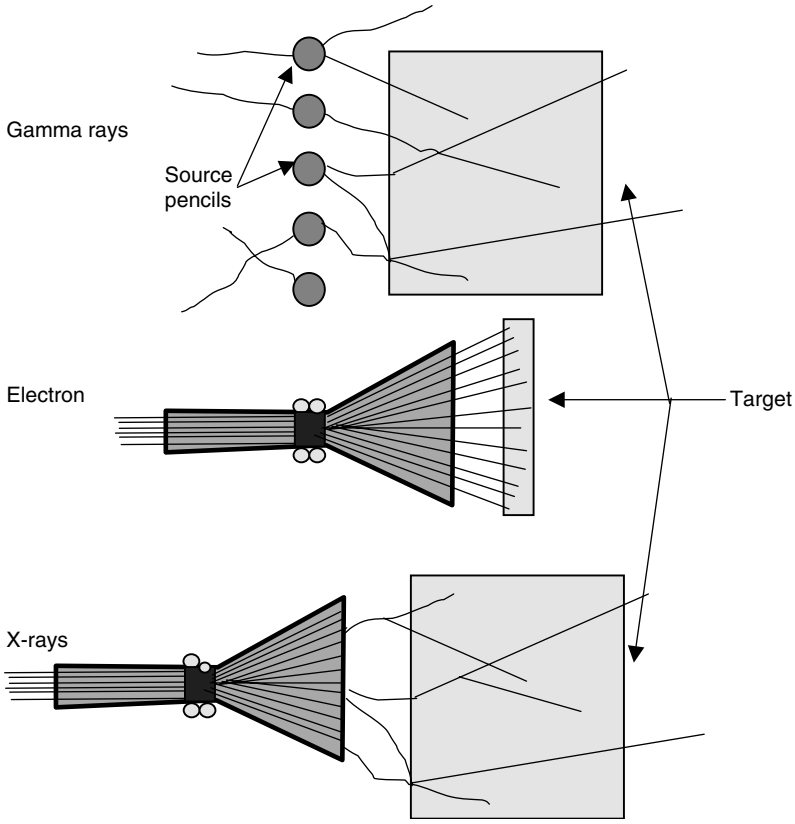

FIGURE 124.1 Typical sources used for food irradiation.

their desired dose. Among the drawbacks to the use of radioactive material is that the isotope source emits rays in all directions and cannot be turned "on" or "off" (4).

B. Cesium-137

^{137}Cs is the only other gamma-emitting radionuclide suitable for industrial processing of materials. It can be obtained by reprocessing spent, or used, nuclear fuel elements and has a half-life of 30 years (6).

The proposed use of ^{137}Cs as a radiation source dates from the early 1970s. It was based on the availability of vast quantities of unprocessed and encapsulated ^{137}Cs from US Government's stock of byproducts from nuclear energy and nuclear weapon production programs. By 1988, the Department of Energy canceled the program and the option of using ^{137}Cs as an ionizing radiation source for food safety was eliminated (5).

C. Electron Beam Accelerators

Some machine sources of radiation are suitable for irradiating certain materials. High energy electron beams can be produced from machines capable of accelerating electrons (accelerators). Electrons cannot penetrate very far into food, compared with gamma radiation or X-rays. However, X-rays can be produced when a beam of accelerated electrons bombards a metallic target. Although X-rays have good penetrability into food, the efficiency of conversion from electrons to X-rays is generally less than 10% (7).

Electron accelerators offer certain advantages over radioactive elements, which make them more attractive for industrialization: (1) The efficiency for direct deposition of energy, (2) the easy variability of electron-beam current and energy to provide flexibility in the choice of surface and depth treatments for a variety of food items, and (3) the ease with which an electron accelerator can be turned off or on.

There are two main differences between gamma rays and accelerated electrons. First, gamma penetration is higher than accelerated electrons, but the penetration capacity of the latter increases with their energy. Electrons at 10 MeV are more penetrating than those at 4 MeV. Second, the gamma dose rate from a typical ^{60}Co irradiator is 1–100 Gy/min, whereas electron beams from an accelerator can produce 10^3–10^6 Gy/sec (5).

A particle accelerator delivers energy to a charged-particle beam by the application of an electric field. Acceleration of charge particles can be divided in two categories: electrostatic and electromagnetic acceleration. An electrostatic accelerator consists basically of two conducting surfaces with a large voltage difference and a particle with charge gains kinetic energy. The peak energy of the beam is limited by the voltage that can be sustained without breakdown (8). The Van de Graaff accelerator is an electrostatic accelerator and will be described below since it is the electron beam source simulated in this work.

Electromagnetic fields are required in order to obtain energies above a few million electron volts. Electromagnetic accelerators can be resonant or nonresonant. Nonresonant are pulsed and are essentially step-up transformers, with the beam acting as a high voltage secondary. Resonant means that electromagnetic oscillations in resonant cavities or waveguides are used to transform input microwave power from low to high voltage. There is also a close coupling between properties of the particle orbits and time variations of the accelerating field. The category of resonance accelerators includes the linac, cyclotron, and synchrotron. Linac is an abbreviation for linear accelerator and the charged particles moves on a linear path and are accelerated by time dependent electromagnetic fields. Likewise, the particle orbit in a cyclotron is a spiral and a circle for a synchrotron (8).

1. Van de Graaff Accelerator

Mechanical transport of electric charges to the inner surface of a hollow electrode underlies the operation of electrostatic generators (Figure 124.2). In this type of accelerator a corona discharge from an array of needles in gas is used as the source of electrons. The electrons drift toward the positive electrode and are deposited in a moving belt. The belt, which is composed of an insulating material with high dielectric strength, is immersed in insulating gas at high pressure. The attached charge is carried mechanically

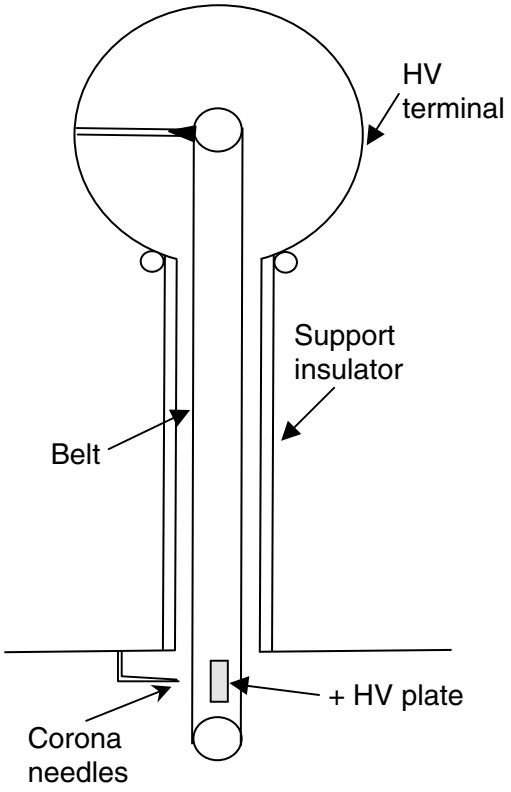

FIGURE 124.2 Schematic of the Van de Graaff accelerator.

against the potential gradient into a high-voltage terminal. The terminal acts as a Faraday cage and there is no electric field inside the terminal other than that from the charge on the belt. The charge flows off the belt when it touches a metal brush and is deposited on the terminal.

The energy to charge the high-voltage terminal is supplied by the belt motor. High-voltage terminals are usually constructed as large, smooth spheres to minimize peak electric field stress. The current available to drive a load (such as an accelerated beam) is controlled by either the corona discharge current or the belt speed. Typical currents are in the range of 10 μA. Van de Graaff accelerators are excellent research tools since they provide a steady-state beam with good energy regulation (8).

Electron beam facilities, widely used to irradiate medical equipment, have been built for food treatment. A conveyor or cart system moves the product to be irradiated under the electron beam at a predetermined speed to obtain the desired dosage. The products move in and out the irradiation area continuously. Energy penetration is about 3.86 cm in food products, so the thickness of items to be treated is limited to about 7.62 cm with double-sided treatment (9).

X-rays are electromagnetic radiation produced when energetic electrons hit a target. In an X-ray machine, the electrons are emitted by a heated cathode whose potential may be the order of 30 to 50 kV above the target (made of a material such as tungsten or molybdenum). For food irradiation electrons from a linear accelerator operating at 5 MeV hit a target to make X-rays and then treat the food.

The early design of accelerator systems provided poor penetration (produced only low energies, −2 MeV), were difficult to control, and were unreliable. So, the irradiation market was taken over by ^{60}Co (gamma).

During the 1970s, several companies, including Varian Associates, Phillips, and Siemens, took a new look at the application of X-ray technology for radiographic and oncology therapy equipment (10). Their involvement in the improvement of durability and reliability of accelerated electron technology raised performance parameters to a new level (11).

Today, industrial e-beam accelerators are characterized by (11):

- Higher energy (10 MeV) and thus better penetration
- High duty cycles (7,000–8,000 hours/year)
- Fully automated electronic control systems featuring programmable logic controllers (PLCs)

The applications of electron beam processing have then increased substantially in the last decades and are still growing.

A comparison among the three basic industrial electron accelerators is shown in Table 124.1 (10): (1) Direct Current machines (DC); (2) Rhodotrons, and (3) Pulsed and Continuous Wave Linear Accelerators (CW LINAC).

TABLE 124.1
Industrial Electron Accelerator Parameters

	Direct Current[1]	Rhodotron[2]	CW LINAC[3]
Energy	10 MeV	10 MeV	10 MeV
Max beam power	50 kW	200 kW	500 kW
Efficiency	<30%	38%	40%
Duty factor	5%	100%	100%
Dimensions	~1.0 × 1.0 × 4.0 m^3	~2.9 × 2.2 m^2	~0.8 × 0.8 × 9.0 m^3
Approximate cost	~$2 M	~$4 M	~$1.5 M

[1] (25) [2] (24) [3] (10).

A Direct Current machine is a linear accelerator that operates at 50 kW to produce up to 10 MeV beams. In a linear accelerator particles move in a linear path and are accelerated by time-dependent electromagnetic fields. The Rodotron technology utilizes a coaxial accelerating cavity of 2 meters in diameter. 10 mA of electrons are sent into the cavity and undergo a first acceleration of 0.5 MeV. Electrons pass through an opening and then emerge into the second part of the cavity, as the electrical field is reversed, they gain once more 0.5 MeV. Around the cavity, window-frame magnets are bending electrons back into the cavity for further acceleration steps. Ten successive crossings would be required to obtain 10 MeV beams (12).

A LINAC may be operated continuously, which is called a continuous-wave (CW) operation or may also be pulsed operated. If the accelerated beam current is small, most of the power in CW operation is not delivered to the beam but is dissipated in the structure walls. If the accelerator is pulsed operated, a larger fractional power is delivered to the beam, and the efficiency is improved (13).

Low-energy applications with electron beams up to ~1 MeV are adequately served by DC machines. These accelerators have high beam power and plug efficiency, but are bulky, thus being difficult and costly to handle. Above ~3 MeV, the CW LINACs have the lowest beam power cost of any commercial accelerator (10).

Electron beam irradiator facilities require shielding and product handling equipment similar to ^{60}Co facilities, although some shielding requirements may be reduced due to the directionality of radiation fields generated by machine sources. In addition, semi permanent facilities may also be developed, because electron beam accelerators can be made transportable (4).

III. EFFECT OF IONIZING ENERGY ON FOOD

Regardless of the source, the effect of ionizing energy on food is identical. Energy penetrates the food and its packaging but with X-rays and gamma-rays most of the energy simply passes through the food, similar to the way

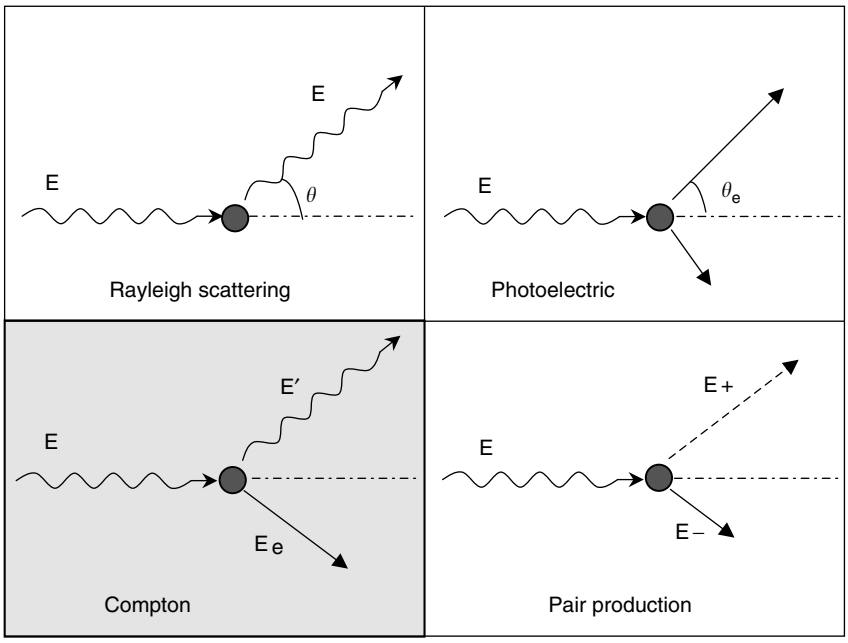

FIGURE 124.3 Basic interaction of photons with matter.

microwaves pass through food, leaving no residue. Most of the energy that does not pass through the food is converted to heat, but some produces DNA strand breaks that inactivate bacteria.

E-beam accelerators work on the same principle as a television tube. Electrons are emitted from a cathode and accelerated by an electric or magnetic field in a vacuum. Instead of being widely dispersed and hitting a phosphorescent screen at low energy levels, the electrons in the accelerator are concentrated and accelerate to higher energy, approximately 99% of the speed of the light. The electrons then pass through a thin metal foil and enter air at normal pressure.

Irradiation efficiency depends on both the accelerator characteristics and irradiation technique, as well as on a number of factors including the type of material, its geometric dimensions, its shape, the packaging material, etc. Processing capacity (kg/h) is directly related to the beam power (kW) and system efficiency, and inversely related to the dose.

The dose unit in the system is the Gray (Gy) — the dose at which 1 Joule of energy is absorbed in each kilogram of substance; the dose rate (D_r) is expressed most often in kGy/s; the total dose absorbed by the irradiated material is directly related to the dose rate and the irradiation time. The dose rate depends on the current (I, mA), the cross-section area (A, m^2) of the irradiation field, over which the electron is scanned, and the energy gradient or the stopping power (T, MeV/g/m^2) of the electrons. In general, the dose rate increases with energy and beam current and decreases as the distance between the accelerator and the irradiated material increases.

The electron range, the distance the electron will penetrate, is inversely proportional to the density of the material being irradiated. The useful range can be increased by 2.5 times by using two-sided irradiation rather than one-sided irradiation (14). In the case of food materials, if radiation is done from one side only a detector can be placed in the back of the material to monitor beam penetration and dose rate.

A. Basic Interaction of Photons and Electrons with Matter

Photons (gamma rays and X-rays) are electrically neutral and do not steadily lose energy as they penetrate food materials. Instead, they can travel some distance before interacting with an atom. Penetration depth of a given photon depends on the specific medium traversed and on the photon energy. When photons interact with matter, they might be absorbed and disappear or be scattered, changing direction of travel, with or without energy loss (Figure 124.3).

By contrast, a charged particle (electron), being surrounding by its Coulomb electric force field, interacts with one or more electrons or with the nucleus of practically every atom it passes (Figure 124.4). Thus, it is convenient to think of the particle as losing kinetic energy gradually in a friction-like process, often referred to as the "continuous slowing-down approximation" (CSDA).

In general, electrons have much less penetration power than gamma and X-rays. Because of the small mass and single negative charge, each time an electron approaches a target, it is deflected from its path by the orbital electrons and the positive atom nuclei. For those reasons, electrons have a poorer penetrability compared to gamma and X-rays.

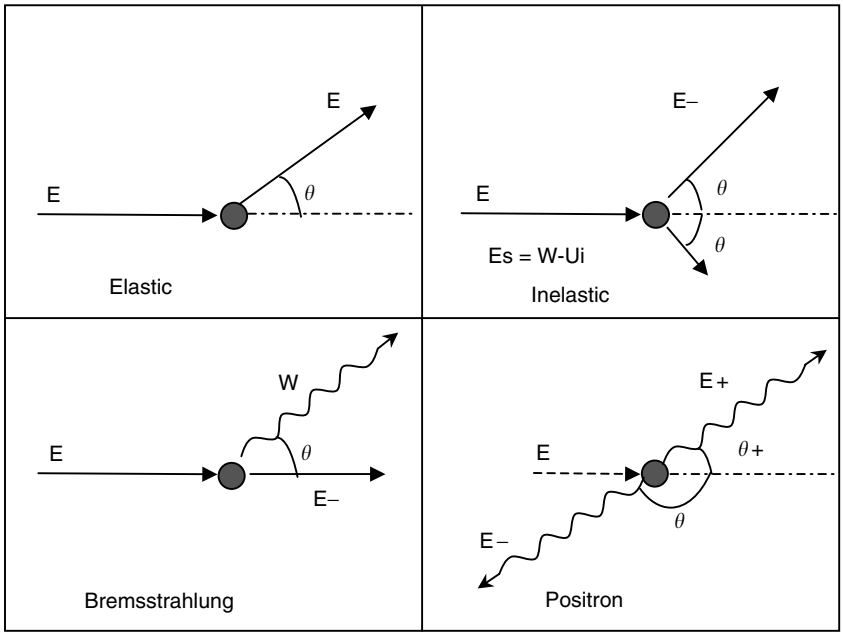

FIGURE 124.4 Basic interaction of electrons with matter.

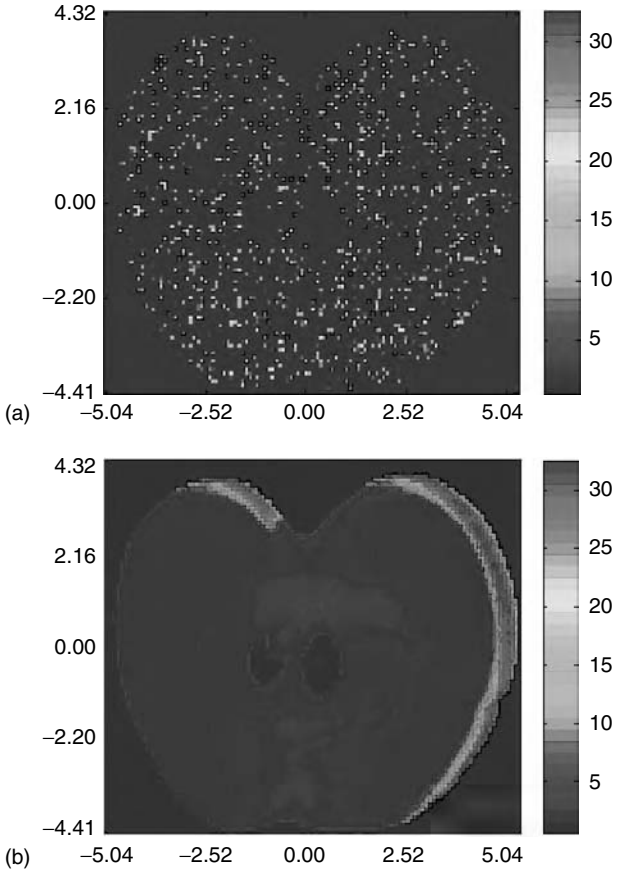

FIGURE 124.5 (a) 1 MeV photons incident in an apple; (b) 1 MeV electrons incident in an apple.

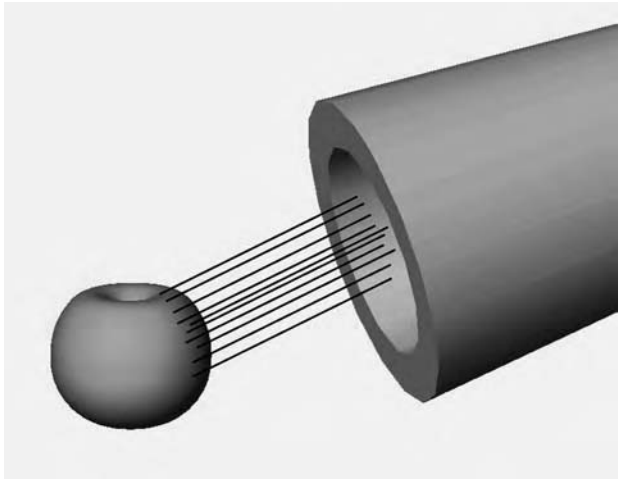

FIGURE 124.6 Source direction in an apple.

Figures 124.5a and 124.5b show the results of a simulation of the energy deposition of 1 MeV photons and electrons, respectively, in an apple with tilted source direction (Figure 124.6). The simulated results clearly show that the absorbed energy is distributed into the entire apple when 1 MeV photons is used as the energy source (Figure 124.5a). However, electrons with the same energy only penetrate about 5 mm of the apple (Figure 124.5b). For surface pasteurization of fruits and vegetables, electrons beams are the preferable source of treatment.

B. The Effect of Ionizing Radiation on Microorganisms

According to the target theory (15), lethality due to ionizing radiation occurs when the irradiated microorganisms are destroyed by the passage of an ionizing particle or quantum of energy through, or in close proximity to, a sensitive portion of the cell. This direct "hit" on the target causes ionization in this sensitive region of the organism or cell and subsequent death.

Bacterial spores are more resistant to ionizing radiation than are vegetative cells. Gram-positive bacteria are more resistant than gram-negative bacteria. The resistance of yeast and molds varies considerably, but some are more resistant than most bacteria.

According to reference (16), the efficacy of a given dose of irradiation to destroy a microbial population depends on the following:

- The kind and species of the organism.
- The numbers of organisms (or spores) originally present. The more organisms there are, the less effective a given dose will be.
- The composition of the food. Proteins, catalase, and reducing substances (nitrites, sulfites, and sulphydryl compounds) may be protective. Compounds that combine with the SH groups would be sensitizing.
- The presence or absence of oxygen. The effect of free oxygen varies with the organism, ranging from no effect to sensitization of the organism. Undesirable "side reactions" are likely to be intensified in the presence of oxygen and to be less frequent in a vacuum.
- The physical state of the food during irradiation. Both moisture content and temperature affect different organisms in different ways.
- The condition of the organisms. Age, temperature of growth and sporulation, and state (vegetative or spore) may affect the sensitivity of the organisms.

C. Chemical and Nutritional Changes in Foods

In general, the irradiation process produces very little chemical change in food. None of the changes known to occur have been found to be harmful or dangerous. Some of the chemical changes produce so-called "radiolytic" products. These products have proven to be familiar ones, such as glucose, formic acid, acetaldehyde, and carbon dioxide that are naturally present in foods or are formed by heat processing. The United States Food and Drug Administration has estimated that the total amount of undetected radiolytic products that might be formed when food is irradiated at a dose of 1 kGy would be less than 3 milligrams per kilogram of food — or less than 3 parts per million (17).

Research has shown that protein, carbohydrates, and fat are relatively stable to radiation doses of up to 10 kGy. Different types of vitamins have varied sensitivity to irradiation and to some other food processing methods. For example, vitamins C and B-l (thiamine) are sensitive to irradiation as well as to heat processing. The evidence suggests that irradiation does not induce special nutritional problems in food (18).

The change in nutritional value caused by irradiation depends on a number of factors including the radiation dose to which the food has been exposed, the type of food, packaging, and processing conditions (temperature during irradiation). Most of these factors are also true for other food preservation technologies.

D. Low Dose Irradiation for Surface Pasteurization of Fresh Produce

Ingestion of raw vegetables and fruits has been linked to outbreaks of food borne illness. Contaminated artichoke, beet leaves, cabbage, carrots, cauliflower, celery, eggplant, endive, fennel, onion, lettuce, mushrooms, potatoes, tomatoes, cantaloupe, watermelon, raspberries, strawberries, apples, etc. have been vehicles for transmission of pathogens (19). The viability of pathogenic organisms on the surface of fresh fruits and vegetables can be significantly reduced by electron beam irradiation. By limiting the irradiation to the surface, changes in the quality of the bulk of the product can be minimized. Electron beams (e-beams) are produced by small accelerators (which do not produce radiation when not in use) and electron energies, which penetrate only a short distance into the tissue, can easily be obtained. The most difficult technical challenge for surface irradiation is the need to achieve a uniform dose over the entire surface. This is particularly difficult if there are deep recesses such as the area of the stem of an apple, or convoluted surfaces such as some type of lettuces.

Most of the investigations in the area of e-beam accelerators to irradiate food have dealt with the effect of this energy source on the inactivation of microorganisms and on the product characteristic changes. No information is available in the literature regarding the development of methods for ensuring reliable quality control of the irradiation process on materials with irregular shapes, for example. Methods to continuously monitor the e-beam characteristics, such as the electron energy, electron current, scanner width, scanner uniformity, penetration depth, and conveyor speed need to be evaluated for these types of product.

IV. DOSE DISTRIBUTION DETERMINATION IN FOOD PRODUCTS

In food irradiation, one way to verify dose distribution in an irradiated product is by using photo luminescent dosimeters (20). This dosimeter is a small plastic wafer that is irradiated with the food. The dosimeter documents the dose received so that the process can be controlled to ensure that the product receives doses within regulatory requirements (dose range). Shining blue light on the irradiated dosimeter induces it to emit a red light (fluorescence). The intensity of the fluorescence is proportional to the dose that the food received.

A chemical dosimeter using a ferrous sulfate (Frike) solution was used by (21) to calibrate e-beam irradiation dose to different foodstuffs. The dosimeter uses the change in ultraviolet absorption caused by dose-dependent oxidation of ferrous to ferric ions. The homogeneity of the dose delivered to the products was verified with an ionizing chamber on a motor-driven scanning system.

The dose can also be predicted by various mathematical methods. Most of the research advances in dose distribution calculations has been in the area of radiation therapy. Although the application and practice of e-beam irradiation techniques vary from one industry to another, engineers tend to learn from each other. Thus, it is worth reviewing some of the radiation applications in the medical industry to learn how to improve the application of this technique to reduce pathogens in the US food chain.

One of the most important steps in radiation treatment planning process is the determination of radiation dose distribution in the body. There are two main methods for calculating the dose in the patient: measurement-based empirical methods and model-based methods (22). The empirical approach relies on experiments with dose measurements in a 'phantom' (a box filled with water). The measured dose is then slightly corrected to account for actual beam shape, patient shape, and density and composition of the body tissue. Measured-based methods are accurate if the beam shape is a simple square, the surface of material to be irradiated is flat and perpendicular to the beam, and the beam travels through homogeneous soft tissue. This method would not be accurate for a more complex 3D structure of foods since it does not adequately account for their inhomogeneity and the lack of secondary electron equilibrium in and around the typical food object.

The model-based methods are then generally used to estimate the dose in a body in radiation treatment planning. Monte Carlo technique is today the most accurate means of dose calculation. Monte Carlo transport simulates the behavior of irradiation particles as they interact with atoms in the body during a typical radiation treatment.

Simulation of radiation transport (electrons and photons) by the Monte Carlo method has been used to simulate radiation treatment machine heads, absorbed dose distribution, energy distributions, and electron-treatment planning (23).

As an example, Figures 124.5a,b show Monte Carlo simulation used to determine the dose distribution at the surface of an apple irradiated with e-beam generated by a Van de Graaff accelerator (1–2 MeV). The dose distribution was used to develop the best irradiation angle while rotating an irregularly shaped food material (the apple) for uniform surface irradiation.

V. CONCLUSIONS

We know that consumption of food contaminated with pathogens (*Salmonella* and *E. coli*, for example) causes serious foodborne illness and even death. Also, foods may be used as vehicles to deliver biological agents to cause disease. The security and safety of our food supply and agricultural production will continue to be topics of widespread international interest in the years to come. Consequently, efforts across the food industry to improve and implement measures to enhance assurances of food safety will always be needed.

Progress in electron beam accelerators will continue to further the development of efficient methods to ensure quality and safety of food products, especially those to be consumed raw or undercooked, to protect consumer health. Satisfactory irradiation of foods will be a common goal for producers, processors, government agencies, and consumers around the world. Advances in dosimetry methods in heterogeneous and irregular shaped materials such as foods will provide accurate, precise, and wide range dosimetry data for effective treatment planning of a wide number of food products.

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125 Microwave Heating in Food Processing

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CONTENTS

I. INTRODUCTION

Microwave heating of food has existed since 1949. Growth in the number of homes with microwave ovens, combined with the industrial use of microwaves, has created a large market for microwave-processed foods and, consequently, has changed food preferences and preparation methods and increased the need for research on the behavior of various types of foods during microwave heating.

Using microwaves as a source of heat in the processing (thawing, heating, drying, etc.) of food materials is advantageous because it offers a potential for rapid heat penetration, reduced processing times, and, hence, increased production rates, more uniform heating, and improved nutrient retention. The use of microwaves represents the use of sophisticated technology in the food industry. Lack of sufficient and unified knowledge of this complex and radically different heating process has been the primary contributor to its unpredictability. Emphasis should be on basic research to better understand the interaction between the microwave energy and product. This chapter will provide fundamentals of microwave heating and description of microwave processes in the food industry.

II. MICROWAVE HEATING

The temperature of a material can be increased either directly or indirectly. The indirect methods are those in

which heat is generated external to the product and is transferred to it by conduction, convection, or radiation. The direct methods are those in which heat is generated within material itself. Dielectric heating is used with electrically non-conducting materials. The material to be heated is placed between two electrodes and forms the dielectric component of a capacitor. Excitation is by means of a high frequency voltage (2 to 100 MHz) applied to the condenser plates. Radio frequency heating, which is at a much lower frequency, has thrived as an industry alongside microwaves over the decades. Radio frequency heating in the United States can be performed at any of three frequencies: 13.56, 27.12, and 40.68 MHz. Microwave heating is a special field of dielectric heating in which very high frequencies (300 MHz to 30 GHz) are applied. Domestic microwave ovens operate at 2450 MHz and industrial processing systems generally use either 2450 MHz or 915 MHz (896 MHz in the UK). A domestic microwave oven is a multimode cavity in which electromagnetic waves form a resonant pattern.

As dielectric materials are poor heat conductors, heat applied from the outside by convection, radiation, or conduction is inefficient. In some cases the heat applied causes a skin or crust to form on the outside which is in itself a thermal barrier. The single most important thing about microwave heating is the unique opportunity to create heat within a material — the volumetric heating effect — not achievable by any other conventional means. No temperature differential is required to force heat into the center of the material. Generation of heat within food products by microwave energy is primarily caused by molecular friction attributed to the breaking of hydrogen bonds associated with water molecules and ionic migration of free salts in an electric field of rapidly changing polarity. Substances that respond to and, therefore, can be processed by microwave energy are composed of polar (e.g., water), ionic, or conductive (e.g., carbon black) compounds. Non-polar substances, e.g., polyethylene and paraffin, are unaffected.

III. DEFINITION OF TERMS AND PROPAGATION OF WAVES

James Clerk Maxwell (1831–1879) developed the classical theory of electromagnetism and correctly predicted that an electromagnetic wave has associated electric field E and magnetic field H. A uniform plane wave characterized by $E = a_x E_x$ propagating in a lossy medium in the +z-direction has associated with it a magnetic field $H = a_y H_y$. The solution to be considered here is that of a plane wave, which for the electric field attains the form

$$E(x) = E_{\max} e^{j\omega t - \gamma z} \tag{125.1}$$

A propagation constant, γ, is defined as

$$\gamma = jk_c = j\omega\sqrt{\mu\varepsilon_c} \tag{125.2}$$

with

$$\varepsilon_c = \varepsilon' - j\varepsilon'' = \varepsilon_0(\varepsilon_r' - j\varepsilon_r'') \tag{125.3}$$

$$\varepsilon_r' \equiv \varepsilon'/\varepsilon_0;\ \ \varepsilon_r'' \equiv \varepsilon''/\varepsilon_0 \tag{125.4}$$

where ε_0 is the permittivity of free space (8.8542E-12 Farad/m). Hence, all the previous equations for nonconducting media will apply to conducting media if ε is replaced by the complex permittivity ε_c. The material's ability to store electrical energy is represented by ε', and ε'' accounts for losses through energy dissipation. ε_r' is often called "relative dielectric constant." This is somewhat inappropriate, as the term "constant" should be used only for true constants. ε_r' varies significantly both with temperature and frequency for many typical workload substances. ε_r'' is called the relative dielectric loss incorporating all of the energy losses due to dielectric relaxation and ionic conduction. The ratio $\varepsilon''/\varepsilon'$ is called a loss tangent because it is a measure of the power loss in the medium:

$$\tan\delta_c = \frac{\varepsilon''}{\varepsilon'} \cong \frac{\sigma}{\omega\varepsilon} \tag{125.5}$$

The quantity δ_c may be called the loss angle.

The propagation factor $e^{-\gamma z}$ can be written as a product of two factors:

$$E(x) = E_{\max} e^{-\alpha z} e^{j(\omega t - \beta z)} \tag{125.6}$$

where α and β are the real and imaginary parts of γ, respectively. Since γ is complex, we write, with the help of Equation (125.3),

$$\gamma = \alpha + j\beta = j\omega\sqrt{\mu\varepsilon}\left(1 + \frac{\sigma}{j\omega\varepsilon}\right)^{1/2} = j\omega\sqrt{\mu\varepsilon'}\left(1 - j\frac{\varepsilon''}{\varepsilon'}\right)^{1/2} \tag{125.7}$$

$$\alpha = \frac{\sqrt{2}\pi f}{c}\sqrt{\varepsilon_r'\left(\sqrt{1 + \tan^2\delta} - 1\right)} \tag{125.8}$$

$$\beta = \frac{\sqrt{2}\pi f}{c}\sqrt{\varepsilon_r'\left(\sqrt{1 + \tan^2\delta} + 1\right)} \tag{125.9}$$

As we shall see, both α and β are positive quantities. The first factor, $e^{-\alpha z}$, decreases as z increase and thus is an attenuation factor, and α is called an attenuation constant. The second factor, $e^{-j\beta z}$, is a phase factor; β is called a phase constant which expresses the shift of phase of the propagating wave and is related to the wavelength of radiation in the medium (λ_m) by $\lambda_m = 2\pi/\beta$ which, in free space, reduces to $\lambda_0 = 2\pi/\beta = c_0/f$.

From Equation (125.6), the first exponential term gives the attenuation of the electric field, and, therefore, the

distribution of the dissipated or absorbed power in the homogeneous lossy material follows the exponential law (Lambert's Law):

$$P_{diss} = P_{trans} e^{-2\alpha z} \tag{125.10}$$

where P_{trans} is the power through the surface in the z direction. Theoretically, the power penetration depth, D_p, is defined as the depth below a large plane surface of the substance where the power density of a perpendicularly impinging, forward propagating plane electromagnetic wave has decayed by $1/e$ from the surface value, $1/e \approx 37\%$ [1]. The absorbed power in the top layer of this thickness in relation to the totally absorbed power (per surface area), is then 63%.

$$D_p = \frac{1}{2\alpha} \tag{125.11}$$

Substitution of Equation (125.8) into Equation (125.11) yields the general expression for the penetration depth:

$$D_p = \frac{c}{4\pi f}\sqrt{\frac{2}{\varepsilon_r'\left(\sqrt{1+\tan^2\delta}-1\right)}} = \frac{\lambda_0}{2\pi\sqrt{2\varepsilon_r'}\left(\sqrt{1+(\varepsilon_r''/\varepsilon_r')^2}-1\right)^{1/2}} \tag{125.12}$$

The skin depth D_s, where the electric field strength is reduced to $1/e$ (and the power density thus to $(1/e)^2$), is twice the power penetration depth, $D_s = 2D_p$.

IV. MICROWAVE POWER DISTRIBUTION

Most practical materials treated by microwave power are nonhomogeneous and very frequently anisotropic; the permittivity of these materials changes with temperature and moisture content (drying process). Thermal losses from the material surface and heat transfer in the bulk of material give additional complications. The generation of heat in food materials is also accompanied by significant moisture migration which, in turn, affects the energy absorption characteristics of food creating a coupling of heat and mass transport that complicates mathematical analysis.

From the physical point of view, microwave heating is a combination of at least four different processes: distribution of power, absorption of power, heat transfer, and mass transfer. The magnitude and uniformity of temperature distribution are affected by both food and oven factors such as:

1. Magnitude and distribution of microwave power where the food is placed;
2. Reflection of waves from the food surface and penetration depth, as characterized by the food geometry and properties;
3. Distribution of absorbed power as well as power dissipated at a particular point (electric field intensity) as functions of the material parameters, temperature, and time (due to drying); and
4. Simultaneous heat and mass transfer.

A. Electric Field Intensity

Electromagnetic waves transport energy through space. The amount of microwave energy absorbed is, in turn, determined by the electric field inside the microwave applicator. It offers an intangible link between the electromagnetic energy and the material to be treated.

For microwave heating, the governing energy equation includes volumetric heat generation that results in a temperature rise in the material:

$$\frac{\partial T}{\partial t} = \alpha\nabla^2 T + \frac{Q_{abs}}{\rho C_p} \tag{125.13}$$

In this equation Q_{abs} (watts/cm^3) corresponds to volumetric rate of internal energy generation due to dissipation of microwave energy. Basically, the apparatus is placed in the oven at the position of interest and the rate of temperature rise, $\partial T/\partial t$, is measured. C_p (cal/g °C) is the heat capacity of the material, and ρ (g/cm^3) is the density of the material. Assuming no temperature gradients in a small mass of dielectric medium, the energy balance can be obtained by simplifying Equation (125.13):

$$Q_{abs} = \frac{P_{abs}}{V} = \rho C_p \frac{\partial T}{\partial t} \tag{125.14}$$

where P_{abs} is the total power absorbed by the dielectric medium (watts). Its relationship to the E-field at the location can be derived from Maxwell's equations of electromagnetic waves [2].

$$Q_{abs} = 2\pi f\, \varepsilon_0 \varepsilon_{eff}'' E_{rms}^2 \tag{125.15}$$

where f is the microwave frequency (2450 MHz), ε_{eff}'' is the dielectric loss factor for the dielectric material being heated, and E_{rms} is the root mean square value of the electric field intensity. By knowing the rate of temperature rise, the heat generation, Q_{abs} can be determined and equated to the electric field, E_{rms}, using Equation (125.15).

$$E_{rms} = \sqrt{\frac{\rho C_p}{2\pi f\, \varepsilon_0 \varepsilon_{eff}''}\frac{\partial T}{\partial t}} \tag{125.16}$$

B. Lambert's Law

In several computational studies of microwave heating, the heat generation has been modeled by Lambert's law, according to which the microwave power is attenuated exponentially as a function of distance of penetration into the sample [3–9]. It must be emphasized that these penetration depth

calculations are valid only for materials undergoing plane wave incidence and for semi-infinite media only and henceforth will be referred to as Lambert's law limit [5, 8]. Although Lambert's law is valid for samples thick enough to be treated as infinitely thick, it is a poor approximation in many practical situations and often does not describe accurately the microwave heating of food in a cavity.

To determine the conditions of the approximate applicability of Lambert's law for finite slabs, Ayappa and others [8, 9] compared it with microwave heating predicted by Maxwell's equation. The critical slab thickness L_{crit} (in cm) above which the Lambert's law limit is valid can be estimated from $L_{crit} = 2.7/D_p - 0.08$. Fu and Metaxas [10] proposed a new definition for the power penetration depth Δ_p, which is the depth at which the power absorbed by the material is reduced to $(1 - 1/e)$ of the total power absorbed. This definition allows a unique value of Δ_p to be found for all thickness and also gives an indication of the validity of assuming exponential decay within the slab. Another approach is used where a spherical dielectric load is assumed to absorb energy from a surrounding radiation field [11]. The power absorption inside of dielectric medium can be estimated in the following way. Assume that the power flux (power per unit area) entering through the surface of the dielectric medium is uniform, and all the waves are transmitted into the medium, i.e., no wave reflection. Then power decays exponentially, $P(x) = P_0 \cdot \exp(-x/D_p)$ where, P_0 is the incident power at the surface. From the Poynting theorem [12], the field energy that dissipates as heat in the enclosed volume is equal to the total power flowing into a closed surface minus the total power flowing out of the same closed surface.

$$\sum P_{eff} = \int \frac{P_0 e^{-x/D_p}}{D_p}\, dx = -P_0\left[\int_0^a \frac{e^{-(a-r)/D_p}}{D_p}\, d(a-r) - \int_0^{-a} \frac{e^{-(a-r)/D_p}}{D_p}\, d(a-r)\right] \quad (125.17)$$

$$P_{abs} = \sum P_{eff} = P_0[1 - e^{-2a/D_p}] \quad (125.18)$$

where a is the radius of the spherical dielectric load, P_{eff} is the effective magnitude of the Poynting vector, and P_{abs} is the total power absorption by the dielectric medium. The use of Lambert's law requires an estimate of the transmitted power intensity P_{trans} (Equation (125.10)), which is obtained from calorimetric measurements [4, 7] or used as an adjustable parameter to match experimental temperature profiles with model predictions [6]. Thus P_{trans} measured by the above methods represents the intensity of transmitted radiation, the accuracy of the estimate depending on the method used. Alternately if P_{trans} is the incident power flux then Lambert's law must be modified to account for the decrease in power, due to reflection at the surface of the sample.

Since Lambert's law does not yield a comprehensive approach, a more accurate estimation of the heating rate based on predicting or measuring the fundamentally nonuniform electric field intensity in a cavity should be the most important subject of current research. How the shape and volume (relative to the microwave oven) of a food material change the rate of heating must be investigated further. The interior electric field, the moisture movement in solid foods, and changes in the dielectric and other properties combined to make designing microwave processes a difficult task.

V. INTERACTION OF MICROWAVE WITH FOOD

Food shape, volume, surface area, and composition are critical factors in microwave heating. These factors can affect the amount and spatial pattern of absorbed energy, leading to effects such as corner and edge overheating, focusing, and resonance. Composition, in particular moisture and salt percentages, has a much greater influence on microwave processing than in conventional processing, due to its influence on dielectric properties. Interference from side effects like surface cooling, interior burning, steam distillation of volatiles, and short cook time alter the extent of interactions.

A. Dielectric Properties

The dielectric properties of foods are very important in describing the way foods are heated by microwaves. The most comprehensive effort on dielectric properties data to date being that of von Hippel [13]. The dielectric properties of foods vary considerably with composition, changing with variation in water, fat, carbohydrate, protein, and mineral content [14]. Dielectric properties also vary with temperature. As indicated earlier, the dielectric properties affect the depth to which microwave energy penetrates into the food to be dissipated as heat. The magnitude of the penetration depth, defined as the depth at which 63% of the energy is dissipated, can be used quantitatively to describe how microwave energy interacts with the food. A large penetration depth indicates that energy is poorly absorbed, whilst a small penetration depth indicates predominantly surface heating.

Dielectric properties data for agricultural products, biological substances, and various materials for microwave processing are widely dispersed in the technical literature [15–18]. Those literature data can provide guidelines, but variability of composition of food products, and other specific conditions for particular applications, often require carefully conducted measurements.

B. Geometrical Heating Effects — Corner, Edge, and Focusing Effects

With conventional cooking methods, heat is transferred from outside to the food product by conduction, convection, or infrared radiation. There is a temperature gradient from the outside to the inside. It is often said that with microwaving, heating takes place from the inside to the outside. This is not true; heating occurs throughout the whole food simultaneously, although it may not be evenly distributed. Probably this misapprehension is due to the fact that surface temperatures tend to be lower than temperatures inside the food (this is because of evaporative cooling and geometrical heating effect). For foods with a high loss factor, most of the microwave energy of a wave impinging on the food will be absorbed near the surface, and penetration and in-depth heating will be limited. In general, the surface will heat more rapidly than the interior, but there are exceptions. Refraction and reflection at interfaces will cause reinforcement of the field pattern near corners and edges of rectangularly shaped foods, resulting in overheating. Core heating effects of the same nature occur in foods of spherical or cylindrical shape at certain dimensions, causing energy concentration and overheating of the central part.

The concentration heating effect means maximum heating occurs in the center for certain spherical and cylindrical geometries [19]. The well-known explosion of eggs during microwave heating is one of the most significant demonstrations of core heating effect. This occurs because center heating cause formation of steam which induces an energy impulse with such high power as to move the surrounding mass parts away from each other. This kind of thermal behavior has already been observed by many people [3, 6, 19, 20] for cylindrical and spherical shaped foods. The maximum heating regions also move slowly from the center towards the surface when the diameter increases. If the diameter is much greater than penetration depth, the temperature profile will be similar to that observed for a "semi-infinite" body. That is, the temperature decreases exponentially from the surface in accordance to Lambert's law which governs the absorption of microwave power. If the diameter is much less than penetration depth, the heating profile will be flat. In between these extremes the focusing effect occurs. Moreover, Mudgett [3] pointed out the effect of salt on drying behavior. With addition of sodium chloride, penetration depth decreases significantly and, therefore, the heating profile could shift from that of focusing and center heating to one of surface heating.

Another reason for uneven heating in lossy products can be traced to the electromagnetic boundary conditions at edges and corners [21]. This is the so called edge and corner effects. In an electric field, where the wavelength is larger than the dimensions of the heated object, field bending will give rise to concentrations at some locations. The convergence of two or more waves at a corner results in a higher volumetric power density than on the flat surface. Higher heating rates will thus be obtained at the corners. If the electric field is strong enough, an arc may emanate from there when the air ionizes [22]. Square containers can cause burning in the corners of the product due to a greater surface area/volume ratio, resulting in more microwave energy absorption. Circular or oval containers help reduce the strong edge and corner effects as energy absorption occurs evenly around the edge but core heating effects may then originate.

C. Microwave Bumping

Another phenomenon during microwave heating is the "bumping" which may occur in microwave cooking. The term, "microwave bumping," also known as microwave popping or microwave splattering, is descriptive of the explosion phenomenon and is characterized by a jostling or shaking of the container, usually accompanied by an audible explosion. When microwave bumping occurs, the explosive sounds which can be heard some distance away are annoying and an unexpected surprise to consumers. Microwave bumping is due to the explosion of food particulates, not localized boiling of the liquid. Increasing the viscosity of the liquid did not result in a significant difference in intensity or frequency of bumping. Degree of microwave bumping is believed to be directly related to local superheating effects. The higher the electric field intensity, the greater the incidence of bumping. Due to edge, corner, and focus heating effects by microwave, container shape influences heating pattern of a food product and location of bumping in the container. Sterilizing vegetable particulates which causes excessive softening and salting food particulates which causes high microwave heating rate are two indispensable conditions to produce microwave bumping [23].

D. Evaporative Cooling and Steam Distillation

During the heating process of foods containing water, the resulting evaporation at the surface causes a depression of the temperature, known as evaporative cooling. The surface of food is seen to be cooler than the region just below the surface and warmer than the surrounding air. This phenomenon is readily seen during the cooking of a meat roast [6, 24].

At the same time, this surface evaporation can cause steam distillation of certain flavor components. Flavor release in microwave cooking is increased by steam distillation. In microwave heating, water vapor (steam) is one of the most important transport mechanisms contributing to movement of flavor compounds within a food matrix.

Individual compounds that make up a flavor which are particularly low molecular weight and water-soluble may be driven off or steam distilled out of the product during microwave heating. Fruit and other "sweet" flavorings are more of a problem. They evaporate easily in foods with high initial water content because they contain a great number of short-chain, volatile flavoring substances. Moreover, they are often of a more hydrophilic character and therefore a great part of the flavoring substances migrates to the aqueous phase of the food, which selectively absorbs the great part of microwave energy [25]. The percent loss may range from less than 10% for high boiling compounds to 95% for very volatile compounds [26]. The latter are the ones that create a strong aroma which is necessary when the flavor is designed to impart a balanced aroma profile in the room during microwave heating [27]. In this case the flavor was added solely for aroma generation and contributed very little to the flavor profile of the microwave product itself. However, this phenomenon, flash-off, often leads to imbalance of flavor concentrations in a finished product with a different character from the flavor that was added before cooking. Formulations that compensate for flash-off may require a highly imbalanced flavor character prior to microwaving. The specific loss is dependent on the types of flavor components used and the food system in which it is incorporated. As the outward migration of water vapor is the most important factor influencing flavor retention in the food product, the flavorings used for microwave application should have low water vapor volatility unless the flavorings are intended to create the "oven aroma" of conventional cooking methods, or to cover undesirable off-notes released during microwave cooking.

E. Lack of Crispness (Texture) and Browning (Color, Flavor) of Microwave Foods

The texture of a microwaveable food may directly affect its acceptance. Toughness or lack of crispness in bread slightly overcooked in a microwave oven may not directly change its flavor, but does influence the consumer's perception of the product. The lack of conventional-styled browning and crisping in microwave ovens is due to the microwave frequency used. At 2450 MHz, the wavelength, 12.2 cm, is too long to create the intense surface heat which occurs at the higher infrared frequencies, limiting the food item to a temperature of approximately 100°C. This is ideal for wet foods like vegetables and stews, but unacceptable for pastry, breaded or batter-coated items, and roast meat. In contrast to the convectively heated food, we have relatively low temperature ambient air (60–75°C) with a rather high relative humidity in most cases during microwave heating. The level of maximum temperature and consequently of maximum water vapor pressure generally lies further below the surface. The main driving force, therefore, is directed towards the surface instead of towards the center [28, 29]. Water vapor generated inside the food continuously migrates to the surface, drawing flavoring substances with it on the way out. As the evaporation rate of water is not high enough to dry out the surface, the evaporated water is continuously replaced by migration of water from the inside [25]. For foods which require a long heating time, e.g., meat joints, the effect can be significant and the resulting moisture loss from the surface of the product can be appreciable. An electromagnetic phenomenon creating "hot" and "cold" spots is inherent in all microwave ovens and is responsible for much of the uneven cooking associated with them. Liquid products quickly dissipate the microwave energy and result in a more uniform product. Solid food products, multiphase systems, or frozen products develop hot and cold spots during heating which further complicate flavor delivery in these systems [27].

During microwave heating the low surface temperature and its much higher water activity (approximately 1.0) and the lack of prolonged baking time have the following consequences: (1) no crust is formed because the necessary physical changes (protein denaturation, starch gelatinization, etc.) are inhibited, and (2) the formation of many flavor compounds and/or pigments (Maillard browning reactions) do not occur to the required extent. Thus, some flavors that typically develop in a conventionally cooked product will not necessarily work in a microwaved product. Van Eijk [25] stated that the differences in flavor generation and the performance of flavoring substances in microwave foods can be explained satisfactorily by the differences in heating pattern, the corresponding differences in water vapor migration, and the resulting physical changes, particularly at the surface of the food. No athermal effects have been observed.

F. Food Ingredients

Some of the ingredients in foods such as water, ionized salts, and fats and oils, in particular, and the distribution of these ingredients in the food product, exert a strong influence on temperature level and distribution. These ingredients interact physically and chemically to an extent dictated by numerous factors including mode of heating.

The dielectric and thermal properties of foods can be modified by adjusting food ingredients and formulations and are manageable within certain limits. Frozen pure water has no microwave dipole relaxation and is therefore microwave transparent. Frozen foods, however, are not microwave transparent since some of the water is still in free liquid form. So when deep-frozen foods are defrosted by microwave energy, particularly difficult problems arise once both ice and water are present. Hot spots and runaway heating may be the consequence in this case. Fats have a low dielectric loss and consequently do not generate as much heat directly from the microwave field. Once

heat has been generated, conduction and convection become the main mechanisms of heat transfer. Fats reach very high temperatures due to their high boiling points, whereas water is limited to a maximum temperature of 100°C. However, since the heat capacity of fats is about half that of water, they heat more quickly in the microwave.

Factors that affect dielectric properties of water, including the presence of other interactive constituents such as hydrogen bonding resulting due to the presence of glycerol and propylene glycol, and sugar and carbohydrate-like polyhydroxy materials will also impact microwave heating [30]. Salts and sugars can be used to modify the browning and crisping of food surface. Heating a sample with higher salt content can change the microwave heating pattern from center heating to surface heating [31]. In addition to direct microwave interactions, lipids, salts, sugar, and polyhydroxy alcohols can also raise the boiling point of water. This allows the food to reach a higher temperature needed for the development of reaction flavors, and Maillard browning reactions.

To obtain useful and meaningful information on the contributions of rates of flavor migration and kinetics of degradation under various conditions, Fu and others [32] designed an apparatus for on-line measurement of flavor concentration, to formulate a thermally stable flavor-dough system and to accomplish isothermal heating. Photoionization detection method [33] and a cold-trap, on-line sampling method [34] were to investigate migration of flavor compounds in a solid food matrix subjected to microwave heating. As the moisture concentration decreased below 0.1 g water/g solid during microwave heating of gelatinized flour dough, a type of encapsulation occurred that prevented flavor from being released. The results of microwave reheating of limonene-formulated dough showed limonene is very stable and no significant limonene concentration profile in the sample and less than 1% overall change in total limonene concentration [35].

VI. MICROWAVE PROCESSING

In the quest for better quality of shelf-stable, low-acid foods, a number of emerging technologies have been considered [36]. Food engineering will continue to evolve. Although alternative processes have been developed over the years, thermally processed food products maintain a clear dominance in the marketplace, primarily as a result of the wealth of theoretical and empirical knowledge that has been developed regarding thermal inactivation of pathogenic microorganisms and their spores [37]. Microwave sterilization is a nontraditional but solely a thermal process and so can be regarded by technologist and regulators as another terminal thermal sterilization technique.

Microwave heating offers numerous advantages in productivity over conventional heating methods such as hot air, steam, etc. These advantages include high speed, selective energy absorption, excellent energy penetration, instantaneous electronic control, high efficiency and speed, and environmentally clean processes [38]. Currently, microwave and radio-frequency both are laboratory or pilot scale and there are no known large operating microwave systems operating in the food industry, except for bacon precooking or tempering. It remains a very exciting processing tool, unmatched by any other technology if attention is paid to their selection.

A. Drying and Dehydration

Microwave drying is rapid, more uniform, and energy efficient compared to conventional hot air drying and sometimes it results in an improvement in product quality. But it is highly unlikely that an economic advantage will be demonstrated if only bulk water removal by microwave heating is desired, such as occurs in the constant-rate region [39]. During the falling-rate period because of the low thermal conductivity and evaporative cooling effect, high product temperatures are not easily obtained using convective drying. Surface hardening and thermal gradients again provide further resistances for moisture transfer. Actually, it has been suggested that microwave energy should be applied in the falling rate period or at low moisture content for finish drying [40–43]. Correspondingly sensory and nutritional damage caused by long drying times or high surface temperatures can be prevented. It is important to understand the dielectric properties of the material with different moisture content during microwave drying. The ability of dielectric heating to heat selectively areas with higher dielectric loss factors and the potential for automatic moisture leveling afford a major advantage for even drying of these types of materials [39].

Because internal microwave heating facilitated a more predominant vapor migration from the interior of the material as compared to that during conventional drying, microwave dried products have been reported to show a higher porosity because of the puffing effect caused by internal vapor generation [44–46]. Similar results are also found for pasta drying. Microwave drying produces a slightly puffed, porous noodle which rehydrates in half the time required for noodles dried by conventional methods [47]. Tong and others [44] investigated temperature and pressure distribution in a dough system with porosity ranging from 0.01 to 0.7 during microwave heating using miniature fiber optic temperature and pressure probes. Pressure build-up to approximately 14 kPa occurred during the initial stages of the heating process when the initial porosity was less than 0.15 and disappeared when the pressure exceeded the rupture strength of the dough. Volume expansion was observed up to the point where the dough sample ruptured, producing visible cracks in the structure. So microwaves produce a pressure gradient that pumps out the moisture [44]. This property can be used to

advantage to speed up the drying process. If the pressure build-up did not exceed the rupture strength of the structure it might be the result of such enhanced porous structure of the samples. So, it is a difficult task for reducing drying time and increasing quality at the same time. Careful studies need to be done by applying the right amount of microwave energy in the process.

Nonuniformities in the microwave electric field and associated heating patterns can lead to high temperature in various regions dried earlier, causing product degradation [48]. Improvement can be achieved by using a fluidized bed dryer, or spouted bed dryers to average the uneven electric field [49, 50]. The combination of microwave and vacuum drying [51–54] or freeze drying [55–59] also has a certain potential. The vacuum process opens the cell structures (puffing) due to the fast evaporation and an open pore structure is generated. Reduced drying time is the primary advantage of using microwaves in the freeze-drying process. But no commercial industrial application can be found, due to high costs and a small market for freeze dried food products.

Pasta and potato chips have been dried successfully. Freeze drying and vacuum drying, in conjunction with microwave energy, have also shown promise and interest from an academic point of view but not meeting the economic criteria. A relative new and successful combination of microwave energy and frying process is used to produce fried goods, such as chips, noodles, and chickens, with 60% reduced time, 50% reduced fat content, and 33–60% energy saving [60].

B. Pasteurization and Sterilization

Pasteurization provides a partial sterilization of substances by inactivating pathogenic microorganisms, notably vegetative cells of bacteria, yeast, or molds. Products have to be refrigerated. Sterilization processes are designed to inactivate microorganisms or their spores. Thermal sterilization is usually done at temperatures in excess of 100°C which means they are usually done under pressure. Industrial microwave pasteurization and sterilization systems have been reported on and off for over 30 years [61–68]. Studies with implications for commercial pasteurization and sterilization have also appeared for many years [69–77]. Early operational systems include batch processing of yogurt in cups [78] and continuous processing of milk [79]. A very significant body of knowledge has been developed related to these processes. As of this writing, two commercial systems worldwide could be located that currently perform microwave pasteurization and/or sterilization of foods [68, 80]. As a specific example, Tops Foods (Belgium) [68] produced over 13 million ready meals in 1998 and have installed a newly designed system in 1999.

Microwave pasteurization can reduce the come-up time, which can shorten to a small fraction of the time used by the conventional process. After this, the microwave heated meals pass into a nonmicrowave hot air tunnel for the hold-time period, and then to the cooler. Microwave is difficult to hold a constant temperature and should not be used. Especially in Europe, food pasteurization by microwave processing has been successfully accomplished for decades. The major advantage of the microwave process is that the product may be pasteurized within a package. A product goes through the line in wrapping continually, package by package, pallet by pallet. Shelf life can be extended from days to over a month without preservatives.

Typical sterilization temperatures in the product may be 121–129°C (250–265°F) with hold times of 20–40 minutes. The come-up time may be significantly reduced by microwaves. This reduced come-up time would provide greater product quality. The enhanced quality retention is due to the fact that quality attributes normally have much lower activation energy (10–40 kcal/mol) than the microbial spores (50–95 kcal/mol). The heat-up time of the microwave process is much faster than that in a retort, so the product's organoleptic (texture, color, and flavor) and nutritional qualities could be considerably improved. Microwave sterilization is more flexible than ohmic heating and aseptic processing. It can sterilize liquids, semi-solid, and solids and it can also sterilize pre-packed food products.

There are several practical concerns and problems that have to be solved before it can be applied at the industrial level. The main issue has been the regulation of process parameters so that commercial sterility can be achieved. For the conventional retort process, by monitoring the time-temperature history at the cold point using a thermocouple thermometer, it is reasonably easy and accurate to determine the microbial lethality through mathematic calculations. But, determining the microbial lethality for a microwave sterilization process is not straightforward. The cold point during microwave sterilization is not always located on the central axis. The problem of providing a uniformly heated product makes it extremely time-consuming and costly to adjust the microwave pattern to produce the quality advantage theoretically possible by using microwaves. Each product could require custom adjustment. The presence of uneven heating (hot and cold spots) makes it very difficult to ensure that all portions of a meal have reached a kill temperature. Microbiological safety is the major reason for the slow acceptance of microwave sterilization. In addition, the technical ability to accurately measure the temperature distribution throughout an entire microwave sterilized product has not been demonstrated. From the engineering point of view, no computer simulation models are available for investigating the feasibility of microwave sterilization. These computer simulation models are not only required by the Food and Drug Administration (FDA) for regulating and

approving microwave sterilization processes, but also highly demanded by the food industry for performing the cost/ benefit analyses. Without the reliable inputs of dielectric properties, thermophysical properties, and boundary conditions, a computer model is completely useless. Unfortunately, literature values on these properties are only available at room temperature to 60°C and not readily available at sterilization temperature.

In Europe, microwave-sterilized foods, primarily pasta dishes such as lasagna and ravioli, are on many grocery shelves with no reported difficulties. Safety regulations are less stringent in Europe. For example, in one implementation [68] the process design consists of microwave tunnels with several launchers in relation to the number of products (ready meals). Microwave-transparent and heat-resistant trays are used with shapes adapted for microwave heating. Exact positioning of the package is made within the tunnel and the package receives a pre-calculated, spatially varying microwave power profile optimized for that package. The process consists of heating, holding, and cooling in pressurized tunnels. The entire operation is highly automated. But, microwave sterilization has not been approved on the use of microwave processing for food sterilization by the Food and Drug Administration in the United States (US-FDA). Today, there continues to be a great deal of interest and some R&D activity in pasteurization and sterilization by microwaves [81–88].

C. Tempering and Thawing

Thawing and tempering of frozen food materials is an important part of some food processes, especially in the meat industry and food service. Reducing thawing time by higher temperatures results in a decrease in product quality such as more dip loss and surface drying in addition to increased risk of microbial growth.

Frozen foods can be considered to be the mixture containing two components: fixed structure of ice and biological material surrounded by monomolecular layer of strongly bound water, and loose liquid water saturated with dissolved salts. Dielectric activity of this mixture is much higher than that of pure ice, but much less than that of the same material at temperatures above zero. The loss factor (ε'') of water is approximately 12; while that of ice is approximately 0.003. The penetration depth in water (1.4 cm) is much lower than in ice (1160 cm) [89]. If the thickness values is much greater than penetration depth, the temperature profile will be similar to that observed for a "semi-infinite" body. That is, the temperature decreases exponentially from the surface in accordance to Lambert's law. Surface layers thus absorb more energy and heat up a little bit faster than the inside of the product. But for thickness values smaller than a certain value, resonance can still not be avoided and inside of a slab can be heated directly at high intensity, resulting in quick thawing. As the loss factor increases with the temperature, the surface heats up faster and faster and the penetration depth continually decreases. Spots of free water and spots that have reached temperature 0°C absorb more energy than crystals of ice, which leads to further acceleration of heating.

Microwave energy penetrates a food material and produces heat internally. The main advantage consists of speed because tempering by microwave takes minutes instead of hours or even dozens of hours. For example, a 20 cm thick piece of beef, frozen to −16°C, thaws more than 10 hours at the surrounding temperature of +4°C. On the other hand, the whole cycle of MW tempering with following slicing, modification, and repeated freezing takes only 30 minutes [90]. There are at least 400 tempering systems operating in the United States alone. Food is heated to just under freezing temperatures, allowing easy chopping, cutting, processing, etc. In the United Kingdom there are several large systems, up to 200 kW, utilized for tempering of frozen beef, as well as butter. The lower frequencies, e.g., 915 MHz band, are used to advantage for MW thawing and tempering of larger blocks of food. For example, when tempering 18 cm thick blocks at 915 MHz frequency, temperature gradient is half of the gradient for 2450 MHz frequency [90]. 915 MHz tempering systems, batch and continuous, are sold worldwide.

Microwave thawing remains a major problem. A main difficulty is formation of wide temperature gradients (run-away heating) within the product. The preferential absorption of microwaves by liquid water over ice is a major cause for run-away heating. Maximum homogeneity is achieved with temperatures slightly above zero. After that the inhomogeneity rises again. Therefore it is advantageous to reduce the thawing process to plain tempering, i.e., to stop the heating at the temperatures −5 to −2°C. Another reason why tempering is preferred is that the progress of energy consumption is dependent on the temperature. With most biological materials and water, the energy consumption starts to rise sharply at temperatures above −5°C; the less fat they contain the higher is the consumption. Since the thawed material has a much higher dielectric loss, microwave penetration depth at the surface is significantly reduced, in effect developing a "shield." Surface cooling helps to reduce the gradient in a frozen food, thus enabling the microwave power to remain on longer to decrease the thawing time. The temperature uniformity during microwave thawing can be improved when appropriate sample thickness, microwave power level, frequency, and/or surface cooling are applied [64, 91–93]. Today, there continues to be a great deal of interest and some R&D activity in thawing and tempering by microwaves [94–97].

D. Baking

Baking, in all cases except unleavened products, involves the creation, expansion, and setting of edible foams through

the use of heat. Proofing is the step of causing the dough to rise and precedes the final baking or frying in the case of donuts. During the baking of raw bread dough significant volume change occurs, and the dough is converted from a viscoelastic material containing airtight gas cells with the ability to expand to a rigid structure which is highly permeable to gas flow. The cell walls are elastic but strong and the increasing gas pressure must cease while the cell walls set. Baking is a complex physicochemical reaction in which all the events must be carefully timed and must occur in a well-defined sequence. All baked products form some sort of crust which acts as a shield, making heat even harder to reach the inside. The heat transfer problems encountered by conventional means can be easily overcome by microwave heating. Pei [98] reviewed heat and mass transfer in the bread baking process and discussed the application of microwave energy. Goedeken [99] investigated microwave baking of bread dough with simultaneous heat and mass transfer. Highly porous products, such as bread, lend themselves well to the use of microwave energy because of greater penetration of microwave energy resulting in more uniform energy distribution within the product. But the microwave application must be carefully controlled or heating and expansion will occur too quickly, and while the cake may look fully expanded and baked, it will collapse to a pancake when the microwave energy is removed.

Bread baking by means of microwave energy was first reported in the literature by Fetty [100]. Decareau [101] noted the possibility of combining microwave energy and hot air to produce typically brown and crusted loaves of bread in a shorter time than by conventional baking methods. One microwave baking process that was quite successful for several years was the microwave frying of doughnuts. Frying times of approximately two-thirds normal time are possible with 20% larger volumes, or 20% less doughnut mix required for standard volume. Fat absorption can be 25% lower than conventional. This proofing system was developed by DCA Food Industries, which operated 2450 MHz and varied in output from 2.5 to 10 kW for production rates of 400–1500 dozen doughnuts per hour [102–104]. One difficulty in the microwave baking process was to find a microwavable baking pan that is sufficiently heat resistant and not too expensive for commercial use. A patent by Schiffmann and others [105] describes microwave proofing and baking of bread in metal pans. This technique utilizes partial proofing in a conventional proofing followed by proofing in a microwave proofer utilizing warm, humidity-controlled air and reduces the proofing time by 30–40%. This was then followed by microwave baking in a separate oven. Four patents by Schiffmann and others [104–107] describe procedures for the baking of bread utilizing metal pans and, in some cases, also provided for partial proofing of the bread in the pans. In the procedure described in the aforementioned patents, the microwave baking process involved the simultaneous application of microwave energy and hot air to both bake and brown the bread, producing thoroughly browned and crusted loaves of comparable volume, gain structure, and organoleptic properties. It was found that the use of either 915 MHz or combinations of 915 and 2450 MHz were quite effective in baking a loaf of bread. The system of microwave frying doughnuts was very successful for quite some time during the 1970s. These doughnuts have longer shelf life, better sugar stability, and excellent eating quality. The larger volume and lower fat absorption provided high profits for the bakery.

To date, some very sophisticated packaging along with advanced susceptor technology has been the predominant solution to the lack of conventional-styled browning and crisping. Susceptors rapidly heat to temperatures where browning readily occurs and thus help produce flavor in the product. However, susceptors solve the flavor-related problems only on the surface. Another possible solution to the lack of browning during microwave cooking is the addition of compounds which give a roasted or toasted reaction flavor. Today, there continues to be a great deal of interest and some R&D activity in baking by microwaves [108–115].

VII. RADIO FREQUENCY PROCESSING

Radio frequency and microwave heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material [2, 116, 117]. Radio frequency heating, which is at a much lower frequency, has thrived as an industry alongside microwaves over the decades. Radio frequency heating in the United States can be performed at any of three frequencies: 13.56, 27.12, and 40.68 MHz. The heating mechanism of radio frequencies is simply resistance heating which is similar to ohmic heating. This lossy dielectric arises from the electrical conductivity of the food and is different from the resonant dipolar rotation of microwave frequencies.

Unlike microwave sources, one cannot purchase an RF high power source. Due to the high impedance nature of RF coupling, the RF source and applicator normally need to be designed and built together. Manufacturers of RF equipment develop the whole system, rather than only the power source. Therefore, developments in RF processing must involve the commercial RF manufacturers. RF equipment is available commercially at much higher power levels than microwave sources. While commercial microwave sources are available only below 75 kW, RF equipments at hundreds of kW are very common. At these high levels, the price per watt of RF equipment is much cheaper than microwaves. In addition to higher power and lower cost, another advantage of RF equipment over microwaves is in the control area. In high power RF systems, the source and the load are commonly locked together in a feedback circuit. Therefore, variations in the load can be followed by the source without external controls [118].

Microwave or radio frequency? For the same electric field, the higher the frequency, the higher the amount of power into the material. This is the reason why microwaves are a conceptually more effective means of heating. However, RF equipment has several advantages which workers in the field of microwave processing may find more suitable for scale-up of some processes. The microwave fields attenuate within the bulk of conductive materials and materials with high dielectric loss. The penetration depth of microwaves is much lower. This is particularly troublesome for larger scale processes. But, this type of nonuniformities are frequency dependent and become less severe as frequency is lowered. Because of much longer wavelengths of radio frequencies, they have better uniformity. Also, the depth of penetration is much higher. So, in cases where uniformity of heating is a critical issue, use of the radio frequencies and 915 MHz microwave frequency may have potential for the future [119, 120].

Working at radio frequencies allows it to process a large range of material types, from the thin wide webs of the paper industry to large three-dimensional objects like textile packages. In general terms, microwave is better for irregular shapes and small dimensions and RF is better for regular shapes and large dimensions. Microwave is more suitable for hard to heat dielectrics. Actually, many applications can be done by either, but RF is cheaper if it fits. RF equipment is easier to engineer into process lines, and can be made to match the physical dimensions of the up- and down-stream plant. In the case of microwaves, in a continuous process, complex arrangements may be necessary to allow the product to move in and out of the enclosure without giving rise to excessive leakage of energy [121]. This is because the wavelengths at microwave frequencies (e.g., 12.54 cm at 2450 MHz) are very much shorter than those at radio frequencies (e.g., 1100 cm at 27.12 MHz).

An overview of food and chemical processing uses of radio frequency can be seen in Minett and Witt [122] and Kasevich [123]. The industrial applications using radio frequency include textiles (drying of yarn packages, webs, and fabrics), food (bulk-drying of grains; moisture removal and moisture leveling in finished food products), pharmaceutical (moisture removal in tablet and capsule production processes), and woodworking (adhesive curing for wood joinery). Radio frequency heating has been used in the food processing industry for many decades. The post-baking of biscuits, crackers, and snack foods is one of the most accepted and widely used applications of RF heating in the food processing industry. A relative small RF unit can be incorporated directly into a new or existing oven line (a hot air oven or conventional baking line) to increase the line's productivity and its ability to process a greater range of products. The benefits of RF-assisted baking are precise moisture control, reduced checking, improved color control, and increased oven line throughput [124]. RF drying is intrinsically self-leveling, with more energy being dissipated in wetter regions than in drier ones [121]. This RF leveling leads to improvements in product quality and more consistent final products. Recently, RF cooking equipment for pumpable foods has been developed. These devices involve pumping a food through a plastic tube placed between two electrodes, shaped to give a uniform heating [125]. The primary advantage of improved uniformity of heating was also shown for in-package sterilization of foods in large packages using radio frequency at 27.12 MHz, although enhanced edge heating continued to be an issue [120]. Defrosting of frozen food using RF was a major application, but problems of uniformity with foods of mixed composition limited the actual use. The interest in RF defrosting has increased again in the last number of years [125].

Today, the use of a more recent 50 Ω RF heating equipment which allows the RF generator to be placed at a convenient location away from the RF applicator gives the possibility of an advanced process control [126]. Whether conventional or 50 Ω dielectric heating systems are used, the RF applicator has to be designed for the particular product being heated or dried. RF post-baking, RF-assisted baking, and RF meat and fish defrosting systems will continue to benefit both existing and emerging food applications and the availability of low cost RF power sources could lead to a major growth in the use of RF heating in the commercial food sectors.

RF heating is well established in industry and, for many applications, it is the standard method. Its equipment is well proven and also reliable. It is an excellent choice where it fits.

VIII. CONCLUSION

The fundamentals of microwave heating should be studied in depth before spending a great deal of effort and time on trial and errors.

Microwave and radio frequency heating all provide a product that is potentially superior in quality to the product produced by conventional techniques. This point is key to almost all industrial processes. The potential synergistic effects of microwaves combined with steam, forced-air convection, and/or infrared will probably lead the future expansion of microwave processing technology.

Microwaves are an extremely expensive way to evaporate water as compared to frying, high-velocity hot air, or infrared. They can be commercially successful if the products are of high intrinsic economic value and can carry the extra cost burden put on them.

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126 Pulsed Electric Field in Food Processing and Preservation

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CONTENTS

Pulsed electric field (PEF) processing is a non-thermal method used to maintain food safety and increase shelf life of foods by inactivating spoilage and pathogenic microorganisms. Many researchers have investigated this problem, including Sale and Hamilton (82, 83), Mizuno and Hori (64), Jayaram *et al.* (36), Qin *et al.* (75), and Pothakmury *et al.* (71). PEF processing is advantageous over other methods because the changes in product color, flavor, and nutritive value during the treatment are minimized (19, 40–42). A high intensity pulsed electric field processing involves the application of pulses of high voltage (typically 20–80 kV/cm) to foods placed between two electrodes. PEF treatment is conducted at ambient, sub-ambient, or slightly above ambient temperatures for less than 1 s, and energy loss due to heating of foods is minimized. For food quality attributes, PEF technology is considered superior to traditional heat treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods (78). Although some studies have concluded that PEF preserves the nutritional components of foods, effects of PEF on the chemical and nutritional aspects of foods must be better understood before PEF can be used in food processing (74).

Some important aspects in pulsed electric field technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature, and the design of electrodes that minimize the effect of electrolysis. The large field intensities are achieved through storing a large amount of energy in a capacitor bank (a series of capacitors) from a DC power supply, which is then discharged in the form of high voltage pulses (108). Studies on energy requirements have concluded that PEF is an energy-efficient process compared to thermal pasteurization, particularly when a continuous system is used (73).

I. TREATMENT CHAMBERS AND EQUIPMENT

Currently, there are only two commercial systems available (one by PurePulse Technologies, Inc. and one by

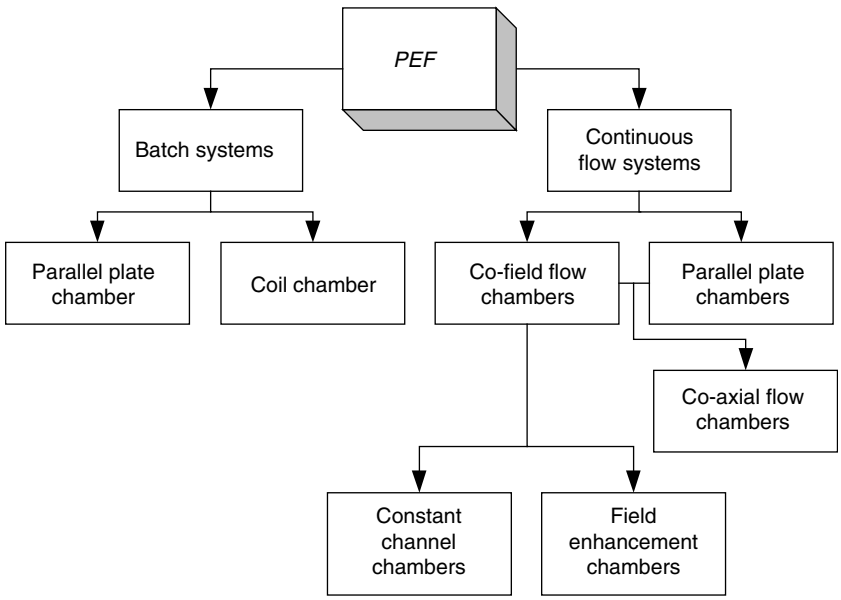

FIGURE 126.1 Classifications of PEF treatment chambers.

Thomson-CSF). Different laboratory- and pilot-scale treatment chambers have been designed and used for PEF treatment of foods. They are classified as static/batch (U-shaped polystyrene and glass coil static chambers) or continuous (chambers with ion conductive membrane, chambers with baffles, enhanced electric field treatment chambers, and coaxial chambers), see Figure 126.1.

A diagram for PEF processing of foods is depicted in Figure 126.2. The test apparatus consists of seven major components (25): a high-voltage power supply, an energy storage capacitor, a treatment chamber(s), a pump to conduct food though the treatment chamber(s), a cooling device, measuring devices (voltage, current, and temperature measurements), and a computer to control operations.

A. Batch Type Processing ("Static") Chambers

1. Parallel Plate Electrode Chambers

This model consists of two carbon electrodes supported on brass blocks placed in a U-shape polystyrene spacer (Figure 126.3a). Different spacers regulate the electrode area and amount of food to be treated. The brass blocks are provided with jackets for water recirculation and controlling temperature of the food during PEF treatment. This chamber could support a maximum electric field of 30 kV/cm. The second chamber model designed by Dunn and Pearlman (19) consists of two stainless steel electrodes and a cylindrical nylon spacer. Another model (3) consists of two round-edged, disk-shaped stainless steel electrodes, with polysulfone used as an insulation material. The effective electrode area is 27 cm^2 and the gap between electrodes can be selected at either 0.95 or 0.5 cm. The chamber can support up to 70 kV/cm. Water circulating at pre-selected temperatures through jackets built into electrodes provides cooling of the chamber.

2. Glass Coil Static Chambers

A model proposed by Lubicki and Jayaram (59) uses a glass coil surrounding the anode. The volume of the chamber is 20 cm^3, which requires filling liquid with high conductivity and similar permittivity to the sample (media — NaCl

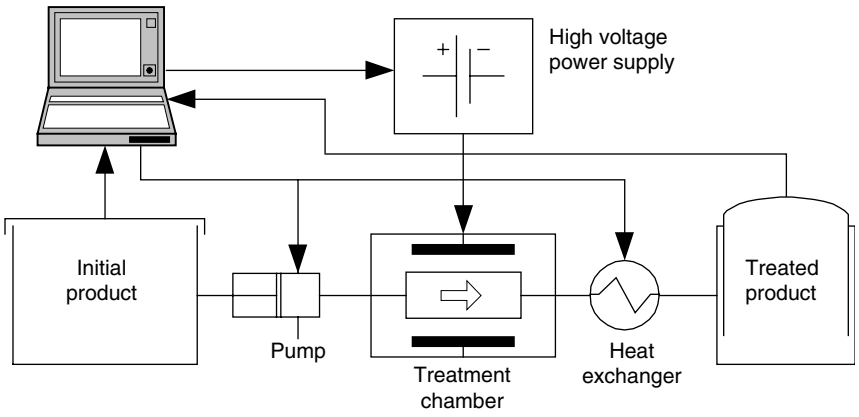

FIGURE 126.2 Flow chart of PEF food processing.

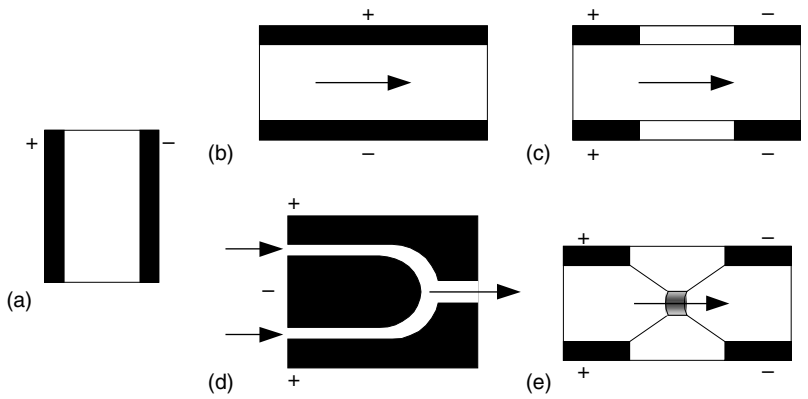

FIGURE 126.3 Different PEF treatment chambers: a — parallel plate chamber, b — continuous flow parallel plate chamber, c — co-field flow chamber, d — coaxial continuous chamber, e — enhanced electric field continuous treatment chamber.

solution, $\sigma = 0.8$ to 1.3 S/m; filling liquid (water) $\sim 10^{-3}$ S/m) to be used because there is no inactivation with a non-conductive medium (silicone oil).

B. Continuous Flow PEF Chambers

Continuous flow PEF treatment chambers (77) are suitable for large-scale operations and are more efficient than the static chambers.

1. Parallel Plate Chambers (Figure 126.3b)

The first experimental chambers were designed to treat a confined, static volume. Some of the first designs incorporated parallel plate geometry using flat electrodes separated by an insulating spacer. The major disadvantage of this type of design is the low productivity of these chambers. Due to the electric field strength limitation it is difficult to increase product load and make this chamber more efficient.

2. Co-Field Flow Chambers

Co-field chambers described by Yin *et al*. (106) have two hollow cylindrical electrodes separated by an insulator to form a tube through which the product flows (Figure 126.3c). Field distribution in a co-field chamber is not expected to be uniform, though some useful advantages may be gained by special shaping of the insulator. The primary advantage of co-field chambers is that they can be designed to operate in PEF systems at lower currents than the coaxial chambers.

3. Coaxial Continuous PEF Chambers

Coaxial chambers are generally composed of an inner cylinder surrounded by an outer annular cylindrical electrode that allows food to flow between them, see Figure 126.3d. A protruded outer electrode surface enhances the electric field within the treatment zones and reduces the field intensity in the remaining portion of the chamber. The electrode configuration was obtained by optimizing the electrode design with a numeric electric field computation. Using the optimized electrode shape, the prescribed field distribution along the fluid path without an electric field enhancement point was determined. This treatment chamber has been used successfully in the inactivation of pathogenic and non-pathogenic bacteria, molds, yeasts, and enzymes present in liquid foods such as fruit juices, milk, and liquid whole eggs (3).

4. Enhanced Electric Field Continuous Treatment Chambers

Yin *et al*. (106) applied the concept of enhanced electric fields in the treatment zones by development of a continuous co-field flow PEF chamber with conical insulator shapes to eliminate gas deposits within the treatment volume (Figure 126.3e). The conical regions were designed so that the voltage across the treatment zone could be almost equal to the supplied voltage.

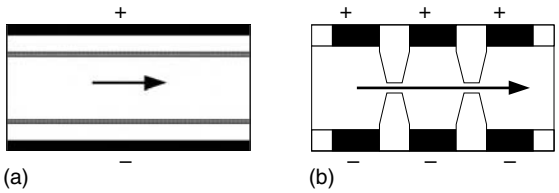

FIGURE 126.4 Special design chambers: a — continuous treatment chamber with ion-conductive membranes separating the electrode and food, b — continuous treatment chamber with electrode reservoir zones.

C. Special Design Flow-Through Chambers

1. Continuous Chamber with Ion Conductive Membrane

One design by Dunn and Pearlman (19) consists of parallel plate electrodes and a dielectric spacer insulator (Figure 126.4a). The electrodes are separated from the food by conductive membranes made of sulfonated polystyrene and acrylic acid copolymers. An electrolyte is used to facilitate electrical conduction between electrodes and ion permeable membranes.

2. Chamber with the Electrode Reservoir Zones

Another continuous chamber described by the same authors (19) is composed of electrode reservoir zones instead of electrode plates (Figure 126.4b). Dielectric spacer insulators have slot-like openings (orifices) between which the electric field enhances. The average residence time in each of these two reservoirs is less than 1 min.

II. MECHANISMS OF MICROBIAL INACTIVATION

The application of electrical fields to biological cells in a medium (for example, water) causes build-up of electrical charges at the cell membrane (84). Membrane disruption in many cellular systems occurs when the induced membrane potential exceeds a critical value of 1 V, which, for example, corresponds to an external electric field of about 10 kV/cm for *E. coli* (15). Several theories have been proposed to explain microbial inactivation by PEF (2, 7, 44, 85, 86, 100, 105). Among them, the most studied (see Figure 126.5) are electrical breakdown and electroporation or disruption of cell membranes (113).

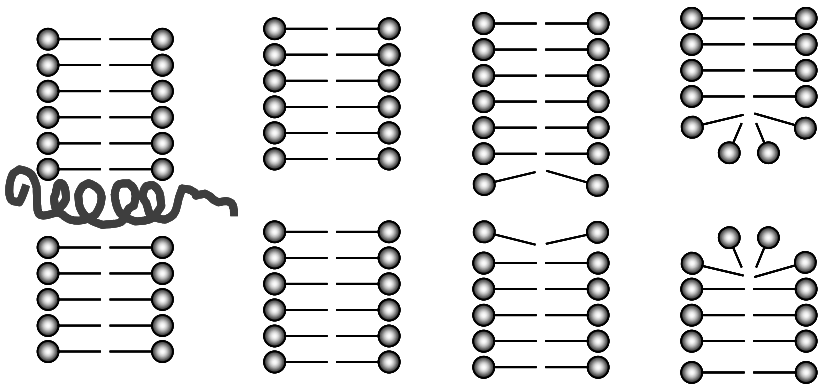

FIGURE 126.5 Schematic diagram of reversible and irreversible breakdown: pore development and cell membrane disruption.

A. Electrical Breakdown

Zimmermann (112) explains what electrical breakdown of cell membrane entails. The membrane can be considered as a capacitor filled with a dielectric (Figure 126.5). The normal resisting potential difference across the membrane V_m is 10 mV and leads to the build-up of a membrane potential difference V due to charge separation across the membrane. V is proportional to the field strength E and radius of the cell. The increase in the membrane potential leads to reduction in the cell membrane thickness. Breakdown of the membrane occurs if the critical breakdown voltage V_c (of the order of 1 V) is reached by a further increase in the external field strength. It is assumed that breakdown causes the formation of transmembrane pores (filled with conductive solution), which leads to immediate discharge at the membrane and thus decomposition of the membrane. Breakdown is reversible if the product pores are small compared to the total membrane surface. With electric field strengths above critical and long exposure times, larger areas of the membrane are subjected to breakdown. If the size and number of pores become large in relation to the total membrane surface, reversible breakdown turns into irreversible breakdown, which is associated with mechanical destruction of the cell membrane.

The corresponding electric field is $E_{critical} = V_{critical}/f_a$, where a is the radius of the cell and f is a form factor that depends on the shape of the cell (84). For spherical cells f is 1.5; for cylindrical cells of length l and hemispheres of diameter d at each end, the form factor is $f = l\,(1 - d)/3$. Typical values of $V_{critical}$ required for the lysing of *E. coli* are of the order of 1 V. The critical field strength for the lysing of bacteria with a dimension of approximately 1 μm and critical voltage of 1 V across the cell membrane is therefore on the order of 10 kV/cm for pulses of 10 microseconds to milliseconds in duration (84).

B. Electroporation

Electroporation is the phenomenon which occurs when a cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes (15, 43, 45, 87, 114). The plasma membranes of cells become permeable to small molecules after being exposed to the electric field, and permeation then causes swelling and eventual rupture of the cell membrane (46, 88, 91, 92). The main effect of an electric field on a microorganism cell is increasing of the membrane permeability due to membrane compression and poration (31, 79, 103–105). Kinosita and Tsong (50) demonstrated that an electric field of 2.2 kV/cm induced pores in human erythrocytes of approximately 1 nm in diameter. They suggested a 2-step mechanism for pore formation in which the initial perforation is a response to an electrical suprathreshold potential followed by a time-dependent expansion of the pore size (Figure 126.5). Large pores are obtained by increasing the intensity of an electric field and pulse duration or reducing the ionic strength of the medium (26, 43).

III. EVENTS OF ELECTROPORATION AND MICROBIAL LYSIS

A. Electric Field-Induced Transmembrane Potential

We now know that when a cell (radius = R_{cell}) suspended in a medium is exposed to external electric field (direct current of strength E_{appl}), there is a rapid redistribution of cations in the vicinity of the plasma membrane, thus generating a transmembrane potential $\Delta\psi_{membr}$ with a rise time, τ_{membr}:

$$\Delta\psi_{membr} = 1.5R_{cell}E_{appl}\cos\theta\,[1 - \exp(-t/\tau_{membr})] \quad (126.1)$$

$$\tau_{membr} = R_{cell} C_{membr} (r_{\text{int}} + r_{ext}/2) \qquad (126.2)$$

Here θ is an angle between the field line and the normal from the center of the spherical cell to a point of interest on the membrane surface; C_{membr}, r_{int}, and r_{ext} are the membrane capacitance (per unit area), and the resistivities of the cytoplasmic fluid and the external medium respectively (17). For biological cells of micrometers in diameter, $\tau_{membr} < 1\,\mu s$ and the exponential term in equation (126.1) approaches zero within 1 μs. Cells of larger diameters have $\tau_{membr} > 1\,\mu s$ (49). The maximum transmembrane potential generated in a cell with the DC electric pulse a few times longer than τ_{membr} is

$$\Delta\psi_{membr,\,\max} = 1.5 R_{cell} E_{appl} \qquad (126.3)$$

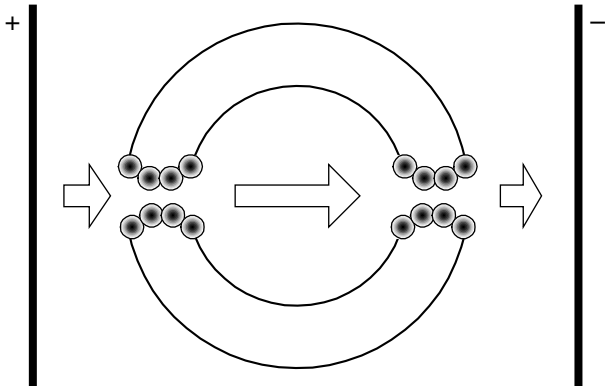

FIGURE 126.6 Electroosmosis-induced hydrodynamic flow toward the negative electrode regardless of whether electropores are in positive-facing or negative-facing hemispheres.

B. Kinetics of Electroporation in Cell Membranes

The plasma membrane of a cell is the first site of the electric interaction. Beside lipids, there are proteins, carbohydrates, and other types of molecules, most of which are either charged or polarizable. Channel proteins are especially sensitive to the $\Delta\psi_{\text{membr}}$, and each type of channel has a range of $\Delta\psi_{\text{membr}}$ in which it becomes conductive. The range of $\Delta\psi_{\text{membr}}$ for opening protein channels is approximately 50 mV, considerably smaller than the dielectric strength of the lipid bilayer, which is in the range of 150–400 mV. Like a lattice defect of the lipid bilayer, once a protein channel is forced to open, a strong current greatly exceeding the normal conductance of the channel will generate local heat sufficient to denature the protein. This denaturation could be reversible or irreversible, depending on the extent of temperature change and the properties of the channel. The time of opening/closing of the protein channel is in the submicrosecond time range (94). Thermal denaturation of a protein takes milliseconds to seconds. Renaturation of a protein occurs in seconds (48). Electropores in lipid domains will reseal within seconds (93). Closing of PEF-perforated protein channels should transpire in milliseconds. However, repairing of a PEF-damaged cell membrane will take minutes to hours (50).

C. Colloid Osmotic Lysis

A major difference between electroporation of lipid vesicles and that of cells is the colloid osmotic lysis of cells (50). A PEF-perforated cell membrane loses its permeation barrier to ions and small molecules but not necessarily to proteins. The electroperforated membrane becomes semipermeable to cytoplasmic macromolecules. The osmotic pressure of these macromolecules causes the cell to swell. This process, known as colloidal swelling, eventually leads to a rupture of the plasma membrane because of the excessive osmotic pressure imposed on the cells.

Colloidal osmotic pressure in the PEF-treated red blood cells was identified as the main cause of the electric field-stimulated hemolysis. Colloidal swelling depends on the osmotic imbalance of the cytoplasm and the suspending medium. When the difference is large, PEF-treated cells will swell in the minute time range. During this swelling phase, electropores in cell membranes also begin to reseal. If the resealing takes place faster than the swelling, cells will shrink again and recover their original volume, thus averting membrane rupture. If, on the other hand, the resealing is slower than the swelling, the plasma membrane of cells will be ruptured. The colloidal osmotic lysis may be prevented by balancing the osmotic pressure of the cytoplasm and the medium.

D. Electroosmosis in Electropores

An electric field parallel to the surface/liquid interface will cause a net hydrodynamic flow in the appropriate direction as long as there is an imbalance in the numbers of the two charges in the layer of liquid adjacent to the charged surface. If electropores, which are expected to be induced closer to the "poles" of the cell that face the electrodes, are viewed as cylinders with an average net negative (from ionized headgroups of phospholipids and ionized amino-acid side chains on integral proteins) charge on this surface and with their axis perpendicular to the plane of the membrane, then a hydrodynamic flow existence would be expected during the electric field pulse (Figure 126.6). It was predicted and experimentally demonstrated that the overall permeabilization difference between both hemispheres would be less than originally thought if an electroporation experiment were conducted to take electroosmosis into account (90).

IV. MICROBIAL INACTIVATION KINETICS

Three types of factors that affect the microbial inactivation with PEF have been identified:

- the process factors (electric field intensity, pulse width, treatment time and temperature, and pulse waveshapes);
- microbial entity factors (type, concentration, and growth stage of microorganisms); and
- treatment media factors (pH, antimicrobial and ionic compounds, conductivity, and medium ionic strength).

Hülsheger and Niemann (32) were the first to propose a mathematical model for inactivation of microorganisms with PEF. Their model was based on the establishing dependence of the survival ratio $S = N/N_o$ (the ratio of living cell count before and after PEF treatment) on the electric field intensity E by the following expression:

$$\ln S = -b_E(E - E_c) \tag{126.4}$$

where b_E is the regression coefficient, E is the applied electric field, and E_c is the the critical electric field value obtained by extrapolating E for 100% survival.

The regression coefficient reflects the gradient of straight survival curves and is a microorganism-media depending constant. The critical electric field was found to be the function of cell size and applied pulse duration. Hülsheger *et al*. (34) proposed an inactivation kinetic model that relates microbial survival fraction (S) with PEF treatment time (t) in the form of

$$\ln S = -b_t \ln \frac{t}{t_c} \tag{126.5}$$

where b_t is the regression coefficient, t is the treatment time, and t_c is the critical treatment time, or extrapolated value of t for 100% survival.

The model proposed by Peleg (69) describes a sigmoid shape of the pathogen survival curves generated by the PEF inactivation. The model represents the percentage of surviving organisms as a function of an electric field and the number of pulses applied. This model is defined by the critical electric field intensity that corresponds to 50% survival (E_d), and the kinetic constant K that is a function of the number of pulses representing the steepness of the sigmoid curve. Generalized equations for both models are combined in Table 126.1.

Small values of the kinetic constants for both models indicate a wide span in the inactivation rate curves and hence lower sensitivity to PEF, whereas large values imply a steep decline or higher susceptibility to PEF.

TABLE 126.1
Inactivation Models

Hülsheger's Model (32)	Peleg's Model (69)
$S = \left(\frac{t}{t_c}\right)^{\frac{-(E-E_c)}{K}}$	$S = \frac{1}{1 + e^{\frac{E-E_d}{K}}}$
E – electric field; t – treatment time; E_c – critical electric field; t_c – critical time; K – kinetic constant.	E_d – electrical field when 50% of population is inactivated; K – kinetic constant.

Lower E_c (or E_d) values indicate lesser resistance of pathogens to the PEF treatment.

A. Microbial Factors in Efficacy of PEF Processing

1. Type of Microorganisms

Among bacteria, gram-positive ones are more resistant to PEF treatment than gram-negative (33). In general, yeasts are more sensitive to electric fields than bacteria due to their larger size, although at low electric fields they seem to be more resistant than gram-negative cells (74, 82). A comparison between the inactivation of two yeast *spp*. of different sizes showed that the electric field intensity needed to achieve the same inactivation level was inversely proportional to cell size. These results are logical but inconsistent with the results obtained by Hülsheger *et al.* (33). Further studies are needed to better understand the effect of microorganism type on microbial inactivation effectiveness.

2. Growth Stage of Microorganisms

Bacterial cells in logarithmic phase are more sensitive to various stresses than cells in lag and stationary phases. Microbial growth in logarithmic phase is characterized by high proportion of cells undergoing division, during which cell membrane is more susceptible to the applied electric field. Gaskova *et al*. (23) reported that the killing effect of PEF for *S. cerevisiae* in the logarithmic phase is 30% greater than for those in stationary phase of growth.

B. PEF Microbial Inactivation

Numerous publications on microbial inactivation present data on vegetative cells, the majority of them from a few genera. Extensive microbial inactivation tests have been conducted to validate the concept of PEF treatment as a non-thermal food pasteurization process (15, 73, 74, 98, 99, 108).

An applied intensive pulsed electric field produces a series of degradative changes in blood, algae, bacteria, and yeast cells (15). The changes include electroporation

TABLE 126.2
Some Bacteria Effectively Inactivated with PEF

Bacillus cereus (70, 81)	*Pseudomonas aeruginosa* (52)
Bacillus subtilis (18)	*Pseudomonas fluorescens* (11, 97)
Bacillus subtilis spores (28)	*Saccaromyces cerevisiae* (111)
Candida famata (102)	*Salmonella* (37, 61)
Escherichia coli (95, 96)	*Staphylococcus aureus (ATCC 25923)* (77)
Listeria innocua (13, 14)	*Yersinia enterocolitica* (60)
Listeria monocytogenes (Scott A) (1, 21, 95, 96)	
Lactobacillus leichmannii (80, 95, 105)	

and disruption of semipermeable membranes, which lead to cell swelling and/or shrinking, and finally to lysis of the cells. The mechanisms for the inactivation of microorganisms include electric breakdown, ionic punch-through effect, and electroporation of cell membranes (75). The inactivation of microorganisms is primarily caused by an increase in membrane permeability due to compression and poration (99).

Castro *et al*. (15) reported a 5-log reduction in bacteria, yeast, and mold counts suspended in milk, yogurt, orange juice and liquid egg treated with PEF. Zhang *et al*. (109) achieved a 9-log reduction in *E. coli* suspended in simulated milk ultrafiltrate (SMUF) and treated with PEF by applying the converged electric field of 70 KV/cm strength, and a short treatment time of 160 μs. These processing conditions and results are adequate for commercial food pasteurization that requires 6- to 7-log reduction cycles. Table 126.2 presents the bacteria reported to be successfully inactivated by the pulsed electric field treatment.

V. PEF PROCESS CALCULATIONS AND VARIABLES

To treat foods with PEF in a continuous system, the liquid food product is pumped through a series of treatment zones in the chamber with high voltage electrodes on one side of each zone and a low voltage electrode on the other side. The PEF process conditions are defined by an applied electric field strength and a treatment time.

A. Electric Field Intensity

It is one of the main factors influencing microbial inactivation (20, 32). The microbial inactivation increases with an increase in the electric field intensity, above the critical transmembrane potential (72). This is consistent with the electroporation theory, in which the induced potential difference across the cell membrane is proportional to the applied electric field. The critical electric field E_c (an electric field intensity below which inactivation does not occur) increases with the transmembrane potential of the cell. Trans-membrane potentials, and consequently E_c, are larger for larger cells (39). Pulse width (duration) also influences the critical electric field; for instance, with pulse widths greater than 50 μs, E_c is 4.9 kV/cm. With pulse widths less than 2 μs, E_c is 40 kV/cm (84).

B. Treatment Time

Treatment time is defined as the product of the number of pulses and the single pulse duration. An increase in any of the two variables improves microbial inactivation (82). As noted above, pulse width influences microbial reduction by affecting E_c. Longer widths decrease E_c, which results in higher inactivation; however, an increase in pulse duration may also result in an undesirable food temperature increase. Optimum processing conditions should therefore be established to obtain the highest inactivation rate with the lowest heating effect (24, 51). The inactivation of microorganisms increases with the treatment time (33). In certain cases, however, the number of pulses that increase inactivation rate reaches saturation. This is the case in *Saccharomyces cerevisiae* inactivation by PEF that reaches saturation with 10 pulses of an electric field at 25 kV/cm (3).

Critical treatment time also depends on the electric field intensity applied (1, 80, 107). At electric field values above E_c, critical treatment time decreases with electric field increase. Barbosa-Cánovas *et al.* (3) reported that for the electric field strength 1.5 times higher than E_c, the critical treatment time would remain constant.

C. Pulse Waveshape

Electric field pulses may be applied in the form of exponentially decaying, square-wave, oscillatory, bipolar, or instant reverse charges (16, 77). Oscillatory pulses are the least efficient for microbial inactivation, and square-wave pulses are more energy and lethally efficient than exponentially decaying pulses (5, 107). Bipolar pulses are more lethal than monopolar pulses, because PEF causes movement of charged molecules in the cell membranes, and reverse in orientation or polarity of the electric field causes a corresponding change in the direction of charged molecules movement (29, 75). The difference between bipolar and monopolar pulses was reported in *Bacillus spp*. (30) and *E. coli* (75) inactivation studies. With bipolar pulses, the alternating changes in the movement of charged molecules cause a stress in the cell membrane and enhance its electric breakdown. Bipolar pulses also offer the advantages of minimum energy utilization, reduced deposition of solids on the electrode surface, and decreased food electrolysis (3).

A study conducted by Zhang *et al.* (110) showed the effect of square-wave, exponentially decaying, and instant-charge-reversal pulses on the shelf life of orange

juice. Square wave pulses were more effective, yielding products with longer shelf lives than those treated with exponentially decaying and charge reverse pulses. In agreement with this study, Love (58) quantitatively demonstrated the stronger inactivation effect of square-wave pulses over all other wave shapes.

D. Treatment Temperature

Experimental results have demonstrated that, in challenge tests, both treatment temperatures and process temperatures impact microbial survival and recovery (8, 107).

PEF treatments at moderate temperatures (50 to 60°C) have been shown to exhibit synergistic effects on the inactivation of microorganisms (19, 36). At the constant electric field strength, pathogen inactivation increases with an increase in treatment temperature (13). Since application of an electric field causes increase in the temperature of the treated foods, proper cooling of treatment chamber is necessary to maintain food temperatures far below those existing during a thermal pasteurization process (10, 57, 68).

Additional effects of high treatment temperatures include changes in cell membrane fluidity and permeability, which increase the susceptibility of the cell to mechanical disruption (34). Also, a low trans-membrane potential decreases E_c and therefore increases inactivation (39).

E. Electrochemistry of a Highly Polarized Electrode/Food Product Interface

Usually, PEF processing is considered as "zero chemistry" treatment with no chemical reactions involved. However, reported changes in the sensory and physical attributes of processed foods are not solely the result of Joule heating and high electric current that passes through the food product. All treatment chambers in existing PEF systems have extremely high electrode surfaces-to-treatment volume ratio due to power supply and electric field strength limitations. Therefore, electrode materials are directly involved in the PEF treatment process. They interact with treated food products by electrochemical reactions that occur at the surface of highly polarized electrodes, and electric double layer assisted reactions of food particulates (solid phase of food product) with the electrode surface. These interactions include (6, 12, 101):

- adsorption of organic and inorganic anions;
- changes in the chamber capacitance due to changes of electric double layer relaxation time;
- electrophoretic deposition of food solids;
- electrocoagulation of solid phase at the electrode surface;
- electrodissolution of electrode material;
- hydrogen/oxygen evolution due to electrode reactions.

Electrode surface interactions with food matrix components have been previously underestimated by the researchers and rarely investigated (5). Due to the importance of the electrode surface properties to the food product behavior in the electrode vicinity and PEF treatment process, electrochemical polarization characteristics of electrode material play the critical role. This is also important because electrical impulses can accelerate electrochemically induced changes in electrode polarization and food properties.

The potential scanning measurements of various food products in the range from 0.5 to 3 V for two widely used electrode materials — aluminum (alloy 2024) and stainless steel — have been performed. Electrochemical potential is the thermodynamic potential that characterizes reaction ability of an electrode in the solution. Increasing of thermodynamic potential by 1 V is equivalent to changing the reaction temperature by 10^3 K. Electrode polarization in the range between 2 and 3 V is considered as extremely high polarization.

Despite the general similarity, both electrodes demonstrate different behavior in acid media (orange juice) (Figure 126.7). In low polarization region aluminum shows the more stable behavior and less corrosive activity. However, to increase electric current through the aluminum electrode one should maintain very high voltage. The stainless steel electrode can support higher current due to lower adsorbance of HO_3 ions at the electrode surface. In the high polarization region both electrodes demonstrate similar volt-ampere characteristics. However, stainless steel has the potential dynamic curve shifted in the direction of higher values of electric current, and therefore is more suitable for PEF applications. For low electric field applications (ohmic heating) aluminum electrodes are more preferred.

The food product composition is a significant factor influencing electrodic processes. Potential dynamic characteristics of stainless steel electrodes in orange juice, whole milk, and tomato soup are depicted in Figure 126.8. Tomato soup has classical corrosion-type characteristics that include Taffel region (adsorption) and electrodissolution (corrosion) of electrode material (12). Orange juice has similar characteristics except the two regions of current-voltage instability, which can be explained with polarized pulp aggregation at the electrode surface. The most unusual potential dynamic curve corresponds to the electrochemical treatment of milk. This type of curve usually corresponds to the passivated metal electrode. At low polarization potentials the behavior of stainless steel electrode in milk does not differ from the other food products. Potential rise leads to the deposition of milk constituents onto the electrode and blockage (passivation) of its surface. In the high polarization regime all three products behave similarly.

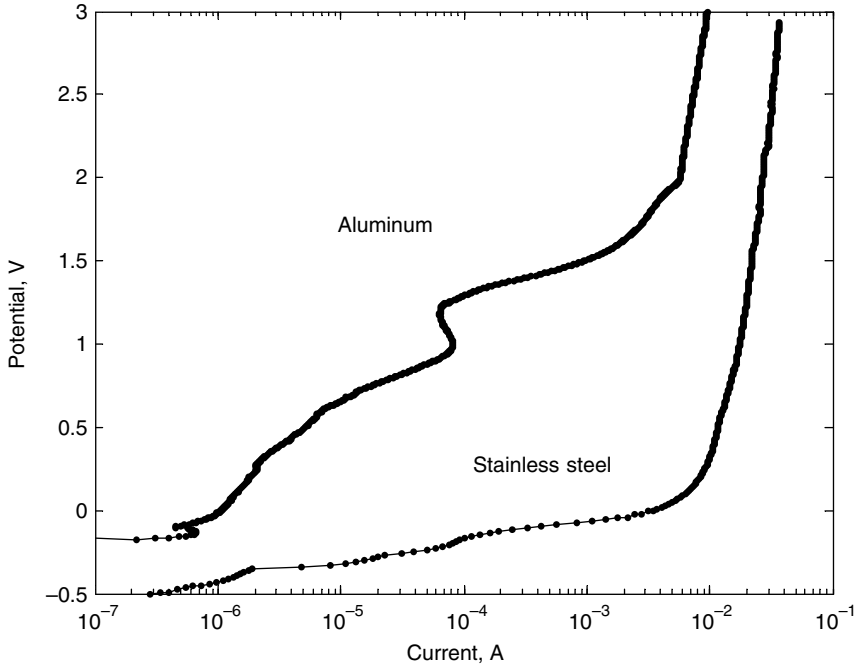

FIGURE 126.7 Polarization of stainless steel and aluminum electrodes in orange juice.

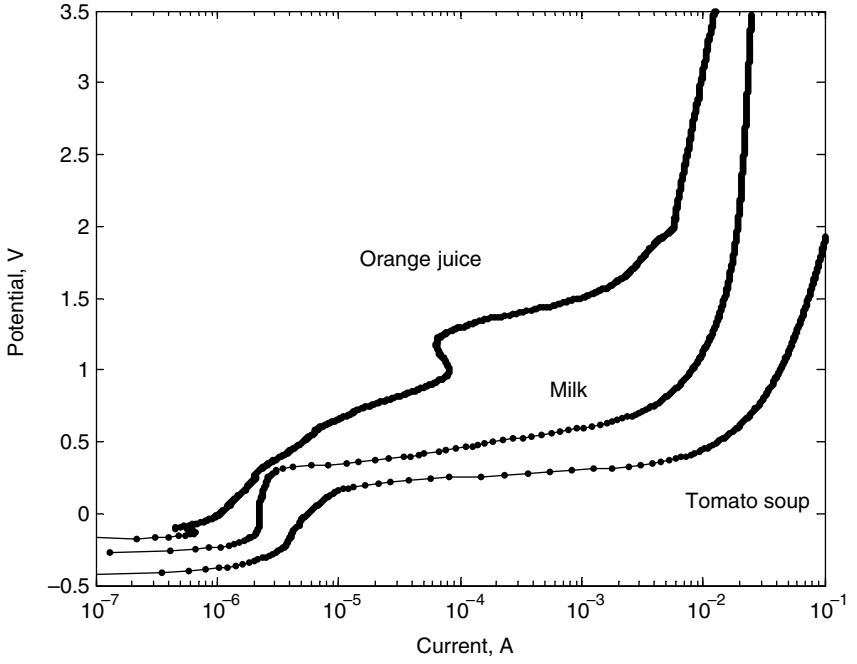

FIGURE 126.8 Potentiodynamic curves of polarized stainless steel electrode for various food products.

V. MATHEMATICAL MODEL OF CONTINUOUS OPERATION (22)

From an engineering point of view, it should be of interest to differentiate between the single pass and recirculation modes of operation of PEF treatment chamber. In both cases, the mathematical model consists of energy and mass balances, kinetic equations, and equilibrium conditions. It is possible to build a large and complicated mathematical model, but that would not be useful. In order to simplify the model, some assumptions may be adopted. Accordingly, plug type flow in the PEF chamber, perfect mixing in the tank, and a first order kinetics for the inactivation of microorganisms were assumed.

A simplified scheme of PEF installation operating in a single pass mode is depicted in Figure 126.2. It can be assumed that the concentration of microorganisms in the feed tank c_T (microorganisms/L) is the same as that at the PEF chamber inlet, and that the rate of microorganism destruction r (microorganisms/(L/s)) follows the first order kinetics with respect to microorganism concentration c (microorganisms/L):

$$r = -kc \tag{126.6}$$

where k (s^{-1}) is the kinetic constant of microorganism inactivation.

Assuming stationary state and plug-type flow in the PEF chamber (56), the microorganism balance gives the following expression:

$$q \ln(c/c_T) = -kV_r t \tag{126.7}$$

where q (L/s) is the fluid flow rate and V_r (L) is the PEF chamber volume.

According to the last equation, the relation between the outlet microorganism concentration c (microorganisms/L) and time t(s) is exponential.

$$c = c_T e^{\frac{-kV_r}{q}} \tag{126.8}$$

The energy balances are more complex. The energy E (J) dissipated during the discharge of the capacitor C (μF) at a voltage V(V) is given by the following equation:

$$E = \frac{1}{2} CV^2 \tag{126.9}$$

Taking into account frequency $f(s^{-1})$ of the pulses, the energy dissipation per second during the liquid flow through the chamber Q (J/s) is:

$$Q = \frac{1}{2} fCV^2 \tag{126.10}$$

However, only one part ϕ of this energy will heat the liquid food (flow q (L/s), density ρ) that passes through the PEF chamber. This ratio ϕ must be less than 1, and strongly depend on the electrical conductivity of the food product. Energy balance for the PEF chamber after the stationary state is reached is represented by:

$$q\rho C_p(T - T_T) = \phi Q \tag{126.11}$$

where q (L/s), ρ (kg/L), and C_p (J/kg°C) are the flow rate, density, and specific heat of the liquid food product, respectively; T_T and T (°C) are the temperature of food sample in the feed tank and in the chamber, respectively. Consequently, the increase in the temperature $T - T_T$ of the liquid food can be estimated as:

$$(T - T_T) = \frac{1}{2} \frac{\phi f CV^2}{q\rho C_p} \tag{126.12}$$

TABLE 126.3
PEF Process Variables

Process Variable	Notation	Dimension
Electric field strength	E	V/m
Total treatment time	t	s
Number of electrode pairs in treatment chamber	n	
Treatment zone diameter	D	m
Mean liquid velocity	μ	m/s
Product electrical conductivity	σ	S m
Product density	ρ	kg/m^3
Product specific heat	C_p	kJ/kg°C

VII. PROCESS CALCULATIONS

The total possible temperature change per pair of electrodes in treatment chamber (ΔT), total energy input during treatment per electrode pair (P), and Reynolds number (N_{Re}) can be calculated using the following equations:

$$\begin{aligned} \Delta T &= (E^2 t\sigma/\rho C_p)/n \\ P &= E^2 t\sigma/n \\ N_{Re} &= \rho D u/\mu \end{aligned} \tag{126.13}$$

The process variables used in the equations are described in Table 126.3.

VIII. PHYSICAL PROPERTIES OF FOOD PRODUCTS FOR PEF PROCESSING

Physical properties of foods that are the most critical for PEF treatment efficacy are the electrical conductivity, density, specific heat, and viscosity of the product. Some useful data can be found in reference (5). Liquid foods contain several ion species that carry an electrical charge and conduct electricity. At a given voltage, the electrical current flow is directly proportional to the electrical conductivity of the food product (108). An increase in the electrical conductivity causes an increase in the overall energy input and change in the product temperature during processing.

The density and specific heat of food product affect the temperature change during PEF treatment. As the density of product decreases, the total temperature change increases (108). Similarly, a decrease in product specific heat also increases the temperature change during PEF processing.

The viscosity of the product determines flow characteristics, which are calculated based on the Reynolds number. For the Reynolds number greater than 5000, the product flow is turbulent, which provides uniform velocity profile in the treatment chamber that, in turn, is likely to provide uniformity of PEF process (8, 57).

A. Conductivity, pH, and Ionic Strength

The electrical conductivity of a medium (σ Ohm^{-1}/m), which is defined as the ability to conduct electric current, is an important variable for PEF processing. Electrical conductivity is the reciprocal of resistivity (r), which is measured in Ohm/m. Foods with large electrical conductivities generate smaller peak electric fields across the treatment chamber, and therefore are not susceptible for PEF treatment (3). Studies on inactivation of *Lactobacillus brevis* with PEF showed that as the conductivity of the fluid increased, the resistance of the treatment chamber was reduced (36), which in turn reduced the pulse width and decreased the rate of inactivation. Since an increase in medium conductivity results from increase of its ionic strength, the latter leads to the decrease in bacteria inactivation rate. Furthermore, increased difference between the conductivities of a medium and microbial cytoplasm weakens the membrane structure due to an increased flow rate of ions across the membrane. Thus, the inactivation rate of microorganisms increases with decreasing conductivity, even at equal input energy (36). Yet another study performed by Dunne *et al.* (20) showed that the resistivity had no influence on *E. coli* and *L. innocua* PEF inactivation effectiveness. These controversial results may be due to the microorganisms or media used.

Vega-Mercado *et al.* (99) studied the effect of pH and ionic strength of the medium (SMFU) during the PEF treatment. The inactivation ratio increased from not detectable (zero) to 2.5-log cycles as ionic strength of the solution was adjusted from 168 to 28 mM. At 55 kV/cm (8 pulses), as the pH was reduced from 6.8 to 5.7, the inactivation ratio increased from 1.45- to 2.22-log cycles. The PEF treatment and ionic strength of the solution were responsible for electroporation and compression of the cell membrane, whereas the pH of the medium affected the cytoplasm when the electroporation was complete. Dunne *et al*. (20) reported that, depending on the microorganism, acidic pH enhanced microbial inactivation, although no specific details were provided (what microorganisms were affected or what range of pH was used).

B. Particulate Foods

Inactivation of microorganisms in liquid-particulate systems has been studied by Dunne *et al.* (20). *E. coli*, *L. innocua*, *Staphyloccocus aureus*, and *Lactobacillus acidophilus* were suspended in a 2 mm diameter alginate beads model system, and the effects of PEF process variables on microbial inactivation were tested. The researchers concluded that the process was effective in killing microorganisms embedded in particulates. However, to achieve more than a 5-log cycle reduction, high energy inputs were needed (70–100 J/ml, depending on the bacteria treated). Qin *et al.* (76) reported that dielectric breakdown occurs when air or liquid vapors are present in the food because of the difference in dielectric constants between liquid and gas.

IX. APPLICATION OF PEF IN FOOD PRESERVATION

PEF has been mainly applied to preserve the quality of foods, such as to improve the shelf life of apple juice (Evrendilek *et al*., 2000a; Simpson *et al*., 1995), cranberry juice (Evrendilek *et al*., 2001a), skim and chocolate milk (Evrendilek *et al*., 2001a), orange juice (Qui *et al*., 1998; Yeom *et al.*, 2000), liquid eggs (Hermawan, 1999), and pea soup (Vega-Mercado *et al*., 1996a).

A. Processing of Apple Juice and Cider

Simpson *et al*. (1995) reported that apple juice from concentrate treated with PEF at 50 kV/cm electric field strength, 10 pulses, 2 μs pulse duration, and maximum processing temperature of 45°C had a shelf-life of 28 days compared to a shelf life of 21 days of fresh-squeezed apple juice. There were no physical or chemical changes in ascorbic acid or sugars in the PEF-treated apple juice, and a sensory panel found no significant differences between untreated and electric field treated juices. Vega Mercado *et al*. (1997) reported that PEF treatment extended the shelf life of fresh apple juice, and apple juice at 22–25°C had a shelf life more than 56 days or 32 days, respectively. There was no apparent change in its physicochemical and sensory properties. Evrendilek *et al*. (2000a) indicated that PEF treatment of apple juice and PEF + mild heat treatment of apple cider improved the shelf life quality of the products compare to control samples at 4, 22, and 37°C without degradation of vitamin C and change in the color measured by L (white if L = 100, black if L = 0), a (−a = green, a = red), and b (−b = blue, +b = yellow) values.

B. Processing of Orange Juice

Sitzmann (1995) reported the reduction of native microbial flora of freshly squeezed orange juice by 3 log cycles with an applied electric field of 15 kV/cm without significantly affecting its quality. The shelf life of reconstituted orange juice treated with an integrated PEF pilot plant system consisted of a series of co-field chambers evaluated by Qui *et al*. (1998) and Zhang *et al*. (1997). It is reported that total aerobic counts were reduced by 3 to 4 log cycles under 32 kV/cm electric field strength. When stored at 4°C, both heat- and PEF-treated juices had a shelf life of more than 5 months. Vitamin C losses were lower and color was generally better preserved in PEF-treated juices compared to the heat-treated ones up to 90 days (storage temperature of 4 or 22°C) or 15 days (storage temperature of 37°C) after processing. In the study of Yeom *et al*. (2000) orange juice was treated by PEF, and with an

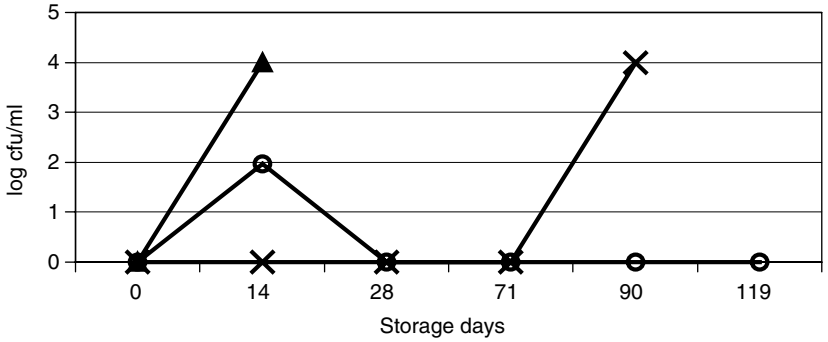

FIGURE 126.9 Total plate count in chocolate milk during storage at 22°C. ○ = PEF + 112°C; X = PEF + 105°C; ▲ = control.

increase in electric field strength longer shelf life is obtained. Compared to heat treatment more flavor components were retained in PEF treated orange juice.

C. Processing of Cranberry Juice

Cranberry juice was treated either by high voltage pulsed electric field at 20 kV/cm and 40 kV/cm for 150 μs, or by thermal treatment at 90°C for 90s. Higher electric field and longer treatment time reduced more viable microbial cells. The overall volatile profile was not affected by PEF treatment, but it was affected by heat treatment. Compared to control samples PEF treatment caused no color change in the samples. When treatment conditions were 40 kV/cm for 150 μs, there was no mold and yeast growth at both 22 and 4°C and no bacterial growth at 4°C (Jin and Zhang, 1999). PEF (32 kV/cm electric field strength, 500 pps frequency, 1.4 μs pulse duration, and 47 μs total treatment time) and PEF + heat (60°C for 32s) processing of cranberry juice revealed that shelf life cranberry juice stored at both 4 and 22°C increased significantly (for 197 days). The PEF and PEF + heat treatments did not cause any significant differences in the color retention of the samples (Evrendilek *et al*., 2001a).

D. Processing of Milk

Inactivation of *Salmonella dublin* and shelf life study with homogenized milk was performed by the electric field strength of 36.7 kV/cm and treatment time of over a 25 min (Dunn and Pearlman, 1987a). *S. dublin* was not detected after PEF treatment or after storage at 7–9°C for 8 days. The naturally occurring milk bacterial population increased to 10^7 cfu/ml in the untreated milk, whereas the treated milk showed approximately 4×10^2 cfu/ml. Further studies by Dunn (1987b) indicated less flavor degradation and no chemical or physical changes in milk quality attributes for cheesemaking.

Fernandez-Molina *et al*. (2001) studied the shelf life of raw skim milk (0.2% milk fat), treated with PEF at 40 kV/cm, 30 pulses, and 2 μs treatment time using exponential decaying pulses. The shelf life of the milk was 2 weeks when it is stored at 4°C; however, treatment of raw skim milk with 80°C for 6 s followed by PEF treatment at 30 kV/cm, 30 pulses, and 2 μs pulse width increased the shelf-life up to 22 days, with a total aerobic plate count of 3.6 log cfu/ml. Reina *et al*. (1998) studied the inactivation of *Listeria monocytogenes* Scott A in pasteurized whole, 2%, and skim milk by PEF. *L. monocytogenes* was reduced 1 to 3 log cycles at 25°C and 4 log cycles at 50°C, with no significant differences being found among the three milks. The lethal effect of PEF was a function of the field intensity and treatment time. Calderon-Miranda (1999) studied the PEF inactivation of *Listeria innocua* suspended in skim milk and its subsequent sensitization to nisin. The microbial population of *L. innocua* was reduced by 2.5 log after PEF treatments at 30, 40, or 50 kV/cm. The same PEF intensities and subsequent exposure to 10 IU nisin/ml achieved 2, 2.7, or 3.4 log reduction cycles of *L. innocua*. Similar to cranberry juice, chocolate milk was processed by PEF (35 kV/cm electric field strength, 600 pps frequency, 1.4 μs pulse duration, and 45 μs total treatment time) and PEF + heat (105 and 112°C for 31.5 s) by pilot plant PEF processing system (Figure 126.4). Compare to control samples, the shelf life of chocolate milk treated by PEF + 105°C and PEF + 112°C increased significantly at 4, 22, and 37°C (Figure 126.9). The PEF + heat treatments did not cause any significant differences in the color retention (Evrendilek *et al*. 2001a).

E. Processing of Eggs

PEF studies in liquid eggs, on heat-pasteurized liquid egg products, and on egg products with potassium sorbate and citric acid (added as preservatives) were conducted by Dunn and Pearlman (1987b). Comparisons were made with regular heat-pasteurized egg products with and without the addition of food preservatives when the eggs were stored at low (4°C) and high (10°C) refrigeration temperatures. The study focused on the importance of the hurdle approach in shelf-life extension. Its effectiveness was even more evident during storage at low temperatures,

where egg products had a final count around 2.7 log cfu/ml stored at both 10°C and 4°C. The samples maintained a low count for 4 and 10 days, respectively, versus a few hours for the heat pasteurized samples.

In addition to color analysis of eggs products, Ma *et al*. (1997) evaluated the density (indicator of egg protein-foaming ability) of fresh and PEF-treated LWE (liquid whole egg) as well as the strength of sponge cake baked with PEF-treated eggs. The stepwise process used did not cause any difference in density or whiteness between the PEF-treated and fresh LWE. The strength of the sponge cakes prepared with PEF-treated eggs was greater than the cake made with non-processed eggs. This difference in strength was attributed to the lower volume obtained after baking with PEF-treated eggs. The statistical analysis of the sensory evaluation revealed no differences between cakes prepared from PEF processed and fresh LWE.

A study reported by Hermawan (1999) indicated that there is a 90% of reduction of *Salmonella enteritidis* inoculated into LWE with circulation mode fluid handling system using 200 pps pulse repetition rate, 2.12 μs pulse duration, and 25 kV/cm electric field strength.

F. Processing of Green Pea Soup

Vega-Mercado *et al*. (1996) exposed pea soup to two steps of 16 pulses at 35 kV/cm to prevent an increase in temperature beyond 55°C during PEF treatment. The shelf life of the PEF-treated pea soup stored at refrigeration temperature (4°C) exceeded 4 weeks, and 22 or 32°C was found inappropriate to store the product. There were no apparent changes in the physical and chemical properties or sensory attributes of the pea soup directly after PEF processing or during the 4 weeks of storage at refrigeration temperatures.

G. Processing of Yogurt Based Product

PEF and mild heat (60°C for 30 s) processing of yogurt based products similar to a dairy pudding dessert and yogurt based drink revealed that the combination of PEF plus mild heat significantly increased the microbial stability of the product at 4 and 22°C without any difference in the sensory attributes. Sensory evaluation of the products indicated that there was no significant difference between control and processed products. Color, pH, and °Brix were not significantly affected by the processing conditions (Evrendilek *et al*. 2001b; Yeom *et al*. 2001).

H. Processing of Rice Pudding

Due to its higher viscosity, rice pudding was preheated to 55°C for 30 s before PEF treatment. Processing conditions were 33 kV/cm electric field strength, 100 L/h flow rate, 1.47 μs pulse duration, and 500 pps frequency. Monopolar negative pulse was applied. Shelf life studies of the product showed that total plate count and a value for color measurement of the PEF treated and control samples were significantly different. PEF treated rice pudding has a shelf life of 94 days, whereas, control samples were spoiled in 10 days (Ratanatriwong *et al*. 2001).

X. PEF AS A HURDLE TECHNOLOGY

In general, controlling the combination of factors (hurdles), such as pH, ionic strength, and antimicrobial compounds of the solution, during PEF treatment can effectively aid in microorganism inactivation. The term *hurdle technology* covers an intelligent use of multiple preservation procedures in combinations specifically relevant to particular types of foods. The concept is pertinent to the control of pathogenic and food spoilage microorganisms, and to almost all food commodities and products. Furthermore, hurdle technologies have been traditionally employed in all countries of the world, although with greatly differing emphasis depending on the history and social characteristics of different cultures (55).

Preservation technologies are based mainly on the inactivation of microorganisms or on the delay or prevention of microbial growth. Consequently, they must operate through those factors that most effectively influence the survival and growth of microorganisms (35). Such factors are not numerous. They include a number of physical factors, some chemical ones, and some that are essentially microbial in that they depend on the nature of microorganisms present in particular products. The most widely quoted classification of those factors derives from the original proposals of Mossel and Ingram (67), updated by Mossel (66). They include:

- *Intrinsic factors*: Physical and chemical factors that exist within a food product, and with which contaminating microorganisms are inextricably in contact.
- *Processing factors*: Procedures that are deliberately applied to foods in order to achieve improved preservation.
- *Extrinsic factors*: Factors that influence microorganisms in foods, but which are applied from or exist outside the food; they also act during storage.
- *Implicit factors*: Factors related to the nature of microorganisms present in food product, and to their interactions among themselves and with the environment during growth.
- *Net effects*: These take into account that many of the factors strongly influence each other, so that the overall effect of combinations of different factors may not be obviously predictable, but may be usefully greater than the perceived effects of the single factors.

Combination (hurdle) effects are the focus of many of the recent developments in the predictive modeling of microbial growth and survival in foods. The limits presented at which these different preservative factors inactivate or inhibit relevant microorganisms must be used to evaluate the effects of these factors on spoilage and food poisoning microorganisms. However, it has to be remembered that the limits listed only apply if all other factors are optimal for the microorganisms in question. But this is hardly the case in any foodstuff. If more than one of the preservative factors (hurdles) is present then an additive or even synergistic effect results, and this is the basis of the hurdle effect and the intentional hurdle technology.

The effective hurdle technologies typically employ multiple hurdles to preserve foods. In the use of such multiple hurdles, a consideration of the stress reactions and adaptations that microorganisms undergo underpins the logic of employing hurdles that affect different targets in the microbial cell. Ideally, the targets should be complementary to gain synergism rather than simply additive effects (55).

If mild heating can be applied to the food, then the injury that would impair the membranes functionality may represent a further sensible target, which should amplify the effects of the previously applied hurdles relying on properly functioning membranes. The potential value of the multitarget approach can therefore be appreciated easily, and perhaps built on more logically in the future. An example of a multitarget novel process is PEF food treatment, which damages the cell membrane, in combination with the application of nisin, so the membrane cannot be repaired due to the membrane-active action of nisin (13, 65). Overall, therefore, homeostasis is interfered with by attacking two distinct targets.

It has been suspected that different hurdles in a food might not just have an additive effect on microbial stability, but could act synergistically (53). A synergistic effect could be achieved if the hurdles in a food hit, at the same time, different targets within the microbial cells and thus disturb the overall homeostasis of microorganisms present in several respects. If so, the repair of homeostasis as well as the activation of "stress shock proteins" in microorganisms becomes more difficult. Therefore, employing simultaneously different hurdles in the preservation of a particular food should lead to optimal stability (54). In practical terms, this could mean that it is more effective to employ different preservative factors (hurdles) of small intensity than one preservative factor of larger intensity, because different preservative factors might have a synergistic effect.

The multitarget preservation of foods is a promising research area, because if small hurdles with different targets are selected, a minimal but most effective preservation of foods could be accomplished. It is anticipated that the targets in microorganisms of different preservative factors will be more fully elucidated, and that hurdles could then be grouped in classes according to their targets. A mild and effective preservation of foods, i.e., a synergistic effect of hurdles, is likely to be achieved if the preservative measures are based on intelligent selection and combination of hurdles taken from different target classes (54). This approach is probably valid not only for the traditional food-preservation procedures, but also for modern processes of food irradiation, ultra-high pressure, or pulsed electric field, or light beam treatments, in combination with conventional hurdles.

a. PEF + hydraulic pressing

Mechanical expression is widely used in the food industry for extraction of fruit juices and vegetable oils, dewatering of fibrous materials, etc. (89). Efficacy of this process can be increased by raw material plasmolysis, cellular damage, or permeabilization prior to its expression. Various methods are traditionally used to increase the degree of raw material plasmolysis: heating, osmotic drying or freezing dehydration, alkaline breakage, enzymatic treatment, etc. (4). Earlier on, the method of electric field treatment (both DC and AC) was also proposed for cellular material plasmolysis. The methods of electro-plasmolysis were shown to be good for juice yield intensification and for improving the product quality in juice production (62), processing of vegetable and plant raw materials (27), foodstuff processing (63), winemaking (47), and sugar production (38). But all these electric field applications are usually restricted by the high and uncontrolled increase in food temperature and product quality deterioration because of electrode material electrolytic reactions, etc.

Bazhal *et al*. (9) investigated the influence of PEF applied simultaneously with pressure treatment on juice expression rate from fine-cut apple raw material. Three main compression phases were observed in the case of mechanical expression. A unified approach was proposed for juice yield data analysis allowing a reduction in data scattering caused by the differences in the quality of samples. PEF application at the moment when the press-cake's specific electrical conductivity reaches the minimum and the pressure achieves its constant value is reported to be the most optimal.

The combination of pressing and PEF treatments significantly enhances the juice yield and improves its quality in comparison with samples untreated by PEF. The PEF treatment intensifies pressing whenever it is applied. The best juice excess yield results at the lowest value of the applied field may be obtained when PEF is applied after some pre-compression period. Such pressure pretreatment before PEF application is necessary for structuring uniformity of the press-cake, removing excess moisture, and decreasing the electrical conductivity of the material. In Bazhal *et al*. (9) the precompression period of 300–400 s and PEF treatment after that period were found

to be optimal for the quality of juice, which was confirmed by its coloration and transmittance. The simultaneous pressure application and PEF treatment reveal the passive form of the PEF-induced cell plasmolysis, which develops very slowly under a low electric field without pressure application. The pressure promotes damage of defective cells, enhances diffusion migration of moisture, and depresses the cell resealing processes.

b. PEF + bacteriocins

Microorganisms in the presence of PEF suffer cell membrane damage, and nisin is a natural antimicrobial known to disrupt cell membrane integrity. Thus the combination of PEF and nisin represents a hurdle for the survival of *Listeria innocua* in the liquid whole egg, which has been investigated by Calderon *et al*. (13). *L. innocua* suspended in liquid egg was subjected to two different treatments: PEF and PEF followed by exposure to nisin. The selected frequency and pulse duration for PEF was 3.5 Hz and 2 ms respectively. Electric field intensities of 30, 40, and 50 kV/cm were used. The number of pulses applied to the liquid whole egg was 10.6, 21.3, and 32. The highest extent of microbial inactivation achieved with PEF was 3.5 log cycles (U) for an electric field intensity of 50 kV/cm and 32 pulses. Treatment of liquid egg by PEF was conducted at relatively low temperatures, 36°C being the highest. Exposure of *L. innocua* to nisin after the PEF treatment exhibited an additive effect on the inactivation of the microorganism. Moreover, a synergistic effect was observed as the electric field intensity, number of pulses and nisin concentration increased. *L. innocua* exposed to 10 IU nisin/ml after PEF exhibited a decrease in population of 4.1 U for an electric field intensity of 50 kV/cm and 32 pulses. Exposure of *L. innocua* to 100 IU nisin/ml following PEF treatment resulted in 5.5 U for an electric field intensity of 50 kV/cm and 32 pulses.

The model developed for the inactivation of *L. innocua* by PEF followed by the exposure to nisin (13) was established to be successful in predicting the extent of microbial inactivation resulting from the combination of PEF and nisin. The combination of these two preservation factors proved to be a hurdle against the survival of *L. innocua* in the liquid whole egg. When energy conservation is a goal, inactivation of *L. innocua* in liquid egg products can be accomplished by selecting the proper combination of PEF intensity and nisin concentration.

Carvacrol was used in another study as a third preservative factor to further enhance the synergy between nisin and pulsed electric field treatment against vegetative cells of *Bacillus cereus* (70). Applied simultaneously with nisin, carvacrol (0.5 mM) enhanced the synergy found between nisin and PEF treatment (16.7 kV/cm, 30 pulses) in potassium-N-2-hydroxy-ethylpiperazine-N-ethanesulfonic acid (HEPES) buffer. The influence of food ingredients on bactericidal activity was tested using skimmed milk that was diluted to 20% with sterile demineralized water. The efficacy of PEF treatment was not affected by the presence of proteins, and the results found in HEPES buffer correlated well with the results obtained in milk. Nisin showed less activity against *B. cereus* in milk, and carvacrol was not able to enhance the synergy between nisin and PEF treatment in milk, unless used in high concentrations (1.2 mM). This concentration in itself did not influence the viable count, but carvacrol did act synergistically with PEF treatment in milk, and not in HEPES buffer. This synergy was not influenced by milk proteins, since 5% milk still allowed synergy between carvacrol and PEF treatment to the same extent as 20% milk.

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127 Nanotechnology and Its Applications for the Food Industry

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CONTENTS

I. INTRODUCTION

Nanotechnology — a term introduced in 1974 to describe ultrafine machining of matter — now can be applied to a wide scope of small-scale engineering (133). Nanotechnology research has emerged as one of the most revolutionary scientific topics in decades. Nanotechnology focuses on the physical/biological structures smaller than 100 nm, which result in unique material properties because of their nanosize. Some of these structures can be manipulated and converted into nanomachines able to perform functions previously not possible. Nanotechnology arises from the exploitation of new properties, phenomena, processes, and functionalities that matter exhibits at intermediate sizes between isolated atoms or molecules (~1 nm) and bulk materials (over 100 nm). The reason that nanoscale materials and structures are so interesting is that size constraints often produce qualitatively new behavior. When the sample size, grain size, or domain size becomes comparable with a specific physical length scale such as the mean free path of the molecules, the domain size strongly affects the corresponding physical phenomena. Figure 127.1 represents the fundamental science and engineering disciplines endowing in the nanoscience and nanotechnology development in their current state.

The objective of this chapter is to show the potential of the nanotechnology field to the food science and technology community.

Food manufacturing will benefit greatly from future developments in nanoscale science, engineering, and technology. For instance, nanoscale synthesis and assembly methods are expected to result in significant improvements in energy-efficient food processing; stronger, lighter materials that increase transportation efficacy; greatly improved chemical and biological sensing; use of low-energy chemical pathways to break down toxic substances for environmental remediation and restoration; and better controls that enhance efficacy of manufacturing processes.

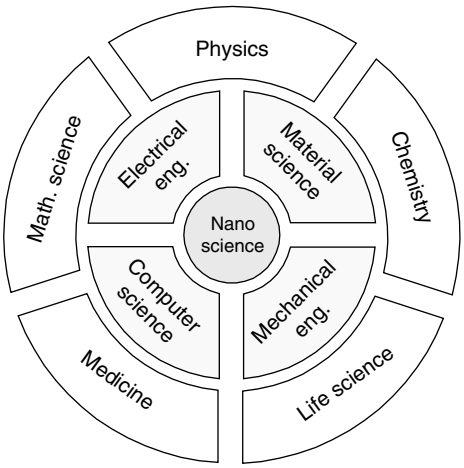

FIGURE 127.1 Nanoscience is the integrative combination of applied and basic sciences.

The study of nanostructures in biological materials of plant and animal origin enables scientists to establish relationships between macroscopic and molecular properties of materials, such as molecular structure, degree of order, and intermolecular forces. Minuscule nanomachines able to circulate through the blood stream and clean out fat deposits from arteries, kill microbes, undo tissue damage, and reverse cancer, could be delivered to the human body through foods. This will put in a new perspective the health promotion role of foods (111). Generation of foods by nonbiological means using advanced nanotechnology could be another future development, meant to ensure enough nutrition for the entire human population with limited resources. In food systems it is possible to envision self-assembling molecules capable of building well-defined food structures; the manipulations of molecular conformation to deliver active compounds precisely to designated sites.

II. NANOTECHNOLOGY IN FOOD SCIENCE

Nanotechnology has the potential to revolutionize world's food system. Agricultural and food systems security, disease treatment delivery methods, new tools for molecular and cellular biology, new materials for pathogen detection, protection of the environment, and education of the public and future workforce are examples of the important links of nanotechnology to the science and engineering of agriculture and food systems. Some overarching examples of nanotechnology as an enabling tool for food industry are:

- Manufacturing, processing, and shipment of food products can be made more secure through the development and implementation of nanosensors for pathogen and contaminants detection.
- The development of nanodevices that will enable maintaining the historical environmental records of a particular product and tracking of individual shipments.
- Systems that provide integration of sensing, localization, reporting, and remote control of food products ("Smart/Intelligent Systems") that can increase efficacy and security of food processing and transportation.

Strategies to apply the achievements of nanoscience to the needs of food industry are quite different from the traditional nanotech applications. Food processing is multitechnology manufacturing with a broad range of raw materials used, high biosafety requirements, and well-regulated technological processes. Four major trust areas in food production can be significantly enhanced by nanotechnology, bringing it to the next technological level: development of new functional materials; micro- and

nanoscale processing; product development; and methods and instrumentation design for improved food safety and biosecurity. Possible nanotechnology applications in food industry, grouped by the target area are depicted in Figure 127.2.

Employing nanoscience in food technology is the complex process not limited to improvement of individual processes and products, but considering the whole food supply chain as a continuous process sequence.

Nanotechnology works at the same scale as a virus or disease-infecting particle, and thus has the potential for very early detection and eradication of pathogens. Nanotechnology holds out the possibility that "smart" treatment delivery systems could be activated long before macro symptoms appear. For example, a smart system could be a miniature device implanted in an animal that samples saliva on a regular basis. Long before an illness develops, the integrated sensing and monitoring system would detect the presence of a disease, and activate a targeted treatment delivery system.

The fundamental processes in agriculture are explored through research in molecular and cellular biology. New tools for molecular and cellular biology that are specifically designed for separation, identification, and quantification of individual molecules are needed. This is possible with nanotechnology and will permit broad advances in agricultural research areas such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful by-products through enzymatic nanobioprocessing, and disease prevention and treatment in plants and animals.

New materials that have special characteristics at the nanoscale will be a tremendous breakthrough in agriculture and food systems for pathogen and contaminant detection. Materials that have self-assembly and self-healing properties will find a multitude of applications in agriculture. Packaging of food in "antimicrobial" containers would prevent food microbial contamination and facilitate food preservation, storage, and distribution.

Protection of the environment through the reduction and conversion of agricultural materials into valuable products is an exciting potential area of nanotechnology advancement. The design and development of nano-catalysts for the conversion of vegetable oils into biobased fuels and biodegradable industrial solvents is one approach already under scientific examination, and would be greatly enhanced with the addition of nanotechnological abilities.

Management of local and environmental emissions is another area of agriculture that could benefit from nanotechnology. Before reaching the dinner table, the lettuce, baked potato, broccoli, and warm wheat bread have survived a formidable number of challenges from the environment. Agricultural crops must be protected against the invasions of wild animals, weeds, insect pests, fungal pathogens, and the whimsy of the weather. Daily "scouting" of crops for potential problems is one of the most important tasks in the agriculture sector. Preventive monitoring and treatment of crops with nanoscale devices (sensors and delivery systems) can improve the quality of food products and durability of post-harvesting processes.

III. NEW PROPERTIES OF MATERIALS AT NANOSCALE

The area of nanomaterials technology presents an unprecedented opportunity to investigate the new properties of materials at the nanoscale (158), and to exploit this knowledge to our benefit. The tools responsible for this

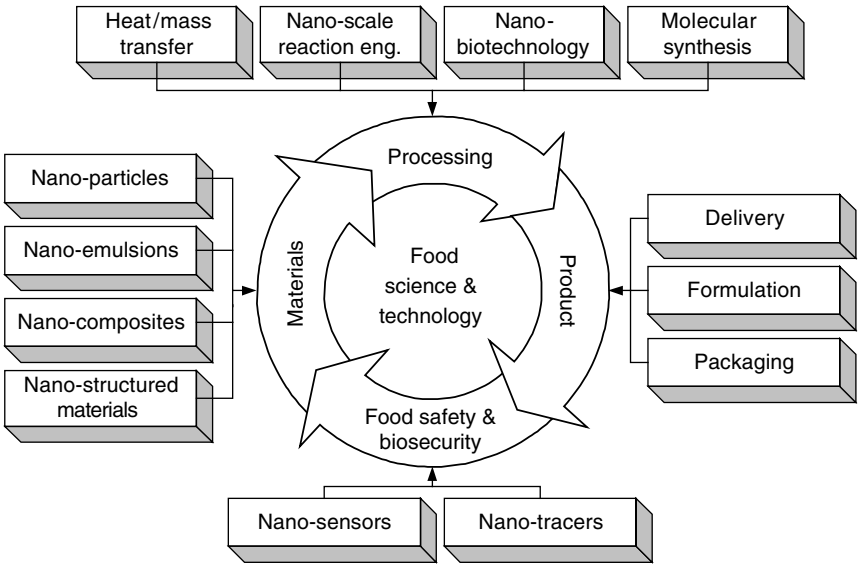

FIGURE 127.2 Application matrix of nanotechnology in food science.

opportunity include synthesis methods that permit atomic and electronic structure of the material to be controlled to the atomic scale, in all three dimensions; instrumental probes that are capable of characterizing nanoscale materials, structures, and their properties on a wide range of length and time scales; and growing computational power which permits theoretical exploration of structures and properties from the atomic to the macroscopic scale.

Nanomaterials can be either created through nanotechnology, or found in nature, such as nanoparticles existing in soil (clays, zeolites, imogolite, iron, and manganese oxides). They provide the potential to manipulate structures at the nanoscale, and to control and catalyze chemical reactions. The shape, structure, and aggregation abilities of individual particles at the nanoscale influence the properties of the material at the macro-level. "Smart fabrics" that can monitor the vital signs of the wearer are an example of the potential new uses envisioned for agricultural fiber products. Nanoparticles are also produced as agricultural by-products: airborne dust and aqueous runoff that cause air and water pollution. Controlling these nanoparticles is in the best interests of cost-effective and environmentally responsible agriculture. Soils are aggregates of nanoparticles, layer particles, organisms, and water. Viewing soil as a nanocomposite, and applying the paradigms and technologies of nanoscale science to it will lead to more efficient and environmentally friendly agriculture.

A. Effects of Size Constraints

Size constraints alone are often responsible for qualitatively new behavior of materials. For example, if the nanoscale structure is smaller than the characteristic lengthscale for scattering of electrons or phonons (the mean free path), qualitatively new modes of electrical current and/or heat transport can arise (49, 148).

Thermodynamic properties, including interface phenomena and phase transitions demonstrate substantial changes when the system size is comparable to the particle size or the coherence length for collective behavior (70). Systems with components sizes ranging from a few tenths to about ten nanometers lie at the fuzzy boundary between the quantum and classical domains. Such systems are also in the size range where thermal energy fluctuations and Brownian motion can have significant effects.

The mechanical properties also change dramatically as the grain size in polycrystalline materials approaches the nanometer scale (3). Changes in the strength of nanoscale structural elements, changes in the nature of friction, and effective modes of fluid flow (hence, of lubrication) all require new design strategies for micromachines (87). Modes of failure also will change, as the size of devices and machines decreases toward the nanoscale. The causes include different mechanical properties that will modify fracture characteristics; the increased importance of surface tension; and, the enhanced role of diffusion and corrosion at the large surface-to-volume ratios that exist in materials at nanoscale (106).

B. Shift of Characteristic Time Scales

Lengthscale change is accompanied by concomitant changes in the characteristic time scale of physical phenomena. In part, this is no more than the increase in characteristic frequencies that follows from the decreased time required to travel shorter distances at a fixed propagation velocity (for phonons, photons, electrons, etc.).

Another time-dependent phenomenon at nanoscale is an increased rate of kinetic processes due to the increased fluctuation rate, as the reduced dimensionality of important structural features (e.g., surface-to-volume ratio) becomes important or dominant. This effect also leads to the increased effectiveness of sensor elements in biological systems.

C. Magnetic Properties

Dramatic quantization and other effects occur in magnetic materials at the nanoscale. The ability to control thin-film growth at the near-atomic level to form epitaxial and heteroepitaxial structures has recently been extended to magnetic nanostructures, including metallic, oxide, and semiconducting phases (51, 116).

D. Thermal Properties

Thermal transport properties of nanostructured materials have received relatively little attention in the past decade. It is well known that polycrystalline materials exhibit lower thermal conductivity than low-defect single crystals of the same material. Investigators have recently realized that this could result in significantly reduced thermal conductivities of nanostructured materials (86, 129), which are expected because of a reduction in the phonon mean free path due to grain boundary scattering (43).

In contrast to the reduced thermal conductivity of nanostructured thin films or coatings, opportunities exist for increasing thermal transport rates in fluids by suspending nanocrystalline particles in them. These "nanofluids" have recently been shown to exhibit substantially increased thermal conductivities and heat transfer rates compared to fluids that do not contain suspended particles (96). Food industry widely employs heat exchangers that require fluids with efficient heat transfer properties. With new "nanofluids" with increased heat transfer rates manufacturers can make heat exchange systems smaller and lighter, and reduce the amount of energy and heat transfer fluid required for the system operation.

E. Energy Conversion and Transport

Energy conversion and transport in nanostructures impacts a variety of fields and applications. Although energy

conversion and transport at macroscales is relatively well understood, it is not at all clear at the nanoscale. For example, it is well known that thermoelectric refrigerators and engines are not as efficient as other energy conversion devices because heat conduction by phonons is too high in thermoelectric materials. There is evidence that nanostructuring can improve electron transport (67–69).

F. Friction Control at Nanoscale

Nano-devices are expected to significantly improve the performance of robots, computers, communication, and other electrical/optical/mechanical devices. However, friction imposes significant limitations on the usage of these tiny devices. As a manifestation of the *nano in the macro*, hundreds of millions of dollars can be lost as a result of wear, friction, breakdowns, and wasted energy at nanoscale. Achievements in the research allowing friction control at the nanoscale will result in highly improved performance in the macro world and can produce significant economic savings (21).

Traditional lubrication methods employ organic substances whose functional groups can adsorb onto polar surfaces to form closed-packed arrangements of almost perpendicularly oriented lubricant chains. Nano-machines lubricant selection is complicated by new considerations. Due to the built-in-place nature of nano-mechanics, lubrication by the conventional means of hidden and contacting surfaces is prohibited. Fluid lubricants may also introduce capillary and viscous shear mechanisms, which would result in energy dissipation. Despite great progress made during the past half century, many basic issues in fundamental tribology such as the origin of friction and the failure of lubrication remained unsolved. Moreover, current reliable knowledge related to friction and lubrication is mainly applicable to macroscopic systems and machinery, and will be of only limited use (if at all) in nano-systems. When the lubrication film thickness is of the same order as the molecular or atomic size, the behavior of the lubricant becomes significantly different (75). Understanding the mechanisms of friction, lubrication, and other interfacial phenomena at atomic and molecular scales can provide designers and engineers with the required tools and capabilities to control friction, reduce unnecessary wear, and predict mechanical faults and failures of lubrication in nano-devices (20, 146).

IV. CONTROLLED SYNTHESIS AND PROCESSING AT NANOSCALE

Nanotechnology is comprised of the large family of phenomena and processes. Only two of them — nanomeasurement and nanomanipulation — are currenty developed and technically proven. Molecular manufacturing is the combination of these two activities. Application of nanotechnology to molecular manufacturing allows that the environmentally clean, inexpensive, and efficient manufacturing of structures, devices, and "smart" products based on the flexible control of architecture and processes at the atomic or molecular scale can be feasible in the near future. The ultimate goal of the molecular manufacturing is to produce complex products on demand from simple raw materials, e.g., inserting basic chemical elements in a molecular assembly factory to yield a common household appliance, perhaps with built-in sensors and actuators to respond to commands or changes in environmental conditions.

A. Nanotechnology: Drawing Inspiration from Nature

Living organisms are not just the collections of nanoscale objects — atoms and molecules; these objects are organized in hierarchical structures and dynamic systems, that are the results of the million years-long Mother Nature's experiments. Tenth-nanometer ions such as potassium and sodium generate our nerve impulses. The size of vital biomolecules, such as sugars, amino acids, hormones, and DNA, is in nanometer's range. Membranes that separate one cell from another, or one subcellular organelle from another, are about five-fold bigger. Proteins can be tens of nanometers across. Every living organism on Earth exists because of the presence, absence, concentration, location, and interaction of these "nano-structures."

The uses of biological molecules are split between two categories:

- Biological molecules can be used in conjunction with other structures to perform just as they do in organisms.
- Biological molecules can be used in conjunction with other structures to perform in a novel manner, quite distinct from their natural function.

Functional nanostructures can incorporate individual biological molecules. For example, biosensors can use natural sugars or proteins as target recognition groups (30). Modified biological structures can be used to act in MEMS devices — for example, modified photosynthetic membranes can split water to hydrogen and oxygen (54). Functional multi-component structures can use molecules in unusual ways. The filament protein actin, found in muscle, can be attached to the enzyme ATP synthase, which is involved in the production of most of the cell's ATP, its "energy currency" (115), and act as a molecular motor.

Many specific functions performed by living systems employ nanometer-size structures in particularly intriguing ways that we would very much like to emulate:

- Living systems utilize **self-assembling** multiple individual parts in a precisely defined functional structure.

- Living systems are hierarchically organized into **complex structures**. For example, collagen, a fiber that rivals steel in strength, is built through successive aggregation of single amino acid strands into triple helices, triple helices into microfibrils, microfibrils into fibrils, and fibrils into fibers.
- Living systems use **template**-based elements, such as DNA, for **reproduction** and recovery (125).
- Organisms can **sense** their molecular surroundings, having developed exquisitely sensitive nanometer-size **sensors** on their outer surfaces. Using the principle of molecular recognition, only the specific desired target can bind to the surface-mounted "receptor" molecules. Upon binding, these receptors change shape in a manner that alerts other components of the system to the presence of the target (78).
- Living systems use nanometer-size structures to act as **highly selective pumps**. The electron transport chain, which is central to the trapping of the energy content of nutrients, pumps protons from one side of the mitochondrial membrane to the other, against the chemical gradient (114). Neurons pump sodium ions out and potassium ions into the cell to prepare it for the next impulse (139).
- Living systems use nanometer-size structures as **switches**. Within 200 msec of the binding of a repellant molecule to a receptor, a phosphoryl group is transferred to a protein and the rotation of the bacterial "tail" is switched from counterclockwise to clockwise. This turns the organism around and allows it to swim away from the repellant (92).
- Living systems use nanometer-size structures to perform **catalysis** with specificity, selectivity, and rate enhancements that are hardly achieved artificially. Enzymes can be selective enough to catalyze a reaction with only one particular molecule from a mix of many, ignoring even its mirror image. Enzymes can selectively catalyze only one of many chemically allowed reactions with that molecule, with rate enhancements up to 10^{16} fold (164).

B. New Manufacturing Paradigm

The concept of manufacturing at the "nano" or atomic scale dates to more than three decades ago. Many developments in biotechnology, chemistry, computational tool building, electrical engineering, and physics have moved the scientific and engineering community closer to operating smoothly on the nanoscale. Manufacturing of new nano-materials with pre-determined functionality is a sequence of macroscale processes combined with the microscale control and energy delivery (see Figure 127.3).

All individual steps in the suggested manufacturing sequence already exist at least in the lab-scale experiments. The aim of the next decade is the integration of individual steps and components into the working system.

Extensive molecular manufacturing applications, if they become cost-effective, will probably not occur until well into the future. However, some products benefiting from molecular manufacturing technology may be developed in the near term. As initial nanomachining, novel chemistry, and protein engineering (or other biotechnologies) are refined, initial products will likely focus on those that substitute for existing high-cost, lower-efficiency products. Likely candidates for these technologies include a wide variety of sensor applications; biomedical products including diagnostics and therapeutics; extremely capable computing and storage products; and unique, tailored, smart materials, including those for food processing and biosecurity applications. Areas that are important to the future of molecular nanotechnology-based advanced manufacturing, and in which successful discoveries could serve other applications, include the following:

- Macromolecular design and folding (32, 59, 94, 172);
- Self-assembly methods (79, 124, 174, 181);
- Catalysis (inorganic, enzyme, and other) (135, 160, 161);

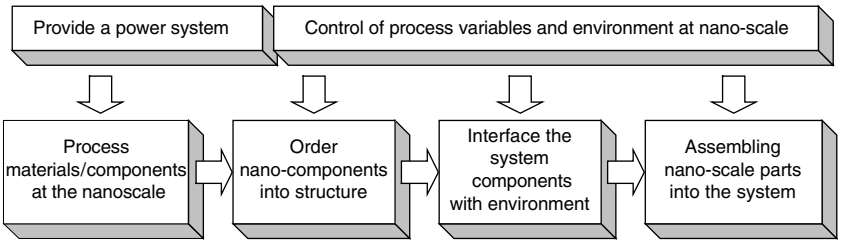

FIGURE 127.3 Manufacturing at nanoscale.

- Dendrimers, fullerenes, and other novel chemical structures development (48);
- Bioenergetics, nanobatteries, and ultrasound-driven chemistry (17, 31, 33, 38, 134);
- Semiconductor-organic/biological interfaces studying (36, 53, 62).

C. Synthesis of Individual Building Blocks

The first step of nanoscale manufacturing process is the fabrication of individual components (building blocks for the entire system) at nanoscale. These components can greatly differ in nature and required processing conditions, but all are suitable for the high-volume production.

1. Nanocrystals

In the last decade there have been significant advances in the preparation of nanocrystals (26, 104). Many common materials, such as metals, semiconductors, and magnets, can be prepared as nanocrystals using colloidal chemistry techniques, which lead to a wide range of applications in unexpected areas, such as in biological tagging (25, 29).

2. Nanotubes and Rods

The exciting discovery of the fullerenes was followed closely by the discovery of nanotubes of carbon (156). Carbon-based nanotubes have the potential to act as a hydrogen storage medium that could exhibit very high storage density per unit weight, which is critical for hydrogen-based transportation systems. A crucial issue is whether or not the hydrogen could be extracted efficiently from such a storage medium at relatively low temperatures. Nanotubes also show tremendous promise as building blocks for new materials.

3. Polymeric Electronic Materials: Dendrimers and Block Copolymers

Tremendous advances in the preparation of organic building blocks of considerable complexity have been made through the last decade (105, 152, 159).

D. Templated Growth and Assembly

High surface area materials with nanoscale dimensions, i.e., small particles and clusters with very high surface-to-volume ratio, can be attained by creating materials where the void surface area (pores) is high compared to the amount of bulk support material (119). Nano-porous inorganic oxides can be an example of such materials. Properties of these materials, e.g., chemical reactivity, magnetic moment, polarizability, and geometric structure exhibit the strong dependence on surface dimensions.

Nanoscale surfaces as well as nano-particles have considerable utility as controlled drug delivery systems and biosensitive molecules carriers (109). Increased surface area of a nano-structured material leads to the increasing of the surface chemical reaction rate and intensification of the electron transfer (i.e., biosensor output signal) through the solid-liquid interface. Furthermore, ordered nanoscale structure of a surface substrate stimulates ordering and self-assembly of deposited specific biological components at the molecular level. The use of nanoscale fabrication techniques for low dimensional devices is being investigated to complement the more traditional fabrication methods (40). These nano-synthesizing techniques take advantage of the self-patterning of natural systems, where the biointerface material is synthesized in the size and shape of the desired nano-structure. These methods include fabrication of nano-structure arrays using self-assembled epitaxial growth (14), chemical synthesis of colloidal nano-structures (2), synthesis of nano-structures in glass and polymer materials (155), and template based chemical synthesis of nano-structures (136). Preparation of mesoporous inorganic solids has been greatly advanced by Antonelli (4). The initial work showed that it is possible to use organic surfactant molecules to prepare complex patterns. These patterns can serve as the templates for the formation of an inorganic phase.

E. Driven Systems

A very promising area for processing of three-dimensional bulk nanoscale structures originates with the recent discovery that mesoscopic structures can be obtained by nonequilibrium processing, such as ion irradiation, implantation, and mechanical working (ball milling, etc.) (11, 88, 103). It is worth mentioning that similar processing schemes perform very important functions in biological systems.

Self-assembly is one of the tools required to build ordered nanostructures. The dynamics underlying the self-assembly process is now well understood (24, 117). A material system embedding enormous complexity dynamically transits through a variety of states, squeezing out free energy along the way, to arrive at a functional (and desirable) configuration. The spontaneous organization of a vesicle and the folding of a protein are notable examples of this phenomenon. Self-assembly, however, is not a completely enabling and sufficient tool. Biological systems make extensive use of both self-assembly and dissipative processes to make important structures or effect adaptive changes (166, 167).

F. Phase Transformations

Many processing schemes involve transformations between different phases of the material. Although thermodynamics ultimately determines the equilibrium phase for a given set of conditions, the answers as to whether and how this phase

is achieved from the metastable precursor phase depend on system kinetics. Since most phase transitions involving nanostructured materials occur under conditions far from equilibrium, the kinetic pathways available to these systems are numerous and not well understood. This problem is further complicated by the large contributions to the free energy of nanoscale materials from interfaces, which substantially shift phase boundaries. It will be important for making progress in the areas of synthesis and processing of nanomaterials to develop better understanding of nanochemistry and the broader general issues of nucleation and growth. One example of how phase transformations are used in the processing of nanostructured materials has already been noted, namely the formation of metallic nanoparticles in silicate glass for non-linear optical devices (98). The processing of bulk nanostructured materials from bulk metallic glass provides another example of using phase transformations in nanotechnology (65, 179). Both materials are now commercially available.

G. Directed Synthesis of Nanoparticles, Nanotubes, and Nanostructured Materials

Nanostructured materials also promise greatly improved structural properties in comparison with conventional metal alloys. For example, small-diameter bundles of single-walled carbon nanotubes have been predicted and observed (126, 141). They have the largest strength-to-weight ratio of any known material with an elastic modulus ~1 TPa, which is approximately 10^2 times that of steel but with only 1/6 its weight. Such materials offer almost unimaginable economic benefits and product opportunities, if only they can be cross-linked to overcome the low shear modulus (141).

H. Nanomechanics and Nano-to-Micro Assembly

The future of nanoscale devices depends upon the abilities of scientific community to physically manipulate the nano-sized parts and to build and integrate larger micro-to-mini scaled devices (42). However, relative significance of fundamental physical forces changes as parts are reduced in size. Figure 127.4 shows the variation of different forces with respect to parts scale. For parts with features of the order of a few millimeters and above, classical mechanical phenomena such as mass and friction dominate the manipulation processes. However, when part sizes are reduced below one millimeter threshold, surface effects such as adhesion due to surface condensation and the electrostatic potential between parts start to dominate in manipulation over the classical inertia effects present in large-scale moving structures.

V. NANO-BIOENGINEERING

The use of natural biological processes to create a desired compound or material from a defined feedstock, e.g., compost material from plant and animal wastes, is called *bioprocessing*. Nanobioprocessing focuses on and utilizes nanoscale technology to achieve the goal of bioprocessing with greater efficacy. The use of molecular probes or development of assays that allow rapid identification of microbes present in a feedstock are examples of the research at nanoscale that can increase the efficacy of bioprocessing. The product itself may be the bulk material or nanomaterial.

Nano-engineering of a biological system is focused on the assembly of nanomaterials to create or enable a specific biological function and/or the subsequent characterization of that function. This can be viewed as a separate

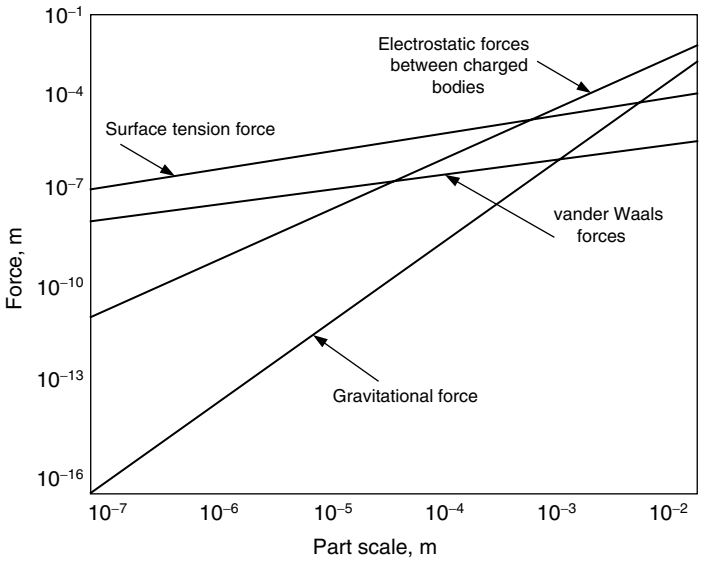

FIGURE 127.4 Forces variation with scale.

project from the study of nano-biomaterials themselves, which is defined by their isolation and characterization or their synthesis from basic building blocks (57, 151, 152).

The extension of nano-engineering to biology includes patterning of 2D templates to direct cell or tissue response for biocompatability or biosensor applications. Applications in medical science include implants, prosthesis, drug delivery, and diagnostics. Other examples include creating a biological input/output device using nano-fabrication techniques to enable communication with individual cells for information technology applications (53, 62, 110, 170). The field of computer science is struggling with the problem of how to integrate information science with biology (73). One approach has been through bioinformatics, where traditional computer science methodology and hardware is applied to manage the enormous amount of information now available from biotechnology (173). Another approach envisions the *integration* of the biology with computer science in creating new hardware and technology to enable direct communication with the biology. If successful, this will provide the platform for the treatment of biology as just another peripheral for sensing, data storage, and information security functions. Other applications of this hybrid system could include communication with individual cells to switch on and off the biomanufacturing of drugs *in vivo*, or the construction of biological–nonbiological hybrids for robotics studies and applications (63, 149, 153).

Nanopatterned surfaces are the environment and location on which most chemical and biological interactions occur (12, 143). A bioselective surface has either an enhanced or reduced ability to bind or hold specific organisms or molecules (128). Bioselective surfaces are important to the development of biosensors, detectors, and catalysts, in the separation or purification of mixtures of biomolecules, and in the processing and packaging of foods (37, 182).

The primary objective of nanoengineering is the engineering of the biological interface (66). The success of two-way communication between electrically active cells and microelectronic devices depends on the proper registration of cells with the microelectrode and their close association or "seal" to this microelectrode (80). The "seal resistance," R_{seal}, is measured between the electrode and the grounded recording media. Low values of R_{seal} are associated with poor electrode-to-cell adhesion resulting in an attenuated and distorted bioelectrical signal, a problem inherent in most extra-cellular recording systems. In contrast, R_{seal} values in the GOhm range, which are typically achieved using glass micropippettes during electrophysiology measurements, permit clearer resolution of bioelectric signals. In tissue this seal is accomplished by the interactions of proteins and other biological macromolecules with the glycocalyx, or outside surface of the cells. The first step in recreating this interface is nanoengineering of the non-biological surface with biologically compatible materials such as proteins, peptides, and biologically active functional groups (47, 83, 89). Surface modification allows a large variety of biomolecules to be attached to a microelectrode surface, which facilitates cell–microelectrode adhesion, increases R_{seal}, and allows for geometric placement of cells (131). For example, it is possible to control nutrients supplied to cells, so that they produce desirable surface groups that allow binding of those cells to specific nonbiological surfaces (175). The immediate benefit is that if a cell is associated with a single microelectrode, it becomes an individual sensor permitting multiple independent assays that then enable statistical analysis. Extracellular recordings of these individual sensor elements would allow long-term, multi-site measurements of electrically active cell bodies and processes for building information technology devices (144).

Modern chemistry and material sciences allow the systematic and parallel patterning of matter on the nanoscale (2). The controlled positioning of atoms within small molecules is of course routinely achieved by chemical synthesis of moles of identical molecules. Nanometer-size objects are much larger entities, comprised of thousands or even millions of atoms. There are many powerful new approaches to patterning on the nanoscale, including atom manipulations by scanning probe tips, and electron beam lithography (132, 165).

VI. CONTROL AND MEASUREMENT PARADIGM AT NANOSCALE

A. Nanoscale Instrumentation

Progress in nanotechnology requires the appropriate tools to observe, characterize, and control phenomena at the nanoscale. A whole new generation of analytical instrumentation and nanoscale devices, capable of providing information about physical, chemical, and mechanical phenomena, and material properties at nanoscale must be developed. Nanotechnology is already benefiting from novel instrumentation developed during the past two decades, for example scanning probe microscopes (SPM, see Figure 127.5), and the new generation of synchrotron x-ray sources capable of studying materials at the nanoscale. On the other hand, recent discoveries in nanoscale science and engineering provide the basis for the development of unprecedented new tools. Thus, both opportunities and challenges in developing instruments for synthesizing nanostructures, as well as for characterizing of existing nanostructures and measuring properties of nanomaterials, will exist during the next decade.

Scanning probe microscopy is a family of techniques, which provide images of the surface topography and, in some cases, surface properties on the atomic scale. The inventions of the scanning tunneling microscope (STM) (16) and the atomic force microscope (AFM) (15) have spawned the development of a variety of new scanning probe

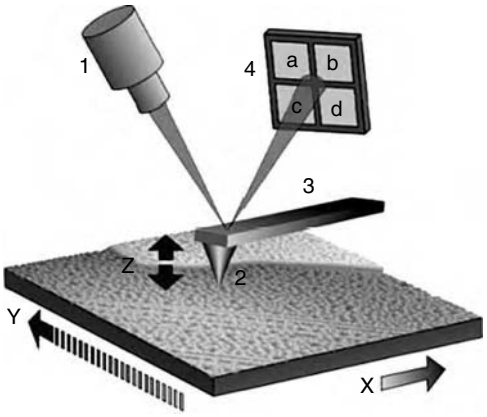

FIGURE 127.5 Schematic representation of Scanning Probe Microscope 1 — Laser source, 2 — tip, 3 — canteliever, 4 — photodetector array.

microscopes (SPMs)(168). Whereas the STM and AFM have found applications mainly in topographical imaging, the other SPMs added more functionality. Examples include the near-field scanning optical microscope (NSOM) (13), scanning thermal microscope (SThM) (99, 100), scanning capacitance microscope (SCM) (171), magnetic force and resonance microscopes (MFM) (72, 138), and the scanning electrochemical microscope (SECM) (7). Some current applications of SPM for nano-materials analysis include a sensor that can measure atomic-level forces that develop between two surfaces as they approach each other and come into contact also has been developed (74). The Interfacial Force Microscope (IFM) utilizes a feedback force sensor that eliminates the snap-to-contact event that is inherent in other scanning force microscope designs. The IFM allows measurement of the full range of adhesive interactions and can be used to image surfaces by controllably hovering out of range of contact.

Understanding of how an atom travels over a surface at different temperatures, and ultimately incorporates into the surface, is crucial to making smaller, faster, smarter nanodevices. A new instrument called "Atom Tracker" (154) can observe an individual atom in motion, and track atomic motions up to 1000 times faster than a conventional STM. Continuous monitoring of the motion of an individual atom as it binds to various sites on a surface allows this diffusing atom itself to become a probe of the surface structure and properties.

In order to understand processes used to produce nano-sized particles deposited on surfaces, the new aerosol instrumentation is needed. Significant progress has been made recently, with instruments now available for detecting particles as small as several nanometers in diameter (145, 150).

Nano-sensors have a high potential for deployment in areas and media that are not readily amenable to probing by traditional devices (177). An example is medical operations requiring minimally invasive surgery. A collection of specialized devices may be needed to provide all necessary functionalities. One can imagine an ensemble of micro- or nanorobots that cooperatively explore, assess, and operate on various locations in an organ. This poses formidable challenges in signal processing, control, and interconnectivity (178). Consequently, as non-destructive, real-time measurements of the physical properties of nanoparticles and nanostructured materials evolve, and as their use to monitor nanomaterials processing is developed, they will provide exceptional opportunities for fundamental and applied investigations and problem-solving at nanoscale (27, 62).

B. Nanosensors

Conventional sensors now can provide an abundance of information about the environmental conditions such as temperature and weather, data on air, land, and sea transportation, chemical contaminants, deceleration for release of airbags in automobiles, and countless other parameters. Biological organisms also have the ability to sense the environment. For example, humans sense the environment through sight, touch, taste, smell, and sound. In living organisms, various sensors operate over a range of scales from macro- (ear drum vibrations), to micro- (nerve cells impulses), to nanoscale (molecules binding to sensors in our noses).

The exciting possibility of combining biology and nanoscale technology into sensors holds the potential of increased sensitivity, and therefore a significantly reduced response time to potential problems (120, 121, 162, 176). It is possible to design a bio-analytical nanosensor that could detect a single virus long before it multiplies and the symptoms become evident in a plant or an animal (133, 163). The potential applications for bioanalytical nanosensors include detection of pathogens (85), contaminants (55), heavy metals, particulates or allergens, and environmental conditions (35).

Thundat *et al.* (157) has demonstrated that the interactions of antigen molecules with their corresponding antibodies, attached to surface of an AFM cantilever, can provide sufficient surface stress to bend the cantilever beam. Such a device is an example of hybridization and integration of nanostructures at several levels, because along with nanocomponents (antibodies bound to a cantilever surface) it has microcomponents (cantilever beams), which can be delivered in a chip (millimeter scale components), that integrates biology and biochemistry with engineering. Boxer (19) has recently developed the method of the deposition of lipid membranes into lithographically defined corrals. Early results suggest that this approach may allow *electrochemical addressing* of photo-defined membrane cells. Consequently, this is the first step towards integrating the functional nanotechnology demonstrated by living cells into robust machine architecture. The design

and assembling of specific structures at near-atomic scales requires precise controlling of the materials according to macro- and mesoscopic specifications, which are actually decided at the quantum level (180).

The development of chips/sensors for rapid detection of biological pathogens is a critical area with applications in the food handling/processing industry, in biological/chemical warfare, and in emerging biosecurity systems with early warning for exposure to air- and water-borne bacteria, viruses, and other antigens (9, 107). Microfabricated chips for DNA analysis (93, 97) and for the detection of polymerase chain reaction (91) have already been demonstrated. The μChemLab (50) has performed the research to incorporate similar structures into a fully autonomous analytical system that can be integrated into on-a-chip architectures. For example, organically functionalized mesoporous structures have been successfully integrated on a micromachined heating and flow stage to provide 1000-fold chemical preconcentration for on-a-chip analysis of chemical warfare agents.

C. Smart Systems Integration: Sensing, Localization, Reporting, and Control

The nanotechnologies can only reach their full potential through integration. The "Smart Systems Integration" is similar to designing and building the logic of a "nervous system" that allows the individual parts to work together (56). Integration of the nanotechnologies into a working food safety management system (whether remotely or automatically controlled) requires successful electronic communication between several components, including sensing systems, reporting systems, localization systems, and control systems (101, 122). The logic to control the subsystems (control algorithms) must be developed and eventually translated into a computer language (35).

VII. APPLICATIONS OF NANOTECHNOLOGY IN FOOD INDUSTRY

A. Nanodevices for Identity Preservation and Tracking

Application of nanotechnology in agriculture brings the opportunities that once were only possible in science fiction. Identity preservation (IP) is a system that provides customers with information about the practices and activities used to produce a particular crop, food, or other agricultural product. Regulatory agencies can take advantage of IP as a more efficient way of recording, verifying, and certifying agricultural practices. Through IP it is possible to provide stakeholders and consumers with access to information, records, and supplier protocols regarding the food product's farm of origin, environmental practices used in its production, food safety and quality, and animal welfare issues.

Each day multiple shipments of different agricultural products are moved all over the world. Currently, there are financial limitations of the number of inspectors that can be employed at critical control points for the safe production, shipment, and storage of foods and agricultural products (64). Quality assurance of agricultural products' safety and security can be significantly improved through the IP. Nanoscale IP holds the possibility of the continuous tracking and recording the history of manipulations, processing steps, and transformations which a particular agricultural product experiences. In the future, these nanoscale intelligent monitors can be linked to recording and tracking devices to improve identity preservation of foods and agricultural products (35).

Originally developed by the 3M Corporation, the MICROTAGGANT® brand identification particle is a microscopic, traceable, anti-counterfeit device that is highly versatile in its applications. The MICROTAGGANT is a distinct numeric code sequence represented in multiple colored layers format. The code becomes a unique "fingerprint" to which the meaning is assigned. Optional fluorescent, magnetic, and other qualities may be added that are detectable by scanners and sensors providing enhanced coding and identification capabilities. These particles can be easily detected in the field using UV/VIS light, 100X magnification glass, magnetic, or laser scanners. The color code sequence in every particle is identical for each specific color code lot (108).

B. Nanodevices for Smart Treatment/Delivery Systems

MEMS technology is based on techniques used in the semiconductor fabrication industry and has generated significant enthusiasm among physicians and surgeons in recent years. At their most basic levels, MEMS are devices with dimensions of micrometers to a few millimeters that combine electrical and mechanical components to acquire data or do work. Implantable and transdermal drug delivery microsystems allow patients both accurate and continuous dosing of medication and allow delivery of drugs directly to their intended sites of action (18, 23).

Today, the application of agricultural fertilizers, pesticides, antibiotics, probiotics, and nutrients is typically performed by spray or drench methods to soil or plants, or through feed or injection systems to animals. Delivery of pesticides or medicines is either provided as "preventive" treatment, or once the disease has multiplied and symptoms are evident in a plant or animal. Nanoscale devices are envisioned to have the capability to detect and treat an infection, nutrient deficiency, or other health problem long before the symptoms become evident at the macroscale. This type of treatment can be targeted to the specific area affected by the disease.

"Smart delivery systems" for agriculture can be defined as a combination of the following: time-controlled, spatially-targeted, self-regulated, remotely regulated, preprogrammed, or multifunctional release of treatment to avoid biological barriers for successful pathogens inactivation (90). Smart delivery systems also can have the capacity to monitor the effects of the delivery of pharmaceuticals, nutraceuticals, nutrients, food supplements, bioactive compounds, probiotics, chemicals, insecticides, fungicides, or vaccinations to people, animals, plants, insects, soils, and the environment (35). Smart treatment delivery systems are envisioned as bioactive systems: drugs, pesticides, nutrients, probiotics, nutraceuticals, and implantable cell bioreactors (34).

C. Nanoparticles Technology

There is currently considerable research interest in the use of microparticles as carriers for poorly bioavailable drugs and vaccines via mucosal (particularly oral) routes (39, 41, 76, 140). A variety of therapeutic moieties, including peptides and proteins, have shown enhanced oral uptake when entrapped within various types of microparticulate system constructs, and this approach has also been used successfully for the oral, nasal, and rectal delivery of a variety of vaccines (8, 48, 71, 123, 137). Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrapment in submicronic colloidal systems (nanoparticles) (22, 123, 137). The rationale behind this approach is to increase antitumor efficacy, while reducing systemic side effects.

Naturally occurring antioxidants in raw fruits and vegetables are thought to provide significant health benefits, such as reduced risk of heart attack, stroke, neurodegenerative diseases, and cancer. Processed foods, which are statistically more likely to be consumed by the high-risk individuals, as opposed to raw fruits and vegetables, typically lose some or all of the potency of their natural antioxidant content. Heat-sensitive nutrients like beta-carotene, Omega-3 fatty acids, and other anti-oxidants are significantly or totally degraded upon pasteurization and canning. In foods that do not naturally contain anti-oxidants, introduction of anti-oxidant compounds is difficult due to these compound's high susceptibility to heat, pH variations, and other conditions existing during food processing.

Now nanoscale engineered materials can protect antioxidants and other health-promoting food components from degradation during manufacturing and storage. Nanoparticles (46) and various types of nano-containers — silica-shell (61) and "nanocochleate delivery vehicles" — offer protection for a wide variety of nutrients that currently cannot be delivered in high-temperature, adverse pH, or other conditions. BDSI's Nanocochleates offer the following benefits for processed food nutrients: pressure and shear resistance; protection from oxidants (air, free radicals); protection from temperature extremes; protection from photodegradation (10).

D. Nanoemulsion Technology

The method of infection control with conventional disinfectants requires a tradeoff: to ensure microorganisms are killed, the toxic chemical must be present at levels that create health and contamination risks. Nanoemulsion formulation works very differently (112, 118, 174). Nanospheres of oil droplets $<1\,\mu m$ are suspended in water to create a nanoemulsion requiring only miniscule amounts of active antimicrobial ingredient (60, 113). The nanospheres carry surface charges that efficiently penetrate the microorganisms' membranes (44, 84, 127, 169). Nanoemulsions are effective against a variety of food pathogens including Gram-negative bacteria (58). The nanoemulsions can be rapidly produced in large quantities and remain stable for many months at room temperatures (6). Nano-emulsions are composed of ingredients, which are either food, e.g., vegetable oils, or are on the FDA GRAS list of food ingredients. They are proved to be effective for decontamination of food processing plants, and for reduction of surface contamination of meat and poultry products (28, 118).

E. Packaging

No longer is packaging expected just to safely contain a product — it may now capture the history of a package, interact with the consumer, have functions other than dispensing (e.g., heating/cooling), communicate with appliances, or allow itself to be tracked through the supply chain (1). Nanotechnology has rised a new packaging paradigm — Smart Packaging (5) — which includes active packaging (147), smart tagging/labeling (52), self-venting films, anticounterfeiting and tamper-proof materials/technologies, RFID devices (81, 82), self-opening packages,

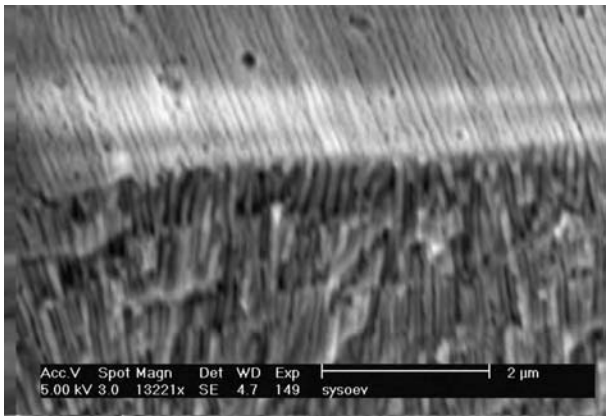

FIGURE 127.6 Nano-patterned aluminum barrier film — multifunctional substrate for sensor/packaging applications.

diagnostic and freshness indicators, responsive labels, time-temperature indicators, self heating/cooling packages, etc.

The addition of reinforcing agents is widely used in the production of packaging films (77, 102, 142). It is expected that the reducing of the added particles size to nanoscale could enhance the performance of these materials (95, 130). The new polymer nanocomposite materials, which can be produced by adding the nanoscale ceramic powders to commercial products, are aimed to substitute more expensive barrier plastic films in food industry (45, 84).

VIII. CONCLUSION

Many other examples can be given to illustrate the close link between fundamental studies of nanoscale phenomena and their technological applications. Although observed at nanoscale changes in material properties can dominantly affect the nanoscale structures, we still have remarkably little experience or intuition for the anticipated phenomena and their practical implications, except for the case of electronic systems. The physics, chemistry, and biology of phenomena occurring at nanoscale is effectively the new subject with its own set of physical principles, theoretical descriptions, and experimental techniques, which we are only in the process of discovering. Thus, there is an urgent need for broad investigations of the phenomena associated with nano-systems and structures, especially in materials and structural contexts where the implications are not at all well understood.

Implementation of nanoscience methods and nanoscale materials in the food industry can bring it to the next technological level. Fundamental changes in food manufacturing and technology due to scientific advances add new qualities into the established industrial practice:

- control of mass and heat transfer at nanoscale;
- improved nanomaterials;
- vastly increased manufacturing capacity due to the miniaturization and combination of processes;
- improved logistics due to the high performance computing and integration of "smart" nanodevices into products packaging and transportation;
- better access to resources and control of energy consumption by introducing micro- and nanoscale power sources and more efficient processes;
- total food quality control by managing food additives and health promoting food components interacting with the product matrix at nanoscale.

In short, nanotechnology has the potential to make as much difference as the discovery of agriculture, steel, germ theory, the assembly line, the colonization of America, electricity, the airplane, computers, and genetic engineering all put together — without further environmental damage! And this is expected to happen within the next few decades.

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128 Biosensor Technology for Food Processing, Safety, and Packaging

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CONTENTS

Biosensor technology is a powerful alternative to conventional analytical techniques, harnessing the specificity and sensitivity of biological systems in small, low cost devices. Despite the promising biosensors developed in research laboratories, there are not many reports of real applications in food safety and quality monitoring. A sensor is the device that can detect a property or group of properties in a food product and respond to it by a signal, often an electric

TABLE 128.1
Sensor Operation Modes

On-line	<5 min	Sensors/detectors operate directly in the process stream, giving a real-time signal related to the quality factor of concern.
At-line	<30 min	Sensors are used, in split-flow measurements, requiring reagent additions or sufficient time for the equilibration or chemical reaction to occur
Off-line	1 hr–24 hr	Sensors/assays are used in the laboratory with extensive periods of time required to performing the measurements

signal. This signal may provide direct information about the quality factor(s) measured, or may have known relation to the quality factor. Usually, sensors are classified according to their mode of use (see Table 128.1).

I. NEEDS OF FOOD QUALITY/SAFETY CONTROL

Food quality control is essential in the food industry; nowadays, an efficient quality assurance is becoming increasingly important. Consumers expect adequate quality of food product at a fair price, long shelf-life, and high product safety, while food inspectors require safe manufacturing practices, adequate product labeling, and compliance with the FDA regulations. Further, food producers are increasingly demanding the efficient control methods, particularly through on-line or at-line quality sensors to satisfy consumers' and regulatory requirements, and also to improve the feasibility of automated food processing, quality of sorting, and to reduce the production time (increase throughput) and the final product cost.

Extensive development of biosensors for food safety and quality control were stimulated by acquiring several new food safety and key quality concepts during the last decade: Hazard Analysis Critical Control Points (HACCP), Total Quality Management (TQM), ISO 9000 Certifications. The wave of terrorist acts and foodborne diseases outbreaks has raised the importance of the food traceability and authentication (77, 99). There are specific safety problems (pathogenic microorganisms, BSE, GMF, pollutants, etc.), which require intensive control, data logging, and data treatments and can be effectively controlled only with the new generations of biodetection systems (46). All these tasks require in-time and on-line sensors for new data analysis systems, warning systems, tight feedback loops for automated processing, etc.

Figure 128.1 explains needs for biosensors in food safety and quality management, showing the sources of biohazard contaminations in foods and their influence on technological, "shelf-life," and perception properties of food products. The major sources of undesirable contaminants and changes in foods can be combined in five groups by their localization and occurrence. Three of them are food

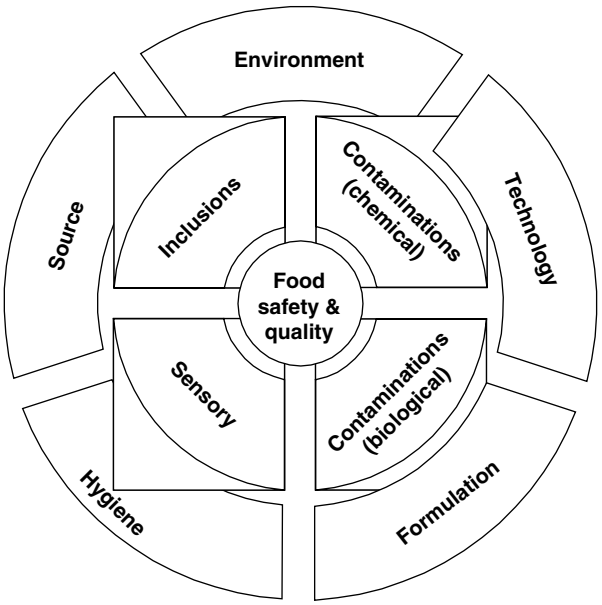

FIGURE 128.1 Needs for food safety and quality control: sources of pollution and contamination.

manufacturing-related: technology (processing and sequence of process operations), industrial hygiene (food safety management at the plant level, HACCP), and formulation (product development, interactions of food additives/ingredients with food matrix, bioavailability). The sources of food raw materials and their quality are the issue of biosafety/biosecurity in the agricultural processing including post-harvesting technologies and logistics. The fifth source of biohazards is the environment in the broadest sense, including pollution, climate changes, and anthropogenic environmental factors.

Below is the brief description of some possible sources of undesirable contaminations and/or changes in food products.

A. Food Processing

It is well known that many important nutrients are denaturalized, altered, or even destroyed by the faulty processing of foods. Food can also become contaminated during processing, handling, distribution, and consumption. Many undesirable or even harmful substances can enter the food as additives and toxic metabolites during its processing and preservation.

B. Food Contamination

Food can become contaminated during every step of food processing sequence, from cultivation to consumption. The contaminants may be:

Microbiological: viral, bacterial, parasitological, and fungal;

Chemical: pesticide residues, nitrates, nitrites, high-salinity, fluorides, arsenic compounds, lead, and other heavy metals. These pose serious and long-term health threats;

Harmful metabolites and biological toxins (e.g., methyl alcohol, estrogen-like substances, hormones, biotoxins including mycotoxins especially aflatoxins, allergens, and carcinogens).

C. Sources/Raw Materials

The major aspect in the area is the utilization of food sources, which were previously wasted or not used. This is mainly to enrich fodder, thus ensuring better recovery for human consumption indirectly.

D. Food Substitutes and Genetically Modified Foods (GMF)

There has been incredible progress in new biotechnology with commercialized products of insulin, human growth hormones, interferon, and recombinant vaccines using human cell culture or "novel" bacteria (90). Unfortunately, some people are allergic to some food ingredients. A reliable system of diagnosis and treatment of infant's milk intolerance exists in all countries. Allergies to natural foods are less common and less serious compared to those to food additives and untraditional or inedible food varieties.

E. Food Industry Hygiene

In addition to the need for development of appropriate policy related to health (135), agriculture, trade, manufacture, and licensing, the rational consumer protection regulatory systems are to be developed and enforced (30).

The next level of the diagram in Figure 128.1 represents the major types of changes in foods caused by the sources of undesirable contaminants. They can be instrumentally controlled, hence represent the primary targets for biosensors development and design. Indeed, the great challenge is to develop the real-time and on-line sensors and data systems suitable for surveying processes and products, controlling automated processes and the raw material stream, sensing the final products quality, typing the product labels with nutritional and health information, and much more.

II. SOURCES OF INFORMATION FOR BIOHAZARDS DETECTION

The detection of biohazards can be performed directly (see Figure 128.2) by measurements of the pollutants/pathogens concentration in a food product with specifically aimed biosensors. Another (indirect) approach to determining the presence/level of biohazard is through the measurements of changes in processing parameters (temperature, pressure, water activity, etc.) that lead to variations in microbial contamination levels. External information can alert the food safety management system on possible increase of bacterial contamination or risk of bioterrorists' attack and/or environmental pollution splash. This information, i.e., expectations of high contamination level, can be used to perform changes in screening and sampling procedures, and extend the range of pathogens to be detected.

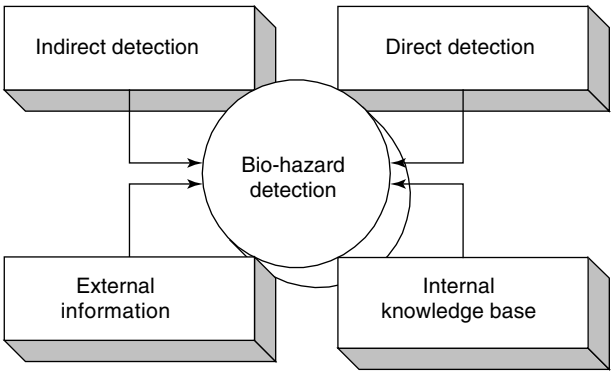

FIGURE 128.2 Sources of information for biohazards detection.

Internal knowledge base ("sensor-free detection") is the set of accumulated data/records giving the correlation between the properties of raw materials, process parameters, and biohazard level in manufactured products.

Today, the most important quality parameters and concepts in food production control are:

- *Sensory*: appearance, flavor, taste, texture, stability, etc.;
- *Nutritional,* including health implications, such as "high in fiber," "low cholesterol," "GMF free," etc.;
- *Composition and labeling*: additives lists, quality and ethical claims (e.g., ecological information), etc.;
- *Pollutants record*: environmental pollutants, veterinary drugs, agricultural chemicals, BSE-prions and mycotoxins;
- *Detection of foreign bodies*, such as stones, glass, or metal fragments;
- *Microbial safety*, in particular *Listeria, Salmonella, Campylobacter*, *E. coli*, and *Yersinia*;
- *Shelf-life*: microbial, sensory, chemical, sterility testing, F_0-values;
- *Production hygiene*: cleaning, decontamination;
- *HACCP*: traceability and authentication;
- *Process parameters control*: machine settings, temperature, pressure, flow, aseptic conditions, and many others;
- *Packaging*: integrity, pinholes, gas permeability, migration control.

III. BIOSENSORS: GENERAL FACTS

Biosensors usually are small analytical bio-electronic devices that combine a transducer with a sensing biological component (biologically active substance). The transducer, which is in intimate contact with the biologically sensitive material, can measure weight, electrical charge, potential, current, temperature, or optical activity of the substance. The biologically active species include enzymes, multi-enzyme systems, antibodies or antigens, receptors, populations of bacterial or eukaryotic cells, or whole slices of mammalian or plant tissue, to name a few. Substances such as sugars, amino acids, alcohols, lipids, nucleotides, etc. can be specifically identified and their concentration measured by these sensors.

A schematic functional representation of a biosensor and the detection principle is depicted in Figure 128.3. The biosensor consists of a biological sensing element integrated with a signal transducer; together they produce a reagent-free sensing system specific for the target analyte.

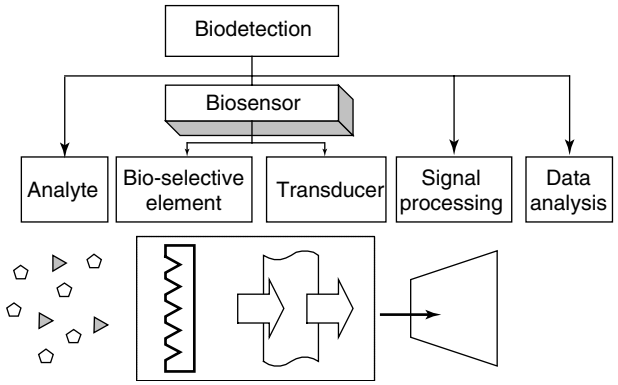

FIGURE 128.3 Schematics of biodetection and biosensor design.

The biological component of a biosensor used for the molecular detection is made of highly specialized macromolecules or complex systems with the appropriate selectivity and sensitivity. Biosensors can be classified according to the biocomponents used for the detection.

The biodetection principle can be schematically described as follows. A chemical, biological, or physical sensor produces a signal (e.g., voltage, absorbance rate, heat, or current) in response to a detectable event, such as binding between two molecules. In case of a biological or chemical sensor this event typically involves a receptor (e.g., macrocyclic ligand, enzyme, or antibody) binding to a specific target molecule in a sample. Physical sensors, on the contrary, measure inherent physical parameters of a sample, such as current or temperature, which can change due to reactions occurring in it. In any case, the signal is then transduced by passing it to a circuit where it is digitized. The obtained digital information can be stored in a memory, displayed on a monitor, or made accessible via digital communications port.

Since it is essential that the sensor's response be detected, it is necessary that an appropriate transduction mode for electrochemical signals, optical signals utilizing changes in the fluorescence or absorbance rate of a sample, or plasmon resonance be available. With most sensors, transduction is accomplished electrochemically or optically.

The transducer transforms the physicochemical variations occurring in the biosensing element as the result of a positive detection event into an electric signal, which is then amplified by an *ad hoc* designed electronic circuit, and used for the control of external devices. The transducers can be electrochemical (amperometric, potentiometric, conductometric/impedimetric), optical, piezoelectric, or calorimetric. Very often this classification is used to identify the type of biosensor (see Figure 128.4).

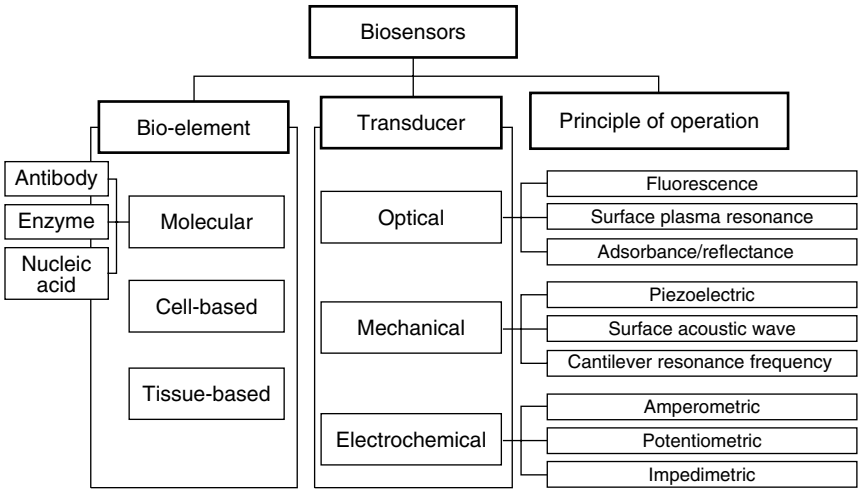

FIGURE 128.4 Biosensors classification.

The bio-specific elements of the biosensor and transducer can be coupled together in one of the four possible ways (80), schematically shown in Figure 128.5: membrane entrapment, physical adsorption, matrix entrapment/porous encapsulation, covalent bonding.

In the membrane entrapment scheme, a semi-permeable membrane separates the analyte and the bioelement, and the sensor is attached to the bioelement (collagen membranes, synthetic preactivated membranes (102), cellulose-acetate membranes). The physical adsorption scheme is depending on a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the sensor surface (52). The porous entrapment scheme is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor (nylon net (60), carbon paste (36) or graphite composites (3)). In the case of covalent bonding the sensor surface is treated as a reactive group to which the biological material can bind (108). One of the bioselective elements most frequently used in biosensors is an enzyme. These are large protein molecules that act as catalysts in chemical reactions, but remain themselves unchanged at the end of reaction.

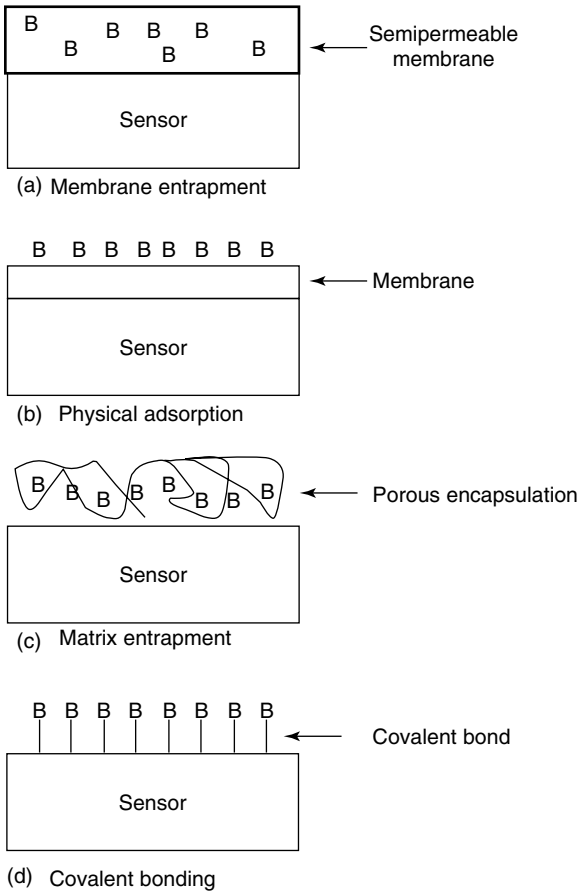

FIGURE 128.5 Coupling of biomaterial with the transducer on a substrate.

IV. TYPES OF BIOSENSORS

A. Mechanical (Resonant) Biosensors

In this type of biosensor an acoustic wave transducer is coupled with an antibody (biosensitive element). When the analyte molecules (antigens) attach to the membrane (cantilever, Figure 128.6), the membrane mass changes, resulting in a subsequent change in the resonant frequency of the transducer (57). This frequency change is detected and measured (80).

1. Sensors Based on Electromagnetic Waves

Electromagnetic sensors may be classified by the wavelength of the electromagnetic waves they use: visible (400–700 nm), ultraviolet (10–400 nm), infrared (700–30,000 nm: NIR (95), FTIR (78), MRI (68)) waves, microwaves (37) (1–10 cm), radiofrequency (59) (1–10 m), X-rays (2) (100 pm–1 nm). Each sensor class may be further sub-divided according to the molecular information that can be obtained through the interaction. For instance, infrared sensors may be subdivided into near-infrared (700–2500 nm), mid-infrared (2500–30000 nm), far-infrared (up to 1,000,000 nm), and thermography (1–15 μm) sensors, which all extract different information from the molecules (sample) interacting with the waves. We may also classify these sensors according to their precise type of interaction: absorbance, transmittance, or reflectance of light.

Sensors based on interactions with electromagnetic radiation waves have been on the market for many years, in particular for laboratory purposes. On-line examples of such sensors are also numerous: x-rays used for foreign body detection (94), visible light sensors for color recognition or machine vision inspections (22), near-infrared sensors for quality inspection and temperature measurements (81), or microwave sensors for the detection of water content (15).

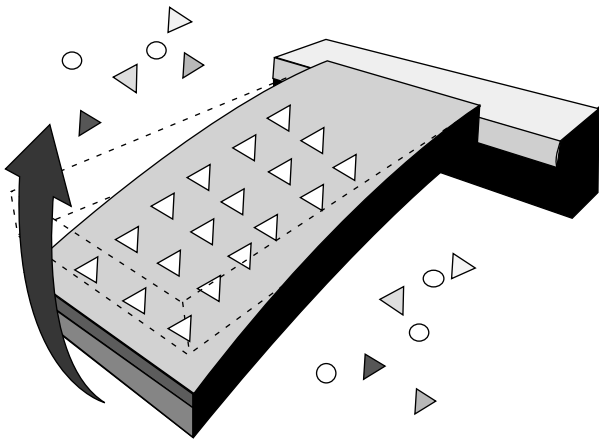

FIGURE 128.6 Cantilever-based systems can detect biomolecules.

B. Optical Detection Biosensors

Strictly speaking, optical biosensors belong to the larger class of electromagnetic detectors, but due to their importance and broad use they are usually considered as a separate group of biosensitive devices. The output signal measured in this type of biosensors is a light signal (119). These biosensors can be made based on optical diffraction or electrochemiluminescence (41).

1. Surface Plasmon Resonance

Surface plasmon resonance (SPR) is another optical phenomenon used in new sensors, often in those involving antibodies or enzymes. The optical range used is most often in the visible part of the spectrum, but may also be in the NIR range. Traditionally, SPR devices (see Fig. 128.7) detect minute changes in the refractive index of the sensing surface and its immediate vicinity. They may detect these changes by a diffraction grating, or with a prism on a glass slide, or through an optical waveguide carrying a thin metal layer (gold). The metal layer carries a sensitizing layer, e.g., immobilized antibodies or other molecules binding the analyte specifically; this layer is in contact with the sample. Inside the device, a collective excitement of electrons in the metal film occurs, and leads at a specific wavelength to a total absorption of light at a particular angle of incidence. This angle depends on the refractive indices on either side of the metal film. Specific molecules binding to the sensitizing layer change the refractive index, thus changing an angle of total absorption; this angle is measured and correlated to the concentration of the analyte.

The SPR detection technique has been used by Hellnaes (69) for on-line and at-line detection of veterinary drug residues (hormones and antibiotics) in dairies and slaughterhouses. Clenbuterol and ethinyl-estradiol in bovine urine, sulfamethazine (SMT) and sulfadiazine (SDZ) in porcine bile, and SMT, SDZ, and enrofloxacin in milk have been successfully detected by the technique. The developed biosensor operates in real time and can simultaneously detect up to 8 different veterinary drugs with a throughput of up to 600 samples per day. The project participants have established a new company to produce and further develop the sensor systems, and several new and elegant designs of SPR sensors are now under development by other groups. The SPR sensor principle has also been used by Patel (113). The sensor developed as a result of this research has been applied to the quantification of mycotoxins, *Listeria,* and markers for growth hormones (recombinant bovine somatotrophin, rBST).

Most current research is focused on the NIR/VIS sensors, SPR sensors (surface plasmon resonance), and NMR sensors (pulsed and low resolution); some work has also been done on fluorescence sensors, MIR and Raman sensors, Fourier transform NIR sensors, thermography based sensors, and sensors combining two or more sensor principles.

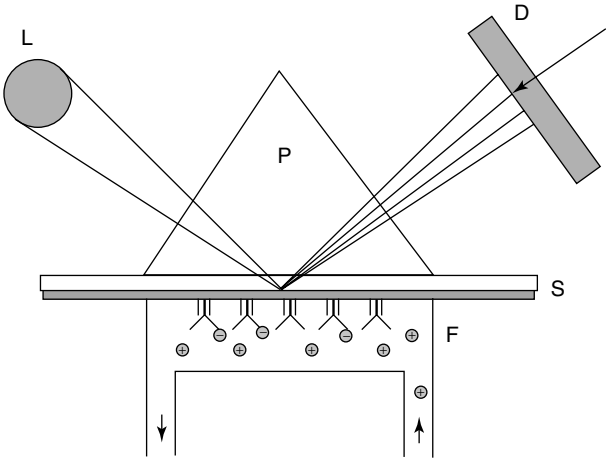

FIGURE 128.7 Surface plasmon resonance detection unit. L: light source, D: photodiode/photodiode array, P: prism, S: sensor surface, F: flow cell.

C. Electrochemical Biosensors

Electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc. The underlying principle of these biosensors is that many chemical reactions produce or consume ions or electrons which in turn cause some changes in the electrical properties of the solution; these changes can be sensed out and measured (75). The electrochemical biosensor can be classified based on the measured electrical parameter as conductimetric, amperometric, or potentiometric (126).

D. Impedimetric/Conductometric Biosensors

Many biological processes involve changes in the concentrations of ionic species. Such changes can be utilized by biosensors, which detect changes in electrical conductivity. The measured parameter is the electrical conductance/resistance of the solution. When electrochemical reactions produce ions or electrons the overall conductivity/resistivity of the solution changes (47). This change is measured and calibrated to a proper scale. Conductance measurements have relatively low sensitivity. The electric field is generated using sinusoidal voltage, which helps in minimizing undesirable effects such as Faradaic process, double layer charging, and concentration polarization (17).

Impedimetric biosensors utilize changes in the electrical conductivity in the frequency domain (impedance) of a biological system for sensing and detection (4, 52, 71). Impedance spectroscopy provides a powerful tool for investigating a variety of bioelectric processes for both electrical and non-electrical applications. In impedance spectroscopy

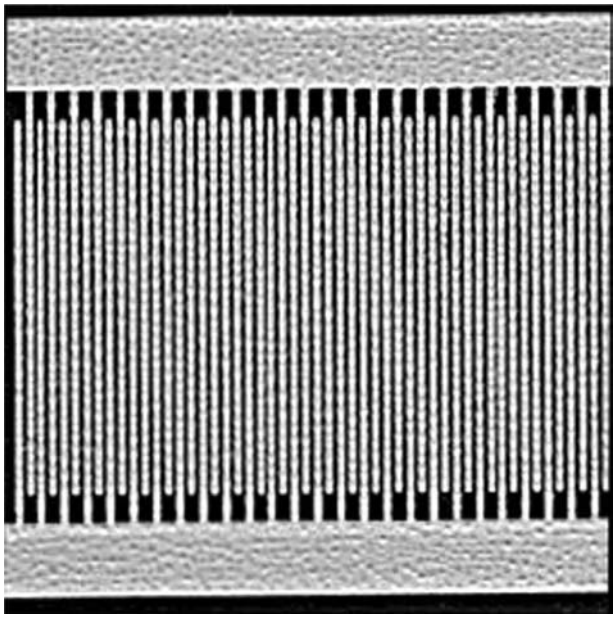

FIGURE 128.8 Conductometric biosensor — interdigitated electrode arrangement.

the current flowing through a sample cell containing a nanoscale patterned bio-interface and the voltage across this cell are measured as a function of frequency (5, 8, 20). Design of impedimetric sensors is very similar to conductivity-based sensors (see Fig. 128.8) (61, 75, 86, 107, 117, 122). Enzyme/antibody immobilization on electrode surface makes these sensors highly selective and sensitive (128).

E. Amperometric Biosensors

This highly sensitive biosensor can detect electroactive species present in biological test samples. Enzyme-catalysed redox reactions can form the basis of a major class of biosensors if the flux of redox electrons can be determined (104). Normally, a constant voltage is applied between two electrodes and the current, due to the electrode reaction, determined. The first and simplest biosensor was based on this principle. It was for the determination of glucose and made use of the Clark oxygen electrode (Figure 128.1). In case of amperometric biosensors, the measured parameter is an electric current. Some of the most recent applications of amperometric biosensors include: glucose sensor for meat freshness (106); glucose sensor for use in fermentation systems (124); rapid cell number monitor (19); monitor for herbicides in surface waters (24, 85); amperometric ELISA method based on the self enzyme amplification system (84); amperometric and novel fluorescent DNA probes (150).

F. Potentiometric Biosensors

In this type of sensor the measured parameter is an oxidation/reduction potential of an electrochemical

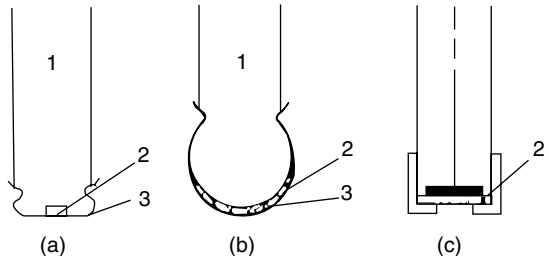

FIGURE 128.9 Amperometric enzyme electrodes. (a) "Clark's" electrode — dialysis membrane electrode with soluble enzyme, (b) entrapped enzyme, and (c) enzyme, membrane electrode. (1) Transducer, (2) enzyme, (3) dialysis membrane.

reaction (13). The simplest potentiometric technique is based on the concentration dependence of the potential, E, at reversible redox electrodes according to the Nernst equation (17):

$$E = E_0 + \frac{RT}{nF} \ln a_s$$

where E_0 — standard redox potential, R — gas constant, T — absolute temperature, F — Faraday constant, n — number of exchanged electrons of the substance S, and a_s — activity of the substance S. Changes in ionic concentrations are easily determined by use of ion-selective electrodes (13). This forms the basis of potentiometric biosensors (4). Many biocatalysed reactions involve charged species, each of which will absorb or release hydrogen ions according to their pK_a and the pH of the environment (88). This allows a relatively simple electronic transduction using the commonest ion-selective electrode (see Fig. 128.9), the pH electrode (121).

1. Field Effect Transistors (FET) and Ion-Selective Field Effect Transistors (ISFET)

Potentiometric biosensors can be miniaturized by the use of field effect transistors (FET). Ion-selective field effect transistors (ISFET) are low cost devices that are in mass production (148). A recent development from ion-selective electrodes is the production of ion-selective field effect transistors (ISFETs) and their biosensor use as enzyme-linked field effect transistors (ENFETs). Enzyme membranes are coated on the ion-selective gates of these electronic devices, the biosensor responding to the electrical potential change via the current output. Thus, these are potentiometric devices although they directly produce changes in the electric current. Figure 128.10 shows a diagrammatic cross-section through an npn hydrogen ion responsive ISFET with a biocatalytic membrane. The build-up of positive charge on this surface (the gate) repels the positive holes in the p-type silicon causing a depletion layer and allowing the current to flow. In (1) Langmuir-Blodgett films containing butyrylcholinestrase

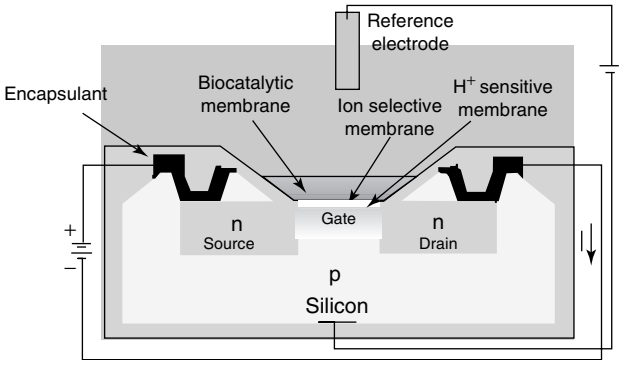

FIGURE 128.10 An ion sensitive FET-based potentiometric biosensor.

(BuChE) are fabricated to realize an ion-sensitive field-effect transistor (ISFET) for the detection of organophosphorus pesticides in water.

G. Cell-Based Biosensors

Cell-based biosensors have been implemented using microorganisms, particularly for environmental monitoring of pollutants (109). Biosensors incorporating mammalian cells have a distinct advantage of responding in a manner that can offer insight into the physiological effect of an analyte (33, 66). Several approaches for transduction of cellular signals (67) are described in the literature: measures of cell metabolism, impedance (129), intracellular potentials, and extracellular potentials (72). Among these approaches, networks of excitable cells cultured on microelectrode arrays (9, 33, 43, 103, 109) are uniquely poised to provide rapid, functional classification of an analyte and ultimately constitute a potentially effective cell-based biosensor technology. Keese and Giaever (82) have designed a biosensor that can be used to monitor cell morphology in tissue culture environment. The sensing principle used is known as electric cell-substrate impedance sensing (ECIS). In this process, a small gold electrode is immersed into tissue culture medium. After cells attach and spread over the electrodes, the electric impedance measured across the electrode chamber changes. These changes in impedance can be used for understanding cell behavior in the culture medium. The attachment and spreading of the cells are important factors for successful use of this biosensor. Unfortunately, some types of cells, e.g., cancerous cells, can grow and reproduce freely in a medium without being attached to any substrate/surface; that makes them impossible to detect with these sensors.

Proposed in (34) biosensor mimics biological sensory functions and can be used with most types of receptors, including antibodies and nucleotides. The technique is very flexible and even in its simplest form it is sensitive to pico-molar concentrations of proteins.

H. Lab-on-a-Chip Systems and DNA Detection Devices

Significant advances have been made in the development of micro-scale technologies for biomedical and drug discovery applications. The first generation of microfluidics-based analytical devices (Lab-on-a-Chip (141)) have been designed and are already functional. Microfluidic devices offer unique advantages in sample handling (45, 65, 142), reagent mixing (23, 79, 140), separation (54, 89, 98), and detection (27). They include, but are not limited to: devices for cell sampling (7), cell trapping and cell sorting devices (9, 12, 16, 44, 152), flow cytometers (49, 105, 142), devices for cell treatment: cell lysis, poration/gene transfection and cell fusion devices (145).

Biosensors used for DNA detection are used to identify small concentrations of DNA (of microorganisms such as viruses or bacteria) in a large sample. The detection relies on comparing sample DNA to a DNA of known microorganism (probe DNA) (28). Since the sample solution may contain only a small number of microorganism molecules, multiple copies of the sample DNA need to be created for proper analysis (31). This is achieved with an aid of the polymerase chain reaction (PCR). PCR starts by splitting the sample's double-helix DNA into two parts by heating it. If the reagents contain proper growth enzymes, each of these strands would grow the complementary missing part and form the double-helix structure again. This happens after the temperature is lowered. Thus, in one heating/cooling cycle the amount of sample DNA is doubled (10). In general, PCR is very power-consuming, so it was previously not possible to fabricate portable biodetectors able to perform PCR. But, using newly developed MEMS devices, such biodectors (also known as lab-on-a-chip systems) have been created. In these MEMS-based devices the amount of reagent used is scaled down (115).

I. DNA-Based Sensors/Assays

The general principle of DNA probe assay is similar to the immunoassay described in Figure 128.11. Indeed, even the applications of DNA probes and monoclonal antibody immunoassay frequently overlap, thus establishing a "competition" between the two possible approaches.

One of the most important applications for DNA probes is the testing for virus infections (96). For probes of infectious disease, it is assumed that all strains can still contain a common DNA sequence region, and thus be identified by a single probe. Recognized by the cell as a foreign body, viruses will induce an antigenic reaction causing antibody generation so they can also be detected in an immunoassay (73).

Another type of biosensor developed by the Naval Research Laboratory (10) uses magnetic field instead of optics or fluorescence. This sensor equipped with magnetic

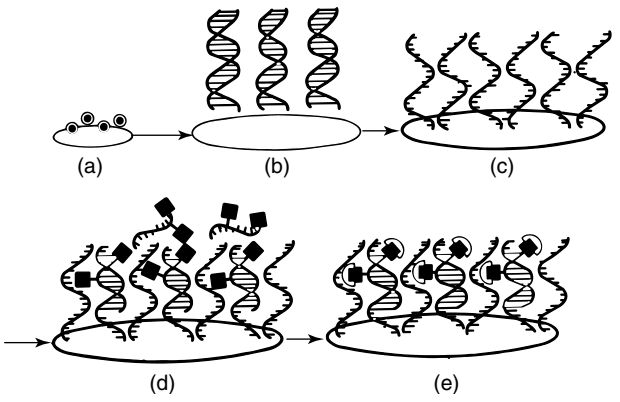

FIGURE 128.11 DNA probe assay. (a) Deposit sample organism on immobilization matrix; (b) release DNA; (c) immobilize DNA to matrix and separate strands; (d) add labeled DNA probes and hybridize; (e) read label.

sensors and microbeads (131) is able to detect the presence and concentration of bioagents. The magnetic sensor (group of sensors) is coated with single-stranded DNA probes specific for a given bioagent or sample DNA. Once a single strand of DNA probe and a single strand of sample DNA find each other, they form a double stranded (double-helix) structure, which in turn binds a single magnetic microbead. When a magnetic bead is present on a sensor surface, its resistance decreases which can be detected and measured.

V. APPLICATIONS OF BIOSENSORS IN FOOD SCIENCE AND MANUFACTURING

Detailed description of all existing biosensors for food applications requires a separate book and is definitely out of the borderlines of this chapter. Instead, this chapter aims to compare needs for biosensors in food safety/biosecurity management systems and existing biosensor technologies. The general classification of sensors, principles of their operation, and some practical examples given in this chapter accomplish this goal. To find more information about specific biosensors and applications readers should refer to other papers. Biosensors for food safety applications are reviewed in references (64, 75, 111, 114). Additionally, the author can recommend a good description of existing market for food safety applications (6). The general review of electrochemical biosensors for food pathogens detection can be found in references (91, 126, 134). Specific details are presented in the following articles about biodetection in poultry industry (93), and pathogen detection in muscle foods (42). The needs for fast, on-line, and accurate sensing, e.g., *in situ* analysis of pollutants in crops and soils, detection, and identification of infectious diseases in crops and livestock, on-line measurements of important food processing parameters (62), monitoring animal fertility, and screening therapeutic drugs in veterinary testing are well-described in another work (138).

A. Sensors for Pathogens Detection

The broad spectrum of foodborne infections keeps changing dramatically over time, as well-known pathogens have been controlled or eliminated, and new ones have emerged. The burden of foodborne diseases remains substantial: one in four Americans is estimated to have a significant foodborne illness each year. The majority of these illnesses is not caused by known pathogens, so more of them remain to be discovered. Among the known foodborne pathogens, the recently identified predominate, suggesting that as more and more is learned about pathogens, they would come under control. In addition to the emergence or recognition of new pathogens, other trends include global pandemics of some foodborne pathogens, the emergence of antimicrobial resistance, the identification of pathogens that are highly opportunistic, affecting only the most high-risk subpopulations, and the increasing identification of large and dispersed outbreaks. New pathogens can emerge because of changing ecology or technology that connects a potential pathogen with the food chain. They also can emerge by transferring the mobile virulence factors, often through bacteriophage (133).

Over the past decade many improvements have been seen in both conventional and modern methods of pathogenic bacteria detection in foods (75). Modification and automation of conventional methods in food microbiology involve sample preparation, plating techniques, counting, and identification test kits. ATP bioluminescence techniques are increasingly used for measuring the efficacy of surfaces and utensils cleaning. Cell counting methods, including flow cytometry and the direct epifluorescent filter technique, are suitable for rapid detection of contaminating microorganisms, especially in fluids. Automated systems based on impedance spectroscopy are able to screen high numbers of samples and make total bacterial counts within 1 day. Immunoassays in various formats make a rapid detection of many pathogens possible. Recently, there have been important developments in the nucleic acid–based assays and their application for the detection and subtyping of foodborne pathogens. The sensitivity of these methods has been significantly increased by employing the polymerase chain reaction and other amplification techniques. Alternative and rapid methods must meet several requirements concerning accuracy, validation, speed, automation, sample matrix, etc. Both conventional and rapid methods are used in the frame of biohazard analysis critical control point programs. Further improvements especially in immunoassays and genetic methods can be expected, including applications of biosensors and DNA chip technology (38).

In recent work of Bokken (18) a surface plasmon resonance biosensor was used to detect a *Salmonella* pathogen through antibodies reacting with *Salmonella* group A, B, D, and E (Kauffmann-White typing). In the assay designed, anti-*Salmonella* antibodies immobilized onto the biosensor surface were allowed to bind injected bacteria, followed by a pulse with soluble anti-*Salmonella* immunoglobulins to intensify the signal. No significant interference was found for mixtures of 30 non-*Salmonella* serovars at 109 CFU ml^{-1}. A total of 53 *Salmonella* serovars were successfully detected at 107 CFU ml^{-1}, except those from groups C, G, L, and P, as expected.

Another sensor technology recently developed uses a micro-electrophoretic system (mFFE) that separates and concentrates the analyte in question by a number of electrophoretic methods: preparative zone, interval zone, isotachophoresis, or isoelectric focusing. The mFFE system can be designed as a plain glass substrate 1.5 mm thick, and a cross-linked polydimethyl-siloxane (PDMS) top layer with micromachined sample channels. The central separation chamber ($12 \times 4 \times 0.15$ mm) is connected to 34 inlet channels for sample injection, and 36 outlet channels for sample collection. The detector unit can be based on several principles. In the case of *Listeria*, the detector unit may be a well-known ATP luminescence detector. For other analytes, the SPR detection system may be used with an immobilized bio-specific layer, e.g., antibodies (111).

A new ion-channel biosensor based on supported bilayer lipid membrane for direct and fast detection of *Campylobacter* species has been reported (76). The sensing element was composed of a stainless-steel working electrode, covered with an artificial bilayer lipid membrane (BLM). Antibodies to bacteria embedded into the BLM are used as channel-forming proteins. The biosensor has a strong signal amplification effect, which is defined as the total number of ions transported across the BLM. The biosensor has demonstrated a very good sensitivity and selectivity to *Campylobacter* species.

A novel assay system for the detection of *Escherichia coli* O157:H7 has been recently developed. The detection is based on the immunomagnetic separation of the target pathogen from a sample and absorbance measurements of p-nitrophenol at 400 nm from p-nitrophenyl phosphate hydrolysis by alkaline phosphatase (EC 3.1.3.1) on the "sandwich" structure complexes (antibodies coated onto micromagnetic beads — *E. coli* O157:H7-antibodies conjugated with the enzyme) formed on the microbead surface (92). The selectivity of the system has been examined, and no interference from other pathogens including *Salmonella typhimurium, Campylobacter jejuni,* and *Listeria monocytogenes* was observed. The sensor's working range is from 3.2×102 to 3.2×104 CFU/ml, with the relative standard deviation of 2.5–9.9%. The total detection time is less than 2 hours.

An improved antibody-coated sensor system based on quartz crystal microbalance analysis of *Salmonella spp*. has been developed, using thiolated antibody immobilization onto the gold electrode of the piezoelectric quartz crystal surface (110). The best results in sensitivity and stability were obtained with the thin layer of a thiol-cleavable, heterobifunctional cross-linker. The long bridge of this reagent can function as a spacer, facilitating antibody–*Salmonella* interaction on the gold electrode. The sensor's response was detected for the microbial suspension concentrations ranging from 10^6 to 1.8×10^8 cfu/ml.

A label-free immunosensor for the detection of pathogenic bacteria using screen-printed gold electrodes (SPGEs) and a potassium hexacyanoferrate (II) redox probe has been reported by Susmel (130). Gold electrodes were produced using screen-printing, and the gold surfaces were modified by a thiol-based self-assembled monolayer (SAM) to facilitate antibody immobilization. In the presence of analyte a change in the apparent diffusion coefficient of the redox probe was observed; it can be attributed to impedance of the diffusion of redox electrons to the electrode surface due to the formation of the antibody-bacteria immunocomplexes. No change in the diffusion co-efficient was observed when a non-specific antibody (mouse IgG) was immobilized and antigen added. The system has been demonstrated to work with *Listeria monocytogenes* and *Bacillus cereus*.

B. Sensors to Monitor Food Packaging and Shelf-Life

In recent work (147) a cell-based biosensor has been used to control meat freshness. Samples of fresh meat stored at 5°C were periodically removed from storage and washed with water for periods of up to 2 weeks. The water was then charged into a flow injection analysis (FIA) system combined with the microbial sensor using yeast (*Trichosporon cutaneum*) as a sensitive element. This sensor has been specifically developed in this work for monitoring the freshness of meat. Relationships between the sensor signals obtained by the FIA system, the amounts of polyamines and amino acids produced from the meat, and the number of bacteria that had been multiplying in the meat during the aging process were investigated. The sensor response has been found to correspond to the increase in amino acid levels and viable counts in the meat during the first stage of aging. This is due to the fact that amino acids produced initially by enzymes in the meat serve as a nutrition source for septic bacteria, and as a result, the amount of bacterial cells increases with an increasing level of amino acids.

1. Foreign Body Detection

The presence of foreign bodies in processed food is of major concern to the producers. Mechanical separation

techniques based on size and weight of different components have been used for many years to help find foreign bodies in powdered and flowing products. Optical inspection techniques were able to extend the range of detectable foreign objects in free-flowing materials with regard to their shape and color. Metal detectors enabled metallic particles inside the product to be found. With recent achievements in sensor technologies advanced foreign body detection systems are becoming available (55).

The working principle and design of an ultrasonic transducer system with auto-alignment mechanism was first described by Zhao (151). The proposed system has been used for detecting foreign bodies in beverage containers. Variations in reflection amplitude were analyzed as a function of the ultrasound beam incident angle to the beverage container surface. It has been concluded that a quadratic relationship exists between the strength of the reflected signal and the incident angle. Furthermore, a calculation for effective angular increment for searching the normal to a curved surface was introduced. Experiments conducted using the sensor prototype have demonstrated that foreign bodies are detectable in containers of various juices. This sensor design is also applicable to nondestructive inspection of canned food products for the presence of foreign bodies.

C. Biosensors for Food Quality/Additives Control

Existing food processing equipment frequently includes microprocessors that are activated by electronic or biological sensors. Recent advances in electronic vision and computer technology have opened the research horizons for greater accuracy in process control, product sorting, and operation. The development of new sensors and instruments in this area is focused on measuring/evaluating the product's internal and external quality and flavor (138).

The aim of food additives control and measurement is to develop, extend, and enhance the instrumental methods in order to improve consumer-perceived macroscopic quality factors. For quality assessment, grading, and sorting of food products, several types of electronic sensors that can provide rapid and non-destructive determination of product internal qualities have been investigated and described in the literature.

A near infrared sensing technique can rapidly determine the sugar content of intact peaches. This technology has been extended to a number of other commodities, including testing avocados for oil content, and kiwifruits for starch and sugar content. NMR method, for example, can be used for nondestructive detection and evaluation of internal product quality factors, such as existence of bruises, dry regions or worm damage, stage of maturity, oil content, sugar content, tissue breakdown, and the presence of voids, seeds, and pits (see Figure 128.12).

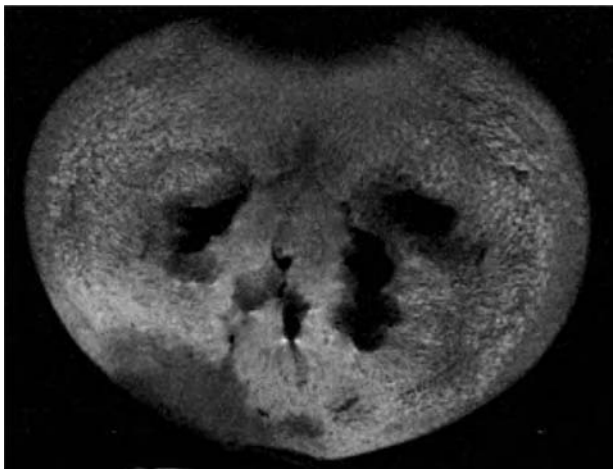

FIGURE 128.12 MRI image of blueberry.

Machine vision for postharvest product sorting and grading is being investigated for a number of commodities. Recent research has included development of a high-speed prune defect sorter, color and defect detector for fresh-market stone fruits, raisin grading, and flower grading machines. In this technology, electronic cameras are used for monitoring the product in various packing-line handling situations. Quality features are computed from digitized images, and the control system allows for product grading and sorting. The NIR/VIS region has been used in several different sensors. Thus, Crochon in his work (35) has presented the design of a glove-shaped apparatus equipped with various miniaturized sensors providing information on fruit quality parameters, i.e., sugar content, maturity, mechanical properties (firmness, stiffness), and internal color. The sugar content and internal color were measured by a miniaturized spectrometer (NIR/VIS) coupled with optical fibers. A sound sensor evaluated the mechanical properties, and the size was measured by a potentiometer placed at the hand aperture. These sensors were coupled to a microcomputer that delivered processed information about the fruit overall quality grade, based on previously established variety and quality classes. The weight of the glove prototype was 400 g, and the electronic devices were held in a rucksack weighing 1000 g. The glove may be used before harvest to control the growth and to estimate the harvest date, at harvest to select fruits with specific qualities, or after harvest to control and measure the quality of the crop.

In (11) chlorophyll fluorescence and reflectance in the NIR/VIS spectrum has been used for the mechanical quality factors assessment of green beans, broccoli, and carrots. Biosensors have been used for evaluating the effects of pasteurization on vegetable quality by measuring the remaining enzymatic activity. Use of mid-infrared (MIR) spectroscopy, as well as Raman scattering, for on-line quality assessment in bakeries, breweries, dairies, and fruit farms has been reported (56).

Another method working in the NIR/VIS range, called time-resolved diffuse reflectance spectroscopy (TDRS), has been used to measure the internal quality of fruits and vegetables (149). The group has developed statistical models for the analysis of relationships between the TDRS signals and the firmness, sugar, and acid content of kiwifruit, tomato, apple, peach, nectarine, and melon. They have also developed the classification models to sort apples, peaches, kiwifruits, and tomatoes into quality classes. Using a pulsed laser diode (70–200 pico-seconds/pulse), the single measurement time was about 100 milliseconds. The absorption coefficient was related to the tissue constituents, while the scattering coefficient was related to the firmness and fiber content. A real-time sensor in the NIR/VIS range can be also used to measure product quality traits, such as maturity, flavor, or internal diseases and defects in potatoes, apples, and peaches (26).

Among the optical sensor systems developed and demonstrated in industrial environments are machine or artificial vision sensors. The system for olives sorting, using a traditional vision camera and three CCD color sensors for the shape, size, and color evaluation, has been described (118). The new algorithm allowed the olives to be sorted into four classes with the speed of 132 olives/sec, and 6 images/sec.

The molecular imprinted polymers (MIP) technology is the new technology used for the development of biosensor substrates (97). The polymers are produced by imprinting the recognition sites of predetermined specificity into cross-linked synthetic polymers. The polymer is consequently able to selectively re-bind the imprinted molecule (100). These sensor materials are called "artificial antibodies" (123). The MIP technology has particular strengths for small molecular analytes up to about 400 Dalton; it may be used to bind and detect many chemicals polluting food products, e.g., pesticides and veterinary drugs in meat and dairy products.

This technology has been successfully employed to develop and optimize plug-in detection cartridge supporting the molecularly imprinted polymer assay (83) for detection of different β-lactam antibiotics in milk. The sensor consists of a micro-fabricated column accommodating an optical detection window. Molecular imprinted polymers in the form of beads were used as packing materials and recognition elements; analyte binding was detected by the fluorescence. The same MIP technology has been used in several other studies, the overall objective of which was to develop novel and robust MIP-based technology that can be used in sensors for real-time measurements of food product contaminants (112, 114, 136). The results of the study indicate that MIP can be used to prepare both selective and general recognition matrices for either individual analytes or groups of compounds, with very good detection reproducibility and stability (136). SPR based sensor shows similar results for dairy product quality applications (48). The MIP developed for clenbuterol has been successfully applied in preparing a novel sensor comprising MIP as the selective element and amperometric detector as the transducer (97). The responses from several sensors were determined to have a variability of 10%. The feasibility for an oxacillin MIP-based sensor was also demonstrated.

At-line immunological sensors using amperometric detection of the resulting antibody-antigen complexes were described (125). The target quality factor assessed in this project was the presence of toxic chlorophenolic fungicides and their chloroanisole breakdown products in potable water, wine, and fruit juices. The electrochemical immunosensor uses monoclonal antibody preparations. The investigations of the effects of liquid food matrices on electrochemical transduction processes indicated that horseradish peroxidase is a suitable label for interrogation of the analyte-antibody immune complex, using amperometry and in-house fabricated screen-printed electrodes. The detection of hormonal substances for growth promotion, also based on immunosensors has been recently reported by Guilbault (58). The sensor has to be used prior to slaughtering, and can detect and measure testosterone, methyltestosterone, 19-nortestosterone, stanozolol, and trenbolone levels in biological fluids (blood). Analysis time achieved was about 30 minutes, compared to 24–36 hours for tests used in laboratories today.

D. Biosensors for Sensory Evaluation of Food Products

"Electronic noses" (139, 146) and "electronic tongues" (32) are the common names of devices responding to the flavor/odor (volatiles) or taste (solubles) of a product using an array of simple and non-specific sensors, and the pattern recognition software system (50). Historically, the sensors used were advanced mass spectrometers or gas/liquid chromatographs, producing a unique fingerprint of the analyte. Nowadays, these sensors have been substituted by arrays of simple electric and/or frequency sensors, or sensors measuring changes in voltage or frequency as a response to the food contact.

Electronic noses and tongues are used in food production and quality control of different products, typically for laboratory tests or at-line control, but may be further developed for in-line operation in the future. Testing times are often in the range of a few minutes, and the largest drawback of these devices is the lack of sensor stability. Examples of claimed successful applications include (14):

- Discrimination between single volatile compounds;
- Tracking of aroma evolution of ice-stored fish or meat;
- Tracking of the evolution of cheese aroma during aging;

- Classification of wines;
- Determination of boar odor (androsterone) in pork fat;
- Classification of peaches and other fruits;
- Differentiation of spices by the area;
- General raw materials control;
- Testing of coffee, soft drinks, and whisky;
- Control of beer quality and faults.

Essentially, each odor or taste leaves a characteristic pattern or fingerprint on the sensor array, and an artificial neural network is trained to distinguish and recognize these patterns (see Figure 128.13). Pattern recognition is gained by building a library of flavors from known flavor mixtures given to the network. Thus, e-noses and tongues are the devices intended to simulate human sensory response to a specific flavor, sourness, sweetness, saltiness, bitterness, etc. (14, 132).

The potentiometric chemical sensors such as ion selective sensors are most often used in the electronic noses. Considerable interest exists in the development of cheap, portable electronic noses to detect, on-line or at-line, odor quality of many foods. For instance, olive oil producers would tremendously benefit from the possibility of detecting oil quality and shelf-life, and classifying the oils by their quality (e.g., Extra Virgin olive oil). This was the objective of a course project in which scientists from olive producing countries developed electronic noses especially for the olive production plants, and tested them with great success (25).

In (40) different tea samples were used to evaluate the applicability of electronic noses for sensory studies. A metal oxide sensor-based electronic nose has been used to analyze tea samples with different qualities, namely, drier month, drier month again over-fired, well fermented normal fired in oven, well fermented overfired in oven, and under fermented normal fired in oven.

Electronic tongues are also widely used to assess taste quality of various products. An electronic tongue based on voltammetry measurements, and a multichannel lipid membrane taste sensor based on potentiometry were compared using two aqueous solutions: detergent and tea (74). The electronic tongue consists of four electrodes made of different metals, a reference electrode and a counter electrode. The measurement principle is based on pulse voltammetry technique in which an electric current is measured during the amplitude change of the applied potential. The taste sensor consists of eight different lipid/polymer membranes. The voltage difference between the electrodes and an Ag/AgCl reference electrode is measured when the current is close to zero. The multichannel electrochemical (potntiometric) sensors have demonstrated better sensitivity, faster dynamic response, but lower reproducibility of the results.

In study performed by Legin (87) the electronic tongue based on a sensor array comprising 23 potentiometric cross-sensitive chemical detectors, and pattern recognition and multivariate calibration data processing tools, has been applied to the analysis of Italian red wines.

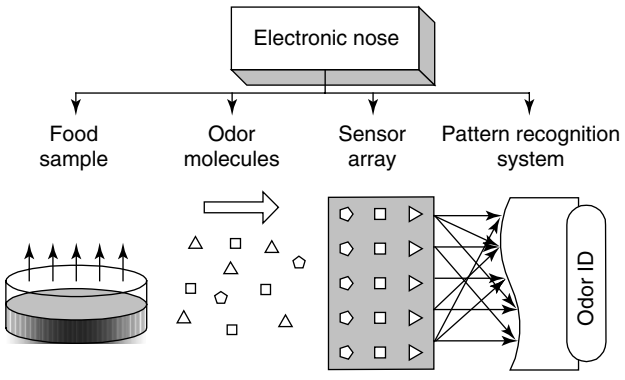

FIGURE 128.13 Principle of the electronic nose operation (42).

VI. ROLE OF BIOSENSORS IN THE FOOD SAFETY MANAGEMENT SYSTEM

A. Biosensors and Biosecurity

Food industry is one of the major potential targets for bioterrorism. The most damage can be attained through: (1) final product contamination using either chemical or biological agents with an intent to kill or cause illness among consumers; (2) disruption of food distribution systems; (3) damaging the food producing cycle by introducing devastating crop pathogens or exotic animal diseases such as foot-and-mouth disease, which could severely impact the food system.

Efforts to develop recognizing preparedness and response strategies for protecting the nation's food supply pose substantial challenges for a number of reasons, including the following (70, 127, 144):

- The food system encompasses many different industries;
- A great variety of biological and chemical agents could potentially contaminate the food supply, and the possible scenarios for deliberate contamination are essentially limitless;
- The public health system is complex, and responsibilities for foodborne diseases prevention and control may overlap, or much worse, fall in the "gray area" between authorities of different agencies.

To achieve an adequate food supply chain and agricultural security, improvement is needed in the activities on bioterrorism prevention, detection, and response. In

addition, appropriate areas for applied research must be identified:

- *Recognition of a foodborne bioterrorism attack.* This may be delayed because of background levels of foodborne diseases and the potential wide distribution of the contaminated product or ingredient.
- *Rapid diagnostic methods* for identifying food-contaminating agents. They are not yet consistently available, and coordinated laboratory systems for pathogens detection are not fully operational.
- *Rapid trace-back procedures* for potentially contaminated products.

B. Biosensors and HACCP

Timely detection of unsafe foods is the main issue that the food safety system should address, providing guidance for the design and integration of such system into the existing food safety management structures, i.e., HACCP. The preventive detection of the biohazard can be accomplished by direct measurements with the biosensors, or indirect detection by the process/environment monitoring and control. Such detection is based on the data from physical and chemical sensors, which are very reliable and allow scale-down, which means the possibility of easy integration into the existing information carriers. The HACCP system for food safety management is designed to identify health hazards, and to establish strategies to prevent, eliminate, or reduce their occurrence. An important purpose of corrective actions is to prevent potentially hazardous foods from reaching consumers. Where there is a deviation from the established critical limits, corrective actions are necessary. Therefore, corrective actions should include the following elements: (a) determine the disposition of non-compliant product; (b) determine and correct the cause of non-compliance; (c) record the corrective actions that have been taken.

Currently, the use of HACCP is voluntary, but it is widely used in the food processing industry as a successful component of comprehensive food safety program. HACCP is a food safety management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution, and consumption of the final product. The terms "HACCP" and "food safety" are used interchangeably in the food industry, implying that HACCP may be the only approach to achieving food safety. HACCP is designed for use in all segments of the food industry from growing, harvesting, processing, distributing, and merchandising, to preparing food for consumption (135).

However, there is a need for enhancement and integration of the existing HACCP system into the total quality management system, and food safety/biosecurity management on higher levels. The system currently includes the mechanisms to decrease the potential for contamination of or damage to the food supply from farm to table (i.e., prevention activities); systems to ensure early detection of deliberate food contamination at any point along the production pathway, including surveillance, rapid laboratory diagnostic, and communication systems; systems to ensure a rapid and thorough response if a bacterial contaminant is detected, including protection of workers and consumers (i.e., emergency response, control, trace-back, and mitigation activities).

The ultimate goal is the integration of sensors and sensor networks into the food safety management structure (see Figure 128.14). Such integration will allow one to perform on-line and "on-shelf" control of the internal and/or external food product quality and package environment.

The integrated sensor information system combines data from multiple sensors (from different packages and/or products) and the information about environmental and process conditions to achieve highly specific information that cannot be obtained by using a single, independent microbiological assay. The emergence of new information carriers and advanced processing methods will make the food safety management system increasingly dependable. A successful biohazard detection system should be able to: (a) identify potential hazards; (b) identify hazards that must be currently controlled; (c) conduct hazard analysis; (d) recommend control factors, critical limits, and procedures for hazard monitoring and verification; (e) recommend appropriate corrective actions if a deviation occurs.

Based on a comprehensive model for multisensor data processing, developed by the US Joint Directors of Laboratories (JDL) Data Fusion Group on DoD request (63), the integrated concept of multiple sensors data processing has been developed for the existing HACCP system of food safety monitoring and biohazard prevention (see Figure 128.14). This model is specifically adapted to the HACCP workflow and utilizes the principle of information

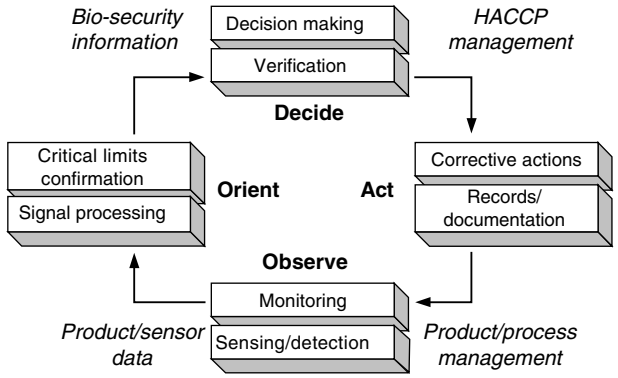

FIGURE 128.14 Schematic process model for integrated HACCP and biohazard detection system.

system cyclic interaction with the environment. The four major steps, including observation/ detection, hazard recognition, decision making, and corrective actions, strictly correspond to the seven HACCP principles. Integration of such a system does not require the redesigning of existing manufacturing and control processes.

The new integrated sensors are able to monitor HACCP control points with corresponding material packaging flow on a continuous basis, or with pre-determined monitoring frequency. Statistically designed data collection or sampling systems lend themselves to this purpose. Issues that need to be addressed when considering implementation of an integrated food safety monitoring system include: where the system would be established; how it would be funded; how the data would be generated, analyzed, summarized, and disseminated; and how "snap surveys" could be utilized as a part of the system.

Microbiological tests are rarely effective for food safety monitoring due to their time-consuming properties and problems with ensuring detection of contaminants. Physical and chemical measurements are preferred because they are rapid and usually more effective for the control of microbiological hazards. For example, the safety of pasteurized milk is based upon the measurements of heating time and temperature rather than on testing the processed milk for the absence of surviving pathogens.

In order to address the issues of connectivity between biosensor devices, the Connectivity Industry Consortium (CIC) has been formed to set up the standardized communication platform for all devices (29). The CIC has identified five requirements: bidirectionality, connection commonality, commercial software interoperability, security, and QC and regulatory compliance (120). Under these standards, new devices should seamlessly link into the existing data management system without additional expenses.

Traditionally, food quality monitoring units consist of a sensor for the particular analyte, an electronic unit to convert the response into a digital signal, and a cable to communicate with the base station. Advances in technology now enable sensors to be integrated with the base station through wireless communication that frees sensors from being physically attached to it. An interest in such freestanding monitoring units is growing rapidly, since they offer the potential for developing integrated networks of sensing devices that can detect, diagnose, and monitor various food safety problems. The merging of computing with wireless communication systems and sensors has led to an increased accessibility to the real-time information in digital form. Due to achievements in communications and connectivity, data from these sensors can even now be easily accessed via personal digital assistants, PCs, mobile phones, and networks. On the other hand, the communications network that has assembled over the past decade and continues to attract huge investments will fuel demands for more sources of health-related information and data.

New technologies do not come into existence easily. It is not just the matter of making conventional laboratory instruments smaller or putting a sensor into the human body. The new sensor devices and networks must satisfy food industry needs by delivering new benefits to users, offering new ways of monitoring food product properties/ contaminations, developing tests that are cheaper, or creating devices that have significant advantages over those already available.

It has been predicted that the trend in biodetection systems development lies in the autonomous sensing technology with the next-generation handheld, portable sensing devices, "smart" sensing, and in-line biodetection. The only limitation to the fast progress in this area is the fact that the sensors — especially chemical and biological ones — lag behind the electronics.

In the future, the evolution of integrated food safety management system may lead to the emerging of a food processing control "nervous system," which will comprise multitudes of sensors and sensing technologies. Such systems could provide the information "nodes" for food safety management and control applications.

VII. FUTURE OF BIOSENSING: DETECTION OF CELLULAR RESPONSE WITH NANOSENSORS

Cells are the smallest functional and integrating communicable units of living systems. Cultured cells transduce and transmit a variety of chemical and physical signals by producing specific substances and proteins throughout their life cycle within specific tissues and organs. Hence, cells and their responses might be usefully employed in screening tools to obtain important information for both pharmaceutical and chemical safety, and drug efficacy profiles *in vitro*. However, cellular signals are very weak and cannot be easily detected with conventional analytical methods. By using novel micro- and nanobiotechnology methods and integrated on-a-chip devices, higher sensitivity to cellular responses and better signal amplification have been achieved (12, 53, 101). Micro- and nanotechnology are now rapidly evolving to suggest new combinations of methods with improved technical performance (53), helping to resolve challenging bioanalytical problems including detection sensitivity, signal resolution, and specificity by interfacing these technologies in micro-scale format in order to confirm specific cellular signals (21, 33, 51, 116, 137, 143). Receiving cell signals in rapid time and small space, and importantly, integration of signals from different cell populations (communication and system modeling), will permit more valuable measuring of the dynamic aspects of cell responses to various chosen stimuli (39). This represents the near future for cell-based biosensing (67).

Concerns over biosafety and security have accelerated the implementation of biohazard control processes, including hazard identification, assessment of its impact on human health, and determination of when, where, and how it would have an impact. Continuous biosafety control is used for assessing the exposure to a hazard, and predicting the necessary dose response.

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129 Genetically Modified Organisms in Food Industry

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CONTENTS

ABBREVIATIONS

ACC	1-amino-cyclopropane-1-carboxylic acid
A. rhizogenes	*Agrobacterium rhizogenes*
ARM	antibiotic resistance marker
APCI	atmospheric pressure chemical ionisation
A. tumefaciens	*Agrobacterium tumefaciens*
B.C.	Before Christ
Bp	basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower Mosaic Virus
cDNA	complementary DNA
CE	capillary electrophoresis
Ct	cycle threshold
CTAB	cetyltrimethylammonium bromide
CTP	chloroplast transit peptide
DNA	deoxyribonucleic acid
ds	double stranded
EDTA	ethylen diamine tetra acetic acid
ELISA	enzyme linked immunosorbent assay
EPSPS	5-enol-pyruvylshikimate-3-phosphate synthase
EU	European Union
FDA	Food and Drug Administration
FRET	fluorescence resonance energy transfer
GM	genetically modified
GMO(s)	genetically modified organism(s)
GUS	β-glucuronidase
HPLC	high performance liquid chromatography
kb	kilobase
LAB	lactic acid bacteria
mRNA	messenger RNA
MS	mass spectrometry
Nalfia	nucleic acid lateral flow immunoassay
NASBA	nucleic acid sequence-based amplification
NIR	near infrared
nm	nanometer
nos	nopalin synthase
nptII	neomycin phosphotransferase II
PCR	polymerase chain reaction
PEG	polyethylene glycol
PG	polygalacturonase
QC-PCR	quantitative competitive PCR
rDNA	recombinant DNA
Ri	root inducing
RNA	ribonucleic acid
RT	reverse transcriptase
sam-k	S-adenosylmethionine
SDS	sodium dodecyl sulphate
spp.	species
ss	single stranded
Taq	*Thermus aquaticus*
T-DNA	transfer DNA
Ti	tumor inducing
UV	ultra violet
Vir	virulent

FOREWORD

The application of biotechnology in food industry is not entirely new. Traditional biotechnology has played a key role in the production of food for thousands of years. For many centuries, the process of fermentation has used microorganisms (yeasts and bacteria) to produce beer, yoghurt and cheese. Naturally occurring microorganisms occur in bread making, beer brewing and vegetable pickling and nowadays, traditional biotechnology techniques are still widely used in the production and preservation of foods.

The basis for modern biotechnology, also referred to as gene technology, is DNA. All organisms are composed of cells containing DNA. This DNA contains the genetic information of an organism. Each organism has its own genetic fingerprint made up of DNA, which determines the regulatory functions of its cells, and thus the characteristics that make it unique. Prior to genetic engineering, the exchange of DNA was possible only between individual organisms of the same species or closely related parent plants to produce offspring, having desirable traits such as disease resistance. The limitations of traditional or conventional biotechnology are time and precision; considerable time may be necessary to achieve the desired traits and the offspring may or may not exhibit the trait of interest, hence the lack of precision.

However, due to improvements in scientific techniques and the advent of genetic engineering in the 70s, scientists have been able to identify specific genes associated with desirable traits in one organism, and transfer those genes beyond the species boundary into another organism. For example, genes from bacteria, viruses or animals may be transferred into plants to produce genetically modified plants with desired characteristics. Through the use of modern biotechnology precision increased and the time to reach the desired trait or characteristic in a cell, animal or microorganism was reduced.

The impact of genetic engineering on the contemporary life has reached unseen heights. Biotechnology continues to be a growing choice among farmers worldwide as the global acreage of crops enhanced through biotechnology increased by 15 percent, or 22 million acres in 2003, according to a report released from the International Service for the Acquisition of Agri-biotech Applications (1).

For the seventh consecutive year, farmers worldwide adopted biotech crops at a double-digit pace, with 2002 global biotech acreage reaching 167 million acres. More than one-fifth of the global crop area of soybeans, corn, cotton and canola acres are now biotech. Nearly 6 million farmers in 18 countries chose to plant biotech crops in 2003, up from 5 million farmers in 13 countries in 2001.

The aim of this chapter is to provide enough information and some examples to give the reader a sound knowledge of the use of plant biotechnology for food purposes. It is not intended to provide an encyclopaedic coverage of the subject though. The text describes the most common technologies that enable the genetic manipulation of crop plants and some applications in food industry. Furthermore, attention is focussed on the detection of genetically modified organisms in food products.

I. HISTORICAL BACKGROUND

Since life began, genes have crossed the boundaries of related and unrelated species in nature. Biotechnology applications by humans date back to 1800 B.C., when people began using yeast to leaven bread and ferment wine. By the 1860s, people started breeding plants through deliberate cross pollination. They moved and selected genes to enhance the beneficial qualities of plants through cross-breeding without knowing the traits for which the genes coded. Most foods, including rice, oats, potatoes, corn, wheat and tomatoes, are the products of traditional cross-breeding. This time-tested practice continues to produce crops with desirable traits.

However, traditional cross-breeding has its limitations. It can only occur in the same or related plant species, so genetic resources available are limited. Moreover, when plants are cross-bred, all plant's genes are mixed, producing random combinations. Since traditional plant breeders ultimately want only a few genes or traits transferred, they typically spend 10 to 12 years backcrossing hybrids with the original plants to obtain the desired traits and to breed out the tens of thousands of unwanted genes. Clearly, this process is not speedy nor precise.

With the advent of recombinant DNA technology in the 1970s, the genetic manipulation of plants entered a new age. Traits previously unavailable through traditional breeding could be acquired through the advance of recombinant DNA technology, developed in 1973. The technique allowed for effective and efficient transfer of genetic material from one organism to another. Genetic engineering of plants began in 1983 when researchers reported that the Ti plasmid of *Agrobacterium tumefaciens*, a common soil bacterium, could be modified to allow transfer of foreign DNA into the plant genome (2, 3). The researchers introduced new genes into plants with the aid of the *Agrobacterium* and also introduced a marker gene for kanamycin resistance to select the transformed cells (2, 4, 5).

The production of genetically modified plants rapidly became an important tool for scientific investigation, and transformation methods for a wide variety of crops were subsequently developed (6–11). Many technological breakthroughs in the laboratory soon followed, including engineered resistance to plant viruses, insect resistance based on expression of *Bacillus thuringiensis* (*Bt*)

proteins, tolerance to various herbicides, control of fruit ripening and softening in tomatoes, engineered male sterility and restoration, modified carbohydrate composition and altered oil composition (12). Elite plant varieties from all of these discoveries at the laboratory bench have now been developed. They have been approved by regulatory agencies and are being prepared or grown for commercial applications.

In the 1990s, the first genetically engineered foods were made available to the public. In 1990, Pfizer Corporation's genetically engineered form of rennet used in making cheese was approved, but it received little public attention. Only four years later, in 1994, the Food and Drug Administration (FDA) gave approval for Calgene Corporation's Flavr Savr Tomato, the first genetically engineered whole food approved for the market (13).

II. TRANSFORMATION METHODS

A transgenic plant is a plant that has received a DNA segment or gene(s) from another organism. The foreign segment of DNA is incorporated into the plant's genome through natural systems present in plant cells. Numerous methods to introduce foreign DNA into plant cells have been developed. A transformation system should allow for (14):

- stable integration into the host genome without structural alteration of the foreign DNA,
- integration of a distinct number of copies of the transforming DNA,
- stability of the new phenotype over several generations,
- eventual tissue and development specific regulation of the introduced gene.

Among the array of genetically engineered plants which currently have been approved, the transformation of choice has been the use of modified plasmids of *Agrobacterium.* Other transformation methods are based on physical and chemical principles.

A. Agrobacterium tumefaciens

Plant transformation mediated by *Agrobacterium tumefaciens*, a soil plant pathogenic bacterium, has become the most used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. Jozef Schell and Marc Van Montagu were the first to discover that the bacterium *A. tumefaciens* transfers a copy of parts of its genetic material into cells of wounded plants, causing the formation of crown gall tumors (3). The ability to cause crown galls thus depends on the ability of *Agrobacterium* spp. to transfer bacterial genes into the plant genome (15).

Virulent strains of *A. tumefaciens* and *A. rhizogenes* contain a large plasmid (more than 200 kb), respectively known as the Ti-plasmid (tumor inducing) (Figure 129.1) and the Ri plasmid (root inducing). These bacteria possess the exceptional ability to transfer T-DNA, a particular mobile DNA segment of the Ti or Ri plasmid, into the nucleus of infected cells where it is then stably integrated into the host genome and transcribed, causing the crown gall disease (Ti) and hairy roots (Ri) respectively (16, 17). The process of T-DNA transfer is mediated by the cooperative action of proteins encoded by genes determined in the Ti plasmid virulence region (*vir* genes) and in the bacterial chromosome.

The initial results of the T-DNA transfer process to plant cells demonstrate three important features for the practical use of this process in plant transformation. Firstly, the tumor formation is a result of the integration of T-DNA into the plant cells and the subsequent expression of the T-DNA genes. Secondly, the T-DNA genes are transcribed only in plant cells and do not play any role during the transfer process. Thirthly, every DNA sequence can be transferred to plant cells, no matter where it comes from. These well-established facts allowed the construction of

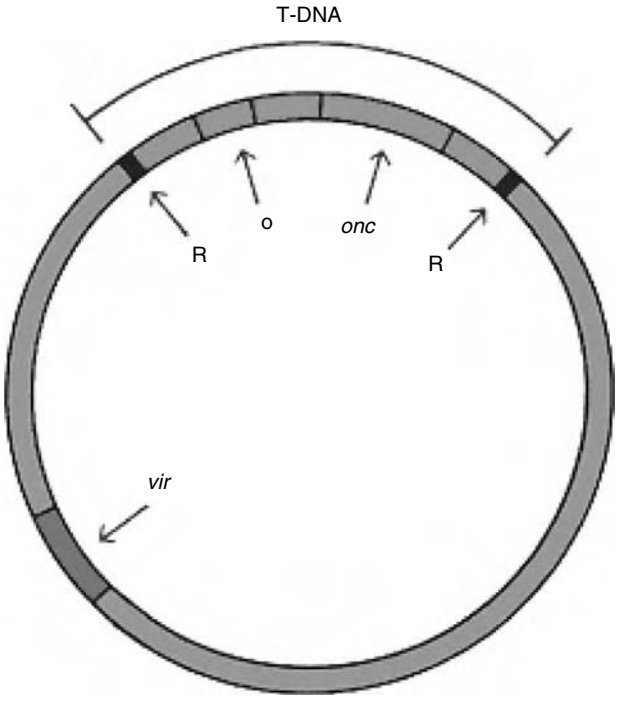

FIGURE 129.1 The Ti plasmid of *A. tumefaciens*. [R: repeat (border) sequence, O: coding for an opine-synthesizing enzyme, *onc*: coding for enzymes that are involved in the biosynthesis of plant hormones, *vir*: controls the transfer of the T-RNA to the host (plant) chromosome.]

the first vector and bacterial strain systems for plant transformation (18).

B. Biolistics

Particle (gun) bombardment, or biolistics, is the most important and most effective direct gene transfer method in regular use. In this method, rapidly propelled tungsten or gold microprojectiles coated with DNA are blasted into target plant material, where the DNA is released and can integrate into the genome (6, 19). The integration of the transgenic DNA though is infrequently. In order to generate transgenic plants, the plant material, the tissue culture regime and the transformation conditions have to be optimised quite carefully and in many cases tissue regeneration is necessary.

Shortly after its discovery, researchers demonstrated the effectiveness of the microprojectile mediated system by successfully transforming monocots, the first of which was Black Mexican Sweet corn (10, 20). This new ability to transform and regenerate monocot plants marked a significant advance in plant transformation.

C. Electroporation

The electroporation of cells can be used to deliver DNA into plant cells and protoplasts. The vectors used can be simple plasmids. The genes of interest require plant regulatory sequences, but no specific sequences are required for integration. Material is incubated in a buffer solution containing DNA and subjected to high voltage electrical pulses. The DNA then migrates through high voltage induced pores in the plasma membrane and integrates into the genome (21). Electroporation has been successfully used to transform all the major cereals, particularly rice, wheat and maize.

D. Microinjection

Simmonds and coworkers reported the use of microcapillaries for the introduction of plasmid DNA into the germ line precursor cells of apical meristems by microinjection (22). Despite a positive indication by PCR amplification of DNA isolated from injected apices, this approach has failed to yield any transgenic plants (23).

E. Polyethylene Glycol

This procedure revolves around the use of protoplasts and their totipotent ability to regenerate into mature plants. Protoplasts are plant cells whose cell walls have been removed leaving only a plasma membrane around the cells. Plant protoplasts can be transformed with naked DNA by treatment with polyethylene glycol (PEG) in the presence of divalent cations (usually calcium). The PEG and the divalent cations destabilise the plasma membrane of the plant protoplast and render it permeable to naked DNA. Once inside the protoplast the DNA enters the nucleus and integrates into the genome (24). PEG treated protoplasts for the most part have been abandoned as a genetic delivery system, because of the low effectiveness.

An advancement to PEG mediated transformation was the liposome mediated transformation technique. Foreign DNA is encapsulated in a spherical lipid bilayer termed a liposome (25). In the presence of PEG, the host protoplast will bind and envelop the liposome through endocytosis (26). After endocytosis, the DNA is free to recombine and integrate with the host genome. The liposomes are formed from neutral lipids similar to those which compose the plasma membrane and can be produced in a variety of sizes ranging from 30–50 nm with a volume of approximately 2 ml. The DNA is packaged *in vitro* and then combined with the target protoplasts. As with other transformation systems, a variety of vectors including viral vectors can be incorporated into this system.

F. Silicon Carbide Fibres

With this technique, plant material is introduced into a buffer containing DNA and silicon carbide fibres, which is then vortexed. The fibres penetrate the cell wall and plasma membrane, allowing the DNA to gain access to the inside of the cell (27). Although the procedure has been utilised with friable callus from maize (28), this type of friable callus is limited only to a few genotypes of maize and oats. Many cereals produce an embryogenic callus that is hard and compact and therefore not easily transformed with this technique. Recently though, some progress has been made in transforming such material, and procedures are being developed to allow transformation of cereals such as rice, wheat, barley and maize without the need to initiate cell suspensions (29).

III. TRANSGENES

Transgenes are not necessarily different from endogenous genes. However, transgenes are often chimeric, that is cobbled together from elements found naturally in different genes. The most important element is usually the protein coding region, which consists of one open reading frame.

A. The Promoter

Promoters mediate the initiation of transcription in a manner that is dependent on tissue type and sometimes other signals. Promoters of variable strength and tissue specificity are available. They can be constitutive (typical) or inducible by environmental or chemical stimuli (rare). The most widely used promoter is the promoter driving

expression of the 35S Cauliflower Mosaic Virus. CaMV 35S is a strong promoter that is active in essentially all dicot plant tissues. Promoters have also been constructed that are inducible by heat shock, copper ions, glucocorticoid hormones, alcohol, antibiotics and other stimuli. Native plant promoters are usually less than 1 kb in length. A plant promoter will often work in many different plant species, but yeast, human or bacterial promoters do not function in plants (15).

B. The Terminator

The terminator serves as a transcriptional stop signal to the polymerase. Various terminators are in widespread use, e.g., one derived from CaMV and one derived from the nopaline synthase (nos-3′) gene of *Agrobacterium*. The various terminators are basically equivalent (30).

C. Structural Genes

The structural genes are the DNA sequences which contain the information encoding the protein of interest. Distinction should be made between target genes and reporter genes (see marker genes).

Many genes have been used for the generation of the currently approved transgenic crops. Some of these genes, such as accd, accS and sam-k (tomatoes) and some genes coding viral coat proteins have only been used in one particular genetically engineered product. As a consequence, the identification of sequences of one of these genes in food would represent a product-specific detection method, provided the actual sample did not contain the natural sources of these sequences (e.g., from bacteriophages or plant viruses) (30).

More common is the endotoxin gene from *Bacillus thuringiensis* (insect resistance — corn, potato, tomato) or the *bar*/*pat* gene, originally isolated from *Streptomyces hygroscopius*, coding for the enzyme phosphinothricin acetyltransferase (herbicide tolerance — chicory, corn, soybean, oilseed rape, sugar beet, rice). Variants of the CP4 EPSPS gene from *Agrobacterium* (herbicide resistance — corn, soybean, cotton, oilseed rape, sugar beet, potato), the β-lactamase gene (tomato) and the polygalacturonase gene (tomato) have also been introduced. Furthermore, the gene encoding for barnase from the bacterium *Bacillus amyloliquefaciens* encodes a ribonuclease which catalyzes the hydrolysis of single stranded RNA molecules. The gene is expressed in the anther only and causes male sterility (chicory, corn, oilseed rape) (30, 31).

Other genetically modified (GM) foods currently available are:

- melon: reduced accumulation of S-adenosylmethionine (SAM), and consequently reduced ethylene synthesis, by introduction of the gene encoding S-adenosylmethionine hydrolase,
- papaya: papaya ringspot virus (PRSV) resistant papaya produced by inserting the coat protein (CP) encoding sequences from this plant potyvirus,
- wheat: selection for a mutagenised version of the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS) or acetolactate pyruvate-lyase,
- squash: cucumber mosiac virus (CMV) and/or zucchini yellows mosaic (ZYMV) and watermelon mosaic virus (WMV) resistant squash produced by inserting the coat protein (CP) encoding sequences from each of these plant viruses into the host genome.

D. Marker Genes

One of the technical problems encountered in attempts at gene transfer is knowing whether a particular gene has actually been introduced into a new host cell and, if transferred, whether it is directing the synthesis of protein. To overcome this problem, reporter or marker genes have been developed, which can be transferred to the plant cell using *Agrobacterium*.

The choice of a selectable marker gene depends on the plant species and the specific genotype of the plant. In general, antibiotic resistance genes make good selectable markers for many dicotyledonous species, such as tobacco or *Arabidopsis thaliana*. In contrast, many monocot species are quite resistant to common antibiotics, and herbicide resistance genes are preferred in this case. Herbicides are also cheaper than antibiotics and they can be applied to soil grown plants.

Marker genes tend to be developed from bacterial genes coding for easily assayed enzymes (32–34). A typical marker gene is the neomycin phosphotransferase II gene (nptII), with kanamycin resistance, as used in the Flavr Savr™ tomato (30, 35, 36). An alternative system uses the gene for a naturally derived enzyme, phosphomannose isomerase (37, 38). This particular enzyme enables plant cells to use mannose as a source of energy. The cells that manage to grow in the presence of mannose have acquired the marker gene and have therefore also taken up the other genes of interest. This system, and similar ones based on other sugars, should allay the fear that GM poses a danger to human health. These should allow a refocusing of effort to tackle the overuse of antibiotics in intensive farming and their overprescription in medicine which pose a far greater threat to our health.

GUS, the *Escherichia coli* β-glucuronidase gene (39, 40), and the luciferase gene, which is obtained either from fireflies (*Photinus pyralis*) or the marine bacterium *Vibrio harveyi* have been very successful as reporter genes too (41).

In the near future, it can be expected that the selection of markers for antibiotic resistance will be avoided. There is no current list though of antibiotic resistance markers that

cannot be used in the genetic modification of plant crops. In Europe, Article 4(2) of Council Directive 2001/18/EC refers to the phasing out of genes expressing resistance to antibiotics which may have adverse effects on human health and the environment or are of use in medical or veterinary treatment. This phasing out must take place by 31 December 2004 in the case of GM crop plants for marketing and by 31 December 2008 in the case of the release of GM crop plants for research and development purposes. In accordance with this requirement the commission has established an expert working group to address the use of antibiotic resistance marker (ARM) genes that genetically modified organisms (GMOs) may contain and will aim to produce a list of ARMs which must be phased out.

IV. STRATEGIES FOR GENETIC MANIPULATION

Enzymes are the products of the majority of transgenes introduced into the currently approved genetically engineered agricultural crops. The expression of these enzymes has conferred novel traits to the respective plants. Proteins without an enzymatic activity, such as toxins, or antisense constructs have also been expressed.

A. Sense Strategy

In order to add a new trait to a crop, one or more genes or their complementary DNA (cDNA) should be added to the genome of the host plant. For this purpose, the gene or cDNA is cloned in a sense orientation in between promotor and terminator. This results in the expression of messenger RNA to a protein.

B. Transgene Silencing

Instability of transgene expression is still a problem encountered in many experiments involving transgenic plants and is often referred to as gene silencing. Gene silencing can involve a variety of methods and is still relatively poorly understood.

When the chromosomal locus harboring a transgene is analyzed carefully, one can find either a single T-DNA or multiple copies of the T-DNA. If multiple copies are present, they can be arranged as direct repeats (sense) or inverted repeats (antisense), or partial copies may be present next to complete copies. Transgene loci with multiple copies, especially inverted repeats, are often associated with gene silencing, meaning that the transgene(s) are poorly expressed.

Gene silencing is thought to represent a highly sequence specific plant genome surveillance mechanism. The plant is able to recognize certain nucleic acids as foreign and it has means to suppress the expression of such genes. Two mechanisms can be distinguished. Either the rate of transcription is reduced (transcriptional gene silencing) or the mRNA is destabilized (post-transcriptional gene silencing). Transcriptional gene silencing occurs when genes share homology in their promoter regions. It usually results in altered methylation patterns and altered chromatin conformation, which results in gene silencing by repressing transcription (42).

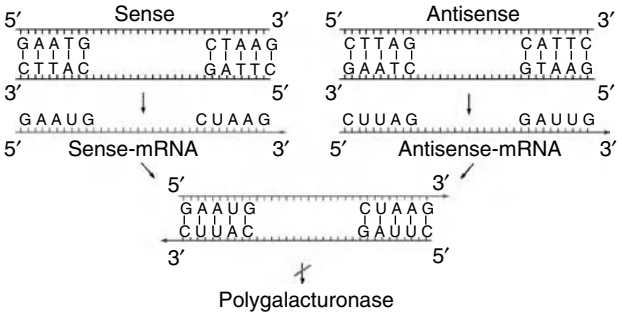

FIGURE 129.2 Construction of the antisense mRNA technology for the polygalacturonase vector.

Sometimes, even endogenous genes that are similar in sequence to the transgene are silenced along with the transgene (co-suppression). This is usually undesirable, although the effect has been exploited to some advantage as well. For example, the delayed fruit softening in the Flavr Savr™ tomato is controlled by co-suppressing the endogenous gene for ethylene production with a transgene of related sequence (43). This tomato contains a gene that is transcribed into a messenger RNA anti-sense to the mRNA from the polygalacturonase (PG) gene (Figure 129.2). The complementary *in vivo* base pairing of these two molecular species results in inhibition of the expression of the gene, with a dramatically decreased PG activity in the transgenic tomatoes. The enzyme PG degrades pectin, a major constituent of the cell wall of the fruit. Its inhibition increases the shelf-life of the tomatoes and prevents them from becoming soft (44).

Unfortunately, it is almost impossible to control whether single or multiple T-DNAs are integrated. One has to generate several independent transgenic lines and screen them for stable gene expression, or stable silencing, as the case may be. It is also possible to find T-DNAs at several unlinked sites in the genome (normally 1–4). Multiple sites are also associated with gene silencing. In this case, genetic backcrossing to wild-type plants should reduce the number of transgene loci and may overcome silencing.

V. APPLICATION OF GENETIC MANIPULATION IN THE FOOD INDUSTRY

A. Benefits of GM Foods

Most of the research in the application of gene technology on food crops has sought to improve product quality and

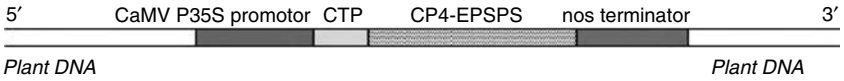

FIGURE 129.3 Constructs introduced in the glyphosate resistant Roundup Ready soybean.

agronomic traits and develop a better resistance to the environment.

Genetic engineering can be used to increase crop yield and reduce crop loss by making plants tolerant to pests, weeds, herbicides, viruses, insects, salinity, pH, temperature, frost and drought. Insect resistant corn (see frame 1), soybean, cotton, potato and apples, virus resistant cantaloupes, squash, papaya, cucumbers and herbicide tolerant corn, tomatoes, potatoes, canola and soybean (see frame 2) have all been produced (45–47). These crops with improved agricultural qualities are considered as the first generation of GMOs, introduced on the market from 1995.

Nowadays, more attention has been paid to the development of GMOs with a clear, direct and significant advantage for the consumer. This second generation of GMOs should gain consumer's interest, trust and acceptance. These foods may have one of the following benefits:

- improved shelf-life, e.g., the Flavr Savr tomato, and organoleptic quality of foods (48),
- improved nutritional quality and health benefits, e.g. oils with an improved fatty acid profile (49), higher lycopene levels in tomato and peppers (47, 50), golden rice with provitamine A (50–54), allergen free rice and peanuts, …,
- improved protein quality and/or quantity (cassava) or increased content in essential amino acids (55–59),
- increase in food carbohydrate content, e.g., potato with a high solids content, which makes it useful for making French fries (60, 61),
- edible vaccines and drugs, e.g., banana with proteins that may be used as vaccines against hepatitis, rabies, dysentery, cholera, diarrhoea or other gut infections prevalent in developing countries (62, 63).

Examples

Bt corn

Bt is a naturally occurring soilborne bacterium that is found worldwide. A unique feature of this bacterium is its production of crystal-like proteins that selectively kill specific groups of insects (Cry proteins). Plant molecular biologists created Bt corn by inserting selected exotic DNA into the corn plant's own DNA. Proteins have been found with insecticidal activity against the Colorado potato beetle (for example, Cry3A, Cry3C), corn earworm (Cry1Ac, Cry1Ab), tobacco budworm (Cry1Ab) and the European corn borer (Cry1Ab, Cry1Ac, Cry9C).

Glyphosate-tolerant soybean

Roundup Ready® soy (Monsanto), the first biotechnologically improved soybean to be marketed, became commercially available in 1996. Glyphosate, the active ingredient in Roundup herbicide, controls weeds by inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is an enzyme in the shikimate pathway for aromatic amono acid biosynthesis in plants. Roundup Ready soybean event 40–3–2 was produced by particle acceleration transformation of active EPSPS isolated from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) into the genome of the soybean cultivar A5403. The insertion of DNA (Figure 129.3) includes the Cauliflower Mosaic Virus (CaMV)-derived 35S promotor with duplicated enhancer, the petunia-derived chloroplast transit peptide (CTP) region, which is responsible for the correct processing of the protein in the cell, the EPSPS gene and the nopaline synthase (nos) sequence to terminate the transcription of the genetic construct.

B. Potential Risks of Genetically Modified Foods

The critics of genetic engineering of foods have concerns, not only for safety, allergenicity, toxicity, carcinogenicity and altered nutritional quality of foods, but also for the environment. The use of marker genes has been restricted to prevent the development of antibiotic resistance. Furthermore, genetic pollution, the creation of superweeds and superpests have to be considered.

1. Food Safety

The introduction of modified foods has led to a shift in the food safety assessment towards a greater need for whole food safety assessment. An important feature in determining the potential risks is whether or not the GMO is able to cause disease to humans, animals or plants.

In the United States, it is the responsibility of the Food and Drug Administration (FDA) to provide oversight for all foods, including those derived from GMOs. More than 15 years of laboratory research and field trials with rDNA-engineered plants indicate that the risks posed by these plants are not any greater than or different from the risks posed by plants produced by traditional breeding methods used for more than 100 years (64).

Various organisations and the biotechnology industry have been working together since 1990 to design a safety assessment strategy for genetically modified crops and

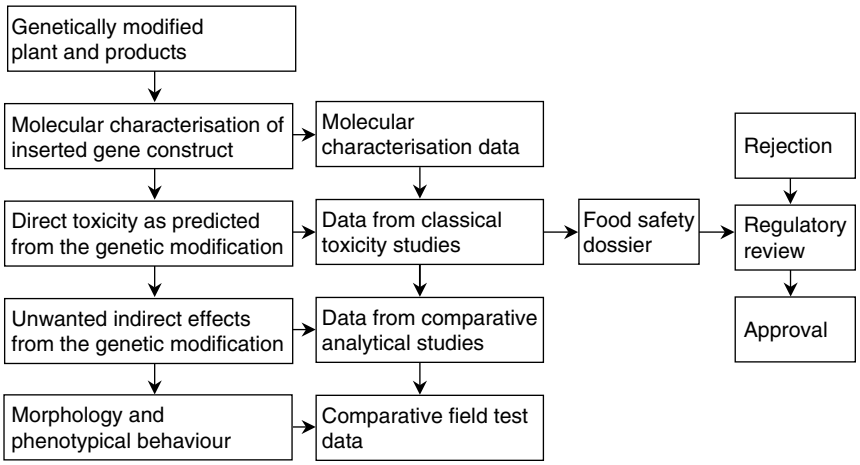

FIGURE 129.4 The current approach for the safety assessment of genetically engineered food (Pedersen *et al.* 2001).

their derived products. The EU legislation has elaborated different rules covering the safety of genetically modified foods: the Directive on the deliberate release into the environment (2001/18/EC) and the Regulation concerning novel foods (258/97/EC).

The food safety assessment of genetically engineered foods should determine whether the modified food is as safe as its traditional counterpart. As a starting point for the safety assessment the concept of 'substantial equivalence' was introduced as a means of establishing a benchmark of safe food. The potential risks associated with the use of a GMO are determined by the characteristics of the organism which receives the modification, the characteristics of the used genetic material and the circumstances under which the GMO is applied.

The food safety assessment of genetically engineered foods is considered to consist of the following parts: (1) a molecular characterization of the insert, (2) determination of any unwanted direct toxicological effects as can be predicted from the nature of the inserted sequences, (3) determination of any unwanted indirect toxicological consequences resulting from the modification and (4) a morphological and behavioral analysis of the plant under relevant field conditions.

The concept of substantial equivalence was applied for the first time in the safety assessment of the Flavr Savr™ tomato before it was placed on the USA market in 1994. In the following years, a lot of experience with the safety assessment of a large variety of genetically modified plants has been gathered. In the EU food ingredients derived from herbicide tolerant soybeans and from several insect and/or herbicide tolerant maize lines, and refined oils derived from several herbicide tolerant rape seed lines were registered and approved according to the legal requirements that have been put in place since 1990 and 1997 respectively.

2. Food Related Concerns

One of the major concerns regarding food safety is the potential allergenicity of genetically modified foods. Well known is the methionine rich protein (MRP) soy from Pioneer Hi-bred International. To increase the protein content of its animal feed, the company incorporated Brazil nut genes into soybeans. This gene modification caused allergic reactions to consumers who were allergic to Brazil nut, so this product was voluntarily recalled in 1996 (65). It is also believed that foreign genes might alter nutritional value of foods in unpredictable ways by decreasing levels of some nutrients while increasing levels of others. Moreover, genetic modification could inadvertently enhance natural plant toxins by switching on a gene that has both the desired effect and capacity to pump out a poison (66).

3. Environmental Concerns

Environmentalists are concerned that transgenic crops will present environmental risks when they are widely cultivated (62). Genetically modified crops with herbicide and insect resistance could cross-pollinate with wild species, creating superweeds (63, 67). These superweeds can become invasive plants with the potential to lower crop yields and disrupt natural ecosystems.

A critical and very controversial aspect of the antibiotic resistance issue is the utilization of antibiotic resistance genes as the selection marker in genetically modified organisms (GMOs). The main safety concern relates to the escape or transfer of the antibiotic resistance genes to sensitive bacterial strains when these GMOs are introduced into the environment. Moreover, the extended exposure to plant produced pesticides could result in the development of a resistance mechanism in the target organism (63).

Plants engineered to contain virus particles as part of a strategy to enhance resistance could facilitate the creation

of new viruses in the environment (53). Plants engineered to express potentially toxic substances such as drugs and pesticides will present risks to other organisms that are not intended as targets. One example includes pollen from transgenic corn, which has been suggested to kill the Monarch butterfly larvae. It has been shown that hybrid corn expresses a bacterial toxin in its pollen, which is then dispersed over 60 meters by wind. In this range, the corn pollen is deposited on other plants near cornfields where it can be ingested by non-target organisms including the Monarch butterfly. These butterflies have been found to eat less, have a slower growth rate and higher death rate (68). On the other hand, research has proven that the Monarch butterfly population has not been affected (69). Since *Bt*-176 corn is no longer available in US maize varieties, the risk to the Monarch butterfly populations from current *Bt* maize varieties is low (15).

4. Other Concerns

The debate involves not only scientific but also political, socio-economic, ethical, religious and philosophical issues (63, 70–73). Some critics of genetic modification argue that patenting which allows corporations to have monopoly control of genetically altered plants or animals violates the justice of life (74).

C. Genetically Modified Microorganisms

Council Regulation 258/97 defines a novel food as food which has not been used for human consumption to a significant degree within the European Community. Within this scope fall foods and food ingredients consisting of or isolated from microorganisms, fungi or algae.

Developments in modern biotechnology facilitate the production of bacterial strains with particular properties. The introduction of such microorganisms into food raises several issues that need to be addressed as part of the safety evaluation. These include: (1) the risk of infectivity and pathogenicity, (2) the potential for colonization and gene transfer within the gastrointestinal tract, and the consequences thereof, (3) their effects on microflora composition and function, including the production of deleterious metabolites and (4) their effects on gastrointestinal mucosa and function (75, 76).

There is huge potential for using biotechnology to develop foods with improved processing qualities. Biotechnology is also likely to be used to produce improved microorganisms, both to improve conventional fermentation processes and to develop new ones. These could include microorganisms for the production of foods (e.g., bread, wine, yoghurt and cheese), or for a wide range of fermentation products for use in food processing (e.g., enzymes, vitamins, amino acids and high-grade chemical additives such as citric acid, a flavouring and acidifying agent).

The major areas currently attracting attention are described in this section.

1. Lactic Acid Bacteria

The vast majority of bacteria used in the food and dairy industries belong to the group known as the lactic acid bacteria (LAB). In general, genetic manipulation of LAB is achieved either by the inactivation of a gene or by the expression/overexpression of a gene (77). Such manipulation may affect a biochemical pathway resulting in different end products or altered yields of end products. This in turn affects the taste, texture, yield or quality of the fermented food.

A commercially important area is the production of new strains of lactic acid bacteria which carry out a faster, more efficient fermentation. For example, using plasmid technology, new strains of *Lactobacillus* spp. have been produced to provide improved starter cultures for cheese production. The potential for the more rapid production and maturation of cheeses, and a whole range of other fermented products, is being exploited. Another important achievement is the introduction into lactic acid bacteria of genes resistant to destructive bacteriophages. Additionally, lactic acid bacteria are excellent producers of peptidases, which are already widely used in food technology. This characteristic is thought to have enormous potential, not least because these bacteria, being associated with the production of traditional foods, are largely recognised as being 'safe.' GM lactic acid bateria have also been used in meat and sausage fermentation (78, 79).

2. Enzymes

Enzymes are very important in food processing. Apart from enhancing nutritional value, they can be used to influence flavour, aroma, texture, appearance and speed of production.

Microorganisms are the most important sources of these enzymes. There is no reason why the ability to synthesise enzymes normally associated only with plants and animals should not be engineered into microorganisms for culture in fermenters. This was achieved some time ago for the enzyme chymosin (rennin), used in cheese manufacture.

Chymosin from transgenic yeast was the first enzyme from a genetically modified organism to gain regulatory approval for food use in 1988. Three such enzymes are now approved in most European countries and the USA derived from *Escherichia coli*, *Kluyveromyces lactis* and *Aspergillus niger*. These proteins behave in exactly the same way as calf chymosin, but their activity is more predictable and they have fewer impurities. Such enzymes have gained the support of vegetarian organisations and of some religious authorities. Chymosin obtained from recombinant organisms has been subjected to rigorous tests to ensure its purity (80).

3. Yeasts

Much attention is currently being paid to the possible use of yeasts as a vehicle in which to express transferred genes. Yeast is an important microorganism in the food industry and has applications in brewing, baking and in the production of fermented foods such as soya sauce.

In recent years, much research has been conducted into the genetic manipulation of *Saccharomyces cerevisiae* in order to enhance endogenous characteristics, such as ethanol tolerance and to obtain expression of foreign genes and the secretion of foreign proteins, some of which are useful to the food industry (81–83). The yeast *Saccharomyces cerevisiae* var. *diastaticus*, whilst not itself suitable for use in brewing, produces an amylase capable of hydrolysing starch residues which normally remain in the brew. The high calorie starch residues are thus converted into fermentable sugar. A gene coding for the enzyme is transferred, via a plasmid, to normal brewing strains of yeast. The transgenic yeast can be used to produce a high alcohol premium product or, alternatively, a greater volume of low calorie 'lite' beer. New yeast strains that are tailored to the barley and hops that are grown in different regions of the world are being developed for use in the brewing industry.

4. Fungal Factories for Enzyme Production

Fungi are of major economic importance as opportunistic pathogens and spoilage organisms but they also have a number of positive uses in food and non-food industries, e.g., the production of catalase, glucose oxidase, lipase and pectinesterase. These useful fungi may be modified genetically to improve their efficiency and enzyme-secreting capacity (84).

VI. DETECTION OF GMOs

A. Labelling of GMOs

There is a need for processors and traders to meet emerging mandatory GMO-labelling requirements in certain countries, in particular the EU, but also in Switzerland, Australia, New Zealand, Japan, etc. The tolerance levels for labelling may differ among countries or still have to be decided. EU legislation on labelling is summarised in the following section.

Since 18 April 2004 two new regulations (1829/2003 and 1830/2003) (86, 87) concerning traceability and labelling of GMOs entered into force in the EU. A harmonised community system is set up to trace GMOs, the labelling of GM feed is introduced, the current labelling rules on GM food are reinforced and a streamlined authorisation procedure for GMOs in food and feed and their deliberate release into the environment is established. Today, labelling of GM food or feed is mandatory, even when the specific DNA or protein of the GMO can no longer be identified in the final product. Adventitious and technically unavoidable presence of GMO are not subject to these labelling provisions as long as this presence is below a 0.9% threshold of the food ingredient individually considered. For the presence of GM material that is not approved in the EU but benefits from a favourable scientific risk assessment by the Scientific Committees or the European Food Safety Authority, a 0.5% threshold is established. These laws also extend the labelling requirements in order to cover food and food ingredients produced from GMOs under the level of detection, e.g., soya or maize oil produced from GM-soya or GM-maize and biscuits with maize oil produced from GM-maize.

It is obvious that the development and application of reliable and quantitative analytical detection methods are of utmost importance for the implementation of these labelling rules. In general, detection methods for GMOs are based on DNA or protein level. The first uses the polymerase chain reaction (PCR), the latter is based on immunoassays. Other detection strategies will be discussed briefly.

B. Detection Based on DNA

1. DNA Isolation

Provided that the laboratory sample is representative for the field sample, batch or lot of the product and that is has been adequately homogenised, even small aliquots of vegetal material are sufficient for DNA extraction, usually between 100 mg (87) and 350 mg (88).

For the extraction of DNA from plant tissues and food products, a vast range of methods is available. An overview has been given by Anklam *et al.* (31). Currently, three different approaches to DNA isolation from plant material and plant-derived products are favoured for GMO detection: the CTAB method, DNA binding silica columns (various commercially available kits) (89) and a combination of these two. In general, DNA extraction from plant material has to accomplish the following steps (31):

1. the breakage of cell walls is usually achieved by grinding the tissue in dry ice or liquid nitrogen,
2. the disruption of cell membranes is achieved by using a detergent (e.g., CTAB or SDS),
3. inactivation of endogenous nucleases is achieved by the addition of detergents and/or EDTA, which binds Mg^{2+}, an obligatory co-factor of many enzymes. Proteinase K may be added for inactivation and degradation of the proteins, particularly in protocols using DNA binding silica columns,
4. separation of inhibitory polysaccharides is possible due to the differential solubility of polysaccharides and DNA in the presence of cetyltrimethylammonium bromide (CTAB),

5. separation of hydrophobic cell constituents, e.g. lipids and polyphenols is attained by extraction with an organic solvent like chloroform,
6. finally, the separation from the detergent and concentration of DNA is carried out by alcohol/salt precipitation.

2. PCR — Principle

The polymerase chain reaction allows the millionfold amplification of a target DNA fragment in a highly sensitive and specific manner. DNA fragments with a length of 100 to 1000 base pairs are amplified with the aid of the polymerase enzyme and two primers. Primers are oligonucleotide sequences complementary to either one of the two strands from the double stranded DNA target.

The kinetics of the PCR are determined by the temperature profile used. Because of the high temperatures required for the denaturation of DNA, the use of a thermostable DNA polymerase is necessary. For this purpose, a polymerase isolated from the thermophylic bacterium *Thermus aquaticus* (*Taq*-polymerase) is used. The PCR protocol exists of a 20 to 50 cycle program, each consisting of the following steps (Figure 129.5):

- denaturation: the single stranded DNA molecules are obtained by heating the DNA solution to a temperature of 94–95°C, enough to break the hydrogen bonds between the strands,
- annealing: by decreasing the temperature to around 55°C, the primers bind to their complementary DNA sequence. Template DNA, primers and DNA polymerase are included in the reaction from the beginning of the PCR,
- extension: a polymerisation step is carried out at around 72°C under the action of the DNA polymerase.

The primer extension products synthesised in one cycle will serve as a template in the next. The repetitive series of cycles results in the exponential accumulation of a specific fragment whose termini are defined by the primers.

After the PCR, the length of the amplification products has to be checked using the electrophoresis technique, although other separation methods such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been used (90, 91). For the separation of large DNA fragments agarose gels are used, while polyacrylamid gels are more suitable for the separation of small fragments. For GMO detection, gel electrophoresis is preferred. Under an electric field, the negatively charged DNA molecules will move through the gel at different rates depending on their size, resulting in a segregation of the different fragments of DNA. The DNA is stained with a fluorescent molecule that binds to DNA and is visualized under UV light.

3. PCR Strategies for GMO Screening and Identification

Any PCR based detection strategy depends on a detailed knowledge of the transgenic DNA sequences and the molecular structure of the GMOs in order to select for the appropriate oligonucleotide primers.

Before conducting a GMO specific or screening PCR assay, the presence of amplifiable DNA in food samples must be determined by using species specific primers. Several (GM) food ingredients have been analyzed using PCR: soy (92), wheat (93), canola, potatoes (94), rice, papaya (40), alfalfa (39), maize, sugarbeet and tomatoes (95).

Nowadays, routine GMO is focused on the detection of the Cauliflower Mosaic Virus (CaMV) 35S promotor (P-35S) and the *Agrobacterium tumefaciens* nos terminator (*nos3′*), which are present in many GMOs currently on the market. However, additional target sequences are needed in order to guarantee a complete identification procedure. Moreover, these target sequences may occur as natural contaminants in the sample (from plant viruses and bacteria). Therefore, specific sequences that are characteristic for the individual transgenic organism should be targeted,

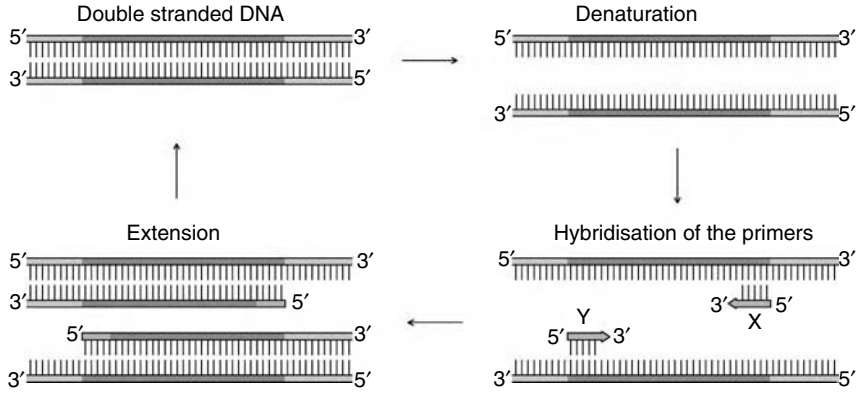

FIGURE 129.5 The polymerase chain reaction.

such as the cross border regions between the integration site and transformed genetic element of a specific GMO, or specific sequence alterations due to truncated gene insertions (i.e., cDNA, or altered codon usage) (96).

If there is any doubt about the fragment identity, the amplified fragment can be checked more precisely using specific endonucleases (restriction fragment analysis). These enzymes cut only the expected DNA sequence into two fragments of known size (97).

With Southern blotting, the sample DNA is isolated and fixed onto nitrocellulose or nylon membranes and probed with double stranded labelled nucleic acid probes specific for the GMO. Hybridisation can be detected radiographically, fluorometrically or chemiluminescently.

Recently, an alternative Southern blot technology has been attempted with near infrared (NIR) fluorescent dyes (emitting at ~700 and 800 nm) coupled to a carbodiimide-reactive group and attached directly to DNA in a 5 min reaction. The signals for both dyes are detected simultaneously by two detectors of an infrared imager (98, 99).

Another strategy for GMO identification recently discussed makes use of amplified fragment length polymorphism (AFLP), a DNA fingerprinting method, which has already been used successfully to discriminate between and identify plant varieties, including processed agricultural materials (100, 101).

Other methods to confirm PCR results are: hybridisation (96), direct sequencing of the PCR product (98, 102, 103), nested PCR (97, 104, 105), anchored PCR (106, 107) and mass spectrometric detection of PCR products (108).

4. Quantitative Detection Methods Based on DNA

Two kinds of PCR strategies are currently being used for the quantification of GMOs in food: end-point PCR (quantitative competitive PCR) and real-time PCR.

a. Quantitative competitive PCR (QC-PCR)

The principle of quantitative competitive PCR is the (co-) amplification of internal DNA standards together with target DNA. A small difference between target and control sequence (<40 bp) makes it possible to distinguish between the two reaction products. Each sample is amplified with increasing amounts of competitor, while keeping the sample volume/concentration constant. PCR products are separated by an appropriate method, such as agarose gel electrophoresis and subsequently quantified by photometric methods. At the equivalence point, the starting concentration of internal standard and target are equal (i.e., the regression coefficient is >0.99 and the slope of the regression line ≈1) (109). Although the presence of PCR inhibitors will be noticed immediately because the amplification of both internal standard and target DNA will be simultaneously affected, competition between the amplification of internal standard DNA and target DNA generally leads to loss of detection sensitivity. QC-PCR has been developed for Roundup Ready soybean, *Bt* and Maximizer maize, the P-35S promotor and nos3′ terminator (110–114).

b. Quantitative real-time PCR (RT-PCR)

The real-time PCR technique was originally developed in 1992 by Higuchi and co-workers, allowing to follow the amplification of the target DNA sequence during the whole reaction by indirect monitoring of the product formation (115). Real-time detection strategies rely on the continuous measurements of the increments in the fluorescence generated during the PCR. Therefore, several formats can be used: (1) the ds-DNA-binding dye SYBR Green I, (2) hybridisation probes or fluorescence resonance energy transfer (FRET) probes, (3) hydrolysis probes (TaqMan® technology) and (4) molecular beacons (116). The number of PCR cycles necessary to generate a signal statistically significant above the noise is taken as a quantitative measure and is called the cycle threshold (C_t). As long as the C_t value is measured at the stage of the PCR where the efficiency is still constant, the C_t value is inversely proportional to the log of the initial amount of target molecules.

More than 150 food products containing GM soy (e.g., baby food, diet products, soy drinks, desserts, tofu and tofu products, cereals, noodles, fats, oils and condiments) have been analysed by TaqMan®, proving this method to be sensitive (112, 117–120). Other research has been done on GM maize (121).

c. PCR-ELISA

PCR-ELISA uses the strategy of real-time PCR and can be quantitative when the PCR is stopped before a significant decrease in amplification efficiency occurs (i.e., before the plateau phase is reached). Then ELISA can be used to quantify the relatively low amounts of PCR products (122, 123).

C. Detection Based on RNA

The RNA based methods rely on the specific binding between the RNA molecule and a synthetic RNA or DNA molecule (primer). The primer must be complementary to the nucleotide sequence at the start of the RNA molecule. Usually binding between the RNA molecule and the primer is followed by conversion of the RNA to a DNA molecule through reverse transcription. Finally the DNA can be multiplied with PCR or translated into as many as 100 copies of the original RNA molecule and the procedure can be repeated by using each copy as a template using nucleic acid sequence-based amplification (NASBA). The specific primers needed for the procedure cannot be developed without prior knowledge of the composition of the RNA molecule to be detected (124).

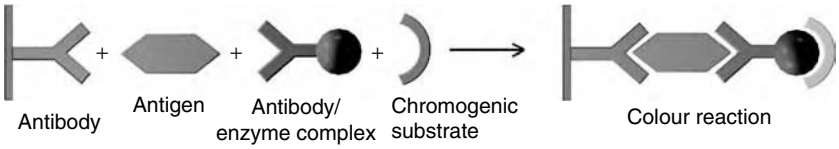

FIGURE 129.6 ELISA principle.

The first consideration when using reverse transcriptase PCR for mRNA analysis is RNA isolation. The RNA should be high quality and free from genomic DNA contamination. However, since most reverse transcriptase PCR methods amplify only a few hundred bases rather than the complete mRNA sequence, the sample RNA can be slightly degraded. The major problem for the use of reverse transcriptase PCR for GMO analysis though is the chemical instability of the RNA molecule and the ubiquitous presence of RNAses.

D. Detection Based on Protein

GMOs are characterised by an altered genome which may lead to the expression of new proteins. Therefore GM foods might be identified by testing for the presence of the introduced DNA or by detecting expressed novel proteins encoded by the genetic material.

Immunoassay technologies with antibodies are ideal for qualitative and quantitative detection of many types of proteins in complex matrices when the target analyte is known. Both monoclonal (highly specific) and polyclonal (often more sensitive) antibodies can be used depending on the amounts needed and the specificity of the detection system (e.g., antibodies to whole protein or specific peptide sequences), depending on the particular application, time allotted for testing and cost (125). On the basis of typical concentrations of transgenic material in plant tissue ($>10\,\mu g$ per tissue), the detection limits of protein immunoassays can predict the presence of modified proteins in the range of 1% GMOs (126).

Both Western blot and enzyme-linked immunosorbant assay (ELISA) techniques have been used for the analysis of protein products of transgenic crops.

1. Western Blot

The Western blot is a highly specific method that provides qualitative results for determining whether a sample contains the target protein below or above a predetermined threshold level (127), and is particularly useful for the analysis of insoluble protein (128). Although developed for the detection of modified soy (129, 130), this method is preferred for research purposes rather than for routine analysis.

2. ELISA

ELISA is the most common type of immunoassay. Antibodies, raised against proteins derived from GMOs, are coated on a microwell plate. For the detection of GMOs, several approaches can be used. In a sandwich ELISA setup (Figure 129.6), the protein extract is spread on the microwell and a specific antigen–antibody binding takes place. After removal of the excess of protein extract, a second enzyme labelled antibody is added, which binds the target antigen. Unbound enzyme labelled antibodies are removed and an enzyme specific substrate is added, which results in a colour reaction if all of the previous reactions have taken place. The intensity of the signal is a measure for the amount of GMO present in the tested sample.

TABLE 129.1
Main Characteristics of DNA and Protein Based GMO Tests (170)

Characteristics	DNA (PCR)	Protein (ELISA/Lateral Flow)
Test sensitivity	High	Medium
Contamination sensitivity	High	Low
Test complexity	High	Medium/low
Test speed	Medium	Medium/high
Universal markers	Yes	No
Test design flexibility	Yes	No
Markers availability	Yes	Low (antibodies)
Automation chance	Yes	Yes/no
Quantification chance	Yes/no	Yes/no
Complex matrices detection	High	Low

One of the major drawbacks of immunochemical assays is that their accuracy and precision can be adversely affected in a complex matrix, such as those found in many processed agricultural and food products. The possible causes for interference from the matrix have been attributed to nonspecific interaction with the antibody by proteins, surfactants (saponins) or phenolic compounds, antibody denaturing by fatty acids and the presence of endogenous phosphatases or enzyme inhibitors. Moreover, detection and measurement may be rendered difficult by low levels of expression of transgenic proteins, the degradation associated with thermal treatments or pH changes, a poor antibody affinity or the commercially available source of antibodies and standards (31, 128, 131).

In summary, the main characteristics and peculiar differences between the DNA based and protein based tehniques for GMO detection are listed in Table 129.1.

Nonetheless, several immunoassay-based methods have so far been developed, such as for the neomycin phosphotransferase II (*npt*II), the EPSPS enzyme (Roundup Ready soy), *Bt* insecticide Cry1Ab and PAT proteins

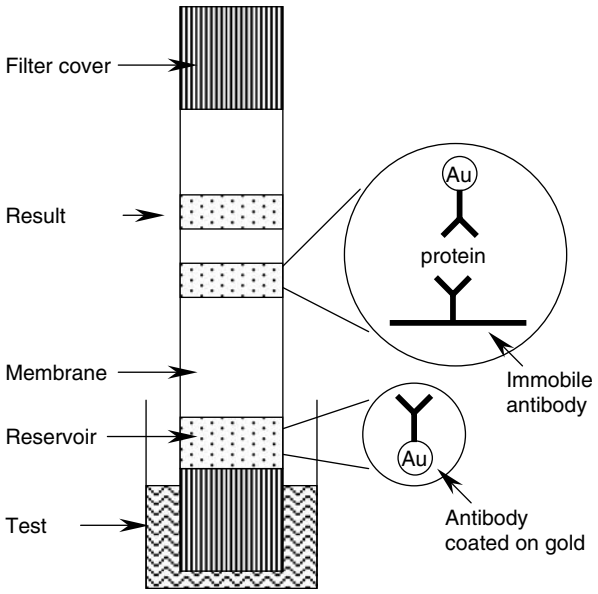

FIGURE 129.7 Schematic representation of the strip test.

(132, 133, 130, 134). Several commercial immunoassay methods are currently available for detection and quantitation of biotech crops expressing Cry1Ab, Cry1Ac, Cry3A, Cry2A, Cry9C, CP4 EPSPS and PAT proteins.

3. Lateral Flow Strip

A variation on ELISA uses strips instead of microtiter wells. A typical strip consists of a reservoir, a result window and a filter cover (Figure 129.7). The reservoir contains (gold) coated antibodies against the target protein. Once the strip has been put into the test solution and this solution reaches the reservoir, the labeled antibodies bind the target proteins. When this complex reaches the area of the second (immobilised) antibodies, a sandwich complex is formed and a colour reaction is observed on the strip, while antibodies are immobilised on the strip. As a positive control, a second band (control line) must be visualised. The lateral flow strip gives results in 5 to 10 min, is economical, consumer friendly and suitable as an initial screening method early in the food chain (125).

4. Other Immunoassays

In addition to microplate ELISA and lateral flow devices, other immunoassay formats are being developed, i.e., in combination with instrumental techniques. For example, in addition to the hyphenated methods, such as immunoassay–mass spectrometry, considerable advances in relative observation of antibody binding to target molecules using biosensors have been reported. Furthermore, immunoassays can be performed with magnetic particles as the solid support surface. The magnetic particles can be coated with the capture antibody and the reaction carried out in a test tube. The particles with bound reactants are separated from unbound reactants in solution by a magnet. Advantages of this format are superior kinetics because the particles are free to move in reaction solution and increased precision owing to uniformity of the particles (128).

E. Other Detection Methods

1. Chromatography

Where the compositon of GMO ingredients, e.g. fatty acids or triglycerides is (significantly) altered, conventional chemical methods based on chromatography can be applied for detection of differences in the chemical profile. This has been demonstrated with oils derived from GM canola for which high performance liquid chromatography (HPLC) coupled with atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) has been applied to investigate the triglyceride patterns (135).

2. NIR Spectroscopy

Recently, NIR has been used in attempts to distinguish Roundup Ready soy from conventional soybean (136). In this study, spectral scans were taken from three spectrometers of whole grains. Results varied slightly, but were promising in alle cases. However, the capability of NIR to resolve small quantities of GMO varieties in non-GMO products is assumed to be low, as is true for the chromatographic methods.

3. Microarray

With the microarray or DNA chip technology microscopic arrays of single stranded DNA of the specific transgene of interest are spotted on a solid support (probe DNA). DNA isolated from the sample of interest (target DNA) is amplified using multiplex PCR. Addition of an exonuclease transforms the double stranded PCR products into single stranded DNA, which is able to hybridise with the spotted ss-DNA. After incubation, fluorescent signals are observed where a positive reaction occured. Analysis of the resulting pattern of spots with a significant degree of hybridisation, and therefore with a significant fluorescent signal, reveals the presence and, depending on the spotted sequence, the identity of GM varieties present in the sample (137).

The method allows fast and simultaneous analysis of several thousand nucleic acids within the very small area of the chip. Therefore, it is very cost saving while maintaining high precision and reproducibility.

4. Nucleic Acid Lateral Flow Immunoassay (Nalfia)

With the Nalfia technique, DNA is amplified through PCR, using primer pairs with different labels. After PCR,

several microliters of the PCR product is pipetted onto a filter (strip), containing an enzyme which interacts with one label of the amplified DNA (originating from one of the introduced labelled primers). The sample then migrates through a nitrocellulose membrane containing another protein, which is able to bind the second labelled primer. If the sample under investigation contains the target gene, the PCR products will contain both labelled primers, which will be sandwiched by the two enzymes from the strip and a colour signal is observed. For the screening of GMOs a prototype of this test has been developed for Roundup Ready soy (138).

VII. THE EFFECT OF FOOD PROCESSING ON THE DETECTION OF GMOs BY PCR

DNA is the preferred analyte for almost any kind of sample (raw materials, ingredients, processed foods) due to the fact that DNA is a rather stable molecule and the most common DNA based detection method, namely the polymerase chain reaction (PCR), is highly sensitive.

The efficiency of the PCR reactions depends on the quality, quantity and purity of the extracted DNA. These factors vary according to the material, the degree of processing of the sample and the DNA extraction method. The DNA quality is determined by its fragment length and its degree of damage due to processing. The quantity of the DNA is determined by the food products itself, the degree of processing and the extraction method applied. The purity of the DNA usually depends on the method of extraction. The detectability of the DNA fragment is also dependent on the PCR approach (nested PCR, real-time PCR, multiplex PCR) and the choice of the primers.

For routine PCR diagnostics in processed foods, amplicon length should be situated below 300 basepairs (30). Damage within the DNA fragments is believed to be caused by the exposure to heat, enzymatic degradation by nucleases, temperature, ionic strength, chemical agents and pH values (139–141). The mechanism of DNA destruction by heat is based on depurination or deamination. At temperatures above 100°C a significant strand scission and irreversible loss of secondary structure occurs (142, 143). The influence of pH may be limited due to cell wall structures protecting the DNA from cleavage. Detectability of DNA template after prolonged incubation at low pH suggests that after initial cell lysis and preliminary DNA destruction, the enzymes responsible for DNA degradation (endogenous nucleases) are destroyed quicker than DNA itself and its further breakdown is avoided (143). In food the rates of these DNA degradation reactions are strongly affected by matrix properties as well as the processing and storage conditions.

Basically no difference between the stability of DNA of a wild type and recombinant organism can be identified. Modifications resulting from methylation or association with DNA binding compounds (e.g., histones, polyamins) might cause minor effects on DNA stability, but these factors apply to any DNA. These conclusions are supported by studies of DNA stability in food such as dairy products, maize polenta, fermented sausages, tofu from soybeans or breads (144).

It is clear that the purity of DNA can be affected by various contaminants in food matrices. These contaminants may originate from the material under examination, e.g., polysaccharides, lipids polyphenols (97, 145, 146) or chemicals used during the DNA extraction procedure, such as CTAB (88, 147). Furthermore, nitrite salts used in sausages (78) and dairy products (148) have been shown to be potent inhibitors of the PCR. A long list of salts, carbohydrates and other compounds frequently used in buffer solutions also decrease the performance of PCR (149, 150). The choice and optimisation of DNA extraction procedures, which eliminate potential inhibitory components may thus be of crucial importance for the success of a given PCR method (151).

Different succesfull DNA extractions have already been published for several GM foodstuffs (30, 85, 88, 92, 97, 141, 146, 152–161).

Although the basic reactions contributing to DNA degradation are already known and many methods have been developed for the detection of DNA sequences of GMOs in foods according to the current legislations, only limited data are available about the release of DNA from cells, as well as its presence and stability during processing and storage of foods.

Bauer *et al*. (144) investigated the kinetics of degradation of plasmid DNA by the process parameters acidic pH and/or temperature using a tomato serum (pH 4.3 and temperature of 65°C). The highest degradation was found for the combined effect of acidic conditions and heating.

Thermal treatment of corn meal at 100°C and potatoes at 80°C contributed to degradation of DNA to fragments smaller than 585 bp and 792 bp respectively (162). A similarly effective thermal DNA degradation was also described for dry corn grains by Chiter *et al*. (163) and for corn gluten and flaked corn by Forbes *et al*. (164). Specific attention has been paid to the degradation of DNA during alkaline boiling (pH 11.0) of corn meal. Alkaline-cooked corn, called nixtamal of corn masa, is an instant product for the production of Mexican corn-based foods such as tortillas, corn chips, taco shells and tamalas. Kharazmi *et al.* (162) reported the failure of amplification of fragments greater than 585 bp while Hupfer *et al.* (140) still detected 1914 bp DNA fragments of corn after boiling at pH 9.0 for 60 min. In a real-time quantitative PCR approach, Quirasco *et al*. (165) were able to detect and

quantify up to 0.1% StarLink corn, in spite of progressive degradation of genomic DNA during processing.

In soybean flour, a rapid decrease in the maximal detectable length of plant DNA under acidic conditions (pH 4.75) was found, even at ambient temperature (144). This is in agreement with the results found by Hupfer *et al*. (140) which illustrated that DNA fragments of 1914 bp were no longer detectable after boiling of Bt-176 maize flour for 5 min at pH 2.0 to 3.0, whereas after boiling for 60 min at pH 8.5 to 9.5 the fragments remained detectable. It was shown for the production of feed that DNA was degraded during ensilaging of Bt-176 maize (pH 3.9–4.1) and fragments of 1,914 bp were no longer detectable after 106 days.

For production of soy milk and tofu, DNA degradation during heat treatment does not significantly contribute to the DNA degradation, the mechanical step of grinding of soaked soybeans is a more crucial DNA degrading step (162). A similar extent of degradation was also observed by Hupfer *et al*. (140) during chopping of whole corn plants. It can be assumed that during grinding or chopping DNA is released and becomes sensitive to the attack of nucleases. Such nucleolytic activities were shown to occur in food matrices, e.g., in bread dough (166) or sugar beet raw juice (167) and it was observed that their action depends on the processing temperature. Klein *et al*. (167) showed that DNA is completely removed during the production of sugar and the overall efficacy of DNA elimination was calculated to 10^{14}.

In addition, the degradation of the plant DNA during production of bread was monitored indicating that temperature and pH are the major effective factors (141, 166).

Still, for some refined and highly processed food products, the detection of DNA remains difficult, resulting in the impossibility to perform a GMO analysis. This category of products contains among others: starch, sugar cane, caramel, dextrose, sorbitol (155, 158), bread with soy sauce (158), refined soybean oil (159, 168), refined corn oil (158), tomato concentrate, tomato puree and tomato ketchup (158), cocoa drinks containing lecithin (97). For these food products, GMO analysis is impossible, or a higher sample volume should be required (160, 161, 169).

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Part O

Packaging

130 Food Packaging: New Technology

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CONTENTS

I. INTRODUCTION

Food packaging is constantly changing to meet new challenges in the market and new needs of the consumer. New technologies continue to emerge with innovations in new packaging materials and packaging techniques that offer new possibilities for manufacturing, packaging, and marketing a wide variety of foods. Today's food package provides not only basic functions (contains and protects) but also offers convenience, facilitates product use, and communicates with intended buyers of the product. The package label informs the consumer of nutrition facts, sells the product through colorful graphics, and addresses environmental concerns such as source-reduction and recycling. Among the innovations and recent developments, major efforts have focused on new barrier materials and technologies for flexible and rigid food containers. Better barrier technology is desirable to enhance quality and safety of food as well as to extend its shelf life. Barrier packaging technology is the main focus in this chapter.

II. EVOLUTION OF PACKAGING

Packaging roles have continuously evolved with social and community development (*1*). Initially bulk packaging was used to deliver goods to retailers where it was received and bagged for merchants. As food processing

technology advanced individual container for consumer products such as canned foods were widely used. By the middle of the 20th century, most consumer goods were individually packed and branded. The small community store that originally sold products manufactured locally evolved into a modern supermarket equipped with refrigeration and accommodated more products. As the number of products increased to hundreds or thousands, the storekeeper could not aid and influence the consumer's purchase. The consumer was left face-to-face with the package. The package had to sell the product as well as to inform the purchaser, calling for the package's motivational and informational roles. To attract the purchaser, package design and graphics needed to be attractive, thus creating a new profession, package design, as well as prompting an evolution in printing and decorating arts.

The post WW II baby boom affected population structure and trends, and subsequently food product and package design. The 1950s marked the emergence of fast food outlets that created a demand for new kinds of packaging, including disposable single-serve packaging and bulk packaging for ready-to-cook food portions. Fast food outlets boomed along with a growing trend toward eating out. This led to formation of the hotel, restaurant, and institutional sector (HRI). The 1960s marked the growth of convenience and prepared food packages, as thermoplastics became available as packaging materials. As use of these plastics increased, the consumer's concern toward environmental problems increased. The 1970s and early 1980s brought changes such as labeling laws, ozone-depleting chlorofluorocarbons (CFCs), standards for the acceptance of new packaging materials, and microwave ovens and microwaveable packaging. Changing demographics of the 1990s were reflected in changes to product packaging. The nutrition labeling law required the package label to include nutrition facts. The aging population became heath conscious and desired food and food products that promote health and living wellness. Families became smaller and the single-person household became common. Married couples have professional careers and higher income levels, calling for "convenient" food products. The food package is required to be environmentally friendly, tamper-proof, and convey detailed information on the nutritional values and ingredients of the food product. Currently, there is a proposed country-of-origin labeling law. Once the law is passed, it will be effective in September 2004 (*2*) and will apply to packaging of meat, seafood and produce, both fresh and frozen. Food packages will continue to evolve in the 21st century.

III. FOOD PACKAGING

Food packaging is the process of wrapping food with a suitable package. The package may be made of one or more materials that provide proper functionalities and properties for holding and protecting the food from the point of production to the consumer, while the quality and safety of the food are maintained. Holding and protecting the food are two major functions for packaging of food. Food is generally sensitive and susceptible to environmental abuse, and deteriorates by chemical, biochemical, and/or microbiological changes that are usually accelerated by environmental factors such as oxygen, water, light, and temperature. With a suitable package, these changes can be prevented or delayed. A suitable package can also prevent contamination by foodborne pathogens, which render the food hazardous and unwholesome for consumption. Food packaging provides wholesome, high-quality, and nutritious food products.

Packaging technology is dynamic as a result of the new challenges and new technologies developed to accommodate new needs in the changing society. Discovery of new packaging materials, new processes, and new techniques have shaped the way we package, deliver, and consume products. The thermoplastics developed in the 1950s have revolutionized the packaging industry. Although many foods are still packaged in traditional glass bottles and tin cans, newer plastic, multilayer, and composite packaging materials are creating opportunities for improved product convenience, presentation, quality, and safety, as well as innovative food product development.

An ideal food package is designed to meet many requirements of the food itself, the processing or preservation methods, distributor and retailer needs, and consumer expectations and acceptance. Modern packaging is driven toward more intensive marketing and globalization. Packaging plays a significant role in motivating purchase. Among competing products that are similar in performance and quality, their packages are different. Package design is then critical in competition, and a new package often helps create uniqueness for its brand.

An increase in market globalization requires a product with an extended shelf life. Better barrier packaging materials and techniques are needed to achieve a desirable shelf life for export. There are two factors influencing the development of food packaging with increased barrier properties. First are the regulatory initiatives that are intended to limit waste generation. Source reduction via downgauging of flexible packaging is one way of reducing plastics' content in solid waste. Second is the improved food preservation that barrier flexible packaging can offer in preventing food losses and spoilage during storage.

IV. RECENT DEVELOPMENTS OF BARRIER PACKAGING SYSTEMS

A barrier can be defined in many ways depending on the desired level of protection from physical damage and chemical and biological changes that affect food quality and safety. Since most food packages are plastics, a barrier

is conceived to be for control of permeation of gases and vapor through the package. Barrier technology has been designed and developed for both flexible and rigid food containers. A desired barrier level can be achieved by using one or more barrier materials for food packages, or by incorporating this barrier material using multi-layer structure, lamination, or coating techniques. Years of research and development have resulted in new barrier technologies for various foods and food products.

V. BARRIER MATERIALS

Although polyethylene terephthalate (PET), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene, (PP), polyvinyl chloride (PVC), and polystyrene (PS) are widely used plastics for food packaging, they provide inadequate barrier properties. Newer barrier materials were desirable. Several barrier materials of current industry interest include polyvinylidene chloride (PVDC), ethylene vinyl alcohol copolymer (EVOH), nylon (PA), modified nylon (MXD6, Selar PA), liquid crystal polymer (LCP), polyethylene naphthalate (PEN), adhesive barrier materials, nanocomposites, and oxygen scavengers. These materials are high priced, so they are used in as small amounts as possible to give the desired barrier properties. Barrier material can be incorporated into a lower cost material by using lamination for a multilayer structure or coating onto a monolayer material.

PVDC is a favored choice for an improved barrier of a food package. It was usually used in multi-layer films and containers before the arrival of EVOH in the 1970s. PVDC and EVOH could be used in co-extrusion of 5-, 7-, and 9- layer cast barrier sheet structures for shelf-stable and retortable food packaging. The basic 7-layer barrier structure is typically a symmetrical arrangement of polyolefin/ regrind/tie layer/EVOH, PVDC, or PA/regrind/tie layer/ polyolefin. EVOH was used for a ketchup bottle that was developed in 1984, and the first multi-layer packaging for retortable packages and microwaveable soup bowls was commercialized in 1987.

Food packaged in shelf-stable, retortable containers is at least one-third more expensive than similar products in metal cans, mostly due to the price of packaging. Despite high packaging prices, the market for the multi-layer cast barrier sheet for shelf-stable and retortable packaging has continued to grow. The growth is especially high for specialty foods, where the package cost is outweighed by the features and benefits that the products provide and the need they meet (*3*). For example, single-service containers of applesauce offer healthier alternatives to most snack foods consumed at school or work, and microwaveable baby foods offer nutrition and convenience in pre-measured, hot meals. Emerging end uses for the multi-layer barrier containers include aseptic packaging for low acid food with particulates, and modified atmosphere packaging (MAP). MAP of meats is going into supermarket chains. Typical MAP is a foamed PS/ tie layer/EVOH/PE or EVA structure with one regrind layer. Shipping meat from fabricating plants in MAP containers could eliminate the need for butcher shops in supermarkets. The expense of packaging, labor, and freight of MAP could be a slightly lower-cost alternative to the in-store butchers.

In the early 1990s, flexible barrier packaging shifted from metalized films, laminates, and rigid containers to films incorporating barrier resins, especially EVOH, along with films produced by new coating techniques that deposit oxides of silicon or metal to obtain clarity (*4*). EVOH use has grown rapidly as a substitute for PVDC coated films and metalized films that are considered less recyclable. Since its introduction, EVOH grades have been developed to overcome delamination problems when used in multilayer structures. Eval Co. of America (Evalca) developed a third-generation delamination-resistant EVOH barrier resin, Eval grade XEP-567, which has better adhesion to PET without a tie layer than the previous grades, MDX6, and other nylons. The XEP-567 grade offers about 50% lower O_2 permeability and about 40% lower CO_2 permeation than Eval XEP-562, a second generation delamination-resistant resin introduced in 1999 (*5*), and offers carbonated soft drinks a shelf life of 16 weeks.

MXD6 is a modified nylon developed by Mitsubishi Gas Chemical in 1986. Selar PA is modified nylon developed by DuPont. Both are often used in multi-layer structure food packages. MXD6 nylon can provide ca. 19–20 times greater barrier capacity than PET. Since it has similar processing temperature to PET, it can be blended with PET but has a drawback of high haze (*6*). MXD6 and liquid crystal polymer (LCP) blend is then preferred for use in multi-layer PET bottles.

Liquid crystal polymer (LCP) is a barrier material superior to PVDC and EVOH. It functions at one-fifth the thickness of EVOH, resulting in overall material saving (*7*). Superex Polymers Inc. developed counter-rotating die technology for biaxially oriented and extruded LCP (*8*). Packaging applications of LCP include beer bottles and blown films. It has more than 200 times greater O_2 barrier than PET and is not moisture sensitive. A monolayer PET bottle is unsuitable for beer because it is neither an efficient oxygen nor flavor barrier, so product shelf life and taste stability are problems. A thin layer of LCP on the PET eliminates both problems. Since LCP has a high price, its use in packaging is minimal and depends on desired product shelf life. A three-layer structure (PET/tie layer/LCP) less than 0.5 mm thick and containing LCP less than 5% of the bottle weight could provide beer with a one-year shelf life. Less LCP could be used if a 6-month shelf life is acceptable, and thus reduce bottle cost. Biaxially oriented LCP film could be laminated or coextruded to produce a lower cost material for barrier packaging.

PEN, a new polyester that has been blended with PET, improves the CO_2 and O_2 barrier properties of the monolayer PET bottle used for beer. As PEN has a glass transition temperature (T_g) of 122°C, far higher than for PET, PEN blends allow the monolayer to sustain pasteurization temperatures for beer. However, the high price of PEN and PET/PEN blends is a major obstacle preventing its widespread use for single-use containers such as beer bottles. Nevertheless, PEN is cost-competitive with refillable glass bottles and less costly than one-way PET bottles when PEN bottles are refilled at least three to five times (*9*). Monolayer PEN bottles are used for marketing of multi-trip refillable beer and water bottles in countries where refillable bottles are accepted, i.e., European countries, Latin America, South Africa, and China.

Blox is the trade name of a new series of clear, tough, highly adhesive barrier polymers from Dow Plastics. They are poly-amino ethers made by polymerizing liquid epoxy resins with an amine to create solid, low melting thermoplastics (*10*). Blox resins can be extruded into films, injection molded, or blow molded. One series of Blox resins can be co-extruded as a barrier layer of multi-layer bottles for juices, beer, or carbonated soft drinks. Their O_2 and CO_2 barriers are about ten-fold higher than that of PEN and in the range of EVOH or MXD6 nylon, depending on humidity level. The clarity and toughness of Blox materials are higher than those of EVOH and MXD6. Blox resins have better adhesion to PET, permitting design of more complex bottle shapes without the risk of delamination, and no tie layer is required.

Nanocomposites are materials where nanometer particles are dispersed in a polymeric matrix, which can be single or multiple phase. A nanoscale particle is a particle with at least one dimension in the nanometer range. Natural and synthetic clays are mainly used as nanoparticles in plastic composites (*11*). However, mixing plastics and clays is not a simple process because the materials are immiscible and tend to form a very light packing of individual clay layers. As a result, clays must be treated with an organic intercalant to improve interactions between clay platelets as well as dispersion of organoclays. An intercalant is an oligomer or polymer that is sorbed between platelets of the layered material and complexes with the platelet surface to form an intercalate. The original concept for these plastic composites began with the invention of polyamide-clay composites by the Toyota Research Corporation in 1985; these were used to make under the hood heat resistant automotive parts that were lighter than metal. The technology has advanced and can be applied to various plastics including thermoplastic olefin (TPO), thermoplastic elastomer (TPE), PP, PET, and nylon. The nanocomposites offer improved mechanical, electrical, gas, and liquid barrier properties. Nanocomposite technology has been migrating from automotive parts to other applications including rigid and flexible packaging. Nanocomposite plastics are usually enhanced by fillers derived from the industrial clay called bentonite (*12*). The fillers form flat platelets that disperse into a matrix of layers, which force gases to follow a tortuous path through the polymer. By increasing the path of diffusion of gases and other molecules, the clay platelets slow gas transmission and increase the barrier properties of the plastics.

Nylon is a preferred nanocomposite additive for making a barrier layer in multilayer PET containers, which are increasingly used to package oxygen sensitive foods and beverages. Nylon has better inherent adhesion than EVOH (an alternative barrier resin), thus sealants are not needed between the nylon and PET layers. With nanoclay additives, the barrier properties of nylon can be doubled or tripled, making it an alternative barrier material to the superior barrier EVOH resins. However, getting nanoparticles to disperse properly to make the tortuous path principle work is not easy. The most promising way is to introduce the clay additive during polymerization, such as with the Aegis's nanocomposite nylon. Eastman Chemical Co., in cooperation with nanoclay producer Nanocor, has developed a nylon composite barrier material, Imperm (*5*). Imperm was designed for use in multi-layer bottles, providing 50–100 times greater O_2 barrier than PET, compared to 10–20 times barrier improvement of MXD6 nylon over PET. A 20-g three-layer PET/Imperm/PET bottle with 4% Imperm in the bottle wall has a 3–5 times greater O_2 barrier than PET and less than 8% haze. A bottle with 10% Imperm has a 6–11 times greater O_2 barrier and less than 10% haze.

Several oxygen scavenger systems have been developed using either oxidizable metal such as iron, various oxidation promoters, and fillers or metal free absorbent systems such as mixtures of organic compounds including quinines, glycol, and phenolics (*13*). In the early 1990s, Toyo Seikan introduced a non-conventional technology, Oxyguard (iron salt-based), for incorporating a high oxygen barrier into blow molded food containers and other rigid packaging (*14*). Oxyguard is an alternative to the EVOH or PVDC coextrusion of a multi-layer structure, where barrier properties decrease under elevated relative humidity and temperature such as during retorting, and they cannot remove oxygen in the headspace of the bottle between the contents and closure. In contrast, Oxyguard's barrier is claimed to be capable of trapping and holding oxygen coming from the headspace instead of simply blocking it.

Amosorb oxygen scavengers developed by Amoco are available in pellet concentrates of PP, PE, PET, and elastomer resins. The oxygen scavenger resins are designed for either retort and hot-fill food applications or non-retort and refrigerated food and beverage packaging, and can be employed in a wide range of packaging structures including rigid containers, films, and closure liners. They are claimed to be heat stable to 320°C and activated by moisture. Amosorb 3000 copolyester was developed for beer, tomato products, fruit juices, and teas. It is an iron-based system that bonds permanently with oxygen that permeates

the package wall, is present in the internal headspace, or is dissolved in the packaged contents. It is transparent and compatible with other polyesters. A multi-layer package containing one or more core layers of Amosorb 3000 is claimed to offer better protection than glass or metal for even the most oxygen-sensitive products.

Hybrid BA-030 copolyester from Mitsui Chemicals was developed for use in beer bottles as part of a 5-layer structure (PET outer layer/BA-030/O_2 scavenger/UV barrier/PET inner layer). The grade is claimed to reduce acetaldehyde levels to as low as 3 ppm, and match or exceed PEN properties when combined with PET (*15*).

AmberGuard polymer from Eastman Chemical was developed for UV light protection. It can be used in a multi-layer container with Eastman's Imperm nanocomposite polymer to provide UV protection and O_2 and CO_2 barriers.

Oxygen absorbing organic ingredients can be copolymerized with monomers of existing packaging polymers to create inherently absorbent structures. Carnaud Metal Box and Crown Cork & Seal developed Oxbar, which is a non-iron-based system that uses nylon and a cobalt salt formulation incorporated into a polyester base, as a chemical trap (*14)*. It is used in three-layer PET bottles for short term applications e.g., single-serve fruit juice and beer as well as wide mouth containers for tomato-based sauces and condiments (*16*).

Southcorp technology developed $ZERO_2$, which is a non-metallic oxygen scavenger system that is activated by UV light after it is incorporated into packaging structures of materials such as PE, PP, PET, and ethylene vinyl acetate (EVA) (*13*). It is used in conjunction with vacuum packing and barrier films.

VI. BARRIER PET CONTAINERS

Polyethylene terephthalate (PET) is one of the most widely used polymers. It was the first polymer to successfully recycle, generating reclaimed materials for a wide range of non-food and food applications. The recyclability of PET material is a factor promoting its use beyond the carbonated soft drink market to include other foods and beverages. It is replacing glass bottles and some bottles made from other plastics such as HDPE, PP, and PS. PET's success is a result of its better barrier and clarity than the plastics being replaced, as well as technological development of the processes used to convert PET into flexible and rigid packages at high outputs, which is crucial for the minimization of packaging costs. Use of PET resins continues to rise because of new applications, as well as the innovation of barrier technologies that help enhance the barrier properties of PET, and thus making it suitable for other demanding applications including use with oxygen sensitive foods. PET is now a commodity polymer competing directly with polyolefins and styrenics in the markets for food and beverage packaging, as well as for other products.

Monolayer PET packages are generally suitable for many food applications but are not suitable for beverages and food products that require better protection or gas barriers. The ideal approach to improve the gas barrier of PET is to design a monolayer PET structure that will provide package design freedom. This approach requires blending a barrier resin, oxygen scavenger, or both with the PET. The monolayer solutions are less practical mainly because suitable materials are high-priced. However, this approach has been the subject of a recent development by Interbrew in cooperation with M&G Group. Interbrew has launched a single-layer PET barrier bottle, Pivopack, for a Russian beer, Klinskoye brand. Pivopack is claimed to be the first monolayer, barrier-enhanced PET bottle, which uses M&G's new Acti TUF, a PET resin made by a proprietary oxygen-scavenging technology that is triggered to react with oxygen only when a container is filled with beverage (*17*). The monolayer PET provides the advantages of allowing the preforms to be manufactured on standard machines. The pricing of these resins is in a range of Eur330–650/ton above that of standard PET (*18)*.

Two other approaches for improving gas barriers are multi-layer structures and surface coatings. Advancements in multi-layer and surface coating technologies are making PET bottles cost competitive with glass bottles and metal cans for beer, carbonated soft drinks (CSDs), oxygen sensitive juices and hot-filled foods. PET bottles for beer were developed in 1999 with at least nine plastic beer bottle programs underway (*19)*. Bottling beer in plastic is difficult due to beer's extreme sensitivity to light and oxygen. Converting beer bottling from glass to PET requires a barrier against carbon dioxide egress and oxygen ingress, while retaining clarity and strength. Beer in bottles requires 120 days of shelf life with less than 15% loss of CO_2 and no more than 1 ppm gain of O_2 (*20*). A major obstacle is a unit cost that is much higher for barrier PET bottles compared to glass. Shifting to barrier PET bottles requires breweries and blow molders to invest heavily in new development. It is not feasible for brewers to drop PET beer bottles into glass bottle lines capable of a high-speed production at a rate of tens of thousand bottles per hour. Another obstacle is that the PET beer bottles fail when they are exposed to thermal stress and pressure at temperatures beyond 62°C, typically used for tunnel pasteurization by 80% of the world's beer filling operations. One solution is heat setting the PET to increase the crystallinity of the material during blow molding. The process produces heavier preforms and slows the process, but the resultant bottle has a thicker wall that can withstand pasteurization at 65°C. Besides a higher cost than glass and cans, one large hurdle to the growth of PET for beer is consumers' perception that beer tastes better in glass (*21*). As PET beer bottles with multi-layers may disrupt the existing monolayer PET bottle recycling stream because of the layers of nylon and PET, the amber color, aluminum cap, and metalized label, some plastic

beer bottle programs have been discontinued. Although recyclability is technically not a problem because studies demonstrated the multi-layer PET beer bottles could be recycled and reused, most recyclers still cannot economically separate the materials (*22*). Due to these complexities, use of both multi-layer and coated PET beer bottles is currently restricted to low-volume breweries, and to concerts and sporting events where there are public safety issues. The barrier PET beer bottles are not as successful as anticipated, but blow molders remain confident that the PET beer bottle will re-emerge in the future. Blow molders are shifting their efforts toward designing barrier PET bottles for less demanding applications in juices, carbonated soft drinks, and hot-filled products.

VII. MULTI-LAYER STRUCTURES

Multi-layer food packaging structures have been used for many decades. Ethylene vinyl alcohol (EVOH) co-extruded with polypropylene (PP) was commonly used in the 1970s. As demand for bottle transparency increased, co-injection blow molding of polyester (PET) with EVOH was developed during the 1990s. However, clarity improvement of multi-layer PP containers is still emerging. Pechiney Plastic Packaging, Inc. (PPPI) has developed a family of PP barrier containers that are claimed to be as clear as multi-layer PET bottles, and to be a cost-effective alternative to multi-layer PET bottles. The three-layer (PP/EVOH/PP) barrier containers are made using modified reheat stretch-blow mold machines (*23*). Today, PET and PP are competing to determine which of these two base polymers, with other barriers, will dominate the barrier plastic bottle market (*24*).

Multi-layer structures are far more prevalent than coatings and account for about 70% of barrier PET bottles (*25*). The technology has succeeded for a decade in the PET ketchup bottle. It is claimed that the multi-layer PET food bottle is the optimal solution in barrier performance, functionality, and cost. Higher productivity currently favors multi-layer preform co-injection systems over coatings. Multi-layer containers can be engineered to survive pasteurization successfully by new bottle design features, processing techniques, and materials modifications.

In a multi-layer structure, a core layer or layers containing higher priced barrier materials are sandwiched by PET structural layers. There are several five-layer PET bottles designed for beverages. A five-layer PET bottle is used for Reallife® line of new-age beverages, non-carbonated flavors. The bottle incorporates virgin PET, EVOH for barrier properties, and post-consumer recycled (PCR) resin within the five-layer structure (virgin PET/EVOH/PCR-PET/EVOH/virgin PET); no adhesives or tie layers are used. Two thin layers of EVOH provide the necessary barrier (*26*). Instead of EVOH, MXD6 is used in a five-layer structure (virgin PET/MXD6/virgin or PCR-PET/MXD6/virgin PET) for Coca-Cola bottles (*27*). Continental PET Technologies supplies a five-layer structure (PET/O_2 scanvenger/PET/O_2 scavenger/PET) single-serve PET bottle for Miller beer (*28*). Krones has developed a five-layer structure (PET/nylon 6/PET/nylon 6/PET) PET beer bottle for a Swiss brewery, while Bass developed a multi-layer (PET/EVOH/PET) PET bottle for Carlsberg Black Label beer.

A. Sealica

Tetra Pak's patented two-stage process, Sealica, was developed for molding a multi-layer PET preform using a new thermoplastic epoxy barrier resin called Blox from Dow Plastics (*20*). Blox is a resin made by the reaction of resorcinol diglycidyl ether (RDGE), a resorcinol derivative used extensively in high performance composites, with monoethanolamine to yield extreme barrier performance (*29*). The preforms are injection molded using less PET, only 60–70% as thick as normal, and subsequently they are overmolded using a thermoplastic barrier material that fills the remaining 30–40% of the preform cavity. The other half of the mold and its cavities are then injected with PET to complete the molding process. The thickness of the barrier can be adjusted to suit the application.

The multi-layer approach is using promising new barrier materials such as nylon-based nanocomposites and passive-active barrier systems. The latter are dual use of a passive barrier material and an active oxygen scavenger that blocks oxygen entry and absorbs this gas from headspace and content.

B. Nylon-Based Nanocomposites

Nanocor in alliance with Mitsubishi Gas Chemical has melt-compounded its own nanoclay additives with MXD6 nylon for making nanocomposite (M9) for use in barrier PET bottles and films. M9 nanocomposite is claimed to improve the CO_2 and O_2 barrier of standard MXD6 by 50% and 75%, respectively, while retaining high clarity and delamination resistance equal to standard MXD6 (*25*). A three-layer (PET/M9/PET) structure extends the shelf life of beer to 110 days in the US and 180 days in Europe. The structure uses a thinner M9 layer than if plain MDX6 were used; this provides a cost saving for a processor even though M9 costs more than MXD6.

Honeywell developed several nylon 6-based nanocomposites, Aegis products, to cover the full spectrum of high barrier food bottles and film applications. Aegis products are reactor-made or melt-compounded blends of nylon 6 with low levels (2%) of nanoclay platelets (*25*). The platelets act as a tortuous path barrier to CO_2 and O_2 gases. To improve the O_2 barrier to the level of glass, Honeywell uses a proprietary oxygen scavenger that involves a polydiene entity dispersed in the nylon 6 without impairing the matrix properties. Aegis OX is a nanocomposite grade that

contains the O_2 scavenger, offering sufficient passive and active CO_2 and O_2 barriers to protect beer. Aegis CSD and HFX are grades optimized for carbonated soft drinks and hot-filled foods, respectively. Aegis CSD extends the shelf life of carbonated soft drinks in 0.5 L containers from 9 to 14–16 weeks. Aegis HFX is a passive-active system that provides a greater O_2 resistance for foods. Both grades also have improved delamination resistance.

C. Passive-Active Barrier Systems

Use of an oxygen scavenger in multilayer packaging is emerging.

1. SurShield Barrier

Owen Illinois has developed a patented process, SurShot, for co-injection molding a patented five-layer plastic bottle for beer (*30*). The outer, middle, and inner layers of the bottles are made of virgin PET. Sandwiched between them are two layers of proprietary passive-active barrier material, SurShield. This barrier system includes MXD6 nylon and an oxygen scavenger in two super-thin layers sandwiched between outer PET layers and a core layer incorporating up to 35% recyclate (*25*). This structure is claimed to improve the CO_2 barrier up by 40%. The bottles are designed for food applications from ketchup to beer.

2. Oxbar Barrier

Developed by Constar International, this uses a three-layer structure with a barrier layer of MDX6 nylon and O_2 scavenger (*25*). This passive-active barrier system is aimed at juices, flavored alcoholic beverages, hot-filled foods, and beer. The Constar bottles are claimed to survive tunnel pasteurization by using extended necks that expand to relieve pressure and base designs that retain shape and strength.

VIII. SURFACE COATING TECHNOLOGIES

Coating basic plastic structures is not new, but the materials and application methods used to achieve desired properties are. Plastic bottle coating technology is evolving. PVDC coating of polyester bottles exists commercially but its use has been slow because there are too many economic issues and environmental concerns, especially in Europe.

A surface coating can be applied to a PET bottle to improve gas barriers. The coating technologies apply a super thin barrier to one surface of a monolayer PET bottle. Coating systems use monolayer PET bottles for their economies, but require costly equipment for integration into complex, high speed filling-capping-labeling lines. Coatings are less prevalent than multi-layer structures because they offer an intermediate barrier and cannot give the barrier of a multi-layer (*31*). However, coated bottles have advantages. They are less expensive on a per bottle basis than the multi-layer ones. Coatings can be applied by end users. Coated bottles that use only one resin are considered far easier to recycle than multi-layers ones. They are less likely to delaminate during handling, avoiding problems in material handling in retail venues like vending machines. Several barrier-coating technologies are available and differ by the type of coating materials, coating placement (interior or exterior), and application method.

Methods for increasing barrier properties in packaging while reducing material use by applying a microscopic layer of silicon on film emerged in the 1990s (*32*). These methods were achieved by coating or by vacuum or plasma deposition on a substrate, and then sandwiching the silicon for protection in a laminated structure. The silicon layer acts almost as a glass-like barrier. Lawson Mardon Packaging Inc. claimed that silicon monoxide (SiO_x)-coated ceramic films yield substantial reductions in oxygen and water vapor permeation. Because the silicon coating is so thin (400–1000 angstroms), recyclability of the films is not an issue. When used in appropriate laminates, the SiO_x barrier maintains a high degree of flex-crack resistance. Silicon coatings can be applied on PET films for retortable and nonretortable laminates, and on other plastics including PP, PS, and polyamide (PA). Applications for films include pouches for dry foods, and liquid and high-moisture content foods. Another process pioneered by AIRCO and PC Materials, Inc. uses a low temperature (40–50°C) plasma-deposition process that applies silica under a low or soft vacuum (750 milli-torr range). The system is a batch, air-vacuum-air process that produces a coating less than 40 nanometers thick. The low-vacuum nature of the process puts less thermal stress on substrates and reduces wear on the coating chamber. Another silicon-oxide QLF (quartz-like-film) barrier coating for films of PET, oriented PP, LDPE, or biaxially oriented nylon was developed by BOC Coating Technology (*33*). QLF coatings are applied in a low temperature, plasma-enhanced chemical vapor deposition (PECVD) process. The clear, colorless silica coatings are only 20–40 nanometers; it is claimed that they improve the O_2 barrier by 120-fold and the moisture barrier by 45 fold of 12.5 micron PET films. These developments have led to the current technology called plasma-enhanced chemical vapor deposition (PECVD). PECVD is most commonly employed to apply silicon oxide barrier coatings on films, sheets, and bottles. The process applies a microscopically thin (40–60 nanometers thick) layer of silicon oxide on plastic surfaces. Under a low vacuum, a silicon containing chemical such as silane, i.e., hexamethyl disiloxane (HMDSO), is exposed to microwave or radiofrequency energy to convert it to a plasma (*34*). The silicon oxide bonds to the plastic and creates a coating that blocks the permeation of gases, water vapor and flavor. Plasma coating may be applied using carbon or silicon oxide on interior or exterior surfaces of packaging.

A. Interior Coating of PET Bottles

1. Actis (Amorphous Carbon Treatment on Internal Surface)

Sidel developed the Actis cold plasma technology that was originally aimed for beer containers. The process uses acetylene gas as a source of carbon coating. The gas is excited by a microwave-assisted process into plasma, which deposits a layer of hydrogenated amorphous carbon about 100 nanometers thick on the bottle's interior. Actis is claimed to improve the CO_2 barrier seven-fold and the O_2 barrier thirty-fold. It reduces acetaldehyde migration to one-sixth of normal (*19).* The Actis system is a stand-alone rotary-style coating machine and offers an advantage over multi-layer structure machine. Actis Lite is a new Actis system designed for less demanding juice, CSD, and hot-filled applications. Actis-treated bottles have a light amber color, providing some UV protection for beer packaging (*35*). Actis coated bottles cost ca. 20% less than multi-layer PET bottles with comparable barriers. Actis received a letter of non-objection from the FDA for beverage contact and commercial application for Mountain Dew's Code Red product to prolong CO_2 retention.

2. Plasma Nano Shield (PNS)

PNS was formally called Diamond-Like-Coating (DLC), was developed by Kirin/Mitsubishi of Japan. The process uses a radiofrequency source plus internal and external electrodes to ionize the gas to produce a coating 20–40 nanometers thick on the internal surface of PET bottles (*34)*. The coating offers an excellent gas and water vapor barrier. Coated bottles outperform PEN bottles in reduced color and flavor sorption and may be refillable (*15*).

3. Glaskin

Tetra Pak developed Glaskin, a silicon-dioxide plasma-coating system for the interior of PET bottles. The coating is created by reacting hexamethyl disiloxane with oxygen (*34*). Microwave energy is used to excite a gas, depositing a thin (10–20 nanometers), clear layer of silicon oxide, essentially glass, on the bottle's interior wall. It is claimed to deliver CO_2 and O_2 barriers equal to glass. The coating is elastic and ensures barrier integrity with crack resistance despite expansion and contraction during the bottle filling operation. It is designed for beer, juice, carbonated soft drink, and hot-fill applications. It delivers 4–12 months of shelf life for beer and juice (*29*) with excellent flavor retention *(28)*.

4. Plasmax

SIG Corpoplast and Schott HiCotec developed Plasmax, which is another silicone oxide (SiO_x) coating system for the interior of PET bottles. The process is based on the plasma impulse chemical vapor deposition (PICVD) technology of Scott HiCotec (*36*). The technology uses a pulsed, cold plasma process that deposits a thin-layer (0.01–1.0 microns thick) of silicon oxide on the interior of PET bottles. The process occurs in a vacuum chamber, where the bottles are held neck down. The bottles are filled with gaseous hexamethyl disiloxane, and are microwaved to decompose this gaseous precursor to form a deposit of SiO_x on the bottle. Byproducts, CO_2 and water, are removed by the vacuum system. The application process uses a rotary coater that enables the system to be integrated into end users' filling line. A transparent adhesive layer is applied before the coating cycle, allowing for good bonding of the barrier layer to the interior wall of the bottle even in case of asymmetrical or other complex bottles shapes. The process creates a barrier to O_2, CO_2, moisture, and chemicals. The SiO_x layer is claimed to improve the oxygen barrier more than ten-fold, and the CO_2 barrier more than seven-fold.

B. External Coating of PET Bottles

Instead of internal coating, an exterior coating can be applied to the PET bottles.

1. Bairocade

The Bairocade system was developed and is supplied by PPG Industries. It is an epoxy-amine coating applied by electrostatic spray, and cured in an infrared oven to thermoset the material on the exterior of the PET bottles. The cross-linked, 1–6 micron thick coating is glossy, offers excellent O_2 and CO_2 barriers, and survives pasteurization (*25*). It is claimed that the shelf life of certain products can be tripled. The coating's lubricity facilitates the treatment of tens of thousands of bottles per hour in a continuous operation. The coating can be removed by aqueous cleaners commonly used in washing reclaimed PET bottle flake. The system can color the clear coating such as amber for beer. Bairocade bottles are currently used most often in hot-filled applications. The technology allows end users to create the barrier for desired shelf life via coating thickness in a range of 6–8 microns, which will increase by 3–5 times the barrier compared to the untreated PET bottles (*37*). It comes in three formulations: 1) one for carbonated soft drinks that keeps CO_2 inside the bottle, 2) one for juice that keeps O_2 outside the bottle, and 3) one for beer that prevents egress of CO_2 and ingress of O_2. Graham Packaging uses the coating on 12- to 20-oz juice bottles. Pepsi uses the coating on single serve CSD bottles sold in Saudi Arabia.

2. BestPET

The process was co-developed by Krones in cooperation with user Coca-Cola. The system uses an energy-intensive

evaporative process to generate ions of silicone-oxide (glass) that coat the exterior of PET bottles for CSD (*34*). This glass coating is claimed to retain good clarity while improving CO_2 and O_2 barriers. A new BestPET Plus version has a topcoat to protect the outside glass coating, and is used for single serve CSD, juice, beer and hot-fill containers.

3. Combustion Chemical Vapor Deposition (CCVD)

The process was developed by Micro-Coating Technologies. CCVD is a new coating system. It is an open-atmosphere, flame-based system that deposits a thin coating made of several organic or inorganic materials onto the exterior of cans, plastic bottles, or films (*34*). Plastic surfaces do not require pre-treatment to achieve an adhesion that is better than conventional plasma deposition. The process atomizes a low vapor pressure coating solution that contains the precursors into a mist in a flame, where they are combusted to generate the coating material. Heat from the flame provides the energy to evaporate the mists and for the precursors to react and to deposit the vapor on the package material substrate, to dry and simultaneously to cure. Exposure time to the flame is so short that no significant increase of substrate temperature is observed. It is claimed to be simpler to operate, have lower cost per bottle, and be more environmentally friendly than other coating systems. CCVD coatings can be thinner to achieve the same barrier as alternative technologies, and improve CO_2 and O_2 barrier properties without impairing clarity. The company's organic coating is adequately flexible to resist much more physical abuse in packaging and distribution than the conventional silica coating.

Markets for barrier coatings include various types of beverages. Carbonated soft drinks currently using monolayer PET bottles represents one of biggest potential markets for coated PET. Soft drink bottlers have two potential advantages when using coated bottles: increased shelf life and decreased material cost. With coatings, the potential exists for downgauging of monolayer PET. If the total saving from downgauging are more than the cost of coatings, the bottlers will use coatings. Another major market for coated PET bottles is beer. Unlike multi-layer structures, interior coatings provide the protective barrier next to the product. Several European brewers have used barrier coating systems including Actis and Glaskin. The coated PET bottles can also be used with other demanding beverages such as nutraceutical or healthy drinks, which are emerging.

IX. OUTLOOK FOR FOOD PACKAGING

Food packaging technology and food package design have rapidly evolved since the 1950s and 1960s when new container alternatives, including flexible packages and new decorating methods, became part of the packaging choices (*38*). Food package design is becoming a focus for branding, the consumer, and public health. Use of multiple-color printing on all package surfaces has increased to improve a marketer's shelf visibility. The development of healthier foods and nutraceuticals is emerging in response to the consumer demand.

There are several reasons for designing a new package, but the main one is for a new food product. A company uses packaging to introduce new products, as well as to give a new life to the company's existing brand products. Packages are redesigned to meet the changing needs of the target consumer group. Today the number one need driving new designs for food products is convenience, for preparation and consumption either at home and/or at work. Consumers want products that are easy to handle and quick to prepare. Many food packages designed for convenience are using barrier packaging systems (39). Technical aspects of barrier packaging systems are related to the type of processing, including hot filling, retorting, aseptic, and controlled atmosphere package and modified atmosphere package (CAP-MAP). A hot-fillable, heat set PET container is an innovative wide-mouth jar. The jar incorporates an easy-grip pinched-in middle to aid pouring, thus allows the container to withstand the vacuum generated as the hot product cools after filling (*40*). MAP is used with ready-to-eat salad mixtures for the consumer who has neither time nor patience to wash and chop salad ingredients. Borden Foods has launched the first shelf-stable cooked pasta that eliminates the need for refrigeration or freezer storage, and requires minimal preparation and cooking time. The pasta is contained in a rotary thermoformed, polypropylene tray sealed with clear, peelable lidstock that holds pouches of cooked pasta and tomato sauce (*39*). Both pouches use the same clear barrier material. This barrier structure and Borden's proprietary processing techniques yield a nine-month shelf life. For the vending channel, Welch Foods has launched 16-ounce bottles for white grape, grape, and 100% apple juices as the first commercial applications of an epoxy/amide barrier coating (Bairocade), which doubles product shelf life compared to monolayer PET. Del Monte Foods developed shelf-stable, ready-to-eat fruits called Fruit To-Go. The fruit is packaged in a clear, 4-oz. plastic cup that is flushed with a mixture of gases and provides up to an 18-month shelf life when stored at room temperature. The fruit is cold-filled and then retorted in a package made of polypropylene to provide heat resistance during retorting, while ethylene vinyl alcohol in the package acts as an oxygen-barrier layer to help maintain product quality and safety.

Innovations in all food package designs must fulfill the basic requirements: to hold the food being contained and provide suitable protection to the food, to offer suitable functional barriers to the permeation of gases and vapors

that cause deterioration of the food, and to be user friendly (ease in opening the package while being tampering-proof). Companies have a significant interest in improving their food products. Like food products themselves, packaging materials are constantly evolving to meet the latest demands of the marketplace. New packaging materials as well as new packaging techniques that offer optimal barrier properties will definitely help the companies meet the challenge of keeping products fresh and extending their shelf life. Trends predict expansion and a promising market for evolving technologies including new barrier resins and oxygen scavengers, low-cost surface coatings, and high-output multilayer PET preforms molding systems (*25*).

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131 Food Packaging: Plastics

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CONTENTS

I. INTRODUCTION

Use of plastics as packaging materials has grown rapidly during the last several decades (Figure 131.1) (1). The development of new plastic resins and the combination of resins in multilayer structures has allowed plastics to substitute for glass and metal, in particular, in a variety of applications. Such changes generally result in smaller and lighter packages that take less space and consume less energy in manufacture, storage, and distribution. Plastics have also substituted for paper in a significant number of applications. In other cases, a combination of paper and plastics, sometimes with aluminum foil as well, has replaced glass or metal. The area of flexible packaging has been a major source of growth for the use of plastics. However, plastics are certainly not confined to such uses. Plastic is also used in crates, boxes, and trays where it usually substitutes for corrugated board, and in pallets where it substitutes for wood. Plastic bottles, drums, and other containers are also widely used.

The focus of this chapter is on the use of plastics for food packaging, and our concentration therefore will be on the primary package — the package that directly contacts the food. First, we will discuss the properties and food-related uses of some of the major packaging plastics. Common plastics additives will be covered briefly. The major processing methods for forming plastic resins into food packages will also be described. For many food products, the barrier ability of the package, especially to water and oxygen, is critical, so we will also discuss package permeability and its relationship to product shelf life.

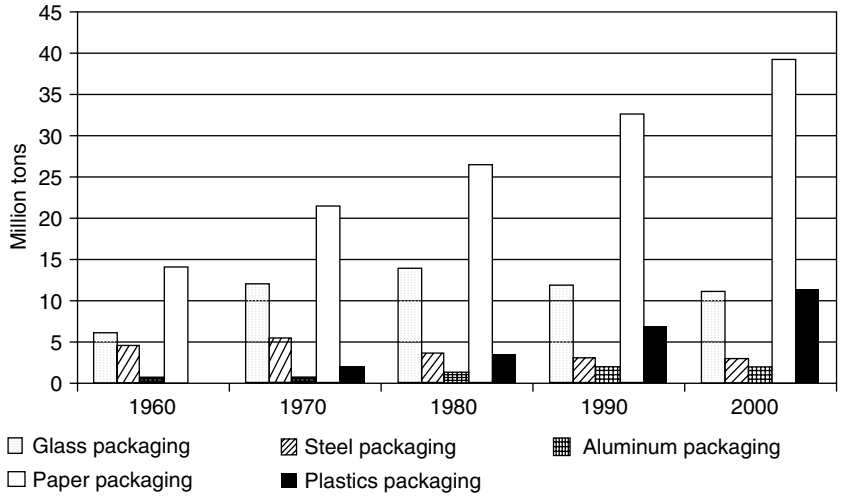

FIGURE 131.1 Packaging material use in the United States (1).

II. BASIC PLASTIC STRUCTURE AND PROPERTIES

Plastics are characterized by being formed by the joining together of small building-block molecules (monomers) into a large chain-like or network structure, in the process known as polymerization. At some point in their manufacture, plastics are capable of being deformed by a combination of heat and pressure. Most of the plastics we use in packaging are thermoplastics, which can be repeatedly softened by the application of heat, and hardened by cooling (think of melting and cooling butter). Most thermoplastics used in packaging are linear, although some branched polymers (low density polyethylene, for example) are very important packaging materials.

A few packaging plastics are thermosets. These plastics undergo a chemical reaction when they are heated, in a process known as curing, which forms them into a network structure, usually three-dimensional, resulting in extremely large molecules, which are no longer capable of flow. These materials cannot be softened and melted once cured (think of cooking an egg). Thermosets are found primarily in can coatings (although some coatings are thermoplastics), and occasionally in rigid closures (although the vast majority of closures are thermoplastics). Because of the preponderant position of thermoplastics in food packaging, we will concentrate on this category of plastics.

Plastics that are formed from only one type of monomer, and hence have a regular repeating unit in their structure, are called homopolymers. Polymers that are formed from more than one type of monomer, so that the units that make up the molecule differ from place to place in the structure, are called copolymers. Copolymerization is used fairly widely as a way to alter the properties of the basic polymer, to fit its performance to application requirements.

Another categorization of plastics is as addition or condensation polymers. In addition (or chain-growth) polymers, generally the whole monomer is added into the polymer structure. The monomers usually have double bonds, and the "opening" of the double bonds allows the monomers to join together in a chain. Condensation (or stepwise-growth) polymers are formed by more "ordinary" chemical reactions such as those between acids and amines, or acids and alcohols, in which a small by-product molecule is eliminated in each step. Despite the definitions of homopolymers and copolymers above, the majority of condensation polymers are made from two different monomers, each containing two identical functional (reactive) groups. Because this results in a polymer with a single repeating unit (formed by the reaction of one type of group with the other and the elimination of the byproduct molecule), these are generally classified as homopolymers. A condensation polymer that is considered a copolymer will be formed from three or more different monomers, so that there are differences between the repeating units.

The processes we use for forming plastics depend, for the most part, on our ability to melt the materials and shape them as desired. An important characteristic of plastics in this regard is their viscoelasticity. Viscoelasticity can be thought of as a plastic's tendency to exhibit viscous flow behavior, characteristic of a liquid, and elasticity, characteristic of a solid, at the same time. In many plastics forming processes, we depend on the ability of a plastic to "hold together" when it flows (melt strength), so that we can shape it in the desired manner while it is in or near a liquid state. At the same time, this flowing plastic has "elastic memory" so that when we remove the deforming force, there is often a response by the plastic to return partially to the shape and dimensions it had before we exerted that force. In the solid state, viscoelasticity is responsible

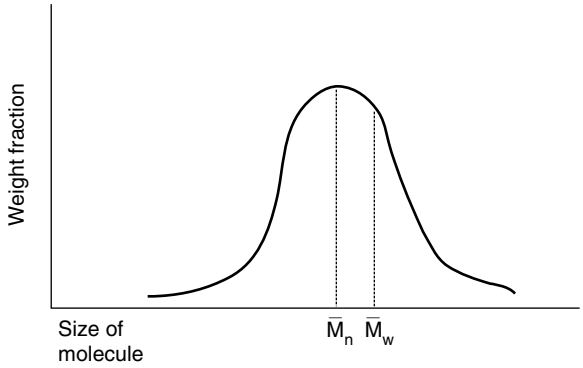

FIGURE 131.2 Example of a molecular weight distribution. $\overline{M}_n$ is the number average molecular weight; $\overline{M}_w$ is the weight average molecular weight.

for our ability to deform the plastic to such an extent that we get limited "flow" of the molecules, resulting in permanent deformation of the plastic, allowing us to modify its shape. Here, too, when the force producing the deformation is removed, we will get an elastic response from the plastic that removes some of the deformation we have imposed. Plastics generally are also characterized by having a viscosity that depends on flow rate, not just on temperature. In other words, they are non-Newtonian fluids. The viscosity of most plastics decreases as the rate of flow (or more accurately of shear) increases.

Another important point about plastics is that many of their properties are influenced by their molecular weight. Unlike materials made up of ordinary small molecules, such as sugar, a plastic resin will contain molecules that differ in size. Whenever we talk about the molecular weight of a polymer, we really mean the average molecular weight. To further complicate matters, these averages can be calculated in different ways, with some being more closely related to certain performance variables than others. Often, we use a viscosity-average molecular weight, $\overline{M}_v$ or $\overline{M}_\eta$, because it is the easiest to determine, and is reasonably closely related to the performance properties of interest for packaging applications. This average is closely related to the weight average molecular weight, $\overline{M}_w$, and is often used to approximate it. In general, when the average molecular weight of a plastic increases, its strength, stiffness, and other mechanical properties improve, while its resistance to flow (its viscosity) increases, as does its cost. Processing is easier for materials with lower viscosity (as long as viscosity is not too low). Therefore, users often must compromise between performance of the finished material and cost and ease of processing.

The behavior of a plastic resin is influenced by its molecular weight distribution, as well as its molecular weight average. Molecular weight distribution (Figure 131.2) refers to the range of sizes of molecules found in the plastic resin. Small molecules contribute to decreased viscosity by, in essence, "lubricating" flow. Large molecules contribute to strength. The proportion of smaller and larger molecules and how much they differ from the average affects performance in much the same ways as does the molecular weight average. Distributions can be characterized as narrow or wide. Polymers with narrow molecular weight distributions tend to have higher strength and other mechanical properties, along with higher viscosity (and cost) compared to polymers with wide distributions. While normally molecular weight distributions approximate the classical normal distribution bell-shaped curve, some polymer resins, typically formed by combining two or more batches of the basic polymer, have bimodal distributions. This is one way to better optimize the mix of mechanical and flow properties of the resin for a particular application.

Plastics can be characterized as crystalline or amorphous. Crystallinity implies the arrangement of molecules (or parts of molecules) in a regular repeating pattern. While materials composed of small molecules such as salt and sugar can be totally crystalline, because of the large size of the molecules in plastics, it is not possible to totally crystallize the material. Thus a crystalline plastic is characterized by crystalline regions linked together by non-crystalline (amorphous) regions. When we refer to a plastic as crystalline, therefore, we mean that it has some significant degree of crystallinity. We classify as amorphous plastics that have no significant crystallinity.

Another difference between plastics and materials composed of small molecules is their melting behavior. While materials such as water melt at a precise temperature, polymers do not. In fact, if a polymer is amorphous, the softening as temperature increases is so gradual that we are unable to clearly distinguish between solid and liquid. Therefore, for amorphous plastics, the melting temperature is not defined. For crystalline plastics, we define the melting temperature as the temperature at which the crystallites (small crystalline regions) break up. Since the size of the crystallites influences how much energy (and therefore what temperature) is required to disrupt the structure, and since the size of the crystallites inside a given plastic resin varies, crystalline plastics melt over a narrow range of temperature, rather than at a precise point. The ability of a polymer to crystallize is determined by its chemical structure. To arrange in a regular repeating order, there has to be a degree of orderliness of the underlying structure. Therefore, some polymers cannot crystallize. If the structure permits crystallization, the way a plastic is processed can have a profound impact on the amount of crystallinity that actually develops. Plastics can crystallize only over a certain temperature range, and the rearrangement of molecules into a crystalline array takes time. If a polymer is cooled rapidly to a temperature below the lower limit for crystallization, it may not develop any significant crystallinity, while the same plastic resin, cooled more slowly, may be highly crystalline. Another factor is that growth of crystals

depends on initiation (nucleation). Think of a supersaturated salt solution that has no crystals. If a seed crystal is dropped into the solution, all at once there can be massive crystallization. Similarly, providing sites that facilitate crystal formation (adding nucleating agents) results in more rapid crystallization.

Another important temperature for understanding polymer behavior is the glass transition temperature, T_g. If a plastic is below its glass transition temperature, it tends to behave like a stiff, brittle material, while above its T_g, it tends to behave like a soft, flexible material. The T_g of a plastic, therefore, serves as a guide to its behavior at any given temperature. All thermoplastics are stiff and brittle if they are cold enough, and soft and flexible if they are warm enough — but the temperatures at which this behavior occurs vary widely.

One important consequence of a change in molecular weight or molecular weight distribution is its effect on melting temperature, and in particular on sealing temperature and sealing temperature range. Polymers with a narrower molecular weight distribution tend to soften and flow (melt) over a narrower range of temperatures. Polymers with a higher average molecular weight tend to soften and flow (melt) at a higher temperature. Since heat sealing fundamentally depends on the flow of molecules, or parts of molecules, from one layer into the adjoining layer, the differences in melting temperature influence the temperature required to produce a seal. At the same time, if the plastic gets too hot, there will be too much flow, resulting in weakening of the material, or even the development of holes. Therefore, if the heat seal range is narrow, greater control over the sealing temperature is required to ensure that it does not deviate too far from the optimum value.

Rather than specifying molecular weight average, molecular weight distribution, or even viscosity, plastic resins are often characterized by their melt index (or melt flow index). The melt index refers to the amount of plastic that will flow through a small orifice of a prescribed size under specified temperature and pressure conditions. Therefore, it is an indirect measure of viscosity; a high viscosity (resistance to flow) means a low melt index, and vice versa. The units normally used for melt index are g/10 min. Plastic resins are often available with a wide variety of melt index. For example, high density polyethylene (HDPE) resins used for milk bottles typically have a melt index of less than one, often in the 0.5 to 0.7 range. HDPE resins are even available that have a melt index of 0 at the usual conditions, so must be subjected to higher pressure to get a measurable value (the result is referred to as a high load melt index). An HDPE resin used for injection-molded margarine tubs, on the other hand, would likely have a melt index of at least 4, and maybe even 90 or so. As would be expected, a high melt index is associated with a wide molecular weight distribution and with a low average molecular weight (low viscosity), and vice versa.

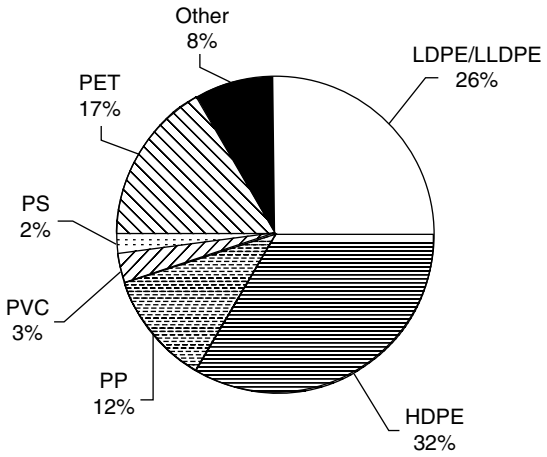

FIGURE 131.3 Plastic packaging used in the United States, 2000 (1).

TABLE 131.1
The SPI Coding System for Plastic Containers

Resin Type	Number	Symbol
Polyethylene terephthalate (PET)	1	PETE
High density polyethylene (HDPE)	2	HDPE
Polyvinyl chloride (PVC)	3	V
Low density polyethylene (LDPE and LLDPE)	4	LDPE
Polypropylene (PP)	5	PP
Polystyrene	6	PS
Other plastics, including multilayer	7	OTHER

III. PLASTICS COMMONLY USED IN FOOD PACKAGING

The most-used packaging plastics, by far, are high density polyethylene (HDPE) and low density polyethylene (LDPE) (Figure 131.3). Polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) are considered the other major packaging plastics. A number of other plastics are used in smaller quantities, for specialty applications or as one component in a multi-resin structure. Important examples include ethylene vinyl alcohol (EVOH), polyvinylidene chloride copolymers (PVDC), nylons, polycarbonate, ionomers, and ethylene vinyl acetate (EVA).

In most states, plastic bottles 16 oz. and larger, and other plastic containers 8 oz. and larger, are required to be marked with their resin type, using the Society of the Plastics Industry (SPI) coding symbol. This symbol consists of a triangle formed by three chasing arrows, with a number inside and a letter code below the triangle. Table 131.1 shows the letters and codes used for the various types of resins used for plastic containers.

A. High Density Polyethylene

High density polyethylene (HDPE) is an addition polymer of ethylene, with a predominantly linear structure that can be represented as $-(CH_2-CH_2)_n-$. The few branches that it contains are short, and do not much influence its properties. Polyethylene has a T_g of about $-120°C$ (estimates vary, for reasons too complex to go into here). Therefore, at the vast majority of use temperatures, HDPE is in the soft, flexible range of behavior. The density of HDPE is actually quite low, about 0.94–0.97 g/cm^3, less than water. It is characterized as high density polyethylene only because its density is higher than that of low density polyethylene, which will be discussed next.

The melting temperature, T_m, of HDPE is also relatively low, at about 128–138°C. Therefore, a characteristic of HDPE is that it maintains its flexibility well at cold temperatures, such as those used for frozen food, but it is too soft to be used for hot-filled products. As the existence of a T_m implies, HDPE is a crystalline plastic. It is able to crystallize over a wide range of temperatures, and generally is 65–90% crystalline. The crystallinity and density of polymers are correlated, as crystalline regions pack a larger number of atoms (and therefore higher mass) into a unit volume of space than do amorphous regions, with their greater degree of disorder.

HDPE has excellent chemical and oil resistance. It is a good water vapor barrier, but a poor barrier to gases such as oxygen and carbon dioxide. Its transparency is poor. For the most part, crystalline polymers tend to have inferior transparency to amorphous polymers, as the crystallites tend to scatter light, interfering with its transmission.

The largest use of HDPE is in containers, especially bottles, although it is also used in film. Its single most common use is for plastic milk bottles. The hazy appearance of these bottles is an example of HDPE's natural appearance. For many applications, HDPE is pigmented, making the containers opaque. This can be done to provide protection for the product against light-induced degradation, or for marketing reasons, to make the container, and hence the product, more attractive.

HDPE is produced by polymerization at moderate temperatures and pressures, using a catalyst to facilitate the reaction. The traditional catalyst systems are in the Ziegler-Natta family. In recent years, there has been increasing use of a new catalyst family, metallocenes. These are capable of providing plastic resins with a narrower and more controllable molecular weight distribution, as well as having other desirable attributes.

B. Low Density Polyethylene

Low density polyethylene (LDPE) is also an addition polymer of ethylene (C_2H_4), but it is polymerized at high temperature and pressure, resulting in a polymer with a highly branched structure, containing both long and short branches (Figure 131.4). While its T_g is the same as HDPE, it is softer and more flexible because of its lower crystallinity, 40–60%. This also gives it better transparency, although it still has a significant degree of haze. Like HPDE, it has excellent oil and chemical resistance, and it is a reasonably good water vapor barrier. Its barrier properties are inferior to HDPE, however. Permeation occurs almost exclusively through the amorphous areas in a polymer, as the crystallites do not have wide enough spacing between polymer molecules to allow the passage of the permeant molecules. Therefore, if other factors are the same, plastics with higher crystallinity will be better barriers.

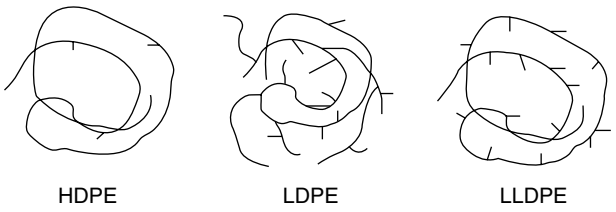

FIGURE 131.4 Illustrations of the structure of HDPE, LDPE, and LLDPE.

LDPE tends to have lower tensile strength than HDPE, but higher impact strength. Tensile strength is increased by HDPE's increased crystallinity, since the crystallites resist both deformation and fracture. On the other hand, impact strength is strongly affected by the ability of the polymer molecules to rearrange without fracture, and in doing so absorb the impact energy. This is facilitated by a greater preponderance of amorphous regions, since the crystallites have very little ability to rearrange without producing fracture.

Applications for LDPE range from stretch wrap for pallet loads of products to bread bags to squeezable drink bottles, but are predominantly in the area of flexible packaging. A common household food bag illustrates the somewhat hazy appearance of LDPE. LDPE is also found in many multimaterial food packages, where it often serves as a heat seal layer. It may also serve as a moisture barrier, or as protection against interaction or chemical attack. For example, in drink box structures, a layer of LDPE on the outside protects the printed paper from exposure to moisture and abrasion, and a layer of LDPE on the inside prevents direct contact of the aluminum foil and the product, as well as providing the ability to form the package by heat sealing. An additional layer of LDPE within the structure serves as an adhesive to bond the foil and paper into a single structure (Figure 131.5).

Discussion of LDPE is complicated somewhat by frequent grouping of the next polymer in our list, linear low density polyethylene (LLDPE), with the type of LDPE we have been discussing, using LDPE to refer to both polymers. The highly branched LDPE described here is produced by

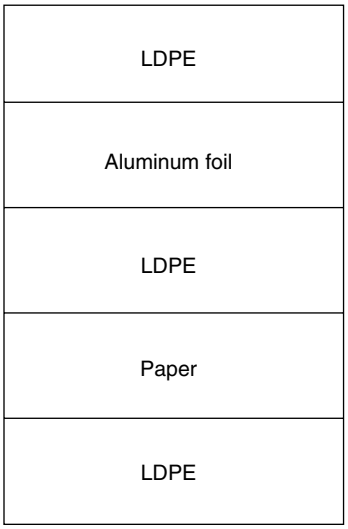

FIGURE 131.5 Structure of a package for aseptic packaging of juice drinks (juice box) (not to scale).

polymerization at high temperature and pressure, and results in the highly branched structure described, having both short and long chain branches.

C. Linear Low Density Polyethylene

Linear low density polyethylene (LLDPE) is a polymer that is formed by a process similar to that used for HDPE, polymerization at moderate temperatures and pressures, with a catalyst, resulting in an essentially linear structure. However, LLDPE has levels of crystallinity, and consequently density, in the same range as LDPE. This is possible because LLDPE is a copolymer. When LLDPE is polymerized, in addition to ethylene, either butene, hexene, or octene are introduced. Where these comonomers are present in the structure, they leave a "tail" hanging off the chain, that looks (and acts) like a short chain branch (see Figure 131.4).

The branches in LDPE cause it to have lower crystallinity than HDPE, because they interfere with the orderly arrangement of the chains — the branches don't fit into the crystal lattice. In just the same way, these side groups in LLDPE interfere with crystallization because they do not fit into the crystal lattice. Therefore, LLDPE is produced with the same range of densities, and the same proportions of crystallinity, as LDPE. The reduction in density depends on both the size and the number of the incorporated comonomer groups. However, because only one type of comonomer is generally used, all the "branches" are identical in size, and their average proportion is readily controlled by varying the amount of comonomer introduced.

The use of moderate temperatures and pressures with a catalyst also permits a greater degree of control over the breadth of the molecular weight distribution. HDPE and LLDPE, in general, have narrower molecular weight distributions than LDPE. This greater degree of uniformity, along with the lack of long chain branches, imparts some significantly different properties to LLDPE than are found in LDPE. Melting temperatures are higher by about 10–15°C, compared to LDPE of the same density. Both tensile strength and impact strength are higher, as well.

Because the comonomers used to produce LLDPE are higher priced than ethylene, LLDPE costs more per pound than LDPE. However, the improvement in performance often means that thinner LLDPE films can be used than would be required to get acceptable performance with LDPE. This downgauging means that fewer pounds of LLDPE are required to package the same amount of goods. The price per unit, as a result, is often less using LLDPE, even though the price per pound is greater. The cost savings that can be gained by using LLDPE have led to it replacing LDPE in a variety of applications.

The primary area where LLDPE is inferior to LDPE is in heat-sealing. The lower melting temperatures of LDPE and broader molecular weight distribution mean heat-sealing temperatures can be lower, and the heat-seal range is wider. Further, entanglement of long-chain branches across the interface between materials occurs faster than entanglement of linear molecules, facilitating the rapid development of sufficient strength in the seal to permit release from the sealing mechanism, and without requiring cooling of the material (hot tack). This combination of improved mechanical properties from LLDPE and better heat seal performance from LDPE has led to these two materials being combined in many applications, either in discrete layers or as blends. Therefore, the term LDPE, as mentioned, sometimes means only the highly branched "true" LDPE, and sometimes means both LDPE and LLDPE, along with materials that are a combination of both resins.

The use of metallocene catalysts, along with other catalysts that are often characterized as "single-site" catalysts, has resulted in the production of a whole new generation of LLDPEs. When Ziegler-Natta catalysts are used, the active sites on the catalyst vary in their reactivity. Some sites produce "average" molecules. Others are not as fast at adding in new monomers to a growing chain, but tend to be relatively better at adding in the larger comonomers, so they produce, on average, smaller molecules with a higher proportion of comonomer groups. Other sites are more efficient at adding in ethylene, but less efficient at adding in comonomers, so they produce, on average, larger molecules with a lower than average proportion of comonomer groups. The "single site" catalysts have active sites that all have identical chemistry and geometry, so they are all equally reactive. Therefore, the polymers produced with these catalysts, although they still vary in molecular weight, etc., are more uniform, both in terms of size and composition, than those produced through typical Ziegler-Natta polymerization.

One other attribute of metallocene catalysts is that they are able to add in monomers that are bigger than octene, which Ziegler-Natta catalysts cannot do. By incorporating larger monomers with a double bond on one end (higher alpha-olefins), it is possible to produce linear polymers with the functional equivalent of long-chain branches. Therefore, better heat-sealing varieties of LLDPE can be produced. Of course, these higher alpha-olefins are also more costly than the smaller ones.

There are also polyethylenes, produced either with Ziegler-Natta or metallocene catalysts, that have a density lower than the range defined as low density polyethylene. These very low density polyethylenes (VLDPEs) incorporate a greater proportion of comonomer, resulting in a lower degree of crystallinity and consequently a lower density. They are used to produce very soft, flexible films, and have so far had only limited applications in food packaging.

D. Polypropylene

Polypropylene (PP) is a close relative of polyethylene, and is a linear addition polymer of propylene. Consequently it has a methyl group attached to every other carbon. While its physical structure is regular, when the molecules are looked at three-dimensionally, three different physical configurations are possible. When the carbons are stretched out in a linear fashion (the fully extended chain conformation), the methyl groups may all be on one side of the chain (isotactic), they may alternate from one side of the chain to the other (syndiotactic), or the side of the chain on which they appear may be random (atactic) (see Figure 131.6).

Although it is not obvious from the two-dimensional view, it is impossible to convert one of these configurations to another without breaking and reforming chemical bonds. If there is no pattern to the placement of the methyl groups, their presence interferes with crystallization in much the same way as does the branching in LDPE. Because the groups appear on every other carbon, this is sufficient to prevent crystallization all together. The result is that atactic PP has very poor performance properties, and is not a desirable packaging material. Fortunately, the Ziegler-Natta catalysts, as well as the metallocenes, tend to add the monomers in a way that results in a preponderantly isotactic configuration (the methyl groups all on the same side of the fully extended chain). Therefore, when we talk about PP for packaging applications (as well as for use in other products), we are almost always talking about isotactic PP, sometimes denoted iso-PP. It used to be very difficult to make syndiotactic PP, and there was little motivation to do so, since for most applications its performance is inferior to the more readily available, and less expensive, iso-PP. With the advent of metallocene catalysts, some interest has emerged in making syndiotactic PP for certain applications. Syndiotactic PP crystallizes less than iso-PP,

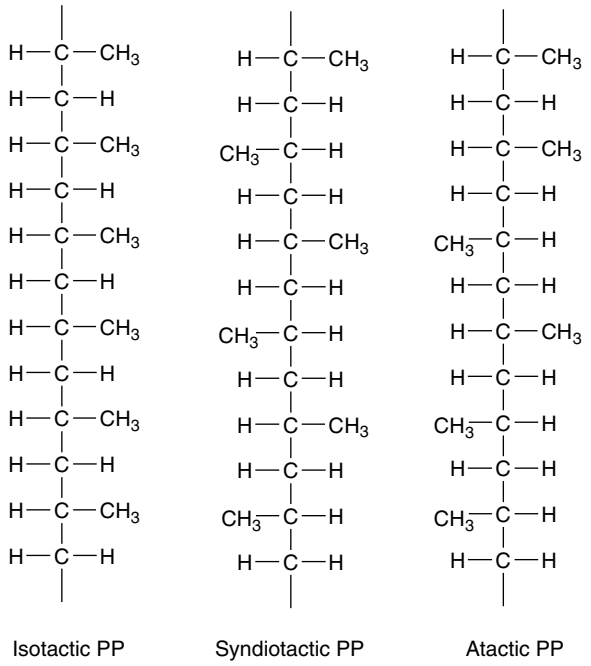

FIGURE 131.6 Illustrations of the structure of isotactic, syndiotactic, and atactic polypropylene.

thereby producing a polymer with increased flexibility and transparency.

Like its cousins in the PE family, PP tends to be chemically inert, have good grease resistance, be a good water vapor barrier, and a poor barrier to gases such as oxygen and carbon dioxide. Its melting point is significantly higher than that of HDPE, about 160–175°C. Its glass transition temperature is much higher than PE, about −10°C. Therefore, PP is significantly stiffer than HDPE. At typical frozen food temperatures, PP is very near its T_g and therefore subject to brittleness which can lead to package failure under impact, such as dropping a frozen microwaveable dinner on the floor. On the other hand, PP can be used at higher temperatures than PE without undergoing excessive deformation. In particular, hot-filling products in PP is possible. The enhanced stiffness of PP also makes it suitable for threaded closures (caps). HDPE cannot be used in such applications because, under load, it will creep too much, causing loosening of the cap and loss of sealing efficacy. PP is much less subject to such deformation, and is by far the most commonly used plastic in closures of all types.

Uses of PP in packaging are divided approximately equally between film, containers, and closures. The stiffness of PP is also an advantage in some film applications. It got its major start replacing cellophane in high-speed packaging lines such as those used for cigarettes. LDPE was too soft for such applications. PP also has better transparency than LDPE; thin PP films generally have excellent transparency.

In thicker gauges, PP often has a somewhat cloudy appearance. This can be modified by blending nucleating

agents into the film. By providing multiple sites for the initiation of crystallite growth, PP with nucleating agents tends to have a larger number of crystallites, but they are smaller, as adjacent crystallites interfere with each other's growth. These small crystallites interfere less with light transmission than larger ones, so the net result is improvement in transparency.

The tendency of PP to be brittle at cold temperatures can be alleviated in two basic ways. One common approach for film is biaxial orientation. Orientation is a process of stretching a plastic film (or container) to partially align the polymer molecules in the direction of the stretch. This tends to enhance mechanical properties and barrier by producing a greater degree of regularity in the structure. Biaxial orientation stretches the plastic in two perpendicular directions, producing alignment in the plane of the stretch. Biaxially oriented PP is called BOPP. Another way to reduce brittleness is to copolymerize propylene with a small amount of ethylene. This introduces ethylene units into the molecule, which bring with them increased flexibility, lowering the T_g of the plastic and reducing its brittleness.

E. Polyethylene Terephthalate

Polyethylene terephthalate (PET) is the plastic that has been growing in use most rapidly. Its largest use remains plastic bottles for carbonated soft drinks, but it is increasingly being used in bottles for a variety of applications, including drinking water, salad dressing, peanut butter, etc. Within the last several years, PET surpassed HDPE as the plastic most often used for bottles. PET also is used in trays, for products varying from croissants to fresh vegetables to meat. PET films and coatings are also used for food packaging applications. One of the earliest uses of PET was as a coating for ovenable paperboard, paperboard trays designed to be useable in both microwave and conventional ovens.

PET is a polyester, and a member of the condensation polymer family, and has the chemical structure shown in Figure 131.7. While there are a large number of different polyesters, products made from PET are often referred to simply as polyester. For example, polyester carpet, polyester clothing, and polyester fiberfill are all made from PET — and often incorporate PET recycled from beverage bottles.

Because condensation polymers have non-carbon atoms (oxygen, in the case of PET) in their main chain, they tend to be more susceptible to chemical reactions leading to rapid decrease in molecular weight than most addition polymers, which generally have only carbon in the main chain. In particular, PET is subject to hydrolysis when exposed to water at high temperature and shear. Therefore, it is important to keep PET dry during processing.

PET is a significantly better oxygen and carbon dioxide barrier than HDPE and PP, but not as effective as a water vapor barrier. The polar bonds in PET result in stronger intermolecular forces, which reduce permeation of non-polar

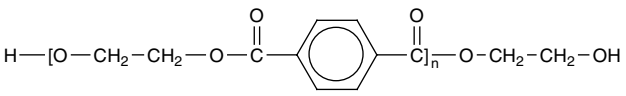

FIGURE 131.7 Polyethylene terephthalate (PET).

substances. However, they also permit greater interaction with water and other polar molecules. Most PET is biaxially oriented for improved performance. As discussed, this orientation improves both strength and barrier properties. Biaxially oriented PET bottles have sufficient CO_2 barrier to provide an adequate shelf life for carbonated soft drinks. While the O_2 barrier of PET is much better than that of HDPE and PP, it is still not sufficient to provide adequate protection for many oxygen-sensitive products, such as ketchup. The most common structure for plastic ketchup bottles, and for plastic containers of other oxygen-sensitive products, combines PET with a barrier resin, usually ethylene vinyl alcohol.

PET has a T_g of 73–80°C. Therefore, at normal use conditions it is on the stiff and brittle side of its behavior. PET is capable of crystallizing, but has a narrow temperature window for crystallization. This permits the degree of crystallinity that develops in PET to be greatly modified by changing the processing conditions. PET films and most PET containers develop only a low degree of crystallinity, with small crystallites that do not interfere substantially with light transmission, resulting in packages with excellent transparency. If increased crystallinity is desired, nucleating agents can be added to facilitate crystallization, resulting in opaque CPET (crystalline PET). Long residence time at temperatures within the crystallization range also produces an opaque white material. Some bottles designed for hot-filling have PET bodies that are transparent, but finishes (the threaded neck area that accepts the closure) that have been crystallized and are opaque white. This results in a bottle neck that is less subject to deformation during the hot-filling process, providing improved sealing.

Because typical hot-fill temperatures are near or even above its T_g, biaxially oriented PET bottles, if unmodified, can undergo a large amount of distortion during hot-filling, as some of the stresses imposed by the orientation are now able to relax. (To see an example, send an empty PET peanut butter jar through a standard dishwasher cycle.) Therefore, containers intended for hot-fill applications have to be stabilized, usually in a process known as heat-setting, where they are subjected to elevated temperature while being held in the desired shape, to allow stress relaxation without permitting deformation.

F. Polystyrene

Polystyrene (PS) is another member of the addition polymer family, with a benzene ring attached to every other carbon (Figure 131.8). Like PP, PS can be either atactic, isotactic,

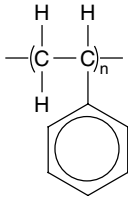

FIGURE 131.8 Polystyrene (PS).

or syndiotactic. The PS used in packaging is atactic. This lack of order in the spatial positioning of the benzene ring means that the PS molecules cannot be packed into an orderly repeating arrangement, so PS is an amorphous polymer. It has a T_g of 74–105°C, so like PET it is stiff and brittle at most use conditions. Since it is amorphous, PS has excellent transparency, but it has poor barrier properties. Transparent grades of PS are often called crystal PS. It should be noted that crystal PS is highly transparent precisely because it is not crystalline!

Despite its high T_g, PS is not suitable for high temperature applications, as it undergoes liquid flow at about 100°C.

The brittleness and low impact strength of PS is a drawback in many food packaging applications, while its relatively low cost and ease of thermoforming are assets. Two distinct approaches are commonly used to modify its brittleness. One of these is foaming. Expanded polystyrene (EPS) uses small bubbles produced by a foaming agent to reduce the density of PS, and also to provide it the ability to better absorb stress without fracture. The low thermal conductivity produced by the bubbles also makes foam PS an excellent insulating material. While the biggest application of foamed PS is as molded or loose-fill cushioning materials, it is also found in such food packaging applications as disposable cups for hot beverages, meat and produce trays, and egg cartons. Since the presence of the bubbles results in light scattering, foamed PS is opaque.

Another way to improve the impact resistance of PS is to modify it with a rubber material that has high impact strength. High impact polystyrene (HIPS) is partially a copolymer and partially a blend of PS with polybutadiene, a synthetic rubber. While HIPS, like PS, is amorphous, the presence of two phases (PS regions and butadiene regions) interferes with light transmission, making HIPS, like foam PS, opaque. Two common applications of HIPS are yogurt containers and disposable cutlery.

G. Polyvinyl Chloride

Unlike most plastic packaging materials, the use of polyvinyl chloride (PVC) in packaging has not been growing. In fact, PVC has lost a number of its packaging markets, mostly to PET. Most of these, however, were in non-food packaging. PVC is FDA-approved only for limited food packaging applications.

PVC is an addition polymer, with a basic structure similar to PP and PS, having, in this case, a chlorine atom attached to every other carbon in the main chain. PVC has a slight tendency to be syndiotactic, but its irregularity is substantial, resulting in a degree of crystallinity so slight that it is often referred to as an amorphous plastic. Because of this low crystallinity, PVC has excellent transparency, with a slight bluish cast. As PVC ages, it tends to yellow, so it is common for PVC containers to have additional blue coloration added, to mask the yellow that will eventually develop.

The T_g of unmodified PVC is 75–105°C, so it is stiff and brittle, and is a reasonably good barrier to oxygen and carbon dioxide. However, PVC used in packaging is generally modified by incorporation of plasticizer. A plasticizer is a component that acts as an internal lubricant in the plastic, getting between the polymer molecules and, by disrupting the attractions between the polymer molecules, increasing their ability to change position, thus lowering the T_g and making the plastic more flexible. PVC, because of its highly polar C-Cl bonds, has a large affinity for plasticizers, so they can be incorporated in substantial amounts. Incorporation of various amounts and types of plasticizer permits the production of a wide variety of PVC resins with significantly differing properties. One of the major uses for PVC in food packaging is in soft, highly flexible stretch films used for meat wrap, etc. In addition to increasing flexibility, incorporation of plasticizer significantly reduces barrier, so these soft PVC films are poor barriers to oxygen, carbon dioxide, etc.

Over the years, PVC has been subject to a number of attacks on environmental and health grounds. The earliest major concern was potential migration of vinyl chloride monomer (the building block for PVC and a carcinogen) into foods or beverages. Changes in production technology greatly reduced the concentration of residual monomer in containers, and hence the potential for migration. The next major concern was the potential for PVC to contribute to formation of dioxins during incineration. This held up the more widespread approval for use in food packaging that was expected following the resolution of the residual monomer problem. Currently, concerns are related primarily to the potentially harmful effects of plasticizers migrating into the package contents. Along the way, the adverse effects on quality of recycled materials from even very slight PVC contamination of recycled PET have also been a concern. Regardless of whether the concerns about PVC are reasonable or not, PVC has suffered from a relatively negative environmental and health image, and PET, with its relatively positive image, has benefited from the comparison.

H. Nylons

Nylons, or polyamides, are a family of condensation polymers made by polymerization of amines and car-

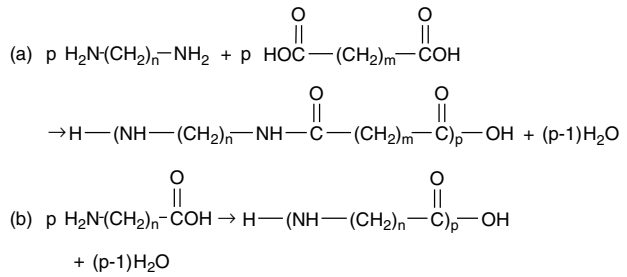

FIGURE 131.9 General scheme for polymerization of nylon: (a) from diamine and dicarboxylic acid, (b) from amino acid. If monomers are linear, structures formed by (a) are named Nylon n, (m + 2), and those formed by (b) are named Nylon (n + 1).

boxylic acids, or by polymerization of amino acids (Figure 131.9). Therefore, they contain nitrogen atoms in the main chain, and attached to the N is a hydrogen atom. This means nylons exhibit hydrogen bonding, resulting in very strong intermolecular attractions, and therefore relatively high T_g and T_m. Nylon 6, for example, has a T_g of 60°C, and a T_m of 210–220°C. The properties of nylons differ, depending on their precise chemical structure. Two of the most common nylons for packaging applications are nylon 6 and nylon 11. Both of these materials are crystalline polymers; as is the case with PET, the amount of crystallinity exhibited is strongly dependent on processing conditions. Some nylon copolymers are amorphous.

Nylons tend to have excellent strength and thermal stability, while maintaining flexibility and strength at low temperatures. They are good gas and oil barriers, and tend to be excellent barriers for odors and flavors. They do tend to be moisture sensitive, however. The combination of low temperature strength and high temperature stability of nylons allows them to be used for applications such as boil-in-bag frozen foods. Nylons are higher in cost than the more common packaging plastics, and therefore are often used in combination with other polymers, in multilayer structures, to reduce overall package cost while still benefiting from nylon's properties.

I. Polycarbonate

Polycarbonate (PC), more properly known as poly(bisphenol-A carbonate) has limited applications in food packaging, primarily due to its high cost. It is a very tough, rigid plastic, which is widely used for 5-gallon refillable water bottles. Recently, however, PET has begun to make inroads into this market. PC has also been used for refillable milk bottles, but this was never a large market and has tended to decrease over time. PC is an amorphous polymer with good impact strength, thermal stability, heat resistance, and good low temperature performance. It is a poor barrier to gases and water vapor, and has relatively poor chemical resistance to alkalis. Like PVC, polycarbonate packaging often incorporates plasticizers, some of which are under attack as hormone mimics or disruptors.

J. Ethylene Vinyl Alcohol

Ethylene vinyl alcohol (EVOH) is another plastic which has grown rapidly in use. It is, in essence, a random copolymer of ethylene and vinyl alcohol, although it is actually made by hydrolysis of ethylene vinyl acetate (EVA), as the vinyl alcohol monomer is unstable. EVOH typically contains 27 to 48 mole % ethylene units.

The O–H groups in the alcohol units of EVOH provide very strong hydrogen bonding between adjacent molecules, and in addition EVOH can crystallize. The –OH and –H groups can both fit the crystal lattice, so the irregularity of the structure does not prevent crystallization in this case. The result is a polymer that is an excellent barrier to gases such as oxygen and carbon dioxide. The most common reason for using EVOH is to take advantage of its excellent oxygen barrier properties. However, the same hydrogen bonding that makes EVOH a good O_2 barrier also makes it highly sensitive to water, and as the polymer absorbs water, its barrier properties decrease. Therefore, EVOH is almost always found in a buried inner layer in a package structure, surrounded by other plastics that can offer protection from exposure to high humidity.

Use of multilayer structures containing EVOH has permitted plastic containers to replace glass and metal in a variety of food packaging applications. The first plastic bottle for ketchup, for example, was a 6-layer structure, PP/regrind/tie/EVOH/tie/PP. The regrind layer is composed of manufacturing scrap from bottle production that is flaked and fed back into the process. The tie layers are a plastic that serves as an adhesive, bonding the EVOH and PP layers together. The current ketchup bottle structure is a 5-layer structure, PET/EVOH/PET/EVOH/PET.

The moisture sensitivity of EVOH is a particular concern for containers that are retorted, as during retorting the package is exposed to the combination of high temperature and high humidity. For such applications, structures have been developed that contain a desiccant in the tie layer, to absorb moisture that gets through the outside package layers and thereby reduce its effect on the EVOH.

K. Polyvinylidene Chloride

The other major oxygen barrier plastic is polyvinylidene chloride (PVDC). The basic structure of PVDC is similar to

PVC, except that instead of a single chlorine on every other carbon, PVDC has two chlorines on every other carbon. One consequence is that PVDC can be highly crystalline. The combination of high crystallinity and strong intermolecular attractions due to the polar C-Cl bonds makes PVDC, like EVOH, an excellent barrier. Since PVDC does not contain hydrogen bonds, however, it is an excellent barrier to water vapor as well as to oxygen, carbon dioxide, odors and flavors, etc. Further, its barrier properties are not much affected by exposure to moisture. A major drawback of PVDC is that its intermolecular forces are so strong that it is very difficult to process. In practice, PVDC must be modified by copolymerization to make it processable; PVDC homopolymers are not used. The degree and type of comonomerization affects PVDC properties, decreasing its barrier. The PVDC polymers with the highest barrier tend to be used as coatings, applied as solutions or emulsions, as they cannot be melt-processed. The melt-processable grades have somewhat poorer barrier properties. Some grades, such as PVDC used for household wrap, have been plasticized, in addition to copolymerization, and consequently their barrier properties are further reduced.

The oxygen barrier of the best PVDC resins is generally inferior to that of the best EVOH resins as long as the EVOH is relatively dry. At very high humidity, PVDC is generally superior in barrier to EVOH. More widespread use of PVDC is limited partially by its cost, and even more by processing difficulties. PVDC copolymers are quite heat sensitive (as is PVC), tending to degrade producing HCl. On the other hand, PVDC coatings on films (or containers) can significantly increase barrier as well as providing heat-sealability.

L. Ionomers

Ionomers are plastics that contain some interchain ionic bonding, most often produced by partially neutralizing ethylene/methacrylic acid or ethylene/acrylic acid copolymers with sodium or zinc bases. The percentage of acid groups is usually between 7 and 30 weight percent, and the amount of neutralization usually is between 15 and 80 percent. The unneutralized acid groups provide hydrogen bonding sites, while the salt ions provide ionic attractions, functioning much like reversible cross-links.

Ionomers, as a result, have increased strength, toughness, tensile modulus, oil resistance, and clarity. Their impact strength and puncture resistance are outstanding. Ionomers excel in difficult heat-seal applications, and are widely used in vacuum packaging of processed meats. Here, their ability to seal through contamination means excellent seals can be obtained even if there is contamination of the seal area with grease. They are also used for packaging of cheese and snack foods. The excellent hot tack of ionomers allows them to be used in form-fill-seal packaging in cases where product is delivered into a pouch before the bottom seal has completely cooled.

M. Other Polymers

Ethylene vinyl acetate is used as an adhesive, as a heat seal layer on other polymers, and as a very flexible but tough cling film. In most food applications, the vinyl acetate content is between 5 and 20 percent. Increasing the vinyl acetate content results in improvement in clarity, increased flexibility, increased impact strength, and increased adhesion.

One of the newest entries in food packaging is polymers based on lactic acid (PLA). PLA plastics tend to have properties somewhat inferior to those of PET, but have the advantage of being biodegradable and compostable. They do not yet have widespread use in food packaging, but this may change in the future.

Polyethylene naphthalate (PEN) is another member of the polyester family. Its properties in general are superior to those of PET, but its cost is much higher, which has greatly limited its use. There is, however, some use of blends and copolymers of PET and PEN, to get some of the advantages of PEN without as much of an increase in cost.

A number of other plastics are used in food packaging applications to a limited extent, because of the properties they can provide.

IV. ADDITIVES

When a plastic resin is formulated, along with the base polymer a number of other components are often added. These additives generally have some potential to migrate to the product, and consequently are regulated by FDA as indirect food additives. It is, therefore, necessary to be sure that food-grade resins are selected for food packaging applications.

A very common class of additives is antioxidants. Some of these are compounds such as BHA and BHT that are also used in food to suppress oxidative deterioration of products. They serve much the same purpose in plastics, usually being targeted primarily at preventing oxidation during processing. These additives can, in some cases, migrate to the product during storage, providing additional product protection. In fact, they have sometimes been added to packaging plastics precisely for this purpose.

Colorants, most often pigments, are used to provide desired colors to plastic packages. For example, certain types of soft drinks are packaged in green PET bottles. Pigments can also be used to prevent transmission of light, or of certain wavelengths of light, in order to minimize light-induced degradation of the product.

Of increasing interest recently are various types of antimicrobial additives, most based on compounds classified as “generally recognized as safe” (GRAS), and intended to inhibit the growth of microorganisms that might otherwise lead to post-processing contamination. Some of these are effective through surface contact, and others act by migrating from the package to the food product. Examples include nisin, potassium sorbate, sorbic acid, natamycin, zeolite-based silver ions, and others.

Other categories of additives include plasticizers, heat stabilizers, UV stabilizers, antiblock agents, slip and anti-slip additives, lubricants, mold release agents, nucleating agents, antifogging agents, antistatic agents, oxygen scavengers, desiccants, etc. Fillers and/or reinforcing materials are also used in some food packaging plastics.

Additives may be mixed uniformly into the resin pellets. Often, however, they are supplied in the form of a "master batch" that has a high concentration of the additive. The amount of master batch added depends on the level of additive desired in the final product. Additives can also be added to the extruder when the plastic is being formed. This is common for fibers and fillers, for example.

V. BASIC PLASTIC FORMING PROCESSES

Most packaging plastics are formed into the desired package shape by melting the plastic and using heat and pressure to shape it as desired. The plastic is generally fed into an extruder in pellet form. The extruder melts and mixes the plastic, so that a uniform stream of melt is available at the desired temperature and pressure for downstream processing. The plastic usually exits through a die, which shapes it to the desired profile.

Plastic film is made in two major ways. For cast film, the melted plastic exits the extruder through a slit die and is brought into contact with a water-cooled chrome roller (or set of rollers). The film may, if desired, then be oriented uniaxially or biaxially. The edges are trimmed off, as relaxation in the plastic makes the edges thicker than the rest of the film. It is then rolled up, and is ready for converting operations, where it may be printed, folded, cut, sealed, etc.

Blown film is made using an annular die, which produces a hollow tube of melted plastic. Increased air pressure inside the tube expands it in diameter, while it is stretched in the lengthwise direction as it is drawn through the machinery. Therefore, blown film is biaxially oriented without requiring an additional orientation step. The tube of plastic may be rolled up as-is, for use in processes such as bag or pouch-making. In this way, it is possible to produce a pouch, for example, that has only top and bottom seams. Often, the tube is instead slit on both edges, producing two rolls of flat film.

Plastic sheet is too thick to be produced by the blown film process, so the cast process is used. For packaging applications, the sheet is usually further modified by thermoforming. In thermoforming, the sheet is reheated and then stretched into or over a mold, using some combination of vacuum and pressure. Foamed plastic sheets, such as polystyrene foam, can be thermoformed, as well as solid plastic sheets. Thermoforming is routinely used for trays, and can also be used for cups and similar shapes. The plastic thins as it is stretched, until it contacts the cool mold and stops stretching. Therefore, the parts of a thermoformed package that have been stretched the most during forming will be the thinnest in the final package. There are a variety of different thermoforming methods; which method is chosen depends on the material used and the package shape being produced. Plug-assist pressure forming, in which a mechanical device (the plug) helps push the plastic into the mold, as well as additional air pressure for forming are used. This facilitates deep draws, can improve the uniformity of wall thickness, and shortens cycle times.

Another way to make plastic containers, closures, etc., is using injection molding. In this process, the plastic is melted in an injection molding machine, which is essentially an extruder, but does not have a die. Instead, the melted plastic is injected into a mold. The mold usually contains multiple cavities, so that several plastic packages or package components (usually identical to each other) are produced in a single step. In injection molding, the plastic fills the entire mold cavity, so there is excellent control over the dimensions of the finished article. Injection molding is limited to shapes that can be removed from the solid core. Therefore shapes such as bottles cannot be made, since there is no practical way to get the solid center out of the molded bottle. For some shapes, innovations such as collapsing cores can be used, where the core is made of multiple pieces that can retract when a center piece is pulled back, allowing their removal. However, this is not practical for bottles. When the plastic flows into the mold block, it passes through a system of runners in order to reach the mold cavities. In packaging applications, these runners are generally heated to keep the plastic inside from solidifying, so that it can be used in the next cycle, rather than needing to be removed and discarded or used as regrind. This process is called hot runner molding. One of the most common applications of injection molding in packaging is in manufacture of threaded plastic closures.

To make containers such as bottles, blow molding is used. There are two major categories of blow molding. In extrusion blow molding, the plastic is extruded as a hollow tube, much like is done for blown film. The tube, or parison, when it is the appropriate length, is cut off and captured in a mold. Air is then blown in to expand the parison into the mold shape. Excess material at the top and bottom of the container is cut off, and generally immediately recycled into the process (this material is known as regrind). Bottles with handles can easily be produced by using a parison that is wide enough to cover both the body and handle area when it is captured in the mold. These handles will be hollow, and have an open connection to the body of the container. The gap between the handle and the body is created by pinching the two plastic layers together during molding, and then cutting out this solid piece of plastic. There is often excess plastic at the sides of the neck of the bottle that must be removed, as well, especially for bottles with handles.

To achieve more uniformity in wall thickness of extrusion blow molded containers, it is common to modify the shape of the die (die shaping) to correct for ovality in the container, and to modify the size of the opening as the parison is produced (parison programming) to control for vertical asymmetry. These techniques can also be used, of course, to produce walls that are thicker at certain points, if this is desired. For example, the thickness in the finish area of the bottle will probably be more than in the body, since greater strength and stiffness are needed there. While these techniques add somewhat to cost, they generally more than make that up by permitting reduced material use.

In injection blow molding, the bottle is produced in two steps. First a parison is produced by injection molding, as was described above. The bottle finish is completely formed in this step. This allows very precise control over finish dimensions. The body of the parison can also be designed to provide varying wall thickness, thus achieving greater wall thickness uniformity in the finished container. In the second step, the parison is placed into the container mold and blown into its final shape, using air pressure. These two steps may be done sequentially in the same machine. In this case, the injection-molded parison is cooled only enough to maintain its shape, with the support of the core rod that shapes the interior of the parison, during the transfer to the blow mold. It is also possible to completely cool the parison and ship it to another location for blow molding at a later time. In that case, of course, the parison must be reheated before it is blown.

It was mentioned earlier that PET soft drink bottles are biaxially oriented. Blow molding naturally produces uniaxial orientation, since the plastic is stretched radially as it is blown. To achieve biaxial orientation, stretching in the vertical direction must be added. This is done by stretch blow molding. While extrusion stretch blow molding does exist, it is rarely used, so we will discuss only injection stretch blow molding. In this process, the parison is produced by injection molding, as already described. However, it is much shorter in length than the height of the final container. The parison is placed in the blow mold with a stretch rod inside. After the parison is carefully reheated to give the desired temperature profile, it is simultaneously blown with air emitted through the stretch rod, and stretched vertically as the stretch rod descends into the cavity, thus producing the desired biaxial orientation.

VI. MULTILAYER PACKAGES

It has become very common to use packages that are made of more than one material. Structures that combine plastic, paper, and/or aluminum and structures that contain more than one type of plastic can efficiently perform functions that cannot be obtained from a single layer structure. There are four basic ways to obtain such multilayer structures.

The first method is coating. We can add a polymer coating to paperboard, for example, to improve its water resistance. We can add a barrier coating to a container to increase the shelf life of an oxygen-sensitive product. When plastic resins are used for coating paper or foil, the most common process used is very similar to that for producing cast film. The plastic is melted in an extruder and then emitted through a slit-shaped die. However, instead of contacting a chrome roller, the plastic goes onto the paper or foil, and adheres to it as the plastic cools. Solvent-based coatings or water emulsions of polymers are sometimes used, instead. The choice depends primarily on the polymer requirements.

The second method is lamination. In this process, two rolls of material (substrates) are joined into a single material by causing them to adhere to each other. Most often the adhesion is achieved by using a third component, an adhesive. The substrates may be plastic, paper, or foil. The choice of the adhesive and how it is applied depends on the requirements of the substrates and the characteristics of the adhesive. If the adhesive is low density polyethylene, for example, it is likely extruded into the gap as the substrates are brought together.

The third method, which has become very common, is coextrusion. In this process, individual plastics are melted, each in their own extruder, and the melt streams are then brought together, either in the die or in a feed block just before the die. The process of bringing the melted plastics together is done very carefully, so that the streams do not mix with each other, but instead flow uniformly in separate layers, each of the desired thickness. Next the multilayer plastic melt is shaped as described already. This can be used to produce multilayer sheet for thermoforming into multilayer structures, multilayer film through either the cast film or blown film process, or multilayer containers through extrusion blow molding.

The fourth method is the newest. Producing multilayer injection-molded objects is more technically challenging than producing multilayer extruded or extrusion-blown objects. Coinjection processes fall into two basic categories. One approach is to use multiple mold cavities and multiple injections, essentially building up the object layer by layer. The more common method, in packaging applications, is to fill the injection mold with a multilayer flow of melted plastic in a single step, again starting with each plastic melted in a separate extruder. Coinjection stretch blow molding, such as is used for PET/EVOH/PET/EVOH/PET ketchup bottles, starts with a coinjection-molded parison, and then uses stretch blow molding to form the final container.

VII. PERMEABILITY AND SHELF LIFE

The length of time a food product remains acceptable once it is packaged is often dependent at least in part on the ability of the package to protect it from external influences

TABLE 131.2
Typical Properties of Plastics Used in Food Packaging

Property	HDPE	LDPE	PP	PET	PS	PVC	Nylon 6	Nylon 11	PC	EVOH	PVDC
T_g (°C)	−120	−120	−10	73–80	74–105	75–105	60		150	48–69	−15 to +2
T_m (°C)	128–138	105–115	160–175	245–265		212	210–220	180–190	265	156–189	160–172
Density (g/cm^3)	0.94–0.965	0.912–0.925	0.89–0.91	1.29–1.40	1.04–1.05	1.35–1.41	1.13–1.16	1.03–1.05	1.2	1.13–1.21	1.60–1.75
Tensile strength (psi)	2500–6500	1200–4550	4500–6000	7000–10500	5200–7500	1490–8020	6000–24000	8000–9500	9100–10500	5400–13600	2800–5000
Tensile modulus (10^3 psi)	89.9–158	24.9–75	165–225	400–600	330–475	600	100–247	185	345		50–80
Elongation at break (%)	10–1200	100–965	100–600	30–3000	1.2–2.5	14–450	300	300–400	110–150	180–330	160–400
WVTR (g mil/100 in^2 d at 100°F, 90% RH)	0.32	0.95–1.3	0.25–0.76	1.0–1.3	4.4–10	1.9–40	9.9–11	2.5–5.1	4.9–5.9	1.40–38.1	0.02–0.61
O_2 permeability, 25°C (cm^3 mil/100 in^2 24 h atm)	100–185	400–540	130–240	3.0–6.1	250–380	9.4–600	1.2–2.6	32	300	0.0067+	0.02–6.9

such as exposure to oxygen or gain of moisture, or from internal changes such as loss of volatile flavor compounds, loss of carbonation, or drying out. In all of these cases, the barrier capability of the plastic, its ability to retard transfer of one or more components through the package wall, is a major factor.

The barrier ability of a plastic is determined by two factors: how soluble the compound in question is in the plastic, and its ability, once in the plastic, to move through it (its diffusivity). Typically, we put these two factors together and evaluate the permeability of the package or package material. A package that is a good barrier has low permeability. As we have seen, plastics can be good barriers for some components of interest, and poor barriers for others. For example, HDPE is a good water vapor barrier but a poor oxygen barrier, while EVOH is a good oxygen barrier but a poor water vapor barrier.

Barrier is enhanced by increasing crystallinity, since crystallites are essentially impervious to permeating molecules. Strong intermolecular forces also increase barrier. Permeation is significantly faster in a polymer above its glass transition temperature than in the same polymer below its glass transition temperature. Above T_g, we have activated diffusion, in which segmental movements of the polymer chain tend to open up pathways for the permeating molecules.

While a thorough discussion of permeability and shelf life is beyond the scope of this chapter, oxygen permeability coefficients for various plastics are presented in Table 131.2. For water vapor, it is traditional in packaging to present water vapor transmission rate (WVTR) coefficients, rather than permeability coefficients. A WVTR is a function of the conditions at which it is measured, so that condition must be taken into account and used to transform the value to a permeability coefficient before it can be used in a shelf life calculation at a differing relative humidity. WVTR values are also listed in Table 131.2. The shelf life of a product depends on how much of the compound of interest can be gained or lost without making it unacceptable, the area available for mass transfer (generally the surface area of the package), its thickness, and the driving force for transfer, in addition to the permeability coefficient. The driving force for transfer is the difference in chemical activity on the two sides of the package (inside and outside), typically expressed as a concentration (partial pressure) difference.

VIII. MIGRATION AND SCALPING

Migration and scalping also involve mass transfer, but instead of transfer between a product and the outside environment, as is the case in permeation, the transfer is between the product and the package itself. By migration, we mean transfer of a component from the package to the product. As mentioned, components that transfer in this way are classified by FDA as indirect food additives. They must either be approved as food additives, have GRAS status, or migrate in such small amounts that they have been determined to pose no real risk. Potential migration of unknown contaminants from a recycled plastic stream is one factor limiting the use of recycled plastics in food packaging. Some recycled resins have been approved for direct food contact, either alone or blended with virgin plastic. Others have been approved provided a "functional barrier" of some minimum thickness of virgin plastic is interposed between the recycled plastic and the food, in a multilayer structure.

Scalping refers to the transfer of a component from the product to the package. Usually, this involves a flavor or odor component, and the transfer is undesirable. Polyethylene, for example, can readily scalp the components that give fruit-flavored cereals their desired smell and taste.

IX. INFORMATION SOURCES

Additional information on plastics used for food packaging, plastic forming processes, additives, mass transfer and shelf life can be found in Refs. 2–4.

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132 Paper and Paperboard Packaging

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CONTENTS

Various materials, functions, forms, and technologies of food packaging are used to protect and maintain product quality during shipping and storage. Recent concern on food safety, health, and resources conservation are evident ever before. Paper and paperboard are made of fibers from renewable, environmental friendly resources. This chapter describes those characteristics of paper and paperboard most relevant to food packaging applications.

I. INTRODUCTION

Paper and paperboard are made of fibers from easily obtained natural, renewable resources such as wood or vegetable fibers. They are useful for packaging, writing, and a variety of other purposes.

There is no distinct difference between paper and paperboard. Paperboard has characteristics of thick caliper. They are pliable, relatively low cost, easy to convert into various shapes, recyclable, biodegradable, and eco-friendly materials. Depending on purpose of packaging, paper and paperboard can withstand conditions of high and low temperatures which are experienced by sterilized food and frozen or chilled foods. Excellent printability and glueability are required for certain purposes. In spite of various advantages of paper and paperboard, they have certain disadvantages such as easiness to burn, and weakness to water, which can be controlled to a certain extent. Therefore, in order to modify properties of paper and paperboard various additives are added to paper or they are laminated with other materials.

Various materials for packaging are used as the science, technology, and machineries are developed, because the packaging market is changing very rapidly. Convenient, high quality, and safe packaging is sought. Share of paper and paperboard in packaging material consumption is about 40% which is higher than plastic, metal, and glass materials. With growing concerns on environment, recyclablility of paper and paperboard packaging materials appeals to customers.

Properties can be controlled by changing raw materials, adding additives, and modifying papermaking processes, or using converting machines depending on purposes.

II. MANUFACTURING OF PAPER AND PAPERBOARD

Paper is manufactured by Fourdrinier, cylinder, or twin-wire former. Fourdriner former has an endless turning wire to distribute fibers evenly on it. Paper produced by Fourdriner former has different sides because of uneven distribution of fillers and small fibers through thickness of paper structure by downward dewatering only. By rotating a cylinder with a wire rotated partially in a fiber suspension, paper is produced on the outside wire and water is drained inward through the wire. The thickness of paperboard by multiple cylinder formers has no limit by depositing one layer on another layer by additional cylinder formers. One directional dewatering limits the speed of Fourdriner former. By impinging fibers solution between two wires and dewatering from both sides of paper, twin-wire former provides even distribution of fillers and small fibers with high speed. Corrugated medium is produced by corrugator and pasted to linerboard to produce corrugated board.

Manufacturing process of paper is composed of two steps, including adding water to fibers then removing water from the sheet structure. Paper is fibrous material of certain thickness with wide area. In order to distribute fibers uniformly, fibers are dispersed in water at low concentration of 0.3–5.0%. Then diluted fibers solution is spread out through a slot, called "headbox," at high speed (about 100 Km/h for modern machine) across a papermaking machine. Water is removed by gravitation, mechanical pressure, and drying energy. Paperboard is a kind of thick paper with several layers of paper in one structure. It is made by making one layer of sheet first, then adding another layer by another headbox, and so on, to make 3–7 layers of sheet.

A. Raw Materials

Cellulosic fibers are used as raw materials for both paper and paperboard. Fibers can be obtained usually from wood or annual plants, but sometimes from animal, mineral, or synthetic for special paper. The fiber is a tubular or cylindrical element several millimeters long and less than 100 micrometers wide. If you tear a sheet of paper and look under a bright lamp at its edge then you can see individual fiber sticking out from the torn zone.

Fiber properties are quite different depending on source. There are many factors that affect final properties of paper and paperboard, but the most profound effect may come from the fiber resources. It is critical to choose proper fibers for specific characteristics development.

Chemical constituents of wood fibers are cellulose, hemicellulose, lignin, and extractives. Cellulose is beta-glucosidic linked glucose chains. Hemicellulose is various polysaccharides which are associated with cellulose, such as glucose, mannose, galactose, xylose, and arabinose. Lignin is phenyl propane unit with complex structure. Extractives are not a part of cell wall structure, but can be removed by neutral solvent such as ether, benzene, alcohol, and water.

Pulping is a process to prepare fibers from wood or vegetables by using mechanical and/or chemical energy to separate individual fibers, because fibers are assembled with lignin as paste that bind fibers together. Pulp means fibers that are separated through pulping. Mechanical pulping uses a grinder to apply friction force to separate individual fibers. Lignin is still in fibers of mechanical pulp (MP), so fibers are brown in color before bleaching. Chemical pulping uses chemicals to dissolve out lignin which binds fibers together; it gives low yield but fibers are strong. Through bleaching chemical pulp (CP) becomes bright in color. A brown grocery bag, the most common chemical pulp, is strong and made of Kraft pulp (KP). Small pieces of wood (called "chips") and chemicals are mixed in a digester to react at certain temperature for a certain period of time, and the reaction conditions

determine the mechanical, morphological, and chemical properties of fibers.

B. Processes and Equipments

Fibers are supplied in a diluted solution of water after pulping. In order to make fibers suitable for paper properties, fibers are treated by mechanical force to develop microfibrils sticking out of the surface so flexibility of fibers is increased. Fibers are mixed well, pumped, and passed through a "headbox" at a very high speed. Papermaking is basically an efficient process of removing water from the diluted solution of fibers, which involves using gravitational force by putting the fiber solution on the wire, using mechanical squeezing compressional force by passing through two rolls, and evaporization drying by contacting paper on steam heated cylinder can. Continuous paper web is wound by winder. A block diagram of the papermaking process is shown in Figure 132.1.

Various pulps from softwood, hardwood, and annual plants are refined to obtain proper fiber properties. Refining is a mechanical bruising process to develop fibrils on fiber surface and delaminate internal layers to make fibers more flexible to bond easily to improve bonding strength. During refining fibers are hit by mechanical force when they pass through stationary or rotating bars. Refined fibers are diluted in water and ejected at high speed from the headbox on a continuously revolving wire. Fibers are retained on wire and water flows through wire without vacuum or with vacuum to form wet web of fibers. By squeezing wet fibers structure water is removed through porous felt and slit roll at press. After removing a certain amount of water by squeezing, mechanical force cannot remove water any more. Then heat energy at a dryer can be used to remove water by evaporation. For printability smooth surface of paper and paperboard is essential. In order to increase smoothness paper and paperboard pass between several rolls called a calender. In continuous web winder, the web is wound in order to prepare for converting in the next process. Web width is about 2–10 m depending on papermaking machine, and total length of web is about 1–10 km. Sheet cutter makes paper sheets rectangular by using a rotary knife or guillotine. For storage or transportation paper rolls or sheets are wrapped with plastic film or vinyl laminated paper.

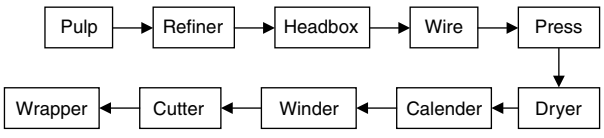

Figure 132.1 Papermaking processes.

III. CONVERTING OF PAPER AND PAPERBOARD

Most papers and paperboards are converted by impregnating, saturating, laminating, embossing, and forming processes to specific shapes and sizes for efficient usage. In food industry, various papers and paperboards are combined with other materials such as aluminum foil, plastic or metallized flexible packaging material, and extruded films.

Representative surface converting processes are coating and laminating. Paper is composed of numerous fibers with many pores in its structure, so its surface is relatively rough. Very fine and white pigment particles are coated on the surface to improve smoothness and brightness of paper. To bind the particles themselves or on the fibers surface, natural or synthetic adhesives are used. Laminating is a combining process of similar or dissimilar webs to impart barrier properties against moisture, oxygen, light, odor and flavor, or to impart special properties.

A. Processes and Equipments

1. Coating

Coating can be divided into aqueous coating and extrusion coating. Aqueous coating, or simply coating, is a process of applying a coating solution on the surfaces of paper and paperboard for enhancing smoothness and printability. Coating is like a makeup on a face for paper. Very fine mineral pigments of 0.1–2 micrometers and adhesives such as natural starch, casein, and synthetic latex are applied on the surface of the paper. To cover coating materials uniformly on paper surface, a very thin knife (called "blade"), an air knife (blowing air), a roll or rod is used to level out across the whole width of paper roll. Extrusion coating is a process that applies high molecular weight polymer on paper by extruder die assembly. It provides heat stability and resistance to water, moisture vapor, some gases, and oil for a paper.

2. Laminating

There are several types of lamination, such as (1) wax and hot melt, (2) wet, (3) dry, and (4) solventless laminations. Critical conditions of lamination are temperatures of web and adhesive, tension of web, humidity of web, and adhesive conditions. Melted wax is supplied from a tank by a geared pump to die or coating station for the wax and hot melt lamination. Wet lamination combines two webs, one of which has wet adhesive on its surface. In dry lamination two webs are combined after drying water or solvent from the adhesives. For solventless lamination, 100% solid adhesives with no volatile components are used and drying is not necessary.

3. Sizing

Pigments or starch solution of low concentration can be applied on paper's surface by passing paper through two or more of rolls (called "size press"). To produce silicone coated baking paper, silicone coating of aqueous emulsion is applied on refined paper fibers at the size press. Silicone coating prohibits sticking of food to the paper during cooking and serving.

Internal sizing is the treatment of fiber slurry. Surface sizing is the addition of starch solution or other materials onto the paper surface.

4. Metallizing

In order to improve appearance and be a barrier to gas and light, a very thin layer of metal (usually aluminum) is deposited on a substrate surface in a vacuum. For satisfactory vacuum-metallized treatment, paper and paperboard should have less than 4% moisture content and be very smooth, and free from pores and voids. The surface of paper is usually treated with polyurethane, acrylic, or polyamide lacquer for proper metallizing.

IV. CLASSIFICATION OF PAPER AND PAPERBOARD

There is no definitive distinction between paper and paperboard, but relatively thick paper is called as a paperboard. Depending on raw materials, converting process, and surface properties, grades of paper and paperboard are classified into various kinds. Usage of paper and paperboard are classified into three categories: packaging, printing and writing, and wiping. In this chapter, packaging grades are discussed.

A. Properties and Test Methods

There are many grades of paper and paperboard depending on purposes. Required properties of paper and paperboard vary depending on production processes, converting processes, and final usage. The moisture content of paper is very important to decide paper quality in many aspects. Therefore properties are measured under standard conditions of temperature and relative humidity since they depend on equilibrium moisture contents.

Properties of paper and paperboard related to food packaging are thickness, grammage, tensile strength, tear strength, impact strength, barrier (vapor or gas barrier), capacity, curl, flexibility, stiffness, static and dynamic friction coefficient, heat sealing, vapor transmission rate, and so on. Chemical properties of food wrapping papers are important, and they must be free of harmful chemicals. Parchment paper for butter should have less than 3 ppm of copper content and 6 ppm of iron content for preventing off-flavor. Properties may be divided into physical, mechanical, strength, chemical, and optical properties.

Physical properties are basis weight, thickness, apparent density, smoothness, porosity, formation, curl, sizing, and printability. Basis weight is a mass of paper per unit area. Thickness of paper is measured as a caliper at the specific pressure as a distance between bottom and top plates where a paper is located. Because detailed contour of paper is not uniform nominal thickness is measured. Apparent density is calculated by basis weight divided by thickness, which is equivalent to mass divided by volume. Smoothness is a measure of how smooth a paper surface is. Smooth surface gives better printability because printing plate contact uniformly on smooth surface. Usually paper has two-sideness which means two sides of paper have different characteristics, because during papermaking process drainage of water occurred in one direction through wire. Top side (felt side) is smoother than bottom side (wire side). Paper structure has a lot of pores in it, and porosity of paper is determined by the degree of fiber refining and filler contents, because filler, small mineral particles, plugs in pores of paper. Porosity may be measured by how fast a specific volume of air can pass through paper structure. Formation is an extent of distribution uniformity of fibers and fillers throughout a paper. Uniformity of paper determines the quality of paper. Paper tends to curl depending on extent of difference in properties of top and bottom side. Paper requires a resistance to ink or liquid. Sizing is an extent of resistance to liquid penetration. Printability may be measured in uniformity of ink density in overall image, sharpness of image, and so on.

Papermaking process is continuous in travel direction that is called machine direction (MD), and perpendicular direction is called cross-machine direction (CD). More fibers tend to align in MD, so paper is stronger in MD and elongates more in CD. The ultimate force a specimen can endure when it is in tension is expressed in tensile strength. Tensile strength may be expressed as the breaking length, which is a length of paper when it is ripped, when a paper roll is unwound to a certain length due to its own weight of paper supported at one end. Burst strength of paper measures the amount of hydrostatic pressure to rupture a piece of paper. Stiffness measures the bending moment of specimen resisting bending. Fold endurance measures number of folding under a specific tensile force before breaking. Tear strength is determined by energy required to tear several sheet of paper at a fixed distance with initial tear.

Chemical contents may be analyzed by various methods of chemical analysis. Optical properties include color, brightness, opacity, and gloss.

For corrugated paperboard, burst strength, edgewise compression strength, flat crush test, pin adhesion, and puncture test are usually performed. For finished

corrugated box, puncture, compression strength, impact resistance, stiffness, and drop test are performed.

Recently the ISO (International Organization for Standardization) issued some global standards for pulp and paper. Each country adopts specific standards to measure the pulp, paper, and paperboard properties. Standard methods such as TAPPI (Technical Association of Pulp and Paper Industry (USA)), SCAN (Scandinavian Pulp, Paper and Board Testing Committee), PAPTAC (Pulp & Paper Technical Association of Canada), PITA (Paper Industry Technical Association), and APPITA (Technical Association of the Australian and New Zealand Pulp & Paper Industry) usually describe the definitions, specimen preparation, conditioning and measuring methods, testing equipment, and reporting method.

B. Functions and Usage of Paper and Paperboard for Food Packaging

Traditional purposes of paper and paperboard were printing and writing. Various packaging and wrapping usage are developed with a variety of characteristics these days. Life cycle of new products (turn-over) becomes shorter than before. There are too much versatile grades to describe here. Only representative paper and paperboard grades are described.

1. Kraft Paper

Bleached or unbleached Kraft pulp (KP), one of the major chemical pulps with high strength, is used in more than 80% for making Kraft paper. Kraft paper is used primarily as a wrapper or packaging material, and its other usage is grocer bags, envelopes, multiwall sacks, butchers wraps, waxed paper, and all types of specialty bags and sacks. Crepe paper is produced by reducing the speed of the press roll to increase elongation rate of 35–200% of its original length. Crepe paper is used for multiwall bags.

2. Greaseproof Paper

By refining chemical pulp severely, fibers bond closely and compactly, and there are few pores in its structure, scattering of light is decreased to have almost transparent appearance. Greaseproof paper is for protective packaging material having a resistance to fat penetration in packaging butter or fatty food with few pores. Greaseproof paper is suitable for lamination and coating with wax or lacquer to improve water resistance. After wax coating, it is used as a wrapping paper of potato chips, dried food, cookies, ice cream, and coffee.

3. Glassine Paper

By very severe calendaring of chemical pulp, paper becomes almost transparent. It has very smooth surface, high density, and low opacity. It also has good grease resistance and gas barrier properties. Transmission of moisture vapor can be enhanced by waxing, lacquering, or laminating the paper. It can be used as a protective wrapper for all kinds of foodstuffs and many purposes where its transparent feature is helpful.

4. Parchment Paper

It is produced by passing paper of cotton fibers or pure chemical pulp through sulfuric acid solution, washing thoroughly, and drying. It is odorless, tasteless, and has good grease resistance and wet strength. It is used for packaging butter, margarine, meat, poultry, and other food products. It can also be used for interleavers for the food (such as meat slice) because of its good releasing property.

5. Waxed Paper

Unsized or sized paper is impregnated or coated with molten wax to make waxed paper. It is used for wrapping or packaging bread and sandwiches, baking cups for cup cakes, and liners for cartons and cracker boxes. To make molten wax, microcrystalline wax and polyethylene are added to paraffin wax.

6. Tissue Paper

Low grammage thin paper is generally called tissue paper. Sometimes it is creped to enhance elongation and softness. It is used as a wrapping tissue, waxing tissue stock, fruit and vegetable wrapping tissue stock, and various specialty purposes. It can be transparent or semi-opaque.

7. Coated Paper

In order to get a high quality printing surface, coating materials such as pigments, adhesives, and additives are used. Clay, calcium carbonate, and titanium dioxide can be used for pigments. Starch, latex, or protein is used for adhesives. Waterproofing agents, plasticizers, rheology control agents, dispersants, defoamers, and dyes can be used as additives to perform specific purposes. Coated paper can be used for labels or bags, and it can be laminated with other material to give special features.

8. Paperboard

Relatively thick paper is called paperboard. Generally it has several layers of paper in whole structure. Properties of each layer of paper determine final properties of paperboard. Various pulps can be used for each layer to save production cost and enhance certain properties. It is classified as containerboard (which is used for corrugated boxes), boxboard (which is used to make cartons), and various paperboards.

9. Corrugated Board

Corrugated board consists of liner board on each side and corrugated medium in between. Various shapes of corrugation determine the compression strength and amount of cushion. Various structures may be produced depending on final usages and required strength. Single wall, double wall, and even triple wall structures may be produced.

V. MANUFACTURING AND USAGE OF PAPER CONTAINERS

A. Paper Bag and Paper Sack

Paper bag is used for packaging dried foods such as sugar or flour is packed in food company and protected during distribution channels of wholesales, and retail stores. Paper bag is also used in grocery stores or retail stores. Recently a high portion of paper bag usage is replaced by plastic bag. Paper sack is made of 2–6 plies of Kraft paper.

B. Paper Box

Paperboard of 0.3–1.1 mm is cut and punctured to produce a paper box. Paper box in folded shape is supplied to a food factory, and final shape of the paper box is produced during the packaging processes. Paper box is used for dried foods such as cookies and snacks in unit packaging. Puncture strength and strength at humid condition are required for frozen food or marine product packaging. Barrier properties and heat sealing properties may be provided by plastic lamination. Paper box is more expensive, but higher in puncture strength, than the corrugated box.

C. Corrugated Box

There are many shapes of corrugated board container. The container may be stored in folded shape for later use, and it is easy to handle for closing and opening. Waterproof properties may be provided for specific end use. This container is used for many purposes including food packaging to protect contained food from impact. Compression strength is required during loading and transportation of box piles. Depending on required burst strength and compression strength, proper shape of container should be provided. Much research is concentrated on reducing raw fibers and use of thinner board for slim packaging in order to reduce the box cost.

D. Liquid Container

For liquid containers, inside or both sides are coated with wax to provide water resistance. Laminated composite paper in pyramid, rectangular hexahedron, or various shapes is used for milk and fruit juice. It is usually used with an aspetic packaging system. PE layer of outside provide protection from water or abrasion. Paper of inside provides strength to support the content.

E. Component Can or Drum

Paper is wound in spiral or normal direction to make a component can or drum that is cheap and easy to discard. It is used to protect salt, pepper, powdered hot pepper, powder, spice, cookies, dried snack, biscuit, doughnut, and so on. Bottom and top are made of metal, plastic, or paperboard. Main body can be also laminated in aluminum foil, plastic film, or high strength paperboard for higher barrier properties and strength. Inside the component can or drum may consist of parchment, waxed paper, aluminum foil, glassine paper, or coated paper to improve protection of contents. Paperboard of 0.25–0.5 mm is used for this purpose.

F. Paper Mould Container

Paper mould is produced in a similar manner as paper. Mechanical pulp, chemical pulp, and recycled pulp are used as raw materials. Screen mould is used to drain water by pressurized extrusion or suction forming method. The container is used for egg tray, shock absorbing packaging, vegetable, and high quality liquor.

VI. NEW TRENDS

Regulations for packaging material are now stricter than ever before. By improving printability for more attractive appearance, increasing strength for better protection and convenient handling, developing efficient recycling method for fewer environmental problems, and reducing raw material for less amount of solid waste, paper and paperboard packaging can be more competent.

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133 Frozen Food Packaging

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CONTENTS

I. FUNCTIONS OF PACKAGING

The basic functions of the package are to contain the food, protect the food, provide convenience, and convey product information. The package protects the food against physical, chemical, and biological damages. It also acts as a physical barrier to moisture, oxygen, volatile compounds, and microorganisms that are detrimental to the food. The package provides the consumer with convenient features such as microwavability, resealability, single serving, and ease of use. The package conveys useful information such as product contents, nutritional values, and preparation instructions. All these functions are applicable to the packaging of frozen foods [1].

The food package can function best when integrated into a food packaging system, which involves certain physical components and operations. The major physical components are the food, the package, and the environment (Figure 133.1). It is useful to divide the environment into internal and external. The internal environment refers

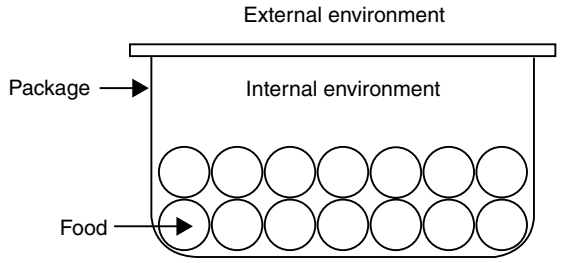

FIGURE 133.1 Physical components of food packaging system.

to the conditions inside the package, which contains the food product and in many cases some air space (also known as headspace). The external environment refers to the conditions outside the package, and it depends on the storage and distribution of the food package. The operations are the manufacturing, distribution, and disposal of the food package. In designing the food packaging system, these physical components and operations must be considered to prevent over-packaging or under-packaging, which results in higher costs, lower quality and in some cases, health risks.

There are several requirements in the selection of packaging materials for frozen foods: temperature stability, barrier properties, thermal insulation properties, consumer appeal, and machine compatibility [2]. Temperature stability is necessary since the packaging materials must be able to withstand the abuses encountered over a broad range of temperatures, including freezer temperatures during transportation and storage as well as high temperatures during the heating of the food package in the microwave or conventional oven. Barrier properties are necessary to minimize deteriorative effects of moisture, oxygen, and light to the food product. Thermal insulation helps maintain low temperatures for frozen foods during distribution, and minimize temperature fluctuations which may cause degradation of the products. Consumer appeal is necessary for successful marketing of the products; the packaging materials should allow high quality printing and graphics. Machine compatibility is necessary to ensure that the packaging materials are compatible with low cost, high speed machineries.

Frozen food packages are typically made using carton machines, form-fill-seal machines, and pouch-forming machines. The constructions and operations of these machines may be obtained from the manufacturers and the literature [3].

II. DETERIORATION MODES OF FROZEN FOODS

In addition to mechanical damages, frozen food products can also fail due to several deterioration modes. The most important deterioration modes of frozen foods are related to the transport of moisture. The water molecules in ice exert a vapor pressure which increases with temperature. Water molecules tend to move from high concentration to low concentration.

Figure 133.2 illustrates the various transport mechanisms of water molecules (small circles in figure) in a frozen food package. Diffusion of water can occur within the food if a concentration gradient exists (in most cases, in the direction from center to surface). Sublimation, evaporation of water from ice to vapor, can occur at the food surface. Precipitation of water vapor as ice crystals can occur on the food surface or on the interior package surface. Permeation is the transport of water vapor across the package

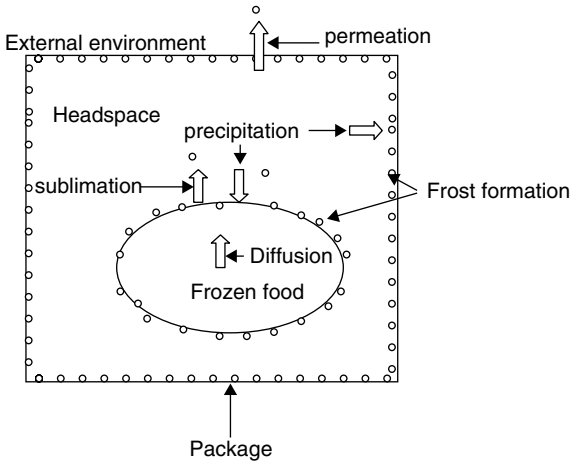

FIGURE 133.2 Transport mechanisms of water molecules.

walls, and the water vapor transmission rate (WVTR) is determined by the permeability of the package. The transport of water molecules can result in dehydration and frost formation, two of the major deterioration modes of frozen foods. In addition to water molecules, the transports of oxygen, flavor, and odor compounds are also important to frozen foods.

Dehydration in frozen foods, also known as freezer burn or desiccation, is the moisture loss at the product surface due to sublimation of ice. The moisture loss results in a drier product surface and a concentration gradient which cause water molecules to diffuse from the food center to its surface. Dehydration is a major deterioration mode in frozen food since it reduces product weight and adversely changes product appearance, texture, and taste. For example, when proteins in meat, poultry, and fish products become irreversibly dehydrated, the tissues become dry and tough. These products frequently contain considerable amount of fats and oils, and dehydration can make these fats and oils more susceptible to oxidation by opening up the tissues and thus making more surface areas available for oxidation.

If the food product is unprotected (i.e., without package), the rate of moisture loss to the external environment is rapid. To retard moisture loss, protecting the product by a good moisture barrier package is necessary. In addition, the package should also have good tensile, tear, and burst strength at low temperatures; otherwise, package damages (such as holes or cuts) can occur and cripple the protective function of the package.

Frost formation is a phenomenon by which water vapor precipitates as frost on the food surface or on the interior surface of the package. Frost formation contributes to the problem of freezer burn since moisture is removed from the product, and it also makes the package less appealing to the consumer. A major factor which affects frost formation is headspace volume: in the

presence of headspace, moisture loss occurs from the food surface to the headspace through sublimation, even when the food is protected by a good moisture barrier package. It is the water vapor in the headspace which is responsible for frost formation. Therefore, an effective packaging technique is to tightly wrap the food product to eliminate the headspace and its water. Another major factor which affects frost formation is temperature fluctuations. Since vapor pressure is temperature dependent, any temperature fluctuations can result in different vapor pressures at different locations, and thus a concentration gradient is created which tends to accelerate the rates of sublimation and precipitation.

Oxidation is another deterioration mode for frozen foods. Although oxidation occurs slowly at freezer temperatures, it remains a problem since frozen foods are often stored for prolonged periods of time and oxygen is more soluble in food at lower temperatures. Oxidative reactions can result in rancidity, off-flavor, and pigment discoloration in frozen meat and seafood products. In general, oxidation reactions accelerate with increasing amounts of oxygen present, but there are exceptions. Different foods have different susceptibility to oxidation; for example, pork and poultry are more susceptible than beef and veal to oxygen. To protect oxygen sensitive frozen foods, the package should have low oxygen permeability.

Flavor loss is also a deterioration mode for frozen foods. Some flavor compounds are volatile and exert considerable vapor pressures even at freezer temperatures. The alternation of flavor profile due to flavor loss may cause the consumer to reject the product. Odor pickup is also a deterioration mode. Trimethylamine, a compound responsible for the objectionable "fishy" flavor, is volatile at temperatures as low as $-23°C$. Therefore, the package should also have low permeability to flavor and odor compounds.

It is clear from the above discussion that packaging is vital for protecting frozen foods. Understanding the deterioration modes can help to develop packaging strategies to extend shelf life of the products.

III. PACKAGING MATERIALS

Packaging materials include paper, plastics, glass, and metal. For packaging frozen foods, paper and plastics are most commonly used, metal is occasionally used (for example, as metal ends in composite cans for frozen concentrated juice), and glass is seldom used. In some package designs, combinations of paper and plastics are used: for example, a frozen meal may be placed inside a plastic tray with a lid, and the tray is placed inside a paperboard carton. The major roles of the package are to protect the products against mechanical damages and deteriorative effects of gas and vapor at low temperatures.

A. Paper and Paperboard

Paper and paperboard are mainly used to provide structural support and protect the frozen food products from mechanical damages. These materials are sometimes used as a light barrier, but their moisture and oxygen barrier properties are poor. These materials are made of wood fibers containing cellulose, hemicellulose, and polymeric residues. They have the advantages of good structural strength, low cost, recyclability, and good printability.

There are several types of paper used for frozen food packaging. Kraft paper is a coarse paper, which may be used in unbleached or bleached form. Greaseproof paper and glassine paper provide good protection against oil and grease. Waxed paper is a good moisture barrier and can serve as a heat-sealable layer. Paper is sometimes used as an insert to separate individual items (such as beef patties) within the same package so that those items do not stick together due to freezing.

Paperboard is commonly used for individual packages and secondary packages (e.g., a box which contains several individual packages). The waxed cartonboard, with a moisture-proof regenerated cellulose film overwrap, was used as earlier packaging for frozen vegetables and fruits [4]. There are two basic folding paperboard designs: skillet and three-flap closed carton [3]. Bleached Kraft carton is often used in packaging for the frozen foods due to its strength and good appearance. To improve moisture and oxygen barrier, paperboard is sometimes coated or laminated with plastics or aluminum. To improve appearance and printing quality, the paperboard is sometimes coated with clay and other minerals. It is quite common that a plastic bag containing a frozen food product is placed inside a paperboard carton. In this case, the plastic bag provides the gas and vapor protection, and the carton provides the structural support and mechanical protection.

B. Plastics

Many frozen food products are packaged in plastics for moisture, oxygen, flavor, and odor protection. Plastics consist mainly of synthetic polymers and small amounts of additives (e.g., antioxidant and pigment) which can be cast, extruded, and molded into various shapes such as films, sheets, and containers. Most polymers used in food-packaging plastics have molecular weight between 50,000 and 150,000. Plastics provide a wide range of properties relating to mechanical strength, gas barrier, printability, heat performance, and machine performance [5].

Plastics have a wide range of gas and vapor barrier properties, which offer many choices for different package requirements. The gas barrier properties of a plastic packaging material are usually quantified in terms of permeability: the lower the permeability, the better the gas barrier. Permeability is a function of the plastic material,

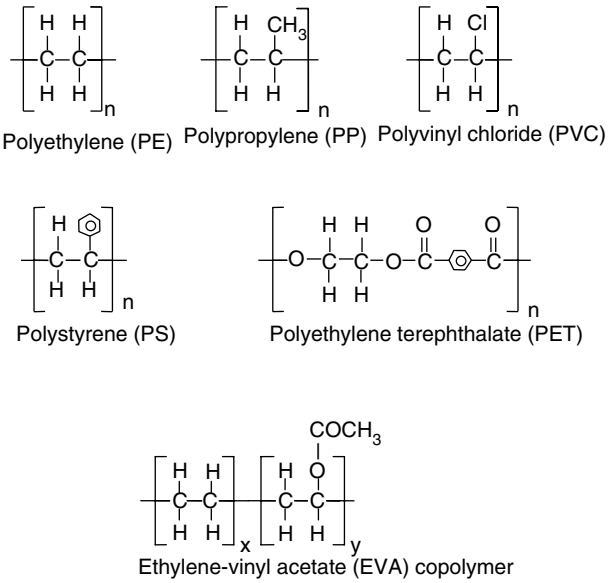

FIGURE 133.3 Chemical structures of some common food-packing polymers.

permeant gas, temperature, and in some cases relative humidity.

In selecting plastic materials for frozen foods, it is necessary to select those which remain flexible at freezer temperatures. Abuse testing (usually includes a combination of shipping, vibration, compression, and drop tests) should be conducted to ensure the package is not brittle and loses its product integrity at low temperatures.

The following is a general discussion of the basic food packaging polymers (Figure 133.3). These polymers are mostly used as bags or pouches for packaging frozen foods. Relatively thick films are used to protect against frozen food products (such as crab legs) which have cutting edges or sharp points. Some packaging films are coextruded or laminated multilayer films consisting of several layers of different polymers. By wisely selecting different polymers, multilayer films can offer the advantages of lower cost and/or better performance.

1. Polyethylene (PE)

PE is a commonly used polymer as plastics bags for individually quick frozen (IQF) foods (e.g., vegetables, fruits, shellfish) [2]. The advantages of PE are low cost, easy processing, and good mechanical and printing properties. PE is usually classified into high-density polyethylene (HDPE), low-density polyethylene (LDPE), and linear low density polyethylene (LLDPE). These classifications differ in density, chain branching, and crystallinity.

HDPE is a linear polymer with relatively few side chains. Its density is typically between 0.94 and 0.97 g/cm^3. It has a higher melting point than LDPE, (135°C versus 110°C typically), and thus it is more suitable for high temperature application such as boil-in-bag applications [2]. HDPE is used in films and containers for frozen foods.

LDPE normally has a density range of 0.91 to 0.93 g/cm^3. It is a branched polymer with many long side chains. LDPE is used mostly as film, an adhesive in multilayer structures, or waterproof and greaseproof coatings for paperboard packaging materials. The film made from LDPE has the advantages of low cost, softness, flexibility, stretchiness, clarity, and heat sealability.

LLDPE is a copolymer with many short side chains. It has LDPE's clarity and heat sealability, as well as HDPE's strength and toughness. Therefore, LLDPE has substituted LDPE in many food-packaging applications.

2. Polypropylene (PP)

PP has the lowest density (~0.9) among all major plastics. It has higher tensile strength, stiffness, and hardness than PE. PP cast film is clearer than PE film and is used in applications where transparency is required.

3. Polyvinyl Chloride (PVC)

PVC is a clear, hard polymer which is often modified with plasticizers (organic liquids of low volatility). Plasticized PVC films are limp, tacky, and stretchable, and the films are commonly used for packaging meat. PVC has better clarity, oil resistance, and barrier properties than those of HDPE.

4. Polystyrene (PS)

PS is a clear, hard, and low impact resistance polymer. High-impact polystyrene (HIPS) is formed by modifying PS with elastomeric molecules such as butadiene. HIPS is more suitable for freezer temperature applications because it has significantly higher impact resistance. Expanded PS (EPS) of various bulk densities are manufactured by adding foaming agents in the extrusion process. Some frozen seafood products (such as lobster tails) are vacuum-skinned down on an EPS tray with a coextruded film.

5. Polyethylene Terephthalate (PET)

PET is the major polyester used in food packaging which can tolerate freezer temperatures and high temperatures. It also provides good resistance to grease and moisture. Biaxial orientation of PET film can improve its clarity and mechanical properties. The crystallized polyethylene terephthalate (CPET) can withstand high temperature up to 220°C, and CPET food trays are suitable for use in microwave and conventional dual ovens.

6. Ethylene-Vinyl Acetate (EVA)

EVA is a copolymer containing 2 to 18% vinyl acetate. It has long chains of ethylene hydrocarbons with acetate

groups randomly throughout the chains. EVA film is tough and tacky, and thus it is often blended with polyethylene to improve sealability, stress resistance, and flex cracking resistance. EVA can be used as bags for frozen foods, and is coextruded with Surlyn ionomer and LDPE for the application in skin packaging.

C. Barrier Properties of Plastics

For foods that are sensitive to moisture or oxygen, gas barrier protection is the major function of the package in providing adequate shelf life, the time period during which the food maintains acceptable quality. Controlling moisture loss is important for frozen foods because moisture loss (sublimation of ice) results in freezer burn and discoloration of the product. Oxidative reaction is also important for some foods even at freezer temperatures.

Transport of gases between the external environment and the headspace through the package can occur by means of leakage and/or permeation. For a properly sealed package in which leakage is not a problem, permeation is the major mechanism of gaseous transport.

Gas permeation is an important consideration in packaging foods with plastics, since food packaging plastics are permeable to moisture, oxygen, carbon dioxide, nitrogen, and other gases (including those that can cause off-odor problems). The gas permeation rate of most interest for frozen foods is water vapor transmission rate (WVTR).

WVTR may be defined as the amount of water vapor transmitted through the package per day [g H_2O/(day(package))] under specific conditions (usually 38°C, 90% relative humidity). WVTR can also be expressed more generally in terms of the amount of water transmitted through 100 in^2 package area per day [g H_2O/(day (100 in^2)] where the package surface area is not specified.

If WVTR is assumed a constant, the shelf life (t_s) can be estimated by

$$t_s = \frac{H_2O,\ max}{WVTR} \tag{133.1}$$

where $H_2O_{,max}$ is maximum allowable water (g H_2O) which can be determined by sensory evaluation. In practice, WVTR is not a constant but decreases with time, because headspace relative humidity decreases and concentration gradient decreases with time. Thus the actual shelf is slightly higher than that predicted by Equation (133.1).

For products that are oxygen sensitive, the oxygen transmission rate (OTR) of a plastic package from the external environment to the headspace can be expressed by

$$OTR = \frac{\bar{P}A}{L}(P_e - P_i) \tag{133.2}$$

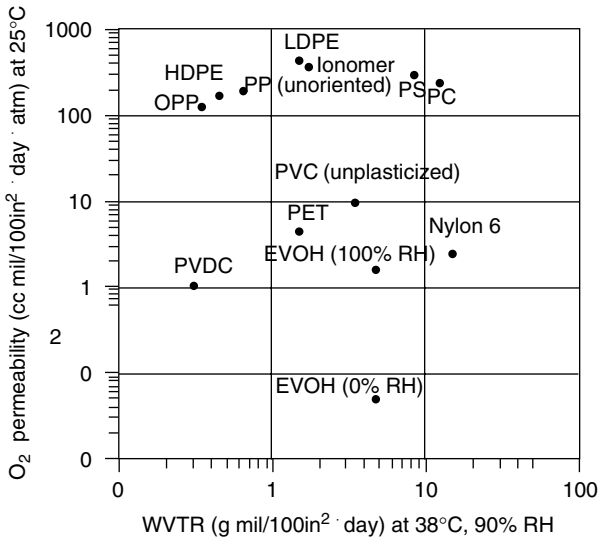

FIGURE 133.4 Gas barrier properties of common food packaging polymers.

where OTR is oxygen transmission rate, cc O_2/day; P is oxygen permeability, cc O_2 (mil)/(100 in^2 (day (atm); A is surface area of package, in^2; L is thickness of package, mil; P_e is oxygen partial pressure in external environment, atm; and P_i is oxygen partial pressure in headspace, atm. The shelf life (t_s) can be calculated using

$$t_s = \frac{O_{2,\ max}}{OTR} \tag{133.3}$$

where t_s is shelf life, day, and $O_{2,max}$ is maximum allowable oxygen, cc O_2. It has assumed in Equations (133.1) and (133.3) that permeation through the package (not the rate of deterioration) is the major factor limiting the shelf life. This is a reasonable assumption when packaging materials of low permeability are used. Combining Equation (133.2) with Equation (133.3) gives

$$t_s = \frac{O_{2,max}\,L}{\bar{P}A\,(P_e - P_i)} \tag{133.4}$$

Equations (133.1) through (133.4) can be used to evaluate many what-if scenarios. For example, according to Equation (133.1), if the thickness of the package is decreased by 20% and the surface area is increased by 20%, then the above equation predicts that the shelf life will be decreased by 33.3%.

An obstacle is that permeability values of packaging polymers are generally not available at freezer temperatures. It is mostly due to the time and cost necessary for measuring permeability at low temperatures. However, literature permeability values are available at higher temperatures, and Figure 133.4 shows the relationship between oxygen permeability and WVTR for some commonly used food packaging plastics [5]. In practice, one can use literature data as

reference when comparing different polymers; better still, one can measure the permeability of interest.

IV. PACKAGING TECHNOLOGIES

A. Vacuum Packaging and Modified Atmosphere Packaging

As mentioned earlier, the headspace is an important factor which affects several deterioration modes of frozen foods. The water vapor in the headspace can cause frost formation, and the oxygen in the headspace can cause oxidation. A technique to control the headspace is vacuum packaging, which simply involves removing air from the headspace. This technique has been shown to help maintain the quality of various frozen products including pizza, seafood, beef, and pork [6]. There are two forms of vacuum packaging depending on rigidity of the package.

The first form of vacuum packaging involves a rigid package (e.g., glass jar) or a semi-rigid package (e.g., plastic container) in which most of the air is evacuated, but a headspace still remains in the package. The removal of air typically reduces the oxygen level in the headspace to as low as 1%, which significantly helps to reduce the problem of oxidation. However, frost formation and freezer burn are still problematic since the headspace exists.

The second form of vacuum packaging involves a flexible package (e.g., a plastic pouch) in which not only the oxygen is removed, but also the headspace is eliminated. Thus both oxidation and frost formation are controlled. This form is also known as vacuum skin packaging, since the food is tightly wrapped by the package. The mechanical stress created by the vacuum also helps to remove air pockets inside the product. This technique has been widely used to package frozen meat and seafood products including meat balls, clam strips, lobster tails, salmon, and farmed rainbow trout [7]. Several types of materials are used for vacuum skin packaging such as a blend of Surlyn ionomer resin with low density polyethylene (LDPE) and ethylene-vinyl acetate (EVA) [8].

Modified atmosphere packaging (MAP) is a technique which involves replacing air (especially its oxygen) in the headspace by other gases such as nitrogen and carbon dioxide. Nitrogen is used as inert gas filler, and carbon dioxide is used because of its ability to inhibit microbial growth. MAP is seldom used for frozen foods because vacuum packaging is often a better alternative in terms of cost (no gas required) and effectiveness (no frost formation). However, MAP is used in some refrigerated and shelf stable food products where the benefit of carbon dioxide is justified or the products cannot withstand the mechanical stress of vacuum packaging.

Both vacuum packaging and MAP requires the use of gas barrier packaging materials; otherwise, the vacuum or the modified atmosphere cannot be maintained for a prolonged period of time. Vacuum equipment or gas flushing equipment is also required.

B. Time-Temperature Indicator (TTI)

It is critical to maintain frozen food products at constant low temperatures. Temperature abuses due to improper handling may result in lower food quality and, even worse, microbial growth if the abuse is severe. Monitoring temperature is a critical control point for frozen foods in designing a HACCP (hazard analysis critical control points) program. While temperature recorders (such as handheld electronic temperature monitoring devices) are often placed in storage rooms and trucks to monitor temperatures, these recorders are not attached directly to the food packages.

A time-temperature indicator (TTI) is a small self-adhesive label that can be attached to a food package for monitoring the package temperatures from the time of production to the time of consumption. The TTI helps to determine whether the product is still fresh at point-of-purchase and at home by providing the consumer with a visual indication.

An important aspect of TTI is the visual indication system which typically involves color change or size change associated with diffusion, chemical reaction, or enzymatic reaction. The visual indication (color change or size change) is correlated to the temperatures or time-temperature history. In order to use the TTI to indicate the shelf life of the product, the kinetics of the TTI and the kinetics of the food must be known, and it is also necessary to match the activation energies of the TTI and food deterioration reaction. The technical details are beyond the scope this chapter but they can be found elsewhere [9].

There are two common types of TTIs. The first type is the temperature limit indicator (or threshold indicator) which triggers an indication when a certain temperature limit is exceeded. For example, if the upper limit is set at $-3°C$, the TTI will trigger a color indication once the temperature limit is exceeded. The second type is the time-temperature integrator which triggers an indication when the time-temperature limit is exceeded. For example, if the indicator is set at 60 days and $-18°C$, the TTI will trigger a color indication once an equivalent of this time-temperature history is exceeded. The equivalent time-temperature history is estimated from the kinetics of the TTI.

Presently several TTIs are available in the market. The LifeLines Fresh-Check® is based on polymerization reaction which responds to cumulative exposure to temperature. The 3M Monitor Mark® is based on dye diffusion which is activated by pulling out an activation strip. Upon exposure to temperatures above the threshold, the activated indicator's window irreversibly turns blue, warning that product quality testing should be performed. The Vitsab® TTI is based on enzymatic color change. More

information about these TTIs can be found on the company websites.

V. CONCLUDING REMARKS

Packaging is essential for protecting frozen foods from mechanical damage, moisture loss, flavor loss, odor pickup, and oxidation. Reducing headspace and air pockets inside of the package by vacuum packaging will help minimize frost formation.

One needs to analyze both the distribution environment and stability of the product in order to formulate the packaging requirements for a specific product. In analysis of food stability, the dominant deterioration modes should be identified. Their kinetics and acceptable limits should also be determined. Once the food stability and required shelf life is known, package requirements for protecting the product from the dominant deterioration modes can be decided.

It is helpful to work with the packaging material supplier and packaging machine manufacturer to design and manufacture the package according to the requirements decided. Other factors including those described earlier in this chapter should also be considered in the package design process.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Frozen foods packaging," by K. L. Yam, H. Zhao, C. C. Lai. In *Handbook of Frozen Foods*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.

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134 Thermal Processing of Packaged Foods

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CONTENTS

I. INTRODUCTION

The term thermal processing has been widely used in food industry to describe the process of heating, holding, and cooling that are required to produce microbiologically safe packaged food products of acceptable quality [1]. Sterilization is a type of thermal processing designed for complete elimination of both spores and vegetable cells. Commercial sterilization does not require complete microbial elimination, but the degree of elimination has to be regulated and optimized under accepted criteria to ensure product safety. Pasteurization eliminates only vegetable cells and thus does not provide shelf-stable food products without other preserving processes such as refrigeration.

A general procedure for evaluating a thermal process of a food package is as follows. First, the heat penetration in the food during the thermal process is determined by measuring the temperature of the food at a specific point (often the slowest heating point) as a function of processing time. Heat penetration parameters such as *f*- and *j*-values may then be determined from this time-temperature profile. Second, a target microorganism is selected based

on the pH, water activity, and other considerations of the food. The thermal destruction properties of the target microorganism including decimal reduction time (D-value) and thermal resistance constant (z-value) may then be obtained from experiments or the literature. Based on these thermal destruction properties and other considerations such as initial microbial load, the minimum heating time (F_r) at a given reference temperature that is required to reduce the target microorganism population to a stated safe standard is determined. Third, the sterilizing value (F-value) of the thermal process is determined. This is the heating time at the reference temperature yielding a microbial lethal effect equivalent to that of the entire actual processing. For commercial thermal processing, the F-value is often set significantly higher than the F_r value to ensure microbiologically stable food products [1].

II. HEAT PENETRATION IN FOOD DURING THERMAL PROCESSING

Figure 134.1 shows typical temperature profiles of heating medium and the slowest heating point of a packaged food during a batch thermal processing. T_{hm} is the holding temperature of heating medium, T_0 is the initial temperature of food, T_1 is the temperature of the slowest heating point at the end of heating phase, and t_{cu} is the come-up time. From these profiles, heat penetration curves of the slowest heating point for the heating and cooling phases may be constructed.

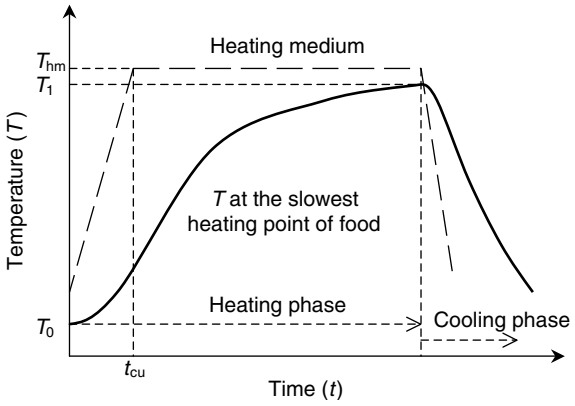

FIGURE 134.1 Typical temperature profiles in a batch thermal processing.

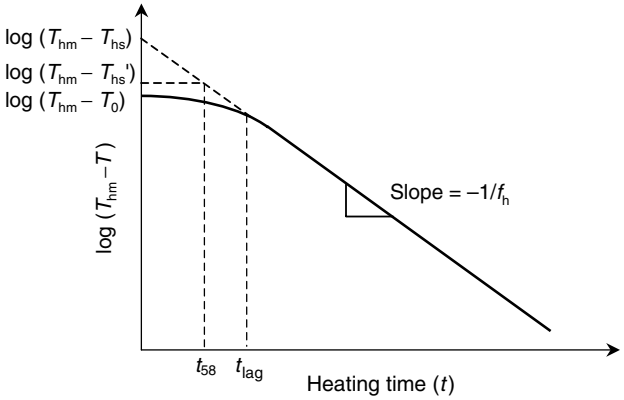

FIGURE 134.2 Heat penetration curve for heating phase.

A. Estimation of Heat Penetration Parameters

For the heating phase, the heat penetration curve is constructed by plotting $\log(T_{hm} - T)$ versus heating time. See Figure 134.2 for the symbols used in the following discussion.

The lag factor for heating phase (j_h) is defined as:

$$j_h = \frac{T_{hm} - T_{hs}}{T_{hm} - T_0} \tag{134.1}$$

where T_{hs} is pseudo-initial temperature obtained by extrapolating the linear line to time zero. The slow come-up of the retort is responsible for part of the lag time (t_{lag}). A widely accepted method to compensate for this come-up effect is to determine a new zero time at 58% of the come-up time (t_{58}) [1, 2]. That is, 42% of the come-up time is added to the process time at T_{hm}. With the new zero time, the j_h-value is redefined as:

$$j_h = \frac{T_{hm} - T_{hs'}}{T_{hm} - T_0} \tag{134.2}$$

where $T_{hs'}$ is pseudo-initial temperature obtained by extrapolating the linear line to time t_{58}. With the j_h-value, the equation for the linear line (for $t > t_{lag}$) can be written as:

$$\log(T_{hm} - T) = -\frac{t}{f_h} + \log[j_h(T_{hm} - T_0)] \tag{134.3}$$

or

$$T_{hm} - T = j_h(T_{hm} - T_0)10^{-\frac{t}{f_h}} \tag{134.4}$$

For cooling phase, plotting $\log(T - T_{cm})$ versus cooling time also yields a negative sloped line with initial curvilinear portion, where T_{cm} is holding temperature of cooling medium. The lag factor for cooling phase (j_c) is defined as:

$$j_c = \frac{T_{cs} - T_{cm}}{T_1 - T_{cm}} \tag{134.5}$$

where T_{cs} is pseudo-end temperature of heating phase obtained by extrapolating the line to the end time of heating phase. The equation for straight line (for $t > t_{lag}$ of cooling phase) can be expressed as:

$$\log(T - T_{cm}) = -\frac{t}{f_c} + \log[j_c(T_1 - T_{cm})] \tag{134.6}$$

or

$$T - T_{cm} = j_c(T_1 - T_{cm})10^{-\frac{t}{f_c}} \tag{134.7}$$

The next section will show that theoretically the *j*-value depends on the location in the food, at which the temperature is measured, when conduction is the main heat transfer mechanism in the food, and the *f*-value depends on the thermal properties and the size of the food.

B. Physical Meanings of f- and j-Values

For a theoretical illustration of the physical meanings of the heat penetration Equations (134.3) and (134.6) as well as the *j*- and *f*-values, two cases of thermal processing are often considered. The first case is when the food is liquid-like and convection is mostly responsible for the heat transfer within the food during thermal processing. The second case is when the food is solid-like and conduction is the main mechanism of heat transfer within the food. The heat transfer resistance due to the packaging material is not taken into account, since the material is assumed to be very thin and have much larger thermal conductivity than that of the food.

1. Convection-Dependent Thermal Processing

If the food being heated or cooled is liquid-like and sufficient convective heat transfer occurs, the temperature gradient within the food may be ignored. Due to the uniform internal temperature, this convection-dependent thermal processing can be analyzed using the lumped capacity method or Newtonian heating or cooling method, which describes the time-temperature history of a solid object when the Biot number (*Bi*) of heat transfer is very small (often less than 0.1) [3]. The mathematical model developed for this method, known as the Schultz-Olson model [1], had been derived by equating the rate of change in internal energy of the food and the rate of heat transfer between the medium and the food. The heat balance equation for heating phase is:

$$\rho V c_p \frac{dT}{dt} = UA(T_{hm} - T) \tag{134.8}$$

where ρ is the density of the liquid food (kg m^{-3}), V is the volume of the food (m^3), c_p is the specific heat of the food at constant pressure (J kg^{-1} K^{-1}), U is the overall heat transfer coefficient at the internal side of the package (W m^{-2} K^{-1}), and A is the internal surface area of the package (m^2). By integrating Equation (134.8) from $t = 0$ to t and $T = T_0$ to T, the Schultz-Olson model for heating phase is obtained:

$$\log(T_{hm} - T) = -\frac{0.434UAt}{\rho V c_p} + \log(T_{hm} - T_0) \tag{134.9}$$

Comparing this model with Equation (134.3), one can obtain theoretical expressions of j_h and f_h for the convection-dependent thermal processing:

$$j_h = 1 \tag{134.10}$$

$$f_h = \frac{2.303\rho V c_p}{UA} \tag{134.11}$$

Equation (134.11) shows that f_h is proportional to the volume and the specific heat of the food but inversely proportional to the overall heat transfer coefficient and the internal surface area of the package. In practice, the value of f_h is smaller than the theoretical value during initial heating, since the initial large temperature difference between the medium and the food yields high U value, and increases gradually to constant theoretical value. Due to the initial small f_h-value, the actual j_h-value is somewhat less than the theoretical value of 1. The meanings of j_c- and f_c-values can be illustrated in a similar manner.

2. Conduction-Dependent Thermal Processing

If a solid food is processed thermally, conduction controls the heat transfer within the food. As an illustration, one may assume a cylindrical food package of radius a and length l, exposed to a constant temperature environment with infinite surface heat transfer coefficient (i.e., infinite *Bi*) and uniform initial temperature. Analytical solution describing the time-temperature history of the cylindrical food during heating phase is found in Cowell and Evans [4] or elsewhere:

$$\frac{T_{hm} - T}{T_{hm} - T_0} = \frac{8}{\pi} \sum_{k=1}^{\infty} \sum_{n=1}^{\infty} \frac{J_0(P_k \rho)}{P_k J_1(P_k)} \times \frac{\sin[(2n-1)\pi\xi]}{2n-1} e^{-AB} \tag{134.12}$$

and

$$\rho = \frac{r}{a}$$

$$\xi = \frac{y}{l}$$

$$A = \left(\frac{P_k}{m}\right)^2 + (2n-1)^2\pi^2, \; m = \frac{a}{l}$$

$$B = \frac{\alpha t}{l^2}$$

where J_0 and J_1 are Bessel functions of the first kind of order zero and one, respectively; P_k is the root of Bessel

functions; r and y are the coordinates of radial direction and length-direction, respectively; and α is the thermal diffusivity of the food. For a large value of B, the first-term approximation of Equation (134.12) may be used:

$$\frac{T_{hm} - T}{T_{hm} - T_0} = \frac{8}{\pi} \frac{J_0(P_1\rho)}{P_1 J_1(P_1)} \sin(\pi\xi) e^{-A_{11}B} \qquad (134.13)$$

where

$$A_{11} = \left(\frac{P_1}{m}\right)^2 + \pi^2 \quad \text{and} \quad P_1 = 2.4048$$

Comparing Equation (134.13) to Equation (134.3), theoretical expressions of j_h and f_h for the conduction-dependent thermal processing can be obtained:

$$j_h = \frac{8}{\pi} \frac{J_0(P_1\rho)}{P_1 J_1(P_1)} \sin(\pi\xi) \qquad (134.14)$$

$$f_h = \frac{2.303}{\alpha\left[\left(\frac{P_1}{a}\right)^2 + \left(\frac{\pi}{l}\right)^2\right]} \qquad (134.15)$$

Equation (134.14) shows that j_h depends on the position in the food, at which the temperature is measured, regardless of the size and the type of the food. At center, the theoretical value of j_h is 2.0397 since $\rho = 0$ and $\xi = 0.5$. Equation (134.15) shows that f_h depends on the thermal diffusivity and the size of the food, but it is the same at any location in the food. The meanings of j_c- and f_c-values can be also illustrated in a similar manner.

III. EVALUATION OF THERMAL PROCESSING

A thermal process should be carefully evaluated to ensure microbiologically safe food products. In most cases, the evaluation requires either of the followings: (1) determination of process sterilizing value (F-value) for a given processing time and a heat penetration curve, or (2) determination of a proper processing time required for a target F-value [1, 5].

A. Sterilizing Value

The F-value is defined as the heating time at a reference temperature, which can yield the lethal effect equivalent to that of the actual process at a critical location, often the slowest heating point. For example, if the reference temperature is 121.1°C and the determined F-value is 3 min for a thermal process, then this means that the process can inactivate the target microorganism as much as the 3 min-heating at 121.1°C at the location. By determining the F-value, different thermal processes can be compared for their lethality.

To determine the F-value, the lethal rate (L) needs to be defined:

$$L = \frac{F_r}{F_T} = \frac{D_r}{D_T} = 10^{\left(\frac{T - T_r}{z}\right)} \qquad (134.16)$$

where F_r and F_T are minimum heating times required for reducing the population of target microorganism at the critical location to a stated safe standard value at a reference temperature T_r and at a certain temperature T, respectively. And D_r and D_T are D-values for the target microorganism at T_r and T, respectively. The fundamental equation for determining F-values is given by:

$$F = \sum_i \left(\frac{F_r}{F_T}\right)_i t_i = \int_0^t L\,dt = \int_0^t 10^{\left(\frac{T - T_r}{z}\right)} dt \qquad (134.17)$$

where t_i is the actual heating time at a certain temperature T, and t is the total processing time. Various methods developed for integrating Equation (134.17) is discussed in Section IV.

The reference temperature T_r is decided depending on the purpose of thermal processing. For sterilization, the value of T_r is in the range 115–130°C. For example, in canning industry, 121.1°C (250°F) is often used. For pasteurization, the range of T_r is 60–100°C. For example, 60°C and 72°C are suggested for beer and milk industries, respectively [2, 6].

B. Guide to Sterilizing Value

Viability of microorganisms depends on various factors such as the type of microorganism, initial microbial load, the pattern and the stage of cell growth, processing conditions, operating procedure, physicochemical properties of food, etc. While F-value should be determined and validated for each food process, some useful guides are available. Holdsworth [1] provides general principles for selecting F-values and summarizes the recommended F-values for meat, vegetable, fish, poultry, and other food products based on various sources including Alstrand and Ecklund [7], Townsend et al. [8], NFPA [9, 10], Hersom and Hulland [11], Codex Alimentarius Commission [12], and reports from UK.

C. Mass Average Sterilizing Value

The concept of mass average sterilizing value (F_s-value) or integrated F-value was introduced by Stumbo [13, 14] for evaluating the lethality of entire food, not just at the slowest heating point. The F_s-value is useful when the location at which the temperature is measured, mostly the geometrical center of the food, is not the slowest heating point. According to Holdsworth [1], the phenomenon is not observed for the domestic sizes of cans, but for large cans the slowest heating location could be a toroidal ring around the center. However, using the F_s-value is not quite

appropriate in evaluating the lethal effect of a thermal processing, since the microbial safety of a food product always depends on the population of a target microorganism at the slowest heating point. A more useful application of the F_s-value can be the quality optimization of thermally processed foods [1, 15–18]. The thermal degradation of heat-sensitive food components, which are distributed throughout the food product, can be evaluated using the F_s-value.

IV. METHODS FOR DETERMINING STERILIZING VALUE

A. General Method

Once the time-temperature profile of the slowest heating point is obtained experimentally or theoretically, the lethal rate (L) at the location can be calculated using Equation (134.16) and plotted with respect to the processing time (Figure 134.3). From the definition (Equation 134.17), the F-value can be determined by calculating the area under the lethal rate curve.

Various methods are available for the area calculation. The simplest method is to count squares or use a planimeter [1]. Another well-known method involves the construction of lethal rate paper [19–22]. Numerical calculations have also been used using trapezoidal rule [23], Simpson's rule [24, 25], or Gaussian integration formula [20].

B. Analytical Method

Analytical solutions of Equation (134.17) could be obtained if the time-temperature profile follows analytical heat penetration equations based on heat transfer theory, such as Equations (134.9) and (134.12). Examples of the solutions were discussed by Holdsworth [1], Hicks [26], and Hurwicz and Tischer [27]. An advantage of the analytical method is that the meanings of the F-value and various processing factors can be better understood and, therefore, it can provide theoretical basis for other F-value determination methods. However, the analytical solutions of Equation (134.17) are not always available, and the assumptions needed for the derivations sometimes greatly reduce the accuracy of the method.

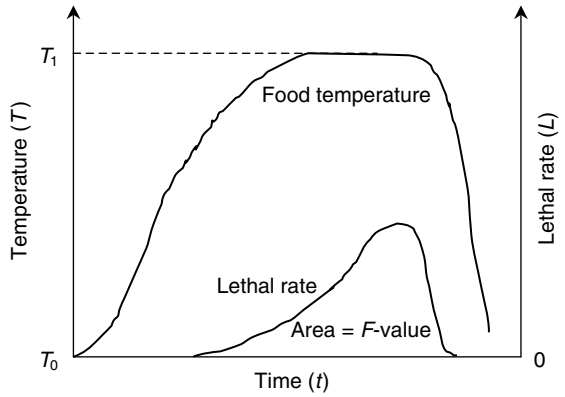

FIGURE 134.3 Typical profiles of temperature and lethal rate at slowest heating point.

C. Formula Method

In the formula method, empirical heat penetration equations such as Equations (134.4) and (134.7) are obtained experimentally and substituted into Equation (134.17) to yield the F-value. The method was first proposed by Ball [28] and further developed by a number of researchers, including Ball and Olson [2], Stumbo [14], Gillespy [29], Jakobsen [30], and Hayakawa [5, 31]. In the following sections, the Ball's method and the Hayakawa's method are discussed.

1. Ball's Method

In the Ball's original method [28], Equations (134.4) and (134.7), which are linear on the semi-logarithmic heat penetration curve (see Figure 134.2), are substituted to Equation (134.17) and integrated to determine the F-value (F_h) for heating phase and the F-value (F_{cl}) for cooling phase, respectively. The F-value (F_{cc}) for the curvilinear portion of the heat penetration curve, observed at the beginning of cooling phase, is also considered.

Therefore, the F-value for the entire thermal process is calculated as:

$$F = F_h + F_{cc} + F_{cl} \tag{134.18}$$

By the substitution and integration, the F_h-value for heating phase is given by:

$$F_h = \frac{f_h}{\ln 10} \cdot 10^{\frac{T_{hm}-T_r}{z}} \left[E_1\left\{ \ln 10 \cdot \left(\frac{T_{hm} - T_1}{z} \right) \right\} - E_1\left\{ \ln 10 \cdot \left(\frac{j_h (T_{hm} - T_0)}{z} \right) \right\} \right] \tag{134.19}$$

where $$E_1(x) = \int_x^\infty \frac{e^{-p}}{p}\, dp \tag{134.20}$$

or $$E_1(x) = -\gamma - \ln x - \sum_{n=1}^{\infty} \frac{(-1)^n x^n}{n \cdot n!} \tag{134.21}$$

Equation (134.20) is useful when $x > 0.01$, because its solution tables are available. Equation (134.21) is used when $x < 0.01$ and γ is Euler's constant (0.577215665). Temperatures are expressed in degree Fahrenheit. For a broken heating curve, the F_h-value is given by:

$$F_h = \frac{1}{\ln 10} \cdot 10^{\frac{T_{hm}-T_r}{z}} \left[f_{h1} \left[E_1\left\{ \ln 10 \cdot \left(\frac{T_{hm} - T_1^*}{z} \right) \right\} - E_1\left\{ \ln 10 \cdot \left(\frac{j_h (T_{hm} - T_0)}{z} \right) \right\} \right] \right.$$

$$+ f_{h2}\left[E_1\left\{\ln 10 \cdot \left(\frac{T_{hm} - T_1}{z}\right)\right\} - E_1\left\{\ln 10 \cdot \left(\frac{(T_{hm} - T_1^*)}{z}\right)\right\}\right]\Bigg] \quad (134.22)$$

where f_{h1} and f_{h2} are f_h-values before and after breaking point, respectively, and T_1^* is the food temperature at breaking point.

For cooling phase, $j_c = 1.41$ is assumed. The F_{cc}-value for the initial curvilinear portion of the semi-logarithmic heat penetration curve is calculated using the following equation:

$$F_{cc} = f_c \cdot 10^{\frac{T_1 - T_r}{z}}\left[0.1435 \cdot e^{-\frac{0.789m}{z}} + 0.1096 \cdot \frac{E}{m} \cdot e^{\frac{0.692m}{z}}\right] \quad (134.23)$$

where

$$E = (2.303n)^2 \int_1^{2.14} e^{-2.303nx}\sqrt{x^2 - 1}\, dx$$

$$m = T_1 - T_{cm}$$

$$n = \frac{0.3m}{z}$$

And the F_{cl}-value for the linear portion is given by:

$$F_{cl} = \frac{f_c}{\ln 10} \cdot 10^{\frac{T_{cm} - T_r}{z}}\left[E_i\left\{\ln 10 \cdot \left(\frac{0.657m}{z}\right)\right\} - E_i\left\{\ln 10 \cdot \left(\frac{m + g - 80}{z}\right)\right\}\right] \quad (134.24)$$

where $g = T_{hm} - T_1$ and

$$E_i(x) = \int_{-\infty}^{x} \frac{e^p}{p} dp \quad (134.25)$$

Tables for Equation (134.25) are also available elsewhere. Ball's original method was further modified by Ball and Olson [2], Hicks [32], Pflug [33], and many other researchers.

2. Hayakawa's Method

Hayakawa [5] introduced the special sterilizing value (U) for determining the F-value. According to his two theorems, the F-value can be calculated using the following equation.

$$F = (U_h + U_c) \cdot 10^{\left(\frac{T_{hm} - T_r}{K_s z_a}\right)} \quad (134.26)$$

The relative z-value, K_s, is defined as:

$$K_s = \frac{z_b}{z_a} \quad (134.27)$$

where z_a is a reference z-value and z_b is any z-value of interest. With Equation (134.27), the F-value can be determined for any z-value. The U-value for heating phase (U_h) and the value for cooling phases (U_h) are given by:

$$U_h = \int_0^{t_h} 10^{-\frac{(T_{hm} - T)}{K_s z_a}}\, dt \quad (134.28)$$

$$U_c = 10^{-\frac{(T_{hm} - T_1)}{K_s z_a}} \cdot \int_0^{t_c} 10^{-\frac{(T_1 - T)}{K_s z_a}}\, dt = 10^{-\frac{(T_{hm} - T_1)}{K_s z_a}} \cdot U_{gc} \quad (134.29)$$

where t_h and t_c are heating and cooling times, respectively. The values of U_h and U_c can be conveniently obtained from the universal tables of U_h/f_h and U_{gc}/f_c, respectively.

The tables were prepared based on the following two observations: (1) the U_h/f_h-values are independent of f_h-values when the j_h- and g ($= T_{hm} - T_1$)-values are fixed, (2) the U_{gc}/f_c-values are independent of f_c-values when values for j_c and for $T_1 - T_{cm}$ are fixed. In the calculations for table preparation, Equations (134.4) and (134.7) were used for the linear portions of heating and cooling curves, respectively. The curvilinear portions of heating and cooling curves were evaluated using the following empirical circular formulas.

a. *Formulas for the curvilinear portion of heating curve*

(i) $0.4 \leqslant j_h < 1.0$:

$$T_{hm} - T = (T_{hm} - T_0)^{\cot(Bt + \pi/4)} \text{ for } 0 \leqslant t \leqslant t_{lag} \quad (134.30)$$

where

$$B = \frac{1}{t_{lag}}\left\{\arctan\left[\frac{\log(T_{hm} - T_0)}{\log\{j_h(T_{hm} - T_0)\} - t_{lag}/f_h}\right] - \frac{\pi}{4}\right\}$$

$$t_{lag} = 0.9 \cdot f_h\,(1 - j_h)$$

(ii) $1.0 < j_h \leqslant 3.0$:

$$T_{hm} - T = (T_{hm} - T_0)^{\cot Bt} \quad \text{for } 0 \leqslant t \leqslant t_{lag} \quad (134.31)$$

where

$$B = \frac{1}{t_{lag}} \cdot \arccos\left[\frac{\log\{j_h(T_{hm} - T_0)\} - t_{lag}/f_h}{\log(T_{hm} - T_0)}\right]$$

$$t_{lag} = 0.7 \cdot f_h\,(j_h - 1)$$

b. *Formulas for the curvilinear portion of cooling curve*

The curvilinear formulas for the cooling curve can be obtained by replacing some symbols in the above formulas in the following ways.

$$f_h \Rightarrow f_c$$

$$\begin{gathered} j_h \Rightarrow j_c \\ T_{hm} - T \Rightarrow T - T_{cm} \\ T_{hm} - T_0 \Rightarrow T_1 - T_{cm} \\ t_{lag} \text{ for heating} \Rightarrow t_{lag} \text{ for cooling} \end{gathered} \tag{134.32}$$

The Hayakawa method is considered as one of the most versatile and reliable techniques [1, 34, 35].

V. FACTORS AFFECTING THERMAL PROCESSING

The severity of a thermal processing, that is, the required F-value, is affected by a number of factors originated from the complexity of food nature and the variety of processing methods and packages. An outline of such factors is well presented in Holdsworth [1].

A. Food Factors

1. The phase and the rheological behavior of the food, the packing of food components in the package determines if the main mechanism of internal heat transfer is conduction, convection, or both, as well as the rate of heat transfer [36].
2. As the initial temperature of the product increases, the processing time and the f_h-value decrease, and the j_h-value increases. The initial temperature is more important in the conduction-dependent thermal processing. Also, the initial temperature distribution in the product affects the j_h-value [37, 38].
3. Initial load of target microorganisms and their z- and D-values are important to determine the process severity.
4. The thermal diffusivity and specific heat of the food, affected by the food composition and the consistency of food components, greatly influences the f-value (Equations (134.11) and (134.15)). Therefore, the change of thermal diffusivity or specific heat with temperature may not be negligible.
5. The pH of the product determines the process severity. The products with lower pH may require lighter thermal processing.
6. Certain additives such as nitrite, salt, sugar, and including various antimicrobial agents can reduce the process severity.

B. Processing Factors

1. As the temperature of heating medium increases, the processing time and the f_h-value decrease, and the j_h-value increases.
2. The rotation of product in the retort can enhance the internal heat transfer to reduce the f_h-value.
3. The longer the processing time, the greater the heat penetration.
4. As the surface heat transfer coefficient between the package and the heating medium increases, the f_h-value decreases and the j_h-value increases [2, 39].

C. Package Factors

1. The thermal conductivity of the packaging material determines the rate of heat penetration.
2. The shape and dimension of the package are important. The f- and j-values depend on the package shape [37]. Equations (134.11) and (134.15) show that the f_h-value depends on the size of the packaged food. The heat penetration into the package is greater for the package of larger surface area and the smaller thickness.
3. The amount of headspace is important for agitation and rotation, which can enhance the internal heat transfer.
4. The position of packages inside the retort and the type of stacking can affect the heat transfer to individual packages.

VI. RETORTS AND HEAT TRANSFER MEDIA

A. Retorts

Currently, a large number of different types of retort are available. In general, the type of retort may be classified into five groups depending on the mode of operation: (1) batch-static (e.g., conventional vertical or horizontal batch retorts without rotation), (2) batch-rotary (e.g., batch retorts with internal rotation of packages), (3) continuous-static (e.g., hydrostatic cookers without rotation), (4) continuous-rotary (e.g., hydrostatic cookers with rotating carrier bars, reel and spiral cookers), and (5) semi-continuous (e.g., Crateless retorts) [1]. Selecting a proper retort requires careful considerations on the three types of factors mentioned in Section V. The pressure balance between the inside and outside of the package is another important factor to be considered, especially for rigid and plastic packages prone to distortion at high temperatures and at high rates of cooling. Beyond the scientific evaluation of the factors, the economic factors such as available area, factory layout, the number of operator, production yield, the cost of installation, operation, and maintenance should be also considered. More detailed considerations on retort systems are presented by Holdsworth [1], Lopez [40], Rees and Bettison [41], and Footitt and Lewis [42].

B. Heat Transfer Media

For heating media, saturated steam, steam-air mixture, hot water, and gas flame, and for cooling media, chilled water, are available. For a proper selection of heat transfer media, the factors presented in Section V, especially surface heat transfer coefficients and packaging materials, as well as various economical, environmental factors must be carefully taken into account.

The heat transfer coefficient at the surface of the package in contact with the heat transfer media is one of the critical parameters to determine the efficiency of heat penetration. Its values for saturated steam, vigorously boiling water, and gas flame are very high and thus the heat transfer resistance at the package surface is often negligible, that is, the Biot number can be assumed infinite. Due to their rapid heat transferring ability, those heat transfer media are suitable for HTST (high-temperature short-time) processes. Other media have much lower values of surface heat transfer coefficient, and therefore, the resistance at the surface is necessary to be considered in the process evaluation. The ranges of the surface heat transfer coefficient (or overall heat transfer coefficient) of the media are well reviewed in Holdsworth [1].

The type of packaging materials is also an important factor to be considered in the media selection. For heating metallic packages, all the existing media are usable. The air heating and cooling of canned products have been also developed, however, their commercial applications have not been appeared yet [1]. For heating retortable laminated pouches or plastic packages, hot water [43] or steam-air mixture [44] can be used. For glass packages, saturated steam, steam-air mixture, and hot water are suitable heating media.

The cooling stage generally uses chilled water of about 10°C, and is slower than the heating stage due to the lower surface heat transfer coefficients. Relatively few studies have been reported on the cooling media and their surface heat transfer coefficients [1].

VII. RETORTABLE PACKAGES

A. Metal and Glass Packages

Metal cans and glass jars have been widely used in thermal processing due to their mechanical strength and thermal stability under high temperature and pressure conditions. The excellent closure integrity of these packages is especially advantageous for protecting high water activity, low acid, meat-based foods against microbial contamination. Metal cans may be two- or three-piece cans made of tin-plated steel, lacquered tin-free steel, or aluminum [1, 45]. An advantage of using glass jars is the visibility of the contents; however, careful operation and handling are required to prevent shock breakage [1].

B. Plastic Packages

Plastic packages are sometimes used for thermal processing of foods, especially ready-to-eat military rations. These packages are usually in the form of pouches or containers. A typical retortable pouch for military rations is made of a foil layer sandwiched between two layers of plastics such as polypropylene. A typical retortable plastic container is made of an oxygen barrier layer such as EVOH sandwiched between two polypropylene layers.

There are several advantages of using plastic packages for thermal processed foods [46]: (1) high surface-to-volume ratio that allows rapid heat transfer under mild thermal conditions, (2) convenience due to light weight, easy opening, easy handling, and microwavability, (3) large surface area for printing, and (4) flexibility in package design. However, plastic packages are not as reliable as metal or glass packages due to the following limitations [46]: (1) lower tolerance to heat and pressure, (2) relatively poor oxygen barrier, (3) heat seal of plastic packages is not as reliable as double seam of metal cans, and (4) seal inspection is more difficult.

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135 Edible Films and Coatings

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CONTENTS

I. HISTORY OF EDIBLE FILMS AND COATINGS

The properties and applications of edible films and coatings (EFC) have been studied extensively during the past few decades. It has been demonstrated that EFC can extend shelf life and maintain quality of various food products.

In fact, applying EFC to a food product is not a new concept. Waxing on fruits to reduce water loss and to provide attractive gloss has been used for thousands of years (1). In the sixteenth century, Englishmen wrapped foods with fat, a process called "larding," to prevent water loss (2). Furthermore, sausage casings made with collagen, which is mainly used for containment of meat batter, is one of the earliest forms of food processing.

II. DEFINITION

Edible films and coatings are classified differently by definition. Edible films are defined as a pre-formed edible layer which can be placed on or between food components. Edible coatings are defined as a thin layer of edible material formed as an integrated coating on food surfaces (3).

III. FUNCTIONS OF EDIBLE FILMS AND COATINGS

A. Retard Moisture Migration

Maintaining an appropriate range of moisture level is crucial for the quality and shelf life of food products. Physical structure of bakery products would collapse if moisture is absorbed. Sensory properties such as texture and flavor are also significantly affected by the changes in moisture content. Furthermore, water loss of food products such as fruits and vegetables, decreases shelf life.

One of the functions of edible coatings is to retard moisture migration. Studies have shown that some EFC are efficient moisture barriers (4). Lipid edible films and bilayer films made with lipid and protein or polysaccharide are generally better moisture barriers than protein and polysaccharide films due to higher level of hydrophobicity. Waxes have the lowest water vapor permeability (WVP) among edible film-forming materials. They have 25 times less WVP compared to common oil films and 100–200 times less WVP than for protein films (5). Raisins pretreated with starch and coated with beeswax had less moisture loss than untreated raisins after five weeks of storage (6). Chocolate brownies coated with beeswax and methylcellulose (MC) had significantly less moisture gain than uncoated brownies. This study also showed that moisture barrier property of MC coating alone is poor, since there is no difference in moisture gain between MC coated and uncoated brownies (7). Besides coating products, edible films can be placed on or between layers of food product to retard moisture migration from a higher moisture level component to a lower moisture level component. Kamper and Fennema (8) developed an edible bilayer consisting of stearic-palmitic acid and hydroxypropyl methylcellulose (HPMC), and placed it between tomato paste and crackers. It significantly delayed water transfer from tomato paste to crackers. Conventionally, waxes are applied to fruits and vegetable surfaces to reduce shrinkage due to water loss (1).

B. Retard Gas Transfer

The levels of carbon dioxide and oxygen that come in contact with a product have to be taken into consideration in order to retain quality of the product and consequently lengthen shelf life (9). The primary deterioration that is involved with gases is rancidity, developed by oxidation of lipids, which results in off-flavors. Products with high content of fat such as nuts and potato chips are susceptible to rancidity (10). Protein and polysaccharide coatings which are highly impermeable to fat, oil, and oxygen are effective in preventing lipid oxidation (11). Wu, Rhim, Weller, Hamouz, Cuppett, and Schnepf (12) reported that wheat gluten, soy protein, chitosan, and carrageenan coatings effectively controlled lipid oxidation of precooked beef patties. Lee, Trezza, Guinard, and Krochta (13) showed that rancidity of whey-protein-coated peanuts was significantly lower than uncoated peanuts. It is desirable to regulate gas transfers during storage of fruits and vegetables, since respiration of fruits and vegetables depletes oxygen and increases carbon dioxide level. If the level of oxygen is too low, anaerobic respiration will occur and lead to abnormal ripening, development of off-flavors, and spoilage. In the presence of oxygen, production of ethylene increases and promotes ripening and senescence, which results in shorter shelf life (14). Internal modified atmosphere can be achieved by applying edible coatings made with materials that have low permeability to gases. Protein and polysaccharide coatings are generally good barriers to gases. Lee, Park, Lee, and Choi (15) reported lower initial respiration rate of apples coated with carrageenan or whey protein than uncoated apples.

Enzymatic oxidative browning is another deterioration that can be prevented by minimizing the uptake of oxygen. When oxygen, polyphenol oxidase, and copper ion are present, enzymatic oxidative browning occurs, which turns phenol, a colorless substance, into a brown substance, melanoidins. This could, thus, lead to the decrease in acceptance of the product. Delayed browning of apple and potato slices coated with calcium caseinate or whey protein solutions were observed by Le Tien, Vachon, Mateescu, and Lacroix (16).

C. Retard Aroma Loss/Gain

Aroma, along with other attributes, such as appearance, taste, and texture is important to the quality of food products. Aroma is perceived when volatile compounds are dissolved in the nasal cavity and perceived by the olfactory system. Volatile compounds that are lost to or picked up from the storage environment could be regulated with edible coatings (11). Debeaufort and Voilley (17) showed that wheat gluten film is an effective barrier for 1-octen-3-ol, which represents a smell of mushrooms. They suggested that it can be used for wrapping cheeses to prevent aroma gain of 1-octen-3-ol from the refrigerator. Oranges coated with cellulose-based coating or commercial shellac coating had retained higher concentration of volatile compounds after storage up to 55 days than uncoated oranges (18). Shellac-coated apples had higher concentrations of fruit-like and apple-like volatiles than uncoated apples, which was due to the reduction in evaporation rate of these volatile

compounds (19). Nisperos-Carriedo, Shaw, and Baldwin (20) also stated that fruits coated with beeswax emulsion and TAL Pro-Long (commercial coating composed of different fatty acids and carboxymethyl cellulose (CMC) sodium salt) effectively retained and increased volatile compounds that were considered important to fresh orange flavor.

D. Retard Lipid Migration

Lipid migration is a major problem in confectionary products. Liquidly lipid such as fatty acid tends to migrate to the surface of chocolate coatings, which results in soft and sticky surface (21,22). Chocolate "bloom" is a result of migration of cocoa butter from chocolate to the surface. Nelson and Fennema (21) investigated lipid barriers of five hydrocolloid films — MC, hydroxypropylmethyl cellulose (HPMC), CMC, carrageenan, and polyethylene glycol alginate (PGA) — and reported that they all were effective barriers to lipid migration.

Methods of reducing fat intake during deep-frying have been investigated extensively due to the rising health concerns of consumers. Studies have shown that edible coatings that had good lipid barrier property could significantly trim down fat intake of fried products. Soy protein isolate (SPI), whey protein isolate (WPI), and MC coatings were listed as the best film-forming materials to be used to reduce fat absorption of fried dough (23,24). Rayner, Ciolfi, Maves, Stedman, and Mittal (25) reported that doughnut mix and potato fries that were coated with soy protein film had considerable fat reduction. They also showed that consumers preferred the coated fries over the uncoated. Balasubramaniam, Chinnan, Mallikarjunan, and Phillips (26) explained that the layer of thermal gel formed by HPMC film controlled the transfer of fat between the meatball and the oil medium, and consequently reduced fat intake.

E. Improve Mechanical Properties

Mechanical strength of products can be improved by EFC. The structure of extruded or molded products can be protected by coating with EFC (9). Waxing of fruits and vegetables minimizes surface abrasion during handling of these products (27). Xie, Hettiarachchy, Ju, Meullenet, Wang, Slavik, and Janes (28) investigated puncture strength of eggshell coated with SPI, WPI, CMC or WG. They showed that eggshell coated with SPI and WPI had the greatest puncture strength, which implied that the SPI and WPI EFC could minimize breakages of eggs during processing, handling, and storage. Guilbert, Gontard, and Gorris (4) stated that food filling coated with edible coating could reinforce the structure and protect the filling. In addition to improving mechanical strength of products, EFC such as mineral oil (29) and hydrocolloid coatings such as pectinates, alginates, and starch (10) were used to minimize stickiness of food products. Improving the mechanical and handling properties can lead to a smoother process operation and reduction in product loss.

F. Carrier of Additives

EFC can provide a cohesive surface structure for additives such as anitmicrobial agents and antioxidants. Antimicrobials are substances that are added to EFC to improve quality and shelf life of products by retarding growth of yeast, molds, and bacteria during storage and distribution. Examples of food grade antimicrobial agents are organic acids and their salts such as benzoic acid, sodium benzoate, sorbic acid (SA), potassium sorbate, propionic acid, lactic acid, and acetic acid (30,31). Cagri, Ustunol, Osburn, and Ryser (32) investigated the effect of adding antimicrobial, p-aminobenzoic acid (PABA) and/or SA into WPI coating for bologna, summer sausage and hot dogs. Zein film coatings with nisin were shown to effectively prevent growth of *L. monocytogenes* on ready-to-eat chicken (33).

Antioxidants may be added to EFC to protect products from oxidation which results in oxidative rancidity, degradation of nutrients, and discoloration. Tocopherols, carotenoids, acids (as well as their salts and esters), and phenolic compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are some examples of food grade antioxidants. Antioxidants such as ascorbic acid and citric acid were added to MC edible films, which were applied to mushrooms and cauliflower. It was found that the coatings significantly decreased oxygen permeability of the vegetables and thus slowed the browning reactions (34). Lee, Park, Lee, and Choi (15) also reported that adding these two antioxidants along with oxalic acid to whey protein or carrageenan coatings helped maintain the color of minimally processed apple slices.

Calcium chloride, known to be a firming agent, was incorporated into EFC to inhibit softening of fruits and vegetables. Lee, Park, Lee, and Choi (15) reported that addition of calcium chloride to whey protein or carrageenan coatings in acidic condition could minimize softening of apple slices. Firmness of kiwifruit slices were also maintained during storage by adding calcium chloride to coating (35).

EFC could also serve as adherence surfaces for seasonings and flavor enhancers (10). Salt and flavorings were added to wheat gluten and dextrin coatings and modified food starches and gum Arabic coatings (36).

IV. COMPONENTS OF EDIBLE FILMS/COATINGS

EFC are made with a high molecular weight molecule as the backbone, a plasticizer, and a surfactant if needed. In this section, macromolecules including protein, polysaccharide,

and lipid that are used to make EFC will be discussed. They are listed in Table 135.1 along with the solvent they are dissolved in to make the film-forming solution.

A. Film Forming Agents

Film forming capability of macromolecules largely depends on their polymer backbone (37). Chain length, number of functional groups, and structure of macromolecules influence the functionality of EFC. For example, wheat gluten film is hydrophobic in nature because it has a large number of non-polar amino acids (38).

Polysaccharides and proteins are hydrophilic in nature, while lipids are hydrophobic. Therefore, EFC made with polysaccharides or proteins have limited water vapor barrier properties while EFC made with lipids have good water vapor barrier properties. Table 135.2 summarizes some of the WVP values of protein and polysaccharides that are investigated in the literature.

Oxygen permeability (OP), in contrast, is high in lipid films while low in polysaccharide and protein films. Krochta (42) summarized that protein EFC such as corn zein and wheat gluten films are better oxygen barriers than polysaccharide films. In general, as the hydrophobicity of the film increases, OP decreases.

TABLE 135.1
Film Preparation for Different Macromolecules

Macromolecule	Solvent
Protein	
Soy protein	Water
Corn zein	95% ethanol
Wheat gluten	Water-95% ethanol
Whey protein	Water
Polysaccharides	
Methyl cellulose	Water-95% ethanol
Hydroxypropylmethyl cellulose	Water-95% ethanol
Hydroxypropyl cellulose	Water-95% ethanol
Lipids	
Beeswax	Melt
Shellac	95% ethanol

TABLE 135.2
Water Vapor Permeability Values of Different Edible Films

Reference	Composition	Thickness[a]	Condition	WVP[b]
105	SPI[c]/GLY[d] (5:3)	0.000254	25°C, 50%RH	2.54
106	WG[e]/GLY (15:6)	0.087	25°C, 50%RH	1.41
69	WPI[f]/GLY (1.6:1)	0.121	25°C, 65%RH	1.39
78	CZ[g]/GLY	—	25°C, 50%RH	0.59
62	MC[h]/PG[i]	0.025	25°C, 52%RH	1.00
107	HPC[j]	0.05	21°C, 85%RH	0.11
39	HPC/SA[k] (1.1:1)	0.019	27°C, 97%RH	0.0005

[a] Thickness in mm, [b] WVP in $\times 10^{-9}\,gm^{-1}\,s^{-1}\,Pa^{-1}$, [c] soy protein isolate, [d] glycerol, [e] wheat gluten, [f] whey protein isolate, [g] corn zein, [h] methyl cellulose, [i] propylene glycol, [j] hydryoxypropylcellulose, [k] stearic acid.

1. Proteins

Proteins can be classified into water-soluble and water-insoluble proteins. Soy proteins and whey proteins are examples of water-soluble proteins, while wheat gluten and corn zein are water insoluble proteins.

a. Soy proteins

Soybean, in the family of Leguminosae, is believed to be originated in Eastern Asia (43). The whole soybean, with 40% protein, 21% fat, and 34% carbohydrate, has much higher protein content than other grains which usually have 8–15% protein (44). Globulin is the major protein group of soy proteins. Soy proteins are further fractionated according to the molecular weight of the protein by ultracentrifugation. The four most widely known soy protein fractions are 2S, 7S (conglycinin), 11S (glycinin), and 15S. Conglycinin and glycinin make up approximately 60–70% of the soybean globulins (45).

Many researches have been conducted to determine the mechanism of soy protein film formation. Okamoto (46) suggested that heat-denatured proteins, which are partially unfolded and more hydrophobic, move to the surface of the film solution, and as water evaporates, protein molecules interact to form film structure. Rangavajhyala, Ghorpade, and Hanna (47) found that aggregation of proteins under heat treatment is through hydrogen bonds and intermolecular disulfide bonds. Kinsella (45) stated that under alkali condition, glycinin breaks down to subunits and unfolds due to disulfide bond cleavage, which is followed by gelation of the protein solution. He also suggested that 11S fraction dissociates into subunits and aggregates under heat treatment.

b. Whey proteins

Whey is a by-product of cheese production. There are five main components in whey proteins: α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins, and proteosepeptones. Different components can be fractionated by differential solubility, electrophoretic, and chromatographic methods. Researchers have shown that EFC can be made with β-lactoglobulin and bovine serum albumin alone (48), as well with WPI and whey protein concentrate (WPC).

Denatured whey protein films are water insoluble due to the intermolecular disulfide bonds induced by thiol-disulfide interchange and thiol oxidation reactions which are promoted by heat treatment (3).

c. Wheat gluten

Wheat gluten is the protein of wheat kernels which accounts for 8–15% of the dry weight (49). Most of the protein

consists of gliadins and glutenins which are only soluble in alcohol. Therefore, wheat gluten films are prepared by dispersing wheat gluten in a mixture of ethanol and water. The pH of wheat gluten film solutions have to be adjusted to the range of 2 to 4 or 9 to 13 to avoid extreme acidic or alkaline conditions, and to avoid isoelectric point (pI) of wheat gluten at which proteins coagulate. Gontard, Guilbert, and Cuq (50) reported that wheat gluten films made in acidic conditions (pH $\approx$ 2 to 6) have better sensory and visual properties than films made in alkaline conditions. However, Gennadios, Brandenburg, Weller, and Testin (51) reported that wheat gluten films made in alkaline conditions have higher tensile strength.

Wheat gluten films are cohesive and elastic (51). Due to the high glutamine content of wheat gluten, highly cooperative protein-protein interactions occur, which contribute to the cohesiveness of the film (52). Hydrogen bonds between hydrated gluten are responsible for film's elasticity (53). Wheat gluten films are very effective oxygen barriers (54). Like other protein-based EFC, wheat gluten films have poor water barrier properties because of the hydrophilic nature of the proteins (42).

d. Corn zein

Corn zein, which is the prolamine fraction of corn proteins, account for about 45–50% of corn proteins (55). It has large amounts of non-polar amino acids, which contribute to the hydrophobic nature of zein (56). The low polar amino acid content and high nonpolar amino acid content also contribute to the insolubility of corn zein in water (57). Therefore, corn zein is generally solubilized in a mixture of water and alcohol (ethanol).

Shukla and Cheryan (55) concluded that corn zein films are glossy, tough, hydrophobic, and good lipid barriers. Tomatoes coated with corn zein had delayed ripening and color development, due to low permeability of oxygen of corn zein EFC, when compared to tomatoes coated with typical shrink wrap films. However, tomatoes coated with corn zein EFC exhibited higher weight loss than the ones coated with typical shrink wrap films, due to high WVP of the zein films (58). They also compared corn zein–coated tomatoes with uncoated tomatoes. Again, they showed that corn zein EFC significantly delayed color development of tomatoes. In this case, corn zein–coated tomatoes reduced softening and weight loss over a period of 8 days comparing to the uncoated tomatoes (59).

2. Polysaccharides

Polysaccharides such as cellulose and its derivatives, starches and its derivatives, pectins, seaweed extracts, and gums are used to form EFC.

Cellulose is a rigid material composed of plant cell walls. It is insoluble in water due to high amount of intramolecular hydrogen bonding in the cellulose polymer (60). Therefore, cellulose is generally dispersed in water and ethanol. MC, hydryoxypropylcellulose (HPC), and HPMC are examples of water-soluble cellulose ethers that are modified by etherification. MC is the least hydrophilic among the soluble cellulose ethers (9,60). It is shown to have better moisture barrier property than other cellulose ether (24,61). Mechanical and barrier properties of MC films with different plasticizers were investigated (62).

Cellulose edible films are tough and flexible (60). They are resistant to oxygen and lipid migration. Cellulose-based films exhibited lower OP than synthetic films, such as low density polyethylene film (LDPE) (40). MC, HPMC, and HPC coatings applied to fried pastry mix and fried potato ball reduced fat uptake (23,24,61). Williams and Mittal (61) showed that HPC and MC films reduced water loss of fried pastry mix. Cellulose-based EFC, like protein-based EFC, are hydrophilic. Therefore, they generally have poor water barrier properties. Park and Chinnan (40) reported that WVP of cellulose films were 100 times greater than LDPE. Cellulose-based films were supplemented with a layer of shellac to form an edible film layer to separate one food phase from another phase (63).

3. Lipid

Lipids that are generally used for making EFC are waxes (i.e., paraffin and carnauba wax), mineral oil, fatty acids, monoglycerides, resins (i.e., shellac), and rosins. EFC made with lipid are cohesive and flexible (64). These characteristics are dependent on molecular weight of both hydrophilic and hydrophobic phases, branching, and polarity of lipid. As hydrophobicity of the film increases, OP decreases. Films that are made with resins such as shellac have lower permeability to oxygen and carbon dioxide than films that are made with waxes. On the other hand, waxes are more resistant to water vapor than other lipid film, due to the tight orthorhombic arrangement of the crystals. Lipid films, in general, are good water vapor barriers due to their hydrophobic nature.

Some example applications of wax and oil coatings are on fruits and vegetables to prevent moisture loss (14), on raisins to prevent moisture migration from the raisin to dry cereal (10), and on confectioneries to provide gloss (64).

4. Composite or Bilayer Film

Fats, fatty acids, and waxes can be added to polysaccharide or protein films to improve water barrier properties (38). There are three main types of composite films: lipid bilayer films, emulsion films, and composite films made of polysaccharide and protein. Bilayer films composed of HPMC and solid lipids such as beeswax (2), and corn zein with a layer of oil or waxes (65) had significantly lower WVP than protein or polysaccharide films alone. Emulsion films developed with whey proteins, beeswax, and glycerin showed

reduction in WVP by 50% compared to films made of whey proteins alone (41). Krochta (42) stated that emulsion films had even lower WVP than bilayer films due to the optimum orientation of fatty acids in emulsion films. SPI and wheat gluten composite film was made by mixing the macromolecules with the plasticizer and solvent to form the film-forming solution. The film was shown to have lower WVP and improved TS than film made with SPI alone (38).

B. Plasticizer

1. Definition and Functions

Plasticizer is defined as "a substantially nonvolatile, high boiling, nonseparating substance, which when added to another material changes the physical and/or mechanical properties of that material" (37). Plasticizers induce flexibility of films by reducing the degree of hydrogen bonding and increasing intermolecular spacing of the polymers (66).

2. Selection of Plasticizers

Selection of plasticizers is based on compatibility of the plasticizer and the substance which they plasticize. For example, water-soluble substance should be plasticized with compound(s) containing hydroxyls (37). The number and position of hydroxyl groups and the number of hydrogen bonds capable of forming with the macromolecule affect the efficiency of plasticization (67). Cho and Rhee (68) stated that hydrophilicity and concentration of plasticizers affect moisture sorption of SPI films. SPI films plasticized with glycerol, which is more hydrophilic than sorbitol, absorbed more moisture than sorbitol-plasticized films. Plasticizer concentration also affected plasticizing effect of whey protein films (69). Addition of plasticizer increases permeability of the film due to the increase in free volume (66). Studies showed that plasticizers increased oxygen and/or water vapor permeability of MC films (62), gellan films (70), whey protein films (71), and gelatin films (72). Increased concentration of plasticizer further increased permeability of edible films (73,74).

Water and polyols such as glycerol, propylene glycol (PG), polyethylene glycol (PEG), and sorbitol are commonly used plasticizers. Glycerol is the most widely used plasticizer, probably because it has small molecule weight, which enables it to incorporate into polymer matrix very easily. Studies have been conducted to investigate the effect of incorporating different plasticizers into polymers on mechanical and permeability properties of edible films. McHugh and Krochta (71) reported that sorbitol-plasticized whey protein film has lower OP than glycerol-plasticized film. Sucrose-plasticized β-lactoglobulin film showed the lowest OP, followed by sorbitol and glycerol, while PEG 200- and 400-plasticized films exhibited the poorest oxygen barrier property (48). Glycerol-plasticized β-lactoglobulin film had higher tensile strength (TS) and percent elongation than sorbitol-, PEG 200-, PEG 400-, and sucrose-plasticized films at equivalent amount of plasticizers (48). Chick and Ustunol (75) reported that sorbitol-plasticized lactic acid casein films were more effective in oxygen and water barrier properties than glycerol-plasticized film.

Addition of plasticizers often decreases TS and increases elongation-at-break of caseinate films (76), MC films (62), peanut films (77), SPI films (68), gellan films (70), and whey protein films (69,71). Effects of using mixture of plasticizers on mechanical and WVP properties were investigated. Park, Bunn, Weller, Bergano, and Testin (78) reported that as the ratio of glycerol to PEG decreased, TS of wheat gluten film increased, while elongation and WVP decreased. A 50:50 mixture of PEG and glycerin plasticizers for protein edible films exhibited the highest TS (78). Environmental factors such as relative humidity and temperature of the room where films are made also affect the plasticizing effect of plasticizers. Gontard, Guilbert, and Cuq (52) reported that the plasticizing effect of water for wheat gluten film is highly temperature dependent.

C. Surfactant

Surfactants are surface-active compounds that have the ability to reduce the interfacial tension between two interfaces since they have both hydrophilic and hydrophobic ends. They are usually incorporated into emulsion systems. Surfactants are chosen according to hydrophilic-lipophilic balance (HLB) and phase inversion temperature (PIT). HLB is a system of values of 1 to 40 according to the hydrophobic and hydrophilic portion of the surfactant. Surfactants with low HLB are used in water-in-oil (w/o) emulsification and surfactants with high HLB values were used in oil-in-water (o/w) emulsification. PIT is the value where emulsions reverse from o/w to w/o depending on the temperature. It is believed that o/w interfacial tension is the smallest at PIT. Naturally occurring emulsifiers, phospholipids, monoglycerides, soy lecithin, sodium stearoyl lactylate, sodium lauryl sulfate, propylene glycol alginate, and paraffin wax are some examples of food grade emulsifiers.

V. METHOD OF MAKING EFC

Two broad categories of manufacturing EFC are: wet processing technologies (i.e., casting) and low-moisture processing technologies (i.e., compression molding and extrusion) (80).

A. Casting

Casting is one of the most common methods to make stand-alone films, which can be used to evaluate physical

and chemical properties of films. Proteins or polysaccharides, in concentrated or naturally occurring form, are dispersed in a mixture of water (with addition of solvent for water insoluble proteins and polysaccharides) and plasticizer(s). After thoroughly mixing the components, pH of the film forming solution is adjusted to the desired range by titrating with acidic or basic solution. Subsequently, the film forming solution may undergo heat treatment for a period of time. Then a controlled amount of solution is filtered and poured into a casting plate. Then this film forming solution is allowed to dry to a stand-alone film at a specific condition (temperature and relative humidity) and for a specific time period (~12 to 24 hr). Finally, the dried film is peeled from the plate and evaluated for various basic film properties. Example of the casting procedure of making soy protein isolate film is showed in Figure 135.1.

Drying temperature of the film should be taken into account since studies have shown that it had an effect on film properties. Thickness of wheat gluten films (81), and whey protein films (82) decreased by increasing the drying temperature. As temperature increases from 70°C to 80°C and 90°C, TS and elongation of peanut film increased while WVP and OP decreased. At 90°C, peanut films had the lowest WVP and OP and the highest TS (77). TS of wheat gluten film investigated by Kayserilioglu, Bakir, Yilmaz, and Akkas (81), on the other hand, decreased as drying temperature increased. Perez-Gago and Krochta (83) explained that WVP of an emulsion film of WPI-beeswax decreased as drying temperature increased, probably due to change in the lipid crystalline morphology and/or lipid distribution within the matrix.

B. Compression Molding

Compression molding is one form of low-moisture processing method used to make EFC. Thermoplastic material, which softens when it is heated, is placed on one half of a mold. Heat and pressure are applied to the mold once it is closed. Film material then fills the mold cavity and polymerization occurs. The film is, then, obtained by cooling the mold. One of the differences between compression molding and extrusion is that flowability of the film-forming material for compression molding can be low, while for extrusion, the material needs to have high flowability. Because compression molding has very limited production amount, it is economical for small production.

Compression molding was used recently as one of the methods to make EFC. Foulk and Bunn (84) showed that SPI films could be produced by compression molding. Films were made according to the method developed by Poly-Med Inc. (84). Mechanical and barrier properties of the SPI films that have various solubility were significantly different. They concluded that compression molded acetylated SPI film could be used as commercial thermoplastic. Slightly yellow and transparent SPI films plasticized with ethylene glycol (EG) formed by compression molding under a pressure of 15 MPa at 150°C was developed by Wu and Zhang (85). Due to the physical cross-linking between protein chains induced by EG, water adsorption of SPI films was reduced. SPI films made by compression molding had improved TS, breaking elongation, water resistance and thermostability, and therefore, they suggested that the thermoplastic materials from SPI could be used commercially for food packaging. Other proteins such as whey proteins (86) and cottonseed proteins (80) were also used to successfully produce compression-molded films.

C. Extrusion

Low-moisture process technologies take less energy and time. One of the advantages of extrusion is that it could be a continuous process in-line and could obtain larger

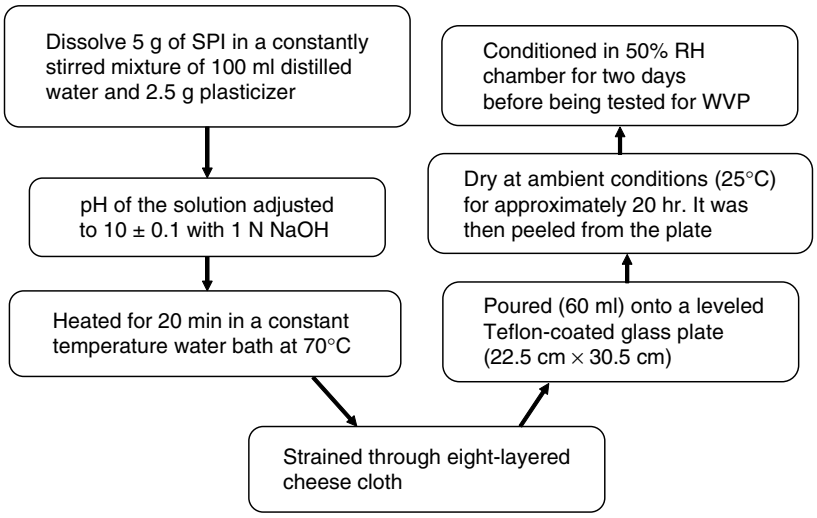

FIGURE 135.1 Flowchart for making soy protein isolate film.

production volume. Extrusion has been used to manufacture industrial polymers such as LDPE (87). A blend of polypropylene and thermotropic liquid crystalline polymer (TLCP), Rodrun LC5000, was fed to a twin-screw extruder and polymerized through a mini-extruder to form a film (88). Biodegradable films manufactured by melt blow extrusion were developed with polyvinyl alcohol (PVA) and collagen hydrolysate (CH) (89). A patent of a soy protein thermoplastic composition containing soy protein, carbohydrate filler, a reducing agent, a plasticizer, and water manufactured by extrusion was granted to Jane and Wang (90). They showed that this soy protein blend has the desirable flowability for processing by extrusion. Feasibility of using other edible film materials such as wheat gluten (91) and corn zein (92) in extrusion was also investigated. Redl, Morel, Bonicel, Vergnes, and Guilbert (93) suggested that extrusion of wheat gluten with plasticizers is feasible under steady-state conditions. Koh and Lim (94) concluded that protein cross-linking is an important characteristic of the leathery structure of extruded wheat gluten. They reported that extruded wheat gluten had higher water absorption capacity and lower protein solubility than unextruded wheat gluten.

Plasticizers were also added to the polymer matrix to improve flexibility of the film for extrusion. Water and glycerol were added to extruded corn gluten meal (92) and extruded soy protein (90).

VI. FACTORS AFFECTING FILM FUNCTIONALITIES

A. pH

Formation of edible films only occurs at a certain pH range (46,95). In general, pH of the film solution should be away from the isoelectric point (pI) of the protein used as the film-forming macromolecule. SPI films formed well at pH range of 7.5 to11, while no films were formed at pH range of 3.5 to 5.5 due to coagulation of soy protein (95). When the pH of the film-forming solution of WPI-beeswax emulsion film was adjusted away from the pI of whey proteins, films had lower WVP (96).

Also, if protein is one of the components of the film forming solution, the pH should be adjusted not to be extremely acidic or extremely alkaline, since intramolecular protein repulsive force develops under extremely acidic and alkaline conditions. Therefore, films formed in these conditions will be less dense and more permeable (51). Film opacity, solubility, WVP, and mechanical properties of wheat gluten films were affected by pH (52). They reported that the films made at pH 5 were the strongest, while films made at pH 6 had the lowest WVP. Jangchud and Chinnan (77) also found that as pH of the peanut film-forming solution increased, protein solubility increased, and the film was darker and more yellow.

Sian and Ishak (95) investigated the effect of pH on the composition of soybean protein-lipid films. They reported that films prepared at higher pH (pH $>$ 7.5) had higher protein proportion and lower fat proportion than films that were prepared at lower pH.

B. Heat Treatment and Irradiation

Heat treatment breaks intramolecular disulfide bonds in proteins and allows the proteins to unfold. Then the unfolded bonds interact and form intermolecular disulfide and hydrophobic bonds, which reduce mobility of protein solution. As temperature of the heat treatment is increased, solubility of SPI films decreased (47). Minimum requirement of heat treatment is different for various macromolecules. Whey protein films need to be heated at 75°C for 30 min in order to form intact water-insoluble films (69). Heat treatment also influences appearance. Heated films are smoother and more transparent than unheated films (97). Cuq, Boutrot, Redl, and Lullien-Pellerin (98) stated that thermal treatment induces inter- and intramolcular cross-linking of proteins which improve mechanical strength. Wheat gluten films developed by Micard, Belamri, Morel, and Guilbert (99) which underwent heat treatment (above 110°C for 15 min and above 90°C) were stronger and more flexible.

When WPI-calcium caseinate and SPI-WPI films underwent γ-irradiation (100), puncture strength and water resistance significantly increased. It is due to protein cross-linkage which contributed to a more ordered and stable structure. Ouattara, Canh, Vachon, Mateescu, and Lacroix (101) also reported that covalent bonds formed between protein molecules under irradiation decreased WVP of caseinate-whey protein films.

C. Concentration and Molecular Weight of Macromolecule

An intact film cannot be obtained if the concentration of the film-forming macromolecule is too high or too low. If the concentration of macromolecules is too high, the film-forming solution will form a gel. If the concentration of macromolecules is too low, film will not form due to the lack of intermolecular interactions (71).

Molecular weight (MW) of cellulose also had influence on film properties. As molecular weight of HPMC increased from 22,000 to 26,000 and 86,000, WVP of film decreased. This suggested that it may be due to decreased mobility of the molecules as MW increased (102).

VII. FUTURE RESEARCH DIRECTION

Basic mechanical and barrier properties of stand-alone edible films have been extensively investigated. Various EFCs have shown high potential to be used commercially.

Composite and bilayer films, especially, received a lot of attention because of the improvement of water barrier property that they offer. Continuous research on understanding and modifying properties of composite/bilayer films are to be expected.

Besides studying the basic properties of EFC alone, the latest interest of EFC research is to investigate the feasibility of applying EFC onto different food products. Physical structure, chemical reaction between the EFC and the product, and sensory properties (103,104) of the product should further be investigated.

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Part P

Ingredients Technology

136 Seasonings and Spices

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CONTENTS

I. SEASONINGS

[1]*Seasonings are compounds, containing one or more spices, or spice extractives, and other flavour ingredients such as salt, sugar, dairy products and flavour enhancers, which when added to a food, either during its manufacture or in its preparation, before it is served, enhances the natural flavour of the food and thereby increases it acceptance by the consumer.*

The compounding of seasoning is considered a specialised, skilful art. The proper blending of such dissimilar components as spice extracts, essential oils, spices, salt, sugars, monosodium glutamate, ribotides, dairy products, and the many other components that enter into complex seasoning mixtures require:

- a high level of technical expertise and a long period of practical experience
- a list of government regulations and restrictions
- a sense of economics in the selection of the ingredients with the optimum use to which they can be used

A seasoning must be compounded in such a way that it increases the natural flavour of the product to be seasoned.

It should not overpower or diminish the product's flavour but add a balanced interest with an odour identity, a smoothly blended, rounded flavour with no perceptible undesirable aftertaste. In general the weaker the flavour of the product to be seasoned the lower the level of added seasoning required to achieve a satisfactory balance of flavour in the finished product.

A. Basic Groups of Ingredients

- Salt
- Sugar/dextrose
- Food acids — citric/sodium diacetate/lactic/malic/tartaric
- Flavour enhancers — MSG/I&G
- Herbs and spices
- Spice extracts
- Vegetable powders — tomato/onion/garlic
- Savoury — HVP/yeast extracts/reaction flavors
- Cheese/dairy powders
- Flavour top notes
- Colours
- Carriers — flour/starch/maltodextrin/lactose etc.
- Free flow agents

1. Salt

Salt is an indispensable flavouring for all snacks, sweet or savoury. In many varieties of foods it is the predominant flavour note. Hundreds of chemical compounds are classified as salts, and most of the water-soluble ones exhibit what we recognise as a salty taste, but only pure sodium chloride gives this flavour in a form not modified by sour, bitter, or sweet tastes.

In addition to having a pronounced and generally agreeable taste of its own, sodium chloride will modify other flavours. In most test situations, it has been found to enhance the sweetness of sugars and decrease the sourness of acids. In some liquid products the addition of small quantities of salt, even below the threshold level, will increase the apparent sweetness of dissolved sucrose.

Wherever practical, salt should be applied to snacks as a topping. This ensures a quickly sensed saltiness which is a primary determinant of consumer acceptability. A sufficient level (super-threshold) should be applied to yield a distinct salty flavour, but gross overstating should be avoided because it can mask or depress desirable flavour notes such as the mild sweetness of potatoes, or accentuate undesirable flavours.

2. Acid

The considerations which govern the choice of acid type and use concentration are extremely complicated. It has been demonstrated that tartness or sour taste of the common food acidulants is directly related to the molar concentration of undissociated acid.

Tartness values for various acids, as reported in the literature, vary considerably, probably reflecting differences in testing conditions. Citric acid and malic acid are quite close in organoleptic tartness value whereas tartaric acid is more tart. One part of citric or malic acid is reported to be equivalent to 0.8–0.7 parts of tartaric acid.

The acids are reported to differ somewhat in their tartness character. Tartaric is slightly bitter, citric acid gives a sharper tartness peak than malic, which gives a smooth and long lasting tartness. Some general observations on the effect of organic food acidulants:

- High viscosity reduces the organoleptic tartness.
- Organoleptic tartness drops when the free acid/sugar ratio is reduced.
- Organoleptic tartness is reduced by higher flavour level.

3. Flavour Enhancers

Savoury or Umami is the descriptive term given to the glutamate found naturally in foods or as added monosodium glutamate. It is also used to describe combinations of glutamate and nucleotides. It usually works as a flavour enhancer, that is, bringing out the flavour of the food itself.

Naturally occurring glutamate is found in foods as diverse as cheese such as parmesan, kelp, tomatoes, anchovies, and potatoes. Naturally occurring nucleotides — the main two being disodium-5′-guanylate (GMP) and disodium-5′-inosinate (IMP) — are mainly found in tuna fish, sardines, bonito, beef, prawns, chicken, and shitake mushrooms.

Nowadays, monosodium glutamate is manufactured by fermentation commercially from molasses. Nucleotides are commercially produced by the natural fermentation of tapioca starch. Commercially, MSG is the more widely used — in various food products. More recently nucleotides have started to be used either in combination with naturally occurring glutamates, or with MSG. The three most common forms of nucleotides used are:

IMP — Disodium inosinate
GMP — Disodium guanylate
Disodium ribotide (I + G) — which is IMP + GMP in a 50:50 mixture

As mentioned before, glutamates can be used alone to impart the Umami flavour whereas nucleotides require a source of glutamate to function as a flavour enhancer. Nucleotides have a very marked synergistic effect with glutamate. The glutamate can be present naturally in the food product or can be added to the food. Even though

foods naturally contain nucleotides and glutamate, they are still used in seasoning as you would use salt. Very small amounts of nucleotides are added to replace some of the MSG to give a magnification of the flavour enhancement effect.

Glutamates and nucleotides are completely water-soluble at use levels. IMP has a low moisture absorption which may be an important point when being used in dry mix products and favours. GMP has the strongest flavour enhancement and both nucleotides have high heat stability and tolerate a wide range of pH without large levels of decomposition.

Nucleotides enable the manufacturer to control excessive saltiness in snack foods such as potato crisps and other snack items. MSG can be used to reduce salt levels and give the same amount of perceived saltiness to a product by bringing out the flavour.

4. Savoury

We have many materials available to use which will enhance and/or add a savoury meaty flavour to seasonings. These products can be derived from yeasts and yeast autolysates, hydrolysed plant proteins, or meat products. Some materials add a savoury meaty flavour with no definite profile, some are specifically tailored to a profile (i.e., Chicken HVP, Beef yeast autolysate, and chicken processed flavours, etc).

Available to us also are vegetable flavoured yeast autolysates, vegetable powders, many different sugars, starches, acids, and an entire range of natural identical flavours.

II. SPICES & HERBS

[1]*Spices and herbs are aromatic natural products that are used to flavor food. Spices are the dried seeds, buds, fruit or flower parts, bark, or roots of plants, usually of tropical origin. Herbs are the leaves and sometimes the flowers of plants, usually grown in a climate similar to the Mediterranean.*

A. Classification of Spices by Sensory Characteristics

Flavour Characteristics	Spices
Alliaceous	Onion, chives, shallots, garlic
Bitter	Celery seed, curry powder, fenugreek, hops, mace, marjoram, nutmeg, oregano, rosemary, saffron, savory, turmeric
Fragrant & delicate	Bail, chives, shallots
Herbaceous	Dillweed, parsley, rosemary, saffron, sage, thyme
Pungent & hot	Capsicum, ginger, horseradish, mustard, black and white pepper
Pungent & sweet	Cassia, cloves, cinnamon
Sour, astringent	Capers
Sweet	Anise, cardamom, fenugreek, star anise
Sulfurous	Garlic, onion
Warm, fruity	Anise, bayleaf, caraway, cardamom, cumin, fennel, rosemary, savory
Warm fragrant & cooling	Basil, oregano, peppermint, spearmint
Warm heavy & aromatic	Cumin
Warm spicy and aromatic	Allspice, basil, caraway, cardamom, cassia, celery, chervil, chilli, cinnamon, cloves, coriander, dillweed, ginger, mace, marjoram, nutmeg, pickling spice, sage, tarragon, thyme, saffron
Woody	Cassia, cinnamon, cloves

B. Main Spices in Asia[1] [2]

1. Cinnamon

Cinnamon is the dried inner bark of various evergreen trees belonging to the genus Cinnamomum. At harvest, the bark is stripped off and put in the sun, where it curls into the familiar form called "quills."

Cinnamon in the ground form is used in baked dishes, with fruits, and in confections. Cassia is predominant in the spice blends of East and Southeast Asia. Cinnamon is used in moles, garam masala, and berbere.

2. Clove

Cloves are the dried, unopened, nail-shaped flower buds of the evergreen Syzygium aromaticum. They are reddish-brown in color and have a strong aroma.

Cloves are an important ingredient in the spice blends of Sri Lanka and North India. They are used in garam masala, biryanis, and pickles. In the U.S., cloves are used in meats, salad dressings, and desserts. Clove is a key flavour contributor to ketchup and Worchestershire sauce seasoning blends. Chinese and German seasonings also depend on cloves to flavour meats and cookies.

3. Cumin

Cumin is the dried seed of the herb *Cuminum cyminum*, a member of the parsley family. The cumin plant grows to about 1 to 2 feet tall and is harvested by hand. Cumin is a key component in both chili powder and curry powder.

The flavor of cumin plays a major role in Mexican, Thai, Vietnamese, and Indian cuisines. Cumin is a critical ingredient of chili powder, and is found in achiote blends, adobos, garam masala, curry powder, and baharat.

4. Fennel

Fennel is the dried, ripe fruit of the perennial *Foeniculum vulgare*. Tall and hardy, this plant has finely divided, feathery, green foliage and golden yellow flowers. Oval seeds form in clusters after the flowers have died and are harvested when they harden.

Fennel seeds are an important ingredient in seasoning blends of the Mediterranean, Italy, China, and Scandinavia. Fennel seeds may be roasted prior to incorporation into seasoning blends to intensify their flavor. Fennel is used in curry blends, Chinese five spice, mirepoix, and herbes de Provence. Fennel is also used to flavor fish, sausages, baked goods, and liquors.

5. Ginger

Ginger is the dried knobby shaped root of the perennial herb *Zingiber officinale*. The plant grows two to three feet tall. Once the leaves of the plant die, the thick roots, about 6 inches long, are dug up. Crystallized ginger is fresh gingerroot cooked in syrup and dried.

Ginger is used in Indian curries, and Chinese, Japanese, and European spice blends.

6. Red Pepper

Red pepper is the dried, ripened fruit pod of *Capsicum frutescens*, one of the most pungent capsicums. It is sometimes referred to as cayenne red pepper, having been named after the high heat chilies grown in the vicinity of the Cayenne River in French Guiana.

Red pepper adds heat and bite to seasoning blends, meats, pickles, seafood, Italian, Indian, Mexican, and Caribbean cuisines. Red pepper is used in seasoned salt, chili powder, jerk, mole negro, and berbere seasoning blends.

7. Black Pepper

Black and white pepper are both obtained from the small dried berry of the vine *Piper nigrum*. For black pepper, the berries are picked while still green, allowed to ferment, and are then sun-dried until they shrivel and turn a brownish-black color. They have a hot, piney taste.

Black pepper adds flavor to almost every food of every nation in the world. It is used in rubs, spice blends, salad dressings, and peppercorn blends.

8. White Pepper

Black and white pepper are both obtained from the small dried berry of the vine *Piper nigrum*. For white pepper, the berry is picked when fully ripe. The outer layer of shunken

skin is removed, leaving the dried, grayish-white kernel. It has a milder, more delicate flavor than black pepper.

White pepper is used whole in pickling spices and marinades. Ground white pepper is used in light colored foods such as sauces and soups. It is especially popular in European cuisine.

9. Bay Leaves

Bay leaves or laurel, are the dried leaves of the evergreen tree, *Laurus nobilis*. The elliptically shaped leaves are light green in color and brittle when dried. They have a distinctively strong, aromatic, spicy flavor. Bay leaves is the approved term for this spice, but the name "laurel" is still seen frequently.

It is used in soups, stews, stocks, pickles, marinades, tomato dishes, and meats. Mediterranean, French, Moroccan, and Turkish cuisines use bay leaves in spice blends such as bouquet garni and curry blends.

10. Chives

Chives, *Allium schoenoprasum*, are the reed-like stems of a perennial, bulbous plant of the lily family. The name "chives" is derived from the Latin *cepa*, meaning onion. Chives are a member of the onion family.

It is used in cold soups, stir-fried items, cheese and cream sauces, dips, potatoes, and as a garnish. Chives are popular in European and Chinese cuisines and in seasoning blend fines herbes.

11. Cilantro

Cilantro is the dried leaves of the herb, *Coriandrum sativum*, an annual herb of the parsley family. Also known as Chinese parsley, cilantro has a distinctive green, waxy flavor. Cilantro is the usual name for the leaf of the plant that is otherwise identified as coriander, and from which coriander seed is obtained.

Cilantro is used in salsas, chutneys, salads, dips, beans, and soups. Cilantro is used in Asian, Mexican, Indian, Tex Mex, Caribbean, and North African cuisines, and is used in seasoning blends such as masala, curry, salsa, and recados.

12. Coriander

Coriander is the dried, ripe fruit of the herb *Coriandum sativum*. The tannish brown seeds have a sweetly aromatic flavor which is slightly lemony. A zesty combination of sage and citrus, coriander is actually thought to increase the appetite.

Coriander is used in lentils, beans, onions, potatoes, hotdogs, chili, sausages, stews, and pastries. It is used in the cooking of North American, Mediterranean, North African, Mexican, Indian, and Southeast Asian cuisines, as well as spice blends, including curry powders, chili powders, garam masala, and berbere.

13. Mint

Mint leaves are dried spearmint leaves of the species *Mentha spicata*. The dark green leaves have a pleasant warm, fresh, aromatic, sweet flavor with a cool aftertaste.

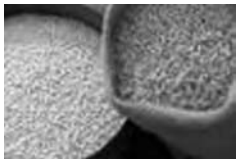
Mint leaves are use in teas, beverages, jellies, syrups, ice creams, confections, and lamb dishes. Mint is used in Afghanistani, Egyptian, Indian, and Mid-Eastern cuisines and spice blends such as chat masola, mint sauce, and green Thai curry.

14. Paprika

Paprika is the dried, ground pods of *Capsicum annum*, a sweet red pepper. It is mildly flavored and prized for its brilliant red color.

Paprika is used in seasoning blends for barbeque, snack foods, goulash, chili, and the cuisines of India, Morocco, Europe, and the Middle East.

15. Sesame Seed



Sesame is the dried, oval-shaped seed of the herb *Sesamum indicum*. Sesame seed is harvested by hand. The seeds have a rich nut-like flavor when toasted. Sesame seed contains 25 percent protein.

Sesame seeds are used to add texture and flavor to a variety of breads, rolls, crackers, and salad dressings. Middle Eastern, Muslim, and Asian seasoning blends use crushed, whole, and toasted sesame seeds for flavour and texture.

16. Turmeric

Turmeric is the dried root of the plant *Curcuma longa*. Noted for its bright yellow colour, it is related to and similar in size to ginger. Turmeric's flavor resembles a combination of ginger and pepper.

Turmeric is a powerful colouring agent. It is used to colour and flavour prepared mustard, pickles, relish, chutneys, and rice dishes as well as butter and cheese. It is also used in spice blends in the Caribbean, India, North Africa, the Middle East, and Indonesia such as curry powder and rendangs.

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2. Tiao Wei Pin Sheng Chan Gong Yi Yu Pei Fang by Zheng You Jun.

137 Sweet Flavor Application

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CONTENTS

Flavors are generally divided into two categories according to their end uses: sweet flavors and savory flavors. Sweet flavors include cola, orange, lemon, apple, strawberry flavors, etc. which are generally applied to sweet foods like beverage, dairy products, bakery products and confectionery. In some multinational companies, oral care flavors like peppermint and spearmint flavors are also divided into sweet flavors. Savory flavors include meat flavors, seafood flavors, mushroom, cheese flavors and so on, which are mainly used in savory foods such as spices, meat products, snack, seasoning, soup, etc.

I. SWEET FLAVOR CLASSIFICATION

Sweet flavors can be divided into four categories according to their existence status:

A. Water-Soluble Flavors

Ethyl alcohol, propyl glycol and water are generally used as solvents for water-soluble flavors. Solvents account for 40%～99% of the composition in the formulae. These flavors are clear and have good topnote, but they are sensitive to heat. Water-soluble flavors are wildly used in beverage, ice cream, water ice, dairy products, pectin jelly, jam, etc.

B. Oil-Soluble Flavors

Corn oil, triacetin and octyl and decyl glycerate (ODO) are used as solvent in this kind of flavors. This kind of flavor has higher concentration of chemicals, longer aroma retention ability and better stability against heating,

but they are water-insoluble. Oil-soluble flavors are generally used in biscuit, confectionery, chocolate, chewing gum, etc.

C. Emulsion Flavors

This kind of flavor is oil-in-water (o/w) flavor which contains two phases: water phase and oil phase. The oil phase is composed of flavor oils, weighting agents, vegetable oils, emulsifiers and antioxidants. The commonly used weighting agents include sucrose acetate isobutyrate (SAIB), ester gum, brominated vegetable oil (BVO), darmmar gum and elemi gum. There is strict legislation restrictions in different countries for each weighting agent. Because the specific gravity of flavor oils are generally between 0.84 and 0.87, and the specific gravity of beverage is above 1.00, weighting agents must be added to increase the specific gravity of oil phase. The specific gravity of several weighting agents is as follows:

SAIB: 1.146
Ester gum: 1.085
BVO: 1.333
Darmmar gum: 1.065
Elemi gum: 1.03

The water phase consists of water, emulsifier (e.g. arabic gum), acid, preservative, thickening agent and antioxidant.

Stokes' Law plays an important part in the formulation of emulsion flavor. The law can be expressed with the equation as follows [1]:

$$V = -\frac{2}{9} \times \frac{gr^2(d_2 - d_1)}{\eta}$$

Where:

V: the velocity of creaming or sedimentation
g: acceleration due to gravity
r: the radius of the droplet
d_2: the density of the dispersed phase
d_1: the density of the continuous phase
η: the viscosity of the continuous phase

According to Stokes' Law, the following measures can be taken to decrease the velocity of creaming or sedimentation of droplets in the finished drinks:

1. Decrease the particle size at the premise of not influencing cloudiness
2. Decrease the difference of specific gravity between the two phases
3. Increase the viscosity of the continuous phase

Emulsion flavors are wildly used in carbonated drinks, juice drinks and sports/isotonic drinks. It also can be used in ice cream, water ice, dairy products and bakery foods.

D. Powdered Flavors

This kind of flavor can be mainly divided into two categories. One is absorptive powdered flavor which is made by absorbing flavor base on carriers like maltose or maltodextrin. The other is encapsulation powdered flavor which is made by emulsifying and spray-drying the flavor base. This kind of flavor is mainly used in powder drinks.

II. FLAVOR DESCRIPTION

It's very important for food developers to give correct and precise description on flavor, which is the basic skills for food developer to create new food products. In flavor houses, primary, secondary and tertiary words are used to describe each flavor. For example, juicy, sweet, peely and other adjective words are used to describe orange flavor, so the primary word is orange, while juicy, sweet, peely and other adjective words are secondary words. If one orange flavor is very sweet with a little juicy note, it can be described as follows:

Orange (Primary)
Sweet (Secondary)
Juicy (Tertiary)

Generally, one can make the correct description on a variety of flavors after receiving at least one year' training on description.

In Table 137.1, a series of secondary/tertiary descriptive words are listed for commonly used flavors.

TABLE 137.1
Descriptive Words for Commonly Used Flavors

Primary Words	Secondary/Tertiary Words
Orange	Sweet, Juicy, Peely, Fresh, Oxidized, Aldehydic, Tangerine, Mandarin, Oily, Candy
Lemon	Juicy, Fresh, Peely, Oxidized, Oily, Candy
Coffee	Roasted, Brewed, Expresso, Sweet, Vanilla-like, Bitter, Fresh, Instant
Cola	Spicy, Citrus, Woody, Oxidized, Vanilla-like
Apple	Red, Green, Peely, Juicy, Delicious, Fresh, Ripe
Vanilla	Vanillin, Hay-like, Creamy, French, Extracted
Banana	Ripe, Green, Candy, Cooked, Spicy
Blueberry	Juicy, Perfumed, Candy, Cooked, Ripe
Grapefruit	Juicy, Sweet, Bitter, Peely
Grape	Concord, Muscat
Honey	Floral, Herbal, Perfumed, Caramellized
Mango	Juicy, Ripe, Green, Floral, Skinny
Lime	Juicy, Peely, Oily, Candy, Fresh, Soapy
Peach	Ripe, Skinny, White, Candy, Canned
Pineapple	Juicy, Ripe, Canned, Candy

III. FOOD FLAVOR, TASTE AND MOUTHFEELING DESCRIPTION AND ANALYSIS

In most cases, food developers tend to describe and analyze their competitors' successful market sample. They are interested in the following information involved in the market sample:

1. Which kind of flavor is used in the product: orange, apple or lemon if the market sample contains only one type of flavor?
2. How many types of flavors are used in this product if the product contains several types of flavor: orange + mango, orange + lemon or orange + mango + peach?
3. Once the developer confirms that the product contains certain flavor (e.g. orange), he wants to know the flavor directions (e.g. juicy, sweet or sweet plus juicy).
4. Flavor solubility. For example, juice manufacturers add orange extracted flavors in juice product, but they may add orange topnote flavor or orange oil to improve the topnote of the juice product. The beverage developer has to gauge from his experience whether it contains orange topnote flavor or orange oil.
5. Food taste, mouthfeeling and texture. Flavors play an important part in food taste and mouthfeeling. Other factors affecting taste and mouthfeeling include sweeteners, acids, emulsifiers, thickening agents, mineral elements, vitamins, etc. The food developers must judge or guess from experience which specific function in the food product is caused by which specific kind of ingredient.

Table 137.2 shows an example that describes a market coffee milk sample.

TABLE 137.2
Flavor, Taste and Mouthfeeling Description of Market Coffee Milk Drink

		Primary*	Secondary*	Tertiary*
Coffee Milk Drink	Flavor	Coffee Milk Vanilla	Roasted Powdered Creamy, extracted	Burnt
	Taste	Very sweet (judge from experience that sugar content is about 8%)		
	Mouthfeeling	Creamy, smooth, flavor lasting		

* Only suitable for flavor description.

IV. FLAVOR SELECTION

Flavor selection is the critical step for a food developer to create high-quality, popular and quality-consistent food. Much attention should be paid to the following factors during the selection of flavors:

1. Flavor supplier.
2. Legal status of flavors in different countries: natural, natural identical, artificial; Kosher or Halal; Does the flavor contain any chemical which is not allowed in the local country?
3. Flavor solubility: water-soluble or oil-soluble?
4. Flavor existence status: liquid, powder or emulsion?
5. Flavor flashpoint. Flavors with the flashpoint below 61°C are classified as dangerous goods, which are easy to fire or explode. These flavors should receive special care during the transportation, storage and usage.
6. Flavor price.
7. Flavor stability against heating, oxygen, light and storage.
8. Flavor dosage and specifications like specific gravity, refractive index, odor, color, etc.
9. Flavor combination.

A food developer generally uses two or more flavors while creating a new food, especially matching a successful food sold well in the market. He has to use another flavor to complement the note which one flavor lacks; or use oil-soluble flavor to produce topnote in juice products although water-soluble flavor has been used. Flavor combination also causes the greatest difficulties for the competitors to match the market successful product.

V. SWEET FLAVOR APPLICATION

In this chapter, we will concentrate on the application of sweet flavors to beverages, dairy products (e.g. yogurt, yogurt drinks, ice cream, water ice) and confectionery.

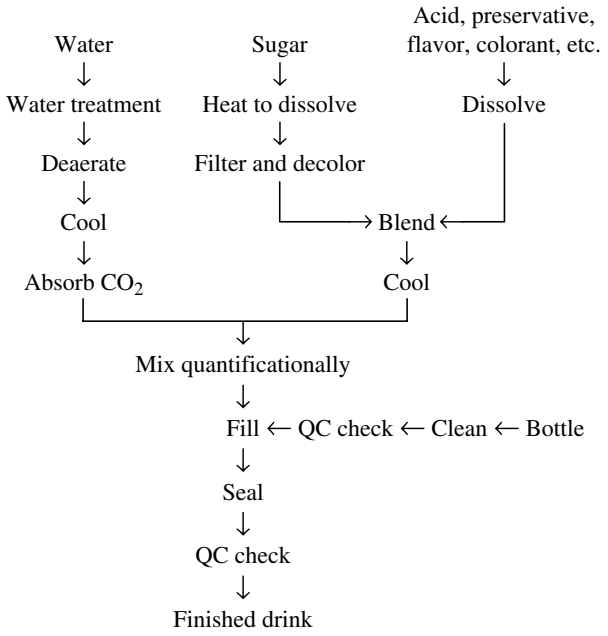
A. In Beverages

The ingredients of beverage consist of water, sweetener, acidulant, colorant, flavor, stabilizer, thickener, antioxidant, concentrated juice, mineral element, preservative, etc. Flavors for beverage must be water-soluble. Generally, the ratio of flavor in the finished drink is between 0.01%~0.3%, but the needed dosage will depend on flavor category, beverage category and other factors. Although flavor only occupies a small ratio in the finished drink, it plays a critical role in the beverage flavor and acceptability.

1. In Carbonated Drinks

a. Process flowchart of carbonated drinks

There are two kinds of process for carbonated drinks: one time filling and two times filling. One time filling is much more popular than two times filling due to its consistent quality of the finished drink. Two times filling has been phased out by most beverage companies because the quality of the finished drink is inconsistent. The process flowchart of one time filling is as follows [2]:



b. Formula examples

Example I: Carbonated cola drink

The syrup:	
Sugar	650.0 g
Phosphoric acid 85%	3.60 g
Caffeine	0.60 g
Color	to suit
Cola flavor	as needed
Water to make	1000 ml
Throw: 1 + 5	

The syrup: brix = 53°
The finished drink: brix = 10.6°; volume of CO_2 = 4.

Example II: Carbonated orange flavored drink

The syrup:	
Sugar	778.0 g
Sodium benzoate	1.50 g
Citric acid	9.00 g
Sunset yellow	0.175 g
Tartarine	0.040 g
Orange emulsion	~1.5 g
Water to make	1000 ml
Throw: 1 + 5	

The syrup: brix = 63.4°; acid% = 0.90%;
The finished drink: brix = 13.1°; acid% = 0.15%.

Two kinds of orange flavors can be used in carbonated drink: orange emulsion and water-soluble orange flavor, but the latter generally can't be used solely in carbonated orange drink because it is a washed flavor and has relative weak flavor profile. The latter is generally used to improve body and bottom note. Orange emulsion can be solely used or combined with water-soluble orange flavor.

2. In Juice Drink

a. Process flowchart of juice drink

Treated water and sugar, acid, juice concentrate, etc. → Deaerate → Homogenize(170/40 bar) → Sterilize (105°C, 15S) → Fill (88°C) → Seal → Cool → QC check → Finished drink.

b. Formula example: 10% orange juice drink

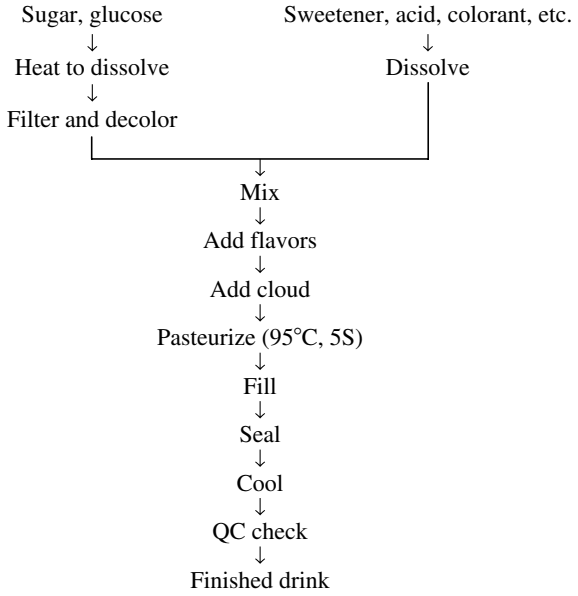
Sugar	90.28 g
Citric acid	1.63 g
Orange juice concentrate (8X)	12.5 ml
CMC(FH9, acid-proof)	1.0 g
β-Carotene emulsion (1%)	to suit
Orange emulsion	~1.0 g
Orange topnote	~0.2 g
Water to make	1000 ml

The finished drink: brix = 9.50°; juice content (%) = 10%.

Three kinds of orange flavors can be used in orange juice drink: orange emulsion, orange topnote flavor and washed orange flavor. Orange topnote flavor is used to improve the topnote, which dosage in the juice drink is 0.01%~0.02%; orange extract flavor improves body and bottom note, which dosage is 0.02%~0.08%.

3. In Sports Drink and Isotonic Drink

a. Process flowchart of sports drink and isotonic drink



b. Formula example: lemon flavored sports drink

Sugar	50.0 g
Glucose	15.0 g
Acesulfame potassium	0.14 g
Potassium citrate	0.30 g
Sodium citrate	0.30 g
Citric acid	1.60 g
Malic acid	0.80 g
Lemon washed flavor	~1.00 g
Cloud	~1.00 g
Water to make	1000 ml

The finished drink: brix = 6.8°; total acid % = 0.24%.

4. In Coffee Mix Drink

a. Process flowchart of coffee mix drink

Sugar and emulsifier → Premix → Dissolve in hot water → Dissolve milk powder and starch → Add coffee extract → Dissolve sodium citrate, etc. → Add flavor → To make 1000 ml → homogenize @170/40 bar → Fill → Sterilize @121°C, 20 min → Cool → QC check → Finished drink.

b. Formula example

Sugar	75.0 g
Whole milk powder	18.0 g
Coffee extract (brix = 51°)	14.0 ml
Sugar ester P1670	0.40 g
Sodium citrate	0.30 g
Sodium ascorbate	0.30 g
Instant textra (Nation starch)	2.0 g
Vanilla flavor	0.40 g
Milk flavor	0.33 g
Coffee flavor	0.85 g
Water to make	1000 ml

The finished drink: pH = 6.6; brix = 11.0°.

5. In Powder Drink

a. Process flowchart of powder drink

Sugar, acid, colorant, anti-caking agent, powder flavor, etc. → Blend → Sieve → Package → QC check → Finished powder drink.

b. Example formula: mango flavored powder drink

Sugar	90.0 g
Citric acid	2.40 g
Ascorbic acid	0.30 g
Lake color #5	0.004 g
Lake color #6	0.006 g
Sodium citrate	0.30 g
Anti-caking agent	0.40 g
Malto dextrin	30.5 g
Powdered cloud	~0.30 g
Powdered mango flavor	~1.0 g
Total	125.0 g

Use: 125 g dilute to 1000 ml for drink.

B. In Dairy Products

Yogurt, yogurt drinks, acidified milk drinks, ice cream and sherbets are classified as dairy products. Raw materials used in dairy products include the following:

Fat: milk fat, cream, butter, butter oil, vegetable oil (sunflower oil, soybean oil, rapseed oil, etc.)
Nonfat milk solids (NMS): proteins, lactose, mineral salts.
Sweeteners: sucrose, glucose, HFCS, etc.
Stabilizers: gelatin, pectin, xantham, carrageenan, agar-agar, CMC, guar, locust bean, sodium alginate, karaya, etc.
Emulsifiers: monoglycerides, diglycerides, etc.
Flavors: commonly used flavors in dairy products include coffee, milk, vanilla, nutty flavors (i.e. hazelnut), fruit flavors (i.e. orange, pineapple, strawberry), chocolate, cream, bean (i.e. green bean, red bean), ube yam, melon, etc.
Water and air
Juice preparations: orange, apple, strawberry, pineapple, banana, etc.
Yogurt cultures
Acids: citric acid, lactic acid, etc.
Colorants
Others

1. In Yogurt Drink

a. Process flowchart of yogurt drink

Skimmed milk
↓
Pasteurize (e.g. 90~95°C for 3~5 minutes)
↓
Culturing to pH 3.8~4.2
↓
Cool to about 20°C
↓
Slowly agitate for minimum 15 minutes
↓
Control pH 3.8~4.2
↓
Pasteurize at 90~95°C for 10~15 seconds
↓
Homogenize at 150~200 kg/cm^2
↓
Cool to filling temperature
↓
Aseptic filling

← Fruit juice, Pectin dispersion, Sugar, flavor, etc.

b. Formula example: strawberry yogurt drink

Skimmed milk yogurt	843 g
Fruit juice (50% sugar)	120 g
Pectin dispersion*	35 g
Strawberry flavor	~1.0 g
Yogurt flavor	~1.0 g
Total	1000 g

*Pectin dispersion: 3.5 g pectin is dispersed in 31.5 g 65% sugar solution at high mixing speed.

The finished yogurt drink:
Fat: 7.6%;
NMS: 8.0%;
Sugar: 0.35%;
Total solids: 15.95%.

2. In Ice Cream

a. Process flowchart of ice cream

Mix at 50~60°C
↓
Pasteurize (e.g. 80~85°C for 20~25 seconds)
↓
Homogenize (e.g. 140/40 bar at 75~80°C)
↓
Cool to 4°C
↓
Age below the temperature 4°C
| ← Add flavor, color, etc.
Freeze (e.g. 5.55°C at the outlet from the freezer)
| ← Incorporation of fruit, nut, cookie solids, ripple, etc.
Harden to −30 ~ −35°C
↓
Store below the temperature −17°C

b. Formula examples

Table 137.3 displays typical formulas for ice cream in different regions.

C. In Confectionery

Market confectionery products can be mainly sorted as: hardboiled candy, chewy sweets (soft candy), gummy candy, pectin jellies, chewy and bubble gums, chocolate, pressed tablet candies, jellies and marshmallows.

Raw materials used in confectionery products involve:

Sweetener: castor sugar, glucose syrup, invert syrup, synthetic and natural sweeteners
Fat: butter, hydrogenated vegetable oil, cocoa butter
Dairy products: fresh milk, butter cream, milk powder, condensed milk
Gelling agents: starch, agar, gelatin, pectin, etc.
Emulsifiers: lecithin, glycerol mono stearate, sucrose esters of fatty acids, span, tween, etc.
Acid: citric acid
Flavors: orange, lemon, strawberry, mint, blueberry, nutty flavors, etc.
Nuts and fruits products
Colorants
Others

TABLE 137.3
Typical Formulas of Ice Cream in Different Regions [5]

Ingredients	U.S.A	Europe	Asia
Milk fat	10.0%	8.0%	6.0%
NMS	10.5%	10.5%	7.0%
Sucrose	12.0%	12.0%	14.0%
Glucose solids (36DE)	6.0%	—	5.0%
Glucose solids (42DE)	—	3.0%	—
Stabilizer/emulsifier	0.4%	0.5%	0.4%
Flavor	~0.1%	~0.1%	~0.1%
Total solids	38.9%	34.0%	32.4%
Overrun	80–100%	100–130%	70–90%

1. In Hard Candy

a. Process flowchart of hard candy

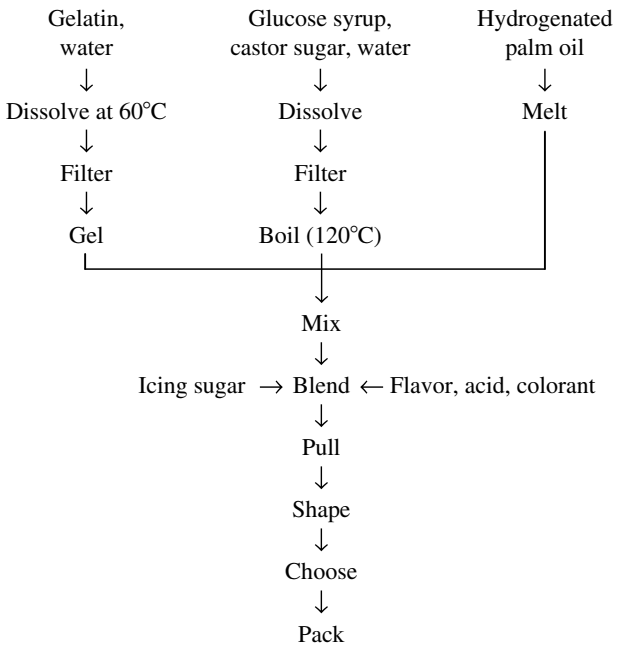
Castor sugar, glucose syrup, water → Dissolve → Filter → Boil → Cool → Add flavor, acid and color → Blend → Cool → Desposit → Cool → Select → Pack

b. Formula example: orange flavored hard candy

Castor sugar	57.0%
Glucose syrup	29.7%
Water	13.1%
Orange flavor	~0.3%
Color	to suit
Total	100%

2. In Chewy Sweets

a. Process flowchart of chewy sweets



b. Formula example: peppermint flavored chewy sweets

Castor sugar	34.36%
Water	18.38%
Glucose syrup	43.23%
Hydrogenated palm oil	3.06%
Emulsifier	0.55%
Gelatin 150 bloom	0.22%
Color	to suit
Peppermint flavor	~0.2%
Total	100%

3. In Chewing/Bubble Gum

a. Process flowchart of chewing/bubble gum

Gum base → Intenerate → Mix → Roll → Shape → Pack
↑
Icing sugar, flavors, other ingredients

b. Formula example: blueberry flavored chewing/bubble gum

Gum base	19.7%
Icing sugar	59.7%
Glucose syrup	19.8%
Glycerin	0.5%
Blueberry flavor	~0.3%
Total	100%

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138 Food Emulsions

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CONTENTS

Only the simplest foods are present as one continuous phase. In practice, much of the texture and other complex behavior of food systems arises from phase heterogeneity. A common and relatively simple form of heterogeneity is when the food is present as a dispersed system — small particles of one material (or phase) in a second continuous phase [1]. We are concerned with that subset of dispersed systems where one of the phases is lipid and the other aqueous, i.e., emulsions. Some examples of water-in-oil food emulsions include milk, ice cream mix, mayonnaise, salad dressings, soups, beverage emulsions, and flavor emulsions. The classic definition of an emulsion requires both phases to be liquid but in foods this definition is frequently expanded to include systems where one or more phases are solid. It is possible to make an emulsion with either a lipid or an aqueous dispersed phase and this distinction largely governs the overall properties of the emulsions (e.g., a lipid-continuous system will disperse poorly in water, and have a low conductivity and a greasy mouthfeel). However, in this work we will focus on aqueous-continuous emulsions.

We will describe the structure of food emulsions and the effect this structure has on the properties of the food. We will consider the mechanisms of emulsion (de)stabilization and finally the effects of food processing on emulsion structure. Our goal in this work is to provide an accessible introduction to the main issues essential to the engineering of food emulsions, but clearly such a brief treatment cannot be comprehensive. In Table 138.1 we provide a selected bibliography of sources that may be helpful for further exploration of the subject.

I. EMULSION STRUCTURE

Figure 138.1 shows an optical micrograph of a coarse food emulsion. The oil is present in spherical droplets in a continuous aqueous phase. The interfacial layer, although insignificant volumetrically, is essential to governing the

TABLE 138.1
Bibliography of Selected Further Reading in Food Emulsions

Reference	Comments
"Colloids in Food" [54]	Although somewhat dated, the treatment of the fundamentals underlying the chemistry of food colloids remains unrivalled.
"An Introduction to Food Colloids" [55]	A simplified text covering many of the topics from #1.
"Foundations of Colloid Science" [27]	Highly technical and comprehensive on the fundamentals of dispersion technology. Particularly useful discussion of surfactants and self-assembled colloids.
"The Colloidal Domain" [16]	A good general reference particularly in the fields of the properties of surfaces and surfactants.
"Advances in Food Colloids" [56]	A useful update to #1.
"Food Emulsions: Principles, Practice and Techniques" [24]	Particularly strong in the application of colloidal force arguments to food systems and analytical methods.
"Physical Chemistry of Foods" [57]	Comprehensive treatment of all of the main aspects of food physical chemistry. Especially relevant are chapters 9–13 on aspects of dispersed systems.

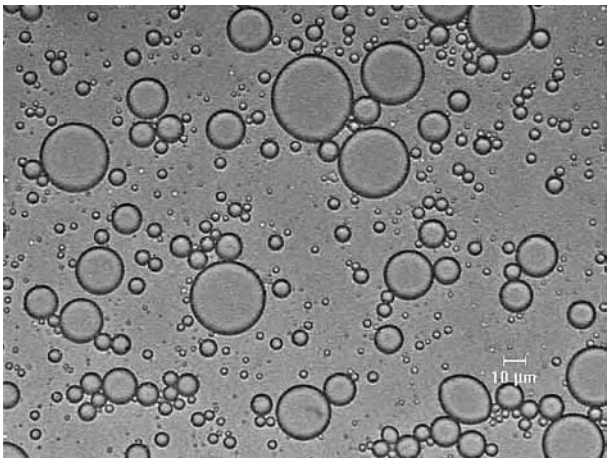

FIGURE 138.1 Optical micrograph of a coarse food emulsion. The oil is present in spherical droplets several 10 s of micrometers in diameter (most food emulsions would be an order of scale finer) surrounded by an aqueous phase. The interfacial layer is seen as a thick line here, but that is an optical artifact. In reality the interfacial region discussed in the text would only be a few nanometers thick.

overall properties of the system. Although all emulsions must have an aqueous and a lipid phase and an interface between them, they may vary widely, and it is useful to develop a vocabulary to describe their microstructure.

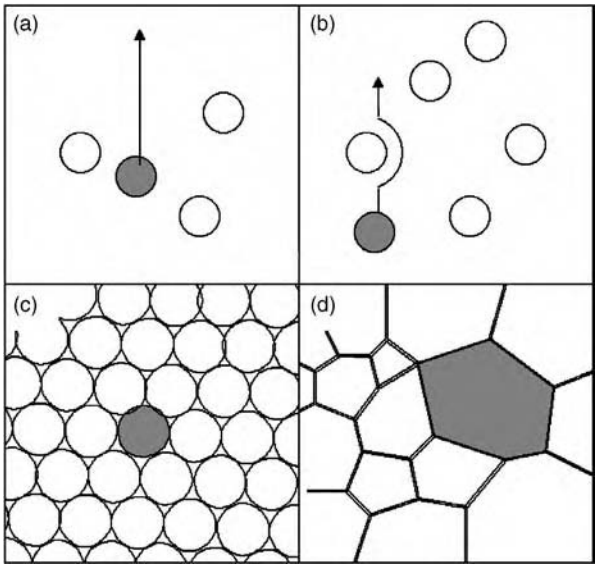

FIGURE 138.2 Schematic diagram showing the effect of volume fraction on the structure and dynamics of an emulsion. (a) A dilute emulsion, the movement of the highlighted particle is not affected by other particles. This would be realistic up to a few percent volume fraction. (b) In a more concentrated emulsion ($\phi \sim 10\%$) the movement of the highlighted droplet would be slowed by interactions with other droplets and the system would be more viscous and slower creaming than predicted from an extrapolation of the properties of the dilute system. (c) An ideally close packed emulsion where the particles are packed into a hexagonal array. The indicated droplet cannot readily move relative to the other droplets and the bulk material may develop elastic properties. Further concentration yields (d), a fluid foam where the droplets are distended into polyhedral shapes separated by narrow lamellae. Note ideal compression of (c) would yield a regular hexagonal structure but the polydisperse structure shown in (d) suggests some of the lamellae have ruptured to allow some coalescence.

First the amount of dispersed phase is typically expressed as a mass or volume fraction:

$$\phi_m = \frac{m_o}{m_o + m_a} \qquad \phi_v = \frac{v_o}{v_o + v_a} \tag{138.1}$$

where ϕ_v and ϕ_m are the volume and mass fraction of dispersed phase and v and m are the volume and mass of the oil (subscript o) and aqueous (subscript a) phases. Mass and volume fractions are readily interconvertable knowing the density (ρ) of each phase. Droplet volume fraction can vary from zero to approaching 100% (Figure 138.2). As the volume fraction increases, the particles increasingly interact with one another until they are close packed (as shown in Figure 138.2). The maximum theoretical close packing of identical spheres is 0.7405, but in reality this type of highly organized structure does not occur and random close packing occurs at much lower volume fractions ($\sim$0.64). Given an appropriate droplet size distribution (including particles approaching zero diameter) it would

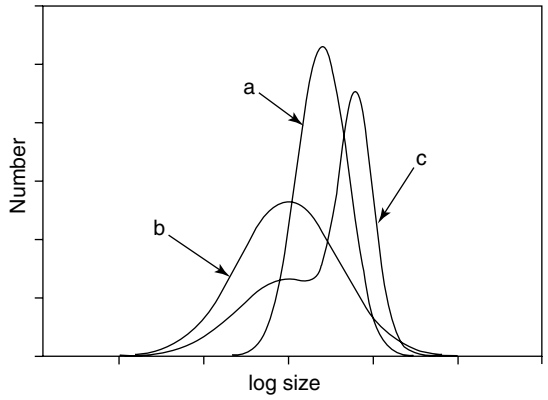

FIGURE 138.3 Typical particle size distributions seen in emulsions. (a) has a larger median diameter than (b) but (b) is more polydisperse and so has more larger particles and may be less stable than (a). (c) is bimodal, very often the larger population of droplets is formed due to the flocculation of smaller droplets.

be possible to achieve a much higher volume fraction but Princen [2] points out that in practice most real polydisperse distributions do not exceed the perfect close packing of homogeneous spheres. Droplet volume fractions beyond close packing are only attainable by deforming the spherical droplets.

Emulsion droplets are spherical (under all but the most extreme conditions) so can be characterized with a single length dimension. Real emulsions are polydisperse, i.e., contain droplets of varying sizes, and thus it may be more useful to represent their size as a distribution or in terms of a mean and polydispersity. For example a typical log-normal size distribution is shown in Figure 138.3a. Figure 138.3b has a lower median diameter, but as it has a broader distribution. It has more large droplets and may be effectively less stable. Figure 138.3c shows a bimodal distribution formed from the overlap of two polydisperse emulsions. Distributions such as Figure 138.3c are often seen in partly aggregated emulsions. Most stable emulsions have average diameters less than a few micrometers, and few are smaller than 100 nm.

A. Lipid Phase

The lipid phase is largely a mix of triacyglycerol molecules of animal or plant origin along with (typically) minor constituents of the lipids including free fatty acids and mono- and di-glycerides. Food lipids typically crystallize over the range of temperatures found in food processing and storage so we may also be concerned with semi-crystalline droplets. In general the melting point of a fat decreases with decreasing chain length and increasing unsaturation. Fats cooled below their melting point may crystallize, but the crystallization process is often very slow. Fat crystallization is further complicated by the fact that there are often several stable solid forms under given conditions and their rate of formation and interconversion may vary widely [3]. In the emulsified state the situation is further complicated as the fat is isolated into many self-contained droplets. In bulk fat it would require in principle only one nucleation event to crystallize all of the fat present but in the emulsified state each droplet must nucleate independently. In the most extreme case, each droplet is effectively pure and nucleation must occur homogeneously in the droplet [4]. Homogeneous nucleation is typically very slow and very large supercooling can occur when the number of droplets is much larger than the number of effective nucleating impurities. More commonly in foods there are sufficient impurities to allow most droplets to nucleate heterogeneously but this often still requires significant supercooling. Moreover, Walstra [5] pointed out that many real food emulsions contain several fat crystals, which implies multiple nucleation events per droplets. He argued this most likely occurs by secondary nucleation, the detachment of nuclei from the surface of a growing crystal.

The pressure of the oil droplet surface also affects the development of crystal structures within the droplet. Lopez and co-workers [6, 7] showed that crystals are more disordered in the emulsion droplets than in bulk, presumably due to the physical constraints of the surface. The effect of the droplets was most strongly seen in the less stable crystal forms. These and other workers have also seen an emulsified fat crystallizes into an α-form more readily than the same fat in bulk [6–8].

A second major reaction we may be concerned with in emulsion lipids is oxidation [9]. Lipid oxidation can lead to rancid off-flavors and -odors that can spoil food when present at very low levels. Further oxidation can lead to the formation of potentially toxic compounds and can also degrade the nutritional value of foods. In general oxidation proceeds through a radical reaction between an unsaturated lipid and oxygen. Radicals are generated by interactions of metals, the action of light, or enzymatic activity and the reactivity of lipids increases greatly with the number of double bonds present. The reactivity of emulsified lipids is notably different from the bulk properties of the same lipid and offers new ways to control the reaction [10]. While oxygen is usually present in significant quantities in both the lipid and aqueous phases, some of the important oxidation catalysts are concentrated in one or either phase. Notable here are aqueous metal ions which are only effective catalysts of lipid oxidation when they can approach the lipid droplets. Consequently emulsion droplets with negatively charged surfaces (e.g., proteins pH $>$ pI, sodium dodecyl sulfate) were much more prone to catalysis by iron cations than positively charged droplets. The actions of antioxidants are also different in dispersed systems. Frankel noted the "polar paradox" in which oil-soluble surfactants are more effective in stabilizing dispersed lipids while water-soluble surfactants are more effective in bulk [11]. He argued that in both cases

the antioxidant concentrates in the portion of the system most prone to oxidation (i.e., the emulsion droplets or the surface of the bulk respectively) where they can be most effective.

B. Aqueous Phase

The aqueous phase contains the water-soluble food ingredients including simple sugars and salts. Other ingredients may be largely water or oil soluble but partition to some extent between the two phases according to their partition coefficients: the ratio of the activity of the solutes in lipid and in water. Very often we are concerned with highly dilute additives such as flavors and antioxidants and it is reasonable to express a partition coefficient in terms of a ratio of concentrations. Lipids and sugars have partition coefficients close to infinity and zero, respectively but many other food ingredients have some solubility in either phase and while their equilibrium concentrations remain constant, individual molecules move dynamically between phases.

The properties of the aqueous phase can also affect the interactions between the emulsion droplets. For example, the change on an ionizable group is a function of pH and so the amount of charge carried on a protein-stabilized emulsion droplets will decrease from a positive to a negative plateau value as the pH is increased (Figure 138.4). The ionic strength ($=\frac{1}{2}\Sigma cz^2$, where c is the concentration of an ion of valence z) decreases the range of effective electrostatic interactions so they will be less important in salty systems. Some other components, particularly sugars, can act as co-solutes and stabilize proteins against thermal denaturation [12]. They achieve this by structuring the water around the hydrophobic groups on the protein and the same effect can reduce the hydrophobic drive to aggregation amongst denatured proteins.

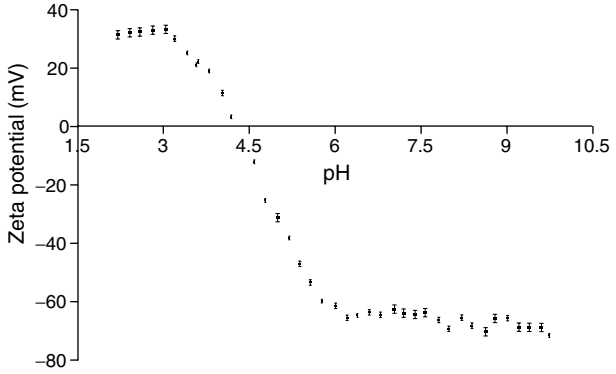

FIGURE 138.4 Effective surface charge (ζ-potential) on fine hexadecane emulsion droplets stabilized with whey protein as a function of pH. The charge on the droplets is governed by the charge on the protein molecules and follows a typical sigmoidal shape with pH.

C. Interface

The fact that oil and water are immiscible and have a clear interface (Figure 138.1) stems from the strong water-water attractive force. A water molecule is so strongly attracted to its peers that it will overcome the entropic drive for mixing with oil and remain as an isolated phase [13]. (In reality there is some small solubility of oil in water and although at the bulk scale an interface is a clear line separating the two phases, at the molecular level there is local mixing and an interfacial region where the concentration of both phases changes from its bulk value to its solubility in the second phase. It is possible to draw a Gibbs interface in this region so that the excess concentration on one side of the interface is equal to the deficit concentration on the other). The tendency of water and oil to demix also leads to a tendency to minimize the interfacial area because each surface water molecule is more exposed to the unfavorable oil phase than a corresponding molecule in bulk water [14]. The energy cost to increase the surface area of a system is manifested in the surface tension, a force opposing surface expansion:

$$\gamma = \frac{dG}{dA} \tag{138.2}$$

where γ is the interfacial tension, G is the surface excess free energy, and A is the surface area. The role of interfacial tension pushing against surface expansion also means droplet interiors are somewhat compressed (i.e., the Laplace pressure). Increased pressure can increase the solubility of lipid components and helps maintain the droplet's spherical shape against applied forces.

The unique molecular environment of a surface allows the accumulation of surface-active ingredients. Surface-active molecules have part of their structure water soluble and part oil soluble. Important examples in food emulsions are predominantly proteins [15] and to a lesser extent small molecule surfactants [16] and some hydrocolloids [17]. If a surface-active molecule is added to a two-phase system it can either remain free in one or both phases (enthalpic disadvantageous) or adsorb to the interface (entropically disadvantageous) [14]. The competition between terms means the amount of adsorbed surfactant will increase non-linearly with added concentration. The maximum amount of sorbed surfactant is defined by packing concerns at the surface but typically for food proteins is in the order of a few $mg\,m^{-2}$. Adsorbed surfactant shields the immiscible water and oil phases from one another and so decreases the interfacial tension from about $30\,mN\,m^{-1}$ for a clean oil-water surface to a surfactant-dependent minimum at surface saturation [1]. The most important functional roles of the interfacial layer in controlling emulsion functionality are to: (i) lower the interfacial tension to ease the formation of the emulsions, (ii) self-repair incipient holes in the lamella separating two approaching droplets via the

Gibbs-Marangoni effect, and (iii) provide a basis for repulsive colloidal forces between droplets. This final point is crucial in limiting destabilization by aggregation (see below).

Surface binding is not instantaneous. The potentially surface active molecule must first diffuse to the surface then "react" and bind. Diffusion is faster for smaller molecules so small molecules will usually develop an interfacial layer in a few seconds while a protein layer would take several minutes for the process to complete [14]. Although the binding of a single hydrophobic group to a surface is a spontaneously reversible event, the multiple binding of several sites on a polymer means that proteins will not typically spontaneously desorb from a surface. However if a more surface active material is added (i.e., small molecule surfactants) it may displace the protein from the interface by a competitive adsorption process. The surface protein concentration is not affected by small amounts of added surfactant, but will be completely desorbed following a small further increase [18]. Gunning and co-workers used atomic force microscopy to image protein displacement from a mica surface and saw that the initial portion of desorption the surfactant accumulated at the surface and pushed the protein into an increasingly thick and dense network surrounding the droplets [19]. At a critical level the surface protein network ruptured and detached from the surface. These workers termed this orogenic process as an analogy to the process of the formation of continents from tectonic plate activity. Oil soluble surfactants are less effective than aqueous surfactants at displacing proteins [20].

When globular proteins bind to a surface they often slightly rearrange their structures to better configure to the interfacial environment and as a result are often denatured. The interfacial protein concentration is relatively high even if the bulk concentration is relatively low and the properties of a concentrated protein solution are seen in two dimensions at the interface [21]. The proteins can cross react and form a viscoelastic two-dimensional gel that can improve the stability to coalescence. The same types of interprotein bonds responsible for bulk protein gelation (e.g., disulfide, hydrophobic) have been identified in the two dimensional surface analogue. Mixed protein films can phase separate in two dimensions just as a thermodynamically incompatible polymer mixture can in bulk [22, 23].

D. Formation of Emulsions

Emulsions can only be formed from bulk oil and aqueous phases through the application of amounts of energy. Some of the energy is used to create oil-water surface free energy but the bulk is "wasted" as heat losses in turbulent flow. Typically a coarse emulsion premix is made by blending or shaking the ingredients together. The particle size of the premix is very large, typically several (1–100) micrometers, and so they have a very short shelf life. However, the goal here is to produce a transiently homogeneous mixture that can be fed through a higher-energy secondary homogenization step for further particle size reduction to the desired final goal [24]. The second stage of homogenization can be achieved by a variety of technologies, e.g., colloid mill, ultrasonication, high pressure valve homogenizer, or microfluidizer, each of which enjoy various advantages and disadvantages but all serve to apply a critical mechanical stress to the droplets.

As noted previously, emulsion droplets are spherical to minimize their surface area and are somewhat pressurized. To disrupt a droplet it must be stretched and eventually fractured and to achieve this the forces input from the homogenizer via the continuous phase must exceed the cohesive forces of the droplets (Figure 138.5) [25]. The cohesive strength of a droplet increases with surface tension and decreasing particle size so one of the main roles of a surfactant in facilitating homogenization is to reduce interfacial tension. The relationship between the applied forces and the operating conditions of the homogenizer depend on the type of flow occurring (i.e., laminar or turbulent) but generally particle diameter is believed to be inversely proportional to either shear rate in laminar flow or (power density) in turbulent flow [24]. It is possible for the homogenizer to very effectively fragment particles but there may be little or no net effect on the particle size because the newly formed droplets take a finite time to become coated in surfactant and may recoalesce before this can happen. Thus the second role of surfactant in homogenization is to rapidly diffuse to and adsorb to newly created

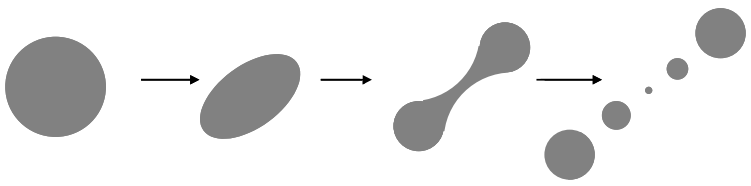

FIGURE 138.5 Schematic diagram showing the effect of applied force on a droplet. Initially the droplet is spherical but deforms into a spheroid under the applied force. Eventually a neck forms in the shape and fractures to form many smaller droplets. The small droplets formed can quickly recoalesce if they are not protected by surfactant or protein diffusion to the interface.

surfaces to protect them before any droplet-droplet collisions occur.

II. PROPERTIES OF FOOD EMULSIONS

We are rarely interested in food microstructure for its own sake but rather as a way to understand the sources of food quality [26]. So for food emulsions we are most concerned with how they affect perceived food texture, color, and flavor.

A. Texture

A dispersed system is always more viscous than the pure continuous phase due to the increased friction between the particle and the liquid layers causing greater energy dissipation. In a highly dilute, uncharged suspension containing non-interacting solid particles (volume fraction ϕ) the relative viscosity (η', i.e., apparent viscosity normalized to that of the dispersed phase) is given by the Einstein relationship (Figure 138.6):

$$\eta' = 1 + 2.5\phi \tag{138.3}$$

The "hard-sphere" assumption may be acceptably valid when a thick surfactant layer limits the transmission of flow from the continuous to dispersed (liquid) flow but in most real foods there are significant particle-particle interactions that will further increase the effective viscosity (Figure 138.2b). Extensions to the Einstein equation are available to account for paired and multi-body interactions.

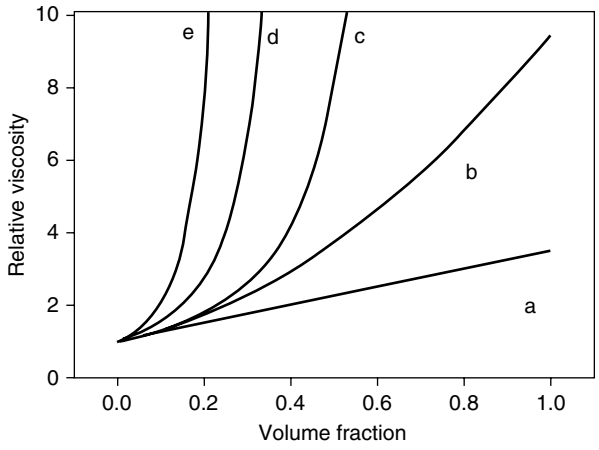

FIGURE 138.6 Theoretical relative (i.e., normalized to the continuous phase) viscosity of an emulsion as predicted by (a) the Einstein equation, (b) a modified version of the Einstein equation including a second order term in volume fraction, and (c) the Krieger-Dougherty relation. (d) and (e) were calculated assuming the emulsion was flocculated. (e) and (f) were calculated assuming the emulsion was flocculated into flocs 10 times the primary particle radius with fractal dimensions 2.8 and 2.6 respectively.

$$\eta' = \sum_{i=0} k_i \phi^i \tag{138.4}$$

where k_i are constants (1, 2.5, ~6, …). Using the first two powers Equation 138.4 reduces to Equation 138.3 and even using the third power it is only useful up to about 15 vol% [24]. At higher concentrations it is common to resort to empirical and semi-empirical expressions such as the Krieger-Dougherty relation:

$$\eta' = \left(1 - \frac{\phi}{\phi_{\max}}\right)^{-2.5\phi_{\max}} \tag{138.5}$$

where ϕ_{max} is a volume fraction approximating (0.6–0.7, Figure 138.2c) close-packing [27]. The ϕ_{max} parameter can be allowed to relax between a high and low shear value to account for non-Newtonian behavior. The Krieger-Dougherty relation tends towards infinity at $\phi = \phi_{max}$ and gives no meaningful solution beyond that point. However highly concentrated emulsions have a real viscosity and also develop significant elastic properties (e.g., mayonnaise). In order to maintain the very high levels of dispersed phase (often >80%) the droplets are distended and the microstructure approaches that of a foam with many polyhedral droplets in very close association (Figure 138.2d) [2].

B. Flavor

Our perception of food flavor comes largely from volatiles released into the headspace in the mouth and detected by nerves at the back of the nose. As well as being volatile, many food flavors have a significant solubility in oil and aqueous phases so the presence of emulsified lipid can affect the kinetics and thermodynamics of their release and therefore our overall flavor perception. Considering the partitioning of a volatile between the oil, water, and headspace in a food emulsion we can quickly see the effects of fat content on the perceived flavor of foods (here presumed to be related to the absolute headspace concentration). Using the subscripts o, w, e, and g to refer to the oil, aqueous, overall emulsion, and headspace gas phases respectively we can define an effective partition coefficient between the emulsion and the headspace) [28].

$$K_{ge} = \frac{K_{gw}}{1 + (K_{ow} - 1)\phi} \tag{138.6}$$

Figure 138.7a shows the effect of oil concentration on the headspace concentration of a number of different flavors with different partition coefficients (partition coefficient data from [24, 28]). The partitioning of the non-polar flavors (octanal, heptan-2-one) between the headspace and the emulsion decreases with oil concentration, the partition coefficient of the more volatile flavor (ethanol) increases. This is logical as oil provides a better reservoir for the non-polar flavors than the polar flavors. While volatile concentration does not necessarily correlate with sensory appreciation of flavor [29], this simple calculation shows the

magnitude of the changes that can be caused by changing the emulsion structure. For example, van Ruth and Roozen [30] studied the headspace concentration of volatiles above an oxidized sunflower oil in water emulsion and noted that while more volatiles partitioned into the headspace above the emulsion than the corresponding bulk fat, the relative change was different for different aromas. In another example fat content was shown to increase the amount of a non-polar flavor (linalool) released more than the polar flavor (diacetyl) [29].

Partition coefficients are thermodynamic constants but very often we are concerned with the dynamics of flavor release. Harrison and co-workers [28] proposed a mathematical model for the release kinetics of flavor from an emulsion. They assumed that as the droplets are relatively small, diffusion in the droplets and hence partitioning between oil and water occurs relatively fast, leaving the transport across the emulsion-headspace barrier as the rate-limiting step. Assuming the emulsion is well mixed they showed the concentration of gaseous volatile (c_g) is given as:

$$\frac{c_g(t)}{c_g(\infty)} = 1 - \exp\left\{-\left(1 + \frac{v_e}{K_{ge}v_g}\right)\frac{h_D A_{ge}}{v_g}t\right\} \quad (138.7)$$

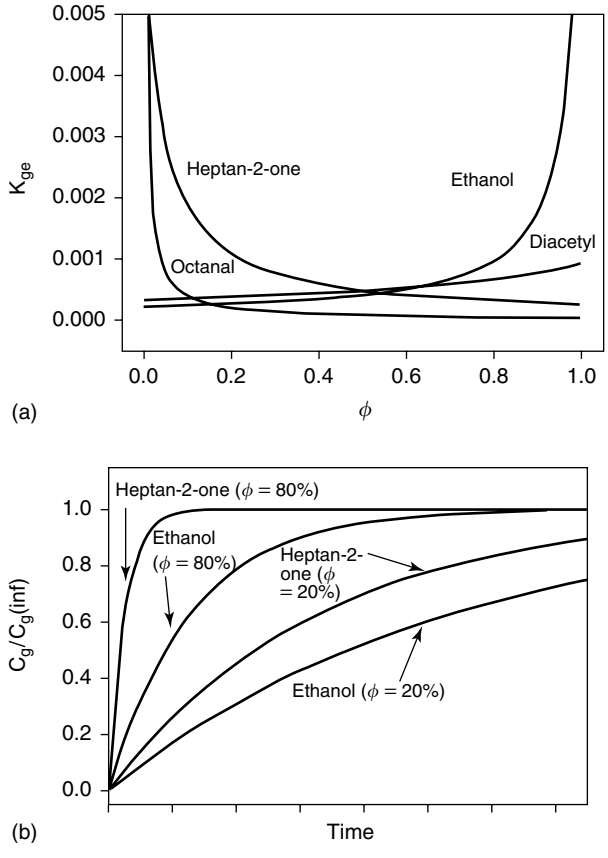

FIGURE 138.7 Calculated (a) partition coefficients and (b) flavor release kinetics for four volatiles as a function of emulsion volume fraction.

where v is the phase volume, K is the partition coefficient, t is time, h_D is the mass transfer coefficient in the emulsion, and A is the interfacial area of the emulsion surface (not the surface of the individual droplets). Emulsion particle size and volume fraction only entered into their formulation as terms controlling the viscosity of the emulsion (and hence h_D). Neglecting this effect, this approach clearly shows the effect of K_{ge} and hence oil content on the release kinetics. Some calculated rates of release of the aroma with partition coefficients calculated are shown in Figure 138.7b. While heptan-2-one is released more rapidly than ethanol in the higher water system ($\phi = 20\%$) the converse is true in the concentrated emulsion ($\phi = 80\%$).

Harrison and co-workers obtained reasonable agreement with their model in a study of the release kinetics of diacetyl and heptan-2-one [28]. However other workers have challenged their assumptions and investigated the effect of particle size and interfacial barrier effects [31]. For example Miettinen and others [29] showed that decreasing droplet size had no affect on the amount of diacetyl flavor (i.e., polar) but increased the release of linalool (i.e., nonpolar) and the effects of surfactant type were quite minor (sucrose stearate vs. modified starch).

The sensory perception of creaminess is associated with food emulsions and while it is related to the flavor present, rheology and particle size also play a complex role and vary by product [32].

C. Color

Fine emulsion droplets have diameters approaching the wavelength of light and therefore scatter light efficiently. Consequently, emulsions containing oil concentrations above a few percent appear turbid and white. Finer emulsions scatter short wavelength light more strongly and therefore take on a bluish tinge. Theory has been developed to calculate the perceived color of an emulsion from droplet size and concentration. First the scattering effects of the droplets are calculated and from this reflectance spectrum and hence the tristimulus values [33].

III. EMULSION STABILITY

Emulsion destabilization is driven by the tendency to minimize interfacial area by lipid droplet coalescence, and by phase separation (under gravity) by creaming. These are both thermodynamic effects and so emulsions will inevitably break down eventually. The goal therefore is not absolute but adequate stability, i.e., we are as much concerned with kinetics as we are with thermodynamics. The product must last as long as expected for that type of food, be it seconds for vinaigrette dressing, days for a dairy product, or years for a beverage emulsion. Some of the main mechanisms are illustrated in Figure 138.8 and discussed further below.

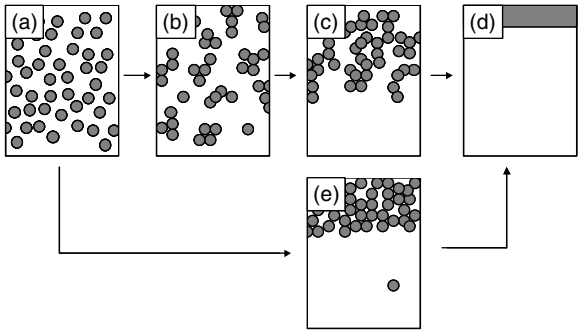

FIGURE 138.8 Schematic diagram illustrating the mechanisms of emulsion destabilization. The primary particles (a) flocculate (b) and rapidly cream (c) into a concentrated layer. The droplets may maintain their individual integrity in this state for a considerable period of time before (d) coalescing into a single phase. This is not the inevitable pathway, in some cases creaming (e) may occur without extensive flocculation. Note the two different creams shown (c and e) have different volumes. This is not the only route to destabilization.

A. Creaming

Oil is usually less dense than the aqueous phase of an emulsion, so it will tend to float to the surface in response to gravity or other applied forces. The movement of oil particles is impeded by viscous drag of the continuous phase and the net rate of particle movement (v_s) can be approximated from a balance of these forces in Stokes' equation:

$$v_s = \frac{d^2(\rho_{water} - \rho_{oil})g}{18\eta_c} \tag{138.8}$$

where d is oil droplet diameter, ρ_{oil} and ρ_{water} are the densities of the respective phases, g is acceleration due to gravity, and η_c is the viscosity of the continuous phase. Equation 138.8 is based on a series of assumptions largely violated in real food emulsions, i.e., the droplets are isolated spheres moving in a Newtonian fluid (Figure 138.2a) but it provides some qualitative guidelines to limit creaming (e.g., minimize particle size, density difference, or increase the viscosity of the continuous phase to reduce the rate of creaming). For example homogenization reduces the size of milk fat droplets approximately tenfold so we would expect homogenized milk to be stable against creaming approximately 100 times longer. As the droplet volume fraction increases, particle-particle interactions become more important (Figure 138.2b), the rate of creaming slows and Equation 138.8 becomes less reliable. An analytical solution is available for moderate concentrations of spherical particles and in principle better fits could be achieved by incorporating higher powers of volume fraction.

$$v = v_s(1 - 6.5\phi) \tag{138.9}$$

However a better description of the effect of volume fraction is achieved by assuming the effective viscosity (in Equation 138.8) experienced by a creaming droplet in a concentrated emulsion is the viscosity of the emulsion (rather than the continuous phase) and calculating that using a Krieger-Dougherty type relation. In this formulation, the emulsion achieved solid-like character as the volume fraction approaches close packing and at this point creaming effectively stops (Figure 138.2c and d, [27]).

Creaming can be readily and temporarily undone by gently shaking an emulsion. It can be measured by visually identifying the junction between a fat rich and fat poor region of a tube of emulsion, but only after creaming is well advanced. Earlier detection is possible using devices that can measure oil concentration as a function of position (e.g., ultrasonic velocity or optical reflectance), but it is often possible to identify systems likely to cream upon long term storage from particle size measurements or by using centrifugation to accelerate the process.

In the remainder of this section we will discuss the aggregation mechanisms responsible for emulsion breakdown. We will first consider the kinetics of aggregation, the various types of aggregation and finally the effects of aggregation on emulsion properties.

B. Aggregation Kinetics

Droplets in an emulsion move either by Brownian motion or under gravitational or other applied forces. The moving droplets collide and may (i) bounce off one another causing no change in the properties of the system, (ii) merge (coalesce) to form a single larger droplet, or (iii) stick together but maintain the shape of two conjoined droplets (i.e., flocculation). Either of the latter two cases will reduce the number of particles in the system and be a step towards destabilization. The rate of aggregation is given by the product of the rate of droplet-droplet collision (β) and the proportion of those collisions leading to reaction (α). Relatively simple terms for collision rates under static (i.e., perikinetic) and sheared (i.e., orthokinetic) conditions were derived by Smoluchowski and are given in Equations 138.10 and 138.11 respectively [34].

$$\beta_{perikinetic} = \left(\frac{2kT}{3\eta}\right) \tag{138.10}$$

$$\beta_{orthokinetic} = \frac{4G}{3}d^3 \tag{138.11}$$

where k is the Boltzmann constant, T is the temperature, η is the viscosity of the continuous phase, d is the droplet diameter, and G is the velocity gradient in the fluid. Smolokowski made several assumptions to derive these expression: (1) particles remain spherical after collision, i.e., complete coalescence, (2) no interparticle forces, (3) no particle or aggregate fracture, (3) fluid motion is exclusively either diffusional or laminar shear, (4) particles are of identical size, and (5) only two-body collisions occur. These

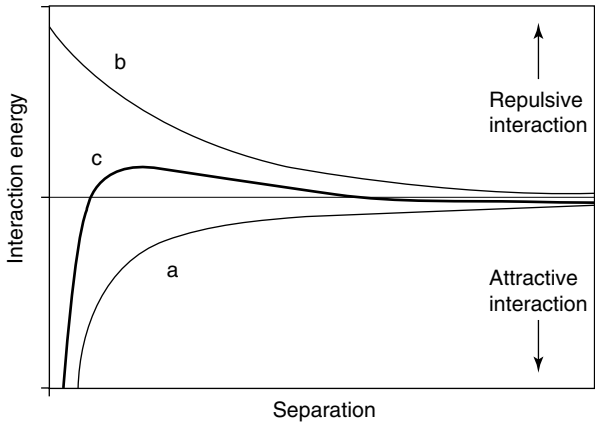

FIGURE 138.9 Schematic illustration of some typical interaction potentials. (a) is attractive at all separation, (b) is repulsive, and (c) shows a barrier to droplet aggregation at intermediate separations.

are clearly violated in real systems so again the full analytical solution can only be used to (at best) give some qualitative guidance. More complete analytical and empirical solutions are available to deal with some of these but the simple equations are still widely used [34].

The collision efficiency parameter α is often used as a fitting parameter to describe flocculation kinetics, but should in principle be relatable to the interdroplet forces acting on the system. If two droplets approaching one another have a significant repulsive force between them they may not collide, whereas if the force is attractive they may veer towards one another and collide even if their original, unmodified trajectories would have caused them to miss. The collision efficiency parameter α would be respectively less than or greater than one in these cases. The interaction forces between droplets vary with range and tend towards zero at long separations. They can also be expressed as interaction potentials — the energy cost of bringing one particle to a given separation from a second particle. Some sample interaction potentials are given in Figure 138.9. The potential is negative at all separations for Figure 138.9a so an approaching particle will tend to be attracted to the second. At very low separations the potential becomes increasingly negative so it will be difficult for the reverse process to occur and the particles will irreversibly coalesce. Figure 138.9b is positive at all separations so the droplets will tend to be repelled from one another. Figure 138.9c is negative or zero at large separations so the particles may approach one another. There is then a positive barrier which can slow the aggregation of particles (if $>kT$) but any particles which have sufficient potential energy to surmount the barrier will permanently coalesce into the potential energy pit at small separations. Interaction potentials can be measured directly or calculated as a sum of attractive and repulsive contributions from the physicochemical properties of the system [24, 35]. The physical bases for a few of the more important interactions are described below:

- **Electrostatic Repulsion.** Most food emulsions are protein-stabilized and under most conditions those proteins have a significant net charge. Consequently protein-stabilized oil droplets act as charged particles. The magnitude of their charge depends on the pH (Figure 138.4) and the amount and type of interfacial protein. However in a given emulsion each droplet is likely to have a similar net charge and thus repel the other droplets. The magnitude of the electrostatic potential force is proportional to the square of surface potential and decreases exponentially moving away from the droplet. The exponential decay is faster in high ionic strength systems so the stability of charged emulsion often decreases in the presence of salt. (Note the ionic strength effect is theoretically independent of ion type. Some specific protein-ion interactions (notable casein and calcium) can also lead to strong droplet aggregation [36].) Demetriades and others [37, 38] studied the effect of salt and pH on the stability of thermally treated whey protein stabilized emulsions. They noted the emulsions thickened and creamed at pH $\sim$ pI and particularly at high salt concentrations. Presumably away from the isoelectric point the electrostatic repulsion was capable of preventing droplet aggregation.
- **Van der Waals Attraction.** Transient dipoles in the bonds within matter lead to a weak colloidal attractive force. The force is relatively long-range, decaying with the reciprocal of distance, and is dependent on the dielectric properties of the component phases.
- **Hydrophobic Attraction.** There is an energetic advantage to moving hydrophobic materials out of an aqueous environment. This is manifested in a strong attractive force between hydrophobic surfaces, which decays exponentially over quite long distances. While this force can be readily measured, until recently there has been relatively poor understanding of the physical basis of the relatively long range of the attraction [35]. However, Pashley [39] recently noted that oil was soluble (as a turbid dispersion) in water if the water was first rigorously degassed by freeze-thaw under vacuum. He proposed that the dissolved gasses were the cause of the long-range hydrophobic effect and once they were removed oil became effectively water-soluble. Emulsion droplets not adequately protected

with surface active material have significant oil-water contact and will quickly aggregate as a result of the attractive hydrophobic forces. Surface protein usually shields the oil from the water and diminishes the hydrophobic attraction but surface denaturation can unfold the proteins and allow some protein-protein hydrophobic attraction to draw the droplets together. For example, Demetriades and others showed that thermally treating whey protein stabilized emulsions led to extensive aggregation [37, 38].

- **Steric Repulsion.** Adsorbed material acts as a barrier to coalescence at very short range (i.e., when the surface layers begin to overlap). The two main contributions to this mechanism are compression (volume exclusion) and mixing (osmotic) effects. Compression is always strongly repulsive but mixing can be attractive at slightly longer range depending on the solvent-polymer interactions [24]. Strong steric repulsion due to the protein layer at very short range is often responsible for the predominance of flocculation over coalescence in many food emulsions.
- **Depletion Interactions**. Non-adsorbing particles are excluded from a region close to a surface equivalent to their hydrodynamic radius. In an emulsion, this sets up an osmotic pressure gradient between particle-rich and particle-free regions of the continuous phase that favors droplet aggregation to reduce the volume of this region. Depletion attraction is particularly important when a non-adsorbing polymer is added to a food emulsion (e.g., xanthan gum, protein aggregates) and can lead to extensive flocculation and creaming [40–42].
- **Hydrodynamic Repulsion.** In sheared systems we can add hydrodynamic interactions to this list. Hydrodynamic forces represent the energy required to make the fluid between the approaching droplets "get out of the way" and so produces a repulsive force. The relative movement of the fluid will also tend to cause approaching particles to rotate and take on a curved trajectory and miss one another. Consequently the presence of hydrodynamic forces will always reduce the collision frequency, sometimes by up to 5 orders of magnitude, compared to rectilinear trajectories [34].

C. Types of Aggregation

Flocculation is the aggregation of oil droplets without mixing of their contents whereas during coalescence the membrane separating the approaching droplets is breached and allows the dispersed phases of the droplet to mix. Once oil is allowed to flow between droplets they quickly revert to a (larger) spherical shape. Coalescence can take place either following droplet collision (see above) or after periods of prolonged storage in a concentrated emulsion (often in a cream layer). The resistance of the interdroplet membrane to rupture depends on the strength of the protein films and the ability of the interdroplet repulsive forces to maintain a thick aqueous layer between the droplets. Surfactant layers can also favor film rupture if their optimal surface curvature favors hole formation [43] and oppose it through the Gibbs-Marangoni effect [44]. Extensive coalescence increases the particle size and therefore the tendency of an emulsion to cream.

In practice, coalescence is rarely the initial cause of food emulsion failure. More common is flocculation whereby droplets collide and stick together but the membrane separating them does not rupture. There is no mixing of oil so the individual droplets retain their spherical shape and a large, porous, and frequently fractal floc develops. A floc immobilizes a mixture of dispersed and continuous phase so the effective volume fraction of a flocculated emulsion, and hence the viscosity (Equation 138.3) is much greater than the corresponding unflocculated emulsion. Coalescence decreases the number of droplets but causes no change in the volume fraction of the emulsion and hence no change in product rheology. Both flocculated and coalesced droplets have larger particle sizes so often cream more readily than the primary emulsion.

Flocs can be characterized in terms of a size and density (fraction of the floc taken up by particles) but in many cases it is possible to ascribe a fractal dimension to the flocculated structure. A fractal object shows some level of self-similarity over several orders of scale. All objects show some relation between length and mass, for Euclidean solids, planes, and lines it is cubic, quadratic, or linear respectively, but for fractal objects it is non-integer. To describe a fractal floc the closer fractal dimension of the floc to three, the more the particles pack together to form a "perfect" Euclidean 3D object, i.e., complete coalescence. The lower the fractal dimension, the more open the structure. Fractal dimension can be used to calculate an effective volume fraction ϕ_{eff}:

$$\phi_{eff} = \phi\left(\frac{R}{r}\right)^{3-D} \tag{138.12}$$

where R is the floc radius, r is the primary particle radius, ϕ is the particle volume fraction, and D is the fractal dimension (= 1–3). The effective volume fraction can be used in the Krieger-Dougherty type relation to link microstructure and viscosity. Plots of viscosity as a function of volume fraction are shown in Figure 138.6d and e for R/r = 10 and D = 2.8 and 2.6 respectively. The looser floc (Figure 138.6e) reaches a critical volume fraction at

lower concentrations. Fully coalesced droplets (D = 3) behave the same as unflocculated emulsion (Figure 138.6c).

Emulsions may flocculate when too little protein is used in their formation. In this case a single protein strand may encompass several droplets and cause them to stick together). Factors which increase attractive colloidal forces or decrease repulsive forces (see above) such as adjusting pH to pI or thermal denaturation of the protein layer will also tend to induce flocculation. This can also be seen as factors that make the aqueous phase a poorer solvent for the protein will tend to favor protein precipitation and hence emulsion destabilization [45].

Related to both flocculation and coalescence is partial coalescence which is a novel mechanism of emulsion destabilization which can occur when the droplets are semi-crystalline (especially in sheared, refrigerated dairy products [44]). The fat crystals penetrate the aqueous layer separating the colliding droplets and then liquid oil flows out to wet the crystal surface and to reinforce the link between the droplets. The solid fat network in each droplet provides a skeleton to prevent the oil flowing completely between droplets and thus prevents full coalescence and maintains the characteristic shape of individual droplets. However if a partially coalesced emulsion is heated so the fat crystals melt they will merge and can lead to significant oiling off. Partially coalesced fat droplets play a role in supporting the foam of whipped cream and ice cream [46].

In conclusion, the typical mode of emulsion destabilization in real food emulsions is flocculation with associated increases in viscosity and often complete or partial phase separation. Later the membranes separating the flocculated droplets may break allowing coalescence and eventual oiling off. (When semi-crystalline droplets are sheared partial coalescence with associated formation of visible clumps or even phase-inversion may dominate.) It is notable that the distinct mechanisms of emulsion destabilization introduced above do not in practice occur in isolation but instead catalyze one another.

IV. EFFECT OF PROCESS CONDITIONS

Foods are processed to improve their safety and quality and sometimes these affect the colloidal forces responsible for the stabilization of emulsions.

Thermal Treatments. Most cooking operations involve some thermal denaturation of proteins. The extent of denaturation depends on the type of protein present at the surface, the temperature and time to which it is exposed, and to a lesser extent the pH and other co-solutes present. Thermal denaturation leads to some protein unfolding and the increased exposure of hydrophobic amino acids to the aqueous phase. The increased hydrophobicity leads to protein aggregation between the surface proteins and either (i) unadsorbed protein, or (ii) adsorbed protein on the same droplets (intradroplet bonding), or (iii) adsorbed protein on other droplets (interdroplet bonding). Of these, (iii) is the most significant as it leads to droplet flocculation and consequent changes in emulsion properties. The prevalence of each case depends on the relative concentration of oil droplets and non-adsorbed protein, and the inter-particle forces acting.

Chilling is important as it induces oil crystallization within the droplets. Droplet oil tends to crystallize more slowly than bulk oil due to the isolation of active catalysts but once there are crystals present there is an increased likelihood of partial coalescence and the formation of visible clumps in the product [44]. Freezing can quickly destabilize many emulsions via a variety of mechanisms. Firstly droplets are forced into very high concentrations in the unfrozen channels between the ice crystals [47]. This can first lead to the conventional forms of droplet aggregation found in concentrated unfrozen emulsions [48], in particular partial coalescence [49]. In addition the dehydrating effect of freezing on the surfactant head groups can alter spontaneous curvature and favor the formation of pores between droplets and eventual full coalescence [47]. In some emulsions the freezing will induce polymer-polymer interactions and droplet flocculation and the formation of a cryo-gel [50, 51].

Other Operations. High pressure treatment can have a denaturing effect on proteins and thereby affect the functional properties of emulsions [52]. The effect of the pressure treatment depends on the nature of the protein and on the solvent conditions, for example, 700 kPa had no effects on the surface pressure of casein but significant effects on whey.

Pulsed electric fields have an antimicrobial effect often exploited to reduce the microbial loads on minimally processed foods but have little effect on the properties of food emulsions, for example, disrupting flocs and rupturing larger droplets [53].

Irradiation inactivates food spoilage organisms by damaging their nuclear material. Extremely high levels of irradiation could in principle trigger more rapid lipid oxidation or damage other ingredients present but it is unlikely the levels used in food processing would have any detectable effect on the physical microstructure.

ACKNOWLEDGEMENTS

We are grateful to Dr. Julian McClements for generously providing a pre-print of parts of the forthcoming second edition of his book and to Ms. Andrea Docking for providing Figure 138.1.

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139 Food Gums: Functional Properties and Applications

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CONTENTS

I. HYDROCOLLOIDS: OVERVIEW OF CHEMISTRY AND FUNCTIONALITY

Hydrocolloids, commonly known as water-soluble gums, are high molecular weight plant polysaccharides, usually with some inorganic or mineral content and low levels of protein. Gums are naturally occurring, water-soluble polymers with thickening, film-forming, and/or gelling properties based on their chemical nature, given specific conditions. Gums as complex carbohydrates exhibit properties that are affected by many factors including the following: active functional groups as substituents, molecular size, orientation, molecular association, water-binding and swelling, concentration, particle size, degree of dispersion, temperature, pH, processing conditions, etc. (1, 2, 3).

Soluble dietary fiber is defined as being resistant to degradation by human digestive enzymes and can help decrease high serum cholesterol levels. Water-soluble gums are good sources of soluble dietary fiber (about 85% on a dry basis) when used as an ingredient in processed foods. Low viscosity gums, such as gum acacia, inulin, hydrolyzed guar, and polydextrose, may be used as fiber source at higher levels in nutraceutical products. Gums can also be used as one of the ingredients in fat mimetic systems due to their ability to bind as much as 100-fold their weight of water. This chapter aims to highlight the functional properties of various commercially available hydrocolloids for applications in food and beverage products. A list of the various categories, examples, and botanical sources of naturally occurring hydrocolloids is shown in Table 139.1A. Examples of the most important chemically modified gums are shown in Table 139.1B.

TABLE 139.1A
Types of Natural Hydrocolloids

Type (Function)	Examples	Botanical Source
Plant Exudates (Emulsifying & Film-Forming Agents)	Gum Arabic Karaya Tragacanth	Acacia sp. Sterculia sp. Astragalus sp.
Seed Gums (Thickeners & Water Binders)	Guar Locust Bean	Cyamopsis tetragonolobus Ceratonia siliqua
Seaweed Extracts (Gelling Agents & Film Formers)	Carrageenan Agar Alginate	Chondrus, Eucheuma spp. Gracilaria, Gelidium spp. Laminaria, Macrocystis spp.
Microbial Gums (Thickener/Gelling)	Xanthan Gum Gellan Gum	Xanthomonas campestris Pseudomonas elodea
Plant Extracts (Gelling/Thickener)	Pectins Inulin Konjac Flour	Apple & citrus fruits Chicory & Artichokes Amorphophallus sp.

TABLE 139.1B
Types of Chemically-Modified Water-Soluble Gums

Type	Examples	Synonym
Cellulose Derivatives (Thickening & Suspending Agents)	Cellulose Gum	Sodium carboxymethyl cellulose
	Methyl Cellulose	Cellulose ether
	Cellulose Gel	Microcrystalline cellulose
	HPMC	Hydroxypropylmethyl cellulose
	EHEC	Ethylhydroxyethyl cellulose
Modified Gums (Emulsifying Agents)	Modified Gum Acacia	Esterified Gum Acacia

A. Hydration Rate, Functional and Rheological Properties

Gums are highly functional ingredients in beverages, salad dressings and sauces, snack foods, cereal products, and other food systems (1, 2, 3). In food applications, they function in a variety of ways (Tables 139.2A and 139.2B), an attribute that may be related to their viscosity characteristics or to their water binding, gelling, and other specific properties. These characteristics ensure the production of high quality food products with extended shelf life. As is evident from Table 139.3, viscosity ranges of the various hydrocolloids can vary greatly due to their chemical nature and degrees of branching and polymerization.

Depending on their chemical nature, type of branching, molecular weight, residual ionic charge, and ability to undergo intermolecular or intermolecular associations, the gums exhibit a variety of rheological properties. Shear-thinning behavior or pseudoplascticity is shown by xanthan gum while gum acacia, a branched, globular arabinogalactan is Newtonian (i.e., does not decrease in viscosity with increasing shear rate) up to 40% concentration. Thixotropic behavior has been observed in some cellulose gum solutions, depending on degree and uniformity of substitution.

To optimize the functional properties of gums, it is essential that the product is completely dispersed and fully

TABLE 139.2A
Functions of Gums in Food Products (1)

Function	Application
Adhesive	Glazes & cereal clusters
Binding agent	Granola bars & sausages
Bulking agent	Sugar-free foods
Clouding agent	Fruit beverages
Crystallization inhibitor	Ice cream, syrup, & candy
Coating agent	Confectionary
Emulsifier	Beverage emulsions & salad dressings
Encapsulating agent	Spray-dried flavors
Fat mimetic	Low-fat cookies & dressings
Film former	Edible films & coatings

TABLE 139.2B
Functions of Gums in Food Products (2)

Function	Application
Foam stabilizer	Whipped toppings, mousse
Gelling agent	Pie fillings, custard, gummy bears
Protein reactive colloid	Whey beverage, acidified milk
Reduce cooking loss	Meat & poultry injection
Suspending agent	Marinades & dressings
Syneresis inhibitor	Cheese, frozen foods
Thickening agent	Sauces, gravies, smoothies
Whipping agent	Whipped yogurt & cream

TABLE 139.3
Relative Viscosities of Gums

Hydrocolloid	Viscosity Range[a]
CMC	4,000–6,000
Guar Gum	3,000–5,000
Locust Bean*	2,500–3,500
Xanthan	1,000–2,000
Carrageenan*	100–1,500
Karaya	300–1,000
Tragacanth	500–750
Sodium Alginate	200–400
Gum Acacia	2–10

[a] Centipoise (cP) after 24 hours at 25°C (77°F) with 1% gum solns. (Brookfield Viscometer), RV4 at 20 rpm, except for gum acacia, LV2 at 60 rpm.
* Requires heating to 180°F to hydrate completely.

hydrated. Factors affecting hydration rate and functionality include the following: (a) chemical nature of the hydrocolloid, (b) particle size distribution, (c) method of incorporation of ingredients, (d) temperature, (e) pH and the presence of other ionic salts, (f) shear rate, (g) duration of mixing, (h) synergy between gums, (i) potential incompatibility between food components, (j) factors required for gel formation including cations and percent solids, and (k) processing conditions and equipment design.

TABLE 139.4
Proximate % Composition of Selected Gums

Gum	Complex Carbohydrates[a]	Protein	Ash	Others[b]	Sodium mg/100 g
Agar	85.0	<1	6.5	8.5	<10
Arabic	85.0	2–3	3.8	9.3	100
Carrageenan	60.0	<1	35.0	5.0	3,000
Guar	85.0	3–6	1.5	9.9	250
Locust Bean	80.0	6.0	1.2	12.8	<10
Tragacanth	80.0	2.5	3.0	14.5	10
Xanthan	85.0	0–2	10.0	5.0	5,000

[a] Soluble dietary fiber.
[b] Primarily moisture.

B. Proximate Composition: High Soluble Dietary Fiber Content

As mentioned earlier, gums have minute quantities of lipids and contain low levels of protein, depending on their source. They consist primarily of complex carbohydrates derived from plants or from the biosynthesis of end products by pure microorganisms (e.g., xanthan gum and gellan gum). They act as soluble dietary fiber (Table 139.4), which has been reported to lower serum cholesterol and improve gastro-intestinal function as well as improve glucose tolerance. Seaweed extracts also contain an appreciable level of ash, which may naturally occur with the gum or may be the consequence of manufacturing conditions.

C. Plant Exudates as Emulsifying Agents

1. Gum Acacia or Gum Arabic

Gum arabic is derived from the plant exudate of *Acacia senegal* or related Acacia species grown mainly in African regions. The general properties of this plant exudate are affected by the age of the tree, the amount of rainfall, type of storage conditions, and other factors. Salts and other electrolytes as well as temperature can affect the viscosity of acacia, which is typically not more than 300 cps at 30% gum level. The highly branched, compact structure may account for its low viscosity. The emulsifying properties are attributed to the protein moieties covalently linked to the polysaccharide (2).

Gum arabic is an anionic heteropolysaccharide consisting of an arabinogalactan complex (about 88%), an arabinogalactan-protein complex (10.4%), and a glycoprotein fraction (about 1.2%). It consists of rhamnose and glucuronic acid, in addition to arabinose and galactose (Figure 139.1). Gum arabic exhibits emulsifying properties and may be used at high concentrations up to 30% in spray dried flavors, due to its unusually low viscosity. When the protein moiety in gum acacia is damaged by high temperature and other specific processing parameters, the emulsifying capacity of the gum may be adversely

affected. Gum arabic is widely used in the food industry due to its emulsifying properties, low viscosity, high fiber content, water-binding capacity, and adhesive and film-forming properties (4).

Gum arabic is reported to be incompatible with vanillin, pyrogallol, vanillin, phenol, thymol, cresols, tannin, etc. However, it is compatible with most other plant hydrocolloids, proteins, carbohydrates, and starches.

2. Modified Gum Acacia: New Emulsifying Systems

The emulsifying properties of gum acacia are significantly enhanced by introducing a covalently linked lipophilic group through reaction with compounds such as octenylsuccinic anhydride (5). Modified gum acacia may be used to replace gum tragacanth and emulsifying starches used in bakery emulsions. Modified guar gum has also been used as a replacement for propylene glycol alginate in emulsion stability studies (5). These new products are members of the series of modified, emulsifying hydrocolloids covered by U.S. Patent No. 6,455,512 (6). A petition for GRAS certification has been approved after a thorough review by a panel of experts. The new products are available for initial evaluation in various formulations including beverage emulsions, cosmetics, and salad dressings.

D. Seed Polysaccharides

1. Guar Specialty Products: Deodorized and Hydrolyzed Guar Gums

Guar gum is a non-ionic galactomannan isolated from the seeds of the shrub that belongs to the species *Cyamopsis tetragonolobus*, Fam. *Leguminoseae*. It is grown in Pakistan and India and may also be cultivated in Texas, Arizona, and other arid regions of the U.S. The structural building blocks of guar are the sugars mannose and galactose at a ratio of 2:1 (Figure 139.2). The protein content ranges from 3 to 6% but the Food Chemicals Codex allows a maximum limit of 10%. It swells in cold water and is one of the highly efficient water thickening agents used in the food industry.

Solutions of guar are non-Newtonian and pseudoplastic or shear-thinning in nature (Figure 139.3). More recent specialty guar types have been developed to reduce the grassy odor and flavor, using a proprietary manufacturing process (7). Reduced odor guar gum is recommended for various food and beverage products with delicate flavor and odor. Hydrolyzed guar gum with lower viscosity (60 to 150 cps at 2%) may also be used to increase the fiber content of the finished product.

The viscosities of two special types of deodorized guar as a function of concentration after hydration for 2 and 24 hours are shown in Figure 139.4. Viscosity loss is reversible when heat is applied and subsequently removed. Guar gum can vary in its viscosity, rate of hydration, and dispersion properties depending on the conditions under which it is manufactured. Being the same as other gums, it has a high dietary fiber content of 80 to 85%.

2. Locust Bean (Carob) Gum

Locust bean gum is a non-ionic polysaccharide obtained from *Ceratonia siliqua*, a tree that belongs to the Family Leguminoseae, and consists of mannose and galactose sugar units in a ratio of 4:1 (Figure 139.5). Unlike guar gum, which hydrates rapidly in cold water, locust bean gum has to be heated to 80°C (176°F) for full hydration. The distribution

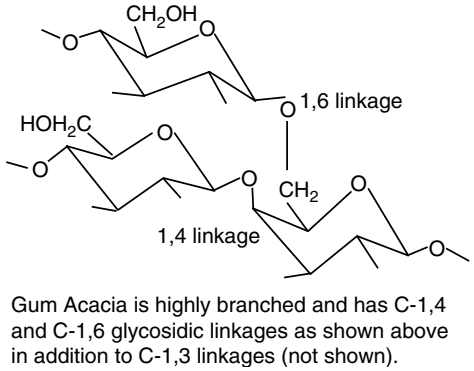

FIGURE 139.1 Glycosidic linkages in disaccharide repeating units in gum acacia.

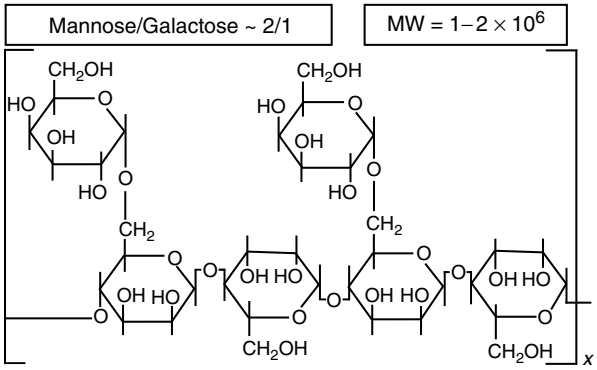

FIGURE 139.2 Structure of guar gum.

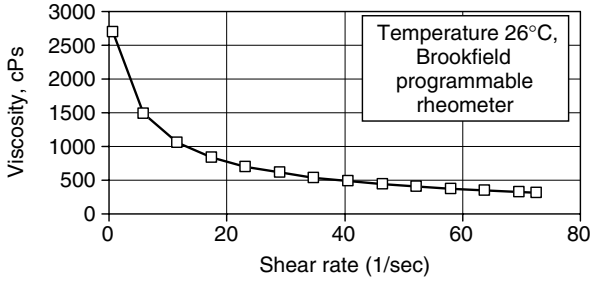

FIGURE 139.3 1% Deodorized guar: shear rate vs. viscosity.

of D-galactosyl groups in guar and locust bean gum has been described by Baker and Whistler (8). Food grade locust bean gum should have a protein content not exceeding 8% as specified in the Code of Federal Regulations.

Solutions of locust bean gum are non-Newtonian with zero yield value and, thus, flow as soon as slight shear is applied. When combined with xanthan, locust bean gum yields heat-reversible, pliable gels. It also acts synergistically with kappa carrageenan to form strong, somewhat elastic gels. Locust bean gum is classified as a direct food additive under FDA regulations. Dilute solution properties of guar and locust bean gum in sucrose have been characterized by Richardson et al. (9).

E. Seaweed Polysaccharides

Some seaweed extracts from the Family *Rhodophyceae* (agar), *Phaeophyceae* (algin), and *Gigartinaceae* (carrageenan) are used as gelling and stabilizing agents in various food products and beverages, based on the optimum gum levels and conditions required for functionality. The seaweeds are typically dried by sun-drying or mechanical means, washed to remove sand, salt, and other debris, mixed with water, and treated with alkali to facilitate the extraction of the polysaccharide. Alcohol is sometimes used to precipitate the concentrated extract. The general principles involved in the purification and isolation of these gums from the seaweeds are essentially similar, but the specific procedures are proprietary in nature and differ slightly for each manufacturer. The structure of the repeating unit of the gelling components from agar and carrageenan are shown in Figures 139.6 and 139.7, illustrating the presence of the ester sulfate groups that account for the anionic character of agar and carrageenan.

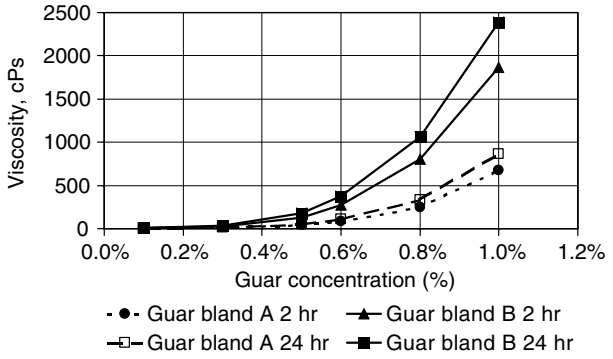

FIGURE 139.4 Deodorized guar levels vs. viscosity.

1. Agar and Agaroid Series as Gelling Agents

Agar and its agarose constituents have been characterized by Armisen (10). Agar consists of a repeating unit of beta-D-galactose attached to 3,6-anhydro-alpha-L-galactose. It is isolated from seaweeds that mainly belong to the *Gelidium*, *Gelidiella*, or *Gracilaria* species. Traditional agar can bind about 100 times its weight of water, and, when boiled to 212°F and cooled, forms a strong gel. It is one of the most potent gel-forming gums known and, unlike most other gelling gums, has a gelation temperature that is far below the gel-melting temperature. A solution of agar (1.5%) congeals in the range of 32 to 39°C (89.6 to 102.2°F) but does not melt below 85°C (185°F). This property is important in many of its applications in the food industry.

A more recently developed type of agar (Agar RS-100) from TIC Gums, Inc. does not require boiling as does the traditional agar (11). The seaweed sources are subjected to a series of manufacturing procedures that yield a product that may be hydrated at 170 to 180°F instead of 212°F. This is a desirable feature, considering the expense involved in boiler operations in the industry.

A series of synergistic systems, the Agaroid Series, that make use of non-boiling agar and other hydrocolloids

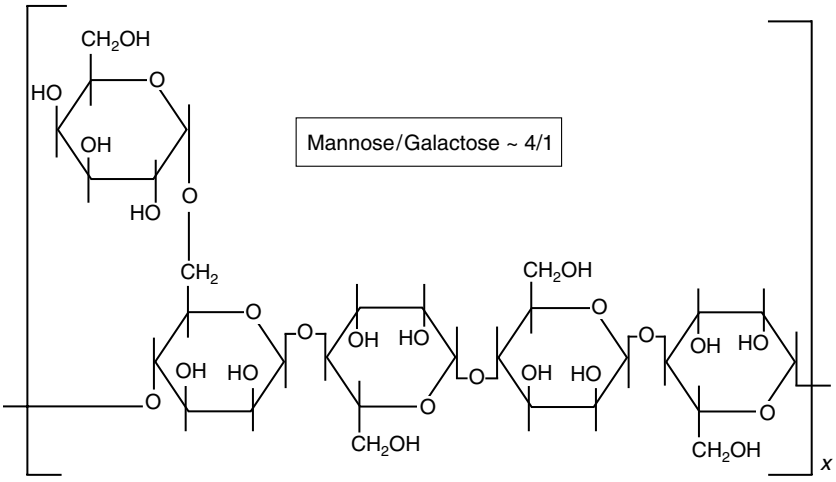

FIGURE 139.5 Structure of locust bean gum.

have been developed (11) to replace gelatin as gelling agent. Gelatin is not acceptable to some religious groups and has been recently associated with the incidence of mad cow disease, which has increased the demand for gelatin substitutes from the food industry.

2. Carrageenans: Protein-Reactive Gelling and Stabilizing Agents

Carrageenan, a water-soluble gum, is a sulfated, linear, anionic polysaccharide composed of D-galactose and 3, 6-anhydro-D-galactose derived from red seaweeds including *Eucheuma*, *Gigartina*, or *Chondrus* species. Carrageenans act as strongly anionic polyelectrolytes, a property that accounts for their high protein reactivity. Due to the presence of the ester sulfate groups, an interaction occurs with charged amino acid groups in proteins above the isoelectric point. The three common types of carrageenans — kappa, iota, and lambda — differ in degree and location of sulfated ester groups and the linkage of the repeating units (Figure 139.7). Bixler (12) conducted studies and reviewed the properties of refined and semi-refined carrageenans.

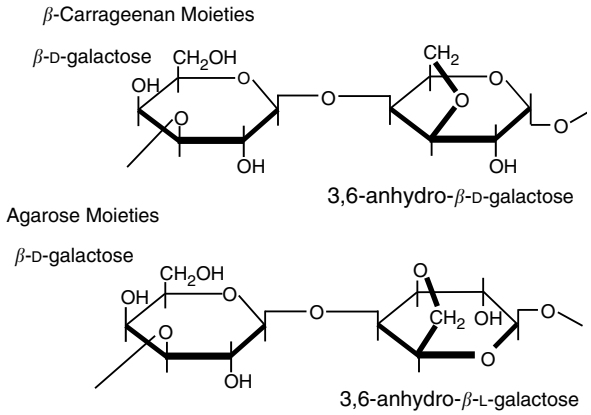

FIGURE 139.6 Repeating chemical units of gelling agents.

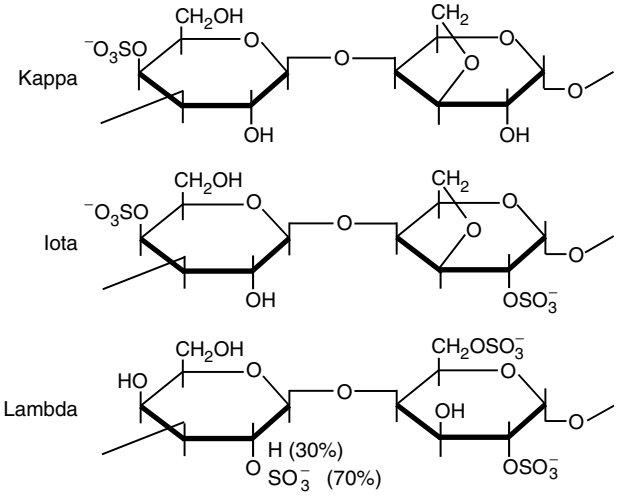

FIGURE 139.7 Repeating chemical units of carrageenans.

In terms of solubility in water, both kappa and iota hydrate at above 70°C, while the lambda type is soluble in cold water. In cold milk, lambda carrageenan disperses and thickens while both the iota and kappa carrageenans are insoluble.

Kappa carrageenan requires potassium ions to gel, while iota carrageenan requires calcium ions to form a heat reversible and flexible gel at 1.5% gum. The non-gelling type, lambda carrageenan is usually used to thicken milk, an action enhanced by tetrasodium pyrophosphate. Thomas (13) described the applications of carrageenans as thickening and gelling agents for food.

3. Alginic Acid Derivatives

Alginic acid is a high molecular weight linear polysaccharide derived from *Laminaria*, *Macrocystis*, *Lessonia*, and other related seaweed species. It consists of homo- and heteropolymic sequences composed of mannuronic and guluronic acid units (Figure 139.8). The guluronic and mannuronic acid content of the alginate affects the nature of the gel that is formed. Sodium alginate in the presence of calcium ions yields gels that are not thermally reversible. The method of addition and type of calcium salt added affects the properties of the final gel. A calcium sequestrant may be used to weaken the gel or delay its setting time. Gel systems may also be prepared using alginates by controlling pH. Neiser (14) characterized the gel formation in heat treated bovine serum albumin-sodium alginate systems.

4. Propylene Glycol Alginate: An Emulsifying Gum

An ester derivative of alginic acid, propylene glycol alginate is widely used as an emulsifier in salad dressings and other types of oil-in-water emulsions. Propylene oxide is reacted with alginic acid to esterify partially the hydrocolloid's mannuronic or guluronic acid units. The use of food colloids and polymers in emulsifying systems has

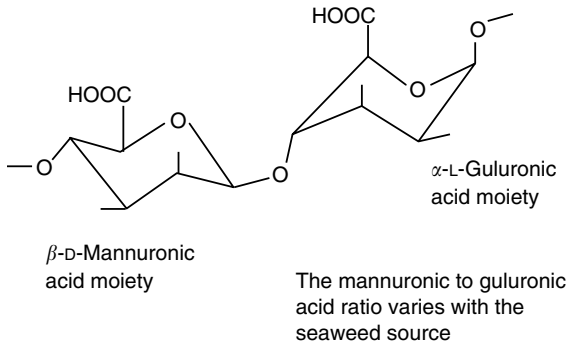

FIGURE 139.8 Glycosidic linkage repeating units in alginates.

been investigated extensively by Dickinson and Walstra (15).

F. Microbial Polysaccharides

1. Gellan Gum

Gellan gum (16) is a fermentation product of *Pseudomonas elodea* grown under aerobic, submerged condition. The active, high-acyl gellan gum consists of a linear sequence of tetrasaccharide repeating units. Cations such as potassium ions, in addition to interchain reactions and hydrogen bonding with water, help stabilize the structure that gives rise to gel formation. The influence of calcium ions, acetate, and L-glycerate groups in the gellan double helix has been investigated by Chandrasekaran and Thilambali (17).

By varying the degree of acylation, a range of gel textures can be generated. The viscosity of gellan gum solutions decrease markedly with increasing temperature, but functionality is retained upon cooling. Ratios of gellan gum and gum arabic at 1:1 exhibit an increase in gel strength by about 60% at 0.5% gum level. Gellan gum is used in food formulations that require gelling properties including jams, jellies, dessert gels, pie fillings, puddings, frostings, and dairy products.

2. Xanthan Gum

Xanthan gum is a highly branched polysaccharide consisting of repeating units of D-glucose, D-mannose, and D-glucuronic acid (Figure 139.9). It is a biosynthetic product of a pure bacterial culture of *Xanthomonas campestris* grown in a special nutrient medium under controlled conditions. It is approved for food use in many countries, including the United States and Canada. Food grade xanthan is also a good source of dietary fiber and should have an ash content that does not exceed 10.0%.

Xanthan gum solutions are extremely pseudoplastic and exceed most common gums in this respect. Viscosity is reduced with increasing shear; viscosity is regained after shear is released. This property is an advantage when pumping gum-thickened liquids.

Xanthan gum is an excellent emulsion stabilizer, although it is not an emulsifier by definition. It is typically used in salad dressing emulsions. Xanthan gum is stable over a wide range of pH (2 to 10) and temperature, which makes it an ideal stabilizer in a variety of applications (3).

G. Pectins

Citrus and apple byproducts have been widely used as sources of pectin. Other sources include tropical fruits such as guava, papaya, mango, etc. Under appropriate conditions, pectins (polygalacturonans) are gel forming. The main component of pectin is D-galacturonic acid partly esterified with methoxyl groups. The degree of esterification is initially high, but the pectin methylesterase enzyme present in most tissues can cause demethoxylation.

Pectins can be classified into high methoxy, low methoxy, and amidated. High methoxy pectins require more than 60% solids and low pH (less than 3.5) to form a heat-irreversible gel. Low-methoxy pectins (between pH 1 to 5) require calcium to gel and may yield a heat-reversible gel at 25 to 35% solids given the proper gelling

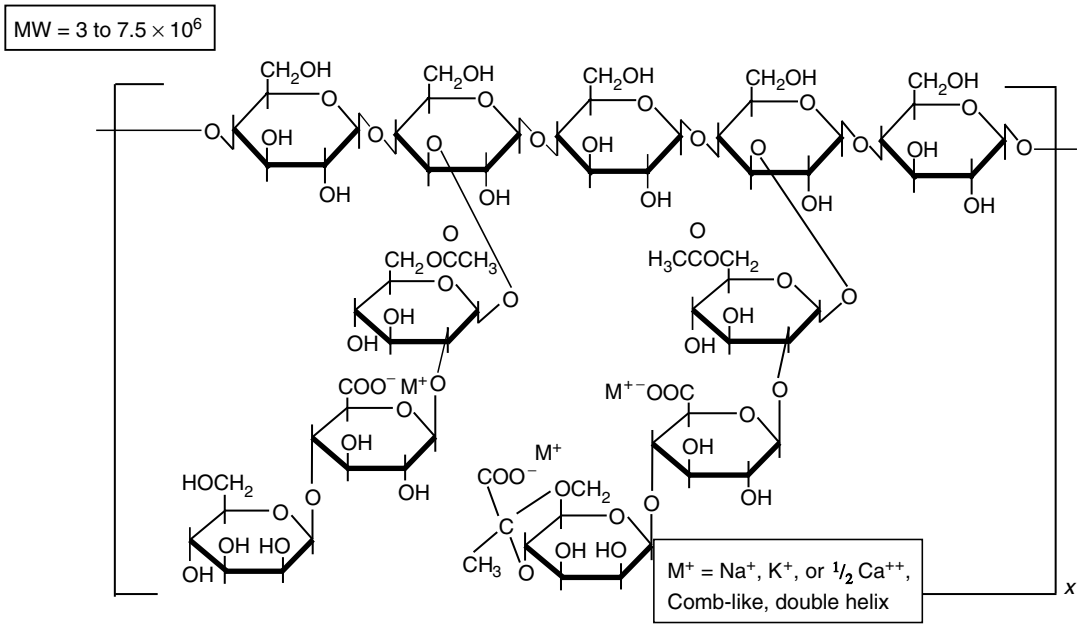

FIGURE 139.9 Structure of xanthan gum.

conditions. Schols et al. (18) describe the structural features of native and commercially extracted pectins.

H. Inulin: A Fructooligosaccharide

A relatively new food ingredient, inulin is isolated from the root of the chicory plant *Cichorium intybus* and other plant sources. It has gained wide use over the past several years due to the following features: readily soluble fiber, "neutral" taste, odor, and color, gelling properties, and ability to stabilize foams and emulsions. Inulin is a combination of fructose chains, the chain length varying from 2 to 60 fructose units. It has also been reported to provide only 1.6 Kcal/g as compared to 4.0 Kcal/g for starch and other carbohydrates (2). In addition, it has a glycemic index of zero, does not stimulate insulin excretion, has been claimed to be 100% soluble fiber, and is recommended in the modern diet for diabetics.

I. Cellulose Gum and Methylcellulose

Cellulose is a linear polymer consisting of beta-D-anhydroglucose units (Figure 139.10). Sodium carboxymethylcellulose (cellulose gum), derived from cellulose, is universally known as CMC. Purified CMC is a tasteless, odorless, and free-flowing powder and is widely used in the food industry. There are various types of CMC manufactured throughout the world. These differ in degree of substitution and viscosity. CMC is recommended in products where clarity is an essential feature. The manufacturer should specify the limits for % insolubles as well as turbidity values and degree of substitution, to ensure that the CMC grade meets the requirement for clarity in the finished product. Other derivatives of cellulose include methylcellulose and hydroxypropylmethylcellulose (HPMC). Methylcellulose, at suitable levels, gels upon heating, a property that makes it useful as a binder for products subjected to heating. Microcrystalline cellulose (MCC) is unmodified, insoluble cellulose that has been reduced to a small particle size and has been used as a fat mimetic in combination with CMC (11).

II. GENERAL APPLICATIONS IN THE FOOD AND BEVERAGE INDUSTRIES

Hydrocolloids, alone or in combination with other gums, other ingredients, and emulsifiers, are widely used in the dairy, bakery, confection, beverage, and snack food industries. They are also used in flavor emulsions, cereal products, candies, and confections. As indicated in the preceding section, their functionality is affected by the chemical nature of the gum as well other factors including pH, temperature, % total solids, presence of cations, and synergy or incompatibility with other ingredients.

Before actual production, planning equipment design, and scaling-up manufacturing procedures, the specific requirements of the gum or gum system should be taken into consideration by the engineer and food technologist. For example, if the filling viscosity is a limiting factor in production, gums that do not attain full viscosity in cold water are required. Shear forces at high temperature should be taken into consideration with some specific gums and starches to avoid excessive degradation. Similarly, high acid and high temperature can cause gum hydrolysis in the food system and should therefore be avoided where possible. For each food or beverage product, all the basic requirements to maximize functionality of the gum and the other ingredients should be adopted to achieve the desired sensory qualities and shelf-life stability. The oral behavior of food hydrocolloids and emulsions, including the relationship of zero shear viscosity and the maximum aroma intensity of guar, xanthan, and sodium alginate solutions, has been extensively investigated by Malone et al. (19).

A. Dairy Foods and Beverages

The conventional, older method of heat processing, batch heating at 145°F for 30 minutes, favored hydration of the gums. With the advent of HTST (high temperature-short time, 171°F for 16 seconds), hydration of gums requiring heat to unfold has become problematic. Ultra-high temperatures, 285 to 302°F for 2–3 seconds, further shorten

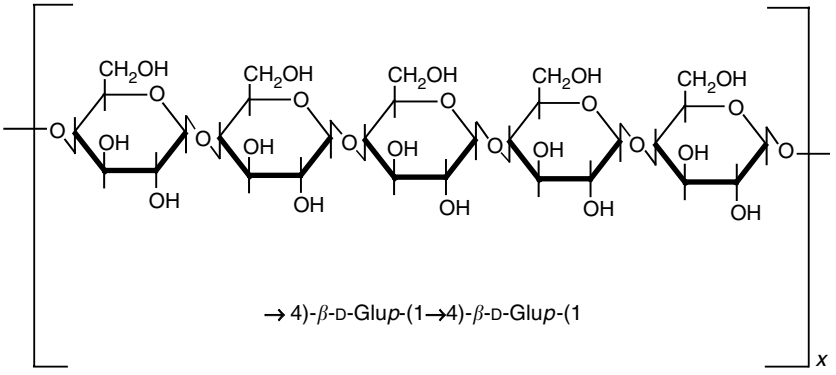

FIGURE 139.10 Structure of cellulose.

the time available for hydration as well as pose stability risks in acidic beverages. The extremely high heat of UHT processing denatures proteins that were not affected by the lower heat treatments. This unfolding can be utilized for additional viscosity, although precipitation problems may arise depending on the isoelectric point of the protein substrate.

1. Ice Cream and Other Frozen Dairy Products

Ice cream (20) is composed of milk ingredients, sweeteners, stabilizing agents, and flavoring. The milk ingredients used may include cream, milk, condensed milk, powdered milk, butter, and frozen cream. Fat is an important component in ice cream due to its mouthfeel and richness of texture. Whey solids are used in lower quality ice creams due to cost considerations. Whey consists of whey protein and other components that are not precipitated by high acidity. A by-product of the cheese industry, whey is now being used in beverages and nutritional sports supplements. Certain types of whey can impart a cheesy or salty flavor to the ice cream. Seventy-five percent of whey solids consists of milk sugar, or lactose. Too high a level of whey in the cream causes a defect, called "sandiness," due to specific structural forms developing. The "sand" or lactose crystals do not melt in the mouth.

Plain ice cream must contain at least 10% milkfat and 20% total milk solids. Air is whipped into the ice cream mix and the structure is quickly frozen. An ice cream with 100% overrun contains 50% air. Too much air causes rapid meltdown and is regulated by the Code of Federal Regulations.

Stabilizers in ice cream, particularly gums, provide smoothness in body and texture, retard ice and lactose crystal growth, and increase freeze-thaw stability. In addition, they contribute to creamier meltdown, lowered sensation of coldness, improved body, overrun control (product uniformity), and resistance to melting. The stabilizer, which increases viscosity and adsorbs to the air lamellae, also aids in the suspension of flavoring particles. Gums stabilize the product by restricting the movement of water and solutes. They hold free water as water of hydration or by immobilization within a gel structure.

In a sucrose-lactose solution simulating the colloid-free phase of an ice cream mix, the effects of CMC and guar gum on ice crystal formation were studied. Freezing profiles, obtained over short-term intervals, showed that the addition of guar gum significantly retarded ice crystal propagation in the sugar solution, whereas addition of CMC showed no effect (21).

Stabilizers are reported to have no significant effect on the amount of freezable water or enthalpy of melting. Although they exhibit no effect on the freezing point depression of ice cream, the gums do limit the growth of crystals during recrystallization (22). The size of ice crystals in ice cream determines the perception of iciness. Properly processed ice cream leaves the filler with small ice crystals. After transport, storage, and home freezing, ice crystal size grows. The consumer considers the ice cream defective, and sensory studies describe the defect as being coarse and icy. Defects in body and texture in ice cream are typically described by the following sensory properties: coarse or icy, gummy, weak, churned or greasy, soggy, and sandy. The use of polysaccharide gum helps inhibit the development of several of these defects.

Carrageenan is an excellent stabilizer and is used in chocolate milk and ice cream. It forms a lattice structure that holds water and keeps it from migrating. The kappa carrageenan component exhibits gel-forming properties. Used with other stabilizers to prevent wheying off, kappa carrageenan reacts with kappa-casein micelles in milk to form a weak pourable gel. It is used in ice cream, evaporated milk, infant formulas, freeze-thaw stable whipped cream, and emulsions in which milk fat is replaced with vegetable oil.

Guar gum is an excellent, cost-effective thickener that is cold-water soluble and is typically combined with locust bean gum and carrageenan at recommended ratios to maximize its functionality in the ice cream product.

Some gum systems form microscopic spherical particles that mimic the rheology and mouthfeel properties of emulsified fat. These systems may include locust bean gum, guar gum, carrageenan, xanthan gum, cellulose gum, and microcrystalline cellulose. Other frozen products in which gums are used include frozen custard or French ice cream, sugar-free ice cream, mellorine, frozen yogurt, fruit sherbets, and water ices. Mellorine is similar to ice cream but the milkfat is fully or partially replaced by vegetable or animal fat. It should contain not less than 6% fat.

2. Cream Cheese and Sour Cream

Gums, particularly combinations of carrageenan, guar gum, and locust bean gum, help minimize or retard syneresis or weeping in cream cheese and sour cream. Xanthan gum is also usually combined with guar gum and locust bean gum to form a synergistic component for cream cheese and similar formulations. The gum is added after the curd is formed by the inoculum, or, as in the case of imitation sour cream, after the protein is curdled with a lactic acid and citric acid mixture.

3. Acidified Milk Beverage

Acidified milk beverages that are subjected to UHT pasteurization may be stabilized by the use of pectin or a combination of pectin and a deodorized guar gum at 0.4 to 0.5%. A prototype formulation is shown in Tables 139.5A and 139.5B. The gum system is dry blended with sugar and added to the milk, then allowed to mix and hydrate. The orange juice and the remaining balance of the milk

TABLE 139.5A
Acidified Milk Beverage (1) (Prototype Formulation, pH 4.2 & 22°Brix)

Ingredient	%
Orange Juice	64.6
Whole Milk	25.0
Sucrose	10.0
Gum System*	0.4
Flavor (as required)	

* Pectin, deodorized guar gum, and propylene glycol alginate.

TABLE 139.5B
Acidified Milk Beverage (2) (Prototype Formulation)

Suggested Procedure

1. Pour milk into a mixing tank.
2. Dry blend sucrose and gum system.
3. Start blending at low speed, add the sucrose-gum blend to the mixer with the milk. Increase speed of the blender by 20%.
4. Let the gum hydrate for fifteen minutes in the mixing tank.
5. Add orange juice slowly and let mix for five minutes, adjust pH to 4.1–4.2 with citric acid, if necessary.
6. Pass through a thermal processing unit at 220°F (104°C) for 20 sec. Cool with chilled water to 40°F (4°C).
7. Package in sterile containers.

are then added to complete the formula. The UHT treatment involves heating at 220°F for 20 sec, after which the mixture is homogenized at 2500 psi and then cooled down with chilled water to 40°F and packaged in sterile containers. The product should show no significant wheying off or separation over a 6-week period. Pectin can also be used to stabilize UHT-treated fruit smoothies, buttermilk, fruit juice, milk drinks, and aseptic yogurt drinks. Pectin, which is anionic, stabilizes the milk protein and protects it from denaturation, thus keeping it suspended.

The effect of carrageenan on sensory properties of milk beverage model systems is described by Yanes et al. (23).

B. Bakery Products, Cereals, and Snack Foods

Hydrocolloids are used in bakery and snack foods (24, 25) due to their ability to bind water and improve the texture of the products as well as act as gelling agents. Gum arabic, used at 0.08 to 0.20 mg/kg of the flour, has been reported to improve the baking properties of rye and wheat flour (3). The anti-staling property of gum arabic and other hydrocolloids, when used in bread and cookies, has also been reported (3). Gum arabic is a main component in some glazing agents due to its adhesive properties. It also yields pliable and stable icing bases. The emulsifying ability of gum arabic is used in baker's emulsions, in combination with other gums such as gum tragacanth. As a surfactant and foam stabilizer, it may be used in whipped cream or toppings. Since gum acacia is high in dietary fiber, it may be used as a texturizer and bulking agent in powdered bakery mixes.

Cellulose gum prolongs shelf life in bread and increases water retention as well as volume of the dough. Comparison of the moisture content of reduced fat oatmeal cookies and cakes prepared with and without gums showed higher moisture levels in gum-treated samples. CMC may also be used as a film-forming coat and adhesive in doughnut glazes (24, 25).

Cellulose gum at 0.10% use level reduces ice crystal growth in frozen dough products and improves freeze-thaw stability. Fruit fillings with CMC in combination with gel-forming systems help reduce syneresis or weeping.

Methylcellulose is another derivative of cellulose that has been reported to increase moisture content and improve the sensory ratings of doughnuts. Microwaveable cakes showed uniformity of moisture distribution attributed to thermal gelation in methylcellulose-treated cake samples.

Carrageenan is used to strengthen and extend the protein ingredients in bread or cake mixes. It is also used as an additive in various dough products to help improve the loaf volume, loaf shape, and texture. The freeze-thaw stability of pasta products is improved by the addition of 0.05 to 0.10% of carrageenan. Kappa carrageenan is used in breading and batter mixes due to its protein reactivity. Lambda carrageenan, a non-gelling type, is used to bind or retain moisture. It also contributes viscosity to sweet dough products. Iota carrageenan, which requires calcium ions to form a heat-reversible and flexible gel, may be used in fruit applications. Cake and doughnut mixes with 0.1% carrageenan show better moisture retention and softer texture in the final product than mixes without the protein-reactive hydrocolloid.

Another important property of kappa carrageenan is its ability to form gels in the presence of potassium ions, and also to form rigid gels with locust bean gum. This gel-forming ability may be used in preparing piping gels, bakery jellies, and similar products. The ice crystal formation in frozen dough products is retarded by the addition of 0.1% carrageenan, thus improving the texture in frozen dough.

Alginates in combination with xanthan may be used to increase batter viscosity and increase cake volume. They also act as a cold-water gel base for instant bakery jellies and instant lemon pie fillings. Freeze-thaw stability of the fillings has been reported to improve in samples treated with alginates. In icings, alginates reduce stickiness and cracking. Alginates stabilize fat dispersion in whipped toppings and stabilize meringue products.

Some of the uses of agar important to the baking industry include its ability to stabilize icings or glazes by preventing water migration. It has also been used to reduce tackiness and to prevent adhesion of the sugar coating to the wrapper. Other applications include its use as a stabilizer

in pie fillings, piping gels, meringues, cookies, and similar products.

Bakery jellies that are heat reversible may be prepared with the use of 1 to 2% amidated pectins at 40 to 65% solids. Weeping or syneresis of pie fillings and glazes is also retarded or inhibited by the use of pectins, in combination with other gums.

Guar gum is used in cake mixtures to improve moisture retention in the finished product. It is a thickening agent and stabilizer for baked goods. Guar also helps to increase volume in yellow cake, probably by aiding in air entrapment. The whipping properties of toppings and icings are enhanced by the addition of 0.1 to 0.2% of guar based on the weight of the finished product.

Guar gum in combination with other hydrocolloids has been used to increase soluble dietary fiber content in bread. In a study, panelists evaluated "internal" scores for bread formulated with guar gum and carrageenan. The internal scores evaluated include grain, mouthfeel, crumb body, and taste aroma. Results of the study show high acceptability of the high-fiber bread with gums (24).

Guar is shown to have water-binding properties when used in bread doughs at 0.15%. When used at 0.1 to 0.2% in fruit pie fillings, it prevents the water from boiling out. Syneresis or weeping is retarded by the use of locust bean gum in gel desserts.

In bakery fillings, guar gum prevents water migration from the filling to the pastry due to its water-binding property. Freeze-thaw stability in frozen doughs is improved by use of xanthan gum. In baked goods, xanthan appears to inhibit starch retrogradation and improves shelf life of the finished product.

C. Salad Dressings and Sauces

"Full fat" salad dressings usually contain 30 to 60% oil and mayonnaise about 70 to 80%. Gums as thickeners, stabilizers, and emulsifiers are widely employed in these types of products industry. The most common emulsifying gum in high-oil salad dressings and sauces is propylene glycol alginate, an esterified form of alginate. Gum acacia may also be used to emulsify the oil but is used in combination with thickeners such as guar gum and xanthan gum. The synergy between xanthan gum and guar gum is well known (3) and utilized in stabilizing salad dressings. Xanthan gum adds acid stability and guar gum provides viscosity at lower cost than other thickeners. In products that contain milk protein such as creamy Italian or ranch dressing, a low concentration of carrageenan (0.05 to 0.10%) may also be used for its protein reactivity.

The formulation for a pourable creamy Italian salad dressing with 10% oil is shown in Table 139.6. The stabilizer used may include xanthan gum, guar gum, propylene glycol alginate, and starch. The sensory evaluation results of salad dressing bases using a 35% oil salad dressing and a 3% oil dressing using a fat mimetic gum system are shown in Figure 139.11. The attributes, opacity, cling, acidity, creaminess, and mouthfeel are comparable using a hedonic rating scale.

TABLE 139.6
Creamy Italian 10% Oil Dressing Formulation

Ingredients	%
Water	66.15
Vinegar (100 gr.)	7.00
Non fat dry milk	5.00
Sugar	4.50
Maltodextrin	4.50
Soybean oil	10.00
Salt	1.50
Xanthan/guar/gum acacia/starch system	1.35
	100.00

Procedure:

1. Add 10 parts to 1 part gum system to form a slurry.
2. Add oil/gum slurry to water while mixing. Hydrate for 10–15 min. with good agitation.
3. Add the rest of the ingredients.
4. Run through a colloid mill at medium setting to form a stable emulsion with small particle size.

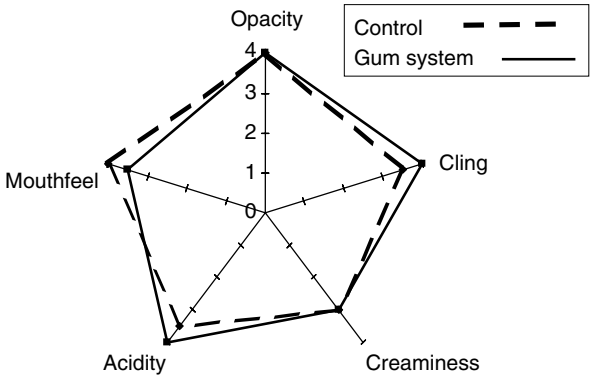

FIGURE 139.11 Sensory analysis of salad dressings (fat mimetic system vs. control).

D. Beverage Emulsions

Oil-in-water emulsions are employed widely in the food, beverage and pharmaceutical industries. Flavor components in beverage can be mainly oil-soluble, water-soluble, or a combination of both, and flavor retention in the beverage requires a good emulsifying system. Beverages mainly consist of water, sweeteners, acidulants (phosphoric acid or citric acid), flavors, and other additives such as coloring agents, weighting agents, vitamins, and minerals. The total solids may vary from 9 to 14% and the beverage may contain alcohol. Clear beverages require that any insoluble components of the citrus oils be removed by special treatment.

Typical beverage emulsion formulations, using a weighted or unweighted emulsion with gum acacia, an emulsifier with an HLB (hydrophile-lipophile balance) value of 10 to 11, are shown in Tables 139.7A, 139.7B, and 139.8, respectively). Weighted emulsions may contain SAIB (sucrose acetate isobutyrate), ester gum (glyceryl abietate) or BVO (brominated vegetable oil) as weighting agents to adjust the specific gravity of the flavor oil.

The emulsification procedure involves homogenization at about 3500/500 psi in a two-stage homogenizer. Homogenizers are special devices that disperse a mixture by forcing it through a tiny orifice under very high pressure. Microfluidizing equipment may also be used to prepare emulsions with very fine particle size (about 90% less than 2 microns).

TABLE 139.7A
Prototype Formulation: Weighted Beverage Emulsion (1)

Ingredients	%
Gum acacia, spray-dried*	15.0
Citrus oil/ester gum/SAIB**	10.0
Sodium benzoate	0.1
Citric acid	0.1
Water (added to make 100 mL)	

* May be replaced by 5% modified gum acacia.
** Sucrose acetate isobutyrate.

TABLE 139.7B
Prototype Formulation: Weighted Beverage Emulsion (2)

Procedure:
1. Add the preservatives to the water and mix thoroughly.
2. While mixing add the gum acacia gradually to the vortex of the solution.
3. Allow the gum to hydrate by mixing for 1 hour.
4. Dissolve the ester gum in the oil thoroughly by mixing for 2 hours.
5. Add the weighted oil from #4 to the gum solution.
6. Mix using a Ross mixer at medium speed for 10 min.
7. Homogenize at 3500/500 psi.
8. Pack into sterile containers.

TABLE 139.8
Prototype Formulation: Unweighted Beverage Emulsion

Ingredients	%
Gum acacia, spray-dried	15.0
Citrus oil	10.0
Sodium benzoate	0.1
Citric acid	0.1
Water (added to make 100 mL)	

Note: Procedure as in weighted emulsion.

E. Confections and Candies

Confectionery products include a spectrum of sweet goods, specifically candies and similar products. Sugar confectioneries include nougats, fondant, caramels, toffees, and jellies (e.g., gum drops and orange slices). Chocolate confections include chocolate and assorted chocolate-covered fruits, nuts, and cremes.

Gum acacia has the ability to retard or inhibit sugar crystallization and is used in the manufacture of pastilles and soft candy where sugar content is very high. In caramels, it will also help emulsify the fat to distribute it more uniformly and prevent oil from forming a rancid oily film. Gum acacia coacervates with gelatin are also used as chewy candy centers in many popular products flavored with peppermint or spearmint.

1. Gelatin Substitutes: Hydrocolloid Gelling Agents

Gelatin substitutes in gummy bears and similar products have been developed in recent years because gelatin is not acceptable to some religious groups and is sometimes associated with "mad cow disease." However, the properties of gelatin, including melting point, flexibility, and mouthfeel, are difficult to simulate with other hydrocolloids and textural differences between products prepared with gelatin and gelatin substitutes can be detected by the consumer. Gummy candies, using gelling agents such as pectin, agar, carrageenan, or combinations of these with modified starches have been introduced in the market, but with limited success. A formulation for a gummy candy without gelatin is shown in Table 139.9. A comparison of the texture profile analyzer (TPA) curves of gummy candy with gelatin and those prepared with some other gum systems containing agar and pectin as gelatin substitutes is shown in Figure 139.12.

F. Meat and Poultry Products

For poultry and meat injection, fine-mesh carrageenan incorporated with the brine significantly reduces cooking loss, thus increasing yield. Marinades may also be thickened and stabilized with guar gum and xanthan gum, a

TABLE 139.9
Gummy Candy with Pectin and Agar System

Ingredients	%
Sucrose	46.0
Glucose syrup	25.0
Agaroid gum system	2.0
Trisodium citrate	0.25
Citric acid (50%)	0.15
Water (to make 100%)	

heat-stable gum. In meat analogs or minced meat products, carrageenan at 0.5% to 1.0% level has been shown to reduce syneresis and act as moisture binder and adhesive (11).

III. FAT MIMETICS AND FUNCTIONAL FOODS

The typical North American diet provides a continuing challenge to the food industry to formulate highly acceptable low-fat products, particularly those low in trans-fatty acids, for the consumer. Unless an integrated approach is used, fat or oil mimetics may yield products that are inferior to the full-fat, high-calorie counterparts. Initially the role of the fat in the specific product has to be determined and then steps taken to simulate or mimic the functionality and sensory qualities of fat or oil being reduced. Some of the important factors to consider include the following: mouthfeel and texture characteristics, impact on threshold value or perception of flavor, functionality of the fat in the product, processing conditions during manufacturing scale-up, shelf life, microbial stability, and water activity. Hydrocolloid systems consisting of gums, starches, and other components may be used as fat mimetics (26). However, it is not recommended that all the fat or oil is replaced in the formulation, since the lipids have special functional properties that the gum systems may not be able to replace. Partial reduction of fat or oil up to 50% replacement may be feasible so as to prevent the significant changes in the threshold value of flavor compounds. The flavor components are significantly affected by the fat or oil content of the finished product as shown by significant changes in threshold value.

A combination of gum acacia, a food starch, and alginates has been successfully marketed for low-fat muffin and other bakery mixes (26). The main component of the system, gum acacia, is an unusual gum that offers advantages over other gums as a fat mimetic for the following reasons. Unlike other gums, it has low viscosity about 100 cps at 25% solids and contains 2 to 3% protein (which provides its good emulsifying properties). It imparts smooth mouthfeel and acts as a lubricant, inhibits ice and sugar crystallization, is high in dietary fiber, has adhesive properties, and may be used for spice adhesion instead of oil.

Fat mimetic systems using gums and starches incorporated in a low-fat cookie formulation have been shown to yield a product with longer shelf life, based on higher moisture content retention and lower hardness values measured using a Steven's Texture Analyzer.

Gelling agents such as carrageenan and agar may also be used in fat mimetic systems to simulate the texture of fat in icings and glazes. In developing low-fat systems, the fat is usually replaced with water and other solids. It is imperative that the formulator minimize the water activity gradient between the substrate and the filling or glaze, to reduce water migration.

The high soluble dietary fiber content of hydrocolloids makes them essential components of functional/nutraceutical foods and beverages. For example, the use of low viscosity guar gum and gum acacia in high-fiber beverages such as smoothies or fruit concentrates is increasing. In granola bars and dry beverage mixes, the health benefits are being augmented by adding gums as sources of soluble fiber including the following: inulin, gum acacia, polydextrose, and hydrolyzed guar.

IV. GUM SYSTEMS: SYNERGY AND INTERACTION

When certain specific gums are used in combination, the functional properties are significantly enhanced or modified due to synergistic action. For example, a combination of xanthan gum and locust bean gum at 1.0% gum level will form a heat-reversible flexible gel whereas the individual gums are not gel forming (27). The interaction of locust bean gum with kappa carrageenan, to yield

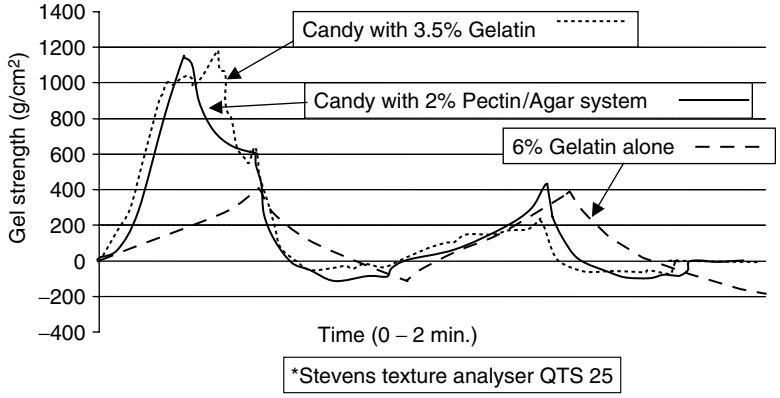

FIGURE 139.12 TPA of gummy candy with pectin/agar system vs. gelatin.

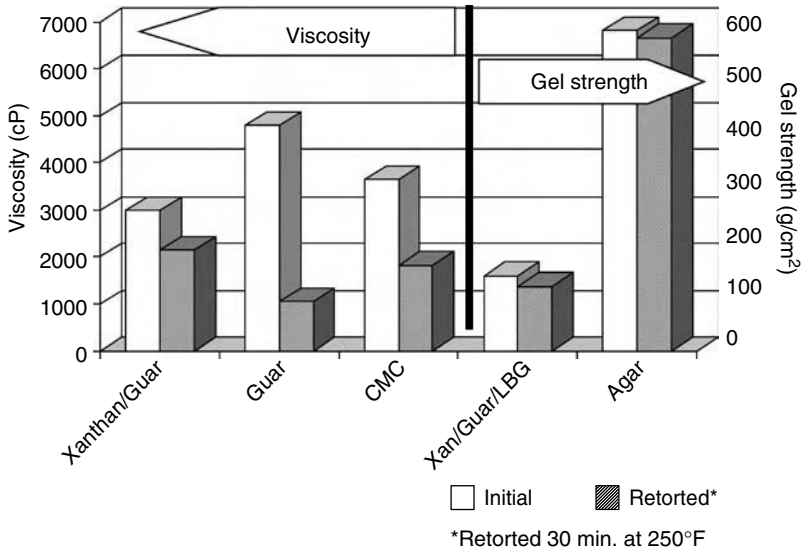

FIGURE 139.13 Effect of heat processing on various gums.

heat-reversible gels with lower degree of syneresis than gels made with kappa carrageenan alone, has also been used in baked goods. Synergy between alginate, gum acacia, and starches has been utilized to formulate fat-mimetic systems as discussed earlier. Guar/xanthan provide strong viscosity synergism when used in combination (1, 2).

A comparison of the effects of heat processing (retort temperature at 250°F for 30 minutes) on some gums and gum blends is shown in Figure 139.13. Some gums, due to their synergy, are more resistant to heat degradation when used in combination with each other.

V. MAXIMUM USAGE LEVELS AND QUALITY SPECIFICATIONS

The maximum permitted usage levels of gums in foods and beverages vary according to the Code of Federal Regulations.

The Food Chemicals Codex of the Food and Drug Administration also establishes the specifications for each gum, and a summary of the FCC standards for guar gum, as an example, is shown in Table 139.10. In addition, the food manufacturer should specify for each gum or gum system specific microbial limits, gel strength, viscosity, particle size distribution, and other relevant parameters, in order to avoid unwarranted variations in the quality of the finished formulation.

VI. PREHYDRATED OR AGGLOMERATED GUMS AND GUM SYSTEMS

Proprietary processes of agglomeration or "prehydration" of gums and gum systems have been developed by a number of ingredient manufacturers (11). The end products hydrate faster, have no lumping problems and are virtually dust-free, resulting in fewer incorporation problems, reduced dusting, mote efficient use of equipment, and production and labor costs. The agglomerated gums such as CMC and gum acacia have been shown to reduce motor load in a twin-screw extruder when incorporated at low levels with the initial dry mix (28). They also improve the mouthfeel and texture of corn cereal extrudates, based on sensory evaluation. These so-called prehydrated gum stabilizers are also preferred for use in low-oil salad dressings and in low-moisture systems.

TABLE 139.10
FCC & USP/NF Standards: Guar Gum

Tests Required	FCC	NF (USP)
Identification	A. Opalescent, viscous solution	
	B. No appreciable increase in visc.	
Acid* insoluble matter	≤7.0%	Same
Arsenic	≤3 ppm	Same
Total ash	≤1.5%	Same
Galactomannans	≥70%	Same
Heavy metals	≤0.002%	Same
Lead	≤5 ppm	≤0.001%
Loss on drying	≤15%	Same
Protein	≤10%	Same
Starch	No blue color produced	Same

* H_2SO_4.

VII. ANALYTICAL METHODS FOR EVALUATION OF GUMS

A laboratory designed to analyze and evaluate individual hydrocolloids and gum systems requires a number of essential instruments and equipment. For the study of viscosity and other rheological properties of gum solutions, a programmable rheometer (e.g., a Brookfield™ model DVIII)

is useful in characterizing the effect of shear rate, temperature, time, and stress. A Bostwick™ flow meter or consistency meter can be used to measure flow of a viscous fluid vs. time.

For the proximate analyses of gums and their basic composition, the laboratory should be equipped with instruments for measuring pH, ash, protein, fat, and moisture. HPLC (high pressure liquid chromatography) equipment with appropriate columns, refractive index measurement capabilities, and UV detectors may be used to analyze monosaccharide ratios of gums and help detect cross-contamination, adulteration, and the presence of bulking agents.

To analyze molecular weight changes and effects of various factors such as pH and temperature on the stability, hydrolysis, or degradation of gums, gel permeation chromatography using various known standards is useful. To save instrumentation costs, independent analytical laboratories may of course be utilized at any time whenever deemed necessary.

For gel strength measurements, programmable texture analyzers that can determine parameters such as hardness, cohesiveness, adhesiveness, and gumminess help evaluate the texture of the gel or the finished gelled product. This may help the sensory evaluation specialist by providing an objective comparison of the texture profiles of products prepared with gelatin with those containing polysaccharide gelling agents.

A useful instrument for evaluating emulsifying systems such as gums for beverage emulsions, is a particle size analyzer (e.g., a Coulter Counter). Preparation of stable emulsions requires a homogenizer that can operate at 2500 up to 6000 psi. A microscope equipped with a digital camera can be used to analyze the morphology of starches and the emulsion particle size of salad dressings, creams, and similar products.

A differential scanning calorimeter may be used to analyze melting point and gelling temperatures and other endothermic and exothermic properties such as glass transition temperatures of various hydrocolloid systems.

VIII. SUMMARY AND RECOMMENDATIONS

Hydrocolloids are highly functional ingredients in foods and beverages. Preferably, they should be incorporated with some of the dry ingredients such as flour or sugar to avoid lumping or incomplete hydration. The gum manufacturer should specify the various requirements for optimum functionality of the gum (pH, % solids, temperature of hydration, salts, co-factors, etc.). The impact of large-scale processing operations and the possible need for special equipment design should be jointly evaluated by the food technologist and the chemical engineer. This can reduce production problems during scale-up from the R & D or applications laboratory to the manufacturing plant.

In developing a fat mimetic system in food products, a systems approach should be used in which a variety of synergistic components, including gums, are used to duplicate the functional and sensory characteristics of the specific full-fat product. The development of a zero fat formulation is generally not recommended since significant changes in threshold value of flavor components in going from a high-oil to oil-free medium make it difficult to simulate the flavor of regular fat products. However, fat or oil reduction and increase in soluble fiber in the average American diet are essential, requiring the food manufacturer to make healthy alternative products and functional foods available to the consumer.

Using an individual gum for a certain application may not be adequate to achieve the desired quality attributes in the finished product. Instead, a combination of the optimum ratio of specific gums that exhibits synergistic properties may be required. The food technologist and the gum supplier should be familiar with the chemical and functional properties of each component in the food system and the resulting interactions between various ingredients and at varying processing conditions.

ACKNOWLEDGEMENTS

The authors would like to thank Steve Andon and Chris Andon, of TIC Gums, Inc., for administrative and financial support; and Ken Kuschwara & Jim Caulfield of the R & D Group of TIC Gums, Inc., for their technical assistance.

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140 Pectins

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Herbstreith & Fox KG, Pektin-Fabrik Neuenbürg

CONTENTS

I. INTRODUCTION

Pectins belong to a group of closely related polysaccharides or pectic substances, located in the middle lamella and primary cell walls of higher plants (dicotyledons). The general term protopectin is often used to designate the native, insoluble pectins in the cell walls that cannot be extracted by methods that are non-destructive or non-degradative. The dominant feature of pectins is a linear chain of α-(1,4)-linked D-galacturonic acid units in which varying proportions of the carboxyl groups are esterified with methanol to methyl esters. This group of pectic substances covers the following:

- different methyl esters (pectins with different degree of esterification — DE), their salts (pectinates)
- unesterified pectic acid, its salts (pectates)
- different neutral polysaccharides linked to the polygalacturonan backbone such as arabinans, arabinogalactans, arabinoxylans, and galactans

Portions of these neutral polysaccharides are a part of the isolated, commercial pectins divided into HM pectins and LM pectins with correspondingly high and low methyl ester content. Amidated pectins are obtained by saponification of HM pectins with ammonia under alkaline conditions.

II. OCCURRENCE OF PECTINS

Beside cellulose, hemicelluloses, glycoproteins, and lignin pectins form a major part of the cell wall of all higher plants. The concentration of pectins is highest in the middle lamella, a tissue responsible for the adhesiveness of cells (1), and decreases from primary to secondary walls, where pectins are almost absent. Pectins participate in plant physiology: water retention, ion transport, porosity, growth, and the size and shape of cells. Pectins are involved in defense mechanisms against infections by plant pathogenic micro organisms, generating by their enzymatic attack (mainly using a polygalacturonase PG) oligogalacturonides with a degree of polymerisation between 10 and 15, which can be recognized by the plant (so called elicitors). This results in an activated metabolism (2). This reaction may be used to activate plant cell cultures for a higher productivity.

The specific functions of pectins in distinct parts of the cell walls or plant tissues are influenced by the amount and nature of specific molecules present (3).

III. CHEMICAL STRUCTURE OF PECTINS

Studies on pectins from many sources have illustrated that pectin is a heteropolysaccharide (see Table 140.1) (4). Pectins consist of a linear zigzag shaped structure of axial-axial linked α-(1,4)-D-galacturonic acid units, a result of the equatorial position of the carboxyl group. The poly-galacturonic acid chain is interrupted by "inserted" α-(1,2)-linked L-rhamnopyranosyl units resulting in a kink that determines the linear portion of the corresponding pectin segment (see Fig. 140.1) (5, 6).

Results from X-ray diffraction analyses fail to confirm:

- if the pectins are right- or left-handed double or single helix
- what the number of repeating units is

Pectin segments or fractions with low content of L-rhamnose are described as smooth regions of pectins or homogalacturonans. Those with high L-rhamnose content are known as hairy regions or rhamnogalacturonans.

The neutral sugar side chains consisting of mainly L-arabinose and D-galactose are bound by covalent linkages to the L-rhamnose units. L-fucose is found as the terminal end of these side chains. As minor sugars also D-xylose, D-glucose, D-mannose, and D-apiose are found next to further rare sugars. The minor sugars can occur as single unit side chain such as D-Xylose or as short side

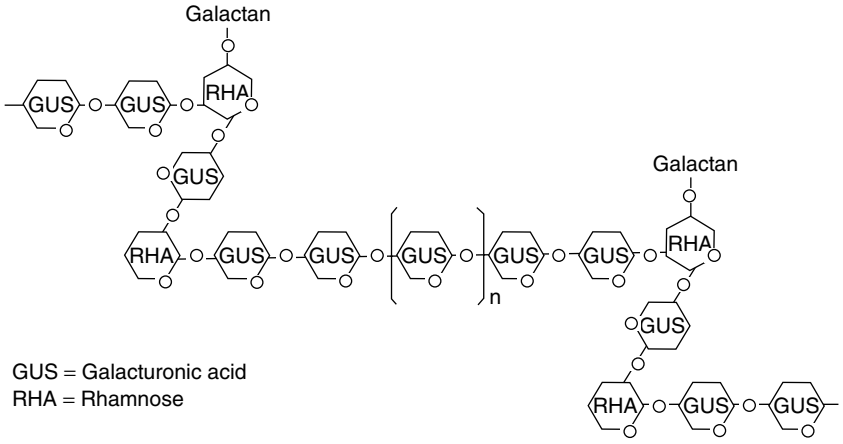

FIGURE 140.1 L-rhamnose in the galacturonan chain.

TABLE 140.1
Galacturonic Acid and Neutral Sugars in Some Pectic Materials (4)

	Apple[a]	Sugar Beet[b]	Carrot[c]	Plum[d]	Potato[e]
Yield	**14[f]**	**11.1[f]**	**13.5[f]**	**28.6[f]**	**13.1[g]**
GalA	58.0	54.9	54.7	43.0	43.6
Rha + Fuc	3.0	3.2	3.8	1.5	1.3
Ara	23.0	12.5	11.7	5.9	7.0
Xyl	1.0	0.2	0.2	0.4	0.4
Man	1.0	—	0.7	1.1	0.5
Gal	5.0	8.1	8.3	15.2	5.5
Glc	3.0	0.3	1.2	3.8	4.5

[a] Pectins extracted with hot water (7).
[b] Pectins extracted by 0.05 M NaOH, 4°C after extraction with water, oxalate, acid (8).
[c] Pectins extracted by 0.05 M HCl at 85°C (9).
[d] Pectins extracted by water at room temperature (10).
[e] Pectins extracted with CDTA, pH 6.8, 20°C (11).
[f] Yield calculated from an alcohol-insoluble residue.
[g] Yield calculated from cell-wall material after SDS and DMSO treatments.

chains whereas L-arabinose and D-galactose form complex structures.

The arabinans are branched polysaccharides with a backbone of α-(1,5)-linked arabinofuranosyl residues with α-(1,2)- and α-(1,3)-linked arabinofuranosyl side chains. Pectins with attached arabinans can be isolated from many fruits and vegetables like apples, sugar beet, apricots, carrots, cabbage, onion, and pears.

Citrus fruits, potato, soy beans, grapes, apples, onions, tomatoes, and others contain arabinogalactans, described as two structurally different forms. Type I consists of a β-(1,4)-linked linear chain of D-galactopyranosyl residues with short chains of linear α-(1,5) arabinans connected to O-3. Type II is a highly branched polysaccharide with ramified chains of β-(1,3)- and β-(1,6)-linked D-galactopyranosyl residues terminated by L-arabinofuranosyl and to a smaller extent by L-arabinopyranosyl residues.

Albersheim and co-workers (12–14) studied the structure of suspension cultured sycomore cells and described rhamnogalacturonan I and II. Rhamnogalacturonan I was analyzed to have a linear structure of alternating α-(1,4)-linked D-galacturonosyl and α-(1,2)-linked L-rhamnosyl residues with a bunch of different neutral sugar side chains. Rhamnogalacturonan II is a very minor and complex heteropolysaccharide consisting of about 30 glycosyl residues.

Using chemical (β-elimination) or enzymatic (endo-PG or endo-PL) degradation techniques to split the polygalacturonan backbone, the following is observed. The L-rhamnosyl residues and the neutral sugar side chains are not homogeneously distributed over the pectin chain. Sequences rich in neutral sugars are interspersed with almost pure poly-D-galacturonosyl blocks. This finding distinguishes pectic polysaccharide as smooth or hairy regions (15–18) which are also homogalacturonans and rhamnogalacturonan I (13, 19, 20). The figures in literature on the length of homogalacturonan blocks vary from 25 (21) to 40–60 (22), and 72–100 galacturonic acid units (17).

But also non-sugar substituents (beside the methyl ester of the carboxyl groups or the amide groups at C-6) bound to C-2 or C-3 of the galacturonic acid like acetic acid and phenolic acids can be found with some pectic substances.

The degree of esterification (DE) or methylation (DM) is described as the percentage of esterified galacturonic acid units. If more than 50% of the carboxyl groups are esterified, the pectin is called high methyl ester (HM) pectin; if less than 50%, low methyl ester (LM) pectin. Pectic acid is defined to have a DE of $<10\%$.

The carboxyl groups (free and esterified) can be distributed statistically (random) or blockwise along the pectin molecules. The distribution pattern influences the reactivity of the pectins with bivalent cations and positively charged proteins. Acid treatment and most microbial pectin esterases (PE) deesterify HM pectins statistically. Plant pectin esterases work blockwise creating high reactive zones of negatively charged sequences. This property is ambiguous when used in commercial pectin applications. Also, this property distinguishes between intra- and intermolecular distribution of carboxyl groups. "Intermolecular" refers to an inhomogeneous pectin preparation.

The degree of acetylation (DAc) is defined as mol% of acetic acid calculated on the content of galacturonic acid. This may result in a DAc higher than 100% because C-2 and C-3 of the galacturonic acid and other sugars of the pectin can be acetylated. Apple and citrus pectins have a negligible DAc whereas beet pectin is highly acetylated. A DAc of about 25% can be found in some commercial beet pectins, e.g., those by Herbstreith & Fox.

Galacturonic acid content (GalA), degree of esterification (DE), and degree of acetylation (DAc; mol/mol) of some pectins extracted from different plants are summarized in Table 140.2 (4).

Fry (30), Rombouts and Thibault (8), and the research group of Ralet and Thibault (31, 32) described ester-linked ferulic acid units in beet and spinach pectin, linked to arabinosyl and galactosyl residues. Fry (33) was investigating the possible role of phenolic compounds of the primary cell wall in the hormonal regulation of growth. By these ferulic esters pectin chains can be cross-linked by phenolic coupling. In plant tissue these phenolic bonds are of big importance and can be used for firming fruits for food application. Also, gels from beet pectin can be formed by oxidative coupling, using phenoloxidase and peroxidase.

Isolated commercial pectins seem to belong to the group of homogalacturonans. However, they are obtained from apples, citrus fruits, sugar beet, or sunflower heads by extraction conditions cleaving covalent bonds to the hairy part of pectic substances firmer bound into plant tissue by neutral sugar side chains.

TABLE 140.2
Galacturonic Acid Content (GalA), Degree of Methylation (DM) and Degree of Acetylation (DAc; mol/mol)[a] of Some Pectins (4)

Origin	Extraction	GalA (%)	DM (%)	DAc (%)	Ref.
Mango	Acidic	54	68	4	(23)
Sunflower	Acidic	81	17	3	(23)
Sugar-beet	0.05 M HCl; 85°C	65	62	35	(8)
Carrot	0.05 M HCl; 85°C	61	63	13	(24)
Grape	Oxalate pH 4.5; 20°C	63	69	2	(25)
Sunflower	Oxalate	83	27	10	(26)
Peach	HCl to pH 2; 80°C	90	79	4	(27)
Siberian apricot	EDTA	64	57	8	(28)
Cythere plum	0.05 M HCl; 85°C	65	65	16	(29)
Potato	HCl, pH 2; 80°C	40	53	15	(4)

[a] Calculated on the assumption that all acetyl groups are bound to galacturonic acid.

IV. BIOCHEMISTRY OF PECTINS

A most recent and excellent description of the biosynthesis of pectins is by Mohnen (34). Pectin is said to be the most complicated polysaccharide in plant cell wall. Since we do not know its exact composition and the synthetic process of various fractions of pectic polysaccharides, we may benefit from a summary of what information is available:

At least 12 activated sugar substrates (nucleotide-sugars), 14 distinct enzyme activities (for their production), and 58 glycosyl-, methyl-, and acetyltransferases are required for pectin synthesis. Mohnen (34) cites comprehensive reviews on:

- pectin structure, pectin and cell wall synthesis (35–40)
- nucleotide-sugar interconversion pathways (41)
- wall biosynthetic genes (38, 42)
- glycosyltransferases (43)

The synthetic process of pectin is often described as follows:

- synthesized as homogalacturonans in the cis-Golgi
- branched in the trans-Golgi cisternae
- highly esterified in the medial and trans-Golgi
- transported as high esterified, branched pectin to the plasma membrane in vesicles, which move along actin filaments via myosin motors, and subsequently inserted into the wall or the cell plate
- followed by deesterification to create calcium-reactive sequences

Other observations confirm that pectin synthesis may differ:

- in different cell types
- in different species
- at different points during development, or
- even at different locations in the same wall (cited in 34)

The precursors of the nucleotide-sugars for pectin synthesis are hexose-phosphates from photosynthesis and stored starch or sucrose. The main sugars of pectic substances are D-GalA, L-Ara, D-Gal, D-Man, L-Rha, D-Xyl, L-Fuc. They are synthesized accordingly:

1. All can be synthesized enzymatically via D-Glc-1-phosphate, uridine-diphosphate-D-glucose (UDP-D-Glc), and their corresponding uridine-diphosphates by different nucleotide-sugar transformation pathways.
2. All can be recycled from walls by the salvage pathway.
3. D-GalA, D-Xyl, and L-Ara can also be synthesised enzymatically by the myoinositol pathway from UDP-D-GlcA and the subsequent and corresponding UDP-sugars/UDP-sugar acids.

Examples of well investigated enzymes in these pathways are:

- UDP-glucose-6-dehydrogenase (EC 1.1.1.22), oxidating UDP-D-glucose to UDP-D-glucuronic acid
- UDP-glucuronate-4-epimerase (EC 5.1.3.6), transforming UDP-D-glucuronic acid into UDP-D-Galacturonic acid
- UDP-xylose-4-epimerase, transforming UDP-D-xylose into UDP-L-arabinose.

UDP-xylose is synthesized from UDP-D-glucuronic acid by UDP-GlcA-decarboxylase (EC 4.1.1.35). L-rhamnose is proposed to be produced from UDP-D-glucose via UDP-4-keto-6-deoxy-glucose (by UDP-Glc-4,6-dehydrogenase) and UDP-4-keto-6-deoxy-L-mannose (by UDP-4-keto-L-rhamnose-3,5-epimerase), with a final conversion via UDP-4-ketorhamnose-reductase.

The nucleotide sugars are transported into the Golgi and used as substrate by a pectin biosynthetic glycosyltransferase that transfers the glycosyl residue onto a growing polymer (34).

V. PECTINS IN CELL WALL ARCHITECTURE

Are pectins and their fractions including neutral sugar side chains covalently linked to other polysaccharides or

glycoproteins in cell walls? Some observations from complex early models suggest such linkages, the existence of which has never been proven (44, 45). Current accumulated reports suggest the following:

1. The existence of several woven but independent networks in the cell walls and pectin network is one of them, situated next to cellulose-xyloglucan-cellulose bridges (46, 47)
2. The existence of independent protein-protein structures (48) instead of the hydroxyprolin-rich protein extension structure crosslinked to pectin

Pectins are linked with each other through Ca^{2+}-bridges (21), via borate esters between apiose residues (40), diferulic acid bridges between arabinoxylans side chains (16), and finally esters between the carboxyl groups of the galacturonic acids (49). In growing plants, cell wall structures undergo a continuous change. Crosslinkages are made and broken, especially when a cell divides, expands, or matures. An in-depth discussion on interactions between pectins and other polymers is reported by Mort (50).

VI. DETERMINATION OF THE PECTIN CONTENT AND KIND OF PECTIC SUBSTANCES

To characterize pectic substances and their change during growth, ripening, storage, and processing in plant tissue a sequence of events is noted.

Pectins are enriched and purified from interfering plant components by washing with hot alcohol to remove alcohol soluble components from the tissue or by fractional extraction techniques. Native enzymatic activities in the plant material are inactivated instantaneously. The repeated washing with hot alcohol results in an AiR (alcohol insoluble residue) composed mainly of cell wall materials, protein, and starch (51). The latter have to be removed by degradation with pure enzymes under conditions not affecting the pectic substances. Starch can also be removed with 90% dimethyl sulfoxide. The cell wall material undergoes further characterization and fractional extraction techniques (chemically and/or enzymatically). Substances, soluble or solubilized under the respective extraction conditions, are yielded as watery extract to be precipitated in alcohol. The alcohol insoluble substance (AiS) is dried, grinded, and analyzed. Note that all extraction and fractionation conditions modify the pectic substances to a certain extent. The evaluation of the different fractions and the knowledge of the enzymatic activity provide us with the ambiguous image of the native pectic substances which we have today.

The chemical fractionation techniques exploit the different properties of pectic substances. A common procedure is the subsequent use of:

1. cold and/or hot water or buffer solutions for already soluble pectins, mainly HM
2. monovalent buffer solutions to solubilize pectins fixed by bivalent cations
3. chelating agents like EDTA, CDTA, oxalate, or hexametaphosphate for HM and LM pectins crosslinked and bound in cell wall by bivalent cations
4. cold and/or hot acid with different pH, temperature, and time regimes for HM pectins crosslinked with cellulose by neutral sugar side chains
5. cold alkali to prevent β-elimination such as sodium carbonate or sodium hydroxide, often in combination with sodium borohydride to protect the reducing end of polysaccharides, suitable for hemicellulose extraction crosslinked with pectins

Other chemicals for extraction are also used (4, 52).

Voragen et al. (4) give an overview on enzymatic extraction and fractionating procedures. One approach is based on the degradation of the rhamnogalacturonan backbone, using endo-polygalacturonase, pectin esterase/endo-polygalacturonase, endo-pectinlyase, or endo-pectatelyase, and recently rhamnogalacturonases. In general two fractions of degradation products are obtained, oligogalacturonides and rhamnogalacturonides, that are rich in neutral sugars. These fragments can be further characterized. Degree of polymerization depends on enzyme combination, sugars, and glycosidic linkages which clarify the structure of the heteropolysaccharides present.

The second approach is to use non-pectolytic enzymes for the extraction of unaltered pectins. This approach is often discussed as an alternative to industrial acidic pectin extraction. According to available data, it is not possible to yield comparable pectin quantities using combinations of cellulases and hemicellulases (53, 54). However, the use of an endo-glucanase in combination with an endo-pectinlyase can result in a significant pectin yield in the laboratory. But the gel strength of this pectin is poor.

VII. CHEMICAL, ENZYMATIC, AND MECHANICAL MODIFICATIONS OF PECTINS

Pectic substances can be attacked at the polygalacturonic main chain, the neutral sugar side chains, or at the methyl, acetyl, and phenolic ester groups. Glycosidic bonds between uronic acids are relatively stable against hydrolysis; arabinofuranosyl bondings are weak in acidic conditions. This difference is used for pectin extraction by

acidic hydrolysis to split the neutral sugar side chains, which connect the pectins with the cellulosic fibrils fixing pectin in the cell walls. This way the insoluble protopectin is converted to soluble pectin. The methyl and acetyl ester groups are partially hydrolyzed by the extraction procedure, which is sometimes used to extract pectins with a smaller degree of esterification.

Pectin solutions are stable at pH 2.5–4.5. At a lower pH the a.m. hydrolysis occurs. This is used to produce pectins with a lower degree of esterification, changing, within the region of HM pectins, their setting behavior from rapid set (DE $>$ 70%) to slow set (DE ca. 60%). Further deesterification results in low ester pectins. At low pH values also acetyl groups from sugar beet pectin are removed and jellifying beet pectins with poor gelling strength are formed.

At low pH, e.g., boiling in 12 M HCl, the galacturonic acid releases CO_2. This reaction quantitates the pectin content (55).

At pH $\geqslant$ 5 and elevated temperature, the glycosidic bonds of esterified polygalacturonic acid main chains are split at the non-reducing side next to a methyl esterified galacturonic acid unit. This is done by β-elimination, resulting in an unsaturated galacturonic acid molecule with double bonding between C-4 and C-5 (56). Because this reaction can occur wherever there is an esterified acid group in the molecular chain, a small amount of degradation can cause a large loss in viscosity, gelling power, and other functional properties. This reaction is slow at low temperatures. Increasing the pH accelerates saponification of ester groups, which competes with β-elimination. Pectic acid is stable in alkaline conditions. We can take advantage of β-elimination to decrease water binding capacity of pectins by soaking legumes in neutral mono-valent buffer solutions and by liming of fruit press cakes after juice production. Such treated residues can be further pressed to increase solids before drying. Pectins can also be degraded by oxidants.

The most common chemical modification is the alkaline amidation of pectins in alcoholic suspension, using ammonia. By this reaction methyl ester groups are converted to acid amid groups ($-CONH_2$). Free carboxyl groups cannot be amidated.

Pectins can also be esterified with methanol at a low pH in methanolic suspension, using sulphuric acid. As a by-product, sodium methyl sulphate can be produced with a limit of 0.1% in commercial pectins per FDA and FCC.

Crosslinking of pectins by epichlorhydrin is successfully used to prepare affinity chromatography columns to separate pectinases. Crosslinking by phenolic coupling was mentioned earlier.

There are several enzymes involved in pectin degradation.

1. glycosidases catalyzing the degradation of neutral sugar side chains (group of arabinanases, galactanases, xylanases, etc.)
2. esterases, pectin esterase (PE) (EC 3.1.1.11), and pectin acetyl esterase
3. enzymes splitting linkages between the galacturonosyl residues of the pectin main chain like endo- and exo-polygalacturonase (PG) (EC 3.2.1.15), endo- and exo-pectate-lyases (PAL) (EC4.2.2.2 and 4.2.2.9), and pectin-lyase (PL) (EC 4.2.2.10)

The reaction products of the hydrolases are identical with those from chemical hydrolysis. The lyases work by a trans-elimination mechanism. PG and PAL split low methyl ester pectins and pectates at an unesterified galacturonic acid unit. PL splits a site next to an esterified group, catalyzing the β-elimination reaction.

More recently a group of rhamnogalacturonases (hydrolases and lyases from *Asp. aculeatus*) were described, acting on highly branched regions of pectins, liberating oligosaccharides consisting of rhamnose linked to galacturonic acid and to galactose (optional) (18, 57).

Commercial pectinases are often mixtures of enzymes mentioned above and several hemi-cellulases, cellulases, and proteases. Producers may use one or both enzymatic systems:

1. a system based on pectin lyase, degrading pectin alone with the disadvantage of creating unsaturated oligogalacturonides that are sensitive to browning
2. a system based on polygalacturonase in combination with pectin esterase, converting HM pectins to LM pectins, the substrate for the polygalacturonase

Bock et al. (58) characterized pectins ground by a vibration mill as follows:

- The molecular weight was decreased by "mechanolysis."
- The degree of esterification, the neutral sugar content, and the reactivity against calcium ions, assuming the pectins could form a gel, was almost unchanged.

Ralet et al. (59, 60) and Ralet and Thibault (61) suggested extrusion cooking as a method for pectin production. The extrusion process converted the pectic substances of the cell walls of apple, citrus, and sugar beet into soluble pectins. The softening of plant tissue after extrusion cooking is also associated with this degradation. Bondar und Golubev (62) announced a new desintegration and extraction technique for pectins, using cavitation.

VIII. PECTINS AS POLYELECTROLYTES

Pectic substances are negatively charged polyelectrolytes. Their charge density and their apparent dissociation

constant pK_a calculated from pH measurement depend on the degree of dissociation. The pK_0 determined for pectins is independent of pectin concentration and of their degrees of polymerization, esterification, amidation, and acetylation. The values obtained are in the range of 3.0 to 3.3, close to that (3.5) of monomeric galacturonic acid (cited in (4)).

At pH $\leqslant$ 3.0, the dissociation of pectins is almost repelled; they are almost undissociated and uncharged. This is an important premise for pectin chain association in the case of jellification of HM pectins.

Monovalent cations (Na^+, K^+, etc. and NH_4^+) are bound to pectins electrostatically only. These ions can weaken pectin association in tissues as in thickened and jelled products. Magnesium ions do not support pectin jellification and follow also only electrostatical theories. The other alkaline earth cations cause pectin chain associations resulting in:

- tissue hardening
- increased viscosity
- gel formation of LM pectins
- precipitation of their pectinates

Such observations or effects are dependent on:

- cation concentration, ionic strength, and pH
- degree of esterification
- distribution of free carboxyl groups (random or blockwise) [for HM pectins]

When HM citrus pectins or HM pectins are slightly deesterified, using a plant, blockwise acting pectin esterase (creating blocks of free carboxyl groups), there is a significant increase in affinity and viscosity with calcium ions. This effect improves protein stabilization and increases the tendency towards syneresis which impairs jellification.

Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, and Pb^{2+} ions influence the circular dichroism behavior of pectins in the same way. That is, associate two pectin chains if blocks of free carboxyl groups with adequate length are present, i.e., 7, 10, and 20 units (21, 63). Practically LM pectins with a degree of esterification of about 40% and less show gelling properties by this mechanism. This chain association is described as "egg-box" gelling mechanism. Two pectin chains arrange parallel and symmetrically and form together (due to the axial-axial glycosidic linkages of the galacturonic acid polymer) negatively charged cavities in which the cations fit as eggs in a box. Thom et al. (64) and Debongnie et al. (65) describe Cu^{2+} ions to behave different, and circular dichroism spectra are also different.

In food processing usually calcium salts of different solubility (like calcium chloride, calcium lactate, calcium citrate, and calcium mono- and di-phosphates together with monovalent buffer salts to influence velocity of chain association) are used together with LM pectins (DE $<$ about 44%) to form gels at reduced soluble solids (sugar content). LM pectins are also described to bind heavy metals as an antidote for heavy metal poisoning and as an ion exchanger to remove heavy metals from effluents.

Calcium pectinate is used as fat replacer forming soft, creamy insoluble particles. Pectins can be precipitated as their aluminium salts (ancient pectin production method) or copper salts (ancient quantitative pectin determination method — Cuprizon method).

IX. RHEOLOGY OF PECTINS

Using pectins for technological reasons means that in general the producer has some means of influencing flow behavior and texture. The ability to form gels under acidic conditions is one such method under the disposal of the producer.

The rheological properties of native pectins are used in food industry. If these properties are negative pectins have to be degraded in the manufacturing process. Pectin degrading enzymes are used to extract and concentrate juices. To achieve a high yield or capacity for the pressing systems, pectinases with other cell wall degrading enzyme activities are used in mash treatment (liquifaction). Stored apples will respond best to treatment with these enzymes for their content of soft tissue and high soluble pectin facilitates the binding of juice in the mash. Berries like black and red currant, grapes, etc. are also treated with pectinases for juice production to increase yield and color intensity. Some of the anthocyanes are bound to pectic substances and will be released into the juice if the pectin is degraded. Tomato is handled in two stages in the cold break process: degrading the pectin and subsequent concentration to tomato concentrate. In the case of manufacturing tomato ketchup, the hot break process is used to maintain pectin quality and flow behavior by inactivating native pectinases immediately after squeezing the tomato juice.

Axelos et al. (66) reported the dividing line between:

- dilute and semi dilute pectin solution in 1 M NaCl: a concentration C* between 0.7% and 1.0%
- semi dilute and concentrated regime: a concentration C** between 8% and 10%

Up to C* the specific viscosity corresponds to $C[\eta]^{1.2}$ and beyond C** to $C[\eta]^{3.3}$. Values of the Huggins coefficient k_h, which characterizes the effect of molecular interaction on the viscosity, vary with the degree of esterification, ranging between 0.37 and 0.6.

Pectins are insoluble in organic solvents and soluble in water to give a viscous solution. Like other viscous gums, it needs care in dispersing the powder rapidly into the water. Lumps of powder easily become coated with a gel layer

which makes further solubility slow and difficult. Appropriate techniques to prepare pectin solutions:

- a high-shear mixer
- separating pectin particles by dilution with soluble powders like sugar (10 fold quantity of pectin)
- dispersing the pectin in high concentrated sugar syrups (at least 50% soluble solids to prevent significant swelling of the pectin) with subsequent dilution to less than 30% soluble solids. Under heating and moderate agitation the pectin will dissolve rapidly.

Pectin will not dissolve completely at high sugar concentrations. Thoroughly dissolved pectin will have no "fish-eyes" on a glass plate and will not feel gritty when rubbed between the fingers. Solutions with 3 to 4% pure pectin can be prepared easily. Industrial processing with more than 10% pectin in hot water will exceed the limit of complete pectin dissolution.

Pectins with a low degree of esterification dissolve better in the form of monovalent metal salts. Bi- and trivalent cations decrease the solubility of all pectins. Pectic acid is only soluble in water as ammonium, sodium, or potassium pectates at $pH > 6$. Pectins are also soluble in DMSO, formamide, dimethylformamide, and warm glycerol (67).

Industrial pectin solutions with unchanged molecular weight exhibit a pseudoplastic flow behavior. Depending on raw material and extraction conditions, the viscosity of pectin solutions varies. Often the viscosity of pectins is standardized with neutral sugars like dextrose and sucrose to achieve constant quality for every batch. Solutions of high esterified pectins show little elastic shares. The viscosity of a pectin solution decreases with increasing pH and ionic strength. In combination with calcium salts and some sugar, a solution of low esterified pectins will form a soft network with measurable elastic properties.

The viscous properties of pectin solutions are used in fruit juices, fruit based products, and soft drinks to increase viscosity, resulting in a better mouth feel and a higher impression of fruitiness and sweetness. The increased viscosity also improves cloud stability and reduces coalescence of oil droplets in emulsions (68). Cloud is also stabilized by bigger hydration if the cloud is positively charged and enveloped by a negatively charged pectin layer with high hydration (69).

Fruit preparations produced with low methyl ester pectins also shows pseudoplastic flow with shear thinning during mechanical stress like pumping and dosing. This lowers the forces acting on the fruits during these processing steps and reduces their destruction. To prevent fruit floating these fruit preparations are produced with pectins giving a significant yield point. In contrast a formulation using only viscosity for thickening will only delay floating. To prevent floating the yield point has to be higher than the lifting force of the fruit pieces. The yield point can be adjusted by the calcium reactivity of the pectin in combination with calcium concentration, soluble solids, and ionic strength. Fruit preparations produced with pectins show also little thixotropy and a high ability to regenerate after removal of the stress.

These factors are very important for fruit preparations for yogurt where fruit preparations are produced and filled aseptically in big containers which are shipped to the dairy plant. There the fruit preparation is pumped out of the container and an even distribution of fruit pieces is a quality criterion. After pumping the fruit preparation has to be mixed with the white mass of the yogurt or metered into the yogurt beaker with subsequent over-layering with yogurt without the reaction between low methyl ester pectin and free calcium ions of the yogurt.

Jams, jellies, and marmalades produced with pectins are visco-elastic solids. The ratio of viscous to elastic shares varies, depending on:

- degree of esterification of the pectins
- pH conditions
- kind of pectin used

Viscous shares increase with increasing pH and decreasing DE of high methyl ester pectins. Apple pectin gels show higher viscous shares with good spreadability whereas citrus pectins have less viscous and more elastic shares. The elastic shares make the products more brittle and less spreadable, with a higher tendency to syneresis. This also can be shown by the smaller linear viscoelastic range of citrus pectin gels compared to apple pectin gels. These figures can be obtained using rheometers with oscillating techniques. The phase displacement angle δ between stress and strain indicates the proportion of elastic (G') to viscous shares (G'') whereas

- pure viscous liquid, $\delta = 90°$
- pure elastic solid, $\delta = 0°$
- viscoelastic solid, $0° < \delta < 90°$.

X. THE MOLECULAR WEIGHT OF ISOLATED PECTINS

The figures on molecular weight of pectin published in the literature vary significantly from about 40.000 to more than 4 million Daltons. This variation is not only influenced by source of raw material, kind of plant tissue, and extraction conditions of the analyzed pectins but also by the analytical method itself. Owens et al. (70) first established that values, determined by viscosity measurements of pectin solutions with different concentrations and extrapolation to zero concentration, depend on the

constants (K) and (a) used in the Mark-Houwink-Sakurada equation $[\eta] = K \cdot M\eta^a$. Values for different pectins can be found in Voragen et al. (4) and literature cited. For the exponent (a) values between 0.8 and 0.9 seem to be confirmed, representing a slightly stiff conformation of pectins.

Polydispersity and aggregation phenomena are reasons for big discrepancies between weight-average (determined by laser light scattering) and number-average (determined by membrane osmometry or end group analysis) molecular weight values. Fishman et al. (71) published a critical re-examination of molecular weight and dimensions for citrus pectin. More detailed information on molecular weight distribution of pectins can be obtained by combinations of size exclusion chromatography (HPSEC) with multi-angle laser light scattering (MALLS) (72). The molecular weight for isolated pectins for food application as thickener or gelling agent is estimated to be 60.000 to 120.000 Dalton.

XI. PECTIN MANUFACTURING

In 1825 Braconnot was the first who isolated a substance from plants, which showed gelling properties. The term pectin has been derived by him from the Greek "πηχτος," meaning to congeal or solidify. It took up to 1917 until Ehrlich described pectic substances being composed of galacturonic acid. The high water binding and the ability to form gels of pectin quickly attracted the interest of the food industry. Before a defined pectin became available, the liquid pectin obtained from concentrated apple juice was used to decrease the influence of the natural content of fruit pectin in the raw material of the fruit processor. The first commercial application of pectin was as a gelling agent for jams, jellies, and marmalades, to balance the different pectin contents of the processed fruit. Kertesz (73) is giving the most comprehensive review of the early work on isolated pectins.

Despite the wide occurrence of pectins in nature only a few raw materials were established as sources for commercial pectins. Reasons for this are the facts that only a few plants contain pectins with suitable properties for food applications, still the main use for pectins, and that the raw material has to be available in a sufficient quantity and constant and storage stable quality. Still pectins are produced mainly from by-products of fruit juice industry, namely apples and citrus fruits whose fresh or carefully dried pomace or peels after washing to remove citric acid and sugars are used. In Central Europe, pectin is produced from dried apple pomace and dried lemon and lime peels, whereas in Central and South America, pectin used to be obtained also from fresh orange, lemon, and lime peels. At present, North America is no longer a supplier of pectin. Instead, it is a major consumer.

Sugar beet chips are of minor importance due to their lower pectin quality. Sunflower heads fail as a pectin source because pectin quality declines rapidly before harvest time.

The careful handling of raw material is essential in the manufacturing process of pectin. For example, in the production of apple pectin, the dried apple pomace from the manufacture of apple juice is the raw material. After the apple mash has been de-juiced, the pomace is dried immediately to prevent the degrading enzymes in the plant tissues from reducing the quality of the pectin. Drying also stabilizes the pomace by reducing micro-organisms to a minimum, permitting the product to be used to produce pectin year-round. Some producers use pectin degrading enzymes to increase fruit juice output and facilitate the de-juicing. The pomace from this operation cannot be used to produce pectin.

Apple pectins, especially from unripe apples are often associated with native starch, which can be removed by enzymatic treatment. The degree of esterification in unripe apples is nearly 100%. Industrial extraction yields 10 to 15% of pectin out of dried apple pomace (or about 0.5% calculated on fresh apples) with a degree of esterification of up to 80%. The distribution of free carboxyl groups is very even. Apple pectins show a higher proportion of neutral sugars than citrus pectins resulting in summary in gels with high viscous shares, low tendency to syneresis, and high internal gel strength.

Citrus pectin production uses the peels of de-juiced and de-oiled fruit. Washing the peels prior to processing is essential in the removal of remnants such as aromatic oils and bitter components. If such remnants cling to the peels and if these peels are used to produce pectin, they may cause an off-flavor, resembling a bitter, rancid taste in the end product. Thus for the production of a product that is stable when shipped and stored, the peels must be dried. If a producer wants to extract pectin from wet peels, he should do it in the vicinity of a citrus processing plant. Also, enzymes in the plant tissues will start to degrade the available pectin if bottlenecks develop in the extraction process or an excess of raw material prevails.

The yield of citrus pectins from dried lemon or lime peels is about 30 to 35%. Orange and mandarin peels are seldom used due to their lower pectin content and pectin quality. Grapefruit pectin is produced mainly for nutritional applications to lower cholesterol (74).

Citrus pectins usually have a lower content of neutral sugars and a higher concentration of D-galacturonic acid as apple pectins. Due to a higher pectin esterase activity in citrus fruits, acting block wise as all plant pectin esterases do, citrus pectins often show blocks of free carboxyl groups which are high calcium reactive. The citrus pectin gels are more elastic and brittle, and have a higher tendency to syneresis and pregelification with calcium ions present in the cooking. The advantage of the higher reactivity with calcium ions is the better protein stabilizing

property especially of heat sensitive casein in acidified milk drinks.

Sugar beet chips are of minor importance. Compared to apple or citrus pectin, pectins from sugar beet have higher neutral sugar content and are partially esterified with acetic acid and ferulic acid. The acetylation is preventing gelation, but even acidic or enzymatic deacetylation does not create proper gelling pectins due to the low molecular weight of beet pectin. However studies have been carried out that show improved gelation of sugar beet pectin through oxidative coupling (75). It could be shown that beet pectins have emulsifying properties due to their hydrophobic (acetyl and methyl groups) and as well hydrophilic (hydroxyl and carboxyl groups) nature (68).

Sunflower heads contain low ester pectins which are also acetylated. Interesting properties can be found in sunflower pectin as long as the sunflower is blossoming until an early mature state of the kernels showing a white and fluffy inflorescence. But when harvesting sun flowers for oil production the heads are dried out, sometimes after a killing frost, turning sunflower pectin almost to pectic acid with a very low solubility.

Several other plant raw material like cabbage, onion peels, mango, cotton, etc. have been investigated as sources for commercial pectins. Up to now all efforts failed to introduce a new source of pectin. Additionally several international pectin specifications limit the sources of pectins to apple, citrus, and beet.

Citrus fruits and apples are not processed with the main purpose of pectin production in mind. One of the main tasks of pectin producers is to secure the supply of high quality raw material by keeping in constant contact with fruit juice producers. The essential know-how of pectin producers is the manufacture and maintenance of pectins with a constant/consistent good quality despite adverse factors, which include variable contents and molecular weights. Such factors, as indicated above, result from differences within the raw materials and production parameters in the fruit juice processing plant.

At first the pectin containing extract is produced in an acidic medium. Here, the insoluble protopectin is converted into its soluble form. Additionally water-soluble ingredients like sugars and phenolic compounds will also be dissolved. The insoluble residue, which mainly contains cellulose and other water-insoluble matter, will be separated and dried and may be used as animal feed. The extract containing pectin is further purified to remove suspended particles. It is then added to an alcohol solution in which the insoluble pectin will precipitate. After the alcohol is removed using standard procedures, the pectin is dried prior to grinding and sieved. The ground and sieved pectin represents the base material for "standardization," i.e., the adjustment of pectin with sugar to ensure constant/consistent functional properties.

Citrus pectin is produced in much the same way as that for apple pectin. In contrast, the use of wet peels is possible for the production of citrus pectin. This reduces one energy intensive working cycle, i.e., drying of the citrus peels. However, this procedure depends on a close proximity between a pectin producer and a fruit juice producer for countries in Central or South America. Differences in the pectin quality within the raw material depend on fruit maturity and enzymatic activity and must be normalized or accounted for in pectin production. In contrast, dried citrus peels are available year round. Important suppliers are countries in Central and South America, Spain, and Italy.

Depending on the extraction conditions and quality of the raw material, large differences in the molecular weights of pectin exist. Nonetheless, the molecular weight of the manufactured pectin is an essential factor for its gelling properties and determines its commercial quality.

The degree of esterification has decisive influence on the gelling behavior and the application of the pectin. Pectin directly precipitated after extraction has, due to its natural properties, a very high degree of esterification. It is also known as rapid set pectin. Other types of pectin, like slow set pectins or low methylester pectins, which have a low degree of esterification, can be produced by adjusting some parameters of the extraction process, e.g., pH and temperature. It is possible to carry out de-esterification during or after the extraction. Pectins are amidated under alkaline conditions, in the presence of ammonia. In this process methylester groups will be transferred to amid groups. These pectins contain three functional groups: methylester, carboxyl, and amid groups.

The term pectin is generally used for a poly-galacturonan, which has been extracted from cell walls. Pectins with a defined structure can be produced via additional processing steps. In order to differentiate the large number of pectins, they can be classified according to their chemical nature (Table 140.3).

Because of the presence of free carboxylic acid groups, pectins have weak acid properties, which can affect and change the pH-value of a food system. Added pectins may

TABLE 140.3
Classification and Definition of Pectins

Description	Chemical Characterization
Protopectin	Water-insoluble native pectin network
Pectic acid	Polygalacturonic acid (degree of esterification <10%)
Pectate	Salts of the pectinic acid
Pectin	Partly or completely methylesterated polygalacturonic acid
Pectinate	Salts of not completely methylesterated polygalacturonic acid
Pectic substances	Collective term of above mentioned susbstances

initiate undesirable reactions during subsequent processing. Pectins can also act as buffer systems in combination with other organic acids to influence the overall buffering capability of the system. This effect will increase considerably with increasing free carboxylic acid groups, i.e., decreasing degree of esterification. Simultaneously, the affinity of pectin against di- or trivalent cations increases with increasing free carboxylic acid groups. In the production of pectins for individual food product applications, the degree of esterification should be adjusted accordingly. Because of the higher affinity towards divalent ions, the low methylester pectins always display additional properties in any application, when compared to high methylester pectins. This difference is one of the major criteria used to select one particular pectin for a specific application.

Low methylester pectins can form a network in the presence of divalent ions to produce gel structures, a fact used in the manufacture of low-calorie fruit preparations. A low methylester pectin with a high affinity towards divalent ions may result in strong cross-linking to form a water insoluble salt, "calcium pectate." Calcium pectate no longer displays any useful functional properties. Another possibility is the use of low methylester pectin as an ion exchanger. For example, pectinic acid loaded with sodium ions may be used as an ion exchanger for divalent cations. This reaffirms the fact that buffer systems and ion strength influence the properties of pectin including gelling.

The affinity of divalent ions is not only influenced by the degree of esterification (DE) but also by the distribution of the free carboxylic acid groups within the pectin molecule. A weak de-esterification of the native pectin can occur in the presence of native pectin esterase in citrus fruit. This behavior is very pronounced in oranges but slight in apples or apple pomace. Since plant pectin sterase acts blockwise, citrus pectin has a more block-by-block structure of free carboxyl groups and thus regions of higher affinity for divalent ions.

Besides traditional pectin types, low methylester amidated pectins offer new possibilities in the application of pectin. The presence of amidated groups plays an important role for the technological properties and will be characterized by the degree of amidation. The degree of amidation is defined as the ratio of amidated carboxylic acid groups to total galacturonic acid content.

One of the most important factors that determine the suitability of pectin as a gelling and thickening agent is its molecular weight. Its viscosity and rheological properties, similar to its gelling strength, depend on the molecular weight. Natural pectin exists in a wide range of molecular weights, which is determined by the different molecular weights of the protopectins prior to extraction. If the raw material is not carefully handled, a loss of pectin quality may occur quickly, due to the enzymatic degradation. Also, the extraction and production process of pectin has a decisive influence on the molecular weight of the final product.

Traditionally, pectins are blended with sugar to standardize their constant gelling, thickening, or stabilizing properties. This step is necessary to adjust the natural deviations of basic pectins and to guarantee consistent properties. To achieve this, a careful quality assurance of the chemical parameters (degree of esterification, degree of amidation, etc.) and the physical properties (gelling strength, viscosity, etc.) is implemented.

XII. PROPERTIES OF ISOLATED PECTINS

Pectins, because of their high molecular weight and molecule structure, are capable of binding water to form viscous solutions. Water binding is only one factor, the second being the formation of convolutions due to linear characteristic of the pectin molecule and the interactions between them. This increases the resistance of pectin to shear and results in shear thinning characteristics.

The most important property of pectin is its capability to form gels. Here a three-dimensional network is formed that is held together by the stable interaction between pectin molecules.

According to Jarvis (76) an aqueous gel consists of three elements:

- junction zones between the polymer molecules
- polymer chain links between the junction zones, which are relatively mobile
- water which is locked and entrapped in the polymer network

According to Figure 140.2, through the interactions between the molecules, junction zones develop and stabilize the network. Links of the molecules nearby not integrated in junction zones are able to bind water.

Important factors which influence the gel formation are (77, 78):

- molecular structure of pectin (pectin type)
- concentration and type of soluble solids (e.g., sugar)
- pH
- ionic strength
- valency and kind of ions
- temperature

Condition for initiating the gelation process at a given pH and a given concentration of soluble solids is a minimum concentration of the pectin. Pectin gels form when the expanded parts of the polymer chains associate to form orderly junction zones, which are stabilized by noncovalent interactions. The length of junction zones is influenced by steric factors. Limiting factors are deviations of

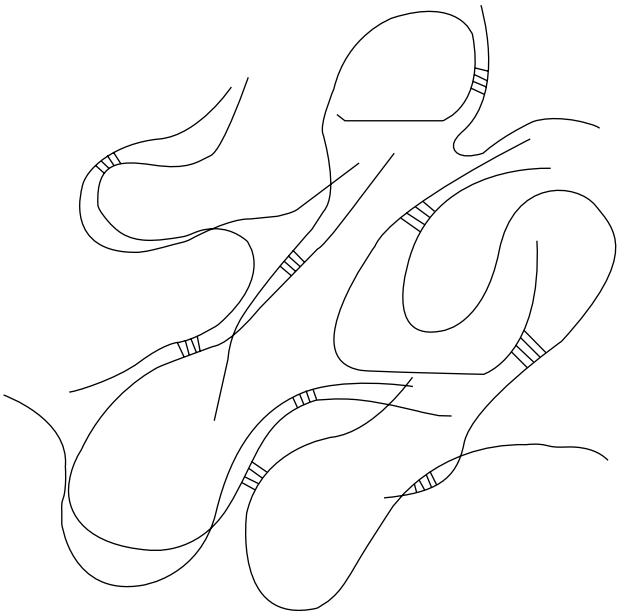

FIGURE 140.2 Schematic model of molecular network of a pectin gel.

the regularly distributed esterified and non-esterified areas as well as bends in the galacturonan chain, caused by L-rhamnose and neutral sugar side chains (79).

The gelling properties of pectin can be characterized in different ways (80–82). Traditionally they are evaluated by the "ridgelimeter" method. A sugar-water-gel is produced and placed in a defined "ridgelimeter glass." After 24 hours, it is removed to determine the sagging or shape change of this gel due to gravity (80, 81). In these experiments, the necessary pectin concentration is the variable and settlement, which will occur, will be evaluated as a function of pectin concentration. From this method, the standardization of pectin of its gelling strength of 150° USA-Sag was developed. The value 150° USA-Sag means that with a pectin concentration of 4.33 g/kg the model gel will sag by 23.5% of a sugar concentration of 649.5 g/kg. In other words: 1 g pectin with 149 g sugar will form a gel (tss: 65°Brix, pH 2.2) with a standard firmness and 23.5% sagging. If a gel with the same pectin concentration settles or sags less than 23.5%, it is assumed that the gel will have a firmer gel structure and the pectin will have a higher degree of USA-Sag.

However, it is not possible to describe the haptic (sensorial impression of texture in the mouth) properties of a gel with this method. For example, only a human taster can describe the wonderful sensory impression from destroying the structure of the gel during the eating process (chewing and swallowing). Another example is the appeal of the product. A visual feedback gives one an idea or a mild sensation about the texture and firmness of the gel structure when the product is taken out of its container or package.

Another possibility is to test the firmness of a gel by inserting a plunger into the sample. Since a dry film on the gel surface will affect the results, the top gel coat should be removed prior to the measurement. Such a film would affect the response of the gel by serving as an additional stress. In the measurement, the force required to submerge the cylinder in the gel will be determined. As a rule, the firmness is defined as the maximum required force prior to structural failure. In 1927 Lüers and Lochmüller (82) suggested a method which avoids this surface effect by determining the breaking point within a gel. This method is the basis of the actual Herbstreith Pektinometer. It is possible to measure an "internal gel strength" by inserting a probe into the gel structure. To measure the internal strength, the probe is first inserted into the gel. After a defined period of time, the measurement is obtained by pulling the probe out of the gel under a constant speed. Comparable to the penetration measurement a measuring device (transducer) will determine the maximum force needed to pull the probe out of the gel. The firmness of the gel structure is the maximum force prior to failure or breaking point of the gel.

Other important measuring devices in the quality control of pectin are rotational rheometers that measure the viscosity of pectin types (e.g., for the use in beverages) and oscillating rheometers that measure differences of gel structures without destroying part or all of the gel, e.g., confectionery products (83). Setting temperature and setting time of pectin formulations can also be measured (84).

A. High Methylester Pectin

High methylester pectins generally form gel structures at $pH < 3.5$ and a soluble solids content of $> 55\%$. It is assumed that the gel formation will take place after the complete dissolution of pectin and after addition of acid which is necessary to reduce the negative charges of dissociated free carboxylic acid groups, which would hinder pectin chains to associate. A high content of soluble solids, e.g., sugar, is necessary to influence the water activity of highly hydrated pectin molecules in order to facilitate interactions between the polymers. This gelling mechanism is also called sugar-acid-mechanism, since these are the main influencing factors for the gelling behavior of high methylester pectins. If any of these factors is not controlled, the gelling of pectins cannot be guaranteed.

The process of gelation can be described as follows. The galacturonic acid, one of the main components of pectin, contains carboxylic acid groups which partly dissociate while the pectin dissolves, causing the molecules to be negatively charged. Thus the pectin molecules repel each other. After the complete dissolution, the pectin molecules are hydrated. The high water binding capacity causes the formation of strong hydration bonds between water and pectin molecules. By decreasing the water activity the hydration will be reduced, so that the pectin molecules

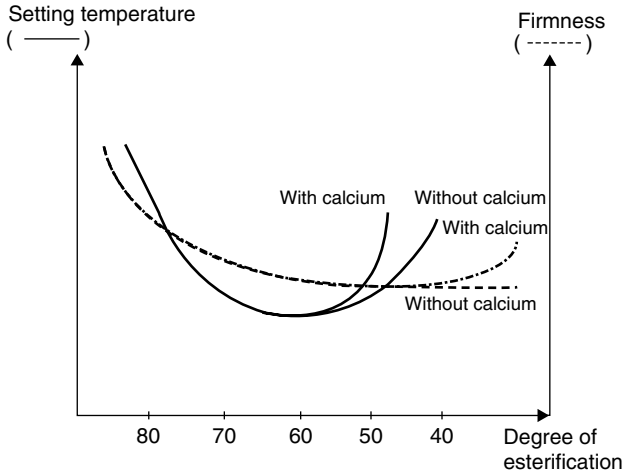

FIGURE 140.3 Gel strength and setting temperature of pectins as a function of the degree of esterification.

approach each other sterically. At this stage, if the pH of the system is higher than 3.5, the repulsive forces between the pectin molecules are so strong that the formation of interactions will not take place. By adding acid the dissociation of the acid groups will be reduced, entailing that the negative charge density and thus the repulsive forces between the molecules will decrease (85). Junction zones between pectin molecules develop through the formation of hydrogen bonds between the carboxylic acid groups of pectins. Hydrophobic interactions between the methylester groups of pectins additionally stabilize the network (86). Moreover, hydrogen bonding can occur between pectin and the secondary hydroxyl group of sugar molecules.

A connection exists between the degree of esterification (DE) and the gel strength, but setting speed or temperature (ST) will also be influenced. Based on a DE of 50% for high methylester pectins, the gel strength will increase with increasing DE and pectins with a DE of 70% will form very firm gels (87, 88). Therefore a setting speed or ST at which the gel is formed must also be monitored and recorded. Figure 140.3 illustrates the following:

- ST decreases as DE increases from 50% to 60%.
- ST rises as DE rises from 60% to 75%.
- A DE of 60% exists as the lowest setting speed or ST.
- Below a DE of 60%, an increasing affinity of the pectins towards divalent ions can be observed, which influences the gelation of these pectins.
- Calcium ions increase the firmness and ST of the pectins.

Based on the effect of degree of esterification on the setting time of high methylester (HM) pectins, one can define three types of commercial HM pectins: rapid set, medium rapid set, and slow set.

The ionic character of a pectin molecule, expressed by its pH, affects the product that is to be gelled and may also affect the structure of the formed gel. Pectins with an increased pH value have a longer setting time. The ionic strength of the product will also affect the setting behavior of the pectin used. If the batch used contains an excess of cations, it may prevent or reduce the repulsive forces due to a reduction in dissociation from the acid added. This results in weaker hydrophilic interactions that assist in the formation of junction zones.

Sugar content, calcium level, pectin concentration, and pH all have a significant influence on the setting temperature and gel strength of pectin gels (89). The gel strength is also influenced by the soluble solids content and the pH of the solution. Because of the water activity decreasing effect of sugar, which also varies based on sugar type, a minimum sugar content of 55% is necessary in order to initiate gelation. Pectins with a very high degree of esterification (>80%) still exhibit gelation properties even if soluble solids content is slightly lower.

Because of the high content of methyl ester groups, bonding zones are mainly formed by hydrophobic interactions between these methyl ester groups. If the soluble solids content is increased, the firmness of the produced gels also increases. Simultaneously, the setting speed of the respective pectin increases. It appears that an upper pH limit exists for the gelation of pectins. In solutions with a rather high pH the number of dissociated carboxyl groups and the negative charge density increases and therefore no gel formation will take place. The repulsive forces are so strong above a pH value of 3.5 that gel formation will not occur. Reducing the pH to just below 3.5 will result in a softer gel that will increase in strength with a further reduction in pH. Simultaneously, the setting temperature of the particular pectin type will increase. This shows that the pH value and the content of soluble solids affect the gelling behavior of pectin. The effects generated by the pH operate within a set of parameters. The same holds true for the content of soluble solids. The two sets of parameters act in opposition as gel strength increases with increasing soluble solids and decreasing pH value.

The application of pectin in products with a relatively high pH has certain limitations. Depending on pH and temperature, a depolymerization of pectin may occur by β-elimination of the glycosidic bonds between the galacturonic acid units. The reaction breaks the bond next to a galacturonic acid which is esterified. Consequently, the low methylester pectins are more stable in solutions with a neutral pH than high methylester pectins. Pectic acid is stable at a neutral pH (90–92).

B. Low Methylester Pectin

Pectins with a degree of esterification below 50% are able to form gels relatively independently of the content of soluble solids and pH value as long as divalent cations are

present. In the food industry calcium ions can serve as the cations. The explanation of the gelation mechanism is mainly based on the egg-box-model of Grant et al. (93). A bonding zone consists of two neighboring pectin molecules bound by calcium ions. Due to the glycosidic bonds between the galacturonic acid units, a zigzag or folded structure of the polygalacturonan chain may form. As a result two galacturonic acid units form a hollow body. The hollow body is so large that a calcium ion can be imbedded. On the opposite side of the dimer is another hollow body, which is formed by the two galacturonic acid units of the second pectin molecule. At least 7 consecutive free carboxylic acid groups within the chain segment should exist to ensure the formation of a bonding zone. Due to steric reasons only every second carboxylic acid group can be linked to a calcium ion. A methyl ester group can only then be incorporated in the bonding zone, if the carboxylic acid group on the opposite side is not esterified. At least 14 galacturonic acid units in the polygalacturonan chain must be present in chain segments which are partly esterified so that a bonding zone after the egg-box-model can form (21).

Thus, pectins with a degree of esterification >50% inter-act with calcium ions, if the chain segments of the pectin contain block-by-block arranged free carboxyl groups.

The calcium ions affinity of low methyl ester pectin depends on the degree of esterification. The gel strength of a pectin at a constant calcium level increases with a decreasing degree of esterification. This is because of the influence of the degree of esterification on the strength of calcium bonding to carboxyl groups of pectin. The density of the electric charge of the macromolecule also changes with the degree of esterification of a pectin. The higher density of an electro-negative charge of very low ester pectin increases the affinity of the pectin towards calcium ions (94). With the same calcium level a gel is formed that is firmer than one from a pectin with a higher degree of esterification (78). The way that calcium ions are added to the system also plays an important role in the gelation behavior of low methylester pectins. Readily soluble calcium salts, e.g., calcium chloride, will cause a quick gelation, whereas adding calcium citrate will result in a slower gel formation. Similar to the gelation of high methylester pectin, gelation of lower methylester pectin will occur at a characteristic temperature. The concentration of calcium ions will influence the setting temperature which increases with increasing amount of calcium added. The addition of an excessive amount of calcium ions may result in a water-insoluble salt, calcium pectinate.

Soluble solids content, pH, ionic strength, and presence of calcium binding substances, in addition to pectin level, will influence the gelation of low methylester pectin. Hence there is a need to determine the calcium requirements of a pectin type in order to obtain a gel with a defined structure. Lowering the pH and increasing the soluble solids content reduces calcium demand whereas the presence of calcium binding matters will increase the calcium requirements (77).

The introduction of amidated groups into the pectin molecule reduces calcium requirement while producing gels with the same gel strength. Thus, amidated pectins can be applied in a product formulation where non-amidated pectins will not form a gel, e.g., in products with a neutral pH. Also, amidated pectins are relatively tolerant towards erratic calcium concentrations.

C. Nutritional Aspects

Pectin is considered a soluble dietary fiber since its nutritional properties meet the definition set up by the American Association of Cereal Chemists (AACC) in January 2001: "Dietary fiber is the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation."

The influence of pectin on the cholesterol level in blood has been analyzed thoroughly. Pectin has the ability to bind cholesterol that the liver develops and secretes into the small intestine. With a pectin-free diet, more than 50% of the cholesterol will be reabsorbed in the colon causing blood cholesterol level to remain at a constant high level. This applies mainly to individuals with a natural high cholesterol production. Another effect of a high-pectin diet is related to blood levels of LDL (low density lipoproteins) and HDL (high density lipoproteins). Pectin can alter the ratio of blood lipoprotein fractions to favor HDL. Low methylester pectins can bind and dispose of heavy metal ions and their radionuclides in the intestine due to their strong ionic character (95).

XIII. FOOD LEGISLATIVE ASPECTS

Pectin is a food additive which is produced from plant material by means of an extraction process. Thus pectins vary in their characteristics which will be balanced and standardized by technology used by pectin producers. This ensures that certain characteristics will remain the same between production runs. It is not possible to eliminate certain built-in variables when one isolates a heterogeneous polysaccharide out of a complex matrix. However, stringent criteria from private and public sources protect the integrity and quality of pectin. The sources originate from pectin producers, legislations, and international recommendations. They define the purity and identity of pectins, including those from the Codex Alimentarius, the European Union (96), Food Chemical

TABLE 140.4
Purity Requirements for Pectin

Criterion	Purity Requirements
Loss on drying	max. 12%
Acid insoluble ash (3 n HCL)	max. 1%
Free methyl, ethyl or isopropyl alcohol	max. 1%
Sulphur dioxide (in dry substance)	max. 50 ppm
Nitrogen content (ash free and dried)	max. 2.5%
Galacturonic acid (ash free and dried)	max. 65%
Degree of amidation	max. 25%

Codex (97), and the Joint Expert Committee on Food Additives (JECFA) of the FAO/WHO of the United Nations (98) can be viewed in Table 140.4.

For pectin to be an approved commercial gelling and thickening agent, the producers are also required to comply with other sanitation and safety criteria such as hygiene, pesticides, etc.

Pectins are generally permitted for use in foodstuffs in which no restriction for the use of permitted additives exists and in foodstuffs in which a limited number of additives may be added and pectins are mentioned as permitted additives. Some countries do issue government regulations for pectin used in such a manner. With the "Organic Directive," the European Union has issued guidelines for the application of pectin in organic products. According to this directive, the maximum level of ingredients from non organic origin permitted to be added is 5% for a prescribed list of food products or ingredients. With the exception of amidated pectins (99), all other pectins belong to that list.

XIV. DISSOLVING PECTIN

Pectins have very high water binding ability and, because of the gelation properties, the dissolution procedure is crucial to activating the entire amount of pectin added. To dissolve pectin, a high shear mixer is used to ensure the separation of all pectin particles in hot or cold water. The mixing tank is filled with hot water and the required amount of pectin is added into the vortex. If a lack of shear is noticed it may be better to mix the pectin with sugar. Depending on the temperature of water and potential shear rate, pectin solutions with a concentration of 8–10% pectin may be produced. Sugar will physically separate pectin particles and prevent lump formation. If high shear is not available, pectin can be mixed with 10 parts sugar or other dry ingredients, which is then added into the cold water or fruit (or fruit juice) under agitation. Pectin will dissolve properly after bringing the cold batch to a boil. A third possibility is the addition of pectin to glucose syrup (tss $>$ 70%). Although pectin will not dissolve because of low water content, an even distribution can be achieved. Pectin will finally dissolve after diluting the batch with water or fruit juice to reach a maximum of 30% for soluble solids content, followed by boiling. For any method of preparing a batch of pectin in a formulation, the producers monitor the content of soluble solids to make sure that it does not exceed 30%.

XV. JAMS, JELLIES, AND MARMALADES

The main application for pectin is in fruit spreads, preserves or jams, jellies, and marmalades. The pectin gives an identity and a remarkable texture to each product. About 50% of all pectin sold is used to manufacture these products. Although traditionally jams and jellies are breakfast items, they have a rather short history in the human diet. One of the main components is sugar which was known since crusaders brought it into the European market. Before then, honey or traded sugar from Arabia was used to sweeten the diet. For a very long time only the rich were able to afford sugar, similar to other spices used today, e.g., pepper. Sugar became commercially available when sugar beet was cultivated in Europe. A long interval elapsed before it was learned that a high concentration of sugar could preserve a food by reducing water activity. At a water activity of $>$0.86, the equivalent of a sucrose solution of 65.6%, the growth of pathogens is suppressed. These products can be described as hardly perishable. With that method it was possible to preserve fruit with sugar, the nutritional value of sugar as an excellent carbohydrate, and the refreshing taste of fruit with its nutritional benefits, for example, of high mineral and vitamin content to be available all year round. For a homemade product, differences in firmness and texture are of little concern. However, a commercial product has to deal with different raw material quality, depending on the fruit type and its pectin content. With consumers' increased expectations on the texture and firmness of fruit spread a demand for a quality product with consistent sensory attributes has been formed.

As for other products, legislation has established a standard of identity for fruit spreads to define quality products. They are jam, preserve, and marmalade. All have a defined sugar and fruit content and a list of permissible ingredients.

A. Traditional Jams and Jellies

To manufacture a traditional jam only fruit, sugar (including sugar syrups), pectin, and organic acids are used. The traditional products have a high content of total soluble solids, achieved by adding ~55% sugar to produce a product which can be stored and transported at ambient temperatures. Jams and jellies produced by major international brands adhere to a high quality standard. The quality of a

finished product is defined by the quality of raw material and the quality assurance of the manufacturing process. The quality of a jam or marmalade is characterized by an appealing appearance, a fresh and fruity taste, and an appropriate and defined texture.

Since no flavor or color is added, the sensory attributes (appearance, taste, etc.) depend on the quality of the fruit that is used. Fruit components to be used may include fresh fruit, frozen fruit, fruit puree or fruit juices, and their concentrates. The fruit should have an optimal ripeness; overripe fruit is less flavorful and disintegrates easily when boiled. Fruits with high water content have less flavorful components. Fresh fruit, mainly a seasonal product, are washed to remove impurities and foreign debris. Most of the fruit used are frozen, canned, or preserved, e.g., with sulfur dioxide. Other quality criteria include a color in the desired range and soluble solids content in agreement with the specification. The soluble solids content will contribute to the total soluble solids content of the finished product, thus influencing the boiling time or the addition of sugar during the manufacturing process. One must consider the acidity of the fruit or its pH value. It is easy to use acid to lower the pH to a desired value when the fruits have an inherent low acidity (high pH value). Fruit with an inherent high acidity (low pH), e.g., red currant, are more difficult to handle. For example, a batch of such fruit, to which pectin has already been added, starts to form a gel once the content of total soluble solids has reached the desired value. In this case the addition of sodium citrate to increase the pH during cooking may be appropriate.

TABLE 140.5
Typical Recipe for a Traditional Preserve

		Refractometer
40–80 g	Pectin solution (5% tss)	(5°brix)
450 g	Fruit (10% tss)	(10°brix)
420 g	Sugar (100% tss)	
200 g	Glucose syrup (80% tss)	(80°brix)
xml	Citric acid solution (50% tss) to adjust the pH value	(50°brix)
Input	Approx. 1150 g	
Output	Approx. 1000 g	
pH value	2.8–3.3 (depending on fruit type)	
Total soluble solids	63%	(63°brix)

Table 140.5 shows a typical jam formulation, in which the content of total soluble solids of the fruit is set to 10% to calculate yield and total soluble solids of the finished product. Next to the recipe, readings of the total soluble solids content taken with a refractometer are shown in °Brix. The yield depends on the content of total soluble solids reached in the finished product after evaporation. Although the used sweeteners have fixed soluble solids content, the soluble solids content of the finished product will depend on the soluble solids content found in the fruit used in the formulation. By using fruit with higher soluble solids content, the yield will be increased. However, it should be noted that the pectin content in the finished product will also be reduced, thus affecting the firmness of the finished product. It is always an option to adjust the addition of sugar if the fruit used has a large difference in soluble solids content.

The soluble solids content of the finished product can be calculated to estimate the yield which will be obtained using Equation (140.1).

The equation in principle can be used for all types of fruit preparations. The necessary pectin dosage depends on:

- desired firmness
- total soluble solids (quantity and type of sugar)
- pH
- fruit content

For products having fruit with a high pectin and fiber content, the use of less pectin may be required. However, fruit with high natural pectin content will be difficult to handle in production since protopectin is extracted in the manufacturing process. This may reduce the influence on the setting behavior of the batch, especially if pectin with rapid setting behavior is obtained. Jellies need a relatively high pectin dosage as the raw material only contains a small amount of soluble fibers. Insoluble fibers and pulp particles, although not part of the gelling system, will make jams firmer and will reduce the product's tendency to syneresis because of their water binding properties. Jams with high fruit content and/or higher content of soluble solids have a lower pectin demand (100).

The natural pectin content in fruit is dependent on:

- the type of fruit
- variety within the type of fruit
- stage of ripeness

$$tss_{Finished\ product} = \frac{(W \cdot tss)_{Pectin\ solution} + (W \cdot tss)_{Fruit} + W_{Sugar} + (W \cdot tss)_{Glucose\ syrup} + (W \cdot tss)_{Citric\ acid\ solution}}{Output} \cdot 100 \quad (140.1)$$

W : Weight
tss : Total soluble solids (%)

TABLE 140.6
Important Parameters of Fruit for the Gelling Properties of Pectin (10)

	Available Carbohydrates (g/100g)	Dietary Fiber (g/100g)	Available Organic Acids (g/100g)	Calcium (mg/100g)
Whole Fruit				
Apple	11.4	2.02	0.46	5.8
Quince	7.32	5.92	0.93	10
Apricot	8.54	1.54	1.40	16
Cherry (morello)	9.88	1.04	1.08	8
Cherry (sweet)	13.3	1.31	0.95	17
Peach	8.89	1.92	0.57	6.3
Strawberry	5.51	1.63	1.05	21
Blueberry	6.05	4.90	1.37	10
Raspberry	4.81	4.68	2.12	40
Red current	4.78	3.50	2.37	29
Black current	6.11	6.78	2.63	46
Cranberry	3.93	n.m.	3.89	14
Rose hip	16.2	23.7	3.10	257
Pineapple	12.4	0.99	0.72	16
Banana	20.0	1.82	0.56	7
Grapefruit	7.41	1.60	1.48	24
Lime	1.90	n.m.	1.90	13
Lemon	3.16	n.m.	4.88	11
Orange	8.25	1.60	1.13	40
Fruit Juice				
Apple	11.1		0.77	6.9
Orange	8.80	0.45	1.23	11
Grapefruit	7.20		1.45	9.6
Grape	16.6		0.38	13
Lemon	2.43		4.75	11

According to Table 140.6, the natural pectin content may influence the amount of pectin needed for getting an appropriate set. Acidity, content of total soluble solids, and calcium content are all important parameters affecting the gelation of pectin.

Sugars are the main constituent of jams, jellies, and marmalades; they extend shelf life and improve the taste and texture of the finished product. Because of the high concentration of sugar used, it is important to select the appropriate type of sweetener in view of potential re-crystallization. Pure dextrose (glucose) is not used because of its high tendency to crystallize. Refined sucrose is used, because of its availability and low tendency to re-crystallize. In the manufacturing process sucrose is partially inverted to glucose and fructose, which is a chemical process supported by low pH of the product. That additionally decreases the tendency of sugar crystal formation. Other influencing parameters are temperature and time of the cooking process. Usually a combination of different sweeteners is used in order to create decisive and characteristic sensory attributes. Sucrose is combined with glucose syrup since glucose syrup is less sweet, resulting in a fresh and fruity taste. Note that the sweetness of sucrose is short and sharp, compared to glucose. Also, high-fructose corn syrup, having the same relative sweetness as sucrose, is used in combination with glucose syrup. Glucose or corn syrups are derived from corn, wheat, or other types of starches by acid or enzymatic hydrolysis and, depending on the manufacturing process, contain glucose, maltose, maltotriose, and dextrins. Fructose containing glucose syrup is obtained by enzymatic induced isomerization of a certain amount of glucose in the syrup. Because of the higher content of fructose, high-fructose containing syrups have a relatively high sweetness (see also Tables 140.7 and 140.8).

In certain dietetic products, sucrose or glucose has to be replaced with other forms of specific sweeteners. For example, fructose is important because of its insulin independent metabolism and digestion. The main group of sugar replacers for dietetic jams and jellies with $>60°$Brix are sugar alcohols, including sorbitol, mannitol, maltitol, and isomalt as shown in Tables 140.7 and 140.8. As an alternative to adding a specific sweetener, syrups from fruits like refined grape juice concentrate or Herbasweet, an apple derived sweetener (Herbafood Ingredients, Germany), may serve to produce an "all fruit product," containing only fruit ingredients.

The choice of the appropriate pectin type to form a gel is determined by textural properties and processing

TABLE 140.7
Common Bulk Sweeteners for Traditional Jams and Jellies

Bulk Sweetener	Relative Sweetness	Concentration of Re-Crystallization
Sucrose	1	65.5%
Glucose	0.75	45%
Fructose	1.2	80%
Sorbitol	0.4–0.5	70%
Isomalt	0.5	25%
Maltitol	0.9	60%
Mannitol	0.4–0.5	12%

TABLE 140.8
Common Liquid Sweeteners for Traditional Jams and Jellies

Sugar Syrup	Relative Sweetness	Containing Sugars	Tendency to Crystallize
Glucose syrup (42 de)	0.35	glucose (17%), maltose (14%), maltotriose (13%)	low
Glucose syrup (62 de)	0.50	glucose (36%), maltose (31%), maltotriose (13%)	low
High fructose corn syrup (42% fructose)	0.95	glucose (53%), fructose (42%), maltose (3%), maltotriose (1%)	high
High fructose corn syrup (55% fructose)	1	fructose (55%), glucose (41%), maltose (2%)	low

parameters. A production using open kettles may have different requirements than a process using vacuum boiling. Finished products have a different color and taste if the ingredients have been exposed to less heat treatment in terms of time and temperature. Color, flavor, and fruit pieces will be much better retained in comparison to cooking at ~100°C (212°F). In a vacuum cooking process the batch starts to boil at ~60°C (140°F), preventing caramelization and excessive loss of flavor from evaporation. Caramelization of sugar occurs at >80°C (176°F). For a vacuum process slow set or medium rapid set pectins have to be used in order to permit an adequate filling time for the finished product. Because of the high cooking temperature, the appropriate pectin type for an open kettle process is determined by the filling procedure of the manufactured product.

A vacuum cooking process is essential if a large quantity of water has to be evaporated from the batch to achieve the desired content of total soluble solids, e.g., when using glucose syrups as a sweetener. Cooking in open kettles may enhance oxidation of certain fruit ingredients, leading to off-flavors. Cooking at ~60°C (140°F) in a vacuum process may not produce a microbiologically stable product. If so, pasteurization prior to filling the finished product into jars is one option. Figure 140.4 provides an illustration of this process.

In principle the addition of ingredients can be done in two ways. Pectin can be added as a powder at the beginning of the cooking process or added as a pectin solution at the end of the boiling process. The pectin solution is added once the fruit-sugar-batch has reached the necessary total soluble solids content. It should be noted that by adding the pectin solution the sugar concentration will be diluted. To induce gelation, citric acid solution is added to adjust the pH. The citric acid should be added at the end of the cooking process to permit a proper control of the filling time. If the total soluble solids content and pH are adjusted, weak interactions between the pectin molecules will commence and the setting process starts. Citric acid is preferably added as a solution. This allows for a better incorporation and an even distribution of the acid can be obtained quickly to bring about a lower pH.

If a high shear mixer is not available, pectin and part of the sugar can be added as a dry mix to the cold fruit batch. Although the dissolving properties of pectin depend on the total soluble solids content, the batch should have no more than 30°Brix to ensure good dissolution/dispersion (see also section 14). Pectin comes into solution by the subsequent heating step. After the rest of the sugar is added and the batch is cooked until a desired total soluble solids content is reached, citric acid solution is introduced.

Two definitions for the manufactured products are important:

- Setting time. The time between adjusting the essential product parameters for gelation and the moment when a gel network is formed.
- Setting temperature. The temperature when the gel network formation occurs is reached by exposing the filled jars to ambient temperature or water in a cooling tunnel, achieving a constant cooling rate.

The duration of the filling process also determines the choice of pectin. If the filling procedure requires a long setting time to fill, for example, portion packs properly, a slow set pectin is recommended. Products filled in large containers also need a long cooling time, since heat exchange by conduction is a slow process. To prevent potential burning in the center, these containers are filled at temperature as low as 60°C (140°F).

In sum, the setting temperature is influenced by:

- pectin type (see previous discussion)
- soluble solids content
- pH
- calcium content

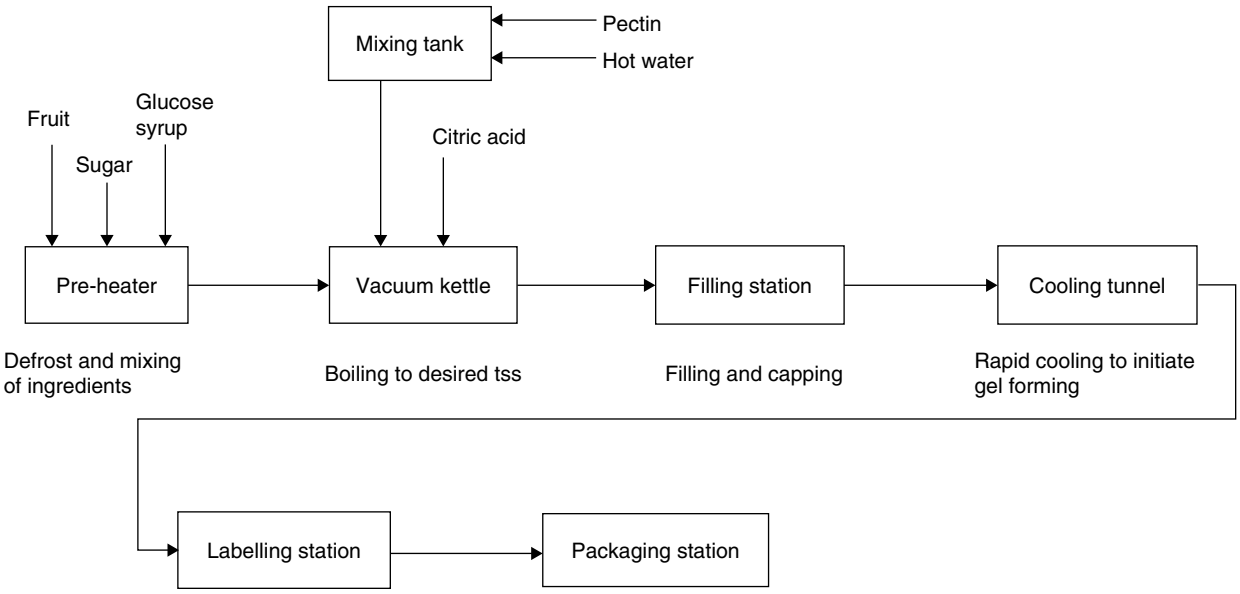

FIGURE 140.4 Manufacturing process of traditional jams and jellies.

An increase of the soluble solids content will lead to an increase of the setting temperature as well as a decrease of the pH from the addition of more citric acid solution. The presence of divalent ions, especially calcium ions, will increase the setting temperature of citrus pectins (see also section 12.1). However, the pectin level used has a relatively small influence on the setting temperature.

Choosing a pectin type that sets too fast for the given manufacturing process, will lead to a quality defect, i.e, a pre-gelled jelly. Pre-gelation is a process in which the pectin forms a network prior to the cooling process of the filled product. A regular network with a structure that can be sliced or cut will form only after filling above the setting temperature. Once the structure is set, it cannot regenerate after being exposed to shear caused by pumping or filling. A pre-gelled structure is softer and cannot be sliced or cut. Also, an increased tendency to syneresis may occur. These factors will lead to some preventive measures after the filling process. For example, filled products should not undergo excessive movements within the first 24 hours. Another important quality defect often results in the product when whole fruit is used. Fruit is naturally high in water and low in total soluble solids. On the other hand the serum during processing (cooking) has a high sugar content, causing a density that is higher than that in fruit. Thus, fruit has positive buoyancy leading to gravitational separation (floatation) after filling. This behavior is easily corrected by cooking the batch for an appropriate period to permit a "reverse" process of migration: water from fruit to serum and sugar from serum to fruit tissues. Additionally a faster setting pectin can prevent separation of the fruit as well as a pectin type that develops a high heat viscosity.

A product may be rejected because of an inappropriate set due to:

- undissolved pectin
- total soluble solids out of gelling range
- pH out of gelling range (see also section 12.1)

In order to prevent rejections, quality assurance from raw ingredients to finished products is of paramount importance. A re-working of the finished product is very difficult, as the sugar-acid-gelation of high methylester pectins is irreversible. Since the crucial point during the manufacturing process is the incorporation of pectin, we must pay attention to the following:

1. A properly dissolved pectin solution should be controlled with a refractometer.
2. The pH should be monitored during the process.
3. The content of total soluble solids should be assessed.
4. The temperature must be carefully regulated.

Automatic readings of the above three variables can be easily achieved with modern equipment such as meters. The temperature of the manufacturing process is important for three reasons:

- to prevent heat damage
- to produce a product that is microbiologically stable
- to avoid pre-gelation or fruit separation/floatation

With a well designed quality assurance program in the processing plant, quality products will be produced. The ultimate test for a quality product is a sensory evaluation or a measurement of the firmness of the product or both.

B. Low Sugar Jams and Jellies

In recent years, jams and jellies with a reduced caloric content have become increasingly popular. This is because consumers know more about nutrition and understand the high caloric content of sugar jams and jellies. To develop a product with a reduced sugar content without preservatives poses a major problem. The products have a short shelf life once opened, with or without refrigeration. Another reason is taste preference. Many consumers prefer less sweetness and more refreshing fruity note. Producers use these concepts also to market products with a high fruit content.

Selecting the appropriate pectin depends mainly on the content of soluble solids and the possibility of using calcium as a firming agent. The pH is of minor influence as long as it is within a typical range of 3.0 to 3.4. A conventional or an amidated pectin will serve as a low methylester pectin. Conventional low methylester pectins require a specific amount of calcium to form a gel. Yet the calcium demand depends on the soluble solids content.

Figure 140.5 shows the gelation behavior of a low methylester conventional pectin in model gels at different soluble solids content and pH as a function of calcium ion concentration. Adding a small amount of calcium to a pectin formula leads to formation of clusters. By increasing the calcium level, a gel may be formed whose firmness depends on the calcium dosage. By adding a high calcium concentration, very firm gels can be obtained with a highly brittle texture (77).

Increasing the calcium also increases the setting temperature of the given formulation. An excessively high dosage can lead to pre-gelation during the final stage of the manufacturing process. Pre-gelling can be detected since the gel is opaque and has a more pasty texture with a lower gel strength and a high tendency to syneresis.

Depending on the degree of esterification, pectin types can be obtained with a varying sensitivity to calcium. A more calcium sensitive pectin has an increased affinity to calcium ions and is forming tighter junction zones. Hence firmer gels will be obtained. Low methylester pectins with a high sensitivity to calcium are preferred for products with a very low soluble solids content.

Recipes with a relatively high sugar content have a lower calcium demand in order to form a firm gel. The calcium demand depends on the sugar and increases from sucrose to fructose to sugar alcohols, e.g., sorbitol. For many products, the calcium content of other ingredients, e.g., fruit, is high enough to act as a calcium donator. For products with lower soluble solids content, the addition of calcium is necessary. If the separate calcium addition is not desired, it is possible to obtain low methylester conventional pectins, that have been already standardized with calcium salts, to obtain a constant gelling behavior and gel strength. Such pectins are available for different soluble solids contents and different textural properties. As an alternative, low methylester amidated pectins can be used. Due to the introduction of amid groups, these pectins have a lower calcium demand. In comparison to conventional pectins, gel formation is achieved without separately adding calcium ions. Therefore amidated pectins are convenient and calcium addition is not a concern. Still, amidated pectins interact with calcium ions and form very firm and elastic gels with a rubbery texture. In comparison to amidated pectins, conventional low methylester pectins form gels with a smoother structure.

An increase in pH will lead to an increased demand for calcium. A higher pH increases the degree of dissociation and the repulsive interactions between the pectin molecules. More calcium has to be added to neutralize the repulsive effects. But the negative repulsive effects can only be partially balanced. At a certain pectin concentration, the calcium induced rate of gelation process is too high and pre-gelation will appear. Other organic acid salts or buffer substances become more negatively charged and interact with added calcium ions, which reduces the amount of calcium ions available for the interaction with pectin. The amount of buffer ions decisively influences the calcium requirement for gel formation.

The required amount of pectin that has to be added depends mostly on the soluble solids content. For products with a content of soluble solids of 50%, ~0.6% pectin is needed. The necessary amount increases up to 1.2% for products with 20% soluble solids. The manufacturing process does not differ from the manufacturing process for high sugar products (Figure 140.5). Depending on the filling facilities, the use of a preservative, e.g., potassium sorbate, may be appropriate. A separate addition of calcium salt is done in two ways. The necessary amount is added to the fruit at the beginning of the manufacturing process. Or, it may be added into the hot batch as a solution at a later stage of the production process.

There is a less tendency of the fruit separating to the surface (floatation), if the content of soluble solids in the serum reaches that of the fruit. There will always be some separation because of the air trapped in the fruit tissue. Filling the finished batch close to the setting temperature ensures an even fruit distribution in the jars. To produce a product that is stable to microorganisms, it is necessary to do the filling at ~85°C (185°F). Therefore, the setting temperature of the pectin has to be adjusted accordingly. If the filling temperature is too low because of the specification of product formulation, a longer cooking is

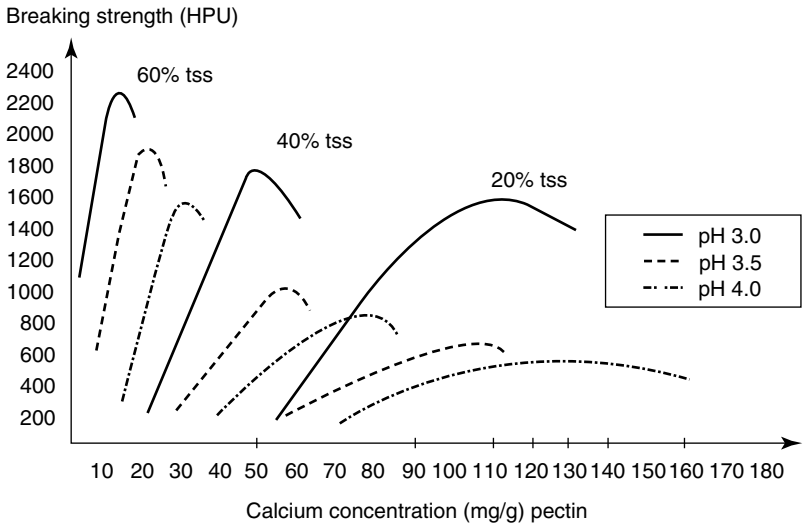

FIGURE 140.5 Firmness of model gels with low methylester conventional pectin as a function of added calcium ions.

required to achieve two goals that can prevent separation of the fruit to the surface, e.g.,

- escape of air bubbles
- equalization of content of soluble solids content in the serum and the fruit tissue

C. Gelling Powder and Gelling Sugar

Home canning of jams and jellies is still popular with consumers since they can use fresh fruits to prepare their own fruit spread. Because of the short boiling time, their preparations have excellent flavor and appearance. The products for home canning contain the proper pectin for a desired sugar content. These products require:

- excellent dissolving properties of the pectin used
- easy handling of the recipe and the manufacturing steps

In Europe, commercial processors offer gelling sugars or gelling powders that focus on the ratio of fruit to sugar, independent of fruit type. Consumers appreciate the convenience because they do not have to worry about the type of fruits used. If the addition of sugar is required, the type of pectin used must form an appropriate set with different fruits with different parameters: acidity, pectin, and sugar contents. The ideal pectin products that offer maximum convenience to the consumers are gelling sugars containing sugar, pectin, and citric acid in a gentle mix (Figure 140.6). Consumers have only to perform two steps: add the gelling sugar cold to fruit and/or fruit juice. Boil the preserve for a defined time. For gelling powders a defined amount of sugar has to be added separately after dissolving the gelling powder in the fruit and/or fruit

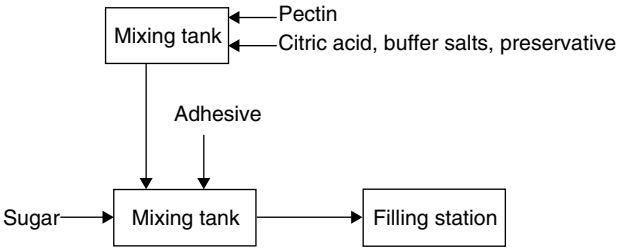

FIGURE 140.6 Typical manufacturing process of gelling sugar.

juice. Though consumers do not work with pH meters and refractometers for compliance with requirements, the producers must make sure that the pectin product has the appropriate gelation properties. Besides powdered products, some producers offer a pectin solution for use by consumers as a gelling agent.

Gelling powders and gelling sugars are manufactured by mixing the required ingredients to an even product mix. For gelling sugar pectin is first mixed with citric acid (often coated with fat to reduce pectin degradation) and then evenly mixed with sugar using an adhesive. For products that are designed to make reduced sugar fruit spreads, a preservative may be needed. A proper mixing process will prevent potential de-mixing of finished product caused by transport and packaging. Standard quality control procedures require testing of the even distribution of the gelling sugar and gelling powder at the respective stages.

XVI. FRUIT PREPARATIONS

Commercial fruit preparations are semi-finished products, tailor-made and specialized for application in different food products. Fruit preparations can be combined with bakery products (biscuits, cakes, etc.) or dairy products.

Glazings are one form of fruit preparations to enhance the appearance of fruit cakes. Specifications of fruit preparations and their manufacturing process are not as regulated as for traditional jams and jellies. Thus producers can make fruit preparations in different ways with numerous ingredients: hydrocolloids, colors, flavors (natural and artificial), and sweeteners. The quality of industrial fruit preparations is still defined by the raw materials, especially the fruit component. Low quality raw ingredients are hardly compensated by good manufacturing practices. Fruit preparations offer flavor, taste, and technical functions. Its important ingredient pectin serves to thicken and stabilize both fruit preparations and often also the final product applied to. Fruit preparations are specialized products and their manufacturing process is batch oriented: However, continuous production is possible if the producer takes into consideration the needs for pectin application. Figures 140.7 and 140.8 show examples of manufacturing processes for pectin based fruit preparations.

Requirements associated with the specific application of a fruit preparation will be discussed below. Filling temperatures have to be adjusted according to the container size to prevent thermal pectin degradation and weakening of texture in the center of the containers (Table 140.9).

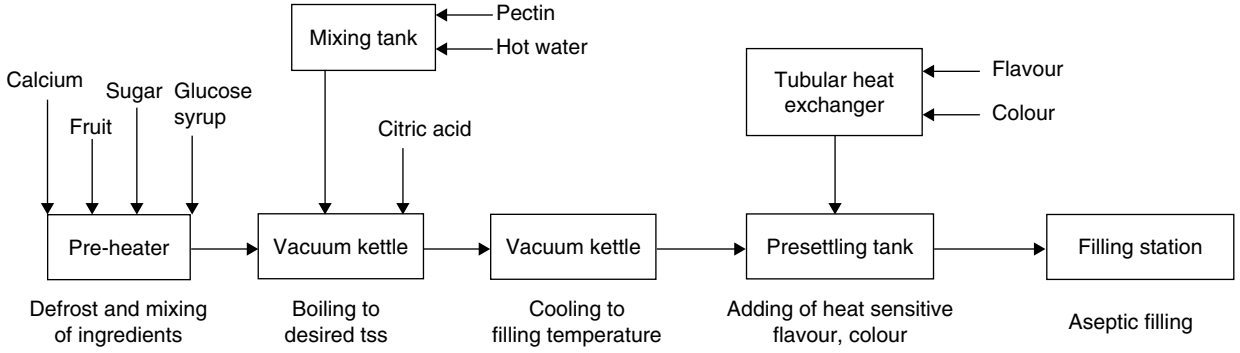

FIGURE 140.7 Batch production of industrial fruit preparations.

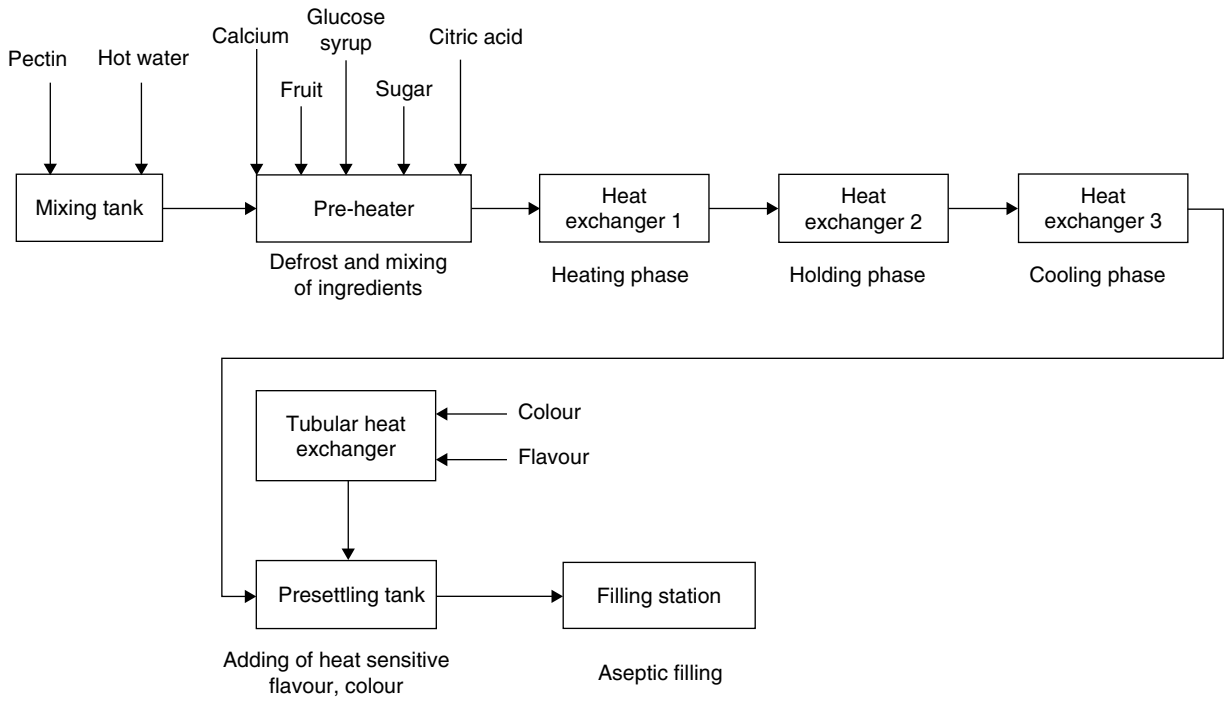

FIGURE 140.8 Continuous production of industrial fruit preparations.

TABLE 140.9
Filling Temperatures of Fruit Preparations According to Size of Container

Container	Container Size	Max. Filling Temperature
Jar	<1kg/<2.5lbs	95°C/203°F
Pail	1–50kg/2.5–110lbs	65°C/149°F
Drum	50–500kg/110–1100lbs	45°C/113°F
Container	500–1000kg/1100–2200lbs	30°C/86°F

A. Baking Stable Fruit Preparations

Fruit preparations for baked products give biscuits a fresh and fruity note. Besides the refreshing property, the use of fruit preparations in biscuits also serves as a source of moisture to prevent drying of the dough, thus increasing the shelf life of the biscuits. But the moisture content must be below a certain level to avoid wetting of the baked products. The fruit preparation can be added before or after the baking process. Normally the fruit preparation is purchased by the bakery and applied mechanically or manually to the dough as a topping or filler. The quality criteria for fruit preparations are two fold: avoid syneresis and apply good manufacturing or handling practices, especially the physical application of fruit preparation, and achieving thermal stability. Baking stability has a wide range of requirements, from high baking stability for biscuits to a certain thermal reversibility for forming a glossy topping. After baking, the thickener has to ensure texture stability during the shelf life of the baked product. Fillings should not show syneresis since this would affect the appearance of the baked product.

Pectin is an ideal ingredient to provide the desired structure and thermal stability for fruit preparations with a soluble solids content of more than 50°Brix. The gelling mechanism with sugar and acid provide the basis of thermo-irreversibility. High methylester pectin can be used in the type of fruit preparations small bakeries manually use in their products. The functions of high methylester pectins are similar to the application in jams that provide the desired firmness for fruit preparations. These fruit preparations are "baking-stable" because of the tight network formed by pectin. Once the structure is broken due to shear, a loss of thermal stability can be observed. This behavior relates to syneresis occurring after the broken gel pieces shrink and baking stability is only seen with the intact gel structure. This also means that fruit preparations have to be filled above the setting temperature for the given formula to receive a regular gel structure with tight bonding zones. A pre-gelled formula will not ensure the desired thermal stability and therefore might only be suitable for filling small containers or pails. Products manufactured with high methylester pectin do not show thixotropic characteristics. Their capability to regenerate after pumping is low. A popular method to achieve thixotropic flow behavior is to combine pectin with modified starch to produce a smooth and creamy fruit preparation. Modified starch, as a thickener, increases viscosity and improves the baking stability of the fruit preparations. The increased viscosity also remains once the fruit preparation has passed the baking process. Instead of starch, other hydrocolloids might be used to increase the gel structure's ability to retain liquid. Formulations and manufacturing processes are similar to those of jams and preserves with a high soluble solids content.

An important quality requirement of industrial fruit preparations, besides being "baking-stable," is the pumpability and the structure's ability to regenerate after pumping. Low methylester pectins provide thixotropic structures and, in combination with calcium ions, the desired baking stability. Low methylester conventional pectins seem to be more appropriate as they interact with calcium ions in a controlled fashion and provide gel structures with enhanced recovery ability. Pectin type and the amount of calcium ions added influence textural properties and thermal stability of the finished fruit preparation. Table 140.10 shows a typical recipe.

In addition to the gelling mechanism with sugar and acid, junction zones, in the presence of calcium ions, will form affecting the setting temperature and the melting temperature of the formula. The calcium concentration for an optimal gelation depends on the pectin type and the parameters for fruit preparation, such as total soluble solids content, sugar alcohols, a_w, pH, and ionic strength. Calcium ions will increase the firmness and the thermal stability, but an excess can interact with pectin to form a non-homogeneous gelation. The full functionality of pectin will be lost resulting in a fruit preparation with an increased tendency to syneresis. Insufficient calcium ions will result in a fruit preparation with unacceptable firmness and heat stability.

TABLE 140.10
Recipe for Bakery Fillings

49%	Sugar
30%	Fruit puree
15%	Glucose syrup
12%	Water
0.8–1.2%	Low methyl ester conventional pectin
0.01%	Sodium citrate
0.01%	Calcium citrate
	Flavor, color, citric acid solution (to adjust pH value)
Input:	Abt. 108%
Output:	Abt. 100%
TSS:	Abt. 65%
pH value	3.5

The addition of calcium salts is adjusted according to the requirements. Although there are many influences on the calcium demand seen, pectin is fairly tolerant for variances of the calcium content in different fruit types, so that it is also possible to obtain from the supplier pectin standardized to thermal stability with calcium salts.

A higher soluble solids content or a lower pH will decrease the calcium demand, whereas an increase of ionic strength and pH in the presence of sugar alcohols increases the required calcium salt addition. Calcium salts can be added at the beginning of the manufacturing process (Figure 140.8). It is also possible to add calcium as a slurry at the end of the manufacturing process, once the pectin has dissolved. If a pectin solution is not feasible, e.g., low water content in fruit preparations, there is an option. Pectin is dispersed in the sweetening syrup with 80°Brix which is reduced to 30°Brix by diluting the batch with fruit and water, in which the calcium has also been dissolved. The dilution to 30°Brix results in a dissolution of all ingredients, forming a homogenous gel structure.

For a fruit preparation with thermal instability, one must identify the proper amount of calcium to be added in combination with a specific pectin type. This is of course the objective of R&D (research and development). A pectin producer will develop the proper combination of calcium addition and manufacturing steps. As proprietary information, it will most likely not be in public record.

B. Cake Glazing

Cake glazings are preparations used to enhance the freshness of fruit cakes by extending the shelf life. A layer on top of the cake will prevent water evaporation from the fruit, giving the cake an appealing glossiness. Cake glazings are finished products with a neutral taste. They may contain fruit to enhance the fresh and fruity character. The total soluble solids content of glazings ranges between 35–50%. At this level of sugar, the products show an excellent melting behavior. Depending on the bakery application, cake glazings are available in many distinct types. Each type has specific requirements for the gelling agent and the manufacturing process. Cake glazing concentrates are re-diluted with water once they are used by the bakery and sprayed or brushed hot onto the cake. The liquid is turned into an elastic gel structure once it drops to ambient temperature. Direct sprayable glazings do not have to be diluted. The product is conveniently connected to a sprayer in which the preparation is heated and sprayed on. Cake glazing concentrates are produced with a water activity low enough to ensure good shelf life properties. Directly sprayable products have to be filled aseptically. Otherwise, preservatives are added. Also, glazings with a thixotropic gel structure can be produced to give the product convenient handling properties. They can be spread at ambient temperature on the cake without addition of acid. Table 140.11 shows a typical recipe for a concentrated cake glaze which can be manufactured as shown in Figures 140.7 and 140.8.

TABLE 140.11
Recipe of Concentrated Cake Glaze to Be Diluted with 30–50% Water

47%	Sugar
37%	Water
18%	Glucose syrup
1.2%	Low methyl ester amidated pectin, buffered
0.05%	Citric acid solution (50% (m/m))
	Flavor, color
Input:	Abt. 104%
Output:	Abt. 100%
TSS:	Abt. 65%
pH value	3.6

Besides the melting property, the re-setting behavior after diluting and heating of the preparation has to be considered. The fruit preparation needs to adhere sufficiently to the fruit to form the protective layer. Syneresis should be absent. A firm gel should be obtained. Products that are sprayed need a low heat viscosity.

For optimal melting behavior, low methylester amidated pectins are used in combination with calcium, acidity regulators, or buffer salts. The pectin type will give the industrial products a characteristic gel structure with a defined melting and re-setting behavior. Calcium ions are used to adjust the melting and setting behavior as well as the firmness of the finished product. All these requirements can be adjusted accordingly and it is also possible to obtain buffered pectin for general use. Directly sprayable glazings need to have the same soluble solids content as the finished product. The manufacturing process of the sprayable glazings requires vigorous agitation during cooling to prevent the gel structure from forming. Thus, the producer obtains a product with low viscosity, possessing good thermal reversible properties.

Glazes can be manufactured for products that do not necessarily need to be heated. With low methylester amidated pectin, thixotropic gel structures can be obtained for products that can be spread directly onto the cakes. Also glazings, which set by the addition of acid can be manufactured. In these products, the gelation properties of high methylester pectin are used to manufacture a liquid semi-finished product, containing pectin and final total soluble solids content. This forms a firm gel based on sugar-acid-mechanisms after the proper amount of acid is added. The setting speed or kinetics can be adjusted through the pectin type and through the addition of retardant substances.

C. Dairy Fruit Preparations

Dairy products, e.g., yogurt and cream cheese, are popular in modern supermarkets, especially in western countries. However, only the combination with fruit offers the large variety of different types of product consumers encounter and prefer. Fruit preparations for dairy products have to overcome two technical problems: sanitation considerations and potential interactions with the dairy phase. Protein and calcium that dairy products contain, apart from fat and lactose, could show potential interactions with a pectin thickened fruit preparation and become a factor in most applications to be avoided.

Dairy fruit preparations can be used in two major ways: layered (bottom or top of the dairy product) or in separate compartments. Consumers can mix the fruit preparation with the dairy product or enjoy the two products separately. Many dairy products contain a fruit preparation stirred into the dairy phase. As these products are produced separately, the mixing behavior between fruit preparation and the dairy ingredient is an important quality requirement. Most dairy products are consumed as a fruit yogurt or cream cheese. The texture of these dairy products, e.g., yogurt, is formed by fermentation and is defined by the casein as the gel forming protein. Protein gels are sensitive to shear stress and are not able to regenerate. High shear stress applied after the fermentation process has finished will lead to a soft texture and loss of mouthfeel. To obtain a fruit yogurt, the mixing process of the dairy product with the fruit preparation will affect the appearance of the finished product. Fruit preparations with shear thinning flow property show a good mixing behavior with the dairy phase. Only low shear has to be used to incorporate the fruit preparation. This has a positive impact on the structure of the finished fruit yogurt.

Low methylester pectin as a gelling agent forms shear thinning gel structures and is an ideal base of a hydrocolloid system that can be used as a thickener and stabilizer. Pectin will yield the desired gel structure and produce fruit preparations that have good pumpability without the tendency to form syneresis. Another quality parameter for a fruit preparation to be filled into a container is an even fruit distribution inside the container. The formation of a weak network with yield stress prevents fruit separation and guarantees a low viscosity during pumping and filling. It also allows easy metering, especially suitable for layered products. Low viscosity during the manufacturing process also decreases shear rates in the fruit preparation as soon as the product is pumped through a tubular heat exchanger. This enhances fruit integrity.

Dairy fruit preparations have a close pH of 3.5–4.0 to the dairy product. This prevents unexpected interactions between the two entities if the pH difference is high. Low methylester pectin is a charged hydrocolloid that can interact with proteins and cations such as calcium ions. Interactions of a dairy fruit preparation with a dairy product can be described as post gelation that is induced by the interaction of negatively charged carboxylic acid groups with calcium ions or protein. This post-gelation process will cause an uneven mixing as well as destabilizing and results in a product with poor appearance and increased tendency for syneresis. In layered products, the migration of calcium ions from the dairy phase to the fruit phase will have an effect on the fruit preparation's texture. Saturating the carboxylic acid groups with, for example, calcium, will prevent profound and unpredictable interactions. The amount of calcium that has to be added to the fruit preparation depends on pectin type, pH, and soluble solids content. The required amount is available from the pectin producer. Or, it may be in the pectins that have been mixed with calcium salts, and standardized to constant flow behavior from the pectin supplier. Table 140.12 shows a typical recipe for a dairy fruit preparation.

A low methylester pectin should be selected as an appropriate pectin type. With a low methylester conventional pectin, a producer can manufacture a fruit preparations with a smooth-viscous gel structure, rich mouthfeel, and enhanced reversibility. For fruit preparations with a short-brittle gel structure, low methylester amidated pectin may be the pectin type of choice. Reversibility of the gel structure will also depend on the calcium affinity of the pectin used and suitable soluble solids content. In fruit preparations with high soluble solids content, low methylester pectin with low calcium affinity is preferred. The advantage is that the finished fruit preparation will not have a tendency for syneresis in case excess calcium is present. Because of possible interactions with the dairy phase, a calcium tolerant pectin is of advantage especially when fluctuations of calcium levels in the fruit occur.

Dairy fruit preparations are specialized products. Therefore, the manufacturing processes are batch oriented. However, for volumes with a respective turnover, a

TABLE 140.12
Recipe for a Dairy Fruit Preparation

40%	Fruit puree
25%	Sugar
22%	Water
14%	Glucose syrup (80% tss)
0.7%	Low methyl ester amidated pectin
0.01%	Calcium citrate (depending on fruit calcium content)
	Flavor, color, sodium citrate, citric acid solution (to adjust pH value)
Input:	Abt. 102%
Output:	Abt. 100%
TSS:	Abt. 40%
pH value	3.6–3.8

continuous production is possible. In the batch production, the fruit is mixed with sugar, liquid sweetener, calcium salt, and acidity regulators. Heat sensitive flavor and color may be added at a later stage. The batch is preheated to defrost the frozen fruit and to dissolve the dry ingredients. Finally the pectin solution is added and the whole batch is pasteurized. Then, the batch is cooled to an appropriate temperature for subsequent aseptic filling into a container. Flavor and color can be added into the sterile tank after a short heat treatment prior to filling. Pasteurization and cooling can take place in one kettle or two separate and specialized kettles to reduce energy cost.

For continuous production of fruit preparations, the soluble solids content of the finished product will influence the process steps. For products with a soluble solids content of <30%, pectin can be added as a dry mix with sugar or as a dispersion in liquid sweetener in the cold batch. The pectin will dissolve completely during subsequent heating. If the soluble solids content exceeds 30%, a pectin solution is made available to be added to the heated batch of product. For example, after pre-heating the fruit, sugar, liquid sweetener, and other dry ingredients, the pectin solution is added, and the batch is pasteurized and filled aseptically after the product is cooled to filling temperature. Figures 140.7 and 140.8 show typical processing steps also valid for dairy fruit preparations.

Quality control in fruit preparations from raw materials to a finished product means the careful supervision of pH, content of soluble solids, and viscosity or flow behavior. Most producers monitor the mixing behavior of the finished fruit preparation with the dairy phase, especially if fluctuations in the quality of the raw materials are observed.

XVII. CONFECTIONERY

The gelling properties of pectin are widely used in the confectionery industry. Confectionery products can be acidic jelly fruits or fruit slices with a firm texture. Also, gummy textured products and aerated products containing egg white, e.g., Snowball, can be manufactured. All confectionery products have a soluble solids content of 75–85%, which is established with a characteristic sweetener profile. To prevent re-crystallization at this high soluble solids content, a combination of sugar and glucose syrup is used. In the production, inversion of sugar and isomerization of some sugar will influence the sweetener profile. The sweetener composition, especially that for glucose syrup, will affect the viscosity of the batch during the manufacturing process and its deposition behavior. Confectionery recipes with pectin have a rather high setting temperature. This means that easy handling during the process is important in influencing the quality of the finished product. The production batch has to be deposited above the setting temperature to receive a firm product with the desired textural properties. Products deposited below the setting temperature will have a structured texture and show a lack of firmness. The short gelling time will give the pectin formulation the advantage of being able to be processed very quickly in order to finish packaging and storage on pallets.

Jelly fruits are manufactured with high methylester pectin standardized to a constant setting temperature. Typically, slow set pectin is used in combination with buffer salts that have retardant properties, e.g., sodium citrate or sodium potassium tartrate, to influence the gelation behavior of the pectin type used. Buffer salts can be added separately to achieve a unique product profile. Pectin types that have been already standardized with buffer salts can also be obtained. Apple pectin will provide soft textures with a smooth-viscous gel structure and are typically tolerant to calcium ions. Calcium ions may originate from the water or other ingredients. Jelly fruits with firm gel structures and a short-brittle texture can be manufactured with citrus pectin. The retarding agent used has also texture influencing properties. Sodium citrate enhances the smooth gel structure, whereas sodium potassium tartrate will result in products with elastic properties. In jelly fruits, typically 1.3% pectin or ~1.7% buffered pectin is used. With a dosage of ~2.5% pectin, gummy-like products with viscous-elastic gel structures and firm chewable textures can be obtained (Table 140.13). For these products, the buffer salt composition may be adjusted to the pectin type used and the manufacturing process selected.

In the manufacturing process the gelation behavior of pectin must be considered. Fruit jellies are manufactured within a pH range of 3.2–3.5. At this range the products have a fresh and fruity character, which emphasizes the release of the flavor by the acidity. In a batch oriented process, pectin is pre-mixed with part of the sugar and buffer salts, assuming the latter has not been incorporated into the original pectin. Cold water is added to the

TABLE 140.13
Recipe for Gummy Jellies

47.5%	Glucose syrup (abt. 70DE)
32%	Sugar
20%	Water
5%	Fructose
2.5%	High methyl ester apple pectin, buffered
0.02%	Sodium citrate (to adjust setting time)
	Flavor, color, citric acid solution (to adjust pH value)
Input:	Abt. 109%
Output:	Abt. 100%
TSS:	Abt. 78%
pH value	3.5

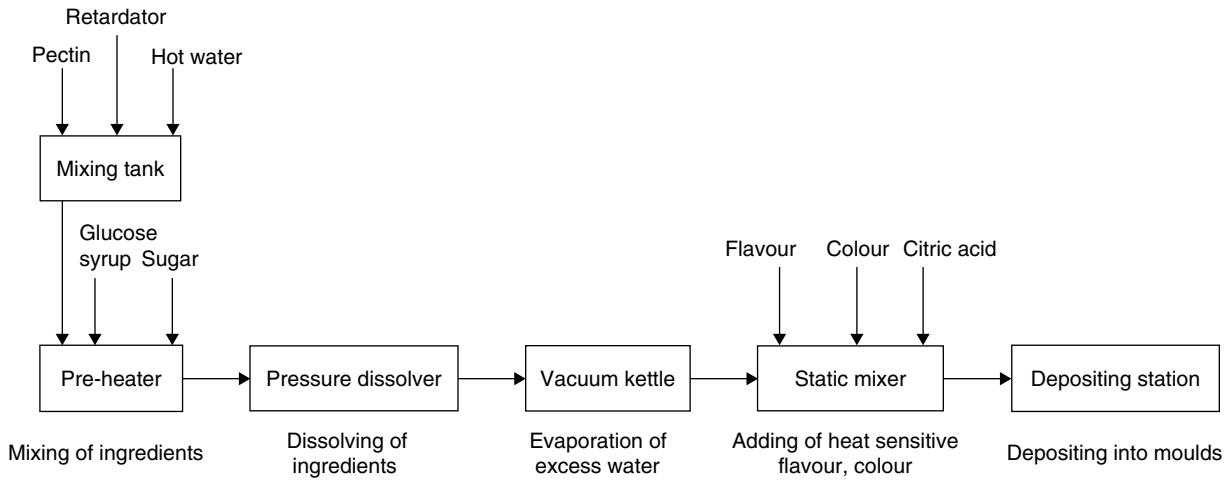

FIGURE 140.9 Continuous production of confectionery products.

cooking vessel and the pectin pre-mix is added while stirring. Heating dissolves pectin and the other dry ingredients. Then, the remaining amount of sweetener, e.g., sugar and glucose syrup, is added. While boiling, the pH should be adjusted to 3.8–4.5. At this pH, pectin will not start to form a gel, but is still stable towards degradation. At a relatively low pH, the tendency to invert sucrose is increased while caramelization will also be increased. Although manufacturing a standard 5% pectin solution may be responsible for adding too much water, a pectin solution with a relatively high content of soluble solids can be prepared in a pressure vessel with a high shear mixer. This method is especially suitable for a continuous manufacturing line (Figure 140.9). After adjusting the soluble solids content, flavor and color are added in the required amounts. Finally the citric acid solution is added to decrease the pH.

As the pectin types used are standardized to a constant gelation behavior, the required amount of citric acid for the pectin type used will be constant, as predetermined during the research and development stage. Depending on the buffer salts used and dissociation of pectin, an equilibrium is reached so that the drop in pH is controlled. The gelling process is irreversibly induced by the addition of acid solution. The batch is then deposited into the molds at 85–95°C (185–203°F). Molds made of plastic have the advantage of permitting the products to be quickly processed further. After depositing the batch into starch based molds, it is possible to run the products through a final drying process to harden the gel structure. Then, after the products are removed and sticking starch cleaned off, they are covered with a parting compound, e.g., sugar or wax. This process can include whipping of the batch before depositing to produce zephyr, a Russian confectionery product.

With the appropriate pectin type, it is also possible to produce non-acid confectionery products. Low methylester amidated pectin is the recommended pectin type for Turkish Delight or other high pH products. Turkish Delight is a soft gelled product covered with chocolate or sugar that has a pH of ~4.5. The manufacturing process is the same as for other pectin based jellies. Also, for aerated confectionery products, e.g., Angel Kiss or Snowball, pectin is an appropriate gelling agent (Table 140.14).

TABLE 140.14
Recipe for Angel Kiss Product

51%	Sugar
24%	Glucose syrup (abt. 40 DE)
22%	Water
10%	Pectin solution (5% low methyl ester amidated citrus pectin, buffered)
0.17%	Egg white
Input:	Abt. 107%
Output:	Abt. 100%
TSS:	Abt. 74%
pH value	Abt. 6

XVIII. BEVERAGES

A. Juices and Soft Drinks

Low caloric soft drinks lack mouthfeel because of the use of artificial sweeteners. However, the mouthfeel contributes decisively to the consumer acceptance of a beverage. A beverage without a mouthfeel offers a watery character not appreciated by a consumer. The addition of more flavoring substances does not reduce this negative response. By adding high methylester (HM) pectin, the viscosity and the mouthfeel of low caloric beverages will be enhanced. The contact between the drink and the taste buds on the tongue is more intense if the viscosity of a soft drink is increased. The enhanced aroma/flavor transfer or detection will create

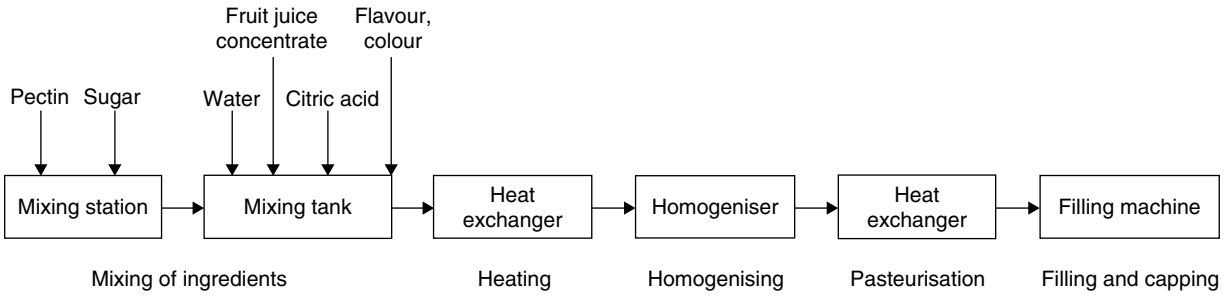

FIGURE 140.10 Manufacturing process of soft drinks.

a better impression of the intensive flavor. By varying the pectin dosage it is possible to increase or decrease several aroma notes, thus affecting indirectly the flavor contribution of the beverage. However, the addition of a large amount of pectin results in a full-bodied product. Thus, the addition of ~0.1% pectin should be sufficient to give the right mouthfeel to a low caloric beverage.

Besides apple and orange juice, exotic fruit juices have become very popular, thus increasing the demand for raw material and the need for fruit juice concentrates. However, beverage bottlers have only limited possibilities to influence the quality of the re-diluted juice. For example, pineapple juice and passion fruit nectar are unstable products, marked by sediment and clear supernatant. This means the consumers usually have to shake the bottle before drinking. The addition of pectin increases the serum viscosity, thus reducing the sediment rate and permitting interaction with the cloud particles to form a stable juice. Additional processing steps, e.g., homogenization, lead to an increase in beverage stability (Figure 140.10) (69). In addition to powder pectin, the pectin industry is able to supply agglomerated pectin to ensure lump-free incorporation without the use of high shear. These pectin types will dissolve during the final pasteurization step.

B. Dairy Beverages and Soy Drinks

Combining milk and other dairy products, e.g., yogurt with fruit, offers nutritional benefits with fresh, fruity, and refreshing beverages. Because of the relatively low pH of the dairy drink, the acid sensitive caseins become unstable and precipitate once the product has been heat treated. Casein at a pH of ~6.8 in milk is embedded in stable protein micelles enabling dairy companies to produce and distribute milk with long shelf life once heat treated. Lowering the pH, e.g., with fruit juice, influences the charge of proteins and subsequently their solubility. Caseins have an isoelectric point at which they become insoluble at pH 4.6. As the pH decreases, the casein slowly loses its hydrate shell and starts to form aggregates. This process is sensitive to temperature changes. At ambient temperatures, casein is able to form a gel if it is not affected by shear at the same time. High temperature and shear that occur during heat treatment will dehydrate casein particles and destabilize the beverage. Casein particles will form sediment in the heat exchanger once the finished product has been filled.

Due to its molecular structure, high methylester pectin is able to bind to casein and form a protective cover (102). Stable interactions are formed by ionic interactions between partially negatively charged pectin molecules and the positively charged casein complex. Also hydrophobic interactions can be observed. In order to get strong interactions, the charge density of casein and pectin has to be considered. Casein has strongly positive charges below pH 4.6, thus enabling interactions with negatively charged pectin molecules. Above pH 4.6, negative charges in the casein molecule will be in the majority, resulting in the repulsion of negatively charged pectin molecules. If the pH of the finished product is adjusted too low, the negative charge density of pectin is reduced, leading to weaker interactions with casein. This observation confirms that a pH of 4.0–4.2 may be the most appropriate pH range, at which a strong protective cover is formed. It is possible to pasteurize the dairy drinks, so that a long shelf life can be guaranteed.

Pectin may be added as a dry mix with sugar or as a pectin solution into the milk prior to mixing with fruit juice or after fermentation of the yogurt (or dissolved in fruit juice). Then, the product has to be homogenized in order to separate already coagulated casein and to enable the formation of a pectin casein complex. After homogenization, the product can be pasteurized or UHT treated to obtain a long shelf life product (Figure 140.11).

The protein content will influence the amount of pectin needed to stabilize the dairy drink. Further influences, e.g., pH and acidity, might be considered as well as the fat content, which has stabilizing properties because it binds casein. Calcium will have an impact on the stabilizing properties of pectin, especially if soluble calcium is used to fortify the beverage. Positively charged calcium might hinder interactions of pectin with casein by blocking negatively charged carboxylic acid groups. Calcium tolerant apple pectin might be selected for these products. The addition of calcium salts to the pectin solution should be avoided.

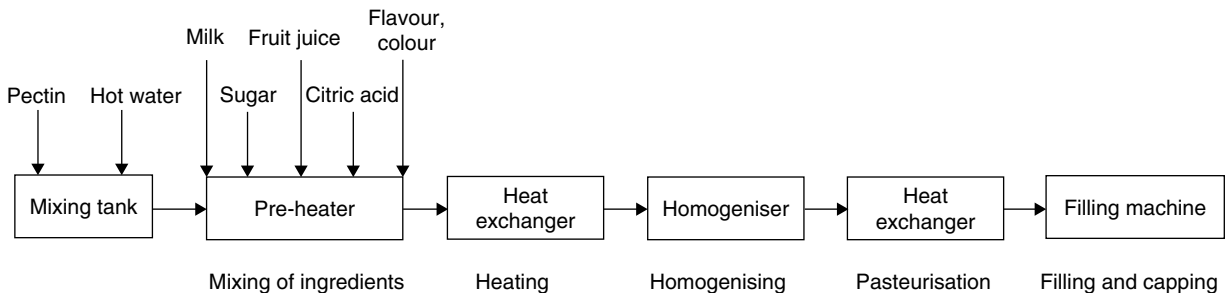

FIGURE 140.11 Manufacturing process of acidified milk drinks.

Soy proteins are, similar to casein, acid sensitive, but they have a different isoelectric point. To obtain a stable product, the pH of soy protein drinks might be more acidic than for dairy drinks and can be adjusted to ~3.8. In the production of soy beverages, the raw material influences the tendency to form sediment. In general dried soy products are used, e.g., soy powder or isolated soy proteins, as raw material. Similar to other dried proteins, soy protein is difficult to re-hydrate. For the development of soy beverages, the product must consider the properties and a proper incorporating technique according to the raw material used. Additionally, soy powder contains a large portion of insoluble fiber, which will form a precipitate eventually. In order to obtain fully hydrated soy protein, an extra operation is necessary. Run a separate processing step with a high shear mixer or homogenizer to manufacture a soy protein solution before it is added to the main process.

XIX. ICE CREAM AND SORBETS

Ice cream is a complex system of different phases and different ingredients that define texture and sensory properties. The liquid phase is an aqueous system that contains dissolved substances, e.g., sugar or hydrocolloids. In the solid phase ice crystals, fruit particles, and fat globules can be found and after aeration air bubbles or nitrogen bubbles form the gaseous phase. The composition of ice cream varies. In dairy ice cream, milk products or derivatives are used to give texture and the decisive taste. Although the fat content is an important factor, oils or fats derived from plant are often used. Proteins and emulsifiers used play an important role in stabilizing the aerated finished product. Sugar or other sweeteners, e.g., glucose syrups, increase the viscosity and mouthfeel of the aqueous phase and improve ice cream structure. Dissolved substances, especially the sweetener used, influence the product's freezing point. The composition of the sweetener determines the rate of lowering the freezing point. Glucose has a stronger effect than sucrose. The freezing point will not only influence the temperature profile of the freezing process, but will also have an influence on the melting behavior and the taste during consumption. Ice creams get their typical smooth structure from the aeration with air or nitrogen in combination with water forming the aqueous phase and the ice crystals. The crystal size affects the structure of the frozen product and defines the mouthfeel. The crystal size is affected by:

- the temperature profile of the manufacturing process
- the soluble solids content
- hydrocolloids added

Because of taste and its influence on the ice crystal size the optimum soluble solids content is 35–40%. Other ingredients, e.g., fruit or fruit preparations, are added as well as other flavoring preparations. Hydrocolloids are added to stabilize the ice structure and have an impact on ice crystal growth. Other functions are the increase of viscosity and mouthfeel. With an appropriate stabilizing system the shelf life of the product will be prolonged. With its flavor enhancing properties and acid stability, pectin can be used as an excellent stabilizer for fruit ice cream and fruit sorbets to control the ice crystal growth.

Crystallization is a time dependent process. Under the right conditions and temperature, a small nucleus is formed which then grows through the deposition of crystals on its surface. The main force holding the ice crystal together is provided by hydrogen bonds. The crystal growth strongly depends on the temperature, mass transport, and effect on the interface. Additionally, re-crystallization can be found to form spherical shaped ice crystals, because of their low interfacial surface energy. Furthermore, a migration process of small crystals towards big crystals can be seen and an agglomeration of ice crystals which are in contact with each other (103). The viscosity does not influence the growing rate of ice crystals, but pectin influences the process, because of its water binding properties to immobilize water molecules. Pectin can influence crystallization by forming hydrogen bonds with the ice crystal and by interactions between negatively charged density of the pectin molecule and the polar charged water molecules. The strong water binding properties of pectin immobilize free water, so that the free water for the ice crystal growth is withdrawn from the system. Pectin also enhances the fruit flavor transfer of the

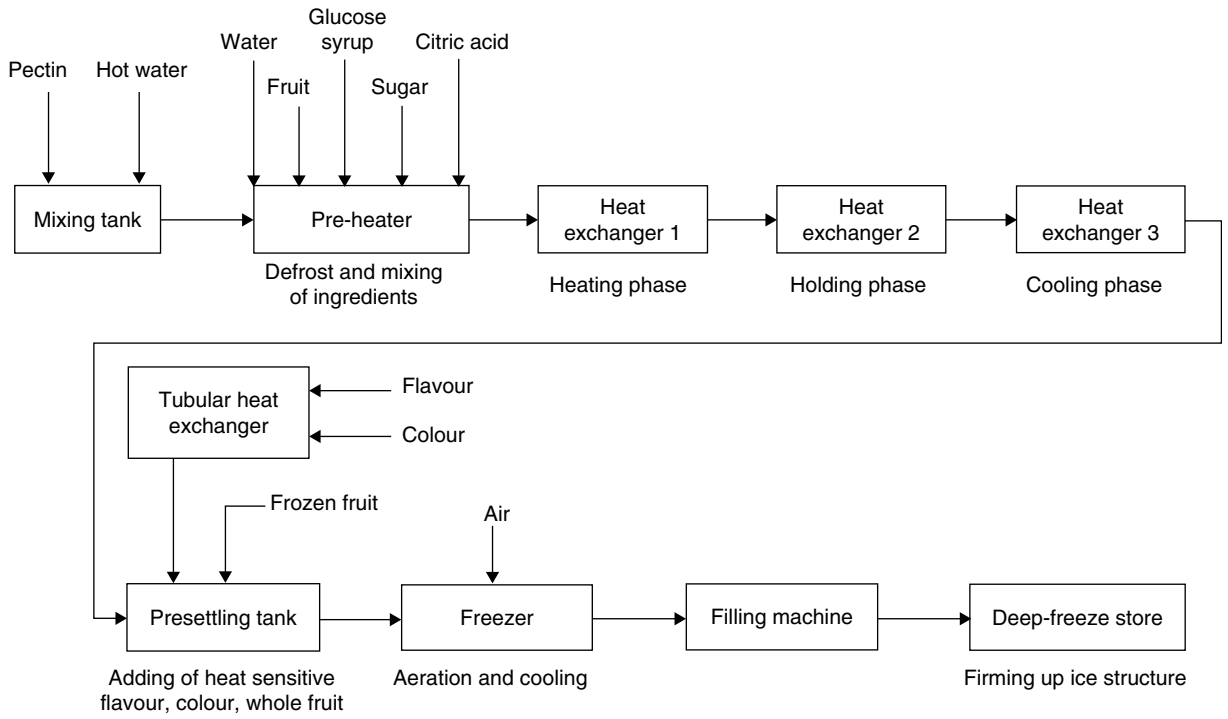

FIGURE 140.12 Manufacturing process of fruit sorbet.

TABLE 140.15
Recipe for Fruit Sorbet

70%	Water
22%	Sugar
10%	Fruit
0.6%	High methyl ester pectin
	Flavor, color
Xml	Citric acid solution (50% (m/m)) to adjust the pH value
Input:	Abt. 102%
Output:	Abt. 100%
TSS:	Abt. 25%
pH value	Abt. 3.0

finished product. So that pectin is an appropriate hydrocolloid to stabilize fruit sorbets and fruit preparations for ice cream fillings. It also serves to stabilize frozen yogurt or other ice cream products with a relatively low pH.

In the manufacturing process of sorbet (Figure 140.12, Table 140.15) all ingredients are mixed and heated to pasteurization, dissolving the dry ingredients. If the recipe contains dairy products and vegetable fat, the batch is homogenized after pasteurization and cooled down to ~2°C (35°F). Whole fruit or other solid ingredients are added to the batch after homogenization. At this stage, heat sensitive flavor, color, and the yogurt base are added. The freezing process is conducted in a continuous system with a freezer in which rotating knives scrape the ice film off the inner wall. Air is introduced into the freezer with excess pressure to aerate the batch. Once the product is removed, the batch is cooled to ~ −5°C (20°F). At this stage the product has a very soft texture, and after filling, is firmed up in the storage freezer at −40°C (−40°F). A product with a long shelf life is produced when ~90% of water is frozen.

Fruit fillings or other preparations can be introduced into the dairy ice cream after the ice cream batch has passed the freezer. In order to prevent freezing or too strong ice crystal growth, such preparations should have a relatively high soluble solids content containing monosaccharides such as glucose or fructose, so that they exert the most lowering effect on the freezing point.

Pectin also enhances the structure of frozen ice pops by providing a softer and crunchy structure for a more tasteful consumption.

XX. OTHER FOOD APPLICATIONS

A. Savory Products

Tomato sauces often need to be thickened and pectin is a suitable stabilizer. Medium methylester to low methylester pectins give similar textures as the natural tomato pectin and a pulpy texture can be obtained with a fruit enhancing mouthfeel. The choice of pectin depends on the application of the tomato product, because it will have a major influence on the melting behavior. A medium methylester pectin will give a low melting point but a smooth texture. Products requiring a higher melting behavior, to be able to consume the product as a hot dish or condiment, employ low methylester pectin, used in combination with calcium. Fruit sauces as condiments are easy to prepare with low

TABLE 140.16
Recipe for Tomato Sauce

45%	Water
23%	Tomato puree (triple concentrated)
22%	Water
7%	Vinegar
2.5%	Salt
1.2%	Low methyl ester pectin
	Bell pepper, pepper, and spices
	Flavor, color
	Citric acid solution (50% (m/m))
Input:	Abt. 104%
Output:	Abt. 100%
TSS:	Abt. 36%
pH value:	3.5

methylester pectin. Although these sauces have a low soluble solids content, pectin can be added to the cold batch and mixed with other dry ingredients. The pectin will then be dissolved in the heating process, in case it is not possible to prepare a pectin solution. Table 140.16 shows a typical recipe for a tomato sauce.

Condiments, like mint sauce or fruit jellies and other fruit condiments, can also be texturized with pectin. The choice of the correct pectin type depends on the soluble solids content and pH of the product. Condiments have a soft and smooth texture to give the meal an appropriate accompanying taste. The quality requirements and the manufacturing process are similar to those for making jams and jellies.

B. Desserts and Other Dairy Products

Pectin can be used in a variety of dessert products. It is possible to gel milk systems, flavored water, or juice (jellies). A very convenient possibility to produce a milk dessert for food service or refrigerated retail products is to combine a fruit preparation containing low methylester pectin with milk. During the cold mixing process low methylester pectin interacts with milk-calcium to form a viscous-elastic gel. The time for the product to set needs to be adjusted to the estimated mixing time. However, a few minutes after mixing, a weak gel is formed which will harden in time when refrigerated. This product concept can also be used to manufacture a fruit preparation that permits a consumer to add the required amount of milk. Depending on the recipe, a fruit preparation can be used as a base for milk desserts or milk shakes.

Water jellies and trifles manufactured with pectin will get a very fruity and refreshing taste. With low soluble solids content, these products require low methylester amidated pectin to form the typical elastic and brittle texture. The addition of calcium might enhance the elastic characteristic of the finished product. In comparison to other commercial gelling agents, e.g., gelatin and carrageenan, pectin is more acid stable, especially when required for acidic flavors. In general the combination of calcium requires increased filling temperatures than for products with gelatin. However, cold-filling of pectin based trifles is also possible. Products can be distributed as dessert powders with different flavors for homemade trifles according to the recipe. After refrigeration the product is ready for consumption. It is also possible to make ready-to-eat retail products, with the possibility of adding a favorite item such as cream, yogurt, and so on.

The interaction of low methylester pectin with calcium and milk protein will provide possibilities to enhance the firmness and creaminess of yogurt products. After fermentation of milk with lactic acid producing bacteria, casein becomes positively charged once the pH of the dairy product drops below 4.6. A weak protein gel is formed, which is stabilized by the interactions between casein micelles. Pectin, being negatively charged, interacts with the positively charged casein as well as with the calcium ions to increase the stability of the protein gel. The addition of low methylester pectin will also enhance the mouthfeel of the yogurt, which is especially suitable for low-calorie products with a reduced milk fat content. However, this technique is limited in application. Low methylester pectin can only be added before the fermentation has taken place. Because of interactions with positively charged casein and calcium in the dairy product, the addition of low methylester pectin can lead to an uneven mixing behavior of the pectin used. Figure 140.13 shows a possible manufacturing process for the application of pectin in low-fat yogurt.

Another limitation is the amount of pectin that can be added to the milk at the beginning of the process. If $>0.2\%$ pectin is used, interactions with milk proteins will result in destabilization of the milk. This is caused by the formation of a pectin-casein precipitate and further processing of the batch will fail.

After fermentation, the yogurts will settle and this will increase their firmness and stability during transport. Pectin will increase the firmness of stirred yogurts but provide a smooth and creamy mouthfeel.

In milk desserts the addition of low methylester pectin will show benefits to increase the firmness and transport stability of puddings. In these products, pectin can be used in combination with other hydrocolloids or starches. The creamy texture and mouthfeel is determined by starch. A low methylester pectin will yield a gelled structure, increasing elasticity of the dessert.

C. Bakery Products

The buying patterns of consumers have changed. The demand for freshly baked bread rolls for breakfast every day of the week expresses this pattern, with a simultaneous demand for convenience products such as frozen dough or part-baked semi-finished dough. Also, food service has an increased demand for convenience products in order to enhance customer service and offer a variety of products. In

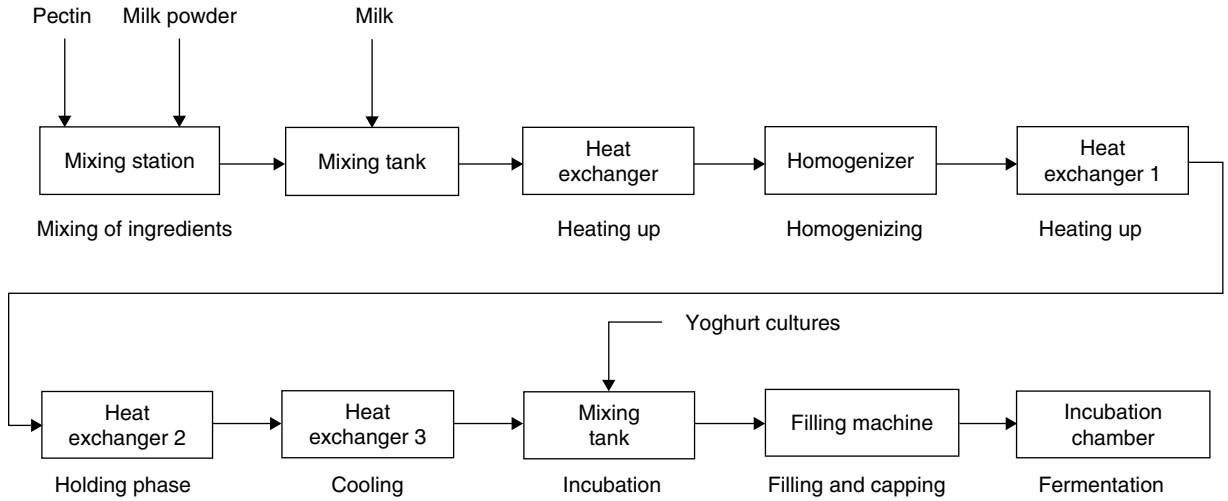

FIGURE 140.13 Manufacturing process of stabilized low fat yogurt.

part-baked products starch tends to undergo retrogradation. This is a process in which the swollen starch recrystallizes and the dough loses its water binding properties. This behavior will have the effect of a rather dry crumb structure. Adding pectin improves the quality of the finished products. In frozen products pectin influences the growth of ice crystals. Large ice crystals obstruct the smooth structure of the dough which may lead to an uneven baking behavior. Pectin also has a positive influence on the retrogradation behavior of starch and in general increases the dough stability. After baking, the finished product shows a thinner dry edge of the crumb which will remain for 24–48 hours. This is a significant extension of freshness, an important consumer preference. Pectin is added at 0.1–0.3% and evenly blended with the flour. After adding the other ingredients, the dough is kneaded and formed.

XXI. SUMMARY

The applications for pectins have changed over the last decades when they were used mainly as a gelling agent for jams and jellies. As a texturizer in fruit preparations, pectin is currently used to manufacture quality products with a high consumer preference. Pectins can affect the taste, enhancing freshness and fruitiness. This is one of the major reasons why they serve as appropriate ingredients to texturize fruit preparations and to gel dessert products or confectioneries. With this premise, pectins have many other applications as a natural texturizer to enhance the appearance and taste of processed food, e.g., low fat margarine or low fat mayonnaise. Other applications including the emulsifying properties of sugar beet pectin (5) and encapsulation of ingredients for food or drug (38) are in research. Another new possibility is in the manufacture of nutraceuticals and functional foods (105). For example, as a soluble dietary fiber with cholesterol reducing properties, pectin will play a major role in a balanced human diet (26). With the increasing demand of processed food, pectin can serve as a functional ingredient in numerous products traditionally not considered.

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Part Q

Modeling

141 Kinetics of Quality and Safety Indicators Under Steady Conditions — Basic Concepts

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CONTENTS

I. INTRODUCTION

Mathematical modelling is a powerful tool in process design, assessment and optimisation. For a given situation it is very important to identify a mathematical model with an adequate functional form, but even the more complex model is of little use if the model parameters are not known with precision and accuracy. The estimation of kinetic parameters from experimental data involves the application of statistical methods in two phases: experimental design

and data analysis. Although much more emphasis is often put on the latter, one should realise that the general value of the information contained in the data is actually established when the experiments are designed and even a very careful data analysis is unable to recover information that is not present in the data (1, 2). As Lenz and Lund (3) stressed, much of the data currently found in the literature could have been obtained with considerable less effort by proper choice of experimental conditions. Furthermore, the lack of quality of the experimental data many times compromises the precision and accuracy of the parameters estimates. This is of utmost importance when microbiological or chemical model parameters are used in process design, assessment or optimisation (4).

This chapter addresses some basic concepts related to the study of kinetics of quality and safety indicators: (i) presentation of experimental design, particularly the concept of optimal designs aiming at improving parameter estimation when the mathematical model is known, (ii) discussion of data analysis strategies, particularly in relation to situations of interest in food research and (iii) presentation of the basic concepts of some fundamental mathematical models (primary models: zero-order, first-order, second-order, Weibull, Logistic, Gompertz and Michaelis-Menten models; secondary models: Arrhenius, Bigelow and Q_{10} models).

II. EXPERIMENTAL DESIGN

In mathematical modelling of experimental data, it is important both to assess whether the model is of the correct functional form and to accurately estimate the model parameters (5). This is clearly affected by the experimental design. The assessment of model adequacy requires the collection of experimental data throughout the whole process, and sampling times are selected on the basis of heuristic designs, such as equally spaced sampling times, equally spaced sampling concentration values and equally spaced logarithmic sampling times. The accurate and precise estimation of model parameters on the other hand usually requires a number of replicates of a limited number of sampling times (often as many as the model parameters) that must be carefully selected. This section discusses mostly experimental designs aiming at improving parameter estimates, assuming that the mathematical model is known, while the use of experimental design for product development and optimisation will be thoroughly discussed in chapter 143 (by Schrevens and Portier).

A. Selection of Sampling Times

Kinetic studies involve the analysis of the decay/growth of a given compound/property/microorganisms over time. Processes can seldom be measured continuously, thus leading to the use of discrete sampling times. The selection of these sampling times is very important as they greatly affect the ability of testing the quality of the fit of the model and parameter estimation.

1. The Concept of Optimal Design

If one is confident that a given non-linear model $E[yi] = f(\mathbf{x_i}; \theta)$ is appropriate for a certain experimental situation, then there is the question of designing the experiment, i.e., choosing the vector $\mathbf{x_i}$ of experimental points (in this case sampling times) so that the unknown parameters θ are estimated in an optimal fashion (6).

Traditionally, the motivation underlying the theory of optimal design is that experiments should be designed to achieve a level of statistical inference as precise as possible. Design optimality was first addressed by Smith (7), and early work in the subject was done by Wald (8), Hotelling (9), and Elfving (10). The major contributions to the area, however, were made by Kiefer (11–13), and Kiefer and Wolfowitz (14), who synthesised and greatly extended the previous work. Reviews on research work on optimal design have been made by St. John and Draper (15), Atkinson (16), and Steinberg and Hunter (17). More recently, Atkinson and Donev (18) wrote a book on optimum design of experiments.

In practice, the application of optimal design theory requires a criterion for comparing experiments and an algorithm for optimising the criterion over the range of possible experimental designs. The classical criteria are derived from linear modelling theory, which assumes that the experimental data can be represented by the equation:

$$Y_i = \mathbf{f(x_i)^T}\,\beta + \varepsilon_i \qquad (141.1)$$

where Y_i is the measured response from the ith experimental run, $\mathbf{x_i}$ is a vector of predictor variables for the ith run (in this case sampling times), $\mathbf{f}$ is a vector of p functions that model how the response depends on $\mathbf{x_i}$, $\boldsymbol{\beta}$ is a vector of p unknown parameters, and ε_i is the experimental error for the ith run, with independent errors and constancy of the error variance.

The variance of the parameter estimate is a natural indicator of the quality of statistical inference with respect to a single parameter. If the errors are uncorrelated and have a constant variance σ^2, the variance-covariance matrix of the least square estimator, $\hat{\boldsymbol{\beta}}$, is (16):

$$\mathrm{var}\{\hat{\beta}\} = \sigma^2(\mathbf{X^T X})^{-1} \qquad (141.2)$$

where $\mathbf{X}$ is the n*p matrix whose ith row is $\mathbf{f(xi)^T}$. The quality of inference may also be assessed in terms of the variance of the estimated response at $\mathbf{x}$, which, from Equation 141.1, is given by:

$$d(\mathbf{x}) = \sigma^2\mathbf{f(x)^T}(\mathbf{X^T X})^{-1}\,\mathbf{f(x)} \qquad (141.3)$$

TABLE 141.1
Most Common Optimality Criteria for the Design of Experiments Under Linear Conditions (According to Steinberg and Hunter (17))

Optimality Criterion	Description	Objective
D-Optimality	A design is said to be *D*-optimal if it minimises $\det(\mathbf{X^TX})^{-1}$, where det denotes determinant.	Minimise the generalised variance of the parameter estimates.
A-Optimality	A design is said to be *A*-optimal if it minimises $\mathrm{tr}(\mathbf{X^TX})^{-1}$, where tr denotes trace.	Minimise the average of the variances of the parameter estimates.
E-Optimality	A design is said to be *E*-optimal if it minimises the maximum eigenvalue of $(\mathbf{X^TX})^{-1}$.	Minimise the variance of the least well-estimated contrast.
G-Optimality	A design is said to be *G*-optimal if it minimises $\max d(\mathbf{x})$, where the maximum is taken over all possible vectors **x** of predictor variables.	Minimise the variance of the predicted response.
I_λ-Optimality	A design is said to be I_λ-optimal if it minimises $\int \mathbf{d(x)}\lambda\,(d\mathbf{x})$, where λ is a probability measure on the space of predictor variables.	Minimise the average integrated variance of the predicted response.

Both Equations 141.2 and 141.3 depend on the experimental design only, trough the full rank $p \times p$ matrix $(\mathbf{X^TX})^{-1}$, and suggest that a good experimental design will be the one that makes this matrix small in some sense. Since there is no unique size ordering of the $p \times p$ matrices, various real-valued functionals have been suggested as measures of "smallness" (see Table 141.1).

The criteria referred in Table 141.1 have been widely applied to the design of experiments for studying systems that follow linear and polynomial models. From those, the most used one is the *D*-Optimality criterion (18). Recently, De Aguiar *et al.* (19) presented a tutorial on *D*-optimal designs, which clearly illustrates the use of this technique.

2. The *D*-Optimal Design

The *D*-optimal design was first proposed by Box and Lucas (20) as an optimum design criterion for non-linear models, based on establishing the sampling conditions (in this case sampling times) that lead to a minimum confidence region, for a standard situation of a number of observations (n) equal to the number of parameters (p). In their pioneering paper on experimental design for non-linear models, Box and Lucas (20) showed how the *D*-Optimality criterion could also be applied to non-linear models, by introducing a linear approximation.

For any choice of the design variable (i.e., the independent variable, t) the size of the parameters joint confidence region is proportional to the Jacobian $|(F^TF)|^{-½}$ of the derivative matrix F (where $F \equiv [f_{i,j}]$, with $f_{i,j} = \partial\eta_i/\partial\theta_j$ evaluated at $t = t_i$, with i ranging from 1 to n; η represents the system response and θ a kinetic parameter). Thus a logical choice of the design criterion is to choose sampling points so that the size of this joint confidence region is minimised, that is, the determinant $D \equiv |F^TF|$ should be maximised.

The main goal of Box and Lucas (20) algorithm is to design a series of n experiments where the levels of the different conditions at which observations are taken (in this case sampling times) are selected in view of optimum parameter precision. Generally some restrictions must be applied, for instance, negative values of time have no physical meaning.

Hill (21) described a non-linear regression model $\eta(\theta,\mathbf{x})$ as being non-linear with a subset of non-linear parameters μ. He showed that the *D*-optimal design for estimating θ depends only on μ. This finding was later confirmed by Khuri (22).

It was also found that, for several chemical reactions involving non-linear models, the optimal design for n experiments (with $n > p$) consists of n/p replications of each of the optimal sets of levels (in this case sampling times) for the case $n = p$ (23–25). This has certainly simplified the evaluation of optimal designs. According to Box and Lucas (20), in the case where $n = p$, the *D*-optimal design can be simplified from the maximisation of $D \equiv |F^TF|$ to the maximisation of $\Delta \equiv \mathrm{mod}(|F|)$ (Δ denotes the modulus of the determinant of the matrix F).

An important drawback of this approach, for a non-linear response function, is that the efficiency of any particular design depends on the initial estimates of the parameters (20, 23, 26). If the estimates upon which the design is based are poor, the design may be inefficient, with the robustness of the criterion to poor estimates depending upon the particular model being studied. The application of the *D*-optimal design may seem strange at first sight, as in order to use the criterion one must initially have estimates of the parameters; after all, it is the purpose of the experiment to obtain such estimates. Actually, this paradox is also common to empirical designs, as when designing any experiment the experimenter is relying either on his/her previous knowledge or on his/her intuition. The efficiency of any design is therefore also dependent on the "guesses" of the experimenter. In the limit, if nothing is known about the experimental situation, then, strictly speaking, no experiment can be planned (27).

Because a good choice of design depends on the true values of the parameters, the idea of sequential design is a natural one. Ford *et al.* (26) have made the distinction between two sequential designs: batch-sequential and

fully-sequential. In batch-sequential design, the total of n observations are divided into b batches, in the ith batch, a set of n_i (the batch size) observations are taken. The sampling values for the (i + 1)th batch are chosen based on the analysis of the first i batches. In fully-sequential design, the batch sizes are all unity, except possibly for an initial larger batch. Ford *et al.* (26) emphasise that sequential designs can pay off, particularly if the initial estimate of the parameters is poor, but that a batch-sequential design with only a few batches is likely to be sufficient.

The non-linear *D*-Optimality criterion was first applied to a first order growth/decay model with Arrhenius temperature dependency at isothermal conditions, the Mitscherlich equation and to a system of two consecutive first-order reactions (20). These models have been further used to illustrate the application of *D*-optimal design to non-linear models (2, 6, 18).

More recently, the criterion was applied to kinetic studies of enzyme-catalysed reactions (28), to the Bigelow model in thermal processing studies (29), to diffusion processes described by Ficks's 2nd law model (30, 31) and to calibrate chemical and enzymic time-temperature integrators (32). An applied example and a more detailed reference of recent applications may be seen in Chapter 144.

3. Heuristic Designs

In practice, scientists tend to use alternative experimental plans that are easier to implement than *D*-optimal (DO) design, such as equally spaced sampling times (ET), equally spaced sampling concentration values (EC) or equally spaced logarithmic sampling times (ELN).

a. ET design

Sampling times are defined in such a way that they are equidistant in the time scale. This plan does not depend on initial estimates of the parameters, and the same measurement-time plan can be used for different levels of a given factor (e.g., at different temperature values). One should be cautious, however, in using this kind of plan because it ignores the functional form of the model and any previous knowledge of the process. In addition, it is necessary to define the period the experiment will cover. If the experimenter does not have a preliminary idea on the process rate, data can be obtained for small extensions of the process only or, quite on the contrary, there may be a lack of information on the early stages of the process, thus compromising the results.

b. EC design

The idea behind this design is to choose the sampling times so that the expected amount of observed degradation/growth between two consecutive times is constant. The EC design depends on: (i) the period defined for following the process, (ii) the parameter estimates and (iii) the "guessed" model (the plan can be very sensitive to differences between the "guessed" and the "true" model). In contrast to the DO design, the EC design has the following advantages: (i) it is easier to compute, (ii) it is not sensitive to small changes in the initial estimates (33) and (iii) when $n > p$, which is usually the case, the plan allows for testing the quality of the fit of the model and allows for testing models other than the "guessed" one.

c. ELN design

The sampling times are defined in such a way that they are equidistant in the log-time scale. This implies that samplings are more frequent at short times that at long times. The rationale for this is that the process rate often decreases with time. Thus, it makes sense to get more observations early when the degradation/growth rate is faster (33). This design shares most of the advantages and disadvantages of the ET design.

B. Selection of Experimental Conditions

Usually, experiments aim at studying the effect of time on the evolution of a certain decaying/growing quality/safety factor, "C," and are performed in a medium with constant characteristics (temperature, pH, water content, etc.). However, it is often important to assess the effect of the experimental conditions on the process kinetics. In food research, temperature is probably the experimental condition most tested. Thus, this section focus on the analysis of temperature effects, although most of the discussion may be generalised to other experimental factors, provided adequate functional relationships between the model parameters and the experimental factor of interest are used.

Methods for estimation of kinetic parameters may be broadly divided into constant and varying-conditions methods, in the case of temperature, isothermal and non-isothermal methods.

The isothermal analysis of kinetic data involves a considerable amount of experimental work at constant temperatures under a specific range of interest, resulting in an expensive and time-consuming procedure. An additional problem may arise if the parameters estimate correspond to a biased solution, that is, although yielding a good fit to the experiments performed are actually quite different from the true values. In this situation, when they are used for predicting processes under non-isothermal conditions, they may lead to significant deviations (34). However, isothermal methods are necessary for assessing the dependency of the kinetic parameters on temperature.

Non-isothermal methods, on the other hand, are based on a single experience in which temperature and the factors under study are recorded as function of time. Thus, kinetic parameters are evaluated from a single test covering the desired temperature range. These methods have significant advantages: minimisation of experimental

requirements, overcoming thermal lag problems and providing a dynamic situation closer to the reality of most thermal processes. Of course, they require the previous knowledge of the functional relationship between the kinetic parameters and temperature.

Isothermal methods are the classical procedure for estimation of kinetic parameters. Despite the advances in data analysis, most kinetic studies in food and pharmaceutical science/engineering still result from isothermal experiments. Thus, techniques for kinetic parameters evaluation are widely and well documented (35).

In the classical isothermal method, rate constants are estimated by fitting the kinetic model to the data sets gathered at different constant temperatures. A model is then identified to describe the dependence of these constants on temperature and its parameters are estimated. The dependence of the rate constants on temperature is usually described by an Arrhenius-type equation. Other models describing the temperature effect are also discussed in the literature (36) and will be further presented.

In terms of defining the experimental conditions, usually a range of temperatures is defined and experiments are taken at temperatures equidistant within this range, with the number of selected temperatures varying from 3 to 6 (see Table 141.2). The concept of the *D*-optimal design may however be also applied to select the temperatures that lead to maximum parameter precision, once the model that relates the kinetic parameters with temperature is known. Box and Lucas (20), suggested for the first-order Arrhenius model, that two experiments should be performed, one at each of the extreme temperature values of the assumed temperature range. Furthermore, at each temperature samples should be taken at a time such that the fractional conversion of the factor under study is equal to e^{-1} (equivalent to a degradation of 36.8% of the initial amount).

At each of the chosen temperatures, the conversion of the decaying/growing factor should be carefully followed. Even when a linear plot is obtained, conclusions must be drawn cautiously from the data, especially if the data points correspond to no more than 10–20% conversion, because mathematical functions are roughly linear over a sufficiently small range of variables (34). According to Arabshahi and Lund (53) reactions should be followed, where possible, through at least 4–5 half-lives. If the method of analysis is not sufficient to measure response values as low as this, the longest heating times possible should be used (3). According to Hill and Grieger-Block (54), one should perform at least experimental runs in which data are taken at 40%, 50% or higher conversions. For example, with regard to nutrient loss, the error in the rate constant, if one goes only to 30% loss, will be greater than ±25% (55). However, for up to 50% loss, the error in the rate constant is less than ±5% (56).

C. Testing Experimental Designs — "Pseudo-Experimental" Data Generation

Parameter estimates greatly depend on the model, on the regression scheme and/or on the experimental design. The first two effects are extensively reported in literature (5, 35, 52, 57, 58).

The comparison of different regression schemes and of different experimental plans requires a great number of experiments and the analysis of the accuracy and precision of the estimated parameters can only be made if the "true"

TABLE 141.2
Typical Temperature Levels Used in Thermal Kinetic Studies Under Isothermal Conditions

System	Temp. Range [°C]	Temp. Increments [°C]	Number of Temp. Levels	Reference
Inactivation of horseradish peroxidase	60–94	4–10	5	(37)
… at different ionic strengths	70–95	5	3–5	(38)
… at different water contents	125–150	5	4	(39)
… at different acid solutions	60–90	5–10	3	(32)
… at different alkaline solutions	40–95	2.5–10	3–4	(32)
Inactivation of α-amylase from *B. amyloliquefaciens*	135–150	5	4–5	(40)
Inactivation of α-amylase from *B. licheniformes*	96–108	4	4	(41)
Inactivation of *B. stearothermophilus*	111–125	2	5	(42)
	110–130	3–5	6	(43)
Inactivation of *C. sporogenes* PA3679	121–140	4–5	5	(44)
	110–121	3–5	4	(45)
Inactivation of *S. uvarum*	35–52	1.5–5	6	(46)
Formation of galactose in milk	90–150	10	6	(47)
Formation of galactose and tagatose in milk	115–135	5	4	(48)
Degradation of thiamin	25–55	5–10	4	(49)
Degradation of anthocyanins	50–80	10	4	(50)
Colour change in pea puree	110–125	5	4	(51)
Quality changes in green peas and white beans	100–122	6–10	4	(52)

TABLE 141.3
Use of Computer Generated Data Sets

Regression Model	Error Structure	Objective	Reference
Isothermal first-order model with Arrhenius temperature dependency	Variance proportional to the response function X_i and $X_i \sim N(X_i, (e \times X_i)^2)$, with $e = 0.08$	Study the influence of three least squares regression methods on the estimation of the Arrhenius model parameters	(5)
Non-isothermal first-order model with linearly increasing temperature profile	Variance proportional to the response function X_i and $X_i \sim N(X_i, (e \times X_i)^2)$, with $e = 0.04$ and 0.008	Study the influence of different integration methods and of two different regression procedures on the accuracy of the parameters	(35)
Fick's 2nd law model with diffusion constant showing a Arrhenius temperature dependency and linearly increasing temperature profile, applied to mass diffusion in spheres	Constant variance and two normally distributed error sets with a standard deviation of 0.05 and 0.06, respectively	Study the influence of the experimental design and of the temperature profile on the estimation of the model parameters	(30) (62)
Fick's 2nd law model considering both internal and external resistance to mass transfer in cubes	Constant variance and error sets drawn from a population of errors, ε, with $\varepsilon \sim N(0, 0.05^2)$	Study the influence of the experimental design and the Biot number on the estimation of the model parameters	(31)
First-order growth model	Constant variance and error sets drawn from a population of errors, ε, with $\varepsilon \sim N(0, 0.05^2)$	Study the influence of the experimental design on the size of the asymptotic joint confidence interval of the model parameters	(6)

values are known. An alternative is to generate data sets by computer simulation (59). Data generation was first applied with the Monte Carlo method. The designation Monte Carlo was first used in World War II in relation to the simulation of stochastic atomic collision processes (60). Since then, it has been widely used to characterise the statistical distribution of a response that is dependent on a number of factors with well known statistical distributions (61). Data generation to compare regression techniques was first used in food research by Haralampu *et al*. (5). Data sets were generated with the following assumptions: (i) data follow a given regression model and (ii) the error structure is known. Since then, similar works have been reported in the literature (see Table 141.3). The use of simulated data eliminates biases in the parameter estimates due to faulty assumptions in terms of the data error structure. Some authors assume a proportional variance, but care should be taken with this approach, as it demands for variable transformation or weighted least-squares, to avoid heteroscedasticity (63). Care should also be taken in the choice of the random number generator.

According to Law and Kelton (64) a "good" computer generator should have the following properties: (i) the generated values should follow a uniform distribution without any auto-correlation, (ii) it should be fast without large memory needs, (iii) it should possess the capability of regenerating the same pseudo-random sequence at least for two times and (iv) it should have a sufficiently long generation cycle (high number of possible outcomes).

Varga (61) presented a comprehensive review on random number generators and developed a computer program in FORTRAN 77 that fulfils the above described properties. This program uses a linear congruential random number generator that generates, from a uniform distribution, random variates following a normal distribution, using the inverse transform method (64).

The computer simulation is divided in three steps:

- An exact set of data is generated according to a certain model using some previously chosen parameter values (these will be considered the "true values") and following a particular sampling plan.
- Using the random number generator, a set of independent and identically distributed (I.I.D.) errors following a normal distribution with a given constant variance is obtained.
- A set of simulated "pseudo-experimental" data is then generated by scattering the exact data with the random normally distributed proportional errors.

An example of simulated "pseudo-experimental" data for the Bigelow model is shown in Figure 141.1.

III. DATA ANALYSIS

When using a given model for predictive purposes, errors may occur because of several reasons: (i) the kinetic model may not have the correct functional form, (ii) it may not consider all the factors that influence the process or (iii) the model parameters may have not be correctly estimated. Data analysis techniques are therefore most important to ensure that the quality of the fit of the model to the data may be truly assessed and that the model parameters are precisely and accurately estimated.

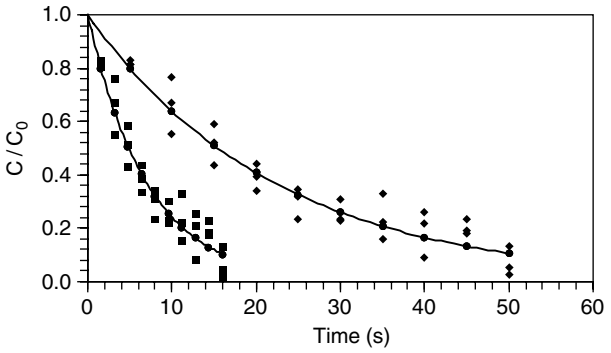

FIGURE 141.1 Computer generated "pseudo-experimental" data for the Bigelow model (Equation 141.19) with D_{ref} = 0.21 min, z = 10°C and T_{ref} = 121.1°C (From Ref. 51). The points show the "pseudo-experimental" data and the lines represent the exact model data. An equally spaced sampling times plan was used and normally distributed random errors, obtained from a population with zero mean and 0.05 standard deviation ("experimental error" = 5%) were added to the exact model data. (♦) T = 115°C; (•) T = 120°C.

A. Basic Concepts

1. Fitting Models to Experimental Data

Fitting procedures are not much different for models with different degrees of complexity, e.g., a simple first-order model or a very complicated reaction network, only the mathematics are more intricate (4). According to Seber and Wild (6), there are different estimation methods, which can be divided in the following groups: least squares estimation, maximum-likelihood estimation, quasi-likelihood estimation, LAM estimation, L1-norm estimation, robust estimation and Bayesian estimation. Least-squares fitting is the most common procedure in food research. This method consists basically on searching the parameters values that minimise the sum of squares of residuals between experimental and predicted values (SSR) (also called the norm, or objective function):

$$SSR = \sum_{i=1}^{n} [y_i - f(x_i, \theta)]^2 \qquad (141.4)$$

where y_i are the experimental data (e.g., reactant concentrations), $f(x_i, \theta)$ is the model function at the experimental design points x_i (x_i is, for instance, time t_i, temperature T_i, or a vector of both, and the model function is, for instance, the first-order model) and θ the parameter(s) (e.g., the rate constant at a given temperature and the activation energy).

At this point, it would be useful to clearly distinguish between linear and non-linear models. A linear model is not necessarily represented by a straight line. A model is linear (in the parameters) if the first derivative(s) of the model in order to the parameter(s) is (are) independent of the parameter(s), and therefore higher order derivatives are equal to zero; in a non-linear model, at least one of the derivatives of the model function with respect to the parameters depends on at least one of the parameters. A model with parameters a, b and c, with the form:

$$y = ax + bx^2 + cx^3 \qquad (141.5)$$

is a linear model (although not linear in x), while a model with the form:

$$y = \frac{ax}{b + x} \qquad (141.6)$$

is non-linear in the parameter b. In general, and in particular in food research, most of the kinetic models are non-linear in relation to the parameters (examples are presented in Section IV).

The linear least-squares method, which is a particular case of the least squares method, has the following advantages: (i) the values of the parameters follow directly from the regression procedure, (ii) the confidence intervals are exactly defined and (iii) there is no need to provide initial "guessed" values of the parameters. Conversely, when the model is non-linear in the parameters, there are no explicit analytical solutions for the parameters or for the confidence intervals, and a solution must be found by linear approximation. This solution is found iteratively (following a specific numerical algorithm — e.g., Gauss-Newton, Levenberg-Marquardt, Quasi-Newton approximation), starting from initial values supplied by the analyst or estimated by a computer program. Resulting confidence intervals for parameters are only approximate. Moreover, the objective function may have local minimum values and the fitting procedure may converge to one of these local minima, rather than to the absolute minimum value. This can be overcome by searching with different starting values for the parameters. If the same parameter values are found with different initial values, a global minimum has likely been found. Good starting values also allow any iterative technique to converge to a solution much faster.

Since linear least squares are easier to solve than non-linear least squares, frequently data is transformed in such a way that linear regression can be applied (e.g., a first-order model can be linearised simply by taking the logarithms of the data). However, this may violate some assumptions required for linear regression, resulting in biased parameter estimates.

Least-squares fitting is very generally used but it is only appropriate when the following assumptions are fulfilled (2, 4):

1. The model (expectation) function is correct;
2. The response is the sum of the model function and a disturbance (the experimental errors);
3. The disturbance is independent of the model function;

4. The experimental errors are normally distributed;
5. The average of the experimental errors is zero;
6. The experimental errors have constancy of variance;
7. The experimental errors are independently distributed.

Because one cannot know *a priori* whether the different assumptions are valid, an iterative approach should be used, as described in Box *et al.* (65): (i) a plausible statistical model for the data is assumed, (ii) the data is analysed on the basis of this model and then (iii) diagnostic tools, such as plots of residuals, are applied to evaluate the assumptions. If the diagnostic indicates failure of assumptions in either the deterministic or the stochastic components of the model, the model must be modified and the procedure repeated from (i) to (iii).

The statistical assumptions above listed encompass different aspects of the regression model. It is also important to recognise that the design of the experiment and the method of data collection can affect the chances of assumptions being valid in a particular experiment (2, 6). There are some important points that should be considered when assessing these assumptions.

a. *The model (expectation) function is correct*

Ensuring the validity of this assumption is, to some extent, the goal of all science. Researchers aim at building up a model able to predict the natural phenomena under study. However, models should be treated, in general, only as a reflection of the reality, as the underlying physical or chemical mechanisms are so complex that the researcher does not have a complete view of the process. A given model may be more appropriate than another to fit a given set of experimental data, but it is possible that neither of them really corresponds to the underlying mechanisms of the process under study. Nevertheless, it is very important that the expectation function includes all the important predictor variables in precisely the correct form, and that it does not include any unimportant predictor variable.

An important aspect that should be discussed in relation to the model is the functional form used for fitting the data. In kinetic parameter estimation, it is quite common that the models are fitted to experimental data in the form of relative concentrations. According to Arabshahi and Lund (53) this is a common mistreatment of kinetic data, as the initial concentration value is also uncertain, as it is measured experimentally. This may be overcome by increasing the number of replicates in the measurement of the initial concentration, so that its experimental error is minimised. Alternatively, the initial concentration may be considered as an additional model parameter. Significant differences between the estimate and the experimentally determined value may indicate a problem with the model specification.

b. *The response ('the y-value') is the sum of the model function and a disturbance (the experimental errors, ε_i)*

$$y_i = f(x_i, \theta) + \varepsilon_i \qquad (141.7)$$

In this case, the probability distribution of y can be estimated from that of the experimental errors. The requirement is thus that residuals are a measure of random uncertainties in data (63). The lack of additivity of the disturbance will manifest itself as a non-constant variance in the diagnostic plots and the corrective action should be either to use weighted least-squares or to use a transformation of the response (2).

c. *The disturbance is independent of the model function*

This implies that the independent variables have no error; only dependent variables contain experimental error. This is seldom true, but may be accepted as such if the errors in x are much smaller than those in y. In most kinetic studies, the independent variable is time, temperature and possibly initial concentrations. It should be possible to control these variables with good precision (4).

d. *The experimental errors are normally distributed*

This assumption is usually fulfilled because the actual experimental error is composed by many small errors. According to the Central Limit Theorem, the resulting error is normally distributed, even if the contributing errors themselves are not (65). The assumption of normally distributed errors can and should be checked by examining the residuals. Care should be taken when transformations of variables are used. The exponential model serves as a good illustration. The model in the natural (untransformed) variables produces an additive error and the transformed variables from the linearised model present a multiplicative error. The only transformations that do not alter noise distribution are addition/subtraction of or multiplication by a constant (66). Incidentally, a transformation may change a non-Gaussian distribution into a Gaussian one, which is actually desirable. For instance, if the error in a first-order model is multiplicative, the model demands for logarithmisation (67). The work reported by Machado *et al.* (68) clearly shows the benefits of such transformation, applied to heteroscedastic data fitted by the Weibull model.

e. *The average of the experimental errors is zero*

This means that systematic bias should be absent. Systematic errors should be removed by the researcher or incorporated in the model function. Frequent calibration, use of reference materials and participation in ring tests are ways to detect systematic errors (4).

f. The experimental errors have constancy of variance

This assumption, when not valid may be simply overcome, because unequal variances (heteroscedasticity) may be dealt with by using weighted least squares methods (63) or by applying transformations. A quite common case of heteroscedasticity is that of constant coefficient of variation (CV). This means that the standard deviation of y_i increases proportionally with y_i. Heteroscedastic error due to a constant CV can be transformed into homoscedastic by logarithmic transformation. However, care should be taken as logarithmic transformation may violate assumption 4. Assumption 6 implies that it is necessary to have an idea of the magnitude of experimental error and how it varies with the response. The only way to get some idea of the experimental error is to perform independent replications.

g. The experimental errors are independently distributed

This means that experimental errors in one measurement do not depend on those in another measurement, i.e., they are uncorrelated. If this assumption is not true, one can correct by considering the covariances of the experimental responses, but the covariances must be determined. Usually, the assumption of uncorrelated errors can be justified, but in kinetic studies correlation can sometimes occur. For example, a reading may be made on a reaction mixture when the measuring device was still influenced by the previous measurement (4). In that case, the error in a measurement may depend on that in the previous one (serial correlation).

2. Confidence Intervals of the Parameters

In data analysis it is also very important to have a realistic measure of the statistical confidence of the parameter estimates. A confidence interval should be specified, and the probability associated to it should be reported. According to Van Boekel (4) three statements must be reported: (a) the most probable value of the parameter, (b) the confidence interval of the parameter and (c) a probability statement about the confidence interval.

For non-linear models, estimation of confidence intervals is not straightforward, and they are not symmetric; the extent of asymmetry depends on the non-linearity of the function, on the number of data and on the experimental design (6). The problem lies in the fact that the parameters are estimated with regression algorithms based on linear approximations and, consequently, the parameter estimates and their confidence intervals are only as good as the linear approximation. Often the calculated confidence intervals are asymptotic and therefore they may underestimate the real confidence intervals (4).

When a model has more than one parameter, the confidence interval of each parameter does not provide full statistical information, because of the correlation that exists between the different parameters. In this case, it is necessary to analyse the joint confidence regions of the parameter estimates. Although joint confidence regions of parameter estimates are well described in statistical books, they are seldom reported in scientific publications. Actually, their use is very recent (69, 70). For linear-models, the joint confidence region, also called joint-confidence interval, may be calculated using the following expression (2, 6, 63, 65):

$$SSR_{x\%} = SSR_{min} * \left(1 + \frac{p}{n - p} * F_{((100-x)\%,p,n-p)}\right) \tag{141.8}$$

where $SSR_{x\%}$ is the sum of squares of residuals at x% confidence level, SSR_{min} is the sum of squares of residuals of the estimated solution, p is the number of parameters, n is the number of experimental data points and $F_{((100-x)\%,\,p,\,n-p)}$ is the value of the F distribution for p and $n - p$.

According to Haralampu *et al*. (5) the extremes of the 90% joint confidence ellipses correspond approximately to the ends of the 95% individual confidence intervals of the parameter estimates (the joint probability of two events at 95% probability is approximately 90%, i.e., $0.95^2 \approx 0.90$). This is illustrated in Figure 141.2, that relates to the fit of the Bigelow model to thermal inactivation of *Bacillus amyloliquefaciens* α-amylase data (71).

For linear models the region defined by Equation 141.8 corresponds to a contour of the SSR. This is not true for most non-linear models, Equation 141.8 providing only asymptotic results. In non-linear models the contours of SSR are often asymmetric about $\hat{\theta}$ and may be twisted, with the shape of p-dimensional bananas (18). The validity of using Equation 141.8 to calculate joint confidence regions for non-linear models may be assessed by analysing the maximum relative intrinsic curvature of the parameters and the maximum relative parameter-effects curvature. Equation 141.8 provides acceptable results when the maximum relative intrinsic curvature of the parameters and the maximum relative parameter-effects

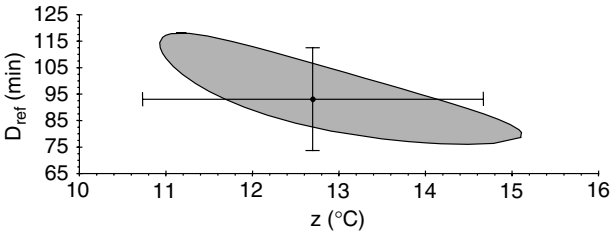

FIGURE 141.2 Asymptotic joint confidence region and individual confidence intervals of the kinetic parameters estimated by fitting the Bigelow model (Equation 141.19) to thermal inactivation of *Bacillus amyloliquefaciens* α-amylase data (From Ref. 71).

curvature are small compared to the confidence region relative curvature. In this situation the solution locus is approximately linear over the confidence region and the parameter co-ordinates projected onto the tangential plane are approximately parallel and uniformly spaced over the confidence region (2).

B. One-Step and Multi-Steps Methods

As earlier referred (Section II-B), when analysing the effect of the experimental conditions on the process kinetics, experiments are often performed at different levels of the factor under study (e.g., temperature). Each experiment allows for estimating the rate constant at a given level of the factor under study and an analysis of the estimates obtained for the different levels allows for identifying how the rate constant depends on that factor. It is therefore important not only to estimate the rate constants, but also the parameters of the model that relates the rate constants to the factor of interest. The estimation of these parameters may be accomplished by two different methods, usually referred to as individual (two-step) or global (one-step) methods. For the sake of simplicity the basic concepts underlying these two methods will be discussed considering temperature as the factor under study, but this discussion may be generalised for other factors.

In the two-steps methods, the rate constants are estimated by fitting the kinetic model to the data gathered at different constant temperatures, this being the first step. In the second step, a model is then identified to describe the dependence of these constants on temperature and its parameters are estimated by fitting this model to the rate constants estimated in the first step. The one-step method consists of combining the kinetic model with the model relating the rate constants to temperature in a single equation, which is then fitted to the whole set of data, directly estimating the parameters of the model that relates the rate constants to temperature. Two-steps (or multi-steps) regression has the disadvantage of applying regression to parameter estimates earlier obtained by regression, thus violating one of the assumptions behind least-squares fitting (assumption 3 in Section III-A-1 — the disturbance is independent of the model function). The two-steps methods also generally result in relatively large confidence intervals of the parameters estimates because of the small number of degrees of freedom (72). Haralampu *et al*. (5) and Cohen and Saguy (57) also showed that parameters estimated by the one-step method were more accurate and precise than those obtained by the two-steps method.

However, as earlier referred, individual fitting of the experiments is required to assess the quality of the fit of the model and to assess the dependence of the rate constants on temperature. Furthermore, this analysis yields estimates that may be used as initial values in the one-step regression procedure, thus accelerating its convergence.

IV. KINETIC MODELS

A. Zero-Order

In zero-order reactions the rate is independent of the concentration. For a degradation reaction:

$$-\frac{dC}{dt} = k \tag{141.9}$$

where C is the concentration, t the time and k the zero-order reaction rate constant, which by integration results in:

$$C = C^i - k \times t \tag{141.10}$$

where C^i and C are, respectively, concentrations at time zero and at any time t.

This may occur in two different situations: when intrinsically the reaction rate is independent of the concentration of reactants, and when the concentration of the reacting compound is so large that the overall reaction rate appears to be independent of its concentration. The second case seems to be the most common situation when this order reaction is observed in food systems (73).

B. First-Order

First-order reaction kinetics is considered by many authors to be an acceptable empirical model for describing chemical and microbiological changes in food processing and storage.

The mathematical expression for a first-order degradation reaction is as follows:

$$-\frac{dC}{dt} = k \times C \tag{141.11}$$

where k is the first-order reaction rate constant. By integration this equation becomes:

$$C = C^i \times e^{-k \times t} \tag{141.12}$$

Although a number of compounds follow this reaction order in a food product, most compounds in a model product will not follow true first-order reaction kinetics but rather, a pseudo-first-order reaction (73). For simplicity sake, pseudo-nth-order reactions are usually referred to as simply nth-order reactions.

C. Second-Order

There are two important types of second-order kinetics: type I, $A + A \rightarrow P$:

$$-\frac{dC_A}{dt} = k \times C_A^2 \tag{141.13}$$

and type II, $A + B \rightarrow P$

$$-\frac{dC_A}{dt} = k \times C_A \times C_B \tag{141.14}$$

where C_A and C_B are concentrations of the reactant species A and B at time t, and k the second-order reaction rate constant. For type I, the integrated expression yields:

$$C_A = \frac{1}{\frac{1}{C_A^i} + k \times t} \tag{141.15}$$

and for type II, it becomes:

$$C_A = C_B \frac{C_A^i}{C_B^i} \times e^{[-(C_A^i - C_B^i) \times k_2 \times t]} \tag{141.16}$$

where C_A^i and C_B^i, C_A and C_B are the concentrations of species A and B at time zero and time t, respectively.

It should be stressed that in the case of type II reactions if one of the components is present in excess the reaction may follow pseudo first-order kinetics with respect to the other component.

D. Dependence of Rate Constants on Temperature

When the effect of temperature is to be assessed, the so-called Arrhenius and Bigelow models are the most common. Both rely on first-order type kinetics.

1. The Bigelow Model

For more than 80 years, the model resulting from the empirical observations made by Bigelow (74) has been the basis for the design of thermal processes used in the canning industry for low acid foods (35). This model is also known as the thermal death time model. It is commonly accepted that at high temperatures and for relatively short times (i) the logarithm of the number of viable cells decreases linearly with time, the slope being the reciprocal of the decimal reduction time (D), and (ii) the logarithm of D decreases linearly with temperature, the reciprocal of the slope being named the z value. The D value at a reference temperature (D_{ref}) and the z value are the basis of the thermal death time method (TDT), which is the current standard in industrial practice for most sterilisation processes (35). Modelling of quality losses in thermally processed foods is also commonly based on a similar kinetic model (e.g., Shin and Bhowmik (51) have used this model to describe kinetics of colour change in pea puree).

The Bigelow model is written in terms of decimal logarithms and of a given temperature (T):

$$\eta_i = \frac{C_i}{C_0} = 10^{-\frac{t_i}{D_r}} \tag{141.17}$$

where η_i is the fractional concentration of colony forming microbial units, or of a quality factor, at time t_i, and D_T is the decimal reduction time at the experimental temperature, T.

$$D_T = D_{T_{ref}} 10^{-\frac{T - T_{ref}}{z}} \tag{141.18}$$

The model has two parameters: $D_{T_{ref}}$ and z, with $D_{T_{ref}}$ being the value of the decimal reduction time at a reference temperature, T_{ref}, that will be further referred to as D_{ref}, and z the thermal death time parameter.

$$\eta_i = 10^{\left[-\frac{t_i}{D_{ref} 10^{\frac{T_{ref} - T}{z}}}\right]} \tag{141.19}$$

2. The Arrhenius Model

For the Arrhenius model natural logarithms are used. Starting with the basic assumption of a first-order model:

$$\frac{dC}{dt} = -k_T C \tag{141.20}$$

Integration leads to:

$$\eta_i = \frac{C_i}{C_0} = e^{-k_T t_i} \tag{141.21}$$

and applying the Arrhenius relationship:

$$k_T = k_0 e^{-\frac{Ea}{RT}} \tag{141.22}$$

This model considers an activation energy (Ea), and an exponential relation of the rate constant (k_0) with the reciprocal of the absolute temperature (K^{-1}) instead of the temperature (°C) used in the Bigelow model. However, the major difference is that this form of the Arrhenius model does not use a finite reference temperature. Hence, k_0 is the reaction rate constant at an infinite temperature, becoming a value with a very large order of magnitude, quite apart from the rate constant values in the range of interest. Some researchers (particularly in chemical reaction engineering and biocatalysis) say that to consider an arbitrary reference temperature is subjective and therefore should be avoided. Besides the generation of values outside and far from the range of interest (usually a narrow temperature range), if an infinite reference temperature is used, the model parameters (k_0 and Ea) will have a very high correlation factor (expressing colinearity), which greatly hinders the regression convergence. This was recognised by Nelson (75), who suggested the use of dummy variables:

$$\delta = (\ln k_0) - \frac{Ea}{R}\beta \tag{141.23}$$

$$\beta = \frac{1}{m} \sum_{j=1}^{m} \frac{1}{T_j} \tag{141.24}$$

where m is the number of temperatures used in the experiments.

Although this was not recognised by this author, this transformation corresponds to using a finite reference temperature, equal to the harmonic average of the temperatures used in the experiments:

$$k_T = k_{ref}\, e^{-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \qquad (141.25)$$

where $T_{ref} = \beta$ and $k_{ref} = e^{\delta}$.

Joint variable-parameter transformations are indeed very common to improve the estimation procedure (2). Frequently transformations aim at centering or scaling the data. The use of a reference temperature is simply the result of considering the model in terms of the reciprocal absolute temperature, rather than temperature, and centering the data on $1/T_{ref}$ (2). This has been used by some researchers (5, 53, 57), but seems not to have been picked up by many food researchers.

If the Arrhenius equation seems to be suitable for describing the temperature dependence, linearisation followed by linear regression for determining Ea and k_{ref} should not be automatic, because errors may not be distributed normally after transformation and the residuals weights are changed. Non-linear regression should be used instead. Also, if the dependence of the rate constants on temperature has been properly assessed, it is better to incorporate Equation 141.25 directly into the rate equations by substituting the right hand side of Equation 141.25 for the rate constant, rather than estimating each k at each temperature studied and subsequently determining Ea and k_{ref} via Equation 141.25 (4). This non-linear regression, often referred to as one-step methodology in opposition to the traditional 2-steps method, has shown not to introduce bias (5). Non-linear least square methods of analysis are thoroughly reviewed by Johnson and Frasier (76).

For the Arrhenius model the use of a reference temperature would yield:

$$\eta_i = e^{\left[-k_{ref}\, e^{\left(-\frac{Ea}{RT}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)} t_i\right]} \qquad (141.26)$$

This allows for a straightforward relation between the Bigelow and the Arrhenius models:

$$k_{ref} = \frac{\ln(10)}{D_{ref}} \qquad (141.27)$$

$$Ea = \frac{R\, T\, T_{ref} \ln(10)}{z} \qquad (141.28)$$

Although Ea and z-values are assumed temperature independent, the two parameters are related to each other trough temperatures T and T_{ref}. Equation 141.28 implies that if Ea is constant over a temperature range, then z cannot be constant in that temperature range and vice versa. Lund (77) suggested that if T is substituted by $T_{ref} - z$, the two models provide similar results over small temperature ranges around T_{ref}. Ramaswamy *et al.* (78) demonstrated that the conversion of z to Ea or vice versa is strongly influenced by the chosen reference temperature and temperature range. According to Hendrickx *et al.* (79), this relationship should be used cautiously, only to estimate the z-value order of magnitude from a known activation energy or vice versa, when raw kinetic data are no longer available.

Several researchers that have directly compared the two models, suggested that both fit experimental kinetic data well (40, 42, 43, 46, 80). Both the Arrhenius and the Bigelow models have merit and have been proven adequate to study degradation kinetics. In spite of the never-ending discussion about the relative merits of each model, both are empirical and the variability of most kinetic data does not allow distinguishing between the quality of the fit of the two models (81).

3. Q_{10} Model

In living systems such as microorganisms, animals and plants, enzymes catalyse most of the reactions. As enzyme activity is highly temperature sensitive, mathematical models have been used in both the food and pharmaceutical sciences to describe how much faster a reaction will proceed if the product is held at some high abuse temperature. If the temperature-accelerating factor is known, then extrapolation to lower temperatures, such as those found in distribution, could be used to predict true product shelf life in those conditions. In the study of food shelf life, this accelerating factor is sometimes called the Q_{10} factor and is defined as:

$$Q_{10} = \frac{\theta_{s(at\ T)}}{\theta_{s(at\ T+10)}} = \frac{k_{T+10}}{k_T} \qquad (141.29)$$

where T is the temperature (°C), and θ_s is the shelf life at the indicated temperatures (82). For any temperature difference Δ, different from 10°C, this becomes:

$$(Q_{10})^{\Delta/10} = \frac{\theta_{s(T_1)}}{\theta_{s(T_2)}} \qquad (141.30)$$

Thus, for example, if the Q_{10} is 3 and the published shelf life θ_s was 6 month at 35°C, the shelf life at 20°C would be:

$$\theta_{20} = \theta_{35} * (Q_{10})^{\Delta/10} = 6 * (3)^{15/10} = 31.2\ months \qquad (141.31)$$

In many cases, the Q_{10} is not given, so one must establish the mathematical relationship (83), which is done by means of kinetic modelling.

E. The Weibull Model

The Weibull distribution is one of many probability distribution functions used to describe the behaviour of systems or

events that have some degree of variability. It was named after the Swedish physicist, Waloddi Weibull, who first used it to represent the distribution of the breaking strength of materials (84, 85) and later for a variety of other applications (86). The close agreement that Weibull demonstrated between observed and predicted data was quite impressive (87).

The probability density function of the Weibull distribution may be described as (67):

$$f(t) = \begin{cases} \frac{\beta}{\alpha}\left(\frac{t}{\alpha}\right)^{\beta-1} e^{-\left(\frac{t}{\alpha}\right)^{\beta}}, & t > 0 \\ 0, \text{ elsewhere} \end{cases} \quad (141.32)$$

with $\alpha > 0$ and $\beta > 0$. The corresponding cumulative distribution is:

$$F(t) = \int_0^t f(t)\,dt = 1 - e^{-\left(\frac{t}{\alpha}\right)^{\beta}} \quad (141.33)$$

This model has an interesting potential for describing ecological, microbial, enzymatic and chemical degradation kinetics (failure of the system after a given time subjected to stress conditions), reliability and life testing (time to failure or life length of a component), considering the scale parameter (α) as a time constant (the reciprocal of the rate constant) and the shape parameter (β) as a behaviour index. The model reduces to a first order decay/growth kinetics for $\beta = 1$ (see Figure 141.3a). As discussed by Hahn and Shapiro (88), Nelson (89), Gacula and Kubala (90) and Johnson *et al.* (87), the failure rate for the Weibull model is an increasing function of time for $\beta > 1$ and a decreasing function for $\beta < 1$. When $\beta = 1$, the failure rate is constant. These authors also emphasise that α is a characteristic time to failure, as it corresponds to the $100 * (1 - 1/e) = 63.2$ percentile of the distribution, regardless of the value of β (see Figure 141.3b).

As a time parameter, α is temperature sensitive and $1/\alpha$ often follows an Arrhenius-type behaviour (91–93):

$$\frac{1}{\alpha_i} = \frac{1}{\alpha_{ref}} e^{\left[-\frac{Ea}{R}\left(\frac{1}{T_i} - \frac{1}{T_{ref}}\right)\right]} \quad (141.34)$$

The β parameter may be expected to be related to the kinetic pattern and thus to be temperature independent, at least within a limited range of temperature. This parameter gives the model a wide flexibility, making it a potential good model to describe different reaction kinetics. This has been verified in published works (92–94). Equations 141.33 and 141.34 may be combined to yield:

$$\eta_i = e^{\left\{-\left(\frac{t_i}{\alpha_{ref}} e^{\left[-\frac{Ea}{R}\left(\frac{1}{T_i} - \frac{1}{T_{ref}}\right)\right]}\right)^{\beta}\right\}}, \quad i = 1, 2, 3 \quad (141.35)$$

Therefore the kinetic parameters of the model are α_{ref}, Ea and β.

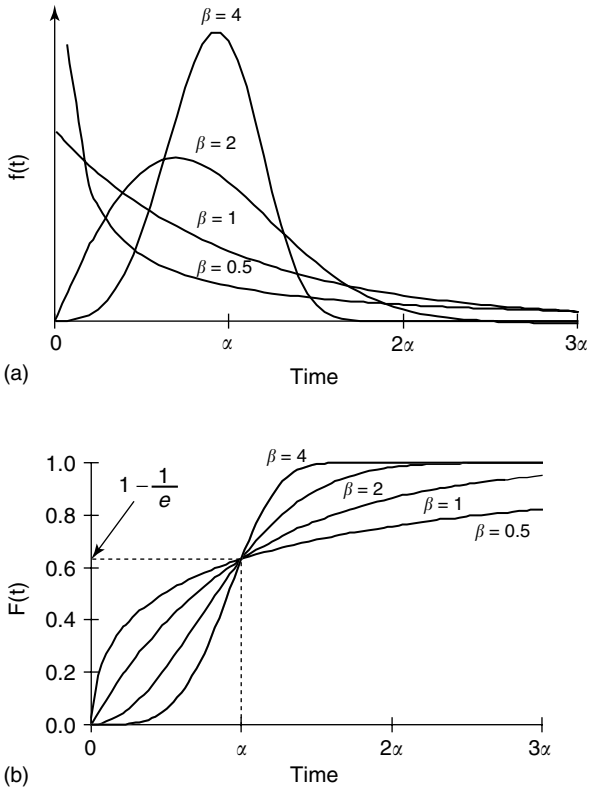

FIGURE 141.3 Effect of the shape parameter β on (a) the Weibull probability density function — f(t), (b) the Weibull distribution function — F(t).

The Weibull distribution was successfully applied to describe shelf-life failure (90, 95). Page (96) developed an equation to describe thin-layer drying of shelled corn, similar to the Weibull probabilistic model that was later used to model thin-layer drying of yellow corn (97, 98), of soybeans (99), of pigeon pea (91) and of adzuki beans (100). This model was also used to describe the drying kinetics of rough rice (101–103) and of Thompson seedless grapes (104). Chhinnan (94) evaluated four mathematical models, i.e., the exponential model, the diffusion model, an approximation of the diffusion model and the Page (Weibull) model, for describing thin-layer drying of in-shell pecans, recommending the Page (Weibull) equation for future modelling studies. Moreover, the Weibull distribution has been used to model failure of apple tissue under cyclic loading (105), grape pomace elutriation (106) and water uptake and soluble solids losses during rehydration of dried apple pieces (92). The Weibull model adequately described moisture uptake and soluble solids losses by breakfast cereals immersed in water (107) or milk (68), after unsuccessful attempts to use the Peleg (108), the diffusion and the first-order models (93). The Weibull model has also been used to describe microbial death kinetics under high-pressure conditions (109), enzyme inactivation under high pressure (110), isothermal heat-resistance of *Bacillus cereus* (111, 112), and shrinkage of potato during frying (113).

Van Boekel (114) made a systematic review of the use of the Weibull model to describe thermal inactivation of microbial vegetative cells (data published for different microorganisms). α and β were calculated from 55 sets of data and it was found that β = 1 in only two cases, which pinpoints the fact that the use of first-order kinetics to model microorganisms death should be the exception rather than the rule.

F. The Logistic Model

The Logistic model is another example of model used to represent growth rate that does not steadily decline, but increases to a maximum before declining to zero. This is shown in the growth curve by an S-shaped, or sigmoidal, pattern.

$$\frac{df}{dx} = \frac{k}{\alpha} f(\alpha - f) \qquad (141.36)$$

where $k > 0$ and $0 < f < \alpha$ (6).

Thus from Equation 141.36 the relative growth rate $f^{-1}df/dx$ decreases linearly in f until f approaches α. Equation 141.35 has a general solution, which can be written as

$$f(x) = \frac{\alpha}{1 + e^{-k(x-\gamma)}} \quad -\infty < x < \infty \quad (141.37)$$

the so-called logistic model (6).

Equation 141.36 is an empirical model derived by Verhulst in 1838 to describe growth in the size of a population or organ. This equation also describes the rate of consumption of a monomolecular chemical substance as it breaks down in an autocatalytic reaction.

Equation 141.37 has asymptotes $f = 0$ as $x \to -\infty$ and $f = \alpha$ as $x \to \infty$. These asymptotes are never actually attained, and that causes modelling difficulties as at time at which growth begins to be monitored $f > 0$. The maximum growth rate is $w_M = k\alpha/4$, and the growth rate is symmetrical about $x = \gamma$. As k acts as a scale parameter it influences the growth rate (6).

Probably the most common parameterisation is

$$f(x) = \frac{\alpha}{1 + \beta e^{-kx}} \quad -\infty < x < \infty \quad (141.38)$$

where $\beta = e^{k\gamma}$ in Equation 141.37.

Bartali *et al.* (115) used multiple logistic models to evaluate whether inadequate intake of selected nutrients could be predicted by nutrition-related difficulties of persons living in two towns of Tuscany (Italy). Jenkins *et al.* (116) described the growth of *Zygosaccharomyces bailii* in acidified products with log-logistic models for time to growth and probability of growth, which is useful for spoilage risk assessment.

G. The Gompertz Model

The Gompertz model is often used for population and animal growth in situations where growth is not symmetrical about the point of inflection. The growth rate is

$$\frac{df}{dx} = kf(\log\alpha - \log f) \ (k > 0, \alpha > 0) \ (141.39)$$

and the relative growth rate declines with log(size). From Equation 141.39,

$$f(x) = \alpha \times e^{\{-e^{[-k(x-\gamma)]}\}} \qquad (141.40)$$

The point of inflection for Equation 141.40 is at time $x = \gamma$ with $f = \alpha/e$, at which point the maximum growth rate, $w_M = k\alpha/e$, occurs (6). For the Gompertz curve log f is monomolecular. The Gompertz function has the property that any power of f is Gompertz. This means that several measures of size such as weight, surface area and length may all provide growth curves of the same shape.

Huang (117, 118) developed a new methodology to describe and classify accurately the bacterial growth as a process using *Clostridium perfringens* as a test organism. He used the Gompertz model to calculate the equivalent growth times under different temperatures and estimated the bacterial population under temperature fluctuations. The model was validated in ground beef maintained under fluctuating temperature conditions.

H. The Michaelis-Menten Model

The theory of enzyme-substrate complex formation, to describe enzyme kinetics was proposed by Michaelis and Menten (119). This is depicted in the following reaction scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P \qquad (141.41)$$

where E is the enzyme, S the substrate, ES the enzyme-substrate complex and P the product. The rate constants for the forward and reverse reactions are k_1, k_2 and k_{-1}, k_{-2}, respectively (120). Both reactions are reversible in general case.

A characteristic of enzyme-catalysed reactions in food is that when the substrate concentration is very small, the forward reaction to form ES is first-order in S, and at very large substrate concentration, this same reaction is zero-order in S as the reaction rate is only dependent on the concentration of enzyme.

Michaelis and Menten (119) applied the steady-state kinetic analysis to Equation 141.41 and derived the following equation to describe the velocity of an enzyme-catalysed reaction:

$$v = -\frac{dS}{dt} = \frac{dP}{dt} = \frac{V_{max}[S]}{K_m + [S]} \qquad (141.42)$$

where v is the velocity, V_{max} is the maximum velocity when the enzyme is saturated with substrate, K_m is the substrate concentration at which $v = 0.5V_{max}$ and $[S]$ is

the substrate concentration at any time t. In this analysis, k_{-2} is assumed to be zero. Most kinetic studies on enzymes are completed at a very small conversion ratio (<5%) in order to remove complications that can take place when substrate concentrations decrease or product concentration increases.

Determination of K_m and V_{max} may be obtained by linearising Equation 141.42, which is done taking the reciprocal of both sides of Equation 141.42:

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \times \frac{1}{[S]} \qquad (141.43)$$

A plot of $1/v$ versus $1/[S]$ results in a straight line with intercept $1/V_{max}$ and slope K_m/V_{max} (121), commonly known as the Lineweaver-Burk equation.

One mechanism of inactivating/controlling the activity of enzymes is the use of inhibitors, compounds that interact with the enzyme reducing its activity. Inhibitors can be reversible or irreversible. Reversible inhibitors may compete in three different ways (120):

a. **competitive** inhibition occurs when the inhibitor binds reversibly with enzyme competing with the substrate for active sites. Slope and y intercept values for the Lineweaver-Burk equation are $\left[1 + \frac{I_0}{K_i}\right]\frac{K_m}{V_{\max}}$ and $\frac{1}{V_{\max}}$, respectively, I_0 being the concentration of the inhibitor and K_i the inhibitor-enzyme complex dissociation constant.

b. **noncompetitive** inhibition occurs when the inhibitor does not compete with the substrate for binding the enzyme but rather both the inhibitor and the substrate can bind to the enzyme simultaneously. Slope and y intercept values for the Lineweaver-Burk equation are $\left[1 + \frac{I_0}{K_i}\right]\frac{K_m}{V_{\max}}$ and $\left[1 + \frac{I_0}{K_i}\right]\frac{1}{V_{\max}}$, respectively.

c. **uncompetitive** inhibition occurs when the inhibitor can bind to the enzyme-substrate or one or more of the intermediate complexes. Slope and y intercept values for the Lineweaver-Burk equation are $\frac{K_m}{V_{\max}}$ and $\left[1 + \frac{I_0}{K_i}\right]\frac{1}{V_{\max}}$, respectively.

A more complex reversible inhibition can occur when the inhibitor binds to the multi-subunit enzymes. Whitaker (122) presents an analysis of complex kinetics, which arise when this kind of inhibition takes place.

Irreversible inhibitors form covalent bonds with enzyme, enzyme-substrate or enzyme-product complexes. Kinetically, irreversible inhibitors can be treated through rate equations and not through Lineweaver-Burk or Michaelis-Menton equations. The effect of temperature on enzyme activity and inactivation is usually treated by the Arrhenius equation (Equation 141.22).

V. CONCLUSIONS

When dealing with kinetics of quality and safety indicators, many different models may be considered; it was the authors' options to present a few of the most representative ones.

This chapter intends to alert the less experienced researcher or student to some of the basic concepts to bear in mind when studying their particular food system, from the experimental design to the data collection and analyses.

The topics here presented will be further developed in subsequent chapters of this handbook.

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142 Kinetics of Quality and Safety Indicators Under Steady Conditions — Shelf Life of Foods

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CONTENTS

I. INTRODUCTION

Shelf life may be defined as the period of time during which the food product will remain safe, be certain to retain desired sensory, chemical physical (1) and microbiological characteristics, and comply with any label declaration of nutrition data, when stored under recommended conditions. The dependence of the kinetics of microbiologic growth/death or of nutrient loss on environmental factors varies from product to product and process to process and must, therefore, be established for each specific case to be evaluated. It is the responsibility of the food manufacturer (together with the retailer for their own label products) to determine shelf life of food.

For forecasting shelf life of a product it is imperative to select adequate shelf life indicators. Shelf life indicators are microorganisms or compounds that have a notorious influence on the product safety and on quality (nutritional value, appearance or flavour characteristics). Critical shelf life indicators are those that first reach the respective threshold levels and thus limit the product shelf life.

The food safety concept is linked with the food preservation by heat, eliminating and reducing to an acceptable level pathogen and spoilage microorganisms, respectively. But this concept cannot be extrapolated to minimally processed foods, as the preservation techniques (non-severe thermal treatments, i.e., up to 100°C) used in these methods

are not directly focused on the total inactivation of the microbial population present in the unprocessed material. Therefore the microbial safety of minimally processed food products, during their shelf life, depends on refrigeration that should prevent the growth and/or toxin production of microorganisms hazardous to the consumer health.

II. SHELF LIFE OF FOODS

There are different types of shelf life studies, in respect to the purpose they serve while the product is being developed:

- Initial shelf life studies (ISLS) are carried out once the concept of a new product has been agreed;
- Preliminary shelf life determinations (PSLD) are the first shelf life determination carried out to the whole product (including package);
- Confirmatory shelf life determinations (CSLD) are carried out at the end of the product development process;
- Routine shelf life determinations (RSLD) are conducted so that the shelf life of any existing product can be monitored and to check if it is still correct for different environmental conditions to which it can be subjected.

The exact test is often product-specific and is selected to measure the end-point of the shelf life. Because these tests are highly expensive (either in house or contracted tests) it is critical that companies use the most cost effective tests in their shelf life studies. Tests may involve one or a combination of sensorial, microbiological, chemical and physical determinations.

It should take in consideration scaling-up factors (any fluctuations that might occur in for a given product formulation), safety and quality aspects.

The final shelf life should be set to give a clear margin of safety, and particularly in the case of a high risk category product a date should be set based on data that relate to the "worst case" manufacturing and storage scenario.

A. Methods for Shelf Life Estimation

Shelf life estimation can be done by means of direct or indirect methods. Direct methods are usually used for products with short shelf life, as shelf life determination is based on monitoring quality factors, chemical and/or microbiological, in a situation that exactly replicates the real conditions of packaging, storage and distribution (2).

When the product shelf life is expected to be relatively long, it is possible that the product may be launched before any confirmatory shelf life determination can be completed. Because of this and economic aspects, it is highly desirable that faster methods for predicting shelf life are available. These indirect methods are generally based on the principles of accelerated shelf life determination. Accelerated tests are done exposing the food product to abusive storage conditions, i.e., high temperature, high oxygen content, or others (humidity, light, etc.) relevant to the deteriorative reaction mechanism involved (1). Accelerated tests in complex food systems present a problem because published kinetic data is scarce, contradictory or even unavailable. Therefore, accelerated tests must be developed in the face of many unknowns. In this respect, foods present more difficult problems than other chemical systems.

Accelerated tests have been used mostly for dried products. A number of reviews on accelerated tests may be found in the literature (3–8).

B. Accelerated Tests for Shelf Life Estimation

1. Accelerated Tests at Elevated Temperatures

Typically foods are subjected to storage at 37 and 51°C, and various correlations (usually based on the Arrhenius relation or the Q_{10} model) are used to extrapolate the results to the expected storage temperatures (6). When more accuracy is desired, several elevated storage temperatures (usually 5 to 6) are used and the Q_{10} value or activation energy is determined experimentally, based on the assumption that they are independent of temperature. However, this is often not the case, leading to erroneous extrapolation. Usually reaction rates are more sensitive to temperature in the range used in accelerated tests, thus implying that shelf life may be overestimated, which causes a major problem to food producers.

2. Accelerated Tests at High Oxygen Pressures

Conducting stability tests at high oxygen pressures can sometimes accelerate reactions involving oxidation. The potential for acceleration is not very great because oxidation reactions typically become independent from the oxygen concentration above some critical concentration level, which varies with temperature and other conditions (6). Nevertheless, traditional accelerated tests for oxidation-susceptible oils use exposure to high oxygen pressures to assess the relative stability of such oils.

One aspect on ASLT, which calls for certain precautions, is the change in the water solubility of oxygen by temperature. In oxygen sensitive products, this has to be corrected for by the use of increased partial oxygen pressure at elevated test temperatures.

C. Predictive Food Microbiology

Predictive food microbiology is an emerging multidisciplinary area of food microbiology. It covers different areas such as mathematics, microbiology, chemistry and engineering to apply mathematical models to predict microbial growth and/or toxin production to specified environmental

variables in order to replace traditional time-intensive challenge studies.

The need to ensure the microbiological safety and quality of increasingly complex food products has stimulated interest in the use of mathematical modelling to quantify and predict microbial behaviour. Buchanan (9) goes through models used in predictive microbiology coupled with 'user-friendly' applications software and the development of expert systems. These applications provide powerful new tools for rapidly estimating the effects of formulation and storage factors on the microbiological relations in foods.

Cheftel (10) reviews the effect of high-pressure on microbial inactivation and food preservation, as it affects biological constituents and systems.

McDonald and Sun (11) reviewed predictive microbiology for the meat industry, with shelf life predictions focusing on *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Heldman and Newsome (12) gathered information and summarized plenary presentations of the IFT second Summit (January, 14–16, 2003) relating to the understanding of microbial inactivation kinetics and models for non-log-linear survivor curves (kinetic models) and identified needs for future research.

III. ASPECTS THAT FIX LIMITS OF FOOD SHELF LIFE AND KINETIC OF NUTRIENT LOSSES — DEPENDENCE ON ENVIRONMENTAL FACTORS AND FOOD COMPOSITION

In the following sections, a brief discussion of kinetics of microbial survival and of the mechanisms of deterioration of nutrients (ascorbic acid, browning and some off-flavours) is presented, with reference to relevant studies reported in literature.

A. Microbiology

The food safety crisis that appeared in the late nineties has made consumers more concerned about food security. Guaranteed food safety is now a priority in the policies of most developed countries. Although the use of mathematical models in food microbiology is not new, developing predictive mathematical models has gained recognition because of its usefulness as a tool that helps ensure food safety. In this context, the main objective of predictive microbiology is to act as a fast microbiological method for controlling changes during processing and predicting the consequences of food storage from a microbiological viewpoint. These predictions are very useful when making decisions under uncertain conditions and when developing risk assessment systems.

As a result of social changes in modern society, consumer demand for fresh-like products is growing throughout the world. Processing products of this kind involves the use of mild heat treatments accompanied by other techniques such as acidification or refrigeration after pasteurisation in order to reduce damage to the products' sensorial and nutritive properties. These preservation conditions have allowed the appearance of new emerging pathogens such as *Bacillus cereus*, spores of which are not totally inactivated by these combined techniques, and surviving spores of this pathogen can grow under refrigeration conditions.

Geeraerd *et al.* (13) thoroughly compare the performances of several models (two models from Casolari, Sapru model, Whiting model, Baranyi model, Chiruta model, Daughtry model, Xiong model and a new model developed by the authors of the paper) to deal with the typical non-loglinear behaviour of survivor curves (*Bacillus cereus*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Lactobacillus sake*) occurring during mild heat treatment of sous vide or cook-chill food products. As the thermal treatment is not as intensive as canning, besides the reduction of pathogenic and spoilage vegetative cells there may occur growing of surviving thermo resistant microorganisms or of their spores, during the later stages of production, storage and distribution. Some models, such as the modified Gompertz growth model, were excluded from this study. Authors explain the requirements and limitations of each model, and conclude that both Baranyi and the author's model fulfill the requirements necessary to describe accurately microbial inactivation during a mild heat treatment.

Despite documented evidence that specific pathogens are capable of survival in fruit juice, until recently it was widely accepted that most low pH/high acid foods were of minimal concern for food poisoning outbreaks. This was based on the knowledge that organic acids have an inhibitory and sometimes microbicidal effect on many bacteria (14). Outbreaks of food poisoning from *Salmonella* and *E. coli* O157:H7 were traced to nonpasteurised orange and apple juices, respectively (15–17).

Many studies have been carried out to model the combined effect of temperature and acidification on the thermal resistance of various strains of microorganisms (18–21). However, models that include parameters with biological significance are scarce. Gaillard *et al*. (22) and Mafart and Leguerinel (23) developed various mathematical models that took into account parameters with biological significance assuming a linear logarithmic relationship between the number of surviving microorganisms and the treatment time.

The Weibull model is gaining increasing importance in microbiology to fit non-linear death curves. Various researchers agree that one valid way of overcoming the lack of fit motivated by the presence of shoulders and tails

in survival curves (24) when describing complex microbiological inactivation kinetics is to use the Weibull distribution (19, 24–31). The effect that external factors such as temperature, pressure or pH have on the parameters of the Weibull model has been studied (19, 24, 27, 28, 32).

Bacillus cereus is a ubiquitous spore. Its presence has been reported in many products, like nuts, dairy products, vegetables, meat, spices and others (33). It is considered a food contaminant, as it is capable of causing an emetic or diarrhoeal syndrome because of its ability to produce two types of toxin (34). Fernández *et al.* (35) developed a model with 4 parameters to describe the effect of temperature and pH on the inactivation of *Bacillus cereus* in vegetable substrate, using parameters (Ea and k) that are common in food engineering or food chemistry but not in microbiological studies. As a continuation of this study, Collado *et al.* (36) developed a model based on the Weibull distribution to describe the combined effect of pH and temperature on the heat resistance of *Bacillus cereus* in carrot juice. It was shown that temperature and pH did not have a significant effect on the shape parameter (β), and the effect of temperature on the scale parameter (α) was modelled by the z concept.

Van Boekel (31) thoroughly studied the application of the Weibull model to describe the thermal inactivation of microbial vegetative cells. He used 55 sets of data (for different microorganisms) taken from the literature and obtained the parameters of the Weibull model. The shape parameter had very different values for the different cases over the temperature range studied. It was found that β equalled one only in two cases, indicating that the classical first-order kinetics approach is the exception rather than the rule. It was concluded that the Weibull model can be used to model nonlinear survival curves, and may be helpful to pinpoint relevant physiological effects caused by the heat.

Shelf stability of some 100% fruit juice products stored at room temperature is maintained by the combination of a heat process and low pH. The high acidity ($pH \leqslant 4.5$) limits the types of microorganisms that can grow in the juice, yet several have been isolated from orange juice (acid-tolerant bacteria, yeasts and moulds) although few of them can cause spoilage (pathogenic) (37). Therefore, in aseptically processed and packed food, microbial growth does not set the storage limit of the product, unless erroneously processed.

Saccharomyces cerevisiae, *Rhodotorula sp*. and *Zygosaccharomyces sp*. are the most common yeasts present in juice (38). *Saccharomyces cerevisiae* is the yeast most commonly associated with spoilage of pasteurised citrus juices. This organism produces an alcoholic fermentation resulting in a fermented off-flavour due to the presence of ethanol and carbon dioxide. Yeasts not capable of alcoholic fermentation may cause turbidity, flocculation and clumping in juice. *Zygosaccharomyces* is an osmophilic yeast and species of this yeast can readily survive the high osmotic pressures and low water activity of frozen concentrated orange juice (39) and it is frequently associated with spoilage of concentrates (38). The presence of *Rhodotorula* may be indicative of poor post-pasteurisation hygiene.

Moulds that have been isolated from orange juice include *Aerobasidium pullulans*, *Aspergillus niger*, *Botrytis sp. Fusarium sp.*, *Geotrichum sp.*, *Muccor sp. Aspergillus fumigatus*, *Cladosporium sp.* and *Penicillium sp.* Moulds are usually of small concern in processed citrus products because of their slow growth rates (40) and although they grow well in acid media, they require abundant oxygen and are generally sensitive to heat treatment and thus easily destroyed by pasteurisation. However isolated cases of mould spoilage by *Penicillium sp*., *Aureobasideum pullulans* and *Cladosporium sp.* occasionally occur (40). With the introduction of long-term chilled storage of single strength orange juice in nonaseptic cartons with oxygen barriers, mould growth in citrus juice has become a more important issue (38).

Acid and acidified foods ($pH \leqslant 4.5$) generally are not heat processed sufficiently to destroy all bacterial spores because that process (sterilization) may adversely affect the quality of the product and is not necessary as most spores will not germinate and grow in such products. The major bacterial spoilage agents in citrus juices are *Lactobacillus* and *Leuconostoc* species (also termed lactic acid bacteria). These organisms are responsible for production of diacetil, which imparts an undesirable buttery flavour to juice (41). *Leuconostoc* and many species of *Lactobacillus* also produce large amounts of CO_2. A thermo-resistant spore-forming acidophilic bacteria was isolated from shelf-stable juices, named *Bacillus acidoterrestris* (42), and later reclassified in a new genus called *Alicyclobacillus* (43). *Alicyclobacillus* are of concern as they can germinate, grow and cause spoilage of products with a pH previously considered below the range of spore-forming bacteria (44). This contamination is introduced into the manufacturing process through unwashed or poorly washed fruit, and it causes a flat sour type of spoilage (no gas production was observed), producing an offensive-smelling compound, guaiacol, and other taint chemicals in orange and apple juices and juice-containing drinks (45). Splittstoesser and co-workers (46) also reported the detection of guaiacol in apple juice contaminated with *Alicyclobacillus*. The worldwide occurrence of alicyclobacilli in non-spoiled fruit juices and rapid growth cycles culminating with sporulation of cells, strongly indicate that spoilage of fruit juices by alicyclobacilli is incidental, requiring a combination of adequate conditions for growth of the organisms in the juice, such as low pH and high temperatures for long periods of time (47). A response surface model was developed to describe the effects of temperature (35 to 55°C),

pH (3.5 to 5.5) and water activity (a_w, 0.960 to 0.992) on germination of *Alicyclobacillus acidoterrestris* spores by Sinigaglia *et al.* (48), was validated with data not used in its development, and results indicated that the model provided reliable predictions of growth of spores.

B. Nutrient Loss

Nutrient loss in juice products is essentially synonymous with vitamin loss. Since consumers derive major vitamin benefits from citrus juices, factors that impact on vitamin retention are of considerable importance (39).

Vitamins can be grouped according to whether they are soluble in water or in nonpolar solvents. The water-soluble vitamins are vitamin C and a series known as vitamin B complex.

Ascorbic acid (vitamin C) is the one of the most important nutrients in orange juice. Some other fruits contain more ascorbic acid than oranges, such as kiwi, strawberries and blackcurrant, but fewer are as popular. Ascorbic acid is essential for the synthesis of collagen, the most abundant protein in mammals, and a lack of this vitamin leads to scurvy (49).

Ascorbic acid is a natural antioxidant and its loss in juices is closely related to the availability of oxygen in packages. The loss of this vitamin in processed citrus juices is due to aerobic and anaerobic reactions of nonenzymatic nature (39) or through catalysed or uncatalysed aerobic pathways (50). Several researchers studied ascorbic acid decomposition, and several studies focused on the influence of oxygen, packaging systems and other chemicals on ascorbic acid contents. It is possible that the various mechanisms of deterioration can operate simultaneously. Which one predominates depends on the storage temperature and on the availability of oxygen, thus highly complicating the treatment of kinetic data (50).

The anaerobic pathway is independent of oxygen (as the name implies) and is mainly driven by the storage temperature. Losses caused by this pathway cannot be prevented by package type (38), lowering the storage temperature is the only way to minimise its rate (51). This mode of degradation is prevalent in hermetically sealed packages. Under anaerobic conditions ascorbic acid decomposes to 2,5-dihydro-2-furoic acid, 3-deoxy-D-pentosone, carbon dioxide and furfural (39, 52, 53).

The aerobic pathway needs oxygen and is therefore related to the presence of headspace oxygen, dissolved oxygen and the oxygen-barrier properties of the package (38). In cases where permeation of oxygen into a package is considerable, headspace oxygen is present or oxygen is dissolved in the product, the contribution of anaerobic degradation to the total vitamin C loss is small compared to aerobic degradation (50).

The scheme shown in Figure 142.1 helps to visualise the influence of oxygen and heavy metals on the route and products of degradation reaction.

In the presence of oxygen, ascorbic acid (H_2A) is degraded primarily via its monoanion (HA^-) to dehydroascorbic acid (A), involving the formation of a metal anion complex, which combines with oxygen to give a metal-oxygen-ligand complex ($MHAO_2^{(n-1)+}$). The exact pathway and overall rate is a function of concentration of metal catalysts ($M^{n+} = Cu^{2+}$, Fe^{3+}) in the system. The

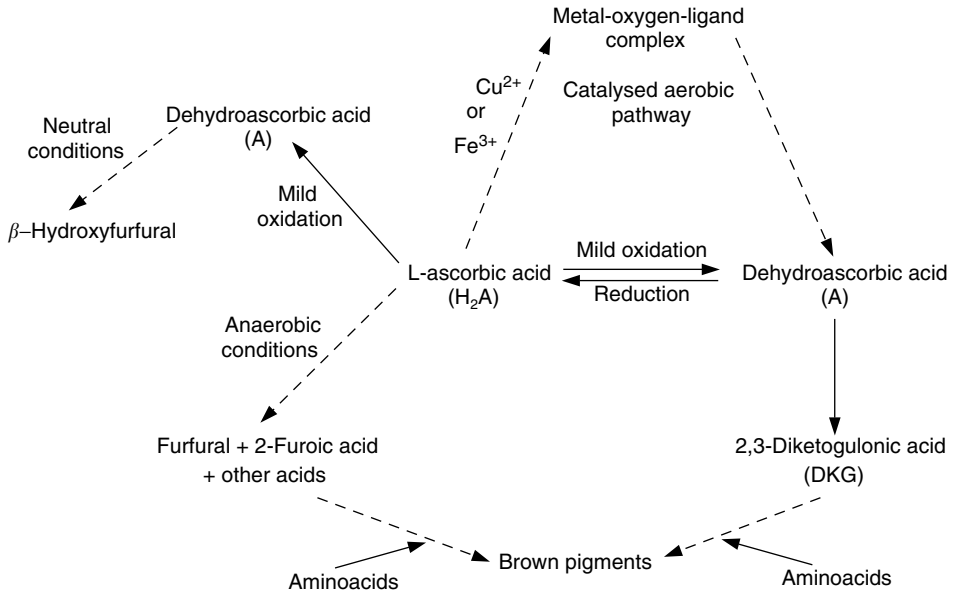

FIGURE 142.1 Schematic representation of degradation pathways of ascorbic acid. Adapted from Tannenbaum *et al.* (55) and Villota and Hawkes (50). Dashed arrows represent simplification of several distinct reactional steps, here grouped as a single one for simplicity sake (from Manso (54)).

rate of formation of dehydroascorbic acid is approximately first-order with respect to $[HA^-]$, $[O_2]$ and $[M^{n+}]$. When the metal catalysts are Cu^{2+} and Fe^{3+}, the specific rate constants are several orders of magnitude greater than the spontaneous oxidation, and therefore even a few parts per million of these metals may cause serious losses of vitamin C in food products (55).

In the non-catalysed oxidative pathway the ascorbate anion (HA^-) is subjected to direct attack by oxygen in a rate-limiting step, to give first the radical anions ($A^{\bullet}$) and ($H_2O^{\bullet}$), followed rapidly by formation of dehydroascorbic (A) and H_2O_2. Thus the catalysed and uncatalysed pathways have common intermediates and are undistinguishable by product analysis. Since dehydroascorbic acid is readily reconverted to ascorbate by mild reduction, the loss of vitamin activity comes only after hydrolysis of the lactone to form 2,3-diketogulonic acid (DKG).

Under anaerobic conditions ascorbic acid degradation yields furfural, 2-furoic acid and other acids (50). The maximum degradation occurs at approximately pH 4, followed by a decrease upon a decrease in pH to 2, followed by a rate increase with an increase in acidity (53).

Many researchers studied ascorbic acid degradation kinetics and factors that affected its rate in food or model food products. Table 142.1 presents a summary of some published studies, stressing the most important findings.

Most studies report L-AA degradation to follow first-order kinetics (56–64). These studies cover a wide range of products (e.g., model food systems, fruit juices, peas, cereals) and conditions (anaerobic, aerobic non-catalytic and aerobic catalytic), as well as different processes (e.g., thermal processing, concentration) and storage in different packages. In many cases the retention of ascorbic acid in the conditions and times tested is relatively high, what may explain the adequability of this simple mathematical model to describe L-AA degradation.

A smaller number of researchers report L-AA degradation to follow zero-order kinetics (65, 66) or second-order kinetics (67–69). Kanner *et al.* (70) reported different reaction orders below 25°C (first-order) and above 37°C (different from first-order). More complex mechanisms have been suggested to describe L-AA degradation by Vieira *et al.* (71), Sakai *et al.* (72), Hughes (73), Lee *et al.* (74), Singh *et al.* (69) and Khan and Martell (75, 76). Manso *et al.* (77) presented a study where a first-order, the Weibull model and a mechanistic model were used to describe the L-AA degradation in orange juice under aerobic conditions, and showed that first-order models could describe well for relatively low conversions (retentions $\geqslant$80%), but if the reaction was followed for longer times, up to total ascorbic acid loss, then it would be preferable to use the Weibull model for predictive purposes.

The dependence of the rate constants on temperature is reported to follow an Arrhenius-type equation. Nagy and Smoot (57) reported two distinct Arrhenius profiles, with a critical transition region between 22 and 26.7°C.

Hsieh and Harris (78) reported that the mechanism of L-AA degradation in canned orange juice depends on oxygen concentration, being different above and below 2 ppm, and Kennedy *et al.* (79) observed that after the dissolved oxygen in juice packed in TB cartons reaches an equilibrium, L-AA decomposition occurs independently of oxygen.

L-AA degradation during storage of lemon juice protected from light was reported to be independent of initial oxygen contents (61).

Similar results found by Singh *et al.* (69) in infant formula protected from light, whereas in samples exposed to light increased initial oxygen led to faster L-AA degradation.

The vitamin B complex group is composed of thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin, a group comprised of pyridoxine, pyridoxal and pyridoxamine (vitamin B_6), pantothenic acid, biotin, folic acid and cobalamin (vitamin B_{12}). All B complex vitamins have been detected in citrus juice, except for vitamin B_{12} (39).

Folic acid is the one found in more significant amounts (38). This vitamin is very important as it is required for DNA synthesis and its deficiency is first expressed in tissues with high rates of cell turnover (49).

Limited studies have been conducted on the stability of the B complex vitamins in citrus juices (80). Folic acid is quite heat-sensitive, but the vitamin C in citrus juices protects it from degradation during heat treatment (38). Davey and Cerf (81) presented a mathematical model to predict the concomitant denaturation of vitamin during sterilization as influenced by combined temperature and liquid pH over a range of exposure times in batch thermal processing. The procedure was illustrated using published data for denaturation of thiamine (vitamin B-1) and ascorbic acid (vitamin C).

C. Colour Stability

Citrus juices colours are primarily due to carotenoids present in plastids located in the juice vesicles (82, 83). Citrus carotenoids are C-40 compounds that can exist as either hydrocarbon carotenes or oxygenated xanthophylls and are primarily responsible for the red, orange and yellow pigments. The main carotenoids in orange juice are α-carotene, β-carotene, zeta-carotene, α-cryptoxanthin, β-cryptoxanthin, antheraxanthin and violaxanthin (84). Juice colour is an important standard for determining quality of orange juice (84). Legally, blending with more highly coloured juice is the only method for enhancing 100% fruit juice colour (85).

Carotenes are relatively unstable and must be protected from oxygen, and stored under refrigerated conditions (86). The colour of processed red and pink grapefruit

TABLE 142.1
Kinetic Studies of Ascorbic Acid Degradation, Nonenzymatic Browning and of Flavour (HMF, Furfural and PVG) Formation

Food/Model System	Package/Storage Conditions	Compounds Under Study	Other Compounds	Compounds/Factors Affecting Degradation	Atm. Cond.	Applied Kinetic	Retention (%)	Fit Quality	Observations	Ref.
Aqueous solution	2 temperatures (T): 0.4, 25°C 7 O_2 contents: 11.1, 8.9, 6.7, 4.5, 2.3, 1.1, 0.4 ppm 9 pH levels: 2–5.5	Ascorbic acid (H_2A)		Cu(II) Fe(III) Temperature Oxygen pH	Aer./ Anaer.	• Spontaneous Oxidation - First order, in relation to HA^- - [0.40; 1.0] atm O_2: $-\frac{dAA_{Total}}{dt} = k_1[HA^-][O_2]$ • Cu^{2+} Ion Catalysed Oxidation - First order, in relation to H_2A, pH = [1.5; 3.5] • Fe^{3+} Ion Catalysed Oxidation - First order, in relation to H_2A, pH = [1.5; 3.85] $-\frac{dAA_{Total}}{dt} = k'_1[H_2A][M^{n+}] + k'_2[HA^-][M^{n+}]$	n.a.	Good*	The rate of the non-catalysed oxidation of AA was found to be proportional to oxygen concentration at 20% or higher. In the pH range investigated [2–5.5] only the monoionic species of AA was found to be reactive toward O_2 A mechanism is proposed whereby O_2 takes part directly in the oxidation of the ascorbate anion, HA^- The rates of the ferric and cupric anion catalysed oxidations were found to be 1st order with respect to O_2 A reaction path involving O_2 bound to metal-ascorbate complex is proposed The catalytic activity of cupric ion was found to be more than that of ferric ion toward the oxidation of the ascorbate anion; in the oxidation of the neutral species, ferric ion is a better catalyst than cupric ion The mechanistic implications of the catalysed and unanalysed reactions are discussed	(75)
Aqueous solution	2 T: 0.4, 25°C Oxygen: 80–5% 5 pH levels: 2.25–3.45	Ascorbic acid (H_2A)		Cu(II)-EDTA Cu(II)-HEDTA Cu(II)-NTA Cu(II)-HIMDA Cu(II)-IMDA Fe(III)-DTPA Fe(III)-CDTA Fe(III)-EDTA Fe(III)-HEDTA Fe(III)-NTA Fe(III)-HIMDA Temperature Oxygen pH	Aer./ Anaer.	Catalytic activity of Cu(II)-IMDA > Cu(II)-HIMDA > Cu(II)-NTA > Cu(II)-HEDTA > Cu(II)-EDTA Catalytic activity of Fe(III)-HIMDA > Fe(III)-NTA > Fe(III)-HEDTA > Fe(III)-EDTA > Fe(III)-CDTA > Fe(III)-DTPA	n.a.	Good*	The metal chelate catalyst is totally inactive for the oxidation of the un-ionised species of AA Mechanism proposed for metal chelate catalysed oxidation of AA: direct participation of metal chelate species in an electron-transfer process A mixed ligand chelate of ascorbate anion, HA^-, and metal chelate, ML^{n+}, is formed in a pre-equilibrium step. This is followed by an electron transfer within the mixed ligand chelate, $HAML^{(n-1)+}$, then dissociates in a fast step to the lower valence metal	(76)

(*Continued*)

TABLE 142.1 (*Continued*)

Food/Model System	Package/Storage Conditions	Compounds Under Study	Other Compounds	Compounds/Factors Affecting Degradation	Atm. Cond.	Applied Kinetic	Retention (%)	Fit Quality	Observations	Ref.
						$-\frac{dAA_{Total}}{dt} = k_1[H_2A][ML] + k_2[HA^-][ML]$			chelate $ML^{(n-1)+}$ and a semiquinone Oxidation of the lower valence metal chelate and semiquinone by molecular O_2 takes place in subsequent steps The catalytic activities of the cupric and ferric chelates were found to be independent of O_2 concentration, which is in agreement with this mechanism	
SSOJ (var. Hamlin, Pineapple, early and late Valencia)	Can (46 oz) 5 T: −18, 4, 16, 21, 35 (°C)	Furfural Flavour evaluation		Temperature	Anaer.	No kinetics for furfural, but by visual observation of the graphs a zero or first-order is not applicable		n.a.	Juices with lower pH the ones with higher % of citric acid produced more furfural at 35°C	(100)
Seaweed Mixed Cereal Orange Juice	Package system: n.a. 4 T: 23, 35, 40, 45°C 7 a_W content: 0.32, 0.51, 0.67, 0.75, 0.84, 0.88, 0.93 10 moisture content: 1.1 to 33.3 $gH_2O/100\,g$ sol	Ascorbic acid		Water activity Moisture content Temperature	n.a.	First-order the rate of AA loss increases with increasing a_w	n.a. ($a_w = 0.93$) 11 ($a_w = 0.84$) 80 ($a_w = 0.32$)	Good*	Ascorbic acid destruction rates increased with increasing a_w Ascorbic acid was more rapidly destroyed in the desorption system than in the adsorption system due to a decrease in viscosity and possible dilution of the aqueous phase	(56)
Infant formula	Closed cells (boxes) 20 hours 3 initial dissolved oxygen content: 1.0, 4.86, 8.71 ppm 5 light intensities: dark, 1071, 2142, 3213, 4284 lux T: 7.2°C	Ascorbic acid		Temperature Oxygen Light intensity	Aer.	Second-order Describes reaction scheme: $AA + O_2 \xrightarrow{k} DHAA + H_2O$ $-\frac{d(AA)}{dt} = k(AA)(O_2)$ $\ln\left[\frac{AA}{O_2}\right] = [AA_0 - O_{20}]\,kt + \ln\left[\frac{AA_0}{O_{20}}\right]$	≈33	n.a.	In samples exposed to light an increase in initial dissolved oxygen content increased the second-order rate constants The rate constants under dark conditions for different initial dissolved oxygen concentration remain unchanged Increasing light intensity above 1756 lux did not increase the rate constant, and below that the rate constant increases linearly with light intensity	(69)

Tomato Juice	Can (8 oz) 6.3 months 4 T: 10, 18.3, 29.4, 37.8°C Initial conc (Cu): 2.6 and 10 ppm pH: 3.53, 3.78, 4.06, and 4.36	Ascorbic acid	Temperature pH Metal catalyst — Cu	Aer.	AA loss is first order in relation to AA AA = f(T) Arrhenius relation Describes 3 reactions involved in the overall destruction of AA: $AH^- \xrightarrow{k_1}$ Product; $AH_2 \xrightarrow{k_3}$ Product $AH_2 + AH^- \xrightarrow{k_1}$ $AH_2.AH^- \xrightarrow{k_2}$ Product $AA_r \xrightarrow{k}$ Product; $-\frac{d(AA)}{dt} = k(AA_T)$	≈60	Good*	The rate of AA destruction was influenced by pH, reaching a maximum near pK of AA The rate of copper-catalysed destruction of AA increased with copper conc. and was affected by pH Ea changes with pH, with a minimum at pH 4.06 (3.3 kcal/mol) A mathematical model was developed for the rate of AA loss as function of T, pH and Cu A computer simulation program was developed to predict AA stability in tomato juice Predictions were in good agreement with results of the shelf-life tests	(74)
SSOJ (Var. early, mid and late Valencia)	Can (6 oz) 11 T: 4.4, 15.6, 21.1, 29.4, 32.2, 35, 37.8, 40.6, 43.3, 46.1, 48.9°C 12 weeks	Ascorbic acid	Temperature	Aer./ Anaer.	First-order Two distinct Arrhenius profiles [4.4, 21.1] and [29.4, 46.1]°C Employment of orthogonal polynomials in the analysis of variance indicates that the mechanism of AA degradation was not the same at all temperatures	92–6	Good $0.97 < R^2 < 0.99$	Values of U.S. RDA of vitamin C are given At each specific temperature all 14 juices, regardless of plant or processing season, showed essentially similar percent vitamin C retention The Arrhenius plot showed two distinct temperatures regions, with a critical transition region between 22 and 26.7°C	(57)
Model system	Mixing bowl (with tight-fit cover); Steady-state experiment: 4 T: 71, 79, 92, 105°C 25 min 3 a_w content: 0.69, 0.80, 0.90	Ascorbic acid	Water activity Temperature	n.a.	Zero-order	≈57.5	Good $R^2 > 0.97$	Rates of AA degradation are dependent upon O_2 availability, which is in turn dependent upon temperature and moisture content The equation derived to predict the amount of AA lost during an unsteady state heating process was successful when tested under conditions approximating a linear T rise (error for the final predicted value <5%)	(66)

(*Continued*)

TABLE 142.1 (*Continued*)

Food/Model System	Package/Storage Conditions	Compounds Under Study	Other Compounds	Compounds/Factors Affecting Degradation	Atm. Cond.	Applied Kinetic	Retention (%)	Fit Quality	Observations	Ref.
									The same equation yielded less accurate predictions of AA losses during extrusion (error for the final predicted value <10%)	
Grapefruit Juice	60 min Initial AA content: 34.8, 112.5, 204.8 mg/100 g °brix: concentrated during the study from 11 to 62 °brix	Ascorbic acid		Added AA concentration °brix	Anaer.	First-order Dependent on T by Arrhenius Eq. Ea = 5 kcal/mol (11°brix) Ea = 11.3 kcal/mol (62°brix) Arrhenius coeff. dependent on °brix Polynomial curve fitting, empirical kinetic equation correlating rate of reaction with temperature and degree of concentration	<85	Good $0.972 < R^2 < 0.999$	Initial AA conc. has no significant effect either on rate of deterioration or mechanism A model combining kinetic data with process variables was developed and proved useful in predicting and optimising vit. C retention processes where grapefruit juice is subjected to any combination of thermal and concentration treatments	(58)
Peas	TDT cans 5 T: 110, 115, 121, 126, 132°C 6 hours	Ascorbic acid	O_2 monitored	Temperature	Aer./ Anaer.	First-order	30–96	Reasonable good*	Discussion on the values of Ea for this study and others, both for aerobic and anaerobic conditions, reaching the conclusion that the differences on Ea indicate that kinetic studies should be conducted for different food systems	(59)
Intermediate moisture food material	Jacketed, stirred reactor with air space above reactants Temperature: 60 to 110°C 3 a_w: 0.9, 0.8, 0.69	Ascorbic acid		Oxygen mass transfer a_w	Aer./ Anaer.	Second-order	n.a.	n.a.	The effect of oxygen transport on degradation rate was determined by comparing the experimental observations with theoretical predictions for a series of four regimes (a regime is characterised by different relative rates of O_2 mass transfer and chemical reaction) The most likely explanation for the experimental data is regime III — the chemical reaction rate is sufficiently fast that all the oxygen reacts in a thin film near the interface between the food and the gas phase, which enhances the mass transfer rate due to chemical reaction	(68)

Orange Grapefruit Tangerine Lemon Lime	Whole Fruit Can	Ascorbic acid	- Production factors - Climate - Position of fruit on the tree - Maturation - Rootstock effects - Citrus variety (and fruit parts) - Processed products: seasonal variability, processing effects, storage time and temperature - Vitamin C destruction: reaction order and reaction rates (aerobic and anaerobic mechanisms) - Effects of container - Influence of juice constituents			First-order			The variability of vitamin C in fresh fruit is due to variety, climate, horticultural practice, maturity stage and storage conditions Processing fruit into juice products results in minimal loss of vitamin C potency but subsequent storage finished of the product at higher temperatures results in considerable loss From the point of view of the consumer, numerous investigations have shown that fresh processed single strength and reconstituted citrus juices may be kept in a refrigerator for a reasonable length of time (4 weeks) without serious loss of vit C; even when juice is stored at room temperature, storage time is limited more by loss of palatability than by loss of vit C Aerobic and anaerobic mechanisms are mainly responsible for loss of vit C in processed products The mode of breakdown of vitamin C can best be explained by a 1st-order reaction but a significant quadratic time effect has been determined by polynomial regression calculations Plots of log rate (vit C loss) vs. 1/T for canned orange juice showed two distinct Arrhenius profiles, whereas canned grapefruit showed only one	(60)
Buffered model system (pH = 6.1)	Closed system with headspace and control of head-space gases 4 T: 30 to 55°C Initial AA conc = 30, 35, 40, 50, 65 g/L 3 O_2 levels = 10, 15, 21%	Ascorbic acid		Temperature Ascorbic acid addition Oxygen	Aer.	Second-order	>75	Good $0.96 < R^2 < 0.99$	Oxygen concentration was maintained throughout the experiment Ascorbic acid autoxidation is dependent on dissolved oxygen concentration It appears that the rate of oxygen dissolution into the AA solution is dependent on both temperature and headspace oxygen levels	(67)
SSOJ (var. Valencia) and OJ concentrate	Can (6 oz) 18 months 6 T (°C) = −18, 5, 12, 17, 25, 37 4°brix = 11°, 34°, 44°, 58	Ascorbic acid	Browning Furfural Sensorial changes	Temperature °brix	Aer./ Anaer.	T ⩽ 25° first-order T = 37°C different from first-order	95–10	Good*	AA loss depends on the degree of concentration of the O. J. (increase with °brix)	(70)

(*Continued*)

TABLE 142.1 (*Continued*)

Food/Model System	Package/Storage Conditions	Compounds Under Study	Other Compounds	Compounds/Factors Affecting Degradation	Atm. Cond.	Applied Kinetic	Retention (%)	Fit Quality	Observations	Ref.
Aqueous solution with AA	Erlenmeyer flasks (open) 4 T = 25, 62, 75, 86°C Initial AA conc = 500 mg/L 90 minutes	Ascorbic acid Dehydro-ascorbic acid		Temperature Continuous aeration.	Aer.	Mechanism of degradation: $\underset{\uparrow k_3}{\text{Prod}}\ \underset{k_1}{}\ \underset{\uparrow k_4}{\text{Prod}}\ \underset{k_2}{}\ \underset{\uparrow k_5}{\text{Prod}}$ $\text{AA} \underset{k_{-1}}{\longleftrightarrow} \text{DHAA} \underset{k_{-2}}{\longleftrightarrow} \text{DKA}$ First-order (reversible) for DHA and diketogulonic acid First-order reaction for AA loss	72.2–50	Good*	The AA-to-DHA-to-DKA mechanism fits the data reasonably well An irreversible path from AA to products appears to exist; the rate constant k_3 appears to be large enough that it may be possible to degrade measurable amounts of AA without any DHA being formed	(73)
Lemon Juice	Glass flasks, 250 mL, covered with AL foil Initial dissolved oxygen content: 0.41, 1.44 and 3.74 mg/L T: 36°C	Ascorbic acid Browning HMF Furfural		Initial dissolved oxygen content Temperature	Aer./ Anaer.	AA: First or second-order Browning: Zero-order with a lag period HMF: first-order reaction Furfural: zero-order reaction	AA: 47.1	AA: 1st order: $0.85 < R^2 < 0.87$ 2nd-order: n.a. Browning: $0.90 < R^2 < 0.95$ HMF $0.96 < R^2 < 0.98$ Furfural $0.98 < R^2 < 0.99$	AA: Initial oxygen content did not affect significantly the rate of AA degradation and furfural formation Correlation between AA and the other compounds was between 0.8 and 0.9 Browning: The lag period before browning increased depended on the initial dissolved oxygen concentration, being greater for the lower initial concentration Highly significant correlations were obtained between browning index, HMF and furfural (>0.96), suggesting that all 3 would be suitable as chemical indices of storage temperature abuse in lemon juices; initial oxygen content did not affect significantly the rate of furfural formation Highly significant correlations were obtained between browning index, HMF and furfural (>0.96), suggesting that all 3 would be suitable as chemical indices of storage temperature abuse in lemon juices	(61)
Aqueous solution with AA	Package system: n.a. 11 hours 2 T: 30 and 71°C Oxygen levels:	Ascorbic acid	β-carotene	Temperature Dissolved oxygen content	Aer.	AA oxidation has a first-order kinetics at one time and zero-order at another time:	17.5–28.2	Good*	Rate constants are independent of initial AA content	(72)

	saturation with air, oxygen, or 10% O_2-90% N_2 B-carotene: 80°C 2 initial AA conc. = 114, 266 mg/L			AA addition Continuous aeration		$A \xrightarrow{k_a} B \xrightarrow{k_b} C$ First order: $k_a > k_b \times (A)_o$ Zero order: $k_a < k_b \times (A)_o$ First order reaction in respect to β-carotene				
Buffer solution	Packaging system: n.a.	Ascorbic acid		Catalytic metals: Fe(III) Cu(II)	Aer.	First-order	n.a.	n.a.	The first order rate constant in an air saturated catalytic metal free solution is less than 6×10^{-7} s^{-1} at pH = 7 Ascorbate can be used in a quick and easy test to determine if the near-buffer solutions are indeed "catalytic metal free"	(62)
Valencia O.J. (Pasteurised at 92°C for 30 sec)	Glass bottles (200 mL) T: 4, 22.5, 35 and 45°C	Ascorbic acid Furfural	Total solids pH, acidity Formol no. Reducing Sugars	Temperature	Anaer.	AA: Zero-order Furfural: Zero-order	AA: 97–47 Furf: n.a.	Good*	At high temperature (45°C) furfural production relates very well to AA degradation (r = 0.96), but for lower T this relation is not so obvious	(65)
Grapefruit juice	Can, bottle 18 weeks 5T (°C): 10, 20, 30, 40, 50	Browning		Temperature	n.a.	Reaction order should be between zero and one, for temperatures <30°C		n.a.	Browning in citrus juices involves a complex group of reactants that produce an assortment of brown pigments of highly unstable characteristics; based on these reasons and results of other researchers, the authors believe that it is inaccurate to define browning by a simple zero or first-order reaction No simplistic models should be applied to define the complex series of events leading to brown discoloration of citrus juices, especially within the temperature region of 30–50°C	(92)
SSOJ (made from concentrate)	TB cartons 64 days Initial dissolved O_2 content = 4.45 ppm T (°C): 4, 20, 37, 76, 75 L-AA addition (supplemented with 0.34 M, 1 mL)	Ascorbic acid		Ascorbic acid Oxygen Temperature	Aer./ Anaer.	Zero, first and second-order were applied (4 to 37°C) Impossible to say which best fits the data	60.4–2	Zero-order: $0.97 < R^2 < 0.985$ 1st-order: $0.93 < R^2 < 0.99$ 2nd-order: $0.97 < R^2 < 0.97$	Initial sudden drop of oxygen, intensified at higher temperatures Initial drop correlates with AA degradation during initial stage of storage After the dissolved oxygen reaches the equilibrium, L-ascorbic acid decomposition occurs independently of oxygen	(79)

(*Continued*)

TABLE 142.1 (*Continued*)

Food/Model System	Package/Storage Conditions	Compounds Under Study	Other Compounds	Compounds/Factors Affecting Degradation	Atm. Cond.	Applied Kinetic	Retention (%)	Fit Quality	Observations	Ref.
Sucrose solution	BOD bottle (300 mL) Initial AA conc = 6.67 g/L ≈700 min (11.7 h) pH: 3.0 to 5.0 3 T: 26.5, 30, and 33°C	Ascorbic acid	Oxygen	Temperature Sucrose pH	Aer.	Second-order in relation to dissolved oxygen Dependent on T by Arrhenius equation	78.5–87	Good $0.98 < R^2 < 0.99$	Above 2 ppm of dissolved oxygen content there is one mechanism of reaction and below 2 ppm there is another Molar ratio between O_2 and AA: ≅ 1 mol of O_2 per mol of AA Ea(AA) was higher in presence of 10% sucrose than in non sucrose controls, and the addition of sucrose reduced the rate of reaction at the temperature tested Effects of sucrose on AA: pH independent physical effect retards AA oxidation; pH dependent catalytic effect accelerates AA oxidation	(78)
Buffer solution	T: 25°C Constant O_2 content 4 pH levels: 2.5, 2.5, 4.5, 6	Ascorbic acid		Cu (II) — citrate complexes pH	Aer.	First-order	n.a.	n.a.	The rate of cupric ion-catalysed oxidation was found to be first order with respect to ascorbic acid. The effects of cupric ion conc. and pH suggests a mechanism involving the formation of a transition complex between monoascorbate ion and Cu(II)-citrate chelate	(63)
Grapefruit juice	Vials (10 mL) 4 T (°C): 90, 100, 110, 120 5 experimental times: 20, 40, 60, 80, 100 min	HMF		Temperature	n.a.	Reaction order, according to empirical kinetics of formation was 0.31		Good*	There was a lag phase in kinetics of HMF formation Ea = 130 ± 7 kJ/mol, kr = 0.0105 ± 0.00040$(mg/L)^{0.69} \times s^{-1}$ (the used reference temperature is not mentioned)	(99)
Sweet aqueous model system: $a_w = 0.94$ and pH = 3.5	Glass flasks: 60 mL (no headspace) ≈210 days at 24°C, 125 days at 33°C, 105 days at 45°C, 140 h at 70°C, 90 h at 80 and 90°C. Initial AA conc = 330 mg/kg Stored in the dark	Ascorbic acid Browning		Temperature Humectants: glucose, sucrose, sorbitol Additives: potassium sorbate, sodium bissulfite	Aer./ Anaer.	AA: First-order Browning: Zero-order	AA: > 20	n.a.	At lower temperatures (24, 33, 45°C) the humectants protected L-AA from destruction (sugars being the most effective due to the structure forming effect they have) At higher temperatures characteristic of processing (70, 80, 90°C) humectants with active carbonyls (glucose, sucrose) promoted AA destruction and nonenzymatic browning reactions AA destruction occurred mainly	(91)

					through anaerobic path as O_2 was depleted in 15 h (70–90°C) or 25 days at low T				
Orange juice	Culture tubes (15 × 1.5 cm) Abuse time/ /temperature protocol T (°C): 75, 85, 95 Time: 15, 30, 60 min pH: 3.1, 3.8, 4.5 Initial O_2: 6.2, 0.6 ppm	Furfural 2-hexanal α-terpineol p-vinyl guaiacol	Temperature pH Initial oxygen	Aer./ Anaer.	Furfural: not modelled 2-hexanal: zero-order α-terpineol: zero-order p-vinyl guaiacol: zero-order Arrhenius equation was used to calculate Ea Ea (2-hexanal) = 14.831 kcal/mol Ea (α-terpineol) = 20.257 kcal/mol Ea (PVG) = 28.258 kcal/mol		Fit not shown R^2(2-hex) = 0.99 R^2(α-ter)= 0.98 R^2(PVG) = 0.98	The pseudo zero order kinetics applied to 2-hexanal is not valid as concentration varies with time in a form far from a straight line pH has the most significant effect on the increase of α-terpineol and PVG, as their concentration doubled at the low pH value for the same temperature; this is in agreement with previous studies from Lee and Nagy (1990) In reality it is the release of ferrulic acid (precursor of PVG) that is acid catalysed, and Blair *et al.* (1952)	(101)
Fresh squeezed O.J. Unpasteurised Frozen	Polyethylene bottle 24 months storage T(°C): −23 Initial AA = 406 mg/L pH = 3.7 °brix: 11.4	Ascorbic acid	Temperature	n.a.	First-order	80.8	Good*	This study did not intend to characterise the kinetics of vitamin C loss Over the 24 month period, the rate of decline was 0.8% per month Based on this storage study, vitamin C content of frozen, fresh squeezed, unpasteurised OJ has been shown to provide 131% of the daily value of vitamin C requirements, even after 24 months of storage; the vitamin C was still within the label declaration which complies with the nutritional labelling law (FDA, 1993)	(64)
Cupuaçu nectar	TDT tubes (with headspace) 6 T: 60, 70, 75, 80, 90, 99°C 240 minutes	Ascorbic acid Dehydro-ascorbic acid	Temperature	Aer./ Anaer.	First-order $AA \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} DHAA \overset{k_2}{\rightleftarrows} DKGA$ After a transformation of variables ($C^*_{AA} = C_{AA} - C^i_{AA}$) the reaction was treated as two consecutive irreversible reactions: $C^*_{AA} \xrightarrow{k_1} C_{DHAA} \xrightarrow{k_2} C_{DKGA}$ $C^*_{AA} = C^{i*}_{AA} \times e^{-k_1 \times t}$ $\frac{C_{DHAA}}{C^i_{DHAA}} = \left\{ \left[\left(\frac{C^*_{AA}}{C^{i*}_{AA}} \right)^{\frac{k_2}{k_1}} \right] \times \right.$	≈75	Good	Reversible first order for AA (Ea = 74 ± 5 kJ/mol, k_{80} = 0.032 ± 0.003 min^{-1}) Mechanistic model for DHAA (with the second reaction having Ea = 65 ± 9 kJ/mol, k_{80} = 0.013 ± 0.003 min^{-1})	(71)

(Continued)

TABLE 142.1 (*Continued*)

Food/Model System	Package/Storage Conditions	Compounds Under Study	Other Compounds	Compounds/Factors Affecting Degradation	Atm. Cond.	Applied Kinetic	Retention (%)	Fit Quality	Observations	Ref.
						$\times\left[\left(-\frac{C^{i*}_{AA}}{C^{i}_{DHAA}}\right)+\left(\frac{k_2}{k_1}-1\right)\right]$ $+\frac{C^{i*}_{AA}}{C^{i}_{DHAA}}\Bigg\}\Bigg/\left(\frac{k_2}{k_1}-1\right)$				
SSOJ (var. Valencia)	Package system: n.a. 6 T: 20, 25, 30, 35, 40, 45°C O_2 contents: [4.7; 5.7] ppm	Ascorbic acid (aa) Browning	Dehydro-ascorbic acid (daa) pH	Oxygen Temperature	Aerobic	AA: - Weibull model - Mechanistic model $\frac{dC_{AA}}{dt}=-k_1C_{AA}+k_2C^2_{AA}C_{DAA}$ $\frac{dC_{AA}}{dt}=-k_1C_{AA}+k_2C^2_{AA}C_{DAA}-k_3C_{DAA}$ Browning: Weibull model	AA:0%	AA: Weibull R^2_{adj} = 0.995 Browning: R^2_{adj} = 0.999	AA: Weibull model: $((1/\alpha)_{32.5}\times 10^3$ =64 ± 1 h^{-1}; Ea = 38.6 ±1.6 kJ/mol, β_{ref} = 1.65 ± 0.35; 0.042 ± 0.007°C^{-1}) Mechanistic model: (Ea_1 = 94 ± 11 kJ/mol, k_{1ref} = 0.33 ± 0.07 h^{-1}, Ea_2 = 107 ± 13 kJ/mol, k_{2ref} = 7.5 ± 1.9 h^{-1}, Ea_3 = 27 ± 9 kJ/mol, k_{3ref} = 0.044 ± 0.005 h^{-1}) Browning: Weibull model: $((1/\alpha)_{32.5}\times 10^3$ = 32 ± 3 h^{-1}; Ea = 66 ± 3 kJ/mol, β_{ref} = 1.6 ± 0.1; C^{∞} = 0.328 ± 0.007 mg/L) The Weibull model provided a good description of the kinetics of AA degradation and the browning index in the range of T and conversions tested, and therefore is appropriate for predictive purposes. The T dependency of the rate constants ($1/\alpha$) was well described by the Arrhenius law. The shape constant (β) was T independent for browning but decreased with T for AA degradation. The later behaviour might be explained by the reconvertion of DAA into AA, following first-order kinetics in relation to DA and second-order reaction in relation to AA and by different sensitivities of the different reaction rate constants to T. Browning appears to follow a single reactive pathway.	(77)

* By visual inspection of the graph; n.a. — not available; Aer. — aerobic; Anaer. — anaerobic.

fades during ambient temperature storage with a simultaneous darkening of the juice. The rate of colour degradation and browning are highly associated with storage temperature for aseptically prepared orange juice. The darkening of citrus juices during storage occurs most often in citrus juices that are stored at ambient temperature in containers that are not effective oxygen barriers and in the absence of antioxidants (87).

Handwerk and Coleman (88) reviewed browning as it occurs in citrus juices and model solutions. The role of amino acids, sugars, ascorbic acid, buffers and catalysts, sulphur dioxide and S-containing amino acids and thiols was discussed. According to the authors, sugar breakdown through the Maillard reaction is initiated by formation of hexose amines from amino acids and sugars present in citrus juice and proceeds through the Amadori or Heyns rearrangement to produce deoxy and amino hexoses. These deoxy compounds go through a series of reactions and through one of two pathways. One of these pathways produces furanones and pyrones, whereas the other produces furfurals and pyrroles. Both pathways are known to be operable because all four types of compounds have been found in citrus juices. However the actual compounds responsible for browning, i.e., the brown pigments, were not identified.

Marcy *et al.* (89) studied concentrate orange juice aseptically packed in flexible bags and stored at 4, 15, 22 and 30°C for 6 months. Nonenzymatic browning was measured monthly using a Hunter colour meter and absorbance at 420 nm. They found the greatest browning for high temperature stored samples and least browning in the samples stored at the lowest temperatures.

Browning compounds are formed during the Maillard reaction, which occurs between sugars and aminoacids, polypeptides, or proteins, and between polysaccharides and polypeptides or proteins (90). There are many pathways and literally thousands of compounds that might contribute to the formation of these coloured compounds, but when it comes to mathematically modeling nonenzymatic browning, most researchers use zero-order reaction kinetics. This linear model may not be the most adequate to describe the complex group of reactions involved in orange juice browning. Nevertheless, it is a very simple model and it proved to be effective to predict the browning of orange juice in a different range of temperatures. Table 142.1 summarises studies reported in literature. Rojas and Gerschenson (91) reported a zero-order kinetic for browning in a sweet aqueous model system. The same order of reaction was reported by Robertson and Samaniego (61) but these authors suggest the existence of a lag period, dependent on initial oxygen content, being greater for the lower initial concentrations. Nagy *et al.* (92) reported that it is inaccurate to define browning by a simple zero or first-order reaction, especially within the temperature region of 30–50°C.

D. Flavour/Off-Flavour Development

Moshonas and Shaw (93) quantified 24 volatile constituents in samples of fresh Valencia and Temple orange juices. Nispieros-Carriedo and Shaw (94) quantified 20 volatile constituents in 15 fresh orange juice samples. Their study is important because it compares volatile flavour compounds in different types of orange juices. Shaw *et al.* (95) used the same technique to quantify 19 volatile compounds in a different set of 4 fresh orange juice samples. Moshonas and Shaw (96) quantified 46 volatile juice constituents in 13 fresh orange juice samples, hand and mechanically squeezed of 6 different cultivars. The results reported in their study provide the most complete database yet determined for quantities of volatile flavour compounds present in fresh orange juice. Nine of the 17 oil-soluble constituents present at higher levels in mechanical obtained juices include α-pinene, myrcene, limonene, octanal, nonanal, decanal, neral, geranial and linalool. From these, only limonene has an established optimum level, and for processed orange juice it is about 135–180 ppm. The seven water-soluble constituents quantified that are considered important for flavour of orange juice include ethyl acetate, ethyl propianate, methyl butanoate, ethyl butanoate, ethyl-3-hydroxylhexanoate, ethanol and (Z)-3-hexen-1-ol. Acetaldehyde and (E)-2-hexanol, two additional components considered important in orange flavour, were identified but not quantified. The components present at highest levels (10-fold or higher above their aroma thresholds) and important in orange flavour are limonene, myrcene, α-pinene, decanal, octanal, ethyl butanoate and linalool.

The highly desirable aroma and flavour of fresh squeezed citrus juices is difficult to stabilize and preserve. Once heat treatment has been applied to the juice to stabilize it from the microbiological and enzymatic point of view, certain chemical processes have been initiated which will ultimately degrade the flavour of the product due to the formation of off-flavours during storage.

Changes that occur during processing and storage of citrus juices can differ widely for the different products because of differences in starting material (juice), processing conditions, packaging materials and storage conditions. For instance, the initial flavour compound contents vary for different types of orange juice (94).

Several factors have been recognized as contributing to the development of off-flavours during thermal processing and subsequent storage. Two aldehydes that are indicative of temperature abuse in fruit juice are 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde (97). Reduction or removal of oxygen from the headspace and minimization of initial dissolved oxygen in the juice may partially reduce the short-term production of off-flavour (39). However, other major off-flavours are formed from the acid-catalysed hydrolysis of citrus oil components, the

formation of sulphur-containing juice components, and the chemical degradation of flavourless precursors to produce off-flavours.

Tatum *et al.* (98) isolated 10 degradation compounds from canned single strength orange juice (SSOJ) stored at 35°C for 12 weeks. Of the compounds identified, 4-vinyl guaiacol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and α-terpineol were found to be the most responsible for the malodorous properties of time-temperature abused juice. The first imparted an old fruit or rotten fruit aroma, the second imparted the pineapple-like aroma typically observed in aged orange juice and the last one was described as stale, musty or piny. Acid degradation of sugars was probably responsible for 2-hydroxyacetyl furan and 3-hydroxy-2-pyrone formation.

These authors also state that furfural was formed from the degradation of ascorbic acid, which was mentioned in Section III.B.1, via its anaerobic degradation pathway (52, 53).

There is little information in literature regarding kinetic modelling of off-flavours, although a number of studies are published showing the effect of the temperature, pH and a_w on sensorial properties (aroma, flavour and threshold values) of these compounds and formation/degradation chemical pathways. Some studies on kinetic modelling of furfural, HMF and PVG are summarised in Table 142.1.

Robertson and Samaniego (61) reported HMF formation in lemon juice to follow a first-order reaction, and Körmendy *et al.* (99) reported a lag phase for the same kinetics. Furfural formation was reported to follow a zero-order kinetic both in orange juice (65) and lemon juice (61). The later researchers concluded that initial oxygen content did not affect the rate of furfural formation. Data reported for furfural (100) no kinetics is presented, though data visualisation suggests that zero or first order are not applicable. Marcotte *et al.* (101) reported PVG formation to follow a zero-order kinetic reaction. The mechanism of formation of PVG has been mainly studied by Fiddler *et al.* (102) and later in citrus by Lee and Nagy (103), Peleg *et al.* (104, 105) and Naim *et al.* (106, 107).

The dependence of the reaction rates of these compounds on temperature was considered to follow an Arrhenius-type relationship.

E. Oxygen

In some situations the amount of oxygen is limited to a level that causes no significant effect in food. In other cases, the total amount of oxygen potentially able to react with nutrients is significant, and the effect of oxygen concentration (or partial pressure) on the rate of oxidative reactions must be considered (7, 108, 109).

The mathematical modelling of oxygen uptake by packaged foods has been mainly applied to dried and fatty foods (5, 110, 111). For liquid foods, several studies report the effect of oxygen on quality degradation during storage, but few of them report changes of oxygen concentration. Scarcer are studies where the changes are mathematically modelled and even scarcer the ones that use a packaged liquid food (in a real package material). Singh (112) developed a mathematical model to describe ascorbic acid and dissolved oxygen concentration changes in an infant formula packaged in glass bottles and stored at 7°C. This model was based on Fickian diffusion of oxygen accompanied by a second order chemical reaction in the liquid food. Barron *et al.* (113) reported a study where a finite element method was applied to the modelling of simultaneous oxygen diffusion and chemical reaction in packaged apple juice, stored at 25°C, using a cylindrical high-density polyethylene package (top and bottom insulated). Hertlein *et al.* (114) presented a study of prediction of steady state oxygen transmission from transient state measurements. Two models were tested, one with an equation describing the transient state, another using a partial pressure pulse instead of a constant change in partial pressure. The study included testing of both models in different films (mono and multilayers) and it was concluded that both models were valid only for ideal conditions. Ahrné *et al.* (115) presented a study of modelling of oxygen changes in liquid foods packaged in aseptic cartons. This study combines oxygen uptake from the outside environment with oxygen consumption by first order oxidative reactions in packed juice during storage. The model was applied to orange juice aseptically packaged in Tetra Brik Aseptic cartons, during storage up to 5 months at six different temperatures, ranging from 4 to 50°C, and was further tested with data reported in literature. It was found that it adequately describes the dissolved oxygen concentration changes during storage.

IV. CONCLUSIONS

The shelf life of a food is the time period for the product to become unacceptable from sensory, nutritional or safety perspectives (116). From the viewpoint of the food industry, shelf life is based on the extent of quality loss that the food company will allow prior to product consumption while ensuring safety. For consumers, the end of shelf life is the time after which food no longer has an acceptable look or taste. Although food systems have very complex mechanisms of deterioration, shelf life of food products can be determined, and subsequently predicted, based on some primary mode of deterioration (116).

This chapter addresses particularly aspects that fix the limits of foods (microbiology, nutrient loss, colour stability, flavour/off-flavour development), as well as kinetics of nutrient losses and their dependence on environmental factors and food composition. Mathematical models are also discussed, particularly in relation to quality characteristics decay.

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143 Experimental Design in Product Development and Optimisation

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CONTENTS

I. INTRODUCTION AND OBJECTIVES

Product development in food engineering involves finding combinations of constituents that produce foods as a result of certain processes. These foods should have desirable properties and should be created in an economical and reliable manner. While achieving these goals can be easy, more often the food engineer has available a wide range of options to choose from when making a product. Finding the specific settings for these factors that satisfy quality, reliability and cost goals can often be difficult.

In this chapter experimental design is introduced with discussion on how this set of statistical tools can be used to optimize quality, reliability and cost by finding the best combinations of constituents and processes for a new product. The concepts and tools discussed here are not new, having been around for over 70 years. These basic methods have proven their utility in a large number of disciplines from food production to medicine and from agriculture to consumer sciences and bio-informatics. As you will see, these methods can be valuable tools in food engineering tasks as well.

This chapter will only introduce a small portion of the full set of statistical design tools available and the interested reader is encouraged to read further in any of the large number of experimental design books available. The reader is assumed to have basic knowledge about statistical concepts of design of experiments and linear modelling. The general objective is to elaborate a methodological framework for product development and optimisation in the food sciences based on optimal experimental design. This framework relies heavily on response surface models to link the experimental factors to the response variable of interest. This presentation is structured into an introductory part in which the basic concepts of experimental design and response surface modelling are elaborated, followed by a discussion of design strategies that accommodate unconstrained and constrained factors. Each section is illustrated with real life examples.

II. A MODELLING APPROACH TO EXPERIMENTAL OPTIMISATION

Experimental optimisation in product development is a multi-step process involving:

1. Identification of the important product ingredients and processes variables involved in producing the target product. The more important factors are selected as the experimental factors and define the region of interest also called the experimental factor space.
2. Development of a predictive mathematical model to describe the relationship between levels of the experimental factors and the produce responses of interest. These process

responses are numeric or categorical product attributes that relate to product quality, cost, physical or chemical feasibility.
3. Construction of an optimal design that spans the experimental factor space in relation to the assumed model.
4. Fit the model to the data and evaluate its fit.
5. Obtain the optimal treatment combination by examination of the mathematical model and or return to step 3 for the next experiment.

Each of the steps above will be discussed in more detail after we introduce some basic concepts of response surface models and associated experimental designs.

III. BASIC CONCEPTS OF RESPONSE SURFACE METHODOLOGIES

Assume a product attribute, denoted y, depends on the levels of a set of numeric factors, coded as $x_1, x_2, \ldots, x_k$. These experimental factors reflect relevant product composition, processing conditions or even socio-economic cues, thought to be important to product objectives. Further assume that the levels of the x's can be controlled by the experimenter with negligible error in comparison with the variance of y. Traditionally, the x's are called independent variables or experimental factors. The response y is called the dependent variable and at this point is considered to be numeric.

The dependence of y on the x's can be formalised in a model

$$y = f(x_1, x_2, \ldots, x_k)$$

Usually the mathematical form of the function f is unknown, but in most cases this function can be approximated satisfactorily, at least within the experimental region, by a polynomial function in the x's. Because polynomials are notoriously untrustworthy when extrapolated, rarely will the polynomial approximation function be used to predict responses for values of x outside the experimental region.

A first order polynomial model, also called a linear approximation model, can be written as:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k$$

This approximation is used when the range of the x's is so small that little curvature is to be expected in the response function f. The fitting of such a linear polynomial is considered as a special case of multiple linear regression. In order to estimate the regression coefficients β_i in this model each independent variable x_i must take at least 2 levels. This model is only able to estimate the main effects of the experimental factors and does not account for either interactions or curvilinear effects. Specific designs for first order models are elaborated in later chapters. For k factors, at least $k + 1$ data points are needed to estimate the $k + 1$ unknown parameters.

Different graphical representations of a first order model are presented in Figure 143.1. Displayed is the response model, $y = 50 + 8X_1 + 3X_2$ over the experimental region of X_1 and X_2 each at levels from -1 to $+1$. Clearly the response y increases with increasing levels of X_1 and X_2, but the main effect, the per unit increase in response for a unit increase in the factor is greater for X_1 than for X_2.

The approximation model can allow more complex relationships by adding to the first order model the 2-factor interaction terms. This model is

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i<j}^{k} \sum \beta_{ij} x_i x_j$$

To fit this model requires that each factor be examined at 2 levels and treatments must be replicated. There are $1 + k + k(k-1)/2$ unknown parameters in the model and hence at least this many observations are required. In Figure 143.2 is displayed the response model $y = 50 + 8X_1 + 3X_2 - 4X_1X_2$. When compared to the first order model, we note that the interaction model accommodates for the mutual influence of the levels of X_1 on the levels of X_2. In other words, both experimental factors interact with each other; the response of y to X_1 depends on X_2 and vice versa. Still, the main effects of X_1 and X_2 on y are linear. In choosing the levels of X_1 and X_2 that will make up the experiment, factorial treatments can be shown to have certain optimality properties.

If the fitting of f involves more curvature, a quadratic or second order model becomes necessary.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \sum \beta_{ij} x_i x_j$$

This model contains linear-, squared- and cross-product terms and is referred to as the full quadratic model. This model requires at least $1 + 2k + k(k-1)/2$ data points and each variable x_i must be measured at a minimum of at least 3 levels. Specific designs to fit second order models are described in later chapters.

Figures 143.3 and 143.4 illustrate two second order models. The first model specifies $y = 50 + 8X_1 + 3X_2 - 7X_1^2 - 4X_1X_2$, and is quadratic in X_1, linear in X_2 and models the interaction between X_1 and X_2 by adding the term X_1X_2 (Figure 143.3).

The second model illustrates $y = 50 + 8X_1 + 3X_2 - 7X_1^2 - 3X_2^2 - 4X_1X_2$, and included quadratic effects in X_1 and X_2 and includes an interaction term X_1X_2 (Figure 143.4).

When a polynomial (either first or second order) accurately approximates the real underlying function f, the values of the X_i that produce the optimal response can

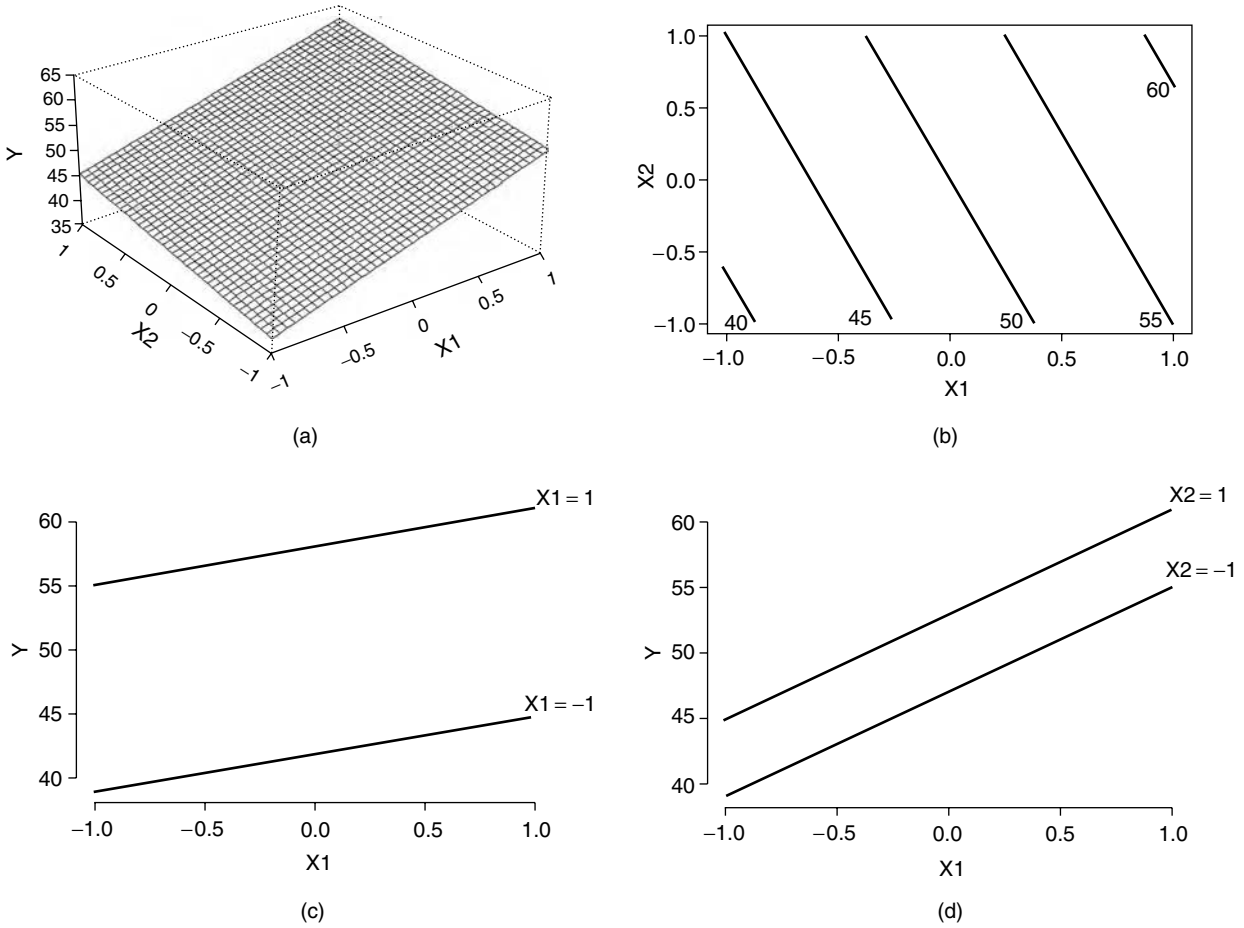

FIGURE 143.1 Different graphical representations of the first order model: $y = 50 + 8X_1 + 3X_2$. Perspective plot (a), Contour plot (b), Line plot $y*X_2 = X_1$ (c), $y*X_1 = X_2$ (d).

be determined mathematically using the fitted function. The precision of this estimate will depend on getting the form of the approximating polynomial correct and estimating the parameters precisely. This being so, the more important problem is determining at which levels of the X_i should the response be measured so that the form and parameters of the model can be most precisely estimated. That is, what factor level combinations should be used as treatments? Before this topic is addressed, the basic concepts of liner regression and experimental design are reviewed. Further discussions on response surface methodology, which is the parent discipline of these models, can be found in Meyers (1), Meyers and Montgomery (2) and Khuri and Cornell (3).

IV. PARAMETER ESTIMATION IN RESPONSE SURFACE MODELS: THE METHOD OF LEAST SQUARES

Without getting too deeply involved in the underlying mathematics, some basic concepts have to be explained as prerequisites for the understanding of some specific properties of experimental designs for response surface models.

The polynomial models described in the previous paragraph can be formulated in matrix notation as follows:

$$Y = X\beta + \varepsilon$$

where Y is an nx1 vector of observations y_i on the independent variable or the response, X equals an n*p matrix of known levels for each individual experimental factor, including cross-product and quadratic terms, β is a p*1 vector of unknown parameters β_i, n is the number of experimental units and ε is an n*1 vector of random errors.

Parameters β_i are estimated using the method of least squares in which we search for the values of the parameters that minimizes the sum of squared differences between the observed response and the corresponding expected value. The sum of squared differences, also called the residual sums of square or error sums of squares, are given as:

$$SSE(b) = \sum_{i=1}^{n} [y_i - E(yi|b)]^2$$

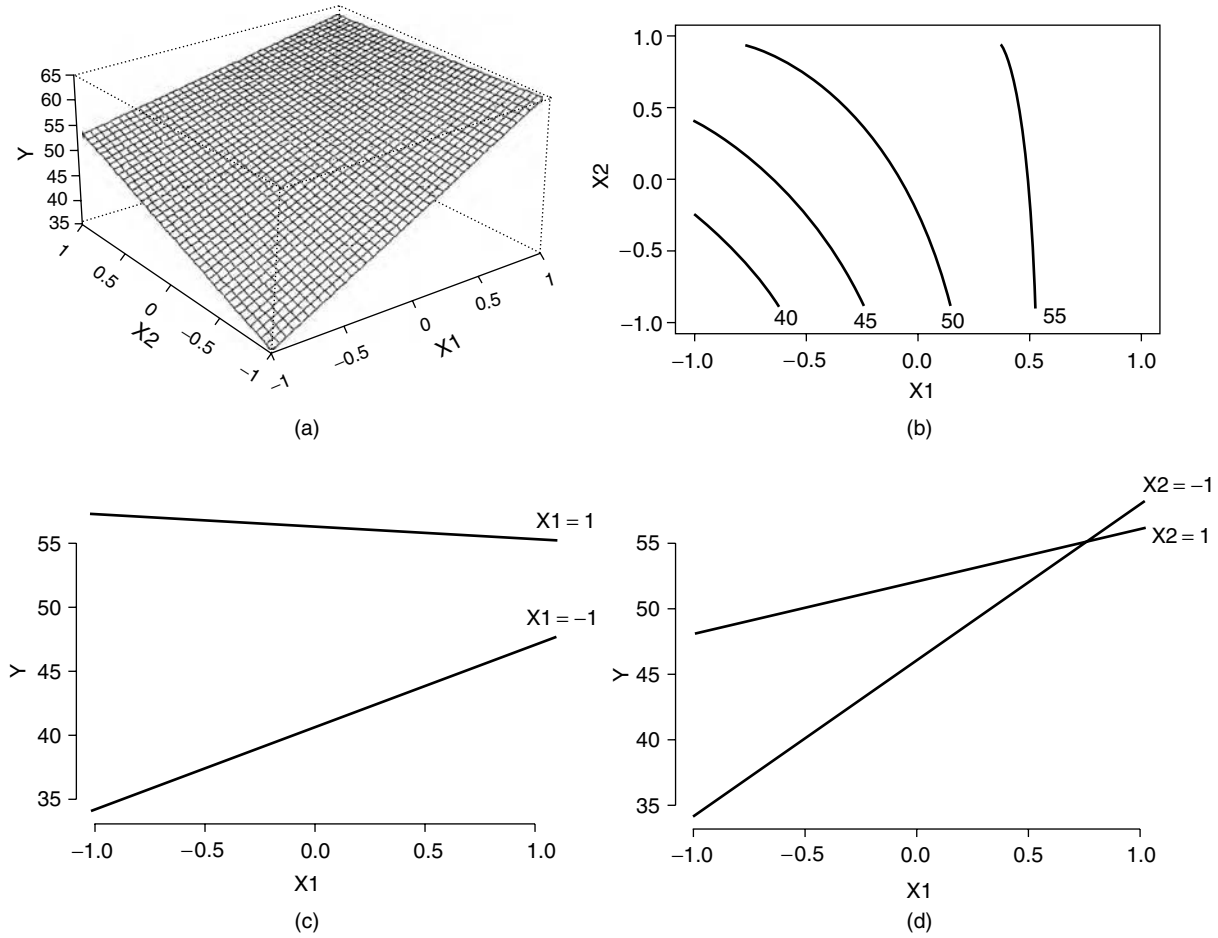

FIGURE 143.2 Different graphical representations of the first order model with interaction term: $y = 50 + 8X_1 + 3X_2 - 4X_1X_2$. Perspective plot (a), Contour plot (b), Line plot $y^*X_2 = X_1$ (c), $y^*X_1 = X_2$ (d).

Because this function is a quadratic in the unknown parameters, finding the minimum is a matter of using calculus to compute the first derivatives of the function with respect to each parameter and then solving the resulting system of equations. The resulting parameter estimates are functions of sums and sums of squares and cross-products of the response, y_i, and the predictors, x_1, x_2, ….

In the case were the $(X'X)$ matrix is not singular, the least squares estimation of the parameters b of β is given by:

$$b = (X'X)^{-1}X'y$$

The variance-covariance matrix of b is expressed in the following equation:

$$\text{var}(b) = \sigma^2 (X'X)^{-1}$$

with σ^2 the error variance. The elements of the matrix $(X'X)^{-1}$ are proportional to the variance and the covariances of the elements of b.

The variance of the prediction in a specific point x is given by:

$$\text{var}(xb) = \sigma^2 x'(X'X)^{-1}x$$

For each factor x_i that is discrete we create a corresponding set of indicator variables that are then used in the multiple-regression model above. So, for example, consider different leavening agents in a biscuit baking process, e.g., effects of yeast, enzymatic fermentation or baking powder on the final weight of the biscuit. For the three levels of leavening agent we would define two indicator variables:

$$z_1 = \begin{cases} 1 & \text{If leaving = yeast} \\ 0 & \text{If not} \end{cases}$$

$$z_2 = \begin{cases} 1 & \text{If leaving = baking powder} \\ 0 & \text{If not} \end{cases}$$

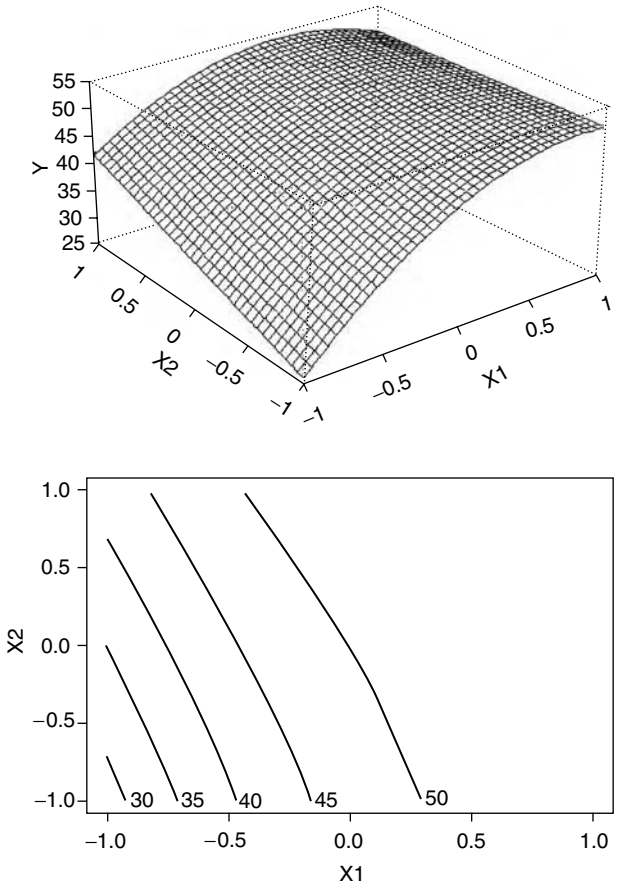

FIGURE 143.3 Perspective and contour plot of the second order model: $y = 50 + 8X_1 + 3X_2 - 7X_1^2 - 4X_1X_2$.

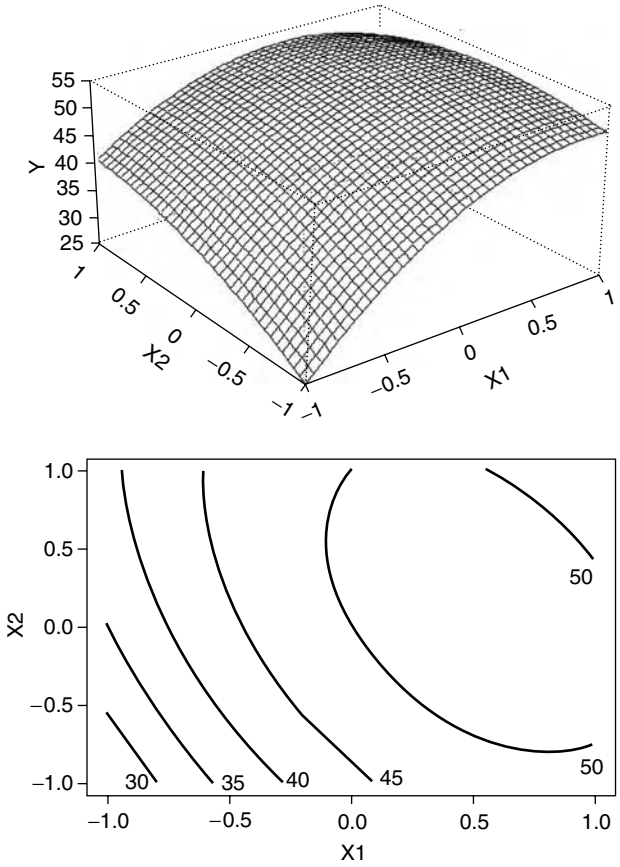

FIGURE 143.4 Perspective and contour plot of the second order model: $y = 50 + 8X_1 + 3X_2 - 7X_1^2 - X_2^2 - 4X_1X_2$.

By creating indicator variables, so called dummy coding, categorical variables can be included in response surface models.

For good reference books see Draper and Smith (4), Gunst and Mason (5) and Neter, Kutner, Nachtsheim and Wasserman (6).

V. BASIC CONCEPTS OF EXPERIMENTAL DESIGN

Experimentation is a basic part of scientific research. In experimentation, the researcher deliberately introduces certain changes in a process or product composition and makes observations to determine the effect of the different changes on a response. The success of the experimentation is a combination of having a good study plan or experimental design and careful and competent implementation of the treatment protocols. The key to a good experimental design is ensuring that the measurements taken will be relevant to the study objectives. Study objectives may be simple, such as getting the correct texture in the final product or may be quite complex involving an array of quality, cost and reliability measures.

All statistical experimental designs have a set of basic concepts in common. Let's elaborate the definitions and basic characteristics (7, 8).

A. Some Definitions in Experimental Design

An experimental factor or independent variable is a fixed variable, which is supposed to determine at least partially the system under study (and thus affects the dependent variable(s)) and which can take at least two different values or factor levels. A factor is denoted x_i for $i = 1, 2, \ldots, k$ and k is the number of experimental factors in the study.

An experimental treatment is a specific combination of a level for each factor. A treatment corresponds with a row vector consisting of a level for each factor ordered in an arbitrary way. Vertical concatenation of all these row vectors gives the treatment matrix.

The number of observations or measurements of the independent variable for each treatment are called replications.

An experimental unit is the smallest subdivision of an experiment to which a treatment is applied in a single trial. An experimental unit corresponds to a row vector representing a treatment.

The design matrix of an experiment consisting of n experimental units and k factors is a n*k matrix, with rows the experimental units.

The region of interest, the factor space of the experiment or the experimental region is the hyper-volume spanned by the points in the treatment matrix. Thus a treatment matrix has a geometrical equivalent. In classical experimental design theory both hyper-cubical and hyper-spherical experimental regions are most common.

The size of an experiment equals the number of treatments multiplied by the number of replications per treatment or the number of experimental units n.

The expanded design matrix is the design matrix extended with a certain number of columns depending on the assumed model. The total number of columns of this matrix equals the number of parameters to be estimated. For instance, for the second order model ($y = 50 + 8X_1 + 3X_2 - 7X_1^2 - 3X_2^2 - 4X_1X_2$) the design matrix consisting of the levels for X_1 and X_2 is augmented with columns representing X_1^2, X_2^2, X_1X_2 and a column of 1's for the intercept.

B. Basic Principles of Experimental Design

1. Replication

A key aspect of a scientific finding is that it can be replicated. In statistical experimental design we formalize this concept by requiring that each treatment be replicated a number of times. Replication allows us to quantify the degree of variability in the response within each treatment. By pooling this variability across all treatments we are able to assess the importance of average response differences among treatments. Without replication we are unable to tell whether the differences in response observed when the treatments are different are due to natural variability in the response of the experimental unit to any treatment or actually reflect a true shift in the underlying process.

Thus replication or measurement of the dependent variables for repetitions of the same treatment has two goals.

Firstly it allows us to obtain an estimate of the experimental error. This estimate of error becomes a basic unit of measurement to determine whether observed differences in the dependent variable are really statistically different. Thus the size of a detectable effect is determined by the number of replications.

Secondly replication permits obtaining more precise estimates of possible differences between treatments.

2. Randomisation: Protection Against Unknown Bias Introducing Factors

By randomisation is meant that both the allocation of the treatments to the experimental units as well as the order in which measurements are taken should be determined at random. The different treatments should be randomised over the experimental units when carrying out the experiment. This means that each treatment should have the same probability of being exposed to bias generating factors not included in the experiment. Thus randomisation of the treatments excludes the introduction of systematic bias into an experiment, by averaging out the possible effects of extraneous factors.

The use of randomisation is the keystone of the application of statistical theory to the design of experiments.

3. Blocking: Protection Against Known Bias Introducing Factors

Often, experimental units are grouped into homogeneous blocks at the start of the experiment. Blocking can be done to control for time or resource constraints, to ease implementation of large experiments or to account for a factor which is known to affect the response but which is not of primary interest. For example, if we are baking cookies in a small oven, the size of the oven places a limitation on how many cookies can be baked at one time. Each batch of cookies baked in the oven at the same time becomes a block. In the analysis of the resulting data, block effects will first be removed before we examine treatment effects. In this way, the final treatment comparisons are said to be independent of or adjusted for the blocking factor.

The blocking factor should be designed as independent (orthogonal) as possible with the other experimental factors. If this factor is not interacting with other factors, the blocking factor has a pure additive effect and is not interfering with the interpretation of the main factors.

4. Factorial Treatment Structures

When there are multiple factors that are of interest in the experiment it is typical that the treatments examined are formed by combining levels of one factor individually with all levels of the other factors: so called factorial experiments. Information obtained from factorial experiments is more complete than that obtained from a series of single factor experiments, because factorial experiments permit one to estimate the effects of several factors simultaneously and allow the evaluation of interaction effects. An interaction effect between experimental factors occurs if the difference in response between the levels of one factor is not the same at all the levels of the other factors. In the context of response surface estimation this involves the presence of significant cross product terms in the model.

A class of factorial designs that are used many times in the first stages of product development, namely for the screening of important experimental factors, are two level factorial designs. This design consists of all possible combinations of a low and a high level for k factors. Geometrically the treatment matrix describes a hyper-cube. For

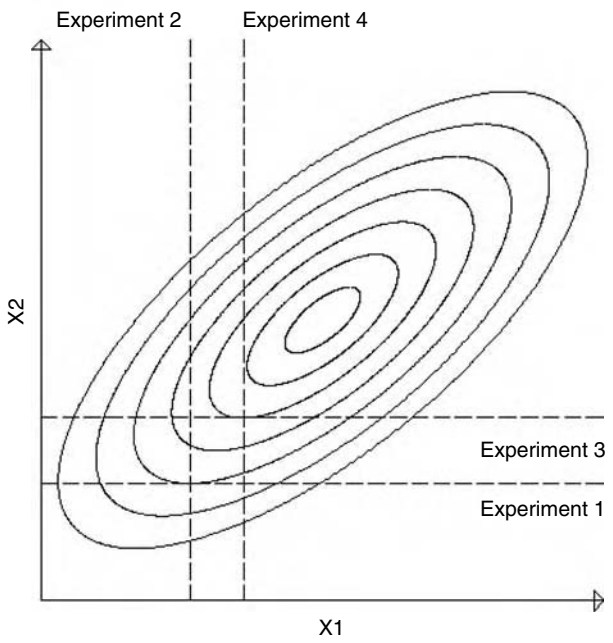

FIGURE 143.5 One-factor-at-a-time approach: an undefined number of experiments is necessary to attain the optimum.

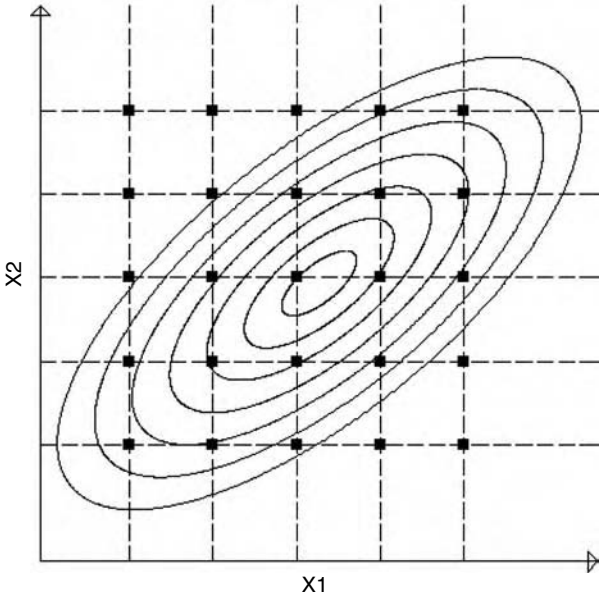

FIGURE 143.6 Factorial approach to optimisation.

example a 2^k factorial design consists of all combinations of the two levels for k factors.

It is natural to wonder why one should experiment with factorial treatments instead of using only simple treatments defined by levels of only one factor. Clearly, such an approach to experimentation is feasible, but the one factor at a time approach has a number of drawbacks. The major drawback is that it does not take into account the interactions that are known to exist among different factors that make up a processed food.

Suppose the contour plot of the real model looks like Figure 143.5. The main objective is finding the values for X_1 and X_2 were y is maximal, in other words find the top of the mountain. Proceeding with the one-factor-at-a-time approach could start with experiment 1. In this experiment X_2 is kept constant and X_1 is varied in several levels, resulting in an optimum value for y. Consecutively an experiment 2 is carried out with X_1 constant at the optimum of experiment 1 and the levels of X_2 changing. Again an optimum for experiment 2 is reached. As demonstrated in Figure 143.5, even for a simple response surface, a large number of experiments is necessary. Depending on the specific shape of the model and the chosen factor levels possibly the optimum is never reached, because this approach is extremely sensitive to local optima.

On the other hand if a factorial type of design, demonstrated by the grid on Figure 143.6, would have been imposed, the approximate shape of the model and the approximate location of the optimum would have been discovered in one experiment.

The sum of all the experimental units of all the one-factor experiments is most of the time much larger than the number of factorial combinations. Taking into account that the one-factor-at-a-time experiment does not guarantee finding the optimum, while the factorial does, it is obvious that factorial type experiments are by far more efficient in optimisation strategies.

Secondly the efficiency of finding significant effects increases with the number of factor combinations included in the experiment. Factorial experiments are very efficient in terms of the resources needed to achieve the experimental objective. In the separate one-factor experiments, the estimate of remaining uncertainty is based on replicates of only a few treatments. The factorial experiment typically uses a larger number of treatments and pools the information on residual uncertainty to produce more precise treatment effect estimates used in factor comparisons.

Finally, factorial treatment structures offer more flexibility in how the full set of treatments are implemented, allowing you to run a different subset of factor combinations in each block yet still incorporate factor interactions in the subsequent analysis.

5. Coding

When fitting first order models to 2^k factorial experiments, it is convenient to code the experimental factors as -1 for the low level and $+1$ for the high level. This corresponds to the transformation:

$$X_i^c = 2\left(\frac{X_i - \overline{X}}{\Delta_i}\right)$$

with X_i^c the coded factor level, X_i the original factor level, $\overline{X}$ the average of X_i and Δ_i the range of X_i.

For instance, if factor X_1 takes values 30 and 50, the coded X_i^c becomes:

$$2\left(\frac{30-40}{20}\right) = -1 \quad \text{and} \quad 2\left(\frac{50-40}{20}\right) = 1$$

Coded in this way, the parameter estimates of the first order model become contrasts in the measured response divided by the number of experimental units. This approach is equivalent to the classical analysis of variance procedures. In the sequel all treatment matrices will be coded like this.

C. Important Properties of Experimental Designs

1. Orthogonality

A design for a first order model is orthogonal when its coded design matrix D is an orthogonal matrix. The product of an orthogonal matrix with its transpose is a diagonal matrix, thus $D'D = I$, with I the unit matrix. Recall that the variance-covariance matrix of the model parameters in a least squares fit of a linear model is:

$$\text{var(b)} = \sigma^2 (D'D)^{-1} = \sigma^2 I$$

with σ^2 the error variance.

In this case the covariances of the model parameters are zero, in other words the parameter estimates can be assessed independently. Thus no confounding between experimental factors arises and effects can be interpreted directly. These designs are especially useful when a large number of experimental factors have to be screened to separate important from less important factors.

How to construct orthogonal designs? Combining each level of each factor an equal number of times always leads to orthogonal factorial designs. Replication of centre points retains orthogonality. A classical example of an orthogonal design is the 2^k factorial.

2. Rotatability

An experimental design is called rotatable if the variance of the predicted response depends on the distance to the centre of the design and not on the direction (7). Since the main aim of fitting a model that relates product attributes to ingredients and process variables is to optimise the predicted response, the variance of this predicted response is most important. The variance of the prediction in a specific point x is given by:

$$\text{var(xb)} = \sigma^2 x'(X'X)^{-1}x$$

The property of equal precision at any given distance from the centre point is desirable because it is not usually known in advance which direction from the centre will lead to optimal conditions. Rotatable designs are especially suited for second order models. The geometry of the discussed rotatable designs is hyper-spheric.

VI. EFINITION OF THE REGION OF INTEREST

A. Determination of the Important Factors and their Ranges

In this context two situations occur:

1. Sub-optimal operating conditions are known. In product development most of the time some 'sub-optimal' operating conditions are available or at least some knowledge exists about important and less important factors and their specific levels in function of the process under study. Starting from the actual operating conditions, namely a specific ingredient composition and/or process conditions, an optimal 'factorial type' design is constructed to explore the neighbourhood of these 'sub-optimal' conditions. In this case the experimental region is defined as a design centred at the 'sub-optimum.' Thus for each factor a range is chosen with as centre the sub-optimum. Some particular combinations of the levels of each experimental factor describe the vertices of a hyper-volume, centered at the sub-optimum. The choice of the actual range per factor depends on a priori knowledge about the effect of the factor on the response variable, on the nature of the factors (mixture or unconstrained) and on the assumed model as will be explained later.
2. No or not enough a priori knowledge about the process is known. When a priori knowledge about the importance of experimental factors or about the approximate location of the optimum is missing, an overall optimisation in multiple steps is necessary. In the first steps screening experiments are used to separate important components from less important ones over a relatively large experimental region, thus involving relatively large ranges for each factor. Once the key factors are determined, the approach in situation 1 can be followed to determine the region of interest.

B. The Choice of the Statistical Model for the 'Factorial Type' Approach

As demonstrated previously experimenting with all important factors simultaneously included in a 'factorial' type experiment is a far better approach than one-at-a-time approaches.

Once this multi-factor experimental region is defined, a model form has to be chosen that will be used to relate the experimental factors with the measured response within the experimental region. In a response surface approach the preferred models are polynomials of first and second degree, namely, linear and quadratic models.

First order models with or without interaction terms, (need at least experiments with 2 levels per factor) are appropriate in three situations:

1. Screening experiments to select the important factors out of a set of possible factors of influence.
2. Experiments in such narrow ranges that the expected effect on the response variable can be assumed to be linear. This approach is especially suitable in the 'Method of Steepest Ascent' (9).
3. When the real model is known to be linear.

A first order model fits a hyper-plane over the experimental region and as a consequence the maximal response will always occur on the border of the region if the plane has slope. This model only provides information about the main effects of the factors. If the fitted surface has no slope the experimental factors have no main effects within the experimental region; in other words a zone of equal response is found.

The model with interaction terms can take into account the fact that the response of one factor differs in function to the levels of other factors, but the response in the main effects is still linear.

Second order models (need at least experiments with 3 levels per factor) provide information about linear, interaction and curvature effects with respect to all or most of the independent variables. These models are widely applicable to describe experimental data in which system curvature is abundantly present. Thus these models are most appropriate to optimise a response over the experimental region, in other words to discover the levels of the ingredients and process conditions of the product that result in optimum product attributes.

The choice of the model is unquestionably linked with the choice of an optimal design and the nature of the factors. Upgrading from a first to a second order model involves an exponential increase of the amount of necessary treatments.

C. Practical Considerations

Optimisation of all the compositional factors of a product, together with all relevant process conditions necessitates a large number of component combinations or treatments, due to the high dimensionality of the experimental region or the high number of possible product ingredients and processing variables.

Of course the number of treatments has to be multiplied by the number of desired replicates per medium to get the real size of the experiments and a good estimate of the work and costs involved.

Although optimal from a theoretical point of view it is obvious that these large sized factorial experiments make no practical sense in optimisation. Although models fitted on these designs will increase the insight in the system, the estimation of the high order interactions is not necessary to find the optimum operating conditions. Response surface modelling emphasises the optimisation of a predicted response with an as 'simple' model approximation as possible. Thus, in most cases, the interpretation of the model parameters is of less importance, because the emphasis is on the estimation of the predicted response, resulting in the optimum conditions.

Moreover, in most practical optimisation situations in the food sciences (and in industry in general) the size of an experiment is restricted to a 'reasonable' number of experimental units due to external factors: product and equipment availability, limited time period to carry out measurements, financial limitations, manpower, etc. Especially in these high dimensional systems the practical feasibility of the experiment is the determining factor for the optimisation strategy, compromising between the number of factors and the complexity of the statistical model.

In practice, methodologies are searched for that have a high probability to find the optimum taking in account as small a number of treatments as possible, resulting in the impossibility to estimate high order interactions. These are defined as screening and response surface modelling designs. Distinction has to be made here between designs for unconstrained experimental regions and designs for constrained regions (mixture systems). What if the wrong model is assumed?

It is good practice to introduce a treatment in each experiment that can be used as a checkpoint to control the lack of fit. A simple approach is to fit the model based on all treatments except the checkpoint and then compare the predicted response in the checkpoint with the real measured value. In particular, this is used to test for possible curvature while fitting a first order model. The statistical approach to lack of fit uses a partitioning of the error sums of squares into pure experimental error and lack of fit as the foundation for formal tests (2).

VII. Optimisation Phase: Obtaining the Optimal Composition by Investigation of the Fitted Mathematical Model

A. Possible Situations

In the optimisation step the fitted response surface is used in the search for the optimum. Different situations result

as a function of the location of the optimum:

1. The optimum is located on the border of the experimental region. In this situation a principal direction of improvement can be defined, directed to the new optimum. A new experiment has to be carried out centered at the new sub-optimum, followed by re-evaluation. This is actually a search for the region of maximal response by consecutively fitting first order models. This procedure in which the experimenter proceeds sequentially along the path of maximum increase in response is called the 'Steepest Ascent Procedure' (9). Once the approximate location of the optimum is found a second order model is used to determine the optimum.
2. Within the experimental region no optimum is found. This means that the response is not affected by the changes of the factors within the range of the experiment. In other words the experimental region can be considered as a region of sub-optimal response. If desirable a new experiment can be set up centered at the sub-optimum but increasing the ranges of all factors. When fitting first order models carry out the lack of fit test to make sure that no curvature is present in the real model.
3. The optimum is found within the experimental region. The objective is reached. Double-check in case of local optima can be necessary. The fitted second order model can be investigated and the optimum can be determined.

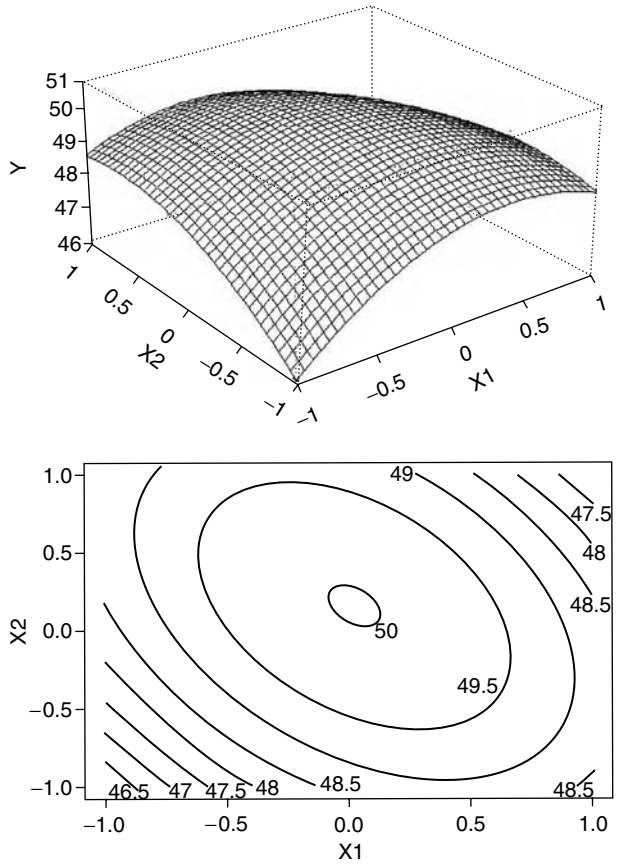

FIGURE 143.7 Perspective and contour plot of the second order model: $y = 50 + 0.2X_1 + 0.3X_2 - 1.5X_1^2 - 1X_2^2 - 1X_1X_2$. The stationary point is a maximum.

B. Determination of the Optimum for a Second Order Model

1. Contour and Perspective Plots

A contour plot is a two-dimensional graph showing contours of constant response in an axes system defined by two experimental factors X_i and X_j, while the other experimental factors are kept constant. In most practical situations the fitted model should be plotted to allow preliminary evaluation of the model and determination of the optimum. Perspective plots are a useful aid in interpretation of fitted models (see Figure 143.7)

2. Mathematical Procedures

If a second order model is fitted, the direct way to find the optimum is by differentiating the response function with respect to the X_i's. Setting all partial derivatives

$$\frac{\partial y}{\partial X_i} = 0$$

will determine the stationary point if it exists. It has to be emphasised that this point can be either a maximum, a minimum or a saddle point of the fitted function (Figures 143.7 and 143.8).

To determine the nature of the fitted surface and the stationary point, a canonical analysis has to be carried out. This analysis consists of two steps. First, the origin of the axes system is translated to the stationary point. Next, the axes system is rotated in such a way that the new axes correspond to the principal axes of the response surface or contour system.

In the new coordinate system, defined by W_i, the response function is given by:

$$y = y_0 + \lambda_1 W_1 + \lambda_2 W_2 + \cdots + \lambda_k W_k$$

where y_0 is the estimated response at the stationary point. This is called the canonical form of the fitted second order function. If all λ_i are negative, a move in any direction from the stationary point results in a decrease in y. Therefore, the stationary point represents the point of maximum response. If all λ_i are positive the stationary point is a minimum. When λ_i differ in sign the stationary point will be a saddle point.

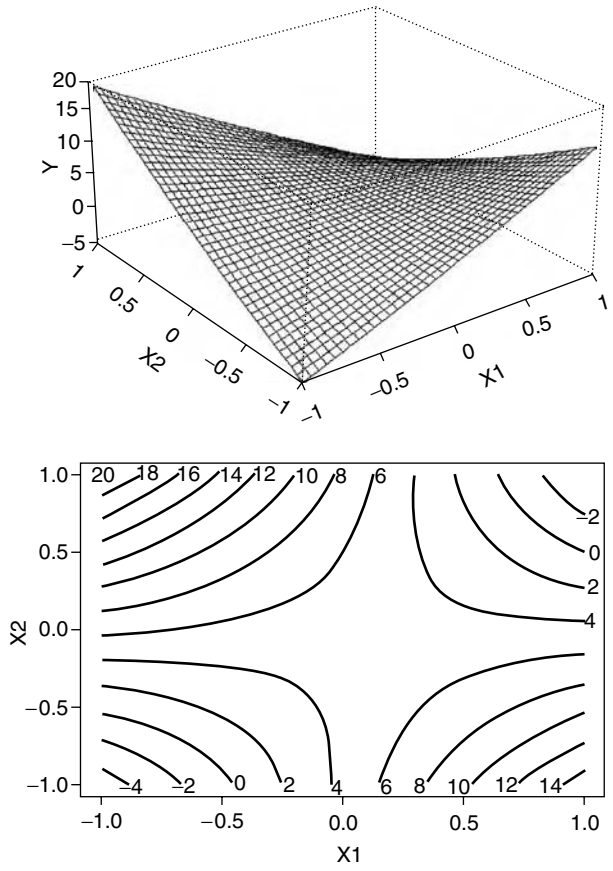

FIGURE 143.8 Perspective and contour plot of the second order model: $y = 5 - X_1 + 1.5X_2 + 0.5X_1^2 + X_2^2 - 11X_1X_2$. The stationary point is a saddle point.

For detailed description of the underlying mathematics the interested reader is referred to the specialised literature (1) (2).

C. Multiple Response Variables

Until now, methodology is described to optimise one response variable. In many situations, however, more than one response variable has to be optimised at the same time. When multiple responses are observed there is a need to reach some type of compromise as far as the optimal product attributes are concerned.

A simple approach is overlaying the contour plots of the fitted models for each individual response variable and then searching for regions of 'satisfactory' compromise. For a few responses this procedure mostly solves the problem. For multiple dimensions in response surface methodology the reader is referred to the specialised literature (1, 2). More general approaches, like multivariate analysis of variance and canonical correlation analysis can be found in multivariate analysis books (10, 11).

VIII. MIXTURE THEORY: IN SEARCH OF OPTIMAL COMPOSITION

Many products consist of a mixture of different ingredients, mixed together in a certain composition, for instance cake dough: a mixture of flour, butter, sugar and baking powder. As a function of the relative proportions of the different components the properties inherent to the final cake product differ. In the general mixture problem, the response that is measured is only a function of the proportions of the ingredients present in the mixture and not of the amount of the mixture. The textural properties of the cake or rheological characteristics of the dough are functions of the relative composition of its constituent and not of the amount of dough prepared.

In this chapter the possibilities and the major advantages of mixture experiments are briefly presented. More detailed information on mixture experiments can be found in the work of Cornell (12), Schrevens (13–15), Goel (16) and De Rijck (17).

A. Basic Concepts of 'Mixture Systems'

1. Whole Simplex Mixture

Mixture experiments can be distinguished by the fact that the independent variables represent proportionate amounts, rather than unrestrained amounts. The proportion of each component must be nonnegative. If the proportions are expressed as fractions, they must sum to unity. For a mixture system consisting of q components, with x_i the fraction of the ith component the following equations are valid:

$$0 \le x_i \le 1 \qquad \text{for } i = 1, 2, 3, \ldots, q$$

$$\sum_{i=1}^{q} x_i = 1$$

The q components of a mixture system are called 'mixture variables.' The proportion of each mixture variable can vary from 0 (the component is not present) to 1, a mixture with only one component, called a 'pure mixture.' If in a q component mixture the proportion of q–1 mixture variables is determined, then the proportion of the qth mixture variable is also determined:

$$x_q = 1 - \sum_{i=1}^{q-1} x_i$$

In this way the mixture equations reduce the q dimensional factor space to a q–1 dimensional simplex (18). This reduction in dimensionality is represented in Figures 143.9 and 143.10 for respectively a two and a three component mixture system. For a two component mixture the simplex factor space is an interval on a straight line (Figure 143.9). If there are three mixture variables the three dimensional space is reduced to a two dimensional surface, resulting in a sim-

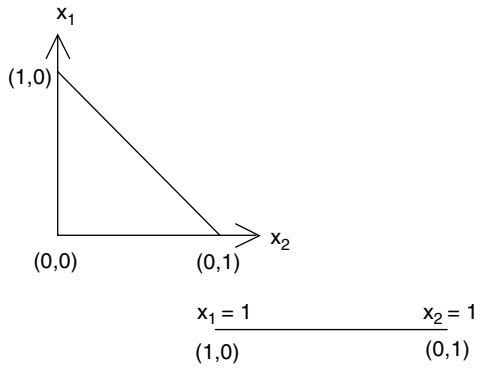

FIGURE 143.9 Simplex factor space for a two component mixture.

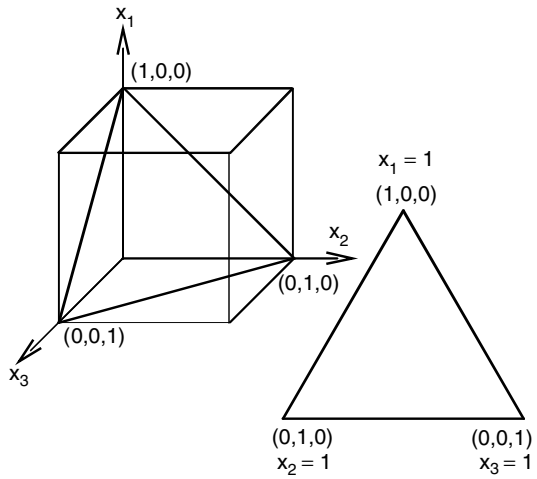

FIGURE 143.10 Simplex factor space for a 3 component mixture.

plex factor space represented as an equilateral triangle (Figure 143.10). Each point within the triangle represents a certain mixture, represented by its co-ordinates. The co-ordinate system used for the values of the mixture components is called a 'simplex co-ordinate system' or 'trilinear co-ordinates.' A four component mixture can be represented by a tetrahedron (Figure 143.11). For a mixture system with more than four components the factor space is a q–1 dimensional simplex or q–1 dimensional hyper-tetrahedron.

A typical whole simplex mixture model is the blending of different fruit juices (orange, pineapple, lemon) to optimise the taste of the mix. In this example pure mixtures, for instance 100% orange juice are possible treatments in the experiment.

2. Constrained Mixture System

In some cases the whole factor space is not feasible. Physical, physiological, chemical or economical considerations in mixture experimentation often impose constraints on the

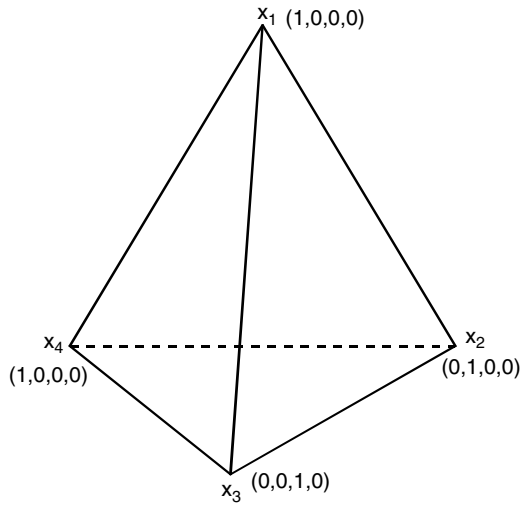

FIGURE 143.11 Simplex factor space for a 4 component mixture.

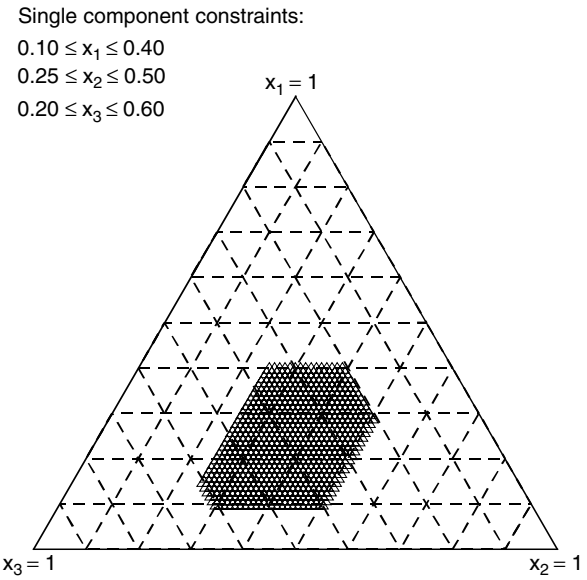

FIGURE 143.12 Subregion of a 3 component mixture.

levels of components in the mixture. These constraints define the region of interest and hence play an important role in the design and analysis of the mixture experiment (19). These constraints reduce the factor space to a sub-region. For instance cake dough as a mixture of flour, butter, sugar and baking powder is a good example of a constrained mixture.

Imposing a certain lower (l_i) and/or upper (u_i) bound on the proportion of the mixture variables, reduces the factor space to a sub-region (Figure 143.12) of the $(q - 1)$ — dimensional simplex, defined by the following equations:

$$0 \leq l_i \leq x_i \leq u_i \leq 1$$

with l_i and u_i respectively the lower and upper bound imposed on the component x_i. Taking into account the mixture equation, the following constraints are imposed on l_i and u_i:

$$\sum_{i=1}^{q} l_i < 1$$

$$\sum_{i=1}^{q} u_i > 1$$

$$l_i \neq u_i$$

If these equations are not satisfied only one component combination is possible ($l_1, l_2, \ldots, l_q$) or ($u_1, u_2, \ldots, u_q$) or none at all. For each $l_i = u_i$, the dimensionality of the factor space is reduced by 1 and the remaining components must sum to $(1 - l_i)$.

If the settings of the other constraints make it impossible for a component to reach its lower or upper bound then the sub-region is called "inconsistent" (19). Single component constraints are consistent if the sub-region contains the points ($x_1, x_2, \ldots, x_q$) so that for each component $x_i = l_i$ and $x_i = u_i$. Consistency of the constraints can be checked, using Piepel's method. Inconsistent single component constraints can be made consistent by decreasing the upper bounds or increasing the lower bounds in such a way that:

$$u_i + \sum_{j \neq 1}^{q} l_j = 1$$

$$l_i + \sum_{j \neq i}^{q} u_j = 1$$

3. Process Variables

If a mixture experiment includes factors besides the mixture components that are not bound by the mixture constraint, these are called 'process variables' (20). Changing the process variables may affect the blending properties of the mixture components. For instance the time of mixing dough may effect the properties of the dough. The mixing time is not a component of the mixture but a process variable of the mixture experiment. The experimental region of a mixture experiment with process variable(s) is the combined region of the mixture components and the process variable(s) (Figure 143.13). The dimensionality of the combined experimental region equals the sum of the dimensionality of the separate experimental regions. The 3 dimensional prism (Figure 143.13a) is the combined experimental region of the two-dimensional simplex (x_1, x_2, x_3) and the one-dimensional experiment in process variable z. In Figure 143.13b a 2^2 factorial design in the process variables is carried out at each of the treatments of a 3 component mixture design.

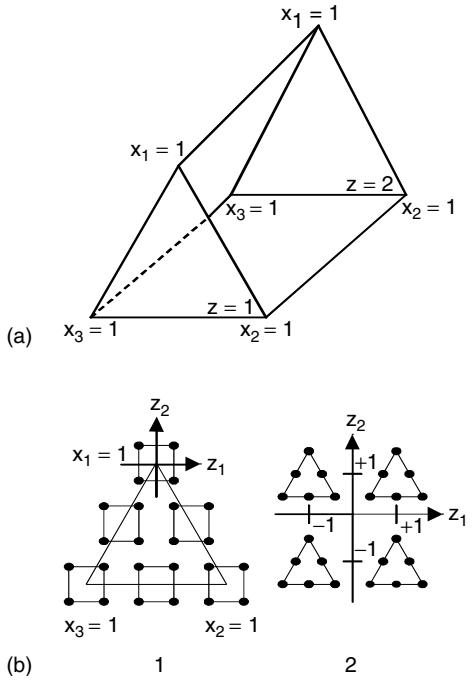

FIGURE 143.13 a: Combined region for a 3 component mixture (x_1, x_2, x_3) and 1 process variable (z); b: Combined region of a 2^2 factorial design and a {3, 2} simplex lattice design. 1: factorial design plotted per lattice treatment. 2: lattice design plotted per factorial treatment.

In some cases the response is a function of the amount of the mixture. For instance the mixing modalities of dough could be related to amount of dough. These kinds of mixture experiments are called 'mixture amount experiments' (21). The mixture amount experiment is a special case of a mixture process variable experiment.

B. Response Surface Models for the Analysis of Mixture Experiments

1. Specific Mixture Models: Canonical Polynomials

The first step in the design and analysis of mixture systems is the choice of a model to describe the response as a function of the mixture variables. As explained earlier polynomials are used to approximate the real model. Incorporation of the mixture constraint (sum of the proportion of the mixture variables equals 1) into polynomial models results in specific mixture models, named canonical polynomials (22).

This renders the following first order canonical polynomial:

$$y = \beta_0 \sum_{i=1}^{q} x_i + \sum_{i=1}^{q} \beta_i x_i = \sum_{i=1}^{q} \beta_i^* x_i$$

with: $\beta_i^* = \beta_0 + \beta_i$ for all i = 1, 2, …, q

Through analogous derivation the second order canonical polynomial is found to be:

$$y = \sum_{i=1}^{q} \beta_i^* x_i + \sum_{i<}^{q} \sum_{j}^{q} \beta_{ij}^* x_i x_j$$

for all i, j = 1, 2, …, q, i < j

In some cases a special cubic canonical polynomial could be of interest:

$$y = \sum_{i=1}^{q} \beta_i^* x_i + \sum_{i<}^{q} \sum_{j}^{q} \beta_{ij}^* x_i x_j + \sum_{i<}^{q} \sum_{j<}^{q} \sum_{k}^{q} \beta_{ijk}^* x_i x_j x_k$$

for all i, j, k = 1, 2, …, q, i < j < k

2. Models for the Inclusion of Process Variables

As defined by Cornell (12), process variables are factors in a mixture experiment that do not form any portion of the mixture, but whose levels, when changed, affect the blending properties of the ingredients. To model the response of the combined experiment, a combination is made of the model in the process variables and the mixture model.

For instance multiplication of a 'first order model with interaction' in two numeric process variables z_1 and z_2 with a second order mixture model in three components and combining the parameters δ_i and β_j in one parameter κ_{ij} renders the following combined model:

Process variable model: $y = \delta_0 + \delta_1 z_1 + \delta_2 z_2 + \delta_{12} z_1 z_2$

Mixture model: $y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$

Combined model:

$$y = \kappa_1 x_1 + \kappa_2 x_2 + \kappa_3 x_3 + \kappa_{12} x_1 x_2 + \kappa_{13} x_1 x_3 + \kappa_{23} x_2 x_3 + \kappa_{11} x_1 z_1 + \kappa_{21} x_2 z_1 + \kappa_{31} x_3 z_1 + \kappa_{121} x_1 x_2 z_1 + \kappa_{131} x_1 x_3 z_1 + \kappa_{231} x_2 x_3 z_1 + \kappa_{12} x_1 z_2 + \kappa_{22} x_2 z_2 + \kappa_{32} x_3 z_2 + \kappa_{122} x_1 x_2 z_2 + \kappa_{132} x_1 x_3 z_2 + \kappa_{232} x_2 x_3 z_2 + \kappa_{112} x_1 z_1 z_2 + \kappa_{212} x_2 z_1 z_2 + \kappa_{312} x_3 z_1 z_2 + \kappa_{1212} x_1 x_2 z_1 z_2 + \kappa_{1312} x_1 x_3 z_1 z_2 + \kappa_{2312} x_2 x_3 z_1 z_2$$

In these combined models, reduction in numbers of terms is desirable and can be accomplished using stepwise- or all-possible-regression model building procedures.

C. Design Strategy to Optimise Mixture Systems

The mixture design used for a specific situation must be constructed to accommodate the region of interest. The shape of this region and all associated constraints has a very strong influence on the final experimental design used.

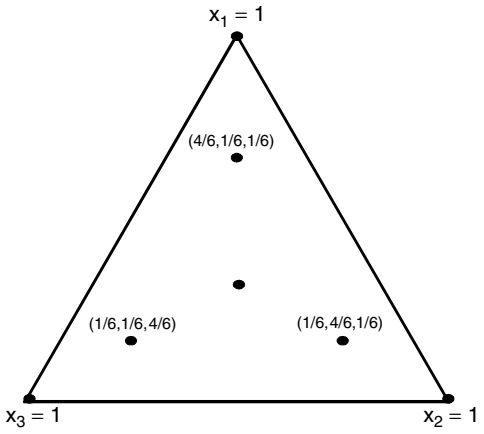

FIGURE 143.14 Three component simplex screening design.

1. Whole Simplex Mixtures

If no additional constraints are imposed on the system the experimental region is defined by the mixture constraint and thus coincides with the whole simplex.

To explore the whole simplex, the designs mostly used are the simplex screening, the simplex lattice and the simplex centroid mixture designs.

a. The simplex screening design

The simplex screening design (23) is appropriate for fitting a first order model. These designs are proposed when more than six components are involved in a screening experiment. These preliminary experiments try to gain more insight into the importance of the mixture components.

A q component simplex screening design consists of:

- q pure components: q permutations of (1,0,0,0,…,0)
- the overall centroid: 1/q, 1/q, 1/q, …, 1/q
- q interior points of the form: $[(2q)^{-1}, (2q)^{-1}, \ldots, (q + 1)/2q, \ldots, (2q)^{-1}]$

With this design q components can be screened with (2q + 1) treatments. An example is shown in Figure 143.14.

b. The simplex lattice design

A design used to explore the whole factor space is the '{q, m} simplex lattice design.' Each mixture variable varies with m + 1 equally spaced values from 0 to 1 (18):

$$x_i = 0, 1/m, 2/m, \ldots, 1 \quad \text{for } i = 1, 2, \ldots, q$$

In the design all possible treatment combinations with these proportions for each mixture variable are used (Figure 143.15). The number of points in the simplex lattice design can be calculated as follows (22):

$$\binom{q + m - 1}{m} = \frac{(q + m - 1)!}{m!\,(q - 1)!}$$

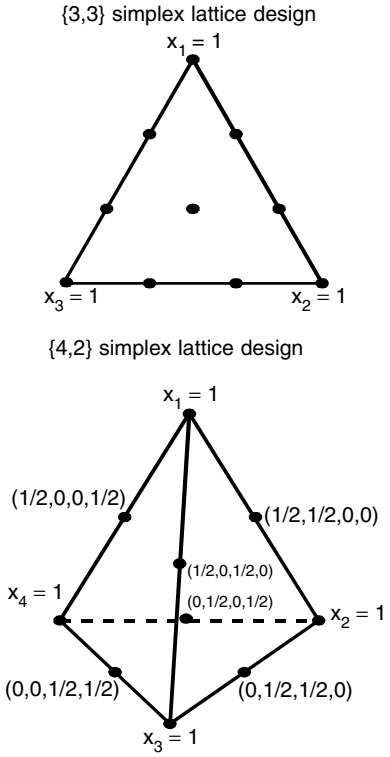

FIGURE 143.15 {q, m} Simplex lattice design.

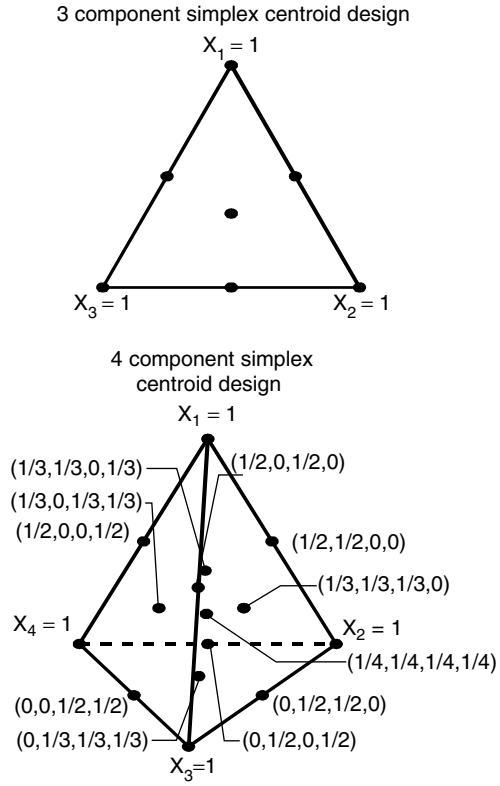

FIGURE 143.16 Simplex centroid designs.

The {q, 1} simplex lattice design is appropriate for first order models, while the {q, 2} and {q, 3} are suited respectively for a second order and special cubic canonical polynomial mixture model.

The {3, 2} simplex lattice design consists of the six points: (1, 0, 0), (0, 1, 0), (0, 0, 1), (½, ½, 0), (½, 0, ½) and (0, ½, ½). The first three mixtures are "pure mixtures" since they only consist of one component. The other mixtures are "binary mixtures" or two component mixtures.

b. The simplex centroid design

The simplex centroid design (20) consists of $2^q - 1$ points or mixtures:

– q pure components: q permutations of (1,0,0,0, …, 0)
– $\binom{q}{2}$ binary mixtures with equal proportions: $\binom{q}{2}$ permutations of ($^1/_2$,$^1/_2$,0,0, …, 0)
– $\binom{q}{3}$ ternary mixtures with equal proportions: $\binom{q}{3}$ permutations of ($^1/_3$,$^1/_3$,$^1/_3$,0, …, 0)…
– one q-nary mixture with equal proportions: the mixture ($^1/_q$,$^1/_q$,$^1/_q$,$^1/_q$, …, $^1/_q$)

The simplex centroid design includes the vertices of the centroid of the simplex and the centroid of all lower dimensional simplices, contained within the (q − 1)-dimensional simplex. For a given number of components there exists only one simplex centroid design, while different simplex lattice designs are possible. As an example a two and three component simplex centroid design is shown in Figure 143.16.

The simplex centroid design is especially suited to fit a special cubic canonical polynomial or to fit a second order model where the q-nary mixtures can be used as checkpoints for lack of fit.

2. Constrained Mixtures

Most mixture experiments are carried out in a sub-region of the total factor space defined by constraints. In functions of the imposed constraints the experimental region can be homomorphic with the simplex or can be a convex irregular polyhedron.

a. Homomorphic experimental region

If the experimental region is homomorphic or has the same shape as the whole simplex, the sub-region can be transformed into the whole simplex using a pseudocomponents transformation. In the transformed sub-region whole simplex designs in pseudo-components can be used as discussed in the paragraph on whole simplex designs.

Two cases occur: lower and upper bound constraints.

b. Lower bound restrictions

If in a mixture system consistent lower bounds are imposed on some or all of the components the resulting sub-region retains the shape of a simplex (Figure 143.17):

$$0 \le l_i \le x_i \le 1$$

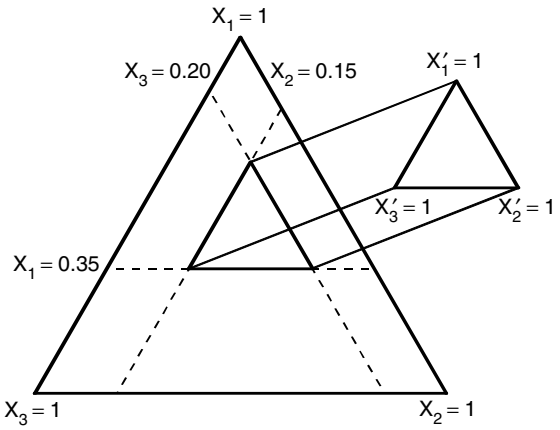

FIGURE 143.17 Transformation to L-pseudocomponents.

$$\sum_{i=1}^{q} l_i < 1$$

Some of the lower bounds l_i can be equal to zero. If the lower bounds are the same for each component, the centroid of the sub-region is the same as the centroid of the factor space. To describe the response surface over the sub-region with a polynomial equation, the model can be written using either the original mixture components with constraints or a lower-bound pseudocomponents transformation. The model calculations are more straightforward and easier to interpret when the lower-bound pseudocomponents approach is used. 'L-pseudocomponents x_i' are defined as follows (24):

$$x_i' = \frac{x_i - l_i}{1 - \sum_{i=1}^{q} l_i}$$

$$\text{with: } \sum_{i=1}^{q} l_i < 1$$

In Figure 143.17 this transformation is demonstrated. The resulting sub-region for a three component mixture system (x_1, x_2, x_3) constrained with the lower bounds $x_1 \geq 0.35$, $x_2 \geq 0.15$ and $x_3 \geq 0.20$, is transformed to L-pseudocomponents (x_1', x_2', x_3'), resulting in a whole simplex in L-pseudocomponents x_1', x_2' and x_3'.

c. *Upper bound restrictions*

Upper bound restrictions on some or all the components in a mixture system can be defined as:

$$0 \leq x_i \leq u_i \leq 1$$

Not all the mixture components need to be constrained by upper bounds. Upper bounds reduce the factor space to a simplex with the opposite orientation of the original simplex and enclosed in the whole simplex if and only if (25):

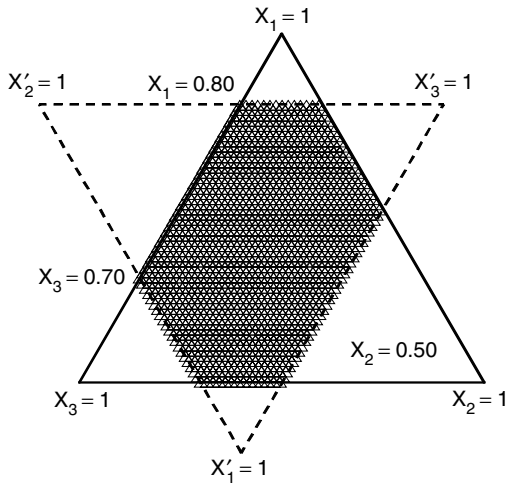

FIGURE 143.18 Transformation to U-pseudocomponents.

$$\sum_{i=1}^{q} u_i - u_{\min} \leq 1$$

with: u_{min} the minimum of the q upper bounds (exception made for the trivial case $q = 2$).

To transform the restricted simplex to a standard simplex the following pseudocomponent transformation can be used:

$$x_i' = \frac{u_i - x_i}{\sum_{i=1}^{q} u_i - 1}$$

Since the pseudocomponents simplex is opposite orientated, the effects of the pseudocomponents will also be opposite to the effects of the original components. If the boundaries of this simplex extend beyond the boundaries of the original simplex, the experimental region is an irregular hyper-polyhedron. The experimental region constrained by the upper bounds $x_1 = 0.80$, $x_2 = 0.70$ and $x_3 = 0.50$ in Figure 143.18 is an irregular hyper-polyhedron with 6 vertices.

d. *Lower and upper bound restrictions*

In practice, mixture components are quite often constrained by both lower and upper bounds. A mixture system constrained at the same time with lower and upper bounds can be defined as:

$$0 \leq l_i \leq x_i \leq u_i \leq 1$$

The resulting sub-region is homomorphic with the total factor space if the ranges ($u_i - l_i$) of all the components of the consistent set of constraints are equal and if the sub-region is positioned within the original simplex. Since

many sets of inconsistent constraint can define the same experimental region, while the region defined by consistent constraints is unique, the consistency of the constraints has to be checked first.

If a consistent set of constraints imposes an equal range on each component, the experimental range for both the lower and the upper bound simplex has to be calculated. The appropriate pseudocomponent transformation will be indicated by the smaller simplex. If this simplex is orientated in the same way as the original simplex, lower bound pseudocomponents are used; in the other case upper bound pseudocomponents are appropriate.

e. Convex irregular hyper-polyhedron

Most mixture experiments are carried out in sub-regions with an irregular shape due to the imposed constraints. In these cases a specific optimal design has to be computed for each specific situation using general optimal design theory. If high dimensional hyper-polyhedrons are investigated, the use of computer-aided design of experiments is essential.

An algorithm is necessary to list candidate points for the design, consisting of the vertices and the centroids of the faces of different dimensions, eventually extended with a number of interior checkpoints. These checkpoints will be used to test the goodness of fit of the model.

Once the candidate points are listed, a second algorithm selects an optimal set of design points from this list of functions of an optimal design criterion, related to the chosen model. This methodology falls beyond the scope of this text (12, 26–31).

D. Examples of Product Development Based on Mixture Theory

Although mixture experimentation strategies have been available since the sixties (oil, pharmaceutical, fertilizer, ceramics and explosives industries) it took until the eighties and even the nineties before these procedures were used in food engineering. Following are some extra examples of mixture product development and optimisation representing real industrial problems, solved by the research group of the main author.

1. The Experimental Region is the Whole Simplex: Optimisation of a Mixture of Different Fruit Juices

In a mixture experiment the consumer preference of a blend of orange-, pineapple- and grapefruit juices is optimised. Since non-linearity in response preference is expected a second order model is used and as a result a design to support the model is chosen. Table 143.1 and Figure 143.19 show a {3, 2} simplex lattice design in the three mixture components and the corresponding observed consumer preferences. A quadratic mixture model is appropriate (Table 143.2). This model has a coefficient of determination of 0.99, meaning that 99 % of the total variability is explained by the model. The contour plot of this model demonstrates that the pure mixture of orange juice is preferred above blends of different juices (Figure 143.19).

TABLE 143.1
{3,2} Simplex Lattice Design in Orange-, Pineapple — and Grapefruit Juice and Observed Average Consumer Preference Scores

Treatment	Proportion Orange Juice	Proportion Pineapple Juice	Proportion Grapefruit juice	Observed Preference. Average of 10 Consumers
1	1	0	0	85
2	0	1	0	78
3	0	0	1	65
4	0.5	0.5	0	80
5	0	0.5	0.5	68
6	0.5	0	0.5	70

2. A Constrained, Homomorphic Experimental Region: Optimisation of the Consumer Preference in Relation to Flour Composition of a Multiple Grain Cracker

In general, crackers are baked with only wheat flour. To develop a new type of cracker, part of the wheat flour was replaced with buckwheat, oats, barley and/or rye flour. To optimise the mixture of these 5 types of flour, the flour in the cracker dough was considered as a 5 component mixture system. Using mixture theory, a multifactorial {5,2} simplex lattice design was set-up in the wheat, buckwheat, oats, barley and rye factor space, resulting in 15 treatments (Table 143.3). The {5,2} simplex lattice design makes it possible to estimate both the main and the interaction effects of the experimental factors. Based on foreknowledge of the food technologist a lower bound constrained experimental region was selected, homomorphic with the total factor space and shifted towards the wheat vertex, meaning that the wheat proportion was partially replaced by the other flours.

The homomorphic experimental region was transformed to the whole factor space with a pseudocomponents transformation, resulting in a minimal confounding structure of the experimental design and thus more efficient parameter estimation.

The complete second order model in pseudocomponents proportions with 15 terms was fitted to the results. Using stepwise regression a reduced model with 6 terms was selected, representing the results with a coefficient of determination of 0.76.

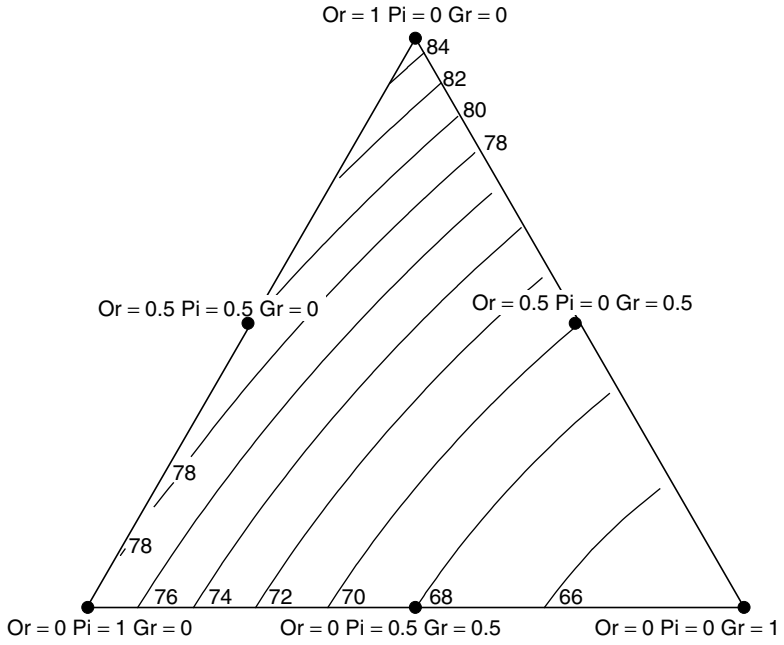

FIGURE 143.19 Contour plot of the quadratic mixture model, relating consumer preference with proportions of orange-, pineapple- and grapefruit juice. The treatments of the {3, 2} simplex lattice are labelled on the simplex.

TABLE 143.2
Parameter Estimate and Statistics of the Quadratic Mixture Model, Relating Preference with Fruit Juice Proportions

Variable	DF	Estimate	Standard Error	t Value	Pr > |t|
Orange	1	85.00000	1.31233	64.77	<.0001
Pineapple	1	78.00000	1.31233	59.44	<.0001
Grapefruit	1	65.00000	1.31233	49.53	<.0001
Or*Pina	1	−6.00000	6.42910	−0.93	0.3691
Or*Grape	1	−20.00000	6.42910	−3.11	0.0090
Pina*Grape	1	−14.00000	6.42910	−2.18	0.0501

This reduced model looks as follows:

$$\text{Preference} = 66.62\ \text{wheat} + 76.51\ \text{buckwheat} + 62.41\ \text{oats} + 40.99\ \text{barley} + 32.81\ \text{rye} - 129.39\ \text{wheat*buckwheat}$$

The reduced second degree model was used to represent the response surface over the experimental region. Since the experimental region is 4 dimensional and also the response has to be presented, a graphical representation demands a 5 dimensional factor space. For this reason the response surface was represented in two figures.

Figure 143.20 presents for 3 wheat and buckwheat levels (major horizontal and vertical axis respectively) and the response surface for oats, barley and rye. Increasing the wheat proportion and/or the buckwheat proportion reduces the maximum proportion of the other grains and consequently the size of the response surface.

TABLE 143.3
Pseudo-Component Proportions of the 15 Experimental Design Points of the {5, 2} Simplex Lattice Design. The Actual Mixing Proportions are Given Between Parenthesis (Proportion = 0 Are Not Marked)

Mixture	Wheat	Buckwheat	Oats	Barley	Rye
1	1 (1)	0	0	0	0
2	0 (0.84)	1 (0.16)	0	0	0
3	0 (0.84)	0	1 (0.16)	0	0
4	0 (o.84)	0	0	1 (0.16)	0
5	0 (0.84)	0	0	0	1 (0.16)
6	0.5 (0.92)	0.5 (0.08)	0	0	0
7	0.5 (0.92)	0	0.5 (0.08)	0	0
8	0.5 (0.92)	0	0	0.5 (0.08)	0
9	0.5 (0.92)	0	0	0	0.5 (0.08)
10	0 (0.84)	0.5 (0.08)	0.5 (0.08)	0	0
11	0 (0.84)	0.5 (0.08)	0	0.5 (0.08)	0
12	0 (0.84)	0.5 (0.08)	0	0	0.5 (0.08)
13	0 (0.84)	0	0.5 (0.08)	0.5 (0.08)	0
14	0 (0.84)	0	0.5 (0.08)	0	0.5 (0.08)
15	0 (0.84)	0	0	0.5 (0.08)	0.5 (0.08)

The more the response surfaces are positioned to the right (top), the higher the wheat (buckwheat) proportion. In each of the 6 response surfaces the oats and the barley proportions are represented. The point where the oats and the barley proportion equals 0, the rye proportion equals 1. The rye proportion is calculated as 1 minus the sum of the proportions of wheat, buckwheat, oats and barley. In each of the six response surfaces the preference can be read as a function of the proportion of the 5 grains.

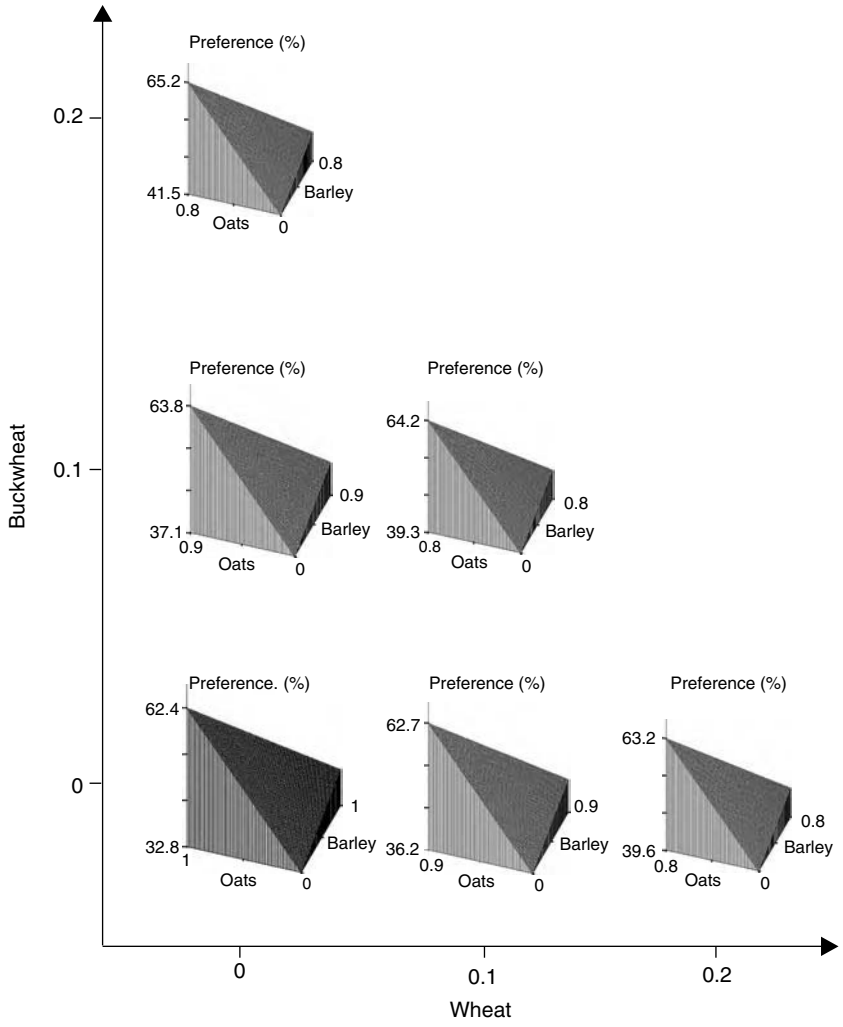

FIGURE 143.20 Preference over the oats, barley and rye factor space for 3 wheat and buckwheat levels.

The oats proportion of the mixture had a strong positive influence on the preference at low rye proportions. Reducing the oats proportion reduced the preference and mainly in the rye direction. Increasing the wheat and the buckwheat proportion from 0 to 0.2 increased the preference. Barley resulted in negligible differences in preference.

Figure 143.21 represents for 3 levels in oats and barley (major horizontal and vertical axis respectively) and the response surface for wheat, buckwheat and rye. Buckwheat had a strong positive influence on preference at low rye proportions. Reducing the buckwheat proportion reduced preference mainly in the rye direction. Buckwheat and wheat interacted antagonistically on preference. Increasing the barley and/or the oats proportion from 0 to 0.2 reduced preference at a high buckwheat and wheat proportion, while preference at a high rye proportion decreased.

The highest preference (76.5%) was obtained with a high buckwheat proportion combined with low wheat, barley, oats and rye; while a high rye proportion yielded the lowest preference (32.8%).

3. A Constrained, Homomorphic Experimental Region, Combined with Two Process Variables: Fat Reduction in the Cream of a Biscuit

This example investigates the possibility of reducing the fat content in the cream of a biscuit from 32 to 27%. The cream consists of 3 ingredients: cacao, sugar and fat. Reduction of the fat proportion involves an increase in the sugar and the cacao proportion. To keep the same high quality of the cream two compensating components are added in very low proportions: aroma and emulsifier. Measurements of intrusion forces in time (texture) are considered as response variables.

The cream is considered as a mixture system, because the total amount of cream should stay constant when cream composition is changed. A {3, 1} simplex lattice screening design (Table 143.4) is proposed to fit a first order model.

To prevent high collinearity between the independent variables due to the small range of the emulsifier (0–1%) and the aroma (0–0.2%), these variables are considered as

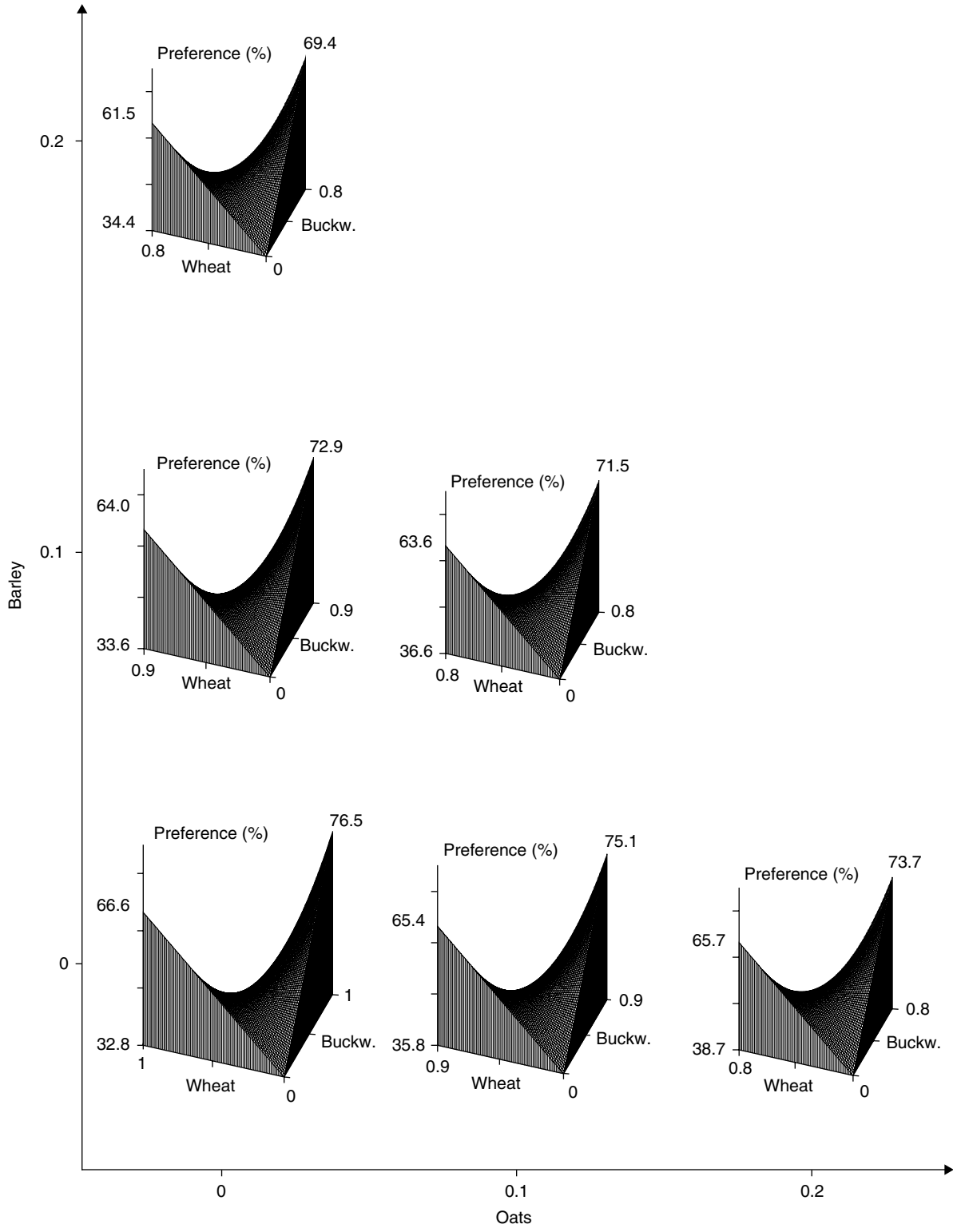

FIGURE 143.21 Preference over the wheat, buckwheat and rye factor space for 3 oats and barley levels.

process variables with a 2^2 factorial design is used for these factors (Table 143.5). The combination of both experimental designs (Table 143.4 and Table 143.5) results in a total of 12 (3*4) treatments and investigates a four dimensional experimental region, determined by 5 independent variables (Figure 143.22).

Each of the 12 creams was prepared 5 times, according to general practice. Penetrometer intrusion force

TABLE 143.4
Proportions of the Experimental Design Points in the Mixture Components

Mixture Treatment	Cacao Proportion	Sugar Proportion	Fat Proportion
1	0.12	0.56	0.32
2	0.17	0.56	0.27
3	0.12	0.61	0.27

TABLE 143.5
Proportions of the Experimental Design Points in the Process Variables

Treatment	Emulsifier	Aroma
1	0	0
2	0	0.002
3	0.01	0
4	0.01	0.002

(force 1) of the cream was measured 1 hour, 1 day and 1 week after preparation of the cream at a temperature of 22°C. In this way, time is considered as a process variable and extends the 4 dimensional experimental region to 5 dimensions.

The complete combined model (Table 143.6) represents the data with a coefficient of determination of 0.84. In the complete combined model only the parameter estimates of the variables cacao, sugar, cacao*time, sugar*time and fat*time are significantly different from zero. Using stepwise regression a reduced model with 8 terms is selected (Table 143.7), still representing the results with a coefficient of determination of 0.81. The reduced model consists of the main effects cacao, sugar and fat, their interaction effects with time and the triple interactions sugar*emulsifier*time and fat*emulsifier*time. The emulsifier interacts antagonistically with sugar and time and with fat and time.

The reduced second degree model is used to represent the response surface over the experimental region. The complete experimental region is 5 dimensional (6 factors). Since aroma has no significant effect on the texture of the cream, this factor can be removed from the experimental design, reducing the experimental region to 4 dimensions.

Figure 143.23 represents for 2 emulsifier and 3 times (major horizontal and vertical axis respectively) and the response surface for cacao, sugar and fat. The right horizontal axis represents the sugar pseudocomponent proportion and the left horizontal axis represents the cacao pseudocomponent proportion. The pseudocomponent proportion of fat equals 1 minus the pseudocomponent proportion of cacao and sugar.

The major changes in force 1 (penetrometer intrusion force) are caused by time. Increasing time increases force 1. Increasing the amount of emulsifier from 0 to 10 grams reduces force 1 and even more as time increases. If no emulsifier is added to the cream, force 1 increases as the sugar proportion increases. If 10 gram of emulsifier is present, the highest force 1 is reached at a low sugar and fat proportion and a high cacao proportion.

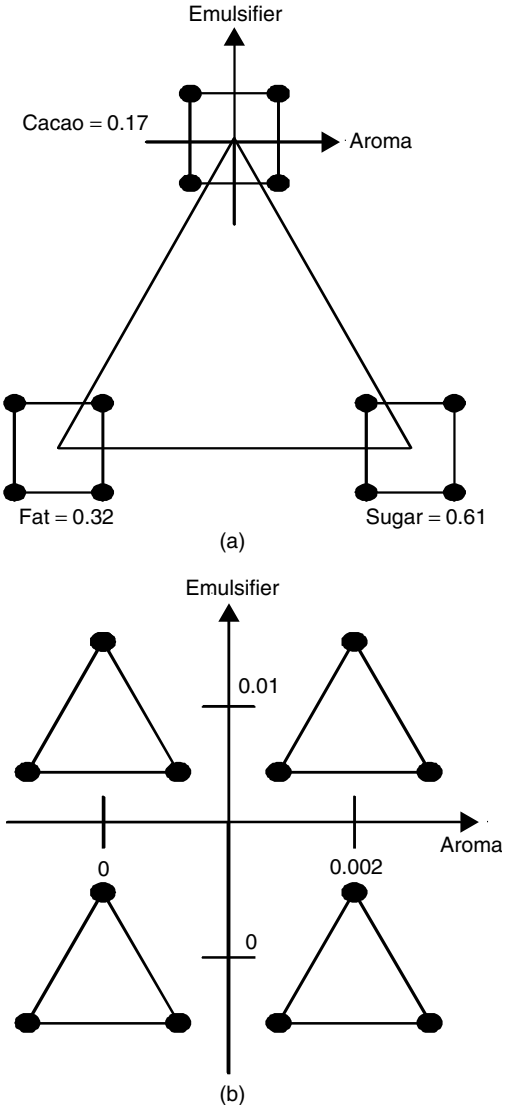

FIGURE 143.22 Combined experimental design of a 2^2 factorial design in emulsifier and aroma and a {3,1} simplex lattice design in cacao, sugar and fat. Factorial design nested in the mixture design (a). Mixture design nested in factorial one (b).

IX. DESIGN STRATEGY TO OPTIMISE UNCONSTRAINED EXPERIMENTAL FACTORS

Typically in the development of a food product, unconstrained experimental factors represent processing conditions or additives. Usually the latter are applied in small amounts and thus do not have to be considered as

TABLE 143.6
Regression Statistics for the Complete Combined Model

Variable	Parameter Estimate	Prob > T
Ca	536.69	0.0215
Su	563.57	0.0168
Fa	299.57	0.1661
ca*em	−34.56	0.2522
su*em	−25.85	0.3858
fa*em	−1.66	0.9549
ca*ar	−90.32	0.7821
su*ar	−123.43	0.7058
fa*ar	121.96	0.7091
ca*ti	4.97	0.0336
su*ti	4.76	0.0404
fa*ti	4.63	0.0452
ca*em*ar	17.52	0.7047
su*em*ar	16.06	0.7282
fa*em*ar	−11.25	0.8075
ca*em*ti	−0.43	0.1700
su*em*ti	−0.41	0.1832
fa*em*ti	−0.38	0.2201
ca*ar*ti	0.87	0.7930
su*ar*ti	0.07	0.9831
fa*ar*ti	−0.20	0.9527
ca*em*ar*ti	−0.10	0.8267
su*em*ar*ti	−0.03	0.9515

TABLE 143.7
Regression Statistics for the Reduced Model

Variable	Parameter Estimate	Prob > T
Ca	362.68	0.0027
Su	414.89	0.0008
Fa	320.84	0.0069
ca*ti	2.99	0.0125
su*ti	5.42	0.0006
fa*ti	4.77	0.0021
su*em*ti	−0.55	0.0029
fa*em*ti	−0.40	0.0256

compositional experimental factors, bounded by the mixture constraint. To optimise these factors classical experimental design theory can be used. Since the optimisation strategy described in this chapter highly relies on response surface fitting, designs for first and second order response surface models are emphasised. General reference books are (7, 8).

A. Designs for First Order Models

As previously explained the fitting of these models involves designs with 2 levels per factor and at least k + 1 treatments (with k the number of experimental factors). For screening experiments with large amounts of components the Plackett-Burman and fractional factorial designs are most appropriate.

1. Placket-Burman Designs

A Plackett-Burman design is an orthogonal screening design, consisting of a fraction of a 2^k full factorial with its main property a tremendous reduction of the amount of treatments (32). As a consequence no interactions between factors are estimable, but all main effects can still be interpreted through a first order model.

In Table 143.8 a 12 treatment Plackett-Burman design is given as an example. To apply these designs, the experimenter assigns a column to each chosen experimental factor under study (columns in the table) and then allocates treatments randomly at least 5 more than the number of experimental factors to allow the estimation of experimental error and valid test statistics about the model.

For instance, to run a 7 factor screening experiment, 7 columns are chosen in Table 143.8 and the 12 treatment combinations are assigned. The data are analysed with a first order polynomial regression model. For 7 to 15 experimental factors the 20 treatment Plackett-Burman is especially suitable. For more than 12 and less than 23 components the 28 treatments Plackett-Burman is appropriate (32). For screening of less than 7 factors the 12 treatment design can be chosen, although for these cases fractional factorial designs are also available. Table 143.9 shows which design to choose as a function of the number of experimental factors.

2. Fractional Factorial Designs

These designs are also fractions of 2^k full factorial designs, but only a few fractions are allowed because these designs emphasise 'balance' in estimating factor effects. In other words the estimation of each specific main effect should consist of geometrically balanced differences of measured responses on high and low factor levels. The geometry of a 2^3 factorial is compared to the geometry of a ½ fraction in Figure 143.24. The reduction in the number of treatments results in higher order interactions, the specific ones depending on the fraction chosen (33). A schematic overview of possible designs is given in Table 143.10. To use these designs in 6, 7 or 8 factors, select respectively the first 6, 7 or 8 columns of Table 143.11 and assign the 16 treatments.

3. Addition of Points in the Centre of Screening Designs

The centre of a coded design corresponds with the treatment coding (0, 0, … , 0). Addition of replicated centre points makes possible lack of fit test that essentially checks whether the real response surface is linear. This lack of fit

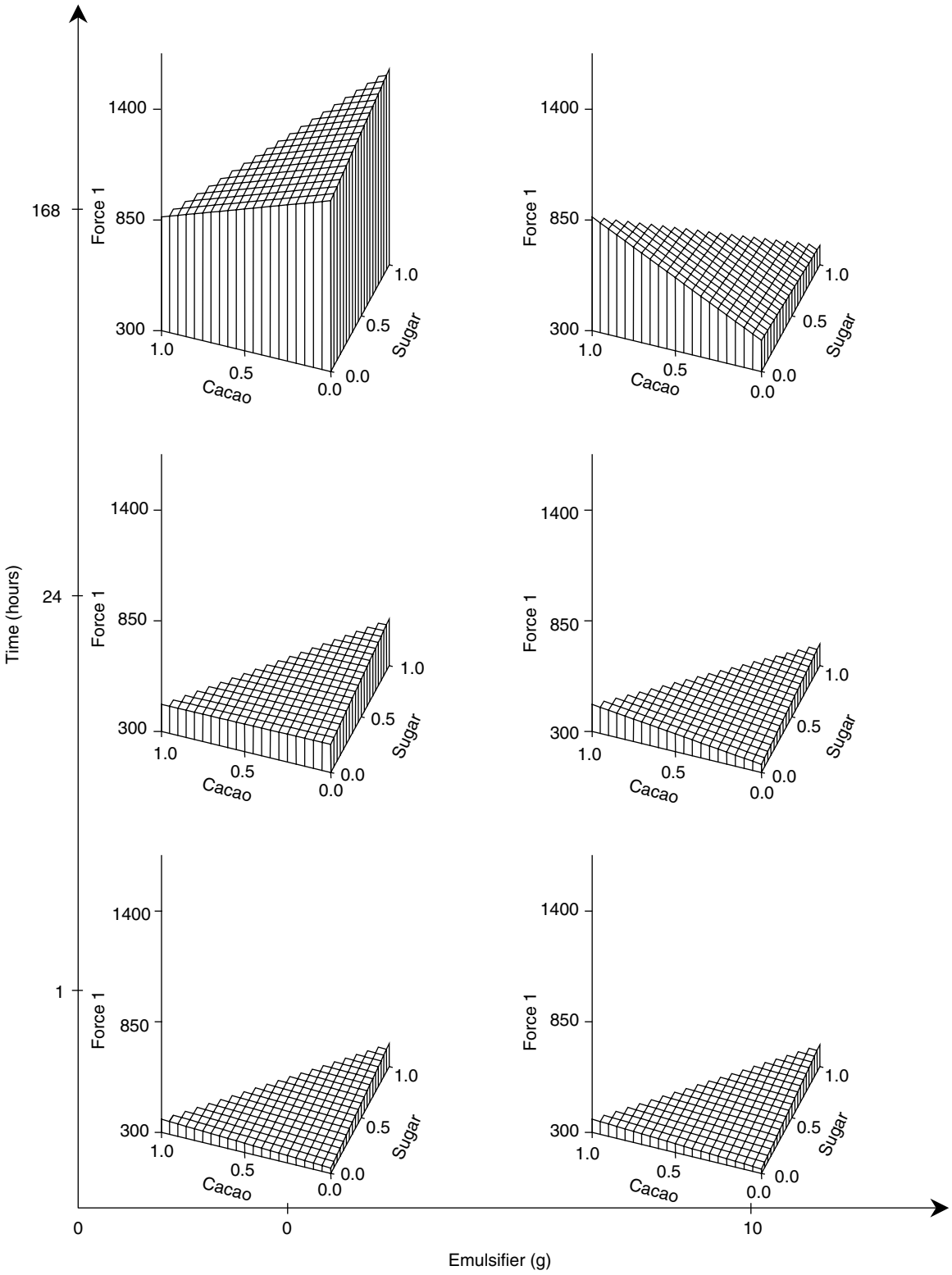

FIGURE 143.23 Intrusion force (force 1) as a function of time, emulsifier and cream composition.

TABLE 143.8
Plackett-Burman Design with 12 Treatments

	Experimental Factors										
Treatment	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
1	+	+	−	+	+	+	−	−	−	+	−
2	+	−	+	+	+	−	−	−	+	−	+
3	−	+	+	+	−	−	−	+	−	+	+
4	+	+	+	−	−	−	+	−	+	+	−
5	+	+	−	−	−	+	−	+	+	−	+
6	+	−	−	−	+	−	+	+	−	+	+
7	−	−	−	+	−	+	+	−	+	+	+
8	−	−	+	−	+	+	−	+	+	+	−
9	−	+	−	+	+	−	+	+	+	−	−
10	+	−	+	+	−	+	+	+	−	−	−
11	−	+	+	−	+	+	+	−	−	−	+
12	−	−	−	−	−	−	−	−	−	−	−

TABLE 143.9
Plackett-Burman Screening Designs

Number of Factors	Possible Design
<7	12 treatments, fractional factorial is better
7–15	20 treatment
13–23	28 treatment

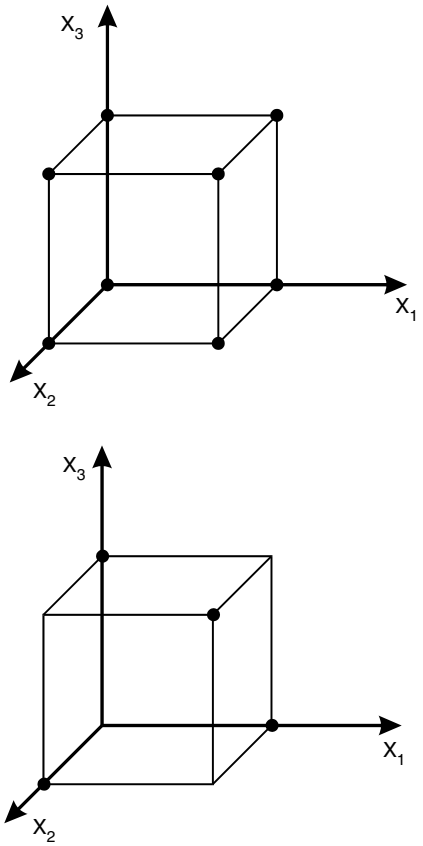

FIGURE 143.24 Geometry of a 2^3 full factorial (a) and a fractional factorial (1/2 replication) (b).

TABLE 143.10
Fractional Factorial Models for Screening Designs

Number of Factors	Possible Design
<4	Full factorial
4	1/2 fraction of 2^4: 8 treatments
5	1/4 fraction of 2^5: 8 treatments
6	1/4 fraction of 2^6: 16 treatments
7	1/8 fraction of 2^7: 16 treatments
8	1/16 fraction of 2^8: 16 treatments

TABLE 143.11
Fractional Factorial Screening Designs for 6, 7 and 8 Factors in 16 Treatments

Treatment	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
1	−1	−1	−1	+1	+1	+1	−1	+1
2	+1	−1	−1	−1	−1	+1	+1	+1
3	−1	+1	−1	−1	+1	−1	+1	+1
4	+1	+1	−1	+1	−1	−1	−1	+1
5	−1	−1	+1	+1	−1	−1	+1	+1
6	+1	−1	+1	−1	+1	−1	−1	+1
7	−1	+1	+1	−1	−1	+1	−1	+1
8	+1	+1	+1	+1	+1	+1	+1	+1
9	+1	+1	+1	−1	−1	−1	+1	−1
10	−1	+1	+1	+1	+1	−1	−1	−1
11	+1	−1	+1	+1	−1	+1	−1	−1
12	−1	−1	+1	−1	+1	+1	+1	−1
13	+1	+1	−1	−1	+1	+1	−1	−1
14	−1	+1	−1	+1	+1	+1	+1	−1
15	+1	−1	−1	+1	+1	−1	+1	−1
16	−1	−1	−1	−1	−1	−1	−1	−1

is calculated as the difference between the mean responses of all design points (equally replicated) and the mean response in the centre point. To test the curvature for specific factors requires a design for a second order model.

B. Designs for First Order Models with Two-Factor Interaction Terms

1. Reflected Plackett-Burman Designs

A reflected Plackett-Burman design arises from a specific Plackett-Burman design augmented with the same design but all '+' changed to '−' and vice versa. Thus the 12 run design is augmented to a 24 treatment design, the 20 run to 40 runs and the 28 to 56 treatments.

The addition of these treatments makes the independent estimation of main effects and 2-factor interactions possible. A summary is given in Table 143.12.

2. Fractional Factorial Designs

When a larger fraction of a 2^k full factorial is taken, not only main effects but also 2-factor interactions can be estimated (Table 143.13). An example for 6 experimental factors is given in Table 143.14. Additional fractional factorial design plans can be found in the literature (34, 35) or in specific software packages for experimental design (36).

C. Designs for Second Order Models

Experimental designs for fitting second order models require at least three levels of each experimental factor so that the parameters of the model can be estimated. An obvious choice for such a design would be a 3^k factorial, namely a factorial experiment with each of k factors at three levels in all possible combinations. For small numbers of factors (2 or 3, resulting in respectively 9 and 27 treatments) this approach is still feasible. For 4 factors, 81 treatments are involved to fit only 15 model parameters (one intercept, four first order coefficients, four full quadratic and six cross-products) in a full quadratic second order model. Starting with 5 factors the number of treatments becomes prohibitively large ($3^5 = 243$). Several authors have suggested specific second order designs that offer a compromise between relative precision in model parameter estimates and the amount of experimental effort measured as the number of treatments. The Box-Behnken and central composite designs are mostly used. Typically, these designs are appropriate for second order models in two to eight factors (37) (9). If more than eight factors are involved the designs become unpractically large.

TABLE 143.12
Reflected Plackett-Burman Screening Designs for Estimation of First Order Models with 2-Factor Interactions

Number of Factors	Possible Design
<7	24 treatments, fractional factorial is better
7–15	40 treatment
13–23	56 treatment

TABLE 143.13
Fractional Factorial Screening Designs for Estimation of First Order Models with 2-Factor Interactions

Number of Factors	Possible Design
<5	Full factorial
5	1/2 fraction of 2^5: 16 treatments
6	1/2 fraction of 2^6: 32 treatments
7	1/2 fraction of 2^7: 64 treatments
8	1/4 fraction of 2^8: 64 treatments

1. Box-Behnken Designs

Box-Behnken designs are subsets of 3^k factorial designs (37). Except for the centre points, all points are centroids of the edges (or faces) of a hypercube with dimensions

TABLE 143.14
Fractional Factorial Design (1/4 Fraction of 2^6) for 6 Factors in 32 Treatments for a First Order Model with 2-Factor Interaction Terms

Treatment	X_1	X_2	X_3	X_4	X_5	X_6
1	−1	−1	−1	−1	−1	−1
2	+1	−1	−1	−1	−1	+1
3	−1	+1	−1	−1	−1	+1
4	+1	+1	−1	−1	−1	−1
5	−1	−1	+1	−1	−1	+1
6	+1	−1	+1	−1	−1	−1
7	−1	+1	+1	−1	−1	−1
8	+1	+1	+1	−1	−1	+1
9	−1	−1	−1	+1	−1	+1
10	+1	−1	−1	+1	−1	−1
11	−1	+1	−1	+1	−1	−1
12	+1	+1	−1	+1	−1	+1
13	−1	−1	+1	+1	−1	−1
14	+1	−1	+1	+1	−1	+1
15	−1	+1	+1	+1	−1	+1
16	+1	+1	+1	+1	−1	−1
17	−1	−1	−1	−1	+1	+1
18	+1	−1	−1	−1	+1	−1
19	−1	+1	−1	−1	+1	−1
20	+1	+1	−1	−1	+1	+1
21	−1	−1	+1	−1	+1	−1
22	+1	−1	+1	−1	+1	+1
23	−1	+1	+1	−1	+1	+1
24	+1	+1	+1	−1	+1	−1
25	−1	−1	−1	+1	+1	−1
26	+1	−1	−1	+1	+1	+1
27	−1	+1	−1	+1	+1	+1
28	+1	+1	−1	+1	+1	−1
29	−1	−1	+1	+1	+1	+1
30	+1	−1	+1	+1	+1	−1
31	−1	+1	+1	+1	+1	−1
32	+1	+1	+1	+1	+1	+1

equal to the number of factors. Thus all these treatments lie on a single hyper-sphere and thus are equally distant from the centre. This geometrical property is associated with rotatability, or the variance of the predicted response depends only on the distance to the centre of the design and not on the direction. These designs have a hyperspheric geometry as can be deduced from Figure 143.25 and are especially useful for experiments in 3 to 7 experimental factors. As an example a design plan for four experimental factors is given in Table 143.15.

2. Central Composite Designs

The central composite designs, introduced by Box and Wilson (9) are by far the most used designs for second order models. These designs consist of a 2^k full factorial or a fractional factorial, augmented with 2^k star points and n_c centre points. If a fractional factorial is used, the main and two-factor interaction effects should be estimable (see designs for first order models with two-factor interaction terms). The star points are located on the main axes of the coded design the same distance from the centre as the factorial points. In other words, both the factorial and the star points are located on a hyper-sphere around the centre of the design. The centre point is replicated to estimate pure error. This design involves five levels for each factor coded as $(-\alpha, -1, 0, 1, \alpha)$, α being the distance from each star point to the centre. To provide the design the desirable property of rotatability, α has to be chosen according to Table 143.16. This choice assures that star and factorial points belong to the same hyper-sphere (Figure 143.26).

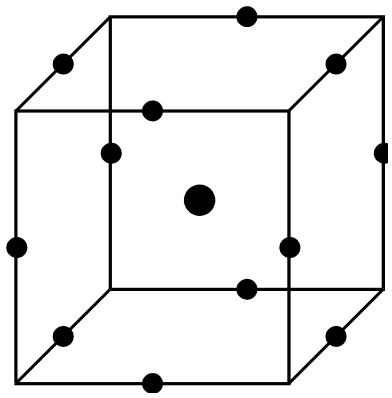

FIGURE 143.25 Geometry of a three-factor Box-Behnken design for a second order response surface model.

TABLE 143.15
Four-Factor Box-Behnken Design (Centre Point Three Times Replicated)

Treatment	X_1	X_2	X_3	X_4
1	+1	+1	0	0
2	+1	−1	0	0
3	−1	+1	0	0
4	−1	−1	0	0
5	0	0	+1	+1
6	0	0	+1	−1
7	0	0	−1	+1
8	0	0	−1	−1
9	+1	0	0	+1
10	+1	0	0	−1
11	−1	0	0	+1
12	−1	0	0	−1
13	0	+1	+1	0
14	0	+1	−1	0
15	0	−1	+1	0
16	0	−1	−1	0
17	+1	0	+1	0
18	+1	0	−1	0
19	−1	0	+1	0
20	−1	0	−1	0
21	0	+1	0	+1
22	0	+1	0	−1
23	0	−1	0	+1
24	0	−1	0	−1
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0

TABLE 143.16
Rotatable Central Composite Designs for Three to Seven Factors

Number of Factors	Full or Fractional Factorial	Value for α	Number of Factorial Treatments	Number of Star Points	Number of Replicated Centre Points	Total Number Treatments
3	full	1.68	8	6	6	20
4	full	2	16	8	6	30
5	full	2.38	32	10	8	50
5 (1/2)	½ replication	2	16	10	8	34
6	full	2.83	64	12	10	86
6 (1/2)	½ replication	2.38	32	12	10	54
7	full	3.63	128	14	10	152
7 (1/2)	½ replication	2.83	64	14	10	88

The sequential nature of these designs is very useful in many problems. Firstly the factorial part of the design, including centre points, can be run to estimate a first order model. If the lack of fit test, based on the replicated centre points, indicates that additional curvature is necessary, in a second step the design can be augmented with the star points to provide degrees of freedom for a second order model. For example, the three-factor central composite design in Table 143.17 consists of a 2^3 factorial (first 8 treatments), augmented with 2*3 star points ($\alpha = 1.68$) and 6 centre points.

D. Example of Process Optimisation for Unconstrained Experimental Factors

1. A Box-Behnken Design for a 3 Factor Optimisation Experiment

Suppose the work on a new product has proceeded to the point where three experimental factors are thought to be important in affecting a quality property. The experimental factors are temperature, pressure and concentration; the independent variable is a numeric quality attribute.

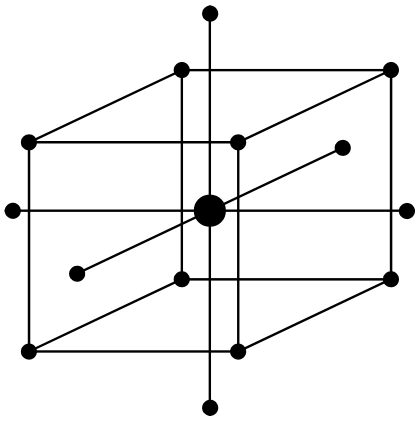

FIGURE 143.26 Geometry of a three-factor central composite design for a second order model.

From preliminary screening experiments the ranges for the factors were set. It is decided to use a Box-Behnken design in search of the optimum response over the experimental region.

Table 143.18 shows the coded design points, the actual values and the measured quality index (response).

A second order full polynomial is fitted by ordinary least squares to estimate the parameters of the model. Model

TABLE 143.17
Three-Factor Central Composite Design (Centre Point Six Times Replicated)

Treatment	X_1	X_2	X_3
1	1.00	1.00	−1.00
2	−1.00	1.00	−1.00
3	1.00	−1.00	−1.00
4	−1.00	−1.00	−1.00
5	1.00	1.00	1.00
6	−1.00	1.00	1.00
7	1.00	−1.00	1.00
8	−1.00	−1.00	1.00
9	1.68 (α)	0.00	0.00
10	−1.68 (−α)	0.00	0.00
11	0.00	1.68 (α)	0.00
12	0.00	−1.68 (−α)	0.00
13	0.00	0.00	1.68 (α)
14	0.00	0.00	−1.68 (−α)
15	0.00	0.00	0.00
16	0.00	0.00	0.00
17	0.00	0.00	0.00
18	0.00	0.00	0.00
19	0.00	0.00	0.00
20	0.00	0.00	0.00

Table 143.18
Box-Behnken Second Order Design for Three Experimental Factors

Treatment	Coded Temp	Coded Pres	Coded Conc	Temperature °C	Pressure Mp	Concentration g/l	Quality Index %
1	1	1	0	90	900	20	44
2	1	−1	0	90	100	20	37
3	−1	1	0	20	900	20	39
4	−1	−1	0	20	100	20	29
5	1	0	1	90	500	30	44
6	1	0	−1	90	500	10	30
7	−1	0	1	20	500	30	17
8	−1	0	−1	20	500	10	38
9	0	1	1	50	900	30	42
10	0	1	−1	50	900	10	48
11	0	−1	1	50	100	30	32
12	0	−1	−1	50	100	10	39
13	0	0	0	50	500	20	50
14	0	0	0	50	500	20	48
15	0	0	0	50	500	20	49

TABLE 143.19
Parameter Estimate of the Full Quadratic Response Surface

Independent Variable	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	11.584652	3.891373	2.98	0.0309
Temperature	1	0.598643	0.070795	8.46	0.0004
Pressure	1	0.024635	0.005406	4.56	0.0061
Concentration	1	1.153598	0.273709	4.21	0.0084
Temperature*Temperature	1	−0.008705	0.000513	−16.97	<.0001
Pressure*Temperature	1	−0.0000555	0.000041831	−1.33	0.2415
Pressure*Pressure	1	−0.0000117	0.000003829	−3.06	0.0281
Concentration*Temperature	1	0.025051	0.001673	14.97	<.0001
Concentration*Pressure	1	0.0000625	0.000147	0.42	0.6887
Concentration*Concentration	1	−0.068750	0.006126	−11.22	<.0001

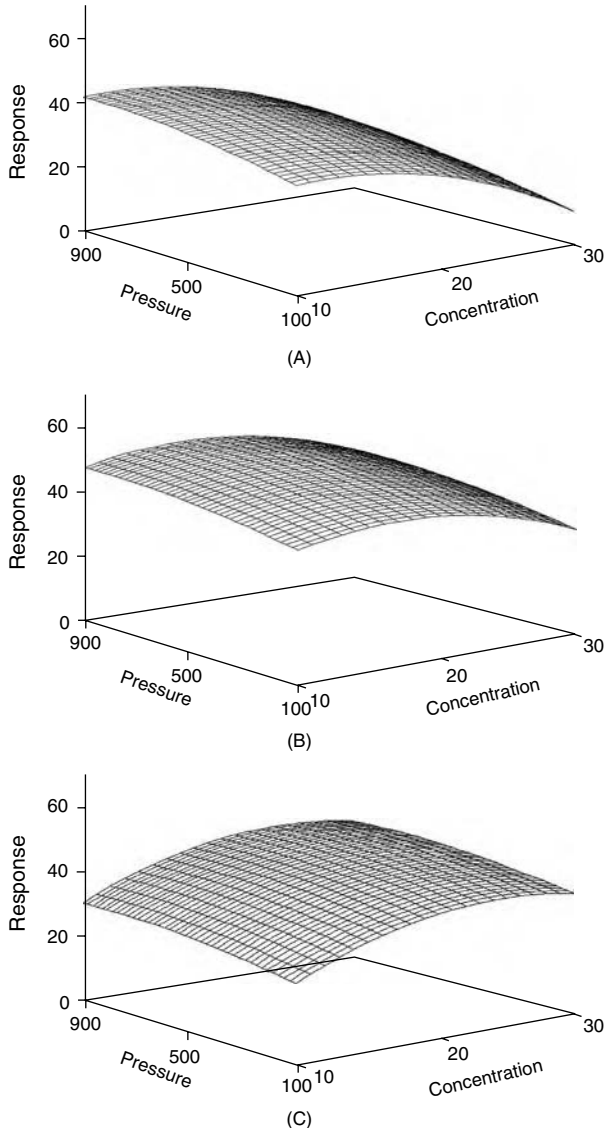

FIGURE 143.27 Perspective plot of response in relation to pressure and concentration at different temperatures. A: temperature = 20°C, B: temperature = 50°C and C: temperature = 90°C.

parameters and statistics are given in Table 143.19. As can be concluded from this table, all parameters are highly significant except the pressure-temperature and the pressure-concentration cross product coefficients. This model is represented as three perspective plots of concentration versus pressure at three different temperatures (Figure 143.27).

A canonical analysis revealed that the optimum is a maximum. At a temperature of 59.6, a concentration of 19.7 and a pressure of 962 the quality index was maximised and reached a value of 52.6%.

This simple example elucidates the design and analysis strategy elaborated in this chapter: determination of the factors of interest, design and model selection in relation to objectives and results of preliminary screening, model fitting and computation of the optimal conditions based on model predictions.

X. CONCLUSIONS

In this chapter experimentation strategies to optimise product attributes in function of ingredients and process conditions during product development and optimisation are elaborated.

The nature of the experimental factors, mixture- or unconstrained components, determines in a large part the design and modelling procedures must be followed.

A generic approach based on response surface methodologies is proposed, due to the high dimensionality of the compositional problem and the high probability of interacting experimental factors.

Emphasis is put on the geometry of experimental regions and the specific designs to explore the region of interest in relation to the assumed model. In addition many designs are given to aid the experimenter and to provide a framework for experimentation.

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144 Model Building

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CONTENTS

I. INTRODUCTION

Food engineering and technology groups a multidisciplinary approach towards safer and better quality food products. This involves the application of methodologies developed in basic sciences (e.g. chemistry, biology, physics and mathematics) and applied sciences (i.e. biochemistry, microbiology and statistics) into the applied field of food. Mathematical modelling of food phenomena has a long tradition with early examples as the development of the Student t-test to study brewing (1) or the Bigelow model in the area of sterilisation (2).

A mathematical model is a representation that captures the interesting properties of the food system of interest. The form of this representation can be: (i) an algorithm, (ii) an equation, (iii) a graphical representation, (iv) some form of experimental data or (v) any combination of them.

Mathematical modelling as such provides powerful tools to (3):

- **Construct** models of the situation under study.
- **Analyse** interactions between components of the food systems.
- **Research** and complement other methodologies to study and master the complexities of the food systems and the complex interactions that happen during the whole food chain (4).

With the advances of analytical and microbiological techniques the paradigm in food technology of studying single food processes analysing their components with powerful analytical and microbiological tools has produced the body of the present technology applied in the food industry and this paradigm has satisfactorily solved most of the problems to both research and the industry. However, with the spread of affordable IT systems that have placed mathematical modelling tools in the hands of the researchers, the former methodology has been complemented with the addition of mathematical modelling techniques. This change has provided an integrating tool able to tackle some of the most complex problems in food technology and engineering (3).

II. OBJECTIVES OF MODEL BUILDING

Mathematical modelling is an objective oriented task where the whole model building process is greatly influenced by the particular application the model is developed for. The objectives of the work, the particular task that the model will perform and even the particular field on which the models are applied will actually determine many of the features of the model. A typical example is the utilisation of Bigelow's model in food microbiology, where this model is widely accepted and used to study the thermal treatment of bacteria/spores (e.g. pasteurisation/sterilisation). Despite the fact that this model is almost equivalent to the first order reaction model with an Arrhenius temperature dependence (used to describe kinetics of chemical reaction and widely extended in many fields of science), the Bigelow equation is still used and will most probably be used in future studies in the areas that have as an objective the study of heat treatment of bacteria (at least while the model and their parameters are useful to transmit information by microbiologists).

Mathematical models are widely used for different purposes in food and engineering, falling usually in one of the following general categories (3).

A. Prediction and Forecast

Model predictions and forecasts are used when an estimation of the property of interest in the future is sought. Generally food phenomena are time dependent and in this way the study of kinetics in food (observing the variation of a property in food during time). Moreover, since most food processes are operated in batch mode, food processing models usually need to include time as an important variable. Some typical examples are:

- The prediction of microbial growth in foodstuff: Mathematical models are generated using previous knowledge and experimental data to be employed in the prediction of the future microbial population in the foodstuff (5, 6, 7) although not exhaustive, provide two typical examples of research work in this area.
- The prediction of food shelf life where mathematical models are used to forecast the time of failure of the critical quality factor(s) of a certain product. The modelling of accelerated shelf life studies can provide an estimate of the product shelf life at retail temperatures (8).
- The modelling of food process phenomena where the food is subjected to different environmental conditions during the time of the process (9).

B. Scenario Modelling

Scenario modelling is used to study the behaviour of the system of interest under new conditions. Some typical examples of scenario modelling are:

- Secondary models in predictive microbiology. The behaviour of microbial growth under different conditions of pH, temperature and other factors is mapped, in order to map the different scenarios of product formulation and storage/distribution that have not been previously tested (9, 10).
- The study of the influence of environmental factors in the processing/storage/retail of foods.

C. Sensitivity Analysis

Sensitivity analysis uses simulation of different scenarios to examine the behaviour of the response(s) in a model with the systematic variation of the different variables involved in it. The main objectives of the sensitivity analysis are:

- To rank the different model inputs by their influence in the response.
- To discover new interactions of the different variables that are involved the model.

Sensitivity studies are closely linked to the study of the derivatives of the models in respect to the input variables. They are usually linked to the further optimisation and control of the particular phenomena and are a particular tool in the area of food engineering (11, 12) where they serve as a preliminary analysis to select the most appropriate variables that can be subjected to optimisation and control.

D. Optimisation and Control

Optimisation and control make use of the mathematical model to meet process/product specifications and direct the phenomena towards target points. The optimisation of food processes is largely based on mathematical models and it has a long tradition (13) since the optimisation of

heat sterilisation from Teixeira et al. (14). Other areas include drying and mass transfer (15, 16). Optimisation and control of food processes presents considerable challenges (13):

- Food phenomena are usually noticeably non-linear and models that are capable of describing fundamentally the phenomena are complex.
- The constraints that have to be fulfilled for an appropriate optimisation and/or control are as well significantly non-linear coming from costing or quality.
- The process is usually affected by many variables that can be global or locally distributed in the product. This complicates the process given that models have to be built to describe the local property variation, usually involving the interaction of several transport phenomena. This usually implies the solution of one or more partial differential equations (PDEs). For example, the drying of spaghetti can involve under reasonable drying conditions a global temperature dynamic, a local moisture content and possibly a local stress field (16).
- Variability and uncertainty in the product and process conditions can present additional difficulties to the optimisation process. An optimal policy for an "average" product may result in significant losses of product that presents different characteristics (17).

One of the most significant benefits of model building is actually to be able to control or to optimise the process/phenomena under study (e.g. 18). This can yield to significant gains in profit and quality of the food products that arrive to the consumer. Despite this fact and the wide appearance of mathematical models in the food engineering area, the exploitation of mathematical models via optimisation and control has yet to become a mainstream activity (13).

E. Metamodelling

Metamodelling is a modelling task that intends to condense the information from complex mathematical models into simpler models. Metamodelling is a modelling tool that makes complex modelling results accessible. Two typical examples of metamodelling:

- Hayakawa et al. (19) studied hygrostress modelling where the information about the stress during the drying of a sphere or a plate was condensed in charts. The charts can be used to produce predictions of stress during a certain drying process without the need of having or knowing how to use simulation software.
- Silva (20) studied optimal sterilisation policies using transient heat transfer PDA in two dimensions together with quality and microbiology thermal kinetics equations. After finding the optimal temperatures for different conditions, polynomial models that allowed for the calculation of the optimal temperature with a reasonable precision were developed. In this way, optimal sterilisation temperatures could be calculated for a new process with a simple spreadsheet and without having to use the heat transfer model.

III. MODEL BUILDING PROCESS

Model building involves the procedure to construct a mathematical model from a particular situation. It usually involves the following tasks (3):

i) **Identification:** *What is the phenomenon to be explored? What is the purpose of a model in these circumstances? What is the information already available? What experiments have to be made? What are the assumptions one can reasonably make? What approach should be taken?*
ii) **Estimation:** *What are the parameters one is looking for? How to obtain parameter estimates for the model? How to solve the problem?*
iii) **Validation:** *Is the model valid for application in the particular situation under study?*
iv) **Application:** *For what is the model needed?*
v) **Maintenance and Iteration:** *Is the problem solved? Does one need to do further work and build more detailed or simpler models? Does one need to revise initial assumptions?*

These steps provide a methodology to develop models applied to a particular situation, and although is rarely the case in practice where they are followed sequentially, they show the different tasks to be taken into consideration. While estimation, validation and application of a mathematical aspects of the model building have been studied by operation research and statistics extensively, model identification, maintenance and iteration of the mathematical model are not yet as developed as the former methodologies.

IV. TOWARDS AN ECLECTIC APPROACH IN MODEL BUILDING

Model building in food has benefited from methodologies already developed to use them for the advance of food technology and engineering. In this way two main

approaches for model building have been laid out from developments in physics and chemistry respectively:

a) Inductive or empirical model building.
b) Deductive of fundamental model building.

Both modelling approaches provide sound methodologies to solve problems in the area of food technology and engineering, with their advantages and pitfalls (21, 22). Gilchrist (23) proposed a somehow "mid-way" between the two of them in an eclectic approach to model building.

A. Inductive Modelling

Inductive modelling uses the *observational approach*, with experiments and inductive modelling techniques to build one or more empirical models that can approximate the particular situation under study. The estimation and validation steps of the model gain a bigger weight, where parameters have to be estimated with a maximum precision to ensure a prediction and the model or the models group is validated to ensure a good model selection process. Given the dependence of the model building process on the experimental data, statistical methods are the basis of inductive modelling (22).

Empirical models can involve different constructions that mimic the behaviour of the system under study and aim at extracting some of the useful information that experimental data from the study can provide. They also can be used to reproduce the behaviour of the system in questions as a *black box* model, to be used for control, optimisation or sensitivity analysis purposes. A particular example of empirical modelling is the classical linear regression model, where a vector response y is expected to behave in a linear fashion in respect to a matrix of X and an error term.

One can hope that this relationship holds under a limited range of study of the independent variables and then can be applied in this range for all uses (24). Other empirical models can include (generalised) linear models, survival models, multivariate statistical models, time series models, modern regression methods, spatial statistics, Markov Chain Monte Carlo models, AI models (neural networks, SOM, Fuzzy logic, etc.), decision trees or data mining techniques (25).

Empirical model building can involve several steps in the building of a model:

i) Experimental design
ii) Parameter estimation
iii) Model assessment
iv) Model validation
v) Model maintenance

1. Experimental Design

The development of an experimental design that will offer reasonable chances to build the model is of prime importance in the model building procedure. Performing experiments is usually a costly task in both academy and industry and often a "one-off" opportunity: Once the set of experiments is done, the possibility of repeating or modifying the experiment is rarely available. In this view, each experiment needs to be rigorously designed to extract the maximum information from the phenomena. The "old-way" of trying-each-thing-at-once has been long time ago demonstrated to be a very inefficient way of laying an experimental design, bound to lose the more complex interactions of the phenomena (24). The statistical design of experiments allows for the minimisation of resource wasting and ensures for precision and lack of bias of the results (24). Statistical experimental design is highly dependent on the type of mathematical model that the statistician wants to use to describe the data and because of this, the planning of an experiment is an essential step in inductive modelling. The way the experiment will be planned will influence the further steps in the model building process. In this way, before planning the experiments the scientist has to gather as much information:

- Rigorous definition of the objective(s) of the work. Without this, it is impossible to design experiments that will contribute to the solution of the problem at hand.
- Selection of the variables of interest in the study. Given that food is a complex biological material in most of the situations, the number of factors that can affect food phenomena can be numerous. A preliminary selection of the most important factors, based on literature, work objectives criteria will help to construct the experimental design.
- If working with continuous variables, the range covered by the study has to be selected. What are the interesting values for the variables? How much will be the response(s) varying? Are the changes in the response measurable?
- Analysis of the expected associated uncertainty and variability of the experiment. What are the sources of uncontrolled variability? Which other factors can contribute to the uncertainty in the experiment? Can those factors be controlled or not?
- Preliminary study of the structure of the model that will be built. Is one devising a linear or a non-linear model? What are the dependencies between the responses and the factors? Can one separate the different levels of the model: For example a modelling step that is concerned with the primary covariate (e.g. time) and another modelling step concerned with the secondary environmental factors in the study (e.g. temperature).

- Planning of the final report design before the experiment is carried out, in order to identify weaknesses in the experimental design.
- Perform trial or preliminary calculations to determine whether the experiment will fulfil the objectives.

The research team will have to spend a reasonable amount of time discussing these aspects of the experiments in order to devise an appropriate experimental design that will contribute to the research work. The main issues that a statistical experimental design will have to deal with are (for a thoroughly description see chapter 50 by Schrevens and Portier):

a) Randomisation to eliminate bias coming from uncertainty in the parameter estimation and prediction.
b) Replication to allow for the estimation of the residual variance and to test the significance of the factors under study.
c) Blocking of the experiments, considering the available experimental units and randomised assignment of the experimental blocks to each of the experimental days. This will:
   - Avoid bias coming from time variation.
   - In the case that random effects are important in the experiment (uncontrollable known factors that affect variability) will help to the estimation of the random effects variance component (26).
d) Factorial treatment structure that will allow studying the effect of different factors in the experiment. It has been demonstrated that experiments that try each of the factors one-at-a-time spend considerable experimental resources compared with factorial designs (26).

a. Experimental design for linear model building

Experimental design is a subjective methodology that depends on the type of model that data is expected to follow. The simplest kind of relationship between a response and a series of factors is to assume a linear relationship, which is an assumption that one can hold in a narrow interval of study (24). The development of experimental design started with the work from Fisher and developed in the context of statistical analysis of laboratory and pilot plant experiments with the ideas from Box and in the design and analysis of industrial scale experiments with the work of Taguchi (26). For a more concise focus on experimental design for product development and optimisation, please refer to chapter 50 (Schrevens and Portier).

Factorial designs

Factorial designs are the most used experimental designs for laboratory and food pilot plant studies when working with linear models. Several different options are available (24):

TABLE 144.1
Example of a Full 2^k Factorial Design with Two Replications to Study the Influence of Pasteurising Temperature (− = 60°C, + = 90°C) and pH (− = 3.0, + = 7.0) in the Shelf Life of a Food Product. The Experiment Had Two Experimental Units That Could Be Used Each Day to Treat the Samples. Blocks Are Not Randomised

Repetition	Block	Unit	Temp	pH
1	1	1	+	+
		2	−	+
	2	1	+	−
		2	−	−
2	3	1	+	+
		2	−	+
	4	1	+	−
		2	−	−

i) When the relationship between the response and the variables is linear and one is happy to explore the main effect and the interaction between the different variables in the response there are several alternatives:
   a) When comparing two different treatments with a single factor randomised block designs can be used to elucidate if the treatment produces a significant effect.
   b) When the objective of the study is to screen the effect of several factors 2^k full factorial designs can be used to help to select the most important variables and their interactions. See Table 144.1 for an example of implementation.
   c) If the number of factors to screen is big and one is not able to perform a full factorial design a 2^{k-p} fractional factorial design may be applied, but some of the interactions in the model will be confounded and the design resolution will decrease. The loss in the ability to estimate further level effects (e.g. two or three factor interactions), will be compensated by the reduction in the experimental runs. Once the fractional design is analysed it would be possible to enhance the design resolution the number by several methods (e.g. via foldover) (24, 27).
ii) In some cases, factors can have more than two levels and sometimes it might be that the effect of the factor in the response is not simply linear. A possible empirical model in this situation is to use polynomial models of different

degrees to model the curvature of the response of the form

$$y = b_0 + b_1x_1 + b_2x_2 + ... + b_kx_k + b_{12}x_1x_2 + ... + b_{nn}x_n^2 \quad (144.1)$$

This models, still fall under the domain of linear regression therefore facilitating parameter estimation, study of effects and ANOVA analysis. They are of special interest if the objective of the study is to find optimum points (maximum or minimum) of the response in the experimental range. In this case, 3^k full factorial designs or 3^{k-p} fractional designs with response surface methodology can be used to estimate the effects of the factors and their interactions in the response (27, 28).

Factorial designs make for the most part of the experimental design in food and agricultural sciences, and they are widely used in empirical modelling, optimisation and response surface analysis of food phenomena. There are, however, some other alternatives.

Latin square designs

Latin square designs are used when the factors of interest have more than one level and when the experimenter is interested in estimating only the main effects, as illustrated in Table 144.2.

Central composite designs

Sometimes the object of the study is to map the effect of changing continuous variables at and around a point of interest. In this case, central composite designs can be a valuable alternative to the 3k-p design (27). A typical central composite design can be observed in Figure 144.1. The analysis of these designs uses the same linear models and ANOVA tables as the conventional factorial designs.

TABLE 144.2
Example of a Latin Square Design for the Analysis of the Effect of the Use of Three Different Sweeteners (Fructose = F, Glucose = G and Aspartame = A) in the Texture of Ice Cream. The Experimenter Can Use One Lot at Each of the Three Ice Cream Factories Available, Is Mostly Interested in the Effect of the Three Sweeteners and Wants to Avoid Bias in the Estimation of the Effects from the Different Plants and the Ice Cream Processing Lines

	Plants		
Processing Lines	**Factory A**	**Factory B**	**Factory C**
1	F	G	A
2	A	F	G
3	G	A	F

Mixture designs

Mixture designs are suitable in the food formulation studies that require more than one ingredient, since the proportion of each ingredient in the mixture depends on the other ingredients, and the sum of all ingredients must complete 100% of the composition of the product (29, 30). An example of a simplex lattice design is shown in Figure 144.2. In the same way as central composite designs, the analysis of these experiments involves ANOVA tables and response surface methodology.

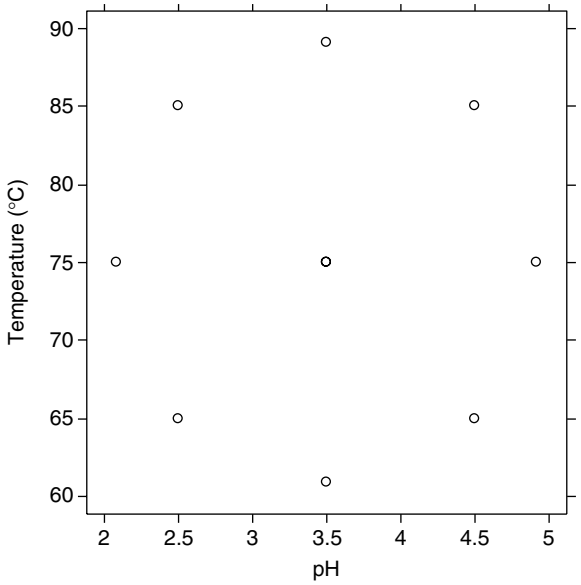

FIGURE 144.1 Example of a central composite design to study the effect of pasteurising temperature and product pH on the shelf life of a pasteurised product at around the 75°C and pH 3.5 region. The design is composed of a 22 full factorial design (cube points) plus the star points. The central point is repeated.

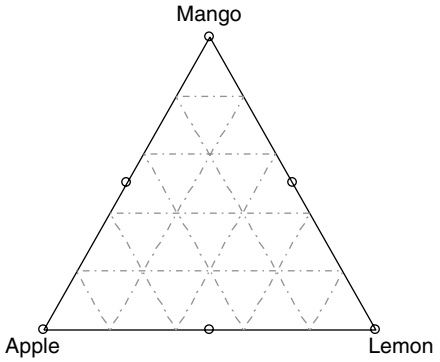

FIGURE 144.2 Example of a simplex lattice design to study the sensorial acceptance of a mixture of mango, apple and lemon in chutney. Each of the points in the graph represents a combination of mango, apple and lemon that complete the mixture.

b. *Experimental designs for linear and non-linear model building: optimal experimental designs*

The application of a design strategy to optimise certain criteria in an experiment (i.e. precision of the prediction, error associated to the estimates) has been extensively studied in applied statistics (31, 32, 33) and several algorithms exist to design different optimal experiments in a single run or sequentially (34).

It is not the purpose of this section to explain the details of the design of optimal experimental designs, as there is specialised literature on it (35, 36, 37) and the reader may find some additional material in chapter 48 (Cunha et al.), but it is well worthwhile to comment and rephrase some of the particular aspects of these designs:

- Optimum experimental designs were introduced first for the design of experiments considering polynomial response surface models over regular regions.
- One of the strengths they had is the development of algorithms for non-standard applications (e.g. constrained experimental domain) and the ease of extension to non-linear models.
- In linear and non-linear model regression, the Fisher information matrix contains the variance and co-variances of the parameter estimates.
- Under usual regression assumptions (see Section "Least Squares Estimation") the Fisher information matrix D is proportional to $X \cdot XT$, where X is the Jacobian matrix of the model, with $X \equiv \lfloor f_{ij} \rfloor$ and $f_{ij} = \partial \eta_i / \partial \theta_j$ (the derivative of the expectation function h evaluated at the i-th design point and the j-th parameter).
- Optimal designs of linear models yield exact designs, but given that for non-linear models $X \cdot XT$ is a Taylor approximation of the information matrix, the designs produced for non-linear models are only locally optimal.
- Another "problem" of the structure of the Fisher information matrix in non-linear models is that the D depends on the actual values of the parameters that are to be estimated. In this way, to design the optimal experiments with non-linear models preliminary estimates of the parameters are needed.
- An optimal design produces a scalar from the information matrix and searches to optimise the scalar to produce the set of experimental points. The most usual experimental designs are:
  - A-optimal designs search to minimise the maximum value of the trace of the Fisher information matrix, looking to minimise the biggest variance associated to one of the parameters in the model.
  - D-optimal designs search the maximisation of the determinant of Δ, with the result of minimising the generalised variance of the parameter estimates. This results with more precise parameter estimates.
  - E-optimal designs search to minimise the ratio of the smallest to the biggest eigenvalue of Δ, with the result of maximising orthogonality and minimising correlation between parameter estimates.

Depending on which one is the objective of the experiment a particular criteria will be chosen to design the experiment.

Example 1. D-optimal design for the Bigelow model under isothermal conditions

A research laboratory is in charge to estimate the thermal stability of different strains of *C. Botulinum* spores. The two properties that characterise the thermal stability of the spores are the D_{121} value (the time in minutes necessary to reduce a certain population one decimal log fold — from 100% to 10%) and the z value (the slope in °C of the thermal death curve). Both parameters are related to the normalised population η (population at any time divided by the initial population at time zero) of colony forming units (cfu) by the Bigelow model (2) where

$$\eta = 10^{\frac{t10^{\frac{T-121}{z}}}{D_{121}}} \qquad (144.2)$$

The following considerations were taken to produce a D-optimal design:

a) Initial estimates of D_{121} and z (1 min and 10°C respectively) were obtained from literature. It is expected that this value will be close to the particular value of each of the strains.
b) The reasonable limits of temperature (110°C to 140°C) and time (from 0 min to 120 min) were assumed as the most appropriate.
c) A number of sampling points equal to the number of parameters to estimate were selected. This type of experiment (two design points) is the minimum set of points necessary to estimate D_{121} and z.

The Jacobian matrix of this experiment is

$$X = \begin{bmatrix} \frac{\partial \eta_1}{\partial D_{121}} & \frac{\partial \eta_1}{\partial z} \\ \frac{\partial \eta_2}{\partial D_{121}} & \frac{\partial \eta_2}{\partial z} \end{bmatrix} \qquad (144.3)$$

Where η_1 and η_2 stand for the remaining spores at the design times 1 and 2 respectively. After solving the partial

TABLE 144.3
Example of D-optimal Design with the Bigelow Model to Estimate the Thermal Destruction Parameters of *C. Botulinum* Spores

	Temperature	Sampling Time
Sample 1	110°C	328 s
Sample 2	140°C	26 s

derivatives in respect to the two parameters, the determinant of Δ is

$$|\Delta| = |X \cdot X^T| = \frac{\eta_1 \ln(\eta_1)\eta_2 \ln(\eta_2)\ln 10}{D_{121}z^2}(T_2 - T_1) \quad (144.4)$$

T_1 and T_2 stand for the temperature of the experimental points 1 and 2 respectively. Looking to the determinant function it can be seen that for given values of D_{121} and z the determinant will be maximised the bigger $T_2 - T_1$, η_1, $\ln(\eta_1)$, η_2 and $\ln(\eta_2)$. Therefore, the experimental points should be each at one of the extreme temperatures in the experimental range. At each of the selected temperatures, the optimal times that maximise the |Δ| correspond to

$$t_1 = \frac{D_{121}10^{\frac{T_1-121}{z}}}{\ln 10} = \frac{D_{T_1}}{\ln 10} \quad (144.5)$$

$$t_2 = \frac{D_{121}10^{\frac{T_2-121}{z}}}{\ln 10} = \frac{D_{T_2}}{\ln 10} \quad (144.6)$$

Using the initial estimates obtained from literature the D-optimal design proposed for this is presented in Table 144.3.

Optimal design in food research

Three research groups in special have applied different optimal designs in food research:

- The group from Oliveira and collaborators have studied the application of D-optimal design on the parameter estimation of food quality, shelf life and mass transfer phenomena in isothermal and non-isothermal experiments (38, 39, 40, 41, 42).
- Nicolai et al. have studied the application of optimal design criteria (A, D and E-optimal) on the design of an experimental set-up to measure thermal properties in non-isothermal experiments (43, 44, 45).
- Finally, Van Impe et al. have proposed the utilization of optimal designs (E-optimal) in the area of parameter estimation of predictive microbiology models (10, 46, 47).

2. Parameter Estimation

This involves the estimation of the unknown parameters of the model from the experimental data. Maximum likelihood, least squares, and Bayesian estimation are the most common ones. The least squares method is widely used nowadays in the food research area in most of the model based estimation methods.

Least squares parameter estimation

For most of the mathematical models describing responses in general science and engineering the maximum likelihood estimates can be found by minimising the residual sum of squares. It is of worth to point out the assumptions used when estimating parameters from a model using least squares (38, 48), previously developed in chapter 48 (Cunha et al.):

a) The expectation function is correct.
b) The response obtained corresponds to the expectation function plus a disturbance.
c) The disturbance in the measurement of the response is independent of the expectation function.
d) Each disturbance has a normal distribution associated.
e) Each disturbance has zero mean.
f) The disturbances have equal variances.
g) The disturbances are independently distributed.

Example 2. Kinetics of thermal degradation of peroxidase during drying

The kinetics of thermal degradation of the enzyme horseradish peroxidase in maltodextrin dextrose equivalent 40 during a drying process was investigated (38).

Looking to the shape of the kinetic curve, the logistic model was identified as the most appropriate to provide a simple description of the situation. In this way:

$$\frac{P}{P_0} = A + \frac{(B - A)}{1 + e^{\frac{xmid - time}{scal}}} + \varepsilon \quad (144.7)$$

Where P/P_0 is the normalised peroxidase activity, time is the drying time, A is the horizontal asymptote at small times (with a fixed value of 1), *B* is the horizontal asymptote at high times, *xmid* is the time at the inflection point of the logistic curve, *scal* a numeric scale parameter on the time and ε a normally distributed error residual.

Figure 144.3 shows that the expectation function seems to describe the data adequately and the residual analysis seems to agree with the assumptions of least squares nonlinear regression (independently normally distributed with a constant variance and no significant autocorrelations). Table 144.4 shows the parameter estimations with their standard errors and regression statistics.

Figure 144.4 shows the bi-variate joint confidence regions of the regression parameters, which are another way of examining the precision of the parameters and the

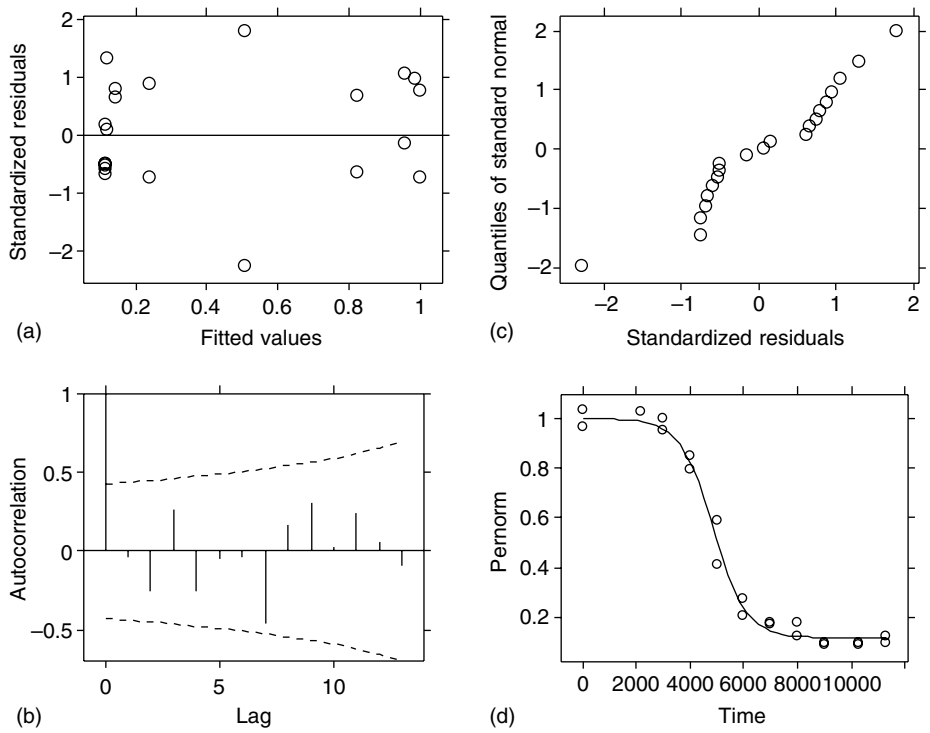

FIGURE 144.3 Diagnostic plots of the regression of the peroxidase data with the logistic model. (a) Standardised residuals vs. fitted values to study if the variance of the residuals is constant. (b) Autocorrelation of the residuals vs. the lags, to help see if there are interdependencies on the residuals. (c) Quantile-quantile plot of the residuals to examine normality. (d) Prediction of the peroxidase data with the logistic model.

TABLE 144.4
Nonlinear Regression Summary of the Peroxidase Data with the Logistic Model

Parameter	Estimate	Std. Error
A	1	Fixed
B	0.12	0.02
xmid	4860	74
scal	620	64
Residual Standard Error	0.04	
Residual Sum of Squares	0.03	

All parameters significant at 95% confidence.

tau-profile (31) that shows the negligible influence of the curvature in the error associated to the parameters of the fit. Table 144.5 compares the fit of the logistic model with the parameter A fixed against a model that has the parameter A estimated (to check if there was a problem in the estimation of the initial enzymatic activity). As those two models are nested, a likelihood ratio test (31) can help to ascertain the necessity of an additional parameter or not. The results of the analysis show that the addition of one more parameter to the model does not improve significantly the fit.

3. Model Assessment

The assessment of the model fit is a statistical analysis of the applicability of the model through analysis of the error term and the expectation functions. Model assessment is usually performed by examining the prediction of the model and by testing that the previous assumptions done in the model building have been fulfilled, generally by making residual diagnostic plots.

4. Model Validation

Model validation can be performed using k-fold validation, bootstrap techniques or external data. Given that the availability of experimental data k-fold and bootstrap are the most common techniques used (24).

5. Model Maintenance

Once models are fit and validated, they can be applied. However, modelling itself is a dynamic activity and models rarely have a long life. As new data and new theories are developed, new models will arise to compete with the previously developed ones. The new models can be compared and selected or discarded using model discrimination criteria like the Akaike and Bayesian information criteria or the likelihood-ratio tests (24) or new experiments can be devised to discriminate between the two models (35) to improve the precision of the forecasts.

B. Deductive Modeling

Deductive modelling uses the *theoretician approach*, with deductive modelling techniques to build a mathematical

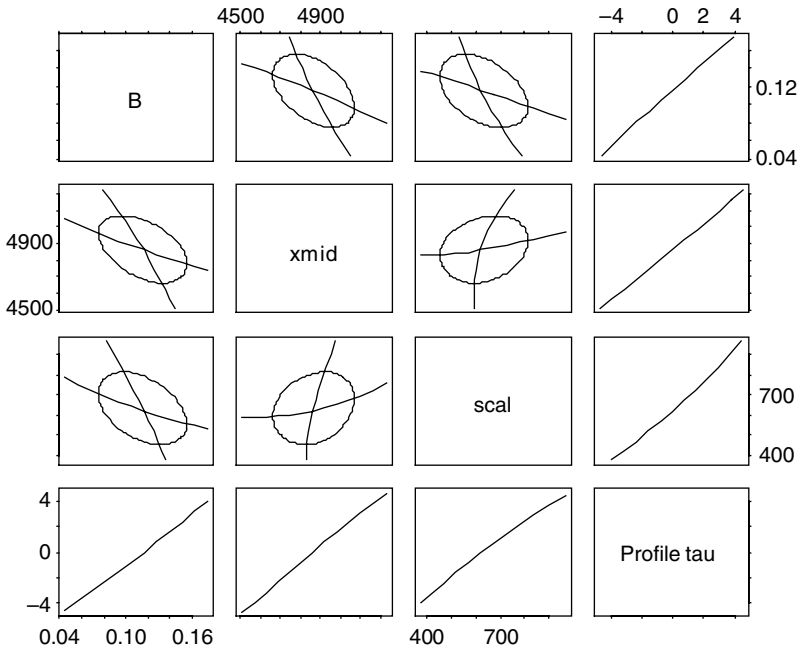

FIGURE 144.4 90% confidence bi-variate regions and tau profiles of the peroxidase non-linear regression (31).

TABLE 144.5
Log-Likelihood Ratio Test to the Addition of the Parameter A to the Fit

Model	RSQ	df	Sum. SQ	F value	Pr (>F)
Logistic fixed A	0.034				
Logistic fit A	0.032	1.00	0.002	0.798	0.384

model from the first principles that govern the phenomena under study. In this way, the food problem is decomposed in a series of components that make a conceptual unit on themselves. Each of the components can then be studied separately and then they can be coupled to describe the integrated behaviour under the study.

For each of the components of the model, the identification process then involves the tasks of:

- Stating the main assumptions that will help to model the problem.
- Identifying the fluxes that govern the phenomena under study.
- Determining the governing equation(s) of the phenomena under study.
- Defining the conditions (initial and/or boundary) that control the particular solution of the governing equation.
- Defining a solution of the problem defined in steps 1–4 or implementing an approximation of the solution using simplification or a numerical scheme.

In deductive modelling the process of estimation and validation becomes more a *calibration* of the *true* model to the particular situation and weights less in the overall model building compared to the model identification task. There is a certain expectation that once the model has been built and validated in a simple system it can be used in different scenarios without much difficulty and that the same explanative behaviour will hold for the new situation (21). Table 144.6 summarises some principles used in deductive modelling of food phenomena.

Example 3. Deductive modelling: Water absorption of breakfast cereals

The water absorption of an extruded cereal product during the storage and consumption dictates the shelf life of the product, given the consumer expectations for a crispy product. Unfortunately, the home conditions vary during the day and extra care has to be taken to build a model that can describe dynamic changes "at home." In order to study this a spherical breakfast cereals were placed under dynamic atmospheric conditions and their weight gain or loss recorded (49).

A typical experiment of water absorption can be seen in Figure 144.5. It seems evident the model to be developed must be very responsive to the changes in relative humidity of the air.

The assumptions to build the model were:

- Water will arrive from the air surrounding the cereal. This implies that the water influx from the atmosphere will define the transport.

- If one considers that the surface of the cereal is in constant equilibrium with the surrounding air and that the main obstacle to the water transport is actually the diffusion of liquid water from the surface of the cereal to the dry core.

TABLE 144.6
Some General Laws Used in Deductive Modelling of Food Phenomena

Fluxes	Law/Equations	Example of Food Phenomena
Heat transfer	Fourier's 1st and 2nd	Sterilisation of solids, refrigeration, freezing, etc.
Mass transfer	Fick's 1st and 2nd	Drying, acidification
Mass transfer	Maxwell	Multi-component mass transfer in food phenomena
Pressure	Darcy's	Transfer of water and gases in porous inert foods
Momentum transfer	Navier-Stokes	Convective flows in food fluids or air fluxes in food systems
Momentum transfer	State equations, mechanical equilibrium equations	Rheology, texture, deformation and fracture of food materials
Chemical species	Zero, First-order, second and n-order reaction	Shelf life of foods, chemical reaction kinetics in foodstuffs, quality prediction
Thermodynamic fluxes	Luikov's	Transfer phenomena in porous media

- A typical volume differential was considered and an effective diffusion coefficient was defined including all the different water transport mechanisms inside the core of the cereal (liquid water diffusion, water vapour transmission by convection and conduction, transport by pressure differentials, etc.).

Under those assumptions, Fick's second law can be proposed as the one that will govern the flow of water from a soaked surface to the core of a spherical breakfast cereal:

$$\frac{dX}{dt} = \frac{1}{r^2}\frac{1}{\partial r}\left(r^2 D_{eff}\frac{\partial X}{\partial r}\right) \qquad (144.8)$$

Where X is the moisture content in dry basis, r is the radius of the cereal, and D is the apparent diffusion coefficient, if the following initial and boundary conditions are considered:

- Constant initial water content in the cereal as the initial condition

$$@\ t = 0,\ X = 0 \qquad (144.9)$$

- Symmetry of the spherical diffusion front from the centre.

$$t > 0,\ r = 0,\ \frac{\partial X}{\partial r} = 0 \qquad (144.10)$$

- Constant flux surface equilibrium through the water sorption isotherm equation of the breakfast cereal (16)

$$t > 0,\ r = R,\ -D_{eff} A\frac{\partial X}{\partial r} = K_g A(P_V - P_{V\infty}) \qquad (144.11)$$

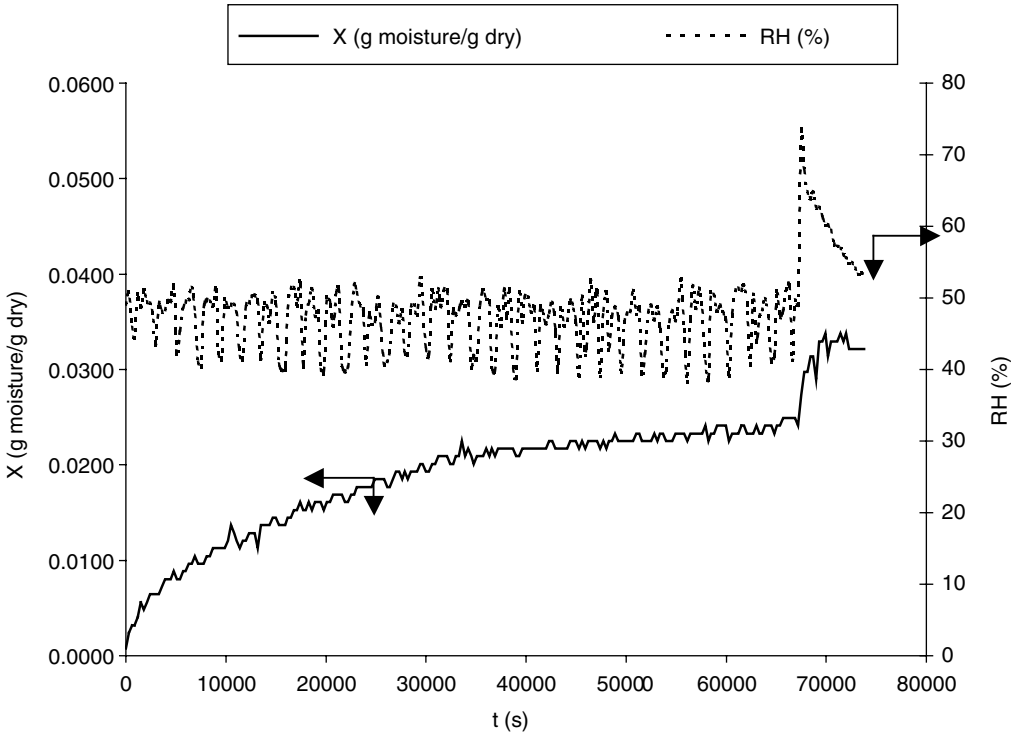

FIGURE 144.5 Plot illustrating the effect of change in relative humidity on equilibrium moisture content.

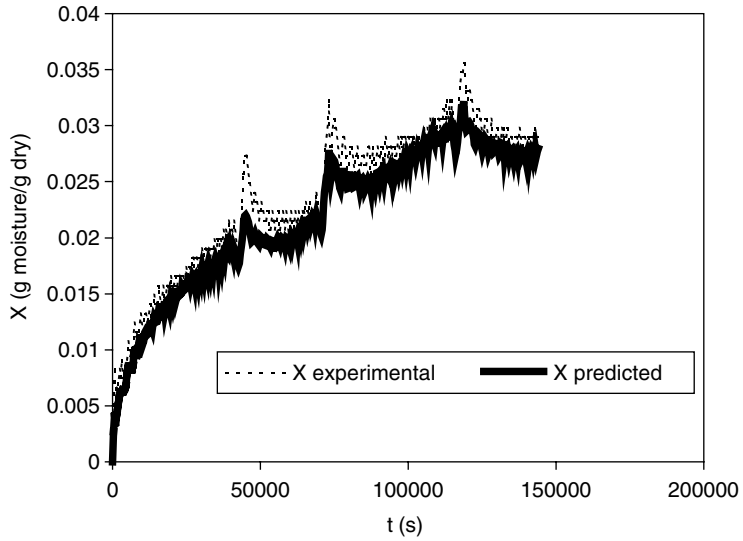

FIGURE 144.6 Prediction of the moisture intake of the breakfast cereals with the model.

where A is the area of the cereal, K_g is the external mass transfer coefficient, P_v is the vapour pressure of the atmosphere surrounding the extruded cereal and P_v is the vapour pressure in the surface of the breakfast cereal that is found from the sorption isotherm equation. The sorption isotherm equation can be described using the GAB model,

$$a_w = \frac{\left(\frac{M_0 C}{X} - C + 2\right) \pm \sqrt{\left(C - 2 - \frac{M_0 C}{X}\right)^2 - 4(1 - C)}}{2(1 - C)kappa} \tag{144.12}$$

with Mo, C and kappa as unknown parameters and with a temperature dependence that follows:

$$\ln\left(\frac{a_{w2}}{a_{w1}}\right) = \frac{E_a}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{144.13}$$

Considering the governing equation and the initial and boundary conditions, an approximation to solve the problem can be built using the method of the lines and a standard ODE solver. If there is no further information from other experiments (i.e. breakfast cereal sorption isotherms), in order to calibrate the model to the particular breakfast cereal the diffusion coefficient and the sorption isotherm parameters have to be estimated from the experimental data. Particular care has to be taken with (50):

- The numerical stability of the scheme that can greatly affect the simulation and the estimated parameters.
- The convergence towards a minimum, ensuring that local minima are avoided.
- The stability of the solution for all the experiments.

Figure 144.6 shows one of the experiments with the model prediction and Table 144.7 shows the parameters found using a least squares method. Both the diffusion and the sorption isotherm parameters are within physical constraints and Figure 144.6 shows the ability of our model to predict a dynamic situation with variation of the outer relative humidity of the air surrounding the cereal.

This mathematical model can then be used to study the water absorption of breakfast cereals under different scenarios of relative humidity and to help predict the shelf life of the breakfast cereals.

TABLE 144.7
Parameters Estimated from the Diffusion Model of Water in Breakfast Cereals

Parameter	Value	95% Confidence Interval
D_{eff} (m^2/s)	1.0633×10^{-10}	1.0616×10^{-10} to 1.0651×10^{-10}
E_a (kJ/(mol K))	6.527×10^4	6.244×10^4 to 6.271×10^4
K_g (kg/m^2Pa s)	3.120×10^{-8}	3.116×10^{-8} to 3.124×10^{-8}
M_o (g water/ g dry)	1.350×10^{-2}	1.348×10^{-2} to 1.352×10^{-2}
C	20.34	20.32 to 20.36
kappa	1.199	1.198 to 1.201

$R^2 = 0.9982$.

C. Eclectic Modelling

The form and the complexity of the mathematical model depend on the purpose of the particular application. A typical example of this is the study of drying of foods:

- Several competing fundamental theories have been developed and models developed from those theories (e.g. 15) co-exist with empirical models (e.g. 16).
- Despite the fact that the theories developed seem to explain thoughtfully many of the interactions in drying systems, it does not seem that empirical models will stop being developed by researchers for particular applications where model building techniques "accommodate."

- In the same way the applied work in empirical drying modelling does not seem to stop new fundamental developments in the area of drying that extend the knowledge on the process.

The co-existence of those two methodologies in several R&D fields seems to indicate that there are areas where one technique or the other can present certain benefits, and certain problems (e.g. optimisation of drying processes) where the two approaches present different solutions that compete in their applicability. In this way inductive and deductive modelling can be complemented in an "eclectic" way to develop:

- Apply the theoretical knowledge to identify model constructions for a particular problem.
- Use powerful inductive statistical techniques to estimate parameters (least squares, likelihood, Bayesian estimation).
- Use inductive validation combined with knowledge based experimental design to compare the model to the experimental data and to demonstrate the domains of applicability of fundamentally based models.
- Use deductive knowledge and statistical criteria to be able to benchmark several different theories and establish the limits and applicability of their models to the particular food phenomena of interest.
- Employ statistical techniques of simulation to apply the fundamental model in new situations.

The statistical procedures to fit a simple empirical model do not differ greatly from the fitting of a complex system of partial differential equations (50) and deductive model building techniques would benefit from the possibility of creating procedures by which different model theories can be compared. In the same way inductive modelling could benefit from the application of knowledge based models to enhance the applicability of the models generated from experimental data.

V. FUTURE PERSPECTIVES

The field of mathematical modelling is in constant advance and there are some topics that will present the opportunity for developing:

a) In the area of inductive modelling:
   - Accurate model formulations for reaction networks present an opportunity to improve the knowledge of complex food phenomena with possible extensions to metabolic studies of biological phenomena in food (51).
   - Multiphysics and the interactions between different transport phenomena (e.g. heat, mass, momentum) may lead to the understanding of complex food problems like (e.g. grain checking, material formation during food processing, influence of rheological properties on food phenomena, etc.) (18).

b) In the areas of estimation and model application:
   - Advances in the integration of system modelling and the development of hybrid simulation can provide the tools for the integrated study of whole food processing systems (52).
   - Bayesian modelling techniques (53) and mixed modelling can help in the estimation of variability in food processes and to integrate food modelling with quantitative risk assessment (17). The Bayesian approach to include previous expertise and experience in the modelling and parameter inference procedure has the potential of producing a fruitful interaction between advance statistics and food technology/engineering. Example 4 provides an illustrative example on an application to build a model that can be used in further risk analysis of the microbiological risks associated with deli shops.

Example 4. Modelling the kinetics of microbial growth in vacuum packaged cooked chicken in deli premises accounting for the uncertainty between premises.

Four sandwich premises (A, B, C and D) where tested where the log of the total viable count (TVC) was measured in chicken pieces (used as an ingredient for ready-made sandwiches) during a working day. The data showing the microbial growth can be seen in Figure 144.7.

Primary modelling

It can be seen that there is a general functional relationship that can describe the growth, with a significant variability between premises. In order to propose a model that could describe in a reasonable way the phenomena, a non-linear mixed effects model was proposed, where the Gompertz model was used to describe the growth of the TVC with time:

$$\log(TVC_{i,j}) = Asym \cdot e^{-b2 \cdot b3^{time}} + \varepsilon_{i,j} \qquad (144.14)$$

where each measurement of the TVC at the premise i in time j is associated to a normal distributed error $\varepsilon_{i,j}$ The fit of a Gompertz model to each individual premise can be seen in Figure 144.8 that serves to validate the model application to model the kinetics of the phenomena. Once this primary model is achieved, one can try to start modelling the variation between premises.

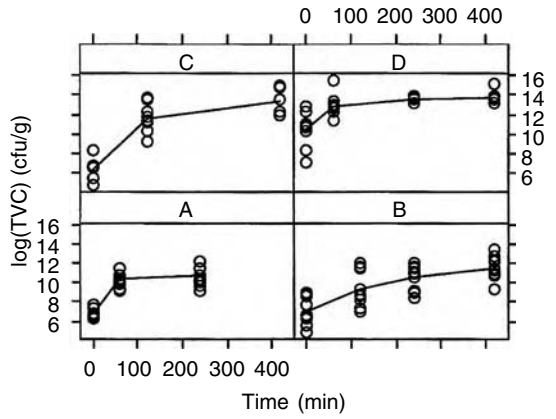

FIGURE 144.7 Growth of total viable count (TVC) in cooked chicken at four sandwich premises.

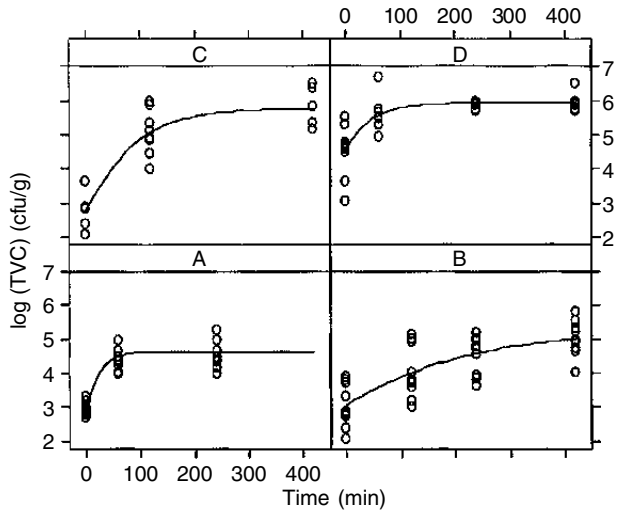

FIGURE 144.8 Individual fit of the Gompertz model to the kinetic data in each of the premises.

Secondary modelling

In order to model the variance of the Gompertz model parameters with the premise variation *Asym*, *b*2 and *b*3 were modelled as unknown parameters which had an associated fixed effect (the behaviour of the "average" premise) and a random effect (the variation between premises) in the form of a random distribution that changes between premises *i*.

$$Asym_i \sim Asym + N(0, \sigma_{Asym}) \quad (144.15)$$

$$b2_i \sim b2 + N(0, \sigma_{b2}) \quad (144.16)$$

$$b3_i \sim b3 + N(0, \sigma_{b3}) \quad (144.17)$$

Mixed effect models convey a hierarchical structure of the error in a model that intuitively suits many food related problems. Using this non-linear mixed effect model, the parameters were estimated via maximum likelihood estimation using the library NLME (54) and the average premise parameters together with the variability between premises can be seen in Table 144.8.

TABLE 144.8
Results of the Model Regression

Parameter	Low 95%	Estimate	High 95%
Average Premises Parameter			
Asym	4.898	5.405	5.911
b2	0.322	0.487	0.652
b3	0.972	0.982	0.993
Between Premises Standard Deviation			
σ_{Asym}	0.216	0.468	1.012
σ_{b2}	0.069	0.153	0.341
σ_{b3}	0.0034	0.0090	0.0241
Within Premises Standard Deviation			
σ	0.444	0.505	0.573
Regression Statistics	**AIC**	**BIC**	**LogLik**
	234.4	254.5	−110.20

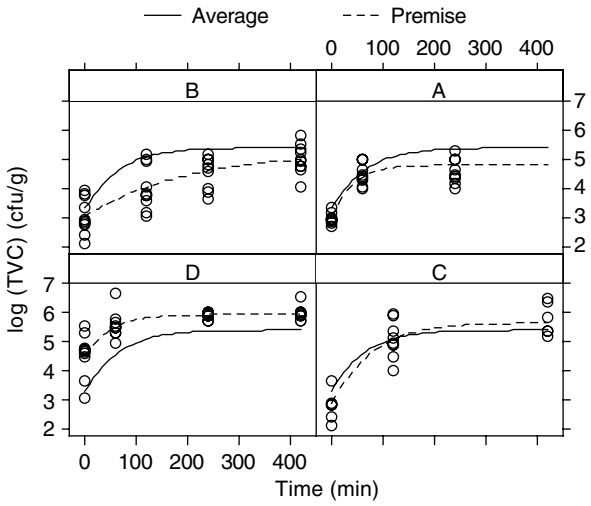

FIGURE 144.9 Fit of the NLME Gompertz model to the data. The continuous line shows the average premises population kinetics and the dotted one the best linear unbiased predictions (BLUP) of the model for the particular premise.

The fit of the model to the experimental data, together with the average behaviour, can be seen in Figure 144.9 showing the agreement with the model. The parameters from Table 144.8 can then be used in stochastic simulations to perform uncertainty analysis of risk assessment on the microbial growth in the sandwiches.

VI. CONCLUSIONS

Model building is an exciting research and development field in food technology and engineering that offers powerful tools to solve complex problems. The main aspects where model building is applied in the food

technology and engineering domain have been outlined. Simple examples have shown the different steps in the building of models from different approaches. This prolific field of food technology and engineering enjoys a healthy development sustained by the new demands of industry and research, and provides exciting tools to investigate food related phenomena.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Nissreen Abu-Ghannam for her kindly offer of experimental data for example 4. Dr. Luís M. Cunha acknowledges financial support from *Fundação para a Ciência e a Tecnologia* (FCT) through "Financiamento Plurianual — POCTI."

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Part R

Waste Management

145 Biological Treatment of Industrial Wastewater: Case Studies and Current Thoughts

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CONTENTS

INTRODUCTION

The purpose of this chapter is to present an overview of major types of engineered wastewater treatment systems that are used by different industries in Canada, with examples of actual systems that have been operated in a diversity of applications ranging from treating food processing and pulp and paper industry wastes to toxic landfill leachates. Regardless of the actual wastewater treatment application, these engineered systems typically combine different physical, chemical and biological unit operations which are selected and matched according to the ultimate treatment

objectives of the treatment system. At the heart of the processes are the biological reactors which employ aerobic and/or anaerobic bacteria to remove a wide variety of soluble substances that may be present in the wastewaters, such as organic matter (biological oxygen demand, BOD_5) and nutrients (typically ammonia and phosphate).

Examples of aerobic engineered biological treatment systems used in Canada and elsewhere include large field-size aerobic stabilization lagoons which typically employ mechanical surface aeration, conventional activated sludge treatment systems, aerobic biofilters (e.g. Acticontact Process), sequencing batch reactors (SBRs), trickling filters and rotating biological contactors (RBCs). Each of these systems requires mechanical aeration to provide needed oxygen to drive the aerobic microbial biodegradation processes. For some applications, anaerobic biological treatment systems such as the upward flow anaerobic sludge blanket reactor (UASB Process) can also be found in Canadian industrial and municipal applications.

The biological treatment unit operations of a wastewater treatment system form what is called the secondary treatment system. To achieve cost-effective and efficient wastewater treatment requires secondary treatment systems to operate in conjunction with other non-biological wastewater treatment unit operations referred to as pre-treatment, primary treatment and tertiary treatment. Pretreatment (e.g. screening, grit removal chambers, comminution (grinders), pH neutralization) and primary treatment (gravity clarification, dissolves air flotation) unit operations are typically located ahead of the secondary treatment system, where they are first to treat the incoming raw wastewater by physical and chemical means. Tertiary (polishing) unit operations are located downstream of the secondary treatment system and are designed to polish the final effluent before final discharge. Examples of unit operations that are used for tertiary treatment include residual phosphate precipitation and removal; break point chlorination for residual ammonia removal, disinfection and dechlorination; and polishing to remove trace metals (e.g. metal precipitation, activated carbon). The pre-treatment steps are designed to remove solid materials such as grit and sand, leaves, rags, bottles, and other large debris. The equipment used in primary treatment on the other hand is employed to remove settleable solids and floatable materials (e.g. fats, oils and grease).

TABLE 145.1
Final Effluent Discharge Compliance Limits for a Large Meat Processing Plant in Ontario, Canada

Parameter	C of A Discharge Limit
Carbonaceous BOD_5 (mg/L)	7.5 mg/L ($t \geq 5°C$); 10.0 mg/L ($t < 5°C$)
TSS (mg/L)	10 mg/L
Ammonia and ammonium (as N) (mg/L)	3 mg/L
Unionized ammonia (as N)	0.02 mg/L
Total phosphate (as P)	0.4 TP (monthly average)
Residual chlorine	0.002 mg/L
Dissolved oxygen	$\geq$ 4 mg/L
Acute toxicity (96 h rainbow trout assay)	Non-toxic (i.e. $\geq$ 50% survival in 100% effluent)

The choice of treatment technologies that will be selected for a particular application will be determined by the treatment objectives of the facility, which are commonly mandated by government regulations through strict, enforceable industrial discharge permits such as Certificates of Approval in Ontario, Canada. Such permits impose very strict final discharge standards which the discharger must meet or else face potential costly fines and/or imprisonment. Table 145.1 presents an example of the tight discharge compliance limits that one meat processing plant in Ontario must currently meet.

This chapter has been written in two parts. The first part presents a description and an analysis of case studies performed during 1990. The second part discusses some current thoughts on new approaches, techniques and methodologies.

PART I. Biological Wastewater Treatment: Case Studies

I. APPLICABILITY OF BIOLOGICAL TREATMENTS FOR THE DETOXIFICATION AND DEGRADATION OF ENVIRONMENTAL AND FOOD PROCESSING POLLUTANTS

Increased awareness and concern over the quality of the environment have led the Federal and Provincial governments in Canada to pass stringent regulations and guidelines on the release or disposal of pesticides, industrial wastes and other pollutants into the natural environment. Failure to comply with such regulations can result in the levy of high fines, costly plant shut down or even jail sentences for responsible offenders. In response to the needs of industry to meet the regulations, there has been a tremendous effort over the past several decades to develop new and improved waste treatment processes that are both efficient and cost effective and which are capable of consistently meeting tight effluent discharge quality criteria. Increased industrial and government funding has sparked fundamental research in the fields of microbial ecology, metabolism, genetics and bioengineering, which in turn has led the way to the development and recent introduction of some novel biological treatment processes for man made synthetic chemicals (xenobiotic) wastes.

A variety of aerobic and anaerobic biological waste treatment processes have been employed for years in Canada for the treatment of domestic wastes such as sewage, food processing wastes and the like. With the widespread manufacture and use of novel xenobiotic substances, many of which have been found to be very recalcitrant to conventional microbiological treatments, came the understanding that the existing biological approaches and processes would have to be refined significantly for the treatment of industrial wastes, landfill leachates and other materials containing hazardous compounds such as dioxins (1).

Many microorganisms have the ability to degrade organic pollutants. Effective treatment in any biological treatment process will be absolutely dependent on the ability to provide the "correct" type(s) of microorganisms in the treatment system and to maintain the treatment microorganism(s) in a satisfactory or optimal physiological state (i.e. environment) to allow their metabolic well being. The latter will be determined by a whole host of chemical, physical, nutritional and biological factors which affect microbial growth, survival and metabolic activity. Failure in the past to understand these fundamental ecological principles has slowed the successful development of biological processes for hazardous waste treatment.

When a natural organic substance is introduced into an environment which is favorable for microorganisms, the substance will eventually become biodegraded. This is not necessarily the case for xenobiotic substances, many of which are highly resistant to biodegradation (e.g. PCBs, dioxins, PHAs). With over 1,200 chemical substances being added annually to the U.S. EPA's list of 60,000 industrial chemicals in 1985 (2) (The Chemical References Complete Index of EPA can be found in http://www.epa.gov/enviro/html/emci/chemref/complete_index.html), the capability of microorganisms to modify and degrade both natural and xenobiotic substances in the environment has been an important topic for research since the 1960s. It is now well recognized that as a group, microorganisms have an enormous capacity to biodegrade natural organic substances, given favorable environmental conditions (3, 4, 5, 6). This is attested by the fact that in the ecosphere, accumulations of natural organic compounds do not exist except where environmental conditions have been adverse to microbial survival (e.g. sites of fossil fuel deposits). In soils or aquatic ecosystems, such adverse conditions may include extremes of pH, salinity, temperatures, or low water availability in soil systems, as a few examples. For a particular waste problem, the operating performance of a biological process (i.e. efficiency, kinetics of biodegradation and detoxification, stability, control of undesirable microorganisms, etc.) will be dictated to a large part by how well these factors are understood (and controlled) by the designers and operators of the system.

The fate of xenobiotic chemicals following their introduction into a natural ecosystem is much more complex than the situation for natural compounds. Such molecules may undergo complete or partial biodegradation or may be entirely recalcitrant to microbiological attack. It is therefore important to distinguish among these possible mechanisms of removal when considering the feasibility of biological treatments.

The major objectives of biological treatment as pointed out by Aitken and Irvine (7) are as follows:

1. Reduction of a substantial fraction of the organic load, to a point where overall treatment costs are

reduced, minimizing the need for more expensive treatment such as activated carbon.
2. Removal of target compounds to specified levels, e.g. for wastes containing low percentages of xenobiotics, the objective is the removal of these compounds.
3. Attainment of a final toxicity standard. The major objectives are to reduce the concentration of priority pollutants in the waste to meet the allowable standards set by the regulatory agencies, as well as the reduction of surrogate parameters such as BOD and COD to acceptable levels. A ratio of BOD to COD will indicate whether the leachate is biodegradable (if less than 1.0) or not.

II. BIOLOGICAL TREATMENT PROCESSES FOR WASTEWATER

Basic biological treatment processes involve the use of microorganisms in a closed environment (a tank, or a man-made lagoon or basin) to remove (biodegrade, bind, entrap, incorporate) the organic matter in the wastewater. Such systems may be anaerobic (with no air included in the treatment), or aerobic (with air to oxidize the organics) as shown in Figure 145.1.

There are numerous types of engineered biological treatment systems which differ in their reactors and equipment designs (e.g. activated sludge treatment (AST), rotating biological contactors (RBCs), sequencing batch reactors (SBRs) and upflow anaerobic sludge blanket (UASB)) and in their operating principles (e.g. aerobic vs. anaerobic). Biological treatment may take place in an open or closed system or even in situ (i.e. right in the ground in contaminated sites). The advantages and disadvantages of several types of systems will be discussed here, as well as factors or guidelines governing the selection of the best process for a particular waste treatment problem.

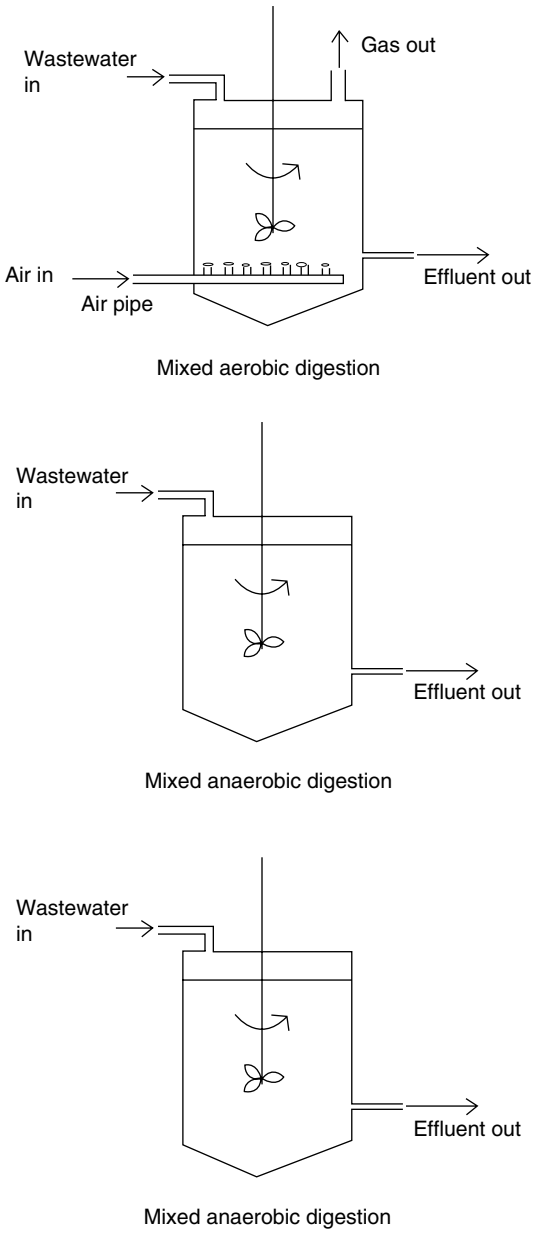

FIGURE 145.1 Basic biological treatment processes.

A. Aerobic Biological Treatment Processes

In aerobic microbiological processes oxygen consuming bacteria metabolize dissolved organic compounds and convert them into carbon dioxide and settleable solids (i.e. microbial biomass). Organic matter may also be removed from such systems by adsorption to, or entrapment within, the microbial biomass, and by incorporation into the microbial biomass itself. For an aerobic process, the rate at which biodegradation occurs is often limited by the rate at which oxygen can be dissolved in the wastewater. The oxygen transfer rate is often the critical parameter limiting the operation of aerobic waste treatment systems. Energy for aeration is also a major operating cost for such treatment systems.

Various types of mechanical aeration technologies are employed for increasing oxygen transfer into wastewater including various types of floating and fixed surface aerators and fine and coarse bubble diffusers fed by mechanical blowers. Figure 145.2 shows an aerial photograph of the activated sludge aeration basin used to treat meat processing wastewater at Canada's largest meat rendering plant, the Rothsay plant owned by Maple Leaf Foods Ltd. in Dundas Ontario. This system has 3 types of aerators in the aeration basin shown: 2 fixed surface aerators, 4 floating surface aerators and an underwater course bubble diffuser. This particular wastewater plant will be taken out of service in late 2005, when a completely new upgraded activated plant comes on line.

FIGURE 145.2 An aerial photograph of the activated sludge aeration basin in Canada.

Oxygen transfer to such systems can also be increased by a number of ways including pumping pure oxygen instead of air into the system (e.g. UnOx process such as is used at several Canadian pulp mills), altering the temperature, adding hydrogen peroxide to the culture, or by increasing the hydrostatic or the back pressure of the system.

1. Aeration Stabilization Basin

Aerobic stabilization basins or lagoons (ASBs) have been used successfully for the detoxification of some industrial pollutants and for organic matter (BOD_5) removal. This type of wastewater treatment technology is widely used in the Canadian pulp and paper industry, where large sections of land are readily available to accommodate these enormous, but simple-to-operate and effective systems. One such system which has been the focus of considerable research is the ASB belonging to the Domtar (formerly E.B. Eddy Forest Products Ltd.) pulp and paper mill in Espanola, Ontario (8). This kraft mill converts approximately 4×10^6 kg of wood chips a day to 1×10^6 kg of kraft pulp. Approximately 68,130 L/min of wastewater containing organic pollutants (BOD_5) and toxic substances (e.g. chlorinated lignin breakdown products, and resin acids) are sent to the mill's waste treatment facility for detoxification and BOD and total suspended solids reduction. Suspended solids (mostly cellulosic fibres and inorganic matter) in the kraft effluent are first removed by gravity in one of two parallel 7.19×10^7 L settling basins. At any one time, only one of the settling basins is operational, since the settling basins must be taken off line annually and de-sludged. The second settling basin therefore becomes operational when the other basin is taken off line for de-sludging. The effluent treatment system consists of two settling basins for primary treatment (each has a volume of 45,000 M^3; one of these is normally on-line, and the other empty), a 2-cell biological aeration lagoon (cascade arrangement) and a final settling basin (Quiescent Polishing zone) for removal of biological solids before final discharge. The capacities of Cell 1 and Cell 2 are 3.03×10^8 L and 3.78×10^8 L , respectively. The 2 aeration cells have a 40 acre surface area and are aerated by twenty two 75 hp mechanical surface aerators, 12 in Cell 1 and 10 in Cell 2. The hydraulic retention time in the system is normally kept between 6–8 days. From the aerobic basin, the treated effluent is sent to quiescent basin with a 12 hour retention time for final polishing (i.e. gravity settling of any remaining suspended solids), and it then is piped to the receiving environment (Spanish River). The entire treatment system is lined with a PVC liner to prevent untreated effluent from escaping into the ground water. In addition, a 2.27×10^8 L emergency spill diversion basin has been constructed as an added precaution. The efficiency of the system and the quality of the treated effluent is monitored closely for pH, conductivity, toxicity towards fish (LC_{50} trout minnow test) and water fleas (*Daphnia magna* 48 h bioassay), BOD, TOC and other physical, chemical and aesthetic parameters. According to two extensive published studies in the scientific literature (9, 8), the quality of the Epanola mill's effluent is within the original design requirements, having a BOD of below 4×10^6 kg/d and being non-toxic to fish.

2. Continuous Flow Activated Sludge Process

Activated sludge treatment (AST) systems are widely used throughout the world for the treatment of municipal and industrial wastewaters. These systems are designed as high rate systems which are able to treat a much larger quantity of wastewater in a much smaller footprint than is possible with aeration stabilization lagoons. This is made possible by the design of these systems which allow them to carry a much higher microbial biomass concentration that is the case with ASBs activated sludge systems typically consist of a mechanically aerated basin or tank followed by a secondary clarifier which separates treated effluent from the floc-forming microbial biomass (Figure 145.3). Most of the latter is returned to the aeration basin as RAS (return activated sludge), except for a fraction that must be wasted in order to maintain an optimal biomass concentration in the system (termed WAS, waste activated sludge). An example of an activated sludge system from a large meat processing plant is shown in Figure 145.2. Such systems are used in many other industries including pulp and paper, petrochemical and food processing.

As with all types of biological treatment systems, it is critical to provide the microorganisms in an AST with a favorable environment to effect the required wastewater treatment objective. For example, in 1986, CANVIRO Consultants of Ontario conducted a study with the Ontario

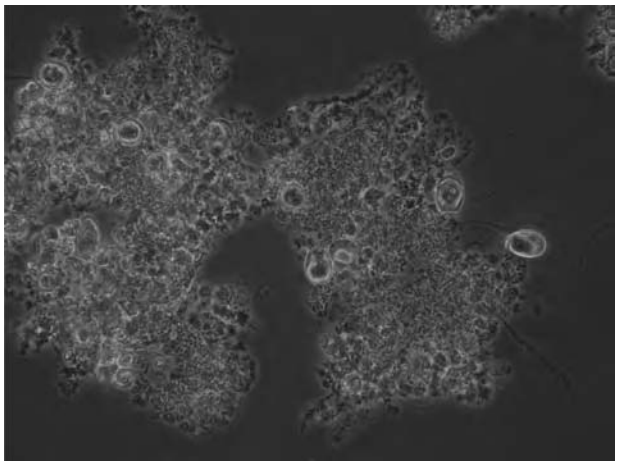

FIGURE 145.3 The floc-forming microbial biomass.

Petroleum Association (OPA), the Ontario Ministry of Environment (MOE) and Environment Canada to determine the relationship between biological treatment process operating conditions and the ability of the process to effect trace contaminant removal (10). The biological treatment (activated sludge system) was carried out at two preselected refineries in Ontario: one at Sarnia (Esso) and the other at Trafalgar (Petro-Canada).

Petro-Canada's Trafalgar Refinery has the capacity to process 1.012×10^7 L of crude oil per day. Major refinery processes at Petro-Canada's Refinery include distillation, reforming, alkylation, desulfurization and blending of crude to produce a variety of petroleum products. Process wastewater treatment involves oil/water separation in API (American Petroleum Institute) separators, equalization, further oil and solids removal in induced air flotation (LAF) separators and in-house biological treatment (aeration tanks-clarifiers-aerobic digester). All tanks are covered and air is supplied by a diffuse air system. There was no nutrient addition to the aeration tanks at the time of sampling in 1988. The activated sludge plant has an average design flow capacity of 6,019 m^3/day.

3. Rotating Biological Contactor (RBC)

An RBC bioreactor consists of a slow rotating disk assembly semi-submerged in an engineered tank or a pond. Several disks are typically attached to the support rotor. The disk is designed to provide a very large surface area for the attachment of microorganisms which grow to form an extensive biofilm on the disk (see Figure 145.4). The slow turning of the disks will enable microorganisms to utilize air oxygen (when the part of rotating disk is in the air) and to consume, bind or entrap the organics in the wastewater (when the part of rotating disk is submerged). A report presented at the HAZMAT Canada '87 Conference showed that the rotating biological disk has been successfully demonstrated for the destruction of phenolics in five

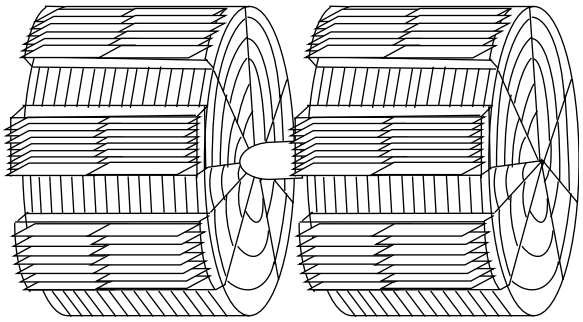

FIGURE 145.4 A rotating biological contactor (RBC) bioreactor.

waste streams, including streams from spray dried resin production, phenol-formaldehyde resin condensate, fibreglass insulation manufacture, pulp and paper mill effluents, and landfill leachates (11).

a. Peel county landfill (Ontario)

In the early 1990s, RBC technology was selected for treatment of chemically contaminated landfill leachate from the Brittania Road landfill site in Peel County, Ontario, following a promising pilot trial of this technology. At the time the landfill had been receiving a mixture of industrial and municipal wastes with a COD of 15000 to 19000 mg/L. According to information we received from the RBC vendor (CMS Rotordisk Inc.), 92% removal of COD was achieved in the pilot study. The decision was made to install a full scale system, designed to treat 2000 m^3 of leachate per month at a loading of 133 kg BOD/day. The total disk surface area for fixed film formation for this system is 11520 m^2.

RBC technology has been used in many other applications world-wide. For example, in Iran, an RBC application for treating bakers yeast wastewater achieved 94.9% and 84% removal efficiencies in COD and BOD in the wastewater, respectively (12). In another example, a rotating biological contactor with volume 14.26 M^3 L was used successfully for treating winery wastewater with maximum COD removal efficiencies up to 95% in South Africa (13).

4. Sequencing Batch Reactor

A sequencing batch reactor (SBR) is a biological reactor quite similar to AST except that all events (aerobic treatment and solids separation) take place in a single tank (i.e. no separate clarifier). SBRs operated in a cyclic batch mode (each cycle consists of 5 discrete events): fill (add wastewater to a tank), react (aeration of the wastewater with stirring to permit thorough mixing of the settled microorganism sludge with the wastewater), settle (an anoxic period in which the mixed liquor and suspended solids (biomass) are allowed to separate by gravity sedimentation), draw

(removal of the clarified supernatant water from the tank), and idle (a period of resting until the next batch of wastewater is ready for treatment) as shown in Figure 145.5. In a two-tank system, one tank is completing "react," "settle," "draw," and "idle" while the other tank is "filling." The SBR is one of several innovative and alternative biological waste treatment systems that has been evaluated by the EPA (14) and the Office of Technology Assessment (15).

A full scale SBR serving the Niagara Falls area (including the nearby industrial belt of St. Catherine in Ontario) has been built and operated at Niagara Falls, New York, since June 6, 1984. The treatment plant typically receives 10,000 m^3 of wastewater each month. Approximately 50% of this leachate is pumped from both active and inactive landfills on the CECOS site; approximately 30% is pumped as part of a remedial groundwater program from an unrelated facility bordering the CECOS site; the remaining 20% is received in bulk or drums from various industries in a 640 km radius. The SBR system uses a two-phase operation: phase 1 is dedicated to oxidation-reduction reactions, acid neutralization, heavy metals precipitation (lime) and de-watering; phase 2 is dedicated to pH adjustment (with HCl), biological degradation, carbon adsorption and batch discharge. The covered SBR (1890 m^3) was situated in the phase 2 operation between the equalization and neutralization basins, and the activated carbon columns. Flow through the SBR averaged 220 m^3/day, giving an 8- to 9-day retention time, and representing 90% of the wastewater processed at the Niagara Falls site. The management at CECOS chose SBR over other aerobic treatment designs because of its simplicity (all the actions in one tank) and its effectiveness in leachate treatment as shown in the pilot studies. They considered anaerobic processes to be more prone to shocks, and it would take the anaerobes a much longer time to recover from shock. Operation of an SBR at CECOS Intl. during the winter months showed a reduction as high as 50% in biodegradation rates. An SBR experiment to simulate cold weather operation showed that a reactor maintained between 5 and 6°C had a TOC value of 800, compared with 1600 for the influent, and 400 for the reactor that was maintained between 21 to 25°C (16). A senior official of CECOS suggested that longer retention time of the wastewater should be maintained for better treatability. Furthermore, if financially possible, each site should have two SBRs to provide more flexibility, such that while one SBR is operating in the reaction step, the other one is discharging treated effluent.

In the early 1990s, there were over seventy-five SBR plants operating in the United States (one in California

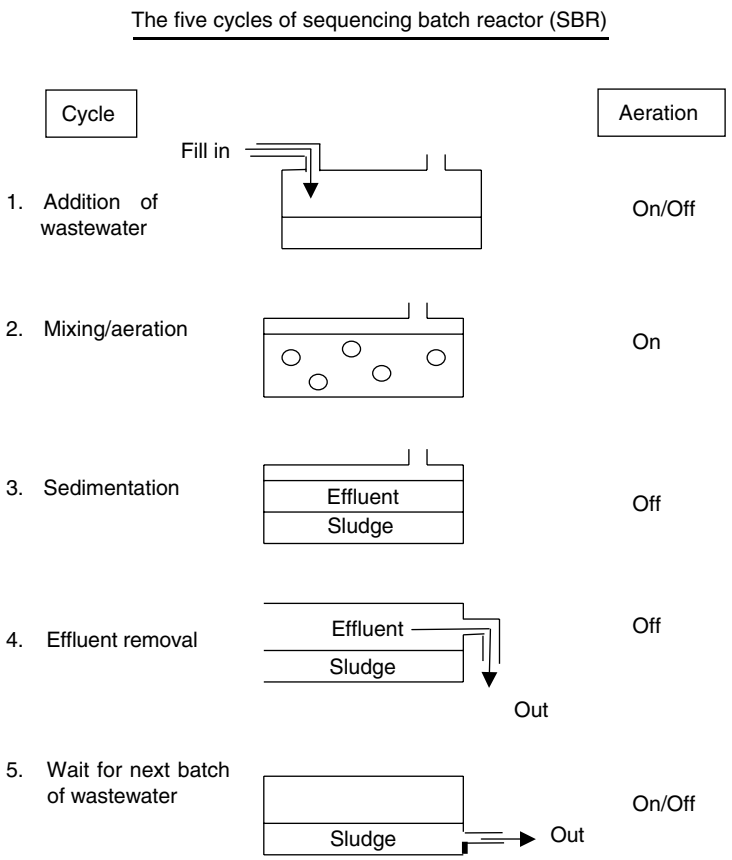

FIGURE 145.5 The five cycles of sequencing batch reactor (SBR).

that treated 1.89×10^7 L per day, and one in Oklahoma City that treated 9.46×10^7 L per day). At this same time in Canada, there was an SBR in operation in Quebec City for the treatment of slaughterhouse wastes. While in Greece, a sequencing batch reactor was operating for the treatment of landfill leachate and domestic sewage. This system was reported to have removal efficiencies of 95% for BOD_5 and 50% for nitrogen (17). Lin and Cheng (18) successfully treated municipal sewage waste with a sequencing batch reactor in Taiwan for removing 93.6%, 91.8% and 53.3% of COD, BOD and ammonia in the wastewater, respectively. On the other hand, a 60 L sequencing batch reactor in Korea removed 97–98%, 96% and 80% of BOD, nitrogen and phosphorus in dairy industry wastewater, respectively (19).

5. Acticontact System

Biofilters such as the acticontact biofilter that was installed in 1995 at the E.B. Eddy Forest Products Ltd. (presently Domtar Inc.) fine paper mill in Ottawa, Canada, are a relatively new technology for high rate aerobic treatment of wastewaters. The E.B. Eddy biofilter was selected after an extensive 10-month pilot trial showed that the system offered many advantages over other treatment technologies that the mill had considered, including limited available space for installation, proximity to neighbouring commercial and governmental buildings, low operating costs and need to meet stringent discharge limits (20, 21). The Acticontact is an upflow aerobic biofilter, in which effluent and air are fed into the bottom of a rectangular concrete tank that is filled with a porous, pebble-like man-made support material called Actilite. The Actilite provides a large surface area for the attachment of large numbers of biofilm-forming microorganisms.

While flowing upwards through and around the Actilite bed, BOD substances in the wastewater are removed and decomposed by the bacteria in the biofilm. The Acticontact can take exceedingly high BOD loadings (3 to 17 allowable BOD loading (kg BOD/m^3/d) compared to 0.6 of activated sludge process). The treatment system is able to consistently meet the BOD discharge limit of the mill, achieving 70–80% BOD reduction across the filter bed with a hydraulic retention time of only 22 min. (21).

B. Anaerobic Biological Treatment Processes

Anaerobic treatment processes are based on microbial biodegradation of organic waste in the absence of oxygen. In these processes, various bacterial species closely interact to effect total mineralization of organic compounds, such as the fermentative, acetogen and methanogen bacteria, etc., as illustrated in Figure 145.6.

1. Halifax, N.S. (Anaerobic Digester)

An anaerobic digester that removed organic-waste from the Halifax-area landfill site leachate could provide an energy-efficient model for other Canadian municipalities to follow (22). This system, the first of its kind in Canada, was officially opened in June, 1987, at the new Regional Landfill Leachate Treatment Plant near Sackville, Nova Scotia. It was designed by the National Research Council and Porter Dillon Ltd., and built by the Metropolitan Authority of Halifax-Dartmouth and the County of Halifax at a cost of $3.5 million. In the early 1990s this system was reported to process over 4500 liters per hour of highly concentrated waste liquid (the leachate is 200 times the strength of normal sewage). At this landfill site, more than 1 million tons of solid waste has been deposited to a maximum depth of 17 m. The site was operated by the Metropolitan Authority and received approximately 1×10^6 kg of refuse per working day. At that time, the site contained about 145 hectares of which 30 hectares are cleared. The daily cover material and liner is excavated from on-site glacial till material with a permeability of 10–6 cm/s or less. The base of the fill is underlain by a herring-bone pattern of leachate collector pipes through which leachate is collected and pumped into the

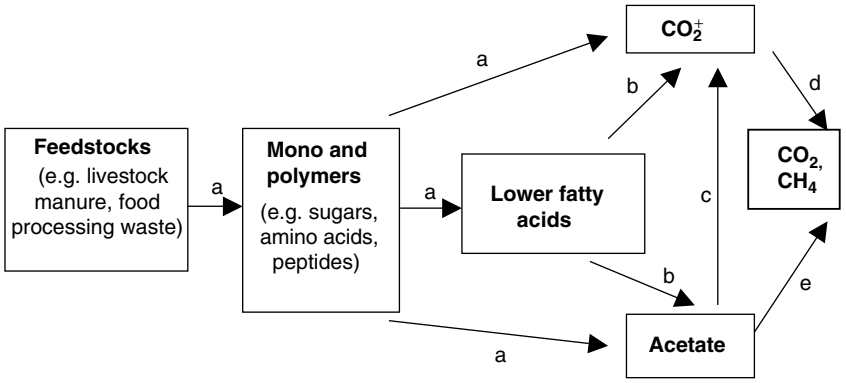

FIGURE 145.6 Flow diagram of degradation of organic wastes by anaerobic biological treatment processes.

closed-system biomass reactor. The leachate is at present treated in two anaerobic lagoons following which it is discharged to a series of four siltation ponds, and then into a swamp adjacent to the Sackville River. The treatment system consists of four stages including a pre-treatment stage, high rate anaerobic-reactors to reduce organic strength, sludge handling facilities and polishing lagoons. The pre-treatment system consists of an equalization tank with a volume of 50 m^3, a caustic feed system (heavy metals are removed by treatment with caustic soda; the high calcium concentration in the raw leachate discouraged the use of lime due to expected scaling problems) and rapid mix, flocculation and settling units. The anaerobic digester constitutes the primary treatment process. This system includes the following components:

a. The anaerobic digesters are fed on a semi-continuous basis by a specially designed feed station. A 1.5 m diameter closed vessel serves as the wetwell (covered and vented through scrubblers to the atmosphere) for the feed pumps.
b. Two upflow anaerobic reactors are used to achieve the reduction of COD in the leachate. The waste effluent is pumped upwards through a dense anaerobic bacterial suspension (i.e. termed the sludge blanket), and are biodegraded in the process. Upward flow anaerobic sludge blanket process (UASB) is an anaerobic bioreactor in which wastewater is introduced at the bottom of the reactor and forced upward to pass through a blanket of sludge (microorganisms). The organics in the wastewater are bioremediated by the microorganisms into carbon dioxide, water, methane and hydrogen gases as shown in Figure 145.7. The biogases can be collected and used as fuel to produce energy. The UASB reactor is designed as a gravity settler to maintain high concentrations of flocculent bacteria inside them. The treated liquid flows over an overflow weir at the top of the reactor, and is then discharged or recycled for more treatment. The concrete cylinders are 5.5 m in diameter and 8 m tall. The effective volume of each reactor amounts to 135 m^3. The bacteria which metabolize the organic waste produce water, carbon dioxide and methane as byproducts.
c. Reactor contents are re-circulated through a drawoff piping network to provide mixing and to maintain the temperature of the reactors at 35°C. The re-circulation ratio of the reactor's contents to influent is established initially at a ratio of 10:1. Duplicate heat exchangers have been provided and sized at capacities of 580,000 Kjoules/hr each, whereas re-circulation pumps have a rated capacity of 26.1 m^3/hr.
d. Methane gas produced during the fermentation is collected at the top of the anaerobic digester and is subsequently used as fuel for the boiler. At the initial and ultimate COD loads of 1,815 kg/d and 2,400 kg/d, respectively, and projected COD removal efficiencies of 95%, methane generation is estimated at 620 m^3/d under initial conditions and 820 m^3/d under

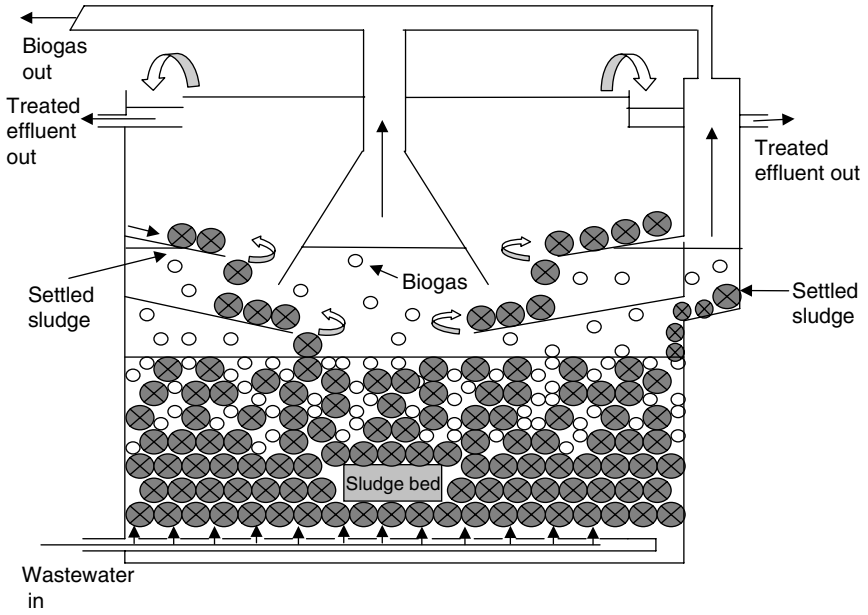

FIGURE 145.7 Upward flow anaerobic sludge blanket process.

ultimate conditions. This gas quantity converts to heating values of 938,000 Kjoules/hr, and 1.27 million Kjoules/hr, under present, and ultimate conditions, respectively. The energy derived from the gas is utilized as a priority to maintain the reactor contents at 35°C with excess gas directed to space heating. The sludge produced during the anaerobic digestion (consisting of microbial biomass, under graded insoluble, etc.) is periodically removed from the bottom of the digester. This material is then dried and returned to the landfill site. The combined sludge from the settling tank and anaerobic reactors are transferred to a covered thickener of 5.5 m diameter and 3 m sidewall depth. Supernatant overflows a perimeter V-notch weir in the thickener to a sump from which it is conveyed by submersible pumps to the equalization tank at the head of the treatment facilities. Thickened sludge is transferred to the dewatering equipment automatically in response to a level signal in the sludge vat of the vacuum filter. A skid mounted fully automated pre-coat vacuum filter system provides sludge dewatering. Dewatered sludge cake is dumped in the landfill, and filtrate from the operation is directed to the aerated lagoons. The final polishing step is carried out in aerated lagoons, which remove residual COD, BOD and ammonia, and precipitate dissolved and suspended solids. According to the information from the Metropolitan Authority of Halifax-Dartmouth and the County of Halifax, the reactor is currently removing 95 to 98% of the incoming waste and is producing enough methane to heat the building it is housed in.

2. Macmillan Bloedel (BIOPAQ)

Currently the (UASB) reactor has been successfully commercialized as a full-scale treatment system in Canada (e.g. the Macmillan Bloedel, Lake Utopia Paper, and Quesnel River Co.). The patented BIOPAQ system (Paques Lavalin, Toronto) is a biological treatment system which is based on the UASB process. The wastewater is pumped into the bioreactor through a number of nozzles that are evenly distributed at the bottom of the reactor. This incoming material then permeates up through the sludge layer, during which time the anaerobic bacteria digest the organics present in the waste, converting it to volatile organic acids, methane, hydrogen and carbon dioxide. Full scale BIOPAQ systems have been built for the pulp and paper, food, textile and petrochemical industries, and for treating domestic sewage.

Following the successful completion of an initial laboratory and pilot scale research, a 19 m^3 Upflow Anaerobic Sludge Bed Reactor was set up for operation under the dynamic mill conditions at Sturgeon Falls (23). The demonstration plant was constructed and was being operated by Paques Lavalin Ltd. There were three major component parts to this treatment system: an 18 m^3 pre-acidification/equalization tank, a 19 m^3 UASB reactor and an operation/control room. The average composition of the wastewater fed into the system was 35% spent sulfite liquor and 65% flotation-clarifier effluent. The two streams were combined, screened and then fed into the pre-acidification tank, where caustic soda (for pH control) and nutrients (required for biological growth) were added to the feed. After the first 8 months of operation, the results obtained showed that the biogas produced in the reactor contained 60–65% methane, 35–40% carbon dioxide and 1.5% hydrogen sulfide. The biogas production rate followed very closely to the volumetric loading rate (VLR) applied, indicating a healthy and acclimatized biomass. Preliminary results indicated net granular sludge growth, and the biomass was not overloaded. At a VLR up to 19 kg COD/m^3/d the plant consistently obtained 85% BOD removal or better. The projected capital cost for 1987 supply and installation of the proposed four reactor BIOPAQ package plant was $5.8 (Canadian) million or about $140 per kg BOD removed. The annual operating costs of the proposed system were estimated to be $0.5 million. The volume of biogas expected from the full-scale plant was estimated to have a value of $900,000/year.

The UASB bioreactor has been installed in many countries for treating different types of wastewaters from different industries, including foods (A. Pauss, Personal Communication). In the United States, an upflow anaerobic sludge blanket bioreactor achieved COD removals of 98% in the confectionery wastewater (24).

III. FOOD WASTEWATER TREATMENT IN HONG KONG

In Hong Kong, there are 12,694 food premises, including thousands of food production companies. By law, these premises (including restaurants) are required to have a basic grease trap (to separate wastewater from oil), and for food production companies they are required to have a wastewater treatment system to treat their food wastewater such that the effluent from the production plant will meet the requirement (such as COD, BOD, oil and grease, pH and suspended solids) of the Environmental Protection Department of Hong Kong. Most of the wastewater treatment systems of the food manufacturers in Hong Kong utilized aerobic systems, including the five government wastewater treatment centers (activated sludge ponds, although the Drainage Service Department is also exploring other pilot plant scale systems, such as the rotating biological contactors).

The followings are the reports of some of the newer development of biological treatment systems in Hong Kong.

1. Wastewater treatment in CFS (A large potato chip processing plant). The treatment plant is operated with physical, chemical and biological processes and it is designed by HKPC. The daily treatment capacity is about 175 cubic meter/day which is treated to comply with the HK discharge standard. All of the daily operation wastewaters are screened first, then collected and pumped to the equalization tank for further treatment. Chemicals are used to have coagulation and flocculation for the sedimentation of suspended solids, then followed by biological treatment process for organic removal. After the processing, the wastewater with sludge are pumped to have dewatering by filter press before deposed as solid waste. The percent of COD and BOD removal efficiency are more than 95%.
2. Immobilized cell bioreactor (a major fast food central kitchen). We had set up an immobilized cell bioreactor model (Figure 145.8) in the wastewater treatment center of a central kitchen of a large fast food chain restaurant (with over 200 branches) to treat their wastewater during 1997–1998. The bioreactor is made of polyvinyl chloride (PVC) with a rectangular working space which has base area of 0.35 m × 0.36 m and a height of 0.52 m. Plastic balls with high porosity which can increase the surface area for microbial attachment were used as the media for immobilization. Chemoheterotrophs were immobilized because of their ability to derive their energy form the oxidation of organic compounds in wastewater, and their ability to resist and recover quickly from organic loading shock and acid shock was noted in our preliminary finding. During the testing period from November 25th 1997 to February 9th 1998, the percent of COD and BOD removal efficiency in the wastewater were increased from 41% and 56% to 76% and 75%, respectively.
3. A counter-current process comprising a series of three batch immobilized cell reactors (R1, R2 and R3) was developed for removing and recovering copper from food industry effluents in Hong Kong. The ability of metal uptake by bacterial biosorbent has caught great attention due to its potential to provide an effective and economic means for removing heavy metals from food industry effluents. Cells of *Micrococcus* sp. were immobilized in 2% calcium alginate and 10% polyacrylamide gel beads. Each reactor consisted of immobilized cells with concentration at 100 grams dry cell per litre of beads. Six hours of reaction time was allowed for each reactor unit. The average copper concentration of the wastewater treated

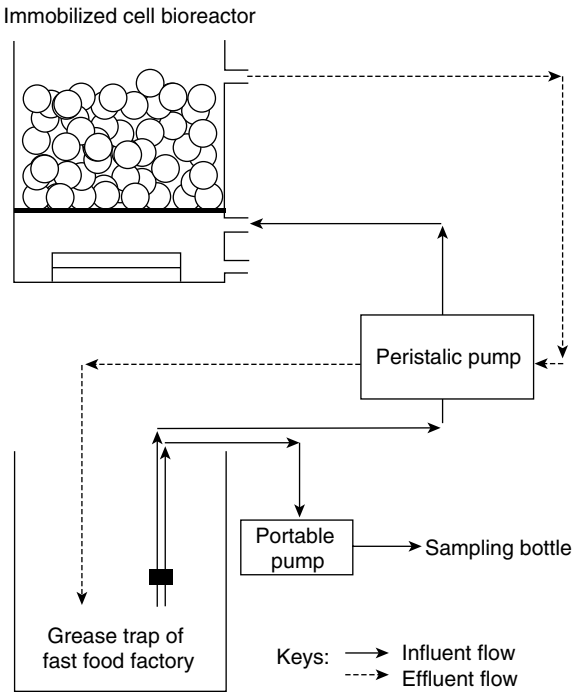

FIGURE 145.8 Immobilized cell bioreactor model.

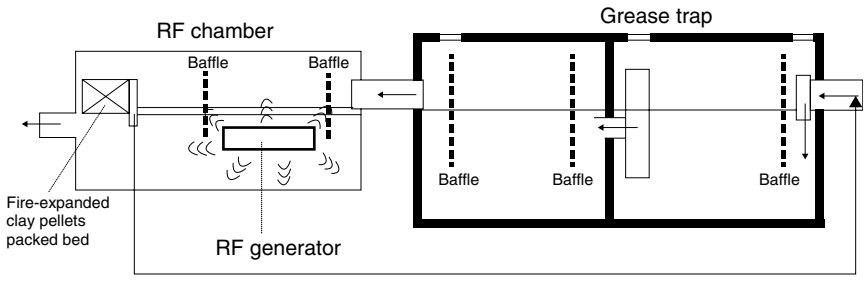

FIGURE 145.9 Radio frequency technology for oil and water separation in Hong Kong.

TABLE 145.2
Removal Efficiencies of COD, Oil and Grease from the Food Catering Sewage of a Local Amusement Centre in Hong Kong

	Inlet (mg/L)	Outlet (mg/L)	Removal Efficiency
COD	1,100	410	30%–70%
	1,100	680	
	1540	490	
	700	370	
	1000	440	
	1000	470	
	900	280	
	1,660	680	
Oil and grease	360	95	70%–80%
	390	102	
	460	114	
	210	62	
	290	79	
	340	96	
	260	70	
	495	128	

with immobilized cells was 5.5 ± 4.8 mg/L. The average copper concentration recovered from the immobilized cells by desorption could be as high as 449 ± 77 mg/L and was ready to be reused. The results show that the immobilized cell system is a promising technology for removal and recovery of copper ions from metal-bearing industrial wastewater.

4. There was a brand new method for oil and water separation (Radio Frequency Technology) for treating the greasy food catering sewage from a local amusement centre in Hong Kong as the conventional greasy traps are incapable of removing those oil and grease that have already been emulsified in the wastewater. Therefore, a novel method with an emulsion breaking device (Figure 145.9) was developed to tackle those emulsified oil molecules. Radiating-wave frequencies (RF) can accelerate the emulsion-breaking process and extract the oil droplets from the emulsified liquid by the vibrating resonance generated from finely adjusting the radio frequency to a specific level, thus accelerating the "demulsification." Desirable effluent without any visible sheen could be obtained after passing through the RF oil interceptor. As shown in Table 145.2, it was found that COD removal efficiency ranged from 30–70% (wt) while oil and grease contents in sewage were reduced 70–80% (wt).

PART II. Biological Wastewater Treatment: Current Thoughts

IV. PRESENT NEW APPROACHES

Conventional biological treatment technologies including anaerobic contact, anaerobic fluidized bed and upflow anaerobic sludge blanket reactors were used frequently in the past. Recently, some newly advanced technologies and some re-modeled technologies were developed.

Using microorganisms for treatment of wastewater was extensively studied in the past. Recently, an ultrasound-assisted bioprocess for wastewater treatment for the food industry was developed in Germany for enhancing the biodegradation efficiency of the microorganisms (25). More than 100% increase of the maximum biological degradation rate has been achieved and the developed bioprocess reduces the overall energy by use of low-energy irradiation below the cavitation level.

Moreover, a fixed-film bioreactor system was designed to increase the efficiency of biological treatment systems for treating oil refinery wastewater (26). The system enhanced the treatment efficiency by (1) allowing greater organic loads, (2) minimizing production of sludge waste by-products, and (3) increasing process stability and resistance to shock loading. It was constructed with a highly porous polyurethane foam which can incubate very high concentration of microorganisms. The support frame which was built from cylindrical plastic pall rings formed a packed bed of "mixed-media" and can provide a high surface area-to-volume ratio. The bioreactor with 8-h hydraulic retention time demonstrated chemical oxygen demand removal rates of 85–90% and near one-third of the sludge waste compared to the traditional activated sludge process.

Another wastewater treatment system treated the wastewater from a food processing factory by bacteria immobilized onto a new type of carrier-ceramic carrier in an aerated system (27). This carrier allowed higher concentration of bacteria to be colonized (2.9×10^9 cfu/g of dry ceramic carrier). The system removed more than 87% of the influent COD when the hydraulic retention time was 30.17 h.

A multi-media-layering (MML) system was developed for food service wastewater treatment (28). The MML system consists of two units. One unit is composed of soil mixed with sawdust plus iron scraps and the other composed of soil mixed with (kenaf plus corncob) plus iron scraps. The mixture layers are arranged in a "brick like" pattern. Aeration significantly increased the efficiency of wastewater treatment in this system. The percentage removal of suspended solid, biological oxygen demand, chemical oxygen demand, total nitrogen, NH_4-nitrogen, total phosphorus and dissolved phosphorus was 71, 90, 70, 91, 76, 90 and 89 with a loading rate of 2301 $m^{-2}day^{-1}$.

V. CURRENT THOUGHTS

In recent years there have been significant advances in the design of wastewater treatment systems having improved performance and operational dependability. In large part, this progress has been driven by the needs of industrial and municipal wastewater discharges to meet ever increasingly stringent effluent discharge regulations that have been promulgated in North America and worldwide. Such regulations include the Ontario Municipal Industrial Strategy for Abatement (MISA) regulations and various United States federal regulations such as the '40 CFR (Code of Federal Regulations) Part 503 (1993) [Sewage Sludge Regulations], National Combined Sever Overflow Regulations (1994) and the Total Maximum Daily Load (TMDL) [2000] Section 303(d) of the Clean Water Act. The TDML is designed to protect ambient water quality, and it represents the maximal amount of a pollutant that a water body can receive and meet the water quality standards.

The net result of this research has been the development of novel high rate treatment systems such as the Acticontact biofilter, membrane bioreactors (MBR) and moving bed biological reactors (MBBR) which offer advantages over conventional treatment system technologies including smaller footprint and greater resilience to microbial upsets such as filamentous bulking. Similarly, for the treatment of contaminated soils and sediments, we have also seen the emergence of many different novel engineered systems such as *in situ* and *ex situ* bioremediation. Each of these systems strives to provide suitable environmental conditions to promote microbial activities which bring about the desired end-treatment result.

With the stricter regulations and the findings of recent research (29), many of the new treatment methods are now being developed to deal with the health and environmental concerns. Filtration is now regarded as a key component in wastewater treatment systems, as filtration is especially effective in improving the effectiveness of disinfection, particularly for ultraviolet (UV) disinfection

systems, because (1) the removal of large particles of suspended solids (which may harbor bacteria) enhances the reduction in coliform bacteria, and (2) the reduction of turbidity improves the transmittance of UV light (29).

Much research has also gone into the study of microbial ecology and into the mechanisms by which microorganisms are able to biodegrade complex xenobiotic substances, with the application of the knowledge gained to the development of improved and novel systems for bioremediation of heavily contaminated sites such as the Superfund sites. There are concerns that the current analytical techniques are inadequate for detecting all environmental microbial and chemical contaminants that may significantly affect health (30). Detection methods of constituent concentrations are becoming more sensitive, and the detection limit has expanded from milligrams per liter (mg/L) to nanograms per liter (ng/L), particularly in the area of pesticides and haloaromatics such as PCBs and dioxin.

From the results of the different types of waste treatment systems studied, it is clear that biological remediation is both technologically and economically feasible for the degradation and removal of chemicals and organics from wastewater and from landfill leachates.

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146 Poultry Waste Management

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CONTENTS

Poultry processing is a typical agro-industry generating a strong wastewater in terms of organics, solids and nitrogen contents and significant amount of solid waste. Crude approaches in the past have often resulted in mixing of the two waste streams and which have resulted in serious treatment and disposal problems. Nowadays, poultry processing requires, like all other agro-industries, an integrated approach in waste management. This approach enables an in-depth look at the processes involved and provides a better perspective for critical issues such as water minimization, waste reclamation and recycling and optimization of treatment and disposal.

In-plant control or source based control of wastes; wastewater and solid waste management are integral parts of a sound waste management policy for the poultry processing industry. The level of in-plant control dictates the amount of wastewaters generated and pollutant loads of the effluents. Solid waste management practices such as the dry collection of wastes on the other hand, directly affect the pollutant loads of wastewaters.

I. SOURCES OF WASTES

Although subject to changes, the general process flow scheme together with the wastes originating from each process is briefly outlined below.

Receiving Area: This is the area in which the live birds are unloaded from the crates. Dirt, feathers and droppings are among the contaminants of receiving areas. Dry removal of solid wastes with subsequent washing operations is recommended for cleaning. In order to deal with fluctuations in delivery, it is sometimes necessary to keep the birds for a period of time in the receiving area. In such cases additional feces can be generated as solid waste.

Killing and Bleeding: Birds hanged from their feet are shocked with electricity. This procedure of stunning can be conducted in either an electrically charged stunning bath or by passing the birds through charged plates. Wastewater is generated when stunning is applied in baths. Then the throats of the birds are slit and blood must be collected in a storage bath as solid waste or by-product.

Scalding: Various types of scalding operations (spray, immersion, etc.) using hot water can be applied to carcasses for the easy removal of feathers. Scalding is one of the main sources of wastewater in poultry processing industry.

Defeathering: The plucked feathers can be transported with water or removed by dry processes. When water flume is applied, a subsequent screening must be performed for the separation of feathers from wastewater. Wastewater can be recycled and feathers can be directed towards a rendering facility or disposed as solid waste.

Evisceration: After removing heads and feet, the birds are spray washed with large amounts of water to reduce microbial contamination during and after evisceration. This produces a large amount of wastewater. Solid waste is generated when viscera is pulled out and sorted as edible

and inedible offal. Offal transport can be conducted by vacuum or water fluming. Water fluming results in additional wastewater.

Chilling: When water chillers are used wastewater with significant levels of organic pollutants is generated.

Filleting, Deboning, Portioning: Filleting, deboning and portioning operations can produce solid waste or by-products.

Area Washdown: Apart from the above mentioned wastewater sources, additional generation of wastewaters due to cleaning purposes should be taken into account.

The sources of wastes in poultry processing industry are summarized in Figure 146.1. However, it should be noted that the shown figure is subjected to variations. When handled as by-products and directed towards rendering facilities, the amounts of solid wastes can be reduced considerably. Similarly, when water usage for solids transportation is avoided throughout the operating steps, wastewater generation can be limited to a significant extent. Besides by-product processing usually done by rendering itself generates and contributes to wastewater loads (1).

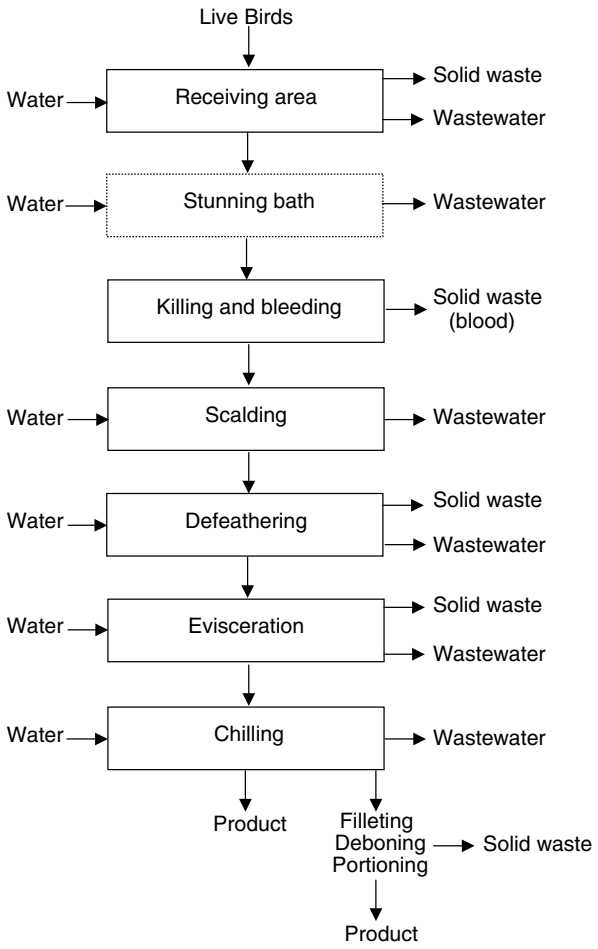

FIGURE 146.1 Sources of wastes.

II. IN-PLANT CONTROL

In-plant control alternatives applicable for poultry processing plants can be grouped under two main headings: i) water use reductions, ii) wastewater reclamation and recycle.

A. Water Use

The water usage varies in a wide range for poultry processing industry as given in Table 146.1.

It should be noted that the large plants generally use less water per bird than the smaller ones, as they are more likely to apply proper in-plant control measures. However, significant variations in water usages can be observed even in industries with same production levels. Table 146.2 tabulates the distribution of water use for a typical large poultry processing plant (1, 2). All the figures given in the table, especially the percentages can be very different for a smaller premise.

The amount of water used for a given plant is a certain value that is independent from the number of the birds processed, as almost all of the water is applied for cleaning operations or processes such as scalding. In this respect the utilization of plant capacity gains importance. The plants operating with full capacity are the most efficient ones in terms of water usage.

TABLE 146.1
Water Uses for Poultry Processing Industry

Water Use (Liters/Bird)	Reference
15–30	1, 2
30	3
30–45	4
21–38	5

TABLE 146.2
Distribution of Water Use for a Large Plant

	%	Liters/Bird
Processing		
• Hanging, stunning, bleeding	1	0.2
• Scalding	17	2.9
• Evisceration, spray washing	33	5.6
• Spin-chilling	12	2.0
Utilities		
• Boilers	1	0.2
• Cooling and refrigeration	7	1.2
Wash-Down		
• Floor & equipment washing	17	2.9
• Crate washing	2	0.3
• Truck washing	3	0.5
By-Products Rendering	5	0.9
Domestic (hand washing, etc.)	2	0.3
Total	100	17.0

Although the following recommendations for reducing the amount of water intake can be applicable to poultry processing plants with all sizes, more effective results can be obtained on large plants:

In order to identify the points where excessive water is used, all the water intakes can be monitored with water meters.
Cold water should be used for cleaning blood spillages. Hot water causes congealing of the blood, making cleaning more difficult and necessitating the use of more water than when cold water is supplied (2).
Dry transport of feathers and viscera should be preferred (1–4, 6).
Dry cleaning with shovels; scrappers or brooms should be conducted prior to washdown. Apart from reducing the water intake, the wastewater pollutant loads can also be significantly lowered with such an application (1–3, 6).
Usage of pressurized water should be applied wherever possible.
Good housekeeping and proper maintenance should be conducted. Leaks in piping should be avoided. Self-closing nozzles should be installed instead of valves to better control total water use.
Staff should be trained about water conservation techniques.

It should be noted that reducing the water intake lowers the amount of wastewater generated and concentrates the wastewater. Therefore it is suggested to check the treatability of wastewaters prior to applying such practices.

B. Wastewater Reclamation and Recycle

Wastewater recycle and reuse can be successfully conducted on poultry processing wastewaters. The important point in such applications is meeting the hygiene requirements. Stream segregation is the first step in wastewater reclamation. Depending on characterization of segregated streams, wastewaters with low pollutant contents can be reused directly or after being subjected to a proper treatment.

The following reclamation and reuse alternatives are recommended:

Streams having low contaminant loads can be reused in floor, vehicle and crate washing after being treated e.g. by membrane processes such ultrafiltration and reverse osmosis (1, 2). Feasibility of using such processes must be checked beforehand.
In plants where removed feathers are transported with water, this water can be reused in feather fluming and offal transport after passing through a screen (1, 2). In order to avoid cross contamination offal flume and feather transport water should be collected in different channels and screening of both wastewaters should be performed with separate screens (3).
Wastewater obtained from chillers can be used as make-up water for the scald tanks (3).
Wastewaters originating from chilling process can be recovered and reused again in chilling operations (3, 8). It is stated that fresh water supply in chilling can be reduced by 80% with recycling (7).
- ozonation (7)
- screening, ozonation, sand filtration, post-ozonation (7)
- screening, diatomaceous earth (DE) filtration, ozonation (7)
- UV-enhanced ozonation (8)

are the proposed treatment methods.

The feasibility of in-plant control applications must be evaluated by comparing the savings obtained on fresh water demand versus possibly elevated end of pipe wastewater treatment costs together with cost of treating reusable streams.

III. WASTEWATER MANAGEMENT

A. Wastewater Generation

Approximately 90% of the water input is converted into wastewater in poultry processing plants (1, 2). It is stated that 15.6 liters of wastewater generation per bird is observed for industries processing more than 10,000 birds/day, whereas for smaller premises a mean wastewater discharge of 19.0 liter/bird is obtained (1).

Table 146.3 outlines the amount of wastewater generated from different operations for a modern large (>10,000 birds/day) poultry abattoir where feather transport water is recycled, inedible offal is transported by use of water flume and efficient blood recovery and collection is applied (2).

B. Wastewater Characterization and Loads

As poultry processing wastewaters are contaminated with fat, viscera, blood, feathers, feces, etc., they can be characterized and distinguished from other industrial wastewaters by their high organic matter, oil and grease and solid contents. Concentrations of main pollutant parameters together

TABLE 146.3
Sources of Wastewaters

Source	Lt/Bird	%
Scalding	2.5	17
Evisceration	5.0	33
Washdown	3.3	22
Miscellaneous	4.2	28
Total	15.0	100

TABLE 146.4
Characterization of Poultry Wastewaters

	Number of Plants Investigated	Size of Plant (Birds Processed/Day)	Total COD (mg/L)	TSS (mg/L)	TDS (mg/L)	pH	Wastewater Volumetric Load (l/Bird)	Ref.
Range	8	<10 000	1100–6780	141–970	477–2170	6.0–8.8	NA	1
Mean			3410	730	1250	7.7	19.0	1
Range	7	>10 000	698–4744	106–1240	332–2040	5.9–10.1	9.6–18.2	2
Mean			1875	442	940	7.2	15.3	2

with volumetric wastewater loads for large and small size poultry processing effluents are outlined in Table 146.4. It is also stated in the literature that the oil and grease concentrations of these wastewaters can reach levels that might adversely affect the subsequent treatment steps (2, 9). Oil and grease might cause adverse effects in treatment units such as aeration tanks, settlers, etc.

Cleaning the bird manure and feathers with water results in the generation of wastewaters containing organic matter and solids in receiving areas. When the period of time for a bird to stay in the receiving area elongates, a proportional increase in wastewater pollutant loads is observed.

The killing and bleeding operation is one of the main sources of organic pollution in the poultry processing industry due to possible blood spillages. Proper blood collection and handling should be applied as part of a sound waste management strategy. In this context, it must be kept in mind that the organic matter content of the blood obtained from one bird is equal to 21,000 mg chemical oxygen demand, COD (2). The end-of-pipe wastewater strength in terms of organic matter can be elevated by 35 to 50% when blood is not collected as solid waste and mixed with other wastewaters sources (3). COD concentrations as high as 100,000 mg/l can be achieved in slaughter areas for small abattoirs with no or very poor blood collection facilities (1). The existence of some proteinaceous materials such as feathers, blood and animal tissues in poultry processing wastewaters is the main reason of getting high effluent total Kjehldal nitrogen (TKN) values (4, 10). In this respect minimizing the blood spillages might at least ease the TKN problem (4). Stunning the birds before slaughtering increases the blood recovery and reduces the organic pollutant loads as the blood spurting due to the birds' movements are minimized.

Pollutants such as blood, feathers, manure, dissolved greases, etc., are associated with the scalding tanks. The contaminants are concentrated at the bottom of the tank. Scalding tank sludges must be collected and separately disposed as solid waste. By doing so, wastewater pollutant loads can be reduced to a significant extent. As scalding tanks are usually continuously replenished with fresh water to maintain a previously set water quality, continuous wastewater is generated as overflows. Apart from this wastewater, the contents of scalding tank are discharged after every batch of operation creating intermittent wastewaters with shock pollutant loads. Wastewaters originating from scalding tanks contribute 4.0 to 15.5% of the total plant suspended solids (SS) and 0.4 to 10.9% of the total plant oil and grease (4).

Feathers that are plucked from the birds are transported either in dry form or by the use of flume in the subsequent defeathering operation. Dry transport does not generate wastewaters other than the ones produced from cleaning purposes. On the other hand when transportation with water is the preferred process, the wastewater originating from defeathering operation must be screened before being directed to drain in order to avoid clogging problems in the treatment system. The reuse of screened feather transport wastewater is recommended as mentioned in the wastewater reclamation and recycle discussion. Feathers exert high organic and TKN loads to wastewaters (4). The contact time of feathers with water is the determining factor in the TKN content of these wastewaters. Longer contact times results in elevated wastewater TKN concentrations (4). Feathers are valuable by-products as they contain protein. Therefore dry collection of feathers and processing them in rendering plants are proposed as part of a proper waste management.

After sorting the obtained viscera in the evisceration step, the offal is transported either by means of vacuum application or water fluming. Depending on the chosen offal transportation method, the amount and pollutant loads of wastewater produced from this operation vary significantly. Dry removal of evisceration wastes not only decreases the volume of wastewater generated, but also lowers the wastewater pollutant concentrations. A reduction that can be as high as 35% in organic strength has been reported in dry transportation of viscera (3). Data ranging from 14.8 liters wastewater production per bird (3), to 5 liter/bird as tabulated in Table 146.3 (2) on wastewater volumes can be found in literature. Microbial contamination is an important issue. Therefore apart from viscera flumes, plenty of fresh water is used for area and worker cleanups and bird washes. The wastewater generated from this source can be characterized with its high organic matter, suspended solids, oil and grease and bacteria (from intestinal tracts of birds) contents. Evisceration processes contribute at least

1/3 of the total wastewater load in terms of the mentioned pollutant parameters (4). It is recommended to use water spray for all sorts of bird washing processes, in order to minimize water use and wastewater generation.

Water chilling operations are the other source of wastewaters. The overflows of water giblet chillers contain significant levels of organic pollutants. However wastewaters originating from chilling processes have a great potential for reuse after being subjected to a proper pretreatment. More information about pretreatment alternatives applicable to chilling wastewaters can be found under the wastewater reclamation and recycle heading.

It was mentioned earlier that especially a significant amount of the solid wastes generated in poultry processing plants such as feathers, blood, offal, etc., can be handled as valuable by-products when they are directed towards rendering facilities. By-product processing can either take place on site or in a separate rendering plant. In this context the best waste management plan to follow for poultry processing plants without rendering facilities, is collecting all recoverable materials and sending them to rendering plants. In order to minimize the organic loads of effluents, it is recommended not to overcharge the cookers of the rendering plants. Otherwise cooker overflow wastewaters with very high COD concentrations (25,000 mg/l) can be obtained (2).

For large poultry plants processing more than 10,000 birds/day, COD and SS loads of 29 g/bird and 7 g/bird, respectively are given in the literature (1). 64,000 mgCOD/ bird and 14,000 mgSS/bird pollutant loads are reported for smaller premises (1). COD loads originating from different wastewater sources are tabulated in Table 146.5 (2). The figures are obtained from a modern large (>10,000 birds/day) poultry processing industry where blood is recovered and collected efficiently, inedible offal and feathers are transported with water and screened feather transport water is reused (2).

It should be kept in mind that different organic loads can be obtained for abattoirs in which feather and inedible offal transport is performed with dry methods. In such cases the COD load originating from washdown processes may constitute 60% of the total organic load (2). On the other hand, concentrations of organic matter differ in a wide range for segregated wastewater streams obtained from different sources. Values as high as 4000 mg/l of COD is reported in literature for giblet chiller effluents, whereas 250 mg/l of COD concentration is given for whole bird washing operations (7).

TABLE 146.5
COD Loads from Different Sources

Source	g COD/Bird	%
Scalding	3.0	11
Evisceration	13.0	48
Washdown	9.5	35
Miscellaneous	1.5	6
Total	27.0	100

C. Treatment Alternatives

Two different approaches can be followed in dealing with the treatment of wastewaters originating from poultry processing industry. The first one involves collecting all wastewater sources in an equalization basin and subsequently treating them by the use of a previously determined treatment scheme. However, it is a well known fact that some wastewater sources contain high concentrations of certain contaminants in this industrial activity. Therefore, it is advantageous to handle wastewater streams separately and apply specific treatment alternatives defined for each of them. By doing so, pollutant loads discharged to end-of-pipe treatment facilities can be reduced, recovery of by-products together with water recovery and reuse applications ease (2, 6). This second approach covering stream segregation concept is of importance especially for new industrial installations.

The following treatment units can be adopted for wastewaters generated from poultry processing industry.

Primary Treatment: Primary treatment may consist of a screen and a primary sedimentation tank to reduce solids loads, an oil and grease removal facility such as dissolved air flotation (DAF) and an equalization unit to minimize the fluctuations in wastewater quality and quantity.

Secondary Treatment: The target of secondary treatment is the degradation of organic matter. The wastewaters respond well to biological processes such as

activated sludge
- conventional activated sludge
- step aeration activated sludge
- high-rate activated sludge
- extended aeration activated sludge
- contact stabilization
- sequencing batch reactors

trickling filter
- high rate trickling filter
- standard rate trickling filter

lagoons
- anaerobic lagoon
- aerobic lagoon
- anaerobic lagoon followed by an aerobic lagoon

biodisks (9, 11–13).

Current understanding of biological treatability covers biodegradability oriented fractionation of the COD parameter. Total COD consists of biodegradable and inert components; both subdivide into further fractions. The total

inert COD consists of soluble inert COD and particulate inert COD both by-passing the biological treatment without being affected from biochemical reactions. Apart from the soluble inert content of wastewater, the level of inert metabolic products generated within the biological treatment processes gain importance in meeting the effluent discharge limitations (14). Such a fractionation was performed on a small poultry processing industry located in a remote area where the wastewater only originated from scalding operations and cleaning processes. The results of this study can be used for better understanding of the nature of COD generated from this industry. According to the mentioned research, the total COD of the poultry processing effluent is around 90% biodegradable, with a practically negligible particulate inert and significant soluble inert portion; the latter together with soluble residual microbial products generation, is likely to impart a residual effluent COD concentration after passing through a biological treatment, not less than 12% of the influent total COD (10).

Tertiary Treatment: When the applied primary and secondary treatment is inadequate in achieving the required treated effluent quality; an additional advanced treatment step can be used.

On the other hand as the major part of poultry processing wastewaters generated from certain processes, such as killing and bleeding and defeathering, is proteinaceous in nature, the recovery of these dissolved and colloidal proteins is an important issue (1, 2). The recovered solids can be rendered and used as animal feed. The following protein recovery methods are the most used ones:

- application of cellulose based ion exchange resins (1)
- use of ultrafiltration (1)
- recovery of protein from generated activated sludge biomass (1)
- precipitation of protein by the use of coagulants before air flotation unit (2). Ferric chloride ($FeCl_3$), sodium hexametaphosphate or lignosulphonate are stated as the most effective coagulants (1, 2). A pH of approximately 4 must be adjusted prior to sodium hexametaphosphate applications (2).

IV. SOLID WASTE MANAGEMENT

The amount of solid wastes generated in poultry processing plants varies in the range of 0.2 to 0.4 kg/bird (2). Feathers, inedible offal, blood, feces and carcasses classified as unsuitable for human consumption are the main contributors of solid wastes for this industrial activity. Table 146.6 outlines the mass distribution of each solid waste source for a typical plant (2).

In small premises without a rendering facility, the majority of the solid wastes can be sold to a rendering plant and the rest must be directed to a solid waste disposal site. Another alternative is to transport all the generated solid wastes for waste disposal. Waste handling practices such as landfills and composting can be applied to solid wastes generated from poultry industries. Solid wastes can also be anaerobically digested (15–17). Poultry processing wastes can be applied as fertilizers in certain agricultural activities after being digested in an anaerobic unit (15). It is reported that compost produced from dissolved air flotation skimming of poultry processing plants can successfully be used in corn production fields (18). Feather composting is an applicable solid waste handling method (19). Feathers are almost made up of pure keratin that is not easily degradable by common enzymes (20, 21). Therefore although biodegradation of feathers can be achieved with native microbial populations, it is possible to accelerate this process by using isolated, target oriented cultures (19–24).

TABLE 146.6
Distribution of Solid Wastes

Source	Kg/Bird	%
Feathers	0.030	10.5
Inedible Offal	0.150	52.6
Blood	0.030	10.5
Unsuitable Carcasses	0.015	5.3
Others	0.060	21.1
Total	0.285	100

When solid wastes are subjected to a rendering process, they are converted to by-products. Protein recovered from wastewaters can be added to by-products to enrich the protein content (2). By doing so, the nitrogen content of the wastewater can be decreased to a level that does not require treatment specific to this pollutant parameter.

In order to improve the solid waste management in a poultry processing plant it is recommended to follow the items below:

Dry collection of solid wastes must be preferred whenever possible. A washing can be carried out subsequently. Such an application eases the solids by-product recovery and reduces wastewater pollutant loads.

Blood must be collected in a properly designed place for minimizing the spillages. This in turn eases the cleaning procedure in the bleeding area, and excess blood contamination of wastewater can be avoided. It must be noted that blood constitutes 6–8% of the bird's mass (2).

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147 Waste Management in Meat Plant

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CONTENTS

I. INTRODUCTION

In the meat industry, plant operators always hope to utilize all they can get from the livestock, but some non-meat wastes still exist and these are called co-products. Co-products can include blood and renderable offal tissues such as viscera, bone, fat trimmings and fine debris; and these materials play a dual role as both assets and liabilities in the meat industry. Thus, how to efficiently recover co-products or how to minimize the waste in a meat plant is an important work and the performances of these actions can create a huge income or minimize disposal cost in a meat plant.

Some unavoidable meat plant waste was being dropped into the bay near-shore or the river, and it was drifted out with the tide, causing unpleasant odors.

TABLE 147.1
The Yield of Wastewater from Different Slaughtering Methods (Skinned and Scalded) for Pork in Meat Plant

	Skinned Method		Scalded Method	
Steps	**Amount of Water (Litter/hog)**	**Amount of Waste Water (Litter/hog)**	**Amount of Water (Litter/hog)**	**Amount of Waste Water (Litter/hog)**
Fasting area	70–100	65–95	70–100	65–95
Stunning	70–120	60–110	70–120	60–110
Scalding	50–60	40–55	0	0
Dehairing	60–80	55–70	0	0
Evisceration	110–250	110–250	110–250	110–250
Viscera washing	150–250	150–250	150–250	150–250
Carcass washing	100–150	99–150	100–150	99–150
Chilling	10	10	10	10
Total	**620–1000**	**589–990**	**560–860**	**494–865**

Source: Adapted from Ref. 4.

Today, with environmental concerns, energy costs and increased volume have together made co-products handling major economic and management problems in the meat industry. Additionally, in many countries' regulations, the air and water effluents from meat plants must be as clean as or cleaner than the water and air entering the plant. It is estimated that there is a total return of 15.07% from co-products out of the total value of product sales in a beef cattle slaughter plant, with inedible offal return of 8.44% and the rendered products return of 1.73% (1).

Many non-meat products with origin from the meat industry enhance modern living. Hence, the use of processing techniques to transform the waste into valuable products that can be used for food, feed or fertilizer in the living environment, or create new products or increase incomes for the meat industry are still an important target in a meat plant. Hansen in 1983 estimated that at least an additional 181 thousand tons of protein would be recovered from meat industry wastes each year in the United States and the worth perhaps exceeded 400 million dollars (2).

II. PRODUCTION

Meat-processing waste consists of solid and liquid portions. Solids include manure, paunch manure, and large amounts of animal tissue. The liquid portion includes blood and other body fluids. In actual practice, most of the solids such as small animal tissue and paunch manure will be mixed with a greater amount of water used for carcass cleaning or rinsing of the equipment, and some blood, and they are discharged into the sewer. Therefore, the best functional filter system should be utilized to separate meat waste into solid and liquid fractions. The solid waste is generally transported to the rendering plant for feed or fertilizer manufacturing; and the liquid waste was sent to the wastewater treatment system.

TABLE 147.2
Percentage and Amount of Inedible Co-Products and Waste Based on Live Weight from Various Livestock Species

	Cattle	**Pigs**	**Sheep**
Inedible raw material %	5–17	6–15	6–22
Waste %	14–20	6–12	
Paunch and manure %	8	4	5.5–9.5
Shrinkage %	2–10		0.5–1.5
Grade	**Choice**	**US No. 1**	**Choice**
Live weight kg	455	45	100
Inedible fats, bone and meat scrap kg	80	10	8
Unaccounted items (stomach content, ahrink, etc.) kg	64	5	12

Source: Refs. 5, 6, 7, 8, 9.

The waste in the meat plant usually comes from slaughtering procedures. A traditional slaughtering method yields approximately one ton wastewater per pig, but only yields 40–50% of this amount in a modern system due to new policy or equipment used (Table 147.1). For example, an automatic spray system can be used for washing carcasses. In red meat plants such as beef, lamb and pork, solid waste includes almost all the inedible co-products and the productions were estimated (Table 147.2). According to a report published by American Meat Institute in 1995, it was reported that total numbers of cattle, sheep and pigs for slaughter in 1994 were 33.4, 5.7 and 85.4 million heads, respectively. Total inedible co-products (inedible tallow and grease, other inedible

products) production was approximately 3,732 thousand tons in the United States in 1994 (3).

III. COLLECTION AND ATTRIBUTES

Generally, meat slaughter and processing plants with rendering facilities must have two separate rendering units. The two units must be separated physically to prevent any inter-transfer of raw materials, products or contamination from inedible rendering area to the edible rendering area. Equipment used to handle, contain or process inedible co-products must be clearly marked "Inedible," and any edible product that comes in contact with any so-marked pan, truck or conveyors immediately is designated as being inedible. The use of the various inedible meat co-products has a profound effect upon the speed at which they must be handled and processed. Inedible co-products from animals as a whole present a heterogeneous collection of tissues, some of which may be subjected to various processing treatments in normal commercial practice. Therefore, each inedible co-product can be expected to exhibit different ways of collection. In addition, undesirable odor, flavor, texture and appearance can be quickly produced in inedible raw materials when these raw co-products are stored under unsuitable temperature treatments and as a result of microbial spoilage. It is usually considered that a temperature of 10°C is adequate to control pathogen proliferation during the collection and processing of inedible raw co-products.

A. BLOOD

Blood contains about 17 percent protein and may be used for meat food products. Thus, blood should be collected because it has high nitrogen content and can be utilized for human consumption. Every effort should be made towards this end, not only because of its enhanced value but also because of effluent problem; thereby reducing the BOD for wastewater with non-blood recovery to about 4480 mg/L (Table 147.3), and for whole blood it is about 200,000 mg/L (10). Small amounts of blood specially collected on the kill floor can also be turned into edible products like black pudding and protein supplements. A closed collection system should be established which requires approval by USDA-FSIS and allows for the disposal of blood from animals whose carcasses are condemned during post-mortem inspection (11). The bacterial quality of edible blood should be less than 2,000 total plate count organisms per milliliter. The blood used in livestock feed, fertilizer or industrial use is collected by an open method and supplied in the form of blood meal and the bacterial quality should be less than 2.5×10^5 organisms per ml of blood (12). It often contains flush water, has a high water content (as much as 85%) and low dry solids value, and is often haemolysed. Therefore, after collection all blood material should be refrigerated at 2°C to minimize bacterial count and maintain good hygiene.

TABLE 147.3
The Wastewater Load Characteristics in Meat Plant with or without Blood Recovery

Items	SS kg/m³	BOD kg/m³	COD kg/m³	Grease kg/m³
Blood recovery	0.64	1.20	1.75	0.16
Non blood recovery	0.64	4.48	10.31	0.16

Source: Adapted from Ref. 4.

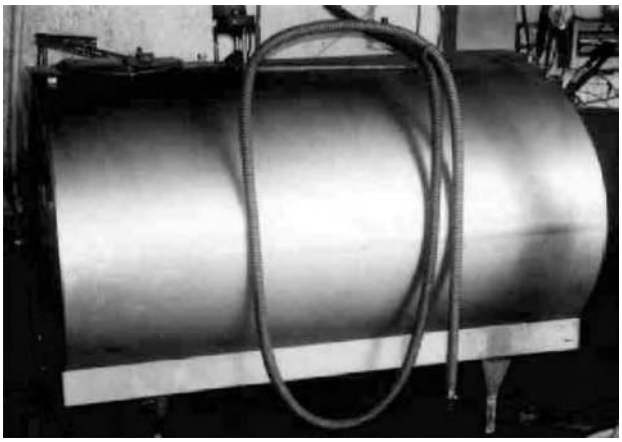

FIGURE 147.1 The blood collection tank with refrigerator.

The blood collection tank with refrigerator is shown as Figure 147.1.

B. ORGANS

It is generally considered that organs are necessarily of poor microbiological quality, with large bacterial loads and a high incidence of contamination with spoilage or pathogenic organisms. A putrid odor and flavor can be produced rapidly when these materials are exposed to improper treatment such as on the kill floor, or stored at a higher temperature (13). Thus, better control of organs handling are clearly necessary, but handling criteria which would be both commercially practicable and generally applicable are difficult to define because circumstances vary so greatly between individual plants. However, the end quality of organs is greatly affected by the manner in which the raw materials are collected, handled and held prior to further processing. The viscera collection is showed as Figure 147.2.

C. DEBONED RESIDUES

The bone yield of beef carcasses, pork carcasses and lamb carcasses is 15, 11 and 16%, respectively (14, 15). In recent years, labor has become more expensive, and as the red meat industry has attempted to salvage more of the adhering meat left on bones, new separating machines have been used. The amount of bone residues depends on the amount of separated red meat recovered from the bone materials. In general, with a requirement of good quality on separated red

FIGURE 147.2 The viscera were collected in an inedible co-product cart.

FIGURE 147.3 Deboning by hand and collecting bone residues in a cart for rendering.

meat, a larger amount of bone residue is obtained. The bone residue yield was from 21 to 80% by species, bone parts and machine types (16, 17, 18). The bone residues are used for meat and bone meals by rendering processes. An example of bone residues collection is showed in Figure 147.3.

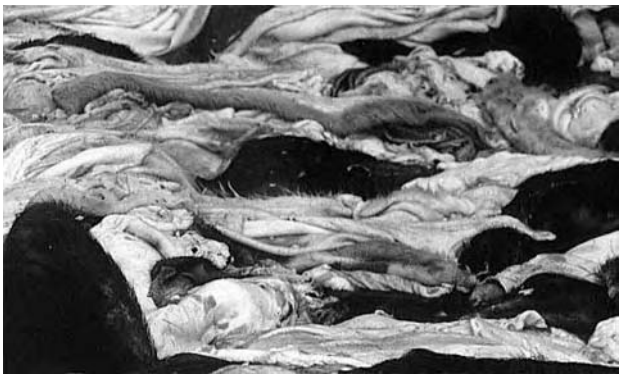

FIGURE 147.4 The hog skins were collected by a peeling system and used for leather making.

FIGURE 147.5 The scalded and dehaired pigskin was collected in a cart for rendering.

D. Skin and Hair

Animal hide is about 4–11% of the weight of the live animal, and one of the most valuable co-products produced by that animal. Hides from cattle sheep and goats are obtained by the hide pulling technique, then preservation, fleshing, trimming, selection, grading, storage and shipping. Average weight of pigskin from a market weight hog is about 4.5 kg. Scalded and dehairing skins are usually obtained in most pork meat plants due to the usage of scalding and dehairing procedures. This heating causes the protein in skin to denature and results in 50% of the pigskins being unsuitable for upper shoe leather. Hogs also can be skinned with a peeling system (Figure 147.4) that pushes the skin and carcass apart and these skins are useful for leather production because it has received no heat treatment. The scalded and dehaired pigskin collection is showed as Figure 147.5.

Hair, a co-product of the slaughtering industry, may be used for making brushes or for fertilizer. Practically, hair is generally collected in a pork plant by the wet method that means hair was mixed with a large amount of

FIGURE 147.6 The pork fat tissues were collected in a carriage for rendering.

water and the wet yield will enlarge by 2.5–3.5%. Most hog hair is not suitable for brush making and is manufactured with other inedible co-products in the rendering plant and sold as meal products.

E. Fat Tissues

Fat tissues are co-products of the meat plant and come from two major sources, one from the preparation of meat for sale, and the other from the manufacture of processed meat products. Lard and tallow are the two major animal fat tissues. Lard is obtained from hog, while tallow is obtained mainly from cattle and sheep. Generally, lard production is limited to certain cut fat from pork. Examples of cutting fats are backfat, belly fat, ham fat, ham trimming fat, fat removed from loin, neck and shoulder and miscellaneous trimming fats (19).

The lard production per hog is approximate 10.1 lbs and total lard production in 1992 was 860 million pounds in the United States (20). The total tallow production in 1990 was 14,727 million pounds in USA; and a total of 4724 million pounds is inedible for factory use, soap and feed (21). The fat tissue collection is shown in Figure 147.6.

IV. SOLID WASTE TREATMENT AND MANAGEMENT

The largest amount of solid wastes comes from inedible co-products and a small amount is animal manure and tankage that comes from wastewater treatment in meat packing house. Most of the inedible co-products will be transported to the rendering plant or unit near the meat plant in a short time to obtain various co-products meals for animal feed or fertilizer in the field.

Rendering is an extraction technique that usually involves the heating or cooking of the raw material to liquefy the fat, and to break down membranes or other structures that may hold the fat. Rendering serves two purposes: (1) to separate fat and protein, and (2) to cook the animal tissue and kill the microorganisms.

Based on sanitary and safety reasons, premises should be situated so that the raw materials can be conveyed to them with minimum handling. They should be spacious, well lit and ventilated, with impervious walls and floors. Floors should be sloped to open channels leading through fat traps to the drains. Abundant amounts of steam, and hot and cold water should be supplied. In addition, a rendering plant for inedible co-products is best located outside the slaughterhouse. The secret in the production of high quality animal co-products lies in prompt treatment of the raw material. Animal wastes decompose quickly and depreciating markedly as a result; it should therefore be an axiom in slaughterhouse control that no raw material left untreated after a day's slaughtering.

A. Rendering Methods

Toady, there are four major methods of processing inedible co-products: wet rendering, dry rendering (batch), continuous dry rendering and continuous low temperature rendering.

1. Wet Rendering Method

The wet rendering method requires a batch pressure cooker (autoclave) in which pre-ground raw material is injected with live steam to a temperature of 140°C under pressure for 3–4 hours. After this time, the pressure is slowly released and the fat runs out into a receiver and is further purified by centrifugation to settle out the water. The proteinaceous solids or graves are emptied from the cooker and the fat removed by pressure and solvent extraction. They are then ground and dried.

2. Dry Rendering Method

This process involves the use of heat in the form of steam and water over a period of 1.5–2 hours at atmospheric pressure to drive off water indirectly from the fat in the cooker (22). The rest of the processes are the same as for the wet rendering process.

3. Continuous Dry Rendering Method

The continuous dry rendering system is similar to a batch dry rendering process. The big difference is that the flow of raw material into and out of the cooker is continuous. The cooker is usually horizontal, steam jacketed and equipped with a hollowed, steam-heated agitator. The duration of the cooking period depends on cooker volume, heat transfer capability and characteristics of the raw material. A screw press conveys and handles the raw material continuously. Remaining solids are pressed to remove

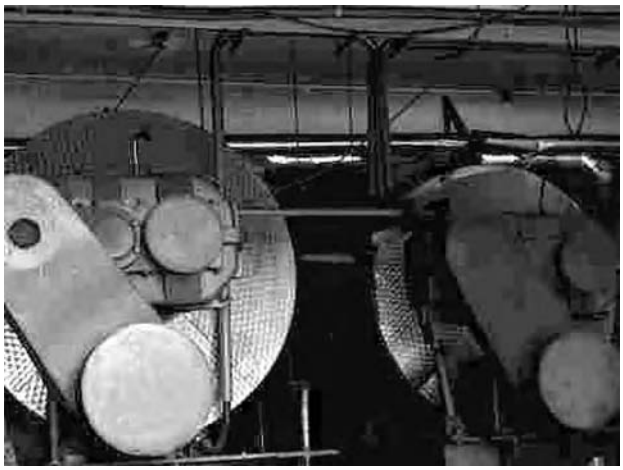

FIGURE 147.7 A dry rendering system usually used in a rendering unit in a meat plant.

FIGURE 147.8 Final rendered end products of meat and bone meal.

residual fat. Rendering processes of livestock by-products are shown in Figures 147.7, 147.8 and 147.9.

4. Continuous Low Temperature Rendering Method

This system is similar to the old wet batch processing but a significant difference is that the bulk of water is removed by mechanical methods made possible by the difference in density between fat, water and solids. The process involves mincing of the raw material, melting by live stem injection at 60–90°C, continuous separation of solids from the liquid fat in a decanter centrifuge, further heating, and centrifugation to remove the fines; and the tallow is used for manufacturing the final products. The solids (which contain about 40% water of the original material) are sent to a cooker/drier that is similar to the unit used in continuous dry rendering. The moisture of solid material is driven off by heat. High quality tallow will be produced in this system because of the relatively low temperature used in the processing.

FIGURE 147.9 A final rendered meat bone meal with package.

B. Use of Rendered Co-Products

1. Inedible Fats

A major use of inedible tallow and oil of animal origin is high-energy additive to livestock and poultry feed. Romans et al. in 1985 stated that inedible animal fat added to animal feed reduces the dust, improves color and palatability, and increases pelleting efficiency (9). An approved antioxidant needs to be added to prevent rancidity when the fat is kept in a storage tank.

Fatty acids and glycerol are obtained from animal inedible fats by a splitting technique that can be finished with the usage of high temperature, pressure and steam. Lard oil and neat's-foot oil are made from White A grease and White B grease, respectively, and can be used for delicate running machines, giving viscosity to mineral oil, and special leather oils, and making candles.

At present, the greatest utilization of inedible fats is still in soap making. Moreover, with environmental concerns, soap is biodegradable and has an advantage over the phosphate-based detergent for the water resources protection in lakes and streams. Additionally, new fat-based cleaning products with detergent-like traits have been developed and are helpful to get a large share of future cleaner markets.

2. Meat Meal, Meat and Bone Meal

Meat meal is used as a source of high quality protein, energy, B vitamins and minerals in animal feed. The bone content of meat meal supplies calcium and phosphorus, thus helps in providing necessary minerals to the animal's diet. It also supplies important B vitamins such as thiamin.

Some special bone meal is fed to poultry for bone growth and provides necessary calcium for eggshells, or

fed to those pregnant or lactating birds requiring higher level of minerals in the diet.

3. Blood Meal

Ockerman and Hansen in 2000 stated that dried whole blood (5–8% moisture) which has been steam-coagulated, dried and coarsely ground is called blood meal (23). If it has been finely ground, it is called blood flour. This product is about 75–83% protein, 1.2–1.6% fat and 3.8–5.6% ash. Blood meal is used in calf starter ration, swine and poultry feeds. It is less digestive than meat meal.

4. The Risk of Rendered Products

The production of end products from these wastes, e.g. bone meal, meat and bone meal, blood meal, poultry co-products meal, feather meal and the use of these for animal feed purposes may cause problem unless safe methods of treatment are employed. One of the problems in processing co-products used for feed is recontamination of the rendered product by incoming unprocessed material. The contaminated rendered product is then fed to animals. This is a special problem with *Salmonella* organisms in poultry feed. Another serious problem is a disease occurring in cattle in recent years (since 1985–86) and its common name is "mad cow disease" or bovine spongiform encephalopathy (BSE) (24).

BSE is defined as one of a group of neurodegenerative diseases categorized as transmissible spongiform encephalopathies (TSEs). The cause of BSE stems from unique self-replicating proteins called "prions" which unlike other infectious agents, contain no genetic material (25). They can survive high sterilization temperatures and remain in infected meat following cooking. The most common form of TSE is called scrapie and has been observed in goats and sheep. It was suspected that meat and bone meal made by rendering scrapie-infected sheep to be used in protein feed supplements for cattle could have been the cause of BSE. BSE was first identified in the United Kingdom in 1985.

V. WASTEWATER MANAGEMENT AND TREATMENT

Meat processing wastewater contains high concentrations of proteinaceous substance (15 kg/m^3) and fat (20 kg/m^3) (26). Therefore, parallel with the development and modification of resource-saving methods for the local purification, problems of cutting down raw material loss with wastewater, reducing specific volumes of unusable waste, exhaust gases and noxious substance escaped are put in the forefront. Fortunately, the technological progress for the management in meat plant can solve these problems. Water expenditures, raw material losses, the composition of wastewater and their loads can be determined for all operations in the meat production performed with water use. The composition of wastewater should also take into account not only their primary parameters [suspended substance (SS), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and fat] to be controlled, but also the specificity of such water. The wastewater source and characteristics of meat plant are summarized in Table 147.4. In general, if BOD, SS and TKN are high in wastewater from a meat-processing plant, other parameters are generally relatively high also (27). BOD is proportional to water usage; as water usage increases, the BOD level in the wastewater from the plant also increases. Thus, paying greater attention to dry cleanup, and recovery of blood, meat scraps and paunch material in a meat plant will have lower sewer and water use bill.

TABLE 147.4
The Wastewater Load Characteristics Per Cubic Meter in Meat Plant

Items	SS kg/m^3	BOD kg/m^3	COD kg/m^3	TKN kg/m^3	NH_3 kg/m^3
Process water	1.20	3.00	4.50	0.35	—
Lairage	11.98	5.13	13.31	1.53	0.80
Total	13.18	8.13	17.81	1.88	0.80

Source: Adapted from Ref. 28.

A better wastewater treatment scheme for a meat plant should be designed based on the following requirements: (a) segregate blood from the wastewater system; (b) isolate solids from wastewater, wherever possible; (c) put flow equalization ahead of the biological treatment in the flow pattern; and (d) remove fat and grease before the biological treatment. The process flow schematic is shown in Figure 147.10 and briefly described below.

A. Preliminary Treatment

1. Sedimentation or Screening

The wastewater from slaughtering pigs has high solid and pig hair loadings comprised of approximately 1/3 of the total solids loading from the primary screening. A 0.5 mm wedge wire rotary screen is suggested in processing wastewater of meat works. A simple sedimentation can be performed to remove solids by gravity (Figure 147.11).

2. Equalization

An equalization tank with adequate mixing and aeration contents is designed to provide adequate blending capacity. It should handle variable volume with high loads associated with slaughtering period (7–8 hours/day), maintain constant and continuous effluent flow into biological treatment unit over 24 hour and maintain enough equalization tank volume (30% of total balancing).

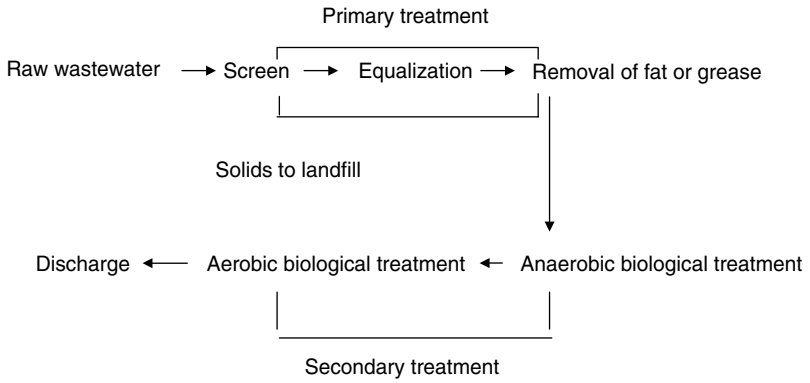

FIGURE 147.10 The schematic of wastewater processing in meat plant.

FIGURE 147.11 A simple and sedimentation and screening for meat plant wastewater.

3. Fat and Grease Removal

Fat and grease removal is done by centrifuges or dissolved by air flotation. Air flotation is a process in which air is dissolved in wastewater under pressure. The wastewater then is moved to a quiescent stage, and air bubbles come out of solution carrying solid with them as they rise to the surface. Air flotation has the advantages in that skimmings can be utilized as inedible grease.

B. Secondary Treatment

This step actually is a combination of anaerobic and aerobic biological treatments.

1. Anaerobic Biological Treatment

Anaerobic biological treatment is the most cost effective, efficient and popular unit process for meat industry and also is able to economically and effectively remove large quantities of organic matter by converting it into biogas, which contains 60–80% methane and 20–40% carbon dioxide. Methane is the major component of natural gas and can be burned to provide heat energy. This treatment can remove up to 82% of the applied BOD when operating properly. Therefore, it is an important part of the treatment process for wastewater containing a high concentration of

FIGURE 147.12 An activated sludge process in a meat plant wastewater system.

organic materials (29). The plug-flow digester or the fixed-firm and slide-blanket digester can be used to replace traditional anaerobic lagoon for economical reasons such as much shorter retention time, reduced area and less maintenance in this treatment (30, 31). The microbial flora balance is very important to keep this treatment stable and effectively operating; therefore, the wastewater should be at pH above 6.8 and temperature maintained around 35°C.

2. Aerobic Biological Treatment

This treatment is excellent in removing odor but needs artificial aeration (mechanically aerated lagoon) and its cost of electricity should be considered. The activated sludge includes aerobic microorganisms and few organic matters from the wastewater in an aerobic treatment (Figure 147.12) and needs to be recycled, meaning these microorganisms are removed by settling and fed back into the incoming wastewater. In this treatment, organic matter in meat plant wastewater is biologically converted primarily into carbon dioxide, water and more microorganisms. Different systems such as a trickling filter, rotary distribution

arms or rotating biological contactor usually are used to reach a high rate of mechanical aeration.

The wastewater is passed through the above primary and secondary treatments, and the solids are clarified by gravity. The final water usually is sufficiently low in BOD for approved discharge into rivers. Many municipalities further require that such water be lightly chlorinated to ensure its freedom from pathogens.

VI. DIMINUTION OF POLLUTION AND FLOW VOLUMES

With increasingly strict standards and restrictions being enforced on how waste can be disposed of, most meat processing plants are looking toward the challenge of improving their waste management practices, keeping cost to a minimum. There are alternatives to conventional processing operations that can significantly lower volumes of wastewater currently being discharged from meat plants. Re-use and recycling of water for less demanding operations within the plant have already been mentioned. Tighter antipollution laws are forcing a variety of innovative processing changes.

Good housekeeping and common sense are necessary for minimization of pollution and flow volumes in a meat plant. Good housekeeping is to avoid disposal of scraps or blood into floor drains and best utilize co-products in high quality products if possible. Two concepts of pollution and wastewater reduction are good housekeeping and water savings. Careful handling of waste and energy resource should be taught to employees and emphasized on a regular basis. A policy or program is composed and a team is needed to perform in the plant to reach the target of pollution and flow volumes reduction. At first, the performing team should consist of engineers, plant manager, maintenance staff and workers in the process. The second step of the program is to identify point of water use, gross spillage and collection of blood or scraps on the floor, etc. The third stage is to get information for decision making as economically and quickly as possible and avoid a large-scale change or measurement program. The last step of the program is to generate a list of possible solutions for each problem in the plant such as broom and shovel pickup, installation of catch troughs, installation of an automatic shutoff and curbing an area to divert water. Finally, the team needs to estimate the improving efficiency by these changes or install new equipment. Berthouex et al. in 1977 stated that increase of nozzles and change of location of shower on the top of the carcass can reduce the volume of water required to clean carcasses. Annual cost due to carcass shower reduction can be saved up to US $3,045 (32).

VII. CONCLUSION

At present, there has been increasing concerns on environmental pollution or large use of water leading to global water shortage and weather alteration in the next quarter century if this vital resource is not conserved and intelligently managed. Hence, how to save water and reduce wastewater flow volume or pollution is still an important responsibility for the managers of food plants. In a meat plant, significant amounts of blood and scrap are lost to the sewer. In addition, usage of more water and energy than needed to finish the given task also usually exists in the processes in meat plants. In consequence, good housekeeping practices and saving in water usage should be taught to all members of the plant. Recovery and upgrading of co-products also need to be developed as tactics to reduce pollution and increase profit.

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ISBN 1-57444-552-9

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CRC Taylor & Francis
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A CRC PRESS BOOK
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6000 Broken Sound Parkway, NW
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270 Madison Avenue
New York, NY 10016

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Abingdon, Oxon OX14 4RN, UK

HANDBOOK OF FOOD SCIENCE, TECHNOLOGY, AND ENGINEERING

Volume 4

Edited by

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CRC Taylor & Francis

Taylor & Francis Group

Boca Raton London New York

A CRC title, part of the Taylor & Francis imprint, a member of the Taylor & Francis Group, the academic division of T&F Informa plc.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-9847-9 (Set)
International Standard Book Number-10: 1-57444-551-0 (Vol 1)
International Standard Book Number-10: 0-8493-9848-7 (Vol 2)
International Standard Book Number-10: 1-57444-552-9 (Vol 3)
International Standard Book Number-10: 0-8493-9849-5 (Vol 4)
International Standard Book Number-13: 978-0-8493-9847-6 (Set)
International Standard Book Number-13: 978-1-57444-551-0 (Vol 1)
International Standard Book Number-13: 978-0-8493-9848-3 (Vol 2)
International Standard Book Number-13: 978-1-57444-552-7 (Vol 3)
International Standard Book Number-13: 978-0-8493-9849-0 (Vol 4)
Library of Congress Card Number 2005050551

Library of Congress Cataloging-in-Publication Data

Handbook of food science, technology, and engineering / edited by Y.H. Hui.
p. cm. -- (Food science and technology ; 148)
Includes bibliographical references and index.
ISBN 1-57444-551-0 (v. 1 : alk. paper) -- ISBN 0-8493-9848-7 (v. 2 : alk. paper) -- ISBN 1-57444-552-9 (v. 3 : alk. paper) -- ISBN 0-8493-9849-5 (v. 4 : alk. paper)
1. Food industry and trade--Handbooks, manuals, etc. 2. Food--Analysis--Handbooks, manuals, etc. 3. Food--Composition--Handbooks, manuals, etc. I. Hui, Y. H. (Yiu H.) II. Food science and technology (Taylor & Francis) ; 148.

TP370.4.H38 2005
664--dc22 2005050551

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Part S

Food Categories

148 Bakery Products

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CONTENTS

Baking is a millennia–old process, and bakery products range in complexity from the simple ingredients of a plain pastry, to the numerous components of a cake. The term baking applies not only to the production of bread, but to all food products in which flour is the basic material, and to which heat is applied directly by radiation from the walls and/or top and bottom of an oven or heating appliance. More particularly, baking includes the production of items such as bread, cake, pastry, biscuits, crackers, cookies, and pies, where flour is the essential and principle ingredient for the base product; baking also includes the toppings, frostings, fillings, and so on that finish the baked product. Despite the fact that many differences in bakery products exist, both important issues of baking technology—baking materials/ingredients and techniques for combining and baking them, will be introduced in this chapter.

I. MATERIALS OF BAKING

A. Ingredients from Wheat

Wheat is one of the world's most important grains, with annual world production of 540~580 million tons. Wheat is the most valuable of all food grains and is widely used in all its stages, from whole to finely milled and sifted. In the bakery, wheat flour is the most important ingredient, which provides bulk and structure to most bakery products, including breads, cakes, cookies, and pastries. Wheat is unique among the cereals, in that its flour possesses the ability to form dough when mixed with water (1). The gluten in wheat dough has the ability to retain the gas produced during fermentation or by chemical leavening, and thus produce a leavened product (2). These characteristics of wheat flour dough or batters are responsible for the preference of bakery products with a light and soft texture.

All wheats belong to the genus *Triticum* of the *Gramineae* family, the 'grass' family. Common wheat (*Triticum aestivum*) and durum wheat (*T. durum*) are the two major wheat groups planted for food use now. For commercial purposes, common wheat is generally classified as hard or soft, red or white, spring or winter. Of these, hard and soft wheat flours are used in the baked goods that make up so many bakery products. Table 148.1 lists the top five wheat exporters in the world trade with the wheat classes produced in these areas. Hard wheat normally has high protein content and its flour is used primarily for yeast-leavened products, such as breads, bagels, croissants, English muffins, Danish sweet rolls, cinnamon rolls and bread type donuts (5). Soft wheat normally has low protein content and its flour is most suitable for making biscuits, muffins,

TABLE 148.1
Top Five Major Countries of Wheat Export (Marketing Year 2001–2002)[a] and Wheat Exports by Class[b]

Top Exporter	1000s Tons	Wheat Exports by Class
1. U.S.	26,139	Hard red spring (HRS)—21%
		Hard red winter (HRW)—44%
		Soft red winter (SRW)—17%
		White (Hard and Soft)—15%
		Durum—3%
2. Canada	16,758	Canada Western Red Spring (CWRS)
		Canada Western Amber Durum (CWAD)
		Canada Western Extra Strong (CWES)
		Canada Prairie Spring Red (CPSR)
		Canada Western Red Winter (CWRW)
		Canada Prairie Spring White (CPSW)
		Canada Western Soft White Spring (CWSWS)
3. Australia	16,494	AWB Prime Hard
		AWB Hard wheat
		AWB Premium White
		AWB Standard White
		AWB Noodle Wheat
		AWB Soft Wheat
		AWB Durum
		Australian General Purpose
4. Eastern Europe	11,494	Winter wheat
		Spring wheat
5. Argentina	11,477	
World Total	**109,751**	

[a] From (2).
[b] From (3).

pastries and cakes (6). The protein content of soft wheat flours ranges from 8% to 10%. The lower protein level produces a cell structure that provides good mouthfeel and a less chewy texture in chemically leavened baked goods.

Numerous bakery products are produced by using specific characteristics of flour that depend on the variety of wheat from which it is milled, the location in which the wheat is grown, and growing conditions. Both the quantity and the strength of the protein in the flour are important indicators of the flour's suitability for various baking applications. The "strength" of flour is generally defined as the capacity of the flour to make a tough, elastic dough, and a low-density loaf of bread has fine, uniform crumb structure (5). Flour contains two proteins of importance to yeast-leavened doughs—glutenin and gliadin. When these are mixed with water, gluten forms and contributes to the dough strength. This creates an elastic, extensible matrix; high-quality gluten entraps large amounts of the carbon dioxide produced by yeast fermentation. Strength of flour is dependent primarily, but not entirely, on the amount and type of protein present in the flour. Therefore, the high protein content of bread flours does not guarantee high-quality bread flour. Additionally, greater water absorption in bread flour results in better dough yield.

1. Wheat Flour Milling

The production of flour from wheat is derived from an ancient process used throughout the Middle Ages, that first separates the husks from the kernels by making use of the wind, which removes the lower density husk ("chaff"), followed by grinding of the kernels between two stone surfaces (7). Since those days, the milling process has developed considerably to enable it to cope with the demands of large industries, many of which require specialized flours. These needs are met by a sophisticated milling process from which various types of flours and by-products are obtained by the use of different types of rollers, sieves, and air classification systems. The separation of wheat components in the milling process relies on the knowledge of the distribution of the various components within the wheat kernel and their physical properties, including their size and shape. In order to produce flours that meet the requirements of the various applications, flour properties need to be correlated with the requirements of each of the end products. With progress of cereal chemistry, millers can benefit more and more from carefully choosing the proper recommended wheat, accompanied by an adequate milling operation.

The wheat kernel consists of three main parts: bran, germ and endosperm. Depending on its source, the wheat endosperm contains about 63 to 73% starch, and 7 to 20% protein (8–10). Flour milling is a complex process by which the interior endosperm of the wheat kernel (flour) is separated from the exterior shell (bran and shorts). In preparation for milling, the grain is thoroughly cleaned, then put through a tempering process where it is moistened and softened. Nowadays, various types of rollers and shaker sieves (e.g., plan sifters) are used to affect the separation. Roller milling is divided into two main categories, namely, "breaking" and "reduction." The breaking rollers are set to flake off the bran layers and germ, and crack the endosperm into coarse pieces. The coarse pieces of endosperm are then broken into smaller pieces by repeatedly shifting and roller reducing. Flour produced throughout the milling process is removed to storage bins for final processing, where it is enriched, graded, and either packaged or shipped in bulk. The remaining bran and germ are used for cereals, baking and animal feed. Figure 148.1 is the simplified scheme of wheat flour milling. Different grades or specific characteristics of flour can be extracted from one type of wheat, or combined from various flour streams that are derived from either a single type of wheat milling or blended wheat milling. For example, flour of medium strength can be achieved by milling and blending hard and soft wheat or blending hard wheat flour and soft wheat flour.

Flour extraction rates are usually used to express the portion of the endosperm that is separated into a particular

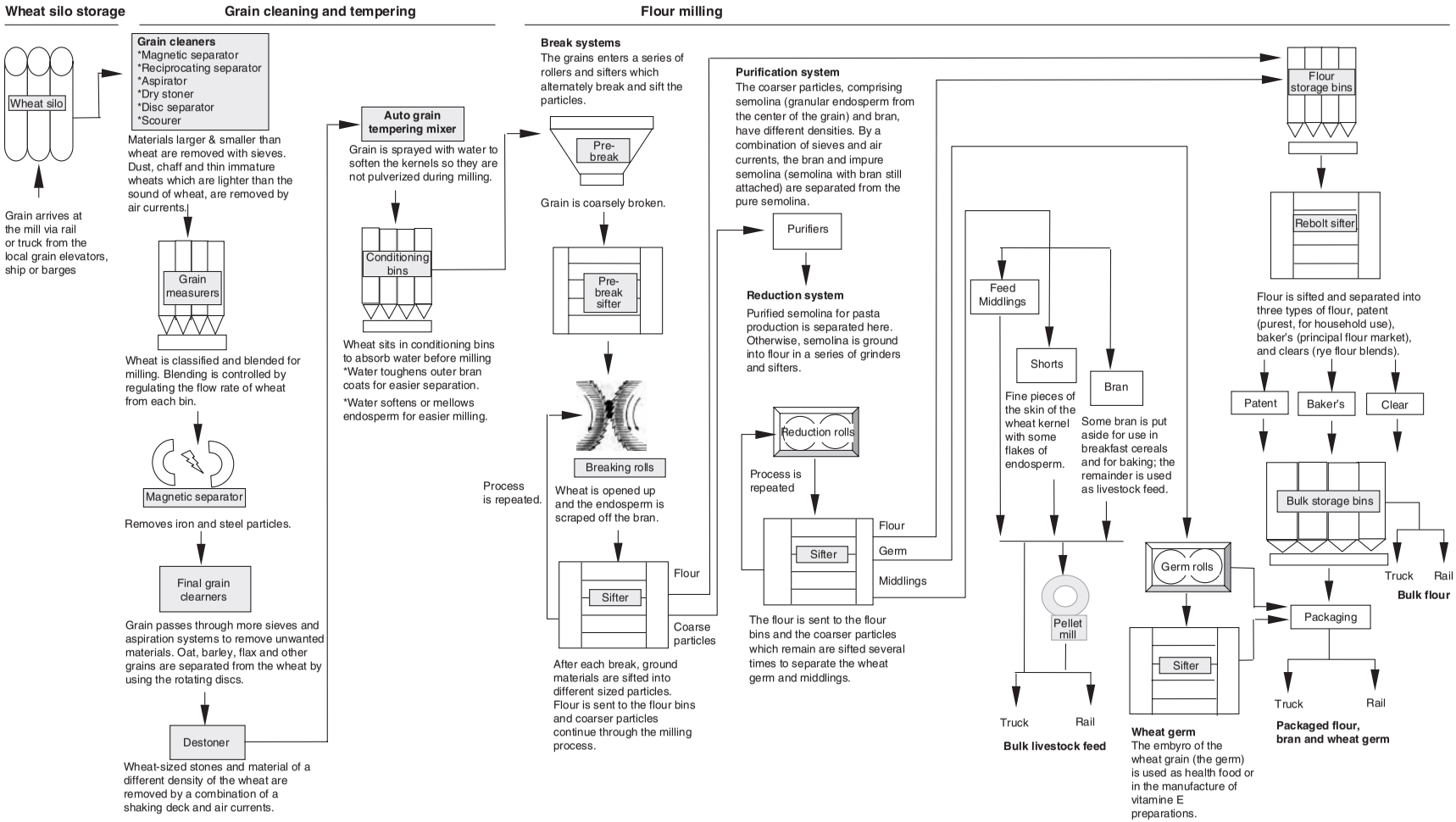

FIGURE 148.1 A simplified flow diagram of wheat flour milling.

grade of flour. Figure 148.2 shows the yields of wheat flour and by-products. The extraction rates are calculated as the percentage of flour obtained from clean, dry wheat. Table 148.2 lists the definitions of terms of milling products commonly used by millers and bakers. Ash content varies with the grade of flour, which depends on when the flour is obtained during milling. Patent flour is the "cut" of flour from the front of the mill and is considered very high quality. Clear flour is the portion of flour remaining after the patent flour has been taken off. Clear flour is further categorized as "first clear" and "second clear." Straight flour is all of the flour extracted from a blend of wheat. The differences in flour from patent to straight grade to clear flours are related to the level of bran and protein quantity or quality in the flour. Patent flour has the least bran and protein content, while second clear flour has the greatest bran and protein content. Commercially, many specialty flours for specific bakery products are usually blended with various flour streams from one or more varieties of wheat milling. For example, high-gluten flour is used in hard-crusted breads and in such specialty products as pizza dough and bagels.

Cake and pastry flours are made of soft wheat. Cake flour is a weak or low-gluten flour, with a lower ash content and smaller particle size, and sometimes is treated with chlorine to lower pH, which improves cake properties. Pastry flour is also a weak flour, but it is slightly stronger than cake flour with the same creamy white color as bread flour. Pastry flour is used for pie doughs, cookies, biscuits, and muffins.

Freshly milled flour is not most suitable for breadmaking, because gluten is somewhat weak and inelastic and the color may be yellowish. After milling, flour usually undergoes additional treatments that can affect performance, including bleaching and maturing. Flour will whiten and mature naturally due to oxidation, which changes its appearance and handling properties. Aging of flour this way is time-consuming. To speed it up, bleaching (maturing) often is done with chemicals. The US Code of Federal Regulations, Title 21, Part 137, Cereal Fours and Related Products, sets a number of requirements for products offered as "flour," in which flour may contain not more than 200 parts per million (ppm) of ascorbic acid as a dough conditioner. Chlorine, nitrosyl chloride, chlorine dioxide, acetone peroxides, benzoyl peroxide mixed with certain specific diluents, or oxides of nitrogen, may be used for bleaching or artificial aging, in quantities not more than sufficient to achieve such effects (12). In contrast to benzoyl peroxide, which only whitens the flour and has little

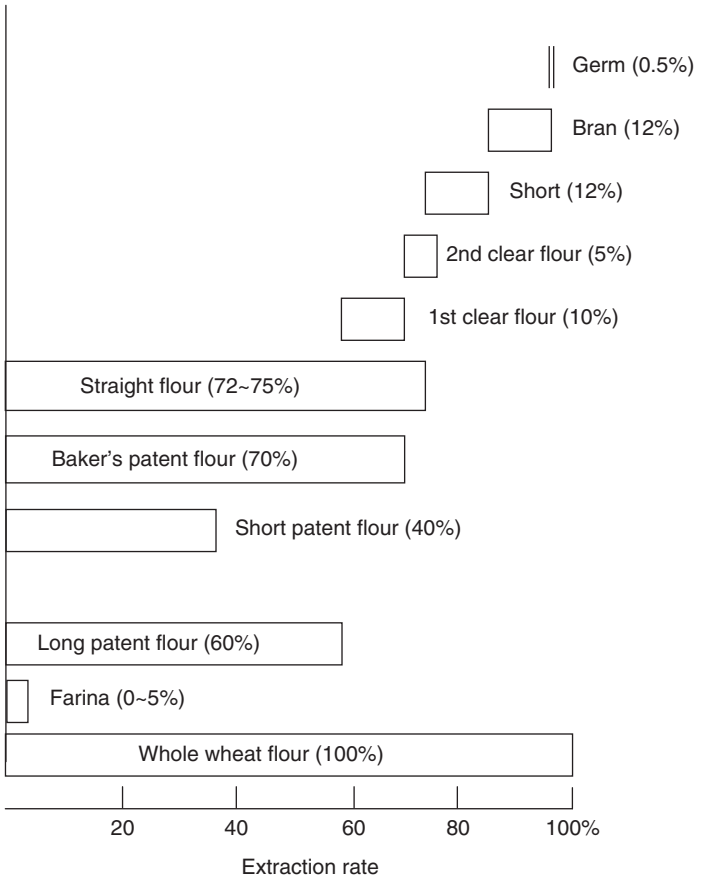

FIGURE 148.2 Milling flour stream of hard wheat and its by-products.

TABLE 148.2
Definition of Terms for Milling Products Commonly Used by Millers and Bakers[1]

Milling Products	Definition
Bleached flour	Flour is chemically treated to improve baking quality and color.
Bran	The broken seed coat of wheat, separated from the flour or meal by sifting or bolting, exclusive of any germ or most of endosperm.
Composite flour	A flour made by blending varying amounts of non-wheat flour with wheat flour and used for the production of baked goods that are traditionally made from wheat flour.
Dusting flour	Flour used on the bench and on machinery to prevent dough from sticking to equipment.
Farina	A very pure wheat endosperm, about the granulation of medium sizings; endosperm particles from hard wheat, in general can be used for breakfast cereal.
1st Clear flour	The portion of flour remaining after a "patent" cut of flour has been taken off. Clear flour is normally higher in ash and protein than patent and marketwise is secondary in value.
2nd Clear flour	The lower grade portion or division of "clear flour" from the tail-end reductions of the milling system, having a higher ash content and poorer color than those included in first clear.
Germ	The most viable portion of the wheat berry, i.e., the embryo extracted from the grain kernels.
Hard flour	This flour is strong in character, requiring prolonged fermentation to ripen, and has considerable fermentation tolerance and stability. Hard flour is produced from hard red winter, hard red spring, or hard white wheat.
Patent flour	The most highly refined flour that is cut off flour (combination of flour streams) from the front of the mill, lower in ash and protein, with good dress and color and marketwise considered highest in value. Short patent flour (SPF) is more highly refined than long patent flour (LPF); while, the SPF and LPF are all relative terms and are not constant from mill to mill.
Red dog	A low grade of flour, actually a mixture of endosperm and bran powder taken from the tail of the mill, which has high ash, high protein, poor dress and is dark in color.
Shorts	An inseparable mixture of bran, endosperm and germ, which remains after flour extraction (milling) has been completed; usually used for animal feed.
Short patent flour	Top-grade flour of any strength. The degree of extraction may vary from mill to mill, but is somewhere in the region of 40% of the total weight of the wheat from which it is milled.
Soft flour	The flour with a low percentage of weak gluten, milled from soft red winter or soft white wheat.
Straight flour	A flour milled from the entire contents of the endosperm available for milling, without division or addition of flour from other runs, excluding only the bran and germ; it is usually 72 to 75% of the wheat berry.
Strong flour	A flour that takes up a relatively large quantity of water and produces a dough that requires a relatively long mixing time for proper development. Usually a flour of high quality for bread-making.
Treated flour	Flour to which some supplement has been added, such as vitamins, calcium, iron, self-rising ingredients, etc.
Weak flour	That which (a) may contain an adequate quantity of gluten but is of inferior quality; (b) contains an inadequate amount of good quality gluten; or, (c) contains gluten that has been affected by proteolysis.

[1] From (11).

effect on its baking performance, bleaching with chlorine gas not only whitens flour, but also functions as a maturing agent. Chlorine modifies the starch and protein, permitting increased starch swelling and hydration while also weakening the gluten structure. Cake flour is usually treated with chlorine to pH values of 4.5–5.2, and cake flour treated with 1.75 oz chlorine/100 lb flour is recommended for optimum baking results (13). All varieties of refined wheat flour can be enriched with nutrients lost during milling off of the bran (e.g., thiamine: 2.9 mg/lb flour, riboflavin: 1.8 mg/lb flour, niacin: 24 mg/lb flour, folic acid: 0.7 mg/lb flour, and iron: 20 mg/lb flour), and it may contain added calcium in such quantity that the total calcium content is 960 mg/lb flour (12).

Commercially, many types of flour were designed for various applications, such as all-purpose flour (alternatively called household flour) for a wide range of applications. This flour is formulated to be slightly weaker than bread flour so that it can be used for pastries as well. Shelf-rising flour is a white flour in which baking powder and sometimes salt have been added. Whole-wheat flour is made by grinding the entire wheat kernel, including bran and germ. It is usually used for wheat bread or whole wheat bread making. The bran and germ are high in fat, which can cause whole-wheat flour to oxidize quickly. They also dilute gluten, so whole-wheat flour produces a denser loaf than bread made from wheat flour. In fact, using all whole-wheat flour results in a very heavy, whole wheat bread loaf; it is best to blend whole-wheat flour with regular wheat flour (50:50 to 20:80, w:w) to make a lighter-textured wheat bread loaf. Bran flour is flour to which bran flakes have been added. The bran may be coarse or fine, depending on specifications.

2. Other Wheat Products

Various types of wheat products beside wheat flour are also available for the making of bakery products. Wheat berries

(whole-wheat grains) or cracked wheat grains are often used for multigrain bread or used for topping of variety breads. Sold as individual ingredients, wheat bran and germ can boost the fiber and nutrients in bakery and cereal applications. Vital wheat gluten isolated from wheat has application in a variety of bakery items to modify the dough properties, resulting in the improvement of baked goods. Another wheat protein isolate, gliadin, can extend the freezer life of buttermilk biscuits and decrease fat absorption in cake donuts; it also provides excellent freeze/thaw stability in frozen doughs and can reduce the toughening of breads and pizza crusts brought on by microwave cooking. Germinating or sprouting the wheat kernel improves digestibility and increases bioavailability of protein, carbohydrates and vitamins. Sprouted wheat is subsequently toasted and used in breads to moisten the crumb and to lend a rich, malted flavor.

B. Ingredients from Other Grains

Products milled from other grains are occasionally used to add variety to baked goods. These include rye flour, corn meal, rice flour, soy flour, potato flour, oat flour and barley flour. In addition to flours, grains can be added in any shape, including whole grain, grit, flake or coarse meal. The term meal is used for products that are not as fine as finely ground flour. All of these products are normally used in combination with wheat flour because they do not form gluten. Table 148.3 is the proximate composition of grain and milled products commonly used in bakery products.

1. Rye

Next to wheat and wheat flour, rye is the most popular flour for bread-making. Although rye is the only other cereal grain with storage proteins capable of forming gluten, rye gluten is weak and inferior compared to wheat gluten. Therefore, bread made with 100% rye flour will be heavy and dense, such as pumpernickel and black bread.

Rye flour is milled much like wheat flour. Light rye extracted from the inner part of the kernel, with a very fine texture and a high percentage of starch but little protein, is correspondent to patent flour. Medium rye is a straight flour, milled from the whole rye grain after the bran has been removed. Dark rye is like the clear flour, which is milled from the part of the rye grain closest to the bran. Rye meal, or pumpernickel flour, is a dark coarse meal made from the entire rye grain. Rye blend is a mixture of rye flour (generally about 25~40%) and a strong wheat flour, such as clear flour, for making a lighter rye bread.

2. Corn

Corn flour, corn masa, corn meal and corn grits are just a few of the many specialty ingredients that can be obtained from corn, mainly from two varieties of yellow and white dent corn (15). Corn meal, or corn grits, comes in coarse and medium grinds for numerous baked goods, including: coatings for deep-fried foods; use in bread doughs; or as a sprinkle on bagels, bread sticks and pizza crust. Corn meal is also a popular ingredient in batter, baking and stuffing mixes. Corn flour, a finer form of corn meal found in pancakes, waffles and some baked goods, or for breading foods, may be yellow or white.

Corn masa, the traditional ingredient of Mexican tortillas, has been substituted by the more popular industrial corn flour, due to the ease of use, lower water requirement, and improved consistency in continuous processing. Masa is dough made from ground white corn or hominy (an old American Indian term for hulled and dried corn kernels

TABLE 148.3
Proximate Composition of Milled Products of Wheat and Rye Commonly Used in Bakery Products[1]

					Carbohydrate		
Grain/Milled Products		Water	Protein	Fat	Starch	Non-Starch Polysaccharide (NSP)	Ash
		g/per 100 g					
Wheat							
Wheat flour	Patent	12.8	11.8	1.8	65.2	22.1	1.6
	White for breadmaking	13.3	11.7	1.4	75.8	3.5	0.6
	White for biscuitmaking	12.8	8.5	1.4	77.5	3.1	0.5
	White, 78% extraction rate	13.0	11.5	1.3	71.6	3.1	0.6
	Wholemeal for breadmaking	13.2	12.7	2.0	65.8	11.8	1.4
	Wholemeal, 100% extraction rate	13.1	12.6	2.3	65.0	12.1	1.4
Wheat bran		10.6	14.6	5.4	22.4	35.4	5.7
Wheat germ		9.3	22.0	7.4	25.1	16.0	3.6
Rye							
Rye flour	Whole	15.0	8.2	2.0	75.9	11.7	N

[1] From (14).

stripped of their bran and germ and soaked in limewater) (12). There are two kinds of masa: softer *masa molida* for tortillas; and coarser, thicker *masa preparada* for tamales. Masa dough can be made using three parts masa flour to one part lard and two parts water, with some salt.

3. Oat

Much like corn, most oats are used for animal feed. This nutritious cereal grain is both a source of high-quality protein and soluble fiber, β-(1→3,1→4)-glucan. In January 1997, the FDA declared that the β-glucan soluble fiber in whole oats is responsible for total and LDL blood-cholesterol-lowering, while helping to maintain good cholesterol (HDL) (13). To qualify for the health claim, the whole-oat-containing food must provide at least 0.75 grams of soluble fiber per serving. The amount of soluble fiber needed for a lowering effect on cholesterol levels is about 3 grams per day. Food products eligible to bear the health claim include oat bran and rolled oats, such as oatmeal, and whole-oat flour.

Oat ingredients come in a variety of forms. The oat kernel, commonly called a groat, is surrounded by an inedible husk, or hull, whose high adherence to the groat makes processing a bit more difficult than with other grains. Whole-oat groats are the whole grain with the tough outer hull removed. Steel-cut oats are groats cut into several smaller pieces. Steel-cut oats are used in thick soups and stews, and add bulk to sausages. Rolled oats, synonymous for oatmeal, are dehulled oats steamed and flattened between rollers, a process that allows them to be cooked quickly, or require no cooking at all, prior to consumption. Rolled oats can be cooked up as hot oatmeal cereal, or added to cold cereal, bread, cookies, muffins and even pancake mixes for flavor, texture and nutrition. They combine very well with other grains in these applications, but can be used as the only grain ingredient in baked and unbaked cookies to produce a chewy texture. In many baked-good formulas, rolled oats can replace up to one-third of the flour at an exchange of one part flour for four parts rolled oats. For a more direct substitution, whole-oat groats can be ground to make whole-oat flour or oatmeal flour, but oats do not contain gluten and will not rise if used alone in bakery applications.

4. Barley

Barley was a staple in the ancient city of Jericho as long ago as 8000 BC, and the Babylonians were brewing with it in 2500 BC (17). Though primarily used as an animal feed, and in the beer and whiskey-brewing industries, some food formulations also use barley. Many varieties of barley are grown, including naked, waxy, and non-waxy. Waxy hullless barley may have 6% to 9% soluble fiber, versus barley that is hulled, which has only 2% to 4% soluble fiber (18). Most forms of whole barley are a good source of protein, vitamin B, and dietary fiber. Whole barley flour is 13.5% dietary fiber, compared to whole wheat's 9% (Table 148.4). The kernels possess a pleasant, nutty taste, and include a protective husk that requires removal before human consumption. Hulled, or whole-grain barley, is simply the whole grain minus the outer hull. Barley flour, ground pearl barley, can be used in bread making.

For brewing, barley is converted to malt through controlled sprouting of the barley grain followed by drying. Malt is a source of the enzyme α-amylase, which hydrolyzes starch to fermentable sugars, such as dextrin and maltose. Malt can supplement flour for baked goods,

TABLE 148.4
Dietary Fiber Content of Grains and Milled Products Commonly Used in Bakery Products[1]

Grain/Milled Product	Moisture (g/100g edible portion)	Dietary Fiber (g/100g edible portion)		
		Total	Insoluble	Soluble
Barley	9.4	17.3	—	—
Barley, bran	3.5	70.0	67.0	3.0
Barley, pearled	10.1	15.6	—	—
Corn, bran	6.5	82.4	80.4	2.0
Corn, flour, whole grain	10.9	13.4	—	—
Cornmeal: whole grain	11.0	11.0	—	—
Cornmeal: degermed	10.3	5.3	—	—
Corn starch	12.0	0.9	—	—
Oat, bran	10.0	22.2	11.7	10.5
Oat, flour	7.8	9.6	—	—
Oats, rolled, or oatmeal, dry	8.8	10.3	6.5	3.8
Rice: brown, long-grain (raw)	11.7	3.9	—	—
Rice: brown, long-grain (cooked)	73.1	1.7	—	—
Rice: white, long-grain (dry)	8.7	1.3	1.0	0.3
Rice: white, long-grain (cooked)	77.5	0.7	0.7	0.0
Parboiled rice: dry	10.4	2.2	—	—
Parboiled rice: cooked	77.0	0.5	—	—
Rice bran	6.1	21.7	—	—
Rice flour: brown	12.0	4.6	—	—
Rice flour: white	11.9	2.4	—	—
Rye, dark	11.1	32.0	—	—
Rye, medium	8.8	14.7	—	—
Rye, light	10.6	14.6	—	—
Wheat, bran	11.6	42.4	40.3	2.1
Wheat flour: white, all-purpose	11.2	2.7	1.7	1.0
Wheat flour: whole-grain	11.5	12.6	10.2	2.3
Wheat germ	2.9	14.0	12.9	1.1

[1] From (19).

increasing the α-amylase content, and the dough's fermentation rate, and improving baking properties. The lack of gluten restricts its use in leavened bread to 10%, or appearance of loaf volume is affected. Extract and syrup forms of barley malt add flavor and color to various applications. Baked goods, such as rolls and bagels, often contain malt syrup at 1% to 3% of the flour weight. Barley flour has a 2.5-fold water-holding capacity compared to wheat, making it an ideal food thickener, binder, or ingredient in oriental noodles. Quick breads can contain up to 30% barley flour. Cakes, cookies, donuts, muffins, and pancakes can be made from 100% barley flour.

5. Rice

Rice is an ancient grain that has been a staple for centuries, and it provides ingredients with unique functional characteristics and health benefits. There are numerous varieties of rice and each possesses distinct flavor and texture characteristics. But overall, most rice grains are quite bland and soft, which is why rice tends to be the first solid food served to babies.

Parboiled rice is steam-treated long-grain white rice. It is more nutritious than long-grain white rice because it is processed prior to hulling, so the grain has the chance to absorb the bran's nutrients before the bran is discarded (20). Brown rice, on the other hand, is hulled (short-, medium- and long-grain) rice with its bran intact. As a result, it requires longer cooking times than polished rice.

Some rice components are sold as food ingredients, such as rice flour, pregelatinized rice flour, and rice bran. Beneficial health effects of rice bran are capturing the attention of the food industry (21). High in dietary fiber, rice bran boosts nutrition and adds texture to cookies or bread doughs. Besides, rice bran is a good source of oil, protein concentrates, protein isolates, and antioxidants (22). Rice flour is polished white rice finely ground to a silky consistency, and it often substitutes for wheat flour in wheat-free applications designed for consumers on gluten-free diets.

6. Soy

Soybeans have been utilized for centuries in Asia; however, worldwide consumption of soybeans has increased. Although soybeans are classified as legumes, their utilization is similar to grain. Human consumption of the whole bean remains low, but components of soybeans show great promise in the coming decade, particularly those derived from soy protein. Soy contains a class of phytochemicals that are referred to as isoflavones, which mimic the female sex hormone estrogen (hence the term phytoestrogen) and has effects on the prevention of post-menopausal syndromes (23). Defatted soy flour, generally by a hexane extraction, is one of the richest isoflavone sources, with levels as high as 2.0 mg per gram of soy protein. Soy concentrate that is aqueous-washed rather than alcohol-washed is another good source at 1.5 mg per gram. In comparison, soymilk can contain as little as 0.1 mg per gram.

Soy proteins are available in three major forms that are all derived from defatted soybean flakes: soy flours and grits; soy protein concentrate; and soy protein isolates. Defatted flours and grits range from 40% to 54% protein, 30% to 32% carbohydrate, 2.5% to 3.5% fiber, and up to 1% fat (24). Functionality of soy flours and grits is related to their capacity to bind water and absorb fat. These characteristics vary according to the particle size and the degree of heat treatment that the flour and grits undergo, resulting in protein denaturation. In general, lower heat treatment and smaller particle size means more functionality. As a general rule, up to 3% of wheat flour can be replaced by soy flour without any adjustments other than water addition, approximately a 1:1 to 1:1.5 ratio based on the weight of the soy flour.

C. Yeast and Chemical Leavening

Leavening is the production or incorporation of gases in a baked product to increase volume and to produce shape and texture. Leavening agents is a term used to indicate a source of gas that causes a dough or batter to rise or spring. These gases must be retained in the product until the structure is set enough by the coagulation of gluten and egg proteins and the gelatinization of starch to hold its shape. Unleavened medium- to high-moisture baked goods are dense and heavy, with virtually no cell structure. Three types of leavening agents, water, yeast and chemicals, can be used in bakery products (Table 148.5).

Hard wheat flour, as is used in bread, is usually leavened with yeast because of the protein content and gluten formation. However, soft wheat flours, used in products like cakes, are usually leavened with chemical leaveners because they do not contain the same level of gluten and cannot retain the gases generated by yeast. Most often, in each case, a yeast or chemical leavening system is used independently, but occasionally a combined system provides the desired result. For example, saltine crackers and pretzels undergo yeast fermentation, but the primary reason is to generate flavor and dough conditioning. The subsequent sheeting action removes much of the gas that is generated, and chemical leaveners are required to provide lift during the bake. Most formulations contain water, which when heated, forms steam and expands. However, water does not contribute significant amounts of leavening in most baked goods, except cream puffs and popovers, and most products require additional leavening, either with yeast or chemical leaveners. In general, chemical leavening is a faster, more convenient, and often more consistent method than yeast. In some frozen

TABLE 148.5
Major Leavening Gases Used in Leavening of Bakery Products

Leavening Gas	Source	Comments	Examples
Air	Mechanical mixing process	Nitrogen solubility in water is low. Forms nucleation sites during mixing.	Whipped egg whites entrap the air in angel food cakes. Beaten whole or fortified eggs incorporate the air into sponge cakes. Creaming of the shortening and sugar leaven the pound cakes.
Water vapor (H_2O)	Water as an ingredient	Because of high boiling point has a limited effect.	Moisture evaporation between the folded fat and dough layers leavens the puff pastry.
Ammonia (NH_3)	Ammonium bicarbonate Ammonium carbonate	Decomposes completely at about 60°C. No residual salts. Used in products that are baked to near dryness, such as cookies and crackers.	Cookies and crackers.
Carbon dioxide (CO_2)	Sodium bicarbonate (major) Potassium bicarbonate Sodium carbonate	Needs acids for appropriate leavening activity. CO_2 is produced faster, thus the products may be baked immediately after dough or batter preparation.	Most cakes and cookies. Cake doughnut.
	Yeast fermentation	CO_2 is produced slower during dough fermentation, thus reasonable fermentation time is required.	Breads, Chinese steamed breads, bread doughnut.

or refrigerated doughs, yeast and chemical leavenings complement each other.

1. Yeast

Yeast (*Saccharomyces cerevisae*) is the leavening agent in bread, dinner rolls, and similar products. Fermentation is the process by which yeast acts on sugars, which can be added sugar or sugar produced by hydrolyzing wheat starch with enzymes, and changes them into carbon dioxide gas and alcohol. This release of gas produces the leavening action in yeast products. The alcohol evaporates completely during and immediately after baking. Because yeast is a living organism, it is sensitive to temperatures (Figure 148.3). Yeast in dough is active between 0°C and 55°C. At temperature less than 20°C and over 40°C, the rate of growth is significantly reduced. Yeast can survive for a few weeks at temperatures as low as −20°C, but it gradually loses its fermentation capacity. The most favorable temperature for yeast to multiply is between 20°C and 27°C; optimum multiplication of yeast is achieved at around 26°C. The optimum temperature for fermentation lies between 27°C and 38°C; yeast ferments best at 35°C (25).

For the baking industry, yeast is available in two forms, fresh and dried. Depending on process, cost, and desired effect in the finished product, various forms of yeast may be used—compressed, fresh in cake or crumbled form, liquid cream yeast, or active dry yeast. The forms and properties of baker's yeast are summarized in Table 148.6.

Determining the correct yeast level is difficult because of variations in yeast activity and in the process. The conversion factors between the fresh yeasts and the dry yeasts are only guidelines and depend dry matter versus the viability. During processing, doughs are traditionally proofed, or allowed to ferment twice. This not only results in an increase in dough volume, but also develops characteristic fermented-yeast flavors. No-time doughs are only proofed once. The finished products are similar in quality to traditional doughs, but increased levels of dough conditioners and yeast are required. Frozen doughs require yeast that retains its activity through extended frozen storage conditions. Ice crystals formed in the frozen dough will disrupt yeast cell walls. Also, as the dough freezes, the amount of available water decreases, and the concentration of the solutes increases. The increase in osmotic pressure can destroy yeast cells. Therefore, yeast with a high osmotic tolerance is required for frozen dough.

Using yeast in bakery products is somewhat challenging, but it creates two main advantages: a distinctive flavor and dough conditioning. As yeast ferments, it forms a number of compounds, including organic acids, alcohols, aldehydes, esters and ketones. Some of these are volatilized during baking, some undergo further reaction, and most contribute to the flavor and odor of the product. Some of the compounds generated during fermentation act as dough conditioners and increase dough extensibility by relaxing the gluten. This is a function of time and pH; the longer the ferment, the more pronounced the effect.

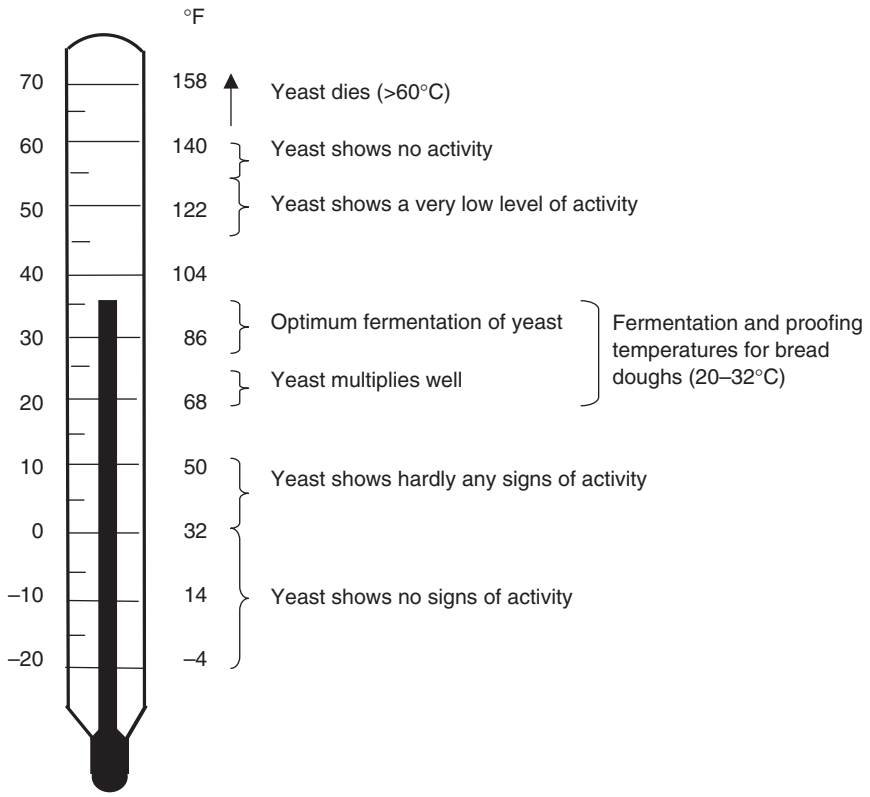

FIGURE 148.3 Effects of temperatures on yeast.

Using certain ingredients in excess—such as the salt that is added for flavor—may limit yeast growth. Extra nitrogen, as part of a dough improver, can be added to ensure optimum yeast growth. This produces the necessary carbon dioxide to yield oven spring, which is the sudden expansion of dough by about one-third its original volume during the early baking stages.

2. Chemical Leaveners

Chemical leavening systems produce CO_2 by one of two means: chemical decomposition through the application of heat, or a reaction of an acid with a base. Sodium bicarbonate (or baking soda) and ammonium bicarbonate are two major gas carriers in chemical leavening systems. For low-sodium applications, potassium bicarbonate may be used, but about 19% more is needed to gain the same effect as baking soda, because it has a greater molecular weight than sodium bicarbonate.

Sodium bicarbonate can be heat decomposed into CO_2, water, and washing soda (Na_2CO_3) as shown in Figure 148.4. Excess washing soda will react with shortening, giving a dark color and undesirable taste to the cake. Therefore, various acids are combined with sodium bicarbonate to accelerate the reaction for gas producing at lower temperature. Sodium bicarbonate is available in several grades differentiated by particle size, which affects the solubility of soda, and therefore, the reaction rate of gas production. Generally, Grade 1 is the standard powder grade. Use of the coarser Grades, 2 through 4, can reduce the tendency for pre-reaction and increase the system stability. Sodium bicarbonate also can be encapsulated with fat-based coatings to increase stability, particularly for refrigerated doughs. If the level of soda is too high in the finished product, it creates soapy off-notes. If the level is too low, it will allow the acidic flavors to come through. Excess levels also result in over-browning.

Ammonium bicarbonate decomposes into ammonia and carbon dioxide when exposed to temperatures above 40°C (104°F), and heat decomposition is complete at about 60°C (140°F), (Figure 148.4). Some gas is released at room temperature, but most is generated during baking. Acidic conditions accelerate the reaction at lower temperatures. The release of gas is rapid, resulting in fairly large cells. Ammonium bicarbonate not only increases the volume or height, but also tends to increase the spread in cookies. After decomposition, it forms ammonia gas, water and carbon dioxide without any residual salt. As it decomposes, it generates basic substances, so it does go through a temporary pH spike. Ammonium bicarbonate dissipates in low-moisture products that have a porous structure, such as cookies and crackers. In higher moisture products, water retains the ammonia, giving the product an undesirable flavor and odor. Ammonium bicarbonate

TABLE 148.6
Types of Baker's Yeast[1]

Yeast Type	Form	Storage Temperature	Shelf-Life	Moisture Content (%)	Gas Power (g Glucose Fermented/g Yeast Solid/hr)			Conversion Factor	Handling Requirements
					Straight Dough	Lean Dough	Sweet Dough		
Fresh compressed yeast	Cake crumbled	2–7°C/36–45°F	3–4 weeks	67–72	2.2–2.3	2.4–2.6	1.0–1.1	1	Weigh and add with other ingredients or disperse in water before mixing.
Fresh cream yeast	Liquid	1–4°C/35–39°F	10–14 days	80–82	—	—	—	1.5–1.8	Meter and add with other ingredients.
Dry active yeast	Granule	Room temperature	2–12 months	6–8	1.4–1.5	1.2–1.3	0.8–0.9	0.4–0.5	Must hydrate at 40–43°C (105–110°F) for 10–15 min before use.
Dry instant active yeast	Granule	Room temperature	1 year plus (depending on packaging)	4–6	1.6–1.8	1.8–2.0	0.8–0.9	0.33–0.4	Blend dry with other dry ingredients or delay addition in mixer.

[1] From (26).

(a) Heat decomposition of baker's ammonia

$(NH_4)_2CO_3 \xrightarrow{\Delta} 2\,NH_3 + CO_2 + H_2O$

Ammonium carbonate

$NH_4HCO_3 \xrightarrow{\Delta} NH_3 + CO_2 + H_2O$

Ammonium bicarbonate

(b) Heat decomposition of baker's soda

$2\,NaHCO_3 \xrightarrow{\Delta} CO_2 + H_2O + Na_2CO_3$

Sodium bicarbonate

(c) Reaction of baking soda and baking acids

$NaHCO_3 + C_4H_5O_6K \longrightarrow CO_2 + H_2O + C_4H_5O_6NaK$

Cream of tartar

$8\,NaHCO_3 + 3\,CaH_4(PO_4)\cdot 2H_2O \longrightarrow 8\,CO_2 + 11\,H_2O + Ca_3(PO_4)_2 + 4\,Na_2HPO_4$

MCP

$2\,NaHCO_3 + Na_2H_2P_2O_7 \longrightarrow 2\,CO_2 + 2\,H_2O + Na_4HP_2O_7$

SAPP

$6\,NaHCO_3 + Na_2SO_4Al_2(SO_4)_3 \longrightarrow 6\,CO_2 + 6\,H_2O + 4\,Na_2SO_4 + 2\,Al(OH)_3$

SAS

FIGURE 148.4 Chemical equations for the reaction of baker's ammonium, baking soda, and baking acids.

can only be used in low-moisture applications, since the ammonia flavor persists at high moistures.

a. Acids

A number of leavening acids can be incorporated into the leavening system (Table 148.7). Natural acids such as lemon juice or sour milk can be used, but typically in a commercial operation, chemical leavening acids are added to provide consistent, controlled gas production. Acids commonly used include monocalcium phosphate (MCP), sodium acid pyrophosphate (SAPP), sodium aluminum phosphate (SALP), and dicalcium phosphate (DCP). Anhydrous monocalcium phosphate (AMCP) is sold with a coating that slows its rate of reaction. Other common leavening acids are sodium aluminum sulfate (SAS), monopotassium tartrate (cream of tartar), and glucono-delta-lactone (GDL).

Choosing the right acidulant(s) depends on the desired effect on the finished product, and on two other major characteristics: the reaction rate and the neutralizing value. The reaction rate is the rate at which the carbon dioxide is released in response to moisture or heat. This can be measured in several ways. The Dough Rate of Reaction (DRR) measures the rate of gas released during mixing and holding. The dough is held at a constant temperature, and the amount of gas is measured, converted to the percentage of sodium bicarbonate, and plotted against time. The reactivity of the acids, or DRR, is mainly a function of solubility in water at different temperatures. Solubility is influenced not only by chemical composition, but also by factors such as particle size, and any coatings or encapsulation techniques used. Dough temperature and rate of temperature change also affect solubility. Reaction rate may also be influenced by the addition of other ingredients, such as sugar and milk (due to calcium) that can slow the rate. Reaction rate affects the finished product's grain, volume and texture. The goal of the product developer is to balance the system so that none of the acid or base remains in the baked good. Residual soda causes a soapy flavor and yellowing of light-colored products. Excess phosphate causes metallic off-flavors and an unusual squeaky-teeth feeling.

The neutralizing value (NV) is the amount of sodium bicarbonate needed to completely neutralize 100 pounds of that acid. In most applications, the goal is to retain little or no sodium bicarbonate or leavening acid in the finished product. However, an excess is sometimes required to provide a specific pH-related effect, such as color or flavor modification in chocolate cakes.

Other ingredients also affect the pH of a product, so adding neutral proportions of leavening acids and bases does not guarantee a neutral pH in the finished product. Changing the pH can affect the speed and reactivity of the leavening system. The degree of flour bleaching can cause the dough to become puffy. Adding ingredients like fruit, buttermilk, or even high-fructose corn syrup and cocoa powder can significantly change the pH of the finished product and reduce its volume. Many ingredients contain organic acids, which will react very quickly with the bicarbonates.

Besides reacting with baking soda, leavening acids affect the structure, color, flavor, and pH of the finished product. Each acid produces a slightly different texture. Ions making up the acid salts influence the crumb structure and texture. Calcium and aluminum ions contribute to a fine grain, and also strengthen the structure of the batter and produce a spongy texture.

TABLE 148.7
Leavening Acids Commonly Used in Bakery Products

Chemical Name	Chemical Formula	Common Name or Abbreviation[1]	Rate at Room Temperature	Neutralizing Value[2] (NV)	Other Characteristics
Monocalcium phosphate monohydrate	$CaH_4(PO_4)_2 \cdot H_2O$	MCP	Very fast	80	Used in combination with slow acids to aid nucleation.
Monocalcium phosphate anhydrate	$CaH_4(PO_4)_2$	AMCP	Slow	83	Slow reacting at room temperature, reacts in oven.
Dicalcium phosphate dihydrate	$CaHPO_4 \cdot 2H_2O$	DCP*	None	33	Slightly alkaline at room temperature. A heat-triggered leavening agent but triggers too late (57–60°C) for most products. Used in frozen cake batters or high sugar baked products.
Dimagnesium phosphate	$MgHPO_4$	DMP*	None	40	The first heat-activated leavening agent (40.5–43.5°C) that does not contain sodium, yet reacts early in the baking process.
Sodium acid pyrophosphate	$Na_2H_2P_2O_7$	SAPP	Slow to fast	72	Wide range of reactivity release CO_2 in the oven, used in production of cake doughnuts. Slow acting: SAPP 21 and 26; used primarily in refrigerated biscuits and cake mixes. Medium acting: SAPP 28; commonly used for commercial baking powders. Fast acting: SAPP 37, 40, 43, and 45; used primarily in cakes and cake doughnuts.
Sodium aluminum sulfate	$Na_2SO_4 \cdot Al_2(SO_4)_3$	SAS*	Slow	100	Little reaction until heated, too slow to be used alone. Used in double-acting household type baking powder. May accelerate fat rancidity.
Sodium aluminum phosphate hydrate	$NaH_{14}Al_3(PO_4)_8 \cdot 4H_2O$	SALPH*	Medium	100	Temperature triggered.
Sodium aluminum phosphate anhydrous	$Na_3H_{15}Al_2(PO_4)_8$	SALPA*	Slow	110	Primarily temperature triggered.
Potassium acid tartrate	$KHC_4H_4O_6$	Cream of tartar	Medium fast	50	First leavening acid developed, relatively expensive.
Glucono-delta-lactone	$C_6H_{10}O_6$	GDL	Slow	55	Very expensive. Some uses in cake doughnuts.

[1] Heat-activated leavening agents are marked as *.
[2] Neutralizing value (NV) defined as parts (by weight) of sodium bicarbonate that 100 parts (by weight) of leavening acid will neutralize.
From (27).

b. Baking powder

Baking powders consist of one or more acids in combination with baking soda and a carrier such as starch. Three major types of baking powders are listed in Table 148.8. The starch physically separates the acid and base and prevents them from reacting during storage. The leavening reaction typically occurs over a period of time rather than all at once, but acids that react mostly at the batter or dough stage are generally categorized as "fast-acting," vs. those that function during baking, frying or griddle cooking and are categorized as "slow-acting." This gas cell nucleation also can occur by incorporating air during mixing. The better the dispersion of these nucleating cells, the finer the grain in the finished product. Often, leaveners are used in combination (or called double-acting) to achieve a specific effect. The initial gas release provides small gas cells that promote uniform expansion during baking. For example, cake doughnuts usually have a combination of a fast-acting SAPP, and a slower leavener such as SALP or GDL, to permit rapid expansion when the batter hits the hot fryer oil.

D. Fats and Oils

Nearly all baked products contain fat—often in significant quantities (10–50%, bakers, percentage). Fat's functionality is very versatile in baked products (Table 148.9). The major functions of fats in baked items are: (1) imparting shortening, richness, and tenderness to improve flavor and eating characteristics; (2) enhancing aeration for leavening and volume; (3) promoting desirable grain and texture qualities; (4) providing flakiness in pie crusts, Danish, and puff pastry; (5) providing lubrication to prevent the wheat gluten particles from adhering together to retard staling; (6) affecting moisture retention for shelf-life

TABLE 148.8
Types of Baking Powder

Type	Definition	Examples	Comments	Applications
Single-acting	Contains only one acid ingredient, either fast or slow.			
Fast-acting	Contains fast acting acids that release a large amount of the gas in a relatively short time during mixing, or while the batter is on the bench.	Cream of tartar Tartaric acid MCP	Increased batter aeration; greater volume; improved texture; most CO_2 released in mixer.	Pancake mixes, cookie mixes, angel food cakes, double-acting baking powder.
Slow-acting	Contains moderately slow acting acids that release a major proportion of the gas in the oven.	SAPP SALP SAS	Used in combination with others; improves tenderness and moistness of baked products.	Various types of donut mixes, refrigerated canned biscuits.
Double-acting	Contains two or more acid ingredients that must have a minimum of one fast and one slow acting, so that some of the gas is released during mixing and on the bench with the remaining gas being released in the oven starting at about 40–43°C (104–110°F).	MCP+SAS MCP+SAPP MCP+SAPP+SALP	Nucleation; late bake reserve; fast oven action; aluminum and calcium ions for batter viscosity and crumb resiliency.	Layer cakes, muffins, pancakes, refrigerated biscuits.

TABLE 148.9
Functionality of Fats on the Bakery Products

Bakery Product	Functionality[a]				
	Shortening	Whipping	Emulsifying	Crisping	Stabilizing
Bread, Roll	++	−	+	−	−
Danish bread	++	−	−	+	+
Cake	+	++	++	−	−
Cookie	+	−	−	++	++
Pie crust	+	−	−	++	+
Doughnut	−	−	−	+	++

[a] ++: highly required functionality; +: required functionality; −: not required functionality.

improvement; and, (7) providing structure for cakes, icing, and fillings (28).

The functional performance and textural quality of fats, and fat-containing products are determined mainly by the balance between the solid and liquid phases (solid fat index, SFI) and the crystal structures of the solid fats. Table 148.10 is the SFI of fats commonly used in baking. The SFI influences how well air is incorporated, as well as the rheology, mouthfeel, shelf-life and other quality issues of many bakery products. The SFI depends on a number of factors, including the degree of hydrogenation and the type of fat. As the fat solidifies, it takes on one of three main crystal forms: alpha (α), beta prime (β'), and beta (β). Each affects hardness, texture, mouthfeel and stability. The β form is the most stable, but β' is generally preferred due to its smoothness and superior creaming properties. The factors that affect the formation of β' crystals include the amount of palmitic acid, the distribution and position of palmitic and stearic acids, the degree of hydrogenation, and the randomization of the fatty acids. Hydrogenation can increase β formation, while randomization of the fatty acids promotes β'. During storage, there is a tendency for the fat to be transformed into the most stable crystal form, which may or may not be desirable. Generally, fats in the α-form are characterized as waxy; fats in the β'-form are characterized as fine-grained with smooth texture; and the β-form crystals are termed coarse and grainy (30).

1. Fat and Oil Processing

Food fats and oils come from a number of different sources, either animal or vegetable. They can come from one source, or consist of a blend that creates a functional or cost benefit. Most food fats start out as refined/bleached/deodorized (RBD) oil. Figure 148.5 shows the typical sequence for fats and oils processing. To achieve improved functionality for bakery applications, solid fat index, melting curves, and crystal structure of fats should be focused. Altering a fat's functionality to meet

TABLE 148.10
SFI of Fats Commonly Used in Baking[1]

Applications	Fat Type	Specification	Melting Point (°C)	SFI 10.0°C/ 50°F	SFI 21.1°C/ 70°F	SFI 26.7°C/ 80°F	SFI 33.3°C/ 92°F	SFI 43.3°C/ 104°F
Croissant	Shortening	Low MP	39	39.0	27.0	22.0	19.0	18.0
Danish, roll-in		Medium MP	45	24.0	20.0	19.0	16.0	11.0
		High MP	52	26.0	20.0	18.0	17.0	14.0
	Margarines	Baker's	45.0	29.0	18.0	16.0	13.0	5.0
	Butter		32.0	34.0	12.0	4.0		
Puff pastry			49.0–53.0	26.0–40.0	24.0–38.0	22.0–34.0	21.0–28.0	17.0–24.0
Pie crust	Lard			29.0	21.5	15.0	4.5	2.9
	Soybean			32.0	25.0	18.0	10.0	7.0
	Soybean + cottonseed oil			26.0	18.0	16.0	10.0	6.0
Cracker	High stability Shortening	Hydrogenated Soybean oil	43.0	43.0	27.0	21.0	9.0	5.0
		Meat fat + vegetable oil	46.0	39.0	28.0	24.0	17.0	11.0
Cookie filler	Coconut oil		24.5	59.0	29.0	0.0	0.0	0.0
			34.0	57.0	33.0	8.0	3.0	0.0
			43.5	63.0	41.0	16.0	7.0	4.0
	Special hydrogenation		38.5	58.0	43.0	34.0	12.0	1.0

[1] From (29).

these needs requires further processing. Fractionation, hydrogenation, and/or interesterification are the processes most often used, but some specialty fats may require other modifications (30).

To increase stability and raise the melting point to accommodate most cookie and cracker applications, many RBD fats must undergo hydrogenation. Hydrogen atoms are added to the unsaturated bonds under high temperatures in the presence of a catalyst. Saturated, or single bonds, resist chemical reactions, including oxidation. As the chain length and the number of single bonds increase, so does the melt point. Hydrogenation may shift the location of the double bond, which slightly affects the melting point. It also promotes *trans* isomers through isomerization, a form where the hydrogen atoms are positioned on opposite sides of the carbon-carbon bond. These configurations also affect the melting point (31). *Trans* fatty acid formation during deodorization is influenced only by time and temperature. Generally, *trans* formation is negligible below 220°C; it becomes significantly between 220 and 240°C, and nearly exponential above 240°C (32).

Manufacturers can also use interesterification to alter properties of fats. This process rearranges the fatty acids on the triglyceride backbone with a catalyst or enzyme to change the fat's properties (33). The lower cost and broad functionalities produced with hydrogenation made this method less desirable, but since it doesn't create *trans* isomers, commercial interest in interesterification has increased significantly in the past several years.

The other processes essential for making oil-based ingredients for baked goods transforms them into a plastic shortening or margarine. Theses process include the use of scraped-surface heat transfer to mix; cooling and controlling crystallization; and incorporating air; making a pliable fat suitable for bakery products (34).

2. Types of Fat and Oil for Bakery Products

Many different fats are available to the baker. These fats have different properties that make them suitable for different purposes. Among the properties a baker must consider when selecting a fat for a specific use are its melting point, SFI (i.e., softness or hardness at different temperatures), flavor, and ability to form emulsions. In the selection process, bakers should take into consideration all of these issues, including functionality, cost, stability and health.

a. Butter

Butter (milk fat) has been legally defined as a food product made exclusively from milk or cream, or both, with or without common salt, and with or without additional coloring matter, and containing not less than 80% by weight of milk fat. The nonfat portion of butter is composed of approximately 16% water, 2.5% salt, and 1.5% milk solids. The soluble components in the fat or triglyceride portion are sterols, pigments, fat-soluble vitamins, and phosphatides or lecithin at approximately 0.2% (35). Butter has traditionally been used for its flavor contribution, but it usually is

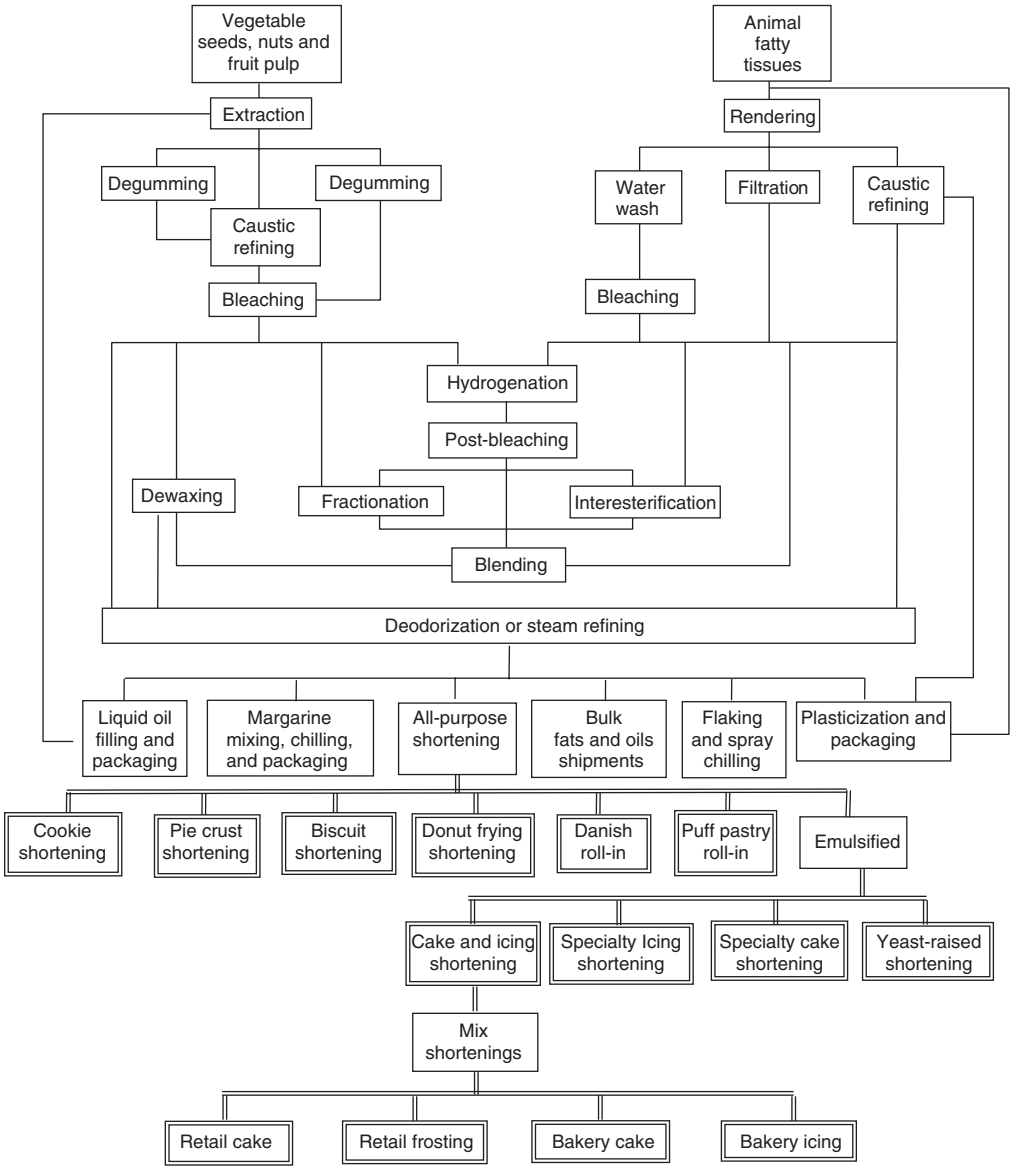

FIGURE 148.5 Flow sequence of fats and oils processing.

not the only source of fat in a product due to the expense. Butter is available salted and unsalted. Unsalted butter is more perishable, but it has a fresher, sweeter taste and is thus preferred in baking. While it can be substituted with margarine or straight vegetable shortening, butter offers an edge in mouthfeel and flavor that helps define a premium dessert. Butter also tends to provide a different end result than margarine, particularly when it is used in pastries and crusts. Both contain about the same amount of fat or oil, but the vegetable fat used in margarine tends to form in small globules or spheres, while the animal fat found in butter forms crystals. This crystal structure is layered flat, almost like shingles. Because of the physical shape of the fat crystals, butter tends to spread and layer more easily and uniformly, forming nice flaky layers with richer flavor notes. Butter is hard and brittle when cold, very soft at room temperature, and it melts easily. Consequently, dough made with butter is much harder to handle than shortenings.

b. Margarine

Margarine is manufactured from various hydrogenated animal and vegetable fats, plus flavoring ingredients, emulsifiers, coloring agents, and other ingredients. It contains 80–85% fat, 10–15% moisture, and about 5% salt, milk solid, and other components (36). Thus, it may be considered a sort of imitation butter consisting of shortening, water, and flavoring. There are two major categories of margarines for bakers: cake margarines (or baker's margarines) and pastry margarines. Cake margarines are soft and have good creaming ability, while, pastry margarines are tougher and more elastic and have a waxy texture that is especially formulated for dough that forms layers, such as Danish

dough and puff pastry. Puff pastry margarine, the toughest of margarines, is sometimes call puff pastry shortening. However, it has significant water content, which helps to give leavening power to the dough when it forms steam.

c. *Shortening*

Shortening was originally a baking term that described the function of a solid fat to give a "short" or tenderizing effect to baked products. Later, the word shortening was used to mean any of a group of solid fats, usually white and tasteless, that had been especially formulated for baking. Today, the term shortening has become virtually synonymous with fat, and includes many other edible fats or oils products designed for all prepared foods, not simply baking (34). Shortenings (or plastic fats) generally consist of nearly 100% fat; however, there are exceptions, such as roll-in shortening, which may contains moisture. Shortenings may be made from vegetable oils, animal fats, or both. During manufacturing, the fats are hydrogenated. Because shortenings are used for many purposes, manufacturers have formulated different kinds of fats with different properties. There are two main types: regular shortenings and emulsified shortenings (Figure 148.5).

Regular shortenings have a fairly tough, waxy texture, and small particles of the fat tend to hold their shape in a dough or batter. They can be manufactured to be of varying degree of hardness. Regular shortenings have a good creaming ability but melt, only at a high temperature. They are usually used for flaky products, such as piecrusts and biscuits. They are also used in many other pastries, breads, and products mixed by creaming, such as pound cakes, cookies, and quick breads.

Emulsified shortenings are soft shortenings that spread easily throughout a batter and quickly coat the particles of sugar and flour. Because they contain added emulsifying agents, they can hold a larger quantity of liquid and sugar than regular shortenings can. They are often used when the weight of sugar in a cake batter is greater than the weight of flour, and this results in a smoother and finer texture, as well as a moister cake. Such cakes are referred to as high-ratio cakes, and emulsified shortening is sometimes called high-ratio shortening. In addition, emulsified shortenings are often used in icings because they can hold more sugar and liquid without curdling.

Roll-in shortening is a harder fat than margarine and is used in sweet doughs. Puff-pastry margarine is harder, and is used to produce croissants and Danish doughs. Shortening for puff pastries is added to dough by a process called booking. A layer of semi-soft margarine is spread across the center of a piece of sweet dough. Each side is folded to the center, forming a book. The dough is retarded (refrigerated) to firm up the fat. After cooling, the dough is rolled out and the process is repeated several more times. The finished dough has between 45 and 60 layers.

Fluid shortenings have some characteristics of plastic shortenings, but are pumpable. Fluid shortenings that produce finished products identical to those made with plastic shortenings have been developed. Fluid shortenings are much more economical to transport, and reduce plant labor. In some applications, lower levels of fats can be used with fluid shortenings.

d. *Vegetable oils*

Vegetable oils used in baking come from a number of sources: canola, cocoa butter, coconut, corn, cottonseed, palm, palm kernel, peanut, safflower, soybean and sunflower. In many cases the primary considerations are price and availability, so soybean, or a blend based on soybean, typically has an edge.

Despite their notoriety, palm oil and the other tropical oils provide a number of positive functional attributes: They can be used as a source for solids to avoid hydrogenation of liquid oils. Palm oil promotes beta prime (β') crystallization. The high palmitic content increases the aeration of fat and sugar mixes. The absence of linolenic acid prevents flavor reversion, or the development of characteristic, often undesirable flavor notes that develop in most oils with time.

3. The Role of Fat in Bakery Products

Examining how the role of fat varies in different products is made clearer by dividing bakery products into the following general categories: yeast-raised products, including breads and rolls; laminated products, such as croissants and pastry; cakes; and cookies and crackers.

a. *Yeast-raised products*

Bread and rolls typically don't have much oil in the formulation, but fat is important both for organoleptic quality and processing. Although fat isn't required for aeration in yeast-raised products, it still is directly related to how well the dough rises. The small amount of fat in bread or roll dough will lubricate the cells so they can slide. This is important both for machining and for lubricity that directly affects product quality. When a dough is proofing, the cells must be able to slide easily to allow even distribution of the fermentation gasses. If the cells cannot move, the loaf will have an irregular shape and bulge in the areas where the gasses have concentrated. The lubricant qualities of the fat also work in the finished yeast-raised product to give a desirable soft texture and grain. If making a product without shortening, such as French bread, a hard crust will result. If making bread with shortening, the softer crumb associated with longer shelf-life will be obtained. As far as lubrication goes, most types of fat will work in yeast raised products because the usage levels are so low. In the Chinese National Standard (R.O.C), the fat content (on the baker's percentage, i.e., based on 100 g of wheat flour) of different types of breads is less than 4% for hearth bread

and roll, 4–10% for soft bread and roll, and more than 10% for sweet roll, based on CNS 3899 (37).

If a solid shortening is used in a bread or roll, it can make the product firmer than one made with oil. Following distribution during winter, or in cooler storage conditions, consumers may feel such a product and think it's stale. Sweet rolls or buns are a little different, because they have higher levels of both fat and sugar. Here, instead of oil, an emulsified plastic shortening may be better. Oil doesn't have anything that will solidify, so the bread stays soft. Because many breads and rolls are delicately flavored, the fat or oil selected also must possess and maintain as neutral a flavor as possible so it doesn't contribute off flavors to the finished product.

b. Laminated products

Laminated products, for example croissants, Danish and pastry, require two types of fat; in-dough fats that provide the function of giving lubricity and enhancing eating quality, and roll-in fats that create the flaky layers of products. Table 148.11 shows the typical formulas for croissant, Danish, and puff pastry doughs. The roll-in fat further enriches the dough to provide flakiness, tenderness and flavor. The fat's flavor, in particular, is critical because of the high fat content in these products. Between the in-dough and roll-in fats, for example, laminated products can have a fat content that ranges from 10 to 40%, based on dough weight. A good quality Danish, for example, will have at least 25% fat for the roll-in alone. At this high use level, many roll-in fats are margarines or other specially formulated fats incorporating rich, butter-like flavors.

The roll-in fat has to be able to be machined and keep its integrity through the machining process. The plasticity of a roll-in fat must be compatible with the dough. If the dough is soft and the roll-in fat is firm, machining will tear up the dough. On the other hand, if the fat is too soft, it will squeeze into the dough and layer integrity will be lost. If it mixes in, it won't create a distinct layer. To be both machinable and maintain integrity, a roll-in fat will have to have a wide plastic range. It should be smooth and workable over a wide range of temperatures and withstand make-up stresses. Such qualities are controlled by the SFI and its processing procedures. Basically, the solid fat profile, votating processing, and subsequent tempering of the product give those characteristics to the fat. Votated fats, which are produced by emulsifying fats with milk, enzyme products, and color with a votator, are soft and pliable over a broad temperature range.

TABLE 148.11
Typical Formula for Croissant, Danish, and Puff Pastry Doughs

Ingredient	Croissant	Danish Bread	Puff Pastry
	% (baker's percentage)		
Strong flour	70	75	100
Weak flour	30	25	
Milk solid	4	6	
Sugar	8	15	3
Salt	1	1.5	
Water	50	45	50
Egg	10	15	7
Yeast (Dry Instant)	1.5	2	—
Vinegar			2
Butter (Dough-fat)		12	
Shortening (Dough-fat)	5		15
Roll-in fat	45	50	85

Croissant dough is softer, therefore requiring a soft roll-in fat. Finished croissants also are a softer eating product, requiring fat with low solids. To maintain the desired softness, croissant roll-in fat also usually has an emulsifier to enhance the shelf-life of the finished product. It's not as chewy as Danish, nor as firm or with a waxy mouthfeel as puff pastry. Generally, the fat going into a croissant will be almost like a table-grade refrigerated margarine with about a 38.8°C (100°F) melting point. These margarines have low solids—they build just enough structure to give croissants the flake. If a shortening with higher solids is used, a very waxy mouthfeel similar to puff pastry will be obtained.

Danish dough is not as fragile as croissant dough, so the plastic texture of the roll-in fat should be slightly firmer. The finished rolls also are somewhat firmer than croissants with a slightly firmer texture. To meet the differences in both makeup and finished product, the roll-in fat for Danish will have a higher level of solids and a higher melting point than those used for croissants. Another noticeable difference between roll-in fats for the two products is that croissant fats are refrigerated margarines requiring the entire roll-in procedure to be conducted under cool conditions. Danish use roll-in fats that don't require refrigeration, so temperature will not be as critical during makeup.

Puff pastry is very different from both croissants and Danish. Here, the flake is very distinct and very crisp. This is achieved by using very little fat in the dough itself—typically not emulsified to avoid softening the flake. Normally, there will be a half percent fat used just to aid in sheeting. Some of the large operations use no fat in the dough at all, making it tough to sheet. With little in-dough fat, the structure of the finished puff will be largely dependent on the roll-in fat. Puff pastry roll-in fats are traditionally higher in melting point, thereby giving the product its distinct flakes and traditional waxy mouthfeel. While the tough puff pastry doughs can withstand machining with such higher solids fats, these fats still must be pliable so they can spread evenly and machine smoothly.

c. Cakes

Cakes are highly dependent on fat for proper aeration. Although the fat will contribute to crumb texture,

mouthfeel and lubricity as it does with other products, a cake simply would not be a cake if the fat didn't have the right aeration qualities. Many fat characteristics contribute to good aeration. This allows for even distribution of leavening gases and water vapor released during baking. The finished results include increased volume, lower specific gravity and a more desirable texture in the finished product. Proper aeration is a function of the solid fat crystals and requires that they be of the right type, size and shape. Many types of crystals exist; and the ones that form will depend on the temperature and rate of cooling when melted fat is plasticized. The most desirable crystal type for aeration is the beta form because of its small size, uniformity, and stability. The shortening having the correct amount of solids at the right melting range will be more critical for cake making.

An oil is not going to trap air in the same way as a solid shortening. Still, a shortening with too high a solids level can go to the other extreme and create a finished cake that is too firm. For some cakes, a liquid shortening with two to three times the level of emulsifier compared with a solid cake shortening can be used. Because of the fact that the fluid shortening is an oil base, a much more tender, moist product will result with about 20% more volume.

d. Cookies and crackers

Cookies and crackers have slightly altered priorities to the basic fat functions. Part of this is because they have lower moisture levels and depend more on the fat for tenderness and mouthfeel. Besides the fact that both have lower moisture levels, cookies and crackers are different products, and fat selection and application differ greatly with each (38). For example, cookies have relatively high levels of in-dough fat, while crackers do not. Nevertheless, crackers frequently have oil sprayed on topically after coming out of the oven to makeup the difference. Lower-moisture products like cookies also tend to have longer shelf-life than other bakery foods. With longer storage times comes the need for the fat itself to resist oxidative degradation.

Most crackers don't have much in-dough fat. Some do have in-dough fat levels as high as 20% based on flour weight; but for the most part, the fat in a cracker dough is at a low level and serves more of a functional purpose, much as it does in yeast-raised products. It smoothens and softens the dough to improve sheeting and cutting. On the other hand, most crackers with higher fat levels have that fat added after the bake as topical spray oil. This enhances the eating characteristics, or can be used to add to the concept when flavorings are added to the spray oil. But the advantages are not exclusively organoleptic. The oil, for example, protects the cracker from picking up too much moisture. Not all oils are suitable for spraying crackers. With many liquid oils, the finished product may appear too glossy and have an oily taste. Many liquid oils also do not possess the required oxidative stability. As a result, most cracker spray oils typically have a certain level of solids—either naturally occurring, or as the result of partial hydrogenation. Some spray oils, such as coconut oil, are solids that are melted prior to spraying. In general, the solids content, the stability, the taste imparted to the product, and the economics are the major concerns for selecting spray oil.

Shortenings have four primary functions in cookies: lubrication, aeration, eating quality, and spread. Lubrication is a function of the liquid oil fraction in the shortening. By coating sugar and flour particles, the oil reduces mixing time, the energy required for mixing, and smoothens the dough. The oils also help reduce gluten development so the finished product is tender. By coating the flour particles, liquid oil helps to increase mixing tolerance. Last, liquid oil's lubrication properties help prevent the dough from sticking to the baking surface. Oil can be used in place of shortening, if lubrication is the only function desired from the fat.

Aeration is the second major function of fats in cookie doughs and occurs primarily during creaming—usually the first stage of a multi-stage mixing profile. The entrapped air serves much as it does in a cake, providing a framework for the leavening gases and water vapor released during baking. The result is increased volume, uniform and fine grain, and tender crumb. Uniform aeration from batch to batch is critical because of its effect on dough density and weight control during forming or depositing.

Eating quality is the third function fats provide to cookies. Wide ranges of differences in eating quality are possible, depending on the selection of the shortening. Consequently, the target eating quality must be clearly defined (i.e., whether the product should be hard, soft, brittle, chewy, or tender) before product formulation and ingredient selection occur. Process requirements affected by texture, such as packaging or sandwiching, also should be established. Distribution also is a concern, since tenderness may have to give way to delivering cookies to consumers in one piece. In general, higher percentages of fat produce more tender cookies.

The fourth functional property of fats and oils is the effect on spread. Increasing the shortening level increases spread, and can do so quite significantly when using partially hydrogenated vegetable oil. This is not necessarily the case with lard, but lard does give a greater spread than vegetable shortening. Like cakes, cookies can be formulated with either a solid or a liquid fat. Solid shortenings are more frequently used, but a liquid shortening may help tenderize a product in a situation where adjusting other tenderizing ingredients is not desirable. While this replacement can be one-to-one in cakes, this is not usually the case with cookies. Because of the lubricity, one has to take a 15 to 20% reduction in fat when going from a solid shortening to oil in cookies.

When combining fats, especially in a filling or coating application, it's important to consider two issues: fat mobility and fat compatibility. When fat is in a liquid state

it travels, seeking equilibrium as long as there is physical contact. Thus, liquid fat can go from the filling to the cookie (or vice versa). If a filling has too high a liquid fat content, the surface of the cookie may become greasy. Combining incompatible fats may result in softening, changes in the crystal structure, and/or fat bloom. A phenomenon known as the eutectic effect occurs.

Fats and oils play a number of widely different roles in cookies and crackers, and they can be divided into dough fats for both cookies and crackers, filling fats, spray oils, and coating or confectionery fats based on their applications.

i. Dough fat

Dough fat is added to cookie dough for its effect on both the finished product and the process. Fat acts as a lubricant. It keeps the dough from sticking to the feeding and forming equipment. It facilitates mixing by lubricating the other ingredients. And it helps the cookies release from the baking surface without sticking. Depending on the product, the fat content of a full-fat product ranges from 20% to 70% (38). Fat helps control the texture of the finished product, its spread, and its appearance. In most cookies containing fat, a plastic (solid or semi-solid) shortening is first combined with the sugar in a creaming stage. This helps to entrap air, and contributes to the structure or grain of the finished product. It also influences the density of the dough. During creaming, the shortening coats the individual sugar particles. The critical point of this procedure is to be able to coat the sugar without any clumping of the shortening or the sugar. If sugar is not coated, then the melted sugar will recrystallize into little chunks.

Normally an all-purpose shortening for cookies has a 44°C (112°F) melt point, but a range from about 35–46°C (95–115°F) is also suitable, depending on the practical limitations. For example, if there were a high shortening level, a 39°C (102°F) shortening may be suitable to reduce the waxy mouthfeel. As a general rule, the more shortening, the more tender the mouthfeel of the finished product. A higher fat cookie will still be crispy without being hard. However, excess fat, particularly liquid, can result in a greasy, soggy cookie. The more fat in a cookie, the more it will spread.

Fat is particularly important in the texture of a low-moisture cookie. Most of these are baked to 2% to 3% moisture, and without the shortening effect the result is rather hard. Certain production techniques, or the addition of emulsifiers such as mono- and diglycerides, can improve the texture. Emulsifiers affect the dough and the finished product differently, depending on the system and the type of emulsifier used. The choice of emulsifier depends primarily on the effect required, in addition to the product characteristics such as moisture level. In a full-fat system, a distilled monoglyceride increases the tenderness of a cracker or cookie while keeping it crisp. In a low-fat application, the same general effect is obtained, but the emulsifier plays a much more important role. Emulsifiers, principally DATEM (diacetyl tartaric acid esters of mono- and diglycerides) will give a fat-sparing effect. They break the fat up into very fine particles. As with cookies, the purpose of fat in cracker dough is lubrication and texture modification. Traditionally the level used has been lower than in cookies (less than 10%). Because of this level and the lack of aeration, fairly good results can occur with the use of liquid shortenings, as well as solid ones.

ii. Filling fat

Approximately two-thirds of a crème filling consists of fats. In a typical sweet filling, the other third is mainly sugar. Savory versions also can be formulated using ingredients such as peanut butter and cheese powders. The key is to have sufficient fat to coat the dry material completely. The fat used must enhance eating quality, serve as an adhesive for the two halves of the sandwich, and function properly in the sandwiching operation. All of these depend on the solid fat index. Either liquid or plastic fat can be used for the crème depending on the result required. The crème, must remain plastic during the sandwiching operation. If the percent of solid fat is too high, the crème becomes brittle and dry and it will not stick to the base cake. If the percent of solid fat is too low, the crème will be too soft, causing a number of problems; for example, the crème will smear, and it will not hold the base cakes together. From an eating quality perspective, nearly all of the fat must be liquid over 33°C (92°F), or the filling will taste waxy rather than creamy. A fairly steep SFI also enhances mouthfeel by giving a cleaner, cooler melt. For both mouthfeel and processing, the standard SFI for filling fats is similar to that exhibited for coconut oil, at 33°C (92°F).

Some crèmes are aerated. The fat must have a fairly high level of solid fat during the mixing and processing step to incorporate air and maintain it during processing. Liquid fat does not retain air. Fats for crèmes also require a melt point and profile that give a rapid melt away in the mouth. The most effective way to aerate filling crèmes is by running them through a continuous scraped-surface heat exchanger prior to sandwiching. Aeration creates a softer crème, and that may affect the process. Because an aerated cream has increased exposure to air, the fat must have a high degree of oxidative stability.

iii. Spray oil

A coating of oil after baking improves the appearance of crackers. It also reduces the pasty mouthfeel generated by a product that consists mainly of flour. The level of spray oil depends on the equipment capabilities and the geometry of the cracker, as well as the desired organoleptic target. Products with a higher surface-to-volume ratio tend to require a higher level of spray oil. SFI plays a role in the fat selection, but with less importance than for a filling fat. The oil must be liquid during the spray operation, which

isn't too difficult, since this takes place at elevated temperatures.

Stability is important in spray oils. Because the oils are held and used at elevated temperatures, any reactions, including oxidative rancidity, occur more quickly. Spraying increases the exposure to air. The cracker will have a fairly high level of oil on the surface of the finished product, and this also increases the exposure to air. Traditionally, coconut oil was used as the preferred spray oil, but health concerns have changed this. Soybean and cottonseed oil have taken the place of coconut oil. Coconut oil has a unique melting characteristic, and it quickly goes from a solid to a liquid, giving it very good eating qualities. It's very stable, but the downside is that it is mostly saturated fat. Coconut oil is very bland. It has higher solids, so it offers a little dryness on the cracker. It has excellent stability because of the level of saturates. Some other the high-stability products, particularly soy oil, have also been used.

iv. Coating fat

Coating fats for cookies closely resemble those for confectionery, especially those used for chocolate or chocolate-like products. The model for coating fats in terms of the SFI is generally cocoa butter; that is, a steep curve with a melt temperature below 36.6°C (98°F). Cocoa butter is used in some high-end products and real chocolate chips, but in many cases, coatings and chips contain fats other than cocoa butter. Traditionally these were known as hard butters, but the fats and oils industry now uses a number of different terms to classify the types of fats used in coating and confectionery applications (32). Cocoa butter alternatives (CBA) are fats designed to replace some or all of the cocoa butter. Cocoa butter equivalents and extenders (CBE) comprise two different categories. The equivalents are completely compatible with cocoa butter and do not change the melting, processing, and rheological properties of the cocoa butter. The extenders can only be combined with cocoa butter to a limited extent, or they will change the properties. Cocoa butter replacers (CBR) have been engineered to replace or extend cocoa butter, and these are often labeled as "partial" or "complete" replacers depending on the level of compatibility. Often this term refers to only non-lauric fats, or those triglycerides that are low in lauric acid. Cocoa butter substitute (CBS) refers to a fat engineered to replace or extend cocoa butter. Some divide CBS into lauric and non-lauric classifications, and subdivide the categories into hydrogenated, fractionated, and/or interesterified fats.

E. Sweeteners

Sweeteners are one of the major ingredients in bakery products, because very few bakery products are made without some added sweetener. Besides providing the sweetness, sweeteners are also used as food for yeast and to develop crust color, enhance flavor and softness, and extend shelf-life in breads. In cakes, cookies, and other soft-wheat products, sugar helps cream air into the fat, maintain moisture, and contribute to spread of cookies. Granulation of sugars affects creaming, spreading of the cookies during baking, and surface texture of the cookies. As cookies are baked, undissolved sugar melts and the dough spreads on the cookie's baking surface. Coarse forms of granulated sugar dissolve less readily than fine granulations. A coarser granulation of sugar results in less spreading of the dough and more surface cracking of the baked cookie. Surface cracking results from the recrystallization of the sugar at the surface of the cookie. During the initial creaming mixing stage, sucrose particles are coated with a layer of fat. When the cookie dough piece warms in the oven, the fat layer melts away, allowing the water to migrate to the sugar and go into solution. As the sugar changes from solid to liquid, it causes the cookie to flow or spread. Table 148.12 shows the sweetness intensity of sweeteners commonly used for baking.

1. Nutritive Sweeteners

Nutritive sweeteners are based on carbohydrates. How a particular carbohydrate affects a formula is directly related to its chemical composition, physiochemical properties, and its physical form. Chemically, they can be mono- or disaccharides, or complex sugars such as oligosaccharides or dextrins. Physiochemical properties include sweetness, texture, solubility, mouthfeel, humectancy, and flavor. Physically, sweeteners can be solids in various granulations, or liquids.

From brown to white, to liquid, granulated, or powdered, there are many choices of sugars or sweeteners. The type of sweetener chosen depends on the degree of sweetness that is needed, the function of the sugars in the dough or batter being mixed, and the appearance or texture of the baked product. Sugars supply varying levels of sweetness, coloring/browning, moisture retention, and body/bulking, and assist in the creaming and aeration process of batters.

a. Sucrose (Regular refined sugars)

Sucrose (commonly called sugar), a non-reducing disaccharide (β-D-fructofuranosyl-α-D-glucopyranoside) refined from sugar cane or beets, provides a clean sweetness in baked goods with no off-flavors. It is used either alone or in combination with other complementary sweetening ingredients, and is still the most common sweetener in baked goods. Refined sugars in bakery are commonly classified by the size of the grains. The most commonly used are regular granulated sugar, also called fine granulated or table sugar, and confectioners' or powdered sugars. Granulated sugars are further classified into ultra fine, very fine, and sanding sugars, depending on the size of the grains. In general, finer granulations are better for mixing into doughs and batters

TABLE 148.12
The Sweetness Intensity of Sweeteners[1]

Type	Sweetener	Sweetness Intensity[2]	Type	Sweetener	Sweetness Intensity[1]
Solid sugar	Dextrose (Glucose)	0.75	*Sugar alcohol*	Sorbitol	0.5–0.7
	Fructose (Laevulose)	1.8		Mannitol	0.5–0.7
	Galactose (Cerebose)	0.3–0.6		Xylitol	1.0
	Sucrose	1.0		Maltitol	0.8–0.9
	Maltose	0.3–0.6		Lactitol	0.3–0.4
	Lactose (Milk sugar)	0.25		Isomalt	0.45–0.65
	Trehalose	0.45		Hydorgenated starch hydrolysates	0.25–0.75
	Molasses	—			
	Solid starch hydrolysates	—			
Liquid sugar	Liquid sucrose	—	*High-intensity sweeteners—Synthetic*	Saccharin	300–600
	Invert sugar	0.9–1.1		Cyclamate	30
	Liquid starch hydrolysates	0.45–0.75		Aspartame	160–220
	Glucose syrup, 42DE	0.5[3]		Alitame	2000
	HFCS 42%	0.92[3]		Acesulfame K	200
	HFCS 55%	1.0[3]		Sucralose	400–800
	HFCS 90%	1.6			
	Honey	1–1.2	*High-intensity sweeteners—Natural*	Glycyrrhizin	50–100
				Thaumatin	2000–3000
				Stevioside	200–300

[1] From (39) and (40).

[2] These values are only approximate and will vary depending on the individual tasting the sweeteners, concentration, temperature, and the product in which the ingredient is added.

[3] Based on solids.

because they dissolve more quickly. Coarse sugars are likely to leave undissolved grains, even after long mixing. These show up after baking as dark spots on crusts, irregular texture, and syrup spots. Also, fine sugars are better for creaming with fats, because they create a finer, more uniform air cell structure and better volume. Coarse sugar, on the other hand, can be used in syrups, where mixing properties are not a factor.

Confectioners' or powdered sugars are ground to a fine powder and mixed with a small amount of starch (about 3%) to prevent caking. They are also classified by coarseness of fineness. 10X being the finest, it gives the smoothest textures in icings. 6X is the standard confectioners' sugar. It is used in icing, toppings, and cream fillings. Coarser types (XXXX and XX) are used for dusting.

b. Invert sugar

Liquid sucrose is available, and is used primarily for yeast-leavened doughs. Invert sugar, made by boiling sucrose with a dilute acid, which breaks the sucrose into its constituent monomers, glucose and fructose, is still popular in bakery. At 75% solids, the invert syrup is as sweet as sucrose. Invert syrup is hydroscopic and resists crystallization, and is used where moisture absorption and retention are important, such as in fillings and icings. It is also brushed on Danish pastries after baking to help seal in moisture.

c. Fondant

Fondant is sugar syrup that is crystallized to a smooth, creamy white mass. When applied as icing, it sets up into a shiny, nonsticky coating. Because it is difficult to make in the bakeshop, fondant is almost always purchased already prepared, either in the ready-to-use moist form, or in the dry form, which requires only the addition of water. During the manufacture of fondant, part of the sucrose is changed to invert sugar to get the right amount of crystallization. This helps keep the sugar crystals very tiny, which makes a very smooth creaming icing with a good shine.

d. Brown sugar

Brown sugar is mostly sucrose (about 85 to 92%), but it also contains varying amounts of caramel, molasses, and other impurities, which give it its characteristic flavor. Basically, brown sugar is regular cane sugar that has not been completely refined. However, it can also be made by adding measured amounts of these impurities to refined white sugar. Its effects on crust and crumb color are related to the darkness of the sugar—the more molasses, the darker the color. It also has softening capabilities due to its solubility and the presence of the molasses, which contains reducing sugars.

e. Molasses

Molasses is the concentrated sugar cane juice, containing larger amounts of sucrose and other sugars (including invert

sugar). It also contains acids, moisture, and other constituents that give it its flavor and color. Darker grades are stronger in flavor and contain less sugar than lighter grades. Its main function in cookies is flavor, but molasses also adds humectancy due to the presence of reducing sugars glucose and fructose. Its natural acids may react with the leavening system when combined with sodium bicarbonate (baking soda) and also affect the spread of cookies.

f. Fructose

Crystalline fructose (β-D-fructofuranose) is another sweetener that may be used. It has sweetness synergy with other sweeteners, including sucrose, that may allow cost savings. Because it is a reducing sugar it can contribute too much color development if not used wisely. Fructose is sweeter than sucrose, on a weight basis, and lowers water activity (Aw) more effectively. It is more expensive than sucrose and is normally used only in bar fillings where low Aw is critical.

g. Starch hydrolysates

Starch hydrolysates, liquid or dried, are also becoming popular because of their diverse properties and reasonable price. Glucose syrup (also known as corn syrup and high fructose corn syrup (HFCS)) is the most common product realized by bakers. The syrups are made from starch via either acid or enzyme hydrolysis. Corn starch is the most common starting material for syrup production, as it can be completely hydrolyzed to glucose. Typical hydrolysis forms a mixture of maltodextrins, polysaccharides, maltose, dextrins and dextrose (i.e., glucose). Degree of hydrolysis is measured in dextrose equivalents, or DE. Glucose syrup generally come in 24, 36, and 42 DE. The lower the DE, the less sweet and more starch-like the syrup, and the higher the DE, the sweeter and thinner the syrup. Enzyme-converted syrups tend to have a cleaner flavor, and are sweeter and remain free-flowing slightly above ambient temperature as compared to acid-hydrolyzed syrups. Corn syrup, especially the lower DE forms, can help control crystallization, an important formulation consideration for icings and glazes. All corn syrups are hydroscopic, which helps retain moisture, thus extending shelf-life.

HFCS is produced from glucose syrup, in which some glucose is isomerized to fructose by the enzyme glucose isomerase. HFCS varies in fructose content from 42% to 90%. The 42% product is the most often used, because the balance of fructose/glucose most closely resembles sucrose. Different types of corn syrup and other sweeteners have different sweetness values, viscosity, and ability to lower Aw. Blending sweeteners—in particular, corn syrup and HFCS—will not exceed the solubility limits of the sweeteners and helps prevent sugar crystallization.

Corn sweeteners with a high level of monosaccharides depress the freezing point of products. This makes it possible to maintain a soft, chewable texture for fruit pieces in fillings used in products that are consumed in the frozen state. Corn syrup will also influence dough viscosity, cookie texture, and finished color. It is possible to obtain excessive flour starch gelatinization by the addition of corn sweeteners, because sucrose will increase the starch gelatinization temperature more than glucose or fructose. If that is the case, where cookie height increases, spread decreases, and the internal texture changes, then the water level should be decreased to rebalance the formula.

The effects of corn syrup will vary based on the type of syrup used; however, the DE cannot be solely used for selection criteria due to varied processing techniques which produce different sugar profiles of syrup. A high-maltose corn syrup, for example, may have a DE of 48, but will have a functional performance that is very different from that of a 48 DE acid-converted corn syrup.

Corn syrup is available as solids by evaporating the moisture in the syrup, but this is not the best form to use for baked goods, especially for the water limited products, i.e., cookies. Replacing solid sweeteners with an equivalent amount of liquid sweeteners, even on a dry solids basis, may not produce the right dough characteristics. Besides, water is limited in cookie systems and, as a result, dissolving the sweetener solids in water will extend the amount of liquids in the system, making doughs stickier.

Adding corn sweeteners adds fructose and dextrose (both reducing sugars) to the formula, which will contribute to browning. Fructose, lactose, maltose, and dextrose (glucose) are all reducing sugars, so they react with amino acids when heated, forming Maillard reaction compounds that give a brown color and characteristic baked flavors and aromas.

h. Honey

Honey is a natural sugar syrup consisting largely of the simple sugars glucose and fructose, in addition to other compounds that give it its flavor. It contains 41% fructose, 34% dextrose, 2% sucrose, and 18% moisture. Honey varies widely in flavor and color based on the nectar source. Honey has some great functional properties as a sweetener, but due to its high cost it is primarily used for flavoring. Composed mostly of invert sugars, it acts as a humectant. Its reducing sugars promote browning and increase spread. To lower cost, it is often found in blends with HFCS and invert sugar syrups, which can be used as a replacement when combined with honey flavors. Honey also is available in free-flowing powders, flakes, granules, and crystals.

Sometimes honey is used as the sweetening agent in cakes. Actually, the use of honey influences more than simply the sweetness of the cake, because of its liquid content and acidity. Because honey is a liquid, it does not provide crystals to aid in formation of a foam during creaming of the fat; thus honey-containing cakes tend to be a bit coarser than those made with only granular sugar. The acidity is quite variable between different honeys; between 1/12 and 1/2 teaspoon of soda may be needed to neutralize

a cup of honey, but the batter is permitted to remain more acidic in most cases. Honey promotes rapid browning because of the abundance of reducing sugars it adds to the batter. Reducing sugars are very susceptible to Maillard browning reactions. The high fructose content of honey is noteworthy in its effect on browning.

i. Malt syrup

Malt syrup, also called malt extract, is used primarily in yeast breads. It serves as food for the yeast and adds flavor and crust color to the breads. Malt is extracted from barley that has been sprouted (malted) and then dried and ground. There are two basic types of malt syrup, diastatic and non-diastatic. Diastatic malt is produced with high, medium, or low diastase content. It is used when dough fermentation times are short, because diastase in malt breaks down starch into sugars that can be a powerful food for yeast. It should not be used when fermentation times are long, because too much starch will be broken down by the enzyme, resulting in a sticky dough and crumb.

Non-diastatic malt is processed at high temperatures, which destroy the enzymes and give the syrup a darker color and stronger flavor. It is used because it contains fermentable sugar and contributes flavor, crust color, and keeping qualities to breads.

2. Alternative Sweeteners

a. Sugar alcohols

Polyols or sugar alcohols, such as sorbitol, maltitol, and isomalt, are produced by hydrogenation of selected sugars, whereby the reducing aldehyde or ketone chemical function is converted into a non-reducing alcohol group. They can be added to replace solids lost when sugar is removed, and used for sweetener for calorie-reduced or sugar-free baked goods. Because many of these products have humectant properties, it will soften the texture and hold on moisture in baked products (39). The sugar alcohols most often considered when designing sugar-free compound coatings are maltitol, mannitol, xylitol, lactitol, isomalt, and erythritol.

Maltitol is the most commonly used polyol in cookies. With 3.0 calories per gram, the organoleptic and technical properties of crystalline maltitol are similar to sucrose, making it effective in baked cookies or crème fillings. Maltitol is noncariogenic and has 0.9 times the sweetness of sucrose, as well as good water solubility (175 g/100 g H_2O). Maltitol has worked extremely well in chocolate and compound-coating applications, both in chocolate-confection applications and in enrobed products, such as nutritional bars.

Mannitol was used in most of the original formulations 10 to 20 years ago, but was eliminated from most general use due to its high cooling effect with very low heat of solution (−28.9 cal/g) and laxation potential (i.e., low laxation threshold, 20 g/day). Mannitol is permitted by FDA at 2% to 5% levels in most foods.

Sorbitol syrup, with 0.6 times the sweetness of sucrose and 2.6 calories per gram, also can be used in both liquid and crystalline forms in baked foods (e.g., cookies) at a maximum of 30%. Sorbitol causes small rise in blood sugar and is widely used as sweetening agent for diabetics. Sorbitol has high solubility (235 g/100 g H_2O at 25°C) and low heat of solution (−26.5 cal/g).

The FDA permits the use of xylitol in baked foods according to Good Manufacturing Practice (GMP). Xylitol is non-cariogenic and has equal sweetness to sucrose. Although xylitol causes a small rise in blood sugar, it also is widely used as sweetening agent for diabetics. Xylitol has high solubility of 200 g/100 g H_2O at 25°C and very low heat of solution (−36.6 cal/g).

Lactitol monohydrate is a sugar alcohol derived from lactose, and is 30−40% as sweet as sucrose. Lactitol the solubility of 140 g/100 g H_2O at 25°C, heat of solution of −13.9 cal/g, and a low degree of hygroscopicity.

Erythritol is produced from glucose by a fermentation process. With a calorie value very close to zero, it is approximately 70% as sweet as sucrose, and is an excellent ingredient for sweetening crème fillings. In some cases, sugar alcohols, such as sorbitol and manitol, take the place of sugar or corn syrup. These are not metabolized the same way as other sugars, and, therefore, provide fewer calories than the sweeteners they replace. However, because they pass through the gut, they can cause digestive problems (diarrhea) if consumed at a high level. Erythritol has solubility of 61 g/100 g H_2O at 25°C and very low heat of solution (−42.9 cal/g).

b. High intensity sweeteners

High intensity sweeteners can also be used to formulate lower-calorie or sugar-free bakery products. However, the formula must be adjusted. Using artificial, or high-intensity sweeteners in place of traditional nutritive sweeteners presents two major problems in preservation and Aw control.

Acesulfame potassium, or acesulfame K, was discovered in 1967 and approved by the FDA in 1989 for use in tabletop sweeteners, chewing gum, dry mixes, and potential baking applications. It is a high-intensity, non-caloric sweetener approximately 200 times sweeter than sugar, and remains stable in the baking process. It has good stability in aqueous media, but has some bitter aftertaste.

Another heat-stable sweetener is sucralose, trichlorogalactosucrose. It has good stability and is 600 times sweeter than sucrose. It was been approved by Canada in 1991 for use in baking.

Aspartame, a synthetic (nutritive) sweetener that combines the amino acids aspartic acid and phenylalanine, was discovered in 1965. It provides 200 times more sweetening than sucrose and imparts 4 kcal/g. It degrades with the heat of baking (41). Encapsulated aspartame is relatively heat

stable and is an option for baking. But change can still occur during storage of the baked goods when the aspartame is no longer protected. Aspartame could be used to make crèmes and fruit purees for filled cookies.

Cyclamate was discovered in 1937 and approved for food use in Canada. It is a non-nutritive sweetener, 30–60 times sweeter than sucrose. It has good heat stability.

Saccharin was discovered in 1879. It is a non-nutritive sweetener, 300–400 times sweeter than sucrose. Saccharin has good heat and pH stability and can be used as a tabletop sweetener in dry beverage blends, soft drinks, canned fruits, gelatin desserts, cooked and instant puddings, jams, jellies, and baked foods.

F. Dairy Products

Next to water, milk is the most important liquid in the bakeshop. Fresh milk, containing 88–91% water, fulfills the function of gluten development. In addition, milk contributes to the texture, flavor, crust color, keeping quality, and nutritional value of baked products. It is probable that bakers use more nonfat dry milk than any other milk-based product. Table 148.13 shows the composition of milk and milk derivatives. Functional characteristics of milk proteins are summarized in Table 148.14.

1. Fresh Liquid Milk

Whole milk is fresh milk as it comes from the cow with nothing removed and nothing added (except when fortified with vitamin D, calcium, folate, or other micronutrients). It contains 3.5% fat (known as milk fat or butterfat), 8.5% nonfat milk solids, and 88% water. Skim or nonfat milk has had most or all of the fat removed. Its fat content is 0.5% or less.

2. Cream

Various types of fresh cream, primarily differing in fat content, are available. Whipping cream has a fat content of 30~40%, such as light whipping cream (30 to 35%) and heavy whipping cream (36~40%). Ultrapasteurized cream will keep longer than regular pasteurized cream, but it will not whip as well. It often contains vegetable gums or other stabilizers to partially compensate for this decreased whipping ability. Light cream, also called table cream or coffee cream, contains 16 to 22% fat, usually about 18%. Half-and-half has a fat content of 10 to 20%, too low for it to be called cream.

3. Fermented Milk Products

Buttermilk, sour cream, and yogurt are fermented milk products. Buttermilk is fresh, liquid milk, usually skim milk, that has been cultured or soured by bacteria. It is usually called cultured buttermilk to distinguish it from the original buttermilk, which was the liquid left after butter making. Buttermilk is generally used in recipes calling for sour milk. When buttermilk is used in place of regular milk in baked goods such as cakes or muffins, the acidity must be neutralized by adding baking soda to the formula. Then, because the soda and acid together release carbon dioxide, this extra leavening power must be compensated for by reducing the baking powder as follows: add 15 g baking soda and subtract 30 g baking powder for each liter (1 kg) of buttermilk.

Sour cream has been cultured or fermented by adding lactic acid bacteria. This makes it thick and slightly tangy in flavor. It has about 18% fat.

Yogurt is milk (whole or low fat) cultured by special bacteria. It has a custard-like consistency. Most yogurt has additional milk solids added, and some of it is flavored and sweetened.

4. Evaporated and Condensed Milk

Evaporated milk is milk, either whole or skim, with about 60% of the water removed. It is then sterilized and canned. Evaporated milk has a somewhat "cooked" flavor.

Condensed milk is whole milk that has about 60% of the water removed and is heavily sweetened with sugar. It is available canned and in bulk.

5. Dried Milk Products

Dried milk is often used because of its convenience and low cost. Dried whole milk is whole milk that has been dried to powder. It has poor keeping qualities, because it still contains the original butterfat which can become rancid. Therefore, it should be purchased in small quantities and always stored in a cool place. Nonfat dry milk (NFDM), also known as nonfat milk solids, is skim milk that has been dried to a powder. It is available in regular form, and in an instant form which dissolves in water more easily. Milk solids, typically used at a level of 5% to 12% in bread, also add flavor and tenderness, and contribute to crust color.

NFDM contains about 52% lactose, which is a reducing sugar, and contributes to Maillard browning. Bakers use high-heat NFDM almost exclusively because of its effect on loaf volume. High-heat NFDM has typically been treated to 87.7°C (190°F) for 30 minutes to denature the milk proteins. This increases their water-binding capacity and gives a finished loaf with good volume. Low-heat NFDM, on the other hand, has less heat treatment and produces a slack dough with low volume.

6. Whey Products

Whey ingredients are often substituted for milk solids, primarily for cost savings. Dry sweet whey is very economical, and contains a lower level of protein and a higher level of lactose than NFDM. A whey protein concentrate of 34% has roughly the same levels of protein and lactose as NFDM, and thus can be substituted for NFDM on a 1:1 basis in most bakery formulations. Products with higher

TABLE 148.13
Composition of Milk and Milk Derivatives

Product		Water	Fat	Protein	Carbohydrate	Ash	Calcium	Sodium
		(%)					(mg/100 g)	
Fluid milk	Whole milk	88.0	3.3	3.3	4.4	0.7	119	49
	Skim milk, nonfat milk	90.8	0.2	3.4	4.9	0.8	123	52
	Evaporated whole milk	74.0	7.6	6.8	10.0	1.6	261	106
	Evaporated skim milk	79.4	0.2	7.6	11.4	1.5	290	115
Sweetened condensed milk	Whole milk (42% sucrose)	27.2	8.7	7.9	54.4	1.8	284	127
	Skim milk (42% sucrose)	28.0	0.2	11.1	16.2	2.5	330	33
Cheese whey		93.2	0.3	0.9	5.1	0.5	—	—
Plain condensed whey		32.0	0.6	10.1	51.3	6.0	—	—
Sweetened condensed whey (38% sucrose)		24.0	0.3	5.6	28.7	3.4	—	—
Cream	Buttermilk	90.1	0.9	3.3	4.8	0.9	116	105
	Light	73.8	19.3	2.7	3.7	0.6	90	37
	Medium	68.5	25.0	2.5	3.5	0.6	90	37
	Heavy	57.7	37.0	2.1	2.8	0.5	65	38
Dried milk	Milk powder, (Dry whole milk solids)	2.5	26.7	26.3	38.4	6.1	912	371
	Nonfat dry milk powder, (Skim milk powder)	3.2	0.8	36.2	52.0	7.9	1257	535
	Dry butter milk powder	3.0	5.8	34.3	49.0	8.0	1184	517
Dried sweet whey		3.2	1.1	12.9	74.5	8.4	796	1079
Whey protein concentrate		2	2.9	20–80	18–60	3–18	—	—

TABLE 148.14
Functional Characteristics of Milk Proteins

Functionality	Casein or Caseinate	Whey Protein
Hydration, water binding	Very high, minimum at pH 4.6.	Water-binding capacity increases with denaturation of the protein.
Solubility	Insoluble at pH 4.6.	Soluble at all pH levels. If denatured, insoluble at pH 5.
Viscosity	High at or above pH 6.	Low for native protein. Higher if denatured.
Gelation	No thermal gelation except in the presence of Ca^{2+}. Micelles gel with rennin.	Heat gelation at 70°C or higher, influenced by pH and salts.
Emulsifying ability	Excellent at neutral and basic pH.	Good, except at pH 4–5, if heat denatured.
Foam formation	Good overrun, κ-casein best, followed by β- and as γ-caseins. Poor foam stability.	Good overrun, β-Lactoglobulin better than α-lactalbumin.
Flavor binding	Good.	Retention varies with degree of denaturation.

levels of whey protein concentrate are also available, which add increased nutrition and functionality, such as dough softening. Major physical and chemical differences in milk proteins is summarized in Table 148.15.

7. Cheese

Two types of cheese are used in the bakery, primarily for the production of cheese fillings and cheesecakes. Baker's cheese is a soft, unaged cheese with a very low fat content. It is dry and pliable and can be kneaded somewhat like a dough. Cream cheese is also soft, unaged cheese, but it has a higher fat content at about 35%. It is used mainly in rich cheesecakes and in a few specialty products. Cream is more important in the production of fillings, toppings, dessert sauces, and cold desserts such as mousses and Bavarian creams.

G. Eggs

Eggs should be well understood by the baker, because they are used in large quantities in bakery products and are more

TABLE 148.15
Major Physical and Chemical Differences in Milk Proteins

Caseins	Whey Proteins
Strong hydrophobic regions	Both hydrophobic and hydrophilic regions
Little cystein content	Both cysteine and cystine present
Random coil structure	Globular structure with helical contents
Heat stable	Easily heat denatured and insolubilized
Precipitate in acidic conditions and insoluble at pH 4.6	Stable in mild acidic environment
Precipitated by di- and polyvalent ions	

TABLE 148.16
Average Composition of Fresh Liquid Eggs

	Whole Eggs	Whites	Yolks
Composition		%	
Water	73	86	49
Protein	13	12	17
Fat	12	—	32
Minerals and other components	2	2	2

TABLE 148.17
Functionalities of Eggs in Bakery Products

Function	Comments
Providing structure	Egg protein, like gluten protein, coagulates to give structure to baked product (e.g., high ratio cakes).
Emulsifying of fats and liquids	Egg yolks contain natural emulsifiers, which help produce smooth batters and contribute to volume and texture.
Leavening	Beaten eggs incorporate air in tiny cells or bubbles, which cause expanding when heated and aid in leavening.
Shortening action	The fat in egg yolks acts as a shortening, an important function in products that are low in other fats.
Moisture	Eggs are mostly water and this moisture must be calculated as part of the total liquid in a formula.
Flavor	Eggs brown easily and contribute to flavor.
Nutritional value	Eggs are nutritious.
Color	Yolks impart a yellow color to dough and batters. Eggs brown easily and contribute to crust colors.

expensive than many of the other high-volume ingredients, such as flour and sugar. For example, half or more of the ingredient cost of the average cake batter is for the eggs.

A whole egg primarily contains a yolk, a white, and a shell. In addition, it contains a membrane that lines the shell and forms an air cell at the large end, and two white strands called chalazae that hold the yolk centered. The yolk is composed primarily of lipids, in the form of phospholipids, triglycerides, and cholesterol. The white portion, or albumen, makes up 60% of the egg's weight and contains approximately 15% protein. Egg white is mostly water and protein, plus a small amount of sugar and inorganic ions. As eggs age, the thickness of the white decreases. The decreasing viscosity correlates with a reduced ability to coagulate and form strong protein networks. Table 148.16 shows the average composition of fresh liquid eggs.

Market forms of eggs are: fresh eggs or shell eggs, frozen eggs (whole eggs, whole eggs with extra yolks, whites, yolks), and dried eggs (whole, yolks, whites). Frozen eggs are usually made from pasteurized high-quality fresh eggs and are excellent for use in baking. To thaw, place them unopened in refrigerator for two days; or place in a defrosting tank containing running water at 10 to 15°C for about 6 hours. Stir well before using. Frozen yolks may contain a small amount of sugar (usually about 10%) to keep the components from separating while frozen. Dried eggs are sometimes used in the bakery, though less often than frozen eggs. The whites are frequently used for making meringue powders. Dried egg products are also used in commercial cake mixes. Dried eggs are incorporated in baked goods in two ways: by reconstituting them with water to make liquid eggs, or by mixing them with the dry ingredients and adding extra water to the liquid portion of the formula.

Most wholesale bakeries use frozen whole eggs to facilitate handling and extend ingredient shelf-life. Egg whites aid in air entrapment, while yolks emulsify and tenderize. Yolks also add a desirable golden color, which gives a rich appearance. Powdered eggs may also be used, but generally do not give the same volume to baked goods as fresh or frozen eggs. A starting level might be to add eggs at the same level as sugar in a sweet dough formulation. The functions of eggs in baking are summarized in Table 148.17.

H. Fruits and Nuts

1. Fruits

Nearly any kind of fresh fruit can be used in the production of desserts. In addition, a wide variety of dried, frozen, canned, and processed fruit products are important ingredients in bakery products. Table 148.18 shows the most important fruit products used in bakery products. Each form of fruit offers its own advantages: fresh and frozen fruits undergo minimal processing; syrup-packed

TABLE 148.18
Fruits Commonly Used in Bakery Products

Fresh	Canned and Frozen	Dried	Candied and Glace	Other Processed Fruits
Apples	Apples, *sliced*	Apricots	Cherries	Apricot glaze or coating
Apricots	Apricots, *halves*	Currants	Citron	Jams, jellies, and preserves
Bananas	Blueberries	Dates	Figs	Prepared pie fillings
Berries	Cherries, *both sour and sweet*	Figs	Fruitcake mix	
Grapes	Pineapple, *rings, chunks, nibs, crushed, juice*	Raisins, *light and dark*	Lemon peel	
Kiwis			Orange peel	
Lemons	Strawberries		Pineapple	
Limes				
Mangoes				
Nectarines				
Oranges				
Papayas				
Passion fruit				
Peaches				
Pears				
Pineapple				
Plums				
Rhubarb (actually not a fruit, but a stem)				

TABLE 148.19
Nut Products Used in Bakery Products

Nut Products	Composition	Applications
Almond paste	Two parts finely ground almonds and one part sugar, plus enough moisture to bring it to the proper consistency.	Cakes, pastries, cookies, and fillings.
Marzipan	A sweetened almond paste. Coarse sugar crystals and freshly blanched almonds ground together until perfectly smooth.	Cake decoration and confectionery work.
Kernel paste	A mass of ground apricot kernels and sugar. It has a strong almond-like flavor.	Used as almond paste but less expensive.
Macaroon paste	A mixture of almonds (or marzipan), sugar, egg whites, and sometimes ground rice or other coarsely-ground cereal, such as maize cones, blended together.	Used as almond paste.
Praline paste	Made from almonds and/or hazelnuts and caramelized sugar, all ground to a paste.	A flavoring for icings, fillings, pastries, and creams.

fruits can be used immediately or stored at ambient conditions; dehydrated fruits can be shipped economically.

2. Nuts

Most nuts are available in-shell, whole, halved, chopped or ground, raw, roasted, or further processed into nut butters. They add both flavor and texture to a finished bakery product. Table 148.19 shows the nut products and their properties commonly used in bakery products.

Nuts supply protein, vitamins, and minerals; in their natural states, they are low in sugar and sodium. They are usually low in unsaturated fats and high in fiber. They should be stored under cool, dry conditions. Temperatures just above freezing are recommended to keep the nuts' unsaturated fats from oxidizing and becoming rancid.

Low humidity limits water activity, thus slowing down oxidation as well as microbial growth.

Almonds, coconut, walnuts, peanuts, and pecans are the most important nuts in bakery products. Other nuts, such as hazelnuts, Brazil nuts, cashews, macadamia nuts, and chestnuts, can also be used. In addition, nut products, such as almond paste, marzipan, kernel paste, macaroon paste, and praline paste, are used in a variety of cakes, pastries, cookies, and fillings.

I. Chocolate and Cocoa

Cacao originated in Mexico, but the Spanish found that the cacao tree (*Theobroma cacao*) would grow in any tropical region within about 20 degrees of the equator. Cacao trees are cultivated today throughout South and Central America, the West and East Indies, and West Africa. West Africa produces nearly 70% of the world's output, pooling the resources of the Ivory Coast (by far the largest producer of cocoa today), Ghana, Nigeria, and Cameroon. Other sources include Indonesia, Brazil, Malaysia, and Ecuador. These trees grow about 20 ft. in height and are susceptible to many diseases, as well as damage from the sun and wind. The average cacao tree produces 20 to 30 pods per year, each yielding only about 2 oz. of dried beans. The pods are cut open on the ground and the beans are immediately fermented, then dried in the sun.

Chocolate and cocoa are derived from cocoa or cacao beans. When the beans are fermented, roasted, and ground, the resulting product is called chocolate liquor, which contains a white or yellowish fat called cocoa butter. The most common chocolate products used in bakery are cocoa, bitter chocolate, sweet chocolate, milk chocolate, cocoa butter, and white chocolate. Pure chocolate and its blends can be used to top or coat bakery products, or become an integral part of the product formula, such as chocolate-chip cookies. Bitter chocolate has a lower cocoa butter content and high chocolate liquor content compared to all other varieties, giving it a more intense flavor. Baking chocolate does not include any sugar, but may be flavored with vanilla.

The color of chocolate cake depends on the color of the cocoa or chocolate used, as well as on certain other variables. The color of cocoa and chocolate is influenced by the variety of the cacao beans from which they are produced, including the extent of roasting of the beans, the addition of alkali, and oxidation. As would be anticipated, the deeper the roast, the darker the color of the resulting chocolate or cocoa. On the basis of processing, natural-processed cocoas and chocolates range between pH 5.1 and 6.2, but Dutch-processed products range between pH 6.0 and 7.8. Using cocoa powder in baked-product applications can also convey chocolate flavor. Cocoa powder is the powder remaining after most of the cocoa butter is removed from chocolate liquor, but it can have a wide range (0% to 24%) of cocoa butter content.

Oxidation of a cacao polyphenol to form a phlobaphene is another factor determining the color of cocoa and chocolate. The phlobaphene is responsible for the reddish color seen in cocoa and chocolate to varying degrees, depending on the extent of oxidation (42). The darker the chocolate, the higher the level of flavonols, such as procyanidins, catechins, and epicatechins. The presence of oxygen also influences the shelf-life of cocoa and chocolate, because of the potential for oxidative rancidity of the fat in these products. The major fatty acids in cocoa butter are stearic acid (35%), oleic acid (35%), palmitic acid (25%), and linoleic acid (3%). Cocoa powder ranks highest in antioxidant content, followed by dark chocolate and milk chocolate.

The pH of chocolate or cocoa-containing cakes differs as a result of the cocoa or chocolate, as well as the presence of leavening ingredients. For a desirable flavor, the pH of the batter should be no higher than 7.9. Chocolate-containing cakes range in color from a definite brown at a pH between 6.0 and 7.0, to mahogany between pH 7.0 and 7.5, with increasing redness above 7.5.

Chocolate coatings offer product designers a more economical and flexible option. Chocolate coatings contain added vegetable fat, either in the form of lauric hard butters (from oils such as coconut and palm seed), or non-lauric (domestic) hard butters (from sources such as soybean and cottonseed oils). The type of fat used will have a direct effect on flavor stability, tempering requirements, cocoa butter content, and the total amount of fat contained in the product. Rather than using chocolate, many coated baked products and nutritional bars opt for a compound coating, which is less expensive and sometimes easier to use. Compound coatings use hard vegetable or "tropical" fats in place of cocoa butter.

Chocolate and compound coating differ greatly in preparation for application to the final product. Chocolate contains cocoa butter, which is polymorphic in nature. Cocoa butter requires tempering to obtain the stable V (or beta) crystal form to maintain the proper gloss and snap of a good coating. To properly temper it, the chocolate mass must be cooled below its setting point, then re-warmed to a temperature higher than the melting point of the unstable beta-prime crystals, but lower than the melting point of the stable beta crystals. This is 31–32°C for milk chocolate (because of the milk protein), and 32–33°C for semi-sweet chocolate. The stable seed crystals are allowed to grow and mature, producing a more stable, compact structure that gives the best gloss and shelf life. Compound coatings, on the other hand, are simply cooled to 3–6°C above the melting point of the coating.

Title 21 of the US Code of Federal Regulations (CFR), part 163, states the industry has "specified requirements for specific standardized cacao products" (43). Standards are defined for: cacao nibs, chocolate liquor, breakfast cocoa, cocoa, low-fat cocoa, cocoa with diacetyl sodium sulfosuccinate for manufacturing, sweet chocolate, milk chocolate,

buttermilk chocolate, skim-milk chocolate, mixed dairy-product chocolates, sweet cocoa and vegetable-fat coating, sweet chocolate and vegetable-fat coating, and mild chocolate and vegetable-fat coating. These definitions include specific required ingredients, optional ingredients, and labeling requirements for certain exceptions.

Milk chocolate must contain at least 12% milk solids and not less than 10% chocolate liquor. As stated in the standard of identity, a sweet or dark chocolate must contain at least 15% chocolate liquor and less than 12% milk solids. German chocolate (not a standardized name), a sweet chocolate often used in baking, originated with an English immigrant, Samuel German, who marketed the product in Massachusetts. Bittersweet, or semi-sweet, chocolate is the darkest eating variety, and must contain at least 35% chocolate liquor. In January 2003, the FDA published the final rule (21 CFR, section 163.124) for white chocolate, which contains no chocolate solids other than cocoa butter. It must contain at least 20% cocoa butter, at least 14% total milk solids, and no more than 55% nutritive carbohydrate sweetener.

J. Salt, Spices, and Flavorings

1. Salt

Salt plays a very important role in baking. It is more than just a seasoning or flavor enhancer. It also has functions of (1) strengthening gluten structure and making it more stretchable, and (2) inhibiting the yeast growth for controlling fermentation in bread doughs. For these reasons, the quantity of salt in a bread formula must be very carefully controlled. If too much salt is used, fermentation and proofing are slowed down. If not enough salt is used, fermentation and proofing occur too rapidly.

2. Spices

Spices are plant or vegetable substances used to flavor foods. Plant parts used as spices include seeds, flower buds (such as cloves), roots (such as ginger), and bark (such as cinnamon). The most important spices in bakery products are allspice, anise, caraway, cardamom, cinnamon, cloves, ginger, mace, nutmeg, poppy seeds, sesame seeds, and zest of lemon and orange.

3. Flavorings

Flavorings in general may be divided into two categories: natural and artificial. Natural flavorings are usually more expensive but have a superior flavor. Because flavorings and spices are used in small quantities, it is not much more expensive to use the best quality.

Extracts are flavorful oils and other substances dissolved in alcohol. These include vanilla, lemon, and bitter almond. Emulsions are flavorful oils mixed with water with the aid of emulsifiers such as vegetable gums. Lemon and orange are the most frequently used emulsions. Their flavor is strong.

Vanilla is one of the most important overall flavors used in both bakery products and ice cream. It also serves and performs as a flavor enhancer, especially in premium and super-premium products, where it is commonly used in conjunction with cocoa or chocolate liquor in ice cream to provide a richer, fuller taste. Pure bourbon vanilla from Madagascar, if made properly, is considered the finest vanilla available. It projects a very clean flavor, and it won't over-flavor as artificial flavorings can.

II. PRODUCTION OF BREADS AND YEAST-LEAVENED BAKERY FOODS

Bread is the most important article of food. It has been used for thousands of years since Egyptians used yeast to produce risen loaves in 3000 BC. Many revolutions in bread-making processes, accompanied with the improvements of baking ingredients, have been employed in making and baking; and as a result, from the first flat cake has come the perfect loaf.

Although the minimal ingredients of bread formula are flour, water, yeast, and salt, many other ingredients and additives are usually included in a typical bread formula for desirable bread products (44). These include sugar, shortening, nonfat dried milk (or a substitute), malt, dough strengtheners, crumb softeners, mold inhibitors (e.g., sodium propionate), and oxidants. Table 148.20 shows the functions of ingredients and additives usually used in breads.

Wheat flour contains primarily gluten and starch, with minor components of pentosans (primarily anabinoxylans) and flour lipids, all of which are important contributors to the characteristics of the process and final product. After mixing, hydrated gluten is the continuous phase of wheat flour dough (46–47). During baking, gluten is denatured, and protein crosslinking occurs via formation of disulfide bonds. The resulting network, combined with partially gelatinized starch granules, is most certainly responsible for the semi-rigid structure of baked products (48–49).

Physical and mechanical mixing, chemical reactions (including enzyme-catalyzed reactions), and thermal effects (baking time and temperature), are factors that influence the nature and properties of breads. The ingredients and mixing effects on the dough rheological properties, which results in the changes of bread quality and storage stability, is a very complex process so that it is still not understood well at the molecular level.

A. Types of Yeast Products

Although all yeast doughs are made according to essentially the same basic principles, using yeast to produce a soft and light bread made from flour of wheat or other cereals, by

TABLE 148.20
Functions of Major Ingredients Used in Breads

Ingredient	Function	Descriptions
Flour	Structure	1. Protein (gliadin and gluten)+ water forms viscoelastic material (gluten) to retain gas, which is formed by sugar fermentation and contributes to structure of dough and bread. 2. Starch + water + heat forms a viscous paste that sets gel after baking. During storage the starch crystallizes (retrogrades) and contributes to firmness (major part of staling) of breads. 3. Protein content for bread flour:11–13% (on 14% moisture basis).
Water	Hydration	1. Hydrates protein to form gluten. 2. Hydrates flour non-starch cell wall polysaccharide (pentosans) and mill-damaged starch granules. 3. Solvent, dispersing agent, and medium for chemical and biochemical reactions. 4. Aids dough mobility.
Yeast	Leavening	1. Produces CO_2 and ethanol by fermentation of fermentable sugars. 2. Conditions dough biochemically. 3. Forms flavor precursors. 4. Rate of fermentation is controlled by temperature, nutrient supply, water level, pH, sugar concentration, salt, and level and type of yeast.
Salt	Flavor enhancer	1. Helps control fermentation. 2. Toughens dough by interaction with gluten. 3. Extends required dough development (delayed addition in dough mixing decreases mixing time by 10–20%).
Mineral yeast food	Controls fermentation	1. Water conditioners—calcium salts. 2. Yeast conditioners—ammonium salts. 3. Dough conditioners—oxidizing agents.
Sugar	Energy source for yeast	1. Fermentable carbohydrates. 2. Flavor—residual sugars, fermentation by-products, Maillard-type compounds during baking. 3. Crust color—results of caramelization (sugars+heat) and nonenzymatic browning (reducing sugar+amino group of proteins, amino acids, etc.).
Shortening	Lubrication	1. Ease of gas cell expansion in doughs. 2. Lubricates slicing blades during bread slicing. 3. Extends shelf life. 4. Tenderizes crust.
Dairy products	Nutrition and crust color enhancement	1. Protein (high in lysine) and calcium. 2. Flavor enhancement. 3. Crust color (browning reaction and caramelization). 4. Buffering effect in doughs and liquid ferments.

[1] From (45).

addition of water and salt, it is useful to divide yeast products based on their formulations into the following categories: lean dough products, rich dough products, rolled-in yeast dough products, and specialty dough products.

1. Lean Dough Products

A lean dough is one that is low in fat and sugar, and can be subdivided into three types based on the fat and sugar contents, or inclusion of other grains.

- Hard-crusted breads and rolls, including French and Italian breads, Kaiser rolls and other hard rolls, and pizza. These are the leanest of all bread products.
- Soft breads and rolls, including white and whole wheat breads and dinner rolls. These have a higher fat and sugar content, and sometimes also contain eggs and milk solids. Because they are slightly richer, they generally have soft crust.
- Bread made with other grains, including rye breads. Many varieties of rye breads are produced, with light or dark flours or with pumpernickel flour, and with various flavorings, especially molasses and caraway seeds.

2. Rich Dough Products

There is no exact dividing line between rich and lean doughs, but in general rich doughs are those that contain higher proportions of fat, sugar, and sometimes eggs.

- Non-sweet breads and rolls, including rich dinner rolls and brioches. These have a high fat

content, but their sugar content is low enough to allow them to be served as dinner breads.
- Sweet rolls or buns, including coffee cakes and many breakfast and tea rolls. These have high fat and sugar content and usually contain eggs. They generally have a sweet filling or topping.

3. Rolled-in Yeast Dough Products

Rolled-in doughs are those in which a fat is incorporated into the dough in many layers by using a rolling and folding procedure. The alternating layers of fat and dough give the baked product a flaky texture. Two major types of breads are non-sweet rolled-in dough (e.g., croissants), and sweet rolled-in doughs (e.g., Danish pastry).

4. Special Dough Products

There are also numerous yeast-leavened breads available in bakeshops, such as fried bread (e.g., bread doughnut), and steam bread. The differences between theses are the ways of heating.

B. Steps in Yeast Dough Production

There are nine basic steps in the production of yeast breads. These steps are generally applied to all yeast products, with some variations depending on the particular product.

(1) Scaling ingredients
(2) Mixing
(3) Fermentation
(4) Punching
(5) Makeup [dividing (scaling), rounding, benching (bench proofing, or intermediate proofing), sheeting, molding, and panning]
(6) Proofing
(7) Baking
(8) Cooling
(9) Packaging and storing.

First, all ingredients must be weighed accurately. Special care must be taken when measuring spices and other ingredients used in very small quantities. This is particularly important with salt, which affects the rate of fermentation. During the mixing of yeast doughs, the three main purposes are (1) to combine all ingredients into an uniform, smooth dough, (2) to distribute the yeast evenly throughout the dough, and (3) to develop the gluten.

Three principal mixing methods are used for yeast doughs: the straight dough method, the modified straight dough method, and the sponge-and-dough method (also called sponge method). The processes and comparison of these methods will be discussed later.

The first two purposes of mixing, combing the ingredients into dough, and distributing the yeast, are accomplished during the first part of mixing. The remaining time is necessary to develop the gluten. For gluten development, the flour proteins must first absorb water, then the gluten forms long, elastic strands after the dough is mixed or kneaded. Properly developed dough results in the desirable viscoelastic properties. As the dough is leavened, the gluten strands capture the gases in cells as a result of rising. Rich doughs are generally undermixed slightly, because a greater tenderness is desired for these products. Overmixing is a common error in bread-making. Gluten that is developed too long has stretched nearly as far as it can and loses its elasticity. It then tears instead of stretching, and molding is more difficult. Overmixed and undermixed doughs have poor texture and volume.

Fermentation is the process by which yeast acts on the sugars and starches in the dough to produce carbon dioxide (CO_2) and alcohol. Gluten becomes smoother and more elastic during fermentation, so that it can stretch farther and hold more gas. An under-fermented dough (young dough) will not develop proper volume and the texture will be coarse. A dough that ferments too long or at too high a temperature will become sticky, hard to work, and slightly sour. An over-fermented dough is called an old dough. Dough with weak gluten, such as rye doughs and rich doughs, are usually under-fermented, or "taken to the bench young." Doughs that are to be made into rolls and loaves requiring a great deal of makeup time should be slightly under-fermented to prevent the dough from being too old by the time makeup is completed.

Punching is the procedure of deflating the dough by pulling it up on all sides, folding it over the center, and pressing it down. The dough is then turned upside-down in the container. The purposes of punching are (1) to expel carbon dioxide, (2) to redistribute the yeast for further growth, (3) to relax the gluten, and (4) to equalize the temperature throughout the dough.

In general, the procedure of dough makeup includes dividing (scaling), rounding, intermediate proofing, sheeting, molding, and panning. During dividing or scaling, the dough is divided into pieces of the same weight, according to the product being made. Approximately 10–13% weight loss due to evaporation of moisture from the dough in the oven (i.e., baking loss) should be considered when scaling. Actual baking loss depends on baking time, size of the unit, and whether it is baked in a pan or free standing. Dividing and scaling should be done rapidly and efficiently to avoid over-fermenting the dough.

After dividing and scaling, the dough pieces are shaped into smooth, round balls by stretching the gluten on the outside of the dough. This procedure, called rounding, can be done by hand or machines. Rounding simplifies the later shaping of the dough and also helps retain gases produced by the yeast.

Rounded dough pieces are usually allowed to rest for 10 to 20 minutes to relax the gluten. The relaxed gluten

makes shaping the dough easier. This procedure calls for benching, benching proofing, or intermediate proofing. The dough is shaped into desired shapes (e.g., loaves or rolls) and then placed in pans or on baking sheets. The procedures of sheeting, molding, and panning can be done by hand in a small operation, or by machines in large commercial bakeries.

Proper makeup is of critical importance to the finished, baked product. All gas bubbles should be expelled during molding. Numerous and even distribution of gas cells' nuclei should be created for good texture of baked breads. For both pan breads and hearth breads, the seam must be centered on the bottom to avoid splitting during baking. For units baked in pans, the pan size must be matched to the weight of the dough. Too little or too much dough will result in a poorly shaped loaf.

Proofing (also called final proofing) is a continuation of the process of yeast fermentation, which increases the volume of the shaped dough. Proofing temperature is generally higher than fermentation temperature. Under-proofing results in poor volume and dense texture. Over-proofing results in coarse texture and some loss of flavor. French bread is generally given a long proof to create its characteristic open texture. Its strong gluten withstands the extra stretching of a long proof. Rich doughs are slightly underproofed, because their weaker gluten structure does not withstand too much stretching.

Many changes take place in the dough during baking. The most important changes are (1) oven spring—the rapid rising in the oven due to production and expansion of trapped gases as a result of the oven heat, (2) coagulation of proteins and gelatinization of starches – resulting in a firm and shaped product, and (3) formation and browning of the crust.

For baking breads properly, the oven temperature and baking time must be carefully controlled. Following are the general rules for the baking, but they should be adjusted depending on actual operation conditions.

- Large units are baked at a lower temperature and for a longer time than small rolls spaced apart.
- Rich doughs and sweet doughs are baked at a lower temperature because their fat, sugar, and milk content make them brown faster.

French breads made with no added sugar and long fermentation times require very high temperatures to achieve the desired crust color. For example, lean breads are baked at 205 to 220°C (400 to 425°F). Some French breads are baked at 220 to 245°C (425 to 475°F). Rich breads are baked at 175 to 205°C (350 to 400°F).

Many yeast products are brushed with a liquid, called a wash, just before baking. The most common washes are water, starch paste, and egg wash. Water wash is used primarily for hard-crusted products, such as French bread. Like steam in the oven, the water helps keep the crust from drying too quickly and thus becoming too thick. Starch paste is used primarily for rye breads. In addition to keeping the crust from drying too quickly, the starch paste helps give a shine to the crust. Egg wash is used to give a shiny brown crust to soft breads and rolls, and to rich doughs and Danish.

C. Types of Dough-Making Processes

1. Straight Dough Method

The straight dough method is the simplest mixing method, consisting of only one step. All ingredients are mixed in one operation, and then given a bulk fermentation time (that is, up until molding and proofing) of 1 to $2^1/_2$ hours. This is called short fermentation straight dough. For rich sweet doughs, the straight dough method is modified to ensure even distribution of the fat and sugar. The fat, sugar, salt, milk solids, and flavorings are mixed first until well combined. Then, the flour and yeast are added and mixed to a smooth dough.

A no-time straight dough is made with a large quantity of yeast, taken from the mixer at a higher temperature (up to 32°C 90°F), and given only a few minutes rest before being scaled and made up. The dough is generally transferred to the dough divider within 10 to 20 minutes after mixing. All further processing is the same as for other doughs. Since no-time doughs are not subjected to bulk fermentation, they do not require degassing prior to dividing. However, since the fermentation process does not condition no-time doughs, this must be done through the addition of extra maturing (oxidizing) agents, such as 60–120 ppm ascorbic acid. Although shortening the dough processing time by eliminating the bulk fermentation period is a significant advantage to the baker, the lack of fermentation has an adverse effect on the flavor and shelf-life of the baked product. This process is usually used only in emergencies.

Long-fermentation doughs are fermented for 5 or 6 hours or longer, sometimes overnight, at a temperature of 24°C (75°F) or lower (retarded fermentation). Retarding means slowing down the fermentation or proof of yeast doughs by refrigeration. This may be done in regular refrigerators or in special retarders that maintain a high humidity. The amount of yeast should be adjusted depending on the fermentation temperature and time for good control of fermentation. The advantage of this method is that the long, slow fermentation greatly enhances the flavor of the product.

The major disadvantage of the straight dough method is that the fermentation is hard to control because of fluctuations in temperature and other factors. Doughs often become over-fermented. Therefore, the straight dough method is usually used in small-scale productions.

TABLE 148.21
Advantages and Disadvantages of Sponge-and-Dough and Straight Dough Methods

Ingredients	Sponge-and-Dough Method	Straight Dough Method
Ingredients		
Yeast level	Less	More
Processing		
Processing time (fermentation time)	Longer	Shorter
Labor, power, and equipment cost	Higher	Lower
Fermentation losses	Increased	Reduced
Scheduling flexibility	Inflexibile	Flexible
Correction of overfermented dough	Yes	No
Products		
Flavor	Stronger	Weaker
Staling	Slower	Faster

2. Sponge-and-Dough Method

The sponge-and-dough method involves a two-stage mixing method. First a sponge is made of water, flour, and yeast and is allowed to ferment. Then, the remaining ingredients are mixed in to form the dough. The finished dough may be given a short fermentation, or if the sponge has had a long fermentation, it may be scaled immediately, like a no-time dough. Table 148.21 shows the advantages and disadvantages of sponge-and-dough and straight dough methods.

3. Chorleywood Bread Process

The Chorleywood bread process (CBP) was introduced in 1961 by British Baking Industries Research Association (now part of the Flour Milling and Baking Research Association). It was discovered that the amount of mechanical work expended on the dough during mixing and development had an important effect on bread quality. The amount of work that gave the best results was independent of mixer design, as long as the work took place rapidly. The best results are obtained when a work input of 11Wh (Watt hours)/kg (40 J/g) is applied during a mixing period of two to four minutes. The optimum level of work input, 11 Wh/kg, is five to eight times the amount of work expended during mixing in the straight dough method. The high speed mixing provides the optimal development of the dough (mechanical dough development). The CBP creates cells by trapping air in the dough during mixing and dividing that air into gas bubbles by the mechanical mixing, action. Molding the dough after fermentation does not create the bubble structure. Intensive mixing causes a dough temperature rise of 14–15°C and a water-chilling unit is needed to cool down the dough temperature to 28–30°C. The CBP needs more yeast (double that of the straight dough method) to give the same dough volume increase in the final proof as the straight dough method. More water is also needed when making a dough using this method because of better hydration of flour during intensive mixing.

4. Sourdoughs

Before commercially prepared yeast was widely available, bread making was often started by mixing flour and water and letting this mixture stand until wild airborne yeasts settled on it and began to ferment it. This 'starter' was then used to leaven bread. A portion of the starter was saved, mixed with more flour and water, and set aside to leaven the next batch of bread. This process is still used today, although the sours are generally started with commercial yeast.

The sour is actually a kind of sponge, except that it is allowed to ferment until it becomes strongly acidic. It may be used in two ways: (1) as a sponge in the production of the bread dough, except that a portion is saved for the next bread production, and (2) added to a straight dough as flavoring, i.e., light sour or rye sour.

Microorganisms in sourdoughs consist of two different types: bacteria and yeast. The end products of bacterial fermentation are primarily organic acids; and yeasts produce mainly alcohol and carbon dioxide. The flavor comes from either the various organic acids produced by bacteria, which may or may not react with each other, or by the alcohol from yeast fermentation which forms flavorful esters during the baking process. The most desirable organic acids are produced by heterofermentative lactic acid bacteria. The kind of acid produced depends on the type and the temperature of the material fermented. Generally, lower fermentation temperatures favor the formation of desirable organic acids, such as acetic, citric, lactic, fumaric, malic, and others. Higher fermentation temperatures seem to favor the formation of butyric and slightly longer chain fatty acids, which tend to result in undesirable flavors. The famous San Francisco sour- dough contained a specific lactic acid bacterium (*L. sanfrancisco*), and a unique yeast strain, which thrives under high acid (low pH) conditions and is unable to ferment maltose.

III. PRODUCTION OF SOFT WHEAT PRODUCTS

The number and types of bakery products made from soft wheat are larger compared to hard wheat. Flour milled from soft wheat normally has low protein content and is most suitable for making crackers, cakes, cookies, and pastries.

A. Crackers

In general, crackers contain little or no sugar and moderate levels of fat (10% to 20%) (50–51). Cracker dough, a

hard dough, generally contains low levels of water (20% to 30%), so baking proceeds quickly. Both yeast-fermented and chemically leavened varieties are common. Usually, oils are sprayed and salts are dusted on the surface of baked crackers to improve appearance and eating characteristics. There are three major types of crackers: saltine (fermented), snack cracker (chemically leavened), and savory crackers.

Saltine crackers are produced from fermented dough and the sponge is fermented up to 19 hours. During sponge fermentation, both yeast and beneficial bacteria grow, causing the consistency of the sponge to change drastically. After fermentation, the sponge is mixed with other dough ingredients, and sodium bicarbonate is added to bring the acidic dough back to pH 7.0. The dough is allowed to relax for 4–6 hours. After relaxation/resting, the dough is laminated by multiple pairs of heavy steel sheeting rolls. The purposes of laminating the sheeted dough (6–8 layers) are (1) to repair a dough sheet with holes or tears, (2) to turn the folded dough through 90°C for more uniform stress in two directions, (3) to accomplish a significant amount of work on the gluten development to obtain the characteristic of saltine crackers—a delicate and layered structure, and (4) to introduce other materials, like fat, between layers of dough to create a characteristic flaky structure after baking (50).

Chemically leavened crackers are not fermented but are leavened by chemical leaveners, and are also called snack crackers. They have a final pH of about 6.5. After mixing and a 2–4 hour rest period, the dough is sheeted to form a continuous ribbon that is laminated with a light application of dusting flour between the layers. The remaining processes are the same for both fermented and chemically leavened crackers. Before baking, the dough sheet is continuously cut into crackers and rolled with docking pins. The docking pins press the dough together to facilitate steam release from the crackers and help keep the layers from separating. The high-temperature, short-time bake puffs the thin dough sheets into crackers by vaporizing internal water. Salting is done prior to baking. If oil is sprayed or seasoning applied to the crackers, this occurs after the bake. Low moisture content in the finished product is important for preserving the crisp and brittle, but tender, cracker texture. Most crackers contain approximately 2% moisture.

Flavored or savory crackers are well accepted in the worldwide market. The appropriate flavoring agents are added directly to the dough or to the surface of the crackers after baking. The formulation and process is basically similar to that of soda crackers, with adjustments made to compensate for the fat and moisture content of the cheese for cheese crackers.

Table 148.22 shows the comparison of parameters or property changes as the recipe becomes enriched with fat and sugar for crackers and cookies.

B. Cookies

The word "cookie" means "small cake," and that's more or less what a cookie is. In fact, some cookies are made from cake batter. For some products, such as certain kinds of brownies, it's difficult to to classify them as cakes or cookies. Most cookie formulas, however, call for less liquid than cake formulas do. Cookie doughs range from soft to very stiff, unlike the thinner batters for cakes. This difference in moisture content means some differences in mixing methods, although the basic procedures are much like those for cakes.

Generally, cookies are produced using soft wheat flour that has relatively weak gluten strength (e.g., pastry flour, a blend of soft red and soft white wheat). The cookies usually consist of high fat and sugar contents, and a low moisture content. Mixing weak gluten flour in two or even three stages can control minimal gluten development, usually a desired property of cookie dough. The weak gluten and the relatively high quantities of fat and sugar in the dough allow plasticity and cohesiveness without the formation of a strong gluten network. Water level and sugar concentration play a role in restricting gelatinization and irreversible swelling of starch in the flour: although internal baking temperatures do not reach a level where this change could occur. Shortening in

TABLE 148.22
The Comparison of Parameters or Property Changes as the Recipe Becomes Enriched with Fat and Sugar for Crackers and Cookies

			Short		
Parameter/Properties	**Crackers**	**Semisweet**	**High Fat**	**High Sugar**	**Soft**
Moisture in dough	30%	22%	9%	15%	11%
Moisture in biscuit	1–2%	1–2%	2–3%	2–3%	3+%
Temperature of dough	30–38°C	40–42°C	20°C	21°C	21°C
Critical ingredient	Flour	Flour	Fat	Fat and sugar	Fat and sugar
Baking time	3 min	5.5 min	15–25 min	7 min	12+ min
Oven band type	Wire	Wire	Steel	Steel	Steel

cookies coats the flour; particles and renders them less available to water.

In general, cookies are formulated with high levels of sugar and vegetable shortening, or butter, and low levels of water. Sugar or other sweeteners determine dough and product properties. Sucrose contributes sweetness and flavor, as well as surface color via caramelization. Granulated sugar has several effects on finished cookies. As sucrose content increases, it acts as a hardening agent, creating a crisp, firm texture. Granulation affects creaming, spreading of the cookies during baking, and surface texture of the cookies. As cookies are baked, undissolved sugar melts and the dough spreads on the cookie's baking surface. Coarse forms of granulated sugar dissolve less readily than fine granulations; a coarser granulation of sugar results in less spreading of the dough and more surface cracking of the baked cookie. Surface cracking results from the recrystallization of the sugar at the surface of the cookie. Chemical leaveners, such as sodium bicarbonate and ammonium bicarbonate, are the most common agents used to leaven cookies. Carbon dioxide generated during heating enhances the leavening action of steam and entrapped air. Leavening cookies produces an increase in total volume of the cookie, and an alteration of the width of the cookie in relation to its height, which is referred to as spread ratio. Spread can also be influenced by flour chlorination, by adding emulsifiers to the dough, or by adding certain enzymes.

Besides the moisture content, granulation and ash content of flour are also important for cookie making. Moisture content of flour should be between 12% and 14% in order to provide proper water absorption. The amount of water used for cookie dough making affects how the gluten develops in the dough, cookie spread during baking, moisture retention, and eating quality of finished-products. High ash content flour contains high percentages of pentosans, which are the components of cell wall material and have high water absorbing qualities. The soluble pentosan content is usually higher in soft white wheat than in soft red wheat. Extra water will be added into the cookie dough for desirable consistency when high pentosan content flour is used.

Cookies come in an infinite variety of shapes, sizes, flavors, and textures. Flour, sugar, shortening, and chemical leaveners are the primary functional ingredients of cookies. Varying the types and quantities of these ingredients yields different dough rheology and finished eating qualities. Table 148.23 lists the factors that contribute to the characteristics of cookie doughs and the finished products. Cookies are crisp if they are very low in moisture; while, soft cookies have a high moisture content in the dough and baked products. Moisture is necessary for chewiness, but other factors are also important. In other words, all chewy cookies are soft, but not all soft cookies are chewy. Spread is desirable in some cookies, while others must hold their shape. Table 148.24 lists the factors affecting the dough spread.

1. Commercially-Made Cookies

Commercially made cookies are commonly classified into three categories: rotary-molded, wire-cut, and cutting machine cookies, based on the way the dough is placed on the baking band.

Rotary-molded cookies are produced by forcing a dough into molds on a rotating roll. As the roll completes a half turn, the dough is extracted from the cavity and positioned on the baking oven band. The doughs are often

TABLE 148.23
Factors that Contribute to the Crispness, Softness, and Chewiness of Cookies

	Crispness	Softness	Chewiness
Ingredients			
Proportion of liquid in the mix	Low—a stiff dough	High—a soft dough	High
Sugar	High	Low Hygroscopic sweetener (i.e., honey, molasses, or corn syrup) usually used.	High
Fat	High	Low	Low
Egg			High
Processing			
Gluten development	Minimum	Minimum	Strong flour or gluten developed during mixing
Dough piece	Small size or thin shape	Large size or thick shape to retain more moisture	
Baking	Long enough to evaporate most of the moisture	Underbaking	
Storage	Tightly packed to prevent moisture absorption	Tightly covered or wrapped to prevent drying	

TABLE 148.24
Factors Affecting the Dough Spread

Increase Spread	Reduce Spread
Flour in the Formulation	
Weak flour	Strong flour
Coarse flour particles	Fine flour particles
	High flour water-absorption value
Sugar in the Formulation	
Coarse granulated sugar	Fine sugar or confectioners' sugar
High sugar content	Low level of sugar
Leavening in the Formulation	
High dough pH	Low dough pH
More ammonium or sodium bicarbonate	Less ammonium or sodium bicarbonate
Fat in the Formulation	
Soft doughs due to higher temperature	Cold doughs
Aeration in the Formulation	
Minimum mixing prior to dough standing	Over-mixing of dough
Creaming together with fat and sugar until light	Blending fat and sugar just to a paste
Dough Age and Dough Piece Weight	
A slack batter with a high liquid content	A stiff dough with a low liquid content
Very fresh dough	Old dough
High dough piece weight	Low dough piece weight
Oven Conditions	
Heavily greased pans or oven band	Flouring of pans or oven band
Cold oven band at time of deposition of dough pieces	Warm or hot oven band at time of deposition of dough pieces
Low oven temperature in first zone	High oven temperature in first zone

high in sugar and shortening, but low in moisture [up to 15% (on flour basis)]. The typical dough is crumbly, lumpy, and stiff, with virtually no elasticity. During baking, dough spread and rise are minimized. For a rotary-molded cookie, such as a sandwich-cookie base, dough is forced into molds on a rotating roll. As the roll turns, the baking band extracts the formed dough piece from the molds. To maintain the molded design of the cookie, rotary doughs contain very low levels of shortening and sugar to prevent excessive spread. Oils do not function well in this case, because they lack shortening's ability to hold dough together during the molding process.

Wire-cut cookies are produced by extruding relatively soft dough through an orifice, and then cutting it to size with a reciprocating wire. The doughs are relatively high in fat, sugar, and water [up to 40% (on flour basis)] so that they spread during baking. The spread is desirable, but it must be closely controlled to have the desired geometry after baking. Wire-cut cookies have more sugar and shortening than rotary-molded cookies. Relatively high amounts of water and eggs are used, so that the dough will hold together during the cutting process. Higher water content means that excessive mixing or handling of the dough can lead to over-development of gluten and decreased tenderness. A tender product is produced by incorporating shortening with the flour into the dough or batter prior to adding water. Any fat retards the adsorption of formula water by the flour, and thereby limits gluten protein development. Examples of wire cookies are the chocolate chip type and fig bars.

Cutting machine dough consists of slightly less fat and sugar but more water [up to 25% (flour basis)] than rotary molded dough. The dough is sent through multiple sheeting rolls and made into a continuous sheet. This method for cookie production is slowly being discontinued.

2. Hand-Made Cookies

a. Dough mixing methods

The cookie mixing methods for hand made or homemade cookies are very much like cake mixing methods. The major difference is that less liquid is usually incorporated, so that mixing is somewhat easier. Less liquid means that gluten will become less developed by the mixing. Also it is a little easier to gel a smooth, uniform mix. There are three basic cookie mixing methods: one-stage, creaming, and sponge. These methods are subject to many variations, due to differences in formulas. The general procedures are as follows.

The one-stage method is the counterpart of the two-stage cake mixing method. There is more liquid in cake batters, so it must be added in two or more stages in order to blend uniformly. Low moisture cookies, on the other hand, can be mixed all in one stage. Because all the ingredients are mixed at once, the baker has less control over the mixing with this method than with other methods.

Therefore, the one-stage method is not frequently used. It can be used when over-mixing is not a great problem, as with some chewy cookies.

The creaming method is nearly identical to the creaming method for cakes. Because cookies require less liquid, it is not necessary to add the liquid alternately with the flour. It can be added all at once. The amount of creaming during the creaming stage affects the texture of the cookie, the leavening, and the spread. Only a small amount of creaming is desired when the cookie must retain its shape and not spread too much. Also, if the cookie is very short (high in fat and low in gluten development), or if it is thin and delicate, too much creaming will make the cookie too crumbly.

The sponge method is similar to the egg-foam methods, for cakes. The procedure varies considerably, depending on the ingredients. Batches should be kept small because the batter is delicate.

b. Types and makeup methods

Cookies can be classified by makeup methods, and grouped into pressed (or bagged), dropped, rolled, molded, icebox, bar, and sheet types.

Pressed or bagged cookies are made from soft doughs. The dough must be soft enough to be forced through a pastry bag, but stiff enough to hold its shape. Like pressed cookies, dropped cookies are made from soft dough. Actually, this method can be considered the same as the pressed method, and many bakers use the term "drop" for both bagging out cookies, and for depositing dough with a spoon or scoop. Usually, a pastry bag is faster, and it gives better control over the shape and size of the cookies. However, using a scoop to drop cookies may be preferred when the dough contains pieces of fruit, nuts, or chocolate that would clog the pastry tube.

Cookies rolled and cut from stiff doughs are not made as often in bakeshops and food service operations as they are in homes, because they require excessive labor. Also, there are always scraps left over after cutting. The advantage of this method is that it allows the making of cookies in a great variety of shapes for different occasions.

Refrigerated dough can be molded into the desired shape. For some traditional cookies, special molds are used to flatten the dough, and at the same time stamp a design onto the cookie. The pieces may also be shaped by hand into crescents, fingers, or other shapes.

The icebox, or refrigerator, method is ideal for operations that wish to have freshly baked cookies on hand at all times. The rolls of dough may be made up in advance and stored. Cookies can be easily cut and baked as needed. This method is also used to make multicolored cookies in various designs, such as checkerboard and pinwheel cookies. This procedure is called the bar method, because the dough is baked in long, narrow strips, which are then cut crosswise into bars. Sheet cookies vary so much that it is nearly impossible to give a single procedure for all of them. Some of them are almost like sheet cakes, only denser and richer; they may even be iced like sheet cakes. Others consist of two or three layers added and baked in separate stages.

C. Cakes

Cakes owe their popularity not only to their richness and sweetness, but also to their versatility. Cakes can be presented in many forms, from simple sheet cakes in cafeterias, to elaborately decorated works of art for weddings and other important occasions.

Producing cake requires as much precision as producing breads, but for completely opposite reasons. Breads are lean products that require strong gluten development and careful control of yeast action during the long fermentation and proofing periods. Cakes, on the other hand, are high in both fat and sugar. The most important consideration is to create a structure that supports these ingredients, and yet keep it as light and delicate as possible. Therefore, good and well-balanced formulas, and in understanding of the basic mixing methods are essential for making good cakes.

1. Types of Cakes

Many types of cakes are made by hand at home, or on a commercial scale, such as sponge goods, Swiss rolls, fruit cakes, cupcakes, etc. Cakes are often classified into three categories: batter type, foam type, and chiffon type, based on their formulations and mixing method. Table 148.25 shows the classification of cakes. Foam cake, featuring a large quantity of foam, results from a light, airy batter, and a baked cake with a somewhat coarse texture with moderately large cells. Chiffon cake is a combination of the batter and foam types, including oil and egg yolk as liquid ingredients, an egg white foam, baking powder, sugar, and cake flour. Cakes can also be classified according to the ratio of sugar and liquid to flour weight. For example, high-ratio cakes, one kind of shortened cakes, generally have a sugar to flour ratio of 1:1 or greater, with the ratio of total liquid (water + egg, or milk + egg) to flour is 1.1:1, or greater.

All egg-foam cakes, such as sponge cakes, angel food cakes, and chiffon cakes, are similar in that they contain little or no shortening and depend on the air trapped in beaten eggs for most or all of their leavening. Egg-foam cakes have a spring texture and are tougher than shortened cakes. This makes them valuable for many kinds of desserts that require much handling to assemble. For example, many European-style cakes or tortes are made by cutting sponge cake layers horizontally into thinner layers and stacking them with a variety of rich fillings, creams, icings, and fruits. In addition, sponge layers in this kind of cake are usually moistened with flavored sugar syrup(s) to compensate for their lack of moisture. Sponge sheets for jelly rolls and other rolled cakes are

TABLE 148.25
Types of Cakes

Types	Major Ingredients	Mixing Method	Examples
Batter type	Flour, sugar, egg, milk		
(High-fat cakes)	Usually containing high fat If fat<0.6 flour (w/w), need baking soda or baking powder for leavening		
High ratio type	Sugar ≧ flour	Creaming method Two-stage method Flour-batter method	Yellow layer cake White layer cake Devil cake Butter cake Pond cake Marble cake
Foam type	Egg, flour, sugar		
(Low-fat cake)	No solid fat		
Meringue type	Using egg white for leavening	Angel food method	Angel food cakes
Sponge type	Using whole egg or the mixture of yolk and whole egg for leavening	Sponge method	Sponge cakes
Chiffon type	The combination of batter type and foam type	Chiffon method	Chiffon cakes

often made without any shortening so that they do not crack when rolled. Flour for egg-foam cakes must be very weak, in order to avoid making the cake tougher than necessary. Corn starch is sometimes added to cake flour for these cakes to weaken the flour further.

2. Cake Formula Balancing

It is possible to change cake formulas, either to improve them or to reduce costs. However, ingredients and quantities can be changed only within certain limits. A cake formula in which the ingredients fall within these limits is said to be in balance. Knowing these limits helps you not only to modify recipes, but also to judge untested recipes and to correct faults.

For the purpose of balancing cake formulas, cake ingredients have been classified into four groups: toughenes, tenderizers, driers, and moisteners, according to their functionalities (Table 148.26). The idea of formula balancing is that toughenes should balance tenderizers, and driers should balance moisteners. In other words, if the amount of toughenes increases in a formula, it must be compensated for by increasing the amount of tenderizers.

Many ingredients have more than one function, sometimes even opposite functions. For example, egg yolk contains protein, which is a toughener, but also contains fat, which is a tenderizer. The general rules for balancing cake formulas are listed in Table 148.27. A common practice in balancing a formula is to decide upon the sugar-flour ratio, then to balance the rest of the ingredients against this ratio (Table 148.28).

TABLE 148.26
Four Groups of Cake Ingredients According to Their Functionalities

Group	Functionality	Ingredients
Toughenes	Provide structure	Flour Eggs (whites and yolks)
Tenderizers	Provide softness or shortening of protein network	Sugar Fats (including butter, shortening, and cocoa butter) Chemical leaveners
Moisteners	Provide moisture or water	Water Liquid milk Syrups Liquid sugars Eggs
Driers	Absorb moisture	Flours Starches Cocoa Milk solids

TABLE 148.27
General Rules for Balancing Cake Formula

Cake Type	Original Formula	Balancing Rules
Creaming method cakes	Made with butter or regular shortening	Sugar ≦ Flour Fat = eggs Eggs + liquids (milk and water) = Flour
High-ratio cakes	Using emulsified shortening	Sugar > flour (110 to 160%) Eggs > shortening Liquid (water + water in milk and eggs) > Sugar

TABLE 148.28
Guidelines for Balancing the Cake Formula

Change Made	Balanced by
Increasing liquid (water or milk)	Reducing eggs and shortening.
Increasing eggs	Increasing shortening.
Adding extra milk solids as an enrichment	Adding an equal weight of water.
Adding cocoa	Adding equal or 75–100% of cocoa (w/w).
Adding cocoa or bitter chocolate	Increasing sugar (up to 180% of the flour (w/w)) in high-ratio cakes. Increasing sugar (up to 100% of the flour (w/w)) in creaming-method cakes.
Baking very large units of cakes	Adding less liquid because less water will evaporate during baking.
Adding liquid sugar (honey, corn syrup, etc.)	Reducing other liquids slightly.
Using large quantities of moist ingredients, such as applesauce or mashed bananas	Reducing the liquid.
Using extra-large additions of moist ingredients	Increasing flour and eggs.
Using creamed batters	Using less baking powder than two-stage batters.

3. Methods of Mixing

Cakes are made on all scales, from the small craft baker to the large scale, highly automated cake bakery. The need to incorporate air into the batter means that most small-scale batch cake mixers have a beater or whisk, and the mixing tool follows a planetary motion during beating. For large-scale production, a continuous mixer (batch fed with slurries) will be used, with air being metered into the batter during mixing.

Beside the selection of high-quality ingredients, a thorough understanding of mixing procedures and precise operation is essential for making a good quality cake. There are many mixing methods available for cake making, but each of these methods is used for particular types of formulas, as indicated in Table 148.25. Three main goals of mixing cake batters are (1) to combine all ingredients into a smooth, uniform batter, (2) to form and incorporate air cells in the batter, and (3) to develop the proper texture in the finished product. These three goals are closely related.

Two of the major ingredients in cakes, fat and water (including the water in milk and eggs), are by nature unmixable. The first purpose of cake mixing is to form a water-in-fat emulsion; that is, the water is held in tiny droplets surrounded by fat and other ingredients. Curdling occurs when the fat can no longer hold the water in emulsion. The mixture then changes to a fat-in-water mixture. To avoid causing curdling during mixing, the following rules should be followed: (1) use high-ratio shortening with emulsifiers, (2) keep temperature of ingredients and batter about 21° (70°F) for stable emulsion formation, (3) mix the first stage of the procedure slowly to form a good cell structure to hold the water, and (4) add the liquids gradually or in stages (that is, a little a time). The secondary purpose of mixing is to create numerous air cells in cake batters, which is important for cake texture and for leavening. A fine, smooth texture is the result of small, uniform air cells. Large or irregular air cells result in a coarse texture. In addition, air trapped in a mix helps to leaven a cake when the heat of the oven causes the air to expand. When no chemical leavener is used, this trapped air, in addition to steam, provides nearly all the leavening. Even when baking powder or soda is used, these air cells provide places to hold the gases released by the chemical leavener.

Correct ingredient temperature and mixing speed are necessary for good air cell formation. Cold fat (below 16°/60°F) is too hard to form good air cells, and fat that is too warm (above 24°/75°F) is too soft. Mixing speed should be moderate (medium speed). If mixing is done on high speed, friction warms the ingredients too much. Not as many air cells are formed, and those that do form tend to be more coarse and irregular. In the cases of egg-foam cakes (sponge, angel food, chiffon), air cells are formed by whipping eggs and sugar. For the best foaming, the egg and sugar mixture should be slightly warm (about 38°/100°F). Whipping may be done at high speed at first, but the final stages of whipping should be at medium speed, in order to retain air cells. Table 148.29 shows the guidelines for making meringues.

Both the uniform mixing of ingredients and the formation of air cells are important to a cake's texture. Another factor of mixing that affects texture is gluten development. For the most part, little gluten development is desirable in cakes when cake flour, which is low in gluten, is used. Some sponge cake formulas call for corn starch to replace part of the flour, so that there is even less gluten (the high percentage of eggs in sponge cakes provides much of the structure). On the other hand, some pound cake and fruit cake formulas need more gluten than other cakes, in order to give extra structure and support to

TABLE 148.29
Guidelines for Making Meringues

1. Fats prevent whites from foaming properly.	*All equipment is free of any trace of fat or grease. *Egg whites have no trace of yolks in them.
2. Egg whites foam better if they are at room temperature than if they are cold.	*Remove them from the cooler an hour before whipping.
3. Do not overbeat.	*Overbeaten meringues look dry and curdled *Overbeaten meringues are difficult to fold into other ingredients and have lost much of their ability to leaven cakes.
4. Sugar makes egg white foams more stable.	*Egg whites can hold only a limited amount of sugar (no more than equal to the amount of egg whites) without sacrificing some volume.
5. Mild acids help foaming.	*A small amount of cream of tartar (15 g/kg) or lemon juice is sometimes added to egg whites for whipping, in order to give them more volume and stability.

the weight of the fruit. Thus, some cake formulas call for part cake flour and part bread flour.

The large number of ingredients in cakes makes it possible to make cakes by a tremendous number of methods, including creaming method, sponge method, and chiffon method.

The creaming method, also called the conventional method, was a traditional and standard method for mixing high-fat cakes. Although the development of emulsified, or high-ratio, shortenings has led to the development of simpler mixing methods for shortened cakes containing greater amounts of sugar and liquid, the creaming method is still used for many types of butter cakes. Fat and sugar are first creamed together to produce a light, fluffy foam. Creaming should stop before the appearance of the fat foam changes toward a frothy or runny consistency. Eggs are thoroughly beaten in the creamed fat-sugar foam. Ideally, the air bubbles are uniform, small, and surrounded by fat. Sifted dry ingredients are usually divided into thirds; the first third is stirred in the egg-fat-sugar mixture, then the first half of the liquid is added. The sifted dry ingredients are stirred in alternately with the liquid. The amount of beating that is needed during these alternating additions varies, depending on the ratio of ingredients in the formula. Rich batters that are high in fat and sugar requires more mixing than do leaner formulas.

In the creaming method, flour is added at or near the end of the mixing procedure, so that there is very little gluten development in properly mixed batters. If the batter is mixed too long after the flour is added, the cakes are likely to be tough. In the two-stage method, the flour is added in the first step. But it is mixed with high-ratio shortening, which spreads well and coats the particles of flour with fat. This coating action limits gluten development. It is important to mix the flour and fat thoroughly for the best results. Also, high ratio cakes contain a high percentage of sugar, which is also a tenderizer.

Modified conventional method is similar to the conventional method, except that the egg white is added as a foam at the end of mixing. The yolks, dry ingredients, and liquids are mixed as in the conventional method. The whites are beaten into a foam in which the peaks just bend over, and this foam is then folded gently and efficiently into the batter just prior to baking. The modified conventional method produces a cake very similar to that made by the conventional method, although the egg white foam has the potential to produce a cake of very slightly larger volume.

The two-stage method was developed for use with modern high-ratio shortenings. High-ratio cakes contain a large percentage of sugar, more than 100% based on the weight of the flour. Also, they are made with more liquid than creaming method cakes, and the batter pours freely. The mixing method is a little simpler than the creaming method, and it produces a smooth batter that bakes up into a fine-grained, moist cake. It is called two-stage, because the liquids are added in two stages. The first step is blending the flour and other dry ingredients with shortening. When this mixture is smooth, the liquids (including eggs) are added in stages. Throughout this procedure, it is important to follow two rules: (1) mix at low speed and observe correct mixing times to develop proper texture, and (2) stop the machine and scrape down the sides of the bowl frequently during mixing to develop a smooth, well-mixed batter.

Although there are many types of sponge cakes, they all have one characteristic in common. They start with egg foam that contains yolks. These are usually whole-egg foams, but in some cases the base foam is yolk foam, and egg white foam is folded in at the end of procedure. In its simplest form, sponge cake batter is made in two basic steps: (1) egg and sugar are whipped to a thick foam, and (2) sifted flour is folded in. Additional ingredients, such as butter or liquid, complicate the procedure slightly.

Angel food cakes are based on egg white foams and contain no fat. For success in beating egg whites, guidelines in Table 148.29 should be followed.

Chiffon cakes and angel food cakes are both based on egg-white foams, but there are some differences in the mixing methods. In angel food cakes, a dry flour-sugar mixture is folded into the egg whites. In chiffon cakes, a

batter containing flour, egg yolks, vegetable oil, and water is folded into the whites. Egg whites for chiffon cakes should be whipped until they are a little firmer than those for angel food cakes, but do not over-whip them until they are dry. Chiffon cakes contain baking powder, so they do not depend on the egg foam for all their leavening.

For the muffin method, the liquid ingredients (including oil or melted fat) are combined, the dry ingredients are sifted together, and then the two mixtures are stirred together until blended sufficiently to develop the necessary gluten, resulting in a cake with coarse texture and limited keeping qualities.

In the pastry-blend method, the flour and fat are creamed (first step); sugar, baking powder, salt, and half the liquid are then added (second step); and the last half of the liquid and the egg are then combined (third step).

The single-stage method is a rapid mixing method in which all of the ingredients, with the possible exception of part of the liquid and the egg, are placed in a bowl and mixed together vigorously to disperse all of the ingredients and develop the gluten. Then the egg and any remaining liquid are added, and the total batter is beaten. Shortenings containing mono- and di- glycerides are important when this mixing method is used, because these emulsifiers help to trap air in the mixture and promote a finer texture. Even then, the single-stage (sometimes called the quick-mix) method is very likely to produce a cake with a somewhat coarse texture and limited keeping qualities.

4. Baking

Cakes undergo remarkable changes during baking. Oven temperatures cause expansion of the air and carbon dioxide already in the batter and generate steam from the liquid. The heat also generates additional carbon dioxide production and expansion. Pressures from these leavening agents expand the cells and hold them stretched, while the proteins are denaturing and coagulating, and the starch is gelatinizing. In addition, some moisture is evaporated. An appropriate amount of pressure must be altered greatly under detrimental conditions. An appropriate amount of pressure must be maintained in the cells until the structure of cell walls is set by permanent changes in proteins (coagulation) and carbohydrates (gelatinization).

Oven temperature has an influence on the quality of cakes. At too low a temperature the volume is poor, because some of the cells collapse while others become quite large with rather thick walls. As the temperature increases, the volume improves and so does the texture. However, if the oven temperature is too high, the crust sets while the interior is still fluid. The pressure generated within the fluid mass presses against the crust, causing it either to begin to hump or to peak, depending on the temperature. A higher temperature can be used for a cake that is high in fat and sugar, because these ingredients elevate the temperature at which the proteins in the crust coagulate. This allows a little more time for the crust to remain somewhat flexible and accommodate the pressures produced within the batter.

Using the right pan for baking is also important for making a good cake. The depth of batter in baking pans influences final outcome. Shallow pans enable the heat to penetrate to the center of the batter more quickly, resulting in a cake of optimal volume. The surface is flatter and lighter in color than that which is produced when the batter is baked in a deeper pan. A cake baked in a deep pan also has a greater tendency to crack in the center than does one baked in a shallow pan. Regardless of the depth of the pan, sufficient batter should be used to almost fill the pan when baking is completed. This usually means filling the pans about half full with the batter. Even the material of which the baking pans are made influences the quality of cakes. Heat penetration is the best if the baking pan is dark and/or dull, so that the heat is absorbed efficiently to promote rapid heating of the batter (52). However, this rapid heating causes the sides of the cake to set quickly before the interior has had time to generate much of its potential gas. Humping and cracking, because of the lack of uniform heating within the batter, can be a problem despite the fact that the total volume is increased. Shiny pans absorb heat more slowly because they reflect the heat, so that there is time for the heat to spread into the interior of the cake before the sides of the cake are set. This leads to a cake that has a gently rounded or flat surface, rather than one that is humped, and browning is more delicate and uniform as a consequence of the slower rate of baking. However, volume is reduced a little, and the cells are a bit coarser and the walls a little thicker. Table 148.30 shows the pan preparation for cake baking.

TABLE 148.30
Pan Preparation for Cake Making

Cake Type	Preferred Pan Type	Pan Preparation
High-fat cakes	Layer pans	Grease with a commercial pan greasing. Dust the greased pans with flour and tap out the excess.
Sheet cakes	Sheet pans	Line the pans with greased parchment.
Angel food cakes Chiffon cakes	Tube pans	Do not grease the pan.
Sponge cake		Layer with little on no fat, grease the bottoms but not the sides of the pans.

D. Pastries

The term "pastry" comes from the word "paste," meaning, in this case, a mixture of flour, liquid, and fat. In the bakeshop, pastry refers both to various pastes or doughs, and to the many products made from these doughs. Pastry, whether it is in the form of pie crust or puff pastry, is a simple baked product that contains few ingredients, yet requires some skill for successful results.

Pies are pastries that consist of two distinct components: a relatively thin crust portion that serves to contain the second component, the filling (53). Piecrust is a very simple product in terms of its ingredients: flour, fat, salt, and water. The same ingredients are used for preparing the multilayered puff pastry. The difference between these two forms of pastry is the fat. The fat differs in kind, amount, and method of incorporation into the dough. The fat usually selected for making puff pastry is butter, and it is used in a flour-to-fat ratio (on a volume basis) of 2:1; that is, twice as much flour as fat, which produces a very rich dough. Pastry for pies usually is made with shortening, but sometimes oil, lard, or butter is selected. Although the ratio of flour to pure fat may vary from one pie dough recipe to another, a volume ratio commonly used is 3:1. Skilled workers can make a satisfactory pastry with the leaner ratio of 4:1.

A good piecrust is both flaky and tender. Yet success or failure depends on how the shortening and flour are mixed, and how the gluten is developed. Ideally, the ratio of ingredients, together with the method of preparation, prevents the formation of a gluten network and results in baked crusts that have a friable texture and flaky structure. That texture and structure is largely a function of the critical mixing step during which fat is incorporated as small, but discrete particles.

There are two basic types of pie dough: flaky pie dough, and mealy pie dough. The difference between the two depends on how the fat is blended with the flour. For flaky dough, the fat is cut or rubbed into flour until the shortening particles are about the size of peas. When water is added, the flour absorbs it and develops some gluten. When the dough is rolled out, the lumps of fat and moistened flour are flattened and become flakes of dough separated by layers of fat. For mealy dough, the fat is blended into the flour more thoroughly, until the mixture looks like coarse corn meal. Compared to the flaky pie dough, the more complete coating of the flour with fat has several results. The crust is very "short" and tender because less gluten can develop. Less water is needed in the mix, because the flour won't absorb as much as in flaky dough. The baked dough is less likely to absorb moisture from the filling and become soggy. Mealy dough is used for bottom crusts, especially in baked fruit pies and soft or custard-type pies, because it resists sogginess. Flaky doughs are used for top crusts and sometimes for prebaked shells.

Puff pastry is one of the most remarkable products of the bakeshop. Although it includes no added leavening agent, it can rise to eight times its original thickness when baked. Puff pastry is a rolled-in dough, like Danish and croissant doughs. This means that it is made up of many layers of fat sandwiched between layers of dough. Unlike Danish dough, however, puff pastry contains no yeast. Steam is created when the moisture in the dough is heated, and is responsible for the spectacular rising power of puff pastry. Puff pastry, or puff dough, is one of the most difficult bakery products to make. Because it consists of over 1000 layers, many more than in Danish dough, the rolling-in procedure requires a great deal of time and care.

Butter is the preferred fat for rolling in because of its flavor and melt-in-the-mouth quality. Special puff pastry shortening is also available. This shortening is easier to work, because it is not as hard when refrigerated, and because it doesn't soften and melt at warm temperatures as easily as butter does. It is also less expensive than butter. However, it can be unpleasant to eat, because it tends to congeal and coat the inside of the mouth. The quantity of roll-in fat varies from 50 to 100% of the weight of the flour. If the lower quantity of fat is used, the dough should be left slightly thicker when rolled out. Puff pastry that is low in fat will not rise as high and may rise unevenly. This is because there is less fat between the dough layers, so that the layers are more likely to stick together.

IV. SHELF-LIFE OF BAKERY PRODUCTS

Bread begins to undergo deteriorative changes commencing with removal from the oven. One of the first changes is thought to be retrogradation (increasing crystallinity caused by crosslinkage of starch molecules) of amylose released from starch granules during gelatinization in the oven. The rather crystalline and firm character of the crumb that develops as bread is stored for a day or more may be the result of retrogradation within the starch granules, and probably involves changes in the physical configuration of amylopectin (perhaps some folding of the branches). This retrogradation is reversible upon reheating of the bread, but recurs when the warmed bread cools once again.

Moisture levels also are involved in the staling process. There is a reversal in the location of water; some of the water that migrated to starch from gluten during baking returns to the gluten proteins. Water also migrates from the center of the loaf toward the crust, causing the crust to gradually increase in leatheriness, while remaining rather soft. This migration occurs even when evaporation from the crust is prevented.

Storage temperature has an effect on staling. Refrigerator storage accelerates firming of the crumb compared with storage at a warm room temperature—the firmness after a day at 8°C being about the same as that after 6 days at 30°C. The advantage of refrigerator storage is only the inhibition of mold growth in breads made without preservatives. Freezer storage is effective in inhibiting the firming of the crumb.

A. The Mechanism of Staling

Bread staling has been a problem for thousands of years, and it is responsible for huge economic losses to both the baking industry and the consumer. In a market of approximately 20 billion pounds of bread produced annually, about 3% (600 million pounds) of them were returned as unsalable because of staling (54). Staling was defined as a term that indicates decreasing consumer acceptance of bakery products caused by changes in crumb, other than those resulting from the action of spoilage organisms (55). The most widely used indicator of staling is measurement of the increase in crumb, firmness, which is the attribute most commonly recognized by consumers. However, bread staling is a very complex process. Neither the bread system nor the staling process is understood well at the molecular level (56–59). To date, water migration and transitions in the starch fractions, and changing starch from an amorphous structure to a partially crystalline one (56) are thought to be the two most important factors controlling the changes in texture of breads during storage.

During baking of bread dough, the starch granules are generally gelatinized, but little else other than restricted swelling followed by collapse happens to them because of the limited amount of water present in the dough system (57). Thus, deformed wheat starch granules can be isolated from the crumb (58). The small amount of amylose that leaches from granules during baking in the limited moisture system of bread dough retrogrades upon cooling and rapidly becomes unextractable (60–61). Even if amylose does leach from granules, by the time bread has completely cooled, any interstitial amylose will have retrograded and is unlikely, therefore, to play a major role in subsequent staling events. Therefore, freshly baked and cooled bread is an elastic system containing swollen wheat starch granules that are still largely intact, but may be deformed.

Bread staling falls into two categories: crust slating and crumb staling (62). Crust staling is generally caused by moisture transfer from the crumb to the crust (63), resulting in a soft, leathery texture, and is generally less objectionable than is crumb staling (64). Crumb staling is more complex, more important, and less understood. The firmness of bread varies with position within a loaf, with maximum firmness occurring in the central portion of the crumb (65).

The major theories on the staling mechanism are reviewed in cursory way in Table 148.31. It summarizes the factors affecting bread staling during storage: (1) starch retrogradation, especially amylopectin retrogradation, which plays an important role, but which alone is not responsible for bread staling, (2) gluten proteins and gluten-starch interaction also play an important role, and (3) moisture migration is also involved in staling. Overall, it is probable that several factors play a role in the bread firming process, but the large volume of data that implicates amylopectin retrogradation as a key factor, and the information that gluten is also involved cannot be ignored. Table 148.32 shows the hypotheses conurning the effects flour components on the staling mechanism.

Bread staling is unquestionably a complex process. In addition to the effects of flour components, formulation, and the processing conditions (103), many other factors affect staling rate of bread during storage: storage temperature,

TABLE 148.31
Factors Affecting Staling Rate of Bread During Storage

Factors	Findings	References
Storage temperature	The rate of staling has a negative temperature coefficient.	(66)
	Bread staling is correlated with starch recrystallization at storage temperature with the maximal rate at 4°C	(67)
Moisture migration	Drying out of the bread does not explain staling, but may accelerate reactions leading to staling.	(68)
Moisture redistribution between crumb and crust	Moisture gradient forms in the loaf as baked bread begins to cool, and moisture migrates from crumb to crust.	(69–70)
Moisture redistribution among components	Transfer of moisture from starch to gluten during aging.	(71–72)
	Transfer of moisture from gluten to starch as the starch crystallizes. The decrease in water mobility in bread upon staling is due to incorporation of water molecules released from gluten into crystalline structure of starch that develops upon staling.	(72–79)
	Gluten undergoes a 1st-order transformation resulting in the release of water from gluten and absorption of this water by retrograding starch.	(80–82)
	Amylopectin crystallization in bread requires both microscopic and macroscopic redistribution of water, so that there is sufficient moisture present at the locus where crystallization take place to plasticize polymer chains, so that they are mobile enough for crystallization to occur and for incorporation into β-type crystal lattices (36 water molecules in the unit cell, compared with α-type which has only 8 water molecules in the unit cell).	(79, 83–84)

TABLE 148.32
Hypotheses Concerning the Effects of Flour Components on the Staling Mechanism

Component	Hypotheses	References
Starch	Gradual change in the starch components from amorphous to crystalline forms is important to the staling process.	(71, 85)
	Degree of crystallinity of bread crumb was inversely related to its firmness, so that starch crystallization and bread firming were separate processes.	(86)
Amylopectin	Amylopectin was the fraction of starch responsible for staling, because heating stale bread above 50°C can restore the loaf to its original freshness.	(87–91)
	Adding 5% retrograded waxy corn starch to bread formula decreased gelatinization and reduced the firming rate.	(92)
	Retrogradation of amylopecitn cannot be the only factor affecting firming.	(93)
Amylose	Amylose fraction was involved in staling of bread through 1 day only, and the role of amylose in bread staling may be merely one of diluting amylopectin.	(94)
	Reoroganization of intragranular amylose enhances the rigidity of starch granules during staling.	(95)
	Amylopectin retrogradation is part of the staling process, but is not solely responsible for the observed changes in texture.	(96)
	Amylose-amylopectin aggregation was responsible for the changes that occur during aging of bread crumb.	(97)
Protein	Starch-gluten interactions are somehow involved in the firming process.	(98–99) (100)
	The differences in molecular mobility in an aged bread are attributed to the role of gluten and/or redistribution of water in the amorphous regions of the bread.	
Pentosans	Water-soluble and –insoluble pentosans retard staling or have no effect on the staling rate.	(60–61)
	Arabinoxylan-fortified breads exhibited a greater rate of starch retrogradation, because of their higher moisture content, while having softer crumbs than did controls.	(101–102)
Lipid	Flour lipids have an effect on the antifirming action.	(102)

moisture migration, moisture redistribution between crumb and crust, and moisture redistribution among components (Table 148.31).

B. Antistaling Additives

Although the mechanism of bread staling is not well understood, baking industries throughout the world have tried to inhibit staling through the addition of ingredients such as enzymes, lipids, emulsifiers, sweeteners, and other chemicals. The addition of sweeteners to provide fermentable solids, influence crust color, improve crumb texture and softness, and increase shelf-life is well documented. For over twenty years, fructose/glucose syrups and corn syrups have shown the ability to retard staling through their ability to bind moisture. α-amylases from malted cereal, fungi, and bacteria, are the most commonly used enzymes for the retardation of bread staling. Numerous research projects have been conducted to understand its mechanisms (Table 148.33). Although the mechanism of the antistaling effect of α-amylases has been debated, it appears that starch hydrolysis products are involved in inhibition of staling. But the hydrolysis products must be of a unique type; perhaps either maltotriose and maltotetraose, or products larger than those present in traditional maltodextrin preparations.

In addition to α-amylase, lipases (specifically 1,3-specific lipases), lipoxygenases (111–112), nonstarch polysaccharide-modifying enzymes (such as endoxylanase, β-xylosidase, and α-L-arabinosidase) (113), and protease are also reported to have a crumb softening effect when enzymes are added. The major purpose of adding proteases to breads is to improve flavor profiles, flow characteristics, machining properties, gas retention, and mixing time (114–115). However, given evidence that protein has a significant role in the bread staling mechanism (98,105,116), it is likely that modification of the gluten network structure via enzyme-catalyzed proteolysis would have an effect on bread staling. It is also possible that liberation of water molecules concurrent with protein hydrolysis could enhance amylase activity (117).

Emulsifiers of various types are widely employed in the baking industry as dough strengtheners and/or crumb softeners (103). Table 148.34 lists that the emulsifiers can be

TABLE 148.33
Effects of α-amylase on Bread Staling

Mechanism	References
α-amylases produce a partially degraded amylopectin that is less prone to crystallize, and its ability to produce partially degraded amylose is responsible for rapid formation of a partially crystalline polymer network that resists later rearrangements.	(95, 104–105)
α-amylases produce low molecular-weight dextrins (DP 19–24) that interfered with the retrogradation of starch.	(17, 63, 106–107)
α-amylases produce maltooligosaccharides (DP 3–11) that disturb water structure and promote ordering and aggregation of starch molecules.	(108–109)
Maltotriose and maltotetraose were directly responsible for retarding retrogradation in bread, suggesting that these oligomers were of the right size to interfere with starch-gluten interactions.	(110)
Maltotriose and maltotetraose might hold water around starch molecules and inhibit starch-starch interactions.	
Maltooligosaccharides (DP 3–8) produced by α-amylases are not themselves responsible for antistaling, but their presence is simply correlated with a key modification of starch granules that is related to reduced staling, possibly by reducing gluten-starch interactions.	(99)

TABLE 148.34
Commonly Used Emulsifiers in Bakery[1]

	Functionality		
Emulsifier	Dough Strengthener	Dough Softener	Aeration Aid
Lecithin (LC) or Hydroxlyated lecithin (HLC)	×		
Mono- and diglyceride (MDG)		×	×
Acetylated MG (AMG)			×
Glycerol-lacto ester of fatty acid (LMG)			×
Diacetyl tartaric acid esters of MG and DG (DATEM)	×	×	
Succinylated MG (SMG)	×	×	
Ethoxylated MG/DG (EMG)	×		×
Stearoyl monoglyceride citrate (SMGC)	×		
Propylene glycol mono- and diester (PGMS)			×
Polyglycerol ester (PGE)	×		×
Sucrose ester (SUE)	×		
Sorbitan monostearate (SMS)			×
Polyoxyethylene sorbitan monostearate (Polysorbate 60, PS 60)	×		×
Polysorbate 65 (PS 65)			×
Lactylic ester of fatty acid (SLA)	×	×	×
Ca-stearoyl lactate (CSL)	×	×	
Na-stearoyl lactate (SSL)	×	×	×
Sodium stearoyl fumarate (SSF)	×	×	

[1] From (120).

used in bakery products based on their functionality. Monoglycerides (MG) are the most commonly used in baking to delay staling in bakery products. The mechanism of the antistaling effect of MG is still unknown, but it is concluded that reduction in crumb firmness is probably the result of the interactions of MG with amylose. The physical state of MG is an important factor in the amylose-MG complexation ability, which decreases in the descending order of MG physical states: α-type crystalline gel > β-type crystalline hydrate > nonhydrated powder (118). Diacetyl tartaric acid esters of monoglycerides (DATEM) were reported to be as effective as antistaling agents in bread as sodium stearoyl lactylate (SSL) or ethoxylated monoacylglycerols (EMG) over 5 days of storage (119).

In addition to enzymes and emulsifiers, ingredients including shortening, hydrocolloids (gums), and damaged or modified starches are also reported as having the ability to retard the staling in bakery products (62, 121).

The uses of hydrocolloids and modified starches are attributed to their great water holding ability, which inhibits the movement of moisture this retards staling because moisture redistribution is a requirement for staling to occur (122).

ACKNOWLEDGMENT

The information in this chapter has been derived from "Bakery products," In: *Bakery Products Manual*, copyrighted ©2004 by H. M. Lai and T. C. Lin. Used with permission.

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149 Chocolate

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CONTENTS

I. HISTORY

Cacao trees (*Theobroma cacao*) originated in the Americas and have been cultivated for at least an estimated 4,000 years where they thrive under conditions of semishade, warmth, and high humidity. Chocolate was important in Mayan and Aztec mythology and used as currency. A crude, unsweetened foamy beverage called chocolatl was served only to the noble ruling class. Early Spanish explorers found this bitter liquid could be improved with the addition of sugar, and it soon became the rage of Spanish nobility. It then spread to Italy, Holland, France, and England.

C.J. Van Houten of Holland patented a cocoa press that was able to remove cocoa butter from the chocolate liquor to produce cocoa powder. This was in response to the difficulty people experienced in digesting the full fat chocolate beverage and dispersing the chocolate in water. Mr. Van Houten is also credited with alkalizing (Dutch process) cocoa, which gives a darker color and a less acid flavor. The pressing of chocolate liquor resulted in an "excess" of cocoa butter. It was found that a superior product could be made when warm water was replaced with cocoa butter. Fry and Son in 1847, and Cadbury in 1849, were among the first to sell solid eating chocolate. The basic formula of chocolate liquor, sugar, and cocoa butter continued until 1875, when Daniel Peter developed a method of adding condensed milk to chocolate liquor and sugar. Moisture was removed from the chocolate "milk crumb" and replaced with cocoa butter to maintain a smooth mouth feel. Thus milk chocolate was born.

II. CULTIVATION, HARVESTING, AND CURING

The commercial cultivation of cacao trees is generally within a 20° latitude of the equator with a rainfall of 45–100 inches (114–254 cm), and a temperature of 70–90°F (21–32°C). Major producing areas include Africa (Ghana, Nigeria, Ivory Coast); South America (Brazil, Ecuador, Venezuela); West Indies (Dominican Republic); Asia and Oceania (New Guinea, Malaysia, Indonesia).

The cacao tree will usually attain its full height in about ten years. Though trees may grow to be 40 feet (1200 cm), they are usually pruned to 15 to 25 feet (450–750 cm) to facilitate harvesting. Flower clusters appear only on the trunk and main branches. Approximately five to six months later, 20 to 30 fruit pods develop. The growth cycle is continuous, so a tree may bear leaves, blossoms, and pods simultaneously. However, the main fruiting seasons occur between October and February.

The ripe pods are elliptical in form, seven to ten inches (18–25 cm) in length, and three to five inches (8–13 cm) in diameter. The pods contain approximately 20–50 seeds surrounded by a mucilaginous pulp. Each dried cocoa bean weighs approximately 1 gram. When dried, the beans from each pod weigh between 1.5–2 ounces (42–56 g). While yields can vary greatly, the average tree produces 20–30 pods; so each tree's output is between two to three pounds (0.9–1.4 kg) of commercial cocoa beans.

The region where the cocoa beans are grown and the variety of the bean can greatly affect characteristics for flavor, color, hardness of butter, etc. There are three major types of cocoa beans: Forastero, which accounts for the majority of the beans; Criollo, the flavor beans; and Trinitario, which are basically a cross between Criollo and Forastero. The bulk beans are generally dark brown in color with a strong, bitter flavor. Flavor beans are lighter in color with a mild, nutty flavor. The beans are blended to produce the desired end product.

Mature cacao pods turn yellow, orange, or purple in color. The pods are split open and the beans and pulp scooped out and heaped into boxes or baskets for fermentation (anaerobic and aerobic). The beans are allowed to ferment for 3 to 6 days, depending upon the type of beans used, batch size, temperature, and aeration. Aeration (turning) of the beans promotes bacterial activity and ensures uniform fermentation. Fermentation and drying processes have major influences on the quality of the beans used in making chocolate.

The beans are subsequently dried (naturally or mechanically) to a moisture content of less than 8 percent to prevent mold growth, with the optimum being 6 to 6.5 percent.

Cocoa beans arriving in the Untied States are usually inspected and fumigated at the ports when received. Beans are inspected for mold, infestation, filth, degree of fermentation, and bean size. To maintain quality, they should be stored in cool, dry, and well ventilated warehouses.

A cleaning step is required to produce a wholesome product with minimal microbiological risks, as well as to remove extraneous materials. On a dry basis, the beans are approximately 87.1 percent nib (cotyledons), 12.0 percent shell, and 0.9 percent germ. The nib contains about 55 percent cocoa butter fat (1).

III. PROCESSING OF COCOA BEANS

Roasting serves to develop the flavor and aroma of the beans. There are several roasting methods, which include whole bean, nib, and chocolate liquor roasting. Roasting can take anywhere from 15 minutes to 2 hours. In whichever roasting method is used, the nibs are separated from the shell (winnowing). Depending upon the degree of roast, nib moisture is about 1.5 to 3 percent. Federal standards allow no more than 1.75 percent shell in the nib portion used for chocolate production. Cocoa bean shells have little commercial value. The germ portion is also removed, since it is hard and gritty.

After roasting, the nibs are ground to a liquid state, which is called chocolate liquor. The heat and friction from the grinding process will rupture the cell walls of the nibs to release the valuable cocoa butter. This liquor (containing approximately 55 percent cocoa butter) will solidify upon cooling.

In the initial stages, the processing for cocoa and chocolate are essentially the same. The beans are cleaned, roasted, and shelled. The manufacturing of cocoa and chocolate though, involve two separate processes. See Figure 149.1.

IV. MANUFACTURING OF COCOA POWDER

Cocoa cake and cocoa butter are produced from chocolate liquor by hydraulic pressing. The hot liquor is subjected to more than 6,000 PSI. The cocoa butter flows out, leaving the cocoa cake. This cocoa butter contains small amounts of cocoa powder, which are filtered out to maintain consistent color and flavor. The butter may be further refined, bleached, and deodorized (via steam distillation) to yield a very bland, clean tasting cocoa butter.

The cake is then ground to the desired degree of fineness — usually 95 to 99 percent will pass through a 200-mesh screen.

The cocoa cake will generally have a fat content ranging from 10 to 24 percent. Powders with less than 10 to12 percent cocoa butter are known as low fat cocoas. Medium fat cocoa contains 10 to 18 percent fat, while high fat or "breakfast cocoa" has 22 to 24 percent fat. Approximately 95 percent of the cocoa powders on the market today are

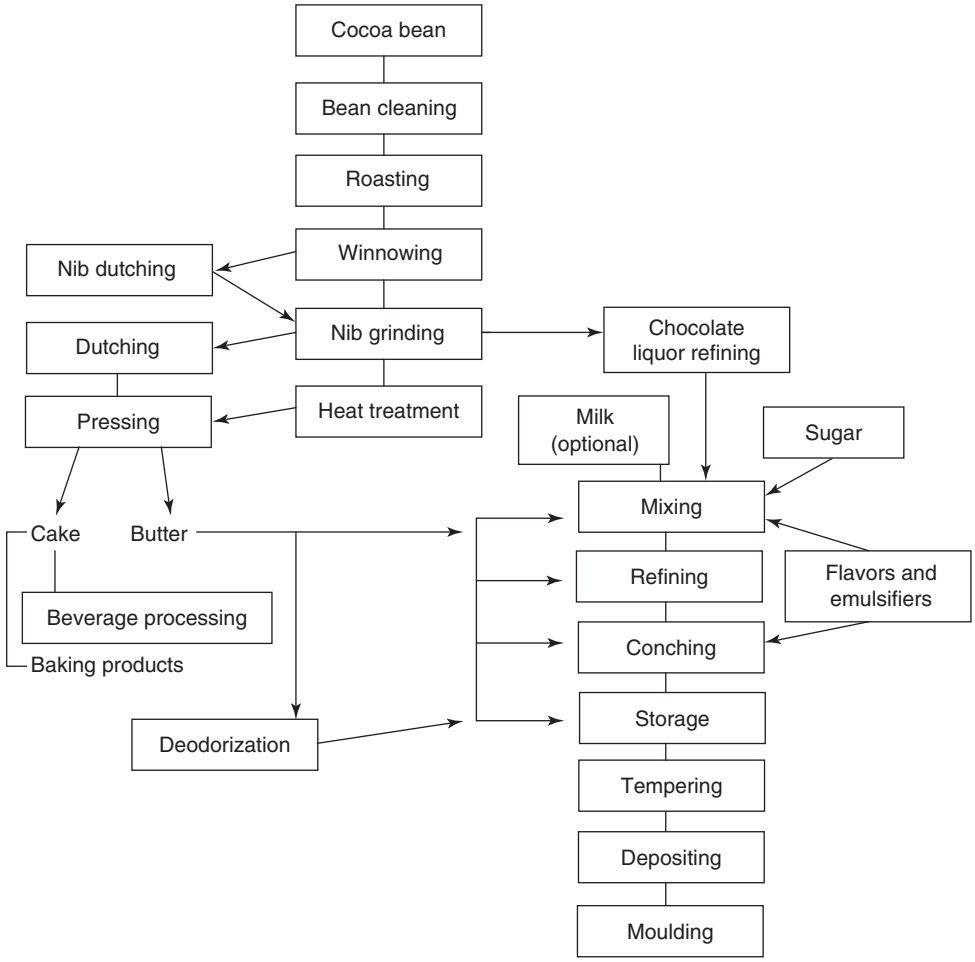

FIGURE 149.1 Typical chocolate production flow chart.

the medium fat variety. The FDA has established standards for various types of cocoas. See Table 149.1.

Cocoa powder not treated with alkali is often referred to as natural cocoa. The addition of alkali was first used by Van Houten in 1828; hence, it is also known as "Dutch" process cocoa.

The alkalization process raises the pH of the cocoa powder. The pH for fermented beans ranges between 5.2 and 5.6. Alkalized cocoas have a pH between 6.8 and 7.5. Black cocoas, used for color, may have a pH as high as 8.5 (3). FDA standards allow a maximum of 3.0 percent potassium carbonate per 100 pounds (45 kg) of nib weight equivalent. The purpose of alkalization is to neutralize the acidity of the cocoa, resulting in a milder, less astringent flavor and a range of colors (from light brown to red, and even black).

Various formulas, equipment, and processes are used to alkalize the nibs, liquor, or powder. This allows for a wide range of cocoa powders to be produced. As such, alkalized cocoa powders are in greater demand than natural cocoa powder. See Table 149.2.

V. MANUFACTURING OF CHOCOLATE

Dark, milk, and white chocolate involve certain basic operations: ingredient mixing, refining, conching, standardization of viscosity, and tempering. Like cocoa powders, the chocolate liquor (white chocolate uses cocoa butter only) may undergo alkalization prior to further processing. The FDA has issued standards of identity for milk, dark, and white chocolate. See Table 149.1.

A. Ingredient Mixing

The basic ingredients, including chocolate liquor, sugar, cocoa butter, milk products (for milk chocolate), emulsifiers, and flavors are blended together. The result is a paste with a rough texture and plastic consistency.

B. Refining

Refining serves to reduce the particle size of the mass, and thus increase the surface area, resulting in a smooth texture.

TABLE 149.1
U.S. Standard of Identity for Cacao Products Code of Federal Regulations (CFR), Title 21- Food and Drugs

21 CFR Reference	Product	Chocolate Liquor (%)	Milk Solids (%)	Comments
163.111	Chocolate Liquor	100	0	50–60% fat Optional ingredients 1. Other names: Unsweetened chocolate, bitter chocolate, baking chocolate, cooking chocolate.
163.112	Breakfast Cocoa		0	22% fat (min) Optional ingredients 1 with the exception of butter or milkfat.
163.113	Cocoa		0	10–22% fat Optional ingredients same as 163.112. Other name: Medium fat cocoa.
163.114	Lowfat Cocoa		0	Less than 10% fat Optional ingredients same as 163.112
163.123	Semisweet/Bittersweet Chocolate	35 (min)	12 (max)	Sweetened chocolate liquor, which may contain one or more specified optional ingredients 2. Traditional bittersweets 50% or more liquor.
163.123	Sweet Chocolate	15–35	12 (max)	Sweetened chocolate liquor, which may contain one or more specified optional ingredients 2.
163.124	White Chocolate	0	14 (min)	20% cacao fat (min) 3.5% milkfat (min) 55% nutritive carbohydrate sweetener (max)
163.130	Milk Chocolate	10 (min)	12 (min)	Sweetened chocolate liquor with dairy ingredient(s), which may contain one or more specified optional ingredients 2.
163.150	Sweet Cocoa and Vegetable Fat Coating		No limit	Same as 163.123, except: mixture of cocoa and chocolate liquor contains a minimum of 6.8% nonfat cacao solids; contains one or more optional ingredients 3.
163.153	Sweet Chocolate and Vegetable Fat Coating		12 (max)	Same as 163.123, except that it contains one or more optional ingredients 3 (except for cocoa).
163.155	Milk Chocolate and Vegetable Fat Coating		12 (min)	Same as 163.130, except that it contains one or more optional ingredients 3 (except for cocoa).

Optional Ingredients 1

- Optional alkalizing ingredients: ammonium, potassium, sodium bicarbonate, carbonate, or hydroxide, or magnesium carbonate or oxide.
- Optional neutralizing ingredients: phosphoric acid, citric acid, and L-tartaric acid.
- Spices, natural and artificial flavorings, ground or whole nut meats, ground coffee, dried malted cereal extract, and other seasonings that do not either singly or in combination impart a flavor that imitates the flavor of chocolate, milk, or butter.
- Other optional ingredients: Cocoa butter and cocoas, dairy butter or milkfat, salt.

When optional ingredients are added, it must be declared on the label.

Optional Ingredients 2

- Optional alkalizing ingredients: ammonium, potassium, sodium bicarbonate, carbonate, or hydroxide, or magnesium carbonate or oxide.
- Optional neutralizing ingredients: phosphoric acid, citric acid, and L-tartaric acid.
- Optional ingredients: cocoa butter, nutritive carbohydrate sweeteners.
- Spices, natural and artificial flavorings, ground or whole nut meats, ground coffee, dried malted cereal extract, salt, and other seasonings that do not either singly or in combination impart a flavor that imitates the flavor of chocolate, milk, or butter.
- Optional dairy ingredients: cream, milkfat, butter, milk (concentrated, evaporated, sweetened condensed, dried), skim milk (concentrated, evaporated, sweetened condensed, nonfat), concentrated or dried buttermilk, malted milk.
- Other optional ingredients: less than 1% emulsifying agents.

When optional ingredients are added, it must be declared on the label.

Optional Ingredients 3

- Cocoa, chocolate liquor.
- Safe and suitable vegetable derived fats, oils, or stearins other than cocoa butter (may be hydrogenated).
- Safe and suitable dairy-derived ingredients; bulking agents, formulation aids, humectants, and texturizers.

Optional Ingredients 4

- Optional dairy ingredients: cream, milkfat, butter, milk (Concentrated, evaporated, sweetened condensed, dried), skim milk (concentrated, evaporated, sweetened condensed, nonfat), concentrated or dried buttermilk, malted milk. Whey or whey products not to exceed 5%.
- Spices, natural and artificial flavorings, ground whole nut meats, ground coffee, dried malted cereal extract, salt, and other seasonings that do not either singly or in combination impart a flavor that imitates the flavor of chocolate, milk, or butter.
- Other optional ingredients: nutritive carbohydrate sweeteners, antioxidants, 1.5% or less emulsifying agents.

When optional ingredients are added, it must be declared on the label.

Source: Adapted from Ref. 2.

TABLE 149.2
Cocoa Powder: Types and Applications

Cocoa Type	pH	Flavor	Applications
Natural–non alkalized	5.2–5.7	Mild, cocoa notes	Compounds, syrups, bakery toppings, confectionery
Alkalized–light dutch	6.5–7.0	Mild, cocoa notes with mild alkali undertones	Dairy, beverage, creams, syrups, toppings, confectionery
Alkalized–red	7.0–8.0	Moderate to strong alkali notes	Bakery, dairy, beverage, creams, confectionery
Alkalized–dark	7.0–8.0	Mild to moderate alkali notes	Bakery, confectionery
Alkalized-black	Up to 8.2	Intensely unique alkali flavors	Cookies, coloring agent

Source: Adapted from Ref. 4.

Steel rollers reduce the size of particles to 10–40 μ. The actual size will depend upon the product desired and type of chocolate, with dark chocolate generally having a smaller finished particle size. During this process the resulting mass often turns into a dry paste or powder.

The manufacture of milk chocolate is similar to that of dark chocolate. The method used to incorporate the milk ingredient plays an essential part in the process. Milk solids are introduced in the form of milk powder or milk crumb, where it is dry mixed with chocolate liquor and sugar.

Milk powder is produced by first concentrating liquid milk and drying it into a powder. The crumb process involves blending sweetened condensed milk and chocolate liquor. It is kneaded and dried. Crumb based chocolates have a unique caramelized flavor based on the Maillard reaction between milk protein and sugars (5,6).

The term "white chocolate" has been used loosely for a number of years. It is basically milk chocolate without the cocoa matter. Cocoa butter is used instead of chocolate liquor and mixed with sugar, milk solids (a white crumb can be made from cocoa butter), emulsifiers, and flavor.

It is important that a mild-flavored cocoa butter be used, since the flavor of white chocolate is quite delicate. White chocolate is also more prone to oxidative rancidity than milk and dark chocolate.

C. Conching

After refining, the mass is transferred to large shear mixers called conches. The machines get their name on the fact that the original equipment resembled a huge conch shell. This is the last manufacturing process where texture and flavor are affected. Time, temperature, moisture control, and shear manipulate the process. Some of the benefits of conching are (1):

- Improved rheology/reduction in viscosity—less cocoa butter needed
- Elimination of harsh volatiles for a mellower taste
- Removal of moisture (reduces lumping and graining)
- Improved mouthfeel (smoothes sharp particle edges).

During this process, additional cocoa butter, flavors, and emulsifier may be added. Conching times will vary depending upon the formulation and final product desired, but can vary from 10 to 12 hours to up to several days. Conching temperatures range from 120°F to 160°F (49–71°C), and sometimes up to 180°F (82°C). The higher conching temperatures give the final product a caramelized flavor that is different from a milk crumb caramelized flavor (7).

D. Viscosity

One of the final steps in manufacturing chocolate is to standardize the product for viscosity. The development of automatic moulding and enrobing equipment requires precise control over the fluidity or viscosity of the chocolate.

It should be noted that *free* moisture in chocolate will increase the viscosity of chocolate. Thus general moisture tests will not necessarily provide pertinent information. Minute amounts of water or steam from leaking equipment, improper storage (humid conditions), and rework can affect the overall performance of the chocolate.

Chocolate is a non-Newtonian liquid, in that its viscosity (internal friction of fluids) is affected by the presence of solids in suspension, as well as by temperature. Once chocolate starts to flow, its viscosity will decrease with an increase in the shear rate (8,9). Chocolate viscosity is still commonly referred to in terms of degrees MacMichael in the United States. In the remainder of the world viscosity is usually measured in centipoise.

The MacMichael viscometer is a single-speed rotational instrument. It works on the following principle: a metal cylinder is suspended on a torsion wire, which in turn, is immersed in a cup of chocolate at a given temperature. As the cup rotates the twisting of the wire is measured by a scale.

The main drawback of the MacMichael method is its inability to provide full information on the flow properties of different chocolates. A Brookfield viscometer can determine plastic viscosity and yield value accurately. Results using a Brookfield (measured in centepoise) can be converted to degrees MacMichael. Table 149.3 shows a range of chocolate viscosities and typical applications.

Viscosity alone will not indicate how the product will handle. Two terms that help to describe flow character are yield value and plastic value. Yield value (YV) is the force required to initiate the flow of chocolate. Plastic viscosity (PV) is the force required to maintain the flow of chocolate once it has started to move.

A high YV is important in maintaining decoration marks and the prevention of "feet" on enrobed goods. A low YV is desirable for moulded products to properly shake out air pockets (8,9).

Viscosity and rheology can be affected by several factors. Smaller particle size in a constant formula will give a higher viscosity. Lecithin exhibits both lipophilic and hydrophilic properties, and is thus an excellent emulsifier. The hydrophilic groups attach themselves to the water molecules on the surface of the sugar particles. This reduces friction, increasing particle mobility, and thus lowering viscosity (8). This reduction in viscosity can also decrease the need for more expensive cocoa butter by as much as 5 percent (9). In the United States, the addition of lecithin or other emulsifying agents is limited to one percent by weight.

E. Tempering

Tempering is the controlled cooling of melted chocolate with agitation that will promote the formation of small stable fat crystals throughout the chocolate. Besides agitation, time and temperature play a key role in the tempering process. Stable cocoa butter crystals will provide the following desired properties (11):

- Snap
- Good Gloss
- Proper texture
- Bloom resistance
- Contraction for demoulding
- Less permeable barrier (shelf life)

The cocoa butter used in chocolate manufacture will affect the physical properties of the chocolate. The origin of the bean will also affect its quality. Attributes affected include hardness, texture, mouthfeel, and melting point of the butter.

Cocoa butter is a polymorphic fat in which its crystals have different characteristics, melting points and stability. There are four major types, γ, α, β', and β, with β being the only stable form. The unstable forms will eventually recrystallize into the stable β form (12,13). See Table 149.4.

Tempering can be done manually or in an automatic tempering unit. There are many types of units available using various methods to achieve the same result: the formation of stable fat "seed" crystals. In general, chocolate tempering involves heating the chocolate to approximately 110 to115°F (43–46°C) to melt the fat crystals. It is then cooled down with agitation to between 80 to 84°F (27–29°C), and subsequently reheated to about 86 to 88°F (30–31°C) before moulding or coating. In general, dark chocolates are tempered about 1 to 2°F (0.5–1.0°C) higher than milk chocolate (11). However, exact temperatures and procedures will depend upon the tempering equipment and type of chocolate used.

During tempering, the viscosity of the chocolate increases in proportion to the increase in seeding; thus chocolate must be used fairly quickly, or carefully heated to remelt some of the seed to prevent overtempering and achieve a steady state of temper. Overtempered chocolate may cause problems such as dull finish, excessive air bubbles, and poor mould release because of reduced contraction of the chocolate. Undertempered chocolate will also have poor mould release and will have a tendency to 'finger print' and have premature blooming.

Once the desired pieces of chocolate are formed, the chocolate should be cooled gradually to prevent future problems. Initial cooling temperature should be approximately 65°F (18°C) with minimal air movement. This is necessary for the continued formation of stable crystals and to prevent case hardening or the formation of a "skin." Temperatures can gradually be decreased to about 45°F (7°C) with increasing air velocity. Final cooling temperature before packing should approach that of the room temperature (about 68°F or 20°C).

When sending product through a cooling tunnel, one must take into consideration the heat load of the product and adjust air temperatures and velocities accordingly. Small pieces of chocolate will set at a faster rate than large moulded blocks. It is also important that the temperature

TABLE 149.3
Chocolate Viscosities

Viscosity	°MacMichael	Used For
Very Thin	< 100°	Enrobing, Panning, Shell Moulding
Thin	100–125°	Solid Moulding, Enrobing, Panning
Medium	125–160°	Enrobing, Hand Dipping
Heavy	160–200°	Moulding, Hand Dipping, Depositing (Chips)

Source: Adapted from Ref. 9,10.

TABLE 149.4
Polymorphic Forms of Cocoa Butter

Form	Approximate Life	Crystallization Temperature (°F)	Melting Range (°F)
Gamma γ	Very Unstable	< 63 (< 17°C)	Up to 63 (17°C)
Alpha α	1 hour	From Gamma	70–75 (21–24°C)
Beta Prime β'	1 month	32–72 (0–22°C)	59–85 (15–30°C)
Beta β	Stable	72–92 (22–33°C)	68–95 (20–35°C)

Source: Adapted from Ref. 14.

on the discharge end be higher than the dew point to prevent condensation on the chocolate.

Although the chocolate may appear solid at this point, only about 70 to75 percent of the cocoa butter is crystallized. It will take about 48 hours for all of the cocoa butter to crystallize, so packing and storage conditions are still an important consideration (11).

VI. PRODUCTION METHODS

A. Enrobing

Enrobing is the mechanized form of hand dipping. The centers to be covered are placed on a conveyor belt, either by manual or mechanical means. The centers are then transferred to a wire belt, where the bottom is coated with a thin layer of chocolate. Not all products go through this prebottoming step; however it serves to reduce problems later on, especially with soft centers. The centers then pass over a cold table to set the chocolate, and then enter the main enrober.

Product entering the enrober should be between 70 to 75°F (21–24°C). Warmer centers may lead to possible bloom problems as the heat tries to escape from the interior of the enrobed product. Cold centers may also cause blooming and cracking of the coating shell due to expansion of the center mass as it warms. It is important that enrobing chocolate have the desired viscosity and rheological properties.

The prebottomed centers are then conveyed on a wire belt through a curtain of tempered chocolate, coating the tops, sides, and bottom as well. The excess coating is removed through the action of adjustable vibratory shakers and forced air blowers. The blowers will often leave a desired rippled decoration on top. Prior to transferring to the conveyor belt, the product passes over a rotating detailer rod. Its main purpose is to remove the trailing "tail" at the end of the enrobed piece. For maximum shelf life, it is important that the centers be completely covered, with no pinholes.

The product will now enter a cooling tunnel to allow the coating to harden. To avoid blooming problems, temperature changes should be gradual. Besides air temperature, attention should also be given to the relative humidity, since if the dew point is lower than the room temperature, moisture could condense on the product and cause sugar bloom during storage.

B. Moulding

Moulded chocolates are popular, since design details and uniformity can be controlled to a greater extent than with enrobed chocolates. The moulding process can be either done manually or mechanically. The main types of moulds include solid, shell, and hollow moulding.

Solid moulds range in size from small novelty items to the 10 pound (4.5 kg) blocks commonly used in industry. Tempered chocolate by itself, or mixed with inclusions such as nuts, raisins, crisp rice, etc., are deposited into metal or the more popular polycarbonate moulds that are the reverse image of the desired end product. The moulds are shaken to distribute the chocolate evenly and to remove air pockets. If necessary, the mould may be scraped to remove excess chocolate before entering the cooling area. After cooling, the hardened chocolate is inverted and the mould twisted or tapped to release the product.

It is important that the chocolate not be overtempered, since overtempered chocolate tends to increase in viscosity, and thus have difficulty flowing into the mould. Overtempered chocolate also exhibits little contraction, and thus may cause demoulding problems.

For solid moulding, a chocolate with a low plastic viscosity (PV) is desired for the proper release of entrapped air. If inclusions are added, a medium viscosity chocolate should keep the particulates in proper suspension.

Shell moulding is a fairly complex operation where a center confection is enclosed in a chocolate shell. Tempered chocolate is deposited in moulds, shaken, and then inverted so that the majority of the chocolate is removed, leaving a thin shell. The chocolate is then cooled and the center mass is deposited. The centers can range from fudges to semi liquid caramels to liqueurs. It is shaken to level out the center and cooled. The mould surface and shell rim are briefly warmed before the final deposit of chocolate is made. This allows for the complete adhesion between the shell rim and the bottom coat of chocolate. Excess chocolate is scraped off before being cooled. After sufficient cooling, the product is demoulded and packaged.

Recent developments in shell moulding equipment allow for the chocolate and filling to be produced in one depositing sequence that is commonly called a one shot line. It is possible to deposit a warm homogenous center mass, such as creme fondant, caramels, truffles and meltaways. Viscosity, tempering process, and nature of the fat phase of the chocolate and of the centers are extremely important.

Hollow moulding is most commonly associated with novelty items such as Easter eggs and rabbits, and Santa Clauses. Tempered chocolate is deposited in a two piece mould which is held together by hinges, clips or magnets. The closed mould is rapidly rotated on a spinner to distribute the chocolate evenly. After cooling, the moulds are opened and the product is removed. Chocolate used for this operation should have a low viscosity and yield value to provide a thin shell.

VII. CONFECTIONERY COATINGS

In the United States, chocolate must conform to a certain standard of identity. If it does not meet the specifications, it cannot legally be called chocolate. The FDA has established standard of identities for cacao products and other

commonly used cocoa-based products. They are listed in Table 149.1.

A. Compound Coatings (Vegetable Fat Coatings)

These are similar to real chocolate coatings, except that chocolate liquor and cocoa butter have been replaced with cocoa powder and less expensive vegetable fat, respectively. The ingredients are dry mixed and refined to a smooth texture, with conching kept to a minimum. White and pastel compound coatings consist of vegetable fat, sugar, and milk powder, with added flavors and colors.

The vegetable fats substituted for cocoa butter may be classified as lauric and nonlauric fats. Lauric fats are short-chained fatty acid glycerides that have physical properties similar to cocoa butter, but are incompatible with cocoa butter. Fractionated coconut and palm kernel oils are lauric fats that have good stability, texture, and flavor release. These oils do not need tempering and are less expensive than cocoa butter. However, when exposed to lipases, especially in the presence of moisture, the free fatty acids released will have a strong soapy taste (15).

The nonlauric fats are composed of longer chain fatty acids and include hydrogenated soy, palm, and cottonseed oils. Unlike lauric fats, the nonlauric fats can be blended with up to 25% cocoa butter and chocolate liquor for a stronger flavor. Due to texture differences, the nonlaurics are used primarily for covering baked goods where a less brittle (or snap) texture is desired. Nonlaurics fats are also susceptible to decomposition by lipases, however, the fatty acids liberated do not have a soapy flavor (15).

B. Sugar-Free Coatings

Sugar-free coatings have been available to the food industry for over 30 years. The original versions were difficult to manufacture and to handle. When compared to "real" chocolate, sensory properties were quite lacking. With the advent of technology and health issues, the quality and quantity of sugar-free coatings and their related products have increased tremendously.

According to the FDA, a sugar-free food contains less than 0.5 g of sugars per reference serving size (16). For most confections, the reference serving size is 40 g, so the 0.5 g/40 g equates to less than 1.25 percent allowed sugars. Sugars are considered to be mono- and disaccharides, and include glucose, sucrose, fructose, and lactose. Confections that are "no sugar added" generally have no added sucrose, but may contain another sugar such as lactose that is found in milk powder.

At the present time, there is no standard of identity for sugar-free chocolates in the U.S. Code of Federal Regulations. From a legal standpoint, this product cannot be called "chocolate." To minimize the sugar level, caseinates are often substituted for the milk, since milk contains sugars in the form of lactose.

The milk powder that is generally used for traditional chocolates can only be used for 'no sugar added' coatings, since the natural lactose sugar is over 50 percent of the non-fat dried milk. This does not meet the requirements of a sugar-free label. Sodium caseinate, and to a lesser extent calcium caseinate, are most commonly used as milk replacers. The total amount needed in the formulation will be less than the amount of milk solids found in standard chocolate, since the lactose and fat portion are removed. Sodium caseinate at a 4 to 9 percent level will be sufficient to replace a traditional milk chocolate containing 12 to 20 percent milk solids (17).

Sugar alcohols as a group have always been considered the key to making a good sugar-free coating. With the exception of xylitol, and in some instances maltitol, they are often combined with high intensity sweeteners such as aspartame, acesulfame K, and sucralose to achieve the same sweetness level as sugar-based coatings.

Aspartame and acesulfame K are approximately 200 times sweeter than sucrose, while sucralose is about 600 times sweeter. The duration of sweetness for sucrose and acesulfame K are similar, while aspartame and sucralose will have a longer sweet sensation. While all of these should be relatively stable in a coating environment, aspartame is more sensitive to heat and acidic conditions. Acesulfame K in high concentration can give a bitter aftertaste. Aspartame has the disadvantage of having to require a 'contains phenylalanine' warning label when used (17).

Most formulations using a high proportion of sugar alcohols will require a label statement of "excess consumption may have a laxative effect." If a sugar-free claim is made, it must also include a "not a reduced calorie food" statement, unless the caloric value is at least 25 percent less than the standard product.

While sugar-free coatings can generally be handled like traditional chocolate, there may be some differences based on formulation and manufacturing processes. Final enrobing/moulding temperatures may be one to two degrees higher or lower and with faster set-up times. They may also appear to be thicker with different flow characteristics.

VIII. CHOCOLATE BLOOM PROBLEMS

Blooming of chocolate is probably the most common problem encountered during chocolate storage. It is a defect which manifests itself as a grayish-white film on the surface of the product. In most instances, the eating quality is not affected; however, the appearance is not very appetizing. Bloom may occur anywhere from a few hours after production, to several months later (18). There are basically two types of bloom–sugar and fat.

A. Sugar Bloom

Sugar bloom occurs less frequently than fat bloom, and its appearance may look like fat bloom. Sugar bloom occurs when the surface of the chocolate is exposed to moisture or high humidity and then dries out. The surface film of water dissolves some of the sugar particles in the chocolate, which then recrystallize upon drying into a dull grayish-white haze. In general, sugar bloom feels dry to the touch and does not melt, as compared to fat bloom.

Sugar bloom commonly occurs when chocolate emerges from a cooling tunnel into a warm and humid room. It also may occur when product is brought out of cold conditions and subjected to warmer, humid conditions without proper packaging and time to gradually bring it up to room temperature.

B. Fat Bloom

Fat bloom is the visible accumulation of large cocoa butter crystals on the chocolate surface, which may give it a greasy surface texture that melts readily when touched. It may be accompanied by numerous mini cracks that gives it a dull grayish-white appearance. These changes are primarily due to the polymorphic nature of cocoa butter and the migration of liquid fat (19). See Table 149.5.

IX. STORAGE AND HANDLING

Like many other foods, chocolate flavor will change over time, resulting in a more balanced flavor profile. In general, most confections should be contained in a non-permeable barrier for protection from oxygen and moisture.

Ideally, chocolate should be stored at 65 to 68°F (18–20°C) and less than 50 percent relative humidity. Dark chocolate is less prone to moisture absorption during storage than milk chocolate (1,20,22). Chocolate should also be stored away from light. Since it is fat-based, it will readily absorb off-odors, so chocolate should be stored in an odor-free environment. Chocolate will also pick up odors from packaging materials and printing inks, as well as odors generated from the heat sealing or shrink-wrapping process. Advances in packaging technology now include water-based cold-seal adhesives, which not only eliminate the potential for heat-damaged chocolates, but are also low in odor, thus eliminating or reducing the possibility for the development of off-flavors or smells (22).

Properly stored, solid chocolate should have a shelf-life of one year of more. Chocolate can be frozen for even longer storage; however, care must be taken when bringing it back to room temperature from the frozen state to avoid condensation.

If chocolate is manufactured from clean beans under sanitary conditions, its microbial count should be low due to its low water activity (A_w) level. However, chocolates may become contaminated from some of the ingredients used and improper storage conditions.

Moisture can be introduced into the product during its manufacture (such as through equipment leaks or improperly dried equipment), or during storage under damp conditions. This may lead to mold and fermentation problems. Molds can survive under low moisture conditions to produce spores, enzymatic reactions, or toxins (such as aflatoxin in peanuts). Fermentation problems are not as common today as in the past, due to better controls on ingredient quality, pH, and A_w of the confection, as well as the use of preservatives such as potassium sorbate. An A_w of 0.68 or less is usually considered stable (20,23).

Rancidity is generally not a problem in milk and dark chocolate because of the natural antioxidant properties of undeodorized cocoa butter. Rancidity can be found on

TABLE 149.5
Some Factors Causing Chocolate Bloom

- Extreme water temperature used in cooling/heating.
- Cooling tunnels/rooms that are extremely cold.
- Cold moulds; warm to 75–80°F (24–27°C).
- Products removed from cold storage to room temperature, not done in steps to prevent condensation.
- Temperature fluctuations during storage.
- Storage temperature exceeding 88–90°F (31–32°C); ideally <68°F (<20°C).
- Too cold or hot center mass when enrobing; ideally 70–75°F (21–24°C).
- Formation of condensate in cooling tunnel.
- Improper formulation of centers that allow moisture or oils to migrate to the outer surface of the coating.
- Packaging/shrink-wrap of finished goods with heat can cause localized bloom.
- Undertempered chocolate.
- Placing chocolates in cold storage before fully solidified. Solidification takes 24–48 hours.
- Introduction of foreign fats (such as coconut oil) into the chocolate.
- Processing line equipment heaters that detemper chocolate.

Source: Ref. 11.

occasion in white chocolate and compound coatings, and is catalyzed in the presence of air, moisture, light, heat, and some metals. With oxidative rancidity, the fats or oils exposed to air produce objectionable flavors due to the formation of aldehydes and ketones. The addition of synthetic antioxidants will help inhibit this reaction, although some fats contain natural antioxidants as well (20).

Hydrolytic rancidity is also known as soapy rancidity, since the off-flavor produced has a soapy taste. Lipases hydrolyze the fat into glycerol and fatty acids. Lipases may be present in ingredients such as coconut, milk products, egg albumen, and cocoa. If possible, it is recommended that ingredients susceptible to hydrolytic rancidity be heat treated to inactivate the enzyme. Lipase activity will not always result in a soapy flavor. Fats containing lauric acid, even a trace amount, can also yield a soapy flavor. Coconut oil and palm kernel oil contain 40 to 50 percent lauric glycerides, while butter fat contains from 2 to 6 percent. Cocoa butter and palm oil do not contain lauric acid (20).

X. NUTRITONAL VALUE

Chocolate has also been noted recently to contain relatively high amounts of polyphenols, in particular the flavanols catechin and epicatechin. Polyphenols are found in the plant kingdom and exhibit antioxidant activity, which is thought to reduce the risk of some diseases of the human body. The levels found in chocolate and related products will depend upon cocoa bean variety, fermentation, and subsequent processing and formulation (24,25).

A food allergy is a reaction by the body's immune system to a substance, usually a protein, in food. An allergy to chocolate itself is uncommon; however, the addition of other ingredients may cause an allergic reaction. According to the National Institutes of Health, approximately seven million Americans (more children than adults) have a true food allergy. Eight food allergens account for 90 percent of all allergic reactions. These include milk, eggs, peanuts, tree nuts, soy, wheat, fish, and shellfish (26,27).

A common belief is that chocolate contains a lot of caffeine. Caffeine belongs to a class of alkaloid molecules known as methylxanthines. Caffeine is the primary alkaloid in coffee, while theobromine is the primary alkaloid in chocolate, and theophylline is the primary alkaloid in tea. Methylxanthines occur naturally in a number of plant species and they exhibit similar pharmacological properties. Methylxanthines are mild stimulants and possess mild diuretic properties (28,29). Table 149.6 shows the USDA nutrient content of several types of chocolate, and also a comparison of the caffeine content with coffee.

While chocolate is not a major source of dietary fat, it nevertheless does contain fat. The majority of the fat comes from cocoa butter and is composed mainly of three fatty acids: 30–37% of the monounsaturated fatty acid oleic acid (18:1), 32–37% of the saturated fatty acid stearic acid (18:0), and 23–30% of saturated fatty acid palmitic acid (16:0). It also contains 2–4% linoleic acid (31). While stearic acid is classified as a saturated fat, it does not appear to raise cholesterol levels the way other saturated fatty acids do (32).

Chocolate is often perceived to be a cause of dental caries. Dental caries occur when bacteria in the mouth metabolize fermentable carbohydrates, resulting in the formation of acids and a decrease in plaque pH, which then causes demineralization of the enamel. Thus the cariogenicity of a food is related to the total amount of fermentable carbohydrates. Unsweetened chocolate is not cariogenic, because it does not contain significant amounts of fermentable carbohydrates. The presence of fats, proteins, minerals, etc., can also modify cariogenicity. Studies have also shown that sweetened chocolate does not increase the incidence of dental caries (33).

Chocolate is one of the most popular flavors, enjoyed by millions of people in a variety of ways, from beverages, to main dishes, sauces, baked goods, and, of course, confections. Eaten in moderation it can be part of a healthful diet.

TABLE 149.6
USDA Nutrient Database for Standard Reference, Release 15 (July 2002)

Item	Kcal	Protein (g)	Fat (g)	Carbo (g)	Calcium (mg)	Sat. Fat (g)	Mono. Fat (g)	Poly. Fat (g)	Cholest. (mg)	Caffeine (mg)	Theobro. (mg)
Semisweet Chocolate	136	1.2	8.5	17.9	9.1	5.0	2.8	0.3	0	17.6	137.8
Sweet Chocolate	143	1.1	9.7	16.9	6.8	5.7	3.2	0.3	0	18.7	120.8
Unsweetened Chocolate	145	2.9	15.7	8.0	21.0	9.1	5.2	0.5	0	57.1	346.4
Milk Chocolate	145	2.0	8.7	16.8	54.1	5.2	2.8	0.3	6.2	7.4	47.9
Cocoa Butter	251	0	28.4	0	0	16.9	9.3	0.9	0	NA	NA
Cocoa Powder	63	5.1	3.7	15.5	31.5	2.2	1.2	0.1	0	22.1	746.7
Brewed Coffee, 8 oz	5	0.2	0	0.9	4.7	0	0	0	0	137.5	NA
Instant Coffee, Prep. 8 oz	5	0.2	0	0.9	7.1	0	0	0	0	76.4	NA
Brewed Coffee Decaf, 8 oz	5	0.2	0	0.9	4.7	0	0	0	0	2.4	NA

Source: Adapted from Ref. 30.

Note: Values are per 1-oz (28.35g) unless otherwise noted.

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150 Mozzarella and Scamorza Cheese

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Arcangelo Esposito
Mozzarella Fresca

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I. INTRODUCTION

Mozzarella and scamorza cheeses originated in the southern regions of Italy, and are typical of a *pasta filata* or stretched curd variety. In fact, the term mozzarella is derived from the Italian verb *mozzare*, meaning to tear, indicative of the stretching process inherent in mozzarella manufacturing. Over the last few decades, mozzarella has experienced substantial growth, given its popularity and role as a topping for pizza. As an ingredient in pizza, as well as in other applications, mozzarella exhibits desirable functional attributes, such as stretching, melting, and browning. These and other critical attributes result from careful control of numerous manufacturing details, such as milk composition, acidification rate and extent, and stretching parameters. In the U.S., mozzarella represents approximately one-third of all natural cheeses and is consumed at a rate of about 9.5 pounds per capita [2]. Mozzarella is

typically manufactured from cow's milk; however, a cheese aficionado would certainly recognize a traditional mozzarella made from domesticated water buffalo milk, i.e., Mozzarella di Bufala.

Mozzarella is considered a fresh cheese and, as such, is known for a typically mild flavor and rubbery texture. Due to its high moisture, elevated pH, and lack of aging, and hence its propensity to support growth of pathogenic bacteria, mozzarella is made from pasteurized milk. In the U.S., mozzarella is generally manufactured according to one of four compositionally and functionally distinct products: whole, low moisture, part-skim, and low moisture part-skim (LMPS).

The word "scamorza" is derived from the manufacturing technique of cutting the melted cheese by hand. The Italian term "scamozzare" means to "behead," reflecting the cutting of the pear-shaped cheese during molding. For centuries, Italian cheese makers used sheep's milk, but as scamorza gained popularity as a semi-hard table cheese, the use of cow's milk became common. Scamorza was also used to preserve small quantities of butter. During the molding process, the cheese maker would include a small sphere of butter (about 10 oz) inside the hot cheese and seal it with the molten curd, thus protecting the butter from spoilage while sharing flavor with the ripening cheese. In the context of U.S. standards of identity, mozzarella and scamorza cheeses are compositionally identical. However, in the U.S., scamorza is somewhat of a rarity compared to mozzarella. Thus, the balance of this work will focus on mozzarella.

A great deal of research has been conducted on mozzarella, and several reviews can be found in the literature [28,41,34]. In general, researchers focused on manufacturing parameters and their impact on functional properties. Such parameters include milk source, milk composition, proteolytic activities, physical treatments, calcium status, and thermal treatments. Given the host of manufacturing complexities, functional properties, and continued positioning into demanding food service applications, mozzarella cheese continues to garner substantial research attention. This chapter will provide a brief overview of mozzarella from historical, manufacturing, and functional perspectives.

II. HISTORY

Mozzarella di Bufala has a springy texture and a pleasantly sour taste, with a faint mossy smell reminiscent of the humid grazing fields of southern Italy. It is porcelain-white in color, spherical in shape and, with a very thin, glossy, edible rind.

Northern Italy fronts the Alps and is bordered by Switzerland and Austria. Between the Alps and the Apennines (a north-south mountain range which traverses nearly the entire length of Italy) is the plain of Lombardy. The Lombardy region is considered the richest and most extensive agricultural area in Italy. Smaller agricultural plains, such as those of Tuscany, Campania, and Apulia, are scattered on either side of the Apennines. Origins of mozzarella begin in the Campania region in southern Italy, along the Mediterranean Sea.

Books by Plinius the Elder, and Lucius Junius Moderatus Columella date cheese technology to the Roman times in the 1st century A.D. The production of mozzarella is linked to that of the water buffalo. Plinius the Elder, in his writings on natural history, classified the water buffalo as a 'bubalus,' or an exotic animal, living in wet and swampy environments. Columella, in the book *De Re Rustica*, described a cheese that after curdling was warmed, cut, scalded in water, hand kneaded, and molded in the shape of a lump. Such descriptions give an indication of the historical presence of "spun" or "stretched" curd cheeses. The rearing of water buffalo in the marshy low lands in the Campania region dates back to the 12th century, but it was not until the 17th century that large scale processing of milk began. The first written records of mozzarella are from the Bishop's Archives in Capua (a city in southern Italy) in the 12th century, which describe a "mozza" or "provatura" as fresh cheese. Initially this cheese was consumed only within the southern region of Campania. The Stock Exchange of Naples and Capua in 1601 quoted water buffalo and its milk products, just like gold or wheat. In the 18th century, mozzarella's popularity spread beyond the Campania region; in particular to the much larger Lombardy region in the north.

At this time, the Bourbon's dominion strongly supported research toward a scientific approach to milk and cheese production. The Lombardy region was already known for its firm Grana-type and blue-veined Gorgonzola cheeses. The Bourbon's Royal Farm in Caserta supported studies to optimize milk production and improve production efficiency. As an artifact of this extensive cheese making, the region supported almost 8,000 free-ranging water buffalo. As production of buffalo and cow's milk mozzarella increased in the north, production decreased in the south. By the 19th century, most industrial cheese production was concentrated in northern Italy.

The reduction of the buffalo herd numbers continued through the mid-1950's. Due to promotional efforts by the northern Italian dairy industry, consumers' interest towards authentic water buffalo milk mozzarella increased. In 1979, the Italian government officially defined and recognized water buffalo milk mozzarella to protect the consumer from the common fraudulent practice of selling cow milk mozzarella at the higher price of water buffalo milk mozzarella.

Mozzarella gained a large section of the retail market several decades ago, and in 1993, became a "DOP" cheese protected and regulated by the "Consortium of Mozzarella di Bufala Campana." In Italy, the origin and the traditional technology are guaranteed by a legal standard of identity.

Only cheese made in specific areas with 100% water buffalo milk can be distributed with the consortium's logo.

In the United States at the turn of the 20th century, Italian immigrants built small cheese factories for the production of soft Italian cheeses, primarily because these high moisture cheeses would spoil during the long sea voyage. Initially, this mozzarella closely resembled the high moisture cow's milk mozzarella of northern Italy. However, changes in the manufacturing procedure altered mozzarella into a firmer, drier, cheese, better suited for transport and cooking saltier. Since the 1920s, the production of mozzarella in the U.S. has increased from less than 50,000 lbs to an estimated 2.8 billion pounds per year in 2002 [2,16]; the vast majority is low moisture, part-skim mozzarella. This change primarily occurred in the Midwestern United States as cheese makers worked toward manufacturing mozzarella with sufficient shelf life for transport to the metropolitan markets on the east coast.

III. DEVELOPMENT OF LOW MOISTURE, PART-SKIM MOZZARELLA

The first U.S. mozzarella manufacturing facilities (in upstate New York) made cheese from standardized milk with no or low levels of starter culture. The starter cultures selected were either an active *S. lactis* at 0.05% (now called *Lc. lactis* subsp. *lactis*), or *S. durans* at 0.50% (now called *Enterococcus faecium*) [10,34]. These cultures do not produce an abundance of acid under the conditions of the high moisture mozzarella protocol. The milk was set, cut (not cooked), drained, cut into small blocks, washed with cold water, and then bundled into cloth bags [33]. After storage at 40°F for 24 hours, these bundles were shipped on ice to stores in New York City markets. It was crucial that acid production up to this point be minimal, and it was not uncommon for acid development to require one to three days. The storekeeper or latticini operator then brought the cheese into a warm room to reactivate the acid fermentation and allow its pH to drop to about 5.3. At this point, the curd was stretched in hot water, molded, and brine salted for the customer.

In the textbook *Cheese Making*, originally published in 1918 [54], Professor J.L. Sammis of the University of Wisconsin-Madison described the manufacturing protocols of Italian cheeses of the time. The text outlines protocols for caciocavallo and provolone, but not mozzarella. These former cheeses are drier cheeses (28–38% and 37–45% moisture, respectively), and are made with one-third skim milk or whole milk, respectively [54]. Sammis describes adding starter (not specified, but presumably *S. thermophilus*) and rennet to milk, then cutting and raising the temperature to 125°F. Curd temperatures were maintained at 125°F for 4 to 5 hours in the summer, or 8 to 10 hours in the winter until the proper acidity was developed for stretching in hot water. In the 1930s the starter culture concept was further developed by Professor Sammis and Louis Rossini, a cheese maker at Stella Cheese Company of Campbellsport, Wisconsin. Sammis and Rossini found that the use of a ratio of *Lb. bulgaricus* (a rod shaped bacterium) in addition to *S. thermophilus* (a coc-cus shaped bacterium) enabled them to make cheese the same day the vat was set [16], greatly increasing production rates.

Italian immigrants in the Midwest also manufactured pasta filata cheeses and, as dairying increased, cheese makers looked to manufacture cheeses for the east coast markets. Of the "spun" Italian-type cheeses, the greatest demand was for mozzarella. Cheese makers found it difficult to maintain the quality of mozzarella manufactured using low acid producing mesophilic cultures. In the 1940s, cheese makers switched from the use of mesophilic to thermophilic cultures, facilitating the completion of mozzarella manufacturing in one day. It is unclear if this new mozzarella is a modified version of caciocavallo (higher moisture) or provolone (lower fat). To optimize acid development from the rod to cocci culture ratio, curd was cooked between 114–116°F. The higher cooking temperatures resulted in a cheese with a lower moisture content than that of the east coast mozzarella variety. Essentially, cheese makers were now manufacturing a low moisture mozzarella that could effectively be shipped long distances to reach new markets. It was noted that cheese made by this method was yellowish, drier, and frequently displayed a slight provolone flavor. This cheese was not considered a table cheese, but a cooking cheese, thus it was called 'pizza cheese,' until the USDA developed the Standards of Identity for Mozzarella in 1965.

A 1964 survey of randomly selected cheeses reported New York mozzarella to be part-skim (~56% moisture, <45% FDM [fat in the dry matter]) and Wisconsin mozzarella to be low moisture, part-skim (~48% moisture, <45% FDM; [50]). Italian Mozzarella di Bufala, by definition, has a minimum of 45% FDM. It is assumed that standardizing cheese milk to a lower fat content in both New York and Wisconsin was based on the higher value of the milk fat for butter making at the time, or to improve cheese shelf-life in the era of poor refrigeration.

IV. MANUFACTURING LMPS PASTA FILATA MOZZARELLA

Most mozzarella manufacturing operations vary in some fashion to accommodate differences in such factors as the production schedule, milk receipt, storage capacity, equipment design, and plant layout. However, there are several core unit operations that are common to most mozzarella manufacturers. A diagram of a typical commercial operation is found in Figure 150.1.

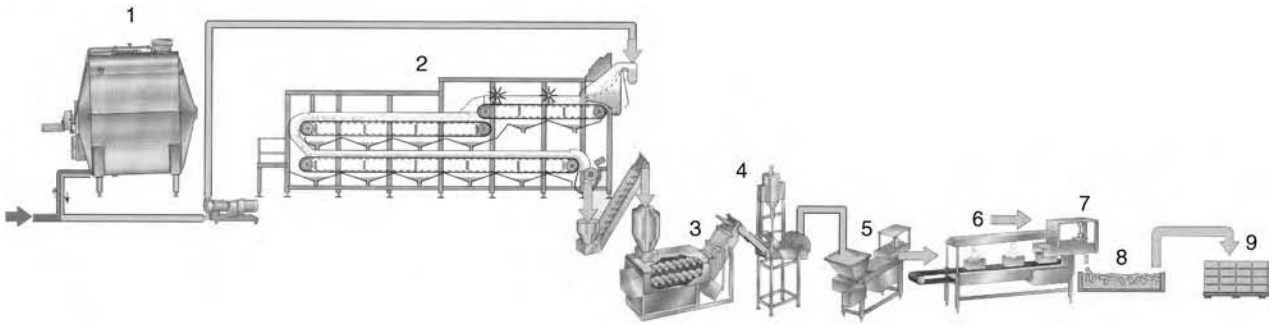

FIGURE 150.1 A typical process line for the commercial-scale manufacture of mozzarella cheese (Adapted from Tetrapak, 1995). 1. Cheese vat. 2. Cheddaring process. 3. Cooker/stretcher. 4. Salting. 5. Molding. 6. Hardening tunnel. 7. De-molder. 8. Brine tank. 9. Palletizing/storage.

A. Milk Quality

Although there is some basis for discussing the raising of water buffalo as a milk source, the vast majority of mozzarella produced is made from cow's milk; hence only this latter source will be addressed. Compositional data of typical cow breeds and water buffalo milks are described in Table 150.1.

In general, milk should be of top quality with low bacterial and somatic cell counts (SCC). As is typical of milk used in the manufacture of any cheese, pH also plays an important rule. Raw, fresh milk should have a pH no lower than 6.50. Milk with an alkaline pH is indicative of udder disease, and will dramatically influence cheese attributes, including the coagulation time and the final curd texture and flavor. The influence of the SCC on the composition of milk and suitability for cheese manufacture has been studied extensively [32,55]. Somatic cells are released from the blood to prevent or reduce udder infections (mastitis). Low numbers of somatic cells are always present in normal milk (i.e., $<$100,000/ml) from healthy animals in mid-lactation. Factors that increase SCC of bulk manufacturing milk include mastitis, advanced stage of lactation, lactation number, environmental stress, and poor nutrition. Milk with an SCC $>$750,000 in the U.S. is considered illegal, and hence is excluded from the commercial supply. An increased milk SCC is associated with marked changes in both the concentrations and state of milk constituents (e.g., degree of proteolysis), and leads to a deterioration of the rennet coagulation properties, curd syneresis, and a noticeable reduction in cheese yield. During cheese making, the curd is weak and subject to extensive shattering during cutting and stirring. This leads to a high quantity of fines, impaired whey drainage, and higher final cheese moisture contents [3,4,19,20,45,46,52,61,64].

TABLE 150.1
Effects of Species and Breed on Milk Composition (Adapted from [56])

Source	Lipid	Protein	Lactose	Ash
Buffalo	6.0	4.5	4.5	0.8
Cow	3.9	3.3	4.6	0.8
Jersey	5.1	3.8	5.0	0.8
Guernsey	4.9	3.9	5.0	0.8
Ayrshire	3.9	3.4	5.0	0.7
Holstein	3.5	3.3	4.6	0.7

B. Thermal Treatment

In certain countries, in an effort to preserve the delicate texture and endogenous microflora, some traditional mozzarella-type cheeses are made with little or no thermal treatment. However, because this practice cannot provide adequate assurance against the presence and outgrowth of microbial pathogens, by regulation, all milk in the U.S. must be adequately pasteurized for the manufacture of mozzarella. In practice, milk for mozzarella is standardized for milk fat (e.g., 2.0–2.4% for LMPS) and casein-to fat ratio (e.g., 1.04 for LMPS). Changes in total milkfat are achieved by centrifugal separation of cream. Casein-to-fat ratios may be manipulated by a number of approaches, including the addition of nonfat dry milk, condensed skim, or unfiltered skim milk. Typical pasteurization treatments are in the range of 163–165°F for 17–20 seconds, which is just above the legal minimum in the U.S. of 161°F for 15 seconds. Such heat treatments are considered sufficient, assuming good quality raw milk, to eliminate pathogens and reduce spoilage and competitive microflora, as well as inactivating several enzyme systems. In this temperature range, there is little change in the nutritive properties of the milk; however, it may develop a slight cooked flavor. Excessive heat treatment of milk is to be avoided, as it results in whey protein denaturation, weak textured curd, and greater moisture retention in the curd [65]. Heat treatment may also denature some enzymes. A notable exception is the enzyme plasmin, which is capable of retaining acti-vity even after significant heat treatments [44]. Plasmin is associated with elevated SCCs and effectively

degrades the casein proteins, resulting in a decrease in cheese yield, the development of off-flavors, and overall degradation of cheese rheological properties [18,58].

C. Curd Formation

The two main events responsible for the coagulation of milk proteins are acid development and rennet-driven proteolysis. In most cases, both processes contribute to the overall destabilization of the casein micelle; the activity of rennet is influenced by pH and setting temperature. The addition of calcium chloride is also a common practice. Each of these processes/practices is detailed below.

1. Calcium Addition

Because curd formation is partially a function of the state and availability of calcium in the milk system, calcium salts may also be directly added as a supplement to ensure adequate gel strength [56]. The level of calcium contributes to proper milk clotting activity; a common practice is to supplement milk at the rate of 3 grams of calcium chloride per 100 liters of milk. Calcium chloride will enhance the rennet properties, namely a reduction in gel time and increased curd firmness.

2. Acid Development

In native milk, the casein micelle is partially stabilized by the repelling effects of a substantially negative net charge. The isoelectric point of the micelle is approximately 4.6. Thus, as acid is produced in or added to milk (native pH 6.4–6.6), the negative charge and resulting repellent force is lessened [65], ultimately destabilizing the micelle and causing curd formation. Acid is introduced into cheese milk for mozzarella manufacture by one of two processes. In one method, milk may be directly acidified by the addition of a food-grade acidulant, such as acetic, lactic, or citric acid. To inhibit the localized destabilization of casein, cold (40°F) pasteurized milk is directly acidified to approximately pH 5.6. Rennet is added, and the acidified milk is heated to approximately 100°F, where it is held as it coagulates [34].

A more traditional method for the acidification of milk for cheese manufacturing is lactic fermentation. In this case, pasteurized milk is tempered to approximately 94°F, whereupon a commercial starter culture is added at the rate of 0.5–2%. The starter culture may be *Streptococcus salavarius* ssp. *thermophilus* only, or a blend of *S. thermophilus* and *Lactococcus delbrueckii* ssp. *bulgaricus.* Starter culture selection is dependent on the desired rate of acid production during the latter steps of cheese making. Detailed descriptions of the primary metabolic capabilities of these bacteria are available in the literature [59]. These bacteria convert lactose to lactic acid, lowering the pH, facilitating moisture loss from the curd, and altering the structure of proteins. In some procedures, mesophilic culture such as *Lactococcus* spp. are used in addition to the thermophilic cultures. In addition to lactic acid, these bacteria produce a caste of metabolites responsible for the pleasant "cultured" flavor and aroma typical of fermented milk products.

3. Rennet-Catalyzed Coagulation

Rennet is a generic term for a preparation containing the enzyme chymosin. Chymosin activities exist in numerous plant and animal sources, including calf stomach, porcine, goat, chicken, and the mold *Rhizomucor meihei* [17]. Due to improved supply volume, reduced costs, improved stability, and performance, the most commonly employed rennet preparations are manufactured using recombinant techniques and microbial expression systems. The primary purpose of chymosin is to enzymatically cleave the hydrophilic κ-casein molecule, thereby rendering the casein micelle unable to remain in aqueous suspension. Most rennet preparations, especially those extracted from plant and animal sources, exhibit several additional enzymatic activities that further catalyze the breakdown of cheese components, ultimately contributing to texture and flavor of the final cheese. Several descriptions of these enzymes and activities are available in the literature [13].

Although the fine detail of the micellar interaction varies, a result of either acid or rennet-catalyzed milk coagulation is that the casein micelles join together in a continuous matrix based on various molecular forces including electrostatic interactions involving calcium phosphate and interactions along protein regions of similar hydrophobicity. The result is a web-like protein structure comprised of open cells containing whey, fat globules, and bacteria. The firmness of the structure depends on the thickness of the chains of micelles, and the space between the chain cells [39]. The state of the milk proteins and their relative concentration to milk fat is also extremely important in order to yield a curd suitable for stretching. An excessive concentration of casein will give a hard, difficult-to-stretch curd. Conversely, higher milk fat levels will produce a soft and slippery curd that would be stretched at slightly lower temperatures to limit milk fat losses.

D. Cutting and Whey Removal

Once the proper gel strength is obtained, the coagulum is gently cut into approximately 1 to 1.5 cm cubes. Immediately, the curd begins to expel whey and shrink. The curd mass is left to rest or "heal" for approximately 5 minutes, and then is gently stirred to prevent clumping. If cultures have been added, the temperature of the curd is increased to approximately 106°F to facilitate acid production and whey expulsion. When an adequate acidity is developed, typically a curd pH of 5.9, the curd is separated from the whey. At this

point, the curd is either allowed to mat together, or it is gently stirred until the curd is ready for stretching (curd pH of 5.25). Prior to mixing, the curd may be pre-salted at a rate of up to 2% (salt to curd ratio). Pre-salting curd is done to reduce brining times by directly incorporating about 1/3 to 1/2 of the total salt.

E. Mixing and Molding

As a descriptor, the term "pasta filata" refers to the process of heating and stretching the curd mass, resulting in the visible alignment of protein fibers within the curd. In practice, the curd is added to hot water (155–165°F), where it warms to approximately 140°F. The heated curd is physically kneaded by an auger system (twin screw or single auger). Protein fibers align and entrap coalesced fat and moisture. The temperature at which the curd is mixed is important, because it influences the residual rennet activity, microbial populations, and the rate that the curd is transformed into a molten mass. All of these factors affect the rate of proteolysis during cheese ripening, thus impacting shelf-life and functional properties. The finished product will have visible fibers similar to cooked chicken breast meat, and a glossy, white appearance.

Next, the cheese mass is distributed into molds of desired shape and size, anywhere from single serving string cheese ropes to large blocks, typically either 6 or 20 pounds in size. The molded cheeses are chilled at ~60°F, then placed into a cold (35–45°F) brine solution. The brine solution typically contains approximately 22–24% sodium chloride salt (~90% saturated), and 0.1% calcium chloride to prevent net calcium loss and the resulting development of a slimy cheese surface due to resolubilized casein. The brine should also be adjusted to a pH of 5.3 to prevent surface defects. Residence time in the brine tank is determined by several factors, including cheese block size, temperature, and salt solution strength. Typical times are on the order of 4–8 hours with pre-salting (12 to 24 hours without) to achieve a final salt level in the range of 1.3–2.0%. Mozzarella is commonly vacuum packaged with plastic or laminant materials.

V. ALTERNATIVE MANUFACTURING PROCEDURES

To this point, the manufacturing procedures discussed dealt with low moisture, part-skim pasta filata mozzarella. The following section will discuss protocols for the production of whole milk mozzarella, as well as a non-pasta filata LMPS mozzarella. Whole milk mozzarella may be manufactured using starter cultures, organic acid, or a combination of the two.

A. Whole Milk Mozzarella with Starter Cultures

When a starter culture is employed to acidify milk in the manufacture of whole milk mozzarella, a relatively high level is used, approximately 2%. Starter culture selections range from solely *S. thermophilus*, to a one-to-one ratio of cocci-to-*Lb. bulgaricus* rods. The milk coagulum is set at 88°F, with a usage level of 1.15 oz/1000 lbs of double-strength milk coagulant. After a 30 minute setting time, the curd is cut into relatively large-sized pieces, allowed to heal, then minimally cooked. The large cutting size, mini-mal cooking temperatures, and short stir-out contribute to the high moisture retention of a whole milk mozzarella. When sufficient acidity is developed, the curd is separated from the whey, rinsed, molded, and brined.

B. Whole Milk Mozzarella with Direct Acidification

Typically, lactic or acetic acid may be used as the acidulant in a direct acid set whole milk mozzarella. At the time of acid addition, the milk should be cold (less than 40°F) to prevent localized clotting of milk proteins and facilitate thorough mixing of the acidulant. Sufficient acid is added to lower the milk pH from approximately 6.6 to 5.6. After thorough mixing, milk temperatures are raised to a setting temperature of 88°F. At this time, 0.25 oz per 1000 lbs of double-strength milk chymosin are added. The coagulum will be firm enough to cut in about 30 minutes. After which, the curd is healed, then gently agitated for approximately 10 minutes. A direct acidified whole milk mozzarella is not cooked. After sufficient curd firming, the curd is separated from the whey stretched, cooled, and brined. The manufacture of direct acidified whole milk mozzarella is a relatively short process, typically requiring an hour from addition of coagulant to chilling after mixing.

C. Non-pasta Filata Mozzarella

A non-pasta filata mozzarella is manufactured without the use of a mixer-molder or brine system. After stirring out, the curd is salted, hooped, and pressed. It is critical that the final cheese has similar composition and functionality to its pasta filata counterpart, meaning the curd should melt and stretch in a similar manner. To ensure similar functionality, strategies to control pH and limit proteolysis need to be incorporated into the non-pasta filata manufacturing schedule. The final pH of the cheese should fall within the range of 5.2 to 5.4 (never below a pH of 5.2). Acidity control may be accomplished by reducing the residual sugar content in the curd (wash treatments), or by reducing the initial lactose content of the milk (e.g., using ultrafiltered milk). Proteolysis is limited in a pasta filata mozzarella by use of the mixer molder. Internal cheese temperatures reached in the

mixer (135–145°F) inactivate residual milk coagulant and decrease starter culture populations. Limiting proteolysis in a non-pasta filata mozzarella may be accomplished by starter culture selection, or a reduction in milk coagulant levels. A non-pasta filata mozzarella manufacturing procedure includes the typical steps in the manufacture of any American-style cheese: setting, cutting, healing, cooking, stirring out, draining, salting, and pressing.

VI. MOZZARELLA FUNCTIONALITY

This section will address the functionality of mozzarella as it pertains to current usage and manufacturing practices. Mozzarella's clean mild flavor and melting characteristics make it well-suited for use as an ingredient in many prepared foods. Mozzarella manufacturers need to know how their cheese will function during high speed shredding or slicing (including the influences of flow agents). Cooking systems that differ from a traditional conduction oven have different affects on cheese (i.e., fast-cooking forced-air or microwave ovens). In addition, as mozzarella finds its way to different markets and food systems, it is more important to control the physical properties so the cheese has specific melt, stretch, cooked color, free oil release, and textural properties. Each of these attributes is addressed in the following text.

A. Shredding

Prior to usage in most cooking applications, block mozzarella is converted into shreds to facilitate uniform distribution and melting. Poor shredding properties were cited as a frequent problem among mozzarella cheese users [51]. Chen [6] identified favorable shred characteristics and textural attributes required for successful conversion of block mozzarella to shreds. Researchers evaluated shred size distribution (long shreds, short shreds, fines), shred dimension (length and width), and visual characteristics (straightness, roughness, wetness) to determine which shredded cheese attributes had the greatest impact on shredded cheese preference. The top three explanatory variables for shred preference were percentage of long shreds (from size distribution), shred thickness (from shred dimension measurements), and shred straightness (from shred character observations). Panelists preferred long, thick, and straight cheese shreds. The percentage of long shreds had the greatest impact on preference, with shred thickness and straightness being of secondary importance.

The most important textural factors in the conversion of block mozzarella to shreds are the cheese firmness and adhesiveness [6]. The relationship between these textural attributes and shred quality are valid, regardless of manufacturing style (pasta filata or non-pasta filata style), composition, or cheese age. Researchers found the firmer and less adhesive the cheese, the higher the quality of shreds. Commercial shredders (e.g., Urschel) use centrifugal force to direct cheese cubes onto stationery blades, thus converting cheese cubes into shreds. A firm textured cheese has less deformation, and blades are able to make cleaner cuts. In addition, a firm textured cheese cube maintains a uniform speed, and blades can cut shreds the length of the cube. On the other hand, as a soft textured cheese comes in contact with the blade, it deforms and bends around the blade, slowing the portion of the cheese cube in contact with the blade. The opposite side of the cheese cube is moving faster and has greater momentum. This momentum directs the cube away from the blade before the blade cuts a full cube length, producing shorter and thinner shreds. The other key textural property, adhesiveness, has similar effects. An adhesive cheese sticks to the blades, slowing the portion of the cheese cube in contact with the blade, and resulting in decreased shred quality (i.e., shorter shreds).

Manipulating the density and structure of the casein matrix can control mozzarella firmness. The more moisture or fat separating casein strands, the lower the protein concentration and the less firm the cheese [26,42]. The structure or strength of the casein matrix relates to the concentration of colloidal calcium phosphate associated with the casein strand [21,40]. Lawrence et al. [36] reported that curd and whey separation is critical. This point determines the basic structure of cheese, because it establishes the calcium content of the curd and the lowest pH that will be attained in the curd.

In general, as mozzarella ages it becomes more adhesive and less shreddable. Chen [6] reported a positive correlation between cheese adhesiveness and proteolysis. Limiting proteolysis can minimize the onset of adhesiveness that occurs during aging, thus maintaining the textural properties required for successful shredding.

B. Flow Agents

The use of flow agents is necessary in commercial shredding operations to ensure controlled distribution of shredded cheese into pouches and to prevent clumping. Flow agents change the shred surface characteristics, making them slide past packaging films and other shreds. This change in the shred surface characteristics affects cheese functionality. Chen and Muthukumarappan [9] investigated how flow agents affect the surface characteristics, melt, and textural attributes of melted mozzarella. The use of flow agents on shreds increased the quantity of blisters and thickness of skinning (a hardened, dehydrated layer of casein on the surface of baked cheese) by about 25%. The use of flow agents on shreds did not affect the cooked cheese color intensity. In addition, flow agents decrease

free oil release by approximately 25%. Flow agent particulates (cellulose or potato starch) adso-rb onto the shredded cheese surface and absorb water from the cheese. The overall water activity of the shreds does change with the use of flow agents. The use of cellulose increased melted cheese hardness by 20%, while the use of potato starch decreased melted cheese hardness by 20%. Panelists noted that melted cheese with potato starch had a slightly slippery mouthfeel. Overall, neither flow agent affected cheese flow rates or chewiness. Also, investigators noted that cellulose, at similar usage rates to potato starch, was visually more apparent on shred surfaces.

C. Cheese Browning

The browning of mozzarella during cooking is due to the Maillard reaction, a heat-induced reaction between sugars (residual lactose or galactose) and protein (free amino groups). The intensity of browning depends on the sugar content of the cheese and the ability of the cheese proteins to remain hydrated during cooking. Johnson and Olson [25] found a positive correlation between residual lactose and galactose levels and brown color intensity when mozzarella was heated. Because of the complex nature of mozzarella as a food product, there are additional factors that influence browning rate and degree. Jaeggi et al. [24] produced skim milk mozzarella with varying levels of a fat mimetic (acid-treated corn starch). These cheeses had higher levels of residual sugar and TCA soluble nitrogen (indicator of proteolysis), which makes for greater browning potential. However, as fat mimetic levels increased, cheese moisture increased, and the degree of browning decreased. The authors presumed that increased moisture helped protect the cheese proteins during cooking, by flashing off and minimizing the heat-induced browning reaction. Rudan and Barbano [53] reduced browning in fat-free and low-fat mozzarella by applying a protective oil coating to shreds prior to cooking. The protective layer prevented protein dehydration and scorching. Forced-air ovens blow hot air onto pizza surfaces, cooking them 2–3 times faster than conventional ovens. Mozzarella cooked in a forced-air oven is more vulnerable to dehydration and skinning.

Moisture protects cheese proteins during cooking. This knowledge can help explain, in part, the relationship between cheese aging and browning intensity. According to Alvarez [1], browning progressively decreased during aging. Oberg et al. [48] reported a relationship between increased cooked color and proteolysis, while Chen and Muthukumarappan [8] found more severe skinning with increased proteolysis. As mozzarella ages, proteolysis breaks casein into peptides and amino acids. With each peptide bond cleaved, two new ionic groups are generated and will compete for available water in the cheese. Creamer and Olson [12] proposed that water previously available for solvation of casein strands becomes tied up with new ionic groups. Due to proteolysis, less water is available to flash off during baking, leaving caseins more susceptible to dehydration and able to participate in heat-induced color reactions or skinning.

D. Melting Properties

During the heating of mozzarella, one can observe several distinct phases. These are described and quantified by a modified squeeze flow method [47]. When cheese is first placed into an oven, the cheese temperature quickly rises, but cheese shape does not change. As the cheese reaches a critical temperature called the softening point, it begins to flow. At this point, the cheese not only continues to rise in temperature, it also changes shape. The cheese matrix collapses, fusing into a semi-solid mass. Flow rate can be measured by calculating the slope of the best-fit line in a cheese height vs. time plot. Next, the melt profile reaches a third critical point called the complete melt point. After this point, cheese height changes are minimal, and cheese temperatures slowly approach the oven temperature.

A study by Chen and Muthukumarappan [8] characterized changes in melt profile attributes over time for pasta filata and non-pasta filata style mozzarella. At all ages, pasta filata mozzarella had lower softening and complete melt temperatures, and higher flow rates than non-pasta filata mozzarella. Softening temperatures decreased throughout aging with the most rapid decrease occurring between two and four weeks. Flow rate and complete melt temperatures were similar: decreasing rapidly during the first four weeks of aging, with relatively small changes between four and eight weeks, then steadily decreasing again between eight and twelve weeks. For all three melt profile descriptors, values changed the least between four and eight weeks. Changes in the melt profile are due to changes in casein-casein interactions within the cheese.

Technically, caseins do not melt, but interactions with each other result in an outcome referred to as melt. As cheese is heated, there is a more rapid relaxation of protein-protein bonds and a greater thermal motion of casein strands, which results in cheese becoming more liquid-like. Mozzarella melt properties are based on the number and strength of casein-casein interactions [40]. Casein-casein interactions may be hydrophobic (attractive forces between casein strands that pull together to keep away from water) or electrostatic [23]. In the pH range of a typical mozzarella (pH 5.15–5.40), we are primarily interested in electrostatic interactions. Electrostatic interaction is the attraction of unlike electric charges; charged calcium phosphate molecules act as a neutralizing bridge between negatively charged clusters on casein strands. To a degree, the higher the colloidal calcium phosphate

levels, the more numerous the casein-casein interactions. This results in a stiffer casein structure and a less meltable mozzarella. Electrostatic repulsion occurs after the solvation of the colloidal calcium phosphate; negatively charged clusters on casein strands push away from each other. The lower the concentrations of colloidal calcium phosphate, the more readily mozzarella melts.

When starter cultures are used in mozzarella manufacturing, a final pH of 5.25 indicates a colloidal calcium phosphate level that produces acceptable melting and stretching. In the case of direct acidified mozzarella, acidulants are added to the milk and there is a lower pH at the time of coagulant addition. In these situations, higher levels of colloidal calcium phosphate are lost into the whey, and sufficient demineralization for melting and stretching occurs at pH 5.6 [26]. At a given pH, the cheese with the least amount of colloidal calcium phosphate will have greater meltability [21].

E. Functionality Related to Melt

Softening and flow characteristics, along with composition and degree of proteolysis, influence the stretch, free oil release, and textural attributes (chewiness, hardness) of melted mozzarella.

Mozzarella's ability to form fibrous strands requires an appropriate flow rate, temperature, and an intact interconnecting casein network. Stretch is the result of casein-casein interactions that are broken and then quickly reformed at different locations in the casein network. When levels of intact casein strands are insufficient due to proteolysis, strands lack continuity and stretch is minimal. If casein-casein interactions are too strong, cheese is more likely to break than form strands. Scenarios include too high a colloidal calcium concentration (electrostatic interactions), or too low a stretching temperature. Stretch-ing occurs at a relatively high concentration of intact casein and within a narrow range of colloidal calcium phosphate [41].

End-user preferences and severe cooking conditions imposed by forced-air type ovens dictate the need for better control of free oil release in melted mozzarella. Free oil release is the separation of liquid fat from melted cheese. It depends on the manufacturing style, cheese composition, and melted cheese flow rates. Low-moisture, part-skim mozzarella manufactured using a non-pasta filata versus pasta filata protocol results in a 50% reduction in free oil release for cheese with similar composition, pH, and degree of proteolysis [7]. During the mixing (plasticizing) of a pasta filata style mozzarella, curd is heated in water to approximately 57°C (milk fat is completely melted and readily coalesces.) Kneading the curd aligns protein strands and entraps coalesced fat and moisture [27,29,60]. During subsequent softening and flow of pasta filata mozzarella, these larger pools of coalesced fat are more likely to leak from the casein matrix. Non-pasta filata mozzarella protocols (stirred curd, direct salted, and pressed) do not have a secondary heating step, resulting in a more continuous protein matrix with smaller pockets of fat and moisture [29]. This structure effectively traps fat during the softening and flow of mozzarella during cooking.

Compositional factors, such as salt content and percentage of fat in the dry matter (FDM), have been reported to affect free oil release. Kindstedt et al [31] proposed that salt (NaCl) influences mozzarella melting and free oil release. Investigators reported a decrease in meltability and free oil release as the concentration of salt increased in blocks of brined mozzarella. They proposed an exchange of sodium ions with casein-bound calcium ions within the casein network. Caseins are much stronger emulsifying agents when dissociated from calcium [57], thus reducing free oil release. The role of fat content on free oil release in melted cheese differs between studies. Investigators Breene et al. [5] and Kindstedt and Rippe [30] reported that cheeses with higher FDM yielded higher free oil levels (Breene et al. FDM 32–50, Kindstedt and Rippe FDM 36–47). A more recent study [9] reported no significant correlation between FDM (range 38 to 46) and free oil release for mozzarella. In the same study, investigators looking at cheddar found the higher FDM (range 50–53) resulted in increased free oil release.

To understand differences in these findings, one must consider how free oil is released during the softening and flow stages of melt, and how FDM affects the meltability of cheese. Thermal motion causes movement of casein strands and the eventual collapse of the casein matrix, allowing exposed fat to leak out and coalesce. Secondly, as temperatures rise, hydrophobic interactions between casein molecules increase. This, in turn, shrinks the casein network, pushes out water, and allows liquid fat to leak out [40]. Olson and Bogenrief [49] reported a biphasic relationship between FDM and meltability. Changes in FDM from 18 to 45% FDM had little effect on meltability, but above 45%, FDM caused a rapid increase in meltability (positive correlation). A FDM of 45% is critical. At this composition, the density of the casein network reaches a point where the solid to liquid ratio influences melt and the ability for casein to hold liquid fat. Both cheese structural elements (strength of casein-casein interactions) and protein density (number of casein-casein interactions) help explain levels of free oil release. Mozzarella can be found in a variety of foods (e.g., pizza, breaded appetizer, breads), each requiring specific functionality. Understanding the relationship between melt profiles and textural attributes enables controlled manufacture, where close tolerances of physical properties are to be expected. Melted cheese cohesiveness can be defined as the degree melted cheese holds together in a mass after chewing. In general, cohesiveness increases over time, with the most rapid changes occurring during the

first four weeks of aging [8]. Hardness and chewiness uniformly decrease over time. Melted cheese hardness correlates best to softening temperature; as softening temperature decreases, hardness decreases [8]. As cheese is heated, thermal energy is stored as movement in the protein, fat, and water molecules, while the cheese structural integrity is not changing. At the softening point, the cheese structure can hold no additional thermal energy and it begins to flow [40]. The amount of thermal energy the cheese is able to store relates to the strength of the casein matrix. Hardness is the force required to bite through melted cheese (rupture the casein matrix). Thus, it follows that the softening temperature (indicator of melted cheese structural strength) correlates well to melted cheese hardness. Melted cheese chewiness correlates best to flow rate; as flow rate increases, chewiness decreases [9]. As mozzarella flows, casein-casein interactions relax (break) and quickly reform [40]. In a mozzarella with greater flow, casein-casein interactions more readily relax, resulting in a more rapid movement of casein molecules past one another. Chewiness is defined as the length of time required to chew cheese before swallowing. As chewiness decreases, casein-casein interactions are more readily relaxing and, over time, not reforming.

VII. RELATED TOPICS

A. Composition

From a compositional standpoint, there are four main types of mozzarella cheese. Due to these compositional and resulting functional differences, there are slightly altered make procedures as well (see [34]). In the U.S., mozzarella cheeses are more precisely defined by standards of identity [62]. These regulations define final compositional requirements (Table 150.2), as well as certain manufacturing procedures. These regulations specify that mozzarella must be made from cow's milk, nonfat milk, cream, or the corresponding buffalo milk products. Cow and buffalo milk may not be blended. The procedure may also include rennet, vinegar (acetic acid), calcium chloride, coloring to mask natural yellow color, salt, and an antimycotic agent. If a product is made from buffalo milk, the label must include the phrase, "made with water buffalo milk."

B. Cheese Yield

The Van Slyke Formula [63], developed in the 1920s, is still a simple and reliable method for estimating cheese yield. Only milk composition (percentage of milk fat and milk casein) and cheese moisture are required in cheese yield calculations. The Van Slyke Formula was originally deve-loped for cheddar, but with a few modifications can readily be used for mozzarella. First, the percentage of fat recovery in the cheese needs to be adjusted. In addition to the fat losses that occur during the formation of curd, a significant amount of fat is lost during mixing and molding. In yield calculations for mozzarella, the value for percentage of fat recovery needs to be decreased from 93% (typical cheddar value) to 86% (mozzarella ranges from 84 to 88%). Secondly, the R-value is a correction factor that takes into account the solids—non-fat, non-casein, which contribute to cheese yield. It includes water soluble minerals, lactic acid, whey proteins, and other minor components. The R-value is dependent on the moisture content of the cheese, since moisture is the mechanism for conveying these contributing factors. Since the formula was originally written for cheddar at ~37% moisture, and a typical mozzarella contains ~48% moisture, this factor needs to be increased from 1.09 to 1.13.

$$\text{Cheese yield} = \frac{(\%\ \text{Milk fat} \times \text{Fat Recovery} + \%\ \text{Casein} \times \text{Casein Recovery}) \times \text{R}}{(100 - \%\ \text{Cheese Moisture})}$$

Assumptions: Fat Recovery (pasta filata style) = 86%
Casein Recovery = 96%
R value (solids non-fat, non-casein factor) = 1.13

TABLE 150.2
U.S. Compositional Standards of Identity for Mozzarella and Scamorza Cheese Types (U.S. CFR, 2003)

Type	Milkfat (% of solids)	Moisture (%)	Labeling Requirement
Mozzarella and Scamorza	≥45	≤60–>52	"mozzarella cheese" or "scamorza cheese"
Low-moisture	≥45	≤52–>45	"low-moisture mozzarella cheese" or "low-moisture scamorza cheese"
Part-skim	<45–≥30	≤60–>52	"part-skim mozzarella cheese" or "part-skim scamorza cheese"
Low-moisture part-skim	<45–≥30	≤52–>45	"low-moisture, part-skim mozzarella cheese" or "low-moisture, part-skim scamorza cheese"

Factors that affect the percentage of fat recovery include initial milk quality, cheese making equipment (vat type, stirring mechanisms), or mixer-molder parameters (type of mixing mechanisms, stretching temperature, finished mozzarella size—string cheese, 6 lb or 20 lb loaf). Factors that affect casein recovery include initial milk quality, milk pasteurization, or milk standardization methods [38].

C. Storage and Shelf Life

Mozzarella cheese will have optimal functional properties between 2 and 6 weeks of age, and a shelf life of up to 4 months. Due to its potentially high moisture and low salt content, there have been some concerns about the safety of mozzarella cheese. For the most part, these concerns are lessened by the regulatory requirement for pasteurized milk, the high temperatures in the mixing-molding operation, and overall improved plant hygiene conditions, especially on the brining and packaging side. An outbreak of *Salmonella javiana* linked to mozzarella cheese consumption prompted a series of studies that examined the microbial lethality achieved during mozzarella manufacturing [14,15,22,37]. Another study identified high levels of the spoilage organism, *Klebsiella pneumonia* in two production lots of mozzarella. This latter microbe is also very heat labile, suggesting that the contamination occurred as a matter of inadequate hygiene after the thermal processes [43]. There are several studies that document the ability of certain strains of Listeria to survive in brine tanks [35]. Other common contaminants include yeasts, molds, and coliform bacteria.

The characteristics of the spoilage of mozzarella cheese are not well described, but may include substantial increases in contaminant microbial counts (e.g., yeast, mold, and coliform), increases in free fatty acids, off-flavor development, curd softening, development of slimy surfaces, poor melt attributes, and visual mold outgrowth [11]. Due to the high enzymatic activities of cultured mozzarella, these products may become softer and whey off more readily that the acid set variety.

VIII. CONCLUSIONS

In spite of its ancient beginnings, understanding the physical, microbial and chemical properties of mozzarella cheeses certainly poses a daunting task. Additionally, the functionality of mozzarella cheeses are continually challenged, as the once simple, fresh cheese is currently expected to withstand rigorous marketing requirements, yet still deliver the desirable characteristics of flavor, melting, browning, and stretching. Although a wealth of knowledge has been generated over the last few decades, work continues to relate the manufacturing variables to the instrumental, chemical, and sensory attributes of significance to mozzarella cheese.

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151 Processed Cheese

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CONTENTS

I. INTRODUCTION

Processed cheese is manufactured by blending shredded natural cheeses of different types and degrees of maturity with emulsifying agents, adding water, and heating the blend under partial vacuum with constant agitation until a homogeneous mass is obtained. Besides natural cheeses, various other dairy and nondairy ingredients may be included in the blend.

While natural cheese varieties were known and appreciated as a valuable food component in ancient Roman times more than 2,000 years ago, processed cheese seems to be a brand-new product, originating in the 20th century, though based on natural cheeses. In Europe, the invention of processed cheese is connected to the year 1912 and a Swiss patent that claimed the introduction of citrate as an emulsifying and calcium-sequestering agent. The industrial production of processed cheese in Europe was then started. This invention was possibly connected to the Swiss national dish, fondue, in which cheese was heat-treated in the presence of wine, and in which tartrate from the wine had the emulsifying effect. Quite independently in 1917, the production of processed cheddar cheese by using the mixture of phosphates and citrates as emulsifying agents (developed by Kraft in Chicago), was started in the United States. Further progress in processed cheese production was the introduction of polyphosphates into the processing procedure.

The basic theory of processed cheese manufacture lies on the change of the state of casein from coarsely dispersed calcium paracaseinate in the raw cheese due to application of heat, agitation and particular salts as emulsifying/peptising agents, into a homogeneous, free flowing condition: the sol state.

In addition to the mentioned patents, there was numerous other research, which deserves attention and has contributed to the creation of completely new cheese-type products. In between, various research attempts targeted to produce long-life cheese by canning and pasteurization have failed. Such procedures were only successfully developed for soft cheese (Camembert), and later for semi-hard cheeses (Dutch cheeses), though were never suitable for hard cheeses. All attempts to pasteurize Emmentaler caused a breakdown of the cheese structure with separation of fat and water. On the other hand, proteolysis and lipolysis in natural hard cheeses always continued during storage, beyond the optimal flavor point, and most often in an accelerated degree. Decomposition of proteins and lipids results in a nutritionally defective product, unsuitable for consumption. This is the problem

if long storage of natural cheeses is expected, e.g., for export to overseas or to the Tropics. The best solution of the long storage problem for all native cheese varieties was the invention of processed cheese.

In addition, processed cheese manufacture enabled the usage of natural cheeses that were difficult or impossible to sell (such as cheeses with mechanical deformations and localized molds), as well as cheese trimmings, to obtain a product of better keeping quality.

The main advantages of processed cheese as compared to natural cheese varieties are:

- Economic advantages: usage of second class raw material (microbially adequate), reduced refrigeration costs during storage and transport, increased keeping quality, as well as adjustable and imaginative packaging.
- Great versatility of type and intensity in flavor: from mild to sharp native (natural) cheese flavor, specific flavoring, or spices of dairy or nondairy origin.
- Milk replacement: excellent source of nutritionally high-valued milk components for children or specific consumption groups.
- Suitability for home use, as well as for fast-food restaurants.

Processed cheese is grouped according to the following characteristics: composition, water content, and consistency. According to these criteria, there are four main groups: processed cheese blocks, processed cheese foods, processed cheese spreads, and processed cheese analogues (1–6) (Table 151.1). Additional subtypes are processed cheese slices and smoked processed cheese.

II. PRINCIPLES

The principles of cheese processing are rather simple, but require a skillful professional to control all the included ingredients, their concentrations in the blend, and processing parameters. Emulsifying agents, known in the processed cheese industry as "melting salts," are central to processed cheese production. They are used in the processing procedure to provide a uniform structure, also affecting the chemical, physical, and microbial quality of the product.

Emulsifying agents are not true emulsifiers according to chemical terminology — they are not necessarily surface-active compounds, although they help in emulsifying fat and stabilizing the emulsion. However, true emulsifiers may be included in commercially produced emulsifying agents that are mixtures of particular compounds. Most common components of the commercial salt mixtures are: phosphates, polyphosphates, and citrates, although compounds such as sodium potassium tartrate, complex sodium aluminum phosphate, or trihydroxyglutaric acid, could be used as well. Generally, polyphosphate application in the emulsifying agent blend results in processed cheese with superior structure and better keeping quality compared to other emulsifying salts as blend components.

The affinity, i.e., sequestering ability, of common emulsifiers for calcium increases in the following order: $NaH_2PO_4 < Na_2HPO_4 < Na_2H_2P_2O_7 < Na_3HP_2O_7 < Na_4P_2O_7 < Na_5P_3O_{10}$. The affinity of protein for the cations and anions of melting salts is determined by the valency of these ions (1,2).

The blend composition is a commercial secret, usually protected by the producer. The main types and roles of emulsifying agents, their characteristics, possible combinations, and commercial producers are listed elsewhere (1–3,6–8).

TABLE 151.1
Some Characteristics of Processed Cheese Types (1)

Type of Product	Ingredients	Cooking Temperature (°C)	Composition	pH
Processed cheese block	Natural cheese, emulsifiers, NaCl, coloring	71–80 80–85 74–85	Moisture and fat contents correspond to the legal limit for natural cheese	5.6–5.8 5.4–5.6 5.4–5.7
Processed cheese food	Same as above, plus optional food ingredients such as milk, skim milk, whey, cream, albumin, skim milk cheese; organic acids	79–85	≤44% moisture, < 23% fat	5.2–5.6
Processed cheese spread	Same as processed cheese food plus gums for water retention	88–91 85–98 90–95	≥44% and ≤60% moisture ≤55% moisture	5.2 5.7–5.9 5.8–6.0
Processed cheese analogue	Sodium caseinate, calcium caseinate, suitable vegetable proteins and fats (soyabean, coconut), emulsifying agent, salt, artificial flavor	As for processed cheese food	As for processed cheese food	

The essential role of emulsifying agents in the manufacture of processed cheese is to solubilize calcium paracaseinate, sequestering calcium and thus dispersing proteins. Calcium in the Ca-paracaseinate complex of natural cheese is removed by the ion-exchange properties of emulsifying agents, solubilizing the paracaseinate, usually as Na-caseinate. Chemically, this reaction could be observed as (Figure 151.1) (2).

Other beneficial effects provided by emulsifying agents are:

- hydrating and swelling of proteins;
- emulsifying fat and stabilizing the emulsion;
- controlling and stabilizing pH;
- forming an appropriate structure after cooling.

Rayan et al. (9) have researched the emulsification of fat during cheddar cheese processing using TEM (transmission electron microscopy). Figure 151.2A shows their findings after 10 minutes of processing. However, SEM (scanning electron microscopy) reveals large fat globules in processed cheese at the beginning of processing (Figure 151.2B).

The quantity of emulsifying agent permitted in the processed cheese blend prepared for processing is prescribed by government regulations, usually not higher than 3–4%. Since the effects of emulsifying agents are responsible for the unique features of processed cheese production, their correct application is of utmost importance for the quality of the final product.

III. MANUFACTURING

The general technological process for processed cheese manufacture consists of the following operations: natural cheese selection, formulation, blending, shredding, emulsifying agent addition, processing (thermal treatment), homogenization (optional), packaging, cooling, and storage (1,2,3). A flow chart of processed cheese manufacture is presented in Figure 151.3 (4).

A. Natural Cheese Selection

Processed cheese quality, particularly taste and flavor, depends on proper natural cheese selection. The most important criteria for cheese selection are: type, maturity, flavor, consistency and structure, nature and character of the additives, legal requirements, and the potential market (1–3,5). Processed cheeses manufactured from only one cheese variety of different degrees of maturity are very popular in some countries, such as: processed cheddar and mozzarella in the USA and Canada; cheddar in the UK and Australia; and Emmental in Western Europe. More frequently, processed cheeses are produced from a mix of various natural cheese types. The result is easier processing and a better flavor balance.

The selection and combination of native (natural) cheeses of proper maturity is of special importance for processed cheese production. Intact casein, present more in young cheese, has an emulsifying effect on milkfat and stabilizes the emulsion, forming a "long structure." This effect is not expressed by partially hydrolyzed protein, predominating in matured cheese ("short structure"). For example, high-fat spreadable processed cheese requires a larger proportion of young cheese in the blend with a correspondingly higher intact casein content (2,3,5,6). Proper selection of natural cheeses of good quality is not a guarantee for obtaining the desired high quality processed cheese, if the process parameters used are improper. However, some cheeses with incorrect physical characteristics can be successfully used in processed cheese manufacture, as it is possible to correct them by skillful blending. Natural cheeses with microbial defects (spore-forming, gas-producing, and pathogenic bacteria) should not be selected for processing.

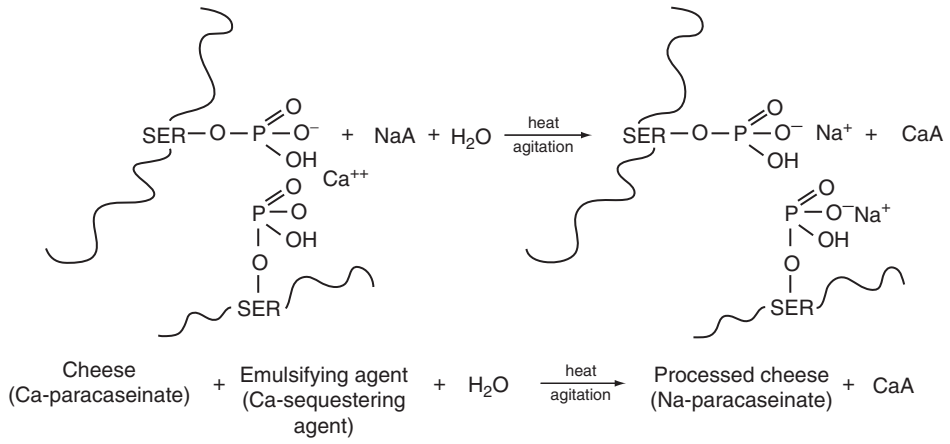
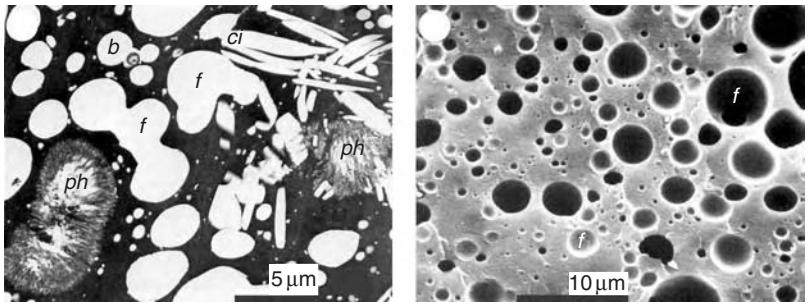


FIGURE 151.1 Chemical reaction during cheese processing (2).



(A) (B)

FIGURE 151.2 Processed cheese microstructure: A. Transmission electron microscopy (TEM) of milkfat emulsification during cheese processing: f-fat; m-protein matrix; ci-crystalline sodium citrate; ph-calcium phosphate crystals; and b-bacterium; B. Scanning electron macroscopy (SEM) of processed cheese: f-large fat particles at the beginning of melting (9).

B. Formulation

Formulation of the ingredients is made in such a way as to yield a desired finished product composition. Formulation is conducted on the basis of fat and dry matter contents of the natural cheeses, including all blend components, added water, and condensate from live steam used during processing. If necessary, additional adjustments of fat and dry matter are possible before processing is completed (1,2).

C. Blending

Blending is influenced by the desired characteristics of the processed cheese type. Generally, in processed cheese spread manufacture the basic raw material in the blend is semi-mature cheese of shorter structure, i.e., approximately 50% semi-mature cheese, 20% mature cheese, and 30% young cheese (10). The recommended formulation for processed cheese block is 70–75% mild cheese and 25–30% semi-mature or mature cheese (6). In contrast, the blend for processed cheese slices must contain 30–40% young cheese, 50–60% mild cheese, and only 10% mature cheese (5,6).

There are some advantages and disadvantages of a high content of young cheese, as well as of a high content of extra-mature cheese, in blends for processed cheese manufacture (1–3). The main advantages if a high content of young cheese is used in the blend are: reduction of raw material costs, possibility of using cheeses with poor curing properties, formation of a stable emulsion with high water binding capacity, and production of a firm body with good slicing properties. The main disadvantages are: production of a tasteless cheese, an emulsifier-like off-flavor, excessive swelling, tendency to harden during storage, and the presence of small air bubbles developed due to the high viscosity of the blend.

A high content of extra-mature cheese in the blend has certain advantages: i.e., development of a full flavor, good flow, and high melting properties. However, there are certain disadvantages: sharp flavor development, low emulsion stability, and soft consistency.

Various other dairy and non-dairy ingredients are used in the production of different processed cheese types. Since the quality of the final product is influenced considerably by all the components present in the blend, the non-cheese components must also fulfill certain qualitative requirements. The most frequently used dairy ingredients are skim milk powder, casein, caseinates, various whey products (whey powder and whey protein concentrates — WPC), and milkfat products.

Skim milk powder improves the spreadability and stability of processed cheese and promotes the creaming properties. If used in quantities exceeding 12% of the total mass, it may adversely affect the consistency or may remain undissolved. Discoloration of processed cheese due to the Maillard reaction is excluded if total lactose content is not over 6% in the final product.

Although whey powder is the most common whey product used in processed cheeses (in concentrations up to 7%), whey protein products with lower mineral and lactose contents are preferable because they yield processed cheeses with better flavor characteristics. However, some other whey products could be successfully used in the processed cheese blend as well, such as: whey concentrate (2–4%), precipitated whey proteins (up to 25% with flavor correction), and native whey protein concentrates obtained by ultrafiltration (5–20%) (1–3,11). The addition of different protein concentrates (denaturated and undenaturated-ultrafiltered) to a processed cheese may lead to a product with particular functional characteristics. Control and manipulation of heating and pH conditions during protein concentrate processing may affect the inherent properties of the protein itself and alter the interactions of the protein with other components of the food system (12). The cheese may be made from 100% UF cheese, containing most and preferably all of the whey proteins from milk, or from a blend of UF and conventional cheese. This process was recently patented in the

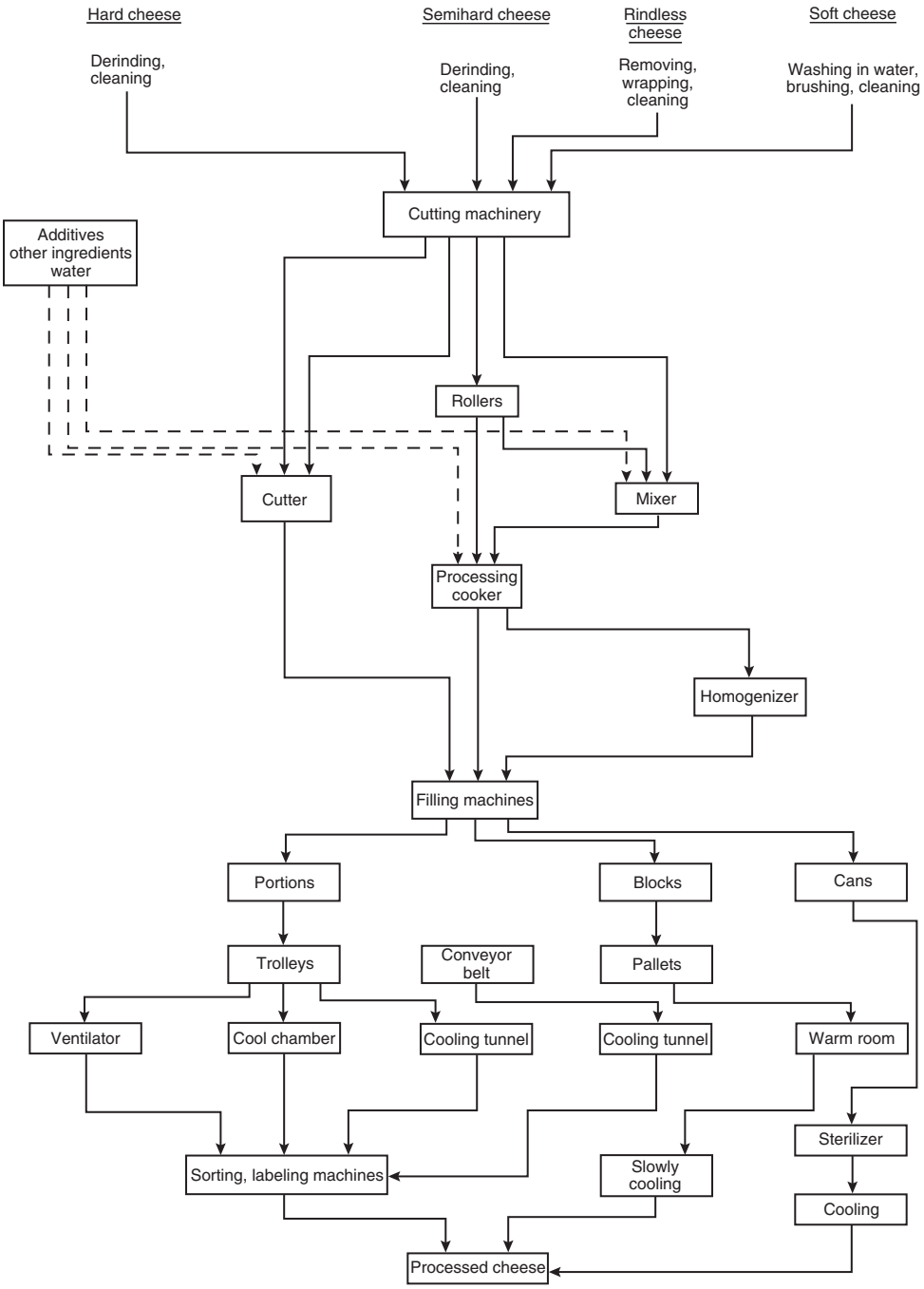


FIGURE 151.3 Flow chart of processed cheese manufacture (4).

USA (13). Generally, whey products incorporated in processed cheese blends promote both the nutritive and economic value of processed cheese.

If the blend contains milk protein coprecipitates, stability of the cheese emulsion and physical characteristics of the processed cheese are increased. Their emulsifying capacity is so high, that they reduce the amount of emulsifying salt added. This is particularly important for dietary and special food products, where limitation of the sodium content may be desirable. Milk protein coprecipitates should not exceed 5% in processed cheeses (1–3).

Butter, cream, and butter oil may be added as milkfat ingredients. All milkfat products used to adjust the fat content of the processed cheese to the desired level must be of high quality and free from off-flavors.

Cheese bases can also be used in processed cheese manufacture. Numerous procedures have been developed for producing economically favorable cheese or cheese

bases intended exclusively for processing. One of these processes for production of a cheese base by ultrafiltration (UF) and diafiltration of whole milk was developed and patented in Germany (14). The retentate (40% dry matter, 4.17% lactose) obtained after ultrafiltration was pasteurized, cooled to 30°C, inoculated with lactic starter, incubated at 25°C until the pH reached 5.2, and packed in plastic bags. The vacuum packaged cheese bases can be stored and later used in processed cheese production in combination with ripe cheese, in an 80:20 ratio.

The ultrafiltration application in preparing raw material for a processed cheese blend pays off the investment costs after only two years of operation.

In Germany, a kind of curd product, called Schmelzpack, is manufactured especially for processed cheese production. Containing 90–100% unhydrolysed casein, it can be blended with natural cheeses of diverse sources, types, and maturity. Good results with the use of chicken pepsin for the peptonization of curd for incorporation into processed cheese have also been reported (1,2,8).

The possibility of successful processed cheese manufacture from cheddar cheese and skim milk powder cheese base has also been investigated (15). Cheese base was produced from reconstituted skim milk powder after ultrafiltration, and was afterwards subjected to accelerated ripening by adding commercial proteolytic enzyme. Obtained processed cheeses were of good quality, if containing less than 40% cheese base.

Relatively new process to achieve the desired flavor in processed cheese is by addition of modified cheese flavors (enzymatically modified cheese — EMC). EMC is produced by inoculation and incubation of particular enzymes with specially designed media, followed by their heat inactivation. Flavor intensity of EMC is 10–30 times greater than the corresponding traditional cheese. The successful substitution of 15% ripe cheese in a processed cheese blend with a mixture of young cheese and whey powder by using cheddar or Parmesan cheese flavor (EMC), has been achieved (11). Headspace analysis showed that experimental processed cheese produced with EMC resulted in higher total aroma content than control samples. Thus, EMC application in processed cheese blends would be economically feasible, resulting in a product of excellent quality (1,2).

Investigations were carried out aimed at improving the economy of processed cheese manufacture by incorporating white cheese in the blend at different concentrations. The easy and rapid manufacture of white cheese makes it a suitable ingredient in processed cheese (16,17). White cheese in concentrations up to 33% blended well with mild and medium ripened cheddar cheese and resulted in an acceptable product. The presence of white cheese in processed cheese can be detected by transmission electron microscopy on the basis of the core-and-shell ultrastructure that is stable during storage (Figure 151.4) (17).

Another dairy-based product that could be included in the blend for processing is precooked cheese (or "rework"). Usually precooked cheese is used in processed the manufacture of cheese spreads to increase the creaming properties of the blend. It is used to improve the texture and stability of the finished product, especially when very young or very mature raw cheese is used for processing. The percentage used in the blend varies from 1 to 30%, depending on the quality of the rework, and the type of processed cheese desired. The presence of rework in processed cheese food has been detected by using electron microscopy (18). Some dairies produce precooked cheese that is stored in large containers.

Methods and composition for fat-free processed cheese manufacture have been patented recently in the USA (19). Processed cheese products with reduced calories and fat are produced by combining high moisture, fat-free, skim milk cheese with a fatty substance which is non-absorbable and non-digestable, and is lower in net caloric efficiency than butterfat, animal fat, or vegetable fat. A suitable fatty substance is a polyol polyester, such as a sucrose polyester.

The nondairy ingredients of the blend for processed cheese manufacture include spices, meat products, vegetables, fruit syrup, cocoa, honey, vanilla, hazelnut, coffee extract, and other components. Their quantities must be properly prescribed for blending. They all must be sterile and of the highest quality with typical flavor (1,2).

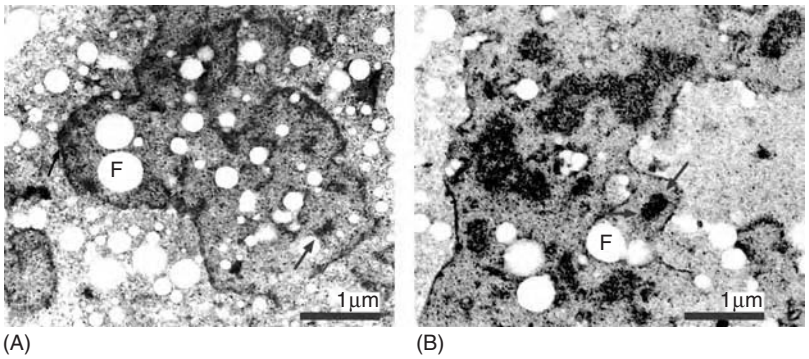


FIGURE 151.4 TEM of processed cheese made with white cheese: A. 16% white cheese; B. 8% white cheese; F-fat particles (18).

D. Shredding

Shredding includes grinding and milling of the components (primarily hard and semi-hard cheeses), enabling better contact between emulsifying agents and blend ingredients. Usually, cheese prepared in this operation is transferred directly to a processing cooker. However, different devices can be used for better blend mixing prior to thermal treatment. This operation can be performed in several ways: shredded cheese is mixed alone, or in combination with other ingredients (butter, milk powder, whey powder); cheese is mixed with water and other ingredients; and cheese is mixed with water, emulsifying agents and other ingredients (20).

E. Emulsifying Agent Addition

The last step in preparing the blend for processing is the addition of emulsifying agents. The type and role of emulsifying agents in processed cheese production is described earlier in the "Principles" section.

F. Processing

Processing involves heat treatment of the previously prepared blend with direct and indirect steam under partial vacuum. The product is constantly agitated when either the continuous or batch method is applied. If processing is carried out in a cooker discontinuously, the temperature reached is 71–95°C for 4–15 min, depending on various parameters (1–3,5). This thermal treatment also provides a pasteurization effect. In newly-developed cookers, it is also possible to reach temperatures up to 140°C. A processing cooker or kettle consists of 2 double-jacketed, round, stainless-steel vessels of various sizes (2–1001), fitted with corresponding lids, three-stage switchable stirring equipment, and fittings for direct steam and vacuum. Double jackets enable indirect steam heating as well. There are specially designed units, similar to cutters used in meat processing, where cutting is completed in the same unit prior to processing, by the aid of rapidly rotating knives with simultaneous heating and homogenization of the product. Two different cooker designs are presented in Figure 151.5.

The horizontal, tube-shaped processing unit, in addition to the most common round design, is also popular, particularly in the United States and Canada. This type of processing unit can process large quantities. It is constructed for batch operation, but also can be continuous. This installation is fitted with one or two mixing arms up to 4-m long. It is fed at one end and the final product is discharged at the opposite end within 4–6 minutes.

By continuous processing, the blend is sterilized at temperatures of 130–145°C for 2–3 s in a battery of stainless steel tubes (1,2,6). Continuous cheese cookers are not commonly used in the processed cheese industry, although they were developed as early as 1920. The main reason is the great versatility in processed cheese products; therefore changing over to a different product in the continuous process, which requires intermediate cleaning which cannot be considered economical. Review of the literature describes a continuous process for simultaneous melting, homogenization, and sterilization in processed cheese manufacture (1,2).

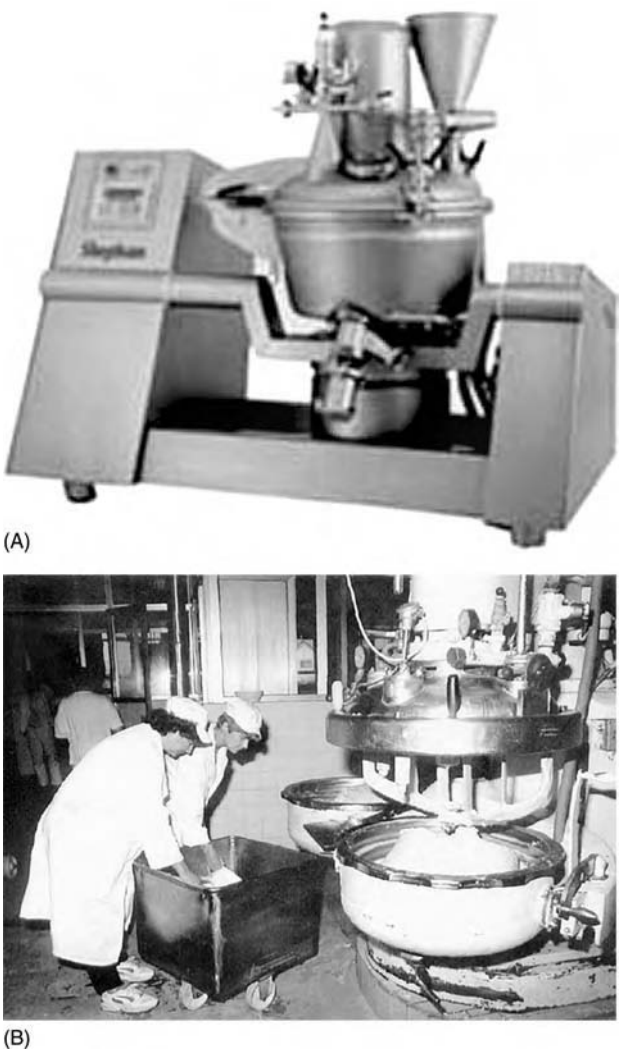


FIGURE 151.5 Equipment for processed cheese manufacture: A. Universal Stephan unit, UM/SK150; B. Voegele machine in Dairy "Mlekoprodukt," Zrenjanin, Serbia.

The most important manufacturing conditions, i.e., chemical, mechanical, and thermal parameters, that affect the processing, and thus the quality of the final product, are:

- temperature: heat induced by direct or indirect steam;
- processing duration: depending on size and construction of the cooker, quality of raw material and blend composition, mechanical treatment, emulsifying agent used, desired keeping quality, etc;

- agitation: slow, at lowest speed of 60–90 rpm for processed cheese block, or fast, at 120–150 rpm for processed cheese spreads;
- acidity (pH): a rather limited pH range; an increase of pH value and decrease of H^+ causes better peptization of casein, but can spoil keeping quality and flavor, whereas a decrease in pH value introduces thickening and solidifying of cheese structure. Higher pH values also favor more rapid product deterioration, in the event of post-pasteurization contamination (1,2).

A new method for processed cheese manufacture has been developed and patented in the USA (21). Processed cheese is made according to the following steps:

- thermal and mechanical treatment such as stirring the initial cheese fractionated into pieces at a temperature less than 60°C;
- cooling the mixture to a temperature less than 50°C;
- if required, mechanically treating the paste to complete the building up of the protein structure and stabilizing the emulsion; and
- forming the paste.

The first two steps are carried out by adding a mixture of emulsifying and chelating agents of calcium and sorbic acid (or a salt thereof) to the initial cheese product.

G. Homogenization

Homogenization improves the consistency, structure, appearance, and flavor of the processed cheese. It also improves the stability of the fat emulsion by decreasing the average fat globule size. It is recommended only for blends with high fat content, because homogenization involves unnecessary additional capital, operational, and maintenance costs. Certain additives or spices must be mixed after homogenization to retain their original form.

H. Packaging

Processed cheese is usually packed and wrapped in laminated foil; tubes, cups, cans, cardboard, or plastic containers; in sausage form; and occasionally in glass jars. Processed cheese in sausage form can be subjected to smoking. The continuous formation, slicing, and packing of cheese slices suitable for sandwiches, is used very often nowadays. Slices may also be obtained by mechanically slicing rectangular processed cheese blocks.

I. Cooling

The method and intensity of cooling depend on the processed cheese type. Processed cheese blocks are cooled slowly, while cooling of processed cheese spreads should be as fast as possible.

J. Storage

Processed cheese should be stored at a temperature range of 5 to 10°C. Sometimes, calcium diphosphate or calcium pyrophosphate crystals may be formed on the surface of processed cheese, at such temperatures, causing a gritty texture.

IV. QUALITY

The influence of blend composition, emulsifying agent type, process parameters, and equipment on the final processed cheese quality has been researched from the beginning of its production in the past century until now. Some data related to processed cheese quality are described, along with processing parameters, in the "Manufacture" section of this chapter. The typical composition of processed cheese is presented in Table 151.2 (3).

New trends in processed cheese technology that should be particularly emphasized are the developments of processed cheese analogues and low-fat processed cheese.

The possibility of substitution of one or more dairy components in processed cheese has been investigated around the world, most intensively in the USA. For example, the most popular processed cheese analogue variants with "mozzarella" cheese flavor and "American" cheese are suitable as toppings in various food industry products.

TABLE 151.2
Typical Processed Cheese Composition (3)

Component	Contents in 100 g of Processed Cheese	
	45% Fat in Total Solids	60% Fat in Total Solids
Water	51.3%	50.6%
Fat	23.6%	30.4%
Proteins	14.4%	13.2%
Natrium (Sodium ?)	1.26 mg	1.01 mg
Potassium	65.0 mg	108.0 mg
Calcium	547.0 mg	355.0 mg
Phosphor	944.0 mg	795.0 mg
Vitamin A	0.30 mg	—
Vitamin D	3.13 µg	—
Vitamin B_1	34.0 µg	40.0 µg
Vitamin B_2	0.38 mg	0.35 mg
Pantotenic Acid	0.52 mg	0.47 mg
Vitamin B_6	70.0 µg	80.0 µg
Biotin	3.60 µg	2.80 µg
Folic Acid	3.46 µg	3.40 µg
Vitamin B_{12}	0.25 µg	0.25 µg
Vitamin C	traces	traces
Energy value (kJ/kcal)	1178/282	1490/339
Utilization (kJ/kcal)	1125/268 (=95%)	1354/323 (=95%)

TABLE 151.3
Most Common Quality Defects in Processed Cheese (2)

Defect	Cause	Correction
	Flavor	
Moldy	Air contamination, moldy raw cheese	Use hermetically sealed foils, eliminate all moldy cheeses from the blend
Acid	Excess phosphates	Reduce emulsifier
Salty	Salty raw cheese or other components, too much emulsifier	Add younger cheese, unsalted cheese, or fresh curd to blend, decrease the quantity of emulsifier
Soapy	High pH value (>6.2)	Add young cheese (with lower pH value), use emulsifying agent with lower pH value
Burned with browning	Maillard reaction (lactose and amino acids); usually when very young cheese or whey products are present	Use processing temperatures < 90°C, cool processed cheese immediately after packaging, avoid large containers, store < 30°C, avoid high pH values in final product
	Texture (Body, Consistency)	
Too soft	High moisture, improper emulsifier, insufficient emulsifier, high pH, fast cooling, excess ripe cheese in blend, prolonged or slow processing	Reduce water content, use suitable emulsifier, increase emulsifier content, decrease pH, slow down agitation and cooling, increase proportion of young cheese in blend, reduce processing time, increase agitation speed
Too hard	Low moisture, improper or excess emulsifier, low pH, slow cooling, improper blend, excessively creamed or overcreamed reworked cheese	Increase water content, use proper emulsifier, decrease emulsifier content, increase pH, speed up cooling, change blend composition, avoid addition of creamed or overcreamed reworked cheese
Hard, with water separation	Collodial change in cheese structure (overcreaming), bacteriological action leading to reduced pH	Remove all factors that affect excess creaming, choose blend components carefully, keep processing temperatures >85°C
Inhomogeneous (grainy)	Unsuitable blend, improper emulsifier, insufficient or excess emulsifier, low pH, short processing time, low processing temperature, improper amount of added water, inadequate agitation, collodial or bacteriological changes caused by improper storage	Add younger cheese, use suitable emulsifier, correct emulsifier quantity, correct pH, prolong processing time, increase processing temperature >85°C, increase the amount of added water, continue agitation during processing and filling, proper cold storage
Sticky (adhering to lid foil)	Sticky foil, insufficiently impregnated, excessively high pH, processed mass left hot too long without agitation	Change aluminium foil, decrease water addition and add in two portions, increase proportions of ripe cheese or cause better creaming, keep pH < 6.0, continue agitation until packaging
	Appearance	
Holes (blown)	Bacteriological changes (growth of *Clostridia*, coliform, or propionic bacteria); physical changes (occluded air, CO_2 from emulsifier mixture), citrates, holes filled up with fluid from emulsifying agent having low solubility, chemical changes (hydrogen from reaction between processed cheese and aluminium foil)	Select cheese blend components, keep processing temperatures >95°C, use proper vacuum, preheat citrate emulsifier before processing, extend processing time, test porosity of aluminium foil and, if necessary, change it
Crystals	Calcium diphosphate and calcium monophosphate crystals (when phosphates are used in emulsifying agent), calcium crystals (when citrates are used in emulsifying agent), crystals due to undissolved emulsifying salt, large crystals due to excess emulsifier, lactose crystal formation caused by excess whey concentrates or low water content, light-colored, grainy precipitate of tyrosine (very mature cheese in blend)	Avoid monophosphates and diphosphates as emulsifying agent, or use in combination with higher phosphates and polyphosphates, exclude citrates from blend, distribute emulsifying agent, exclude sandy reworked cheese, use better emulsifying agent, increase processing time, add emulsifying agent in solution, use prescribed quantity of emulsifier, reduce level of whey products and increase water content, exclude raw cheese that contains tyrosine crystals

Processed cheese analogues are produced with similar processing parameters and identical equipment as conventional processed cheese. Blends for this processed cheese type can be tailored to obtain a final product of desirable chemical, physical, and nutritive characteristics, as well as lower costs. In processed cheese analogue manufacture, the essential ingredients are: milk proteins — casein, caseinates, whey proteins; vegetable proteins — soya (predominately), gluten, coconut, etc.; vegetable fats; flavorings; vitamins; minerals; emulsifying agents; and lactic/citric or other food-grade acid (for pH correction). The newly developed procedures for processed cheese analogue manufacture are mainly patented. The results of numerous investigations, as well as the composition of blends intended for processed cheese analogue manufacture, are summarized in publications by Caric´, Kalab (1) and Caric´ (2).

Investigations were also carried out aimed at developing processed cheese with a natural cheese appearance. The French cheese company, Fromageries Bel, has patented a process which includes the following steps: melting a blend of cheeses, casein, skim milk, butter, and emulsifying agent; agitating the mixture under an inert gas (N_2); defoaming of the obtained product; shaping; and rapid cooling. The final processed cheese has an open texture with visible eyes (aproximately 0.5 mm in diameter), similar to conventional cheeses (22,23).

Another modern trend in the processed cheese industry is the development of low-fat and low-sodium processed cheese products (1,2,24). Low-fat sliced processed cheese with natural or herbal flavor and 50% less fat has been developed. No significant natural or herbal flavor losses were noticed compared to full-fat processed cheese. The manufacture of virtually fat-free processed cheese, and virtually fat-free processed cheese analogues, have also been patented. Blends for virtually fat-free processed cheese (ca. 1.67% fat) consist of skim milk cheese, skim milk powder, whey, and buttermilk powder (25). Innovators found the body, texture, and eating properties of virtually fat-free processed cheese analogues to be identical to classic processed cheese (26).

Good quality processed cheese types (Table 151.1) should have typical flavor, shiny surfaces, homogeneous and smooth structure, uniform color, and no fermentation gas holes. The most common quality defects, their causes, and corrections are summarized in Table 151.3 (2).

Generally, causes of quality defects of processed cheese are unsuitable blend, irregular quality or quantity of emulsifying agent(s), or improper processing. Many of the listed quality defects originate from the natural cheeses used in the blend. Some of them can be avoided by adequate processing. Crystal formation (caused by emulsifying agent type and quantity), and discoloration (result of inadequate process parameters), are most the often visible physicochemical quality defects of processed cheese (1–3).

Processed cheese with minor defects can be recovered by reworking small quantities of it into subsequent batches. Processed cheese with more serious defects that cannot be corrected, such as presence of metal ions, excessive Maillard browning, microbial changes (presence of *Cl. botulinum*), should not be used for human consumption.

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152 Yogurt

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CONTENTS

I. INTRODUCTION

The first time fermented milk (yogurt) was consumed is not known. Fermented milk foods are the predecessors of cheese, which originated about the time dairy animals were first domesticated, 8–10,000 BC (1).

Belief in the healthful aspects of yogurt for human beings has been noted in many civilizations for centuries. Ilya Ilitch Metchnikoff was the Director of the Pasteur Institute. He received the Nobel Prize in medicine and physiology in 1908 for his classical work on phagocytes and phagocytosis, which formed a basis for the theory of

immunity. He is credited for his studies on consumption of fermented milk and longevity. Metchnikoff was not the first to promote curdled milks like yogurt. Hippocrates, in the 4th century B.C., in his "Application of Hygenic and Dietary Measures," spoke highly of yogurt (2). Leben, a fermented milk of the Middle East, is mentioned in the Book of Job (10:10), 1520 years before the birth of Christ (2).

In India, people have consumed yogurt called Dahi for thousands of years. A significant amount of milk produced in India is consumed as yogurt. A few years ago, Lassi, a sugared yogurt drink, was declared the state drink for the parliament in Punjab, a province in Northern India. In different countries, fermented milk is called by different names. However, the word "yogurt" is gaining popularity. In the developing countries, yogurt is an indigenous milk product. In the peri-urban and rural areas of Delhi, India, fresh raw milk is available from small local dairy farms with 40–50 buffaloes (3). Most households make their own yogurt from morning and evening milk. Raw milk is boiled and allowed to cool. When it feels warm to touch, it is inoculated from the previous day's fermentation. Some samples from these household fermentations have been examined in the author's laboratory. They contained mesophilic lactococci, including diacetyl producers, thermophilic cocci, thermophilic lactobacilli, yeast, and some coliforms.

In the past 30 years, annual per capita yogurt consumption in the United States has grown sixfold to 2.31 kg (Table 152.1). The consumption of fermented milk products (yogurt, sour cream, and buttermilk) in the United States is very low compared to other developed countries (Table 152.2). Actual consumption of yogurt and related products in the U.S.A. is a bit higher than reported. Many ethnic groups that consume large quantities of plain yogurt ferment their own. There are a number of adults who do not eat yogurt because they do not tolerate lactose in milk. Incidence of lactose intolerance in several ethnic groups is high (6). However, feeding trials with humans (7), and with rats (8), showed that yogurt containing viable microflora could be tolerated by individuals determined to be lactose intolerant. Other research workers demonstrated that the lactase (beta-galactosidase) activity of the yogurt culture remains in the intestinal tract after the culture has lysed, and continues to break down lactose (9). This activity can be detected in the duodenum and terminal ileum after consumption of viable yogurt (10).

TABLE 152.1
Per Capita Consumption of Yogurt in the United States (kg)

1970	0.36	1980	1.13	1990	1.81
1971	0.50	1981	1.09	1991	1.91
1972	0.59	1982	1.18	1992	1.91
1973	0.64	1983	1.45	1993	1.95
1974	0.68	1984	1.63	1994	2.13
1975	0.91	1985	1.81	1995	2.31
1976	0.95	1986	1.91	1996	2.18
1977	1.04	1987	1.95	1997	2.31
1978	1.13	1988	2.04		
1979	1.09	1989	1.91		

Source: Ref. 4.

TABLE 152.2
Consumption of Fermented Milk Products other than Cheese in Selected Countries

Country	1993 Per capita (kg)*
Finland	38.02
Netherlands	29.64
Sweden	28.54
Iceland	25.85
Denmark	20.66
Switzerland	18.66
France	17.06
Federal Germany	14.77
United States of America	6.00**

* Adapted from ref. 5.

** For 1998, ref. 4.

Fermented milk products include yogurt, sour cream, and buttermilk.

The 2.9 billion dollar yogurt industry is growing at about 3 to 4 percent every year. The segment catering to kids under the age of 12 has shown huge gains. For example, Danimals, a yogurt product targeting children, has grown 137.9% (11). Excluding private labels, the top nine refrigerated yogurt brands are dominated by Yoplait and Dannon. In a recent consumer survey of 40 yogurts, the quality varied from flavor to flavor in a single brand (12). Many of the yogurts were characterized as astringent, thick, gummy, having jam and candy-like flavors, or as being chalky and slightly bitter. Fruit containing yogurts also contain large amounts of sugar. This is a huge problem for an estimated 16 million Americans with Type 2 diabetes, and another 20 to 30 million Americans with impaired glucose tolerance (13). Yogurt formulated for this segment of population can offer a big growth opportunity.

This brief chapter on yogurt is written to bring a clear understanding of this simple product, which is now becoming a vehicle for introducing probiotics and prebiotics believed to impart good health by maintaining a balanced and healthy gut. For a more extensive discussion on yogurt, see Tamine and Robinson (5).

II. DEFINITION OF YOGURT

Yogurt is an acid gel made from the fermentation of a standardized milk mix by *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) (14). The solid-not-fat component of milk may be raised to 12%

or higher to yield a custard-like gel. The fat in the product is adjusted to qualify for lowfat or fat-free yogurt.

A. Yogurt Description

Yogurt is the food produced by culturing one or more of the optional dairy ingredients with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. One or more of the other optional ingredients specified below may also be added before the culturing process. All ingredients used are safe and suitable. Yogurt, before the addition of bulky flavors, contains not less than 3.25 percent milkfat, and not less than 8.25 percent milk solids-not-fat, and has a titratable acidity of not less than 0.9 percent, expressed as lactic acid. The food may be homogenized, and shall be pasteurized or ultra-pasteurized prior to the addition of the bacterial culture. Flavoring ingredients may be added after pasteurization or ultra-pasteurization. To extend the shelf-life of the food, yogurt may be heat-treated after culturing is completed to destroy viable microorganisms.

Optional Ingredients: Vitamins: (a) If added, vitamin A shall be present in such quantity that each 946 milliliters (quart) of the food contains not less than 2,000 International Units thereof, within limits of current good manufacturing practice. (b) If added, vitamin D shall be present in such quantity that each 946 milliliters (quart) of the food contains 400 International Units thereof, within limits of current good manufacturing practice. Optional dairy ingredients are cream, milk, partially skimmed milk, or skim milk, used alone or in combination.

Other optional ingredients include: (a) Concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, or whey, modified by partial or complete removal of lactose and/or minerals, to increase the nonfat solids content of the food; provided, that the ratio of protein to total nonfat solids of the food, and the protein efficiency ratio of all protein present shall not be decreased as a result of adding such ingredients. (b) Nutritive carbohydrate sweeteners. Sugar (sucrose), beet or cane; invert sugar (in paste or syrup form); brown sugar; refiner's syrup; molasses (other than blackstrap); high fructose corn syrup; fructose; fructose syrup; maltose; maltose syrup, dried maltose syrup; malt extract, dried malt extract; malt syrup, dried malt syrup; honey; maple sugar; or any of the sweeteners listed in part 168 of this chapter, except table syrup. (c) Flavoring ingredients. (d) Color additives. (e) Stabilizers.

1. Lowfat Yogurt

Lowfat yogurt has a similar description as given earlier, except the milkfat of the product before addition of bulky flavors is not less than 0.5% and not more than 2% (14).

2. Nonfat Yogurt

Nonfat yogurt is the product as per the previous description of yogurt, except the milkfat content before the addition of bulky flavors is less than 0.5% (14).

B. National Yogurt Association (NYA) Criteria for Live and Active Culture Yogurt

According to the NYA (15), live and active culture yogurt is the food produced by culturing permitted dairy ingredients with a characterizing culture, in accordance with the FDA standards of identity for yogurt. In addition to the use of ST and LB, live and active culture yogurt may contain other safe and suitable food grade bacterial cultures.

Heat-treatment of live and active yogurt with the intent to kill the culture is not consistent with the maintenance of live and active cultures in the product. Producers of live and active culture yogurt should ensure proper practices of distribution, code dates, and product handling conducive to the maintenance and activity of the culture in the product.

Live and active culture yogurt must satisfy the following requirements (15):

1. The product must be fermented with both *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus.* The cultures must be active at the end of the stated shelf-life-as determined by the activity test described in item 3. Compliance with this requirement shall be determined by conducting an activity test on a representative sample of yogurt that has been stored at temperatures between 32 and 45°F for refrigerated cup yogurt. The activity test is carried out by pasteurizing 12% solids of nonfat dry milk (NFDM) at 92°C (198°F) for 7 min, cooling to 110°F, adding 3% inoculum of the material under test, and fermenting at 110°F for 4 h. The total organisms are to be enumerated in the test material both before and after fermentation by IDF methodology. The activity test is met if there is an increase of 1 log or more during fermentation. In the case of refrigerated cup yogurt, the total population of organisms in live and active culture yogurt must be at least 10^8 cfu/g at the time of manufacture. It is anticipated that if proper distribution practices and handling instructions are followed, the total organisms in refrigerated cup live and active culture yogurt at the time of consumption will be at least 10^7 cfu/g.

C. Frozen Yogurt

Frozen yogurt resembles ice cream in its physical state. Both soft-serve and hard-frozen yogurts are popular. These are available in nonfat and lowfat varieties. These products are not very acidic. The industry standards require minimum titratable acidity of 0.30%, with a minimum contribution of 0.15% as a consequence of fermentation by yogurt bacteria (15,16). Technology for production of frozen yogurt involves limited fermentation in a single mix, and arresting further acid development by rapid cooling; alternatively, using a standardization of titratable acidity to a desirable level by blending plain yogurt with ice cream mix containing fruit/syrup base, stabilizers, and sugar, and then freezing the mix in a conventional ice cream freezer. The mix is frozen at −6°C and hardened at−40°C. The finished product pH may vary between 5.5 and 6.0, depending on the consumer acceptance. Typical composition of nonfat hard-pack frozen yogurt may contain 0% fat, 13% milk-solid-nonfat, 13% sucrose, 6% corn syrup solids 36DE, and 2% maltodextrin 10DE, 1.2% stabilizer (16). For further discussion see references (5,16,18).

III. YOGURT STARTER ORGANISMS

For manufacture of legal yogurt in the United States, *Streptococcus thermophilus* and *Lactabacillus delbrueckii* subsp. *bulgaricus* must be added for basic fermentation of the yogurt mix. Additional optional organisms, preferably of human intestinal origin, may be incorporated in the yogurt either through the starter culture, or blended in after fermentation is complete (14,15). The optional organisms may be selected from a long list of candidates (Table 152.3). Some characteristics of organisms encountered in yogurts are listed in Table 152.4.

TABLE 152.3
Optional Cultures for Addition to Yogurt

Lactobacillus acidophilus
Lactobacillus rhamnosus
Lactobacillus reuteri
Lactobacillus casei
Lactobacillus gasseri
Lactobacillus johnsonii
Lactobacillus plantarum
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium adolescentis
Bifidobacterium infantis
Bifidobacterium lactis
Bifidobacterium longum
Enterococcus faecalis
Enterococcus faecium
Sacchromyces boulardi
Pediococcus acidilactici
Propionibacterium freudenreichii

Source: Adapted from ref. 18 and 19.

TABLE 152.4
Selected Characteristics of Organisms Found in Yogurt

	% Lactic Acid in Milk	Lactic Acid Isomer	Growth		Carbohydrates Fermented							
			15°C	45°C	Glucose	Inulin	Galactose	Fructose	Lactose	Sucrose	Ribose	Raffinose
Lb. delbrueckii ssp. bulgaricus[a]	1.8	D	—	+	+		—	+	+	+	—	—
Lb. lactis[a]	1.8	D	—	+	+		[d]	+	+	+	+	—
Lb. helveticus[a]	3.0	DL	—	+	+		+	[d]	+	—	—	—
Lb. casei[b]	0.8	L	+	—	+		+	+	±	+	+	—
Lb. paracasei[b]		DL	+	[d]	+		+	+	+	+	+	—
Lb. rhamnosus[b]		DL	+	+	+		+	+	+	+	+	—
Lb. plantarum[b]		DL	+	—	+		[d]	+	+	+	+	+
Lb. acidophilus		DL	—	+	+		+	+	+	+	—	[d]
Streptococcus thermophilus[a]	0.7	L	—	+	+		—	+	+	+	—	—
B. bifidum		L	—	—	+	—	+	+	+	[d]	—	—
B. longum		L	—	—	+	—	+	+	+	+	+	+
B. infantis		L	—	—	+	[d]	+	+	+	+	+	+
B. breve		L	—	—	+	[d]	+	+	+	+	+	+
B. adolescentis		L	—	—	+	[d]	+	+	+	+	+	+

[a] do not grow in 2.5% salt. [b] can grow in 6 to 8% salt. [d] 11 to 89% of strains are positive.
Adapted from reference 5, 20 and 21.

A. Streptococcus thermophilus

This organism is a Gram-positive, catalase negative anaerobic cocci, and it is largely used in the manufacture of hard cheese varieties, mozzarella and yogurt. It does not grow at 10°C, but grows well at 45°C. Most strains can survive 60°C for 30 min (22). It is very sensitive to antibiotics. Penicillin (0.005 Iu/ml) can interfere with milk acidification (20). It grows well in milk and ferments lactose and sucrose. Two percent sodium chloride may prevent growth of many strains. These streptococci possess a weak proteolytic system. It is often combined with the more proteolytic lactobacilli in starter cultures. Most ST grow more readily in milk than lactococci, and produce acid faster. These streptococci strains possess β-galactosidase (β-gal) and utilize only the glucose moiety of lactose, leaving galactose in the medium (23).

The proteolytic activities of nine strains of ST and nine strains of LB cultures incubated in pasteurized reconstituted NFDM at 42°C as single and mixed cultures were studied (24). Lactobacilli were highly proteolytic (61.0 to 144.6 μg of tyrosine/ml of milk), and ST were less proteolytic (2.4 to 14.8 μg of tyrosine/ml of milk). Mixed cultures, with the exception of one combination, liberated more tyrosine (92.6 to 419.9 μg/ml) than the sum of the individual cultures. Mixed cultures also produced more acid (lower pH). Of 81 combinations of LB and ST cultures, only one combination was less proteolytic (92.6 μg tyrosine/ml) than the corresponding LB strain in pure culture (125 μg tyrosine/ml). *Streptococcus thermophilus* requires fewer amino acids than lactococci and lactobacilli. Only glutamine and glutamic acid, along with sulfur amino acids, are essential for all of the strains that have been tested (25,26). Recently it was shown that ST possesses a branched-chain amino acid (leucine, isoleucine, and valine) biosynthesis pathway as an essential pathway for optimal growth in milk. This pathway is thought to play a role in maintaining the internal pH of the organism by converting acetolactate to amino acids (26). These organisms also have urease that produces ammonia from urea in milk to counter the acid effects. Dairy lactococci do not have this pathway (26).

B. Lactobacillus delbrueckii subsp. bulgaricus

These organisms are Gram-positive, catalase negative, anaerobic/aerotolerant homofermentative, and produce D-(−) lactate (1.8%), and hydrogen peroxide (27). These cultures have β-galactosidase activity. Only the glucose moiety of lactose is utilized and galactose is released in the medium (28). LB has a high level of protease activity in milk that reaches its maximum during the log phase; while ST produces highly active peptidase instead of protease (29,30). In a recent study (30), it was demonstrated that LB reached maximum protease activity between 4–8 h after incubation, and then declined rapidly. When grown singly in pasteurized reconstituted NFDM at 42°C, ST and LB were less proteolytic compared to the mixed culture growth (24).

C. Associative Growth of ST and LB

A symbiotic relationship exists between ST and LB in mixed cultures (31); carbon dioxide, formate, peptides, and numerous amino acids liberated from casein are involved. Associative growth of rod and coccus results in greater acid production and flavor development than the single culture growth (32,33). It has been established that numerous amino acids liberated from casein by proteases of LB stimulate growth of ST (34,35). In turn, ST produces CO_2 and formate which stimulates LB (35–38). During the early part of the incubation, ST grows faster and removes excess oxygen, as well as produce the stimulants noted above. After the growth of ST has slowed because of increasing concentrations of lactic acid, the more acid tolerant LB increases in number (29,39,22). For a one-to-one ratio of rod and coccus and inoculum level, time and temperature of incubation must be controlled and bulk starter should be cooled promptly.

D. Bifidobacteria

This Y-shape organism was isolated from infant stool in 1899 at the Pasteur Institute by Tissier (21). There are 24 species in this group. Nine of these are of human origin, and the other 15 come from animals (40). These Gram-positive organisms are strictly anaerobic. The degree of tolerance to oxygen depends on the species and the growth medium. It appears that the strains of *B. bifidus* are relatively aerotolerant (40). Optimum growth temperature for species of human origin is 36–38°C; no growth at 20°C. They do not tolerate heat; *B. bifidus* is inactivated at 60°C. Two moles of glucose are fermented by the fructose-6-phosphate phosphoketolase pathway to 2 moles of L (+) lactate and 3 moles of acetate. Some formic acid and ethanol may also be produced. Bifidobacteria of human origin synthesize several vitamins, thiamine (B1), riboflavin (B2), pyridoxine (B6), folic acid (B9), cyanocabalamine (B12), and nicotinic acid (P) (40).

It has been observed that the number of bifdobacteria falls significantly in the stools of adults and the elderly. The proportions of various species of bifidobacteria vary with the age of humans. It appears that *B. infantis* and *B. breve* are favored in breast-fed infants, and *B. adolescentis* predominates in bottle-fed infants and in adults (40). Allergic infants were reported to be colonized mainly by *B. adolescentis* species, with a lower mucous binding capacity than bifidobacteria from healthy infants (41). The Bifidobacteria population may be reduced after the administration of a Western diet. All strains of bifidobacteria do

not behave similarly. Morinaga Milk Industry Co, LTD claims that their *B. bifidum* (BB536) is hardier and superior to other strains (42).

E. Starter Culture Propagation

These days, yogurt manufacturing plants are large and highly automated. Trouble-free functioning of these operations requires predictable and dependable starter culture performance in the context of types of products and their sensory attributes. Following are some helpful hints.

1. Milk should be of good microbiological quality, free of antibiotics and inhibitors of bacterial origin.
2. Fresh milk, heat-treated at 90 to 95°C for 5 minutes, or 85°C for 30 minutes, tends to give a balanced growth with a 1:1 or 2:1 ratio for ST:LB. When cultures are propagated in reconstituted NFDM, STs tend to show abnormally large cells within a chain. More severe heat treatment, such as autoclaving of milk, is somewhat inhibitory to ST and favors the growth of LB, causing culture imbalance in favor of LB. It should be noted that ST first initiates growth, followed by LB (22). A low population of ST will delay the completion of starter or yogurt fermentation. The heat-treatment of 90°C for a few minutes generally inactivates bacteriophages present in the milk.
3. Many yogurt producers prefer to use skim and condensed skim milk over nonfat dry milk to raise solids in the growth medium. Handling of nonfat dry milk is labor intensive and invariably results in dusting of plant equipment and overhead fixtures. Under these conditions, keeping the plant clean is difficult and can result in higher incidence of yeast and mold. If nonfat dry milk is used, the area should be enclosed and isolated. The exhaust air should be filtered.
4. It is important to cool the starter when appropriate pH/% TA is attained. Higher acidity tends to reduce the ST count (22).
5. At any given time, the availability of phage-unrelated cultures suitable for specific yogurt attributes is rather small. These cultures should be handled carefully to grant them long life in the plant.
6. Monitor phage in the starter and in the environment on a regular basis.
7. Many of the defined cultures may contain up to six strains of lactobacilli. The culturing conditions should be carefully verified and controlled for uniform culture activity. If the starter contains probiotic cultures or other adjuncts, their numbers should be verified in the starter and the product.

The plants should work closely with the culture suppliers for phage monitoring and culture performance.

1. Bulk Starter Preparation

This is one of the key operations and should be attended to carefully by trained personnel dedicated to this duty. The starter tank valves, pipes, and hatch with gasket should be assembled and sterilized with live steam at low pressure (3 to 5 lbs). Keep the bottom valve open for the condensate to drain. Continue to steam the tank for 30 minutes after the surface temperature in the tank has reached ~99°C. Turn the steam off and close the bottom valve.

Skim milk with total solids raised to 10 to 12% is either pumped cold and heated to 90°C and held for 60 minutes, or the starter mix is heated to 90°C in a plate heat exchanger, and then held at that temperature in the tank for 60 minutes. Some plants prefer to use reconstituted nonfat dry milk at 10–12% TS for their bulk starter. In certain operations, where large amounts of starter are used for yogurt inoculation, yogurt base is used for starter culture preparation. This brings ease of operation, and eliminates yogurt composition variation. Cool the mix to 43°C. Close the chilled water valve early enough that the temperature does not fall below 43°C. For 500 gallons, thaw a can (350 ml) of frozen culture concentrate (10^{10} cfu/g) in 5 gallons of tepid water containing 100 ppm chlorine. Inoculate the tank and stir for five minutes. Turn off the agitator and let it incubate quiescently for 6–8 hours to reach 0.9% titratable acidity. Cool the starter to 5°C using slow agitation. Then turn off agitation. Turn on agitation for a few minutes before the starter is to be pumped. This rate of inoculation yields ~10^6 cfu/g of starter mix. For a healthy culture, it may take 8 to 10 hours to reach 0.9% acidity. Starter has 2–5 $\times$ 10^8 cfu/ml. Time to 0.9% titratable acidity also depends on the strain composition of the frozen culture concentrate.

It is advisable to make a Gram-strain of the fresh starter and run an activity test as described in section II. B., using 1% inoculum. Such data is invaluable in tracking the performance of culture(s), and in preventing failed yogurt fermentation.

IV. YOGURT MIX PREPARATION

Fermented milk products have a delicate flavor and aroma and require milk of good microbiological quality. A variety of yogurt mix can be formulated and standardized from whole milk, partially skimmed milk, condensed skim, nonfat dry milk, whey compositions, and cream. These mixes should be formulated to comply with regulations and meet consumer expectations. Clarified, fat-adjusted milk at 50°C should be blended with appropriate dry ingredients using

a powder funnel. It should be allowed to circulate for a few minutes. The solids content of separated milk or whole milk can also be raised to 12% and 15% respectively by evaporation. The increased solids content prevents whey separation and improves the texture.

A. Sweeteners

Sweeteners may be added to yogurt as part of the mix before fermentation, and/or through fruit preserved with sweeteners. Sucrose (sugar) is widely used in yogurt production. It provides a clean sweet taste which has no after taste or other odors. It complements flavors, and contributes to desirable flavor blends (17). It can be used in a dry, granulated, free-flowing, crystalline form, or as liquid syrup (67% sucrose). Inclusion of more than 5% sucrose in yogurt mix of 16 to 20% total solids may cause culture inhibition and lack of flavor development (5,16). Several corn syrup preparations and other sweeteners are also available (5,17). Non-nutritive sweeteners, such as Aspartame or Nutrasweet® are used in light products. These sweeteners have a lingering aftertaste (5) and have not faired well in consumer acceptance (12). Several newer sweeteners, Actilight®, Acesulfame-κ, Natren, Neohesperidine, and Thaumatin are available. These could be used alone or in combination. The choice of sweetner(s) is determined by availability, cost, and its legal status for use in yogurt (5).

B. Stabilizers

The set yogurt gel structure results from an acid-casein interaction, where casein micelles at or near their isoelectric point flocculate, and the colloidal calcium phosphate partially solubilizes as acidity increases. During the fermentation of milk, the pH gradually declines to around 4.5, and destabilized micelles aggregate into a 3-dimensional network in which whey is entrapped (43,44). Appearance of whey on the surface of yogurt gel is due to syneresis, separation of serum from curd. In yogurt, this defect is called wheying-off (45).

In stirred style yogurt, the 3-dimensional network is disturbed when fruit and flavors are mixed into the plain yogurt. The texture and physical properties of the yogurt depend on the fruit, stabilizer, and the rate of cooling (45).

Stabilizers are added to prevent surface appearance of whey, and to improve and maintain body, texture, viscosity, and mouthfeel. Yogurts with lower milk solids have a greater tendency to synerese. Numerous stabilizers are available on the market. Generally a combination of several stabilizers is included in the formulation to avoid defects that may result from the use of a single stabilizer. A partial list of stabilizers used in yogurt include:

1. Gelatin is a protein of animal origin. It is derived from the hydrolysis of collagen. Only high bloom gelatin should be used in yogurt making, due to improved gelatin/casein interactions, its higher melting point, and its higher stabilizing ability (5). The term "bloom" refers to the gel strength. It disperses in cold, but requires heat for activation. It is used at a 0.3 to 0.5% level. Microstructure of yogurt made with 0.5% gelatin under scanning electron microscopy did not show the gelatin, and the structure did not differ from that of a plain, unfortified yogurt. This yogurt was rated smooth in sensory evaluation (46).
2. Whey protein concentrates (WPC) are used at 1 to 2% of protein addition. In a study with skim milk yogurt fortified with dairy-based proteins, the yogurts made with casein-based products were coarser and inferior compared to those made with WPC at 1 to 1.5%. It was recommended that WPC should be used along with other stabilizers (47).
3. Gums are water soluble or dispersible polysaccharides and their derivatives. In general, they thicken or gel aqueous systems when used at low concentration. Gums are used to stabilize emulsion and prevent wheying-off. Food gums are tasteless, odorless, colorless, and non-toxic (48). All are essentially non-caloric and are classified as soluble fiber. These are used at 0.2 to 1.5%, depending on the application.

Locust bean gum is a seed gum. It has low cold water solubility. It is generally used where delayed viscosity development is needed. Dispersion of this gum, when heated to about 185°F and then allowed to cool, is high in viscosity. It works synergistically with carrageenan in some applications (48).

Guar gum is very similar to locust bean gum, but is more soluble in cold water. It hydrates readily at pH 6–9. Its solubility is not affected by pH in the 4.8 to 5.0 range. It does not cross-link well with carrageenan (48).

Carrageenan is derived from red seaweed. It is a mixture of various types, kappa, iota, and lambda. It may contain 60% of kappa form and 40% lambda. The kappa type forms a gel, whereas, lambda does not. The polymer is stable at pH above 7.0, and has a tendency to degrade slightly at pH 5–7, degrading rapidly below pH 5.0. The potassium salt of this gum is the best gel former, but the gels are brittle and prone to syneresis. This defect is prevented by the addition of a small amount of locust bean gum. It interacts with casein in milk, and promotes stabilization of the yogurt gel (48).

Xanthan is produced by microbial fermentation. It is readily soluble in cold and hot water. It is not affected by pH changes. A synergistic increase in viscosity results from interaction of xanthan with κ-carrageenan and locust

bean gum. These gels are prone to shear thinning. It also gives sheen to products, which may not be desirable in yogurt (48).

Protein, starch, modified starch, and tapioca-based starches can be used without affecting the flavor of yogurt. The stabilizer used in yogurt is generally a blend of stabilizers incorporated at 0.5 to 0.7% or less. The amount used also depends on the milk solids level (5). A recent consumer survey of marketplace yogurts has indicated that products are gummy, perhaps due to over-stabilization (12).

C. Fruit and Fruit Flavorings for Yogurt

The growth and popularity of yogurt is largely due to fruit and sugar. For fruit-containing yogurt, the primary component of yogurt taste is the perceived degree of sweetness. This attribute of yogurt is believed to be responsible for its spectacular growth popularity (49). In an earlier study, 79% of consumers preferred flavored yogurts (50). The surveys, done in the United Kingdom, indicated that 90% of yogurt sales were of fruit and flavored varieties (51).

Many fruit flavors, single or blended, are popular, and these may vary with the season. Fruit preparations are added to 10 to 20% in the final product. A fruit preserve consists of 55% sugar and 45% fruit (16). These are cooked until the final solids reach 65 to 68%. The pH of these preparations is adjusted to 3.0 to 3.5 with citric acid or other food-grade acid. The processed fruit, in most cases, is filled aseptically in totes and shipped to yogurt plants. Transfer of fruit to yogurt should be done through sterile equipment to avoid yeast and mold contamination. The blending and the filler areas are very crucial to the microbiological quality of the yogurt with respect to yeast and mold. These areas should have HEPA-filtered air to keep out airborne yeast, mold, and other contaminants. Cardboard boxes should not be brought into these areas. Also, high pressure water hoses should be avoided while fruit blending and packaging is going on.

D. Heat Treatment

The high solids mix is given a higher heat-treatment than the conventional pasteurization. Generally, milk is heat-treated at 85 to 95°C, and then held for 10 to 40 minutes. At these temperatures, bacteriophages and vegetative bacterial cells are inactivated, and the growth of starter bacteria improves. Up to 60°C, there is no effect on whey protein. At 60° to 100°C, the whey proteins interact with each other and κ-casein. This interaction decreases dissociation of α_s- and β-casein, and increases κ-casein dissociation (51). When skim milk was preheat-treated at 85°C for 30 min and 90°C for 2 min, whey protein denaturation was 76.5% and 55.0%, respectively (52). This heat-treatment and resulting interactions increase the water binding capacity of the protein system.

E. Homogenization

Homogenization of mix is carried out in two stages; the first stage is at 10–20 mega Pascal, and the second stage is at 3.5. It reduces the fat globule size to less than 3 μm, which gives a rich mouthfeel. This prevents creaming of the mix upon storage. Homogenization also improves gel strength upon fermentation, due to greater protein-protein interaction.

V. YOGURT MIX INOCULATION AND INCUBATION

The inoculation rate may vary from 0.5 to 6%, depending on the type of yogurt and system set up. For yogurt fermented in the cup (set style), small surges of mix, 20–100 gal, may be inoculated at 5% and packaged and then incubated at 43–45°C. Yogurt may reach pH of 4.7 to 4.8 in less than 2 hours, and is then sent out of the hot room. If more time is available, mix can be inoculated at 0.5 to 1.5%, which may take 6 to 10 hours to reach pH 4.4 to 4.5.

The inoculation rate may also vary from 0.5 to 6%, depending on the plant layout and equipment available. Two kinds of fruited yogurts are popular in the United States.

1. Set style, fruit-on-the-bottom. In this kind of yogurt, cups receive about 2 oz fruit preparation, followed by inoculated mix at ~44°C in the filler room. The cups are placed in cases, and pallets are moved to the hot room which is maintained at ~48°C. The pH of the product reaches 4.7 to 4.85 in ~100 min., then it is moved to the cooling tunnel. At the end of the tunnel, the temperature of the yogurt is 16 to 18°C. It is then moved to the cooler.
2. Stirred style or Swiss style yogurt. In this type of product, fully fermented, plain cooled yogurt at pH 4.3 to 4.4 is cooled to ~20°C, blended with the fruit preparation, and filled in the cups. The palletized product is placed in the cooler. The texture and physical properties of the yogurt depend on the fruit, stabilizer, and the rate of cooling (45).

A. Yogurt Fermentation

Yogurt fermentation is a homolactic fermentation. Glucose metabolism by ST, LB, and *Lb. acidophilus* proceeds by the EMP pathway. Lactose utilization in ST, LB, and bifidobacteria involves lactose transport into the cells via cytoplasmic proteins (permeases). This translocation of lactose takes place without chemical modification. This unphosphorylated lactose is hydrolysed by β-galactosidase to glucose and galactose. Glucose is catabolized and galactose is secreted from the cells (23,53). The lactose permease is an active transport system, and the energy is provided in the

form of a proton motive force developed by expulsion of protons. The excreted amount of galactose is proportional to the amount of lactose taken up. The current model for lactose transport in these bacteria is that a single transmembrane antiport permease simultaneously translocates lactose molecules into the cytoplasm, and galactose out of the cell. The energy generated through galactose efflux thus supports lactose uptake into these cells. The average lactose content of a yogurt mix of 13% milk solids-not-fat was about 8.5% (54). During fermentation it was reduced to about 5.75%. The initial galactose content of the mix was a trace, but increased to 1.20% during fermentation. Only a trace amount of glucose was noted (54). In commercial yogurts, lactose ranged from 3.31 to 4.74%, and galactose varied from 1.48 to 2.50% (54).

There are a number of inhibitors for yogurt cultures that can impede or slow down lactose fermentation. Some of these are listed here. For further details, refer to (53,27,20,5). These inhibitors are: heat-sensitive lactenins, lactoperoxidase/thiocyanate/hydrogen peroxide (LPS), agglutinins, mastitic milk, antibiotic residues, hydrogen peroxide, detergents and santizer residues, and bacteriophages. Many of the inhibitors mentioned here may be seasonal and sporadic, or accidental. Bacteriophages on the other hand are pernicious, and can be devastating if not managed properly.

B. Bacteriophages

Bacteriophages (phages) are viruses that can infect bacteria and destroy one or more components of the yogurt culture. Phages are differentiated into virulent (lytic) and temperate phages, which reflect different growth responses in the bacterial host. Phages that infect and lyse the host cell are called virulent phages; whereas those which do not necessarily lyse their bacterial hosts, but instead insert their genome into the host cell chromosome, are called temperate phages. The propagation of virulent phages in the bacterial cell is called the lytic or vegetative cycle of phage multiplication, and results in the release of new infectious phage progeny (55). Bacteriophages multiply much faster than the bacteria. A bacteriophage with a burst size of 100 can destroy a culture within a couple of generations. This can cause huge economic losses, and result in inferior yogurt. Due to explosive growth of yogurt and mozzarella cheese production, a greater incidence of phages against ST has been reported (29). It is also noted that phages for lactobacilli appear less frequently (29,56). Recent work has shown that ST phages are closely related, at both the genetic and morphological level, making differentiation difficult. Electron microscopy studies revealed that both temperate and lytic phages were nearly identical, having small isometric heads and long non-contractile tails (57,58). Some lysogenic strains were autolytic at 45°C (58). New strategies to develop phage-resistant strains include antisense RNA technology, and origin-conferred phage-encoded resistance (PER) (57).

Both lytic and temperate bacteriophages have been found in *Lactobacillus delbrueckii* subsp. *bulgaricus* and subsp. *lactis* (59,57). Strains of *Lactobacillus acidophilus* isolated from dairy products harbored temperate phages, and some produced bacteriocins. One induced phage lysed nine other dairy lactobacilli, including LB (60). Some of the LB were also sensitive to the bacteriocin produced by *Lb. acidophilus*.

Primary yogurt cultures, ST and LB, should be carefully chosen and evaluated with respect to phage and compatibility with other adjuncts. Since many of the organisms used in yogurt may harbor temperate phages, propagated starter culture, yogurt, and environment should be monitored for phages. Use of aseptic techniques for propagation and production by properly trained personnel, along with proper mix heat-treatment sufficient to kill phages, are essential to keep phages under control.

VI. FLAVOR OF YOGURT

Flavor of yogurt depends on the milk, its heat-treatment, the starter strains used, incubation temperature, and the balance of the organisms in the yogurt. Biochemically, flavor compounds of yogurt include, but are not limited to, lactic acid, acetic acid, formic acid, propionic acid, butyric acid, acetaldehyde, acetone, diacetyl, acetoin, and several other compounds. In milk, ST produces formic, acetic, propionic, butyric, isovaleric and caproic acid, diacetyl, acetone, and some acetaldehyde. Lactobacilli, on the other hand, produce large quantity of lactic acid, acetaldehyde, diacetyl, peptides, and amino acids. Many of the compounds are derived from lactose, and some from other components in milk (22). The flavor of yogurt can turn acidic and bitter during storage and through out shelf-life. Protein degradation can continue during cold storage of yogurt, and some peptides released may be bitter (22,61). Incubation temperature of yogurt below 30°C may also cause bitterness (61). It is again emphasized that the cultures used should be carefully selected to deliver quality attributes of yogurt through out shelf-life.

VII. NUTRITIONAL AND HEALTH ASPECTS OF YOGURT

Nutritional value of yogurt is derived from milk. The value of milk and its products was recognized long ago. An example is a reference to the Promised Land as a land "flowing with milk and honey" (62). Yogurt has higher nutrient density, at 13–18% milk solids, compared to milk, at 12.3%. In literature, a number of healthful benefits have been assigned against various disease states (16,19,62).

These disease states are: colitis, constipation, various kinds of diarrhea, gastric acidity, gastroenteritis, indigestion, intoxication (bacterial toxins), diabetes, hypercholesteremia, kidney and bladder disorders, lactose intolerance, liver and bile disorders, obesity, skin disorders, tuberculosis, vaginitis and urinary tract infections, cancer prevention, and prevention and treatment of *Helicobacter pylori* gastritis and irritable bowel syndrome. Yogurt is a good source of calcium. One 8 oz. serving will provide about 400 mg of calcium (62). It is recommended that milk and yogurt be consumed with oxalate rich foods, such as spinach and soy products. A diet of products rich in calcium decreases urinary oxalate and helps in the prevention of kidney stones (62).

In 1908, in explanation to his longevity-without-aging theory, Metchinkoff stated that lactic acid bacteria in sour milk displaced toxin producing bacteria, thus, promoting health. At about the same time (1899), Tisser isolated bifidobacteria from the stools of infants, and recommended administration of the same to infants suffering from diarrhea (63). Since that time, numerous studies on the use of lactic cultures in foods have continued throughout the centuries. These studies have yielded variable results with regard to the health benefits of probiotics. In the past few years, the use of probiotics in yogurt and fermented products has exploded.

A. Definition of Probiotics

Probiotics are a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (63). Considerable work is going on, where a combination of molecular biology techniques and phenotypical analysis are being used to deciphe the participation and the role played by the probiotics. This will certainly challenge our current concepts.

B. Benefits of Probiotics

Health benefits imparted by probiotic bacteria are strain-specific and not species or genus specific. The strains *Lactobacillus rhamnosus* GG (Valio), *Saccharomyces boulardii* (Biocodex), *Lactobacillus paracasei* Shirota (Yakult), and *Bifidobacterium* BB12 (Chr Hansen), have the strongest human health efficacy data against some or all of the following: lactose intolerance; rotaviral diarrhea, antibiotic-associated diarrhea, and the associated *Clostridium difficile* diarrhea; as well as some other bacterial diarrheas and infections. Travelers' diarrhea may also be alleviated in some people by *Lactobacillus* GG. It is not yet possible to relate probiotic intake to prevention of bowel cancer in humans. However, there is stronger evidence for effects on bladder cancer in the Japanese population using the Shirota strain (65). Probiotics may also help in atopic eczema, irritable bowel syndrome, inflammatory bowel disease, *Helicobacter pylori* infections, and, possibly, in arthritic conditions (65). For early evaluation and comparison of probiotics, in vitro laboratory tests and standards for good clinical tests must be followed. These are listed in Tables 152.5 and 152.6. Over the years, lots of studies have been done with different strains; but there are only a few strains for which peer-reviewed published evidence from human clinical trials is available. The evidence from these trials is presented in Table 152.7 (65). In a 15-month long study with human subjects consuming a probiotic milk product containing *Lb. rhamnosus* DR20, it was observed that the population of *Lactobacillus* and enteroccal content of the feces was transiently altered without markedly affecting biochemical or other bacteriological factors (66). Based on their work, the authors have proposed a new definition of probiot: "Probiotics contain microbial cells which transit the

TABLE 152.5
In Vitro Tests for Probiotic Bacteria as Indicators of Human Health

- Acid/pepsin (pH 2.0 for 105 min) and bile tolerance (physiological concentrations)
- Fast growth rate and active metabolism
- Acid production (rate of production, types of acids, and regio-specificity)
- Production of bacteriocins
- Immune response (local and systemic)
- Vitamin production (e.g., folate, B group)
- Colonization and adhesion ($CaCo_2$ and HT29 cells, mucus)
- Antibiotic sensitivity patterns
- Bile deconjugation
- Inhibition of pathogens (e.g., *Salmonella utyphimurium, Escherichia coli, Clostridium difficile, Clostridium perfringens, Candida albicans*)
- Fecal enzyme concentrations (e.g., β-glucuronidase)
- Safe history

Source: Ref. 65. Reproduced with permission from Playne (2002).

TABLE 152.6
Standards for Good Clinical Trials on Probiotics

- Each strain identified by molecular methods, and properties fully documented
- Extrapolation of data from strains of the same species is not acceptable
- Double-blind, placebo-controlled randomized studies, with a "crossover" design where possible
- Adequate number of treatments to avoid "confounding" of data by other ingredients and organisms
- Study end-points should be unequivocally stated prior to commencement
- Currently acceptable statistical tests must be used
- Confirmation of findings by one or more independent research groups
- Publication in peer-reviewed journals of international standing
- Efficacy of the probiotic strain in different products should be assessed separately for each product against each health condition

Source: Ref. 65. Reproduced with permission from Playne (2002).

TABLE 152.7
Strains of Probiotic Bacteria for Which There is Published Peer-Reviewed Clinical Data. Strains are Listed in Decreasing Order of Clinical Evidence

Strain	Conditions
Lactobacillus rhamnosus GG (Valio)	1, 2, 4, 5, 6, 7 8, (12), 14, 15
Saccharomyces cereviseae Boulardii (Biocodex)	2, 3, 4, 5, 7, 11
Lactobacillus paracasei Shirota (Yakult)	2, 5, 6, 9, (10), 11, (12), 15
Bifidobacterium lactis BB12 (Chr Hansen)	1, 2, 3, 4, 5, 6, 11, 15
Lactobacillus reuterii (Biogaia)	1, 5, (10), (12)
Lactobacillus johnsonii La1 (Nestle)	6, 11, 14, 15
Enterococcus faecium SF68 (Cemelle)	2, 5, 10, (12), 13
Lactobacillus acidophilus La5 (Chr Hansen)*	2, 4, 5, 6, 11
Bifidobacterium longum BB536 (Morinaga)	2, 5?, 11, (12), (15)
Bifidobacterium breve (Yakult)	(1), 5
Lactobacillus acidophilus NFCM Rhodia (USA)	1?, 5, 6, (12)
Lactobacillus plantarum 299v (ProViva, Sweden)	5, 13

Condition: 1 = rotaviral diarrhea; 2-antibiotic-associated diarrhea; 3 = *Clostridium difficile* pseudomembranous colitis; 4 = travelers', diarrhea; 5 = other acute bacterial diarrhea; 6 = lactose intolerance; 7 = bacterial vaginitis; 8 = atopic eczema and food allergy; 9 = bladder cancer; 10 = cholesterol; 11 = chronic constipation; 12 = bowel cancer; 13 = irritable bowel syndrome; 14 = *Helicobacter pylori*; 15 = immune response modulation.
? = doubtful evidence; ( ) = animal data and/or biomarkers only.
* Data for this strain uncertain, as it was co-administered with *B. lactis*, BB12 usually.
Source: Ref. 65. Reproduced with permission from Playne (2002).

gastrointestinal tract and which, in doing so, benefit the health of the consumer." This definition certainly calls into question some of the criteria set forth in Tables 152.5 and 152.6 for an organism to qualify as a probiotic.

It is generally agreed that *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptooccus thermophilus*, the yogurt bacteria, do not adhere to the mucosal surfaces of the intestinal tract during their transit through the gut (67,68,5). These organisms do not survive in the human gastrointestinal tract because of their low tolerance for bile salts (69,70). A combination of molecular methods has been used for the detection of lactic acid bacteria in the human gastrointestinal tract (41). *Lactobacillus delbrueckii* group was detected, but not *Streptococcus thermophilus* (41). Recent feeding trials with Gottingen minipigs appear to indicate that these yogurt organisms do survive the passage to the terminal ileum (71). The numbers detected (10^6 to 10^7 cfu/g of chyme) are considered to be high enough to be considered for potential probiotic status. It is believed that the gut is home to 400 to 500 species of organisms. Recent studies have indicated that the total microbiota of each adult individual had a unique pattern reflecting their differences in composition, which is partly dependent on the host genotype (41). What happens to the balance of these in different human beings, with different dietary habits and age, is not known. How the organisms get established in the gut in different segments is also not known. Lots of useful information is coming forth as this matter is studied more seriously.

C. Prebiotic

A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thereby improving the host health (18). The beneficial effects of the presence of bifidobacteria in the gut are dependent on their viability and metabolic activity. Their growth is stimulated by the presence of complex carbohydrates known as oligosaccharides. Some of these are considered as prebiotics (72). Fructo-oligosaccharides (FOS) are well-known prebiotics found in 36,000 plants (73). FOS may contain 2 to 8 units in a chain. Inulin, a type of FOS extracted from chicory root, has a DP (degree of polymerization) up to 60. FOS and inulin occur naturally in a variety of fruits, vegetables, and grains, especially chicory, Jerusalem artichoke, bananas, onion, garlic, asparagus, barley, wheat, and tomatoes (73). Recommended levels of prebiotics begin at 5 g/day (73), and may be as high as 15–20 g/day (18).

1. Benefits of Prebiotics

Prebiotic fermentation leads to health benefits, such as increased fecal biomass, and increased stool weight and/or frequency. Prebiotics are fermented by bifidobacteria with the production of short chain fatty acids (SCFA), mainly acetate, propionate, and butyrate, and hydrogen and carbon dioxide. Production of SCFA leads

to lower pH in the colon, which facilitates absorption of calcium, magnesium, and zinc (18,73). Lower pH also restricts pathogenic and other harmful bacteria, thus reducing or eliminating precarcinogenic activity (73).

2. Potential Prebiotics

Several materials are under study as prebiotics. These are: oligosaccharide (FOS Rafitilose P95), inulin, pyrodextrine, galacto-oligosaccharides, soy-oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides, lactulose, transoligosaccharides, Rafitaline, HP, Frutafit EXL, and Fruitofit IQ (73,74). In a recent study of 54 strains of lactic acid bacteria, lactose-derived galacto-oligosaccharide utilization was linked to the presence of β-galactosidase in bifidobacteria studies (75).

3. Synbiotic

Synbiotic refers to a product in which a probiotic and prebiotic are combined. The synbiotic effect may be directed towards two different regions, both large and small intestines. The combination of pre- and probiotic in one product has been shown to confer benefits beyond those of either on its own (76).

4. Survival of Probiotics in Yogurt

It is known that bifidobacteria and lactobacilli are members of the human gut flora. Also, a large number of yogurt brands claim the addition of probiotics. For the probiotics to be effective, the organisms have to be alive (19). In market-bought samples, the fresh product had 10^6 to 10^7 cfu/g. Their numbers declined to less than $\leq 10^3$ cfu/g in some products (77,78). Some strategies to improve probiotics numbers in yogurt include reduction of regular ST and LB in the product, and the addition of cultured probiotics to yogurt (78,79). Since, the dietary probiotics appear to be transitory, feeding of prebiotics, inulin and FOS, may be sufficient to favor the growth of healthy organisms in the gut (80).

VIII. FUTURE TRENDS

The market for probiotic drinks is booming, and clinical work with probiotics continues to provide support for their benefits. Yakult from Japan, is the world's largest probiotic dairy beverage, followed by Actimel from Dannon (81). The dividing line between yogurt and cultured milks is becoming thinner. Probiotic strains may become the sole fermenting agents in yogurt and fermented milk (82,83). Biogaia and Farm Produce Marketing Ltd. have signed an agreement to market and sell drinkable yogurt with Biogaia's delivery system, Life Top™ straw. The straw will be attached to the side of the package and contain *Lactobacillus reuteri* (Reuteri TM) (84). Under the category of healthy and functional yogurts, new fruits and flavors are being offered (85). Some of these are:

- Yogurt containing fruit pieces and flavors like melon and pink grapefruit
- Yogurt with herbal extracts, including ginger and green tea extract
- Yogurt with passion fruit enhanced with elder flower extract
- Strawberry/rhubarb fitness yogurt, fortified with vitamins B, C and E

There is a growing interest in offering premium and indulgent yogurts as desserts (85). Some of the creations listed include:

- Bourbon Vanilla
- Pear and Butterscotch
- Goat yogurts containing mandarin and ginger, lemon and lime zest, and summer berries

We have come a long way in the development of yogurt into a respectable dairy product with noted health benefits. Producers and marketers of this product are making every effort to keep the yogurt category growing through product development and packaging innovations, while delivering a good-for-you flavorful product suited for all occasions of gastronomic indulgence.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Yogurt," by K. R. Nauth, in *Handbook of Food and Beverage Fermentation Technology*, Editors: Y. H. Hui et al., Marcel Dekker, NY 2004.

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153 Egg Biology

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CONTENTS

I. INTRODUCTION

The egg is both a biological and chemical entity. Eggs are unique in the sense that the nutrients are already in the egg, and are balanced in such a way as to support a new life upon incubation. The egg also provides a perfectly packaged, portion controlled, highly nutritious food containing vital nutrients essential for maintaining human health. This chapter describes, briefly, the formation, composition, and nutrient content of the egg, the egg's role as a functional food, nutrient modulation strategies, and also the uniqueness of egg in nutrition research.

II. PHYSIOLOGY OF EGG FORMATION

The ovary of an immature pullet has small-undifferentiated follicles that are not visible to the naked eye. At puberty, these follicles increase in size to form an array of small follicles (known as the ovarian stroma), as well as a hierarchy of large follicles that vary in size (1,2). Growth of follicles can be divided into a slow phase (months to years), a rapid phase increasing in protein content (2 month period), or a final phase (7 to 11 days before ovulation), when the majority of yellow yolk and lipids are deposited (1). During the final phase, a follicle will increase in size from 0.08 g to 16 g in weight. The production of eggs is orchestrated through the action of organs, such as the brain (endocrine), liver (lipoprotein), and skeletal system (calcium). Light stimulation causes the release of follicle stimulating hormone from the pituitary, causing an increase in the growth of ovarian follicles. Upon reaching sexual maturity, the ovum is released by the action of leutinizing hormone secreted by the pituitary gland. A list of major hormones and their role in egg production is listed in Table 153.1. The liver plays an active role in yolk lipid accretion, as it produces lipoproteins that contribute to egg yolk lipids. Yolk is deposited into follicles as they proceed through the hierarchy to become mature. Other nutrients, such as water, sugars, proteins, vitamins, and minerals are also deposited into the yolk from the hen's blood. Follicles are said to be "mature" when they are capable of producing progesterone, which is released from the largest follicle (3). Release of progesterone will trigger the ovulation process. Follicular maturation typically takes longer than 24 hours, and so the ovulatory cycle is set back slightly each day (4). The matured follicle is ovulated and drops from the ovary into the egg-laying tract as the pullet reaches laying age (around 20 weeks). The process of laying an egg takes approximately 25 hours from the time of ovulation until the egg is laid.

The avian oviduct is a tubular organ extending from the ovary to the cloaca. In laying hens, the oviduct is 40–80 cm long, consisting of five portions: infundibulum, magnum (albumen-secreting portion), isthmus, uterus, and vagina (1) (Figure 153.1). The oviduct ensures smooth passage of the egg and secretes extracellular matrix components to surround the egg albumen. The yolk enters the oviduct and is surrounded by two layers of thin white, and two layers of thick white. Initiation of calcification occurs in the shell gland (uterus). The egg stays in the uterus for about 21–26 hr to complete the process of calcification (5). The high rate of calcium secretion returns to basal level approximately 2 hr before expulsion of the egg. It takes approximately 2 grams of calcium to form an eggshell. Prior to calcification, the egg takes up water and pigments into the albumen from the tubular glands. Pigment deposition occurs in the last three to five hours the egg spends in the shell gland. It is believed that light colored eggs spend a shorter amount of time in the shell gland, and therefore have less shell deposition. The laying of an egg is known as oviposition, which occurs in sequences. Sequences are consecutive days of laying that are separated by a "pause"

TABLE 153.1
Major Hormones Involved in Egg Production[a]

Hormone	Location of Production	Function
Luteinizing Hormone	Anterior Pituitary	Ovarian hormone production
Follicle Stimulating Hormone	Anterior Pituitary	Early follicle development
Progesterone	Ovarian Follicle (Granulosa cells)	Oviduct growth. Initiate ovulation through feedback

[a] Reference 1,3.

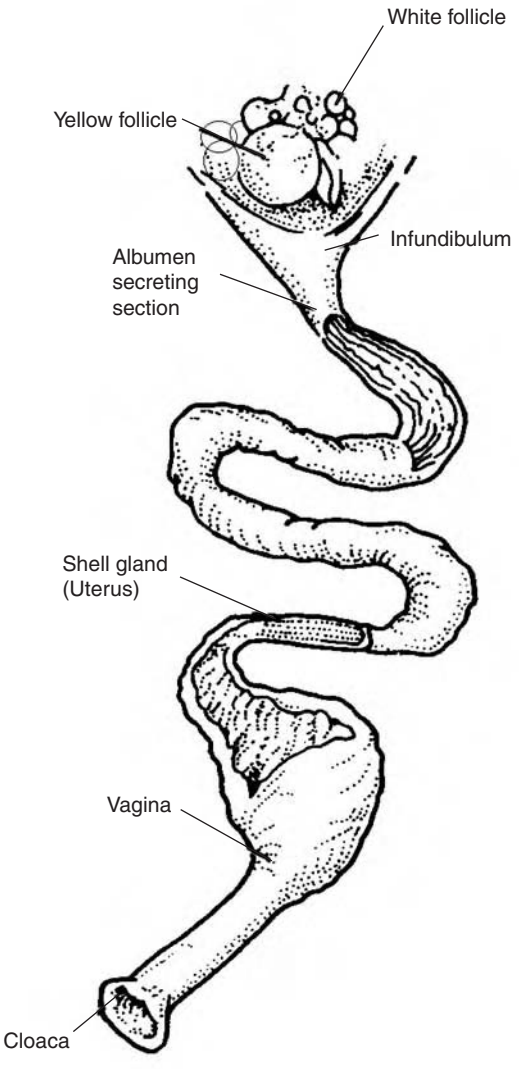

FIGURE 153.1 Oviduct of laying hens.

of 40 to 44 (or more) hours duration. Sequence length changes through the egg production year. The longest sequences are seen during the time of peak production at about 30 to 35 weeks of age (3,4).

III. EGG COMPOSITION

The egg is comprised of four main parts: yolk, egg white or albumen, shell membranes, and shell. The composition of eggs, reported decades ago, consisted of 58% white, 31% yolk, and 11% shell (6). Dietary manipulation of egg nutrients, strain of the bird, and genetic selection for egg size and production may lead to changes in egg components. Recently, there has been a tremendous interest in specialty eggs. The composition of such specialty eggs reveals a wide variation in egg components, such as yolk, white content, edible portion, and yolk:white ratio (7). A comparison of the egg components of supermarket eggs to those from hens fed special diets is shown in Table 153.2. In general, the shell contributes 9 to 11%, the yolk 25 to 33%, and the egg white 56 to 64% in chicken eggs (7). The total edible portion is 89 to 91%. Cooking leads to minor changes in egg nutrient content (8).

A. The Egg Yolk

The yolk is the first part of the egg to develop and comprises about 30–31% of the total weight. The yolk is comprised of 51–52% water, 16–17% protein, 31–33% fat including cholesterol, fat soluble vitamins, pigments, and some minerals.

1. Lipids

The lipids in eggs have attracted attention both at the scientific and consumer level, due to the link between high dietary fat consumption and coronary heart diseases. The fat in the egg is exclusively in the yolk, and comprises 5.5 to 6 g in an average 60 g egg. Almost all lipids are present as lipoprotein complexes associated with yolk. Trace levels of lipids have been observed in the whites. Yolk lipids are made mainly of triacylglycerol, phospholipid, and free cholesterol. Triacylglycerol and phospholipids are the major components of yolk lipids, comprising up to 65 and 32%, respectively (9). Fatty acids are the most prevalent components of triacylglycerol and phospholipids. Fatty acids may be of different chain lengths and degrees of saturation, as well as different configurations. The degree of unsaturation of fatty acids is of interest to researchers, because of its effect on health. Consequently, the most significant characteristic of dietary lipids is the content of different types of fatty acids. Fatty acids are classified into three families: saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (PUFA).

TABLE 153.2
Comparison of Egg Components in Regular and Speciality Eggs

Egg Components	Control		Specialty Eggs			
Yolk	30.2^{b}	29.7^{b}	25.9^{c}	33.4^{a}	25.6^{c}	29.8^{b}
White	59.6^{b}	59.4^{b}	63.7^{a}	56.3^{c}	64.5^{a}	60.7^{b}
Shell	10.1^{ab}	10.8^{a}	10.3^{ab}	10.2^{ab}	9.9^{b}	9.5^{b}
Edible Portion	89.8^{ab}	89.2^{b}	89.7^{ab}	89.7^{ab}	90.1^{a}	90.5^{a}
Yolk: White	50.6^{b}	50.0^{b}	40.6^{d}	59.0^{a}	39.6^{d}	49.0^{c}
Total Lipids	25.1^{abc}	27.7^{a}	24.6^{bc}	26.2^{a}	22.9^{c}	25.9^{ab}

Different specialty eggs are collected from hens raised on a vegetarian diet or as free range. Reported as percentage of egg weight.

[a–d] within a row with no common superscripts indicates significantly different ($P < 0.05$), $n = 6$.

Adapted from Reference 7.

a. *Saturated fatty acids*

The predominant saturated fatty acids in eggs are palmitic (C16:0) and stearic (C18:0). The content of these two fatty acids in chicken eggs may range from 22 to 26% and 8 to 10%, respectively. There are also other minor amounts of C14 and C20. The total saturated fatty acids in eggs may constitute up to 30 to 35%.

b. *Monounsaturated fatty acids*

The monounsaturated fatty acids in eggs are constituted by C16:1, and C18:1, which constitutes to 42–46%. Oleic acid (C18:1) is the major monounsaturated fatty acid in chicken eggs. Oleic acid has been reported to be hypolipidemic, reducing both cholesterol and triacylglycerol without decreasing high density lipoprotein cholesterol in human patients (10). The content of egg monounsaturated fatty acids is affected by dietary lipids. Addition of high oleic acid sunflower seeds in feed has been reported to increase the content of oleic acid in eggs up to 3.2 g, compared with 1.9 g in regular egg (11). Recent studies reported a significant decrease in egg oleic acid when diets contained conjugated linoleic acids (CLA) (12,13,14).

c. *Omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids*

There are two families of PUFA in the egg, namely n-6 and n-3 fatty acids. The predominant n-6 PUFA in egg lipids is C18:2n-6 (linoleic acid). Other n-6 fatty acids in eggs may include C20:4n-6, C22:4n-6, and C22:5n-6. The content of long chain n-6 PUFA (LCPUFA) (>20-carbon) may vary from 1 to 2%, and is reflected by the type of laying hen diet (15). The content of n-3 fatty acids in eggs is contributed by α-linoleic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acids (DHA, 22:6n-3). Among these, DHA is the major n-3 fatty acid in the egg. The α-linoleic content in regular eggs is under 1% of the total lipids. DHA may constitute

between 1 and 3%. The content of n-3 PUFA is a reflection of dietary fat. Addition of flax, fish oil, and marine algae in laying hen diet leads to significant increases in α-linoleic acid and DHA in eggs (15,16).

d. Sterols

The major sterol in yolk lipids is cholesterol, and is found in the free form. The cholesterol content may vary from 11–14 mg/g yolk or 200 to 220 mg/average egg. The cholesterol content of chicken eggs has received far more attention than any other component of eggs from the media, consumers, and health professionals, due to the reported role of serum cholesterol in cardiovascular diseases. Although diet has very little effect on egg cholesterol (17), other factors like egg weight, yolk size, and strain of bird may affect the cholesterol content (19). Most reported studies indicate an enhancement of high density lipoprotein ('good') cholesterol in humans by egg consumption (20).

e. Phospholipids

Phospholipids are an important component of egg yolk lipids and constitute 28 to 30%. The egg phospholipids are constituted by phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, and sphingolipids (9). The amphipathic nature of phospholipids aids in their good emulsifying properties, and is widely used in the food, pharmaceutical, and cosmetic industries. Egg phospholipids are also rich in polyunsaturated fatty acids, such as DHA (22:6 n-3) and arachidonic acid (20:4–6), and are getting attention as a source of long chain PUFA for the infant formula industry, due to their role in central nervous system development (21).

2. Yolk Proteins

Egg proteins distributed in the yolk exist as lipoproteins. Low-density lipoprotein (LDL) is the major protein, and accounts for up to 65% of the total yolk proteins, and may contain up to 80–89% lipids. The high density lipoprotein exists as a complex with phosphoprotein as 'phosvitin.' About 80% of phosphorus in eggs is located in phosvitin, which is derived from vitellogenin formed in the liver (22). Other yolk proteins include livetin, a water-soluble, non-lipid glycoprotein, and riboflavin-binding protein, a hydrophilic phosphoglycoprotein.

3. Yolk Vitamins

The chicken egg is considered a good source of most vitamins, except vitamin C. Egg vitamins include both fat soluble and water soluble (Table 153.3) (23). Most fat-soluble vitamins are concentrated in the yolk. Although several factors, such as age, strain of bird, and age of bird are involved, diet is the most important factor, regulating egg vitamin content. Transfer efficiency of a vitamin depends on vitamin level in the diet, feed intake, rate of egg production, and egg weight. The transfer efficiency may vary between vitamins. For example, vitamin A has a transfer efficiency between 60 and 80%; riboflavin, pantothenic acid, and biotin have a transfer efficienies between 40–50%; vitamin E and vitamin D have a transfer efficiencies between 15–25%; and vitamin K, thiamin, and folacin have 5 to 10% transfer efficiencies (24).

TABLE 153.3
Vitamin Content of Eggs[a]

Nutrient	Whole Egg	White	Yolk
Vitamin A (IU)	317	—	323
Vitamin D (IU)	24.5	—	24.5
Vitamin E (mg)	0.70	—	0.70
Vitamin B12 (μg)	0.50	0.07	0.52
Biotin (μg)	9.98	2.34	7.58
Choline (mg)	215.1	0.42	215.9
Folic Acid (μg)	23	1	24
Inositol (mg)	5.39	1.38	3.95
Niacin (mg)	0.037	0.03	0.002
Pantothenic Acid	0.63	0.04	0.63
Pyridoxine (mg)	0.07	0.001	0.065
Riboflavin (mg)	0.25	0.15	0.106
Thiamine (mg)	0.03	0.002	0.028

[a] http:/www.aeb.org/food/nutrient.html (21).
Based on 59 g shell weight, with 50 g total liquid whole egg, 33.4 g white, and 16.6 g yolk.

4. Yolk Minerals

The egg yolk contains 1% minerals, and phosphorus is the most abundant mineral component. More than 60% of the total phosphorus in egg yolks is contained in phospholipids. The major inorganic components of egg white are sulfur, potassium, sodium, and chlorine (Table 153.4). Phosphorus, calcium, and magnesium are next in importance. Table 153.3 shows the content of major minerals in eggs (23).

5. Yolk Carbohydrates

The content of carbohydrates in egg yolk is about 1%. The majority of carbohydrates are oligosaccharides bound to protein. The remaining 30% are free carbohydrates in the form of glucose, and constitute to 0.8 and 0.7% in the whole egg, albumen, and yolk respectively. The total carbohydrate in the whole egg, white, and yolk constitute to 0.61, 0.34, and 0.30 g, respectively. Sialic acid is a functional carbohydrate in egg with pharmacological potential (25), due to its involvement in cell adhesion, receptor functions, and defense mechanisms.

6. Yolk Pigments

Several pigments are present in all parts of the egg. The yolk has the highest pigment content constituting 0.02%.

TABLE 153.4
Mineral Content (mg) of Egg Edible Portion[a]

Nutrient	Whole Egg	White	Yolk
Calcium	25	2	23
Chlorine	87.1	60.0	27.1
Copper	0.007	0.002	0.004
Iodine	0.024	0.001	0.022
Iron	0.72	0.01	0.59
Magnesium	5	4	1
Manganese	0.012	0.001	0.012
Phosphorus	89	4	81
Potassium	60	48	16
Sodium	63	55	7
Sulfur	82	56	25
Zinc	0.55	—	0.52

[a] Reported in mg (21). http:/www.aeb.org/food/nutrient.html. Based on 59 g shell weight, with 50 g total liquid whole egg, 33.4 g white, and 16.6 g yolk.

The yolk carotenes are classified as xanthophylls and carotenes. Leutin, zeaxanthin, and cryptoxanthin belong to the xanthophyll group, and β-carotene belongs to the carotene group. The yolk color is of aesthetic importance in some parts of the world, with preferences ranging from deep yellow to light-colored yolks. Feed containing yellow corn, canola, flax, or alfalfa produces medium yellow yolks, while feed containing wheat or barley produces lighter color yolks. Natural yellow-orange substances, such as marigold petals, are added to feeds to enhance the yolk color. The yolk pigments are attracting a lot of interest, due to their potential role in sight and antioxidant actions.

B. Egg White

1. Egg White Proteins

The protein value of whole egg protein is considered to be 100, and is used as a standard for measuring nutritional quality of other food proteins. Addition of two eggs in the diet provides 12 g of protein, which will meet 30% of recommended dietary allowance in the United States. Protein is the major component of egg white, which contains about 11% proteins consisting of more than 40 different kinds of proteins. Due to their functional and pharmacological properties, egg proteins are desirable ingredients in many baked foods and in the drug industry. Ovalbumin is the major protein, and constitutes up to 54% of total egg white proteins, serving as a major source of amino acids for the developing embryo. Ovotransferrin and ovomucoid constitute about 12 and 11% of proteins, respectively (22). Ovotransferrin is implicated in the transfer of iron to target cells, and could be used as a nutritional ingredient in iron-enriched food products. Other proteins include ovomucin, ovoglobulin, ovomacroglobulin, ovoglycoprotein, flavoprotein, ovoinhibitor, avidin, and cystatin. Egg white also contains enzymes, such as lysozyme, phosphatase, and catalase. Among these enzymes, lysozyme constitutes about 3.5% of egg white, and is widely used in the food industry due to its antibacterial properties.

C. Egg Shell

Egg shell is a complex compound composed of 95% minerals, of which calcium is more than 98%. Other inorganic components include phosphorus, magnesium, and trace amounts of iron and sulfur comprising less than 0.05%. Egg shell powder is considered to be a good source of highly bioactive calcium, and could be used as an ingredient for human consumption. Egg shell is composed of a thin film cuticle, a calcium carbonate layer, and two shell membranes. Egg shell membrane contains several bacteriolytic enzymes, such as lysozyme and N-acetyl glucosaminidase as well as other membrane proteins that have been thought to have beneficial effects in treating injuries. Carbohydrates in egg shell are composed of glycosaminoglycans that are anionic polysaccharides consisting of hyaluronic acid (48%) and galactosaminoglycan (52%) (27). These carbohydrates have wide application in the cosmetic, pharmaceutical, and food industries.

IV. EGGS AND HUMAN HEALTH

Although chicken eggs are a rich source of vital nutrients essential for maintaining human health, the per capita consumption of eggs has been declining steadily over the past four decades (Figure 153.2). Changing lifestyles, more choices of food products, and consumers' increasing health consciousness are some of the contributing factors. The egg, with its high source of fat and cholesterol, has been an easy target in the control of dietary fat intake. Due to its relatively high cholesterol content, the egg has been used as an exclusive source of dietary cholesterol in both

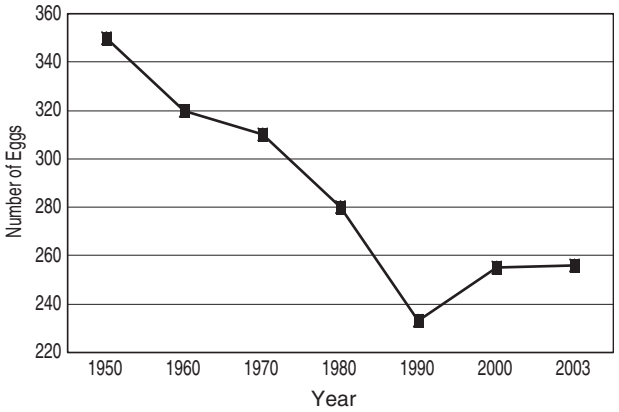

FIGURE 153.2 Shell egg consumption patterns in the U.S. during the last 50 years.

animal and human experiments, and has become the visual icon of high cholesterol. The results from some of the studies claimed cholesterol as a promoter of diet-related chronic diseases, resulting in damage to the egg's reputation as a nutritious food item. However, recent research has shed more insight into the beneficial health effects of eggs, and egg consumption has observed an increase since the late 1990s (Figure 153.2).

A. Egg Consumption, Blood Lipids, and Coronary Heart Disease

In a large prospective cohort study that evaluated the food consumption patterns of more than 100,000 people, Hu et al., 1999 (28) reported no overall association between egg consumption (up to one egg per day) and the risk of coronary heart disease. The authors speculated that the positive health effects associated with egg consumption were attributable to health-promoting nutrients, including PUFA, antioxidants, folates, and other B vitamins. However, an adverse effect of egg consumption on diabetic subjects was observed by these authors. The increased risk of coronary heart disease may be associated with abnormal cholesterol metabolism in diabetics (28). The effect of dietary egg cholesterol on the ratio of total cholesterol to high density lipoprotein (HDL) cholesterol was investigated by Weggemans et al., 2001 (20). After reviewing the data from 1974 through 1999, these authors concluded that egg cholesterol increases the ratio of total to high density lipoprotein cholesterol, thus adversely affecting the human cholesterol profile. Investigating the nutritional significance of eggs in American diets, Song and Kerver, 2000 (29) conducted a cross-sectional and population-based study (n = 27, 378) during 1988–1994. Nutrient intake, egg intake, and blood cholesterol concentrations of subjects were evaluated according to the frequency of egg consumption. These researchers reported no association between egg consumption and blood cholesterol concentration. In addition, consuming eggs contributed significantly to other nutrients, such as vitamin B6, saturated and PUFA, and vitamins A, E, and B12 (29).

V. HENS' EGGS AS FUNCTIONAL FOOD

Functional foods have been defined as foods that, by the virtue of the presence of physiologically active components, provide health benefits beyond basic nutrition. Consumers are highly interested in functional foods that prevent or ameliorate the disease progression process. The egg is a storehouse of several biologically active functional ingredients, like PUFA, carotenoids (leutin, zeaxanthin), cholines, sphingolipids, and antioxidant vitamins, providing new and exciting avenues for developing health-value-added egg and functional foods. The health-enhancing nutrients in eggs could also be modified through the hens' diet, making eggs an excellent choice as a functional food.

A. Eggs: Role in Sight

Age–related macular degeneration is a debilitating eye disorder of the elderly, resulting in irreversible blindness. Two carotenoids, leutin and zeaxanthin, accumulate in the macular region of the eye and have recently received attention for their potential role in delaying age–related macular degeneration (30). Leutin and zeaxanthin are able to absorb blue light striking the retina, which is thought to initiate degeneration of the retinal membranes. Egg yolk contains 292 and 213 μg of leutin and zeaxanthin, respectively; and they are more bio-available from this source than from plant sources of carotenoids. Eating eggs resulted in respective 50 and 114% increases in plasma leutin and zeaxanthin (30). Leutin and zeaxanthin also possess antioxidant properties and protect the PUFA-rich retinal membranes from phototoxic damage. In view of the potential role of these carotenoids in delaying macular degeneration, including eggs in the diets of the elderly should be reconsidered.

B. Eggs as Brain Food

Phospholipids are an important component of egg yolk lipoproteins, and also the major constituent in cell membranes of all living organisms. Phospholipids provide the necessary interface for nutrient transport, due to their amphipathic properties, and are rich in long chain PUFA, such as DHA (22:6n-3) and arachidonic acid (20:4n-6). Neural tissues, such as those in brain and retina, are highly concentrated, with DHA constituting over 50 and 70% of fatty acids in brain synaptosomal membranes and rod outer segments of retina, respectively DHA is involved in maintaining membrane fluidity, signal transduction, protein signaling, and gene expression (31). Accretion of DHA in the human brain is also high during periods of brain growth spurt, which starts during the third trimester of pregnancy and extend through the first 2 years of life (21). An average egg could provide over 300 mg of long chain PUFA, and could well be included in the diet of pregnant and nursing women as well as the diet of weaning toddlers. The content of egg DHA and arachidonic acid is highly dependant on dietary PUFA, and can be increased by feeding appropriate feed sources, which are discussed later in the nutrient modulation section.

C. Source of Choline

Chicken eggs are a rich source of choline (associated with phosphatidyl choline in phospholipids). Choline is responsible for the structural integrity and signaling function of phopholipid-rich cell membranes. One large egg contains about 300 mg of choline, which provides 60% of the recommended daily intake of choline for adults,

including pregnant and lactating women (32). Animal studies have found that choline plays an essential role in the development of brain function, memory and learning ability in mice (33).

D. Source of Sphingolipids

Sphingolipid content of eggs has been reported to be the highest of any food at 2250 umol/kg (34). Sphingolipids are critical for the maintenance of membrane structure and modulate the behavior of extracellular matrix proteins. Sphingolipids also function at the binding site for microorganisms, microbial toxins, and viruses. Inhibition of early stage colon cancer and a decrease in the proportion of adenocarcinoma have been reported in mice fed sphingolipids (35). An inverse correlation between tissue and plasma concentrations of sphingolipids and cholesterol in normal and pathological conditions has also been reported. Sphingolipids have been reported to alter cholesterol metabolism and, consequently, to affect cholesterol balance in the cell. A 30% reduction in plasma cholesterol in rats fed semipurified diets supplemented with a 1% mixture of sphingolipids and glycosphingolipids was reported (36). However, very little information is available on the health effects of egg sphingolipids. Further research in this area is warranted.

VI. EGG NUTRIENT MODULATION

Animal fats including egg lipids are important sources of many nutrients. In a typical Western diet, 58% of dietary fat, 74% of saturated fat and 100% of cholesterol are supplied through animal products such as eggs, meat and milk. Modern agriculture with its emphasis on production resulted in drastic changes in fatty acid content of food products resulting in an imbalance of polyunsaturated and saturated fatty acids, antioxidant vitamins and a wide ratio of n-6:n-3 fatty acids in the current food supply including eggs. The dietary recommendations set forth by health agencies include reduction in total fat intake to 30% of calories with an increase in monounsaturated fatty acids and PUFA to 15 and 10% of calories and a balance of PUFA to saturated fatty acids close to 1 (37). The food industry is encouraged to intensify the effort to develop and market products that would facilitate adherence to the dietary guidelines. The egg industry has been very responsive to these recommendations and dietary guidelines by producing and marketing eggs with more balanced PUFA containing both n-3 and n-6 fatty acids. Chickens are monogastric and the lipid nutrient composition of the diet alters the content of fatty acids and other fat-soluble vitamins and pigments in eggs. The enrichment of other minor nutrients through feed manipulation has also been reported. This section highlights some recent research in nutrient manipulation of chicken eggs.

A. Designing Egg Constituents

1. Omega-3 (n-3) Fatty Acids

The past decade witnessed a tremendous interest in the health benefits of the n-3 family of PUFA. Several studies have indicated that increased consumption of n-3 PUFA offers potential for reducing the risk of coronary heart disease, atherosclerosis, hypertension, and other cardiovascular diseases (37). These health-promoting effects of dietary n-3 fatty acids have invoked considerable efforts during recent years to create alternate sources by enriching chicken eggs with n-3 fatty acids (38). The technology has been focused on increasing the n-3 fatty acid content of eggs through incorporation of different oils or oil seeds in the hens' diet (37). Addition of ground flax seed has been used as a common practice in the production of n-3 PUFA-rich eggs (18,37). Eggs from hens fed diets containing 10 to 15% flax seed could provide up to 550–600 mg of n-3 PUFA, compared to 60 mg of n-3 PUFA in regular eggs (38). The amount of n-3 PUFA supplied from n-3 fatty acid modified eggs is equivalent to a 100 g serving of fish. Other oil seeds, such as canola seeds, contain 18:3 n-3 and also increase n-3 fatty acid content to a lesser extent (38). Other source of n-3 PUFA for fatty acid enrichment include marine oils and algae. A comparison of total n-3 fatty acids in eggs from hens fed different levels of flax oil (3.5%) or menhaden oil (3.5%) is depicted in Figure 153.3.

2. Conjugated Linoleic Acids

Conjugated linoleic acids (CLA) is the generic name for a group of positional and geometric conjugated dienomic isomers of linoleic acid. CLA has received considerable attention for its anticarcinogenic, antiatherogenic, hypocholesterolemic, immunomodulatory and body fat

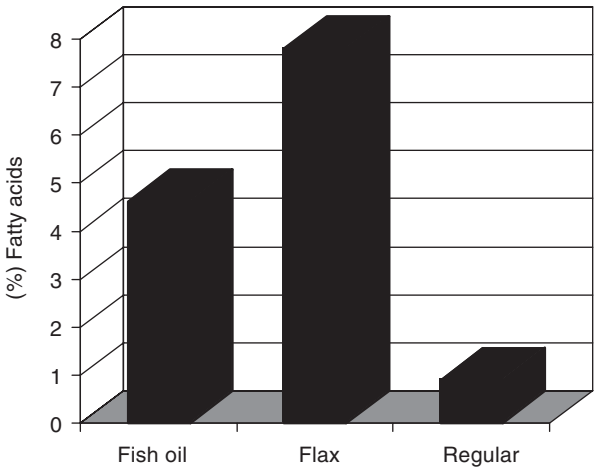

FIGURE 153.3 Omega-3 fatty acid content of eggs from hens fed 3.5% fish oil or flax oil, compared to regular eggs. (Adapted from Reference 52.)

reduction properties (40). Current intake of CLA is estimated to be several hundred milligrams/day (41). Based on animal data, it is estimated that approximately 3 g/day of CLA would be required to produce beneficial effects in humans (42). Humans do not have the ability to synthesize CLA, which be supplied through their diet. Dietary CLA is contributed by food products of ruminant origin, such as dairy products and beef (42). Poultry products contain relatively low CLA concentrations (less than 0.9 mg CLA/g of fat, respectively (43)). Increasing the concentration of CLA in poultry foods is a possible way for humans to increase their CLA intake and obtain the potential health-benefits. A significant incorporation of CLA in eggs by dietary manipulation has been reported (44,45). The diet-induced incorporation of CLA in eggs is dose-dependent. Diets containing 5% CLA resulted in about 15% CLA in egg lipids (46). From the research reported, consumption of an average chicken egg weighing 60 g could provide over one-third of the daily CLA recommendation (3 g) for an adult human (46). Cherian et al., 2002 (48) used menhaden fish oil (as a source of long chain n-3 PUFA) along with CLA in the diet of laying hens to produce n-3 PUFA-CLA rich eggs. Such eggs from hens fed a diet containing 1.0% fish oil and 2.0% CLA oil could provide over 300 mg of CLA along with 230 mg of n-3 fatty acids to the human diet.

3. Antioxidant Vitamins

a. Tocopherols, tocotrienols, retinols, and carotenes

In recent years, the term "antioxidant vitamins" (tocopherols, retinols, carotenes) has caught the attention of consumers. Both clinical and epidemiological studies have reported the protective effect of dietary antioxidants against the biggest health threats in the US, such as heart disease, cancer, and stroke. The Food and Nutrition Board of the National Academy of Sciences has increased the recommended daily amounts of certain antioxidant micronutrients. The consumption of health-enhancing antioxidant nutrients may benefit public health and reduce medical costs. Of the natural antioxidants, vitamin E has attracted much attention, due to its powerful antioxidant activity both in vivo and in vitro. In biological systems, vitamin E can function both as an intracellular and intercellular antioxidant, thus neutralizing free radicals and preventing oxidation. In addition to tocopherols, other tocols, such as tocotrienols and carotenoids also exhibit health benefits in certain diseases, such as cancer, coronary heart disease, and immune functions (49,50). Incorporation of tocols in the hens' diet has been reported to increase the vitamin E content of chicken eggs, providing a natural source of health-enhancing vitamins through food (51,52,53). Cherian et al., 1996, reported a total of 7 to 8 mg vitamin E per average egg from hens fed diets containing 400 μg/g of vitamin E (52). These results corroborate with the 400 μg/g of yolk tocopherols when hens' diets were supplemented with 800 mg/kg tocols (53). Enrichment of other minor tocols like tocotrienol and retinol alteration in eggs through diet has been reported (54).

Carotenoids constitute a group of lipid-soluble organic compounds. Carotenoids are of biological importance for their light-absorbing properties, antioxidant functions, and immunomodulatory functions. Carotenoids have been used for many years in the poultry industry as a means of pigmenting eggs and meat. Over the past few years, there has been tremendous interest in the role of carotenoids and other pigments, in particular lutein and zeaxanthin, in prevention of eye disorders. Feeding diets containing palm oil to laying hens resulted in a significant incorporation of carotenoids in eggs (54,55). Other natural products, like tomato byproducts, have been reported to increase carotenoid pigments in eggs (56). Marigold meals are used as premixes in pigmenting eggs, and contain natural yellow xanthophylls. As plant-derived natural forms of antioxidant nutrients are gaining popularity, and since poultry products contribute a major part of animal products in the western diet, altering the micronutrients and pigments in eggs and meat or further-processed poultry products may provide an alternate source of such health-promoting nutrients to consumers.

4. Vitamin D

Vitamin D is needed for normal bone formation. A deficiency of Vitamin D can lead to osteomalacia in adults and rickets in children. A less severe form of vitamin D deficiency can happen in elderly women leading to postmenopausal osteoporosis (57). Vitamin D deficiency may also occur in Europe in winter months due to restricted sun exposure (58) and because few foods are rich in vitamin D. One way to ease this problem is through fortification of eggs with vitamin D. A recent study by Mattila et al., 2004 reported up to 34 μg/100g of Vitamin D3 in vitamin D–enriched egg vs. 2.5 to 5.0 μg/100 g in control eggs (59).

5. Other Minor Nutrients

a. Selenium

Selenium (Se) is an essential component of several antioxidant enzymes such as Se-dependant GSH-Px, superoxide dismutase, and catalase. These enzymes are involved in providing cellular antioxidant protection against free radical damage produced during normal physiological processes at the cellular level. Evidence from epidemiologic, clinical and experimental studies have reported a chemopreventive potential of selenium (60). In particular, the results of recent clinical intervention trials have shown strong protective effects of selenium-enriched yeast for cancers of the lung, colon and prostate. The average intake of Selenium from food sources is reported to be between 50–70 μg/day. Levels of over 100–200 μg are

reported to have beneficial effects. With the knowledge of the effects of these selenocompounds as anticarcinogenic agents, it became of interest to investigate the most appropriate methods for delivery to the general population. One obvious approach was to investigate additional methods for expeditious ways to deliver these protective agents through the food system. When laying hens were fed high dietary levels of selenium, a significant deposition of selenium in eggs was observed (61,62). Additions of graded amounts of selenite up to 4 mg/kg diet resulted in a linear increase in the selenium content in eggs, reaching up to 30 µg of Se, which is about 50% of the daily requirement.

b. Folic acid

The term folate compounds encompasses a number of different water-soluble compounds, each based on the structure of folic acid. Folic acid does not naturally occur in appreciable amounts in foods. The recommended dietary allowance of folate is 400 mg/day (63). In 1998, the US enacted legislation requiring cereal fortification with folic acid. However, additional strategies may be needed to ensure that all segments of the population are consuming adequate folates. Eggs naturally contain 22 µg folate per large egg (Table 153.2), which provides 6% of the newly established adult daily requirements for folate (63). Recent studies by House et al., 2002, reported 41 µg/egg, providing 12.5% of the recommended dietary allowance of folate for adult humans (64).

B. Omega-3 Fatty Acid Modified Eggs and Human Health Effects

1. Effects on Blood Lipids and Blood Pressure

Several authors investigated the effects of n-3 fatty acid enriched eggs on human blood lipid parameters. In general, a significant increase in plasma n-3 fatty acids and platelet phospholipid n-3 fatty acids, as well as a decrease in platelet aggregation have been reported after the consumption of n-3 eggs (for review, see Reference 65). A reduction in systolic blood pressure was also noticed with the daily consumption of two n-3 PUFA modified eggs for four weeks (65).

2. Omega-3 Fatty Acid Eggs and Breast Milk Lipid Composition

In human infants, most DHA and arachidonic acid accumulates during the last intrauterine trimester and during early stages of life, suggesting that long chain PUFA may be indispensable for neural development of the newborn (21). During the early stages of life, infants consume n-6 and n-3 fatty acids both as C18:2n-6 and C18:3n-3 if fed on formula. Breast milk, however, reflects the mother's diet and because modern diets are low in PUFA, it is assumed that infants at present are receiving less long chain n-3 or n-6 fatty acids from breast milk. In view of the importance of n-3 PUFA for newborn infants, Cherian and Sim, 1996, used n-3 PUFA rich egg in the mothers' diet as a vehicle for supplying n-3 PUFA to nursing infants (66). Consuming two n-3 eggs as a part of their normal daily meal for six weeks resulted in a significant ($P < 0.05$) deposition of total n-3 fatty acids at 3.6%, compared with 1.9% for the pre-test milk, and a reduction in n-6:n-3 ratio (6.7 vs 3.0). The long chain n-3 PUFA comprised 1.2% compared with 0.4% in the pre-test milk ($P < 0.05$). Consuming n-3 eggs did not ($P > 0.05$) alter the C20:4n-6 or the total n-6 fatty acid content of breast milk. Infants nursed from women consuming n-3 eggs could have over 300 mg of long chain n-3 fatty acid, such as 20:5n-3, 22:5n-3, and DHA (66). Thus, a diet supplemented, with n-3 eggs for nursing women should provide an alternate way of increasing the dietary consumption of n-3 fatty acids.

3. Fatty Acid Modified Eggs: Alternate Food Uses

a. n-3 fatty acid eggs for infant formula enrichment

Although there has been a significant increase in breast-feeding in the last decade, infant formulas remain the major source of nutrition for many infants during the first 12 months of life. Fat sources in the formulas are derived from one or more vegetable oils, usually coconut, soy, safflower, or corn oil. All vegetable oils differ from human milk in such a way that they do not contain long chain n-3 or n-6 fatty acids. The ability for the in vivo formation of DHA from C18:3n-3 is also limited especially in premature infants. Therefore, optimal postnatal accretion may depend on the dietary supply of preformed DHA and other long chain PUFA to the newborn. One average n-3 fatty acid modified egg contains 6 g of lipids, with DHA constituting to about 2 to 4%, along with other long chain n-3 and n-6 PUFA. Thus, eggs enriched with LCPUFA would be a useful weaning food for infants fed formulas that do not have DHA (67). The fatty acid composition of the n-3 fatty acid enriched egg yolk lipids and breast milk lipids show similarities in their saturated, and monounsaturated fats, as well as PUFA (Table 153.5). Thus, egg oil, once extracted could be useful as a natural lipid source to be included in infant formula preparations (67). Agostoni et al., 1995 and Carlson et al., 1996, reported improved visual acuity and higher developmental quotients in term infants fed formulas containing egg phospholipids, when compared to term infants fed unsupplemented formulas (68,69). These results demonstrated the uniqueness of egg lipids in providing essential nutrients for the newborn.

VII. EGGS AS A UNIQUE MODEL FOR NUTRITION RESEARCH

In oviparous species, the developing embryo is absolutely dependent on nutrients stored in the egg yolk. Thus, the developing chick is in a nutritionally 'controlled' environment not influenced by maternal supply of nutrients

TABLE 153.5
Comparison of Fatty Acids in Human Breast Milk, n-3 Fatty Acid Enriched Egg Yolk and Commercial Infant Formula[a]

Fatty Acid (%)	Breast Milk	Egg Lipids	Commercial Formula
Total saturated fatty acids	38.3	34.2	52.8
Total monounsaturated fatty acids	45.0	38.4	19.6
Total long chain (>20-carbon) omega-6 fatty acids	1.1	1.4	0.0
Total long chain (>20-carbon) omega-3 fatty acids	1.1	3.3	0.0

[a] Adapted from Reference 67.

through the placenta as in mammals. This biologically self-contained model allows a close relationship between nutritive substances and their physiological utilization. Thus, the laying hen and the chick (avian model) can be useful models for studying the net transfer of nutrients from maternal sources and its effect on the progeny (70). In this model, chicks with severe deficiency or excess of a nutrient could be obtained through diet manipulation. Upon hatching, the newly hatched chick can be used to study the effect of maternal (yolk/laying hen) or neonatal (chick) dietary nutrients (excess or deficient) on metabolic effects and/or other behavioral changes in the progeny. Furthermore, as incubation takes only 21 days, the time span involved in raising multi-generation progeny with severe deficiency of a particular nutrient (e.g., essential fatty acids) can easily be reduced. The hatched chick, like newborn infants, must obtain the bulk of its nutrients from dietary supply.

A. Fertilized Eggs and Hatched Chicks for Infant Fatty Acid Nutrition Research

Because fat composition can be manipulated by diet, the egg is a unique research tool in studying the role of maternal dietary fatty acids on the lipid metabolism in the progeny. In mammals, the nervous system is the organ with the greatest concentration of lipids after adipose tissue. These lipids are structural and are high in long chain PUFA of the n-6 and n-3 series. DHA is the predominant n-3 fatty acid, and arachidonic acid is the major n-6 fatty acid in the central nervous system of mammals and avians. Deficiency of long chain PUFA has been reported to cause impaired visual acuity, abnormal electroretinogram, and reduction in intellectual performance. In the human brain, the last intrauterine trimester is the most active period of brain tissue growth and DHA accumulation. During prenatal life, the accretion of long chain PUFA in the human brain is of quadratic type, the increase being most rapid towards the end of gestation and continuing into early life, plateauing by two years of age, by which time 90% of human brain growth is completed (71). Considerable similarities exist between mammalian and avian species in the accretion of LCPUFA during embryonic development (72). The effect of maternal diets high or low in n-3 essential fatty acids on the PUFA metabolism of the brain tissue of developing progeny was investigated using laying hens, eggs, and hatched chicks (73). Regardless of dietary supply, an intense transfer of lipids, DHA, and arachidonic acid from maternal supply (yolk sac) resulted in a preferential incorporation of DHA and arachidonic acid in the chick (73). The changes in maternal dietary source of fat during development of brain tissue can affect the fatty acid composition of the progeny in mammals (74). Thus, despite the obvious developmental difference between mammals and avians, the subsequent usage and metabolism of PUFA is similar. This is evidenced by accretion and preferential uptake of long chain PUFA by the chick brain during the last week of incubation, suggesting that the egg and the hatched chick are unique research models in studying the effect of maternal diet on the metabolism of PUFA in the brain.

Considering the uniqueness of egg in offering several nutrients and other biologically active components that contribute to human health and the health and development of a new life during its 21-day incubation, eggs are nature's first and original functional food. Much work remains to be done before the nutritional significance of this biological and chemical entity will be fully known.

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154 Ice Cream and Frozen Desserts

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CONTENTS

This chapter is focused on frozen desserts, dairy or non-dairy, that are characterized by being concomitantly whipped and frozen in a scraped surface freezer, and subsequently consumed in the frozen state. There are many product variations on this category, ice cream and lower fat versions being the most common, but also including sherbets and sorbets, frozen yogurt, soy-based frozen desserts, etc. Thus we begin with definitions and formulations of the major products within this category. However, there are many features of these products that are similar, hence many other aspects can be treated collectively. We will review the sources and functional roles of ingredients, mix manufacturing, including formulation calculations, the dynamic freezing process, including structure and structure formation, the static freezing (hardening) process, product storage and distribution, and finally, a review of shelf-life and quality aspects. Although we use "ice cream" in the generic sense throughout this chapter, all of these topics are relevant to all products within this category.

It is not possible to provide a complete coverage of all aspects of ice cream and frozen desserts in one chapter. However, various aspects are covered in numerous books (1,2), book chapters (3–8), and review papers (9–11).

I. FORMULATIONS AND INGREDIENTS

A. Product Definitions and Formulations

1. Ice Cream

The most common product within the category of frozen desserts is ice cream. The legal definition of ice cream is controlled by regulations and varies with jurisdiction, but it is generally a sweetened product containing milkfat and milk solids-not-fat (msnf), and is frozen while being whipped. The general composition of most ice cream products is shown in Table 154.1.

Some of the factors affecting the choice of composition include legal requirements, which must be met, the quality desired in the finished product (increasing fat and total solids are usually associated with increasing quality), and the cost to be borne by the consumer. Premium products usually command a higher price. There are no specific definitions of common industry-accepted terms, such as premium or super-premium ice cream, but a relationship between fat content, total solids content, air content, and cost (also affected by quality and proportion of inclusions and marketing issues) exists, as illustrated in Table 154.2.

Suggested formulations for a range of ice cream products are presented in Table 154.3. Several trends are evident. There is usually an inverse relationship between fat and total solids compared to msnf. As discussed in Section I.B.2, the lactose component of the msnf is quite insoluble and above its saturation level in ice cream, so with increasing lactose content in a decreasing quantity of water, the risk of lactose crystallization increases. There is also generally an inverse relationship between corn syrup solids (starch hydrolysate sweetener, sometimes referred to as "glucose solids") levels and total solids. The corn syrup solids will contribute to a firmer, chewier texture, which is more desirable when there are less solids present. Likewise, as total solids increases, there is less requirement for stabilizer. This is generally due to the fact that increasing stabilizer-in-water ratios lead to enhanced gumminess, which becomes undesirable at high levels. Also, a reduction in the water content means there are diminished problems associated with ice recrystallization. Additionally, as fat levels in a mix increase, there is generally less need for emulsifier in order to optimize the extent of partial coalescence of the fat. Further discussion on many of these aspects of formulations can be found in the appropriate sections of the chapter.

Soft-serve ice cream is very similar to its hard-frozen counterpart in composition, but is sold at a different point

TABLE 154.1
The General Composition of an Ice Cream Mix

Component	Range of Concentration
Milkfat	>10–16%
Milk solids-not-fat	9–12%
Proteins, lactose, minerals	
Sweeteners	
Sucrose	10–14%
Corn syrup solids	3–5%
Stabilizers	0–0.25%
Guar, locust bean gum (carob), carrageenan, carboxymethyl cellulose (cellulose gum), micro-crystalline cellulose (cellulose gel), sodium alginate, xanthan, gelatin	
Emulsifiers	0–0.25%
Mono- and di-glycerides, Polysorbate 80	
Water	55–64%

TABLE 154.2
Average Values for Fat and Total Solids Content, Overrun, and Cost among the Categories of Ice Cream

Component	Economy	Standard	Premium	Super-Premium
Fat content	Legal minimum, usually 10%	10–12%	12–15%	15–18%
Total solids	Legal minimum, usually 36%	36–38%	38–40%	>40%
Overrun	Legal maximum	~100%	60–90%	25–50%
Cost	Low	Average	Higher than average	High

TABLE 154.3
Suggested Mixes for Hard-Frozen Ice Cream Products

	Percent (%)						
Milk Fat	10.0	11.0	12.0	13.0	14.0	15.0	16.0
Milk Solids-not-fat	11.0	11.0	10.5	10.5	10.0	10.0	9.5
Sucrose	10.0	10.0	12.0	14.0	14.0	15.0	15.0
Corn Syrup Solids	5.0	5.0	4.0	3.0	3.0	—	—
Stabilizer *	0.35	0.35	0.30	0.30	0.25	0.20	0.15
Emulsifier *	0.15	0.15	0.15	0.14	0.13	0.12	0.10
Total Solids	36.5	37.5	38.95	40.94	41.38	40.32	40.75

*Highly variable depending on type; manufacturers' recommendations are usually followed.

in its production stage, and usually with a much lower overrun content. Suggested formulations are shown in Table 154.4 for soft-serve ice cream, but it should also be recognized that much of the soft-serve on the market today falls into the low-fat, or ice milk category, with fat contents typically around 4%. Generally, while the fat content is kept lower, the msnf content is generally higher than for hard-frozen products. Lactose crystallization is not a problem in these products, as they are consumed immediately after freezing. Corn syrup solids are often used, but can lead to an enhanced sensation of gumminess. Stabilizers are also generally used for viscosity enhancement and mouthfeel, but their function in ice recrystallization is no longer needed. Dryness and shape retention, however, are a big concern in soft-serve products, hence the emulsifier content is generally kept high.

2. Reduced Fat Products

Ice milk was the traditional lower fat ice cream product for many years; but this category has been reclassified by many regulatory jurisdictions to include three reduced fat categories: light ice cream, lowfat ice cream (the traditional ice milk), and non-fat ice cream. Light or "reduced fat" ice creams are usually in the range of 5–7.5% fat. Lower fat versions are usually in the range of 3–5% fat. It has generally been possible to produce lower fat products, as low as 4% fat, with traditional ingredients, but further fat reductions have generally involved the incorporation of fat-replacers. These are discussed further in Section I.B.1. Suggested formulations for light and low-fat ice creams are presented in Table 154.5.

3. Sherbet

Sherbet is usually taken to be a frozen dairy dessert made from a milk product, but containing a low (usually legally-defined maximum, e.g., 5%) level of milk solids, including milk fat, a high level of sweeteners (sugar and corn syrup solids, 30–35%), and added acidity (usually to greater than a legally defined minimum, e.g., 0.35%, expressed as lactic acid). Suggested formulations are given in Table 154.6. Because of the acidified nature of sherbets, they are most suited for typical acidic fruit flavors, e.g., citrus. The sugar and acid levels in fruits or fruit purees have to be considered in the final formulation, and are included in the numbers suggested above. Acidity is usually added in the form of citric or tartaric acid, and this level of acidity modifies the perception of sweetness that would otherwise be created by the high level of sugars. Acid should not be added to ice and sherbet mixes until just before freezing. Heating of some stabilizers in the presence of acid will reduce their effectiveness. Adding acid to a sherbet mix in which the milk solids have been included, may result in aggregation/precipitation

TABLE 154.4
Suggested Mixes for Soft-Frozen Ice Cream Products

	Percent (%)	
Milk Fat	10.0	10.0
Milk Solids-not-fat	12.6	12.0
Sucrose	13.0	10.0
Corn Syrup Solids	—	4.0
Stabilizer *	0.15	0.15
Emulsifier *	0.20	0.20
Total Solids	36.0	36.3

*Highly variable depending on type; manufacturers' recommendations are usually followed.

TABLE 154.5
Suggested Mixes for Low-Fat Ice Cream or Ice Milk Products (3–5% Fat) and Light Ice Cream Products (6–8% Fat)

	Percent (%)				
Milk Fat	3.0	4.0	5.0	6.0	8.0
Milk Solids-not-fat	13.0	12.5	12.5	12.0	11.5
Sucrose	11.0	11.0	11.0	13.0	12.0
Corn Syrup Solids	6.0	5.5	5.5	4.0	4.0
Stabilizer *	0.35	0.35	0.35	0.35	0.35
Emulsifier *	0.10	0.10	0.10	0.15	0.15
Total Solids	33.65	33.45	34.45	35.5	36.0

*Highly variable depending on type; manufacturers' recommendations are usually followed.

TABLE 154.6
Suggested Sherbet Mixes Showing Typical Components

	Percent (%)	
Milk fat	0.5	1.5
Milk Solids-not-fat	2.0	3.5
Sucrose	24.0	24.0
Corn Syrup Solids	9.0	6.0
Stabilizer/emulsifier	0.3	0.3
Citric acid (50% sol.)	0.7	0.7
Water	63.5	64.0
Total	100.0	100.0

of the protein. Sherbet generally requires the addition of milk solids, and at least some fat (~0.5%) is desirable, as it tends to lubricate the dynamic freezer and provides a slightly more pleasant mouthfeel than non-fat products. In many multi-product manufacturing settings, ice cream mix is widely used as a source of milk solids, and the amount added will depend upon the level of milk solids desired. Overrun should be kept much lower in sherbet than that in ice cream, usually 30–35%.

4. Frozen Yogurt

Yogurt is a well-established dairy product, is generally perceived to be characterized by developed acidity (lactic acid) from fermentation of lactose by bacterial culture, and may or may not include live culture. The acidity destabilizes the casein micelles in the milk, and they, in turn, establish the typical acid gel. Frozen yogurt, therefore, should be much like the unfrozen version, and be also characterized by developed acidity from fermentation. The example formulation in Table 154.7 is typical of a more traditional frozen yogurt. However, in most legal jurisdictions, frozen yogurt is not standardized, so a wide range of products exists, including those in which the acidity is not developed by bacterial culture, but has been added in the form of citric acid.

To make a traditional frozen yogurt, as in Table 154.7, the processing occurs in two steps: the manufacture of a fermented yogurt-like ingredient, and the blending of this product with the rest of the ingredients. For example, 20% of the mix in Table 154.7, consisting of skim milk and skim milk powder, blended to give 12.5% msnf, is pasteurized at 85–90°C, cooled to 40 to 43°C, inoculated with a yogurt culture (typical of yogurt processing), and incubated as the yogurt portion. When the fermentation is complete (to the desired acidity), the "yogurt" is cooled. To make the "sweet" mix, the cream, sugar, stabilizer, and the balance of the skim milk powder and skim milk are combined, pasteurized, homogenized, cooled (typical for ice cream processing), and then blended with the "yogurt." The completed frozen yogurt mix is then aged and prepared for flavoring and freezing.

TABLE 154.7
Suggested Frozen Yogurt Formulation

	Percent (%)
Milk Fat	2.0
Milk Solids-not-fat	14.0
Sugar	15.0
Stabilizer	0.35
Water	68.65
Total	100.0

5. Fruit Ices and Sorbets

"Ice" or "sorbet" is likewise typically not defined in legal regulations, but is generally taken to be much the same as sherbet, except that milk solids are not included. Sorbets are generally frozen in a swept surface freezer, while ices are generally frozen quiescently in molds. Both sorbets and ices are usually fruit-based, and ingredients include combinations of fruit and/or fruit juices, sugar, stabilizer, and water. Overrun is very low, as aeration is difficult to achieve without protein or emulsifier. To make water ice or sorbet mixes from the above suggested sherbet formulae, delete the fat and msnf.

B. Sources and Functional Roles of Ingredients

1. Fat

The fat component of frozen dairy dessert mixes increases the richness of flavor, produces a characteristic smooth texture by lubricating the palate, helps to give body, and aids in producing desirable melting properties (1,6). The fat content of a mix also aids in lubricating the freezer barrel while the ice cream is being manufactured. Limitations on excessive use of fat in a mix include cost, a hindered whipping ability, decreased consumption due to excessive richness, and high caloric value. Fat contributes 9 kCal/g to the diet, regardless of its source. During freezing of ice cream, the fat emulsion that exists in the mix will partially coalesce (destabilize) or churn as a result of emulsifier action, air incorporation, ice crystallization, and high shear forces of the blades (6,12). This partial churning is necessary to set up the structure and texture in ice cream, which is very similar to the structure in whipped cream (13). This process will be discussed in Section II.B.4. The fat content is an indicator of the perceived quality and/or value of the ice cream. Ice cream must have a minimum fat content of 10% in most legal jurisdictions. Premium ice creams generally have fat contents of 14 to 18%. It has become desirable, however, to create light ice creams, $<$10% fat, with the same perceived quality. In addition to

structure formation, fat contributes a considerable amount of flavor to ice cream, which is difficult to reproduce in lowfat ice creams. Fat content must be altered by at least 1% before any noticeable difference appears in the taste or texture (1). Several recent papers have examined the effect of source and quantity of milk fat on sensory and textural characteristics of ice cream (14–20).

Milkfat as a fat source for ice cream formulations is in widespread use in North America, Australia and New Zealand, and parts of Europe. The triglycerides in milkfat have a wide melting range, +40° to −40°C. The crystallization patterns of milkfat are also very complex, due in part to the large variation in fatty acids and large numbers of different triglycerides present (21). Consequently, there is always a combination of liquid and crystalline fat at refrigeration and subzero temperatures. Alteration of this solid: liquid ratio at freezer barrel temperatures, through natural variation or fat fractionation, may affect the ice cream structure formed. The best source of milkfat in ice cream for high quality flavor is fresh sweet cream, from fresh sweet milk (1). Other sources of milkfat include sweet (unsalted) butter, frozen cream, or condensed milk blends. Whey creams have also been used, but may lead to flavor or texture problems.

Vegetable fats are used extensively as fat sources in ice cream in the United Kingdom and parts of Europe, but only to a very limited extent in North America. Three factors of great interest in selection of fat source are the way in which the fat crystallizes, the temperature-dependent melting profile of the fat, especially at refrigerator and freezer temperatures, and the flavor and purity of the oil (6). For optimal partial coalescence during freezing, it is important that the fat droplet contain an intermediate ratio of liquid:solid fat at the time of freezing. Crystallization of fats occurs in three steps: subcooling of the oil (below the equilibrium crystallization temperature) to induce nucleation, heterogeneous or homogeneous nucleation (or both), and crystal propagation. In bulk fat, nucleation is predominantly heterogeneous, with crystals themselves acting as nucleating agents for further crystallization, and subcooling is usually minimal. However, in an emulsion, each droplet must crystallize independently of the next. For heterogeneous nucleation to predominate, there must be a nucleating agent available in every droplet, which is often not the case. Thus in emulsions, homogeneous nucleation and extensive subcooling are expected (6). Blends of oils are often used in ice cream manufacture, selected to take into account physical characteristics, flavor, availability, and cost. Hydrogenation is often necessary to achieve the appropriate melting characteristics. Palm kernel oil, coconut oil, palm oil, sunflower oil, peanut oil, and fractions thereof with varying degrees of hydrogenation are all used to some extent. Tong and co-workers (22) substituted a portion of milkfat in ice cream with safflower oil, a highly unsaturated oil, in an attempt to lower the saturated fatty acid content of the final product. They reported that increasing concentration of safflower oil decreased overrun, but had little effect on the extent of fat destabilization at lower substitution levels.

There has been a great interest in the marketplace for the development of lower fat alternatives to traditional ice cream products. As a result, a large amount of product development time has been used in searching for a combination of ingredients that will replace the textural and flavor characteristics of fat in ice cream (17,18,23). These often involve the use of fat substitutes. Such products may be formulated with starch or other polysaccharides, proteins, or lipids, but their main requirement is to provide less calories to the product than traditional fat sources in the diet. A great deal of technical literature exists on the various properties of the products being marketed by a number of commercial firms. Schmidt and co-workers (24) studied the rheological, freezing, and melting properties of ice milks manufactured with protein-based or maltodextrin-based fat alternatives. They concluded that the carbohydrate-based alternatives resulted in greater affects on mix rheology, while the protein-based alternatives were more similar to ice cream, due in part to the functional contributions of proteins to food systems, especially in the area of emulsification and air incorporation. Ice cream products are very complex systems, both in structure and flavor. In creating products that are meant to deliver to the consumer the same attributes but with less fat or calories, it is imperative that the structural element of fat be considered to the same extent as flavor in order to deliver high quality products and develop market share for these products.

2. Milk Solids-not-Fat

The milk solids-not-fat (msnf) or serum solids improve the texture of ice cream, aid in giving body and chew resistance to the finished product, are capable of allowing a higher overrun without the characteristic snowy or flaky textures associated with high overruns, and may be a cheap source of total solids (25). The msnf contain the lactose, caseins, whey proteins, minerals (ash), vitamins, acids, enzymes, and gases of the milk or milk products from which they were derived. The content of msnf used in a mix can vary from 10 to 14% or more. Whole milk protein blends contain both caseins and whey proteins, and this category includes most of the traditional sources of milk msnf, fresh concentrated skimmed milk, or spray dried low-heat skim milk powder. However, most ice cream formulations now use another source or sources of msnf or milk protein to replace all or a portion of skim milk solids, for both functional and economical reasons (26).

When assessing replacements for skim milk solids, an important consideration is the levels of protein, lactose, and ash in the ingredients being assessed (27). Lactose is not very sweet and not very soluble, and therefore, during

freezing of ice cream, it is freeze-concentrated beyond maximum solubility (supersaturated) and thus potentially prone to crystallization. Lactose crystals are very undesirable in ice cream, causing the defect known as sandiness. Lactose, being a disaccharide, also contributes to freezing point depression in the mix, so its concentration must be closely controlled. In addition, the milk salts affect both the flavor and texture of ice cream. Also, when replacing skim milk solids, sufficient total solids must be added to limit the water content of the mix and meet legal minimum total solids requirements. For these reasons, it is often desirable to replace skim milk solids with a product(s) with similar concentrations of lactose and protein. Lactose can be reduced through ultrafiltration, or modified by limited hydrolysis to its constituent monosaccharides; either change will affect the concentration of the ingredient that can be used and the subsequent protein level achieved in the ice cream. Buttermilk solids have often been cited as a useful substitute for skim milk solids. Buttermilk contains a higher concentration of fat globule membrane phospholipids than skim milk. Thus, it can be used for its emulsifying properties to reduce the need for emulsifiers, or in formulations where it is undesirable to add emulsifiers (1).

It is possible to produce concentrated protein products from the casein portion of milk proteins, the most common for use as a food ingredient being sodium caseinate. The use of sodium caseinate in ice cream has been investigated, and a small percentage may be useful in contributing to functional properties, particularly aeration and emulsification (28,29). However, the functionality of sodium caseinate is different than that of micellar casein, the form in which it is found in milk ingredients, and this needs to be considered when proposing its use. It can contribute positively to aeration, but may lead to an emulsion that is too stable to undergo the required degree of partial coalescence. It is therefore most desirable in the serum phase, rather than at the fat interface.

There has been a great deal of attention to the use of whey products in ice cream. Whey contains fat, lactose, whey proteins, and water, but very little, if any, casein. While skim milk powder contains 54.5% lactose and 36% protein, whey powder contains 72–73% lactose and only about 10–12% protein. Thus, it can aggravate some of the problems associated with high lactose. However, an increasing number of whey products are available that have higher protein and lower lactose contents, mostly processed by membrane technology. Many of these can provide much higher quality than the traditional whey ingredients (26,29). Whey protein concentrates with similar protein and lactose contents to skim milk solids can be produced. Protein content can vary from low values of 20–25% to 75% or more. In addition, the level of lactose can be modified by hydrolysis, although the freezing point depression effect of the higher monosaccharide content must be considered. Ash content can be reduced by demineralization. Whey protein isolates, which contain no lactose, are also available for blending with other ingredients to form the msnf content of ice cream formulations.

Proteins contribute much to the development of structure in ice cream, including emulsification, whipping, and water holding capacity (8,30). The interfacial behavior of milk protein in emulsions is well documented, as is the competitive displacement of proteins by small molecule surfactants (31–35). In ice cream, the emulsion must be stable to withstand mechanical action in the mix state, but must undergo sufficient partial coalescence to establish desirable structural attributes when frozen. These include dryness at extrusion for fancy molding, slowness of melting, some degree of shape retention during melting, and smoothness during consumption. This implies the use of small molecule surfactants (emulsifiers) to reduce protein adsorption and produce a weak fat membrane that is sensitive to shear action (7,11,12,29,36–41). Bolliger and coworkers (42) showed that protein adsorbed to the fat droplets (mg m^{-2}) in ice cream mix correlated with major characteristic analyses describing the fat structure in ice cream (fat agglomerate size, fat agglomeration index, solvent extractable fat) (Figure 154.1). The loss of steric stability from the globule, which was contributed from micellar adsorption, accounts for its greater propensity for partial coalescence during shear. Partial coalescence is responsible for establishing a three-dimensional aggregation of fat globules that provide structural integrity (see Section II.B.4). This is especially important if such integrity is needed when the structural contribution from ice is weaker (i.e., before hardening or during melting). Variables that affect the destabilization of fat in ice cream have been well studied (43–46).

With respect to protein contribution to fat globule integrity, it is obvious from the studies to date that a weak surface layer is most desirable (8). Segall and Goff (47) examined the susceptibility of ice cream emulsions to partial coalescence during shear when the emulsion was prepared with varying concentrations and type of protein, while still retaining sufficient quiescent emulsion stability. The membranes of fat globules stabilized by whey protein isolate were more susceptible than those made from sodium caseinate or casein micelles, while those made from partially hydrolyzed whey proteins did not show sufficient quiescent emulsion stability. However, when casein was added to the whey protein-stabilized emulsion, after homogenization, further casein adsorption to the whey protein membrane was rapid. Nevertheless, an understanding of protein structures and protein:surfactant interactions at the fat interface may lead to better control over the extent of partial coalescence desirable in the finished product.

Milk proteins are well known for their foaming properties, and during the manufacture of ice cream, air is

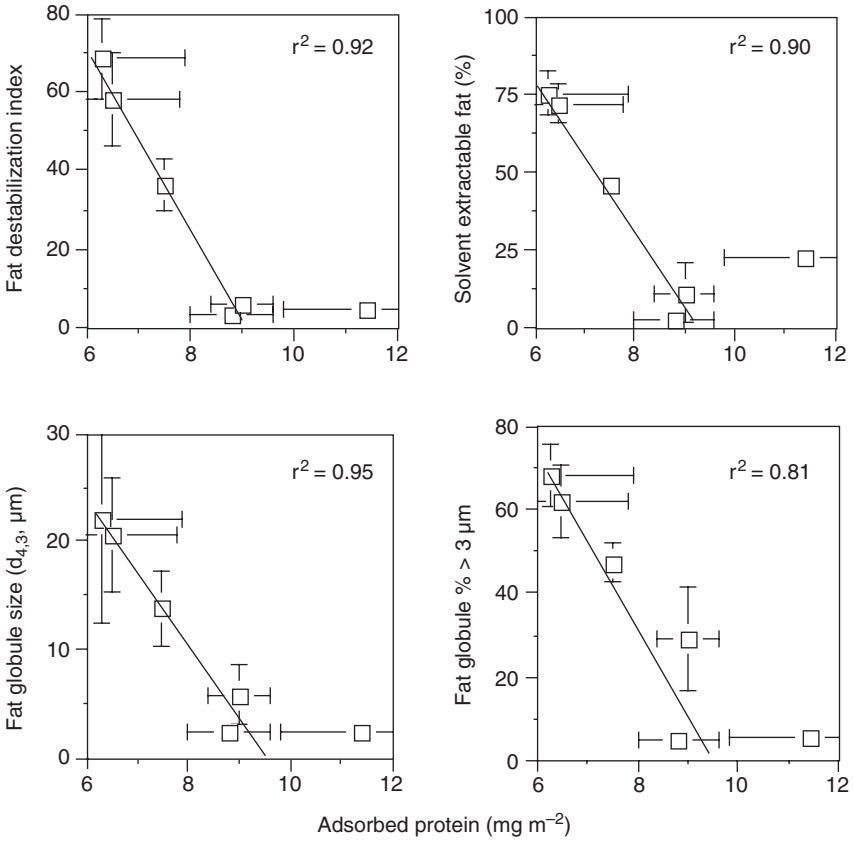

FIGURE 154.1 The effect of protein adsorbed to the fat globules in the mix on the fat destabilization index, solvent extractable fat, and fat agglomerate size in ice cream (42).

incorporated to about 50% phase volume. Thus, it should not be surprising that milk proteins contribute to stabilizing the air interface in ice cream. This air interface is very important for overall structure and structural stability (48). Loss of air can lead to a defect known as shrinkage (see Section III.B.3), the occurrence of which is fairly common and very significant for quality loss and unacceptability of the product (49). The process of whipping heavy cream includes an initial protein adsorption to the air interface, and a subsequent adsorption of fat globules and their associated membrane to the existing protein air bubble membrane (13). Globular fat adsorption to air interfaces is known to stabilize air bubbles from rapid collapse (50). Proteins at the fat interface have also been shown to play an important role during the aeration of emulsions (51). However, the actual contribution of protein to ice cream aeration and its interaction with both the added emulsifying agents (which are also surface active) and partially coalescing fat at the air interface has been less well studied. Incorporation of air into ice cream is rapid, within seconds, and at the same time, viscosity of the surrounding matrix is increasing exponentially due to freezing, such that air bubbles after formation become physically trapped into a semi-solid matrix, making their collapse quite difficult.

Similar to the role of milk protein in aeration, the role of this protein in the unfrozen aqueous phase is recognized but less well studied than the role at the fat interface. Milk proteins interact with water, and the subsequent hydration is responsible for a variety of functional properties, including rheological behavior. Thus, freeze-concentration of proteins in ice cream must lead to a sufficient concentration to have a large impact on the viscosity of the unfrozen phase and its subsequent effect on ice crystallization, ice crystal stability, and solute mobility (52). Jonkman and co-workers (53) studied the effect of ice cream manufacture on the structure of casein micelles and found that the micelles *per se* were not affected by the process. Although the stability of the micelle was expected to be affected by low temperature, this was offset by an increasing concentration of milk salts in solution during freeze-concentration, such that the micelle remained intact in a similar state to that found in mix.

Polysaccharides are also added to ice cream mix to enhance solution viscosity and to impact ice crystallization behavior. Commonly used polysaccharides can be incompatible in solution with milk proteins, leading to a microscopic or macroscopic phase separation (54), a phenomenon that has been studied in milk and ice cream-type systems (55–57). Goff and co-workers (58) examined the

interaction between milk proteins and polysaccharides in frozen systems using labeled polysaccharides and fluorescence microscopy, and demonstrated a clear phase separation between the two, leading to discernable networks created by freezing from both locust bean gum and milk proteins. It has also been shown in ice cream that when in solution with polysaccharides, the casein aggregates into distinct networks (58). Flores and Goff (59) demonstrated that milk proteins had a large impact on ice crystal size and stability. It thus appears that microscopic phase separation of the milk protein induced by polysaccharides, and "aggregation" of casein into a weak gel-like network, promoted also by freeze-concentration, may be at least partly responsible for ice crystal stability, and thus improvement of texture during consumption.

Lactose, or milk sugar, is a disaccharide of glucose and galactose that does not contribute much to sweetness of ice cream, since it is only 1/5 to 1/6 as sweet as sucrose (21). Lactose is relatively insoluble and crystallizes in two main forms, α monohydrate and β anhydrous, depending on conditions. The α monohydrate crystals, which take on a characteristic tomahawk shape, lead to the defect known as sandiness when they are allowed to grow sufficiently large (about 15 μm). Lactose content of ice cream mix is about 6% if no whey powder has been used in the formulation. Levels of lactose in ice cream mix in excess of this leads to reduced freezing point, causing a softening of the ice cream and the potential for development of iciness, a greater potential for lactose crystallization or sandiness, and salty flavors (60). The lactose solubility in water at room temperature is about 11% (21). During freezing, this concentration is exceeded as a result of freeze concentration (water removal in the form of ice). When 75% of the water is frozen in a mix consisting originally of 11% msnf (6% lactose), the lactose content in the unfrozen water corresponds to ~40%. Probably much of the lactose in ice cream exists in a supersaturated, amorphous (non-crystalline) state, however, due to extreme viscosity (61). Stabilizers help to hold lactose in a supersaturated state due to viscosity enhancement.

3. Sweeteners

Sweet ice cream is usually desired by the consumer. As a result, sweetening agents are added to ice cream mix at a rate of usually 12–17% by weight. Sweeteners improve the texture and palatability of ice cream, enhance flavors, and are usually the most economical source of total solids (1). Their ability to lower the freezing point of a solution imparts a measure of control over the temperature-hardness relationship (see Section II.B.1). In determining the proper blend of sweeteners for an ice cream mix, the total solids required from the sweeteners, the sweetness factor of each sugar, and the combined freezing point depression of all sugars in solution must be calculated to achieve the proper solids content, the appropriate sweetness level, and a satisfactory degree of hardness (5,6,62). The most common sweetening agent used is sucrose, alone or in combination with other sugars. Sucrose, like lactose, is most commonly present in ice cream in the supersaturated or glassy state, so that no sucrose crystals are present (6,61).

It has become common practice in the industry to substitute sweeteners derived from corn starch or other starch sources such as rice, for all or a portion of the sucrose (1,4). A typical sweetener blend for an ice cream mix usually includes 10–12% sucrose and 4–5% corn syrup solids (corn starch hydrolysate syrup, commonly referred to as "glucose solids") (1,4). The use of corn syrup solids in ice cream is generally perceived to provide enhanced smoothness by contributing to a firmer and more chewy texture, providing better meltdown characteristics, bringing out and accentuating fruit flavors, reducing heat shock potential which improves the shelf-life of the finished product, and providing an economical source of solids (62,63).

During the hydrolysis process, starch, a high molecular weight polymer of the monosaccharide glucose (dextrose), is continually and systematically cleaved by enzymes (α amylase, glucoamylase, and β amylase) to produce mixtures of medium and low molecular weight units (Figure 154.2). The medium molecular weight saccharides (dextrins) are effective stabilizers and provide maximum prevention against coarse ice crystal formation, which is reflected in improved meltdown and heat shock resistance. They also improve cohesive and adhesive textural properties. The smaller molecular weight sugars provide smoothness, sweetness, and flavor enhancement. With the appropriate use of enzyme technology, corn syrup manufacturers have the ability to control the ratios of high to low molecular weight components, and the ratios of maltose, the disaccharide, to glucose, the monosaccharide. Glucose monosaccharide offers sweetness synergism with sucrose, but, being half the molecular weight, has greater freezing point depression than either sucrose or maltose. The ratio of higher to lower molecular weight fractions can be estimated from the dextrose equivalent (DE) of the syrup. Figure 154.2 shows that as the DE decreases, the sweetness increases, but the freezing point decreases (more freezing point depression) and the contribution to viscosity and chewiness in the mouth also decreases. Thus, optimization of DE and concentration of corn sweeteners are required for the most beneficial effects. Ice cream manufacturers usually use a 28 to 42DE syrup, either liquid or dry (1,62). High maltose syrups modify the effect of dextrose on the freezing point (62,63). With further enzyme processing (glucose isomerase), glucose can be converted to fructose (Figure 154.2), as in the production of high fructose corn sweeteners. The resultant syrup is much sweeter than sucrose, although it has half the molecular weight, and thus contributes more to freezing point depression than sucrose. These modifications to properties would also

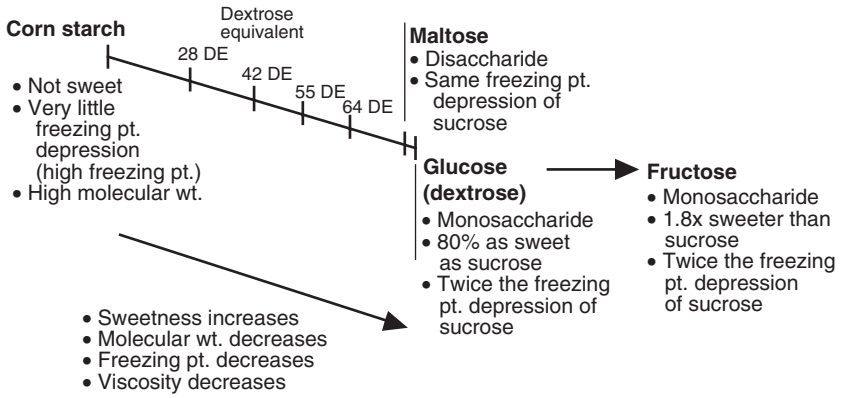

FIGURE 154.2 An illustration of the products that result from the hydrolysis of corn starch and their properties relevant to ice cream manufacture.

require optimization of all sugars for appropriate use of HFCS, although it has been shown that blends of high fructose syrup, high maltose syrup, and low DE syrup can be utilized to provide appropriate sweetness, freezing point depression and total solids in the absence of sucrose (62,63).

4. Stabilizers

Ice cream stabilizers are a group of ingredients (usually polysaccharides) commonly used in ice cream formulations. The primary purposes for using stabilizers in ice cream are to produce smoothness in body and texture, retard or reduce ice and lactose crystal growth during storage, especially during periods of temperature fluctuation, known as heat shock (64), and to provide uniformity to the product and resistance to melting (1,4). They also increase mix viscosity, stabilize the mix to prevent wheying-off (e.g., carrageenan), aid in suspension of flavoring particles, produce a stable foam with easy cut-off and stiffness at the barrel freezer for packaging, slow down moisture migration from the product to the package or the air, and help to prevent shrinkage of the product volume during storage (65). Stabilizers must also have a clean, neutral flavor, not bind to other ice cream flavors, contribute to acceptable meltdown of the ice cream, and provide desirable texture upon consumption (65). Limitations on their use include production of undesirable melting characteristics, excessive mix viscosity, and contribution to a heavy, soggy body. Although stabilizers increase mix viscosity, they have little or no impact on freezing point depression.

Gelatin, a protein of animal origin, was used almost exclusively in the ice cream industry as a stabilizer, but has gradually been replaced with polysaccharides of plant origin due to their increased effectiveness and reduced cost (1). Stabilizers currently in use include: a) carboxymethyl cellulose, derived from the bulky components or soluble fibre of plant material; b) locust bean gum (carob bean gum) which is derived from the beans of the tree *Ceratonia siliqua*, grown mostly in the Mediterranean; c) guar gum, from the guar bush, *Cyamoposis tetragonolba,* a member of the legume family grown in India and Pakistan for centuries, and now grown to a limited extent also in the USA; d) xanthan, a bacterial exopolysaccharide produced by the growth of *Xanthomonas campestris* in culture; e) sodium alginate, an extract of seaweed, kelp, or brown algae; or, f) carrageenan, an extract of *Chondus crispis (*Irish Moss), a red algae, originally harvested from the coast of Ireland, near the village of Carragheen. Each stabilizer has its own characteristics, and often two or more of these stabilizers are used in combination to lend synergistic properties to each other and improve their overall effectiveness. Guar, for example, is more soluble than locust bean gum at cold temperatures, thus it finds more application in HTST pasteurization systems. Carrageenan is a secondary colloid used to prevent wheying-off of mix, which is usually promoted by one of the other stabilizers (1,6); hence it is included in most blended stabilizer formulations.

The mechanisms by which ice cream stabilizers affect freezing properties or limit recrystallization (see Section III.B.1) have been extensively studied, but are as yet not fully understood. Ice recrystallization in ice cream has recently been reviewed (10,66). It appears from the literature available to date that stabilizers have little (67) or no (68,69) impact on the initial ice crystal size distribution in ice cream at the time of draw from the scraped surface heat exchanger. They also has little or no impact on initial ice growth during quiescent freezing and hardening (52,70,71), but do limit the rate of growth of ice crystals during recrystallization (59,67–69,72–78). They have no effect on the freezing properties of an ice cream mix, e.g., freezing point depression (79,80), amount of freezable water or enthalpy of melting (71,81,82), or heterogeneous nucleation (83), and thus may not have been expected to

affect initial ice crystallization processes. With respect to recrystallization, there has not been a demonstrable correlation between viscosity of the unfrozen mix and recrystallization rate (74,79,80,84). The protective effect of stabilizers also appears not to be related to a modification of the glass transition (74,82,84,85). However, it has been suggested that they modify the ice crystal serum interface, either through surface adsorption to the crystal itself (68,69,76,78); by modifying the rate at which water can diffuse to the surface of a growing crystal during temperature fluctuation, or the rate at which solutes and macromolecules can diffuse away from the surface of a growing ice crystal (67,85); or by some other modification of the ice serum interface (86). It must be remembered that freeze-concentration of the unfrozen phase results in a polysaccharide concentration several times higher than what was present in the original mix. Most polysaccharides are also incompatible in solution with milk proteins, which leads to further localized concentrations. Recent research by Goff and co-workers (58) has focused on the ability of at least some stabilizers to form a cryo-gel and entrap ice crystals within this gel. Phase separation of polysaccharides and proteins also appears to be related. Control of ice recrystallization may then relate to microstructural differences in solute concentration at the surface of the crystal.

5. Emulsifiers

Emulsifiers have been used in ice cream mix manufacture for many years (87,88). They are usually integrated with the stabilizers in proprietary blends, but their function and action is very different than that of the stabilizers. They are used for: improvement of the whipping quality of the mix; production of a drier ice cream to facilitate molding, fancy extrusion, and sandwich manufacture; smoother body and texture in the finished product; superior drawing qualities at the freezer to produce a product with good stand-up properties and melt resistance; and more exact control of the product during freezing and packaging operations (1,87–89). Their mechanism of action can be summarized as follows: They lower the fat/water interfacial tension in the mix, resulting in protein displacement from the fat globule surface, which in turn reduces the stability of the fat globule to partial coalescence that occurs during the whipping and freezing process, leading to the formation of a fat structure in the frozen product that contributes greatly to texture and meltdown properties (12). The extent of protein displacement from the membrane, and hence the extent of dryness achieved, is a function of the emulsifier concentration (6,90). Their role in structure formation will be described further in Section II.B.4.

Egg yolk was formerly commonly used as an ice cream emulsifier. Emulsifiers used in ice cream manufacture today are of two main types: the mono- and diglycerides, and the sorbitan esters. Mono- and diglycerides are derived from the partial hydrolysis of fats of animal or vegetable origin. The sorbitan esters are similar to monoglycerides, in that the sorbitan esters have a fatty acid molecule, such as stearate or oleate, attached to a sorbitol molecule; whereas the monoglycerides have a fatty acid molecule attached to a glycerol molecule. To make the sorbitan esters water soluble, polyoxyethylene groups are attached to the sorbitol molecule. Polysorbate 80, polyoxyethylene sorbitan monooleate, is the most common of these sorbitan esters. Polysorbate 80 is a very active drying agent in ice cream (12), and is used in many commercial stabilizer/emulsifier blends.

II. MANUFACTURING AND STRUCTURE OF FROZEN DESSERT PRODUCTS

A. Mix Manufacture

Ice cream processing operations can be divided into two distinct stages: mix manufacture and freezing operations (Figure 154.3). Ice cream mix manufacture consists of the following unit operations: combination and blending of ingredients, batch or continuous pasteurization, homogenization, and mix aging.

1. Blending

Ingredients are usually preblended prior to pasteurization, regardless of the type of pasteurization system used. Blending of ingredients is relatively simple, if all ingredients are in the liquid form, as automated metering pumps or tanks on load cells can be used for measurement, and tanks, usually conical-bottom and agitated, are used for mixing. When dry ingredients are used, powders are added through either a pumping system under high velocity, or through a liquifier, a large centrifugal pump with rotating knife blades that chop all ingredients as they are mixed with the liquid (3).

2. Mix Calculations

The general object in calculating ice cream mixes is to turn the formula, which is based on the desired components, into a recipe, which is based on the actual ingredients to be used to supply the components and the amount of mix desired. The formula is given as percentages of fat, msnf, sugar, corn syrup solids, stabilizers, and emulsifiers. The ingredients to supply these components are chosen on the basis of availability, quality, and cost. Table 154.8 illustrates the relationship between the major components, the main ingredients that supply the major components, and the minor components that are supplied with the major ones for each ingredient.

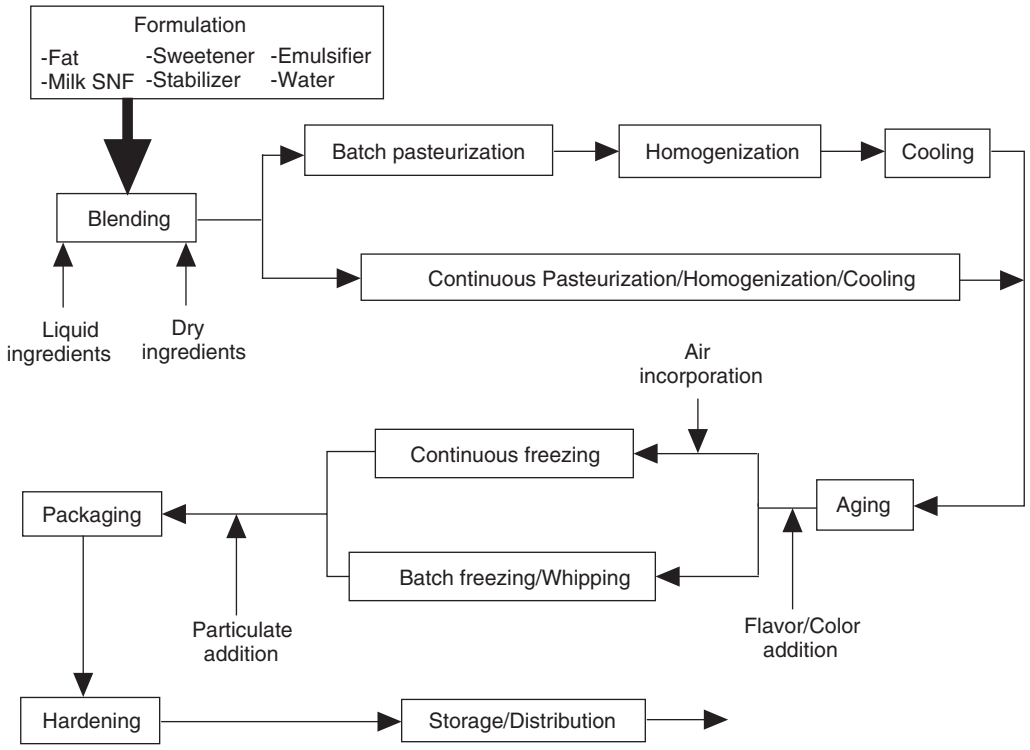

FIGURE 154.3 A schematic illustration of the processing steps in ice cream manufacture.

The first step in a mix calculation is to identify the composition of each ingredient. In some cases the percentage of solids contained in a product is taken as constant or provided by an ingredient supplier, while in others, the composition must be obtained by analysis (e.g., the fat content in milk or cream). If there is only one source of the component needed for the formula, for example, the stabilizer or the sugar, it is determined directly by multiplying the percentage needed by the amount needed, e.g., 100 kg of mix @ 10% sugar would require 10 kg sugar. If there are two or more sources, for example 10% fat coming from both cream and milk, then an algebraic method may be utilized. Computer programs developed for mix calculations generally solve simultaneous equations based on mass and component balances. For manual calculations, a method known as the "Serum Point" method has been derived (1,4). This method has solved the simultaneous equations in a general way so that only the equations need to be known and not resolved each time. In the Serum Point method, 9% msnf is assumed in the aqueous (serum), non-fat portion of all milk ingredients. Thus, the msnf content of milk or cream is calculated as (100 − percent fat) × 0.09. This section will illustrate mix calculation solutions using algebraic techniques and the Serum Point method.

EXAMPLE PROBLEM 1 - Mix using cream, skim milk, and skim powder (three sources of msnf, three sources of water), solution shown by both the Algebraic and Serum Point Methods.

Desired : 100 kg mix @ 13% fat, 11% msnf, 15% sucrose, 0.5% stabilizer, 0.15% emulsifier.

On hand: Cream @ 40% fat; skim milk; skim milk powder @ 97% msnf; sugar; stabilizer; emulsifier.

TABLE 154.8
Sources of the Major Components in Ice Cream Mix, as well as the Minor Components Supplied by these Ingredients

Component	Ingredients to Supply (but also supplies)
Milkfat	Cream (msnf, water)
	Butter (msnf, water)
Milk solids-not-fat (msnf)	Skim powder (water)
	Condensed skim (water)
	Condensed milk (water, fat)
	Sweetened condensed (water, sugar)
	Whey powder (water)
Water	Skim milk (msnf)
	Milk (fat, msnf)
	Water
Sweetener	Sucrose
	Corn syrup solids
	Liquid sugars (water)
Stabilizers/ emulsifiers	

Solution via an algebraic method:
Solution (Note: only one source of fat, sugar, stabilizer, and emulsifier, but two sources of msnf):

Cream

$$100 \text{ kg mix} \times \frac{13 \text{ kg fat}}{100 \text{ kg mix}} \times \frac{100 \text{ kg cream}}{40 \text{ kg fat}}$$

$$= 32.5 \text{ kg cream}$$

Sucrose

$$100 \text{ kg mix} \times \frac{15 \text{ kg sucrose}}{100 \text{ kg mix}} = 15 \text{ kg sucrose}$$

Stabilizer

$$100 \text{ kg mix} \times \frac{0.5 \text{ kg stabilizer}}{100 \text{ kg mix}} = 0.5 \text{ kg stabilizer}$$

Emulsifier

$$100 \text{ kg mix} \times \frac{0.15 \text{ kg emulsifier}}{100 \text{ kg mix}} = 0.15 \text{ kg emulsifier}$$

Skim milk and skim powder, Note: two sources of the msnf
Now, let x = skim powder, y = skim milk.

MASS BALANCE (All the components add up to 100 kg, so skim powder + skim milk = 100 − mass of other ingredients)

$$x + y = 100 - (32.5 + 15 + 0.5 + 0.15)$$

MSNF BALANCE (Equal to 11% of the mix and coming from the skim milk, the skim powder, and the cream, so the portion from the skim powder and skim milk = 11 kg − the contribution from the cream). The msnf portion of the skim milk and cream are taken as 9% of the non-fat portion, i.e., 9% in the case of the skim milk and (100−40) × 0.09 = 5.4% in the case of the cream.

$$0.97\,x + 0.09y = 0.11(100) - (0.054 \times 32.5)$$

Once the appropriate equations have been written, they need to be solved algebraically.

$$x + y = 51.85 \quad \text{so} \quad y = 51.85 - x \qquad \text{from the mass balance}$$
$$0.97\,x + 0.09\,y = 9.245 \qquad \text{from the msnf balance}$$
$$0.97\,x + 0.09\,(51.85 - x) = 9.245 \qquad \text{substituting}$$
$$0.97\,x - 0.09\,x + 4.67 = 9.245$$
$$0.88\,x = 4.58$$

$$x = 5.20 \text{ kg skim powder}$$
$$y = 46.65 \text{ kg skim milk}$$

The above shows the simultaneous solution of 2 equations with 2 unknowns. Likewise, if there were 3 unknowns, e.g., fat, msnf, and the total weight, then three equations could be written, one for each of fat, msnf, and weight. However, the above problem could also be solved with the Serum Point method, and the solution of the above example by that method, along with the derivation of the equations, follows. The Serum Point calculation assumes 9% msnf in skim milk and the skim portion of all dairy ingredients. It then solves the calculation beginning with the most concentrated source of msnf first. It is advisable to solve a problem with the Serum Point method on the basis of 100 kg, and then scale it up to the desired mix quantity by multiplying by the appropriate factor, e.g., solution for each component for 100 kg × 50 = solution for 5000 kg.

Solution of Problem 1 via the Serum Point method:

1. Amount of skim milk powder needed is found by the following formula:

$$\frac{\text{msnf needed} - (\text{serum of mix} \times 0.09)}{\%\ \text{msnf in powder} - 9} \times 100$$

$$= \text{kg skim powder} \qquad (154.1)$$

The derivation of Equation 154.1 is shown at the end of the problem. For now, just assume that this equation will work!

The serum of the mix is found by adding the desired percentages of fat, sucrose, stabilizer, and emulsifier together and subtracting from 100 [i.e., "serum" = msnf (or "serum solids") + water]. In the present problem then,

$$100 - (13 + 15 + 0.5 + 0.15) = 71.35 \text{ kg serum.}$$

Substituting in Equation 154.1 we have:

$$11 - \frac{(71.35 \times 0.09)}{97 - 9} \times 100 = \frac{4.58}{88} \times 100$$

$$= 5.20 \text{ kg skim powder}$$

2. The weight of cream (since there is only one source of fat) will be

$$13 \text{ kg} \times \frac{100 \text{ kg cream}}{40 \text{ kg fat}} = 32.5 \text{ kg cream}$$

3. The sucrose will be 15 kg/100 kg mix.
4. The stabilizer will be 0.5 kg/100 kg mix.
5. The emulsifier will be 0.15 kg/100 kg mix.
6. The weight of mix supplied so far is,

Cream	32.50 kg
Skim powder	5.20 kg
Sucrose	15.00 kg
Stabilizer	0.50 kg
Emulsifier	0.15 kg
	53.35 kg

The skim milk needed therefore is

$$100 - 53.35 = 46.65 \text{ kg.}$$

It is always important to check your solutions to ensure they give the desired result. Such a proof is shown below, where the quantities of each ingredient and the quantities

of each component in each ingredient are laid out in a table and summed. The total mass of each component divided by the total mass of mix should yield the desired percentage.

Proof:

Ingredients	Kilograms	Kgs. Fat	Kgs. msnf	Kgs. T.S.
Cream	32.50	13.0	1.75	14.75
Skim milk	46.65	—	4.20	4.20
Skim powder	5.20	—	5.05	5.05
Sucrose	15.00	—	—	15.00
Stabilizer	0.50	—	—	0.50
Emulsifier	0.15	—	—	0.15
Totals	100.0	13.0	11.0	39.65

Derivation of the Serum Point equations:
Problem 1 is resolved again using simultaneous equations in a general way to show where the serum point equations come from.

On hand: cream @ 40% fat
(supplies fat, water, and msnf, therefore can be thought of as a mixture of fat and skim milk)
skim milk @ 9% msnf
(supplies water and msnf)
skim milk powder @ 97% msnf
(supplies water and msnf)
sucrose
stabilizer
emulsifier

Solution

- Only one source of fat, sucrose, stabilizer, and emulsifier

$$\text{kg fat} = 100 \text{ kg mix} \times 13 \text{ kg fat}/100 \text{ kg mix} = 13 \text{ kg fat}$$

(The explanation for this assumption becomes clearer in a moment!)

$$\text{kg sucrose} = 100 \text{ kg mix} \times 15 \text{ kg sucrose}/100 \text{ kg mix} = 15 \text{ kg sucrose}$$

$$\text{kg stabilizer} = 100 \text{ kg mix} \times 0.5 \text{ kg stab.}/100 \text{ kg mix} = 0.5 \text{ kg stabilizer}$$

$$\text{kg emulsifier} = 100 \text{ kg mix} \times 0.15 \text{ kg emul.}/100 \text{ kg mix} = 0.15 \text{ kg emulsifier}$$

- Two sources of msnf

Let X = skim powder (kg)

Let Y = skim milk (kg) + skim milk in cream (kg)

MASS BALANCE

$$X + Y = \text{Total mix} - \text{components already added}$$

$$X + Y = 100 - (13 + 15 + 0.5 + 0.15)$$

(the "Serum of the Mix")

$$X + Y = 71.35$$

$$(\text{so } Y = 71.35 - X)$$

MSNF BALANCE

$$0.97X + 0.09Y = (0.11 \times 100)$$

"Serum solids fraction in powder" *"Serum solids fraction in skim"* *"Serum solids fraction in mix"*

$$0.97\,X + 0.09\,(71.35 - X) = 11$$

$$0.97\,X + (0.09 \times 71.35) - 0.09\,X = 11$$

$$0.97\,X - 0.09\,X = 11 - (0.09 \times 71.35)$$

$$X = \frac{11 - (0.09 \times 71.35)}{0.97 - 0.09}$$

Which is equal to:

kg skim powder

$$= \frac{\text{msnf needed} - (0.09 \times \text{serum of mix})}{\%\text{ msnf in powder} - 9} \times 100$$

(which is Equation 154.1!)

$$X = \frac{4.58}{0.88} = 5.20 \text{ kg powder}$$

$$\text{kg cream} = 13 \text{ kg fat} \times 100 \text{ kg cream}/40 \text{ kg fat} = 32.5 \text{ kg cream}$$

$$\text{kg skim} = 100 - 32.5 - 15 - 0.5 - 0.15 - 5.2 = 46.65 \text{ kg}$$

EXAMPLE PROBLEM 2 - Mix using cream, milk, and skim powder (three sources of msnf, three sources of water, and two source of fat); solved by both the Algebraic and Serum Point Methods.

Desired: 100 kg mix containing 14% fat, 9.5% msnf, 15% sucrose, 0.4% stabilizer, 1% frozen egg yolk.

On hand: Cream 30% fat, milk 3.5% fat, skim milk powder 97% solids, sucrose, stabilizer, and egg yolk (50% solids).

The solution to this problem will be shown by the simultaneous solution of 3 equations, since there are three sources of msnf, three sources of water, and two source of fat, and by the Serum Point method. Both produce the same results. Follow whichever method you prefer. Computer programs exist that solve simultaneous equations; writing

the equations, however, requires an understanding of the objectives of the problem.

Solution via the algebraic method:

$$\text{Sucrose: } 100 \text{ kg mix} \times \frac{15 \text{ kg sucrose}}{100 \text{ kg mix}} = 15 \text{ kg sucrose}$$

$$\text{Stabilizer: } 100 \text{ kg mix} \times \frac{0.4 \text{ kg stabilizer}}{100 \text{ kg mix}} = 0.4 \text{ kg stabilizer}$$

$$\text{Egg yolk: } 100 \text{ kg mix} \times \frac{1 \text{ kg egg yolk}}{100 \text{ kg mix}} = 1 \text{ kg egg yolk}$$

Now, let x = skim powder, y = milk, and z = cream.

MASS BALANCE All the components add up to 100 kg, so the sum of the three unknowns = 100 − the sum of the known mass of the other components.

$$x + y + z = 100 - (15 + 0.4 + 1)$$

MSNF BALANCE Equal to 9.5% of the mix and coming from the milk, the skim powder, and the cream; assume 9% in the skim portion of the milk and cream so that the msnf of the milk = 0.09 × (100 − 3.5) and of the cream = 0.09 × (100−30)

$$0.97x + 0.08685y + 0.063z = 0.095\ (100)$$

FAT BALANCE Equal to 18% of the mix and coming from the milk and cream

$$0.035y + 0.3z = 0.14\ (100)$$

These equations could now be solved to produce the final outcome:

$$x = 3.7 \text{ kg skim powder}$$

$$y = 37.7 \text{ kg milk}$$

$$z = 42.3 \text{ kg cream}$$

Solution via the Serum Point method:

1. Determine the amount of skim milk powder required by using Equation 154.1:

$$\frac{\text{msnf needed} - (\text{serum of mix} \times 0.09)}{\%\ \text{msnf in powder} - 9} \times 100 = \text{skim powder}$$

Serum of mix = 100 − (14 + 15 + 0.4 + 1.0) = 69.6.

Substituting we have,

$$\frac{9.5 - (69.6 \times 0.09)}{97-9} \times 100 = \frac{3.236 \times 100}{88} = 3.68 \text{ kg powder}$$

2. Amount of sucrose required is 15.0 kg.
3. Amount of stabilizer required is 0.4 kg.
4. Amount of egg required is 1.0 kg.
5. Determine amount of milk and cream needed.

Materials supplied so far are 3.68 kg powder, 15 kg sucrose, 0.4 kg stabilizer, and 1 kg egg yolk, a total of 20.08 kg. 100 − 20.08 = 79.92 kg milk and cream needed.

6. Determine the amount of cream by following formula:

$$\frac{\text{kg fat needed} - (\text{kg cream and milk needed} \times \frac{\%\ \text{fat in milk})}{100}}{\%\ \text{fat in cream} - \%\ \text{fat in milk}} \times 100 \quad (154.2)$$

Note: Equation 154.2 is derived from a generalized fat balance, in much the same way that Equation 154.1 was derived.

Substituting we have,

$$\frac{14 - \left(79.92 \times \frac{3.5}{100}\right)}{30 - 3.5} \times 100 = \frac{11.20}{26.5} \times 100 = 42.26 \text{ kg cream.}$$

7. Amount of milk needed = 79.92 − 42.26 = 37.66 kg of milk.

Proof:

Ingredients	Kilograms	Kgs. Fat	Kgs. msnf	Kgs. T.S.
Cream	42.26	12.68	2.66	15.34
Milk	37.66	1.32	3.27	4.59
Skim powder	3.68	—	3.57	3.57
Sucrose	15.00	—	—	15.00
Stabilizer	.40	—	—	.40
Egg yolk	1.00	—	—	.50
Totals	100.00	14.00	9.50	39.40

With Equations 154.1 and 154.2, most complex mix problems can be solved. There are additional complications for the use of condensed skim or whole milk, and for liquid sugars. See Ref. 1 for further details.

3. Pasteurization and Food Safety Issues

Pasteurization is the biological control point in the system, designed for the destruction of pathogenic bacteria. If raw milk or cream are used as ingredients, it could be

that these are contaminated with a human pathogen from the dairy farm. Therefore, it is essential that pasteurization be carefully designed and closely monitored. If raw dairy ingredients are not used, contamination from a human source could also occur, and thus the use of pasteurization conditions that eliminate pathogens is mandated by most legal jurisdictions. In addition, it serves a useful role in reducing the total bacterial load, and in solubilization of some of the components (proteins and stabilizers). Both batch and continuous (high temperature short time or HTST) systems are in common use (3). In a batch pasteurization system, blending of the proper ingredient amounts is done in large jacketed vats equipped with some means of heating, usually saturated steam or hot water. The product is then heated in the vat to at least 69°C (155°F) and held for 30 min to satisfy legal requirements for pasteurization, or equivalent times and temperatures as determined by the local legal jurisdiction. The heat treatment must be severe enough to ensure destruction of pathogens and to reduce the bacterial count to a maximum (e.g., 10,000 per gram), depending on the legal jurisdiction. Following pasteurization, the mix is homogenized using high pressures and then is passed across some type of heat exchanger (plate heat exchanger or double or triple tube heat exchanger) for the purpose of cooling the mix to refrigerated temperatures (4°C).

Continuous pasteurization is usually performed in an HTST heat exchanger following the blending of ingredients in a large, insulated feed tank. Some preheating, to 30 to 40°C, may be necessary for solubilization of the components. The HTST system is equipped with a heating section, a cooling section, and a regeneration section (Figure 154.4). Mix first enters the raw regeneration section, where cold or preheated mix is heated to as warm as possible on one side of a plate heat exchanger, while the pasteurized hot mix is cooled to as low as possible running countercurrent on the opposite sides of the plates. Following the raw regeneration section, the mix enters the heating section where a minimum temperature of 80°C is obtained. The mix is held at this temperature for 25 sec by flowing either through a series of holding tubes or through an additional set of plates in the HTST unit. Holding times much longer than the minimum can be accomplished with longer holding tubes. Holding times of 2 or 3 min may produce superior mixes that retain many of the advantages of batch pasteurization (4,6). After leaving the holding tube, the mix enters the homogenizer, depending upon the particular configuration, then flows back through the pasteurized side of the regeneration section and enters the cooling plates, where a chilled brine solution or chilled water bring the mix down to around 4°C.

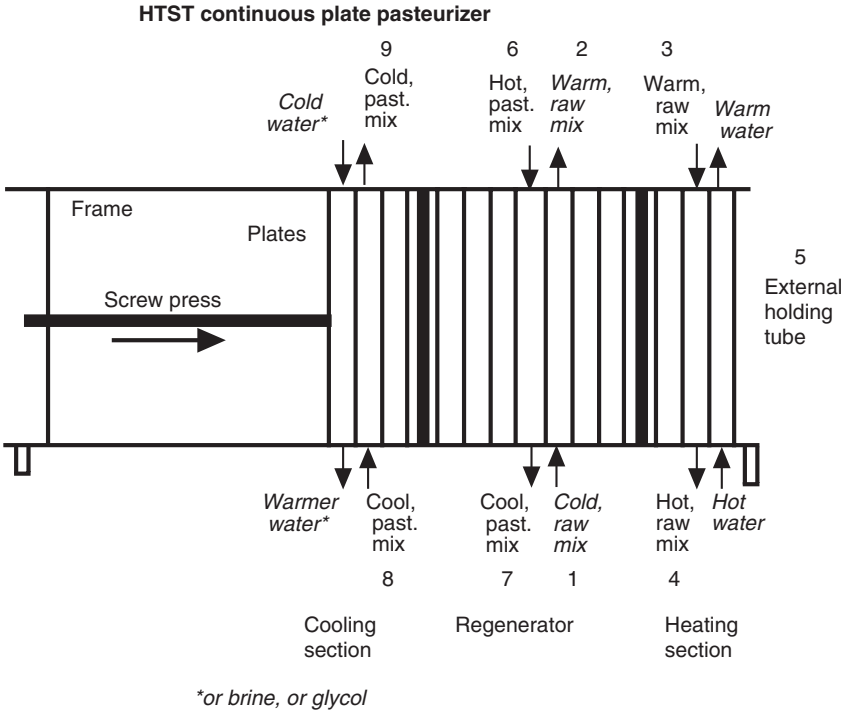

FIGURE 154.4 A schematic illustration of the side view of a plate heat exchanger used for HTST pasteurization of frozen dairy dessert mixes. Numbers indicate the sequence of flow of mix. Italics are used to differentiate the material on one side of a section from the material on the other.

4. Homogenization

Homogenization is responsible for the formation of the fat emulsion by forcing the hot mix through a small orifice under pressures of 15.5 to 18.9 MPa (2000–3000 psig), depending on the mix composition. The actual mechanism of fat disruption within the homogenizer is thought to result from turbulence, cavitation, and velocity gradients (energy density) within the valve body (91). The 4–8 fold increase in the surface area of the fat globules is responsible in part for the formation of the fat globule membrane, comprised of adsorbing materials attempting to lower the interfacial free energy of the fat globules (92,93). Koxholt and co-workers (94) have recently shown that sufficient fat structure in the mix for optimal ice cream meltdown was created by homogenization pressures on the first stage of 10 MPa, in mixes with up to 10% fat content, and that higher pressures were not required. With single stage homogenizers, fat globules tend to cluster as bare fat surfaces come together or adsorbed molecules are shared. Therefore, a second homogenizing valve is commonly placed immediately after the first, with applied back pressures of 3.4 MPa (500 psig) (3), allowing more time for surface adsorption to occur. However, Koxholt and co-workers (94) have recently shown that two-stage homogenization is not necessary for ice cream mixes up to 10% fat content in order to achieve optimal fat structuring and ice cream meltdown. The net effects of homogenization are in the production of a smoother, more uniform product with a greater apparent richness and palatability, and better whipping ability (1). Homogenization also decreases the danger of churning the fat in the freezer, and makes it possible to use products that could not otherwise be used, such as butter and frozen cream.

5. Aging

An aging time of four hours or greater is recommended following mix processing prior to freezing. This allows for hydration of milk proteins and stabilizers (some viscosity increase occurs during the aging period), crystallization of the fat globules, and a membrane rearrangement, to produce a smoother texture and better quality product (6,11). Non-aged mix is very wet at extrusion and exhibits variable whipping abilities and faster meltdown (1,6). The appropriate ratio of solid:liquid fat must be attained at this stage. This is a function of temperature and the triglyceride composition of the fat used; as a partially crystalline emulsion is needed for partial coalescence in the whipping and freezing step, as discussed in Section II.B.4. Emulsifiers generally displace milk proteins from the fat surface during the aging period (12,36,95), and this is also discussed in detail in Section II.B.4. The whipping qualities of the mix are usually improved with aging. Aging is performed in insulated or refrigerated storage tanks, silos, etc. Mix temperature should be maintained as low as possible (at or below 4°C) without freezing.

B. Dynamic Freezing

In a continuous, scraped surface freezer, numerous processes take place that ultimately influence the overall quality of the ice cream. One of the most important steps, of course, is freezing water into ice. At the same time as ice is being formed, there is also air incorporation, leading to development of air cells and the desired overrun. In addition, destabilization of the fat emulsion (partial coalescence, see Section II.B.4) takes place during freezing, which promotes incorporation and stabilization of the air cells. All of these processes take place simultaneously in the minute or less that ice cream spends in the dynamic freezing step. Following this initial phase of ice formation in a dynamic freezer, where about half of the water is turned into ice, there is a static freezing step, often called hardening (see Section II.D).

The mechanisms that lead to ice formation in an ice cream freezer are quite complex. Ultimately, the product exiting the freezer contains numerous, small ice crystals. As seen in Figure 154.5 (96), the ice crystals in ice cream at the exit of the freezer are somewhat block-shaped and vary in size from a few microns to over 100 μm. A typical size distribution for hardened ice cream is shown in Figure 154.6 (6). The large number of very small ice crystals, estimated to be 4×10^9 crystals per liter (97), gives ice cream its smooth, cool character. The ice crystals must remain below some threshold detection size, often given as about 50 μm mean size (1), for the ice cream to remain smooth. When crystals become larger than this, the ice cream may be considered coarse. Control of ice crystallization to produce the desired number and size of crystals is critical to producing high quality ice cream.

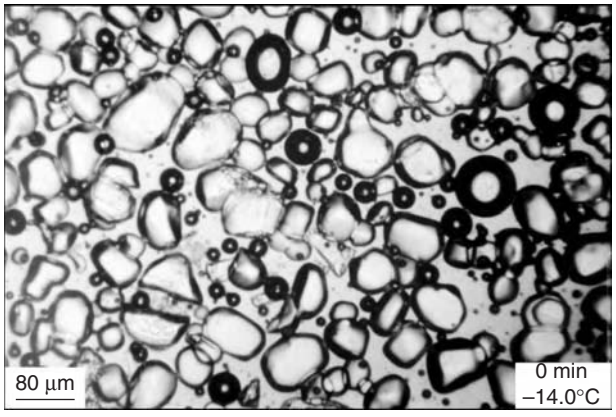

FIGURE 154.5 Ice crystals in ice cream, as observed using light microscopy (96).

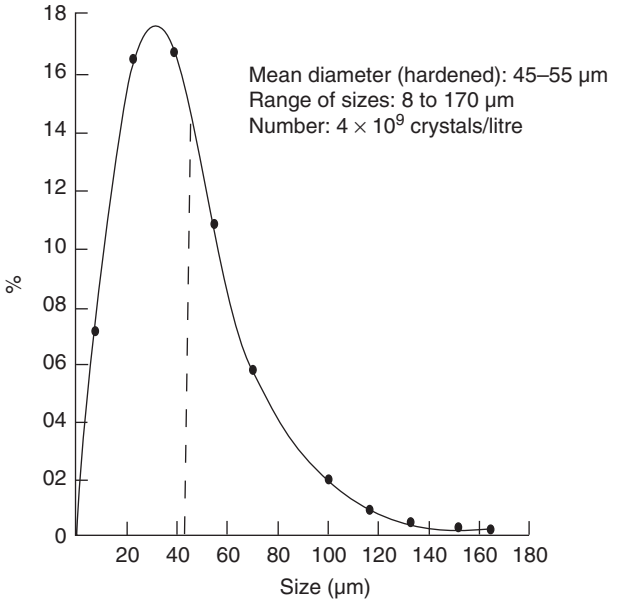

FIGURE 154.6 Typical ice crystal size distribution for hardened ice cream (6).

1. Principles of Ice Crystallization

When ice freezes or crystallizes from any solution, several steps must take place. First, the solution must be cooled below the freezing (melting) point of the solution. The temperature difference between the actual temperature and the freezing point temperature of the mix is the driving force for freezing. Once an appropriate driving force has been attained, formation of the solid ice phase from the liquid molecules must occur. This step is called nucleation, where tiny bits of crystalline ice have just started to form. Once these nuclei begin to form, they continue to grow until some phase equilibrium has been obtained. In freezing, ice continues to form until a thermal equilibrium between the freezing product and its environment has been reached. The total amount of ice that forms (at any storage temperature) depends on the system. For pure water, all of the water is converted to ice as long as the temperature is below 0°C. In ice cream, however, the other ingredients influence the freezing process and determine how much water turns to ice (the ice phase volume) at any temperature. Both the total amount of ice as well as the nature of the ice dispersion (size, shape, etc.) influence the physical properties of the final ice cream product.

After the product is frozen, the ice phase continues to undergo recrystallization. Recrystallization is a term used for a combination of several events, including melting, growth and ripening, that occur after the initial ice crystal phase has been developed. Recrystallization leads to changes in the distribution of ice crystals within the system based on the thermodynamic difference in melting point between large crystals and small ones. Typically, recrystallization occurs with no change in ice phase volume.

In continuous ice cream manufacture, mix is pumped into the freezer and flows along the length of the barrel. As the ice cream moves from the inlet to the outlet, ice is frozen, fat is destabilized and air is injected, as shown in Figure 154.7. The mix enters the freezer barrel at a temperature between 0 and 4°C and begins to freeze as it contacts the metal wall cooled by expanding refrigerant (ammonia or Freon). Ice forms at the barrel wall since this is where the driving force for freezing is the highest. However, the ice layer that forms is rapidly scraped off of the wall and dispersed into the center of the freezer barrel where the ice changes form depending on temperature conditions and mixing parameters. As the mix moves axially along the freezer barrel, the amount of ice formed increases as the bulk average temperature of the slurry decreases. At the draw (exit) of the freezer, approximately half of the initial water in the mix is frozen into ice and the product is a pumpable slurry of partially frozen ice cream. The change in temperature along the length of the freezer for a typical ice cream operation is shown in Figure 154.8 (98). The final temperature and the amount of ice formed depends on the rate of freezing within the barrel of the freezer. This is controlled by the flow of refrigerant (ammonia or Freon) on the outside of the barrel, the throughput rate of ice cream through the freezer and the type of mixing device used within the barrel of the freezer. In general, conditions in a scraped surface freezer

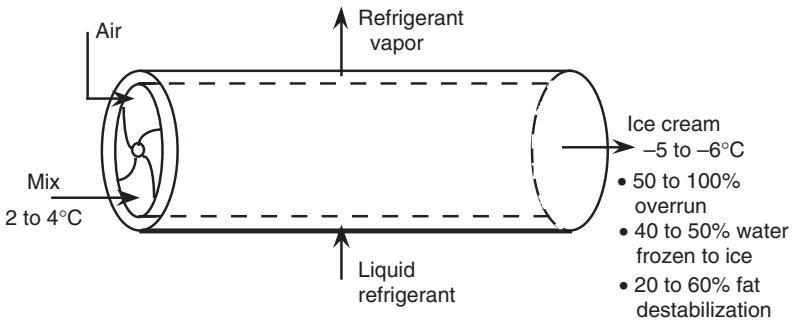

FIGURE 154.7 A schematic diagram to represent inputs and outputs during the continuous freezing of ice cream.

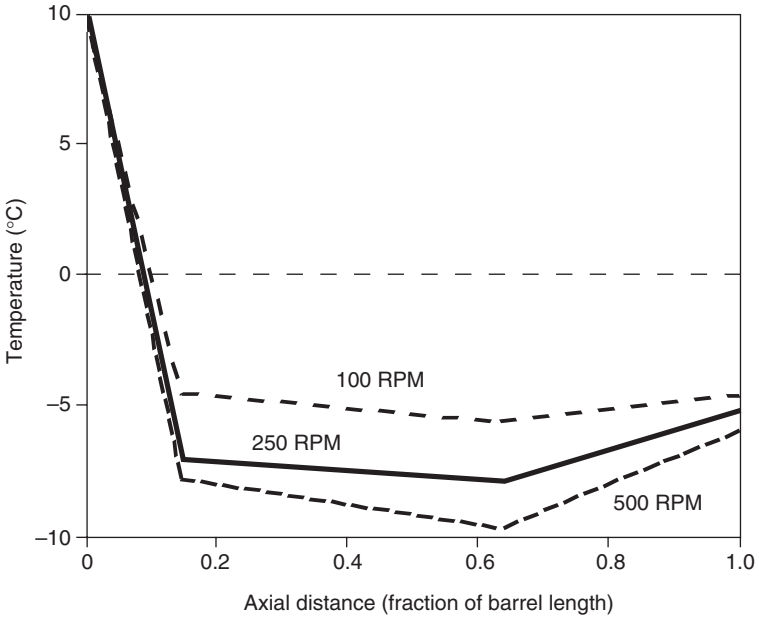

FIGURE 154.8 Axial profile of ice cream temperature as a function of dasher speed within the barrel of a scraped-surface ice cream freezer (98).

are controlled to give a compromise between the draw temperature (amount of ice frozen) and the stiffness of the ice cream exiting the freezer. The ice cream should be as frozen as possible (since here is where control of ice formation occurs), yet be sufficiently fluid to incorporate inclusions and/or fill the containers without leaving air gaps. This compromise depends to some extent on the type of product being produced and its final form.

a. Phase/state behavior

Freezing Point Depression. In order for ice to freeze, the temperature of the solution has to be lowered below its freezing point. The temperature at which a solution freezes, or the freezing point, is determined by the concentration and type of solutes present in the mix. The presence of dissolved salts and sugars causes the freezing point of water to be lowered. This freezing point depression occurs because the solute molecules interact with water and inhibit the ability of the water molecules to come together and form an ice crystal lattice (or freeze). The extent of freezing point depression is based on the number of solute molecules and their size. Small molecules have the greatest effect; the higher the concentration of these small molecules, the lower the freezing point. Thus, ice cream mixes made with a high concentration of milk salts and lactose, with high sugar content, or with high content of low-molecular weight sweeteners, have lower freezing points. For example, use of high fructose corn syrup as a sweetener gives a lower freezing point (compared to the use of sucrose) due to the addition of lower molecular weight sugars. Mixes made with high levels of msnf have a low freezing point due to the addition of milk salts and lactose.

The freezing point of the ice cream mix is an important quality control parameter since it governs the amount of ice that can form at a given temperature, which affects the quality and textural attributes of the ice cream. As seen in Figure 154.9 (99), melting rate increases and firmness decreases with increasing freezing point (as indicated by osmolality) (99,100). As the freezing point of the mix goes down (osmolality increases), ice cream contains less ice and more unfrozen water at any given temperature, which leads to ice cream that is less firm and melts at a faster rate.

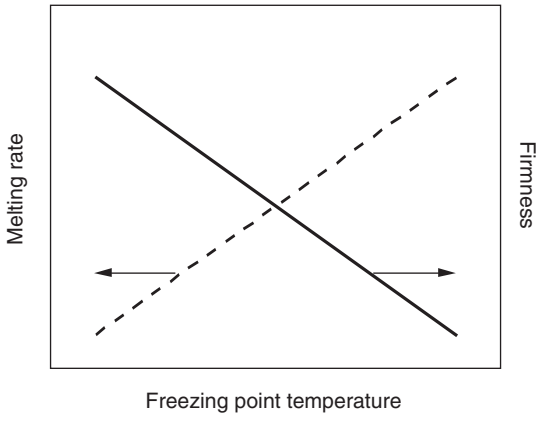

FIGURE 154.9 Effects of freezing point of ice cream mix on melting rate and firmness of final product (based on data from Ref. 99).

Freezing point depression also can be calculated based on principles of thermodynamics (96), assuming ideal solutions and dilute concentrations. At the point where the two phases (solid ice and liquid water) are in equilibrium, the chemical potentials of the two phases are equal and the following equation can be developed.

$$\frac{\Delta H}{R}\left[\frac{1}{T_0} - \frac{1}{T}\right] = \ln(X_w) \qquad (154.3)$$

Here, ΔH is the latent heat of fusion, R is the ideal gas constant, T_0 is the freezing point of pure water, T is the freezing point of solution with mole fraction of water of X_w. For aqueous foods, equation (154.3) may be modified to give:

$$(T_f - T_0) = K\frac{C}{MW} \qquad (154.4)$$

where, T_f is freezing point (°C) of a solution with concentration C (in g/100 g water), MW is the molecular weight of the dissolved solute, and K is a conversion factor (equal to 1.86 for water).

In simple systems, Equation 154.4 gives a good estimate of freezing point and can be used to show the relationship between freezing point and solute content. For example, the freezing point depression curves for several sugars are shown in Figure 154.10 (96). Note that fructose has a lower freezing point than sucrose at any equal concentration (wt %), because it has lower molecular weight and there are more molecules of fructose added (at equivalent mass of sugar). Conventional corn syrup solids (42DE), which contain numerous longer-chain saccharides, have a higher freezing point than sucrose. In more complex food formulations, the sum of each of the components that impact freezing point depression is needed. In ice cream mix, it is the combination of sweeteners and milk ingredients used in the formulation that leads to the specific freezing point depression curve for any mix. Sugars (from sweetener and msnf) and salts (from msnf) are the main components that impact freezing point depression of an ice cream mix.

Typically, freezing point depression of an ice cream mix is calculated from Equation 154.4 by taking the sucrose equivalents of all the important components that influence freezing point. Sucrose equivalency values for common sweeteners have been developed (101) for use in ice cream formulations. The contributions of both sweeteners and salts on freezing point are then summed (102) to obtain the initial freezing point of the mix. Equation 154.4 can also be used to calculate the amount of water frozen into ice for a given ice cream at any temperature by varying the concentration since freeze concentration of the unfrozen phase occurs during freezing. Based on the approximate freezing point depression curve and the assumption of slow freezing, the amount of water converted to ice at any temperature can be calculated by a mass balance. For a typical ice cream, a relationship between temperature and the amount of water frozen into ice is obtained, as shown in Figure 154.11 (103). Since Equation 154.4 technically only works for dilute, ideal solutions, it does not apply very accurately at higher concentrations found in the unfrozen phase of ice cream. Thus, correction factors have been developed based on experimental data for frozen sucrose solutions (104).

To calculate the freezing point of a given mix, the effects of sweeteners and salts must be summed. The effects of sweeteners are obtained by summing the

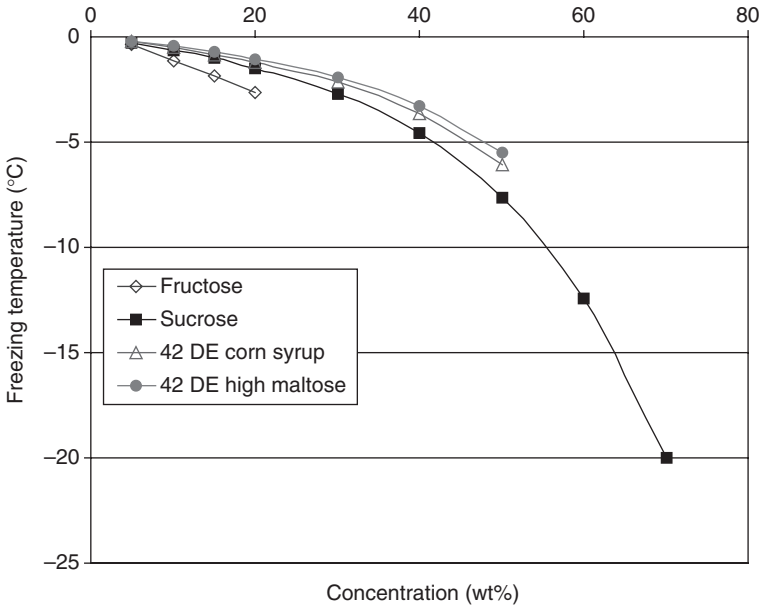

FIGURE 154.10 Freezing point depression curves (freezing temperature as a function of concentration) for several sugars (96).

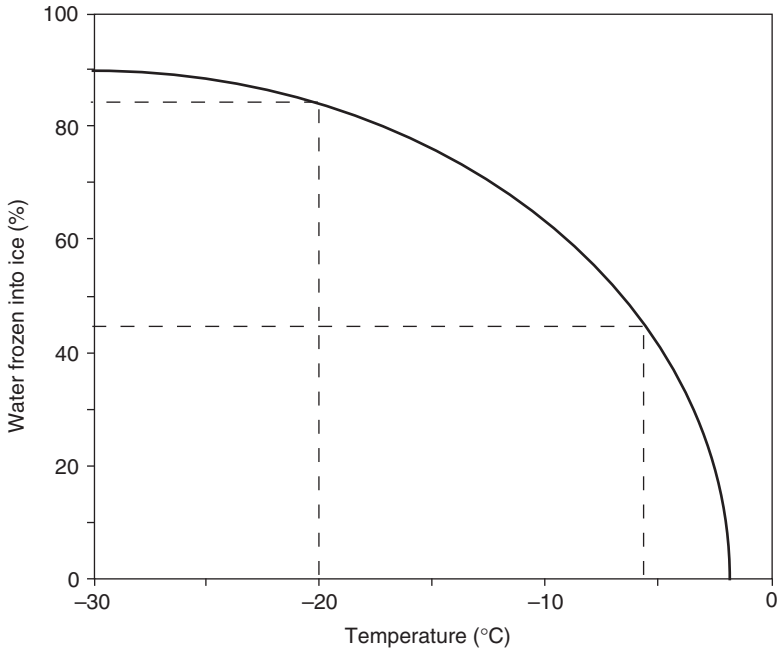

FIGURE 154.11 Examples of the approximate amount of water frozen into ice for ice cream of standard formulation at given temperatures, based on an equilibrium freezing curve for that formulation (based on Ref. 103).

contributions of sucrose, lactose (from msnf) and any corn syrups added. For an ice cream mix containing only sucrose, Equation 154.5 is used (1).

$$SE_{sw} = \frac{[(msnf \times 0.545) + S]100}{W} \qquad (154.5)$$

Here, SE_{sw} is the sucrose equivalence from sugars, S is sucrose content, W is water content (100 – total solids, %) and 0.545 is the percentage of lactose typically found in msnf. To obtain the freezing point depression associated with this level of sugars, FPD_{sw}, Table 154.9 is used (1). The contribution to freezing point depression from salts in msnf is found from Equation 154.6.

$$FPD_{sa} = \frac{msnf \times 2.37}{W} \qquad (154.6)$$

Here, FPD_{sa} is the sucrose equivalence for salts contained in msnf, and the constant 2.37 is based on the average molecular weight of the salts present in msnf. To obtain the freezing point depression of the ice cream mix, FPD_t, the two contributions are summed.

$$FPD_t = FPD_{sw} + FPD_{sa} \qquad (154.7)$$

EXAMPLE PROBLEM 3:

Calculate the initial freezing point of an ice cream mix containing 16% sucrose, 12% msnf, and 60% water (40% total solids). First, calculate the sucrose equivalents from Equation 154.5:

$$SE_{sw} = \frac{[12 \times (0.545) + 16]100}{60} = 37.57$$

TABLE 154.9
Freezing Point Depression in Sucrose Equivalents (1)

	Freezing Points	
Sucrose Equivalent (%)	(°C)	(°F)
0	0.00	32.00
5	−0.42	31.25
10	−0.83	30.50
15	−1.17	29.90
20	−1.50	29.30
25	−2.08	28.25
30	−2.67	27.20
35	−3.58	25.55
40	−4.39	24.10
45	−5.69	21.75
50	−7.00	19.40

Now, find the freezing point depression for this level of sucrose equivalent from Table 154.9. By interpolation,

$$FPD_{sw} = 2.31°C$$

For salts, from Equation 154.6:

$$FPD_{sa} = \frac{12 \times (2.37)}{60} = 0.47°C$$

Find the total freezing point depression for the mix from Equation 154.7:

$$FPD_t = FPD_{sw} + FPD_{sa} = 2.31 + 0.47 = 2.78°C$$

Thus, the initial freezing point temperature for this ice cream mix is −2.78°C.

Freezing Curve. In order for ice to form, the temperature of the system (T) must be below the freezing point (T_f) of the mix. The extent of subcooling ($\Delta T = T_f - T$) determines the rate of freezing, as discussed in the next section. Once freezing occurs, though, several things take place. The change in phase due to formation of ice causes a release of heat (latent heat of fusion), which increases the temperature in the vicinity of the phase change; this heat is removed by the refrigerant. At the same time, removal of water from the mix in the form of ice causes an increase in concentration of the remaining unfrozen phase, which has a lower freezing point due to the higher concentration. Thus, in the vicinity of the ice crystals, the temperature increases and the freezing point decreases. This leads to a freezing profile (Figure 154.12) dependent on the rate of freezing (96). For slow freezing, once nucleation starts, the temperature increases to approximately the melting point, due to fast release of latent heat, and then begins to decrease as further heat is removed and the concentration increases. Slow freezing results in a freezing profile that essentially follows the freezing point depression curve.

As freezing continues, the unfrozen phase becomes more and more concentrated and temperature continues to decrease. This leads to an increase in viscosity of the unfrozen phase until ultimately, the viscosity is sufficiently high that the freeze-concentrated unfrozen phase becomes glassy. That is, at some low temperature (the glass transition temperature, T_g), the unfrozen phase solidifies into a glassy state. Note that this is not a true solid (in the sense of a crystalline solid), but rather it is a high viscosity fluid that acts like a solid for as long as the temperature remains low. The point where the glassy state is formed during slow freezing is called the maximally freeze-concentrated temperature (T_g'), as seen in Figure 154.12. For various ice cream mixes, T_g' has been found to be around −30 to −35°C (85,105). For slow freezing, the amount of ice formed at any temperature is obtained as described in the previous section since the system follows the freezing point depression curve. If freezing is very rapid, the temperature and concentration of the solution falls somewhere below the freezing point depression curve, as shown in Figure 154.12. In this case, Figure 154.11 no longer applies and the amount of ice formed at any temperature is less than that shown in Figure 154.11 and is dependent on the rate of freezing.

b. Nucleation

The driving force for freezing is the temperature difference between the actual temperature of the system and the freezing (melting) point ($T - T_f$). At higher subcooling, freezing occurs more rapidly; that is, the rate of ice formation is a strong function of the thermal driving force (ΔT). The onset of nuclei formation is the point when the water molecules convert into molecules in an ice crystal lattice. When the temperature driving force is sufficiently high (temperature sufficiently below the freezing point), there is sufficient energy for the water molecules to overcome the energy barrier needed to form an ice crystal surface (the interface between crystal and liquid). Typically, ice formation begins on a surface that catalyzes the formation of ice crystals. This surface may

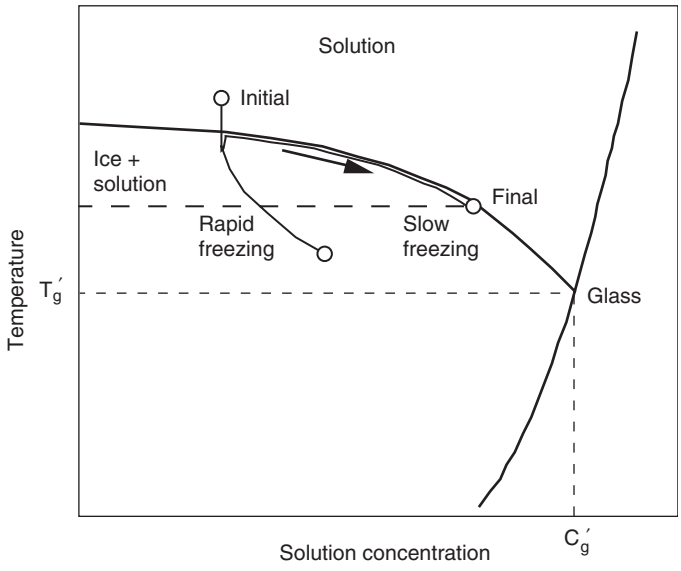

FIGURE 154.12 A phase diagram for solutions (e.g., ice cream mix) showing the path of freezing (temperature and solution concentration) for freezing at different rates. Figure shows schematic representation of freezing point depression and glass transition curves (adapted from Ref. 96). T_g' and C_g' represent point of maximally freeze-concentrated solution.

be that of the vessel that contains the solution or particles distributed throughout the solution that provide sufficient energy to order the water molecules in solution and promote nuclei formation. In commercial ice cream manufacture, it is likely that nucleation initially occurs by formation on the metal surface (inner barrel wall) exposed to the refrigerant, since that is where the driving force (ΔT) is highest. The rate of nucleation (number of nuclei formed per unit volume per unit time) for melt systems has been described by Equation 154.8 (96,106).

$$J = A\exp\left\{-\frac{BT_f^2}{(\Delta H_f)^2(T_f - T)^2} + \frac{\Delta G_v'}{kT}\right\} \quad (154.8)$$

Here, J is nucleation rate, A is a frequency factor (or preexponential term), B is a constant depending on the solutes present, T_f is freezing (melting) point, k is Boltzman's constant, ΔH_f is latent heat of fusion, T is system temperature and $\Delta G_v'$ is a diffusion-limited term that describes the mobility of water molecules.

Equation 154.8 clearly shows the dependence of nucleation rate on operating parameters, particularly the temperature driving force. When the system temperature, T, is close to the freezing point temperature, T_f, the temperature driving force (ΔT) and nucleation rate are low. In fact, at temperatures close to T_f, nucleation is so slow that the system may effectively remain unfrozen for long times, even though the temperature is below the freezing point of the solution. However, when ΔT is sufficiently high, or when system temperature falls sufficiently below T_f, Equation 154.8 predicts a sudden onset of nuclei formation. As the driving force (ΔT) increases, the rate of nuclei formation increases precipitously, giving rise to the spontaneous nature of freezing once it has initiated. When ΔT increases to too high a value, nucleation rate once again decreases due to the limited mobility of water molecules. As temperature goes down, the viscosity increases substantially, until eventually the system becomes glass-like. At this point, the $\Delta G_v'$ term overwhelms the ΔT term in Equation 154.8, and nucleation rate again goes to zero. Thus, there is a maximum in the nucleation rate curve as shown schematically in Figure 154.13 (9).

In a commercial scraped-surface freezer, the primary temperature driving force for nucleation occurs at the barrel wall. On the jacket side of this metal wall, liquid refrigerant (either ammonia or Freon) is vaporizing to provide the cooling effect. Vaporizing refrigerant removes heat from the ice cream mix nearest to the barrel wall and creates a high degree of subcooling in the mix at that region (9), as seen in Figure 154.14. Ice forms on the metal surface of the barrel wall where the temperature driving force is highest and catalytic nucleation sites exist (microscopic imperfections in the wall itself). Without agitation and scraping, this ice layer would continue to grow and increase in thickness until a thermal equilibrium

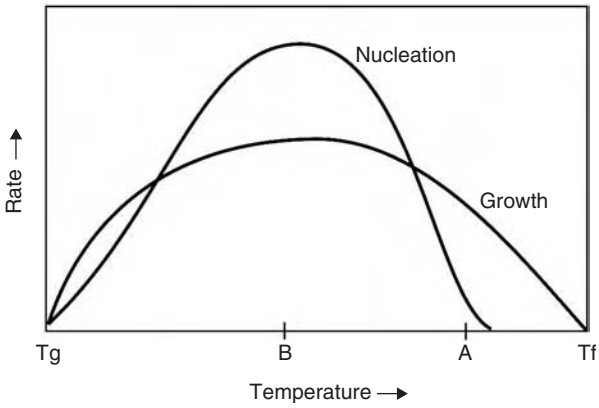

FIGURE 154.13 Rates of nucleation and growth of ice over the temperature range from glass transition temperature (T_g) to melting point (T_f). A and B represent temperatures where nucleation rate is low and high, respectively (9).

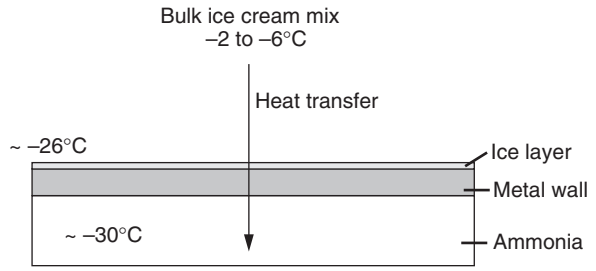

FIGURE 154.14 Approximate driving force (ΔT) for freezing of ice cream in a continuous freezer with vaporizing ammonia as refrigerant.

was attained between unfrozen mix and the coolant. In commercial freezers, the rotating scraper blades repeatedly clean off the metal surface of the barrel wall. Based on an agitator speed of 200 RPM and a six-bladed agitator, it can be calculated that the metal surface is scraped every 0.05 s. Thus, ice has very little chance to build up on the barrel wall. Recent studies (107,108) using videomicroscopy to observe ice formation on a cooled surface suggest that the scraper blade effectively cleans most of the ice off the metal wall at each scraping. Small pockets or shards of ice left on the wall serve as seeds for subsequent growth of the ice layer between scrapings. These studies suggest that the ice layer initially grows out along the surface to fill an ice layer on the metal wall rather than initially growing out into the solution. Most likely, the scraper blade removes the regrown ice layer before substantial growth into the solution (away from the wall) has occurred. The ice layer that is scraped off the metal wall is dispersed into the bulk mix circulating around the agitators.

The nature of the ice layer scraped off the metal wall in a commercial freezer has been the subject of much discussion in the past decades. Based on work by Schwartzberg and co-workers (109,110), it has been suggested that the ice layer in a scraped-surface freezer forms as dendritic (or needle-shaped) ice crystals extending into the solution (9). The scraper blade then removes these dendrites from the surface and disperses them into the center of the barrel, where subsequent recrystallization and ripening occur. Recent experiments suggest a different form of ice crystals at the barrel wall. Rather than dendrites extending into the solution, it appears that ice initially grows horizontally along the metal surface since this is the most favorable direction for heat transfer (107,108). The ice crystals in this layer are most likely needle-like although this has not been shown conclusively. Because growth is extremely rapid at the low temperatures of the metal wall, this ice layer is comprised of multiple ice crystals surrounded by concentrated mix. Before this layer has a chance to form more perfect crystals and exclude solvent molecules from the mix, it is scraped off by the blades and dispersed into the bulk of the freezer. In "slushie" machines that produce iced fruit drinks, the first evidence of ice formation when the refrigeration unit is turned on is thin "flakes" of ice removed from the refrigerated metal surface. Apparently, the scraper blade removes a layer of slush composed of ice and concentrated mix that temporarily maintains its integrity in the bulk, appearing as a thin layer or flake of ice approximately 0.5 to 1 cm in diameter. A submersed microscope in a batch scraped-surface freezer initially catches large (about 250 μm across) sheets of ice that take a hexagonal form (111). Similar forms have been seen growing horizontally along a cooled metal surface (108).

These polycrystalline ice flakes are distributed into the bulk of the freezer by the action of the scraper blades. What happens next depends to some extent on the nature of the bulk phase within the barrel of the freezer. For freezers with open dashers and internal mixers, the ice layer is mixed well with the warmer mix farther away from the refrigerated barrel wall. Here, the blades of the internal dasher can break the ice "flakes" into smaller shreds or pieces. In addition, melting, growth and ripening take place due to fluctuations in temperature that arise from the heat being removed by the barrel wall and the latent heat associated with melting and growth. A complex heat and mass transfer environment exists in which the ice crystals ultimately grow to product size and shape. Ice crystals exiting the scraped-surface freezer are typically disk-shaped with sizes ranging from a few microns to over 50 μm. In a closed dasher (one with high displacement of barrel volume), where the ice cream essentially flows in an annular space between the two cylinders (barrel and dasher), there is much less internal mixing and less opportunity for melting, growth and ripening. Nevertheless, enough of these processes take place that the ice crystals exit the freezer as disk-shaped crystals (as seen in Figure 154.5).

c. Growth, ripening, and equilibration

Within the barrel of the scraped-surface freezer, several complex processes related to freezing take place simultaneously. Furthermore, each process affects the nature of the other processes, primarily through influences on heat transfer. The thin layer of polycrystalline ice and slush that is scraped off the barrel wall is colder than the fluid at the center of the barrel. Thus, the first thing that happens is that the colder slush flake cools the surrounding environment as it, in turn, is warmed up. This warming, coupled with mechanical agitation, causes the flake to be broken down into smaller shreds, as has been observed by submersible microscope in a batch freezing apparatus (111). The polycrystalline ice crystals contained within the slush flakes are dispersed into the bulk solution where they melt, grow or ripen according to the conditions in their immediate environment. In regions where temperature is slightly higher than the slush from the wall, the ice crystals begin to melt. However, melting takes heat out of the solution as latent heat, which subsequently cools the surrounding environment. The direction of heat transfer determines which regions get the most cooling effect. In the regions where temperature is a little lower than the slush from the wall, ice crystals grow due to the temperature driving force. However, growth causes a release of latent heat, which warms the surrounding environment.

The rate of ice crystal growth is primarily influenced by two mechanisms. Ice crystal growth depends on the rate of counter-diffusion of solute molecules away from the growing interface and on the rate of heat transfer removal from the environment through either the solution or the ice crystal itself (112). The solute molecules present in the ice cream mix (i.e., sugars, salts, proteins, hydrocolloids, etc.) must diffuse away from the growing surface to allow incorporation of water molecules into the existing crystal lattice structure. The rate of diffusion of these solutes depends on the molecular size (larger molecules diffuse more slowly) and the concentration gradients existing during growth. Once water molecules are incorporated into the crystal lattice, there is a release of the latent heat of fusion, which must be removed by conduction and/or convection mechanisms. In an agitated environment, heat transfer generally occurs most rapidly by convective processes with fluid movement carrying away the heat from the growing crystal surface.

Further complicating these dynamics of melting and growth within the freezer barrel is the thermodynamic mechanism of ripening (112). Ripening is based on the slight difference in equilibrium (e.g., freezing temperature) between crystals of different size. It is well known that very small crystals (less than about 5 μm for ice) have

a slightly lower freezing point than large crystals (96). Thus, very small crystals may actually melt at the same time (in the same environment) that larger ice crystals continue to grow. In fact, it is this principle of ripening that leads to changes in ice crystals due to recrystallization in storage.

d. Controlling freezing

The principles of freezing discussed in the previous section are applied in commercial ice cream manufacture to make products with the desired number and size distribution of ice crystals for the highest quality. In the continuous commercial freezer described above, conditions are controlled to maximize production of numerous, small ice crystals. A low-temperature refrigerant (vaporizing ammonia or Freon) is used to lower the temperature of the mix quickly to about –25°C at the surface of the freezer barrel. This low temperature (high temperature driving force for nucleation) causes nucleation to occur rapidly, and results in formation of many small nuclei. Even though these nuclei ripen and grow as they make their way to the exit of the continuous freezer, they remain quite small (20 to 25 μm).

Compare the commercial situation above to that in a small batch home freezer. In both cases, ice forms on a cold metal surface in contact with a refrigerant, with a scraper blade periodically removing the ice layer formed at the wall. In the batch freezer, an ice-brine solution is made to lower the temperature of the ice cream mix. However, this brine reaches temperatures of perhaps only −10° to −12°C. This warmer temperature means that nucleation occurs at a significantly lower driving force than in the commercial freezer (liquid ammonia at about −30°C). According to Figure 154.13, the rate of nucleation is significantly lower in the batch freezer, due to the lower ΔT, than in the continuous freezer and thus, fewer ice crystals are formed. When the final ice cream products are hardened to the same temperature, the product from the batch freezer, which contains fewer crystals, ends up with significantly larger ice crystals (and potentially coarser ice cream) than the product from the continuous freezer, which has many more smaller crystals. This principle is described schematically in Figure 154.15 (113).

2. Operation of the Freezer Barrel

In larger ice cream manufacturing plants, ice cream mix is initially frozen into a semi-frozen slurry in continuous freezers. These units are scraped-surface freezers designed to carefully control ice formation, air incorporation and fat destabilization. Small-scale operations may utilize a batch freezer, where a single batch of ice cream is frozen at a time. In small soft-serve ice cream and custard stands, batch freezers are sometimes used that involve

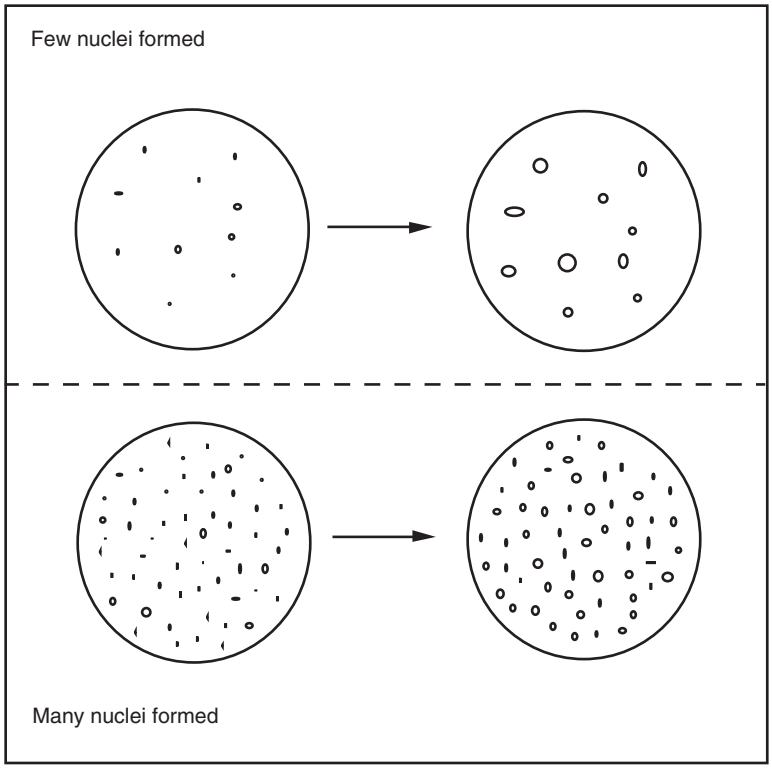

FIGURE 154.15 Schematic depiction of ice crystal size distributions obtained from batch (top) and continuous (bottom) ice cream freezers, based on nucleation rate (113).

discontinuous freezing, where ice cream is produced on an as-needed basis.

a. Continuous, scraped-surface freezer

A schematic of a commercial, continuous freezer is shown in Figure 154.16. Ice cream mix at a temperature of 0 to 4°C is pumped into the main barrel of the scraped-surface freezer under a pressure of 4–5 atmospheres (3) where it is frozen and aerated at the same time. The pressure inside the barrel is applied to reduce air phase volume and hence increase heat transfer. Refrigerant is introduced to the outside wall of the annular space between the two concentric cylinders, where vaporization of the refrigerant occurs to provide the refrigeration effect. Heat is removed from the ice cream as it freezes inside the barrel through the walls. Typically, either ammonia or Freon, kept at high pressure to maintain the liquid state, is pumped into the freezer where a lower pressure allows it to expand and vaporize to provide the refrigeration effect. Vaporized refrigerant is removed from the freezer and recompressed in a mechanical refrigeration system. Refrigerant pressure is controlled to maintain the desired temperature (about –30°C) and driving force for heat transfer removal. The rotating dasher, operating at 150 to 300 RPM within the freezer, holds scraper blades that contact the metal wall and scrape away the slush freezing on the inside of the barrel wall.

As the mix enters the freezer barrel, several things take place at the same time: water freezes in the mix, air is incorporated and the fat emulsion becomes partially coalesced. Control of these multiple factors is necessary to make ice cream with the desired physical and sensory characteristics. As discussed in the previous section, freezing of water occurs in the barrel and control of ice crystal formation is critical to product quality and shelf-life. Since the mix enters the freezer slightly above its freezing point, sensible heat must be removed to lower the temperature to the point where nucleation occurs. This occurs first at the barrel wall with vaporizing refrigerant separated from the ice cream mix by only a thin layer of metal. At the wall, the mix is quickly cooled below the freezing point and nucleation occurs. It has been estimated that the temperature just on the inside of the barrel wall is about −26°C, based on heat transfer resistances of the metal wall and perhaps a thin layer of ice present on the inside of the barrel wall (9). Since the initial freezing point of the mix is about −2°C, there is a significant driving force {(−2) − (−26) = 24°C} for nucleation at the wall and freezing occurs rapidly. Since the refrigerant temperature is maintained along the length of the freezer, the temperature at the barrel wall along the length of the freezer does not change significantly. That is, temperature just at the inside of the barrel wall is likely to be close to −26°C along the length of the entire freezer barrel.

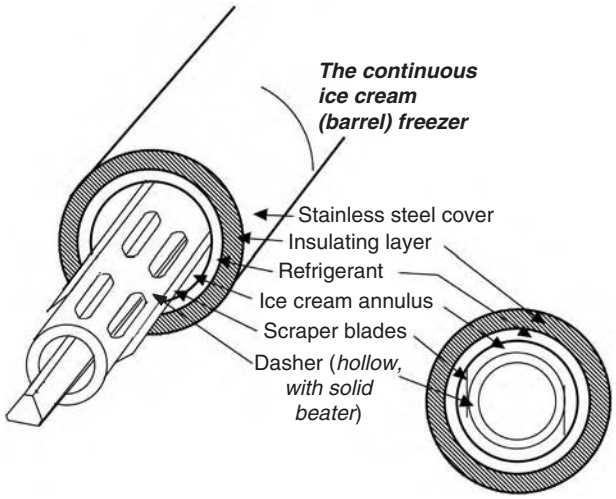

FIGURE 154.16 Schematic of the main components of the heat exchanger in a typical continuous ice cream freezer.

In the center of the barrel, however, the mix temperature is quite different from at the wall and a temperature gradient in the radial direction exists. Temperature in the center of the barrel may remain above the freezing point for some time as the mix works its way from the inlet to the outlet of the freezer. Eventually, as more and more ice scraped from the wall is mixed in with the warmer mix at the center of the barrel, the temperature in the center gradually decreases. It is at the center of the barrel where melting, growth and ripening occur, as discussed in the previous section. Thus, temperature at the center is essentially adiabatically controlled, based on the complex interactions (melting, growth, ripening, etc.) that take place.

It is thought that the decrease in temperature along the length of the barrel at the center of the freezer follows approximately the freezing point depression curve as more and more water is removed in the form of ice (9). Russell and co-workers (98) measured the temperature profile along the length of an experimental freezer and found that temperature decreased rapidly initially (near the inlet), decreased more slowly in the middle section and then increased slightly towards the outlet of the freezer, as seen in Figure 154.8 (98). At higher (500) dasher RPM, the temperature decreased to a greater extent than at lower (100) dasher RPM, which suggests that convective mixing from the colder environment near the wall is better with higher agitation rates. However, the mechanical energy input at the wall of the freezer with a higher agitation rate decreases the efficiency of nucleation and leads to ice cream with larger mean ice crystal size (98). There was slight increase in temperature of the ice cream just prior to the end of the barrel where the ammonia jacket ended and no longer provided a cooling effect. This indicates that the ice cream within the freezer barrel was slightly subcooled below the freezing point and the

release of latent heat at the end of the freezer caused the temperature to go up slightly. Once the ice cream was removed from the freezer, however, no temperature changes were observed when the ice cream was held adiabatically. This indicates that no additional crystallization took place once the ice cream was removed from the freezer and suggests that ice cream as it exits the freezer is at a point nearly on the freezing point depression curve for that temperature. Thus, estimates of the amount of water frozen into ice at any temperature that are based on freezing along the freezing point depression curve are essentially correct.

The importance of surface nucleation of ice at the barrel wall was shown by attempts to promote nucleation of ice through addition of ice-nucleating bacteria in a commercial continuous ice cream freezer (114). Ice-nucleating bacteria (*Pseudomonas syringae*) were added to an ice cream mix, and the mix was frozen under typical operating conditions in a pilot plant freezer. Ice crystal size of the ice cream exiting the freezer was identical for the control mix and the mix containing the ice nucleator. Since these nucleators promote nucleation in the bulk solution, this result suggests that the rate of ice nucleation at the wall of the freezer barrel was so high that the presence of ice nucleators had no effect on the total number of crystals formed in the freezer.

At the same time as freezing is taking place within the barrel, changes are also occurring to the lipid phase and air component. In commercial scraped-surface freezers, filtered compressed air is injected under pressure through a diffuser at the end of the barrel where the mix is input (3). The fine air bubbles formed in the diffuser are incorporated within the mix as the dasher rotates within the barrel. The air cells are broken down into smaller and smaller bubbles based on the shear forces within the freezer as the ice cream is formed (115). Dispersion of air into fine bubbles (about 20 μm in size after draw) requires that freezing occur at the same time to increase the shear forces within the freezer. Whipping air into ice cream mix without freezing results in lower amounts of overrun incorporated and larger air bubble sizes (115).

The fat emulsion also undergoes important changes in the barrel of the scraped-surface freezer (see Section II.B.4). Emulsifiers are added to the ice cream mix to decrease the stability of the emulsion droplets and allow partial destabilization during freezing. The shear forces within the freezer result in breakdown of the fine (< 3 μm) emulsion droplets and lead to partial coalescence of the fat globules. In this case, partial coalescence of the emulsion results in clusters of fat globules that are attracted to the air-serum interface. These partially-coalesced fat globules provide stabilization to prevent coalescence of the air cells so that many small air bubbles remain intact within the ice cream. It is this network of clusters of fat globules that provides meltdown resistance to the finished ice cream.

The refrigeration effect needed for ice cream freezing has been estimated by treating the distinct phases of the freezing process (116). The total energy required may be estimated as the sum of the energy required to cool the mix from the initial temperature to the freezing point, the energy associated with the latent heat needed to convert a certain amount of water into ice and the energy needed to cool the slush to the draw temperature (1). Although this approach gives only an approximation of the true refrigerant requirements for freezing ice cream, based on the simplifying assumptions, the values obtained give a starting point for calculating refrigeration load in an ice cream facility.

b. Batch freezer

Operation of a batch freezer proceeds in much the same manner as for a continuous freezer with several notable differences. That is, similar events take place in batch freezing as just described for continuous freezing, with the ice cream remaining in one place rather than moving along the length of the freezer barrel as in a continuous freezer. One notable difference in batch freezing is that there is typically a lower ratio of heat transfer surface to volume of ice cream. Thus, heat transfer is generally not as efficient in batch freezers compared to continuous freezers. Another typical difference between continuous and batch freezing is the nature of the refrigerant used. In commercial batch freezers, as found for soft-serve or custard-type freezers, vaporizing Freon may be used to provide the refrigeration effect. In this case, the temperature differential at the wall of the freezing cylinder may be as low as those found in continuous freezers. Hence, very small ice crystals are formed at the wall, scraped off by the mixing blades and then dispersed into the mix at the center of the cylinder. The temperature profiles at the wall and center of the freezing cylinder are very similar to those found in continuous freezers, except the temperature changes with time during freezing. When the temperature of the bulk of the ice cream reaches the desired draw temperature, or when the consistency of the ice cream within the barrel reaches some preset or desired value, the ice cream is drawn from the freezer. Typically, draw temperatures from batch freezers are similar to those in continuous freezers. However, due to the quantity of mix to freeze, the residence time required to achieve this draw temperature is much longer than in the continuous freezer, typically 15–30 minutes compared to approximately 1 to 2 min, and the resulting slower rates of freezing result in more recrystallization events in the barrel, larger crystal sizes, and slightly coarser texture when first frozen.

Another significant difference between batch and continuous freezing involves the nature of air incorporation. In batch freezers, the mix is allowed to whip at atmospheric pressure. Hence, whipping properties of the mix are very important and overrun is more variable, being controlled simply by the headspace remaining after the mix charge is put into the barrel. In the continuous freezer, air is injected through controlled valves, so whipping properties of the mix are perhaps less important and overrun control is

exact. Air distribution occurs under pressure in the continuous freezer, and it is the rapid expansion of the air bubbles at draw that establishes the air bubble interface.

Soft-serve ice cream freezers contain a swept-surface barrel freezer similar to the batch freezer, but they also contain a mix hopper that permits entry of a charge of mix each time a portion of the semi-frozen ice cream is removed. Thus, the complete barrel is only emptied on shut-down. The air handling systems of some large installation soft-serve ice cream freezers are a hybrid between batch and continuous freezers, in that the air inlet and barrel itself are pressurized to allow more exact control of overrun.

3. Overrun Calculations

Overrun is the industrial calculation of the air added to frozen dessert products, and it is calculated as the percentage increase in volume that occurred as a result of the air addition. The following examples will show calculations of overrun by volume and by weight, without and with the addition of particulates, and will also show calculations of target package weights. When packages are being filled on a processing line, package weights should be closely monitored. Deviations can be attributed to variations in the fill level of the package (packaging machine adjustment), variations in the ratio of ice cream to particulate addition (ingredient feeder or ripple pump adjustment), or variations in the overrun of the ice cream (freezer barrel adjustment).

Determining manufacturing overrun by volume, no particulates:
The equation for overrun determination of a production run, based on the definition of overrun as above, is as follows:

$$\% \text{ Overrun} = \frac{\text{Vol. of ice cream produced} - \text{Vol. of mix used}}{\text{Vol. of mix used}} \times 100\% \qquad (154.9)$$

Example. 500 L mix gives 980 L ice cream, using Equation 154.9:

$$\frac{980 - 500}{500} \times 100\% = 96\% \text{ Overrun}$$

Any flavors added, such as chocolate syrup in the next example, that become homogeneous with the mix can incorporate air and are thus accounted for in the following way.

Example. 80 L mix plus 10 L chocolate syrup gives 170 L chocolate ice cream, using Equation 154.9:

$$\frac{170 - (80 + 10)}{(80 + 10)} \times 100\% = 88.8\% \text{ Overrun}$$

Determining manufacturing overrun by volume, with particulates:
Example. 40 L mix plus 28 L pecans gives 110 L butter pecan ice cream, using Equation 154.9:
110 − 28 = 82 L actual ice cream surrounding the nuts.

$$\% \text{ Overrun} = \frac{\text{Vol. of ice cream} - \text{Vol. of mix used}}{\text{Vol. of mix used}}$$

$$= \frac{82 - 40}{40} \times 100\% = 105\%$$

The pecans do not incorporate air. This type of a determination might be useful if, for example, defects in a given mix were known to show up at >115% overrun. Otherwise, in a calculation of total output, a calculation similar to the one above, with no particulates, may be more useful.
Determining package overrun by weight, no particulates :

$$\% \text{ Overrun} = \frac{\text{Wt. of mix} - \text{Wt. of same vol. of ice cream}}{\text{Wt. of same vol. of ice cream}} \times 100\% \qquad (154.10)$$

Must know density of mix (wt. of 1 L), usually 1.09 − 1.1 kg /L (*see example below*).

Example. If 1 L of ice cream weighs 560 g net weight (exclusive of package), assuming a density of 1.09 kg/L, using Equation 154.10:

$$\% \text{ Overrun} = \frac{1090 - 560}{560} \times 100\%$$

$$= 94.6\% \text{ Overrun}$$

Determining package overrun by weight, if the ice cream has particulates in it, gives very little information because both the ratio of ice cream to particulates and the air content of the ice cream affect the final weight.

Determining mix density:
The density of mix can be calculated as follows:

$$(\text{Wt. per litre of water}) \Big/ \left[\frac{\% \text{ fat}}{100} \times 1.07527 + \left(\frac{\% \text{ total solids}}{100} - \frac{\% \text{ Fat}}{100} \right) \times 0.6329 + \frac{\% \text{ Water}}{100} \right] = \text{Wt./L mix} \qquad (154.11)$$

Example. Calculate the weight per litre of mix containing 12% fat, 11% msnf, 10% sugar, 5% corn syrup solids,

0.30% stabilizer, and 38.3% total solids, using Equation 154.11:

$$\frac{1.0 \text{ kg/L}}{0.12 \times 1.07527 + (0.383 - 0.12) \times 0.6329 + 0.617} = 1.0959 \text{ kg/L of mix}$$

Determining target package weights, no particulates:

Use the following formula:

$$\text{Weight of given vol. of ice cream} = \frac{\text{Wt. of same vol. of mix}}{\frac{(\text{Desired overrun} + 1)}{100}} \quad (154.12)$$

Example. Desired 90% overrun, mix density 1.09 kg/L, using Equation 154.12

$$\text{net wt. of 1 L} = \frac{1.09 \text{ kg}}{\frac{(90 + 1)}{100}} = 573.7 \text{ g}$$

Also, the density of ice cream can be calculated in a similar manner from Equation 154.12,

$$\text{Density of ice cream} = \frac{\text{density of mix}}{\frac{(\text{Overrun} + 1)}{100}}$$

Example: Density of mix 1100 g/L,

$$@100\% \text{ Overrun, density of ice cream} = \frac{1100 \text{ g/L}}{\frac{(100 + 1)}{100}} = 550 \text{ g/L}$$

Figuring target package weights, with particulates:
Example. Ice cream with candy inclusion; density of mix 1.1 kg/L; overrun in ice cream 100%; density of candy 0.748 kg/L*; candy added at 9% by weight, (i.e. 9 kg to 100 kg final product).

In 100 kg final product, we have:

$$9 \text{ kg of candy } \left(\text{or } \frac{9 \text{ kg}}{0.748 \text{ kg/L}} = 12.0 \text{ L}\right)$$

$$91 \text{ kg of ice cream } \left(\text{or } \frac{91 \text{ kg}}{1.1 \text{ kg/L}} = 165.4 \text{ L}\right)$$

$$\left(\frac{100}{100} + 1\right)$$

So, 100 kg gives a yield of 12 + 165.4 = 177.4 L

$$1 \text{ L weighs } \frac{100 \text{ kg}}{177.4 \text{ L}} = 564 \text{ grams}$$

In many cases, ice creams of different flavors are manufactured to provide the same weight per package for the consumer. As a result, overrun of the actual ice cream in the product varies from flavor to flavor, depending on the density and addition ratio of the particulate ingredients.

4. Fat Destabilization and Foam Stabilization

The texture of ice cream is perhaps one of its most important quality attributes. It is the sensory manifestation of structure; thus, establishment of optimal ice cream structure is critical to maximal textural quality. While the dynamic freezing process is generally associated with the formation of the ice phase, aeration and agitation during this process are also responsible for the formation of colloidal aspects of structure, viz., the formation of air bubbles and the partial coalescence of the fat into a major structural element (Figure 154.17). The colloidal structure of ice cream begins with the mix as a simple emulsion, with a discrete phase of partially crystalline fat globules surrounded by an interfacial layer comprised of proteins and surfactants (Figure 154.18) The continuous, serum phase consists of the unadsorbed casein micelles in suspension in a solution of sugars, unadsorbed whey proteins, salts, and high molecular weight polysaccharides. During the "freezing" stage of manufacture, the mix emulsion is foamed, creating a dispersed phase of air bubbles, and is frozen, forming another dispersed phase of ice crystals (Figure 154.19). Air bubbles and ice crystals are usually in the range of 20 to 50 μm and are surrounded by a temperature-dependent unfrozen phase (60). In addition, the partially-crystalline fat phase in the mix at refrigerated temperatures undergoes partial coalescence during the concomitant whipping and freezing process, resulting in a network of agglomerated fat, which partially surrounds the air bubbles and gives rise to a solid-like structure (Figure 154.18) (12,40,43,44,117).

The development of structure and texture in ice cream is sequential, basically following the manufacturing steps. To properly describe the role of fat in the structure, it is necessary to begin with the formation of the emulsion and the role of the ingredients present at the time of homogenization, with particular reference to the fat, proteins, and emulsifiers. After preheating or pasteurization, the mix is at a temperature sufficient to have melted all the fat present, and the fat passes through one or two homogenizing valves. Immediately following homogenization, the newly

* Note: density of particulate pieces containing void spaces must be determined by first crushing the material to eliminate void spaces, given that ice cream will fill in the voids after incorporation.

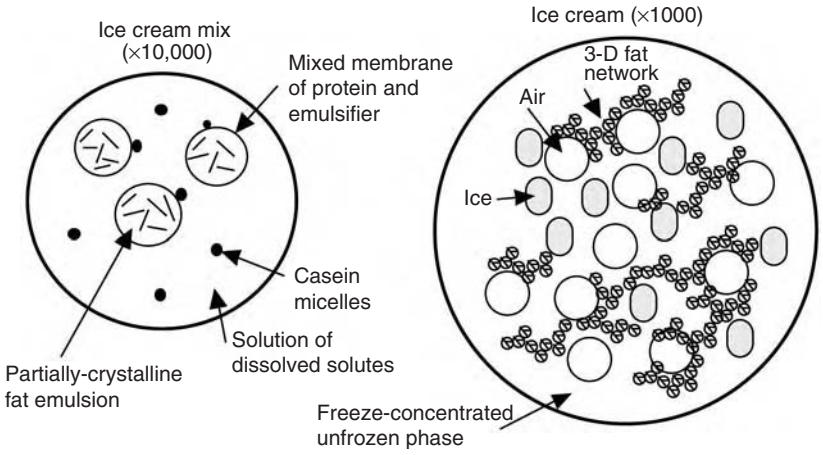

FIGURE 154.17 A schematic representation of the structure of ice cream mix and of ice cream.

formed fat globule is practically devoid of any membranous material and readily adsorbs amphiphilic molecules from solution (93). The immediate environment supplies the surfactant molecules, which include caseins, undenatured whey proteins, phospholipids, lipoprotein molecules, components of the original milkfat globule membrane, and any added chemical surfactants (6,93). These all compete for space at the fat surface. By controlling the adsorbing material present at the time of homogenization, it may be possible to predetermine the adsorbing substances and thus create a membrane with more favorable functional attributes, utilizing natural proteins rather than relying on the chemical surfactants (47). The membrane formed during homogenization continues to develop during the aging step and rearrangement occurs until the lowest possible energy state is reached (95). The transit time through a homogenization valve is in the order of 10^{-5} to 10^{-6} seconds (91). Protein adsorption or unfolding at the interface may take minutes or even hours to be complete (21). It is clear, therefore, that the immediate membrane formed upon homogenization is a function of the microenvironment at the time of its creation, and that the recombined membrane of the fat globule in the aged mix is not fully developed until well into the aging process (12).

Emulsifiers are not needed in an ice cream mix to stabilize the fat emulsion, due to an excess of protein and other amphiphilic molecules in solution (87,88). If a mix is homogenized without any emulsifier, both the whey proteins and the caseins will form this new fat globule membrane, with the caseins contributing much more to the bulk of the adsorbed protein. However, if added emulsifiers are present, they have the ability to lower the interfacial tension between the fat and the water phases lower than the proteins. Thus they become preferentially adsorbed to the surface of the fat (12,32,95). As the interfacial tension is lowered and proteins are eliminated from the surface of the fat, the surface excess (quantity of adsorbed material, mg/m^2) is reduced (42) and the actual membrane becomes weaker to subsequent destabilization. This is due to the fact that the protein molecules, and particularly the caseins, are considerably larger than the emulsifier molecules, such that a membrane made up entirely of emulsifier is very thin (Figure 154.18). This results lower surface excess, although the emulsion is thermodynamically favored due to the lowering of the interfacial tension and net free energy of the system.

Crystallization of fat also occurs during aging, creating a highly intricate structure of needle-like crystals within the globule (Figure 154.18). The high melting point triglycerides crystallize first, and continue to be surrounded by liquid oil of the lower melting point triglycerides. It has been reported that fat crystallization of emulsified milkfat at refrigerated temperature reaches equilibrium within 1.5 hours (6). A partially crystalline fat droplet is necessary for clumping to occur. van Boekel and Walstra (118) found emulsion stability of a paraffin oil in water emulsion to be reduced by six orders of magnitude when crystals were present in the dispersed phase. This has been attributed to the protrusion of crystals into the aqueous phase causing a surface distortion of the globule (118). The crystal protrusions can then pierce the film between two globules upon close approach. As the crystals are preferentially wetted by the lipid phase, clumping is thus inevitable. This phenomenon may account for partial clumping of globules under a shear force. The clusters thus formed actually hold the ice cream serum in their interstices resulting in the observed dryness. These fat globule chains may also envelope the air cells thus improving overrun (36), however, fat crystals are also known to impair overrun development in whipped cream (21).

The next stage of structure development occurs during the concomitant whipping and freezing step. Air is incorporated either through a lengthy whipping process (batch freezers), drawn into the mix by vacuum (older continuous freezers) or injected under pressure (modern continuous freezers) (1). This process causes the emulsion to undergo partial coalescence or fat destabilization, during which clumps and clusters of the fat globules form and build an

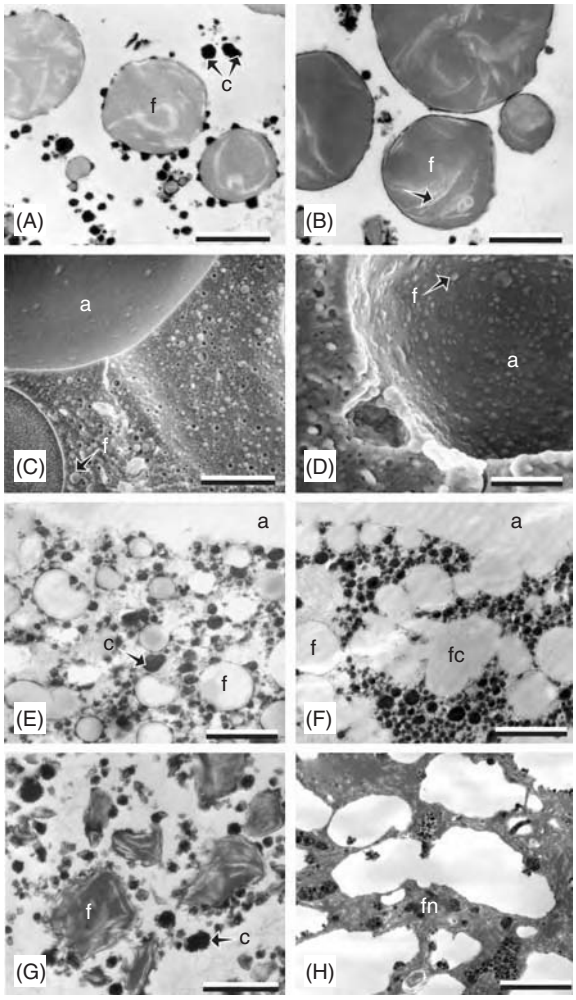

FIGURE 154.18 The effect of added emulsifier/adsorbed protein on structure of ice cream mix, ice cream, and melted ice cream. A-B, ice cream mix with no emulsifier and with added Polysorbate 80, respectively, as viewed by thin section transmission electron microscopy; f=fat globule, c=casein micelle, arrow (in B)=crystalline fat, bar= 0.5 μm. See Ref. 36 for methodology. C-D, ice cream with no emulsifier and with added polysorbate 80, respectively, as viewed by low temperature scanning electron microscopy; a=air bubble, f=fat globule, bar= 4 μm. See Ref. 61 for methodology. E-F, ice cream with no emulsifier and with added Polysorbate 80, respectively, as viewed by thin section transmission electron microscopy with freeze substitution and low temperature embedding; a=air bubble, f=fat globule, c=casein micelle, fc=fat cluster, bar= 1 μm. See Ref. 121 for methodology. G-H, melted ice cream with no emulsifier and with added Polysorbate 80, respectively, as viewed by thin section transmission electron microscopy; f=fat globule, c=casein micelle, fn=coalesced fat network, bar= 1 μm in G and 5 μm in H. See Ref. 36 for methodology.

internal fat structure or network into the frozen product (1,6) in a very analogous manner to the whipping of heavy cream (13). During the initial stages of whipping of cream, air bubbles have been shown to be stabilized primarily by beta casein and whey proteins with little involvement of fat (13). Adsorption of fat to air bubbles occurred when the fat globule membrane coalesced with the air water interface. Only rarely did fat spread at the air water interface. The final cream is stabilized by a cross-linking of fat globules surrounding each air cell to adjacent air cells, thus building an infrastructure in the foam (119). In skim milk foams, the initial air water interface is also formed by the serum proteins and soluble β-casein, with little involvement of micellar casein. Micelles become attached as a discontinuous layer, but are not deformed or spread (21). It can be postulated that air cell incorporation into ice cream mix follows a similar mechanism. Cross-linking of fat globules from one air cell to the next, thus forming an infrastructure, is less likely due to the reduction in dispersed phase volume from the heavy cream system to the ice cream mix system. However, it must also be borne in mind that the air bubbles, fat globules, and aqueous phase are being freeze-concentrated at the same time.

The fat globule clusters formed during the process of partial coalescence are responsible for surrounding and stabilizing the air cells and creating a semi-continuous network or matrix of fat throughout the product, resulting in the beneficial properties of dryness upon extrusion during the manufacturing stages (aids in packaging and novelty molding, for example), a smooth-eating texture in the frozen dessert, and resistance to meltdown or good stand-up properties (necessary for soft serve operations) (6,120). Fat destabilization is enhanced by the emulsifiers in common use (12,88). When the emulsion is subjected to the tremendous shear forces in the barrel freezer, the thin membrane created by the addition of surfactant is not sufficient to prevent the fat globules from colliding and coalescing, thus setting up the internal fat matrix (36). If an ice cream mix is subjected to excessive shearing action or contains too much emulsifier, the formation of objectionable butter particles can occur as the emulsion is churned beyond the optimum level. Polysorbate 80, having a small molecular weight and producing the lowest interfacial tension compared to mono- and diglycerides displaces more protein, resulting in a very thin membrane, and thus produces the maximum amount of fat destabilization (36).

The extent of fat destabilization can be quantified in several ways. It is sometimes presented as a % change in turbidity as measured by a spectrophotometer on diluted samples of mix and ice cream (12). It can also be determined based on a solvent extraction technique using a mild solvent, since coalesced fat becomes increasingly susceptible to extraction, whereas emulsified fat does not (95). As well, it can be presented as a change in size distribution of fat globules as measured by laser light scattering techniques (e.g., %>3 μm, since 0% of the mix emulsion was greater than 3μm) (42).

Gelin and co-workers (37) demonstrated through light scattering measurements of fat globule size distribution

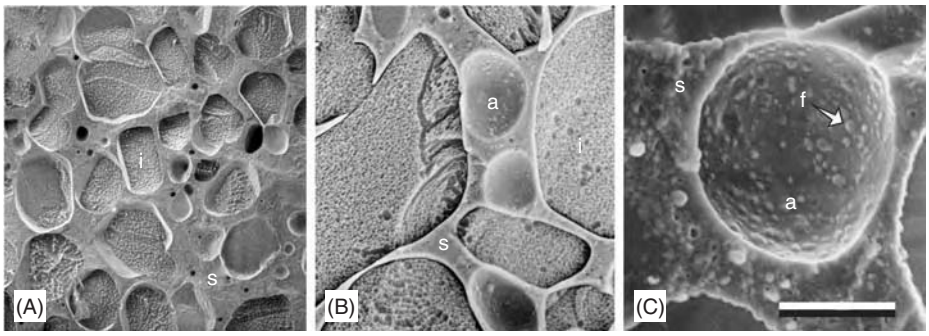

FIGURE 154.19 Low temperature scanning electron micrographs of the overall structure of ice cream. A) General overview of spatial distribution of ice crystals (i) within the unfrozen phase (s). Bar (in C) = 100 μm. B) Higher magnification showing air bubbles (a) and ice crystals (i) embedded into the unfrozen serum (s) as discrete phases. Bar (in C) = 40 μm. C) High magnification picture of an air bubble, showing fat globules (f) adsorbed at the air interface and also dispersed in the unfrozen phase (s). Bar = 20 μm.

and aggregation that the freezing step is responsible for considerable fat aggregation. This aggregation is initially reversible through dissociation with SDS, but not after fat crystal sintering has occurred. They have also shown the changes occurring to the protein distribution between the aqueous and adsorbed states. It was obvious from their study that the homogenization step accounted for a large amount of adsorbed protein, and that casein was preferentially adsorbed over the whey proteins. The aging and freezing-hardening-thawing steps each accounted for subsequent protein desorption, again mostly of the caseins. The sequential process of partial coalescence during ice cream freezing has also been examined (12). The incorporation of air alone, or the shearing action alone, independent of freezing, are not sufficient to cause the same degree of fat destabilization as when ice crystallization occurs concomitantly. The freezing process causes an increase in concentration of the mix components, such as proteins and mineral salts, in the unfrozen water phase. It is believed that the ice crystals contribute to the shearing action on the fat globules, due to their physical shape, and that the concentration of components also leads to enhanced destabilization. However, to create the desired extent of fat destabilization, whipping and freezing must occur simultaneously (87).

Goff and co-workers (121) examined air interfaces in ice cream and fat:air interactions using transmission electron microscopy with freeze-substitution. The structures created by increasing levels of fat destabilization in ice cream (achieved through increased emulsifier concentration in the mix in both batch and continuous freezing) were observed as an increasing concentration of discrete fat globules at the air interface (Figure 154.18), and increasing coalescence and clustering of fat globules, both at the air interface and within the serum phase (Figure 154.18). Air interfaces at the highest levels of fat destabilization were not completely covered by fat globules. It has been suggested that the air interface in ice cream may be covered by a thin layer of non-globular liquid fat (6). However, there was no evidence of a surface layer of free fat in the work of Goff and co-workers (121). Further, air interfaces in a fat-free ice cream formulation showed a very similar, continuous membrane as those from a formulation containing fat. This further suggests that the air bubble membrane itself is comprised of protein, with discrete and partially-coalesced fat globules subsequently adsorbed.

C. Flavors and Flavor Addition

Ice cream and frozen dessert manufacturers offer a wide variety of flavors and particulate ingredients to consumers, which are often the basis upon which consumers make selection choices. Some of the major flavors and flavor categories, based on consumption in North America, are shown in Table 154.10. Ingredients are added to ice cream in three ways during the manufacturing process: in the mix tank prior to freezing (for liquid flavors, colors, fruit purees, flavored syrup bases, or anything else that will become homogeneous within the ice cream); through a variegating pump (for ribbons, swirls, ripples, revels, etc.); or through an ingredient feeder (for

TABLE 154.10
Ice Cream Consumption by Flavor, 2002 Annual, Canada and the U.S.

Flavor	Percentage of Production Volume
Vanilla	28.4
Nut Flavors	10.4
Chocolate	8.0
Fruit flavors	7.6
Neapolitan	7.4
Bakery Flavors	5.8

Data from the International Dairy Foods Association.

particulates — fruits, nuts, candy pieces, marshmallows, cookies and bakery pieces, etc.). In the case of the latter two, this equipment is added in series after the continuous freezer, when the ice cream is already semi-frozen. Often, these may be placed in sequence for complex flavors involving multiple components, e.g., a variegating pump and an ingredient feeder or two ingredient feeders. Ingredients added into the semi-frozen ice cream should be as cold as possible, either refrigerated or stored at subzero temperatures, so as not to cause any melting and recrystallization of the ice crystals at this point in the process.

Vanilla. Vanilla is the most popular flavor for ice cream in North America. Vanilla ice cream is used to make milkshakes, sundaes, floats and other types of desserts at the retail level, and is often an a accompaniment to other desserts, such as cakes or pies. Vanilla is also used in many other flavors as a flavor enhancer, e.g., chocolate flavor is improved by the presence of a small amount of vanilla.

Vanilla comes from a plant belonging to the orchid family called *Vanilla planifolia*, grown typically in Mexico, the islands off the east coast of Africa (particularly Madagascar), Tahiti, South America (Guadeloupe, Dominica, Martinique), and Indonesia (Java). Bourbon beans from Madagascar are often considered the finest and account for over 75% of world production. From each blossom of the vine that is successfully fertilized comes a pod that reaches 15–25 cm in length, picked at 6–9 months. It requires temperatures of 24–29°C day and night throughout the season, as well as frequent rains with a dry season near the end for development of flavor. Pods are immersed in hot water to stop biological activity of the seed (which also serves to increase enzyme activity), then fermented for 3–6 months by repeated wrapping in straw to "sweat" and then uncovering to sun dry. 5–6 kg green pods produce 1 kg cured pods. Beans are then aged 1–2 years. Enzymatic reactions during aging produce many compounds, of which vanillin is the principal flavor compound. However, there is no free vanillin in the beans when they are harvested. It develops gradually during the curing period from glucosides, which break down during the fermentation and "sweating" of the beans. Extraction takes place as the beans are chopped (not ground) and placed in a stainless steel percolator. Cold alcohol (no heat involved) and water are pumped over and through the beans until all flavoring matter is extracted. Vacuum distillation takes place for a large part of the solvent. The desired concentration is specified as twofold, fourfold, etc. Each multiple must be derived from an original 10g beans/100 mL of alcoholic extract.

Vanillin can be and is produced synthetically to a large extent. Vanillin is contained in many types of woods and thus is a by-product of the pulp industry. Compound flavors are produced from combinations of vanilla extract and vanillin. Vanillin may be added at one ounce to the fold for compound flavors. The number of folds plus number oz. of vanillin equals the total strength, e.g., 2 fold + 2 oz. = 4 fold vanilla-vanillin. However, more than 1 oz to the fold is deemed imitation. Usage level in the mix is a function of purity and concentration. Typically a single fold natural vanilla is recommended at 3–6 mL/L mix, a two fold vanilla-vanillin at 2–3 mL/L mix. Some vanillin may improve flavor over pure vanilla extract, so often natural and artificial compound flavors are more desirable than pure natural flavors; however, too much vanillin results in harsh flavors.

Chocolate and Cocoa. The cacao bean is the fruit of the tree *Theobroma cacao* ("Cacao, food of the gods"), which grows in tropical regions such as Mexico, Central America, South America, West Indies, and the African West Coast. The beans are embedded in pods on the tree, 20–30 beans per pod. When ripe, the pods are cut from the trees, and after drying, the beans are removed from the pods and allowed to ferment for 10 days (microbiological and enzymatic fermentation). Beans then are washed, dried, sorted, graded and shipped for processing. Figure 154.20 shows a flow diagram for the processing of chocolate and manufacture of cocoa. At the processing plant, beans are roasted, the seed coat is removed and the interior of the bean, called the nib, is ground. Friction melts the fat and the nibs flow from the grinding as a liquid, known as chocolate liquor. The composition of chocolate liquor is about 55% fat, 17% carbohydrate, 11% protein, 6% tannins and many other compounds. After the cocoa butter is pressed from the chocolate liquor, the remaining press cake is now the material for cocoa manufacture. The amount of fat remaining determines the cocoa grade: medium fat cocoa, 20–24% fat; low fat 10–12% fat. There are many types of chocolate that differ in the amounts of chocolate liquor, cocoa butter, sugar, milk, other ingredients, and vanilla. Imitation chocolate is made by replacing some or all of the cocoa fat with other vegetable fats. For ice cream, this provides improved coating properties and enhanced resistance to melting. White chocolate is made with cocoa butter, milk msnf, sugar, but no cocoa or chocolate liquor.

There are two types of cocoa available, namely, American (domestic) and Dutch (alkalized). The latter is treated with an alkali (sodium hydroxide, etc.) to increase solubility, darken the color, and modify the flavor. The Dutch type is usually preferred in ice cream because it gives a darker, less red color but the choice depends upon consumer preference, desired color (Blackshire cocoa may also be used to darken color), strength of flavor, and fat content of the ice cream (19). For chocolate ice cream manufacture, cocoa is more concentrated for flavoring than chocolate liquor (55% fat) because cocoa butter has relatively low flavor. Hence, low fat cocoa powders are usually utilized at 2–3% (w/w) in the mix. Cocoa is usually added with other dry ingredients at the blending

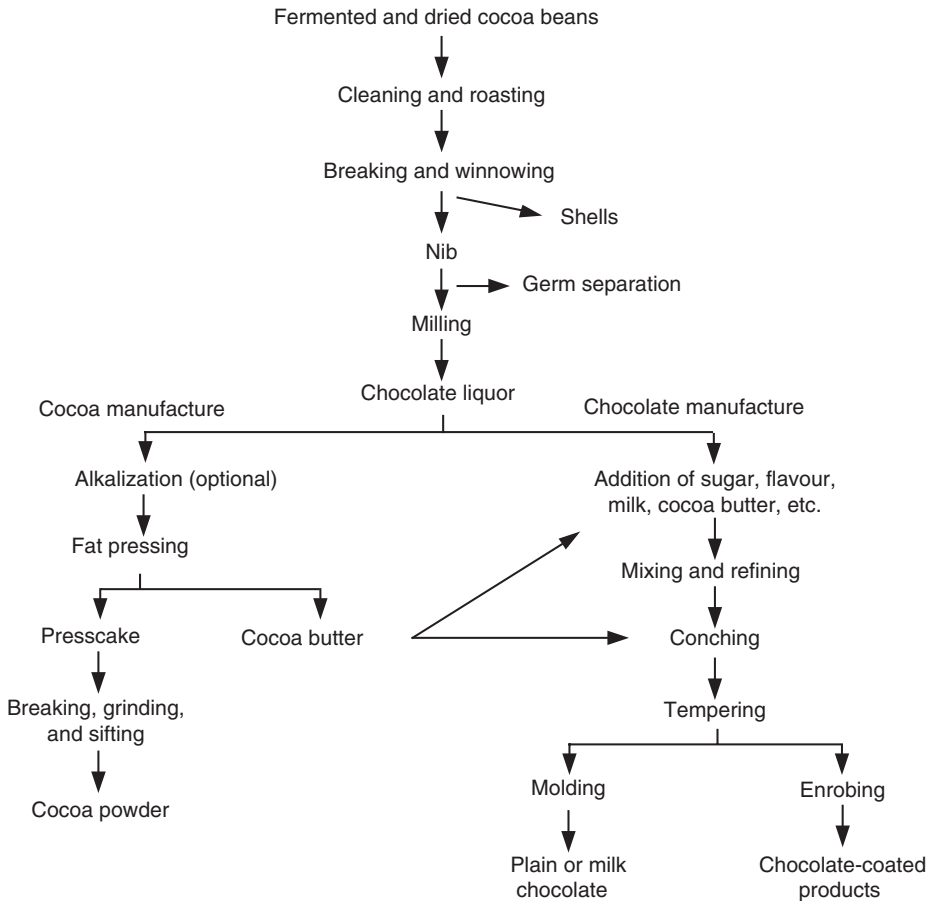

FIGURE 154.20 The processing of cocoa into ingredients typically used in chocolate ice cream.

stage, and pasteurized and homogenized with the rest of the mix. Blends of cocoa (2–3%) and chocolate liquor (2%) or chocolate liquor alone (5%) can also be used to produce a chocolate ice cream with enhanced smoothness and with the typical full-fat flavor of chocolate products. Chocolate mixes have a tendency to become excessively viscous, so stabilizer and corn syrup solids content and homogenizing pressure need to be slightly lowered to account for the enhanced viscosity. Sucrose content is generally increased by 2–4% (w/w) in the mix to offset the slight bitterness from the cocoa.

One frequent defect with chocolate ice cream, particularly soft-serve, is chocolate specking. Cocoa becomes entrapped in partially coalesced fat, which then darkens. Alleviation of excessive fat destabilization usually alleviates this problem.

Fruit Ice Cream. Fruit flavors are quite popular in ice cream. Fruit for ice cream can be utilized as fresh fruit, raw frozen fruit, "open kettle" processed fruit, or aseptically processed fruit cooked in swept-surface heat exchangers. Fruit additions should use sufficient fruit (15–25% w/w) of choice quality for best fruit ice cream. The more highly flavored the fruit, the less required in ice cream. Fruit should be kept in large pieces in the ice cream where possible, and that is usually a function of the incorporation method. Ingredient feeders are used with continuous freezers to add the fruit pieces or sugared fruit preparations, while a portion or all of the fruit juice, as appropriate when straining of fruit is employed, is added directly to the mix. In the batch freezer, fruit juice is added with the mix at the start of the batch, and the fruit pieces are added when the mix has been partially frozen or at draw.

Some small-scale ice cream processors may find it desirable, for a variety of reasons, to use fresh fruit. Such use involves all of the preparation steps of washing, sorting, peeling, destoning, etc. If fresh fruit is being added to ice cream, it should be prepared with sugar in such a way as to allow the sugar to penetrate the fruit. Otherwise, it will freeze to form solid lumps in the ice cream. Sugar draws out juice by osmotic dehydration. If fruits are to be pureed, this will not be necessary, although sugar does help to bring out flavor. With strawberries it is advisable to slice in half and treat with sugar at the rate of at least 20–30% sugar, allowing the berries to stand in a cool temperature until sufficient sugar has been absorbed. Sugared fruit can

either be strained to separate juice from pulp, or can be cold-stabilized with the use of pectin or starch prior to adding to the ice cream. In this way, the juice and pulp can be added at the same time through the ingredient feeder.

Frozen fruit for ice cream is usually frozen with the addition of a suitable content of sugar, usually 25–30%. Frozen packs must be thawed before use. Forced thawing with heat will cause rupture of the fruit with resulting poor appearance. Where discrete fruit pieces are not desired in the ice cream, forced thawing may be used. Thawing usually results in juice separation, unless the product has been cold-stabilized with starch or pectin, and if so, this juice should be strained and added to the mix before freezing. Polysorbate 80 (see Section I.B.5) is sometimes added to the mix prior to the freezing of fruit ice cream, particularly if the fruit is "wet." This aids in producing a dry ice cream to help incorporate the fruit addition. Depending on the strength of flavor of the fruit preparation and the concentration utilized, it may be necessary to augment fruit flavors with the addition of natural or artificial flavors. Also, sometimes the addition of citric acid to the mix is desirable.

Fruit can be processed by cooking in a syrup with added sugar to a total sugar content (°Brix) of 50–60%, and is often stabilized with pectin or starch. This processed fruit moves the problems of procurement, variability, and quality from the ice cream manufacturer to the fruit manufacturer/supplier. The fruit manufacturer can source fruit from around the world and blend it from a variety of sources to achieve year-round supply and consistency. Fruit preparation ensures removal of debris, stones, pits, skins, etc., and cooking ensures microbial safety. By cooking in sugar, the fruit will not freeze as a solid in the ice cream, which provides a more pleasant texture. For the ice cream manufacturer, this product is available in a ready-to-use form, with no need for thawing, straining, etc., so it involves no product loss. Fruit processed by open kettle methods, however, often provides a cooked flavor that detracts from the natural fruit flavor desired by the ice cream manufacturer and consumer. The processing of such fruit aseptically in scraped surface heat exchangers provides the opportunity to offer an improved flavor and color, a more consistent product, no preservatives, and a longer shelf-life.

Variagates. Variagates are injected through a positive pump connected to a small-diameter nozzle or nozzles within the stream of ice cream from the continuous freezer. They are available as a prepared base, e.g., chocolate, butterscotch, marshmallow, strawberry, cheese cake concentrate, etc., and are usually incorporated at 10% (w/w) of ice cream. Almost any flavor can be variegated into ice cream in a variety of contrasting ice cream flavors and colors. A good variegating syrup should not settle out or run into pools in the ice cream. It must not become icy during storage.

Nuts in Ice Cream. Nut-flavored ice creams are also very popular, although concern for consumers with nut allergies has meant strict segregation of nuts from non-nut products and declaration of possible cross-contamination with nuts, and has limited the use of nut flavors in recent years. Nuts should be used in generous amounts, usually around 10% (w/w), and kept in large pieces. Commonly used are walnuts, pecans, filberts, almonds, and pistachios. Brazil nuts and cashews have been tried without much success. Pecans are usually roasted with butter and incorporated into a butter pecan ice cream. Pistachios may be treated in somewhat the same manner as pecans, or may be used in the characteristic pistachio ice cream, which is usually colored green and is flavored with bitter almond. Raw walnuts may be preferred to roasted for flavor, but some form of heat (oven) treatment should be given to walnuts to eliminate surface microbial contamination. Walnuts are often used with a maple flavoring. Almonds are commonly dry roasted to a point just before burning, and are added to the mix flavored with vanilla or almond flavoring. Filberts are roasted dry to a light brown color. The skins are removed (blanched), and the nuts reduced in size by chopping. They are added to a mix mildly flavored with vanilla.

Due to potential contamination with extraneous (e.g., shells) and foreign matter, nuts require extensive cleaning and screening. Nuts must be processed in a clean sanitary premise following good manufacturing practices. Nuts should be either oil roasted or heat treated to reduce any bacteria. Microbiological testing for Standard plate count, coliforms, *E. coli*, yeasts, molds, and *Salmonella sp.* is carried out randomly but routinely, and testing for aflatoxin (mold toxin from *Aspergillus flavus*) is performed on peanuts. Nutmeats should be stored either at subzero temperatures in a freezer, or at least at 2–4°C to maintain freshness and reduce problems with lipid oxidation in the nuts.

Color in Ice Cream. Ice cream should have a delicate, attractive color that is closely associated with the type of flavoring material that has been added. In some instances, ice cream mix may be slightly colored to give it the shade of the natural product, e.g., 15% (w/w) fruit produces only a slight effect on color and may need to be augmented. Some fruit solid packs may already be colored by the fruit manufacturer, for convenience to the ice cream manufacturer. Most colors are of synthetic origin and can be purchased in liquid or dry form. Color solutions can easily become contaminated, and therefore must be fresh.

D. Packaging and Static Freezing

Once the ice cream exits the freezer as a partially-frozen slush, particulate flavors can be added, and then it is pumped into a package, sealed, and hardened. When the semi-solid ice cream exits the continuous freezer, it should have the correct stiffness, or ability to flow, for its

intended use. For ice cream intended for direct packaging, about half of the water is frozen to ice when the ice cream exits the freezer, and it should still be sufficiently fluid to flow and completely fill a package without leaving void spaces. If the draw temperature of the freezer is too low, or the mix is otherwise frozen too much, the ice cream exiting the freezer will be too stiff for proper packaging. In some cases, as for frozen novelties, this high degree of stiffness may be desired so that the ice cream maintains its shape prior to hardening.

Packages of ice cream are sent to a hardening room or tunnel for further freezing. The aim of hardening is to remove heat so that the ice cream cools quickly to temperatures below –18°C. The time required for hardening primarily depends on the size of the package entering the hardening facility, and the nature of the refrigeration process within the hardening facility. Very small containers, as in 0.5L or smaller cups, may take as little as 30 minutes to harden properly; whereas larger bulk-sized containers may take 24 hours. If cartons of ice cream are collected on a pallet prior to hardening, the time for the center-most container to reach hardening temperatures may be substantially longer than 24 hours. Most commercial facilities allow between 12 and 24 hours in the hardening facility to ensure proper freezing.

As the ice cream cools, additional ice freezes in accordance with the freezing point depression curve. It is important to note that, typically, no new ice crystals (nuclei) are formed during hardening, since the thermal driving forces are generally too small to promote nuclei formation. Thus, the increase in ice content (ice phase volume) comes about through a general increase in the size of all existing ice crystals. Clearly, the number of ice crystals formed in the initial freezing step will have a big impact on the ice crystal size of the final hardened ice cream. Typically, ice crystals increase in size about 10 to 15 μm during hardening. That is, the mean ice crystal size after drawing from the continuous freezer may be about 25 to 30 μm, but the mean size after hardening is more likely to be between 40 and 45 μm.

The speed of cooling has a significant impact on the ice crystal size, and this may vary through the container. The ice cream near the outside of the package cools the fastest. The ice cream near the center is insulated by the rest of the ice cream and cools much more slowly. For example, Donhowe (122) followed the temperature decrease at different locations in a half-liter cylindrical container of ice cream during hardening, as shown in Figure 154.21 (10). The surface cooled most rapidly, with the center taking nearly 10 minutes to even start cooling. During that 10 minute delay, the ice crystals at the center of the package were undergoing recrystallization at a rapid rate due to the high temperature. The result is that the ice crystals in the ice cream at the center of the container had substantially larger mean size than the ice crystals in the product near the surface, as seen in Figure 154.22 (10). This effect becomes even more dramatic when larger-sized containers are hardened. For example, the ice cream at the center of a pallet of containers may remain at elevated temperatures for substantially longer than the 10 minutes in this example, and the mean size can get considerably larger. Proper hardening is critical to maintaining the highest quality of the ice cream.

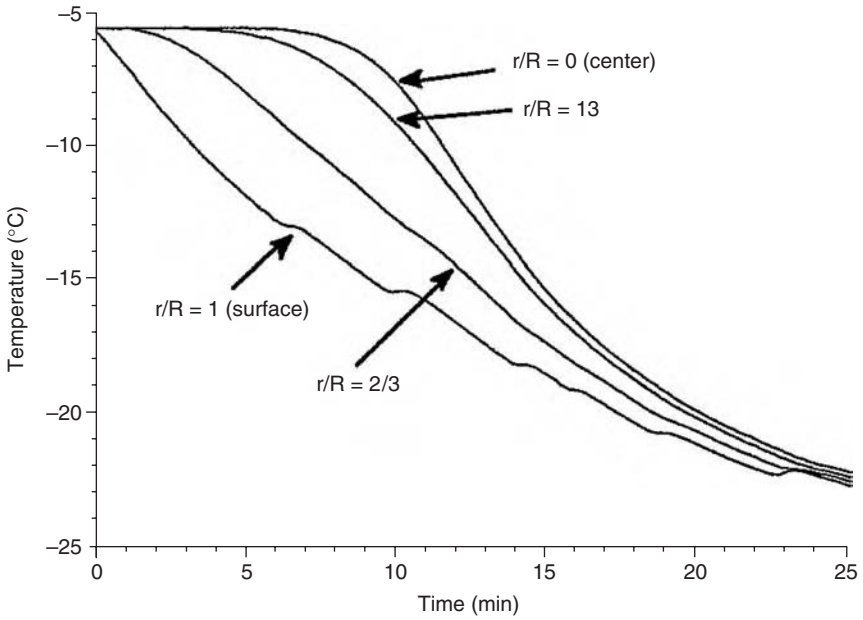

FIGURE 154.21 Temperature profiles as a function of time at different distances from the center (relative radial dimension, r/R) during hardening of a half-liter cylindrical container of ice cream at –30°C (10).

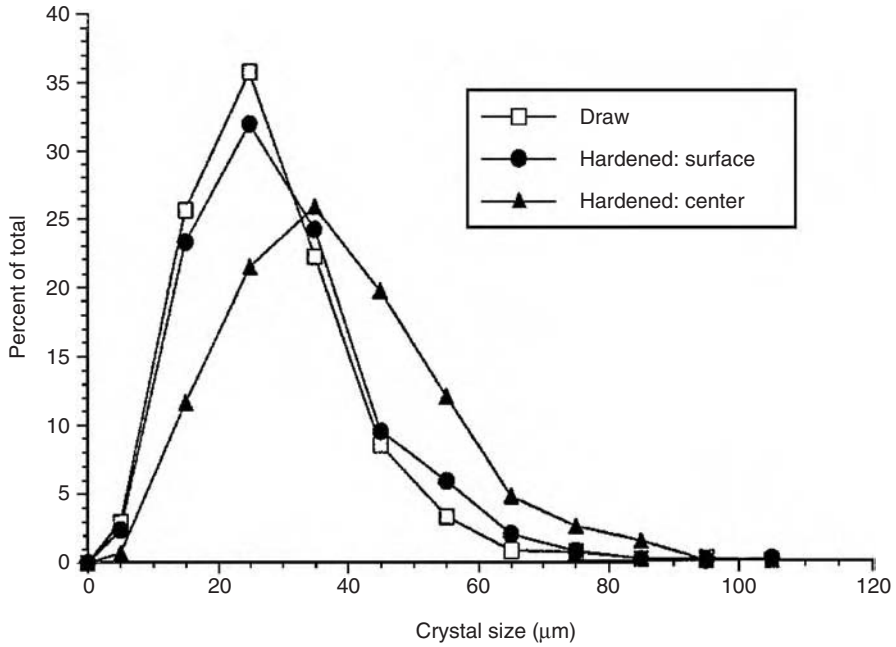

FIGURE 154.22 Ice crystal size distributions for ice cream at different points within a half-liter container after hardening to –30°C. Points correspond to container positions in Fig. 154.21 (10).

The speed of cooling in the hardening facility also depends on the type of refrigeration system chosen. There are numerous options for hardening ice cream. The choice of hardening facility depends on many factors, including the size of the operation, the types of ice cream products being frozen, as well as other economic factors. In some cases, as in small operations, the packages of ice cream may simply be transported to an air blast freezer for hardening. In this case, cold air blowing across the packages removes heat from the ice cream as it freezes further. Typically, air at –30°C, cooled by a mechanical refrigeration system, blows past the packages. Good air flow across each individual package is necessary to obtain the fastest rate of cooling.

In larger operations, packages of ice cream are placed on a conveyor (e.g., spiral configuration) and transported through a hardening tunnel to provide rapid convective cooling. The tunnel is maintained at −35° to −40°C and with very high air velocity. The residence time of a package on the conveyor may be between 40 and 160 minutes, which is sufficient to lower the temperature to about –18° to −25°C (1). Again, cold air (−30 to –40°C) blowing across the individual packages provides a rapid rate of cooling in the hardening tunnel. Product exiting the tunnel is then transported to a storage freezer for further distribution.

Another type of hardening system is the plate freezer, which works well for products in containers with flat sides. In the plate freezer, the containers come in contact with a metal surface (the plates) on both sides (top and bottom). The plates are cooled internally with circulating refrigerant so conductive heat transfer is excellent between plates and ice cream. Hardening in a plate freezer can be accomplished in as little as 2 hours (1).

The choice of packaging material is based on many considerations. From a heat transfer standpoint, the package should have a sufficiently high heat transfer rate that the ice cream cools rapidly in the hardening facility, so that ice crystals are maintained as small as possible. However, during storage and distribution of the ice cream, a good insulating package is desired to minimize thermal fluctuations (and minimize recrystallization during storage). Thus, a compromise on the type of packaging material used is necessary, and often the choice comes down to marketing considerations and the price of the packaging material, with heat transfer and product concerns essentially ignored.

E. Novelty/Impulse Product Manufacture

Ice cream products designed for single servings are widely available, and are often purchased to be handheld items, eaten immediately after purchase. Many of these items are designed specifically for the children's market, so a vast array of shapes exist, and new introductions and variations occur frequently. As a result, this category of products is often referred to either as novelty or impulse products, and account for a larger share of the ice cream and frozen dessert market in many countries of Europe and Asia than do packaged items designed for home consumption. Examples include stick or stickless bars, cups, and cones. They can be made of many types

of frozen desserts, including ice cream with its various fat contents, frozen yogurt, sherbet, puddings, tofu, sorbet, gelatin, and fruit ices. To these are frequently added chocolate, baked items such as wafers and cakes, and numerous kinds of fruit. Recent advances in novelty manufacture equipment have greatly increased the number of products available. This equipment is usually high-speed for mass production, but at high capital cost, so production of such items is a specialty market. Strict portion control is a common attribute of modern equipment. Marketing of these items is a large factor in their success.

Novelties can be formed in any of several ways. Most novelty freezing equipment uses ice cream direct from a continuous freezer, at various draw temperatures, in order to get the appropriate consistency for the next step. Different configurations of novelty items include direct filling into a preformed single-service cup or edible cone, layering ice cream between biscuits, as in ice cream sandwiches, filling into molds and then quiescently freezing the molds, or extruding ice cream through various shapes or dyes (1).

In the molding method (Figure 154.23), unfrozen mix, such as juice or fruit ice formulations, or ice cream from the continuous freezer, usually at higher than normal draw temperature so it is not too stiff, is transferred to molds that are immersed in or sprayed with chilled brine or glycol. After the product has been partially frozen, sticks are inserted and freezing is completed in the molds. The molds then progress to a section where they are lifted from the secondary refrigerant and briefly exposed to heat (warm brine or water) to loosen the bar. An extractor there picks up the novelty by the stick and passes it to the next station. This station can be an enrober, decorator, or packaging apparatus. Individual packaged items are typically placed in bags or boxes, which may be packed in cartons. Because they typically are very hard when packaged, it is unnecessary to transfer them through a hardening tunnel before sending them to cold storage. Some flexibility with external shapes is possible, however with the use of metal molds, the mold shape must allow for the product to be extracted. Some machines are equipped with flexible molds that peel off the surface of the frozen product during extraction, allowing for more surface features. It is also possible to produce "splits," products with multiple layers from exterior to inner core, on molding machines by filling the mold with the first layer (e.g., fruit ice), allowing for partial freezing of this layer, then sucking the remaining unfrozen material from the inner core and refilling with another material (e.g., ice cream). In belt-type molding equipment, as in Figure 154.23, the molds are then cleaned prior to refilling. Mold freezing equipment is also available in a rotary table-type configuration.

The extrusion method (Figure 154.24) involves extraction of ice cream from a continuous freezer at

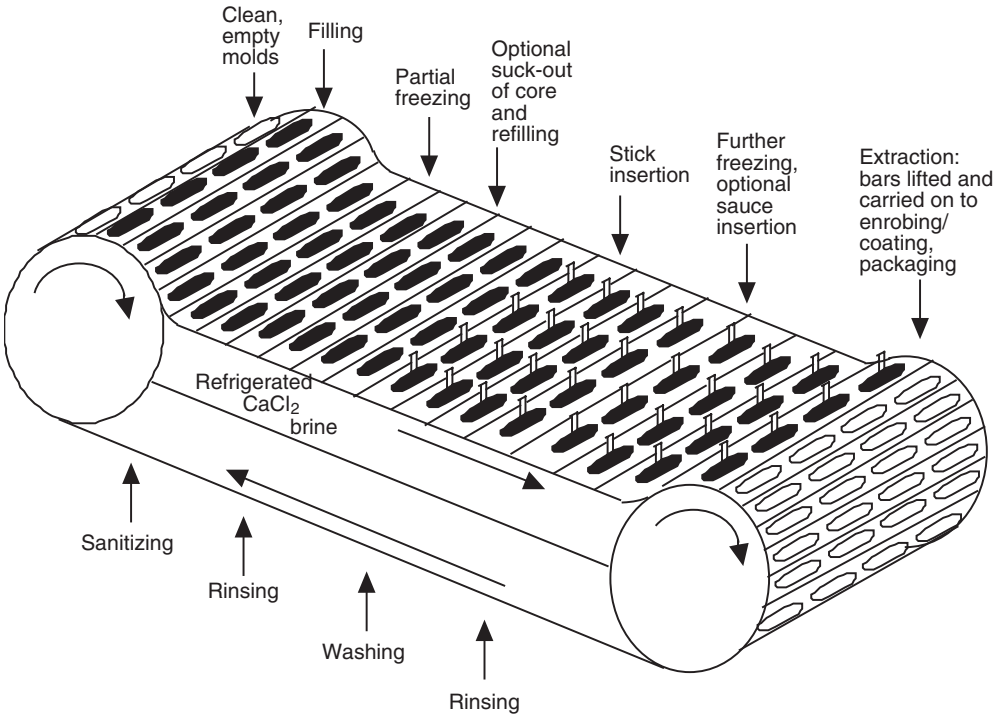

FIGURE 154.23 A schematic illustration of molded novelty freezing equipment used in the production of molded ice cream novelties.

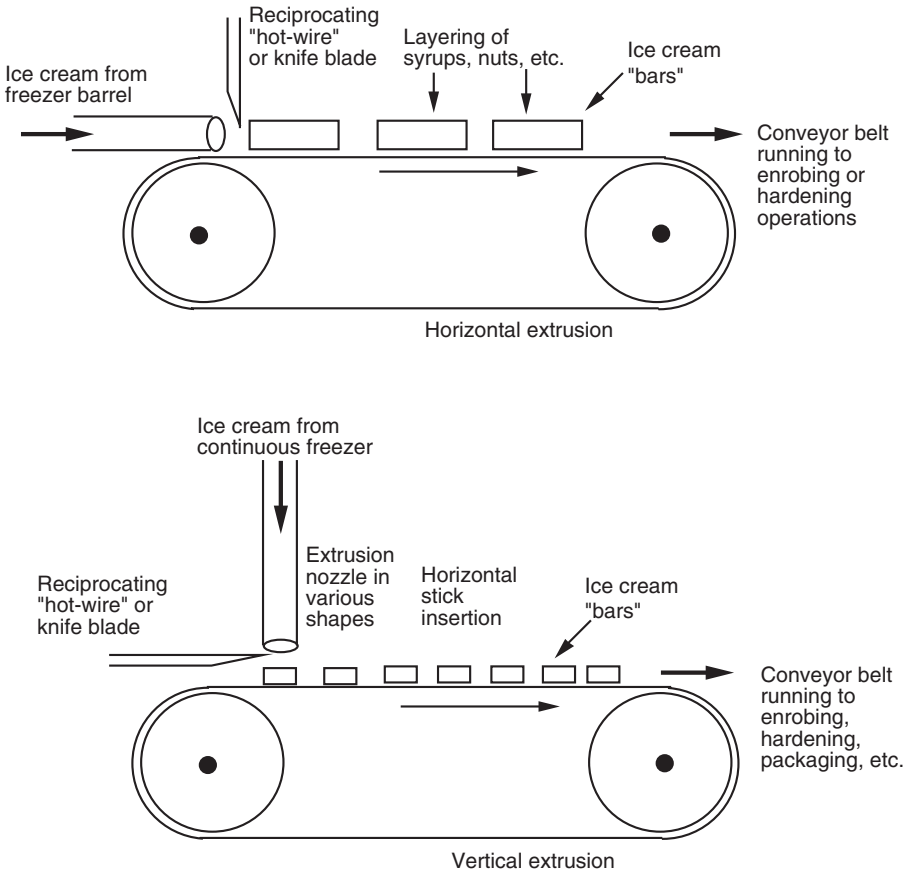

FIGURE 154.24 A schematic illustration of horizontal and vertical extrusion and continuous belt-type freezing equipment used in the production of extruded ice cream novelties.

lower-than-average draw temperatures, about −6° to −8°C. The ice cream is then pumped through an extruder nozzle and sliced into portions by an electrically heated wire cutter. The extruder may take a horizontal or vertical form (Figure 154.24). The external contour of the slice may be almost any desired shape, as is dictated by the shape of the extruder nozzle. By placing different extrusion nozzles inside each other, intricate designs can be formed. Complex extrusions in which multiple flavors or colors are extruded require the use of multiple continuous freezers. Cold-forming or pressing of the extruded item is also possible, allowing complex shapes, designs, patterns, words, etc., to be embossed into the frozen item. If a stick item is desired, the stick is inserted in the extruded ice cream. The pieces are formed on or dropped onto carrier plates and pass through a freezing chamber at −40°C, with rapid air circulation for fast freezing. Each piece is removed from the carrier plate as it emerges from the freezing chamber. Alternatively, a liquid nitrogen dip can be utilized for rapid setting of surface layers. Portions to be coated with chocolate or other coating are then transferred to an enrober, then through a chill tunnel to set the coating.

F. Storage and Distribution

Once ice cream has been frozen and hardened, it then goes through a storage and distribution system designed to get the product to the point of commercial use. This may be a retailer's freezer cabinet, and ultimately, in the case of take-home packaging, the consumer's freezer; or it may be another retail outlet like a scooping shop. Whatever the case, the steps and sequence of storage and distribution are critical to maintaining the highest possible quality of the ice cream.

Once the ice cream comes out of the hardening facility, it is typically stored in a low-temperature (−25 to −30°C) freezer within the plant itself until it is shipped to its next destination. It is difficult to generalize the series of distribution points for ice cream, since this depends on many factors, including the size of the ice cream manufacturer, the radius of distribution, and the facilities available. Some companies have their own distribution resources, including refrigerated trucks, whereas other companies must rely on contractors for distribution. In some cases, the ice cream goes first to a central warehouse; whereas in other cases the product may go directly

to retail outlets. Everington (123) shows a typical time-temperature history for distribution of ice cream.

Keeney (124) reported on a survey of ice cream manufacturers and presented some typical time scales for storage at several points in the distribution chain. The time ice cream spent in the factory before shipping varied from 1 to 4+ weeks, with 2 weeks being most common (36%). The next stage in distribution was a warehouse or distribution center, where most companies (64%) reported that the ice cream spent over 4 weeks before being shipped to the point of purchase. The majority of ice cream (68% of respondents) was purchased within 2 weeks at the retail outlet and used within 2 weeks of the consumers getting the product to their homes. However, in both the retail and consumer stages, some respondents (21%) reported that the ice cream was kept for greater than 4 weeks. Since temperatures are typically more variable in retail outlets and the consumer's freezer than in the factory or warehouse freezers, ice cream that spends a long time at warmer temperatures is more prone to becoming coarse, as the ice crystals continue to get larger by recrystallization.

Ben-Yoseph and Hartel (125) report some typical conditions and storage times at various stages in ice cream distribution, as shown in Table 154.11. These numbers were obtained from anecdotal reports from various sources, and are only meant to indicate the range of conditions that might be found (122). Ben-Yoseph and Hartel (125) used data on recrystallization of ice cream, coupled with rates of heat transfer into a half-gallon container of ice cream, to predict the increase in size of ice crystals at various locations within the container (center to surface) as it progressed through the distribution system presented in Table 154.11. Not surprisingly, the retailer's outlet and the consumer's freezer were two of the most significant sources of quality loss. However, any point of transport from one center to another is cause for concern, as temperature spikes (heat shock) due to lack of control can cause significant product damage in a short time.

TABLE 154.11
Approximate Distribution Sequence for Ice Cream (125)

Storage Site	Storage Time	Mean Air Temperature	Fluctuation[a] Amplitude
Manufacturing plant	2 weeks	−22.0°C	2.0°C
Distribution vehicle from plant	6 hours	−19.0°C	2.8°C
Central warehouse	4 weeks	−24.0°C	6.0°C
Distribution vehicle from warehouse	3 hours	−19.0°C	2.8°C
Supermarket storage	1 week	−15.6°C	2.8°C
Consumer vehicle from supermarket	0.5 hour	21.0°C	0°C
Home freezer	1 week	−12.0°C	2.8°C

[a] Approximate amplitude of temperature fluctuations.

III. PRODUCT QUALITY AND SHELF-LIFE

A. Flavor Defects

There can be numerous flavor and textural defects associated with ice cream. Excellent reviews on ice cream defects can be found in Refs. 1 and 126. Flavor defects are classified according to origin, and include those associated with the flavoring system (lacks fine flavor, lacks flavor, too high flavor, unnatural), the sweetening system (lacks sweetness, too sweet, syrup flavor), the dairy ingredients (acidic, salty, lacks freshness, old ingredient, oxidized/metallic, rancid, whey), processing (cooked), and others (absorbed from storage, stabilizer, neutralizer, foreign).

The dairy ingredients give rise to many of the common flavor defects in frozen dairy dessert products. Acid flavors may develop due to microbial growth in the dairy ingredients used in the manufacture of mix or in mix before freezing. However, off-flavor development due to microbial growth is dependent on the type of organisms present. Acidity is developed by lactic-acid organisms, but the organisms that grow at refrigerated temperatures are mostly psychrotrophs, and off-flavors associated with their growth are usually fruity and/or bitter in nature, due to peptides derived from proteolysis. Salty flavors may arise from formulations that are too high in msnf, especially if whey powder is used. Whey powder tends to be higher in natural milk salts than does skim milk powder. However, it should also be recognized that salt is often an ingredient in mix formulations, for flavor enhancement, and too much salt may have been used. Another source of high salt flavor may be salted butter, used in error rather than sweet butter.

Defects in ice cream flavor associated with the fat phase are usually related to either lipolysis of free fatty acids from triglycerides by the action of lipases (known in the dairy industry as rancidity), or autoxidation of the fat resulting in oxidized flavors (oxidative rancidity as distinct from lipolytic rancidity). These defects tend to be present in the raw ingredients used in ice cream manufacture, rather than promoted by the manufacturing process itself. However, similar precautions to the processing of milk must be taken to ensure that these flavor defects are not present.

Oxidation of milk and other fats proceeds by the well-known autoxidation reaction in three stages: initiation, propagation, and termination. In milk, the initiation reactions involve phospholipids present in the fat globule membrane. Free radicals formed from phospholipids are then able to initiate oxidation of triglycerides, especially in the presence of copper and proteins (21). During propagation, antioxidant compounds, such as tocopherols and ascorbic acid, are depleted, while peroxide derivatives of fatty acids accumulate. Peroxides, which have little flavor, undergo further reactions to form a variety of carbonyls, some of which are potent flavor compounds, especially

some ketones and aldehydes. Most methods available to monitor lipid oxidation are unsuitable as an early index of oxidized flavor development in milk: measurement of peroxides is not useful because peroxides are unstable intermediates; tests based on colorimetric reaction of thiobarbituric acid with malonaldehyde show some correlation to sensory values, but are rather insensitive; and direct measurement of oxygen uptake is only suitable for controlled experimental conditions.

Milk may oxidize as a result of either factors extrinsic or intrinsic to the milk (21,127). Important extrinsic factors include contamination with metals, temperature of storage, oxygen tension, heat treatment, agitation, light, and acidity. Both copper and iron may catalyze lipid oxidation, but probably only copper is significant in milk. Added copper is much more potent than natural copper, because a significant portion of added copper goes directly to the fat globule (21). Significant intrinsic factors affecting milk fat oxidation include metallo-proteins, such as milk peroxidase and xanthine oxidase, endogenous ascorbic acid which acts as a co-catalyst with copper to promote oxidation, endogenous copper content, and endogenous antioxidants, mainly tocopherols. Fresh forage is well known to control spontaneous oxidation, as indicated by obvious seasonal effects on the incidence of oxidized flavor. This effect is probably due to increased levels of endogenous antioxidants.

Hydrolysis of fatty acid esters by the action of lipases results in the common flavor defect known as lipolytic or hydrolytic rancidity, and is distinct from oxidative rancidity (127,128). Lipolysis in dairy fats can be extremely detrimental, due to the number of highly volatile, short chain fatty acids present, especially butyric acid. Lipases are unique among enzymes, in that they are active at the lipid-serum interface. In milk, lipases are ineffective unless the fat globule membrane is damaged or weakened in some way. Lipolysis may be caused by the lipoprotein lipase (LPL) that is endogenous to milk, or by bacterial lipases. The properties of the fat globule membrane are most important to lipolysis. Mastitis, which alters milk composition, also increases sensitivity of the fat globule to lipolysis. Other factors that destabilize the fat globule membrane, especially agitation and/or foaming, also promote lipolysis. Lipolysis is accelerated by the replacement of the native membrane with surface active material (mainly casein micelles and whey proteins) from the plasma (128). This effect is at least partly due to redistribution of LPL from the plasma to the fat globule membrane, and accounts for greatly increased lipolysis after homogenization. In the milk from some animals, lipolysis may proceed without subsequent thermal or mechanical activation. This effect, frequently referred to as spontaneous lipolysis, is unlikely to occur in herd or pooled milks, because it is prevented by the mixing of affected milk with three to five times its volume of normal milk. The major conditions that influence spontaneous lipolysis are late lactation, insufficient fresh forage, and low yielding cows.

Cooked flavors in dairy products, including ice cream mix, are caused by using milk products that have been heated to too high a temperature, or by using excessively high temperatures in mix pasteurization. The flavor is typified by scalded milk, and is caused by sulfhydral groups from denaturation of disulfide bonds in whey proteins. If it is mild, it can dissipate with time as the sulfhydral groups oxidize, so it is most often noticeable directly after heat processing. A mild cooked flavor is not objectionable, but intense heating can cause the defect to linger and become increasingly objectionable.

Ice cream can sometimes absorb off-flavors from its storage environment. Volatile compounds like smoke, ammonia, paint or diesel fumes have been known to be detectable in ice cream after inadvertent exposure to these odors. It is thus important to recognize that storage environments must be kept free of strongly volatile materials.

B. Texture Defects

Considerable effort goes into processing ice cream so that the final product has the desired consumer appeal. From a structural standpoint, this involves controlling ice crystallization, air incorporation, and fat destabilization. During storage, however, significant changes can occur to the structural elements that lead to loss of quality. Textural defects common to ice cream include recrystallization of ice crystals, lactose crystallization (sandiness), and shrinkage.

1. Recrystallization

In ice cream, numerous small crystals are desired for the smooth texture that they impart. Thermodynamically, however, this state is inherently unstable due to the very high surface area of ice crystals. In principle, this system would be in a lower energy state if the ice phase took the form of a single, very large crystal to minimize the surface area (or more correctly, the surface energy). Thus, there is a thermodynamic driving force for the small crystals in ice cream to disappear, leaving fewer and larger ice crystals. Recrystallization is seen as an increase in mean size and widening of the range of sizes (Figure 154.25), and is accompanied by a decrease in the number of crystals (96).

The driving force for this rearrangement is based on the Kelvin equation, which states that the equilibrium temperature of a crystal surface is dependent on its radius of curvature. Thus, smaller ice crystals have a slightly lower equilibrium temperature than larger crystals. In a mixture of ice crystals as found in ice cream, the small crystals are less stable than the larger ice crystals. During storage, the smaller ice crystals melt away at the same time that the larger ice crystals grow larger, as shown

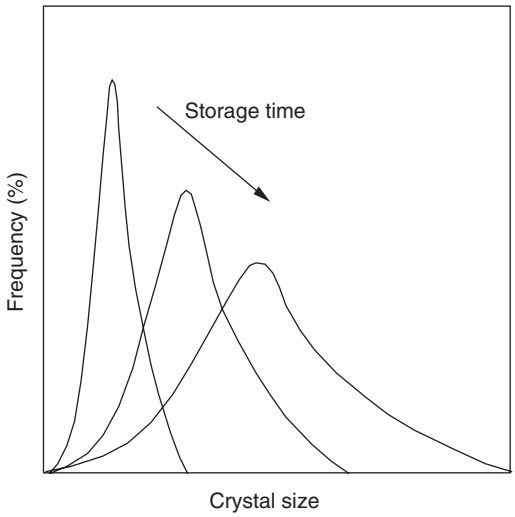

FIGURE 154.25 Typical changes in crystal size distribution during storage (96). The arrow represents a decrease in frequency of crystals found within a size range with increasing mean size.

schematically in Figure 154.26(A). This increase in size of larger ice crystals at the expense of smaller crystals is often called Ostwald ripening, or simply ripening.

However, calculations of the difference in equilibrium temperature between small and large ice crystals in ice cream show that this difference is only significant for very small crystals (10,122). The difference in driving force, expressed as a difference in equilibrium temperature, between crystals of only 1 μm in radius is less than 0.05°C. For a crystal of 10 μm radius, the temperature difference is less than 0.005°C. Thus, the driving force for Ostwald ripening of ice crystals in ice cream is very small. In fact, Donhowe and Hartel (72) did not observe true Ostwald ripening in extensive studies of mechanisms of ice recrystallization during storage of ice cream under accelerated recrystallization conditions on a microscope slide. It was found that other mechanisms were more important in ice cream. Nevertheless, it is this slight difference in equilibrium temperature between large and small crystals that, over long periods of time, can lead to significant changes in the state of ice crystals in ice cream (and other frozen foods).

The main static (constant temperature) mechanisms for recrystallization of ice crystals during storage of ice cream include accretion and isomass rounding (10). When storage temperature is constant, these two mechanisms are responsible for recrystallization of ice crystals in ice cream (72). Isomass rounding is very similar to Ostwald ripening, but is based on regions of a single crystal with different radii of curvature. A spherical ice crystal would not undergo isomass rounding, since the radius of curvature is uniform at all points of the sphere. In other words, a sphere has the minimum surface area to volume ratio. Ice crystals in ice cream are not spherical in nature (see Figure 154.5), so have a higher surface area to volume ratio. Ice crystals in ice cream are somewhat irregularly shaped, based on the mechanisms of ice formation in the freezer barrel. Thus, there is a driving force for the sharper edges (protruberances) to melt away, and for the flatter sides to grow out until the ice crystal approaches a more spherical state (Figure 154.26(B)). This process has been observed for ice crystals in ice cream held at relatively warm temperatures (−5°C) (72). In this case, the ice crystal dispersion in ice cream progressed from the initial irregular-shaped crystals

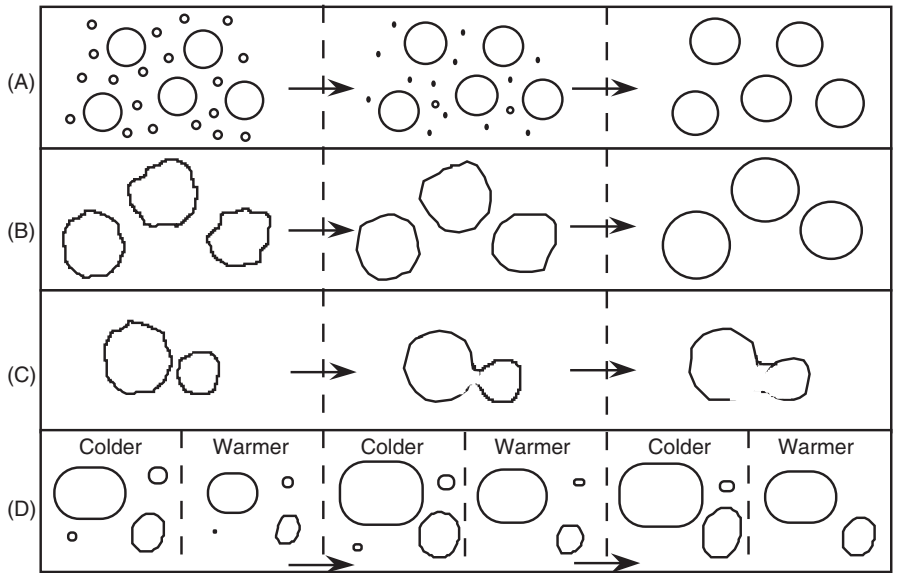

FIGURE 154.26 Mechanisms of recrystallization: (A) Ostwald ripening, (B) isomass rounding, (C) accretion, (D) melt-refreeze.

to essentially spherical crystals over time. Because the driving force for this transition is very small (the differences in size characteristics are very small), the process is relatively slow compared to other recrystallization mechanisms.

Another important mechanism of recrystallization under constant temperature conditions is accretion. It has been estimated, based on the physical number and sizes of ice crystals and air cells, that ice crystals in freshly hardened ice cream are separated, on average, by a serum film that is less than 10 μm thick (6). This close proximity leads to an instability in the region between the two crystals that leads to bridge formation, and eventually to accretion (Figure 154.26(C)). Accretion has been found to be the main mechanism of recrystallization during the initial stages when ice crystals are closely packed together. Once the crystals have become larger and more separated, the importance of accretion diminishes (72,75).

Although it is informative to understand these static mechanisms for recrystallization, ice cream is rarely (if ever) stored under conditions where temperature is constant. As documented in Section II.F, temperatures are continuously changing during storage and distribution of ice cream. Even when stored under "constant" temperatures, most refrigeration systems evoke some temperature fluctuation as compressors cycle on and off. Thus, the process of melting and refreezing is continually occurring, and this process can have a dramatic impact on the ice crystals. In fact, the melt-refreeze mechanism of recrystallization is probably the most important process leading to the change in ice crystals in ice cream during frozen storage (59,72). As temperature fluctuates in ice cream, the amount of ice (phase volume) changes accordingly. If the temperature fluctuations are relatively slow, the ice phase volume changes according to the equilibrium freezing point depression curve. This can be seen schematically in Figure 154.27 (96). When temperature increases, the amount of ice present decreases according to the freezing point depression curve. All ice crystals melt away to some extent, but the smallest crystals melt away a little faster (due to the lower equilibrium temperature) and may eventually disappear (melt away completely). Once a crystal has disappeared, it no longer returns and no new crystals nucleate (driving force is too low). The mass initially contained in that ice crystal must now be redistributed on the remaining crystals when the temperature is lowered and the ice phase volume increases. This process is seen schematically in Figure 154.26(D). The melt-refreeze mechanism is the primary mechanism for recrystallization in ice cream under conditions where temperature is changing (59,72).

The rate of recrystallization in ice cream during storage and distribution is dependent on numerous factors, including the initial state of ice crystals in the ice cream, storage temperature and fluctuations, and formulation factors (10). Extended shelf-life requires that the ice crystals are maintained as small as possible for as long as possible.

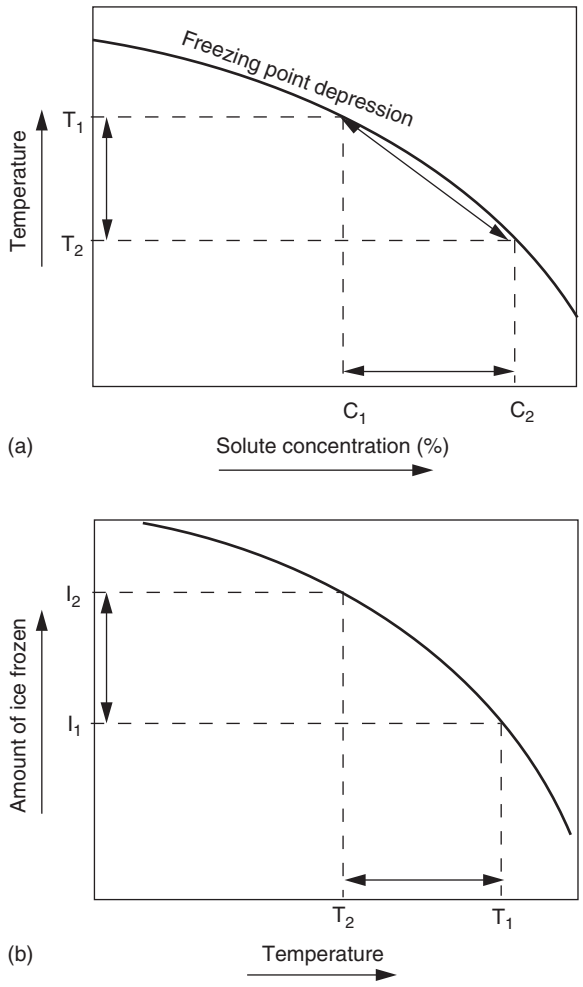

FIGURE 154.27 Effects of fluctuations in temperature (from T_1 to T_2) on a) change in the concentration of the unfrozen phase (C_1 to C_2), and b) change in amount of ice frozen (I_1 to I_2) (96).

Of the parameters that influence recrystallization, storage conditions and formulation factors are two of the most important.

The rate of recrystallization is a strong function of temperature, with the rate decreasing significantly as storage temperature decreases (59,72). Each of the mechanisms of recrystallization described above progresses more slowly as the temperature is decreased. The result is that the rate of recrystallization decreases as storage temperature decreases. In fact, if ice cream is stored below its glass transition temperature, molecular mobility will be sufficiently low, and the recrystallization rate effectively goes to zero. The glass transition temperature of ice cream is about –32°C (85,105). However, the rate of recrystallization typically is quite low if storage temperature is maintained below about –20°C (72). The extent of temperature fluctuations also influences the rate of recrystallization through the effect on the melt-refreeze mechanism. Based on Figure 154.26, the effect of temperature fluctuations depends on the storage

temperature since the change in ice phase volume with a given change in temperature decreases as temperature decreases (72). Thus, storage at –20.0 ± 2.0°C has much less effect on recrystallization than storage at –8.0 ± 1.0°C. A heat shock index can be used to quantify this effect (129).

Since the temperature changes during the various stages of storage and distribution, the rate of recrystallization changes during storage according to the local temperature and fluctuations. Furthermore, different points within a single package experience different thermal conditions and undergo recrystallization at different rates. Donhowe and Hartel (73) showed that ice crystals at the center of a half-gallon container of ice cream remained the smallest, whereas ice crystals near the package surface experienced the greatest rate of recrystallization. The thermal insulating capacity of ice cream, in effect, protects the interior of ice cream from external temperature fluctuations. Ben-Yoseph and Hartel (125) used typical temperatures and times in different stages of distribution of ice cream, and the rates of heat transfer into a package, to predict the ice crystal size at any point in a container of ice cream based on the recrystallization kinetics of Donhowe and Hartel (72). The effects of storage temperatures on ice crystal size at different points in the distribution system were clearly demonstrated.

Of the formulation factors that influence recrystallization, stabilizer and sweetener types are the two most important. In fact, stabilizers are added to ice cream primarily to control recrystallization during storage. However, it is still not clear exactly how stabilizers affect recrystallization (see Section I.B.4). Several potential mechanisms have been hypothesized for the effect of stabilizers on recrystallization (10). These include (1) an increase in viscosity of the unfrozen phase, (2) specific inhibition of ice crystal growth rates, (3) physical obstruction due to formation of a weak gel structure (58,71), (4) a change in thermal properties of ice cream due to addition of stabilizer (82), and (5) a decreased perception of iciness due to addition of stabilizers (81). It is possible that each of these potential mechanisms plays a role in the effect of stabilizers on recrystallization. However, further work is needed to verify exactly how stabilizers act to inhibit ice recrystallization during storage of ice cream.

The type of sweetener used in the mix formulation has also been found to influence the rate of recrystallization during storage of ice cream (74,84). The effect of sweetener type, however, is primarily related to the amount of water frozen into ice at any temperature. Hagiwara and Hartel (74) correlated recrystallization rate during storage of ice cream with the calculated amount of water frozen into ice for ice creams made with different sweeteners. Recrystallization rate decreased proportionally as the amount of water frozen into ice increased. Since the amount of water frozen at any temperature is directly related to freezing point, recrystallization rate also was seen to decrease as the freezing point temperature increased. Since recrystallization is a diffusion-limited process (based on migration of water molecules), more ice at a given temperature (and less water) leads to slower recrystallization, due to the lower mobility of the water molecules. The lower mobility correlates with an increase in glass transition temperature of the ice cream (74).

2. Lactose Crystallization

The problem of "sandiness" in some ice creams during storage has been related to crystallization of lactose from the milk solids in the formulation (1,130). It is not only that lactose crystals appear in ice cream during storage, but that these lactose crystals must grow to sufficient size that they can be detected by the palate and distinguished from ice crystals (131). Based on various sources, it has been estimated that the critical size for lactose crystals in ice cream is about 15 μm. Above this size, their presence can be detected as a sandy or grainy characteristic that is different from the coarse texture associated with large ice crystals. When present in ice cream, lactose crystals dissolve at a much slower rate than ice crystals melt. Thus, the lactose crystals remain in the mouth even after the ice cream has melted; hence, the sandy mouthfeel.

Lactose in ice cream crystallizes when the concentration in the serum phase (unfrozen concentrate) exceeds the solubility concentration of lactose. Since the solubility of lactose is very low (and decreases as temperature goes down), lactose is supersaturated and prone to crystallize at almost any level in ice cream stored at common freezer temperatures. In fact, thermodynamically, lactose should crystallize in just about all ice cream, since it is in the supersaturated state at storage temperatures. The fact that lactose does not crystallize in all ice cream during storage may be attributed to the slow kinetics of lactose nuclei formation at these conditions. The viscosity of the unfrozen phase is sufficiently high that lactose nucleation is inhibited for extended periods of time (and may not occur within the shelf-life of an ice cream product).

Thus, two competitive forces are at work that govern crystallization of lactose in ice cream. The first is the increase in concentration driving force as temperature is decreased, which tends to promote lactose crystallization at lower temperatures. Working against this, however, is the decrease in molecular mobility as the temperature is decreased. Thus, there is a storage temperature where lactose crystallization is at a maximum. For a wide range of commercial ice creams, this temperature occurs at about −10 to −12°C (130,132,133). Storage in this temperature range leads to the most rapid lactose crystallization in ice cream. Storage at both higher and lower temperatures requires longer times for onset of lactose nuclei formation (132).

Of the formulation factors responsible for lactose crystallization, the initial milk solids level in the mix is probably the most important. An upper limit of 15.6 to 18.5% msnf has been suggested to prevent lactose crystallization, with the higher limit for products that move

quickly through the distribution chain (1). The presence of sucrose and stabilizers may have an inhibitory effect on lactose crystallization, perhaps through their effect on viscosity of the unfrozen phase during storage. However, addition of powdered or particulate ingredients (e.g., nuts) after initial freezing tends to promote lactose crystallization through two potential mechanisms. Any particulate material added may act as nucleation sites for lactose and promote graining, and it is widely recognized that agitation of a supersaturated sugar solution enhances the likelihood of nucleation (134).

3. Shrinkage

In some situations, ice cream that has been improperly handled exhibits shrinkage, where the ice cream pulls away from the walls of the container. Many parameters have been implicated in the mechanism of shrinkage, including formulation factors like improper use of proteins, emulsifiers and stabilizers, and external factors like atmospheric pressure (49). Shrinkage results from a loss of discrete air bubbles as they coalesce and begin to form continuous channels, eventually leading to collapse of the product itself into the channels (48). Shrinkage tends to occur most often after the ice cream experiences a decrease in pressure, as when ice cream is shipped across mountains or transported by plane, which first causes a volume expansion followed by collapse. The extent of air channeling, and hence a measure of ice cream susceptibility to collapse and shrinkage, can be measured by determining the response in volume of the ice cream to pressure changes, given that the volume of discrete bubbles will correlate directly to pressure changes, while the volume of air channels will not (135).

According to the ideal gas law, the size (volume) of an air bubble is related to the external temperature and pressure, assuming the volume is free to change. As temperature is decreased, at constant pressure, the volume of an air bubble will decrease. As pressure is increased, at constant temperature, the air bubble should also contract. For example, when ice cream exits the draw of a continuous freezer, pressure is reduced (pressure within the freezer is higher than atmospheric pressure), and all of the air bubbles should expand slightly. At this point, though, the viscosity of the ice cream is sufficiently low that this expansion can easily be accommodated by the surrounding matrix, and the air bubbles approach an equilibrium at atmospheric pressure. Cartons of ice cream are filled to their final weight and volume at this point, and any changes in volume during later storage and distribution may lead to negative changes in the ice cream appearance. After hardening, when the surrounding matrix has stiffened considerably, subsequent changes in pressure (or temperature) can lead to changes in the forces between the air cells and the surrounding matrix. Expansion or shrinkage, depending on the conditions, may be the result.

Goff et al. (136) reported on the effects of vacuum storage on expansion and shrinkage of ice cream. Containers of ice cream at –16°C were exposed to reduced pressure (8 in Hg) for 3 hours and then stored for 6 days at –16°C. Volume changes were measured 3 hours after release of vacuum, and again at the end of 6 days of storage. Expansion of the ice cream was observed after the vacuum storage, in accordance with the ideal gas law. However, after 6 days of storage those same ice creams exhibited shrinkage. In all cases, ice creams made with higher overrun had the greatest expansion and subsequent contraction. At –16°C, the unfrozen matrix must still be sufficiently pliable that a change in atmospheric pressure can cause a change in volume of the ice cream. Interestingly, although the period of vacuum exposure caused expansion, the ultimate result when pressure was brought back to atmospheric was shrinkage of the ice cream volume. This suggests that the unfrozen matrix expanded with the increased air bubble size initially, and then relaxed to a smaller volume than originally found. Goff et al. (135) related this to the nature of the interface between the air bubble and the unfrozen serum. They suggested that components like proteins, stabilizers, and emulsifiers play an important role in determining the viscoelasticity of this interface and subsequent changes in ice cream volume during pressure or vacuum storage.

IV. CONCLUSIONS

Ice cream is one of the most complex food products, since it contains multiple phases (ice crystal dispersion, foam, emulsion, viscous unfrozen matrix, and potentially, a weak gel system and a glass). Formation of the different phases is controlled during freezing, but the process of forming one phase generally influences the formation of the other phases. Thus, manufacturing of ice cream requires careful control of both ingredient formulation and processing conditions. Since ice cream and related products are some of the few food products consumed in the semi-frozen state, the freezing process is most important to ultimate smooth texture. As ice cream readily undergoes ice recrystallization, especially during periods of temperature fluctuation, precise control of frozen storage and distribution conditions is also critical for the preservation of optimal textural quality. For all these reasons, ice cream type products present processing, storage, and distribution characteristics that are unique amongst the frozen foods.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Ice cream and frozen desserts," by H. D Goff and R. W. Hartel, in *Handbook of Frozen Foods*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.

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155 Edible Fats and Oils Processing and Applications

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CONTENTS

I. INTRODUCTION

Fats and oils have been recovered for thousands of years from oil bearing seeds, nuts, beans, fruits, and animal tissues. These raw materials serve a vital function in the United States and world economics for both food and nonfood applications. Edible fats and oils are the raw materials for oils, shortenings, margarines, and other specialty or tailored products that are functional ingredients in food products prepared by food processors, restaurants, and in the home. The major nonfood product uses for fats and oils are soaps, detergents, paints, varnish, animal feeds, resins, plastics, lubricants, fatty acids, and other inedible products. Interestingly, many of the raw materials for industrial purposes are by-products of fats and oils processing for food products; however, some oils are produced exclusively for technical uses due to their special compositions. Castor, linseed, tall, and tung oils are all of vegetable origin and are produced for industrial uses only. The USDA Economic Research Service statistics indicate that, of the 27.472 billion pounds of edible fats and oils used in the year 2000, 76.6% was for food products and 23.4% was for nonfood products [16].

Fats and oils occur naturally in a wide range of sources and each source provides a separate and distinctive material. There are hundreds of oil bearing seeds and fruits, all animals produce fat, and marine sources provide oils; however, only a few are of economic importance. All edible fats and oils are water insoluble substances which consist predominantly of glyceryl esters of fatty acids, or triglycerides, with some nonglyceridic materials in small or trace quantities. The terms "fats" and "oils" are used interchangeably and the choice of terms is usually based on the physical state of the material at ambient temperature and tradition. Generally, fats appear solid at ambient temperatures and oils appear liquid. In the final analysis, it is the chemical composition that defines the characteristics of the individual fat or oil, which in turn determines the suitability of this ingredient for various processes and applications.

II. FATS AND OILS CHARACTERIZATION

Both the chemical and physical properties of fats and oils are largely determined by the fatty acids that they contain and their position within the triacylglycerol molecule. Chemically, all fats and oils are esters of glycerin and fatty acids. Nevertheless, the physical properties of natural fats and oils vary widely. This is because (i) the proportion of the fatty acids vary over wide ranges, and (ii) the triacylglycerol structures vary for each individual oil and fat. Fats and oils are commonly referred to as triglycerides because the glycerin molecule has three hydroxyl groups where a fatty acid can be attached. All triglycerides have the same glycerin unit, so it is the fatty acids which contribute the different properties. The fatty acid components are distinguished in three ways: (i) chain length, (ii) the number and position of the double bonds, and (iii) the position of the fatty acids within the glyceride molecule. Variations in these characteristics are responsible for the chemical and physical differences experienced with edible fats and oils.

The structure of a fatty acid is commonly denoted by a systematic name after the nomenclature of its parent hydrocarbon, by its common name, or by a convenient shorthand designation showing the number of carbon atoms and the number of double bonds. The fatty acids carbon chain lengths vary between 4 and 24 carbon atoms with up to three double bonds. The most prevalent saturated fatty acids are lauric (C-12:0), myristic (C-14:0), palmitic (C-16:0), stearic (C-18:0), arachidic (C-20:0), behenic (C-22:0), and lignoceric (C-24:0). The most important monounsaturated fatty acids are oleic (C-18:1) and erucic (C-22:1). The essential polyunsaturated fatty acids are linoleic (C-18:2) and linolenic (C-18:3).

The triglyceride structure of an edible fat or oil is affected by the fatty acids present and the point of attachment of each fatty acid to the glycerin. Triglycerides with three identical fatty acids are called monoacid triglycerides. Triglycerides containing more than one type of fatty acid are called mixed triglycerides. A mixed triglyceride containing three different fatty acids has three regioisomeric forms and six stereo-isomeric forms, depending on which fatty acid is in the middle, sn-2, or beta position of the glycerol portion of the molecule and which fatty acids are in the alpha or outer positions (sn-1 and sn-3). The distribution of the fatty acids is considered to be nonrandom when the saturated fatty acids are positioned predominately in the sn-1 and/or sn-3 positions and the unsaturated fatty acids are positioned predominately in the sn-2 position [17].

The fatty acid compositions of natural fats and oils vary significantly depending not only on the plant or animal species but also within the same species. Among the factors that affect the vegetable oil fatty acid compositions are climate conditions, soil type, growing season, plant maturity, plant health, microbiological, seed location within the flower, and the genetic variation of the plant. Animal fats and oils composition vary according to the animal species, diet, health, fat location on the carcass and maturity [12].

A. Edible Fats and Oils, Nonglyceride Components

The primary constituents of extracted fats and oils are triglycerides but they also contain varying amounts of nonglyceride materials. Some of the nonglyceride components are undesirable, and can be considered a food safety hazard, while others are very beneficial. Therefore, the objective in all of fats and oils processing is to remove the

objectionable materials with the least possible damage to the desirable constituents. In most cases, free fatty acids, phospholipids, moisture, color pigments, oxidation products, waxes, trace metals, proteins, pesticides, meal, dirt, and other gross impurities are the materials that need to be removed. Most vegetable oils contain tocopherols, which are natural antioxidants that protect the oils from oxidation and should be retained. For some products, neither the color pigments nor waxes are detrimental and need not be removed. The major product quality concerns are with free fatty acids, phospholipids, oxidation products, proteins, and trace metals; all materials that affect the odor, flavor, and flavor stability of edible fats and oils products. In the U.S., fats and oils color is usually a major concern from a cosmetic sense to include pigments adsorption as a major impurities concern especially for products marketed directly to consumers. The major food safety concerns are with residual pesticides, mold, bacteria, and impurities developed during processing or with mishandling.

III. SOURCES OF FATS AND OILS

Humans have survived as hunters and gatherers for a majority of their known existence on earth. It was only during the last 10,000 years that they learned to domesticate plants and animals. During this period, the evolution of cultivated plants has been shaped to the needs of modern man. Today's agricultural crops are mankind's creation. Humans cannot survive without them, nor can the crops that have been developed survive without human care. The combined largest source of vegetable oils are the seeds of annual plants grown in relatively temperate climates. Most of these annual plants are cultivated not only as a source of oil, but are also utilized as protein-rich foods.

A second source of vegetable oil are oil-bearing trees. Olive, coconut, and palm oils are extracted from the fruit pulp rather than the seed of the fruit. Palm also has seeds, which provide palm kernel oil. All of the oil-bearing tree fruits require a relatively warm climate; i.e., tropical for coconut and palm and a warm climate for olive trees. Most of the oil-bearing tree fruits and kernels provide the highest oil yields. Oilseeds are annual plants which must be replanted each year, whereas the fruit oils are harvested from trees with long life spans. Olive trees are the most hardy and can live several hundred years. Coconut trees start to bear fruit after 5 to 6 years; their life expectancy is as long as 60 years. Palm trees start to bear fruit after 4 to 5 years and continue for another 20 years.

Edible meat fats are supplied almost entirely by three kinds of domesticated animals, i.e., lard from pigs, tallow from cattle and sheep, and milk fat or butter from cows. These animals are raised in the greatest quantities, where they thrive the best, in temperate climates. Animal husbandry has evolved to the stage that these domestic animals not only require a temperate climate but also intensive agriculture to provide a plentiful supply of foodstuffs to produce the desired quality and quantity.

IV. GENETICALLY MODIFIED VEGETABLE OILS

Plant breeding to modify the genetics of crops has been practiced for centuries. Historically, plant breeders have used crossing and selection techniques to enhance yields, oil contents, climate adaptation, and to effect changes in oil quality, composition, and resistance to pests or pesticides. Introduction of high-oleic safflower in 1964 and low erucic acid rapeseed oil, which became known as canola oil in 1978, are examples of successful fatty acid composition modifications using this technology. Mutagenesis, another plant breeding technique, where the seed is treated with a chemical or gamma-radiation to alter its physiological functions, was utilized by the Russians to develop high-oleic sunflower oil. These traditional tools used by plant breeders have been combined with biotechnology to broaden their capabilities.

The traditional breeding methods cause thousands of genes to be transferred at each cross, whereas molecular genetic engineering can now transfer or alter a single gene. Genetic engineering can also transfer a gene from one species to another, which is impossible with the traditional methods. The genetic modification of oilseeds by conventional breeding techniques, combined with molecular genetic transformations, provide a much broader array of possibilities to improve food products. One of the first modified oil compositions produced commercially with this process was high-laurate canola oil. High-laurate canola was engineered by inserting a single gene from the California bay laurel tree that provided a substantial quantity of lauric fatty acid (C-12:0) in the oil [5]. This genetic engineering feat proved that a gene from one plant could be transferred to another to produce an oil with specific fatty acid groups in selective positions for either performance or nutritional effects. Agronomically, high-laurate canola was a total success but it failed in the marketplace. Two reasons were suggested for its failure. First, potential customers for genetically modified products were reluctant to commit because of consumer opposition to genetically modified crops. Second, the specialty oil was marketed at a premium price, which was twice that of most other oils [1].

Genetic varieties have been developed to modify the oilseed's fatty acid profile to create new value-added oils. Regardless of the oilseed variety, most of these efforts have followed the same directions: (i) low-saturates for dietary needs; (ii) low-linoleic for flavor stability; (iii) high-oleic for health and oxidative stability; and (iv) high-saturates to replace hydrogenation. Currently, most of these modified varieties have captured very little market share or have never been commercialized. The major

reason these improved oils have not found acceptance is the high cost. Some of the key factors which drive up the costs for these modified oils are [9]:

- Lack of competitive field yields—most modified oilseeds provide only about 85 to 95% of the yield potential of the regular oilseed variety. Farmers require a high premium to grow these lower yielding varieties.
- Identity preservation systems—separate handling systems are required at every stage from seed handling, planting, growing, harvesting, transportation, storage, extraction, and final processing.
- Low trait stability—environmental effects have caused inconsistent oil compositions in modified oilseed products.

Currently, the commercially available genetically modified oilseed crops are primarily varieties with improved agronomic traits, such as herbicide tolerance and pest resistance. In the U.S. these bioengineered herbicide-tolerant or pest resistant soybeans, cotton, canola, corn, and sunflowers have the same oil and protein compositions as their traditional counterparts, and do not require post-harvest segregated handling. Global acreage devoted to growing these genetically modified (GM) crops continues to rise. The United States produces 68% of the GM crops worldwide; Argentina, 22.5%; Canada, 6.1%; and China, 2.9%. Estimates, by the USDA National Agricultural Statistics Service, indicate that 74% of the soybean crop, 32% of the corn acreage, and 71% of the cotton planted in the United States in 2002 were GM hybrids [2].

V. PROCESSING FLOW SEQUENCE

The fats and oils, extracted from the oilseeds, nuts, beans, fruits and animal tissues, vary from pleasant smelling products that contain few impurities to very offensive smelling, highly impure materials. Fortunately, researchers have developed technologies for processing the fats and oils products to make them more suitable for foods and other applications. Developments in lipid processing technology have produced ingredients that have been instrumental in the development of many of the current food products available that provide the functional and nutritional requirements of discerning and better informed consumers. Processes have been developed to make them flavorless and odorless and lighter in color, modify the melting behavior, rearrange the molecular structure, remove potential disease causing impurities, capture possible harmful materials, and provide other changes to make them more desirable for the intended application.

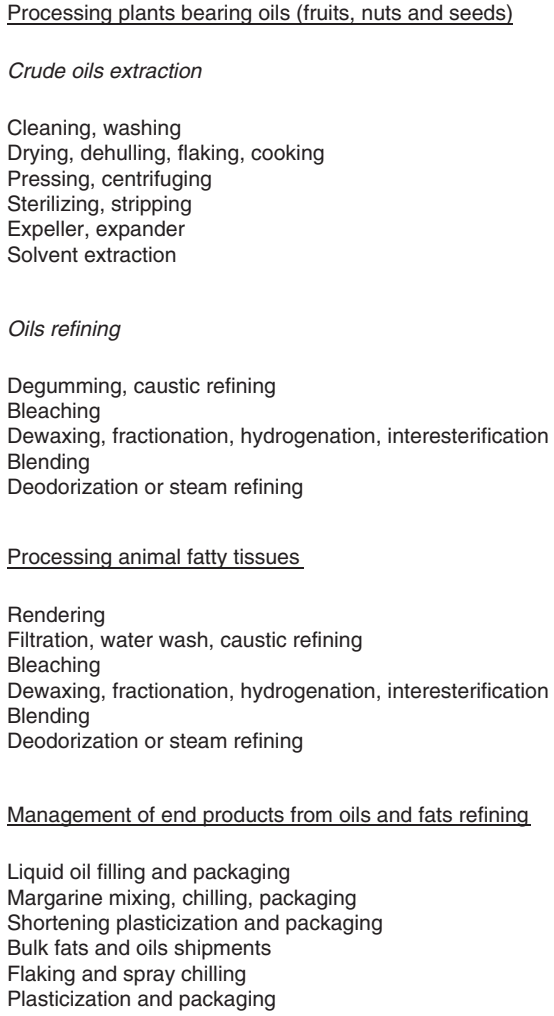

FIGURE 155.1 General steps in processing oils and fats from plant and animal products.

Edible fats and oils processing involves a series of processes in which both physical and chemical changes are made to the raw material. Figure 155.1 illustrates most of the potential processing flow sequencing to produce the various fats and oils products. Processing of fats and oils is initiated by an extraction or rendering process to remove the fat or oil from the seed, bean, nut, fruit, or fatty tissues. Vegetable oil's processing after extraction almost always includes neutralization or refining, bleaching, and deodorization with the major differences being the choice of equipment and techniques utilized. Rendered animal fats are normally clarified to remove impurities, bleached and deodorized, again with differences in equipment and techniques providing the major differences. Clairification, neutralization, bleaching and deodorization are all purification processes which affect the flavor, flavor stability and appearance of the fat or oil product while removing harmful impurities. A review of the major fats and oils processes follows.

A. Extraction

Cleaning is the first step in the processing of vegetable oils. Typically, oilseeds contain stems, pods, leaves, broken grain, dirt, sand, small stones, and other extraneous seeds. These foreign materials reduce the oil content, adversely effect oil quality and increase the wear and damage potential to the extraction equipment. Shaker screens are used to separate the particles on the basis of size, whereas aspiration separates on the basis of density and buoyancy in a stream of air. Tramp iron, extraneous metal acquired during harvesting, storage or transportation is removed to prevent damage to the equipment by the placement of magnets in chutes just ahead of vulnerable processing equipment.

Extraction of oil from materials of plant origin is usually done by pressing with the use of a continuous screw press or by extraction with volatile solvents. Prior to 1940, mechanical pressing was the primary method used. Mechanical pressing had limits because the oil recovery is poorer than with solvent extraction and the high temperatures generated damaged both the oil and the meal. The solvent method allows a more complete oil extraction at lower temperatures. Solvent extraction plants can be either batch or continuous. The continuous extraction plants can be percolation, immersion or direct extraction plants. Generally, the oilseeds may be divided by oil content; above and below 20% oil content. In most cases, oilseeds with a low oil content are subjected to both continuous and batch solvent extraction. High oil content seeds are normally extracted in two stages; first pressing and then solvent extraction; however, many single step continuous direct solvent extraction systems are in current use.

To be used legally in the United States, oilseed extraction solvents and food processing substances must have been subjected to an approval by the U.S. Food and Drug Administration (FDA) or the U.S. Department of Agriculture (USDA), be generally recognized as safe (GRAS) for this use, or be used in accordance with food additives regulations promulgated by the U.S. FDA. Commercial hexane has been in major use since the 1940s as an oilseed extraction solvent on the determination that it is GRAS, and it may also be subject to a prior sanction. Like many other food-processing substances, there is no U.S. FDA regulation specifically listing n-hexane as GRAS or having prior sanction. However, it has been cleared as a solvent in a number of other food products, one of them a cocoa butter substitute with a 5 ppm maximum limit. Because edible fats and oils are subjected to deodorization and other purification processes as a part of the manufacturing process before being used as a food product, they should not contain any of the extraction solvent, if proper practices are followed [19].

B. Rendering

The fatty tissue from meat animals which is not a part of the carcass or that trimmed from the carcass in preparation for sale is the raw material from which lard and tallow are obtained. Separation of fat from the fatty tissues of animals is called rendering. The rendering process consists of two basic steps. First, the meat by-product is heated to evaporate the moisture, melt the fat present and condition the animal fibrous tissue. Two alternative cooking temperatures are used: fat temperatures below 120°F and fat temperatures above 180°F. A more complete separation of the fat and protein is accomplished with the higher temperature processing but a better quality protein is obtained with the lower temperature processing. Normally, the value of the protein dictates that the lower temperature poorer separation technique be used which probably leaves trace quantities of protein in the rendered lard or tallow. After cooking, the fat is separated from the solid proteinaceous material. In batch rendering the cooked material is allowed to separate and the fat to drain followed by filtration to complete the separation. Continuous rendering, introduced to replace the batch systems, normally consists of a continuous cooker which requires less cooking time and is more energy efficient with better quality control [11].

C. Refining Systems

Processors have the option of approaching edible oil purification in two ways; either chemical or physical refining. The two systems utilize very similar processes with the major difference being the method used for free fatty acid removal. Chemical refining, the conventional method used for removal of the nonglyceride impurities from edible fats and oils, consists of optional degumming, caustic neutralization, bleaching and deodorization. The alkali refining process produces good quality oil and is flexible with the ability to treat different oils and different qualities of individual oils. However, caustic refining has three major drawbacks: (1) the soap produced promotes a tendency for emulsion formation which will occlude neutral oil to increase oil losses; (2) oil losses are particularly high when processing oils with free fatty acids over 3.0%; and, (3) disposal of the soapstock produced has become more difficult.

The second process, which has become known as physical refining, consists of removing the fatty acids from the oil by steam distillation under vacuum after the phosphatides have been removed by a degumming process followed by a pretreatment process before bleaching. The major advantages for physical refining are the elimination of soapstock, lower capital costs and fewer processes to operate and maintain. The objective of the initial processing step in either refining method is the

removal of phosphatides, color bodies, and trace metals. Removal of these non-triglyceride impurities is crucial to ensure good product quality. Herein lies the major drawback for the physical refining system; i.e., complete phosphatide removal with degumming and bleaching is very difficult. Some of the other problems with physical refining systems can be: (1) additional bleaching earth is usually required; (2) pesticides are co-distilled with the fatty acids during steam refining; (3) phosphoric acid treatment may darken the gums produced and incomplete removal can produce off-flavors in the oil after deodorization; (4) steam distillation or deodorizer units must be designed to handle higher concentrations of free fatty acids; (5) cottonseed oil cannot be physically refined because the gossypol pigment must be removed with alkali refining; and, (6) it may be necessary to steam refine before hydrogenation or other processing to adjust melting characteristics followed by a second deodorization step. Physical refining is favored for processing high free acidity oils with low phosphatide contents; it has been demonstrated to produce good quality product from coconut, palm kernel, palm, lard, tallow, and some of the seed oils [20].

D. Degumming

Degumming is the treatment of crude vegetable oils with water, salt solutions. or dilute acids such as phosphoric, citric, or maleic to remove phosphatides, waxes, and other impurities. Degumming converts the phosphatides to hydrated gums, which are insoluble in oil for separation as a sludge by settling, filtering, or centrifugal action. Phosphatide removal is the first process for the physical refining system, and can also be used in chemical refining. However, with chemical refining the processor has the option of removing the phosphatides for their by-product value as lecithin or treating them as impurities to be removed along with free fatty acids during caustic neutralization.

E. Caustic Neutralization

The conventional caustic neutralization process is the most widely used and most well known purification system. The addition of an alkali solution to a crude oil brings about a number of chemical and physical reactions: (1) the alkali combines with the free fatty acid present to form soaps; (2) the phosphatides absorb alkali and are coagulated through hydration; (3) pigments are degraded, absorbed by the gums, or made water soluble by the alkali; and, (4) the insoluble matter is entrained with the other coagulable material. Efficient separation of the soapstock from the neutralized oil is a significant factor in caustic neutralization which is usually accomplished with centrifugal separators. The conventional caustic soda neutralization systems have the flexibility to efficiently refine all of the crude oils presently utilized for food products [8].

Caustic neutralization is ordinarily accomplished by treating the fat or oil with diluted sodium hydroxide. This treatment forms soapstock with the free fatty acids, phosphatides, trace metals, pigments, and other nonglyceride impurities that can be separated by settling or centrifugal force from the neutralized oil. The neutral oil is usually water washed and again separated by settling or centrifuged to remove trace impurities and residual soaps from the neutralization and separation processes. After water washing, the oil is either dried with a vacuum dryer or immediately bleached to remove the trace quantities of remaining water.

F. Bleaching

Edible fats and oils bleaching is popularly and correctly regarded as the partial or complete removal of color; however, bleaching is also an integral process in both the chemical and physical refining systems. Bleaching is relied upon to clean up the traces of soap and phosphatides remaining after caustic neutralization and water washing for the chemical refining system. For physical refining, the technical feasibility depends upon bleaching as a pretreatment to remove phosphatides, trace metals, waxes, and the color pigments. Another, very important function of bleaching in both refining systems, is the removal of peroxides and secondary oxidation products.

The usual method of bleaching is by adsorption of the pigments and other nonglyceride impurities on bleaching earth. In a typical process, the bleaching materials are added to the oil in an agitated vessel, either at atmospheric pressure or under a vacuum. The oil is heated to bleaching temperature and held to allow contact time with the bleaching earth. After the adsorbent has captured the color pigments, soap, phosphatides, trace metals, and polar materials, it becomes an impurity which must be removed from the oil with a filtration system. Control point impurities analyses are used to monitor the removal of the potential food safety hazard.

G. Animal Fat Purification Systems

Traditionally, the method used to purify meat fats has been a form of physical refining. The two main impurities in meat fats are proteins carried over from the rendering process and free fatty acids. The pretreatment phase for meat fats is the removal of the proteinaceous materials. Typically, this is easily accomplished by adding small amounts of diatomaceous earth and/or bleaching earth followed by filtration. An alternative clairification or pretreatment method is to water wash the fat to remove the proteins. This method also requires bleaching or at least drying to remove the moisture remaining in the oil after water washing. A third method for meat fat clarification is caustic refining. Chemical refining is usually reserved for poor quality animal fats or for specialty products used undeodorized to preserve the characteristic meat fat

flavor. The caustic refining system consists of caustic neutralization, water washing and vacuum drying.

H. Hydrogenation

The hydrogenation process is an important tool for the edible fats and oils processor. With hydrogenation, liquid oils can be converted into plastic or hard fats more suitable for a particular food product. There are two reasons to hydrogenate a fat or oil; (1) to change the physical form for product functionality improvement, and (2) to improve oxidative stability. Hydrogenation involves the chemical addition of hydrogen to the double bonds in the unsaturated fatty acids. The reaction is carried out by mixing heated oil and hydrogen gas in the presence of a catalyst. After the hydrogenation end point has been achieved, the hardened oil is cooled and filtered to remove the nickel catalyst.

Most hydrogenations are performed in batch reactors due to the variation in raw materials and the desired end products. Normally, batch hydrogenation is performed in an agitated tank reactor with heating and cooling capabilities designed to withstand pressures of 7 to 10 bar. First, the catalyst is suspended in the oil. Then, hydrogen gas, dispersed as bubbles, must be dissolved in the oil to reach the surface of the catalyst. The three reaction variables, pressure, temperature, and rate of agitation, are controlled to reduce batch-to-batch variation for preparation of the desired hydrogenated product or basestock. The typical analytical evaluations for endpoint control which measure consistency are refractive index, iodine value, and various melting points. A food safety control point would be the incomplete removal of the nickel catalyst after the reaction is completed; however, this is not a critical control point because the post bleaching process immediately following hydrogenation is designed to remove the remaining trace catalyst impurities.

I. Post Bleaching

A separate bleaching operation, immediately following the hydrogenation process, has three purposes: (1) insurance that all traces of the prooxidant hydrogenation catalyst that have escaped the filtration system after hydrogenation have been removed; (2) to remove undesirable colors generally of a greenish hue, accentuated during hydrogenation by heat bleaching of the red and yellow pigments; and, (3) removal of peroxide and secondary oxidation products. Post bleach systems are usually batch systems for the same reasons as for hydrogenation systems; production of a wide variety of hydrogenated basestocks.

J. Fractionation

Edible fats and oils are fractionated to provide new materials more useful than the natural product. Fractionation may be practiced to remove an undesirable component, which is the case with dewaxing and winterization, or to provide two or more functional products from the same original fat or oil, as is the case with cocoa butter equivalents or substitutes and high stability oils.

The three fractionation process types practiced commercially to produce the value-added products are: (1) dry fractionation; (2) solvent fractionation; and, (3) aqueous detergent fractionation. Dry fractionation, which includes winterization, dewaxing, hydraulic pressing, and crystal fractionation processes, is probably the most widely practiced. Solvent or aqueous detergent fractionation processes provide better separation of specific fractions for the more sophisticated fats and oils products. All of these fractionation processes practice the three successive stages of fractionation: (1) cooling the oil to supersaturation to form the nuclei for crystallization; (2) progressive growth of the crystalline and liquid phases; and, (3) separation of the crystalline and liquid fractions. A food safety control point identified for the solvent fractionation system would naturally be removal of the solvent used. Complete solvent removal is assured with steam distillation in the deodorization process which is downstream.

K. Interesterification

The interesterification process can alter the original order of distribution of the fatty acids in triglyceride-producing products with melting and crystallization characteristics different from the original oil or fat. Unlike hydrogenation, interesterification neither affects the degree of saturation nor causes isomerization of the fatty acid double bond. It does not change the fatty acid composition of the starting material but rearranges the fatty acids on the glycerol molecule. The process of interesterification can be considered as the removal of fatty acids from the glyceride molecules, shuffling them, and then replacement on the glyceride molecules at random. This change in the distribution of the fatty acids affects the structural properties and melting behavior of the fats and oils. Commercially, the interesterification process has been utilized for the production of confectionery fats, margarine oils, cooking oils, frying fats, shortenings, and other special application fats and oils products.

Two types of chemical interesterification process are practiced: random or directed. Random rearrangement of fats and oils can be accomplished using either a batch or continuous process. Both random interesterification processes perform the three important rearrangement steps: (1) pretreatment of the oil; (2) reaction with the catalyst; and, (3) deactivation of the catalyst. In the directed rearrangement process, one or more of the triglyceride products of the interesterification reaction is selectively removed from the ongoing reaction. Continuous processes are normally used for directed rearrangements for better

control. Trisaturated glycerides are crystallized and separated from the reaction which upsets the reaction equilibrium so that more trisaturated glycerides are produced.

L. Blending

Different stocks are blended to produce the specified composition, consistency, and stability requirements for the various fats and oils products, such as shortenings, frying fats, margarine oils, specialty products, and even some salad or cooking oils. The basestocks may be composed of hydrogenated fats and oils, interesterified products, refined and bleached vegetable oils, purified animal fats, and/or fractions from winterization, dewaxing, or another form of fractionation. The products are blended to meet both the composition and analytical consistency controls identified by the product developers and quality assurance. The consistency controls frequently include analytical testing for solids fat index, iodine value, various melting points, fatty acid composition, and other evaluations designed to insure compliance with customer requirements. The blending process requires scale tanks and meters to proportion the basestocks accurately for each different product. The blend tanks should be equipped with agitators and heating to assure a uniform blend for consistency control [8].

M. Deodorization

With conventional edible oil processing, deodorization is the last in a series of process steps used to improve the taste, odor, stability, and food safety of the fats and oils by the removal of undesirable substances. In this process, the fats and oils products are steam-distilled under vacuum. The object is to remove the volatile impurities from the oil. The foremost concern from a quality aspect is the volatile impurities which have objectionable flavors and odors; however, deodorization is also very important from a food safety aspect. Steam distillation removes any trace pesticide and "heavy" metals contents obtained during the growing process. Deodorization is primarily a high-temperature, high-vacuum, steam distillation process to remove volatile, odoriferous materials present in edible fats and oils. It is the last major processing step through which the flavor and odor and many stability qualities of a fat or oil product can be changed. From this point forward, efforts must be directed toward retaining the quality that has been built into the fat and oil product with all of the preceding processes [8].

The odoriferous substances in fats and oils are generally considered to be free fatty acids, peroxides, aldehydes, ketones, alcohols, and other organic compounds. Experience has shown that the removal of flavor, odor, and other undesirables correlates well with the reduction of free fatty acids. Therefore, all commercial deodorization consists of steam stripping the oil for free fatty removal. Currently, batch, semicontinuous, and continuous systems of various designs are utilized by edible fats and oils processors to produce deodorized oil. All of the systems utilize steam stripping with four interrelated operating variables: (1) vacuum, (2) temperature, (3) stripping steam rate, and (4) holding time.

N. Liquid Oil Filling and Packaging

Most salad and cooking oils are packaged shortly after deodorization in containers for home, restaurant, or large food processor use. The processing necessary for most oils are oxidative stability preservation measures, such as nitrogen protection, temperature control, light avoidance, and the addition of any additives required by the individual products. The oil is filtered for a final time in-line to the bottle filler. The effectiveness of this final filtration is monitored with laboratory filterable impurities testing of packaged product samples obtained utilizing a statistical sampling plan. Food safety concerns for retail liquid oils were lessened with the packaging change from glass to plastic containers. Glass breakage and contamination of other containers were major concerns when glass bottles were used. Exposure of the oil to the atmosphere is limited to a micro-second for most filling lines with a tamper-evident seal applied to the container before the cap is applied.

O. Shortening Plasticization and Packaging

Plasticized shortening products can be defined as fats with a consistency that can be readily spread, mixed, or worked. Considerably more is involved in the plasticization of shortening and margarine than merely lowering the temperature to cause solidification. Slow cooling of these products produces a grainy, pasty, non-uniform mushy product that lacks the appearance, texture, and functional characteristics associated with plasticized products. Development of these characteristics are a function of controlled crystallization or plasticization. The final consistency of a shortening is the culmination of all the factors influencing crystallization and plasticization: chilling, working, tempering, pressure, and gas incorporation.

The plasticization process involves the rapid chilling and homogenization of a shortening mixture. Most shortenings are quick-chilled in closed thin-film scraped-wall heat exchangers with extrusion valves to deliver a smooth homogeneous product to the package at 17 to 27 atm pressure. Nitrogen is injected at 13 ± 1% into most shortenings to increase the product's workability and provide a white, creamy appearance. After packaging, many processors temper shortenings at temperatures slightly above the packaging temperature to allow the crystal structure of the hard fraction to reach equilibrium and form a stable matrix. After tempering, shortenings are usually stored and shipped at controlled temperatures of

70 to 80°F (21.1 to 26.7°C) to avoid crystal change and loss of the plastic properties [7].

P. Margarine Mixing, Chilling, and Packaging

Margarine was developed as and continues to be a butter substitute. It is a flavored food product containing 80% fat, made by blending selected fats and oils with other ingredients, such as milk, salt, color and fortified with vitamin A, to produce a table, cooking, or baking fat product that serves the purpose of dairy butter, but is different in composition and can be varied for different applications. Now, spreads have been developed as margarine substitutes. The major difference between spreads and margarine is that spreads are not required to contain a minimum of 80% fat.

Processing for margarines and spreads begins with the preparation of an emulsion of the ingredients. Emulsions are prepared by adding the oil soluble ingredients to a heated margarine oil formulation in an agitated emulsion tank. Concurrently, a pasteurized aqueous phase is prepared by mixing all of the water soluble ingredients together in another vat. The water phase is then added to the oil phase to make the emulsion. The emulsion is rapidly chilled with scraped-wall heat exchangers similar to those used for shortening products. The plasticized products are then formed into prints, or filled into the various containers for consumer, restaurant, or food processor use. Most margarine and spread products are stored at refrigerator temperatures immediately after packaging, with the exception of some specialized baking products [8].

Q. Flaking and Spray Chilling

Fat flakes describe the higher melting fat and oil products solidified in a thin flake form for ease of handling, quick remelting, or for a specific function in a food product. Chill rolls and processed oil formulations have been adapted to produce several different flaked products that can provide distinctive performance characteristics in specialty formulated foods. The flaked products, produced almost exclusively for restaurant and food processor consumers, are hardfats or stearines, shortening chips, icing stabilizers, confectioners fats, hard emulsifiers, and other customer-specific products.

The flake products are solidified on a chill roll, which has been described as an endless moving chilling surface held at a temperature below the crystallization point of the applied fat or oil product to form a congealed film on the outer surface. Specifically, chill rolls are usually 4 foot diameter hollow metal cylinders, in various lengths, with a machined and ground smooth surface, internally refrigerated, that revolve slowly on longitudinal and horizontal axes, with several options for feeding the melted oil onto the surface. After application, a thin film of liquid fat is carried over the roll, and as the revolution of the roll continues, the fat is partially solidified. With all chill roll designs, the solidified fat is scraped from the roll by a doctor blade positioned ahead of the feeding mechanism. Flakes are packaged in kraft bags, corrugated cartons with vinyl liners, or other suitable containers for storage and shipment [7].

Spray chilled or powdered fats are specialized products developed for ease of incorporation, handling, melting efficiency, uniform delivery with addition systems, encapsulation, and other special purpose uses. The spray chilling process consists of atomizing a molten fat in a crystallization zone or tower, maintained under temperature conditions where a very fine mist of the melted fat is contacted with cooled air or gas to cause crystallization without marked supercooling [8].

R. Bulk Fats and Oils Shipments

Food processors that use fats and oils in large quantities often have the facilities to handle this liquid ingredient in bulk. All of the products packaged for shipment and use can be provided to the customers in tank cars or tank trucks, except margarine and spread mixes, which contain milk and salt. The customers for these bulk products must have fats and oils bulk handling systems to receive, store, and handle the liquid products.

VI. U.S. EDIBLE FATS AND OILS CONSUMPTION

Climate and availability certainly influenced the eating habits of our ancestors. Inhabitants of central and northern Europe obtained their edible fats from animals, while people in southern Europe, Asia, and Africa acquired their edible oils from vegetable sources. The food products developed in these different regions used the available fats and oils products. Consequently, the cuisine of the central and northern Europe countries developed around the use of solid fats like butter, lard, and tallow for breads, pastries, and many other baked products. Similarly, the diets of inhabitants from the warmer climates were developed around liquid oils for food products like sauces, dressings, etc. These trends appear to continue to be the preference of their descendants.

Immigrants to the United States brought their food preferences with them and introduced them to others from different regions of the world. Fats and oils technology has further increased the varied and rich American diet by improvement of existing products and development of new food products. The resultant North American eating habits have made the U.S. a consumer of almost every available fat and oil. The American consumer is offered these fats and oils as a liquid oil, margarine, shortening, or as an ingredient in a prepared food product. The fourteen

TABLE 155.1
U.S. Edible Fats and Oils Usage and Per Capita Consumption

	Millions of Pounds					
Year	**1950**	**1960**	**1970**	**1980**	**1990**	**2000**
Canola					577	1774
Coconut	129	172	788	1032	897	968
Corn	223	310	445	673	1149	1711
Cottonseed	1445	1225	891	523	851	674
Olive	79	51	67	58	211	455
Palm		1	182	299	256	375
Palm Kernel	26	53	94	NR	362	243
Peanut	103	62	193	112	197	244
Safflower			100		58	102
Soybean	1446	3011	6253	9114	12164	16210
Sunflower				64	200	357
Lard	2050	1889	1645	1023	825	962
Tallow	156	328	518	995	955	1498
Butter	1327	1113	1075	1017	1095	1022
Total	6984	8215	12251	14910	19797	26565
Per Capita Consumption, pounds						
Vegetable Oils	24.0	26.7	39.0	45.0	52.8	63.1
Animal Fats	21.9	18.5	14.1	12.2	9.4	11.5
Total	45.9	45.3	53.0	57.2	62.2	74.6

NR = not reported.

major U.S. fats and oils sources are listed in Table 155.1, which reviews the annual usage of both animal and vegetable oils over the past 50 years [14–16].

Fats and oils consumption has been categorized into visible and invisible sources. Visible fats and oils are those isolated from animal tissues, oilseeds, or oil fruits and used for food preparation as shortening, margarine, or salad oil. Invisible fats and oils are consumed as part of meats, poultry, eggs, dairy products, fish, fruits, or vegetables, and account for approximately 60 percent of fat consumption. The pounds per person values reported in Table 155.1 are those from visible sources only. Visible fats and oils usage has more than tripled in the United States over the past 50 years, not only due to population increases, but also from increased consumption. Average per person consumption has increased by almost 50% during this period. The fats and oils usage data in Table 155.1 reflect some distinct trends: (a) a move away from animal fats to vegetable oils; (b) replacement of previously established fats and oils with different source oils; (c) introduction of new vegetable oils; (d) a rise and fall of some individual source oils; (e) source oil changes reflecting the results of medical studies; (f) introduction of new oil seed varieties; and more.

VII. EDIBLE FATS AND OILS UTILIZATION

Fats and oils are the raw materials for margarine, shortening, liquid oil, and other specialty or tailored products that become essential ingredients in food products prepared in the home, restaurants, and by food processors. Butter, lard, and tallow are fats that are used as raw materials for margarines or shortenings, as well as for direct use with little or no processing. The direct usage of animal fats has decreased considerably since 1950, as shown in Figure 155.2 [14,15]. Butter usage decreased 58% from 1950 to 2000. Lard and tallow direct use also had a substantial decrease (82.5%) through 1990, but rebounded for only a 53% overall decrease from 1960 to 2000. Margarine, developed as a butter substitute, has also experienced a decrease (40%) in popularity since 1980. Shortening usage more than doubled between 1950 and 1990, but slowed to only a 3.6% increase for the decade ending 2000. The usage rate for liquid oils almost tripled since 1950 to absorb all of the other fats and oils product losses and then some. Overall, the per capita fats and oils visible consumption rate

Fats and oils products, per capita consumption data

	1,950	1,960	1,970	1,980	1,990	2,000
Butter	8.6	8	5.3	3.6	3.5	3.6
Margarine	4.9	9	11.0	9.1	8.7	6.6
Lard & tallow	12.6	8	4.7	3.4	2.2	5.9
Shortening	11.0	13	17.3	18.3	22.3	23.1
Liquid oils	8.6	9	15.5	21.3	24.2	33.7
Other	0	2	2.4	1.5	1.2	1.6

FIGURE 155.2 Fats and oils products per capita consumption during 1950–2000.

increased 63% for the last half of the twenty-first century, with a move toward liquid oils. The increased popularity of liquid oils is more than likely due to:

1. Diet modifications to reduce saturated fats, trans isomers, and cholesterol
2. Awareness of the high polyunsaturated or essential fatty acid content of liquid oils
3. More convenient handling of the liquid oils than that of solid fats
4. Improved product formulations and processes to accommodate liquid oils
5. Reduced dependence on the crystalline properties of solid fats for functionality through the use of emulsifiers

A. Shortening Products

Originally, shortening was the term used to describe the function performed by naturally occurring solid fats like lard and butter in baked products. These fats contributed a "short" or tenderizing quality to baked products by preventing the cohesion of the flour gluten during mixing and baking. Shortening later became the product identification used by all vegetable oils processors in the United States to abandon the lard substitute concept; hence, shortening was an American invention. As the shortening product category developed, the limited application also expanded to include all baked products. Today, in the U.S., shortening has become virtually synonymous with fat to include many other types of edible fats designed for applications other than baking. In most cases, products identified as shortening will be 100% fat; however, there are exceptions such as puff pastry and roll-in shortenings which may contain moisture. Generally, a fat product containing at least 80% fat and the required vitamin A content, is margarine. Products that do not meet this criteria have been identified as shortening since shortening does not have a U.S. Standard of Identity. Currently, a description for shortening would be: processed fats and oils products that affect the stability, flavor, storage quality, eating characteristics and the eye appeal of prepared foods by providing emulsification, lubricity, structure, aeration, a moisture barrier, a flavor medium, or heat transfer [8].

Most shortenings are identified and formulated according to usage. The packaged shortening forms that have emerged to satisfy the requirements of the consumers and the food industry are plasticized, pumpable liquid, flakes, powders, chips, and beads. Almost all of these shortening products can also be shipped to large customers in liquid bulk quantities. A brief description of each of the shortening forms follows [7]:

- Plasticized Shortenings - General purpose plasticized shortenings are still identified as all-purpose, unemulsified, emulsified, animal-vegetable blends, all vegetable, or the like; while the trend is to formulate foodservice and food processor shortenings to perform a specific function for the intended food product. These shortenings are also identified by their intended usage, i.e., a baking application such as cakes, icings, puff paste roll-in, and others; frying applications, specific dairy analog products, household use, and so on.
- Liquid Shortenings - The pumpable liquid shortening designation covers all fluid suspensions that consist of a hard fat, usually beta tending, and/or a high melting emulsifier dispersed in a liquid oil. This shortening type was developed to pour or pump at room temperature for volumetric measurement or metering for either packaged or bulk-handled products.
- Flakes - Hardfat, hardbutter, hard emulsifier, and stabilizer flakes are high melting fats- and oils-based products solidified into thin flake form for ease in handling and quick melting, and are used to perform many different functions in food products.
- Chips - Shortening chips are made thicker and larger than flakes for incorporation into baked products to provide a flaky product similar to danish pastry without the labor intensive roll-in process.
- Powders - The higher melting fats and oils products can be spray chilled to produce powders. Most of the products flaked can also be powdered for ease in handling or encapsulation of a food product for protection and/or delayed release in a finished food product.
- Beads - Shortening beads have irregular granular shapes that can be metered at more uniform rates with vibratory or screw feeders and resist stratification or separation in mixtures with other granular materials.

B. Margarine and Spread Products

Margarine is a prepared food product developed because of a butter shortage in France. Its evolution to a highly accepted table spread and ingredient for cooking, baking, and prepared foods is a prime example of fats and oils technology. Margarine has evolved from an imitation of dairy butter to a nutritive food which provides a concentrated source of energy, a uniform supplement of vitamin A, a source of essential fatty acids, satiety, a universally accepted flavor, and a compliment to other foods. United States FDA and USDA regulations define margarine as a plastic or liquid emulsion food product containing not less than 80% fat and 15,000 international units per pound of vitamin A, and may contain optional ingredients with

specific functions. The usual optional ingredients are water, milk or milk products, emulsifiers, flavoring materials, salt and other preservatives, and colorants.

Low fat spreads, originally introduced in the 1960s as diet margarines, are available with a multitude of fat levels between 20 and 70%. Low calorie and low fat marketing created a consumer interest in spreads after the diet margarines had been rejected by consumers for poor melting and eating characteristics. Functionally, the spread products are intended to be used as a tablespread or for cooking and most packages have a statement that the spread is not intended for baking or frying.

1. Consumer Margarines and Spreads

Margarine prints generally in quarter pound and one pound solids were the basic margarine products available until soft margarines were introduced in 1962. The soft margarines, with higher unsaturated fatty acid levels, were packaged in plastic tubs in both regular and whipped versions. The whipped margarines were easier to spread and provided less calories per serving due to the 30% nitrogen content. The nutritional appeal of the soft margarines was carried even further with the introduction of spreads, which began to capture market share in the 1970s; the spreads market share increased from less than 5% in 1976 to more than 74% in 1995.

The major uses for consumer margarines and spreads continue to be as a tablespread, cooking ingredient, seasoning agent. The consumer-directed functional aspects of the margarine and spread products are spreadibility, oiliness, and melting properties. Spreadibility continues to be one of the most highly regarded attributes of consumer margarine products, second only to flavor. Oil-off is the most serious for print products, as the inner wrappers become oil soaked and oil may even leak from the outer package. The melting properties of the margarine oil ingredient, the emulsion tightness, and the processing, tempering, and storage conditions which help determine crystal development and stability have a direct affect upon the mouth feel and release of the flavoring materials, as well as the consistency.

Scratch baking in the home decreased considerably with the introduction of good quality prepared mixes and frozen ready-to-eat products. Nevertheless, for any home baking, the U.S. consumer will usually choose shortening or a print margarine. Measurement of soft margarine requires a different scale than stick products because of the creaming gas content: 5% in regular and 30 to 35% in whipped soft margarines. Also, spreads have exceptionally poor baking functionality due to the high moisture/low fat content.

2. Industrial Margarines and Spreads

Foodservice and food processor margarine and spreads are usually considered industrial products. The most popular foodservice margarine is the consumer stick margarine formulation packaged in 1-pound solids, which is used for cooking and seasoning. Individual serving or portion control spread products are also popular foodservice dining room products. Additionally, a bakers' margarine formulated with an all-purpose shortening base is used by many foodservice kitchens for their baking requirements.

Food processor margarine and spread products are formulated for more specific uses than either the foodservice or consumer products. The stick margarine formulations are packaged in 50 pound cube cases for use in prepared foods. Margarines are also formulated and plasticized with Danish pastry roll-in capabilities, like the shortening products discussed in section V.A, to take advantage of the flavor, color and moisture incorporated into the emulsion. Spread type products were used by food processors before the consumer had accepted them, but for different applications. One of the applications is for self-basting of meat and poultry products during baking. Another is a biscuit topping with special dairy flavor notes and buttermilk curd. Others employ different flavors, spices, or other special ingredients for specific applications, products, or processes.

C. Liquid Oils

A liquid oil is usually identified by its physical state at ambient temperature, irregardless of whether the source material is animal, vegetable, or marine. Some source oils appear to disagree with this designation until the mean temperature at the place of origin is considered. For example, oil products from palm and coconut trees are a solid at ambient temperatures in cool climates, but a liquid at the prevailing temperatures in the tropical climates where these plants grow. Therefore, the definition of a liquid oil would be: any oil that is a clear liquid without heating.

Liquid oils are further classified by their functionality traits; cooking, salad, and high stability. The definition for each of these classifications is:

- Cooking Oil - An edible oil that is liquid and clear at room temperature, or 75°F (23.9°C), that may be used for cooking. Cooking oils are typically used for pan frying, deep fat frying, sauces, gravies, marninates, and other non-refrigerated food preparations where a clear liquid oil has application. Cooking oils usually congeal or solidify at refrigerator temperatures.
- Salad Oil - An edible oil that is suitable for the production of a mayonnaise or salad dressing emulsion and which is stable at low temperatures. This requirement has been refined to require that, in order to qualify as a salad oil, an oil sample must remain clear without clouding for at least 5½ hours while submerged in an ice bath.

- High Stability Oil - An edible oil that possesses an exceptional oxidative or flavor stability, and is a clear liquid at room temperature. The measure of oxidative stability used for high stability oils is the Active Oxygen Method (AOM) or AOCS Method Cd 12-57. High stability oils will withstand the AOM abuse for periods in excess of 75 hours, and some longer than 300 hours, as opposed to the 8 to 20 AOM hours for cooking and salad oils.

1. Consumer Liquid Oils

Cooking and salad oils available for home use are bottled and marketed through grocery stores and other retail outlets. The source oils available to the retail consumer are canola, corn, cottonseed, olive, peanut, safflower, soybean, sunflower, blends of these source oils, and some other specialty oils. Most of the oils are only refined, bleached, and deodorized, with the exception of those that require dewaxing or winterization to remain clear liquids on the grocery store shelves, like canola, corn, cottonseed, and sunflower.

A steady growth in the consumption of cooking and salad oils is evident from the USDA Economic Research Service statistics in the Oil Crops Situation and Outlook Yearbook. In fact, salad and cooking oils were the sole fats and oils growth area for the year 1997. The trend away from solid fats to liquid oils indicates that the U.S. consumer is reacting to the cautions of the medical profession regarding the relationship of fats and oils to coronary disease. As a result, consumers have replaced solid shortenings and margarines with liquid oils.

2. Industrial Cooking Oil Applications

Cooking oils are utilized wherever liquidity is permissible or important and the application does not require a clear liquid oil at cool temperatures. Cooking oils may be used for pan frying, deep fat frying, gravies, and other applications. Cottonseed oil has a unique flavor property that makes it a desirable frying oil for snack foods. Corn oil is regarded as exceptional in flavor and quality, with a healthy image for incorporation into processed foods, and also for snack foods frying. Peanut oil maintains a respectable cooking oil market for snack frying and foodservice frying operations, especially for fish and chips. Some of the other applications for cooking oils are packing canned meats and fish products, pan-release products, bread, buns, and sweet doughs.

3. Industrial Salad Oil Applications

Salad oils are required in most dressing products, sauces, and other food products prepared or stored at cool temperatures. Salad oils were developed for use in mayonnaise, and are a necessity for the preparation of other salad dressings, sauces, and other food products that are emulsions prepared at cool temperatures or must withstand clouding or congealing at refrigerator temperatures after preparation. Most of these products require high quantities of oil in the formulation, 30 to 80%, to provide the eating characteristics and consistency desired.

4. High Stability Oils

The primary prerequisites of a high stability oil is liquidity at ambient temperatures and resistance to oxidation. Most oils which are liquid at room temperature contain high levels of unsaturated fatty acids. They are most susceptible to oxidation, which limits application to products where an extended shelf-life is not a requirement. Technology has identified two techniques to enhance the stability of liquid oils: (1) hydrogenation and fractionation to separate the hard fraction from the liquid oil fraction, which retains a high stability, and (2) the use of plant bleeding techniques to produce liquid oils with very high monounsaturated fatty acid levels. The applications established for the high stability oils are [8]:

- Deep Fat Frying - The high stability oils have substantially increased frying stability by limiting the opportunities for oxidation due to the absence of polyunsaturates. Frying stability for the high stability oils is near the performance for heavy duty frying shortenings, with the convenience and fried food appearance of a liquid oil.
- Protective Barrier - Surface application to food products with the high stability oils provides protection from moisture and oxygen invasion, prevents clumping, and imparts a glossy appearance. Specific applications include raisins and other fruits, breakfast cereals, nut meats, snacks, croutons, bread crumbs, spices, and seasonings.
- Carrier - Colors, spices, flavors, and other additives may be blended in the high stability oils to preserve the flavor, color, and activity without development of off-oil flavors for long periods.
- Pan-release Agents - As a major ingredient in the preparation of oxidative stable spray or brushing lubricants for baking pans, confectionery products, and other materials.
- Food Grade Lubricants - The high stability oils are food grade alternatives to the mineral oil products for lubrication of equipment that contacts food products.
- Compatibility - Unlike solid fats, the high stability oils are compatible with all types of fats and oils since crystal type is not a concern.

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CONTENTS

Increasing the degree of saturation of fatty oils via hydrogenation is the most important process of the fatty oil industry, particularly in the production of edible fat products. This reaction makes possible the synthesis of food products such as shortening and margarine. Vegetable oils are too soft for margarines because of their liquid nature, while on the other hand saturated fats are too hard. Depending on the end use, most shortening fat systems require an intermediate hardness. Industries use this process to turn cheap oils into simulated butter products. Hydrogenation is an excellent process for consistency change and prolongs shelf stability. However**,** there are medical side effects due to the *trans* isomers produced that can cause many health problems if too much is consumed.

Hydrogenation of edible oils and fats are chemical processes in which hydrogen is added to double bonds of unsaturated fatty acids in lipid. Lipid is usually in the form of triacylglycerol, but other structures containing ethylenic linkages can be also hydrogenated [7]. The general aim of hydrogenation processes is to adjust their melting properties and improve their stability [32]. Besides fat and hydrogen, this process requires a catalyst — usually nickel deposited on a silicate support. Though already a classical process, widely applied since the early 20th century, it is not yet possible to a predict the molecular composition of the hydrogenated oil as a function of feed stock composition, catalyst type and concentration, reaction pressure, temperature and time. This is partly due to the complexity of the process, with a large number of hydrogenation reactions occurring in parallel, and partly due to the simultaneous occurrence of isomerization and double bond conjugation reactions. The complexity of the reaction is further illustrated by noting that the partial hydrogenation of soybean oil results in the production of a minimum of different linoleic, linolenic and oleic esters, the *cis* and *trans* forms of which could produce more than 4000 different triacylglycerols.

Understanding hydrogenation is important because it is a major reaction, which leads to many everyday products. Besides margarine and shortenings, hydrogenated oils end up in such things as ice cream, candy, chocolate, potato chips and baked goods.

I. HISTORICAL BACKGROUND

Although reactions involving catalytic hydrogenation of organic substances were known prior to 1897, the property of finely divided nickel to catalyze the fixation of hydrogen on hydrocarbon double bonds was discovered by the French chemist, Sabatier. Thus, unsaturated hydrocarbons in the vapor state could be easily converted into saturated ones when passing hydrogen gas over a catalytic metal. Soon after this report a liquid-phase hydrogenation of fatty oils was patented in 1903 in England by German chemist Normann [23] and this title was passed to the British firm Joseph Crossfield and Sons. In 1909, Procter and Gamble Company acquired the American rights to the Crossfield patents. Soon after that Procter and Gamble introduced its hydrogenated shortening, "Crisco," on the market.

The first hydrogenated products were a blend of totally hydrogenated cottonseed oil and refined liquid cottonseed oil. This created a product that had the consistency of lard but which was less likely to liquefy at warmer temperatures. The technique of partial hydrogenation was developed in the 1930s and it complemented the development of

a high-yield solvent extraction method to render fats from vegetables and seeds.

The advent of hydrogenation led to possibilities for a new branch of science — oleochemistry. In the mid-1930s, a stainless steel stirred autoclave for hydrogenating tallow fatty acids was constructed. One of the earliest, and still exceedingly important oleochemical products, was fabric softener, whose principal ingredients were fatty amines, manufactured by hydrogenating fatty nitriles derived from fatty acids. Esterification of fatty acids, followed by hydrogenolysis, produces fatty alcohols, another of the major products in the manufacture of oleochemicals.

II. BASIC PRINCIPLES OF EDIBLE OIL HYDROGENATION

The basic chemical equation for hydrogenation of an unsaturated carbon-carbon double bond is shown below. While it appears very simple, in reality it is extremely complicated.

$$-CH = CH- + H_2 \rightarrow -CH_2 - CH_2-$$

It includes the following steps:

1. Transfer and/or diffusion of individual reactants (unsaturated fatty acids and hydrogen);
2. Hydrogen adsorption on the surface of a catalyst;
3. Addition of hydrogen on double bonds accompanied with their *cis*/*trans* and positional isomerization;
4. Desorption of reactants from catalyst surface and *trans*fer to the bulk of liquid oil.

During a hydrogenation reaction three different modifications can occur:

1. A double bond can be changed to a single bond, e.g., changing a 2-polyunsaturated fatty acid into a monounsaturated fatty acid, or a monounsaturated fatty acid into a saturated fatty acid.
2. The location of the double bond can be moved up or down the fatty acid chain, and/or the configuration of the double bond can be changed to either *cis* or *trans*.
3. Highly polyunsaturated fatty acids are the most susceptible to the process of hydrogenation, as they contain more double bonds than other fatty acids.

Depending on the conditions applied during the process, hydrogenation can be classified as either a selective or non-selective process. Reaction selectivity is defined as the conversion of a diene to a monoene, compared with the conversion of a monoene to a saturate. The simple reaction model for triene hydrogenation was published by Bailey in 1949, and later mathematically defined by Albright [1]. This model considered each reaction to be first order and irreversible.

It could be used for measuring the relative reaction rate constants (K) for each hydrogenation step during the batch hydrogenation of oils containing triene fatty acids.

$$\text{Linolenic} \xrightarrow{K_1} \text{Linoleic} \xrightarrow{K_2} \text{Oleic} \xrightarrow{K_3} \text{Stearic}$$

$$Ll = Ll_0 e^{-K_1 t}$$

$$L = Ll_0 \left(\frac{K_1}{K_2 - K_1}\right)\left(e^{-K_1 t} - e^{-K_2 t}\right) + L_0 e^{-K_2 t}$$

$$Ol = Ll_0 \left(\frac{K_1}{K_2 - K_1}\right)\left(\frac{K_2}{K_3 - K_1}\right)\left(e^{-K_1 t} - e^{-K_3 t}\right) - Ll_0 \left(\frac{K_1}{K_2 - K_1}\right)\left(\frac{K_2}{K_3 - K_2}\right)\left(e^{-K_1 t} - e^{-K_3 t}\right) + L_0 \left(\frac{K_2}{K_3 - K_2}\right)\left(e^{-K_2 t} - e^{-K_3 t}\right) + Ol_0 e^{-K_3 t}$$

Where:
Ll_0 = concentration of linolenic acid in time t = 0
L_0 = concentration of linoleic acid in time t = 0
Ol_0 = concentration of oleic acid in time t = 0
K_{1-3} = relative reaction rate constants (K) for each hydrogenation step
t = time

In a theoretical sense, an oil hardened with perfect preferential selectivity would, first of all, have its linolenic acids reduced to linoleic acids before any linoleic was reduced to oleic acid; then all linoleic acids would be reduced to oleic, before any oleic acids were saturated to stearic acid. Unfortunately, this does not happen in the actual practice. For practical application, if the relative reaction rate constant calculated for linoleic to oleic divided by the reaction rate constant for oleic to stearic is 31 or above, the hydrogenation is termed selective, if below 7.5, it is non-selective.

Hydrogenation can be partial or complete. This reaction does not proceed at room temperature, and requires a catalyst to overcome activation energy. These catalysts can be heterogeneous, such as nickel, platinum, or palladium, or homogeneous, such as the Wilkinson's catalyst. These reactions give off heat, which can determine the degree of saturation and predict stability as well. Transition metal complexes make good catalysts, since they bring the reactants together and break the hydrogen bond. These reactions are stereoselective, in that the hydrogens are added in such a way as to predict the outcome.

Hydrogenation can take place only when the liquid unsaturated oil, the solid catalyst and the gaseous hydrogen have been brought together in a heated stirred reactor. Interaction between chemisorbed hydrogen on the catalyst surface and the double bond of fatty acyl is the first step of heterogeneous hydrogenation. Another hydrogen atom may add to the adjacent position and the saturated molecule is desorbed; or if there is no hydrogen atom available, hydrogen may be removed from a chain carbon atom by the catalyst. Addition of one hydrogen atom results in the opportunity of free rotation to reorient the geometry of the molecule from *cis* to the thermodynamically more stable *trans* configuration. Whether *cis* or *trans* is formed depends on the geometric positioning of the carbon chains attached to the double bonded carbons. The double bond in the original position may also be converted to *trans*. The double bonds in the new position may also be shifted. As hydrogenation proceeds, the isomerized double bonds tend to be shifted farther and farther along the chain, and the *trans* isomer content increased until the monoenes are saturated.

Hydrogenation of the polyunsaturated fatty acid chain is similar to that for the saturated fatty acid chain. During partial saturation of polyenes**,** besides hydrogenation of one double bond, the positional migration or *trans* formation of the other bonds can occur. Methylene-interrupted dienes on the catalyst surface can be conjugated or hydrogenated to saturated equivalent. If the mixture to be hydrogenated contains monoenes, dienes, and polyenes, there will be competition among the different unsaturated systems for the catalyst surface. By simple arithmetic probability, an ethylenic linkage from one of the more unsaturated esters will be preferentially adsorbed from the oil to the catalyst surface, isomerized and/or hydrogenated, and then desorbed to diffuse to the main body of the oil.

Factors that affect the hydrogenation and consequently the resultant products, are the temperature of the oil mixture, hydrogen gas pressure, catalyst activity, catalyst concentration, agitation of the mixture, and time duration of the process [7]. The degree of selectivity in hydrogenation also affects the crystal stability of the resulting fat. Commonly, the selective reaction conditions cause more isomerization to *trans* isomers and less stearic acid development to effect high solids at the lower temperatures [24]. Since the *trans* form of an unsaturated fatty acid has a higher melting point in comparison to the *cis* form, the occurrence of the *trans* form in the product helps to create desirable solid levels. Although it has a beneficial effect on the quality of the product, it also increases the risk of coronary heart diseases [27].

A study carried out by Yap [36] showed that selectively hydrogenated canola oil formed a mixture of beta-prime and beta crystals, whereas non-selective hydrogenation resulted in the beta form of crystals. Incorporation of *trans* fatty acids through selective hydrogenation favors beta-prime crystallization. Therefore, it becomes difficult to obtain desirable acceptability in terms of melting profile, low *trans*-acids, and favorable polymorphic behavior (and indirectly rheological behaviour) by sticking to only one technique of hydrogenation. Thus, hydrogenation conditions are manipulated to choose the most desirable set of processing parameters.

III. EFFECTS OF PROCESS CONDITIONS

In the hydrogenation process, the composition and properties of the final product depend on various operating factors. Temperature has a significant influence on the rate of hydrogenation, selectivity and *trans*-isomer production. It has been reported that selectivity is directly proportional to the temperature applied during hydrogenation [7]. This fact is the consequence of different activation energies for various hydrogenation reactions. Reduction of double bonds in the unsaturated fatty acid chain is an exothermal process. σ bonds formed between the carbon and hydrogen atoms are, together, stronger than the hydrogen-hydrogen σ bond and π bonds being broken. The heat of hydrogenation can be measured and is simply the amount of heat evolved when one mole of the unsaturated compound becomes hydrogenated. Increasing temperature accelerate**s** the rate of saturation and influence on hydrogen solubility of oil. The common temperature interval for partial catalytic hydrogenation of edible oils is between 160–210°C. High temperature of the oil during hydrogenation favors greater selectivity and thus results in more *trans* fatty acid generation.

Heat of hydrogenation is an important measure of stability. The *trans* isomer gives off the least amount of heat, while the hydrogenation of the terminal double bond gives off the most. The *cis* isomer is in the middle of the two. All three consume one mole of hydrogen and yield the same product. The *trans* isomer is usually more stable than the *cis* isomer because the substituents are farther apart in the *trans* isomer than in the *cis*, and it is sterically more favorable.

Among important factors influencing the hydrogenation process is the type of catalyst. Currently, the most widely-used commercial catalyst for edible oil hydrogenation is active nickel supported on an inert substance [6], [35]. High catalyst concentration favors selectivity with large amounts of *trans* isomer formation. There is, however, some concern about the toxicity of traces of nickel remaining in the oil [31]. Other catalysts including copper on silica [15], nickel–silver [17] or copper chromite [29] have been investigated. Noble metal catalysts are not generally used because of their high cost. However, their high activity in small quantity and the possibility of reuse with fixed bed reactors may offset the cost disadvantage. Palladium on carbon has been used for some commercial hydrogenation because of its high linolenic and linoleic activity at low temperature [22]. Platinum on carbon had been found to be highly active but with low selectivity

producing the saturated fat [5]. A more recent report [16] found that a modified platinum on carbon catalyst by incorporating a small amount of ammonia in the hydrogen has high selectivity with low *trans*-isomerization.

Rubin et al. [30] reported on the first mixed system containing both homogeneous and heterogeneous catalysts for edible oil hydrogenation. Using Ni and methyl benzoate chromium tricarbonyl [$MeBeCr(CO)_3$], they showed it was possible to retain the advantages of both catalysts while using them in combination. $MeBeCr(CO)_3$ is highly stereoselective toward *cis*-monoenes. However, because it hydrogenates via a cisoid mechanism in which methylene interrupted double bonds must be conjugated prior to hydrogenation, the reaction rate is limited by the slow conjugation reaction. When $MeBeCr(CO)_3$ was paired with Ni, minimal *cis–trans* isomerization was maintained while higher hydrogenation rates, more characteristic of Ni, were observed.

Polymer stabilized noble metal nanoclusters for selective hydrogenation of small molecule substrates such as unsaturated aldehydes and hydrocarbons have been studied [34]. These catalysts were also reported to possess high enantiomeric selectivity [38]. Polymer stabilized platinum has been found to have ~5 times higher activity than Pt/C with increased selectivity for partial hydrogenation of allyl alcohol at 25°C and atmospheric pressure. In general, metal nanoclusters have properties and activity that are quite different from the corresponding conventionally prepared supported and unsupported metal catalysts. Their potential as catalysts for selective hydrogenation for the oil and fat in the oleochemical industries is only scarcely being explored.

Hydrogen solubility in oil is directly proportional to its pressure. Mattil [19] reported that high hydrogen gas pressure during hydrogenation increased the rate of hydrogenation and caused a decrease in the selectivity of the reaction. Such conditions favor less TFA (*trans* fatty acids) formation. Change of hydrogen pressure from 103 to 310 kPa for example can reduce hardening time by at least 60%. All edible oils contain trace amounts of poisons deactivating reaction sites of catalyst. The minimum amount of catalyst necessary for their neutralization is called the threshold concentration. Once this threshold level has been reached, additional catalyst increases reactivity in a mathematically predictable manner. Under conditions of high temperature and pressure, a more than doubling of catalyst concentration (from 0.005% nickel to 0.0125%) increased the reaction rate above 50%. This is a very important factor in keeping solid catalyst in oil bulk and facilitating solubilization of hydrogen in the oil. Increases in the degree of agitation favors non-selectivity hydrogenation and suppresses the formation of high melting *trans*-isomers [3]. Beal and Lancaster [4] studied the effect of agitation and batch size on the rate of hydrogenation, and on the stability of the fat. They observed that the rate of hydrogenation increased with an increase in the degree of agitation of an oil or mixture of oils. Furthermore, the stability of the fats increased with an increase in the hydrogenation batch size. Achieving the optimum degree of mixing is not difficult in the laboratory; but it can be difficult in the plant.

Researchers conducting optimization tests of the hydrogenation process, have investigated reactor configurations [12], alternative energy sources such as microwave, magnetic, ultrasonic, etc., in addition to heat [11] or alternative sources of hydrogen, such as metal hydrides or soluble hydrogen donors [22]. In 1992, Yusem and Pintauro [37] developed an edible oil hydrogenation electrolytic process using atomic hydrogen produced at the cathode. Since hydrogen was generated *in situ* directly over the catalytic surface, it eliminated the need for enhancement of hydrogen transfer rates. As a result, high temperatures and pressures were not required.

IV. HYDROGENATED FAT IN HUMAN NUTRITION

The medical viewpoint of hydrogenation is strongly discussed, especially for the role of *trans* fatty acids (TFA). In 1993, a report published in Lancet by Willett et al. [35] an extensive study of more than 85,000 nurses concluded that women who ate four or more teaspoons of margarine a day had more heart attacks than women who rarely ate margarine. The main goal of this controversial study was the correlation of dietary vegetable oil-based *trans* fatty acids intake with coronary heart disease. The results started biochemical, toxicological and epidemiological research aiming to elucidate the real nutritional and health impact of *trans* fatty acids. On the other hand, it is known that cows' milk or dairy products contain *trans* isomers of fatty acids because of intestinal bacterial activity [18]. Depending on the diet, milk fat has 2–9% total TFA isomers vaccenic acid [28]. There are many other side effects of *trans* fatty acids including allergic reaction, arteriosclerosis, increased risk of cancer, decrease in insulin response, lowered quality of breast milk and slight immune dysfunction. Because of the effects of TFAs on the metabolism of gamma-linolenic and arachidonic acid [13], ingestion of *trans* isomers can affect the metabolism of prostaglandins and other eicosanoids and may alter platelet aggregation and vascular function as well [2]. TFAs also show competitive interactions with essential fatty acid metabolism (EFA) by inhibiting its incorporation into membrane phospholipids and reducing the conversion of EFAs to eicosanoids. In 1991 Koletzko [14] supposed that isomeric *trans* fatty acids could actually reduce tumor growth and metastasis. Up to the present time, there is little evidence that TFAs are related to the risk of cancer at any of the major cancer sites [10]. On the contrary, association between some forms of cancer in humans and the intake of hydrogenated vegetable fats has also been reported [8]. The general conclusion is that increasing the intake of TFAs (at expense of *cis* fat) does not produce an adverse outcome with respect to cancer risk.

It has been demonstrated that TFA ingestion increases low-density lipoprotein (LDL) cholesterol to a degree similar to that of saturated fats [20]. The increase in LDL concentration has been attributed in part to the down-regulation of the LDL receptor. In contrast to other forms of fats, *trans* isomers decrease high-density lipoprotein (HDL) cholesterol [9]. This can be responsible for a markedly increased risk for coronary heart disease, a relationship that is different and must not be confused with serum low-density or high-density lipoprotein (LDL or HDL) cholesterol levels [21]. *Trans* fatty acids can make platelets stickier, which increases the chance of a clot in a blood vessel. This is the cause of strokes, heart attacks and circulatory occlusions in other organs like the lungs.

Our body, fortunately, has natural ways to protect itself from massive TFA intake. There are certain enzymes which recognize the conformational difference and reject the *trans* fatty acids. Enzymes can refuse to use these molecules for processes for which they are not suited. Our brain is also protected from the *trans* fatty acids and, as well, an unborn child will have no exposure to the side effects imposed by them, as the placenta is impermeable to *trans* fatty acids [25]. Our body will also break them down as quickly as possible for energy use to ensure that the *cis* fatty acids are reserved for more important, vital roles.

Strong hydrogenation of soybean oil allows for the formation of variable small amounts of conjugated linoleic acids (CLA). They have been claimed to affect immunomodulation and body composition alteration, and to prevent or cure atherosclerosis and stomach, colon, skin, and prostate cancer. CLA has also been linked directly to increased insulin sensitivity, normalized glucose tolerance, improved hyperinsulinemia and lowered levels of circulating free fatty acids. Recent findings suggest that not only does CLA affect many different pathways, but that individual isomers of CLA act differently. Several studies have demonstrated that the *cis*-9, *trans*-11 isomer is responsible for the anticarcinogenic effects of CLA [26], [33]. Obviously, we have to wait for human clinical studies to confirm all these claims.

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157 Manufacture of Asian (Oriental) Noodles

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CONTENTS

I. INTRODUCTION

The term "Asian (oriental) noodles" is used very broadly to describe mostly noodle-like products produced mainly in Eastern, Southeastern or Pacific Asian countries using common wheat flour, rice (or rice flour) or other starch materials as the main structural ingredient. Even though the terms "noodles" and "pasta" are often used interchangeably, they are technically different. The common wheat-based "Asian or oriental noodles" differ from the Western style pasta that uses durum wheat flour as the main structural ingredient. The term "noodles" also differs from the US definition of noodles that contains egg solids as part of the Standard of Identity, and readers for this chapter should be aware of these differences. At this time, there is no Standard of Identity for Asian (oriental) noodles in the USA.

Asian noodles vary considerably in size, appearance (color and shape), ingredients, chemical properties, and methods of manufacturing. These variables are introduced briefly in this chapter. There are a few excellent reviews on Asian (oriental) noodles available and readers should consult these references for further information (1–8). Factors affecting the production of these Asian (oriental) noodles are also studied to some extent, and some references are provided at the end of this chapter for further information (9–37). It will not be surprising that considerable amounts of literature in Japanese, Korean, and Chinese are also available, but not easily accessible at the time of this writing.

It is generally believed that noodles originated in China several thousand years ago, and the present day form of noodle was developed at least two thousand years ago. In the Chinese language, the term "mien (mian)" is used to describe noodle-type products (with a few exceptions in shape) made from common wheat flour (main structural ingredient). In fact, the Chinese character for noodles has "mia (wheat)" on its side as part of its character structure. Similar products made from rice, mung bean, and other ingredients are all grouped under the term "fen" (rice noodles), for example, "mi fen" (rice noodle), "tung fen" (mung bean threads), and "ho fen" (oily rice stripes). The Chinese character for "fen" is written with "mi (rice)" on its side as part of its character structure, indicating it originated from rice or starch material.

It is also believed that "mien" and "fen" spread from China to its neighboring countries. This is supported by the terms with similar sounds used in these

countries: "men" (Japan); "mie" (Indonesia); "mee" (Thailand, Singapore, and Malaysia), "Pho" (Vietnam).

II. SIZE CLASSIFICATION, TYPES OF WHEAT-BASED ORIENTAL NOODLES AND SOME CHEMICAL PROPERTIES

Table 157.1 shows the classification of "Asian noodles" in two ethnic groups with examples: Chinese and Japanese. The two classifications are similar.

Table 157.2 shows the appearance of various types of wheat-based oriental noodles with typical examples, components, and quality of cooked products. It should be noted that these are only examples, and they may fall into different categories when some ingredients are substituted or omitted. For example, Chinese cat's ear noodles can be made with or without buckwheat. When they are made with buckwheat, they fall into the brownish buckwheat category, and when they are made with just common wheat flour, they fall into the white-salted category.

Table 157.3 compares the basic chemical properties of some common wheat-based Asian noodles. The major properties include protein content in the wheat flour, amount of water used in making the dough, amount of salt used in the formulation, and presence or absence of alkaline agents (sodium and/or potassium carbonate). All these factors affect the eating quality of the final product. It is obvious, with the addition of alkaline agent(s), the pH of the final product will be shifted to the alkaline range.

The addition of alkaline salts to the formulation not only alters the pH and color, but also improves the water absorption properties of the final product. In addition, addition of alkaline agents improves the texture of the cooked product, making it more chewy with less of a tendency to soften and paste after cooking. The flavor of the cooked product is also typical of an alkaline odor. The majority of consumers in Hong Kong, for example, prefer this type of Asian (oriental) noodles to the common white-salted noodles. However, the reverse is true for most of the consumers in central and northern China.

Oil is used to coat a few freshly-made oriental noodle-type products (see below). For instant noodles, the range of oil content in the final product depends on whether the noodles are oil-fried after steaming.

TABLE 157.1
Classification of Wheat-Based Noodles from Two Ethnic Groups

Ethnic Group	Class	Examples
Japanese	Very thin noodles	Somen
	Thin noodles	Hiya-mugi
	Standard	Udon
	Flat noodles	Kishi-men, Hira-men
Chinese	Very thin noodles	Longxu mian (China), Yinsi (silver threads) mien (Hong Kong)
	Thin noodles	Xi mien (China, Hong Kong, Taiwan)
	Flat noodles	Yangchun mien (China) Kuan (broad) mien (China, Taiwan)
	Wide flat noodles	Dai mien (China) Cu mien (China, Hong Kong, Taiwan)
	Thick noodles	Cu mien [Shanghai (China)], similar to Udon

III. GENERAL PROCEDURES IN THE MANUFACTURE OF WHEAT-BASED ASIAN (ORIENTAL) NOODLES

In general, wheat-based Asian (oriental) noodles are made by sheeting and rolling procedures in small factories

TABLE 157.2
Comparison of Major Types of Dry Wheat-Based Noodles

Types	Typical Examples	Components	Quality of Cooked Product
White-salted	Japanese udon Regular plain noodles	Common wheat flour, salt, water, egg (optional)	Soft, elastic texture and smooth surface
Yellow-alkaline	Cantonese-type noodles Taiwanese-type noodles	Common wheat flour, water, alkaline salts (sodium and/or potassium carbonate), salt (optional), egg (optional), yellow coloring (optional)	Firm, chewy, springy texture, and bright yellow appearance
Brownish buckwheat	Japanese soba, Chinese cat's ear noodle	Buckwheat flour, water, limewash or alkaline salts (optional), salt (optional), yam flour (optional)	Firm, chewy, tender texture
Instant	Japanese ramen, Cantonese E-mien (deep-fried noodle)	Common wheat flour, water, salt, alkaline salts (optional), oil/fat (optional),	Elastic, chewy
Savory	Cantonese shrimp egg noodle, Imitation	Common wheat flour, salt, water, alkaline salts (optional), savory ingredients	Firm, chewy, springy texture, color dependent on savory ingredient used

TABLE 157.3
Chemical Properties of Various Types of Dry Wheat-Based Noodles Ingredients

Types	Protein in Flour	Water Added	Salt Added	Alkaline Agent(s)	pH	Oil Added
White-salted	8 to 10%	30 to 35%	2 to 3%	No	6.5 to 7	None
Yellow-alkaline	10 to 12%	30 to 35%	Variable	Yes	9 to 11	None
Brownish buckwheat (60–70 parts common buckwheat to 30–40 parts wheat flour)	12 to 14 %	45 to 48%	Variable	Yes/No	6–5 to 7	None
Instant	8 to 12%	30 to 36%	Variable	Yes/No	6.5 to 7	15 to 21.5% (fried-type)
Savory	10 to 12%	30 to 35%	Variable	Yes/No	9 to 11% (alkaline type) 6.5 to 7% (regular type)	1.5 to 1.8% (dried type)

that is different from the extrusion procedure used commonly in the production of pasta (see chapter on dried pasta in this handbook). Figures 157.1–157.7 show the activities in the production of the dough, sheeting, rolling, and cutting of the dough sheets into noodle stripes. It should be noted that dough development is achieved to some degree in these sheeting and rolling processes (Figure 157.4).

FIGURE 157.1 Putting flour in the mixer.

FIGURE 157.3 Sheeting of dough into thin dough sheets.

FIGURE 157.2 Making of a soft dough from various ingredients.

FIGURE 157.4 Rolled dough sheets ready for further processing.

FIGURE 157.5 Double rolling of dough sheets to form the gluten structure.

FIGURE 157.6 Connecting the upper rolls of dough sheets to the bottom roller.

IV. MANUFACTURE OF DRY ASIAN (ORIENTAL) WHEAT- AND RICE- BASED NOODLES

Dry Asian (oriental) noodles are common products with the advantages of stability and being easy to transport. However, they take a longer time to cook than the fresh product. Examples of selected wheat-based and rice-based Asian (oriental) noodles are described below with comments.

A. Wheat-based Noodles

The majority of Asian (oriental) noodles are sold in the dry form in plastic/cellophane wrappings or in cardboard boxes with cellophane wrapping. Chinese people are the major consumers of Asian (oriental) noodles, especially the regular white-salted and rice noodles.

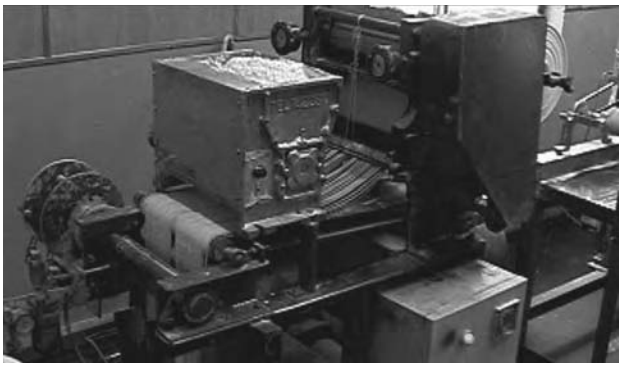

FIGURE 157.7 Cutting of dough sheets into raw noodle stripes.

Table 157.4 is a generalized scheme for production of dry noodles in China. It should be noted that savory-type noodles are also available in many varieties. The generalized scheme for production of dry noodles is a procedure that involves mixing, resting, sheeting, rolling, cutting, and drying. Similar products, such as Japanese somen, udon, and buckwheat sobo are produced in a similar manner, except that in some cases, extrusion procedures are used instead of sheeting and rolling.

Table 157.5 describes a procedure used in Taiwan to produce dry "La Mien" (stretched noodles). Making traditional "La Mien" (hand-swung noodles) manually is a very skillful technique (see below), accomplished by swinging the dough into very thin noodles. The "La Mien" thus produced is usually consumed right away by cooking in boiling water, followed by addition of seasonings, soup and other ingredients. However, due to the popular demand of such products, a dry form has been developed by varying the manufacturing process from hand-swinging to manually stretching, and the La Mien thus produced is dried for easy transportation and long term storage. It also can be produced in much larger quantity than the traditional hand-swinging process. Figures 157.8 to 157.15 show the unique operations in the production of dry Taiwanese La Mien (hand-stretched noodles) in a small factory.

Table 157.6 is a generalized production scheme for dry Taiwanese Yi-mien, an alkaline-type noodle with the addition of alkaline agent(s). This production scheme is also similar to that for Cantonese alkaline noodles except for the ingredients used (egg is used fairly commonly for Cantonese alkaline noodles) and the addition of a shaping process (also a common procedure in Cantonese alkaline noodles). Shaping is not common in other forms of oriental noodles except instant noodles. Figure 157.16 shows the sun drying procedure in the production of Taiwanese Yi-mien in a small factory.

Deep-frying the freshly-made noodles not only removes the moisture, but also cooks and alters the structure of the final product. The frying process makes the structure very porous, allowing it to more easily absorb

TABLE 157.4
Generalized Scheme for Production of Dry Wheat-Based Noodles in China

Weigh out the basic ingredients:
- wheat flour (100 parts)
- water (25–32 parts, dependent on gluten content of wheat flour, at 30°C)
- salt (2–3 parts)
- alkaline agent (optional, 0.1 to 0.2% of flour weight)

Weigh out additional ingredients (optional):
- Egg – 10% of flour weight (fresh egg); 8% of flour weight (frozen shelled egg), or 12.5% of flour weight (egg powder)
- Milk – 14 to 25% (fresh milk), or 2 to 3% (milk powder)
- Dried meat floss – 5% by flour weight plus 3% salt
- Tomato sauce – 5%
- Soymilk – made from 15 kg soybean for each 50 kg of flour
- Fish stock – made from 2.5 kg of fish plus 1 kg salt for 50 kg of flour
- Mung bean milk – made from 15 kg mung bean for each 50 kg of flour
- Chili powder – 1.5% by weight of flour plus 3% salt
- Monosodium glutamate (MSG) – 0.5 to 1.0%
- Egg white
- Egg yolk
- Butter
- Beef powder
- Prawn meat
- L-lysine hydrochloride
- Chicken broth
- Spinach juice
- Calcium powder

Mix up the flour, water, and other ingredients fully for 10–15 minutes to allow for hydration of the protein, starch, and other biological components to a uniform-colored, crumbly mixture without any pockets of dry flour. Linear velocity of dough mixer is adjusted to 2–3 m/sec.

Rest the dough for 10–15 minutes by mixing at low speed of 0.6 m/sec (10 rpm) at room temperature.

Sheet and roll the dough 6 to 7 times to reduce the dough to desirable sheet thickness (0.6, 0.8, 1.0, or 1.5 mm).

Cut the dough sheets to desirable width (0.8 to 1.0, 1.5, 2.0, 3.0, or 6.0 mm).

Dry the noodle stripes at a temperature below 50°C for about 2 hours, or at 38°C for about 7.5 hours (at controlled relative humidity between 70 to 80 %), or at 45°C for 3.5 hours (at 80% relative humidity) to 13.5 to 14% moisture content.

Machine-cut the dried noodles.

Weigh the noodles at 250 or 500 g each.

Pack the weighed noodles into appropriate plastic bags, followed by sealing of the bags.

Adapted from Refs. 3, 4, 5.

boiling water. The fried product puffs up and expands considerably during the frying process. This makes the final product very easy to rehydrate in the cooking process, generally in a matter of seconds after putting it into boiling water. Cantonese E-mien (E-fu-mien) has been a delicacy among the Cantonese community for decades. It is now available in regular laminated packs or

TABLE 157.5
Generalized Scheme for Production of Dry Taiwanese La Mien (Stretched Noodles)

Weigh out appropriate amount of medium strong wheat flour (9–10% protein)

Weigh out appropriate amount of salt (6%)

Mixing of the wheat flour and salt with appropriate amount of water (32–36%)

Sheet the dough and cut the sheets into threads

Coil dough threads onto two sticks (like spinning cotton into yarn) 20 cm apart, followed by stretching to 40 cm and then 60 cm, respectively

At 60 cm in distance, age the noodle stripes are aged (matured) for 2.5 hours

Stretch the noodle stripes again to 120 cm in distance

Sun dry or mechanically dry the noodle stripes to about 30% moisture

Fold the noodle stripes 4 times

Further reduce moisture content to about 13%

Packaging of final product in plastic or laminated packages

FIGURE 157.8 Dough stripes in a bowl.

FIGURE 157.9 Pulling out the dough stripes from the bowl of noodle stripes.

FIGURE 157.10 Coiling the dough stripes on two sticks 20 cm apart.

FIGURE 157.11 Extending the noodle stripes to 40 cm apart.

FIGURE 157.12 Extending the noodle stripes to 60 cm apart.

FIGURE 157.13 Extending the noodle stripes to 120 cm apart for sun drying.

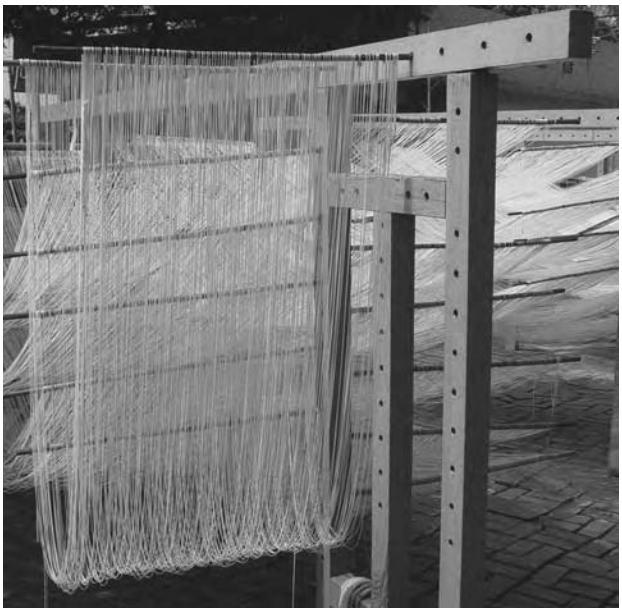

FIGURE 157.14 Partially sun-dried noodle stripes folded back to 60 cm apart.

as a vacuum-packed product in supermarkets in the US. It is also available as a freshly-made product directly from the factory. Table 157.7 shows a generalized production scheme for Cantonese E-mien (E-fu-mien).

Nissin Foods Company in Japan first invented Instant Chicken Ramen (noodles) in 1958, and packaged in laminated/cellophane pouches. This product that can be rehydrated in a bowl with boiling water in 3 minutes. It is produced by first steaming the noodle portions, followed by

FIGURE 157.15 Partially sun-dried noodle stripes folded back to 30 and 15 cm (final), respectively.

TABLE 157.6
Generalized Scheme for Production of Dry Taiwanese Yi-Mien

Weigh out appropriate amount of strong wheat flour (>11% protein)
Mix the four with 0.4 to 0.6% alkaline agent (sodium and/or potassium carbonate) and water to form a weak dough
Cover the dough and leave the dough for maturation for about 30 minutes
Sheet and roll the dough to desirable thickness (about 2–3 mm)
Fold the dough into plates of dough sheets
Cut the multi-layered dough sheets to make the noodle stripes
Noodle stripes are then shaped and sun-dried or mechanically dried to about 13% for long term storage (*see* also scheme for Taiwanese La Mien production)

FIGURE 157.16 Sun drying of Yi-mien on bamboo trays to reduce the moisture for long term storage.

brief frying in tropical oil. The seasoning was packed in a small packet. The fried noodle and the seasoning then were packed in a laminated/cellophane pouch. This form of package was later improved by using Styrofoam cups. However, both types of packaging are available today. Because of the concern in consuming tropical oils, a modified production process of drying the steamed noodles instead of frying was introduced. A separate packet of oil is included in the package for optional use by the consumer. Various flavors are available now. Table 157.8 is an example of a generalized production scheme for instant noodles in Taiwan, adapted from the Japanese procedure.

TABLE 157.7
Generalized Scheme for Production of Cantonese E-Mien (Deep-Fried Noodles)

Weighing of ingredients [wheat flour (medium strength), salt, water, egg (optional, potassium carbonate (optional)]
Mixing of dry ingredients for a short time
Adding water to adjust moisture content to about 36%
Mixing of dough for 10–15 minutes
Sheeting the dough through sheet rollers to reach 1–2 mm in thickness
Cutting of sheeted dough to strips of 1 mm in width to form long strings of noodles
Weighing of noodles to standard portions
Frying of noodle portions in a continuous oil fryer
Cooling of oil-fried noodles
Packaging of fried noodles into cellophane bags, cardboard boxes, or Styrofoam trays then wrapping with cellophane

TABLE 157.8
Example of Generalized Process for Dry Instant Noodle Manufacturing in Taiwan

Weighing of ingredients [wheat flour (medium strength), salt, water]
Mixing of dry ingredients for a short time
Adding of water to adjust moisture content to about 36%
Mixing of dough for 10–15 minutes
Sheeting the dough through sheet rollers to reach 2 mm in thickness
Cutting of sheeted dough to strips of 1 mm in width to form long strings of noodles
Weighing of noodles to 100 g portions
Steaming of portioned noodles for 2–3 minutes in temperature-controlled steamer (95°C)
Frying of steamed noodle blocks at 150°C for 1.5 minutes in a continuous oil fryer (fried-typed instant noodles) (net weight 80 g)
or
Drying of steamed noodle blocks in controlled temperature chamber to 80 g net weight
Cooling of oil-fried or dried instant noodle
Packaging of fried or dried noodles in plastic/cellophane bags, or Styrofoam cups
Adding packages of seasonings (dry, wet, and/or oils) into container
Sealing of container

The Instant Ramen developed by Nissin Foods Company was well accepted by the Japanese. It expanded its operation into other countries in the late 1960s and early 1970s. It is one of the most popular snack or regular meal items in many countries. Currently, instant rice noodles ("mi fen") are also available.

In comparing the production schemes for Cantonese E-mien and instant noodles, the instant noodle process can be considered as an improvement of the Cantonese E-mien process, as the noodle volume of instant noodle remains essentially the same, as compared to the expanded volume in Cantonese E-mien (3–4 times). This has the advantage of much smaller product volume. Also, seasoning are included in the product package. In addition, dried and not fried instant noodles are available. This satisfies the health-concerned consumers. However, Cantonese E-mien has the advantage of much reduced cooking time and unique texture. It probably will remain as a unique cuisine-type product, as compared to the instant noodles which are part of the mainstream food chain.

B. Rice-based Dry Asian (Oriental) Noodles

Rice noodles ("mi fen" or rice sticks) are a deviation of Asian (oriental) wheat-based noodles in that rice is the basic structural component. In addition, traditional procedures for the production of rice noodles involve the wet-milling of rice to remove the soluble constituents in the rice kernel, and the gelatinization of the rice starch. This process is tedious, and involves the problem of liquid waste disposal, even though it is not high in biological oxygen demand. A modified procedure is to use rice flour directly instead of the wet-milled rice flour, thus avoiding the liquid waste disposal problem. It should be noted that dry milled rice flour is not the same as the wet-milled rice flour, and the quality of the final product is not expected to be the same. It is generally believed that wet-milled rice flour has a smoother texture after gelatinization. Table 157.9 describes the general steps involved in the production of dry rice noodles. Consumers should be aware that in recent years, some manufacturers have begun to use corn starch or other starches partially or completely instead of rice flour to make similar products, and still call them "rice sticks." They have similar properties, but are not as good as the original rice noodles (rice stick).

TABLE 157.9
Generalized Scheme for Production of Dry Rice Noodles in Taiwan

Process A
Clean polished high-amylose rice to remove foreign matters
Soak rice kernels in water for 3 hours
Grind the soaked rice into a slurry with a suitable amount of water, avoiding the production of excessive damaged starch granules
Pour the rice slurry into a cloth bag and press the filled bag with a mechanical press to remove water
Mix the de-watered rice solids in a heated mixer for 50 minutes and partially cook the rice solids to a soft mass (addition of corn starch and/or wheat is optional)
Mix the soft rice mass a second time to further soften
Transfer the soft rice mass to a presser to form thick sheets, followed by extruding the sheets into rice noodles, cooling and loosening the extruded rice noodles immediately to avoid sticking together.
Steam-cook the extruded rice noodles for about 50 minutes
Cut the cooked rice noodles with a knife when still warm
Shape the cut rice noodles into bundles or blocks
Load the shaped rice noodles onto trays in carts
Mechanically dry the shaped rice noodles for 8 hours
Cool thoroughly before packaging into specific containers for retail or storage
Process B
Instead of soaking, grinding, and de-watering the rice, rice flour is used directly in mixing and partial cooking
The rest of the steps are essentially the same as Process A

V. MANUFACTURE OF FRESH ASIAN (ORIENTAL) WHEAT- AND RICE-BASED NOODLES

Considerable amounts of fresh Asian (oriental) noodles are produced for the retail market, restaurant trade, and at a household level. The makings of some of these products are described below.

Won Ton Mien is one of the alkaline wheat-based noodles produced for the fresh retail markets common in Taiwan. Similar products are also produced elsewhere. The production steps are similar to production of dry white-salted noodles, with basic mixing, sheeting, rolling, and cutting steps. It should be noted that a differently-shaped product called "pian er mien" (sheeted mien) is produced by similar procedures with or without alkaline salts; instead of cutting the dough sheets into thin stripes, the sheets are cut into about 1 inch squares. Also, the fresh Won Ton Mien can be dried into dry Won Ton Mien, like the other wheat-based "mien." Table 157.10 describes the basic steps in the production of Taiwanese Won Ton Mien. Figures 157.17 and 157.18 show the typical operation procedures in its production.

Fresh oily wheat-based "mien" are unique, in that oil is added to the cooked "mien" or "fen" to provide a special mouthfeel and *al dente*. They are common in southern China and southeastern Asian countries. The oily, alkaline wheat-based "mien" are sometimes called Hokkien-type noodles. The procedures for preparing the raw "mien" are essentially the same as other alkaline wheat-based "mien." They are then boiled once or twice, or steamed until they are completely cooked before coating with oil and food coloring. Table 157.11 describes the procedures of manufacturing Taiwanese oily wheat-based noodles in a small

TABLE 157.10
Generalized Scheme for Production of Fresh Taiwanese Won Ton Mien

Weigh out appropriate amount of strong wheat flour (>11% protein)
Mix the four with 0.35% alkaline agent (sodium and/or potassium carbonate) and water to form a weak dough
Cover the dough and leave for maturation for about 30 minutes
Sheet and roll the dough to desirable thickness (about 1 mm)
Fold the dough into plates of dough sheets
Cut the multi-layered dough sheets to make the noodle stripes or noodle squares
Noodle stripes and noodle squares are then sold fresh

FIGURE 157.17 Sheeting of dough to 1.0 mm thick sheet.

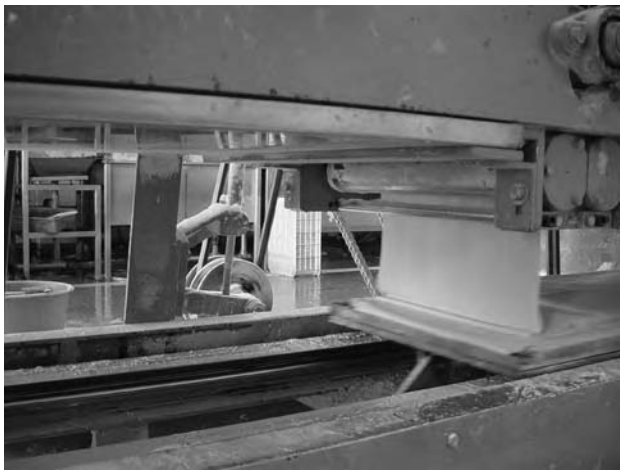

FIGURE 157.18 Dough sheets folded back and forth before cutting.

factory. Figures 157.19 to 157.22 show the unique steps in the manufacture of such product.

Another oily product is the Cantonese oily rice-based "ho fen" (rice stripes). It is produced first by preparing a rice

TABLE 157.11
Generalized Production Scheme for Fresh Oily Noodle Production

Weigh out appropriate amount of medium strong wheat flour (9–10% protein)
Weigh out appropriate amount of sodium carbonate (about 0.35 to 0.5%)
Mix wheat flour and sodium carbonate with water (about 32–36%) to make a weak dough
Leave the dough for maturation for about 30 minutes
Sheet and roll the dough to desirable thickness (about 2 mm)
Cut the dough sheets into noodle stripes (about 2–3 mm wide)
Steam the noodle strips for about 1.5 minutes until the noodle stripes are completely cooked
Cool the cooked noodles to room temperature
Mix the cooked noodles with small amounts of yellow coloring and liquid oil
Package the oily noodles in suitable containers for marketing

FIGURE 157.19 Cutting of dough sheets into noodle shape.

FIGURE 157.20 Cut noodles on conveyor belt.

FIGURE 157.21 Cooling of cooked noodles for a short time in a water bath.

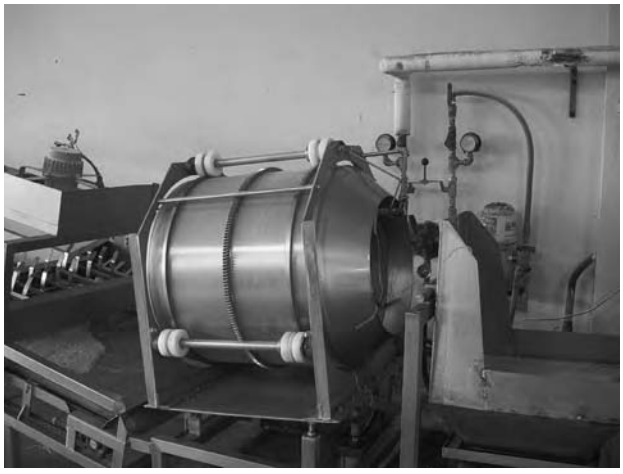

FIGURE 157.22 Mixing of cooked noodles with liquid oil in a rotating drum.

slurry from rice flour, followed by steaming a thin layer of the slurry on an oil-coated stainless tray or bamboo sheet. The gelatinized "fen" is then folded into layered slabs, followed by cutting of the slabs into stripes. These noodles are much thicker and broader than the Chinese "hand-cut mien" (see later) or Japanese "udon." Oily rice-based "ho fen" is very soft and smooth in texture. The granular size of rice flour used has a definite effect on the quality of the final product, as the difference in granular size can be detected easily. The original "ho fen" was made with wet-milled rice flour with a very fine texture. However, it is much more costly to make, and has the liquid waste disposal problem similar to dry rice noodles (rice sticks). Table 157.12 describes the basic steps in the making of Cantonese "ho fen."

Original Chinese hand-swung La Mien (La Mian) was made by skillful masters or chefs. They are considered a delicacy, as they take skill to make. The hand-swung La Mien is considered a fresh "mien," as it is cooked right after making. The addition of alkaline agent(s) is optional. Table 157.13 describes the procedures used in the making of hand-swung La Mien. It should be noted that the process can be modified to make the production less labor intensive, as described earlier.

TABLE 157.12
Generalized Production Scheme for Fresh Cantonese Oily "ho fen" (Thick Rice Stripes)

Prepare a rice slurry with rice flour and water.
Put a small amount of oil on stainless or bamboo trays to coat the trays evenly.
Pour rice slurry on the trays to form a thin layer (about 1–2 mm thick).
Place the trays on racks in the steamer.
Steam the trays of thin layers of rice slurry to gelatinize the starch.
Remove the trays from the steamer and cool.
Roll up the gelatinized rice sheet from each tray with a spatula (about 10 cm in depth).
Cut the layered rice sheets into 1 cm wide stripes ("ho fen").
Wrap the rice "ho fen" in paper.

TABLE 157.13
Generalized Scheme for Production of Hand-Swung La Mien in China

Weigh out medium strong wheat flour (about 10% protein, 1 kg), salt (20 g), water (550 g), and sodium carbonate (10 g)
Put wheat flour in a bowl
Dissolve salt in 500 ml (500 g) water with temperature at about 25°C (summer) to about 35°C (winter)
Add salted water gradually to the flour
Mix wheat flour and salted water to a crumbly consistency and knead the dough until uniform
Cover the dough with a clean cloth and rest for 30 minutes at 20–30°C
Dissolve sodium carbonate in 50 ml (50 g) water
Add the sodium carbonate solution to the rested dough and knead the dough until the sodium carbonate solution is uniformly distributed into the dough
Roll the dough into a long rope
Hold each end of the dough rope with each hand and swing the dough rope up and down
The elongated dough rope is folded and twisted
Repeat the swinging, folding and twisting 6 to 7 times to create a uniform dough piece
Place the dough piece on a table and dust the dough rope evenly with wheat flour
Hold the two ends of the dough rope with one hand and insert the four fingers of the other hand into the loop
Stretch by gently shaking the rope until the rope is 10–16 cm long
Repeat the dusting and stretching until 6 stretches with 64 strands of La Mien is achieved (for very thin La Mien, up to 12 stretches are used)
The La Mien then can be put into boiling water for a few minutes before consumption by mixing with other ingredients

Adapted from Refs. 1, 4, 5.

Noodles of various shapes other than the traditional stripes are made commonly by the Chinese people, especially those in the rural areas in the northern provinces. Table 157.14 describes three common Chinese hand-cut "mien." The addition of whole egg, egg white, or egg yolk is optional, depending on consumer preference in different locations. The hand-sheeted and cut noodle is the most common. "Cat's ear mien" and "knife-cut mien" are less common, and are considered specialty products. It should be noted this "knife-cut mien" has a thicker center, providing a special *al dente* texture.

VI. MANUFACTURE OF PRE-COOKED ASIAN (ORIENTAL) NOODLES

In recent years, precookd, ready-to-eat udon in sterilized pouches has been available. The cooked udon is sealed with water in pouch-type containers with lactic acid or sodium benzoate used as preservatives, and sterilized for long term storage.

Frozen precooked "saimen" (alkaline noodles) have also been available for some time. The "saimen" is precooked in water or steamed, cooked, cooled, packaged and sealed in plastic bags before freezing. The shelf-life of this product is good.

TABLE 157.14
Generalized Scheme of Hand-Cut Wheat-Based Noodles

Hand Sheeted and Cut Noodles
Weigh out special wheat flour (1000 g), cold water 400 g, and potato starch (50 g)
Mix the flour with cold water to make a dough
Knead the dough to a smooth and even condition
Flatten the dough with a rolling pin on a smooth surface into a rectangular shape by rolling in different directions to extend the dough evenly
Dust the dough sheet with potato starch
Repeat the rolling and dusting several times to stretch the dough to about 3 mm thick
The dough sheet is then folded accordian-like before cutting to 1.5 mm wide
The noodles are shaken to remove the potato starch
The noodles are then cooked in boiling water until done before consumption by mixing with other ingredients
Cat's Ear Noodle (Mao-er-dao)
Procedure similar to above for making dough
Cover the dough with a damp cloth and let stand for 20 minutes
Sheet and roll the dough to 1 cm thick
Cut the sheets into 5 cm parallelograms or squares
Pinch the small slanted squares into the shape of a cat's ear before cooking in boiling water
Knife-Cut Noodles (Dao Xian Mian, Paring Noodles with a Knife)
Weigh out appropriate amount of wheat flour (e.g., 600 g)
Mix the flour with appropriate amounts of salt (5 ml) and water (10 ml)
Knead the wheat flour, salt and water into a stiff dough cylinder
Hold the dough cylinder in one hand and pare long noodle stripes out of the dough with a knife. (Note: The stripes are not of even thickness, thicker in the center and thinner on the sides.)
Cook the noodle stripes in boiling water

Adapted from Refs. 3, 4, 5.

VII. MANUFACTURE OF SELECTED NON-WHEAT- OR RICE-BASED ASIAN (ORIENTAL) NOODLES

For decades, there have been products on the market that are made from materials other than wheat or rice, but are included in the Asian (oriental) noodle category. Table 157.15 lists some of these examples with their ethic origin and main ingredients. These products are sometimes called "starch noodles" as they are made from starch of various origins.

One of the most fascinating Asian (oriental) noodles made from materials other than wheat or rice are mung bean threads, sometimes called cellophane or transparent noodles by Westerners. This product has the unique property of being transparent like clear cellophane after it is cooked. The best mung bean threads can stay in their original shape and remain intact for about 2 days after being cooked and kept in the soup. This is because of their unique starch gelling properties, which also provide very good *al dente* properties. Table 157.16 describes the procedures used to make traditional mung bean threads.

True (pure) mung bean threads are made from mung bean only. However, the products today on the market may contain mung bean and other starch materials like broad beans and other starches. The bottlenecks of making true mung bean threads include the intensive labor required and liquid waste disposal. The liquid waste is fairly rich in nutrients as it contains all the vitamins, minerals and proteins in the mung bean. In the past, this waste was used as animal feed. Attempts have also been made to recover the protein from this liquid waste. Because of these problems, it is understandable that materials other than mung beans, and improvements in technology are being considered.

Korean sweet potato vermicelli ("dang myun") is a product similar to mung bean threads with transparency after it is cooked. However, the vermicelli is colorless, but kind of light brownish green. It also has very good *al dente* properties. Japanese "harusame" is also a similar starch noodle product as it is made also made from starches from potato, sweet potato, rice or mung bean.

Another unusual Asian (oriental) noodle is the translucent Japanese "Shirataki" noodle made from devil's tongue yam (elephant yam or konjac/konjak yam). This product is marked as a form different from others. It stays in liquid in a sealed container, is pasteurized and has to be kept refrigerated. This product is considered a low-calorie

TABLE 157.15
Oriental Noodles Made from Materials other than Wheat Flour and Rice Flour

Name	Ethnicity	Main Ingredient(s)
Bean threads	Chinese	Mung bean starch
cellophane noodles		
transparent noodles		
Chinese vermicelli		
fen si, fen sai, fun sai		
soo hoon, suhoon		
green bean thread noodles		
tung boon		
su un	Indonesian	
panit sotanghon	Tagalog (Philippines)	
won sen	Thai	
bun tau	Vietnamese	
tanghoon	Malaysian	
Harusame	Japanese	Potato, sweet potato, rice, or mung bean starch
Sweet potato vermicelli	Korean	Sweet potato
dang myun (tang myun)		
Korean buckwheat noodles	Korean	Buckwheat, potato starch
naeng myun		
Shirataki	Japanese	Devil's tongue yams
sirataki		
ito konnyaku		
yam noodles		
devil's tongue noodles		
Soba	Japanese	Buckwheat, common wheat
Buckwheat noodles		
Tapioca sticks	Vietnamese	Tapioca starch
tapioca starch noodles		
hu tieu bot loc		
Tientsin fen pi (sheets)	Chinese	Mung bean

TABLE 157.16
Generalized Scheme for Traditional Production of Dry Mung Bean Threads ("fun see," Cellophane, or Translucent Noodles)

Clean mung beans to remove foreign matter
Soak mung beans in water for 4 to 5 hours in summer and 10 hours in winter
Finely grind the soaked mung beans with added water using a stone grinder
Dilute the bean slurry with three times the amount of water and let sit for about 8–9 days to ferment (dissolving the nitrogenous and other undesirable matter)
  Remove the liquid when it gets foamy, leaving the sediment at the bottom
  Add clean water back to the sediment and mix
  Repeat the process for 7–8 days until the mung bean starch is pure
Filter out the mung bean starch in cloth bag by gravity
Divide the mung bean starch into two portions
Add small amount of cold water to the first half of mung bean starch to make a slurry
Add boiling water to make a thin paste
Mix in the second half of mung bean starch with stirring to form a sticky and elastic paste
Transfer the sticky and elastic paste to a perforated funnel
Press the paste in the funnel so that the paste is extruded out through the small openings
Drop the extruded mung bean threads into boiling water immediately and gelatinize the starch into transparent threads
Scoop out the transparent mung bean threads and spread them on bamboo trays, keeping the threads in an orderly arrangement
Sun dry the mung bean threads to dryness
Package the dried mung bean threads into suitable containers for retail or storage

food as it utilizes the gums (hydrocolloids) in the devil's tongue yam as the main structural material. Fresh elephant yam contains glucomannan (a soluble dietary fiber) and starch at a ratio of about 2:1, and has excellent water holding capacity. It is popular in Japan and Taiwan, and also available in the oriental markets in the US. Elephant yam is the original material used to make Konjac/kojac gum, a GRAS food ingredient.

VIII. FACTORS AFFECTING PRODUCT QUALITY OF ASIAN (ORIENTAL) NOODLES

In the manufacture of Asian (oriental) noodles, one or more of the following common procedures are applied, depending on the kinds of product to be produced:

- Selection of raw materials
- Mixing of ingredients to form a dough, slurry or paste
- Resting of dough
- Sheeting and rolling
- Extruding
- Shaping
- Pressing of slurry or paste through perforated funnel
- Steaming
- Boiling of cut noodle stripes
- Frying of noodles
- Cooling
- Drying of final product

In addition to the above-mentioned variables in a manufacturing process, the producer has to consider production cost, environmental issues, consumer preference, and market competition, as well as proprietary formulations and practices. This makes it very complicated to compare quality of similar types of product. Scientific measurements are helpful, and these have been applied. However, a food technologist or scientist is more interested in the composition of ingredients, kinds of oil used and amount absorbed, application of food additives to prolong shelf-life, rheology of dough, consistency of slurry, work needed to knead the dough, and other factors. Table 157.17 is a summary of the major factors that could affect the quality of Asian (oriental) noodles based on the literature published (9–37). The list of references at the end of this chapter does not answer all the questions a food technologist or scientist wants to ask, but it will provide some indications of what is known and what needs to be studied in the future.

Asian (oriental) noodles, with beginnings in a primitive, cottage-type industry, is now a big industry, especially for instant noodles. These noodles have captured the attention of consumers worldwide. With the improvement in technology, it is expected that this industry will grow further and gain further acceptence by more consumers in various regions.

TABLE 157.17
Factors Affecting Product Quality of Oriental Noodles

Group I. Ingredients	
	Wheat flour used (especially protein content)
	Buckwheat flour used
	Rice flour used (especially amylose to amylopectin ratio)
	Mung bean used
	Water quality
	Amount of salt used
	Kinds of frying oil used
	Amount of coating oil used
	Amount and kinds of alkaline salts used
	Kinds and amount of additional ingredients used (starchy materials, savory ingredients)
Group II. Dough Quality	
	Rheology of dough
	Viscosity of slurry
Group III. Processing Conditions	
	Mixing of ingredients
	Kneading of dough
	Drying temperature, duration and condition
	Dough resting condition and duration
	Cutting actions for dough or noodle stripes
	Stretching actions on dough
	Relative humidity in drying chamber and environment
	Steaming temperature and duration
	Frying temperature and duration
	Sheeting and rolling actions
	Starch extraction conditions
	Extruding condition
	Cooking conditions

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158 Extruding and Drying of Pasta

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CONTENTS

I. INTRODUCTION

Before the 1800s, pasta was made by hand. The first mechanical devices for pasta manufacturing were invented in the 1800s (1). Around 1850, the first hand-operated pasta press was built. By the early 1900s, mixers, kneaders, hydraulic piston-type extrusion presses, and drying cabinets were available for batch manufacturing of pasta. In 1933, the first continuous single-screw pasta press was invented. Before 1974, pasta was dried using low temperature drying profiles that mimicked open-air drying conditions typical of the region around Naples, Italy. It required 18 to 20 hours to dry pasta when using a low temperature drying profile. High temperature drying (60 to 80°C) of pasta was introduced in 1974 and ultra-high temperature (80 to 100°C) drying was introduced in the late 1980s. Drying at high or ultrahigh temperatures has reduced drying time of long goods (e.g., spaghetti) to about 10 and 6 hours, respectively. Today, pasta manufacturing is totally automated with pasta presses capable of producing spaghetti at 3,500 kg/h and macaroni at 8,000 kg/h.

II. INGREDIENTS

A. Semolina

1. Durum Milling

Semolina is the primary product of durum milling. A durum mill consists of a break-roll system, a sifter system, and a purifier system. Each break-roll consists of a pair of corrugated rolls that rotate at different speeds. The speed differential between the two rolls, typically 2.5:1, provides a shearing action that removes the bran and germ from the endosperm (2,3). Granulation of semolina is dependent on the number of corrugations per unit length. The sifter system sizes the granulated material through a series of sieves and returns large particles to a break-roll for further reduction. The fine material is collected as flour. The intermediate material goes to the purifier system, which uses air and sieves to further size and clean the semolina. Aspiration separates bran particles and other impurities from the semolina. Bran has a greater surface area to unit weight than semolina. Sieves associated with the purifier system size the semolina into specific particle size ranges (millstreams).

The final granulation depends on the durum mill and the millstreams collected. The coarse granulation of semolina results in better flow properties than does the very fine particle size of flour ($\leq$212 μm). Federal Code of Regulation (4) defines semolina as the food prepared by grinding and bolting cleaned durum wheat to such fineness that, when tested by a prescribed method, it passes through a No. 20 sieve (840 μm), but not more than 3% passes through a No. 100 sieve (149 μm). It is freed from bran coat, or bran coat and germ, to such an extent that the percent of ash therein, calculated to a moisture-free basis, is not more than 0.92%, and its moisture content is not more than 15%.

Semolina used for pasta processing is much smaller than the 840 μm maximum size and typically ranges in particle size from 450 to 150 μm. The particle size distribution of semolina from five commercial mills shown in Table 158.1 indicates that 60 to 70% of the semolina granules are between 425 and 250 μm. The short mixing times used by new pasta presses require a fine granulation because small granules hydrate quicker than large granules. However, the hard grinding required for fine granulation can result in starch damage, which can increase cooking losses and decrease cooked firmness (5). Uniform granulation is important for even hydration. Hydration of semolina having a wide range of particle sizes will tend to result in the small particles absorbing too much water and the large particles absorbing too little. Both over-hydration and under-hydration will adversely affect dough development and will result in poor pasta quality.

2. Semolina Quality

Semolina quality is determined by speck count, color, grit content, ash content, moisture content, protein content and quality, and microbial load. Defects in semolina are directly transferred to the pasta product (6,7).

a. Physical quality

Speck count is a quality control measure for semolina commonly determined at the mill. Specks in semolina are generally brown or black. Bran is a common source of brown specks. Black specks originate from bran of diseased kernels, weed seeds, dirt, and insect parts. Black specks are more noticeable than brown specks in semolina and in pasta. Speck counts of five commercial semolinas ranged from 17 to 30 specks/10 in^2 (Table 158.1). Speck counts greater than 40 specks/10 in^2 are considered undesirable (8). High speck counts indicate a possible problem with grain cleanliness and grain quality and/or with the mill.

Speck count is typically determined by a visual count of specks in a given area. The visual count of specks in semolina is generally determined under a constant light source for a fixed area of semolina that has been packed flat. Visual speck counts can vary greatly depending on the individual (9). Some mills have begun using digital image analysis to determine speck count (9,10).

Ash content reflects the purity of the semolina. Semolina with high ash content generally will have a high speck count. Bran, weed seeds, and soil are high in ash.

TABLE 158.1
Quality and Particle Size Distribution (%) of Semolina from Different Mills[1,2]

Commercial Sample	Mesh size, μm[1] 600	425	250	180	149	<149	Protein (%)	Ash (%)	Specks no/10 in^2
A	0.2	12.6	61.9	17.0	4.0	2.0	12.7	0.78	24
B	0.3	18.0	53.7	17.8	3.8	3.0	11.9	0.74	21
C	0.0	13.5	69.8	13.0	1.6	2.5	13.8	0.79	17
D	0.0	12.2	69.8	13.8	1.6	2.6	12.7	0.74	23
E	0.8	29.3	50.6	14.5	1.3	2.8	13.7	0.77	30

[1] Corresponding mesh size and sieve number: 600 μm mesh = No. 30 sieve; 425 μm mesh = No. 40 sieve; 250 μm mesh = No. 60 sieve; 180 μm mesh = No. 80 sieve; 149 μm mesh = No. 100 sieve.

[2] Protein and Ash based on 14% moisture.

For this reason, ash is commonly used in semolina specifications to ensure low contamination. In general, ash contents up to 0.80% are acceptable (11).

Grit is metal, stone, or glass particles found in semolina. Grit can clog screens, block the die orifice, and/or damage the Teflon coating of the die. Damage to the die will result in defects in pasta such as grooves or tears. Grit that contaminates pasta poses a health threat as it can damage consumers' teeth.

b. Compositional quality

Semolina contains up to 80% starch and 2 to 3% nonstarch polysaccharides (12,13). Durum starch is composed of 70 to 75% amylopectin and 25 to 30% amylose (14,15). The impact of variations in amylopectin-amylose ratio on pasta extrusion is probably minimal, since dough temperature during extrusion ranges from 45 to 50°C, which is below gelatinization temperatures for durum starch. Starch is important in determining cooking quality of pasta (16), as variations in starch properties impact water uptake, gel consistency, and gluten matrix integrity during cooking.

Starch damage in the semolina is a result of milling and of α-amylase catalyzed breakdown of starch during preharvest sprouting. Starch damage after milling is generally $\leq$5% (2,17). Some starch damage can occur during extrusion (18,19). Damaged starch is associated with increased water absorption and increased cooking losses.

Nonstarch polysaccharides are primarily composed of arabinoxylan. Nonstarch polysaccharides have high water binding capacity. For example, nonstarch polysaccharides isolated from hard red spring wheat absorbed 6.3 to 6.7 times their weight of water (20).

Semolina contains 1 to 2% lipid (21). Semolina lipids affect cooking loss and are important for color. Fatty acids can complex with amylose, which reduces water solubility of amylose and lowers cooking loss. Carotenoid pigments, particularly xanthophylls and lutein, are found in semolina and give pasta its characteristic yellow color (22,23). Semolina color can be evaluated by quantifying pigment concentration or by using a reflectance colorimeter. Johnston et al. (24) reported that reflectance values corresponded well with spectrophotometer values for pigment content. The apparent color of semolina varies with particle size. The yellow appearance decreases with a decrease in particle size, even though the yellow pigment content remains the same. The decrease in yellow color is due to greater light scattering with small than with large particles.

Pasta quality is greatly affected by semolina protein content and quality. Semolina typically contains 12 to 16% protein (25,26). For good pasta quality, protein content of at least 12% on a 14% mb is preferred. The target minimum used by the durum wheat breeding program at North Dakota State University for advancing durum lines is 12.5% protein on a 14% mb.

Semolina protein is composed of about 20% metabolic proteins (enzymes) and about 80% storage proteins. Storage proteins are composed of two classes of proteins, gliadins and glutenins, which, when hydrated and mixed, form gluten. Gluten gives dough its unique viscoelastic structure.

Gliadin proteins are monomeric and range in size from 30,000 to 80,000 kDa (27). Gliadin proteins provide cohesion to the gluten matrix and are responsible for extensibility and viscosity of gluten (28,29,30).

Glutenins are polymeric proteins consisting of subunits ranging in size from 12,000 to more than 130,000 kDa and can form polymeric protein complexes with molecular weights ranging from a few hundred thousand to several million (31). Unlike gliadins, subunits of glutenin proteins are capable of aggregation due to the formation of intermolecular disulfide bonds between subunits (32). Glutenins are responsible for the strength and elastic properties of gluten (33).

c. Gluten/dough quality

Gluten quality is assessed by the wet gluten/gluten index (approved Method 38-12) and sodium dodecylsulfate (SDS) microsedimentation tests (34,35). The wet gluten/gluten index test involves washing semolina with a 2% salt solution. During washing, the semolina is kneaded to develop the gluten and to help remove the starch. After washing, the remaining wet gluten is centrifuged in a special centrifuge tube that contains a perforated plate. Gluten index is the ratio of the weight of gluten remaining on the top of the perforated plate divided by the weight of the wet gluten. The stronger the gluten, the less likely the gluten will be forced through the perforations during centrifugation. Gluten index has been used to identify weak, strong, and very strong gluten lines (36). In general, gluten index values $<$5 indicate weak gluten and gluten index values $\geq$85 indicate very strong gluten. Durum grown in North Dakota and Montana typically has gluten index values of 45 to 55.

The SDS microsedimentation test involves hydrating ground durum wheat in a SDS/lactic acid solution. Proteins of high molecular weight and those having strong interactions with starch are insoluble in the SDS/lactic acid solution and form a sediment. The height of the sediment is measured. A high SDS microsedimentation value is associated with good protein quality. A SDS microsedimentation value $<$30 indicates weak gluten while a value $\geq$35 indicates strong gluten.

Dough properties of semolina generally are assessed by mixograph in North America and by farinograph or alveograph in other parts of the world. Mixograph records the torque transmitted through the dough, which is recorded by a pen on chart paper (37). Mixograms from semolina of four cultivars grown in the Northern Plains are presented in Figure 158.1. Mixogram peak represents maximum dough

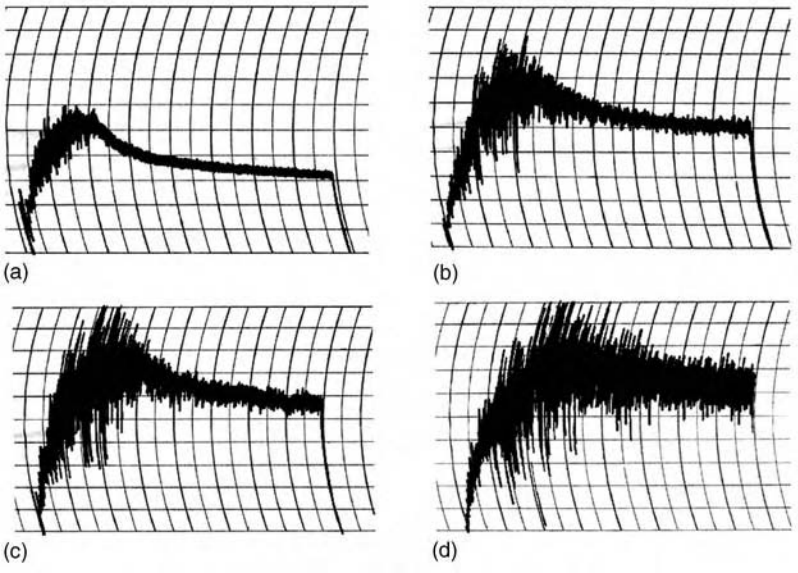

FIGURE 158.1 Mixograms for semolina from durum wheat cultivars: Rugby (a), Munich (b), Lebsock (c), and Belzer (d). Durum wheat cultivars were grown in North Dakota.

development. Time to peak gives an indication of time required to fully develop the dough. The drop in the curve after the peak gives an indication of dough stability. Both peak height and width of the curve at peak reflect dough strength. Rugby (Figure 158.1a) is known to be a weak gluten cultivar. Even though Rugby has excellent protein content, it has weak dough properties (low peak height and narrow band width) compared to Belzer (Figure 158.1d). Most cultivars grown in North Dakota, Montana, and Canada are considered to have strong gluten, and produce mixograms similar to Munich and Lebsock (Figure 158.1b,c).

B. Other Ingredients

Other ingredients sometimes are added to semolina to improve its nutritional quality or to make specialty pastas. In the United States, pasta is enriched with vitamins (thiamin at 8.8 to 11 mg/kg, riboflavin at 3.75 to 4.85 mg/kg, niacin or niacinamide at 59.4 to 74.8 mg/kg, and folic acid at 1.98 to 2.64 mg/kg) and minerals (iron at 28.5 to 36.3 mg/kg and calcium (optional) at 1100 to 1375 mg/kg). Wheat bran, oat flour, and barley flour are examples of materials added to pasta to increase dietary fiber content (38,39,40). Flours of edible legumes, buckwheat, amaranth, and lupin have been added to improve the content and nutritional quality of protein in pasta (41,42,43). Vital wheat gluten, disodium phosphate, surfactants, and lipids have been added to improve cooking or textural quality of pasta, particularly pasta that is refrigerated, frozen, or canned (44,45). A variety of non-wheat and non-cereal products have been added to pasta to improve its nutritional quality (46). Code of Federal Regulations (21 CFR Part 139) provides a list of ingredients that can be added to pasta products (4).

C. Water Quality

Water used in pasta processing should be pure, without chemical (including heavy metals) or bacterial contamination, have no off-flavors, and be slightly acidic, pH 6.6 to 6.9 (44). Water can have a maximum mineral content of 400 to 500 mg/L. Presence of iron salts should be avoided. Mineral impurities should not exceed 180 to 200 mg/L calcium and magnesium carbonates, 70 to 90 mg/L sulfates, 25 to 30 mg/L silicates, 5 to 10 mg/L chlorides, and 10 to 40 mg/L of organic matter (44,47,48).

III. PASTA PROCESSING

Modern pasta presses are capable of producing 3,500 kg/h of long goods (spaghetti, vermicelli, and linguine) and up to 8,000 kg/h of short goods (macaroni, rigatoni, and ziti). Pasta processing can be divided into four stages: mixing, kneading, shaping, and drying.

A. Mixing

1. Hydration

The goal of the mixing stage is to uniformly blend and properly hydrate ingredients. Semolina is typically hydrated to 30 to 32% moisture content. The hydration level often has to be adjusted higher or lower to obtain proper consistency of dough containing nontraditional

ingredients during extrusion (42,49). When nontraditional ingredients are present, the amount of water added will depend on the overall moisture content and the water binding properties of the various ingredients. For example, nonstarch polysaccharides have a high water binding capacity, which can affect water distribution in dough systems during pasta processing and drying. Due to high levels of nonstarch polysaccharides in bran, whole wheat and bran-semolina mixtures can require greater hydration to achieve proper dough development during extrusion than does semolina. Thus, it might take more water and time to properly hydrate whole wheat, compared to semolina, during the hydration/mixing stage of pasta processing (49,50,51).

Rate of hydration is promoted by warm water, warm semolina, and small semolina particle size. Semolina temperature is generally only a concern in winter, as semolina stored in unheated bins can be very cold. Cold temperature will slow the rate of hydration. Semolina containing fine granulation will hydrate quicker than semolina with coarse granulation, because of the increased surface area and increased starch damage associated with small granulation.

Uniform granulation is important for proper hydration. When semolina contains both large and small particles, the small particles tend to over-hydrate and large particles tend to under-hydrate. Over-hydration results in a soft, sticky dough. Pasta extruded from over-hydrated semolina requires more energy to dry and can stretch and stick together on the drying rods. Under-hydration results in a stiff dough which requires more energy for extrusion, generates more heat during processing, and can result in breakage problems for long goods hung on drying rods, as well as cutting problems for short goods.

Complete hydration of the semolina particles is very important for proper dough development. Protein must be hydrated before gluten can form. As the storage proteins (gliadin and glutenin) hydrate, they change their conformation and begin to unfold and interact by forming intra-molecular and inter-molecular bonds. Without adequate hydration, regions in the dough and pasta will exist where no gluten is formed, which results in discontinuity of the gluten matrix. These regions of discontinuity are areas of structural weakness and will appear as white starchy areas in extruded pasta.

2. Mixing Equipment

The mixer is divided into two sections: premixer and main mixer. The flow of dry ingredients into the premixer is regulated by volume or weight using a volumetric or gravimetric feeder, respectively. Warm water (35 to 40°C) is sprayed onto the semolina in the premixer. It is important that the water not be sprayed onto the metal parts of the premixer, as semolina will accumulate on wet metal. Ingredient buildup in the mixer represents a potential source of microbial activity. High speed premixers are commonly used to rapidly and evenly coat the semolina particles with water before entering the main mixer.

The principal function of the main mixer is to provide time for thorough hydration. Most mixers contain two counter-rotating parallel shafts with paddles set so that they move the hydrated material forward (Figure 158.2). The retention time in the mixing chamber depends on the speed of the paddles and the length of the mixing chamber. The time must be sufficient to allow for proper absorption of water by the semolina or semolina-ingredients. Conventional mixers have a retention time of 10 to 20 min. New systems are being developed that reduce the retention time to 2 to 3 min or less (52). Pasta lines using short retention time mixers require fine granulation of semolina to achieve rapid hydration.

Most dry pasta manufacturers apply a vacuum (−63 to −80 kPa) either at the mixer or just before the extrusion barrel (53). Vacuum promotes hydration by eliminating surface tension associated with air and reduces pigment oxidation by lipoxygenase enzymes (23). Vacuum also prevents air from being trapped inside the developing dough. Air trapped inside extruded pasta will appear as a series of very fine bubbles resulting in a hazy appearance. If dried at high or ultra-high temperatures, these bubbles will act as focal points for stress and ultimately result in checking of the finished pasta.

Fresh or frozen pasta manufacturers generally do not use a vacuum system in the process. Their product is opaque. The air bubbles in the product do not seem to have any significant impact on the end product appearance or cooking quality. The added equipment to produce a vacuum, combined with the maintenance required, do not seem to justify the cost.

B. Extruding

Hydrated semolina passes through several zones in the extruder: conveying, compacting, kneading, relaxing, and

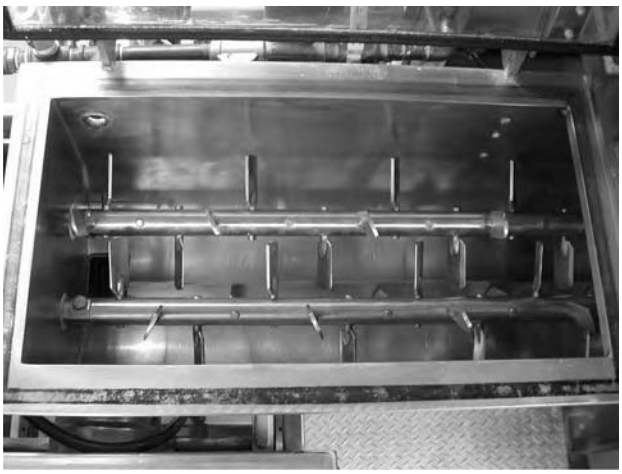

FIGURE 158.2 Mixing chamber. (Photograph courtesy of Northern Crops Institute, Fargo, ND.)

extruding. Conveying, compacting, and kneading are associated with different regions of the extrusion screw. At the end of the screw, an extension tube often is used to allow the dough to relax before being extruded through the die.

1. Screw Design

The extrusion screw is made of stainless steel or chrome-plated steel. Traditionally, extrusion screws used in pasta processing were deep flighted with constant root diameter and uniform pitch the entire length of screw (Figure 158.3). Deep flights provided high screw conveying capacity and allowed high back pressure flow in the screw. Harper (53) reported a flight angle of 12 degrees for pasta extrusion. A sharper flight angle would increase mixing and decrease the conveying efficiency. Extrusion screws are designed with length-to-diameter ratios between 6:1 and 9:1 (53). The long length-to-diameter ratio results in low mechanical energy/unit throughput. Screws used to extrude pasta typically have diameters ranging between 12 to 20 cm.

Extrusion screws have become more sophisticated. Some newer pasta presses have screws with variable pitch and variable root diameter. These screws often begin with a large dough cavity and gradually get smaller in pitch and larger in root diameter toward the end of the screw (52). As pitch decreases, the number of flights on the screw increases, which increases the screw surface-to-volume ratio and increases the conversion of mechanical energy to heat through friction. Similarly, increasing root diameter decreases flight depth, which increases the amount of energy and pressure applied to the dough. Thus, the new screws increase the amount of work applied to the dough, which has allowed a reduction in screw speeds from 20 to 40 rpm with traditional presses, to typically 18 rpm, and still maintain output (52).

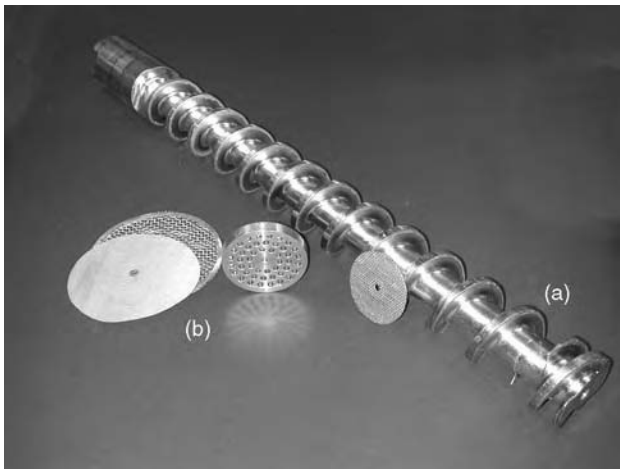

FIGURE 158.3 Extrusion screw (a) and kneading plate (b). (Photograph courtesy of Northern Crops Institute, Fargo, ND.)

2. Screw Function

The beginning of the screw is involved with conveyance. This part of the screw is not fully encased by the extruder barrel, but is exposed to allow the hydrated semolina from the mixer to be deposited onto the screw. The screw is choke-fed. The hydrated semolina is conveyed forward into the barrel as the screw turns.

Compaction occurs once the hydrated semolina is conveyed inside the barrel. Pressure rapidly increases from 0 to 2 MPa after about two turns of the screw (54). Hydrated semolina exposed to 2 MPa begins to transition from a granular material into a fully compacted dough. Temperature changes during compaction are due to heat dissipation by friction against the barrel, which is then transmitted to the hydrated semolina. Le Roux et al. (54) reported that temperature rise was localized near the barrel wall and that the average temperature increase was about 5°C for a channel length of 10 cm under typical experimental conditions.

After compaction, the remaining length of the screw is involved in kneading and conveyance of the dough toward the die. Pressure continues to slowly increase as the dough progresses toward the end of the screw. Deep flights provide high conveying capacity and high back pressure (up to 12.7 MPa) in the screw (48). At the end of the screw, pressure declines slightly as the dough moves into the extension tube, where it is allowed to relax before being extruded through the die. For a commercial press, the pressure at the die is generally 10 MPa.

3. Dough Flow

Dough is conveyed in the channel of the screw. Dough moves toward the leading edge of the flight next to the cylinder wall and flows away from the flight near the root of the screw, which causes the dough to spiral down the channel. Forward flow and back pressure act together to knead the dough. During kneading, the gluten molecules are stretched and aligned according to rotational movement of the screw (55).

The flow of dough is not uniform in the channel. Dough near the root of the screw moves much more slowly than the material near the cylinder wall (55,56). The material against the metal wall is worked and heated more than the material at the center of the screw channel. Le Roux et al. (54) did not observe any recirculating regions within the channel, but noted that a large zone existed at the channel bottom where the product moved very slowly, less than 10 mm/s when the barrel velocity was 50 mm/s.

The uneven flow rate results in irregular dough development in the extrusion screw. At the end of the screw, the protein matrix is irregular, but is interconnected with some alignment of starch granules along the direction of flow (55). To reduce the heterogeneity of the dough, some

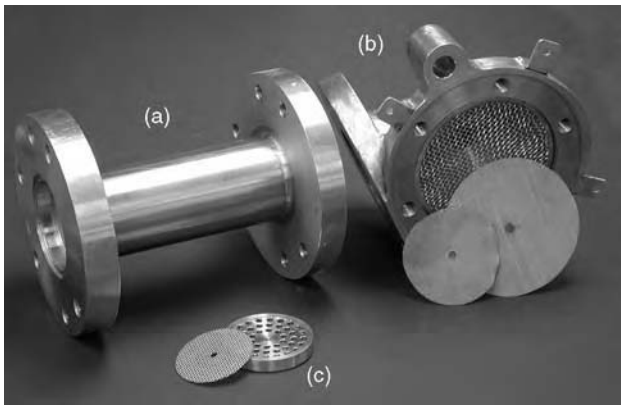

FIGURE 158.4 Extension tube (a), extrusion head (b), and kneading plate (c). (Photograph courtesy of Northern Crops Institute, Fargo, ND.)

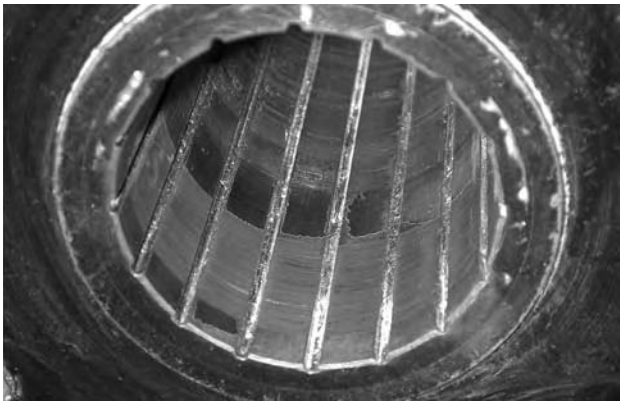

FIGURE 158.5 Longitudinal grooves machined along the inner surface of the extrusion barrel. (Photograph courtesy of Northern Crops Institute, Fargo, ND.)

screw designs include a cut-flight or a kneading plate at the end of the screw (Figure 158.3). Kneading plates are stainless steel with small holes. Kneading plates split the dough into streams that recombine on the other side of the plate. An extension tube is sometimes placed after the kneading plate when extruding long goods (Figure 158.4). The dough flowing through the kneading plate enters the extension tube, which allows the dough a brief rest before entering the extrusion head where the dough flow is diverted downward 90° and forced through a die. At the beginning of the extension tube, after going through the kneading plates, the protein matrix is quite continuous and starch granules are clearly aligned. By the end of the extension tube, the dough is translucent and cohesive and is considered fully developed (55).

Friction between the dough and the extrusion barrel and between the dough and the screw is necessary for compression of the dough and for conveyance of the dough through the extrusion barrel. Without friction, the screw would turn and the dough would remain stationary relative to the screw. To have proper conveyance of the dough, the friction associated with the barrel must be greater than the friction associated with the screw. Longitudinal grooves are machined along the inner surface of the extrusion barrel (Figure 158.5) to enhance the friction between the dough and the barrel. To reduce friction between the dough and the screw, the screw surface is made of polished stainless or chrome-plated steel. Experimental values of the coefficient of friction corresponding to the screw smooth surface have been estimated to be between 0.2 and 0.4. Le Roux et al. (54) reported that a screw with a deep flight and a uniform pitch and root diameter needed a barrel coefficient of friction greater than 0.5.

4. Frictional Heating

Heat is generated by friction between the dough and metal surfaces of the barrel and the screw. The target dough temperature during extrusion is 45 to 50°C (48,57). Protein begins to denature at dough temperatures >50°C. Denatured storage proteins (gliadins and glutenins) are unable to form gluten. High dough temperature during extrusion will cause a soft sticky product when cooked. Excess heat generated by friction during extrusion is removed by use of a water jacket which surrounds the extrusion barrel. A high volume of warm water is used to maintain both the barrel and dough temperature near 45°C. Circulating cold water in the water jacket would result in overcooling the dough at the barrel surface, which would adversely affect dough viscosity.

Frictional heating of dough can be calculated by knowing the moisture content of hydrated semolina and the specific mechanical energy (SME, kJ/kg) for pasta extrusion. Heat capacity of wheat dough can be estimated using the equation presented by Baird and Reed (58),

$$c_p\ (\text{kJ/kg°C}) = 1.44 + 2.74X_w$$

where c_p is heat capacity and X_w is moisture content.

If the moisture content of hydrated semolina is 31%, then the heat capacity of the hydrated dough would be 2.29 kJ/kg°C. If the SME is assumed to be 70 kJ/kg (59,60), then the heat generated during extrusion would be 70 kJ/kg divided by the heat capacity of the hydrated dough (2.29 kJ/kg°C) to give a temperature rise of 31°C. If the hydrated semolina entered the extrusion screw at 35°C, it would exit the extruder at 66°C. With a target dough temperature of 45°C, two thirds of the heat would need to be removed by a circulating water jacket. These results are similar to those reported by Harper (53) and Hoskins (56).

5. Dough Viscosity

Dough systems have non-Newtonian flow properties. Dough is a viscoelastic system. Viscoelastic properties of

dough are dependent on gluten strength of the semolina used, temperature, hydration, and amount of work applied to the dough. Dough viscosity decreases with increased temperature and hydration. Dough viscoelastic properties are decreased when overworked. This can be seen in the mixograms in Figure 158.1, where dough strength declines with time after reaching peak height.

Apparent viscosity of a dough system can be described by:

$$\mu_a = m v^{n-1}$$

where μ_a = apparent viscosity, m = consistency, v = shear rate, and n = flow index.

Using a dough made from semolina, Le Roux et al. (54) demonstrated that the power law remained valid over a wide range of shear rates, typically 0.1 to 1000 s^{-1}, which encompasses the range expected in pasta extrusion. A typical shear rate for a commercial pasta press has been estimated to be 5 s^{-1} (59). Power law (or flow) index, n, increased with hydration, but remained within 0.4 to 0.5 (54). Food extrudates exhibit flow indices between 0.25 and 0.5 (61). Within normal extrusion temperatures, 45 to 55°C, consistency, m, varies with hydration and temperature according to exponential laws.

6. Die Assembly

The die is attached to the end of the extrusion head (Figure 158.6). A die is composed of a support and multiple inserts (Figure 158.7). Die support is made from bronze or stainless steel. The support must be capable of withstanding a tremendous amount of pressure over time without yielding. Stainless steel can tolerate higher pressures, but tends to retain more heat than bronze (62). Most industrial die supports are made from stainless steel. Inserts are miniature dies that are housed inside holes that have been bored into the supports (Figure 158.7). Inserts are generally made from bronze, due to their low heat retention. The advantage of using inserts is that worn inserts can be replaced easily without having to return the entire die to the manufacturer.

The number of inserts in the die will determine production output and must be balanced with the pasta press. Too many inserts can weaken the die, which could cause the die to bend under pressure, could reduce the density of the product, and could cause strands to overlap during extrusion. Too few inserts can cause excessive back pressure that can damage the die and/or the extruder and can reduce production output.

Die supports are typically 100 mm thick, and their inserts are typically 20 mm thick (63). Thus, dough flows into the hole in the die support, then into and through the orifice in the insert. Pasta comes in hundreds of shapes, which are determined by the flow of dough through the die orifice during extrusion. For example, inserts for spaghetti contain a round orifice, whereas inserts for shells have a horizontal orifice that is slightly larger at the center. The dough will flow faster at the center than at the ends of the orifice in the shell die, which causes curvature (62,63).

The orifice in the insert is often coated with a fluorocarbon polymer such as Teflon. A Teflon-coated orifice will have a low coefficient of friction, which will reduce back pressure needed for extrusion and increase the rate of extrusion (53,56). Dies with Teflon inserts are used when a smooth translucent surface is desired. Surface texture affects cooking and culinary properties of the product. Pasta with a rough surface has a greater exposed surface area, which tends to absorb water quicker and retain more sauce when compared to pasta with a smooth surface.

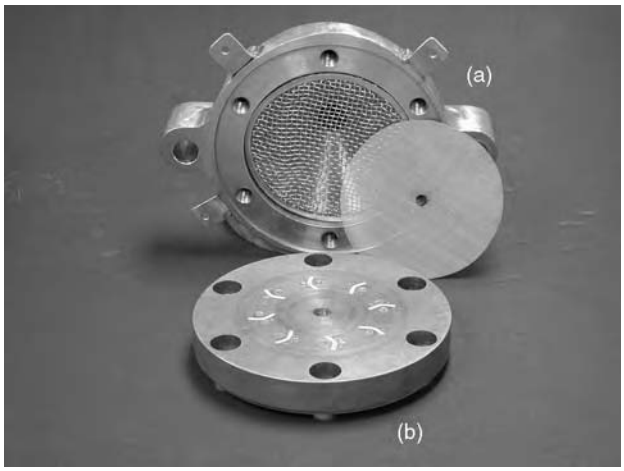

FIGURE 158.6 Extrusion head (a) and die (b). (Photograph courtesy of Northern Crops Institute, Fargo, ND.)

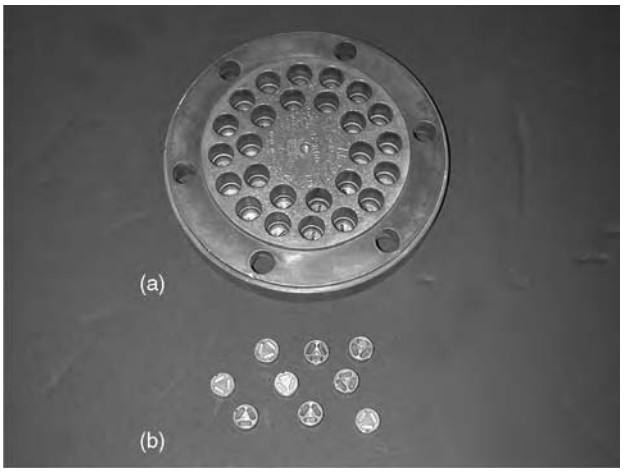

FIGURE 158.7 Die support (a) and inserts (b). (Photograph courtesy of Northern Crops Institute, Fargo, ND.)

The shape of the die support depends on the product produced. Short goods generally have a round die support, while long goods generally have a rectangular support. Extruded long goods are collected by a spreader, which spreads long goods on sticks and cuts the long goods from the die. Since product discharge is not uniform across the die, the strands are cut to a uniform length and the trim is reintroduced into the mixer via the trim return system. Pasta hanging on the sticks is conveyed to the dryer. Dried pasta is cut to length and the trim is reground and reintroduced into the semolina at amounts up to 15% (64,65). Extruded short goods are cut by a special rotary cutter with one or more blades. The cut pasta falls onto trays or a conveyer where it moves into the dryer. Short good length is determined by extrusion rate and speed of the rotary cutter. An uneven rate of discharge across the die will result in variable lengths of the short goods.

7. Extruder Output

In-depth mathematical descriptions and discussion of extrusion can be found in several references (53,66,67).

Extruder output is described by the following equation:

Extruder output = drag flow − pressure flow − leakage flow.

Drag flow is the forward movement of the dough due to the relative motion between the screw and the barrel (68). Drag flow increases with increased screw speed and flight depth to screw diameter ratio. Pressure flow is the backward flow of dough in the screw channel due to a pressure gradient. Leakage flow is the backward flow between the flights and the extruder barrel due to a pressure gradient. Pressure flow is proportional to the pressure gradient across the screw length (69). An increase in flight depth to screw diameter ratio increases drag flow more than pressure flow. Flight depth to screw diameter ratio for a commercial pasta press is typically 0.25 to 0.33 (59). Both drag flow and pressure flow increase with increased pitch angle (70). Leakage flow is related to pressure flow. Leakage flow occurs within the clearance between screw flights and the barrel and within the longitudinal grooves in the barrel wall. A typical clearance between the screw and barrel is 0.5 mm (66). The clearance will increase over time due to wear. An increase in clearance will be manifested by a decrease in output and an increase in energy transferred to the product, with associated increase in dough temperature.

Hoskins (56) estimated that the ratio between pressure and drag flow in a pasta press screw is about 0.6. If pressure flow and leakage flow are combined, then drag flow and pressure flow can be estimated if press output is known.

For example, assume that a pasta press extrudes 3,500 kg/hr of long goods.
Then: Drag Flow − Pressure Flow = 3,500 kg/hr and Pressure Flow/Drag Flow = 0.6.
Drag Flow × 0.6 = Pressure Flow
Drag Flow − Drag Flow × 0.6 = 3,500 kg/hr
Drag Flow (1 − 0.6) = 3,500 kg/hr
Drag Flow = 8,750 kg/hr
Pressure Flow = 5,250 kg/hr

The amount of energy transferred to the product during extrusion is designated as SME (kJ/kg). SME transferred to the pasta is calculated as the mechanical energy (kJ/s) to extrude pasta divided by the amount of pasta processed (kg/s). Mechanical energy required to operate the empty press is subtracted from the mechanical energy required to operate the press under load. Dough temperature and hydration level greatly affect SME required for extrusion. Abecassis et al. (60) reported that an increase in dough temperature or hydration decreased dough viscosity and subsequently decreased SME. A SME of 70 kJ/kg is typical for pasta extrusion, under normal operating conditions (54,59,60).

C. Pasta Drying

Like many other foods, pasta is dried to give a longer storage time. Shelf-life of pasta products is commonly listed as two years. Vitamins degrade over time. When the vitamin claim on the nutritional label is no longer valid, then the processors must declare the pasta expired. Therefore, fortification level and the levels claimed on the label determine the shelf-life.

Typically, industry will dry pasta to 12% moisture, although the Federal Code of Regulations (4) allows the moisture content to be as high as 13%. Pasta is a difficult product to dry. Pasta's low moisture, coupled with dimensional changes during drying, can result in checking (stress cracks in the product). Checking occurs when the stresses in the product exceed the strength of the pasta. As drying temperatures increase, the properties of the pasta change, due primarily to inactivation of enzymes and protein denaturation. A list of changes can be found in *Pasta Technology Today* (47) and in *Pasta and Noodle Technology* (71).

The pasta drying process is generally divided into three main stages: predrying, final drying, and cooling/stabilizing stages.

1. Drying Stages

a. Predrying

The predrying stage begins when the product exits the die. Typically, ambient air (possibly heated) flows at the face of the die, which provides some surface drying of the

product. This is referred to as the initial predrying. Initial predrying serves several purposes. The primary purpose is to dry the surface of the product sufficiently to prevent pieces from sticking together. Initial predrying increases the rigidity of the pasta surface, which minimizes collapsing or deforming of hollow products (such as elbows, shells, and ziti) when they drop onto a solid surface or have the weight of other pieces resting on them. The amount of moisture removed during the initial predrying step is small (<1%), but is a necessary step for maximum pasta quality.

After initial predrying at the die, the product is conveyed to the predrying section of the dryer. In the case of long goods, the product is collected onto rods, which are mechanically conveyed directly into the dryer. In the case of short goods, the product can either be dropped directly into the dryer (if the extruder is positioned above the dryer) or conveyed to the dryer, usually by a mesh belt conveyor or other conveying system able to keep airflow on the pasta.

The moisture content of the pasta entering the predryer section of the dryer is ~29 to 31%. The predrying stage is ~10% of the total drying time and removes about one-third of the total water in the pasta. The high drying rate is possible because the pasta is still in a plastic state, which prevents a buildup of stress in the pasta. Depending on the temperature of the dryer, the pasta will remain plastic as moisture content is decreased to 18% (for ultrahigh temperature, ≥80°C, drying) or 21% (for low temperature, <60°C, drying). Water is one plasticizing agent, which allows the product to deform without creating residual stresses. Temperature is another factor that helps plasticize the product. The moisture content at which the pasta transitions from plastic to elastic state decreases as temperature increases.

The predrying stage (and the final drying stage) will generally have a "resting" stage (sweating stage) scheduled in the drying profile or built into the dryer design. During the "resting" stage, the driving force for the drying (temperature and humidity of the air) is low enough that the product is not losing water to the surrounding air. The moisture inside the product redistributes during this time. The core, which has higher moisture content than the surface of the product, will lose water to the surface of the pasta product. Redistribution of the moisture helps even out the dimensional change and minimizes stress due to moisture loss in the product. If enough moisture reaches the surface, the surface becomes plastic and stresses are relieved.

b. Final dryer

The product will have ~18 to 21% moisture content upon entering the final dryer and will exit the dryer at ~12%. The rate of moisture removal in the final dryer is critical, because the product is in an elastic state. If drying is too fast, the stresses near the surface of the product will exceed the strength of the pasta and checking will occur. While stress accumulated during the final drying can result in checking, the root cause of stress in the product may be due to events that occur earlier in pasta processing. For example, product thickness will increase as the insert wears during extrusion. A thick product is more difficult to dry; and if no changes are made in the drying profile, checking will occur.

c. Cooling/stabilization stage

The product is brought to near ambient temperature and exposed to ~50% relative humidity during the cooling/stabilization stage. The product is equilibrated with the ambient environment to minimize the possibility of checking due to environmental stresses. Moisture is more evenly distributed as the product moves through the cooling stage. Some of the water near the core of the product will migrate toward the surface, which will relieve some of the stresses that accumulated during the drying process.

One difficulty in humid environments is that the surface of dried pasta will begin to absorb moisture from the atmosphere. Stress occurs as the absorbed moisture causes the surface of the product to expand. This stress, coupled with the residual stresses in the product from drying, may result in checking of the product. Even with little or no residual stress, checking will occur in pasta if the relative humidity is ≥75% (72). To prevent checking in humid environments, dried pasta may need to be packaged within 3 hours of exiting the dryer.

Advances in drying technology have resulted in three drying intensity categories that describe processing temperature and relative humidity ranges: conventional drying, high temperature drying, and ultrahigh temperature drying. Conventional drying (low temperature drying) imitates the conditions that occur in the Mediterranean region of Italy, the origin of pasta. The drying of pasta originally took place in the open air or in a ventilated room. High temperature drying is a drying cycle where the maximum temperature applied is between 60 and 80°C. Whereas, ultrahigh temperature (very high temperature or tres haute temperature) is a drying cycle where the temperature applied reaches ≥80°C.

As drying temperature increases, the relative humidity of the drying cycle increases and drying time decreases. Spaghetti (a thick product) may take about 24 hours to dry with low temperature drying, 12 hours with high temperature drying, and 5 hours or less with ultrahigh temperature drying. The exact drying cycle used is dependant on the product being dried and the equipment manufacturer, so exact drying profiles are not provided here. The drying profiles used by the Durum Wheat Quality and Pasta Processing Laboratory in the Department of Cereal and Food Sciences at North Dakota State University can be found in Yue et al. (14).

2. Moisture Migration during Pasta Drying

Factors that have a strong influence on drying rate include pasta thickness (doubling the thickness of the pasta will reduce the drying rate 75%) and air temperature, while factors that have a small influence on drying rate include relative humidity and airspeed (73).

Pasta is a dense, continuous product with very few capillaries. Like similar products (soap, gels, pastes), pasta drying is unsteady-state and can be modeled using Fick's second law:

$$\frac{\partial X}{\partial t} = D\frac{\partial^2 X}{\partial x^2} \quad \text{(for a slab) or}$$

$$\frac{\partial X}{\partial t} = D\left|\frac{\partial^2 X}{\partial r^2} + \frac{1}{r}\frac{\partial X}{\partial r}\right| \quad \text{(for a cylinder)}$$

where X = moisture content, t = time, x = ½ the thickness of the product, and r = radius of the product.

Fick's second law describes drying in materials where liquid water is diffusing toward the surface of the material. Research indicates that Fick's second law is not a perfect model for the pasta drying process, because the moisture gradient in the pasta is steeper than the Fickian model predicts (74,75). However, Fick's second law may be a reasonable starting point.

A wide range exists for diffusivity values (at least two orders of magnitude between the highest and lowest reported values), but the majority of results include diffusivity values in the range of 25×10^{-12} m^2/s to 50×10^{-12} m^2/s (73,74,76). Diffusivity is unaffected by total pressure in the drying environment, indicating that moisture migrates in pasta as liquid or adsorbed water and not as water vapor (76).

3. Maillard Reactions during Pasta Drying

Maillard reactions result in the development of a red or orange color, which is significantly different from the golden color expected in pasta. Maillard reactions can also result in the development of off-flavors in pasta. The Maillard reaction requires a reducing sugar and a free amino group. The reducing sugars for the Maillard reaction are provided by damaged starch. The damaged starch can be the result of growing conditions, milling, mixing with water, or extrusion (77,78,79). The free amino group is generally from a lysine residue in protein.

Maillard reactions can occur during high temperature and ultrahigh temperature drying. Water activity of 0.75 to 0.80 (80) and/or a moisture content of ~15% (77) are optimum moisture conditions for the Maillard reaction to occur in pasta. Pasta in equilibrium with a high relative humidity environment (97% rh, water activity = 0.97) is unlikely to develop an off-color during drying (79). The development of off-colors also may be limited by not applying high temperature in the dryer until the pasta reaches a water activity of 0.7 (81).

The Maillard reaction is and will continue to be a concern in pasta production. The industry has accepted high temperature and ultrahigh temperature drying as optimal drying cycles, producing the highest quality pasta. This higher quality comes with the risk of its own defect: "browning" during drying. Most pasta produced under high temperature and ultrahigh temperature drying cycles will not have the browning defect, but there are factors that can increase the likelihood of Maillard reactions occurring. Factors directly caused by the semolina or the drying cycle have been researched to some degree (77,79,80). However, there are factors not easily anticipated in a research environment, such as "holding" of product in the dryer due to other equipment problems, effect of equipment wear on the amount of starch damage in the product, and poorly calibrated sensors. While the Maillard reaction may not be a concern for most pasta processors today, it would be unusual not to see some reddish-colored pasta on the shelf in a local supermarket.

4. Checking in Pasta

There are two types of checking in pasta: predryer checking and final dryer checking. Predryer checking, as the name implies, occurs in the predryer section. This defect will appear as spots in the finished product. The spots will generally be round and be near the surface of the product. These spots may appear similar to spots due to insufficient vacuum during extrusion. Final dryer checking is easily identifiable. In the spaghetti, final dryer checking will appear as an oval on the surface of the spaghetti, and can be described as a "crescent," "canoe," or "half-moon" (Figure 158.8). In other products, final dryer checking

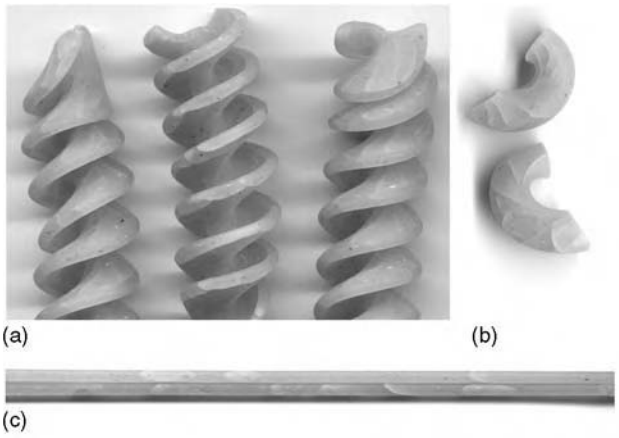

FIGURE 158.8 Final dryer checking in rotelle (a), elbow macaroni (b) and spaghetti (c). Note partial and full oval shaped checking in spaghetti and checking on the outside and on the face of vanes of rotelle.

will be visible as cracks in the pasta. The stresses causing final dryer checking are created during final drying, but the actual checking of the product may not occur for up to 10 days after drying.

Both predryer and final dryer checking are caused by removing moisture too quickly from the pasta. Predryer checking can be corrected by increasing the relative humidity and or the residence time in the drying chamber. These changes may ensure that the moisture content going into the final dryer is appropriate for that dryer. Because each dryer is different, knowing the operating conditions and properties of the product when the production line is running correctly cannot be over-emphasized. While general values of moisture content at various stages in the drying can be found for "typical" dryers, knowing the exact conditions in the dryer that you are trying to troubleshoot is extremely valuable. Final dryer checking can be corrected by increasing the relative humidity in the final dryer section. Increased relative humidity in the final dryer section may slow drying enough to require increased drying time. Final dryer checking can be due to improper predryer conditions. Final drier checking may also be due to pasta leaving the predryer at too high of a moisture content.

Frequently, only minor changes in the dryer will correct a checking problem. For example, a pasta manufacturer was producing a penne product that was checking badly. The plant producing this product eliminated the checking by increasing the relative humidity in the final dryer section by 3%. The root cause of this plant's problem appeared to have been worn die inserts. The thickness of the product had increased enough to cause these drying problems.

5. Pasta Defects/Troubleshooting

Defects in pasta production do occur, and being able to locate the source of the problem is important in solving the defect problem. Having accurate data on the optimal drying parameters as measured and controlled in the process being investigated cannot be over-emphasized. Having a "base-line" for comparison will help to solve many problems quickly. Table 158.2 lists some of the more common defects found in dried pasta along with the likely cause(s).

IV. GOOD QUALITY PASTA

The goal of pasta production is to make a dried pasta that is translucent (although some portions of the market prefer pasta extruded through a bronze die, which will result

TABLE 158.2
Troubleshooting Pasta Defects

Defect	Possible Cause	Possible Solution
Specks in the pasta	Raw ingredients	Check for grit and black or brown specks. Ensure it meets the plant's specifications.
White spots and streaks on the pasta (see Figure 158.9)	Wide semolina particle size distribution	Narrow the size distribution of semolina
	Very dry semolina	Use semolina with a higher moisture content
	Insufficient hydration time	Increase residence time in the mixing chamber
Circular bubbles in the pasta	Loss of vacuum on the extruder	Make sure vacuum system is clean and operating properly
	Predryer stress	Ensure relative humidity the product is exposed to from the die and through the predryer is correct
Deformed pasta	Varying dough moisture at die	Ensure semolina and water flow rates are consistent
	Poor die pressure distribution	Check for possible obstructions to flow at the die
Cracks (checking) in pasta	Drying too fast in the final dryer section "canoe" or "crescent" shape in spaghetti (see Figure 158.8c). Cracks in other products (see Figures 158.8a,b)	Check conditions in the final dryer, possibly increase relative humidity to slow the drying process
		Ensure product does not drop far or onto too solid a surface when conveyed
	Mechanical damage (sections of pasta chipped out of the ends of tube products (see Figure 158.10)	Post-dryer checking can occur if ambient relative humidity is too low (<10%) or too high (>75%) and the product is exposed to the conditions for 3 hours or more
	Post-dryer checking	
	Rough mechanical handling	Pasta may be dropped from too great a height, fracturing the product. In tubular products, this will result in squares or triangles being knocked out of the ends, instead of cracks on the entire length of product
"Red" or "orange" color in the pasta	Maillard reaction occurring in the pasta	Check RH and temperature in the final dryer section
		Check semolina for excessive sugars or ash (indicating a high semolina extraction)

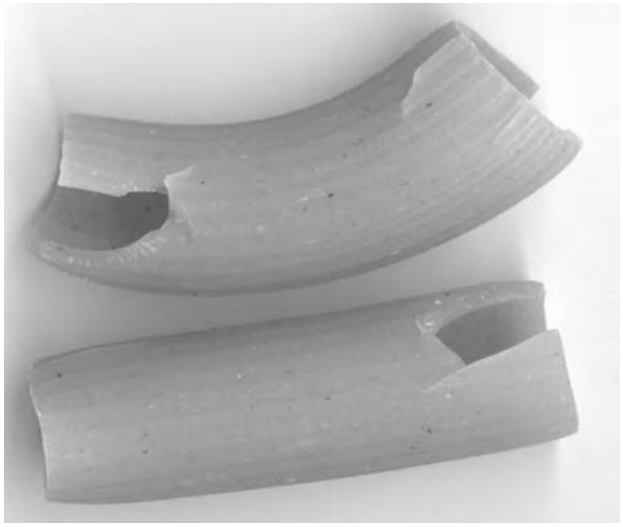

FIGURE 158.9 Rigatoni showing mechanical damage. Cracks in the pasta are only at the ends of the pasta and broken areas are roughly rectangular.

FIGURE 158.10 Hydration problem. Rotelle viewed from the end. Note hydration streaks about 1/3 the distance from the center of the product.

in a rough surface) and free of visual defects such as checking, hydration spots, and specks. This pasta should cook to a non-sticky, firm product with little cooking loss, and resist overcooking.

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159 Seafood Products – Science and Technology

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CONTENTS

I. GENERAL INFORMATION

Dr. George Pigott coined the phrase "aquatic food products" because it was difficult to find one word to adequately define edible animals and plants from the aquatic environment. "Seafood" denotes only food from the sea or marine environment, omitting freshwater plants and animals. Similarly "fish" is not an all encompassing term for all of the different animals (both vertebrate and invertebrate) and plant products humans take from the water and eat. The FDA recently defined fish to mean:

> "fresh or saltwater finfish, crustaceans, other forms of aquatic life (including, but not limited to, alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, where such animal life is intended for human consumption." (Title 21 of the Code of Federal Regulations. Part 123.3(f)).

Over 350 species of mollusca (*e.g.* clams, oysters, snail, octopus), arthropoda (*e.g.* lobsters, crabs, shrimp and crayfish), reptilia (*e.g.* turtles, alligators), amphibia (frogs), gastrapoda (whelks), holothurians (sea cucumbers), and chordata (finfish) are used as food (1,2). Furthermore, aquatic plants and marine mammals are important components in the diets of people from many cultures.

Interesting, unconventional, unique and somewhat bizarre traditional foods are made from aquatic products. Numerous tissues are consumed besides the muscle from aquatic animals. Some examples of the wide variety of aquatic food products are presented in Table 159.1 using

TABLE 159.1
Examples of Aquatic Foods

Item	Source	Examples
Whole animal	Mollusc	Clams, oysters, octopus, limpet
	Crustacea	Small crab
	Fish	Sardines, smelt
	Insects	
Plants	Marine plants, various	Sea vegetables, nori
	Marine plants	Food additives (agar, carrageenan)
Liver/hepatopancreas	Finfish, various	Cod, pollock, salmon
	Marine mammals	Seal, walrus, whale
	Crustacea	Lobster, crab
Tongue	Finfish, various	Cod, halibut
	Marine mammal	Seal, whale
Heart/kidney	Finfish, various	Salmon, tuna
	Marine mammal	Seal, whale
Stomach/throat	Finfish, various	Cod
Brain/head	Finfish, various	Salmon, cod halibut (cheeks)
	Marine mammal	Whale
Spinal column	Finfish, various	Sturgeon
Eyes	Finfish, various	Salmon
Gills	Finfish, various	Salmon
Gonadal tissue, roe	Mollusk	Sea urchin
	Fin fish	Sturgeon, herring, salmon
	Crustacea	Lobster, crab, shrimp
	Reptile	Turtle eggs
Gonadal tissue, milt	Mollusk	Oyster
	Finfish	Salmon
Testicles	Marine mammal	Seal
Penis	Marine mammal	Seal (East Asian medicinal)
Skin	Finfish, various	Salmon, rockfish
Connective tissue	Finfish, various	Shark (cartilage)
Adipose tissue	Marine mammal	Seal, whale
Oil (head or adipose)	Finfish, various	Herring, salmon, shark, cod
	Marine mammal	Whale (blubber), seal (blubber and extracted oil)
Bodily fluids	Mollusks	Squid (ink)
	Finfish	Blood
Fin	Finfish, various	Shark
Shell	Mollusk, various	Oyster (as nutritional supplement)
Exoskeleton	Crustacea, various	Shrimp
Bone	Finfish, various	Smelt, sardine

species common to Westerners. This is nothing close to an exhaustive list of the different types of products made from aquatic plants and animals, but it provides some insight into how diverse aquatic food products can be.

For many species the muscle tissue plus certain organs are eaten together. For example, lobster, crab or scallop meat is eaten with the hepatopancreas and roe (if present) by individuals who like it.

The entire organism is commonly consumed (*e.g.* oysters, limpets, some clams, small fish). For some species only certain organs are consumed, such as sea urchin roe. Roe is eaten in a salted or seasoned form as caviar from a variety of different fish and invertebrates. The most common vertebrate species for the production of roe products are flying fish roe (for tobiko used on sushi), sturgeon and paddlefish for black caviar, salmon for salmon caviar or ikura, herring roe for kosunoko, cod roe for tarako, and pollock roe for mentaiko (also spelled mentiko). Salted fish roe from cod or capelin are used as a component of pastes or spreads. Lower grade sturgeon can also be pressed and sliced or mixed with butter or soft cheese and used as a spread. Roes can be partially dehydrated; for example, sujiko, which made by salting and then pressing skeins of sockeye salmon roe. A dried roe product is made from mullet roe and is called karasumi. High levels of wax esters give mullet roe a unique chewy texture. Flavored or seasoned roes are becoming increasingly popular. For example, chili flavored mentaiko is a widely used

condiment in Korea, and salmon roe treated with soy and other seasonings is popular in Japan and throughout Asia. Sauces and salad dressings often contain fish roe. Fish roe and milt are served in soup or lightly sautéed. Milt can be smoked and made into spreads. Uni, is a colored, flavored alum treated gonadal tissue from the sea urchin used primarily for sushi.

Steamed fish, soups and stews are made from the whole fish, with tissue from the head (such as the cheeks and tongue) and the eyes reserved for special guests.

Cheek tissue, particularly from larger fish such as halibut are commonly served separately. Halibut cheeks are the muscle from underneath the eye of the fish. Since halibut can reach up to 250 pounds, the cheeks can be several ounces each. In the recent past, halibut cheeks were sometimes mislabeled as scallops or sea scallops, but with the current high demand, halibut cheeks are a popular food in their own right.

Certain tissues of a fish may be fried (skin, intestines) and served as such. Skin and intestine are often incorporated into stews, soups, or ground meat preparations. These tissues are dried for use in a seasoning or base. In addition, fish skin can be cured into leather for belts, wallets and shoes. The most common types of leather in Western markets are from shark and skate, sturgeon, eel and salmon.

Specialty dishes use fish connective tissue as a featured ingredient. Fish maw soup (from finfish stomach and throat tissue) and shark fin soup both take advantage of the thickening properties of these connective tissues for the preparation of various dishes. Gelatin can be recovered from fish skin and connective tissue for use in traditional foods and, if appropriately manufactured, as kosher gelatin. Shark cartilage is both a food component, and when dried, a nutritional supplement.

A wide variety of condiments, sauces and seasoning are made from aquatic animal tissues. Most common are fish oils forming the base for margarine, dressings, sauces and condiments. The omega-3 fatty acids in high quality stabilized fish oil are a nutritional supplement commonly sold in capsule form and more recently as a stabilized ingredient in functional foods. The importance of fish oil in the diet is a continuation of a longstanding trend. Prior to the widespread availability of vitamin supplements and fortified milk, cod liver oil was a primary source of vitamin D. Oil from marine mammal tissue (*e.g.* seal oil or ooksook, and whale blubber) remains popular in the traditional diets of people above the 47th parallel in reflecting the dietary patterns of the samoyed peoples who migrated throughout Alaska, the Pacific Northwest, Scandinavia, British Isles, Russia and Japan. Marine mammal oils can be rendered at room temperature or rendered with heat. These oils may be consumed fresh or aged. These oils serve as a condiment or dipping sauce and are used in a similar fashion as olive oil in Mediterranean cuisines.

Aquatic animal foods are valuable, and in some cases, incredibly expensive. Because of the value, widespread poaching of shark and sturgeon have led to severe restrictions and closures in these fisheries. Fortunately, sturgeon can be cultivated, hopefully saving important Caspian Sea species from extinction. Reasonably good Beluga sturgeon caviar at retail routinely runs $200 per ounce or more. The most expensive aquatic food product however is gold “Almas” caviar from Beluga sturgeon (*Huso huso*) purported to be over 100 years old, is packaged in 24 karat gold tins and sells for US$ 23,000+ per kilogram (3).

There have been few efforts to cultivate shark. Sharks are slow growing and breed after several years of age. Some sharks bear live young, meaning that the number of offspring from each female is small. Fishing pressure on large sharks is threatening the viability of sub-populations, particularly in the waters surrounding Southern Asia. The value of dried shark fin varies greatly by size and appearance, but for good specimens, the value exceed hundreds of dollars per pound.

A variety of nutritional supplements and traditional medicines are made from aquatic organisms. Besides fish oil, calcium supplements are made from oyster shell. Oyster milt and other reproductive tissues are purported to have medicinal properties. This includes the use of the seal penis in Asian medicine, a suitable, albeit a misbranded substitute for tiger penis in traditional Chinese medicine. Numerous marine plants and their extracts are common sources of vitamins, minerals, and phytoactive components with alleged beneficial properties.

Aquatic products have been consumed from ancient times and were probably the first animal sources of protein in the human diet. Humans settled near water and have captured fish and harvested mollusks since prehistoric times. Commercial fishing was part of the ancient culture of Egypt where ancient fishing vessels have been unearthed that vary little from designs of vessels used in artesian fisheries today. Asian and European civilizations have consumed fish for thousands of years before recorded history. Native Americans can trace their use of fishery products back 3,500–10,000 years. Numerous archeological sites containing bone, shell, fishing hooks from bone and stone net weights have been recovered in the United States (4) with important sites at Lake Ozette in Washington state verifying the use of cedar bark nets among coastal tribes going back several hundreds of years. In addition to wild harvest, culturing fish and ranching or impounding fish in ponds dates back a thousand years or more. Among the Makah, live fish were harvested off the northern most tip of the continental United States and then placed into ponds and held until consumed. This technique provided the people with a source of food when the weather was too rough to fish. It also provided a means to have fish species that would otherwise be out of season. Similarly aquaculture and impounding of fish have been practiced for centuries on the islands of Hawaii and in

Polynesia. Commercial aquaculture and fishing were key industries in ancient Asian socities and in the West during the Roman Empire with archeological sites indicating the widespread use of aquaculture in Italy and North Africa.

Today aquatic food products are the major source of high quality animal protein to a quarter of the world's population. The consumption of aquatic food products is increasing internationally, with aquaculture being the source of any new product to meet market demand. By 2020, a deficit of 10% or more in the world-wide supply of aquatic foods is predicted, even with the growth of cultured product increasing rapidly.

International trade in aquatic food products is critical to the balance of trade of many countries. For example, the US imports 3.9 billion pounds of aquatic food products from over 160 different countries with half of this from China, Ecuador, Chile, Canada and Thailand. Imported seafood constitutes more than half of the seafood consumed in the United States (5). Rights to fish and the control of critical fisheries (*e.g.* cod in the North Atlantic) have provided the basis for numerous protracted trade disputes, and sometimes, all out war.

II. PROCESSING TECHNOLOGIES

The primary advantages of preservation of seafood products are to extend product shelf life, ensure product safety and nutritional value, and maintain product quality. Specific market advantages obtained by preserving aquatic food products include: the ability to distribute food over long distances far from the point of harvest; to hold product when it is no longer in season, or to hold it in a form suitable for later processing or consumption.

III. COLD STORAGE

To make these products widely available, aquatic food products are often refrigerated or frozen. The widespread use of refrigeration, freezing, and cold storage has meant that aquatic food products, normally available only seasonally and within a small region, can now be sent around the world any time of the year. Because of reliable integrated transport, live and high value products can be air shipped from relatively remote locations to major metropolitan areas within 48 hours of harvest. Shipping costs often exceed the value of the product.

Until quite recently, aquatic foods were primarily harvested and consumed locally. These foods were generally available only during a limited season. These limited seasons or "fish runs" were critical to the human survival in many parts of the world. Entire cultures developed around the annual salmon runs in Asia, Alaska and the Pacific Northwest. Also important to the survival of many cultures have been the seasonal migrations of huge schools of fish such as herring, smelt, sardines or anchovetta and the larger pelagic fish and the mammals that feed upon them. Harvests were primarily restricted to coastal areas as there were few methods available for preserving fish on-board high seas fishing vessels. An exception to this was the North Atlantic salt cod fishery that started in the 14th century and continues today.

Animal food products deteriorate rapidly at ambient temperatures, and aquatic food products are generally even more susceptible to deterioration. Refrigeration works by slowing metabolic processes. Reducing temperature slows the growth of pathogenic and spoilage microorganisms and reduces the rate of deteriorative biochemical and chemical reactions in the muscle and other edible tissues (6). However, many animals and plant foods from the aquatic environment, particularly marine fish, are poikilothermic and are adapted to living at low temperatures (−1–10°C). Tropical fish are also poikilothermic since water temperatures for these species rarely exceeds 20°C. The endogeneous enzymes in these cold adapted "naturally" work at refrigeration temperatures, so spoilage reactions occur at a relatively rapid rate since refrigeration does little to impede them. In addition, spoilage bacteria associated with poikilothermic organisms continue to grow. This is why products from aquatic animals and plants deteriorate more quickly than foods from terrestrial sources and must be processed quickly and held under proper conditions to maintain highest quality.

IV. LIVE HANDLING

With the exception of aquaculture where the fish can be harvested with limited stress, finfish are most commonly "stressed" when captured. In the capture fisheries, finfish are literally still "hunted" and capture techniques still include the use of nets (for example, seine, trawl, and gill nets to catch salmon, herring, or pollock); hooks and lines (for example, to catch halibut or swordfish); traps (for crab and shrimp), even harpoons (for bluefish tuna and marlin). This harvest induced stress leads to a reduced level of glycogen in the flesh when the fish are brought on board. New methods of reduced temperature and moderate amounts of carbon dioxide as an anesthesia prior to slaughter can reduce the stress to fish and improve muscle quality.

As the fish pass through rigor, the ultimate pH of the fish tissue is higher than for meat, generally pH 6.4–6.6. Little glycogen is left in the muscle tissue for conversion to lactic acid during the glycolytic process that accompanies rigor. In contrast, land animals are generally rested prior to slaughter and have higher levels of glycogen, and a lower ultimate pH, around 5.5 for mammalian muscle and 5.9 for chicken. The higher ultimate pH in fish is one reason why fishery products are relatively more susceptible to microbial spoilage than other muscle foods stored under the same conditions. The endogeneous enzymes in

the fish muscle and viscera of most commercially important species are highly active at refrigeration temperatures. Also, the microbes that grow on the external surfaces, gills, and in the viscera are adapted to growing at relatively low temperatures and can cause rapid spoilage.

Other factors specific to the biology of aquatic animals causes the muscle tissue to be in less than prime condition when harvested. These biological factors make proper refrigeration and freezing critical for maintaining product quality. Salmon, for example, are commonly captured as they return from the ocean to spawn in a fresh water stream, often many hundreds of miles inland. With salmon, the fish have stopped eating, and have also had to physiologically "readapt" to swimming in fresh water. The fish must mobilize their energy reserves (adipose fat, muscle fat, and muscle protein) for migration as well for producing roe (eggs) or milt (sperm). At a certain point during the spawning process, the salmon flesh becomes pale, soft, and flavorless. This severity of this problem is species, gender, and run dependent.

Any aquatic food product should be refrigerated or cooled on ice as soon as possible after harvest. Live mollusks should be placed in refrigerated seawater, held in cold storage at 10°C or lower, or be placed in salt water ice. Live marine mollusks can be placed ON THE SURFACE of fresh water ice; however, placing live marine mollusks in fresh water ice will kill them. Mollusks can remain alive under these conditions for five days or more.

V. HANDLING AND SHIPPING LIVE FISH

Handling and shipping live invertebrate fish is a common practice. Molluscan shellfish (clams, scallops, oysters, mussels, snails), crustaceans (crabs and lobsters) and other invertebrates (limpets, sea cucumber) are shipped by keeping the animals moist by wrapping them in seaweed, moist paper or liners, and reducing temperature slightly. Often these creatures are placed into circulating water tanks at the point of sale. The large marine Alaskan and Tasmanian king crab are available for retail sale as live animals and sell for over $100 per animal.

Live transport of finfish is becoming more common. Here, fish are shipped in temperature controlled tanks with air or oxygen circulation and then transferred and held in "live tanks" at retail facilities or in restaurants. More exotic foods animals including live frogs and turtles are transported in a similar fashion. Most of the recent advances in oxygen permeable packaging for life fish transport have come from the pet trade with these techniques becoming more important for food fish.

VI. HARVESTING

"White fish" such as cod, pollock, or whiting, are commonly harvested on the high seas by trawl and held on board in refrigerated seawater until they have gone through rigor. Certain species of larger and high value fish are harvested by hook and line including swordfish and halibut. Pacific cod are harvested using a long line technique, headed and gutted and frozen shipboard within 2.5 hr at −20°F in a prerigor state. Product frozen prerigor is preferred in the Japanese market. If handled properly, fish caught by hook and line can be of higher quality than those harvested by net.

Warm water aquacultured fish such as catfish or tilapia are collected from ponds and stunned by dropping the water temperature. Another way of stunning the fish is to place the fish into carbon dioxide saturated water (600 ppm or greater). After this, the fish are bled by cutting the gill rakers or by cutting the artery anterior of heart. This allows the heart to remain functioning and pump the blood out of the body. The fish are then placed circulating ice water for 5–20 min. so the blood can be completely removed, further processed for the fresh market or frozen. Bleeding a fish greatly improves muscle color, storage stability and flavor.

Clearly the post harvest stress in cultured fish is less, since struggle can be reduced when the fish are harvested. To improve quality, cultured fish can be fasted for up to a couple of days before harvest, reducing the metabolic activity of the digestive enzymes. Fish generally pass through rigor "whole" and still retain visceral enzymes, and if these are present at high levels they can cause deterioration of the meat during storage.

Besides stress, and the affect of harvest method used, seasonal variations play an important role. Wild caught fish can vary greatly in product quality depending upon when in the breeding cycle harvesting occurs. For Alaska pollock (*Theragra chalcogramma*), the fish harvested during the breeding season for mentaiko (or mentiko) have poorer quality flesh than fish harvested at other times during the year. Similarly, large variations in muscle quality are seen in salmonids. For example, the flesh texture of pink salmon (*Oncorhynchus gorbuscha*) tends to remain firmer for male compared to female fish in the same run, as a result of the mobilization of fat and energy reserves by the female fish for egg production. Also, salmon within the same run harvested at a mouth of a river contain more fat and have a much higher quality than fish harvested closer to the spawning grounds. Fish harvested at the mouth of a river from short runs also tend to be of lower quality than fish from longer runs. This is because fish with longer migration patterns require larger energy stores for migration and tend to have a higher fat content and a richer flavor.

Normally, fish are allowed to pass through rigor before they are processed and are commonly held at 4–10°C until rigor has resolved. For the best quality product, fish should be processed as soon after resolution of rigor as possible. Because these animals and their accompanying microflora are adapted to cooler temperatures, deleterious biochemical reactions can occur quickly in fish. Care must be

taken to ensure that butchering operations are as clean and sanitary as possible to avoid cross contamination between viscera and meat. Eviscerating must be conducted under cool conditions. Often fish processing facilities are kept at 45–50°F to maintain product quality.

VII. REFRIGERATION

For this chapter, *refrigerated* is used to describe product temperatures above 0°C and *frozen*, product temperatures below 0°C. Holding and shipping live fish is *technically* a refrigeration process since products are held at temperatures between 3–10°C, depending upon the species.

Rigor begins (onset of rigor) in fish within one to two hours depending upon species and ambient temperature. Onset of rigor is temperature dependent and occurs sooner at higher temperatures. Extremely large fish such as bluefin tuna weighing several hundred pounds go through rigor slowly like other large animals. As a comparison, the onset of rigor in beef muscle is within 10–24 hr postmortem at room temperature, in chicken in 2–4 hr, and in whale muscle, 50 hr (7).

Fish pass through rigor within hours and are generally processed post-rigor. Fish should pass through rigor (resolution of rigor) before fillets are frozen to avoid toughening, shrinkage and to reduce drip loss when the product is thawed out and used (thaw rigor). One exception to processing pre-rigor for certain at-sea longline processors that process high value fish prerigor, is freezing fish within two or three hours of harvest. Another exception is in aquaculture, where fish are often processed prerigor.

Fish must be carefully handled post rigor, since rough handling can tear the muscle tissue causing the myotomes to separate. This phenomenon is called gaping. Gaping is an important quality consideration in finfish harvested from cold waters. Gaping is most prevalent in fish allowed to pass through rigor at elevated temperatures (>17°C). Other manifestations of rough handling include discoloration and softening as a result of bruising, caused by rupturing blood vessels within the muscle tissue. Also, fractures of the vertebrae introduce blood spots into the muscle tissue.

Controlling the temperature of muscle foods is important for maintaining quality during storage. Muscle fibers contract postmortem at physiological temperatures. However, the amount of contraction decreases and is lowest around 10–20°C. At temperatures lower than 10°C, muscle contraction increases again. Contraction of muscle fibers at low temperatures causes the quality defect of cold shortening making muscle tissue tough. Cold shortening occurs in prerigor muscle (below 10°C) because the sarcoplasmic reticulum cannot efficiently store calcium ions at these lower temperatures. Fish muscle, with the exception of that from large pelagic species, is not highly susceptible to cold shortening. However, a related problem is thaw shortening, which occurs when muscle is frozen prerigor and then thawed rapidly. Because ATP is not depleted in the muscle cells when tissue is frozen prerigor, the muscle fibers contract rapidly during thawing releasing large amounts of tissue fluids (drip loss) with accompanying muscle toughening.

There are a number of methods for storing fish at reduced temperatures (15°C or less) including: crushed ice, slush ice (water ice dispersed in water alone or in water containing additives (*e.g.* salt, organic acids, antimicrobials, sugar)), champagne ice (slush ice with gaseous carbon dioxide) and mechanical refrigeration.

Even with refrigeration, aquatic food products have a limited shelf life (Table 159.2). Eviscerated ("dressed" or gutted) whitefish such as cod or halibut, and salmon have a shelf life of a week or less at 4°C, but fatty fish in the "round" containing visceral contents such as mackerel or herring should be stored no longer than a couple of days. The shelf life can be extended significantly using tightly controlled storage conditions at lower temperatures by a process called superchilling. This technique involves holding the product at 0 to −1°C with variations of holding temperature less than ± 0.5°C. Most fish muscle does not freeze above −2°C.

A. Vacuum Packaging

Vacuum packaging also increases shelf life of certain products. Storage of chilled vacuum packaged meats including smoked fish up to 10 weeks is possible at 4°C. The primary, but somewhat unwarranted concern with vacuum packaged seafood products, is the growth of *Clostridium botulinum* type E. This organism can grow at refrigeration temperatures 38°F (3°C) and relatively high concentrations of water phase salt (4.5–6%)(6). However, most products will have signs of decomposition before the risk of botulism becomes significant.

Recent concern among regulatory agencies about the safety of vacuum packaged fresh fish and smoked fish products from *Listeria monocytogenes* is also misplaced.

TABLE 159.2
Refrigerated Shelf Life of Fresh and Cured Aquatic Food Products

	Approximate Days Remaining in Good Condition	
Products	**32°F**	**60°F**
Cod, fresh	14	1
Salmon, fresh	12	1
Halibut, fresh	14	1
Finnan haddie	28	2
Kippers	28	2
Herring, salted	1 yr	3–4 mo
Cod, dried salted	1 yr	4–6 mo

Adapted from Pigott and Tucker, 1990 (4).

Packing these foods under vacuum or with nitrogen flushing maintains product quality longer and also provides a packaging that is tamper evident. Concerns with thermal abuse can be addressed by labeling: "keep refrigerated at 38°F or less" and "use or freeze by" dating. Modern time temperature indicators or recorders can be used to ascertain whether a product has been thermally abused possibly jeopardizing its safety, and these monitoring techniques are becoming more common.

VIII. FREEZING

One of the earliest food patents was issued in 1842 for refrigerated fish. However, mechanical refrigeration/freezing did not become a significant method for preserving aquatic food products until the early 1950's. The development of shipboard refrigeration and freezing systems made high seas fisheries possible by permitting vessels to harvest finfish and crustaceans from distant areas and bring these aquatic food products to shore-based processing facilities and distribution centers. In a similar manner, the development of practical freezing technologies and refrigerated/frozen transportation systems allowed shore plants to be constructed near fishing grounds while providing service to worldwide markets (8).

The recent rapid international expansion of aquaculture now provides fresher and less expensive aquatic foods to consumers throughout the year. Important cultured species including salmonids (Atlantic and Pacific salmons, rainbow trout), catfish, tilapia, sea bream, halibut, eels, sole/flounder, striped bass, molluscan shellfish, shrimp, and sea vegetables (*e.g.* nori, the common covering for sushi rolls) are commonly available around the world at any time. This would not be possible if it had not been for the development of practical refrigerated/frozen processing, sophisticated supply chain management and reliable air transportation. Unfortunately *high quality* frozen or refrigerated (fresh) aquatic foods are too often unavailable because of poor handling, poor processing, or inadequate temperature control (9). This is a problem that still plagues the industry and has since its inception.

Refrigeration and freezing also made it possible to introduce new and extremely valuable products into commerce, for example caviar and fish roe products. Caviar products are cured with salt, but with few exceptions, refrigeration or freezing is required to maintain product safety and quality. Other extremely valuable aquatic food products which would not otherwise be available without freezing include king crab with the shell on, giant prawns, magaro (sashimi tuna or tuna to be consumed raw) and lox (lightly salt cured, cold smoked, effectively raw, salmon).

In general, deterioration can be reduced if the temperature is 4°C or less and by ensuring holding temperatures are well controlled. To halt deterioration, the mobility of water within the food must be reduced. This is particularly significant for aquatic food products because the water content is high. Finfish contains 60–80% water on a weight basis and some aquatic products contain over 90% water. Individuals within the industry often remark that they sell some of the most expensive water in the world. Water retains the ability within any food product to serve as a solvent or reactant until a temperature of −40°C is achieved and maintained. Even below −40°C, product quality will still be affected by surface dehydration unless protected by packaging or physical barriers such as an ice glaze.

Freezing muscle foods permits storage for one year or longer at −20°C, assuming that temperature fluctuations in the storage freezer can be controlled [Table 159.3]. Rapid freezing is required for aquatic food products, even more so than for muscle tissue from terrestrial animals. Muscle proteins in fish are less tolerant to changes in the ionic strength of intracellular fluids that occur during freezing than other types of muscle food. Small intracellular ice crystals will form in rapidly frozen samples with less visible tissue damage. For slowly frozen samples, large intracellular ice crystals form which rupture cell membranes, increase drip loss and damage texture.

Fish muscle myotomes are more susceptible to mechanical damage during freezing than the muscle tissue of terrestrial animals. This is due in part to the orientation of the myotomes and to the relative weak connective structures that hold them together. Rapid freezing is also critical for maintaining the quality of animals frozen whole such as shrimp, lobster and molluscan shellfish. These animals are often frozen without eviscerating, so it is critical to freeze tissue rapidly with as little tissue damage as possible to limit digestive enzymes from being released into the flesh. Usually, after the fish has been frozen, it is protected with a water glaze to limit surface dehydration. Packaging materials that permit moisture retention and exclude light are preferred.

Freezing and on-board refrigeration has made it possible to expand commercial fisheries to new species that were not widely utilized until the late 1970's. The

TABLE 159.3
Practical Storage Life for Aquatic Foods (Months) (4,9)

	Temperature		
	−12°C/ 10°F	−18°C/ 0°F	−24°C/ −12°F
Fatty fish, glazed	3	5	> 9
Lean fish	4	9	> 12
Lean fish fillets	—	6	9
Lobster, crab, shrimp in shell	4	6	> 12
Shrimp, cooked peeled	2	5	> 9
Clams, oysters	4	6	> 9

Adapted from Institut International du Froid, 1986 (9).

development of a factory trawler fleet and growth of whitefish fisheries around the world for surimi, fillets, and fish blocks production would not have been possible without the ability to harvest tons of fish at a time and keep them in refrigerated seawater storage until the fish could be processed on board. Similarly, without recent developments in freezing technology it would not be possible to hold the millions of pounds of frozen processed product on board ship until it can be delivered hundreds of miles to shore, and from there to consumers.

A. Types of Freezing

Different freezing methods are employed in seafood production. Some of these are outlined in Table 159.4, with common temperature and air velocity parameters provided for freezing different food products. Most aquatic food products are blast frozen, or frozen under conditions in which product is packaged and placed upon shelves inside a chamber. Very cold air at high velocity is blown around the chamber by powerful fans near the ceiling. After the product is frozen, it is removed from the blast freezer and placed in a storage freezer.

Sometimes large fish, such as salmon, are frozen in a blast freezer without being packaged first. Glazing is also used to extend the shelf life of frozen whole, dressed fish, fillets, whole shrimp or molluscs. Glazing involves dipping or spraying water or an aqueous solution on the product after the surface has been frozen. Sometimes a cryoprotectant such as fructose, sucrose or sorbitol, an antioxidant such as ascorbic acid, or a thickening agent (*e.g.* alginate) are added to the glaze. Levels of glaze on whole fish can be as high as 9% by weight. The glaze sublimes during frozen storage, protecting the product from surface dehydration or freezer burn. The glaze also keeps oxygen from migrating into the food limiting lipid oxidation. The presence of a good glaze on seafood is positive factor, however to prevent economic fraud, seafood products are sold by weight after the glaze has been removed (or weight net of glaze).

TABLE 159.4
Freezing Methods for Different Muscle Food Products (9)

Product	Freezer Type	T	Air Velocity (m/s)
fish, bulk	air blast/batch	−30–−40	17
	air blast/continuous	−40	
fish	tunnel	−30	
	plate	−40–50	
fish	cryogenic nitrogen	−196	
	carbon dioxide	−78.5	

Adapted from Institut International du Froid, 1986 (9).

Contact plate freezers are commonly use for freezing products which can be marketed as uniform slabs such as blocks of fish fillets, fish mince, fish roe, and surimi. Plate freezing is used upon factory processors because it is compact, efficient, and has relatively low operating costs. In a contact plate freezer, the product is placed in a rigid pan between two large metal plates that contain circulating refrigerant. These plates are pressed down upon the product as it freezes. Plate freezing is *required* for products that must have uniform dimensions including fillet block, mince/block, or mince used for sandwich portions, fish sticks, or nuggets. Very uniform dimensions are required by the secondary manufacturer who cuts the blocks into portions of uniform size and weight. Plate frozen products are frozen in aluminum pans of very specific dimensions. These pans are lined with coated paperboard block liners folded to fit inside the freezer pan. The fish product is arranged inside the liner, and the lid of the liner folded over and closed. The product is packed by weight. These pans are placed into a contact plate freezer. Commercial freezers on ships can be 10–12 plates and contain dozen of blocks per layer. It takes approximately 2–2.5 hours to freeze a 7.7 kg block of fish in a commercial plate freezer (−28°F).

Cryogenic freezing or immersion freezing in liquid nitrogen or a carbon dioxide "snow" are popular methods for freezing high value items such as shrimp and molluscan shellfish. The freezing rate is extremely rapid, and for some products, this can cause the food to crack or split. The carbon dioxide forms a snow on the food, and then sublimes. Carbon dioxide is often preferred, since there is less thermal shock than with liquid nitrogen and less physical damage to the product. For seafood, the product is placed on a conveyor and passed through a carbon dioxide snow. For nitrogen freezing systems, the product is cooled with gaseous nitrogen before the liquid nitrogen is sprayed on it. After the product is frozen, it is packaged in plastic and allowed to equilibrate to the frozen storage temperature before it is transferred to a storage freezer. These products are generally glazed. Often vacuum packaging is used.

Individually quick frozen shrimp are commonly frozen in carbon dioxide snow in South American plants, and in spiral blast freezers in Asian facilities. Each type of freezing can produce an excellent product and freezing rate is rapid. Shrimp are glazed with a spray of water after freezing. After the glaze sets, the shrimp are packaged in plastic barrier film of various types, packaged in a cardboard master case and held in a storage freezer, preferably at –20°C although this not always possible.

Rapid freezing is critical for fish fillets or steaks to limit the formation of large intracellular ice crystals. Contact plate freezers would be used for frozen block, but tunnel freezer (blast freezer) operated as a batch or continuous system could also be used successfully. Chemical changes, specifically lipid oxidation, occur in fish tissue

during frozen storage. Even though the lipid content of "white fish" is less than 1%, the membrane lipids are highly susceptible to oxidation. This oxidation can lead to stale and rancid off-flavors. Gadoid fish including Atlantic and Pacific cod, hakes and haddock, contain high levels of trimethylamine oxide (TMAO). This compound is broken down by enzymes active during frozen storage that cause proteins in the muscle to cross-link and cause toughening. These enzymes are more active when the tissue has been damaged, which is another reason careful freezing is important. "Fishy" off flavors are a result of microbial decomposition occurring BEFORE the fish were frozen.

Frozen storage temperature must be carefully controlled to limit ice crystal growth and water migration in the fish. Wide fluctuations in storage temperature enhance the rate of deleterious chemical and biochemical reactions in the fish that lead to off flavors. For other products, poor frozen storage conditions would result in the liberation of proteolytic and lipolytic enzymes from the viscera which would cause loss of quality during storage and after the product is thawed.

B. Packaging

A wide variety of packaging materials are used for frozen aquatic food products. For frozen fish fillets, headed and gutted Pacific salmon, and frozen glazed crab, the product is loosely wrapped in plastic and placed inside a cardboard carton for shipment to distribution centers. Shrimp, individually quick frozen fillets, and breaded products, are commonly marketed in heat sealed plastic bags. Some large products, such as whole tuna, are not packaged at all. Certain traditional foods, including uni [sea urchin roe brined and treated with alum] and sujiko [brined, colored, whole skeins of salmon roe] are still marketed in small wooden boxes. Frozen Dungeness crab meat (muscle removed from cooked crab) and razor clams are packaged in cans with double seamed metal ends although there is a trend to package these products in plastic because of food safety concerns. People mistakenly believe that products in metal cans are shelf stable, which raises food safety concerns.

IX. CURED AND SALTED PRODUCTS

The addition of salt is the initial step in the production of cured, smoked, fermented and many dehydrated products. The purpose of curing is to reduce water activity through the addition of the salt itself and by the dehydrating effect the salt has by removing water from tissue being treated. After salt is added, products are placed on inclined or perforated surfaces to drain. This step removes water, and although is not enough to make a product shelf stable, will increase shelf life or, if drying is to follow, allow this step to be more efficiently conducted.

Salted aquatic foods are ancient products and still widely consumed today as specialty foods. In Japan, the most common salted fish are sardine, mackerel, salmon and roe, herring and roe, pollock and roe and squid. In China small pelagic fish, cuttlefish, shrimp, squid, jellyfish and molluscs are salted and dried. Marine fish of all types, most commonly cod, haddock, salmon, sardine, anchovies and herring are important products in Europe and North America and are cured and salted

Cured products are ready-to-eat raw foods. The most common are from salmon. Gravlax is made by adding spices, sugar, salt and herbs to salmon fillets and allowing these to cure for several days under refrigeration. In Japan, teijin is made by dry salting or brining salmon for no more than a few hours. Lox is made by dry salting or brining salmon for several minutes to a couple of hours, and then smoking the product for a short period of time at temperatures less than 90°F. These foods are not normally cooked prior to consumption. Because of the potential for parasite contamination in wild-harvested salmon, it is recommended that only frozen fish be used.

Fish roe products are another type of cured ready to eat foods (3). For a detailed description of roe products see Chapter 161 in this series. Production of roe products is still an art to some degree. Sturgeon roe is made by simply blending singled eggs with 4–5% finely ground salt by weight for a number of minutes at or somewhat below room temperature. The salted eggs are drained on a fine screen, during which time the eggs are carefully inspected and any defective eggs or connective tissue removed. After this, the product is packaged into cans that have slots in the side to allow fluid to drain from the container. The product is generally aged for at least 30 days prior to sale to develop oxidized flavor notes and a darker color, although there is a growing market for the highly desirable freshly salted eggs. Salted salmon roe or ikura were traditionally made using a dry cure method. But more common now is a process which carefully suspends singled out eggs in a saturated salt solution for two to less than ten minutes depending upon the species of eggs, degree of maturity, and desired final salt concentration. For sujiko, whole skeins are used. The skeins are soaked in saturated brine containing flavors, sodium nitrite (100 ppm) and hydrolyzed vegetable protein for about 20 minutes. After brining, the skeins are layered with fine salt in a plastic (formerly wood) container. Weights are placed upon these containers to compress the sujiko and to remove moisture. Barako is a similar product made by recovering broken salmon skeins from sujiko processing. Barako is sold as singled eggs and is not compressed.

Herring roe or kazunoko [*Clupea pallasii* (Pacific herring), *Clupea harengus* (Atlantic or Baltic herring)] are also a product in high demand in Asia, particularly in Japan. Kazunoko or "yellow diamond" roe is made by curing whole herring egg skeins and is commonly

prepared as sushi or as a garnish for rice dishes. The herring roe is not immediately removed from the fish. Instead, the fish are frozen, preferably by brine freezing, or a combination of brine freezing and blast or plate freezing, the objective being to preserve the natural shape and form of the roe sacs within the fish. The frozen herring is then shipped to processing plants where the kazunoko is produced. The freezing and frozen storage of the herring is part of the process of "conditioning" the herring making roe removal easier.

At the processing plant, the herring are thawed, tempered, or "slacked out" by placing the fish in fresh water that is exchanged several times during the 24-h thawing process. This helps to remove blood and other undesirable constituents from the fish. The skeins are then removed (or "popped") from the herring. While skein removal is commonly done by hand, automated systems are now used at many facilities both to sort the fish by gender and then to remove the egg skeins from the female fish. The skeins are sorted, brined, cured, and then packed in an approximately 5-gal plastic pails in 100% brine solution, which is topped off with a scoop (500 to 750 g) of loose salt. The product is then shipped and held under refrigerated temperatures of −4°C or lower. The brining process traditionally involves many steps in which the skeins are held in totes of brine of increasing strengths, finishing with a saturated brine solution. All in all, the brining process normally takes 5 to 7 days with daily changes of brine. A primary purpose of this process, in addition to curing, is to remove any discoloration in the skeins due to blood, enzymatic activity, or contaminants. In some instances, hydrogen peroxide is used by secondary processors to bleach discolorations. Following brining, skeins are sorted by quality and size. Most kazunoko is shipped to Japan where it is drained, inspected once again, and packaged for retail sale. Gift packs consisting of individual matched pairs of skeins sell for $10+ per pair (approx. 5 oz).

Individual brined herring eggs (capelin, cod, or tobiko also have the same application) are added to sea vegetable salads and to seafood salads containing, among other things, marine plants (sea vegetables), clams, limpets, or marinated octopus. Another product from herring roe is tarama, a mayonnaise-like condiment manufactured from emulsified fish eggs. Acceptable tarama can be produced from damaged skeins and from overly mature roe.

A most interesting herring roe product is kazunoko kombu or herring roe on kelp and is a garnish for a variety of dishes, most commonly soups, salads or side dishes. It can be very expensive, often over $100 per pound. For the highest quality kazunoko kombu, a uniform, dense layer of herring eggs of similar size and color covers both sides of a piece of kelp. Traditionally, kazunoko kombu is harvested when herring spawn. Schools of herring release their eggs simultaneously, and the eggs adhere to kelp until the fish larvae hatch. Kazunoko kombu is still harvested in the wild; however, most is now produced by harvesting live herring just prior to spawning and placing them into pens (called "pounds") in which cut kelp has been suspended. When the fish spawn, the eggs adhere to this kelp to a thickness of up to one-half inch per side (or an inch in total thickness). The fish are then released back to the wild and the egg-coated kelp is washed, trimmed, cut to market size, and packed in brine. Due to a shortage of natural kazunoko kombu, there have been several attempts at developing acceptable substitutes. One of the somewhat successful attempts at such uses a surimi-based paste as an adhesive in the highly labor-intensive operation of attaching a layer of herring eggs to pre-cut pieces of kelp. The coated kelp is then placed in a form under slight pressure and heat to set it and then packed in light brine.

Other aquatic plants are salted and used as food. Brown kelp (*Laminaria japonica*) is commonly washed, boiled, salted, dried, salted and repackaged. The largest market is China. Seasoned kelp is a popular snack in Taiwan and Japan prepared by cooking dried kelp in soy sauce, sugar, salt and spices. Wakame (*Undaria pinnatifida* and *U. peterseniana*) is a salted dehydrated brown seaweed reconstituted and added to miso soup (10).

Salted jelly fish is made with a multistep salting process by treating brine and alum in increasing concentrations with a final step in dry salt. Uni, from sea urchin gonadal tissues, is also made with salt and alum.

X. SMOKING

Smoking is a form of dehydration and is an ancient form of food preservation. Smoke imparts a flavor and color to the food but limited preservative effect. Fish with a higher fat content such as salmon, black cod or sablefish are the most popular smoked products in addition to specialty products such as cold smoked oysters. Fortunately, *Listeria monocytogenes* is somewhat sensitive to phenolic components in smoke. The shelf life of smoked product is similar to that of fresh product. Therefore, unless the smoked product has also been thermally processed (commercially sterile, canned or retort pouch), or dehydrated to a water activity low enough to inhibit pathogen growth (Aw≤0.85) it must be refrigerated (6).

Cold smoked products are ready-to-eat products that have not received a 'cook step' or pasteurization treatment. To cold smoke fish, cured fish is exposed to smoke at ≤ 90°F (6). Fish can also be cold smoked without brining. Product prepared in this fashion is held refrigerated and then broiled or grilled. It is a preparation that works especially well for halibut, black cod and salmon and is popular for restaurants.

A common smoking process is to treat fish fillets, steak or pieces of larger fish, or butterflied smaller fish

with dry salt or in a salt brine until a salt content of 1–3% is reached. Then the fish are drained and permitted to cure for several minutes or several hours depending upon the product. Curing allows intracellular fluids to drain from the fish, and permits further equilibration of salt within the muscle tissue. During curing, a pellicle or slightly hardened surface layer on the fish is formed from the migration of soluble proteins to the surface coupled with surface dehydration. After curing, the fish is exposed to smoke. The type of product will dictate the type of wood used, the heat, time and humidity of the smoking operation. With few exceptions, hard woods are used for smoking (*e.g.* oak, hickory, alder, maple, cherry, apple, mesquite). The key with cold smoking, is for the temperature remain low enough so that microbial growth is kept to a minimum. Smoking can take a couple of hours for lox to several days for traditional Indian smoked products, some of which are still made over fires in traditional drying sheds.

Cold smoked products are sometimes thermally processed in jars. Thin strips of cold smoked salmon, eel or lamprey are packed in jars with oil and then subjected to a commercial sterilization process.

Hot smoked products are cured and smoked as described above, then, in addition, receive a pasteurizing treatment. A heating cycle follows at the end of the smoking cycle. Depending upon the product, smoke may or may not be applied during this heating step. Hot smoked products are commonly vacuum packaged. Either hot or cold smoked product may also be canned or processed in retort pouches producing a shelf stable product.

XI. DEHYDRATION

Dried fish has historically been a critical item of commerce. Even today, as much as 25% of the world's fish harvest is dried. Access to cod fishing grounds was the cause of major international disputes throughout the 19th century. This is because dried salt cod (bacalao) was the major protein source for workers at sea, during the period of slave trading in the New World, and for the military in 16th–19th century Europe. Dried fish is light and easy to transport and did not decompose without refrigeration. Markets are still strong for dried cod in southern Europe, West Africa, Brazil and the Caribbean. Per capita consumption of dried fish is high in island states, Subsaharan Africa, and in nations with a high per capita fish consumption such as Portugal, where there has also been a historically large cod fishery.

Dehydrated product includes dried and flaked fish and are popular products in Asia. Flaked fish is usually reconstituted prior to use, primarily in soups. Dried bonito flakes form the base for popular condiments in Thailand and Japan. Dried fish (including shrimp, squid and cuttlefish) are often consumed as a snack in Asian communities, among Native Americans, and to a more limited degree in Africa and South America in areas where fish consumption is high.

Salted and dried fish are traditionally made by layering fish with dry salt at levels of 1:4 by weight of salt to fish, allowing the fish to cure and drain from anywhere from a couple of days to a few weeks. Then the fish are removed from the brine, the surface liquid removed, and placed on drying racks, often outside in the sun, until enough water is removed to make the product shelf stable.

Often, fish are strung through the tail or impaled on small wooden sticks to dry, commonly without being salted in advance. In earlier times, fish were split and placed upon rocks to dry in the sun and wind. Sun drying is still used today with major commercial operations for drying cod in the cold North Atlantic winds off the Lofoten Islands in Norway. Drying racks are up to sixty feet high. Similar operations for air drying fish are found in Newfoundland and Labrador. In Hong Kong and in other parts of Asia, small fish to large squid are commonly sun or air dried. In fact, the vast majority of dried fish products are still 'naturally' sun or air dried.

Even though this drying technology is simple it is not without sophistication. Multistage drying processes are conducted in facilities of simple construction. Among the Native American tribes along the Columbia River, salmon fillets are sun or air dried until a certain desired consistency is reached, then the fish are cut into strips, sometimes salted, and placed on dowels in drying racks. Drying, usually in combination with smoking, occurs over a number of days inside a drying chamber or shed. Ambient temperature and wind velocity control drying time.

Often times, dehydration is combined with other food preservation processes such as curing or salting. Jerkies are thin hard or somewhat pliable strips of fish made by a cold smoking fish treated with salt or salt-sugar mixtures.

Aquatic plants are often dehydrated. Nori or laver (*Enteromorpha* spp; *Ulva* spp., *Monostroma nitidum*) are green seaweeds sold in dried sheets that form the outer surface of sushi rolls. Small slices coat the surface of rice crackers and are included in dried seasonings for rice, soups and sauces. Nori is either mechanically or sun dried and is sometimes toasted. Dried or toasted nori is a component in seasoning pastes containing soy sauce and sugar.

Drying is a less important preservation method for fish in developed nations than in the past. Reconstitution and use of salt-preserved foods, such as salt cod or dried cuttlefish, in food preparation is involved and labor intensive. Many dried, salted foods have fallen out of favor in part because current meal preparation times are short – in the West averaging less than 15 minutes per meal! However, the increasing popularity of authentic Asian, Spanish and Caribbean cuisine are exposing more individuals to traditional salted and dried fish products, reintroducing these foods to many people.

All known mechanical drying methods have been used for fish products. The highest value are for freeze dried products such as small shrimp used in instant soups. Microwave drying technologies may replace freeze drying as a cost effective alternative to these methods are further developed. Fluidized bed drying makes a highly acceptable product as well. Flaked fish is prepared on drum dryers and by convective drying methods.

XII. FERMENTATION

Aquatic animal tissues are commonly fermented (9) at both low and higher salt contents. Lactic acid fermentation are utilized to some degree in the production of Asian fish sauces, fish balls and sausages. Fish sauce (from whole fish) or fish pastes (shrimp paste) are salty and highly flavored, and clearly an acquired taste for the Western palate – like single malt scotch and cigars. Fish sauce with specific flavor profiles, clarity and color are national and regional specialties in Thailand (nam-pla), Vietnam and Kampuchea (nuoc-mam), Indonesia (petis), Malaysia (budu), Philippines (patis), China (yu-lu) and Japan (shotturn). Traditionally, fish sauce is made by layering whole small fish with salt (20–40% by weight) in a ceramic crock with a perforated bottom. A weight is placed on top of the fish to remove air and to force the liquid produced during fermentation to drain off. The crock is held for several days at high ambient temperatures. To control fermentation temperature in certain regions, fermentation vessels may be buried in the ground, in this case, preparation of fish sauce may take a number of months. Fish sauce may be filtered or a certain amount of insoluble residue may remain in it. It may or may not be pasteurized. Common types of fish are sardines, anchovies, ambassids and shrimp. In Japan, fish sauce preparations may also contain soy sauce or wheat koji, small clams, or oysters to modify the flavor and aroma. Japanese fish sauces may also contain added sources of proteolytic enzymes to assist with the fermentation process.

Fish pastes can be made from the residue remaining after fish sauce production or by fermentation of fish and wheat bran by *Aspergillus oryzae*. Fish sauce residue is mixed with red rice and fermented producing the pink condiment, bagoong, popular in the Philippines. Similar products are made in southeast Asia by blending fish sauce residue with glutinous rice, or roasted rice and molasses (mam-cho).

Proteolytic fermentations using endogeneous microflora conducted at cold temperatures include fermented whole fish and fish viscera in Asia, and the stink fish and stink eggs of the Northwest US coast up through Alaska and into Siberia. Fermented organs (*e.g.* sea urchin gonad) or muscle (fermented squid) are popular foods in Japan (9). Fermented salmon belly flaps and viscera (the traditional lomi lomi in Hawaii) or fermented pyloric caeca are specialty items in Japanese and Filipino communities. These fermentations are generally conducted with little or no added salt. Among the Native people of the Pacific Northwest, Alaska and into Siberia, seal flippers, marine mammal muscle, and fish heads were traditionally packed into seal skins and buried in the ground for several months. Fermented seal flippers prepared by salting the flippers and packing them into barrels remains a popular food in the Pribilof Islands. Problems arose in these Native communities with the advent of plastic bags and central heating, when people began making these traditional foods indoors within a shorter period and at a higher temperature creating a risk for *Clostridium botulinum* intoxication. After this problem was discovered, practices were modified again to make the traditional foods safely. Stink eggs are another traditional food of the indigenous peoples of the Northwest. In one type of preparation, salmon eggs are fermented by placing them into a small cloth sack coated with flour to exclude air. After several days, the eggs become liquified producing a condiment.

Marine plants are incorporated as ingredients alcoholic beverage production in Japan.

XIII. THERMAL PROCESSING

By the mid 19th century, fish was being thermally processed in metal, lead soldered, and sealed cans. Thermal processing for aquatic foods includes retort pouches and more recently microwave sterilization. Canned tuna and salmon are still common, and new convenient forms of these products are making inroads into lunch markets. Canned tuna remains the most popular fish product by volume, consumed in the United States.

Improvements in the quality, availability and price of fresh and frozen fish products, along with cheaper poultry products, have negatively impacted the canned seafood market, particularly salmon, which is still widely consumed around the world and remains an important product for the Alaskan fishing industry. Producers of canned salmon are seeking new markets in the food service sector with retort pouch products, and with new market forms of canned salmon including seasoned products, canned smoked products, and skinless/boneless products and microwave processed shelf stable portions. The market for canned salmon is not expected to grow, with the possible exception of high-end pet foods. Likewise, the markets for other types of canned seafood products (clams, shrimp, tuna, anchovies, sardines, etc.) are expected to remain stable or to decline.

Recent innovations in commercial sterilization should create new markets for shelf stable aquatic foods. Salmon processed in a microwave retort with overpressure has properties similar to fresh steamed salmon. The dramatic improvement in quality is a result of the dramatically

reduced come-up time for the thermal process that reduces overheating at the surface of the container and resulting loss of texture and flavor. Similarly, dielectric processing using microwave (915 MHz) or radio frequency (27 MHz) energy shows similar promise for pasteurizing heat labile aquatic foods such as caviars and smoked fish that begin to thermally denature around 70°C.

XIV. SUMMARY

Aquatic food products are among the most varied and interesting food products we consume. Improvements in live haul and refrigerated transport have made year round availability of fresh aquatic food products a reality in world markets. Traditional dried, fermented and cured products are becoming popular in more markets as people become exposed to foods from other areas and regions. The aquatic environment, particularly aquaculture, will be the major source for increased sustainable production of protein foods as world population grows, making technical advances applicable in this area critical to food self sufficiency.

ACKNOWLEDGMENTS

We appreciate the support of the International Marketing Program for Agricultural Commodities and Trade (IMPACT) at Washington State University, National Fisheries Institute, and Aquaculture Idaho/Washington for this work and for development of aquatic food products technology.

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160 Surimi and Surimi Analog Products

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I. BACKGROUND

Surimi is composed of the myofibrillar proteins recovered by washing minced fish and was developed in the early 1960's as a source of fish muscle protein for neriseihin or kneaded seafood a popular food in Japan (1,2,3). Japan remains the primary market for surimi where various types of boiled, grilled or fried fish patties, cakes or balls constitute up to one-third of all seafood consumed. Pigott (3) describes the most common product forms as follows:

Kamaboko–washed fish flesh mixed with flavorants, possibly color (usually pink) and gelling agents; shaped, and steamed. It is commonly sliced and added to udon soup.

Chikuwa (broiled kamaboko) – is an open cylinder of kamaboko placed onto a skewer and broiled.

Satum-age (fried kamaboko) – kamaboko is shaped into different forms and sizes and fried. May or may not have other ingredients added.

In Western markets, surimi is most commonly recognized as the base material for production of imitation crab or kanibo. But because surimi is a protein gel, it can be used in all sorts of food products. The most common are breaded, broiled or fried surimi-containing hors d'oeuvres or entrée items. Surimi forms the base for flavored meat, seafood, or vegetable mousses, spread and dips. It can also be extruded into different shapes mimicking shrimp, scallops or lobster and vegetables such as mushrooms. Surimi has been used in low fat imitation cheeses and dairy desserts, fish sausages and fish-based hams. Ham and sausage kamaboko, the first analog products replacing pork with fish and adapting products which are part of Asian cuisines to Western tastes. Other objectives have been to reduce the fat or caloric content of the beef or pork products they imitate. Emulsions using surimi or washed or formed salmon mince or chunks, can also resemble ham or hard sausages. Reduced fat pepperoni and hard sausages have been made from fish, but are formulated most successfully when tallow or pork fat is added, defeating one of the major purpose for using fish as a base, since the level of saturated fat remains high.

Surimi can replace dairy emulsifiers and binding agents in foods where allergens are a concern. A novel application for surimi is the production of a kosher artificial black (sturgeon, lumpfish or paddlefish) caviar. A few of these foods have had limited commercial success, and hopefully more will follow.

Prior to the development of surimi, kamaboko was made from fresh fish in coastal communities in Japan including the conger eel, lizardfish and croaker with records for its production to the 12th century AD (2). Guild artisans produced surimi using a traditional labor intensive process involving a careful filleting of fish, removal of bone, skin, viscera, and the dark stomach lining. The fish was minced, washed several times with water to remove soluble proteins, salts, and lipids. Washing improves gel strength and elasticity, essential properties for high quality analog products (1). In the traditional process, the washed fish mince is drained and forced through a fine sieve. This last step removed any remaining bone and skin fragments. The resulting fish cake was ground with salt in a stone mortar to solubilize the myofibrillar proteins. Flavorings and starch are also blended into the fish muscle matrix forming a pliable dough, which is shaped into half cylinders upon a flat base consisting of a small piece of a native Sawara cypress making kamaboko. The wood contributes an aromatic flavor, and provides a cutting surface for the kamaboko after it is cooked. The kamaboko sets into a firm gel by cooking it in steam.

Recovery of the Japanese economy after World War II significantly increased kamaboko consumption, with production climbing to 268,000 tons in 1954, and to 408,000 tons by 1960 (2) and by 1965, there were over 40 shore-based kamaboko manufacturing plants in northern Japan (3). Accompanying this growth in demand was less availability of fish from in-shore grounds. Kamaboko requires relatively fresh fish and fish frozen at sea produces a less satisfactory product. Further, it was not economically feasible to produce any of the myriad of formulated consumer products, such as kamaboko or chikuwa, on board fishing vessels. A combination of these factors led to the development of the intermediate product, surimi.

In modern commercial operations, surimi is made by recovering the myofibrillar proteins by washing minced fish muscle, stabilizing the washed mince with a combination of cryoprotectants and then freezing it. Cryoprotectants are added to retain the functional properties of the muscle proteins during frozen storage. A typical cryoprotectant combination includes: 4–5% sucrose (granular cane sugar), 4–5% sorbitol (in powdered form), and a 0.3% blend of phosphate salts. These cryoprotectant blends are usually proprietary. Other additives may include calcium lactate, sodium bicarbonate, ascorbic acid, or antioxidants. With cryoprotectants, surimi can be stored frozen for a year or more at −20°F.

Other factors led to an increase in the demand for surimi analogs in the 1980's, most importantly a shortage of king crab which led to the development and commercial production of kanibo or imitation crab, which remains one of the most popular analog products on the world market in the form of "crab legs" and "crab meat" salad pieces or chunks is a popular product in the United States. "Crab legs" are made from small ropes or logs of texturized, colored, crab flavored surimi. Other "imitation" seafood products are much less common in US markets including imitation scallops, shrimp, lagnostino, and lobster.

Unfortunately, surimi production is one of the most inefficient uses of fish and one that comes with a high capital and operating cost. Yields have improved and can exceed 20% recovery on well run lines. The market is currently moving away from pollock surimi production, due to low market prices for surimi, although other species are becoming more common as a source of raw material. The demand for fillets and fish portions compete with surimi for raw material.

Fish that are not used for surimi production are used for individually quick frozen or shatter pack fish fillets (specifically deep-skinned fillets), fillet blocks, or minced fish blocks. Most at sea processing vessels have the capacity to make both surimi and fillet products. Many vessels also produce fishmeal from the meat that cannot be efficiently recovered as well as the skin, bone, viscera. The ratio of surimi to fillets manufactured will depend upon the market for the respective products. Currently, the market for fillets is stable and the price for surimi is low causing a bias toward fillet production.

II. SURIMI MANUFACTURE

Concurrent with development of offshore surimi production was the emergence of the Alaska pollock (*Theragra chalcogramma*) fishery. Alaska pollock is still the most common source of fish for surimi although other fish are commonly used. Alaska pollock could be harvested in large quantities with limited amounts of by-catch and could be purchased for a low price compared to other "white fish" such as Pacific cod (*Gadus macrocephalus*), and during part of the year provided a lucrative roe (mentaiko or mentiko) market. Alaska pollock is currently the most plentiful commercially harvested species in the world with an exploitable biomass estimated at over 6,800,000 metric tons and a target catch weight in most years exceeding one million metric tons. Surimi production is stable at roughly 200,000 metric tons per year. Surimi manufactured at sea on board a factory trawler is generally superior to that produced in shore-based facilities because the fish is fresher. The yield of surimi from at-sea processors also tends to be higher than for shore based plants because of the higher quality of fish. Shore based processors were established in the 1990's for political reasons tied to fishery allocations and not necessarily with the best interest of the fishers or the resource in mind.

To hopefully obtain better control over management of the fishery, the United States foreclosed foreign fishers from the North Pacific fishing grounds off the coast of Alaska in the late 1970's. The Fishery Conservation and Management Act (1976) and the International Fishery Conservation Act, extended the sovereignty of coastal nations over fish from 3 miles to 200 miles leading to the creation of a factory trawler fleet, that in the mid 1990's had over 60 vessels. In response, Japanese companies formed joint ventures with American companies to produce surimi and exert market control over the pollock resource. As part of this effort, U.S. subsidiaries were established to operate shore-based surimi processing plants in Alaska. The shore-based plants could be completely foreign controlled, whereas factory trawlers were required by law to have at least 75% US ownership.

To make matters worse, The Fishery Conservation and Management Act (1976) was amended in 1998, shifted a larger portion of the harvest to these foreign owned shore based processors. This significantly reduced the number of fish available to US owned at-sea processing vessels. This amendment was passed under the guise that a reallocation of fish would create more jobs in the local Alaskan economy; however, it has instead resulted in

the loss of much of the US fleet along with the high wage jobs they created. Currently less than half of the pollock surimi production occurs on at-sea processors, the reverse of the situation in 1997.

Pollock is currently harvested in the US in three to four seasons. In the first or "A" season, roe bearing pollock are harvested. The pollock roe (*mentaiko* or *mentiko)* is a very valuable product and is in high demand in Japan and Korea. During the "A" season in January–April, the muscle tissue is in relatively poor condition because the fish are spawning; therefore the surimi is also of a poorer quality. The pollock harvested in the "B" season during late summer, are not spawning and produce a higher quality surimi. The "C" season runs during the fall, and late in this harvest, some fish are beginning to spawn. A "D" season for early summer is also proposed during some seasons.

In a typical operation, Alaska pollock is trawl-caught and held on-board in refrigerated seawater (RSW) until they have progressed through rigor (Figure 160.1). The fish are then processed as soon as possible post-rigor but never longer than twenty-four hours after harvest. When surimi is to be manufactured at a shore based facility, the fish are held on-board in refrigerated seawater (RSW) at approx. 2°C, and delivered to the plant within 48 hours. Product held more than this will not make high quality surimi. The fish are off-loaded by pumps, and held at the plant in RSW until the fish pass through rigor, which takes around 5 hr.

Other fish species besides Alaska pollock are playing a more significant role in surimi production (see Table 160.1). The international demand for Alaska pollock has increased, both as a source for surimi and also as a relatively inexpensive source of fish fillets that can substitute for cod.

One species that has received particular attention in the United States is the Pacific whiting (*Merluccius productus*). This fish is commonly harvested in Washington, Oregon and British Columbia coastal waters. A relatively large biomass and low price, lack of competing uses and developed markets, and the proximity of the principal harvest, processing areas and markets, led to the utilization of Pacific whiting for surimi production. Recent Pacific whiting landings range between 310,000–350,000 metric tons. Management of the whiting fishery provides for separate in-shore and off-shore harvest allocations that, in turn, encouraged joint ventures by both U.S. fishing companies and Japanese firms with local shore processors in Oregon and Washington. In the Canadian whiting fishery, Canadian fishers harvest fish which are then processed on board foreign processing vessels or at on-shore facilities within 24 hr of harvest. For political reasons, US vessels are currently excluded from this fishery.

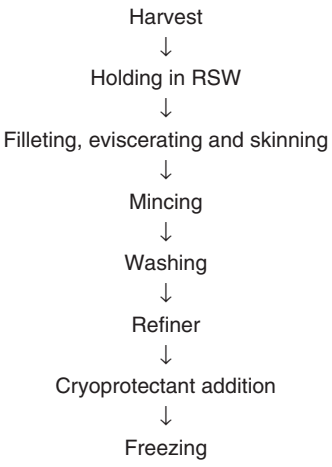

FIGURE 160.1 Outline of a Surimi Manufacturing Process.

The quality of Pacific whiting is affected by seasonal variations in muscle quality and in variations in pH as a result of spawning. A unique quality problem with Pacific whiting is an endogeneous protease produced in reaction to a muscle parasite (*Myxosporidian proteinas*). This parasite causes problems with proteolytic muscle disintegration reducing the gel-forming ability of myofibrillar proteins in whiting surimi. This protease is a cysteine cathepsin and weakens Pacific whiting surimi gel structure by hydrolyzing myosin, it is most active around 55°C (3). Fortunately, protease inhibitors (ca. 1% by weight) can be added to the minced whiting muscle in conjunction with the usual cryoprotectants (sucrose, sorbitol and phosphate salts) to maintain gel forming ability. Calcium lactate may also enhance the effectiveness of the inhibitors. Calcium salts, such as calcium lactate, enhance gel formation by accelerating myosin heavy chain crosslinking via cross-links between negatively charged groups on protein molecules. The most effective protease inhibitors include bovine blood plasma proteins, egg white, potato-based inhibitors (4,5) or whey added to a cryoprotectants blended at up to 1.5%. Recent concerns with "mad cow" disease (BSE) have resulted in the collapse of the blood plasma additive market. Allergen labeling requirements affect the attractiveness of egg white as an inhibitor. Together, these factors may create a demand for a new generation of surimi additives.

The production of surimi is highly mechanized and closely resembles the first few traditional steps in kamaboko manufacture. The yield of surimi can vary significantly from 14 to 30% of the original weight of the fish. A common process for surimi manufacture is outlined in Figure 160.1.

In the initial steps for surimi production, bones from fish fillets are removed mechanically in a "deboner." This is a perforated drum that minces the fish and removes any bones by forcing the tissue through 3–5 mm perforations. The muscle tissue passes through to the inside of the drum, and the bone and any remaining skin remains on the outside. This material may be recovered for pet food or fish meal production. Other types of meat-bone separators

TABLE 160.1
Some Fish Species Evaluated for Surimi Production

Atlantic croaker (*Micropogonias undulatus*)	White croaker (*Argyrosomus argentatus*)
New Zealand Hoki (*Macruronus novaezelandiae*)	Lizard fish (*Synodus* sp.)
Southern blue whiting (*Micromesistius australis*)	Antarctic whiting (*Merluccius australis*)
Pacific whiting (*Merluccius productus*)	Hake (*Merluccius merlussius*)
Atlantic cod (*Gadus morhua* L.)	Pacific cod (*Gadus macrocephalus*)
White hake (*Urophycis tenuis*)	Red hake (*Urophycis chuss*)
Arrowtooth flounder (*Atherestes stomias*)	Yellowfin sole (*Limada aspera*)
Pacific herring (*Clupea harengus pallasi*)	Capelin (*Mallotus villosus*)
Atlantic menhaden (*Brevoortia tyrannus*)	
Atlantic mackerel (*Scomber scombrus*)	Jack mackerel (*Trachurus murphyi*)
Sardine (*Sardina pilchardus*)	Sardine (*Sardinops melanosticus*)
Horse mackerel (*Trachurus japonicus*)	Bluefish (*Pomatomus saltatrix*)
Yellow striped trevally (*Selaroides leptolepis*)	Unicorn leatherjacket (*Alutera monoceros*)
Pink salmon (*Onchorhynchus gorbuscha*)	Chum salmon (*Onchorhynchus keta*)
Northern squawfish (*Ptychocheilus oregonensis*)	
Marlin (*Kakaira* sp.)	
Thresher shark (*Alopias pelagicus*)	Spotted shark (*Galeocerdo curier*)
Silvertip shark (*Carcharhinus albimarginatus*)	Silvertip shark (*Carcharhinus brachyurus*)
Hammerhead shark (*Sphyma lewini*)	Dogfish shark (*Squalus ancanthias*)
Milkfish (*Chanos chanos*)	Threadfin bream (*Nemipterus tolu*)
Sea bass (*Diplectrum fresum*)	Bacoco (*Pomadasys branicki*)
Weakfish or sea trout (*Cyanoscion nothus*)	Lisa (*Mugil cephalus*)
Ronco (*Micropropagon undulatus*)	

have also been used to a lesser degree including sieve type separators. Minced fish is composed of approximately 2/3 myofibrillar proteins and these proteins can form strong gels under the right conditions. In protein gels, the myofibrillar proteins are solubilized by blending, chopping or stirring salt into the minced muscle tissue.

Soluble muscle proteins (sarcoplasmic proteins) including enzymes and heme proteins, blood, and lipid are removed during the washing steps (4) and these must be removed during surimi production if a high quality gel is to be formed. Sarcoplasmic proteins and residual lipids impede gel formation and can accelerate protein denaturation during frozen storage. The washing step is conducted two to four times under agitation at 5–10°C. Typically, in shipboard operations, the fish mince is washed with 1.8 to 3.6 volumes of (5–10°C) water in a countercurrent two-step washing system. Water is removed from the washed minced fish by passing the mixture through rotating screens or through a decanting centrifuge. Having excess washing hydrates the meat and makes water removal difficult during dewatering steps and impedes gel formation as well. Salt (0.01–0.3%) may be added to the final wash to make water removal easier (3). The recovered minced tissue contains approximately 25% solids. The salt content must be lower than that required to solubilize actomyosin prematurely forming a protein gel. Sodium bicarbonate ($NaHCO_3$) may also be added to the rinse water to increase net recovery and to assist in the removal of unwanted constituents (6).

Refining is the final impurity removing stage of processing. A screw drive is used to force the washed mince through a cylindrical screen that has fine perforations. In a refiner, the washed fish mince is passed through a screw press achieving a final moisture content of 72–75%.

Yield is low in surimi processing with as much as one-third of the fish flesh lost during the washing steps. Typically, less than 25% of the "round" (whole, unevisceratedd) fish is recovered as surimi. Although much has been made of the potential to manufacture surimi from pollock frames and trim, this has yet to be successfully accomplished commercially. Almost all surimi is produced from the fillets, and the remainder of the fish muscle tissue is converted to fishmeal or discharged as waste (see Table 160.2). Recovery of additional solids by decanting wastewater streams and though secondary refining can increase overall recovery, but these steps must be conducted carefully if product quality is to be maintained.

Cryoprotectants are blended into the surimi to maintain the gel forming properties of the myofibrillar proteins during frozen storage. The cryoprotectants commonly added are: approximately 5% sorbitol, 4–5% sucrose, and 0–0.3% phosphate (generally in the form of a blend of tetrasodium pyrophosphate and sodium tripolyphosphate). High quality pollock surimi contains approximately 72–75% water, 18% protein, 4% sucrose, 4% sorbitol and 0.3% polyphosphates.

TABLE 160.2
Material Recovery in Surimi Production from Pollock (Data from (3))

Step	Yield
Whole fish	100%
Deheading and eviscerating	60%
Mechanical deboning	47%
Washing	45%
Refining	22%
Dewatering/screw press	20%
Mixing	24%
Extruding	24%

Sorbitol and sucrose act as cryoprotectants and stabilize the protein gel network in surimi during freezing. Sucrose inhibits ice crystal formation and water migration from proteins during frozen storage. Sorbitol and sucrose promote preferential hydration of protein molecules and effectively increase the surface of area of the protein. Other monosaccharides used include glucose, lactose and fructose. Initially 10% sucrose was used as a cryoprotectant, however this produced surimi that was too sweet for Western palates and resulted in the formation of brown off colors during frozen storage from Maillard reactions. Sorbitol, which has a bland flavor, was substituted for half of the sucrose, and found to be effective.The level of sucrose and sorbitol vary depending upon the type and condition of fish and the desired flavor characteristics of the finished product.

Gelling properties of surimi are improved by adding phosphate salts. Phosphates partially decouple actin myosin complexes formed during rigor. By adding phosphate, the gel forming ability and functional properties of a gel approach that of pre-rigor tissue. Phosphate addition can counteract loss of gel strength resulting from starch addition during the surimi analog process. Phosphates also increase moisture retention and increases the ability of a protein to reabsorb liquid when the surimi is thawed or tempered. Phosphate will increase the pH slightly, which will also lead to improved gel forming ability, gel strength and cohesiveness due to an increase in water holding capacity at a higher pH. Phosphate will also sequester magnesium, iron and zinc ions that interfere with gel formation. It can also sequester calcium, and this may or may not impact gel-forming ability. Polyphosphates added at 0.5% provide the greatest gel strength, but 0.3% is optimal for gel strength and flavor (3,6) with sodium tripolyphosphate (STP) and trisodium pyrophosphate (TSPP) used in combination (4).

Sodium bicarbonate ($NaHCO_3$) is also used in the leaching steps of darker fleshed fish to aid in the removal of solubles and lipids in herring surimi and as cryoprotectant in pollock and whiting surimi. Antioxidants have been added to maintain protein functionality during frozen storage, the most common being ascorbic acid which may promote disulfide bond formation.

Normally, surimi is extruded into plastic bags (often 17 b) and frozen in contact plate freezers for 2.5 to 3.0 hours, with a target temperature of −20°C. After freezing, the surimi may be packed two bags to a case and transferred to a storage freezer.

III. ANALOG MANUFACTURE

A common formulation for a gelled fish product will have approximately 60% surimi. However, by adding certain starches imitation crab analogs containing as little as 33% surimi can be commercially produced. A protein content of 11% will provide a strong gel suitable for most analog products. To make an imitation seafood product from surimi, the surimi is tempered to slightly less than 0°C, blended with salt and other additives, including flavors and colorants; formed, extruded, or texturized by multiple folding or the use of spinnerets; and heat set, and packaged, generally under vacuum. These products are ready to eat. As a safety precaution, almost all analog products produced for sale in the United States, are pasteurized and are held either refrigerated or frozen. A common process is outlined in Figure 160.2.

Surimi is tempered, or taken from a frozen state to a condition where it is pliable, under controlled conditions until it can be easily mixed in a silent cutter. This is a large bowl shaped device with moving blades which chop the protein gel into pieces and incorporate salt and other additives before the surimi mix is extruded to form myofibrillar protein gels, similar to what occurs in sausage manufacture.

The texture and flavor of the surimi-based product is affected by the quantity and type of salts added, and the pH of the surimi. Therefore, tempered surimi is combined with salt to solubilize actomyosin. Starch is an extender

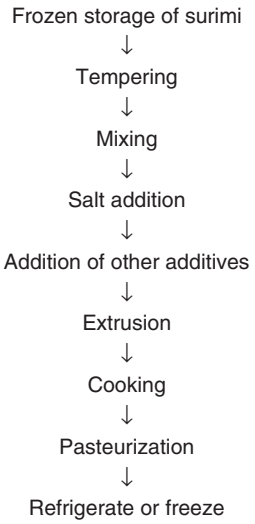

FIGURE 160.2 Surimi analog production.

for surimi and reduces formulation cost. More importantly however, starch can strengthens the gel, and participates in gel formation as a dispersed phase. Egg white also increases gel strength and gel elasticity. Egg white can improve color and appearance of the surimi-based product after extrusion. Flavors, flavor potentiators, colorants and additional phosphate, sweeteners, mirin (Japanese rice vinegar) and antioxidants such as ascorbic acid (Vitamin C) may be added depending upon the desired product traits. After addition of various components, the surimi gel is transformed into a thick paste or protein gel that is subsequently heat set. When heat set, this results in the formation of a three-dimensional, continuous structural matrix.

Salt (NaCl) solubilizes actomyosin. Complete solubilization is required to make a resilient surimi gel. Salt binding to myofibrillar proteins creates electrostatic repulsive forces between protein molecules, loosens the structure of a protein network and permits greater water binding. The optimal salt content varies with the fish species used. Generally, 2.5–3% salt provides optimal gel strength, however 2–2.5% produces products with a more acceptable flavor. The preference for salt content is product and market dependent. Higher concentrations of salt reduce the thermal stability of fish proteins allowing them to gel at lower temperatures. Very high levels of salt decrease gel strength due to protein precipitation, a phenomenon known as "salting out." Freeze denaturation of the proteins in surimi during frozen storage will reduce protein solubility and increase the amount of salt required to solubilize actomyosin during analog manufacture.

The minimum concentration of salt for extracting actomyosin from fish muscle is pH dependent. At pH 7.0, approximately 2% salt is needed. Salt solubilizes myofibrillar protein forming a thick sol or paste. The presence of the chloride ion may shift the isoelectric pH to a lower value increasing protein solubility at the existing pH.

In surimi starch modify texture and improve gel strength of low quality surimi. Adding starch can reduce formulation cost by increasing water retention in the surimi product. It can also bind water, improve adhesion and stabilize the surimi gel. Adding excess starch makes surimi based gels brittle. Up to 20% starch can be added without adverse affects on gel strength.

The mechanism for interaction between starch and protein in surimi gels is not well understood. Swollen starch granules are dispersed within the protein gel matrix. Starch granules swell but complete gelatinization does not normally occur. The surimi gels set at temperatures below the starch gelatinization temperature. Potato and wheat starches are commonly used. Potato starch has a low gelatinization temperature and produces high strength surimi gels. Pre-gelatinized tapioca starch and thin boiling starch produce surimi with reduced gel strength (3).

Some surimi analog products contain egg white. Egg white modifies the rubbery texture of surimi caused by the addition of starch. It also provides the surimi with a whiter and glossier appearance. Egg white makes the partially heat-set analog more elastic and stretchable, this is an advantage for "ropelike" products like imitation crab legs. The amount of egg white added depends upon the fish species used and the quality of the fish used.

Egg white added at 10% produces a gel with a high yield stress, gels containing up to 20% are softer, and greater than 20% there is a decrease in gel strength and gels become brittle. Egg white contributes to the structure of surimi analog gels by filling interstitial spaces in the fish protein network.

Because egg white is an allergen, it must appear on the label of surimi analog products. Class II food recalls of analog products have been initiated in the United States because of failure of companies to list egg white on the ingredient statement.

Carotenoproteins in crab turn orange-red when crab is cooked. This is the reason a colorant is used on the surface of kanibo [imitation crab]. A natural or artificial color is added to surimi paste and applied to the outside surface of ropes of crab leg analog following extrusion.

Flavors are added as liquid concentrates, pastes, or free flowing powders to the surimi mix during blending. Natural and artificial flavors are used. Crab meat extract and extracts from shell and processing by-products have been used. Crab-like flavors derived from non-shellfish sources have also been used to produce a kosher imitation crab.

The level of salt, level and type of phosphate, and level of sugar and sorbitol have an impact on the flavor of surimi analogs. Hydrolyzed protein, dipeptides, or amino acids added to provide meaty, sweet or slightly bitter flavor to analog products. Monosodium glutamate (MSG) may be added as a flavor enhancer. Nucleotides are also added as flavor modifiers, commonly guanosine (0.0035%) and inosine monophoshate (0.01%). Nucleotides enhance flavor potentiating properties of MSG.

Heat setting occurs in 3 stages represented by distinct textural changes. At 40°C, the setting is attributed to hydrophilic interaction of protein molecules. At 60°C, the gel weakens somewhat due to action of endogeneous proteases. Intermolecular and intramolecular protein bonding occurs around 80°C increasing gel strength.

For the production of crab meat analogs, the surimi paste containing the desired additives (with the exception of red colorants) is sheeted in a thin layer and then heat set. After this first heat set, the sheet is scored with a device that looks like a large comb. The sheet is not cut completely through. This scoring forms long thin strips that resemble crab muscle fibers. Several of these strips are rolled together to form "muscle fiber bundles." These are set and then a portion of the outside surface is colored red with a blend of surimi and food coloring. The ropes

are then cut into logs (approx. 4 inches in length), or into small cylinders or diagonal cut product for salad chunks.

A second extrusion process involves extruding spaghetti thin strips of a surimi containing mixture into an acid bath. This sets the surimi and when this material is cut into small pieces and reformed, has the texture and mouthfeel of muscle fibers. This product is commonly mixed with salad chunks for use in seafood salads.

Kamaboko is formed into half-rolls, surface dyed, and heat set. Other products, such as chikuwa (which look like a huge fish ziti or rigatoni) are extruded and cooked.

Surimi texture is affected by how the product is heated and by the heating rate. Cooked surimi based analogs are more stable in the frozen state than surimi. Surimi based products pass through three stages of gel formation during conventional heat processing. The conventional isothermal method of producing analogs is divided into three stages of gel formation. These three stages are treated as separate unit operations called: forming, heat-setting and cooking. The first stages of gel formation occur as the surimi is initially heated. The gel increases in strength up to 13°C. In the range from 13°C to approximately 30°C, few changes in gel properties are observed. A second heat set occurs between 30–40°C and a final cook between 50–90°C during which time the maximum gel strength is realized.

Imitation crab products are commonly vacuum packaged in plastic or nylon packaging. The products are pasteurized inside the package in a hot water bath. This is a food safety precaution because surimi analogs are ready to eat foods. In addition to killing vegetative cells of bacterial pathogens, pasteurization reduces the number of spoilage flora leading to an extended refrigerated shelf life.

Surimi-based products are distributed as refrigerated or frozen foods. For frozen product, individual packages are frozen in a blast freezer, packaged into cases and held in frozen storage, preferably at −20°C. Surimi-based products have a shelf life of approximately 1 year under these conditions. Quality changes, which occur to the product during frozen storage, include flavor changes resulting from oxidation of lipids and lipid soluble constituents, toughening, and loss of product integrity and product texture.

The gel-forming ability of fish is affected by the frozen storage treatment as well as the fish species, freshness, and biological conditions of the fish prior to harvest. Poor frozen storage can encourage enzymatic lipid oxidation, cause protein denaturation and negatively impact gel formation.

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161 Caviar and Fish Roe

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CONTENTS

I. INTRODUCTION

Fish roe products are popular traditional foods, often of very high value, with growing domestic and international markets. Caviars are the salt-cured and preserved eggs of finfish or aquatic invertebrates. Fish eggs are commonly called "roe," particularly when they are present in skeins. Roe products are usually refrigerated or frozen although some are shelf stable, as a result of thermal processing, pickling, salting or dehydration. Roe are sometimes consumed along with other tissue in a single dish for example whole scallops in the shell, shrimp or lobster with coral (or roe), rock sole with roe, and she crab soup.

Caviars are generally made after the eggs have been singled out by screening or otherwise separated from any supporting connective tissue. The eggs are then brined and cured, and sometimes flavored and/or colored. The most widely recognized and valued caviar is made from sturgeon harvested from the Caspian Sea region. Only sturgeon caviar can be labeled in the U.S. simply as "caviar." Caviar from other fish or aquatic animal species must be identified with a qualifying term including the common name of the fish used. For example, caviar from salmon (or ikura) must be labeled "salmon caviar."

II. PROCESSING ROE INTO CAVIAR

Roe products are to be made from wholesome, undamaged eggs, have a proper color and glossiness, texture, a desirable mouth feel, and their characteristic flavor with limited fishy, bitter, or oxidized flavor notes. The preferred mouth feel varies with species. In the case of ikura or salmon roe, a distinct fracture or "pop" when the egg is broken with the teeth or palate, a smooth, honey-like mouth feel is desired, while with sturgeon caviar a buttery texture that tends to melt in the mouth is desirable.

Often hundreds or thousands of individual eggs are enveloped within ovarian membranes and these skeins or whole ovaries can be processed into products such as sujiko. More commonly, the individual eggs are separately recovered, brined and cured. Pastes or spread are common products made by blending eggs with butter, mayonnaise or salad dressing bases, lemon juice or soy or miso based sauces. Roe can be dehydrated. Dried mullet roe is one such food possessing an odd rubbery texture. Technically, caviar should only be used to describe fish eggs that are separated from the connective tissue of the ovaries and then salted and cured.

Roe, regardless of the type, must be an optimal level of maturity to produce caviar. Immature roe tend to be bitter (*e.g*., herring, salmon) and may not take up salt uniformly. If salt uptake is not uniform, the product can readily spoil during storage since psychotropic spoilage micro flora, specifically certain types of lactic acid bacteria can grow within the lower salt pockets of the stored product. Overly mature roe may be soft, lose its elasticity, and may not form a plump, full egg after brining. Overly mature salmonid roes develop tough, rubbery outer shells, while the sturgeons become soft when overly mature. Also, the flavor and consistency of the lipid moieties and proteins change with maturity making the mouth feel of caviar prepared from overly mature roe less desirable.

III. RECOVERY AND YIELD OF ROE

Recovery of roe from whole fish can vary a great deal and is dependent upon the species, method of reproduction, stage of maturity, availability of appropriate feed, the level of stress and various environmental elements. For a female pink salmon, the yield of eggs is approximately 15%, and for a gravid sturgeon as high as 25%. Normally salmon roe is measured as the percent recovered from the entire harvest including both males and females or bucks and hens as they are referred to in trade. For midseason (mid- spawning) pink salmon runs recoveries are as low as 3% (range 3 to 10%, average 6%) (6). Mature roe herring are purchased by the ton on a basis of 10% roe recovery. A herring harvest testing out higher than 10% would receive a premium and one of less than 10%, a reduction in the final price. A more detailed comparison of egg yields for a number of species may be derived from the values presented in Table 161.1.

IV. STURGEON CAVIAR

More than 20 species of sturgeon are harvested for caviar. All are important sources of high quality and expensive sturgeon caviar. The most famous are the caviars produced from the Caspian white or beluga (*Huso huso*), Osetra (or Osietr or Ossietre) (*Acipenser sturi* or *A. guldenstadti* (1), Sevruga (*A. stellatus* or *sevru*), and Chinese or Kaluga sturgeons (*Huso dauricus, A. dauricus* or *A. mantschuricus.* Other important sources of caviar are the Russian sturgeon (*A. gueldenstaedti*), Amur River sturgeon (*A. schrenki*), ship sturgeon (*A. nudiventris*), and Siberian sturgeon (*A. baerii dauricus,* or *A. mantschuricus*) (2). Depending upon the species and environmental conditions, it can take 15 to 20 years for a female fish in the wild to become sexually mature and suitable for caviar production. A single one-ton Beluga sturgeon can produce 350 pounds of caviar worth hundreds of thousands of dollars on the wholesale market (10).

Prices for premium Caspian Sea beluga caviar have always been high, and through 2003 prices of US$700/100 g with prices of US$150–400/100 g for osetra and sevruga caviars depending upon quality common in high end retail markets around the world. Prices for the world's most expensive and extremely rare white or gold colored "Almas" or diamond Beluga caviar from fish, possibly 100 years old or more, was US$2,330/100 g in 2002. Almas caviar is traditionally sold in 24 karat gold tins. It is unclear whether Almas caviar is still available in the marketplace. Current prices of all forms of sturgeon caviar have skyrocketed following new CITES restrictions placed upon trade in beluga caviar in 2005. Current prices

TABLE 161.1
Egg Yield and Size for Fish Roe

Species	Yield (Ave % wt)	Diameter (mm)
White Fish		
Whitefish (*Coregonus* sp.)	14	0.9–1.4
Cod (*Gadus morhua*)		1.3–1.4
Alaska or walleye pollock (*Theragra chalcogramma*)	14	1.3–1.5
Herring (*Clupea harengus*)	18	0.9–1.5
Haddock (*Melanogrammus aeglefinus*)		1.2–1.4
Whiting (*Merlangus merlangus*)		1.0–1.1
Saithe (*Pollachius virens*)		0.9–1.1
Capelin (*Mallotus vilosus*)		1.0–1.2
Carp (*Cyprinus* sp.)	20	0.8–1.6
Sand eel (*Ammodytes lancea*)		~0.3
Pike (*Esox* sp.)		2.5–2.8
Flounder (*Psuedopluronectes* sp.)		0.8–1.2
Lumpfish (*Cyclopterus lumpus*)	23	2–5
Tobiko (*Cheilopogon furcatus*)		<2
Salmon		
Chum (*Onchorynchus keta*)	8–13	4–5
Pink (*Onchorynchus gorbuscha)*	7–11	3.5–5
Coho (*Onchorynchus kisutch)*	7–12	3.5–4
Sockeye (*Onchorynchus nerka)*	6–8	4–4.5
Chinook (*Onchorynchus tshawytscha)*	10	6–7
Sturgeon		
Cultured beluga (*Huso huso*)	25 (max)	
White sturgeon (*Acipenser transmontanus*)	20	<2.2–>2.5 mm

Data from: (6)(15).

(March 2005) for sturgeon caviar at Caviar House, Heathrow Airport, London, UK were as follows:

Osetra: US$220–635/100 g
Sevruga: US$250–400/100 g
Beluga: US$612–665/100 g

Despite a strong demand for sturgeon caviar, supplies of wild harvested product have been decreasing. Over the last few decades, sturgeon (*Huso* spp.) harvest from the Caspian Sea has dwindled, and currently production cannot keep up with consumer demand. Naturally occurring populations of *Acipenser* sp. in Europe and Central and Eastern Asia have also decreased in recent years, resulting in part from political instability in Iran and new states formed from the republics of the former Soviet Union. Because of this, cultured fish species particularly *Acispenser* have received increased attention as sources of black caviar. Sturgeon culture is currently employed to restore natural runs, enhance natural run through hatcheries, or by production of adult animals in culture facilities for meat and roe. These efforts will hopefully ensure that sturgeon fisheries remain viable or become viable again. Currently there are culture operations for *Huso* and *Acispenser* sp. in the nations surrounding the Caspian Sea, Eastern Europe, China, North and South America. However, in addition to CITES, the US Department of Fish and Wildlife has taken the short-sighted position of barring sale of beluga caviar in the US, effectively stopping culture efforts of *Huso* sp. in the United States, because the agency is concerned that it will not be able to differentiate whether or not caviar is from these culture operations or from wild harvested product.

Regulation of caviar production is nothing new, with the fishery highly regulated for centuries. Russia and Iran are the largest producers of black caviar in what have been historically tightly controlled politicized fisheries. The Russian government has regulated production of black caviar since 1675 when Tsar Alexi prohibited Cossacks from direct marketing their caviar to foreigners (4). Many individuals associated with caviar production were executed who objected to Soviet control of the industry in the early 1920s. Fortunately, the Soviet Union was able to maintained reasonable control of caviar harvest and production, maintaining product quality and supporting close monitoring of fishing effort to protect fish stocks during this era. But after the break up of the Soviet Union, management of the fishery fell apart.

Iran is the other major producer of sturgeon caviar. Their territorial waters do not appear to have suffered to the same extent from poaching and boot leg production as areas under the control of the former USSR republics including Russia. Sturgeon caviar is a subject of Persian lore back to biblical times, but the fishery was not highly regulated until 1893. In 1893, the Lianozov brothers managed to secure a fishing concession from the Persian government to harvest sturgeon and process the roe into caviar under a quality control system similar to that in place in Russia. The Soviets seized the Lianozov caviar operations in 1925, ignoring the fact that the operations were in Iran, not the USSR, creating a series of conflicts with Reza Shah Pahlavi who took umbrage at this incursion. By 1927, the Persians signed an agreement with the USSR government and caviar operations recommenced (4). The ouster of Mohamed Reza Shah Pahlavi in 1979 precipitated over 20 years of political chaos in Iran including an eight year war with Iraq that following directly upon the heels of the Islamic Revolution. Governmental control of caviar production in Iran deteriorated after the Revolution along with programs to protect the fishery. Fortunately, the situation has improved in recent years. Iran continues to be a major producer of caviar with well established markets in Europe and recently in the U.S. markets following a two decade trade embargo.

Unlike Iran, the current situation in the former Soviet Union is grim. The construction of dams on the Volga River and the devastation to the fishery caused by virtually uncontrolled poaching after the breakup of the USSR has brought the Caspian sturgeon fishery to the point of collapse with extinction of the beluga, osetra and sevruga

sturgeon becoming a very real threat. Projections are that 90% of the beluga sturgeon population has been lost in the past 20 years. In late 2001 only 28 beluga sturgeon were found over an entire Caspian Sea collection area with over 85% of the fish harvested being immature suggesting a highly depleted population (5).

Although there are several well intended efforts to restore the depleted runs, the generally poor economic conditions in the effected countries has resulted in a reduction of funding to maintain sturgeon hatcheries in Russia and the former Soviet republics, and this has made matters worse. Demands for the surrounding range states to cooperate in setting realistic quotas and for conducting comprehensive surveys of sturgeon stocks have fallen on deaf ears. Recent harvest quotas were largely arbitrary and do not reflect fishing pressure on the Caspian Sea stocks (5).

The situation with poached sturgeon has become so desperate that Russia has considered establishing a governmental monopoly on sturgeon harvest as a means to control poaching to some extent and maintain a viable fishery. Predictions are that illegal fishing generates $2 to $4 billion dollars a year in Russia and the former Soviet republics (Anon, 2001). Interpol has been involved in assessments of illegal trade in sturgeon although currently available data on illegal harvesting, trade and enforcement is very limited, citing the "Paris Agreement" CITES SC45 Doc 12.2 (5).

Control of the fishery is difficult. For example, the Dagestani Coast Guard confiscated 64 tons of fish and 184 kg of caviar from poachers in 2000 and 10 tons of sturgeon through April 2001. In response, a crowd of at least 300 poachers stormed a coast guard station in Izberbash, Dagestan, to forcibly retrieve their confiscated boats and fishing nets in a well-organized attack that local officials described as part of the ongoing war with the local "caviar mafia" (6). Poachers used their wives and children as human shields in this attack. The seizure was a result of a newly instituted Russian restriction on sturgeon fishing and the caviar trade. More problems are sure to follow in light of new fishing restrictions. Mature and immature sturgeon are commonly available in bazaars and for sale on the street, with most of this harvest unregulated.

As a result of the threat to the fishery, international organizations have called for measures from severe restrictions to the complete prohibition of import/export sales of caviar from Caspian Sea species. The UN's Convention on International Trade in Endangered Species (CITES) and Russia, Kazakhstan, Turkmenistan and Azerbaijan reached agreement in January 2002 to severely restrict production of caviar from their waters (7). Regardless, much bootlegged product remains on the market.

The US imports roughly 80% of all beluga caviar (5) and there have been numerous incidents of caviar being smuggled into the U.S. in recent years. In 2000, the U.S. Fish and Wildlife Service seized one ton of illegal imported product through its enforcement powers under the Endangered Species Act, destroyed it, and fined the importers over 10 million dollars. Misbranded and adulterated products are also common in the US market. Decomposed, adulterated, and misbranded osetra, kaluga, sevruga, and beluga caviars have made their way to U.S. markets, and, when detected, have been seized.

In 2002, the US Fish and Wildlife Service proposed a ban on beluga caviar imports into the United States stating that: "Despite the CITES listing, beluga sturgeon populations have continued to decline, and the population structure is increasingly skewed towards sub-adult fish, with a critical lack of spawning-age adult female fish." The US Fish and Wildlife Service proposed to list the species as endangered[1] due to "...loss of habitat throughout historic spawning areas due to dam construction and river-modification projects, over-harvest, widespread poaching and illegal trade, and pollution, [which] imperil the continued existence of this species."

The net effect of the turmoil in the Caspian Sea fishery has been an increase in aquaculture or farmed produced sturgeon caviar. The product is accepted as a responsible way to continue the production of this delicacy and to protect the remaining wild stocks. Although captive breeding programs for sturgeon in Russia began in the 1930s, development was much later in other countries. There are currently commercial aquaculture operations for sturgeon in Germany, Hungary, Romania, Italy, France, Spain, Portugal, Israel, Chile, Argentina, Russia, China, Iran, the Czech Republic, Uruguay and the United States. France and the Czech Republic each produce roughly 10 tons of caviar annually, with French production expected to double in the next 2 years (6). Most of these operations rely upon primary species such as beluga while commercial hybrids of the beluga include the bester, a cross between a female beluga sturgeon and a male sterlet (*Acipenser ruthenus,* also listed as *Acerpensiformes ruthenus*) are also cultured. There are also emerging commercial aquaculture operations of the single strain sterlet (*Acipenser ruthenus*) in Hungary, Poland, other European countries, and Florida directed toward meeting the international demand for gold caviar.

[1]An endangered species is any species which is in danger of extinction throughout all or a significant portion of its range (16 U.S.C. 1532(6)). Factors which support listing the beluga sturgeon as endangered include: (1) loss of 85–90% of its historic spawning habitat through dams, river channelization etc., (2) over fishing, (3) disease causing tumors, reproductive abnormalities and large fish kills throughout their range, (4) inadequacy of existing regulations. For example, arbitrary catch limits, no maximum size limits which would protect spawning fish, poor control of by-catch mortality, insufficient penalties for poaching and illegal trading, and (5) an additional problem is lack of genetic diversity in hatchery stock in which fewer than 10 adult females may provide the base for regional hatchery operations for any given year class. 67 Fed Reg. 49657-49660.ecting sturgeon li.

Commercial harvesting of sturgeon in North America appears to have started about 1750 in New Jersey, however, the production of caviar did not enjoy much attention until the mid-1800's. Initially starting on the Atlantic coast using Atlantic sturgeon (*Acipenser oxyrhynchus*), the production expanded to the Pacific coast from California to British Columbia where the white sturgeon (*Acipenser transmontanus*) was most common. The industry was virtually eliminated in just 50 years by overharvesting. US caviar production is currently seeing a significant revival primarily due to the farming of white sturgeon primarily in California and Idaho.

The most common source of black caviar in North America is the white sturgeon (*Acipenser transmontanus*). This is also the most common sturgeon found on the North American continent. However, the production of caviar from the native wild stock is not normally permitted due to the near extinction of certain sturgeon species and subspecies in some North American watersheds. Therefore, almost all commercial production of sturgeon for either caviar or meat is from cultured sturgeon. Captive breeding programs for white sturgeon along with federal, state, and tribal management projects for wild stocks in the Snake, Columbia, and Missouri River systems should reverse a decline in wild fisheries stocks. In the Pacific Northwest, Native American tribes, along with US Fish and Wildlife, are the primary leaders in sturgeon restoration efforts. The Nez Perce, Kalisbell, and Yakama Nations have wild white sturgeon broodstock in captivity and are developing fishery enhancement programs for the sturgeon. Some of the tribal organizations are expanding their restoration efforts to include production and growout facilities for fish suitable for caviar production. By coupling restoration efforts with production, there is a greater likelihood that programs will remain viable because they would become financially self-sustaining.

U.S. culture of sturgeon began in earnest in the late 1970s. In 1979, the U.S. began an intensive aquaculture program for white sturgeon as part of the Aquaculture and Fisheries Program at the University of California, Davis (8). For this program, wild female brood stocks were harvested and their eggs surgically removed. The eggs were fertilized and the first hatchlings produced in 1980. After this first success, commercial aquaculture firms began raising sturgeon for caviar from *A. transmontanus* possible between 6 to 10 years of age (8); other sources indicate 8 to 9 years (9). Dozens of sturgeon farms have been started in California (10) and Idaho. Southern states, including South Carolina, Florida and Louisiana, began evaluating programs for culturing Atlantic and Gulf sturgeons during the 1980s with current production and pilot operations in place. Cultured sturgeon are harvested at 1.5 to 6 years for meat production and 7 to 10 years for caviar. The Gulf of Mexico sturgeon (*Acipenseroxyrinchus* desotoi) a subspecies of the Atlantic sturgeon distributed from the Mississippi River to Tampa Bay, was commercially harvested for caviar in small quantities in inland Florida (12). As with other North American sturgeons, culture programs have been established for the Gulf sturgeon.

Unfortunately, determining the sex of even mature sturgeon can be relatively difficult. Currently, the most practical method of sex determination requires a small incision in the upper part of the fish's abdomen and a visual examination of the gonadal tissue. Male fish are generally diverted to harvest for meat and the female fish are returned to growout facilities where they remain until they have reached sexual maturity. At maturity, female fish may weigh from 40 to 80 kg. Premium caviar from farm-raised sturgeon is valued at a price equal to that of imported osetra or sevruga with the lowest prices around $36.00 per ounce (3) and production in California farms projected at 30,000 pounds annually.

A. Processing Sturgeon Roe

Preferably, black caviar processing begins with the removal of the roe immediately after the fish has been killed. Wild sturgeon are generally harvested by seining and are transported alive to the processing facility. In some instances, the roe is removed from the sturgeon on board the harvest vessel. It is not necessary to kill the fish however to take the roe. Russian technologists long ago discovered that as much as two thirds of the roe can be removed by Cesarean section and after a short recovery period the fish can be returned to the wild or to the aquaculture tanks (6), thus roe can be harvested again from these fish during later spawning cycles. There are many instances in which roe has been successfully harvested over ten times from the same Beluga female and Sternin reported that eggs were taken from a single bester seven times over a period of 15 years (11).

Processing caviar from cultured sturgeon begins when the fish are removed from the growout tank and transported immediately to the processing room where they are stunned and the roe is immediately removed. Bleeding the fish prior to roe recovery improves quality. The roe is then normally controlled in lots which identify the individual fish from which the roe was harvested. The roe is kept on ice and then processed as rapidly as possible under sanitary conditions in a cool environment. The total processing time from extraction to primary packing is normally less than 2½ hours.

Following extraction, the first step in caviar production is the separation of the individual eggs from the connective tissue. This is normally accomplished by rubbing the skein on single stainless steel screen over a stainless steel bowl. The opening dimensions of the screen are approximately equal to the size of the eggs being separated. Traditionalists in Russia still prefer linen thread screens. Nylon has also been used with success. In contrast to singling out of salmon eggs where two or even

three layers of screens are used, only a single screen is commonly used for sturgeon eggs.

The singled eggs are then inspected, picked over, and briefly washed in a chilled, mild saline solution (ca. 3%) to remove extraneous debris. This rinsing step should not be more than 15–20 seconds or the quality and the shelf life of the end product may be reduced. The eggs should be briefly drained following the rinsing.

The eggs are then placed in a stainless steel bowl, weighed and 3–5% by weight of fine, non-iodized salt, is added and blended by hand. They are then immediately spread out in a 2–5 cm thick layer on a stainless steel, fine mesh, and screened rack for curing. The curing step is very brief and need not be more that 5–15 minutes. From curing the caviar is placed in two-part, specially coated, tins for aging and sale. The tins are slightly overfilled and the top placed on and pressed down to gently compress the caviar so as to drive out any trapped air. A broad rubber ban is placed about the connecting segment of the can and cans are placed in refrigerated storage, initially on their sides, for continued draining and curing. This step takes approximately 28 days for the caviar to develop a mild and distinctly oxidized flavor favored by caviar aficionados. It should be noted, however, that many Americans prefer the flavor of the caviar present immediately after the initial 15 minute curing which leads us to another subject. Just what is good caviar?

Salinity (total salt) of 3 to 3.5% is achieved in the final product after the excess fluids are drained off (11). The final salt concentration will vary depending upon egg maturity, freshness, brining temperature, and brining time. Salting affects the physical characteristics of the egg and increases the hardness of the egg sheath. Egg sheath assays show that the hardness doubles after brining and curing.

Sturgeon caviar can also be pressed (pausnaya). Small or damaged eggs may also be lightly salted and compacted into a product that resembles a thick marmalade and is used as a spread. Damaged eggs are also added to butter or soft cheese and can be incorporated into sauces or pâtés.

Fine caviar has much akin with fine wine and individual preference can be very different. Superb caviar to one person may not be so to another. In general, however, the best caviar should not taste salty, fishy or musty. It should have a mild flavor described by some as being slightly "nutty." The texture should be similar to that of butter and should almost melt in one's mouth. High quality caviar should have no detectable membrane or residual shell material present and a pleasant aftertaste. There should be no metallic or other off-flavors.

Caviar also tends to readily absorb off-flavors, particularly from contact with certain metals. Traditionally, therefore, it is consumed with an ivory, mother of pearl or horn spoon and served in glass or porcelain bowls. Caviar should be stored in the refrigerator, tightly closed in the jar or specially coated metal tin in which it was purchased. Some individuals like to place the container inside of a sealed plastic bag as well.

A few additional notes on processing; for maximum quality and storage stability, sturgeon roe should be handled using exemplary sanitation practices and processed at as low a temperature as possible. The processing, curing and storage areas should be as clean as an operating theater and the temperature of the area kept well below 50°F while processing. The eggs should not be allowed to come in contact with metals other than stainless steel. The water used in the processing should be as aseptic as possible and not contain chemicals that will impart any off-flavors. Only non-iodized salt should be used and salt that has not been coated so as to reduce caking. Kosher-style salt seems to work quite well.

The flesh of harvested sturgeon is first reduced to what is referred in the seafood industry as a "bullet." A bullet is formed by removing the fins, scutes (the armored plates on the sides of the fish), gills, blood, intestines and the spinal cord from the fish. The bullet is commonly processed further into fillets, steaks, roasts, and hot or cold smoked meat products. The skin can be tanned into rather durable leather.

B. Paddlefish Caviar

Caviar from the sturgeon's poor cousin, the paddlefish, is showing significant growth in the Southeastern and Western US as a source of black caviar. In North America, paddlefish (*Polyodon spathula*), the shovel-nose catfish (*Hemisorubim platyrhynchos*) in addition to fresh water sturgeons (*Acipenser* sp.) are harvested from wild stocks for black caviar production. Paddlefish are found mainly in the Mississippi–Missouri River systems, reaching as far north as Minneapolis and St. Paul, Minnesota; as far east as Pittsburgh, Pennsylvania; and as far south as New Orleans, Louisiana.

Their roe is processed in a similar manner as sturgeon roe and the quality of these products can be very high, although the products are generally less expensive than sturgeon caviar, ranging between $7 to $15 per ounce (3). However, wholesale prices of U.S. domestic paddlefish caviar (2002) reached these levels with retail sales prices being in the range of $20–35/oz. Restriction on sturgeon caviar should cause prices for these products to increase. Domestic production of North America paddlefish roe is roughly 60,000 pounds. The commercial culture of paddlefish is viable. The fish are raised both for their meat and roe. The meat is commonly hot smoked. Paddlefish feed on plankton, and this has led to a unique method of polyculture for these fish in the U.S. Paddlefish can be placed at a relatively low density in catfish ponds where they feed on the natural algal blooms and on blooms resulting from the breakdown of uneaten feed and fecal material. This type of polyculture has the potential to reduce the problem with benthic off-flavors in catfish

resulting from consumption of geosmin containing algae. The paddlefish must large enough before introduction into ponds to discourage predation by the catfish.

The roe from paddlefish harvested from recreational fisheries in Montana are sold by non-profit civic organizations and the government to commercial processors and the derived funds are used to support local fisheries and wildlife programs.

V. OTHER FISH ROE PRODUCTS

The increased popularity of sushi, coupled with a heightened interest in haute, international, and fusion cuisines, has spurred the development of expanded markets and new products from fish and fish roe. Important quality parameters for roe are a small sized egg (generally); a mild flavor and an appropriate mouth feel including a good "pop." The most marketable roe products can withstand distribution and handling procedures, frozen storage, and have a reasonable shelf life under refrigeration. Fish from many species can be colored or flavored to match popular products such as black caviar or tobiko, which are in short supply. Data for size and yield of various fish roe are presented in Table 161.1.

A. Catfish Roe

Channel catfish (*Ictalurus punctatus*) has been evaluated as a black caviar substitute (13). Catfish roe from *Ictalurus sp.* and *Clarius* sp. resembles that of paddlefish or sterle and ranges in color from dark charcoal to gray and rarely to a light gold. The eggs have a greater variation in average diameter than do most other species and many are of a smaller size. The eggs also tend to be much more difficult to separate at earlier stages of maturity, however, can be made in to an excellent spread that has a number of gourmet applications.

B. Salmon Roe

"Red" or salmon caviars, called "ikura" in Russia and Japan are popular around the world. Ikura is the style of salmon caviar where individual eggs are separated from connective tissue and cured. Other forms of salmon roe preparations include marinated roe, smoked and flavored roe products, barako, and sujiko. Sujiko are salted and flavored whole skeins. The majority of salmon caviar is produced from Pacific salmon, with chum salmon (*Oncorhynchus keta)* and pink salmon (*O. gorbuscha*) being the most popular. Salmon caviar is also produced from the other Pacific salmon: coho (*O. kisutch*), sockeye or red (*O. nerka*), and king or Chinook (*O. tshawytscha*). Both the largest volume and value is from chum salmon with ikura production at 2000 to 3000 MT per year. Salmon eggs are a major source of income for Alaskan harvesters and processors; the price paid for chum and pink salmon is often so low as not to warrant harvest or processing, however, the roe always has value. It is against the law in North America to simply harvest the roe and not to utilize the remainder of the carcass, a process known as "roe stripping;" however, some jurisdictions have permitted the practice in times of severe economic depression. The practice is not as harmful to the salmon runs as one might think at first glance, as these salmon are at the termination of their life cycle and spawn but once and then die. The harvest size is calculated to not adversely impact future runs. Still the practice of discarding the carcasses should be avoided and some use should be found for the fish.

Atlantic salmon (*Salmon salar*) and Arctic char (*Salvelinus alpinus*) are used to a lesser degree for caviar production (6). The Pacific masu or cherry salmon (*O. masou* masou) is a minor source of ikura, restricted to small regions of Japan and Korea (11).

Returns of salmon are cyclic with a peak in the late 1990's followed by a collapse in 2000, putting the valuable roe in incredibly short supply. Chum salmon returns for the 2001 harvest season were similarly grim. Harvests increased in 2002 and 2003, but prices were somewhat depressed due to a poor economy and near deflationary conditions in important East Asian markets. The market potential for 2004 was brighter, due to a stronger economy, and to the resurgence in interest in wild harvested salmon resulting from a number of unjustified food scares tied to cultured salmon in 2003 and 2004. High protein low carbohydrate fat diets spurred a trend for greater salmon consumption and bolstered prices for wild salmon. This trend is expected to continue through 2005.

Most of the salmon roe harvested in Alaska is exported, primarily to Japan, Korea, China and Western Europe although the US domestic market is growing. Some is processed into ikura in Alaskan facilities, but a large quantity of the "green" or unprocessed roe is simply packed in bulk, frozen, and then exported.

Farmed Atlantic salmon (*Salmo salar*), coho salmon (*O. kisutch*), rainbow trout (*O. mykiss* formerly *Salmo gairdneri*), and its ocean run variant steelhead trout (also *O. mykiss* and referred to as salmon trout) are available in large quantities, exceeding 1 billion pounds per year. Due to the current glut of salmon on the world market, Atlantic salmon culturists in Norway and Finland are holding fish to sexual maturity, harvesting the roe, and producing a very high quality salmon caviar. The market for the product is growing, particularly in Europe and in South America. Salmon trout is already a popular source of red caviar in Europe where much of the product is from cultured fish. There have been some complaints in Japanese ikura markets that roe from cultured fish have an "aquaculture" smell, but that the appearance, color and sheen are excellent (6). Any legitimate issues surrounding the flavor of the aquacultured product can be addressed by altering fish diet. If there continues to be an oversupply of

salmon on the world market this will lead to even a greater proportions of the harvest being directed toward the production of roe products.

1. Processing Salmon Roe-Sujiko

Eggs from salmonid fish are much larger than sturgeon roe, for chum, the eggs range from 4 to 5 mm diameter to as large as 7 mm for Chinook salmon (Table 161.1) and are generally processed with less added salt. Salmon roe have a less "fishy" flavor and oxidized flavor than many sturgeon caviars (6) because salmon roe products are not aged.

Salmon roe are most commonly processed into cured, individual eggs (ikura) or as whole egg skeins (sujiko). Sujiko is prepared by brining whole roe skeins for approximately 20 min in a solution of salt, nitrites, polyphosphates, and other additives and seasonings. After brining, the skeins are sorted by quality and size, and then alternately layered with fine salt in plastic or wooden containers (most commonly containing 5 kg of the finished product). The curing process involves compressing the skeins under a weight for 3 to 5 days at temperatures below 50°F (11°C). The freshly brined product contains approximately 20 ppm nitrite and after curing, the finished product should have no more than 5 ppm, which is the maximum concentration allowed for import into Japan. At this time, this is not an approved use of sodium nitrite in the United States, therefore, all product thus processed in the U.S. must be exported.

Sockeye or red salmon is the primary species used for sujiko production, although chum and pink salmon are also used in large quantities. The early summer run of sockeye salmon in Bristol Bay constitutes the largest single supply of sujiko, and every effort is made to speedily process and ship it to Japan each summer for the Japanese summer festival of Obon at which sujiko is traditionally consumed. A byproduct of sujiko production is "barako," or singled-out eggs from broken or rejected skeins of sujiko. To make barako, the broken skeins are removed following the sujiko brining process, and the eggs are simply singled by mechanically separating them from the skein membrane using a screen.

2. Processing Salmon Roe-Ikura

a. Separation of eggs from skein material

The process for separating the eggs from each other and from skein material is called screening. Screening is normally a manual, laborious, and time-consuming process. Using the conventional method, the roe is rinsed with 3% salt brine. Then the individual eggs are removed from the skein by mechanically forcing the eggs gently through specially designed three tiers of nylon or stainless steel screens. Enzyme-based processes for removing the connective tissue surrounding the eggs for ikura production decreases hand labor and may increase recovery.

b. Enzyme processes for egg recovery

Enzyme preparations for roe recovery include high concentrations of collagenase. The enzymatic process mimics the release of collagenases and other activities by the female fish to dissolve the connective tissue surrounding mature roe when the eggs are released into the water column for fertilization. Recovery of a high yield of good quality eggs varies greatly with enzyme treatments. Proteolytic enzyme mixtures for removing the membranous egg skein material from the individual eggs have been developed for salmon roe.

c. Brining and curing processes for ikura

Brining and curing fish eggs is still an art and requires a great deal of skill. Salmon caviar usually contains 3 to 4% total salt for "malasol" or lower salt caviar. Higher salt products in the range of 4 to 6% are also common and are becoming a regulatory requirement in some markets due to concerns about *Clostridium botulinum* growth. Products with a salt content (2.8 to 3.5% salt) are becoming more popular as consumer preferences change, but these products tend to have a shorter storage life and must be prepared with this consideration in mind.

For ikura, the separated eggs are agitated in brine (for saturated brine the egg/brine ratio is usually 1:3 (v/v) or less). The eggs are brined, generally 2 to 6 minutes between <8 to 12°C, until the desired salt content is reached. The shell of the egg becomes fairly firm after brining. The best process control for ikura manufacture is achieved using saturated brine. Salt uptake of the egg varies between species of salmon. Salt uptake also depends upon the condition and degree of maturity of the eggs and often is affected by whether the fish are "early" or "late" run. Salt uptake varies with eggs in a given lot by 0.5% or more (26). Salt uptake is also faster in frozen or enzyme treated roe, compared to fresh roe, assuming that roe are of the same quality initially and consistent salt uptake is difficult to control (6).

After brining the roe is cured. During the curing process for ikura, the roe is placed on inclined stainless steel screens or in perforated plastic baskets, allowing the excess salt brine to drain off. Salmon roe is cured at refrigeration temperatures for at least 30 min and up to 12 h. This allows excess surface and cellular fluid to be released from the eggs and drained off. Also, the curing permits the salt concentration within the egg to equilibrate and the eggs sheath to harden slightly. Efforts to speed up the curing process by the using fans, etc. results in a product of lower quality.

3. Processing Salmon Roe, Marinated Roes and Other Products

Once the predominant salmon roe product in Japan, the popularity of sujiko is decreasing and being replaced by

lightly salted ikura and flavored/marinated salmon roes. Marinated roe constitutes up to 80% of some Japanese ikura markets (6). These are prepared by taking singled out green roe and marinating them in a solution of garlic, mirin or Japanese sweet rice wine vinegar, soyu (soy sauce), sugar, or other seasonings. The product may be prepared in a commercial food processing plant or in the home or restaurant just a short time before serving. Roe treated in this fashion is used in a number of dishes, including sushi, in sushi-rice combinations such as domboro, or in a number of other Asian or Pan-Asian preparations. The popularity of marinated roes and ikura is also increasing in Europe, North America, China, and other Asian countries.

In Europe and North America, ikura or "red caviar" is commonly consumed as a hors d' oeuvre. It is also added to cream or white sauces in pasta dishes, as a garnish on seafood salads, fish or poultry entrees, and served as a condiment with egg dishes. The traditional wild salmon caviar markets in Europe have been in Germany, Spain, France, and Scandinavian countries. New growth markets are in Eastern Europe and in the Commonwealth of Independent States (former Soviet republics). Western chefs have began to discover many uses for ikura and other roes and the caviars are increasingly found on the menus of white tablecloth restaurants

Traditional Pacific Northwest Native American and Native Alaskan salmon roe products include compacted and dried whole skeins (salmon egg cheese) or "stink eggs." Stink eggs are produced by covering the skeins in animal skins and burying them in the ground, allowing them to ferment under anaerobic conditions and reasonably cool temperatures. Other preparations involve holding salmon eggs in flour coated cloth sacks at temperatures somewhat below ambient for a period of time sufficient to obtain the desired flavor development. Eggs are also dried or smoked and used in soups.

C. Lumpfish Roe

Lumpfish (*Cyclopterus lumpus*) roe is an extremely popular, moderately priced 'red' or 'black' caviar and in much the same fashion as more expensive caviar products. Flavor should be mild and somewhat salty, but with no strong fishy flavor notes.

In its natural state, lumpfish roe is a small 2- to 5-mm egg and ranges in color from gray-white for the immature roe, to purple to red (mature roe), to reddish-orange for overly mature roe (14). The eggs are dyed black or red. Female lumpfish release approximately 100,000 eggs as a sticky mass that attaches to rocks or benthic organisms in the ocean environment during spawning. The male guards the nest of eggs until hatching (10). Lumpfish roe can survive pasteurization better than other caviar products and can also be produced at a high enough salt content and a low enough water activity to make a shelf stable food commonly found in supermarkets. Gum tragacanth may be added to reduce the water activity. Sodium benzoate is commonly added to inhibit mold growth during refrigerated storage.

1. Processing of Lumpfish Caviar

Lumpfish roe is mechanically separated out and the resultant caviar salted to 3 to 5% salinity and packed immediately, or salted to 10 to 14% and then delivered for reprocessing, desalting, and repacking for later retail sale (11,14). Salt levels of 5.8 to >11% may be found (14). After addition of the dry salt, the eggs stick together and must be mixed until uniform dispersion is formed from the eggs and the brine created from expressed intracellular fluid. The eggs are drained and cured. Curing is important for achieving the desired viscosity of the inner fluids of the egg.

An alternative process involves rinsing roe recovered by a mechanical screening process in 5% salt to remove blood spots, ovarian membrane, and other foreign material. At this stage, the product can be stored at 0 to 3°C in vacuum sealed packages for curing at a later date.

Lumpfish caviar is dyed as part of or after the curing process. Most lumpfish is dyed black using coal tar based dyes but 10 to 15% of the world's production is dyed red. Commonly for each 100 g of roe, 0.4–0.5 g of a black dye, 5g salt and 22 ml water are added and held at 4 to 7°C for 1 h. The caviar is then rinsed with an acidified brine (20 g salt, 5.2 g 8% acetic acid, 0.12 g citric acid (per 100 ml)), drained for 30 min, and then vacuum packaged and refrigerated (6,14).

D. Tobiko or Flying Fish Roe

A popular caviar used in sushi preparations is flying fish roe or tobiko [*Cheilopogon furcatus* (spot fin flying fish)]. This is a small, crisp, golden orange roe with an egg size of 2 mm or less. It is sometimes flavored with chili. Wasabi (Japanese horseradish) tobiko is a popular condiment where tobiko is mixed with wasabi and dyed a light spring green. Sometimes tobiko is dyed red. As with salmon caviar or ikura, tobiko may also be flavored with Japanese rice wine vinegar (mirin), a fermented soybean paste (miso), soyu, or other seasonings. In these forms, it is generally served with rice or sushi. Caribbean Islanders commonly fry flying fish, and make a pie from this fish which also includes the roe.

Because tobiko is in short supply, faux tobiko is often prepared from the mature or immature herring roe (*Clupea* sp.), from the eggs of small capelin (*Mallotus villosus*), smelt (*Spirinchus lancerolatus)*, and other fish that have small, bland, light colored roe. For making faux tobiko from immature herring roe, whole herring roe skeins are brined, then graded. The salt content is reduced

by rinsing or soaking the skeins in weak saline solutions or water. Various colorants or flavors can be added at this point. The individual eggs are recovered by rubbing the skeins on a plastic or metal screen.

E. Whitefish Roe and Similar Products

Other fish with roe of small size sold commercially are mullet (*Mugil cephalus*) smelt, and the roe from various white fish including: Atlantic whitefish (*Coregonus huntsmani*), Lake whitefish (*Coregonus clupeaformis*), Mountain or Rocky Mountain (golden)whitefish (*Prosopium williamsoni*). Other fish with small light colored roe used in similar applications as tobiko or lumpfish are the: lavaret (*Coregonus lavaretus*); roach (*Rutilus rutilus*); perch (*Perca fluviatilis*); Pacific herring (*Clupea pallasii*), Atlantic or Baltic herring (*Clupea harengus*), smelt (*Spirinchus lancerolatus*), and burbot (*Lota lota*). There is also a limited roe fishery for the Pacific sardine (*Sardinops caerulea*) (16)(17). Most of these roes are brined, cured, flavored and/or seasoned, and sometimes smoked.

A special note should be made regarding smelt roe or "masago" which is experiencing an increased market again as a substitute for tobiko and similar small size, mid-priced caviars. This roe is commonly subjected to a number of flavored or seasoned variants including green tea, wasabi, chili oil, teriyaki, and combinations thereof.

Another small roe is vendace (*Coregonus albula*) the eggs of which are tiny and pink, and green or unprocessed vendace roe is sometimes consumed as a paste or spread with no salt added. Roe products are consumed in Central and South America from the hake (*Merluccius hubssi* and *M. antarcticus*), mullet (*Mugil cephalus*), orange roughy (*Hoplostethus atlanticus*), and white fish (*Coregonus albula*) (17,18).

F. Cod Roe

Atlantic cod (*Gadus morhua*) roe has long been used for a variety of products by fishers throughout its population range. The skeins may be simply breaded and fried or used in soups. A very popular product in Scandinavian homes consists of a blend of cod eggs and cream cheese or another soft cheese, with or without additional seasonings or herbs, which is served as a pâté or spread. The cheese based products are commonly sold in tubes and may not require refrigeration. Cod roes are also consumed alone or blended with butter. Sometimes roe is a component of meat spreads. Additional uses include the use of cod roe as a condiment in poultry or egg-based dishes, pasta dishes or salads. Other small white fish roe may also be treated in a similar manner and have become more common in areas where cod are scarce.

Salted cod roe [*Gadus macrocephalus* (Pacific cod), *G. morhua* (Atlantic cod)] are sometimes flavored with sugar, treated with nitrite, and smoked. Oil, potato flour, and other ingredients may also be added to salted cod roe. Salted roe may be aged for several months before sale.

Pacific cod roe (*Gadus macrocephalus*) or "tarako" is prepared in a similar fashion as pollock roe (*Theragra chalcogramma*) (see below), including spicy roe preparations. Alaska pollock roe has replaced cod roe in many product lines due to erratic availability and the market preference for a consistently small sized egg. Commonly now, most tarako will be made from pollock roe.

G. Shad Roe

An unusual product, shad roe (*Alosa sapidissima*) is harvested from this anadromous member of the herring family. Colonists transplanted the fish from the rivers originating in the Great Lake region and Northeastern US rivers to the Sacramento River in 1871 and the Columbia River in 1876. Shad now range from Cook Inlet, Alaska, to the US/Mexican border. Because the runs happen within a very short period, it has been difficult to develop a successful commercial fishery in the Columbia basin, although there is a great deal of interest in removing this introduced species from the Western watersheds. Shad roe can be eaten fresh; it is also frozen, and thermally processed in oval metal cans resembling large sardine cans. Commercial harvest is primarily in the West with recreational fisheries in the eastern United States. Shad is a popular spring dish in East Coast markets. Shad are harvested with roe during spring runs, with the fillets prepared separately, and then dressed with the sautéed roe. Shad roe is also breaded and fried, used as a spread or pâté, or incorporated into egg or meat-based entrees.

H. Mullet Roe

Mullet (*Mugil cephalus*) roe (karasumi) is consumed either as a salted product similar to ikura or salted and dried (similar to sujiko). Dried mullet roe has a yellowish red color and a rubbery chewy mouth feel due to the large quantity of wax esters, which runs as high as 60 to 70% of the extracted oil (19). Making mullet roe involves coating the roe with dry salt for 4 to 5 h, removing the salt, compressing the roe during the curing process, and air drying the compressed roe until 30% of the original product weight is lost. Mullet roe is also served as a pickled food ("Botargo") in Italy and other Mediterranean countries (19,20).

I. Orange Roughy Roe

Another roe with a high concentration of wax esters is the orange roughy (*Hoplostethus atlanticus*) (21). Orange roughy lipids have become a suitable replacement for sperm whale (*Physter macrocephalus* and *P. catadon*) oil or jojoba oil for industrial applications. Sale of the roe is not common. Applications are similar to tobiko.

J. Herring Roe or Kazunoko

Herring [*Clupea pallasii* (Pacific herring), *Clupea harengus* (Atlantic or Baltic herring)] are also a product in high demand in Asia, particularly in Japan. Kazunoko or "yellow diamond" roe is cured whole herring egg skeins, and kazunoko kombu is herring roe on kelp. Herring produce small, creamy white to yellow eggs (1.3 to 1.5 mm diameter) (15) in skeins about 8 cm long and 3 cm in width. The highest value kazunoko is a perfectly matched pair of skeins. A perfect pair of skeins has no surface blemishes or discolorations, large veins, nicks, or other visual defects, with both skeins of nearly identical size containing eggs of a consistent degree of maturity and a uniform, natural color. Herring harvesting methods are critical for good product quality. The skeins are vascularized, and the more the fish struggles during harvest, the greater the likelihood that the skeins will become bruised and discolored (6).

The herring roe seine fishery in Sitka Sound, Alaska, has the distinction of being one of the shortest fisheries in the world. Generally, the fishery is open for a few days at best. Among the shortest openings was the 1986 season, with the first opening of only 3 h and 10 min, and the second opening, held about a week later, of 2.5 h long (10). Several seiners may join forces to hire aircraft to spot schools of herring. Meanwhile, fisheries biologists sample the herring and permit an opening only when the fish reach the exact, desired degree of maturity and an average roe content of 10% by weight. Biologists signal the start of the opening by radio. State-commissioned aircraft then fly over the harvest area determining the number of successful catches. When the target allowable catch has been reached, the fishery is closed, again signaled by radio.

The herring roe is not immediately removed from the fish. Instead, the fish are frozen, preferably by brine freezing, or a combination of brine freezing and blast or plate freezing, the objective being to preserve the natural shape and form of the roe sacs within the fish. The frozen herring is then shipped to processing plants where the kazunoko is produced. The freezing and frozen storage of the herring is part of the process of "conditioning" the herring to make removal of roe from the fish easier.

At the processing plant, the herring are thawed, tempered, or "slacked out" by placing the fish in fresh water that is exchanged several times during the 24-h thawing process. This helps to remove blood and other undesirable constituents from the fish. The skeins are then removed (or "popped") from the herring. While skein removal is commonly done by hand, automated systems are now used at many facilities both to sort the fish by gender and then to remove the egg skeins from the female fish.

The skeins are sorted, brined, cured, and then packed in approximately 5-gal plastic pails in 100% brine solution, which is topped off with a scoop (500 to 750 g) of loose salt. The product is then shipped and held under refrigerated temperatures of –4°C or lower. The brining process traditionally involves many steps in which the skeins are held in totes of brine of increasing strengths, finishing with a saturated brine solution.

All in all, the brining process normally takes 5 to 7 days with daily changes of brine. A primary purpose of this process, in addition to curing, is to remove any discoloration in the skeins due to blood, enzymatic activity, or other contaminants. In some instances, hydrogen peroxide is used as a bleach to remove discolorations. Following brining, the skeins are sorted by quality and size. Specialized automated weighing machines are used in some instances.

Most kazunoko is shipped to Japan where it is drained, inspected once again, and then packaged for sale. Often individual matched pairs of skeins are packaged in gift packs that sell at retail for $10 or more per pair (approx. 5 oz). Kazunoko is commonly prepared as sushi or for dombori.

Individual herring eggs (capelin, cod, or tobiko can also be used) are added to sea vegetable salads and to seafood salads containing, among other things, marine plants (sea vegetables), clams, limpets, or marinated octopus. Another product from herring roe is tarama, a mayonnaise-like product manufactured from emulsified fish eggs that is used as a condiment or salad dressing. Acceptable tarama can be produced from damaged skeins and from overly mature roe.

A most interesting herring roe product is kazunoko kombu or herring roe on kelp. Kazunoko kombu is used in a variety of dishes, most commonly soups, and can be very expensive, oftentimes over $100 per pound. For the highest quality kazunoko kombu, a uniform, dense layer of herring eggs of similar size and color covers both sides of a piece of kelp.

Traditionally, kazunoko kombu has been harvested when herring spawn. Schools of herring release their eggs simultaneously, and the eggs adhere to kelp until the fish larvae hatch. Kazunoko kombu is still harvested in the wild; however, most is now produced by harvesting live herring just prior to spawning and placing them into pens (called "pounds") into which cut kelp has been suspended. When the fish spawn, the eggs adhere to this kelp to a thickness of up to one-half inch per side (or an inch in total thickness). The fish are then released back to the wild and the egg-coated kelp is washed, trimmed, cut to market size, and packed in brine. The finished product is commonly used in soups, salads, or as a side dish.

Due to a shortage of natural kazunoko kombu, there have been several attempts at developing acceptable substitutes. One of the more innovative attempts uses a surimi-based paste as an adhesive in the highly labor-intensive operation of attaching a layer of herring eggs to pre-cut pieces of kelp. The coated kelp is then placed in a

form under slight pressure and heat to set the product, which is then packed in light brine.

K. Pollock Roe or Mentaiko

Another roe product popular in the Japanese/Korean markets is mentaiko (also spelled mentiko). Mentaiko is the Japanese name for Alaska or walleye pollock roe (*Theragra chalcogramma*). The most valuable form of mentiko is whole, matched pairs of skeins, defect free and preferably with the oviduct intact. Mentaiko is brined and cured. It may be dyed (mostly red) and/or flavored.

There are literally dozens of grades of mentiko, depending on the type and degree of the defect (Table 161.2).

Other defectives include: hemorrhages or bruising, crushed roe skeins, large veins or unattractive veining, fracture of the oviduct connection between the two skeins, paired skeins of non-uniform size, and skeins that are not uniform in color or no longer connected together. Contact with sea water can reduce the quality of the roe, resulting in a two-tone discoloration on a roe sack. Sea water can also toughen or impart a rubbery texture to the skin surrounding the roe sac.

Tarako is the term used for smaller salted roe sacs. Although the literal translation of the Japanese word "tarako" is salted cod roe, most tarako is actually produced from pollock roe. This is a popular food item and served as a topping for rice or rice balls (musubi). Pollock roe is a component in ready-to-serve sauces for noodles or spaghetti marketed to children and young adults using Digimon cartoon characters.

Lower-grade pollock roe is commonly seasoned with salt, sugar, monosodium glutamate, garlic and other spices, sesame, chili or other flavored oils, soyu (soy sauce), or sake. Sodium nitrate may be added for products destined for export from the U.S. The product is called "spicy roe" or "mentai" and is marketed as a condiment in Korean markets.

Karashi mentaiko incorporates ground cayenne or flaked chili pepper. Mentaiko may also be treated with sodium nitrite and ascorbic acid and/or nicotinic acid to produce a product with a pink coloration. In some cases, fresh roe may be treated with a solution containing salt and 25% ethanol at pH 4.0 to 4.2, and brined in solution containing salt, nitrite, and antioxidants (22).

Pollock roe may also be used as an ingredient in salad dressings, in pastes or spreads, and in preparations where single eggs (barako) are desirable. Dehydrated tarako or mentaiko are used in seasonings in soups and sauces. Popular 'Hello Kitty' characters have been used in marketing campaigns for dry mix preparations and condiments containing pollock roe.

TABLE 161.2
Typical Alaska Pollock Roe Grades

Name	Description
Mako	No defects
Kireko	Defective, generally; broken skeins, skeins with cuts or tears
Aoko	Blue green discoloration from contact with bile
Kuroko	Dark colored roe
Iroko	Orange stains from contact with digestive fluids
Gamako	Immature
Mizuko	Overly mature
Yawoko	Soft

L. Hake Roe

Roe skeins from the Southern Atlantic hakes (*Merluccius hubbsi* and other species) are boiled in water and seasoned and are a traditional food in Uruguay served during the autumn (18).

M. Rock Sole Roe

Rock sole (*Lepidopsetta bilineata*) with roe is a unique, high risk winter fishery in the North Pacific often under severe weather conditions. In 2001, the catcher-processor vessel F/V *Arctic Rose* was lost at sea with all hands while participating in this fishery. Fish may be frozen whole and shipped in this form, headed, and sliced diagonally in strips (kirimi) containing both flesh and roe, or the roe may be removed and processed separately on-board. The roe is quite valuable.

N. Sea Urchin Roe (Uni)

The gonadal tissue of invertebrates, specifically the sea urchin is a popular food in Asian and Mediterranean cuisine. Both roe and milt from sea urchins are consumed. Several species of sea urchin are commercially harvested primarily by divers, including the highly valuable green urchin (*Strongylocentrotus pulcheriius*), the red sea urchin (*S. franciscanus*), and purple urchin (*S. intermedius*). *Pseudocentratus depressus* and *Heliocidaris crassispina* are used for fermented uni (23) as are lower grades of the *Strongylocentrotus* spp. Sea urchins are cultured in the Mediterranean, Ireland, China, and Japan and more recently in the US. Wild harvested animals may also be held in raceways and fed a high-nutrient diet. Proper husbandry and nutrition can increase yield 40% or more in gonadal tissue production.

Sea urchin may be consumed raw, but is more commonly steamed, baked, or sautéed. Uni, a preparation for sushi, is brined sea urchin gonadal tissues treated with alum. It may also be colored ranging from mustard yellow to bright orange. Color and condition are important for determining grades of uni, and a bright orange product is the most desirable.

The salted gonadal tissue from the sea urchin may also be fermented, producing a paste (neri uni). Also, a

more watery preparation, mizu uni, is prepared using a dry cure process. Doro uni is made by washing the gonadal tissue with dilute alcohol, draining it, then mixing it with salt.

O. Sea Cucumber Roe

Fresh and dried gonadal tissue from the sea cucumber (*Stichopu* spp.) is also consumed (10). The gonads can sell for 50 times the price paid for sea cucumber muscle tissue in China. Culture of this organism in China and elsewhere in Asia, and experimental efforts in North America are currently underway.

P. Roe from Crustaceans

Roe or "coral" from gravid female shrimp, lobster, and crab are popular delicacies in European and Asian cuisines. The roe from these crustacea are incorporated into traditional preparations of boiled or steamed lobster or crab. Stir-fried or deep fried dishes and soups are also common. She-crab soup, available as a canned product in many countries, is an example of a product where gravid female animals are targeted.

VI. CHEMICAL COMPOSITION OF CAVIAR PRODUCTS

The chemical composition of caviar varies from species to species, with the condition and maturity of the eggs, within and between runs or harvest areas, and from year to year. Caviar composition also differs slightly due to the variation between eggs within a single skein. This is because the degree of the maturity of the eggs suitable for caviar is greatest in the anterior end of the skein, with the eggs becoming more immature as you progress from the head to the tail of the fish.

The caloric content of caviar is between 370 and 320 calories/100 g and can vary with the quality and fat content (6). Salmon caviar products are highly digestible and have been used traditionally in Russia to aid the recovery of surgical patients and to treat rickets in children. Black caviar (osetra) has 251 to 282 kcal/100 g, with other varieties having a somewhat lower caloric content (220 to 255 kcal/100 g) (6). Caviar has formed the basis for fat diets over the years. Caviar is a recent addition to salon hair care products and cosmetics because of alleged benefits from the protein, lipid conditioners and vitamin content.

Fish roe are also a rich source of vitamins. Salmon eggs contain 50 to 3000 IU/g vitamin A, 5 to 25 IU/g vitamin D, 10 to 80 IU/100 g vitamins B1, B2, and B12 and 10 to 30 IU/100 g vitamin C. The vitamin content of catfish roe includes: vitamin A −2 mg/100 g, vitamin C −0.26 mg/100 g, vitamin D −0.2 mg/100 g, vitamin E −0.1 mg/100 g (13). Mineral nutrients include calcium, iron, magnesium, manganese, phosphorus, potassium, copper, and zinc (13)(24).

A. Proximate Composition of Fish Roe

Like poultry eggs, fish roe have high concentrations of lipid and protein. In general fish roe products are high in protein (16 to 30%). Crude lipid content can vary from less than 5 to 20% with an average value for salmon of around 10%. The lipid content of fish roe can double on a dry weight basis as the roe matures. After the roe is brined, the percentage of moisture decreases, the protein content increases because water is removed during the brining process. The ash content increases because of the added salt, and the lipid content increases proportionately as moisture is removed during brining and curing.

Like poultry eggs, the distribution of macronutrients within individual eggs is not uniform. For example, the interior fluid of coho salmon (*O. kisutch*) eggs constitutes 98% of the volume of the egg and is primarily a clear to translucent protein solution. A waxy low-density lipid fraction and oil droplets constitute the remaining volume. For chum salmon (*O. keta*) the volume of the oil droplet comprises up to 10% of the volume of the individual egg with lipids in the mature roe constituting about 13.5% of the wet weight of the egg.

The protein quality of fish roe is high with methionine/cystine or typtophan/tyrosine being the limiting amino acid. Table 161.3 shows the proximate composition of the unprocessed roe several roe producing aquatic animals along with data for muscle tissue and processed products.

B. Lipid Composition of Fish Roe and Fish Roe Products

Lipid composition and fatty acid profiles have been used to evaluate caviar quality and to determine the species of origin. With few exceptions, the predominant lipid components in fish roe are triglycerides and phospholipids. Also, lipid classes and the ratio of different lipid classes have been evaluated as a basis for objective quality assessment traits. In fish with a total lipid content of 10 to 15%, the majority of the lipid (ca. 70%) is polar lipid (15). For example, Baltic herring (*Clupea harengus*) and roach (*Rutilus rutilus*) have polar lipid contents of 75 to 90% (17). Table 161.4 provides compositional information for lipids for selected roe products and muscle tissue.

For fish with a high level of polar lipids, phospholipids may serve as an energy source rather than triglycerides, being more easily mobilized in the eggs. The requirement for alternative sources of energy for the egg is influenced by different species dependent incubation periods for individual fish. Fish from the same order or family can have widely differing ratios of triglycerides to phospholipids in the roe, for example chum salmon

TABLE 161.3
Proximate Composition of Roe, Roe Products, and Muscle Tissue (Average Values)

Species	Moisture (%)	Protein[1] (%N × 5.7)	Crude Lipid[1] (%)	Total Ash[1] (%)	Cholesterol (mg/100 g)
Salmonids					
Salmon, chum (*O. keta)*					
Roe	53	31	16	1.6	450
	57	27	14		40
Muscle	75	18			
Salmon, pink (*O. gorbuscha)*					
Roe					
Salted and cured ikura	55	31	13	2.0	
	50	32	11	7.0	
Salmon, sockeye (*O. nerka)*	57	25	11	1.2	
Salmon, Chinook *(O. tshawytscha)*	60	28	13	1.5	
Trout, rainbow (*O. mykiss)*	—	—	8	—	200
Sturgeons					
Caspian, *Huso* sp.	67	25	15	1.5	
Pollock and Related Species					
Alaska pollock (*Theragra chalcogramma*)					
Roe	67	26	5	—	310
	72	56	5	5.9	—
Muscle					
Salted "tarako"	80	18	1	—	37
	65	45	4	13	—
Cod, Pacific (*Gadus morhua macrocephalus*)					
Roe	68	26	4	—	310
Muscle	80	17	1	—	30
Cod, Atlantic (*Gadus morhua)*	79	18	0.5	2.0	—
Hake (*Merluccius hubbsi*)	67	—	7		380
Mullet (*Mugil cephalus*)					
Roe	52	—		—	—
	62	23	14	1.8	440
	50	30	20	—	490
Muscle	70	20	10	—	50
Salted and dried	30	35	25	5.4	—
Herring, Pacific (*Clupea pallasii*)					
Roe					
Muscle	77	20	3	—	300
	78	20	3	—	32
Herring (*Clupea* sp)					
	75	—	—	—	—
Invertebrates					
Crab (*Protunus truberculatus*)					
Roe					
Muscle	55	30	13	—	500
	77	18	4	—	90
Sea urchin (*Hemicntrotus pulcherrimus*)	75	16	8	—	310

[1] Percent by weight on an as-is basis.

Data from (6).

TABLE 161.4
Lipid Composition of Roe for Selected Species

Species	% Lipid (wet wt)	Phospholipid % Total Lipid	Triglycerides % Total Lipid	ω-3 FA % Total Lipid	ω-6 FA %Total Lipid	Wax + Sterolesters % Total Lipid
Salmonids						
Rainbow trout (*O. mykiss)*	6.5–8.8	49–54	42–49	37	6.3	0–0.3
Chum salmon (*O. keta*)	13.5	30	63	—	—	—
Steelhead (*O. mykiss)*	9.2	21–39	30–55	0.8	13	1.4–2.7
Sturgeon						
Gulf sturgeon (*Acipenser oxyrinchus* desotoi)						
Cultured	—	—	—	14–30	6–10	—
Wild	—	—	—	9–15	4–6	—
White Fish						
Whitefish (*Coregonus* sp.)	6.6–9.8	31–35	59–66	44	16	80–83
Vendace (*Coregonus albula*)	6	—	—	44	13	—
Cod (*Gadus morhua*)	9.8	71–76	12–13	31	3	4
Burbot (*Lota lota*)	6–7	11–14	3–5	37	13	1.3–2.7
Capelin (*Mallotus vilosus)*	18–19	49–52	4–5	33	2–3	—
Roach (*Rutilus rutilus*)	3.3–4.2	73–85	10–15	—	—	0.1–0.2
Saithe (*Pollachius virens)*	11	65–68	14–15	31	3–4	
Hake (*Merluccius hubbsi*)	5–8	14	26–42	2.6	—	27.6
Haddock (*Melanogramus aeglefinus)*	9–10	69–79	8–9	44	5	5.3
Whiting (*Merlangus merlangus*)	9	60–62	6–8	40	3–4	—
Other Fish						
Mullet (*Mugil cephalus*)	13.7	—	—	—	13	—
Baltic herring (*Clupea harengus)*	2–11	68–89	6–16	31	2–3	1.3–2.7
Perch (*Perca fluviatilis*)	4.1	11–17	1–2	—	—	80–87
Catfish (*Ictalurus punctatus*)	8	—	—	12	11	—
Orange roughy (*Hoplostethus atlanticus*)	5–6	20.7	51	11–32	1–3	2.9
Blue mackerel (*Scomber autralasicus*)	7–8	27	36	32	3.4	26
Kahawai (*Arripis trutta*)	12	29	33	22	4.9	32
Hoki (*Macruronis novaezelandiae*)	12	22	39	21	2.9	26
Red cod (*Pseudophycis bacchus)*	9–10	31	36	17	3.0	15
Sand eel (*Ammodytes lancea)*	12–13	19–28	45	38	3–4	—

Data from (6).

(*O. keta*) vs. steelhead (or sea) trout (*O. mykiss*) and cod (*Gadus* sp.) vs. hake (*Merluccius* sp.).

Diet can affect the lipid profile of caviar products. For example, cultured Gulf sturgeon roe (*Acipenser oxyrinchus* desotoi) has a different fatty acid composition than that of the wild Gulf sturgeon (12). The cultured sturgeon had a higher ω-3 fatty acid content (13.8 to 30.2) than wild caught sturgeon (8.86 to 15.5) and a different lipid profile generally, but these conclusions are based upon very limited data.

A high relative proportion of neutral lipids are often used to define roe ripeness (6),(17),(20),(21),(25). The higher lipid content, in general, of the Salmonidae roe compared with Clupeidae roe may be a result of differences in energy needs for the fertilized eggs (17), even though the muscle tissue of the Clupeidae is among the food fish with the highest muscle fat content. The lipid content of the roe from either Salmonidae or Clupeidae does not vary greatly with the degree of maturation or from year to year (17). For whitefish (*Corengonus albula*) (total lipid: 6.6 to 9.8%, 14% salt), there are proportionately greater triacylglycerols (66%) content compared with phospholipids (31%). Lecithin is the major phospholipid (90%). These products contain high cholesterol, 1.3 to 1.6% of total lipid (6).

Certain roes have an unusual lipid profile. Sand eel (*Ammodytes lancea*) and capelin (*Mallotus vilosus*) roe have high total lipid contents and a higher percentage of neutral lipid, over 75%. Capelin and sand eel also have high concentrations of steryl esters at a level that exceeds the free sterol content (15). These two fish have longer incubation times, roughly twice as long as many other

Northern marine fish, and the high neutral lipid content serves as an energy reserve for embryonic and early larval development. Levels of wax esters and steryl esters are high in mackerel (*Scomber japonicus*) and in mullet (*Mugil cephalus*) (20). The role of wax esters and steryl esters is unclear, but these may be used to determine the degree of maturity for some types of roe. Wax and steryl esters may play a role in buoyancy, permeability control, serve as insulation, or as an energy reserve for the eggs during incubation (17). These esters may also serve as a fatty acid reserve for modifying structural lipids after the fertilization of the eggs has occurred.

C. Cholesterol Content of Fish Roes

The cholesterol content of roe is about one-fourth of that of chicken eggs. The cholesterol content of vertebrate fish roe ranges from 200 to 500 mg/100 g. Some fish such as smelt, kahawai, and channel catfish are higher with cholesterol values 550 to 640 mg/100 g (Table 161.3). The cholesterol content of the roe is substantially higher than muscle tissue, generally by a factor of 10 for finfish but less for invertebrates (Table 161.3). Salmonid roes contain variable amounts of cholesterol (Table 161.3).

VII. GRADING AND QUALITY ATTRIBUTES OF ROE PRODUCTS

Upon completion of the curing process, caviars are graded and packaged. Grading can be a very labor-intensive process. During grading, any crushed or broken eggs are removed, along with "bullets," which are bitter, dark, undeveloped eggs. The amount of effort spent grading a product is proportionate to the final product quality and to the overall value of the product.

Roe may be graded according to flavor. Salmon eggs from sockeye, coho, or Chinook have a slightly bitter taste, a main reason the popularity of these roes is lower than for pink and chum salmon eggs, which tend to be sweeter. Salmon from stocks with longer migration patterns may tend to produce roe with higher lipid content a smoother mouth feel and a richer flavor profile. High quality product from early salmon runs have high value as these are the first new products of the season. Sensory properties of roe from different regional fisheries, *e.g.,* Hokkaido, Eastern Russia, and Alaska are different, although there have been no systematic studies to substantiate these variations. Little effort has been made to take advantage of these differences between strains for wild harvested products, or to investigate how to improve flavor and texture profiles for roe from culture operations. This will provide opportunities for market differentiation.

Taste is a primary consideration for grading products. With sturgeon, eggs may have a grassy or muddy aftertaste (11). The taste of the roe is dependent upon proper processing, the fish's feed, water conditions, maturity, harvest area, or culture practices and characteristics of the individual species. There is research currently underway investigating how the incorporation of vitamin E into sturgeon diets may to improve the flavor stability of black caviar. The flavor/taste of the eggs may change, be masked, or disappear after salting.

Color of roe is also critical to grade. There are color standards for many species of fish. This is particularly important for salmon, as the color ranges from light orange to a bright reddish orange. Because the color of roe can vary widely within a single lot from the same species, product is sorted to match. Consistent color for ikura in the Japanese market is very important to a high price, so both sujiko and ikura are matched in retail units whenever possible.

The color of sturgeon eggs is also highly variable, ranging in shades of gray (light to dark), black, yellowish gray, brownish gray, greenish gray, and even a dark golden color (11). Eggs from other fish species are pale shades of gray, yellow, pink, green, or brown. Depending upon the product, some roe are colored and sold as an imitation of the roe of another species (*e.g*., dyed herring roe for flying fish roe (tobiko); lumpfish roe for "black" sturgeon or paddlefish caviar). Regardless, the highest quality product involves uniform coloration.

Some caviar products are also graded by size. Sturgeon eggs, due to their high value, are typically size graded. Sturgeon eggs have three sizes: small (diameter $<$2.2 mm), medium (diameter = 2.2 to 2.5 mm), and large (diameter $>$2.5 mm). High-quality ikura (salmon caviar) may also be graded by egg size. Generally, larger fish produce larger eggs. The size of salmonid eggs varies: 3 to 5 mm diameter and larger for chum salmon, 3 mm or larger for pink, sockeye, and coho roe, and 6 to 8 mm for Chinook roe. Maturity, feed quality and nutritional status affect egg size.

Shell or sheath toughness is another factor considered when grading roe products. Manual or mechanical tests, which squeeze the egg until it breaks, test for product toughness. This process works best with larger eggs that come from salmon and sturgeon because their outer membranes are not as strong as smaller eggs from herring or carp. Toughness provides an indication of maturity although many factors can be involved. In salmon and most other species, soft eggs are an indication of immaturity. The situation for sturgeon is not so simple, with changes in egg texture varying less dramatically as the egg matures. Eggs from all fish will become soft as a result of enzymic degradation or microbial decomposition. Frozen eggs also tend to be softer than fresh ones.

Controlled proteolysis is an important feature for flavor development in roe products such as tarako and karasumi from pollock or cod roe and for traditional products made

from salmon roe. Sturgeon caviar is commonly aged under refrigeration for a month or more prior to retail sale for flavor and color development. Sturgeon caviar darkens and develops an oxidized paint-like and somewhat fishy flavor during aging which is desirable is some markets. Changes during aging result from endogenous and most likely microbial enzymes in the caviar under microaerophilic conditions. Psychrotropic lactic acid bacteria may be involved in flavor development as well as a souring form of decomposition. A new market for unaged sturgeon caviar is being developed. This product should be popular with the US market and others that prefer a mild flavored product. Production of unaged product is now possible as a result of a wider availability roe from the controlled harvest of cultured sturgeon.

VIII. PACKAGING ROE PRODUCTS

Vegetable oil (olive or cottonseed for sturgeon caviar) or glycerol is sometimes added to the packaged eggs to give a bright, shiny appearance and to prevent the eggs from sticking together. Salmon caviar is commonly packaged in plastic containers often with double bottoms, with the layer closest to the product perforated, to allow for draining, curing and drying. Traditional cotton lined wooden boxes (500 g to 3 kilo) are still popular in the Japanese market although plastic is replacing it due to food safety considerations. After curing, the packaged roe is compressed to remove additional water and reduce the water activity somewhat. This also removes entrained air and reduces spoilage from lipid oxidation and microbial growth. Traditional dehydration methods for caviar exposed the product to airborne bacteria; other methods sometimes used are centrifugation or drying with controlled currents of clean air (6). Modified atmosphere packaging and/or vacuum packaging are becoming more popular. Some products are pasteurized. Ikura has a shelf life of approximately 5 days at refrigeration temperatures, but can be stored for a year at −20°F.

Sturgeon caviar is often packaged into 2-part metal tins of 1.8 kilos (4 lb) that have a slot for drainage along one side wall. Lids are placed onto containers that have been overfilled by 10–15% and pressed down. The tins are laid on their sides to allow fluid to drain from the product during curing. After 2 h, the tins are placed under weight to further compress the caviar and remove excess liquid and air. Finally, a thick rubber band is placed around the circumference of the tin to secure the lid. Product is aged for a number of months at super chilled conditions of –2–8°C. For retail sale, the caviar is repackaged into smaller containers generally glass jars (1, 2, and 4 oz) with screw cap, crimp seal, or press-on vacuum closures. Retail tins up to 100 g and wholesale tins of up to 1 kg are also sold. Caviar is also packaged in crystal and in gold plated containers. Interestingly, an American, Harry Dolbro developed the familiar two part metal can plus rubber band packaging system for aging black caviar around 1900. He also developed the concept of marketing caviar in small 2 and 4 oz glass jars. Prior to this, the standard packaging for black caviar was a 135 pound wooden keg (4).

After the caviar is packaged, it is stored at −2 to −3°C. Depending upon the microbial load of the product, the shelf life can be several months. Frozen storage of a year or more is also possible. Addition of a variety of preservatives, including borate (unfortunately not permitted in US), antioxidants, chlorous acid, and nisin can extend shelf life. Unapproved compounds including formaldehyde have been added to improve storage stability. New processes are currently being evaluated to extend product shelf life and enhance product safety including various combined technologies (e.g. antimicrobials and mild heating), dielectric thermal processing methods, and cryogenic freezing. High hydrostatic pressure processes are most likely not feasible for pasteurizing caviar product, as the roe become elastic and loose translucency at pressures above 45 KPSI.

Lumpfish roe is generally packed in 10.5-kg plastic barrels. Iceland and Canada are the major producers of lumpfish caviar (6). Icelandic companies process a large percentage of the green roe into ready-to-eat lumpfish caviar products. The most common product forms are dyed cured roe packaged in 50- and 100-g glass jars with screw- or lug-type closures.

Alaska pollock roe (mentaiko) is sold in several different forms. The highest quality is defect-free matched skeins in which both ovaries are of uniform size with the oviduct intact, with no bruises, no prominent dark veins, no discolorations, and no cuts. Intact skeins of pollock roe, which include defects, are of lower value. Retail packages can be as small as a single vacuum-packaged pack containing a set of matched skeins.

Other product forms include 4, 8, and 16 oz plastic trays (traditionally black in color with a clear lid), 500 g or larger boxes of skeins, nicely arranged, or marinated products sold in glass jars. Sometimes mentiko is packaged in flat 100-g (3.5 oz) cans for retail sale.

Broken skeins of mentaiko are of the lowest value. This product is shipped frozen in bulk primarily to Japan or Korea. It may be marinated in salt or highly spiced with hot chili ("spicy roe"). In addition to pollock, spicy roe can be made from either pollock, cod, capelin, herring, mullet, whiting, Southern blue whiting (*Micromesistius australis*), hoki (*Macruronus novaezelandiae*), flying fish, or sometimes lumpfish roe. Spicy roe is usually packaged in small 30–250 g glass or plastic containers for retail sale.

IX. FOOD SAFETY ISSUES ASSOCIATED WITH ROE PRODUCTS

Salted fish roe products are often ready to eat. However, roe are heat labile and can only be heated to a temperature of 70°C without the eggs becoming dull or loosing color

(6)(11)(27). Irreversible protein denaturation occurs between 70 to 80°C. Some caviar can be pasteurized, but this is not yet a widely used practice and the pasteurized product is not of as high a perceived quality as unpasteurized. Refrigerated caviar products are often packaged in the same way as pasteurized products (*e.g.*, in glass jars), and the possibility of inadvertent or unintentional thermal abuse is a risk and can occur if products are either not adequately labeled or label instructions are ignored by the retailer or consumer. Market requirements for time temperature indicators may improve this situation.

The United States Food and Drug Administration requirement for roe products call for a combination of refrigerated temperature and either a pH at or below 4.6, a water phase salt content at or above 3.5% or a water activity at or below 0.97. The water phase salt content at or above 3.5% is actually a value identified for cured and smoked foods, and FDA guidance (28) actually recommends 5% for caviar products despite a lack of scientific justification for this higher value. Only the combination of refrigerated temperature and the 0.97 water activity value appears to be practical for caviars if one wishes to retain the flavor and textural and other qualitative attributes associated with these highly valued products. All products we have tested with a water phase salt content of at least 3.5% have also met the water activity requirement of 0.97. The purpose of these measures is to protect the product against *Clostridium botulinum* growth.

The salt concentration and storage temperature are the factors relied on to preserve and make caviar safe, yet salt concentration varies widely among caviar products of the same type (Table 161.4). Adjusting pH is not an appropriate preventive measure for *Clostridium botulinum* or *Listeria monocytogenes* growth as suitable products cannot be made at pH $<$ 4.6. The pH of roe falls between 5.7 to 6.5 (6) and can decrease up to 0.5 unit during refrigerated storage. The pH of lumpfish caviar ranges from 4.8 to 5.9 (6)(14). At least for lumpfish caviar, products with a higher pH had greater acceptability, appearance, and flavor. A compilation of salt, pH, and water activity values for different caviar products are provided in Table 161.5.

Because caviar is a ready-to-eat product, the lack of suitable preservatives for caviars for most markets is a safety concern. Borax (0.l3%) and boracic acid (0.1%), urotropin(e) (0.1%), sorbic acid ($<$0.1%), sodium benzoate ($<$0.1%) and formic acid ($<$0.05%) may be added

TABLE 161.5
Salt Content, Water Activity and pH for Various Roe Products

Product	Storage Conditions	N[1]	Salt (% wt)	Water Activity	pH
Lumpfish	Refrigerated	4	6.33[2]	0.96	5.6
		2	7.74[2]	0.95	5.7
		4	6.81[2]	0.96	5.6
		4	4.24[2]	0.97	5.4
		3	8.6[2]	0.94	5.3
		6	12.9[2]	0.91	5.4
		31	9.5	—	5.5
		—	6.0–20.7	—	4.6–6.8
Salmonid Roe					
Salmon	Refrigerated	3	5.36[2]	0.96	5.6
		13	20	—	5.8
		—	6.5–25.0	—	5.5–6.3
Pink Salmon	Refrigerated	2	4.7–6.7	0.98	6.1
Chum Salmon	Refrigerated	—	13.8	—	—
		—	3.5–25.5	—	—
Steelhead Trout	Refrigerated	—	2.3–7.0	—	6.6–6.7
Sturgeon	Shelf stable	2	7.74[2]	0.94	5.5
		5	14	—	5.5
		—	11.2–17.3	—	4.8–5.8
Whitefish		—	4	—	5.5
		—	—	—	4.8–5.9
Herring	Refrigerated	—	—	—	5.7–6.5
Smoked cod roe		—	17.2	—	5.5
		—	13.2–38.8	—	4.8–5.9
Cod roe paste	Refrigerated	4	18.6V	0.77	5.5

[1] Number of samples tested. Reported values are averages. Data from (6).

[2] Salt concentration in brine. Other values are % weight in the edible portion.

to sturgeon caviars. Formaldehyde is also found in some non-North American products. Nisin (<0.1%) is also a permitted additive in Russia. Recent research indicates that nisin in combination with a mild heat treatment is effective in both inactivating listeria and in preventing its outgrowth in sturgeon caviar (27).

Adulterated and misbranded products containing unapproved preservatives and color additive are routinely imported into the U.S. Borax is particularly problematic as it can mask decomposition. Adulterated and misbranded salmon caviar is somewhat less common, however, salmon roe product containing nitrate/nitrite can be found at retail.

The salt content and storage temperature of caviar are key preventive measures for producing a safe caviar product. In general, salt content alone may not be high enough to inhibit the germination of *C. botulinum* spores. *Clostridium botulinum* type E can grow at 4.5 to 6% salt and at temperatures of 3.3°C (28). Water phase salt concentrations of 10% will inhibit *C. botulinum* germination for low acid foods held at room temperature. It is unlikely that type E would germinate and grow in caviar products with a aw<0.97. Somewhat disconcerting are results from inoculated pack studies chum salmon ikura with *Clostridium sporogenes* (PA 3679) spores showing that at a water phase salt concentration of >13% with 200 ppm potassium nitrate, spores may still germinate and grow.

Pasteurization processes could improve caviar product safety. Pasteurization is the destruction of pathogenic vegetative cells. Thermal pasteurization processes have been used to extend the shelf life. Batch pasteurization in steam or water bath of air tight containers or vacuum packaged containers of caviar have been attempted with limited success. Because protein denaturation occurs in caviars between 70 to 80°C, long pasteurization times at 50 to 70°C are feasible, but cause loss of product quality. Within this range of temperature, the fish egg proteins do not undergo substantial coagulation and the appearance of the product remains visually the same. However, as a result of pasteurization, salmon caviar becomes slightly paler and soft, and immature salmon eggs lose their shape. At 56 to 60°C, only slight changes in pink salmon caviar color are noticeable (11). At 71°C, all the eggs appear dull; at 72°C, the egg yolk is completely coagulated and the caviar converts into a chewy, boiled egg mass. Conventional thermal pasteurization of sturgeon and whitefish caviar should be conducted at temperatures less than 65°C. For salmon and sturgeon, process temperatures up to 70°C can be used (27).

Development of a high temperature-short time pasteurization using radiofrequency pasteurization (27 MHz) shows great promise. Pasteurization extends the shelf life of the caviar, and allows short term storage at refrigeration temperatures (2 to 3 weeks). The D values of *Listeria* spp. for roe are within the range for meat and egg products. The D60°C value for *L. innocua* is 10 min for chum salmon (*Oncorhychus keta*) caviar (0.5% salt), 4.8 min for 2.5% salt. The D60°C for white sturgeon caviar (*Acipenser transmontanus*) (approx. 3% salt) range from 10 to 19 min.

X. CONCLUSION

The roe of literally hundreds of aquatic animals are used for food. Most of these roe products are brined or cured. Some are flavored or colored. The appearance, flavor and texture are all important sensory characteristics for these foods. Roe products are most commonly used in relatively small quantities as a hors d' oeuvres, as a condiment, a garnish, or as an ingredient. They are often associated with ethnic or gourmet foods and may be served with rice or in preparations with other aquatic foods, meat products or eggs.

Despite the importance of these products in international commerce, there is relatively little technical information available about the chemical composition, product quality, and food safety attributes of roe products in the scientific literature. As a result, all too often regulatory agencies have promulgated regulations or guidelines that are contrary to the consumer desired attributes of these products and which may not contribute to increased food safety. It would appear that increased education about and research on these products is warranted.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the National Fisheries Institute, the International Marketing Program for Agricultural Commodities and Trade (IMPACT) at Washington State University, Aquaculture Idaho-Washington, and the Western Regional Aquaculture Consortium along with the in-kind and moral support for our research from Mayco Fish Co., Tacoma, WA, Stolt Sea Farms, Elvira, CA, the Southern Southeast Regional Aquaculture Association (SSRAA), Ketchikan, AK, Prince William Sound Aquaculture Association, Cordova, AK, Signature Seafood's, Seattle, WA, American Pride Seafoods, Seattle, WA, Clear Springs Foods, Buhl, ID, Trout Lodge, Inc., Orting, WA, Trident Seafoods, Seattle, WA, and Ward's Cove Packing Co., Ketchikan, AK.

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162 Thermal Processing of Meats

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CONTENTS

I. INTRODUCTION

Heat transfer is one of the most important unit operations in the food industry; it is the cheapest and most efficient method of preservation. Almost all processes include supplying or removing heat by physical, chemical or biological methods. The objective of food heat treatment is the destruction of microbial populations and enzyme inactivation in order to prevent spoilage and proliferation of pathogens and spoilage microorganisms. Sanitation is ensured after heating; practically all microorganisms are destroyed or irreversibly damaged by heat (1). Process conditions – time and temperature – are the decisive applied factors according to the expected shelf life of the product, although heating also causes changes in physicochemical and biological food characteristics (2). Therefore, in order to ensure thermal processing efficiency several variables must be considered such as microbial survival rate and physicochemical composition and structure.

Heat processing also aims enzyme destruction. Intrinsic and extrinsic parameters leading to microbial destruction are practically the same to those involved in enzyme inactivation as microbial death is due to destruction of at least one enzymatic system resulting from denaturation of the protein moiety (3). Most foods, particularly meats, are consumed after heating as chemical constituents react improving sensory and nutritional characteristics. Conversely to microorganisms and enzymes, heat processing aims the least nutrient destruction (4). Even though, the same factors destroying or inhibiting microbial growth also accelerate nutrient loss. Therefore thermal processing of foods must reach a compromise between sanitation and quality.

II. THERMAL PROPERTIES OF FOODS

In order to calculate thermal processing efficiency, the following information is necessary:

- Thermal resistance of a given microorganism, as calculation basis (z- and F- values) (5).
- Temperature profile of the product. Thermal processing includes two transport phenomena: heat transfer where heat flows proportionally to the driving force and inverse of the resistance to flow; mass transfer within the food material and resulting from reactions among food component (lipids, proteins, minerals, etc.) (6).

In any food, thermal properties are determined by heat distribution within the product. These are mainly related to transference (thermal conductivity and specific heat) and physical properties (density and geometry) (7). Thermal conductivity in a food material depends on the thermal flow rate through the product. Heat is defined as energy transferred as a result of temperature gradients, the larger the temperature difference the higher the flow rate. In solid foods temperature difference between the product surface and the center determines the heating rate.

Heat penetration depends on the transfer mechanisms within the foods. Food heating depends on the surface heat transfer coefficient; physical properties of the food and container, if any; temperature difference between the heating medium; and food initial temperature and container size (8). However, food thermal processing assumes that heat transfer in the surface is very high; therefore resistance is only due to the food composition and structure. Knowing the type and extent of the driving forces involved transport parameters can be calculated (9).

The main mechanisms involved in heat transfer in food processing are conduction and convection. Conduction is transmitted within a solid due to vibrations of adjacent molecules. In meat canning, conduction occurs in meat chunks or in gelled canned pastes, such as luncheon meat. Heat transference rate through a uniform material depends on the area (A) and temperature gradient (ΔT) but inverse to the thickness of the material (L); it also depends on the thermal conductivity of the canned food and tin (k) (7). Fourier's law indicates this relationship

$$q = k\,(A\Delta T/L)$$

In meats, k is very low (1.89 kJ/h m°K) as compared to stainless steel (59.47 kJ/h m°K) (10). This makes conduction in food materials very inefficient.

Convection heating is mostly related to fluids, such as soups, brine, milk, etc., as a result of movement of differential densities when the fluid is heated or cooled. Convection can be accelerated if stirring is applied reducing the temperature difference. In this mechanism, heating depends on the area of transference (A), temperature difference (ΔT) and a constant, h, which depends on flow properties, type of surface and flow rate. For boiling water h = 1898 to 25308 kJ/h m°K, whereas for air h = 3.16 to 31.63 kJ/h m°K (11). Convection is based on Newton's law:

$$q = h\,A\Delta T$$

Canned foods with low viscosity or with small particles, such as soups or sausages in brine, have higher transfer coefficients as heat penetration follows a convection mechanism increased by can rotation. The mechanism can change from convection to conduction during heating of heat-induced gels, such as luncheon meats. As convection rate is higher than conduction, heating rate varies during processing (6).

Conductivity in meats depends on the direction in which heat is transferred. Pérez and Calvelo (12) reported that thermal conductivity in lean beef at 78.5% humidity and 0°C, applying thermal flow perpendicular to the meat fiber, is 0.411 kcal/m h°K, whereas under the same conditions, in lean beef at 75% humidity, if the flow is parallel, conductivity is 0.422 kcal/m h°K.

Heat transfer also depends on characteristics of the heating medium (Table 162.1). High coefficients mean high heat transference rates to the product surface. According to these figures, free convection in a smokehouse has the lowest transference rate; forced convection with a fan significantly increased heat transference rates.

III. THERMAL PROCESSES

Heat transfer principles can be applied to any material, including foods. However every thermal process has a specific aim, its severity varies accordingly. Hurdle effects are the result of particular event interactions (13). In order to alter food quality to a minimum extent, only necessary hurdles to obtain a microbiologically safe food with considerably extended shelf life must be applied. Therefore severity of heat treatments also depends on intrinsic microbial controls such as low pH, presence of bacteriostatic compounds, application further preservation methods such as refrigeration, etc. Because heat processing may alter food quality attributes, it is advisable it is as mild as possible, without

TABLE 162.1
Heat Transfer Coefficients of Heating Media (11)

Heating Medium	Coefficient (kcal/h m² °K)
Free convection in gases	2.5–25
Forced convection in gases	10–100
Forced convection in water	500–5000
Boiling water	1500–20000
Condensing steam	5000–15000

compromising quality or sanitation. If other antimicrobial hurdles are present, they must be taken into consideration.

There are four heating processes applied to food materials, based on temperature increase:

A. Scalding

It is generally applied to tissues before freezing, drying or canning. Conditions depend on the subsequent process. If scalding is applied before canning, the objective is to remove gases from tissues, to increase tissue temperature and to provide initial cleaning (14). When applied to meats, scalding usually results in volume reduction. Scalding temperatures are around 65°C (15).

B. Cooking

It is applied to improve sensory characteristics of the food material, although it also destroys a number of microorganisms and inactivates some enzymes. In meat processing "cooking" implies several heating methods: oven cooking, roasting, frying, boiling, steaming and grilling. How heat is applied to meats depends on the method. Oven cooking, roasting and grilling are carried out with dry heat and high temperature (around 100°C), whereas boiling and steaming are applied in water. Frying temperatures are above 200°C (8). Cooking is also a preservation method, if recontamination is prevented. In addition to enzyme destruction and reduction of microbial populations, cooking also destroys toxins present in the meat or from microbial origin and improves digestibility. However, it also promotes adverse changes such as nutrient depletion (5).

C. Pasteurization

In most cases, the aim of pasteurization is to destroy pathogens. Vegetative cells may survive, therefore a further preservation method, such as refrigeration, addition of antimicrobials, packaging or fermentation must be applied. Pasteurization time-temperature relationship depends on specific thermal resistance of a given strain and on food heat sensibility (14). Pasteurization temperatures are 140 to 150°C for 1 to 45 seconds, or 70 to 73°C for 15 to 20 seconds (8). Optimization of a pasteurization process depends on relative destruction rate of a given microorganisms without considerable altering quality factors.

Vegetative cells are destroyed at temperatures slightly higher than their maximum growth temperature, whereas spores can survive at much higher temperatures. Processing conditions vary depending on the microbial growth interval; pasteurization applies temperatures higher than those where microbial growing can occur (16). Meat products are generally pasteurized in water baths. The packed product is placed in stainless steel tanks and heated with water; cooling is carried out with cold water. Continuous pasteurization equipment consists in a long tank; the product is transported through the water in a conveyor (5).

D. Sterilization

A sterile product does not contain any viable microorganisms therefore the shelf life of sterilized foods is considerably extended even without the application of additional preservation methods. Because sterilization temperatures are above maximum to allow bacterial growth, this process destroys vegetative cells but not spores; sterilization process calculations are based on spore survival. However food sterilization in not practically achieved as, strictly speaking, sterility is the destruction of all spores or vegetative cells that can grow in normal storage conditions (5). Therefore, although pathogens are destroyed some non-pathogens may be inactivated preventing them to growth and reproduce. This is called commercial sterilization and depends on the type of food; storage conditions after heat treatment; cell or spore resistance; heat transfer characteristics of the food, container and heating medium; and initial microbial load. Time-temperature relationship of the sterilization process depends on the thermal resistance of a given microorganism, taken as indicator. *Clostridium botulinum* and *Clostridium sporgenes* are indicators for meat products (16).

Sterilized foods are packed in hermetically sealed containers in order to prevent recontamination. Under these circumstances aerobes do not grow and spores of strict anaerobes are less heat resistant than those of anaerobe (17). Even though, in some foods such as cured canned meats oxygen is not completely removed from the product. Spoilage due to anaerobe growth, such as *Bacillus subtilis* and *Bacillus mycoides* may occur. In this situation, in addition of moderate heat processing other preservation methods are necessary, such as curing or smoking (18).

IV. THERMAL PROCESSING OF MEATS

Basically, meat is subjected to two types of thermal treatment: cooking and canning.

A. Cooking

Meat cooking is carried out in forced convection ovens in batch and continuous operations. Convection and conduction are the dominant heat transfer mechanisms (8). Conduction is the main heat transfer mechanism within the product, starting from the product surface inwards in a transient state as temperature changes with time in any point within the product. Convection occurs from the heating medium to the product surface due to mixing of the heating medium. In free convection, fluid movement is due to density gradient resulting from temperature variation. Force convection is promoted when the fluid is moved using any

device, such as a fan (14). Convection from the heating medium to the product surface, and conduction from the product surface to the inner part of the food occur at the same time, for example, meat batters, stuffed in water permeable casing, or cuts such as ham, ribs or loins in stockings cooked in a forced convection oven (19).

Process variables in forced convection are cooking time, air velocity and relative humidity (dry bulb-wet bulb temperature) (5). If the heat transference coefficient to the product surface is small, convection from the heating medium to the product surface is the limitant force whereas if the coefficient is high the limitant is the conduction within the product (10).

Cooking can be also considered as a pasteurization process, as it is carried out at temperatures below 100°C. Meat in batch operations are manually placed and removed to and from the oven. Small ovens can process 180 kg of boneless ham; large ovens up to 25,000 kg. In a continuous operation the product is automatically transported in a conveyor through one or several cooking zones and through a cooling area (20).

A variation in meat oven cooking is smoking. It implies two processes taking place at the same time; with the exception of cold smoking where the aim is to impart flavor and to add preservative compounds such as phenols, the main process is cooking, smoking being a secondary procedure.

B. Canning

The basic purposes in canning are:

(a) All microorganisms (cells and spores) feasible to grow and produce toxins must be eliminated. Canned meat, to be safe from the public health point of view, must be free of *Cl. botulinum* the most dangerous agent producing a fairly heat-stable toxin (16).
(b) Spoilage microorganisms must be reduced to a safe limit.

Thermal processing is carried out in two ways: aseptic processing where the food is heated at conditions of commercial sterilization and placed in sterile containers which are subsequently sealed; and canning where the food is placed in the container, then sealed and finally sterilized. Process conditions are the same for both cases (21).

From the commercial point of view, any canned food is sterile if it is free of spoilage microorganism such as *Bacillus stearothermophilus* or *Clostridium perfringens* (commercially sterile). Spore-forming thermophiles such as *Cl. sporogenes* must be considered only when storage temperatures are high, as 40°C is their maximum growing temperature. However, *Clostridium thermosacaroliticum* spores, a spoilage bacterium, can survive at temperatures as high as 450°C. Heat treatment eliminating *Cl. botulinum* and *Cl. sporogenes* renders heat-stable foods with considerably long shelf life (22). Processing conditions to destroy vegetative cells are shown in Table 162.2.

Commercial sterilization consists in four stages: food preparation, can filling, can closing and sealing, and thermal processing (24). At industrial level it is carried out in batch and continuous operations, both are based on the heat transfer principles described before. The batch or retort method consists in loading the retort, closing and heating with vapor. The temperature is controlled throughout the process depending on calculated processing conditions. Pressure difference is also controlled to avoid deformation of large cans or lid blowing. The heat transfer mechanism is convection. Heating medium in continuous retorts is also vapor; as the cans are continuously moving in this process the heat transference rate is higher. In some systems cans are fed into the continuous retort through a pressure lock, moving along the system in a U-shaped conveyor where heating and cooling are applied (5).

V. TIME-TEMPERATURE PROFILE CALCULATION

As mentioned before, thermal process conditions are calculated on the basis of several considerations such as composition of the food material, expected shelf life, transportation and storage conditions, initial microbial load and specific present microflora, among others.

As thermal processing is aimed to destruction of microbial population responsible of spoilage or a health hazard, process calculation must take into consideration how the food material will be handled. Microbial associations in refrigerated meats consists of Gram negative, rod shaped, non-fermentative psychrotrophs of genus *Pseudomonas, Alcaligens, Flavobacterium, Shewella* and *Moraxella* (23). This association changes during curing, becoming dominant Gram positive microorganisms of genus *Micrococcus, Lactobacillus, Carnobacterium* and *Brochothrix* (25). Table 162.3 shows the growth interval of several microorganisms associated with meat spoilage.

TABLE 162.2
Processing Conditions to Destroy Vegetative Cells (8, 10, 15, 16)

Microorganism	Z-Value (°F)	D_{250} Value (min)
B. stearothermophilus	12.6	4.0
B subtilis	13.3 to 23.4	0.48 to 0.76
B. cereus	17.5	0.0065
B. megaterium	15.8	0.04
Clostridium sporogens	23.4	0.15
Cl. botulinum	17.8	0.21
Cl. thermosaccharolyticum	16–22	3.0 to 4.0

TABLE 162.3
Microorganisms Associated to Meat Spoilage (26)

Microorganism	Growth Interval
Psychorphiles	−5 to 35°C
Pseudomonas sp.	
Achromobacter	
Mesophiles	15 to 45°C
E. coli	
Bacillus subtilis	
Facultative thermophiles	24 to 54°C
Streptococcus thermophilus	
Clostridium perfringens	
Thermophiles	45 to 75°C
Clostridum thermosaccharolyticum	
Bacillus stearothermophilus	

TABLE 162.4
Pathogens Associated to Meats (8, 10, 14, 21)

Microorganism	Lethality (min)
Clostridium botulinum	$D_{65°C} = 0.1$
Vibrio sp.	$D_{70°C} = 0.3$
Aeromonas hydrophila	$D_{55°C} = 0.17$
Listeria monocytogens	$D_{60°C} = 1.9$
Salmonella sp.	$D_{60°C} = 0.2$
E.coli 0157:H7	$D_{60°C} = 4$
Staphylococcus aureus	$D_{60°C} = 0.4$

Several inactivation parameters have been developed as mathematical tools to obtain a time-temperature relationship necessary to achieve a successful treatment.

(a) D- and z-values. If a microbial population is subjected to temperatures slightly above those for its maximum growth temperature, vegetative cells or spores are destroyed due to the inactivation of enzymes present in the microorganisms. The destruction follows the exponential equation:

$$-(dc/dt) = kc$$

That is, cell concentration decreases (dc) with time (dt) in a direct proportion of cell concentration (c). In other words, 90% of the microorganisms are destroyed in a given time interval if constant temperature is applied. The time interval is different for each microorganism, and is called decimal reduction time (D). It represents the minutes necessary to destroy 90% of a given microbial population at constant temperature. Table 162.4 shows D values for some pathogens possibly associated to meats.

Therefore it is possible to compare thermal destruction of different microbial populations. D values are expressed at a given temperature ($D_{120°C}$). For example when heating at 110°C, 90% of the population of *Cl. sporogenes* (i.e. from 10^5 to 10^4) is reduced if heating is maintained for 10 min ($D_{110°C} = 10$ min). If the same population is heated at 115°C, the time necessary to reduce the population one logarithmic cycle at 115°C is 3 minutes ($D_{115°C} = 3$ min), and at 120°C it requires only 1 min ($D_{120°C} = 1$ min) (20).

Heat resistance for a given microorganism is given by z-values, indicating the temperature required decreasing D-values in 1/10.

(b) F-values. Calculation, evaluation and comparison of different heat treatments are achieved by the so-called F-value. This value represents the extent of thermal death of microorganisms and severity of the treatment in order to predict the product's shelf life. The practical importance of F-values is that the individual effect of each part of the process is additive. As it is impossible to rise the temperature in the container to 120°C in every point at the same time F = 1 concept is applied. It is the lethality effect of heating at 120°C for 1 min. F-values increase, depending on the severity of heat treatment required for given meat. F_s is the sum of all F-values in every parts of the container.

According to the F-value concept, each temperature above 100°C has a given lethal effect; it increases with temperature's increment. For instance, heating must be applied during certain time and temperature in order to have similar heat damage: 100 min at 101°C, 10 min at 110°C, 1 min at 120°C or 0.1 min at 130°C (8). Thermal treatments therefore depend on a time-temperature relationship. Increasing the temperature for 10°C, the time necessary to achieve the same thermal effect is 1/10. Heat treatments are also calculated taking into consideration the survival of spores from two of the most damaging bacteria in meat products: *Cl. botulinum* and *Cl. sporogenes*.

However, as heating is not homogenous in the entire can geometry, calculations are always done considering the temperature rise at the cold point (where heating is the slowest). In this point, the sum of all lethal effects is F_c. The position of the cold point is determined by the type of food material, therefore by its main heat transfer mechanisms, and to a certain extent by the agitation of the cans in the retort.

In conduction heating, the cold point is located in the geometrical center of the container. For viscous meat, with cans rotating during the heating cycle, the cold point is close to the geometric center. Rotation in this case does not substantially increase the heating rate.

In static heating of liquid or semisolid products, such as meat pieces in brine, where the leading heat transference mechanism is convection, the cold point is on the

vertical axis, one-third from the can bottom end (10). F_c is always lower than F_s due to the fact that heat effect in the center is always lower than in the rest of the container. When a thermal process is calculated for the first time, the cold point is located experimentally, using thermocouples (Figure 162.1).

A simple method to calculate the lethal effects during the heating and cooling phases consists of measuring with thermocouples the temperature at the cold point, and to calculate the corresponding F-values.

The relationship between D and F, taking into account the amount of cells before and after heat processing is:

$$F = D (\log a - \log b)$$

where a = initial cell load; b = final cell load.

It is assumed that low acid foods, as are most meat products, are heated at a temperature that assures total absence *Cl. botulinum* spores, and are microbial safe. In this case, spore counts must be reduced from 10^{12} to 10^{0} (24), that is, reducing the count 12 log cycles or 12D. This means that heating must be enough to find only 1 *Cl. botulinum* spore in 10^{12} cans, i.e. one spore per gram of meat or $1/10^{12}$. *Cl. botulinum* types A and B are reference microorganisms for D values at 120°C and 0.21 min., as follows:

$$F = 0.21 (\log 1 - \log 10^{-12})$$

$$F = 2.52$$

For *Cl. botulinum*, $D_{121°C} = 0.21$ min and z = 10°C. In order to reduce an assumed number of *Cl. botulinum* cells 12 log cycles, heat must be 12 times higher during 0.21 min, that is 2.52 min at 120°C. Heat processing of food around F = 2.5 is called "botulinum cook."
Lethality is calculated by the equation:

$$(\log t - \log F) / (\log 10) = (250 - T) / Z$$

where log 10 = 1; therefore log (t/F) = (250 − T) / Z.
The destruction rate per minute of a given microorganism at a temperature T in the process corresponds to the time, t, needed for the destruction of microorganism at that temperature. For every minute, lethality can be calculated at a given temperature, obtaining a curve. The area under the curve represents the total lethality of the process.

Another way to calculate the lethality necessary in a given process is by adding all F-values during the heating and cooling phases; this gives F_{total}, the sum of all F values.

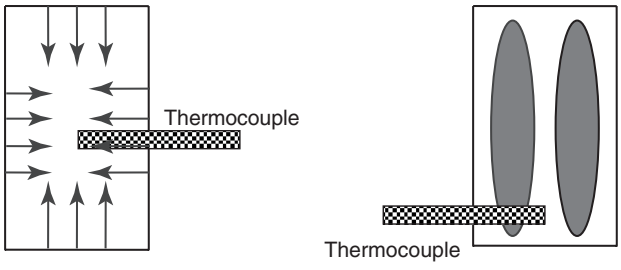

FIGURE 162.1 Location of cold point in conduction (left) and convection (right) mechanisms.

VI. EFFECT ON MEAT QUALITY

Quality improvement or deterioration of heat-treated foods depends on three factors:

- Type and amount of microorganisms in the food. Insufficient heat treatment may results in microbial survival and presence of metabolites such as gas, acid or off-odors and flavors. Inadequate cooling after heat treatment in processes such as canning encourages thermophile growth (26). Recontamination can also occur from microorganisms in cooling water lacking of suitable sanitary conditions.
- Chemical reactions of food components with one another or with the packaging material (27) can result in can or thermoformed package blowing.
- Physical alterations due to inadequate equipment or process operation, such as rapid increase in retort pressure, insufficient vacuum in packages or excessive package or can filling of the processing equipment.

Heat treatment severe enough to destroy *Cl. botulinum* or *Cl. prefringens* ensures the production of a stable food without the need of applying further special storage conditions. However, as severe heating can alter sensory characteristics, it is necessary to achieve a compromise between preservation and alteration of sensory attributes. Heat treatment can also improve sensory characteristics of meat, such as texture due to alteration of the muscle fibers, and flavor due to generation of aroma-related compounds (23).

VII. EFFECT OF MEAT PHYSICOCHEMICAL CHARACTERISTICS ON MICROBIAL GROWTH

Thermal properties in foods are altered due to changes in chemical components, such as protein denaturation, fat melting and water evaporation. Thermal conductivity in a meat batter is related to water content and increases with temperature and humidity. Carbohydrate, protein and fat protect microorganisms against thermal destruction due to their low heat transfer coefficients (28).

On the other hand, microbial resistance to destruction can be increased by physiochemical characteristics of the food material such as water, fat, carbohydrate, and protein and salt content; pH; and presence of inhibitory compounds. Quality and sanitary risks depend on microbial access to the food, a function of microbial characteristics of the contaminant strain; food intrinsic antimicrobial attributes; storage extrinsic conditions in particular temperature, oxygen availability and time; and microbial interactions (29). Physicochemical attributes that must be also considered when thermal processing is applied are:

A. Water Activity and Redox Potential

It is directly related to microbial growth. Dry meat products do not require further heat processing. Limiting a_w for *Cl. botulinum* is 0.97 for psychrothrophic species and 0.95 for mesophiles (30). There is also a correlation between a_w and redox potential. Raw meat has a redox potential around –50 mV; it changes after heating, for example, sausages have potential from +20 to –100 mV, depending on the degree of grinding and ingredients added. Vacuum and addition of reducing agents can further decrease the redox potential (21).

B. Oxygen Tension

It is particularly important for strict anaerobes such as *Clostridium* sp. or microaerobes as lactic acid bacteria. Canning cured meat, where some oxygen remain in the product, implies applying mild heat treatment conditions although other preservation methods such as curing or refrigeration are used. In this situation spoilage may occur due to the presence of aerobes such as *Bacillus subtilis* or *Bacillus mycoides*. Carbon dioxide addition displaces oxygen; together with low temperature storage carbon dioxide reduces Gram negative bacterial growth (32).

C. pH

Meat and meat products, with the exception of fermented meats, are a low acid food (>4.5). In this case, heat treatment must be more severe as potential pathogens or spoilage microorganism can grow in this environment. In most cases *Cl. botulinum* grows and produces toxins at pH close to neutrality (8,16).

It is a determinant factor for several inactivation processes; generally highly acidic (pH , 3.7) or acid (pH 3.7 to 4.5) foods do not represent a sanitary risk. Growth of Cl. botulinum does not occur at pH # 4.5. Only foods with moderate acidity (pH . 4.5) may present a risk due to Cl. botulinum growth; at this pH level it produces toxins and heat-resistant spores. This anaerobe can be present in canned foods, where low acidity and absence of oxygen are suitable to growth and produce toxins. Its spores are heat resistant; that destruction is the calculation criteria for thermal processing of low-acidity foods, such as meats. A and B are the most heat-resistant toxins but are destroyed heating at 100°C for 10 min. Preventing conditions leading to toxin and/or spore presence in the food is the process calculation criteria (33). In low acidic foods such as most meat products, processing is based on inactivation of *B. stearothermophilus*, spores, 20 times more resistant than *Cl. botulinum* spores and responsible, when germinating, of producing sour taste and gas.

D. Temperature

Meat storage of meats at 0–4°C promotes the growth of *Pseudomonas* and *Acinetobacter* spp.; at 20–25°C *Micrococcus* grows in addition to the previous two microorganisms; at 25–40°C there is a dominance of *Enterobacter, Clostridium* and *Bacillus* spp.; above 40°C *Clostridium* y *Bacillus* spp. proliferate (17).

E. Antimicrobial Agents

Such as added benzoate and sorbate, or chemical components of the food (organic acids, lysoszyme, medium length chain fatty acids) (34,35,36).

F. Physical Structure

Physical structures are at the same time a barrier and a way to migrate to the internal part of a food. Microbial growth starts initially in the food surface; this is particularly true with meats, where microbial contamination starts in the carcass or cut surface, finding its way to the inner part of the muscle through the perimysium. However, when meat is cut or minced, microbial population is rapidly distributed throughout the food (32).

G. Food Composition

As foods in general, and particularly meats, have more than one constituent (oil/water emulsions, protein gels, collagen casings, connective tissue, muscle fibers) heterogeneity must be considered (37,38). It promotes different habitats due to a variety of physical structures and chemical compositions that influence growth and colonization of specific microbial populations. Meat smoking establishes a concentration of phenol and other compounds, at the same time a humidity gradient is established from the sausage center outwards promoting different microbial ecologies throughout the product. Heat processing must be calculated to reduce a given microbial type and counts in the food section where microbes are most protected (16).

VIII. MICROWAVE HEATING

It is based in alignment of water dipoles when exposed to an electric field. As the microwaves change the direction of the electric field at rate around 5×10^9 per second, water dipole alignment also changes causing friction and producing thermal energy. The same direct heating, microbial inactivation is the result of enzyme destruction. However, the microwave may cause overheating of some areas due to excessive energy absorption as a result of food heterogeneity (8). Microwaves frequency is between 300 Mhz and 300 Ghz, wavelengths between 1 mm and 1 m, although domestic and industrial microwaves are between 915 Mhz and 2450 Mhz (11). At present, microwave heating is applied to meat and meat products only for cooking purposes.

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163 Frozen Meat: Processing Equipment

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CONTENTS

I. INTRODUCTION

Meat freezing is a necessity that has emerged and grown with the opening of world level markets. Meat industry, in its quest to become more competitive, has found itself constrained to establish marketing channels at a long distance and for extended periods. Even though refrigeration is one of the most commonly used preservation methods, it is insufficient to cover such needs. Therefore, freezing becomes a solution in spite of its high cost compared to refrigeration and a decrease of the price of thus preserved meat, which results in a paradox.

The primary need required for a meat processor is to have and to be acquainted with an equipment adapted in cost function and quality production. However, such need cannot always be covered due to the fact that the election criteria employed in its selection is based on company's capacity or facility to acquire such equipment. Nearly 80% of the water contained in fresh meat submitted to a −5°C (23°F) freezing temperature is actually frozen; but it will rise to nearly 90% if it is frozen at −30°C (−22°F) (1).

The different types of equipment that have been utilized for the freezing of meat and processed products are presented in this chapter. Freezing equipment is classified according to its heat transference system (air, contact, immersion); its operation procedures (batch, continuous or in line) and by product handling presentation (before or after packaging).

II. FREEZING EQUIPMENT

There are three basic red meat freezing methods applied at commercial level: freezing by air, freezing by immersion in a coolant medium and freezing by contact. The type of equipment will depend on the method utilized. References for different types of equipment and their utilization on meat are shown in Table 163.1.

A. Freezing by Air

Such a freezing method is quite common; air is the refrigerating medium in it. Its particular compatibility with food even before packaging is one of its multiple advantages, and regardless of form or product dimension, it can be perfectly adapted to the processor requirements (3). This last issue turns into a very important matter in meat freezing because of large bulks handled in certain cases (meat carcasses or large product boxes intended for ulterior transformation) (4). While freezing, cold air presents a low specific heat (1 kJ/kg°C) requiring just a small amount of energy to increase its temperature, which should be reflected in a low heat transference coefficient (25 to 35 $W/m^2°C$) that is translated in a lack of heat transference in an air-product interphase. Air freezing devices are determined to be regularly voluminous due to these air properties (5).

Ventilation is an important factor in air freezing, because as the air speed increases, the amount of heat relinquished by the food by time unit increases as well, due to an increase of heat transfer coefficient. Therefore, to absorb the heat being relinquished by a product while freezing, great amounts of air must be handled. The energy cost required by a ventilator determines the practical limitations which indicate air maximum speed. It is interesting to analyze if this energy cost has an impact in the process, considering that this cost rises due to an increase on air speed

TABLE 163.1
Equipment Utilized in Freezing of Meat

Freezing Method	Equipment	Application	Reference
By air	Chamber with plates	Meat carcass, cuts (with or without packaging)	(3) (4) (7) (11)
By air	Tunnel continuous	Meat carcass, cuts (with or without packaging)	(3) (9) (11)
Immersion	Liquid nitrogen	Meat in pieces, fish and chicken	(3) (6) (9) (11)
Cryogenic	Nitrogen atomizer	Meat, fish and fruits	(8) (9) (11)
Contact	Horizontal plates	Meat cuts (packaged)	(3) (11)
Contact	Vertical plates	Meat cuts (packaged)	(3) (11)

upon third potency approximately. In practice, frozen air will normally be expelled at −30°C if the fluid being frozen by the air (cryogenic fluid) reaches a −40°C temperature. In order for the air to reach a −40°C temperature it would be necessary to duplicate the freezer energy consumption so the freezer can lower its temperature (5).

Regarding red meat freezing by air, it can be carried on by utilizing some of the following procedures:

a) Cold storage room. There is no standard design equipment. They are chambers built specifically according to volume of the meat to be frozen (cuts or carcasses) and presentation (with or without package). Basically, they are rooms designed to operate at low temperature levels. Airflow within chambers can be controlled in them, but not the air flowing over the product (6). It is worth mentioning that due to air effect a product tends to dehydrate on its surface, which results in a mass loss and turns into an economical loss, especially if the meat has not been properly packaged.
   Beef carcasses can be frozen in forced air freezers, which are usually refrigerated from 16 to 48 hours before freezing at temperatures from −10 to −30°C and air velocities from 1 to 3.5 m/s. At temperatures lower than bovine carcasses (−30°C to −40°C) pork can also be frozen with no previous refrigeration and so can lamb carcasses (without refrigeration) at −20°C at a speed from 0.5 to 1 m/s for 12 to 18 hours. Regarding meat cuts freezing, either for processing or separate selling, the most common form is by using individual packaging or grouped by muscle type placed in 15 cm width boxes. Temperatures of −30°C and air velocities of 12 m/s are the conditions utilized (5).
b) Tunnel type: These are chambers equipped with vaporizers and ventilators, where cold air circulates through the products located on trays or in boxes from 20 to 30 kg. Such boxes and trays are prepared on stationary shelves or on cars that travel through the tunnel, either isolated or in series, separated enough so the air can freely circulate. As red meat freezes, it is suspended over transporters or rails (6)(7). Cold air flows perpendicularly to a product movement, providing air in the tunnel in continuous sections. This represents an advantage of having only one-way in and out for its unloading. Having a perpendicular cold air direction allows a heat exchange reduction between the air within and outside the tunnel. Another advantage is that thermal conditions to keep humidity level high in each section can be controlled avoiding evaporation and preventing dehydration on a product surface, which leads to burn by freezing. Thermal transportation in this type of freezing depends on dimensions and product form. A tunnel freezing equipment turns very flexible and adaptable to products of all dimensions and forms, packaged or not (4)(8).

B. Freezing by Immersion

A direct contact between the food or the container that holds the product and the coolant is established in this type of freezer. The methods to freeze can be either by submerging the food in a cold liquid or spraying the liquid on it, using brines and syrups as well as calcium chloride, ethylenglycol, propylenglycol and alcohol among others (6). This method has been in practice since the beginning of the freezing process. In freezing by direct immersion, coolants selection becomes limited, especially when the food is not covered by package. Thus the coolant must fulfill some characteristics, such as non-toxic, certain purity, free of coloring, flavors and strange odors, etc. Also, when it comes to packaged foods, it is also important that the coolant does not contain toxic products or substances that could corrode the container (9). Nowadays, this freezing method is employed in irregular form products such as meat, fish and chicken (4).

C. Cryogenic Freezers

In cryogenic freezing a direct contact between the coolant and the food takes place. It has been proven to be a good conservation method. In this equipment the coolant is not provided by a static cooling system, but it is a consequence

of the change phase from a heat-transmitting agent. Such agent can be a sublimatory solid (solid carbon dioxide) or a cryogenic liquid (a liquefied gas with an extremely low boiling point) such as liquid nitrogen at −195°C and carbon dioxide at −78°C (5)(10).

The liquid carbon dioxide under pressure is employed to reinforce conventional freezing lines by cold air or to provide a total refrigerating medium in a freezing room. A mixture of cold carbon dioxide and carbonic snow (solid) is produced when this liquid atomizes, which can establish contact with the food to freeze. Since liquid nitrogen boiling point is −196°C (at atmospheric pressure), freezing velocities are extremely fast. This provides a quality hardly reached by any other freezing method known. High heat transference coefficients between a solid and a coolant fluid can be obtained by using liquid nitrogen. Irregular formed products can be frozen as easy as rectangular blocks, as they can equally be frozen individually (Individually Quick Frozen, IQF). A frozen food production by this method offers an advantage, since it allows the consumer to acquire individual portions from a package, storing the rest until needed (3)(11).

Nitrogen is the most important cryogenic liquid used in freezing by immersion. It is employed for meat cuts, fish fillets, seafood, fruits, etc. The employment of this cryogenic liquid is common in freezing equipment through tunnels. A reference to several types of equipment used appears on Table 163.1.

The most commonly used equipment for this type of freezing is the one where a product enters from one side of the tunnel and nitrogen is sprayed from the other; when it vaporizes, cold nitrogen gas runs through the tunnel until reaching the product. The gas lowers product temperature before the sprayed nitrogen gets to it. Once in contact with the spray, its superficial temperature goes down to a value close to nitrogen temperature (−195°C). The product passes to an equilibrium zone where cold surface temperature as well as the hottest core temperature are equaled in order to reach uniform temperatures. Finally, water spraying is applied to induce the cold stored within the product to form a thin frost layer, which will protect it from storage dehydration. Immersion freezing equipment is more commonly used to freeze packaged white meat (chicken and turkey) than for red meats.

Generally, compared to any other method, liquid nitrogen freezing causes less losses by freezing dehydration and thawing drainage. This can represent up to 5% of the weight from some foods. If analyzed, this freezing process though expensive in some cases can prove profitable considering the quality and volume obtained; for instance, a large scale beef patties production. An advantage of this type of equipment is that its installation expenses are lower than others; approximately 30% less compared to any freezing system of the same capacity. Weight loss by the product dehydration is reduced (8).

D. Freezing by Contact

Freezing by contact includes methods in which food or food containers are in contact with a surface cooled by a refrigerant. When this method is utilized, the food or container is not in direct contact with the coolant (5)(12). Generally, it requires a flat surface or an almost flat surface to be in contact with the refrigerated plates for solids or packaged products. Thus, a contact between these plates and 1 or 2 surfaces from the food or food container can be established (9). A good operation from this type of equipment is achieved if the heat transmission coefficient between food and coolant is high and uniform. This is possible if there is a deep contact from the food to the package material, that is if the vessel material is completely full of food. Aluminum compounds employed for plates manufacture has improved freezers plates capacity significantly (3).

Contact equipment freezing time periods are shorter than for air freezing systems; for instance, cardboard boxes with 27 kg of meat are frozen from 14 to 16 hours instead of 20 to 24 hours in a freezing tunnel (13).

Freezing by contact can be made with equipment that operates with plates provided in a horizontal or vertical way. In both cases such devices assure a good thermal transmission in a short freezing period, assuming that the product itself is a good heat conductor. The advantage of this transfer is reduced when product thickness increases; this procedure is more frequently applied to a thickness that does not surpass 5 or 6 cm, limiting its use to meat cuts packaged over trays (4)(6). Although it is possible to place solid foods over an ice block or dry ice, it is rarely done at a commercial level. What is actually done is placing the food over plates, trays, transporting bands or even more cold walls frozen by a circulating coolant so that the food is kept in contact with a cold wall but indirectly in contact with the coolant. A more detailed description of equipment provided with horizontal and vertical plates is presented next (4)(10)(14).

a) Horizontal plates: In this case, a product is placed on trays or metallic tables, which can be accomplished manually or automatically. Automatic operation is carried out by doing the following: The battery from the plates can be moved upward or downward in an elevating system; at a load carrier level plates are separated and the packages accumulated on the conveyor are pushed among the plates, unloading simultaneously frozen packages on the opposite side. The operation is repeated until all the frozen packages have been replaced; the two plates are adjusted again afterwards, the set goes back up and a new cycle begins.
b) Vertical plates: They are mainly used for products in blocks from 10 to 15 kg, such as entire

fish or meat cuts. This equipment contains a set of cooled plates, which at intervals form the compartments where the product is placed. The frozen blocks are unloaded sideways, upwards or downwards.

Certain freezing processes by indirect contact must not be mistaken with direct immersion. In order to distinguish one from the other, a direct contact from the food or its vessel with its coolant must be apparent. It will be a direct immersion freezing if a slice of meat or a slice of meat covered by a film is submerged in a brine bath or if it is sprayed with it. If the same meat is placed in a metallic bucket and immersed, the food and its package are being frozen by indirect contact.

III. EQUIPMENT SELECTION

A wide range of equipment to freeze foods is available in the market. Thus, there is a long list of characteristics that must be fulfilled in order to select a method and freezing equipment. Until now a final selection has depended basically on the cost-benefit analysis that it represents. However, there are many factors affecting the adoption of a correct cooling installation and it is difficult to make the right decision. Among the main factors are (6)(7)(15):

a) An appropriate size or capacity for the production.
b) Space requirements.
c) Cost and benefits for equipment installation and maintenance.
d) Quantity of products and presentations that can be frozen.
e) Cooling power (Q) required.
f) Operation temperature from the cooling fluid (T∞).
g) Heat transmission coefficient (h) achieved for freezing operation (Table 163.2).
h) Energy recovered as well as freezing as part of the process in line.
i) Feasibility and method comparison regarding evaporation losses.

TABLE 163.2
Heat Transfer Coefficient of the Surface Layer According to Freezing Method

Freezing Method	Heat Transfer Coefficient W/(m²°K)
By Airflow	
Static	6–9
Air at 3 m/s	18
Air at 5 m/s	25–30
By Immersion	
In freon	500
In liquid nitrogen	1500
By Direct Contact	
Plates	100

Adapted from (8)(15).

Furthermore, some inherent characteristics must be considered:

1) Process: Damage to the product, hygiene, and safety (6).
2) Product: Composition (humidity, w), thermal conductivity (λ), specific heat (C), specific weight (ρ) and geometry (5)(12).

The equipment selection definitely depends also on the size of the company in a sense that a smaller company will give more credit to a direct cost–benefit analysis along with characteristics of the freezing product to freeze. On the other hand, in a medium size company, besides these factors, the listed points before must be analyzed and prioritized according to the expected benefits. In big size companies, these factors are completely taken into account and include some more of their own as to effort, personnel, machinery and storage time saving policies. However, all these factors become obsolete if an inventory of the freezers is not done. Good Handling Practice, which is using the right procedures or systems for in and out product control (Just On Time, In and Out frozen products, etc.). It is common to hear that "freezing is useless because it decreases products quality with its usage" when problems arise, which is not completely accurate. Therefore, it is important to train the commercail user on the equipment, operating conditions and products to freeze.

IV. CONCLUSIONS

The different types of equipment utilized in meat freezing were described in this chapter. Regarding carcass freezing and boxed meat, the use of air remains the most commonly used method at commercial level, especially because it represents a lower cost concerning the volumes handled. It is also worth emphasizing that the utilization of contact freezing and the use of cryogenic agents offer greater advantages based on the ultimate quality of the product being frozen. Therefore, its usage is still limited to cut meats, where the operation cost is covered by the sale of the product. In conclusion, a meat freezing equipment selection must be very carefully carried on and consider not only cost-benefit, but also priority factors according to company size and policies.

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164 Dry-Cured Ham

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CONTENTS

I. INTRODUCTION

The origin of dry-cured ham is lost in ancient times, when humans used salting as a useful preservation tool for times of scarcity. The evolution of this product has followed a traditional route over the centuries, with oral transmission of the techniques involved from generation to generation but it was produced very empirically, with a rather limited knowledge of the process technology (1,2). In the last decades of the 20th century, rapid advances in the scientific knowledge of the chemistry, biochemistry, and microbiology involved in the process were made (3); this knowledge prompted successful developments in technology and significant progress in quality standardization (4).

The wide variety of processing technologies (with important variations in the conditions for drying, ripening, smoking, etc.) as well as the important influence of the hams used as raw material (genetic type, feed, rearing system, etc.) make for important variations in quality, especially in sensory characteristics. Main types of hams and the most important processing technologies are described in this chapter.

II. TYPES OF PRODUCTS

Some of the most important and well-known hams are listed in Table 164.1. Iberian hams are produced in the

TABLE 164.1
Main Characteristics of Dry-Cured Hams World-Wide

Dry-Cured Ham	Country of Origin	Approx. Length of Process (Months)	Smoking
Iberian	Spain	24–36	No
Serrano	Spain	9–18	No
Bayonne	France	9–12	No
Corsican	France	24	No
Parma	Italy	12–18	No
San Danielle	Italy	9–18	No
Katenschinken	Germany	3–5	Yes
Westphalia	Germany	3–5	Yes
Country-Style	U.S.	3–9	Yes
Sauna	Finland	2–4	Yes
Ching Hua	China	3–6	Yes
Yunnan	China	3–6	Yes

Source: Ref. 3.

southwest region of Spain by a long process that usually takes 2–3 years and gives an unique typical flavor. Hams originate from autochthonous heavy pigs (around 150 kg live weight), grown in extensive system and fattened with acorns (2). Similarly, Corsican hams take a long time (18 months) to produce in Corsica (France) and are made from autochthonous heavy pigs grown in extensive system and fattened with chestnuts, although the output is restricted due to the low number of pigs.

Hams from certain crossbreeds of white pigs constitute the raw material for Spanish Serrano, Italian San Danielle, and French Bayonne dry-cured hams. These pigs are slaughtered at 110 kg live weight whereas those used for Italian Parma hams are heavier (150 kg live weight). Dry-cured hams may receive different labels in the European Union (EU) area, such as Protected Designation of Origin, Protected Geographical Indication, or Traditional Speciality Guaranteed, depending on the specific region and particular regulations (e.g., type of crossbreeds, type of feed, slaughter age, processing technology). These hams are controlled by consortiums, such as the Parma Consortium or the Serrano Foundation, that verify the fulfillment of the specific requirements. All these hams can be eaten and require no further smoking or cooking (5).

Country-style ham is produced in the United States, particularly in Kentucky and Virginia. Hams are salted, dried for at least 70 days or even longer for better flavor development, and then smoked. The traditional German Westphalian ham, the German cold smoked ham (Katenschinken), and the Finnish ''sauna'' hams are dry-salted, left for a few weeks in vats or wooden barrels, and then smoked (6). Hams are also produced in other areas such as China, where typical hams like Ching Hua or Yunnan enjoy great acceptance (7).

III. PROCESSING TECHNOLOGY

Traditionally, pigs were reared at home and slaughtered by the end of November or early December so that hams could be salted and then left for salt diffusion during the coldest months. During the spring and summer, hams were ripened and dried, becoming ready for consumption by autumn (almost one year of total process). The production sites were usually located in the mountains, with cool and dry weather conditions favoring this process. The windows of the rooms were opened or closed depending on visual and tactile assessment by an experienced operator. Of course, this method was transmitted from fathers to sons, but the subjective assessment resulted in a great variability in the final quality. Today, most modern factories use computer-controlled drying chambers that allow a full control of air speed, temperature, and relative humidity. The final quality depends on the length of the process because time is needed for the enzymatic and chemical development of flavor, as will be discussed later. In general, the process is as schematized in Figure 164.1 and consists of the following stages.

A. Reception

Pork legs are classified when they arrive at the factory in order to facilitate their correct processing. This classification depends on the particular area but is usually based on ham weight, pH, and fat thickness (3). The composition of fatty acids in the fat mainly depends on the animals's feed (8) and, to a minor degree on the crossbreed used (9) but is of extreme importance for correct flavor development. Depending on the composition of certain polyunsaturated fatty acids, hams may develop an adequate flavor or may experience undesirable oxidation and develop rancid off-flavors. Fat may be controlled through the iodine index (as an indicator of unsaturation) and the acid index (as an indicator of freshness).

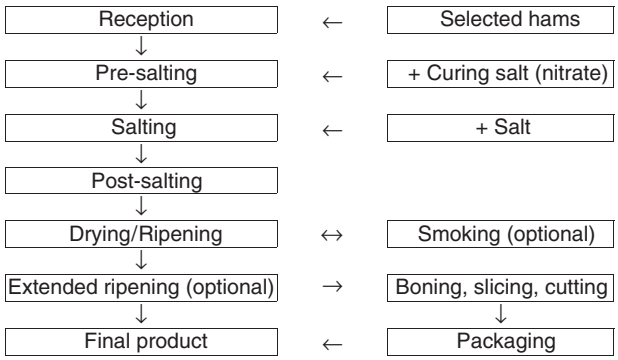

FIGURE 164.1 Process flow diagram for the processing of dry-cured hams.

The exudative hams, with a condition known as pale, soft, and exudative (PSE), have a low water-binding capacity and may sustain important weight losses, substantially higher than normal hams (10). In addition, PSE hams have a pale color and a wetted surface that facilitates the dissolution and penetration of the added salt but, on the other hand, results in an excessive salt uptake and unpleasant salty taste. Other groups of hams, those having high ultimate pH and known as dark firm, and dry (DFD), must be rejected in order to avoid microbial contamination.

Although the modern meat industry uses standard pigs, hams produced from older pigs usually are of better quality due to the higher amount of myoglobin (improved color) and different enzyme profile (better flavor profile) (11,12).

The skin is partially removed, leaving an area where salt will penetrate and water will evaporate. Hams are then registered to facilitate traceability, subjected to pressing rollers for bleeding, and left for 1 or 2 days under refrigerated storage (2–4°C) to reach a uniform temperature. Frozen hams are allowed to thaw until a temperature of about 4°C is reached inside the ham.

B. Presalting

This is a short stage during which nitrate is added to the hams in the form of a curing salt (sodium chloride with a small amount of potassium nitrate in order to get a final nitrate concentration of 150 mg kg^{-1} inside the ham) for a few minutes within a rotary drum (i.e., Spanish Serrano hams). The curing salt may be directly applied in the salting stage (i.e., for French and country-style hams). Nitrate and/or nitrite are used as protective agents against botulism (13) and slowly diffuse throughout the entire ham. Nitrate is reduced to nitrite by the action of nitrate reductase, a bacterial enzyme present in the natural flora (i.e., *Micrococcaceae*) of ham. This reduction is slow due to the low bacterial counts. Further formation of nitric oxide is achieved at slightly acid pH, as found into the ham and favored by curing adjuncts, such as ascorbic or erythorbic acids, that act as reducing substances. The maximum amount allowed in the European Union is 150 ppm potassium nitrate or 300 ppm for combination of potassium nitrate + sodium nitrite, and in the United States 156 ppm sodium nitrite (1/4 ounce per 100 pounds of meat). In some cases, the use of nitrate and/or nitrite is banned (i.e., Italian Parma ham).

C. Salting

Salt inhibits the growth of spoilage microorganisms by reducing the a_w; it also imparts a characteristic salty taste and increases the solubility of myofibrillar proteins. The main objective of the salting stage is to supply the necessary amount of salt to the outer surface of the hams. Absorbed salt is then slowly diffused through the whole piece during the postsalting stage. The amount of salt may be tightly controlled, on a weight basis, allowing time enough for its penetration into the piece (exact salt supply). So, hams are weighed one by one and the exact amount of salt per kilogram of ham is added on the lean surface. For instance, Parma hams receive 20–30 g medium-grain salt per kilogram on the lean surface and 10–20 g of wet salt per kilogram on the skin (14). Then, salt is hand-rubbed and left to be absorbed into the ham (14–21 days, depending on size).

In other cases, the amount of salt is undetermined but time of salting is strictly controlled. Hams are entirely surrounded by rough sea salt or refined mineral salt and then placed by layers into stainless steel bins with holes for the elimination of drippings. Salt may be rubbed onto the lean surface, and the hams are placed on shelves. This stage may last up to 13 days under refrigeration with 3–4% weight losses. In some cases, hams are salted again. Once the salting stage is finished, the excess salt is removed by brushing and water rinsing.

D. Post-salting or Resting

The main objective of this stage is to achieve salt equalization through the entire piece. Salt and nitrate must diffuse through the whole ham piece as shown in Figure 164.2. The required time may vary between 40 and 60 days, depending on many variables such as the size of the ham, pH, amount of fat, and conditions in the chamber. The relative humidity in the chamber is progressively reduced with time, and the typical weight losses are around 4–6%.

E. Smoking

The use of smoke is one of the oldest preservation technologies, and it is used for short term processed hams like American country-style or German Westphalia ham. The use of smoking is typical in areas where drying was originally more dificult (i.e., Northern countries) and gives a particular flavor and color to the hams. The smoke compounds also protect hams against molds or yeasts growth due to their bactericidal effects (15).

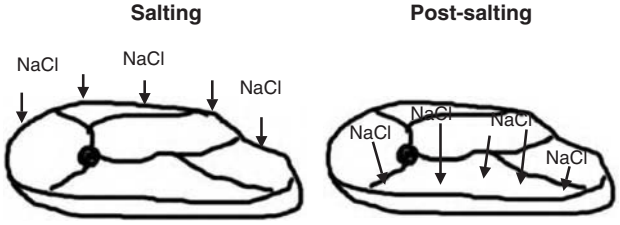

FIGURE 164.2 Scheme showing the uptake of salt and nitrate during the salting and then its diffusion during the post-salting stage.

F. Ripening-Drying

Hams are placed into modern computer-controlled drying chambers; some may contain up to 30,000 hams per chamber. Temperature, relative humidity, and air speed must be as homogeneous as possible and are carefully controlled and registered. Each type of ham has a specific set of variables along time of processing. For instance, Spanish hams are subjected to a progressive slow increase in temperature whereas French hams are heated to 22–26°C just after the post-salting stage. In all cases, these conditions allow the action of the endogenous enzymes, as will be described later. The length of the process depends on the type of ham (pH, size, amount of intramuscular fat, etc.) and drying conditions. Water is slowly diffused towards the outer part of the ham, specially the lean, for further evaporation once it reaches the ham surface as shown in Figure 164.3. The final expected weight loss (around 32–36%) is usually achieved within 6 to 9 months. Then, hams are covered with a layer of lard to avoid further dehydration and prevent any growth of molds and/or yeasts on the outer surface. Hams quality is monitored through a sniff test consisting of the insertion of a small probe in a specific area of the ham prone to spoilage and immediately smelled by an expert for detection of any off-flavor (14). The rapid development of commercial electronic noses and probes to get an objective assessment of flavor quality has led to their increased use for quality classification of hams (15).

G. Extended Ripening

Hams of high quality are further ripened in cellars for several months under mild conditions in order to get a full, rich flavor development. This is the case with Iberian hams, which may undergo 24 to 30 months of total processing time.

H. Final Product

Hams may be sold either as an entire piece (usually those of higher quality) or boned. Commercial distribution of sliced ham in vacuum-packages or under controlled atmosphere is increasing very fast (17). Boned hams are usually vacuum-packaged and distributed through retailers for final cutting into pieces or slices (see a slice in Figure 164.4). Hams are sliced by retailers or directly by consumers at home.

IV. MICROBIAL EVOLUTION

The increased concentration of salt and progressive reduction in water activity constitute limiting factors for microbial growth (18). In fact, low bacterial counts have been found inside the hams (19). Some species of the Kocuria family with nitrate reductase activity are present in ham. Some microorganisms, such as *P. pentosaceus* and *S. xylosus*, which are present in the natural flora of ham, have been studied for their enzyme activity but no significant endoprotease activity was detected, only a minor exopeptidase activity (16) although *S. xylosus* also showed an important nitrate reductase activity. The enzyme profile of some lactic acid bacteria has been also studied, because they could be used as microbial starters to accelerate the process. In fact, *L. sakei*, *L. curvatus*, *L. casei*, and *L. plantarum* have shown good endo- and exo-proteolytic activity against myofibrillar and sarcoplasmic proteins (21–24).

Molds can grow and develop on the outer surface of the ham due to the humidity and temperature conditions in the curing chambers when no precautions are taken. The most common mold is Penicillium (25), but some yeasts, mainly *Candida zeylanoides* in early stages and *Debaryomices hansenii*, also may grow (26). The isolated molds (around 75%) have shown good antimicrobial activity against *Staphylococcus aureus* and are able to inhibit its growth (25).

V. PHYSICAL AND CHEMICAL CHANGES DURING THE PROCESS

Main chemical changes are the result of changes in composition due to water loss and salt penetration as shown in Figures 164.2 and 164.3. The diffusion of water through

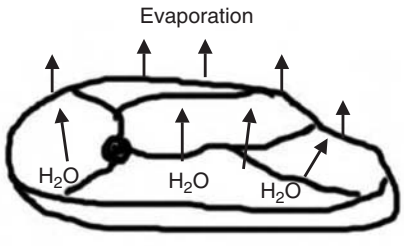

FIGURE 164.3 Scheme showing the diffusion of water towards the outer lean areas and then its evaporation once it reaches the surface.

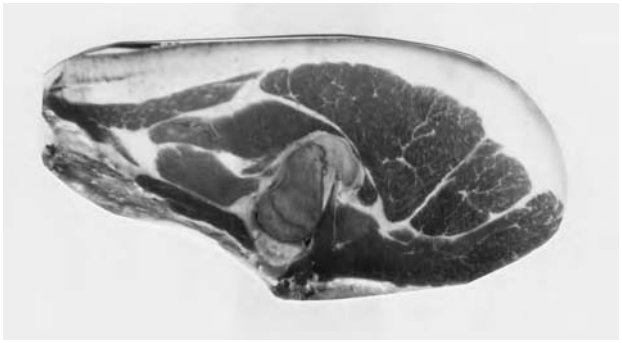

FIGURE 164.4 Cross-section of a typical dry-cured ham.

the ham and its evaporation when reaching the surface is a slow and difficult process. Both rates must be equilibrated to achieve adequate drying and, in this sense, it is very important to have the water sorption isotherms to predict the required time for drying. Diffusion of salt is also very slow and is affected by many variables such as temperature, size of the ham, pH, amount of moisture, and intramuscular fat. It takes around 3–4 months to get full salt equalization through the entire piece, although the salt profile may change a little depending on the particular moisture content in each muscle. pH increases from initial values of around 5.6–5.8 to values near 6.4 toward the end of the process. PSE hams have a pH evolution similar to normal ones (10,27). This evolution constitutes a narrow range where all the enzymes and chemical reactions operate (28). However, even slight variation in pH might affect the action of muscle enzymes; for example, more intense proteolysis has been reported in low-pH hams (29).

VI. BIOCHEMICAL CHANGES DURING THE PROCESS

Many biochemical changes have been reported during the processing of dry-cured ham, most of them bring a consequence of enzymatic reactions. Some of these changes are restricted to the beginning of the process; such is the case with nucleotide breakdown reactions or the glycolysis-related enzymes and subsequent generation of lactic acid. Proteolysis and lipolysis constitute two of the most important enzymatic phenomena, responsible for the generation of compounds with direct influence on taste and aroma.

A. PROTEOLYSIS

Proteolysis consists of the progressive degradation and breakdown of major meat proteins (sarcoplasmic and myofibrillar proteins) and the subsequent generation of peptides and free amino acids. The result is a weakening of the myofibrillar network and generation of taste compounds; but its extent depends on many factors. One of the most important is the activity of endogenous muscle enzymes, which depends on the original crossbreeds (9,30) and the age of the pigs (11,12). Main muscle enzymes involved in these phenomena and their main properties are listed in Table 164.2. These enzymes show a great stability in long dry-curing processes like hams (19,31). Other important factors are related to the processing technology: for instance, the temperature and time of ripening will determine the major or minor action of the enzymes, and the amount of added salt, which is a known inhibitor of cathepsins and other proteases, will also regulate the enzyme action (32–35). Excessive softness in ham has been correlated with high cathepsin B activity and low salt content (36,37).

Great amounts of small peptides, in the range of 2700 to 4500 Da or even below 2700 Da, are generated during the process (38–40) although this generation may be depressed by the level of salt that inhibits muscle peptidases (41). Some of these peptides give characteristic tastes (38). Recently, several tri- and dipeptides have been isolated in flavorful fractions and sequenced (42). Final generation of free amino acids by endogenous muscle aminopeptidases is very important, reaching impressive amounts as high as several hundreds of milligrams per 100 g of ham (43–46).

TABLE 164.2
Proteolytic Muscle Enzymes and Main Properties

Enzyme	EC Number	Main Action	Main Substrate	Product	Opt. pH	Opt. Temp (°C)	Stability	Effect of Salt	References
Cathepsin B	3.4.22.1.	Endo-protease	Proteins	Polypeptides	6.0	37	Years	Inhibition	33
Cathepsin L	3.4.22.15.	"	"	"	6.0	30	Years	"	31
Cathepsin D	3.4.23.5.	"	"	"	4.0	40	Months	"	32
Cathepsin H	3.4.22.16.	Amino/endo	"	Amino acids	6.8	37	"	"	33
Calpain I	3.4.22.17.	Endo-protease	"	Polypeptides	7.5	25	Days	Activation	47
Calpain II	3.4.22.17.	"	"	"	7.5	25	"	"	47
TPP I	3.4.14.9.	Exo-protease	Polypeptides	Tripeptides	4.0	37	Months	Inhibition	3
TPP II	3.4.14.10.	"	"	"	7.0	30	"	"	3
DPP I	3.4.14.1.	"	"	Dipeptides	5.5	50	"	No effect	48
DPP II	3.4.14.2.	"	"	"	5.5	65	"	Inhibition	49
DPP III	3.4.14.4.	"	"	"	8.0	45	"	"	50
DPP IV	3.4.14.5.	"	"	"	8.0	45	"	"	51
Methyonyl	3.4.11.18.	Aminopeptidase	Peptides	Amino acids	7.5	40	Years	"	52
Alanyl	3.4.11.14.	"	"	"	6.5	37	"	"	53
Leucyl	3.4.11.1.	"	".	"	9.0	45	"	No effect	54
Pyroglutamyl	3.4.19.3.	"	"	"	8.5	37	Weeks	Inhibition	35
Arginyl	3.4.11.6.	"	"	"	6.5	37	Months	Activation	55

B. Lipolysis

Lipolysis consists of the breakdown of triacylglycerols by lipases and phospholipids by phospholipases, resulting in the generation of free fatty acids. These fatty acids may contribute directly to taste and indirectly to the generation of aroma compounds through further oxidation reactions. The relative amounts of released fatty acids will depend on the initial composition in fatty acids of both triacylglycerols and phospholipids. Fatty acid composition is strongly affected by dietary fat (56,57) and slightly affected by cross-breeding (58–60). Main lipolytic enzymes located in muscle and adipose tissue and involved in these phenomena are listed in Table 164.3. These enzymes show good stability through the full process (61–63). Although their activity also depends on pH, salt concetration, and water activity, the conditions found in the hams favor their action (64). The generation rate of free fatty acids in the muscle, especially oleic, linoleic, estearic, and palmitic acids, increases for up to 10 months during the process (65). Most of these fatty acids proceed from phospholipids degradation (3). After this time, a reverse trend is observed due to further oxidative reactions (62,66). In the case of adipose tissue, the rate of generation, especially of oleic, palmitic, linoleic, stearic, palmitoleic, and myristic acids, is also high up to 6 months (63). In the same way, a decrease in 14% of the triacylglycerols is observed (67).

C. Oxidation

The generated mono- and polyunsaturated fatty acids are susceptible to further oxidative reactions, giving rise to volatile compounds. The beginning of lipid oxidation is correlated to an adequate flavor development (68). In contrast, an excess of oxidation may lead to off-flavors. In fact, the generation of the characteristic aroma of dry-cured meat products correlates with the beginning of lipid oxidation. Free-radical formation is catalyzed by muscle oxidative enzymes, like peroxidases and cyclooxygenases, external light, heating, and the presence of moisture and/or metallic cations. The next step in oxidation is the formation of peroxide radicals (propagation), by reaction of free radicals with oxygen. The formed hydroxyperoxides (primary oxidation products) are flavorless but very reactive, giving secondary oxidation products that contribute to flavor (69). Oxidation is finished when free radicals react with each other. Main products from lipid oxidation (70–72) are aliphatic hydrocarbons (poor contribution to flavor), alcohols (high odor threshold), aldehydes (low odor threshold), and ketones. The last two groups are related to the aroma of dry-cured ham in French-type hams (73) and Spanish hams (74,75). Alcohols may interact with free carboxylic fatty acids, giving esters, especially when nitrate is not used, as in Parma ham, in which esters are generated in greater amounts and are well correlated with its aged odor (76).

VII. DEVELOPMENT OF SENSORY CHARACTERISTICS

A. Color

The color of dry-cured ham mainly depends on the concentration of its natural pigment myoglobin, which depends on the type of muscle and the age of the animal (77,78). So, myoglobin concentration is higher in muscles with oxidative pattern and in older animals. The typical bright-red color is due to nitrosomyoglobin, a compound formed after reaction of nitric oxide with myoglobin. About 10–40% of total myoglobin is transformed into nitrosomyoglobin (79). Those hams without added nitrate present a pinky-red color. Some surface colors on smoked hams may result from the pyrolytic decomposition of wood.

TABLE 164.3
Lipolytic Muscle and Adipose Tissue Enzymes and Main Properties

Enzyme	Main Action	Main Substrate	Opt. pH	Opt. Temp (°C)	Stability	Effect of Salt	References
Muscle							
Acid lipase	Lipase	Triacylglycerols	5.0	37	Months	Activation	61,62
Neutral lipase	"	"	7.5	45	Years	Inhibition	61,62
Phospholipase A	Phospholipase	Phospholipids	5.0	37	Months	Activation	3
Acid esterase	Esterase	Triacylglycerols	5.0	30	Years	Inhibition	61,62
Neutral esterase	"	"	7.5	20	"	"	61,62
Adipose Tissue							
Hormone-sensitive lipase	Lipase	Triacylglycerols	7.0	37	Months	Activation	61,63
Monoacylglycerol lipase	"	Monoacylglycerols	7.0	37	"	"	1
Lipoprotein lipase	"	Lipoproteins	8.5	37	"	Inhibition	1
Acid esterase	Esterase	Triacylglycerols	5.0	60	Years	"	62,63
Neutral esterase	"	"	7.5	45	Years	"	62,63

B. Texture

The texture of dry-cured hams depends on several factors, such as the extent of drying (loss of moisture), the extent of proteolysis (degree of myofibrillar protein breakdown), and the content of connective tissue (80). In fact, major structural proteins such as titin, nebulin, and troponin T are fully degraded whereas myosin heavy chain and α-actinin are partly proteolyzed (31,81). Some small fragments (150, 85, 40 and 14.4 Kda) appear as a consequence of proteolysis (1,28). The content of intramuscular fat also exerts a positive influence on some texture and appearance traits (82).

C. Flavor

1. Generation of Taste Compounds

Taste is closely related to the enzymatic breakdown of proteins and peptides into smaller peptides and free amino acids as summarized in Table 164.4. Glutamic and aspartic acids impart an acid taste and sodium salts give ham salty and umami tastes. Bitter taste is mainly associated with aromatic amino acids such as phenylalanine, tryptophane, and tyrosine; sweet taste with alanine, serine, proline, glycine, and hydroxyproline (38). The generation of all these free amino acids is extremely important in dry-cured ham (40,43,45) and is somehow affected by levels of salt (45,83). For instance, lysine and tyrosine are well correlated to an improvement in the aged taste of Parma ham (76), although in other cases, such as in French-type dry-cured ham, only a small effect on flavor development has been reported (73). An excess of proteolysis (proteolysis index higher than 29–30%) is undesirable because it may give a bitter or metallic aftertaste (76,84). Some sourness may be expected from the generation of free fatty acids if they are not followed by further reactions like oxidation. Specific tastes for dry-cured ham have been found after fractionation by gel filtration chromatography in several fractions with low molecular mass, below 2700 Da, accompanied by some nucleotides and a few compounds from protein-lipid interaction (38). Some of these tasty peptides, mainly di- and tripeptides, have been successfully purified and sequenced (42). Free amino acids may also serve as a source of volatile compounds during further ripening (85) or when the ham is heated, like the country-style ham (86).

2. Generation of Aroma Compounds

Aroma development in dry-cured ham is a very complex process involving numerous reactions such as chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides, and free amino acids (71,89). In fact, more than 200 volatile compounds have been reported in dry-cured hams (70,74,90–92) as summarized in Table 164.5. Some volatile compounds, such as 2-methyl propanal, 2-methyl butanal, and 3-methyl butanal, arise from Strecker degradation of the amino acids valine, isoleucine, and leucine, respectively. Some pyrazines formed through Maillard reactions between sugars and free amino acids, although in low amounts, also impart some characteristic aromas like nutty, green, earthy, and so forth. Final flavor depends on the mixture of characteristic aromas and odor thresholds for each compound, although, in general, ketones, esters, aromatic hydrocarbons, and pyrazines are correlated with pleasant aroma of ham (93). Some correlations have been found between some volatile compounds and specific characteristics of the process. For instance, the correlation of aged flavor of Parma ham with short-chain methyl-branched aldehydes, esters, and alcohols (76,94); hexanal, 3-methyl butanal, and dimethyl disulfide with short drying

TABLE 164.4
Main Groups of Non-Volatile Compounds Generated during the Processing of Dry-Cured Ham

Groups Non-Volatile Compounds	Main Origin	Characteristic Taste
Tri- and di-peptides	Proteolysis of proteins and larger peptides	Different tastes depending on amino acid composition
Phenylalanine, tyrosine, tryptophane, leucine, valine and isoleucine	Proteolysis of proteins and peptides	Bitter taste, may be strong when high concentration of these amino acids from intense proteolysis
Alanine, serine, proline, glycine and hydroxyproline	"	Sweet taste that depends on the reached concentrations
Sodium glutamate and aspartate	"	Umami taste
Inosine monophosphate	ATP degradation	Slight taste enhancement
Glutamic and aspartic acids	Proteolysis of proteins and peptides	Slight sour taste
Lactic acid	Postmortem glycolysis	Slight sour taste
Free fatty acids	Lipolysis of triacylglycerols and phospholipids	Very slight sour taste
Inorganic compounds	Salt, mainly NaCl, added during salting	Strong salty taste

Source: Refs. 3, 38, 42, 87, 88.

TABLE 164.5
Main Groups of Volatile Compounds Generated during the Processing of Dry-Cured Ham

Groups Volatile Compounds	Main Origin	General Characteristic Aromas
Aliphatic hydrocarbons	Autooxidation of lipids	Alkane, crackers
Aldehydes	Oxidation of free fatty acids	Green, pungent, fatty
Branched aldehydes	Strecker degradation of amino acids	Roasted cocoa, cheesy-green
Alcohols	Oxidative decomposition of lipids	Medicinal, onion, green, alcoholic
Ketones	β-keto acid decarboxylation or fatty acid ß-oxidation	Buttery, floral, fruity
Esters	Interaction of free carboxylic acids and alcohols	Fruity
Nitrogen compounds	Maillard reaction of amino acids with carbohydrates	Meaty, nutty, toasted nuts
Sulfur compounds	Sulfur containing amino acids	Dirty socks
Furans	Reaction of sulfur containing amino acids with carbohydrates	Ham-like, fishy

Source: Ref. 3.

processes (74); or methyl-branched aldehydes, secondary alcohols, methyl ketones, ethyl esters, and dimethyl trisulfide with nutty, cheesy, and salty descriptors (95).

VIII. ACCELERATED PROCESSING OF DRY-CURED HAMS

Many attempts have been made to accelerate the process, especially in country-style hams. Most of them try to accelerate the penetration and diffusion of salt into the hams, such as by boning and skinning of hams (96–98), mechanical tenderization through blade penetration prior to dry cure (99), tumbling in a revolving drum with bafles (100), or the direct use of nitric oxide instead of nitrate or nitrite (101). In other cases, more intense biochemical changes through papain injection (102), microbial inoculation (103), or membrane disruption by prefreezing and thawing of hams (104,105). Other attempts for reduction of the salting time have consisted on the substitution of the salting stage by using simultaneous brine thawing-salting method, that can be even further accelerated by performing vacuum impregnation (106). Future developments will include the addition of microorganisms with a determined enzyme profile or just a purified enzyme, microencapsulated in lyposomes or rubbed onto the outer surface of the hams, with specific roles to reinforce the action of the endogenous enzymes during the process.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Dry-cured ham," by F Toldrá In: YH Hui, LM Goddik, J Josephsen, PS Stanfield, AS Hansen, WK Nip and F Toldrá, eds. Handbook of food and beverage fermentation technology. New York: Marcel-Dekker, 2004.

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165 Poultry Carcass Evaluation

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CONTENTS

I. INTRODUCTION

The low relative price of poultry meat and shifting consumer preferences have expanded poultry meat share of global meat output. In 2001, broad-based output gains were realized in most regions with that of developing countries growing by 3 percent. Global per capita consumption inched up slightly, from 11.2 to 11.3 kilograms/caput. While consumers in developing countries increased their intake slightly to 7.7 kg in 2001, shifting meat consumption preferences in the European Community, a region that accounts for only 13 percent of global consumption but accounted for almost 30 percent of the consumption gains in 2001, pushed up the average intake in developed countries from 24.1 to 24.8 kilograms/caput. Consumer preferences for poultry prompted global poultry trade to increase 4 percent in 2001 to 7.6 million tones, pushing up poultry's share of global meat trade to 44 percent. Asia, mainly China, is one of the largest poultry markets, resulted from lower domestic prices, as excess supplies built up due to Avian Flu problems that limited exports (1).

II. SLAUGHTER

Slaughter begins with the bird catching and preparation to transport from the poultry yard to the slaughterhouse. Since transport could be a very traumatic experience for any animal, many troubles could result from this operation. Main welfare problems in poultry (broiler, turkey and duck) industries are leg disorders, overstocking, underfeeding and cannibalism, inadequate bird inspection, catching and transport, and stunning. Although deaths during transport are low, congestive heart failure (CHF) has been related to 47% of bird's deaths, appeared to stress associated with loading and transport that precipitate the heart attack in broilers with CHF. In its severe form, CFH leads to ascites (water belly), and ascitic birds are not fit for human consumption. Another cause of death

is a different kind of traumas (Table 165.1), which account for about 35% of the total deaths (2).

In the slaughterhouse process line, the birds are unloaded and hanged from the legs in hooks. The conventional method of poultry processing involves shackling live birds and use electrical stunning using a multiple bird water bath stunner or directly to neck prior to bleeding. Generally the electric shock is between 100 to 500 volts. Alternatively, another novel method of killing poultry is by using gas mixtures, named controlled atmosphere stunning, using 90% of inert gases in air or a mixture of 30% carbon dioxide and 60% inert gases in air (3). Immediately the neck is sectioned for the bleeding, followed by an automatic plucking. The carcasses are washed and ready for the first inspection (4). Depending on the line process design, there are two ways to make the evisceration. In the first, generally an incision in the abdomen is made to retire intestines and the crop, gizzard, liver and heart are extracted and examined. Evisceration process is modified when the plant has a general inspection, where after the abdominal incision the liver and heart are exposed for inspection. Figure 165.1 is a check-point diagram of the general poultry process industry from the reception to process line. An automatic on-line inspection system developed in the Agricultural Research Service, where the system quickly diagnoses all physical or nonmicrobial, biological conditions that cause an inspector to remove a chicken from the processing line (5).

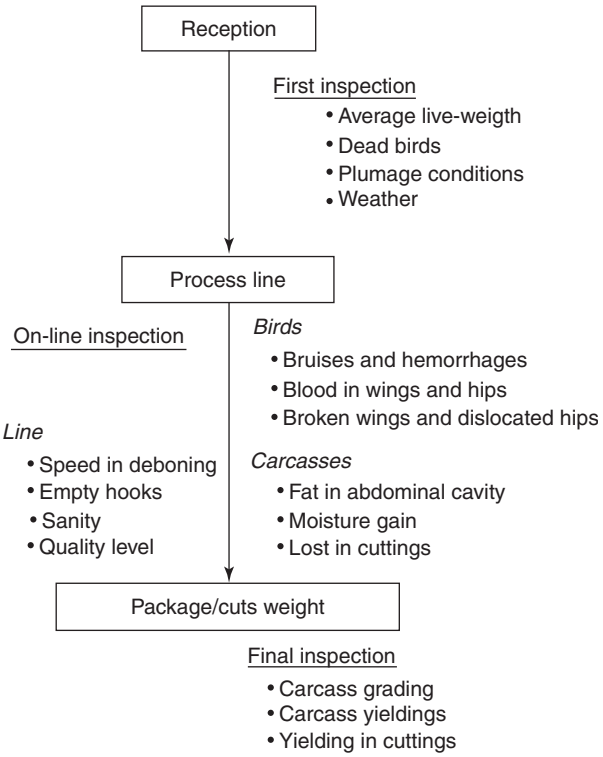

FIGURE 165.1 Check point by area in poultry processing.

III. MAIN FACTORS AFFECTING CARCASS QUALITY

A. Microbial Contamination

An important problem in poultry meat is the higher microbial contamination. Each carcass could contain bacteria from poultry yards, skin, pens and cross-fecal contamination during process, and microbial population in live birds can be as high as 10^4 cfu/cm^2 (6). To eliminate fecal contamination, the poultry processors employ interior and exterior washers. Fecal material could be difficult to remove, especially in two areas: wing and below the fat deposits. Many methods for poultry carcass decontamination have been suggested, as irradiation, electromagnetic waves, microwaves (7), but washing is the most common practice, including some chemical agents such as clorhexidine digluconate (8). Many studies have been made to reduce poultry carcass pathogens, such as *Salmonella* (9) *Salmonella enteritidis* (10) and *Escherichia coli* (11).

TABLE 165.1
Main Causes of Trauma that Cause Poultry Death

Dislocated or broken hip	76%
Liver or internal hemorrhages	14%
Head trauma	8%
Others	2%

B. Color

Some of the poultry muscles are white and others red due to myoglobin concentration. Because of different muscle activity, the oxygen demand will be different. Red and pink color and the blood in carcasses are the main problems in poultry plants. These colors are due to conditions before the slaughter and punches during birds handling. The stress due to heat and excitation can produce an abnormal red color and a high myoglobin concentration (12). Heat-shortening turkey meat is less red probably because the haem pigments are denatured by the high temperature-acid conditions, and this type of pale meat in turkey is similar to pale, soft, exhudative meat in pork (2). The handling and bleeding could cause bruises. There are two important forms of ante-mortem bruising in poultry: breast bruising and red wingtip, caused mainly by inadequate transport conditions (2). Figure 165.2 shows the location of common bruises.

C. Texture

Poultry breast muscle is an important eating quality criterion. The major factors affecting texture are the rate of

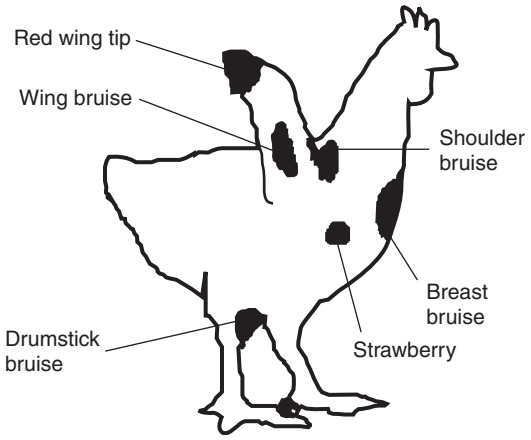

FIGURE 165.2 Location of common sites of bruising in poultry carcasses.

rigor development, rate of carcass chilling and filleting time. Stunning methods can affect texture mainly by altering the time course of rigor development and the extent of muscle contraction and relaxation occurring during early post-mortem (3).

If the birds are stressed before slaughter and this causes depletion of their muscle glycogen, the meat is likely to be more tender. On the other hand, if the birds struggle and flap their wings excessively just before slaughter, this will cause a build-up of lactic acid in the breast muscle and the meat is likely to be tough. In this last case the muscle glycogen is not depleted and becomes acidic whilst it is still hot. Meat toughness increases as the lactic acid content in the muscle at the time of slaughter increases. The increase toughness is due to more extensive muscle contraction during rigor development, and this effect is temperature dependent: the higher the temperature as the muscle goes into rigor, the stronger will be the contraction, named heat shortening. Breast meat is more prone to heat shortening than thigh meat. In turkeys, hot shortening is likely to be paler as well as having a lower water-holding capacity. In general, toughness in heat-shortened poultry meat does not resolve when the meat is left to age for 48 hours (2).

D. Poultry Carcass Electric Stimulation

An electric current applied three minutes after the plucking and cooling resulted in the ATP fall and pH reduction, increasing the meat tenderness (13). Electrical stimulation by breast contact has been commercially implemented in poultry plants, but there was difficulty in maintaining contact between the bird and the metal rub bar. When the birds contracted due to the stimulation, they pushed off of the bar, losing contact with the electrical current and decreasing the tenderizing effect. Using electrically stimulating carcasses at the neck in a saline bath could reduce the aging time, decreasing the costs (14).

IV. CARCASS EVALUATION METHODS

The objective of the estimation of carcass composition is to determine the edible part of the carcass. The differences among poultry carcasses have been of great economic significance for the meat industry. The most valuable carcasses are those that meet the highest quality standards and highest yields. To evaluate quality and yield is required to estimate and measure some characteristics of the carcass. Poultry can be commercialized in carcass or pieces. Figure 165.3 shows the main cuts for poultry.

A. Ante-mortem Methods

1. Ultrasound

The same equipment employed to monitored gestation, can be employed to determine the carcass composition. The images are received in real time and some measurement can be made to determine fat and muscular tissue. This can be made in live animals or birds and carcasses (15,16).

2. Live Weight

The live weight without the gastrointestinal content gives the empty body weight used for the carcass yielding. Poultry producers influence on carcass composition looking for a higher muscle proportion together with a convenient fat proportion and minimum bone (17). Table 165.2 show the carcass yielding of some poultry species (17,18,19,20). As observed, broilers and capons had the highest carcass yielding (up 90%) as compared with the other species. Geese and turkey had similar yielding (80–87%), followed by ducks. Ostrich had the lowest yielding, probably due to its bigger size and weight with more viscera content.

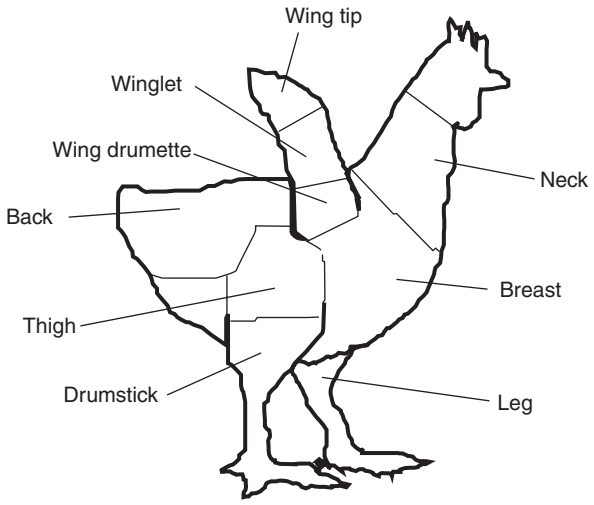

FIGURE 165.3 Main cuts in poultry carcass.

TABLE 165.2
Carcass Yielding for Some Poultry Species

	Live Weight (kg)	Hot Dressed (kg)	Yielding (%)
Broiler	1.92	1.71	88.89
Capons	3.92	3.51	89.66
Turkey	8.28	7.61	91.85
Pekin duck	2.57	2.18	85.09
Roaster	3.69	3.29	89.02
Geese	5.48	4.77	87.06
Ostrich	80.00	48.00	60.00
Duck	2.32	1.75	75.46

B. Post-mortem Methods

1. Electric Conductivity

The electric conductivity in the meat will be different as a function of the fat content. The higher the fat content the lower electric conductivity, because the fat and water content are inversely proportional. A fatty muscle has less water. These results are used to determine the fat, water and protein muscle composition. Total body electrical conductivity (TOBEC) is a technique that relies upon the differences in electrical conductivity and dielectric properties of the fat free and fat tissues to estimate body composition. The instrumentation used is an adaptation of the commercial device developed for determination of lean tissue in meat and live animals (21). The oscillating field with induces an electrical current in any conductive material placed within the coil. The actual measurement consists of the difference between the coil impedance when empty and that when the subject is inserted (22).

2. Optical Fibers

Fiber-optic light guides can be used for spectrophotometry within the carcass. But for illuminating and receiving apertures locates behind the cutting tip of the probe, light guides are restricted in their minimum radius of curvature and require a relatively thick shaft. A thick probe is difficult to push into the carcass. A beam splitter can be used to both illuminate and gather light from meat (23).

3. Multispectral Images

Recently, real-time multispectral image processing algorithms were developed for on-line poultry carcass inspection. The classification accuracy using dual wavelength spectral images was much higher than single wavelength spectral images in identifying unwholesome poultry carcasses. The classification accuracy was 91.1% for wholesome and 83.3% for unwholesome carcasses (24). In the system, which has been tested in commercial process poultry plants, when a chicken carcass on a hook dangling from a moving chain passes through a light beam, the interruption triggers a scan with a light probe from about an inch away. The reflected light is analyzed by a computer using Agricultural Research System-developed "Automated Poultry Inspector" software to identify variations in external skin color and texture and tissue composition, which are clues to problems (5).

V. INTERNATIONAL LEGISLATION ON POULTRY CARCASS GRADING

Generally speaking, quality refers to the appearance of carcass or meat and has become more important in determining consumer acceptability and retail price. Some of the more commons carcass and meat quality defects are: red wingtips or feather tracks, engorged wing veins, hemorrhagic wing veins or shoulders, in breast or/and legs, and dislocated or broken pectoral, wing or leg bones. The commercial significance of these defects varies according to market outlet. However, the prevalence of appearance defects can also lead to downgrading and loss of value of fresh carcasses (3). Normally, the categories are reduced to three, being the flesh in breast and thighs, ending of the breastbone, number of broken bones in carcass, exposed flesh, etc. (19). Figure 165.4 despite the main classification of poultry carcasses.

A. United States of America

In the United States, there are Standards for Quality of Ready-to-Cook Poultry (25), with three quality gradings: *A*, *B* and *C*. In the A Quality the carcass or part is free of deformities that detract from its appearance or that affect the normal distribution of flesh. Slight deformities may be present, with a well-developed layer of fat in the skin. The carcass or part shall have a clean appearance, especially

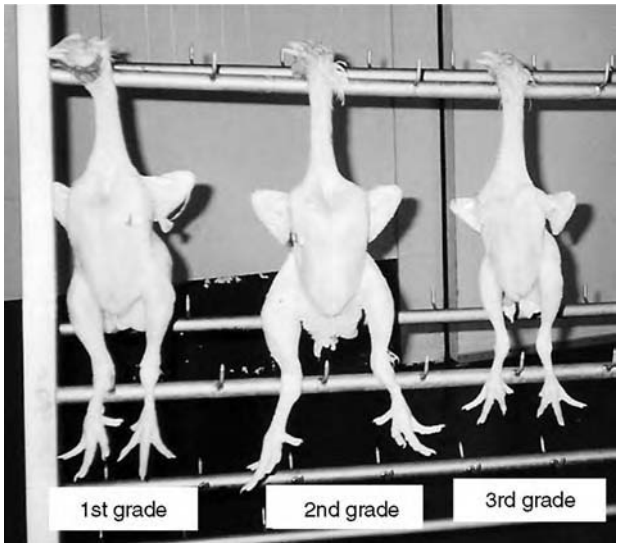

FIGURE 165.4 Poultry carcass grading or classification.

on the breast and legs. For all parts, trimming of the skin along the edge is allowed, provided that at least 75 percent of the normal skin cover associated with the part remains attached, and further provided that the remaining skin uniformly covers the outer surface in a manner that does not detract from the appearance of the parts, and is free of broken bones and free of disjointed bones. Discolorations that do not detract from the appearance of the product are allowed. Evidence of incomplete bleeding is not permitted. In the B Quality the carcass or part may have moderate deformities, such as a dented, curved, or crooked breast, crooked back or misshapen legs or wings, which do not materially affect the distribution of flesh or the appearance of the carcass or part. Any parts may be disjointed, but are free of broken bones, two disjointed bones, or one disjointed bone and no protruding broken bones. Discolorations are limited to moderately shaded areas and the carcass or part is free of serious defects. Evidence of incomplete bleeding shall be no more than slight. The skin and flesh shall have a sound appearance, but may lack brightness. The carcass or part may have a few pockmarks due to drying of the inner layer of skin. Carcasses that do not meet the requirements for A or B quality may be of C quality if the flesh is substantially intact. Both wings may be removed or neatly trimmed. Trimming of the breast and legs is permitted, but not to the extent that the normal meat yields is materially affected. The back may be trimmed in an area not wider than the base of the tail and extending from the tail to the area between the hip joints.

B. Canada

In Canada there are three grades of poultry carcasses: *Canada A*, *Utility* and *C* (26). The carcass of the grade Canada A is not deformed except for a slightly crooked keel bone that does not interfere with the normal placement of the meat. In the case of poultry, other than a turkey, the breast, thighs and back show evidence of fat cover. In the case of a turkey, the carcass has deposits of fat in the main feather tract on each side of the breast as indicated by a pronounced thickening at the center of each such area. The carcass has no prominent discolorations and no broken or dislocated bones; and does not have more than 3 cm of exposed flesh at the posterior end of the keel bone. Canada Utility carcass is not missing more than the wings, one leg including the thigh or both drumsticks, the tail, small areas of flesh, and skin not exceeding an area equivalent to one half of the area of the breast; where no flesh has been removed, the carcass breast has sufficient fullness of flesh on both sides of the keel bone to prevent a sharp falling away of flesh from the anterior to the posterior end and the keel bone does not project more than 3 mm beyond the flesh with a minimum fat cover to prevent the flesh from appearing prominently through the skin. The carcass has no dislocated bones other than in the wings or legs; and has no broken bones. In poultry carcass Canada C the wings or any part of them has been removed elsewhere than at a joint; and only a part of the drumsticks has been removed. Mature chicken may be graded as C if the carcass weighs more than 1.8 kg and the carcass breast has not sufficient fullness of flesh on both sides of the keel bone to prevent an extremely sharp falling away of flesh from the anterior to the posterior end, with prominent discolorations.

C. Mexico

In Mexico poultry carcasses are classified in three categories: *First* or *Mexico-Extra*, *Second* or *Mexico-1*, and Out of classification (27). The first category is defined by a well-developed musculature without deformations and a uniform fat distribution, besides no bruises or broken wings present, and an average weight of 1.950 kg including viscera and feet. The second are carcasses with a moderate musculature and enough fat to cover the muscle in breast, leg and thigh. This category allows two articulations separated in the wings, but with no severe damages. The minimum weight is 1.750 kg including viscera, head and feet. Finally, the carcasses out of classification are: a) those dead before slaughter; b) birds out of the Sanitary Legislation; c) birds with fracture, mutilations, hemorrhages, exposed flesh, out of the limits; d) pigmented with forbidden substances; e) present the incorporation of substances for weight gain; and f) pigmented birds by immersion. The approved and graded poultry carcasses can be commercialized in fresh or frozen. The main poultry in Mexico is consumed fresh.

Nonetheless, Mexican poultry grading for the international trades shall be similar to the described above. Poultry eligible for export under NAFTA rules would likely be processed in existing facilities and would represent a small percentage of Mexico's total production, besides U.S.-origin poultry carcasses and parts or products of poultry carcasses that are shipped to Mexico for processing and then returned to the United States (28).

D. European Community

The European Community (29) established regulations applied to the commercialization of poultry in the community territory, grading in two categories in agreement with carcass conformation and aspect. Poultry meat is included in Category *A* or *B* in agreement to carcass conformation and parts aspect. The category A is divided in *A1* and *A2*, taking in account the state of the carcass and fat cover, damage or contusions. Poultry carcass are commercialized in fresh, freeze or ultra freezing. To be classified into grade *A* or *B*, carcasses shall meet the following standards: carcass or part shall have to be intact, with a clean appearance,

without foreign material or blood. Some little protruding feathers and hairs are permitted on breast, thigh and back. Discrete discoloration or contusions are permitted as well. Wing tips can be missing. Fresh carcass shall have no evidence or previous freezing. Freezing and ultra freezing carcass have to be free of frozen burns, accepting little or discrete burns but not in breast or thighs. In the A category there are three classifications depending on the carcass weight: A or European chicken, no heads, no legs, ready to cook (1.0–1.1 kg); B or big chicken (1.8–2.2 kg) and C or small chicken (1 kg). A fourth grading of residue carcasses is not commercialized.

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166 Canned Poultry Products: Turkey Ham

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CONTENTS

I. BACKGROUND INFORMATION

A. Canning as a Preservation Food System

Canning is the technique of preserving food in airtight containers through the use of an extensive heat treatment that inactivates enzymes and kills microorganisms responsible for deterioration during storage. The airtight packaging protects the food from recontamination following sterilization, thus permitting storage at room temperature for many months without spoilage. In general canned meat products may be described as a convenience food, because they offer several advantages as they contain little or no additives, retain most of the nutritive value of raw materials, are ready-to-eat, shelf-stable, and easy to consume and handle [29].

This process is the basis of a large segment of the commercial food industry, a position that probably will continue despite the development of other means of preserving food. However, the food canning industry needs to renew itself continuously, in order to keep consumers' attention; especially nowadays, when market globalization politics

provide new opportunities for process and trade a great range of products from all over the world. Therefore, canners most offer safe and nutritive products; but also, use attractive containers, with opening features, heating instructions using microwave, several size serving portions, etc. In addition, the use of friendly environmental packaging materials that provide the same stability and safety of those conventional metal cans may be more attractive for consumers who demand healthy foods [23].

Developments in thermal processing involve new packaging materials as flexible retotable pouch and plastic containers. Addtionally, control systems for retorts are becoming more advanced, permitting real-time calculation of process lethality, and optimization of temperature and time required without excessive heating [8].

B. Poultry Meat

Poultry refers to any domesticated avian species, and poultry products can range from whole carcasses, cut-up carcasses, portions, boneless meat, or any further processed meat. Poultry production and consumption all over the world have increased considerably during the last years as it is shown in Table 166.1 with data obtained from U. S. Department of Foreign Agricultural Services [30]. Several variables influence buying decisions and eating patterns in short or long-term bases, including ethnic or religious traditions, diet and health concerns, as well as price and availability. Recently, consumer attitude about eating meat has been greatly influenced in relation to the saturated fat and cholesterol intake and their contribution to arteriosclerosis and heart attack. Additionally, recent outbreaks of Bovine spongiform encephalopathy (BSE) or foot-and-mouth disease have modified consumers' attitude against red meats [19].

Processed meats, including canning products now contain less fat (under 25% or lower), where poultry, especially chicken and turkey are popular as an alternative to manufacture healthy low fat meat products. U.S. trade projections (Figure 166.1) show an increasing demand for fresh poultry and processed poultry products, over other meat species such as pork or beef. This may be explained as poultry meat posseses a "plain" flavor easy to adapt to most recipes and it is an excellent choice to develop low-calorie products with the advantage of being a low cost protein source. Although, the presence of Avian Influenza and Sudden Acute Respiratory Syndrome (SARS) outbreaks in 2003 joined with stricter quarantine and sanitary requirements with a zero tolerance for salmonella and *E. coli* have had a stagnant effect on the poultry trade market all over the world.

All meat and meat products, including poultry must be subjected to inspection and declared suitable for human consumption; most countries have national laws and regulate inspections with specific requirements for conditions in which animals are reared, transported, slaughtered, and how products are prepared, distributed and sold. In the United States, poultry and poultry products are subject to the Poultry Products Inspection Act, which is enforced by the FDA Food Safety and Inspection Service. In Mexico, SAGARPA (Secretary of agriculture and rural development) dictates all regulations for food processing and distribution [30].

TABLE 166.1
Poultry Meat Production 2000–2004

Country	2000	2001	2002	2003 (p)	2004 (f)
	(Thousand metric tons; ready to cook or equivalent)				
Angola	8	8	8	nd	nd
Argentina	870	870	640	670	780
Brazil	5,980	6,567	7,449	7,560	7,825
Canada	877	927	932	915	930
China	9,269	9,278	9,558	9,844	10,129
European Union	8,394	8,599	8,605	8,425	8,410
Hong Kong	65	59	61	60	58
Japan	1,091	1,074	1,097	1,120	1,110
Korea	394	413	437	425	434
Mexico	1,948	2,080	2,201	2,297	2,412
Russia	387	430	500	580	640
Saudi Arabia	390	424	510	520	530
South Africa	707	730	760	790	805
Thailand	1,070	1,230	1,205	1,290	1360
United Arab Emirates	25	28	31	33	35
United States	16,122	16,523	17,052	17,349	17,834
Yemen	67	78	78	nd	nd

Source: United States Department of Foreign Agricultural Services (February, 2004). (p = preliminary data, f = forecasted, nd = no available data.)

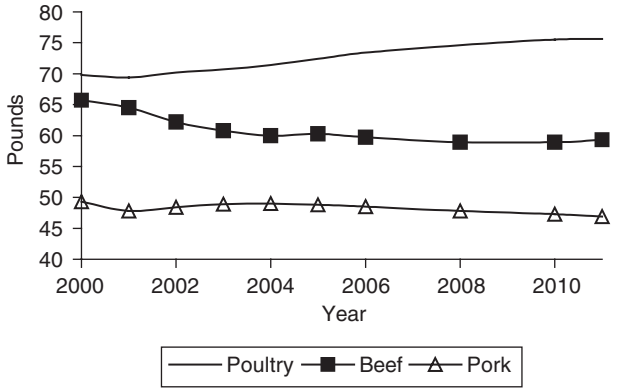

FIGURE 166.1 U.S. per capita boneless meat consumption projection (USDA National Agricultural Statistical Service, Jan. 2003).

C. Types of Commercial Canned Poultry Products

Canned poultry products include a wide variety of products such as reformed and emulsion type products, or puree and soups formulated with chicken and turkey cubes. But most of them claim to be low fat products. Examples are cured breast of turkey, ham turkey, chunky chicken, chicken & vegetables, Vienna sausages, chicken soup, and chilorio (Mexican spiced poultry) that are shown in Figure 166.2.

1. Formed Products

Formed poultry products are boneless and uniform in composition; examples are hams, loaf and restructured products. They may be produced from sectioned muscle pieces or from grounded or chopped meat and shaped into a specific portion and size. Texture varies according to the initial material; for example, hams are primarily produced from intact muscles and have more a "whole-muscle" texture, while restructured products have a smaller particle size as they are produced from grounded or chopped meat.

Formed products are prepared from defatted whole muscle pieces bounded together after marinating, tumbling and cooking. Proteins form a network between meat pieces during heating to form a continuous body. Non-meat binders can be used to enhance cohesivity between meat pieces to obtain a whole meat-like texture; such as soy protein, casein or hydrocolloids, among others [26].

FIGURE 166.2 Variety of canned poultry products (includes sausages, soups, ham, pâté, chilorio, etc.).

2. Emulsified Products

Emulsified or comminuted poultry products include frankfurters, bologna or loaf items, and are usually prepared from chilled or frozen mechanically deboned poultry or turkey. Meat is homogenized in a cutter bowl with iced water, salt, cure, alkaline phosphates, starch, sodium erythorbate, milk or soy proteins, starch, gums and spices; to an end temperature of 15°C to avoid melting of fat that might result in fat caps or fatting-out after heating. Batter is then vacuum encased and cooked and after peeling, sausages are canned [26].

D. Composition and Physicochemical Properties of Meat

The process of canning begins with the selection of high-quality raw materials, where skeletal muscle is the main constituent. Turkey meat contains 75% moisture, 23% protein, 1.2% lipids and 1% minerals. Table 166.2 shows the principal skeletal muscle proteins, which are classified according to their solubility and location as: sarcoplasmic, myofibrillar and stroma fractions [17]. The myofibrillar or salt soluble fraction comprises more than 20 distinct proteins and represents about 60% of the total poultry

TABLE 166.2
Main Poultry Skeletal Muscle Proteins [17]

Protein Fractions	Content (% of total protein)
Myofibrillar (Salt Soluble Proteins)	
Myosin	29
Actin	13
Tropomyosin	3.2
Troponins C, I, T	3.2
Actinins	2.6
Desmin	2.1
Conectin	3.7
Sarcoplasmic (Water Soluble Proteins)	
Myoglobin and other heme-proteins	1.1
Glycolytic enzymes	12
Mitochondrial enzymes	5
Lysosomal enzymes	3.3
Stroma (Insoluble Proteins)	
Collagen	5.2
Elastin	0.3
Reticulin	0.5

muscle protein. Myofibrillar proteins can be divided into three groups based on their function as: contractile (responsible of muscle contraction), regulatory (involved in regulation of contraction) and cytoskeletal proteins (responsible of myofibril integrity). The contractile proteins, myosin and actin have a large influence in muscle functionality; these proteins are usually forming the actomyosin complex in *post-rigor*, and contribute to functionality for comminuted and formed processed poultry products. The ratio of actin to myosin, as well as the ratio of free myosin to actomyosin, also influences the functional properties of poultry meat [17,23].

Sarcoplasmic proteins play a minor role in meat protein functionality, although myoglobin and other water-soluble compounds may have a great influence in color. Myoglobin consists of a heme group bound to the histidyl (His^{93}) residue of a single polypeptide chain as shown in Figure 166.3 [4]. The amount of myoglobin varies within species, age, and muscle fiber distribution; for instance, dark muscles in turkey thigh are mainly comprised of red fibers which containing more myoglobin than light breast muscles. White turkey meat contains from 0.1 to 0.4 mg of myoglobin/g, whereas dark meat ranges 0.6 to 2 mg/g. Moreover, mechanically deboned turkey (MDT) contains some bone marrow and will have higher pigment levels than manually deboned meat [12,26].

Stroma proteins are related to meat tenderness; collagen is the major stroma protein present in flesh and in poultry skin. When present in a high concentration in the

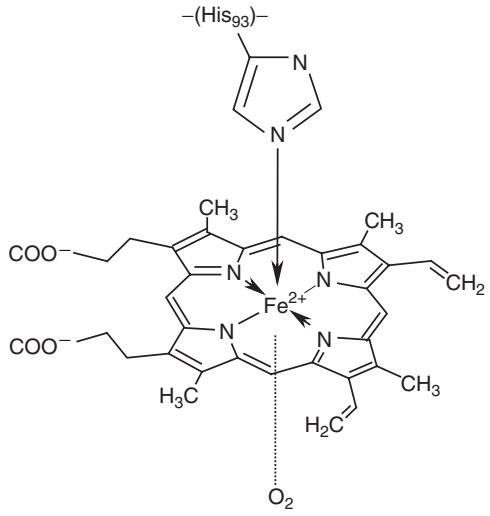

FIGURE 166.3 Myoglobin heme group bound to a histidyl (His_{93}) residue of the peptide chain and oxygen. (Adapted from Belitz and Grosch, 1999.)

poultry product formulation, collagen shrinkage may reduce functionality of myofibrillar proteins, reducing binding between meat pieces in formed products, and may reduce fat and water retention in comminuted products, especially when cooked at high temperatures [17,26].

Meat functionality is based on the physicochemical properties of proteins and determines its behavior during processing, storage and consumption. Poultry meat

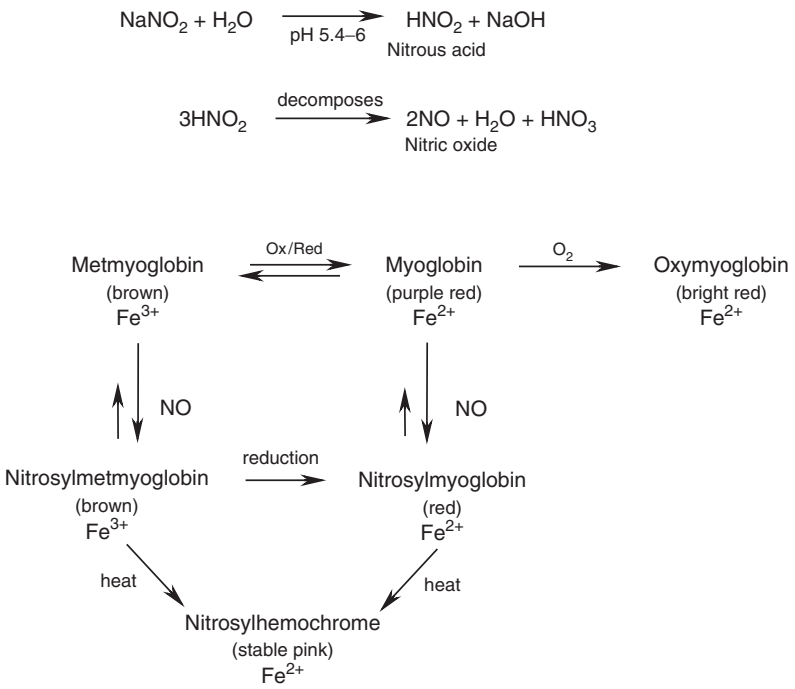

FIGURE 166.4 Reaction pathway leading to the formation of nitric oxide (NO) and nitrosylhemochrome pigment. (Adapted from Pearson and Gillett, 1999, and Smith and Acton, 2001.)

functionality is based on three types of molecular interactions as: protein hydration and water holding capacity (WHC), depending on protein-water interactions; while cohesivity and gelation are based on protein-protein interactions, and emulsifying is based on protein surface related properties. All these interactions are affected by intrinsic and extrinsic factors such as the type of protein, distribution of hydrophobic and hydrophilic groups on the protein surface, charge, and molecular flexibility. Extrinsic factors include pH value, salt concentration, phosphate salts, temperature, and integrity of meat, processing and addition of other non-meat additives [17].

The amount of total myofibrillar protein, the ratio of moisture to total protein, and the physicochemical condition (PSE or DFD) determine the functional properties of raw materials. Lean poultry meat contains 19 to 23% protein, while mechanically deboned poultry without skin has 14 to 16% protein and with skin 11 to 12% protein. Therefore, both poultry product formulation and processing must be designed in order to improve protein functionality and the final product quality [10].

1. Water Holding Capacity

Water holding capacity is the ability of meat to retain or absorb added water in the presence of an external force; this functional property is based on protein-water interactions. Water is held by muscle proteins, and physically entrapped within the muscle structure in the inter-filament spaces of myofibrils. Factors such as pH, salt concentration, process, and temperature influence the protein-water binding and the quality of the poultry meat product network [10,17].

Protein-water interactions are highly related to the state post-mortem. At the isoelectric point (pH ~ 5.1), myofibrillar proteins have no net charge and tend to aggregate. However, as the pH increases during resolution of rigor mortis, proteins become more negatively charged, with an increase in repulsive forces between myofibrils that leads to swelling, allowing more water to interact with proteins, and therefore, protein solubility and water holding capacity increase as proteins become more negatively charged.

Addition of salt up to 0.6 M (2–3.5%) NaCl reduces electrostatic interactions between proteins increasing protein extractability, solubility and water binding in both breast and tight muscle. In addition, alkaline phosphates in combination with salt and mechanical work increase pH and myofibrillar protein extraction and solubilization. Additionally, chopping or tumbling disrupts the muscle allowing the muscle fibers to absorb water and swell. However over-chopping or tumbling can lead to an excessive disintegration of muscle fibers and can induce protein denaturation, as it is also associated with an increase in temperature and excessive shearing. Denatured proteins as in PSE muscle form aggregates that have low water affinity and reduced emulsification and foaming abilities [17,23].

2. Cohesivity

During cooking muscle proteins denature and form a continuous cross-linked gel network, stabilized by a series of protein–protein interactions, such as electrostatic and hydrophobic interactions, hydrogen and disulfide bonds. Muscle protein gelation involves a series of steps. First, when muscle proteins are heated up to a critical temperature they unfold; in a second step, unfolded molecules aggregate to form an increasing viscous solution, then when the gelling point is reached, molecules aggregate into a continuous gel. Myofibrillar proteins form irreversible strong gels that are responsible for the textural and sensory properties, as well as the cooking yields of poultry products. Nevertheless, connective and sarcoplasmic proteins may interfere with the ability of myofibrillar proteins to form a gel [10,14].

3. Emulsifying

Poultry comminuted products such as sausages may be referred to as emulsions, as the fat tissue is comminuted and dispersed in small particles into a continuous salt/protein/water matrix. The stability of this system is influenced by pH value, ionic strength, melting range of the lipid, soluble protein content, and by temperature of processing.

Soluble and extracted meat proteins form a monomolecular film around the fat globules, proteins rearrange where polar regions are oriented towards the water phase, while non-polar regions align towards the fat droplets to minimize free energy [15,26].

Meat proteins show different emulsifying responses that decrease in the following order: myosin > actomyosin > sarcoplasmic proteins > actin. The hydrophobic heads of the myosin dip into the fat globules, while the tails interact with actomyosin in the continuous phase. Actomyosin binds water and contributes to stabilization of emulsion because of its viscous, elastic and cohesive properties. Comminuting is necessary to extract proteins, disrupt fat and to form an emulsion; also, concentration of protein must be sufficient to form continuous and stable film around the fat globules. During the emulsification stage, poultry batter temperature and chopping times should be monitored to avoid melting of the fat globules. Stable emulsions require at least 45% myofibrillar protein in the formulation with a maximum of 30% sarcoplasmic and connective proteins should be limited to less than 25% of total protein [15].

II. RAW MATERIALS PREPARATION

Canned turkey ham is a boneless-formed product, made from cured meat pieces bound together into a specific shape in a sealed container, and heat processed. This

TABLE 166.3
Basic Curing Brine Formula [23]

Component	%
Salt	2
Phosphate	0.5
Sucrose	0.5
Carrageenan	0.3
Sodium nitrite	156 ppm
Sodium erythorbate	450 ppm

product retains most of the nutritional value of raw materials, is ready-to-eat, shelf-stable, and convenient for consumers [15]. Process for the manufacture of canned turkey ham involves several stages such as meat conditioning, brine injection, vacuum tumbling, can filling, exhaustion, closing and sterilization [23].

Turkey ham may be prepared from boneless breast, legs, thighs, desinewed drumsticks, and MDT with or without skin. These raw materials may be chilled or frozen but without off-color, off-odor, or apparent microbial growth. The internal temperature of fresh cuts should not be above 4.4°C, while frozen materials should be below −18°C when received. Frozen cuts must be kept packaged during thawing to prevent dehydration, and to avoid the risk of microbial contamination until reaching −3.3 to −2.2°C. Turkey meat can be sliced, cubed or ground, according to the desired final texture, but up to 33% of the meat may be finely comminuted to provide good binding that resembles a whole "meat-like" texture and good water retention. Nevertheless, temperature must be kept below 10°C during these operations to avoid the risk of microbial growth [15,26,27].

III. CURING AND BRINE INJECTION

Meat is usually cured by injection of brine under pressure using a multineedle system, which facilitates and accelerates incorporation of the curing solution (see Table 166.3) [21]. Salt improves flavor, and in conjunction with phosphates extracts myofibrillar proteins producing a sticky surface that will bind meat chunks during thermal processing. Sodium chloride increases protein negative charge as well as protein repulsion, allowing more water to bind within the muscle fibers. On the other hand, alkaline phosphates increase pH and ionic strength allowing protein to uncoil exposing hydrophilic sites; therefore phosphates act in a synergistic way with sodium chloride to increase WHC and protein extraction [9,15,27].

Sodium nitrite is a multifunctional ingredient. It prevents the outgrowth of the anaerobic spore former *Clostridium botulinum*, which grows under anaerobic conditions as those created during canning. Some spores can survive normal heat processes and generate vegetative cells that produce lethal toxins [9,31]. The antibacterial properties of nitrite are based of the reaction of nitric oxide with SH groups to form nitroso thiols (R-S-NO), and by depriving anaerobic spore formers from available iron compounds with a key role in biochemical mechanisms [24]. Nitrite is also responsible for the development of the distinctive color and flavor of cured processed meats. Nitric oxide derived from sodium nitrite reacts with the heme iron of myoglobin and metmyoglobin to form nitrosylmyoglobin and the heat-stable pink nitrosylhemochrome after cooking [27] (see Figures 166.3 and 166.4). Although, color intensity depends on the myoglobin content of the raw material [4,11,21]. Nitrite also contributes to the flavor stability preventing warmed-over-flavors by complexing the heme iron, which could promote lipid oxidation reactions [31]. It has been reported that the non-heme iron is a potent prooxidant and it is released during heat processing as a result of porphyring breakdown, thus heating accelerates the release of iron from the heme complex [4]. Legal limits of initial nitrite levels are 200 ppm and 156 ppm for pasteurized and sterile canned hams, respectively; with residual levels of 100 and 120 ppm[6]. Finally, the addition of reducing agents such as sodium erythorbate accelerates curing, promotes formation of nitrosylhemochrome, and contributes to the flavor and color stability [15,21,31] (Figure 166.5).

IV. VACUUM TUMBLING

Injection followed by non-continuous tumbling cycles maximizes the quality of the product, as it permits a uniform distribution and absorption of curing ingredients, and extraction of salt-soluble proteins [15,16,21]. A vacuum tumbler consists of a large rotating tank with paddles

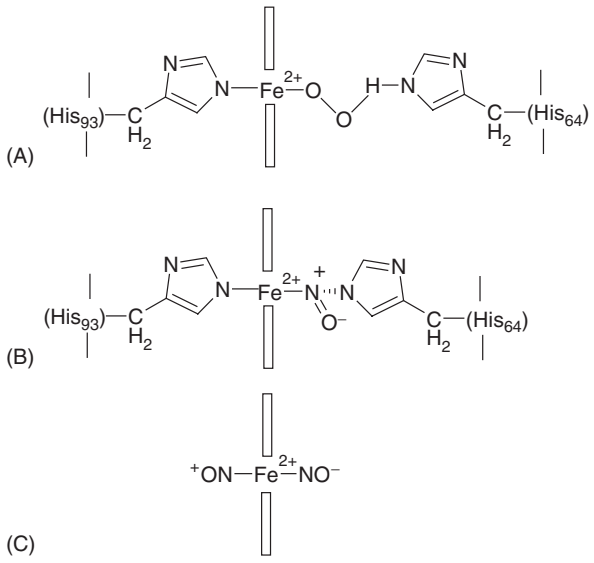

FIGURE 166.5 Heme ligands of (A) oxi-myoglobin, (B) Nitrosylmyoglobin and (C) Nitrosylhemocrome. (Adapted from Larousse and Brown 1997.)

inside, and jacketed walls to cool the product while tumbling. The temperature should be kept between 4 and 8°C, and the rotation rate between 3 and 15 rpm; a higher speed can cause cell breakdown and temperature increase thus reducing the quality of the final product. Vacuum tumbling has the advantage of speeding up the brine uptake, and avoiding formation of air bubbles within the product [9,16,21].

V. CONTAINER FILLING

Containers should be clean and washed before filling, the cured meat mix is then transferred into appropriate containers using an automatic vacuum filler as well as pressed to ensure elimination of air pockets [13,16,29]. Selection of the appropriate container is vital to maintain stability and organoleptic properties and avoid damage during transportation, storage and distribution. Packaging materials include metal, glass, and laminated containers. Semi-rigid containers made of nylon, surlyn, and other ethylene/vinilacetate copolymers are used for cook-in products that receive a pasteurizing treatments [29].

A. Metal Cans

Containers for poultry canning can be made in a variety of forms that include flat rectangles, tall and thin, squat, pot, pot-bellied, wasp-waist, parallelepiped, twisted, embossed, etc. Bodies may be fabricated from steel or aluminium with protunding or flush end seams, preprinted or bright. These materials are cheap and provide excellent barrier properties against gases, water vapor, light and odors. Additionally, they can be used in on-line filing processes, have high mechanical resistance and excellent thermal conductivity, are suitable for sterile products, and can be hermetically sealed and recycled. Metal cans have some disadvantages, for instance, shipping empty cans takes up a lot of space. Also during storage cans must be protected from moisture and/or humidity (if not lacquered or coated), and cans may be considered old fashioned by the modern consumer because they are not suitable for use in the microwave oven [6,29].

Metal containers usually have an internal and sometimes external enamel coating to avoid corrosion during storage, and prevent interaction of the can with the food product; while the external coating provide both decorative and protective function. Coating materials include natural oleoresins or synthetic products, such as epoxy, phenolic, acrylic and polyester lacquers and coatings. Synthetic resins have better performance and are available in a wide range of materials and specially designed for use in different foods. In addition, a release agent is applied to facilitate product removal after opening [21,29]. Coatings should impart no odor or flavor to the food, must be non-toxic and protect the can and contents during the required shelf-life; must not flake of the plate during can manufacture or storage, be easy to apply and quickly cured, and resist all temperatures encountered during processing and storage [29].

1. Fabrication

Cans are fabricated into the three-piece can or into the two piece can. The three-piece can is composed of a body and two ends. The body is usually cylindrical (but can be rectangular, pear-shaped and oblong) and after it is formed, the two edges are brought together and sealed. The ends are made of tinplate, one end is applied by the can manufacturer, and called the manufacturer's end, whereas the other is prepared by the canner and called the canner's end. Round and no round two-piece containers are made from a precut aluminum alloy or steel sheet by stamping out in a cupping press, or by combination of stamping and deep drawing. Ends or covers are made from aluminum alloy, tinplate or tin-free steel (TFS), coated on both sides [29].

Application of ends is critical, as the end provides mechanical resistance to support the internal pressure differentials during thermal processing and cooling. It is also a barrier against all type of contamination and ensures food safety throughout storage. Sealing forms a double seam into three operations. First, the can body and the cover are brought together and clamped on a seaming chuck by a load applied vertically to the base plate; the end curl is tucked under the can flange and interlocks it. In a second operation, the interlocked layers of metal are compressed and the seaming compound is squeezed into voids to complete the hermetical seal [29].

B. Glass Jars

Glass jars are used less often for meat products because of their fragility. They consist of a glass body, and a metal lid. In households, glass jars with glass lids are often used. The seaming panel of the metal lid has a lining of synthetic material. Glass lids are fitted by means of a rubber ring.

C. Retort Pouches

The retort pouches are flexible lightweight and easily disposable laminated containers for preserving foods. Heat-resistant plastic pouches, which are closed by clip, are usually made of polyester (PETP) and used ready-to-eat dishes. Laminated films made of polyester/polyethylene (PETP/PE) or polyamide/polyethylene (PA/PE) are relatively rigid containers, which are used for filling with pieces of cured ham or other kinds of prepared meat. While, round containers formed out of a laminate of aluminium foil and polyethylene (PE) or polypropylene (PP) are widely used for small portions, as PE or PP permit the

heat-sealing of these containers, which can then even be subjected to intensive heat treatment. Retort pouches offer some advantages over other typical food containers used in canning, because they are easy to handle and the thermal process is faster and therefore produces less flavor and texture changes. Also the consumer can easily heat the pouch in boiling water before eating and save shelf space [6,8,18].

VI. EXHAUSTION AND CLOSING

Once containers have been properly filled and pressed, they are sealed under mechanical vacuum, or by using steam to create a vacuum while the product is cooled. Air evacuation from the headspace as well as from the bulk of the turkey ham is necessary to achieve good heat penetration; also to minimize alterations in color, flavor and texture during processing and storage. Afterwards, the exterior of the container should be cleaned and closure must be checked prior to sterilization [16,21,29].

VII. STERILIZATION

Canned foods are preserved by application of a heat treatment, which inactivates enzymes, pathogens, and microorganisms that cause deterioration during storage. Turkey canned ham is considered as a commercially sterile food, because the sterilization step ensures that the product is free from viable microorganisms capable of reproducing in the food under normal no refrigerated condition of storage and commercialization. Sealed cans protect the product from recontamination after sterilization, allowing storage at room temperature for several months without spoilage [21]. Canning includes two steps; first, the product is heated in a retort to high temperature for enough time to destroy spoilage and pathogenic microorganisms, followed by a rapid cooling to room temperature, where additional microbe destruction is achieved [9,31]. Most non-spore-forming organisms are heat labile; but some spores can survive even after heating to 120°C; however, addition of salt and nitrite decreases the thermoresistance of microorganisms [9].

A. Thermal Destruction of Microorganisms

Thermal destruction of bacteria is expressed in terms of its exposure to a specific temperature for a period of time, at higher temperatures, shorter periods of time are required to get the same destruction. However, the amount of microbial destruction within the food matrix depends on several factors such as the number and kind of microorganisms and the growth conditions of the microorganisms of concern. As well as, composition, viscosity, moisture, and pH of the food, presence of preservatives; as well as can size and shape, among others. Thus, successful sterilization requires knowledge of the rate of heat penetration at the coolest point, since heating is not homogeneous throughout the entire can due to its geometry [24]. For example, food in small container is heated more rapidly than in a large container; also the center of a solid product or near the end in a liquid caned food may be the coldest point within the food matrix. There are several mathematical relationships that describe the thermal destruction of microorganisms, and the rate at which a food is heated, and the temperature of the coldest point. The number of microorganisms destroyed by the thermal process can be estimated by incorporating the destruction rate of the microorganism of concern into the heat transfer model for a food system. However, not all food systems are easily modeled. Therefore, actual time-temperature measurements can be used to establish the amount of microbial destruction during the process. In addition, microbial destruction can also be measured by inoculation of an indicator organism and then measuring the remaining population after the thermal process [2].

The rate of inactivation of microorganisms increases in a logarithmic rate with increasing temperature and tends to follow a first order rate reaction. The D-value is the time in minutes during which the number of a specific microbial population exposed to a specific temperature is reduced by 90% or 1 log. It is expressed as D_T = t min, where T is the temperature and t is the time for 1 log reduction of the microbial population. In addition the F-value represents the amount of time in minutes required to completely destroy a given number of microorganisms at a reference temperature (121.1°C for spores and 60°C for cells) [24].

Several authors [2,16,21] indicate that changes in microbial populations as a function of time can be described by the following equation (166.1); while equation (166.2) describes a first–order kinetic model, if k is the slope of the natural logarithm of survivors at any time for the microbial population, then equation (166.2) can be integrated into equation (166.3) to describe the reduction of microbial populations:

$$\log [N/No] = -t/D_T \quad (166.1)$$

where:
N = microbial population at any time, t
No = initial microbial population
D_T = decimal reduction time required for a 1−log cycle reduction in the microbial population.

$$dN/dt = -kN \quad (166.2)$$

$$\ln [N/No] = -kt \quad (166.3)$$

Consequently, the decimal D-values and the constant k can be correlated by equation (166.4):

$$k = 2.303/D_T \quad (166.4)$$

TABLE 166.4
Thermal Resistant Parameters for Some Microorganisms Having Public Health Significance [2]

Microorganism	D (min)	k (1/min)	Z (°C)	Temperature (°C)
S. typhimurium	2.13–2.67	0.86–1.08		57
E. coli O157:H7	4.1–6.4	0.36–0.56		57.2
E. coli O157:H8	0.26–0.47	4.9–8.86	5.3	62.8
C. jejuni	0.62–2.25	1.0–3.72		55–56
L. monocytogenes	1.6–16.7	0.14–1.44		60
S. aureus	2.5	0.921		60
Bac. cereus	1.5–36.2	0.064–1.535	6.7–10.1	95
Clo. perfringens	6.6	0.349		104.4
Clo. botulinum B	1.19–2.0	1.152–1.935	7.7–11.3	110
Clo. botulinum E	6.8–13	0.177–0.339	9.78	74
Clo. botulinum 62A	1.79	1.287	8.5	110
Bac. subtilis	32.8	0.0702	8.74	88

D = decimal reduction time, k = rate constant, Z = thermal resistant constant.

Both parameters k and D-values describe the microbial population reduction only at a constant and specific temperature. In order to measure the influence of temperature, the thermal resistant constant (Z-value) must be incorporated in the thermal death time curve (log F vs. T) or thermal resistance curve (log D vs. T) as shows equation (166.5) and (166.6), respectively:

$$\log [D/D_R] = -(T - T_R) / Z \qquad (166.5)$$

$$\log [F/F_R] = -(T - T_R) / Z \qquad (166.6)$$

The thermal death time (TDT) involves graphical integration of time-temperature data at the coolest heating point during thermal processing, and measures the microbial death rate in relation to temperature. Thus the Z-values indicate the number of degrees the temperature must be increased to decrease the microbial population by one log cycle, and measure the microbial heat resistance; Table 166.4 shows the thermal kinetic parameters for some microorganisms having public health significance, where it can be seen that spore-forming microorganisms have the largest D and Z-values.

The rate of inactivation of microorganisms increases in a logarithmic rate with increasing temperature. Thus D and F values will decrease logarithmically with increase in temperature. Z is the change in temperature that accompanies a 10-fold change in the time for inactivation. The z value is calculated by plotting log (D) against temperature and increasing the temperature of a thermal process by the z value results in a 10-fold reduction in the time required to obtain the lethality of the original process. Conversely reducing the process temperature by the z value necessitates a 10-fold increase in the processing time to achieve the original lethality [2].

Commercial sterilization ensures destruction of microorganisms growing in the product under normal storage conditions. The low acid or high pH foods require a severe heat treatment to guarantee microbial destruction to an F value ≥ 3 [9,21]. The heat treatment for this type of food is based on the total destruction of *Clostridium botulinum* type A or B spores (the most heat resistant spores of a pathogen) by applying the 12-D concept or "botulinum cook." This refers to the heat process necessary to reduce the number of surviving spores of *Cl. botulinum* from 10^{12} to 10^{0}, i.e., to reduce the number of surviving spores by 12 log cycles. Canned turkey ham falls in the category of low-acid foods as it has a pH higher than 4.8. The reference temperature for canned low-acid foods for measuring the destruction of *Cl. botulinum* is 121°C; at this temperature the destruction time for 12 log cycles is designated as F_0. The high acid or low pH-food (pH $\leq$ 4.6) requires lower heat treatments, since *Clo. botulinum* cannot germinate and outgrow at this low pH [5,9,13,16,26].

Heat penetration tests are performed to determine appropiate heating parameters to design thermal processes. Determinations of the coldest point known as point of lowest lethality within the container and runs to measure temperature in the coolest spot at various time intervals allow the establisment of a thermal process. An accepted practice for heat penetration testing is to use an exaggeration or worst case approach by using data collected from the slowest heating container with the largest heating rate index (f_h-value) and the corresponding lag factor (j_h-value) or to conduct the test on samples that reflect the most adverse operating conditions; however, data varies with the number of test containers and test runs. Some authors have suggested the use of statistical approaches to reduce variations in heating parameters when designing processes, using an univariate and a

TABLE 166.5
Processing Steps and Application Principles of Canned Poultry Ham

Stages of Processing		Application and Principles
1	Raw meat deboning and conditioning	Texture Water holding capacity Color Lipid oxidation Particle size
2	Curing and brine injection	Water holding capacity Color Lipid oxidation Flavor
3	Vacuum tumbling	Water holding capacity Protein solubility Color Lipid oxidation
4	Can filling	Particle size
5	Exhaustion and closing	Heat transfer
6	Sterilization	Protein binding Maillard browning Lipid oxidation Water holding capacity Flavor Color Heat transfer Microbial destruction by heating

bivariate statistical method that also enable to estimate the minimum number of heat penetration measurements to give reliable values to calculate safe thermal processes [28].

B. Texture and Flavor Changes during the Thermal Process

During cooking the extracted muscle proteins denature and form a continuous cross-linked network, which is stabilized by electrostatic and hydrophobic interactions, hydrogen and disulfide bonds, giving rise to the characteristic product texture. Myofibrillar proteins are mainly responsible for meat binding and textural properties, as well as the product yield [21,26]. Nevertheless, stroma and sarcoplasmic proteins may interfere. In particular, collagen, a major stroma protein present in flesh and skin, diminishes WHC and binding when it is present at high concentrations due to shrinkage and conversion into gelatin during cooking [9,26,27].

Heating also promotes changes in flavor, since uncooked meat has little aroma and blood-like taste. During cooking, lipids and water soluble non-volatile precursors and/or their breakdown products generate a large number of intermediates and volatiles that contribute to flavor [7,20]. The main reactions that take place during heating are the Maillard reaction, thermal degradation of lipids, and degradation of thiamine, that lead to a complex mixture of chemicals, such as aldehydes, ketones and sulfur compounds that provide the meaty, toasted, roasted, fatty, fruity and sulfurous meat aroma [1,3,25].

VIII. FINISHED PRODUCT

Canned poultry products can suffer several alterations due to chemical, enzymic and microbial activity which diminish the quality. Production of H_2, CO_2, browning and corrosion of cans are due to chemical reactions; while liquefaction and discoloration are due to enzymic reactions. Improper processing and handling, as well as storage at elevated temperatures are the main factors associated to spoilage of canned products. Alteration can take place before heat treatment due to microbial growth, chemical reactions with the container and physical alterations when there is a delay prior to heat processing [26]. Therefore, it is recommended that heat processing must be applied within 20 minutes of can closure. Spoilage is usually associated with defects and mechanical damage as improper pressure control during retorting and cooling operations may stress the seam, resulting in poor seam integrity and subsequent spoilage. Therefore, quality control of the finished product involves: pH determination of the product, gas analysis of can headspace, microbiological testing, and complete external can examination for any leakage, pinholes, dents, buckling, and general exterior conditions [18].

A. Microbial Spoilage

Depending upon the thermal treatment, microbial cells and spores can be sublethally injured or dead. The sublethally injured cells and spores are capable of repair and can multiply [24]. Canned products can have spores of thermophilic bacteria organisms (such as *Bacillus stearothermophilus*, *Bac. coagulans*, *Clo. thermosaccharolyticum*), but if stored at 30°C or below, the spores do not germinate to cause spoilage; but if the cans are stored under temperature-abused conditions to 40°C or higher, the spores germinate, multiply and spoil the product. Microbial spoilage is generally due to germination and growth of thermophilic spore-forming bacteria, because of inadequate cooling after heating or high storage temperature. On the other hand, growth of survival mesophilic microorganisms is associated with an inappropriate heat treatment and microbial contamination from outside [24].

Insufficient thermal treatment or insufficient cooling allows the survival of thermophilic spores that can germinate when cans are temperature abused at 40°C even for a short period; once germinated, some can outgrow and multiply at temperatures as low as 30°C and generate acid with or without gas [13,16]. Germination and growth of the facultative anaerobic *Bac. stearothermophilus* is accompanied by acid without gas due to fermentation of carbohydrates;

while the growth of the anaerobic *Clo. thermosaccharolyticum* produces H_2 and CO_2 gas and swelling of cans. The sulfide stinker spoilage is caused by the Gram-negative anaerobic sporeformer *Desulfotomaculum nigrificans* which is characterized by flat container but darkened product with the odor of rotten eggs due to production of H_2S from the sulfur containing amino acids that dissolve in the liquid and reacts with iron to form the black color iron sulfide. Spoilage can be either from the breakdown of carbohydrates or proteins. *Clostridium spp*., *Clo. butyricum* and *Clo. pasteurianum*, ferment carbohydrates to produce volatile acids and H_2 and CO_2 gas causing swelling of cans. Proteolytic microorganisms such as *Clo. sporogenes*, *Clo. Putrefaciens* or *Clo. botulinum* metabolize proteins and produce H_2S, mercaptans, indol, skatole, ammonia, CO_2 and H_2 [24].

Proper sterilization process ensures the destruction of bacteria; however gas and other microbial metabolites can remain in the can. Additional microbial contamination can take place after thermal processing due to improper sealing, or in damaged and leaky containers or because the use of poor sanitary quality water during the cooling-down stage. Those conditions will allow different types of microorganism to get inside from the environment after heating. They can grow in the food and cause different types of spoilage depending upon the microbial types, care must be taken because contamination with pathogens will make the product unsafe. Therefore, cans that undergo abnormally high pressure or excessive filling can suffer physical deformation, and must be discarded to avoid the risk of leak [13].

B. Corrosion

Corrosion of metal containers can be both internal and external, it can be initiated during the can manufacture or at any point during processing. External corrosion is evident by formation of a reddish-brown ferric oxide, and may be induced by corrosive water conditions or by poor conditions during storage or shipment. Internal corrosion is not visible until it produces leakages or swelling of the container. This phenomenon is associated with elevated oxygen levels in the headspace and aggressive foods. Bubbles or loose flaps in the internal coating may occur followed by corrosion at the point of detachment, if the base metal is exposed, then the corrosion will be more extensive. The presence on nitrites also may induce internal corrosion [16].

IX. APPLICATION OF PROCESSING PRINCIPLES

See Table 166.4.

ACKNOWLEDGMENT

The information in this chapter has been derived from a chapter in: *Food Chemistry Workbook*, edited by J. S. Smith & G. L. Christen, published and copyrighted by Science Technology System, West Sacramento, California, 2002©. Used with permission.

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167 Processed Poultry Products: Nugget and Pâté

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CONTENTS

I. POULTRY NUGGETS

A. Background Information

Poultry nuggets are restructured meat products made from poultry meat or mechanical recovered meat (MRM) where the frying process will reduce product humidity and develop color and texture, but with a considerable increase in fat content. Nuggets are usually small (bite-size) and are covered or breaded with a mixture of flour and spices to give characteristic flavors, color, and a crispy texture. Nuggets are preformed with a precooking step and then fried. Frying is considered one of the oldest cooking methods in existence, especially in countries where oil plays an important role (1). The kind of oil used depends on cultural traditions and the kind of crops in the region, such as olives in Mediterranean countries, corn or soy oil in the United States,

and peanut or canola oil in Asian countries. The immersion frying process is also called deep-fat drying (2). During frying many chemical reactions occur from heat and mass transfer. Breading nuggets with batter improves texture due to starch gelatinization and results in a unique color from Maillard reaction compounds formed during the crust layer formation. Figure 167.1 shows the general flow chart and Table 167.1 describes the principal steps of the process.

B. Raw Materials Preparation

Poultry meat is chopped to liberate myofibrillar proteins that will act as "glue" to bind meat pieces. The addition of a binder enhances and improves nugget texture, and breading is the most common practice. Binders can also be another protein, such as egg white. A great variety of ingredients can be added for a specific or ethnic flavor. Oil temperature must be hot enough to maintain a constant heat transfer during the frying process. Temperatures around 130–150°C are recommended. The cleanliness of oil is important because impurities may accumulate on parts of the nugget or its cover. In addition, air and water escaping from the food during frying can cause many chemical alterations.

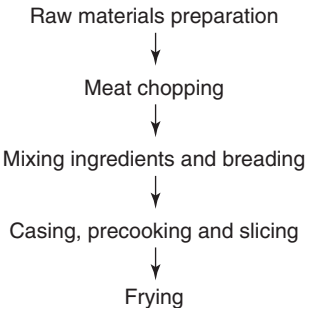

FIGURE 167.1 Flow chart for poultry nuggets elaboration.

TABLE 167.1
Processing Steps and Application Principles of Poultry Nuggets Elaboration

	Stages of Processing	Application Principles
1.	Raw materials preparation	Oil preheating
2.	Meat chopping	Particle size reduction (protein liberation) Homogenization
3.	Mixing ingredients and breading	Poultry nuggets batter homogenization Flour addition
4.	Casing, precooking and slicing	Mechanical entrapment to give form Moderate heat treatment Slicing
5.	Frying	Cooking process Microbial population reduction Color and texture development

C. Processing Stage 1: Meat Chopping

Functional properties of meat myofibrillar proteins, mainly myosin, are responsible for many meat products sensory characteristics. In poultry nuggets, myofibrillar proteins act like emulsifying agents mainly to bind the meat pieces in a restructured product. Egg white proteins have the same function. The binding and gelling capacity will give form to the nuggets in the precooking stage. Using MRM implies the use of another protein or flour to give form to the nugget.

D. Processing Stage 2: Mixing Ingredients and Breading

The homogeneous mixture of the ingredients is important for the distribution of proteins that will form matrix-entrapping water, fat, and other components. This protein matrix is different than in an emulsified meat product, where the proteins act like emulsifiers stabilizing the fat droplets in the water-protein-salt system. The fat content in this kind of batter is low. So, proteins have the function of holding together the small pieces of meat. Flavor ingredients must be well distributed in order to have a quality product.

The breading will contribute to nuggets yield and help to develop crispy texture and characteristic color. Breading is composed of wheat flour, corn flour, or whole-wheat flour or a combination of two or three of these flours. Other ingredients may be used to provide the needed adhesion and functional properties and to produce the desired appearance, color, texture, crispness, and flavor, with a great increase in product yield. Breading for chicken nuggets is recommended not to exceed 30 percent by weight.

E. Processing Stage 3: Casing, Precooking and Slicing

A preforming stage, where proteins will form a restructured gel, is an important step before applying the cover ingredients or breading. The homogeneous batter is put into casings of the desired diameter and cooked until it reaches an internal temperature of 58°C. After cooling, the product is cut into slices with a height of 1–1.5 cm. The height and size of the nugget are important due to the heat necessary to reach the center of the nugget without producing an overheating of the cover ingredients, resulting in an extra golden color.

F. Processing Stage 6: Frying

The result of deep fat frying produces food with a different structure and properties: textural changes, attractive

and tasty surface, crust, increased palatability and browning reactions. During deep fat frying, the fat is continuously or repeatedly used at a high temperature. Oxidative transformations usually accompany and probably precede the thermal transformation of the frying medium. The fried food absorbs this heated fat and contributes considerably to the fat ingested by consumers (3).

During frying the oil undergoes many reactions, resulting in oxidative and hydrolytic degradation. The oil is exposed to molecular tension and changes due to the energy applied to heat the oil (4). In the frying process the high temperature employed (180°C) promotes fatty acid hydrolysis and many oxidative reactions take place. Oxidative reactions, polymerization and hydrolysis occur rapidly during frying, depending on process conditions like temperature, time and aeration (5). The complex series of reactions include those such as oxidation, polymerization, hydrolysis, isomerization, and cyclization (6).

G. Finished Product

Fried foods absorb a considerable quantity of oil. The oil imparts a great part of the flavor, odor and color properties. The oil flavor is given to the food. The quantity of oil absorbed depends on food composition (humidity, porosity and surface exposed to the frying oil). Fat or oil quantity has an effect on nuggets quality during storage. Higher fat content increases the flavor, but the product tends to be softer with storage time (7). Absorption of fat during frying should be kept to a minimum because fat-soaked foods are less palatable and carry more calories. Keeping the contact time and the surface of the food exposed to the fat at a minimum reduces absorption (8).

H. Application of Processing Principles

The main applications during poultry nuggets process are heat and mass transfer during frying. During immersion frying of foods, there are two distinct modes of heat transfer – conduction and convection. Conductive heat transfer, under unsteady-state conditions, occurs within a solid food. The rate of heat transfer is influenced by the thermal diffusivity, thermal conductivity, specific heat and density. Convective heat transfer occurs between a solid food and the surrounding oil. The surface interactions between the oil and the food material are complicated because of the vigorous movement of water vapor bubbles escaping from the food into the oil. In addition, the water vapor bubbles entrapped on the underside of the food material prevent efficient heat transfer between the bottom side of the food and the oil. The amount of water bubbles escaping food material decreases with longer frying times as a result of the decrease in the remaining moisture of material. During frying the temperatures inside a food material are restricted to values below the boiling point of liquid. Since the liquid present in foods is mostly water with some solutes, the boiling point of the liquid inside a food is slightly elevated above the boiling point of the water. As the frying process proceeds, more water evaporates from the outer regions of a food material. Consequently, the temperature of the dried regions begins to rise above the boiling point (2).

Deep-fat frying has long been a means of food preparation for achieving desired texture and flavor attributes of a variety of food products. The breading enhances the texture, flavor, and appearance of the food. It acts as a moisture barrier to diffusion of water vapor from inside and thereby contributes to juicy meat during the holding of the product. In some products, it acts as the major carrier of the seasoning and thus the flavor system. The physicochemical phenomenon of heat-induced texture of breading (denaturation of protein and gelatinization of starch) is the combined effect of several multiple-order chemical reactions. However, such reactions can be modeled as a pseudo first-order reaction affecting breading texture, strength, water-holding capacity, etc. The texture of the breading in a piece of chicken will largely be dependent on the temperature history, pressure during frying and the specific ingredient included, such as type of flour, browning agent, protein content, and other functional agents. It is well known that using positive pressure during frying imparts a softer texture to the breading, while using atmospheric pressure results in a crispy texture (9).

Figure 167.2 shows the principal process pathways during frying and the main alteration caused in oil during frying. On the left side, the four sequential distinct processes that take place during frying are described (10). During the first step, lasting a few seconds, Initial Heating, the surface of the food heats to a temperature equal to the boiling point of oil. The mode of heat transfer between the oil and nugget in the first seconds is by natural convection, and no

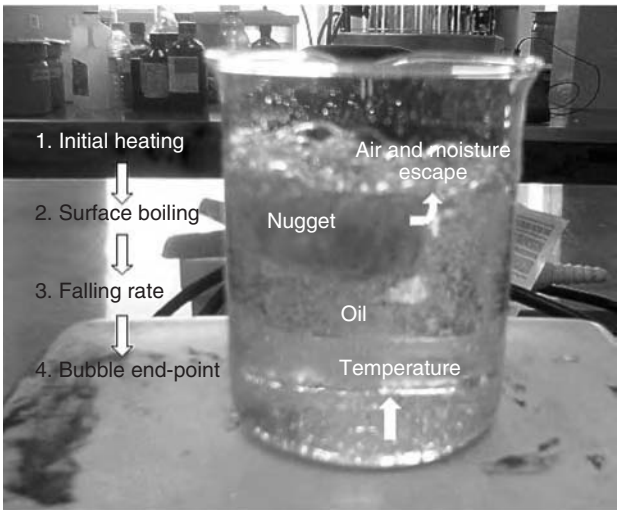

FIGURE 167.2 Distinct stages during frying process.

vaporization of water occurs from the food surface. Second step is Surface Boiling, when the surface boiling state due to the vaporization process starts. Here, the convective heat transfer changes to a forced convection because the presence of turbulence in surrounding oil. The crust or dry region begins to form at the surface of the food. The Falling Rate is the stage during which more internal moisture leaves the food, and the internal core temperature rises to the boiling point. Additionally, several physicochemical changes (e.g., starch gelatinization and cooking) take place in the internal core region. The thickness of crust layer increases and after sufficient time and more removal of moisture, the vapor transfer at the surface decreases. Finally, during the Bubble End-Point stage, after a considerable period of time, the rate of moisture removal diminishes and no more bubbles are seen escaping from nuggets surface. As the frying process proceeds, the thickness of crust layer increases. As food material undergoes frying, several important changes take place in the surrounding oil. These reactions cause the formation of a great number of decomposition products, both volatile and non-volatile. The rate of deterioration of the heated fat is greatly influenced by its degree of unsaturation, the conditions of frying, the nature of the fried food and the presence of chemicals that minimize darkening and polymerization during prolonged heating. The significance of the unsaturation and distribution of fatty acids in the triacylglycerol molecule as well as the effect of antioxidants, silicon and phytosterols on the stability of heated oils is discussed in terms of the mechanism proposed for antioxidant activity (6). The alterations that frying oil can undergo are due mainly to three factors: air, temperature and moisture. Air and moisture are produced when the food surface begins to boil, reflecting the moisture escaping by the increase in internal core temperature. Oxygen and humidity condensation accumulates in oil during successive frying, producing oxidative and hydrolytic alterations. Oxidative alterations produce fatty acids, monoglycerides, diglycerides and glycerol. Hydrolytic alterations produce oxidized monomers, oxidative dimmers and polymers, non-polar dimmers and polymers and volatile compounds (hydrocarbons, aldehydes, ketones, alcohols, acids, etc.). The compounds that can be formed during frying by thermal alteration are cyclic monomers, dimmers and polymers (11).

The heat transferred from oil into a food causes several chemical and physical changes such as starch gelatinization, protein denaturation, water vaporization, and crust formation (color and flavor development). Mass transfer during frying is characterized by the movement of oil into the food and the movement of water in the form of vapor from the food into oil. Frying oil becomes contaminated with component of food materials leaching into oil, water vapor condensing in oil. thermal breakdown of oil, and oxygen absorbed at the oil-air interface. These contaminants reduce oil surface tension acting like surfactants. When the level of surfactants increase the wetting of food surface by the oil is also increased, influencing the heat and mass transfer processes. The surfactants entering the food with the oil are suspected to influence the moisture pickup by the food during subsequent storage, hence reducing its shelf life (2).

II. POULTRY PÂTÉ

A. Background Information

Liver from poultry and mammals is the most widely used organ, resulting in many styles of processed meats such as liver sausage and paste (12). Pâté or liver sausage is a ready-to-eat cooked sausage, with the special feature that the meat batter can be worked at relatively high temperature. In meat batters, the temperature is important in order to maintain the integrity of the solubilized protein-salt-water matrix. The muscle proteins (myosin, mainly) must be liberated and activated during meat chopping and the addition of ice and sodium chloride. The fat must be dispersed in the batter with the cutter, to induce protein matrix gelation, entrapping fat and water. This results in juiciness and textural attributes in sausages. In liver pâté, the amount of lean meat is low as compared with sausage formulation (15% versus 50–60% in the average sausages). The meat added must be precooked. This reduces the protein functionality and makes the product less thermostable, where fat can be released or color and flavor affected by excessive heat. Thus, liver proteins act like the main emulsifying agent in pâté elaboration. In the same way, fat is an important part in the formulation (47–50%), and higher as compared with the fat amount in sausages (10–15%, depending on formulation). Also, fat in pâté has different functions that in regular sausage, where the juiciness, flavor, and texture are affected by fat. In pâté, the fat is responsible for the product spreadability. That is, certain type of protein must be added to the formulation to improve emulsification. Normally, milk protein is added (1–2%) to do this and enhance flavor.

Heat treatment is the other important process in pâté production. Heat is applied to: form the protein gel matrix, destroy microbial population, extend shelf life, and make the product safe for consumption. Heat transfer is usually achieved by conduction in water or by steam in autoclaves. Two important criteria in this step: the internal temperature in the pâté or the heating effect (F value) necessary to destroy the microbial population. Figure 167.3 shows the general flow chart and Table 167.2 describes the principal steps of the process.

B. Raw materials Preparation

Fresh poultry liver is used for pâté formation, because old livers lose emulsifying capacity. Livers must be clean, with no fat or vessel. They can be chopped or cut in half-inch cubes and frozen if not used in the next 24 hours. Lean meat cut in one inch cubes is parboiled in salt (one third of

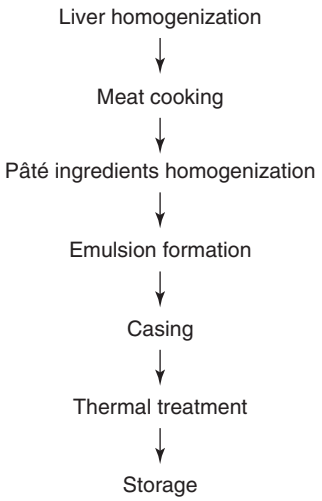

FIGURE 167.3 Flow chart for poultry pâté elaboration.

TABLE 167.2
Processing Steps and Application Principles of Poultry Pâté Elaboration

	Stages of Processing	Application Principles
1.	Raw materials preparation	Precooking
2.	Ingredients homogenization	Particle size reduction (protein liberation)
		Homogenization
3.	Emulsion	Fat globules dispersion
		Homogenization
4.	Casing	Mechanical entrapment to give form
		Heat transfer
5.	Thermal treatment	Microbial population reduction
		Fat and water entrapment in the gelled protein-water matrix
6.	Packaging	Extend shelf life

the total weight), not exceeding 80°C for 30–60 minutes. The broth is left to settle and concentrated by boiling, clarifying and kept at 60°C until addition to batter (13).

C. Processing Stage 1: Ingredient Homogenization

1. Liver Homogenization

Raw livers are comminuted in the cutter with half of the salt to obtain a uniform mass. The cooked meat is added and roughly disintegrated using the lower cutter speed. The percentage of liver in the formulation has a direct influence on the emulsion stability. The higher the amount of liver, the more stable emulsions will be formed, with a constant fat content (14).

2. Pâté Ingredients Homogenization

After the meat is cooked and the fat scalded (>65°C) they are placed together in the cutter and homogenized at low speed. The speed is then increased, adding the rest of the salt. If milk proteins are used, half is added at the beginning and the rest at the end of the operation (13).

D. Processing Stage 2: Emulsion

1. Emulsion Formation

Hot broth is added gradually during homogenization to maintain the mixture at a constant temperature of 58–60°C, with a final temperature reaching 45°C. When the batter is thoroughly homogenized, the homogenized liver (step IV-1) is added and well distributed. The sausage emulsion is then ready. In order to improve flavor, spices such as onion can be added. If desired, the raw pâté or liver sausage mass may be passed through an emulsifying mill (13). Liver proteins will act like an emulsifying agent. Proteins are adsorbed at the fat/water interface, reducing interfacial tension to prevent fat globule coalescence. The parameters that influence meat batter formation and stability are: chopping time (fat globule size), protein concentration (emulsifying agent concentration) and mixing speed (energy input and batter heating). The last factor, i.e., temperature is the most important factor to control during emulsification (15).

2. Emulsion Temperature

The temperature of meat ingredients used in processing is a decisive factor in pâté production. Fat and lean meat must be heated >5°C to melt fat and denature proteins. Raw livers should be added when the meat-fat-broth mixture falls <60°C in order to avoid liver protein denaturation, but >45°C to ensure that the fat is melted (13). The final fat content in the pâté has an important effect on the texture, slicing, spreading and color. Products with a high fat percentage have a fine texture and are more spreadable but with less stability in the emulsion (16).

On the other hand, high fat content meat products, e.g., poultry pâté, have other problems. For example, fat not completely entrapped in the protein matrix tends to move outside the product as water. If so, liquids and melted fat occupy the empty air spaces, improving heat transfer. Furthermore, the pate surface will be affected by the presence of fat and liquids (17).

E. Processing Stage 3: Packing (Casing)

1. Casing

Molded meat products can be processed in the same mold or casing. Heat and energy are transferred first to the casing and then to the batter. Air is an excellent insulator,

and if air bubbles are present in the batter inside the casing, the heat transfer will be interrupted provoking temperature differentials in the same product and extending the heat treatment due to the low efficiency of heat transfer. If the pâté batter is in a water impermeable casing, humidity losses will cause an important reduction in final volume (17).

F. Processing Stage 4: Thermal Treatment

Thermal process can be reviewed in standard literature. We are interested in its effect on poultry pâté and meat products. Thermal treatment is crucial in killing or reducing pathogens. If so, an evaluation and a recording of cooking temperatures become an important part of any food safety program, including the Hazard Analysis Critical Control Points (HACCP) requirements and should be regulated (17). The shelf life of cooked meat products is achieved through reducing growth of, or inactivating, microorganisms by a thermal process. Heat or thermal treatment is done by submerging the products in cooking vats or pressure cookers that contain hot water or steam or a mixture. It can be performed under pressure in retorts or autoclaves in order to reach temperatures above 100°C (sterilization). In contrast, temperatures up to 100°C can be achieved in vats (pasteurization). A certain amount of microorganisms resist the moderate heat treatment and the resulting pasteurized products must consequently be stored under controlled low temperatures to retard microbial spoilage (18).

The intensity of thermal treatment can be defined in physical terms. The term widely used under practical conditions is the F-value, with which the lethal effect on microbial population can be defined. The thermal death time for different microorganisms calculated at 121°C and expressed in minutes is used as the reference value. For example, the thermal death time for spores of *Clostridium botulinum* at 121°C is 2.45 minutes, or, a F-value of 2.45 is needed to inactivate the spores in the product at 121°C. This pathogenic microorganism is the most resistant and serves as reference for low pH foodstuffs (18).

1. Organoleptic, Physical and Microbial Aspects of Thermal Treatment

The intensity of heat treatments has not only decisive impact on the inactivation of microorganisms, but also on the organoleptic quality of the final product. There are products that undergo intensive temperature treatment without significant losses in quality. On the other hand, some products may deteriorate considerably in taste and consistency after heat treatment. One objective of heat treatment is to destroy microorganisms. Since some resist moderate heat treatment, the resulting pasteurized products must be stored under controlled temperatures. Proper preventive measures must be taken to avoid protein denaturation or fat release due to emulsion destabilization from excessive heat application.

2. Internal Temperature

A uniform batter is necessary to obtain the same internal temperature. When variations in composition are present in the product, final temperature will be affected. Final internal temperature only can be obtained if all the product or casing is at the same temperature. Casing surface will receive the same heat quantity at the same rate in different points if the heat is applied uniformly on the casing surface (17).

3. F Value

By measuring the temperature of the product periodically during thermal treatment, the final F-value can be determined. It is obvious that during thermal treatment the product temperature will rise. The temperature taken in the center of the container or casing after each minute of heat treatment corresponds to a certain F-value. These partial F-values are added up and the sum is the overall F-value of the product. The exact F-value is of special importance to the meat producer because it ensures appropriate thermal treatment of the product, thus avoiding undercooking. It also enables the product storage time to be determined (18).

G. Processing Stage 5: Packaging

Handling after processing is considered the primary cause of contamination in cooked meat and poultry products. Quantitative information that the processing plan meets the specific lethality performance standard for each product is necessary in order to implement a zero tolerance presence of pathogens in this kind of product. In poultry pâté the advantage is that the casing in which the batter is cooked is also the final package.

Storage time and temperature, apart from the packaging method, greatly affects the shelf life of meat products. The purpose of packaging is primarily to protect foodstuffs during the distribution process, including storage and transport, from contamination by dust, microorganisms, mold, yeast, toxic substances or those influences affecting smell and taste or causing loss of moisture. Packaging should help to prevent spoilage, weight loss and enhance consumer acceptability (18). Therefore, the type of material for the casings must be chosen correctly to comply with the specification in order to avoid oxygen intake and humidity loss.

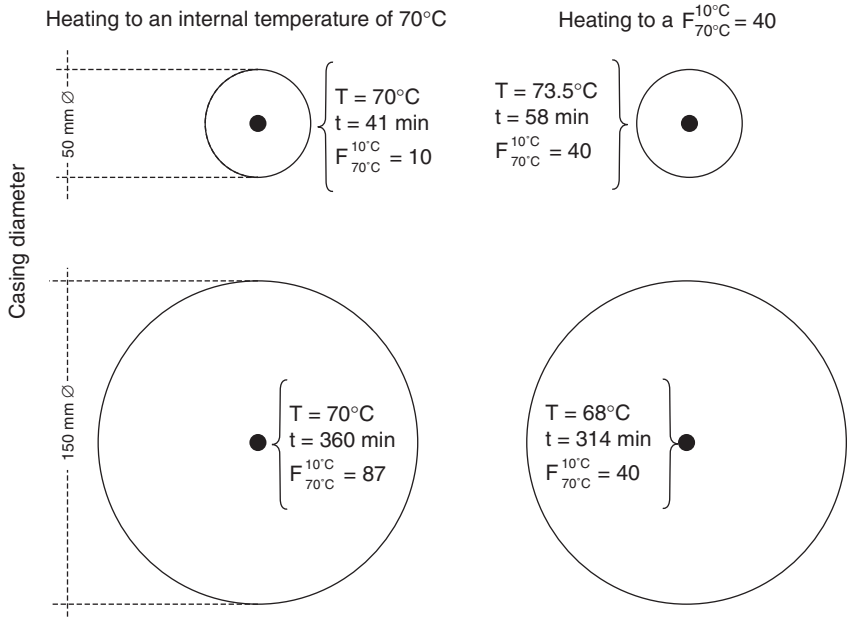

FIGURE 167.4 Comparison between internal temperature heating and $F^{10°C}_{70°C} = 40$ thermal treatment for different casing diameters of pâté sausages.

H. Finished Product

Factors that limit shelf life can be intrinsic, i.e., pH value and water activity (aw), and extrinsic, i.e., oxygen, microorganism, temperature, light and humidity loss (18).

Intrinsic parameters, pH and aw are controlled in the correct selection and managing of raw materials before and after process, together with a correct heat treatment (for both microbial reduction and gel formation). The other parameters should be controlled after the poultry pâté is finished and packed in the casing. The storage temperature for the pâté (1–4°C) is important to prevent microorganism spoilage and fat rancidity or protein denaturation.

The finished product shelf life is the reflection of the quality of the raw materials and the handling of the product during all the process steps, and the right storage conditions to guarantee a high quality poultry pâté.

I. Application of Processing Principles

Emulsion formation and heat treatment are considered the most important aspects of the manufacture of pâté or liver sausage. This means:

1. Form the protein matrix entrapping fat and water.
2. Ensure a physicochemical, microbiological and sensory stable shelf life.

Heat is necessary to destroy microbial population (denoted by F value) (19). The temperature in the foodstuff may be inadequate if one considers data for thermal treatments above or below 100°C, because:

- The prediction of the overall heating effect cannot be done, due to dependence on the food dimension (diameter and length), the cooking temperature magnitude and the cooking time.
- Maintaining a determined internal temperature for a given time cannot be done.
- The microorganisms destruction is not lineal, but exponential. Thus, the microbial count reduction depends on the applied temperature and time.
- Changes in sensory (color, flavor, texture) and other parameters (fat separation, cooking loss, protein coagulation) also depend on time and temperature.

If so, some advantages in the use of F values on the internal temperature are the following:

- The lethal effect on microorganisms can be significant with different food or casing sizes.
- Unnecessary alterations by cooking are not produced due to higher casing capacities or size of the containers.
- A comparison between different heating temperatures can be done by referring to heat alterations (fat losses, weight loss, sensory attributes).

F value has many advantages:

- F values can compare the lethal effect reached with different temperatures
- In new product development, the reference values are available from similar products and bibliographic references
- A comparison between different sizes and shapes can be done
- Different heating procedures can be compared

F value in the meat industry is employed only in sterilized products. To improve the heat treatment, the use of $F_{70°C}^{10°C}$ is recommended. Comparing casing sizes (i.e., 50 and 150 mm ∅), different F values are obtained if we use a heating temperature of 75°C until reach 70°C in the geometric center, or, we apply an $F_{70°C}^{10°C} = 40$ (Reichert, 1988). For 50 mm ∅ casings, takes 41 minutes to reach an internal temperature of 70°C, and only a $F_{70°C}^{10°C} = 10$ was reached. In contrast, if a $F_{70°C}^{10°C} = 40$ is applied, the internal temperature reach 73.5°C in 58 minutes, ensuring a higher microorganisms destruction. In casing of higher diameter (150 mm), the excessive heat is more important due to sensory changes in final product. It takes 360 minutes to obtain an internal temperature of 70°C, with a $F_{70°C}^{10°C} = 87$. Using a $F_{70°C}^{10°C} = 40$, the internal temperature only reach 68°C in 314 minutes. Heating to the same internal temperature provokes a higher lethal effect in higher casing sizes, causing more heat damage with loss of the quality. A heating effect of $F_{70°C}^{10°C} = 40$ should be enough in many cases. Try to reach a given internal temperature, the F value could be not enough and a short shelf life will be expected if the microbial population is not low. Instead, if a given F value is followed during thermal treatment, unnecessary alteration by the heat can only be justified if the contamination of the raw materials is high (19) (Figure 167.4).

ACKNOWLEDGMENT

The information in this chapter has been derived from two chapters in: *Food chemistry workbook,* edited by J. S. Smith & G. L. Christen, published and copyrighted by Science Technology System, West Sacramento, California, 2002©. Used with permission.

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168 Extruded Snacks

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I. BACKGROUND

American Heritage dictionary defines snack as "hurried or light meal" or "food eaten between meals." Traditional snack foods appeal to consumers on a number of levels. Snacks can be considered a treat or reward. Snack foods have always been a significant part of modern life, and they represent a distinct and constantly widening and changing group of food items, whose sales in 2002 was over $22 billion (1), and per-capita snack consumption was 22 pounds. According to a rough working estimate, annual worldwide sales including US is $30–35 billion.

Designing snack foods today can be a complex process to meet changing consumers' taste and expectation, e.g. "good for your health," "rich source of soy protein," "offering a unique flavor" and the elusive search for something unique that also appeals to a wide variety of people. Most snack manufacturers use some form of existing technology as the basis for creating snack products, but incorporate variation that increase the resulting snack's health image appeal by lowering fat and calories or adding nutrients (2). This can be accomplished by using extrusion technology.

II. WHAT IS EXTRUSION?

Food extrusion is a process in which a food material is forced to flow, under one or more varieties of conditions of mixing, heating and shear, through a die which is designed to form and/or puff-dry the ingredients (3). The food extruders can be visualized as a high temperature short time (HTST) device that can transform a variety of raw ingredients into intermediate and finished products. During extrusion, the cooking temperature could be as high as 180–190°C, but residence time is usually 20–40

seconds (depends on types of extruders and speed of the shaft). For this reason the extrusion process could be called as HTST process. It is very important to understand the extrusion terminology, since each manufacture likes to use their own terminology based on their equipment. Extrusion technology provides several different advantages over the traditional methods of food and feed processing. Some of the advantages based on Smith (4) and Riaz (5) with modification include the following.

Extrusion technology provides the opportunity to process a variety of food products by just changing a minor ingredient and processing condition on the same machine. Several different shapes, texture, color, and appearances can be processed by minor changes in the hardware and processing conditions. Extrusion process is energy efficient, and low cost compared to other processes. Presently, most of the extruders are available with automation, which can increase the productivity. Since extrusion process is considered HTST the product quality is much better than other processes, since cooking is done in a very short time and less destruction takes place to the heat sensitive ingredients. It is very easy to scale up the extrusion process for larger installation.

III. DIFFERENT TYPES OF EXTRUDERS

Food or snack extruders are generally divided into two major categories: single screw and twin screw. Single screw extruders can further be divided to different classification (6):

1. Low shear forming extruder;
2. Low shear cooking;
3. Medium shear cooking; and
4. High shear cooking.

Whereas twin screw extruders can be divided into four different categories (7):

1. Co-rotating intermeshing,
2. Co-rotating non-intermeshing,
3. Counter-rotating intermeshing, and
4. Counter-rotating non-intermeshing.

In the past the most common extruders in the snack food production have been the single screw extruders. Still most of the expanded snack is made from single screw extruders because of the economic point of view. Many common snack food such as fried or baked are corn-based and produced by single screw extruders. However, with the new and complex ingredients, different snack shapes and color, the twin-screw extruders have begun to take the place of single screw extruders. Snack products like half products or third generation snacks, may require more advanced extruders like twin screw.

IV. SNACK CONSUMPTION PATTERNS

The snack food market is constantly changing relative to product types, and although most snacks are not primarily consumed for their nutrients, many snacks are made with nutrition in mind. The snack food industry is experiencing extraordinary changes from the consumers point of view. Consumers want snacks to not only taste good, but also smell good, feel good, and look good. Snacks should give the consumer a homemade/fresh feel. Some of the snacks are developed with a special theme in mind like world soccer. These snack pellets are soccer ball shaped, which upon frying or microwaving become soccer balls.

Snacking overall is on the rise in the US owing to an increasingly hectic lifestyle with more time spent at work. Currently more than two-thirds of US women work outside the home and the number of two income families in the US has doubled over the last 20 years. Snacking is increasing from factors such as increases in one-person households, and more school age children obtaining their own meals and refreshments, a highly mobile population, and availability of snack foods in vending machines and convenience markets. Snacking now provides approximately 30 percent of many American's daily calories (8). Various products that were once consumed mainly on impulse, are becoming accepted as side-dish items, for example, corn chips or potato chips served in place of mashed potatoes. The established position of snack foods in the diet is demonstrated by the continuous growth in sales.

In the last ten years, changes in life-style and eating patterns have led to a gradual increase in demand for snack foods. The pattern of snacking in different countries can be affected by several factors such as the lifestyle in each area, the economic climate, rival foods and public receptiveness of current views on nutritional matters. Snacks can provide an increased dietary intake of essential amino acids and other nutrients for developing countries.

V. TYPES OF SNACK FOODS

Although it is not possible to discuss all types of snack foods available in the market in this article, only extruded snacks (expanded and pellets snacks) will be discussed in detail. A broad variety of snacks made by different processes are available in the market. These include potato chips, tortilla chips, corn chips, ready-to-eat popcorn, extruded snacks, pretzels, snack nuts, meat snacks, pork rinds, party mix, multi-grain chips, granola products, variety packs etc. Most recently we have seen a variety of health snacks made with soy protein. These soy-based snacks contain 6.25 grams of soy protein per serving to qualify for the health claim of the Food and Drug Administration. On the same lines, snacking on soy nuts

is becoming very popular among the health conscious consumers. Nowadays, we can find soy nuts with different flavors in most grocery stores. Snacks with different spices and flavors are becoming very popular with US consumers. There is a substantial growth in ethnic snacks from Mexico and India in the markets. Snacks from India are mostly fried lentils, chickpeas, and similar types of pulses with different flavors (mostly with chilli powder) are entering the US market.

Each snack processor may use a specific unit operation and somewhat different technologies to produce unique snacks. There are many ways to classify the snacks. Snack manufacturers use three main terms to identify the snacks:

1. First generation snacks: In this category all the natural products used for snacking, nuts, potato chips and popped popcorn are included;
2. Second generation snack: Majority of the snacks fall in this category. All the single ingredient snacks, simple shaped products like corn tortilla chips and puffed corn curls and all directly expanded snacks are included in this category;
3. Third generation snacks also called half-products or pellets: In this category, multi ingredients formed snacks and pellets, made by extrusion cooking are included.

VI. PRODUCTION OF SNACKS

It is not possible to discuss every snacks manufacturing procedure in detail. Only major snacks manufactured using extrusion technology will be discussed here.

VII. EXTRUDED SNACKS

This category has the greatest potential for growth among the snack foods. The snacks can be made to produce innovations that capture the consumer imagination. Some of the examples are three dimensional snacks, a variety of animals, cartoon, and alphabet shapes etc. Producing a successful snack is a fine balance between the consumer's needs, like tastes and interests vs. a manufacturer's production abilities, economics and quality control. Raw material cost plays an important role in the finished product's selling price. Therefore, it is an advantage to use the lowest cost raw material to produce a successful snack.

VIII. COMMON INGREDIENTS USED FOR EXTRUDED SNACKS

Presently, snack products are being made from a variety of ingredients. However, the selection of the ingredients was limited by the equipment availability. By the introduction of extrusion process, other processing equipment and better knowledge of extrusion technology have led to more diverse and complex formulations for snack foods. The most common source of ingredient is corn, wheat, rice, potato, tapioca, and oats. This is not an inclusive list and one should not limit his/her snack food formulation based on these ingredients. There are several other sources of ingredients for snack food all over the world. A major ingredient in snack food formulation is starch. In its natural form, the starch is insoluble, tasteless, and unsuited for human use. To make it digestible and acceptable it must be cooked.

IX. CEREAL SOURCES

Almost any cereal can be extruded, but if expansion is a major objective, the numbers of functional cereals are limited to degermed corn/grits and rice. Cereals that have high amounts of lipids, are more difficult to expand due to dough slippage within the extruder barrel. This type of cereal usually requires high moisture and high temperature before significant puffing will occur. In general, starches with 5–20% amylose content will significantly improve expansion as well as texture of the snack foods. The most common cereals used in snack food formulations are described below (9).

A. Corn

Extruded snacks are a growing segment of the corn-based market. Corn (also called maize) is a primary ingredient for corn collets and many pellet products. For most corn-based extruded snacks, dry-milled corn meal is used. Large quantities of corn meal are used in puffed extruded snack production and some is used in corn chips. Cornmeal, corn grits, corn flour and corn cones are all a different form of dry-milled dent corn, and in general vary only in particle size distribution. Selection of the granulation depends upon the type of snack and type of extruder. For example, for fine texture and cell structure, or softer bite, a fine granulation of corn meal should be used. Whereas for crunchy texture with a slightly large cell structure snacks, more coarse granulation of corn meal is desired. Similarly, a twin screw extruder can handle fine as well as coarse granulation corn flour, while collect extruders require coarse granulation. Mostly, degermed corn is used in extruded snacks because it expands better than a whole corn. Yellow and white corns are most commonly used in snack foods. Corn starch granules are medium in size (5–20 μm) and have very good expansion characteristics. Protein content of corn ranges from 6–10%. Snack food formulations with corn have a definite flavor. Corn starch is usually cooked at a medium to higher temperature during extrusion. The function of the starch in snack foods is to achieve various textural attributes and characteristics. These attributes can be changed by changing the amylose/amylopectin ratio in the starch.

Today, we can find corn starches with high amylose or high amylopectin in the market. High-amylose corn starches are used when crunchiness and strength is required in the snack. To increase the expansion of the snack, high amylopectin corn starches (waxy starch) can be used. Waxy corn contains very little amylose, whereas the normal corn contains approximately 25–35% amylose. Under high shear and high temperature cooking, a cross-linked waxy corn starch is recommended in snack foods, since it exhibit an improved property of resistance to amylopectin breakdown.

B. Wheat

In general, wheat can be classified into two types: hard and soft. Hard wheat is higher in protein, produces a stronger flour, and is better for bread making. On the other hand, soft wheat is lower in protein and yields a weaker flour, which is better for cake making. In the snack food industry, wheat flour is used in formulation for making baked and fried snacks, flavored crackers, snack cakes, pretzels, bread, and the like. Semolina (coarse particle) usually produced from hard wheat milling, is also used in snack food formulation. The semolina product had an expansion ratio and bulk density about the same as corn meal. Snack foods with all-semolina will produce a very crispy texture. Wheat starch granules are fairly large (20–40 μm) as compared to other cereal grain starches. In wheat, amylose and amylopectin are found within a narrow range of 20–25% amylose. It gives good expansion during extrusion cooking. Wheat is relatively high (8–15%) in protein than other cereals. Sometimes it is difficult to expand due to the presence of gluten. In extruded snacks, wheat gluten provides nutritional value, crispness and desired texture. In general, 1–2% wheat gluten is used in snack foods. Hard wheat is commonly used in bread roll, pretzels, fabricated or pellet type snacks. Wheat varieties with a lower gluten level, will give more tender expanded product than semolina, or hard varieties. Snack products made with wheat usually have mild flavor and white to off white color. It needs medium to low cooking temperature during extrusion cooking. Milling by-products (bran) can be used with soy protein and some other ingredients to produce expanded snack foods of high nutritional and fiber value. The use of wheat in snack foods formulation is limited because of cost.

C. Rice

Rice is one of the largest crops grown in the world. Four types of rice are produced in the United States: long, medium, short and waxy grain. In the US, rice ingredients are not commonly used in snack food formulation. In Japan, most of the snacks are made with rice or rice flour. One major reason is the cost of the rice as compared to the other snack food ingredients. Broken rice can be used as ingredients in expanded or puffed snack products, since rice has good expansion qualities. Rice starch granules are the smallest (2–8 μm) of all grain starches and it digests very easily. Its functional properties are very different from corn or wheat starches. The primary difference is in amylose-to-amylopectin ratio in the starch. Flours from different rice varieties have major differences in physical and chemical properties, which can affect the snack cell structure and expansion. For example, long grain rice flour can increase the crispiness in snack foods, whereas waxy rice flour can reduce chip hardness and at the same time can provide a melt-in-the-mouth texture usually achieved with extra fat. Rice is commonly used as a carrier product for other flavor, since it is bland in flavor. Rice requires the highest temperature during extrusion to cook a snack. Selection of the rice starch in the snack foods formulation will depend upon the amylose content of the common rice varieties. Long grains have 22–23%, medium grain 15–19%, and waxy grain $<1\%$ amylose (10). This difference in amylose-to amylopectin ratio greatly affects the gelatinization temperature of rice flour. The protein content of rice ranges from 6–8%. Rice flour could be used for texture improvement in multi-grain snack foods. Rice flour can be mixed with masa flour, potato flakes, or bean flakes. Chips made with 100% rice flour absorb 20–30% less oil during frying. In a formulation where rice and potato blend is used, the potato flavor and texture remains distinctive even though it is mixed with the less costly rice blend. A mixture of bean flake and rice flour produce a distinct visual appearance of the beans while creating a well-blended bean flavor with no bitter aftertaste.

D. Oats

In general, oats are marketed as rolled oats or as an ingredient for breakfast cereal. Oats have not been used in grain-based snacks as wheat and corn. Recent discoveries that oat bran can reduce serum cholesterol level in humans have boosted the market for oats in the snack food industry. The major problem with oats is high oil content (7–9%) and lipase enzyme. Before using oats in the snack food formulation, it is desirable to inactivate the lipase. Otherwise lipase will catalyze the hydrolysis of oil, which would lead to the production of bitter tasting free fatty acid. Oat starch granules are comparatively small (2–12 μm) in size as compared to other starches. Amylose content of oats varies from 16–27%. Oat starch has a very strong flavor and it gives light brown color to the product. It requires a relatively low gelatinization temperature, but a higher amount of energy input for cooking because of higher amounts of oil content. Oats contain high levels of fiber. Snacks extruded with oat starch expand poorly. For this reason, it has only found its way into product at low level. By using longer barrel extruders with preconditioner, a higher level of oats can be used in snack foods. Among the snacks that have

traditionally included oats in their formulation are cookies and granola. With new technologies and more interest in oats due to health claims, oat-based snack products may be popular in the future.

E. Barley

Barley is used in small quantities in some snack food formulations. It has a mild flavor and nutritionally it is almost the same as wheat, except it contains considerably more fiber. Barley starch granules are medium to large in size as compared to other cereals. A reasonable amount of expansion can be obtained during extrusion of snack foods using barley starch. It gives light brown to gold color to the product. Snack food formulation containing barley starch needs a low cooking temperature during extrusion. Barley fiber can be used in healthy snack foods, as a fiber supplement. Sometimes, manufacturers use barley in multi-grain snack foods in order to add one extra cereal on the label.

X. OTHER CEREAL SOURCES

Cereal such as rye, sorghum, millet, amaranth, and triticale have been used in snack foods. Presently, these cereals are not major ingredients in the snack food formulation.

A. Tuber Sources

Roots and tubers belong to the class of foods that basically provide energy in the human diet in the form of carbohydrates. According to a recent FAO estimate, virtually every country in the world grows some species of root crop. Potato and tapioca (also call cassava) are two main tuber crops used for extruded snack foods.

B. Potato

Different forms of potatoes (granules, flakes, flours and starches) are used in snack food formulations. Potato starch is often used in snacks to provide extra expansion. Potato starch has a wide range of sizes with some larger granules (60–100 μm) than the other cereals. This starch contains 20–25% amylose and has very low oil contents. Potato starch develops high viscosity during extrusion cooking. It has an excellent swelling and binding power. In snack food it has a definite flavor and it gives gold to light brown color to the product. It requires low cooking temperature since its granules breakdown easily. Potato flour is the major ingredient for two common snack products, i.e., direct expanded snack (product looks like French fries) and fabricated chips.

C. Tapioca

Tapioca (cassava) is a basic source of low calories or a supplement to cereal. In general, tapioca starch is used in third generation snack foods formulation. Tapioca starch grains vary in shape, and size from 5–35 μm. The amylose content is about 17%. Good quality starch should have a pH of 4.7–5.3, a moisture content of 10–13.5% and should be uniformly white in color. Tapioca starch develops very high viscosity and it is an excellent binder. It has a bland flavor and requires moderate cooking temperature during extrusion cooking.

XI. EXPANDED SNACKS

The majority of extruded snacks are in this category. This group is also referred to as "collet" or "second generation snacks." In general, expanded snacks are made on high-hear extruders. These are high-fiber, high-protein, and low calorie snacks. Some examples are corn curls, onion rings, three dimensional snacks, and potato sticks. These types of snacks can be seasoned with a variety of different flavors, oils, salt, sugars, etc. The quality of an expansion-cooked product depends upon the conditions of operation of the extruder and the main raw material used in the formulation. Several other factors can influence the degree of puffing of snacks during extrusion, i.e., amount of moisture in the feed material, dough residence time in the extruder barrel, and cereal particle size.

A. Fried Collets

These are the most familiar extruded snacks in the market. A special die arrangement gives the product a twisted puffed shape. These collets are made on collet extruders. The product is then fried in vegetable oil, and coated with cheese and some other flavor. During frying the moisture level reduces from 8% to 1–2% in this product. The most common material used for fried collet is corn meal. Typical corn meal specifications are given in Table 168.1. Some other cereal grains can also be used for this type of product.

B. Baked Collets

Baked collets are another example of the expanded extruded snacks. This includes products such as baked corn curls,

TABLE 168.1
Typical Corn Meal Specifications for Fried Collets

Granulation (Mesh)	Percent Retained on Screen
16	0
20	0–2
25	0–10
30	25–50
40	45–65
50	0–8
60	0–2
Moisture	11–13
Fat	<1

Reference 11.

TABLE 168.2
Typical Formula for Baked Snacks (Second Generation Snacks)

Ingredients	Amount (%)
High Protein Snack	
Rice flour	35
Wheat flour	35
Soy concentrate	20
Sugar	6
Corn starch	2
Vegetable oil	2
Potato Stick Snack	
Potato granules	64
Degermed corn meal	35
Vegetable oil	1
Corn Curls	
Degermed corn meal or grits	100

Reference 11.

onion rings and potato sticks. Baked collets can be made with different cereal grains and tuber flours. Protein, fibers, cellulose, and bran can be blended with cereal grain up to 20% to make healthy snacks (11). Potato sticks usually made by mixing potato flour with corn or rice flour. A typical formulation for baked snacks is given in Table 168.2.

XII. THIRD-GENERATION SNACKS

Third generation snacks (3G), also referred to as "half products" or pellets provide an alternative to fully prepared puffed snack foods. Third generation snacks, or half products are extrusion cooked, and formed at low pressure to prevent expansion, and then dried to a final moisture content of about 10% to form a glassy pellet. In developing third generation snacks, "half" of the process is completed to prepare "pellets" which are shelf-stable for periods of up to a year without refrigeration, provided they are properly packed to retain their moisture. Many types of proteins and protein enrichments may be added to third generation snack type recipes such as meats (whole fresh shrimp, fresh chicken, beef, etc.), dairy products (cheese, yoghurt, milk solids) and legume proteins (soy, pea, bean). Up to 30 to 35% levels may be added and still maintain high quality final products (12). Several minor ingredients have very useful effects on the texture, quality and flavor of the final products. Salt is very useful in assisting with uniform moisture migration throughout the third generation pellet after drying during the moisture equilibration period. Baking soda will give special flavor and textural attributes to the finished products after frying, puffing or microwaving. Oils or emulsifiers reduce stickiness during cutting and other processing steps (13). Drying is very critical in the production of good quality third generation snacks. Proper drying will reduce the moisture content of the pellet to approximately 12 percent. Temperatures of 70–95°C and retention times of one to three hours are required. These products are economical to run and have built-in-marketability due to their highbulk density. Third generation snacks can be prepared in homes or restaurants. Unlike typical snack foods, half-products do not yet contain oil that can oxidize to give off-flavor to the products. These pellets can be shipped from a central manufacturing distribution point, held until needed for the market, and then puffed, flavored and packed fresh and locally. New variations of the third generation snacks expand using infrared heating, hot air, or microwaving. The use of hot air systems reduces the oil uptake that occurs in frying and allows a controlled addition of oil to be made as required for flavoring. With consumer concerns about fats and oils, a half product

TABLE 168.3
Typical Third Generation Snack Formulations

Ingredients	Amount (%)
Corn Based	
Hard, Crunchy Texture	
Ground corn	94.5
Corn starch	5.0
Monoglyceride	0.5
Soft, Frothy Texture	
Corn starch	55.2
Wheat starch	27.5
Tapioca starch	14.0
Liquid shortening	2.5
Monoglyceride	0.8
Potato Based	
Hard & Crunchy	
Potato flakes	49.0
Durum flour	30.0
Wheat starch	20.0
Monoglyceride	1.0
Crispy	
Potato flakes	47.0%
Drum flour	30.0%
Wheat starch	20.0%
Vegetable oil	3.0%
Soft	
Potato flakes	49.0
Corn flour	30.0
Wheat starch	20.0
Monoglyceride	1.0
Speciality Snacks	
Fresh Shrimp Recipe	
Tapioca starch	64.0
Fresh shrimp	20.0
Rice flour	10.0
Vegetable oil	3.0
Salt	3.0
Pepper seasoning	1.0

Reference 13.

snack that expands using hot air offers snack food manufacturers an oil free snack with perceived health benefits. Elimination of frying oils reduces calories and allows a marketing opening for snacks with a "lite" image. Typical flow diagrams for the production of third generation snacks are shown in Figures 168.1 and 168.2.

With the multidimensional snack system, a wide range of raw ingredients can be used to blend together to make an excellent formulation for many types of third generation snacks. The extruder feed must contain a high level of starch to maximize expansion of the collet during exposure to hot oil or air. Levels of 60% or less total starch in the formula give only slight expansion in the puffing step and yield a final product with a crunchy, hard texture. Wheat, corn and tubers are widely grown crops in developing and industrialized countries, and they are cheaper and more available in the market than the other cereal crops. Several formulations for third generation snacks are given in Table 168.3.

XIII. CO-EXTRUDED SNACKS

This is a relatively new technology introduced in 1984 for the snack food industry. In this process two different materials are extruded from one die. The two materials can come from two extruders or from one extruder and one pump. This process can produce a snack with two

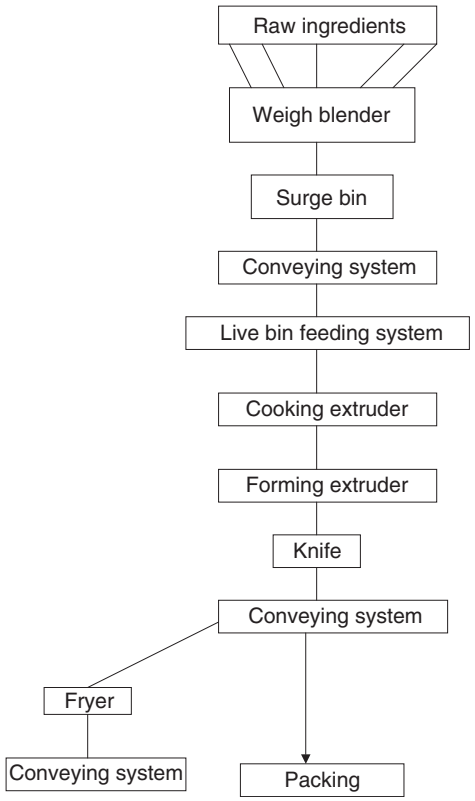

FIGURE 168.1 Flow diagram for 3G snacks.

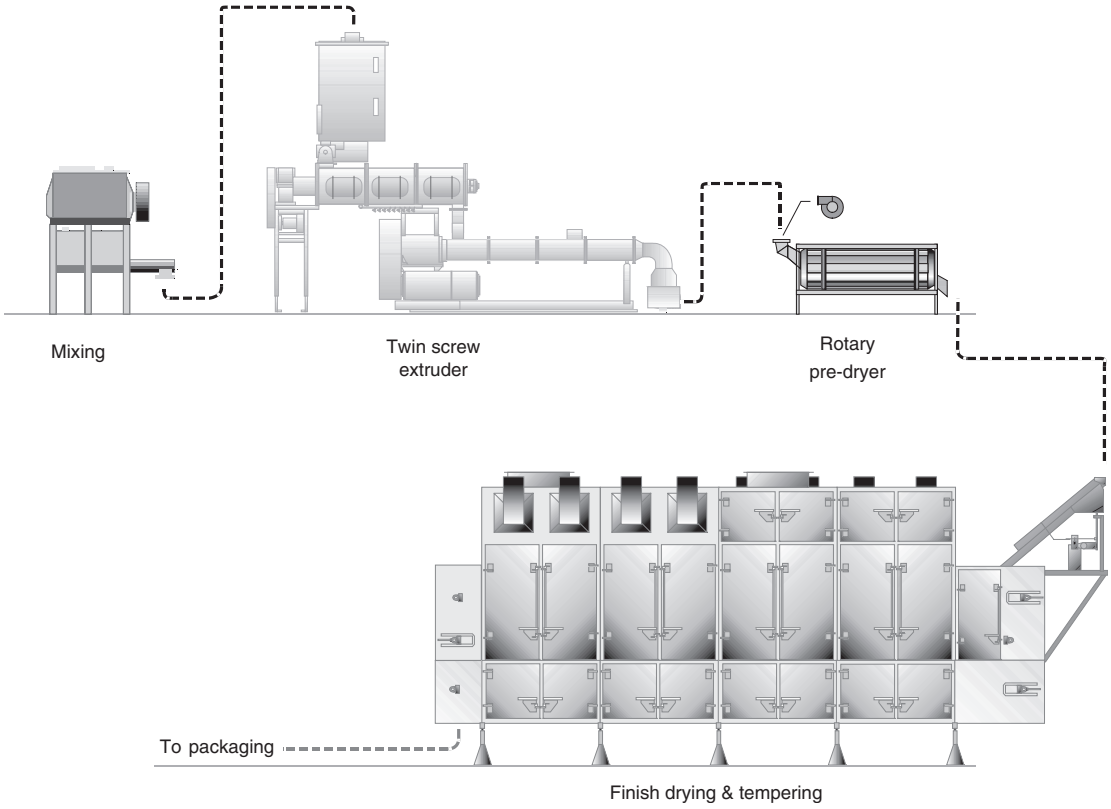

FIGURE 168.2 Flow diagram for third generation snacks. Courtesy of Wenger Manufacturer.

TABLE 168.4
Typical Formulation for Co-Extruded Snacks

	Ingredient (Sweet Snack)	Amount (%)		Ingredient (Savory Snack)	Amount (%)
Tube:	Wheat flour	70	Tube:	Corn meal	80
	Sugar	20		Wheat bran	10
	Milk powder	9		Milk powder	8
	Salt	1		Salt	2
Filling:	Powered sugar	50	Filling:	Cheese powder	24
	Vegetable oil	21		Vegetable oil	30
	Corn starch	11		Shortening	14
	Shortening	11		Corn starch	10
	Cocoa powder	7		Milk powder	10
				Dairy powder	10
				Salt	2

Reference 14.

different flavors, or two textures or two colors. The most common snack produced by co-extrusion is a cereal-based outer tube with a cheese filling inside. There are three basic types of co-extruded snacks in the market: cereal-based tubes with cereal-based fillings, cereal-based tubes with fat-based fillings, and cereal-based tubes with water-based filling. The shelf life of these snacks is limited, because of migration of moisture and/or oil from the filling to the outer shell. A typical formulation of co-extruded snacks is given in Table 168.4 (14).

In conclusion, snacks can be processed by a variety of different methods and techniques. Several new raw materials containing nutraceutical and functional properties are being introduced in the market every day for snack food products. Snacks can be made with a combination of different raw materials containing different properties. The role of snacks in a healthy lifestyle is only starting to be developed. The recognition of snacks as healthful will increase as industry changes products from merely good taste to nutritious.

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169 Coating Snack Foods

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CONTENTS

I. THE SNACK FOOD INDUSTRY

The snack food industry includes everything from chips to confections, from salty to sweet. In the United States, sales of savory snack foods reached $21.8 billion in 2001, according to the Snack Food Association's State of the Industry report. If cookies, crackers and snack bars are included, sales top $34 billion. Potato chips, America's favorite salty snack, accounted for $6 billion of those sales. Overall, the snack market ranks third in total dollar sales in supermarkets, which comes to around 22 pounds per person, per year. Snack foods are sold everywhere from retailers to prisons. They're eaten at every mealtime, and in between, from breakfast pretzels to midnight chips, and they're popular in every demographic age group from the cradle to the grave (1).

Much of the success of snack foods is due to the coatings that are applied. Coatings are added to a variety of foods because they add novelty, give an attractive appearance, define the product shape, improve taste, increase shelf life, protect ingredients, prevent migration of core constituents, create texture, or maintain structural integrity. Coatings are especially important in the snack food industry where the snack base (which frequently consists of items such as corn chips, popcorn, and extruded corn starch) often has an unattractive appearance and tastes bland, mealy, sticky, dusty or dry. The secret to the popularity of these snacks is coatings which give an appealing color and flavor.

For most snacks, it is the way they are coated that produces their pleasant mouth sensations and taste profile (2). The majority of flavored snack products are flavored topically, that is, the seasoning is applied onto the surface of the product. Some flavorings are incorporated into the main body of the snack, but the high temperatures involved in baking, frying or extruding drive out most of the volatile flavor compounds, making it necessary to apply the bulk of the flavors to the surface of the product.

II. ADHESION OF THE SEASONING

Since most seasonings are applied on the surface of the product, it is critical that the seasoning adhere to the surface for the entire shelf life of the snack. For fried snacks, seasonings are typically applied as a powder. The snack goes straight from the fryer to the coating line so that the product is still hot and the surface of the snack is still wet with oil when the seasoning is applied. Thus, no additional tack agent is required. The temperature of the snack has only a small effect on the adherence of the seasoning. Comparing potato chips at 300 and 100°F, the adherence decreased from 81 to 68% (3).

Alternatively, oil can be applied directly as a tack agent (Figure 169.1). The dry product is sprayed with oil, then seasoning is applied and adheres to the oil. Other tack agents include water, sugar water and hydrocolloids. For these solutions, a final drying step is required, which may be undesirable with dry snack foods because of the increased expense from the additional processing step.

Some products, such as fried corn chips, absorb most of the surface oil after frying, so they require a secondary spray

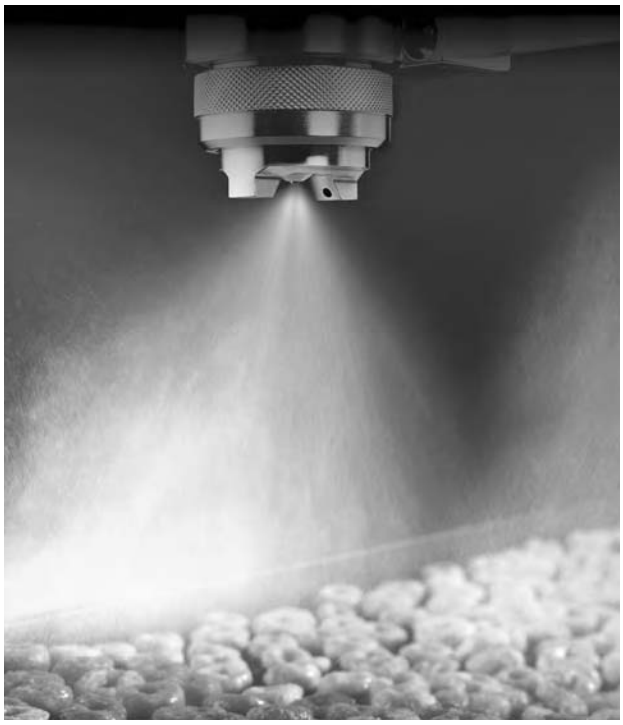

FIGURE 169.1 Application of liquids onto the surface of foods. (Photo courtesy of Spraying Systems Co.)

FIGURE 169.2 Over the belt seasoner. (Photo courtesy of FMC Food Technologies/Allen Systems PPM.)

of oil after the powder is applied, to hold the seasoning to the surface. A top coating of oil is also added to low or no fat snacks, to add palatability. Without the surface oil, these products are unpleasantly dry in the mouth.

III. COATING APPLICATION METHODS

Coatings can be applied to snacks on a conveyor belt or inside a tumble drum, in coating pans or by enrobing. Each method has its own advantages and disadvantages, and produces different coating characteristics.

In a conveyor belt system, the seasoning is dispensed over the belt, so that only one side of the snack is coated, unless there is a device to flip the pieces over. In some foods, such as potato chips, the chips are layered two or three layers deep, so that more seasoning lands on the top layers. One study found that adherence dropped from 60% on the top layer to 30% on the middle layer and 10% on the bottom layer (3). Thus, a conveyor belt system may not produce snacks that are as evenly coated as other methods, but requires much less space. In some factories there is not enough ceiling height to allow for tumble drums to be installed, and so conveyor belts are used instead. In addition, some companies use conveyor belts because they want to apply seasoning to only one side of the snack, such as for crackers. If the snacks are only one layer deep and one-sided coating is desired, this method can give very even coating. However, more waste is typically produced because the food does not cover the entire surface of the belt, unless a waste recovery system is used. An example of a seasoner that can be placed over a conveyor belt is shown in Figure 169.2.

Many foods are coated in a tumble drum because the tumbling action mixes the product as the seasoning is being applied, producing even coating on both sides. Tumble drums are horizontal cylinders with one end raised so that product flows from one end to the other. Flights are set inside the drum which lift the product, causing the product layers to be rearranged as they move down the drum. As the layers of product slide over each other there is some transfer of seasoning, producing more even distribution of powder. Spray nozzles and dispensing tubes can be installed inside the drum, so that the snack may be sprayed with oil in one section, then coated with powder in the next. Popcorn being salted in a tumble drum is shown in Figure 169.3.

Another method is coating pans, which are a slow, batch process, but have become highly automated. The panning process consists of building up successive layers of coating on a core material. The core is commonly a nut, dried fruit or candy, and the layers of coating are most commonly sugar or chocolate. The product is tumbled in a smooth pan which comes in different shapes. The round, or apple shape is the least effective shape for coating because it doesn't move the product from the front to the back of the bowl. The doughnut shape is for coating nuts with sugar (Figure 169.4, left). The pear shaped bowls move product around the most effectively and are the work horse of the coating industry (Figure 169.4, right). Coating is sprayed

FIGURE 169.3 Popcorn being coated with salt in a tumble drum.

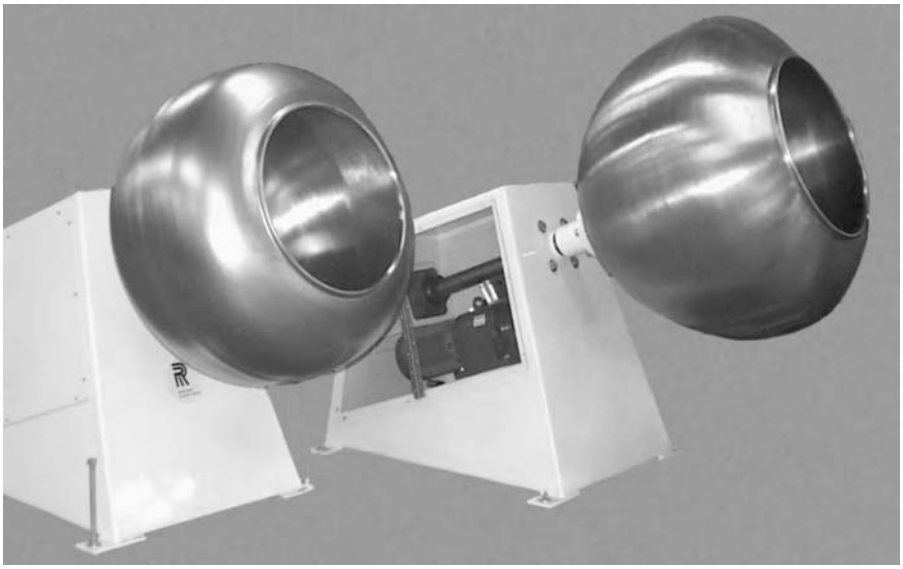

FIGURE 169.4 Donut shaped (left) and pear shaped (right) coating pans. (Photo courtesy of Royce Metal Products Limited, Toronto Canada.)

onto the core material then dried with forced air, and the process repeated until the desired number of layers has been built up. This is popular for approximately round, evenly coated snacks, such as yogurt covered peanuts. These products have smooth surfaces caused by the polishing action of the pan. If a rough surface is required, this can be produced by spraying on maltodextrin to produce a bumpy surface.

Enrobers work by dipping, pouring or spraying a liquid coating onto a core material (Figure 169.5). Products that are dipped may be individually dipped, such as caramel apples, or carried between two belts to keep the product submerged as it is carried through the coating. Other products are coated by passing underneath a curtain of molten coating, especially for chocolate. With a curtain, a pan

FIGURE 169.5 An enrober. (Photo courtesy of Macintyre Chocolate Systems/Ladco.)

beneath the curtain catches the excess coating and recirculates it to the top. Air knives are used to remove excess coating and control the thickness of the coating. This is followed by a shaker to remove further coating. Disks, wires or rollers are used to apply a final decoration on the top of the product (Figure 169.6). A pre-bottomer may be used with a curtain system, especially for chocolate coatings. In a pre-bottomer, the core material is passed through the coating on a conveyor belt, then passed over a cooling plate to set the coating, before proceeding through the curtain.

A subset of enrobing is battering. Batters are produced from flour, starch, sugar, fat and water, with some flavorings added in, though the majority of the flavor is usually applied as an oil slurry onto the outside of the batter. If a smooth surface on the batter is desired, the product is tumbled in a coating drum with smooth walls to produce the desired surface smoothness (2).

An even smoother coating can be produced by spraying on a hydrocolloid solution. Gum arabic is common, as well as modified starch. Once dried, the coating has a glossy, crunchy surface. The solution itself has a bland or starchy flavor, so flavorings are sometimes added into the coating solution to produce a more desirable flavor.

IV. POWDER COATING

Powdered seasonings can be dispensed from several different types of distributors. Powdered seasoning can be applied by gravity feed out of a tube, pneumatically conveyed by forced air or centrifugally applied by spinning cones or disks. Many systems are gravity fed due to the simplicity of the design. In a seasoning tube, the seasoning is conveyed down the tube along the flights of a rotating screw (Figure 169.7). Seasoning may be dispensed only out of the end of the seasoning tube, or the tube may have small openings along its length, allowing seasoning to fall out over the whole length of the tube. The curtain of seasoning produced by the powder falling out of holes along the length of the tube can be seen in Figure 169.3. Another gravity fed device is a roll salter, which employs a slotted roller to dispense salt or seasoning over unseasoned snacks on a conveyor. Systems using air currents or centrifugal force to dispense the powder are especially useful for cohesive powders that tend to clump and produce erratic feed rates in gravity fed systems.

Snack manufacturers put 30–50% more seasoning than actually needed onto a product because of the expected waste (4). Also, to produce more even coating, flavorings are often diluted with a bulking agent so that excess powder can be added to the coating system (5). During processing, the extra coating falls off and leaves the food with a more uniform coating than would be possible if only the desired amount was applied to the food initially. A recycling system is installed under some coating systems to capture the powder that falls off, remove the fines and clumps and reuse the rest of the seasoning.

V. LIQUID COATING

Sometimes oil or other liquid coatings are applied to the surface of a product (Figure 169.8). Oil-soluble flavors and colors may be dissolved in oil and applied directly to

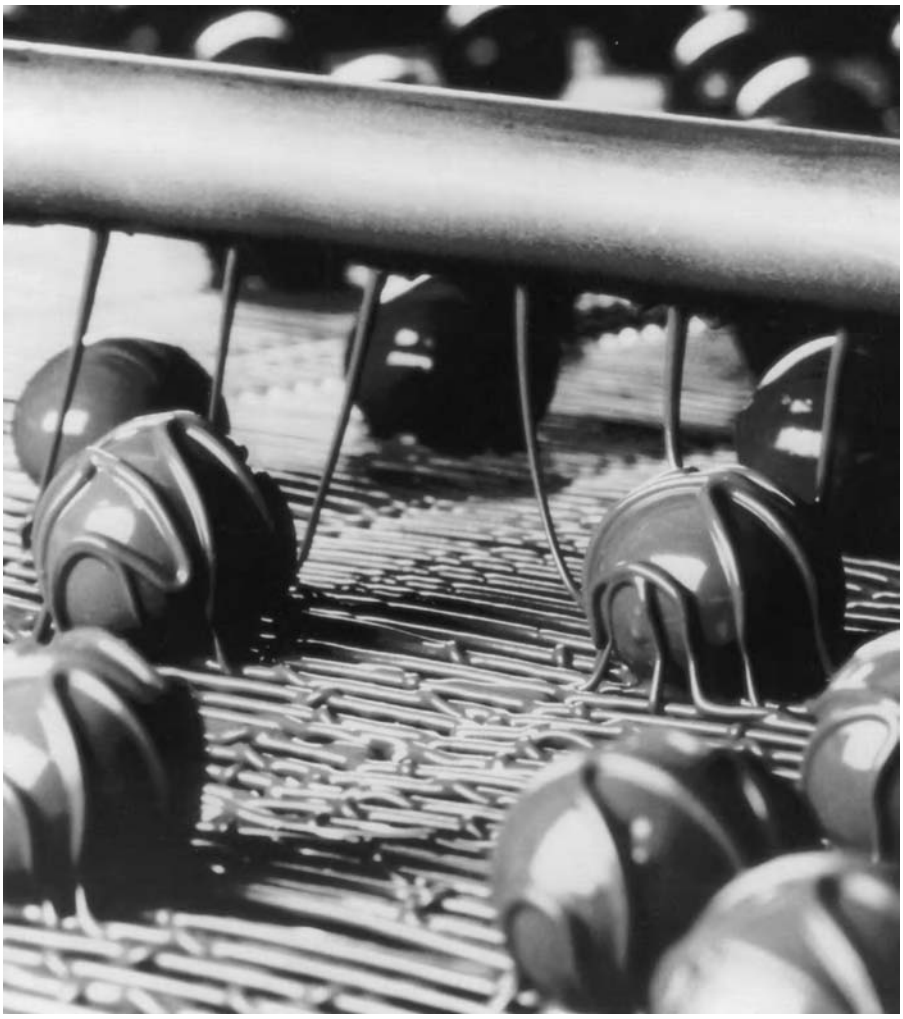

FIGURE 169.6 Adding the final decoration. (Photo courtesy of APV Baker.)

FIGURE 169.7 A screw-type seasoning dispenser. (Photo courtesy of Spray Dynamics, Inc.)

the surface of the product. Sugar solutions are also commonly sprayed onto the surface, especially for breakfast cereals. When applying sugar, solutions of 80% sugar are often made so that less water needs to be removed from the final product. The solution needs to be kept heated to keep the sugar in solution since it is above the saturation point at room temperature. For snacks that are not fried or have a dry surface, an oil slurry can be used to apply seasonings. The powder is suspended in oil in a ratio of 80:20, oil to powder (2). The slurry is sprayed onto the product, applying the seasoning and tack agent at the same time.

FIGURE 169.8 Spraying of oil onto the surface of bread. (Photo courtesy of Spraying Systems Co.)

The biggest challenge with liquid application is to produce an even spray pattern across the surface of the

conveyor belt or within a tumble drum. Nozzles have been designed to produce circular, donut, fan and other spray patterns in an attempt to produce more uniform application. In comparison to powder application, liquid application is usually very even and the amount of liquid delivered is very reproducible.

VI. ELECTROSTATIC COATING

The basis of electrostatic coating is that by charging the seasoning powder as it is dispensed, it is attracted to the food product, which is grounded through contact with the conveyor belt or tumble drum underneath it. The charged particles tend to separate and distribute themselves evenly as they coat the food item because of repulsion between the particles. Electrostatic attraction has been claimed to produce a more homogeneous coating even on difficult shapes, reduce seasoning fall off and make it possible to lower the oil content by up to 20% because of improved adherence (6). The charged powder coats not only the surface of the work piece facing the coating source, but also the more difficult, hidden regions, due to the wrap around effect of the space-charged field (7). The attraction of the charged coating particles towards a ground also reduces dust that would otherwise be produced (8). The use of electrostatics has been shown to increase transfer efficiency an average of 68% and decrease dust 65% (9). A labeled example of an electrostatic system is shown in Figure 169.9.

A comparison of an electrostatic salter with a mechanical salter found that the electrostatic salter maintains uniform spacing of particles during dispensing by inducing a negative static charge on their surfaces, causing the particles to mutually repel each other and producing a more even distribution of salt (10). Other foods that are electrostatically coated include seasoning corn and tortilla chips (4), putting salt onto crackers and pretzels (11), and flavors onto confections (12). Liquid smoke applied to meat electrostatically improves flavor uniformity and decreases waste (13). The bactericide added to control pathogens on the meat surface can be reduced by as much as 90% using electrostatic coating because electrostatics improves coating evenness and eliminates overspray (14).

In the flavor industry, some flavor houses perceive electrostatic application as a reduction in revenue, however some producers have experienced an increase in market share and thus require more flavor to meet the demand (15).

VII. SALT

The most common snack food seasoning is salt. Typically, salt is the first flavor variety sold and if the product is successful, line extensions are developed using other seasonings. But even with other seasonings, salt is added to accentuate the flavor. The Snack Food Association recommends a salt level of 1.75% plus or minus 0.25% for salted potato chips. Between 1.5% and 1.7%, the saltiness curve is ascending, so small differences in concentration can make a larger difference in saltiness (16). There is a plateau region between 1.7% and 2.5% where additional salt adds very little to the intensity of taste.

Most salt in the food industry is produced by thermal evaporation of artificial salt brines. Salt is available in different shapes. Purified brine is evaporated in a vacuum pan to produce a concentric or cubic form of crystalline salt, which is then screened into different size fractions (Figure 169.10, top). A second salt shape is dendritic salt. Dendritic salt is a porous variation of the cubic crystal. Its crystal formation is interrupted by sodium ferrocyanide, also known as yellow prussiate of soda (YPS). Low levels of YPS added to the brine prevent normal crystallization. The crystals form as aggregates of attached cubes,

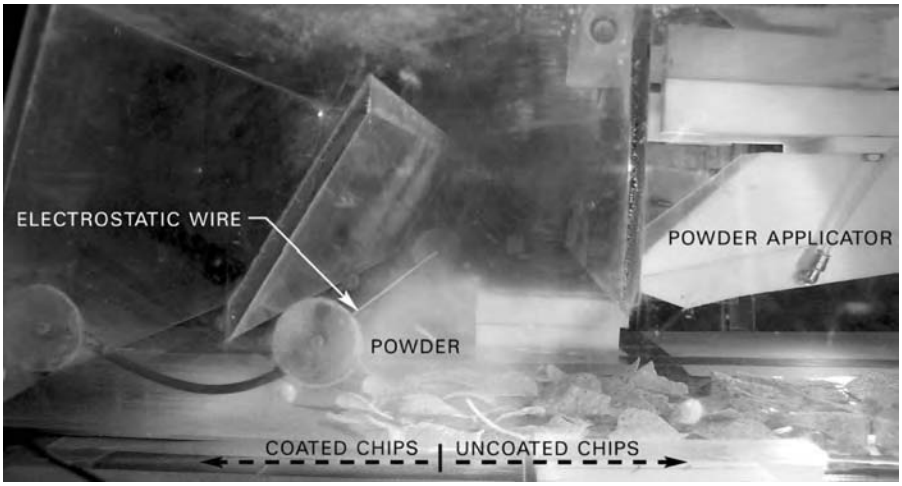

FIGURE 169.9 Electrostatic coating of tortilla chips. (Photo courtesy of Terronics Development Corp.)

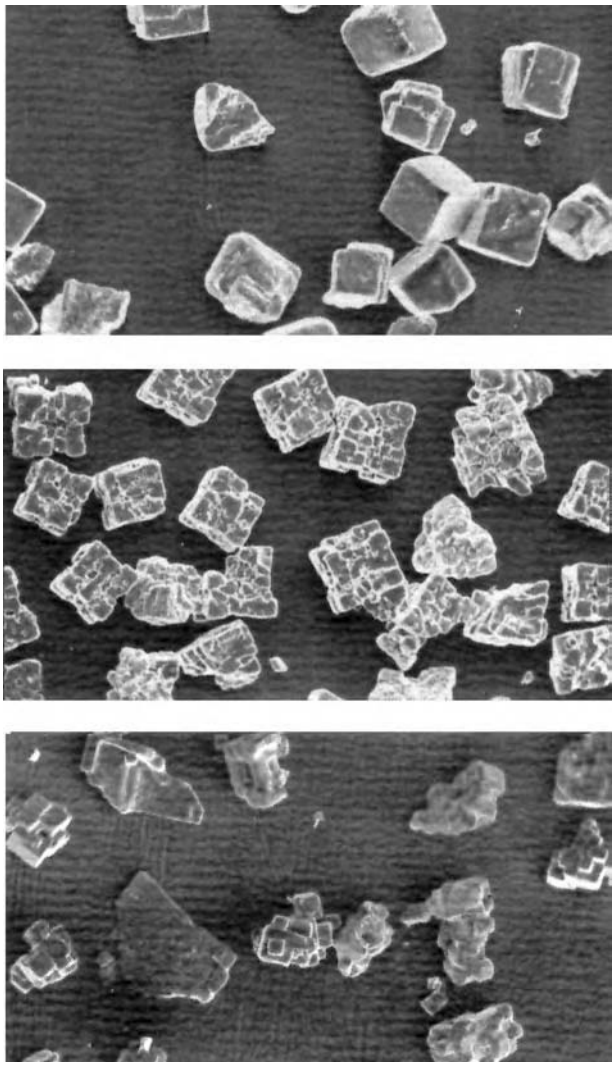

FIGURE 169.10 Cube (top), dendritic (middle) and grainer (bottom) salt. (Photo courtesy of Morton Salt Co.)

providing a porous structure with large surface area and good adherence (Figure 169.10, middle). A third type of salt is produced by evaporating salt in the grainer, or modified Alberger process (16,17). Due to evaporation at the surface of the brine, the uppermost layer of the brine becomes saturated before the lower, causing the crystal nuclei to be produced entirely at the surface. The crystals are held there by surface tension but, being heavier than the brine, are partially immersed into it. Because crystallization proceeds at the surface, the crystals grow laterally, causing them to sink more deeply. The result is a hollow quadrilateral pyramid, floating inverted, with thin sides and a relatively heavy bottom point (Figure 169.10, bottom).

Salt is also available in a range of sizes. Size can be determined by laser diffraction, but it is most commonly sold based on sieve size. Generally, the percent retained in each sieve of a series of sieves is reported, though in some cases a single sieve size is reported. When a single sieve size is reported, it is the sieve that stops at least 50% of the salt. The sieve size is the number of wires per square inch, thus the larger the sieve size, the smaller the salt. Salt is available from coarse or pretzel salt at a 20 sieve size, down to extra fine with a 325 sieve. Flour salt, originally developed to mirror the size of wheat flour, is about 70 mesh. The size is adjusted to match the size of the powder it is blended with. The size can be reduced by grinding rolls or screening out the smallest size fractions. If the salt is formed with grinding rolls, it no longer has a regular form (18).

The size of the salt affects a number of factors. The taste perception of salt is affected by the size of the salt crystals. Small salts dissolve quickly because they have a large surface area to weight ratio, creating an intense salty sensation, but then disappear quickly. Larger salt particles provide a longer lasting salty perception because they dissolve more slowly (16). Fat decreases the intensity of saltiness, so in snacks with a large amount of surface fat, small particles of salt are enrobed in fat, losing their intensity. For that reason, large salt is typically used for potato chips and high fat snacks (16). Dry snacks with less than 30% oil, such as tortilla chips, work best with a small particle size flour salt because the small salt has better adherence, more uniform distribution and high solubility.

Size also affects salt's flow characteristics. The ability of the salt to flow is crucial to produce an even, reproducible coating on snacks. Flowability is improved by low humidity in the environment, a free flowing structure such as for cubic salt, and the use of anti-caking agents, such as tricalcium phosphate (TCP). Smaller salts can be more evenly applied, though this size benefit may be offset because smaller salts are more cohesive and will clump readily unless an anticaking agent is used.

It is important that the size and density of the salt be the same as the other ingredients in a mixed seasoning blend to minimize stratification during handling. The density of salt can be decreased by the addition of sodium ferrocyanide to interrupt the crystal structure and produce a more porous structure. Also, the more irregular the shape and the more porous the structure, the lower the bulk density. Since salt is denser than most food powders, the goal is typically to reduce the salt's bulk density and match its size to the other powders.

The solubility of salt is important in some food systems because it determines how rapidly the salt will dissolve in the food system. When high solubility is desired, a porous structure, such as in dendritic salt, will be more soluble. The irregular surfaces found in Alberger and pulverized salt also make them go into solution more rapidly than cube salt. In some cases, a lower solubility is desired. For example, large, rock salt is frequently added to pretzels for visual appeal. However, the partially dissolved salt raises the local heat capacity, creating a weak spot in the dough. This is not a problem for large pretzels, but for small, thin pretzels, flake salt is used instead because it is

less soluble, causes less of an increase in heat capacity and therefore is less likely to cause blisters (16).

Once the salt reaches the snack, it must adhere to be of any value. Flakes give better adhesion than cubes because of their larger surface area. For crackers especially, adhesion can be difficult because of their smooth surface. Crackers are frequently oiled then flaked or dendritic salt is applied because they have the best adherence to the smooth cracker surface.

VIII. OTHER FLAVORS

The most popular flavors for salty snacks are cheese, BBQ, sour cream and onion, and ranch (5). Typical seasoning levels are 8–10%, depending on the seasoning and snack base (3). These flavors are complex, and change over the years as consumer preference changes. The flavor base frequently combines cheese, tomato, onion and garlic. Monosodium glutamate, yeast extracts, disodium inosinate and disodium guanylate can be added to accentuate savory, salty flavors. Botanical extracts may also be added to boost savory notes, as well as acting as antioxidants. Maltol is a sweetness enhancer, but it also enhances and modifies savory flavors, adds mouthfeel, increases creaminess and dairy notes and decreases bitterness. At low levels, salt enhances sweetness as well as rounding out flavors. Fillers such as maltodextrin, whey powder, wheat flour, or corn flour are also used to add bulk (5). Finally, a free flow or anticaking agent such as silicon dioxide or tricalcium phosphate is used to prevent clumping.

Anticaking agents are frequently added because of the hydrophobic nature of many food powders. These are very small powders that are added to another powder to inhibit its tendency to cake, improving the flowability (19). Most anticaking agents are insoluble in water but many of them can absorb considerable amounts of water as a result of their large surface area. The host particles are coated with a layer of these anticaking particles and are physically separated. Thus the points of contact are between anticaking particles, which are chosen primarily for their low attractivity. This prevents the rest of the powder from clumping.

Another problem with flavors is their tendency to lose intensity quickly during storage. Flavors deteriorate on snacks primarily because of oxygen and ultraviolet rays. To minimize loss, snacks can be packed in nitrogen flushed, aluminum foil laminate bags. Greater than 2% moisture in potato chips also accelerates oxidation of both the flavors and the oils. Flavors can be encapsulated to extend shelf life but this is not common due to the cost.

IX. POWDER PROPERTIES

The effect of size and shape on taste, adherence and flowability has already been mentioned in the section on salt. The increased flavor intensity as the particle size decreases is used in a number of applications, such as powdered sugar on donuts (20).

In a typical gravity fed tumble drum application, the most efficient coating is produced by small, free flowing powders (21). Larger particles are more likely to be unevenly distributed, and are more likely to fall off of the product. Free flowing powders produce a more reproducible flow rate and are evenly distributed across the surface of the product. If the powder is cohesive or ambient humidity gets too high, spices clump, and uniformity in application suffers. Many flavor powders are very hygroscopic and agglomerate easily. Thus the addition of anticaking agents is important to keep powders free flowing. The smaller the powder, the more cohesive it is (22), thus anticaking agents are especially important for small powders. In a pneumatically fed conveyor belt system, free flowing powders are still the most efficient, but efficiency increases as particle size increases. This is because smaller particles are more likely to be lost off the conveyor belt unless a powder recovery system is used.

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CONTENTS

I. INTRODUCTION

Vegetables are increasingly recognized for their values in human diet. A commercial vegetable business is an important sector of the agricultural industry. Several important fresh vegetable commodities (e.g., potatoes, tomatoes, sweet corn, peppers – red and green, onions, lettuce, cabbage, and cucurbits) are currently important in the international market. Production and consumption of processed vegetables are also increasing in the world market.

Vegetables are highly perishable fresh food material. Water loss and post-harvest decay account for most of their losses. The post-harvest technology for treatment and storage of vegetables is critical to minimize wastage and to obtain the maximum benefits from the harvested crops.

Post-harvest technologies include harvesting, handling, maturity, low temperature storage and environmental control (controlled/modified and hypobaric storage), irradiation, use of chemicals and fungicides, packaging techniques and processing of fresh vegetables into suitable products with improved storage characteristics, etc.

II. CLASSIFICATION

Vegetables grown for their leaves or stems are cabbage, kohlrabi, collards, asparagus, rhubarb, salad crops and all leafy greens or potherbs. Vegetables used for their fruits include tomatoes, melons, eggplant, okra, peppers, beans and peas; and those grown for their flower parts include cauliflower and broccoli. Vegetables grown for their underground portions (roots, tubers, bulbs, and corms) are potatoes, sweet potatoes yams, taro, cassava, beets, carrots, parsnips, radishes, turnips, onions and garlic. Crops within a family may vary widely in their physiological characteristics, growth conditions, and requirements for cultural practices. Potatoes and eggplant, for example, belong to the same family, *Solanaceae*, but their requirements are very different. However, other crops in this family, such as tomatoes, eggplant and peppers have similar requirements. While only a few vegetables belong to the class Monocotyledonneae (e.g. sweet corn, onion, garlic, or taro), most other vegetables belong to the class Dicotyledonneae (two seed leaves), including crucifers, peas and beans, and cucurbits. All crops belong to the *Solanaceae* or *nightshade* family.

III. PRODUCTION

With the continuous increase in demand, the world vegetable production has been largely expanded in the past two decades (1). Total acreage of land used for vegetable production was about 47 million ha in 2002, which was 74% increase from 1982. Meanwhile, yield per ha has also increased by 26% within the same time scale, with the adoption of improved management practices, such as, new varieties, optimized fertilization, irrigation, and weed management. Both expansion of land acreage and increases of yield per ha have contributed to the substantial increase of the total production.

The present annual world production of vegetables is close to 800 million metric tones, 2.2 times of what it was in 1982 (1). China is the largest producer of vegetables in the world, accounting for about 47% of the total vegetable and melon production, followed by India (10%), United States (5%), and Turkey (3%). China and India together produce more than half of the world total production.

The production of several vegetable crops in the less-developed countries includes roots and tubers (the cassava, potato, sweet potato, yam and aroid root), which form the majority of the total vegetables produced in the tropical countries providing staple food for over 500 million people. The comparison of total agricultural area covered by the vegetable production, of average yield and import and export figures in the developing and developed countries clearly indicates that there is a great scope to increase the vegetable production by improvements in pre and post-harvest technology. Although the total vegetable area of the developing countries in the world is about six times larger than that of the well developed countries, the mean yield per hectare is only one half. Export of vegetables by developing countries is low and there is a lack of uniformity in the quality of vegetables.

The high production of vegetables in most developing countries is mainly due to the increase in area under cultivation. Owing to poor harvesting, handling, processing and marketing of these perishables, high post harvest losses occur in tropical and subtropical countries, which vary widely according to commodity and areas.

IV. PHYSIOLOGY OF VEGETABLE

A. Seed Storage and Germination

Most vegetable crop growth and development begins with seeds. Physiological aspects of seeds related to storage, germination, quality and enhancements are practically important. Seeds are stored at low water content and they have unique characteristics that permit them to withstand desiccation. The period of storing time from harvesting to sowing can vary from a few months to several years. Two major environmental factors influencing seed storage are seed moisture content and temperature.

Moisture content is determined by the storage relative humidity and by seed characteristics. Seed components determine the water binding status in seed tissues, which governs the types of reactions, enzymatic or non-enzymatic, that may occur during the storage. Since the variation of components in seeds from different vegetables, the water binding status in seeds can be variable, and is classified into three types. Type 1, water is bound tightly and interacts very strongly with charged groups of proteins (2). Type 2, water is less tightly bound and condenses over the hydrophilic sites of macro-molecules (3). Type 3, water is bound with very small energy and forms a bridge over hydrophobic moieties (2).

Temperature has a direct and significant influence on longevity of storage. The rate of seed deterioration increases with increases in temperature at a given relative humidity.

Seed aging (e.g., loss of germination) during storage for a particular species maintained under a given moisture content and temperature can be predicted by using the Ellis and Roberts equation as follows (4):

$$v = K_i - p/\{10\exp[K_E - (C_w \times \log m) - (C_H \times t) - (C_q \times t^2)]\}$$

where v is the probit of the germinability (%) after a storage period of p days; K_i represents the probit of the initial germinability; K_E, C_w, C_H, and C_q are species-specific constants; m is the seed moisture content expressed on a fresh weight basis; and t is the storage temperature (°C). The storability of vegetable seeds varies largely amongst the species.

Seed germination is the transition period between the resting and the growing stages of the plant. Germination

starts with water uptake, of which the process can be explained using the terminology of water potential, the sum of the matric, osmotic and pressure potential. Water uptake in vegetable seeds can be classified as a three-phase pattern, 1) the initial rapid uptake phase, 2) a lag phase, and 3) a second increase in moisture content. Phase I is known as imbibition which is a physical process that happens in both living and dead seeds (5). The water uptake during this phase proceeds very rapidly (such as tomato seeds are fully imbited in a period of 4–8 hours), and is caused by cell wall components and proteins, which form the negative matric potential. With the process of imbibition, the seeds swell due to the expansion of hydrophilic compounds, such as proteins, pectic substances, and cellulose (6). During phase II, the osmotic and pressure potentials regulate the total water potential, because the matric potential is very small. During this phase, enzymes and membranes are functional in the fully hydrated cells as seed advances to the completion of germination. The duration of phase II is largely species dependent and is influenced by the environmental conditions, especially temperature. There is little water uptake in phase II. Phase III of water uptake starts with visible germination. In this phase the seed coat is ruptured by the emerging radicle which forms the root system. Radicle growth is caused by cell elongation, and then followed by shoot growth. Consequently, the seed becomes a seedling and meanwhile losses its ability to withstand desiccation. Drying seeds during phase I and II is not injurious to the viability of the seed, while drying seeds after visible germination will result in death of the radicle.

Factors affecting seed germination include water, oxygen, temperature, and seed quality. While plant population is not a factor, seed vigor is important for yield potential in many vegetable crops, especially those harvested in the vegetative or early reproductive stages.

B. Transplanting

Transplanting is a practice which is often used with small-seeded vegetable crops, particularly those which are slow or difficult to germinate, or need special germination conditions**.** In the areas where growing season is short transplanting technique is also often used. Factors controlling the growth rate of seedlings and transplant size include the size of container cell, above ground space among individuals, supply of water and mineral nutrients, light quality, growth-retardant chemicals, day-night temperature differences and transplant pruning.

The growth rate of seedlings is proportionate to the volume of the container cell. Big container size means that loosely spaced individuals have more total light available which is richer in far-red light than do individuals close to each other. Seedling plants develop elongated stems and petioles when illuminated with light having relatively high amounts in the far-red (725 nm) compared to the red (650 nm) part of spectrum. The more growing space available to the plant, the larger it becomes, and the more rapidly it attains particular stage of growth. Sufficient supply of both macro- and micro-nutrients (N, P, K, Ca, Mg, S, B, Mn, Cu, Zn, Mo, Fe, Cl) is necessary for satisfactory growth of plants in transplant containers. Stem extension is favored with higher day temperature relative to night temperature. Removal of leaves from transplants in early days would reduce moisture loss from the plants in the field and result in more rapid establishment.

Satisfied transplants expected are the seedlings which are capable of withstanding stress during transplanting and of hastening the recovery of active growth in the field. Many researchers have found that early yield of tomatoes is enhanced by producing seedlings in large rather than small containers. However, for production systems in which earliness is not important, such as production for processing tomatoes, transplants grown in small cell volume can give satisfactory yields.

C. Development, Growth and Yield Formation

In general, the entire period of plant development and growth can include two stages, vegetative stage and reproductive stage. Sowing is the start of the development and growth processes, after which plants emerge, grow, develop with or without transplanting. Vegetables may be harvested in the vegetative (such as cabbage and lettuce) or reproductive phase (such as tomatoes and green pepper). After transplanting growth phase or the equivalent phase if directly seeded, the plants will experience the exponential growth phase and the linear growth phase. If the vegetables are grown for reproductive organs, the plants will continue to experience fruit growth phase (flower bud differentiation, anthesis, fertilization, concentrated or continuous fruiting) and grain filling phase. All the physiological processes and consequently the formation of harvesting yield are affected individually and/or interactively by a number of variables, including soil factors and climatic conditions.

1. Soil Factors

Major soil variables affecting vegetable growth and development are nutrients, moisture, aeration and temperature.

Nitrogen is the essential component for amino acids, proteins, and enzymes or co-enzymes. Nitrogen deficiency generally reduces photosynthesis and limits plant growth. In the early stages of nitrogen deficiency, carbohydrates accumulate in leaves and in the root system. The reduction of photosynthetic rate caused by nitrogen deficiency is not apparent until later, so the plants temporarily have a surplus of carbohydrates, which may be available to continue root growth. Induced nitrogen deficiency can cause increases in levels of ABA, which consequently leads to the reduced expansion of leaves. In general, nitrogen

deficiency has relatively larger effect on vegetative growth than on reproductive growth.

The most essential function of phosphorus in plants is in energy storage and transfer. An adequate supply of phosphorus at the early stage in plant life is important in the development of its reproductive parts. A large quantity of phosphorus is found in seed and fruit, and is considered essential for seed formation. A good supply of phosphorus is also associated with increased root growth, greater straw strength, enhanced resistance to disease and other adverse environmental conditions and early maturity of crops.

Potassium, not like nitrogen, phosphorus and most other nutrients, forms no coordinated compounds in the plant. Instead it exists solely as K^+, either in solution or bound to negative charges on tissue surface through radicals. Potassium is involved in activation of 80% of enzymes in plants. Potassium provides much of the osmotic "pull" that draws water into plant roots and maintains plant turgor, which is essential to the proper functioning of photosynthetic and metabolic processes. Potassium deficiency causes plant lodging due to the weaken straw, increases in crop damage by bacterial and fungal diseases, insect and mite infestation, and nematode and virus infection.

In general, the reaction of the plant to deficiencies of particular nutrients will depend on the specific effects that those nutrients have on shoot growth, the rate of photosynthesis, the export of assimilates and their on-time proper distribution. There are interactions among nutrients which can be either positive or negative. For instance, excessive phosphorus uptake may reduce zinc uptake and thus cause zinc deficiency. Increased nitrogen uptake can increase crop phosphorus demand accordingly.

Soil moisture content is another important soil factor influencing vegetable growth and yield formation. Excessive water supply reduces soil aeration and respiration rate of crop roots which consequently decreases water and nutrient uptake, while shortage of soil water decreases nutrient availability to crops and leads to the development of deep-rooting systems.

2. Climatic Conditions

Typical factors of climatic conditions affecting plant growth and development are temperature, precipitation (both quantity and temporal distribution), radiation and CO_2 concentration.

a. Temperature and radiation

Both production and partitioning of assimilates are influenced by light quality and intensity. The photosynthetic active radiation is from 400 to 700 nm. When a plant is transferred to a low light condition, growth of the shoots closest to the light source is relatively less decreased than root growth. Meanwhile, leaves become thinner to maximize the capacity for light interception per unit plant biomass. Partitioning of assimilates for some plants can be affected by light quality. Storage of root-bearing crops by exposing to far-red light enhances stem growth.

It has been long certain that a given genotype of any annual crop has its own definite optimal requirements of temperature and light, called photoperiod. The plant can not proceed at an economically desirable rate to flower formation, flowering and the production of seeds, if the photoperiod is not reached. All crops of tropical or subtropical origin are essentially short-day plants, whereas the majority of crops temperate or Mediterranean in origin are long-day plants. In addition, many species of crops also include genotypes which are intensive to photoperiod, named day-neutral plants.

b. Precipitation

The quantity, intensity, and seasonal distribution of rainfall in a certain area are directly related to the timely supply of soil water to the growth and development of vegetable crops. In order to meet the crop demand for maximized crop production, irrigation is often required when possible. In addition to springer irrigation, drip irrigation or fertigation has been widely used to increase the water use efficiency and to minimize the adverse effects of agricultural chemicals and nutrients on surface and ground water quality.

c. CO_2 concentration

Carbon is a main component of plant biomass, making up 40–45% of total dry matter. Plants adsorb CO_2 mainly from atmosphere, with a small amount from the soil solution, and form carbohydrates through photosynthesis. Increase of CO_2 concentration in the atmosphere enhances the reactions of photosynthesis and consequently the crop yield. Fertilization of CO_2 has been largely used in vegetable production under controlled environment, such as greenhouse.

V. COMPOSITION AND NUTRITIONAL VALUE OF VEGETABLES

Leafy vegetables (or edible leaves) form the second important category of vegetables. They are the most important sources of essential minerals, vitamins and dietary fiber for the regulation of digestive tract. The third important category of vegetable, "fruit-type vegetables," including tomato, eggplant, pumpkin, squash, gourd, also provides protein, vitamins, and minerals, apart from their greatest value in adding variety to the human diet. The immature or green grain legumes (peas and beans) are the most important sources of protein. With some crop types, in addition to their pods, leaves, flowers and sprouted dried seeds are also utilized.

The nutritional value of vegetables as a food source of essential minerals, vitamins, dietary fiber, and as a source of many health-promoting compounds has been well recognized. Vegetables not only supply fair amounts of carbohydrates, proteins and energy, but also play an important

role in human nutrition in supplying certain constituents. Dietary fiber and secondary metabolism constituents in vegetables are increasingly realized for their health benefits in recent years. The possible beneficial effects of crude fiber and secondary metabolism constituents derived from vegetables on human health are being examined by extensive research *in vivo* and *in vitro* trials.

Vegetables are fair suppliers of calcium, phosphorus, and iron for the needs of human body. Leafy greens are rich sources of calcium (or calcium oxalate), phosphorus and iron. Vitamin C is an important constituent from vegetables. Human's dietary vitamin C is obtained mostly from vegetables. Some vegetables are rich sources of vitamin A, thiamin, niacin and folic acid. Vitamin A (beta-carotene) is essential for the normal functioning of the visual processes and structure of the eye. Vegetables do not contain an active vitamin A compound, retinol, but certain carotenoids such as beta-carotene are converted by man to active retinol in the body. The carrot, sweet potato, and spinach are rich in vitamin A. Carbohydrates in vegetables is in the form of cellulose, hemicellulose, pectic substance and lignin. The human body lacks some enzymes like cellulose, hemicellulase and pectinase. The epidemiological evidence shows that dietary fiber is critically important to cure several diseases of human body. Most vegetables, especially the leafy vegetables, such as celery, cabbage, spinach and lettuce, contain a high percentage of cellulose (fiber). Vegetables also supply protein, lipid, organic acids, and volatiles giving a characteristic flavor or aroma to the produce.

While cucurbits are generally low in carbohydrates, starchy vegetables such as potatoes, sweet potatoes, taro and cassava are high in carbohydrates. Most vegetables contain about 2% protein and some, such as dried seeds of beans, peas and lentils, are rich sources of proteins. The lipids comprise less than 1% of most vegetables and are associated with protective cuticular layers on the surface of the produce and with cell membranes. Spinach, legumes, potatoes and tomatoes contain a relatively higher proportion of citric acid; and melons, broccoli, carrots, celery, lettuce and onions are rich in malic acid.

Another special group of components in vegetables that needs to be pointed out is the toxicants, which are defined as the incidence of adverse effects in humans or in animals. The range of toxicants in vegetables is large, but mainly including lectins, glycoalkaloids, *S*-methylcysteine sulfoxide (SMCO), isoflavones, glucosinolates, nitrate, furanocoumarins and oxalates.

Glucosinolates are thioglucosides which are widely distributed in vegetables, particularly among members of the cruciferae. Over 100 different glucosinolates have been identified. They all share a common structure, being composed of a thioglucose group attached to an R-group. The R-group can be within a wide range. In cabbage, for instance, the main glucosinolates are sinigrin, progoitrin and glucobrassicin. These three glucosinolates also predominate in a variety of other brassica vegetables, including broccoli, cauliflower, etc. In addition, brassica vegetables also contain a non-protein sulfur amino acid in the form of SMCO. Contents of SMCO in brassica vegetables can be very different. For instance, concentrations of SMCO are 14, 18, 19, and 68 mg/100g fresh weight in cauliflower, cabbage, broccoli, and Brussels sprouts, respectively (7). Highest levels of SMCO are generally found in the young leaves and growing shoots, but increases have also been observed as the brassica crop reaches maturity.

TABLE 170.1
Concentrations of Nitrate in Some Vegetables (*Source*: Lyons et al. 1994)

Vegetable	Nitrate-N (mg/kg fresh weight)
Cabbage	70–370
Lettuce	65–330
Parsley	10–330
Beetroot	290–650
Radish	145–790
Broccoli	30–85
Brussels sprout	10–15
Cauliflower	40–75
Celery	295

Vegetables are almost the greatest source of nitrate in the human diet, accounting for 76% of the total intake (8). Concentrations of nitrate in vegetables vary very widely, because of the physiological variation of each species, soil nitrogen status, fertilization rate and timing, as well as the effects from environmental factors (temperature and precipitation). Levels of nitrate tend to be negatively correlated with light intensity and temperature. A summary of data relating to nitrate contents of different vegetables is presented in Table 170.1. Based on the nitrate concentrations, various vegetables can be grouped into three categories: 1) high in nitrate content, including celery, lettuce and beetroot; 2) medium in nitrate content, including spinach, cabbage and potatoes; and 3) low in nitrate content, mainly including tomatoes.

Lectins are present in the seeds of a variety of plants, including kidney beans (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*) and jack bean (*Canavalia ensiformis*).

Although the above components have been believed to be toxic, it has been reported that moderate intakes of some of them may be beneficial in the prevention of cancers, such as SMCO and isoflavones.

VI. POST-HARVEST TECHNOLOGY – HARVESTING, STORAGE AND TRANSPORTATION

The post harvest technology includes different commodity treatments, such as the use of physical (low and high

temperatures, modified atmosphere, correct humidity, magnetic fields, preservation by ionizing radiations, good sanitation and development of wound barriers) and chemical (waxes, fungicides, growth regulators, etc.) methods, improvements in packaging techniques, and processing of vegetables into durable products. The vegetables are harvested, packed, transported, prepared for market and ripened and stored or sold in retail markets. Post-harvest biotechnology facilities to minimize losses of vegetables. An efficient management of production, handling, storage, pricing and marketing will increase the economic development of vegetable production and distribution system.

A. Climate

The climate factors influence the vegetable crop production and post-harvest losses. The major factors are inadequate distribution systems, insufficient marketing and storage facilities. Numerous pre- and post-harvesting practices can be adopted to minimize post-harvest losses of vegetables. Pre-harvest factors include harvesting and handling, packaging and transportation, curing, refrigeration, phytosanity and sprout control.

B. Harvest Time

Vegetables reach the peak of quality at a definite time, depending upon the variety, time of planting, location, temperature, soil type, available water, fertilizers and cultural practices. The time of harvesting is critical for quality and the manner of harvesting and handling is economically critical. Vegetables at the peak of quality are highly perishable and should be harvested, handled and processed within a few hours. The texture, sugar content, color, and volume of peas and corn are criteria for determining the time of harvesting. Serious losses occur when harvesting is earlier or later than the optimum.

C. Vegetable Sorting

Leafy vegetables are tumbled in drum-type cleaner to dislodge sand and soil particles, but these mechanical cleaning operations can bruise or crush delicate tissues and increase respiration and loss of vitamin C through oxidation. The losses of nutrients that occur during such processing depend upon the vegetable crop, cultivar, maturity, freshness and season.

The most common types of conveyors for handling individual vegetables are flighted or cupped belts. However, both types present problems when each individual vegetable must be handled separately from the others.

D. Vegetable Handling

The most obvious losses after harvest are due to mechanical injury, moisture loss, decay and aging. Abusive, rough handling and holding or transporting at undesirable high or low temperature increase these losses. Losses can be substantially reduced by following approved packaging, transport and handling practices. After over maturity the next most important factor in lowered quality of vegetables for processing is deterioration during handling. The principal hazards are:

1. Metabolic changes associated with respiration, heating, ripening and aging- changes in composition, texture, color and flavor.
2. Bruising and other mechanical injury.
3. Moisture loss, with resulting wilting and shriveling.
4. Mold, mildew and other microorganisms by heat of respiration.

E. Moisture Loss

Loss of moisture with consequent wilting and shriveling is one of the obvious ways in which freshness is lost. Serious wilting may occur within an hour when leafy vegetables are harvested on a hot sunny day. Moisture losses of 3 to 6% are to cause marked loss of quality for many kinds of vegetables. Moisture losses may be reduced by lowering the air temperature, raising the relative humidity, reducing air movement or by protective packaging. Vegetables with greater exposed surfaces per unit volume have a faster rate of water loss. The type of protective surface is also important. Carrots have less protective waxy covering than apples or pears and consequently lose water faster. Tomatoes have a relatively impermeable skin and lose moisture mainly through the stem scar.

F. Vegetable Storage

The only reasons for holding vegetables for processing in common or refrigerated storage are to provide a regular supply and to allow the ripening to even up. Chilling injury may occur with certain vegetables during rapid cooling. Transportation and distribution of vegetables are the most important areas of post-harvest losses. Physical and mechanical injuries can occur during the hauling, grading and packaging operations before shipment, which may not be readily apparent in vegetables such as potatoes until they have been stored for a period of time. The greatest increase in injury occurred between the time the potatoes were in the wholesale warehouse and the time they were placed on retail shelves for sale. The actual transporting of potatoes from shipping point to destination increased hard and serious bruises by 5%. During distribution, which causes more bruising than any other operation, the hard and serious bruising increased by 12.8%. While the vegetables seriously injured during harvesting, handling and storage may still be processed into some form of human food, the vegetable

products like potato tubers seriously injured during transportation and distribution may be lost completely for human use. Potatoes can be stored for about 1 year with small losses, due to recent developments in storage technology.

Refrigeration or low temperature storage can most effectively extend the shelf life of horticultural produce and reduce their post-harvest losses. Refrigeration is produced mechanically by the evaporation of a compressed, liquefied gas (e.g., ammonia, Freon) in a closed system. Refrigeration is the most widely used short-term preservation method for a variety of vegetables. It is used throughout the distribution chain by commercial firms, retail outlets and the consumer. Vegetables can be kept in good condition for several months in cold storage.

Although most vegetables are processed right after harvest, asparagus, carrots, bell peppers, onions, potatoes and others are sometimes stored under controlled temperature and humidity for some time prior to processing in order to suit the operating capacity of the plant. The recommended temperature and humidity for storage prior to processing are designed to suit the operating capacity of the plant.

Leafy vegetables should be promptly cooled to about 32F and held there until processing. Wilting and disorders increases in severity as temperature increases. Vacuum cooling is the most common method of pre-cooling although hydro-cooling is used on some leafy vegetables. High humidity (about 95%) is essential to prevent wilting.

Root crops such as beets, carrots, radishes and turnips, are generally stored at 32°F to retain their quality. Decay can cause substantial losses in root crops during even a week or ten days if they are held above 40F. Rapid cooling of root crops to storage temperature is not as critical as for leafy vegetables. However, roots should be brought into desirable temperature range promptly after harvesting due to respiration can rapidly reduce quality. A high relative humidity is essential for storage of cold requiring roots if desiccation is to be avoided particularly during prolonged storage of root crops.

Storage at a few degrees above their freezing point is desirable for all of the commonly grown leaf, stem, and bud vegetables and, for the most part, the root vegetables. Storage life of the leafy vegetables is only a few days, even in optimum environments. The root crops, including carrots, parsnips, turnips, beets and horseradish, which are adapted to storage for several months, are commonly held in ventilated storage. Other vegetables, such as potatoes, carrots, sweet potatoes, turnips, dry onions and cabbage are usually stored under ventilation and may be refrigerated to extend their storage life beyond their growing season. Vegetables including cucumbers, eggplant, green beans, okra, sweet potatoes, squash and tomatoes are native to subtropical or tropical areas are not adapted to low temperatures.

Controlled Atmosphere (CA) storage is one of the most important technologies in vegetable storage systems. Controlled atmosphere storage is a system for holding fresh vegetables in an atmosphere that differs substantially from normal air in respect to the proportion of nitrogen, oxygen and carbon dioxide. The composition of the atmosphere may be altered by restricted venting of the storage room or the container by scrubbing the atmosphere of carbon dioxide or oxygen or by adding individual gases to the container while reducing the proportion of others. This method if combined with refrigeration retards respiratory activity and may delay softening, yellowing, changes in quality and other deteriorative processes by maintaining an atmosphere of higher carbon dioxide and oxygen than in normal air. The limits of tolerance vary according to a function of the temperature, duration of storage, concentrations of supplemental gases, type and physiological age, and anatomy and morphology of the vegetable produce involved.

CA storage has proved beneficial to extend the life for vegetables that deteriorate rapidly or those that complete ripening after harvest. Temperature and duration of storage and commodity to be stored are the interdependent factors, each influencing the decision of use of the gas mixture in CA storage.

VII. VEGETABLE PROCESSING TECHNOLOGIES

Processing begins in the field with many vegetables. This is true with mobile cutters for greens, viner/shellers for peas and beans, juice extractors for tomatoes, and graders/washers for many vegetables. Advantages are that harvesting/processing can be stopped or started at any point to accommodate variation in the field. There is no lag in time in which freshly harvested sometimes badly bruised product can deteriorate while waiting for the next step in processing and most of the refuse including vines, shells, leaves and other organic waste is scattered over the field to be incorporated into the soil. There are several steps common to the preparation of most vegetables for processing.

Harvest in the immature tender stage, before any portion becomes fibrous and tough. Corn should be full grown, but tender; peas and beans should be green; stalks of asparagus, stems of greens and shells of snap beans should be without fiber. There is a tendency for most vegetables to be harvested after the peak of quality.

Grade to remove trash, over mature, diseased, insect infested vegetables, and other materials that would impart an off flavor to the product. This may be with a roller grader/sizer, a blower, or rod/shaker, followed by hand inspection. Bruising or cutting which might cause loss of juices should be avoided.

Weigh to ascertain pay rate, yield and production rate. Rinse in water to remove surface dirt, insects and small trash not removed by the blower. A detergent may be used on vegetables taken from the soil, such as potatoes, sweet

potatoes and turnips and leafy vegetables like spinach and turnip greens, provided it is thoroughly rinsed off.

Prepare as required for individual vegetables. This includes peeling, shelling, shucking, trimming and cutting and dicing.

Vegetable processing steps include handling, transportation, refrigeration, molding, washing, trimming, blanching, freezing, canning, drying, packaging, use of chemicals, radiation, storage and ultimately cooking or heating.

Vegetables are processed into more stabilized products that can be used the whole year round in the world market by canning, freezing, dehydrating and ionizing radiation. Some processed products are canned soups, chutney, catsup, instant dehydrated mashed potatoes, etc. The processing of vegetables is as follows:

1. Removal of water- to reduce moisture content of vegetables to lower activity of microorganisms;
2. Heat sterilizing- to destroy or inactivate enzymes and microorganisms (Suitable packaging prevents entry of microorganisms into processed products.);
3. Lowering temperature- to slow down most biochemical reactions that can deteriorate food;
4. Providing a chemical environment that will not permit certain deteriorative actions to proceed;
5. Sterilizing with ionizing radiation to destroy enzymes and microorganisms.

In most cases, combinations of preservation technologies are recommended. For example, heat sterilizing effectively destroys microorganisms, then sealing in a container prevents further contamination, and storage of the canned product under refrigeration reduces deterioration normal for any canned product, cold storage of dried products extends their storage life, etc.

A. Blanching

Blanching can fulfill one or several of the following purposes:

1. Inactivation of enzymes prevents discoloration or development of unpleasant taste during storage. Colors caused by the presence of chlorophyll or carotenoids are also protected from enzymatic degradation.
2. Proteins are forced to coagulate and shrink under liberation of water. If this shrinkage happened during sterilization of canned products, they would appear to be underweight. Also, starch that could otherwise cause a cloudy appearance can be removed.
3. Oxidation during frozen storage will be reduced.
4. Many products obtain a clearer color.
5. Defective parts become more visible so the product can be sorted more effectively.
6. The microbial status is improved because vegetative cells, yeast and mold are killed.
7. Cooking time of the finished product is shortened. If the blanching periods for the individual components in mixtures are adjusted, the heating requirement for the preparation of the finished product is equalized.

When water or steam is used for heating, leaching of vitamins, flavors, colors, carbohydrates, and other water-soluble components takes place. If products are going to be frozen after blanching, a chilling step will generally take place before transporting the product into the freezer. A good blanching technique must fulfill the following essential demands:

1. A uniform heat distribution to the individual units of the product.
2. A uniform blanching time to all units of the product.
3. No damage to the product during the entire blanching and cooling process.
4. A high product yield and quality.
5. Low consumption of energy and water.
6. Tough, reliable design, giving an operation without problems even when running in continuous three-shift operation. This also includes quick and proper cleaning.
7. The design must also provide a pleasant working environment for the staff without any unnecessary noise, heat radiation, and loss of steam and process water with foam and product particles flushing on the floor.

When blanching vegetables before freezing, inactivation of the enzymes is dependent upon both time and temperature of the heat treatment. Other effects of heating, which may respond differently to time and temperature conditions, include color changes, softening, gelling of starch and extraction of various soluble components.

B. Canning

Canning is the major method of preserving vegetables, and is founded on the most effective method of destruction of microorganisms. In canning process, the vegetable is placed inside the container, the air is removed by vacuum, and the cans are hermetically sealed. The cans are placed in the retort, and sterilized with steam. The basic heat penetration characteristics are convection and conduction. The constancy of the rate of heat penetration for a given product in a given size container and the ability to measure these constants with accuracy have

resulted in the remarkable safety of commercially canned vegetables.

C. Freezing

There are three types of freezing processes. The individually quick-frozen vegetables are frozen by the fluidized bed process and then packed in suitable containers. The second process involves placing the unfrozen prepared vegetables in a container and then freezing it. The individual particles do not cohere and the final package contains individual portions of the food rather than a solid block. Commercial freezing is conducted with moving air, termed blast-freezing. Air is accelerated by fans and passed over the vegetables. With advances in refrigeration engineering, many plants are now freezing foods at −40°C.

The third freezing process is that of immersing the vegetables in a liquid. Some of the liquids used for vegetables freezing are liquid nitrogen, liquid carbon dioxide and Refrigerant 12. Vegetables freeze extremely rapidly in contact with such liquids. Refrigerant 12, a fluorinated hydrocarbon, can be adjusted to various temperatures. The use of such liquid nitrogen, liquid carbon dioxide and Refrigerant 12 meets food safety regulation.

D. Fermentation

Fermented vegetables are the food products by the fermentation of lactic acid bacteria, which are preserved primarily by acidification. Olive products and sauerkraut are solely produced by fermentation. Some cucumber pickles are produced by fermentation, in which spices and salt are added before fermentation and fermentation occurs in the presence of these seasonings. This is called the genuine dill fermentation, but is rapidly being displayed by salt stock and fresh pack pickling methods in which fermentation does not occur and the acid is added directly to the product in the final container. A wide variety of vegetables are pickled commercially. Pickling is usually combined with some type of heat treatment in the final container to prevent secondary fermentation and subsequent spoilage. A recent development in the pickled vegetable industry has been the development of the fresh pack or pasteurized cucumber and other vegetable pickles. For example, cucumbers are packed directly into jars, then covered in brine which is made of salt and vinegar, preferably containing some lactic acid and seasonings. The residual sugar in the cucumber can be fermented by the action of the lactic acid bacteria.

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171 Chemistry and Technology of Tofu Making

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CONTENTS

I. INTRODUCTION

China is the birthplace of soybeans. In Oriental countries such as China, Korea and Japan, soy foods have been consumed for thousands of years. In the1800s, soybeans were introduced to America, but large-scale production of soybean began only after World War II. In the first part of the 20th century, soybeans were known to the Westerners as an oilseed and feed-stuff only. Aside from the image problem, the major obstacles in the utilization of whole soybean for foods in the Western society include the beany flavor and the flatulence factor. Substantial use of soybeans for

foods did not take place until recent decades, where a small part of defatted soy meal was used or processed further for human foods. However, since 1980s, soybeans have been used for making a variety of soy foods in America. Hence, some soybeans are known as vegetable legumes and soy foods are popular vegetable foods. In very recent years, because several soybean components have been discovered to possess health benefits, hence, soy foods have become health foods. Consumption of soy foods in America has increased dramatically in the last few years. Soy foods are finding their ways in the main stream supermarkets. The total retail value of soy foods has exceeded 4 billion US dollars. The United States of America produces one half of the world soybeans, which are estimated to be approximately 159 million metric tons (1). Only approximately 5% of the US soybeans are used for making foods. However in the last three years, it was estimated that the annual soy food market had increased by 25% per year. Soymilk, tofu and meat analogs are the three major soy food types in the USA. The increases in soymilk and tofu retail values in the USA are about 40–50% and 15–20%, respectively, each year since 1997 (P. Golbitz, personal communication, 2000), while the overall growth of the US food industry has been only 3% per year. It has been predicted by the United Soybean Board that by the year of 2010, soyfoods' retail value will reach 100 billion dollars in the United States.

Soy protein has long been known to have a good nutritional quality. Recent discoveries of potential health benefits of soy foods include reducing the risk of cardiovascular diseases, preventing certain cancers, reducing postmenopausal syndromes, and increasing bone mass density all contribute to the recognition of soybean as a health food. US Food and Drug Administration has approved a health claim for processed foods containing soy proteins that states 'consumption of 25 gm soy proteins per day in conjunction with a low cholesterol diet would reduce the risk of heart disease.' All of these will continue to enhance the consumption of this ancient Oriental food crop in America and other parts of the world.

Tofu has found its history dated back to the China's Han Dynasty approximately 2000 years ago. Tofu has been an integral part of the Chinese food culture; it is indispensable in the diets of Chinese and the people of several other East Asian countries, including Japan and Korea. Soybeans have contributed to the health of Chinese people in the history. We believe that a large-scale prolonged protein malnutrition has never occurred in China might be attributed to the ready availability of soy foods. Soy foods are not only nutritious but also very delicious, which have been included in thousands of dishes of Chinese foods. Because of the functional properties and health benefits reported in recent years, soy foods are gaining acceptance increasingly in the Western society.

Since its original invention, tofu manufacture process has been improved greatly. Many manufacture and utilization methods have been developed in various countries and regions. Japan has taken the leadership role in the advancement of the science and technology for tofu making. It is well known that making tofu is not a difficult task. Many people can claim that they can make tofu. However, making excellent quality tofu consistently is not an easy matter. The principles of tofu making are simple, which consist of two main stages: (a) the preparation of soymilk and (b) the coagulation of soymilk to form bean curd that is then made into various types of tofu. However, many factors involve in the processing of tofu and raw bean components affect substantially the quality of tofu. There have been several great books related to tofu making, including the "The Science of Tofu" (2), the "Tofu & Soymilk Production, the Book of Tofu, Volume 2" (3), and the "Soybeans: Chemistry, Technology and Utilization," (4). In this chapter, we focus on recent studies related to soybean quality and tofu making and try to organize available information together to show how various factors affect tofu quality.

II. TOFU PROCESSING METHODS

Tofu manufacturing requires a series of unit operations. Generally, three steps are critical in determining product type: (a) soymilk extraction and solid content, (b) coagulation method (types of coagulants, breaking or not after curd formation), and (c) pressing or not. However, all methods for making various tofu products begin with similar steps for soymilk as shown in Figure 171.1. The traditional Chinese method separates raw soymilk from the okara (residue) before heating. In the Japanese process, heating the slurry "go" prior to separation facilitates soymilk extraction and increases tofu yield. However, Beddows and Wong (5) reported that yield and quality of silken tofu made by a laboratory scale with the slurry filtration prior to heating are better than that with heating prior to residue separation. Both Chinese and Japanese methods for extracting soymilk are known as the traditional Oriental methods because of the presence of beany flavor in the final soymilk product. Regarding the beany flavor, several modern methods aimed at improving soymilk taste and flavor have been developed in the past decades. We will discuss this in more detail in the lipoxygenase section. In tofu industry, many manufacturers worldwide have adopted the Japanese process because of higher tofu yield, available tofu manufacturing machines, and a lower beany flavor than the Chinese process. Some industries wash the okara one or two times to extract residual proteins/soluble solids and use the wash water to grind the soybeans to improve yield.

After soymilk is produced, various steps are used to manufacture different types of tofu. Tofu in the market is

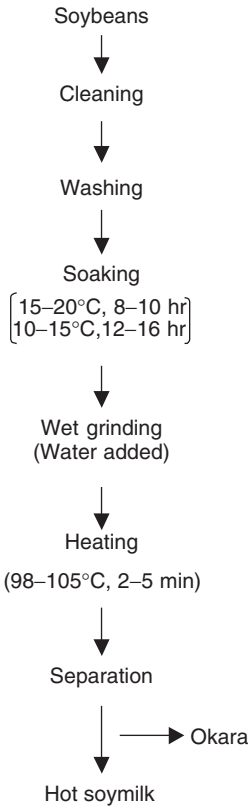

FIGURE 171.1 Initial steps in the preparation of soymilk for tofu making.

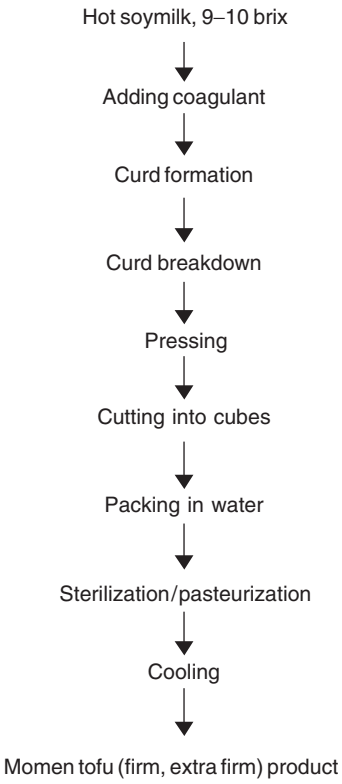

FIGURE 171.2 Scheme for momen tofu production.

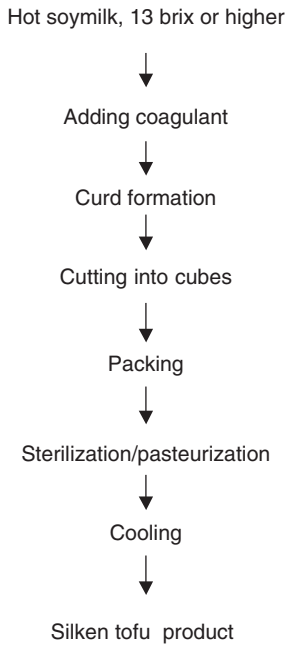

FIGURE 171.3 Scheme for Silken (Kinugoshi) tofu production (no curd breaking and no pressing).

generally classified into soft, firm, and extra firm tofu, based on water content and textural properties. Tofu is classified into momen (regular), kinugoshi (silken), soft, packed silken, and aseptic tofu, depending on processing methods. Figures 171.2 to 171.5 describe the methods used in the tofu industry for the production of momen tofu, silken (Kinugoshi) tofu, filled packed silken tofu, and soft tofu, respectively.

A. Soaking

After proper washing, soybeans are soaked in water to soften their cellular structure for water grinding. Soaking time depends on water temperature, the soybean variety, and the age of soybeans. Temperature is the main factor affecting the rate of water uptake, with higher rate associated with higher temperature (6). Generally, soaking in ambient water takes 8–10 hours in summer and 16–18 hours in winter. After soaking, the beans weigh approximately 2.2–2.3 times of their initial weight (3).

B. Grinding

After soaking, soybeans are ground with water into slurry using a stone-mill or a stainless steel grinder. The amount of water added during grinding depends on the type of final products. For example, the water dosage for silken tofu, soft tofu, and regular tofu is 5, 7–8, and 10 times of raw soybean weight, respectively (2). Proper grinding gives appropriate small particle sizes in the slurry and

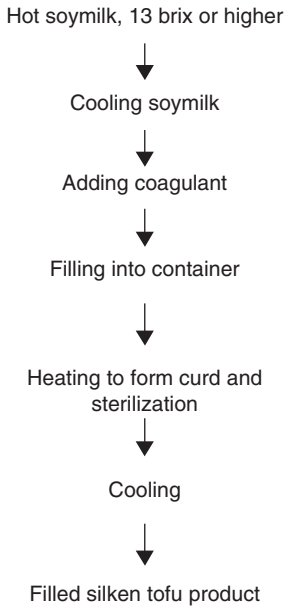

FIGURE 171.4 Scheme for manufacturing filled silken tofu (cold filling and curd formed in container).

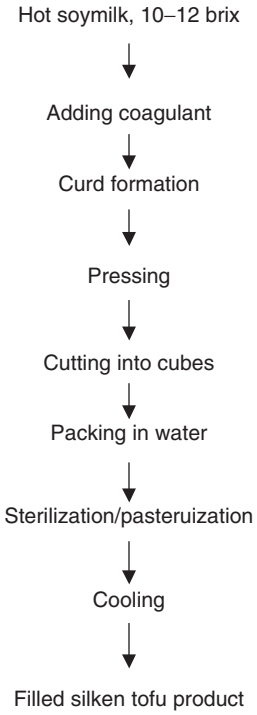

FIGURE 171.5 Scheme for manufacturing soft tofu (no curd breaking).

facilitates the extraction of solid and nutrients into the soymilk. The smaller the particles, the better the extraction, but okara (residues) becomes more difficult to separate. The water temperature of grinding affects not only the flavor of soymilk, but also the texture of tofu. Tofu firmness decreases as the water temperature of grinding increases between 0°C and 50°C (7). They found that the relationship is related to the content of sulfhydryl groups (-SH) in soymilk. The decrease of –SH group may be caused by a lipid oxidation activated by lipoxygenases in soybeans. Since water composes of 88–90% of tofu weight, it plays an important role in determining the tofu taste. The source and quality of water are always an important concern to tofu manufacturers. Water, containing a proper amount of minerals (about 100 mg/L) including calcium, magnesium, sodium, potassium, iron, and manganese, provides a harmonious and mellow taste (2).

C. Heating

The step of heating is essential during the tofu processing not only for killing microorganisms in the slurry, improving nutritional quality by inactivating trypsin inhibitor (TI), and reducing beany flavor, but also for denaturing proteins so that they can coagulate into curd in the presence of a coagulant. Before heating, soy protein molecules maintain their native globular structures in which the hydrophobic regions are wrapped inside. Upon heating, the soy proteins are denatured resulting in native molecules unfolded, and their hydrophobic groups expose to outside, consequently protein solubility decreases due to aggregation. What extent of heat treatment is considered adequate for soymilk and tofu making? Hackler et al. (8) conducted a study of heat treatment on nutritive value of soymilk protein fed to weaning rats, and found that heat treatment should be sufficient to inactivate 80–90% of trypsin inhibitors for maximizing nutritive values. Trypsin inhibitors are heat resistant. At 100°C, 14 min are required to inactivate 80% TI or 30 min to have 90% TI destruction. Wilson (9) suggested that the time/temperature requirement for soymilk be based on 85% TI inactivation. Trypsin inhibitors are water-soluble proteins, a part of which may be released in the whey during pressing of tofu making. Thus, the slurry used for tofu making requires shorter heating time than that for soymilk as the final product. Watanabe (2) recommends that boiling at 100°C for 3–5 min is required for tofu making.

The optimum heating time of soymilk for making tofu corresponds approximately to the maximum amount of sulfhydryl groups. If heating is not adequate, soy proteins do not dissociate into subunits; but in excessive heating, sulfhydryl groups are oxidized by air (10). Tofu prepared with soymilk, which has been heated at 100°C up to 60 min, is softer than that from usual preparations (100°C, 3 min) (11). This may be due to the oxidation of sulfhydryl groups of soy protein during excessive heating, resulting in the decreases of sulfhydryl group content and tofu hardness. In the Japanese method, cooking the slurry for about 7 to 14 min at 100°C gives the best soymilk solid and protein recovery, especially for tofu (12). In the Chinese method, the hardness of tofu increases slightly

from 0 to 12 min of boiling soymilk, but decreased significantly after 30 min and 60 min of boiling (13). Amino acid composition of soymilk has no significant changes when heating at 93°C, but the amount of cystine and tryptophan decrease while heating at 121°C from 0 to 121 min (14). Approximate 30% of cystine and methionine are destroyed after 30 min of boiling soymilk (15). In some tofu factories, the slurry is heated by a continuous pressure cooker at various pressure increasing from 80°C to 105°C for times ranging from 4–20 min.

D. Separation of Soymilk

In the Japanese process, soymilk is extracted from the slurry after heating. The separation of a small volume of slurry can be done by filling the slurry in a cotton cloth bag then pressed by hands. Industrial processing can be done by drum pressing, screw pressing, centrifugation, or shaker-filtration. The efficiency of soymilk separation depends on the extraction pressure and pressing time, the pore size of the filter or screen, the particle size of slurry, and whether the okara is re-washed or re-pressed. When the okara is re-washed and re-pressed to extract more protein and solids, the yield of tofu can increase by 15–20%. Okara contains 76–80% of moisture (24–20% solids) after being well pressed. About 29% of the solids and 17% of the protein in the original soybeans are remained in the pressed okara that has not been washed (3). On dry weight basis, okara comprises 25–28% protein, 9–11% lipid, 40–44% insoluble fiber, 13–15% soluble fiber, and 4–5% soluble carbohydrate (16). Most of total fiber in soybeans is concentrated in okara.

E. Coagulation of Soymilk

Coagulation is the most important and the most difficult step in tofu making because it depends on the complex interrelationship of many variables, including soybean variety, soymilk concentration and pH, temperature, type and amount of coagulant, and coagulation method. Hot soymilk is usually coagulated to form curd by adding a salt or an acid coagulant. Tofu coagulants are classified into four basic types: (A) nigari-type or chloride-type coagulant, including magnesium chloride, calcium chloride, and sea water; (B) sulfate-type, including calcium sulfate and magnesium sulfate; (C) glucono-delta-lactone (GDL); (D) acidic coagulants including citrus juices, vinegar, and lactic acid (3). Each type of coagulant has its advantages and disadvantages.

1. Type of Coagulants

Nigari or chloride-type coagulants include natural nigari, refined nigari, calcium chloride, and seawater. Natural nigari, known as 'bittern' in the West, is extracted from seawater by removing most or all of the table salt (NaCl) and water. It consists primarily of $MgCl_2 \cdot 6H_2O$ (92.3%) plus all of the other salts and trace minerals in seawater (3.8% $MgSO_4$, 1.7% NaCl, 1.2% KCl, and 1% $CaSO_4$). Refined nigari is a relatively pure magnesium chloride (99.5%). Calcium chloride ($CaCl_2$) is not found in seawater, it is not a traditional nigari-type coagulant. However, it gives tofu excellent flavor, almost identical to that made from natural nigari or magnesium chloride nigari. Moreover, it is the cheapest nigari-type coagulant and the food-grade type produced in the U.S. and on the GRAS (Generally Recognized as Safe) list. In Japan, most tofu industries use nigari-type coagulants in combination with calcium sulfate rather than alone. Nigari-type coagulants make the most delicious tofu, prized for its wonderful subtle sweet flavor and aroma. However, they have some disadvantages compared with calcium sulfate and GDL. They react very rapidly with soymilk, so their use requires more skill and attention and must be added slowly. Due to the extremely quick reaction of nigari with soymilk, the coagulated bean curd is destroyed while agitation is still going on. The nigari-coagulated bean curd does not incorporate so much water as the sulfate type, consequently produce tofu with lower yields and coarser texture than that made with calcium sulfate. Nigari alone is not suitable for making silken tofu from hot soymilk, because the high temperature and high solids content of soymilk make it extremely difficult to solidify uniformly with nigari in such a short reaction time. However, filled silken tofu can be made with nigari if the soymilk is cooled to a low temperature (e.g. 4°C) prior to coagulant addition, and followed by heating to slowly coagulate the proteins.

Sulfate-type coagulants (especially calcium sulfate known as gypsum) are the most widely used tofu coagulants in the world. They have low water solubility (3.0 g/L at room temperature), which is an important factor in determining the speed of the coagulation reaction. Because of their low solubility, they react slowly with the soymilk; consequently allow the formation of bean curds with a high water-holding capacity. Thus, they give 15 to 20% higher bulk yields than nigari. The resulting tofu has a soft and smooth texture. Calcium sulfate can be used to make regular, firm, soft, silken, and even packed (package-filled) tofu, whereas it is not easy to make the latter two types with nigari. Calcium sulfate is easy to use even by relatively unskilled tofu makers. Even if agitation is carried out slowly or the dosage varies slightly, the differences in tofu yield and texture are not very large. Calcium sulfate as tofu coagulant produces tofu with a mild or bland flavor; however, the taste is slightly inferior to that of nigari tofu.

GDL is an oxidation product of glucose. It is manufactured from corn starch by a fermentation process, and was first used for silken and packed tofu production during the 1960s in Japan (3). GDL is fundamentally different from nigari and gypsum-type coagulants, in which an acid

rather a salt does the coagulation. Upon being dissolved in water, it is slowly hydrolyzed (about 2–3 hr) to gluconic acid by water. The pH of 1% fresh aqueous solution at room temperature is 3.5, it drops to 2.5 due to the conversion to gluconic acid within 2–3 hr. For packed and silken tofu, GDL is dissolved in previously cooled soymilk and forms gluconic acid gradually, then heat coagulates soymilk with this acid to form homogeneous solidified curd with rich water holding property in the container. The GDL gives tofu a slightly acidic flavor and tender jello-like texture. For better flavor and texture, GDL is often used in combination with a calcium sulfate.

The acid-type coagulants, including lactic acid, acetic acid, and lemon juice, work well as natural coagulants. However, the yield of tofu is low, the texture is slightly crumbly, and the flavor of tofu is a little tart, when compared with nigari and calcium sulfate as coagulants (17,18).

2. Soymilk Concentration

The solid concentration of soymilk is related to "water-to-bean ratio" that is defined as the total weight of water added to the beans during soaking, grinding, and cooking divided by the original weight of the soybeans (3). The ratio of water:beans can be very critical to affect protein extraction yield and the properties of tofu . In the range of 9:1 to 14:1, 10:1 gave the best result in protein recovery (19). For making regular tofu, the best water-to-bean ratio is about 10:1 which results in a soymilk with 6.0 to 6.3% solids and 3.0% protein (12). However, 5:1 to 7:1 ratios are required for making soft tofu or silken tofu (10). The amount of coagulant required to reach the optimum coagulation varies with the concentration of soymilk. Watanabe et al. (12) reported that more coagulant is required for more concentrated soymilk (in the range of 3% to 8%) to reach the same level of whey transparency, and the dosage for calcium sulfate was about 10–20% more than that for nigari. In our laboratory, we found that the coagulant concentration required to reach the optimum coagulation of silken tofu increases linearly with the soymilk solid content in the range from 6% to 11%, and the concentration for magnesium chloride is about 13–15% more than that for calcium chloride (unpublished data).

3. Coagulant Concentration

The amount of coagulant added into soymilk affects greatly the yield, texture, taste, and aroma of tofu. In general, by observing curd formed and whey produced during pressing, tofu makers could tell whether the amount of coagulant is appropriate or not. When a proper amount of coagulant is used, the whey is transparent and amber or pale yellow. In the case of too much coagulant used, the whey is yellowish color and the curds have a coarse or crumbly texture. If too little coagulant is added, the whey is cloudy and some uncoagulated soymilk may be remained. Several methods have been compared to determine the optimum amount of coagulant in tofu making, including light transmittance of whey (%T), whey volume, pH of whey, and conductance of whey (19). Among these methods, they found that whey transmittance and conductance correlated with coagulant concentration, and concluded that measuring the conductance of the coagulating soymilk was faster and more reproducible than obtaining pH and transmittance values of the whey. A rotational viscometer (viscograph) is applied by researchers to measure the optimum concentration of coagulant for the coagulation of soymilk (20,21). Among five coagulants studied, with an increase in coagulant concentration from the minimum required concentration (from 0.15% to 0.5% of soymilk volume depending on coagulant), there is an increase in whey volume and decreases in the moisture of tofu (22). Sun and Breene (23) found negative correlations between calcium sulfate concentration and both yield and protein recovery of tofu from five soybean varieties.

4. Coagulation Temperature

The soymilk temperature for adding coagulant affects coagulation rate as well as tofu quality. The yield and moisture content of tofu decrease as the temperature of coagulation increases, whereas the hardness and elasticity increase (15). When soymilk is at high temperature, proteins possess high active energy that can lead to fast coagulation, resulting in the formation of curd with low water holding capacity, consequently, tofu has hard texture and low bulk yield. The hotter the soymilk at the time of coagulation, the less the amount of coagulant required. When tofu is coagulated at a high temperature, a small increase in the amount of coagulant may lead to a large decrease in yield. In the tofu industry, the temperature of coagulation varies from one factory to another, depending on the type of coagulant used. Generally, coagulation temperature ranges from 68°C to 95°C for those using nigari, while those using calcium sulfate prefer the range from 70°C to 80°C in Japan. Beddows and Wong (5) reported that the optimum coagulation temperature is 75–80°C for silken tofu with gypsum as coagulant in a small bench scale. Shih et al. (24) reported that the optimum coagulation temperature is 85–91°C for soft tofu with modified nigari (mostly $CaSO_4$) in a medium scale. The operational temperature of coagulation also varies from one region to another. In America, tofu makers prefer a relatively high temperature, 85°C, for using nigari and calcium sulfate, since less coagulant is required, the curd forms quickly, and the tofu has firm and dense texture but no significant drop in yield.

5. Coagulation Methods

The addition method, the stirring speed at which the coagulant is added, and the continuous stirring time after

the coagulant is added have very definite effects on the tofu yield and quality. Traditionally, calcium sulfate in a suspension is added to soymilk, which has been stirred vigorously by hand with a paddle, and the mixture continues to be mixed 6 to 8 more times. Nigari-type coagulant could be divided to three portions and added in three steps in order to coagulate soymilk slowly and get high yield and smooth texture. The first portion of nigari is poured from a height of several feet into the soymilk being swirled with a paddle. Coagulation starts from the bottom of the container and slowly works up, while the uncoagulated soymilk constantly rises to the surface. The second portion is sprinkled over the soymilk surface that is covered and stand for about 5 min, then the last portion is also sprinkled over the surface, and allow to stand for 15–20 min to solidify completely (3). Generally, the controlling techniques of this coagulation rely on the experienced tofu maker's judgement.

Stirring soymilk by a motorized stirrer and the effect of mixing speed and time had been investigated by researchers (25,26). By using a small scale for silken tofu (250 mL soymilk), Beddows and Wong (25) found that the stirring speed during coagulant addition was critical and the optimum speed for tofu yield, texture, and protein recovery was 240–280 rpm for 30 sec. By using a medium scale research equipment for soft tofu (4.5 L soymilk per batch, mold size is shown as (Figure 171.6), tofu made at 285 rpm stirring speed of a stirrer (Model RZR1, Caframo LTD, Wiarton, Ontario, Canada) equipped with a paddle (7 cm × 7 cm) has lower yield but higher firmness than tofu made at 207 rpm stirring speed (26) (Table 171.1). We found that yield decreased when stirring time increased to 30 sec, and tofu texture was affected as stirring time increased to 25 sec. By using a medium scale and a stirrer fixed at 285 rpm, we have determined the optimum combinations of soymilk solids, coagulant concentration, soymilk temperature for adding coagulant, and stirring time after adding coagulant for soft tofu making (24). Tofu yield is affected mainly by soymilk solid content and coagulant concentration. Tofu solids and protein content are affected by soymilk solids, coagulant concentration, and stirring time. Solid content of soymilk is the most important factor affecting textural properties of tofu. The optimum combinations are soymilk 11.8 to 12.3 °Brix, coagulant 0.27 to 0.32% of soymilk volume, stirring temperature 85 to 91°C, and stirring time 5 to 11.3 sec (24).

FIGURE 171.6 The tofu mold used by the medium-scale method.

F. Pressing of Curd

For momen tofu and soft tofu, pressing the bean curd to expel the soy whey is necessary as shown in Figure 171.2 and Figure 171.5. The pressures and duration of pressing can influence moisture content, yield, and texture of tofu. A range of values has been used for both parameters among different researchers (Table 171.2). Generally, silken tofu is not pressed after coagulation, however, Beddows and Wong

TABLE 171.1
Effects of Stirring Speed and Time on Tofu Yield and Hardness*

	Tofu Yield (g/100g soybeans)**		Tofu Hardness (g)	
Stirring Time (sec)	Stirring at 207 rpm	Stirring at 285 rpm	Stirring at 207 rpm	Stirring at 285 rpm
10	533 ± 0[a]	535 ± 12[a]	2005 ± 186[a]	2120 ± 199[a]
15	539 ± 8[a]	535 ± 9[a]	1993 ± 302[a]	2232 ± 211[a]
20	541 ± 8[a]	532 ± 10[a]	1838 ± 109[a]	2016 ± 158[a]
25	540 ± 10[a]	511 ± 14[a]	1597 ± 142[b]	1528 ± 122[b]
30	513 ± 10[b]	462 ± 38[b]	1580 ± 47[b]	2187 ± 92[a]

Source: Data adapted from Hou et al. (1997).

* Data are expressed as means 6 s.d. and are means of three replicates.

** Data of yield are on wet weight basis.

[a,b] Means within the same column not followed by same letters are significantly different ($p < 0.05$).

TABLE 171.2
Pressure and Duration of Tofu Processing Applied by Tofu Investigators

Investigators	Pressure	Duration
Pontecorvo and Bourne, 1978	9 kg/cm^2	20–30 min
Lu et al., 1980	2.58 g/cm^2	2–3 hr
Skurray et al., 1980–81	5.56 g/cm^2	2 hr
Wang et al., 1983	10.0 g/cm^2	1 hr
deMan et al., 1986	31.4 g/cm^2	15 min
Beddows and Wong, 1987c	4.0–6.0 g/cm^2	—[†]
Gandhi and Bourne, 1988	4.79–19.1 g/cm^2	15 min
Wang and Cavins, 1989	10.0 g/cm^2	1 hr
Lim et al., 1990	15.7 g/cm^2	15 min
Sun and Breene, 1991	10.0 g/cm^2	2 hr
Metussin et al., 1992	2.78 g/cm^2	30 min
Wang and Chang, 1995	7.6 g/cm^2	40 min
Hou et al., 1997	21.8–65.4 g/cm^2	50 min
Torres-Penaranda et al., 1998	2–6 kg/cm^2	15 min
Moizuddin et al., 1999	1–3 kg/cm^2	8 min

[†] No data presented.

(25) reported the optimum pressure applied to silken tofu was 4 to 6 g/cm^2 for pressing until dripping ceased. They found that below 4 g/cm^2 the tofu was very soft with little or no retention of cut shape and that above 8 g/cm^2, tofu was hard and rubbery. Gandhi and Bourne (27) showed that when the pressure increased from 4.79 to 19.1 g/cm^2, the moisture content of tofu decreased from 82% to 60% and yield decreased from 2.0 kg to 1.2 kg per kg whole dry soybeans. From a commercial standpoint, most manufacturers apply a light initial pressure of 2 to 4 g/cm^2 for about 5 to 10 min and a stronger pressure of about 5 to 15 g/cm^2 for 10 to 15 min to make soft tofu; for firm tofu, a pressure of 20 to 100 g/cm^2 is used for 20 to 30 min (3). In our laboratory, we apply a pressure of 21.8 g/cm^2 for 10 min, followed by 43.6 g/cm^2 for another 10 min, and adding to 65.4 g/cm^2 for 30 min for making soft tofu by a medium scale (24,26). Because of several variables, tofu-making process differs in various research laboratories, which reported data that were difficult to compare. Therefore, there is a need to standardize the procedures for determining quality of soybeans for tofu making.

III. ROLES OF SOY PROTEINS IN TOFU MAKING

A. Storage Proteins of Soybeans

Soy proteins constitute about 35–45% of the soybeans on a dry basis. Approximately 90% of the proteins are storage protein and are extractable with water or dilute salt solutions. Most of the storage proteins in soybeans are globulin. As soybean seeds mature, many organelles, such as nucleus, mitochondria, and endoplasmic reticulum, disappear and storage proteins deposit in large protein bodies that are surrounded by many small oil bodies. Storage proteins have no biological activities and 90% of them locate in cotyledons (28). Soy proteins consist of discrete groups of polypeptides that have a wide range of molecular size. A typical ultracentrifuge pattern of water-extractable soy proteins has four major fractions designated as 2S, 7S, 11S, and 15S on the basis of their sedimentation rates. Each fraction is a complex mixture of proteins. The 7S and 11S proteins are the two major storage proteins in soybeans, which comprise approximately 70% of storage protein. The 2S fraction accounts for about 20% of the extractable proteins, which contain protease inhibitors (the Kunitz and the Bowman-Birk trypsin inhibitors) and cytochrome C (29). The 7S fraction has been classified into three major components with different physicochemical properties named β-conglycinin, γ- conglycinin, and basic 7S globulin (30, 31). β-conglycinin is the most prevalent of these three and accounts for about 30% to 35% of the total seed protein, which is used interchangeably with 7S protein since it is the major 7S protein. The 11S fraction, designated as glycinin, accounts for an additional third of the total seed protein and is generally simple protein. The 15S fraction accounts for approximately 10% of the total seed protein, which is an aggregate of 11S protein (32).

B. β-conglycinin (7S Protein)

β-conglycinin (7S) is a complex protein which exhibits polymorphism in its subunit composition. It is a trimer with a molecular mass of 150–200 kDa. Four subunits are identified: three major (α, α', and β) and one minor (γ) (33). β-Conglycinin exhibits molecular heterogeneity, in which 7 molecular species are isolated and their subunit composition identified as α'ββ, αββ, αα'β, ααβ, ααα', ααα, and βββ (34,35,36). β-conglycinin is a glycoprotein, its α and α' subunits contain two carbohydrate moieties and the β subunit one (33). β-Conglycinin undergoes a complex association-dissociation phenomenon in response to changes in ionic strength and pH. At neutral pH, β-conglycinin is a 7S-form globulin when the ionic strength is ≥ 0.5, but as a 9S dimer at ionic strength of ≤ 0.2 (37). The subunits of β-conglycinin are held primarily by hydrophobic forces (38). The molecular mass of subunits of β-conglycinin are estimated to be 57–59 kDa for α and α' subunit and 42–44 kDa for β and γ subunits by gel electrophoresis and gel filtration (33).

C. Glycinin (11S Protein)

Glycinin is a major storage protein of soybeans and accounts for approximately 35% of the total seed protein. It is a hexamer with a molecular weight of around 300–380 kDa. Each subunit is composed of an acidic polypeptide (A_n) with a molecular mass of approximately 35 kDa and a basic polypeptide (B_n) with a molecular mass of approximately 20 kDa. The acidic and basic polypeptides

are linked together by a single disulfide bond shown as A_n-S-S-B_n (39). It is known that initially a single polypeptide precursor is synthesized and then processed posttranslationally to form the acidic and basic polypeptides (40). The disulfide linkage between the acidic and basic polypeptides forms after subunit synthesis and may help stabilize the subunit after posttranslational modification. Five subunits are identified by Nielsen et al. (41) and Utsumi et al. (42): $A_{1a}B_{1b}$ (G1), A_2B_{1a} (G2), $A_{1b}B_2$ (G3), $A_5A_4B_3$ (G4), and A_3B_4 (G5). Among these subunits, two groups can be separated based on sequence homologies (39). Group I subunits, $A_{1a}B_{1b}$, $A_{1b}B_2$, A_2B_{1a}, are uniform in size (~58 kDa), relatively rich in methionine and cysteine, and exhibit about 90% sequence homology. Group II subunits, A_3B_4 and $A_5A_4B_3$, exhibit a smaller level of homology (about 60–70%), and contain less methionine and cysteine, but are larger (~62–69 kDa) than group I. The $A_5A_4B_3$ (G4) subunit is synthesized as a single polypeptide precursor similarly to the others, but the acidic polypeptide is cleaved to produce A_5 and A_4 polypeptides (40). Most major subunits of glycinin are present in most soybean varieties except the subunit $A_5A_4B_3$ (39). In Japan, about 20% of soybean varieties are absent of subunit $A_5A_4B_3$ in glycinin (43). Glycinin, having different subunit compositions, exhibits distinguishable functional properties.

Glycinin hexamers can dissociate to their constituent polypeptides, subunits, and half-molecules under various pH, ionic strength, and temperature (42). At pH 7.6 and an ionic strength of 0.5, glycinin forms hexamer complexes (11S), whereas at pH 3.8 and an ionic strength of 0.03, glycinin exists as trimers (7S) (44,45). The dissociation of 11S to 7S seems to be correlated with significant changes at the secondary and, to a lesser extent, the tertiary structures. When ionic strength is below 0.2, the basic polypeptides shift more to the exterior of the molecule (44).

D. Gelation of Purified Soy Proteins

Generally, denaturation is essential for proteins to form gel, which results in an altered conformation of the protein and changes in physical and biological properties. Upon heating, soy proteins initially undergo a stepwise dissociation of subunits, followed by unfolding of the polypeptides that subsequently associate and aggregate to form precipitates or progels (46). Glycinin and β-conglycinin exhibit apparent denaturation temperatures of 90°C and 75°C, respectively. The difference in the thermal transition temperatures of these two proteins results from inherent differences in their structures. Glycinin is more heat-stable than β-conglycinin (47).

The gel-forming ability induced by heating soy proteins is one of the most important functional properties with respect to their usage in the food systems. Glycinin and β-conglycinin show different gel-forming properties and their gelation mechanisms are different. A soluble aggregate model describes how glycinin forms the gel structure (48,49,50). The model could be regarded as a three-stage process. When glycinin solution (5%) is heated, glycinin aggregates (MW 8000 kDa) are formed; then on subsequent heating, it undergoes association resulting in gel formation; finally, the gel network structure is stabilized through further formation of non-covalent bonding (such as hydrophobic interaction and hydrogen bonding) and disulfide cross-links by subsequent heating. The network structure of β-conglycinin heat-induced gel is hypothesized as a randomly aggregated assembly of clusters (51). Upon heating of β-conglycinin (7.5%), soluble aggregates are formed (MW about 1000 kDa), then associate with each other randomly to form a cluster; finally clusters aggregate randomly to form a gel. The gelation rate of β-conglycinin is slower than that of glycinin (52). 11S gels prepared in the presence of calcium sulfate are harder and show larger breaking stress, breaking strain, and young modulus than crude 7S gels (52,53). The 11S gel has a higher water-holding capacity, higher tensile value, and higher hardness and expands more on heating than the 7S gel (54). Sulfhydryl-disulfide interchange reaction is important in the formation and maintenance of the structural matrix of 11S-globulin gel. No SH/S-S exchange reaction participates in the 7S-gel formation, whereas hydrophobic interactions and hydrogen bonds play an important role in the formation and maintenance of the gel network of 7S protein (55). The gel formed by β-conglycinin is transparent in contrast to the turbid gel of glycinin globulin (51).

For glycinin, the rates of gelation and the hardness and turbidity of gels are affected markedly depending on the subunit composition (56). Subunit $A_5A_4B_3$ is closely related to gel formation because of an easy cleavage of hydrophobic bond between A_5 and A_4 polypeptides during heating. Cultivars containing the A_4 polypeptide in glycinin form glycinin gels faster than cultivars without A_4 (57). Soybean cultivars without the A_4 polypeptide that is identified as A_5 by Nishinari et al. (58) produce a harder and more solid-like protein gel than those cultivars with the A_4 polypeptide in glycinin (59). Subunit A_3B_4 is related to the gel hardness because the A_3 acidic polypeptide plays an important role in increasing the hardness of the gel. Nakamura et al. (57) found that the hardness of glycinin gels is different among varieties, depending on the percentage of A_3, which is the largest constituent acidic polypeptide of glycinin. However, Tezuka et al. (60) reported that tofu curd made from soybeans containing glycinin with only subunit $A_5A_4B_3$ is the hardest among those made from soybeans containing subunit A_3B_4 or Group I subunit ($A_{1a}B_{1b}$, $A_{1b}B_2$, A_2B_{1a}) in their glycinin protein. The roles of glycinin and β-conglycinin subunits in influencing certain characteristics of tofu texture remain to be clarified in the future.

The turbidity of the gels is positively related to the numbers of the free -SH residues, and is caused by the basic polypeptides that are dissociated from glycinin during heating (61). The turbidity of the gel from the glycinin globulin containing A_3B_4 subunit is the smallest because of fewer -SH groups in this subunit. The contribution of the constituent subunits of β-conglycinin to the physical properties of β-conglycinin gels is not clear. In a mixed system, glycinin is related to hardness and unfracturability of the gels, while β-conglycinin largely contributes to the elasticity of the gels (42). During gel formation, glycinin and β-conglycinin interact each other through association between basic polypeptides of glycinin and β-subunits of β-conglycinin to form composite aggregates (62).

E. Soy Proteins in Tofu Making

Generally, crude proteins constitute more than 50% of the total solids of tofu on dry basis. Soy proteins are the dominant components in tofu dry matter, which provide the major network structure of tofu gel. Soy proteins form gel by a combination of heating and the addition of a coagulant, which is either an acid or divalent salt or a combination of both. Tofu is a complex food system that is very different from the thermally induced purified protein gels. Although both types of gels require protein denaturation, the exact mechanisms for tofu gelation are not identical. Tofu is made from heated soymilk that is a turbid solution containing approximately 5% protein and 3% lipid. Therefore, tofu is an emulsion gel system. The tofu emulsion is permanent since heating is not able to separate lipids from the protein system. For tofu making, a coagulant is required, while heat-induced protein gel does not need a coagulant. Besides protein and lipid, other components in soymilk such as phytate, isoflavones, saponins, and lipoxygenases also may play important roles in coagulation of proteins during curd formation. Because of the complexity of the soymilk-tofu system, the mechanisms of tofu formation are also complex, and are not fully understood. To clearly identify the mechanisms that affect the tofu gel properties by using real soymilk is very difficult. Some studies have been conducted to understand the interactions between non-protein constituents (e.g. phytate and lipid) and proteins on coagulative reaction in tofu making (53,63). However, there is a lack of a comprehensive approach to put all factors together in one picture to understand the gel formation in tofu making.

Early researchers found that isolated glycinin-rich proteins produce firmer gels than β-conglycinin-rich proteins by either heat or calcium (53,54). Recent studies have shown that various soybean cultivars have various ratios of 11S/7S proteins that may influence the textural quality of tofu. In our laboratory, we found that the β-conglycinin (7S) and glycinin (11S) contents in 13 varieties are 17.2–23.1% and 36.3–51.3% of total proteins, respectively, and the 11S/7S protein ratio varied from 1.64 to 2.51 among the varieties (64). Furthermore, we found positive correlations existed between tofu firmness and the 11S/7S ratios in various (13 to 16) soybean cultivars (64,65). However, conflicting results on the relationships between 11S/7S ratio and tofu firmness have been reported by other researchers (55,59,66,67). The conflicting report may be partly due to different methods used for processing, because of a lack of standard methods for tofu research. We have found that processing methods affect 7S- and 11S-protein content of tofu and their contribution to tofu hardness, yield and sensory quality. Thus, processing methods have an impact on the relationships between 11S/7S ratios and textural quality since different coagulation processes and pressing steps are used for preparing tofu (64).

Yagasaki et al. (68) reported that cultivars, having a higher glycinin/β-conglycinin ratio, had a higher gel firmness than that with a lower ratio. However, on a closer examination of their report, it is apparent that firmness does not increase above 1.3 of the 11S/7S ratio in four cultivars (Figure 171.7). Therefore, in a complex system such as soybeans/soymilk (which is very different from the purified protein system), there is a limit of the firming effect due to the increase in 11S/7S ratio. In other words, above certain ratio, the tofu firmness does not increase. A similar phenomenon has been observed in one of our studies (69). We added purified 11S protein to the soymilk systems prepared from three cultivars while maintaining a constant protein concentration in soymilk for tofu making. The results showed that the increase in firmness was cultivar-dependent and the increase was not substantial (Figure 171.8). Therefore, in the soymilk system as opposed to the purified protein system, other biochemical constituents may play important roles in determining the tofu yield and quality. Further research is needed to continue to elucidate the effect of individual components and their interactions to understand the fundamental biochemistry of tofu making.

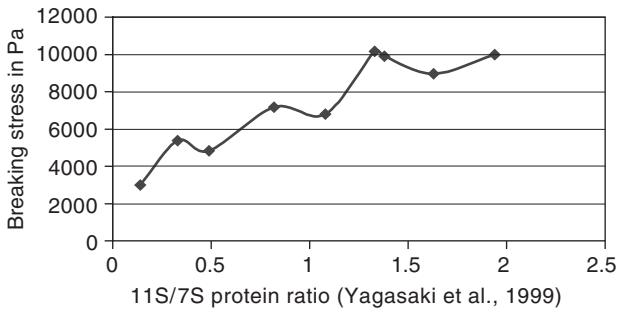

FIGURE 171.7 Hardness of tofu made from various 11S/7S ratio in soybeans.

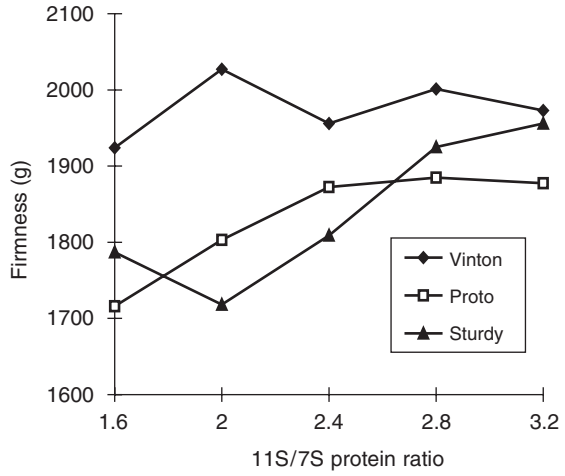

FIGURE 171.8 Firmness of tofu as affected by modified 11S/7S protein ratio in soymilk (means of two treatment replicates).

IV. MOLECULAR MODELS FOR TOFU CURD FORMATION

A. Role of Protein Charges

The gelation process of tofu has been studied in a mixed protein system by adding glucono-δ-lacton (GDL) or calcium sulfate (70). For both GDL and calcium systems, protein mixture containing higher 11S proportion has faster gelation. 11S-protein forms a continuous matrix and 7S is a discontinuous filler. The roles of 11S and 7S are interchanged with each other at a lower 11S proportion. The gelation mechanism of tofu gels induced by coagulants in an imitative 7S and 11S protein mixture has been hypothesized (70). The negatively charged groups of soy proteins denatured by heat are decreased by adding cations through the action of coagulants such as GDL, calcium sulfate, and magnesium chloride, then the neutralized protein molecules are able to aggregate due to a reduction in electrostatic repulsion. Finally, the gel network is stabilized by the formation of hydrogen bonds and hydrophobic interactions.

B. Role of Lipids in Curd Formation

Soymilk and tofu contain approximately 30% lipids on a dry basis. Lipids influence the gelation of soy proteins and play an important role in texture and sensory quality of the products (71,72). In raw soymilk, approximately 60% of total lipid are associated with the protein particles, however, only 3% of total lipid are found in the protein particles of cooked soymilk (73). After being heated to 65°C, a part of lipids and almost all α and α' subunits of β-conglycinin in the particulate fraction begin to liberate to soluble fraction. Above 90°C, almost all neutral lipids in the protein particles of raw soymilk are liberated to a floating fraction, but about one half of the phospholipids remain in the particles (73,74). Coagulation of soymilk depends on the concentration of coagulant, the pH of soymilk, and temperature, which is an external factor and accelerates the soymilk coagulation. In fact, the addition of coagulant causes not only protein coagulation and gelation but also the incorporation of lipids into the protein gel (75). It has been observed that when soymilk coagulates and forms a gel, the lipid droplets are located in the networks of the protein gel (53).

C. Role of Phospholipids in Curd Formation

The polar phospholipids are believed to play an important role in combining the particulate proteins with neutral lipids (73). Some significant amount of lipids exist in isolated 11S and 7S proteins from hexane-defatted soy meal (0.8% and 2.3%, respectively), more than 50% of these lipids are phospholipids. Phospholipids bind to the hydrophobic sites of β-conglycinin (76). Phospholipids bind stronger to the 7S protein than to the 11S protein, because 7S protein is more hydrophobic than 11S protein. The removal of lipids, particularly the phospholipids, from the surface of 7S proteins by extraction with chloroform:methanol solution makes 7S proteins vulnerable to form insoluble aggregates thus decreasing the ability to complex with protein particles. Adding phospholipids to soymilk increases the formation of protein particles. Lecithin-supplemented gels exhibit a fine network structure. Soy proteins depleting in phospholipids could be damaging to their neutral lipid binding ability. Therefore, soybean curd network structure could be promoted by phospholipids that act to combine neutral lipids in the protein network.

D. Hypothesized Molecular Model of Tofu Curd Formation

A protein particle theory with the incorporation of soy lipids in the protein network for understanding the mechanism of tofu formation has been proposed (73,74,77–80). In raw soymilk, proteins could be separated to particle and soluble fraction by centrifugation. The particle fraction is composed of large (> 100 nm) and medium (100–40 nm) particles. The soluble fraction is considered as the supernatant proteins (< 40 nm). The large particles, in which 70% are 11S globulin, constitute 40% of the total proteins in raw soymilk. The medium-sized particles are formed by a combination of the supernatant proteins with each other. Lipids are mainly present in the particle (large) fraction. The protein particles play an essential role for tofu curd formation with calcium chloride; the content of the particles in soymilk determines the density of the network; the more particles, the finer network are formed (78). Tezuka et al. (81) reported that 11S protein-rich cultivars contain more protein particles than the 7S protein-rich cultivars. Therefore, the glycinin (11S) is essential for protein particle

formation in soymilk. The cultivars having greater protein particle contents produce firmer tofu than that with lower protein particle contents (60).

As mentioned above in the section on lipids, lipids of soymilk can be separated in the floating fraction by centrifugation after heating. When $CaCl_2$ is added to soymilk, the floating fraction (lipids) decreases, which occurs before the formation of protein aggregates. When a half of the proteins coagulate with coagulant, almost all the lipids in soymilk are trapped and become inseparable. The decreases in the floating fraction (lipids) with addition of $CaCl_2$ are parallel to the increases of the coagulation of particulate proteins. This indicates that lipid conjugates with particulate and soluble proteins and become inseparable by the association with particulate protein. The protein particles are essential for the incorporation of lipids into aggregates. The lipid incorporation due to the conjugation of lipids and protein particles explains why lipids incorporated are stable against oozing and separation of the oil phase from the continuous hydrophilic phase in further storage and cooking of tofu.

The pH decrease and calcium binding with proteins also play important roles on the formation of tofu curd. A decrease of pH was observed when calcium chloride combined with soy proteins (53). When a coagulant such as calcium chloride is added into soymilk, the protein particles precipitate at lower concentrations of calcium than that of the soluble proteins, resulting in protein solubility decreases and accompanying with a pH decrease. The pH decrease promotes proteins aggregation by reducing the electric repulsion, and liberates the hydrated water of proteins, while calcium ions bind to proteins through the carboxyl groups of the glutamyl and aspartyl residues and the imidazole groups of the histidine residues. The binding of calcium to proteins brings the association of protein molecules and accelerates the formation of curd.

According to the studies reviewed above, the mechanism of tofu curd formation could be summarized in following three steps. (a) In raw soymilk before heating, the total of the large and medium-sized particles constitute more than 50% of the proteins in soymilk. Most of the particles are large particles. Most lipids are present in the protein particles. (b) When the soymilk is heated up to 90°C, the proteins denature and the lipid droplets are liberated to floating fraction. Approximate three quarters of the large particles are degraded to supernatant proteins, but the medium-sized particles increase due to the combination of β subunit of 7S and the basic polypeptide of 11S from the supernatant proteins that contain mainly 11S and 7S globulin. (c) The addition of coagulant (calcium ion, magnesium ion or GDL) to the heated soymilk is the key step. At low concentration of coagulants, protein particles combine with lipid droplets and the gel network is first formed through the binding of calcium to protein particles to neutralize the negatively charged protein molecules and to cause protein aggregation due to a reduction in electrostatic repulsion. When a half of the proteins are coagulated with coagulant, almost all the lipids in soymilk are trapped and become inseparable by the association with particulate proteins. Further addition of coagulants leads to a decrease in pH. The soluble proteins aggregate at a higher concentration of coagulant and bind to the protein particles-oil droplet complex to form a stable tofu-curd emulsion network. These reactions are illustrated stepwise in the postulated models from left to right (Figure 171.9).

V. ROLE OF PHYTIC ACID IN TOFU MAKING

Phytic acid content in seeds varies widely, it comprises 1~2% of soybeans on a dry basis and accounts for about

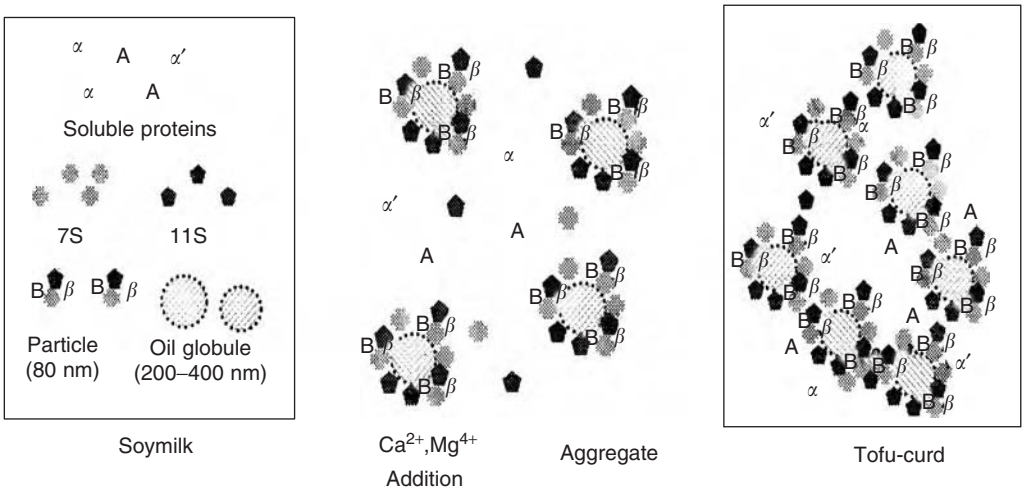

FIGURE 171.9 Postulated molecular models of the formation of tofu-curd (79).

70%~80% of the phosphorus in seeds (82). Phytate is structurally integrated with the protein bodies as phytin,a mixed potassium, magnesium, and calcium salt of inositol (83). It is reported that phytate attaches to the glycinin at pH between 2.5 and 5.0, and the extent of binding increases with decreasing pH; above the pI (isoelectric point) of glycinin (pH 4.9), no binding is found (84). Phytate has strong chelating ability with multivalent metallic ions, especially zinc, calcium, and iron. According to Graf (85), calcium ion can bind to phytic acid over a wide pH range (pH 4.8 to 10.4); the degree and tightness of binding are affected by pH, temperature, ionic strength, and size and valence of the cation. The affinity of phytic acid for calcium increases sharply with pH; the higher the pH (alkaline), the higher affinity; the affinity in pH 10.4 is a thousand-fold higher than in pH 4.8. A portion of phytate in soymilk is bound to particulate and soluble proteins (about 35% and 23%, respectively), the others are present in the free form (about 42%) (78). Therefore, when calcium is added to soymilk, it binds to phosphate groups of phytate and binds to proteins as well. The role of phytic acid in the coagulation step during tofu-making has been related to a decrease in pH after calcium salt is added (78,81). When calcium is added, the phytate-calcium salts form at approximately the neutral pH (6.6), and hydrogen ions are liberated, which are bound originally with phosphate groups in phytate. Therefore, the pH decrease upon the addition of calcium may be mainly due to the formation of phytate-calcium salts. The decrease in pH of soymilk from approximately 6.6 to 5.8 after adding calcium chloride allows the use of a lower concentration of Ca salts for coagulation. When calcium is present, it binds simultaneously to proteins and phytate, calcium binding to proteins can retard the decrease of pH because of less phytate-calcium formation. Tofu curd contains both types of calcium bound to protein or phytate.

Phytate is very important in relation to the speed of coagulation during tofu making. It has been found that higher content of phytic acid results in a slower coagulation of soymilk during tofu making, and gives a higher tofu yield (53). Therefore, the phytate content in soymilk can affect the textural properties of tofu. In other words, a decrease in phytate increases the speed of coagulation and makes the gel harder. We have found that phytic acid contents in soybeans and in soymilk are correlated positively to tofu yield, but negatively to tofu hardness ($p < 0.01$) (86). Significant positive relationships exist between soybean phytate and tofu yield ($r = 0.93$) and between soymilk phytate and tofu yield ($r = 0.95$). Negative correlations between soybean phytate and tofu hardness and brittleness are observed ($r = -0.92$ and -0.84, respectively). Negative correlation coefficients are found between soymilk phytate and tofu hardness and brittleness ($r = -0.94$ and -0.86, respectively).

VI. ROLES OF ISOFLAVONE AND SAPONINS IN TOFU MAKING

Isoflavones are a subclass of the more familiar flavonoids and have an extremely limited distribution in nature. Soybeans and soy foods are the major foods containing significant amount of isoflavones. The main isoflavones found in soybeans are genistein, daidzein, and glycitein, each of which exists in four chemical forms, as an aglycone form (genistein, daidzein, and glycitein), a β-glucoside form (genistin, daidzin, and glycitin), a malonylglucoside form (6”-*O*-malonylgenistin, 6”-*O*-malonyldaidzin, and 6”-*O*-malonylglycitin), and an acetylglucoside form (6”-*O*-acetylgenistin, 6”-*O*-acetyldaidzin, and 6”-*O*-acetylglycitin). Saponins are widely distributed in plants, which are glycosides and composed of a sapogenin that makes up the aglycone moiety, and a sugar. The sapogenin is a triterpeneoid alcohol; at least 5 sapogenins have been found in soybeans (87). Xylose, arabinose, galactose, glucose, rhamnose, and glucuronic acid have been found in the glucoside portion of soy saponins. Saponins exist in two groups, A and B (88,89,90). The group A saponins consist of 6 different kinds of saponins (Aa, Ab, Ac, Ad, Ae, and Af), which are acetyl-soyasaponins. The group B saponins, on the other hand, consist of 8 kinds of saponins (Ba, Bb, Bb’, Bc, Bd, Be, BdA, and BeA), which are not acetylated and are different from the group A. BdA is the major natural soybean saponin in soybean seeds (90). Saponins are polar compounds because of the associated sugars (oligosaccharides), which are found in the soybean meal in amounts of approximately 0.5% of the dry weight (91).

Isoflavone content varies among soybean varieties, which contain approximately 1 to 4 mg/g soybean. The isoflavone content of soybeans is markedly affected by crop year and growing condition (92,93). Isoflavones are quite heat stable. Although isoflavones are not destroyed by heat in conventional food-processing operation, heating causes a change in the conjugation profile of the isoflavones in soy products. Baking or frying of isolated soy protein and textured vegetable proteins does not alter total isoflavone content but increases the β-glucoside conjugates at the expense of 6”-*O*-malonylglucoside (94). Wang and Murphy (92) reported that cooking did not influence the isoflavone retention during tofu making, but it did alter the distribution of isoflavones by dramatically decreasing in malonylglucoside forms and increasing in acetylglucoside forms. Minimal heat processing can convert substantial amounts of malonylglucoside to the β-glucosides. Total isoflavone content in soy products decreased most likely due to leaching of isoflavones into water during processing. Wang and Murphy (95) reported that 61%, 44%, and 53% of total isoflavones were lost during the processing of tempeh, tofu, and soy protein isolate, respectively.

It is known that isoflavones and saponins impart the bitter and astringent aftertastes in the flavor of soy

products. Okubo et al. (96) reported that glucoside forms of saponins and isoflavones are the major compounds that cause objectionable aftertaste in soybeans, and saponin A groups contribute most strongly to the undesirable taste. The undesirable taste becomes weaker when saponins decompose from glucoside forms to aglycone forms, while isoflavone glucosides show a reverse tendency. The aglycones of isoflavones have stronger objectionable aftertastes than those of glucosides (96,97).

Saponins, glycitin, and glycitin derivatives are present primarily in hypocotyl, and all of these substances can give objectionable aftertaste. The previous consideration has been to remove these compounds by processing methods. In Japan, good-taste tofu has been prepared by the Namashibori technique by squeezing raw extract, which is prepared from seedcoat-removed and hypocotyl-removed soybean materials (2). In the method, the soybean is first cracked, and seedcoat and hypocotyl are removed. The resulting materials are soaked in water for a short period of time and then ground to make the slurry "go" and filter. The raw soymilk is boiled and the brown foams (which contain saponins) are scooped away. However, in light of potential health benefits of these isoflavone and saponin compounds (98), a different strategy may be needed to preserve these compounds to produce soy foods with the maximum health benefits.

There are many excellent reviews (99,100,101,102) addressing the potential benefits and adverse effects of consuming diets containing isoflavones from soy foods. Four major potential benefit effects of isoflavones include (a) heart disease prevention (103,104); (b) cancer prevention, particular with respect to breast (105,106,107), prostate (108,109), and colon (110) cancers; (c) bone mass density increase to prevent osteoporoosis (111,112); and (d) reducing post-menopausal syndromes in women (99). In animal study, soybean isoflavones can reduce experimental metastasis of melanoma cells in mice (113). The two major concerns are potential adverse effects in infants having a high intake of isoflavones from soy-based formula (114,115) and possible reproductive disorders in adults having a high isoflavone intakes (116). However, there is no direct evidence to show the adverse effects of isoflavones in human. Among 12 isoflavones, genistein has been reported as the most potent inhibitor of cancer-cell growth (117). Aglycone forms of isoflavones have been found with faster absorption and in higher amount than their glucosides in human gut (118,119). Genistein has been reported having the highest antioxidant activity than genistin, daidzin, and daidzein do (120).

VII. EFFECT OF LIPOXYGENASE ON TOFU MAKING

Lipoxygenase is an iron-containing dioxygenase, which catalyzes the oxidation of polyunsaturated fatty acids such as linoleic acid, producing unsaturated fatty acid hydroperoxides which are broken to produce cis-n-hexenal, which is the major source of grassy-beany off-flavor in soy food. Soybeans are the most abundant lipoxygenase source known by researchers, which contain four lipoxygenase isozymes, identified as L-1, L-2, L-3a, and L-3b. The L-3a and L-3b are so similar in behavior and composition that they are often considered a single type as L-3 (121). Lipoxygenase separates with the 7S fraction in the ultracentrifuge with a molecular weight of about 100,000, which contains one atom of iron per mole of protein. The lipoxygenase L-1 is heat stable, which activity loses 50% by heating at 69°C for 25 min (122); it has an optimum pH at 9, and is most active on free fatty acids, but is not activated by calcium ions. In contrast, L-2 and L-3 are less heat stable, with activities losing 50% by heating at 69°C for only 0.7 min or less (122), with pH optimum at 7. Lipoxygenase L-2 and L-3 are more active on fatty acid esters and triglycerides than on free fatty acids, and their activities are increased by calcium ions. It has been reported that L-3 is the most abundant one in mature soybeans. The L-2 is the least abundant but has the highest specific activity, which is mainly responsible for the production of grassy-beany flavors (123). The lipoxygenase isozymes show differences in their product region specificity. When linoleic acid is a substrate, the L-1 prefers the 13 position as the site for hydroperoxidation, whereas the L-2 prefers equally the position 9 and 13, and the L-3 prefers more the position 9 (65%) than the position 13 (35%) (124,125).

The lipoxygenases are rapidly activated when the substrate is available and in the presence of water. Therefore, the beany flavor is mainly developed in the step of grinding because the enzyme and lipid are liberated, as well as excess water is present. Wilkens et al. (126) found that as the temperature of slurry increases, both the number and the volume of volatiles decrease; when beans are ground at 80°C or above, no volatiles are formed. Heat inactivation of lipoxygenases during grinding in the presence of hot water and/or in the absence of air/oxygen is very critical in eliminating beany flavor from soybeans, since beany flavor is difficult to be eliminated after it is formed. Additional vacuum treatment of hot soymilk may reduce beany flavor formed. However, using heat to inactivate lipoxygenases in whole soaked beans may cause a decrease of protein solubility, loss of protein functionality, and loss of solid recovery. Therefore, several techniques involving milder heat treatment of soybeans, adjusting moisture or pH, or using aqueous alcohol to soak soybeans, or their combinations have been developed to make non- or low-beany flavor soy product (4). Genetic elimination of lipoxygenases from the seeds provides a new approach to eliminate beany flavor (127,128). Lipoxygenase-null soybean variety has the functional properties of normal soybean variety but with less beany

flavor in soy foods (129). Soymilk made from lipoxygenase-free soybeans has less cooked beany aroma, less cooked beany flavor and less astringency, and is rated darker and more yellow than that made from soybeans with normal lipoxygenase (130).

In addition to potential deterioration of tofu flavor, lipoxygenase has been found to affect tofu texture (7,131). Grinding soaked soybeans in the temperature range of 2 to 50°C promotes lipoxygenase activity, which oxidizes lipids to hydroperoxides and subsequently oxidizes the free –SH group to disulfide bonds, and possibly cysteinic acid or cysteic acid. The oxidation of –SH affects its availability to participate in the interchange of free –SH groups with disulfide bonds during heating to form protein networks, thereby decreasing the firmness of the tofu product. Firmer tofu products could be prepared by grinding soybeans under an anaerobic condition. Lipoxygenases can degrade sulfhydryl group in soymilk during grinding even at low temperature (2°C) or in a nitrogen atmosphere (N_2) (131). Among lipoxygenases, L-2 isozyme has the greatest SH-degrading capability.

VIII. EFFECT OF SOYBEAN STORAGE ON TOFU MAKING

Soybeans are subject to transportation and storage after harvest before processing into various soy products. Soybeans may be stored up to one year or longer after harvest in a wide variety of environmental circumstances before they are processed. It has been understood that both the quality of edible soybeans and the viability of soybean seeds decrease gradually with prolonged storage. The process of storage-induced biological changes in soybean seeds is generally known as aging. The mechanism of soybean aging has not been completely understood. A commonly acceptable hypothesis is that lipid peroxidation plays an important role in the initial stage of seed aging process (132,133,134). Hydroperoxides, which are highly reactive free radical compounds generated from lipid peroxidation of polyunsaturated fatty acids in the presence of oxygen, can abstract hydrogen from adjacent hydrocarbon chains resulting in not only destruction of the lipid itself, but also damage to cell membranes and other cellular components. In addition, hydroperoxides can break down to form secondary volatile oxidation products, which may contribute to the off-flavor formation in soy products during storage of soybean (135). Both enzymatic and non-enzymatic oxidation may be involved in the deterioration of the aged seeds.

The magnitude of the quality deterioration of seeds depends upon storage conditions, including time, temperature, relative humidity (RH), and microbial contamination. Among these factors, relative humidity/water activity is the most important. Low humidity may effectively preserve the original bean qualities even at a high temperature (136). The reported changes of components in soybeans induced by storage include surface discoloration that may be caused by enzymatic reactions such as polyphenolase on tannins and by non-enzymatic Maillard reactions between reducing sugars and free amines, a loss in protein extractability (137, 138), an increase in the acidity or decrease in pH (139), and a decrease in phospholipid content (140). Phospholipids are completely destroyed by storing beans at 14% moisture, and 40°C for four weeks (141). Nakayama et al. (142) also found that phospholipids were decreased during soybean storage at 35°C. Storage of soybeans influences physicochemical properties of proteins including decreases in nitrogen solubility index (NSI), decreases in extractability of glycinin and β-conglycinin, and changes in subunit composition of glycinin (59,143). When soybeans are stored in adverse conditions, soymilk quality is significantly decreased by a darkened color and a lower solid extractability, and the yield and quality of tofu are decreased by having off-flavor and a coarser texture (139). The deterioration of functional properties of soy proteins, including viscosity, gel forming ability, and emulsion stability during storage has been reported (144). Soybeans stored in adverse conditions (84% RH and 30°C) deteriorate significantly after 2 months, in which mold appears and off flavor is generated, tofu yield decreases significantly, and texture of tofu becomes coarse and hard. However, soybeans in conditions of 57% RH 20°C, cool 4°C, or in an uncontrolled ambient temperature condition in North Dakota could retain their soymilk and tofu qualities for up to 18 months (86).

In general, whole soybeans are more resistant to deterioration during storage than soy meal or damaged beans including split and seedcoat cracking. Usually, the amount of broken or damaged beans tends to increase with prolonged storage, especially when moisture content is low ($< 13\%$) (144). Yield of tofu decreases significantly beyond 30 days of storage in the condition of 85% relative humidity and 30°C for both whole and physically damaged soybeans. Furthermore, higher damage ratios cause greater losses in tofu yield (139). The off-flavor of tofu develops as soybean storage time increases. Since soybeans contain a high amount of polyunsaturated lipids, oxidation of unsaturated lipids caused by lipoxygenases, the secondary products of hydroperoxides caused by lyase, and volatile materials derived from the Maillard reactions may play important roles in off-flavor formation of tofu during storage of soybeans in adverse conditions. Besides flavor deterioration, the hydroperoxides as well as their secondary products may interact with food proteins or amino acids through protein-protein crosslinks, protein scission, protein-lipid adducts, and amino acids damage to cause deterioration (145).

Locher and Bucheli (146) stored soybeans under conditions of 4°C, 45% RH and 30°C, 82% RH to assess the degradation of soluble sugars and their relationship with seed deterioration. They reported that substantial hydrolysis of stachyose, raffinose, and verbascose occurred under

conditions of 30°C, 82% RH, and that reducing sugar content in soybeans was first reduced and later non-reducing oligosaccharides in the soybeans were hydrolyzed. A part of the reducing sugars formed by hydrolysis of the oligosaccharides may participate in the non-enzymatic glycosylation and in the Maillard reactions with the amino residues in the soy proteins (147). Sugar content in soybeans and tofu has significance in color and sweetness of the products. As mentioned in previous section, phytic acid affects the coagulation of soymilk during tofu making by decreasing pH after calcium salt is added. We found that phytate in soybeans degrades gradually with storage time in the adverse environment. However, under the mild or cold conditions, hydrolysis of phytate also could occur, but at a lesser degree (86). The hydrolysis of phytate in soybeans during storage contributes not only to the decrease of soymilk pH, but also to the loss in chelating ability with calcium ions, and subsequently affects protein coagulation behavior to lead to a reduction in the product yield and changes of textural quality (Table 171.3).

The enzyme, β-glucosidases, can hydrolyze glucosides of isoflavones to their aglycones (148). Storage of soybeans may affect the activities of β-glucosidases in the conversion of glucosides of isoflavones to more bitter aglycones. In raw soybeans, malonylgenistin is the major isoflavone form, representing 48% of overall total isoflavones, followed by malonyldaidzin, malonylglycitin, and genistin, respectively (149). The three malonylglucosides compose of 87% of overall total isoflavones. As the storage time is prolonged in adverse conditions such as 84% relative humidity and 30°C, the contents of malonylglucosides are significantly decreased to less than 1% of the overall total isoflavones after 9 months. In contrast, the content of aglycones shows a significant increase along with storage time. In the beginning, aglycones compose only 1% of the overall total isoflavones, whereas they compose 80% in 5 months, then up to 97% of the overall total isoflavones after 9 months of storage under adverse conditions (Table 171.4). Tofu made from soybeans stored under high humidity and high temperature conditions would have stronger aftertaste than that made from soybeans stored under mild conditions, because the former contains more aglycone isoflavones (genistein and daidzein), which have much stronger aftertaste than the corresponding glucoside isoflavones (genistin and daidzin). Soybeans for tofu making could be kept in cold or mild conditions (57% RH, 20°C) for a long period of time (up to 18 months) without increasing the aftertaste because of only very little conversion occurred from malonylglucosides to aglycones (Table 171.5).

IX. EVALUATION OF SOYBEAN CULTIVARS FOR TOFU MAKING

It is well known that good tofu can only be prepared from good soybeans. Cultivar, location (environment of growth) and handling practice at harvest, and storage practices post-harvest can affect soybean chemical compositions, which affect curd formation and sensory properties of tofu. The differences in tofu properties may be truly from the soybeans themselves, but there is a possibility due to differences in preparation methods. Soybean cultivar is one of the factors in influencing the quality of tofu. Soybean cultivar, soybean quality, cultivation environment, and

TABLE 171.3
Phytate Content of Soybean and Tofu Yield and Textural Properties Made from Soybeans Stored in 84% RH, 30°C

Storage Month	Soybean	Tofu		
	Phytate*, %	Yield**, g/100 g	Hardness, g	Fracturability, g
0	1.332 ± 0.030	512 ± 5	2090 ± 42	1020 ± 57
1	1.199 ± 0.013	503 ± 3	2182 ± 12	1033 ± 81
2	1.178 ± 0.074	481 ± 3	2465 ± 21	923 ± 25
3	1.163 ± 0.033	435 ± 25	2704 ± 107	873 ± 11
4	1.101 ± 0.063	382 ± 12	3080 ± 113	920 ± 57
5	1.056 ± 0.017	372 ± 35	3287 ± 39	945 ± 14
6	1.054 ± 0.034	232 ± 28	> 5000	> 5000
7	0.993 ± 0.011	71 ± 1	> 5000	> 5000
8	0.932 ± 0.083	NA#	NA#	NA#
9	0.873 ± 0.011	NA#	NA#	NA#

* Data are expressed as percentage of means ± sd of three replicated on a dry weight basis.

** Data of yield are means of two replicates on a wet weight basis (5.4% moisture).

Tofu did not form.

Source: Data are adapted from Hou and Chang (86).

TABLE 171.4
Isoflavone Content of Soybeans Stored in 84%RH 30°C for up to 9 Months (μg/g dry weight)[1]

	Glucosides			Malonylglucosides			Acetylglucosides			Aglycones			Total Individuals[3]			Overall
Month	Din[2]	Gin	Gly	Din	Gin	Gly	Din	Gin	Gly	Dein	Gein	Glein	Dein	Gein	Glein	Total[4]
0	142^{d}	180^{d}	121^{c}	1248^{a}	1837^{a}	251^{a}	17	3	13	12^{i}	7^{f}	1^{g}	734^{d}	1072^{d}	218^{abc}	2024^{f}
1	254^{b}	368^{b}	143^{b}	1089^{b}	1758^{b}	199^{b}	0	12	2	33^{h}	27^{f}	4^{g}	734^{d}	1174^{c}	201^{d}	2109^{e}
2	376^{a}	481^{a}	172^{a}	949^{c}	1489^{c}	194^{b}	2	4	0	100^{g}	114^{e}	11^{f}	807^{c}	1187^{bc}	223^{ab}	2217^{c}
3	232^{c}	315^{c}	142^{b}	531^{d}	884^{d}	123^{c}	0	1	0	430^{f}	558^{d}	71^{e}	838^{b}	1213^{ab}	227^{ab}	2278^{b}
4	113^{e}	145^{e}	85^{d}	296^{e}	571^{e}	70^{d}	0	0	0	594^{e}	794^{c}	133^{d}	812^{b}	1181^{bc}	224^{abc}	2216^{c}
5	30^{f}	45^{f}	57^{e}	99^{f}	274^{f}	26^{e}	0	0	0	784^{c}	1045^{b}	174^{c}	852^{a}	1215^{a}	224^{abc}	2292^{a}
6	15^{gh}	16^{h}	49^{e}	31^{h}	106^{h}	9^{fg}	0	0	0	820^{b}	1107^{a}	176^{c}	845^{a}	1172^{ab}	212^{bc}	2230^{bc}
7	21^{g}	32^{g}	60^{e}	65^{g}	173^{g}	14^{f}	0	0	0	761^{d}	1040^{b}	178^{c}	806^{b}	1149^{bc}	224^{ab}	2179^{cd}
8	12^{gh}	14^{h}	51^{e}	29^{h}	94^{h}	8^{fg}	0	0	0	827^{b}	1081^{ab}	196^{a}	849^{a}	1138^{bc}	233^{a}	2220^{c}
9	8^{h}	0^{i}	34^{f}	4^{h}	17^{I}	0^{g}	0	0	0	854^{a}	1106^{a}	188^{b}	861^{a}	1115^{bc}	210^{c}	2186^{cd}

[1] Values are the mean of two replicates and in the same column with different superscripts are statistically different at $p \leq 0.05$.

[2] Din = daidzin; Gin = genistin; Gly = glycitin; Dein = daidzein; Gein = genistein; Glein = glycitein.

[3] Total individuals = moles of isoflavone × molecular weight of aglycone form isoflavone.

[4] Overall total = sum of total individuals of aglycones.

Source: Data from Hou and Chang (149).

TABLE 171.5
Isoflavone Content of Soybeans Stored in 57%RH 20°C for up to 18 Months (μg/g dry weight) [1]

	Glucosides			Malonylglucosides			Acetylglucosides			Aglycones			Total Individuals[3]			Overall
Month	Din[2]	Gin	Gly	Din	Gin	Gly	Din	Gin	Gly	Dein	Gein	Glein	Dein	Gein	Glein	Total[4]
0	142^{f}	180^{f}	121^{d}	1248^{b}	1837^{b}	251^{ab}	17	3^{e}	13	12^{d}	7^{c}	1	734^{c}	1072^{d}	218^{b}	2024^{d}
3	181^{e}	233^{e}	150^{b}	1398^{a}	1987^{a}	262^{a}	9	10^{d}	9	16^{c}	13^{b}	2	833^{ab}	1193^{b}	241^{a}	2267^{ab}
6	239^{d}	292^{d}	137^{c}	1256^{b}	1830^{b}	223^{cd}	9	17^{c}	8	19^{c}	16^{b}	0	800^{b}	1155^{c}	210^{b}	2165^{c}
9	297^{c}	397^{c}	151^{b}	1206^{b}	1832^{b}	237^{bc}	10	21^{b}	8	23^{b}	14^{b}	0	816^{ab}	1221^{b}	227^{b}	2264^{ab}
12	328^{b}	478^{b}	161^{b}	1123^{c}	1717^{c}	220^{d}	10	24^{b}	10	24^{b}	13^{b}	0	793^{b}	1214^{b}	225^{b}	2231^{bc}
18	477^{a}	652^{a}	180^{a}	1015^{d}	1538^{d}	190^{e}	6	38^{a}	7	41^{a}	33^{a}	4	845^{a}	1258^{a}	223^{b}	2326^{a}

[1] Values are the mean of two replicates and in the same column with different superscripts are statistically different at $p \leq 0.05$.

[2] Din = daidzin; Gin = genistin; Gly = glycitin; Dein = daidzein; Gein = genistein; Glein = glycitein.

[3] Total = moles of isoflavone × molecular weight of aglycone form isoflavone.

[4] Overall total = sum of total individuals of aglycones.

Source: Data from Hou and Chang (149).

processing conditions all affect the resulting tofu. Over the years, substantial interest has been placed on the understanding of the quality of various soybean cultivars for tofu making. This has a practical importance in soybean trading since a good quality identity-preserved soybean cultivar would commend a premium. Several researchers have reported the differences in the quality of various soybean cultivars for making tofu. Soybean cultivars with higher protein content have generally lower oil and total sugar content. The chemical composition of soybeans is closely related to that in soymilk and tofu (150–152). The higher protein content in soybeans, the higher protein contents in soymilk or in packed tofu. Soybean cultivars vary in chemical composition resulting in significant differences in textural properties of tofu, and the cultivars with higher protein contents may not produce tofu with harder texture, because protein content alone is not adequate to explain the observed to hardness. Therefore, a thorough understanding of the protein structures in various cultivars is important to relate to tofu quality. The structures of soy proteins may be affected by cultivar as well as storage of soybeans.

In marketing soybeans for foods, the buyers and processors are interested in knowing the suitability of soybeans for making tofu because soybean quality will affect the processing procedures, tofu yield, consumer

acceptability, sale and profit of the tofu products. The desire for good quality of soybeans by the processing industries have led the soybean breeders to breed specialty soybeans for tofu uses. Tofu makers prefer large size, round shape, yellow soybeans with clear hilum, high protein, high sugar, and high nitrogen solubility for tofu making (153). These physical and chemical characteristics are associated with variety. However, color of hilum is not related to color of tofu. Tofu with white and less reddish color is preferred in Japan. Even though large-size soybeans are preferred in the market, size of soybeans does not affect tofu yield and quality (151,154–156). Some small size beans could also make good tofu. Uniformity is important. Soybeans fail to be hydrated will adversely affect the yield of tofu.

There is no standard method to evaluate soybean quality for making tofu. It is important that an evaluation method has the ability to detect the differences of soybeans with different quality characteristics, and such a method could produce a similar trend of results in a large-scale tofu manufacture process. Since manufacturing processes of tofu vary from manufacturer to manufacturer, one evaluation method can not be applied to all manufacturers. However, for a simple purpose of comparison among different varieties for tofu making one method developed for a specific tofu product may be appropriate. Most methodologies reported for tofu making are small-scale methods and have not been described in detail. Except the method reported by our lab (154) all other reported studies have not compared the reported small-scale method with a large-scale method.

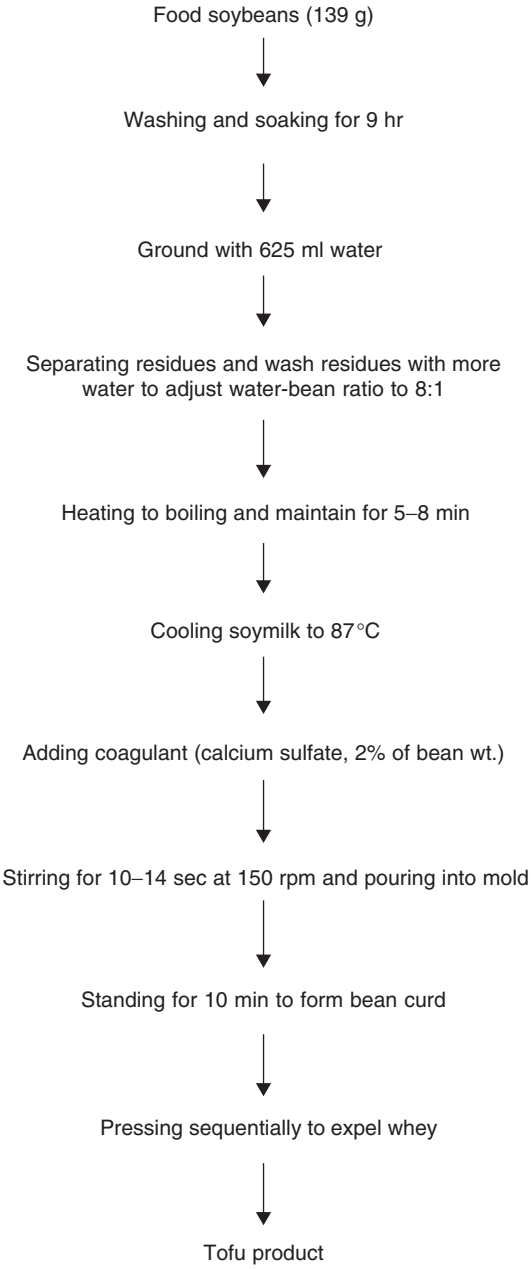

FIGURE 171.10 Procedures of tofu making for a small-scale method.

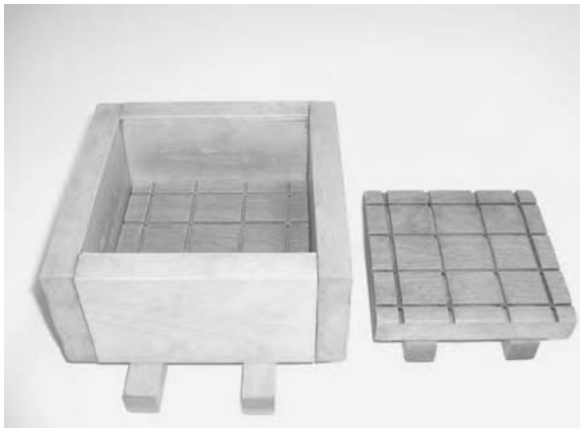

FIGURE 171.11 The tofu mold used by the small-scale method. Right: top piece for pressing.

FIGURE 171.12 The tofu mold used by the production-scale machine equipped in North Dakota State University.

FIGURE 171.13 The automatic production-scale machine (made by Ta-Ti-Hsing Machinery Co., Taoyuan, Taiwan) for soymilk and tofu production equipped in North Dakota State University.

Small-scale (139 g bean per experiment) and large-scale (6500 g bean per experiment) processing methods were developed and applied for making soymilk and soft tofu from 13 soybean varieties (154). A diagram for making soft-tofu with small scale is shown in Figure 171.10. The tofu molds for small and large scale are shown in Figure 171.11 and Figure 171.12, respectively. The automatic production-scale soymilk and tofu machine in our laboratory is shown in Figure 171.13. The results revealed that the small bench and the large-scale method correlated significantly ($p \leq 0.05$) in tofu yield, color, texture, and chemical composition (moisture, protein, lipid, ash, calcium and magnesium) (Table 171.6). Since tofu quality made by the small-bench scale was well correlated to the production method, the bench scale method may be used for determining the quality of soybeans for making tofu. Our research indicated that the quality and yield of tofu were significantly affected by soybean cultivars and processing methods (Table 171.7). Although a production-scale method has been suggested by Murphy et al. (59) for determining the soybean quality for suitability of commercial processing, the bench scale method developed in our laboratory is appropriate for evaluating soybean quality using a small quantity, since tofu quality made by the small-bench scale is well correlated to a production method.

TABLE 171.6
Correlation Coefficient of Soymilk and Tofu Physico-Chemical Properties between Small Bench-Scale Method and Large-Scale Methods

Physicochemical Property	Correlation Coefficient (r)[a]
Soymilk	
Protein	0.94***
Lipid	0.71**
Ash	0.93***
Tofu	
Yield	0.82***
Moisture content	0.78**
Protein	0.80***
Lipid	0.65*
Ash	0.96***
Calcium	0.72**
Hardness	0.54*
Elasticity	0.60*

[a] Significant levels: *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$.

Source: Data adapted from Cai et al. (154).

TABLE 171.7
Effect of Processing Method and Soybean Variety on Tofu Yield and Tofu Hardness[a]

	Yield (g/100g raw bean)		Hardness (g)	
Variety	**Bench**	**Production**	**Bench**	**Production**
Proto	551.5a*	434.8ab	1460e	3058a
T5	521.9ab*	438.1a	2122bcde	3023a
Corsoy-79	229.3f*	341.5g	3766a	3035a
Vinton	522.7ab*	402.1cd	2006bcde	2807ab
Kato	480.9bc	430.3ab	1587de	2638abc
Hardin	469.8c	411.8cd	1742cde	2307cd
Sturdy	404.1d	358.1fg	1937bcde	2592abc
SBB100ND	471.3c*	424.3abc	1707cde	2431bcd
SBB100SD	387.8de	373.2ef	2547bc	3105a
Stine 2220	411.3d*	335.3g	2227bcde	3045a
Stine 1590	351.4e	346.8g	2350bcd	2763abc
Stine 0380	395.9d	359.7fg	2683b	3082a
Stine 1570	499.0bc*	393.6de	1602de	2057d

[a] Data are means of two replicates (one determination per replicate except that hardness on production scale had four determinations per replicate). Yield is based on the wet weight basis. Means within the same column followed by different letters are significantly different ($p \leq 0.05$).

* Means are significantly different ($p \leq 0.05$) from the production counterparts.

Source: Data are adapted from Cai et al. (154).

ACKNOWLEDGMENT

- The information in this chapter has been modified from "Science and Technology of Tofu Making," by K. C. Chang and H. J. Hou. In *Handbook of Vegetable Preservation and Processing*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.
- Funding for this study was provided by the National Science Foundation EPSCoR Doctoral Fellowship project # 4414 and USDA CSREES Hatch project ND 2707 and USDA CSREES NRI 2001-10853.

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172 Biochemistry and Fermentation of Beer

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CONTENTS

I. INTRODUCTION

The production of alcoholic beverages is as old as history. Wine may have an archeological record going back more than 7.5 thousand years, with the early suspected wine residues dating from early to mid-fifth millennium B.C. (1). Clear evidence of intentional winemaking first appears in the representations of wine presses that date back to the reign of Udimu in Egypt, some 5000 years ago. The direct fermentation of fruit juices, such as that of grape, had doubtlessly taken place for many thousands of years before early thinking man developed beer brewing and, probably coincidentally, bread baking (2). The oldest historical evidence of formal brewing dates back to about 6000 B.C. in ancient Babylonia: a piece of pottery found there shows workers either stirring or skimming a brewing vat.

Nowadays, alcoholic beverage production represents a significant contribution to the economies of many countries. The most important beverages today are beer, wine, distilled spirits, cider, sake and liqueurs (3). In Belgium ("the beer paradise"), beer is the most important alcoholic

beverage, although the beer consumption declined the last 40 years: from 11,096,717 hl in 1965 to 10,059,513 hl in 2001 (4). In this time frame, wine consumption doubled from 1,059,964 hl to 2,215,579. Another trend is the spectacular increase in waters and soft drinks consumption (from 5,215,056 hl to 24,628,781 hl).

In this chapter, the biochemistry and fermentation of beer is reviewed. Firstly, the carbohydrate metabolism in brewer's yeast is discussed. The maltose metabolism is of major importance in beer brewing since this sugar is in a high concentration present in wort. For the production of a high quality beer, a well-controlled fermentation needs to be performed. During this fermentation, major flavor-active compounds are produced (and some of them are again degraded) by the yeast cells. The metabolism of the most important fermentation by-products during main and secondary fermentation is discussed in detail. The latest trend in beer fermentation technology is the process intensification using immobilized cell technology. This new technology is explained and some illustrative applications – on small and large scale – are discussed.

II. THE BEER BREWING PROCESS

The principal raw materials used to brew beer are water, malted barley, hops and yeast. The brewing process involves extracting and breaking down the carbohydrate from the malted barley to make a sugar solution (called "wort") which also contains essential nutrients for yeast growth, and using this as a source of nutrients for "anaerobic" yeast growth. During yeast fermentation, simple sugars are consumed, releasing energy and producing ethanol and other flavoring metabolic by-products. The major biological changes, which occur in the brewing process, are catalyzed by naturally produced enzymes from barley (during malting) and yeast. The rest of the brewing process largely involves heat exchange, separation, and clarification which only produces minor changes in chemical composition when compared to the enzyme catalyzed reactions. Barley is able to produce all the enzymes which are needed to degrade starch, β-glucan, pentosans, lipids and proteins which are the major compounds of interest to the brewer. An overview of the brewing process is shown in Figure 172.1, where also the input and output flows are indicated. Table 172.1 gives a more detailed explanation of each step in the process.

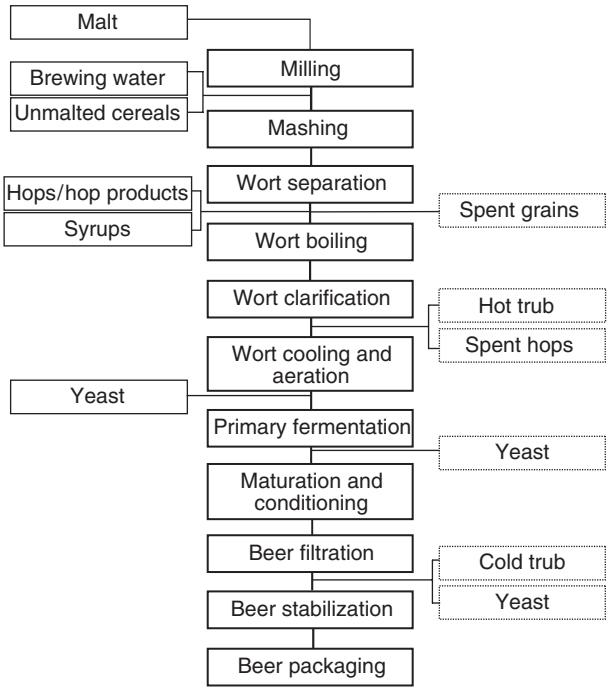

FIGURE 172.1 Schematic overview of the brewing process (input flows are indicated on the left side and output flows on the right side).

III. CARBOHYDRATE METABOLISM – ETHANOL PRODUCTION

A. Carbohydrate Uptake

Carbohydrates in wort make up 90–92% of wort solids. Wort from barley malt contains the fermentable sugars sucrose, fructose, glucose, maltose and maltotriose together with some dextrin material (Table 172. 2). The fermentable sugars typically make up 70–80% of the total carbohydrate (6). The three major fermentable sugars are glucose, and the α-glucosides maltose and maltotriose. Maltose is by far the most abundant of these sugars, typically accounting for 50 to 70% of the total fermentable sugars in an all-malt wort. Sucrose and fructose are present in a low concentration. The unfermentable dextrins play little part in brewing. Wort fermentability may be reduced or increased by using solid or liquid adjuncts.

Brewing strains consume the wort sugars in a specific sequence: glucose is consumed firstly, followed by fructose, maltose and finally maltotriose. The uptake and consumption of maltose and maltotriose is repressed or inactivated at elevated glucose concentrations. Only when 60% of the wort glucose has been taken up by the yeast will the uptake and consumption of maltose start. Maltotriose uptake is inhibited by high glucose and maltose concentrations. When high amounts of carbohydrate adjuncts (*e.g.* glucose) or high-gravity wort are employed, the glucose repression is even more pronounced, resulting in fermentation delays (7).

The efficiency of brewer's yeast strains to effect alcoholic fermentation is dependent upon their ability to utilize the sugars present in wort. This ability very largely determines the fermentation rate as well as the final quality of the beer produced. In order to optimize the fermentation

TABLE 172.1
Overview of the Brewing Processing Steps: from Barley to Beer

Process	Action	Objectives	Time	Temperature (°C)
Malting				
Steeping	Moistening and aeration of barley	Preparation for the germination process	48 h	12–22
Germination	Barley germination	Enzyme production, chemical structure modification	3–5 d	22
Kilning	Kilning of the green malt	Ending of germination and modification, production of flavoring and coloring substances	24–48 h	22–110
Milling	Grain crushing without disintegrating the husks	Enzyme release and increase of surface area	1–2 h	22
Mashing + wort separation	Addition of warm/hot water	Stimulation of enzyme action, extraction and dissolution of compounds, wort filtration, to obtain the desired fermentable extract as quick as possible	1–2 h	30–72
Wort boiling	Boiling of wort and hops	Extraction and isomerization of hop components, hot break formation, wort sterilization, enzyme inactivation, formation of reducing, aromatic and coloring compounds, removal of undesired volatile aroma compounds, wort acidification, evaporation of water	0.5–1.5 h	> 98
Wort clarification	Sedimentation or centrifugation	Removal of spent hops, clarification (whirlpool, centrifuge, settling tank)	< 1 h	100–80
Wort cooling and aeration	Use of heat exchanger, injection of air bubbles	Preparing the wort for yeast growth	< 1 h	12–18
Fermentation	Adding yeast, controlling the specific gravity, removal of yeast	Production of green beer, to obtain yeast for subsequent fermentations, carbon dioxide recovery	2–7 d	12–22 (ale) 4–15 (lager)
Maturation and conditioning	Beer storage in oxygen free tank, beer cooling, adding processing aids	Beer maturation, adjustment of the taste, adjustment of CO_2 content, sedimentation of yeast and cold trub, beer stabilization	7–21 h	−1–0
Beer clarification	Centrifugation, filtration	Removal of yeast and cold trub	1–2 h	−1–0
Biological stabilization	Pasteurization of sterile filtration	Killing or removing of micro-organisms	1–2 h	62–72 (past.) −1–0 (filtr.)
Packaging	Filling of bottles, cans, casks and kegs; pasteurization of small volumes in packings	Production of packaged beer according to specifications	0.5–1.5 h	−1–room temp.

efficiency of the primary fermentation, a detailed knowledge of the sugar consumption kinetics which is linked to the yeast growth kinetics, is required (8).

1. Maltose and Maltotriose Metabolism

The yeast *S. cerevisiae* transports the monosaccharides across the cell membrane by the hexose transporters. There are 19, or possibly 20, genes encoding hexose transporters (9). The disaccharide maltose and the trisaccharide maltotriose are transported by specific transporters into the cytoplasm, where these molecules are hydrolyzed by the same α-glucosidase yielding two or three molecules of glucose, respectively (10,11).

Maltose utilization in yeast is conferred by any one of five *MAL* loci: *MAL1* to *MAL4* and *MAL6* (9,12). Each locus consists of three genes: gene 1 encodes a maltose transporter (permease), gene 2 encodes a maltase (α-glucosidase) and gene 3 encodes a transcriptional activator of the other two genes. Thus, for example, the maltose transporter gene at the *MAL1* locus is designated *MAL61*. The three genes of a *MAL* locus are all required to allow fermentation. Some authors persist in using gene designations such as for the *MAL1* locus: *MAL1T* (transporter = permease), *MAL1R* (regulator) and *MAL1S* (maltase). The five *MAL* loci each map to a different chromosome. The *MAL* loci exhibit a very high degree of homology and are telomere linked, suggesting that they evolved by translocation

TABLE 172.2
Carbohydrate Composition of Worts (Adapted from (5))

Origin	Danish	Canadian	British
Type of Wort	Lager	Lager	Pale Ale
Original Gravity	1043.0	1054.0	1040.0
Fructose (g/l)	2.1	1.5	3.3
(%)[a]	2.7	1.6	4.8
Glucose (g/l)	9.1	10.3	10.0
(%)[a]	11.6	10.9	14.5
Sucrose (g/l)	2.3	4.2	5.3
(%)[a]	2.9	4.5	7.7
Maltose (g/l)	52.4	60.4	38.9
(%)[a]	66.6	64.2	56.5
Maltotriose (g/l)	12.8	17.7	11.4
(%)[a]	16.3	18.8	16.5
Total ferm. sugars (g/l)	78.7	94.1	68.9
Maltotetraose (g/l)	2.6	7.2	2.0
Higher sugars (g/l)	21.3	26.8	25.2
Total dextrins (g/l)	23.9	34.0	25.2
Total sugars (g/l)	102.6	128.1	94.1

[a] percent of the total fermentable sugars.

from telomeric regions of different chromosomes (13). Since a fully functional or partial allele of the *MAL1* locus is found in all strains of *S. cerevisiae*, this locus is proposed as the progenitor of the other *MAL* loci (14). Gene dosage studies performed with laboratory strains of yeast have shown that the transport of maltose in the cell may be the rate-limiting step in the utilization of this sugar (15). Constitutive expression of the maltose transporter gene (*MALT*) with high-copy-number plasmids in a lager strain of yeast has been found to accelerate the fermentation of maltose during high-gravity (24°P) brewing (16). The constitutive expression of *MALS* and *MALR* had no effect on maltose fermentability.

The control over *MAL* gene expression is exerted at three levels. The presence of maltose induces, whereas glucose represses, the transcription of *MALS* and *MALT* genes (17–19). The constitutively expressed regulatory protein (*MALR*) binds near the *MALS* and *MALT* promotors and mediates the induction of *MALS* and *MALT* transcription (20–22). Experiments with *MALR*-disrupted strains led to the conclusion that MalRp is involved in glucose repression (23,24). Relatively little attention has been paid to posttranscriptional control, i.e., the control of translational efficiency, or mRNA turnover, as mechanisms complementing glucose repression (25). The addition of glucose to induced cells has been reported to cause a 70% increase in the lability of a mRNA population containing a fragment of *MALS* (17). The third level of control is posttranslational modification. In the presence of glucose, maltose permease is either reversibly converted to a conformational variant with decreased affinity (26,27) or irreversibly proteolytically degraded depending on the physiological conditions (28,29). The latter phenomenon is called catabolite inactivation. Glucose repression is accomplished by the Mig1p repressor protein, which is encoded by the *MIG1* gene (30). It has been shown that Mig1p represses the transcription of all three *MAL* genes by binding upstream of them (31). The *MIG1* gene has been disrupted in a haploid laboratory strain and in an industrial polyploid strain of *S. cerevisiae* (32). In the *MIG1*-disrupted haploid strain, glucose repression was partly alleviated; i.e., maltose metabolism was initiated at higher glucose concentrations than in the corresponding wild-type strain. In contrast, the polyploid Δ*mig1* strain exhibited an even more stringent glucose control of maltose metabolism than the corresponding wild-type strain, which could be explained by a more rigid catabolite inactivation of maltose permease, affecting the uptake of maltose.

Recently, the gene A*GT1*, which codes for a α-glucoside transporter has been characterised (33). *AGT1* is found in many *S. cerevisiae* laboratory strains and maps to a naturally occurring, partially functional allele of the *MAL1* locus. Agt1p is a highly hydrophobic, postulated integral membrane protein. It is 57% identical to Mal61p (the maltose permease encoded at *MAL6*) and is also a member of the 12-transmembrane domain superfamily of sugar transporters (34). Like Mal61p, Agt1p is a high-affinity, maltose/proton symporter, but Mal61p is capable of transporting only maltose and turanose, while Agt1p transports these two α-glucosides as well as several others including isomaltose, α-methylglucoside, maltotriose, palatinose, trehalose and melezitose. *AGT1* expression is maltose inducible and induction is mediated by the Mal-activator.

Brewing strains of yeast are polyploid, aneuploid, or, in the case of lager strains, alloploid. Recently, Jespersen and co-workers (35) examined 30 brewing strains of yeast (5 ale strains and 25 lager strains) with the aim of examining the alleles of maltose and maltotriose transporter genes contained by them. All the strains of brewer's yeast examined, except two, were found to contain *MAL11* and *MAL31* sequences, and only one of these strains lacked *MAL41*. *MAL21* was not present in the five ale strains and 12 of the lager strains. *MAL61* was not found in any of the yeast chromosomes other than those known to carry *MAL* loci. Sequences corresponding to the *AGT1* gene (transport of maltose and maltotriose) were detected in all but one of the yeast strains.

Wort maltotriose has the lowest priority for uptake by brewer's yeast cells and incomplete maltotriose uptake results in yeast fermentable extract in beer, material loss, greater potential for microbiological stability and sometimes atypical beer flavor profiles (7). Maltotriose uptake from wort is always slower with ale strains than with lager strains under similar fermentation conditions. However, the initial transport rates are similar to those of maltose in a number of ale and lager strains. Elevated osmotic

pressure inhibits the transport and uptake of glucose, maltose and maltotriose with maltose and maltotriose being more sensitive to osmotic pressure than glucose in both lager and ale strains. Ethanol (5% w/v) stimulated the transport of maltose and maltotriose, due in all probability to an ethanol-induced change in the plasma membrane configuration, but had no effect on glucose transport. Higher ethanol concentrations inhibited the transport of all three sugars.

B. Wort Fermentation

Before the fermentation process starts, wort is aerated. This is a necessary step since oxygen is required for the synthesis of sterols and unsaturated fatty acids, which are incorporated in the yeast cell membrane (36). It has been shown that ergosterol and unsaturated fatty acids increase both in concentration as long as oxygen is present in the wort (*e.g.* (37)). A maximum concentration is obtained in 5–6 hours after pitching, but the formation rate is dependent upon pitching rate and temperature. Unsaturated fatty acids can also be taken up from the wort, but all malt wort does not contain sufficient unsaturated lipids to support a normal growth rate of yeast. Adding lipids to wort, especially unsaturated fatty acids, might be an interesting alternative (38,39). The oxygen required for lipid biosynthesis, can also be introduced by oxygenation of the separated yeast cells.

Different devices are used to aerate the cold wort: ceramic or sintered metal candles, aeration plants employing venturi pipes, two component jets, static mixers, or centrifugal mixers (40). The principle of these devices is that very small air (oxygen) bubbles are produced and quickly dissolve during turbulent mixing.

As a result of this aeration step, carbohydrates are degraded aerobically during the first few hours of the "fermentation" process. The aerobic carbohydrate catabolism takes typically 12 h for a lager fermentation.

During the first hours of the fermentation process, oxidative degradation of carbohydrates occurs through the glycolysis and Krebs (TCA) cycle. The energy efficiency of glucose oxidation is derived from the large number of $NADH_2^+$ produced for each mole of glucose oxidized to CO_2. The actual wort fermentation gives alcohol and carbon dioxide *via* the Embden-Meyerhof-Parnas (glycolytic) pathway. The reductive pathway from pyruvate to ethanol is important since it regenerates NAD^+. Energy is obtained solely from ATP-producing steps of the Embden-Meyerhof-Parnas pathway. During fermentation, the activity of the TCA cycle is greatly reduced, although it still serves as a source of intermediates for biosynthesis (41).

Lagunas (42) observed that during aerobic growth of *S. cerevisiae*, respiration accounts for less than 10% of glucose catabolism, the remainder being fermented. Increasing sugar concentrations resulting in a decreased oxidative metabolism is known as the Crabtree effect. This was traditionally explained as an inhibition of the oxidative system by high concentrations of glucose. Nowadays, it is generally accepted that the formation of ethanol at aerobic conditions is a consequence of a bottleneck in the oxidation of pyruvate, *e.g.* in the respiratory system (43–47).

A reduction of ethanol production can be achieved by metabolic engineering of the carbon flux in yeast resulting in an increased formation of other fermentation product. A shift of the carbon flux towards glycerol at the expense of ethanol formation in yeast was achieved by simply increasing the level of glycerol-3-phosphate dehydrogenase (48–51). The *GDP1* gene, which encodes glycerol-3-phosphate dehydrogenase, has been overexpressed in an industrial lager brewing yeast to reduce the ethanol content in beer (52). The amount of glycerol produced by the *GDP1*-overexpressing yeast in fermentation experiments – simulating brewing conditions – was increased 5.6 times and ethanol was decreased by 18% compared to the wild-type strain. Overexpression did not affect the consumption of wort sugars and only minor changes in the concentration of higher alcohols, esters and fatty acids could be observed. However, the concentrations of several other by-products, particularly acetoin, diacetyl and acetaldehyde, were considerably increased.

IV. METABOLISM OF BIOFLAVORING BY-PRODUCTS

Yeast is an important contributor to flavor development in fermented beverages. The compounds which are produced during fermentation, are many and varied, depending on both the raw materials and the microorganisms used. The interrelation between yeast metabolism and the production of bioflavoring by-products is illustrated in Figure 172.2.

A. Biosynthesis of Higher Alcohols

During beer fermentation, higher alcohols (also called "fusel alcohols") are produced by yeast cells as by-products and represent the major fraction of the volatile compounds. More than 35 higher alcohols in beer have been described. Table 172.3 gives the most important compounds which can be classified into aliphatic (n-propanol, isobutanol, 2-methylbutanol (or active amyl alcohol), and 3-methylbutanol (or isoamyl alcohol)) and aromatic (2-phenylethanol, tyrosol, tryptophol) higher alcohols. Aliphatic higher alcohols contribute to the "alcoholic" or "solvent" aroma of beer, and produce a warm mouthfeel. The aromatic alcohol 2-phenylethanol has a sweet aroma and has a positive contribution to the beer aroma, whereas the aroma of tyrosol and tryptophol are undesirable.

Higher alcohols are synthesized by yeast during fermentation *via* the catabolic (Ehrlich) and anabolic pathway (amino acid metabolism) (62–64). In the catabolic pathway,

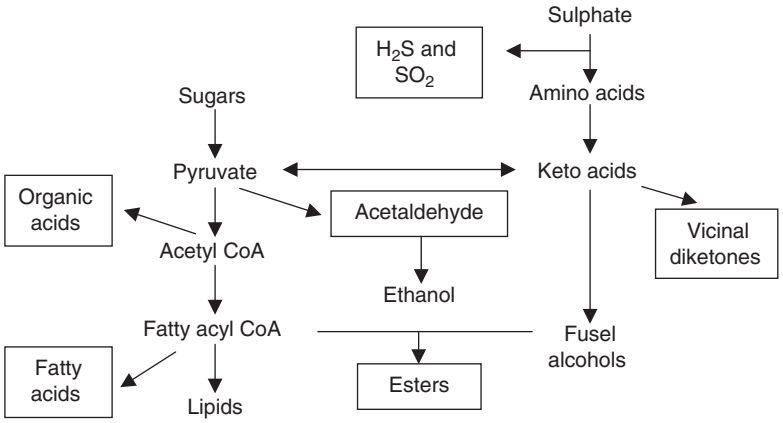

FIGURE 172.2 Interrelationships between yeast metabolism and the production of flavor active compounds (53).

TABLE 172.3
Major Higher Alcohols in Beer (54)

Compound	Flavor Threshold (mg/l)	Aroma or Taste[b]	Concentration Range (mg/l) Bottom Fermentation	Concentration Range (mg/l) Top Fermentation
n-Propanol	600[c], 800[b]	Alcohol	7–19 (12)*[f]	20–45[i]
Isobutanol	100[c], 80–100[g], 200[b]	Alcohol	4–20 (12)[f]	10–24[i]
2-Methylbutanol	50[c], 50–60[g], 70[b]	Alcohol	9–25 (15)[a]	80–140[i]
3-Methylbutanol	50[c], 50–60[g], 65[b]	Fusely, pungent	25–75 (46)[a]	80–140[i]
2-Phenylethanol	5[a], 40[c], 45–50[g], 75[d], 125[b]	Roses, sweetish	11–51 (28)[f], 4–22[g], 16–42[h]	35–50[g], 8–25[a], 18–45[i]
Tyrosol	10[a], 10–20[e], 20[c], 100[d,g], 200[b]	Bitter chemical	6–9[a], 6–15[a]	8–12[g], 7–22[g]
Tryptophol	10[a], 10–20[e], 200[d]	Almonds, solvent	0.5–14[a]	2–12[g]

* Mean value.

[a] (55); [b] (56); [c] (57); [d] (58); [e] (59); [f] Values in 48 European lagers, Dufour (unpublished data); [g] (60); [h] (61); [i] Derdelinckx (unpublished data).

the yeast uses the amino acids of the wort to produce the corresponding α-keto acid *via* a transamination reaction. The excess oxoacids are subsequently decarboxylated into aldehydes and further reduced (alcohol dehydrogenase) to higher alcohols. This last reduction step also regenerates NAD^+.

Dickinson and co-workers looked at the genes and enzymes, which are used by *S. cerevisiae* in the catabolism of leucine to isoamyl alcohol (65), valine to isobutanol (66), and isoleucine to active amyl alcohol (67). In all cases, the general sequence of biochemical reactions is similar, but the details for the formation of the individual alcohols are surprisingly different. The branched-chain amino acids are first deaminated to the corresponding α-ketoacids (α-ketoisocapric acid from leucine, α-ketoisovaleric acid from valine and α-keto-β-methylvaleric acid from leucine). There are significant differences in the way each α-ketoacid is subsequently decarboxylated. Recently, the catabolism of phenylalanine to 2-phenylethanol and of tryptophan were also studied (68). Phenylalanine and tryptophan are first deaminated to 3-phenylpyruvate and 3-indolepyruvate, respectively, and then decarboxylated. These studies revealed that all amino acid catabolic pathways studied to date use a subtle different spectrum of decarboxylases from the five-membered family that comprises Pdc1p, Pdc5p, Pdc6p, Ydl080cp and Ydr380wp. Using strains containing all possible combinations of mutations affecting the seven *AAD* genes (putative aryl alcohol dehydrogenases), five *ADH* and *SFA1* (other alcohol dehydrogenase genes), showed that the final step of amino acid catabolism can be accomplished by any one of the ethanol dehydrogenases (Ahd1p, Ahd2p, Ahd3p, Ahd4p, Ahd5p) or Sfa1p (formaldehyde dehydrogenase).

In the anabolic pathway, the higher alcohols are synthesized from α-keto acids during the synthesis of amino acids from the carbohydrate source. The pathway choice depends on the individual higher alcohol and on the level of available amino acids available. The importance of the anabolic pathway decreases as the number of carbon atoms in the alcohol increases (63) and increases in the later stage of fermentation as wort amino acids are depleted (69). Yeast strain, fermentation conditions and wort composition all have significant effects on the combination and levels of higher alcohols which are formed (69).

Conditions which promote yeast cell growth – such as high levels of nutrients (amino acids, oxygen, lipids, zinc, …), increased temperature and agitation – stimulate

the production of higher alcohols. The synthesis of aromatic alcohols is especially sensitive to temperature changes. On the other hand, conditions which restrict yeast growth – such as lower temperature and higher pressure – reduce the extent of higher alcohol production.

B. Biosynthesis of Esters

Esters are very important flavor compounds in beer. They have an effect on the fruity/flowery aromas. Table 172.4 shows the most important esters with their threshold values which are considerably lower than those for higher alcohols. The major esters can be subdivided into acetate esters and C_6–C_{10} medium-chain fatty acid ethyl esters. They are desirable components of beer when present in appropriate quantities and proportions but can become unpleasant when in excess. Ester formation is highly dependent on the yeast strain used (72,73) and on certain fermentation parameters such as temperature (74–76), specific growth rate (75), pitching rate (75,77,78) and top pressure. Additionally, the concentrations of assimilable nitrogen compounds (53,76,79), carbon sources (80–83), dissolved oxygen (76,84–86) and fatty acids (87,88) can influence the ester production rate.

Esters are produced by yeast both during the growth phase (60%) and also during the stationary phase (40%). They are formed by the intracellular reaction between a fatty acyl-coenzyme A and an alcohol:

$$R'OH + RCO\text{-}ScoA \rightarrow RCOOR' + CoASH \quad (172.1)$$

This reaction is catalyzed by an alcohol acyltransferase (or ester synthethase). Since acetyl CoA is also a central molecule in the synthesis of lipids and sterols, ester synthesis is linked to the fatty acid metabolism (see also Figure 172.2).

Alcohol acetyltransferase (AAT) has been localized in the plasma membrane (89) and found to be strongly inhibited by unsaturated fatty acids, ergosterol, heavy metal ions and sulphydryl reagents (90). Subcellular fractionation studies conducted during the batch fermentation cycle, demonstrated the existence of both cytosolic and membrane-bound AAT (91,92). In term of controlling ester formation on a metabolic basis, it has further been shown that ester synthesizing activity of AAT is dependent on its positioning within the yeast cell. An interesting feature of this distribution pattern is that specific rates of acetate ester formation varied directly with the level of cytosolic AAT activity (93).

The *ATF1* gene, which encodes alcohol acetyltransferase, has been cloned from *S. cerevisiae* and brewery lager yeast (*S. cerevisiae uvarum*) (94). An hydrophobicity analysis suggested that alcohol acetyltransferase does not have a membrane-spanning region that is significantly hydrophobic, which contradicts the membrane-bound assumption. A Southern analysis of the yeast genomes in which the *ATF1* gene was used as a probe, revealed that *S. cerevisiae* has one *ATF1* gene, while brewery lager yeast has one *ATF1* gene and another, homologous gene (*Lg-ATF1*). The AAT activities have been compared *in vivo* and *in vitro* under different fermentation conditions (95). This study suggested that ester synthesis is modulated by a repression-induction of enzyme synthesis or processing the regulation of which is presumably linked to lipid metabolism.

The ester production can be altered by changing the synthesis rate of certain fusel alcohols. Hirata *et al.* (96) increased the isoamyl acetate levels by introducing extra copies of the *LEU4* gene in the *S. cerevisiae* genome. A comparable *S. cerevisiae uvarum* mutant has been isolated (97). The mutants have an altered regulation pattern of amino acid metabolism and produce more isoamyl acetate and phenylethyl acetate.

Isoamyl acetate is synthesized from isoamyl alcohol and acetyl coenzyme A by AAT and is hydrolyzed by esterases at the same time in *S. cerevisiae*. To study the effect of balancing both enzyme activities, yeast strains with different numbers of copies of *ATF1* gene and isoamyl acetate-hydrolyzing esterase gene (*IAH1*) have been constructed and used in small-scale sake brewing (98). Fermentation profiles as well as components of the resulting sake were largely alike. However, the amount of isoamyl acetate in the sake increased with increasing ratio

TABLE 172.4
Major Esters in Beer (70)

Compound	Flavor Threshold (mg/l)	Aroma	Concentration Range (mg/l) in 48 Lagers
Ethyl actetate	20–30, 30[a]	Fruity, solvent-like	8–32 (18.4)*
Isoamyl acetate	0.6–1.2, 1.2[a]	Banana, peardrop	0.3–3.8 (1.72)
Ethyl caproate (ethyl hexanoate)	0.17–0.21, O.21[a]	Apple-like with note of aniseed	0.05–0.3 (0.14)
Ethyl caprylate (ethyl octanoate)	0.3–0.9, 0.9[a]	Apple-like	0.04–0.53 (0.17)
2-Phenylethyl acetate	3.8[a]	Roses, honey, apple, sweetish	0.10–0.73 (0.54)

* Mean value.

[a] (71).

of AAT/Iah1p esterase activity. Therefore, it was concluded that the balance of these two enzyme activities is important for isoamyl acetate accumulation in sake mash.

The synthesis of acetate esters by *S. cerevisiae* during fermentation is ascribed to at least three acetyltransferase activities, namely alcohol acetyltransferase (AAT), ethanol acetyltransferase and isoamyl AAT (99). To investigate the effect of increased AAT activity on the sensory quality of Chenin blanc wines and distillates from Colombar base wines, the *ATF1* gene of *S. cerevisiae* was overexpressed. Northern blot analysis indicated constitutive expression of *ATF1* at high levels in these transformants. The levels of ethyl acetate, isoamyl acetate and 2-phenylethyl acetate increased 3- to 10-fold, 3.8- to 12-fold, and 2- to 10-fold, respectively, depending on the fermentation temperature, cultivar, and yeast used. The concentrations of ethyl caprate, ethyl caprylate and hexyl acetate only showed minor changes, whereas the acetic acid concentration decreased by more than half. This study established the concept that the overexpression of acetyltransferase genes such as *ATF1* could profoundly affect the flavor profiles of wines and distillates deficient in aroma.

In order to investigate and compare the roles of the known *S. cerevisiae* alcohol acetyltransferases, Atf1p, Atf2p and Lg-Atf1p, in volatile ester production, the respective genes were either deleted or overexpressed in a laboratory strain and a commercial brewing strain (100). Analysis of the fermentation products confirmed that the expression levels of *ATF1* and *ATF2* greatly affect the production of ethyl acetate and isoamyl acetate. GC-MS analysis revealed that Atf1p and Atf2p are also responsible for the formation of a broad range of less volatile esters, such as propyl acetate, isobutyl acetate, pentyl acetate, hexyl acetate, heptyl acetate, octyl acetate, and phenyl ethyl acetate. With respect to the esters analyzed in this study, Atf2p seemed to play only a minor role compared to Atf1p. The *atf1*Δ*atf2*Δ double deletion strain did not form any isoamyl acetate, showing that together, Atf1p and Atf2p are responsible for the total cellular isoamyl alcohol acetyltransferase activity. However, the double deletion strain still produced considerable amounts of certain other esters, such as ethyl acetate (50% of the wild-type strain), propyl acetate (50%), and isobutyl acetate (40%), which provides evidence for the existence of additional, as-yet-unknown ester synthases in the yeast proteome. Interestingly, overexpression of different alleles of *ATF1* and *ATF2* led to different ester production rates, indicating that differences in the aroma profiles of yeast strains may be partially due to mutations in their *ATF* genes.

Recently, it has been discovered that the Atf1 enzyme is localized inside lipid vesicles in the cytoplasm of the yeast cell (101). Lipid vesicles are small organelles in which certain neutral lipids are metabolized or stored. This indicates that fruity esters are possibly by-products of these processes.

C. Biosynthesis of Organic Acids

Important organic acids detected in beer include acetate, lactate, succinate, pyroglutamate, malate, citrate, α-ketoglutarate and α-hydroxyglutarate (102). They influence flavor directly when present above their taste threshold, and by their influence on beer pH. These components have their origin in raw materials (malt, hops) and are produced during the beer fermentation. Organic acids which are excreted by yeast cells, are synthesized *via* amino acid biosynthesis pathways and carbohydrate metabolism. Especially, they are overflow products of the incomplete Krebs cycle during beer fermentation. Excretion of organic acids is influenced by yeast strain and fermentation vigor. Sluggish fermentations lead to lower levels of excretion. Pyruvate excretion follows the yeast growth: maximal concentration is reached just before the maximal yeast growth, and is next taken up by the yeast and converted to acetate. Acetate is synthesized quickly during early fermentation and is later partially reused by the yeast during yeast growth. At the end of the fermentation, acetate is accumulated. De reduction of pyruvate results in the production of D-lactate or L-lactate (most yeast strains produce preferentially D-lactate). The highest amount of lactate is produced during the most active fermentation period.

The change in organic acid productivity by disruption of the gene encoding fumarase (*FUM1*) has been investigated and it has been suggested that malate and succinate are produced *via* the oxidative pathway of the TCA cycle under static and sake brewing conditions (195). Using a NAD^+-dependent isocitrate dehydrogenase gene (*IDH1*, *IDH2*) disruptant, approximately half of the succinate in sake mash was found to be synthesized *via* the oxidative pathway of the TCA cycle in sake yeast (103).

Sake yeast strains possessing various organic acid productivities were isolated by gene disruption (104). Sake fermented using the aconitase gene (*ACO1*) disruptant contained a two-fold higher concentration of malate and a two-fold lower concentration of succinate than that made using the wild-type strain. The fumarate reductase gene (*OSM1*) disruptant produced sake containing a 1.5-fold higher concentration of succinate, whereas the α-ketoglutarate dehydrogenase gene (*KGD1*) and fumarase gene (*FUM1*) disruptants gave lower succinate concentrations. In *S. cerevisiae*, there are two isoenzymes of fumarate reductase (FRDS1 and FRDS2), encoded by the *FRDS* and *OSM1* genes, respectively (105). Recent results suggest that these isoenzymes are required for the reoxidation of intracellular NADH under anaerobic conditions, but not under aerobic conditions (106).

Succinate dehydrogenase is an enzyme of the TCA cycle and thus essential for respiration. In *S. cerevisiae*, this enzyme is composed of four non-identical subunits, i.e., the flavoprotein, the iron-sulfur protein, the cytochrome b_{560}, and the ubiquinone reduction protein

encoded by the *SDH1*, *SDH2*, *SDH3*, and *SDH4* genes, respectively (107–110). Sdh1p and Sdh2p comprise the catalytic domain involved in succinate oxidation. These proteins are anchored to the inner mitochondrial membrane by Sdh3p and Sdh4p, which are necessary for electron transfer and ubiquinone reduction, and constitute the succinate:ubiquinone oxidoreductase (complex II) of the electron transport chain. Single or double disruptants of the *SDH1*, *SDH1b* (which is a homologue of the *SDH1* gene), *SDH2*, *SDH3* and *SDH4* genes have been constructed and shown that the succinate dehydrogenase activity was retained in the *SDH2* disruptant and that double disruption of *SDH1* and *SDH2* or *SDH1b* genes is necessary to cause deficiency of succinate dehydrogenase activity in sake yeast (111). The role of each subunit in succinate dehydrogenase activity and the effect of succinate dehydrogenase on succinate production using strains which were deficient in succinate dehydrogenase, have also been determined. The results suggested that succinate dehydrogenase activity contributes to succinate production under shaking conditions, but not under static and sake brewing conditions.

D. Biosynthesis of Vicinal Diketones

Vicinal diketones are ketones with two adjacent carbonyl groups. During fermentations, these flavor-active compounds are produced as by-products of the synthesis pathway of isoleucine, leucine and valine (ILV pathway) (see Figure 172.3) and thus also linked to amino acid metabolism (112) and the synthesis of higher alcohols. They impart a "buttery," "butterscotch" aroma to alcoholic drinks. Two of these compounds are important in beer, i.e. diacetyl (2,3-butanedione) and 2,3-pentanedione. Diacetyl is quantitatively more important than 2,3-pentanedione. It has a taste threshold around 0.10–0.15 mg/l in lager beer, approximately 10 times lower than that of pentanedione (113).

The excreted α-acetohydroxy acids are overflow products of the ILV pathway which are non-enzymatically degraded to the corresponding vicinal diketones (114). Tetraploid gene dosage series for various *ILV* genes have been constructed and the obtained yeast strains were used to study the influence of the copy number of *ILV* genes on the production of vicinal diketones (115,116). It was shown that the *ILV5* activity is the rate limiting step in the ILV pathway and responsible for the overflow (Figure 172.3). The non-enzymatic oxidative decarboxylation step is the rate-limiting step and proceeds faster at a higher temperature and a lower pH (117,118). The produced amount of α-acetolactate is very dependent on the used yeast strain. The production increases with increasing yeast growth. For a classical fermentation, 0.6 ppm α-acetolactate is formed (119). At high aeration, this value can be increased to 0.9 ppm and in cylindro-conical fermentations tanks even to 1.2–1.5 ppm.

Yeast cells posses the necessary enzymes (reductases) to reduce diacetyl to acetoin and further to 2,3-butanediol, and 2,3-pentanedione to 2,3-pentanediol. These reduced compounds have much higher taste thresholds and have no impact on the beer flavor (120). The reduction reactions are yeast strain dependent. The reduction occurs at the end of the main fermentation and during the maturation. Sufficient yeast cells in suspension are necessary to obtain an efficient reduction. Yeast strains which flocculate early during the main fermentation, need a long maturation time to reduce the vicinal diketones. Diacetyl can be complexed using SO_2. These complexes can not be reduced, but diacetyl can again be liberated at a later stage by aldehydes. This situation is especially applicable to yeast strains, which produce a lot of SO_2. Worts which are produced using much adjuncts can be

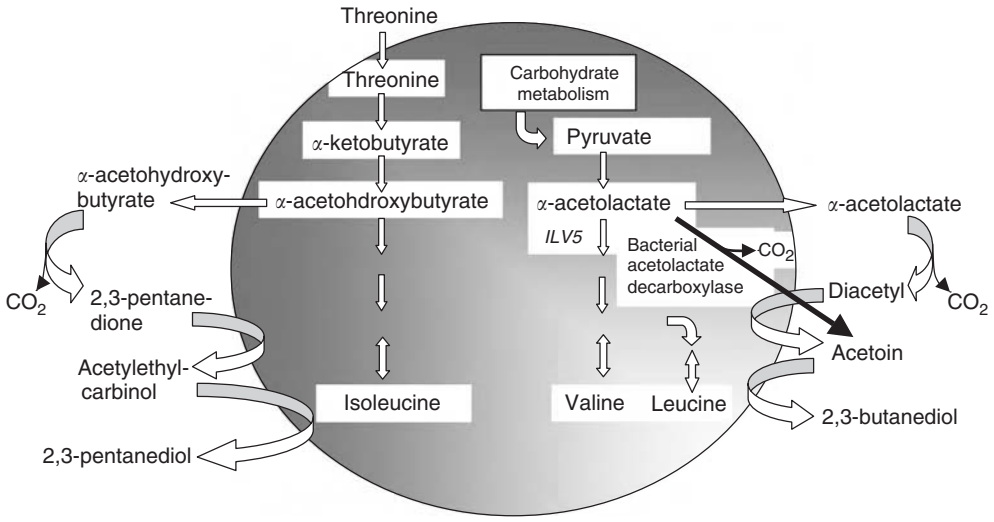

FIGURE 172.3 The synthesis and reduction of vicinal diketones in *S. cerevisiae*.

low in free amino acid content. These worts can give rise to a high diacetyl peak at the end of the fermentation.

There are several strategies which can be chosen to reduce the vicinal diketones amount during fermentation:

1. Since the temperature has a positive effect on the reduction efficiency of the α-acetohydroxy acids, a warm rest period at the end of the main fermentation and a warm maturation are applied in many breweries. In this case, temperature should be well controlled to avoid yeast autolysis.
2. Since the rapid removal of vicinal diketones requires yeast cells in an active metabolic condition, the addition of 5–10% Krausen (containing active, growing yeast) is a procedure that gives enhanced transformation of vicinal diketones (54). This procedure can lead to overproduction of hydrogen sulphide, depending upon the proportions of threonine and methionine carried forward from primary fermentation.
3. Heating up the green beer to a high temperature (90°C) and holding it there for a short period (ca. 7–10 min) to decarboxylate all excreted α-acetohydroxy acids. To avoid cell autolysis, yeast cells are removed by centrifugation prior to heating up. The vicinal diketones can be further reduced by immobilized yeast cells in a few hours (typically at 4°C) (see further).
4. Adding the enzyme α-acetolactate decarboxylase (121,122). This enzyme decarboxylates α-acetolactate directly into acetoin (see Figure 172.3). It is not present in *S. cerevisiae*, but has been isolated from various bacteria such as *Enterobacter aerogenes*, *Aerobacter aerogenes*, *Streptococcos lactis, Lactobacillus casei, Acetobacter aceti* and *Acetobacter pasteurianus*. It has been shown that the addition of α-acetolactate decarboxylase from *Lactobacillus casei* can reduce the maturation time to 22 h (121,123). An example of a commercial product is Maturex L from Novo Nordisk (Denmark) (124). Maturex L is a purified α-acetolactate decarboxylase produced by a genetically modified strain of *Bacillus subtilis*, which has received the gene from *Bacillus brevis*. The recommended dosage is 1–2 kg per 1000 hl wort, to be added to the cold wort at the beginning of fermentation.
5. Using genetic modified yeast strains:
   a. Introducing the bacterial α-acetolactate decarboxylase gene into yeast chromosomes (125–132). Transformants possessed a very high α-acetolactate decarboxylase activity, which reduced the diacetyl concentration considerably during beer fermentations.
   b. Modifying the biosynthetic flux through the ILV pathway. Spontaneous mutants resistant to the herbicide sulfometuron methyl have been selected. These strains showed a partial inactivation of the α-acetolactate synthase activity and some mutants produced 50% less diacetyl compared to the parental strain (133).
   c. Increasing the flux of α-acetolactate acid isomeroreductace activity encoded by the *ILV5* gene (134). Since α-acetolactate acid isomeroreductace activity is responsible for the rate-limiting step, increasing its activity reduces the overflow of α-acetolactate. A multicopy transformant resulted in a 70% decreased production of vicinal diketones (135), whereas an integrative transformant gave a 50% reduction (136). A tandem integration of multiple *ILV5* copies resulted also in elevated transciption in a polyploidy industrial yeast strain (137).

V. SECONDARY FERMENTATION

During the secondary fermentation or maturation of beer several objectives should be realized:

- sedimentation of yeast cells,
- improvement of the colloidal stability by sedimentation of the tannin-protein complexes,
- beer saturation with carbon dioxide,
- removal of unwanted aroma compounds,
- excretion of flavor-active compounds from yeast to give body and depth to the beer,
- fermentation of the remaining extract,
- improvement of the foam stability of the beer,
- adjustment of the beer color (if necessary) by adding coloring substances (*e.g.,* caramel),
- adjustment of the bitterness of beer (if necessary) by adding hop products.

In the presence of yeast, the principal changes that occur are the elimination of undesirable flavor compounds – like vicinal diketones, hydrogen sulphide and acetaldehyde – and the excretion of compounds enhancing the flavor fullness (body) of beer.

A. Vicinal Diketones

In traditional fermentation lagering processes, the elimination of vicinal diketones required several weeks and determined the length of the maturation process. Nowadays, the maturation phase is much shorter since strategies are used to accelerate the vicinal diketones removal (see above). Diacetyl is used as a marker molecule. The objective during lagering is to reduce the diacetyl concentration below its taste threshold (< 0.10 mg/ml).

B. Hydrogen Sulphide

Hydrogen sulphide plays an important role during maturation. Hydrogen sulphide, which is not incorporated into

S-containing amino acids is excreted by the yeast cell during the growth phase (see Figure 172.4). The excreted amount depends on the used yeast strain, the sulphate content of the wort and the growth conditions (139). At the end of the primary fermentation and during the maturation, the excess H_2S is re-utilized by the yeast. A warm conditioning period at 10–12°C may be used to remove excessive levels of H_2S.

Brewing yeasts produce H_2S when they are deficient in the vitamin pantothenate (140). This vitamin is a precursor of coenzyme A, which is required for metabolism of sulphate into methionine. Panthothenate deficiency may therefore result in an imbalance in sulphur-amino acid biosynthesis, leading to excess sulphate uptake and excretion of H_2S (141).

Sulphite is a versatile food additive used to preserve a large range of beverages and foodstuffs. In beer, sulphite has a dual purpose, acting both as an anti-oxidant and an agent for masking of certain off-flavors. Some of the flavor stabilizing properties of sulphite are suggested to be due to complex formation of bisulphate with varying carbonyl compounds, of which some would give rise to off-flavors in bottled beer (142). Especially, the unwanted carbonyl *trans*-2-nonenal has received particular attention since it is responsible for the "cardboard" flavor of some types of stale beer. It has been suggested that it would be better to use a yeast strain with reduced sulphite excretion during fermentation and to add sulphite at the point of bottling to ensure a good flavor stability (143). Therefore, a brewer's yeast disabled in the production of sulphite has been constructed by inactivating both copies of the two alleles of the *MET14* gene (which encodes for adenylylsulphate kinase). Fermentation experiments showed that there was no qualitative difference between yeast-derived and artificially added sulphite, with respect to *trans*-2-nonenal content and flavor stability of the final beer.

The elimination of the gene encoding sulphite reductase (*MET10*) in brewing strains of *Saccharomyces* results in increased accumulation of SO_2 in beer (144). The inactivation of *MET2* resulted into elevated sulphite concentrations

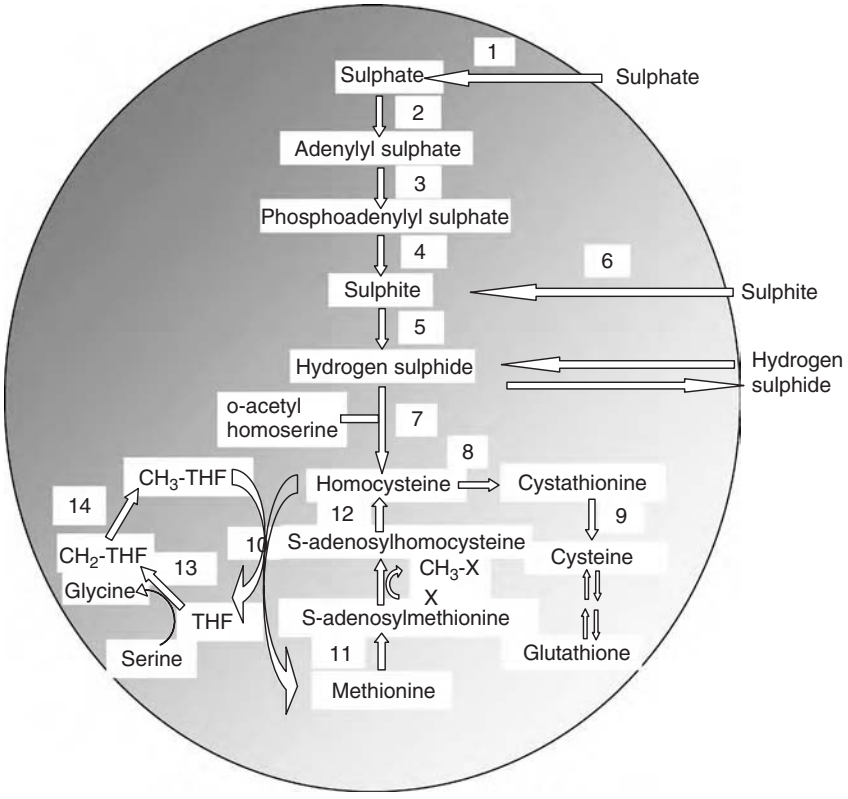

FIGURE 172.4 The re-methylation, transulfuration, and sulfur assimilation pathways. Genes and enzymes catalyzing individual reactions are: 1. sulphate permease; 2. ATP sulphurylase; 3. *MET14*: adenylylsulfate kinase (EC 2.7.1.25); 5. *MET10*: sulfite reductase (EC 1.8.1.2); 6. sulphite permease; 7. *MET17*: *O*-acetylhomoserine (thiol)-lyase (EC 2.5.1.49); 8. *CYS4*: cystathionine β-synthase (CBS; EC 4.2.1.22); 9. *CYS3*: cystathionine γ-lyase (EC 4.4.1.1); 10. *MET6*: methionine synthase (EC 2.1.1.14); 11. *SAM1* and *SAM2*: *S*-adenosylmethionine synthetase (EC 2.5.1.6); 12. *SAH1*: *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1); 13. *SHM1* and *SHM2*: serine hydroxymethyltransferase (SHMT; EC 2.1.2.1); 14. *MET12* and *MET13*: methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20). "X" represents any methyl group acceptor; THF, tetrahydrofolate; CH_2-THF, 5,10-methylenetetrahydrofolate; CH_3-THF, 5-methyltetrahydrofolate (partly adapted from (136)).

in beer (145). Beers produced with increased levels of sulphite showed an improved flavor stability.

The production of H_2S could be reduced by the expression of cystathione synthase genes from *S. cerevisiae* in a brewing yeast strain (146).

C. Acetaldehyde

Aldehydes – in particular acetaldehyde (green apple-like flavor) – have an impact on the flavor of green beer. Acetaldehyde synthesis is linked to yeast growth. Its concentration is maximal at the end of the growth phase, and is reduced at the end of the primary fermentation and during maturation by the yeast cells. As with diacetyl, levels may be enhanced if yeast metabolism is stimulated during transfer, especially by oxygen ingress. Removal also requires the presence of enough active yeast. Fermentations with early flocculating yeast cells can result in too high acetaldehyde concentrations at the end.

D. Development of Flavor Fullness

During maturation, the residual yeast will excrete compounds (i.e., amino acids, phosphates, peptides, nucleic acids, …) into the beer. The amount and "quality" of these excreted materials depend on the yeast concentration, yeast strain, its metabolic state and the temperature (54). Rapid excretion of material is best achieved at a temperature of 5–7°C during 10 days (147).

When the conditioning period is too long or when the temperature is too high, yeast cell autolysis will occur. Some enzymes are liberated (*e.g.* α-glucosidase) which will produce glucose from traces of residual maltose (54). At the bottom of a fermentation tank, the amount of α-amino-nitrogen can rise to 40–10000 mg/l, which account for an increase of 30 mg/l for the total beer volume. The increase in amino acid concentration in the beer has a positive effect on the flavor fullness of the beer. Undesirable medium chain fatty acids can also be produced in significant amounts if the maturation temperature is too high (148). Measurement of these compounds indicates the level of autolysis and permits the determination of the most appropriate conditioning period and temperature.

VI. BEER FERMENTATION USING IMMOBILIZED CELL TECHNOLOGY

The advantages of continuous fermentation – such as greater efficiency in utilization of carbohydrates and better use of equipment – led also to the development of continuous beer fermentation processes. Since the beginning of the 20th century, many different systems using suspended yeast cells have been developed. The excitement for continuous beer fermentation led – especially during the 1950s and 1960s – to the development of various interesting systems. These systems can be classified as: (i) stirred *versus* unstirred tank reactors, (ii) single-vessel systems *versus* a number of vessels connected in series, (iii) vessels which allow yeast to overflow freely with the beer ("open system") *versus* vessels which have abnormally high yeast concentrations ("closed" or "semi-closed system") (5,149–151). However, these continuous beer fermentation processes were not commercially successful due to many practical problems, such as the increased danger of contamination (not only during fermentation but also during storage of wort in supplementary holdings tanks which are required since the upstream and downstream brewing processes are usually not continuous), changes in beer flavor (152) and a poor understanding of the beer fermentation kinetics under continuous conditions. One of the well known exceptions is the successful implementation of a continuous beer production process in New Zealand by Morton Coutts (Dominion Breweries) which is still in use today (5,150).

In the 1970s, there was a revival in developing continuous beer fermentation systems due to the progress in research on immobilization bioprocesses using living cells. Immobilization gives fermentation processes with high cell densities, resulting in a drastic increase in fermentation productivities compared to the traditional time-consuming batch fermentation processes.

The last 30 years, immobilized cell technology has been extensively examined and some designs have reached already commercial exploitation. Immobilized cell systems are heterogeneous systems in which considerable mass transfer limitations can occur, resulting in a changed cell yeast metabolism. Therefore, successful exploitation of ICT needs a thorough understanding of mass transfer and intrinsic yeast kinetic behavior of these systems.

A. Carrier Materials

Various cell immobilization carrier materials have been tested and used for beer production/bioflavoring. Selection criteria are summarized in Table 172.5. Depending on the particular application, reactor type and operational conditions, some selection criteria will be more appropriate. Examples of selected carrier materials for particular applications are tabulated in Table 172.6.

B. Applications of Immobilized Cell Technology (ICT) in the Brewing Industry

1. Flavor Maturation of Green Beer

The objective of flavor maturation is the removal of diacetyl and 2,3-pentanedione, and their precursors α-acetolactate and α-acetohydroxybutyrate, which are produced during the main fermentation (see above). The conversion of α-acetohydroxy acids to the vicinal diketones is the rate-limiting step. This reaction step can be

TABLE 172.5
Selection Criteria for Yeast Cell Immobilization Carrier Materials (153)

High cell mass loading capacity
Easy access to nutrient media
Simple and gentle immobilization procedure
Immobilization compounds approved for food applications
High surface area-to-volume ratio
Optimum mass transfer distance from flowing media to center of support
Mechanical stability (compression, abrasion)
Chemical stability
Highly flexible: rapid start-up after shut-down
Sterilizable and reusable
Suitable for conventional reactor systems
Low shear experienced by cells
Easy separation of cells and carrier from media
Readily up-scalable
Economically feasible (low capital and operating costs)
Desired flavor profile and consistent product
Complete attenuation
Controlled oxygenation
Control of contamination
Controlled yeast growth
Wide choice of yeast

TABLE 172.6
Some Selected Applications of Cell Immobilization Systems Used for Beer Production

Carrier Material	Reactor Type	Reference
Flavor maturation		
Calcium alginate beads	Fixed bed	(154)
DEAE-cellulose	Fixed bed	(155)
Polyvinyl alcohol beads	Fixed bed	(156)
Porous glass beads	Fixed bed	(129,157)
Alcohol-free beer		
DEAE-cellulose beads	Fixed bed	(158,159)
Porous glass beads	Fixed bed	(160)
Silicon carbide rods	Monolith reactor	(161)
Acidified wort		
DEAE-cellulose beads	Fixed bed	(162)
Main fermentation		
Calcium alginate beads	Gas lift	(163)
Calcium pectate beads	Gas lift	(164)
κ-Carrageenan beads	Gas lift	(165)
Ceramic beads	Fixed bed	(166)
Gluten pellets	Fixed bed	(167)
Polyvinyl alcohol beads	Gas lift	(156)
Porous glass beads	Fixed bed	(168)
Porous chitosan beads	Fluidized bed	(169,170)
Silicon carbide rods	Monolith reactor	(171)
Spent grains	Gas lift	(172)
Wood chips	Fixed bed	(173,174)

accelerated by heating the beer – after yeast removal – to 80–90°C during a couple of minutes. The resulting vicinal diketones are subsequently reduced by immobilized cells into their less-flavor-active compounds.

The traditional maturation process is characterized by a near-zero temperature, low pH and low yeast concentration, resulting in a very long maturation period of 3 to 4 weeks. Using immobilized cell technology this long period can be reduced to 2 hours. An ICT maturation process using a packed-bed bioreactor with DEAE-cellulose beads has been successfully integrated in Synebrychoff Brewery (Finland) for the treatment of 1 million hl per year (175). Alfa Laval and Schott Engineering developed a maturation system based on porous glass beads (176). This system has been implemented in several breweries in Finland (179), Belgium and Germany. The German company Brau & Brunnen has also shown an interest in the Alfa Laval maturation technology. In 1996, a 30000 hl/year pilot scale system was purchased and installed in their plant (178). The Alfa Laval maturation system has been implemented in a medium-sized German brewery (Schäff/Treuchtlingen) (179). De obtained beers yielded overall good analytical and sensorial results.

2. Production of Alcohol-Free or Low-Alcohol Beer

The classical technology to produce alcohol-free or low-alcohol beer is based on the suppression of alcohol formation by arrested batch fermentation (180). However, these beers are characterized by an undesirable wort aroma since the wort aldehydes have only been reduced to a limited degree (158,181,182). The reduction of these wort aldehydes can be quickly achieved by a short contact time with the immobilized yeast cells at a low temperature without undesirable cell growth and ethanol production. A disadvantage of this short contact process is the production of only a small amount of desirable esters.

Controlled ethanol production for low-alcohol and alcohol-free beers have been successfully achieved by partial fermentation using DEAE-cellulose as carrier material which was packed in a column reactor (158,183). This technology has been successfully implemented by Bavaria Brewery (The Netherlands) to produce malt beer on an industrial scale (150000 hl/year) (162). Several other companies – i.e., Faxe (Denmark), Ottakringer (Austria) and a Spanish brewery – have also implemented this technology (178). In Brewery Beck (Germany), a fluidized-bed pilot scale reactor (8 hl/day) filled with porous glass beads was used for the continuous production of non-alcohol beer (157,160,184). Yeast cells immobilized in silicon carbide rods and arranged in a multichannel loop reactor (Meura, Belgium), have been used to produce alcohol-free beer at pilot scale by Grolsch Brewery (The Netherlands) and Guinness Brewery (Ireland) (185).

Nuclear mutants of *S. cerevisiae* which are defective in the synthesis of tricarboxylic acid cycle enzymes – i.e. fumarase (186) or 2-oxoglutarate dehydrogenase (187) have been immobilized in calcium pectate gel beads and used in a continuous process for the production of non-alcoholic beer (188). These strains produced minimal amounts of ethanol and they were also able to produce much lactic acid (up to 0.64 g/dm^3).

3. Production of Acidified Wort Using Immobilized Lactic Acid Bacteria

The objective of this technology is the acidification of the wort according to the "Reinheidsgebot," before the start of the boiling process in the brewhouse. An increased productivity of acidified wort has been obtained using immobilized *Lactobacillus amylovorus* on DEAE-cellulose beads (162,189). The pH of wort was reduced below a value of 4.0 after contact times of 7 to 12 min using a packed-bed reactor in downflow mode. The produced acidified wort was stored in a holding tank and used during wort production to adjust the pH.

4. Continuous Main Fermentation

The Japanese brewery Kirin developed a multistage continuous fermentation process (166,190,191). The first stage is a stirred tank reactor for yeast growth, followed by packed-bed fermenters, and the final step is a packed-bed maturation column. The first stage ensures adequate yeast cell growth with the desirable free amino nitrogen consumption. Ca-alginate was initially selected as carrier material to immobilize the yeast cells. These alginate beads were later replaced by ceramic beads ("Bioceramic®"). This system allowed beer to be produced within three to five days.

The engineering company Meura (Belgium) developed a reactor configuration with a first stage with immobilized yeast cells where partially attenuation and yeast growth occurs, followed by a stirred tank reactor (with free yeast cells) for complete attenuation, ester formation and flavor maturation (171,192). Silicon carbide rods are used in the first reactor as immobilization carrier material. The stirred tank (second reactor) is continuously inoculated by free cells which escape from the first immobilized yeast cell reactor.

Labatt Breweries (Interbrew, Canada) in collaboration with the Department of Chemical and Biochemical Engineering at the University of Western Ontario (Canada) developed a continuous system using κ-carrageenan immobilized yeast cells in an airlift reactor (165,178,193). Pilot scale research showed that full attenuation was reached in 20–24 hours with this system compared to 5–7 days for the traditional batch fermentation. The flavor profile of the beer produced using ICT was similar to the batch fermented beer.

Hartwell Lahti and VTT Research Institute (Finland) developed a primary fermentation system using ICT on a pilot scale of 600 l/day (174). Woodchips were used as the carrier material that reduced the total investment cost by one third compared to more expensive carriers. The results showed that fermentation and flavor formation were very similar compared to a traditional batch process, although the process time was reduced to 40 hours.

Andersen and co-workers (194) developed a new ICT process in which the concentration of carbon dioxide is controlled in a fixed-bed reactor in such a way that the CO_2 formed is kept dissolved, and is removed from the beer without foaming problems. DEAE-cellulose was used as carrier material. High gravity beer of acceptable quality has been fermented in 20 hours at a capacity of 50 l/h.

ACKNOWLEDGMENT

The information in this chapter has been derived from "Beer," in: *Alcoholic Beverages Manual,* copyrighted 2004© by R. Willaert. Used with permission.

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CONTENTS

I. INTRODUCTION

The technology of fermentation is a valuable asset of the Chinese culture. This chapter introduces the fermentation technology of Chinese wine or "jiu" (alcoholic beverages) in order to promote the Chinese wine culture, strengthen the cultural exchange by joining together the ancient tradition and modern cultures on alcoholic beverages, and enhance the development of fermentation industry. Readers should note that the term "jiu" used in this chapter refers to Chinese alcoholic beverages (a very broad term including those made from fruits, cereals, animal milks, distilled spirits, and various specialty alcoholic beverages and liqueurs). The term Chinese wine in this chapter will not comply with U.S. regulations that will be mentioned where applicable to provide readers a frame of reference.

History of Chinese alcoholic beverages. Alcoholic fermentation has a long history in China. The most primitive Chinese "jiu" was made from the piling of wild fruits and natural fermentation; it was called "hou jiu," which mean "ape/monkey wine," as the fruits most likely were collected by the apes or monkeys. Historic records on "jiu" fermentation showed that humans developed this technology at least 4000 to 5000 years ago. There were lots of artifacts related to "jiu" fermentation unearthed from the Dawenkou Cultural Relics in Shandong Province in China. These discoveries showed that man already knew how to make "jiu" at that time. This period was about 2300 BC, and approximately coincided with the Chinese legend that the daughter of Emperor "Shun" commanded the capture of yellowed-haired monkey to make "jiu." There was the "jiu" meaning "wine/alcoholic beverage" on the carvings on bones from the "Shang" Dynasty, indicating that the alcoholic fermentation industry was already well developed at that time. In the "Yueling" or "Orders of Months" chapter in the "Lizi" or "the Book of Manners," there was already descriptions on the processes of making "jiu" in Ancient China. Later, there was also "The Six Must-Have Method:"

- must have all the cereals;
- must have the proper starter(s) and its/their carrier(s);
- the soaking must be sanitary;
- the underground water must have pleasant flavor;
- the ceramic vessels must be of good quality; and
- the heating must be proper.

In the ancient times, in order to obtain the flavor-specific "jiu," it was common to utilize the fragrances from flowers, either alone or in combination in the fermentation. However, the above descriptions on "jiu" was limited to regularly fermented "jiu;" there is no accurate record on when distilled alcoholic beverages (spirits) started to appear. In the Ming Dynasty, the famous herbist, Lee Shizhen, pointed out in his "Ben Cao Gang Mou" or "Outline of Herbs" that distilling "jiu" was not an ancient methodology; it was invented in the Yuan Dynasty. The strong "jiu" and fermented mash were heated in containers, forcing the alcohol to evaporate, and the condensed droplets (of alcohol) were collected. Whether distilled spirit was really "invented" in the Yuan Dynasty, there is no conclusion. However, distilling the strong "jiu" and fermented mash is a fermentation technology unique in the whole world, and it is an invention of ancient Chinese.

Utilizing starch as the raw material in "jiu" making requires two important biochemical steps: saccharization and fermentation. These two steps are accomplished by using the "jiu qu" or "starter." "Jiu qu" is an important invention in the Chinese fermentation technology; it is the world's earliest "compound fermentation reagent" containing multi-microorganisms. In the "Shuo Ming" or "Description of Life" chapter of the "Shang Shu" or "the Book of Highest Honor," it was recorded that the "qu" and "nie" (sprouted cereals) were required to make "jiu" and "li" (sweet wine), respectively. The "qu" contains many beneficial microorganisms providing the necessary starch hydrolyzing enzymes and the enzymes required for converting the sugars into alcohol in the fermentation of "jiu."

The ancient technology of making "qu" is being improved gradually. It started out from the individual "qu" to "shenyi" or "overcoat," and then finally to the current "qu" ball or cake. The quality of "qu" also is improved gradually. In order to inhibit the undesirable or harmful microorganisms and develop the beneficial microorganisms, the ancient Chinese even added various herbs or juices from selected plants in the production of "da (large) qu" and "sui (small) qu." These techniques are still preserved today. For example, the addition of laliaocao

(Polygonanum hydropiper L.) in the making of "xiao qu" with even more than 90 various herbs in the "xiao qu" for "Dong jiu," and more than 40 herbs in the "da qu." "Hong qu" ("red qu") is a big branch of technology in making "qu." "Outline of Herbs," and "Tian Gong Kai Wu" or "Development of New Products," recorded the production of "hong (red) qu." At the present time, the main "qu" series in China are the "da qu," "xiao qu" and "hong qu." There are both saccharifying agents and fermenting agents. This way of making "qu" not only is suitable in the cultivation and selection of beneficial microorganisms, but also effective methodology in preserving the stocks of microorganisms, and their enzymatic activities. This invention is unique to the Chinese in the technology of making "qu" and "jiu" fermentation. In other countries of the world, saccharification by malt followed by the addition of yeast are still being used in the production of alcoholic beverages from cereals such as beer and whiskey. However, in China, the production of "jiu" using "qu" reflects a parallel action of saccharification and alcoholic fermentation. It was not until the 19th centuries that Europeans understood the action of Chinese "qu," and called it "starch fermentation."

In the ancient times, the Chinese also has stringent requirements on the water used and fermentation season. In the "Qi Ming Yao Shu" or "Important Technologies in Governing the Qi People," it was indicated that river water was best. If the location for making "jiu" is far from the river, use water from wells with sweet taste; water with slightly salty taste is not good or suitable. The season for making "jiu" is when the new crop is ripe, i.e., in the Fall after harvest. The "jiu" with origins from the ancient past that are still being made are "da qu bai jiu" or white "jiu" made with "da qu," "xiao qu huang jiu" or yellow "jiu" made with "xiao chu," "tian jiu niang" or sweet fermented "jiu," and others. Their fermentation methods are Chinese unique technologies, and they are also the pride of the Chinese race. The "bai jiu" and "huang jiu" are the most famous traditional "jiu," and they can be called the Chinese national "jiu."

II. FERMENTATION OF "BAI (WHITE) JIU"

A. Characteristics and Classification of Chinese "Bai Jiu"

"Bai jiu" are distilled spirits made from raw materials with starch and fermentable sugars after fermentation and distillation. The alcohol content is high, and therefore is called "lei xing jiu" or "shao jiu" (potent "jiu" or "burning "jiu"). A poem from the Tang Dynasty has the verses as follows: "fresh litchi is red as the cock's comb; the first odor of "shao jiu" is like the fragrance of amber." This showed that "shao jiu" originated before the Tang Dynasty. According to old Chinese literature and subsequent speculations, the distilled "bai jiu" may originate from the modification of "huang jiu." The production characteristics of Chinese "bai jiu" are the wide variation of raw materials, unique technology, numerous products and wide variations in flavor.

Chinese "bai jiu" usually are classified by two methods. The first method is based on the "qu" used. They can be classified into four (4) types — "Da qu jiu," "Xiao qu jiu," "mixed Da-xiao method qu bai jiu" and "gluten qu bai jiu."

The second method is based on the aroma: they can be classified into five (5) types.

- The first kind has paste-like aroma, e.g., "Maotai jiu." Its flavor characteristics are exceptional paste aroma, fragrant, rich body, and long-lasting — the flavor can last overnight, and the flavor stays in the glass after it is emptied.
- The second kind is rich odor type "jiu," e.g., "Luzhou da qu jiu," "Wulangyi" or "Five grain liquid." Their main characteristics are their rich flavor from the fermentation tank, sweet taste, balanced flavor, and long-lasting. The rich odor of this "jiu" is due to mainly capronyl ethyl ester.
- The third kind is light-odor "jiu," e.g., "Shanxi Fen jiu." Its characteristics are light, pure aroma, with balanced taste, sweet and soft, and its residual taste is clean and pure. The aroma comes from the combination of acetyl ethyl ester and lactyl ethyl ester.
- The fourth kind is rice-aroma type such as "Quilin Sanhua (three flowers) Jiu," and "Guangtong mi (rice) jiu" with clear, sweet, clean and pure flavor. Their characteristics are honey-type odor, clear, soft, clean, with pleasing aftertaste. The odor is like honey-type with the combination of fermented "Huang jiu" and lactyl ethyl ester. The main flavoring compounds are β-benzyl ethyl ester, and capronyl ethyl ester.
- The fifth kind is a combination of two or more of the above-mentioned types, such as "Meijiang jiu" (with a combination of "Luzhou dai qu jiu" and fruity flavors), and the "Bai Sha Yi Jiu" from Shanxa, Wunan Province (a mixture of "Luzhou Da Qu Jiu" and "Maotai Jiu" with its typical flavor).

In the northern provinces, there are some "jiu" with bland flavor. They are produced by new technology utilizing artificially-constructed old-fermentation tanks. They carry a light "Lozhou Da Qu Jiu" flavor. With improved quality, they may be considered as similar to "Fen Jiu" and "Lozhou Dai Qu Jiu." Besides the above-mentioned, the "Chen Liang Jiu (Prolonged, Fermented Jiu)" from the

Laolong Winery in Xanyang Province carries a woody odor that is unusual. In general, except for the "Fen Jiu" which carries a clean light odor, most "bai jiu" carry to some extent the flavor of "Luzhou Da Qu Jiu."

B. "Da Qu Jiu"

"Bai Jiu" made with "Da Qu" as the saccharifying and alcoholic fermentating agents are called "Da Qu Jiu." Most of the famous Chinese "Bai Jiu" and the local quality "Jiu" are produced using the "Da Qu" method. The technology is described as follows:

1. Preparation of the "Da Qu"

a. Raw materials and proportions

Raw materials. Raw materials for "Da Qu" usually include wheat, barley, peas, and other beans/peas and other cereals.

Proportions. Proportions of raw materials vary among factories. In general, a ratio of 120–130 kg barley to 38 kg wheat is used for "Da Qu" type "Jiu." For the lighter "Fen Jiu" and "Xi Feng Jiu," the "Da Qu" is prepared with 60% barley and 40% green peas on a weight basis. For the stronger type such as the "Luzhou Da Qu" and the paste-aroma type "Maotai Da Qu," the "Da Qu" is prepared using only wheat.

b. Time to prepare the "qu"

In general, "qu" is prepared in late Spring to before mid-Autumn. For example, the "Fen Jiu Qu" is prepared in Qing Ming (April) to the 10th month in the Chinese lunar calendar (about November), and it is called "Tao Fa (peach flower) Qu." The "Luzhou Qu" is prepared in the Summer, and is called "Fu (hot summer) Qu." The "Maotai Jiu Qu" is prepared starting from the 5th month (about June) in the lunar calendar. However, most of the "qu" is now prepared all year round. With proper control of the preparation requirements, "qu" can be prepared all year round without difficulty.

c. Technology of "qu" preparation

Based on the incubation temperature, the technology can be divided into the "high temperature qu" and "intermediate temperature qu."

i. High temperature "qu" technology (40–45 days)

Figure 173.1 is a schematic flow chart of preparing "high temperature qu."

Grinding of the wheat. "High temperature qu" is prepared with wheat only. The husk should be removed before the grinding process. The wheat kernels are soaked (wetted) in 5–10% water for 3–4 hours before grinding with a steel grinder, with the bran flattened and the endosperm disintegrated into coarse wheat flour.

Mixing of the "qu" ingredients. The ingredients such as the wheat bran and flour, water and inoculum are added continuously in appropriate ratios into the mixer. After thorough mixing, the mixture is conveyed into the presser for the "pressing" process. The water added depends on the moisture content of the wheat component, and weather during the preparation period (temperature and relative humidity). In general, the water added for barley and green pea "qu" is controlled within the range of 45–56%, pure wheat "qu" at 37–40%; and barley, wheat and pea "qu" about 40% of the weight of the raw materials. Temperature of added water is controlled at 30–35°C in the winter, and 14–16°C in the summer. The traditional procedure of preparing "high temperature qu" is to add a fixed amount of inoculum during the mixing process. This procedure is still being used today. The amount of inoculum is 4–5% of the wheat flour in the summer, and 5–8% in the winter. In general, it is believed that the residual "qu" from the previous year containing abundant microorganisms with white "qu" is a better choice.

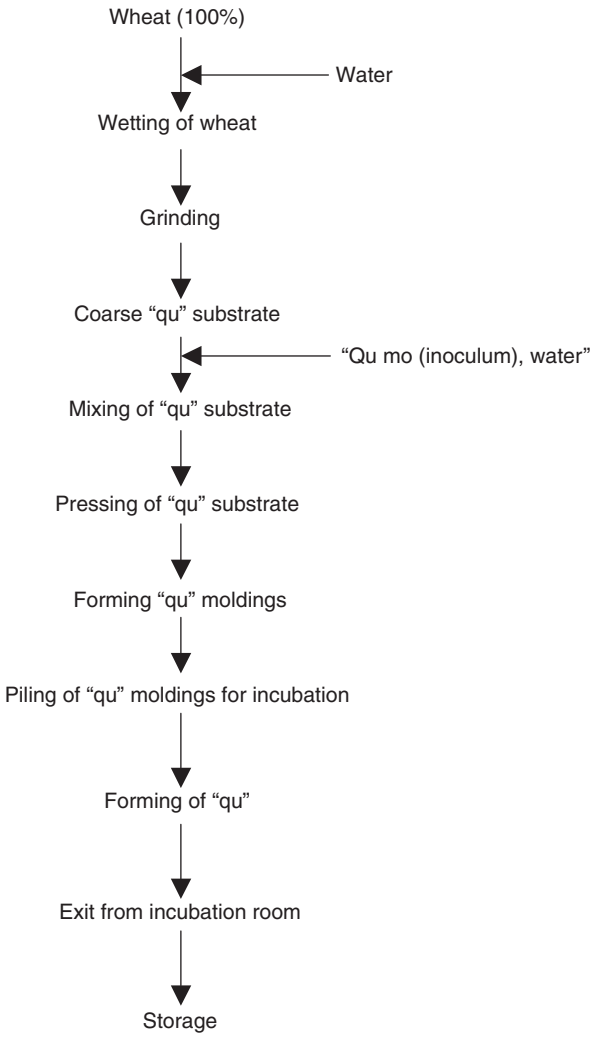

FIGURE 173.1 Generalized schematic flowchart for manufacturing of high-temperature "qu."

Pressing of the "qu" bricks. The purpose of pressing is to press the "qu" mixture into brick form. In the past, it was done manually. The requirements for pressing "qu" are as follows: the angles must be proper, same thickness, smooth and uniform surface, uniform moisture inside and out, and on the hard side so that they will not shatter during transportation. For a good brick of "qu," the sides must be tightly packed with a looser interior so that the microorganisms can penetrate and reproduced. This manual pressing process is mostly replaced by mechanical means today.

Piling and inocubation of the "qu" bricks. This process includes four (4) steps: piling, covering with hay and water sprinkling, turnover, and breaking. After the "qu" bricks are made, they are left in the open air for 2–3 hours to smoothen and dry out the surface. The bricks are then firmer and are ready for transfer to the incubation room. The floor areas around the walls should be covered with hay, about 15 cm thick in order to maintain the temperature. The bricks are then laid with three bricks in one direction and another three bricks in the opposite direction in one row. These bricks are separated 2 cm apart with hay to facilitate mold development. When one layer is completed, hay is laid on top of these bricks, about 7 cm thick. Another layer of bricks is put on top, with bricks laid in a position opposite to the lower layer so that there is good air circulation. These bricks are piled to 4–5 layers high. Other rows are then piled up until enough space is left for turnover later.

Covering with hay and sprinkling with water. The top layer of "qu" bricks is covered with hay to maintain the temperature and relative humidity. The relative humidity is maintained by sprinkling water on the hay. Amount of sprinkling water usually is more in the summer than in the winter, and the amount is limited to not letting the water get into the bricks.

Turnover. When the piles of "qu" bricks are covered with hay and water sprinkled on to it, the windows and doors in the incubation room are closed. The microorganisms then start to grow on the surface of the "qu" bricks, and the temperature of the bricks starts to increase. The brick temperature will reach to about 63°C in 5–6 days in the summer, and 79 days in the winter. Relative humidity is almost saturated in the room. At this point, the "qu" bricks are covered with a coat of microorganisms. After that, the bricks are turned over the first time. After another week, the bricks are turned over the second time to dry up the bricks faster. The purpose of turning the bricks is to adjust the temperature and relative humidity to allow even maturation for each brick. When the bricks are turned over, the wet hay between bricks and on the floor should be replaced with dry hay to facilitate air circulation. In order to facilitate the maturation and drying, the distance between bricks can be increased, and the bricks can be stood on their ends. Most of the mycelia penetrate inward after the bricks are turned over; the drying process is then the process for the mold to penetrate inside the brick. During this period, if the moisture level in the bricks is too high, this will delay the rate of mold development. The timing for turnover should be carefully controlled. Too early turnover decreases the highest achievable temperature in the "qu" brick. The "Da qu" produced then has too much white mold; too late turnover causes the development of black mold. For proper production, the requirement is to have lots of yellow mold. At the present time, the time to turn over the bricks is determined by the temperature and the taste of the "qu" bricks. That is, when the brick reaches a temperature of about 60°C and a sweet taste, it is time to turn the bricks.

Breaking the "qu" bricks. After the "qu" bricks are turned over, the temperature usually will decrease by 7–12 °C. After 67 days, the temperature will again reach to its peak, and then decreases gradually. The bricks start to dry up. About 15 days after turned over, the doors and windows can be opened slightly to exchange the air. After 40 days (50 days in winter), temperature of "qu" bricks drops down to about room temperature. The bricks are almost completely dry. They can then be broken up and transferred out from the incubation room. If wet and heavy bricks are noticed at the bottom of the piles (moisture content above 15%), these bricks must be placed in a ventilated area or hut to complete the drying process.

Storage of matured "qu." There are three colors for the high temperature "qu:" yellow, white and black. Traditionally, the golden yellow "qu" with chrysanthemum (yellow) center or red center have the "qu" paste odor and flavor. White "qu" has strong saccharifying activity, but not as good as the yellow "qu." After the "qu" bricks are broken, they should be stored for three months; these are called aged "qu." The enzymatic activities of aged "qu" are lower than the newly-made "qu," but aged "qu" has better odor. Also, during fermentation, rate of temperature increase is slower, and less acid is produced in the final product.

ii. Technology of preparing intermediate temperature "qu" (20–30 days)

Figure 173.2 is a schematic flowchart in preparing the intermediate temperature "qu."

Grinding of raw materials. Mix 60% barley and 40% peas (weight basis), and grind them together. Powder going through a 20 mesh screen should be 20% in the winter, and 30% in the summer.

Pressing of "qu" bricks. The powered raw material is mixed with water and filled into the pressing machine to make "qu" bricks. The "qu" bricks contains 36–38% water, and weighs 3.2–3.5 kg each. The formed bricks should have smooth surface, proper angles, and uniform thickness.

Incubation of "qu." The procedure in preparing "Qing Cha Qu" is used as an example.

- Lining the room. The incubation room should be pre-adjusted to room temperature of 15–20°C, with temperature lower in the summer. The floor

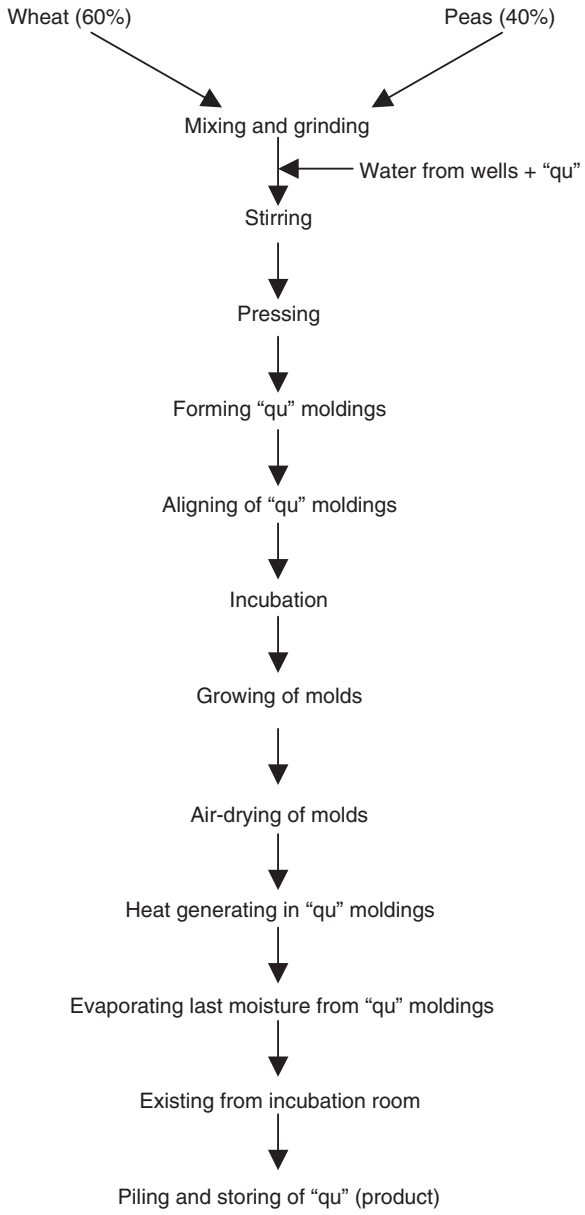

FIGURE 173.2 Generalized schematic flowchart for production of medium-temperature "qu."

of the incubation room is lined with hay. The "qu" bricks are laid on the side in rows with space of 2–3 cm apart, closer in the winter and farther apart in the summer. Each row is 34 cm apart. Above each layer is laid with reed and bamboo rods. There are three layers of "qu" bricks arranged in "empty squares and inverted cone" shapes.

- Growth of molds. The "qu" bricks are naturally dried briefly after the alignment. The bricks are then covered above and around with mats or hemp bags to maintain the temperature. Evaporation is rapid in the summer time; cool water can be sprinkled on top. Doors and windows are then closed. Temperature inside the room starts to increase. Generally after one day, molds start to grow on the surface of the bricks with appearance of white mold mycellium and spots. Temperature can rise to 38–39°C in about 36 hours in the summer, and about 72 hours in the winter. In practice, the temperature rise in "qu" bricks should be gradual to provide proper growth. At this time, rhizomoid type molds and sporadic molds with powdery spots start to appear; also there are pin-head sized creamy or yellowish yeast colonies. If the designed temperature is reached, but the molds on the surface do not developed properly, a remedy is to uncover part of the mats slowly to release the heat and extend the incubation time for a few hours to allow proper mold growth. However, it is important to maintain the relative humidity.
- Drying of the mold. When the "qu" bricks reach a temperature of 38–39°C, the window and doors in the incubation chamber should be opened to ventilate the moist air and lower the room temperature. At the same time, the mats should also be removed. Layers of "qu" bricks should be turned over (reversed) and the space between bricks should be increased to help reduce the moisture and temperature. This is to control the growth of microorganisms on the brick surface, and avoid too much mycelia. This process will fix the shape of the bricks, and is called the "drying of molds." The "drying of molds" should be done at the right time. If it is done too late, the mycelia will be too long, the brick surfaces will wrinkle and inhibit the evaporation of moisture from the interior of the bricks. If the drying is done too early, the amount of mycelia is not enough, and affects the proper growth of microorganisms inside the bricks as the bricks are not porous enough. The initial temperature for "drying of molds" is 28–32°C, allowing more extensive cross ventilation, and avoiding the dried up and cracking of the brick surface. The period of "drying of molds" is 2–3 days; stacking the brick layers once everyday; the first stacking increases the brick layers from 3 to 4, and the second stacking from 4 to 5.
- Rise in temperature. Two to three days after "drying of molds," the bricks are no longer sticky on the surface. The windows and doors are closed and temperature rise stage begins. After entering the incubation room for 5–6 days, the bricks start to increase in temperature. When the temperature reaches 36–38°C, turnover of

bricks starts. The reeds are removed, and the layers of bricks increases from 5 to 6, and the bricks are aligned in the "mirror image of λ" shape. The bricks are turned over twice each day. At this time, the bricks are further dried twice, with the windows and doors opened and closed twice in the daytime and at night. Temperature of "qu" bricks rises and drops twice, with temperature starting from 38°C increasing gradually to 45–46°C. This will take 4–5 days. After this period, it enters the high temperature stage, and the bricks are stacked to 7 layers.

- High temperature stage. At this stage, the growth of microorganisms is still vigorous. The mycelia penetrate from the outside inward, and the moisture and heat migrate from inside outward. The room temperature is maintained at 44–46°C for 7–8 days by opening and closing the windows and doors. The temperature cannot be higher than 48°C, or lower than 28–30°C. At this high temperature stage, the bricks are turned over once everyday. At the end of this high temperature stage, basically 50–70% of the bricks are mature (ready).
- Post-high temperature stage. At this stage, the bricks gradually dry up with brick temperature dropping gradually from 44–46°C to 32–33°C, until the brick surface no longer feels hot. Then the bricks enter the holding period. Three to five days after this post-high temperature period, moisture from the centers of bricks continues to evaporate and this helps dry the bricks further.
- Holding period. At the post-high temperature period, the brick centers still have 10–20% moisture, and require additional heating to evaporate; because at this stage the bricks themselves will not generate any more heat. Supplemental heating to maintain 32°C room temperature and 28–32°C will evaporate the residual moisture from the bricks completely.
- Exit from incubation room. The "qu" bricks, after exiting from the incubation room are piled up together, with 1 cm space in between.

Characteristics of three kinds of intermediate temperature "qu." In the "jiu" manufacturing process, three kinds of "qu" [Qingcha (clear, soft center), post high temperature and red center] are used in definite proportion. The procedures for producing the three kinds of "qu'' are the same, except that there are differences in controlling the brick temperature.

- "Qingcha qu." The highest temperature for the "qu" bricks is 44–46°C, and the cooling temperature is limited to 28–30°C. It requires mild heating and extensive cooling.
- "Post-high temperature qu." The highest temperature in the bricks from the "rise in temperature" stage to "high temperature" stage is 47–48°C. The bricks are held at the high temperature for 5–7 days. The cooling temperature is limited to 30–32°C. It requires extensive heating and moderate cooling.
- "Red center qu." In the "qu" preparation stage, the cooling and "rise in temperature" stages are not that clearly distinguishable. The rise in temperature to 38°C is rapid. The windows and doors are opened and closed twice during daytime and at night, causing the temperature to rise and drop twice. Temperatures of the bricks are controlled by adjusting window openings. Starting from the "rise in temperature" stage to the "high temperature stage," the highest temperature is 45–47°C, with the lowest cooling temperature limit at 34–38°C.

2. Fermentation with "Da Qu"

The world famous Chinese "jiu" ("Maotai jiu," "Fen jiu," and "Luzhou da qu jiu") are made using "da qu" as the saccharifying and fermenting agents. They differ not only from other "bai jiu," but also they differ among themselves in the fermentation processes. They are representative, and at the same time unique.

3. Fermentation of "Fen Jiu"

"Fen jiu" is produced in the Xinghua village, Fenyang County, Shanxi Province. It was claimed to have a history of about 1,500 years. This "jiu" has alcohol content of 65° Proof. It is bright, transparent, clear to mouthfeel, smooth, fragrant, and long lasting in the mouth after consumption. It is one of the world famous alcoholic beverages – it won the Gold Medal at the 1915 World Expo in Panama, and the title of "National Famous Jiu" four times in the National "Jiu" Evaluation Conference in the past 50 years.

The technology of "Fen jiu" production has "seven musts:"

- people must know the essence;
- cereal must be edible;
- water must be sweet;
- "qu" must be made at the right time;
- utensils must be clean;
- crocks must be moist;
- fire (heating) must be gradual.

Its characteristics are: "da qu" made from 60% barley and 40% peas, locally grown "Yibazhua variety" sorghum, and "double distillation of raw material methodology." It differs from the "continuous mash" methodology that continuously produces raw materials in the mash

from the previous distillation, and at the same time utilizes the effect of the bran. In the "double distillation methodology," bran is not used in the first fermentation, but after the first fermentation. Bran is added in large quantity, up to 25% or 50%, mixed well before the distillation. The raw material is used only twice. The main reason is that the "jiu" odor may be different from different batches.

"Fen jiu" is produced by "double fermentations." In the first fermentation, the completely steamed raw material and the "qu" are put in a small-mouthed big-belly ceramic croak and sealed. The croak is buried in the ground and fermented for 21 days. The mash is then distilled. "Qu" is added to the residual mash, fermented again for 21 days, and distilled. The distillates from the two distillations are used to formulate "fen jiu." Figure 173.3 is a general flowchart of the process.

Wetting of the raw material. Ground sorghum (500 kg) and 200–300 kg water (60–65% of the weight of raw material) are mixed and rested for 7–8 hours to allow the penetration of water into the raw sorghum. Temperature of water is 50–65°C in the winter and 28–32°C in the summer.

Steaming of raw materials. After wetting of the raw material, The sorghum used in "fen jiu" production is steamed separately in croaks. After steaming, water (100 kg, 30% of the raw material) is added immediately, and cooled. The steamed sorghum must be cooked thoroughly but not sticky, with the typical cooked sorghum odor without any foreign odor.

Addition of "qu." When the cooked sorghum is cooled down to 20–30°C, "qu" is added. The ratio is 250 kg sorghum to 30 kg "qu," and the ratio of the three kinds of "qu" is "Qingcha:" post-high temperature: red center, 30:30:40. 20–25°C is the preferred temperature for addition of "qu."

Loading to croak and fermentation. Ceramic croaks of 225 kg or 127 kg capacity are used. They are buried under the ground, with the croak mouth leveled with the ground. For 1,100 kg raw material, 8 croaks of 225 kg capacity or 16 croaks of 127 kg capacity are used. Distance between croaks is 10–24 cm. Croaks must be washed thoroughly and rinsed with huajao (Chinese pricky ash) water once before use. The loading temperature of mash is 10–16°C, with a lower temperature in the summer time. The moisture in the mash is controlled at 52–53%. The croaks are covered with a stone slab and sealed with the residual mash after distillation. The cover is then covered with rice husk to maintain temperature.

The whole fermentation process can be divided into three stages: initial, intermediate and final with control of intermediate temperature and gradual fermentation; temperature during fermentation being gradual increased in the initial stage, maintaining high temperature in the intermediate stage and gradual decline in the final stage. This is what is called "the gradual initial, steady intermediate, and gradual decline final rhythm." The traditional fermentation period

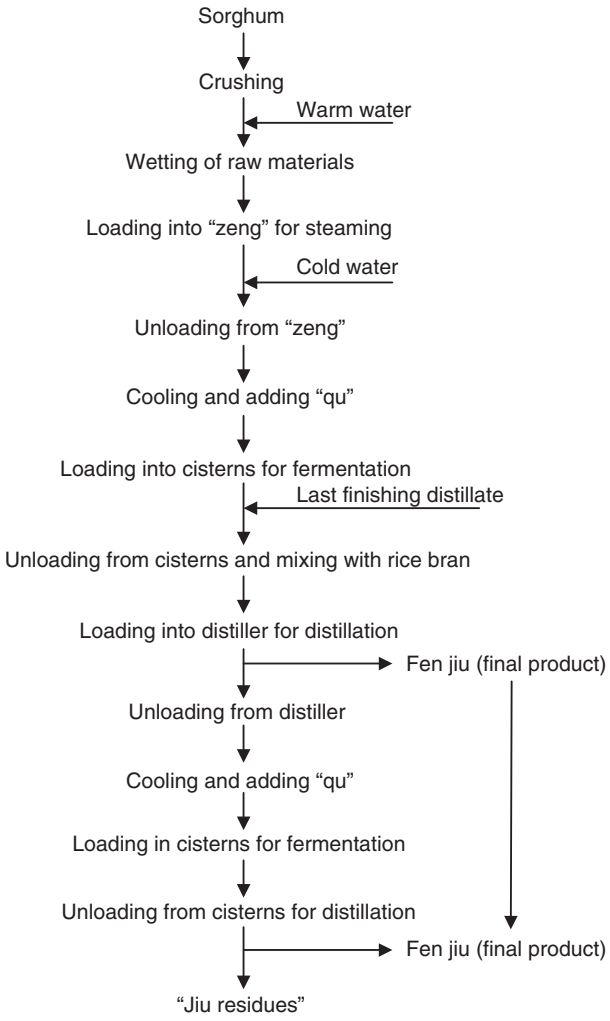

FIGURE 173.3 Generalized schematic flowchart of production of "fen jiu."

was 21 days, and is now extended to 28 days to increase the aroma. During the fermentation, control of temperature is very important. Because area in the fermentation room is much large than the size of the croak, effect of room temperature is more significant, especially in the summer. Traditionally, the temperature of the croak is cooled by surrounding the croaks in the ground with cool water; and in the winter, cover the croaks with barley stalks. During the 21 or 28 days of fermentation, the fermentation has to be checked every other day. Usually, this is conducted in 1–12 days. After this period, inspection is not necessary. If an apple aroma is noticeable in the fermentation room, it is indicative of normal fermentation. As the fermentation progresses, the solid residual matters will gradually sediment to the bottom of the croaks. The more residual matter sediment to the bottom, the more "jiu" will be produced. In normal situations, the residual sediment will sink to the bottom to about a quarter depth of the croak.

Unloading from croak and distillation. The sediment from the first fermentation is recovered and laid on the

cooling platform, followed by addition of cereal bran, about one quarter of the sediment. After mixing, the mixture is loaded to the special earthenware for distillation. The procedure is also specific for "Fen jiu" production. Water is first boiled from the bottom of the earthenware. The amount of water is added, and a fine-toothed comb is placed in the earthenware, followed by the residual sediment-bran (4 inches thick). Then the residual "jiu" from the last distillation is added. When steam/alcohol starts to appear from the mixture, more sediment-bran mixture is added. When the mixture starts to get hot by touch, another 4 inches of mixture is added, until the whole earth ware is full. The mixture in the earthenware is placed in a flat but a concave manner.

The "jiu" obtained from the first distillation is also called "first residual Fen jiu." Its main composition is as follows: total acids 0.0413 g/100 ml, total esters 0.549 g/100 ml, total aldehydes 0.00924 g/100 ml, and alcohol 48.5–80.5 v%. From the above composition analysis, it is clear that the esters content is fairly high, with strong flavor/fragrance. This is related to the long fermentation time.

Second fermentation. The residues left from the first fermentation has residual starch content of 14–16%. It has to be fermented for the second time. The procedure is as follows:

- collect the residue from the earthenware after distillation,
- add 25–30 kg warm water (at 35°C), (the so-called cover up liquid),
- cool rapidly to 30°C,
- add to "da qu" at the rate of 10% of the residue quantity,
- mix well and wait until the temperature lowers to about 20°C,
- load into the croak,
- seal the croak for the second fermentation.

The water content is controlled at 59–61%. Because the substrate has less starch and more bran, it is looser than the first fermentation. Lots of air is carried into the croak, and this is not favorable for the fermentation. Therefore, this mash must be pressed appropriately, and sprayed with the distillate from the first fermentation. The second fermentation takes 28 days. The mash from the second fermentation is called "second mash." After it is taken out from the croak, bran/husk is added to it before distillation. After the second fermentation, the residue will not go through another fermentation, but sold as wine mash residue. A comparison of the compositions of the mash before and after fermentation, respectively, is as follows: starch content 16.57–22.5% vs. 8.85–11.03%; moisture content 56.0–60.4% vs. 58.5–67.2%; acidity 0.92–1.45% vs. 1.92–2.85%; sugar content 2.9–3.78% vs. 0.31–0.338%; alcohol none vs. 5.2–5.8%.

Second distillation. After the second fermented mash is recovered from the croak, small amount of rice husk is added before distillation like the first distillation. The distillate is called "secondary Fen jiu." Its main composition is as follows: total acids 0.059 g/100 ml, total esters 0.2777 g/100 ml, total aldehydes 0.01012 g/100 ml, and alcohol 50–80 v%. The first and second distillates are stored for three years before formulation according to specifications. The main chemical composition of "Fen jiu" is as follows: alcohol 65 v%, total acidity <0.1 g/100 ml; total aldehydes $< 0.03\%$ g/100 ml; total esters <0.30 g/100 ml; higher (long chained) alcohols <0.02 g/100 ml; methanol <0.04 g/100 ml; furfural < 0.0008 g/100 ml; lead < 0.3 ppm; solids < 0.04 g/100 ml.

4. Fermentation of "Maotai Jiu"

Figure 173.4 is a generalized schematic flowchart of "Maotai jiu" manufacturing. The production of "Maotai jiu" is an example of "continuous mash" fermentation methodology. It has its own unique fermentation technology.

First fermentation. Grain sorghum is ground to contain 80% coarse particles and 20% fine particles, the "qu" is ground to a powder. For 100 kg of ground sorghum, 45 kg of water at 80°C is needed. Water is sprinkled in

Sorghum → Grinding → Wetting (45%) → Steam cooking → Cooling of cooked sorghum → Mixing with "qu" (32%) → First fermentation in tank → Unloading from tank → Mixing with raw sorghum (1:1) → First steam distillation → First distillate + First residue → Cooling of first residue → Mixing of first residue with "qu" (40%) → Second fermentation in tank → Unloading from tank → Mixing with raw sorghum (1:1) → Second distillation → Second distillate + Second residue → Cooling of second residue → Mixing with "qu" (32%) → Third fermentation → Third distillation → Third distillate + Third residue → Cooling of third residue → Mixing with "qu" (20%) → Fourth fermentation → Fourth distillation → Fourth distillate + Fourth residue → Cooling of fourth residue → Mixing with "qu" (15%) → Fifth fermentation → Fifth distillation → Fifth distillate + Fifth residue → Cooling of fifth residue → Mixing with "qu" (15%) → Sixth fermentation → Sixth distilation → Sixth distillate + Sixth residue → Cooling of sixth residue → Mixing with "qu" (8%) → Seventh fermentation → Seven distillation → Seven distillate + Seventh residue → Cooling of seventh residue → Mixing with "qu" (8%) → Eighth fermentation → Eighth distillation → Eighth distillate + Eighth residue (to be discarded).

FIGURE 173.4 Generalized schematic flowchart of "Maotai Jiu" manufacturing.

with continuous turning over of the ground sorghum to allow even absorption of water. Let stand for 20 minutes before loading the wetted sorghum to earthenware for steaming with high heat for 2–3 hours. When the grain sorghum is completed cooked, they can be taken out from the earthenware, and let cool to 32°C before mixing with the "qu" powder. For 100 kg sorghum, 32 kg "qu" powder is used. After mixing thoroughly, the mixture is loaded to the fermentation tank, sealed with yellow earth, and ferment for 1 month.

Second fermentation. For 100 kg of groundup sorghum, sprinkle with 45 kg of water, and leave aside. Take out the mash from the first fermentation. For 100 kg of fermented mash, add 100 kg pre-wetted sorghum. Mix thoroughly and load the mixture to earthenware for distillation. The distillate from this process is not for sale. When the residue after fermentation is cooled to 32°C, the distillate is sprinkled back to the mash. For 100 kg of this mash, add 40 kg "qu," and mix thoroughly before loading to the fermentation tank, and seal with yellow soil. This second fermentation lasts for 1 month.

Third fermentation. The fermented mash is taken out and mixed with wetted raw material (1:1) thoroughly before loading to earthenware for fermentation. This collected distillate is called "Huisha Mao jiu." When the mash after distillation is cooled to 32–33°C, for 100 kg add 32 kg "qu." The fermentation tank is again loaded, sealed, and fermented for 1 month.

Fourth fermentation. The fermented mash is distilled. The distillate is called "Dahui Mao Jui" with exceptionally strong flavor. When the residual mash after distillation is cooled to 32–33°C, 100 kg of mash is mixed with 20 kg "qu," and fermented for 1 month.

Fifth fermentation. The fermented mash is distilled. This distillate is call "Original tank Mao Jiu," and it has exceptionally good quality with very fragrant odor. The cooled mash after distillation is again mixed with "qu" at a ratio of 100 kg to 15 kg. Again, the mixture is fermented for 1 month.

Sixth fermentation. The fermented mash is distilled. This distillate is called "Huizao Mao jiu." This distillate has fragrant odor. The distilled mash is again cooled to 32–33°C, mixed with "qu" at a ratio of 100 kg to 15 kg, and fermented for 1 month.

Seventh fermentation. The distillation procedure is repeated, and the distillate is still called "Huizao Mao jiu." This distillate has a lower quality with a bitter dregs taste. The cooled, distilled mash is again mixed with "qu" at a ratio of 100:8, and fermented for 1 month.

Eighth fermentation. The mash is distilled and the distillate is called "Zhuizao jiu." The taste of the distillate is strong in the bitter dregs taste. The cooled, distilled mash is again mixed with "qu," and fermented for 1 month. The mash is distilled for the last time and the distillate is also called "Zhuizao jiu" with even stronger bitter "tank" taste. The fermented residue is not used for further fermentation, and is sold as animal feed.

The distillates are stored in barrels separately according to their quality, and aged. The aged distillate is at least 3 years old and can be over 20 years. They are then formulated according specification. The main chemical composition of "Maotai jiu" is as follows: alcohol 52.8 v%, total acids 0.115 g/100 ml; total aldehydes 0.0631 g/100 ml; total esters 0.2886 g/100 ml; fusel oils 0.3300 g/100 ml; furfural 0.0025 g/100 ml.

"Maotai jiu" is produced in Maotai town, Renhuai County, Guizhou, China. It has a history of over 200 years. The alcohol content is 60 v% with unique fragrant, mainly taste odor. It is smooth, with the long-lasting residual odor and taste, particular the emptied glass gives a comfortable and loved paste taste, and lasts for a long time. The fermentation methodology is also unique. The "jiu" went through addition of raw material twice, eight distillations, and eight times addition of "qu." Every addition of "qu" will go through a 1-month fermentation period. Therefore, it takes 8–9 months, sometimes even a year to complete the whole process. Because it takes a long fermentation period, the product has good fragrance. However, it take much larger amount of "qu" as compared to other "jiu," and 1 kg of "jiu" takes 27 kg of sorghum, and 3 kg or more "qu." The rate of "jiu" produced is low and therefore cost more to produce.

5. Fermentation of "Luzhou Da Qu Jiu"

Luzhou Old Cellar Special "jiu" is a product of the "Qu Jiu Factory" in Luzhou City, Sichuan Province. It has a long history, and has a reputation that spans over 200 years ago. This "jiu" belongs to the rich fragrance type, and is a representative in this category. The raw materials are sorghum, and intermediate heat "da qu" made from wheat as the saccharifying and fermenting agents. It utilizes mixed steaming and mixed heating, "thousand year" old mash, 45–60 days fermentation, and distillation by layers. The distillate is collected by quality, stored and aged, and carefully formulated into the final product. The "jiu" is colorless, transparent, possesses rich cellar fragrance, smooth and rich body, sweet and clean taste, long-lasting unique flavor. The alcohol content is 59–61 v%.

"Luzhou da qu jiu" is famous for its old cellar. The oldest cellar is claimed to be 380 years old (the Wen Yong Sheng Factory). The so-called cellar is the fermentation tank. It is a deep tank dug out from the ground of various sizes. After the tank is formed, it is surrounded with a layer of yellow soil. It is the general perception of consumers that old cellars are good. This is because after long fermentation periods, the "jiu" will penetrate through the surface layer of soil in the cellar. Therefore, the soil around old cellars, about 1 meter around, turned black because of the "jiu" penetration. When the new raw materials mixes

with the old mash and interacts with soil that has changed quality, it gives the "jiu" a special fragrance. This fragrance is not produced in new cellars. All the new cellars carry a new soil flavor. Production of "Luzhou da qu jiu" applies the traditional continuous residual methodology. It is typical of its mixed-steaming and mixed-heating old cellar continuous residual methodology. The production technology is close to the "Laowu Jan" (old earthenware) methdology. Figure 173.5 is a generalized schematic flowchart for the production of "Luzhou da qu jiu."

Treatment of raw materials. The sorghum is ground to pass the 20 mesh sieve, with 28% coarse particles. The "da qu" is ground to a power.

Unloading of mash and mixing with raw materials. Production of "Luzhou da qu jiu" applies continuous mash methodology, and the mash is called "thousand year old" mash. This mash is mixed with new raw materials. In normal situation, the old cellar has four (4) earthenware loads of old mash, it is unloaded to mix with one earthenware load of new raw materials. After distillation, four earthenware loads of residue are loaded to the cellar, and one earthen ware of the residue is discarded. At the bottom of the cellar, the mash contains more water than the upper layers. After the upper layers of mash are unloaded for mixing with new raw materials, the bottom three layers of mash have to go through a "water reduction" process. The common ratio of mash and raw materials is as follows: for each earthern ware, 500 kg of mash, 20–130 kg of ground sorghum, and 20–25% (weight of raw materials) of rice husk.

Loading of earthenware, steaming raw materials, and distillation. The mash and the raw materials are well mixed. Let it stand for an hour to hydrate the raw materials. After loading to the earthenware, the steaming of raw materials and distillation of mash are conducted at the same time. Before loading the earthenware, enough water is added, and 1 kg of rice husk is spread on the bamboo comb. Th mash loaded to the earthenware should be loose, and the heating should be gradual. The criteria for the procedure are light, loose and even. When steam starts to come up, heating should be turned down. The quantity of steam should be controlled, without pressure, and no escape. The mash in the earthenware should be higher on the sides and lower in the center. Usually, it takes 35–45 minutes to load an earthenware, The temperature of the liquid "jiu" is 35°C. Collect the first 0.5 kg of "jiu," and

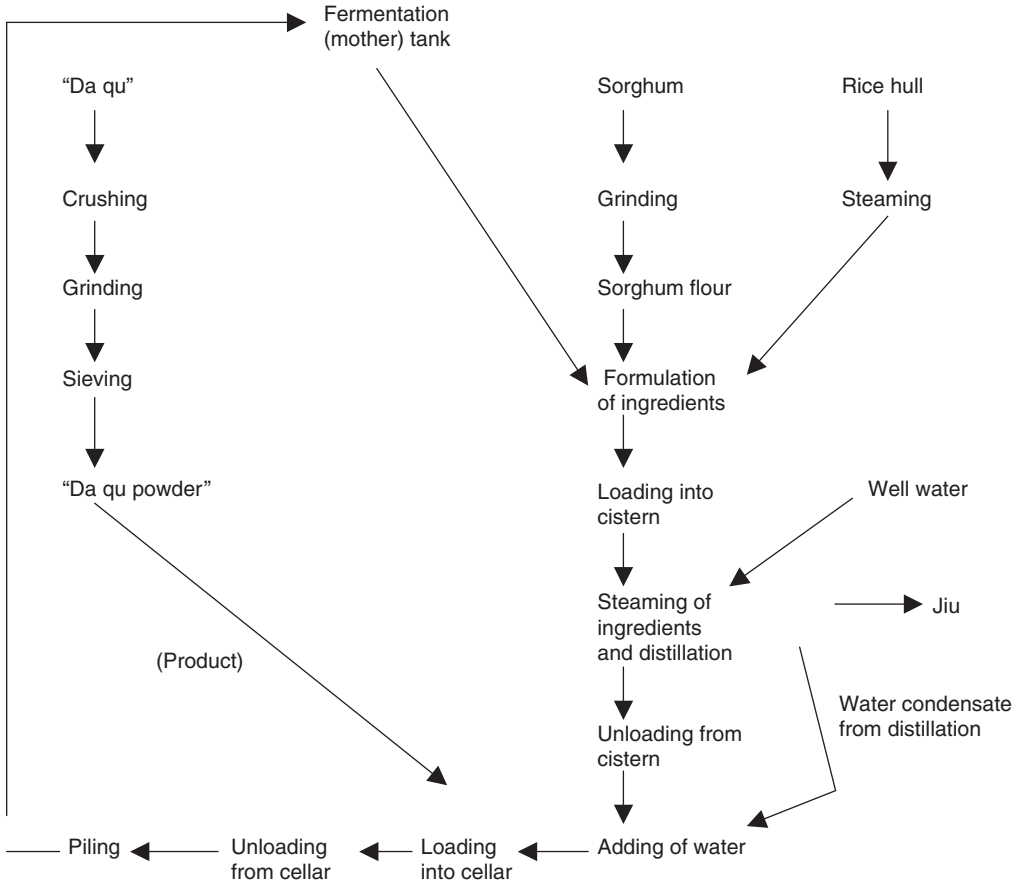

FIGURE 173.5 Generalized schematic flowchart for production of "Luzhou Da Qu Jiu."

the time required is about 15–20 minutes. The rate of "jiu" coming out is about 34 kg per minute. The guidelines are gradual steaming in distillation, and extensive steaming at the end. The average alcohol content of the "jiu" for storage is controlled at 61 v%.

Unloading from earthenware, adding water and spreading of "qu." The traditional process is to add the cooling water collected from the distilling process at a ratio of 100 kg sorghum flour to 70–80 kg water. In this process, hot water (above 80°C) is sprayed onto the mash to allow proper water absorption. In the cellar, water is not added to the bottom two layers, and the other layers have water added a different levels. In general, the water content is controlled at 53–55%. The mash is put on screens to lower its temperature to 13°C (2–3°C below room temperature in the summer). Then the "da qu" power is added to the mash. The amount is 20% of the sorghum power in the mash; however, the dregs returned to the earthenware require only half of the amount of "da qu" needed for the mash. Because returned dregs do not have new raw materials, the temperature entering the cellar is 20–21°C, and it is 18–21°C for the mash. The changes in the composition between the entering mash and the exiting mash, respectively are as follows: moisture 53–54% vs. 55–58%; starch concentration 14–16% vs. 8–10%; acidity 1.4–1.8%, vs. 2.3–3.4%; mash esters 0.43–0.6 g/100g vs. 0.6–0.8 g/100 g; moisture content before mixing with raw materials 53–57% vs. 48–49% after mixing.

Fermentation in the cellar. Fermentation facility. Factories that produce "Luzhou da qu jiu" use earthen cellars, the capacity is 8–12 cubic feet. The phrase "thousand years old cellar, and ten thousand years old dregs" means the older the cellar, the better the product. The earthen cellar already adapts to the activities of butyric bacteria with formation of butyric and caproic acids, and their esters. The older the cellar, the more microorganisms and metabolites are there, and the richer the flavor in the final "jiu." The cellar is made with yellow soil, with the bottom of the cellar built with yellow soil preferred. On the walls are inserted bamboo "nails." The cellar walls are covered with a 10 cm thick "plaster" of fine, soft yellow soil without any sand particles mixed with the "yellow water (liquid)" sipped out from the fermented mash. The cellar is covered with a firmly pressed layer of 30 cm yellow soil. After 7–8 rounds of fermentation, the yellow soil in the newly constructed cellars start to turn black. After a year of fermentation, the soil will change gradually to "blackish white" in color with texture changing from soft to hard and brittle. Quality of the product improves gradually with time and changes in the soil quality. After twenty more years, the soil quality again change from hard and brittle to soft and brittle, and the color is again changed from "blackish white" to black, with red and green colors. This product has a rich fragrance. At this stage, the product reaches the standard of "old cellar," and the quality of the product also improves gradually, getting better every year thereafter.

Criteria for "entering the cellar." The following criteria for "entering the cellar" in the production of "Luzhou da qu jiu" must be strictly controlled:

- starch concentration, 14–16% in the summer and 16–17% in the winter;
- temperature, 1–2°C lower than room temperature and about 18–20°C;
- moisture content, 57–58% in the summer and 53–54% in the winter;
- acidity, below 2% in the summer and 1.4–1.8% in the winter.

Managing the fermentation. After loading, the mash (equivalent to two layers on the earthenware) must be pressed firmly to reduce air in the cellar and inhibit growth of aerobic microorganisms, and gradually induce the normal fermentation. The factory that produces "Luzhan da qu jiu" limits the period of fermentation to 60 days. The longer the fermentation period, the higher the ester content in the final product. During the fermentation period, temperature of the mash increases by 10–15°C. In order to improve the product quality, the factories nowadays generally apply the "returned 'jiu' " and "dual bottom rotation fermentation" technologies to fully utilize the end distillates, and let part of the mash ferment twice in order to increase the rich fragrance. After the mash enters the cellar, they are sealed with pressed yellow soil. Room temperature is checked regularly. Rice husk covers the soil to maintain temperature. "Luzhou da qu jiu" is characterized with its rich fragrance and odor. The main components in "Luzhou da qu jiu" are as follow: alcohol 60 v%, total acids 0.108 g/100 ml; total aldehydes 0.069 g/100 ml; total esters 0.3784 g/100 ml; fusel oils 0.330 g/100 ml; furfural 0.002 g/100 ml.

"Luzhou da qu jiu" applies fermentation in old cellars, mixing of new raw materials and old mash, and contact with soil that has changed quality. These give the "jiu" a rich and unique fragrance. Production of "Lozhou da qu jiu" is a typical example of using "mixed steaming and mixed heating" procedure. By using this procedure, small amounts of esters, ketones, and fragrant materials from the new raw materials are carried over into the "jiu" through the distillation and steaming of the raw materials. This procedure increases the fragrance in the "jiu," the so-called "raw material fragrance."

C. "Xiao Qu Bai (White) Jiu"

"Xiao qu" is used as the saccharifying and fermenting agents to conduct the fermentation, and after fermentation, the collected distillate is "xiao qu jiu." The application of "xiao qu" to produce "jiu" has a long history in

China. However, the production of the distilled "xiao qu jiu" today was started around the Yuan Dynasty. Its unique methodology is well known not only within China as an effective fermentation technology, but also internationally. "Xiao qu jiu" is very popular in the southern provinces of China, particularly preferred by the working class in Fujian, Guangdong, and Guangxi. It is also popular among the overseas Chinese in Southeastern Asia, and in Hong Kong and Macau. It is a light fragrant "bai jiu." When compared to the "da qu jiu," it is different not only in its production methodology, but also in the flavor of the products. Its characteristics are incubation of the microorganisms with the rice kernels, semi-solid fermentation, use of small amount of "qu," short fermentation period, smooth "jiu" quality, and high "jiu" production rate. "Guilin sanhua jiu" in Guangxi (58 v% alcohol), and "Xiangsan (mountain in Hunan province) jiu" are well known "xiao qu jiu."

"Xiao qu" is the saccharifying and fermenting agents for production of "xiao qu jiu." It possesses the dual purpose of saccharifying and fermenting actions. It is made from rice flour or rice bran as the raw material, with addition of Chinese herbs, inoculation with starter (original inocula), and incubation. The microorganisms in the "xia qu" consist of mainly Rhizopus, and Mucor molds, and yeast. From the microbial incubation standpoint, this is natural growth incubation. The handling of raw materials and addition of Chinese herbs can provide effective microorganisms a beneficial growing environment; in addition, it applies the inoculation of starter from long-term natural incubation. In recent years, there are also pure cultures of Rhizopus microorganisms and yeast available for inoculation; this can further guarantee the extensive growth of microorganisms. "Xiao qu" not only produces "bai jiu'' (white wine), it can also be used to produce "huang jiu" (yellow wine). "Xiao qu" such as Sichuan "Qionglai lai mi (rice) qu and kang (bran) qu," "Fujian Xiamen bai yao," "Zhejiang Ningbo bai yao," "Shaoxing jiu yao," "Suzhou tian (sweet) jiu yao" are all famous "bai yao (xiao qu)." "Xiao qu" applied Chinese herbs originated from various sources, and the numbers of herbs also varied, from 1 to 15, including precious ones. In the Ming Dynasty, over 100 kinds of herbs were used. In the old days, there were reasons for using a few herbs. It is now proven through production practices, use of a few herbs, or even without using herbs can also produce good "jiu."

1. Production of "Xiao Qu"

"Xiao qu" is made from rice flour or rice bran as the raw material, with the addition of Chinese herbs, inoculation of starter and incubation. There are all kinds of "xiao qu," but they can be grouped into "yao (herb) xiao qu" (jiu qu ball, or cake), "herbless bai qu," "pure cultures mixed qu," "concentrated tian jiu yao." There are differences among various places in the use of raw materials and production technologies; but, in general, grain(s) are the main ingredient, and the Chines herb(s), and "guan yin earth (bentonite)" as supplemental materials. Some also use rice and others use only the rice bran. The shapes of "xiao qu" may be perfect cubes, round, and round cakes, and also different sizes. For example, "Zhijiang Wuyi (black coat) hong (red) qu" can be 0.1 to 160 g each.

a. Production of "yao xiao qu"

"Yao xiao qu" is called "jiu" herb, or jiu qu" balls. Its characteristics are the use of raw rice flour as the substrate, addition of Chinese herbs and starter (inoculum). Some also have white clay powder as the bulking agent. The addition of herbs and their amount vary with places, some use only one herb, called "single herb xiao qu;" and some use up to 10 herbs. This is called "multi-herb xiao qu." There is also pure culture "yao xiao qu." "Guilin jiu qu" balls are "single herb xiao qu;" it is made from raw rice flour with addition of one herb and inoculation with the starter and incubation. Figure 173.6 is a generalized schematic flowchart on the production of "Guilin Sanhua jiu qu" balls.

Production of the raw "qu" balls. Pearled rice is soaked for 3–6 hours, dripped dry and crushed for production of "qu" balls. For every batch of 15 kg rice flour, add 13% Chinese herb powder, starter 2%, and water 60%. The ingredients are mixed, made into cakes, and pressed flat. Cut the cakes into approximately 2 cm cubes, and sieve them into balls. These balls are evenly coated with 5 kg of fine rice flour, 0.2 kg starter powder. After coating, these balls are the raw "jiu yao balls." They are loaded into small bamboo sieves, and placed in the "qu" room for incubation.

Incubation of "qu" balls. This is managed in three stages. In the first stage, the room temperature is maintained at 28–30°C for 20 hours, and the mold growth is extensive. When the mold mycelia start to fall, surface of the "raw jiu yao balls" shows white bubbles. It is time to lift up the mats from the "jiu yao balls." The temperature at this time is generally about 33–34°C, but cannot be over 37°C. In the second stage, after 24 hours, yeast growth is extensive, and room temperature should be controlled within 28–30°C, and the product temperature cannot be over 35°C. This stage should be maintained for 24 hours. For the third stage of 48 hours, product temperature drops gradually, and the "jiu" balls are mature, and they can be transferred from the incubation room to the drying room for dehydration, or solar dried. These mature "jiu" balls are stored until use. The whole process takes 5 days to complete.

Quality criteria. The sensory quality of "jiu" balls is judged by its white or slightly yellow color with no black color appearance, porous structure, and possesses the

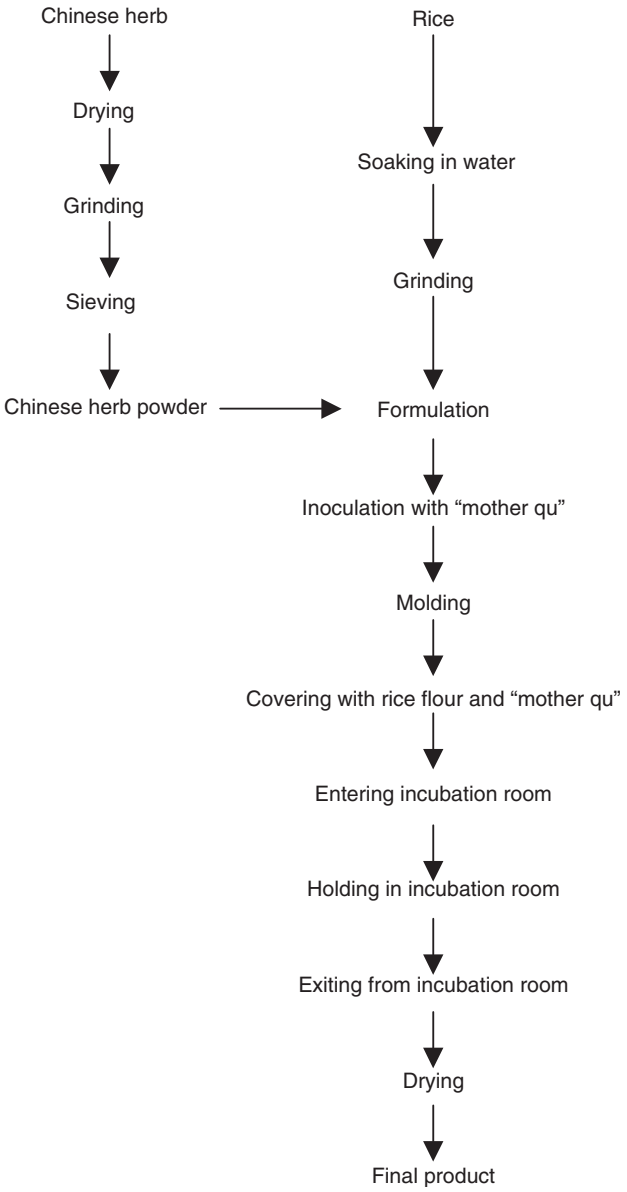

FIGURE 173.6 Generalized schematic flowchart on production of "Guilin Sanhua Jui Qu ball."

characteristic fragrance of "jiu yao." The chemical indices are moisture 12–14%, and total acidity not higher than 0.6 g/100 g.

2. Production of "Jiu Qu Cakes"

"Jiu qu cakes" are also called "da (large) jiu cakes." They are made from rice and soybeans with addition of Chinese herbs and white clay soil, followed by inoculation and incubation. These "jiu cakes" are cubical in appearance, $20 \times 20 \times 30$ cm in dimension, each piece weighing 0.5 kg, containing mainly Rhizopus molds and yeasts. Examples are "jiu cakes" for the production of Guangdong "Chunxiang mi jiu (pure fragrance rice jiu)," and "Xiwei (soy paste-flavored) yubingshao jiu." Figure 173.7 is a generalized schematic flowchart for production of "jiu qu cakes."

Ratio of raw materials is rice 100 kg, soybeans 20 kg, "jiu starter" 1 kg, "jiu herbs" 10 kg (small tangerine leaves 9 kg and cinnamon barks 1 kg), bulking agent 40 kg. The rice should be cooked under low or ambient pressure with 80–85% (weight of rice) water. The soybeans are cooked thoroughly under ambient pressure for 16–20 hours. The cooked rice is spread on the "bed" for cooling to about 36°C and mixed with the cooled, cooked soybeans, "qu," "qu herbs," and bulking agent. The mixture is transferred to a machine for pressing into cake forms. When these cakes are at a temperature of 29–30°C, they are transferred to the incubation room and left there for 7 days. The incubation process depends on the changes in weather and the quality of raw materials. The temperature and relative humidity are adjusted accordingly. When the incubation is complete, they are transferred to the drying room at 60°C for a three-day dehydration to moisture content of 10% to complete the process.

3. Fermentation of "Xiao Qu Jiu"

Polished rice quality is very pure, absent of bran, and low in lipid and protein contents. This is beneficial for low-temperature, progressive fermentation, producing a pure quality with unique fragrance. There are many quality "jiu" produced in the southern provinces in China. They are manufactured using rice as the raw material and application of "xiao qu" in the fermentation process. Examples such as "Guilin sanhua jiu," and "Guangdong yuebingshao jiu" enjoy a very good reputation.

a. Fermentation of "Guilin sanhua jiu"

"Guilin sanhua jiu" is produced in the Guilin City of Guangxi province. The alcohol content is 56–58% and belongs to the "rice fragrance type xiao qu jiu." Its characteristics are a very clear body, smooth, and long-lasting pleasant. In 1957, it was awarded the 1st prize among the "xiao qu jiu" in China, and also awarded as one of the quality "jiu" in the 3rd and 4th National "Jiu" Evaluation Conference. This product is sold to Japan, Southeastern Asia countries, Hong Kong and Macau, and enjoys popularity.

The fermentation methodology is a common practice since the Tang and Song Dynasties. It is believed that quality of "jiu" can be determined by the appearance of bubbles produced; long-lasting and agglomeration of small bubbles are considered as having high alcohol content, and also good quality. Thus, many manufacturers practice the triple distillation process to get to 56–58 v% alcohol stage. The "jiu," because of surface tension, produced three levels of various sized bubbles, then disappears gradually. In the industry, it is termed "triple

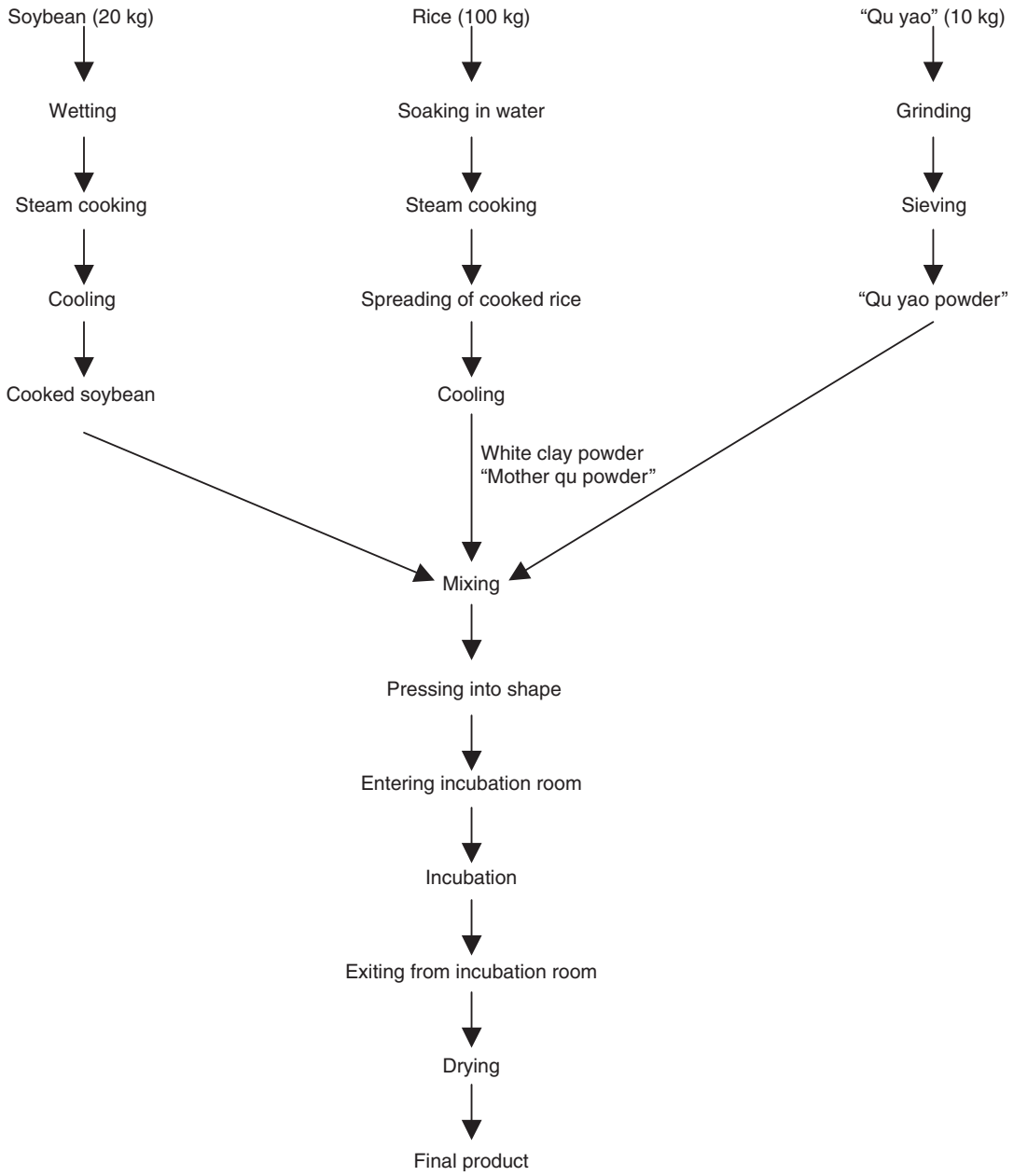

FIGURE 173.7 Generalized schematic flowchart on production of "jiu qu" cake.

distillation" or "piling of three levels of bubbles," thus the name "sanhua" meaning the three levels of bubbles. This term is still used today.

"Sanhua jiu" uses high quality rice as the raw material, the traditional "jiu yao," and semi-solid fermentation technology by cultivating the microorganisms, followed by saccharification and alcoholic fermentation. The water comes from wells in "Li jiang (river)." The mash is sealed in ceramic croaks and stored in caves for over two years. The product is then carefully formulated. Figure 173.8 is a generalized schematic flowchart on the production of "Guilin sanhua jiu."

Steaming the rice. The rice is washed briefly, steamed thoroughly, and contains 62–63% moisture. The rice is then loaded into the grinder, disintegrated and cooled to 36–37°C. The "xiao qu" powder is added at a rate of 0.8–1.0% of the raw material and mixed thoroughly.

Loading to croaks and culturing of the microorganisms. After mixing the raw materials, the mixture is loaded to the culturing croaks, about 15–20 kg each raw material juice. The rice is loaded at a thickness of 10–13 cm. Dig a hole in the center to facilitate aeration. When the mixture is cooled to 32–34°C, cover the croak with a bamboo sieve, and proceed with the saccharification. After about 20–22

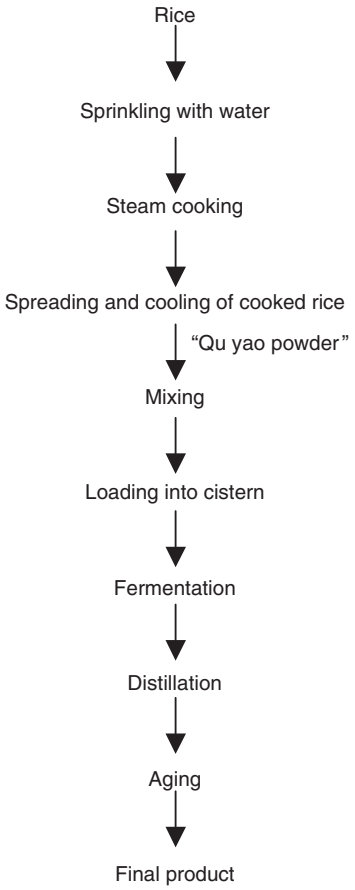

FIGURE 173.8 Generalized schematic flowchart on production of "Guilin Sanhua Jiu."

hours, intermediate product temperature should increase to 37–39°C. It is important to maintain and lower the temperature, if necessary and not to exceed a maximum of 42°C. After 20–24 hours, degree of saccharification should reach 70–80%.

Transferring to croak for fermentation. After the saccharification process, water is added and mixed to lower the intermediate product temperature to 35–36°C. The amount of water is 120–125% of the raw material. In normal situation, the mash after water addition contains 9–10% sugar, total acids not higher than 0.7%, alcohol content of 2–3 v%. After mixing with water, the mash is transferred to a larger croak, two croaks of cultures for each fermentation croak. In the fermentation room, it is important to control the temperature. In normal situation, after 6–7 days of fermentation, sugar content in the mature mash is close to zero, with 11–12 v%, total acids not higher than 1.5%. The mash is ready for distillation.

Distillation. Traditionally distillation is conducted in a distillation kettle in the earthen hearth. Besides using the traditional kettle, "Guilin sanhua jiu" production also applies the vertical or horizontal distillation equipment. The distillation technology usually is stepwise, discarding the beginning and the end distillates. For the first 2–2.5 kg, the distillate contains more impurities, and should be collected separately. The main distillate is collected in a separate croak until the alcohol content is 58 v%. When the distillation is below 58 v%, it is considered as the end distillation. It is collected separately and is added to the second distillation. During distillation, the heating should be even to avoid burning of the mash, or raising the pressure too high and causing mash loss. Temperature of the cooler should be maintained not higher than 55°C, to avoid temperature of the "jiu" too high and the loss of alcohol through vaporization. When the color of the beginning distillate is yellowish with burnt or other odor, it is close to acceptable.

Aged fermented product. When the distillate has been inspected for acceptable quality, it is stored for production of "aged fermented product." Criteria of product to be acceptable are as follows:

- Sensory quality: colorless, good odor, smooth, and long-lasting taste.
- Physicochemical indices (g/100 ml): Alcohol 58, total acids 0.06–0.10, total aldehydes < 0.01, fusel oils < 0.15, total esters > 0.12, methanol < 0.05, turbidity $< 50°$, total solids < 0.1, lead 1.0 mg/liter.

b. Fermentation technology of "Yubingshao jiu"

"Soy-taste flavored yubingshao jiu" is a unique product from the Guangdong Province. This product is different from all the "bai jiu" produced in China. The raw material is rice. The technology is semi-solid fermentation with partial saccharification and partial fermentation progressing at the same time. This fermentation technology is common in the southern provinces in China. The most unique characteristics in this product are low alcohol content (29.5 v%), and a special meaty odor from soaking fat animal meat in the collected distillate for 3 months. Because the fat meat is soaked in the distillate, it absorbs the undesirable taste. Therefore, the product is smooth and not spicy hot. Figure 173.9 shows a generalized schematic flowchart for the production of "yubingshao jiu."

Steaming rice, cooling and mixing with "qu." Criterion for steaming rice is that it must be thorough without any uncooked centers. This is to increase the alcohol production rate. The cooled rice is spread loosely on the rice bed for cooling to 35°C in summer, and 40°C in winter. The temperature of the cooled rice must be even. There should not be lumps of rice. After cooling, "qu" is mixed in at a rate of 1.8–2.0 kg of "qu cake" for every 100 kg of uncooked rice. The "qu cake" is ground to powder before sprinkling on the cooled rice. After mixing, it is ready for loading to croaks.

Loading of croaks and fermentation. Before loading, the croaks are filled with 6.5–7 kg of water. The

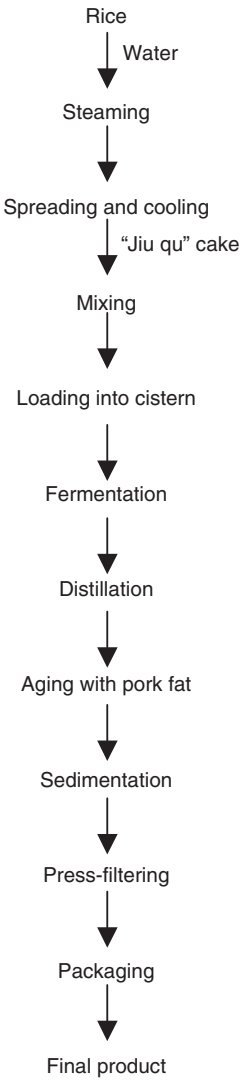

FIGURE 173.9 Generalized schematic flowchart on production of "Yubingshao Jiu."

rice-"qu" mixture is loaded at a rate of 5 kg of rice per croak. The croaks are sealed and stored in the fermentation room. During fermentation, temperature in the fermentation room should be controlled at 26–30°C, and product temperature below 30°C, and not over 40°C. Fermentation time is 15 days in the summer, and 20 days in the winter.

Distillation. When the fermentation is complete, the croaks are taken out for distillation. Removing the beginning and end distillates reduces the impurities with high boiling point, and guarantee the smoothness of the product.

Aging with fat meat. When the collected distillate is filled into the croak, fat pork is added for the soaking process. For every 20 kg "jiu" in a croak, 2 kg of fat pork is soaked for 3 months. The pork fat is gradually dissolved with absorption of impurities and induction of the esterification reaction. The degree of maturation is increased, the "jiu" is smooth and pleasant to taste. At the same time, it provides an unique "soy paste" flavor.

Quality indices of final product. The quality indices for "yubingshao jiu" are as follows:

- Sensory quality:
- Color – Clear, transparent, colorless or slightly yellowish, no suspensions and sediments.
- Odor – Pure fragrant, possessing the unique soy paste flavor in the "yubingshao jiu."
- Taste – Smooth to the taste, also having soy paste-meaty flavor, without bitter or other undesirable taste.
- Physicochemical indices: alcohol 29.5 v% (in croaks) and 30.5 v% (in bottles), total acids $<$ 0.08 g/100 ml, total esters $>$ 0.15 g/100 ml, total aldehydes $<$ 0.1 g/100 ml, amino acids $<$ 0.002 g/100 ml, methanol $<$ 0.06 g/100 ml, fusel oils $<$ 0.2 g/100 ml, Solids $<$ 0.02 g/100 ml, cyanides $<$ 0.5 ppm, lead $<$ 1 ppm.

D. Famous Chinese "Bai Jiu"

In the 4th National "Jiu" Evaluation Conference conducted in Taiyuan City, Sanxi Province in 1984, 13 "jiu" were given the Chinese Famous "Bai Jiu" title. They are Maotai Jiu, Fen Jiu, Wuliangyi, Gujing Gong Jiu, Luzhou Da Qu Jiu, Dong Jiu, Jiannan Chun Jiu, Xifeng Jiu, Quanxiang Da Qu Jiu, Yanghe Da Qu Jiu, Shuanggou Da Qu Jiu, Lang Jiu, and Huanghelou Jiu. The fermentation technologies for "Moutai Jiu," "Feng Jiu," and "Lozhou Da Qu Jiu," together with "Guilin Sanhua Jiu" and "Yubingxiao Jiu" have already been described. Readers interested in the production of other "jius" should consult the references at the end of this chapter.

E. Quality of "Bai Jiu"

In general, quality determination of "bai jiu" relies on sensory evaluation of its color, odor, taste and body to differentiate their quality, and thus determine their classification. Physicochemical analyses are then applied to evaluate their quality.

1. Color of "Bai Jiu"

Sensory evaluation of "bai jiu" color basically includes the evaluation of the color hue, transparency, suspended matter and sediments by visual observation.

- Colorless. "Bai jiu" belongs to distilled spirits, and should be colorless, transparent, absent from suspended matters and sediments. However, some "bai jiu" after long periods of aging, may be slightly yellowish. If this color does not affect the clearness and transparency, it is considered normal.

- Colors. When the "bai jiu" is contaminated, it may be yellow, black, blackish brown, brown, or blue in color; and they are considered as abnormal.
- Loss of luster. The loss of luster in "bai jiu" may be due to the following reason: in formulating "bai jiu," water was added, thus decreases the solubility of higher alcohols, higher fatty acids and esters, and cause diffraction. If the original "jiu" is high in alcohol with minimal amount of impurities, it is not easy to lose luster and show the appearance of milkiness, and not transparent when water is added.
- Sediments in "bai jiu." "Bai jiu" showing sediments of white, brown, bluish black or grayish white color is considered as abnormal.

2. Odor of "Bai Jiu"

Many odorous components have been identified from "bai jiu." These components include esters, acids, carboxyl compounds, and sulfur-containing compounds. Their presence in "bai jiu" is very limited. However, under proper formulation, can give "bai jiu" pleasant and unique fragrance. The characteristic fragrance in different "bai jiu" is an indication of the balance of the different odor components.

- Esters. Esters are the main group of components in "bai jiu." Common fragrant "jiu" or famous "jiu" contains a higher esters content, on the average of 0.2–0.6 g/100 ml. C_1–C_4 straight chain fatty acid ethyl esters are the major esters in "jiu." When the ethyl acetate is strong, they show the odors of apple and banana, and pear and pineapple odors when they are weak. When the ethyl butyrate is strong, its shows unpleasant odor, even objectional; however, when they are weak, they show the odor of rum. When the ethyl caproate is strong, it gives a spicy hot and objectional odor; when they are weak, they give the unique "cellar odor" in "bai jiu." Ethyl lactate is not that significant in odor, but increases the rich sensation.
- Alcohols. Besides ethyl alcohol, the main alcohols in "bai jiu" are the higher alcohols with isopentyl alcohol as the main one and others such as butyl alcohol, propyl alcohol, isopropyl alcohol, isobutyl alcohol, amyl alcohol, normal hexyl alcohol, and heptyl alcohol. Small amounts of higher alcohols gives "bai jiu" its characteristic odor, and support the ester odor. This combination gives a more complete odor in "bai jiu" and forms the characteristic odor on "bai jiu." Alcohols and fatty esters interact and form esters, and can increase the odor to some extent. However, if the concentration is too strong, it will give objectional odor. The β-benzyl ethyl ester in the alcohols is an essential component in the odor of "bai jiu." The concentration of higher alcohols in "bai jiu" should be under 0.3 g/100 ml.
- Aldehydes. Small amount of aldehydes can assist the vaporization of the odors, and give the "jiu" a pleasant odor, such as acetylaldehyde, an important compound in the odor of the beginning distillate. The furfural is a harmful substance giving an irritating odor; however, when its concentration is lower than 0.003%, it can give an "explosive" pleasant odor. All the better "jiu" contains certain amount of furfural, on the average of 0.002–0.003 g/100 ml. The other aldehydes such as isopentyl aldehyde, hexyl aldehyde, and vanillin that can also allow the "jiu" to form a good flavor. However, if the aldehyde content is too high, it will give the "jiu" a taste too "spicy hot," and irritating, in addition to the harmful effect. Propenyl aldehyde also irritates the eyes to tear. This compound, and crotyl aldehyde, and glyceryl aldehyde all give irritating odor.
- Acids. Fatty acids are the major acids in "bai jiu." Generally, acetic acid is the most abundant, followed by propionic acid, lactic acid, succinic acid, caproic acid, citric acid, butyric acid, and isobutyric acid. In addition, there are citronellic acid, oleic acid, pyruvic acid, et al. Organic acids provide the traditional odor to the "jiu," and also give "jiu" the proper taste. Small amounts of organic acids with few carbons help the odor in "jiu." For example, acetic acid gives the pleasant acid odor and taste. Butyric acid has the cellar soil odor and slightly sweet. Caproic acid has the soil odor and slight spicy hot. Propionic acid has a sharp odor, and sour with slightly sweet taste. Lactic acid has a mild acid odor and gives the "jiu" a rich and smooth taste, too much will give a stringent taste. Succinic acid balances the "jiu" odor and taste. These organic acids balance the "explosive" action of "jiu;" when the contents are balanced, the "jiu" gives the consumer a cool, clear, smooth and long-lasting sensation. If the contents are too high, the acid odor is strong and irritating. Fatty acids, starting from the propionic acid, give objectional odor; buytric acid has a sweat odor; amyl acid, caproic acid, and heptylic acid have strong sweat odor. However, these objectional odors will decrease with the increase in carbons in the molecules. Caprylic

acid is only slightly objectional in odor, and in fact shows a weak pleasant odor. Acids with more than eight carbons have weaker acid odor, and have slight resinous odors. Total acids in "bai jiu" should be about 0.1 g/100 ml.

- Phenols. At the present time, research on the effect of phenolic compounds on the odor of "jiu" is still very limited. Whether vanillyl phenol and other phenols are related to the soy paste odor is still not conclusive. However, it is generally believed that these kinds of compounds are beneficial to the long-lasting properties; for example, vanillyl has a mild and creamy odor.
- Alpha-di-keto compounds. When the contents of compounds such as diacetyl and acetoin in "jiu" are limited, they give "jiu" a honey-like sweet and pleasant odor. When the content is excessive, they give out odor like sour milk.
- Objectional odors commonly occurred in "bai jiu." In is fairly common to have objectional sweat odor in "bai jiu" due to excessive butyric acid and its esters. Using excessive amount of rice bran or the bran not cleaned and cooked properly will produce objectional furfural odor. Too much fusel oil will also cause objectional odor. When decayed raw materials or supplementary materials are used directly in the production process, the "bai jiu" will show these decay odors. When the raw materials contain too much fatty substances, these substances will oxidize and develop rancid odor. Use of rubber tubing in the transportation of distillate or use of rubber lining in bottle caps will give the product rubber odor. Acrolein, crotyl aldehyde, thioalcohol, and hydrogen sulfide will give rotten odors. When the raw materials contain too much protein, interaction with alkaline substances will also give rotten odor. Formaldehyde and related substances, particularly acrolein, will irritate the nose and give eyes a hot sensation and induce tears.

No matter how complicate the odorous substances are in "bai jiu," there are always one main odorous substance combined with other supplementary substances to give the characteristic odor. Research on "jiu" odors always consider the "jiu tone" and divide the various "jiu" odors into various categories, such as "Luzhou da qu jiu" odor, "Maotai jiu" odor, "Fen jiu" odor, and "Mi (rice)" odor. The sensory quality of "bai jiu" odor is a balance of the various odors, providing a pleasant sensation with outstanding main odor. At the same time, the overflow and residual odors should be considered. "Jiu" with good overflow odor give the consumer an overflow sensation of odors once the "jiu" is in the mouth, like a hiccup manner, indicating the "jiu" contains more low-boiling point odorous substances. "Jiu" with long-lasting characteristics give the consumer a residual sensation after the "jiu" is swallowed or even when hiccup takes place. This indicates that the "jiu" contains more esters, particularly the high boiling-point odorous substances.

3. The Taste of "Bai Jiu"

The taste of "bai jiu" is determined by the tasting organ (tongue). Basically, "bai jiu" taste can be categorized into sweet, sour, spicy hot, bitter and salty. The sensory quality of "jiu" taste should be evaluated, besides the odor, based on its balanced taste sensation.

- Sweet taste. The sweet taste of "bai jiu" comes basically from the alcohols, particularly the poly-alcohols. All poly-alcohols have sweet taste functional groups and assist in sweet taste functional groups. For example, glycerol, 2, 3 butanediol, erythritol, normal amyl alcohol, and mannitol, with the increase in hydroxyl groups, will increase the sweet taste. The sweet taste of erthritol is important in the sweet taste in fruits. These poly-alcohols not only contribute the sweet taste, (twice as sweet as sucrose, and mannitol having very sweet taste), but also they are viscous, and can contribute to "jiu" its the rich body, giving a mouth feel of smoothness. Maotai is particularly smooth, and this is related to its high glycerol content of 9.5–9.7 ppm. Besides the alcohols, diacetyl possesses the honey-like sweet odor and taste. Acetoin and diacetyl both can contribute to the rich sensation in "jiu." Sweet taste compounds are present in the raw materials themselves and are produced during fermentation. For example, the phytic acid in corn, is hydrolyzed during fermentation into inositol and phosphoric acid, the former being a sweet taste compound in "jiu," and the latter can facilitate the production of glycerol.
- Sour taste. Sour taste is due to the stimulation of hydrogen ions. Acids are the major sour compounds in "jiu." With limited amount of acids, the "jiu" has a light taste, and short aftertaste; excessive acids give the sour sensation, and coarse taste in the "jiu." Appropriate amount of acids will balance the "jiu" taste, and during aging, gradually form the aromatic esters. Acids also affect the sweet taste of "jiu," too much acid reduces its sweet taste, and affects its sweet aftertaste. The acids in "jiu" can be divided into volatile acids and non-volatile acids. Formic acid, acetic acid, propionic acid, butyric acid,

valeric acid, caproic acid, caprylic acid and others are volatile acids. The larger the molecular weight, the softer the smoother taste. The smaller the molecule, the more irritate it is. Lactic acid, citric acid, tartaric acid, succinic acid, and gluconic acid and others are non-volatile acids. They can increase the rich sensation of the "jiu."

- Bitterness. Bitterness in "jiu" is due to mainly the excessive amount of fusel oils, excessive succinic acid, small amount of tannins, too much phenolic compounds and furfural. Fusel oils formed from higher alcohols are important odorous compounds in "jiu," but too much is the source of bitterness. Isobutanol has bitter taste; normal propanol is very bitter, dilute n-butyl alcohol is a very good odorous compound, but in high concentration it is bitter. Most of the alkaloids are bitter. The bitterness on one hand is carried in from the raw materials, such as the tannins in sorghum; on the other hand, the bitterness is related to the technology involved. For example, the ipomeamarone in diseased sweet potato is a bitter substance. It can be carried over during the distillation process into the final product, and give strong bitter taste. Excessive use of "qu," particularly the "qu" made from wheat bran, can carry over large amounts of spores from the *Aspergillus niger* and give a strong bitter taste. Use of "qu" containing green penicillium mold or too little water in the cellar, bottom mash too acid, inappropriate sealing of cellars/croaks, mash temperature too high entering the cellar or croaks all are possible sources of bitterness.
- The spicy hot and pungent taste. A strong irritation of the taste nerve will give a spicy hot sensation. High concentration of alcohol gives a spicy hot sensation, also it can irritate the nerve system to give the nose the irritation sensation. The main components in "bai jiu" causing the spicy hot sensation comes from the aldehydes. Very minute concentration of acetaldehyde can cause the spicy hot sensation. Glyceraldehyde facilitates eye tears and also causes spicy hot sensation. Excessive amounts of acetal, furfural, and higher alcohols also cause spicy hot sensation. Newly distilled "jiu" after a period of storage or distilled over bulking agent can reduce the spicy hot sensation.
- Salty taste. All halogenic ions give the salty sensation. All alkaline sulfates, nitrates and organic acid salts have salty taste. During the fermentation production process, if the water quality is not good with high degree of hardness will also give " bai jiu" the alkaline ions and salts and thus causing slight saltiness and coarseness in the final product. However, presence of limited amount of salts (such as sodium chloride) can facilitate the tasting sensation, and give the "jiu" a rich taste.
- Astringency. Some compounds can cause coagulation of the proteins on the surface of the tongue, and then the stringency action and thus the stringency sensation. For example, presence of minute amount of the oxidative product of tyrosine, 2,5-dihydroxyl acetate, can cause bitter stringent sensation. Excessive amount of lactic acid, ethyl lactate, higher alcohols, and tannins all can cause bitter astringency in "bai jiu." When excessive rice bran is used in the fermentation process, this mishandling not only gives the "jiu" the furfural odor, but also the astringent taste. Lower fermentation temperature in the winter, and incomplete fermentation, give the distilled "jiu" significant stringent sensation on the tip of the tongue.

The taste of "bai jiu" emphasizes on the "taste tone." Excessive sweetness, sourness, bitterness and astringency all will lower the quality of "jiu." Good "jiu" must be balance on its taste; in addition, it must also possess richness, smoothness, sweetness, and long-lasting characteristics.

4. The Body of "Bai Jiu"

When odorous and tasting components are mixed together, it is called the flavoring components. When the flavoring compounds dissolve in water, they form the volatiles and solid matters. Alcohol, water, volatiles, and solid matter together form the "jiu" body. The main component in the "jiu" body is water, then the alcohol, volatiles, and solid matter, including the minute amounts of aromatic compounds and taste components. Because of the differences in raw materials in the production of "bai jiu," the kinds of "qu," and fermentation technologies, the kinds of matters and amounts forming the "jiu" body are also different, thus forming the various characteristics in different "jiu" bodies. In sensory evaluation, the components in "bai jiu" are reflected in the color, odor and taste of the product, then through the combined sensation on these characteristics and abstract judgement, the "jiu" body is determined. In general, the requirements for "jiu" body are normal color, odor, and taste, and the components forming the "jiu" body are properly balanced with characteristic flavor.

5. Sanitary Indicators for "Bai Jiu"

Sanitary indicators for "bai jiu" production must follow the National Standard GB2757-81 of the People's Republic of China (Table 173.1).

TABLE 173.1
Sanitary Standards for Distilled Spirits and Formulated "Jiu" GB2757-81 (China)

Item	Index
Methanol (g/100 ml)	
Cereals as raw materials	≤0.04
Root crop(s) as raw materials	≤0.12
Fusel oils (g/100 ml)	≤0.15
Rice as raw materials	≤0.20
Cyanides (mg/100 L, as HCN)	
Casava as raw materials	≤5
Casava substitutes as raw materials	≤2
Lead (mg/L, as Pb)	≤1
Manganese (mg/L, as Mn)	≤2
Food additive(s)	According to GB 2760-81 (China)

Notes:

1. Distilled spirit means the "bai jiu" collected through distillation, using sugar or starch as the raw materials gone through saccharification and fermentation.
2. Formulated "jiu" means using fermented "jiu" or distilled spirits as the basal "jiu," with addition of edible supplementary substance(s).
3. Sensory indices: Clear and transparent liquid [formulated "jiu" can have color], absence of precipitate, foreign matters, and objectional odor.
4. Indices as based on 60 v% alcohol distilled spirit; if above or lower than 60 v%, adjust accordingly.

6. Harmful Materials in "Bai Jiu"

In the production of "bai jiu," some harmful materials are produced. Some of these harmful materials come from the raw materials, and some are produced during the fermentation process. Proper procedures must be taken to lower the contents of these harmful materials.

- Fusel oils**.** Fusel oils are aromatic components in "jiu." However, when the content is too high, it is harmful to consumers. The poisoning and anesthetic effects of fusel oils are stronger than ethanol (they can cause the nerve system full of blood, and cause headache). The toxicity increases with the increase in molecular size of the fusel oils. The oxidation of fusel oils in the body is slower than ethanol, and stay in the body longer. The main components in fusel oils are isoamyl alcohol, amyl alcohol, isobutanol, propanol et al., with the toxic effects for isobutanol and isoamyl alcohol the greatest. When there are more protein contents in the raw materials, the fusel oils content is also higher. The boiling points of fusel oils are generally higher than ethanol (78°C for ethanol, 97°C for propanol and 131°C for isoamyl alcohol). During distillation, careful control of the distillation, and removal of the beginning and end distillates can reduce the fusel oil content in the final product.
- Aldehydes**.** The aldehydes in "jiu" are the result of oxidation of various sized alcohols, and are also produced in the fermentation process. Lower boiling point aldehydes include formaldehyde, and acetaldehyde; high boiling aldehydes include furfural, butyl aldehyde, amyl aldehyde, hexyl aldehyde. The toxicity of aldehyde is greater than alcohols, and formaldehyde is more toxic; its toxicity is 30 times greater than methanol. It is natural toxic compound, and can coagulate proteins. Ten grams of formaldehyde can be fatal. In acute toxicity, there appears coughing, chest pain, burning fever, dizziness, loss of consciousness and vomiting. Furfural is also toxic to the body. When rice husk, corn cobs, and wheat bran are used as supplemental materials, the furfural and other aldehydes are higher in the distillates. In order to lower the aldehyde contents in "bai jiu," the following procedures should be followed: lesser amounts of rice bran and rice husk should be used, or these materials are distilled separately; strict control of distillation temperature; and removal of beginning and ending distillates.
- Methanol. The toxicity of methanol is fairly high in the human body, 4–10 g will show significant toxicity effect. The oxidation products of methanol, formic acid and formaldehyde are even more toxic. The toxicity of formic acid is 8 times higher than methanol, and formaldehyde is 30 times higher than methanol. Consuming too much "bai jiu" will lead to accumulation of methanol in the body and not easy to excrete, and its metabolites are the formic acid and formaldehyde. Even small amount of methanol can cause toxicity. Acute toxicity of methanol will show the following symptoms: headache, vomiting, stomachache, and blurred vision, followed by shortness of breath, paralysis of the breathing central mechanism, coma and even death. Chronic toxicity will show irritated membrane symptom, dizziness, lethargic sleep, headache, indigestion, blurred vision, and buzzing in the ear, followed by blindness. The source of methanol is the hydrolysis of pectic substances. In the selection of raw materials for fermentation, it is important to select those with low pectic substances. In addition, it is critical to separate the methanol out from the rest of the "jiu."

- Lead. Lead is a very toxic heavy metal, 0.04 g can lead to acute toxicity, and 0.20 g can lead to death. Lead toxicity through "jiu" consumption is not common, mainly in chronic toxicity. When 10 mg is absorbed everyday, toxicity is shown in a short time. Nowadays, the regulation is a maximum absorption of 0.2–0.25 mg in 24 hours. With the increase of lead absorption in the body, symptoms include headache, dizziness, loss of memory, poor sleep, weakness in hand holding capacity, anemia, full-stomach, and constipation. The main cause of lead in "bai jiu" is the dissolution of lead from distillation apparatus, cooling apparatus, tubing, and holding containers. The higher lead content in the equipment, the more lead dissolved from them.
- Cyanides. The cycanides in "bai jiu" come mainly the raw materials such as cassava, and wild plants. In the fermentation process, they are hydrolyzed to form hydrocyanic acid (hydrogen cyanide). Light toxicity includes dripping of saliva, vomiting, diarrhea, rapid breathing. The more serious situation is hard to breathe, whole body seizure, coma, and death within a few minutes to 22 hours. The ways to remove cyanides are as follows: extended soaking of raw materials; enough ventilation during steaming to remove the vapor; drying of the raw materials to vaporize the cyanides; or adding 2% *Aspergillus niger*, maintaining 40% moisture, mixing at 50°C, holding at this temperature for 12 hours, and steaming for 45 minutes to vaporize the hydrocyanic acid. The finer the raw material, the better the removal effect.
- Mycotoxins. Due to decay with quality changes in wheat, rice, corn, peanut, et al., they may be contaminated with mycotoxins. Some of the metabolites from mycotoxins are toxic. When foods are made from these raw materials, consumers can ingest carcinogens at the same time. It is important to store the raw materials properly to avoid mold growth and quality changes.
- Agricultural chemicals (pesticides). If excessive agricultural chemicals are applied in the production of cereals and root crops, they will be absorbed and stay as residues in the fruits or tubers. In the production of "jiu," these harmful substances will be carried over to the "jiu," special attention must be paid to particularly the organic chloro- and phospho-compounds. In order to avoid poisoning from agricultural chemicals, the raw materials must be carefully inspected; and the promotion of biologically control and appropriate use of agricultural chemicals.

III. FERMENTATION OF "HUANG (YELLOW) JIU"

"Huang ju" is not only one of the oldest alcoholic beverages in China, but also in the world. About 3,000 years ago, fermentation of "huang jiu" was already common in China. In "Shi Jing (Book of Poems)," it was proven that "rice is harvested in the tenth month (lunar calendar), and this is used to make "jiu" for the Spring." There are a wide variety of "huang jiu," and their names are quite different, some based on color such as "yuanhong (winner red) jiu," some based on geographic location, such as "Shaoxing jiu," some based on the fermentation technology such as "Jia fan (additional rice) jiu." At the present time, all these "huang jiu" have a bright yellow color; that is why they are called "yellow (huang) jiu." There are many kinds of "huang jiu." The current classification system is based on the sugar content and they are divided into three groups: sugar content over 10% is called "sweet huang jiu" such as "Shaoxing xiangxue (flavored) jiu" and "Fujian michenchen (very sweet) jiu;" sugar content between 5–10% is called semi-sweet "jiu" such as "Shaoxing shanniang (carefully fermented) jiu," "Fujian lao (old) jiu;" sugar content below 5% is called dry "huangjiu" such as "Shaoxing yuan hong jiu," "Ja fan jiu," and common "huang jiu."

A. Raw Materials and Methodology

1. Water Used in Fermentation

Many places famous for their "jiu" are related to the quality of their water. The world famous "Shaoxing jiu" cannot be separated from the water in the "Jian Lake." It is best to use spring water, river water away from towns and cities, centers of wide, clean rivers or lakes, or water from wells. Clarified tap water can also be used. However, tap water contains too much iron; if this is used, it is important to remove the iron, and meet the requirements for "jiu" fermentation.

2. Cereal and Its Treatment

The raw materials can be either rice or millet. In the southern provinces of China, it is common to use rice. Rice is again divided into glutinous rice, nonglutinous rice and long grain rice. In the northern provinces of China, it is more common to use millet. Either rice or miller can be used to make "jiu," however, it is best to use glutinous rice. All the famous "huang jiu" nowadays are made from glutinous rice, and more important good quality glutinous rice. That is, the rice should be soft in texture, large kernels, white centered, and containing high percentage of starch. In addition, it is important to use a new crop of rice, because aged rice is broken easily, and its lipids can get rancid and affect negatively the "jiu" quality.

Treatment of the rice. The rice used for production of "huang jiu" must be polished, soaked, steamed, and cooled. Polishing of rice removes the outer layers of cells containing the proteins, lipids, ash and vitamins. Temperature during fermentation rises gradually, with production of high purity "jiu." In addition, polished rice absorbs water rapidly, thus can be steamed, gelatinized, and saccharified. The traditional method in making "Shaoxing jiu" is to soak the rice for 18–20 hours, so that the rice can absorb water and expand, and more importantly, the availablity of sour soaking water. For steaming of rice, the requirements are: soft interior but with firm exterior, no white centers, loosely separately and not sticking together, cooked thoroughly but not mushy, and uniform. The cooked rice must be cooled rapidly to the temperature for microorganisms to grow. The cooling process can be "sprinkle cooling" and "spread cooling." In the production of the starter, it is customary to use "sprinkle cooling" and for the actual fermentation, "spread cooling" is used. It is required that cooling is rapid and even, and no lumps of hot rice.

B. The "Qu," "Jiu Yao" and "Jiu Mo (Mother Jiu)"

The "qu" and "jiu yoa" are the "da qu" and "xiao qu" described earlier. They are commonly called the saccharifying agents. In the production of "huang jiu," a fixed amount of "qu" is added, and "jiu yao" is only used in the production of "sprinked rice jiu mo." The traditional production technology for "Shaoxing jiu" is to use "qu" and "jiu yao" at the same time. The new technology of "large tank fermentation" uses "qu" as the saccharifying agent, and does not use "jiu yao," but uses pure cultured "jiu mo" to replace the "sprinkled rice jiu mo" as the fermenting agent. There are many kinds of "qu" and jiu yao" with various names. "Qu" can be "wheat qu" or "rice qu," based on the source of raw material. Within wheat "qu," there are "cake qu," "grass-wrapped qu," and "hanging qu," and within the "rice qu," there are "red qu," "black coat qu," and "yellow coat qu," "Jiu yao" can be "white yao" or "black yao."

1. Production of "Wheat Qu"

In the traditional fermentation technology of producing "huang jiu," the "qu" used are either "grass-wrapped qu," "cake qu" or "hanging qu." They are all naturally incubated "qu" with better quality. Figure 173.10 is a generalized schematic flowchart for the production of "wheat qu." The procedures are discussed as follows.

Rolling and crushing. Wheat kernels are sieved, rolled, and crushed into 3–5 pieces per kernel.

Mixing with "qu" and shape forming. The rolled broken wheat is loaded to the barrel to be mixed with "qu." First, water (20%) is added, mixed with the broken wheat kernels to wet the wheat pieces to 23–25%. They are rapidly turned over to let water absorbed evenly. In order to increase the quality of "qu," small amount of aged "wheat qu" is added as seeds. The mixture is then mixed well and spread in boxes to be pressed into cakes. The cakes must be firmly pressed so that they will not fall apart. The cakes are then taken out from the boxes and cut into smaller cakes.

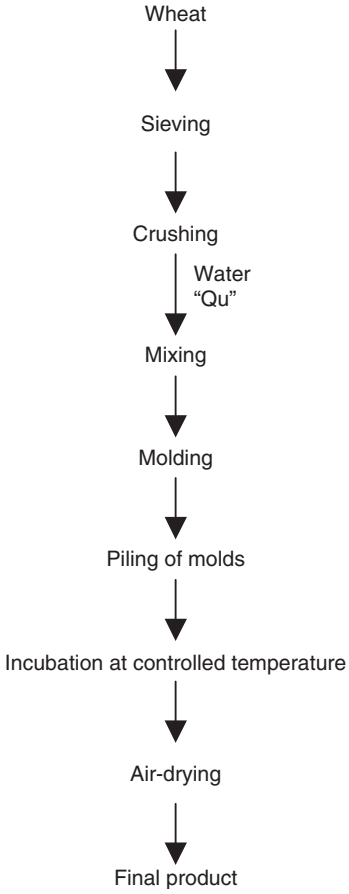

FIGURE 173.10 Generalized schematic flowchart on production of wheat "qu."

Piling of "qu." The incubation must be sanitary. The walls are sanitized with lime slurry. The floor is covered with rice husk and bamboo mats to maintain temperature. The small cakes are then transferred into the room gently without breaking them. They are aligned and stood in T-shape to facilitate air circulation and growth of saccharifying fungi to grow.

Incubation under controlled temperature. After the raw materials are transferred into the room, the windows and doors are closed to maintain the temperature. In general, the entering temperature is about 26°C. After 3–5 days, growth of mold mycelia is abundant, the highest temperature can reach 50°C. At this time, lowering of temperature should be practiced by removal of temperature

maintaining materials and appropriate opening of windows. As incubation continues, product temperature drops rapidly. After 20 days, the "wheat qu" forms hard cakes. They should be piled in # shape for ventilation and drying. Excellent "huang qu" has small amount of greenish yellow spores, the "qu" cakes are hard and porous with moisture content of 15–18% and high saccharifying activity.

There are also procedures to produce pure cultured "wheat qu" using thick layers and ventilation. Generally, they include stepwise culture of the pure microorganism from test tube culture, to Erlemeyer flask culture, to enlarged capacity culture of "wheat qu."

2. Production of "Hong (Red) Qu"

"Hong qu" is a famous typical Chinese product. It is made by reproducing the "hong qu" on rice. In the production of "hong qu," the "Gutian hong qu" and "Jianou hong qu" from the Fujian Province are the most famous. Because of the differences in raw materials and managing methods, "Gutian hong qu" can be divided into three groups: "Ku (storehouse) qu," "Qing (light) qu," and "Se (color) qu." Specific gravity of "Ku qu" is larger, and is used mainly for production of "jiu;" "Qing qu" is lighter, and is used production of "jiu" and for dyeing; "Se qu" is used mainly as a food coloring. "Hong qu" is also used in the production of soy cheese, "dregs fish," "dregs meat," and in medication. There are also pure cultures of "hong qu" available nowadays. The source "qu" for "Gutian hong qu" comes from "Jianou tu (earth) qu." "Tu qu" is also called "Black-coat hong qu." Its outer appearance is pitch black with red shade; its cross-section is red in the center with a pitch black with red shade exterior. Isolated microorganisms from it include *Monascus*, *Aspergillus niger*, and yeast. Therefore it has very strong starch liquefying activity and fermenting activity. In the past, "tu qu" was used as the original starter for "Gutien hong qu." Nowadays, some people just break up the "hong qu" and use these broken pieces as original starter. "Qu gong (male qu), and "qu mo (female qu)" are the most original starters for "Gutien hong qu."

a. "Jianou tu qu"

Preparation of raw materials. The "qu gong" and "qu mo" usually are prepared in the summer and stored for later use.

- "Qu gong." One hundred (100) kg of rice is washed, soaked, steamed, and spread cooled to 40°C. Eighty (80) grams of "qu gong powder," and 500–800 g "qu mo slurry" are mixed into the cooled rice. Let the temperature rise to 43°C, turn over and transfer to the incubation room. Temperature is then maintained at 38–40°C. Water is sprayed on once. After 4–5 days, the "qu" is taken out for drying. A good product should be hard particles with pure greenish red color.
- "Qu mo." One hundred (100) kg of rice is washed, soaked, steamed, and spread cooled to 40°C. Ten to twenty (10–20) grams of "qu gong" powder," and 1,600 g "qu mo slurry" are mixed into the cooled rice. Let the temperature rise to 43°C, turn over and transfer to the incubation room. Temperature is then maintained at 38–40°C. After 4–5 days, the "qu" is taken out for drying. A good product should be hard particles with slightly red color.
- "Qu mo slurry." Wash 1–1.5 kg of rice, add about 7.5 kg water, cook to a porridge, cooled to 32°C, and mix with 1 kg of "qu mo powder." After 7 days of fermentation, the slurry should show alcoholic odor, and spicy hot taste. The "qu mo slurry" is then ready for use.
- Rice. Select good quality rice.
- For production of 100 kg "tu qu," it takes 200 kg rice, 80 g "qu gong powder" and 1.5 kg "qu mo slurry."

Figure 173.11 is a generalized schematic flowchart for production of "jianou tu qu."

Technology essentials.

- **Spreading the rice and mixing with starter.** Rice is soaked for 12 hours after washing, and drip-dry. The rice is then loaded to earthenware ("zeng"), steamed until steam comes out from the "zeng," and the "zeng" is then covered. Continue the steaming for 20 minutes to a stage of completely cooked but not mushy. Cooked rice is transferred to a cooling bowl, spread out, turned over and cooled to 30°C. Fixed amounts of "qu mo powder" and "qu mo slurry" are mixed in evenly. After mixing, the mixture is loaded to a stacking cart, covered with clean and sterilized hemp bags, and transferred to the incubation room.
- **Culturing at controlled temperature.** In general, steaming of rice and inoculation are done in the morning, then transferred to the incubation room at controlled temperature at noon and left overnight. Product temperature rises to about 40°C. The next morning, the "qu"-rice mixture is transferred to a convex earthenware and pile up the mixture to a hip form. The mixture is turned over once at noon to release the heat and provide even contact with the mold mycelia. In the afternoon, spread out the "qu"-rice mixture flat on the earthenware evenly. At this time, growth of mold mycelia is vigorous. On the third day, turn over the mixture once in the morning and once in the afternoon. At this time, the rice grains start to show a red shade on white background. On the

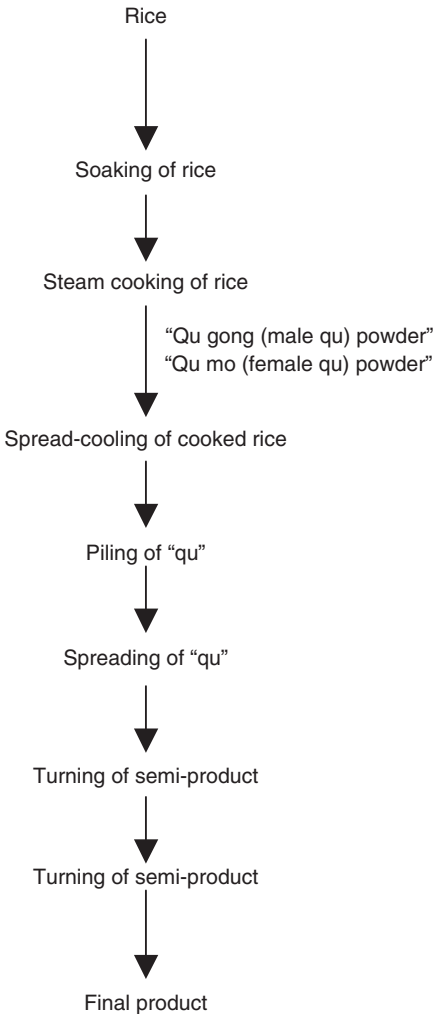

FIGURE 173.11 Generalized schematic flowchart on production of "Jian Ou Tu Qu."

fifth day, take out the mixture, load it on a bamboo (slightly porous) container and for soaking in water, drip-dry, and again transfer to the incubation room and maintain the temperature. The mixture is again turned over in the afternoon. On the morning of the sixth day, collect the mixture in bamboo containers, soak in dilute lime solution, drip-dry, and transfer to the incubation again. Product temperature is maintained at 32–37°C. On the seventh day, mixture is turned over once in the morning and once in the afternoon. At this time, *Aspergillus niger* starts to develop on the exterior, the surface is greenish black, and interior center is reddish with dull black shade. On the eighth day, the mixture is taken ot from incubation, and sun-dried to form the final product.

b. "Gutian hong qu"

Raw materials. The main ingredients are "qu starter," quality vinegar, and rice. The "qu starter" is the "Jianou tu qu." Ferment the rice with "tu qu." The residue is saved for later use. In the production of "Ku qu," and "Qing qu," it is appropriate to use vinegar aged for one year; however, in the production of "se qu," because it takes a long time, the quality requirement for vinegar is even higher. Vinegar aged over three years is needed, because of its sweetish sour taste, and mild and long lasting characteristics. In the production of "qing qu," it is better to use polished nonglutinous rice; however, in "ku qu" production, carefully polished long grain rice should be used, and the cross-section of this rice shows a shade of blue, and is commonly called "blue boned rice."

Production flowchart. Figure 173.12 shows a generalized schematic flow chart for the production of "Gutien hong qu."

Technology essentials.

- **"Qu starter" ratios and "qu" mixing.** Rice after soaking, steaming and cooling to 40°C is ready for "qu" mixing. In the production of "ku qu," 100 kg of rice is mixed with 7.5 kg of "jiu" residue. In the production of "qing qu," 100 kg of rice is mixed with 7.5 kg "tu jiu" residue, and 10.75 vinegar. In the production of "se qu," 100 kg of rice is mixed with 10 kg of "tu qu" residue, and 15 kg vinegar. First, the "tu qu" residue is mixed with vinegar to form the "vinegar residue;" then this "vinegar residue" is mixed with a part of the rice; and finally, this portion of the rice is mixed with the rest of the rice. It is important that the mixing is thorough.
- **Management inside the incubation room.** The well-mixed rice is transferred to the incubation room and piled up on the "qu" beds. They are covered with clean hemp bags for 24 hours to maintain temperature. Mold mycelia grow abundantly, and the rice temperature rise gradually. At 35–40°C, the rice is kneaded and flattened. The kneading is conducted every 4–6 hours to adjust the temperature. After 3–4 days, mold mycelia penetrate gradually to the rice interior, showing red spots. At this time, the rice is loaded to bamboo containers, rinsed with water for 10 minutes to allow water absorption. After drip-drying, the rice is piled again for 12 hours till the temperature rises again to "hot" stage, the rice is then spread loosely and flatly. After this stage, the rice is turned over every 6 hours. When the mold mycelia are abundant and start to excrete red pigment, and the rice kernels show a dry state

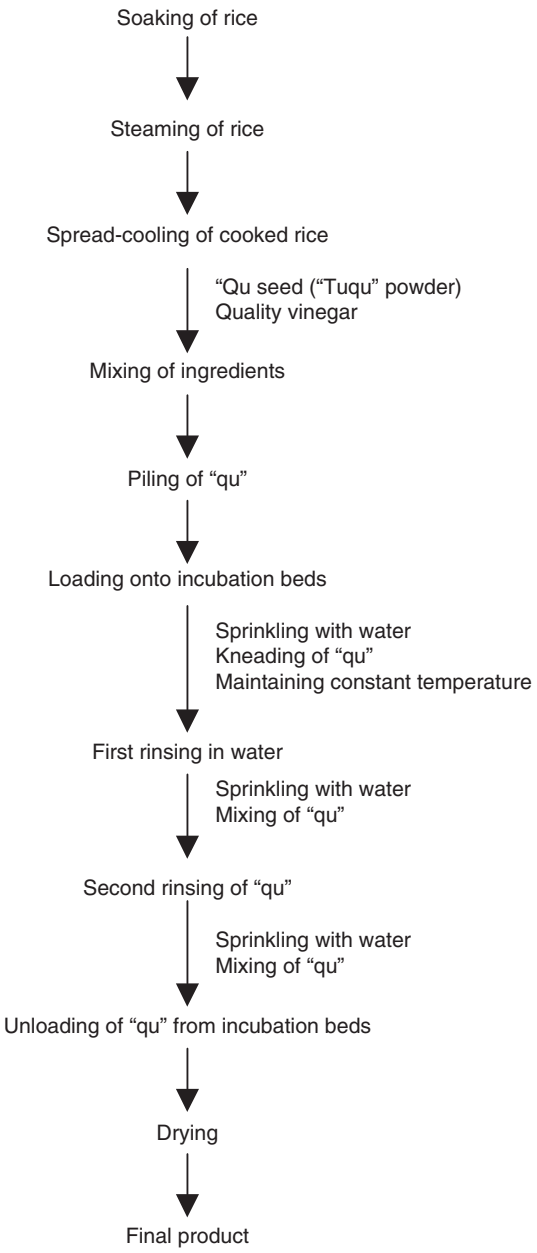

FIGURE 173.12 Generalized schematic flowchart on production of "Gutian Hon Qu."

(making noise when turned over by hand), appropriate water can be sprinkled on. Pay attention to adjustment of room temperature controlling the product temperature to 28–30°C. This stage takes 3–4 days, and the rice turned bright red. This is called "first watering." After this stage, water is sprinkled on appropriately to maintain proper temperature and relative humidity. If too much water is sprinkled, temperature will be too high, and the "qu" start to decay or produce very long mycelia. When it is too dry, the "qu" mold does not grow properly. Therefore, temperature and relative humidity must be controlled carefully. The "qu" is turned over every 6–8 hours, and last for 3–8 days. This is called "second watering." The "qu" particles show transparent red on the exterior with characteristic "qu" odor. At this stage, they can be taken out for drying. In general, the production rate yield (to the rice) is 50% for "ku qu," 33% for "qing qu" and 25% for "se qu."

c. "Jiu yao"

"Jiu yao" is a traditional, innovative method of preserving the quality "qu" culture in China. It is the saccharifying agent for producing "sprinkled rice jiu mo." "Jiu yao" has the white "yao" and the black "yao," with the white "yao" being more common. The white "yao" is made from coarse powder of dry long grain rice, "Laliaocao" powder, and water as the raw materials through a natural cultivation process.

Figure 173.13 is a generalized schematic flowchart for the production of "Ningbo bai (white) yao." Raw materials include 1875 kg of rice flour, 126–157 g, "Laliaocao" powder, 400–500 g "qu mo," and 10.5–11.5 liter of water. The mixture is incubated under controlled temperature. The procedure is similar to the "Gulin yao xiao qu" (see Figure 173.6).

d. "Jiu mo"

Based on the source of lactic acid, "jiu mo" can be divided into two kinds. The first kind is lactic acid produced by the Rhizopus and Mucor molds in the "sprinkled rice jiu mo." The other kind is "rapid fermentation jiu mo" with artificially added lactic acid and high temperature saccharifying "jiu mo."

e. "Sprinkled rice jiu mo"

"Sprinkled rice jiu mo" is also called "jiu niang (ferment or mother)." The names came from the sprinkling of cold water on cooked rice. Figure 173.14 is a generalized schematic flowchart for the production of "sprinkled rice jiu mo."

Raw materials. For every "gang" (earthen ware), use 125 kg glutinous rice, "jiu yao" 0.187–0.25 kg, 19.5 kg "wheat qu," and 375 kg water.

Loading of "gang," nest forming and saccharification. Drip-dry the sprinkle-cooled rice before loading into the "gang" (container). Before use, the "gang" should be sun-dried, and sanitized with lime solution and boiling water. Loading of rice is controlled at 27–30°C, or even as high as 32°C on cold days. The rice is mixed twice with the "jiu yao" powder and evenly. The rice-"jiu yao" mixture is loaded in a concave shape inside the "jang," with additional "jiu yao" powder sprinkled on it. Room temperature should be controlled. The Rhizopus mold will grow

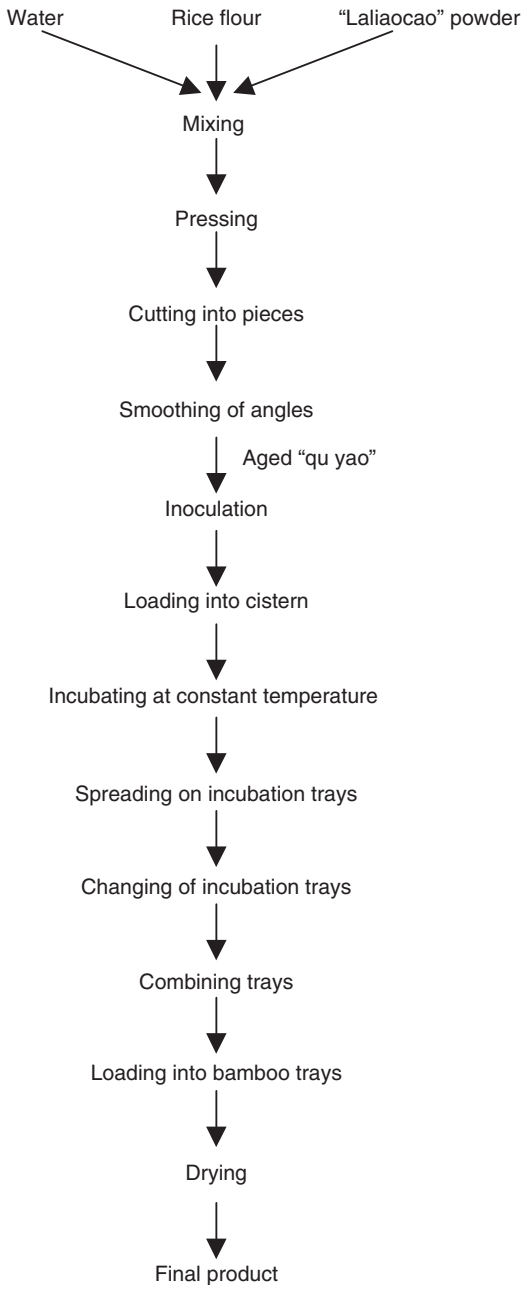

FIGURE 173.13 Generalized schematic flowchart on production of "Ningbo bai yao."

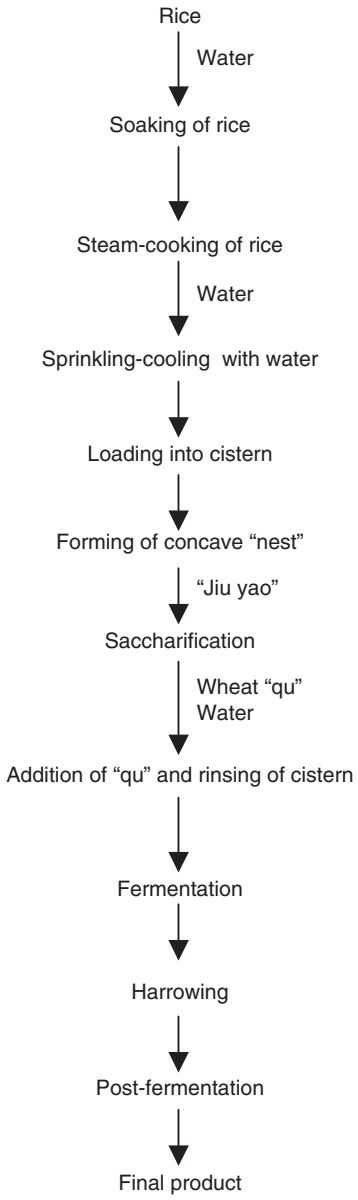

FIGURE 173.14 Generalized schematic flowchart onsprinkled rice "Jiumo (mother jiu)."

rapidly on the rice kernels. In a short time, the white mycelia on the rice kernels will be visible with excretion of saccharifying enzymes to saccharify the starch. Usually after 36–48 hours, the concave bottom starts to have a sugary liquid.

Addition of "qu" and rinsing of "gang." Because of the availability of sugars and the presence of Rhizopus and other molds, sugars are converted to lactic and other organic acids. This appropriately adjusts the pH of the sugary liquid, inhibits growth of undesirable microorganisms, and allows the reproduction of yeast followed by the production of alcohol. When the sugary liquid rises to the height of four-fifths of the rice, the broken "wheat qu" and water can be added and mixed thoroughly. Rinse the interior of the "gang," dilute the fermenting mash, increase the nutrients and oxygen, and facilitate the extensive reproduction of yeast.

Fermentation and harrowing. Because the yeast is reproducing extensively, the alcoholic fermentation is vigorous, and increases the product temperature. When the temperature raises to 28–30°C, use a wooden harrow to mix the mash. This is commonly called "first harrowing." The purpose of this harrowing not only lowers the temperature and has an even temperature for the rice and the sugary liquid, but also removes the carbon dioxide, provides fresh air, facilitate the yeast reproduction, and

avoids the overflow of the mash, and contamination of undesirable microorganisms. Therefore, harrowing is one of the important steps in the fermentation of "huang jiu." After the first harrowing, harrowing is conducted every 3–5 hours to maintain the product temperature maintained at 26–30°C . Generally the number of harrowing is based on the room temperature and changes in the product.

Post-fermentation. About seven days after the loading of "gang," the "gangs" are filled up and sealed. This procedure is to reduce the contact with air, continue the post-fermentation to produce more alcohol and increase the quality of "jiu mo." The complete fermentation process takes 20–30 days and the alcohol content is 15 v% or above. The mash is then used as "jiu mo."

"Sprinkled rice jiu mo" can also be used as a commercial product for direct consumption. This is commonly called "Kuai (rapid) jiu." However, the product flavor is fairly simple.

f. "Rapid fermentation jiu mo" and "high temperature saccharifying jiu mo"

Both the "rapid fermentation jiu mo" and "high temperature saccharifying jiu mo" are pure-cultured yeast. The principle of pure culturing of yeast is to inoculate the yeast to "huang jiu" yeast with a single cell, then gradually increase the culturing to reproduce large amount of yeast and form the "jiu mo." The scale-up process is as follows: Original microorganism → Slant culture in test-tube → Erlemeyer flask culture → Karl's jug culture → "jiu mo."

C. Fermentation and Pressing

The uniqueness of "Huang jiu" fermentation is open fermentation, simultaneous saccharification and alcoholic fermentation. The mash is a high concentration mash fermented at a low-temperature for a long time, thus producing a high concentration of alcohol. On the basis of procedures, the fermentation methodology generally can be divided into three categories: "sprinkled rice" method, "spread rice" method, and "feed rice method." The "sprinkled rice" method has already been described earlier under the section of "jiu mo" production. The following describes the traditional "spread rice" and "fed rice" methods.

1. "Spread Rice" Method

"Yuan hong jiu" is the most popular, and largest in quantity produced "Shaoxing jiu" by following the traditional production technology. Its fermentation technology is a combination of "sprinkled rice" and "spread rice" methodologies.

a. Equipment

The main equipment includes the large cistern (croak), and small jugs. Generally, large cisterns are used for the initial and main fermentation, and the small jugs are used for the slow post-fermentation.

b. Flowchart

Figure 173.15 is a generalized schematic flowchart for the production of "yuan hong jiu."

c. Procedures

- **Raw materials.** The following raw materials are used for each cistern: 144 kg glutinuous rice, 2.5 kg of "wheat qu," 5–8 kg of "sprinkled rice," 84 kg of "starchy water (slurry)," and clear water 112 kg. Traditionally, a ratio of 3 portions of starchy water vs. 4 portions of clear water is practiced. The "starchy water" contains total acids of 0.7%. When a ratio of 3 portions "starchy water" vs. 4 portions of clear water is used, the total acids concentration is about 0.3–0.4%, which is just right for yeast reproduction.
- **Procedures before loading cistern. See above.**
- **Loading of cistern.** The tools for loading the cisterns must be sanitary or even sterilized. After adding the clear water, it is followed by the rice, "jiu mo," mashed "wheat qu," and lastly the "starchy water." The mixture is mixed thoroughly. Temperature at loading should be maintained at 24–26°C, with a maximum of 28°C.
- **Saccharification and alcoholic fermentation.** After loading the cisterns, saccharification and alcoholic fermentation start to proceed. In the initial stage of fermentation, it is basically the reproduction of yeast, and the temperature increase is slow. Temperature should be maintained carefully. After 12 hours, the yeast population in the mash already reaches to a large number, and the main fermentation is initiated. Because of the vigorous yeast fermentation, large amount of sugars are converted to alcohol and carbon dioxide, with release of heat; temperature rise is more rapid, even with "si si" sound. Bubbles will push the mash to the top of the liquid, forming a thick layer. At this time, the taste of the mash is sweet with mild "jiu" flavor. The temperature is 4–7°C higher than loading the cisterns. At this stage, it is important to check the temperature. When the temperature reaches a certain level, harrowing should be started.

Traditionally, the initiation of harrowing can be either the high temperature process or the low temperature process. For the high temperature process, the mash should reach 35°C for the first harrowing in order to lower the temperature. For the low temperature process, harrowing of the mash is initiated at 30°C. Fermentation temperature should not be higher than 30°C. Because of the differences

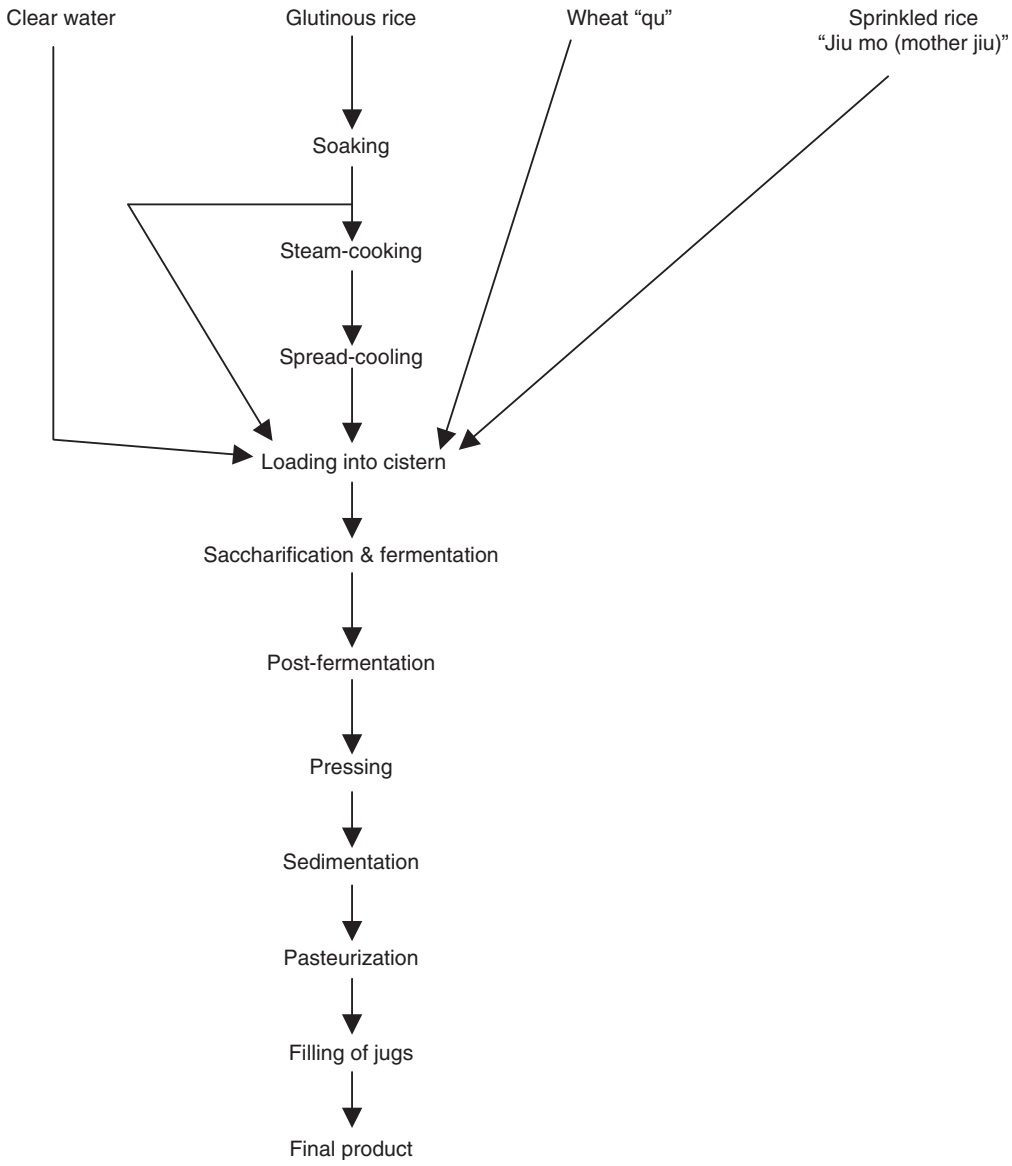

FIGURE 173.15 Generalized schematic flowchart on "Yuanhong Jiu" production.

in the initiation of harrowing, the flavor of the final products is affected. For the high temperature process, because of the higher temperature, yeast ages early with a decrease in fermentation activity. The final product has more solutes with a rich sweet taste. This is commonly called the "hotly made jiu" or " sweet to the mouth jiu." In the low temperature process, the fermentation is complete, with lower acid and higher alcohol contents. This process can easily reach the sugarless stage, and the product is dry, and is commonly called the "coolly made jiu."

Consumers usually prefer the "hotly made jiu." Therefore, it is common to harrow the mash at high temperature. After the first harrowing, product temperature starts to drop; thereafter, the following harrowing depends on the situation. If the room temperature is low, and the product temperature rises slowly, the time in between for harrowing should be extended, and vice versa. After the fourth harrowing, it is customary to harrow the mash once in the morning and again in the evening. The main reasons are to lower product temperature and proceed the saccharification and alcoholic fermentation evenly. However, under low room temperature, the number of times on harrowing should be reduced in order to reduce the loss of alcohol. After 6–8 days, when the product temperature and room temperature are getting close, and the residues are sinking to the bottom, it is the end of the main fermentation. Harrowing should also be terminated and the mash is transferred to jugs to proceed the post-fermentation.

- **Post-fermentation.** After the main fermentation, the alcohol content in the mash is already very high, but some starch and sugars are still not fermented. Therefore, it requires post-fermentation and continues the fermentation to increase the alcohol content. Since the alcohol content is already in the range of 13–14 v%, fermentation is very slow, and therefore requires a long time to achieve, usually 70–80 days. After the long fermentation period, the "jiu" flavor is also better. Post-fermentation usually is conducted in jugs as their mouth is small and is easy to seal off, avoiding the excessive evaporation of alcohol and the invasion of undesirable microorganisms. The procedure to fill the jugs is to transfer the mash from the cisterns to clean and sanitized small jugs, each holding 25 kg of "jiu." They are piled in cellars or outdoors for post-fermentation. Every three jugs are piled up together, and their mouths are sealed with paper, then the ceramic cover. The product temperature fluctuates with the outdoor temperature. In cold days, the jugs can be piled up facing the sun, and in warm days, they should be piled in the cool shade or outdoor to avoid too high a temperature that can cause acid spoilage.

2. "Feed Rice" Method

This method is to divide the raw materials into batches. The first batch is used to produce the "jiu mo," and the rest of the raw materials are added in batches to continue the fermentation. The characteristics of this method is as follows:

- Because of numerous feeding of rice to the mash, more raw materials can be used. This allows the use of small amount of "jiu mo" and produce large amount of "huang jiu."
- The yeast will continuously receive new nutrients, and conduct the reproduction several times. Thus, as compared to common "jiu mo," more new yeast cells are produced and they don't age easier. The fermentation is always vigorous.
- Because the raw materials are added in installments, the yeast and acids in the "jiu mo" are not extensively diluted at once. Yeast dominates in the mash and prevents the growth of undesirable microbes. The fermentation can be conducted safely.
- It is easier to control the fermentation temperature and other technologies. This is beneficial for the control and adjustment, and easier to adapt to the environment.
- Because of the vigorous fermentation, the mash is also turned over vigorously. Fermentation in large cistern thus facilitates the natural harrowing. It has been proven that "feed rice" method fermentation is beneficial in lowering the product temperature and controlling the fermentation temperature. The mash is not easy to have acid spoilage. Yeast has a stronger fermentation capacity, and the fermentation is more complete with increases in "jiu" production rate and "jiu" quality. Thus, the "feed rice" method is getting more popular in the "huang jiu" factories.

In the Zhejiang area of China, there are more factories using the "feed rice" method. In Japan, the production of sake also applies the "feed rice" method. The detail procedures vary with the number of feedings and the ratio of raw materials fed to the mash. Based on the experiences in China and other countries, the following points need special attention:

- It is better to limit the number of feedings to 2 to 3.
- The time between each feeding should be 24 hours.
- Among the various feeding, the ratio is less at the beginning and more afterwards. With the gradual increase in the amount of rice, it will also increase the reproduction of yeast. In the later stages, with larger amount of rice added, the final product is also sweeter.
- For the first feeding, the amount should not be too big to avoid the sudden proportional decrease of acids and yeast. If the undesirable microorganisms are not properly inhibited before fermentation, acid spoilage occurs more often.
- Temperature at the beginning of fermentation should be lower, and increase gradually. After the completion of the final rice feeding, temperature should reach to the maximum as specified.
- Even though the total amounts of water and "qu" added are the same, the appropriate amounts added at the time of feeding can be adjusted according to the actual situation. However, the increased or decreased amount should be proper and not vary too much.

3. Pressing, Filtration, and Sterilization

After the long fermentation, even though the mash contains the basic ingredients of "huang jiu," the "jiu" and its residues are mixed together, this mash can not be considered as a final product. In order to separate the liquid and solids, it is necessary to proceed with pressing,

and filtration. Sterilization and packaged in hermetically sealed containers are also necessary to avoid quality change and spoilage.

D. Famous Chinese "Huang Jiu"

"Shaoxing jiafan (additional rice) jiu" and "Fujian chengang (sediment in cistern/croak) jiu" have been rated as "National Famous Jiu" in every "Jiu" Conference in China. There are many kinds of "huang jiu," and their fermentation technologies also vary considerably. The following are examples of fermentation technologies on Chinese famous "jiu" and some with typical location characteristics.

1. "Shaoxing Jiu"

There are many variations of "Shaoxing jiu." Their fermentation technologies basically are the "sprinkled rice" method and the "spread rice" method. Based on the ratios of their raw materials, there can be over 10 different kinds, such as "Yuanhong (winner red) jiu," Shanniang (well fermented) jiu," Xiangxue (flavored) jiu," et al. The technology for "Yuanhong jiu" fermentation has already been described (see above). The following are the technologies for three other "Shaoxig jiu."

a. "Shaoxing jiafan jiu"

"Shaoxing jiafan jiu" is produced in the "Shaoxing Jiu Fermentation General Company" in Shaoxing City, Xijiang Province, China. "Shaoxing" is famous for its "jiu" production. It was mentioned in one of the oldest classics on Chinese history " Lu's Chun Qiu (Dynasties)" over 2,000 years ago, and has been subsequently mentioned in the "Jiu Jing (Book of Jiu)" in the Tang Dynasty. "Shaoxing jiu" also enjoyed a good reputation in the Ming and Qing dynasties. It won the Gold Medal in the World Expo in Panama in 1915. Since then, it was world famous and is now exported to over 50 countries.

"Shaoxingjiafan jiu" uses glutinous rice as the raw material, and the "wheat qu" and jiu yao" as the saccharifying and fermenting agents. After soaking, steaming, saccharification and alcoholic fermentation, pressing and storage, the "jiafan jiu" is thus produced. The storage period is at least 3–5 years, with some 10–20 years. This "jiu" is nutritious, with 17 amino acids, among them 7 are essential; this exceeds beer and grape wines. It is named as "liquid cake" and is equivalent to the Japanese national alcoholic beverage, sake. The amino acid content in "Shaoxing jiafan jiu" is 5,600 mg per liter, this is over 30% higher than the Japanese sake. "Shaoxing jiafan jiu" belongs to the dry type "huang jiu," the sugar content is less than 1%, with alcohol content 16.5 to 17.5 v%, and total acids < 0.45 g/100 ml. The product is orange-yellow in color, transparent with luster, rich odor, savory, sweet and smooth with long-lasting taste.

"Jiafan jiu" has in the ingredients additional cooked rice. In reality, it is a thick mashed fermented "jiu."

- *Ingredients*. The standard formula for each cistern has the following ingredients: glutinous rice 144 kg, "wheat qu" 25 kg, "starchy water" 50 kg, clear water 68.6 kg, and 50 v% residual "jiu" 5 kg.
- *Fermentation technology*. The fermentation technology is basically the same as "yuanhong jiu" but with the following variations:
  - Because the amount of rice is larger, the mash viscosity is larger, and the temperature during the main fermentation rises faster. Therefore, the temperature of raw materials entering the cistern should be 2–3°C lower than in "yuan hong jiu," and the temperature maintenance criterion is less stringent. Besides, for the ease of temperature control, this product can be fermented in the very cold period of the year.
  - Because of the longer peiod of fermentation, it takes 10–15 days for the rice kernels to be completely settled to the bottom of the cistern, before loading to the small jugs for post-fermentation. Before loading into the jugs, they are filled with 5 kg of 50 v% "residue jiu" and small amount of mash liquid from the "sprinkled rice jiu mo." This is to increase the alcohol content and the fermentation capacity, and prevent the possibility of acid spoilage. The post-fermentation period takes 80–90 days.
  - Because the mash is more viscous, the fermentation usually is not complete, resulting in more residues and causing difficulty in pressing. The pressing time usually is double of that for "yuanhong jiu."

b. "Shaoxing shanniang jiu"

"Shanniang jiu" is also called "tao (formulated) jiu," and uses "jiu" to replace the water in the "jiu" fermentation. It is a semi-sweet "jiu." The flavor of this "jiu" is sweet, rich, and unique. Its nutritional values and mouth feel are equivalent to high-quality sweet grape wine. In 1979, it was named as one of the excellent quality "jiu."

Ingredients. For every cistern, the ratio of ingredients is as follows: glutinous rice 144 kg, "wheat qu" 25 kg, "starchy water" 50 kg, "Sprinkled rice qu mo" 15 kg, "yuanhong jiu" (aged for 3 years) 100 kg.

Fermentation technology. The technology is similar to "yuanhong jiu." The difference is that yeast reproduction is inhibited and the fermentation is slow due to the large amount of "yaunhong jiu" added, and the mash has an alcohol content of over 16%. The sugars cannot be

digested and remain at over 7% during the whole process. In order to facilitate the yeast reproduction and fermentation, it is required that the loading temperature for the cistern is 1–2°C higher than that in the fermentation of "yuanhong jiu;" also the temperature maintenance should be strengthened. Besides, because of the alcohol content, fermentation is slow, and the fermentation time requires 80 days. Because of the mash viscosity, the pressing time also takes longer.

c. Shaoxing xiangxue jiu"

The fermentation technologies for "xiangxue jiu" and "shanniang jiu" are similar, both replacing water with "jiu" in the production process; however, "residue jiu" is used instead of "yuanhong jiu." In the production process, "Sprinkled rice" method is used in the fermentation of "xiangxue jiu." Because "xiangxue jiu" is made with sweet "jiu niang" together with "residue jiu," both the alcohol and sugar contents are increased. The production is not limited by the weather, and a warm environment is very suitable for the production of "jiu niang;" therefore, it is commonly produced in the summer.

Even though the "xiangxue jiu" is made with "residue jiu," with its long fermentation, the mouth feel is sweet and rich; it does not show the spicy hot taste as in "bai jiu," but provide the characteristic, rich "Shaoxing jiu" flavor. The key to "xiangxue jiu" production is its appropriate time for saccharification. That is, when the sugar is accumulated to a fairly high level, a large amount of "residue jiu" is added, and inhibits the fermentation by yeast. The sugar content in the mash is basically fixed, and forms a "jiu" with very high levels of sugar and alcohol. The fermentation technology is as follows:

Figure 173.16 is a generalized schematic flowchart of Shaoxing xiangxue jiu" production.

For each cistern, the ingredients are as follows: glutinous rice 100 kg, "wheat qu" 10 kg, 50 v% aged "residue jiu" 100 kg, and "jiu yao" 0.187 kg.

Fermentation technology. The procedures on forming the concave shaped raw materials are the same as the "sprinkled rice jiu." Thirty-six to forty hours after this stage, the sugary liquid in the concave raw materials fills up the cavity. Ground up "wheat qu" is then added to the sugary liquid, mixed with the rest of the raw materials, and continues to maintain the temperature to facilitate the saccharification. After another 24 hours, the sugars have accumulated to a high level, and the 50 v% "aged residue jiu" can be added. The mash is harrowed evenly, covered and left standing without disturbance. The mash is harrowed every 3 days. After 2–3 harrowings, the cistern is covered with another clean cistern. The exterior two mouths of the cisterns are covered with lotus leaves as the cushion, and sealed with a mixture of salt solution (nigari) and earth. After 3–4 months, the mash is recovered for pressing.

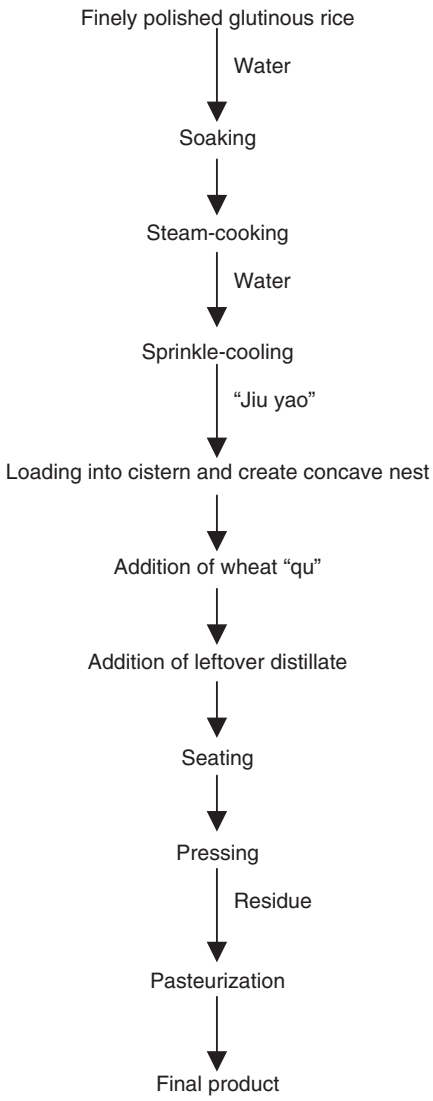

FIGURE 173.16 Generalized schematic flowchart on production of "Shaoxing Xiangzue Jiu."

"Xiangxue jiu" does not require addition of caramel for coloring and shows a transparent golden yellow color. The filtrate is heat-treated to coagulate the colloidal materials and clarify the "jiu liquid." Because the sugar and alcohol contents are fairly high, they can be filled into jugs without sterilization.

Table 173.2 is a comparison of the major chemical components in several kinds of "Shaoxing jiu."

2. Fujian Chengang Jiu

"Shenjang jiu" is a product of "Longyan Jiu Factory" in the western part of Fujian Province, China. The production of this "jiu" has a history of about 160 years. Currently, the factory utilizes the water from Luopan Fountain for the fermentation process. In the 2nd and 3rd "Jiu" Evaluation Conference, this "chengang jiu" was

TABLE 173.2
Main Components in "Shaoxing Jiu"

Components	Yuanhong Jiu	Jiafan Jiu	Shanniang Jiu	Xiangxue Jiu
Specific gravity	0.992	0.995	1.0349	1.073
Alcohol (%, v/v)	15.60	17.80	16.70	19.40
Solubles (mg/100 ml)	3.325	4.450	15.65	24.44
Sugar (g/100 ml)	0.38	0.78	6.5	20.00
Total acids (g/100 ml)	0.48	0.46	0.46	0.28
Volatile acids (g/100 ml)	0.06	0.027	0.054	0.056
Nonvolatile acids (g/100 ml)	0.42	0.43	0.406	0.22

named one of the famous "jiu" in China. The fermentation technology for this "jiu" is unique using glutinous rice, "hong qu" and specially made "yao xiao qu" as the saccharifying and fermenting agents, and precise procedures in its production. In its production process, the mash has to rise three times and settle three times. Lastly the residue sediments sink to the bottom, thus the meaning of "shenjang" or "sediment to the bottom of the cistern jiu." This "Longyan chengang jiu" is a sweet "huang jiu," with sugar content as high as 24%, about 20% alcohol, and rich in amino acids. It is nutritious, with reddish brown and lustrous color, clear and transparent, rich and smooth with a full body, balanced in flavor and long lasting. The fermentation technology of this "jiu" is similar to "xiangxue jiu," utilizing the "sprinkled rice" method for the initial fermentation, followed by the addition of distilled rice "jiu" to form the sweet "jiu" with high alcohol and sugar contents, but not very sweet.

a. Ingredients

For each cistern, the formulation is as follows: glutinous rice 40 kg, "jiu yao" 0.186 kg, "hong qu" 2 kg, "bai qu 0.065 kg, 53% distilled rice "jiu" ("bai jiu") 34 kg.

b. Fermentation essentials

The "bai jiu" is added in two increments. At the beginning, only small amount of "bai jiu" is added, this is beneficial for the saccharification and fermentation to proceed. With proper control of temperature and prevention of acid spoilage during fermentation, the rest of the "bai jiu" is added to meet the specified alcohol content and fix the sugar content in the mash. The production procedure for this product is to wait until the sugary liquid is about three-fifths in the concave "nest," then add in "hong qu" and 17.6% or 6 kg of the 53 v% distilled rice "jiu" ("bai jiu"). The saccharification process is allowed to proceed for 3–4 days, and the rest of the "bai jiu" (26 kg) is then added. Let stand for 50–60 days, then the mash is pressed, the filtrate is heated, filled in the jugs, sealed, and stored for a fixed period to form the product.

E. Quality of "Huang Jiu"

1 The Color, Odor and Taste of "Huang Jiu"

a. Color

The color of "huang jiu" varies with the product, ranging from light yellow to reddish brown or black. The color of "huang jiu" may come from the red pigments of glutinous rice and wheat, pigments from the "qu" such as the "red pigments in "hong qu," and the melanoids formed during the storage period. Besides, large proportion of the "huang jiu" has caramel added to improve its color. Iron, copper and manganese ions can also form compounds that give or facilitate the color.

b. Odor

There are many odorous compounds in "hunag jiu," and they vary considerably. However, there is no single compound discovered that is outstanding, and these odorous compounds among themselves can increase or decrease the odor of the product. Therefore, the odor of "huang jiu" is very complex, and quality "huang jiu" has its own odor requirements with absence of undesirable odor. The main odorous compounds in "huang jiu" are made up of mixtures of esters, acids, carboxyl compounds, and phenolic compounds.

c. Taste

The taste of "huang jiu" is a balance of sweetness, sourness, bitterness, astringency, and spicy hot sensation.

- **Sweetness.** Sweetness comes mainly from the sugars, with dextrose the highest in content. General "huang jiu" contains only limited sugars, and is not too sweet. However, sweet "huang jiu" contains more sugar, and its sweetness is significant. Besides, "huang iu" contains gycerol, 2,3-butanediol, and alanine that give sweet taste, and a rich sensation to the product.
- **Sourness.** A common saying is that if there is no acid, there is no "jiu." Acids decrease the sweetness and intensify the rich taste. The acids

in "huang jiu" are mainly organic acids, and they are formed mainly during the yeast fermentation that produces alcohol. Lactic and succinic acids have the highest contents. Succinic acid not only gives "jiu" the sour taste, but also together with glutamic acid, guanylic acid, and inosinic acid gives the umami (savory) taste. General "huang jiu" should have total acids about 0.4%, and for sweet "huang jiu" the total acids can be higher.

- **Bitterness and astringency.** The bitterness in "huang jiu" comes mainly from some amino acids, peptides, butanol, 5′-methyl sulfonic acid, and amines. Some "hunag jiu" has burnt rice or caramel that can also give bitterness. The astringency in "huang jiu" comes from lactic acid and amino-butyric acid, the added lime also carries over to "jiu" the astringent taste. Very small amounts of bitter and astringent substances do not give the "jiu" undesirable taste, but instead give "jiu" a rich and balanced mouth feel. Most of the "huang jiu" do not carry significant bitter or astringent taste.
- **Spicy hot sensation.** The spicy hot sensation comes mainly from the alcohol, and also the fusel oils. Newly fermented "jiu" usually gives an irritating spicy hot sensation. After long term storage, the spicy hot sensation decreases, and instead gives a rich and smooth flavor. This is due to the oxidation of alcohols to form aldehydes, and then organic acids. The ethanol and fusel oils react with the organic acids to form esters. In addition, the binding of alcohol molecules to the water molecules also reduces the spicy hot sensation.

2. Quality Standards of "Hunag Jiu"

Currently, Chinese national standards for "huang jiu" do not exist. However, the "huang jiu" standards from the Xijiang Province, China can be used for reference (Table 173.3).

3. Sanitary Standards of "Huang Ju"

"Huang jiu" is fermented "jiu," and fermented "jiu" must meet the national GB2758-81 Fermented "Jiu" Sanitary Standard (see Table 173.4).

IV. FERMENTATION OF "GUO (FRUIT) JIU"

A. Traditional Chinese "Guo Jiu"

1. Selection of Raw Materials

All fruits contain sugars; with suitable treatment, they all can be fermented into "jiu." However, the cultivar and quality of the fruit used for fermentation have significant effects on the final products. The flavor components in "guo jiu" is very complex, and there are two sources for these compounds. One source is fermentation: the growth activity of yeast and its related chemical reactions produce esters, alcohols, aldehydes and organic acids. Another source is from the components of essential oils from the fruits themselves; they are carried into the "guo jiu" and contribute to the total flavor of the final products. Until now, the flavor profiles of "guo jiu" can not be completely duplicated, and true "guo jiu" with good flavor can not be made with other raw materials.

Although all fruits can be used for fermentation, the use of quality raw materials is essential in the production of quality "guo jiu." The meaning of quality raw materials

TABLE 173.3
Quality Standard for "Huang Jiu" in Zhejiang Province, China

Product	Sensory Indicators			Alcohol (%, v/v)	Total Acids (g/100ml)	Sugars (g/100 ml)
	Color	Aroma	Taste			
Shaoxing Yuanhongjiu	Orange-yellow, clear	Typical "huang jiu" smooth aroma, absence of objectional odor	Absence of acid spoilage & objectional odor	15–16.5	≤0.45	None
Shaoxing Jiafan jiu	Same as above	Same as above	Same as above	16.5–17.5	<0.45	None
Shaoxing Shanniang jiu	Same as above	Same as above	Same as above	<15	<0.55	<6
Shaoxing Xiangxue	Same as above	Same as above	Same as above	18–22	<0.4	<19
Wuyi Hongqujiu	Same as above	Same as above	Same as above	15–16.5	<0.5	None

TABLE 173.4
Sanitary Standard of Fermented "Jiu," GB2758-81 (China)

Item	Index			
1. Sensory evaluation	Clear liquid, absence of precipitates, foreign matters, objectional odor and taste.			
2. Physicochemical analyses				
Residual sulfur dioxide, (g/kg, free sulfur dioxide)	≤0.05			
Aflatoxin B_1 (μg/kg)	≤5			
3. Bacterial numbers	*Draft beer*	*Regular beer*	"*Huang jiu*"	*Grape wine*
Total bacterial counts (unit/ml)	None	≤50	≤50	≤50
E. coli colonies (unit/100 ml)	≤50	≤3	≤3	≤3

Note: Fermented "jiu" is an alcoholic beverage without distillation using sugar or starch as the raw materials gone through saccharification and fermentation.

is that the fruits must meet certain criteria: the cultivar and kind of fruit must have high sugar content and lots of juice, and it is easy to extract the juice; in addition, the quantity of acids, tannins, and pectins should also be considered. Because the sugars are fermented into alcohol, they are related to the production rate and quality of the final product. Appropriate amounts of acids are beneficial to the reproduction of yeast, inhibit the invasion of undesirable microorganisms, facilitate the solution of plant pigments and ferment the raw materials into tasty "guo jiu." It can also combine with the alcohols to form esters in the storage and aging processes. "Guo jiu" with too little acids has a flat taste, and too much acids give the "guo jiu" a sour taste and lower the quality of the product. The amount of tannins when appropriate can also inhibit the growth of harmful microorganisms, and also facilitate the clarification of "guo jiu." However, if the tannin content is too high, it inhibits the reproduction of yeast, and the "guo jiu" will have an astringent taste, which is also undesirable. With too much pectins, it creates difficulty in juice extraction and clarification; in addition, it also gives too much methanol in the final product.

From the standpoint of world production of alcoholic beverages from fruits, the majority is wine made from grapes. However, from the standpoint of chemical composition, color, odor and taste, other fruits such as citrus fruits (oranges, mandarin oranges, and tangerines), lizhi (litchi, lychee), and pears are also suitable raw materials. The fruits used for fermentation must be fully ripe in order to obtain high sugar contents and their rich flavor and color. Decayed fruits, in principle, are not suitable for the production of "guo jiu."

2. Flowchart for Production

In the production of "guo jiu," it is generally divided into two categories, one utilizing the pigments in the fruit skin, and the other having the residues removes. They are schematically shown in Figure 173.17.

3. Technology Essentials

a. Extraction of juice

Generally, in the production of "guo jiu," juice is extracted before fermentation. However, in the production of colored "guo jiu" like the "yang mei (plum) jiu," the extraction or pressing process is conducted after the fermentation; the fruits are crushed followed by fermentation to extract the pigments from the skin. Before extraction of fruit juice, wash the fruits to remove the dust, soil, and microorganisms. For fruits on which pesticides have been sprayed, this is very critical. Besides, the fruit skin, hearts and seeds have complicated composition, and give undesirable flavor to the product. Therefore, removal of skin, hearts, and seeds is a necessary step to improve product quality. Fruit juice is usually extracted by pressing the crushed fruits mechanically.

b. Improving the fruit juice

Sugar and acid contents in fruits usually are not at the desirable levels and ratios. In order to guarantee the proper fermentation with expected product quality, it is necessary to adjust the sugar and acid contents. Fruit juice generally contains 10–15% sugars, and every 1.7 g glucose (or 1.6 g sucrose) is needed to produce 1 ml ethanol. This amount can only produce "guo jiu" with 6–9 v% alcohol. "Guo jiu" with alcohol content lower than 10 v% is called weak "jiu;" not only the taste is flat, it is also difficult to preserve. The standard "guo jiu" to go with meals should have alcohol content above 14%, and fruit acids 0.5%. In order to meet these requirements, the sugar content in fruit juices should be 25% or higher with 0.5% fruit acids. It is very difficult for natural fruit juice to reach these requirements and therefore, adjustment of these contents is common. It is also true for colored "guo jiu" fermentations including the residues (skin, hearts and seeds).

The adjustment of sugar contents can be accomplished in three ways: addition of sugar, addition of alcohol, and increasing the raw materials (jiu niang). In the production of "guo jiu" with alcohol content in the 16–18 v% range,

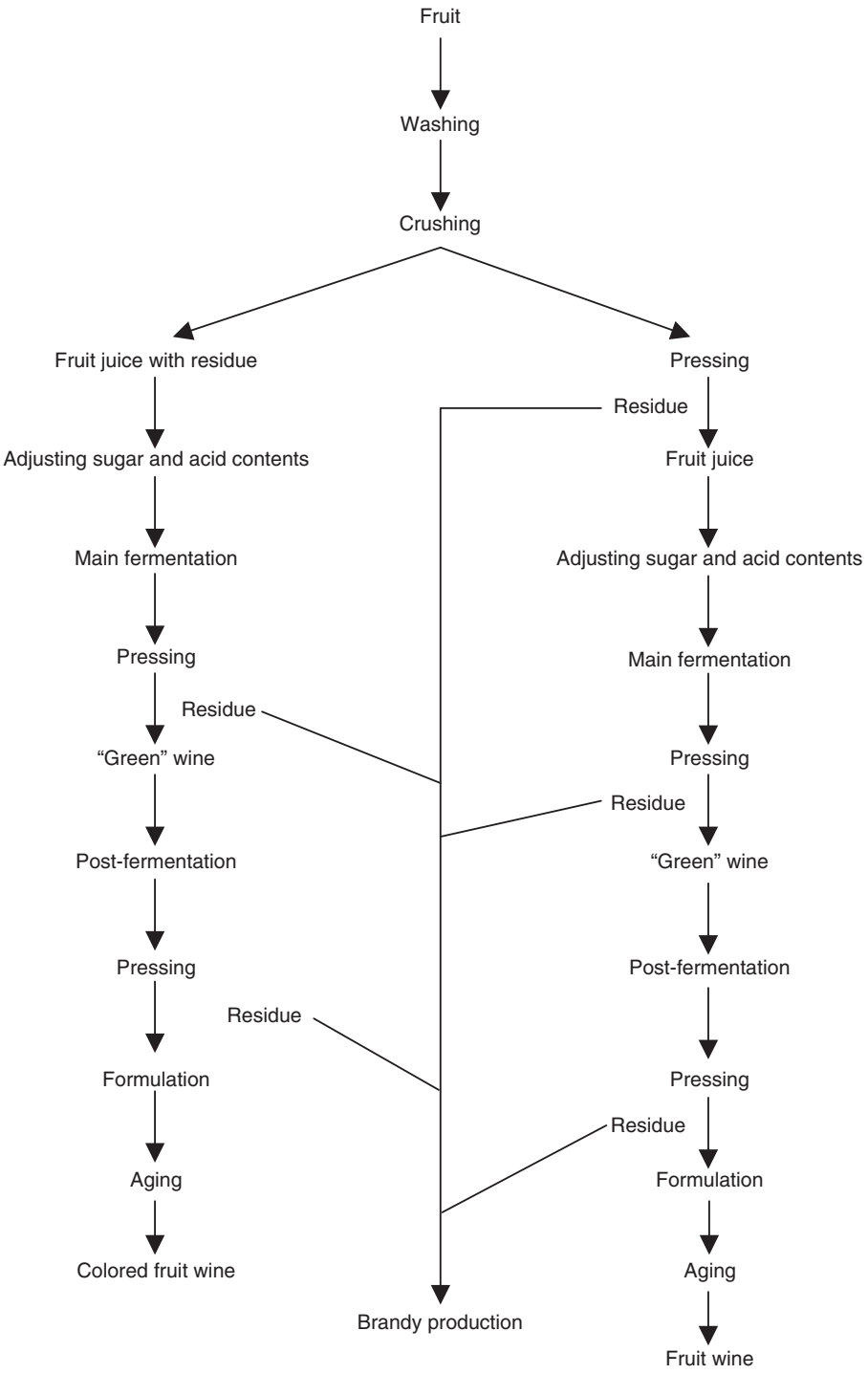

FIGURE 173.17 Generalized schematic flowchart on fruit wine production.

the only way is appropriate addition of alcohol or brandy to increase the alcohol content, and sugar cannot be added before the fermentation. This is because yeast in "guo jiu" fermentation cannot reproduce and produce alcohol in an environment of 15–16 v%. Addition of sugar to 27% is practiced only in the production of sweet "guo jiu" so that the residual sugar stays in the "guo jiu," giving the product a sweet taste. In the production of this kind of "guo jiu," when the alcohol content reaches to the desired alcohol content, the mash is cooled immediate or is treated with sulfur dioxide to terminate the fermentation, and proceeds with the aging process. In the production of high quality "guo jiu," the sugar content is achieved by vacuum concentration of the fruit juice.

The adjustment of acidity in fruit juice is accomplished by measuring first the total acids and converting this total acidity into citric acid for most fruit juices, and tartaric acid for grape juice, then adjusting the acidity accordingly by various means. If the fruit juice is low in sugar and acidity lower than 1%, addition of sugar is good enough without adjusting the acidity. If the fruit juice has normal sugar content but slightly higher acidity, the acidity can be adjusted by neutralizing the acid with calcium carbonate. For the removal of every 0.1% acidity in 100 liters of fruit juice, addition of 78.2 g calcium carbonate is needed. If acidity is not that high, it is better to remove the acids after fermentation. In the presence of suitable amount of acids, this is beneficial for the yeast reproduction and fermentation, and at the same time, inhibits the invasion of harmful microorganisms. In addition, this will facilitate the solubilization of pigments, and have a positive effect on flavor formation. In the production of colored "guo jiu," water can be added to dilute the substrate followed by addition of sugar, because addition of calcium carbonate will change the color of the anthocyanin pigments. If acid concentration is too low, citric acid, malic acid or tartaric acid can be added according to the fruit variety to total acids of 0.5% standard.

4. Fermentation of the Fruit Juice

a. Fermentation equipment

- **Fermentation room and storage room.** Fermentation rooms should have the following criteria: room temperature maintained at about 20°C with relative humidity at 75%, good ventilation, no direct sunshine, and easy drainage. Criteria for storage rooms are similar with room temperature maintained at 10–15°C. They are usually constructed at the lower level of fermentation rooms.
- **Fermentation containers.** Tanks for main fermentation and barrels for post-fermentation storage can be made of hard wood. The fermentation tanks can also be constructed with concrete, stones, or bricks. For smaller factories, "gang," jugs, or ceramic containers can be used.
- **Fermentation systems.** Open systems are used for fermenting juice with fruit residues, and closed systems are used for fermenting juice without fruit residues.
- **Fermentation methods.** Fermentation methods can be natural, semi-natural, or artificial. In natural fermentation, the fruit juice is not sterilized and without addition of yeast, it relies on the wild yeast adhered to the fruit surface. In the semi-fermentation method, fruit juice is not sterilized but yeast is added. In the artificial method, the fruit juice is sterilized with addition of pure-cultured yeast. The oblong strains of yeast for beer fermentation and the yeast for grape juice fermentation (*Saccharomyces urarum*) are suitable yeasts for "guo jiu" production. Fruit juices can be sterilized by heat treatment or sulfur dioxide treatment. Heat treatment is pasteurization. In the sulfur dioxide sterilization, enough sulfiting agent(s) are added to the crushed fruits or fruit juice at one time. The sulfiting agent(s) can be 6% sulfurous acid, liquid sulfur dioxide, or potassium thiosulfite. Table 173.5 is a comparison of sulfiting agent(s) for various treatments.

Potassium bisulfite continues 57.5% sulfur dioxide in the solid form. When added to fruit juice, it can release sulfur dioxide. Six hours after addition of potassium bisulfite, it can achieve the sterilizing effect. At this time, 5% yeast slurry can be added. The treated fruit juice contains 100 ppm sulfur dioxide. It can inhibit 99.9% of the wild yeast, bacteria, and mold; but the tamed yeast can tolerate this amount of sulfur of dioxide. The yeast slurry can be cultivated gradually to a larger scale through four expansion stages.

- **Fermentation process and management.**
  Main fermentation. Eight to twelve hours after inoculation, yeast reproduction is very vigorous, continuously producing carbon dioxide with bubbles coming up to the surface. When main fermentation enters the intermediate

TABLE 173.5
Amount of Sulfur Dioxide Used before Fermentation (per 1,000 Liters of Fruit Juice)

Condition of Fruits	6% H_2SO_3 (Liters)	Liquid SO_2 (g)	$K_2S_2O_5$ (g)
Fruits in good clean condition, about 20°C, total acids ≥0.8%	1.1	65	12
Fruits in good clean condition, about 20°C, total acids 0.6–0.8%	1.5	90	180
Fruits in cracked, individually moldy, above 25°C, total acids ≤0.6%	4.2	250	380

stage (large bubbles not common at this stage), the containers must be sealed tightly with bolts. During main fermentation, controls of fermentation temperature and air conditioning are important steps; room temperature should be about 20°C, and product temperature is maintained at 25–28°C. The fermentation must be ventilated properly in order to introduce clean air into the fermenting mash. For smaller fermentation containers, they can be stirred or transferring to different containers to achieve the introduction of air into the mash. At the end of the intermediate stage of main fermentation, there is no need for introduction of air. Under normal situation, fermentation with residues in mash takes 8–10 days to complete the main fermentation with vigorous fermentation for 3–4 days. With residue removed, it takes 12–15 days to complete with 5–6 days of vigorous fermentation. At the end of main fermentation, sugar content is about 1%, and the concentration of the mash is close to °Brix degree. Product temperature is the same as room temperature, with minimal carbon dioxide bubbles, and no noise heard. Residue settles to the bottom of the container, and the fermenting fluid is fairly clear. The bottom of the fermentation container has large amount of yeast sediment, indicating the end of the main fermentation.

Post-fermentation. With the main fermentation completed, the fluid is transferred to different containers promptly to remove the dead yeast cells, insoluble minerals, proteins, pigments, et al. The main purpose is to allow the "guo jiu" absorb a certain amount of oxygen which is beneficial to post-fermentation; avoiding the autolysis of dead yeast cells which can create turbidity and undesirable odor in the product; and permitting part of the tannins and pigments to oxidize and sediment. When the "guo jiu" is transferred to the storage barrels, the containers should be filled as full as possible to exclude air and prevent growth of acetic bacteria, newing bacteria, and other harmful microorganisms. After the containers are bolted, they are stored at 10–15°C in the stored room to conduct the post-fermentation. This takes 20–30 days. In this post-fermentation period, room temperature and product temperature are the same, with minimal amount of carbon dioxide produced. At the end of the process, no carbon dioxide is produced; yeast cells, proteins, and tannins combined together form the sediment; insoluble pigments and micro-fruit tissue particles also settle. The liquid is clear, and the flavor is mature with increase in pleasant odor but still "spicy hot."

Clarifiction of "guo jiu." Clarity is an important indicator in "guo jiu." Therefore, clarification of the "go jiu" must be conducted through either the natural or artificial method. Natural clarification takes a long time, so artificial clarification is applied in most cases. Artificial clarification can be conducted through one of the following methods:

Filtration. Filtration applies porous materials to separate the solids from the liquid; in "guo jiu," the suspended particles are separated from the final product. Common filtration medium usually presents a cloudy phenomenum in "guo jiu;" when it is used in combination with centrifugation, a better effect can be achieved. However, if pretreatment such as coagulation and chilling are not applied, the resulting "gui jiu" even though it has been clarified, its clarity is not stable.

Clarification. Clarification by means of coagulation is a common practice in the production of "guo jiu." The principle is the addition of hydrophilic coagulant(s) such as gelatin, egg white, or marine colloid to interact with the suspended particles in "guo jiu" and precipitate out the suspended matter.

Enzymatic method. With "guo jiu" containing more pectic substances such as apple jack, citrus (i.e., orange or tangerine) "jiu," application of pectic enzymes to hydrolyze the pectic substances is very effective in clarifying the "guo jiu."

- **Formulation and aging.**

Formulation. Standard "guo jiu" is also called dry "guo jiu" containing 14% alcohol, less than 1% sugars, and 0.5% acids. Some consumers do not appreciate it. Therefore, it is common to add sugar or brandy (or purified alcohol) to formulate products containing alcohol higher than 14% and sugar content 5% or above.

Aging. Newly fermented "guo jiu" has a spicy hot sensation and the flavor is not that good. They should be filled into storage rafters and aged in low-temperature cellars to improve the flavor. The product is called aged "guo jiu." "Liquid jiu" after post-fermentation, if clarified, can be transferred into other containers. The residues has to be removed, the

liquid "jiu" filled into bottles, and corked with brandy as a liquid seal. If the "jiu" has evaporated, it is replenished as needed to avoid contamination of harmful microorganisms, and the aging process is conducted. In this aging period, "guo jiu" experiences a series of slow oxidation, esterification, and synthesis, and improves the flavor of "guo jiu." For colored "gui jiu," the insoluble pigments, tannins, et al., precipitate out during this long aging period, and give the "guo jiu" a transparent yellow or bright red color. In addition, the solubilization of compounds from the wooden rafters can also facilitate the flavor. Therefore, aged "guo jiu" has a smooth and pure taste, with rich fragrance, and attractive color. In addition, because of the binding action of alcohol with other compounds, the irritating properties have decreased considerably, and the product is smooth and has a pleasant mouth feel. The ideal environment for aging "guo jiu" is 10–15°C, relative humidity in the air is about 75%. Aging for "guo jiu" takes a longer time, at least 6 months, with some 2–3 years. This is not economical from the production standpoint. Process used to shorten this aging period is called accelerated aging. The methods can be heat-treatment (25 days at 50°C), cold treatment (0.5°C which is higher than the freezing point of "guo jiu" for 3–5 days), alternate cold and heat treatment, or microwave treatment.

Bottling and sterilization of "guo jiu." When the "guo jiu" is mature, it can be bottled, heated to 65°C in hot water for 15 minutes, sealed when it is still hot, and have the mouth covered with a plastic envelop. If the "guo jiu" has higher alcohol content, or has a reservation unit of 80 or higher, it can be preserved without changes and does not require sterilization. It can be bottled and sealed directly.

5. Examples of "Guo Jiu" Fermentation

a. Citrus "jiu"

Citrus production is large. Citrus includes mandarin oranges, tangerine, oranges and lemon. Except for lemon that is sour, all the other citrus fruits are suitable for fermentation. The edible flesh in citrus fruits is about 65%, and their chemical compositions differ because of kinds, cultivars and maturity. As the fruit juice contains too little sugar (8–10%), and too much acid (about 1%), it must be adjusted for fermentation. The bitter substances (neohesperidin and naringin) in the citrus flesh must be removed during fermentation. The aromatic compounds in citrus fruits are very complex, and include water-soluble substances such as formic acid, acetaldehyde, ethanol, acetone, and esters of olefine alcholols (enols), phenyl acetic acid, formic acid, acetic acid and capric acid. Besides, small amount of the essential oils in the citrus peels (mainly capric and caprylic aldehydes) is mixed into the juice. The fruit flesh also contains water-soluble pigments such as anthocyanins and flavones, and fat-soluble carotenoids that are oxygenated derivatives and show a yellowish color. Fruit flesh is rich in vitamin A precursor and vitamin C.

Fermentation technology. Select citrus fruits (such as mandarin orange, tangerine and orange) that are ripe, not damaged, juicy and suitable for fermentation. The peels are removed, fruit flesh are crushed but avoid crushing the seeds (as citrus seeds contain the bitter substances naringin and limonin), and juice extracted. The juice extraction rate is about 70%, modified as needed, and sterilized or not sterilized before filling the fermentation containers. Add yeast at a rate of 5% and maintain the mixture at 20–25°C. After 12 hours, fermentation starts with the vigorous period in 5 days, and completes in 15 days. The mash can then be transferred to other containers for post-fermentation at 10–15°C for about 1 month. With the completed fermentation, the "jiu" is transferred for the second time, and according to needs, add citrus brandy and sugar to adjust the contents (for dry "jiu," sugar is not added), and proceed with the aging process for 6 months or longer. The product "jiu" is clear, transparent, golden yellow, with sweet and cool taste and characteristic citric aroma.

b. Lizhi (litchi, lychee) "jiu"

Lizhi is a typical Chinese fruit with fruit aroma like sweet osmanthus, and very juicy. The sugar content is on the high side (about 12–25%), and sugar-acid ratio is also suitable for fermentation with minimal modification. It is a better common fruit for fermentation. Lizhi "jiu" is rich in aroma, smooth and has good mouth feel. It should be pointed out that lizhi season is the hot summer. Lizhi has less tannins, and the risk in fermentation is higher. Special attention is needed.

Fermentation technology. Select thick fleshed, undamaged, and ripe fruits. After removal of shell and seeds, the juice is extracted, modified accordingly, sterilized with sulfur dioxide, filled into containers, and stirred evenly. After 6 hours of still standing, add lizhi shell previously cleaned and soaked in alcohol for 30 minutes. The amount is 1% of the fruit juice to increase the tannin in the fruit juice. At the same time, seed yeast slurry of 5% is added to initiate the fermentation. After 6 hours, fermentation starts, and enters the vigorous period in 24 hours. After 3–4 days, the fermentation slows down. The lizhi shell can be dragged out, and after about 7 days, the main fermentation is completed. The "jiu" is then transferred to other containers for post-fermentation.

The chemical composition of litchi varies considerably among varieties. In general, the fruit flesh is about 72% of the fruit, the juice recovery rate is about 80%, and the alcohol production rate is about 85%. For small schedule fermentation of "lizhi jiu," temperature control is more difficult, and spoilage is fairly common. From preliminary investigation, use of high sugar concentration (33%) for fermentation, or even better use of lizhi syrup to dissolve the sugar, and inoculation with high temperature-tolerant yeast can provide successful fermentation. With post-fermentation completed, the "jiu" does not require additional adjustment. The flavor of this kind of "lizhi jiu" is better than the "jiu" produced by fermentation followed by adjustment. This method is also suitable for preliminary preservation of peak season production of lizhi. That is, enough sugar is added to the lizhi flesh at a ratio of 1 to 0.5–1, and proceeds with natural fermentation. This can maintain the flavor, and this mixture can then be used for fermentation to produce "lizhi jiu."

c. Plum "jiu"

Plum has purplish red color and is juicy, with 8% sugars, and 1.2% acids. It is also suitable for "jiu" fermentation. The fermentation method can follow the technology with residues, or follow the technology with residues removed, and the latter is better. The uniqueness of this fermentation is that the juice has more acids, and they cannot be neutralized with alkaline. To avoid color change, the options are addition of sugar to adjust the acidity, or dilution of juice to modify the composition. The "guo jiu" produced has bright red color with suitable mouth feel and characteristic light aroma, light red grape wine (Figure 173.18).

d. Longan "jiu"

Longan is also called "dragon eye," and is also one of the typical Chinese fruits. The longan "jiu" produced has a light aroma and a prospective future. The chemical composition of longan varies with cultivars. In general, the fruit has about 55% flesh, 20% shell, and 25% seed. The juice rate is about 70%, with about 15% sugars. Acids are on the low side, (usually less than 0.3%), and have slightly too much nitrogen ammonia (about 0.1%). Special attention should be paid to these characteristics.

Fermentation technology. Basically it is similar to the technology for litchi "jiu." Because the juice rate is low and has more nitrogen ammonia, warm water in the same amount of the juice is added to the residual pulp, mixed and soaked for 1 hour for the second extraction. The two extractions are combined, and adjust for sugar and acid contents. Potassium dihydrogen phosphate (0.05%) and a suitable amount of sulfur dioxide should also be added before filling the fermentation rafters. After 6 hours of still standing, yeast slurry (5%) is added. Proceed with fermentation. For each (metric) ton of longan, 650 kg of longan "jiu" can be produced.

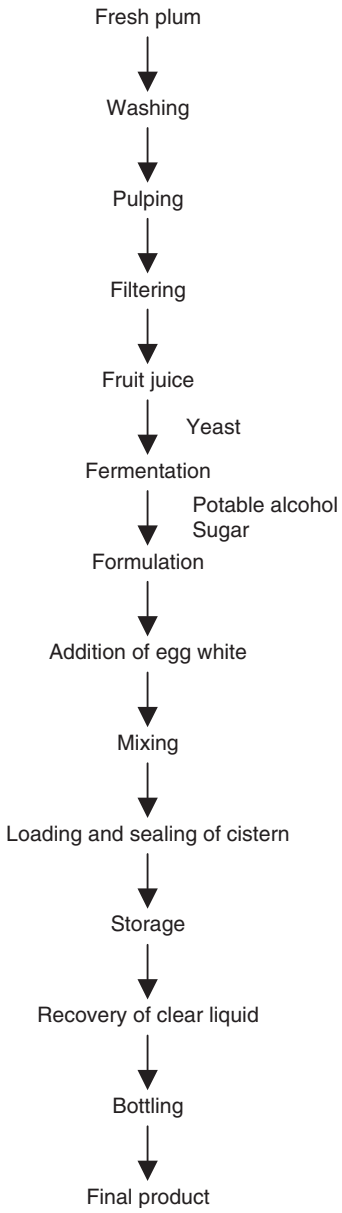

FIGURE 173.18 Generalized schematic flowchart for production of plum "jiu."

B. Chinese Grape Wines

1. Classification

There are numerous kinds of grape wines. There are four ways of classifying them:

- **Based on color.** Red grape wines are produced with grapes having pigments in their colored skins and/or flesh. They can be dark red, bright red, or ruby red. White grape wines are produced with grape juice from green or red grapes. Their colors can be either light yellow or golden yellow.

- **Based on sugar content.** According to the requirements established at the International Grape Wine Conference in Madrid, Spain in 1973, they can be categorized into:
  - sweet grape wine (sugar content higher than 5%);
  - semi-sweet wine (sugar content between 1.2–5.0%);
  - dry grape wine (sugar content less than 0.4%); and
  - semi-dry grape wine (sugar content between 0.4–1.2%).
- **Based on fermentation method.** Based on the fermentation methods, grape wines can be naturally fermented or enhanced. For natural fermented grape wines, they are produced using grape juice only without the addition of alcohol (brandy) or sugar. The enhanced fermentation utilizes addition of alcohol (or brandy) to increase the alcohol content (enhanced dry grape wine), or addition of alcohol and sugar to increase both the alcohol and sugar contents (enhanced sweet grape wine).
- **Based on the carbon dioxide content.** Based on the carbon dioxide content, grape wines can be categorized into still wines without any carbon dioxide or sparkling wines containing carbon dioxide. The latter is again divided into naturally sparkling (like the French champagne) or artificially adding carbon dioxide (generally called sparkling wine).

2. Fermentation of Red Grape Wine

Production of red grape wine can follow the technology on wine with residues. Its technology includes the following major steps:

- **Crushing and removal of stem.** Select good quality red grapes for the fermentation. Use a pulper to crush the grapes and remove the stems. The pulp is checked for sugars and acids, and adjusted accordingly, followed by addition of sulfur dioxide. The adjusted pulp is filled into fermentation tanks or barrels, about three-fourths deep to avoid overflow of the skins in the mash due to production of carbon dioxide.
- **Main fermentation.** Six hours after the filling the fermentation tanks, a 5–10% cultured yeast slurry is added, and sealed with bolts. Fermentation temperature is controlled at 20–25°C. If temperature rises above 33°C, cooling to lower the temperature is initiated. The fermenting mash is stirred regularly, and periodically checked for temperature, sugar and alcohol contents. Main fermentation usually takes 5–6 days at 25°C, 10–15 days at 20°C, with residual sugar of about 1%.
- **Post-fermentation.** After the main fermentation, the liquid "jiu' is immediately transferred to the fermentation rafters with proper bolting to conduct the post-fermentation. Post-fermentation is weak, and should be conducted at about 20°C. After 15–20 days, there should not be any more carbon dioxide released, and the sugar content is lowered to 0.1%. The post-fermentation is then completed.
- **Aging.** Three to five days after the post-fermentation, the residues in the fermenting mash should be removed by transferring fermentation rafters or barrels. During the transfer, the "jiu" is in contact with air. This allows the dissolved carbon dioxide to escape. After the removal of residue, the containers are again bolted to proceed with the aging process at temperature controlled between 10–15°C. The second transfer of fermentation containers should be conducted 1.5–2 months after post-fermentation, usually in early winter. At this point, the newly fermented "jiu" is basically clear and transparent. After this stage, the "jiu" is transferred to another container at the end of spring and early winter each year. The production rate of red grape wine from completely sound grapes is 80 kg per 100 kg of grapes: 70 kg from the original fluid "jiu," and 10 kg from pressing the residues.

3. Fermentation of White Grape Wines

Green grapes or red grapes with white flesh can be used for fermenting white grape wines. The fermentation technology usually follows that for fermentation with residues. Management of the fermentation is basically similar to red grape wine, but more precise. The technology is as follows:

- **Crushing, sprinkling with juice and pressing.** The grapes are crushed with a crusher, but the stems are not removed. Sprinkling with juice is generally conducted in a rotary sprinkler, the juice recovered is generally slightly cloudy with recovery rate of 50–55%. Pressing is usually conducted with a continuous presser.
- **Sulfiting, addition of tannins, and inoculation with yeast.** Grape juice recovered through the sprinkler and presser are combined for fermentation. Because green grape juice is deficient in tannins, when it is used for fermentation, 4–5 g of tannins per 100 liters of juice is added followed by immediate sulfiting, the sulfiting amount varies with the kinds of

juice available. If the juice is clearer, 25–30 g of sulfur dioxide per 100 liters of juice is needed and the treated juice is transferred to the fermentation tanks. Six hours after, 5% yeast slurry is added. For cloudy juice, it has to be clarified in a clarifying tank once before usage. For 100 liters of juice, 40–50 g sulfur dioxide is added and let stand for 24–48 hours of clarification. The cleared liquid is then transferred to the fermentation tanks with 8–10% yeast slurry added to proceed with the fermentation.

- **Main fermentation.** Fermentation is conducted in a closed system. Main fermentation starts 12–24 hours after the tanks are filled. Fermentation temperature is lower than that for red grape wine, at 18–20°C. Low temperature fermentation results with a product lighter in color with strong aroma. The main fermentation takes 10–15 days. With the peak fermentation (about 4–5 days after loading the tanks) passed, the container are bolted. When the fermenting mash shows a specific gravity of 1.005 to 1.006, the fermentation almost stops. The newly fermented liquid is transferred to another fermentation tank with removal of the sediment. The residue can be used for distillation of brandy.
- **Post-fermentation and aging.** The white grape wine transferred to the new tanks still has small amount of sugar, about 2–3%. The fermentation is continued until the specific gravity is dropped to 0.992–0.996 with a dry white grape wine essentially free of sugar. Post-fermentation takes about 20–30 days to complete. Its indicator, besides specific gravity, is its clarity. At this time, the white grape juice is transferred to another tank until full, and bolted to proceed with the aging process. When the weather is coldest, the "jiu" is transferred to another fermentation tank. Thereafter, the "jiu' is transferred to different tanks twice each year. The residues left from the old tanks and the turbid "jiu" can be used for distillation of brandy.

4. Fermentation of Sweet Grape Wines

In the fermentation of sweet red and white grape wines, the initial procedures are the same as that for dry red and white grape wines. When they are fermented to the required sugar contents, brandy or potable alcohol is added to increase the alcohol content to 16% or higher, and stop the fermentation. If the sugar content is not high enough, concentrated grape juice is added to adjust accordingly. For products with requirements that are not that specific, sugar or inverted sugar can also be used for adjustment.

One month after fermentation, the "jiu" is transferred to another fermentation tank for aging. The "jiu" is transferred again to a new tank at the end of spring next year. If the "jiu" is not clear, it can be clarified by a coagulation process with appropriate coagulant, and the aging process is then continued. Even though artificial accelerated aging is generally not used in dry grape wines, the alternate heat and cool process is used commonly in the aging of sweet grape wines to significantly increase the quality.

5. Famous Chinese Grape Wines

There are many grape wines nationally recognized in China and some received international awards. These are

- Yantai red grape wine: sweet, alcohol content 16 v%, acidity 0.7%, and sugar content 12%.
- Qingdao white grape wine: sweet, alcohol content 13 v%, and sugar content 12%.
- Beijing Chinese red grape wine: sweet, alcohol content 16 v%, and sugar content 12%.
- Minquan white grape wine: sweet, alcohol content 12 v%, sugar content 10%, total acidity 0.6%.
- Tianjing Wangchao white grape wine: semidry and semisweet, alcohol content 12 v%, sugar content 0.5–1.2%.
- Shacheng white grape wine: dry, alcohol content 12 v%, sugar content less than 0.4%.

C. Quality of "Guo Jiu"

1. Sensory Quality Standards of Grape Wines and "Guo Jiu"

- Appearance: clear, transparent, no sediment and suspended matter, no loss of luster.
- Color: bright, balanced, lustrous, no loss or change of color.
- Aroma: possess the original fruit's aroma, soft and balanced, long-lasting rich aroma, absence of objectional odor, typical characteristics.
- Taste: pure, completely balanced, smooth, clean, long-lasting, no objectional taste, typical characteristics.
- Characteristics: typical for the product.

The above standards are suggested for grape wines and "guo jiu." When all the criteria are met, the product is considered as having a full score.

2. Sanitary Standards of Grape Wines and "Guo Jiu"

Both grape wines and "guo jiu" are fermented products, their processes should meet the GB2758-81 Fermented "jiu" sanitary standards.

V. FORMULATED "JIU"

Formulated "jiu" includes essence "jiu," flavored "jiu," and Chinese herbal "jiu" or wines. Essence "jiu" is again subdivided into "fruit essence jiu" and "flower essence jiu." The former is produced by adding fruit juice and other ingredients to alcohol or "bai jiu;" the latter is produced by soaking flowers in alcohol or "bai jiu," or distillating flowers with alcohol or "bai jiu" and formulated.

A. Treatments of the Various Raw Materials

1. Alcohol and "Bai Jiu"

In the common alcohol, there are fixed amounts of methanol, fusel oils, aldehydes, and ketones forming undesirable odor and taste. They should be removed completely or reduced. Industrial ethanol must be purified to the potable level before use. Generally, the crude alcohol is treated with 0.01–0.05% potassium permanganate (predissolved in 10% solution), added slowly into the alcohol, and stirred thoroughly. The mixture is let stand overnight; 0.08–0.09% sodium hydroxide is then added before distillation. The beginning and ending distillates should be discarded, with beginning distillate being 7% of total alcohol, and ending distillate 8% of total alcohol. The middle section is retained for use in formulation. For a further purification, the alcohol collected can be treated by activated carbon to remove odor at a range of 100–200 g per 100 liters and left standing 36 hours before filtering. The so-treated alcohol must meet the GB 394-81 National Standard for Potable Alcohol. (Table 173.6)

Good quality "bai jiu" (especially brandy) does not require purification. Common "bai jiu" particularly those made from root crops and mollasses have to be purified. The method is the same as that for potable alcohol. However, because the alcohol content is lower (about 60 v%), the amounts of potassium permanganate and sodium hydroxide used should be less, about two-thirds.

2. Fruit Juice

Fresh fruits are washed, drip-dried, crushed and pressed to make the fruit juice. The fruits can also be cut into small pieces and soaked in water or "jiu," and then filtered. For dried fruits, they generally are cut into pieces, and soaked to extract the essence. The fresh or dried fruit pieces should not be too small to avoid cloudy infusion liquid. Fruit juices or extracts should be clarified before use.

3. Natural Spices

Rose flowers, clove, and mandarin orange or tangerine peels, et al. can be extracted with alcohol and distilled, or cooked in water to tender to form liquid essence.

4. Sugar and Organic Acids

Sugars and organic acids can be dissolved in water or alcohol to form solutions, or added directly. If the fruit juice has too much acids, avoid the use of neutralization, and use dilution method in the formulation.

5. Water

Distilled water or potable water that has been boiled and cooled.

6. Color(s)

Caramel or other colorings can be dissolved in small amount of alcohol to form a solution, and added accordingly.

TABLE 173.6
Standards for Potable Alcohol (Molecular Formula C_2H_5OH, M. W. 46.07) in China, GB 394-81

	Class		
Name of Index	Excellent	1st Class	2nd Class
Appearance	Transparent	Transparent	Transparent
Color, number	≤10	≤10	≤10
Odor	Absence of objectional odor	Absence of objectional odor	Absence of objectional odor
Alcohol, (%, v/v)	≥96.0	≥95.5	≥95
Sulfuric acid, number	≤10	≤15	≤100
Oxidation test, points	≥30	≥25	≥15
Aldehydes, % as acetaldehyde	≤0.0004	≤0.0010	≤0.0030
Fusel oils, % as isoamyl alcohol, isobutanol	≤0.0004	≤0.0025	≤0.010
Methanol, %	≤0.06	≤0.12	≤0.16
Acids, % as acetic acid	≤0.0015	≤0.0015	≤0.002
Esters, %, as ethyl acetyl ester	≤0.0025	≤0.0038	None
Nonvolatile matters, %	≤0.0020	≤0.0020	≤0.0025

Note: % = g/100 ml, except alcohol.

B. Formulation Methods

In the formulation of "fruit essence jiu" it is important to do preliminary formulation and sensory evaluation to determine the ratios of various ingredients and essence solution. After all the ingredients are mixed together to form a concentrate, it is left 1–2 days before filtration. Lastly, the pigment and essence solutions are added.

C. Examples of Production of "Fruit Essence Jiu"

1. "Lizhi (Litchi, Lychee) Essence Jiu"

- **Formula.** Lizhi juice 100 liters, and 90 v% alcohol 22 liter.
- **Procedure.** Select ripe lizhi fruits, remove the shell, and extract the juice. Twelve grams of sulfur dioxide per 100 kg fruit flesh can be added to prevent spoilage. For 100 liters of fruit juice, add 22 liters of alcohol. Mix thoroughly, store in sealed containers for two months, filter and pasteurize before bottling and capping.

Essence "jiu" of loquat, pineapple, mango, et al. can be produced the same way. If the juice is not sweet enough, sugar can be added accordingly. The "lizhi essence jiu" from Zhangzhou City, Fujian is of supreme quality.

2. "Logan Essence Jiu"

- **Formula.** Logan fruit flesh 500 g, "bai jiu" 1 kg.
- **Procedure.** After removing the shells and seeds, 500 g of the flesh is soaked in 1 kg of quality "bai jiu" in sealed containers. Recover the clear liquid after 15 days. The flavor is even better if aged. This "jiu" is known in Traditional Chinese Medicine as beneficial to the heart and spleen. It can calm oneself and cure insomnia, besides other claims.

3. "Loquat Essence Jiu"

- **Formula.** Loquat 350 g, vanilla beans 2 g, lemon peels half of a fruit, sugar 250 g, potable alcohol 300 g.
- **Procedure.** Put the loquat fruits and sugar in hot water (enough to cover), mash, and add the vanilla beans. When the mixture is cooled, pour into glass jugs, add alcohol and shredded lemon peels, and seal the containers. Share the containers regularly. After one month, pour the liquid into dark-colored bottles, seal and store for another 4 months. This "loquat essence jiu" helps digestion, sooths the throat and stops the coughing. Alcohol content is 40 v%. It is a good after-meal liqueur.

4. Green Plum "Jiu"

- **Formula.** Potable alcohol (65 v%), 27.75 liters, green plum essence 27.5 liter, 2.4 g, citric acid 15 g, glycerol 300 g, sugar 16 kg, a green pigment, distilled water enough to make up 100 liters.
- **Procedure.** The green plum essence is produced using the following procedure. Green plum or dried green plum 17.65 kg in a cloth bag together with 100 liters of 65 v% alcohol are put in a kettle-type distiller for reflex of 1.5 hours. The distillate of 50 v% is the "green plum essence." Distillate below 50 v% is stored for another distillation. After mixing 27.75 liters of potable alcohol and the "green plum essence," sugar is added followed by water to make syrup. Glycerol and pigment are then added. Enough distilled water is added to make 100 liters. After thorough mixing, it can be stored for 3 months, filtered and bottled.

5. Strawberry "Jiu"

- **Formula.** Strawberries 100 kg, potable alcohol, 60 kg, sucrose 6 kg, citric acid appropriate amount, and water.
- **Procedure.** Sound strawberry fruits that are bright red, ripe, with proper sweetness and sourness are used. Cultivars such as Shanghai, Yantai Jiguan (cock comb red), Baojiao Early Cultivar, et al., are suitable cultivars in China. The alcohol should be the residual distillate recovered from the production of "huang jiu" – "jiu han (sweat)." Its characteristic is its pure taste.

  Fresh fruits should be processed immediately to preserve its color and flavor. Strawberries, the "jiu han" and sugar are mixed with a ratio of 10:6:0.6. The alcohol content after pressure filtration should be controlled at about 20 v%. The filtered strawbery "jiu" is stored for a definite time to achieve the aging effect. The main components in the "jiu" such as alcohol, sugar, and acid must be prepared at the proper ratio. Except for addition of citric acid to adjust the acid concentration, other additions are not permitted to highlight the strawberry flavor, and natural color. Brown bottles should be used to prevent the color-fading effect of sunshine and strong light.

  This strawberry "jiu" has a light brown color, clear and transparent. Its has a strong strawberry aroma and also the pleasant "jiu" aroma. The taste is pure and soft, with proper sugar-acid ratio, pleasant mouth feel, and is absent from undesirable odor. The product has 16.5–17 v% alcohol, 8–8.5% sugars, and 0.45–0.50% total acidity.

D. Examples of "Flower Essence Jiu"

"Flower essence jiu" utilizes alcohol or "bai jiu" as the base; the flowers from various plants are soaked or distilled. The "flower essence jiu" is made after proper formulation. The various treatments to produce "flower essence jiu" are basically similar to "fruit essence jiu." The difference is that either fresh flowers or dried flowers are used as the aromatic materials.

1. "Meiguilu (Rose Essence Jiu)"

Rose flowers have been claimed to reduce heart pain, hyperchlolesterolemia, and chest pain, et al. Drinking of rose flower tea is said to help the complexion, strength the body, and stop diarrhea. "Meiguilu'' is a combination of the rose aroma and the "jiu" aroma, giving a product rich in rose aroma and long-lasting, and slightly sweet.

a. Production technology

- **Flower selection and picking.** There is a wide variation of color, size, and aroma in roses due to cultivar differences. In order to better guarantee the unique "Meiguilu" flavor, only cultivars with main pedals plump and red are used. The flowers are harvested in May, the peak flowering season, and processed. The flowers are harvested early in the morning when the morning fog has not disappeared, the flowers are just beginning to open and full of dews. These flowers have the richest aroma because the sun has not shone on them and evaporated their aroma.
- **Soaking of flowers.** Harvested flowers are sealed in containers and shipped back to the factory for processing. The flowers are spread in a cool, shaded area with good ventilation to lower the temperature and prevent the petals from getting soft and separating from the stem. The flowers are then mixed with "jiu" at a fixed ratio, mixed thoroughly and packed tightly in the porcelain storage containers and sealed with a plastic wrap, excluding air and preventing decay to occur. After several months of soaking, the "flower jiu" is transferred to smaller jugs and then sealed. They are stored for the next year's use.
- **Preparation of the original aroma source.** Distillation is conducted in a copper distiller. The completely "jiu"-soaked rose flowers are loaded into the distiller, heated slowly to boiling to completely extract the rose aromatic compounds. It is even better if the beginning and ending distillates are removed, with distillate maintained at 62 v% or above. The product will have a delicate, conservatively soft aroma with minimal undesirable odor.
- **Selection of basal alcohol.** It is very important to select the proper basal "jui" with quality so that the delicate rose flavor can be detected easily. It is better to use deodorized high quality potable alcohol or "bai jiu" made from cereals with clean aroma as the basal "jiu." "Jiu" with rich aroma or soy-paste aroma are not suitable because the delicate rose aroma can easily be covered by the rich cellar or soy-paste aroma, and not show off the typical rose aroma.
- **Formulation of various components.** Before formulation, calculations should be conducted based on the physiochemical indicators of the various components. Then follow the proper sequence of combining the various components together to formulate the final product. This proper sequence of combination is important as a different sequence can affect the physicochemical indices of the final product, especially the mouth feel and appearance.
- **Formulation procedures.** The basal "jiu" is pumped into the mixing chamber, then add the rose distillate and mix thoroughly. The sugar is dissolved in water in a double-jacketed steam kettle, cooked to boiling to form a 65% syrup. After filtering out the impurities, it is pumped to the mixing chamber to mix with the basal rose "jiu" with continuous stirring. Citric acid is formulated to a 50% solution with additional heating, and then added to the mixture. Distilled water is then added to make up the volume.
- **Storage and aging.** The various components in the newly formulated "Meiguilu" is not completed synchronized, with alcohol and undesirable odor. In order to give the "Meiguilu" a delicate aroma with smooth taste, it should be sealed and stored. Impurities in newly formulated "Meiguilu" will sediment in 7 days; and after three months of aging, the quality is stabilized.
- **Filtration.** When the storage period is completed, the "Meiguilu" is filtered through diatomaceous earth to clarify the product. The product should not be stored for a long time to prevent the contact with oxygen and oxidized to form extensive aged sensation. This is because the main aromatic components in rose aroma are the terpinenes. They can interact with the alcohols, aldehydes, acetones and form derivatives that are easily oxidized, and thus affect the aroma and taste of the product.

b. Quality standards

The final product should be a clear transparent liquid with pleasant rose aroma. The flower aroma and "jiu" aroma are synchronized. It should be slightly sweet with long-lasting aroma. Its physicochemical indicators are: alcohol $\geq 18 \pm 1\%$ or higher, total sugar $\leq 8 \pm 1$ g/100 ml, total acids (calculated as citric acid) ≤ 0.4 g/100 ml. The sanitary standard should meet the GB2758-81 standard of PRC.

2. "Yulan Xianglu (Magnolia Aroma) Jiu"

- **Formula.** Air dried magnola flower petals 100 g (fresh petal should be double), "gaoliang (sorghum) jiu" 5 kg, rock sugar small amount, glycerol small amount.
- **Procedure.** The magnolia petals are soaked in the basal "gaoliang jiu" overnight, and scooped out. They are steamed to extract the aroma by collecting the distillate. The rock sugar and glycerol are then added to taste. This intermediate product is sealed in containers for a few months to age. The final product has alcohol 41 v%, and sugar 3%. The color is light yellow, clear and transparent, slightly sweet, and has magnolia aroma and "jiu" aroma with a balanced soft aroma.

3. "Juhua (Chrysanthemum) Jiu"

- **Formula.** Chrysanthemum 80 g, sugar 40 g, "bai jiu" 650 g, honey small amount.
- **Procedure.** The chrysanthemum flowers are cleaned, drip-dried before putting into a container. Sugar, honey and "bai jiu" are added. The container is sealed and left in a cool and shady area. After three months, filter through cheesecloth, and store the filtrate for 15 days. This product has typical chrysanthemum's light aroma, and the taste is rich and slightly sweet. It is claimed that this "jiu" has certain curing effect for headache and dizziness, and is good for the eyes and stomach, improve appetite and relieve fatique.

4. "Guihua (Sweet Osmanthus) Jiu"

- **Formula.** Sweet osmanthus flowers 16 kg, 65 v% alcohol 330–350 kg, sugar 150 kg, citric acid 3 kg, glycerol 2 kg, distilled water enough to make up 1,000 liters.
- **Procedure.** Remove foreign matters from collected sweet osmanthus flowers. Add 32 kg 65 v% alcohol to the flowers, soak and seal the container for 7 days, filter, and soak the residue again with 20 kg alcohol for one day, then filter. Combine the two filtrates. The sugar is dissolved to make a 86 °Brix syrup. The citric acid is dissolved in 60°C hot water and is added together with the glycerol to the flower infusion. Mix thoroughly. Add the rest of the alcohol, filter and make up to 1,000 liters with distilled water. Store for three months. Filter again before bottling.

5. "Guihua (Sweet Osmanthus) Chen (Aged) Jiu"

- **Formula.** Good quality white grape wine 100 kg, sweet osmanthus flowers appropriate amount.
- **Procedure.** Aged "guihua jiu" utilizes aged white grape wine as the basal "jiu" with sweet osmanthus flowers as the flavoring. The sweet osmanthus flowers are of the common "jingui (golden sweet osmanthus)" cultivar from the Suzhou and Hanzhou areas, China. The fresh budding flowers are collected during the flowering season in November. The collected flowers after processing (rinsing and removal of foreign matters) are soaked in the aged white grape wine. With careful formulation fully balancing the aroma and taste to meet the established standard, the product has the required characteristics. The characteristics of this "Guihua chen jiu" are as follows: golden yellow, crystal clear, transparent, balanced fresh sweet osmanthus aroma and the rich aroma of white grape wine, slightly sweet and slightly sour, pleasant and aromatic to mouth feel with long-lasting aromatic taste. The product shows the unique sweet osmanthus aroma, with 15 v%, sugar 14%, and total acids 0.65 g/100 mg. This product contains glucose, fructose, organic acids, and various vitamins. The product is claimed to activate the blood circulation system, smooth muscles, increases calories, help digestion, increase appetite, and relief of fatique. It is a product that does not contain any medicinal herbs, but is a nutritious drink.

E. "Jiawei (Flavor-added) Jiu"

"Gawei jiu" utilizes alcohol, "bai jiu," and fruit wine as the basal "jiu" with the addition of Chinese herbs or seeds (dry nuts) as the aromatic material(s), and soaking of the aromatic materials to produce. It is one of the formulated "jiu." The treatment of the raw materials are basically similar to the fruit essence "jiu." These aromatic components give the products different flavor and claimed medical effects. They are unique.

1. Yantai Weimeisi (Vermouth) Jiu"

"Yantai Weimeisi jiu" is produced by the Zhangyu Grape Wine Company in Yantai, Shandong Province, China. In the early 20th century, it was called "Qiongyaojiang." It won the Golden Medal Award in the World Expo in Panama, in 1915. The name was later changed to "Weimeisi (vermouth)." It has been known as a famous "jiu" in China for a long time. Since 1952, it was named as a "National Famous Jiu" many times in the "Jiu" Evaluation Conferences. Besides its national first–class quality, its characteristics are also recognized worldwide as a Chinese vermouth, and enjoys an international reputation.

Chinese "Weimeisi jiu" utilizes superior grape cultivars such as "Longan," "Baiya," "Guirenxiang," "General Li," "Baiyu" to extract the juices for the production of grape wine. This grape wine is the basal "jiu," has to be stored for two years before formulated with valuable Chinese herb extracts such as "honghua (Carthamus tinctorius L.)," "doukou (Amomum cardamomum L. or Alpinia katsumadai Hayata)," cinnamon, clove, "dahuang (Rhei Rhizoma, Rheum palmatum L., R. tanguticum Maxim. Ex Reg., or R. officinale Baill)," "baichi (Angelicae Radix, Angelica dahurica Benth et Hook, A. anomala Lallem, or A. taiwaniana Boiss)," "longdanchao (Gentianae Radix, Gentiana scabra Bunge, G. triflora, or G. manshurica Kitag.)," chrysanthemum, et al. The extracts are prepared individually by soaking and extraction to ensure the high-quality flavor, and the continuity of the product.

"Yantai Weimeisi jiu" is dark brown in color, clear, transparent, lustrous, has a balanced aroma from plants, "jiu," and fruits, and the taste is sweet, slightly sour, and slightly bitter that are balanced and unique. The alcohol is 18 v%, with sugar 15%. This "jiu" is claimed to be appetizing, strengthens the digestive system, drives off the "wind" (Traditional Chinese Medicine terminology) and the cold, relaxes the tendons, activates the blood circulation system, and strengthens the blood and "qi (vital energy)." It is promoted as a healthy drink, and good for longevity.

2. "Zhuyeqing (Green Bamboo Leaf) Jiu"

"Zhuyeqing jiu" is one of the famous "jiu" produced in the Fen Jiu Factory, Xinghua Village, Fenyang County, Shanxi Province, China. It is an old and common "jiu" in China. It is also produced in many provinces and cities in China. It was also mentioned frequently in old Chinese literature. However, the one produced in Fen Jiu Factory of Shanxi Province enjoyed the best reputation from the old days, like the "Fen jiu." Since 1962, it has continuously won the Golden Award at the National "Jiu" Evaluation Conference, and is one of the famous "jiu" titles in China.

Before the Tang and Song Dynasties, "Zhuyeqing jiu" was produced in various places in China using "huangjiu" as the basal "jiu." The "Zhuyeqing jiu" produced in Xinghua Village, Shanxi Province utilizes its "Fen jiu" as the basal "jiu." Fresh bamboo leaves from southern China are chosen, with the addition of Chinese herbs: mandarin orange peels, male clove flowers, "muxiang (Saussurea lappa Clarke)," "tanxiang (sandal wood, Santalum album L.)," "linglingxiang" (basil, Ocimum basilicum), "zhizi (Gardenia jasminoides Ellis), "shannai (Kaempferia galanga Linn.), "danggui [Angelica sinensis (Oliv.) Diels.]," and "sharen (Amomum villosum Lour., A. longiligulare T. L., or A. xanthiodes Wall), and rock sugar. They are soaked together in the "fen jiu," and processed scientifically.

This "jiu" can be consumed independently, or as a health tonic to improve body function. "Zhuyeqing jiu" is light yellow with a green shade, clear, transparent and lustrous. It has unique aroma and "jiu" aroma, comfortable and balanced. Its taste is softly sweet, and long-lasting. It is said to be exceptional in color, aroma and taste. It has alcohol 45 v%, and sugar 10%. This "jiu" is claimed to balance the body organs, remove obstruction in "qi," support the blood system, calm the heat, reduce inflammation, detoxify, be diuretic, nourish the liver, and strengthen the digestive system. It is also claimed to be beneficial to cardiovascular disease, hypertension, and arthritis.

3. "Yuanlinqing Jiu"

"Yuanlinqing" is a product produced by the Yuanlinqing Jiu Factory, Qianjiang City, Hubei Province. It is another national famous "jiu." "Yuanlinqing jiu" utilizes sorghum as the raw material to produce a quality, lightly aromatic "Kongming daqu jiu" as the basal "jiu." Precious Chinese herbs such as "tanxiang" (sandal wood, Santalum album L.), clove, "sharen" (Amomum villosum Lour., A. longiligulare T. L., or A. xanthiodes Wall), "danggui" [Angelica sinensis (Oliv.) Diels.]," "zhizi" (Gardenia jasminoides Ellis), and other herbs, together with sugar are used to formulate this "jiu." This "jiu" combines "jiu," herbs, and sugar into one liquid, and at the same time combining the color, aroma, and taste together. Consuming it before meals can increase appetite, and consuming it after meals can increase the digestion. It is claimed that long-term consumption at suitable quantities can nourish the liver and lung, strengthen the digestive system and the body, supplement the "qi" and vitalize the blood. "Yuanlinqing jiu" has golden yellow color with a shade of green. It is clear and transparent. Its taste is a balance of the herb aroma and the "jiu" aroma; delicate, richly sweet and long-lasting, smooth and clear to the mouth, and overall long-lasting taste after consumption. It has 39 v% alcohol.

4. "Yinxing (Gingko Nut) Jiu"

- **Formulation.** Gingko nut (shell removed), 15 kg, "Hancai" (Rorippa montan) 22.5 kg.
- **Procedure.** Soak the crushed gingko nut in 65% alcohol for 24 hours, and then drip off the infusion at a rate of 5 ml per kg per minute. The ethanol is recovered for later use. The "Hancai" is cooked in water twice for 2 hours each time. The liquid is combined, filtered and concentrated to a ratio of 1:2. After cooling, alcohol is added, mixed thoroughly, rested for 24 hours and the clear liquid at the top is filtered out. Alcohol is recovered from the filtrate for later use. The filtrate is combined with the gingko nut infusion, mixed thoroughly and let stand for 3 days to precipitate the residues before filtration. The filtrate is saved for later use. Ten kg sugar is dissolved to form a syrup. Together with a suitable amount of menthol, almond essence oil, preservative and alcohol are added to the filtrate. Add hot water to a volume of 31,500 ml. The product has a specific gravity of 1.15–1.18. This product is claimed to suppress coughing, dissolve phlegm, and stop asthma. It is used in acute or chronic bronchitis, inefficient removal of phlegm, and long-lasting coughing and asthma in Traditional Chinese Medicine.

F. Chinese Herbal Wines

Chinese herbal wines have a very long history. It was mentioned in one of the oldest classics in Traditional Chinese Medicine, "The Neijing Suwan," as a solvent for medications. In Traditional Chinese Medicine, alcohol was believed to improve blood circulation, enhance the effect of medications, warm the intestine and stomach, and overcome the "wind" and "coldness." Thus, herbal wines have been viewed as important remedies by Traditional Chinese Medicine practitioners, and also enjoyed by consumers. The formulations for herbal wines are included in the many Traditional Chinese Medicine literatures, or handed down as folklore. It is not the intention of this chapter to have an extensive coverage of Chinese herbal wines, and readers should refer to references in this area for further information. However, a brief introduction of the topic with a few common examples is described below.

Chinese herbal wines can be viewed as a subcategory of formulated wines, as their preparations are very similar. The active compounds in the herbs are extracted by soaking them in the basal wine. The tincture is then recovered accordingly by filtering out the liquid and discarding the solid herbs. Sometimes, the herbs are extracted for a second time to extract the residual active components. The basal wine can be the "bai jiu" (distilled spirits), "huang (yellow) jiu" (rice wines), or fruit wines. The herbs used vary extensively among the various herbal wines, from one herb to ten or more herbs (see below) depending on the usage or effect expected. They can be tonics, remedial, or just for pleasure in nature. However, it is important to understand the nature of the formulations and match the herbal wines with the consumers' body chemistry, as the wrong match can have the negative effect, and may be harmful.

Chinese herbal wines can be sub-divided into different groups based on their intended usage, such as those used in internal medicine, and for treatment of obstetrics-gynecology, dermatitis, tumors, orthodonics, artheritis, various organs, OB, and tonics. The herbs selected are similar to those commonly used in Traditional Chinese Medicine formulations. Readers interested in this topic should consult The English-Chinese Encyclopeida of Practical Traditional Chinese Medicine, Vol. 9 Maintaining Your Health edited by Xu Xiangcai et al. (1991), and other references on Chinese Herb Formulations available elsewhere.

Several Chinese herbal tonic wines have been produced commercially for decades, such as the "Wujiapei Jiu (or Wu Chia Pi Chiew)," "Ginseng Wine," and the "Shiquangdaibu Jiu (Or, Sze Chuan Dah Poo Chiew, Ten complete herbs big tonic)." "Wujiapei Jiu" contains the herb "Wujiapei" (Acanthopanax bark, Acanthopanacis Cortex, Acanthopanax senticosus, A. gracilistylus, or A. giradii); Ginseng Jiu contains the herb ginseng; and the Shiquangdaibu Jiu contains ten herbs: Radix Ginseng (ren shen), Rhizoma Atractylodis Macrocephalae (bai zhu), Sclerotium Poriae Cocos (fu ling), Honey-fried Radix Glycerrhizae Uralensis (zhi gan cao), Radix Rhemanniae Glutinosae Conquitae (shu di huang), Radix Paeoniae Lactiflorae (bai shao), Radix Angelicae Sinensis (dang gui), Radix Ligustici Chuengxiong (chuan xiong), Cortex Cinnamomi Cassiae (rou gui), Radix Astragali Membranacei (huang qi).

Others are produced as needed according to the practitioner's guidance for special treatments. These are usually not produced commercially as the formulation is designed for individual patient's condition, even though some formulations have been available in the literature. Some consumers also produce their own herbal wines for their own consumption.

VI. "JIU" FROM THE MINORITY GROUPS IN CHINA

China is a multi-racial country with over 50 ethnic groups, and each ethnic group has its own customs and a long history of "jiu" culture. For example, in Mongolia, it is customary to have the whole family sitting in their tents at the end of the year and drink through the night with "manai-jiu (koumiss)" being their favorite. In Tibet, before their New Year, it is customary for every family to brew the

"qingkejiu (jiu made from a kind of wheat in Tibet, *Avena nuda* L.)." This "qingkejiu" is slightly yellow in color, sweet and sour, and the alcohol content is not high, like the "huang jiu," and is called by their foreign guests the "Tibetan beer." The other minority groups also have their own customs and "jiu" in their culture. In general, every minority ethnic group loves to make and consume "jiu." After all these centuries, there are considerable valuable experiences in their fermentation technology with abundant contents. However, the knowledge is only known at the layman's level, and lacks a systematic organization. Some of the products are not commercialized but only made at the household level for consumption. The following are some better known examples.

A. "Ru Jiu (Milk Wine, Kefir)" and "Marujiu (Mare's Milk Wine, Komuiss)" of the Mongolian Ethnic Group

"Ru (milk) jiu" is a group of fermented, alcoholic liquid milks. It has its unique position between fermented milks and alcoholic beverages. The common examples are "kefir" in the Mongolian Self-governing District and "koumiss" in the Xinjiang Weiwuer Self-governing District in China. These alcoholic fermented milks contain small amounts of alcohol, and can facilitate metabolism, and adjust the circulation and nerve systems. They have special physiological functions. In the rehabilitation hospitals of the former Soviet Union, these alcoholic fermented milks are used extensively in the treatment of gastrointestinal diseases, diabetes, hypertension, and cardiovascular diseases. In the Xilinhaote City of Inner Mongolia, there were also factories specially built for the industrial production of alcoholic fermented milks to meet the rehabilitation, dietary therapy and tourism industries.

In fact, kefir is a general name for "ru jiu" (milk wines), it can be made either from cow's, goat's or mare's milk; with the addition of kefir grains, the kefir grains will conduct a special fermentation to form the alcoholic fermented milk. Kefir originates in Europe, and has a long history. In Japan, commercial production of kefir has already been achieved. Initially, the alcohol content in kefir reached 1.2%; however, with the modern technology, the alcohol content can be reduced to 0.01–0.05%. In China, there is no commercial production of kefir, and most of the kefir is produced by the herbsman, and is consumed as they are produced. It is basically a homemade drink. Therefore, with inheritance of the ancestor's valuable experience, the development and utilization of kefir has a prospective market in the future. The modern production of kefir is as follows:

1. Microorganisms in the Kefir Grains

Kefir is produced through the fermentation of kefir grains. The key is in the kefir grains. There are many kinds of microorganisms in kefir grains with the presence of the following microorganisms being known: lactostreptococcus, lactobacillus, yeast, leuconostoc bacteria, and acetic acid bacteria. There exists a very complex relationship among these microorganisms. Under the condition of digesting the peptone with the formation of vitamins, the yeast and the acetic acid bacteria activate the lactic acid bacteria; and only under the presence of lactic acid bacteria, the non-lactose dependent fermentative yeast and acetic acid bacteria can grow. With the large amount of lactic acid accumulated, it provides a favorable environment for the yeast and acetic acid bacteria to grow. When the fermentative lactic acid bacteria is inhibited, the accumulation of alcohol from yeast also decreases.

2. Preparation of the Kefir Grains

The kefir grains from commercial suppliers usually are freeze-dried products. They have irregular shapes and sizes, some as large as walnuts, and the small ones as small as wheat kernels. They show a yellow color with some individual ones being white and creamy in color. A fixed amount of kefir grains is soaked in sterilized saline solution at a ratio of 1:10, and incubated at 20°C for 5 hours. The then expanded kefir grains are filtered through metal sieves and rinsed with sterile water. The rinsed kefir grains are then inoculated into sterilized milk at a ratio of 1:15, and incubated for 24 hours at 25°C, until grains are formed. The kefir grains are then sieved again, rinsed, and are incubated again (sometimes requiring several inoculations). Kefir grains from the last incubation are preserved for later use, and the filtrate is used for the inoculum for the production of kefir.

3. Procedures for Kefir Production

Good quality milk must be used as the raw material for kefir production. The raw material is generally not standardized. First, the milk is heated to 70°C, homogenized at 15 MPa pressure. The homogenized milk is then heated to 95°C for 10–15 minutes. The purposes for the heat treatment are: sterilize harmful microorganisms, and denature all the serum albumin. The denatured serum albumin has a high water affinity. This avoids the separation of serum in the product, and the viscosity can also be increased. Ultrahigh temperature can only denature the serum albumin partially, and therefore is not applicable in this situation. After heat treatment, the milk is cooled to 22–25°C. Inoculation fluid at the rate of 3–5% is added and the mixture is incubated at 22–23°C for 8–12 hours. When the total acidity reaches to 90–100 T, the mixture is shared, and slowly cooled for 10–12 hours to 4–6°C. The product can then be packed and stored at low temperature.

The main products at the end of kefir fermentation are lactic acid, ethanol, and carbon dioxide. There are also traces of acetaldehyde, diacetyl, and acetone. Kefir is

generally viscous, with a certain amount of bubbles; the product has a fresh sour taste and the light aroma of yeast. This product is suitable for lactose intolerant consumers, at the same time, prevents constipation and increases appetite. The most valuable component in kefir is the presence of 0.2–0.7% kefiran that can stimulate protein digestion in the human body.

Strictly speaking, kefir is made from cow's milk, and koumiss is made from hare's milk, and the lactose content in hare's milk is higher. Another alternative is to use skim milk with addition of serum-type lactose to increase the total solids. After fermentation, because it is also the action of lactic acid bacteria and lactose fermenting yeast, the product has lactic acid 0.8–1.0%, and alcohol about 2%, as compared to 0.6–0.9% lactic acid and about 1% alcohol if cow's milk is used as the raw material.

B. "Jiangmijiu" [Tianjiuniang (Sweet Jiu Fermentation)]

1. Formulation

Good quality "jiangmi (glutinous rice)" 1.5 kg, "tian jiu yao" (fermenting agent) 1 piece, and a small amount of sweet ormanthus essence.

2. Procedures

- The "jiangmi" is cleaned from foreign matters, soaked in a container for 24 hours, scooped out and washed cleanly.
- The washed clean "jiangmi" is flatly loaded onto a steamer lined with a piece of cloth. The rice is steamed to completely cooked using high heat.
- The cooked rice is sprinkled with water from deep wells once. If the temperature of the cooked rice is too high, it can be sprinkled again to lower the temperature. When the temperature of cooked rice is lowered to about 30°C, the rice is transferred to another clean wide-mouthed ceramic container.
- The "tian jiu yao" is ground to a fine powder, and sprinkled on the cooked rice. The rice is then pressed firmly, with a deep trough in the center. The sweet osmanthus essence is sprinkled on the trough, and then some "tian jiu yao." Lastly, some cooled, boiled water is sprinkled on it. The container cover is put on, and the container is wrapped with a cotton comforter, and left in a warm place. In the northern provinces, they can be put on the warming plates to increase the temperature, and shorten the fermentation time. After 2–3 days, the fermentation is mature. In the southern provinces, the wrapped containers can be stored by the side of cooking stoves.

3. Product Characteristics

The rice kernels are soft, and sweet with rich "jiu" aroma. When it is consumed, it should not have sour taste; instead, it should have a sweet aromatic taste. The product can be consumed as such; it is clean, cool, and sweet, especially good in the summer time. In the summer time, it can also be mixed with mung bean soup, refrigerated and consumed as a cold dessert. It has a clean aroma. This "jiangmijiu" can also be mixed with wheat flour to make cakes or steamed buns with pleasant "jiu" flavor. It is also commonly consumed boiled with eggs poached in it; this way of consumption is claimed to be good for the lactating mother with ample milk excreted. In the minority ethnic groups, it is common to treat honored guests with "jiangmijiu" as snacks.

C. "Chongyang Jiu" in the Dong Minority Ethnic Group

The "chongyang jiu" in the Dong Minority Group is basically made in the same way as the "jiangmijiu." Using glutinous rice, it is fermented in the ninth month of the lunar calendar and consumed in the new year (lunar calendar), thus the name "chongyang" meaning the ninth day in the ninth month. The only exception is that it is aged in a warm environment for extended fermentation for about 4 months. Its characteristics are that the product is very viscous and forms threads when scooping out, even sweeter than honey, rich in "jiu" aroma and pleasant to mouth feel. For consumers with small alcohol capacity, it is easy to get drunk by not noticing its potency.

D. "Zhubei (Bamboo Cup) Shui (Water) Jiu" in the LiSu Minority Ethnic Group

Both males and females in the Lisu Minority Ethnic Group enjoy drinking the "shui jiu." It has its unique way of manufacturing and consumption. "Shui jiu" is made in every family in the ninth and tenth months (lunar calendar) after the crops are harvested.

- **Formula.** Raw materials include corn, sorghum, barnyard grass or others. It is best made with barnyard grass.
- **Procedure.** The raw materials are crushed, steamed, and inoculated with "jiu yao." They are filled into containers, sealed and stored for fermentation. After a few score of days, they can be opened and diluted before consumption.
- **Method of consumption.** After the fermentation container is opened, place a cooking pot on a fire, add the pre-heated warm water in the pot. The contents from the fermentation container are scooped out, put into the warm water in the

cooking pot, and stirred briefly. The host first tastes the "shui jiu" for temperature and flavor. If the "shuijiu" is acceptable, it is filtered and filled into specially made "zhubei (bamboo cups)" and served to the guest(s). When one pot is consumed, the residue can be repeated 2–3 times. This product is sweet and rich in taste with pleasant aroma; alcohol content is not high, and it is clean to the mouth feel.

E. "Mageli Jiu" of the Korean Minority Ethnic Group

The manufacturing process for "Mageli jiu" is similar to that for the "tianjiuniang" described earlier. It is a homemade product using regular rice as the raw material in the families of the Korean Minority Ethnic Group. The product is light yellow, sweet with aroma, alcohol content lower than regular "baijiu," but higher than beer.

F. "Xiaobo (Small Bowl) Guihua (Sweet Ormanthus) Jiuniang"

This "xiaobao guihua juiniang" is made similar to the "tianjiuniang" described earlier except that it is made in small bowls and the sweet ormanthus is added later.

G. "Qingke (a Kind of Wheat in Tibet, *Avena nuda* L.) Jiu" in the Tibet and Qinghai Minority Ethnic Groups

- **Formula.** "Qingke" 500 g and suitable amount of ferment "qu."
- **Procedure.** The "qingke" is washed clean. After it is cooked, the "jiu qu" is added and mixed, allowing for saccharification to proceed and form the sweet mash (product). Fermentation temperature should be maintained at 15–18°C; too high a temperature will lead to production of too much acid, and too low a temperature will lead to flat taste product that is not sweet. It is important to cook the "qingke" thoroughly and soft, the cooked "qingke" should look like fruit flesh with a light "jiu" aroma.
- **Product characteristics.** The sweet mash is the final product. It is sweet and thick, with yellow color, and the aroma is light and sweet. It is appetizing and helps digestion, with rich highlander's flavor. In Qinghai province, the product is consumed as a common homemade product; however, the pressed filtrate is what the Tibetans will drink regularly, it is sweet and soothing.

H. Quality Standard of "Jiu" from the Minority Ethnic Groups

At this time, there is no national standards for 'jiu" from the Minority Ethnic Groups. However, the sanitary standard must follow the GB2757-81 (see Table 173.1) for distilled "jiu" and "formulated "jiu," or meet the GB2758-81 (see Table 173.4) for fermented "jiu."

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174 The Tailoring of Designer Grapevines and Microbial Starter Strains for a Market-Directed and Quality-Focused Wine Industry

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CONTENTS

I. INTRODUCTION

It is the nature of change that big events erupt suddenly and noisily, grab the headlines and shake the world. Changes that often have more impact on mankind's long-term future tend to take place much more slowly and quietly. It is in our nature to pay too much attention to short-term events and tentative viewpoints; larger, seismic shifts happen on a scale too great for us to easily bring into focus. Futurists concur that the world at the start of the third millennium is on the verge of an era of massive and unprecedented change, change so dynamic and far-reaching as to make many facets of society virtually unrecognisable during the next few decades. We are already starting to feel tremors from what will be tectonic transformations. In this context, we might remind ourselves of the adage 'the future belongs to those who prepare for it.'

In an attempt to establish a compass with which the wine industry can navigate through the enormous challenges of the 21st century, a number of major wine-producing countries have made in-depth analyses of global trends in the wine business environment, and have begun to plan for the most probable scenarios. With the threat of growing wine surpluses hovering ominously, they have formulated visions and put together long-term strategies to deal with the confusing fog of technological innovations and shifting consumer preferences, recognising the consequences, both for their industry and their countries, of failure to adjust.

Change in the wine industry is not new. The fundamental principles of vineyard cultivation and vinification have remained largely unaltered over the past 7000 years, while the role of wine has changed completely. At the start of the 17th century, wine was regarded as a practical beverage, wholesome and uniquely storable. Today, consumers consider wine to be a luxury product associated with a convivial lifestyle. This dramatic shift in image and function has placed wine in the center of a high voltage field between the forces of *market pull* and *technology push*. Here tradition and innovation must become properly polarized to attract rather than repel each other to meet the demands of wine producers and the preferences of wine consumers. On the one hand, the continued existence and welfare of the producer is directly dependent on the sustainable profitability of the wine industry; on the other, the increasingly health- and environment-conscious wine consumer is looking for a diverse range of quality products at the right price. The weight is increasingly moving to the side of demanding consumer preferences, and this places the producer under pressure.

There is an increasing gap between wine production and wine consumption in today's competitive, global economic system. This mismatch of demand and supply is exacerbated by two major factors: a worldwide decrease in the consumption of alcoholic beverages, and a rapid expansion of wine production in the New World countries (Argentina, Australia, Canada, Chile, New Zealand, South Africa, USA, etc.). These countries, which now produce about 20% of the world's wine, have been quick to respond to market forces and shifting perceptions of wine quality. As a result they have gained significant market share in the past few decades, moving from 2 to 15% of the world export market (1). During the same period, the per capita consumption of wine in the Old World producing countries (France, Italy, Spain, Portugal, Germany, etc.), which now produce slightly more than half of all the world's wine, has fallen by 40 to 50% (1). There is now a fundamental realignment of wine-producing countries around the global wine league table. Leading traditional, *production-oriented* wine industries need to transform themselves into *market-driven* enterprises.

Approximately 27 billion liters of wine are produced annually from about 8 million hectares of vineyard throughout the world (2–4). This is about 5000 million liters more than the market can absorb, and the surplus, which fluctuates between 15 and 20% of the total production, has given rise to keen competition for market share (1). This excess is concentrated in the basic bulk wine category, an indication that there has been a significant shift in consumer preference from basic commodity or 'economy' wines to premium, super-premium, ultra-premium and even icon wines. Globalization and the information explosion have given today's consumers a far more sophisticated understanding of product quality and value, and they have high expectations in all price categories. The demand for artisan wines, currently only 2–3% of the market (5), is growing, yet another indication, if one is needed, that the market is driven by consumer choice, not by producers, and surpluses are nothing more than bad management. In the wine industry, gone are the days typified by Henry Ford's famous quote 'you can have any color you want as long as it is black' (or in the case of wine, white or red).

The marketplace for today's wine industry is image conscious and price sensitive; the rules have changed to

the degree that *quality* is defined as *sustainable customer and consumer satisfaction* (1). It is a market that demands products with strong brand names and continuous renewal with regard to wine style, quality, purity, uniqueness and diversity. The successful and enterprising winemaker has become consumer driven and customer responsive, striving for products of outstanding quality that can be offered at every price point. Innovation at all levels of the total value chain is no longer an option for the wine industry, but a necessity; it is the only route to the consumers, who relentlessly vote with their wallets. A profitable and socially responsible wine industry will make a total commitment to quality, style and innovation from the vineyard to the consumer's palate.

It is clear that technological innovation is one of the cornerstones on which the successful wine industries of the 21st century will build toward global influence and sustainable profitability. Market-oriented, biotechnological innovation will focus on overcoming problems in the wine industry and creating and realizing new, strategically important opportunities. Biotechnological innovations can harmonize meaningfully with a complex of market, cultural, social, environmental and technical factors, without stripping the ancient art of winemaking of its charming mysticism and romanticism.

Despite the current scepticism of some consumer groups about genetically modified organisms (GMOs) and GM products, there is no doubt that the application of gene technology in the wine industry holds great potential. To be technologically equal to the tremendous consumer challenge, the world's pacesetting wine industries increasingly are focusing on the genetic programming and improvement of the three main organisms involved in the production of wine, namely grapevine, wine yeast and malolactic bacteria.

This overview highlights the most important examples of the way in which *Vitis vinifera* grape varieties, *Saccharomyces cerevisiae* wine yeast strains and *Oenococcus oeni* malolactic bacterial strains are currently being designed with surgical precision to meet market demand for the cost-effective, sustainable and environmentally friendly production of healthy, top-quality grapes and wine.

II. THE POTENTIAL OF GENETICALLY IMPROVED GRAPEVINE CULTIVARS

A. Grapevine Species and Cultivars

Economically, the grapevine constitutes the most important fruit species globally and has been linked to agricultural and religious activities in the earliest writings and chronicles. This ancient species has evolved over the ages from a bushy, sun-loving plant to a trailing climber. The grapevine has been domesticated with ease, giving rise to approximately 8 million hectares of intensely pruned and manicured grapevines that are typical of vineyards across the world (3,4).

Grapevines are classified into the genus *Vitis*, consisting of two sub-genera, *Euvitis* and *Muscadinia*, of which the former comprises the bulk of the *Vitis* species. A single *Vitis* species, *Vitis vinifera*, originated in Europe, whereas more than 30 species are native to China and a further ~34 species have been characterized in North and Central America. The scientific record of the origin of grapevine cultivars is at best rather fragmentary, but it is generally accepted that *V. vinifera* (the most cultivated *Vitis* species) comprises approximately 5000 true cultivars used in the wine, table and dried-grape industries of the world (6). Improvements to these cultivars initially relied largely on arbitrary selections of natural mutations that enhanced cultivation or some aspect of fruit and/or wine quality and were later followed by the more directed, clonal selection schemes. Grapevine improvement has curiously been "untouched" by classical breeding programs, in the sense that relatively few new cultivars became commercial successes, especially in the wine industry, where a few select and ancient cultivars are relied on for commercial production. However, breeding programs significantly impacted on the development of rootstock varieties resistant to soil-borne pests and pathogens, as well as to negative abiotic conditions (7).

When cultivar improvement is considered, the table/dried-grape and wine industries have different goals. The former industries market their products directly and have to provide the consumer with new, exciting and excellent-quality products, whereas the wine industry typically relies on established varietal names and predictable wine styles to sell its products. Genetic transformation technology has been heralded as having high potential in grapevine improvement programs in all three of these industries. Some of the advantages linked to this technology and its application in grapevine production will be discussed in the following sections.

B. Genetic Features and Techniques for the Analysis and Development of Grapevine Varieties

The fact that several plant genomes have been fully sequenced and that genome-wide, proteomic and metabolomic analyses are becoming more accessible confirms that a more advanced phase of plant improvement through molecular biology and genetic transformation is dawning. The accessibility of the grapevine genome in terms of molecular biology applications is currently much more restricted than that of wine yeast due to the size of the genome (ca. 470–483 mb divided into 38–40 chromosomes) and its complexity (only 4% of the

genome is transcribed) (4). The grapevine genome, however, is currently targeted for intense study, with multinational consortia collaborating in several initiatives to render molecular markers as well as the complete sequencing of the *Vitis* genome (8).

The technology to add genes of interest under the control of regulatory elements of choice into plant genomes has opened up various possibilities for plant improvement, and a wide range of economically important plant species have been targeted in this regard. *Agrobacterium*-mediated and biolistic bombardment technologies have ensured that an ever-growing list of plant species are accessible for genetic transformation. Noteworthy, however, is the fact that the grapevine has been considered recalcitrant to genetic transformation due to various difficulties, amongst others in obtaining regenerable tissue-culture systems that can withstand *Agrobacterium* or biolistic transformation and the subsequent selection regimes (3,4,9).

The first significant progress was made when embryonic cell lines were used as target tissue for grapevine transformations, leading to several laboratories (both private and public) routinely producing transgenic grapevines. In this regard, the term ''routinely'' includes only a few commercially important grapevine scion and rootstock cultivars. Since 1989, when the first successful grapevine transformations were performed, the focus has gradually shifted from the development of grapevine transformation technology to the implementation of the technology in the generation of useful plant lines.

C. Targets for the Genetic Improvement of Grapevine Varieties

Integrally linked to the prospect of rendering genetically improved grapevines is the use of molecular biology to study the fundamental processes in grapevine that underpin the physiological responses targeted for improvement. Initially, when limited genetic resources were available, genes with known function were introduced into plant species in the hope of developing ''improved'' phenotypes. This shotgun approach has taught us valuable lessons, especially about complications regarding transgene silencing, but has also proven that true and sustainable progress can only be made when basic knowledge is combined with application. Grapevine transformations are co-entering an exciting era with the plant sciences, as a growing list of genes and their regulatory sequences are becoming available from economically important species, including grapevine. Table 174.1 lists important focus areas of studies relevant to the potential targets for the grapevine improvement.

1. Improving Grapevine Health

It is generally accepted that plant disease is the exception rather than the rule, due to the very efficient mechanisms of plants defending themselves against pests and pathogens (10). Agricultural monoculture, however, is under constant threat by various pathogens and pests, and mechanisms to curb viral, bacterial, fungal and insect pathogens remain the major focus of the genetic engineering of crop plants. The current approach of single-gene transfers into plant genomes is perhaps also best-suited to the aim of enhanced disease tolerance, since single genes can confer disease resistance to plants.

Several different approaches have been used to enhance disease tolerance in plants, but almost all of them make use of some part of the natural interaction between host and pathogen (11). This interaction is complex and highly fluid due to the fact that the host and pathogen coevolve in the battle for survival. Most transformation strategies involve a gene product with known antipathogen activity that is introduced at high copies or in an inducible manner into the host of choice in an attempt to optimize parts of the plant's innate defence response. Examples of pathogenesis-related defence proteins in plants are glucanases, chitinases, thaumatin-like proteins, defensins, cyclophilin-like proteins, polygalacturonase-inhibiting proteins (PGIPs) and ribosome-inactivating proteins (RIPs) (12–14). Obtaining transgenic plants resistant to pathogenic bacteria has been less successful; the expression of the lytic peptide Shiva-1 has been reported (15), and induced expression of several defence genes have been detected in response to bacterial pathogens (13,16).

The other major approach of manipulated disease tolerance in grapevine (and other plants) relies on pathogen-derived resistance (PDR) and various applications thereof. In this approach, a pathogen-derived gene and its encoding product is expressed at an inappropriate time or in an inappropriate form or amount during the infection cycle, thus preventing the pathogen from maintaining infection (10). Most of the antiviral strategies rely on some aspect of PDR and constitute a major portion of the activity in the genetic transformation of grapevine varieties. One example of the PDR approach is the expression of a Closteroviral coat protein in grapevine in an attempt to combat leafroll disease (17). Some research groups are also endeavoring to express antisense to viral movement and replicase proteins to curtail the spread of viral infection in plants (18).

A range of transgenic plant species has been developed by this approach, with varying success. Some of the first transgenic grapevines expressing heterologous antifungal and antiviral genes are currently undergoing field-testing (19). These first "prototypes" of manipulated disease tolerance in grapevine, as in other plant species, are the beginning of a new era in plant cultivation, as old problems are being addressed in new ways. The technology will undoubtedly improve in sophistication, with the possibility of multiple gene transfers and the long-term and stable expression of transgenes, with the use of highly specific inducible regulatory sequences.

TABLE 174.1
Targets for the Genetic Improvement of Grapevine Cultivars and Rootstocks

Desirable Properties	Focus Area	Examples of Current and Potential Target Genes
Improving Grapevine Health		
Fungal tolerance	Grapevine defense and defense signaling in response to fungal pathogens; pathology of the various fungal pathogens; innate resistances (molecular basis) of various species towards fungal pathogens	Glucanase- and chitinase-encoding genes from fungi, yeast and plants; ribosome inactivating proteins (RIPs); thaumatin-like protein (*Vvtl1*); antifungal peptide encoding genes from plants and insects; genes encoding polygalacturonase-inhibiting proteins (PGIPs), stilbene phytoalexins (stilbene synthases*: stsy*, *vst1, vst2*); phenylalanine ammonia lyase *(pal) CuZnSOD* (putative CuZn superoxide dismutase); detoxification enzyme-producing genes (NADPH-dependent aldehyde reductase, *Vigna radiata*-Eutypine reducing enzyme)
Bacterial tolerance	Grapevine defense and defense signaling in response to bacterial pathogens; pathology of the various bacterial pathogens; innate resistances (molecular basis) of various species towards bacterial pathogens	Anti-microbial peptides (lytic peptide, Shiva-I, defensins); dysfunctional import and integration protein encoding gene (*virE2delB*) from *Agrobacterium*
Viral tolerance	Epidemiology of virus infections and vectors; molecular biology on infecting virus; pathogen-derived resistance strategies (coat-proteins; movement proteins)	Virus coat proteins (translatable, anti-sense, non-translatable); virus movement proteins (anti-sense); replicase (RNA-dependent RNA polymerase), proteinases (2,5 oligoadenylate synthase)
Improving Grapevine Cultivation		
Resistance to water stress	Aquaporins; isolation of root-specific promoters	TIPs (tonoplast integral proteins); PIPs (plasma membrane integral proteins)
Oxidative damage	Carotenoid biosynthesis and control (several putative genes and promoters have been cloned); anaerobiosis	Carotenoid biosynthetic genes; *Adh* (alcohol dehydrogenase) genes; SODs (cystosolic CuZnSOD, chloroplast-residing CuZnSOD, mitochondrial-residing MnSOD)
Osmotic stress and other abiotic stresses	Proline accumulation; polyamines and their role in stress	*Vvp5cs* (Δ^1-pyrroline-5-carboxylate); *Vvoat* (δ-ornithine aminotransferase); FeSOD, glycine betaine, antifreeze genes from Antarctic fish (freezing tolerance)
Improving Grape Quality		
Color development	Ripening-related processes and signals, anthocyanin biosynthesis and control (several genes and some promoters have been cloned); isolation of berry-specific promoters	*ufgt* (UDP-glucose:flavanoid 3-*O*-glucosyltransferase) and/or regulatory sequences of *ufgt*; production of pelargonidin-based anthocyanins for novel berry color; anthocyanin methyl-transferases
Sugar accumulation and transport	Phloem loading/unloading; invertases; sugar transporters; isolation of berry-specific promoters	Invertases from plants and yeast to study phloem loading/unloading; sucrose transporters (*Vvsuc11, Vvsuc12*,*Vvsuc27); h*exose transporters (*Vvht1*, *Vvht2*)
Reduced browning (table and dried grapes)	Oxidation reactions	Silencing of polyphenol oxidase
Seedlessness (table grapes)	Seed-formation; isolation of seed-specific promoters	Baranase gene

Much knowledge has been gained about the nature of plant-pathogen interactions and the disease resistance pathways that operate in plants by generating and analysing transgenic plants. Model plants transformed with the various targeted genes become important resources if the nature of the manipulations and their effect *in planta* are further analysed with state-of-the art technologies, such as proteomics and microarray chips (20). A range of *Arabidopsis thaliana* mutants blocked in certain pathways of pathogen defence also provide extremely valuable information regarding the functions of genes (21). The research has developed to the point where the disease pathways are fairly well characterized and much emphasis is currently placed on the elucidation of the trigger systems of defence and the subsequent signal transduction processes leading to the various forms of defense.

2. Improving Grapevine Cultivation

Genetic transformation technology carries with it the tantalizing prospect of developing plant lines able to adapt to adverse climatic conditions, enhanced by greater understanding of stress tolerance in plants and basic knowledge of key aspects of plant growth and development. Ongoing efforts to develop transgenic grapevines with improved cultivation prospects are focusing on processes such as carbon-partitioning, modes of sugar translocation, water transport and the role of aquaporins, as well as the regulation of these processes. These efforts also deal with important limitations to cultivation such as drought and salt stress, photo-damage and freezing tolerance (22).

To date, a transgenic grapevine expressing antifreeze genes from Antarctic fish has been reported as a mechanism to provide cold tolerance (22). However, plant stress responses are complex pathways of interacting proteins driven by a range of signals attenuated or amplified by equally complex processes. This biological interaction is more difficult to manipulate with single or even multiple gene additions; knowledge of the control mechanisms and alterations thereof might prove more feasible. With this view, the accumulation of proline and polyamines are two examples in grapevine that have been studied. The regulation of key genes in these biosynthetic pathways is providing insight into their involvement in abiotic stress (23,24). Furthermore, the expression of several stress-related genes has been shown to be activated during grape ripening (25,26).

Crop prediction is another area of attention. One of the most difficult issues facing viticultural production is the understanding and management of crop load and quality in the face of seasonal and environmental variation and change in market demand. Most recently, a study of how related genes in grapevine are organized and interact was made possible by the identification of the genes involved in flowering and fruitfulness in *A. thaliana*. For example, the chimera that results in pinot meunier can be separated into a conventional pinot and a mutant pinot form in which fruitfulness is dramatically increased even in juvenile plants. This dramatic change is the result of a single DNA base change in a single gene affecting vegetative and floral development of the vine (27).

3. Improving Grape Quality

The description of quality in grapevine products differs in the three grapevine industries. The wine industry regards small, well-colored fruit complying with optimal ripeness indicators (sugars/acids/phenolics) as desirable, whereas the appearance and optimal size of table grape bunches are of prime importance. Basic quality factors, such as good color and sugar development, are of generic importance and are currently targeted in grapevine molecular biology. The basic processes of berry ripening and, more importantly, the elusive ripening signal(s) are being researched (28,29). The hormonal, environmental and biochemical signals impacting on the key ripening processes, such as pigment production, sugar accumulation and transport, as well as aroma component formation, are studied in grapevine as an example of a non-climacteric fruit. The ultimate aim of this type of approach is to change the metabolic flux through the important biosynthetic pathways that are active in the ripening berry to increase the formation of desirable or novel metabolites linked to the quality parameters of grapes.

The means of glucose/fructose accumulation during ripening is coming to light with the identification and analysis of grape berry genes encoding invertases and sugar transporters (30). The expression of flavenoid biosynthetic genes, responsible for color development, coincide with hexose accumulation in berries (31–33). The importance of UDP glucose flavonoid-3-glucosyl transferase (UFGT) in the control of berry color has been highlighted by comparison of gene expression in red and white grapes (34,35), and recent results indicate that Myb genes are involved in regulating UFGT (36). A number of genes that influence grape berry softening have also been isolated (37–39). Auxin and abscisic acid (natural hormones regulating growth and physiology) affect the expression of genes involved in the ripening process and have been implicated in the control of grape berry ripening (40). The aim of this approach is to meet the quality parameters of grapes with the formation of desirable or novel products by changing the metabolic flux through the important biosynthetic pathways active in the ripening berry.

Grapevine biotechnology, however, is in its infancy in this regard (as in most other crops) and will have to draw on significant elucidation of the underpinning physiological processes as well as improvements in transformation technology to reach these goals. Targeted gene insertion and deletion technologies are some of the tools that would make these and other innovative prospects, such as the manipulation of biochemical pathways to produce novel products and metabolites, more feasible.

III. THE POTENTIAL OF GENETICALLY IMPROVED WINE YEAST STRAINS

A. Yeast Species and Strains

Yeasts are predominant during wine fermentation. In spontaneous fermentations, there is a progressive growth pattern of indigenous yeasts originating from the surfaces of grape berries and the winery equipment (41–43). Yeasts of the genera *Kloeckera*, *Hanseniaspora* and *Candida* predominate in the early stages, followed by several species of *Metschnikowia* and *Pichia* in the middle stages,

when the ethanol rises to 3–4%. The latter stages of spontaneous wine fermentations are invariably dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae,* which therefore is known universally as the "wine yeast" (41,44). The indigenous yeasts present in spontaneous wine fermentations are thought to produce wines with a fuller, rounder palate structure. However, spontaneous fermentations are usually protracted and the outcome is highly unpredictable. Therefore, spontaneous fermentations are most often used in some Old World and *boutique* wineries that depend more on vintage variability and that are willing to accept these risks to achieve distinct styles of wines that reflect the yeast diversity of that specific region.

In modern, large-scale wineries, where rapid and reliable fermentations are essential for consistent wine flavor and predictable quality, specially selected starter culture strains of *S. cerevisiae* with known ability are used (41). In addition to the primary function of these active dried wine yeast starter culture strains to catalyze the rapid, efficient and complete conversion of grape sugars to alcohol without the development of off-flavors, today's pioneering winemakers demand starter culture strains with a whole range of specialized properties that can add value to the final product. This quest for wine yeast strains that are optimized for specific tasks set by winemakers has led to dedicated yeast breeding and genetic engineering. For reviews see (1,2,41,42,45–58).

B. The Genetic Features and Techniques for the Analysis and Improvement of Wine Yeast Strains

The majority of laboratory-bred strains of *S. cerevisiae* are either haploid or diploid, whereas industrial wine yeast strains are predominantly diploid or aneuploid, and occasionally polyploid. The nucleotide sequence of the entire genome of *S. cerevisiae* is known. It has a relatively small, compact genome (ca. 13 000 kb), a large number of chromosomes (16 linear chromosomes varying in length from 200 to 2200 kb), a small number of genes (ca. 6000 protein-encoding genes), little repetitive DNA and few introns (59–62).

Powerful classical and molecular genetic methods exist with which wine yeast strains can be analyzed and modified. Initially, tetrad analysis with the aid of a micromanipulator was used for the genetic identification, characterization and mapping of yeast genes. Recently, technology has been developed to provide a direct link between the genome (full set of genes) and the transcriptome (full set of transcripts) of a wine yeast strain. The genomic sequence has been used to design and synthesize high-density oligonucleotide arrays to monitor the levels of expression of nearly all the genes of yeast cells grown under fermentation conditions (reviewed in (63)). Furthermore, when the current deciphering of the function of the 6000 *S. cerevisiae* genes is completed in the near future, the entire proteome (full set of proteins) will become accessible for the unlocking of wine yeast's complex metabolome (metabolic activities and metabolites) (50,64–67).

The information obtained from the analysis of the entire genomes, transcriptomes, proteomes and metabolomes of wine yeasts undoubtedly will increase the specificity of the current methods with which starter strains are genetically selected and tailored for the production of particular types and styles of wine. At the moment, the classical strain selection and modification methods, such as variant selection, mutagenesis, hybridization (mating, spore-cell mating, rare mating, cytoduction and spheroplast fusion), are based mainly on a "shotgun" approach (68). With this approach, random genes are affected in an unpredictable way, or large genomic regions or entire genomes are recombined or rearranged. These methods, therefore, are not specific enough to modify wine yeasts in a well-controlled manner and they may bring an improvement in some of the yeast strain's properties, while compromising other desired traits. The only advantages of these methods are that they can be used to improve and combine traits under polygenic control and that they do not give rise to products that are included in the statutory definition of GMOs. Therefore, variants, mutants, hybrids, cytoductants and fusants are not subject to the same strict statutory regulations that pertain to GMOs and are also not treated with the same level of public suspicion as are wine yeasts that have been transformed with a single gene. However, genetic engineering remains the only reliable method that offers the possibility to modify an existing property, to introduce a new characteristic and to eliminate an unwanted trait without adversely affecting other desirable properties. Several effective transformation methods and plasmid vectors, as well as expression and secretion cassettes for the expression of heterologous genes and the secretion of their encoded proteins, have been developed for *S. cerevisiae*. This has offered wider applicability and a higher degree of specificity in the development of improved wine yeasts (2).

C. Targets for the Genetic Improvement of Wine Yeasts

Generally, the targets of yeast strain development all relate to improved economics of production and wine quality. Table 174.2 highlights some of the improvements that can be achieved using genetically engineered wine yeasts. These targets include increasing the efficiency of the fermentation process, the processing of wine and control of microbial spoilage, as well as enhancement of the wholesomeness and sensory quality of wine (2,54,55).

TABLE 174.2
Targets for the Genetic Improvement of Wine Yeast Strains

Desirable Properties	Focus Areas	Examples of Potential Target Genes
Improving Fermentation Performance		
Improved general resilience and stress tolerance	Stress response, sterol, glycogen and trehalose accumulation	Modification of glycogen or trehalose metabolism [for example acting on *GSY1* and *GSY2* (glycogen synthase), *TPS1* (trehalose-6-phosphate synthase), *TPS2* (trehalose-6-phosphate phosphatase)]
Improved efficiency of sugar utilisation	Hexose transporters, hexose kinases	Overexpression and modification of *HXT1-HXT18*, *SNF3*, *FSY1* and use of heterologous transporters and kinases
Improved efficiency of nitrogen assimilation	Improved utilisation of less efficient N-sources	Proline catabolism [*PUT1* (proline oxidase) and *PUT2* (pyrroline-5-carboxylate dehydrogenase)] and use of heterologous catabolic genes
Improved ethanol tolerance	Sterol formation, membrane ATPase activity	Modification of the expression of *PMA1* and *PMA2* (ATPase), sterol anabolic genes
Increased tolerance to antimicrobial compounds	Resistance to killer toxins, sulfur dioxide, agrochemicals	Inclusion of *KIL2* (zymocin and immunity factor), overexpression of *CUP1* (copper chelatin)
Reduced foam formation	Cell surface proteins	Deletion of *FRO1* and *FRO2* (froth proteins)
Improved Wine Processing		
Improved protein clarification	Proteases	Overexpression of *PEP4* (protease A) and secretion of other proteases
Improved polysaccharide clarification	Glucanases, pectinases, xylanases, arabinofuranosidases	Overexpression of *END1* (endoglucanase), *EXG1* (exoglucanase), *CacEL1* (cellodextrinase), *BGL1* (β-glucosidase, cellobiase), *PEL5* (pectate lyase) and *PEH1* (polygalacturonase), *XYN1-5* (xylanases), *ABF2* (arabinofuranosidase)
Controlled cell sedimentation and flocculation	Flocculins	Late expression of flocculation genes (*FLO1, FLO5, MUC1*/*FLO11)* under control of promoters (*HSP30*) imparting desired expression
Controlled cell flotation and flor formation	Cell wall hydrophobic proteins	Late expression of *MUC1*/*FLO11* under control of promoters (*HSP30*) imparting desired expression pattern
Improving Wine Preservation		
Wine yeasts producing antimicrobial enzymes	Lysozyme, glucanases, chitinases	Expression of *HEL1* (hen egg white lysozyme), *CTS1* (chitinase), *EXG1* (exoglucanase) and other antimicrobial enzymes
Wine yeasts producing antimicrobial peptides	Bacteriocins	Expression of *PED1* (pediocin), *LCA1* (leucocin) and other heterologous bacteriocin and zymocin genes
Wine yeasts producing sulfur dioxide	Sulfur metabolism and SO_2 formation	Overexpression of *MET14* (adenosylphosphosulfate kinase) and *MET16* (phospho adenosylphosphosulfate reductase), and deletion of *MET10* (sulfite reductase)
Improving Wine Wholesomeness		
Increased production of resveratrol	Stilbene synthesis	Expression of *4CL9/216* (co-enzyme A ligase), *VST1* (stilbene synthase)
Reduced formation of ethyl carbamate	Amino acid metabolism, urea formation	Deletion of *CAR1* (arginase) or expression of *URE1* (urease)
Reduced formation of biogenic amines	Bacteriolytic enzymes, bacteriocins	Expression of *HEL1* (hen egg white lysozyme), *PED1* (pediocin), *LCA1* (leucocin) and other bacteriocins
Decreased levels of alcohol	Carbon flux, glycerol metabolism and glucose oxidation	Overexpression of *GPD1* and *GPD2* (glycerol-3-phosphate dehydrogenase), modification of *FPS1* (glycerol transport facilitator), expression of *GOX1* (glucose oxidase)
Improving Wine Sensory Attributes		
Enhanced liberation of grape terpenoids	Glycosidases, glucanases, arabinofuranosidases	Overexpression of *END1* (endoglucanase), *EXG1* (exoglucanase), *CEL1* (cellodextrinase), *BGL1* (β-glucosidase, cellobiase), *PEL5* (pectate lyase) and *PEH1* (polygalacturonase), *ABF2* (arabinofuranosidase)

(*Continued*)

TABLE 174.2
(*Continued*)

Desirable Properties	Focus Areas	Examples of Potential Target Genes
Enhanced production of desirable volatile esters	Esterases	Modified expression of *ATF1* (alcohol acetyl transferase) and other alcohol transferases, *IAH1* (esterase) and other esterases
Optimized fusel oil production	Amino acid metabolism	Deletion of the *ILE*, *LEU* and *VAL* genes
Enhanced glycerol production	Glycerol metabolism	Overexpression of *GPD1* and *GPD2* (glycerol-3-phosphate dehydrogenase), *FPS1* (glycerol transport facilitator), and deletion of *ALD6*
Bio-adjustment of wine acidity	Maloethanolic and malolactic fermentation, lactic acid production	Expression of *MAE1* (malate permease), together with *MAE2* (malic enzyme) or *mleS* (malolactic enzyme), or *LDH1* (lacticodehydrogenase)
Optimization of phenolics	Phenolic acid metabolism	Modified expression of *PAD1* (phenyl acrylic acid decarboxylase), *pdc* (ρ-coumaric acid decarboxylase), *padc* (phenolic acid decarboxylase)
Reduced sulfite and sulfide production	Sulfur metabolism, hydrogen sulfide formation	Deletion of *MET14* (adenosylphosphosulphate kinase) and *MRX1* (methionine sulfoxide reductase)

1. Improving Fermentation Performance

Wine fermentations generally proceed at a rate greater than desired and are usually controlled by lowering the fermentation temperature. "Runaway" fermentations have a commercial implication, as fermentor space is reduced because of foaming and volatile aroma compounds are lost by entrainment with the evolving carbon dioxide. On the other hand, wine fermentation sometimes ceases prematurely or proceeds too slowly. The financial losses caused by "stuck," "sluggish" or "incomplete" wine fermentations are usually attributed to inefficient utilization of fermentor space and wine spoilage as a result of the low rate of protective carbon dioxide evolution and high residual sugar content (41). Therefore, the predictability of fermentation and the quality of the wine are directly dependent on wine yeast attributes that assist in the rapid establishment of numerical and metabolic dominance in the early phase of wine fermentation and that determine the ability to conduct an even and efficient fermentation with a desirable residual sugar level. Many factors affect the fermentation performance of wine yeasts. Among the general targets for the improvement of fermentation performance are increased resilience and stress resistance of active dried yeast cells; improved grape sugar and nitrogen uptake and assimilation; enhanced resistance to ethanol and other microbial metabolites and toxins; resistance to sulfite, heavy metals and agrochemical residues; and reduced foam formation (2).

As sterols, trehalose, glycogen and aquaporins fulfil multiple roles in increasing the survival of *S. cerevisiae* cells exposed to several physical and chemical stresses, they have important implications for the general stress tolerance, resilience, fitness and vigour of active dried wine yeast starter cultures upon reactivation (69). As a result, there is a strong incentive to develop wine yeast strains with a superior ability to accumulate these compounds and proteins. However, due to the complex stress response mechanisms in yeasts, it is not yet clear whether the deletion of the *ATH1* trehalase gene and the modification of the expression levels of the genes involved in the metabolism of trehalose (*TPS1, TPS2, ATH1*) (70,71), glycogen (*GSY1, GSY2*) (72) and sterols (*ERG1*, *ERG11*, *SUT1, SUT2*) (73), and in the synthesis of aquaporins (*AQY1, AQY2*) (74) will result in an improvement in yeast viability and vitality.

An imbalance in the high levels of carbon and low levels of nitrogen in grape must is the most common cause of poor fermentative performance (41). Sluggish or stuck fermentations occur because nitrogen depletion irreversibly arrests hexose transport. The main focus to ensure the efficient utilization of grape sugar (glucose and fructose) under conditions of nitrogen limitation is to increase the rate of glycolytic flux by replacing any nonfunctional mutant alleles of genes encoding the key glycolytic enzymes, to enhance the efficiency of hexose (especially fructose) uptake, and to alleviate the assimilation of proline and arginine (accounting for 30 to 65% of the total amino acid content of grape juice) from nitrogen catabolite repression (2,54,55). In the case of incomplete fermentations, the preference of wine yeasts for glucose over fructose can lead to excessive residual fructose levels that compromise the quality of the wine. It is hypothesized that the rate of alcohol production by wine yeast is limited primarily by the rate of sugar uptake, especially the uptake of fructose in the presence of high sugar levels during the early phase of fermentation and during the final stages of nitrogen depletion coupled to nutrient limitation. Therefore, the research of several laboratories focus on phosphorylation by the *HXK1*- and *HXK2*-encoded hexokinases and the *GLK1*-encoded glucokinase, as well as on hexose transporters encoded by *HXT1-HXT18* and *SNF3*. The low affinity hexose transporter Hxt3p and high

affinity transporters Hxt6p and Hxt7p play particularly important roles in wine fermentation (75). It is possible that increased expression of these transporters will decrease the occurrence of stuck fermentation. Two hexose transporter homologues, Snf3p and Rgt2p, are required for glucose sensing. Snf3 is specifically required for inducing a number of *HXT* genes under low glucose conditions. Dominant *SNF3* mutants constitutively express hexose transporters and are resistant to translational inhibition upon glucose withdrawal, providing a potential mechanism for wine yeast improvement (76,77). Furthermore, genes encoding fructose-specific transporters could also be sourced from other yeasts, such as *S. pastorianus, S. bayanus* and *Zygosaccharomyces bailii.* For instance, the *S. pastorianus FSY1*-encoded fructose/H^+ symporter could be overexpressed in wine yeast together with the other *HXT3, HXT6, HXT7* and *SNF3*-encoded hexose transporters and the *HXK1*-encoded hexokinase (with the highest affinity for fructose, but still a significantly lower affinity than for glucose), thereby attempting to improve glucose and fructose uptake during wine fermentations. Regarding nitrogen assimilation during wine fermentations, the deletion of the *URE2*-encoded repressor of the *PUT1*-encoded proline oxidase and *PUT2*-encoded pyrroline-5-carboxylate dehydrogenase represents the first step towards the development of wine yeasts that can efficiently assimilate the abundant supply of proline and arginine in grape juice under fermentative conditions (78–80).

Another thrust to improve the fermentation performance of wine yeasts is to increase their resistance to toxic microbial metabolites (e.g., ethanol, acetic acid, medium chain fatty acids, etc.), zymocins (yeast-derived killer toxins), chemical preservatives (e.g., sulfite) and agrochemicals containing heavy metals (e.g., copper). For example, modification of the expression of the *SUT1*, *SUT2*, *PMA1* and *PMA2* genes results in increased sterol accumulation and cell membrane ATPase activity, thereby increasing the resistance to ethanol. Also, the mycoviral determinants and other genes encoding killer toxins (zymocidal peptides) and immunity factors can be incorporated into wine yeasts to make them insensitive to the zymocins of contaminating wild yeasts (81–83). With respect to resistance to agrochemicals, an increase in the copy number of the *CUP1* copper chelatin gene enables wine yeasts to tolerate higher levels of copper residues in the grape must (2,54,55).

2. Improving Wine Processing

The main objectives of fining (addition of an adsorptive compounds followed by settling or precipitation) and clarification (e.g., sedimentation, racking, centrifugation, filtration, etc.) during wine processing include the removal of excess amounts of certain components and microbial cells to achieve clarity and to ensure the physicochemical stability of the end product (2). The fining and clarification of wine often includes expensive and laborious practices that generate large volumes of lees for disposal, thereby causing a loss of wine and removing important aroma and flavor compounds from the remaining wine. To minimize the disadvantages of these harsh fining and clarification practices, an increasing spectrum of relatively expensive commercial enzyme preparations (e.g., proteases, pectinases, glucanases, xylanases, arabinofuranosidases, etc.) are frequently added to the grape must and wine (84,85). As an alternative strategy to the addition of costly enzyme preparations that often contain unwanted contaminating or side activities, wine yeasts are being developed to secrete proteolytic and polysaccharolytic enzymes that would remove haze-forming proteins and filter-clogging polysaccharides, respectively. To this end, the overexpression of several bacterial, fungal and yeast genes resulted in the development of proteolytic, pectinolytic, glucanolytic and xylanolytic wine yeast strains. Under winemaking conditions, proteases have been unsuccessful in degrading haze proteins (86). However, a number of glycoproteins have been found to visibly reduce haze formation in wine, including yeast invertase (*SUC2*) (87) and two mannoproteins from *S. cerevisiae* known as haze protection factors (*HPF1* and *HPF2*) (88).

A second target for the improvement of clarification and filtration aims at efficiently removing all yeast cells from the liquid phase of the tank or barrel. Regulated expression of the flocculation genes is important to guarantee a high suspended yeast count for a rapid fermentation rate during the fermentation process, while efficient settling is needed to minimize problems with wine clarification at the end of sugar conversion. Yeast flocculation is especially important for the production of bottle-fermented sparkling wine, and the controlled onset of yeast flocculation at the appropriate time during sparkling wine production can simplify this costly process. The expression of the *FLO1* flocculin gene, linked to the late-fermentation *HSP30* promoter, can be induced by a heat-shock treatment (89), confirming that controlled flocculation is indeed possible during fermentation. Cell aggregation also plays a key role in the production of flor sherry, during which a related cellular process results in the flotation of the yeast cells, thereby forming a velum (biofilm) on the surface of the wine. By placing the *MUC1* (also known as *FLO11*) mucin gene under the control of the *HSP30* promoter, the formation of the biofilm can be promoted at the end of fermentation, thereby simplifying the development of the flor (90,91).

3. Improving Wine Preservation

Uncontrolled microbial growth before, during or after wine fermentation, can alter the chemical composition of the end

product, thereby detracting from its sensory properties of appearance, aroma and flavor. Healthy grapes, cellar hygiene and sound oenological practices are the cornerstones of the winemaker's strategy against the uncontrolled proliferation of spoilage microbes. Added safety is provided by the addition of chemical preservatives, such as sulfur dioxide, dimethyl dicarbonate, benzoic acid, fumaric acid and sorbic acid, which control the growth of unwanted microbial contaminants. However, excessive use of these chemical preservatives is harmful to the quality of the wine and is confronted by mounting consumer resistance. Consumer preferences have shifted to products that are less heavily preserved with chemicals, less processed, of higher quality, more natural and healthier. Therefore, biopreservation with yeast-derived metabolites (e.g., formation of sulfite or hydrogen peroxide during wine fermentations), antimicrobial enzymes (e.g., lysozyme, chitinases, endoglucanases, etc.) and peptides (zymocins and bacteriocins) is currently being considered as an alternative strategy to chemical preservation (92). However, the use of purified antimicrobial enzymes and bacteriocins is expensive, resulting in an increase in retail costs. This problem might be circumvented by expressing effective antimicrobial enzymes and peptides in wine yeast starter culture strains, thereby addressing the wine industry's call for wines of higher quality and purity. To this end, the hen egg white lysozyme gene (*HEL1*), the *Pediococcus acidilactici* pediocin gene (*PED1*) and the *Leuconostoc carnosum* leucocin gene (*LCA1*) have been used to engineer bactericidal yeasts (93–95). The antifungal yeast *CTS1*-encoded chitinase and *EXG1*-encoded exoglucanase have also been overexpressed in *S. cerevisiae* (96). The main approach in the construction of zymocidal strains entails the inclusion of a combination of mycoviral killer toxin determinants of *S. cerevisiae* (e.g., a K_1/K_2 double killer) and zymocin-encoding genes from other yeasts (e.g., *Hanseniaspora, Kluyveromyces, Pichia, Willopsis*, etc.) (97) into wine yeasts. The ideal would be to incorprate all of these antimicrobial activities into a single wine yeast, thereby counteracting all contaminating spoilage bacteria (e.g., *Acetobacter, Gluconobacter, Lactobacillus, Pediococcus*, etc.), yeasts (e.g., *Brettanomyces/Dekkera, Pichia, Zygosaccharomyces*, etc.) and molds (*Aspergillus, Botrytis, Penicillium, Trichoderma*, etc.) in winemaking.

4. Improving Wine Wholesomeness

It is generally accepted that moderate wine drinking can be socially beneficial and can be effective in the management of stress and the reduction of coronary heart disease. The principal protective compounds found in wine include the phenolic compounds, resveratrol, salicylic acid and alcohol (98). However, prudent wine drinkers are increasingly fastidious about the presence of undesirable compounds in wine. These unwanted compounds include ethyl carbamate, biogenic amines, and chemical preservatives (92). The most finicky among these fussy wine drinkers are even concerned about high levels of alcohol in wine. When wine yeast strains are developed, it therefore is of the utmost importance to focus on these health aspects and to develop yeasts that may enhance the benefits (e.g., production of resveratrol, carnitine, etc.) and reduce the risks (e.g., eliminating ethyl carbamate and biogenic amines, and reducing the levels of alcohol) associated with moderate wine consumption.

With regard to the production of resveratrol during fermentation, progress has already been made by constructing a wine yeast that expresses the *4CL9/216* co-enzyme A ligase and *VST1* stilbene synthase genes (99). The development of a bactericidal yeast, which is deleted for the *CAR1* arginase gene (blocking the secretion of urea, the precursor for the formation of ethyl carbamate) (100) or which is transformed with heterologous urease genes (enabling the degradation of urease) would reduce the requirement for added sulfite, yeast-derived ethyl carbamate and bioamines formed by bacterial contaminants (101,102). The bioreduction of the levels of alcohol in fermented beverages can be achieved by redirecting the carbon flux away from ethanol formation and towards the production of glycerol and gluconic acid. A significant increase in the level of extracellularly accumulated glycerol and concomitant decreases in ethanol concentrations have been achieved by the overexpression of the endogenous *GPD1* and *GPD2*-encoded glycerol-3-phosphate dehydrogenase isozymes of *S. cerevisiae* (103–105), together with the constitutive expression of its *FPS1*-encoded glycerol transport facilitator. Similar decreases in ethanol levels have been achieved by the expression of the *Aspergillus niger GOX1* glucose oxidase gene in *S. cerevisiae* (106).

5. Improving Wine Sensory Attributes

The single most important factor in winemaking is the organoleptic quality (appearance, aroma and flavor) of the final product. The endless variety of flavours stem from a complex, completely non-linear system of interactions among many hundreds of compounds. The bouquet of a wine is determined by the presence of a well-balanced ratio of desirable flavor compounds and metabolites and the absence of undesirable ones (107). With the exception of terpenes in the aromatic grape varieties and alkoxypyrazines in the herbaceous cultivars, perceived flavor is the result of absolute amounts and specific ratios of many of these interactive compounds, rather than being attributable to a single "impact" compound. Subtle combinations of trace components (accumulated secondary metabolites) derived from the grapes usually elicit the characteristic flavor and aroma notes of wine, whereas the products of yeast fermentation (e.g., esters, alcohols, etc.) contribute to the generic background flavor and aroma, as well as to the complexity and

intensity of the aroma and taste of the final product (5). Yeast can also be responsible for the production of unwanted byproducts, such as hydrogen sulfide.

There is an obvious need for the development of wine yeasts that could impart specific desirable characteristics to a wine. To this end, significant progress has been made in the construction of yeasts producing color- and aroma-liberating enzymes (e.g., pectinases, glycosidases, glucanases, arabinofuranosidases, etc.) (85,108) and ester-modifying enzymes (e.g., alcohol acetyl transferases, esterases, isoamyl acetate hydrolysing enzyme, lyases, etc.) (109–111). Furthermore, yeasts producing optimal levels of glycerol (the overexpression of *GPD1*, *GPD2* and *FPS1*, together with the deletion of the *ALD6* acetaldehyde dehydrogenase gene) (112), fusel oils (e.g., isobutyl alcohol, isoamyl alcohol, etc.), and phenolic acids (modified expression of the yeast *PAD1* phenyl acrylic acid decarboxylase gene, as well as the expression of bacterial *pdc p*-coumaric acid decarboxylase and *padc* phenolic acid decarboxylase genes) have been developed (113). In addition, wine yeasts carrying disrupted alleles of the *MET14* adenosylphosphosulfate kinase or *MRX1* methionine sulfoxide reductase have been constructed (114).

The bioadjustment of acidity in wine can be achieved by recombinant wine yeasts containing combinations of genes cloned from *Schizosaccharomyces pombe* and lactic acid bacteria. A wine yeast that contains the *S. pombe mae1* malate permease gene and the *mae2* malic enzyme gene converts malic acid to ethanol (maloethanolic fermentation) (115), whereas a transformant carrying the *mae1* gene together with the *Oenococcus oeni* (*mleA*), *Lactococcus lactis* (*mleS*) or *Lactobacillus delbrueckii* (*mleS*) malolactic enzyme gene converts malic acid into lactic acid (malolactic fermentation) (116). The maloethanolic wine yeast would be preferred for low pH wines from the cooler wine-producing regions, while the malolactic wine yeast would provide the best solution for high pH wines from the warmer regions. In the case of high pH wines, the production of additional lactic acid during fermentation can be achieved by incorporating the *Lactobacillus casei LDH1* lacticodehydrogenase gene into the malolactic wine yeast strain (117). These yeasts also preclude the requirement for the use of bioamine-forming malolactic bacteria in red wine and certain styles of white wine that are required to undergo malolactic fermentation.

IV. THE POTENTIAL OF GENETICALLY IMPROVED MALOLACTIC BACTERIA

A. Lactic Acid Bacterial Species and Strains

Malolactic fermentation, conducted by malolactic bacteria, is an important step in the grape vinification process, particularly in red wines. Most often malolactic fermentation will occur after the alcoholic fermentation; however, it is not limited to this stage of winemaking. The role of malolactic fermentation is threefold: wine deacidification by the conversion of L-malic acid to the ''softer'' L-lactic acid, microbial stability and wine flavor modification.

The wine bacteria associated with spontaneous malolactic fermentation belong to the family of lactic acid bacteria, encompassing four genera, *Lactobacillus* (*Lb.*), *Leuconostoc* (*Lc.*), *Oenococcus* (*O.*) and *Pediococcus* (*P.*). The species in these genera can be characterized by their ability to tolerate low pH, high ethanol concentration and to grow in wine. Those most commonly associated with wine are *Lb. brevis*, *Lb. plantarum*, *Lb. hilgardii*, *Lc. mesenteroides*, *O. oeni, P. damnosus* and *P. pentosaceus*. Even though it is postulated that species of *Lactobacillus* and to a lesser extent *Pediococcus* species may conduct the deacidification reaction in spontaneous malolactic fermentation, species of these two genera are more likely to be associated with spoilage of wine than with positive sensory attributes. *Oenococcus oeni*, formerly known as *Leuconostoc oenos* (118), is the species most commonly responsible for malolactic fermentation, as it is particularly well adapted to the harsh wine environment (low pH, high ethanol content, low nutrients) (119). Several selected strains of *O. oeni* have been commercialized to initiate malolactic fermentation.

B. Genetic Features and Techniques for the Analysis and Development of Malolactic Bacteria

The sequence of the *O. oeni* genome is almost complete, adding to the limited number of genes that have been characterized (120–123). Though *O. oeni* is an important organism in winemaking, knowledge of its genetics is limited. To date, however, a genetic transfer mechanism into *O. oeni* does not exist.

Considerable research has been done on other lactic acid bacteria, particularly those from the dairy industry. Substantial work has been devoted to the genetics of *Lactobacillus* species but to a lesser extent in *Pediococcus* species. Numerous vectors have been constructed to introduce modified genes into *Lactobacillus* species and strains. These vectors are designed to enable easy movement between Gram-positive and Gram-negative organisms utilizing dual replicons (origin of replication) and antibiotic markers. Also available are temperature sensitive features on a few of these plasmids. Some of these plasmids/vectors also function in *Leuconostoc* species, which is the closest lactic acid bacterial genus to *Oenococcus*. Many of these vectors may be suitable for *O. oeni*.

Another approach to improving industrially important *O. oeni* strains is the use of a new technology known as genome shuffling (124,125). This technique involves using a classical strain improvement method to generate populations with subtle improvements. Next, these populations are shuffled by recursive pool-wise protoplast

fusions. Genome shuffling has been successfully applied to improve acid tolerance in a poorly characterized industrial *Lactobacillus* strain (126) and appears to be broadly useful for the rapid development of tolerance and other complex phenotypes in industrial organisms.

C. Targets for the Genetic Improvement of Malolactic Bacteria

The ability to genetically alter the malolactic bacterial genome will depend upon the development of a suitable genetic transfer system (transformation, conjugation or transduction), which currently is unavailable. Potential improvements to malolactic fermentation by the genetic modification of *O. oeni* are summarized in Table 174.3. It would be of obvious interest to construct an *O. oeni* strain which is able to ward off potential competitors, improve its ability to cope with the harsh wine environment, increase its efficiency in the bioacidification of wine - conversion of malic acid to lactic acid, and/or to provide mechanisms to improve the organoleptic qualities of wine. Such an organism would not necessarily possess all these attributes, but generating a selection of improved *O. oeni* would be of great benefit to the wine industry.

1. Improving Malolactic Fermentation Performance

It has been demonstrated that *O. oeni* responds to various environmental stresses, such as high alcohol, acid and sulfur dioxide concentrations, by producing heat shock and stress proteins (*hsp18*, *clpX* and *trxA*) (127), as well

TABLE 174.3
Targets for the Genetic Improvement of Malolactic Bacteria

Desirable Properties	Focus Area	Examples of Potential Target Genes
Improved Fermentation Performance		
Increased ethanol, low pH, non-optimal temperature resistance	Protease associated with the ATPase complex, heat shock proteins and stress proteins	*hsp 18, clpX, trxA, fstH*
Efficient malic acid degradation	Malolactic fermentation and increased strain viability	*mleA, mleR, mleP* and genes involved in oleic acid assimilation
Improved tolerance to antibacterial compounds	Resistance against bacteriocins, sulfur dioxide, agrochemicals	Desired bacteriocin resistance gene
Improved tolerance against growth-inhibitory compounds such as tannins, fatty acids	Protease associated with the ATPase complex	*ftsH*
Improved Processing Efficiency		
Improved protein clarification	Proteases	Target genes not yet identified
Improved polysaccharide clarification	Glucanases, pectinases, arabinofuranosidases	Target genes not yet identified
Improved Wine Flavor or Other Sensory Attributes		
Optimized production of glycerol	Glycerol metabolisms	Glycerol dehydratase enzyme
Enhanced liberation of monoterpenes	β-glycosidases, glucanases, arabinofuranosidases	*bgl* (β-glycosidases) and other genes required for sequential release of monoterpenes from glycoside
Optimized levels of aldehydes	Enzymes involved in acetaldehyde metabolism and catabolism	Target genes not yet identified
Optimized levels of phenolics	Phenolic acid metabolism	Modified expression of *pdc*
Optimized levels of diacetyl	Enzymes involved in citrate fermentation	*alsS*, *alsD* (α-acetolactate synthase and decarboxylase), diacetyl reductase, citrate permease (*citP*), citrate lyase
Optimized levels of esters	Lipases and esterases	Increase in acyl transferases and decrease in esterase activities
Improved Wine Wholesomeness		
Reduced formation of biogenic amines	Amino acid decarboxylases	Deletion of *hdc* and *tdc* genes
Reduced formation of ethyl carbamate	Arginine degradation	Deletion of the arginine deiminase and ornithine carbamyl transferase
Reduced formation of glyoxal and methylglyoxal levels	Metabolism of glyoxal and methylglyoxal	Target genes not yet identified

as the protease-associated with the ATPase complex (*fstH*) (128). The manipulation of these genes could lead to improved tolerance of *O. oeni* to wines with conditions at the upper limit of *O. oeni* tolerance. The elevated expression of these stress proteins may also aid the commercial production of *O. oeni* for direct inoculation into wine, by better preparing the bacterial cells for the harsh wine environment.

The genes for malate metabolism have been cloned from *O. oeni* and three genes *mleR* (regulator), *mleA* (enzyme) and *mleP* (permease), have been identified (115). In order for malate metabolism to be initiated and to proceed efficiently in wine, a bacterial population density of at least one million cells/mL is needed. To increase the conversion efficiency of L-malate to L-lactate, it is necessary to understand the regulation of *mleA* by *mleR* and determine the rate-limiting step for malate metabolism, especially important at low cell density. A rapid adaptation of the *O. oeni* cell to its harsh wine environment may also ultimately enhance the catabolism of L-malate.

2. Improving Wine Wholesomeness

The role that malolactic fermentation plays in ethyl carbamate formation remains unclear. Arginine, a quantitatively important amino acid of grape must and wine, is a precursor to ethyl carbamate. Lactic acid bacteria vary in their ability to degrade arginine; experiments conducted in a synthetic and a laboratory vinified wine demonstrated a correlation between arginine degradation, citrulline production and ethyl carbamate formation during malolactic fermentation conducted by an *O. oeni* and *Lb. buchneri* strain (129–131). The arginine catabolism (*arc*) gene cluster of *O. oeni* has been cloned and characterized, thus providing a basis for manipulating the *arc* genes and reducing the potential of ethyl carbamate production (132).

Biogenic amines have undesirable physiological effects when consumed at high concentrations. The major biogenic amines in wine are histamine, phenylethylamine, putrescine and tyramine (133–135). Their concentration is lowest after alcoholic fermentation and increases variably in most wines during malolactic fermentation (136). Wine-associated lactic acid bacteria, including *O. oeni,* have been shown to decarboxylate amino acids to their corresponding amines. This decarboxylation reaction is purported to favor growth and survival in acidic media, since it induces an increase in pH and can also provide energy to the lactic acid bacteria (102,137). Lactic acid bacteria vary in their ability to produce the various amines.

The *O. oeni* histidine decarboxylase gene (*hdc*) has been cloned and characterized (138). A PCR-based test has also been developed for the detection of amino acid decarboxylating genes in lactic acid bacteria (139). The manipulation of the *hdc* gene in *O. oeni* strains with other desirable characteristics would ensure that reduced concentrations of histidine are present in wine.

The removal or reduction of spoilage bacteria (in particular *Lactobacillus* and *Pediococcus* species) which can potentially produce off-flavors and undesirable biogenic amines during grape vinification could be beneficial to the final wine product. The use of bacteriocin-producing species/strains of lactic acid bacteria in other food industries, especially the dairy industry, has met with considerable success. A pediocin producing *Lb. plantarum* strain was shown to efficiently combat the spoilage of cheese by *Listeria monocytogenes* (140). Furthermore, the expression of pediocin PA-1 from *Pediococcus acidilactici* or plantaricin 423 from *Lb. plantarum* by the introduction of the respective genes into *S. cerevisiae* has been shown to effectively eliminate lactic acid bacteria from an alcoholic fermentation (94). A similar strategy could be used to introduce the necessary genes into *O. oeni*.

3. Improving Wine Sensory Attributes

One of the most important flavor compounds associated with malolactic fermentation is diacetyl (a diketone, 2,3-butanedione), which can impart a ''buttery'' or ''butterscotch'' flavor to wine (141–143). Diacetyl is principally formed during malolactic fermentation by the bacterial metabolism of citric acid. This diketone is also an important flavor compound in the dairy industry, providing the characteristic ''buttery'' flavor of many fermented milk products.

The metabolism of diacetyl is well understood (144–146) and the environmental factors that influence its formation and degradation in wine have been established (147,148). The diacetyl pathway has been amenable to genetic manipulation in *Lactococcus lactis*, leading to overproduction of diacetyl (149). With the inactivation of the lactate dehydrogenase gene (*ldh*) in *L. lactis*, there was an accompanying alteration to the metabolic flux, eliminating lactic acid as a metabolic end product and producing ethanol, formate and acetoin. Acetoin is a degradation product of diacetyl and is considered flavorless in wine because of its high aroma threshold. The over expression of α-acetolactate synthase (*ilvBN* genes in *L. lactis*) leads to an increased production of acetoin (the reaction is driven towards acetoin and away from diacetyl), whereas the inactivation of the *aldB* gene (encoding α-acetolactate decarboxylase) resulted in an increased production of α-acetolactate and diacetyl at the expense of acetoin. The latter scenario is quite desirable in the dairy industry. The analogous genes [*alsS* (α-acetolactate synthase) and *alsD* (α-acetolactate decarboxylase)] have been cloned and sequenced from *O. oeni* (150) providing a means of altering diacetyl concentrations in wine. An alternative strategy would be to inactivate the citrate permease gene (*citP*), thus removing the

initial substrate for diacetyl. Such a natural mutant of *O. oeni* has been isolated and is commercially available.

Many potential aroma and flavor compounds are found in grapes and wine as glycosidically bound aglycons (monoterpenes, norisoprenoids, benzene derivatives and aliphates). Though β-glucosidase activity has been demonstrated in *S. cerevisiae* and *O. oeni* (151), both have limited ability to release the disaccharide glycosides. Improving release of glycosides in *O. oeni* strains following malolactic fermentation using similar methods to those described in yeast could lead to enhanced grape variety aroma in the wine.

Various sensory studies pre- and postmalolactic fermentation have shown that the fruity qualities of a wine may be enhanced after malolactic fermentation (152,153). An improved understanding of the formation and further metabolism of esters [acyl alcoholtransferase (ester synthesizing enzymes) and esterases (ester catabolizing enzymes)] in *O. oeni* could lead to the enhancement of specific, desirable esters.

Bitterness is a spoilage problem, primarily in red wine, which can be associated with lactic acid bacteria, including *O. oeni* strains. The fermentation of glycerol can lead to the formation of acrolein, which when it reacts with phenolic hydroxyl groups, results in wine bitterness. The manipulation of glycerol catabolism, for example the glycerol dehydratase, may lead to reduced acrolein formation. The presence of certain peptides is also a potential cause of bitterness in wine. Bitterness due to proteolytic action and formation of short peptides has been studied extensively in dairy-associated lactic acid bacteria. There appears to be minimal proteolytic activity associated with *O. oeni* and the peptide transport system of *O. oeni* is poorly understood. A better understanding of potential bitter wine peptides and their formation could reduce the occurrence of bitterness in some wines.

Numerous malolactic fermentation sensory studies and anecdotal evidence point to changes in the texture and body of the wine following malolactic fermentation, with reports indicating a fuller, richer, longer aftertaste (152). The chemical changes contributing to these favorable mouth-feel properties are poorly understood. The deacidification process itself contributes substantially to changes in mouth-feel, with the consequent increase of pH and decrease in titratable acidity. In addition, lactic acid feels softer than malic acid. Other contributors to wine mouth-feel or taste perception have been suggested, including polysaccharides, glycerol and mannoproteins. The contribution of malolactic fermentation in this area remains to be studied.

Malolactic fermentation has great potential to further enhance wine qualities by retaining or altering the aroma/flavor profile of the wine as well as by conferring microbial stability. However, the genetics of *O. oeni* for all these processes are still not well understood. With the availability of the complete *O. oeni* genome sequence, the development of an efficient gene transfer system into *O. oeni* and the understanding of the various pathways, "tailor made" *O. oeni* strains will become possible.

V. CHALLENGES FACING THE COMMERCIALIZATION OF GENETICALLY IMPROVED GRAPEVINE CULTIVARS AND MICROBIAL STARTER STRAINS

A. Scientific and Technical Hurdles

Despite the strong and persuasive scientific case for the use of gene technology in the improvement of grapes and wine, the wine industry has entered the 21st century without a transgenic grapevine variety or a recombinant microbial starter strain being used on a commercial scale (4). This is partially due to the complex chemical origins of wine quality, which has made the definition of specific and detectable genetic quality improvements difficult. An additional complexity in improving grapevine and wine yeast and bacteria entails the kaleidoscopic sequences of metabolic changes that occur throughout the successive stages of grape ripening and fermentation, respectively. However, considerable progress has been made over the last few years to overcome the technical hurdles in defining the wine industry's requirements genetically and improving grapevine varieties, wine yeast strains and strains of malolactic bacteria accordingly. The development of genetic transformation methods for *S. cerevisiae* (in 1978) and *V. vinifera* (in 1989), and the advent of technology with which entire genomes, transcriptomes, proteomes and metabolomes can be analyzed, have undoubtedly opened up new horizons for the wine industry. However, it is important to note that the information and technology that currently exist for model plant, yeast and bacterial systems have yet to be expanded to the much more complex genomes of grapevine and industrial strains of wine yeast and malolactic bacteria before all of the requirements and concerns of the producers, consumers and regulatory authorities can be addressed satisfactorily (4). The recent promising "prototypes" of genetically engineered grapevine cultivars and microbial starter strains have brought these objectives within the realms of possibility.

B. Legal and Regulatory Hurdles

The initial problems with statutory approval for the use of genetically engineered plants and organisms in the agro-industry are now slowly being dissolved by a growing consensus that risk is primarily a function of the characteristics of a product, rather than the use of genetic modification *per se* (2). The concept of "substantial equivalence" is widely used in the determination of safety by comparison with analogous conventional food and beverage products. When substantial equivalence can be

demonstrated, no further safety considerations usually are necessary. When substantial equivalence is not convincingly shown, the points of difference must be subjected to further safety scrutiny.

The legislation and regulations, although differing in detail, are broadly similar in most countries. Guidelines for the approval of GM products and the release of GMOs usually require a number of obvious guarantees. These include a complete definition of the DNA sequence introduced and the elimination of any sequence that is not indispensable for expression of the desired property; the absence of any selective advantage conferred on the transgenic organism that could allow it to become dominant in natural habitats; no danger to human health and/or the environment from the transformed DNA; and a clear advantage to both the producer and the consumer (2).

C. Intellectual Property and Patenting Hurdles

Patents covering many of the genetic tools (e.g., DNA sequences, gene promoters, marker genes, vectors, etc.) and methods (e.g., transformation protocols) commonly used in genetic engineering leave little ''freedom to operate'' (3,4). It is therefore imperative to address intellectual property issues such as patents or other forms of protection of genes, promoters and technologies through formal agreements (19). If ownership of a genetically improved grapevine, wine yeast or malolactic bacterium is in dispute, the release of such genetically improved grapevine plantlets and microbial strains might cause serious impediment to the commercialization process. On the other hand, genetically tailored grapevines, wine yeasts and malolactic bacteria (with "sufficiently distinct" properties) must also be protected in some way by the developer. However, whether improved grapevine varieties or strains of wine yeast and malolactic bacteria can be patented itself or protected in other ways may also depend on the legislation and regulations in each wine-producing country (19).

D. Political and Economic Hurdles

It is well known that economies are driven by different forces and therefore go through life cycles (54,55). For example, in terms of resources, the *Industrial Economy* was ''the economics of scarcity,'' because everything that fueled the economy was in short supply and available to only a few nations. The current *Information Economy*, which was built on the successes of the *Industrial Economy*, is driven by "the economics of plenty" and, thanks to communications, computer technologies and the Internet, information is no longer a scarce resource. Furthermore, it is already being speculated that the *Information Economy* is only the first phase of the *Bioeconomy*, which rests on the pillars of both Information Technology and Biotechnology (4). There is ample evidence that the *Info-Bioeconomy* has already brought about more economic transformation in the past few decades than was brought by the *Industrial Economy* in the previous centuries. Not everybody perceives all of these transformations as positive changes. Some critics and activists are whipping up public alarm and fueling political agendas and protests against globalization and a universal "borderless" economy. Certain lobby groups also claim that patents on genetically engineered organisms confer an unfair advantage to certain producers, thereby concentrating economic power in the hands of a few large multinational producers (2). Therefore, it can be expected that the commercialization of genetically improved grapevines and wine yeasts would not escape political meddling from the vested interests of economic and agricultural protectionism. The swelling tide in an overflowing ocean of wine is likely to increase the temptation for some to twist scientific data and misuse consumer confusion to justify trade bans and technical barriers to free trade.

E. Marketing Hurdles

The marketing of wine relies a great deal on label integrity and product identity. Therefore, it is of the utmost importance that genetically improved grapevines and yeasts do not interfere with the established varietal names and predictable wine styles. For example, the wine industry relies heavily on a few select cultivars and would therefore be very hesitant to introduce new varietal names (3,4,19). In the most profitable market segments, the varietal name (especially the names of the so-called "Big Five," namely Cabernet Sauvignon, Shiraz, Merlot, Chardonnay and Sauvignon Blanc), together with the origin of production and the vintage, form the cornerstones of the information that is presented on the bottle label to the increasingly brand-conscious customers and consumers. The outcome of the current debate on the description and naming of transgenic grapevines therefore will determine not only the procedure for the description of genetically modified grape varieties, but also, to a large extent, their acceptance by grapegrowers and winemakers and their commercial value in the marketplace (3,4).

This debate on the naming issue entails a number of factors, such as the source of gene(s) introduced into a particular grapevine, the "true-to-typeness" of the transgenic vine when compared to the original cultivar/clone and the organoleptic and sensory qualities of the resulting wine (3,4). Given the immense marketing value contained in varietal names, there is an urgent need for consensus that genetically modified grapevines are little different to grapevine clonal selections, which have been selected on the basis of beneficial, spontaneous genetic variations (e.g., a change in plant performance). When clonal selections are used, the identity may be known to the grape

grower, but the wine is still marketed under the varietal, and not the clonal (typically specified by a clone number) name (19).

It is important to note that many of our modern and most highly regarded wine grapevine cultivars (e.g. Cabernet Sauvignon, Chardonnay, Syrah, etc.) are the product of accidental crosses between varieties grown close to each other, probably in the same vineyard, a common practice in medieval times. The parents of many of today's cultivars are often obscure and sometimes humble varieties. Therefore, we do well to remember that much of the wine that we drink and value today is made from varieties that were already "genetically modified" many centuries ago. However, it remains to be seen whether transgenic grapevines with altered fruit qualities, such as improved color and flavour compound composition, will have to be assigned a new varietal name or just a new clonal number. These uncertainties, together with the impractical, but strong, calls for all products that are produced by gene technology to be labelled specifically, aggravate the wine industry's hesitance to adopt transgenic grapevines and recombinant strains of wine yeast and malolactic bacteria in the face of those who cannot resist riding the dangerous "backlash" market with labels stating that a particular wine product is "GM free" (2,4).

F. Traditional and Cultural Hurdles

The future application of gene technology in the wine industry will have to overcome some more specific hurdles. Foremost, national and, even more relevant, regional wine industries possess strong identities and deep cultural roots, as illustrated by proudly maintained local traditions. Furthermore, wine is generally viewed as a natural beverage produced by time-honored procedures. As a consequence, the industry is less receptive to technologies that promise revolutionary changes. In this context, it is also feared that gene technology may accelerate the tendency to standardize wines to satisfy large supermarket chains and the "average" international consumer, leading to loss of local identity, variety and uniqueness (4). The successful application of recombinant DNA technology in the wine industry will depend on assuring commercial users of transgenic grapevines and recombinant microbial starter strains that existing, desirable characteristics have not been damaged, that the requirements of beverage legislation are met and that the engineered cultivar and strain will be stable in practice, with suitable procedures for monitoring. Once the traditionalists are convinced of a clear organoleptic, hygienic or economic benefit of a transgenic grapevine variety or recombinant microbial starter strain, they would be in a strong position to implement such a vine or yeast, because most of the wine enterprises are fully integrated agro-industries that could exert direct control over the development of new, specialized niche markets for "GM wine products." Wine consumers in such types of niche markets are frequently passionate, well informed, well educated and, above all, very curious. Therefore, GM wines produced by a limited number of interested producers would certainly attract widespread attention and could possibly create a new, successful niche market. Based on such small beginnings, the broader benefits conferred by GM technologies could become apparent to grapegrowers and winemakers, and the technology could move rapidly from satisfying niche markets to general acceptance (4).

G. Public Perception Hurdles

The emotive, fear-mongering qualms and myths of the immorality of "unnatural" genetic interference with Nature, of unsafe "Frankenfood" and global havoc caused by GMOs have spread more readily than good sense or wise science, and far enough to masquerade in the cultural folklore as truth (2). Therefore, public perception of risk with regard to GM food has, so far, outweighed its view of possible benefits. Regulatory authorities appear more willing to approve the use of GMOs than the public is to use them. A significant proportion of the public still suspects that GM food will prove unhealthy in the long term and that the escape of GMOs will damage the environment and result in a loss of biodiversity (154). They also doubt that there is sufficient legal and practical protection against accidents involving GMOs.

It is clear that consumer education is essential to remove this fear of the unknown. Scientists must consistently inform the public and remain open about experiments, research and products. The consumer should be reassured of first-class, transparent regulatory systems and the meticulous implementation of biosafety legislation with clear technical standards and definitions with respect to GM products. The consumer should be persuaded by proper risk assessment and clear demonstration of safety, and thus be empowered to make informed decisions. Assurance must be given that GM wine and other grape-derived products will not be "force-fed" to consumers' for profit when there is no clear advantage for the consumer (4).

VI. CONCLUSIONS AND FUTURE OUTLOOK

The image of wine is romantic and appealing, a harmonious blend of nature, art and science. Wine has been called bottled poetry. But underneath the romance is tension, brought about by the classic clash between tradition and innovation, and no tension in the business of making wine is greater than that created by 21st century grape and wine biotechnology (5).

The production of wine requires a unique integration of artistic, scientific and economic aspects, individual creativeness and innovative technology pursuing perfection

(5). Most modern beverages rely upon bold, consistent flavors to attract and maintain a customer base; by contrast wine's attraction is a subtle assortment of changing sensations that makes its charming romanticism difficult to define (5). Historically the definition of wine quality has been the prerogative of the wine producer, but, in the modern globalized market, the control of this definition has shifted to the consumer. Consumers are purchasing *sensory experiences* when they buy wine, not standardized commodities; the winemaker with the best understanding of the biology of human perception and flavor and olfactory preferences will be most able to satisfy the consumer (5). There is even increasing evidence that different evolutionary pressures have shaped the chemosensory repertoire of the various human population groups (155). This will become an important factor for the exploration of untapped markets, Asia in particular, and for winemakers to offer styles customised for the different "noses and tongues" of the emerging markets.

In addition to wanting a product that is enjoyable in all its sensory aspects, today's consumers increasingly expect wines to be healthful and produced in an environmentally sustainable manner (5). The wine producers of the third millennium must understand the fundamental motivation that underpins consumer choice, and to produce wines of enhanced attractiveness while simultaneously developing and implementing sustainable production practices for both grapegrowing and winemaking. The stakes of success in meeting these consumer expectations are high; wine exports are crucial to the economies of many wine-producing countries.

As winemaking continues to evolve from cottage industries in a few countries into today's global network of producers, ultimate success (read profitability) will increasingly become reliant on biotechnological innovations (54,55). Expanding knowledge of senso-chemistry, fundamental molecular genetics and bioinformatics (including genomics, transcriptomics, proteomics and metabolomics) will continue to accelerate the pace of improving grapevines and microbial starter strains for a market-driven and quality-focused industry. Genetically tailored grapevine cultivars, wine yeast and malolactic bacteria promise to help the wine industry meet the consumers' demand for nutritional benefits and sound environmental practices.

There is a multitude of complex scientific, technical, economic, marketing, safety, legal and ethical issues to be overcome (2,4). To our knowledge, no transgenic grapevine, wine yeast or malolactic bacterium has been used for the production of commercial wine; this will continue to be the case until both consumers and industry are satisfied that GM products are safe, of high quality and beneficial. Given current concerns, it is perceived as commercial suicide to prematurely market the first wine made from transgenic grapes or fermented with recombinant yeast.

It would, however, be crippling to the entire wine sector to ignore the phenomenal potential of gene technology; it could propel the wine industry with quantum leaps into the era of "*designer*" products. That benefit will be realized, however, only if the application is judicious, systematic, and done with high regard for the unique nature of the product. The first GM wine products should unequivocally demonstrate minimal resource inputs, low environmental impact and organoleptic, hygienic and economic advantages for the wine producer and the consumer (54,55). Furthermore, wine's most enthralling and fascinating aspect, its diversity of style, should never be threatened by the use of tailored grapevines and wine yeasts. In fact, gene technology should rather be harnessed to *expand* the diversity of high quality wines. These are very large challenges that will require a substantial and sustained effort to overcome. The rewards for success in this endeavour, however, are correspondingly large (54,55).

ACKNOWLEDGEMENTS

The authors thank Elsevier for granting permission to reproduce the content of two articles that were previously published in Trends in Biotechnology (volume 20, pp. 426–432 and vol 21, pp. 472–478). This chapter is therefore a merged, updated and expanded version of these two publications by Pretorius and Bauer (2002) and Vivier and Pretorius (2002).

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Research conducted at The Australian Wine Research Institute is supported by Australia's grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Federal Government. Research conducted in the Institute for Wine Biotechnology is supported by grants from the National Research Foundation (NRF) and the South African Wine Industry (Winetech).

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175 Manufacture of Whisky

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CONTENTS

I. INTRODUCTION

Whisky is one of the world's most loved alcoholic drinks. The word 'whisky' itself derives from the Gaelic '*uisge beatha*,' or 'water of life.' The traditional Latin name for a distilled spirit is aqua vitae, 'water of life' is equivalent to the Gaelic '*uisge beatha.*' Before the 11th century, alchemists invented by accident and gave this name (1).

The Irish were drinking a form of whisky in the 12th century. Henry II of England said that the distillation of the spirit 'usquebaugh' was well established in Ireland when he invaded there in 1172 (2). Distilling was certainly then brought from Ireland to Scotland in the early Middle Ages, although it seems to have been known in the Far East at a very early date among alchemists (3).

Scotch whisky was well established in Scotland by the end of the 15th century. The first record of a commercial transaction involving the supply of whisky (aqua vitae) was in the year 1494. The manufacture of whisky was brought to America in the 17th to 18th century and further developed to the manufacture in Canada. The whisky manufacture was also brought to Japan in the early 20th century. These five countries produce about 95% of the total production in the world and, therefore, they are known to be the five largest international whisky-distilling countries (4,5).

The appellation of whisky, spelled without an 'e' in Scotland, Canada and Japan but with an 'e' in Ireland and America, is the generic name for a distilled product made from saccharified and fermented cereal extracts.

Figure 175.1 is an exhibition of the tools used by the alchemists to make whisky.

II. SCOTTISH MALT WHISKY

The word 'Scotch' is, of course, a contraction of 'Scottish.' It was first used in the mid-19th century (6). Scotch whisky is a spirit produced by human art well established in Scotland by the end of the 15th century and now sold in vast quantities all over the world (2).

Barley, water and peat are the materials to produce the malt whisky of Scotland. Local barley grown in the north-east of the country was originally used and some of the greatest Highland distilleries today use the imported barley. Barley with high starch and low protein is preferred.

FIGURE 175.1 Exhibition room of alchemists in Suntory Whisky Museum (Japan). (Courtesy of Suntory Hakushu Distillery.)

The first process in making the whisky is malting, the turning of barley into malt. Malt is essentially barley that has been promoted to germinate by soaking in water and has then been dried by the application of heat (2). The soaking period in the tank named 'steep' is usually 2 to 3 days, then the water is drained off and the grain is spread out to a depth of 20 to 30 cm on a concrete floor in the malting house. Water content of barley raises to approximately 43% by this soaking. Barley starts to develop the germination on the malting floor and generates energy that rises the temperature. Maltman turned the barley every 4 or 6 hours in order to maintain temperature to be approximately 16°C and to prevent growing roots tangling each other. After 7 to 10 days, the growing stem of the barley becomes half to five-eighths of the length of the seed (4). Some distilleries now adopt huge revolving drums with which aeration and temperature are automatically controlled by computer. These labor-saving drums hold 10 to 50 tons of barley (in case of floor malting 8 to 12 tons).

The germinated green malt is transferred to the drying kiln that has a unique chimney-like oriental pagoda. The floor of the kiln is a drain board made of perforated iron or wire mesh. The green malt is spread on this floor at a depth of 70 cm to 1 m and dried in the smoke arising from a peat fire below the floor (4). Figure 175.2 is a sample of the peat to dry malt.

Besides peat, anthracite and coke are also often used for the fuel and the barley is dried for 40 to 55 hours until the moisture content becomes 3 to 4%. The peat smoke gives a special smoky flavor to the malt and to the final product mature whisky.

A growing number of distilleries no longer do their own malting, but buy their malt ready-made from maltsters, peated to the desired degree. The building of kiln is kept for the symbol of such distilleries (7).

The dried malt is moved to the maltmill and ground to fine grist that consists of husk, grits and flour in the ratio of 2:7:1, respectively (4). The malt grist is placed in a container known as a mash tun and hot water is added. The shape and size of mash tuns vary and they are made from stainless steel, copper and cast iron and usually have a lid (Figure 175.3). The malt is extracted for three or sometimes four times with hot water, each time at a different temperature, ranging from about 60°C, for the first to 100°C for the last (2). Mashing with hot water dissolves the starch from the malt and also activates the amylase that decomposes the starch to maltose.

The resultant liquid, wort, is drawn off from the base of the mash tun through the finely slotted bottom, cooled, and passed into fermentation vessels, or washbacks. The solids, draff, are removed from the mash tun and used as cattle feed (8). Mashing technology has made considerable advances in the brewing industry where the factors of conversion and filtration have tended to become distinct processes (7). The wort is run into a refrigerator and immediately cooled to about 21°C. If the wort is not cooled properly, the maltose would be decomposed and the yeast used in the next process, fermentation, would be killed by the heat.

FIGURE 175.3 Mash tun made from stainless steel. (Courtesy of Suntory Yamazaki Distillery.)

FIGURE 175.2 Peat used to dry malt. (Courtesy of Suntory Yamazaki Distillery.)

FIGURE 175.4 Wooden fermentation vessel, washback. (Courtesy of Suntory Yamazaki Distillery.)

Yeast is added in liquid or solid form to the liquid wort and fermentation takes place at 17 to 35°C for an average of 48 hours in large wooden or stainless steel washbacks (Figure 175.4). The lid of washback incorporates a rotating blade that prevents the foam pouring over the sides.

The inoculated yeast converts maltose into glucose, and then alcohol and carbon dioxide are produced from glucose. Yeast also produce small amounts of other compounds such as wide range of esters, aldehydes, acids and higher alcohols. Many of these are flavor elements. The fermented wort, wash, is a sweet peaty beer with an alcoholic content of 7 to 8% (4). The next process, distillation, is what actually produces the whisky.

Scotland malt whisky has been traditionally distilled in pear-shaped stills (pot stills) which are large copper kettles with narrow necks called lyne arms or lyne pipes that curved and enter the condenser. The shape and size of the pot still affect the quality of the whisky produced. Stills come in three basic designs; the 'onion' being the most common, the 'boil-ball' and the 'lantern' shape (Figure 175.5). The way these designs are interpreted - as to capacity, height, method of heating, angle of the lyne arm etc. differs from one distillery to another, and varies on the quantity of volatiles that will end up in the spirits (9,10). While many distilleries have retained or even switched back to direct firing either by coal or gas, a large number of distilleries now heat the stills by means of internal steam exchangers (7).

Another key aspect of still design is the area of copper that comes into contact with the wash and low wines. Copper dissolves easily and has an important influence on the quality of the spirit, since it removes sulfury or vegetable aromas by chemical reaction (10).

Since malt whisky is usually produced after two times distillation (occasionally a third still is installed), every distillery must have at least two such stills.

After fermentation, the wash passes through the wash charger, into the wash still (or singling still) for the first distillation. The charged volume is between half and two-thirds capacity, in order to allow for the expansion of the wash and the froth which builds up as it heated (10). In the still, the wash is heated and the alcoholic vapors that are boiling at a lower temperature (78.3°C) than water pass up the neck of the still and down through the worm (coiled copper pipe) within a water jacket (worm tub or condenser). The distillate from the wash, low wines, consists of 21 to 24% by volume of alcohol (ABV). A wash still of 30 000 liters capacity, charged with 20 000 liters, will remove alcohol and water at about 1,000 to 1,500 liters per hour. At the end of the run about a third of the wash will have become low wines (10).

This distillation process is repeated in the low wine still (or double still) which is smaller than wash still. During the second distillation, the stillman monitors its progress carefully as it runs through the spirit safe (Figure 175.6). The spirit safe contains several glass bowls into which the spirit can be directed using external faucets by the stillman. The stillman will start to test the spirit as soon as the condensed vapors pass through the spirit safe. The first liquid to the glass bowls, known as foreshots or head, turns cloudy when water is added (called demisting test) as they are still impure. The aldehyde, esters, furfural and other compounds of hydrogen, oxygen and carbon formed in the process of distilling the wash and known generally as congeneric are present in excess in the foreshots but they also give pot-still malt whisky its special bouquet and flavor (2). The foreshots are directed into the low wine charger to be redistilled in the low wine still. The stillman will continue to test the spirit in the spirit safe by adding water at regular intervals and checking the specific gravity.

As soon as the spirit ceases to turn cloudy with the addition of water, the stillman will immediately turn the faucets on the outside of the spirit safe and direct the spirit into the spirit receiver (8). When the spirit starts to weaken, this weakened spirit, called feints or tail, is run into the feints receiver. Stillmans stop collecting the spirit at the point when the alcohol content is as high as 69% by

FIGURE 175.5 Pot stills with various shape and size. (Courtesy of Suntory Yamazaki Distillery.)

FIGURE 175.6 The spirit safe — the apparatus to control the distillation. (Courtesy of Suntory Yamazaki Distillery.)

volume of alcohol for the lighter whisky character while as low as 60% for the heavier whisky character (10). The collected foreshots and feints will be added to the next wash for the distillation. The residue in the low wine still, spent lees, is little more than water, with a very small amount of copper from the still in solution: it is of no use or value and is gone to waste (10).

The spirit directed to the spirit receiver is called hearts or middle cut which consists of about 70% by volume of alcohol. The amount of the hearts is approximately a third of the spirit distilled. The immature spirit, called new pot or new spirit, is piped to the filling station, where it is diluted with water until its strength is reduced to 63.5% by volume of alcohol (4), prior to being run into casks which are made from American white oak (*Quercus alba*) or European oak (*Quercus robur*). Oakwood is thought to be desirable for maturing whisky because of its chemical complexity. The casks are second-hand that have once stored mainly either sherry or bourbon, and in rare cases port or other wines.

New spirits filled in casks are matured in bonded warehouses called 'dunnage' warehouses at least three years and after that they can be legally called Scotch whisky. If they are to be used for 'single malt' or for 'deluxe blend,' the cask will be stored for at least 10 to 15 years (8). But there is always the danger of the whisky acquiring a slightly 'woody' flavor after 15 years (2).

Whisky maturation is influenced by the factors such as the nature and history of the cask, the style of warehouse and its location, the outside microclimate of casks and maturation period. Oak casks that have previously been filled with sherry give a splendid mellowing effect (color, softness, richness, and smoothness) to the mature whisky while bourbon casks give a typically vanilla-like aroma. The process of charring the inside walls of bourbon cask prior to their first use also contributes to the flavor of malt whisky. This releases vanillin into the spirits and assists in removing undesirable off-notes. Oakwood also facilitates oxidation, which remove harshness, increases fruitiness and adds complexity (10).

The smaller the cask, the faster the maturing and the larger the amount of whisky lost through absorption by the porosity of the wood of cask. The degree of humidity in the warehouse where the casks lie during the maturation also affects the degree the loss of maturing whisky both its volume and its strength: the greater the humidity the more it loses strength, and the drier the more it loses volume (2). This is known as "angel's share," losing 1 to 3% every year (4). After ten year's maturation, alcohol content decreases to around 60%; the volume becomes 80% of the initial filling. The longer whisky is matured in the cask, the more changes of color and flavor will take place. Whisky is further diluted with water until its strength is reduced to 40 to 60% by volume of alcohol, prior to being run into bottles. Soft water free from organic and mineral impurity is the preferred water used for the whisky production.

Once run into the bottles, whisky does not further mature but some chemical changes will occur.

Since whisky when distilled is almost colorless, some degree of coloring is often added. Although malt whisky may acquire a certain amount of color while it is being matured in old oak sherry casks, it is an open secret that this can also be due to caramel or even sherry (11). Recently, a light colored whisky is becoming popular in the United States and Japanese markets. The manufacturer will also bear in mind the color of whisky preferred by the consumer.

Single malt whisky is the product of the single distillery. The age shown on the bottle label means the length of the youngest whisky's maturation period.

A vatted malt whisky is the product that is married together with various malt whiskies from different distilleries. A vatted malt whisky is labeled 'Pure Malt' or 'Scotch Malt Whisky.' It is impossible to determine the optimum age for whisky in general terms; so much depends on the individual case.

There are about 110 malt whisky distilleries in Scotland and about 80 to 90 distilleries are now in operation (12–14).

The traditional division of Scotch whisky is into Highland Malts, Lowland Malts, Campbeltown and Islays (15–18).

III. SCOTTISH GRAIN WHISKY

Scottish grain whisky is produced largely from maize with a small amount of malted barley that is not dried over peat fires. (The unmalted barley is also sometimes used with the maize.) The unmalted cereal is crushed and then pressure-cooked in batch cookers to gelatinize the starch so that it can be released and solubilized. Wet ground malted barley is added one-fifth of the amount of the unmalted cereal, then hot water; and the mixture is stirred (2). Amylase from malted barley converts the starch to fermentable sugars, maltose.

While many distillers retain this system, there is an increasing use of continuous operation for the cooking and conversion process (7). Fermentation of the wort is done in the same way by the action of inoculated yeast as the case of the making of malt whisky. The fermented wort, wash, is pumped into the patent still or Coffey still which can distillate with a different system from the distillation with pot still.

The patent still was first invented by Robert Stein in 1826 and was improved by Aeneas Coffey in 1831. According to the invention of patent still, making whisky has become quicker, cheaper, and in greater amounts. Its product has no peaty flavor, for the malt has not been dried over peat fires (2,4). The patent still basically consists of two linked copper columns, 12 to 15 m high; these are the analyzer and the rectifier. Each is divided horizontally into tens of chambers by perforated copper plates (Figure 175.7). Steam is led into the analyzer and proceeds up the

FIGURE 175.7 A copper plate of patent still. (Courtesy of Suntory Hakushu Distillery.)

FIGURE 175.8 A large plant of patent still. (Courtesy of Sun Grain Ltd.)

analyzer and then through the linking pipe into the rectifier.

The wash is continuously pumped into the rectifier that comes down in a coiled copper pipe and then by the connecting wash pipe into the top of the analyzer. The heated wash moves down, chamber by chamber in the analyzer and all the alcohol contained is vaporized before the wash reaches to the base of the analyzer. The steam and alcohol vapors, which rise up through the analyzer, go down the connecting vapor pipe (which emerges from the top of the analyzer) into the base of rectifier. As the alcohol vapor and the steam come more up to the rectifier, chamber by chamber, the new incomes wash, coming down the rectifier in its winding pipe, is then cooled, so that condensation takes place (2,16). Around 95% alcohol can be obtained when the vapor is drawn off through a cooling worm to the stainless steel spirit receivers. [Alcohol strength for making whisky is regulated below 94.8% by the law of Scotland (4).] Figure 175.8 shows one of the modern plants of patent still which uses more than two columns.

The spirit is then diluted with water around 70% by volume of alcohol, prior to being run into the secondhand oak casks that were previously used for the mature of malt whisky. The spirit-filled casks are stored in the warehouse for at least 3 years, the same as malt whisky (4). The grain whisky, being nearer neutral spirits than pot-still malt whisky, takes less time to mature and changes less in maturing. There are two types of grain whisky, one is single grain whisky produced in one distiller and the other is that produced by vatting the whiskies distilled in different distilleries. Only eight grain-distilleries are in operation and seven distilleries among these are located in Lowland (6).

IV. SCOTTISH BLEND WHISKY

Blending can involve combining around 20 to 50 different malt whiskies with 2 to 5 grain whiskies to produce a whisky with a more rounded character (2,16). Blends are central to the Scotch whisky market, outselling malt whiskies at a ratio of around nine bottles of blended whisky to one bottle of malt whisky. Andrew Usher, in Edinburgh pioneered blending in the early 1860s (4). Today, blending provides the distiller with an opportunity to create a product with unique and recognizable flavor attributes. It also enables product consistency. The proportion is 60 grain to 40 malt, and that the proportion of grain has risen since the earlier day of blending, when it was more likely to be 50:50 (2). Deluxe blended whiskies generally contain a higher ratio of malt to grain than standard blends (1).

The blended whisky is stored in oak casks (generally plain oak) for between 6 months and a year in order to optimize the product quality. Some blenders ‘marry’ the malts and the grains separately and bring them together only in bottling. Prior to bottling, the whisky is usually diluted to market strength, caramel is added to ensure continuity of coloring, and finally the spirit is filtered to prevent cloudiness from developing when water is added by the consumer (1). As for blends, the age (if any) stated on the label is the age of the youngest whisky in the blend. A flow diagram for the production of Scotch whisky is shown in Figure 175.9.

V. IRISH WHISKEY

Irish whiskey is whiskey distilled in Ireland. It is well known that the whiskey distilling originated in Ireland. The Irish were drinking a form of whiskey in the 12th century and the skills of whiskey making were brought to Scotland (1,2,16). The extra ‘e’ in the name is traditional, but gives no special meaning (6).

Malt and unmalted barley are what is needed to produce the malt whiskey of Ireland. Much of the current productions also use oats, wheat, and rye with barley. The

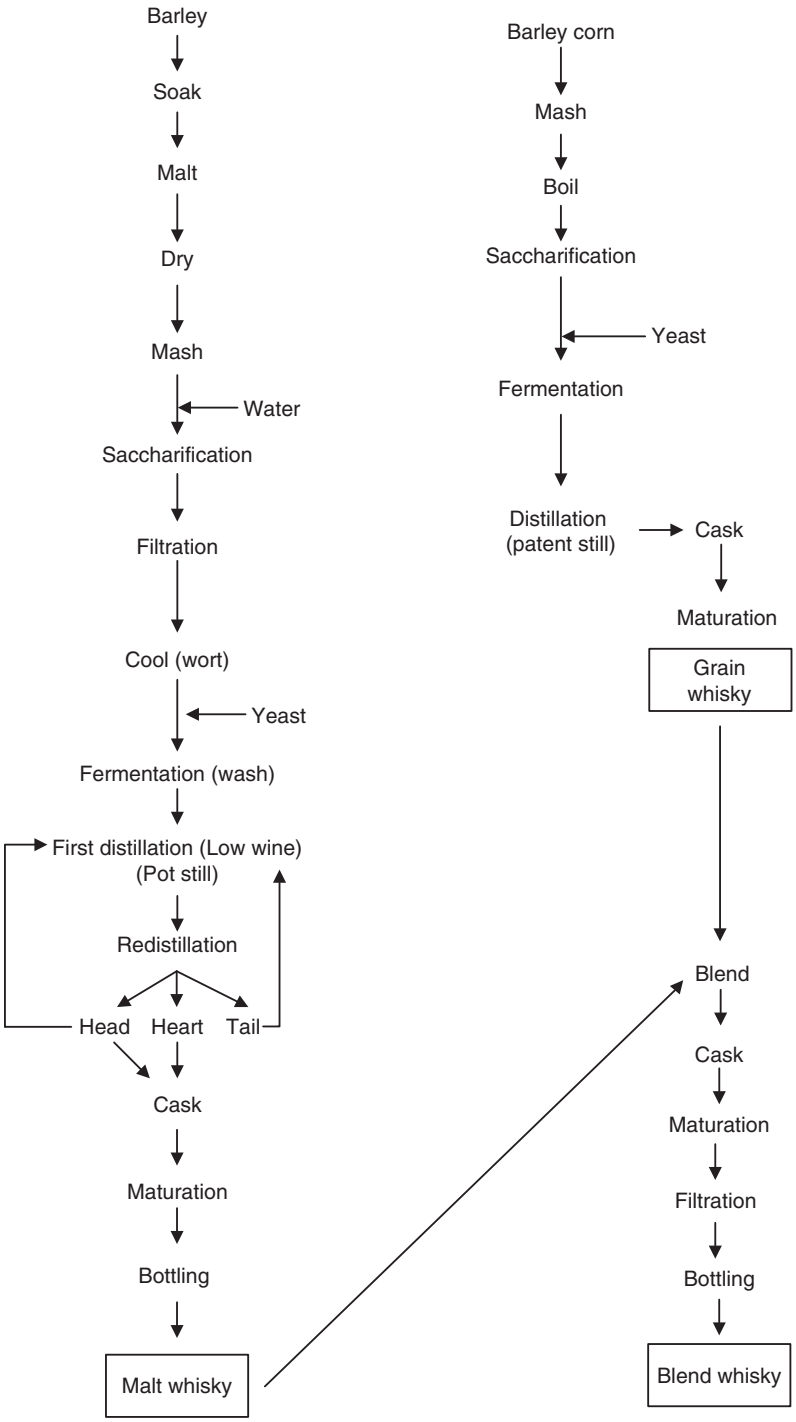


FIGURE 175.9 Flow diagram for the production of Scotch whisky.

size and design of Irish pot stills are quite different from Scottish pot stills. The size of pot stills in Scotland is in the range of 2,000 to 30,000 liters, while Irish stills are in the range of 50,000 to 150,000 liters. Irish stills are attached with the purifiers that remove congenerics such as the aldehydes, esters, furfural and other compounds of hydrogen, oxygen and carbon formed in the process of distilling of wash (2).

The distillation profiles of Irish whiskey are also different from that of Scotch whisky. The low wines obtained from the wash still are separated into two fractions according to the difference of alcohol concentration. The

weak low wines are distilled in a low wines still to obtain further fractionation, strong and weak feints. The weak feints are redistilled with the next batch of weak low wines, while the strong feints are added to the strong low wines for the third distillation (Figure 175.10).

The alcohol concentration (about 80% ABV) of the new spirit is much higher than the equivalent Scottish malt (6). New spirits that are reduced to 63.5% ABV are filled into secondhand casks of bourbon, rum and sherry for at least 3 years (usually 5 to 7 years). These straight Irish whiskeys are blended with grain whiskey before bottling. The more temperate climate of Ireland produces whiskeys with a slightly spicier taste and a crisp finish (8). Although the market of Irish whiskey is limited, it is internationally recognized as a distinct generic brand (5).

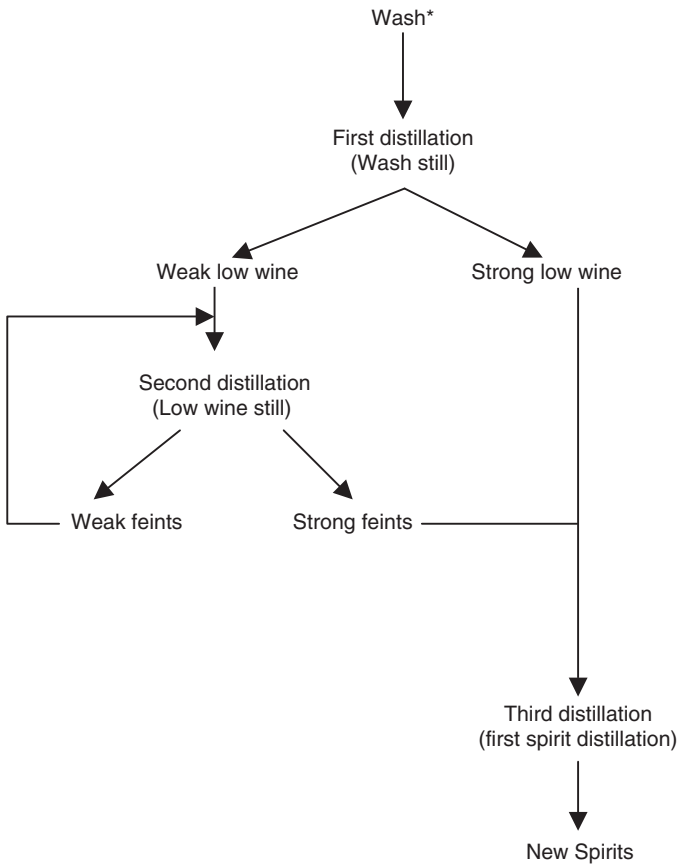
VI. AMERICAN WHISKEY

Contrary to what many people believe, Bourbon is one of a number of American whiskeys and its name was taken from Bourbon County in the state of Kentucky. By law, Bourbon must be produced from a mash of not less than 51% corn grain, and is usually made from between 70% and 90% corn, with some barley malt and rye or wheat (6,19).

The sour mash technique is one of the distinctive styles of American whiskey. The lactic acid bacteria is inoculated in order to prevent the bacterial contamination, then the residue (called backset or setback) of former distillation is added prior to yeast fermentation. If no sour mash is added at the start of fermentation, the result is called sweet-mash straight whiskey (6).

The distillation is done in column stills (patent stills) which is in common with nearly all American whiskeys. Spirits are collected at relatively low alcohol concentrations from 64 to 70% by volume. Therefore various flavor components are also included, hence the product has distinct aroma. The spirit is matured at least for 2 years in new white-oak barrels that have been charred. Charring has been used as a means of disinfecting barrels since the early 19th century. In bourbons, the definite characteristics are the tones of vanillin and caramel that the whiskey takes from the wood. It is bottled at over 40% by volume of alcohol (Figure 175.11).

Tennessee whiskey, which is legally classified as bourbon, is produced in the state of Tennessee in the same way as the method of Kentucky bourbon. The new spirit is filtered through the maple charcoal, prior to the maturation in the barrel. It has a mellow taste.



* See Figure 175.9 on flow sheet of Scotch whisky making for production of Wash.

FIGURE 175.10 Flow diagram for the production of new spirits for Irish whiskey.

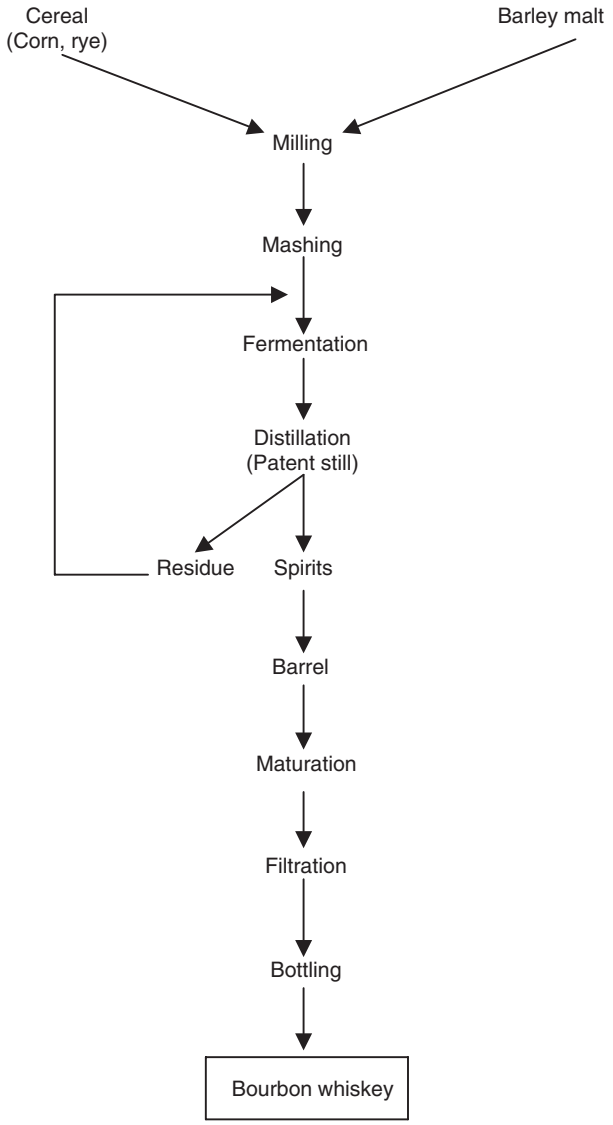


FIGURE 175.11 Flow diagram for the production of bourbon whiskey.

Rye whiskey is made from a mix of grains containing a minimum of 51% rye and straight rye is matured at least for 2 years in the charred white-oak barrels.

Corn whiskey is made from a mixture of grains containing a minimum of 80% corn. The product matured at least for 2 years in the oak barrels is called straight corn whiskey (16).

A neutral grain spirit is also made in the United States and it is exclusively used in blends.

VII. CANADIAN WHISKY

Canadian whisky is the whisky that is distilled in Canada. Hiram Walker in 1858 founded the first distillery and the product was launched as Canadian Club brand in 1884 (6). It is produced from corn, rye and barley malt. When rye is used over 51%, its product can be named 'rye whisky.'

There are two types: one is the flavoring whisky that is produced mainly from rye and the other is the base whisky that is made mainly from corn. Both are distilled in column stills. Flavoring whisky is matured in a charred barrel (new or old) and the base whisky is matured in regenerated bourbon barrels. Both are matured in the small barrels (smaller than 180 liters) for at least 3 years, then blended to make various brands. Canadian whisky is characteristic as flavory, light and mild, as compared to other whiskies (17).

Canadian whisky has been industrially developed to supply mainly the USA market. Today, there are a dozen or so distilleries across the country (6).

VIII. JAPANESE WHISKY

Although the whisky industry of Japan was not founded by Scottish/Irish immigrants, Japanese and Scottish distillers have strong ties. The first distillers were trained in Scotland and took their newly found knowledge to traditional sake distilleries in Japan (Figure 175.12) (5,8).

The first Japanese distillery was established in 1923 in the valley of Yamazaki on the outskirts of Kyoto. Its first whisky was launched in 1929. This whisky was a blended product of malt and unmatured neutral spirit and the neutral spirit has been replaced with a matured grain whisky made from North American maize.

The Japanese distilling industry have renewed their bonds with Scotland by buying into Scottish distillery operations. Other Japanese businesses and trading houses have also participated in the growth of whisky distilling, either by setting up joint venture companies or by importing Scotch whisky. They set out to produce the best raw materials, distilling technology, and maturing conditions required in producing a 'Scotch malt' type of whisky.

FIGURE 175.12 Suntory Whisky Building in Yamazaki Distillery (Japan). (Courtesy of Suntory Yamazaki Distillery.)

The landscape of the northern island, Hokkaido, is very similar to that of the Highland of Scotland with peat bogs, mountains, and cool, fresh streams, which flow over granite rocks (5,7). The peat produces a less intensive aroma than Scotland peat. Yoichi distillery of Nikka Company on the island Hokkaido east of the city Sapporo makes the most peaty of Japanese single malts (6).

Malt is imported, with a large proportion coming from Scotland and an increasing amount from Australia. Malt whisky was (and still is) fermented in wooden wash backs with cultured yeast, and distilled in traditional copper wash and spirit pot stills.

The grain whisky is made using North American maize, which is mashed and distilled conventionally. As in Scotland, malt and grain whiskies are matured in oak casks before blending (Figure 175.13).

The most popular brands are blends, but the Suntory and Nikka companies have been successfully developed single malts. They are indeed whiskies in their own right, not just as copies of Scotch as some people claim, even if a small number of Japanese blends and pure malts are based on Scotch single malts (6).

Most Japanese whisky businesses now fully own distilling, maturation and blending facilities in Scotland, setting a consumer trend that will continue into the future (Figure 175.14) (5).

IX. CONCLUSION

Whisky is world's most consistently successful and popular alcoholic drink that was commenced in Scotland in 1494 according to documentary records. Scotland, Ireland, the United States, Canada and Japan are now considered to be the five major whisky-producing countries. They developed their own individual style of whisky into something approaching a fine art.

FIGURE 175.13 Casks in the warehouse of Suntory Yamazaki Distillery (Japan). (Courtesy of Suntory Yamazaki Distillery.)

FIGURE 175.14 A display of the whisky made in the five major whisky-producing countries. (Courtesy of Suntory Yamazaki Distillery.)

Recently the 'light' whiskies that have light flavor, color and body are pushed mainly in the United States and Japanese markets. And the proportion of single malts to blends consumed, even in Scotland, is very small; but there is nevertheless a steady rise in the availability and the consumption of single malts both in Britain and in certain overseas countries (2). The marketer must have eyes to lookout the consumer trends and make an effort to meet their needs. However, each country's particular style will remain basically unchanged.

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Part T

Food Fermentation

176 Fermentation: Principles and Microorganisms

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CONTENTS

I. INTRODUCTION

The use of microorganisms to process or preserve foods is an ancient technique. Yeast was the first microorganism used in the production of wine, beer and the leavening of dough. These techniques have been known for at least 4,000–5,000 years. When these processes are underway, bubbles form as in gentle boiling. This bubbling is due to the liberation of carbon dioxide gas from the degradation of sugar. The word fermentation signifies the gentle bubbling or boiling condition in these processes.

The nature of the fermentation reaction did not become clearly understood until the late part of the 19th century when Louis Pasteur discovered the relationship between living cells and fermentation. In 1854, Pasteur demonstrated the relationship between yeast and this reaction. The word fermentation became associated with microorganisms. Pasteur also showed that true

fermentation occurs only in the absence of free oxygen. He called life without air anaerobiosis. Actually, the definition of fermentation in biochemistry is the extraction of energy from carbohydrates and other organic substrates without using O_2 as an electron acceptor. Hence fermentation is an energy-yielding catabolic pathway that proceeds with no net change in the oxidation state of the products compared to that of the substrate. The common usage of the word fermentation frequently overlooks the strict biochemistry definition. A broad sense was adopted, that is, a process in which microorganisms produce chemical changes in organic substrates through the action of enzymes produced by these microorganisms. According to the common usage, the term "fermented foods" is used to describe a special class of foods that contain a complex mixture of carbohydrates, proteins, fats, etc., undergoing simultaneous modification under the action of a variety of microorganisms and enzymes. Reactions involving carbohydrates and carbohydrate-like materials are referred to as "fermentative." Changes in proteinaceous materials are designated "proteolytic," and the breakdown of fatty substances are described as "lipolytic." When complex foods are "fermented" under natural conditions, they invariably undergo different degrees of each type of change. Whether fermentative, proteolytic, or lipolytic end products dominate will depend upon the nature of the food, the types of microorganisms present and the environmental conditions affecting their growth and metabolic patterns.

The basic concept of fermentation is to facilitate the proliferation and predomination of desirable microorganism in the raw plant materials. The desirable microorganisms will metabolize sugars into chemicals such as lactate, ethanol and acetate that infuse the plant materials with various characteristics. The addition of salt and the inoculation of a defined microbial culture are the two basic methods for controlling the growth of microorganisms during fermentation. In this chapter, we will describe the predominant bacterial strains occurring in some popular fermented vegetables, illustrate their sugar metabolic reactions, and discuss how fermentation is manipulated with these organisms.

II. THE FERMENTATION OF VEGETABLES

At present only cabbage (sauerkraut and Korean kimchi), cucumbers (pickles), and olives are of real economic importance. In this chapter, the discussion is focused on these vegetable products. In addition to these vegetables, fermented carrots, the potential new products, and fermented bamboo shoots will be described.

A. Cabbage Fermentation

Sauerkraut is a fermented product made from fresh cabbage. In the cabbage fermentation process lactic acid bacteria are favored. The addition of 2.25–2.5% salt restricts the activities of undesirable gram-negative bacteria. The fermentation is started by *Leuconostoc mensenteroids*. This bacterium converts sugar to lactic acid, acetic acid, alcohol, CO_2, and other products that contribute to the flavor of sauerkraut. CO_2 helps maintain the anaerobic conditions necessary in fermenting cabbage. As the acids accumulate, *Leu. mensenteroids* is inhibited, but the fermentation continues with *Lactobacillus brevis, Pediococcus cerevisiae*, and finally, *Lactobacillus plantarum*. *Lb. plantarum* and *Lb. brevis* effect the final stages of sauerkraut production. *P. cerevisiae* and *Enterococcus faecalis* may also contribute to product development (1).

Kimchi is a traditional Korean fermented vegetable product. Kimchi fermentation is the Korean method for preserving a fresh and crispy vegetable texture for consumption during the winter when fresh vegetables are not available. Although the history of kimchi fermentation in Korea can be traced to the 3rd and 4th centuries, the earliest description of the processing methods is found in 17th century works of literature (2). The fresh cabbage is cut in half or shredded, soaked in brine with an approximately 10% salt concentration overnight and then washed and drained. The minor ingredients (garlic, red pepper, green onion, ginger) are chopped and mixed with shredded radish and stuffed between the salted cabbage leaves. The kimchi is packed in an earthen jar, buried in the ground, and pressed with a stone placed inside in order to keep the ingredients immersed in the juice. Before ripening, *Leu. mensenteroides* is the dominant microorganism, while *Lactobacillus* spp. are the major organisms in overripened kimchi. *Lactobacillus* species may be dominant in the later stages of kimchi fermentation depending on the temperature (2).

The difference between sauerkraut and kimchi is the of fermentation end-point. The best-tasting kimchi is attained before *Lb. brevis* and *Lb. plantarum* overgrowth occurs with an optimal pH of 4.5. The *Lb. brevis* and *Lb. plantarum* overgrowth diminishes the product quality, but sauerkraut production depends on these organisms.

B. Cucumber Fermentation

In the natural fermentation of pickles, selected cucumbers are placed in brine with about 5% NaCl. The brine strength is gradually increased during fermentation until it reaches around 16% NaCl. The sugars that diffuse from the cucumbers are fermented sequentially by *Leu. mensenteroides*, *P. cerevisiae*, *Lb. brevis*, and *Lb. plantarum*. Depending on the of fermentation condition, about 0.6 to 1.2 % lactic acid is formed in about 7 to 14 days. When the pH is lowered to 3.2, the metabolism of *Lb. plantarum* is inhibited and the fermentation is completed. In this process, the high salt level is used to protect against spoilage. The fermented cucumber must be desalted before being used in products.

However, the NaCl level in the desalting solution creates a serious dumping problem. Procedures have been developed for brining cucumbers in closed anaerobic tanks at substantially lower salt concentrations (3). This approach to fermentation has the potential to allow cucumber fermentation and storage at sufficiently low salt concentrations that require no desalting.

In natural fermentation, bloating in defective pickles often occurs. Bloating is due to the accumulation of CO_2 gas inside the cucumber during fermentation. The respiration of cucumber tissue and fermentation by *P. cerevisiae* and *Lb. plantarum* produce sufficient CO_2 to cause bloating (4). The degradation of malic acid to lactic acid is a major source of CO_2 when *Lb. plantarum* ferments brined cucumbers. Research has demonstrated that using a mixed culture with a malolactic-deficient mutant and normal malolactic strain of *Lb. plantarum* in brined cucumber fermentation could reduce the level of released CO_2 (5).

In cucumber fermentation, yeast has conventionally been viewed as undesirable because it produces CO_2. However, when N_2 is used in purging cucumber fermentation tanks to prevent bloater damage, using yeast (*Saccharomyces cerevisiae* or *S. rosei*) in the mixed culture can facilitate complete sugar metabolization (6).

Softening in defective pickles is another problem. Softening is attributed to pectinolytic enzymes that degrade the cucumber tissue. The source of these enzymes may be the microorganisms growing in or on the cucumbers. To reduce fermentation defects, a controlled fermentation process is used. The controlled fermentation method employs a chlorinated brine with 25° salinometer, acidification with acetic acid, the addition of sodium acetate, and inoculation with *P. cerevisiae*, and *Lb. plantarum* (7).

C. Olive Fermentation

Olive fermentation is similar to that in sauerkraut except that the olives are soaked in a 1.6 to 2.0% lye solution before brining. The lye treatment is necessary to remove oleuropein, a bitter factor in the olives. The olives are brined in containers following the complete removal of lye by rinsing the olives in fresh water. The brine concentration varies from 5 to 15%, depending on the variety and size of the olives (8). Lactic acid bacteria become prominent during the intermediate stage of fermentation. *Leu. mensenteroides* and *P. cerevisiae* are the first lactic acid bacteria to become prominent. These bacteria are followed by lactobacilli, with *Lb. plantarum* and *Lb. brevis* being the most important (9). The lye treatment may affect the microbial flora. Inoculation with *Lb. plantarum* may be required. A study has showed that using a strain of *Lb. plantarum* with the capability to produce bacteriocin as a starter controls lactic acid fermentation much better (10). The entire fermentation process may take two weeks to several months. The acid content of the final product varies from 0.18 to 1.27% (11).

D. Carrot Fermentation

Carrots are not a traditional vegetable for fermentation. Until 1969, carrots were fermented using a home-based process (12). Fermentation provides a simple method to preserve raw carrots. The raw carrot slices contain a high level of reducing sugar that might cause Maillard reactions and produces dark compounds with a burnt smell during thermal processes. Using lactic acid fermentation, the reducing sugar content in the raw carrot can be decreased to a level that allow the carrot slices to be processed using high temperature deep-frying to yield chips. The deep-fried carrot chips have a light red-yellow color and pleasant taste that makes them a potential new product (13).

A mixed culture of *Lb. plantarum*, *Lb. brevis*, *P. cerevisiae* and *Leu. mesenteriodes* is used to ferment carrots (14). Use of carrot-adapted inocula significantly reduced the lag period for early acid production despite the salt concentration. The repressive effects of increased salt concentrations on the rate of fermentation is that carrots treated with the lowest level of salt, 1.5%, require only 10 days incubation to produce a 1.0% acid level, whereas a 3.0% salt concentration requires 18 days incubation to reach a similar acid value. The acidic properties of fermented brines resemble the fermentation properties of the cabbage head brining solution (15).

A new process for carrot fermentation using an alkaline treatment with lye before inoculating a pure culture of *Lb. plantarum* was developed (16). The alkaline treatment helps inoculum establishment over the natural flora in the fermentation. However, most of the sucrose remains unmetabolized after 7 days of fermentation. Thus, long-term stability in the fermented carrots is not ensured. A high risk of secondary fermentation may present in the package product. This process was further modified using a mixed culture of *Lb. plantarum* and *S. cerevisiae* to replace the single culture of *Lb. plantarum*. The result indicated that the mixed culture was able to completely use up all of the sugars and, at same time, improve the flavor of the fermented carrots (17).

E. Bamboo Shoot Fermentation

People in the bamboo-growing regions of Asia have traditionally consumed fermented bamboo shoots. Dried Ma-bamboo (*Dendrocalamus latiflorus*) shoot is a special product of Taiwan (18). Mesu is a similar product from India (19). Both are produced by using non-salted fermentation with natural cultures.

Using mesu as a pickle and as the base for curry is a tradition in the Darjeeling hills and Sikkim area of India. A study has shown that a total of 327 strains of lactic acid

bacteria, representing *Lb. plantarum, Lb. pentosaceus* were isolated from 30 samples of mesu. These species were present in all of the raw bamboo shoot samples tested. Mesu is dominated by *Lb. plantarum* followed by *L. brevis*. *P. pentosaceus* was isolated less frequently and recovered from only 40–50% of the mesu samples. Fermentation is initiated by *P. pentosaceus*, followed by *L. brevis*, and finally succeeded by *L. plantarum* species. During the fermentation, the titrable acidity increased from 0.04 to 0.95%, resulting in a decline in pH from 6.4 to 3.8 (20). Ma-bamboo shoots are fermented using a traditional natural culture. After 10 days of fermentation, the fermented bamboo shoots contain about 10^9 cfu/g of lactic acid bacteria, and 10^4–10^6 cells/g of yeast and mold. The final pH was 3.3 to 4.1, and the titrable acid was 1.05–1.20% (19).

III. FERMENTATION TECHNIQUES

The procedures for vegetable fermentation are varied and complicated. Basically, vegetable fermentation can be considered as a three-staged process.

A. Stage 1 Consists of the Pretreatment Steps

In this stage, the common operations include sorting and grading raw vegetables, cleaning the selected vegetables, specific pretreatment, such as peeling carrots, blanching green beans, shredding cabbage, or lye-treating olives.

B. Stage 2 Is the Fermentation Environment Adjustment Operation

Adding salt and inoculating the defined starter culture are two methods to set up a suitable environment around the vegetables to allow the desirable microflora proliferate and predominate.

Salt addition is necessary in most kinds of vegetable fermentation. The major contributions of salt are: to inhibit the growth of pathogens and destroy spoilage microorganisms; to exert a selective effect on the microorganisms present on vegetables; to enhance the release of tissue fluids from the fermenting vegetables, and to impose a special flavor on the fermented vegetables. The amount of salt used depends on the particular vegetables. In the fermentation of cucumbers and olives, the salt concentration is 5–8% at equilibrium. For cabbages the salt concentration is less than 2.5% at equilibrium. The difference in salt concentration between that used in sauerkraut fermentation and pickle fermentation probably accounts for the difference in the types of lactic acid bacteria that grow in each fermentation environment (21).

The application of a defined starter culture is an another method to facilitate the predomination of desirable microflora in the fermenting vegetables. The lactic acid bacteria used for this purpose include *Lactobacillus* species (*Lb. plantarum* and *Lb. casei* are the most often used.), *Lactococcus lactis*, and *Leu. mensenteroides*. The defined starter cultures are capable of growing rapidly and are highly competitive under the environmental conditions used to ferment products.

C. Stage 3 Is the Vegetable Fermentation Process

Temperature, pH value and anaerobiosis maintenance are major factors that influence the course of fermentation. The temperature range for vegetable fermentation is 16 to 35°C. Vegetables fermenting at 10°C lead to good-quality products. Usually, the optimal temperature is between 15 and 20°C. Various microorganisms may dominate a mixed fermentation depending on the temperature. For sauerkraut fermentation, the preferred temperature is 18°C or lower. The predominant strain *Leu. mensenteroides* grows optimally at a lower temperature than the homofermentative *Lb. plantarum*, presumably resulting in a higher ratio of volatile/nonvolatile acids than at higher temperatures. For cucumber fermentation, the predominant cultures of *P. pentosaceus* and *Lb. plantarum* are capable of rapid growth at 18°C (22). The optimal temperature for vegetable fermentation depends on the predominant cultures during the fermentation.

The buffering capacity of the vegetable affects the extent of proliferation of the predominant culture used to ferment the natural sugars. Several methods have been adopted to maintain the pH during fermentation. Sodium acetate (23) and calcium acetate (24) have been used as buffering agents to assure complete sugar utilization during the primary fermentation of cucumbers. Acid neutralization during fermentation with a pH controller has also been used to assure complete sugar utilization (25). In the fermentation of carrots, sodium hydroxide treatment of peeled and trimmed carrots is a useful alternative to pasteurization to achieve controlled fermentation. Subsequent neutralization of the NaOH by adding acetic acid to the brine could lead to the formation of a buffer system in the brine. The buffer system benefits greater utilization of the fermentable sugars by the starter culture (26). For preserving fermented vegetables for long periods of time, the pH should be controlled below 4.0 (27).

During fermentation, to maintain anaerobic conditions the plant materials must be totally covered by the brine in the vessels. Open filled vessels are normally covered with plastic sheets or wooden plates weighted down with stones or heavy matter to exclude oxygen from the air. For cucumber fermentation, anaerobic tanks provide more suitable anaerobic conditions (23). Anaerobic tanks replaced open tanks in the olive fermentation industry of the USA and Spain many years ago (28).

IV. VEGETABLE FERMENTATION MICROORGANISMS

Fresh plant material harbors numerous and varied types of microorganisms. The microflora in vegetables and fruits is largely made up of *Pseudomonas* spp., *Erwinia herbicola*, *Flaeobacterium*, *Xanthomonas*, and *Enterobacter agglomerans* as well as various molds. Lactic acid bacteria, such as *Leu. mesenteroids* and *Lactobacillus* spp., are also commonly found, as are several species of yeasts (29). Between 40% and 75% of the bacterial flora in peas, snap beans and corn was shown to consist of leuconostocs and streptococci, whereas many of the gram-positive, catalase-positive rods resembled corynebacteria (30,31). An analysis of 30 different samples of white cabbage from four growing seasons has shown that the microflora normally is dominated by aerobic bacteria(e.g., pseudomonads, enterobacteria, and coryneforms) and yeasts, while lactic acid bacteria represent 0.15 to 1.5% of the total bacterial population (32). Vegetable fermentation involves controlling specific microorganisms or a succession of microorganisms that dominate the microflora in vegetables. Although lactic acid bacteria are present as a small population, the metabolic activities of this microorganism are indispensable in the vegetable fermentation process. Lactic acid fermentation is the most important contribution to the fermentation of vegetables.

A. The Major Lactic Acid Bacteria in Vegetable Fermentation

The major lactic acid bacteria involved in vegetable fermentation are located in three genera, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. Among the lactobacilli, several species and strains have been isolated from fresh vegetables. These include the homofermentative species *Lb. plantarum*, *Lb. casei, Lb. arabinosus*, and *Lb. homohiochii*, and the heterofermenters *Lb. brevis*, *Lb. fermentum*, and *Lb. buchneri.* The genus *Pediococcus* comprises two species, *P. pentosaceus* and *P. acidilactici.* Currently, *Leuconostoc* comprises a single species, *Leu. mensenteroides* (33).

The lactic acid bacteria share some common features: Gram positive; mesophilic but some can grow at temperatures as low as 5°C or as high as 45°C; growing at pH 4.0–4.5, some are active at pH 9.6 and others at pH 3.2; generally weakly proteolytic and lipolytic and require preformed amino acids, purine and pyrimidine bases and B vitamins for growth; do not contain a citric acid cycle or a cytochrome system no energy derived from oxidative phosphorylation, but energy is obtained via substrate level phosphorylation during the fermentation of sugars into lactic acid, ethanol or acetate, and CO_2.

There are four important species of lactic acid bacteria associated with vegetable fermentation: *Leu. mensenteroides, Lb. brevis, P. pentosaceus*, and *Lb. plantarum.* These species are successively predominant during sauerkraut fermentation in the approximate order listed (7). *Lb. brevis, P. pentosaceus*, and *Lb. plantarum* have also been reported to ferment cucumbers (34) and olives (35). The properties of these four species are described as follows.

1. *Leu. mensenteroides*

The colorless bacterial cell is spherical or egg-shaped and appears usually in pairs. The size of the bacterium is 0.5–0.7 μm. *Leuconostoc* is distinguished among the lactic acid bacteria in being heterofermentative and also in lacking aldolase, a key enzyme in glycolysis. Under anaerobic condition, this bacterium metabolizes glucose via phosphoketolase pathway, and produces D-lactate. At the temperature range of 20 to 25°C, this bacterium produces dextrans from sucrose. This bacterium is capable of metabolizing citrate into CO_2 and diacetyl, which is an important flavor component in many dairy products.

2. *Lb. plantarum*

Lb. plantarum is the final and predominant lactic acid bacterium species at the completion of fermentation in many vegetables. This is attributed to its metabolic diversity and tolerance for low pH conditions. The optimal growth temperature for this bacteria is 30°C. The bacterial cell is a short to medium rod usually in single, but sometimes in pairs or short chains. The size of the bacterium is 0.9–1.2 (width) × 3–8 (length) μm. This bacterium is classified as a facultative heterofermenter according to the metabolism of hexoses (36). This bacterium possesses both aldolase and phosphoketolase. Its homofermentative action on glucose with aldolase results in producing up to 1.5% DL-lactate. The lactate can be further metabolized to acetoin, formate, and acetate under certain conditions. In the heterofermantation of pentose via the phosphoketolase pathway, this bacterium produces lactate, acetate and CO_2. Strains are often adopted as acid producers in starter cultures. This bacterium is the first species recognized to possess the unique ability to protect against oxygen-free radicals by a nonenzymatic superoxide reduction mediated by manganese (37).

3. *Lb. brevis*

This bacterium has a short rod shape, occurring singly or in short chains. The size of the rod cell is 0.7–1.0 (width)× 2.0–4.0 (length)μm. The optimal temperature for growth is 30°C. This bacterium is heterofermentative, and metabolizes glucose to DL-lactate, ethanol, acetate, and CO_2. This bacterium is able to reduce fructose to mannitol.

4. *P. pentosaceus*

This is a spherical bacterium, occurring in pairs, tetrads, or cluster. The size of the coccus is 0.8–1.0 μm in diameter.

This bacterium cannot grow at temperatures over 45°C. The optimum growth temperature is in the range of 28–30°C. The optimum and final pHs are 6.5–6.0 and 4,0 respectively. This bacterium is homofermentative and produces DL-lactate from glucose. Most strains ferment arabinose, ribose, maltose, fructose, galactose and glucose to produce DL-lactate. Strains that are capable of fermenting xylose and lactose are known. Some strains produce bacteriocins during fermentation.

B. The Lactococci in Vegetable Fermentation

Although lactococci are not major lactic acid bacteria in the fermentation of vegetables, this bacteria support the fermentation with proteolytic activity (38) and the capability to break down citrate (39). Recently, some strains of *Lactococcus lactis* subsp. *lactis* with the capability of producing bacteriocin were isolated from minimally processed fresh vegetable and fruit products. Some researches have used these bacteriocin-producing lactococcal cultures as biopreservative in minimally processed fresh vegetables and fruits (40,41). Lactococci are spherical or ovoid cells that occur singly, in pairs, or as chains. They grow at 10°C but not at 45°C. They are homofermentative and produce L-lactate as the predominant end product of sugar fermentation.

C. The Yeasts in Vegetable Fermentation

Various groups of yeast are present at the beginning of vegetable fermentation. Sometimes, these yeasts become predominant in the fermentation. For example, in the fermentation of olives, yeasts are the predominant microorganisms in spontaneous fermentation, because the polyphenol compounds in olives affect the microflora by inhibiting the growth of lactic acid bacteria but not yeasts (42). As a result, the final product has a shriveled form, a high salt content and the principle bitterness does not disappear completely from the product. To limit the negative effects of yeast on the product quality, it is necessary to remove the polyphenols, acidify the cover brine and lower the salt content to enhance lactic acid bacterial growth (43). It is well known that yeast can proliferate under low pH conditions which usually inhibits the growth of lactic acid bacteria. In the spontaneous fermentation of tart carambola, which contains affluent organic acid with a pH is as low as 1.37–2.01, the yeasts *Candida pelliculosa, C. inconspicua, C. ciferrii,* and *S. cerevisiae* become predominant in the brine with 7–10% salt (44). In the cucumber fermentation, yeasts can affect the fermentation by utilizing sugars that would otherwise be metabolized to lactic acid by the lactic acid bacteria. The yeasts can also utilize the produced lactic acid, raise the pH, and allow other microorganisms to grow. The yeasts produce large amounts of gas. This is associated with pickles that bloated or have hollow defects. Although fermentative yeasts have been viewed as undesirable in vegetable fermentation, yeasts may facilitate the removal of fermentable sugars. In the cucumber fermentation, when N_2 is used to purge the cucumber fermentation tanks to prevent bloater damage, a selected yeast (*S. cerevisiae* or *S. rosei*) in a mixed culture with *Lb. plantarum* can help to exhaust the fermentable sugars rapidly and improve the quality of the products (6).

V. FERMENTATION BIOCHEMISTRY

Lactic acid bacteria are the dominant microflora in most fermented vegetables. Under normal food fermentation conditions, the main product from lactic acid bacteria metabolism is lactic acid with other products formed as by-products, such as acetic acid, acetaldehyde, ethanol, and diacetyl. All of these products contribute to control the growth of spoilage microorganisms and the specific flavor of the fermented products. Lactic acid bacteria are divided into two groups based on glucose metabolism end products. Those that produce lactic acid as the major or sole product of glucose fermentation are designated homofermentative. *Pediococcus, Lactococcus*, and some lactobacilli belong to this group (homolactics). Those organisms that produce equal molar amounts of lactate, carbon dioxide and ethanol from hexoses are designated heterofermentative. *Leuconostoc* and some lactobacilli are heterofermentative (heterolactics). The major metabolitic pathways of these organisms are described as follows.

A. Carbohydrates Metabolism

In general, approximately 75% of the solids in plants are carbohydrates. Total carbohydrates generally consist of simple sugars, starches, pectic substances, lignin and cellulose. Cellulose, pectic substances and lignin occur in all plants as the principal structural component of the cell walls. These structural polysaccharides contribute greatly to the characteristic texture of plant foods. These structural polysaccharides are usually not fermentable. The most common fermentable carbohydrates in vegetables are glucose, fructose, sucrose and starch.

1. Glucose Metabolism

Glucose is the major fermentable sugar in vegetables. Homolactics metabolize glucose via the glycolytic pathway to yield pyruvate. Pyruvate is further reduced to lactic acid via the enzyme lactate dehydrogenase. The pathway that converts glucose to lactic acid is called lactic acid fermentation.

Heterolactics produce lactic acid via the phosphoketolase pathway. This pathway involves the initial splitting of CO_2 from the glucose molecule, followed by a further splitting of the resulting pentose (xylulose-5-phosphate) into two-carbon and three-carbon fragments in a phosphoroclastic reaction catalyzed by phosphoketolase, yielding glyceraldehyde-3-phosphate and acetylphosphate respectively. The three-carbon fragment is eventually

reduced to lactate in the same way as homolactics, and the two-carbon fragment is reduced to ethanol. Products other than lactate are generated, the pathway is, therefore, called the heterofermentative pathway. Because lactic acid bacteria lack functional heme-linked electron transport chains and a functional Krebs cycle, they obtain energy via substrate level phosphorylation. In the heterofermentative pathway, 1 mole of ATP is produced per mole of glucose metabolized compared with 2 mol in the homofermentative pathway. Thus, the fermentation of glucose via the heterofermentative pathway is only half as efficient as in the homofermentative pathway.

2. Fructose Metabolism

Fructose is the second major sugar substrate for lactic acid fermentation in vegetables. Lactic acid bacteria contain fructokinase and phosphoglucoisomerase to phosphorylate fructose to fructose-6-phosphate and then isomerize to glucose-6-phosphate. In the homofermentative pathway, glucose-6-phosphate is further metabolized to pyruvate via glycolysis. The pyruvate is then reduced to lactate via lactate dehydrogenase. In contrast to homolactics, heterolactics contain mannitol dehydrogenas which catalyze the reduction of fructose to mannitol and oxidize NADH under anaerobic conditions (45). In this reaction a small amount of fructose is used as an electron acceptor with the remaining fructose converted to lactate, ethanol, acetate, and CO_2 (46).

3. Sucrose Metabolism

The sucrose content is less than the glucose and fructose content in most vegetables. The metabolism of sucrose in vegetable fermentation is usually incomplete in the final stage. For example, when beets containing 3.8% sucrose were fermented using *Lb. plantarum* for 12 days, the fermented beets still contain 2.8% residual sucrose. Using the same starter to ferment carrots for 35 days, 1.96% of the sucrose was reduced to 0.91% but the glucose and fructose in the fermented carrots were exhausted at the same time (47). Sucrose does not seem to be an optimal fermentable sugar for lactic acid bacteria. Actually, only a few lactic acid bacteria possess the ability to ferment sucrose. A screen test report showed that among 14 strains of lactic acid bacteria *Lb. cellobiosus* appeared to be the only species that metabolizes all of the sucrose in green bean juice. Under the same conditions, *Lb. buchnerii, Lb. fermentum,* and *Leu. mensenteroides* ferment about half of the sucrose in green bean juice (48). These lactic acid bacteria are able to hydrolyze sucrose with β-glucosidase. These products, glucose and fructose, can be metabolized via the pathways previously mentioned.

4. Starch Metabolism

The starch content in most fermented vegetables is limited, hence the amylolytic ability of lactic acid bacteria is a characteristic with little demand. Although hydrolyzing starch to simple sugars is not important in traditional fermented vegetables, a few amylolytic lactic acid bacteria have been isolated from starchy raw materials. An investigation of Mexican pozol, a fermented maize dough, indicates that lactic acid bacteria accounted for 90–97% of the total active microflora. Strains of lactic acid bacteria were isolated and identified including *Leu. mesenteroides*, *Lb. plantarum*, *Lb. confusus, L. lactis* and *L. raffinolactis* (49). From sour cassava starch fermentation *Lb. plantarum* and *Lb. manihotivorans* were isolated. *Lb. manihotivorans* grow and convert starch into lactic acid more rapidly and efficiently than *Lb. plantarum* (50) (51). During fermentation, these amylolytic lactic acid bacteria degrade the starch first, and then the resulting sugars allow a secondary flora to develop. An acidophilic starch-hydrolysing enzyme secreted from a strain of *L. plantarum* was isolated and partially purified. This enzyme has a molecular mass of approx. 230 kDa and is capable of hydrolysing soluble starch, amylopectin, glycogen and pullan. The major reaction products from soluble starch were maltotriose, maltotetraose and maltopentaose. These reaction products suggest that this enzyme may hydrolyses both α-1,6- and α-1,4-glucosidic linkages (52).

B. Organic Acids Metabolism

Citrate and malate are the most abundant organic acids in plants. Citrate metabolism is important in fermented dairy products while malate metabolism is important in wine.

The organisms responsible for citrate metabolism in starter cultures are leuconostoc and Cit^+ lactococci. Citrate is hydrolyzed to oxaloacetate and acetate by citrate lyase. Citrate lyase is inducible in leuconostocs and constitutive in Cit^+ lactococci (53). The oxaloacetate is decarboxylated to pyruvate, which can undergo several further transformations to diacetyl, acetoin, and 2,3-butylene glycol (54).

Malic acid is fermentable by lactic acid bacteria. Both homolactics and heterolactics are able to decarboxylate malic acid to lactic acid and CO_2. Minimal CO_2 production has been ascribed a beneficial role in maintaining anaerobiosis in sauerkraut. In cucumber fermentation, CO_2 production causes bloater damage. The decarboxylation of malic acid is undesirable in cucumber fermentation. *Lb. plantarum* produces most of CO_2 during cucumber juice fermentation via the decarboxylation of malic acid (55). Strains of *L. plantarum* that do not decarboxylate malic acid (MDC^-) might improve cucumber fermentation. Some MDC^- mutants have been obtained through N-methyl- N′-nitro-N-nitrosoguanidine mutagenesis of MDC^+ parent strains. These mutants did not produce significant amounts of CO_2 when they fermented cucumber juice containing native malate (56).

C. Biogenic Amines Biosynthesis

Fermented vegetables usually contain small amounts of biogenic amines. An excessive intake of biogenic amines may

cause food poisoning. Biogenic amines may also be considered carcinogens because of their ability to react with nitrites to form potentially carcinogenic nitrosamines (57). Most biogenic amines present in fermented vegetables are formed by the action of microorganisms through the decarboxylation of amino acids during fermentation. Sauerkrauts and sauerkraut-like products are popular fermented vegetables in many countries. Thus, sauerkraut could represent an important source of biogenic amines in daily diets. The biogenic amine content in commercial sauerkraut products is approximate 540 mg/kg (58). The main biogenic amines in sauerkraut are histamine, tyramine, putrescine and cadaverine derived from histidine, tyrosine, ornithine, and lysine, respectively. Biogenic amine formation in the initial stage of a spontaneous fermentation is correlated with the growth of *Leu. mensenteroides. Pedicoccus* species are also responsible for the formation of biogenic amines. It was observed that the production of histamine was associated with the vigorous growth of the *Pedicoccus* species (32). *Lb. plantarum* starter cultures were able to suppress the formation of tyramine, putrescine, and cadaverine by raising the pH to impede the growth of biogenic amine producers (59). Hence, it is possible to decrease the biogenic amine content in fermented vegetables by using lactic acid bacteria inoculates.

VI. STARTER CULTURE IMPROVEMENT

In vegetable fermentation starter cultures with certain beneficial bacteria are desirable. Cultures with desirable characteristics can be achieved through genetic modifications. Artificial mutagenesis and DNA recombination techniques are two available methods to generate genetically modified strains.

References on using mutagenesis to improve starter cultures for the vegetable fermentation are rare. One (60) of these researches describes procedures to obtain *L. plantarum* mutants that have lost the ability to decarboxylate malic acid (MDC^-) from the parent strain (MDC^+). In this research, the parent strain was mutagenized with N-methyl-N′-nitro-N-nitrosoguanidine, and the derived mutants were then screened with designed media to select the MDC^- mutants (60). The MDC^- mutants do not produce CO_2 from the degradation of malic acid. When using MDC^- culture in cucumber fermentation, the cucumbers were shown to be less susceptible to bloating (61). Another research involving mutation involved cultivating variant *L. delbrueckii* strains that were able to tolerate high concentration of lactic acid (62). Treatment with ethyl methanesulfonate mutated the parent strain. The lactic acid tolerant mutants were then selected using an acclimation and selection procedure. This procedure was reported to be successful in consistently producing stable mutants with enhanced lactic acid production capacity.

Recently the DNA recombinant technique has superseded classic mutagenesis in the field of industrial strain improvement. In industrial food fermentation, new genetic techniques have already been applied to lactic acid bacteria to generate desirable starter cultures (63). No customized genetically modified strains for vegetable fermentation have yet been developed. However, some *Lb. plantarum* strains used as grass silage starters have been genetically modified by introducing heterologous genes to gain desirable attributes. Because *Lb. plantarum* is also a dominant microorganism in the fermentation of vegetables the genetic modification of the silage starter *Lb. plantarum* will be a useful model for cultivating desirable vegetable fermentation cultures in the future.

It has been customary to add soluble carbohydrate to silage to facilitate rapid fermentation. If a starter with the ability to hydrolyze cellulose was used in the fermentation of silage, rapid fermentation might be achieved even without adding carbohydrates. According to this thinking, a genetically modified *Lb. plantarum* with the ability to degrade cellulose was cultivated. An *Lb. plantarum* strain was transformed by inserting the *cel*E gene coding endoglucanase from *Clostridium thermocellum* (64). A transformed *Lb. plantarum* strain possessing endoglucanase activity may be useful in improving the fermentation of olives and cabbage by producing acids rapidly, because this strain is able to supply mono- and disaccharides through the hydrolysis of cellulose.

A combination of polysaccharides metabolism and lactic acid fermentation trait is desirable for a starter strain to ferment plant material. For this objective, an *Lb. plantarum* silage starter strain was transformed by electroporation with plasmids containing an α-amylase gene from *Bacillus stearothermophilus* and an endoglucanase gene from *Clostridium thermocellum* (65). The transformed *Lb. plantarum* is a turely cellulolytic and amylolytic silage starter bacterium with the ability to produce lactic acid from the fermentation of cellulose and starch materials.

Increasing ethanol levels in lactic acid fermentation may be valuable in developing vegetable juice products. A strain of *Lb. plantarum* deficient in both D- and L-lactate dehydrogenase activity was constructed by using a two homologous recombination processes (66). Following cloning a alcohol dehydrogenase gene and pyruvate decarboxylase gene originating from *Zymomonas mobilis* in this lactate dehydrogenase-negative strain resulted in an ethanol production of more than 400 mM (almost 2%).

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177 Fermented Food and Starter Cultures

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CONTENTS

I. INTRODUCTION

Fermentation is a process in which microorganisms, in the absence of oxygen, generate energy by oxidizing carbohydrates and related compounds. It has been used since ancient times as an important method for preserving food. Vegetables, fruits, cereals, milk and other raw materials have been treated in special ways in order to promote the growth of beneficial microorganisms while inhibiting the growth of deteriorating and pathogenic microorganisms. Fermentation will preserve the food and enhance the taste, aroma, texture and nutritional value of the product. The preservation effect is the result of synthesis of lactic acid and by heterofermentation also, acetic acids and, some times, antimicrobial substances. Besides lowering the pH level, the organic acids are also toxic for many microorganisms. It is also important that the fermentable carbohydrates are completely utilized by the fermenting microorganisms and thereby made unavailable for the undesirable microorganisms. In some product the addition of salt will increase the shelf life of the products by lowering of the water activity. The natural habitats of lactic acid bacteria, yeast and mold are most often plant materials. However, the type of organisms can vary considerably [1], depending on type of plant, climatic conditions, and available nutrients in the raw material. During some fermentation (e.g., fermentation of plant material such as cabbage, cucumbers, olives, soya beans, and coffee), several different types of microorganisms are required at the various stages of the fermentation process. In other fermentations (e.g., production of yogurt and beer), only a few different microorganisms are required.

Even though the involvement of microorganisms and their importance for the fermentation process was not

known until relatively recently, it was found by practice that for some fermentations the addition of a portion of a previous fermentation was beneficial for the process. With the utilization of pasteurization of the milk in the late 19th century, it was discovered that bacteria was necessary for the souring of milk for production of butter. In 1878, Joseph Lister isolated a pure culture from sour milk and named it *Bacterium lactis* [2]. Later Sigurd Orla-Jensen (see Figure 177.1) classified this bacteria bacterium as *Streptococcus lactis* (see Figure 177.2) [3]; today it is classified as *Lactococcus lactis* subsp. *Lactis* [4]. In the late 19th century Vilhelm Storch (see Figure 177.1) in Denmark, Herman Weigmann in Germany and H.W. Conn in USA [5] independently introduced the addition of pure cultures to milk in order to improve fermentation. In 1896 Storch was granted a USA patent on production of starter cultures. The Danish pharmacist Chr. D. A. Hansen (see Figure 177.1) pioneered the production of commercial starter cultures as he initiated the production of liquid and dried cultures as early as the late 1880s. Most of the bacteria used for milk fermentations were named lactic acid bacteria (LAB) because they mainly produce lactic acid by their catabolism of the milk sugar (lactose) and because they were used to start or begin the fermentation, they were termed starters or starter cultures [6].

A breakthrough in the history of wine occurred when Louis Pasteur described "life without oxygen" and of brewing fermentation when Emil Christian Hansen (see Figure 177.3) at Carlsberg brewery (Copenhagen) in the late 19th century isolated a pure yeast culture from single cells. Alfred Jørgensen (see Figure 177.3), another Dane, managed to introduce pure yeast cultures as starter cultures in brewing all over the world. For wine making the concept of inoculating wine fermentations with pure yeast starter cultures was introduced by Müller-Thurgau in 1890 and in 1965 the first commercial dried starter cultures were produced for a large Californian winery [7]. In 1920 the "fed-batch" process was introduced for the production of baker's yeast and that resulted in a significant increase in the industrialised production of baker's yeast [8]. The involvement of molds (mycelial fungi) in food fermentation goes back to the first records on blue and white molded cheeses. The first records for the production of well-known cheeses such as Gorgonzola and Roquefort

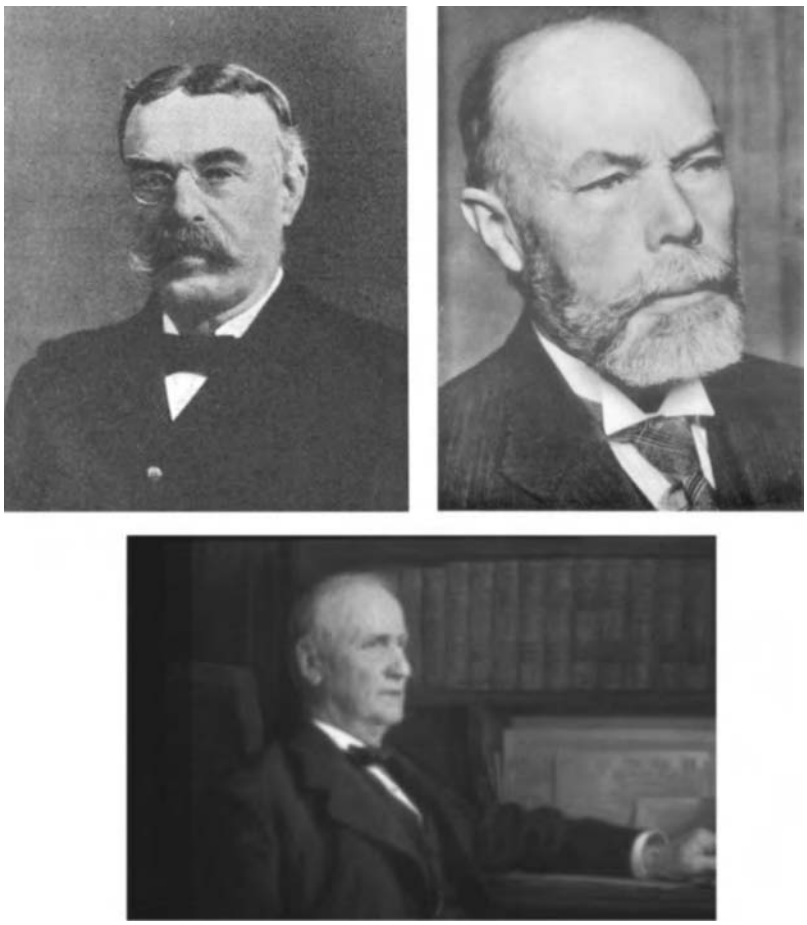

FIGURE 177.1 Photos of the Danish scientists Vilhelm Storch (1837–1918), Sigurd Orla-Jensen (1870–1949), and Chr. D.A. Hansen (1843–1916) important for isolation, identification and classification of lactic acid bacteria and their utilization as starter cultures.

FIGURE 177.2 Micrographs of different lactic acid bacteria made by S. Orla-Jensen in 1919 (From ref. [3]). The magnification is 1000 times.

is dated to 879 and 1070 respectively [8]. Eventually, the cultures involved in the fermentation were purified and starter cultures are now commercially available for cheese and meat even though not developed to a great extent.

Over the years the concept of utilization of starter cultures for the production of fermented food, especially of bread, beer, fermented milk products and cheeses, and for the production of sausages has gained increased attention.

II. FERMENTATION PROCESSES

Fermentations can basically be performed either by spontaneous fermentation, by back-slopping or by addition of starter cultures. By spontaneous fermentation the raw material, and its initial treatment, will encourage the growth of an indigenous flora [9]. For most spontaneous fermentations a microbial succession takes place, quite often LAB will initially dominate followed by various species of yeasts. Molds will only grow aerobically which limits their occurrence in certain types of fermented products. LAB produce lactic acid and other antimicrobial substances that will inhibit the growth of harmful bacteria, along with reducing the sugar content and, thereby, prolonging the shelf-life of the product. Yeasts mostly produce aroma components and alcohols [10]. When molds are involved in the fermentation they generally contribute by producing both intra- and extracellular proteolytic and lipolytic enzymes which highly influence the flavour and texture of the product [8].

In back-slopping, a part of a previous batch of a fermented product is used to inoculate the new batch. This procedure produces a higher initial number of beneficial microorganisms than found in raw material and ensures a

Emil Chr. Hansen

Alfred Jørgensen

FIGURE 177.3 Photos of the Danish scientists Chr. Emil Hansen (1842–1909) and Alfred Jørgensen (1848–1925) significant for isolation of a pure culture of yeast and its utilization as starter cultures within brewing.

faster and more reliable fermentation than occurs in spontaneous fermentation. This procedure probably also favors the growth of bacteria producing antimicrobial substances, ensuring the growth of the same bacteria every time. Examples of back-slopping are home-made fermentation of milk, vegetables and cereals. Industrial production of sourdough bread is often also done by back-slopping; a sample of the previous days sourdough is used to inoculate a new batch of dough [8].

Addition of starter cultures is most often used when it is possible to inactivate the indigenous flora by heat treatment of the raw material, permitting the growth of only the added starter microorganisms. However, it is not always possible to heat treat the raw material (e.g., fruits and vegetables) without influencing the texture of the final product [11]. Nevertheless, the addition of starter cultures—especially those containing a bacteriocin-producing strain alone or in combination with selected bacteriocin-resistant strains—may in fermentations of plants yield a greater possibility that the desirable flora will dominate in the fermentation [12–14]. Starter cultures can basically be classified as shown in Table 177.1.

Single strain starter cultures are primarily used for yeasts and molds in the production of beer and wine, and LAB for the production of a few dairy products, sausages and sauerkraut. Multiple starter cultures are used for dairy products, sourdough, sausages and wine. Mixed undefined bacterial starter cultures, also called traditional or artisanal starters [15], are primarily used in the dairy industry and in sourdough production.

III. BACTERIAL STARTER CULTURES

A. Introduction

Starter cultures can also be classified according to their optimal growth temperature as shown in Table 177.2. The most important bacteria for food fermentation are designated lactic acid bacteria (LAB). They are gram-positive rods or cocci, non-spore formers, catalase negative, obligatory fermentative, microaerophilic, usually non-motile bacteria having extensive growth requirements. They produce mainly lactic acid from glucose fermentation. The first classification of LAB was made in 1919 made by S. Orla-Jensen (see Figure 177.2) [3]. However, utilization of DNA technology and molecular typing methods has had a great impact on the taxonomy of bacteria and has led to a great deal of taxonomic revision. Table 177.3 shows a list of common LAB which have received new names within the last two decades. Table 177.4 shows the genera into which the LAB are presently divided. Table 177.4 also shows which pathways LAB use for fermentation of glucose.

Homofermentative LAB mainly makes lactic acid as their final product, whereas heterofermentative LAB makes equal amounts of lactic acid, acetic acid and CO_2. Other bacteria like *Acetobacter, Bifidobacterium, Micrococcus,* and *Staphylococcus* are also used as starter cultures for food and beverage fermentations; *Brevibacterium* and *Propionibacterium* are used as secondary or adjunct cultures. Some *Lactobacillus* species are also used as adjunct cultures.

TABLE 177.1
Definitions of Starter Cultures

Name	Contains
Single strain starter	A single well defined strain with known technological properties
Multiple starter cultures	2–6 well defined strains with known technological properties
Mixed strain starter	An unknown number of undefined strains

TABLE 177.2
Types of Starter Cultures

Type	Optimum Temperature	Typical Growth Temperature
Mesophilic	25–34°C	18 to 30°C
Thermophilic	37–44°C	40 to 44°C

TABLE 177.3
Name Changes of Common Lactic Acid Bacteria Resulting from Taxonomic Reversions*

New Names	Old Names
Carnobacterium divergens	*Lactobacillus divergens*
Carnobacterium piscicola	*Lactobacillus carnis*
Lactobacillus delbrueckii subsp. *bulgaricus*	*Lactobacillus bulgaricus*
Lactobacillus delbrueckii subsp. *lactis*	*Lactobacillus lactis*
Lactobacillus rhamnosus	*Lactobacillus casei* subsp. *rhamnosus*
Lactobacillus sakei	*Lactobacillus sake* and most strains of *Lactobacillus bavaricus*
Lactobacillus sanfranciscensis	*Lactobacillus sanfrancisco*
Lactococcus lactis subsp. *cremoris*	*Streptococcus cremoris*
Lactococcus lactis subsp. *lactis*	*Streptococcus lactis*
Lactococcus lactis subsp. *lactis* biovar. diacetylactis	*Streptococcus diacetylactis*
Leuconostoc mesenteroides subsp. *cremoris*	*Leuconostoc citrovorum*
Leuconostoc mesenteroides subsp. *dextranicum*	*Leuconostoc dextranicum*
Oenococcus oeni	*Leuconostoc oeni*
Streptococcus thermophilus	*Streptococcus salivarius subsp. thermophilus*
Tetragenococcus halophilus	*Pediococcus halophilus*
Weissella confusa	*Lactobacillus confusus*
Weissella paramesenteroides	*Leuconostoc paramesenteroides*

* Not all of these listed bacteria are used as starter or adjunct cultures; some have only been isolated from fermented food and beverage products. An updated list is present on http://www.bacterio.cict.fr.

TABLE 177.4
Genera of Lactic Acid Bacteria

Name	Old Name	Number of Species	Sugar Fermentation
Lactococcus	lactic or group N *Streptococcus*	5	Homo
Enterococcus[1]	faecal *Streptococcus*	14	Homo
Streptococcus[2]		39	Homo
Leuconostoc	*Betacoccus*	9	Hetero
Oenococcus	*Leuconostoc*	1	Hetero
Pediococcus		6	Homo
Tetragenococcus	*Pediococcus*	1	Homo
Lactobacillus		>60	Group I: Homo Group II: Facultative hetero[3] Group III: Hetero
Carnobacterium	*Lactobacillus*	6	Homo
Weisella	1 previously *Leuconostoc*, 5 previously *Lactobacillus*	7	Hetero

[1] Several are pathogenic.

[2] Many are pathogenic.

[3] Ferment glucose by the homofermentative pathway and pentoses and 6-P-glyconate by the heterofermentative pathway.

B. Cultures for Milk Fermentation

The utilization of starter cultures in industrial milk fermentation is widespread. The most important starter cultures are the LAB. These cultures often consist only of mesophilic or thermophilic LAB, however, mixtures can also occur. Mesophilic starter cultures originate from North and East Europe. They consist primarily of *Lactococcus lactis* subsp. *cremoris* (*L. cremoris*), *Lactococcus lactis* subsp. *lactis* (*L. lactis*), *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (*L. diacetylactis*), *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lacti*s. Especially, *L. cremoris and L. cremoris* are capable of rapid acidification of milk. *L. diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis* can catabolize citrate into CO_2 and diacetyl. CO_2 is responsible for the production of holes in the cheeses, while diacetyl, the characteristic flavor of butter, is important for the flavor of many cheeses and fermented milk products. The diacetyl producing organisms are often called the aroma producers or *L. lactis* subsp. *lactis* (citrate +). *Lactobacillus paracasei* and *Lactobacillus casei* are the most frequent mesophilic lactobacilli found in many cheeses and are in some cases used as adjunct cultures [16–19]. *Lactobacillus rhamnosus, Lactobacillus plantarum* and *Lactobacillus curvatus* are also found in many chesses [17,18,20]. The dairy propionibacteria *Propionibacterium shermanii* and *Propionibacterium freudenreichii* are used in some Swiss type cheeses such as Emmental, Gruyère and Comté, in which they slowly catabolize lactate to propionate, acetate and CO_2. This is important for the production of holes and taste of the cheeses [20,21]. *Micrococcacaceae* and

Brevibacterium are used as surface flora in various chesses [22], they are important for cheese ripening.

Different types of mixed starter cultures have been developed. The composition of the different mesophilic starter cultures, and examples of products for which they are used is shown in Table 177.5. The most abundant cheese produced is cheddar cheese. It is commonly produced by the use of a multiple strain starter culture of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* with or without *Streptococcus thermophilus*. However, a mixed O-culture, TK5, has also been developed [23,24].

Thermophilic cultures originate from South and East Europe. They consist mainly of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *lactis, and Lactobacillus helveticus.* The thermophilic LAB are used for rapid acidification or as adjuncts in cheeses. *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and some *Lactobacillus delbrueckii* subsp. *lactis* catabolize lactose into lactate and galactose, which may be secreted. The residual galactose can give problems (e.g., growth of undesirable bacteria) in cheese and influences the browning of pizza cheese. *Lactobacillus helveticus* can use galactose as a carbon source and thereby remove the residual galactose. Furthermore, some strains of *Lactobacillus helveticus* are very proteolytic, thereby influencing the taste and texture of cheese [25,26]. Thermophilic LAB can also produce acetaldehyde, which is the characteristic flavor of yogurt. The composition of these starter cultures varies. Both defined single or multiple starter strain cultures and mixed undefined cultures are used. Mozzarella and yogurt is commonly produced by single or multiple strain starters which contain one or more *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains, but many cooked cheeses are produced with mixed cultures. They are used either as milk cultures or whey cultures, with or without rennet. Thermophilic cultures and some examples of their products are shown in Table 177.6.

Other cheeses or fermented milk products are made with both mesophilic or thermophilic LAB starter cultures, or by addition of other LAB such as *Enterococcus faecium, Lactobacillus acidophilus* or other genera of bacteria (e.g., *Bifidobacterium*). Yeast and mold can also be added. Table 177.7 gives examples of dairy products provided with different combinations of microor-ganisms.

TABLE 177.5
Composition of Different Types of Mesophilic Starter Cultures and Some Examples of Their Products

Type	Organisms	Composition	Products
O	*Lactococcus lactis* subsp. *lactis*	5–10%	Cheddar
	Lactococcus lactis subsp. *cremoris*	90–95%	Cottage cheese
			Feta
			Quarg
L	*Lactococcus lactis* subsp. *lactis*	5–10%	Lactic butter
	Lactococcus lactis subsp. *cremoris*	80–90%	Feta
	Leuconostoc mesenteroides subsp. *cremoris* + *Leuconostoc lactis*	5–10%	Cheddar
D	*Lactococcus lactis* subsp. *lactis*	5–10%	Lactic butter
	Lactococcus lactis subsp. *cremoris*	70–85%	
	Lactococcus lactis subsp. *lactis* biovar. *diacetylactis*	10–20%	
DL	*Lactococcus lactis* subsp. *lactis*	5–10%	Continental cheese
	Lactococcus lactis subsp. *cremoris*	60–80%	(with eyes)
	Lactococcus lactis subsp. *lactis* biovar. *diacetylactis*	10–20%	Mold ripened cheese
			Lactic butter
	Leuconostoc mesenteroides subsp. *cremoris* + *Leuconostoc lactis*	5–10%	Cultured buttermilk
			Creme fraiche, ymer

1. Starter Culture and Bacterophage

One of the disadvantages of using pure bacterial starter cultures is that they are more sensitive to bacteriophages than undefined mixed starters [23] or indigenous flora, where there will always be strains present that can survive phage attack and continue fermentation. Lactic acid fermentations of milk for cheese production are especially susceptible to

TABLE 177.6
Composition of Different Thermophilic Cultures and Some Examples of Their Products

Organisms	Products
*Streptococcus thermophilus**	Yogurt
Lactobacillus delbrueckii subsp. *bulgaricus* +/- *Lactobacillus helveticus*#	
Streptococcus thermophilus,	Mozarella cheese
+/- *Lactobacillus delbrueckii* subsp. *bulgaricus* or *Lactobacillus helveticus*	Pizza cheese
	Provolone
*Streptococcus thermophilus**	Emmental, Comté, Gruyère Jarslberg
+/- *Lactobacillus delbrueckii* subsp. *lactis*	Hartkäse, Berg-Alpkäse
+/- *Lactobacillus helveticus*	Pecorino Romano
*Streptococcus thermophilus**	Gorgonzola
+ *Lactobacillus delbrueckii* subsp. *lactis* or *Lactobacillus helveticus*#	Bleu de Bresse
	Bleu de Gex
	Fourme d'Ambert

* some of the *Streptococcus thermophilus* ought to be classified as *Streptococcus macedonicus* in the future.

a Gal- variant of *Lactobacillus helveticus* previous designated *Lactobacillus jugurti* is sometimes used for yogurt and Gorgonzola.

TABLE 177.7
Examples of Fermented Milk Products and the Composition of Their Added Starter Cultures

Fermented Milk Products	Microorganisms
Acidophilus milk	DL starter, *Lb. acidophilus*
Villi	DL starter, Geotricium candidum
Langfil	D, L or DL culture
Cultura, AB-milk	*Lb. acidophilus, Lb. johnsonii, Lb. gasseri, Bifidobacterium*
Yogurt variants	*S. thermophilus, Lb. acidophilus*
Yogurt variants	*S. thermophilus, Bifidobacterium*
Gaio	*S. thermophilus, E. facium*
Yakult	*Lb. casei*

phages and special precautions have to be taken in order to exclude them. The use of phage resistant starter cultures, a high level of cleaning and sanitation, use of closed vats equipped with filters, specially designed pipelines, equipments and facilities, minimal access of persons and high personal hygiene are all recommended to achieve this. In this way it was possible to use a mixed O-culture, TK5, for production of cheddar cheese in Denmark for 11 years before bacteriophages able to inhibit acidification appeared [27,28]. Unfortunately, the phages became so virulent that after 12 years of production it was not possible to use the TK5 starter culture any longer in the dairy [23].

C. Cultures for Fermentation of Vegetables, Fruits and Grains

Plant fermentations involve either lactic acid, acetic acid or alcoholic fermentation or a combination of these fermentation types. In alcoholic fermentation it is mainly yeast (*Saccharomyces cerevisiae*) and fungi (*Aspergillus oryzae*) that participate; however, lactobacilli and *Pediococcus* can also be involved. This fermentation is described in more detail in this chapter section IV. Acetic acid fermentation, used for production of vinegar, is a two stage fermentation process, in which the first stage includes an alcoholic fermentation followed by the oxidization of ethanol via acetaldehyd to acetic acid [29]. The typical raw materials are grapes, potatoes or rice. Different subspecies of *Acetobacter* (*A. acati, A. pasteurianus* and *A. hansenii*) and *Gluconobacter oxydans* are used for vinegar production. Pure cultures are not widely employed in the acetic acid fermentation industry [29,30]. Interestingly, Nanda et al. [31] found that the *Acetobacter* strain responsible for the rice vinegar "Komesu" and "Kurosu" spontaneously established an almost pure culture during its long production time.

Traditional fermentations of vegetables, fruits and grains most often include a lactic acid fermentation involving many different species of LAB which are active at different stages of the fermentation process; this is followed by fermentation by yeast and mold [30,32–34]. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are the major microorganisms, however, many other LAB e.g. *Lactobacillus species* and *Pediococcus* may be involved.

Fermentation of vegetables is difficult to control [35] because it depends on the quality of the raw material, the harvesting condition and the temperature, which are vital in providing the optimal conditions for growth of the desirable microorganisms. The addition of salt, either as dry salt (2–3% w/v) or in solution (4–10% w/v) (called brining) and the creation of an anaerobic condition is commonly the initial step in fermentation of vegetables. One obstacle is that raw vegetables cannot normally be pasteurized without adverse influence on the product texture [11]. Another issue is that vegetable fermentation often relies on a very complex process in which many different bacteria succeed each other in very specific ways.

Many different plant fermentations are commercially produced [35] and most often on a small scale. Currently only olives, pickled cucumbers, sauerkraut and kimchi are industrially produced in economically important large amounts [36]. Commonly, the fermentations are performed by spontaneous fermentation or back-slopping. In a few cases utilization of LAB as single-strain cultures has been tried successfully. For production of sauerkraut, *Lactobacillus plantarum* [37,38], *Lactobacillus curvatus* [37], and *Leuconostoc mesenteries* alone [30] or combined with *Lactococcus lactis* [39] have been tried. *Lactobacillus plantarum* and *Lactobacillus pentosus* have been used in olive fermentation [40–44]; *Lactobacillus plantarum* [45–48], *Lactobacillus pentosus* [48], *Pediococcus pentosaceus* [48] successfully for pickled cucumbers. Examples of plant fermentations in which starter cultures have been used are shown Table 177.8.

Different grains (e.g., maize, rice, sorghum [49–64]) and legumes (e.g., soybeans, lupins, peas, lentils [65] can be fermented and most do not involve addition of starter cultures, but are fermented by spontaneous fermentation or back-slopping. However, starter cultures are used for production of sourdough from wheat or rye [30]. They are used as either single- or multiple-strains starters, with or without the addition of yeast. The LAB used in starter cultures are shown in Table 177.8. However, back-slopping using a batch of dough derived from a previous fermentation to inoculate the next batch of dough is still a common practice in industrial production in Denmark, Finland and Germany. Another way to start the fermentation is by addition of dried dough [66]. Many different LAB (especially *Lactobacillus* species) have been isolated from sour dough [67–69].

D. Cultures for Meat Fermentation

Starter cultures for meat fermentation are mainly used in the production of fermented sausages. These cultures are either single- or multiple-strain cultures of LAB and/or

TABLE 177.8
Examples of Lactic Acid Bacteria Used as Starter Cultures or Occurring Spontaneously in High Number in Fermented Plant Material

Raw Material	Dominating Microorganisms or Starter Culture	Products
Cabbage	*Leuconostoc mesenteroides*, Lb. plantarum*, Lb. curvatus*, Lb. brevis, P. cerevisiae*	Sauerkraut
Cucumber	*Lb. brevis, P. cerevisiae¸ Lb. Plantarum*, Lb. pentosus*, P. pentosaceus, yeast*	Salted/pickled cucumber
Olives	*Lb. brevis, P. pentosaceus¸ Lb. plantarum*, Lb. pentosus, yeast*	Olives
Fruit juice	*Lb. casei, Lb. plantarum, Lb. xylosus, Lb. sakei*	Fruit juice
Wheat and rye	*Lb. sanfranciscensis*, Lb. brevis*, Lb. plantarum*, Lb. fermentum*, Lb. fructivorans*, Lb. delbrueckii**,	Sourdough

* Have been used as starter culture.

TABLE 177.9
Examples of Fermented Meat Products and Composition of their Added Starter Cultures

Products	Microorganisms	Comments
Semidry sausages	*Staphylococcus carnosus*	
	+/- Lactobacillus pentosus	
	+/- Pediococcus pentosaceus	
Dry sausages	*Staphylococcus xylosus*	
	+/- Pediococcus pentosaceus	
Special cultures	*Lactobacillus pentosus*	
	Lactobacillus sakei	Bioprotection
	Pediococcus pentosaceus	
	Pediococcus acidilactici	High temperature
	Pediococcus acidilactici	
	Lactobacillus curvatus	Enhanced safety
	Lactobacillus plantarum	[1,2]
	Lactobacillus sakei	[1,2]
	Lactobacillus curvatus	[1,2]

+/-, with or without.

staphylococci. However, sausages may also be produced without the addition of starter cultures and rely instead on the indigenous microflora in the meat [70]. However, starter cultures provide technological advantages such as rapid and uniform acidification, good texture and sliceability, production of desirable flavor compounds, enhanced safety [71,72], good color formation and stability, and better control over the fermentation process. Staphylococci are important for the aroma and color formation and stability; LAB are central for the other properties. Because meat contains extremely low amounts of sugar, the addition of carbohydrate influences the final pH. There are two main types of sausages, the northern European type in which the sausages are smoked and dried, and the southern European sausages that are dried with or without mold present [35]. Generally the sausages from southern Europe are drier than the sausages from northern Europe (semidry). Most fermentations are carried out at 17–24°C, although variations occur. For example, the American pepperoni sausages are fermented at 40°C. Table 177.9 shows which microorganisms are used as starter cultures for the production of sausages.

IV. YEASTS USED AS STARTER CULTURES

A. Introduction

Yeasts are involved in both spontaneous and controlled fermentations. For spontaneous fermentation processes the yeasts are introduced by either the raw materials or via the process equipment [74–76]. When yeast are used as starter cultures, they are in general used as single cultures and may be introduced either to initiate the fermentation process or at a later stage in the fermentation to ensure optimal aroma production and so forth. Most yeast species are able to grow under both aerobic and anaerobic conditions. However, some yeast species are specifically respiratory yeasts whereas others are fermentative yeasts for which respiration is repressed even at aerobic conditions. Primarily, yeasts utilize carbohydrates as carbon source, which are converted into alcohols and CO_2 as well as a number of secondary metabolites such as esters, organic acids, aldehydes, ketones a.o. [77].

Yeasts involved in the fermentation of foods and beverages belong primarily to the ascomycetous yeasts. Among these, the most well described yeast species is undoubtedly *Saccharomyces cerevisiae*. This species is used worldwide for the production of bread, wine, beer, cheese etc. and is overall the predominant starter culture in use. Other important yeast species are *Saccharomyces pastorianus,* used for production of lager beer, and *Debaryomyces hansenii*, used for production of cheese and fermented meat products. A microscopic picture of *D. hansenii* cells is shown in Figure 177.4. Yeast species other than the above mentioned are potential starter cultures and do often occur in high numbers during spontaneous fermentations (Table 177.10). The evolvements of DNA technologies and molecular typing techniques have over the recent decades influenced significantly the taxonomic position of many yeast genera and further reorganizations are expected in the future. For a current taxonomic description of yeast species the taxonomic keys of Kurtzman and Fell [78] and Barnett et al. [79] should be consulted.

The benefits obtained by moving from spontaneous fermentations to controlled fermentations are many and, therefore, there seems to be a growing interest in the use

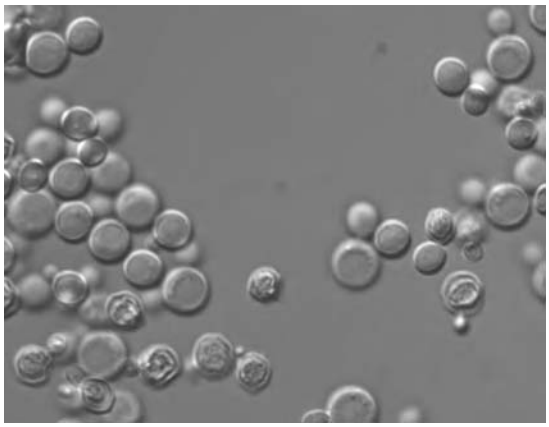

FIGURE 177.4 Cells of *Debaryomyces hansenii.* The magnification is 1000 times.

of yeasts as purified starter cultures, not only in the control of existing fermentation processes but also in the development of new food products. In controlled fermentation, the habitat of the yeast species as well as the various functions of different yeast species should be taken into consideration as well as any probiotic property [80] [81] or possible pathogenic hazard [82] [83]. Also the taxonomic position of the starter cultures must be clarified and methods for typing at subspecies level introduced.

Especially for brewing, wine and baker's yeasts, efforts have been made to improve the technological properties of the strains by formation of genetic modified organisms (GMO). However, in most cases the GMOs have not been used in industrial food fermentations due to consumer resistance and statutory regulations [7].

B. Brewing Yeasts

The brewing industry has a long tradition of the use of single starter cultures of brewing yeast based on single cell cultures. Worldwide, up to a thousand different brewing yeast cultures have been described. The brewing yeast strains vary in their technological properties including aroma production, rate and degree of attenuation, flocculation, oxygen requirement and reproduction [84]. During brewing fermentation maltose is the most dominant carbon source but sucrose, glucose, fructose and maltotriose will also be present and utilized. Brewing yeast strains have been shown to vary in their ability to utilize maltose and genotypic variations in their number of maltose transporter genes have been reported [85].

Two types of *Saccharomyces* yeasts are involved in beer fermentation: ale yeasts (also known as top fermenting yeasts) and lager yeasts (also known as bottom fermenting yeasts) [84]. Ale yeasts have, since the last century, been classified as *S. cerevisiae,* whereas lager yeasts have been known under a variety of names such as *Saccharomyces carlsbergensis, Saccharomyces uvarum* and *S. cerevisiae*. The development of molecular typing techniques has revealed several genetic differences between ale and lager brewing yeasts [86–90], and according to recent classifications, lager yeasts are now considered to belong to *Saccharomyces pastorianus* [91] even though they often are still referred to as *Saccharomyces carlsbergensis* [92]. However, some confusion still exists regarding the phylogenetic relationship between lager yeasts and other yeast within the genus *Saccharomyces*. It appears to be generally accepted that lager yeasts are alloploid and contain parts of two divergent genomes [93,94], one from *S. cerevisiae* and one from another *Saccharomyces* species, most likely *S. bayanus* [88,95,96] or to a specific strain of *Saccharomyces monacensis* [92,97], which according to recent taxonomic keys, now also belongs to *S. pastorianus* [91].

Starter cultures of *S. cerevisiae* have also been reported to be used in the production of South African sorghum beer [98] and in addition to its use as an industrial starter culture *S. cerevisiae* has been isolated from a variety of different indigenous spontaneously fermented beers or beer-like beverages [75].

C. Winery Yeasts and Yeasts Used for the Production of Distilled Alcohols

Traditionally, wine is produced by spontaneous fermentation and several yeast species have been reported to be involved in the fermentation. The predominant microorganisms on the grapes vary according to the grape variety, climatic conditions, soil quality, development and physical quality of the grapes as well as the amount of fungicides applied to the vineyards. Nevertheless, the predominant yeast genera on grapes are reported to be *Kloeckera* and *Hanseniaspora,* whereas *Saccharomyces cerevisiae* is not observed or observed at only very low concentrations on healthy undamaged berries. The yeast genera associated with wine making include: *Candida*, *Cryptococcus*, *Debaryomyces*, *Dekkera* (teleomorphic form of *Brettanomyces*), *Hanseniaspora* (teleomorphic form of *Kloeckera*), *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces* and *Zygosaccharomyces*. Some of these yeast genera are thought to be essential for the wine fermentation, while others are regarded as transient organisms. Due to the low pH and high sugar content of grape juice a natural yeast selection will take place during spontaneous wine fermentation. At the early stages of the fermentation yeast of the genera *Candida*, *Hanseniaspora* and *Kloeckera* will dominate, followed by species of *Metschnikowia* and *Pichia.* The latter stages of the fermentation will be dominated by alcohol-tolerant strains of *S. cerevisiae* [7].

TABLE 177.10
Examples on Yeast Species Used as Starter Cultures or Occurring Spontaneously at High Numbers in Fermented Products

Fermented Foods and Beverages	Yeast Species[a]	Products
Beer	*Saccharomyces cerevisiae**	Ale, stout, porter,
	*Saccharomyces pastorianus**	Pilsner
Wine	*Saccharomyces cerevisiae**	Red and white wine,
	*Saccharomyces bayanus**	sherry
	Candida spp.	
	Hanseniaspora spp.	
	Kloeckera spp.	
	Metschnikowia spp.	
	Pichia spp.	
Indigenous fermented beverages	*Saccharomyces cerevisiae**	Kaffir beer,
	Candida spp.	plantain beer,
	Galactomyces geotrichum (*G. candidum*)	palm wine,
	Hanseniaspora uvarum (*K. apiculata*)	sugar cane wine, sake
	Kluyveromyces africanus	
	Pichia spp.	
	Rhodoturula spp.	
	Saccharomyces spp.	
	Schizosaccharomyces pombe	
	Schizosaccharomyces japonicus	
	Torulaspora delbrueckii (*C. colliculosa*)	
Distilled alcohol	*Saccharomyces cerevisiae**	Whisky, rum,
	Schizosaccharomyces pombe	aquavit
Bread	*Saccharomyces cerevisiae**	Wheat bread,
	Saccharomyces exiguous (*C. holmii*)	rye bread
Cheese	*Debaryomyces hansenii* (*C. famata*)*	Surface-ripened cheeses,
	Galactomyces geotrichum (*G. candidum*)*	Camembert,
	*Saccharomyces cerevisiae**	Gorgonzola and
	Yarrowia lipolytica (*C. lipolytica*)	other blue veined cheeses
	Kluyveromyces lactis (*C. spherica*)	
	Kluyveromyces marxianus (*C. kefyr*)	
Fermented milk	*Galactomyces geotrichum* (*G. candidum*)*	"Villi," kefir,
	Candida spp.	indigenous sour milk
	Kluyveromyces marxianus (*C. kefyr*)	
	Saccharomyces unisporus	
	Saccharomyces spp.	
	Torulaspora spp.	
Meat products	*Debaryomyces hansenii* (*C. famata*)*	Sausages,
	Candida zeylanoides	cured ham, bacon
	Debaryomyces polymorphus	
	Pichia guilliermondii (*C. guilliermondii*)	
	Pichia membranifaciens (*C. valida*)	
	Cryptococcus spp.	

[a] * indicate that commercial starter cultures are available; anamorph form is given in parentheses.

Within the last decades there has been in wine-making an increasing interest in the use of starter cultures and today most large-scale productions are carried with starter cultures of primarily *S. cerevisiae.* Several different physiological variants of *S. cerevisiae* have been reported for the production of different types of wine and *S. bayanus* has been used as a starter culture especially for wine partially produced at low temperatures (e.g., Sauternes, Tokay, Muscat and Amarone) [99,100]. Recently, genera other than *Saccharomyces* have been reported to be beneficial in the production of wine in order to enhance the taste and flavor of the wine [101] [102].

Yeasts are involved in the production of several special other types of alcoholic beverages, besides wine, including a large number of indigenous alcoholic beverages produced by spontaneous fermentation [9]. In most cases, the yeast species responsible for the fermentation is *S. cerevisiae.* However, yeast species belonging to genera

other than *Saccharomyces* have been reported – for example, for the production of sherry where a secondary fermentation by so-called flor yeast occurs. At the early stage of the secondary fermentation the yeast forms a surface film on the top of the wine; several yeast species, including *Pichia anomala* and *Pichia membranifaciens* yeast have been reported to be involved in this secondary fermentation. However, during the fermentation a microbial succession takes place and *S. cerevisiae* is now believed to be the most important flor yeast for sherry production [103]. Non-*Saccharomyces* yeasts have been reported to be especially involved in the early stages of indigenous, spontaneously fermented beverages [104]. But, at the later stages of the fermentations *S. cerevisiae* will nearly always dominate, as is the case for brewing of sake where isolates of *S. cerevisiae*, previously known as *Saccharomyces saké,* is used [105]. For distilled alcohols the vast majority of modern distilleries use starter cultures of *S. cerevisiae*; exceptions are *Schizosaccharomyces pombe,* used for production of specific spirits and lactose-fermenting yeasts, used in the production of neutral spirit from whey [106].

D. Baker's Yeast

Baker's yeast is traditionally used throughout Europe and the United States as starter culture for the production of a large variety of wheat-based breads. In Scandinavia and the northern part of Europe, sourdough bread made from rye by back-slopping is also popular. The European tradition for production of bread seems to have spread now all over the world, including Southeast Asia and Africa [8]. In all cases the dominant yeast used as starter culture is *Saccharomyces cerevisiae* Meyen ex. E.C. Hansen, as described by Vaughan-Martini and Martini [91] and Barnett et al. [79]. As an alternative baker's yeast *Saccharomyces exiguus* has been used [107].

Baker's yeast is produced as a bulk product. The global yearly production amount to 2 million tons and a yearly growth of approximately 4% has been reported [108]. The propagation of baker's yeast is based on a fed-batch process characterized by aerobic conditions and low carbohydrate concentrations [109]. Except for the utilisation of carbohydrates, optimization of baker's yeasts seems to focus on other aspects quite different from those of brewing yeasts. Also, the number of commercially available baker's yeast appears to be limited compared to brewing yeasts. Beside efficient utilization of maltose, maltotriose and other fermentable carbohydrates present in the dough, the important technological properties of baker's yeasts appear to be biomass yield, formation of CO_2, influence on dough structure, aroma formation and a high resistance to stress conditions such as oxidative stress, drying, freezing and thawing [8,110,111]. The latter results in a demand for high yeast concentrations of trehalose as a protective agent [112]. Also, the ability of the yeast strains to adapt, from the conditions during propagation and production, to the conditions in the dough needs to be considered [109].

Types of fermented cereals other than traditional wheat bread are seen especially in areas where European style bread is not traditionally produced as e.g. in a great part of Africa. Examples of indigenous fermented cereals are products based on fermented maize, millet and sorghum. These products are most often produced by spontaneous fermentation where *S. cerevisiae* is the dominant yeast species, in co-existence with LAB especially [9,50,74,113].

E. Yeasts Used for Dairy Products

For dairy products yeasts are mainly used in cheese production but may also be involved in the production of fermented milk. Yeasts are primarily used as single-starter cultures but many products are still produced by back-slopping or spontaneous fermentation. A mixture between the used starter culture and a dominant indigenous flora is also seen in many dairy products. Yeasts are in most cases used as secondary starter cultures in order to enhance the aroma production or to facilitate the growth of other microorganisms. In general the functions of yeasts during cheese production and their influence on the cheese quality are poorly investigated [114].

The occurrence and functions of yeasts have been especially studied for the production of surface ripened cheeses such as Brick, Limburger, Port Salut, Taleggio, Tilsitter, Trappist, and the Danish Danbo cheese. The surface smear of these cheeses is found to consist of a mixed flora comprising both yeasts and bacteria. For cheese such as the Danish Danbo, the osmotolerant yeast *Debaryomyces hansenii* has been found almost exclusively [76], whereas in other types of surface ripened cheeses, yeasts such as *Candida zeylanoides*, *Yarrowia lipolytica, Kluyveromyces lactis* and others have been found [115–117]. The yeasts initiate the ripening process by degradation of lactate, thereby increasing the pH on the cheese surface and allowing the growth of a more acid-sensitive bacterial flora comprising, amongst others, *Brevibacterium linens* [118]. It should be mentioned that the species *D. hansenii* has been divided into two varieties, *D. hansenii* var. *hansenii* and *D. hansenii* var. *fabryii* [119]; apparently the predominant variety seen in cheese production is *D. hansenii* var. *hansenii* and isolates of this variety have been introduced as starter culture [76,118]. *K. lactis* has further been found to occur in high numbers in soft cheeses such as Camembert [20]. *S. cerevisiae* has also been used as a starter culture in the production of especially Gorgonzola, but it apparently also occurs spontaneously, together with other yeasts, as an integral part of the

microflora of both blue-veined cheeses and some types of soft cheeses [20,120]. *Saccharomyces cerevisiae* has been reported to stimulate mycelial growth and conidia formation of *Penicillium roqueforti* as well as influencing aroma formation and having some proteolytic activity [120,121].

Even though previously considered as a mold, *Galactomyces geotrichum* (*Geotrichum candidum*) is now considered as a yeast species [122] and will be considered as such in the following. *G. geotrichum* is associated with milk and the dairy environment. It is known as a starter culture for several types of mold-ripened cheeses (e.g., Camembert), surface ripened cheeses and cheeses as the French St. Albray. On the other hand, *G. geotrichum* has also been shown to be a potential spoilage organism. *G. geotrichum* is sometimes used in combination with other microorganisms such as *Penicillium camemberti* or *Brevibacterium linens* for the production of surface-ripened cheeses.

Even though not used as a starter culture in the dairy industry, *Yarrowia lipolytica* is often found in soft, blue veined and surface-ripened cheeses. *Y. lipolytica* is characterized by having a quite pronounced lipolytic and proteolytic activity [123] that might be difficult to control if used as a starter culture for cheese production. Furthermore, *Y. lipolytica* is known to produce brownish pigments in cheeses.

For fermented milk yeasts within the genera *Candida*, *Galactomyces*, *Kluyveromyces*, *Saccharomyces* and *Torulaspora* are generally used [124]. *G. geotrichum* (*G. candidum*) is used as commercial starter culture in the production of viili, a Scandinavian fermented milk product and *Saccharomyces unisporus* and *Kluyveromyces marxianus* (*Candida kefyr*) are used as commercial starter cultures in the production of milky kefir. *S. cerevisiae* has also been reported to be involved in the fermentation of a number of indigenous African fermented milk products known under names such as *amasi*, *nono* and *rob* [125,127].

F. Yeasts Used for Meat Products

The use of yeasts in the fermentation of meat products is not well developed. An exception is the use of the osmotolerant yeast *D. hansenii* for the production of sausages and a few other meat products [128]. *D. hansenii* is aerobic and, therefore, primarily found on the outer parts of the fermented meat products; its depletion of oxygen has a color stabilizing effect. Further, *D. hansenii* is reported to have proteolytic activity against sarcoplasmic proteins and several peptides [128] and to have lipolytic activity against pork fat [129]. Several meat products, including high quality products such as Parma and Serano ham, are still spontaneously fermented and yeasts are also involved in these types of fermentation. In addition to *D. hansenii*, *Candida zeylanoides* has been found to be involved in the ripening of Iberian dry-cured ham [130], *C. zeylanoides* and *Pichia membranifaciens* in British sausages [131] and *Cryptococcus laurentii*, *Cryptococcus humicolus*, *Debaryomyces polymorphus* and *Pichia guilliermondii* in Portugese cured ham and bacon [132].

V. MOLDS USED AS STARTER CULTURES

A. Introduction

In Europe starter cultures of molds are primarily used for the production of cheese and meat, and worldwide the production of a variety of indigenous foods is based on spontaneous mold fermentations (Table 177.11). Molds only grow in the presence of oxygen which limit their

TABLE 177.11
Examples of Mold Species Used as Starter Cultures or Occurring Spontaneously at High Numbers in Fermented Products

Fermented Foods and Beverages	Mold Species[a]	Products
Cheese	*Penicillium roqueforti**	Roqueforti, gorgonzola, Danish blue, camembert
	*Penicillium camemberti**	
Meat	*Penicillium camemberti**	Meat sausage, dry cured ham
	*Penicillium chrysogenum**	
	*Penicillium nalgiovense**	
	Penicillium aurantiogriseum	
	Penicillium commune	
	Penicillium olsonii	
	Penicillium solitum	
	Eurotium rubrum	
Wine	*Botrytis cinerea*	Sauternes, Tokay
Indigenous fermented foods	*Aspergillus oryzae**	Soy sauce, tempe, Chinese soybean paste ("sufu" or "furu"), Japanese "miso" and "shoyu"
	*Aspergillus sojae**	
	Actinomucor spp.	
	Mucor spp.	
	Rhizopus oligosporus	
	Rhizopus oryzea	
	Rhizopus spp.	
Fermented fish	*Aspergillus penicillioides*	Indonesian dried salted fish
	Aspergillus wentii	
	Eurotium rubrum	
Indigenous fermented beverages	*Aspergillus oryzae*	Sake, Chinese, Indian and Thai spirits, wine and beers
	Aspergillus spp.	
	Mucor spp.	
	Rhizupus spp.	

[a,*] indicate that commercial starter cultures are available.

applications. In general molds are known as effective producers of enzymes and their proteolytic and lipolytic activities are often high; several molds also have extracellular glycoamylase activity. Furthermore, molds are known to produce a variety of different aroma compounds of which the best known are alcohols and organic acids. Molds preferentially grow on carbohydrates but may also grow in protein-rich media without carbohydrates in which they use amino acids as carbon source [133]. The taxonomy of molds is primarily based on their micromorphology and growth characteristics on different media, but their production of secondary metabolites can also be used as taxonomic characters [134]. Unfortunately, DNA technology and the use of molecular typing techniques have not developed as fast for molds as for yeasts and the typing of molds used as starter cultures is still based primarily on phenotypic criteria. A taxonomic description of food borne fungi is given by Samson et al. [135].

B. Molds Used for Dairy Products

The use of such molds as *Penicillium roqueforti* and *Penicillium camemberti* has a long history in the production of cheese and their use as starter cultures goes back to the beginning of 19th century. *Penicillium roqueforti* is used as a secondary starter culture in the production of blue-veined cheeses but it may also occur spontaneously in a number of other foods and for some types of cheeses it is regarded as a contaminant. The fact that *P. roqueforti* is able to grow at high NaCl and low O_2 concentration and at a relative high CO_2 concentration makes it suitable for the production of blue-veined cheese. During cheese maturation *P. roqueforti* produces a number of extracellular peptidases and proteinases that are mainly responsible for the extensive proteolysis of blue-veined cheese. Large differences in proteolytic [136] and lipolytic activity [137] have been reported between different commercial strains of *P. roqueforti*. The lipolytic enzymes especially seem to be responsible for the characteristic flavor and taste of blue-veined cheeses through their production of high concentrations of methyl ketones [137]. *P. camemberti* is used as a starter culture for the production of Camembert and similar types of surface-ripened cheeses, and is highly restricted to the production of cheese and is seldom observed in other foods or in the environment [8]. *P. camemberti* is able to grow on the cheese surface due to its high NaCl tolerance. On the cheese surface, it degrades lactate, resulting in an increased pH. When the lactate is depleted, the proteinases from *P. camemberti* then degrade casein, resulting in a further increase in pH and the release of ammonia. Besides its pronounced proteolytic activity, *P. camemberti* also produces lipases that are involved in aroma formation. Figure 177.5 shows the mycelial growth of *P. camemberti* on a soft cheese surface.

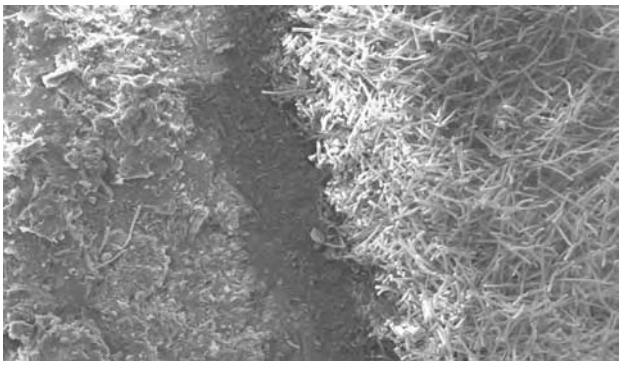

FIGURE 177.5 Growth of *Penicillium camemberti* on a soft cheese surface. The magnification is 250 times.

C. Molds Used for Meat Products

The use of molds as starter cultures for fermentation of meat is mainly concentrated in such European regions as Italy, Spain, France, Germany and Hungary. *Penicillium* spp. are mainly involved in the fermentation and the meat products produced typically are sausages and ham. Common molds used for fermentation of meat include *Penicillium nalgiovense*, *Penicillium chrysogenum* and *Penicillium camemberti* [8,138,139]; but also other species might be used. *Penicillium commune* and *Penicillium olsonii* have been reported to be involved in the spontaneous fermentation of Spanish meat sausage [140], *Penicillium aurantiogriseum* in the fermentation of dry sausages [141] and a variety of *Penicillium* spp. including *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. commune*, *Penicillium echinulatum* and *Penicillium expansum* have been found during spontaneous fermentation of dry-cured Iberian ham [142]. For the latter, nontoxigenic strains of *P. chrysogenum* have been recommended as starter cultures [142]. *Eurotium rubrum* and *Penicillium solitum* were found to be the dominant species during production of traditional Tyrolean smoked and cured ham [143]. *Aspergillus* spp. are not used as starter cultures for meat fermentation but may be observed during production of cured ham where they can grow at low water activity.

Besides influencing the appearance, molds contribute to the characteristic aroma and flavor of the product by production of extracellular proteinases and lipases [144]. Also, the molds inhibit the growth of unwanted microorganisms and have an antioxidative effect. Strains of both *P. chrysogenum* and *P. nalgiovense* are known penicillin producers and the latter, at least, has been shown to be able to produce penicillin when growing on meat surfaces and to secrete it into the product [145]. Therefore, starter cultures of molds must be carefully analyzed by both chemical and biological tests to ensure that they do not form neither penicillin nor mycotoxins in the product [139].

D. Molds in Wine Production

Molds are not used as starter culture for wine production, but the growth of *Botrytis cinerea* may be required for the production of certain types of wine. In general the growth of *B. cinerea* is unwanted, as it will rot the grapes. However, for the production of sweet white wine such as French Sauternes and Hungarian Tokay, the development of *B. cinerea* on the matured grapes is required and known as "noble or vulgar rot;" this results in overripening and dehydration leading to increased sugar content in the grapes. Also, the growth of *B. cinerea* adds a characteristic flavor to the wine produced from these grapes. Specific environmental conditions such as alternating dry and humid periods are required for reaching the perfect stage of maturation and the development of *B. cinerea* [146,147]. Biological control of the growth of *B. cinerea* on matured grapes has been obtained by use of the yeast *Pichia membranifaciens*; its antagonistic effect against *B. cinerea* appears to be related to its secretion of exo- and endo-β-1,3-glucanases [148].

E. Molds Used for Fermentation of Indigenous Fermented Foods and Beverages

The most well known indigenous fermented products, where molds are involved in the fermentation, are probably soy sauce and tempeh. Soy sauce is of Chinese or Japanese origin and is produced by an initial solid stage mold fermentation of equal parts of cooked soybeans and roasted and crushed wheat, inoculated with conidia of *Aspergillus oryzae* and *Aspergillus sojae*. After fermentation for 3 days the material is placed in brine (22–25% (w/v) NaCl) for 6–8 month. During this phase halophilic LAB and yeasts (*Zygosaccharomyces rouxii*) occur either spontaneously or are added as starter cultures. Finally, the soy sauce is harvested by pressing and is then pasteurized. Tempeh originates from Java and Indonesia and is a solid-stage mold fermentation of soaked and cooked soybeans or other leguminous seeds or cereals. After boiling and cooling, the beans are inoculated with *Rhizopus oligosporus* and *Rhizopus oryzae* and packed. After fermentation for 24–48 hr the beans are tighten together due to mycelial growth. The fresh tempeh can be eaten after cooking or frying [146]. Other examples of indigenous mold fermented foods are *sufu* or *furu*, a Chinese soybean pasta produced by *Actinomucor* spp., *Mucor* spp. or *Rhizopus* spp. [149], and Japanese *miso* and *shoyu* produced by *Aspergillus oryzae* and *Aspergillus sojae* [150]. Xerophilic molds such as *Eurotium rubrum*, *Aspergillus wentii* and *Aspergillus penicillioides* have also been reported to be involved in the fermentation of Indonesian dry salted fish [151].

Especially in the Asia, species of *Aspergillus*, *Mucor* and *Rhizopus* are used as amylase producers for fermented beverages that are based on rice or wheat [150]. An example is the production of sake in which spores of *Aspergillus oryzae* are used to breakdown starch to fermentable carbohydrates that are then converted to alcohol by *S. cerevisiae.* Similarly, *Aspergillus* spp., *Mucor* spp. and *Rhizopus* spp are used in the production of Chinese, Indian and Thai spirits, wines and beers [152].

ACKNOWLEDGMENT

The information in this chapter has been modified from "Fermented food and starter cultures," by J. Josephsen and L. Jespersen, in *Handbook of Food and Beverage Fermentation Technology*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.

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178 Manufacture of Fermented Product

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CONTENTS

I. INTRODUCTION

The availability of fermented foods has a long history among the different cultures. Acceptability of fermented foods also differs because of cultural habits. A product highly acceptable in one culture may not be so acceptable by consumers in another culture. The number of fermented food products is countless. Manufacturing processes of fermented products vary considerably owing to variables such as food group, form, and characteristics of final products; kind of ingredients used; and cultural diversity. It is beyond the scope of this chapter to address all the manufacturing processes used to produce fermented foods. Instead, this chapter is organized to address fermented food products based on food groups such as dairy, meat, cereal, soy, and vegetables. Within each food group, manufacturing processes of typical products are addressed. This chapter is only an introduction to manufacturing processes for selected fermented food products. Readers should consult the references below and other available literature for detailed information.

II. FERMENTED DAIRY PRODUCTS

A. Ingredients and Kinds of Products

Fermented dairy products are commonly produced in milk-producing countries and by nomadic peoples. These products are highly acceptable in these cultures. They have been gradually accepted by other cultures because of cultural exchange. It is generally accepted that most fermented dairy products were first discovered and developed by nomadic peoples. The production of a fermented dairy product nowadays can be a highly sophisticated process. However, the production of another fermented dairy product can still be conducted in a fairly primitive manner in another location. The quality of a fermented dairy product varies due to the milk, microorganisms, and other ingredients used in the manufacturing process. Many factors affect the gross composition of milk (15, 32, 34, 55, 62). The factors most significant to the processing of milk products are breed, feed, season, region, and herb health. Reviews of animals' milk are available in the literature. Table 178.1 lists the approximate composition of cow's milk (15, 32, 34, 48, 55, 62). In industrial countries, milk composition is standardized to meet a country's requirements. However, it is understood that the requirements in one country may not be the same as those in another; thus, the composition may vary for the same product. International agreements to standardize some products are now available. However, products produced in different locations still can vary because of microorganisms and culturing practices used in their production.

Fermented dairy products can be grossly divided into three big categories: cheeses, yogurts, and fermented liquid milks. Within each of these categories, there are

TABLE 178.1
Approximate Composition of Milk

Components	Average Content in Milk (% w/w)	Range (% w/w)	Average Content in Dry Matter (% w/w)
Water	87.1	85.3–88.7	
Solid-not-fat	8.9	7.9–10.0	(69)
Fat in dry matter	31	22–38	(31)
Lactose	4.6	3.8–5.3	36
Fat	4	2.5–5.5	31
Protein	3.25	2.3–4.4	25
Caesin	2.6	1.7–3.5	20
Mineral substances	0.7	0.57–0.83	5.4
Organic acids	0.17	0.12–0.21	1.3
Miscellaneous	0.15		1.2

Source: References 15, 32, 34, 48, 55, 62.

TABLE 178.2
Kinds of Fermented Dairy Products with Examples

Kinds	Examples
Fermented Liquid Milks	
Lactic fermentation	Buttermilk, Acidophillus,
With alcohol and lactic acid	Kefir, Koumiss
With mold and lactic acid	Villi
Concentrated	Ymer, Skyr, Chakka
Yogurts	
Viscous/liquid	Yogurt
Semisolid	Strained yogurt
Solid	Soft/hard frozen yogurt
Powder	Dried yogurt
Cheeses	
Extra hard	Parmesan, Romano, Sbrinz
Hard with eyes	Emmental, Gruyere, Swiss
Hard without eyes	Cheddar, Chester, Provolone
Semi-hard	Gouda, Edam, Caerphilly
Semi-hard, internally mold ripened	Roquefort, Blue, Gorgonzola
Semi-soft, surface-ripened with bacteria	Limburger, Brick, Munster
Soft, surface mold ripened	Brie, Camebert, Neufchatel
Soft, unripened	Cream, Mozzarella, US-Cottage

Source: References 15, 32, 34, 48, 55, 62.

TABLE 178.3
Ingredients for Fermented Dairy Food Production

Ingredients	Fermented Liquid Milk Products	Yogurt	Natural Cheese	Processed Cheese Products
Milk				
Raw	Optional	Optional	Optional	Optional
Standardized (fat and milk solids)	Preferred	Preferred	Preferred	Preferred
Milk powders	Optional	Optional	Optional	Optional
Microorganisms				
Starter bacteria	Required	Required	Required	Required
Mold	Optional	Optional	Optional	Optional
Yeast	Optional	Optional	Optional	Optional
Genetically modified microorganisms	Optional	Optional	Optional	Optional
Coagulant				
Rennet	Preferred	Preferred	Preferred	Preferred
Acid	Optional	Optional	Optional	Optional
Microbial protease(s)	Optional	Optional	Optional	Optional
Common salt (sodium chloride)	No	No	Required	Required
Sugar	Optional	Optional	No	No
Vitamins	Preferred	Preferred	Preferred	Preferred
Buffering salts (calcium chloride hydroxide phosphates, sodium or potassium phosphates)	Optional	Optional	Optional	Optional
Bleaching (decolorizing) agents	No	No	Optional	Optional
Antimicrobial agents	Optional	Optional	No	Preferred
Dyes (coloring agents)	No	No	Optional	Optional
Flavoring compounds (spices, spice oils, fruits, fruit flavors, artificial smoke)	Optional	Optional	Optional	Optional
Stabilizers	No	Preferred	No	Preferred
Emulsifiers	Optional	Optional	No	Preferred

Source: References 15, 32, 34, 48, 55, 62.

subcategories. Table 178.2 presents examples for each of these categories (15, 32, 34, 48, 55, 62).

In the manufacturing of fermented dairy products, various ingredients such as the milk itself, microorganism(s), coagulants, salt, sugar, vitamins, buffering salts, bleaching (decolorizing) agents, dyes (coloring agents), flavoring compounds, stabilizers, and emulsifiers may be used. The use of these ingredients in fermented liquid milks, yogurts, and natural and processed cheeses are summarized in Table 178.3 (15, 32, 34, 48, 55, 62).

Various microorganisms such as lactic acid bacteria, yeasts, and molds are used in the manufacturing of fermented dairy products to produce the various characteristics in these products. Table 178.4 lists some of the more common dairy microorganisms and their uses in fermented liquid dairy products, yogurts, and cheeses (12, 31, 49).

Cultures of the different microorganisms are available in various forms, such as liquid, frozen, or freeze-dried. Examples of their usage in the manufacturing of fer mented dairy products are listed in Table 178.5 (15, 32, 34, 48, 55, 62).

Because the starter cultures are available in various forms, the preparation steps for these cultures, before inoculation, are different. Table 178.6 lists some of the preparation procedures used in the industry for different forms of starter cultures (15, 32, 34, 48, 55, 62).

Different microorganisms have different temperature requirements for their optimum growth and functioning. Some fermented dairy products, such as mold-ripened cheeses, may require more than one microorganism to complete the manufacturing process. These molds function best during the long ripening period and therefore have standard incubation temperatures in the refrigerated range. This is also true for some cheeses that require long ripening periods. Microorganisms requiring higher incubation temperatures are used in the production of fermented liquid milks that require only a short incubation time. Table 178.7 lists some of the dairy microorganisms used in some products and their incubation temperatures (12, 17, 31, 35, 42, 52, 54).

B. Cheeses

Cheeses can be classified into different categories based on their moisture, the way the milk is processed, and the types of microorganisms used for the ripening process (Table 178.8) (15, 32, 48).

TABLE 178.4
Some Common Organisms Used in Fermented Milk Products

Microorganisms	Buttermilk	Cream	Fermented Milk	Yogurt	Kefir	Cheese
Bifidobacterium bifidum			X	X		X
Enterococcus durans						X
Enterococcus faecalis						X
Geotrichum candidum						X
Lactobacillus acidophilus			X			
Lactobacillus casei						X
Lactobacillus delbrueckii subsp. *bulgaricus*	X	X				X
Lactobacillus heleveticus						X
Lactobacillus kefir					X	
Lactobacillus lactis						X
Lactobacillus lactis biovar. *diacetylactis*		X				X
Lactobacillus lactis subsp. *cremoris*	X	X				X
Lactobacillus lactis subsp. *lactis*						X
Lactobacillus lactis var. *hollandicus*						X
Leuconostoc mesenteroidis subsp. *cremoris*						X
Leuconostoc mesenteroides subsp. *dextranicum*						X
Propionibacterium freudenreichii subsp. *shermanii*						X
Penicillium camberberti						X
Penicillium glaucum						X
Penicillium roqueforti						X
Streptococcus thermophilus				X		X

Source: References 12, 31, 49.

TABLE 178.5
Dairy Starter Cultures

Physical Form	Usage
Liquid cultures in skim milk or whole milk (antibiotic free)	For inoculation of intermediate cultures
Liquid culture—frozen	For inoculation of intermediate cultures For inoculation into bulk cultures
Dried culture—from normal liquid culture	For inoculation of intermediate culture
Spray dried cultures	For inoculation into bulk cultures For direct-to-vat inoculation
Frozen cultures in special media (frozen at −40°C)	For inoculation into bulk cultures For direct-to-vat inoculation
Frozen concentrated culture (in sealed containers at −196°C)	For inoculation into bulk cultures For direct-to-vat inoculation
Single strain lypholized cultures (in foil sachets with known activity)	For inoculation into bulk cultures For direct-to-vat inoculation

Source: References 15, 32, 34, 48, 55, 62.

TABLE 178.6
Types of Starter Cultures and Their Preparation Prior to Usage

Kinds	Preparation Steps	Timing
Regular starter culture	Preparation of starter culture blanks	8:00 a.m.
	Storing milk blanks	11:00 a.m.
	Activating lypholized culture powder	3:00 p.m.
	Daily mother culture preparation	3:00 p.m.
	Semibulk and bulk starter preparation	3:00 p.m.
Frozen culture and bulk starter application	Store frozen culture at −40°C or less	
	Warm to 31°C and use directly	
Reconsitituted milk or whey-based starter	Reconstitution	8:00 a.m.
	Heating and tempering	8:30 a.m.
	Inoculating and incubating	10:00 a.m.
Bulk starter from ultrafiltrated milk	Ultrafiltration	1:00 p.m.
	Heating and tempering	3:30 p.m.
	Inoculating and incubating	5:00 p.m.

Source: References 15, 32, 34, 48, 55, 62.

TABLE 178.7
Temperature Requirements and Acid Production for Some Dairy Microbes

Microorganisms	Product Group[a]	Standard Temperature for Incubation,°C	General Maximun Titratable Acidity Produced in Milk, %
Bacteria			
Bifidobacterium bifidum	1	36–38	0.9–1.0
Lactobacillus acidophilus	1	38–44	1.2–2.0
Lactobacillus delbrueckii subsp. *bulgarius*	1	43–47	2.0–4.0
Lactobacillus lactics subsp. *cremoris*	2	22	0.9–1.0
Lactobacillus subsp. *lactis*	2	22	0.9–1.0
Leuconostoc mesenteroides subsp. *cremoris*	2	20	0.1–0.3
Streptococcus durans	2	31	0.9–1.1
Streptococcus thermophilus	2	38–44	0.9–1.1
Molds			
Penicillium roqueforti	3	11–16	NA
Penicillium camerberti	3	10–22	NA

Source: References 12, 17, 31, 35, 42, 49, 52, 54.

[a] Product group: 1 = yogurt, 2 = fermented liquid milk, 3 = cheese.

In the processing of cheese, the amount of curd used for each block of cheese to be made differs considerably, (Table 178.9) (15, 32, 34, 35, 42, 48, 52, 54). Harder cheeses have much larger blocks than the soft cheeses. This may be due to the ease of handling after ripening.

Cheeses are packaged in different forms to satisfy consumer consumption patterns and, to some extent, to be compatible with the way the cheese is ripened and for marketing purposes. The various packaging materials are selected to protect the cheeses in a sanitary condition, extend shelf life, and delay the deterioration of the final products. Table 178.10 lists some of the requirements of cheese packaging materials (15, 17, 34, 42, 48, 52, 54, 55, 62).

All cheeses produced must be coagulated from acceptable milk to form curd, followed by removal of the whey. Most cheeses are made from standardized and pasteurized milk. Nonpasteurized milk is also used in some exceptional cases, provided they do not carry pathogens. The majority of cheeses are made from cow's milk. Milks from other animals are also used for specialty products. The coagulation process is conducted through the addition of coagulant (rennin or chymosin) and incubation of appropriate lactic acid bacteria in milk to produce enough acid and appropriate pH

TABLE 178.8
Classification of Cheese According to Moisture Content, Scald Temperature, and Method of Ripening

Hard Cheese (Moisture 20–42%; Fat in Dry Matter, 32–50%, Minimum)				
Low Scald, Lactic Starter	*Medium Scald, Lactic Starter*	*High Scald, Propionic Eyes*	*Plastic Curd, Lactic Starter or Propionic Eyes*	
Gouda	Cheddar	Parmesan	Provolone	
Cheshire	Svecia	Beaufort	Mozzarella	
Semi-Hard Cheese (Moisture 45–55%; Fat in Dry Matter, 40–50%, Minimium)				
Lactic Starter	*Smear Coat*	*Blue-Veined Mold*		
St. Paul	Limburg	Roquefort		
Lanchester	Munster	Danablue		
Soft Cheese (Moisture >55%; Fat in Dry Matter, 4–51%, Minimum)				
Acid-Coagulated	*Smear Coat or Surface Mold*	*Surface Mold*	*Normal Lactic Starter*	*Unripened Fresh*
Cottage cheese (USA)	Brie	Camembert	Quarg	Cottage (UK)
Quesco-Blanco	Bel Paese	Neufchatel	Petit Suisse	York

Source: References 15, 32, 48.

TABLE 178.9
Approximate Weight of Cheese Varieties

Cheese Variety	Approximate Weight (kg)
Hard to Semi-Hard or Semisoft	
Wensleydale	3–5
Caerphilly	3–6
White Stilton	4–8
Single Gloucester	10–12
Leichester	13–18
Derby	14–16
Sage Derby	14–16
Cheddar	18–28
Cheshire	20–22
Dunlap	20–27
Double Gloucester	22–28
Lancashire	22
Internally Mold-Ripened (Blue-Veined) Cheese	
Blue Wensleydale	3–5
Blue Vinney	5–7
Blue Stilton	6–8
Blue Cheshire	10–20
Soft Cheese	
Colwich	0.25–0.50
Cambridge	0.25–1.0
Melbury	2.5

Source: References 15, 32, 34, 35, 42, 48, 52, 54.

TABLE 178.10
Requirements of Cheese Packaging Materials

Low permeability to oxygen, carbon dioxide, and water vapor
Strength and thickness of film
Stability under cold or warm conditions
Stability to fats and lactic acid
Resistance to light, especially ultraviolet
Ease of application, stiffness, elasticity
Ability to seal and accept adhesives
Laminated films to retain laminated
Low shrinkage or aging unless shrinkage is a requisite
Ability to take printed matter
Should not impart odors to the cheese
Suitability for mechanization of packaging
Hygienic considerations in storage and use
Cost effectiveness as a protective wrapping

Source: References 15, 17, 34, 42, 48, 52, 54, 55, 62.

for curdling the milk. After the casein is recovered, it is salted and subjected to fermentation, with or without inoculation with other microorganisms to produce the desirable characteristics of the various cheeses. Variations in the different manufacturing steps a wide variety of cheeses with various characteristics. Table 178.11 summarizes the basic steps in the cheese manufacturing process (12, 15, 31, 32, 34, 42, 48, 52, 54, 55, 62). Table 178.12 summarizes the ripening conditions for various cheeses. Selected examples are introduced below to provide an overview of the complexity of cheese manufacturing (12, 15, 31, 32, 34, 42, 48, 49, 50, 52, 54, 55, 62).

1. Cottage Cheese Manufacturing

Cottage cheese is a product with very mild fermentation treatment. It is produced by incubating (fermenting) the standardized and pasteurized skim milk with the starter

TABLE 178.11
Basic Cheese Making Steps

Standardize cheese milks
Homogenize cheese milks
Heat-treat or pasteurize cheese milks
Add starter
Add color and additives
Coagulation/Curdling:
Cut coagulum/curd
Stir and scald
Wash curd cheese
Salt cheese
Press cheese
Coat, bandage, and wrap cheese
Let cheese ripen
Package for retail
Store

Source: References 12, 15, 31, 32, 34, 42, 48, 49, 52, 54, 55, 62.

TABLE 178.12
Cheese Ripening Conditions

Types of Cheese	Storage Period (days)	Temperature (°C)	Relative Humidity (%)
Soft	12–30	10–14	90–95
Mold ripened	15–60	4–12	85–95
Cooked, e.g., Emmental			
Cold room	7–25	10–15	80–85
Warm room	25–60	18–25	80–85
Hard, e.g., Cheddar	45–360	5–12	87–95

Sources: References 12, 15, 31, 32, 34, 42, 48–50, 52, 54, 55, 62.

lactic acid bacteria to produce enough acid and appropriate pH for the curdling of milk. The curd is then recovered and washed, followed by optional salting and creaming. The product is then packed and ready for marketing. No further ripening is required for this product. This is different from most fermented cheeses that require a ripening process. Table 178.13 lists the various steps involved in the production of cottage cheese (15, 34, 42, 48, 52, 55, 62).

2. Cheddar Cheese Manufacturing

Cheddar cheese is a common hard cheese without eyes used in the fast-food industry and in the household. Its production process is characterized by a requirement for milling and cheddaring of the curd. This cheese can be ripened with a wax rind or rindless (sealed under vacuum in plastic bags.) It is also categorized into regular, mild, or sharp based on the aging period (45–360 days). The longer the aging period, the sharper the flavor. It is packaged as a large block or in slices. Table 178.14 lists the basic steps in the manufacturing of cheddar cheese (15, 34, 42, 48, 52, 55, 62).

TABLE 178.13
Basic Steps in Making Cottage Cheese

Standardize skim milk			
Pasteurize milk with standard procedure and cool to 32°C			
Inoculate with Active Lactic Starter, Add Rennet, and Set Curd:			
Rennet addition—at 2 ml single strength (prediluted, 1:40) per 1000 kg milk within 30 minutes of starter addition			
Specifications	Short Set	Medium Set	Long Set
Starter concentration	5%	3%	0.5%
Temperature of milk set	32°C	27°C	22°C
Time from setting to cutting	5 hr	8 hr	14–16 hr
Final pH and whey titratable acidity—4.6 and 0.52%, respectively			
Cut curd with 1.3, 1.6, or 1.9 cm wire cheese knife			
Cook Curd:			
Let curd cubes stand for 15–30 minutes and cook to 51–54°C at 1.7°C per 10 minutes			
Roll the curds gently every 10 minutes after initial 15–30 minute wait			
Test curd firmness and hold 10–30 minutes longer to obtain proper firmness			
Wash Curd:			
First wash with 29°C water temperature			
Second wash with 16°C water temperature			
Third wash with 4°C water temperature			
Drain washed curd (by gravity) for about 2.5 hours			
Salt and cream at 152 kg creaming mixture per 454 kg with final 0.5–0.75% salt content and 4% fat content (varies with products and optional)			
Package in containers			
Storage at refrigerated temperature			

Source: References 15, 34, 42, 48, 52, 55, 62.

3. Swiss Cheese Manufacturing

Swiss cheese is also a common cheese used in the fast-food industry and in the household. It is characterized by having irregular eyes inside the cheese. These eyes are produced by *Propionicbacterium freudenreichii* subsp. *shermanii,* which produces gases trapped inside the block of cheese during fermentation and ripening. A cheese with eyes like Swiss cheese has become the icon for cheese in graphics. Swiss cheese is also characterized by its propionic acid odor. The salting process for Swiss cheese utilizes both the dry- and brine-salting processes. Like cheddar cheese, it can be categorized into regular, mild, and sharp, depending on the length of the curing process. Table 178.15 lists the basic steps in the manufacture of Swiss cheese (15, 34, 42, 48, 52, 55, 62).

4. Blue Cheese

Blue cheese is characterized by its strong flavor and by blue mold filaments from *Penicillium roqueforti* inside the cheese. It is commonly consumed as cheese or made into a salad dressing. In the manufacturing of blue cheese, as in that of Swiss cheese, salting is accomplished by the application of dry-salting and brining processes. It is characterized by a cream bleaching step to show off the blue

TABLE 178.14
Basic Steps in Making Cheddar Cheese

Standardize cheese milk.
Homogenize milk.
Pasteurization and additional heating of milk.
Cool milk to 31°C.
Inoculate milk with lactic starter (0.5–2% active mesophilic lactic starter).
Add rennet or other protease(s)—198 ml single strength (1:15,000) rennet per 1000 kg milk. Dilute the measured rennet 1:40 before use.
Agitate at medium speed.
Set the milk to proper acidity—25 minutes.
Cut the curd using 0.64 cm or wider wire knife. Stir for 5 minutes at slow speed.
Cook the curd at 38°C for 30 minutes with 1°C for every 5 minute increment. Maintain temperature for another 4–5 minutes and agitate periodically at medium speed.
Drain the curd at 38°C.
Cheddar the curd at pH 5.2–5.3.
Mill the curd slabs.
Salt the curd at 2.3–3.5 kg salt per 100 kg curd in three portions in 30 minutes.
Waxed Cheddar Cheese:
Hoop and press at 172 kPa for 30–60 seconds then 172–344 kPa overnight.
Dry the cheese at 13°C at 70% RH for 2–3 days.
Paraffin the whole cheese at 118°C for 6 seconds.
Rindless Cheddar Cheese:
Press at 276 kPa for 6–18 hours.
Prepress for 1 minute, followed by 45 minutes under 686 mm vacuum.
Remove and press at 345 kPa for 60 minutes.
Remove and vacuum seal in bags with hot water shrinkage at 93°C for 2 seconds.
Ripen at 85% RH at 4°C for 60 days or longer, up to 9–12 months, or at 3°C for 2 months then 10°C for 4–7 months, up to 6–9 months.

Source: References 15, 34, 42, 48, 52, 55, 62.

TABLE 178.15
Basic Steps in Making Swiss Cheese

Standardize cheese milk to 3% milk fat—treatment with H_2O_2-catalase optional.
Pasteurize of the milk.
Inoculate with Starters:
Streptococcus thermophilus, 330 ml per 1000 kg milk
Lactobacillus delbruechii subsp. *bulgaricus,* 330 ml per 1000 kg milk
Propionibacterium freudenreichii subsp. *shermanii,* 55 ml per 1000 kg milk
Add rennet, 10–20 minutes after inoculation—154 ml single-strength (1:15,000) rennet extract per 1000 kg milk, prediluted 1:40 with tap water before addition. Stir for 3 minutes.
Let milk set (coagulate) for 25–30 minutes.
Cut the curd with 0.64 wire knife; let stand undisturbed for 5 minutes; stir at medium speed for 40 minutes.
Cook the curd slowly to 50–53°C for about 30 minutes and stir at medium speed, then turn off steam and continue stirring for 30–60 minutes with pH reaching 6.3–6.4.
Allow the curd to drip for 30 minutes.
Press the curd—with preliminary pressing, then at 69 kPa overnight.
Salt the Curd:
First salting—in 23% salt brine for 2–3 days at 10°C
Second salting—at 10–16°C, 90% RH. Wipe the cheese surface from the brine soaking, then sprinkle salt over cheese surface daily for 10–14 days
Third salting—at 20–24°C, 80–85% RH. Wash cheese surface with salt water and sprinkle with dry salt 2–3 times weekly for 2–3 weeks
Rinded Block Swiss Cheese:
Cure—at 7°C or lower (USA) or 10–25°C (Europe) for 4–12 months.
Package in container and store at cool temperature.
Rindless Block Swiss Cheese:
Wrap or vacuum pack the blocks.
Cure stacked cheese at 3–4°C for 3–6 weeks.
Store at cool temperature.

Sources: References 15, 34, 42, 48, 52, 55, 62.

mold filament with a lighter background and by needling the block of curd to spread the blue mold filaments. It also has a soft and crummy texture due to the needling process and to the gravity draining procedure used to drain the curd. The curing period of two to four months is shorter than for hard cheeses. Its shelf life of two months is also shorter than that of its harder counterparts. Table 178.16 lists the basic steps in the manufacture of blue cheese (15, 34, 42, 48, 52, 55, 62).

5. American Style Camembert Cheese

American style camembert cheese is categorized as a soft cheese. It is characterized by a shell of mold filament on the surface produced by *Penicillium camembertii*. Brie cheese is a similar product. Addition of annatto color is optional. Like blue cheese, it is gravity drained. Therefore it has a soft, smooth texture. This cheese is surface salted and has a total curing period of three weeks before distribution. It is usually cut into wedges and wrapped individually for direct consumption. Table 178.17 lists the basic steps in the manufacture of American style camembert cheese (15, 34, 42, 48, 52, 55, 62).

6. Feta Cheese Manufacturing

Feta cheese is a common cheese in the Mediterranean countries. It is a soft cheese characterized by its brine curing (maturation) process, which is not common in cheese making. Instead, it has a similarity to the manufacture of sufu (Chinese fermented tofu, see below in this chapter). Like other soft cheese, the curing period is only two to three months. Table 178.18 lists the basic steps in the manufacture of Feta cheese (50).

C. YOGURT

Yogurt can be considered as a curdled milk product. Plain yogurt is yogurt without added flavor, stabilizer, or

TABLE 178.16
Basic Steps in Making Blue Cheese

Milk Preparation:
- Separate cream and skim milk.
- Pasteurize skim milk by HTST, cool to 30°C.
- Bleach cream with benzoyl peroxide (optional) and heat to 63°C for 30 seconds.
- Homogenize hot cream at 6–9 mPa and then 3.5 mPa, cool, and mix with pasteurized skim milk.

Inoculate milk at 30°C with 0.5% active lactic starter. Let stand for 1 hour.
Add rennet—158 ml single strength (prediluted 1:40) per 1000 kg milk. Mix well.
Let Coagulate or set, 30 minutes.
Cut curd with 1.6 cm standard wire knife.
Cook curd at 30°C, let stand 5 minutes, and then agitate every 5 minutes for 1 hour. Whey should have 0.11 to 0.14 titratable acidity.
Drain whey by gravity for 15 minutes.
Inoculate with *Penicillium roqueforti* spores—2 kg coarse salt and 28 g *P. requeforti* spore powder per 100 kg curd followed by thorough mixing. Add food grade lipase (optional).

Salting:
- First salting—dip the curd in 23% brine for 15 minutes, then press or mold at 22°C, turning every 15 minutes for 2 hours and every 90 minutes for rest of day.
- Second salting—salt cheese surface everyday for 5 days at 16°C, 85% RH.
- Final dry salting or brine salting in 23% brine for 24–48 hours. Final salt concentration about 4%.

Incubate for 6 days at 16°C, RH
Wax and needle air holes or vacuum pack and need air holes.
Mold filament development in air holes at 16°C for 6–8 days.
Cure at 11°C and 95% RH for 60–120 days.

Cleaning and Storing:
- Strip off the wax or vacuum packaging bag.
- Clean cheese, dry, and repack in aluminum foil or vacuum packaging bags.
- Store at 2°C.

Product shelf life—2 months.

Source: References 15, 34, 42, 48, 52, 55, 62.

TABLE 178.17
Basic Steps in Making American Style Camembert Cheese

Standardize milk.
Homogenize milk.
Pasteurize milk at 72°C for 6 seconds.
Cool milk to 32°C.
Inoculate with 2% active lactic starter followed by 15–30 minutes acid ripening to 0.22% titratable acidity.
Add annatto color at 15.4 ml per 1000 kg milk (optional).
Add rennet —220 ml single-strength (prediluted 1:40) rennet per 1000 ml, then mix for 3 minutes and let stand for 45 minutes.
Cut curd with 1.6 cm standard wire knife.
Cook curd at 32°C for 15 minutes with medium speed stirring.
Drain curd at 22°C for 6 hours with occasional turning.
Inoculate with *Penicillium camemberti* spores by spray gun on both sides of cheese once.
Press and mold curd by pressing for 5–6 hours at 22°C without any weight on surface.
Surface salt cheese; let cheese stand for about 9 hours.
Cure—at 10°C, 95% RH for 5 days undisturbed, then turn once and continue curing for 14 days.

Packaging, Storage, and Distribution:
- Wrap cheese and store at 10°C, 95–98% RH for another 7 days.
- Move to cold room at 4°C and cut into wedges, if required, and rewrap.
- Distribute immediately.

Source: References 15, 34, 42, 48, 52, 55, 62.

TABLE 178.18
Basic Steps in Making Feta Cheese

Standardize milk with 5% fat, enzyme treated and decolorized.
Homogenize milk.
Pasteurize by standard procedure and cool to 32°C.
Inoculate with 2% active lactic starter as cheddar cheese and allow to ripen for 1 hour.
Add rennet at 198 ml single-strength rennet (prediluted, 1:40) per 1000 kg milk and let set for 30–40 minutes.
Cut the curd with 1.6 cm standard wire knife and let stand 15–20 minutes.
Allow curd to drip for 18–20 hour at 12–18 kg on 2000 cm^2, with pH and titratable acidity developed to 4.6 and 0.55%, respectively.
Prepare cheese blocks of 13 × 13 × 10 cm each.
Salt in 23% salt brine for 1 day at 10°C.
Can and box cheese blocks in 14% salt brine (sealed container).
Cure for 2–3 months at 10°C.
Soak cured cheese in skim milk for 1–2 days before consumption to reduce salt.
Yield—15 kg/100 kg of 5% fat milk.

Source: Reference 50.

coagulant. Its acceptance is limited to those who really enjoy eating it. With the development of technology, other forms of yogurt, such as flavored and sweetened yogurt, stirred yogurt, yogurt drinks, and frozen yogurt, are now available. Its popularity varies from location to location. It is considered as a health food when active or live cultures are added to the final product. Table 178.19 lists the basic steps involved in the manufacture of yogurt. Table 178.3, presented earlier, should also be consulted for reference to other ingredients (9, 58).

Most commercially produced yogurt and its products contain sweeteners, stabilizers, or gums (Table 178.20); fruit pieces; natural and synthetic flavors (Table 178.21); and coloring compound (Table 178.22) (9, 58).

Different countries also have different standards on the percent fat and percent solids-not-fat (SNF) contents in their yogurt products (Table 178.23) (9, 58).

The different variables described above make the situation complicated. The term "yogurt" in one country may not have the same meaning in another country. This creates difficulties for international trade. Consensus or agreement among countries, and proper labeling are needed to identify the products properly.

TABLE 178.19
Basic Steps in the Production of Yogurt

Standardize liquid milk.
Homogenize liquid milk.
Heat-treat or pasteurize liquid milk at 90°C for 5 minutes or equivalent.
Cool pasteurized milk to 1–2°C above inoculation temperature.
Add starter (inoculation), 1–3% operational culture.
Add flavor, sweetener, gums, and/or color (optional).
Incubate at 40–45°C for 2.5–3.0 hours for standard cultures.
Break curd (optional).
Cool to 15–20°C in 1–1.5 hours.
Add live culture (optional).
Package.
Store at ≤10°C.

Source: References 9 and 58.

D. Fermented Liquid Milks

In milk-producing countries, it is common to have fermented milk products. These products were first discovered or developed by accident. Later, the process was modified for commercial production. Fermented liquid milks are similar to plain yogurt drinks. It is basically milk that has gone through an acid and or alcoholic fermentation. The final product is maintained in the liquid form rather than in the usual soft-gel form of yogurt. There are different fermented liquid milks available, but only sour milk, kefir, and acidophilus milk are discussed below. Readers should refer to the references listed below and other available literature on related products.

1. Sour Milk Manufacturing

Table 178.24 presents the basic steps in the manufacturing of the most basic fermented liquid milk, sour milk. The milk is standardized, pasteurized, inoculated, incubated, homogenized, and packaged. It is a very straightforward procedure compared to those for the other two products, kefir and acidophilus milk (12, 15, 31, 32, 34, 49, 55, 62).

TABLE 178.20
Some Common Gums that Could Be Used in Yogurt Manufacturing

Kind	Name of Gum
Natural	Agar
	Alginates
	Carrageenan
	Carob gum
	Corn starch
	Casein
	Furcelleran
	Gelatin
	Gum arabic
	Guar gum
	Karaya gum
	Pectins
	Soy protein
	Tragacanth gum
	Wheat starch
Modified gums	Cellulose derivatives
	Dextran
	Low-methoxy pectin
	Modified starches
	Pregelatinized starches
	Propylene glycole alginate
	Xanthin
Synthetic gums	Polyethylene derivatives
	Polyvinyl derivatives

Sources: References 9 and 58.

2. Kefir Manufacturing

Kefir is a fermented liquid milk product characterized by the small amount of alcohol it contains and its inoculant, the kefir grains. It is a common product in the Eastern

TABLE 178.21
Some Common Flavors for Yogurt

Retail Flavor	Natural Characteristic—Impact Compound	Synthetic Flavoring Compound Available
Apricot	NA	g-Undecalactone
Banana	3-Methylbutyl acetate	NA
Bilberry	NA	NA
Black currant	NA	*trans- and cis- p-Methane-8-thiol-3-one*
Grape, Concord	Methyl antranilate	NA
Lemon	Citral	15 compounds
Peach	g-Decalactone	g-Undecalactone
Pineapple	NA	Allyl hexanoate
Raspberry	1-p-Hydroxyphenyl-3-butanone	NA
Strawberry	NA	Ethyl-3-methyl-3-phenylglycidate

Source: References 9 and 58.

TABLE 178.22
Permitted Yogurt Colorings

Name of Color	Maximum Level (mg /kg)
Intigotine	6
Brilliant black PN	12
Sunset yellow FCF	12
Tartrazine	18
Cochineal	20
Carminic acid	20
Erythrosine	27
Red 2G	30
Ponceau	48
Caramel	150

Source: References 9 and 58.

TABLE 178.23
Existing or Proposed Standards for Commercial Yogurt Composition [% Fat and % Solid-Not-Fat (SNF)] in Selected Countries

	% Fat			
Country	Low	Medium	Normal	% SNF
Australia	NA	0.5–1.5	3	NA
France	0.5	NA	3	NA
Italy	1	NA	3	NA
Netherlands	1	NA	3	NA
New Zealand	0.3	NA	3.2	NA
UK	0.3	1.0–2.0	3.5	8.5
USA	0.5–1.0	2	3.25	8.5
West Germany	0.5	1.5–1.8	3.5	8.25–8.5
FAO/WHO	0.5	0.5–3.0	3	8.2
Range	0.3–1.0	0.5–3.0	3–3.5	8.2–8.5

Source: References 9 and 58.

TABLE 178.24
Basic Steps in Sour Milk Processing

Standardize milk.
Heat milk to 85–95°C, then homogenize.
Cool milk to 19–25°C and transfer to fermentation tank.
Add 1–2% start culture (inoculation).
Allow shock-free fermentation to pH 4.65–4.55.
Homogenize gel.
Cool to 4–6°C.
Fill bottles, jars, or one-way packs or wholesale packs.

Source: References 12, 15, 31, 32, 34, 49, 55, 62.

European countries and is considered to have health benefits. Among all the fermented dairy products, only this and similar products contain small amounts of alcohol. Also, in all the other fermented dairy products, pure cultures of bacteria, yeasts, and/or molds are used, but in kefir, the kefir grains are used and recycled. Kefir grains are masses of bacteria, yeasts, polysaccharides, and other

TABLE 178.25
Basic Steps in Kefir Processing

Preparation of Mother "Kefir"
Standardize milk for preparation of mother "kefir."
Pasteurize milk at 90–95°C for 15 minutes and cool to 18–22°C.
Spread kefir grains at the bottom of a container (5–10 cm thick) and add pasteurized milk (20–30 times the amount of kefir grains).
Ferment for 18–24 hours, mixing 2–3 times. Kefirs grains float to the surface.
Filter out the kefir grains with a fine sieve, wash the grains with water, and save for the next fermentation.
Save the fermented milk for the next-step inoculation.

Preparation of Drinkable Kefir
Blend fermented milk from above with 8–10 times fresh, pasteurized, untreated milk.
Pour into bottles, then close the bottles and ferment mixture for 1–3 days at 18–22°C.
[Another option is to mix the fermented milk with fresh milk at 1–5% and ferment at 20–25°C for 12–15 hours (until pH 4.4–4.5 is reached), then ripen in storage tanks 1–3 days at 10°C. Product is not as traditional but is acceptable.]
Cool to refrigerated temperature.
Store and distribute.

Source: References 12, 15, 18, 31, 32, 34, 48, 49, 55, 62.

products of bacterial metabolism, together with curds of milk protein. Production of kefir is a two-step process: (1) the production of mother kefir and (2) the production of the kefir drink. Table 178.25 lists the basic steps in kefir manufacturing (12, 15, 18, 31, 32, 34, 48, 49, 55, 62).

3. Acidophilus Milk

Acidophilus milk is considered to have probiotic benefits. Like yogurt, it is advertised as having live cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (optional). These live cultures are claimed to provide the benefit of maintaining a healthy intestinal microflora. Traditional acidophilus milk has a considerable amount of lactic acid and is considered to be too sour for the regular consumers in some locations. Therefore, a small amount of sugar is added to the final product to make it more palatable. This later product is called sweet acidophilus milk. Table 178.26 lists the basic steps in the manufacture of acidophilus milk (12, 15, 31, 32, 34, 48, 49, 55, 62).

III. MEAT PRODUCTS

A. Ingredients and Types

Fermented meat products such as ham and sausages have been available to different cultures for centuries. It is interesting to learn that the ways these products are produced are basically very similar in different cultures. Besides the meat, nitrite and salt, and sugar (optional), pure cultures are

TABLE 178.26
Basic Steps for Sweet Acidophilus Milk Processing

Procedure 1:
Standardize milk.
Heat milk to 95°C for 60 minutes, cool to 37°C, and hold for 3–4 hours; reheat to 95°C for 10–15 minutes, then cool to 37°C.
Inoculate with 2–5% bulk starter.
Incubate for up to 24 hours or to 1% lactic acid.
Cool to 5°C.
Pack and distribute.
Procedure 2:
Standardize milk.
Homogenize milk at 14.5 MPa.
Heat to 95°C for 60 minutes.
Cool to 37°C.
Inoculate with direct vat inoculation (DVI) starter.
Incubate for 12–16 hours or to about 0.65% lactic acid.
Heat at ultra high temperature (UHT), 140–145°C for 2–3 seconds to eliminate undesirable contaminants.
Cool to 10°C or lower.
Package and distribute.

Source: References 12, 15, 31, 32, 34, 48, 49, 55, 62.

sometimes used, especially in fermented sausages. Microorganisms do not merely provide the characteristic flavor for the products; the lactic acid bacteria also produce lactic and other acids that can lower the pH of the products. Pure cultures are sometimes used in hams to lower the pH and thus inhibit the growth of *Clostridium botulinum.* The raw meat for ham manufacturing is basically a large chunk of meat, and it is difficult for microorganisms to penetrate into the center, unless they are injected into the interior. Microbial growth is mainly on the surface, and the microbial enzymes are gradually diffused into the center. By contrast, in sausages the cultures, if used, are mixed with the ingredients at the beginning, and the fermentation is carried out without difficulty. Besides, sausages are much smaller than hams. Table 178.27 lists some of the ingredients used in the manufacture of hams and sausages (6, 24, 28, 29, 51, 53, 60, 61, 64).

TABLE 178.27
Raw Ingredients for Fermented Meat Products

Ingredient	Ham	Sausage
Meat		
Pork	Yes	Yes
Beef	No	Optional
Casing	No	Yes
Salt	Yes	Yes
Sugar	Optional	Optional
Starter microorganisms	Optional	Optional
Lactobacillus sakei, L. curvatus, L. plantarum, L. pentosus, L. pentoaceus		
Pediococcus pentosaceus, P. acidilactic		
Staplyococcus xylosus, S. carnosus		
Kocuria varians		
Debaryomyces hansenit		
Candida famata		
Penicillium nagiovense, P. chrysogenum		
Spices	Optional	Optional
Other flavoring compounds	Optional	Optional
Moisture retention salts	Optional	Optional
Preservatives	No	No

Source: References 6, 24, 28, 29, 51, 53, 60, 61, 64.

TABLE 178.28
Basic Steps in Dry Cured Ham Processing

Prepare pork for dry curing.
Mix the proper ratio of ingredients [salt, sugar, nitrite, and inocula (optional)].
Rub the curing mixture into the meat.
Stack the green ham for initial dry curing at 36–40°C.
Rerub the green ham and stack for additional curing at 36–40°C.
[The ham should be left in the cure for the equivalent of 3days per pound of meat.]
Soak the cured ham for 2–3 hours, then thoroughly scrub.
Place green ham in tight-fitting stockinette and hang in smokehouse to dry overnight.
Smoke at about 60 or 80°C with 60% RH for 12–36 hours.
Cool.
Vacuum pack and place in cool storage.

Source: References 6 and 61.

B. HAMS

Hams, as indicated earlier, are made from large chunks of meat. Western cultures manufacture ham using either a dry cure and or a brine cure process, sometimes followed by a smoking process. Tables 178.28 and 178.29 list the basic steps involved with the dry cure and brine cure of hams, respectively. These two processes are similar except for the salting step (6, 61).

Chinese hams are basically manufactured using a dry curing process. Procedures differ slightly, depending on

TABLE 178.29
Basic Steps in Brine Cured Ham Processing

Prepare pork for brine curing.
Mix the proper ratio of ingredients (salt, sugar, and nitrite with inocula optional): 5 gallons of brine for 100 pounds meat.
Soak the meat in the prepared brine, or stitch pump the brine into the meat (10% of the original weight of the meat) followed by soaking in the brine for 3–7 days vacuum tumbling or massaging (optional).
Remove the meat from the cover brine and wash.
Place green ham in tight-fitting stockinette and hang in smokehouse to dry overnight.
Smoke at about 60 or 80°C and 60% RH for 12–36 hours.
Cool.
Vacuum pack and place in cool storage.

Source: References 6 and 61.

TABLE 178.30
Basic Steps in Chinese Jinghua Ham Processing

Select pork hind leg, 5–7.5 kg.
Trim.
Salt, 7–8 kg salt per 10 kg ham.
Stack and overhaul at 0–10°C for 33–40 days.
Wash with cold water and brush.
Dry in the sun for 5–6 days.
Ferment (cure) for 2–3 months at 0–10°C (harmless green mold will develop on surface).
Brush off the mold and trim.
Age for 3–4 months, maximum 9 months; alternate aging process in temperature-programmable room with 60% RH for 1–2 months.
Grade.
Package and distribute.
(Yield: about 55–60%.)

Source: References 28 and 64.

the regions where the hams are made. The most famous Chinese ham is the Jinghua ham made in central China. Yunan ham, from southern China, also has a good reputation. In the old days, without refrigeration facilities during processing, transportation, and storage, it is believed that the ham completed its aging process during the transportation and storage stages. Today, with controlled temperature and relative humidity rooms, the hams are produced under controlled conditions. Table 178.30 lists the current process used in China for Jinghua ham (28, 64).

C. Sausages

Many European-type sausages are manufactured using a fermentation process. These sausages have their own characteristic flavors due to the formulations and curing processes used. It is not the intent of this chapter to list the various formulations. Readers should consult the references in this chapter and other references available elsewhere. Commercial inocula are available. Bacteria and some yeasts grow inside the sausage during the ripening period, producing the characteristic flavor. Molds can grow on the surface during storage if sausages are not properly packaged and stored in the refrigerator. Because these sausages are not sterilized, fermentation is an ongoing process, and the aged sausages carry a stronger flavor. Table 178.31 lists the basic steps in the manufacture of dry fermented sausages (24, 29, 51, 60).

IV. FERMENTED CEREAL PRODUCTS (BREADS AND RELATED PRODUCTS)

A. Kinds of Products and Ingredients

In wheat-producing countries or areas, baked yeast bread is a major staple in people's diets. This is common in the major developed countries. In other countries, other forms of bread may be the major staple. Baked bread may come in different forms such as regular yeast breads, flat breads, and specialty breads. Today, even retarded (chilled or frozen) doughs are available to meet consumers' preference for a semblance of home-cooked food. For countries or areas with less available energy, other forms of bread such as steamed bread and boiled breads are available. Fried breads are consumed mainly as breakfast or snack items. Table 178.32 lists some examples of different types of breads (8, 22, 27, 45–47).

Today, as a result of centuries of breeding selection, there are different types of wheat available to suit production environments in various regions with diverse climatic conditions. Wheat used for making bread is hard wheat, soft wheat, or a combination of both to meet product specifications. Wheat kernels are milled with removal of the bran and germ and further processed into wheat flour. Traditionally, this flour is the major ingredient for baking bread. For some health conscious consumers, whole wheat flour is the flour of choice for making bread nowadays. Wheat bran is also added to increase the fiber content of the product. Table 178.33 lists the proximate

TABLE 178.31
Basic Steps in Dry (Fermented) Sausage Processing

Select meat for processing.
Chop and mix chopped meat with spices, seasonings, and inocula at temperature of about 10°C.
Stuff the mixture in suitable casings.
Make links.
Cure or dry for 1–3 months in rooms with temperature, relative humidity, and air circulation regulated according to the type of sausage being produced.
Package and place in cool storage.

Source: References 24, 29, 51, 60.

TABLE 178.32
Types of Bread and Related Products

Type	Examples
Baked Breads	
Regular yeast breads	Bread (white, whole wheat or muti-grain)
Flat (layered) breads	Pocket bread, croissants
Specailty breads	Sourdough bread, rye bread, hamburger bun, part-baked bread, Danish pastry, stuffed bun
Chilled or frozen doughs	Ready-to-bake doughs, retarded pizza doughs, frozen proved dough
Steamed breads	Chinese steamed bread (mantou), steamed stuffed buns
Fried breads	Doughnuts
Boiled breads	Pretzels

Source: References 8, 22, 27, 45, 46, 47.

TABLE 178.33
Composition of Wheat, Flour, and Germ

Material	Mositure %	Protein %	Fat %	Total CHO %	Fiber %	Ash %
Wheat						
Hard red spring	13	14	2.2	69.1	2.3	1.7
Hard red winter	12.5	12.3	1.8	71.7	2.3	1.7
Soft red winter	14	10.2	2	72.1	2.3	1.7
White	11.5	9.4	2	75.4	1.9	1.7
Durum	13	12.7	2.5	70.1	1.8	1.7
Flour, Straight						
Hard wheat	12	11.8	1.2	74.5	0.4	0.46
Soft wheat	12	9.7	1	76.9	0.4	0.42
Flour, Patent						
Bread	12	11.8	1.1	74.7	0.3	0.44
Germ	11	25.2	10	49.5	2.5	4.3

Source: References 8, 22, 45.

composition of wheat and some of their common wheat products (8, 22, 45).

In the manufacture of various wheat-based breads and related products, the major ingredients are wheat flour, yeast, sourdough bacteria (optional), salt, and water. Other ingredients vary considerably with the types of products produced. These may be grossly classified as optional ingredients, additives, or processing aids. Each country has its own regulations and requirements. Table 178.34 lists basic ingredients, optional ingredients, additives, and processing aids used in the manufacturing of bread and related products (8, 22, 45).

B. Regular Bread

Table 178.35 lists the basic steps in bread manufacturing (8, 22, 45).

There are three basic processes in commercial bread making: straight dough process, sponge-and-dough process, and continuous-baking process. The process to be used is determined by the manufacturer and the equipment available in the baking plant. Table 178.36 lists the basic steps in the different processes. The major difference is in the way the dough is prepared and handled (8, 22, 45).

Because the dough may be prepared in various ways, the amounts of ingredients used differ accordingly. Table 178.37 lists two formulations, comparing the differences in ingredients that arise from differences in the dough preparation processes (8, 22, 45).

C. Retarded Dough

As indicated earlier, retarded dough is also available to some consumers. This type of dough is more accessible where refrigerators and freezers are more common. Dough is prepared so that the fermentation is carefully controlled, and the dough is packed inside the container. Storage of

TABLE 178.34
Bread Making—Functional Ingredients

Kind	Examples
Basic Ingredient	
Wheat flour	Bread flour, whole wheat flour
Yeast	Compressed yeast, granular yeast, cream yeast, dried yeast, instant yeast, encapsulated yeast, frozen yeast, pizza yeast, deactivated yeast *Saccharomyces cervisiae, S. carlsburgenis, S. exisguus*
Salt	
Water	
Optional Ingredients	Whole wheat flour, gluten, soya flour, wheat bran, other cereals or seeds, milk powder, fat, malt flour, egg, dried fruit, vitamins Sourdough bacteria: *Lactobacillus plantarum, L. brevis, L. fermentum, L. sanfrancisco* Other yeasts
Additives	
Emulsifier	Diacetylated tartaric acid esters of mono- and di- glycerides of fatty acids (DATA esters), Sodium stearyl-2-lactylate (SSL), distlled monoglyceride, lecithin
Flour treatment agents	Ascorbic acid, L-cysteine, potassium bromate, potassium iodate, azodicarbonamide
Preservatives	Acetic acid, potassium acetate, sodium diacetate, sorbic acid, potassium sorbate, calcium sorbate, propionic acid, sodium propionate, calcium propionate, potassium propionate
Processing Aids	Alpha-amylase, hemicellulose, proteinase, novel enzyme systems (lipases, oxidases, peroxidases)

Source: References 8, 22, 45.

TABLE 178.35
Basic Steps in Regular or Common Bread Making

Prepare basic and optional ingredients.
Prepare yeast or sourdough for inoculation.
Mix proper ingredients to make dough.
Allow to ferment.
Remix dough (optional).
Sheet.
Mold and pan.
Proof in a temperature and relative humidity controlled chamber.
Decoratively cut dough surface (optional).
Bake, steam, fry, or boil.
Cool.
Package.
Store.

Source: References 8, 22, 45.

TABLE 178.36
Various Bread Making Processes

Straight Dough Baking Process:
Weigh out all ingredients.
Add all ingredients to mixing bowl.
Mix to optimum development.
Allow first fermentation, 100 minutes, room temperature, or at 27°C for 1.5 hours.
Punch.
Allow second fermentation, 55 minutes, room temperature, or at 27°C for 1.5 hours.
Divide.
Allow intermediate proofing, 25 minutes, 30–35°C, 85% RH
Mold and pan.
Allow final proofing, 55 minutes at 30–35°C, 85% RH
Bake at 191–232°C for 18–35 minutes to approximately 100°C internal temperature.

Sponge-and-Dough Baking Process:
Weigh out all ingredients.
Mix part of flour, part of water, yeast, and yeast food to a loose dough (not developed).
Ferment 3–5 hours at room temperature, or at 21°C for 12–16 hours.
Add other ingredients and mix to optimum development.
Allow fermentation (floor time), 40 minutes.
Divide.
Allow intermediate proofing, 20 minutes, 30–35°C, 85% RH, or 27°C for 30 minutes.
Mold and pan.
Allow final proofing, 55 minutes, 30–35°C, 85% RH
Bake at 191–232°C for 18–35 minutes to approximately 100°C internal temperature.

Continuous-Baking Process:
Weigh out all ingredients.
Mix yeast, water, and maybe part of flour to form liquid sponge.
Add remaining flour and other dry ingredients.
Mix in dough incorporator.
Allow fermentation, 2–4 hours, 27°C.
Pump dough to development chamber.
Allow dough development under pressure at 80 psi.
Extrude within 1 minute at 14.5°C and pan.
Proof for 90 minutes.
Bake at 191–232°C for 18–35 minutes to approximately 100°C internal temperature.

Source: References 8, 22, 45.

this package is also carefully controlled. When the package is open, consumers can just follow the instructions on the package to bake their own bread. The technology is proprietary to the manufacturers, but there are some guidelines available (Table 178.38) (8, 22, 45).

D. Flat (Layered) Bread

Flat bread is a general term for bread products that do not rise to the same extent as regular bread. Flat breads are common commodities in Middle Eastern countries and in

TABLE 178.37
Sample Bread Recipes

White Pan Bread (Bulk Fermentation or Straight Dough Process):

Ingredients	Percent of Flour Weight
Flour	100.0
Yeast	1.0
Salt	2.0
Water	57.0
Optional Dough Improving Ingredients	
Fat	0.7
Soya flour	0.7
Malt flour	0.2

White Pan Bread (Sponge and Dough Process):

Sponge Ingredient	Percent of Total Flour
Flour	25.0
Yeast	0.7
Salt	0.5
Water	14.0
Dough Ingredients	**Percent of Total Flour**
Flour	75.0
Yeast	2.0
Salt	1.5
Water	44.0
Optional Improving Ingredients	
Fat	0.7
Soya flour	0.7
Malt flour	0.2

Source: References 8, 22, 45.

TABLE 178.38
General Guidelines for Retarded Dough Production

Reduce yeast levels as storage times increase.
Keep yeast levels constant when using separate retarders and provers.
Reduce yeast levels as the dough radius increases.
Reduce yeast levels with higher storage temperatures.
The lower the yeast level used, the longer the proof time will be to a given dough piece volume.
Yeast levels should not normally be less than 50% of the level used in scratch production.
For dough stored below −5°C, the yeast level may need to be increased.
Reduce the storage temperature to reduce expansion and weight loss from all dough pieces.
Lower the yeast levels to reduce expansion and weight losses at all storage temperatures.
Dough pieces of large radius are more susceptible to the effects of storage temperatures.
The lower freezing rate achieved in most retarder-provers, combined with the poor thermal conductivity of dough, can cause quality losses.
Proof dough pieces of large radius at a lower temperature than those of small radius.
Lower the yeast level in the dough to lengthen the final proof time and to help minimize temperature differentials.
Maintain a high relative humidity in proofing to prevent skinning.

Source: References 8, 22, 45.

TABLE 178.39
General Production Scheme for Flat Bread

Ingredient preparation.
Mixing of ingredients (dough formation).
Fermentation.
Dough cutting and rounding.
Extrusion and sheeting (optional).
First proofing.
Flattening and layering.
Second proofing.
Second pressing (optional).
Baking or steaming.
Cooling.
Packaging and distribution.

Source: References 46 and 47.

countries or areas with less accessible energy. In developed countries, flat breads are considered specialty breads. The making of the dough is similar to that of regular bread. But, the dough is flattened and sometimes layered before it is baked directly inside the hearth or in an oven. Table 178.39 lists the general production scheme for flat breads (46, 47).

E. Croissants and Danish Pastries

Croissants and Danish pastries can be considered as products that result from modifications of the basic bread making process. The dough preparation steps are similar, but the ingredients are different. Table 178.40 compares the ingredients used in making croissants and Danish pastries. From this table, it is clear that even within each group, the ingredient formulation can vary considerably, producing a wide variety of products available in the market (8, 22, 45).

F. Steamed Bread (Mantou)

Steamed bread is common in the Chinese community. Plain steamed bread is consumed as the major staple in the northern provinces of China. However, stuffed steamed breads are consumed as specialty items in various parts of China. Manufacture of steamed bread differs from that of regular bread mainly in the dough solidification process. Regular bread uses a baking process, whereas in steamed bread, steaming is used instead of baking. Consequently, in steamed bread, there is no brown crust on the bread surface because the temperature used is not high enough to cause the browning reaction. Steamed bread is always consumed hot or held in a steamer because the bread is soft at this temperature. Sometimes the bread is deep-fried before consumption. Steamed bread hardens when it cools down, making it less palatable. Various procedures are available for the production of steamed bread. Table 178.41 lists the basic steps in steamed bread processing in China (27).

TABLE 178.40
Formulations for Croissant and Danish Pastries

Ingredients	Croissant	Danish Pastries
Flour	100	100
Salt	1.8–2.0	1.1–1.56
Water	52–55.4	43.6–52
Yeast (compressed)	4–5.5	6–7.6
Shortening	2–9.7	6.3–12.5
Sugar	2–10	9.2–25
Egg	0–24	5–25
Skimmed milk powder	3–6.5	4–6.25
Laminating margarine/butter	32–57	50–64

Source: References 8, 22, 45.

V. FERMENTED SOY PRODUCTS

A. Kinds of Products and Ingredients

Soybeans have been available to the Chinese for centuries, and various fermented soy products were developed and spread to neighboring countries. These countries further developed their own fermented soy products. Soy sauce originating in China probably is the most famous and widely accepted fermented soy product. The credit for this wide acceptance also goes to the Kikkoman Company from Japan, which has helped spread soy sauce worldwide through their marketing strategy. Fermented whole soybeans such as ordinary natto, salted soybeans (e.g., Japanese Hama-natto and Chinese dou-chi), and tempe (Indonesia); fermented soy pastes (e.g., Japanese miso and Chinese dou-pan-chiang); and fermented tofus (e.g., sufu and stinky tofu or chao-tofu of Chinese origin) are

TABLE 178.41
Basic Steps in Steamed Bread Processing

Selecting flour and ingredients such as milk powder and sugar (optional).
Mixing dough.
Fermentation:
Full fermentation—1–3 hours
Partial fermentation—0.5–1.5 hours
No-time fermentation—0 hours
Remixed fermentation dough—remixing of fully fermented dough with up to 40% of flour by weight.
Neutralizating with 40% sodium bicarbonate and remixing.
Molding.
Proofing at 40°C for 30–40 minutes (no-time dough).
Steaming for about 20 minutes.
Steamed bread is maintained at least warm to preserve quality.

Source: References 27.

TABLE 178.42
Raw Ingredients for Fermented Soy Products

Ingredient	Soy Sauce	Natto	Soy Nuggets	Soy Paste	Tempe	Soy Cheese	Stinky Tofu
Major Ingredients:							
Soy							
Soybean	Yes	Yes	Yes	Optional	Yes	Yes	Yes
Soybean flour	Optional	No	No	Yes	No	Optional	Optional
Salt	Yes	Yes	Yes	Yes	No	Yes	No
Wheat	Optional	No	No	No	No	No	No
Rice flour	No	No	No	Optional	No	No	No
Major Microorganism(s):							
Mold							
Aspergillus oryzae	Yes	No	Yes	Yes	No	Optional	No
Aspergillus sojae	No	No	No	Optional	No	No	No
Mucor hiemalis, M. silivaticus	No	No	No	No	No	Yes	No
M. piaini	No	No	No	No	No	Yes	No
Actinomucor elegans	No	No	No	No	No	Yes	No
A. repens, A. taiwanensis	No	No	No	No	No	Yes	No
Rhizopus oligosporus	No	No	No	No	Yes	No	No
R. chinesis var. chungyuen	No	No	No	No	No	Yes	No
Bacteria							
Bacillus natto	No	Yes	No	No	No	No	No
Klebsiella pneumoniae	No	No	No	No	Yes	No	No
Bacillus sp.	No	No	No	No	No	No	Yes
Streptococcus sp.	No	No	No	No	No	No	Yes
Enterococcus sp.	No	No	No	No	No	No	Yes
Lactobacillus sp.	No	No	No	No	No	No	Yes
Halophlic yeasts							
Saccharomyces rouxii	Yes	No	Yes	Yes	No	No	No
Torulopsis versatlis	Yes	No	Yes	Yes	No	No	No
Halophilic lactic bacteria							
Pediococcus halophilus	Yes	No	Yes	Yes	No	No	No
Bacillus subtilus	Yes	No	Yes	Yes	No	No	No
Other Ingredients:							
Additional flavor added	Optional	No	No	No	No	Optional	No
Preservative added	Optional	No	No	No	No	No	No

Source: References 16, 37–39, 56, 57, 59, 63, 65.

more acceptable to ethnic groups. Consumers worldwide are gradually accepting these products through cultural exchange activities. The manufacturing of these products varies widely. Table 178.42 summarizes the ingredients needed for the manufacture of common fermented soy products (16, 37–39, 56, 57, 59, 63, 65).

B. Soy Sauce

There are many types of soy sauce, depending on the ratio of ingredients (wheat and soybeans), the fermentation and extraction procedures, and the flavoring ingredients (caramel and others) used. However, the procedures for manufacturing are similar. Basically, soy sauce is made by fermenting cooked soybeans in salt or brine under controlled conditions to hydrolyze the soy proteins and starches into smaller flavoring components. The soy sauce is then extracted from the fermented soybeans for standardization and packaging. Table 178.43 lists a generalized scheme for the manufacture of soy sauce. More detailed information is presented in references listed in this chapter and available literature elsewhere (16, 37–39, 57, 65).

C. Fermented Whole Soybeans

1. Ordinary (Itohiki) Natto

Ordinary natto is a typical Japanese fermented whole soybean product. The sticky mucilaginous substance on the surface of soybeans is its characteristic. It is produced by a brief fermentation of cooked soybeans with *Bacillus natto*, and it has a short shelf life. Table 178.44 lists the basic steps in the manufacture of ordinary natto. For

TABLE 178.43
Production Scheme for Soy Sauce

Select and soak beans.
Cook clean or defatted soybean under pressurized steam at 1.8 kg/cm^2 for 5 minutes.
Cool cooked bean to 40°C.
Roast and crush wheat.
Mix prepared soybeans and wheat.
Inoculate with *Aspergillus oryzae* or *sojae.*
Incubate mixture to make starter koji at 28–40°C.
Add brine (23% saltwater) to make moromi (mash).
Inoculate with halophilic yeasts and lactic acid bacteria (optional).
Brine fermentation at 15–28°C.
Add saccharified rice koji (optional).
Age moromi (optional).
Separate raw soy sauce by pressing or natural gravity.
Refine soy sauce.
Add preservative and caramel (option).
Package and store.

Source: References 16, 37–39, 57, 65.

TABLE 178.44
Production Scheme for Itohiki (Ordinary) Natto

Start with clean, whole soybeans.
Wash and soak at 21–25°C for 10–30 hours.
Cook soybean under pressurized steam at 1–1.5 kg/cm^2 for 20–30 minutes.
Drain and cool soybean at 80°C.
Inoculate with *Bacillus natto*.
Mix and package in small packages.
Incubation:
40–43°C for 12–20 hours, or 38°C for 20 hours plus 5°C for 24 hours.
Final product.
Refrigerate to prolong shelf life.

Source: References 38 and 39.

detailed information on ordinary natto, please refer to the references in this chapter (38, 39, 65).

2. Hama-natto and Dou-chi

Hama-natto is fermented whole soybeans produced in the Hama-matsu area of Japan. Similar products are produced in Japan, prefixed with different names taken from the production location. A very similar product in the Chinese culture is "tou-chi" or "dou-chi." It is produced by fermenting the cooked soybeans in salt, brine, or soy sauce, and then drying them as individual beans. Hama-natto includes ginger in its flavoring, whereas the inclusion of ginger flavoring is optional in dou-chi. Table 178.45 lists the basic steps in the production of Hama-natto and dou-chi. For further information, readers should refer to the references in this chapter and other available literature (37–39, 65).

TABLE 178.45
Production Scheme for Soy Nuggets (Hama-natto and Dou-chi)

Start with clean, whole soybeans.
Wash and soak for 3–4 hours at 20°C.
Steam cook soybean at ambient pressure for 5–6 hours or at 0.81.0 kg/cm^2 for 30–40 minutes.
Drain and cool soybean to 40°C.
Add alum (optional for douchi).
Mix with wheat flour (optional for Hama-natto).
Inoculate with *Aspergillus oryzae.*
Procedure 1 (Hama-natto):
Incubate for 50 hours at 30–33°C.
Soak inoculated soybean in flavoring solution for 8 months.
Incubate under slight pressure in closed containers.
Procedure 2 (dou-chi):
Incubate at 35–40°C for 5 days.
Wash.
Incubate for 5–6 days at 35°C.
Remove beans from liquid for drying.
Mix with ginger soaked in soy sauce (Hama-natto only).
Package final product (soy nuggets).
Refrigerate to prolong shelf-life (optional).

Source: References 37–39, 65.

D. Fermented Soy Pastes

Both the Chinese and Japanese have fermented soy pastes available in their cultures, and they are made in similar manner. However, the usage of these two products is quite different. The Japanese use their fermented soy paste, miso, in making miso soup, and to a lesser extent, for example, in marinating/flavoring of fish. Miso soup is common in traditional Japanese meals. The Chinese use their fermented soy paste, dou-pan-chiang, mainly as condiment in food preparation. Dou-pan-chiang can also be made from wing beans, and this is beyond the scope of this chapter. Table 178.46 lists the basic steps in the manufacture of miso. For detail information on miso and dou-pan-chiang, readers should consult the references for this chapter and other literature available elsewhere (16, 37–39, 56, 57, 65).

E. Fermented Tofu

1. Fermented Soy Cheese

Sufu, or fermented soy cheese, is made by fermenting tofu that is made by coagulating the soy protein in soy milk with calcium and/or magnesium sulfate. It is similar to feta cheese in its fermentation process. Both products are matured in brine in sealed containers. Some packed sufu contains flavoring ingredients. Table 178.47 lists the basic steps in the manufacture of sufu. For detail information, readers should refer to the list of references in this chapter and the other available literature (37–39, 59).

TABLE 178.46
Production Scheme of Fermented Soybean Pastes (Miso)

Start with whole, clean soybeans.
Wash and soak at 15°C for 8 hours.
Cook at 121°C for 45–50 minutes or equivalent.
Cool and mash the soybeans.
Prepare soaked, cooked, and cooled rice (optional).
Prepare parched barley (optional).
Inoculate rice or barley with *Aspergillus oryzae* (tane-koji, optional).
Mix koji and rice or barley mixture.
Add salt to koji and rice or barley mixture and mix.
Inoculate halophilic yeasts and lactic acid bacteria (optional).
Pack mixture (mashed soybean and koji) into fermenting vat with 20–21% salt brine.
Ferment at 25–30°C for 50–70 days.
Blend and crush ripened miso.
Add preservative and colorant (optional).
Pasteurize (optional).
Package and store.

Source: References 16, 37–39, 56, 57, 65.

2. Stinky Tofu

Stinky tofu is a traditional Chinese food made by fermenting tofu briefly in "stinky brine." The tofu is hydrolyzed slightly during this brief fermentation and develops its characteristic flavoring compounds. When this raw stinky tofu is deep-fried, these compounds volatilize and produce the characteristic stinky odor, thus the name "stinky tofu." It is usually consumed with chili and soy sauces. Stinky tofu is also steamed with condiments for consumption. Table 178.48 lists the basic steps in the manufacture of stinky tofu. Readers should consult the references in this chapter for further reading (37–39, 59).

TABLE 178.47
Production Scheme for Sufu (Chinese Soy Cheese)

Clean whole soybeans.
Soak.
Grind with water.
Strain through cheesecloth to recover soymilk.
Heat to boiling and then cool.
Coagulate soymilk with calcium and/or magnesium sulfate.
Cool to 50°C.
Press to remove water (formation of tofu).
Sterilize at 100°C for 10 minutes in hot-air oven.
Inoculate with *Mucor*, *Actinomucor*, and/or *Rhizopus* sp.
Procedure 1:
Incubate in dry form for 2–7 days, depending on inocula.
Incubate (ferment in 25–30% salt brine) for 1 month or longer.
Brine and age in small containers with or without addition of alcohol or other flavoring ingredients.
Procedure 2:
Incubate at 35°C for 7 days until covered with yellow mold.
Pack in closed container with 8% brine and 3% alcohol.
Ferment at room temperature for 6–12 months.
Final product (sufu or Chinese soy cheese).

Source: References 37–39, 59.

TABLE 178.48
Production Scheme for Stinky Tofu

Clean whole soybeans.
Soak.
Grind with water.
Strain through cheesecloth to recover soymilk.
Heat to boiling and then cool.
Coagulate soymilk with calcium and/or magnesium sulfate.
Cool to 50°C.
Press to remove water (formation of tofu).
Press to remove additional water.
Soak in fermentation liquid for 4–20 hours at 5–30°C.
Fresh stinky tofu, ready for frying or steaming.
Refrigerate to prolong shelf life.

Source: References 37–39, 59.

F. Tempe (Tempeh)

Tempe is a traditional Indonesian food consumed commonly by its people. It is made by fermenting cooked soybeans wrapped in wilted banana leaves or plastic wraps. The mold *Rhizopus oligosporus* produces its mycelia, and these mycelia penetrate into the block of soybeans. The mold mycelia also surround the block. This kind of fermentation is similar to molded cheese fermentation. Tempe is gradually being accepted by vegetarians in the West as a nutritious and healthy food. It is generally consumed as a deep-fried product. Table 178.49 lists the basic steps in the production of tempe (38, 39, 63, 65).

TABLE 178.49
Production Scheme for Tempe

Start with whole, clean soybeans.
Rehydrate in hot water at 93°C for 10 minutes.
Dehull.
Soak with or without lactic acid overnight.
Boil for 68 minutes.
Drain and cool to 38°C.
Inoculate with *Rhizopus oligosporus* w/o *Klebsiella pneumonia.*
Incubate on trays at 35–38°C, 75–78% RH for 18 hours.
Dehydrate.
Wrap.

Source: References 38, 39, 63, 65.

TABLE 178.50
Raw Ingredients for Fermented Vegetables

Ingredient	Sauerkraut	Western Pickles	Jalapeño Peppers	Kimchi	Oriental Vegetables
Vegetable					
Head cabbage	Yes	No	No	Optional	Optional
Chinese cabbage	No	No	No	Major	Optional
Mustard green	No	No	No	Optional	Optional
Turnip	No	No	No	Optional	Optional
Jalapeño Pepper	No	No	Yes	Optional	Optional
Chili pepper	No	No	No	Yes	Optional
Pickle/cucumber	No	Yes	No	Optional	Optional
Salt	Yes	Yes	Yes	Yes	Yes
Starter culture (lactic acid bacteria)	Optional	Optional	Optional	No	No
Added vinegar	No	Yes	Yes	No	Optional
Added spices	No	Optional	Optional	Optional	Optional
Other added flavors	No	Yes	No	Optional	Optional
Preservative(s)	No	Optional	Optional	Optional	Optional

Source: References 1, 3, 5, 10, 13, 14, 19, 25, 36, 43.

VI. FERMENTED VEGETABLES

A. Kinds of Products and Ingredients

Fermented vegetables were produced in different cultures in the old days to preserve the harvested vegetables when they are not available or due to climatic limitations. Some of these products started as traditional cultural foods but became widely accepted in other cultures. It is interesting that most of these processes are similar. Salt is used in the production of the product or the salt stock. Natural lactic acid fermentation, to produce enough lactic acid to lower product pH, is the major microbial activity in these processes. With the amount of salt added and lactic acid produced, these two ingredients create an environment that can inhibit the growth of spoilage microorganisms. Available leafy vegetables, fruits (commonly used as vegetables), and roots are used as the raw materials. Starter cultures are used occasionally. Vinegar is used in some products. Chili pepper and other spices are used in many products. Preservatives may also be used to extend shelf life after the package is opened. Table 178.50 compares the ingredients used in different fermented vegetable products (1, 3, 5, 10, 13, 14, 19, 25, 36, 43).

B. Sauerkraut

The term sauerkraut literally means sour (*sauer*) cabbage (*kraut*). It is a traditional German fermented vegetable product that has spread to other cultures; it is used on its own or in food preparations. Its sequential growth of lactic acid bacteria has long been recognized. Each lactic acid bacterium dominates the fermentation until its end product becomes inhibitory for its own development and creates another environment suitable for another lactic acid bacterium to take over. The fermentation continues until most of the available fermentable sugars are exhausted. The production of sauerkraut is not risk-free and sanitary: precautions must be taken to avoid spoilage. Table 178.51 presents the basic steps in sauerkraut processing (1, 13, 19, 25).

TABLE 178.51
Basic Steps in Sauerkraut Processing

Select and trim white head cabbage.
Core and shred head cabbage to 1/8 inch thick.
Salt with 2.25–2.50% salt by weight with thorough mixing.
Store salted cabbage in vats with plastic cover, weighed with water to exclude air in the cabbage.
Ferment at 7–23°C for 2–3 months or longer to achieve an acidity of 2.0% (lactic).
Heat kraut to 73.9°C before filling the cans or jars, then exhaust, seal, and cool.
Store and distribute.

Source: References 5, 13, 19, 25.

C. Pickles

Western-style pickles are produced by salting the pickling cucumbers in vats in salt stocks for long-term storage, followed by desalting, and bottling in sugar and vinegar, with or without spices. The fermentation is still lactic acid fermentation. However, it is more susceptible to spoilage because air may be trapped inside the slightly wax-coated cucumbers. In the salt curing of cucumbers, spoilage can occur, and precautions should be taken to avoid its occurrence. Because of their high acidity and low pH as well as their high salt content, the products are generally mildly

TABLE 178.52
Basic Steps in Fermented Pickles Processing

Size and clean cucumbers.
Prepare 5 (low salt) or 10% brine (salt stock).
Cure (ferment) cucumbers in brine for 1–6 weeks to 0.7–1.0% acidity (lactic) and pH of 3.4–3.6, dependent on temperature, with salinity maintained at a desirable level (15% for salt stock). Addition of sugar, starter culture, and spices is optional.
Recover pickles from brine, then rinse or desalt (salt stock).
Grade.
Pack pickles into jars filled with vinegar, sugar, spices, and alum, depending on formulation.
Pasteurize at 74°C for 15 minutes, followed by refrigerated storage; exhaust to 74°C at cold point, then seal and cool; or vacuum pack and heat at 74°C (cold point) for 15 minutes, then cool.
Store and distribute.

Source: References 1, 3, 5, 13, 14, 19.

heat-treated to sterilize or pasteurize them. Table 178.52 lists the basic steps in the production of Western-style pickles (1, 3, 5, 13, 14, 19).

D. KIMCHI

Kimchi is a traditional Korean fermented vegetable. Most kimchi is characterized by its hot taste because of the fairly high amount of chili pepper used in the product and its visibility. However, some kimchis are made without chili pepper, but with garlic and ginger as well as other vegetables and ingredients. Vegetables used in making kimchi vary with its formulation: Chinese cabbage, cucumber, and large turnip are more common. Either chili pepper, or garlic and ginger can be used to provide a hot sensation. Other ingredients may also be added to provide a typical flavor. The fermentation is still lactic acid fermentation. Traditionally, kimchi was made in every household in rural areas in Korea to provide vegetables for the winter, when other fresh vegetables were not readily available. Today, it is a big industry in Korea, and kimchi is available year-round. Even small kimchi refrigerators are now available to meet the demands of consumers living in cities. In other parts of the world where Koreans are residents, kimchi is available either as a household item or as a commercial product. Kimchi is usually not heat sterilized after packaging in jars. Pasteurization is optional. Kimchi is considered perishable and is stored refrigerated. Table 178.53 lists the basic steps in the manufacture of kimchi (36, 43).

TABLE 178.53
Basic Steps in Kimchi Processing

Select vegetables (Chinese cabbage, radish, cucumber, or others).
Wash vegetables.
Cut vegetables, if necessary.
Prepare 8–15% brine.
Immerse vegetables in brine for 2–7 hours to achieve 2–4% salt in vegetable.
Rinse and drain briefly.
Add seasoning.
Ferment at 0°C to room temperature for about 3 days.
Package (can also be done before fermentation).
Store at 3–4°C.

Source: References 36 and 43.

TABLE 178.54
Basic Steps in Fermented Chinese Vegetables

Select and clean vegetables.
Cut vegetables (optional).
Procedure 1:
Wilt vegetables for 1–2 days to remove moisture.
Dry salt vegetables in layers with weights on top (5–7.5% salt).
Ferment for 3–10 days.
Wash.
Dry or press fermented vegetables (optional).
Add spices and flavoring compounds.
Package.
Sterilize (optional).
Procedure 2:
Wilt cut vegetables.
Rinse fermentation container in hot water.
Fill the container with cut vegetables.
Add 2–3% brine and other flavoring compounds (optional).
Ferment at 20–25°C for 2–3 days.
Ready for direct consumption or packaging and cool storage.

Source: References 10 and 36.

E. CHINESE PICKLED VEGETABLES

The Chinese also manufacture a wide range of pickled vegetables. Various kinds of vegetables are used as raw materials. The fermentation can be either a dry-salting or a brining process, depending on the product to be manufactured. However, the fermentation is still lactic acid fermentation. The major difference between Chinese-style pickled vegetable products and Western-style pickles is that desalting is usually not practiced in the manufacture of Chinese-style pickled vegetables. The desalting process is left to the consumers, if needed. Also, some Chinese-style vegetables are made into intermediate moisture products that are not produced in their Western-style counterparts. Table 178.54 lists some of the basic steps in the manufacturing of selected Chinese pickled vegetables (10, 36).

VII. APPLICATION OF BIOTECHNOLOGY IN THE MANUFACTURING OF FERMENTED FOODS

With the advances in biotechnology, microorganisms with special characteristics for the manufacturing of fermented

foods have become available. The most significant example is the approval by the FDA of Chy-Max (chymosin produced by genetic manipulation) used in the production of cheese. Its availability greatly reduces the reliance on chymosin from young calves and produces economic savings. Other products with similar or other properties are also available in the market. Genetically modified lactic acid bacteria and yeasts used in fermented food production are also available nowadays to reduce production costs. Gradual acceptance by consumers is the key to the further development and success of biotechnology (2, 15, 20, 26, 31, 34, 52, 55, 62). Readers should refer to the references in this chapter and other references available for further information.

VIII. PROCESS MECHANIZATION IN THE MANUFACTURE OF FERMENTED FOODS

Fermented foods produced by traditional methods are labor intensive and rely a great deal on the experience of the manufacturers. The main drawback is product inconsistency. In most developed countries, products such as many cheeses, yogurts, breads, sausages, and soy sauce are now made by highly mechanized processes to standardize the products (4, 7, 11, 21, 23, 30, 33, 40, 41, 44). This not only provides product consistency, but also reduces production costs. Consumers benefit from these developments. However, some consumers, even in developed countries, still prefer the traditional products, even at an increased cost, because of their unique product characteristics. There are also fermented products that are still made by traditional or semimechanized processes because mechanization processes have not been developed for them.

ACKNOWLEDGMENT

The information in this chapter has been derived from *Food Manufacture Manual*, copyrighted and published by Science Technology System, West Sacramento, California, ©2004. Used with permission.

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179 Sour Cream and Crème Fraîche

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CONTENTS

I. INTRODUCTION

Sour cream is a relatively heavy, viscous product with a glossy sheen. It has a delicate, lactic acid taste with a balanced, pleasant, buttery-like (diacetyl) aroma (1). Various types of sour cream are found in many regions of the world. The products vary in regard to fat content and by the presence or absence of non-dairy ingredients. Furthermore, both cultured and direct acidification is utilized to lower pH. This chapter will cover sour cream as it is produced in the US and its French counterpart — crème fraîche.

II. SOUR CREAM

A. Definition

The US Food and Drug Administration (21CFR 131.160) defines sour cream as follows (2): "Sour cream results from the souring, by lactic acid producing bacteria, of pasteurized cream. Sour cream contains not less than 18 percent milkfat; ……. Sour cream has a titratable acidity of not less than 0.5 percent, calculated as lactic acid." If stabilizers are used, the fat content of the dairy fraction must be at least 18 percent fat and above 14.4 percent of the entire product.

Consumers' desire for decreasing dietary fat content has created a market for low fat sour creams. Among these products, the reduced fat (at least 50% fat reduction), and non-fat are common**,** in part due to FDA's labeling requirements for low fat products (21CFR101). Sales data over the past 25 years for the US market (3) are illustrated in Figure 179.1. The trend clearly shows increased sales. In 2000, nearly 400 million kg of sour cream were sold. Per capita sales of sour cream and dips were 1.4 kg. In comparison, per capita sales for yogurt, heavy cream, and half and half were 2.1 kg, 0.9 kg, and 1.7 kg, respectively (3).

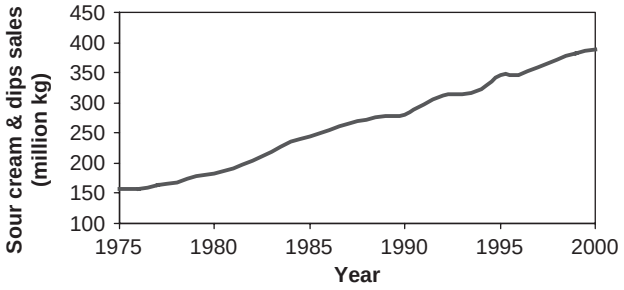

FIGURE 179.1 Sale, in million kg, of sour cream and dips in the US between 1975 and 2000.
Source: USDA, Agricultural Marketing Service.

B. Sensory Characteristics

Traditionally, the flavor of sour cream was well characterized by "sour." However, the trend for cultured dairy products is toward a milder flavor (4), which permits the sensation of aromatic compounds produced by lactic acid cultures. Lindsay et al. (5) found that important flavor compounds in sour cream include diacetyl, acetic acid, acetaldehyde, and dimethyl sulfide. Sour cream is highly viscous and should be smooth and free of particulate matter. As for appearance, a homogenous, glossy surface is preferred and no whey separation should be visible in the container (6).

C. Utilization

Sour cream is predominantly utilized as an accompaniment with warm entrees such as baked potatoes, burritos etc. This usage imposes certain demands on the sensory characteristics of the product, especially in regard to texture when in contact with warm surfaces. Sour cream must remain viscous without whey separation when placed on warm food. Some have even requested that baked potatoes can be reheated in the microwave with sour cream already added, and the sour cream should remain unaltered by this treatment. In addition, flavor characteristics become less significant when mixed with high intensity savory flavor notes such as those encountered in the Mexican cuisine. In fact, for some usages the absence of off-flavors may be considered as the primary flavor attribute. This general shift in emphasis away from flavor toward texture has led to a renewed interest in a "back to basics" sour cream such as crème fraîche, which is described later in this chapter.

III. FERMENTATION

As with all fermented dairy products, the choice of starter culture is crucial for the production of high quality sour cream (7). Mixed strains of mesophilic lactic acid bacteria are used for sour cream. In general, both acid and aroma producers are utilized. Acid producers include *Lactococcus (Lc) lactis* ssp. *lactic* and *Lc lactis* ssp. *cremoris. Lc lactis* ssp. *lactis* biovar *diacetylactis* (or *Cit*$^+$ *Lactococci*) and *Leuconostoc mesenteroides* ssp. *cremoris* are commonly used aroma producers.

The acid producers convert lactose into L-lactate through a homofermentative pathway. They can produce up to 0.8% lactic acid in milk (8) and are responsible for lowering pH in the fermented product.

In contrast, aroma producers are heterofermentative and can convert lactose into D-lactate, ethanol, acetate and CO_2. In addition, these strains convert citrate into diacetyl which is one of the major flavor compounds responsible for typical sour cream flavor. Diacetyl is subsequently partially converted into acetoin, which is a flavorless compound (9). Extensive research at starter culture companies have led to the development of *Leuconostoc* strains that show less of a tendency to convert diacetyl into acetoin**,** thus retaining high levels of diacetyl (D. Winters, personal communication. 2002). Use of such strains can extend the shelf-life of sour cream**,** as it takes longer for the product to turn stale. *Leuconostocs* also reduce acetaldehyde to ethanol (10,11). In fact, acetaldehyde has been shown to promote the growth of *Leuconostoc mesenteroides* ssp. *cremoris* (12,13). Acetaldehyde is typically associated with yogurt flavor (green apple), but is considered an off-flavor in sour cream.

The choice of starter cultures will affect product texture as well. Strains of acid producers have been developed which increase viscosity through the production of exopolysaccharides (14). These polysaccharide chains contain galactose, glucose, fructose, mannose and other sugars. Quantity and type depend on the bacteria strain and growth conditions (15,16). The exopolysaccharides interact with the protein matrix creating a firmer network and increasing water binding capacity. The importance of this behavior was confirmed by Adapa and Schmidt (17) who found that low fat sour cream, fermented by exopolysaccharide producing lactic acid bacteria, was less susceptible to syneresis and had a higher viscosity.

Production of high quality sour cream requires a fine balance of acid, viscosity, and flavor producing bacteria. While this balance varies among commercially available strains, a typical combination would be 60% acid producers, 25% acid and viscosity producers, and 15% flavor producers (D. Winters, personal communication, 2002).

IV. GEL FORMATION

Fermentation leads to a significant increase in viscosity. Two physicochemical changes cause this behavior (18,19). The casein submicelles disaggregate because of solubilization of colloidal calcium phosphate. In addition, the negative surface charge on the casein micelles decrease as pH approaches the isoelectric point. This creates the opportunity for casein micelles to enter into a more ordered system. Besides the protein network, cream gains viscosity from the formation of homogenization

clusters (20). Following single stage homogenization at room temperature, milk fat globules will cluster and these clusters may contain up to about 10^5 globules (21). Casein molecules adsorb onto newly formed fat globule membranes and, in the case of high fat content, form bridges between fat globules. Clustering increases viscosity because 1) serum is entrapped between the globules and 2) formation of irregular shaped clusters.

V. STABILIZERS

The gel structure may not be sufficiently firm to withstand abuse during transportation, handling, and storage. This could result in a weak bodied sour cream and whey syneresis in the container. These defects are especially noticeable for low fat products. To ensure consistent firm texture dairy processors often choose to add non-dairy stabilizers (22). Stabilizers commonly found in sour cream include polysaccharides and gelatin.

Stabilizers must be food grade and approved. The type and quantity used vary widely dependant on fat content, starter culture, and required sensory characteristics of the final product. Types and quantities of potential stabilizer mixtures used in sour cream are outlined in Table 179.1. Especially the non-fat formulation contains other ingredients such as emulsifiers, color, and protein.

Polysaccharides bind water and increase viscosity. Commonly used plant polysaccharides include carrageenans, guar gums and cellulose derivatives. Modified starches are frequently utilized as well. It is necessary to fully hydrate these polysaccharides to optimize their functionality. Depending on the ingredient, this may require efficient blending systems for incorporation of the ingredient into the cream, though care should be taken to avoid churning the cream. Complete hydration can sometimes only be accomplished following heating and cooling steps, which conveniently are done by the pasteurization process. Time may also be a factor for hydration to occur. Besides binding with water molecules, polysaccharides may also interact with milk proteins and form a network, which limits the movement of water and increases viscosity. A short description of the stabilizers is provided below:

a) Carrageenans: Extract of seaweed. Three types of carrageenans are commercially available, lambda, iota, and kappa, which differ based on the amount of sulfate. They have low viscosity at high temperature but viscosity increases during cooling. Lambda has the highest sulfate content, is soluble in cold milk, and forms weak gels. Iota is soluble in hot milk (55°C) and prevents syneresis. Kappa only dissolves in hot milk (<70°C) and forms brittle gels (23).
b) Guar gum: Endosperm of seed from Cyanopsis tetragonolobus plant. Different types of guar gum are available to fit processing conditions. Maximum viscosity develops over time. All are soluble in cold milk. The main component is mannose with attached galactose units.
c) Methylcellulose: A cellulose which improves freeze-thaw stability and prevents melt upon heating (22).
d) Gelatin: In contrast to the polysaccharides described above, gelatin consists primarily of protein (84–86%) and is derived from animal sources such as skin and bones (24). Gelatin is an excellent gelling agent but some off-flavors are perceived when used at excessive concentrations.

TABLE 179.1
Example of Stabilizer and other Ingredients Used in Sour Cream

Product	Ingredients	Usage Level
Sour Cream	Modified food starch, grade A whey, sodium phosphate, guar gum, sodium citrate, calcium sulfate, carrageenan, locust bean gum	1.5–1.8%
Low fat sour cream	Same as above	1.75–2.0%
Non-fat sour cream	Modified food starch, microcrystalline cellulose, propylene glycol monoester, gum Arabic, artificial color, cellulose gum	6.2–6.6%

Source: Adapted from 27.

VI. PROCESSING

Throughout the processing of sour cream, extra care should be taken to protect the cream. Prior to pasteurization, rough cream treatment could lead to rancid off flavors due to lipolysis. Following fermentation, it is important to treat the coagulum gently to retain body and texture. This includes use of positive displacement pumps instead of centrifugal pumps, round pipe elbows instead of 90° angles, and use of gravity feed wherever possible. In addition, special cream pasteurizers may be used (Figure 179.2).

Ingredients can be incorporated directly into standardized cream by mixing equipment such as a triblender. Another option is to incorporate the dry ingredients into the milk portion before standardizing the cream. The mix is preheated and homogenized (~ 65°C, 10–25 MPa) (25,26) immediately prior to pasteurization. Dairy homogenizers are normally double stage to prevent homogenization clusters. However, in sour cream production single stage homogenization is preferred to build up the body of the product. Additional viscosity is obtained if the cream is homogenized downstream from the pasteurizer though such a process increases the potential for postpasteurization contamination. Pasteurization is done at relatively

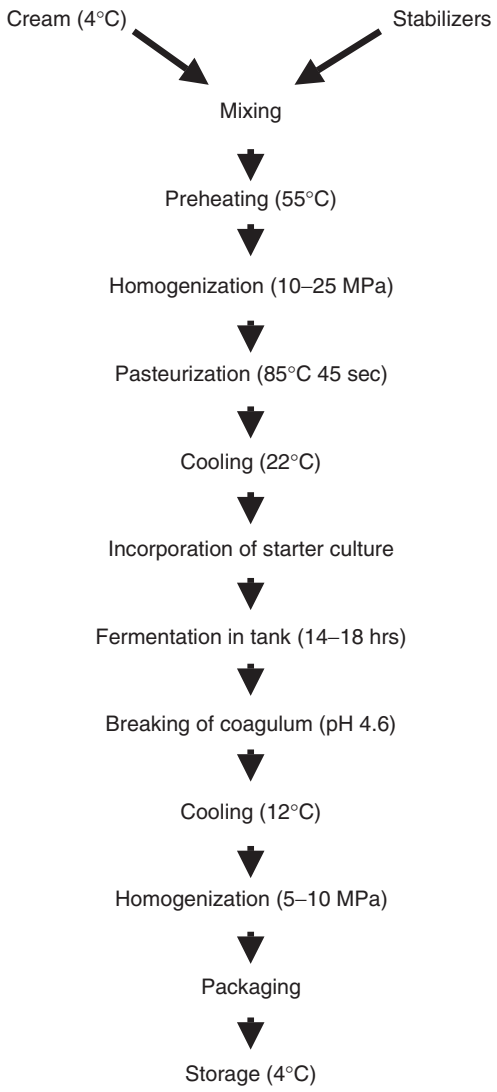

FIGURE 179.2 Process flow chart of typical sour cream process.

high temperatures (85–90°C for 10–45 sec), well above what is required for destruction of pathogens. The more severe heat treatment lowers the potential for oxidative and rancid off-flavors during storage as well as it may help improve product viscosity. The cream is cooled to 22–25°C, pumped into the fermentation tank and starter culture is added. Gentle mixing should continue until culture and cream are properly mixed (maximum 30 min). At this point mixing is stopped until fermentation is complete. The fermentation tank may be double-jacketed to allow for better temperature control. However, in reality this is not essential if the temperature of the processing room remains relatively constant around 22°C. Fermentation temperature may vary slightly from plant to plant. Higher temperatures lead to faster fermentation and potentially a more acidic product while lower fermentation temperatures may give a more flavorful product. The fermentation is slowed down/stopped by cooling when the desired acidity (~pH 4.5 or titratable acidity around 0.7% – 0.8%) is achieved. Typically this takes 14–18 hours. The coagulum is broken by gentle stirring and the product is cooled either by pumping cooling water into the double jacketed area of the tank or by pumping the cream through a special plate cooler. The cream should be cooled to around 8–12°C, which slows starter culture activity before packaging. Prior to packaging, it can also be passed through a homogenizer screen (smoothing plug) or similar type of flow restrictor to smooth and improve texture (27). The final cooling to around 4°C must occur slowly in the package in the cooler in order to allow the cream to obtain the appropriate viscosity. It is essential that the cream not be moved during this cooling step.

The above process assumes large-scale production. However, numerous process variations exist.

A. "Short Cuts"

Throughout the process described above, special attention is focused on gentle treatment of the product to assure proper body and texture. In reality, the stabilizers used today permit more flexibility in the process. A certain amount of product abuse can be tolerated without lowering the product quality because the stabilizers, when properly used, create a firm texture and prevent whey separation.

B. Low Quantity

It is possible to significantly simplify the process when producing small quantities of product. Sour cream can be made with a double-jacketed pasteurization tank, a pump, and a fermentation tank with gravity feed to the filler. The absence of a final in-line cooling step would require an efficient cooling procedure for the packaged product.

C. Chymosin Addition

Low quantities of chymosin may be added at the same time as the starter culture. This creates a more "spoonable" sour cream. Lee and White (28) found that chymosin addition (e.g. 0.066 ml/L) to low fat sour cream resulted in increased viscosity and whey separation. Sensory scores were lower for the chymosin containing sour cream in regard to flavor, body/texture, and appearance. This indicates that it may be preferable to modify the stabilizer mixture rather than to add chymosin when trying to increase product viscosity.

D. Set Sour Cream

The standardized, pasteurized cream can be mixed with starter culture and immediately filled into the package. The cream is then fermented within the final package which leaves the coagulum undisturbed. When the appropriate acidity is obtained, the products are cooled either by passing through a blast cooler or by placement in a cooler.

The advantage of this method is the possibility to lower or eliminate stabilizers and yet obtain excellent body and texture. The disadvantages are the large space requirement for fermenting the packaged product and the relatively slow cooling.

E. Direct Acidification

A product somewhat similar to sour cream can be obtained by direct acidification by organic acids such as lactic acid instead of fermentation. However, Kwan et al. (29) and Hempenius et al. (30) found that sensory panelists preferred cultured sour cream instead of chemically acidified cream. Product temperature at the time of acidification is critical and should be around 20–25°C. Higher temperatures increase the likelihood that graininess occurs and lower temperatures increase the time required for gel formation (27).

F. Low Fat and Non-fat Sour Cream

Vitamin A fortification is required in these products. The processes are often similar to traditional sour cream though non-fat sour cream mix should be homogenized at much lower pressure. The main difference is observed in the stabilizer mix as described above in section V.

VII. SHELF-LIFE

Sour cream should have a shelf-life around 25–45 days. One study documents that, when properly stored undisturbed at 4°C, sour cream has an acceptable shelf-life for up to 6 weeks (31). In another study, Folkenberg and Skriver (7) evaluated the change of sensory properties of sour cream during storage time. As storage time approached 28 days the intensity of prickling mouthfeel, sour odor, and bitter taste increased. The samples were stored under ideal conditions, which suggest that real life distribution and storage temperature abuse would likely decrease the shelf-life of this product below 28 days.

The single most important factor determining shelf-life remains cream quality. Unless the cream is of excellent quality, the sour cream quickly develops off-flavors. Two parameters that impact cream quality are 1) raw milk quality, and 2) pretreatment of milk. Good quality raw milk has a low bacterial content (low standard plate count) and comes from healthy cows (low somatic cell count). Even good quality raw milk spoils unless quickly cooled and kept at low temperatures until pasteurization. Furthermore, the time interval between milking and pasteurization should be as short as possible. Other factors to consider are proper cleaning and sanitation of all milk contact surfaces, well installed and sized pumps, and no unnecessary milk handling.

Assuming that high quality cream is utilized the parameters that limit shelf-life tend to be associated with either flavor defects or surface growth of yeast and molds. When using appropriate stabilizers the body and texture should remain adequate throughout the shelf-life. A guide on how to prevent flavor defects is included below. Yeasts and molds are controlled by improving sanitation throughout the process. As with many other dairy products sanitation trouble spots are often associated with the filler machines, which are difficult to clean.

VIII. SENSORY DEFECTS IN SOUR CREAM

A. Flavor

The high lipid content makes sour cream extremely vulnerable to lipid associated off flavors such as rancidity and oxidation. Other flavor defects include flat, lacks cultured flavor and high acid.

Rancid: Hydrolytic rancidity or lipolysis is caused by the release of free fatty acids from the glycerol backbone of triglycerides. The reaction is catalyzed by the lipase enzyme, which can be a native milk lipoprotein lipase or can originate from bacterial sources. Triglycerides are generally protected from lipase activity as long as the milk fat globule remains intact. However, damage to the globule will lead to rapid lipolysis because lipase, which is situated on the surface of the globule, can access the triglycerides. Therefore, precautions must be taken to prevent damage to the milkfat globule until pasteurization, which denatures most types of lipase. This means that raw milk/cream must be pasteurized before or immediately after homogenization to assure denaturation of lipase. Likewise, it is strongly recommended never to recycle pasteurized milk/cream back into raw milk/cream storage, which is essentially an issue of rework handling. Cream, from poor quality raw milk, can also develop rancid off-flavors during storage, as some bacterial lipases are quite heat stable and do not denature during pasteurization.

Oxidized: Autoxidation of milk fat is a reaction with oxygen that proceeds through a free radical mechanism. Unsaturated fatty acids and phospholipids are the prime substrates that are broken down into smaller molecular weight compounds such as aldehydes and ketones. Oxidized cream exhibits off flavors and aromas that have been characterized as cardboardy, metallic, oily, painty, fishy, and tallowy (6). Oxidation is catalyzed by divalent cations such as iron or copper. Thus, the best prevention is to avoid contact of milk/cream with these metals. This requires attention to details, as a single fitting or pipe made of these metals can cause significant autoxidation.

Lacks fine flavor/lacks cultured flavor: Both flavor defects tend to be associated with the choice of starter culture. It may be possible to improve flavor by switching to culture systems with more aroma producing capacity or to strains that retard the transfer of diacetyl into acetoin. It is also possible to add low concentrations of citric acid (below 0.1%), which is then converted to diacetyl by the aroma producing starter cultures. The defect can also result from

flavors imparted by stabilizers. Lowering the stabilizer dose or changing to another stabilizer system may be required.

High acid: If the final product pH is very low (e.g. around pH 4.0) the product gets an unpleasant sour flavor. While it is possible to stop the fermentation at a higher pH, this does not necessarily solve the problem because slow fermentation continues in the cooled and packaged product. Therefore, it is often preferable to change the starter culture mixture to lower the ratio of acid producing bacteria.

Bitter: Bitter off flavors are often indicators of excess proteolytic activity. Poor quality raw milk may contain heat stable proteases that remain active throughout storage. The defect is especially noticeable at the end of shelf life. Improving raw milk quality, increasing pasteurization temperature, or shortening code dates are possible solutions.

B. Body and Texture

As described above, texture is an essential quality parameter. Sour cream must remain highly viscous when in contact with warm food surfaces such as baked potatoes.

Too firm or weak: Improper choice of stabilizers can cause over-stabilized sour cream that clings to the spoon. Alternatively, the sour cream can be weak bodied and "melt" on the hot food surface.

Grainy: Grainy is primarily a mouthfeel problem, though it can be visually distracting as well in extreme cases. Grainy sour cream can be an indication of poor blending or incomplete hydration of ingredients. A different choice of stabilizers or a modification of incorporation procedure may improve the product. Another solution is to pass the product through a single stage homogenizer valve prior to packaging. Grains can also indicate that the fermentation was stopped at too high a pH and the caseins are at their isoelectric point around pH 4.6.

Free whey: Whey syneresis on top of the sour cream in the package is considered a significant quality defect. There are three solutions available to solving the problem. 1) Change or increase the concentration of stabilizer. 2) Increase fat content. Higher fat sour creams have a better water binding capacity. 3) Reevaluate the entire process and eliminate points of product abuse. This would primarily include all steps following fermentation.

IX. CRÈME FRAÎCHE

Crème fraîche or more correctly crème fraîche épaisse fermentée is the European counterpart to the US sour cream product. Crème fraîche has a fat content around 30–45% and has a mild, aromatic cream flavor. The differences between the two products originate in the manner of usage. The usage of sour cream is described above. Crème fraîche is used cold on desserts such as fruit or cakes, or warm as foundation in cream sauces which are commonly used in the French cuisine. This double usage creates a unique demand for specific product attributes. The dessert utilization requires a clean, not too sour (4), cultured flavor, that doesn't overpower flavors from other dessert components. The cultured flavor should be refreshing so that it covers the impression of fat in the product. This emphasis on flavor has led to significant research at starter culture companies and dairy processing companies to develop starter cultures that cause optimum flavor development. The body and texture should be smooth and less firm than sour cream. Crème fraîche should be "spoonable," not "pourable," and should spread slightly on the dessert without being a sauce.

The incorporation of crème fraîche into warm sauce requires thermostability, otherwise the protein would precipitate and flocculate in the sauce. For regular crème fraîche (>30% fat) flocculation is rarely a problem. In contrast, low fat crème fraîche (~15% fat) is less stable when heated. Addition of stabilizers such as xanthan gum can stabilize low fat crème fraîche. However, based on European labeling legislation a crème fraîche cannot contain stabilizers and a stabilized product would therefore need to be marketed under another name.

Crème fraîche is produced by a process similar to that of sour cream, with the exception that no ingredients are added. Without stabilizers, it becomes a challenge to obtain good body and texture. Each processing step requires attention to producing and maintaining high viscosity. In this case the homogenizer becomes an essential tool for building viscosity. Only single stage homogenization is utilized. The product is sometimes homogenized twice, either in subsequent runs before pasteurization, but more commonly both before and after pasteurization. Homogenization after pasteurization promotes better viscosity and, equally important, better thermostability. An additional homogenization following fermentation gives a homogeneous product with a smooth mouthfeel. Homogenization downstream from the pasteurizer (i.e. after pasteurization) should raise concerns in regard to post-pasteurization contamination. Ideally, an aseptic homogenizer should be used. However, the high price of such homogenizers makes this an unsuitable alternative. Instead, great emphasis must be placed on proper cleaning and sanitizing of the downstream homogenizer. In addition, food safety issues are normally controlled because of the high content of lactic acid bacteria and the low pH.

There is some discussion as to the final pH of crème fraîche fermentée. Kosikowski et al. (25) and Kurmann et al. (32) state that the cream is fermented to pH 6.2–6.3. However, commercially it is commonly fermented to an end pH around 4.5. The mild flavor is not obtained by a higher pH but rather through selection of aroma producing starter cultures. It is the combination of aroma compounds and the high fat content that mask the sour flavor in crème fraîche.

Crème fraîche is a new product on the US market. The high fat content and small scale processing contribute to a retail price which is at least twice as expensive as traditional sour cream. Nevertheless, sales are growing. Its increasing popularity is an indication of changing culinary habits promoted by growing population diversity and exposure to European culture. While crème fraîche is far from being a mainstream product on the US market, it is an interesting addition to the dairy case and can be found in many specialty stores.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Sour cream and crème fraîche," by L.M. Goddik, in *Handbook of Food and Beverage Fermentation Technology*, Editors: Y. H. Hui et al., Marcel Dekker, NY 2004.

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180 Quality Control and Sanitation of Cheese

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CONTENTS

I. INTRODUCTION

Production of cheese is a process of concentrating milk by the interaction of the milk, starter cultures and in most cases rennet. Traditionally cheese has been produced in small vats, but during the last 3 to 4 decades, processing has become increasingly industrialized. Before industrialisation, little attention was directed toward the hygienic aspects of cheesemaking, partly because the batch sizes were small and partly because methods of analysis were not well developed, consumption of cheese would normally cause only a few disease cases. In the recent years, more attention is being given to the hygienic aspects for several reasons: Methods for detecting pathogens have improved; more focus on emerging pathogens like *Listeria* and *E. coli* H7:O157; and the larger batch sizes increase risk to larger numbers of consumers, if pathogens are present. Furthermore, because of the large batches, economical losses will be substantial if the quality is not acceptable. Finally an unacceptable quality in just a few batches from a producer may lead to loss of market shares. All these reasons have led to a considerable increase in attention on the hygienic aspects of cheesemaking. These aspects include a range of factors such as

hygiene, environmental and technological factors, interactions between microorganisms, and the setting up control systems in order to prevent contamination, or at least inhibit the growth of pathogens.

Complications in doing this arise from the fact that there are many different cheese varieties, such as yellow- (with or without surface ripening), fresh-, blue veined-, white molded and cottage cheese, each with their own risks for the presence or growth of pathogens or spoilage microorganisms. In this chapter, the most important physical, chemical and microbiological factors required for inhibiting or avoiding pathogens or spoilage microorganisms are described. The creation of a comprehensive control system is discussed.

II. GENERAL ASPECTS OF CHEESE

Cheeses comprise a huge number of varieties and thus the composition also varies. The chemical composition of a cheese results from production under either high or low acidification, depending on the type of cheese, and the starter culture, which also plays a role in formation of the metabolic profile. As an example, the starter culture is able to form lactic acid as the major component, diacetyl, ethanol, acetic acid, benzoic acid and bacteriocine (1).

Depending on the type of cheese, the water content varies from very low in grana cheeses to very high in cottage cheese and the pH may vary from very low in blue veined cheeses and feta (4.6–4.8) to very high in queso fresco (6.2–6.5). As pH varies, so does the lactic acid content. The sodium chloride content varies from very high (4–6%) in some blue veined cheeses and feta to very low (0.8–1.0) in cottage cheese. The sodium chloride content, dry matter and other salts in cheese are responsible for the water activity, which is a very important growth determinant for microorganisms.

Regarding growth of microorganisms on the surface of cheeses, the packaging conditions are very important because the oxygen barrier varies, depending on the packaging material. The choice of packaging material depends on the type of cheese to be packed. Curing times for some cheeses may vary from short (1–6 days) or longer (up to 2 years) time intervals. This factor challenges the hygienic conditions in the curing rooms in relation to the chemical composition of the cheeses. Another challenge in this respect is the variation in temperatures that may occur in curing rooms. The temperature may vary from very high (20–22°C), for example for some Swiss type cheese varieties, to very low (2–5°C) for fresh cheeses or special varieties. During the curing time, temperature is elevated or lowered depending on the cheese type to be produced. Finally, the addition of nitrate or lysozyme to prevent growth of primarily *Clostridia* is an antimicrobial factor to be considered (2).

From a hygienic point of view, the cheese process in itself is a stabilizing factor. Starter culture is added to the cheese milk at 30°C and, together with the action of rennet, the milk coagulates to form a gel. As pH drops through the formation of lactic acid from the starter culture, the water binding capacity of the proteins drops. This, together with cutting of the formed gel, separates the milk into cheese and whey. After about 90 minutes, depending on type of cheese to be produced, the pH has dropped from 6.7 in fresh milk to about 6.0. After this initial cheese process, the cheese mass is pressed and anaerobic conditions are created. After pressing, the cheeses are left to complete acidification to the minimum pH (5.2), typically requiring 24 hours. Most cheese types are then cured in curing rooms at different temperatures and they may be ripened with or without a surface ripening culture. Some cheeses are packed in different foils in the curing room. Some fresh types of cheeses, however, are packed directly, then stored at 5°C and consumed within a few weeks.

III. ENVIRONMENTAL AND TECHNOLOGICAL FACTORS

A. Organic Acids and pH

The starter culture consists of lactic acid bacteria (LAB), and within 24 hours, the minimum pH is usually achieved. The minimum pH may vary, but in most cheeses the minimum pH is about 5.2 or lower; for cheddar pH 5.0 is normal, and in feta the pH may be as low as 4.6. While the buffer capacity in the cheeses is high due to the high protein content, the amount of lactic acid formed in the cheeses is also very high, up to 1.5% for some cheese types. This amount of lactic acid and the relatively low pH, achieves inhibition of many pathogens and spoilage microorganisms, especially gram negatives. However, the gram positives will also be inhibited under these conditions. Yeast and molds are only affected a little by the low pH and high amount of lactic acid. Depending on the type of starter culture, certain amounts of other organic compounds will also be formed (1). When gas producing mesophilic LAB are used as starters, diacetyl is formed in amounts that are able to cause a little inhibition of pathogens and spoilage microorganisms. Due to the metabolism occurring in the cheeses, the starters will also form acetic acid, up to 250 ppm is normal. This amount is not enough to prevent the growth of pathogens or spoilage microorganisms, and has a little impact. Other organic compounds like benzoic acid and ethanol may also have an impact on the growth of pathogens and spoilage microorganisms.

While the amounts of organic compounds formed are difficult to control, it is easy to control pH and it is important to keep it as low as possible without altering with the desirable organoleptic properties of the cheeses.

B. Temperature

Temperature and curing duration are important variables from a technological and hygienic point of view. While the curing temperature and time may improve the organoleptic properties of the cheese, it may also possibly lead to microbial growth. At 1°C, given the right conditions, *Listeria* is able to grow (3), while others such as *Clostridium tyrobutyricum* are not able to grow below 8°C (4). Therefore it is important to monitor the interaction between the curing temperature and time, in relation to the growth of selected microorganisms, and the cheese's organoleptic properties. From a hygienic point of view the temperature should be kept as low as possible.

C. NaCl and Water Activity

At a high NaCl content, and/or low water content, many microorganisms are prevented from growing (5). In such cheeses, *Staphylococci, Listeria* and yeast are chief concerns, as they are salt tolerant (6). In fresh cheeses of which the water activity is high and the NaCl content is about 0.8–1.0, the risk of growth is high these are physiological conditions. It is not possible to lower the NaCl amount because it originates from the milk, and, in many cases, it is not possible to elevate the amount due to changes in the organoleptic properties. In these cases, other means must be used to prevent growth of pathogens and spoilage microorganisms.

D. Nitrate and Lysozyme

Nitrate and lysozyme are often added to cheese milk in order to prevent late blowing from *Clostridium tyrobutyricum* (2–7). In most cases these additives also inhibit the growth of other microorganisms. But it is worth noting that the activity of the starter may also be slightly inhibited, causing a slower decrease in pH during the fermentation process, resulting in less inhibition of pathogens and spoilage microorganisms during the acidification process.

IV. ANTAGONISTIC/SYMBIOTIC ACTIONS IN CHEESES

For several years, nisin, a bacteriocin produced during fermentation has been recognized as preservative in a variety of cheeses. Nisin is produced by *Lactococcus lactis* subsp. *lactis*, one of the species used for acidification. The ability to produce nisin is strain dependant. Nisin can be added to cheese milk or processed cheese as a powder for inhibiting gram positives. Use of a living nisin producing *Lactococcus* in cheese production is not widespread because of inhibition of the starter culture may be a problem. Other bacteriocins are known (8–9). The starter culture used in the production of surface ripened cheeses, consists of a mixture of yeast, *Brevibacterium linens*, other coryneform bacteria, *Micrococcus, Staphylococcus* (primarily *equorum* and *xylosus*) and gram negatives in limited numbers (10). Bacteriocins from *B. linens* and *Staphyloccus* have been reported, and this is considered to be one way to control *Listeria* on surface ripened cheeses (11–13). *Enterococcus* sp. has also been reported to produce bacteriocins, and this production may also have an impact on the control of harmful gram positives on cheeses.

Apart from producing organic inhibitors and bacteriocins, the starter culture may also inhibit other microorganisms by direct competition for substrate. The starter culture ferments lactose into lactic acid/lactate, and thus inhibits the growth of harmful lactose fermenting microorganisms like coliforms or spoilage bacteria e.g. heterofermentative lactobacilli. Other substrates converted by the starter culture during cheesemaking are citrate and protein fragments, which mean that these compounds can't serve as substrate for pathogens. The formation of lactic acid/lactate will in turn promote the growth of lactate fermenting microorganisms (e.g. certain *Clostridia*). The best known is *Clostridium tyrobutyricum* which causes late blowing in cheeses; however, there are means available to prevent this (see Section VI.A).

V. HYGIENIC ASPECTS OF EQUIPMENT

A. Bactofugation

Bactofugation is widely used as a mean to remove sporeformers from the milk; well functioning bactofugation removes up to 98% of the spores (14). During the autumn and winter seasons, when cows are fed with silage, the spore content of *Clostridium tyrobutyricum* may be as high as 4000 per liter milk; As few as 10–20 spores per liter may cause late blowing in cheese. With a removal efficincy of 98% by bactofugation, the number of spore formers remaining is about 80 per liter, thus bactofugation is not completely effective in preventing late blowing. A relatively new process involving double bactofugation, is usually enough to prevent late blowing. Normally bactofugation is able to remove about 70% of the non-sporeforming flora, but this is far from sufficient removal of the non-sporeforming microorganisms.

B. Microfiltration

A better, but also more expensive, way to remove bacteria in general is microfiltration. Microfiltration will remove about 99.9% or more of the microbial flora present, including spore formers. By this the quality of the cheese milk is improved and the risk of the presence of microorganisms will decrease considerably. In cases where it is crucial that special spoilage microorganisms are absent, it is appropriate to perform microfiltration prior to pasteurization.

C. Pasteurization

Pasteurization is the ultimate step for removal of pathogens. Low pasteurization is defined as the combination of time and temperature and is sufficient to kill all vegetative pathogens. Still, it should be noted that not all microorganisms are killed by low pasteurisation. Spores of *Bacillus* and *Clostridium* sp. will survive along with a few spoilage microorganisms such as heterofermentative *Lactobacillus*.

In the pasteurization process it is important to control the temperature. During pasteurization the temperature will oscillate from the set point and it is crucial that the lower temperature be above 71.8°C. Controlling this requires an accurate temperature detection system. Such a system should be able to register the temperature rapidly and with high frequency. Another issue in pasteurization is the temperature differences between the components in the pasteurization unit. For example if the differences in the regenerative system are too big, fouling may occur, leading to lower efficiency in the pasteurization unit. The operation time for the pasteurization unit is also of importance. With the demands for high production efficiency, running times tend to increase, but this is often compromised due to biofilm formation. Finally of course, the cleaning of the pasteurization unit is important considering the concentration of the cleaning agents and the temperature used.

D. Cheese Vats

The cheese process is normally conducted at 30°C, with a cooking temperature range from 35–55°C. These temperatures are the normal interval in which pathogens or spoilage microorganisms are able to grow or survive. It is, therefore, necessary that the cheese vats are maintained in a high hygienic condition. There should not be any dead ends in the vats and the interfaces between the cheese vat and pumps, stirring systems, etc. should be secured properly. Finally, it is important that cleaning is easy to perform either as a "cleaning in place" (CIP) system or manually.

E. Brines

In most cases cheeses are subjected to brine with a NaCl content of about 21%. Direct salting may also be used, for example, in cheddar and cream cheeses. Due to the high salt content only a few microorganisms represent a risk; yeast (as spoilage microorganisms), *S. aureus* and *Listeria monocytogenes* are the only microorganisms of concern. It is also important to note that an infection in the brine leads only to surface contamination, as the cheese at this stage is already formed and the surface has been closed during pressing.

F. Curing and Packaging

There are three methods for curing cheeses: Packaged in bags or foil; unprotected on shelves with surface ripening; and unprotected on shelves without surface ripening. If the cheeses are packaged, the risk of contamination and/or growth is small. In general, packed and subsequently cured cheeses, keep their characteristic low pH, which along with the packaging protects against contamination.

Cheeses that are not packed, and without surface ripening, have a higher risk of contamination. For surface ripened cheeses the risk of contamination is higher than if they are also packed. The microorganisms used for surface ripening will develop into a thick layer and, hence, protect against contamination partly by producing antagonistic compounds such as methanthiol and bacteriocins, and substrate competition.

G. Distribution and Cheeses on the Market

Obviously the risk of contamination is very low when cheeses are distributed packaged and will only be contaminated by damaging the packaging. The risk is if pathogenic or spoiling microorganisms are already present in low amounts. They may grow if the cooling chain is broken. This factor is often seen in the cooling desks at the supermarkets especially, where the temperature often is as high as 15°C, 10°C above the required 5°C; precautions should be taken to keep the temperature at 5°C or below.

VI. IMPORTANT MICROORGANISMS

There are many species of pathogens or spoilage microorganisms in and on cheeses to be considered, in and on cheeses. However, many microorganisms are not found in cheese or will not grow during the cheese process. Absence of other microorganisms is controlled by veterinarian authorities in the primary (at the farmhouse) production. Among other *Brucella, Mycobacteria* and *Tuberculosis* are under veterinarian control in most countries.

A. Clostridia

Clostridia are widespread in nature and occur in raw milk. Only very few cases of illness due to *C. botulinum* can be attributed to cheese, thus the major concern is spoilage due to *C. tyrobutyricum* which causes late blowing of hard or semihard cheeses. Late blowing occurs when the number of *C. tyrobutyricum* in the cheese milk exceeds 10–50 spores per liter and the pH is 5.2 or higher (15). During late blowing lactate is converted into butyric acid, carbon dioxide and hydrogen; spoiling is characterized by extreme eye formation, split defects and off flavors. Prevention of spores in cheese milk can be achieved, to some extent, by bactofugation (16), but bactofugation is not adequate to prevent late blowing. Double bactofugation or microfiltration is, however sufficient to prevent late blowing. If it is not possible to bactofugate or microfiltrate, the addition of nitrate or lysozyme is an alternative, but the legal amounts

allowed of these compounds may not be sufficient to prevent late blowing. An effective alternative is to cool the cheese down below 8°C, at which the spores will not develop.

B. *E. coli*

Normally *E. coli* should not occur in cheeses, although 10–1000 *E. coli* per gram can be allowed from time to time depending on the cheese type. The major concerns are the pathogenic *E. coli* types. These are divided into enterohemorrhagic (EHEC), enteropathogenic (EPEC) enteroinvasive (EIEC) and enterotoxigenic (ETEC) (17). They can cause serious disease and have been reported to cause foodborne diseases in at least five outbreaks (18–20). Most concern is with the EHEC *E. coli H7:O157* that was involved in an outbreak in cheese produced from raw milk. In order to prevent pathogenic *E. coli* it is crucial to pasteurize the cheese milk. This will assure that they are not present in the milk, although postcontamination may occur. Good manufacturing practice is normally sufficient to prevent such contamination. If postcontamination does occur, it is important to prevent its growth. The activity of the primary starter should be controlled to assure a fast pH drop to below 5.5, which will inhibit *E. coli* growth. If hard cheeses are produced, the water activity should be held as low as possible, because growth of pathogenic *E. coli* does not occur at a water activity below 0.96. Such conditions are present in some blue veined cheeses like Danish blue and roquefort.

C. *Salmonella*

As for pathogenic *E. coli*, only a few cheese related *Salmonella* outbreaks have been reported (21–23). Because *Salmonella* are very heat and salt sensitive, they are not likely to grow in cheese. Thus, prevention of their contamination of cheese milk is crucial. Properly pasteurized milk is sufficient to eliminate *Salmonella* and the same precautions as described for control of *E. coli* should be taken. Fast acidification and good manufacturing practices along with maintaining as low water activity as possible is usually enough to produce safe *Salmonella* free cheeses.

D. *Listeria*

Listeria is widespread in nature and can be found in up to 50% of milk samples from raw milk silotanks, depending on geographical and seasonal variations. *Listeria monocytogenes*, a gram positive pathogen, has caused a few outbreaks of disease. Two of these outbreaks have caused higher rates of mortality (24,25). In 1985, 48 people died due to consumption of a Mexican style cheese (25). The reason for the contamination was a leak in the pasteurization equipment, resulting in a mixing of raw and pasteurized milk. In the mid-1980s 34 people died due to consumption of Vacherin Mont d'Or from Switzerland (24).

Listeria is difficult to control due to the relatively high heat stability (D10 at 69°C is about 15 sec). The temperature range for growth is 0–45°C, at pH 4.4–9.5, and up to 10% NaCl. In order to prevent growth it is important to pasteurize efficiently, which means ensuring that the cheese milk has been heated to at least 72°C for 15 sec. In the outbreaks, the products had characteristics that favored growth of *L. monocytogenes*. In the Mexican style cheese, for example only a weak acidification took place and the NaCl content was low. In the case with Vacherin Mont d'Or, insufficient hygiene, coupled with a rise in pH and a low NaCl also favored growth of *L. monocytogenes* (24). It is also worth noting that bacteriocin-producing starters and surface ripening cultures may inhibit the growth of *L. monocytogenes* (12). The infectious dose of *Listeria* is high compared to *Salmonella* and pathogenic *E. coli* which makes it easier to control it in the products. But on the other hand, *Listeria* is more likely to grow in the final product, depending on the type of cheese, because of its high resistance to low pH, NaCl and low temperature.

E. *Staphylococcus aureus*

S. aureus is associated with milk due to its close association with cows. It is relatively salt tolerant but sensitive to pH. The infectious dose is high about 10^5 pr ml. Disease arises from heat stable toxins, which means that even though no living *S. aureus* may be detected, the toxins may still be present (26). Thus, it must be assured that the number of *S. aureus* transferred from cow to product does not exceeded 10^5 pr ml, which is assured by a good manufacturing practice on the farms including assuring cooling in milk tankers and raw milk silotanks. Because *S. aureus* does not grow below about 8°C, the holding temperature should be held below 8°C, especially if the milk is stored for a long time in a silotank. *S. aureus* will only grow in cheeses with low acid content or if the cheese surface pH rises during curing. Good hygiene is normally enough to assure either the absence or low numbers in or on cheeses.

F. Others

Other microorganisms may cause spoilage or diseases in addition to the discussed above. Yeast and heterofermentative *Lactobacilli* may cause gas production or off flavors in cheeses, although the problem is generally easily solved by cleaning. In both cases, spoilage is due to heavy contamination in the dairy environments, and normally it is easy to control the environments to prevent heavy contamination. There is also the possibility for mold growth, which may be a problem on the surface of cheeses.

Formation of molds should be avoided partly because the damage to the product is severe and partly because formation of molds in some cases leads to formation of mycotoxins. Packaging in a modified atmosphere and in a material which creates a high oxygen barrier will prevent growth of molds. The risk from growth of molds is the formation of mycotoxins, but normally if molds become apparent, it will be destroyed. Mycotoxins are not able to penetrate the entire cheese, but are normally located in the outer rind, about 0.5–1.0 cm in depth depending on the cheese type and water activity.

VII. CONTROL SYSTEMS

In building up a control system, it is important to consider in each step of cheese production from the farm to the final product the microorganisms of concern. These steps are called critical control points and by introducing hazard analysis of critical control points (HACCP) it is possible to introduce a very high safety level of the products. Critical control points are production steps where some of the physiological or chemical conditions could cause a change conductive for growth of unwanted microorganisms. This means that careful evaluation should be performed if the microorganisms of concern can survive or grow at each control point. The most important parameters to evaluate are temperature; process time at the given temperature; pH and possibility of pH to change; water activity; and addition or formation of inhibitors.

There are different tools for determining whether a control point is critical. One is Predictive Modeling. Predictive Modeling is based on subjection of a large dataset on the growth of different microorganisms under different conditions into a database system. The growth data on the microorganisms derived from laboratory research, challenge tests and real product experiences are analyzed statistically and a program formed that predicts the microorganisms growth at selected temperatures, water activities, pH values, and often under other conditions such as the presence of additives.

Another critical point is the cleaning system. In most dairies, cleaning in place (CIP) is used. Sodium hydroxide with a pH of about 11 and a temperature at 70–80°C is used to wash away most of the milk components from the equipment followed by flushing with water. Nitric acid at pH about 2 is then used to remove acid soluble components from the equipment. It is expected that the strength of the CIP and the temperature will drop during the long transportation distances. In both cases, the cleaning efficiency and the direct killing effect on microorganisms will be less. Thus CIP is an important critical control point. Once the critical control points are established, limits for accepted values are determined and controlled with selected intervals.

VIII. CONCLUDING REMARKS

In order to control the presence and growth of pathogenic microorganisms, it is important to do whatever is possible to prevent their occurrence from farm to cheese product and to ensure that good manufacturing practice is implemented throughout the production. Implementation of HACCP is an excellent tool to control the pathogens or spoilage microorganisms. Raw milk should be of good quality, and its storage should be at low temperatures, especially if storage times are long. Pasteurization is also critical, thus the pasteurisation plant should be under careful control: The temperature must be stable and not below 72°C, the pasteurizer should be cleaned at required intervals and it must be assured that there are no dysfunctions such as mixing raw and pasteurized milk. The activity of the starter must be high; this will lead to a fast drop in pH, helping to control pathogens. If possible, the temperature should be kept as low as possible and the salt content as high as possible. During the process, hygienic precautions should include good personal hygiene, high water quality used for the production and adequate cleaning efficiency. It must be ensured that the CIP system be optimal with regard to strength of the sodium hydroxide and acid used, as well as the temperature employed during the cleaning step.

It should be emphasized that foodborne outbreaks in cheese consumption seldom occur. It is encouraging that considering the huge amount of cheeses consumed worldwide, only a few outbreaks have been documented. One of the reasons for this is that cheese is a well conserved system, creating a protective chain of hurdles against pathogenic and spoilage microorganisms.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Quality control and sanitation of cheese," by S. Lillevang, in *Handbook of Food and Beverage Fermentation Technology*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.

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181 Meat Fermentation

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CONTENTS

I. INTRODUCTION

Fermentation is one of the oldest preservation practices used by man and is applied to a wide variety of foods. The term fermented meat is very generic and involves a wide variety of meat products based on a mixture of minced meat and fat, with salt and/or sugar, which is stuffed into a casing, fermented and dried or smoked (1). The evolution of fermented meats has followed a traditional route, with oral transmission from generation to generation over the centuries but very empirically, with a rather poor knowledge of the process technology (2). It was just in the latest decades of the 20th century when rapid advances in the scientific knowledge of the chemistry, biochemistry and microbiology involved in the process were reached (3). This knowledge prompted successful developments in technology and a significant progress in quality standardization.

There is a wide variety of processing technologies (with important variations in the conditions for drying, ripening, smoking, etc.) as well as an important influence of the meats used as raw materials (genetic type, feed, rearing system, etc.) and microorganisms selected for the fermentation, all of this giving important variations in quality, especially in sensory characteristics. Main types of fermented meats and the most important processing technologies are described in this chapter.

II. TYPES OF PRODUCTS

Some of the most important and well-known products are listed in Table 181.1. Based on the moisture content, most of fermented meat products may be classified as dry (weight loss higher than 30%), semi-dry (weight loss lower than 20%) or just fermented sausages when no drying is applied. Some of the typical Mediterranean sausages are French saucisson, Spanish chorizo or Italian salami. On the other hand, German or Hungarian style salamis represent some of the typical northern European products. There are basic differences between both groups of products (4). So, the Mediterranean sausages, which are not smoked, undergo a slow process with nitrate addition and very mild temperatures for both fermentation and drying. On the other hand, only nitrite is used in northern European sausages with faster processes and final smoking in most cases — i.e. up to 95% of German raw sausages are smoked (5). The pH drop, reduction in water activity and drying are main factors responsible for shelf-life of these products. In general, and depending on the total processing time, three main groups of fermented sausages can be established (4): Rapid (less than 7 days), regular (around 3 weeks) and slow (up to 3–4 months).

III. RAW MATERIALS

A. Ingredients

The main ingredients are chilled raw meat from skeletal muscle tissue, either porcine alone or mixed with bovine. Other species like chicken may be used. Frozen fat tissue, usually firm pork back fat, with low content of polyunsaturated fatty acids is preferred. Highly unsaturated fat may experience undesirable oxidations and result in off-flavors, color oxidation and an unpleasant melting fats appearance on the cut surface. Fat kept under frozen storage for long time may also experience an intense oxidation and thus must be rejected for the same reasons.

TABLE 181.1
Examples of Fermented Meats Based on the Extent of Drying (3,6,7)

Extent of Drying	Weight Loss (%)	Type	Examples
No drying	< 10	Spreadable	German teewurst
"	"		Frische mettwurst
Short drying	< 20	Sliceable	Summer sausage
"	"		Lebanon Bologna
			Saucisson d´Alsace
Long drying	> 30	Sliceable	Hungarian & Italian salami
"	"		Pepperoni
"	"		Spanish salchichón
"	"		French saucisson

B. Other Ingredients and Additives

Salt constitutes the most typical curing agent. It is added within the range 2–3% and plays several important functions: It exerts a partial bacteriostatic action, contributes to an initial reduction in a_w to 0.96 and to partial solubilization of the myofibrillar proteins and, finally, imparts a typical salty taste. Nitrite, and sometimes nitrate, are also added to the curing mixture. Nitrite is a well-known microbial preservative with a specific protection against pathogens, especially *Cl. botulinum*. Another important role of nitrite is the development of the typical cured meat color (8,9). In addition, nitrite also helps in preventing oxidation and contributes to cured meat flavor (10) although the full chemical mechanisms are not fullly understood due to the complex number of compounds in the sausage and the high reactivity of nitrite. Ascorbic and erythorbic acids or their sodium salts are used to favor nitrite reduction to nitric oxide, exert antioxidative action and inhibit the formation of nitrosamines (3). Carbohydrates are added as substrate for microbial growth and development. The choice of the carbohydrate and its amount depends on the type of desired fermentation and ripening time. The fermentation rate will be rather faster or slower depending on the type of carbohydrate. Monosaccharides are rapidly fermented while disaccharides and more complex polysacharides take longer and pH drop is thus delayed. Sometimes, glucono-delta-lactone, that hydrolyzes to gluconic acid, may be used as an alternative way for a non-microbial pH drop but the quality of the product is rather poor (11). Spices, like ground pepper, paprika, garlic, etc., are used to give a typical and characteristic flavor, and sometimes color, to the fermented meat (12). Most of them are also effective antioxidants (13).

C. Starters

Traditionally, the fermentation was held at mild temperatures with the selective growth of the indigenous flora or the inoculation of flora from a previous succesful fermentation, a technique known as back-slopping. But these practices resulted in a wide variability in both safety and quality of the final products. The use of micrbial starters had a rapid development and application in the second half of the last century. Most of these cultures are based on lactic acid bacteria (*Lactobacilli* or *Pediococcus* strains) to ensure a rapid acidulation and *Micrococcaeae (Kocuria* or *Staphylococcus* strains) to have a good sensory profile (14–18). Proteolytic and lipolytic enzyme activities are important for flavor development. Other important enzyme activity in *Micrococcaceae* are nitrate reductase which contributes to the reduction of nitrate to nitrite, and catalase that mediates the degradation of hydrogen peroxide (19–21). Yeast may be used as a complement in starter cultures due to its growth on the outer area of the sausage and its important deaminase/deamidase and lipolytic

activity. Certain molds may be used as starters for the external surface of the mold-ripened sausages (22).

D. Casings

Casings are available in many materials but all of them must exhibit a good permeability to water and air. Traditional sausages have typically used natural casings that are irregular in shape but give good elasticity, tensile strength and permeability. Collagen-based casings shrink with the sausage and have good permeability. Synthetic casings are usually based on cellulose and, although non-edible, they allow good standardization due to the uniform shape and controlled pore size (23).

IV. PROCESSING TECHNOLOGY

The main stages in the processing of fermented sausages are briefly described below.

A. Comminution or Chopping

Chilled meat pieces, usually from pork and beef, and frozen fat tissues are comminuted in a meat grinder, usually in a 2/1 ratio. The size of the particles may be regulated depending on the holes of the grinder (see Figure 181.1), Then, additives (salt, nitrate/nitrite, carbohydrates, microbial starters, spices and optionally sodium ascorbate or erythorbate) are added and the whole mass is mixed for homogenization. This operation is carried out under vacuum to remove as much oxygen as possible (see Figure 181.2). Once fully homogenized, the batter is stuffed into the casings by using vacuum filling devices. A general view of stuffing machines in a fermented sausage industry is shown in Figure 181.3. Once the sausages are stuffed, they are hung in racks and placed in air-conditioned rooms with computer control of temperature, relative humidity and air flow rate. Local traditional sausages, which are produced in an artisanal way, are stuffed in natural casings and placed in either natural or air-conditioned ripening rooms.

FIGURE 181.1 Grinding of meats and fats. There are many sizes of grinder plates in accordance to the required particle size.

FIGURE 181.2 Detail of the batter after mixing in a vacuum mixer massager.

FIGURE 181.3 General view of a sausage manufacturing plant. Stuffing machines and vacuum mixer massaging can be observed. Courtesy of Tabanera Company, Segovia, Spain.

B. Fermentation

The main goal of the fermentation stage consists in the growth and development of the microbial flora, either naturally present in the meat or added as starter in the mixing. Simultaneously, different biochemical changes consisting in the enzymatic breakdown of carbohydrates, proteins and lipids take place. Other changes consist in the acid gelation of meat proteins as a result of pH drop, an initial moisture loss as a consequence of water release from meat proteins and a reduction in the redox potential through the combined action of the muscle and lactic acid

bacteria enzymes (24). An example of fermentation in an industrial computer-controlled chamber is shown in Figure 181.4.

The time required for fermentation depends on the type of product but is mainly a function of temperature and specific microorganism used as starter. There are two clear technologies for meat fermentation that decide the type of starter to be used as well as fermentation conditions (2). In the USA fermentation, starters such as *L. plantarum* or *P. acidilactici* are used for fermenting at high temperatures, (i.e., up to 40°C). The result is a rapid lactic acid generation that accumulates and produces a pH drop below 5.0. The spoilage microorganisms are rapidly inhibited but flavor formation is somehow restricted by low pH values due to inhibition of exopeptidases and lipolytic enzymes, most of them active at neutral pH. In Europe, milder fermentation temperatures, around 22–26°C, are used although other differences may be found between Mediterranean and northern European countries. For instance, the use of nitrate and long ripening, with no smoking, are typical of Mediterranean sausages. The shelf life mainly depends on drying and low water activity. On the other hand, northern European countries use nitrite, short ripening and smoking (25).

FIGURE 181.4 Example of a fermentation chamber with computer control of temperature, relative humidity and air rate. Courtesy of Tabanera Company, Segovia, Spain.

C. Ripening and Drying

There are two main objectives in this stage: Drying and development of sensory properties. Sausages are hung in racks and placed in either natural or air-conditioned ripening rooms. Special care must be taken with relative humidity in the chamber as an excessive dryness in the environment may result in an excessive dehydration of the sausage surface, known as case hardening. Recommended relative humidity in the environment should not exceed in more than 0.1 units the water activity value in the sausage and air speed must be kept to values as low as 0.1 m/s which are enough for environment homogenization (2). A periodic change in the circulation of the air also allows a good exchange of the air between the sausages and the fresh blown-in air (26) The length of the ripening/drying period depends on the kind of product and its diameter, ranging from 7 days to 3–4 months. The length and conditions of the process, that allow for an intense and prolonged microbial and enzymatic action, as well as the optional smoking, have a strong influence on the sensory properties. In some Mediterranean dry fermented sausages, a mold layer is grown on the outer surface, giving a particular appearance and contributing to ammonia generation and pH increase through deamination of amino acids (see Figure 181.5).

D. Smoking

Traditionally, smoking has been applied in areas with cold and humid climates for preservation, due to its bacteriostactic effect on yeasts, molds and certain bacteria. In addition to its antimicrobial and antioxidative effects, its main role has changed to the development of sensory properties that are appreciated by consumers (27). Smoking has several advantages such as giving a characteristic color

FIGURE 181.5 Example of a ripening chamber with computer control of temperature, relative humidity and air rate. Courtesy of Tabanera Company, Segovia, Spain.

and flavor to the product, preservation due to the bactericide and bacteriostatic effect of smoke compounds and antioxidative properties due to the phenols in the smoke (28). Smoking can be applied, by controlled combustion of oak wood, before or after fermentation.

V. SAFETY

Preservation of fermented sausages is achieved through a chain of succesive events known as hurdle effect (29). Nitrite added to the mass exerts its bactericidal effect, reinforced by oxygen removal during the mixing under vacuum, being aerobic bacteria inhibited by low redox potential. Lactic acid bacteria grow during fermentation and generate large amounts of lactic acid, that produce a pH drop inhibitory of acid-sensitive spoilage microorganisms, and other metabolites like acetic acid or hydrogen peroxide that contribute to preservation (30). In addition, many strains of lactic acid bacteria, associated with meat fermentation, are producers of bacteriocins, which are biological active proteins or peptides, active at micromolar concentrations (31), with a bactericidal action against other microorganisms (32,33). These bacteriocins act by adsorption to specific or non-specific receptors on the cell surface resulting in cell death (34,35). Finally, the reduction in water activity values as a consequence of dehydration during the drying/ripening stage also contributes to the stability of the product, especially in dry fermented sausages. The combination of these hurdles restrict the activity of most food-borne pathogens although strict care must be taken. For instance, *Salmonella* can be inhibited by pH 5.0 and $a_w < 0.95$ (6,36). *Staphylococcus aureus* is sensitive to acid pH but its toxin, that is produced in aerobic conditions, might be produced in the elapsed time before pH drop depending on the conditions that need to be controlled (7). *Clostridium botulinum* is restricted by the presence of lactic acid bacteria and nitrite together with a rapid pH drop and low a_w (6). The combination of low pH, specific starter cultures and $a_w < 0.90$ limits the growth of *Listeria monocytogenes* (37) and *Escherichia coli* (38). It is important to adopt and implement a hazard analysis and critical control points (HACCP) plan as a system of preventive controls to improve the safety of fermented meats. Hazards would include both biological and chemical contaminants.

Biogenic amines constitute another group of toxic substances that can cause disease in humans. Several factors such as the presence of microorganisms with decarboxylase activity, favorable processing conditions for the growth of these microorganisms, the production of the enzyme involved in decarboxylation of amino acids and the availability of free amino acids as substrate, contribute to the generation of amines (39). Main amines are tyramine (from tyrosine), phenylethylamine (from phenyalanine), histamine (from histidine), tryptamine (from tryptophane), putrescine and cadaverine (from ornithine and lysine, respectively). The amine levels in different types of sausages were recently reported (40) but some variability was observed probably due to variations in the manufacturing process and the type and quality of meat used (41) but, in general, the concentrations were relatively low. Tyramine is the amine generated in larger amounts through the decarboxylation of tyrosine, activity found in strains of *Lactobacillus* and *Enterococcus* (42,43). This amine is involved in increased cardiac output and migraine (44) but, fortunately, the estimated tolerance level is higher than for other amines (45,46). Cadaverine and putrescine may appear when meats of poor hygienic quality have been used as raw materials (47). Reduced risk for amine generation implies the use of raw materials of high quality, good manufacturing practices during the whole process and the use of starter cultures with no decarboxylase activity but, if possible, competitive against amine-producing microorganisms (36). In general, the low amounts of nitrate and/or nitrite initially added to the mix and the low nitrite residual levels reduce the possibility for nitrosamines generation to negligible levels (48).

VI. CHANGES DURING THE PROCESS

Many biochemical changes have been reported along the processing of fermented meats, being most of them as a consequence of endogenous and/or microbial enzymatic reactions. Some of these changes are restricted to the beginning of the process which is the case of nucleotide breakdown reactions or the glycolysis-related enzymes and subsequent generation of lactic acid. Proteolysis and lipolysis constitute two of the most important enzymatic phenomena, responsible for the generation of compounds with direct influence on taste and aroma (3,24) (Figure 181.6).

A. Glycolysis

Lactic acid is the main product resulting from carbohydrate fermentation. Once the added carbohydrates (glucose, sucrose, etc.) are transported into the cell, they are metabolized via the glycolytic or Embden-Meyerhof pathway. The ratio of the enantiomers L and D lactic acid depends on the species of lactic acid bacteria present and, more specifically, on the action of the L and D lactate dehydrogenases, respectively, and the lactate racemase. There are some key enzymes in the carbohydrate metabolism like aldolases, that generates glyceraldehyde-3-phosphate, pyruvate kinase, that generates pyruvate from phosphoethanol pyruvate and lactate dehydrogenase that generates lactic acid from pyruvate (11). Glucose is mainly metabolized through a homofermentative way but some other end products like acetate, formate, ethanol and acetoin, with an impact on sausage aroma, may be produced in trace amounts from alternative heterofermentative pathways (25). The pH drops as a consequence of

lactic acid accumulation and contributes to the preservation of the sausage by preventing the growth of undesirable microorganisms (49). The generated lactic acid also contributes directly to acid taste and indirectly to aroma, due to the formation of metabolites, and sausage consistency due to protein coagulation as pH approaches the isoelectric point of most of the myofibrillar proteins (50).

B. Proteolysis

Proteolysis consists in the progressive degradation and breakdown of major meat proteins (sarcoplasmic and myofibrillar proteins) and the subsequent generation of peptides and free amino acids. The result is a weakening of the myofibrillar network and generation of taste compounds but its extent depends on many factors. One of the most important is the activity of endogenous muscle enzymes, which depends on the original crossbreeds (51,52) and the age of the pigs (53,54). Main muscle enzymes involved in these phenomena are cathepsins B, D and L that show a great stability in long term dry-curing processes, good activity at acid pH values and are able to act against myofibrillar proteins (55–57). Other important muscle endopeptidases like calpains exhibit poor stability and its optimal pH near 7.0 is far from that in the sausage (58). Muscle enzymes exert a combined action with microbial proteases although different enzymatic profiles may be found depending on the microorganisms used as starter cultures (59). One of the major challenges is just to establish the relative role or percentage of contribution of endogenous and microbial enzymes to proteolysis. The proteolytic system of different *Lactobacillus* has been studied and contains endopeptidases able to degrade sarcoplasmic and myofibrillar proteins (60–63) as well as exopeptidases like dipeptidylpeptidase (64), tripeptidase (65), dipeptidase (66) and aminopeptidases (67,68). However, some studies (40,69) revealed that protein degradation, especially myosin and actin, is initiated by cathepsin D, a muscle endopeptidase very active at pH values near 4,5 and able to degrade both proteins. Cathepsins B and L would be more restricted to actin and its degradation products. The latter stages of proteolysis would be predominantly by bacterial peptidases and exopeptidases.

Other important factors are related with the processing technology. For instance, the temperature and time of ripening will determine the major or minor action of the enzymes, the amount of added salt, which is a known inhibitor of cathepsins and other proteases, will also regulate the enzyme action (70,71) and thus the proteolysis and taste (72).

The generation of small peptides may be depressed by the level of salt which inhibits muscle peptidases (73–75) although intense levels of non-protein-nitrogen, up to 20% of the total nitrogen content, may be reached. Some of these peptides give characteristic tastes (76). Final proteolysis steps by aminopeptidases, especially from microbial origin, are very important (77,78). These enzymes release free amino acids along the process and a substantial increase in the concentration of free amino acids is usually observed (79,80).

C. Transformation of Amino Acids

The released free amino acids as a consequence of proteolyisis are then subject of a number of enzymatic and/or chemical transformations that produce different compounds that will affect the sensory characteristics of the product (12). So, microbial decarboxylation of amino acids may produce biogenic amines. Transamination consists in the transference of the α-amino group of the first amino acid to the α carbon atom from an α-keto acid generating a keto acid from the first amino acid and a new amino acid. Dehydrogenases transform the amino acid in the corresponding keto acid and ammonia. Deamidation also generates ammonia (81). The microbial degradation of the amino acid side chain by liases may lead to phenol and indole formation (82). The Strecker degradation of amino acids produces branched aldehydes, like 3-methylbutanal, 2-methylbutanal and phenylacetaldehyde from leucine, isoleucine and phenylalanine, respectively, through oxidative deamination-decarboxilation reactions (83).

D. Lipolysis

Lipolysis consists on the breakdown of tri-acylglycerols by lipases and phospholipids by phospholipases resulting in the generation of free fatty acids. These fatty acids may contribute directly to taste and, indirectly to the generation of aroma compounds through further oxidative reactions. Main lipolytic enzymes, located in muscle and adipose tissue, in combination with microbial lipases, are involved in these phenomena (84). Although it is difficult to establish a relative role of endogenous and microbial enzymes to lipolysis, the percentage of contribution of endogenous lipolytic enzymes to total fat hydrolysis is estimated around 60 to 80% with the rest due to microbial lipases (69). The most important lipases located in muscle are the lysosomal acid lipase and acid phospholipase while in adipose tissue are the hormone sensitive lipase and the monoacylglycerol lipase (85). These enzymes show good stability through the full process (86–88). Although their activity also depends on pH, salt concentration and water activity, the conditions found in the sausages favor their action (89). The generation rate of free fatty acids, especially oleic, linoleic, estearic and palmitic acids, increases during the process. Most of these fatty acids proceed from phospholipids degradation (3) although some of them generate volatile compounds through further oxidative reactions (90). In the case of adipose tissue, the rate of generation, especially of oleic, palmitic, linoleic, stearic, palmitoleic and myristic acids, is also high.

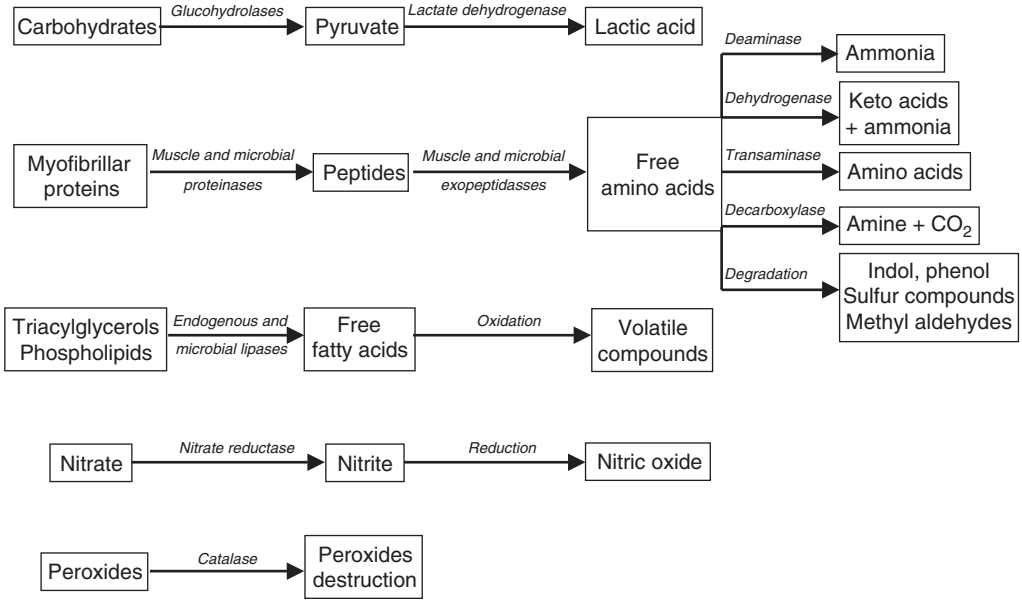

FIGURE 181.6 Scheme showing the most important reactions by muscle and microbial enzymes affecting sensory quality of fermented meats.

E. Oxidation

The generated mono and polyunsaturated fatty acids are susceptible to further oxidative reations to give volatile compounds. The beginning of lipid oxidation is correlated to an adequate flavor development. On the contrary, an excess of oxidation may lead to off-flavors (91). In fact, the generation of the characteristic aroma of dry-cured meat products is in agreement with the beginning of lipid oxidation. Free radical formation is catalyzed by muscle oxidative enzymes, like peroxydases and ciclooxygenases, external light, heating and the presence of moisture and/or metallic cations. The next step in oxidation is the formation of peroxide radicals (propagation) by reaction of free radicals with oxygen. The formed hydroxyperoxides (primary oxidation products) are flavorless but very reactive giving secondary oxidation products that contribute to flavor (92). The oxidation is finished when free radicals react each other. Main products from lipid oxidation are aliphatic hydrocarbons (poor contribution to flavor), alcohols (high odor threshold), aldehydes (low odor threshold) and ketones. Alcohols may interact with free carboxylic fatty acids giving esters, especially when nitrate is not used.

VII. DEVELOPMENT OF SENSORY CHARACTERISTICS

A. Color

The color mainly depends on the concentration of its natural pigment myoglobin, that depends on the type of muscle and the age of the animal (93,94). For instance, myoglobin concentration is higher in muscles with oxidative pattern and in older animals (94). The typical bright-red color is due to nitrosomyoglobin, compound formed after reaction of nitric oxide with myoglobin. About 10 to 40% of total myoglobin is transformed into nitrosomyoglobin (95). Nitric oxide is produced by reduction of nitrite and is favored by ascorbic acid. Those sausages made with nitrate need its reduction to nitrite by nitrate reductase activity in *Micrococcaeae*. However, this bacteria is inhibited at pH values below 5.2, being necessary to control the pH drop during fermentation to ensure that nitrate reductase can reduce nitrate to nitrite (2). Nitrosomyoglobin is susceptible to oxidation, especially at low pH and redox potential, conditions found in dry fermented sausages. So, it is very important to avoid oxidants (i.e.- peroxides) and thus the convenience of the presence of antioxidants to preserve color.

B. Texture

Texture depends on several factors like the extent of drying (loss of moisture), the extent of proteolysis, especially by cathepsin D (degree of myofibrillar protein breakdown) and the content in fat and connective tissue. The lactic acid accumulation produces the coagulation of myofibrillar proteins, the release of some water and the formation of a gel. The bonds are stabilized and the matrix of the sausage is developed (96). The consistency is accelerated during the drying period. The content in fat also exerts a positive influence on some texture and appearance traits. Textural characteristics such as firmness, hardness and cohesiveness of meat particles are continuously developed during drying. Shear values

are corelated with sausage diameter, moisture content, drying time and sometimes the initial grinding (97). In general, a good consistency is desired to facilitate the sliceability.

C. Flavor

1. Generation of Taste Compounds

Main contributors to taste are listed in Table 181.2. D and L lactic acid, especially the D enantiomer, and acetic acid are the main compounds responsible for the sour taste (43). The generation of free amino acids is the result of the combined action of muscle and microbial aminopeptidases. These enzymes are active at neutral pH, being partly inhibited at acid pH. This is the reason why those fermented meats with an intense pH drop lack significant generation of free amino acids (80). Salt is the main compound responsible for salty taste. Other compounds like glutamic and aspartic acids impart a sour taste while its sodium salts also impart a salty taste. Bitter taste is mainly associated with aromatic amino acids like phenylalanine, tryptophan and tyrosine and sweet taste with alanine, serine, proline, glycine and hydroxyproline (98,99). The generation of all these free amino acids is extremely important in fermented meats (55,79) and somehow the generation rate is affected by levels of salt as the involved enzymes (peptidases and aminopeptidases) are partly inhibited (73,74,100). Some taste enhancement may be expected from ATP-derived compounds like inosine monophosphate (IMP) and guanosin monophosphate (GMP).

2. Generation of Aroma Compounds

Aroma development is a very complex process involving numerous reactions like chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and free amino acids (50). In fact, a substantial number of volatile compounds have been reported in fermented sausages (101–105). Main groups of volatile compounds are listed in Table 181.3 and a scheme of flavor generation routes is shown in Figure 181.7. Final flavor depends on the mixture of characteristic aromas and odor thresholds for each compound although, in general, ketones, esters, aromatic hydrocarbons and pyrazines are correlated with pleasant aromas (25). Aliphatic aldehydes, ketones, alcohols and esters are typical products of different lipid oxidation reactions. Some volatile compounds like 2-methyl propanal, 2-methyl butanal, and 3-methyl butanal arise from Strecker degradation of the amino acids valine, isoleucine and leucine, respectively (105). Branched-chain acids and alcohols are secondary products. Dimethyldisulfide proceed from the Strecker degradation of sulfur containing amino acids like methionine. Compounds like diacetyl, acetoin and acetaldehyde are typical products of pyruvate microbial metabolism (25). Some pyrazines are formed through Maillard reactions between sugars and free amino acids and, although generated in low amounts, they also impart some characteristic aromas like nutty, green, earthy, etc. The spices have an intense impact on aroma. Some sulphur compounds are derived from garlic, some terpenes from pepper, 3-hexenol in paprika, etc. (12). Ammonia is released through enzymatic deamidation and deamination reactions (81).

Several hundreds of volatile compounds have been identified in the aroma of fermented meats and several techniques have been used to estimate their relative importance (105). The most important compounds contributing to aroma may be determined by comparison of a certain amount with its sensory threshold value in a similar matrix, by correlation of the respective amounts to the sensory profile of the fermented meat or by gas chromatography coupled to olfactometry (104). Some interesting correlations have been found between some volatile compounds and specific characteristics of the process. For instance, a

TABLE 181.2
Main Compounds Contributing to Taste during Meat Fermentation

Reactions Involved	Group of Compounds	Main Compounds
Proteolysis	Nitrogen compounds	Small peptides
"	"	Free amino acids
Lipolysis	Fatty acids	Long and short chain fatty acids
ATP degradation	Nucleotides/nucleosides	Inosine monophosphate, inosine
Glycolysis	Acids	D, L lactic acid, acetic acid
Addition	Carbohydrates	Glucose
"	Inorganic compounds	Salt

TABLE 181.3
Main Volatile Compounds Contributing to Aroma during Meat Fermentation

Reactions Involved	Group of Compounds	Main Compounds
Oxidation	Aliphatic aldehydes	Hexanal, pentanal, octanal
Strecker degradation	Branched-chain aldehydes	2- and 3-methylbutanal
"	Branched-chain acids	2- and 3-methyl butanoic acids
"	Branched-chain alcohols	2- and 3-methyl butanol
"	Sulfides	Dimethyldisulfide
Oxidation	Ketones	2-pentanone, 2-octanone
"	Alcohols	Ethanol, butanol
Interactions	Esters	Ethyl acetate
Lipids autoxidation	Hydrocarbons	Pentane, heptane
Pyruvate metabolism	Dicarbonyl compounds	Diacetyl, acetoin, acetaldehyde
Deamination/ deamidation	Nitrogen compounds	Ammonia

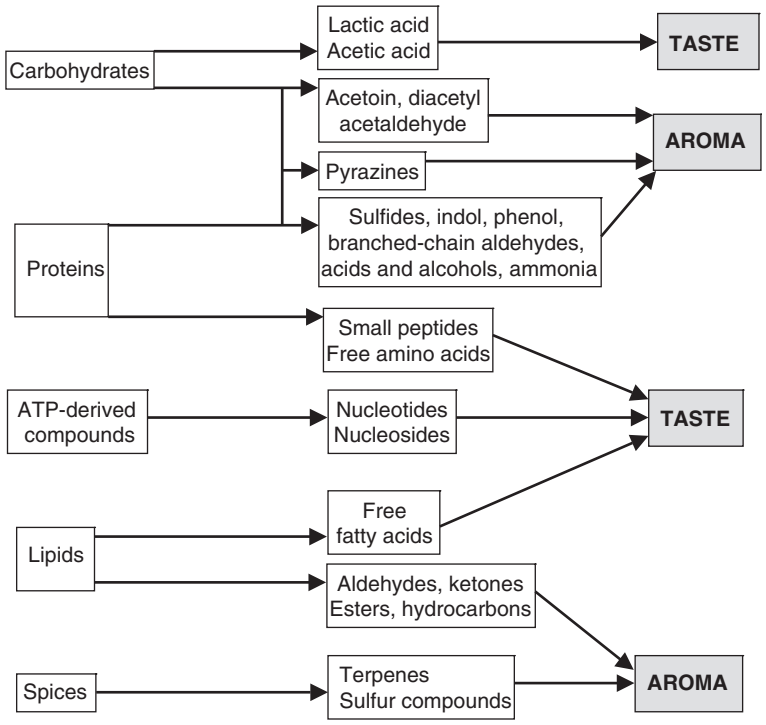

FIGURE 181.7 Scheme showing the contribution of fermentation to flavor compounds.

stronger and typical flavor has been reported in nitrite-containing dry-fermented sausages (106). In small diameter sausages, with mild processing conditions and short ripening time, most of the volatiles are produced by lipid autooxidation (107). Ketones, aldehydes, esters and terpenes are the volatile compounds usually found in Spanish and Italian sausages (51). Medium aged Italian Milano salami with low production of lactic acid, and thus higher pH, is preferred (108). The type of starter also has a strong influence on flavor like a lower rancidity when using *S. saprophyticus*, curing odor correlated with 2-pentanone, 2-hexanone and 2-heptanone when using *S. carnosus* in combination with either *P. acidilactici*, *L. sakei* or *P. pentosaceus*, or butter odor correlated with acetoin, diacetyl, 1,3-butanediol and 2,3-butanediol when using *S. saprophyticus* and *S. warneri* (109). Many aroma volatile compounds have been reported to be produced by *S. xylosus* (110) and *S. carnosus* (111). Flavor may be affected not only by time of ripening but also by packaging (112). Pre-ripening exerts a beneficial sensory effect in dry fermented sausages (113).

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182 Technologies for Jalapeño Pepper Preservation

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CONTENTS

I. INTRODUCTION

Nutrition of Aztecs and other cultures living in prehispanic Mexico was based on corn, beans, hot pepper and a type of zucchini. Hot pepper (*Capsicum annuum*) is an excellent source of vitamins A and C; the compound responsible for irritation ("hotness") is capsaicin located in the fruit placenta. In addition to the pungent effect, capsaicin stimulates appetite, increases saliva secretion and is considered to have beneficial effects on gastric fluid production. Ever since the Aztec empire, chili is added to a number of Mexican traditional foods. This cultivar is widely acceptance in Europe, Asia and Africa where Spanish traders took it as commercial item after the conquest of Mexico. Today, chili is distributed and consumed worldwide (1–3).

The most important chili cultivars in Mexico are "ancho" (wide chili): poblano, mulatto and miahuateco; Jalapeño: classic Jalapeño, candelaria or peludo ("hairy") and espinalteco; serrano and mirasol (known as guajillo or cascabel when dried). These cultivars represent 70 to 80% of chili national production. Sweet peppers, those with low concentration or none of capsaicin, are exported. These are mainly moron, and in a lesser extent anaheim, caribe, fresno and cherry (4).

Asia was the main chili producer in 2001 (8,238,000 MT) followed by Mexico (1,961,000 MT) and the United States (885,630 MT). A high percentage of chili production undergoes processing such as freezing, canning, dehydration and pickling (5).

II. PROCESSING OF JALAPEÑO PEPPER

Jalapeño pepper is the most popular in North America. Its name, jalapeño, refers to the city of Xalapa, situated in the Mexican state of Veracruz. This is a fleshy, pungent fruit; harvested when unripened, with green bright color (Figure 182.1). Most of the harvest (60%) is pickled and canned; about 20% is consumed raw. If the fruits are harvested when ripened (red), they are dried and smoked; this product is known as chipotle (2,4,6).

FIGURE 182.1 Fresh Jalapeño pepper.

III. JALAPEÑO PEPPER PRESERVATION BY FERMENTATION OR PICKLING

Pickled Jalapeño pepper is widely consumed in Mexico. This is a scalded, pasteurized product, generally merchandized in cans or glass jars, with brine to which spice and vinegar have been added. However, Jalapeño pepper shelf-life extension by fermentation is carried out only at a very small industrial level. Information regarding fermented or pickled vegetables is scattered and there is no clear differentiation between pickled and fermented products (7). This section describes the processing of fermented and pickled Jalapeño pepper merchandised in cans or glass jars.

Pickling and fermentation are preservation methods extending fruit and vegetable shelf lives via a simple and

inexpensive technology. The processed material undergoes transformation resulting in a highly acceptable food to the consumer. Pederson (8) pointed out the various methods for fruit and vegetable preservation:

1. Pickling without undergoing fermentation
2. Fermentation in a low concentrated brine
3. Fermentation in a highly concentrated brine
4. Preservation by drying and salting at low salt concentration

However, there is a controversy regarding whether the terms "pickling" and "fermentation" are equivalent. According to Pederson and Luh (9) pickled products are those added with edible acids, either lactic or acetic (vinegar); on the other hand, fermented products are such that acid was produced from sugars by bacterial metabolism. Both pickled and fermented vegetables are mainly preserved by the action of acid, also improving sensory characteristics and possibly increasing its nutritive value. According to these definitions, Jalapeño pepper can be either fermented or pickled.

Undesirable microbial growth is inhibited by acid as well as by salt concentration (10). In addition of reducing populations of spoilage microorganisms shelf-life extension of fermented or pickled vegetables also depends on inhibition of plant enzymatic activity involved in the ripening process. Control of both spoilage mechanisms, microbial and enzymatic, in Jalapeño peppers is achieved by pickling and fermentation.

IV. FERMENTED JALAPEÑO PEPPER

Acid is produced by fermenting sugars through the action of lactic acid bacteria, such as *Lactobacillum plantarum,* although the presence of *Leuconostoc mesenteroides* also has a marked effect upon fermentation and product quality (11,12). In addition to lactic acid bacteria activity, other fermentative bacteria, such as acetic acid-producing microorganisms also carry out vegetable fermentation enhancing shelf life and sensory characteristics (12). Undesirable microorganisms are inhibited by various mechanisms. Salt addition allows the growth of naturally present lactic acid bacteria, but the combined salt and acid action allows the selection of microflora associated with vegetable preservation. In some cases, sugars are added to enhance the fermentation process (10). At the same time, fermentation reduces carbohydrate concentration and increases acid production (13). The most important conditions for an adequate vegetable fermentation are: anaerobiosis, salt concentration, temperature, and the used of suitable starters. Lactic acid bacteria can be present as native microflora in the pepper, but to assure a uniform fermentation, selected starters are usually added.

To obtain the best fermented jalapeño pepper quality, the raw material (*Capsicum annuum)* cv. Jalapeño must be recently harvested, still green, and without wounds or peduncle. Figure 182.2 shows the general flow diagram of fermented Jalapeño pepper processing (14).

A. Preliminary Operations

Raw Jalapeño peppers are selected according to their size and quality. They are washed, and small incisions are made in order to facilitate brine diffusion to the central part, and to eliminate gas formed during fermentation. Washing and blanching diminishes hot pepper fermentative ability; therefore it is necessary to add a starter culture (*Lactobacillum plantarum*).

V. FERMENTATION

Fermentation is carried out by facultative anaerobic homofermentative strains such as *Lactobacillum plantarum* and *Pediococcus cereviseae*. *L. plantarum* produces acetic acids as well as ethanol and gas (CO_2 and H_2). Peppers are immersed in 10% brine for 4 to 6 weeks, and 0.5 to 1% sucrose is added although pepper cell fluid contains carbohydrates as well as nitrogen compounds and minerals. The fruit cell fluid, however, tends to dilute the brine. For this reason, it is also necessary to add 1% salt daily during the first week, and three times a week during the rest of the immersion time in order to keep the desired brine concentration (18–20%). The peppers must be completely covered by the brine at all times.

Fermentation takes place in 4 to 6 weeks. It is carried out in closed tanks, with a vent to allow the gas formed during the process to dissipate. At the end of the fermentation period, the peppers, originally bright green, turn into olive green. The plant tissue also changes, taking a translucent aspect. Acid concentration increases from 0.8 to 1.5% (expressed as lactic acid) promoting a decrease in pH. The peppers are then washed to eliminate salt excess, classified according to their size, placed in glass jars or plastic bags, mixed with other vegetables, usually carrots and onions, and covered with vinegar.

Fermented Jalapeño peppers are highly perishable if the vinegar has less than 3% acetic acid. In this case, pasteurization is necessary. It is carried out over 30 min at 71°C (for glass jars containing 280 g of product). Finally, the product is labeled, packaged and stored in a similar way as for pickled (non-fermented) Jalapeño peppers.

VI. PICKLED (NON-FERMENTED) JALAPEÑO PEPPERS

The most widely merchandized Jalapeño peppers in producing countries such as Mexico, are pickled non-fermented products. They are sold in different can sizes and consist of whole cut peppers, mixed with scalded onions, carrots, mushrooms and vinegar to which spices have been added (Figures 182.3 and 182.4).

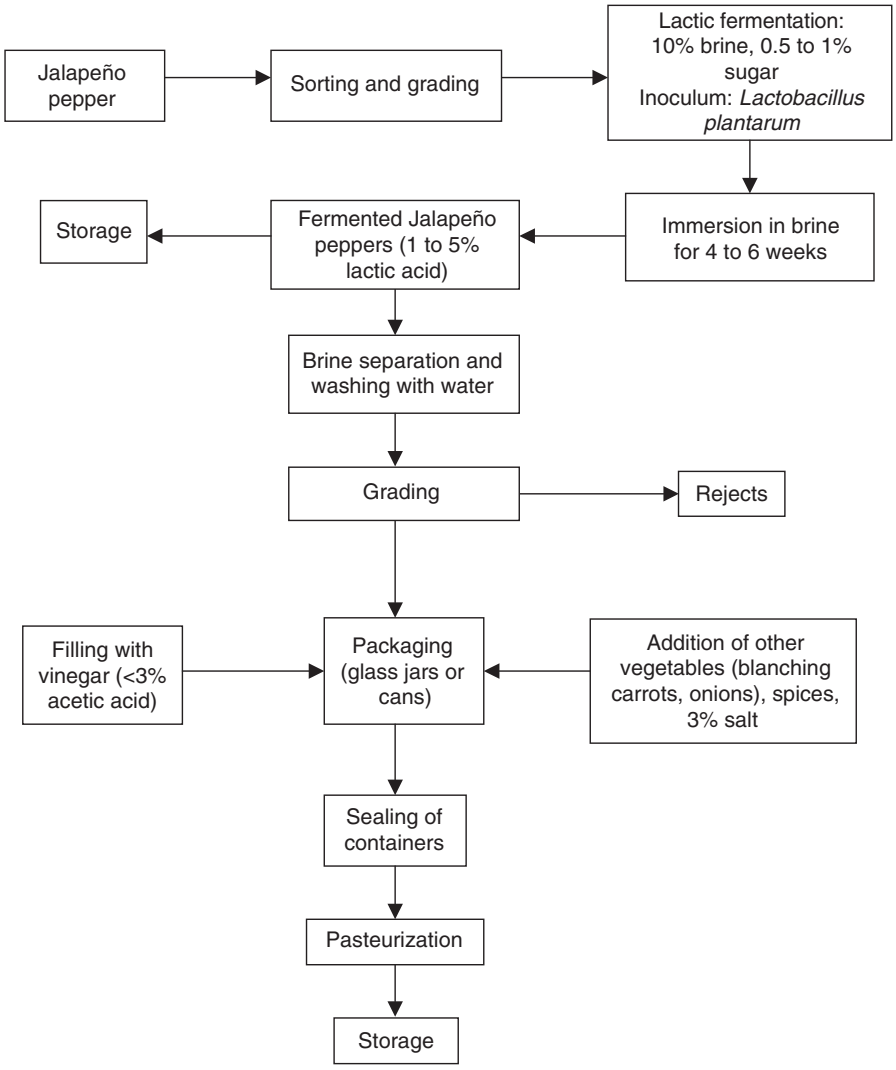

FIGURE 182.2 Fermented pickled Jalapeño pepper processing.

FIGURE 182.3 Pickled and sliced lengthwise Jalapeño peppers.

FIGURE 182.4 Pickled and sliced Jalapeño peppers ("nachos").

The main difference between this product and fermented peppers is that the raw material is fresh peppers or preserved with salt (brine). According to its acidity, the product is then heat-treated (15). As it is an acidic food spoilage can take place, therefore a further preservation method is necessary. The flow diagram for this process is shown in Figure 182.5. At domestic level or in small industries, pickled Jalapeño peppers are prepared by mixing scalded carrots, onions and other vegetables with Jalapeño peppers cut lengthwise, adding vinegar previously flavored with pepper, cinnamon, marjoram, thyme and clove, and other condiments (onion, garlic and laurel fried in vegetable oil) (16).

A. Preliminary Operations

Contaminants or inedible components can be present when Jalapeño peppers, carrots and onion are selected for this process. Added vegetables undergo following operations: washing, selection, classification, size reduction and scalding. Canning also ensures an adequate edible quality of the product.

Jalapeño peppers, carrots and onions are transported into the processing plant and selected for processing or storage, according to their quality and degree of ripeness. Washing by immersion, spraying, or combined methods are carried out in order to eliminate contaminants (17,18).

1. Washing

During immersion washing dirt adhering to the vegetable surface is softened and eliminated together with stones, sand and other abrasive material that may damage the equipment during further operations. Immersion tanks are made of metal, mortar or building materials suitable for easy cleaning and disinfection. In order to improve the washing efficiency, stirring is provided. Detergent or chlorine is also added to decrease the microbial load. During spray drying, vegetables are exposed to pressurized water applied if water supply is restricted.

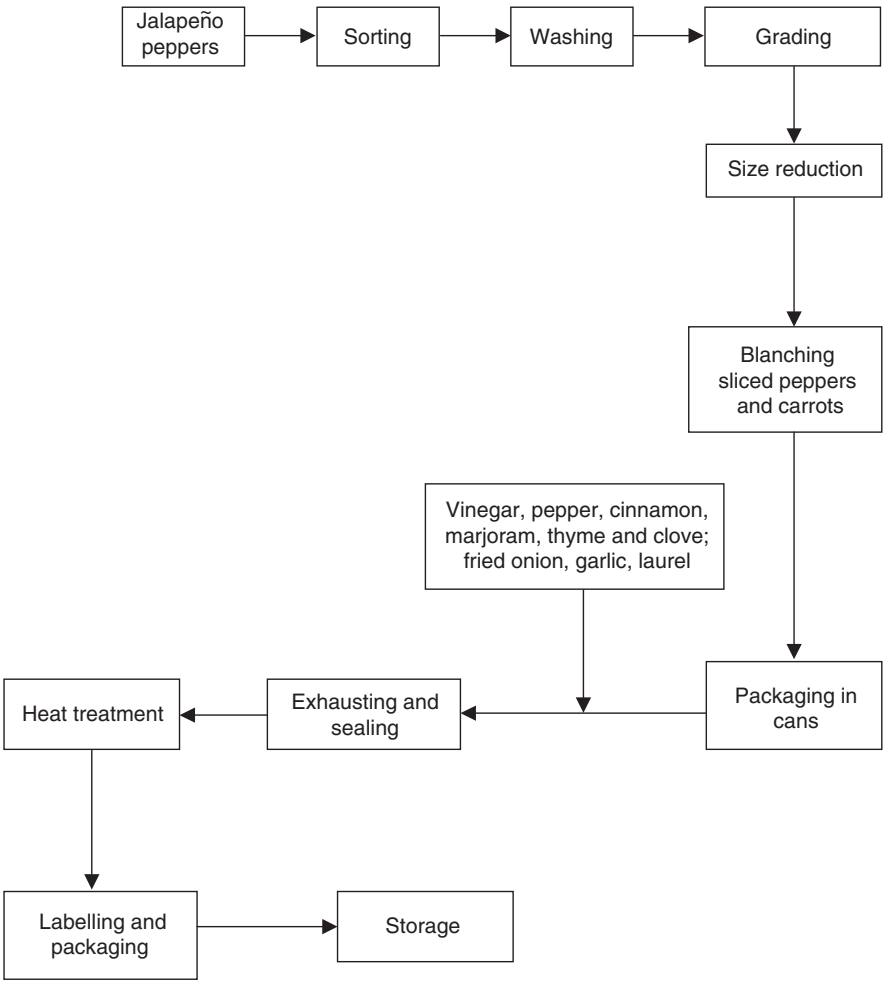

FIGURE 182.5 Non-fermented pickled Jalapeño pepper processing.

2. Sorting and Grading

Deterioration occurs during harvesting, transportation to the processing plant, or cleaning. These operations are carried out to discard products unsuitable for processing: damaged, unripe, overripe or deformed vegetables.

Vegetables are selected according to size and quality and are directed to processing or direct consumption. In general, classification consists in simultaneous evaluation of various physical properties. For hot peppers, onions, and carrots, classification is manually carried out. By this procedure simultaneous evaluation of several attributes is achieved, difficult in an automatic procedures. Classification renders having uniform material directed to a specific operation, such as peeling, size reduction, or blanching. Grading also homogenizes the product, improving heat-processing efficiency. Trained personnel generally carry out quality classification.

3. Peeling

Peeling is a necessary operation during carrot and onion processing to improve product appearance and to eliminate inedible parts, although it is important not to discard large portions of the vegetable. There are different peeling methods:

Abrasion: Carrot peeling is done by abrasion. Skin is removed by friction; the product is in contact with carborundum rollers or placed into containers coated in the inside with abrasive materials such as silicon or carbon. The abrasive surface detaches the carrot skin, later removed by a water stream.

Flame peeling: Applied mainly to onions, it consists of placing the vegetables on a conveyor moving through an oven at >1000°C. As vegetables pass, the outmost layer and fine roots are burnt and eliminated by high pressure water spraying.

4. Size Reduction

In this operation the average size of a solid food material is reduced by the application of external forces such as impact, compression or abrasion (17). In the case of Jalapeño peppers they are cut lengthwise into four parts and the peduncle and seeds eliminated. Cutters consist of a series of rotating blades, and centrifugal force holds the product against the blades.

5. Spoilage Enzymes

Enzymes, endocelular, exocelular or microbial, assume an active role in food deterioration. Microbial enzymes are also able to act on the food substrate even when the microbial cell is inactivated or dead (19). Insufficient scalding can result in an increase in food spoilage as heat applied can disrupt the tissues, liberating the substrate but not inactivating the enzymes. Scalding efficiency in vegetables is measured by inactivation of two enzymes: catalase and peroxidase. Jalapeño scalding in water is carried out for 8 to 10 min at 95°C; carrots are scalded for 6 to 8 min, water at 95°C (15).

6. Blanching

It is applied prior to processing in order to inhibit enzymatic activity and to decrease microbial populations. Blanching can be combined with other operations such as peeling or cleaning (17–19). Efficient enzyme inactivation is carried out by heating at given temperature-time conditions, and further fast cooling to room temperature. The two blanching methods commonly used are: applying saturated steam and immersion in hot water. At industrial levels, steam blanching is the most widely applied (17), it consists in applying steam to the vegetables placed on a conveyor moving through a steam tunnel. Varying the conveyor speed controls the residence time in the tunnel. In some cases water spray is applied at the start and end of the conveyor in order to condense excess steam. During hot water blanching vegetables are held for a given time at 70–100°C, with a further draining-cooling period.

B. Packaging

The aim of this operation is to keep the product, from processing to the consumer, in the same hygienic and quality conditions. Cans are made from three-piece tin sheets, coated on the inside with an epoxyphenolic enamel (Figure 182.6). The lids are also made of tinfoil and coated with the same material used in the can. The lid also has two or three circular expansion rings, providing resistance against deformation due to an increase in the internal pressure (20).

C. Pickle

According to Mexican regulations (21), pickle is a mixture of vinegar, vegetable oil, onion, carrots, laurel, garlic,

FIGURE 182.6 Three-sheet tin can, with internal porcelain enamel covering.

salt, sugar and optional spices. Vinegar has 2% acetic acid and 5% sodium chloride.

D. Blanching Vegetables

Cut peppers, carrots and onions must not be less than 60% total product weight, peppers must be included in a higher proportion.

E. Filling

Vegetable mix is first added to the can, previously washed with hot water; the brine (pickle) is then added at 82 to 86°C. Filling must be carefully controlled in order to assure that the correct amount of vegetable mix and pickle is added, and to fulfill specifications. Headspace must be 10% of total can volume. Filling is done when transported by the conveyors, which carry the cans to the vegetable mix filler and then to the liquid one.

F. Exhausting

When air is evacuated from the headspace before sealing internal pressure is decreased during sterilization. At the same time oxygen evacuation prevents tin corrosion and oxidation. During this operation air is replaced by vapor promoting a partial vacuum in the headspace after condensing. Exhaustion is carried out in tunnels (or exhausters), as shown in Figure 182.7. Another way to promote exhaustion is by using steaming equipment, which inject steam to the headspace before closing the cans (20).

G. Sealing

Sealing is carried out in steaming machine. According to the design and speed of the operation, the basic stages of the operations are: (a) folding edges; (b) pressing the folded tin to form an hermetic seal impermeable to air (22).

FIGURE 182.7 Vapor tunnel or exhauster.

H. Heat-Treatment

Cans or glass jars are subjected to heat treatment to sterilize or pasteurize their contents. It can be done in batches or by continuous retorting. Cans are heated at a time-temperature condition in vapor or hot water. Pasteurization of pickled Jalapeño peppers destroys microorganisms resistant to high acetic acid concentrations, able to promote product alteration. Heat treatment also inhibits vegetable or microbial enzymes (23). Heat treatment of 93.3°C and 10 min are recommended for acid pickles (pH 4.3 to 4.5). However, a time-temperature process depends on type of container, volume, and heat processing equipment.

1. Batch Processing

During this operation retorts are saturated with vapor and containers are placed in baskets. Retorts can be horizontal or vertical, and the cans can be still or rotating during the process. Can rotation promotes heat transfer, so that processing time is reduced and higher temperatures can be achieved.

2. Continuous Retorting

This type of equipment is fitted with hydrostatic closings before and after the pressurized sections. Processing can be also carried out by can rotation, where the cans move in and out of the pressurized section through hydrostatic column seals, equilibrating the internal pressure.

A variation of this equipment is the flame retort, operated at atmospheric pressure throughout the process. Flame retort equipment is fitted with direct heating, applied to the rotating retort. An advantage of this type of retorting is a high product quality due to mild heating conditions.

In all heat treatments the final part is can cooling with water to reach final temperatures not less than 38°C. Because the cans are not completely cooled down, water is eliminated from the outside, avoiding corrosion.

I. Marking, Labeling and Packaging

Once the containers undergo heat treatment, each can or jar is marked with a code, a production date, a batch number and a plant code. The label includes the product name, the commercial name, the drained and net weight, the ingredients and other specifications required by the country's regulations (21). Packing is automatically carried out in cardboard boxes or high-density polyethylene bags, or other suitable packaging material with enough resistance to protect the product and containers.

J. Storage

Heat-treated Jalapeño peppers keep their quality characteristics at 18 to 21°C. At a higher temperature, acid products in cans without inner coating consume oxygen in the

headspace faster than in coated cans. The result is a considerable loss in ascorbic acid content and fast product oxidation (23,24). On the other hand, canned Jalapeño peppers have a longer shelf life if stored at 0 to 5°C (23).

VII. REGULATIONS

A. Mexican Specifications

NMX-F-121-1982, 21 is Mexico's quality bylaw (Norma Mexicana) regulation for pickled Jalapeño or serrano peppers. This regulation includes six consumer presentations or styles and two quality levels.

The presentations are: whole peppers, peppers without seeds, peppers in halves, peppers cut lengthwise, pepper cut in rings and chopped peppers. There are two quality classifications for whole peppers only; for the rest of the presentations there is one quality classification. Table 182.1 shows physical and chemical specifications.

These specifications also include microbial characteristics, chemical contaminants, optional ingredients, sampling and specificity of quality degrees, labeling, containers and packaging. Among optional ingredients are: garlic, pepper, cinnamon, cloves, ginger, laurel, marjoram, thyme and nutmeg. In defining the Mexican official specifications the main Jalapeño pepper processing industries took part, such as Productos Del Monte, La Costeña, Hérdez, Conservas San Miguel, Conservas Guajardo and Elías Pando.

B. International Specifications

The processed fruit and vegetable Committee of the Codex Alimentarius Commission FAO/OMS, has elaborated a General Specification project for pickled products. At present, this project is at the sixth stage, that is revision by all member countries. However, the project does not include pickled cucumbers or kimchi (25).

During the 21st session of the Codex Committee on Processed Fruits and Vegetables, held in San Antonio, Texas on 2002, it was agreed to stop the draft Codex standard on the sixth stage of Codex Alimentarius normalization procedure. This decision was taken on the basis of the product nature, expressed by several member countries, with respect to the edible characteristics of the covering medium or if it should be eliminated, as well as pH, salt concentration and processing conditions (scalding, lactic fermentation, heat treatment before or after container filling, etc.) (25). The Draft Codex Standard for Pickled Products (26) includes the following specifications:

TABLE 182.1
Specification of Jalapeño Peppers (Mexican Legislation NMX-F-121-1982, 21)

Specification	Minimum	Maximum
Acidity (as acetic acid) (%)	0.75	2.0
Chloride (as sodium chloride)(%)	2.0	7.0
pH		4.3
Filling (%)	90	
Headspace (%)		10
Vacuum (mm Hg)	76.2	

1. Scope

This standard applies to edible fruits, vegetables, cereals, legumes, spices and condiments which have been cured, treated or processed to produce an acid product and which are offered for direct consumption in oil, brine or acidic media.

2. Product Definition

Due to the wide variety of pickled or fermented products, this chapter deals with a general definition.

Pickled products are:

a) Prepared from sound, clean and edible fruits, vegetables, cereals, legumes, spices and condiments.
b) Subjected to curing and processing with ingredients appropriate to the type in order to ensure preservation of the product and its quality.
c) Processed in an appropriate manner in order to ensure the quality and proper preservation of the product
d) Preserved in an appropriate manner in a suitable packing medium with ingredients appropriate to the type and variety of pickled product.

3. Essential Composition and Quality Factors

This section includes basic ingredients such as edible fruits, vegetables, cereals, legumes, spices and condiments in a liquid medium in a combination with one or more of the optional ingredients.

The optional ingredients are nutritive sweeteners, unrefined nutritive sweeteners, edible vegetables oils, vinegar, citrus juice, dried fruits, malt extract, salt, brine, chilies, seasoning (of plant origin and animal origin).

Some specific requirements are:

Pickled products in edible oils: Oil must not be less than 10% by weight.

Pickled products in brine: Salt in the covering liquid must not be less than 10% by weight, if salt is used as the main preservation agent.

Pickled products in acidic media: Acid must not be less than 2% by weight, expressed as acetic acid. Accepted food additives are shown in Table 182.2.

TABLE 182.2
Authorized Food Additives (Draft Codex Standard for Pickled Products, 26)

Preservatives	Maximum Level
220 Sulphur dioxide 221 Sodium sulphite 222 Sodium hydrogen sulphite 223 Sodium metabisulphite 224 Potassium metabisulphite	30 mg/kg (as sulphur dioxide)
211 Sodium benzoate 212 Potassium benzoate	250 mg/kg (as benzoic acid)
200 Sorbic acid 202 Potassium sorbate	1000 mg/kg as sorbate
Acidity Regulator	
260 Acetic acid (glacial)	Limited by GMP

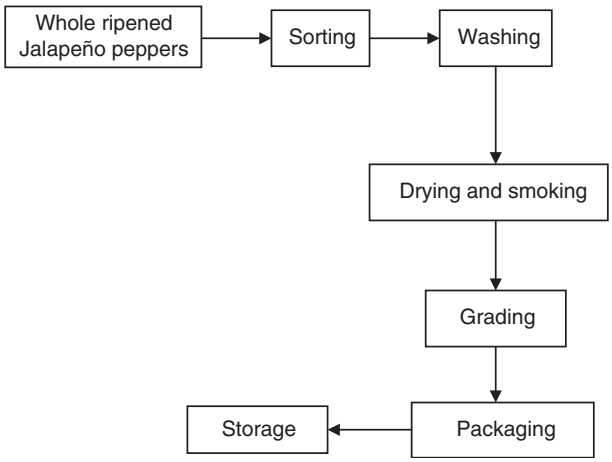

FIGURE 182.8 Drying of ripened Jalapeño pepper.

4. Weights and Measures

Pickled products in edible oil, in brine and in acid media: basic ingredient in the final product (drained weight) must not be less than 60% by weight.

This draft standard also includes other chapters on contaminants, hygiene, labeling and methods of analysis and sampling, referring to relevant legislations of other related Codex Committees.

VIII. RIPENED JALAPEÑO PEPPER DRYING (CHIPOTLE PEPPER)

Jalapeño pepper is a conic fruit of approximately 6 cm long, 4 cm width, tasty and consistently, in general the surface is scorched. This is a highly acceptable characteristic as it prevents cuticle removal during pickling. However, if it is excessive, the fruits are directed to chipotle fabrication (2,3). A flow diagram depicting this process is shown in Figure 182.8.

A. Preliminary Operations

1. Sorting

Jalapeño peppers, if directed to dehydration and smoking, must be healthy, ripened and showing intense red color.

2. Washing

Fruits are washed by immersion in tanks added with detergent or chlorine to reduce microbial loads.

3. Seed Removal

The fruits can be processed complete, with seeds, or once the seeds are removed, in this case the product has a higher commercial value. In addition, seeds are used for the next seeding season. Seed extraction is carried out before smoking; chilies without seeds are called "capones" (capons) (3).

B. Drying

This operation removes through evaporation by heating procedures most of the water present in fruits and vegetables. The main objective is to extend the shelf life by reducing water activity. Microbial growth and enzymatic activity is considerably reduced due to the reduction of available water. Drying also reduced food weight and volume; therefore transportation and storage costs are also reduced (17). Drying and smoking, or applying hot air carries out dehydration of Jalapeño peppers.

1. Drying and Smoking

It consists in applying hot smoke in ovens, located close to the producing area. In small processing plants, the oven consists of two brick chambers linked together with a tunnel; in one chamber smoke is generated by burning sawdust or wood, chilies are placed in a second chamber on a 1.5 to 2.5 cm thick, 2 × 2 m long rack made generally of bamboo, with 20 cm × 2 m wood supports (27). Chilies on the rack in 18 to 20 cm thick layers are in direct contact with the smoke; they are constantly turn over using a 1.5 m long shovel to reach homogeneous drying and smoking of the product. The combined effect of heat and smoke is an efficient drying. Process duration varies depending on the desired final moisture and smoke content, average duration is 3 to 6 days obtaining a dark brown product with smoke-like flavor taste (Figure 182.9) (2,27).

In large processing plants ovens can held 800 a 900 kg of raw peppers, producing a 60 to 70 kg dehydrated product. If more than 900 kg raw chilies are processed, fruits can be damaged or broken, reducing product quality (3).

FIGURE 182.9 Chipotles (dried and smoked Jalapeño peppers).

The final product quality (chipotle pepper) depends on ripening stage of the harvested raw material as well as drying conditions.

2. Hot Air Drying

In this method the fruits are dried in hot air tunnels with isolated walls, allowing continuous operations and high production capacity (18). Jalapeño peppers are placed in trays stocked in moving racks, with enough separation to allow drying air to circulate around them. The racks are introduced in the drying tunnel at suitable intervals. When one rack is introduced in the "wet" end of the tunnel, another is removed from the "dry" end. Air is forced by fans though heaters, producing forced convection; this sir is then horizontally fed into the tunnel and trays, although some turbulence is also produced by air circulating between trays. Air is applied at rates between 2.5 to 6 m/s; each rack contains 15 trays with a total of 350 to 400 kg raw pepper, following a 25 m total tunnel length with a rectangular or square 2 $\times$ 2 m transversal section and capacity for 22 moving racks; air counter currently fed. After 8 hours drying, the tunnel is at 55 to 60°C; drying of the first racks fed is achieved after 36 hours; after this time 2 to 3 racks are dried every 4 hours (18,27). Dried fruits should have 6% to 10% moisture content (2,17,23).

3. Grading

The dried product is classified into three categories:

First quality: Peppers of largest size, uniform color, without any deterioration or breakage

Second quality: Peppers of the same size as previous ones, but non-uniform color.

Third quality: Broken or damaged peppers, non-uniform color.

C. Packaging

In general, dried peppers are packaged in 55 to 60 kg sacks.

D. Storage

If the product has been properly packed and protected against oxygen humidity and light, it can reach 1 or 2 years shelf life (22). Oxygen presence deteriorates carotenoides present in the skin. Pigment oxidation increases if other extrinsic factors are present such as high storage temperature, light, metal ions, oxygen or peroxidases (28). These factors affect color, aroma and composition, and, consequently, considerably reduce the commercial quality.

IX. CANNED CHIPOTLE PEPPER IN "ADOBO" (SPICY SAUCE)

Canning, still as the main food preserving method, is based on the premise of microbial destruction by heat, and on recontamination prevention. With the exception of certain heat-tolerant bacteria, lethality starts at about 46–49°C. In conventional canning, food is placed inside containers, air is removed and cans are hermetically sealed and placed in a retort to be sterilized with steam. The rate at which heat penetrates into the canned product must be measured from the slowest-heating part of the can, or cold point. The basic heat penetration processes are convection and conduction, and a combination of the two (29).

The quality of canned fruits and vegetables is affected not only by the heat process but also by the method used to prepare the food material. Such preparation involves washing, trimming, sorting, blanching, filling into containers, and maintenance of the headspace in the can upon vacuum closing.

Canned chipotle peppers are of great demand in Mexico; they are consumed directly from the can or used as meat or sauce seasoning. Cans are merchandized in different sizes and formats; chipotle peppers are usually canned in a spicy sauce, or "adobo," made of other peppers (anchos, or "wide," and mulattos) tomato, garlic, onion, cumin, oregano, salt, sugar and vinegar (Figure 182.10).

FIGURE 182.10 Adobo ("spicy sauce") chipotle peppers.

A. Preliminary Operations

Canned chipotle pepper in "adobo" is shown in Figure 182.11. Whole dried chipotle peppers, as well as dried ancho and mulatto are transported to the processing plant in 50 to 60 kg sacks.

1. Sorting

Dried peppers are sorted in order to eliminate broken peppers, seeds or leaves. Tomatoes, onions, garlic are also sorted to remove any material, unsuitable for processing.

2. Washing

Pepper and other vegetables washing is carried out by immersion in stirred tanks, or by water spraying.

3. Grading

It is manually carried out.

4. Peeling

Garlic and onion peeling is carried out applying the flame method already described for non-fermented pickled peppers. Chipotle peppers peduncles are removed; seeds, veins and peduncles are also removed from ancho and mulatto peppers.

5. Blanching

Tomato blanching is carried out in water during 1 to 2 min at 95°C; blanching and peeling is carried out at the same time. Fast cooling at room temperature improves the efficiency of both operations.

Dried pepper scaling softens the disuse, reduces pungency, removes seeds, improves color and decreases microbial loads. Chipotle pepper is scalded in water during 10 to 15 min at 95°C; ancho and mulatto peppers are scalded in water at 95°C for 15 to 20 min. At the industrial level, scalding with steam is generally applied.

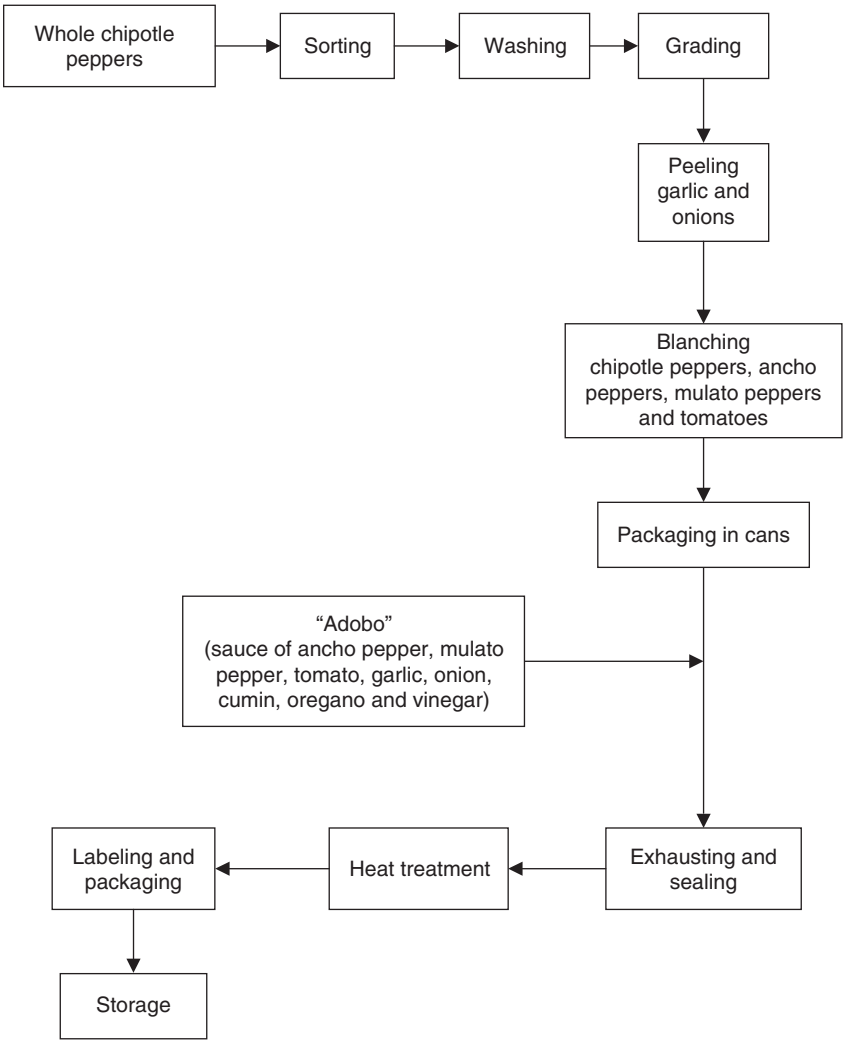

FIGURE 182.11 Canning of adobo ("spicy sauce") chipotle peppers.

B. Packing

Packaging is carried out in 3-piece tin cans with epoxy phenol enamel; lids are made of the same material, fitted with an easy-opening ring and three expansion moldings (Figure 182.12).

C. Adobo ("Spicy Sauce")

To date, there is no Mexican standard (NMX) for chipotle peppers in adobo. Therefore, adobo formulation varies from one food processor to other, mainly regarding the amount of mulatto pepper sometimes using other pepper (guajillo) as well as amount and type of spices included. Several industries buy the adobo from specialized plants that formulate and dry this sauce.

D. Blanching Vegetables

Whole chipotle peppers, as well as sliced onion must be 60% minimum weight of total net weight, although chipotle peppers must be the dominant vegetable present. The covering medium is adobo sauce.

E. Filling

Chipotle peppers and onion slices are placed in previously washed cans; adobo is then added at 82–86°C. Headspace must be 10% total can volume. Filled cans are exhausted, closed, pasteurized, marked, labeled, packed and stored in a similar way as with non-fermented pickled Jalapeño peppers.

FIGURE 182.12 Three-piece easy-opening can.

ACKNOWLEDGMENTS

- The author thanks Dr. Isabel Guerrero Legarreta for the manuscript revision.
- The information in this chapter has been modified from "Jalapeño pepper preservation by fermentation or pickling," by R. M. Galicia-Cabrera, in *Handbook of Food and Beverage Fermentation Technology*, Editors: Y. H. Hui et al., Marcel Dekker, New York, 2004.

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183 Sourdough Bread

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CONTENTS

I. INTRODUCTION

Sourdough is used as an essential ingredient in the production of wheat and rye bread and mixtures hereof. Sourdough has been used for leavening of bread dough for several hundreds of years, and sourdough bread was made in Egypt as early as 3000 BC [1]. The sourdough was a piece of dough from the previous baking which was kept

until the next baking, where it was mixed with flour, salt and water to make the bread dough. The intervals between baking could be from one day in bakeries to one month in home baking. If the time between baking was long, salt could be added to the surface of the sourdough to avoid wrong fermentation. While this piece of dough was saved, lactic acid fermentation took place due to multiplication and metabolic activity of lactic acid bacteria (LAB) originally present in the flour. During this fermentation, selection and multiplication of yeasts from the flour also occurred. The natural content of LAB and yeasts from the sourdough was responsible for the leavening capacity of the bread dough primarily due to their production of carbon dioxide. Yeast from beer or wine production could also be added to the dough to increase the leavening capacity until production of commercial baker's yeast began during the 19th century [2]. The sourdough still holds a place of honour in many households throughout the world, and small portions are passed on to the daughters at marriage [2].

A. Wheat Sourdough

Sourdough is used as an important ingredient in the production of wheat bread [3,4] as well as crackers and the Italian sweet baked products as Pandoro, Colomba and Panatone [5,6]. The tradition of making wheat bread with the addition of sourdough is widely used in the Mediterranean area such as Italy [5,7], Greece [8,9], Spain [10], Egypt [11] and Morocco [12]. The tradition is also known from The Netherlands [13], Iran [14] and the San Francisco bay in the US [15,16].

The cereal intake in the traditional diet of Greece is mostly in the form of sourdough bread rather than pasta [8]. In Italy, sourdough is used in more than 30% of bakery products, which include more that 200 different types of sourdough bread. In some regions of southern Italy, most of the bread including sourdough bread is made from durum wheat instead of common bread wheat [7]. In Morocco, commercial bakeries supply only part of the population with bread, while most people eat homemade bread made with traditional sourdough, which has been carefully kept in every family. Addition of baker's yeast is used mainly in towns and villages where refrigeration can be employed [12].

B. Rye Sourdough

Sourdough is essential in rye bread making and the tradition of rye sourdough fermentation correspond to the rye-growing areas in the north, central and eastern European countries including the Baltic states, where rye bread constitutes a considerable amount of the bread consumption. Rye sourdoughs have been characterised from Finland [17], Sweden [18] and Denmark [19,20], Germany [21–23], Austria [24], Poland [25], Czechoslovakia [26], Russia [27] and Portugal [28].

Bread made from mixed wheat and rye is very common in many European countries, and sourdough should be used to enhance the sensory properties of the bread and prolong the microbial shelf life if more than 20% of the flour is from rye [29]. One of the most famous rye sourdough bread still produced today is the pumpernickel named after the Swiss baker Pumper Nickel. The bread originates from 1443, where there was a significant scarcity of wheat in Europe [2].

The tradition of production of rye bread without the addition of baker's yeast has continued even in large-scale bakeries until today, and the leavening capacity of the sourdough is still very important in rye bread production. In the sixties and seventies, the time between baking and consumption of bread increased due to changes in the society, and in some bakeries, preservative compounds such as vinegar, propionic acid or sorbic acid were added to the dough for the prevention of moulds. However, the natural content of yeasts from the sourdough is also inhibited by those preservatives, resulting in decreased leavening capacity, and it was necessary to add baker's yeast to increase the bread volume. The use of propionic acid as a preservative in bread is prohibited in many countries today. Stringent hygiene in bakeries makes it possible to produce bread with long shelf life without added preservatives, if sourdough is added.

C. Why Is Sourdough Used?

The advantages of using sourdough for bread production include the possibility of leavening bread dough with little or no baker's yeast added, improved dough properties, and the achievement of a better and more aromatic bread flavor and texture compared to bread only leavened by bakers yeast (Table 183.1). Sourdough flavor is developed by a long fermentation process that requires 12–24 hours, while fermentation by baker's yeast has to be finished within 1–2 hours. The addition of sourdough can also extend the shelf-life of bread by several days by increasing the mold-free period of bread and retarding the development of rope. The nutritional value of sourdough bread made from high extraction flour is enhanced

TABLE 183.1
The Advantages of Using Sourdough in Bread Making

Leavening of dough
Improved dough properties
- inhibition of α-amylase
Increased flavor and taste of bread
Improved nutritional value of sourdough bread
- higher bioavailability of minerals
- lower glycemic index
Extended shelf life of sourdough bread
- longer mold-free period
- prevention of rope in bread
- antistaling

compared to bread made without sourdough due to a higher content of free minerals, which are separated from phytic acid during the long fermentation processes.

Interest in using sourdough in bread production has increased considerably in many European countries during recent decades [4,30,31]. Today, a larger part of the consumers prefer healthy bread with aromatic taste, good texture, and long shelf-life without the addition of artificial preservatives. The demand for organic food is also on the rise, and a larger part of the bread made from organically grown cereals is made with sourdough due to its higher quality and better image. More consumers are also interested in food with history, and sourdough bread is related to traditional and original food.

II. CHARACTERIZATION OF SOURDOUGH

High quality sourdough bread is dependent on a consistent and microbial stable sourdough. Good fermentation capacity of the sourdough is influenced by the microbial flora (lactic acid bacteria and yeasts) in the sourdough, flour type (wheat/rye, flour extraction rate, activity of enzymes), flour/water ratio (dough yield), and the process parameters. The process parameters such as temperature, initial pH, quantity of added sourdough starter, time of fermentation, and type of production system (batch/continuous) have to be strictly controlled.

A. Definition of Sourdough

Sourdough is a mixture of flour and water, in which LAB have caused a lactic acid fermentation to occur. It is in general accepted that the LAB should still be able to produce acids, when flour and water are added (metabolically active). The sourdough also has a natural content of sourdough yeasts, which are important for the leavening capacity of the dough. However, no official definition of sourdough exists, but it should include all different types of sourdough products with "living" LAB and exclude artificial sourdough products. According to Lönner [32], a sourdough should contain more than 5×10^8 metabolically active LAB/g, and have a pH value below 4.5.

B. Types of Sourdoughs

Sourdoughs can be started as follows:

a) during spontaneous fermentation
b) by adding a piece of mature sourdough (mother sponge)
c) by adding a defined starter culture

Most sourdoughs used in both wheat and rye bread baking are still initiated by adding a piece of mature or ripe sourdough also called mother sponge, but there is a tendency to use defined starter cultures with specific fermentation patterns. This tendency increases as these cultures become commercially available.

1. Spontaneous Fermentation

When dough made from flour and water is left for one to two days at ambient temperature, a spontaneous fermentation will take place due to the naturally occurring microorganisms in the flour. The dough will become acidified due to lactic acid fermentation. During the fermentation there is a successive favoring of the Gram-positive LAB from the flour at the expense of the Gram-negative bacteria, which dominate the microflora of the flour [21,32]. The microflora of some spontaneously fermented rye sourdoughs were dominated by a homofermentative *Lactobacillus* spp. and *Pediococcus* spp. [32,33]. The level of LAB in sourdoughs was up to 3×10^9 colony forming units (CFU)/g and the number of yeasts about 10^6 to 10^7 CFU/g. However, spontaneous sourdoughs do not always succeed and may result in products with off-flavor.

2. Mature Sourdough

Sourdoughs used by artisan bakers and in bakeries have traditionally been based on spontaneous fermentation, during which the sourdough has been kept metabolically active and probably microbial stable for decades by the addition of flour and water daily, the so called "freshening" of the dough based on "back-sloping" (Figure 183.1). The fermented sourdough is used for bread production, but part of it is used as starter by initiating a new sourdough. The terminology for sourdough and starter in different countries is listed in Table 183.2.

In commercial rye bread baking, the bakeries can use their own adapted sourdough or, if they have quality

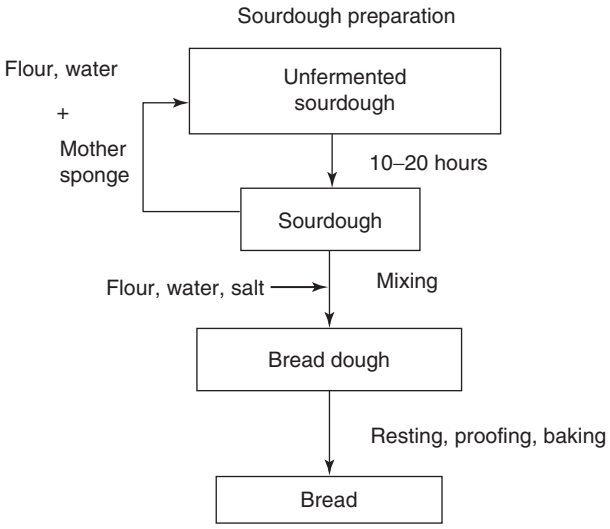

FIGURE 183.1 A schedule for production of sourdough.

TABLE 183.2
Terminology for Sourdough in Different Countries

	English	German	French	Spanish	Italian
Sourdough for bread production	Sourdough Leaven	Sauerteig	Levain natural	Masa madre (Masa agria)	Lievito naturale (impasto acido)
Sourdough used as starter for a new sourdough	Mother sponge Starter	Anstellgut Reinzuchtsauer®	Le chef	Pie	Madre, Capolievieto

Modified after [5].

problems due to unstable process control, they can add a commercial sourdough as a starter. Most bakeries in Germany and Denmark regularly add commercial sourdoughs composed of a well-adapted microflora derived from natural sourdough fermentation. Examples of commercial sourdoughs are the Sanfrancisco sour for wheat bread production [34] and the Böcker-Reinzucht-Sauer® for rye bread production. Some products sold as sourdough have no living microorganisms, and these products will not contribute to a natural acidification and development of flavor compounds in the dough.

3. Starter Cultures of Pure Strains of LAB

Starter cultures for sourdough fermentation are pure cultures of dried or freeze-dried LAB, or a mixture of LAB and sourdough yeast. They should be mixed with flour and water, and kept for several hours for multiplication and fermentation of the microflora. This fermented dough can then be used as a sourdough. The microorganisms have been selected due to their ability to acidify dough in a short time and result in acceptable bread flavor when used in bread baking. Cultures containing *Lactobacillus sanfranciscensis*, *L. plantarum*, *L. brevis* and *L. fructivorans* or *L. brevis*, *L. pontis* and *Saccharomyces cervisiae* are available [35]. Use of defined starter cultures with specific properties gives rise to new interesting opportunities for controlling and regulating of the sourdough fermentation. The term "starter culture" is sometimes used in the literature for a mature sourdough which has to be mixed with flour and water to ferment, or for commercial sourdoughs.

C. Sourdough Parameters

A sourdough can be characterized by the chemical parameters pH, content of total titratable acids (TTA), content of lactic and acetic acid, and the microbial parameters such as number and species of LAB and yeast. The microbial parameters are described in the following section.

The final pH of a mature sourdough is 3.5–3.8 in most rye and wheat sourdoughs [3,36,37]. Sourdough pH values show less variation and differences than TTA values. The TTA values in sourdoughs are dependent on the fermentation temperature, extraction rate of the flour, and the water content. In wheat sourdoughs, TTA has been found to vary between 8 and 11 in sourdoughs made from low extraction flour and 16 to 22 in wholemeal sourdoughs [3,38]. Rye sourdoughs are often made from flours with higher extraction rate than wheat flour and TTA values vary between 15 and 26 [21,36].

The content of lactic and acetic acid in sourdoughs is very important for the taste and flavor of the sourdough bread [31,39]. The fermentation quotient (FQ), the molar ratio between lactic and acetic acid, is used as a measure in German studies of sourdoughs for the balance in production of those acids. The FQ should be around 4 in sourdough to result in a harmonic taste of bread. A low content of acetic acid results in a high FQ with a too little flavor, whereas a low FQ results in too strong an acid flavor [21]. However, acetic acid has a more efficient antimicrobial effect against mold- and rope-producing bacteria compared to lactic acid [40].

III. MICROBIOLOGY OF SOURDOUGH

The microflora of the sourdoughs includes adapted LAB and yeasts that have optimal conditions for growth and fermentation similar to the conditions for the sourdough (temperature, water content, pH), and which probably produce antimicrobial compounds [41]. The microflora in bakery sourdoughs remains remarkably stable in spite of the use of non-aseptic fermentation conditions [20,23,34,42]. The LAB and yeasts isolated and identified from wheat and rye sourdoughs are listed in Tables 183.3 and 183.4, respectively.

Early systematic studies of the microflora responsible for the sourdough fermentation were made on sourdoughs from Germany by Hollinger in 1902, from Russia by Seliber in 1939 (cited in [43]), and from Denmark by Knudsen in 1924 [19]. Spicher and co-workers have carried out many profound investigations concerning the identification of the microflora from different types of sourdoughs, both adapted sourdoughs from bakeries and in commercial starter cultures for sourdough [4,21]. Recent investigations on sourdough fermentation have mainly dealt with interactions between sourdough microorganisms, identification of new species, inhibitory substances of sourdough LAB, and induced specific enzymatic activities.

LAB are mainly responsible for the acidification of the sourdough, whereas the sourdough yeasts are very

TABLE 183.3
Lactic Acid Bacteria and Yeasts Isolated from Rye Sourdoughs

Country	Lactic Acid Bacteria		Yeasts	Authors	Year	Reference
	Homoferm.	Heteroferm.				
Russia	*Streptobacterium plantarum* (*L. plantarum*)	*L. brevis* *L. fermenti*	Non-identified species	Seliber	1939	Cit. from [43]
Czechoslovakia	*Str. plantarum*	non-identified species	*S. cerevisiae*	Pokorny	1955	Cit. from [43]
Germany	*L. delbrueckii* *L. plantarum* *L. leichmanii*	*L. brevis* *L. fermenti*		Spicher	1959	[43]
Germany	*L. alimentarius* *L. plantarum* *L. acidophilus* *L. casei* *L. farciminis*	*L. brevis* *L. brevis* ssp. *lindneri* *L. plantarum* *L. fermentum* *L. fructivorans*		Spicher, Schröder	1978	[119]
Germany			*C. krusei* *S. cerevisiae* *Pichia saitoi* *T. holmii*	Spicher, Schröder, Schoellhammer	1979	[120]
Austria	*L. alimentarius* *L. casei* *L. rhamnosus*	*L. brevis* ssp. *lindneri* *L. büchnerii* *L. fermentum* *L. fructivorans*	*S. cerevisiae* *C. krusei*	Foramitti, Mar	1982	[24]
Russia	*L. plantarum* (firm) *L. plantarum* (liquid) *L. leichmanii* *L. casei var. casei* *L. delbruckii* (48–52°C)	*L. brevis* *L. fermenti* *L. brevis* *L. büchnerii*	*S. minor/S. exiguus* *S. minor* *S. exiguus*	Kazanskaya, Afanasyeva, Patt	1983	[27]
Finland	*L. acidophilus* *L. plantarum*	*L. büchnerii* *L. cellobiosus* *L. viridescens*		Salovaara, Katunpäa	1984	[17]
Finland			*Torulopsis holmii* *S. cerevisiae* *T. unisporus* *T. stellata* *Endomycopsis fibuliger* *Hansenula anomala*	Salovaara, Savolainen	1984	[57]
Sweden	*L. acidophilus* *L. plantarum*	*L. brevis* *L. brevis* ssp. *lindneri*		Spicher, Lönner	1985	[18]

(*Continued*)

TABLE 183.3 (*Continued*)

Country	Lactic Acid Bacteria: Homoferm.	Lactic Acid Bacteria: Heteroferm.	Yeasts	Authors	Year	Reference
	L. farciminis *L. casei rhamnosus* *L. delbruckii* (53°C)	*L. fermentum* *L. viridescens*				
Poland			*S. cerevisiae* *S. exiguus* *T. candida* *C. krusei*	Wlodarczyk	1986	[25]
Germany		*L. brevis* *L. sanfrancisco*		Böcker, Hammes	1990	[121]
		L. curvatus		Okada, Ishikawa, Yoshida, Uchimura, Ohara, Kozaki	1992	[122]
		L. sanfrancisco				
Germany		Non-identified species closely related to *L. fermentum* and *L. reuteri*	*S. cerevisiae*	Strohmar, Diekmann	1992	[42,123]
Germany		*L. brevis*		Vogel, Böcker, Stolz, Ehrmann, Fanta, Ludwig, Pot, Kersters, Schleifer, Hammes	1994	[49]
		L. sanfrancisco *L. fermentum* *L. fructivorans* *L. pontis* sp. *nov.*				
Portugal (rye and maize)			*S. cerevisiae* *Torulaspora delbrueckii* *Issatchenkia orientalis* *Pichia anomala* *P. membranaefaciens*	Almeida, Pais	1996	[58]
Germany		*L. panis* sp. *nov.*		Wiese et al.	1996	[50]
Finland			*C. milleri* *S. cerevisiae* *S. exiguus*	Mäntynen et al.	1999	[55]
Germany		*L. frumenti* sp. *nov.*		Müller, Ehrmann, Vogel	2000	[52]
Denmark	*L. acidophilus* (liquid) *L. amylovorus*	*L. panis*	*S. cerevisiae*	Rosenquist, Hansen	2000	[20]
Germany	*L. mindensis* sp. *nov.*			Ehrmann et al.	2003	[53]

L., Lactobacillus; Str., Streptobacterium; S., Saccharomyces, C., Candida; T., Torulopsis.

TABLE 183.4
Lactic Acid Bacteria and Yeasts Isolated from Wheat Sourdoughs

Product	Lactic Acid Bacteria		Yeasts	Authors	Year	Reference
	Homoferm.	Heteroferm.				
San Francisco Bread		*L. sanfrancisco*	*S. exiguus*	Kline, Sugihara, McCready	1971	[15]
			S. inusitas	Sugihara et al.	1971	[34]
Balady bread				Abd-el-Malek, El-Leithy, Awad	1974	[11]
Sangak bread	*L. plantarum*	*L. brevis*	*T. collucolasa*	Azar, Ter Sarkissian, Ghavifek, Ferguson, Ghassemi	1977	[14]
		Lc. mesenteroides	*T. candida*			
Wheat bread	*L. plantarum*	*L. brevis*		Spicher, Lönner	1985	[18]
Wheat bread	*L. plantarum* *L. farciminis* *L. casei*	*L. brevis*		Spicher	1987	[124]
Wheat bread		*L. brevis var. lindneri* *L. sanfrancisco*	*S. exiguus*	Nout, Creemer-Molenaar	1987	[13]
		L. brevis var. lindneri	*S. exiguus*			
Swiss panettone/cake		*L. brevis var. lindneri*		Spicher	1987	[124]
Swiss bread		*L. brevis var. lindneri*		Spicher	1987	[124]
Panettone, brioches, wheat bread, crackers	*L. plantarum*	*L. sanfrancisco* *L. fermentum*	*S. cerevisiae* *C. stellata*	Galli et al.	1988	[125]
	Pediococcus	*Lc. mesenteroides*	*C. milleri*			
Wheat bread	*L. plantarum* *Lc. mesenteroides*	*L. brevis* *L. cellobiosus*	*S. cerevisiae* *C. boidinii* *C. guilliermondii* *Rhodutorula glutinis* *Pichia polymorpha* *Tricocporon margaritiferum*	Barber, Baguena	1988	[10]
Wheat bread	*L. plantarum* *L. delbrueckii* *Lactococcus casei*	*L. brevis*	*C. milleri* *S. cerevisiae*	Boraam et al.	1993	[126]
Pannettone, bread	*L. plantarum*	*L. brevis var. lindneri*	*S. cerevisiae*	Gobbetti, Corsetti, Rossi, Rosa, Vincenzi-S-de	1994	[127]
	L. farciminis		*S. exiguus* *C. krusei*			
bread			*S. cerevisiae* *S. exiguus* *C. krusei* *Pichia norvegensis*	Rossi	1996	[59]

(*Continued*)

TABLE 183.4 *(Continued)*

Product	Lactic Acid Bacteria		Yeasts	Authors	Year	Reference
	Homoferm.	Heteroferm.				
			Hansenula anomala			
Maize bread	*L. delbrueckii* *L. curvatus* *L. plantarum* *L. lactis* spp. *Lactis*	*L. brevis*	*S. cerevisiae*	Rocha and Malcata	1999	[28]
Wheat bread	*L. paralimentarius* sp. *nov*			Cai, Okada, Mori, Benno, Nakase	1999	[51]
Cakes: panettone, colomba, brioche		*L. sanfranciscensis* *L. brevis*	*C. holmii*	Foschino, Terraneo, Mora, Galli	1999	[60]
	L. delbrueckii	*L. sanfranciscensis*	*S. cerevisiae*			
Bread: durum wheat and bread wheat	*L. alimentarius*	*L. sanfranciscensis*	*S. cerevisiae*	Corsetti, Lavermicocca, Morea, Baruzzi, Tosti, Gobbetti	2001	[7]
	L. plantarum	*L. brevis* *Lc. citreum* *L. fermentum*				
Wheat bread	*L. paralimentarius*	*L. sanfranciscensis*		De Vuyst, Schrijvers, Paramithiotis, Hoste, Vancanneyt, Swings, Kalantzopoulos, Tsakalidou, Messens	2003	[9]
	Weissella cibaria	*L. brevis*				

L., Lactobacillus; S., Saccharomyces, C., Candida; T., Torulopsis.

important for the production of flavor compounds and for a harmonic bread flavor in combination with the acids. The levels of LAB in sourdoughs are 10^8–10^9 cfu/g and yeasts are 10^6–10^7 cfu/g, respectively [4]. The LAB: yeast ratio in sourdoughs is generally 100:1.

A. Lactic acid Bacteria

1. Identification

LAB are a group of Gram-positive bacteria, which are catalase-negative, non-motile nonspore-forming rods or cocci which produce lactic acid as the major end product during the fermentation of carbohydrates. They are strictly fermentative, aero-tolerant or micro-aerofile, acidophilic, salt-tolerant and have complex nutritional requirements for carbohydrates, amino acids, peptides, fatty acids, salts, nucleic acids derivates and vitamins [44,45].

The LAB have traditionally been classified taxonomically into different genera based on colony and cell morphology, sugar fermentation, growth at different temperatures, configuration of lactic acid produced, ability to grow at high salt concentration, acid tolerance or cell wall analyses [46]. Genera of LAB identified from sourdoughs are *Lactobacillus*, *Leuconostoc, Pediococcus* and *Streptococcus*, and the majority of the sourdough LAB belongs to the genus *Lactobacillus*. The taxonomy of LAB is still under revision. *Lactobacillus* have been divided into three groups according to their carbohydrate fermentation patterns [46]:

- Obligately homofermentative LAB:
  Hexoses are almost completely fermented to lactic acid (>85%) by the Embden-Meyerhof-Parnas (EMP) pathway. Fructose is also fermented, but neither gluconate nor pentoses are fermented.
- Facultatively heterofermentative LAB:
  Hexoses are almost completely fermented to lactic acid by the EMP pathway. Pentoses are fermented to lactic acid and acetic acid by an inducible phosphoketolase.
- Obligately heterofermentative LAB:
  Hexoses are fermented to lactic acid, acetic acid (ethanol) and CO_2. Pentoses are fermented to lactic and acetic acid. In general, both pathways involve phosphoketolase.

Lactobacillus isolated from sourdoughs are divided into the three groups shown in Table 183.5. When the LAB are only divided as homofermentative or heterofermentative LAB, the facultative heterofermentative LAB are grouped as homofermentative due to the fermentation pathway of glucose (Tables 183.3 and 183.4).

In the presence of oxygen or other oxidants increased amounts of acetate may be produced at the expense of lactate or ethanol [45]. Various compounds such as citrate, malate, tartrate, quinolate and nitrate may also be metabolized and used as energy sources or electron acceptors [44,45].

The techniques used for classification of LAB are not reliable for many LAB, and they have often led to misidentification [47]. Some organisms badly grow on laboratory media and may escape isolation and can therefore not be identified by standard procedures. For rapid classification, a set of molecular probes was developed. These include hybridization- and PCR based techniques as well as recognizing specific sequences in the ribosomal genes [47]. Nevertheless, the use of probes at the species level is restricted, as their specificity may be lost during discovery of new species sharing the same part of an RNA sequence [48]. Alternatively, the taxonomic method Random Amplified Polymorphic DNA (RAPD) allows elucidation of strain biodiversity below the species level, and the resulting electrophoretic patterns can be clustered and compared to a database [48]. The consequent application of 16S rRNA sequence analysis and DNA-DNA hybridization experiments have led to identification of many new species. *L. pontis* [49] and *L. panis* [50] were isolated from rye sourdoughs, and *L. paralimentarius* [51] was isolated from wheat sourdough. Recently described species isolated from sourdoughs are *L. frumenti* [52] and *L. mindensis* sp. *nov* [53].

2. Occurrence

Heterofermentative LAB play a major role in sourdough fermentation compared to other fermented food systems. *L. sanfranciscensis* (former names *L. brevis var. lindneri* and *L. sanfrancisco*) is by far the most dominant LAB in both wheat and rye sourdoughs (Tables 183.3 and 183.4). *L. brevis* and *L. plantarum* also occur frequently in both types of sourdoughs. Some strains initially classified as *L. brevis* were renamed as *L. pontis* [49]. Several other *Lactobacilli* have been identified from rye sourdoughs e.g., the homofermentative *L. acidophilus, L. alimentarius*, *L. amylovorus*, *L. casei, L. delbrueckii, L. farciminis, L. leichmanii, L. rhamnosus*, and recently *L. mindensis*, and the heterofermentative *L. büchnerii, L. cellobiosus, L. curvatus, L. fructivorans, L. fermentum, L. viridescens* including the new identified species *L. panis*, *L. frumenti* and *L. pontis*. Strains of *L. pontis* utilize only a very limited number of carbohydrates and they are found in close association with *L. sanfranciscensis*, from which they are difficult to separate physically.

Fewer different *Lactobacilli* have been identified from wheat sourdoughs, such as *L. alimentarius, L. casei, L. cellobiosus, L. curvatus L. delbrueckii, L. farciminis, L. fermentum L. lactis* and the recently identified *L. paralimentarius* (Table 183.4). However, the homofermentative *Pediococcus* and *Weissella* and the heterofermentative *Leuconostoc* have also been isolated from wheat sourdoughs.

TABLE 183.5
Groups of Lactobacillus Isolated from Sourdoughs

Characteristics	Obligately Homofermentative	Facultatively Heterofermentative	Obligately Heterofermentative
Growth at 15°C	−	+	+/−
45°C	+	−	+/−
Pentose fermentation	−	+	+
CO_2 from glucose	−	−	+
CO_2 from gluconate	−	+[a]	+[a]
FDP[b] aldolase present	+	+	−
Phosphoketolase present	−	+[c]	+
Lactobacillus	*L. acidophilus*	*L. alimentarius*	*L. brevis*
	L. amylovorus	*L. casei*	*L. buchneri*
	L. delbrueckii spp. *bulgaricus*	*L. curvatus*	*L. fermentum*
	L. delbrueckii spp. *delbrueckii*	*L. paralimentarius*	*L. fructivorans*
	L. farciminis	*L. plantarum*	*L. frumenti*
	L. helviticus	*L. rhamnosus*	*L. panis*
	L. leichmanni		*L. pontis*
	L. mindensis		*L. reuteri*
			L. sanfranciscensis[d]
			L. viridescens

[a] when fermented; [b] FDP fructose-1,6-diphosphate; [c] inducible by pentoses.

The table is modified after Kandler and Weiss,1986 [46].

Former names of some of the bacteria: [d] *L. brevis* spp. *lindneri* and *L. sanfrancisco.*

The variation in the composition of the microflora depends on the fermentation conditions such as flour type, extraction rate, water content, fermentation temperature, fermentations time and how the sourdough is refreshed. Most sourdoughs are fermented about 30°C, but *L. delbrueckii* has been isolated from rye sourdoughs with a fermentation temperature above 50°C [18,27]. Hammes, Stolz, and Gänzle [35] found that the most predominant LAB in firm sourdoughs with fermentation temperature between 23 and 30°C are *L. sanfranciscensis* and *L. pontis*. However, *L. fructivorans*, *L. fermentum* and *L. brevis* were also identified from this type of sourdough.

Some industrial sourdoughs are characterized by high water content to fluid conditions (suitable for pumping), elevated fermentation temperature (>30°C), and shorter fermentation time (15–20 hours). Fluid sourdoughs can be produced in large volumes — often by continuous fermentation systems, and they can be cooled for storing in silos up to one week. The microflora in this type of sourdough is dominated by *L. panis* [50], *L. reuteri, L. sonfranciscensis* and *L. pontis* [35]. The development of sourdough yeast is poor in fluid sourdoughs and consequently it is necessary to add baker's yeast is to the bread dough.

Sourdoughs kept at ambient temperature will continue acidification. The LAB are sensitive to low pH in longer time and the LAB will thus die off. Therefore dried sourdough preparations are preferred for commercial sourdough samples. However, LAB are rather sensitive to preservation by drying, and LAB present in commercial sourdoughs must survive drying. *L. plantarum*, *L. brevis*, *Pediococcus pentosaceus* have been identified from dried commercial sourdough preparations, and dried starter cultures containing strains of *L. sanfranciscensis* have only recently become commercially available [35].

B. Yeast in Sourdoughs

Several species of yeasts have been isolated from bakery and commercial sourdoughs. However, the taxonomy of yeasts has been gradually changed since the 1970s, and various synonyms have been used (Table 183.6). The traditional systematization and identification of yeasts have been based on biochemical tests as well as morphological and physiological criteria [54], but imperfect fungi cannot be studied using traditional genetics. New molecular characteristics have defined and changed the taxonomy of yeasts [55]. The physiological features of industrial yeasts have been shown to alter when changes occur in growth conditions, and species of *Saccharomyces cerevisiae*, *S. exiguous* and *Torulopsis delbrueckii* have been found to intermix genetically with each other [55].

The most frequently isolated yeast species from rye and wheat sourdoughs are *S. cerevisiae* (Tables 183.3 and 183.4). Other yeast species often isolated from sourdoughs are *S. exiguus, Candida milleri (C. holmii)*, *C. krusei* (*Issatchenkia orientalis*). The yeast species *Pichia saitoi*, *P. norvegensis* and *Hansenula anomala* and some *Saccharomyces* spp. have occasionally been isolated from sourdoughs.

TABLE 183.6
Yeasts Isolated from Sourdoughs and Their Synonyms

Perfect Fungi	Imperfect Fungi	Synonyms
Saccharomyces cerevisiae		
S. exiguus	*Candida holmii*	*Torulopsis holmii*
		Torula holmii
		S. rosei
	Candida milleri	*Torulopsis holmii*
S. delbrueckii		*Torulaspora delbrueckii*
S. uvarum		*S. inusitatus*
Issatchenkia orientalis	*C. krusei*	*S. krusei*
		Endomyces krusei
Pichia anomala	*C. peliculosa*	*Hansenula anomala*
P. membrafaciens	*C. valida*	
P. norvegensis		
P. polymorpha		
P. satoi		
Endomycopsis fibuligera		*S. fibuliger*

C., Candida; P., Pichia; S., Saccharomyces.
The table is modified after Kurtzman and Fell [128] and Barnett, Payne and Yarrow [129].

Candida spp. are members of *Deuteromycetes* (fungi imperfect), because they have lost their ability to undergo sexual development. *C. milleri* is a non-sporulating form of *S. exiguous* and was first described by Yarrow in 1978 [56]. *C. milleri* is physiological similar to *C. holmii*, but is different according to DNA identification. Some strains identified as *Torulopsis holmii* in the literature before 1978 have subsequently been assigned to *C. milleri* [55].

T. holmii and *S. cerevisiae* were the dominating yeasts in bakery rye sourdoughs from Finland, whereas *S. cerevisiae* dominated in rye sourdoughs used for home baking [57]. A later study showed that the yeasts isolated from rye bakery sourdoughs in Finland were similar to *C. milleri* [55]. Wlodarczyk [25] found that *S. cerevisiae* accounted for 99% of all yeasts found in starters from three industrial rye bread bakeries in Poland, whereas the sourdoughs from the smaller bakeries contained a wider range of yeast strains.

Traditional Portuguese sourdoughs prepared from maize and rye were dominated by *S. cerevisiae* and *Torulaspora delbrueckii* [58], and *S. cerevisiae* and *C. pelliculosa* [28], respectively. *S. cerevisiae* was also the

dominating yeast in wheat sourdoughs from Italy, followed by *S. exiguous*, *C. krusei*, *P. norvegensis* and *Hansenula anomala* [59]. Addition of baker's yeast is widely used in some Italian wheat sourdoughs [7], whereas many sourdoughs are also prepared without sourdoughs [60].

The yeasts present in sourdoughs are generally acid-tolerant. Strains of baker's yeast *S. cerevisiae* have poor tolerance of acetic acid in sourdoughs [61], whereas strains of *S. cerevisiae* isolated from sourdoughs can grow on MYGP broth acidified with acetic acid to pH 3.5 [37].

C. Microbial Interactions

The high stability of sourdoughs used for a longer period might be caused by production of inhibitory substances [41], but also microbial interaction between the LAB and the sourdough yeasts are of importance. Several sourdough LAB produce inhibitory substances against spoiling microorganisms. These compounds are organic acids in particular acetic acid, carbon dioxide, ethanol, hydrogen peroxide, and diacetyl [62]. The inhibition, however, can also be caused by bacteriocins that are low molecule-mass peptides, or proteins, with a bactericidal or bacteriostatic mode of action, in particular against closely related species [41].

Microbial interaction was demonstrated early for the Sanfrancisco sourdough. The sourdough yeast *T. holmii (C. millery)* does not assimilate maltose [34,63], whereas *L. sanfrancisco* hydrolyses maltose and excretes one of the glucose molecules to be used for the sourdough yeast [64]. The glucose uptake of the yeast cell can induce an outflow of amino acids, and this liberation of amino acids has made growth of *L. sanfranciscensis* possible even in a medium initially deficient in essential amino acids [65]. Several LAB increase the acidification of sourdoughs when the sourdough are added to the sourdough yeasts *T. holmii* or *S. cerevisiae* [63,66]. However, LAB might also multiply more slowly and decrease the production of acids in mixtures with yeasts [67].

A real risk of bacteriophage contamination of sourdoughs exists as bacteriophages with activity against *L. fermentum* have been isolated from an Italian sourdough [68].

IV. TECHNOLOGICAL ASPECTS

A. Production of Sourdough

Sourdough can be made with variations in the following parameters: flour type — wheat/rye, flour extraction rate, flour/water ratio, temperature, time and amount of starter. Sourdough can also be made in one to three steps. The one-stage process is the basic way to make a sourdough and is widely used. Two- and three-step sourdoughs have traditionally been used in rye bread production in many German bakeries [21]. Industrialization in bakeries has also included the sourdough production, where the time consuming multiple-stage processes have changed to the work-saving one-stage process. Traditional rye sourdoughs have often been based on firm sourdoughs, but in automated large scale bakeries firm sourdoughs are difficult to handle, and they have been replaced by pumpable semi-fluid to fluid sourdoughs which are suitable for automated fermentation systems. Today, continuous fermentation plants are used in many bakeries in Europe. The following deals with how sourdough fermentation can be influenced by the flour type, flour extraction rate, fermentation temperature, water content in sourdough, and by the amount of added ripe sourdough.

B. Flour Type

The flour in the sourdough is the substrate for the fermenting microorganisms. Wheat and rye flour are mostly used for sourdough making, but maize flour can also be used [28,69]. The amount of fermentable carbohydrates in the flour varies with the type of cereal, but in particular with the activity of endogenous enzymes in the flour. The activities of amylases, xylanases and peptidases are important for liberation of the fermentable low molecular weight carbohydrates and amino acids. On dough stage, the α-amylase can not degrade intact starch granules, but some granules are damaged during the milling process and may be partly degraded in the dough.

Starch is generally not degraded by LAB, and the content of fermentable mono- and disaccharides in rye flour is up to 5% with maltose (3%) as the main part [70]. Savola found that this content of free sugars decreased by 3% during sourdough fermentation. However, Henry and Saini [71] found only small amounts of low molecular weight sugars in rye (0.7% sucrose and $< 0.1\%$ of glucose, fructose, raffinose and stachylose). The content of pentosans (arabinoxylans) in rye flour is high (6.5 to 12.2%) [72] compared to wheat flour (2–3%) [73], and they can be degraded to the pentoses xylose and arabinose by the corresponding enzymes during the bread making processes [74].

The content of fermentable carbohydrates in wheat flour is 1–2% [67,75]. The content of maltose increased during the sourdough fermentation from 1.5 to 2.4%, and the content of fructose from 0.05 to 0.45% in a sourdough fermented with *Lc. mesenteroides* [75]. The content of glucose was unchanged at the level of 0.17% as a result of a balance between bacterial consumption and hydrolysis by the enzymatic activity. No sucrose was detected in the samples, so the increase in fructose could not be caused by yeast invertase.

Most *Lactobacillus* isolated from sourdoughs are non-amylolytic, but amylolytic strains have been isolated from African fermented cereal products made from maize such as ogi, mawé and kunu-zakki [76].

1. Extraction Rate

The extraction rate of the flour is one of the most important factors for determining the character of sourdough [77,78]. With a high extraction rate (80–100%), the content of nutrients such as B-vitamins and minerals increases compared to low extraction rate flour (65–75%), as does the buffering capacity of the flour primarily due to the phytic acid from the aleurone layer of the cereals. These factors can stimulate the growth and biochemical activity of the microflora in the sourdough followed by a higher production of acids and flavor compounds. Rye flours have a generally higher extraction rate than wheat flours.

A linear relationship between ash content and TTA was found in wheat sourdough. The final TTA in sourdoughs made from wholemeal flour (ash 1.5%) was almost double the value compared to sourdoughs made from straight-grade flour (ash 0.55%), and the final pH was reached in less time in sourdoughs made from the low extraction flours [3].

C. Water Content

The water content in the sourdough determines the firmness of sourdoughs, and it can be expressed as the dough yield (DY), which is the amount of sourdough per kg per 100 kg flour. DY varies from 150 in firm sourdoughs to 300 in fluid sourdoughs. The development in TTA is lower in fluid rye sourdoughs compared to firm sourdoughs, but if the acidity is measured per gram dry matter it will be lower in firm sourdoughs [21,36]. This indicates that the nutrients are better used by the LAB in fluid sourdoughs compared to firm sourdoughs. The production of lactic acid is not influenced by the DY, whereas the production of acetic acid is generally lower in fluid sourdoughs [21,36]. The water content in sourdoughs influences the acidification of the dough more than the temperature [79].

The content of LAB was not influenced by the firmness of rye sourdoughs, whereas the yeast propagation was low in the firm sourdoughs with levels below 10^3 cfu/g in six of the seven sourdoughs [36]. However, a surface layer of yeast cells was seen on the firm sourdough fermented with *L. plantarum*.

D. Temperature

The temperature of the sourdough is influenced by the temperature of the flour, the water and the mother sponge, and it is often adjusted and regulated by the water temperature. In practice, the temperature increases 6 to 8°C during industrial fermentation, if the temperature is not thermostatically regulated. So it is important that the temperature of the water is not too high. The temperature of the sourdough greatly influences the microbial propagation and production of acids, as the optimal temperature for growth and acidification varies for the different species of LAB. Spicher [80] found that the lowest generation time was 20 min for *L. brevis* at 35°C and *L. plantarum* at 40°C, and 60 min for *L. fructivorans* at 30°C and *L. fermentum* at 40°C. Changes in the fermentation temperature from the optimal conditions increased the generation time considerably, and the generation time for *L. fermentum* was prolonged to 120 min at 40°C and 140 min at 25°C.

The optimum temperature for growth of the LAB is close to the optimal temperature for acid production, and most LAB have temperature optima between 30 and 35°C [21]. In general, the final pH is reached more quickly at higher temperatures (30–35°C) compared to lower temperatures (20–25°C) [21,79,81]. Some species, mostly heterofermentative, can grow below 15°C, such as *L. farciminis, L. plantarum, L. rhamnosus, L. brevis, L. fructivorans, Lb. sanfranciscensi*. The highest temperature for growth is between 45 and 55°C and most species which can tolerate high temperatures are homofermentative, such as *L. acidophilus, L. amylovorus, L. delbrueckii*. However, also the heterofermentative species of *L. pontis, L. rhamnosus, L. fermentum* and *L. reuteri* can grow above 45°C [45].

The optimum temperature for growth of sourdough yeasts has not been intensively investigated, but it seems to be lower than for the LAB. The optimum temperature for growth of *C. milleri* was determined to be 27°C [44], while *C. milleri* and *S. exiguous* do not grow at temperatures above 35°C [56]. The minimum temperatures for growth of LAB and yeast are important when sourdoughs are stored by cooling, as the sourdough should not develop during the storage. The minimum temperature for growth of most sourdough yeasts has been found to be 8°C [55].

The content of acids produced in sourdough increases with increased fermentation temperature due to higher production of lactic acid, whereas the production of acetic acid is only negligible influenced by the temperature [21,81]. This confirms the general rule that the relative content of acetic acid is higher in cold sourdoughs compared to warmer sourdoughs [21].

Investigation of the influence of the fermentation temperature on the production of flavor compounds in rye sourdoughs showed that the starter cultures themselves produced few volatile compounds, whereas the production of iso-alcohols and ethyl acetate increased considerably with higher temperature in sourdoughs fermented with homofermentative LAB due to activity by the propagating yeasts [81].

E. Amount of Mother Sponge

The amount of mother sponge to be mixed with flour and water for a new sourdough should be so high that the content of LAB in the sourdough is able quickly to decrease the pH to inhibit the growth of the gram-negative bacteria in the flour. The amount of mother sponge influences the pH-lowering capacity in a sourdough, as low pH is

reached more quickly when the amount of added mother sponge is high [79]. However, higher levels of acids are produced when a lower amount of mother sponge is added, as the fermentation time is longer before the pH drops to the critical pH level [20]. The recommended amount of mother sponge is generally 10–20% for both rye and wheat sourdoughs [21,79]. The Sanfrancisco sourdough is rebuilt every eight hours or at least two to three times a day, seven days a week. The amount of mother sponge used in preparing a new sourdough is 25–40% of the sourdough [15]. This high amount of mother sponge makes the sourdough very stable, and this sourdough has been continued for more than a century.

V. DOUGH PROPERTIES AND BREAD QUALITY

A. Dough Properties and Bread Texture

1. Wheat Dough and Bread

Incorporation of sourdoughs in wheat bread making influences the gluten proteins and the viscoelastic behavior of doughs due to the drop in pH value caused by the organic acids produced. Several investigations have shown that the addition of acid to wheat dough decreased the dough stability during mixing, and the acidified doughs became considerably softer than a non-acidified control dough [82–84].

Dough stability was also decreased when it was prepared with the addition of sourdough [85] [86]. The dough consistency was unchanged when the sourdough was fermented by a heterofermentative culture and softer if a homofermentative culture was used [86]. Proteolytic breakdown of proteins was enhanced at low pH during fermentation of wheat dough, and major effects were attributed to changes in pH rather than to microbial proteolytic activity from the sourdough [87].

In spite of the decreased stability in doughs with added sourdough increased bread volumes are reported for bread containing up to 20% sourdough [31,86]. The crumb structure of bread containing up to 20% sourdough has been comparable to standard bread without sourdough, whereas inferior crumb structure was observed in bread containing 40% sourdough [88].

2. Rye Dough and Bread

The main component of rye and wheat is starch, and its content has a crucial influence on the bread texture. It becomes sticky and pasty if the starch is degraded during the bread making due to too high activity of amylases. This problem is greater in rye bread making than for wheat bread, as the activity of the sprout-induced enzyme α-amylase is highest in rye [89]. This is caused by rainy summers in the rye-growing area. Furthermore, the period from harvest to possible sprouting is extremely short for rye; it can even sprout in the fields [89]. One of the main functions of sourdough in rye bread making is inactivation of the α-amylase activity, and a general rule in bakeries is to add a larger amount of sourdough when the activity of enzymes in the flour is high. Bread with a rye content of more than 20% normally require the addition of sourdough to prevent degradation of starch [88,90]. Rye starch begin to swell as low as 52°C and subsequently the α-amylase can degrade the starch until it will be heat-inactivated at 80°C [91] (s.169, 171). Rye α-amylase has pH-optimum at pH 5.5 [92] and the activity is totally inactivated in sourdough at pH below 4. Wassermann and Dörfner [93] found that the viscosity of rye doughs (rye flour and water) was lowest at pH 5.

The activity of α-amylase is not only reduced considerably in the sourdough, but also in the rye dough with added sourdough. The activity of α-amylase was totally inactivated in an imitated sourdough acidified to pH 3.5 (TTA 32) by lactic and acetic acid [94]. The activity of α-amylase in the bread dough after resting (pH 4.5), with 20% sourdough added, was about half the activity in the flour.

Pentosans (arabinoxylans) play a key role in the viscosity of rye doughs due to high water-binding capacity. The viscosity of sourdoughs decreases during the sourdough fermentation due to the activity of the pentosan-degrading enzymes at the beginning of the fermentation. However, those enzymes are inactivated in the fermented sourdough [94]. Rye proteins are different from wheat proteins, as they do not form gluten structure. Kratochvil and Holas [95] found that proteolytic activity in rye sourdough was caused by enzymes from the flour.

B. Flavor and Taste

The flavor of bread crumb depends mainly upon the flour type and the enzymatic reactions taking place due to yeast and sourdough fermentations, whereas the flavor of bread crust is more influenced by the thermal reactions during the baking process. Including sourdough in the bread recipe is recommended for a more aromatic bread flavour [31,96] and sourdough bread has higher content of volatile compounds [31,39,97–99] and higher scores in sensory tests [31,100,101]. The content of volatile compounds produced during sourdough fermentation depends on the flour type (wheat, rye, maize), the extraction rate of the flour, the fermentation temperature, the water content in the sourdough and the microorganisms in the sourdough. Generally, the LAB in the sourdough are mostly responsible for the acidification of the dough, and the sourdough yeasts for the production of flavor compounds. Factors that favor the propagation of yeasts will also result in higher content of yeast fermentation products.

The extraction rate of the flour and the water content in the sourdough mostly influences the acidification of the sourdough. Higher extraction rate of the flour results in

higher production of lactic and acetic acid [38,102], however, sourdoughs fermented with heterofermentative cultures have much higher content of ethyl acetate [38]. The production of acids calculated per gram dry matter is higher in fluid sourdoughs than in firm sourdoughs. Higher water content in the sourdough and increased fermentation temperature result in higher propagation of yeasts and in higher content of iso-alcohols [36,81,102].

Sourdoughs fermented with heterofermentative LAB have, aside from much higher content of acetic acid and ethanol, a higher content of ethylacetate and ethyl-hexanoate compared to sourdoughs fermented with homofermentative LAB, which have higher contents of diacetyl and some other carbonyls [36,81,102,103]. The production of acetic acid in sourdoughs can be increased in heterofermentative cultures with the addition of fructose as a hydrogen acceptor [102,104]. When sourdough yeasts are added in the preparation of the sourdough, the production of ethanol, iso-alcohols, esters and diacetyl increase considerably [37] [103].

In sourdough bread, the content of esters are very low compared to the corresponding sourdoughs [31,39]. Sensory evaluation of rye bread crumb shows that the most intense and bread-like flavor is associated with 2-propanone, 3-methyl-butanal, benzylalcohol and 2-phenylethanol [39]. However, vanillin, 2,3-butandione, 3-hydroxy-4,5-dimethyl-furanone and methylbutanoic acids also contribute to the overall crumb flavor [98]. The perceived taste of salt is enhanced in sourdough rye bread compared to wheat bread, so less salt can be added in sourdough rye bread [105].

Sensory evaluation of wheat bread crumb showed that bread made with sourdough fermented with the heterofermentative *L. sanfranciscensis* had a pleasant mild, sour odor and taste. Bread fermented with *L. plantarum* had an unpleasant metallic sour taste, but when the sourdough was also supplemented with the sourdough yeast *S. cerevisiae,* the bread acquired a more aromatic bread flavor. That bread had a higher content of methyl-butanol, methylpropanoic acids and 2-phenylethanol which may, in part, cause the more aromatic flavor [31]. Mixed cultures with both Lab and yeast are recommended for an aromatic and pleasant sourdough bread flavor [31,103,106]. A lexicon for description of the flavor of wheat sourdough bread has been developed [107].

C. Longer Shelf Life

During storage of bread, several different physical and microbiological changes occur, lowering the quality of bread. The bread crumb becomes hard, the bread crust changes from crispy to leathery, and the characteristic and favorable bread flavor disappears. All these changes are characterized for the staling process. Within few days the bread might be spoiled due to contamination and growth of molds on the surface or development of rope in the bread crumb caused by *Bacillus* spp. Addition of sourdough in the bread recipe can be used to retard the staling process of the bread, prevent the bread against ropiness and prolong the mold free period. Sourdough addition is the most promising procedure to preserve bread from spoilage, since it is in agreement with the consumer demand for natural and additive free food products.

1. Anti-Mold Activity of Sourdough Bread

Mold is the most frequent cause of bread spoilage. Addition of sourdough in the bread recipe increases the mould-free period both for rye bread [21,32] and wheat bread [78,108]. The length of mold-free period was prolonged from 4 days in wheat bread to 6 to 8 days in sourdough bread [78], or from 9 days in wheat bread supplemented with a prefermented dough to 20 days in bread supplemented with 20% sourdough [108]. No correlation was found between pH and bread shelf life.

The mold-free period was prolonged 1 to 3 days in slices of sourdough rye bread inoculated with *Aspergillus glaucus* when the sourdough was fermented with heterofermentative LAB compared to homofermentative LAB, or bread without addition of sourdough [32]. The antimicrobial effect of the heterofermentative LAB was supposed to be their production of acetic acid.

Two hundred and thirty two strains of sourdough LAB belonging to nine different species were screened for production of anti-mold substances against *Aspergillus niger*, *Fusarium graminearum*, *Penicillium expansum* and *Monilia sitophila* using agar-well-diffusion assay [109]. The anti-mold activity varied very much among the strains and was mainly detected within obligately heterofermentative LAB. *L. sanfrancisensis* had the largest spectrum of anti-mold activity. Not only the acetic acid had inhibitory effect, but the LAB produced also formic, propionic, butyric, n-valeric and caproic acid, and a mixture hereof was responsible for the anti-mold effect.

2. Prevention of Rope Spoilage

Ropiness is spoilage of wheat bread noticed as an unpleasant odor similar to that of over-ripe melons, followed by the occurrence of a discolored sticky bread crumb and sticky threads, that can be pulled from the crumb. This bread spoilage is caused by heat-resistant strains of *Bacillus* and occurs particularly in summer when the climate favors growth of the bacteria. It is mainly caused by *Bacillus subtilis,* formerly referred to as *B. mesentericus*, because the heat resistant spores can survive the baking process, sporulate and multiply in the baked bread. The rope symptoms can be recognized when the level of *Bacillus* in bread crumb is 10^8 bacillus/g [110]. Its incidence has increased during the last decade, presumably because most bread is now produced without preservatives and often with the addition of raw materials such as oat products, wheat bran and sunflower seed with a high contamination level of *Bacillus*

spores [110]. Even a low level of the heat-resistant spores (10^1–10^2 bacillus/g) in raw materials resulted in a level of 10^7 bacillus/g bread in two days.

One potential way to prevent development of rope is to include sourdough in the bread recipe. Addition of 10% sourdough inhibited the natural *Bacillus* contaminants in wheat dough, but it was insufficient to inhibit the *Bacillus* strains inoculated at a level of 10^6 spores/g [40]. Addition of 15% sourdough was more efficient as the strains of rope producing *Bacillus* were effectively inhibited by sourdough fermented by strains of *L. sanfranciscensis*, *L. brevis, L. maltaromicus* or by three different strains of *L. plantarum*. In this investigation *B. subtilis* tended to be inhibited, if the TTA value in the sourdough was more than 10 and when the pH of the bread crumb was below 4.8. Röcken [29] demonstrated that sourdough effectively decreased the heat resistance (D_{97}-value) of a rope-producing strain of *Bacillus*. He found that the heat resistance was reduced from 143 min without the addition of sourdough to 5.9 min and 6.9 min with the addition of 10% and 20% sourdough, respectively.

3. Bread Firmness and Staling Rate

Bread becomes firmer during storage, and retrogradation of starch towards a more crystalline form is considered to be the primary cause of this bread staling. Several sourdoughs have been investigated for their potential effect on delaying the development of bread firmness and staling rate of wheat bread, but most investigations did not find any influence on staling rate by the sourdough compared to yeast- and sponge-leavened bread [108,111]. However, delayed staling rate has been observed in sourdough bread [112]. The rate of starch retrogradation was not influenced if the acidification was rather low, whereas a standard sourdough (*L. sanfranciscensis* 57, *L. plantarum* 13, *S. cerevisiae* 141) was able to retard the staling rate. The staling rate was mostly influenced if the starter culture had amylolytic activity (*L. amylovorus* or a genetic modified strain *L. sanfranciscensis CB1 Amy*).

In some investigations the addition of sourdough resulted in lower bread firming. However, sourdough wheat bread has higher bread volume [31,86,88] and the measured resistance will thus be lower.

VI. NUTRITIONAL VALUE

The addition of sourdough to the bread recipe has a positive influence on the nutritive value of the bread, as the minerals become bioavailable [113], and the blood glucose and insulin responses are lowered after eating sourdough bread compared to wheat bread [114].

A. Reduced Phytate Content by Sourdough

Whole meal cereals are good sources of minerals such as K, P, Mg, Fe and Zn, but without treatment the bioavailability is poor for those minerals which are stored as phytate, an insoluble complex with phytic acid (myoinositol hexa-phosphoric acid, IP6). The content of phytate is 6 mg/g rye grain [115], 3–4 mg/g in flour of soft wheat and 9 mg/g in hard wheat flour [116]. Phytate accounts for more than 70% of the total phosphorus in cereals, and it can be degraded during the bread making process due to the activity of endogenous phytase and thus liberate the bound minerals when the ester-bound phosphoric acids are hydrolyzed. The pH-optimum of rye phytase is found to be at pH 6.0 [115].

Sourdough fermentation has been shown to be more efficient than yeast fermentation in reducing the phytate content in whole bread (−62% and −38% respectively) [113]. The prolonged fermentation with sourdough enhanced the acidification and led to increased solubility of Mg and P. Five different strains of LAB isolated from sourdoughs have been tested for their ability to degrade phytic acid, but no difference was observed among the strains in the levels of phytic acid hydrolyses [117].

B. Reduced Glycemic Response with Sourdough Bread

Conventional wheat bread products are rapidly digested and absorbed, thus giving rise to high blood glucose and insulin responses. Eating wholemeal sourdough bread resulted in both lowered blood glucose and insulin response compared to wholemeal bread made without sourdough [118]. This nutritional positive effect was possibly due to a reduced gastric emptying rate caused by the lactic acid produced during the sourdough fermentation [114].

ACKNOWLEDGMENT

The information in this chapter has been modified from "Sourdough bread," by A. S. Hansen, in *Handbook of Food and Beverage Fermentation Technology*, Editors: Y. H. Hui et al., Marcel Dekker, NY 2004.

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Part U

Food Microbiology

184 Food Microbiology and Safety: Basic Requirements

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CONTENTS

I. INTRODUCTION

The objective of food processing and preparation is to provide safe, wholesome, and nutritious food to the consumer. The responsibilities for accomplishing this objective lie with every step in the food chain; beginning with food production on the farms, and continuing through processing, storage, distribution, retail sale, and consumption. Producing safe food is a continuum, where each party has certain obligations to meet and certain reasonable expectations of the other parties involved in the process. No single group is solely responsible for producing safe food, and no single group is without obligations in assuring the safety of food.

Food producers have a reasonable expectation that the food he or she produces will be processed in such a manner that further contamination is minimized. Food producers are an integral part of the food production system, but are not solely responsible for food safety. It is not practical to deliver fresh unprocessed food that is completely free of microorganisms, whether the food in question is of animal or plant origin. The environment in which the food is produced precludes the possibility that uncontaminated food can be grown or produced. However, appropriate methods can be utilized to reduce, to the extent possible, this level of background contamination. These methods are referred to as "Good Agricultural Practices" (GAPs) (1). Alternately, producers have an obligation to use these same reasonable practices to prevent hazards from entering the food chain. As an example, when dairy cattle are treated with antibiotics for mastitis, producers have an obligation to withhold milk from those animals from the normal production lot.

Milk from these animals must be withheld for the specified withdrawal time, so that antibiotic residues will not occur in milk delivered to dairies. In contrast, production of salmonellae-free poultry in the United States has been an elusive goal for poultry producers. While it is not a reasonable expectation for producers to deliver salmonellae-free birds to poultry processors, it is reasonable to expect producers to use good livestock management practices to minimize the incidence of *Salmonella* within a flock.

Food processors have reasonable expectations that raw materials delivered to the processing facility are of reasonable quality and not contaminated with violative levels of any drugs or pesticides. In addition, processors have a reasonable expectation that processed food will be properly handled through the distribution and retail chain, and that it will be properly prepared by the consumer. The latter is particularly important, as processors have responsibility for products because they are labeled with the processor's name, even though the food is no longer under processor control once it leaves the processing facility. Processor obligations are to process raw foods in a manner that minimizes growth of existing microorganisms as well as minimizes additional contamination during processing. These obligations extend from general facility maintenance to the use of the best available methods and technologies to process a given food.

Clearly, consumers have an important role in the microbiological safety of foods. However, it is not reasonable to expect every consumer to have a college degree in food science or microbiology. Consumers have an expectation that foods they purchase have been produced and processed under hygienic conditions. They also have a reasonable expectation that foods have not been held under unsanitary conditions, or that foods have not been adulterated by the addition of any biological, chemical, or physical hazards. In addition, consumers have an expectation that foods will be appropriately labeled, so that the consumer has information available on both composition and nutritional aspects of products. These expectations are enforced by regulations that govern production, processing, distribution, and retailing of foods in the U.S. The vast majority of foods meets or exceeds these expectations, and the average consumer has relatively little to be concerned with regarding the food they consume.

Some consumers have advocated additional expectations, which may or may not be reasonable. For example, some would argue that raw foods should be free of infectious microorganisms. Initially, this would appear to be reasonable; however, in many cases technologies or processes do not exist in a legal or practical form to assure that raw foods are not contaminated with infectious agents. Two recent examples are the outbreaks of *Cyclospora* epidemiologically linked to imported raspberries and *Escherichia coli* O157:H7 in raw ground beef. With the exception of irradiation, technologies do not exist to assure that either of these foods would be absolutely free of infectious agents while still retaining desirable characteristics associated with raw food. Therefore, in some cases, the expectation that raw foods should be free of infectious agents may not be reasonable.

Consumers have several obligations regarding food safety. As part of the food production to consumption chain, consumers have similar obligations to food processors. Namely, not holding foods under unsanitary conditions prior to consumption and not adulterating foods with the addition of biological, chemical, or physical agents. Improper food handling can increase foodborne illness risks by allowing infectious bacteria to increase in numbers or by allowing for cross contamination between raw and cooked foods. In addition, consumers have an obligation to use reasonable care preparing foods for consumption, as do personnel in food service operations. As an example, consumers should cook poultry until it is "done" (internal temperature at or above 68°C) to eliminate any concerns with salmonellae.

Consumer education on the basics of food safety in the home should be a priority. Every consumer should understand that food is not sterile, and the way food is handled in the kitchen may affect the health of individuals consuming it. Although our long-term goal is to reduce or eliminate foodborne disease hazards, in the near term we need to remind consumers of what some of the potential risks are and how consumers can avoid them. In the end, it is the consumer who decides what they will or will not consume.

II. ADMINISTRATIVE REGULATION

Several regulatory groups are involved in the regulation of food safety and quality standards, from local and state agencies to international agencies. Since there is tremendous variation within and between local and state agencies, this discussion will be confined to the national and international agencies that regulate food. At the national level, two federal agencies regulate the vast majority of food produced and consumed in the United States; namely, the U.S. Department of Agriculture (USDA) (2) and the Food and Drug Administration (FDA) (3).

A. U.S. Department of Agriculture

USDA has responsibility for certification, grading, and inspection of all agricultural products. All federally inspected meat and meat products, including animals, facilities, and procedures, are covered under a series of meat inspection laws that began in 1906 and have been modified on several different occasions, culminating in the latest revisions in 1996 (4). These laws cover only meat that is in interstate commerce, leaving the legal jurisdiction of intrastate meats to individual states. In the states

that do have state inspected meats, in addition to federally inspected meats, the regulations require that the state inspection program be "equivalent" to the federal program. Key elements in meat inspection are examination of live animals for obvious signs of clinical illness and examination of gross pathology of carcasses and viscera for evidence of transmissible diseases. The newest regulations also require the implementation of a HACCP system and microbiological testing of carcasses after chilling. Eggs and egg products are also covered by USDA inspection under the Egg Products Inspection Act of 1970 (5). This act mandates inspection of egg products at all phases of production and processing. USDA inspection of meat processing is continuous; that is, products cannot be processed without an inspector or inspectors present to verify the operation.

B. U.S. Food and Drug Administration

FDA has responsibility for ensuring that foods are wholesome, safe, and have been stored under sanitary conditions, as outlined by the Food Drug and Cosmetic Act of 1938. This act has been amended to include food additives, packaging, and labeling. The last two issues relate not only to product safety and wholesomeness, but also to nutritional labeling and economic fraud. FDA is also empowered to act if pesticide residues exceed tolerances set by the U.S. Environmental Protection Agency. Unlike USDA inspection, FDA inspection is discontinuous, with food processing plants being required to maintain their own quality control records while inspectors themselves make random visits to facilities.

C. Milk Sanitation

Perhaps one of the greatest public health success stories of the 20th century has been the pasteurization of milk. The U.S. Public Health Service drafted a model milk ordinance in 1924, which has been adopted by most local and state regulatory authorities and has become known as the Grade A PMO (Pasteurized Grade A Milk Ordinance) (6). This ordinance covers all phases of milk production, including but not limited to animal health, design and construction of milk processing facilities, equipment, and most importantly, the pasteurization process itself. The PMO sets quality standards for both raw and processed milk, in the form of cooling requirements and bacteriological populations. The PMO also standardizes the pasteurization requirements for fluid milk, which ensures that bacteria of public health significance will not survive in the finished product. From a historical perspective, it is interesting to note that neither the public nor the industry initially embraced pasteurization, but that constant pressure from public health officials finally succeeded in making this important advance in public health almost universal.

D. International Administration

The Codex Alimentarius Commission, created by the Food and Agriculture Organization and the World Health Organization, has the daunting task of implementing food standards on an international scale (7). These standards apply to both general and specific food categories and also set limits for pesticide residues in foods. Acceptance of these standards is voluntary and at the discretion of individual governments, but acceptance of the standards requires that the country apply them equally to both domestically produced and imported products. The importance of international standards is growing daily as international trade in food expands. Many countries find that they are both importing and exporting foods, and a common set of standards is critical in establishing trade without the presence of non-tariff trade barriers.

III. PRE-REQUISITE PROGRAMS

In order to achieve the goal of producing a safe food product, food processors should have in place a variety of fundamental programs covering the general operation of the process and the processing facility. These programs are considered "pre-requisites," as without these basic programs in place, it is impossible to produce safe and wholesome foods, irrespective of the available technology, inspection process or microbiological testing. These pre-requisite programs fall generally under the term "good manufacturing practices" (GMPs), but also include sanitation, equipment and facility design, personal hygiene issues, and pest control.

A. Good Manufacturing Practices (GMPs)

GMPs cover a broad range of activities with the food-processing establishment. Although there is general guidance in the Code of Federal Regulations (8), GMPs are established by the food processor, and are specific to their own operation. There is also general guidance on GMPs available from a variety of organizations representing specific commodities or trades. Specific applications of GMPs are discussed in the following sections, but GMPs also apply activities that affect not only the safety of the product, but also the quality. As an example, a refrigerated holding or storage temperature may be set by a GMP at a point below that which is actually required for product safety, but is set at that point for product quality reasons. Conversely, if a raw material or partially manufactured product, which under normal circumstances would be kept refrigerated, were subsequently found to be at a higher temperature, it would be deemed to be out of compliance with the GMP.

GMPs may also focus on the actual production processes and controls within those processes. GMPs may be viewed as rules that assure fitness of raw materials and

ingredients, rules that maintain the integrity of processed foods, and rules to protect the finished product (foods) from deterioration during storage and distribution. Other GMPs may address the presence of foreign materials in the processing area, such as tramp metal from equipment maintenance or broken glass from a shattered light bulb. These GMPs are established to provide employees with specific guidance as to the company's procedures for addressing certain uncommon but unavoidable issues.

While GMPs by their nature cover broad areas of operation, the individual GMP is usually quite specific, presenting complete information in a logical, step-wise fashion. An employee should be able to retrieve a written GMP from a file, and should be able to perform the required GMP function with little or no interpretation of the written material.

B. Training and Personal Hygiene

Personnel who are actually involved in food processing operations should also understand the necessity for proper cleaning and sanitation, and not simply rely on the sanitation crew to take care of all issues. In addition, all employees must be aware of basic issues of personal hygiene, especially when they are in direct contact with food or food processing equipment. Some key elements, such as hand washing and clean clothing and gloves, should be reemphasized on a periodic basis. An important aspect of this is an emphasis on no "bare handed" contact with the edible product, using utensils or gloves to prevent this from occurring. This information has been outlined by the U.S. Food and Drug Administration in the Good Manufacturing Practices section of the Code of Federal Regulations (8).

C. Pest Control

Pests, such as insects and rodents, present both physical as well as biological hazards (10). While the consumer would undoubtedly object to the proverbial "fly in the soup," the concerns with the introduction of biological hazards into the foods by pests are even greater. Integrated Pest Management (IPM) includes the physical and mechanical methods of controlling pests within the food processing environment and the surrounding premises. At a minimum, the processing environment and the area surrounding the processing plant should be evaluated by a competent inspector for both the types of pests likely to be present, and the potential harborages for such pests. A comprehensive program should be established that addresses flying insects, crawling insects, and rodents, the objective of which being to prevent access to the processing environment. Given that it is impossible to completely deny pest access to the processing environment, internal measures should be taken to reduce the numbers of any pests that enter the processing area. Since it is undesirable to have poisonous chemicals in areas surrounding actual food production, active pest reduction methods should be mechanical in nature (traps, insect electrocuters, etc.).

Record keeping is an important aspect of pest management. Documentation of pest management activities should include maps and maintenance schedules for rodent stations, bait stations, insect electrocutors, an inventory of pesticides on the premises, and reports of inspections and corrective actions. There should be standard operating procedures for applying pesticides, and they should only be applied by properly trained individuals. Many food-processing establishments contract with external pest control operators to address their pest control needs.

IV. SANITATION

Sanitation is the fundamental program for all food processing operations, irrespective of whether they are converting raw products into processed food or preparing food for final consumption. Sanitation impacts all attributes of processed foods, from organoleptic properties of the food to the safety and quality of the food itself. From a food processors perspective, an effective sanitation program is essential to producing quality foods with reasonable shelf lives. Without an effective program, even the best operational management and technology will ultimately fail to deliver the quality product that consumers demand.

Sanitation programs are all encompassing, focusing not only on the details of soil types and chemicals, but the broader environmental issues of equipment and processing plant design. Many foodborne microorganisms, both spoilage organisms and bacteria of public health significance, can be transferred from the plant environment to the food itself (11). Perhaps one of the most serious of these microorganisms came to national and international attention in the mid-1980s, when *Listeria monocytogenes* was found in processed dairy products. *Listeria* was considered to be a relatively minor veterinary pathogen until that time, and not even considered a potential foodborne agent. However, subsequent research demonstrated that *Listeria monocytogenes* was a serious human health concern, and more importantly was found to be widely distributed in nature. In many food processing plants, *Listeria* were found to be in the general plant environment, and subsequently efforts have been made to improve plant sanitation, through facility and equipment design as well as focusing more attention on basic cleaning and sanitation.

A. Sanitary Facility Design

Some of the basic considerations of food processing facility design include the physical separation of raw and processed products, adequate storage areas for nonfood items (such as packaging materials), and a physical layout that minimizes employee traffic between raw and

processed areas. While these considerations are easily addressed in newly constructed facilities, they may present challenges in older facilities that have been renovated or added on to. Exposed surfaces, such as floors, walls, and ceilings, in the processing area should be constructed of material that allows for thorough cleaning. Although these surfaces are not direct food contact surfaces, they contribute to overall environmental contamination in the processing area. These surfaces are particularly important in areas where food is open to the environment, and the potential for contamination is greater when temperature differences in the environment result in condensation (12). As an example, a large open cooking kettle will generate some steam that may condense on surfaces above the kettle. This condensate may, without proper design and sanitation, drip back down into the product carrying any dirt and dust from overhead surfaces back into the food. Other obvious considerations are basic facility maintenance as well as insect and rodent control programs, as all of these factors may contribute to contamination of food.

B. Sanitary Equipment Design

Many of the same considerations for sanitary plant design also apply to the design of food processing equipment. Irrespective of its function, processing equipment must protect food from external contamination and from undue conditions that will allow existing bacteria to grow. The issue of condensate as a form of external contamination has already been raised. Opportunities for existing bacteria to reproduce may be found in the so-called "dead spaces" within some equipment. These areas can allow food to accumulate over time under conditions that allow bacteria to grow. These areas then become a constant inoculation source for additional product as it moves through the equipment, increasing the bacteriological population within the food. Other considerations of food equipment design include avoiding construction techniques that may allow product to become trapped within small areas of the equipment, creating the same situation that occurs in the larger dead spaces within the equipment. As an example, lap seams that are tack welded provide ample space for product to become trapped. Not only does this create a location for bacteria to grow and contaminate the food product, it also creates a point on the equipment that is difficult if not impossible to clean.

C. Cleaning and Sanitizing Procedures

Cleaning and sanitizing processes can be generically divided into five separate steps that apply to any sanitation task (13). The first step is removal of residual food, waste materials, and debris. This is frequently referred to as a "dry" clean up. The dry clean up is followed by a rinse with warm (48° to 55°C) water, to remove material that is only loosely attached to surfaces and to hydrate material that is more firmly attached to surfaces. Actual cleaning follows the warm water rinse, which usually involves the application of cleaning chemicals and some form of scrubbing force, either with mechanical brushes or with high-pressure hoses. The nature of the residual food material will determine the type of cleaning compound applied. After this, surfaces are rinsed and inspected for visual cleanliness. At this point, the cleaning process is repeated on any areas that require further attention. Carbohydrates and lipids can generally be removed with warm to hot water and sufficient mechanical scrubbing. Proteins require the use of alkaline cleaners, while mineral deposits can be removed with acid cleaners. Commercially available cleaning compounds generally contain materials to clean the specific type of food residue of concern, as well as surfactants and, as necessary, sequesterants that allow cleaners to function more effectively in hard water (14).

When surfaces are visually clean, a sanitizer is applied to reduce or eliminate remaining bacteriological contamination. Inadequately cleaned equipment cannot be sanitized, as the residual food material will protect bacteria from the sanitizer. One of the most common sanitizing agents widely used in small and medium sized processing facilities, is hot water. Most regulatory agencies require that when hot water is used as the sole method of sanitization, the temperature must be at or above 85°C. While heat sanitization in effective, it is not as economical as chemical sanitizers because of the energy costs required to maintain the appropriate temperature. Chlorine containing sanitizers are economical and effective against a wide range of bacterial species, and are widely used in the food industry (15). Typically, the concentrations of chlorine applied to equipment and surfaces are in the 150 to 200 parts per million range. Chlorine sanitizers are corrosive and can, if improperly handled, release chlorine gas into the environment.

Iodine containing sanitizers are less corrosive than chlorine sanitizers, but are also somewhat less effective. These sanitizers must be used at slightly acidic pH values to allow for the release of free iodine. The amber color of iodine sanitizers can give an approximate indication of concentration, but can also leave residual stains on treated surfaces. Quaternary ammonium compounds (QACs) are noncorrosive and demonstrate effective bactericidal action against a wide range of microorganisms. These sanitizers are generally more costly and not as effective as chlorine compounds, but they are stable and provide residual antimicrobial activity on sanitized surfaces. Food processing plants will frequently alternate between chlorine and QAC sanitizers to prevent development of resistant bacterial populations or will use chlorine sanitizers on regular production days and then apply QACs during periods when the facility is not operating (for example, over a weekend).

Another element in food plant sanitation programs is the personnel who perform the sanitation operations as well as the employees who work in the processing area.

Sanitation personnel should be adequately trained to understand the importance of their function in the overall processing operation in addition to the training necessary to properly use the chemicals and equipment necessary for them to perform their duties.

V. HAZARD ANALYSIS CRITICAL CONTROL POINT SYSTEM (HACCP)

The basic concept of HACCP was developed in the late 1950s and early 1960s as a joint effort to produce food for the manned space program. The U.S. Air Force Space Laboratory Project Group, the U.S. Army Natick Laboratories, and the National Aeronautics and Space Administration contributed to the development of the process, as did the Pillsbury Company, which had a major role in developing and producing the actual food products. Since that time, the HACCP system has evolved and been refined, but still focuses on the original goal of producing food that is safe for consumption (16).

Since development, HACCP principles have been used in many different ways. However, recent interest in the system has been driven by changes in the regulatory agencies, specifically the U.S. Department of Agriculture–Food Safety and Inspection Service, and the U.S. Food and Drug Administration. USDA-FSIS recently revised the regulations that govern meat inspection to move all federally inspected meat plants to a HACCP-based system of production and inspection (4). FDA has also changed the regulations for fish and seafood, again moving this to a HACCP-based system for production (17). It is likely, given current trends by federal agencies, that most commercially produced foods will be produced under HACCP systems within the next ten years.

The goal of a HACCP system is to produce foods that are free of biological, chemical, and physical hazards (18). HACCP is a preventative system, designed to prevent problems before they occur, rather than trying to fix problems after they occur. Biological hazards fall into two distinct categories, those that can potentially cause infection and those that can potentially cause intoxications. Infectious agents require the presence of viable organisms in the food and may not, depending on the organisms and the circumstances, require that the organism actually reproduce in the food. As an example, *Escherichia coli* O157:H7 has an extremely low infectious dose for humans (possibly less than 100 viable cells), and as such the mere presence of the bacterium in foods is a cause for concern. In contrast, organisms involved in intoxications usually require higher numbers of the organism in the food to produce sufficient amounts of toxin to cause clinical illness in humans. However, some of the toxins involved in foodborne diseases are heat stable, so that absence of viable organisms in the food is not necessarily an indication of the relative safety of the food. *Staphylococcus aureus* is a good example, where it typically requires greater than 1,000,000 to 10,000,000 cells per gram of food to produce sufficient toxin to cause illness in humans (19). However, because the toxin itself is extremely heat stable, cooking the food will eliminate the bacterium but not the toxin, and the food can still potentially cause an outbreak of foodborne illness.

Chemical hazards include chemicals that are specifically prohibited in foods, such as cleaning agents, as well as food additives that are allowed in foods but only at regulated concentrations. Foods containing prohibited chemicals or food additives in levels higher than allowed are considered adulterated. Adulterated foods are not allowed for human consumption and are subject to regulatory action by the appropriate agency (USDA or FDA). Chemical hazards can be minimized by assuring that raw materials (foods and packaging materials) are acquired from reliable sources that provide written assurances that the products do not contain illegal chemical contaminants or additives. During processing, adequate process controls should be in place to minimize the possibility that an approved additive will be used at levels not exceeding maximum legal limits for both the additive and the food product. Other process controls and GMPs should also insure that industrial chemicals, such as cleaners or lubricants, will not contaminate food during production or storage (8).

Physical hazards are extraneous material or foreign objects that are not normally found in foods. For example, wood, glass or metal fragments are extraneous materials that are not normally found in foods. Physical hazards typically affect only a single individual or a very small group of individuals, but because they are easily recognized by the consumer, are sources of many complaints. Physical hazards can originate from food processing equipment, packaging materials, the environment, and from employees. Physical contaminants can be minimized by complying with good manufacturing practices and by employee training. While some physical hazards can be detected during food processing (e.g., metal by the use of metal detectors), many non-ferrous materials are virtually impossible to detect by any means and so control often resides with employees.

VI. HACCP PLAN DEVELOPMENT

Prior to the implementation of HACCP, a review should be conducted of all existing pre-requisite programs. Deficiencies ion these programs should be addressed prior to the implementation of HACCP, because a HACCP plan presumes that these basic programs are fully functional and effective. Development of a HACCP plan begins with the formation of a HACCP team (20). Individuals on this team should represent diverse sections within a given operation, from purchasing to sanitation. The team is then responsible for development of the plan. Initial tasks that the team must accomplish are to identify the food and

method of distribution, and to identify the consumer and intended use of the food. Having done this, the HACCP team should construct a flow diagram of the process and verify that this diagram is accurate.

The development of a HACCP plan is based on seven principles or steps in logical order (21). With the flow diagram as a reference point, the first principle or step is to conduct a hazard analysis of the process. The HACCP team identifies all biological, chemical, and physical hazards that may occur at each step during the process. Once the list is completed, it is reviewed to determine the relative risk of each potential hazard, which helps identify significant hazards. Risk is the interaction of "likelihood of occurrence" with "severity of occurrence." As an extreme example, a sudden structural failure in the building could potentially contaminate any exposed food with foreign material. However, likelihood of the occurrence of such an event is small. In contrast, if exposed food is held directly below surfaces that are frequently covered with condensate, then the likelihood of condensate dripping on exposed food is considerably higher. An important point in the determination of significant hazards is a written explanation by the HACCP team regarding how the determination of "significant" was made. This documentation can provide a valuable reference in the future, when processing methods change or when new equipment is added to the production line.

The second principle in the development of a HACCP plan is the identification of critical control points (CCPs) within the system. A CCP is a point, step, or procedure where control can be applied and a food safety hazard can be prevented, eliminated, or reduced to acceptable levels (18). An example of a CCP is the terminal heat process applied to canned foods after cans have been filled and sealed. This process, when properly conducted according to FDA guidelines, effectively eliminates a potential food safety hazard, *Clostridium botulinum*. Once CCPs have been identified, the third principle in the development of a HACCP plan is to establish critical limits for each CCP. These limits are not necessarily the ideal processing parameters, but the minimum acceptable levels required to maintain the safety of the product. Again, in the example of a canned food, the critical limit is the minimum time and temperature relationship to insure that each can has met the appropriate standards required by FDA.

The fourth principle, following in logical order, is to establish appropriate monitoring requirements for each critical control point. The intent of monitoring is to ensure that critical limits are being met at each critical control point. Monitoring may be on a continuous or discontinuous basis. Presence of a physical hazard, such as metal, can be monitored continuously by passing all of the food produced through a metal detector. Alternately, presence of foreign material can be monitored on a continuous basis by visual inspection. Discontinuous inspection may involve taking analytical measurements, such as temperature or pH, at designated intervals during the production day. Some analytical measurements can be made on a continuous basis by the use of data recording equipment, but it is essential that continuous measures be checked periodically by production personnel.

The fifth principle in the development of a HACCP plan is to establish appropriate corrective actions for occasions when critical limits are not met. Corrective actions must address the necessary steps to correct the process that is out of control (such as increasing the temperature on an oven) as well as addressing disposition of the product that was made while the process was out of control. A literal interpretation of the HACCP system and a CCP is that when a CCP fails to meet the critical limits, then the food product is potentially unsafe for human consumption. As a result, food produced while the CCP was not under control cannot be put into the normal distribution chain without corrective actions being taken to that product. Typically this means that the product must be either re-worked or destroyed, depending on the nature of the process and the volume of product that was produced while the CCP was out of control. This argues for frequent monitoring, so that the actual volume of product produced during each monitoring interval is relatively small.

The sixth principle in the development of a HACCP plan is verification. Verification can take many forms. Microbiological tests of finished products can be performed to evaluate the effectiveness of a HACCP plan. Alternately, external auditors can be used to evaluate all parts of the HACCP plan, to ensure that the stated goals and objectives are being met. A HACCP plan must also be periodically reviewed and updated, to reflect changes in production methods and use of different equipment. Another critical aspect of verification is education of new employees on the HACCP plan itself. As HACCP is phased in to many food-processing environments, many employees who are unfamiliar with the concepts and goals of HACCP will have to be educated on the necessity of following the plan. In one sense, USDA-FSIS regulations have guaranteed that meat processors will follow HACCP plans, as the penalty for not following the HACCP plan can be as severe as the loss of inspection at an establishment. However, HACCP is an excellent system for monitoring and improving production of food products, and many food processors will discover that HACCP plans offer many benefits, well above and beyond the legal requirements of the regulatory agencies.

The seventh principle in the development of a HACCP plan is the establishment of effective record keeping procedures. In many respects, a HACCP plan is an elaborate record-keeping program. Records should document what was monitored, when it was monitored and by whom, and what was done in the event of a deviation. Reliable records are essential from both a business

and regulatory perspective. From the business perspective, HACCP records allow a processor to develop an accurate longitudinal record of production practices and deviations. Reviewing HACCP records may provide insight on a variety of issues, from an individual raw material supplier whose product frequently results in production deviations, to an indication of an equipment or environmental problem within a processing plant. From a regulatory perspective, records allow inspectors to determine if a food processor has been fulfilling commitments made in the HACCP plan. If a processor has designated a particular step in the process as a CCP, then they should have records to indicate that the CCP has been monitored on a frequent basis and should also indicate corrective actions taken in the event of a deviation.

VII. SUMMARY

The intent of food processing is to deliver safe and wholesome products to the consumer. Basic food safety programs, including GMPs and sanitation, are the minimum requirements to achieve this goal. HACCP is a logical extension of these programs, and focuses on the prevention of hazards before they occur, rather than waiting for a failure to occur, and then addressing the problem. HACCP provides the most comprehensive approach to food safety in the processing environment, but is not foolproof. Perhaps the most challenging aspect is that, even with the best designed and implemented HACCP plan, it may not always be possible to "prevent, eliminate or reduce to acceptable levels" the pathogen of concern. This is particularly true with foods that are purchased by the consumer in their raw state, and then cooked. A specific example is *Escherichia coli* O157:H7 in ground beef. Irrespective of the preventative efforts of the processor, it is not possible to assure that the product is free of the bacterium, and there is no "acceptable level" of this organism in ground beef.

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185 Conventional Microbial Testing Methods and Microscopy Techniques

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CONTENTS

I. INTRODUCTION

Microbial testing has been a concern since it was determined that microbes play an integral role in food quality and safety. One of the earliest volumes that discussed standardized testing methods was Food Adulteration and its Detection (1), which was followed by numerous other texts. Today many similar volumes are in use by a variety of regulatory agencies, industries and scientific groups. These include, but are not limited to the Food and Drug Administration's (FDA) Bacterial Analytical Manual (BAM), U.S. Department of Agriculture's (USDA) Microbiological Laboratory Guidebook (MLG), AOAC's Official Methods of Analysis and the American Public Health Association's (APHA) Compendium of Methods for the Microbiological Examination of Foods.

This chapter will briefly review some of the basic concepts surrounding food microbiology, microscopy, and testing. It will touch upon a variety of microscopy- and media-based methodologies, but will exclude antibody or enzyme linked immunosorbent assays (ELISA) and genetic-based testing.

II. MICROORGANISMS

Microorganisms are single to multi-celled microscopic living units that are generally categorized as bacteria, viruses, yeasts, molds, algae, and protozoa. Microorganisms are ubiquitous, including but not limited to humans, animals, plants, soil and water. They first appeared on earth over 3 billion years ago and have coexisted with humans providing both beneficial and detrimental consequences on our food supply (2). Of most concern for food quality and safety have been bacteria, yeasts, and molds. While other microorganisms are still significant, this chapter will focus on the aforementioned three.

A. Bacteria

Although bacteria are extremely diverse with great differences in their structural and biochemical components, they share a basic cellular organization. Bacteria are the smallest and fastest growing of all living cells. They have physical shapes that are generally classified as cocci (round) or rods. Rods can be straight, spiral, or curved in

appearance. Often, the types of morphological forms observed depend upon environmental conditions in which the bacterial cells are cultured. Depending on the conditions present, cells may differentiate into spores, a dormant state, or form actively growing cells referred to as vegetative (3). Spores, commonly referred to as endospores, are produced as a bacterial survival strategy. Some vegetative cells form spores during periods of environmental stress such as depletion of nutrients or moisture needed for growth. Spores exhibit no metabolism and are capable of withstanding adverse conditions such as heat, radiation, disinfectants, desiccation, and ultraviolet light. When optimum environmental conditions are introduced, spores germinate and form a single vegetative bacterial cell. Common spore-formers that are important to the food industry are Gram-positive members of the *Bacillus* and *Clostridium* genera (4).

B. Yeasts

Yeasts are members of a higher group of microorganisms called fungi. They are single-celled organisms of spherical, elliptical, or cylindrical shape. Their size varies greatly but is generally larger than bacterial cells. Yeasts may be divided generally into two groups according to their method of reproduction; those that reproduce by budding only (asporogenous), and those that reproduce by both budding and spore formation. Unlike bacterial spores, yeasts form spores as a method of reproduction (3).

C. Molds

Molds are filamentous, multi–celled fungi with an average size larger than both bacteria and yeasts (10×40 μm). Each filament is referred to as a hypha. Mats of hyphae that spread over a food substrate are called mycelia. Molds may reproduce either asexually or sexually, sometimes both within the same species (3).

Some mold species produce only one type of asexual spore, while others are capable of producing several types. Most mold species produce asexual spores called conidia. Some molds reproduce asexually through a process of fragmentation, in which the hyphae separate into individual cells called arthrospores. Other mold asexual spore states include zoospores or chlamydospores among others. Asexual spores may be produced in the tip of fruiting hyphae, along the length of the hyphae, or in swollen structures called sporangia. When reproducing sexually, sexual spores are produced by nuclear fission in times of unfavorable conditions to ensure survival.

III. MICROBIAL GROWTH FACTORS

There are a number of factors that affect the survival and growth of microorganisms in food and in culture media. Intrinsic factors inherent to the food include but are not limited to the following: nutrient content; water activity; pH; reduction-oxidation (redox) potential; temperature; humidity; available oxygen; biological structures; and antimicrobial constituents. These factors all play a role in the survival, growth and subsequent recovery of microorganisms (3).

A. Nutrient Requirements

Microbial growth is achieved through the synthesis of cellular components and energy from the catabolism of nutrients. All of the nutrients necessary for growth are obtained through the immediate environment of the microbial cell. Essential nutrients required for microbial growth include a carbon source such as sugar, or compounds with sugar moieties, a nitrogen source such as ammonia, nitrates or other compounds, and growth factors such as vitamins, minerals, and water. From these building blocks, microorganisms can synthesize carbohydrates, proteins, and lipids needed to produce complex cellular structures like the cell wall, membranes and organelles (4).

B. Moisture Content

Water is one of the most important factors involved in the degradation of food. Water activity (a_w) is a measure of free unbound water and is defined as the ratio of the vapor pressure of water in a food, P, to the vapor pressure of pure water, P_0, at the same temperature. Most fresh, raw food products have a_w levels of 0.98 or higher, which will support growth of most microorganisms. Generally, Gram-negative bacteria have the highest a_w requirement ranging from 0.99–0.88, while some yeasts and molds are capable of growing at much lower a_w than bacteria (4). There is a correlation between a_w, temperature, and nutrition. At any given temperature, microorganisms have a reduced ability to grow when the a_w is lowered. Also, when nutrients are present, there is an increase in the range of a_w in which microorganisms are able to survive (3).

C. pH

The term pH is defined as the logarithm of the reciprocal hydrogen ion concentration in solution. The measure of the hydrogen ion concentration, or pH, generally corresponds to the degree of acidity in a sample (5). The majority of microorganisms thrive in a neutral pH (6.6–7.5), although some microorganisms are capable of growing below a pH of 4.0 (3). Other bacteria, such as *Alicyclobacillus* spp., are known as acidophiles and grow only at lower pH levels found in acidic foods such as fruit juices.

D. Redox Potential (Eh)

The reduction–oxidation or redox potential (Eh) measures the potential difference generated by a coupled reaction in

which one substance is oxidized and the second substance is reduced simultaneously. The reduced substance gains electrons while the oxidized substance loses electrons. When electrons are transferred, there is a potential difference created which can be measured electrometrically with most pH meters (measured in millivolts or mV). Microorganisms can be grouped according to the Eh range capable of supporting growth. Aerobes grow between the range of +500 and +300 mV, facultative anaerobes grow best between +300 and −100 mV, and anaerobes are most capable of growing between +100 and −250 mV and less (2). Redox potentials are dependant on pH and are useful in determining the degree of anaerobiosis. Eh can also be estimated by using common redox dyes such as methylene blue and resazurin (5). The redox potential of a specific food can be influenced by several factors: the characteristic redox potential of the original food, the poising capacity, the oxygen tension of the atmosphere around the food and the access of the atmosphere to the food (3).

E. Temperature

Microorganisms are capable of growing over a wide range of temperatures. The simplest way of organizing these organisms is placing them in three categories: psychrophiles, mesophiles and thermophiles. Psychrophiles have an optimum growth temperature ranging from 10°C to 15°C, but can also grow well at or below 7°C and may grow at sub 0°C temperatures. These bacteria may produce spoilage or safety issues in refrigerated foods. Mesophiles grow well between 20°C and 45°C with an optimum between 30°C and 40°C. Although mesophiles are often found on foods held under refrigeration temperatures, they are usually not capable of proliferation at those temperatures. Those mesophiles that can grow under refrigeration are called psychrotrophs. Their growth is best at mesophilic temperature and very slow under refrigeration. Thermophiles require high temperatures for growth with an optimum range between 55°C and 65°C. The bacteria included in this category are of special interest in the canning industry (3). It should be noted that some microorganisms of importance to food spoilage are true psychrophiles ("cold loving") and will only grow under refrigeration conditions. An example, *Mrakia frigida*, is a fermentative yeast isolated from some spoiled, chilled fruit juices.

F. Relative Humidity

Relative humidity is important with respect to the a_w within foods and the growth of microorganisms at the surface of foods. Relative humidity and temperature have a relationship that should be carefully considered when storing foods. Generally, an inverse relationship exists in that the higher the temperature, the lower the relative humidity, and vice versa. It is important that foods under storage conditions are exposed to an appropriate relative humidity that inhibits microbial growth. The best way to prevent surface spoilage of foods such as chicken and beef is to store them at a low relative humidity (3).

G. Atmosphere

It is possible to classify microorganisms by oxygen requirements that facilitate their growth and survival. Obligate aerobes require oxygen, while facultative organisms are capable of growing in the presence or absence of oxygen. Microaerophilic organisms grow best at very low levels of oxygen. Aerotolerant anaerobes do not require oxygen for growth but are not harmed if oxygen is present. Obligate anaerobes can only grow in complete absence of oxygen and if present oxygen can be lethal (6).

There are two important atmospheric gases that exhibit antimicrobial properties. The most important is carbon dioxide (CO_2), which is commonly used as a food preservative. It has been known since 1882 that raising the CO_2 concentration in the immediate environment of fresh meat will increase the shelf life of the product (3). In a study utilizing modified atmosphere packaging with chilled storage temperatures, growth of *Aeromonas hydrophila* on fresh turkey and pork slices was strongly inhibited at 1°C, especially in those meats packaged with CO_2. No growth of *A. hydrophila* was observed on the pork or turkey at 40/60 CO_2/O_2 concentrations (7).

Ozone (O_3) is another important atmospheric gas that displays antimicrobial properties. This gas has been shown to be effective against many microorganisms. Since ozone is a strong oxidizing agent, it should not be used on foods that have high lipid contents due to the likelihood of rancidity development from the oxidized fats. In 1997, ozone was granted GRAS (generally recognized as safe) status in the United States for food use (3). While *Escherichia coli* O157:H7 can be destroyed within 20 to 50 minutes upon exposure to ozone at 3 to 18 ppm in culture media (8), its usefulness in foods is not fully realized. One reason for this may be that ozone concentration in the processing plant environment and subsequent employee exposure to the compound must be carefully monitored and controlled to ensure employee safety.

H. Biological Structures

There are many foods that have natural exterior barriers that provide protection from spoilage microorganisms. Some examples are the shells of nuts and eggs, the hides of animals, and the outer coverings of fruits and vegetables. Once these protective barriers have been compromised, the interior substances are generally susceptible to the invasion of microorganisms (3).

I. Antimicrobial Constituents

As part of the natural protection against microorganisms, many foods contain constituents such as essential oils that may have antimicrobial properties. Some examples of these

oils are eugenol in cloves and allicin in garlic. Cow's milk contains antimicrobial substances such as lactoferrin, conglutinin, and the lactoperoxidase system (3). Lactoperoxidase is an enzyme that is naturally present in raw milk, saliva, colostrums, and other natural secretions. An antimicrobial compound is formed when this enzyme reacts with thiocyanate in the presence of hydrogen peroxide. This reaction is commonly referred to as the lactoperoxidase system (4). Ground beef studies have demonstrated that the lactoperoxidase system is effective against food-borne pathogens. A broad range of food products may be treated with the lactoperoxidase system in order to prevent the growth of pathogenic microorganisms (9).

IV. SAMPLING PLANS

Since it is not practical to examine an entire lot of food for the presence or absence of microorganisms, an appropriate sampling plan must be employed. Statistical methods of population probability must be utilized to determine the number and size of sample units needed to produce statistically valid analytical results (4).

A. Attribute Plans

When shipments of food are received, very limited information may accompany the shipment about the processing methods. Attribute plans are most appropriate for this type of situation. One of the simplest ways to choose whether to accept or reject a food lot can be based upon the results from a microbiological test performed on several sample units (n). Attribute plans test for the presence or absence of a microorganism. Concentrations of microorganisms can be allocated to a specific attribute class by observing whether they are above or below a preset concentration (10).

B. Variable Plans

Unlike attribute plans, variable plans are utilized when the distribution of microorganisms is known or can be estimated. Variable plans can be considered more useful than attribute plans because they determine microbial counts instead of assigning counts to categories or ranges. The greatest advantage of variable plans is that fewer samples are required, which in turn results in a lower cost to obtain the same protection as a single attribute plan. The most significant disadvantage includes the numerous calculations involved in evaluating a lot, the multiple calculations needed for each variable, and the requirement that a probability distribution must be known or assumed for each measurement (10).

V. MICROSCOPY

One of the first steps in the identification of microorganisms is the direct examination of the isolated organism using microscopy. The organisms can be viewed live or as prepared stained isolates. The following summarizes the common types of microscopy used by researchers and the food industry.

A. Light Microscopy

The invention of the light microscope is first accredited to Antony van Leeuwenhoek in 1668. It consisted of a single lens that was moved by a screw mechanism. Today there are an enormous variety of microscopes that utilize numerous light microscopy techniques to study microbes and their environment. The light microscope has many limitations, but this form of microscopy is usually the first employed for microbiological evaluation of food and water. A light microscope consists of three essential components, the eyepiece, the objective, and an illumination source, with the last two components having the greatest influence on image quality (11). A major disadvantage of the microscopic examination of foods is that particulate matter often interferes with the observation.

B. Phase Contrast Microscopy

Presently, there are small and easily useable phase contrast microscopes that are capable of quickly identifying microbial characteristics such as morphology, motility, and spores. A limiting factor for this type of microscopy is that there must be a fairly high bacterial count, greater than 10^5 CFU/mL, before the cells would be visible. Utilizing a Petroff-Hauser cell counter or other suitable counting device, it is possible to conduct a direct microscopic count to estimate the cell density viewed with a phase contrast microscope (2).

C. Epifluorescence Microscopy

Epifluorescence microscopy or EFM is a visual set-up for a fluorescence microscope where the objective lens is used to focus ultraviolet light on the specimen and collect fluorescent light. This form of microscopy has a greater efficiency than transmitted fluorescence, in which a separate lens or condenser is utilized to focus ultraviolet light on the sample. Epifluorescence allows for fluorescence microscopy to be merged with another type of microscopy in the same device (12). A recent study comparing the effectiveness of virus enumeration using an epifluorescence microscope, transmission electron microscope, and flow cytometry demonstrated that the epifluorescence microscope displayed the greatest accuracy and precision. The epifluorescence microscope deviated less than 5% from the true and relative errors. It was also shown that an EFM is considerably more time and cost efficient than a transmission electron microscope (13).

D. Transmission Electron Microscopy

A transmission electron microscope (TEM) consists of an electron gun, which generates electrons, a series of

condenser lenses that focuses the electron beam onto the specimen, a goniometer stage that manipulates the specimen under the electron beam, another series of lenses that produces a magnified image, and a phosphor screen on which the image of the specimen being observed is projected. There are several disadvantages to TEM that restrict its usefulness. These include specimen stability in vacuum, the need for very thin samples to produce an image, and damaging effects on the specimen produced by the high current density of the electron beam (14).

E. Scanning Electron Microscopy

The scanning electron microscope (SEM) uses a thinly focused electron beam to scan the surface of a sample and produce high quality images with magnification up to 100,000X. SEM also has a large depth of field that enables the entire surface of the specimen to be focused (14). With the use of TEM and SEM, it is possible to observe the effects of various technologies to inactivate microorganisms present in foods. With these advanced microscopes, changes in the cell cytoplasm and cell membrane can be clearly observed (15).

F. Low-Voltage Field-Emission Scanning Electron Microscopy

Field-emission scanning electron microscopes (FESEM) are capable of producing clear sharp images of superior resolution to conventional SEM. One distinct advantage to FESEM is that low kinetic energy electrons are able to probe close to the specimen surface to produce high quality, low voltage images with minor electrical charging of samples. With FESEM, there is no need to introduce conducting coatings on insulating materials (16). Testing procedures for bacterial microfiltration membranes often utilize FESEM to observe entrapment of bacteria in the membrane matrix (17).

G. Variable-Pressure Scanning Electron Microscopy

A scanning electron microscope functions in high vacuum mode by scanning a focused beam of high-energy electrons over the surface of a sample. The advantage of variable pressure modes is that they allow for the microscopy of wet, oily and nonconductive specimens in their natural state without the addition of conventional sample preparation and coating (18).

H. Cryo-Scanning Electron Microscopy

The most direct approach for electron microscopy of organic material is through the imaging of fast-frozen samples. This method does not require chemical fixation and drying of artifacts that might influence results. One advantage to this method is that charged artifacts and the effects of beam damage are greatly decreased. This method is particularly suitable for high-pressure frozen samples (19).

I. Environmental Scanning Electron Microscopy

A major advantage of ESEM is that it is capable of being operated with a low vacuum in the specimen chamber. Conventional scanning electron microscopy requires a moderately high vacuum in the specimen chamber in order to avoid atmospheric interference with primary or secondary electrons. This method is termed "wet mode" imaging. This method allows the specimen chamber to be isolated from the rest of the vacuum system by valves, pressure-limiting apertures, and a large-diameter bypass tube. The most commonly used imaging gas is water vapor and is controlled by a separate vacuum pump in the specimen chamber. The electron beam of ESEM consists of primary electrons and ejects secondary electrons from the surface of the sample. As a result, the secondary electrons collide with water molecules, functioning as a cascade amplifier to deliver the secondary electron signal to the positively biased gaseous secondary electron detector (GSED). Due to the loss of electrons in this exchange, the water molecules become positively ionized and are attracted to the specimen which may be nonconductive and uncoated and acts to neutralize the negative charge produced by the primary electron beam (20).

J. Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) has many advantages over light microscopy in that there is improved contrast at high resolution, improved resolution in fluorescent specimens, and improved depth resolution which allows for optical sectioning of the specimen being observed. Compared to transmission electron microscopy, CLSM has poorer resolution but requires less specimen preparation and is capable of relaying three-dimensional information about internal structures with greater convenience. Regarding biological applications, CLSM was mainly developed with fluorescent staining and especially for localization with fluorescent markers used to derive three-dimensional images (21). In a recent CSLM study, viable and nonviable cells of *E. coli* O157:H7 that were labeled with a fluorescent antibody were observed on fresh fruits and vegetables (22).

VI. DETECTION AND IDENTIFICATION

There are numerous methods that can be utilized for the evaluation or detection of microorganisms. They are broadly grouped as quantitative or qualitative methods. The quantitative methods are used to enumerate or to

directly or indirectly estimate the microbial load in a test sample. Some examples of quantitative methods used are aerobic plate counts, coliform counts, yeast and mold counts, direct microscopic counts, and most probable number protocols. Qualitative methods are intended to determine if a sample contains a specific microbial species amidst the total microbial population based upon a presence/absence determination. These methods are commonly used to detect the presence of certain foodborne pathogens including *Salmonella*, *E. coli* O157:H7, and *Clostridium botulinum* (2).

A. Indicator Organisms

Groups of microorganisms, such as Enterobacteriaceae, total coliforms, thermotolerant coliforms, and *E. coli*, are sometimes utilized to indicate potential contamination of food and water by enteric pathogens. Testing for thermotolerant coliforms is often used as a presumptive test for *E. coli*, which often indicates fecal contamination. Although testing for total coliforms is sometimes used as an indicator for the possible presence of *E. coli*, the usefulness of this test method is questionable since there are many coliforms that are not of fecal origin. Standard methods for the enumeration of indicator organisms rely on the use of specific microbiological media and protocols that isolate and enumerate viable cells in the sample (23).

B. Culture Media

Bacteria are differentiated using several major characteristics, including cultural requirements of each microorganism. The nutrients that each organism requires for optimal growth can be used to identify it from closely related organisms.

1. Selective Media

Many media contain selective components that prevent the growth of non-target microorganisms. Selective media are useful in the isolation of specific microorganisms from mixed populations. In many media, compounds are included as sole sources of carbon or nitrogen so that only a few types of microorganisms can proliferate. Selective toxic compounds are also frequently added to select for the cultivation of particular microorganisms. These toxic compounds are incorporated into media to suppress the growth of the background microbiota while allowing for the cultivation of the target microbe. Examples of toxic chemicals are bile salts, azide, sodium lauryl sulfate, and various dyes such as crystal violet and methylene blue (24).

2. Differential Media

The use of certain chemicals, food sources, and reagents can result in a pattern of growth or change in expression that can be used to differentiate between different types of microorganisms (23). An example of this type of media is blood agar plates. Some bacteria can hemolyze the red blood cell, resulting in a clear zone on the agar. These bacteria can easily be distinguished from microorganisms that do not produce hemolysins. In this example, the blood-agar plates serve both as a differential medium and as a source for enrichment.

3. Synthetic Media

A synthetic medium is one that is composed completely of chemically defined nutrients. Most synthetic media contain a mineral base, which provides the inorganic nutrients necessary for microbial growth. This base can then be supplemented, as required, with a carbon source, a nitrogen source, and any required growth factors. These supplements will vary with the nutritional properties of the particular organism being cultured (25).

4. Complex Media

Complex media is one that contains ingredients of unknown chemical composition. An example would be potato dextrose agar, which contains a potato extract of unknown composition. Complex media are useful for the cultivation of a wide range of microorganisms, including those whose precise growth-factor requirements are unknown. Even when the growth-factor requirements of a microorganism have been precisely determined, it is often more convenient to grow that organism in a complex medium, especially if the growth-factor requirements are numerous (25).

5. Liquid Media

Many larger-celled bacteria, protozoa, and algae are not capable of growth on solid media. Often, these microorganisms are easily isolated by the use of liquid media. The simplest procedure of isolation in liquid media is the dilution method. The sample is serially diluted into a sterile medium, with the goal being to inoculate a series of tubes with a microbial suspension so dilute that the probability of introducing even one individual into a given tube is very small. From this, if a tube shows any growth, there is a high probability that the growth resulted from the introduction of a single organism (25).

6. Enrichments

When a mixed microbial population is introduced into a liquid selective medium, competition for nutrients among that population will arise. Liquid enrichment media will select the microorganism of highest growth rate among all the members of microbial population. The resulting growth from enrichment can be greatly modified by variation of

other factors such as temperature, pH, aeration or source of inoculum (25).

7. Commercially Available Agars

Several companies market selective and differential agars targeting common pathogens. These have been used for the rapid screening of pathogens found on food. ALOA™ is a prepared selective and differential medium for the isolation of *Listeria* spp. from foodstuffs and other samples and for the presumptive identification of *Listeria monocytogenes* (26). The selectivity of the medium is due to lithium chloride and the addition of an antimicrobial mixture. The differential activity is due to the presence in the medium of the chromogenic compound X-glucoside as a substrate for the detection of beta-glucosidase enzyme, common to all *Listeria* species. The specificity is obtained by detecting the metabolism of a substrate by an enzyme (phospholipase), which is only present in the *L. monocytogenes* species. The combination of both substrates allows the differentiation of non-*monocytogenes Listeria* spp., which develop blue colonies, from *Listeria monocytogenes*, which develops blue colonies surrounded by an opaque halo.

ASAP™ is a selective medium for the isolation of *Salmonella* from foodstuffs, clinical, and environment samples. The activity of the C8-esterase, which is found in all *Salmonella* species, is detected using a chromogenic substrate. The enzymatic activity of *Salmonella* is visualized by the pink to purple coloration of their colonies.

Biolog, Inc. manufactures several types of differential media such as Rainbow® Agar O157 and Rainbow® Agar *Salmonella*. The selective and chromogenic properties of Rainbow® Agar O157 make it particularly useful for isolating pathogenic *E. coli* strains. The medium contains chromogenic substrates that are specific for two *E. coli*-associated enzymes: β-galactosidase and β-glucuronidase. Rainbow® Agar *Salmonella* utilizes a formulation designed to take advantage of H_2S production common among *Salmonella* spp. Black colonies are formed by even weak H_2S-producing strains. In addition, other compounds increase the recovery rate of *Salmonella* while inhibiting the growth of other microorganisms and inhibiting H_2S production by *Citrobacter* and other H_2S positive species (27).

Chromagenic *Shigella* spp. Plating Medium (CSPM) (28) is a selective medium (bile salts, antibiotic supplementation) that offers an alternative to differentiation methods that are based on lactose fermentation. Instead, differentiation on CSPM is based on proprietary agents consisting of select carbohydrates, pH indicators, and chromagens. *Shigella* spp., which are negative for the select carbohydrates and the chromagens, produce white to clear colonies on CSPM. Colonies of *Enterobacter* spp., *Klebsiella* spp., and *Acenitobacter* spp. are blue on CSPM, while colonies of *Citrobacter* spp. are green.

C. Culturing Methods

Almost all measurements in microbiology are method dependent. When selecting the methods to be used in the laboratory, it is vital to utilize published standard methods. Sources of standard culture methods that are commonly used by food microbiologists include the Official Methods of Analysis of AOAC International, Compendium of Methods for the Microbiological Examination of Foods published by APHA, FDA's Bacteriological Analytical Manual, and USDA FSIS's Microbiological Laboratory Guidebook.

1. AOAC

The Association of Analytical Communities (AOAC) International is a worldwide provider and facilitator in the development and use of validated analytical methods and laboratory quality assurance programs and services. Primarily, the AOAC focuses on the validation of chemical and microbiological analytical methods. AOAC International also acts as the primary source for knowledge exchange, networking, and high-quality laboratory information for its members. In order to establish these goals, AOAC International has three method validation programs: AOAC® Official Methods℠ Program®, Peer-Verified Methods℠ Program, and AOAC® Performance Tested Methods℠ Program. Methods validated by AOAC International are utilized worldwide by various governments and industries for the analysis for a variety of products, especially those related to food, agriculture, public health and safety, and the environment (29).

2. American Public Health Association Standard Methods

The Compendium of Methods for the Microbiological Examination of Foods is widely utilized by food microbiologists as a collection of standard methods for microbiological detection and enumeration techniques. It is published by the American Public Health Association and is a comprehensive and all-inclusive reference for protocols related to specific food products. The Compendium presents information on standard enumeration/detection procedures, sampling plans and analysis preparations, indicator organisms, microbiological aspects of specific food products, and methods related to specific spoilage and safety-related microorganisms.

3. Food and Drug Administration's Bacteriological Analytical Manual

FDA's Bacteriological Analytical Manual or BAM is a compilation of methods and techniques preferred by analysts in the U.S. Food and Drug Administration laboratories for the detection of pathogens in food and cosmetic

products. Methods included in the BAM have been analyzed and peer reviewed by FDA scientists and other researchers (30). BAM methods have been organized into an accessible spreadsheet format and are complimentary in the hopes of increasing awareness of microbial safety of food and water (31). These methods are available on line at http://www.cfsan.fda.gov/~ebam/bam-mm.html.

4. United States Department of Agriculture, Food Safety Inspection Service's Microbiology Laboratory Guidebook

The Microbiology Laboratory Guidebook (MLG) is a manual created by the USDA agency FSIS to aid in the microbiological analysis of meat, poultry, and egg products. The MLG contains techniques that FSIS prefers to employ for the analysis of these foods. Because USDA does not endorse or approve these techniques for use in the food industry, inclusion of a specific method in the MLG should not be interpreted in this manner (32). These methods are available on line at http://www.fsis.usda.gov/ophs/microlab/mlgbook.htm.

VII. ENUMERATION OF VIABLE CELLS

Standard plate counts, Most Probable Number (MPN), membrane filtration, plate loop methods, and spiral plating are techniques that allow for the estimation of viable cells. These methods can be successfully utilized in the food industry to enumerate fermentation, spoilage, pathogenic, and indicator organisms (6).

Several pathogens of interest such as *Salmonella*, *Campylobacter* spp., pathogenic *E. coli* and *Vibrio* spp. are not capable of sporulation; however, they may exist in a viable but non-culturable (VNC) state in which they cannot be cultured using normal microbiological techniques (4). VNC cells are typically severely injured and incapable of reproduction under standard protocols. Pathogenic *Vibrio parahaemolyticus* is known to demonstrate a VNC state when subjected to low incubation temperatures and starvation. Previously, it was unknown whether this microorganism was capable of resuscitation or if regrowth of a few remaining culturable cells occurred. Recent studies have shown that the VNC cells are capable of being resuscitated after plating onto agar containing H_2O_2-degrading compounds such as catalase or sodium pyruvate (33). It is unknown whether VNC pathogens are capable of causing illness, but there is concern that this physiological state could result in disease outbreaks from foods that have yielded false negative detection results for specific pathogens.

A. Direct Enumeration

The direct microscopic count or DMC is utilized to obtain a gross estimation of cell density that includes both viable and nonviable cells. DMC is most efficient when there are a large number of microorganisms in the sample (greater than 10^5 CFU/mL). Due to the fact that DMC cannot differentiate between live and dead cells and requires a large number of cells in the test sample, its use for food analyses is limited for quality issues in which a gross, highly variable enumeration is adequate (4).

B. Indirect Enumeration

Unlike direct enumeration of microbes by microscopy, indirect enumeration can be accomplished by plate counts or statistical estimation. The plate count technique involves spreading a sample on a nutrient agar surface or incorporation of the sample within the agar. If organisms are present and if they are plated on a suitable medium, each will grow a viable unit called a colony. Each colony-forming unit (CFU) can be counted and thus theoretically relates to the viable number of microorganisms in the sample. Statistical estimations, such as the Most Probable Number assay, are useful when the microorganisms in question need an enriched environment to grow or are in very low numbers.

1. Plate Counts

Aerobic plate counts (APC) indicate the level of microorganisms in a product. Aerobic plate counts generally do not relate to food safety hazards, but sometimes can be useful to indicate quality, shelflife and post-heat-processing contamination. The plating medium used in an APC can affect the number and types of bacteria isolated because of the variation in nutrient and salt requirements of various microorganisms (34).

The commercially available 3M Petrifilm™ plate decreases labor by eliminating the need for media preparation. Another benefit of this product is it delivers consistent easy-to-read results, creating a lesser chance for error than with conventional agar methods. There are numerous formulations that allow for several common types of microbial testing, including coliform, aerobic, *E. coli*, *Staphylococcus aureus,* and yeast and mold counts. 3M Petrifilm™ plates enable food processors to test products and equipment easily, which can assist in rapid detection and resolution of problem areas. These sample-ready microbial testing products reduce the possibility of human error in test preparation to produce consistent results (35). They have been particularly useful in field application due to the reduced need for incubator space. One disadvantage of Petrifilm™ is that samples with numerous particles may be difficult to read.

2. Most Probable Number (MPN)

The Most Probable Number or MPN is a broth dilution technique that is especially useful when establishing low

concentrations of microrganisms, specifically less than 100 per gram. The method as described in the classic paper by Oblinger and Koburger (36) has several advantages over other enumeration procedures. One advantage is that the sample is prepared to ensure that bacteria are distributed randomly and do not clump together thereby giving an equal opportunity for any one sample to contain viable cells if they are present in the food. Also, culture medium and incubation conditions are carefully chosen to encourage even one viable organism to generate detectable growth. The amount of food sample that produces growth at each dilution will suggest an estimate of the original, undiluted concentration of bacteria in the sample (30).

Isogrid is a hydrophobic grid membrane filter method utilized for the detection and quantification of target microorganisms such as *Salmonella* spp., *Listeria* spp., total *E. coli* and *E. coli* O157:H7, coliforms, *S. aureus*, yeast and mold, and total bacteria count. A sample's total bacterial count is enumerated through a membrane filter containing a grid of 1,600 squares. Diluted samples are filtered through a 5 μm stainless steel prefilter to eliminate any food particles. Samples are then filtered through a hydrophobic membrane, and the membrane is placed on Tryptic Soy Fast Green Agar (TSFGA). TSFGA is specially formulated to provide a total bacterial count. After incubation the membrane filter is examined, and all squares containing one or more green or blue colonies are counted. The total number of positive squares is converted to the corresponding Most Probable Number (MPN) determined by the Isogrid manual (37). As with Petrifilm™, samples that contain large numbers of particles may obscure the results. This system has been used for milk, meat, black pepper, flour, mushrooms, seafood, and oysters (38–41).

The SimPlate™ system includes specific formulations of media and a patented plating device. The SimPlate™ device has a broad counting range to minimize the number of dilutions needed for accurate counts. This unique combination of media and plating device offers advantages over some other techniques, including greater accuracy, ease of use, and faster time to obtain results. SimPlate™ tests are available for the quantification of total plate counts (TPC), total coliforms and *E. coli*, yeast and mold, and *Campylobacter*. Another benefit of the SimPlate™ is that positive and negative results can be easily distinguished by simply counting the number of positive wells and referring to the SimPlate™ Conversion table provided with the kit. SimPlate™ results are usually available in 24 hours, with the exception of *Campylobacter* and yeast and mold, which takes 48–72 hours, still days faster than other methods. SimPlate™ media comes pre-measured and ready to hydrate for single or multiple tests. While agar plate and film counting ranges may be limited to 300 CFU or less, SimPlate™ devices are available in two sizes with maximum counting ranges of 738 or 1659 CFU per plate. The number of dilutions and reruns are minimized, saving labor, time, and material costs (42).

VIII. METABOLIC ACTIVITY MEASUREMENT

Dye reduction tests, acid production, electrical impedance, and batteries of other metabolic assays are all used to determine cell population sizes and to identify specific isolates. The level of bacterial activity can be used to assess the quality and freshness of food products. Toxin levels can also be measured to indicate the presence of toxin producing pathogens (6). Measurable undesirable metabolites produced from the action of specific microorganisms on certain food products include histamine in canned tuna, lactic acid in canned vegetables, cadaverine and putrescine in vacuum-packaged beef, and mycotoxins in various cereal and fruit based products (4). Specific substrate utilization can be used to determine the type of microorganism in question. The following sections will discuss common tests used for this purpose.

The AOAC has developed a comprehensive list of test kits that are available to measure or detect specific chemicals or microorganisms commonly referred to as analytes. The performance tested certified kits have been extensively reviewed by the AOAC Research Institute, a subsidiary of the AOAC International. All performance tested certified kits have been evaluated for accuracy, precision, detection limits, false positive/negative rates, stability and compared to an already existing method. There are several test kit analyte categories consisting of potential biological and chemical agents, microbiological, antibiotic, toxin, hormones, chemical, biochemical, and genetically modified organisms. The biochemical test kits include analytes such as ATP, sugars, enzymes, DNA, and many others (43).

A. VITEK

VITEK by bioMérieux is an automated instrument that offers rapid results within 2 to 6 hours, random or batch processing, and a quality control module. This instrument is used for bacterial and yeast identification, antimicrobial susceptibility testing, and urine screening. Since the VITEK is an automated system, this allows for greater safety and eliminates repetitive manual operations to quicken response time and provide faster results (44).

B. API Strips

Another reliable product used for the rapid identification of microbes is API Strips, which generally contain 20 miniature biochemical tests and databases. To date, there are 16 identification products covering most bacterial groups and more than 550 different species. There are test

kits to identify Gram-positive and negative bacteria and also yeasts. API Strips are economical when compared to the cost of preparing numerous biochemical media, are generally user-friendly, and have a long shelf-life (45).

C. BBL Crystal

There are many different BBL Crystal miniature identification kits available. Those utilized most for the identification of microorganisms are the Gram-Positive ID Kit, Rapid Gram-Positive ID Kit, and the Anaerobe ID Kit. The Gram-Positive ID kit is the most comprehensive Gram-Positive ID System available. Both the Gram-Positive ID System and the Rapid Gram-Positive ID System utilize modified conventional, fluorogenic, and chromogenic substrates. These kits are capable of identifying 121 Gram-positive bacteria including cocci and bacilli. The Gram-Positive ID System can be completed in 18 hours while the Rapid Gram-Positive ID System can be completed in 4 hours. The Anaerobe ID kit is a miniaturized 4 hour identification method for clinically significant anaerobes (46).

D. Impedance

Impedance is described as the resistance to the flow of a sinusoidal alternating current through a conducting material. Detection of cells by electrical impedance is determined from changes in the growth medium associated with the increase in biomass of metabolically active microorganisms. The components of growth media such as proteins, carbohydrates, and lipids are uncharged or weakly charged substrates. As microorganisms metabolize these compounds, they are converted into more highly charged molecules such as amino acids, lactate, and acetate. Consequently, these metabolic products increase the conductivity of the growth medium. Because this increase is small, a microbial level of at least 10^6 CFU/mL must be attained before significant changes can be observed. There are also disadvantages to electrical impedance method. The most critical disadvantage is that this method cannot function under high salt concentrations that are often found in particular growth media (5). In a recent study to determine the efficiency of a modified impedance method, numerous samples of ground beef were inoculated with 12 different serotypes of *Salmonella*. The method proved to be a reliable way for rapid detection of different *Salmonella* serotypes in fresh meat (47).

There are several commercial systems available that utilize a color system to monitor levels of contamination. Red generally depicts samples that are "highly contaminated"; yellow signifies "caution zones" and green signifies "acceptable" levels. Common impedance-monitoring devices are the Bactometer, the RABIT, the Malthus and the BacTrac (5).

The Bactometer by bioMérieux can be utilized to test raw materials and finished products. It is used to make important quality control decisions quickly, prevents unnecessary delays in production, reduces testing costs, and helps protect companies' reputations. The Bactometer consists of a processing unit (BPU), computer, and printer. The Bactometer analyzes samples that are simultaneously incubated and read by the BPU every six minutes. The computer continuously monitors operations and interprets the results. The terminal allows all results to be displayed quickly at any time. The Bactometer can be used to assay for total microbial counts, coliforms, yeast and mold, lactic acid bacteria, shelf-life testing, and environmental monitoring. Typical testing times can be reduced to less than 48 hours while conventional methods can take up to 5 days. Additional benefits associated with the Bactometer are a 512 sample capacity, performing different tests simultaneously, and working with a wide temperature range, all of which increase laboratory efficiency (50).

E. BioSys

The BioSys system is a computerized instrument designed to rapidly detect microbial contamination in industrial samples. It can be applied to foods, beverages, dairy, wine, cosmetics, toiletries, and nutraceuticals (48,49). The system has applications for the detection of the presence of various groups of microorganisms in food samples or swabs (e.g., Total Viable Count, Enterobacteriaceae, Coliforms, Yeast, Lactic Acid Bacteria, *E. coli*, etc.). Other tests performed by the system are the detection of spoilage microorganisms, shelflife assessment, microbial limits and preservative challenge test, and environmental sponges and swabs for the presence of certain organisms such as *Listeria* spp.

BioSys system uses vials consisting of a nutrient broth with an agar plug at the bottom. The system measures microbial growth by monitoring changes in pH or other biochemical reactions, thereby resulting in a color change as microorganisms grow and metabolize. Color changes in the agar mirror the color change in the broth, without letting the sample particles or turbidity influence the measurements.

Light from light emitting diodes passes though the agar and a photo diode on the other side of the vial reads the color change as microbial growth occurs. A measurement is taken every six minutes. As soon as a color change is detected, the time of such detection is recorded. Detection times (DT) are inversely related to the number of organisms in the sample.

F. Bioluminescence

Bioluminescence is defined as the production of light by living organisms. Common bioluminescent organisms

include certain fungi and bacteria that continuously emit light. The production of bioluminescent light is formed as a result of the conversion of chemical energy to light energy (51). Adenosine triphosphate (ATP) is the main source of energy in all living organisms. Two hours after cell death ATP completely disappears. One of the simplest techniques that measures ATP is the firefly luciferin-luciferase test. When ATP is present, luciferase produces light that is measured with a liquid scintillation spectrometer or a luminometer. The amount of light that is produced by the luciferase is directly proportional to the amount of ATP. The luciferase method is an example of a rapid and sensitive test that indicates the presence of bacterial cells (3).

A rapid system has been developed using bioluminescence to detect bacteria with minimal cultivation. Food samples are cultivated to produce a sufficient amount of ATP and are sprayed with luminescent reagents. Bioluminescent cells appear as spots to indicate the presence of bacteria in the sample. This method of detection is fast and is useful in hygiene testing for food, beverages, and water (52). In a recent study, ground beef containing *E. coli* O157:H7 was analyzed using a luciferin-luciferase assay. It was determined that in the presence of glucose, the cellular ATP of the *E. coli* increased causing significant luciferin-luciferase bioluminescence in beef hamburger. This method, coupled with an immunomagnetic capture technique allowed for rapid detection of viable *E. coli* O157:H7 cells in certain food samples (53).

G. ATP Test Kits

There are many different ATP test kits available that are used as a measure of total hygiene after sanitation or are used to detect presence/absence of microorganisms in samples. Bev-Trace™ is an example of an ATP test specially designed for the rapid detection of microbial contamination in beverages, such as beer, wine, and soft drinks (54). Results from ATP tests can be obtained faster than traditional techniques, saving time and money. The luminometers that are commonly used with this type of test are usually lightweight, easy to operate, and might be able to provide printed results. Some key features of these tests are earlier detection of contamination, decreased chance of recall, brand protection, and reduced stock holding requirements.

Another type of ATP test kit is used as a sanitation check on equipment surfaces. An example is the PocketSwab (55). These types of test kits are generally self contained, single service hygiene tests that use the firefly luciferin-luciferase enzyme system to emit light when unclean surfaces are detected. Tests are capable of verifying within intervals as short as 30 seconds if a surface is clean enough for production. ATP from food residues, microorganisms, biofilms, and human contact are detected to provide a quick check for cleaning effectiveness that enhances overall food safety.

IX. OTHER DEVELOPMENTS IN MICROBIAL TESTING

Rapid methods for microbiological analysis of foods also include improvements in sample preparation. One of the more useful instruments developed for sample preparation is the Seward/Tekmar Stomacher™ (Norfolk, UK) and similar devices (56). After transferring a food sample to a sterile plastic bag that contains appropriate diluents, the sample is placed within the stomacher where two paddles massage the bag for 1 to 5 minutes or longer using alternate strokes. The massage action simulates activity of the human stomach to mix the sample and dislodge microorganisms for further microbiological analysis, such as plate counting of viable cells.

Conventional viable cell count, or standard plate count, is tedious and time consuming. As a result, alternative methods to enumerate colony forming units within a food sample have been developed. One particular instrument, the spiral plater has gained wide acceptance and has become an essential element in many food microbiology laboratories (56). This instrument spreads a liquid sample onto an agar surface in an Archimedes spiral pattern. The sample is applied such that there is a concentration gradient starting from the center and decreasing as the spiral progresses outward on the plate. After the liquid containing the microorganism is spread, the plate is incubated to allow colonies to develop. The colonies are then counted either manually or electronically. The sample gradient is spread across the agar surface in such a manner that the equivalent of three standard dilutions can be read from one plate. This substantially decreases sample preparation and plating time and reduces supplies needed to enumerate microorganisms in a sample.

Major advancements in spiral platers include automation and plate readers. Automated spiral platers require little more than the presentation of a liquid sample followed by the press of a button. This activates the plater to apply an appropriate size sample to an agar surface after which the instrument goes through a clean and sanitation cycle. Despite the benefit of automation, there have been complications involving clogging of the dispensing stylus by large food particles. This problem was largely eliminated with the use of sterile sample preparation bags with filters that separate large food particles from the liquid sample (56).

Automated plate readers are designed to allow real-time enumeration of colonies on a plate. Scanners originally used with the early spiral platers were based on laser technology. Automatic readers today often utilize camera imaging coupled with sophisticated software to detect and count colonies. These readers can be utilized with any type of plating method; pour, spread, or spiral.

X. SUMMARY

No review can completely cover all the methods currently available, especially when considering that products continuously enter and leave the marketplace. It is critically important for safety and quality reasons to conduct microbiological analyses of food samples. Some microorganisms that are occasionally found in food products can cause illness or death, while other microorganisms produce spoilage and economic losses. Numerous variables are considered during the selection of a method to detect or enumerate microorganisms. The analyst must decide if a qualitative or quantitative test is required, and which microorganisms are of importance for the sample. If regulatory issues are involved, an analyst might want to consider the type of sampling plan used, and other factors such as speed, specificity, cost, and ease of use. Relatively rapid methods include direct microscopic observations, ATP assays, and test kits to detect or enumerate specific microorganisms or groups of microorganisms. Conventional methods of enumeration (plate counts or MPNs) generally consume more time and supplies than rapid methods, but are often less expensive and can yield more accurate data.

XI. NON-ENDORSEMENT OF COMMERCIAL PRODUCTS AND SERVICES

References, hypertext links and images to all products and services are provided for information only and do not constitute endorsement or warranty, express or implied, by the authors and/or their employers or the publishers of this work, as to their suitability, content, usefulness, functioning, completeness, or accuracy.

ACKNOWLEDGMENT

This is Florida Agricultural Experiment Station Journal Series number R-09807.

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186 Immuno-Based Methods for the Detection of Bacterial Pathogens

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CONTENTS

I. INTRODUCTION

According to the Centers for Disease Control and Prevention (CDC), there are over 250 known different foodborne diseases (1). These diseases are caused by bacteria, viruses, chemicals, toxins, and fungi. In the United States, where the food supply is one of the safest in the world, the number of food related illnesses is estimated to result in 76 million sick individuals, and nearly 5,000 deaths yearly. Many of these pathogens, such as *Campylobacter jejuni*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*, were not recognized as major causes of foodborne illness until recently (2).

One of the main reasons for the emergence of foodborne pathogens is the increased complexity of food products and processes. Each year, hundreds of thousands of new food products are introduced into the market place, and as a result different and more complex food matrices are produced. Other reasons that foodborne disease outbreaks appear to be increasing are: increasing consumer demand for fresh, unprocessed, and minimally processed foods that are inherently less safe than pasteurized or processed counterparts; public health officials have established national surveillance programs that are more sensitive at outbreak detection; there are increasing imports of foods from countries that may not have programs that would decrease contamination levels; and, innovative packaging and processing techniques may allow for much longer shelflife of sensitive foods, thereby allowing a very small pathogen population to proliferate to infective levels. Despite efforts by the government and the food industry to curtail illnesses, such as through the

use of programs like Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practices (GMPs) there is still a need for rapid and efficient microbiological testing.

There have been many improvements in the last few decades in both the conventional and newer microbiological techniques. Driven by food safety issues and economics, advanced pathogen detection and identification protocols make use of rapid and automated microbiological analyses. This includes the use of microbiological, biochemical, immunological, and serological methods for improving the isolation, early detection, characterization, and enumeration of microorganisms and their products. Whether these methods were originally developed for a clinical, industrial, or environmental setting, such innovative technology has been adopted by the food industry over the past decade.

II. IMMUNOLOGICAL METHODS

Immunological methods rely on the specific binding of an antibody to an antigen. An antigen is a substance that is capable of eliciting the production of antibodies in a living organism (the host). Figure 186.1 illustrates the Y-shaped structure of an antibody, with the antigen binding sites on the arms of the Y structure. It is these binding sites that account for the specificity of the antibody, particularly the regions termed the light and heavy chains. The suitability of the antibodies for food application toward a particular microbiological target depends on their specificity, including whether they are monoclonal or polyclonal. Polyclonal antibodies contain an assortment of antibodies, each with different specificities for specific antigens. Monoclonal antibodies react with only one antigen. Improvements in monoclonal antibody production have led to better supplies of these potentially powerful diagnostic tools (3). Typically, monoclonal kits are portrayed as having less variability than polyclonal-based kits.

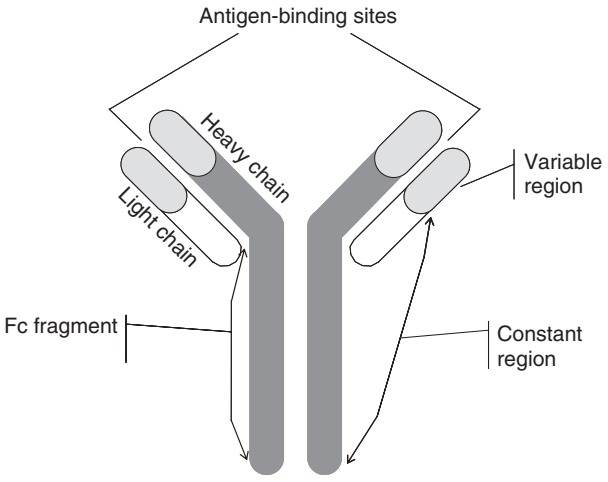

FIGURE 186.1 Antibody Structure. The immunoglobulin molecule consists of two identical light and heavy chains. Binding occurs at the variable regions.

Commonly used immunological techniques include enzyme-linked immunosorbent assay (ELISA) (sometimes referred to commercially as an enzyme immunoassay, or EIA), immunomagnetic separation (IMS), latex agglutination, precipitation assays, immunostaining, biosensors and automated systems. Numerous commercial products to simplify immunodiagnostics are based on these detection systems.

III. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) METHODS

In an enzyme-linked immunosorbent assay (ELISA), an enzyme is used as a label on an antigen or antibody, which will then bind to the antigen (or antibody) of interest (the analyte). After binding, the enzyme portion can be assayed, which allows for the detection of an immune reaction and the estimation of the analyte (4).

Occasionally, the term enzyme immunoassay (EIA) will be encountered, rather than the term ELISA. EIA is a nonspecific term used to refer to all ELISA-like assays, including those designed to detect nucleic acids as well as antigens or antibodies. Thus, the terms EIA and ELISA are oftentimes used interchangeably.

There are two general forms of enzyme immunoassays, the heterogeneous and the homogeneous, typically differentiated by the use of an incubation period and a wash step. The most commonly used form, and the one most often associated with the term ELISA, is the heterogeneous enzyme immunoassay method. In heterogeneous ELISA, the antibody or antigen is bound either covalently or noncovalently to the solid matrix. The unreacted antigen or antibody in the heterogeneous method is removed by washing or centrifugation. Unlike the heterogeneous assay, the homogeneous ELISA has no separation of the immune complex and the free reactants via a wash step.

Heterogeneous ELISA can be simple or complex. In simple ELISA, there is just binding of the labeled antibody to the antigen, followed by a detection step. The more complex sandwich ELISA, in which a primary antibody is "sandwiched" between a bound antigen and a second labeled antibody, is commonly used to detect bacterial antigens in foods. Figure 186.2 is a schematic diagram of the sandwich ELISA and the components involved in the assay. A list of ELISA test kit manufacturers can be seen in Table 186.1.

A. Microtiter Plate Format

These assays are usually performed on plastic microtiter plates. These are trays containing a fixed number of wells,

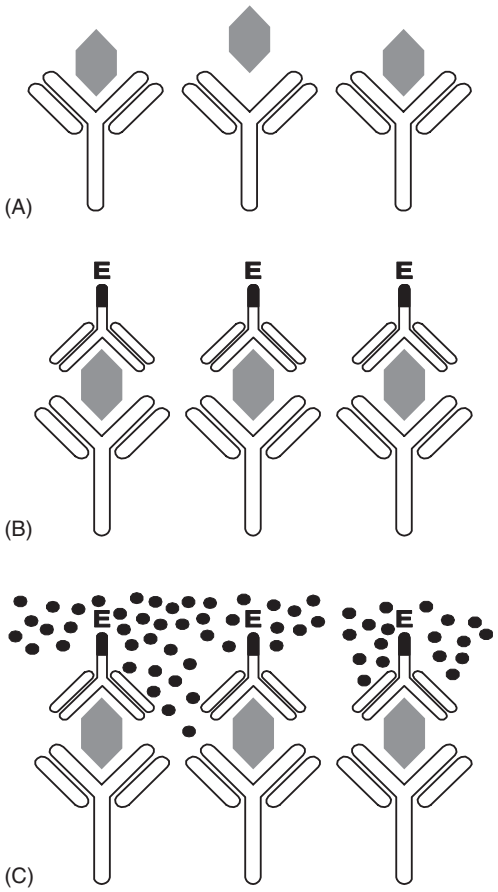

FIGURE 186.2 The double antibody sandwich methods for the detection of specific antibodies. A) Antibody specific for target antigen(s) is bonded to surface (e.g., plate, bead, paper, etc.). Sample is added and antigen binds with antibody. B) Enzyme-linked antibody specific for the target antigen(s) is added and binds to antigen. C) Enzyme substrate is added and reaction produces a visible color change. (Reprinted by permission from Tecra Diagnostic, Ltd.)

typically 24, 48, or 96. When the antibody (or antigen) is added to the well, it then binds to the inside surface of the well. This binding is due to the hydrophobic interaction between the hydrophobic residues on the protein and the nonpolar plastic surface. Once it is bound, the protein cannot be easily washed from the surface of the well. After application of the sample and subsequent binding of labeled antigen or antibody, the response, either enzymatic or isotopic, is then read via the plate reader.

One commercially available ELISA, manufactured by BioControl Systems, Inc. (Bellevue, WA) uses a mixture of monoclonal antibodies specific for *Salmonella* detection (BioControl). Another manufacturer, TECRA Diagnostic Ltd. (NSW, Australia) uses polyclonal antibodies rather than monoclonal antibodies for the detection of pathogens (5,6). Figure 186.3 shows an example of a standard ELISA test kit and Table 186.1 gives a list of the various manufacturers and/or distributors of ELISA based detection systems.

TABLE 186.1
Partial List of Commercially Available, ELISA-Based Assays for the Detection of Foodborne Pathogens

Organism/Toxin	Trade Name	Assay Format[a]	Manufacturer
Campylobacter	VIDAS	ELFA[b]	bioMérieux
	ALERT	ELISA	Neogen
	VIA	ELISA	TECRA
	Assurance Gold EIA[d]	ELISA	BioControl
	Transia Plate	ELISA	Transia
EHEC[c] O157:H7	Assurance EIA[d]	ELISA	BioControl
	VIA[d]	ELISA	TECRA
	ALERT	ELISA	Neogen
	Transia Plate	ELISA	Transia
	VIDAS	ELFA[b]	bioMérieux
Listeria	VIA[d]	ELISA	TECRA
	Assurance EIA[d]	ELISA	BioControl
	Transia Plate	ELISA	Transia
	ListerTest	ELISA	Vicam
	Pathalert	ELISA	Merck
	Listeria-TEK[d]	ELISA	bioMérieux
	VIDAS[d]	ELFA[b]	bioMérieux
	VIDAS (monocytogenes II)	ELFA[b]	bioMérieux
Pseudomonas	VIA	ELISA	Tecra
Salmonella	VIA[d]	ELISA	TECRA
	ULTIMA	ELISA	TECRA
	ALERT	ELISA	Neogen
	Assurance EIA[d]	ELISA	BioControl
	Assurance Gold EIA[d]	ELISA	BioControl
	Transia	ELISA	Transia
	Bioline	ELISA	Bioline
	VIDAS[d]	ELFA[b]	bioMérieux
	Salmonella-TEK[d]	ELISA	bioMérieux
Staphylococcus aureus	VIA	ELISA	TECRA

[a] Abbreviations: ELISA, enzyme linked immunosorbent assay; ELFA, enzyme linked fluorescent assay.
[b] Automated System.
[c] EHEC - Enterohemorrhagic *E. coli*.
[d] Adopted AOAC Official First or Final Action.
This table has been adapted from the FDA Bacterial Analytical Manual.

IV. IMMUNOPRECIPITATION

Immunoprecipitation, also called immunochromatography, is another method that is antibody-based. These assays use the technology originally developed for home pregnancy tests. It utilizes the same "sandwich" technology described in the section on ELISA assays. The main

FIGURE 186.3 Typical ELISA kits for the detection of a bacterial pathogen. In addition to detecting pathogens such as *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*, some test kits will test for the presences of bacterial toxins (e.g., *Staphylococcus aureus*). (Reprinted by permission from Tecra Diagnostic, Ltd.)

difference between the two systems is that instead of enzyme conjugates, the detection antibody is bound to latex beads or to colloidal gold, which produces a color change. The sample is placed on the device and is wicked across media. The antibodies for the specific components in a sample are then bound to an antibody. It is this binding of the antibody/antigen complex which results in a visibly detectable line known as a precipitation band (7).

A. Lateral Flow Devices

These assays combine the recognition ability of an immunoassay with the separation power of chromatography. In the lateral flow device (LFD) an extracted sample is introduced onto one end of a membrane strip, usually encased in a plastic holder. The sample is drawn through to a reagent zone containing labeled antibodies specific for the target analyte. A positive reaction occurs when the analyte in the sample extract combines with the labeled antibody where it stops at a line of anchored antibodies. This zone also has antibodies specific for the target analyte. Here they form an antibody-antigen-antibody "sandwich" that is visualized as a line. A second "control" zone also wicks from the reagent zone and forms a second line further along the device (Figure 186.4). One benefit of this class of tests is that they can be read visually. Strategic Diagnostics, Inc. (Newark, DE) has developed rapid screening tests for *E. coli* O157 (including O157:H7) and for *Salmonella* via their RapidChek™ kits. The RapidChek™ *E. coli* O157 system has been approved by the AOAC for use in ground beef, boneless beef, and apple cider (7). Neogen Corporation (Lansing, MI) has also developed commercially available lateral flow devices for the detection of *Salmonella*, *Salmonella enteritidis, E. coli*

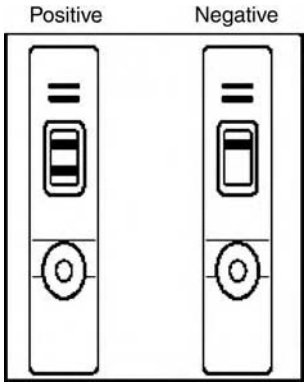

FIGURE 186.4 The RapidChek™ Lateral Flow Assay is an immunoassay which employs a combination of anti-pathogen antibodies and colloidal gold conjugate coated on the surface of a membrane encased within a plastic cassette. (Reprinted by permission from Strategic Diagnostics, Inc.)

O157:H7, as well as *Listeria* in their line of Reveal® kits (8,9). BioControl Systems, Inc. (Bellevue, WA) produces the VIP® test kit for *Salmonella*, *Listeria*, and *E. coli* O157:H7 (10). These as well as other commercially available lateral flow kits are listed in Table 186.2.

B. Dip Stick Assays

Dip stick assays, such as Tecra Diagnostics Ltd.'s *E. coli* 0157 Immunocapture and UNIQUE™ test kits, offer yet another iteration of the ELISA methodology. These assays use an antibody-coated dipstick to capture target antigens from an enriched sample. All steps take place within a self-contained module that contains all necessary reagents and eliminates media preparation (11). These and other commercially available dip stick assays are listed in Table 186.2.

C. Immunodiffusion

BioControl Systems, in addition to its VIP™ line of immunoprecipitation tests also produces the 1–2 test used for detection of motile *Salmonella* in food. The method, sometimes referred to as an immunoimmobilization assay (12), utilizes a unique two-chamber unit. One unit contains the enrichment media while the other is used for the immunoimmobilization process. Anti-*Salmonella* antibodies are added to one chamber as the motile *Salmonella* migrate in the other. At the point where the diffusing antibodies contact the advancing microorganisms, a visual "immunoband" is formed when a positive result is present.

V. IMMUNOMAGNETIC SEPARATION

Immunomagnetic separation is the selective concentration of a target organism using antibody-coated magnetic beads or other devices to selectively trap the target

TABLE 186.2
Partial List of Immuno-Based (Non-ELISA) Assays for the Detection of Foodborne Pathogens

Organism/Toxin	Trade Name	Assay Format[a]	Manufacturer
Campylobacter	Campyslide	LA	Becton Dickinson
	Latex	LA	Microgen
	Campylobacter	LA	Oxoid
EHEC[c] O157:H7	Prolex	LA	PRO-LAB
	Wellcolex O157	LA	Murex
	VIP[e]	LFD	BioControl
	Reveal	LFD	Neogen
	Immunocapture™	DS	Tecra
	Reveal	LFD	Neogen
	Transia Card	LFD	Transia
	Dry Spot	LA	Oxoid
	Dynabeads	Ab-beads	Dynal
Listeria	Latex	LA	Microgen
	Listertest	Ab-beads	VICAM
	Dynabeads	Ab-beads	Dynal
	VIP[e]	LFD	BioControl
	Reveal	LFD	Neogen
	UNIQUE™	DS	Tecra
	UNIQUE PLUS™	DS[b]	Tecra
Salmonella	Immunocapture	DS	Tecra
	UNIQUE™	DS	Tecra
	UNIQUE PLUS™	DS[b]	Tecra
	VIP[e]	LFD	BioControl
	Reveal	LFD	Neogen
	Reveal (*enteritidis*)	LFD	Neogen
	Capture-TEK	Ab-beads	bioMérieux
	Transia Card	LFD	Transia
	Latex	LA	Microgen
	Salmonella Latex	LA	Oxoid
	Wellcolex	LA	Murex
	Dynabeads	Ab-beads	Dynal
	Screen/Verify	Ab-beads	VICAM
	Screen/SE Verify	Ab-beads	VICAM
	1–2 Test[d]	Diffusion	BioControl
	UNIQUE PLUS™	DS[b]	Tecra
Shigella	Wellcolex	LA	Murex
Staphylococcus aureus	Staphyloslide	LA	Becton Dickinson
	Staphaurex	LA	Remel
	Staph Latex	LA	Wampole Labs
	Dry Spot	LA	Oxoid
	Prolex	LA	PRO-LAB

[a] Abbreviations: RPLA, reverse passive latex agglutination; LA, latex agglutination; ab-beads, immunomagnetic; Ab-ppt, immunoprecipitation; DS, dip stick; LFD, lateral flow device.

[b] Automated System.

[c] EHEC - Enterohemorrhagic *E. coli*.

[d] Adopted AOAC Official First or Final Action.

This table has been adapted from the FDA Bacterial Analytical Manual.

microorganism (13). The beads are typically uniform polymeric particles coated with a polystyrene shell, providing a smooth hydrophobic surface (15). This allows for the facilitated absorption of the immunoglobulin molecules. It can be effectively used to reduce background flora and eliminate interfering food particles, but is sometimes labor intensive and therefore not well suited for high-volume users. Both VICAM (Watertown, MA)

and Dynal Biotech (Oslo, Norway) have successfully developed magnetic beads coated with various antibodies for the detection of specific pathogens, such as *Salmonella* (*Salmonella* Screen/*Salmonella* Verify™) (15) and enteropathogenic *E. coli* (Dynabeads®) (16). VICAM also produces beads for *Listeria* detection under the name of ListerTest®. Immunomagnetic bead separation technology can rule out the possible presence of pathogens in less than 24 hours, with similar or better sensitivities as conventional methods. Upon use of this method, further microbiological procedures, such as direct plating or ELISA tests, can be performed on the charged beads. This is quite useful for foods with very low numbers of target pathogens such as *Listeria* and *Salmonella* (5). Magnetic bead separation has been used for detection of pathogens in many food and environmental matrices, such as *Cryptosporidium* species in water samples, *Bacillus* spores in food and environmental samples, and *Staphylococcus* species in milk (17–20). Commercially available immunomagnetic kits are listed in Table 186.2.

VI. LATEX AGGLUTINATION

Another relatively fast and simple immunological method for food pathogen detection involves latex agglutination. This method, sometimes referred to as the slide test, involves the reaction of latex particles coated with a specific antibody to the corresponding pathogenic antigen (21). A positive test yields agglutination, or visible clumping of the test reagents, while a negative control is indicated by the absence of clumped particles. Latex agglutination has been developed for many foodborne pathogens as well as their toxins; commercial products are available for *Salmonella*, pathogenic *E. coli*, *Staphylococcus aureus*, and *Clostridium.* These test kits have similar or better sensitivities than conventional or ELISA methods (21–25). Oxoid Ltd. (Scotland) produces various commercial latex agglutination kits for microorganisms such as *Bacillus cereus* (BCET-RPLA), *E. coli* and *Vibrio cholerae* (VET-RPLA). Other latex agglutination products that are readily available can be found in Table 186.2.

VII. IMMUNOSTAINING

Immunostaining, also known as immunoblotting, is the transfer of antigenic material from one surface onto a nitrocellulose membrane, and is often used in conjunction with ELISA. Once antigens are transferred to the membrane, antibodies specific to the antigen are added. The resulting membrane containing the antigens and antibodies is then assayed as with the ELISA method (12).

Few commercial immunostaining kits are currently in use for food pathogen detection. 3M Corporation had developed a system for the detection of *E. coli* O157:H7, which involved the inoculation of a Petrifilm™ Test Kit-HEC plate. If the target bacteria were present, colonies would form on the plate. Colony antigens were then transferred from the plate to a reactive disc. If the *E. coli* O157:H7 antigens were present, they would be transferred to the disc and would capture the enzyme-labeled antibody, which was added later. The antibody-antigen complex was then visually detected via the presence of a black spot on the plate. This system is no longer available and is only mentioned to provide historical background, although this technique is still used as a research tool for the detection of bacteria in food matrices (12).

VIII. BIOSENSORS

A biosensor is a compact analytical device incorporating a biological or biologically derived sensing element. These can include enzymes, antibodies, or DNA, either integrated within or associated with a physiochemical transducer (27). The biological compounds can be used to detect changes in the environment, such as the presence of microorganisms, and can vary from simple temperature sensitive paint, to very complex DNA-RNA probes (28). Biosensors provide a means for production of very sensitive, miniaturized systems that can be used to detect microbial activity or the presence of biological compounds.

There are two commonly used definitions for the term biosensors. The first definition refers to any device or instrument consisting of a biological sensing element combined or attached to a transducer (29,30). Thus enzymes, antibodies, cells, DNA, and tissues are considered the sensing element while typical transducers for these molecules can consist of electrochemical, calorimetric, optical, acoustical, or even mechanical means. The second definition for biosensors refers to a self-contained analytical system that is capable of responding both directly and selectively to biological species (29,30).

Enzymes are the most commonly used biological elements. Biosensors using microorganisms are called microbial biosensors and they exploit the metabolic functions of living microorganisms to effect detection and measurements of analytes (31). Immunosensors are biosensors that use antigens or antibodies as sensing elements, and are analytical devices based on the affinity and specificity of the antigen-antibody reaction.

At Georgia Institute of Technology, a biosensor has been developed that operates with three primary components – integrated optics, immunoassay techniques, and surface chemistry tests (32). It indirectly detects pathogens by combining immunoassays with a chemical sensing scheme. In the immunoassay, a series of antibodies selectively recognizes the target bacteria. An antibody, termed the "capture antibody" is bound to the biosensor and captures the target bacterium as it passes nearby. Then a set of "reporter" antibodies, which bind with the same target pathogen, contain the enzyme (urease), which breaks down

urea that is then added and produces ammonia. The chemical sensor detects the ammonia, affecting the optical properties of the sensor and signaling changes in transmitted laser light.

Biosensor development has been accelerated by improvements in materials research and miniaturized technologies. Biosensors' specificity for the biological binding reaction is derived from numerous types of interactions and affinities (33,34). Some common interactions that have been studied for biosensor applications include antigen/antibody, enzyme/substrate/cofactor, receptor/ligand, energy transducer systems, synthetic chemical interactions, and nucleic acid hybridization.

Funded by the United States Department of Agriculture, a group at the University of Rhode Island's Fiber Optic and Biosensor Research lab has developed sensors that use vibrating quartz crystal or fiber optic probes in conjunction with antibodies for the detection of *Salmonella* (35). Another group at Cornell University has used nucleic acid sequences to detect pathogens (36). In this system, the biosensor consists of disposable microchannels with areas for capture and detection. DNA probes complimentary to the target pathogen RNA serves as the biorecognition element. To obtain a signal, two other probes are used, one coupled to dye encapsulated liposomes, and the other coupled to superparamagnetic beads for target capture. The probe then hybridizes to the target RNA and the liposome-target bead complex is captured on a magnet.

Biosensor techniques are still commercially limited, but hold promise with applications in the poultry, beef, and seafood industries (32).

IX. AUTOMATED SYSTEMS

The latest immunological detection methods involve the development of automated systems. These allow for the rapid testing of multiple samples concurrently. Typically, these automated systems are comprised of an analytical module, a computer, and a recording device such as a printer. The analytical module is capable of automatically performing all stages of the analysis, such that all that is required for analysis is the addition of the sample to the analyzer.

VIDAS® (bioMérieux) and mini VIDAS® are two currently available autoimmuno-analyzers that use enzyme linked fluorescent assays (37). VIDAS® is capable of analyzing four different modules concurrently, thus allowing it to run 240 tests per hour. The mini VIDAS® is a smaller version of the VIDAS® and contains a built in computer, keyboard, and printer. It has two independent sections capable of analyzing a total of 12 samples simultaneously. This system has been used for the detection of *E. coli* O157 in cheese, and *Listeria* in milk (38,39). The Tecra Diagnostics, Ltd. UNIQUE PLUS™ system automates the steps required for the UNIQUE™ dip stick test kit. The system currently supports assays for *Salmonella* and *Listeria* (11). Figure 186.5 shows the UNIQUE PLUS™ automated system by Tecra Diagnostics, Ltd.

X. SUMMARY

Immunological assay methods, particularly ELISA and latex agglutination, are routinely used for the detection and analysis of pathogens in food products. The use of automated systems and biosensors is not as common, but should increase in popularity as sensitivity increases and costs decline with these systems. Most developments in immunological assays have come about within the last few decades and future improvements are likely to continue at the same pace, as immuno-techniques have potential for superior accuracy and can provide rapid analytical results.

XI. NON-ENDORSEMENT OF COMMERCIAL PRODUCTS AND SERVICES

References, hypertext links and images to all products and services are provided for information only and do not constitute endorsement or warranty, express or implied, by the authors and/or their employers or the publishers of this

FIGURE 186.5 The UNIQUE PLUS™ utilizes the UNIQUE™ ELISA format for pathogen screening. Modules are plugged into UNIQUE PLUS™ and results collected. The automated system performs all the steps of UNIQUE™ ELISA which are normally done by a technician. (Reprinted by permission from Tecra Diagnostic, Ltd.)

work, as to their suitability, content, usefulness, functioning, completeness, or accuracy.

ACKNOWLEDGMENT

The authors would like to thank the following individuals for providing product literature and art that was instrumental in the preparation of this manuscript: Tony Vagnino, Strategic Diagnostics and Laura Gleeson, TECRA Diagnostics Limited. This is Florida Agricultural Experiment Station Journal Series number R-09806.

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187 Genetic-Based Methods for Detection of Bacterial Pathogens

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CONTENTS

I. INTRODUCTION

The food industry is witnessing a tug-of-war as processors make a slow transition from the use of traditional microbiological methods for quality control/quality assurance of foods, which are essentially designed around the recovery and enumeration of viable bacteria in the food matrix, to miniaturized rapid methods and molecular tools that achieve the same purpose with greater sensitivity, specificity, and in less time. In order to successfully counter increasing consumer demand for high quality food, to remain abreast of emerging food-associated pathogenic bacteria, and in keeping with Hazard Analysis and Critical Control Point (HACCP) implementation, significant resources are being invested in monitoring food during and immediately following processing to preclude spoilage and/or pathogenic bacteria from contaminating food, or detect them prior to shipment for retail sale (1).

Novel means of detecting and enumerating bacteria of interest are continually being reported. Some of these strategies still rely heavily on traditional, relatively inexpensive microbiological methods, but the majority of new assays in the literature entail a molecular component that affords rapid (8–48 hour), sensitive, and specific results for detection of particular target microbes (or their products). Such techniques may offer the food processor higher sample throughput, greater assay versatility, and speed compared to traditional (albeit more widely accepted) manual methods (2,3). This chapter will present a comparative overview of many of the commonly used

approaches in food microbiology for enumeration and/or detection of bacteria in foods, including many of the emerging molecular-based technologies currently flooding the literature.

II. BACKGROUND ON TRADITIONAL MICROBIOLOGICAL METHODS IN FOOD

Of the more than 200 known diseases transmitted through food in the United States yearly, at least 5,000 deaths are recorded on average from over 75 million reported cases. Surveillance and detection of food-associated pathogenic bacteria is complicated by emerging strains not routinely encountered, and the unclear route of transmission of many bacteria, which may be problematic in being spread by water or direct contact, in addition to contaminating specific foods (4). Routine screening of an increasingly diverse array of fresh and processed foods obligates pre- and post-harvest food safety measures be dynamic, sensitive, specific, as well as versatile and cost-effective for large sample numbers. In order to assess the microbiological quality of foods, detection of viable bacteria is traditionally performed by implementing a means of culturing/measuring growth of individual microorganisms. Hundreds of commonly used bacteriological media used in the food industry are met with unique ways of applying them to best monitor for spoilage and/or pathogenic bacteria in food (5). The use of routine nonselective media such as trypticase soy agar or standard methods agar, known as the aerobic plate count (APC) or standard plate count (SPC), offer low cost and ease of use. However, these approaches are not sensitive below levels of approximately 10^2 viable cfu/ml or gram of suspect food, require extended incubation times, and do not adequately address the presence of key virulence determinants in specific food-associated pathogens that may or may not be present in target bacteria. The widespread use of these traditional approaches is being superseded by molecular tools that are protein- and nucleic acid-based. The latter, in particular, lend themselves well to not only sensitive and specific detection of spoilage or pathogenic bacteria, but DNA typing/fingerprinting, quantitation, and differentiation of viable from dead microorganisms, frequently in real-time depending on the format of the assay. Following are descriptions of some of the most common nucleic acid-based methods used in quality control/quality assurance within the food industry, as well as some specialized DNA and RNA technologies that have demonstrated potential for application in food safety.

III. PULSED FIELD GEL ELECTROPHORESIS

Nucleic acid-based analysis of food-associated spoilage and pathogenic bacteria encompasses a diverse array of methods, many of which have had their origins in the clinical arena. One such method dates back to the early 1980s and is now the basis for the PulseNet molecular subtyping network of bacterial foodborne disease surveillance. Pulsed field gel electrophoresis (PFGE) is a fundamental method in molecular biology for separation of high molecular weight DNA for typing bacterial strains and tracing foodborne disease outbreaks through standardized protocols and data sharing.

In 1984, Schwartz and Cantor (6) described PFGE and demonstrated the ability of this technique to resolve yeast chromosomal DNA fragments and in doing so, raised the upper limit on the size of nucleic acids able to be separated electrophoretically. Following this initial high-profile study, a battery of subsequent papers reported on the utility of PFGE in genetic analyses of other organisms, as well as improvements on the protocol itself (7,8,9,10). In principle, PFGE is based on the physics of high molecular weight DNA fragments (i.e., chromosomal DNA or bacterial genomic DNA) not being resolved when exposed to constant voltage, but if the DNA is forced to change through periodic polarity inversion during electrophoresis, the mobility of large molecular weight DNA is altered and separation as distinct bands may be obtained (11). Various instruments and protocols are commercially available that reorient the DNA at unique angles depending on the specific experimental objectives, in an effort to obtain optimal separation within particular size ranges, but the separation principle is essentially the same (12,13,14).

In an effort to standardize protocols for molecular subtyping of bacterial food pathogens, PFGE has been developed as the tool of choice for characterizing and epidemiologically tracing isolates associated with foodborne illness outbreaks. In 1996, the Centers for Disease Control and Prevention in Atlanta, GA, and several state health departments established PulseNet with just 10 laboratories focusing on a single pathogen – *Escherichia coli* O157:H7 – following an outbreak of hemorrhagic colitis from contaminated ground beef consumed in a fast food restaurant (15). PulseNet now encompasses nearly every state in the US, and several provincial Canadian laboratories in an effort to meet the growing frequency of documented foodborne illness outbreaks with information on strain designations, tracing outbreak clusters, and sharing improvements on DNA extraction and end point analysis methods (16,17,18,19). PulseNet is also established overseas, and implements the same PFGE technology in the molecular subtyping of bacteria associated with foodborne illness outbreaks in Japan (20). In terms of applicability to food safety, PFGE has been useful for typing *Listeria monocytogenes* (21,22), *Staphylococcus aureus* (23,24), *Shigella flexneri* (25), *Campylobacter jejuni* (26), *Salmonella* spp. (27), *Clostridium perfringens* (28), and pathogenic *E. coli* (29,30).

IV. POLYMERASE CHAIN REACTION (PCR)

A. General Principles of PCR-Based Detection

Ideally, the development of a commercially viable detection assay for spoilage or pathogenic bacteria would be supplemented by a molecular approach with the potential for extreme sensitivity and specificity, while still maintaining low cost per assay. The polymerase chain reaction (PCR) has been in use in the food microbiology arena for over 10 years, with many variations of the common theme of this technique (Figure 187.1), some of these manifesting themselves as commercial assays (31,32) (Table 187.1). PCR has the potential to significantly reduce the necessary time for detection and screening of foods for pathogenic or spoilage bacteria, with a myriad of offshoot technologies that afford real-time, fingerprinting, quantitative, and/or RNA-based virulence gene expression assessment in a variety of data generation and collection formats (33,34).

The specificity of PCR usually relies on DNA sequence-specific oligonucleotide primers that initiate repetitive rounds of *in vitro* replication of a target gene fragment through denaturation, primer annealing, and new strand synthesis (35) (Figure 187.1). The end product, or amplicon, is traditionally analyzed by agarose gel electrophoresis, and visual confirmation establishes that the expected size fragment has been amplified from DNA extracted from contaminated food. The general PCR technique has been used in many applications for pathogen detection in food, enough to be previously well reviewed in a variety of sources (36–46).

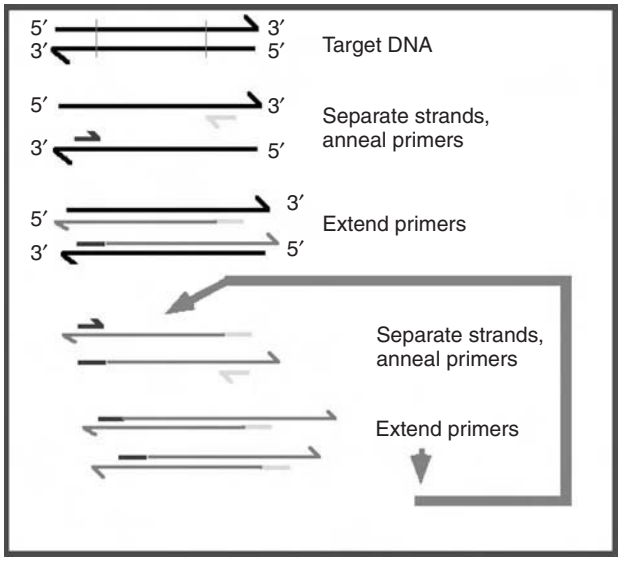

FIGURE 187.1 Process of polymerase chain reaction (PCR), showing template strands, sequence-specific primers (usually) employed that anneal to complementary base sequences, and extension (new strand synthesis) using *Taq* DNA polymerase. Each cycle of PCR theoretically doubles the amount of DNA in the reaction tube, but only within the region spanned by the forward and reverse primers.

TABLE 187.1
Commercially Available Nucleic Acid-Based Rapid Methods for Pathogen Detection in Food

Nucleic Acid Assays		
Organism	**Trade Name**	**Manufacturer**
Clostridium botulinum	Probelia	BioControl
Campylobacter	AccuProbe	GEN-PROBE
	GENE-TRAK	GENE-TRAK
Escherichia coli	GENE-TRAK	GENE-TRAK
E. coli O157:H7	BAX	Qualicon
	Probelia	BioControl
Listeria	GENE-TRAK[b]	GENE-TRAK
	AccuProbe	GEN-PROBE
	BAX	Qualicon
	Probelia	BioControl
Salmonella	GENE-TRAK[b]	GENE-TRAK
	BAX	Qualicon
	BIND[a]	BioControl
	Probelia	BioControl
Staphylococcus aureus	AccuProbe	GEN-PROBE
	GENE-TRAK	GENE-TRAK
Yersinia enterocolitica	GENE-TRAK	GENE-TRAK
Any (NASBA) assay	Nuclisens	Organon Teknika

The composition of the food medium directly impacts PCR assay sensitivity, however, and thus no universal DNA extraction procedure exists; rather, each food matrix presents its own set of challenges according to composition and must be addressed on a case-by-case basis (47,48). In fact, many factors affect efficiency of DNA template purification from food matrices, subsequent PCR amplification robustness, or both. For example, in dairy products and meats lipids, proteases, divalent cations, carbohydrates, or a host of undefined organic material may drastically interfere with PCR assay detection sensitivity (49–53). Rarely is one able to apply a PCR-based detection assay that lacks sample processing and template cleanup prior to setting up reactions (54). In virtually all foods under scrutiny by PCR-based analyses, debris and other inhibitory components may be at least partially sequestered or minimized using specialty buffer or detergent cocktails, solvent extractions, PCR additives, or a combination of these (55–61).

Several means have been described for removing the target spoilage or pathogenic bacterium from the food medium prior to DNA extraction as an additional efficient way to obtain PCR template of higher quality than solvent-extracted DNA. Immunomagnetic separation (IMS)

is perhaps the best described method, and has been used to concentrate *E. coli*, *Salmonella*, *Campylobacter* spp., *Bacillus* spp., and other food-associated pathogenic bacteria from a variety of foods, including fruit juices, dairy products eggs, seafood, ethnic foods, chicken, and meat homogenates (62–85). Additionally, insoluble metal hydroxides have been employed with great success for bacteria and spore immobilization in food suspensions for subsequent removal, resuspension, and DNA purification in a cleaner environment more conducive to high template yields (68,86–88). Metal hydroxides, essentially a supersaturated suspension of zirconium, titanous, or hafnium chloride in pH-adjusted ammonium hydroxide and sterile saline bind to the negatively-charged bacterial surface and sequester the cells from polluted suspensions such as food slurries or other contaminated liquid matrix. Once the cell-free supernatant is decanted following a low speed centrifugation, the cells may be rinsed, plated, or subjected to DNA extraction (89). The various factors that may antagonize DNA extraction and yield, template purity, or PCR amplification efficiency have been comprehensively reported (90) for clinical, food, and environmental DNA template purification applications.

B. Multiplex and Nested PCR

In order to balance PCR assay versatility with optimal levels of detection sensitivity and specificity under varied contaminated food matrices, multiple primer sets may be employed to simultaneously detect two or more target bacterial DNA sequences followed by agarose gel electrophoresis (91). This approach, called multiplex PCR, reduces the incidence of false-negative results. If one or more of the DNA fragments are visible, the sample is presumably positive. In most cases, multiplex PCR targets two gene sequences at once, although several target sequences may be possible (92–94). The notion of multiplex PCR may lend itself well to high throughput sample processing because once experimental conditions are optimized for a specific food/bacteria system, reactions may be in large part prepared ahead of time and stored in bulk, frozen until needed (95).

When designing primers for multiplex PCR reactions, one needs to ensure that the primers will have minimal tendency to form primer dimers or secondary structure elements when placed together in the same tube. Primary sequence homology and GC content of each primer should be analyzed individually and in concert with the other primers to be used in the assay to confirm that the T_m of each is within a few degrees for optimal annealing efficiency. Additionally, the multiplex amplicons should be different enough in size to resolve using agarose gel electrophoresis (91). Multiplex PCR affords increased assay versatility, but sacrifices assay sensitivity. Multiplex detection of *Salmonella* and *Vibrio* spp. in shellfish and mussels (93,96), and enterotoxigenic *Staphylococcus aureus* in skim milk and cheddar cheese (97) have been documented.

The use of multiplex PCR for detection of food-associated bacteria is quite prevalent in the literature, and in many cases this tool as a rapid method is partially negated by the need for a selective or nonselective enrichment from the food matrix that may add up to 20 hours to the assay (53,98–102) while other multiplex regimes follow confirmation plating steps on selective media (103), or are not demonstrated in a food system, limiting their potential applicability in a food processing environment and with a diverse array of foods (101).

While multiplex PCR techniques decrease the likelihood of obtaining false negative results, assay sensitivity may be improved with a nested PCR approach. This method utilizes sequence-specific primers for an initial round of amplification that when analyzed using agarose gel electrophoresis, may not yield visible amplicon bands. By using some of the PCR product in a second round of reactions with primers internal in annealing position with respect to the first set, a reamplification is done, with the goal of obtaining a visible amplicon (albeit one smaller than the original) on the gel (33).

Although nested PCR has largely been supplanted by faster and more sophisticated real-time methods, the basic technique has merit in terms of sensitivity, being used for detecting *Listeria monocytogenes* and *Yersinia enterocolitica* in raw milk (105–107), *Campylobacter* spp. (108), verotoxigenic *E. coli* in ground beef (109), and *Vibrio vulnificus* in fish (110), to cite but a few representative studies. As in standard PCR detection assays applied in food systems, nested PCR protocols generally implement an enrichment step and typically yield sensitivity on the order of 10^1–10^2 cfu per m contaminated food following such steps.

C. RAPD-PCR and Rep-PCR for DNA Fingerprinting

The application of random amplified polymorphic DNA (RAPD) analysis for typing of food-associated spoilage and pathogenic bacteria is widely reported in the literature. This technique employs relatively short (~10 bases) arbitrarily designed primers (one or two) in PCR reactions having a much lower annealing temperature than standard sequence-specific amplifications. Such conditions allow for the generation of PCR amplicons that represent more or less a DNA fingerprint of particular bacterial strains under that set of defined conditions (111). Although potentially useful as a rapid screening tool for tracking contaminants by the RAPD banding pattern on gel electrophoresis, this technique has limitations, including difficulty in obtaining reproducible amplicon banding profiles within replicates. The issue of variable results due to

random primer design and low annealing temperatures used, negate the utility of the technique as a feasible quality assurance tool in the food industry. Nevertheless, RAPD-based PCR detection assays have been reported in the detection of food-associated bacteria ranging from arcobacters in poultry (112) to assessment of *Bacillus cereus* ecology and contamination in processing facilities and commercial dairy powders, (113–115). RAPD analysis has also been reported for the typing pathogens such as *E. coli* O157:H7 (116), *Salmonella* (117–119), *Vibrio* spp. (120,121), *Campylobacter* spp. (122–125), and *L. monocytogenes* (126–129) in food matrices as diverse as cheese, seafood, pork, and beef.

A unique PCR-based approach to characterizing pathogens, repetitive element palindromic-based PCR (rep-PCR), has been applied in clinical settings to differentiate the genetic diversity of bacterial pathogens from hospitals (130). Rep-PCR is used to amplify repetitive, noncoding DNA sequences interspersed within bacterial genomes using primers specific to the repeated elements (Figure 187.2). Differences in the resulting banding profile are used to categorize new isolates, or identify strains based on known DNA banding patterns, or fingerprints (131–135) (Figure 187.2). Rep-PCR has been recently employed in typing *Bacillus sporothermodurans* and other *Bacillus* spp. isolated from milk (136,137), and to differentiate *Bacillus anthracis* strains (138,139) and enterotoxigenic *Bacillus* spp. from nonenterotoxigenic strains in contaminated milk (140). Rep-PCR shows broader species applicability and better discriminatory power than biochemical profiling and RAPD analysis and allows consistent pattern formation and storage of strain typing information in a database as a digitized image. Because this technique is sequence-specific, rep-PCR fingerprints are highly reproducible, unlike RAPD analyses. Unknown strains characterized by rep-PCR can be compared against the stored databases across laboratories for identification purposes and to monitor changes in microbial populations (141), similar to the PulseNet infrastructure using pulsed-field gel electrophoresis (PFGE) as the means of generating strain typing data.

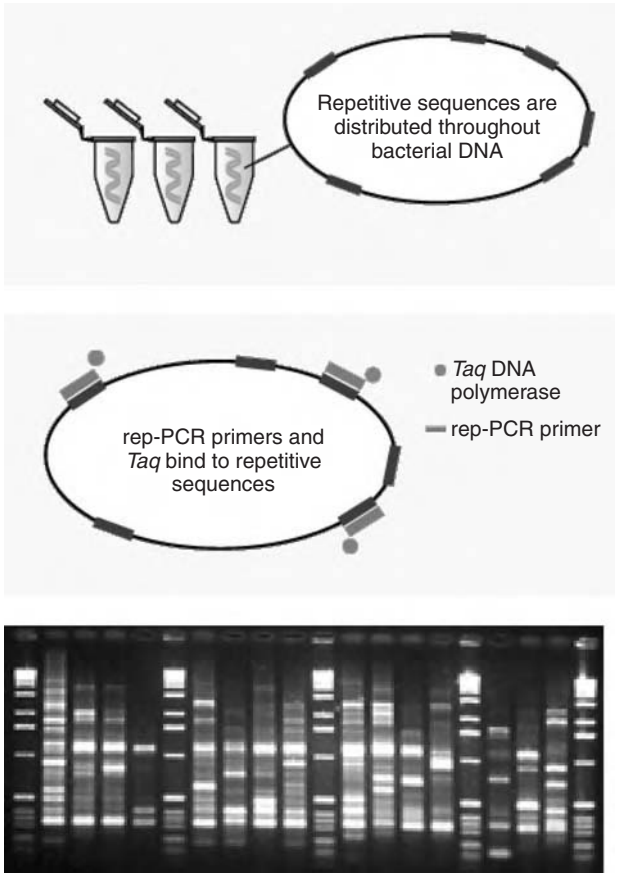

FIGURE 187.2 Repetitive element palindromic polymerase chain reaction (rep-PCR). Primers in rep-PCR are specific for conserved noncoding DNA elements found throughout the genome of bacteria, and as such generate muliple, but reproducible amplicon banding patterns useful for molecular subtyping following electrophoretic separation.

D. Quantitative-Competitive (QC-) PCR

For detection and enumeration of target genome equivalents or bacterial numbers in foods, including viable-but-nonculturable (VBNC) state cells, QC-PCR may be used (142). Enumeration of cells is possible through the coamplification of the target sequence with a shorter fragment (the competicon) containing the same primer annealing regions, allowing amplification of both target and competicon to occur with equal efficiency (143). By assembling QC-PCR reactions using titrations of competicon DNA concentrations but constant levels of target DNA, a series of doublet bands result following electrophoresis, with band intensity of competicon fragments decreasing with inversely increasing band intensities of the slightly larger target DNA sequence. The concentration of competicon that is equal in band intensity to that of the target fragment is calculated by scanning densitometry of the gel image and/or generation of a regression plot. Genome equivalents are determined and converted to a value for cell number in the suspect food sample. QC-PCR has been applied to a few food-based systems, such as quantitation of *E. coli* O157:H7 (142), and for GMO screening in grains (144). Because of the logistical difficulty in optimizing QC-PCR assays, the approach has limited potential for large-scale applications in the food industry as a rapid method, particularly in light of the many real-time chemistries in use.

E. Real-Time PCR

Despite the specific advantages as a sensitive tool for detection and/or screening suspect foods for the presence

of spoilage or pathogenic bacteria, PCR and the variations discussed above all have one caveat in common — the need to analyze the data using traditional end point analysis (i.e., agarose gel electrophoresis). While this technique is well-understood by many and does not require expensive equipment, analysis of amplicon band intensity in a gel following a cycle run adds 1–2 hours to the assay, may not be quantitative, and possesses a narrow dynamic range when attempting to detect differences in amplification efficiency among multiple samples. Moreover, post-PCR processing is necessary if one wishes to confirm amplicon identity by restriction enzyme digest analysis or a hybridization assay, either of which would completely negate the effect of using PCR as a rapid method for pathogen detection by stretching the protocol from hours to days.

The nature of PCR chemistry dictates that the exponential phase of amplification is the most accurate stage for quantification of products, rather than the plateau phase when reaction conditions are suboptimal and the relative amplicon band intensities of a set of templates that were at varying concentrations prior to PCR are now essentially equivalent (33). PCR assays that measure the reaction progress during each amplification, rather than after reaching a plateau, represent an attractive means of obtaining real-time quantitative data for rapid and sensitive detection using uniplex, multiplex, nested, or fingerprinting-based variations on the common theme of PCR to detect DNA (or RNA) (145,146). Currently, a number of real-time chemistries are commercially available for use in PCR. These can be divided into those that are not sequence specific—such as DNA minor groove binding dyes, and those approaches that are sequence-specific and may even afford simultaneous detection and confirmation of target amplicon during the PCR reaction.

1. Nonspecific Real-Time Chemistries

The standard method for nonspecific real-time detection of PCR amplicons is use of fluorescent double-stranded (ds) DNA intercalating dyes such as SYBR Green™ I or SYBR Gold™. Both of these commercial dyes are DNA minor groove binding dyes that fluoresce after interacting with dsDNA (Figure 187.3). Most real-time PCR instruments are programmed to read near the emission and excitation wavelength spectrum of SYBR Green™ (495 and 537 nm, respectively). This dye is very light sensitive, degrading quickly following dilution to working concentrations, but when fully active, affords the user the ability to obtain real-time fluorescence emission data (relative fluorescence units on the y-axis of a plot) as a function of cycle number on the x-axis. Since relative fluorescence units for each sample are plotted during the exponential phase of amplification, results are quantitative and thus useful for determining copy number and genome equivalents from

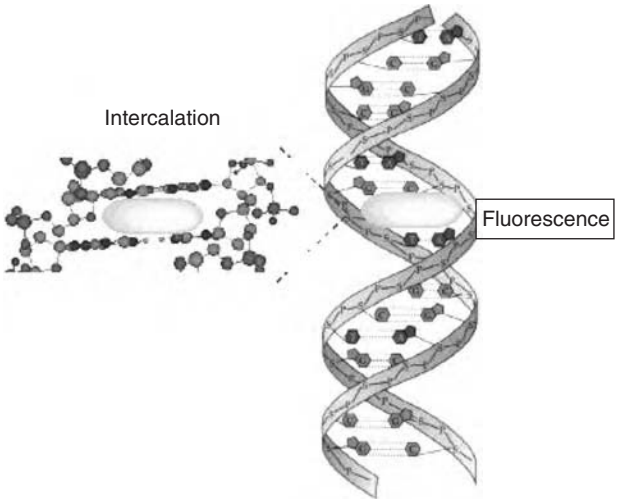

FIGURE 187.3 Interaction of SYBR Green™ I intercalating dye with double-stranded DNA and subsequent fluorescence under appropriate wavelength. The interaction is not sequence-specific.

template DNA purified from food. SYBR Green™ I has been used as an alternative to ethidium bromide for staining DNA in agarose gels, but is also useful for real-time PCR detection assays in food systems, such as quantification of enterotoxigenic *S. aureus* in cheese (147), *E. coli* O157:H7 in a multiplex design (148), and for GMO screening in grains (144). Due to the logistical difficulty in optimizing QC-PCR assays, the approach has limited potential for large-scale applications, particularly in light of many of the real-time chemistries.

In addition to simply quantitative detection of target pathogenic or spoilage bacteria in foods, intercalating dyes such as SYBR Green™ I allow one to discriminate among amplicons in a multiplex PCR reaction by using melt curve analysis. This approach subjects the PCR reactions to slow and continual heating to 95°C while monitoring fluorescence over time. Since each amplicon of a varying length and/or GC content will melt at a slightly different temperature, fluorescence will decrease incrementally according to the population of products in the reaction tube. Once conditions are optimized, the negative derivative of the fluorescence vs. temperature line will allow for small sequence differences, and certainly differences in length of products to become apparent (33) (Figure 187.4). Melt curve analysis has been applied primarily for mutation screening in specific clinical pathogens, but also may be useful for food pathogen detection.

2. Specific Real-Time Detection Chemistries

A diverse array of fluorescently labeled probes are in use clinically and industrially for sequence-specific detection of target DNA or RNA, and many of these have been applied in food analysis. The primary category of these

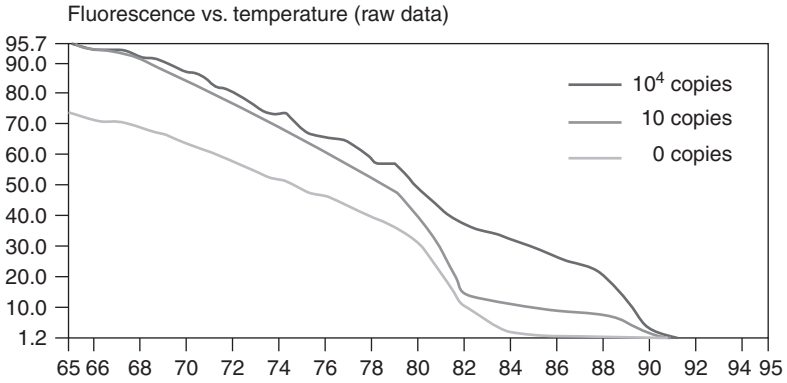

FIGURE 187.4 Melt curves of typical multiplex PCR amplicons showing the typical patterns that may be generated as fluorescence decreases with increasing temperature of samples. Melt curve profiles are useful in distinguishing amplicons in multiplex PCR reactions, or for mutation screening.

involves fluorescence resonance energy transfer (FRET) between a specific fluorophore and a quencher group. Perhaps the most widely used FRET conjugate pair for real-time PCR assays includes the fluorophore FAM (fluorescein) and the quencher TAMRA. The resonance energy from the fluorophore is passed to the appropriate quenching moiety, and if in close proximity (as described below for specific primer and probe regimes), generates low levels, if any, detectable fluorescence as measured by a PCR cycler with fluorimeter capabilities. If separated or alone in solution, the fluorophore will not be quenched and the resonance energy will be emitted as a detectable fluorescent signal at the appropriate wavelength. Depending on the format of the PCR assay, the signal generated will be directly correlated with the amount of target DNA present or amplicon concentration (Figure 187.5). Regardless of the specific means in which the fluorophore/quenching pair is applied, the basis remains the same, and includes the added advantage of sequence specificity that dsDNA intercalating dyes do not offer.

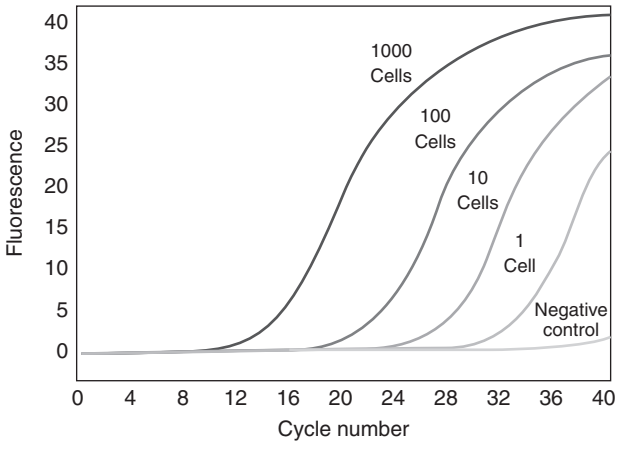

FIGURE 187.5 Real-time fluorescence plot of multiple samples at varying target cell densities analyzed using FRET-labeled probes. Relative fluorescence units are plotted as a function of time or cycle number on the x-axis.

One of the earliest uses for the FRET-based probe approach was the 5′-nuclease (TaqMan) assay, first described as a radioisotopic system, but soon modified to be based on fluorogenics (149). The 5′-nuclease activity incorporates a target gene-specific primer set and a dual-labeled probe that will hybridize to a region on one of the template strands within the primer annealing sites (Figure 187.6). During the extension phase of a PCR cycle, the 5′-3′ exonuclease activity of *Taq* polymerase will cleave the 5′ fluorophore from the terminal end of the hybridized probe, separating it from the quenching moiety, eliciting fluorescence at a specific wavelength (150) (Figure 187.5). Depending on the instrument being used for real-time detection, the investigator may choose to use multiple TaqMan primer and probe combinations in the same reaction tube for multiplexing, with each being detected in a unique optical channel at the respective wavelength. Regardless, TaqMan is a specific and sensitive assay for detection of pathogenic and/or spoilage bacteria in food. In recent years, the TaqMan approach has been reported for *E. coli* O157:H7 in raw milk and other foods (151,152), *Salmonella* spp. in meat and seafood (153,154), *Campylobacter jejuni* from poultry, shellfish, and other commodities (155,156), *Vibrio cholerae* in raw oysters (157), *Yersinia enterocolitica* in raw meats and tofu (158), *Clostridium botulinum* in MAP-packaged Japanese mackerel (159), enterotoxigenic *Bacillus cereus* from nonfat dry milk (160), and *L. monocytogenes* in dairy foods (161,162). These representative studies illustrate the versatility of the TaqMan assay for a very diverse array of foods to detect pathogens to levels as low as 10^1 cfu per ml, although frequently following several hours of preenrichment.

Though not as prevalent in the literature for applications in foods, another interesting variation on the use of double dye FRET-based probes for real-time PCR is the use of molecular beacons. Molecular beacons, first described

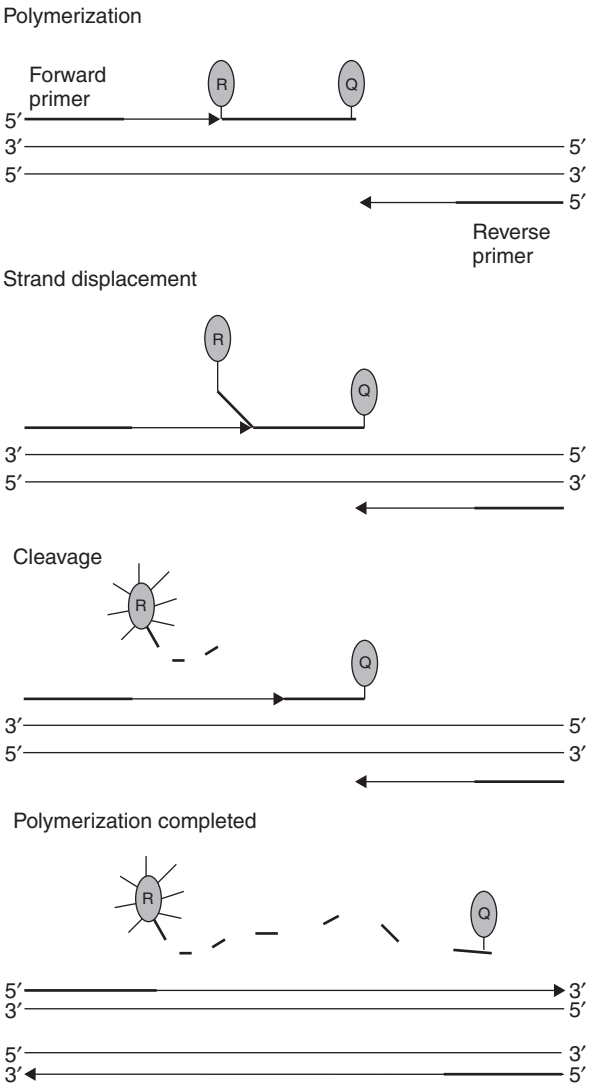

FIGURE 187.6 Mechanism of TaqMan 5′ nuclease assay for real-time detection of PCR products using FRET-labeled probe internal to the sequence-specific primers. R denotes the reporter dye while Q represents the quenching moiety.

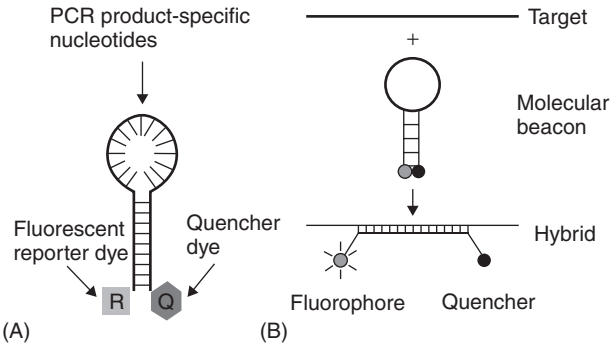

FIGURE 187.7 (A) Molecular beacon stem-loop conformation that forms by intramolecular base pairing when in solution without the presence of complementary target nucleic acid. (B) When in the presence of target DNA or RNA, the molecular beacon unfolds because the bases comprising the loop (probe) region form more numerous and more stable base pairs than those allowing the stem-loop secondary structure to form. A single base mismatch between the target nucleic acid and the probe portion drastically decreases stability of molecular beacon interaction and may preclude it altogether.

by Tyagi and Kramer (163), are short ssDNA probe molecules that are complimentary to target DNA sequences within the gene (or transcript) under study (Figure 187.7). Beacons are comprised of a loop region (the probe sequence) flanked by stem sequences 4–6 bases in length. The loop is comprised of bases with complete complementarity to the target DNA or RNA, and must match with perfect identity to the nucleic acid sequence being detected. The stem portions are designed to be complementary to each other and frequently are comprised of a majority guanine and cytosine bases. A fluorophore reporter dye is conjugated to one end of the molecular beacon and a quencher is attached to the other end (164). When the labeled probe is in solution alone, the beacon assumes the secondary structure conformation by forming intramolecular base pairs involving the stem portion. In this state, the beacon does not fluoresce, or fluoresces at baseline levels. When in the presence of the target nucleic acid, the loop attraction to the target sequence is stronger than the C/G bonds holding the beacon as a stem-loop, resulting in an unfolding of the probe, separating the quencher and reporter dye and emitting detectable fluorescence (163,165).

Although not widely employed in foods to date, molecular beacon technology offers many advantages, including simultaneous detection and confirmation of target nucleic acid when incorporated in PCR reactions flanked with sequence-specific primers. The stability of the stem structure helps to ensure that unfolding and hybridization will only occur in the presence of perfectly complementary base pairs, making the use of molecular beacons essentially a solution-based fluorimetric Southern blot. FRET-labeled beacon probes have been used as a clever means of assessment for ribonuclease H activity *in vitro* (166), but have recently been demonstrated on pathogens relevant to the food industry, including *Salmonella* and *E. coli* O157:H7 (167–169). Although extremely specific and capable of multiplexing, molecular beacons are still fairly cost-prohibitive, a feature likely to delay extended use as a means of rapid pathogen detection in foods. Specific variations of the FRET chemistries exist commercially, such as the Scorpion® primer (Eurogentec, Belgium) approach that relies on a quenched hairpin loop-based PCR primer that unfolds following the extension step and elicits fluorescence (170). Although not widely used yet in the food industry, such proprietary spin-off technologies offer great utility beyond the clinical arena. Regardless of the real-time chemistry selected, a number of commercial real-time instruments are

available such as the ABI Prism® 7000 (Applied Biosystems), RotorGene (Corbett Research), Cepheid's Smart Cycler® II System, and the BioRad iCycler iQ Real-Time Detection System. Most of these offer 2–4 optical channels to allow for multiplex capabilities, as well as interactive software for user-friendly data analyses.

V. RNA ASSAYS—MONITORING VIRULENCE GENE EXPRESSION IN FOOD PATHOGENS

A. The VBNC Dilemma

Although DNA (i.e., virulence determinant gene sequence) is the most frequent choice of target molecule when designing a PCR-based detection assay for foodborne pathogens, differentiation of living from dead bacteria is not possible, as DNA may be quite persistent in dead cells (171–175). Moreover, traditional culture-based approaches for enumeration of sublethally injured and/or viable-but-nonculturable (VBNC) bacteria are not accurate, as the selective media employed prevents many such bacteria from growing to visible, countable colonies. Specialized approaches utilizing viability dyes that interact specifically with DNA from dead cells and prevent it from being amplified by PCR may be augmented using additional fluorescent tags that allow for quantitation with confocal laser scanning microscopy are not practical for high-throughput sample analyses (176). Therefore, in order to accurately detect and monitor pathogenic foodborne bacteria (particularly VBNC cells) as well as virulence gene expression, RNA-based methods must generally be used.

When selecting RNA as a determinant of cell physiological state (177), one must bear in mind that ribosomal RNA (rRNA) is not an appropriate target, as bacterial ribosomes are stable for at least 48 hours after cell death (174). Ultimately, only mRNA is ideal to use as an indicator of either the metabolic status of bacteria or assessment of VBNC pathogens that must be assumed to still pose a threat if ingested by the consumer in contaminated food (178,179). Initial studies reporting the detection and measurement of gene expression in foodborne pathogenic bacteria implemented reverse transcriptase PCR (RT-PCR) as the means to the qualitative end of mRNA analysis. This labor-intensive protocol involves total RNA extraction from enrichment cultures, DNase-I treatment to eliminate genomic DNA (to preclude the incidence of false positive results), reprecipitation of RNA, and a reverse transcriptase step to enzymatically convert target transcripts to cDNA using a sequence–specific primer. The product is eventually converted to dsDNA in traditional PCR cycling using a second (forward) primer flanking the region of interest. In addition to samples, one needs to prepare a no reverse transcriptase control reaction to confirm the absence of gDNA carryover, as well as a no template control as a contamination screen (both of which should never yield an amplicon on the gel).

In practice, mRNA amplification using RT-PCR has been used to monitor cell viability in bacteria of relevance to the food industry (172,173,179–181). However, because of the inherent sensitivity issue (owing to the laborious process of RNA recovery), as well as moderate sample-to-sample variations in yield, RT-PCR is not a feasible means of high throughput gene expression analysis for the food industry, even with the onset of new commercial products such as single step RT protocols designed to streamline the process.

B. Nucleic Acid Sequence-Based Amplification (NASBA)

A more rapid means of RNA analysis has been applied in studies of virulence gene expression in bacteria and viruses for clinical microbiology, and also lends itself particularly well for viable cell determination (182,183). First described by Compton (184) and Fahy et al. (185), nucleic acid sequence-based amplification (NASBA) is an isothermic cyclical series of reactions utilizing RNA as template (either purified using acidic/phenol or 'whole cell' NASBA starting template) combined with an enzyme cocktail (Figure 187.8). NASBA begins with first-strand cDNA synthesis catalyzed by AMV reverse transcriptase using a transcript-specific forward primer. RNase H activity digests only the RNA half in the RNA-DNA heteroduplex, leaving ssDNA. Second-strand DNA synthesis then occurs by way of a second sequence-specific (reverse) primer containing a T7 RNA polymerase promoter sequence engineered on the 5′ end, extended with the DNA polymerase activity of the AMV reverse transcriptase. Double-stranded

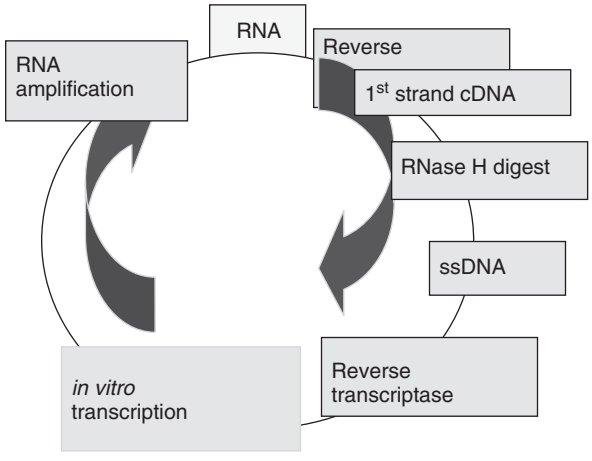

FIGURE 187.8 Nucleic acid sequence-based amplification (NASBA) for RNA amplification (i.e., virulence gene expression studies). Details of this isothermic process are explained in the text.

DNA results, allowing *in vitro* transcription to occur using T7 RNA polymerase (interacting at the promotor site on the second primer, incorporated into the dsDNA extension products), generating many mRNA copies of the original transcript template. A typical 90 minute NASBA reaction series is performed at 42°C and may amplify mRNA some 10^{15}-fold as *in vitro* transcription products serve as template for subsequent rounds of NASBA. The amplified RNA may be visualized via subsequent RT-PCR, or any of a number of real-time chemistries with appropriate fluorophores.

Because of the promotor-containing reverse primer utilized in NASBA assays, this procedure may be performed in a DNA background, unlike RT-PCR. Therefore, NASBA is more rapid than traditional methods for RNA detection, and if linked with real-time detection chemistry such as FRET probes (i.e., molecular beacons), has the ability to detect virulence gene expression in any pathogen relevant to the food industry. NASBA has recently been applied in the study of *Campylobacter jejuni* in foods (186,187) and to monitor enterotoxin gene (*hblC*) expression in three strains of toxigenic *Bacillus* spp. in contaminated milk (188). Although seemingly a very specialized technology at first glance, the speed and versatility of NASBA to be modified with a variety of uniplex or multiplex real-time chemistries make this method an attractive option when one wishes to assess virulence gene expression in target foodborne pathogens. To date, only one commercial supplier (Organon Teknika, Durham, NC, USA & Markham, Ontario, Canada) manufactures a NASBA assay in kit form (Nuclisens®), although the individual enzymes are available from virtually any supplier of molecular biology reagents for individual optimization sample-to-sample.

C. Microarrays

It is widely believed that thousands of genes and their products (i.e., RNA and proteins) in a given living organism function in a complicated and orchestrated way that creates the mystery of life. However, traditional methods in molecular biology generally work on a one gene in one experiment basis, which means that the throughput is very limited and the whole picture of gene function is hard to obtain. In the past several years, a new technology, called DNA microarray, has attracted tremendous interests among biologists and offers much in the way of high throughput analysis of virulence gene expression in food-associated pathogenic bacteria (189). This technology promises to monitor the whole genome on a single chip so that researchers can have a better picture of the interactions among thousands of genes simultaneously. An array is an orderly arrangement of samples. It provides a medium for matching known and unknown DNA samples based on base-pairing rules and automating the process of identifying the unknowns. An array experiment can make use of common assay systems such as microplates or standard blotting membranes, and can be created by hand or make use of robotics to deposit the sample (189,190). In general, arrays are described as *macroarrays* or *microarrays*, the difference being the size of the sample spots. Macroarrays contain sample spot sizes of about 300 microns or larger and can be easily imaged by existing gel and blot scanners. The sample spot sizes in microarrays are typically less than 200 microns in diameter and these arrays usually contains thousands of spots. Microarrays require specialized robotics and imaging equipment that generally are not commercially available as a complete system (Figure 187.9).

There are two major application forms for DNA microarray technology: 1) Identification of sequence (gene/gene mutation); and 2) Determination of expression level (abundance) of genes. In the former, a cDNA probe (500–5,000 bases long) is immobilized to a solid surface such as glass using robot spotting/lithography and exposed to a set of targets either separately or in a mixture. This method, traditionally called DNA microarray, is widely considered as developed at Stanford University (191,192). The second method is likely to prove more directly useful in the food industry over the next several years as a means of global gene expression analysis. In this variation, an array of oligonucleotides (20–80-mer oligos) are synthesized either *in situ* (on-chip) or by conventional synthesis followed by on-chip immobilization. The array is exposed to labeled sample DNA, hybridized, and the identity/abundance of complementary sequences are determined using quantitative instrumentation (Figure 187.9). DNA microarray technology has been a most powerful technique in areas of clinical and environmental microbiology since its inception, and will likely demonstrate great potential in the food industry as a sensitive means of detecting gene expression in a battery of target pathogens.

VI. SUMMARY

In an effort to stay abreast of heightened public awareness of food safety, and in light of contemporary concerns of food bioterrorism, research and development of nucleic acid-based molecular tools for pathogen detection, enumeration, and subtyping must expand. Such DNA- and RNA-based assays offer versatility in reducing the incidences of false negative quality assurance screening measures, sensitivity in processing heterogenous food matrices, specificity in differentiating among closely related target bacterial strains, and speed, as in the case of many of the real-time fluorescent chemistries flooding the market (3). Although some of these nucleic acid-based technologies are unlikely to supersede conventional culture, biochemical, or antibody based testing regimes completely, most offer significant

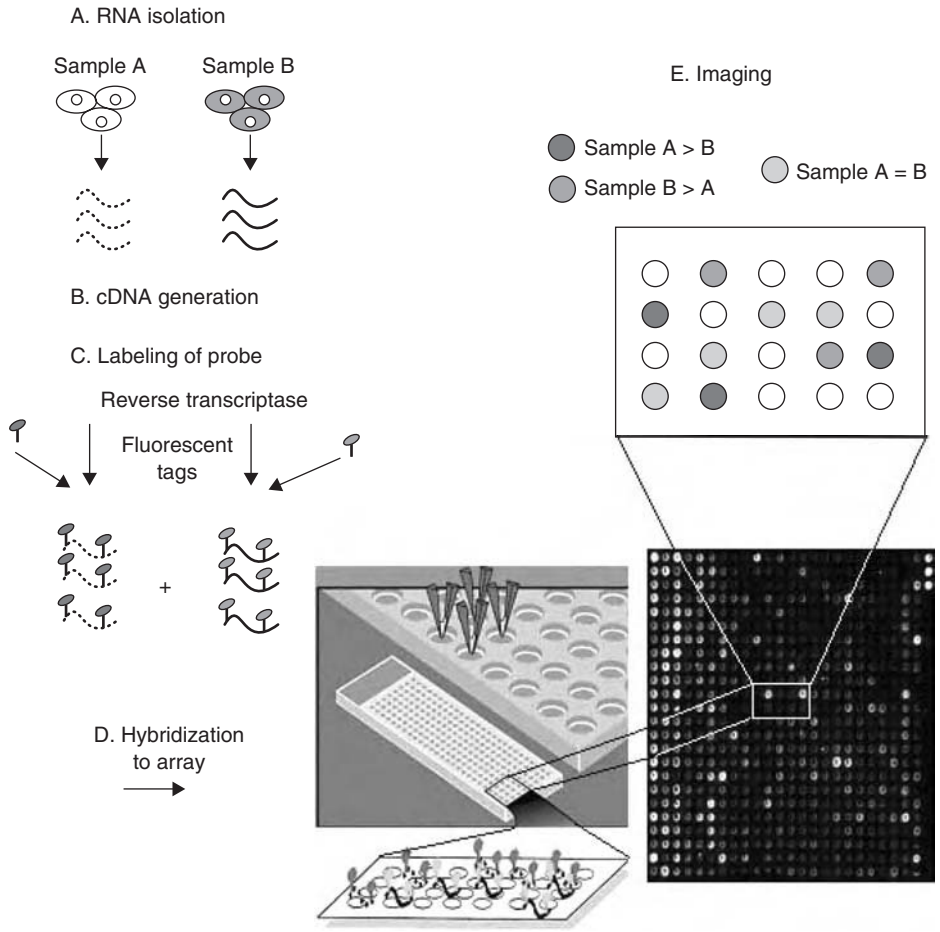

FIGURE 187.9 DNA microarray showing the steps in preparing oligonucleotide fragments that are subsequently probed using complementary sequences for quantitative large-scale, high throughput screening of gene expression using fluorescence. Details are explained in the text.

potential for high throughput and reliability, advantages that offset the cost of initial equipment needs and ongoing maintenance.

The notion of quality control by performing a PCR assay and sample analysis by agarose gel electrophoresis is obsolescent. The need to perform an enrichment step in order to increase cell numbers prior to the appropriate assay will likely be replaced with molecular approaches that are powerful enough to elicit reliable data in the midst of carbohydrates, lipids, and cellular debris from varied food matrices. Whether this will involve a spin-off of one of the existing approaches discussed on the previous pages remains to be seen (193), but in a time when the public demands both fresh ready-to-eat foods and a wide safety margin, detection of existing and emerging bacterial foodborne pathogens, and quantitation of spoilage microbes for accurate shelf life prediction is vital to meet an ever-increasing demand for a growing spectrum of convenience as well as fresh, ready-to-eat foods. The food industry's proverbial tug-of-war between conventional microbiological techniques for ensuring food safety and the implementation of molecular approaches must be carefully staged and stringently refereed.

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188 Methods for the Detection of Viral and Parasitic Protozoan Pathogens in Foods

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CONTENTS

I. INTRODUCTION

Both the human enteric viruses and the parasitic protozoa are now recognized as significant causes of human disease, perhaps being responsible for as much as 68% and 3% of all foodborne illness in the U.S., respectively (1). Although they have been recognized for years, the human enteric viruses and parasitic protozoa could be considered "emerging" agents of foodborne disease, largely because scientists have only recently been able to detect these pathogens. In fact, prior to the advent of molecular biological techniques, epidemiological criteria were the primary means by which cases of enteric viral and parasitic illness were recognized. Unfortunately, epidemiology had several limitations including the fact that the diseases caused by most gastrointestinal viruses and parasites were (and are) not reportable in the U.S.; only the largest, most severe, and/or most widespread outbreaks were (and are) investigated, leaving smaller outbreaks and sporadic disease underestimated; and early detection capabilities, even for clinical (fecal and blood) specimens, were severely limited. These early detection methods, which sought to

directly detect either the virus particle or parasite cyst or oocyst, were based mostly on some form of microscopy. Later methods relied on detection of antigen (enzyme immunoassay) in the stool, or alternatively, on seroconversion, i.e., a rise in specific antibody titer against the pathogen. The microscopic methods had poor sensitivity, and the reagents necessary for the serological methods were not always available to clinical laboratories. Among other factors, the absence of dependable detection methods contributed to an underestimate of the true scope and significance of foodborne viral and parasitic protozoan infections.

The human enteric viruses replicate in the intestines of infected human hosts, are excreted in the feces, and are therefore transmitted by the fecal-oral route through contact with human fecal pollution. In some instances, the parasitic protozoa are less species specific and can therefore be transmitted by the fecal oral-route through contact with either human and animal feces. Both viruses and parasites can also be spread by person-to-person contact, a phenomenon which is frequently responsible for the propagation of primary foodborne outbreaks. Contamination may occur directly, through poor personal hygiene practices of infected food handlers, or indirectly, via contact with fecally contaminated waters or soils. Since both types of agents must survive the pH variations and enzymes present in the human gastrointestinal tract, they are regarded as highly environmentally stable, allowing virtually any food to serve as a vehicle for their transmission and enabling them to withstand a wide variety of food processing and storage conditions. Neither viruses nor parasitic protozoa are able to replicate in contaminated foods. Furthermore, when found in foods, they are likely to be present in low numbers, but since their infectious doses are presumed to be low, any level of contamination may pose a public health threat.

The human enteric viruses of primary epidemiological significance include the hepatitis A virus (HAV) and the Noroviruses, formerly known as the Norwalk-like viruses (NLVs) and before that, as the small round structured viruses (SRSVs) (reviewed in ref. 2). The Sapoviruses (previously called Sapporo-like viruses) which are genetically related to the Noroviruses, have also caused cases of viral gastroenteritis in humans. Both the Noroviruses and the Sapoviruses are members of the *Caliciviridae* family, an antigenically and genetically diverse group of gastrointestinal viruses. The other viruses that can cause food and waterborne disease include the astroviruses, the human enteroviruses (polioviruses, echoviruses, groups A and B coxsackieviruses), hepatitis E virus, parvoviruses, and other relatively uncharacterized small round viruses. The rotaviruses, which are the leading cause of infantile diarrhea worldwide, are transmitted primarily by contaminated water but can on occasion be foodborne.

The parasitic protozoa of primary foodborne significance include *Cyptosporidium parvum, Giardia lamblia, Cyclospora cayetanensis*, and *Toxoplasma gondii* (reviewed in refs. 3–6). The former three organisms cause predominantly gastrointestinal manifestations, while the latter organism is associated with severe birth defects in infants whose mothers become infected during pregnancy. All of the parasitic agents can cause serious disease in immunocompromised hosts. Like the viruses, parasitic protozoa are obligate intracellular parasites that produce environmentally stable forms that serve as the vehicle for infection. Both *Cryptosporidium* and *Giardia* are primarily transmitted by waterborne routes, but foodborne infections have been reported. Toxoplasmosis has long been recognized as an uncommon but nonetheless severe foodborne infection that can be transmitted by the consumption of contaminated meats, offal, or unpasteurized milk, as well as by waterborne routes and contact with cat feces (5). *Cyclospora cayetanensis*, which to date has been almost exclusively foodborne, has been associated with the consumption of contaminated imported produce items (7).

Historically, the detection of human enteric viruses from food concentrates has been based on virus infectivity assays using susceptible, live laboratory hosts. Host systems employed were mainly mammalian cell cultures of primate origin, particularly primary and secondary human embryonic kidney and monkey kidney cell cultures. However, it is critical to note that to a large degree, the epidemiologically important human enteric viruses, including the Noroviruses and wild-type hepatitis A virus, cannot be propagated in mammalian cell culture systems and so these are not viable detection options. For the parasitic protozoa, many of the same considerations exist. For instance, cell culture and animal models for the propagation of *C. cayatanensis* are in developmental phases only (3). Although *C. parvum* can be assayed for infectivity using either cell culture or the mouse bioassay, neither method is very practical for the routine detection of this pathogen.

Likewise, immunological and DNA hybridization-based assays are not practical approaches for the detection of viruses and parasitic protozoa in foods. For viruses, this is due in part to the unavailability of immunological reagents, particularly for the antigenically diverse Noroviruses. While acid fast and immunologically based fluorescent staining techniques (or autofluorescence for *Cyclospora*) may be considered the "gold standard" for the detection of parasitic protozoa in environmental samples, and many effective kits exist for their detection in clinical specimens (8), these methods are laborious, require highly trained personnel, and are subject to interpretive problems when sample matrix components interfere with the assay. Furthermore, immunological methods tend to have relatively poor assay detection limits ($>10^3$–10^5 detection units/sample) which restricts their applicability to food samples, which are likely to be contaminated with small numbers of pathogens. Much the same can be said for DNA hybridization methods.

Without question, nucleic acid amplification methods have emerged as a promising approach when it comes to methods to detect enteric viruses and parasitic protozoa in foods. Methods such as the polymerase chain reaction (PCR) have the theoretical ability to replace standard cultural enrichment methods with faster nucleic acid enrichment. For the detection of viruses and parasitic protozoa, where cultural enrichment methods are virtually not feasible, this is a tremendous improvement. The purpose of this chapter is to discuss recent developments in molecular detection methodology that are enabling scientists to begin detecting viruses and parasitic protozoa in foods, and to identify research needs that must be effectively addressed before this effort can become a routine reality.

II. GENERAL DETECTION CONSIDERATIONS

There are significant impediments to the development of effective virus and parasitic protozoan detection methods as applied to food commodities. Similar to bacterial pathogens, these agents are likely to be present at low levels in contaminated foods. However, unlike bacterial pathogens, both viruses and protozoa require live mammalian cells in order to replicate, so the traditional food microbiological techniques of cultural enrichment and selective plating cannot be used. The general concept, then, is to separate and concentrate the agents from the food matrix prior to detection. In summary, in order to effectively detect viruses and parasitic protozoa from foods, one must consider the following restrictions: (i) the agents are inert in the food; (ii) they are likely to be present in very low numbers or intermittently in the product; (iii) because of (ii) above, it is necessary to process relatively large sample volumes to assure representation and promote detection; and (iv) the food matrix possesses inhibitory substances or interferences that can later compromise detection. In general, molecular detection schemes for viruses and parasitic protozoa in foods rely on five sequential steps, which can be designated (i) sampling; (ii) pathogen concentration and purification; (iii) nucleic acid extraction; (iv) detection; and (v) confirmation. These will be discussed in detail below.

III. SAMPLING

For effective sampling, a large and representative sample size is needed. A sample size of 25−100 g is usually recommended, which is large enough to provide adequate representation yet small enough to work with in the laboratory. To further increase the chances for detection, the analyst may choose to obtain multiple samples of the suspected or implicated product. For complex food products such as sandwiches, it may be easier to divide the product into its component parts, processing each part separately for pathogen concentration and detection. For instance, some investigators have processed dissected digestive tracts in an effort to improve virus recovery efficiency from contaminated raw molluscan shellfish. Although the viruses and parasites may be relatively stable in the food matrix, food samples should nonetheless be refrigerated upon collection and processed immediately for virus recovery upon receipt by the testing facility. Freezing may inactivate parasitic protozoa and should hence be avoided if attempting detection of these pathogens (9).

IV. PATHOGEN CONCENTRATION

The most common molecular amplification method applied for the detection of pathogens, including the parasitic protozoa, is the polymerase chain reaction (PCR). Since the nucleic acid for the enteric viruses is RNA, the PCR must be preceded by a reverse transcription step, producing cDNA which can then be readily amplified in a subsequent PCR, hence the designation RT-PCR. When using PCR or RT-PCR for the detection of viruses or parasitic protozoa in foods, one must consider pathogen concentration as a prerequisite to detection. In actuality, appropriate sample preparation prior to detection is even more important when applying molecular methods because of the small sample volumes ($<$10 µl) used in nucleic acid amplification reactions, as compared to 0.5–1.0 ml volumes used for cell culture infectivity assays or ELISA methods. Therefore, the pathogens and/or their nucleic acids must be concentrated and purified from food matrices before applying detection methods such as PCR or RT-PCR. The challenges of high sample volumes, low levels of contamination, and the presence of residual food components that can act as enzymatic inhibitors (10–14) must all be considered in designing these assays.

A. Principles of Virus Concentration in Foods

The purpose of virus concentration methods is to reduce sample volume and remove matrix-associated interfering substances, all the while recovering most of the viruses that are present in the food sample. Sample manipulations undertaken during concentration utilize the property of the viruses to behave as proteins in solutions, and their ability to remain infectious at extremes of pH or in the presence of organic solvents such as chloroform, trichloro-trifluoro ethane (Freon), and the more environmentally friendly solvent Vertrel® (DuPont Chemical Company). Almost all of the early work in virus concentration and purification from foods was limited to bivalve molluscan shellfish, largely because of their frequent association with viral foodborne disease outbreaks. More recent efforts have targeted a wider variety of at-risk foods.

As applied to shellfish, two general schemes for virus concentration have been reported, designated

extraction-concentration and adsorption-elution-concentration (15). These methods were developed in the decades between 1970 and 1980 and produced concentrates that could be assayed for virus infectivity using mammalian cell culture techniques. Both schemes employ conditions that favor the separation of viruses from shellfish tissues, primarily through the use of filtration, centrifugation, adsorption, elution, solvent extraction, precipitation, and organic flocculation. All protocols begin with sample blending in some type of buffer, usually containing amino acids and an elevated pH. Further processing may sometimes be preceded by a crude filtration step, through a mesh material such as cheesecloth, to remove particularly large sample particulates. A general theme in all of these scenarios is that viruses, since they are so small, do not sediment unaided, even at high centrifugation speeds (10,000 × g). This means that that routine centrifugation can be used without substantial virus loss as long as the virus-containing supernatant is recovered in the process. By pH manipulation or the addition of precipitation agents, conditions can be created such that viruses adsorb to the shellfish tissues, and when followed by centrifugation, the adsorbed viruses will sediment with the tissues and residual fluids are discarded with the supernatant. This is usually followed by an elution step, whereby virus desorption from the tissues is facilitated by further pH and/or ionic manipulations and subsequent centrifugation, discarding the precipitated tissue in the process. In this case, a large proportion of the food matrix can be disposed of while retaining a relatively clear solution that contains most of the recovered viruses and smaller amounts of matrix-associated organic material.

Other sample manipulations are designed to further remove matrix-associated organic materials and reduce sample volume, all the while optimizing recovery of viruses. For instance, a variety of organic solvents can be used to remove lipid materials, capitalizing on the fact that virus infectivity remains intact even after exposure to organic solvents. Virus precipitation can be accomplished using pH reduction, called acid precipitation, or else through the use of polyethylene glycol (PEG). Both methods are based on the fact that viruses behave as proteins in solution; by reducing the pH to that approximating the virus isoelectric point, the virus will precipitate, along with other matrix-associated proteins. Polyethylene glycol essentially removes water, allowing proteins to fall out of solution. A related precipitation method is organic flocculation. Used extensively in water treatment, flocculating agents interact with organic material in the matrix, causing the formation of a gelatinous "floc" to which the viruses adsorb. In the case of acid and PEG precipitation, and in organic flocculation, the virus-containing solid materials can be readily harvested by centrifugation. Finally, methods such as ultrafiltration can further reduce same volumes. The same general principles of filtration, centrifugation, adsorption, elution, solvent extraction, precipitation, and organic flocculation are used when extracting and concentrating viruses from food commodities other than shellfish. For purposes of illustration, a candidate virus concentration and detection protocol is illustrated in Figure 188.1. Note that this is only one of literally hundreds of iterations of the basic techniques used for the concentration of viruses from foods.

With the advent of molecular biology methods and their use in detection, additional virus concentration methods have been reported. For instance, alternative

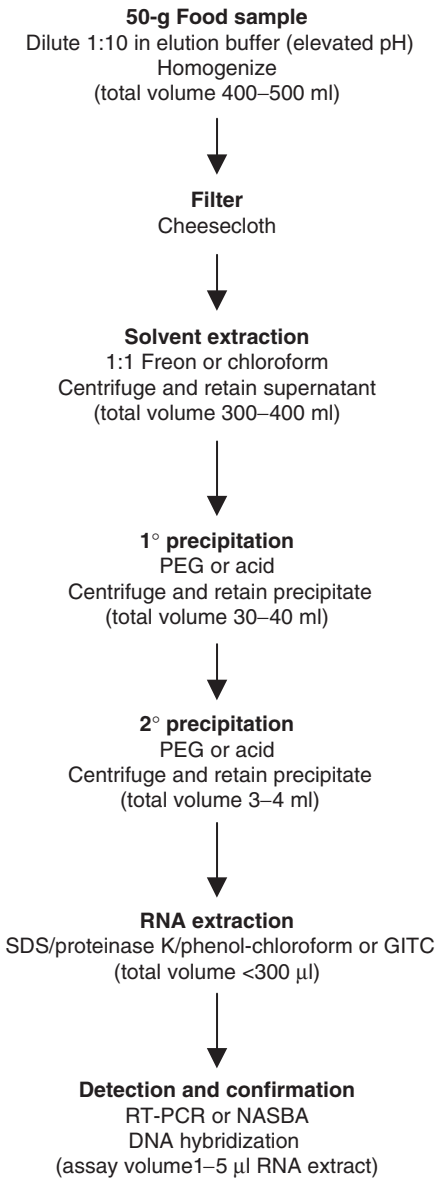

FIGURE 188.1 Representation of virus concentration and detection scheme for foods, consisting of sequential steps of filtration, solvent extraction, primary and secondary precipitation, RNA extraction, detection and confirmation.

commercial virus purification agents such as ProCipitate™ and Viraffinity™ (LigoChem, Inc., Fairfield, NJ 07004) (11,16,17) have been found to eliminate polysaccharides, an important matrix-associated inhibitor in shellfish and produce. Likewise, the cationic detergent cetyltrimethylammonium bromide (CTAB) (16,18–20) also promotes the removal of polysaccharides. Several other protocols utilizing Sephadex (21), cellulose (22), or Chelex (23) effectively eliminate salts and small proteins. D'Souza and Jaykus (24) used zirconium hydroxide to concentrate enteric viruses in a method resembling organic flocculation. Another active area of research has been immunomagnetic separation (25–28).

1. Virus Concentration Methods for Shellfish

Historically, two different approaches have been applied to prepare food samples for nucleic acid-based methods to detect enteric viral contamination. The first and most common approach relies on some form of virion concentration prior to release or extraction of nucleic acid and subsequent amplification. The second circumvents the need for virus concentration methods altogether by resorting to direct nucleic acid extraction of the food matrix. As with the early virus concentration work that was done to facilitate detection in cell culture, most methods to concentrate viruses in preparation for molecular detection were initially developed and evaluated for shellfish commodities.

In the case of virion concentration, the investigator aims at concentration of viruses and removal of inhibitors prior to the application of nucleic acid amplification, with or without prior nucleic acid extraction. In early work, Atmar et al. (19,20) processed artificially contaminated shellfish samples using an initial concentration and purification scheme consisting of solvent extraction and PEG precipitation, followed by nucleic acid extraction and subsequent amplification. Cetyltrimethylammonium bromide (CTAB) was added to remove residual inhibitors from crude nucleic acid extracts, and the resulting solution was amplifiable using RT-PCR. These investigators were able to further improve detection limits by dissecting the oysters, discarding the muscle tissue and processing only the digestive diverticula. This has remained the sampling method of choice for a number of other investigators (29–35). Other investigators have used also used glycine buffer elution, single or sequential PEG precipitation, and/or Freon extraction to initially purify the viruses from large sample sizes of shellfish, followed by extraction of total nucleic acids and subsequent RT-PCR amplification (33,36–42).

Some investigators have focused on optimization of virus extraction methods from shellfish. For instance, Croci et al. (43) evaluated four different elution buffers (PBS, pH 9.5; glycine, pH 9.5; and 0.3 or 3% beef extract, pH 9.5) and four different concentration techniques (acid precipitation, PEG precipitation, ultracentrifugation, and ultrafiltration) in an effort to optimize RT-PCR detection of viruses from mussels. Muniain-Mujika et al. (44) looked at the effect of alternative elution buffers (borate buffer, pH 9.5; glycine buffer with conductivity changes, pH 7.5; nutrient broth with Tween 80; and glycine buffer, pH 10) on the recovery of viruses from shellfish, finding the latter to be the most efficient. Likewise, Traore et al. (45) investigated four methods of elution (borate buffer, gycine buffer, saline beef extract, and beef extract-Freon) and three methods of concentration (PEG 6000, PEG 8000, and organic flocculation) to prepare mussel samples for the detection of three enteric viruses using RT-PCR. These investigators found that borate or glycine buffers performed best, as did both PEG 6000 and organic flocculation as concentration methods.

Other investigators have started with an adsorption-elution-precipitation method for the concentration of viruses from 50-g samples of shellfish, resulting in an aqueous solution of 10–20 ml in volume (46,47). This has been followed by additional virus concentration steps which included two consecutive extractions using Freon followed by virus precipitation with polyethylene glycol and further removal of matrix-associated inhibitors, particularly polysaccharides, using proprietary agents such as ProCipitate™ or Viraffinity™ (11,16,48). In a recent study, Mullendore et al. (49) reported a modified virus concentration method that consisted of acid adsorption at pH 4.8, two sequential elutions using glycine and threonine-based buffers, respectively, two PEG precipitations, and two chloroform extractions. After nucleic acid extraction and RT-PCR, detection limits were 1 PFU HAV/g oyster meat.

Others have used an antibody capture step to further concentrate and purify viruses from shellfish extracts prior to detection using RT-PCR. In its first application, Desenclos et al. (50) implicated hepatitis A virus in oyster outbreak specimens by immunocapture of the virus, heat release of viral nucleic acids, and subsequent RT-PCR detection. Building upon the clinical work of Jansen et al. (51), other investigators coated paramagnetic beads with anti-HAV IgG, mixed these with one ml volumes of oyster extract initially processed for virus concentration using a combination of elution, polyelectrolyte flocculation, filtration and/or ultrafiltration, and then captured the HAV to provide further virus concentration (25,27). Antibody capture steps have been used as a final virus concentration step by others as well (28).

Some investigators have used a direct nucleic acid extraction RT-PCR method that involves extraction of total sample RNA, including viral RNA, without any prior sample manipulations. Although perhaps best suited for simple sample matrices such as the surfaces of fresh fruits and vegetables, Legeay et al. (29) reported a method that involved enzymatic liquefaction of shellfish digestive tissues, followed by clarification using dichloromethane

extraction. This method produced a concentrate that could be extracted directly for nucleic acid isolation and subsequent virus detection using RT-PCR.

2. Virus Concentration Methods for Other Foods

Gouvea et al. (52) were the first to systematically develop a method to detect Norwalk virus and rotavirus from representative food commodities other than shellfish, including orange juice, milk, lettuce, and melon. By blending or washing, clarification by centrifugation, and RNA extraction with further removal of inhibitors using a Freon extraction, these investigators were able to detect both viruses in various foods. Schwab et al. (53) reported the use of TRIzol, a proprietary RNA extraction method, to wash deli meats, including samples artificially contaminated with Norwalk virus and ones linked to an outbreak of Norwalk-like viral gastroenteritis, for subsequent detection by RT-PCR. While the TRIzol surface wash method was reportedly simple, nucleic acid amplification inhibition persisted unless sample concentrates were diluted 10- to 100-fold.

Leggitt and Jaykus (17) developed a prototype method employing homogenization, filtration through cheesecloth, Freon extraction (hamburger only) and two sequential PEG precipitations to concentrate poliovirus, hepatitis A virus, and Norwalk virus from 50 g samples of artificially contaminated hamburger sandwiches and lettuce. The sequential precipitations were done at increasing PEG concentration and resulted in a 10- to 20-fold sample volume reduction from 50 g to approximately 2.5 ml. When appropriate, the resuspended PEG precipitate could be assayed for virus recovery by mammalian cell culture infectivity assay, with virus recoveries approximating 50% for poliovirus but only 4% for hepatitis A virus. Subsequent nucleic acid extraction resulted in an additional 100-fold sample volume reduction and detection could be achieved at initial inoculum levels of $\geq 10^2$ infectious units/50-g food sample. Similar to the TRIzol wash method of Schwab et al. (53), the final RNA concentrates obtained in this study were compatible with nucleic acid amplification after a one- to two-$\log_{10}$ dilution (17).

Most recently, Bidawid et al. (54) reported a method for the concentration of hepatitis A virus from lettuce and strawberries. After washing to remove the viruses, the wash solution was passed through a positively charged filter, eluted, and further concentrated by immunocapture. The investigators reported that as few as 10 PFU of cell culture-adapted hepatitis A virus per piece of lettuce or strawberry could be detected by RT-PCR, but residual amplification inhibition remained problematic.

It should be clear from the above discussion that by preceding nucleic acid amplification with virus concentration and sample purification steps, the investigator can achieve significant sample volume reductions with recovery of infectious viruses. The antibody capture methods may require even fewer sample manipulations, making these methods perhaps simpler than others. This strategy also has advantages in that a positive reaction demonstrates the presence of antigen-associated viral nucleic acid, which may be more highly associated with infectious virus than detection of free viral nucleic acid (25). However, concentration methods are not without their disadvantages, including the need for multiple sample manipulations which may result in substantial virus loss during the extraction steps, impacting assay detection limits. While the specificity of antibody capture methods may be appealing, limited reagent availability and the fact that only a single virus type can be targeted in a specific antibody capture reaction are considered limitations by some. Schwab et al. (26) used human hyperimmune serum in immunocapture methods to target a wider variety of enteric viruses, but these reagents can be costly and are not available to all laboratories. The direct nucleic acid extraction methods, while simpler, almost always result in residual RT-PCR inhibition and may not provide adequate sample volume reduction.

When taken together, virus concentration methods result in sample volume reductions ranging from 10- to 1000-fold, which means that a 25-g sample theoretically can be reduced to 25 µl–2.5 ml volumes with recovery of infectious virus. In the food matrix, the yields after virus concentration have ranged from as low as 1–2% to as high as 90%. Yield is almost always virus-specific, and a general rule of thumb is that recovery efficiency for hepatitis A virus is usually quite low, that for the human enteroviruses (poliovirus as a model) are quite high, and that for the *Caliciviridae* is in an intermediate range. Further volume reductions can be achieved through effective nucleic acid extraction, and this is discussed below. It is important to remember that there are many methods for the extraction and concentration of viruses from foods, all of which use the same general sample manipulations in varying combinations. Interested readers are advised to consult Jaykus et al. (55) for a more detailed review of methods to detect enteric viruses in shellfish or Jaykus et al. (13) for a more detailed discussion of specific virus detection methods in foods. There are, however, limitations to all of these virus extraction and concentration approaches that ultimately impact the ability to detect the relatively low levels of virus that might be anticipated in naturally contaminated foods. Tables 188.1 and 188.2 highlight the detection limits reported by a number of investigators in their efforts to develop methods to detect viruses from artificially contaminated shellfish and other food commodities, respectively.

B. Principles of Parasitic Protozoa Concentration in Foods

Like viruses, parasitic protozoa must also be concentrated and purified from the food matrix prior to detection. Almost exclusively, the early parasite concentration

TABLE 188.1
Detection Sensitivities for Human Enteric Viruses in Food (Shellfish) Commodities

Sample Type	Virus	Detection Limit	Comments	References
Clams	HAV	10 PFU/g	Spiked samples only	79
Oysters	PV[1]	2 PFU/g	Spiked samples only	19
Clams/oysters	HAV	67 PFU/g	Spiked samples only	19
Clams/oysters	NV/SRSV[2]	3–7 PCRU/g	Spiked and field samples for SRSV	20, 16
Various	NV[3]	2–20 PCRU/g	Spiked samples; nested RT-PCR	52
Cockles	PV	20 MPNCU/g	Spiked and field samples for PV; nested RT-PCR	139
Cockles	HAV	20 $TCID_{50}$/g	Spiked and field sample; nested RT-PCR	139
Cockles	Rotavirus	100 FF/g	Spiked samples; nested RT-PCR	139
Oysters/mussels	PV	2 PFU/g	Spiked samples only	37
Clams	PV, HAV	2 PFU/g	Spiked samples only	11
Clams	NV	20 PCRU/g	Spiked samples only	11
Shellfish	PV	3–30 $TCID_{50}$/g	Spiked samples; nested RT-PCR	109
Oysters	HAV	8 PFU/g	Spiked samples only	155
Oysters	HAV	0.5 PFU/g	Spiked samples only	27
Oysters	PV, HAV	0.02 PFU/g	Spiked and field samples	16, 48
Oysters	NV	90 PCRU/g	Spiked samples only	16
Clams	PV, HAV	20 PFU/g	Spiked samples only	11
Clams	NV	2 PCRU/g	Spiked samples only	11
Oyster	HAV	$\geq$ 1 PFU/1g	Spiked samples only	49
Mussels	PV1	4 pfu/g	Spiked samples only	156
	HAV	1.8–18 CPU/g		
Hardshell clams	PV1	$<$0.02 PFU/g	Spiked samples only	28
	HAV	$<$0.2 PFU/g		
	NV	0.02–0.22 PCRU/g		
Hardshell clams	HAV	0.004 PFU/g	Spiked and outbreak specimens	36
	NV	6 PCRU/g		
Mussels	HAV	0.1 $TCID_{50}$/g	Spiked samples; nested RT-PCR	40
Mussels	HAV	0.4 $TCID_{50}$/g	Spiked samples; nested RT-PCR	42
Oysters	HAV	0.33FFU /g	Spiked samples only	38
Mussels	HAV	20 PFU/g	Spiked samples, dissected digestive tissues	29
Oysters	HAV	20 PFU/g	Spiked samples, dissected digestive tissues	
Mussels	PV	1 $TCID_{50}$/ml	Spiked samples and nested RT-PCR	39

*PCRU, RT-PCR amplifiable units.

[1]PV1 poliovirus type 1.

[2]SRSV small round structured virus.

[3]NV Norwalk virus.

methods were developed for environmental water samples and focused on the two important waterborne parasitic protozoa, i.e., *C. parvum* and *G. lamblia*. As applied to environmental waters, detection has included three basic steps, e.g., pathogen concentration, purification, and detection (3). Both filtration and centrifugation approaches are effective for the concentration and purification of protozoa, largely because the cysts or oocysts are much larger than viruses or bacteria, so they can be readily captured on filters and will sediment at relatively low centrifugal forces. Filtration is a common first step, and both cartridge and membrane filters have been used, the former being an excellent choice when processing large sample sizes of environmental waters. After elution of the pathogens from filters, centrifugation approaches have been used for further pathogen concentration and purification. These include differential centrifugation, density gradient centrifugation, and/or immunomagnetic separation. The latter are available commercially for the further concentration of *Cryptosporidium* oocysts (8).

1. Parasitic Protozoa Concentration Methods for Foods

Relatively less work has been done in developing methods to concentrate and purify parasitic protozoa from a

TABLE 188.2
Detection Sensitivities for Human Enteric Viruses in Food Commodities Other Than Shellfish

Sample Type	Virus	Detection Limit	Comments	References
Lettuce	HAV	0.5 PFU/1ml	Spiked samples only	54
Strawberries	HAV	0.5 PFU/1 ml	Spiked samples only	
Ham	HAV	0.5PCRU/g	Spiked samples only	53
	NV[1]	5 PCRU*/g	Spiked samples only	
Turkey	HAV	0.5 PCRU/g	Spiked samples only	
	NV	0.5 PCRU/g	Spiked samples only	
Roast beef	HAV	5 PCRU/g	Spiked samples only	
	NV	0.5PCRU/g	Spiked samples only	
Frozen raspberries	NV	40 PCRU/g	Spiked samples only	81
	HAV	0.4 $TCID_{50}$/g	Spiked samples only	
Strawberries	NV	40 PCRU/g	Spiked samples only	
Lettuce	NV	40 PCRU/g	Spiked samples only	
Tomatoes	NV	40 PCRU/g	Spiked samples only	
Mashed raspberries	NV	150 PCRU/g	Spiked samples only	
Hamburger	HAV	<1.7 PFU/g	Spiked samples only	70
	NV	<0.83 RT-PCRU/g	Spiked samples only	
Lettuce	HAV	<16.7 PFU/g	Spiked samples only	
	NV	<8.3 RTPCRU/g	Spiked samples only	

*RT-PCR amplifiable units.
[1]NV Norwalk virus.

potentially contaminated food matrix. In part, this is because of a general lack of epidemiological data linking foods as a critical vehicle of transmission for the common enteric protozoa. However, food matrices may at times be more complex than environmental waters, further complicating the development of concentration and purification methods. Most of the developmental work in this area has been done on shellfish matrices, with only a few studies attempting pathogen concentration from other potentially contaminated foods. In the few instances when these techniques have been applied to shellfish matrices, investigators have recovered hemolymph and used it directly for immunofluorescent staining or PCR for detection. Alternatively, oocysts have been recovered by PBS washing of dissected gill tissue (56–60) or else by using an alkaline digestion method (61). In recent work, Graczyk et al. (60) processed whole mussels by grinding in PBS, sieving the homogenate with recovery of the finest particle fraction (<100 μm), followed by overnight sedimentation at 4°C and cesium chloride density gradient centrifugation to achieve further concentration and purification of oocytes. Deng et al. (62) used an immunomagnetic separation method to concentrate *C. parvum* from apple juice and milk. These investigators carried out a primary concentration step using sucrose flotation, and then treated the concentrate with rabbit anti-*C. parvum* antibodies, which were subsequently captured using magnetic beads coated with anti-rabbit IgG. After subsequent elution, the oocysts were visualized by fluorescent microscopy.

With the emergence of *C. cayetanensis* in the 1990s, several investigators developed prototype methods to concentrate and purify the oocyctes of this organism from contaminated produce items in preparation for detection by molecular amplification methods. In the first study of its kind, Ortega et al. (7) eluted the oocytes by washing large sample sizes of raspberries using distilled water, then centrifuged at approximately 2000 × g to sediment the oocytes, and used the wash sediment (designated RWS) for further testing or processing. A similar sample concentration method was used by Steele et al. (63), but in this case, applied to artificially inoculated basil and mesclun lettuce as well as to berries. The RWS could be further processed for *Cyclospora* concentration using sucrose flotation (63) or glass wool column chromatography (64). Robertson and Gjerde (65) processed fresh fruits and vegetables for detection of a variety of parasitic protozoa using surface elution aided by sonication, followed by further sample concentration using centrifugation and, when appropriate, immunomagnetic separation.

Like the virus concentration methods, the methods for parasitic protozoa concentration suffer from many of the same limitations. These include the potential for poor recovery of parasites after completion of the concentration steps, the inability to recover very low levels of contamination, and a general lack of available immunocapture reagents for pathogens such as *C. cayetanensis*. Because so much less work has been done to develop effective concentration and purification

methods for parasitic agents in foods, at least when compared to virus methods, there is much that remains to be done in this field.

V. NUCLEIC ACID EXTRACTION

The reliability of nucleic acid amplification methods, as applied to the detection of pathogens in all types of samples (clinical, environmental, and foods), depends in large part on the purity of the target template and the number of target molecules. Extraction of nucleic acids from food samples is therefore one of the most important steps preceding the detection of pathogens using molecular amplification methods. It should be noted that since the viral genomes are almost always composed of RNA, the nucleic acid extraction methods preceding virus detection are RNA extraction methods. For the parasitic protozoa, either DNA or RNA can be used as the target nucleic acid. The purpose of these sample processing steps are (i) to make the nucleic acids available for amplification and detection; (ii) to provide for further sample concentration; and (iii) to remove any residual matrix-associated inhibitory substances that might remain even after the initial pathogen concentration steps are completed. Molecular methods can be inhibited by a wide variety of compounds, including divalent cations, matrix-associated components which degrade the target and/or primer nucleic acids, and by matrix-associated compounds which inactivate or inhibit enzymes (10,66,67). For instance, several reports have demonstrated that proteoglycans (68), polysaccharides (18,19,69), glycogen (16,19) and lipids (53,70,71) all can inhibit molecular amplification methods. Unfortunately, many of the methods to extract and concentrate pathogens from foods result in the simultaneous co-extraction of these inhibitory substances. Nucleic acid extraction methods, like virus concentration methods, must therefore be designed to further concentrate the nucleic acids while removing these inhibitory substances, without degrading the nucleic acid target or contributing additional inhibitory substances in the process.

A. Nucleic Acid Extraction of Food Concentrates — Viruses

In early work, nucleic acids from shellfish concentrates were often released by heat, making them available for nucleic acid amplification while circumventing the need for RNA extraction (11,16,72). At the same time, other early studies used SDS-proteinase K digestion to release nucleic acids from shellfish concentrates, followed by phenol chloroform extraction with or without the addition of cetyltrimethylammonium bromide (CTAB) for further removal of inhibitors (20,35,38,73). With the introduction of guanidinium thiocyanate (GuSCN)-based methods, this became for many investigators the RNA extraction method of choice, largely because the method is effective at deproteinization of nucleic acids while providing ample protection of RNA against the effects of native RNases. In fact, many guanidinium-based protocols have been commercialized over the last decade and a variety of these commercial kits have been used in more recent studies (33,39,53,70,74).

In many instances, multiple extraction methods have been used in combination to purify nucleic acids. Boom et al. (75) developed a simple and rapid protocol for the purification of nucleic acid that uses a combination of the chaotropic agent GuSCN and silica particles. The Boom method has been modified and commercialized by bioMerieux (Durham, NC) for use in RNA extraction prior to nucleic acid amplification using the NucliSens® Basic Kit Nucleic Acid Sequence-Based Amplification (NASBA) assay. Others have used a GuSCN method followed by RNA binding to glass powder in order to provide further purification (37,76). Over the last several years, a number of investigators have compared alternative RNA extraction methods for fecal or food matrices. For instance, Hale et al. (77) compared four different RNA extraction methods to precede RT-PCR detection of Noroviruses in fecal specimens, finding that the GuSCN/silica (Boom) method was best at removal of inhibitors, while RNA extraction methods using the metal chelating agent Chelex-100, or alternatively, Sephadex G200 column chromatography, provided the best detection limits. Svensson (78) reported similar results in that guanidinium thiocyanate is the most efficient at removal of inhibitory substances.

In another study, seven RNA extraction methods were compared to prepare hepatitis A virus RNA from stool and shellfish concentrates for RT-PCR detection (74). In this case, GuSCN-silica methods were found to be the most suitable for their rapidity and low cost. In very recent studies, total sample RNA has been extracted using phenol-chloroform-based methods followed by further selection for viral RNA using magnetic poly(dT) beads (33,36,42,79).

A small number of studies have described RNA extraction and purification for virus concentrates originating from other type of foods. Gouvea et al. (52) used deproteinization with GuSCN, adsorption of RNA to hydroxyapatite and sequential precipitation with CTAB and ethanol to purify RNA from shellfish and other selected foods. Phenol-chloroform based methods have also been used for more complex commodities such as hamburger, deli sandwich and deli meats (53,80). More recently, commercial RNA extraction kits have been used on concentrates from fruits and vegetables (54,81) and those from oysters (49). Sair et al. (70) compared multiple RNA extraction methods with respect to their efficacy in removing RT-PCR inhibitors from model food commodities (hamburger sandwiches and lettuce) in preparation for RT-PCR detection of enteric viruses. The methods compared included GuSCN, commercial microspin columns, the QIAshredder™ Homogenizer and TRIzol™, and various combinations thereof. These

investigators found that the use of TRIzol™ followed by further sample preparation using the QIAshredder™ Homogenizer yielded the best detection limits (<1 RT-PCR amplifiable units/reaction) for Norwalk virus in food samples.

When considered as a whole, the RNA obtained after extraction steps may be of relatively high purity, yet food-related amplification inhibitors frequently remain. Also, multiple sample manipulation steps can result in incomplete recovery and/or potential degradation of RNA during the extraction procedure (55,82). Another problem with RNA extraction is that, in destroying the integrity of the virion, one loses the ability to directly correlate infectivity to RT-PCR detection limits, at least when effective cell culture-based methods are available. Researchers continue to work on refining extraction procedures to provide higher nucleic acid yields and improved purity in an effort to further improve molecular amplification assay detection limits.

B. Nucleic Acid Extraction of Food Concentrates — Parasitic Protozoa

Template preparation for the detection of parasitic protozoa is perhaps more complicated than that for viruses due to the nature of the organisms, which are largely resistant to disruption and lysis. Three methods used frequently to disrupt cysts or oocysts for detection from fecal specimens, and these include sonication, freeze-thaw, and glass bead disruption; these can be followed by DNA binding in the presence of chaotropic agents for further template purification (83). Jinneman et al. (84) investigated various methods to prepare DNA template from *Cyclospora* oocysts, including multiple freeze-thaws in liquid nitrogen to release the nucleic acid, followed by direct DNA extraction with or without the addition of various proprietary enhancement agents. Basicially, PCR detection from raspberries artificially seeded with *C. cayetanensis* could be achieved, but no one template preparation method outperformed another. Orlandi and Lampel (64) reported a method to prepare template from the pathogenic parasitic protozoa using FTA filters. These filters are impregnated with denaturants, chelating agents, and free radical traps, causing most cells to lyse on contact and in turn, binding the DNA to the filter matrix. Other matrix associated inhibitory substances are removed by washing the filter, and the filters can be used directly in nucleic acid amplification reactions. These investigators showed that the FTA filters effectively lysed the cysts or oocysts of various parasitic protozoa and when applied to artificially contaminated raspberry extracts, the DNA template could be detected by PCR at initial seeding levels of 30 *C. cayetanensis* oocysts per 100 g of berries. Higgins et al. (85,86) reported on the efficacy of various commercial DNA extraction kits for the preparation of *C. parvum* DNA for PCR after its initial release by multiple cycles of freeze-thaw, finding that some commercial methods outperformed others. Most recently, Steele et al. (63) reported *C. cayetanensis* template preparation from produce washes using a combination of glass bead disruption followed by three freeze-thaw cycles and final template preparation using a commercial DNA extraction method originally designed for stool samples. In the only study of its kind, Warnekulasuriya et al. (87) were able to detect *T. gondii* in artificially contaminated ready-to-eat cured meats at levels of 10^3 trophozoites/g by applying a simple SDS-proteinase K DNA extraction method directly to emulsified meat specimens. Similarly, Deng et al. (62) used three sequential proteinase K/phenol extractions to isolate PCR-amplifiable genomic DNA from *C. parvum*.

VI. DETECTION

Virtually all of the molecular approaches for the detection of viruses in foods originated from the clinical literature. For both clinical and environmental samples, the sensitivity and the specificity of molecular amplification methods is largely dependent on the choice of primers. Due to the tremendous genetic diversity in the *Caliciviridae* family, primer design for the detection of the Noroviruses is quite complicated. The first Norovirus primers, NV 5'/3' and NV 36/35, were based on sequences in the prototype Norwalk virus genome and were extremely specific (21,88). As more sequence information on related viruses was collected, more broadly reactive primers were designed (31,89–92). Typically, the highly conserved RNA-dependent RNA polymerase region is the amplification target for the detection of the Noroviruses, although on occasion the capsid region has been chosen for primer design (93–96).

For a long time, the genogroup I (GI) and genogroup II (GII) primer sets developed by Ando et al. (89) were the "gold-standard" with respect to Norovirus detection and strain discrimination in clinical (fecal) and food samples (95,97,98). Degenerate primers, a mixture of oligonucleotides varying in nucleotide sequence but having the same number of nucleotides, have also been developed and used for the detection of the Noroviruses (30,31,90,99). The advantage of using degenerate primers lies in the fact that every possible combination of nucleic acid sequence that could code for a given amino acid sequence can be generated and used for PCR amplification. Currently, combinations of the Ando et al. (89) GI and GII primers, as well as one or more degenerate sets, are routinely used in Norovirus epidemiological investigations (70,100,101).

By and large, there is no consensus for the choice of the primer pair(s) to detect the Noroviruses and optimal detection protocols may actually change by year or with locale (97). In a review of nine sets of PCR primers used for the detection of the Noroviruses, no single primer pair

could be used to detect all strains, but the NV110/NV36 primer set was the most efficient of the nine sets tested in the study (101). More recently, in an effort to address international harmonization of Norovirus detection methods, five laboratories in five countries evaluated the performance of different RT-PCR protocols on a coded panel of 91 fecal samples (102). Although no single assay was superior by all criteria of sensitivity, detection limit, assay format, and successful implementation, the authors recommended the assay based on the Boom extraction method and using the JV12/JV13 primer set for laboratories seeking to newly initiate Norovirus diagnostics. It should be clear from the above discussion that Norovirus primer design is a work in progress and is likely to change as more sequence information becomes available. Unlike the great diversity of primers for the detection of the Noroviruses, primers used for the molecular epidemiological studies of hepatitis A virus target the VP1/2A junction sequence (51).

Because it is so highly conserved and is frequently used in phylogenetic analysis, the target gene for most PCR-based methods to detect the parasitic protozoa is that corresponding to the small subunit (SSU) 18S ribosomal RNA gene. For instance, Relman et al. (103) developed 18S rDNA-based primers for the detection of *C. cayetanensis*, and these have been used subsequently by others (63,64,84,104). Likewise, a similar gene target has been used for the PCR-based detection of *Cryptosporidium* (57,64,85,86). On a few occasions, alternative targets have been used, including genes corresponding to *Cryptosporidium* oocyst wall protein (62,85,86) and the *Toxoplasma* p30 gene (87).

A. RT-PCR Detection of Viruses in Foods

In complex matrices such as food systems, the choice of primer is even more critical since the matrix can be responsible for non-specific amplification and the potential for false positive results. Alternatively, matrix inhibition can cause false negative assays result. Those consequences are especially important in food samples in which the level of contamination is comparatively lower than in clinical samples. The criteria for primer selection when attempting to detect viruses in food should include (i) a reasonably high annealing temperature; (ii) a relative lack of primer sequence degeneracy; and (iii) broad reactivity. These criteria are essential since high stringency and primer specificity are necessary to prevent the non-specific amplification that readily occurs with food and environmental sample matrices. An additional consideration for the genetically diverse Norovirus group is the use of primers that are broadly reactive and hence will be able to detect as many genetically distinct strains as possible in a single assay.

Table 188.3 summarizes various *Caliciviridae* primers that have been used in studies to develop RT-PCR methods to detect the Noroviruses in different food matrices. As for clinical samples, all of the primers reported in Table 188.3 target either the viral RNA-dependent RNA polymerase or the capsid protein genome regions. Several investigators have used the NV 36/35 primer set for Norovirus detection in shellfish and deli meat samples (19,20,35,53,76,105). Previously, our group has used the NV-3/5 primer set to detect the Norwalk agent in shellfish and ready-to eat foods such as hamburger and lettuce (11,16,17,72). The NI/E3 primer set has been used for the detection of the GII Noroviruses in shellfish (12,76,106). Shieh et al. (107) used primer sets targeting both the RNA polymerase and capsid genes, Mon381/383 and SR33/46, respectively, to identify a GII Norovirus in oyster samples implicated in a California outbreak. Recently, Dubois et al. (81) used a newer primer pair that detects both Noroviruses and Sapoviruses, as applied to the detection of viruses in artificially contaminated produce. Sair et al. (70) systematically compared four Norovirus primer pairs, reporting the best detection limits for the NVp110/NVp36 primer combination as applied to virus detection in various food matrices (hamburger sandwiches and lettuce). Furthermore, Honma et al. (101) found that this same primer pair was broadly reactive with a range of Noroviruses, without requiring separate amplifications for the two Norovirus genogroups. Other researchers have also used those primers together or in association with NI or NVp69 for the detection of Noroviruses in shellfish (30,31,33). As for the Noroviruses, most of the methodological approaches described for the detection of hepatitis A virus in foods have been applied to artificially contaminated shellfish species (Table 188.4).

To improve assay sensitivity, "nested" RT-PCR has been used for the detection of Noroviruses (26,52,76,108) and hepatitis A virus (36,40,76,109–111) in foods. While nested reactions can improve assay sensitivity, they have rightfully earned a reputation of being risky from the standpoint of contamination. To bypass those issues, novel single-tube nested RT-PCR methods have been described. For instance, Ratcliff et al. (112) separated the reagents required for the nested amplification in a "hanging drop" that was intended to be introduced by centrifugation after the first RT-PCR amplification. Burkhardt et al. (113) compartmentalized the nested RT-PCR cocktail in a "tube-within-a-tube" (TWT) device using inexpensive and simple materials such as a pipette tip and a microcentrifuge tube.

In all, methods such as these have been used by many investigators for the detection of viruses in environmental and food samples, with detection limits ranging from approximately 1–100 infectious units/g food. Some assays have used internal amplification standards to simultaneously evaluate RT-PCR inhibition and/or to provide a semiquantitative assay (20,31,34,73,99,105, 114,115).

TABLE 188.3
RT-PCR Detection Methods of Members of the Family *Caliciviridae,* Including Noroviruses and Sapoviruses in Foods

Primer	Sequence (5′→3′) (Polarity)	Location (bp)	Size (bp)	Viruses	Food Sample	Reference
NV-5	CAAATTATGACAGAATCCTTC (+)	4601–4621	260	NV[1]	Lettuce and	17
NV-3	GAGAAATATGACATGGATTGC (−)	4840–4860			hamburger	72, 16
					Oysters	11
						28
					Clams	
					Clams	
NV 36	ATAAAAGTTGGCATGAACA (+)	4475–4494	470	NV, UK[2],	Shellfish	35
NV 35	CTTGTTGGTTTGAGGCCATAT (−)	4923–4944		SMA[3], TV[4]	Delicatessen	53
					Oysters	20, 73
					Shellfish	
NI	GAATTCCATCGCCCACTGGCT (+)	4756–4776	113	Norovirus GII	Shellfish	12
E3	ATCTCATCATCACCATA (−)	4869–4853			Shellfish	76
					Shellfish	106
M5	CACCACCATAAACAGGCTG (+)	2197–2214	224	NV	Shellfish	36
M3	AGCCTGATAGAGCATTCTTT (−)	2438–2421				
P290	GATTACTCCAAGTGGGACTCCAC (+)	4568–4590	319	Noroviruses	Fruits and	81
P289	TGACAATGTAATCATCACCATA (−)	4865–4886		and Sapoviruses	vegetables	
NP1	GCAGATTATACAGCATGGGAC (+)	4552–4572		NV, Semi-	Shellfish,	52
NP2	GAGTGAGGCGGGCTGGGTC (−)	4910–4891	358	nested with	orange juice,	
NP4	CAGGTGATAAACCAGTGG (−)	4819–4802	268	NP1	milk, cole slaw, melon, lettuce	
Mon381	CCAGAATGTACAATGGTTATGC (+)	5362–5383		Norovirus GII	Deli sandwich	80
Mon383	CAAGAGACTGTGAAGACATCATC (−)	5661–5683	322			
Mon382	TGATAGAAATTGTTCCTAACATCAGG (−)	5559–5584	223	Nested with 381	Shellfish	107
NVp110	AC(A/T/G)AT(C/T)TCATCATCACCAA (−)	4865–4884		Noroviruses	Clams	33 (4 primers)
NVp36	ATAAAAGTTGGCATGAACA(+)	4487–4501	398		Lettuce and	70 (p36 + p110)
NI	GAATTCCATCGCCCACTGGCT (+)	4768–4788	150		hamburger	31
NVp69	GGCCTGCCATCTGGATTGCC (+)	4733–4752	116		Oyster	30
					Shellfish	
SRI-1	CCAACCCARCCATTRTACAT (−)	5659–5640	316	Norovirus GI	Seafood	109
SRI-2	AAATGATGATGGCGTGTA (+)	5344–5361				
SRI-3	AAAAYRTCACCGGGKGTAT (−)	5584–5566	241			
SRII-1	CGCCATCTTCATTCACAAA (−)	5357–5339	514	Norovirus GII		
SRII-2	TWCTCYTTYTATGGTGATGATGA (+)	4844–4866				
SRII-3	TTWCCAAACCAACCWGCTG (−)	5046–5028	203			
SR33	TGTCACGATCTCATCATCACC (−)	4856–4876			Oysters	114
SR48	GTGAACAGCATAAATCACTGG (+)	4754–4773	123	GI[5]: UK2, NV	Oysters	30
SR50	GTGAACAGTATAAACCACTGG (+)	4754–4773				114
SR52	GTGAACAGTATAAACCATTGG (+)	4754–4773			Shellfish	30 (with p110)
SR46	TGGAATTCCATCGCCCACTGG (+)	4754–4773	123	GII[6]: UK1,		80
SR33IN	CACGATCTCATCATCACCAGTA(−)	4853–4873		UK3, UK4	Deli sandwich	14, 107
SR46IN	AATTCCATCGCCCACTGGCTTCG (+)	4757–4776	117	(TNA[7],UK4	Oysters	
				(TNA,HWA[8], SMA)		

[1] Norwalk virus.
[2] UK: Antigenic groups UV1, UK2, UK3, and UK4.
[3] SMA: Snow Mountain agent.
[4] TV: Toronto virus.
[5] GI: Genogroup I Noroviruses.
[6] GII: Genogroup II Noroviruses.
[7] TNA: Taunton agent.
[8] HWA: Hawaii agent.

TABLE 188.4
RT-PCR Detection Methods of Hepatitis A Virus in Foods

Primer	Sequence (5′ → 3′) (Polarity)	Location (bp)	Size (bp)	Food Sample	Reference
H1	GGAAATGTCTCAGGTACTTTCTTTG (−)	2390–2414	247	Fruits and vegetables	81
H2	GTTTTGCTCCTCTTTATCATGCTATG (+)	2167–2192		Lettuce and strawberry, on-tube	54
				Shellfish	74, 157
				Shellfish	29, 155
				Oysters	30
				Shellfish	27
				Oyster	
HAV-R	CTCCAGAATCATCTCAAC (−)	2208–2226	192	Oyster	48
HAV-L	CAGCACATCAGAAAGGTGAG (+)	2035–2054		Oyster	49
				Lettuce and hamburger	17 16
				Oyster	48
				Oyster	11
				Clams	38
				Oyster	28
				Clams	
				Lettuce and hamburger	70
H2	GTTTTGCTCCTCTTTATCATGCTATG (+)	2167–2192	210	Shellfish	139
H3	TCCTCAATTGTTGTGATAGC (−)	2358–2377			
2870	GACAGATTCTACATTTGGATTGGT (+)	2986–3004	534	Clams	33
3381	CCATTTCAAGAGTCCACACACT (−)	3381–3360			
Down	CTCCAGAATCATCTCC (−)	2226–2211	207	Oyster	19
Up	ACAGGTATACAAAGTCAG (+)	2020–2037			
BG7	CCGAAACTGGTTTCAGCTGAGG (−)	7125–7104	276	Produce and shellfish, nested	79
BG8	CCTCTGGGTCTCCTTGTACAGC (+)	6850–6871			
BG7a	CTGGTTTCAGCTGAGGYA (−)	7120–7102	264		
BG8a	GGTCTCCTTGTACAGCTT (+)	6856–6873			
D	GGAAATGTCTCAGGTACTTTCTTTGCTAAA AAACTGGATCC (−)	2389–2413	248	Shellfish, one-step	74, 157 45
E	GTTTTGCTCCTCTTTATCATGCTATGGAT GTTACTACAC (+)	2167–2205		Mussels	
HAVp3	GGAAATGTCTCAGGTACTTTCTTTG (−)	2413–2389	248	Shellfish	20
HAVp4	GTTTTGCTCCTCTTTATCATGCTATG (+)	2167–2192		Delicatessen	53
				Shellfish	35
2949	TATTTGTCTGTCACAGAACAATCAG (+)	2949–2973		Shellfish, nested	36
3192	AGGAGGTGGAAGCACTTCATTTGA (−)	3168–3145	267		158
dkA24	CTTCCTGAGCATACTTGAGTC (−)	3139–3119?		Oysters, non-nested	
dkA25	CCAGAGCTCCATTGAACTC (+)	2986–3004	200		
HAV4	ATTCTACCTGCTTCTCTAATC (−)	6716–6696	412	Seafood, semi-nested	109
HAV1	TTTGGTTGGATGAAAATGGTT (+)	6305–6325			
Primer 1	CAGACTGTTGGGAGTGG (+)	762–778		Shellfish, nested	40
Primer 2	TTTATCTGAACTTGAAT (−)	1131–1147	385	Mussels, nested	110
Primer 3	CAAGCACTTCTGTTTCCCCGG (+)	780–797			
Primer 4	ATTGTCACCATAAGCAGCCA(−)	1092–1109	329		
P1	CAGGGGCATTTAGGTTT (−)	669–685		Fresh produce, nested	111
P2	CATATGTATGGTATCTCAACAA (+)	1063–1084	415		
P3	TGATAGGACTGCAGTGACT (−)	807–825			
P4	CCAATTTTGCAACTTCATG (+)	1000–1018	211		
HAV240	GGAGAGCCCTGGAAGAAAGA (−)	194–213	170	Bivalve molluscs	143
HAV68	TCACCGCCGTTTGCCTAG (+)	43–60			
A1	AGCATGGAGCTGTAGGAGTG (+)	291–309	271	Lettuce	144
A2	TAGAGACAGCCCTGACAATC (−)	543–562			
HAV1	TTGGAACGTCACCTTGCAGTG (+)	332–353		Shellfish, nested	141
HAV2	CTGAGTACCTCAGAGGCAAAC (−)	680–700	368		
neHAV1	ATCTCTTTGATCTTCCACAAG (+)	371–391			
neHAV2	GAACAGTCCAGCTGTCAATGG (−)	641–661	290		

B. PCR Detection of Parasitic Protozoa in Foods

In general, considerably less effort has been put into using molecular amplification methods for the detection of parasitic protozoa in foods. In the first study of its kind, Fayer et al. (57) screened commercial oyster harvesting sites from the Cheasepeake Bay region by PCR for the presence of *C. parvum*-specific DNA. Using an 18S rRNA gene-based nested assay, these investigators identified *Cryptosporidium* from all sites surveyed but only two genotypes (human and bovine) were found. The IMS-PCR method of Deng et al. (62) was able to achieve detection limits of 10 oocysts of *C. parvum* per 100 ml of apple juice or milk. A series of studies to develop methods to detect various parasitic protozoa in produce have resulted in PCR detection limits ranging from 10–40 *C. cayetanensis* oocyts/100 g raspberries (63,64) and 1000 *C. cayetanensis* oocyts/100 g mesclun lettuce (63). Finally, Warnekulasuriya et al. (87) developed a PCR-based assay to detect *T. gondii* in ready-to-eat cured meat products with a detection limit of 5×10^3 trophozoites/g. It is important to note that almost all of these studies have required nested amplification reactions in order to achieve adequate detection limits for artificially contaminated foods.

C. Alternative Nucleic Acid Amplification Methods

Nucleic Acid Sequence-Based Amplification (NASBA) is a nucleic acid amplification method specifically designed to detect RNA. The system utilizes three enzymes, (i) a reverse transcriptase (AMV-RT); (ii) an RNase H; and (iii) a T7 RNA polymerase, all of which act in concert with two oligonucleotide primers specific to the target (116). One of the primers (P1) contains the T7 RNA polymerase promoter sequence at 5' terminal. The transcription-driven NASBA reaction is carried out at a single temperature (41°C) and theoretically amplifies the RNA target more than 10^{12}-fold within 90 min. The final product of the amplification is mostly single-stranded RNA, which can be readily detected by probe hybridization without the need for a denaturation step.

From a clinical standpoint, NASBA commercial kits (NucliSens®, bioMerieux, Inc. Durham, NC) are available for the detection of human pathogens such as HIV and cytomegalovirus in blood or plasma. NASBA assays have also been developed for the detection of foodborne enteric viruses (Table 188.5). For instance, Jean et al. (117) developed a NASBA-based method to detect hepatitis A virus which performed adequately when tested on artificially contaminated lettuce and blueberry samples. The same group (118) also developed a NASBA assay to detect human rotavirus. Most recently, Greene et al. (119) applied the NucliSens® Basic Kit NASBA protocol for the detection of Norwalk virus RNA in stool using primers targeting the RNA polymerase region of the Norwalk virus genome. This NASBA assay could consistently detect 10^4 RT-PCR detectable units of NV RNA. When applied to stool specimens from human challenge studies and outbreaks of foodborne disease, the NASBA assay yielded 100% sensitivity, 50% specificity and 67% concordance using RT-PCR as the "gold standard."

In general, the NASBA method is more rapid (6–8 hr) and may demonstrate 10- to 100-fold improvements in detection limits when compared to RT-PCR. Although the NASBA is likely to be impacted by many of the same restrictions as RT-PCR (e.g., contamination control, sample volume considerations, matrix-associated reaction inhibitors), it is likely to remain an important alternative detection method for foodborne pathogens.

VII. CONFIRMATION

After any nucleic acid amplification reaction, the amplicons generated should be detected and confirmed to assure the sensitivity and the specificity of the assay. The most common confirmatory testing tool is agarose gel electrophoresis followed by Southern blot hybridization. In the case of RNA products (for NASBA), Northern blot analysis using labeled internal oligonucleotide probes has been used (101,120). Interestingly, an oligonucleotide array dot-blot format for the simultaneous confirmation of Norovirus amplicons and strain genotyping has recently been reported, offering the promise of providing both detection and strain typing in a single test (121). It is, however, important to note that Southern and Northern hybridization are largely being replaced by more rapid and user-friendly hybridization methods based on enzymatic, chemiluminescent, or fluorescent endpoints.

DNA enzyme immunoassay (DEIA) methods are an appealing confirmation approach because they can be readily automated and are familiar to many individuals working in testing laboratories. For most of these methods, a capture probe is immobilized to a microtiter plate well. A labeled amplicon can then by detected directly, or alternatively, an unlabeled amplicon can be hybridized to a second labeled detector probe followed by detection after the addition of an enzyme-conjugate and appropriate chromogen substrate. After each step, unbound compound is removed by washing; for colorimeteric, luminescent, or fluorescent endpoints, absorbance is read using a conventional microtiter plate spectrophotometer or fluorescent plate reader, and the intensity of the signal may be approximately proportional to the concentration of amplicon. Microtiter plate assay sensitivity is usually equal to or better than Southern hybridization and has the advantages of ease of interpretation and more rapid (4 hr) amplicon confirmation (35,118).

Electrochemiluminescence (ECL) detection technology has been developed by bioMerieux for the detection

TABLE 188.5
NASBA Detection Methods for the Detection of Enteric Viruses

Primers	Sequence (5′ → 3′) (Polarity)	Location (bp)	Size (bp)	Samples	Reference
HAV					
BB1	CAGATTGGCTTACTACACA	1000–1018	474	Lettuce, Blueberry, turkey	117, 159
BB2	AATTCTAATACGACTCACTATAGGGAGACATGCAACTCCAAATCTGT	1428–1446			
GI					
NVP1	AATTCTAATACGACTCACTATAGGGAGAAGGATCTCATCATCACCATA	4592–4605	371	Lettuce, turkey, stool	119, 160
NVP2b	GATGCAAGGTCGCATATGAGATACCACTATGATGCAGATTA	?			
GII					
NVP1	AATTCTAATACGACTCACTATAGGGAGAAGGATCTCATCATCACCATA	4592–4605	165	Lettuce, turkey	160
NVP2a	GATGCAAGGTCGCATATGAGGAATTCCATCGCCCACTGGCT	4505–4515			
Rotavirus					
Rota-1	GTAAGAAATTAGGTCCAAGAG	794–814	286	Lettuce, Blueberry	118, 159
Rota-2	AATTCTAATACGACTCACTATAGGGAGAGGTCACATCGAACAATTC	1045–1062			

of NASBA amplicons generated using the NucliSens® Basic Kit. The technology involves a liquid sandwich hybridization employing two specific oligoprobes; a capture probe immobilized to streptavidin-labeled magnetic beads and a generic detection probe complexed to a ruthenium chelate. The hybrid is drawn into the ECL flow cell, magnetic particles are trapped on an electrode, and unbound reagents are washed away. Application of a voltage trigger to the electrode induces the ECL reaction, and the emitted light is detected and the signals interpreted by the NucliSens® Reader and associated software. ECL technology has been used by Fox et al. (122) as applied to the detection and confirmation of enterovirus NASBA amplicons obtained from clinical samples and by Greene et al. (119) for the detection Norwalk virus in stool samples. The NASBA-ECL system usually allows for confirmed detection results in a single working day.

Other amplicon confirmation methods have included specific "nesting" reactions (52,76,108,109) and restriction endonuclease digestion of RT-PCR products (52,109). As nucleic acid sequencing equipment became more widespread, direct amplicon sequencing for the confirmation of RT-PCR products has become the method of choice (31,53,76). However, all of these confirmation methods require significant amounts of sample manipulation and remain time-consuming.

In the case of parasitic protozoa, confirmation of PCR amplicons has been achieved by classic DNA hybridization (for *C. parvum*) (123), nested reactions (for *C. parvum*) (57,64,85,86), restriction enzyme digestion of amplicons (for *C. cayetanensis*) (63,84), or by oligonucleotide ligation assay (for *Cyclospora*) (84). For the most part, species and strain specific typing methods for *Cryptosporidium* and *Cyclospora* are based on polymorphisms in either the 18S rRNA gene or intervening transcribed spacer regions between rRNA genes (124–126). Accordingly, a second group of investigators have used PCR-Restriction Fragment Length Polymorphism (RFLP) assays for both confirmation and genotyping of *C. parvum* (57) and *C. cayetanensis* (104).

A. Real-Time Detection

Several achievements have been made during the last decade enabling nucleic acid amplification and hybridization technologies to be linked so that detection and confirmation occur simultaneously, resulting in so-called "real time" detection. There are currently five main chemistries used for real-time amplification and detection technologies, including DNA binding fluorophores, the 5' endonuclease assay, adjacent linear and hairpin oligoprobes and self-fluorescing amplicons (127). Recently, investigators have developed real-time PCR systems for the detection of pathogens in foods (128–134). Prototype real-time PCR and RT-PCR amplification technologies have been developed for the detection of hepatitis A virus (135), the Noroviruses (136), *C. cayetanensis* (137), and *C. parvum* (138), although none of these methods has yet been applied to the detection of these pathogens in food matrices.

VIII. DETECTION OF VIRUSES AND PARASITIC PROTOZOA IN FIELD AND FOODBORNE DISEASE OUTBREAK SPECIMENS USING MOLECULAR METHODS

It should be clear from the above discussion that much of the developmental work in the detection of viruses and parasites in foods has focused on applications to

TABLE 188.6
Recent Investigations in Which Viruses Have Been Detected in Foods Epidemiologically Linked to Foodborne Disease Outbreaks

Agent	Food	Samples Tested	Methods	Conclusions	Reference
NLV	Shellfish	Outbreak samples (food only)	RT-PCR (single and nested) sequencing	4/4 outbreak samples Positive	76
NLV	Oysters	Outbreak samples (clinical and food)	RT-PCR (nested) sequencing	Co-existence of two different NLV genogroups in single oyster specimen by RT-PCR and sequencing	108
NLV (G II)	Oysters	Outbreak samples (food only)	RT-PCR sequencing	2/3 recalled outbreak oysters samples positive	14
NLV (G II)	Deli meats	Outbreak samples (clinical and food)	RT-PCR (nested) sequencing	Identical nucleotide sequences of amplicons from food and clinical samples	53
NLV	Raspberries	Outbreak samples (clinical and food)	RT-PCR southern hyb. sequencing	Identical nucleotide sequences of amplicons from food and clinical samples	140

artificially contaminated shellfish species. Only a few of the early methods were applied to naturally contaminated shellfish (12,14,20,48,50,76,109,139). Successful detection of viruses in food samples epidemiologically linked to disease has been reported only rarely and as applied to shellfish (14,31,33,50,76,108), deli meats (53), and raspberries (140) (Table 188.6). Many investigators have found it necessary to use nested amplifications to achieve the sensitivity needed to detect viruses in naturally contaminated products (12,31,39,42,53,76,106,108,109,141). Interestingly, recent survey work originating from Europe, including France (30), Italy (42,142), Spain (106,141,143), Brazil (38), and Greece, Sweden, and the United Kingdom (106) has reported consistent detection of hepatitis A virus, the Noroviruses, human enteroviruses, adenoviruses, and rotaviruses from field shellfish harvesting sites, sometimes at prevalence rates exceeding 40% (30,141,143). A field study of market lettuce from Costa Rica demonstrated sample pools that were positive for both hepatitis A virus and rotavirus during a period of high diarrhea prevalence in that country (144).

Protozoan parasites such as *Giardia* and *Cryptosporidium* species may be present in shellfish-growing waters and the ability of shellfish to concentrate *Giardia* and *Cryptosporidium* was first demonstrated by Toro et al. (145). The first isolation of *Giardia* from market bivalves was also reported by this same group (61). Later studies postulated the potential role of oysters in the epidemiology of *Cryptosporidium* infection (146) and were followed by studies that reported oocyst stability for 4–12 weeks in artificial seawater (56) with detection of *Cryptosporidium* ocysts in Eastern oysters harvested from commercial sites in the Chesapeake Bay (56). *Cryptosporidium* oocystes have also been recovered from Bent mussels (*Ischadium recurvum*) (58) and from various clams, mussels, and oysters harvested in Europe (147).

With respect to non-shellfish food commodities, Warnekulasuriya et al. (87) reported finding viable *T. gondii* in one of 67 ready-to-eat cured eat samples screened in their study. Ortega et al. (7) found a prevalence of 14.5% and 1.8% of *C. parvum* and *Cyclospora* oocysts, respectively, in their market survey of vegetables collected from a Peruvian periurban slum. Robertson and Gjerde (65) reported that 6% of the fresh fruit and vegetables sampled in their survey were contaminated with either *Cryptosporidium* or *Giardia*, although the pathogen concentrations were generally low.

IX. DISCUSSION AND CONCLUSIONS

Although the advent of molecular methods has revolutionized our ability to detect viral and parasitic contamination in foods, these methods are far from perfect. The reality remains that detection of these agents in foods is done infrequently, usually only in response to known or suspected foodborne disease outbreaks, and rarely are these methods applied routinely. Although there are many reasons for the hesitance to routinely use these assays, the most important among these are: (i) the association (or lack thereof) between detection and viability; (ii) the lack of widely accepted, collaboratively tested methods; and (iii) the expense and need for highly trained personnel.

One of the more serious limitations of molecular methods is their failure to discriminate between viable and inactivated viruses or parasitic protozoan cysts or oocysts. This is of critical importance because the inactivated forms of these pathogens pose no real public health threat, and yet the pathogens might be expected to be at least partially inactivated during routine processing and storage of foods. For instance, some investigators have reported that free viral RNA is stable for days to weeks and that its detection by RT-PCR is not necessarily an indication of infectious

virions (71,148). To avoid positive RT-PCR results that might occur with inactivated viruses, Nuanualsuwan and Cliver (149) pre-treated virus-containing samples with proteinase K and ribonuclease before RT-PCR amplification, finding that amplification was inhibited when inactivated viruses were pre-treated with these enzymes. Others have developed combined mammalian cell culture-RT-PCR methods for virological analysis, attempting to confirm infectivity with the cell culture step (48,150). Unfortunately, this approach will not work for the epidemiologically important non-culturable enteric viruses.

For the parasitic protozoa, the issue of viability is no less important. In general, a dependable indicator of cyst or oocyst infectivity is needed. Although the use of vital dyes and *in vitro* excystation assays have been used to establish the viability for *C. parvum*, recent evidence indicates that these are not always reliable (4). Although not always practical, this organism may also be tested for infectivity using the mouse model (9,56). Molecular tests for *Cryptosporidium* viability have been attempted, including fluorescent *in situ* hybridization (151) and cell culture followed by RT-PCR (152,153). Recently, Jenkins et al. (154) estimated the viability of *C. parvum* oocysts by RT-PCR using a mRNA amyloglucosidase target. The issue is even more complicated for *C. cayetanensis*, for which cell culture and animal models are only in developmental phases (3). It should also be noted that an additional hurdle is the necessity to differentiate between *Cryptosporidium* genotypes that are pathogenic to humans as compared to those that are not. In the past, this has been approached by PCR-RFLP or sequencing analysis of antigen, structural and/or housekeeping genes (4), although recently this has been facilitated by real-time PCR (138).

There is a need to develop more universal sample extraction methods. Although virus and parasitic protozoa concentration from foods is likely to remain at least in part product dependent, research is needed to develop and refine the prototype methods into collaboratively-tested protocols. This has only been done on a limited basis for viruses in shellfish (73) and not at all for the parasitic protozoan methods as applied to foods. In general, as investigators attempt to refine methods, they must continue to seek effective methods to concentrate the pathogens from the food matrix, all the while removing matrix-associated inhibitors. Furthermore, additional experimentation is needed to establish the relationship between detection using molecular amplification techniques and the presence of infectious virus particles or parasitic protozoan cysts and oocysts, in shellfish, other foods, and environmental samples. Since the concepts of viral and protozoan pathogen detection differ so dramatically from those used for the detection of bacteria in foods, scientists need to continue to educate food safety professionals about the principles of pathogen concentration, and provide technology transfer to bring these methods into the commercial realm. However, even with the best developmental efforts, the methods will probably never be perfect and will always require a high degree of sample manipulation on the part of the laboratory personnel. We are hopeful, though, that with sufficient improvement, effective methods for the rapid detection of human enteric viruses and parasitic protozoa in foods can become more widely available to the food safety community.

ACKNOWLEDGMENTS

This effort was supported in part by a grant from the USDA National Research Initiative, Competitive Grants Program, Ensuring Food Safety Division, contact #2002-35201-11610. The use of trade names in this paper does not imply endorsement by the North Carolina Agricultural Research Service nor criticism of similar ones not mentioned.

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189 Methods for Detection of Molds and Mycotoxins

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I. INTRODUCTION

Fungi are a large and diverse group and include the yeasts and molds. They are widely distributed in nature. Due to their versatile nutritional and environmental requirements, fungi are common contaminants of agricultural commodities, foods, beverages, and feeds. The overall economic losses of foods and crops due to mold growth are high (1,2). In addition to economic losses, mold growth on foods and crops presents a health hazard to humans and animals by producing mycotoxins. Before the discovery of aflatoxins, scientists and consumers considered fungal spoilage undesirable only because of economic losses and the unaesthetic and organoleptic changes that result from fungal spoilage. However, after the Turkey X outbreak disease of 1960 that killed 100,000 turkey poults in England, scientists and regulatory officials became aware of the health hazards of mycotoxins (1,3,4,5).

Mycotoxins are naturally occurring compounds produced by some mold species. Mycotoxins can be toxic to humans and animals. The origin of the word mycotoxin is from "mykes," a Greek term for fungus, and "toxicum," a Latin word for poison or toxin. The exposure of humans and animals to mycotoxins has been documented since ancient times. In the Middle Ages ergotism, also known as "St. Anthony's Fire," killed thousands of people in some European countries. The cause of their death was attributed to ergot alkaloids produced by *Claviceps purpurea* that infested rye and other grasses. In the late 1800s and early 1900s a combination of *Penicillium* toxins caused what was called "Yellow rice" outbreak, characterized by severe liver damage in farm animals (3,6,7).

During World War II a human outbreak known as Alimentary Toxic Aleukia (ATA) occurred in Russia due the consumption of moldy grains. The disease was characterized by severe dermal necrosis, hemorrhaging, decrease in leukocytes, and bone marrow degeneration. In 1960 the investigation of the "Turkey X-disease" outbreak led to the discovery of mycotoxins produced by *Aspergillus flavus* subsp. *parasiticus*, known as aflatoxins (for *Aspergillus flavus* toxins) (3,4). The production of mycotoxins occurs in the field, and during distribution, processing, and storage. The production of mycotoxins presents a unique challenge to food safety experts due to the fact that mycotoxins are natural toxins and their production is sometimes unavoidable, and depends on various environmental factors in the field and during storage conditions. The main mold producers of mycotoxins include *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* (4,8,9,10). Various environmental factors affect the growth of molds and the production of mycotoxins, including temperature, pH, moisture content, oxygen levels, carbohydrates, trace elements, the mold strains, and microbial competition.

There is no direct relationship between the presence of molds in foods and crops and the production of mycotoxins. On the other hand, the absence of mold fragments and/or mold spores in foods and crops does not guarantee that they are free from mycotoxins, because these toxins are heat resistant and can survive long after the molds have been destroyed or inactivated. A number of mycotoxins produced by different mold species have been reported in the literature. The majority of these toxins have been investigated

under laboratory conditions, and only limited numbers of them have been encountered in nature.

The major mycotoxins that are of concern to human and animal health include aflatoxins, ochratoxins, patulin, zearalenone, fumonisins, moniliformin, and trichothecenes (Table 189.1).

Aflatoxins are secondary metabolic products produced by *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* (11) in a number of commodities, especially corn and peanuts. Other nuts and grains are susceptible to aflatoxins as well. The main aflatoxins of concern are B_1, B_2, G_1, and G_2. Generally these toxins are found together at various levels. Aflatoxin B_1 is considered the most potent hepatocarcinogen to animals and presumably to humans. Generally young animals are more susceptible to aflatoxins than older animals (12).

Ochratoxin A is a naturally occurring mycotoxin produced by certain species of the genera of *Aspergillus* and *Penicillium*, such as *Penicillium verrucosum* and *Asepergillus ochraceus*. Ochratoxin A is linked to kidney damage (nephrotoxic) in animals and humans. A number of investigations have reported the presence of ochratoxin A in animal tissues, human blood serum, and milk. Ochratoxin A also has been reported to affect the immune system. Ochratoxin is commonly found in corn, barley, wheat, oats, and coffee beans (6,13). Another mycotoxin that usually coexists with ochratoxin is citrinin, which is produced by some *Aspergillus* and *Penicillium* species and causes kidney damage and mild liver damage.

Patulin is a low molecular weight mycotoxin that is produced by a number of *Penicillium* species (especially *P. expansum*), *Aspergillus*, and *Byssochlamys* species. *P. expansum* is the usual mold causing apple rot (6). These molds are the most important producers of patulin in apples, apple juice, and other apple derived products. Patulin has been reported to have immunological, neurological, and gastrointestinal effects on animals. The potential presence of patulin in products that are consumed by children and adults, such as apple juice and apple cider, constitutes a significant a health risk.

Fumonisins, especially Fumonisin B_1 and Fumonisin B_2, are produced in corn by *Fusarium moniliforme*, *F. proliferatum* and other *Fusarium* species. Fumonisins were found to be extremely toxic to horses, causing what is known as equine leucoencephalomalacia (ELEM), which is characterized by loss of appetite, brain liquefaction, liver damage, and respiratory complications (6,8,14). A number of studies have also linked fumonisins and the occurrence of esophageal cancer in humans in northern Italy, South Africa, and China.

Trichothecenes are a group of 148 related compounds produced by the following *Fusarium* species: *F. tricinctum*, *F. sporotrichioides*, *F. poe*, and *F. equiseti*. The most common trichthecenes isolated from agricultural commodities include T-2 toxin, HT-2 toxin, neosolaniol, deoxynivalenol (DON), 3-acetyldeoxynivalenol, and nivalenol. Trichothecenes have been isolated from corn, oats, peanuts, rice, sorghum, wheat, barley, rye, corn meal,

TABLE 189.1
Important Mycotoxigenic Molds and Their Mycotoxins Found in Foods

Mycotoxin	Primary Mold Species	Food Commodity
Afaltoxins B_1,B_2, G_1, G_2	*Aspergillus flavus* *Aspergillus nomius*	Peanuts, corn, dried fruits, spices
Afaltoxins B_1, B_2	*Aspergillus parasiticus*	Peanuts, corn, dried fruits, spices
Aflatoxins M_1, M_2	*Aspergillus flavus*	Milk and dairy products
Ochratoxins	*Penicillium verrucosum* *Aspergillus ochraceus*	Cereals, coffee beans, beer, nuts
Patulin	*Penicillium expansum*	Apples, apple juice…
Citrinin	*Penicillium verrucosum*	Cereals
Deoxynivalenol	*Fusarium graminarium*	Cereals
Nivalenol	*Fusarium culmorum*	
Trichothecens		
Fumonisins	*Fusarium moniliforme* *Fusarium proliferatum*	Corn, corn products
Zearalenone	*Fusarium graminearum* *Fusarium culmorum*	Cereals
Moniliformin	*Fusarium proliferatum* *Fusarium subglutinans*	Cereals
Alternariol	*Alternaria alternata* *Alternaria tenuis*	Tomatoes, fruits, oil seeds, cereals

breakfast cereals, bread, and snack foods. Trichothecenes are toxic to animals at the cellular and organic system level. They inhibit the synthesis of proteins. Some of the common symptoms include refusal to eat, weight loss, digestive disorders, dermal irritation, vomiting, diarrhea, hemorrhage of the large intestine, and weakening of the immune system (6,8).

Moniloformin is another common mycotoxin that is produced by *Fusarium* species. It has been detected in cereals such as wheat and rice. Moniloformin produces intestinal hemorrhage and heart problems in laboratory mice. It has been linked to Keshan disease in humans in certain areas of China.

Alternaria toxins are a group of compounds produced by *Alternaria* species including *A. citri*, *A. alternata*, and *A. tenuissima*. These are common contaminants of crops, especially fruits, vegetables, and oilseeds at the preharvest stage. The most commonly detected *Alternaria* toxins are tenuazonic acid, alternariol, and alternariol monomethyl ether. These toxins have been linked to a number of disorders in humans and animals (3,6).

Other mycotoxins have been reported in the literature. These include citreoviridin, gliotoxin, ergot alkaloids, mycophenolic acid, penicillic acid, PR toxin, roquefortine C, Rubratoxin A, vioxanthin, and xanthomegnin.

II. MOLD DETECTION AND ENUMERATION: GENERAL CONSIDERATIONS

Accurate enumeration and identification of spoilage and mycotoxigenic molds is essential to the control of these fungi in the food supply (11,15–17). The current food mycology techniques that are used to enumerate mold spores and propagules consist of microscopic examination and plate count techniques, which are inherited from food bacteriology. Measurement of mold growth is more complicated than bacterial counts. The significance of mold count has to be carefully interpreted, since the mold colony counts can result from mold fragments and/or individual mold spores. A heavy mold sporulation that can result in a significant increase of viable counts does not automatically reflect an increase in the fungal biomass. In addition the blending of samples can easily increase the counts by breaking mycelial fragments into smaller filaments. The results of mold counts should take into consideration a number of points including the history of the food product, its chemical composition, and the sampling technique. Recently, progress has been made in the development of potential rapid methods for the detection of molds in foods and feed. These potential rapid techniques can be grouped in the following categories: chemical methods, direct methods, immunological methods, and genetic methods (18).

Any mycological methods should use a proper sampling method. The sampling plans developed by the International Commission on Microbiological Specifications of Foods (ICMSF) are suitable for food mycology (2,19).

Direct plating and dilution plating are the two main methods used to detect and enumerate molds in foods. Direct plating is generally more accurate than other plating techniques. In direct plating, solid food particles are deposited on solid media. Sometimes the food particles such as grain kernels are disinfected in chlorine solution to eliminate surface contamination. The results of direct plating are expressed as percentage of infected particles. Dilution plating is recommended for powdered and liquid foods. Generally the sample is blended for two minutes using a stomacher. To dilute powdered foods and grains intended for flour manufacturing, peptone diluent (0.1%) is recommended. After serial dilutions, 0.1 ml aliquots are surface plated on an agar surface, with plates incubated at 25°C for five days. It is recommended that plates should not be stacked more than three high and they should not be inverted. The plates should not be disturbed during the incubation period. After incubation, plates containing between 10–150 mold colonies are counted.

III. SELECTION OF MEDIA

The selection of agar media is critical in the success of mold detection and enumeration in foods and feeds. A general purpose mycological medium should satisfy a number of considerations: inhibition of bacterial growth without affecting fungal growth, suppression of rapidly spreading fungi such as *Rhizopus* species, and support the growth of the target fungi. Generally these media contain both differential and selective ingredients. To inhibit bacterial growth, antibiotics such as chloramphenicol and chlorotetracycline are used. To slow colony spread, rose Bengal and 2,6-dichloro-4-nitoalinin (dichloran) are used. Another important consideration is the water activity of the agar medium and the food to be analyzed. For help in proper selection of mycological media the reader is referred to Table 189.2. To simplify preparation, 3M Microbiology Products (Minneapolis, MN) developed Petri film yeast- and mold-count plates (Petrifilm YM). Comparison studies have shown that Petrifilm YM plates to be comparable to traditional methods, with the advantage of time savings, because there is no need for media preparation and addition of antibiotics (19–24).

IV. ENUMERATION AND DETECTION OF MYCOTOXIGENIC MOLDS

The versatile nature of molds in their environment and their unique nutritional requirements allow them to grow in many agricultural commodities and food products. Molds are generally aerobic microorganisms; however, a number of mold species can grow at very low oxygen level. Their pH requirements are broad, ranging from pH

TABLE 189.2
Recommended Agar Media for the General Detection, Enumeration, and Isolation of Molds

Food Products	Type of Molds	Medium*
High water activity fresh foods (milk, fruits, cheese)	Molds in general	DRBC, MA PDA, PCA + antibiotics
Cereal grains and nuts freshly harvested	Molds in general	DRBC
Dried foods including stored cereals	Molds in general	DG18
Flour	Molds in general	DG18
Confectionary	Xerophiles	MY50G
	Extreme xerophiles	MY70GF
Salty foods	Molds in general	DG18
	Halophilic fungi	MY5-12 My10-12
Any food	Afaltoxigenic molds	AFPA
Any food	Ochratoxigenic molds	DRYS
Any food	*Fusarium* species	CZID

*AFPA- *Aspergillus flavus* and *parasiticus* agar; CZID – Czapek iprodione dichloran agar; DRBC – Dichloran rose Bengal chloramphenicol; DG18 – Dichloran 18% glycerol agar; DRYS – Dichloran rose Bengal yeast extract sucrose agar; MEA – Malt Extract Agar; MY50G – Malt extract yeast extract 50% glucose agar; MY70GF – Malt extract yeast extract 70% glucose fructose agar; MY5-12 and MY10-12 – Malt extract yeast extract 5% (or 10%) salt 12% glucose agar; PCA – Plate count agar; PDA – Potato dextrose agar.
Source: Pitt and Hocking (2), Tournas et al. (19).

2 to 9.5. The majority of molds can grow at water activity (a_w) of 0.85 or less. The majority of molds will grow at temperature range of 10–35°C. There are a number of *Penicillium* and *Cladosporium* species that can grow at refrigeration temperatures. Other factors that affect mold growth and mycotoxin production include the nutritional status of the substrate, its physical state, presence of preservatives, and competitive microorganisms.

The three major mycotoxin mold producers are *Aspergillus*, *Penicillium*, and *Fusarium* species. *Aspergillus* and *Penicillium* species mainly attack commodities during storage, while *Fusarium* species are generally considered field fungi. Mycotoxin production is more prevalent in commodities such as corn, peanuts, nuts, wheat, and barley. Due to the fact that mycotoxins are natural fungal toxins, the total prevention and control of their production in the field and storage is almost impossible. However, an accurate detection and identification of mycotoxigenic molds, in combination with other measures, is essential in minimizing the problem of mycotoxins (2,25).

During the last 20 years, food mycologists are increasingly developing various selective and differential media to enumerate and detect specific mold species.

Selective mycological media will help the isolation and the identification of mycotoxigenic molds such as *A. flavus* strains, producers of aflatoxins. Although good progress has been made in the development of selective media, a significant level of research is still needed to improve the existing media and to develop new ones. One of the first selective media for *A. flavus* was called *Aspergillus* differential medium (ADM). The main ingredients include 1% yeast extract, 1.5% tryptone, and 0.05% ferric acid. In this medium *A. flavus* and *A. parasiticus* are identified by their characteristic production of reverse orange-yellow color (9,25,26). Only a few other molds such as *A. niger* produce the same reverse coloration. It was demonstrated that the production of the orange-yellow color is due to the reaction of aspergillic acid that is produced by the mold and the ferric ammonium citrate (27). ADM was reformulated to develop *Aspergillus flavus-parasiticus* agar (AFPA). When AFPA is incubated at 30°C for 42–48 hours, aflatoxigenic molds (*A. flavus*, *A. parasiticus*, and *A. nomius*) are diagnosed by their production of bright orange-yellow reverse color prior to their production of olive green conidia. *A. niger* and *A. ochraceus* have been found to produce false positive results. However, after 48 hours incubation, these mold species start to produce their characteristic colors that are different from the color of the aflatoxigenic molds. AFPA is generally used to detect aflatoxigenic molds in nuts, corn, peanuts, various seeds, animal feed, and other commodities.

Frisvad (28,29) developed a selective medium (DRYES) for molds producing ochratoxin A, by adding 50 mg/L chloramphenicol, 50 mg/L chlorotetracycline, 2 mg/L dichloran, and 25 mg/L rose bengal to yeast-extract

sucrose agar. The main producer of ochratoxin, *P. verrucosum* produces yellow pigment on the colony reverse. DRYES was reported to distinguish between producers of ochratoxin A and citrinin (*P. verrucosum*) and producers of xanthomegnin and viomellein (*P. viridicatum* and *P. aurantiogriseum*). It is recommended to incubate DRYES at 20°C for 7–8 days.

To isolate *Fusarium* species, the following selective media have been reported (2,25,30,31): Nash-Snyder medium, modified Czapek-Dox agar, Czapek iprodione-dichloran (CZID) agar, potato-dextrose-iprodione-dichloran (PDID), and dichloran-chloramphenicol-peptone agar (DCPA). In Nash-Snyder medium, pentacholoronitrobenzene (PCNB) is the selective ingredient. However, the potential carcinogenic properties of PCNB limited its use in food mycology laboratories. DCPA is recommended when *Fusarium* species are dominant due to their high production of macroconidia. When these selective media for *Fusarium* species were compared, PDID and CZID were found more effective. Identification of *Fusarium* isolates in CZID is more difficult; therefore, their culture on Carnation Leaf Agar (CLA) is recommended.

V. DETECTION OF XEROPHILIC MOLDS

Xerophilic fungi are characterized by their ability to grow under low to very low water activity (a_w). Moderately xerophilic molds can grow on normal media with high a_w, while extreme xerophiles need a medium with low a_w. Media for xerophilic fungi should be able to inhibit the growth of non-xerophilc microorganisms due to addition of various humectants such as glucose, sucrose, sorbitol, and glycerol. These media are useful for the analysis of high-sugar food products. Addition of sodium chloride to media is recommended for the analysis of high-salt foods. Dichloran –18% glycerol agar (DG18) is recommended as a general medium for the isolation of the moderately xerophilic molds in dry products. Fastidious xerophiles, such as *Xeromyces bisporus, Chrysosporium* spp., and *Eremascus*, grow poorly on DG18 and require other media such as malt extract-yeast extract –50% glucose (MY50G) agar. Pitt and Hocking recommend the use of malt extract-yeast extract –70% glucose-fructose (MY70GF) agar to isolate fastidious xerophiles (2,32–34).

VI. DETECTION OF HEAT RESISTANT MOLDS

Heat resistant molds such as *Byssochlamys, Talaromyces*, *Neosartorya,* and *Eupenicillium* species, are a unique group of fungi in that they can cause the spoilage of shelf stable foods and can survive standard heat processing operations. The isolation of heat resistant molds from heat processed food products such as fruit juices and concentrates is accomplished by using a plating method or direct incubation method after heating the samples at 75–80°C for 30 minutes. It is recommended to heat a 100 g sample that then should be dispersed in an equal volume of double strength agar medium of a defined composition. Generally, Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) and Oxytetracycline Glucose Yeast Extract (OGY) supplemented with antibiotics are used. The plates are incubated at 25–30°C for two to four weeks (2,35,36).

VII. RAPID DETECTION METHODS

Current methods, such as direct plating and dilution plating, that are used to detect and enumerate molds in food and feed are time consuming and do not offer rapid assessment of the quality of foods and ingredients. In the last 10 to 15 years, a number of potential rapid techniques have been investigated. These techniques include chemical and biochemical techniques, electrical impedence, and immunological methods (18,37–39). The chemical and biochemical techniques are based on the detection and quantification of specific components of the molds, such as chitin, ergosterol, and adenosine triphosphate (ATP).

Chitin, a polymer of N-acetyl-D-glucosamine, is one of the most frequently occurring polysaccharides in mold spores and mycelia. One of the major non-fungal sources of chitin are insects. However, chitin does not exist in bacteria and in the absence of insect infestation is not found in food and feed products. Therefore, the detection of chitin in foods and feeds can indicate fungal activity, even if the mold contaminants have been destroyed. There is a good correlation between chitin or its breakdown compounds (glucosamine, N-acetylglucosamine) and mycelial growth in cereal grains. Methods such as colorimetry, chromatography (HPLC, GC), microscopy with fluorescent and non-fluorescent dyes, and infrared spectroscopy have been used to detect chitin. The disadvantages of these assays are that sophisticated instruments are used, and the methods have low sensitivity and reproducibility problems (40–43).

Ergosterol is another fungal chemical that has been suggested for use to measure fungal growth in cereal grains. Ergosterol is the principal sterol in fungal membranes. Ergosterol does not occur in high quantities in plants. There is some correlation between ergosterol level and mold growth and associated mycotoxin production. The methods of analysis for ergosterol are based on direct saponification, extraction with hexane, and quantification using high-pressure liquid chromatography (HPLC), thin liquid chromatography (TLC), liquid chromatography (LC), or spectrophotometric techniques (UV, infrared) (41,44–50).

Research on the use of immunological techniques to detect food-borne fungi has also been active for the last 10 to 15 years (51). These techniques are based on the binding of fungal antigens and their corresponding antibodies.

Fungal antigens have been found to be heat resistant, and they can last after the destruction of the mold species. The fungal antigens used were mainly mold spores, fungal proteins, extracellular polysaccharides, and ergosterol. One of the major issues related to the detection of molds using immunological techniques is the lack of specificity and cross reactivity with food components (38,52–57).

Other potential rapid detection techniques include genetic methods (i.e., DNA fingerprinting, polymerase chain reaction, and DNA-DNA hybridization), electrical impedence methods, automated hydrophobic grid membrane filter techniques, and detection of fungal volatile compounds (58–64). More research is needed in order for all these promising methods to be applicable for routine and online analysis.

VIII. DETECTION OF MYCOTOXINS

There is no single method that is appropriate for the detection of all mycotoxins. These methods depend on many factors including the chemical nature of the mycotoxin and the substrate. Appropriate sampling methods are critical for the accurate detection and quantification of mycotoxins in foods and feeds. The routine analytical steps for the analysis of mycotoxins include sample preparation, extraction, clean up, qualitative detection, confirmation, and quantification.

Mycotoxin extraction is generally done using polar solvents such as methanol, acetone, and chloroform. For some mycotoxins a combination of organic solvents and water or water alone are used. Usually these extracts are cleaned to remove impurities by using liquid-liquid partitioning or column clean up, such as immuno-affinity cartridges. These cartridges contain monoclonal antibodies that are specific to the target mycotoxin(s). Different mycotoxins or other chemical components in the sample are separated by thin-layer chromatography (TLC), gas liquid chromatography (GLC), or high performance liquid chromatography (HPLC). Quantification is done using fluorodensitometry or fluorescence detection with HPLC. Other detection methods use U.V. absorbance, electrochemical, or flame ionization. Naturally occurring mycotoxins should be determined using the methods outlined in the Official Methods of Analysis (25,65,66). Recently a number of immunoassay methods, such as enzyme-linked immunosorbent assays (ELISA) have been developed and are commercially available (Table 189.3). The big advantage of these immunoassay techniques is that they are simple and practical to use for qualitative screening of a large number of samples.

IX. SUMMARY

Despite recent advances in the detection of foodborne molds, many practitioners still use very traditional methods (67). Much of these methods are based on macroscopic and microscopic techniques. As such, there is potential risk that this knowledge will not be adequately passed on to the next generation of mycologists. Perhaps more modern methods will replace the traditional techniques before food microbiologists are pondering the need for a fungal analysis.

TABLE 189.3
Commercially Available Immunoassay Test Kits

Company	Test Kit	Mycotoxin
Neogen Corporation (www.neogen.com)	Microwell ELISA	Aflatoxin, Vomitoxin, Fumonisin, Ochratoxin, Zearalenone, T-2 toxin
Vicam LP (www.vicam.com)	Immunoaffinity Column	Aflatoxin, Vomitoxin, Fumonisin, Zearalenone, Ochratoxin
R-Biopharm (www.rbiopharm.com)	Microwell ELISA	Afaltoxin, Vomitoxin, Fumonisin, Zearalenone, T-2 toxin
Editek (Medtox Laboratories) (www.medtox.com)	Card ELISA	Aflatoxin, Zearalenone, T-2 toxin, Ochratoxin
International Diagnostic Systems (www.ids-kits.com)	Cup ELISA	Aflatoxins, Fumonisin B_1
Romer Laboratories (www.romerlabs.com)	Clean-up	Aflatoxin, Ochratoxin, Patulin, DON, T-2 toxin, Zearalenone, Fumonisins, Sterigmatocystin

Source: Bullerman and Gourama (25).

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Part V

Water Technology

190 Water: Sources and Properties

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CONTENTS

I. INTRODUCTION

Source water quality is not the same all over the world. Arguably, it has the most variability of any raw material associated with the food industry. Deep wells, shallow wells, reservoirs, lakes, streams, rivers, even the oceans themselves are potential sources of useable water. The control and management of these water sources can vary along a spectrum from completely private control (for example, a plant well) to completely public control (for example, a municipal water source). Irrespective of whether a food plant has its own well, or it draws its water from a modern municipal treatment plant, each individual water supply presents its own particular problems. This chapter discusses the importance of water sources to the food industry and provides information on minimizing risk through the use of source selection and protection strategies.

II. WHY SOURCE WATERS ARE CRITICAL TO THE FOOD INDUSTRY

A. Contaminants in Water Supplies

Contaminants in water supplies present a danger to the taste, odor and appearance of food products. In extreme cases there are potential risks regarding public health.

Physical discrepancies in water, such as high turbidity, off-colors, off-odors or off-tastes, can have an almost immediate effect on the flavor, appearance or shelf-life of the food products it is used to manufacture. Chemicals and minerals present in the water supply can also have adverse effects. For example, high salts content can change the taste of the food products, thereby making them unpalatable and unacceptable to the consumer. Another example is high alkalinity which can quickly neutralize the delicate acidity of carbonated beverages, lessening their "tang" and making them more susceptible to spoilage. Microorganisms, algae, or even small amounts of organic matter in the water can cause unsightly sediment to develop in food products and lead to premature spoilage. A particular danger when using a surface water supply (for example, reservoir, lake, stream or river) is where seasonal changes result in "blooms" of algae or "inversion," where organic debris rises from the basin. In addition to the debris that is produced, microbial metabolites from several types of algae can produce highly sensory-active organic compounds. Two of the more common compounds are *geosmin* and methylisoborneol which are sensory active at nanogram per liter (ng/l) concentrations and have been a major cause of sensory complaints at municipal water works and some food plants. These concerns, in addition to a growing number of organic compounds being detected in water supplies historically considered safe and of high quality, make the quality of source water and its appropriate treatment extremely important to the food industry.

B. Regulations

On a world-wide basis, our knowledge regarding the effects of contaminants in water is increasing. Much of this advancement is due to the huge progress in the area of analytical chemistry. Water testing instruments are becoming more and more sensitive and sophisticated. Coupled with this is the fact that water has such a simple sample matrix. The result is the measurement of trace materials in water at levels previously impossible. Test results reported in parts per billion and parts per trillion are now commonplace. Regulatory agencies, understanding the importance of water quality to the health of the population, and now having analytical tools to support them, are striving to make water supplies as safe as possible. As a result, drinking water regulations around the globe are in a period of change and refinement. Source waters for products such as natural mineral waters, or other bottled water categories, often must meet extremely stringent requirements and the allowable treatments are often limited.

It is important that food plants be in compliance with all regulations regarding their water source, as well as those relating to water treatment and treated water quality. As analytical capabilities increase and the limits that instruments can detect decrease, regulators, suppliers and end users of water will be forced to continually learn and refresh their understanding of the nature and adverse effects of all potential contaminants.

C. Consumer Awareness and Sensitivities

Water is like no other food industry raw material in terms of consumer knowledge and sensitivity. Water quality-related issues are increasingly apparent in the public domain. Almost any day newspapers will contain some water-related issue. *Cryptosporidium parvum* or *Giardia lamblia* outbreaks, *E. coli* contamination, algae blooms, high arsenic levels, methyl-tertiary-butyl-ether in groundwater, disinfection by-products such as trihalomethanes or bromate and depletion of water resources are some of the more common examples of this. This media coverage has helped increase the global awareness of the quality of water, something that for centuries has been taken for granted. This heightened consciousness underscores the need for water and its environmental and ecological considerations to be valued and adequately addressed by food companies.

D. Treatment Selection

The list of possible contaminants in a given source water supply could literally take all day to review. We should recognize this variability and know as much as possible about the water source that is used at each food processing location. Chemical, physical, microbiological and radiological analytical data can provide valuable information with respect to the characteristics of a given source water supply. Historical data, seasonal variation and plans for development of the source and surrounding areas can help determine its consistency. Water treatment system design at each food plant should be based on this complete understanding and is source specific.

E. If Something Goes Wrong, Alternative Water Sources Are Usually Limited

Water is not like other raw materials that can usually be procured from an alternative supplier if there is a problem. Droughts can result in reduced availability of water at certain times of the year. In some coastal areas, it is not uncommon that intensive pumping of fresh groundwater will cause salt water to intrude into fresh water aquifers. Contamination from accidental spills, waste disposal, industrial effluent, run-off and numerous other events can potentially compromise the quality of a plant's source water supply. Intentional contamination, such as terrorist activity, could easily result in widespread contamination, as relatively small amounts of a contaminant can have substantial effects. For example, the U.S. Environmental Protection

Agency estimates that less than one gallon of gasoline can contaminate one million gallons of groundwater to the point where it is unusable for drinking purposes (1).

III. WATER SOURCE PROPERTIES

Clearly, both the quality and quantity of water available to food plants need to be preserved. The selection, protection and subsequent treatment design are paramount to helping assure that only a safe, consistent, high quality treated water is used. This requires a thorough understanding of the characteristics of the source water supply. The following paragraphs will provide an overview of different types of source water supplies and their attributes.

A. Comparison of Surface and Groundwater Sources

Water sources may be classified into two major categories, namely groundwater and surface water. Examples of surface waters include reservoirs, lakes, streams, rivers and oceans. Examples of groundwater include confined and unconfined aquifers, artesian supplies and springs. The two categories differ greatly in the characteristics of the water they supply, as does water from groups even within the same category. Due to this wide variability, it is not possible to describe with certainty what a "typical" surface or ground water looks like in terms of its chemistry. The best we can do is provide generalities, see Table 190.1, with the condition that there are truly exceptions to every rule.

TABLE 190.1
Relative Comparison of Ground and Surface Water Supplies

Parameter	Ground Water	Surface Water
Total Dissolved Solids (TDS)	Higher	Lower
Suspended solids	Lower	Higher
Turbidity and color	Lower	Higher
Alkalinity	Higher	Lower
Total Organic Carbon (TOC)	Lower	Higher
Microbiology		
• Protection from bacteria and viruses	Highly protected	Highly susceptible
• Protection from protozoa	Highly protected	Highly susceptible
• Presence of iron and/or manganese bacteria	Common	Rare
Hydrogen sulfide gas	Common	Uncommon
Aeration/dissolved oxygen	Lower	Higher
Temperature	More consistent	More variable
Flow rate	Very slow	Very fast
Flow pattern	Laminar	Turbulent
Susceptibility to pollution via surface run-off	Low	High
Time for a contaminant plume to resolve	Very Long	Usually short

Source: Adapted from Bena (2001).

B. Surface Water Sources

In general, surface supplies can be highly variable in every respect—chemically (total dissolved solids, alkalinity, etc.), microbiologically (bacteria, viruses, protozoan "cysts," etc.) and physically (turbidity, color, etc.). Many surface waters are subjected to contamination which may present in many forms, including:

1. Bacteria and other organisms from animal wastes via direct introduction (animals) or indirect introduction (poorly or untreated wastewater)
2. Algae blooms, which are typically acute, seasonal events
3. "Natural" chemical contamination as evidenced by high levels of natural organic matter (primarily the humic substances from decaying vegetation and animal waste)
4. "Synthetic" chemical contamination from surface run-off (outflow) of agricultural chemicals (pesticides, herbicides, insecticides, etc.)
5. Intentional human contamination (sabotage, terrorism, war, etc.).

Again, it must be underscored that the characteristics above, and those that follow, are intended to provide general trends in terms of water composition and characteristics. Exceptions to virtually every characteristic described have been observed.

1. Lakes

Due to their relatively stagnant flow patterns and long water residence time, natural lakes are usually of consistent composition relative to other types of surface supplies. One major climatic event which may have drastic changes in lake water quality is seasonal "inversion." This refers to the phenomenon whereby water at the surface of the lake reaches a temperature at which it is most dense (3.98°C, or 39.2°F). The water below it has not yet reached this state, and density and temperature gradients are therefore formed. This "heavy" water then begins to descend, and displaces the water below it. The displaced water then inverts (hence the term "inversion") and moves from the bottom of the lake toward the upper portion. The resulting agitation brings with it much of the sediment, and associated unwanted components, which make municipal and industrial monitoring even more critical during this time. As you might imagine, both the phenomenon of inversion and its related thermal stratification, are actually much more complex. Their effects on aquatic life and the eutrophication process (basically, the

nutrient enrichment of a body of water, usually lakes or ponds, which results in algae growth or "blooms") have been studied under many other scientific disciplines. Unfortunately, industrial waste effluent and sewage treatment plant discharge are often re-introduced into the same body of water that originally supplied the influent water (this is also often the case for many large streams). These practices are coming under more scrutiny and there is ever increasing political pressure for regulatory reform.

2. Reservoirs

Reservoirs, or "man-made" lakes, are similar in overall characteristics to those described above for natural lakes. They are often regarded as huge storage reservoirs for municipal water supplies prior to treatment. As surface supplies go, reservoirs are of fairly consistent quality, reasonable turbidity (due in large part to natural oxidation and settling mechanisms) and often afford lower bacteria counts than other surface supplies (2). However, as a consequence of their relatively low flow patterns and lack of agitation, algae blooms are often a problem.

3. Streams

In general, streams are often of reasonable chemical/physical composition. Due to their locations and physical dimensions they offer easy access for a multitude of animal life. With this comes the frequent introduction of microorganisms of fecal origin, in addition to appreciable amounts of organic matter. This organic material is typically considered to be the precursor material of trihalomethanes and a host of other chemical by-products which can be formed once this water supply is disinfected. Smaller streams are often influenced by rain events, whereby their flows are increased, with subsequent increase in suspended solids and turbidity. Larger streams are generally at higher risk of having industrial waste (often poorly or inadequately treated) discharged into them. They are often more of a concern, due in part to their larger surface area, in terms of accepting surface run-off and sub-surface drainage.

4. Rivers

Rivers represent perhaps the most difficult of the surface water supplies to address. In general, they are of highly inconsistent quality, very high turbidity and suspended solids, prone to considerable temperature fluctuations and vary widely with respect to their flow patterns (that is, areas of excessive turbulence and areas of minimal movement may exist within close proximity to each other). Rivers, as a result of their great length and flows, are recipients of surface run-off from many types of areas. For example, a river might flow across areas of agrarian activity, with the potential to incorporate pesticides, herbicides, nitrate, and other contaminants along its route. This same river might later flow through an industrial zone, pick up run-off from poorly contained chemical storage tanks, drains, sewers, etc. and possibly even meet the discharge of one or more waste treatment plants! Imagine the contaminant "soup" that would result. For this reason, any methods of treatment for river water must be preceded by thorough characterization of the supply. The treatment itself must be capable of addressing a wide range of quickly changing water qualities.

C. Groundwater Supplies

In comparison to surface supplies, groundwater supplies are generally more consistent in every respect—thermally, microbially, chemically and physically. They have historically been considered a much safer supply, or one which produces "purer" water. Two hundred years ago, this blanket statement might have been more universally true. However, along with the development of the chemical and related industries came the increased potential for groundwater contamination. With increasing industry, came increasing underground chemical storage, increasing contaminant spills, increasing numbers of poorly located and/or poorly constructed septic systems, along with a host of other problems for the groundwater environment that needed to be addressed (in fact, many still do).

As with surface water supplies, there are different classifications of groundwater, with some being more preferred than others in terms of providing consistently good quality water. A brief discussion of each, in terms understood by the novice hydrogeologist (which is a discipline becoming increasingly important for anyone involved with water operations to know), follows below.

1. Aquifers and the Underground Environment

An aquifer may be defined as "a geologic formation with sufficient interconnected porosity and permeability to store and transmit significant quantities of water under natural hydraulic gradients"(3). Critical terms in this description are "store," "transmit," and "significant quantities." All three should be satisfied to consider a supply an aquifer. The first two are straightforward. For example, huge amounts may be stored but unable to be transmitted, in which case this supply, however large, should not be considered an aquifer. The third term, "significant quantities" is more nebulous and linked to the intended use of the supply, such as a residential well, a large municipality, or multiple industrial users tapped into a community well. Clearly, "significant quantities" would be defined differently for these three applications.

Figure 190.1 describes the different layers or zones encountered as we move downward from the surface of the ground to the water bearing strata below it. As we move from the surface, the first zone encountered is the

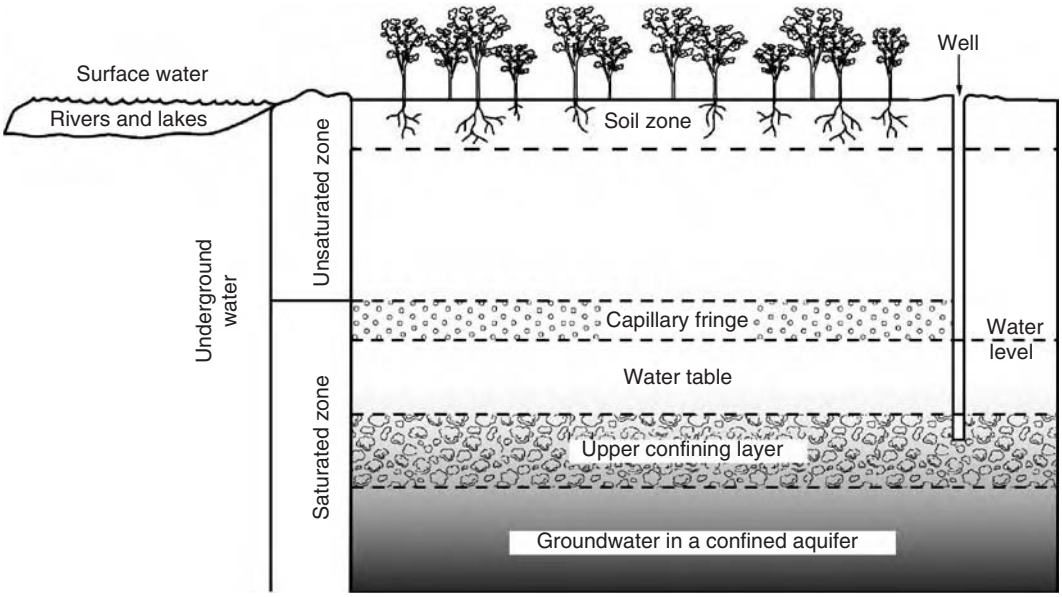

FIGURE 190.1 The underground environment.

"unsaturated zone" (also referred to as the "vadose zone"). In this area, the geologic media (dirt, clay, sand, etc.) contains a mixture of water and void spaces with air—hence, the terms "unsaturated" or "variably saturated." Continuing downward, we reach the "capillary fringe," which is generally considered the beginning of the "saturated zone," but is sometimes considered a distinct entity. This interface between unsaturated and saturated zones is not completely understood and is the subject of much study with regard to movement of certain contaminants within it. The "saturated zone" is the area where air is at a minimum and water is at a maximum. The geologic media here are saturated with water. Dissolved oxygen in the saturated zone is extremely rare. Many deep groundwater formations exist under anaerobic or hypoxic conditions. It is within the saturated zone where we find our aquifers and where most production wells are placed. Throughout the saturated zone, many strata of varying permeability will be found. Most importantly, this is where groundwater supplies are found.

2. Unconfined and Confined Aquifers

Aquifers may be grouped into the two broad categories of "unconfined" and "confined." Unconfined aquifers (sometimes referred to as "water table aquifers") are those water bearing, geologic formations which are under atmospheric pressure at their upper boundary. The "water table" (sometimes referred to as the "phreatic surface") is the upper boundary of the saturated zone. Water levels in wells which tap unconfined aquifers should be the same as the level of the water table. The plane which connects the upper levels of water in all wells which penetrate unconfined aquifers is known as the "potentiometric surface." Confined aquifers (sometimes called "artesian aquifers") are those water bearing geologic formations whose upper and lower boundaries are comprised of geologic material of low permeability, and which are under pressure greater than atmospheric.

In the case of confined aquifers, due to the internal pressures, the water levels in wells which tap them may often exceed the level of the water table (which may result in a "flowing artesian well" or "spring"). In the food industry, conversational knowledge of confined and unconfined aquifers will account for nearly all of the hydrogeologic discussions into which a food technologist might become engaged.

In addition to aquifers being classified as confined or unconfined, the geologic material of the aquifer may be described as consolidated or unconsolidated. "Unconsolidated" deposits are formed from loose geologic material, such as sand, clay, silt, gravel, and even sea-shell remains. "Consolidated" deposits are formed by mineral particles combining from heat and pressure, or via chemical mechanisms. They include sedimentary rocks, such as limestone, dolomite, shale, and sandstone; igneous (formed from molten) rocks, such as granite and basalt; and metamorphic rocks, such as limestone and gneiss (4). Fractured rock formations almost always refer to fractures or fissures in consolidated deposits. Ground water and contaminant flows through this type of formation are highly unpredictable, since it is difficult to determine

which route the water will take through this hard rock maze. Carbonate aquifers (also known as "karstic formations") are formations of limestone and other water-soluble rocks whose fractures have been widened by erosion to form sinkholes, caves, or tunnels (5). As you might expect, with such little resistance, the flows through fractured and carbonate formations can be rapid enough to rival surface water sources. Flows, though rare, have been reported up to 450 meters (approximately 1500 feet) per day (5).

D. Municipal Supplies

Municipal supplies of water may come from either surface or groundwater supplies, or a mixture of the two. In many cases, the regulatory oversight of municipal water treatment plants and distribution systems affords water of normally very high quality. However, this should not be depended upon as a guarantee. Even if a food plant is lucky enough to have a normally high quality municipal supply, it is still necessary for them to monitor their source water and have appropriate water treatment systems. This is largely due to the fact that problems can occur with municipal treatment and distribution, as will be discussed below. It is also partly because municipalities have different treatment objectives and requirements to food industry plants. For example, high total dissolved solids, high alkalinity levels and disinfectant residuals (e.g. chlorine) might be acceptable in municipally treated water but can adversely impact the quality of many food products.

Some of the chemicals used by municipalities can lead to problems with food plant water treatment systems. For example, chloramines which are often used for disinfection purposes by municipalities (or may form unintentionally through municipal chlorination if ammonia is present in the source water supply) can negatively affect food plant water treatment operations, especially those which operate conventional coagulation water treatment systems. Conventional coagulation systems are also especially prone to gross upset to the floc formation if polyphosphate use is instituted by a municipality for corrosion control within their distribution system. Even the simple act of flushing out distribution lines and fire hydrants, which can occur on a periodic basis, can stir up loose particles of iron, debris and/or organic material leading to short periods of highly undesirable water quality entering the food plant water supply. Flushing of municipal distribution systems can also introduce contaminants from the scouring agents which are regularly used. Other problems for which the municipal treatment sometimes does not adequately address include sensory issues, such as those related to algae blooms (see above discussion on "inversion"), high levels of disinfection byproduct formation (for example, trihalomethanes, bromate, etc.) and accidents (such as cross-connections or damaged valves/pipes that can potentially introduce contamination such as that from septic sewage). At the time of writing there are still cities in the U.S. (most notably New York) and in Europe (for example, Belfast and Manchester) that receive surface water from protected catchments that have essentially had no treatment other than addition of a disinfectant, fluoridation and corrosion control. The view that catchment control alone provides water that is safe after disinfection is becoming less and less acceptable as there have been too many outbreaks of waterborne diseases (especially those associated with protozoan parasites such as *Giardia lamblia* and *Cryptosporidium parvum*). In addition, there is increasing pressure for recreational access to protected catchments.

Many municipal treatment problems occur in the developing world where there are important differences in water priorities compared to developed countries. Waterborne diseases are one of the greatest health hazards across much of the world and the high incidence of such diseases is one of the key factors that cause high infant mortality rates. The priorities in the developing world is thus to provide water from reliable supplies of microbially safe water at an affordable cost. That is not to say that physiochemical quality criteria are ignored, but they are considered with due regard to the supply of a microbially safe water at an affordable cost. Unfortunately, water treatment processes in the developing world all to often fail, even with their primary objectives.

It is highly advantageous for food plant personnel to understand the challenges faced by their municipal water supply authority. This allows them to be prepared with the appropriate in-plant monitoring and water treatment. Maintaining a good relationship can also be very useful so as to be informed if and when changes to the municipal water treatment process are being planned or if flushing of municipal water distribution systems is to occur near the food plant. Municipalities can also provide valuable analytical data which helps a food plant determine the characteristics and consistency of its source water supply.

IV. WATER SOURCE SELECTION CONSIDERATIONS

The categories above briefly discussed the major sources of water supplies to food plants. Obviously, there are others (oceans, lagoons, glaciers, etc.) and many possible combinations of supplies. The keys to any consideration of a water source are:

1. First and foremost—what is its microbial quality? As discussed above, in some areas of the world, the potability of a supply, even a municipal supply, may not be guaranteed.
2. Chemical/physical quality—is it safe? Is it too high a risk to even consider? Can it be treated economically and within regulatory guidelines?

These questions must be answered on a case-by-case basis, and will depend on the degree of due diligence desired by a parent company, presiding regulations, corporate policies and a risk assessment of the impurities themselves.

3. Consistency of composition—is it consistent? Will water quality vary beyond the capability of the proposed treatment? Gathering any and all available data will aid in answering this question. Make use of municipal monitoring data, plant testing data, third party testing laboratory data, rainfall data and hydrogeologic or surface water surveys.
4. Its recharge—is run-off a concern? The volume of recharge, or replenishment, of the aquifer is important, as is the quality and origin of the water being used for that recharge.
5. Volumes/supply—can it currently meet the plant's needs? Will it in the future? In addition to the quality and safety components, supply is a key parameter to help ensure that the volume of water will be available for the long-term needs of the business. In many areas, the volume of withdrawal of water from an aquifer falls under government control or guidance, and this must be considered.
6. Future plans for the source or surrounding areas—is a municipality planning to develop the source and treat it? For example, is there planned construction or industrial entry to the area?

These questions highlight the value of considering water a dynamic ingredient throughout its supply chain. Irrespective of surface vs. ground, or private vs. municipal, absolute diligence in the selection, qualification and on-going monitoring of water sources and treatment systems, in addition to vigilant physical control of these areas (fenced, locked access, etc.), are paramount in helping to minimize the risk to the food industry and its brands.

V. WATER SOURCE PROTECTION PROGRAMS

From the standpoint of water source protection, the ideal situation is a completely fenced in watershed owned by the food plant or by the municipal water authority. Unfortunately, this is not usually possible. When examining alternative source protection activities there are two categories to consider, namely groundwater and surface water.

A. Groundwater Source Protection

Private wells are most common wherever water distribution systems are not reliable or simply do not reach industrial users. The use of private groundwater sources also represent important cost savings opportunities where the full cost of high quality water and wastewater services apply. As a result, private wells are the most common water source for food plants in many international markets. It is widely considered good practice for food plants using private wells to develop a well protection program to reduce the vulnerability of water supplies from a quality, availability and economic standpoint. The well protection program steps indicated below were adapted from those suggested for municipal drinking water companies by governmental agencies such as the U.S. Environmental Protection Agency (7,8). They have been amended to apply to the needs of food plants.

1. Form a team or at least identify the person(s) responsible for coordinating the plant's well protection program. This team will gather map information, contacts with local authorities and consultants, if necessary.
2. Delineate the area to be protected. The water may travel through hundreds of meters of surface and sub-surface land before becoming available at the well. Ideally, the protection perimeters would be defined with the help of local regulatory officials, hydrogeologists, and others. Realistically, checking the land immediately surrounding the well-head is often the most practical method to define the capture zone for small systems (arbitrary or calculated fixed radius).
3. Identify and locate potential sources of contamination. This is critical. Often, potential contaminant sources, which may be quickly and economically addressed, go unnoticed until it is too late.
4. Manage the plants well protection area. This may range from removing or stabilizing leaking underground storage tanks, to educating the farmers adjacent to the plant on the use of alternative fertilizers or pesticides. This step must be approached from both a strategic point-of-view and an economic one. Strategically, build the relationships that would help this program over time (local regulators, government officials, testing labs, etc.). Economically, include the appropriate expenses in the business plan [for example the costs for re-grouting or re-digging a well or constructing security measures around the well-head (shed, lockable cover, sampling ports, fences, etc.)].
5. Develop contingency plans with responses for the most likely scenarios identified. At this point, it may be important to list all water supply alternatives for the case of major disruptions.

Contingency plans must also secure funding sources and trained responders.

6. Monitor results and evaluate the well protection plan. It is critical in any planning process to compare results with the goals and assess the success or failure of the effort. Monitoring water quality and quantity is the key factor and may require maintaining a network of monitoring wells in addition to pumping tests and water analysis. Evaluation of the management activity outlined in step 4, both from an individual point-of-view and from the plan itself, must be conducted every year to ensure that the objectives are being achieved and the plan is adjusted to new realities. The team should take the time to reflect on its contribution to the protection of our main ingredient. Recognition from management and/or from the community for its achievements help sustain the effort over time.

These six steps should be considered as general information. Some parts can be immediately implemented, while others will be longer term. The key is keeping everyone informed and involved throughout the process, since the success of the plan relies heavily on the awareness and support from the public.

B. Surface Water Source Protection

A watershed management program can be a useful tool to help reduce risks associated with surficial supplies. Managing the watershed is typically the responsibility of the local water authority. The best way for the authorities to enforce protective measures is to have the backing of its citizens. Public education and news media play a critical role and public relations opportunities allow businesses to exercise good citizenship. Users of large quantities of water, like many food manufacturing plants, are especially encouraged to support the community where they operate and eventually lead the process, if necessary, in order to ensure the continuity and quality of the water supply.

Whenever a surface water source is used, the boundary of the entire "hydrographic" basin delineates its ideal protection zone. Topographic maps indicating features such as vegetation, roads, open land, sewers and industrial activities are particularly useful for outlining the area. A knowledge of potential sources of contamination within the area is critical. Community engagement has proven very effective in obtaining this type of information (resident associations, senior citizens, environment protection groups, etc.). Overlaying maps containing information about the points of water withdrawal, animal populations, potential erosion, sewered areas, zoning, land use and development plans provide a good understanding of the overall vulnerability of the watershed. The risk assessment step is more complex for surface water supplies than for groundwaters as it must take into account all current and potential values of the surface water resources to a given community (cultural, environmental, recreational, spiritual, aesthetic, as well as economic values).

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191 Water: Purification and Distribution

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CONTENTS

I. INTRODUCTION

Abundant, clean and safe water is essential for good public health. Of all the advancements made possible through science and technology, the purification and distribution of water for safe use is truly one of the most significant. For example, before cities began routinely treating drinking water, cholera, typhoid fever and hepatitis A had reached epidemic proportions. The implementation of effective water purification technologies has virtually eliminated these diseases in developed countries.

An overview of some key dates in water treatment is provided in Table 191.1. Archeological evidence suggests that as early as 2000 BC, people living across the Tigris, Euphrates and Nile rivers possessed the desire to improve their water quality (1,2). It is thus clear that water purification is not a modern concept. Four thousand years later we still strive for the same goal.

TABLE 191.1
Some Key Dates in Water Purification

Approximate Date	Drinking Water Treatment Development
Circa 2000 B.C.	Use of alum for water clarification in ancient Egypt (1)
1804	First municipal water filtration plant constructed in Paisley, Scotland (1,2).
1855	Dr. John Snow proves that cholera was transmit ted via contaminated drinking water (2). Slow sand filters coming into widespread use (3).
1880s	Louis Pasteur develops theory of germs (3)
1890s	First application of chlorine disinfectants to treatment of drinking water begins in England (4)
1906	Ozone used as a water disinfectant in Nice, France (1).
1908	US cities began routinely treating drinking water with chlorine (starting with Chicago and Jersey City) (4)
1915	First U.S. drinking water bacterial standard (4)
1952	The first recorded law was passed, in London, requiring filtration for all drinking waters (2).
1959	Reverse Osmosis membrane treatment invented (5)
1990s	Widespread use of granular activated carbon and ozone in Europe, primarily to remove pesticide residues (2, 6).

Developed by the author.

Effective treatment ensures that the water used in producing food products meets all standards and requirements. Many parameters need to be managed by water treatment, some for regulatory compliance, some for product safety, some for process control and others for product sensory performance and stability. Stringent water treatment processes are necessary to consistently meet these water quality needs.

II. REASONS FOR WATER PURIFICATION

If water appeared clean two centuries ago then it was considered suitable for drinking. In addition, there were very few environmental concerns relating to its use for waste disposal. By the early 19th century, urbanization and industrialization led to the gross pollution of rivers serving the larger cities and waterborne diseases became commonplace. Intensive agriculture ensued, and with it the widespread use of agricultural chemicals. More recently, with increasing scientific research, terms such as genotoxin and carcinogen have been used to classify a seemingly endless number of compounds in relation to their health effects. Water quality standards and regulations were developed to combat these threats to public health. The water industry has responded in admirable fashion developing many water treatment technologies. A testimony to this advancement is that the technology is now available to treat almost any water supply to potable drinking water standards. However, a continuing hurdle to the provision of safe drinking water to the entire global population is the affordability of these technologies.

As scientific knowledge in relation to the different aspects of water quality increases, our expectations as to what constitutes safe and consistent water quality is changing. For example, a food plant might have a treatment process that produces water hitherto considered potable. But now the treated water at this plant might be unacceptable because of, for example, a failure to comply with new turbidity, trihalomethane or bromate standards. The importance of effective and reliable treatment has been reinforced by the threat from *Cryptosporidium parvum* for which the U.S. Environmental Protection Agency has set a treatment standard (7). This is a prime example of public confidence relying on effective water treatment.

Demanding numerical drinking water quality standards throughout the world provide the framework for what is considered safe water. Regulatory compliance, including the expectations for water treatment and quality, is required by whatever regulatory agency has jurisdiction. Historically, water for food applications needs to meet, as a minimum, the water standards that apply to potable drinking water. For example, in the United States, this means adherence to U.S. Environmental Protection Agency primary drinking water standards (8), in addition to all applicable local standards and company/product specific water standards. Around the globe, the foundation of many drinking water quality standards is the World Health Organization Guidelines for Drinking Water Quality (9). National drinking water standards are usually enforced by means of close scrutiny from various governmental agencies.

In addition to meeting regulatory standards, a specific water quality is often required to make individual food products in the way they were designed. As food products require ingredient water of varying composition, the food plant water treatment process needs to be designed to consistently deliver the desired water quality. First and foremost, treated water must be absolutely safe to drink, from a physiochemical, microbiological and radiological perspective. Second, the receptors in our bodies are sensitive to certain chemical substances contained in the food we eat and air we breathe. Because food products contain large amounts of water, adherence to key water treatment principles ensures a consistent taste, odor and appearance in food products, no matter where in the world they are manufactured. Food plants are also required to deliver water of a quality that is suitable and operationally efficient for use as a process aid. This includes use in applications such as rinsing, cleaning, sanitation, lubrication, warming, cooling and boiler operations.

III. PRIMARY WATER TREATMENT TECHNOLOGIES

Many water treatment technologies can be used to purify water. There may not necessarily be a single correct process chain to treat a particular raw water supply, but a number of possible options. The on-site water treatment technologies most widely used in the food industry are conventional coagulation, membranes and ion exchange, each configured with the appropriate pre- and post-treatment unit operations. In some cases the food plant source water source might already meet the necessary water quality requirements and none of the primary water treatment technologies will be needed. Table 191.2 provides an overview of the effectiveness of the primary treatment technologies, along with some of the pre-and post-treatment unit operations, with respect to chemicals that are of health significance in drinking water (10).

The actual on-site water treatment process that is selected at a given food plant facility will depend on several factors, including:

1. The nature of the source water, especially its physiochemical, microbiological and radiological characteristics and consistency. A suitable water treatment system assures consistency by easily addressing source water or distribution system problems, often without the plant staff even knowing that these problems had occurred.
2. The desired treated water quality. The treatment selection to achieve the desired quality is highly dependent on source water attributes, the nature of the food product being produced and company treated water requirements. This protects the designed sensory aspects of the food product and helps ensure it has an

TABLE 191.2
Treatment Effectiveness for Chemicals that are of Health Significance in Drinking Water

	Primary Treatment Systems			Pre- and Post-Treatment Unit Operations		
	Coagulation	Membranes	Ion Exchange	Activated Carbon	Chlorination	Ozonation
Naturally Occurring Chemicals That Are of Health Significance in Drinking Water						
Arsenic	+++	+++	+++			
Barium	+	+++	+++	+		
Fluoride	++	+++				
Manganese	++	+++			+++	+++
Selenium	++	+++	+++			
Uranium	++		+++			
Chemicals from Industrial and Human Sources That Are of Health Significance						
Cadmium	+++	+++	+++			
Mercury	+++	+++		+++		
Carbon tetrachloride	+	+++		+++		
1,2-Dichloroethane				+++		+
1,1,1-Trichloroethane				+++		
1,2-Dichloroethene				+++		+++
Trichloroethene				+++		+++
Tetrachloroethene				+++		
Benzene				+++		+++
Toluene				+++		+++
Xylenes				+++		
Ethylbenzene	+			+++		+++
Styrene				+++		
Monochlorobenzene				+++		+++
1,2-Dichlorobenzene				+++		+++
1,4-Dichlorobenzene				+++		+++
Trichlorobenzenes (total)				+++		+++
Hexachlorobutadiene				+++		
Edetic Acid (EDTA)				++		++
Nitrilotriacetic acid						+++
1,4-Dioxane						

(*Continued*)

TABLE 191.2 (*Continued*)

	Primary Treatment Systems			Pre- and Post-Treatment Unit Operations		
	Coagulation	Membranes	Ion Exchange	Activated Carbon	Chlorination	Ozonation
Chemicals from Agricultural Activities That Are of Health Significance in Drinking Water						
Nitrate		+++	+++			
Nitrite					+++	+++
Alachlor		+++		+++		++
Aldicarb		+++		+++	+++	+++
Aldrin/Dieldrin	++	+++		+++		+++
Atrazine	+	+++		+++		++
Bentazone		+++		++		+++
Carbofuran		+++		+++	+	
Chlordane				+++		+++
Chlorotoluron				+++		+++
Cyanazine		+++		+++		+
2,4-Dichlorophenoxyacetic acid (2,4-D)	+			+++		+++
1,2-Dibromo-3-Chloropropane (DBCP)				++		
1,2-Dibromoethane				+++		
1,2-Dichloropropane (1,2-DCP)		+++		+++		+
Dichlorprop		+++		+++		+
Dimethoate				++	+++	++
Diquat				+++		+++
Endothall	+			+++		+
Endrin	+			+++		
Fenoprop						+++
Heptachlor		+++		+++	+	
Heptachlor Epoxide		+++		+++	+	
Hexachlorobenzene	+			+++		+++
Isoproturon		+++		+++	++	+++
Lindane				+++		++
4-Chloro-2-Methylphenoxyacetic acid (MCPA)				+++		+++
Mecoprop				+++		+++
Methoxychlor	++			+++		+++
Metolachlor				+++		++
Pentachlorophenol				+++		
Simazine		+++		+++	+	++
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	++			+++		+
Terbuthylazine (TBA)	+			+++		++
Trifluralin		+++		+++		
Pesticides Used in Water for Public Heath						
Dichlorodiphenyltrichloroethane (DDT) and metabolites	+++	+++		+++	+	+
Pyriproxygen				+++		

A blank entry in the table indicates that either the process is completely ineffective or there is no data on its effectiveness.

+ indicates limited removal.

++ indicates 50% or more removal.

+++ indicates 80% or more removal.

Source: Adapted from WHO Guidelines for Drinking Water Quality, 3rd Edition, Draft, 2004.

adequate shelf-life in the market. In this respect, the plant water treatment process protects the trademark and helps build consumer confidence on a continuous basis.

3. A consideration of capital and operating costs. This includes the initial capital investment, replaceable costs based on anticipated life (e.g. carbon, membranes, etc.), service agreement costs, operating supplies and their availability, chemicals, labor and energy.
4. Projected water volume demand. Often the flexibility to upgrade the process for future

plant expansions is a key consideration at the outset of a plant design.
5. The space available to construct the water treatment plant.
6. Restraints due to the need to upgrade an existing plant can result in limitations on the processes that can be used.
7. Source water costs. The cost of source water can be an important factor to take into consideration. For example, a reverse osmosis water treatment system is typically 75% efficient in terms of the amount of treated water that is produced from its feed-water, the other 25% ending up in the concentrated waste or "brine" stream. In fact, the % efficiency of reverse osmosis may sometimes be lower than 75% depending on source water quality and pretreatment. By contrast, a conventional coagulation system is often greater than 99% efficient in terms of water usage.
8. The costs associated with disposing of sludge or liquid waste discharge to the drain (for example, sewer surcharges) also need to be considered. In particular, total water volume to drain, total dissolved solids, chemical residuals (e.g. chlorine), effluent license restrictions, etc., can influence selection of treatment. Effluent costs can be very significant, and have often been the key factor for food plants deciding on one treatment technology over another.
9. Often, company or personal preferences are a major influence.

A. Conventional Coagulation

Conventional coagulation treatment is also commonly referred to by other descriptions such as conventional lime treatment systems (CLTS), the cold lime process and batch coagulation/flocculation. Note that the two terms coagulation and flocculation are often used interchangeably by water technologists, which is incorrect. For clarity, coagulation is the destabilization and coalescing of colloidal particles which occurs extremely quickly. Flocculation, on the other hand, is a longer term process whereby larger particles are formed by aggregation of smaller particles that are formed by the coagulation process. In the past, conventional coagulation was at the core of many food plant water treatment systems, and was applied to treat source water of virtually any quality. Indeed, this system does address a broad range of water contaminants including particulates, alkalinity, hardness, metals, natural organic matter, bacteria, viruses and protozoan populations as well as off-colors, off-tastes and off-odors. Conventional coagulation is often adequate as a primary treatment for many food plant water purification applications, but may also be used as a superb pre-treatment for membrane processes, ion-exchange, or electrodialysis.

A major disadvantage of conventional coagulation is that it does not effectively reduce chloride, sulfate or nitrate concentrations which means that it cannot be used alone if reduction of these parameters is part of the water treatment objectives. Additional difficulties that may be encountered include the requirement for sludge disposal, a high potential for formation of trihalomethane compounds (which are strictly regulated in most jurisdictions) under certain conditions and difficulties operating with some water supplies that have low levels of dissolved solids. Membrane-based processes have decreased the dependence on this technology. Despite the limitations and the availability of cost competitive membranes, conventional coagulation arguably remains the most important primary water treatment process for the food industry around the globe.

Simply put, conventional coagulation involves mixing three primary chemicals in a reaction tank and allowing a contact time of at least two hours for the ensuing reactions to proceed. The three primary chemicals typically used are 1) a coagulant; 2) hydrated lime; and 3) a chlorine source. Less commonly applied chemical treatments are 4) the application of calcium chloride or sulfate, 5) the addition of soda ash, 6) the use of coagulant aids and 7) the use of chemicals (other than hydrated lime) for pH adjustment. Coagulation jar testing can prove helpful to determine the optimal type and dosage of treatment chemicals. The coagulant, with the assistance of the chlorine, hydrated lime, and/or any other chemical used, forms a heavy floc which entraps particles of debris, dirt, organic matter and other undesirable material in the water, and settles slowly toward the bottom of the tank. While the floc is forming, the precipitated alkalinity also settles slowly toward the bottom of the tank. The water in the tank flows toward the draw-off pipe located at the top of the reaction tank and the floc, because of its own weight, is left behind, continually trapping particles of debris as it settles. The treated water flows upward toward the treated water draw-off pipe and out of the tank toward the post-treatment system.

1. The Coagulant

The primary purpose for coagulant addition is to cause particles to coagulate. The coagulant also assists in the precipitation of the insoluble alkaline salts created by the lime-based reactions. The coagulant, aided by steady non-turbulent agitation, overcomes the repulsive negative charges present on colloidal matter in the water supply. This charge neutralization and subsequent destabilization allows the coagulant to form a floc of ever-increasing size and density. As it forms, it incorporates the precipitating calcium carbonate, along with a host of other contaminants present

in the incoming water. Two commonly used coagulants are ferrous sulfate and ferric sulfate. They both provide good coagulation over a wide range of water conditions, especially at the high pH typically encountered where hydrated lime addition occurs (pH 9.6 to 10.5). Anecdotal data suggest that, when ferrous sulfate is used, oxidation of ferrous to ferric in the reaction tank results in appreciable silica removal. Aluminum sulfate, or "alum," can be used but its effectiveness is optimum in the 5.5 to 8.0 pH range which is typically cited as too low for alkalinity reduction. Despite this, there is an appreciable amount of plant operating data where alum is used effectively with hydrated lime at pH values near 10. Research into inorganic coagulants in the 1960s led to polymeric aluminium salts such as polyaluminium chloride and polyaluminium silicate sulfate.

2. Hydrated Lime

It might seem ironic that in order to reduce the water's hardness and alkalinity levels, hydrated lime is added which contains both hardness and alkalinity. Note that the word lime is often loosely used to cover both calcium hydroxide ($Ca(OH)_2$, slaked lime or hydrated lime) and calcium oxide (CaO, quicklime or lime). Calcium oxide is rarely used due to difficulties with its handling and control. Hydrated lime increases the pH of the water in the reaction tank to above 9.6, and at this pH soluble hardness and alkaline salts are converted into insoluble ones which precipitate out of solution. That is, it forces the conversion of bicarbonates to carbonates. The chemical reactions that occur to precipitate calcium carbonate are:

$$Ca(OH)_2 + Ca(HCO_3)_2 \rightarrow 2CaCO_3 + 2H_2O$$

$$Ca(OH)_2 + Mg(HCO_3)_2 \rightarrow MgCO_3 + CaCO_3 + 2H_2O$$

and also

$$Ca(OH)_2 + MgCO_3 \rightarrow Mg(OH)_2 + CaCO_3$$

At this high pH, naturally occurring alkalinity, usually present in the form of bicarbonate alkalinity, converts to carbonate alkalinity. The calcium carbonate is virtually insoluble in water at this pH, and precipitates from solution.

3. Chlorine

The third primary chemical applied in conventional coagulation is chlorine, which can be supplied from liquid sodium hypochlorite, solid calcium hypochlorite or chlorine gas. Residual chlorine levels are typically maintained in a range of 6 to 8 ppm free chlorine in order to ensure an effective kill of microorganisms, including bacteria, yeast and algae, at the high pH ranges typically encountered in the lime treatment system. Protozoans, like *Cryptosporidium parvum* and *Giardia lamblia*, are more resilient to chlorine and will be less affected by the chlorine at the high pH employed in conventional coagulation treatment systems. However, the chlorine, aided by the physical entrapment and removal of these organisms during the coagulation process, will still typically result in a substantial reduction in their numbers. Indeed, conventional coagulation is widely regarded as being one of the effective barriers in a multiple barrier approach against protozoans. In addition to the microbial disinfection effects of chlorination, the following benefits also result from its use:

- Improved coagulation by oxidizing organic matter, thereby assisting in its removal. This is preferred as organic matter may hinder the coagulation process and impart objectionable odor and taste to the water.
- Removal of components which contribute to off-taste, off-color, and off-odor.
- Facilitate the removal of soluble metals like iron, manganese, arsenic and others through oxidation.
- Reduction of chloramines through breakpoint chlorination.
- Oxidation of ferrous hydroxide to ferric hydroxide when ferrous sulfate is used as a coagulant.

4. Calcium Chloride or Calcium Sulfate

In some cases, water contains sodium alkalinity. This essentially means that the sodium salts of bicarbonate ($NaHCO_3$) or carbonate (Na_2CO_3) are present, instead of the calcium and magnesium salts of bicarbonate (e.g. $Ca(HCO_3)_2$) or carbonate (e.g. $CaCO_3$). The presence of sodium alkalinity is indicated by the water hardness being less than the alkalinity, both expressed as $CaCO_3$ equivalents. Where this happens, the lime-based reactions may need the assistance of some form of calcium or magnesium (usually calcium chloride or magnesium chloride) in order to effectively reduce the total alkalinity. The calcium provided to the reaction tank allows the calcium carbonate to precipitate out of the water. The following reaction is involved where calcium chloride is added to effect alkalinity reduction:

$$Na_2CO_3 + CaCl_2 \rightarrow CaCO_3 + 2\ NaCl$$

Notice that, even though the alkalinity will now decrease (through precipitation of calcium carbonate), the chloride level (or sulfate level if calcium sulfate is used) will increase. This must be accounted for in the dosing so that treated water chloride levels remain within acceptable thresholds.

5. Soda Ash

Non-carbonate or permanent hardness will not be reduced with lime addition alone. The addition of soda ash (sodium

carbonate; Na_2CO_3) is often required to reduce non-carbonate calcium hardness. Reduction of non-carbonate magnesium hardness may also require additional hydrated lime. Addition of soda ash should be done with care as it will also result in an increase in treated water sodium levels.

6. Coagulant Aids

When a chemical is used to strengthen or enlarge flocs formed by coagulation, it is normally referred to as a coagulant aid. Coagulant aids are not normally needed for food plant conventional coagulation operations. Sodium aluminate is sometimes used only in systems that use aluminum sulfate as the coagulant. When needed, calcium carbonate may be used at 50% of the lime dosage to aid floc settling. The term polyelectrolyte is used as a generic term to describe high molecular weight organic polymers that may be used as coagulant aids. For example, bentonite and Fuller's sand can be used as so-called "weighters" to assist removal of colloidal matter. For a properly sized and designed system, coagulant aids should not be necessary on a long-term basis.

7. pH Adjustment

Optimum coagulation is generally, but not always, obtained between pH 9.5 to 11 when using ferrous or ferric sulfate and between pH 6.5 to 7.5 when using aluminum sulfate (alum). For systems that use aluminum sulfate as the coagulant (i.e. where hydrated lime addition does not take place), soda ash or caustic is sometimes used for pH adjustment in place of hydrated lime. Acid-enhanced coagulation refers to the practice of lowering pH during coagulation to enhance the removal of natural organic matter. A food grade mineral acid such sulfuric acid or hydrochloric acid is most often used for this application. While acid-enhance coagulation is widespread in relation to municipal water treatment, it is rare in the food industry as it would work against the precipitation of calcium carbonate.

The design of the coagulation reaction tank can be important in relation to the effective operation of a conventional coagulation system. A depiction of a conventional coagulation reaction tank is provided in Figure 191.1. The basic design includes a minimum two hours retention and appropriate agitation using mixing paddles. Sludge recirculation can sometimes be important in the treatment of waters with low bicarbonate concentrations. This results in enhanced flocculation through "seeding" which involves the production of larger particles that will precipitate more easily. Some settling basins are equipped with settling tubes (angled at 60°) or a Lamella-type inclined plate design, which promote the settling of suspended floc. Temperature can also play a role in terms of coagulant effectiveness. For example, a Canadian study on cold weather treatment revealed that ferric and ferrous salts might be more effective than alum for some Canadian waters during winter. Alum, on the other hand, seemed more effective in summer according to the same study (11).

Conventional coagulation and "in-line" coagulation (also commonly referred to as direct filtration) are two very different types of treatment, and should never be confused or regarded as equivalent. In-line coagulation is typically viewed as a sort of "enhanced filtration." It uses a

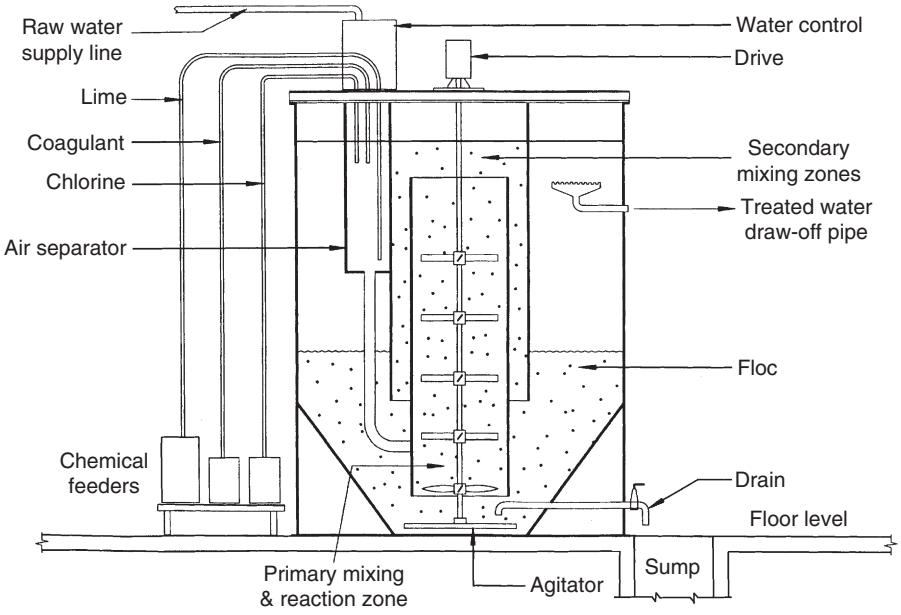

FIGURE 191.1 Conventional coagulation reaction tank.

coagulant, which is already oxidized (like ferric sulfate), which is injected in-line ahead of a static mixer. This forms a floc, which is captured atop a media filter. Typically, in-line coagulation affords little to no alkalinity reduction and should only be considered when the incoming water is already of consistent, high quality. However, it can be a useful pre-treatment to water treatment systems such as reverse osmosis where colloidal fouling of the membranes might be a potential issue. The flocculation and subsequent capture of in-line coagulation systems takes place over the course of approximately 6 to 8 minutes, and is not nearly as effective at removing contaminants as is conventional coagulation which typically has a reaction time of 2 hours or greater.

B. Membrane Processes

The term "membrane process" applies to technologies that use membranes to remove particulates, colloids, microorganisms, molecules and ions from water. The types of membrane water treatment process encountered in food plant operations are, in order of decreasing pore size, microfiltration, ultrafiltration, nanofiltration and reverse osmosis. Thus, the use of membranes may range from the use of a simple five-micron microfilter to help remove granular activated carbon fines, to reverse osmosis which has pores so small that molecular ions are removed. Figure 191.2 provides a visual representation of the relative particle size removal of the each membrane process. The driving force behind each membrane process is pressure. When pressure is applied across the membrane, purified water is forced through the membrane, leaving unwanted impurities behind. Table 191.3 compares the typical removal percentages of reverse osmosis, nanofiltration and ultrafiltration for a variety of water impurities (12).

Ultrafiltration, nanofiltration and reverse osmosis can all afford removal of many microbial impurities. Absolute rated microfilters of 0.45 microns (1 micron is 1×10^{-6} meters) or less are also effective at removing many microorganisms. However, a critical point which must be understood is that none of these processes, not even reverse osmosis, produces a commercially sterile water. Believe it or not, this is still a common misconception among many in the food industry. Owing to the nature of cross-flow technology, the high pressures used, the integrity of the seals and the variability in the pore structure of the membrane materials, these membrane treatment operations will remove a large percentage of the microorganisms to which they are introduced, though not all. As a consequence, additional disinfection of the water supply is usually undertaken (for example, chlorination, ozone, ultraviolet irradiation, etc.).

1. Microfiltration

Microfiltration is a direct extension of conventional particulate filtration into the sub-micron range. Microfiltration membranes have a filtration range that is typically from 12 to 0.01 microns. It rejects suspended and some colloidal material at operating pressures of approximately 15 to 30 pounds per square inch (psi). Due to their controlled pore size distribution (when absolute rated) microfilters are often used for mechanical removal of bacteria from water streams. For this application, a stepped removal approach is often employed which will include filters of decreasing pore size oriented in series, so as to minimize the plugging potential of the smallest pores (for example, a 5 micron particulate filter, followed by a 1 micron microfilter and finally a 0.2 micron microfilter which helps assure adequate bacterial reduction).

2. Ultrafiltration

Ultrafiltration is excellent for removing particulate matter, large organic matter (which includes humic acids, fulvic acids and related materials that comprise the natural organic matter in many water supplies) and microorganisms such as bacteria, yeast, mold, viruses and protozoa. The usual ultrafiltration range is from greater than 0.01 to 0.1 microns. The pore sizes of these membranes are not small enough to remove dissolved inorganic salts. Typical operating pressures range from 80 to 150 psi.

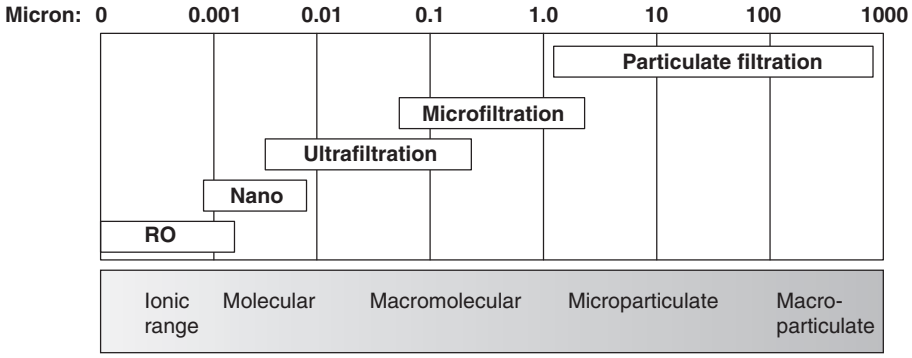

FIGURE 191.2 Range of particle sizes associated with membranes.

TABLE 191.3
Comparison of Approximate Removal Percentages of Ultrafiltration, Nanofiltration and Reverse Osmosis Membrane Processes

Component	Ultrafiltration	Nanofiltration	Reverse Osmosis
Alkalinity	None	50 to 70%	95 to 98%
TDS	None	50 to 70%	95 to 98%
Particulates	nearly 100%	nearly 100%	nearly 100%
Organic matter	some >2000 MW	most >200 MW	most >100 MW
THM precursors	30 to 60%	90+%	90+%
Sodium	None	35 to 75%	90 to 99%
Chloride	None	35 to 60%	90 to 99%
Hardness	None	50 to 95+%	90 to 99%
Sulfate	None	70 to 95+%	90 to 99%
Nitrate	None	20 to 35%	90 to 95%
Protozoa	near 100%	near 100%	near 100%
Bacteria	near 100%	near 100%	near 100%
Viruses	near 100%	near 100%	near 100%
Operating pressure	80 to 150 psi	100 to 200 psi	200 to 450 psi

Actual performance is system-specific.

Source: Adapted from Brittan (1997).

3. Nanofiltration

For removal at the level of dissolved inorganic salts, nanofiltration and reverse osmosis are the only two feasible options. Both are very similar technologies, so similar, in fact, that nanofiltration used to sometimes be referred to as "reduced pressure reverse osmosis." Two major differences are that the percent rejection of nanofiltration membranes (30 to 70%, in general) is much lower than that of reverse osmosis membranes, and the operating pressures for nanofiltration systems, which typically range from 100 to 200 psi, are generally lower than for reverse osmosis systems. Nanofiltration is an excellent technology where inorganics (salts) removal needs are less important (approx. 50% removal) than removing organics and microorganisms. There is less concentration of salts into the concentrated waste-water effluent which, in some cases, can be critical (e.g. for regulatory compliance). As there are so many similarities between nanofiltration and reverse osmosis, the discussion below in relation to mechanisms of how reverse osmosis works and membrane fouling is also applicable to nanofiltration systems.

4. Reverse Osmosis

Reverse osmosis, or in some countries termed inverse osmosis, can afford removal of from 95 to greater than 99% of many dissolved salts, resulting in a treated water exiting the system with a total dissolved solids concentration often below 10 mg/L. Significantly, it reduces inorganics not reduced by coagulation, such as sodium, chloride, sulfate and nitrate. It reduces large organic molecules and microorganisms (bacteria, mold, viruses and water-borne parasites) at efficiency of greater than 99%. Typical operating pressures range from 200 to 450 psi. The major disadvantage of reverse osmosis is the volume of concentrated waste-water that is produced (typically 20–25%). This can be costly on both ends i.e. where cost of source water is high and where sewer surcharges are high for concentrate disposal. Membrane materials of construction include cellulose acetate, polyamide and thin film composite, the latter having become predominant in the food industry in recent years. A comparison of the attributes of each type of membrane is provided in Table 191.4.

The mechanism of operation of reverse osmosis systems can become complicated, but the principle is fairly straightforward. In normal osmosis, water flows from a less concentrated salt solution through a semi-permeable membrane into a more concentrated salt solution. By applying pressure in excess of the osmotic pressure, this process is reversed, and water will flow through the membrane, leaving most of the salts, organics and microbial life to remain in the high salts solution (or concentrate). The purified water (or permeate) then goes on to be used for production. Several mathematic models exist which describe the movement of water and its components across the reverse osmosis membrane, but the key point is to design the system in such a way so as to maximize the water flux (flow through the membrane per unit of surface area in a given time). Water flux will naturally decrease as the membrane ages, but the key to any pre-treatment operations is to minimize membrane fouling, thereby minimizing the flux reduction and maximizing the useful life of the membrane. The pre-treatment processes will normally include the in-line dosing of acid, antiscalant, or both. These steps help prevent a loss of membrane

TABLE 191.4
Comparison of Cellulose Acetate, Polyamide and Thin Film Composite Membranes

Parameter	Cellulose Acetate	Polyamide	Thin Film Composite
Operating pH range	4–8	1–11	1–12
Langelier index, preferred	Slight negative	Slight negative	Slight negative
Chlorine tolerance, free, mg/l	0.2–1.0	0.0	0.0, or slightly higher
Bacterial resistance	Very low	Very high	Very high
Required silt density index, %	< 5	< 5	< 5
Overall rejection, %	90–99	95–99+	95–99+
Turbidity, NTU	< 1	< 1	< 1
Temperature (operating), °C	4–30	4–30	4–30
Life expectancy, years	3	5	5
Membrane cost	Medium	High	High

Source: Adapted from Pepsi-Cola Water Training Manual (2001).

performance due to metal oxide fouling, scaling or related processes which might occlude the membrane pores.

"Membrane Fouling" is generally defined as any process that results in a decline of permeate flux. Some processes are chemically or hydrodynamically reversible, others are not. Any fouling processes must be avoided to protect the reverse osmosis membrane. This damage to the membrane can proceed via several mechanisms, but is most often related to membrane degradation [bacterial, hydrolytic (pH), or oxidative (disinfectants)] or membrane blockage [suspended solids, silicates, metal oxides/hydroxides or scaling]. Proper pre-treatment design, which will vary depending on system specifics, can avoid any of the fouling mechanisms described above.

5. Electrodialysis

Electrodialysis is not a true filtration or osmotic process. It makes use of different types of membranes than those used for reverse osmosis and nanofiltration. Electricity is used to divert cations and anions through a semi-permeable membrane to the waste stream. Electrodialysis systems are designed such that treated water total dissolved solids, alkalinity and ion concentrations are reduced by greater than 80%. Similar to reverse osmosis and nanofiltration, electrodialysis removes inorganic ions not removed by coagulation technology. Organics and organisms are not effectively removed by electrodialysis.

C. Ion Exchange

Ion exchange has long been used in the food industry for treating water. Its early use was not usually for treating water that was in direct contact with food products, but instead for treating water that was used in plant utilities (boilers, heat exchangers, etc.) in order to minimize scaling which would impact their operational efficiency. However, the development of many new ion exchange resin technologies has led to its widespread use in processing water for many food applications.

As its name implies, ion-exchange uses natural or synthetic resin material to exchange less desirable ions with more desirable ions of like charge. The reactions are reversible, which means that the resin can be regenerated to be used repeatedly for many years. Many naturally occurring materials have been found to exhibit ion-exchange properties to some degree. Soil, for example, can be an effective ion-exchange material. Although many natural resin materials are still used today, many synthetic resin materials are also available. Ion-exchange resins for most food plant water treatment operations may be divided into two major categories, namely cation resins, which remove positively charged ions (cations) like calcium, magnesium and sodium; and anion resins, which remove negatively charged ions (anions) like nitrate, sulfate and chloride. Each of these categories can then be further subdivided into "weak" or "strong" depending on their affinity of the chemical functional group attached to the resin material for cations or anions. Therefore, there are four major classifications of resins:

1. Strong acid cation (SAC) exchange
2. Weak acid cation (WAC) exchange
3. Strong base anion (SBA) exchange
4. Weak base anion (WBA) exchange

The resin manufacturers prepare the resin in a particular form, depending on its intended use. For example, cation resins can be purchased in either the hydrogen form (H^+) or the sodium form (Na^+). Anion resins can be purchased in either the hydroxyl form (OH^-) or the chloride form (Cl^-). The ions that are attached to the resin (in this example, either the hydrogen, sodium, hydroxyl or chloride) are attached in a very weak fashion. Because of this, other ions in the water, with a stronger attraction to the resin, will compete with the ions that were originally attached, and displace them. In this way, the ions originally on the resin are exchanged for the ions in the water. Hence, the ions on the resin to begin with are the ions that will end up in the treated water.

Ion exchange systems are often referred to as co-current and counter-current. These terms refer to the direction of flow during normal operation and regeneration. Where water flows downwards during normal operation, it is a counter-current system if regeneration is carried out with an upward flow of regenerant solution. Conversely, if regeneration involved downward flow the system would be co-current. The significance of this is that regeneration will be most effective in those parts of the resin bed which are treated with the freshest regenerant solution. The bottom of the resin bed for a co-current system would be the area least effectively regenerated, so there will tend to be some leakage of ions from this area of the bed during normal operations. In practice, counter-current regeneration is preferable for demineralization applications, but there is generally little difference for other types of ion exchange applications (13).

Applications of ion exchange include: 1) demineralization (near complete or partial removal of total dissolved solids to required level); 2) zeolite softening of water (the removal of calcium and magnesium to an acceptable level); 3) dealkalization (reduction of alkalinity); and, 4) specialty applications such as removal of nitrate, arsenic, iron silica or other targeted impurities.

1. Near-Complete and Partial Demineralization

It is possible to use combinations of resins to remove almost all of the inorganic components in a water supply resulting in a TDS of less than 1 mg/l. This is often referred to as either complete demineralization or near-complete demineralization. In some cases too much of the inorganic component is removed, in which case a flow bypass may be operated whereby the resins are by-passed with partially treated water, and then both streams are combined later in the water treatment chain (partial demineralization). Demineralization includes reduction of sodium levels to meet any necessary labeling requirements for specific products (such as "sodium free" or "very low sodium"). In a demineralization process, a strong acid cation (SAC) resin in the hydrogen removes cations, and a strong base anion (SBA) resin in the hydroxide form removes anions. Separate columns are normally installed for each type of resin (i.e. "two bed" systems). However, there are many commercially available "mixed-bed" deionization systems, so called because the two resin types are intimately mixed together in one column. Regeneration requires the use of an acid for the SAC resin, usually sulfuric or hydrochloric acid, and caustic (sodium hydroxide) for the SBA resin. Regeneration is initiated after a preset number of gallons have been de-ionized, or when a signal from the conductivity (or resistivity) monitor indicates regeneration is required. The effluent from these regenerations usually requires neutralization prior to discharge from the plant.

2. Zeolite Softening

Zeolite softening is the simplest and most common form of ion exchange used in the food industry. It is now mostly used for the treatment of water for auxiliary plant uses (boiler, heat exchangers, etc.) to prevent mineral scale buildup. Softening is also sometimes required so as to not hinder the effectiveness of food preservatives, to prevent precipitation in sensitive beverages (for example, oxalic acid containing teas which are prone to calcium oxalate precipitation) or as a pre-treatment to unit operations such as reverse osmosis (to inhibit membrane fouling).

This process typically uses zeolite SAC resins to remove the calcium and magnesium hardness ions, replacing them with sodium ions. Resins are regenerated with brine (sodium chloride) prior to reaching their capacity limits. Typically, the regeneration mode is initiated after a pre-determined volume of water has passed through the resins. Keep in mind that zeolite softeners increase the sodium concentration in treated water by 46 mg/l for every 100 mg/l of $CaCO_3$ removed. Therefore, if used for food applications then compliance to all sodium related product requirements and regulations must be ensured (e.g. labeling requirements, product standards, local regulations, etc.).

3. Dealkalization

Dealkalization involves the use of a WAC exchange resin in the hydrogen ion (H^+) form. This resin will exchange the hydrogen ion for weakly bonded cations in the water. The predominant cations removed are those that comprise hardness (primarily calcium and magnesium). In addition, some sodium may be removed in the process, but only after the hardness is removed. As the hardness is removed, and exchanged for hydrogen ions, these hydrogen ions combine with the naturally occurring bicarbonate alkalinity in the water to form carbonic acid (H_2CO_3). This newly formed carbonic acid quickly dissociates into water and carbon dioxide, and the carbon dioxide is usually removed with a downstream degasifier. By forming the carbonic acid and removing the carbon dioxide, the alkalinity of the water is reduced proportionally. Hence, dealkalization using weak acid cation exchange resin removes both hardness and alkalinity in one treatment unit. Typically, regeneration takes place after a pre-determined volume of water has passed through the resins. If sulfuric acid regenerant is used, the concentration is normally kept below 0.5–0.7% to prevent calcium sulfate precipitation in the resin.

4. Specialty Applications

Many specialty ion exchange applications are being offered by suppliers, including the removal of nitrate, arsenic, iron, silica, or other targeted impurities. The

difference between many of these specialty applications and those outlined above is that it is likely that only a small percentage of the total ions present will be ions that are targeted for removal. The challenge, therefore, is to develop resins that will preferentially remove some ions as opposed to others. In weak solutions, the main factor is valency, with ions of higher valence being preferred. However, the size of the ion is especially noteworthy as ion exchange is not simply a surface phenomenon with up to 99% of the ion exchange reactions occur inside the resin bead (14). Thus, it is possible to optimize the resin for the removal of specific ions.

The ionic composition of the water being treated is critical as there will be competition between it and other ions in water. For example, in the case of many traditional strong base anion exchange resins, the order of selectivity is sulfate, nitrate, chloride and bicarbonate – sulfate will be exchanged first, and bicarbonate will be exchanged last. If the sulfate content in the water being treated is low, the next anion of preference is nitrate, so nitrate will be effectively removed. If sulfate levels are high, then the SBA's capacity for nitrate will be low, and other options should be explored (for example, a more nitrate-selective ion exchange resin).

IV. PRE- AND POST-TREATMENT TECHNOLOGIES

Many unit operations are intended not as primary treatment but rather to augment the treatment to result in a robust, complete treatment chain designed to deliver a high quality treated water that is required for food and beverage plant applications. A wide variety of pre- and post-treatment unit operations may be grouped into this category, including those depicted in Figure 191.3. This chapter will not address all of the possible pre-and post-treatment unit operations, but will address some of the more common ones that are used in the food industry. The choice of proper pre- and post-treatment operations is a key considerations when selecting and designing a food plant water treatment system.

The primary goal of pre-treatment processes is to protect the integrity of primary treatment operations, such as reverse osmosis membranes or ion exchange resins, which often represent a substantial portion of the capital cost. The membranes and resins are susceptible to a variety of processes and impurities which may foul or degrade them, thereby rendering them inefficient, ineffective, or, in the worst case, totally destroyed. Post-treatments include additional steps of disinfection, organics removal and polishing which are needed to provide a consistently high quality treated water. The unit operations discussed herein are granular activated carbon filtration, chlorination, ozonation, ultraviolet irradiation, media filtration and particulate filtration. They may be used for both pre- and post-treatment applications.

A. Granular Activated Carbon Filtration

Granular activated carbon (GAC) filtration is a common unit operation in food plant water treatment processes.

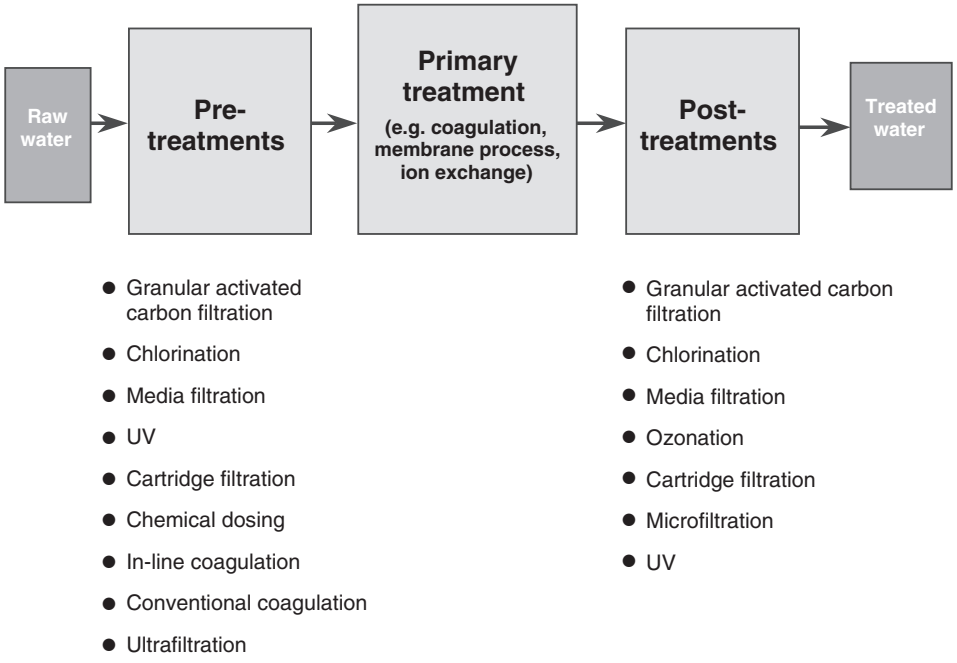

FIGURE 191.3 Pre- and post-treatment unit operations. (*Source*: Developed by the author.)

The primary water application of activated carbon has been to effect the removal of the chlorine species used to disinfect the treated water. The other major application of organic impurity removal, which is relatively new to the food industry, is the provision of broad protection against a wide range of possible contaminants through the removal of pesticides and other trace organic chemicals (for example, see Table 191.2), taste and odor compounds, algal toxins and natural organic matter. In fact, granular activated carbon is recognized as the "best available technology" (B.A.T.) by many regulatory agencies, including the United States Environmental Protection Agency, for removing a variety of natural and synthetic organic contaminants.

A critical distinction must be drawn between the catalytic mechanisms carbon employs to dechlorinate and its adsorptive mechanisms of organic contaminant removal. The catalytic mechanism refers to the formation of a surface oxide on the activated carbon medium as a result of the reaction of the carbon with hypochlorous acid, according to the following reaction:

$$HOCl + C^* \rightarrow HCl + C^*O$$

Hypochlorous acid + carbon → hydrochloric acid + carbon surface oxide

The catalytic reaction is not reversible, and, in fact, carbon bulk is destroyed in the process.

Adsorption refers to the adhesion, bonding and other chemical attractive forces that retain impurities on the surface of the carbon and within its pores. Unlike the catalytic mechanisms, adsorption is usually a reversible process, to varying degrees.

Carbon may be obtained from a variety of different starting materials, including coal, lignite, peat, coconut shells and wood. The carbon is activated either thermally (steam) or chemically. Steam activation, the more common, involves two steps: 1) carbonization, and 2) activation. Carbonization involves the conversion of the raw material into a disordered carbon structure with a very low volatile content. Carbonization is done at elevated temperatures in an oxygen-lean environment which keeps it from burning. In activation, some carbon atoms are vaporized, leaving behind the highly porous structure. Steam activation is carried out in temperatures of approximately 1,800°F (982°C). At these conditions, carbon reacts with steam to form carbon monoxide and hydrogen, which exit as gases. The result: a highly porous carbon material. Chemical activation is used to produce very high pore volume in wood-based carbons, particularly in the medium-size pore range. The most common process consists of mixing wood dust or some other cellulose-based material with a strong dehydrating agent and then heating to a designated temperature. The activating agent not only extracts moisture but also helps prevent collapse of the pore structure during activation (15).

Activated carbon is generally available in the powdered or granular form, but granular activated carbon (GAC) is used for the vast majority of water treatment applications in the food industry. In addition to the pesticide and volatile organic impurities mentioned above, and the removal of chlorine and chloramine, GAC also affords treated water protection against adversely sensory-active compounds, like the microbial metabolites geosmin and 2-methyl isoborneol. These compounds may be odor active in nanogram per liter concentrations and represent a substantial proportion of off-odor complaints to municipal water treatment works (16).

The activated carbon unit operation must be diligently maintained and sanitized as part of normal food plant operations. Many microbial complaints and sensory excursions in the food industry have been linked, at least in part, to inadequate carbon bed management practices. One of the reasons for this required diligence is that the core of the GAC bed has the potential to provide optimal conditions for the growth of troublesome microorganisms—specifically, the chlorine is absent, the environment can vary in its level of air or dissolved oxygen, and, in most cases, the organic microbial nutrients abound (since GAC is so proficient at removing organic compounds, including natural organic matter, from water supplies). These conditions combine to make carbon an excellent medium for the support of microbial growth. Once established, the extremely large surface area within the carbon pores can make control of an unwanted microbial population a daunting task. Routine and diligent backwashing and sanitization of the carbon bed should be viewed as an absolute requirement for any food plant water treatment system. Hot water, steam, or a combination are generally employed to sanitize the carbon filters, provided their material of construction can withstand the temperature needed. When performed regularly, this helps prevent a biofilm from becoming firmly established in the bed and avoids problems often associated with poor carbon maintenance, including high bacteria counts, off-odor production within the bed, poor dechlorination or chlorine breakthrough and loss of adsorptive capacity.

B. Chlorination

The commercially available forms of chlorine for the food industry are chlorine gas, solid calcium hypochlorite pellets and sodium hypochlorite solutions. The traditional and most common form that is used for disinfection of treated water by the food industry is the last, sodium hypochlorite solutions, although the others are also employed at some locations. Chlorine gas is usually reserved for large-volume food plants, and considerable drawbacks to its use are the strict transport, handling,

storage, metering, permitting, and use requirements being enforced by many regulatory agencies. Calcium hypochlorite is utilized, but in many international markets it is more costly than sodium hypochlorite. Irrespective of which form is chosen, once in aqueous solution, the chlorine chemistry becomes essentially the same.

The chlorine species, when dissolved in water, will eventually dissociate into primarily two active forms: 1) hypochlorous acid (HOCl); and, 2) the hypochlorite anion (OCl^-). The ratio of these two chlorine species varies as a function of pH, with hypochlorous acid predominating at acidic pH, and the hypochlorite anion predominating at alkaline pH. One critical concept to link with these chlorine equilibria is that hypochlorous acid (predominant at lower pH) has been described as 80 to 100 times more potent a germicide than the hypochlorite anion (predominant at higher pH). As a result of this, the World Health Organization suggests a pH of less than 8.0 to help assure effective disinfection of water with chlorine (9,10). In conventional coagulation water treatment systems, where the operating pH in the reaction tank is often above 10.0, the chlorine equilibrium favors the existence of hypochlorite anion, which is why, in addition to allowing adequate floc settling time, a minimum two hours of retention must be designed in these systems.

In summary: 1) chlorine is an effective disinfectant against bacteria and viruses, although it is less effective against protozoan organisms like *Giardia* and *Cryptosporidium*; 2) the effectiveness of chlorine varies markedly with pH, owing to the distribution between the more effective hypochlorous acid, and the less effective hypochlorite anion; and, 3) the preferred operating range for chlorine disinfection is roughly pH 6.0 to 7.5; below this, corrosion may occur, above this, its effectiveness declines. Although the actual disinfection criteria for individual applications must be decided within each plant or corporation, a long-standing industry practice for water disinfection using chlorine in conventional treatment systems is to maintain a free chlorine residual of 6 to 8 mg/L over the course of a two-hour contact time. For other treatments, where the pH is lower, the free chlorine residual concentration and/or contact time is often decreased.

C. Ozonation

Ozone (O_3) is an unstable, colorless gas that is an allotrope of oxygen (O_2). It has a distinctive pungent odor, from which its name is derived (from Greek *ozein*, "to smell"). Ozone has been used for municipal scale treatment of drinking water since 1906 (Nice, France) (1). It is formed naturally in air by the ionizing effects of environmental lightning, and in the earth's stratosphere by ultraviolet irradiation. It is also formed during combustion in automobile engines, thereby contributing to the troublesome phenomenon of "photochemical smog." Ozone safeguards us from the damaging effects of the sun by inhibiting the penetration of much of the sun's UV waves, preventing them from reaching the earth's surface. The major use of ozone in the food industry currently is the treatment of bottled water. Its use in the treatment of water for wider food industry is still uncommon.

TABLE 191.5
Relative Effectiveness of a Variety of Disinfectants against *Cryptosporidium*

Disinfectant	Effectiveness	Estimated CT-99
Free Chlorine	Poor	7200 mg-min/L
Chloramine	Poor	7200 mg-min/L
Mixed Oxidants	Fair	1000 mg-min/L
Ozone	Good	5–15 mg-min/L
Chlorine Dioxide	Good	80 mg-min/L
UV Irradiation	Excellent	2–5 mJ/cm^2

Note: CT-99 is the Concentration × Contact time required for 99% *Cryptosporidium* Inactivation.

Source: Aquionics UV Systems, Inc. (2001).

Most ozone for commercial applications is produced on-site via the Corona Silent Arc Discharge process (17). With the Corona Discharge, a gas feed of dry oxygen or dry air passes through an electrode pair (high and low voltage) where free electrons are of sufficient energy to split the diatomic (i.e. two atoms) oxygen molecules apart. The "single" atomic oxygen species then recombine with other diatomic oxygen to form a molecule with three atoms of oxygen, ozone (O_3). The net result is that three oxygen (O_2) molecules become two ozone molecules (O_3).

In ozone generation by Corona discharge, it is essential that the feed air or oxygen be dry. Moist air fed into ozone generators may result in formation of nitric acid inside the generator (through the reaction of nitrous oxides with water). In addition to reduced ozone output, nitric acid can corrode ozone generator internals and cause generator failure. Feed gas must also be free of particulates, coalescible oil (hydrocarbon) mists and gas impurities (methane, ammonia, etc.) all of which reduce ozone yield. When compressed purified oxygen gas is used as the feed, in place of treated air, roughly twice the amount of ozone is produced for the same energy input. For most bottled water applications (where ozone is frequently used), even despite the increased output, the cost of the compressed oxygen usually makes its use uneconomical. There are many designs of ozone generators, including tubular, plate, water-cooled, refrigerated and air-cooled.

As with any chemical disinfectant, when ozone is used for disinfecting water supplies, the ozone demand must first be met, and then a residual ozone concentration established, and maintained for the desired contact time. An industry practice, which dates back to data nearly four decades old, is to utilize an ozone CT value of 1.6 mg-min/L (CT refers

to the ozone concentration multiplied by its contact time). This is generally done by maintaining a residual of 0.4 mg/L ozone for 4 minutes. A CT of 1.6 mg-min/L is adequate to provide at least a two-log reduction in bacteria, virus, and *Giardia lamblia* populations. However, as with chlorine, *Cryptosporidium parvum* remains resilient to inactivation, so higher CT values will be necessary to address this organism (18).

Operationally, the three major drawbacks to the more widespread application of ozone to the food industry include the fact that: 1) it must be generated and used immediately on-site and cannot be stored; 2) due to its short half-life, it does not provide adequate residual disinfectant activity; and 3) there is a high potential to form bromate, a strictly regulated disinfection by-product of ozonation, which can result if bromide is present in the water. As with other disinfectants, the dose and half-life of ozone will vary as a function of pH, temperature, organic matter and other variables, but somewhat unique to ozone is its behavior at varying levels of total dissolved solids (TDS). The lower the level of total dissolved solids, the longer the ozone residual will last (19). Finally, ozone is a very powerful chemical oxidant and can be extremely aggressive toward equipment, both in air and in aqueous phase. Care must be taken to ensure that all materials used are suitable for ozone contact and that all employee safety precautions are observed.

D. Ultraviolet Irradiation

UV radiation energy waves are 100 to 400 nm long, lying between X-ray and visible light spectrums in the electromagnetic spectrum. The division of UV radiation may be classified as Vacuum UV (100–200 nm), UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm). In terms of germicidal effects, the optimum UV range is between 245 and 285 nm. UV disinfection typically utilizes a mercury source, although Xenon sources are also commercially available. UV systems for food plant applications are usually in the form of either: 1) low-pressure lamps that emit maximum energy output at a wavelength of 253.7 nm; 2) medium-pressure lamps that emit energy at wavelengths from 180 to 1370 nm; or, 3) lamps that emit at other wavelengths in a high intensity "pulsed" manner. Pulsed UV is a relatively new technology to the food industry and is not widely employed at present. While both low and medium pressure designs have their own advantages and disadvantages, they have both proven to be adequate for water disinfection applications.

The degree to which the destruction or inactivation of microorganisms occurs by UV radiation is directly related to the UV dose. The UV dosage, D, is calculated as the arithmetic product of intensity, I, in milli- or micro-watt seconds per square centimeter, and time, t, in seconds. Internationally, the dose is often expressed in millijoules per square centimeter, which is exactly equivalent to milliwatt-seconds per square centimeter (1 mJ/cm^2 = 1 mW-s/cm^2 = 1000 microW-s/cm^2). Research indicates that when microorganisms are exposed to UV radiation, a constant fraction of the living population is inactivated during each progressive increment in time. This dose-response relationship for germicidal effect indicates that high intensity UV energy over a short period of time would provide the same kill as a lower intensity UV energy at a proportionally longer period of time. The UV dose required for effective inactivation is determined by site-specific data relating to the water quality and log removal required (20).

The mechanism of inactivation of microorganisms by UV is complicated, but has been reported many times in the literature. Fundamentally, the organism's genetic material (for example, bacterial deoxyribonucleic acid, or DNA) absorbs the UV radiation, which results in a chemical disruption of the DNA's chemical bases. Though many various photoproducts form as a result of this, the major rearrangement is the dimerization of the thymine base. This change renders the organism unable to replicate their DNA, and, therefore, cannot reproduce. As you might expect, ultraviolet disinfection does not provide any residual disinfectant activity.

Just as with ozone and chlorine, ultraviolet energy also has an accepted industrial "rule of thumb" which has been established for decades. A typical UV system used for the disinfection of food plant water treatment is sized to deliver a dose of at least 30 mJ/cm^2 at the end of its service life (typically 8,000 hours). To achieve this, since UV intensity naturally decreases over time as the lamp ages, the initial dose design is usually on the order of 60 mJ/cm^2. This design has traditionally been credited with providing at least a three-log (99.9%) inactivation of bacteria, yeast, and virus populations. Data published in late 1999 (21) and confirmed since then in several industry journals, suggest that the same 30 mJ/cm^2 dose also provides at least a three-log inactivation of *Cryptosporidium parvum*, a protozoan organism highly resistant to disinfection by chlorine and, to a lesser extent, ozone. This is promising news for the water treatment and food industries, since it confirms the effectiveness of UV as part of a multiple barrier water treatment process to help ensure the microbial safety of our treated water.

E. Media Filtration

The passage of water through any of a variety of coarse filtration materials can be referred to as media filtration. Applications of media filtration for the food industry includes the removal of particulates (for example from source water or the overflow of conventional coagulation reaction tanks), prevention of occlusion of carbon pores, avoiding the fouling of ion-exchange resins and providing

the required contact time for chlorine and coagulant (for in-line coagulation systems). Traditionally, the most common medium for food plant water treatment was simple filtering sand, supported by a bed of gravel. The major intent of these sand filters is to provide a coarse straining of the water stream. Sand filters may be located at different points in the treatment chain, but for conventional lime treatment systems, the industry practice is to locate the sand filter downstream of the reaction tank. Logically, this was done to capture any loose floc carryover, precipitated calcium carbonate, or other particulates that might not have settled adequately in the reaction tank. In some applications, like in-line coagulation, the sand filter which is used serves not only to filter the floc which is intentionally formed in-line, but to provide part of the contact time of the water with the chlorine disinfectant.

In addition to sand, garnet, anthracite and diatomaceous earth are common media used as part of a dual or mixed media filter bed. Other media are commercially available to suit a variety of applications. For example, some reverse osmosis and nanofiltration membrane systems might incorporate "greensand" as one of the pre-treatment operations. Greensand is a naturally occurring mineral that consists largely of dark greenish grains of glauconite, possesses ion-exchange properties (22) and is used for the removal of soluble iron and manganese from water streams to prevent fouling of downstream membrane systems.

Media filtration, like granular activated carbon filtration and virtually all water treatment processes, requires diligent maintenance in order to help assure its on-going performance. Since media filters remove particulate matter from the water, these impurities must then somehow be removed from the filter itself. This is accomplished by frequent backwashing of the filter medium, often with treated water, to suspend and expand the filter bed into the freeboard of the filter vessel, thereby allowing collected impurities to be washed to the drain. Some media filters, more commonly in municipal applications, augment the water backwash by the injection of air. This air scouring also helps suspend the bed and conserves the overall volume of water necessary for the backwash cycle. The frequency and conditions of backwash depend on the quality of water/ amount of debris going into the filter, the type of filter media, the vessel design, supplier recommendations and corporate policies. The range of operating conditions usually include a frequency of anywhere from daily to monthly, and a backwash rate of from two to five times the normal flow. Intimately linked with the maintenance described above is the routine sanitation of the media filters. Several methods may be used, including heat and chemical sanitizers, but this critical operation must not be overlooked. It is possible for bacterial populations to become established in media filter beds and for biofilms to form, which may be extremely resistant to remove.

F. Cartridge Filtration

Cartridge filters were once considered only as a point-of-use water treatment method for removing larger particulate matter. However, breakthroughs in filter design, such as the controlled use of blown microfiber filters (as opposed to wrapped fabric or yarn-wound filters), have tremendously broadened cartridge filter utilization (5). Cartridge filters are usually disposable, cost-effective, have a high flow capability and are commercially available in the particle removing range of 100 to 0.1 microns. Particulates, and other matter that are removed from the water, are held within the media matrix and are not generally subject to release, even where system fluctuations occur. There are two broad categories of cartridge filters, namely "depth" and "pleated."

Depth style cartridge filters have become a standard tool of the food industry to remove particulate matter and reduce turbidity to low levels. In a depth style cartridge filter, water flows through the thick walls of the filter where the particles are trapped throughout the complex openings in the filter media. The filters are often constructed of blown microfibres of polymers, such as polypropylene, or from cotton, cellulose or synthetic yarns. Filter media is usually provided with continuously graded fiber density. This yields progressively finer particulate retention through the depth of the media. Generally, they are not an absolute method of filtration since a small amount of particles within the micron range may pass into the filtrate. Depth style filters are used as both pre-treatment and post-treatment unit operations. Their main pre-treatment function in food industry water treatment applications (typically sized at 5 micron nominal rating) is to condition water prior to reverse osmosis membranes or ion exchange resins to prevent particulate fouling. They are also widely used as a pre-treatment to ultraviolet irradiation systems to ensure minimal turbidity is present which diffract incident ultraviolet light thereby reducing the dose and resulting disinfection performance. The main post-treatment application (typically sized at a 5 or 10 micron nominal rating) is to "polish" treated water. For example, they remove small floc particles, oxidized iron, granular activated carbon "fines," or precipitated calcium carbonate that might have carried over from the primary treatment process.

Absolute rated pleated cartridge filters can perform in the microfiltration range (see previous discussion on membrane processes) providing protection against bacteria and many water parasites. They use flat sheet media, either membranes or non-woven fabric materials. They typically act as surface filters with the media being pleated to increase the usable surface area. Pleated cartridge filters are not usually cost effective for particulate filtration applications as particles greater than 1 micron quickly plug them. However, they serve well for water that has already passed through depth cartridge filters, or other treatment steps, in applications such as sub-micron

particulate, bacteria and protozoan removal filtration. The National Sanitation Foundation (NSF International) has published a standard for cartridge filters, Standard 53, which assures removal of a large percentage of protozoan oocysts ("cyst reduction filters").

V. DISTRIBUTION

A variety of factors can affect water quality in municipal and plant distribution systems. This includes the quality of water entering the distribution system, contaminants formed or added by the system, cross connections, maintenance, construction and restoration. There are many ways in which municipal water quality deteriorates on the way from the municipal water works to the food plant or domestic consumer (23). A discussion on this topic is beyond the scope of this chapter. However, keep in mind that even the simple act of a municipality flushing out distribution lines, which can occur on a periodic basis, can stir up loose particles of metals, debris and/or organic material leading to short periods of highly undesirable water quality entering the food plant water supply. At the food plant, close monitoring of incoming municipal source water quality helps identify many of these issues (24). In addition, having the necessary in-plant treatment that can address upsets in municipal water quality is paramount.

The water distribution system within the food plant can also lead to a deterioration in water quality. Monitoring the microbial quality of water throughout a food plant water distribution system can indicate a variety of conditions that need correction. For example, water entering the system may have poor microbial quality resulting in inferior water quality in the plant system (this underscores to need for adequate monitoring of the incoming water supply), the filtration process may not be functioning correctly (filtration performance can be usefully monitored using turbidity, differential pressure and microbial monitoring measurements), there may be microbial biofilms in the distribution system (especially where dead-legs, unprotected storage and/ or rough internal surfaces are present), microbial contamination of one or more of the water treatment unit operations (highlighting the need for diligent maintenance and sanitation practices for water treatment system unit operations, especially granular activated carbon filters), the disinfection step(s) in the process might not be adequately functioning or is under-designed (insufficient concentrations and/or contact times of chemical disinfectants, improper UV operation, etc.), or there is a cross-connection within the plant distribution system (operator error, poor system design and malfunctioning valves may be possible causes). Closely monitoring the plant distribution system for microbial indicator organisms and identifying and monitoring potential corrosion products helps a plant to recognize emerging problems.

A prevalent problem at food industry plants is where inferior or unsuitable materials of construction are used, either in food plant or in the municipal water distribution systems that are largely outside of plant control, resulting in extensive corrosion and/or leaching of metals from internal into the plant water. It is important for food plants to use materials that have smooth surfaces and high resistance to oxidation and corrosion and to have adequate treatment processes to effectively deal with potential issues from the municipal distribution system. 316 stainless steel is recommended by many suppliers and food industry corporations for plant treated water distribution applications (22). If adequate materials are not used, corrosion of internal surfaces of the distribution system can introduce contaminants to the water, reduce flow capacity and provide environments for biofilm growth. For example, iron pipe corrosion has led to complaints of red water, caused fouling of membranes and ion exchange resin, caused taste complaints in water and food products, and the increasingly rough surfaces provide harborage sites for microorganisms. Corrosion from brass pipes, valves and fittings can result in leaching of lead, copper and zinc. Where galvanized iron pipes and plumbing might be used, leaching of iron, zinc and cadmium can often be problematic. Corrosion can result from a variety of causes. The water supply may be aggressive to pipes and plumbing because of its particular chemical makeup. In particular, water with very low pH and TDS (such as RO permeate or ion exchange effluent) and/or high levels of chlorine, ozone, chloride, sulfate, carbon dioxide, or other constituents, can result in increased corrosion. Though corrosion is generally difficult to predict and quantify, its results are easily observed through regular inspections of equipment as part of the plant preventative maintenance schedule.

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192 Water: Chemistry and Analysis

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CONTENTS

I. INTRODUCTION

Pure water has virtually no color, taste or smell. On the face of it, what could be more boring? But the hidden qualities of this substance make it a most interesting subject. In fact, absolutely pure water is never found in nature. Instead, water is often described as the "universal solvent" largely because of the seemingly infinite number of substances that dissolves in it. Some of these substances are considered contaminants, others are considered therapeutic, and still others are considered essential for life. The chemistry and microbiology of key water quality parameters will be discussed, in broad terms, in this chapter including the general approach adopted for their analysis by the food industry. This work is not intended to provide detailed chemistry, microbiology and instruction for how to test each parameter as this would entail a large book unto itself.

II. SUSPENDED MATTER

Water contains complex and shifting combinations of suspended substances. They may be derived from mineral, geologic, biologic and organic materials and particle sizes can vary widely in size, as is outlined in Table 192.1 (1).

TABLE 192.1
General Classification of Suspended Particle Sizes

Description	Approximate Particle Size (micron)
Gravel	64,000 to 2,000
Sand	2,000 to 62
Silt	62 to 4
Clay	4 to 0.24

Source: Adapted from United States Geological Survey at http://ga.water.usgs.gov/edi/dictionary.html

The amount of particles being carried by a given body of water is highly dependent on the speed of water flow. Fast-flowing water is capable of picking up and suspending more material than calm water. As surface water supplies have higher flow rates (in particular, rivers often have flow rates as high as one meter per second or more), they are generally considered to be more prone to high levels of suspended matter. However, keep in mind that even with a groundwater that has a typical flow of one meter per day, suspended matter might be present. An example of this is a groundwater for which there has been improper selection of the well screen sizing (length, diameter and size of openings of the screen) (2). Total suspended solids (TSS) is a parameter that provides a gross measurement of the amount of particulate matter present in a water sample. To test for TSS, a well mixed sample is filtered through a weighed standard glass fiber filter and the residue retained on the filter is dried in an oven. The increased weight of the filter represents the TSS (3).

A. Turbidity

Turbidity is an expression of the amount of solid particles that are suspended in water that cause light rays shining through the water to be scattered and absorbed, rather than transmitted. Thus, turbidity makes the water cloudy or even opaque in extreme cases. According to the Standard Methods for the Examination of Water and Wastewater (4), turbidity in raw water "...is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms." Turbidity measurement can detect potential problems in a food plant treated water, including microbiologic contamination, floc carryover, filter channeling, carbon fines leakage and increase in iron/manganese levels. In general, any disinfection process will be more effective on waters of low turbidity than on waters of high turbidity. In the case of ultraviolet disinfection, the particles that cause turbidity in effect "shield" the microorganisms from the ultraviolet bombardment and cause the beam to scatter. For chemical disinfectants such as chlorine and ozone, the turbidity particles provide harborage sites for the microorganisms thereby decreasing the surface contact and germicidal effectiveness.

Water turbidity is typically measured utilizing the principle of nephelometry and is reported in nephelometric turbidity units, or NTU. This technique provides high accuracy, precision, and stability over a wide range of turbidity levels. It is based on the measurement of the amount of light scattered at a 90 degree angle to the incoming (or incident) light beam. This right angle-scattered light is then correlated to the actual level of turbidity in the sample (4). Many companies offer devices that can measure turbidity in-line in the plant water treatment process.

B. Colloids

Colloids are characterized as small particles, including silica, clays, algae, bacteria, viruses and natural organic matter, that do not settle in water. The majority of colloidal particles in water have a slight negative charge that are stabilized by a cloud of positive counter-ions. This means that they repel each other, preventing them from coalescing together to form larger settable particles. Although colloids are too small to see with the naked eye, they are increasingly important from an operational perspective for the food industry due to the sensitivity of reverse osmosis membranes, and related water treatment unit operations, to colloidal fouling. When water that has high levels of colloidal material is fed to a membrane treatment unit, particulate matter tends to collect on the membranes and in the inter-membrane passages, causing reduced performance.

The Silt Density Index test, or SDI, is a widely used test to measures the quantity of colloidal contamination in waters above 0.45 micron (5). It measures how fast a sample of water pumped at a pressure of 30 psi will plug a 0.45 micron filter disc. The time it takes to collect a fixed volume (usually 500 ml) of filtrate through a clean disc is compared with the time it takes to collect the same volume after 15 minutes of filtration through the same disc. Obviously, the dirtier the water, the longer it will take to collect the final volume. In general, the higher the SDI, the more frequently chemical or physical cleaning will be required to restore reverse osmosis membrane performance.

SDI is not the perfect indicator of membrane fouling potential. The zeta potential, a measure of the electrical charge on the particles, and hence their fouling potential, will be altered by pH, ionic strength of the water and by inhibiting agents added. As result of these variables, there are cases where high SDI waters do *not* foul membranes and cases where low SDI waters *do* cause fouling. Despite these limitations and exceptions, however, SDI measurements are an important tool for tracking the consistency of incoming water with respect to colloids, and for tracking the performance of membrane pre-treatment equipment.

III. DISSOLVED SOLIDS

Concentrations of dissolved solids in water vary considerably in different regions due to differences in the local geology and environment. The bulk of the dissolved residue is typically inorganic minerals. In addition, water typically contains small amounts of dissolved organic matter. An aggregate measurement of the dissolved solids in water is provided by total dissolved solids, or TDS, testing. This test does not measure a single, specific component, but provides instead a single measurement for a variety of components. TDS is useful to gauge the overall consistency of the mineral content in the water. For operations using reverse osmosis (or similar membrane processes), TDS measurement provides a critical indication of the overall rejection characteristics of the membrane. For food plants using ion-exchange, TDS is important to determine both overall effectiveness of the resin and adequate blend ratios if a flow by-pass is operated.

Most official methods for measuring TDS, as recognized by regulatory agencies and certified laboratories, involve a "gravimetric" approach. In these methods, a water sample is coarsely filtered to remove suspended solids, then evaporated to complete dryness in an oven and the remaining residue is accurately weighed (6). This residue is expressed as the mg/l of total dissolved solids in the original water sample. These methods, however, routinely require anywhere from two to four hours before a result can be obtained. An alternative "conductometric" procedure was developed primarily for its speed and simplicity as compared to the "official" methods (7). The method has gained wide acceptance as the standard in the food industry. It is based on the fact that most of the components of TDS, when present in a water sample, will conduct an electric current. Remember that pure water, with almost no TDS, is a very poor conductor of electricity. Conversely, salt water, with a high TDS content, conducts electricity very well. Correlations have been found between the measure of the conductivity of a solution, and the actual level of TDS determined by drying. It is best to develop a local factor through empirical testing specific to the local water supply at each plant location, and these factors typically range from 0.5 to 0.9. Many automatic TDS meters, however, come already calibrated with an internal factor. It is important to know what the factor is so that results among laboratories may be compared.

A. Derived from Minerals

Minerals account for the vast majority of dissolved solids in water. They may be categorized into cations and anions.

1. Common Cations

Common cations, or positively charged ions, in water include the ions of calcium, magnesium, sodium, iron and manganese.

Dissolved calcium and, to a lesser extent, magnesium cause hardness in water. Water hardness is discussed in more detail later in the chapter. Sodium, along with the associated anions, may be a major contributing factor where high levels of total dissolved solids are determined. It should be noted that some water softeners can add significantly to the sodium content of treated water. This is because softeners remove the cations that cause hardness, calcium and magnesium, by replacing them with sodium cations (which do not cause water hardness). Sodium usually only becomes a concern for the food industry with regard to label claims (for example, "very low sodium," "sodium free," etc.). However, concentrations of sodium in excess of 200 mg/l may give rise to unacceptable taste in water (8), and this can also impact the taste of any food product it is used to manufacture.

Iron and manganese concentrations can often reach several mg/l in water, high levels being more prevalent in groundwater than surface water supplies (8). Iron may also be present in drinking water as a result of the use of iron-containing coagulants or the corrosion of steel or cast iron pipes during water distribution. If adequate treatment is not in place, the presence of high levels of iron and/or manganese may be problematic for the food industry for many reasons. These include taste impacts in the water and products it is used to manufacture, water and product discoloration, potential catalytic activity with chemicals in food product matrices and undesirable deposits that create havoc with valves and piping in the plant water distribution systems. In addition, without adequate pre-treatment, iron and manganese can result in fouling of plant water treatment membranes and ion exchange resins. Certain bacteria can further complicate iron problems. Organisms such as *Crenothrix*, *Sphaerotilus* and *Gallionella* use iron as an energy source. These iron reducing bacteria eventually form a rusty gelatinous sludge that can plug a water pipe (9). Concentrations of iron below 0.1 mg/l and manganese below 0.05 mg/l are typically acceptable for food industry applications.

Concentrations of individual cations may be measured using sophisticated analytical instrumentation such as flame atomic absorption (AA) spectrometry and ion coupled plasma (ICP) emission spectrometry (10–13). With AA, a sample is aspirated through a flame and atomized. A source lamp composed of that element's characteristic absorption wavelength is used. The amount of energy absorbed at the characteristic wavelength is proportional to its concentration in the sample. ICP uses a flowing stream of argon gas that is ionized by an applied radio frequency field. A sample

aerosol is generated in the nebulizer and spray chamber which is carried into the plasma. This subjects the constituent atoms to temperatures of approximately 6000 to 8000°C which results in the atoms producing ionic emission spectra. The efficient ionic excitations of ICP, coupled with use of precise monochromators and polychromators, allow effective multi-element detections that can provide greater sample throughput.

Hardness (calcium and magnesium) can be measured using the traditional hardness test which involves a titration with a standard solution of ethylenediaminetetraacetic acid (usually abbreviated as EDTA) (14). Hardness may also be calculated directly from the concentration of calcium and magnesium ions which might be determined using AA or ICP instruments. The formula ($[Ca^{2+}]*2.50$) + ($[Mg^{2+}]*4.12$) is used, the result being expressed as the equivalent quantity of calcium carbonate (15). Convenient iron and manganese on-site test kits are widely used throughout the food industry. The iron test kits typically use the phenanthroline method (16), while the preferred field method for manganese detection is often the persulfate method as it controls interferences from limited chloride ion concentrations (17).

2. Common Anions

Common anions, or negatively charged ions, in water include the ions of chloride, sulfate, nitrate and bromide.

Chlorides are present in water from natural sources, but sewage and industrial effluents, urban run-off containing de-icing salt, and saline intrusion can lead to increased levels. Excessive chloride concentrations increase rates of corrosion leading to elevated concentrations of leached metals in the water (e.g. iron or lead). Chloride concentrations in excess of about 250 mg/l can give rise to detectable taste in water (8). Sulfate is widely distributed in nature. As with chloride, high sulfate can contribute to the corrosion of distribution systems. Taste thresholds for sulfate in water have been found to vary with the nature of the associated cation, ranging from 250 mg/l for sodium sulfate to 1000 mg/l for calcium sulfate (8). The nitrate concentration in water is normally low but can reach high levels as a result of run-off from erosion of natural deposits, agricultural fertilizer use, refuse dump run-off, or contamination with human or animal wastes (8,18,19). High nitrate levels (typically well above 50 mg/l, expressed as nitrate ion) are implicated with methaemoglobinaemia, more commonly referred to as "blue-baby syndrome". Nitrate is reduced to nitrite in the stomach of infants, and nitrite is able to oxidize hemoglobin to methaemoglobin, which is unable to transport oxygen around the body. This is thought to be partly due to the larger proportion of fetal hemoglobin present in infants which is more easily oxidized (20). Bromides have only recently become widely recognized as a potential raw water quality problem. When water containing bromide is ozonated, there is a risk of bromates forming for which the allowable concentrations are typically very low.

Chloride, sulfate, nitrate and bromide can be measured in water by ion chromatographic (21,22) and capillary ion electrophoresis (23) methods for concentrations above 0.1 mg/l. A commonly employed wet chemistry analytical technique for chloride in water is a titration with silver nitrate solution (24). It is not uncommon to determine sulfate in aqueous solutions by means of a gravimetric method in which sulfate is precipitated as barium sulfate by the addition of barium chloride (25). For nitrate determinations, a common method uses ultraviolet to measure the absorbance of the nitrate ion at 220 nm (26). A widely used colorimetric method for determination of bromide uses phenol red indicator solution (27).

B. Natural Organic Matter

Natural organic matter refers to the gross content of organic matter in the water. This includes humic acids, fulvic acids and related materials that can originate from plant or animal residues, or substances made by living organisms. All are based upon carbon compounds. It is often difficult to treat waters that contain high natural organic matter content. For example, chlorination can lead to the production of high levels of disinfection by-products such as the trihalomethanes (THMs). This topic is further discussed below under the "Impurities Added to or Formed by Water Treatment" heading.

Total organic carbon, or TOC, is a widely used measure of natural organic matter in water. To determine the quantity of TOC, the organic molecules must be broken down and converted to a single molecular form that can be measured quantitatively. Two techniques that are used to achieve this for TOC analysis are high temperature combustion (28) and persulfate oxidation (29). The measurement of chemical oxygen demand, or COD, can provide relative indications of organic matter (30). Likewise, a UV spectrophotometer may be used to track the relative organic loading of waters by monitoring the absorbency at 253.7 nm (31). While not directly correlated to total organic carbon or natural organic matter, COD or UV monitoring do provide useful screening techniques for food plants, especially to track seasonal variations for surface water supplies. Assimilable organic carbon (AOC) is yet another surrogate measure of organic levels. AOC is used to measure the potential of a particular water for biological regrowth in the distribution system. The AOC determination is made by measuring the maximum growth level of a particular microorganism in a sample of water from which the original bacteria have been eliminated (32).

C. Organic Pollutants

In Figure 192.1, the message is clear…"nothing disappears from the earth." This applies to all industrial processes,

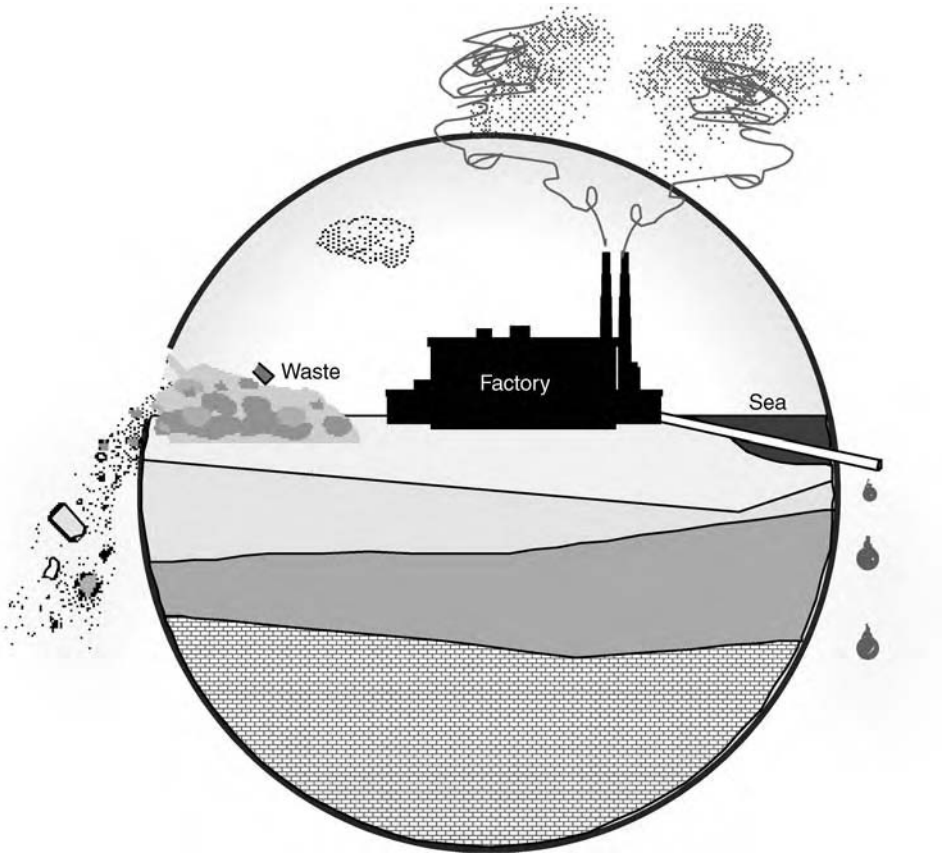

FIGURE 192.1 Nothing disappears from Earth. (*Source*: Adapted from KT Cullen & Co., training course material, Dublin, Ireland, 2000.)

including water treatment. Chances are, our food plant is either treating a contaminant that someone else introduced—maybe decades ago—or, we are contributing our own to the overall watershed by way of our wastewater effluent. Compounds like petroleum-based chemicals, pesticides and herbicides all too often make their way to water supplies either via run-off to rivers and streams, or down the well casing into groundwater aquifers. Pollution from such compounds can be a serious and costly matter for food companies in terms of consumer safety, adverse media attention, product recalls, remediation, loss of brand equity and regulatory concerns. Keep in mind that a very small amount of an organic pollutant can contaminate a very large amount of water. For example, the U.S. Environmental Protection Agency estimates that less than one gallon of gasoline can contaminate one million gallons of ground water to the point of losing potability.

1. Volatile Organic Compounds

Volatile Organic Compounds, or VOCs, are a category of compounds that includes many petroleum-based chemicals. For example, VOC compounds such as benzene, toluene, ethyl benzene and the xylenes (ortho-, meta- and para-xylene) are often implicated in the contamination of ground water by fuel (gasoline, kerosene, or some other petroleum-based energy source) (33–35). It is worth remembering that microbial soil mechanisms may transform the VOC contaminant in groundwaters. For example, under certain conditions, the common solvent tetrachloroethene (also known as tetrachloroethylene, perchloroethylene, PERC) can be transformed in soil first to trichloroethene, then to dichloroethene, and finally to vinyl chloride (monochloroethene). So, if vinyl chloride is detected in a groundwater sample, it might actually be the result of a tetrachloroethene spill from the dry cleaner several miles away!

The most common laboratory methods for analysis of VOCs use purge and trap gas chromatography using either a combination of photoionization detection (PID) and electrolytic conductivity (ELCD) detectors in series (36) or a mass spectrometer (MS) detector (37). With PID/ELCD, identification is by elution time only and can be partially confirmed by the use of a dissimilar chromatographic column. The MS detector provides more reliable identification.

2. Pesticides and Herbicides

Although the use of pesticides has led to many advances in agricultural output throughout the world, they have

been found to occur in surface and groundwater supplies all too frequently. Several of these compounds are bioaccumulative and reactively stable, as well as toxic or carcinogenic (8). Categories of pesticide residues include the organochlorine pesticides and the carbamate pesticides. Agricultural chemicals for weed control can also be routinely found in water supplies. Common types of these herbicides include chlorinated phenoxy acid herbicides, other types of carboxylic acid compounds and glycophosate herbicides (the latter is sold under registered trade names such as Roundup and Rodeo). Treatments such as granular activated carbon filtration, reverse osmosis, and to a lesser extent oxidation via chlorine and ozone, can play a vital role in controlling contaminant levels. However, it can be a challenge to food plant water treatment systems to meet the strict regulatory limits of some countries, especially if there are not controls governing the local use of pesticides and herbicides.

The type of test method utilized for pesticide and herbicide analysis in water often depends on the type of residue in question and the detection limit required. Sample preparation steps (which extract and concentrate the residue from water) and quality control checks can be time intensive. Analysis of organochlorine pesticides typically involve liquid-liquid extraction of water samples, followed by a capillary column gas chromatographic technique (38). Detection by either mass spectrometer (MS) or Electron Capture Detector (ECD) is commonplace. Degradation compounds of pesticides can also be detected by this method. Carbamate pesticides are heat sensitive and labile, and hence are not amenable to analysis by gas chromatography. Therefore, analysis of carbamate pesticides is often based on high performance liquid chromatography (HPLC) in conjunction with a post-column derivatization system and a fluorescence detector (39). A common method for measuring acidic herbicide compounds uses a micro liquid-liquid extraction method that is simpler than other extraction procedures and does not require large volumes of solvent extract (40). Glycophosate herbicides can be measured in water using liquid chromatography with a post column reaction/fluorometric method (41).

IV. MICROBIOLOGY

Typhoid, cholera, hepatitis A and E viruses, *Legionella* (often associated with piped water distribution systems) and *E. coli* O157 are examples of organisms that can cause infectious diseases by transmission through water. Others such as the protozoan organism *Cryptosporidium parvum* result in what are usually less severe, though uncomfortable, outcomes such as self-limiting diarrheal disease (8). According to the World Health Organization, "infectious diseases caused by pathogenic bacteria, viruses and protozoa or by parasites are the most common and widespread risk associated with drinking water (8). In addition to waterborne organisms of health concern, microorganisms can cause aesthetic and/or operational concerns. Despite these concerns, it is not practical to attempt to test all of these and other possible threats to a food plant water system. Consequently, the focus of the food industry, supported by most major regulatory organizations, is to monitor recognized "indicator organisms" of water quality.

A. Indicator Organisms

Total plate count bacteria testing provides a measure of the total number of viable bacteria. Proactively addressing increasing counts can avert microbial problems in the water treatment system. The corrective action(s) is dependent on the individual situation at each food plant. Areas that a plant might focus on include disinfectant residual levels, filtration operations, sanitation processes, ultraviolet radiation performance, materials used in water treatment operations, equipment performance and changes in the microbiologic quality of the incoming water.

Coliforms are rod-shaped bacteria that are widely found in the environment. Total coliform testing is widely used as an indicator of the general sanitary quality of treated drinking-water. The food industry is particularly interested in bacteria that may be present in excreted feces from the human gut, or from the gut of other mammals, which prosper in the condition found in the human digestive system. This is an acidic environment that has a temperature of approximately 98.6°F (37°C). The primary indicator tests of such fecal contamination in water are *Escherichia coli* (*E. coli*) or thermotolerant coliform bacteria.

Despite many benefits, coliforms do have shortcomings as indicator organisms. Among these is the fact that under certain circumstances (for example, following disinfection) they fail to indicate the presence of resistant pathogens such as viruses and protozoan parasites in treated drinking-water supplies. Keep in mind that total coliform bacteria and *E. coli* bacteria are part of the normal intestinal flora of healthy individuals, and are not always themselves implicated with adverse health effects. However, a pathogenic strain of *E. coli* known as *E. coli* O157 has received much recent discussion in the public domain. For example, an incident occurred in May 2000 where *E. coli* O157 contaminated the supply system of a farming community in Ontario, Canada. Five people died and 27 were hospitalized (42).

Mold is present throughout the environment (air, dust, soil, water) and can be transferred into a food manufacturing environment by people (direct contact with dirty hands, clothing, shoes, etc.), air ventilation systems, open doors and poor manufacturing practices in general. Sometimes mold issues in food or beverage products can be traced back to a water root cause. Although yeast is

rarely a problem with water, high yeast counts are occasionally detected and may be indicative of environmental issues at the plant. In addition to testing processed water, yeast and mold tests are sometimes conducted on sanitation rinse waters (assuming no disinfectant is present in the rinse water) to provide an early indication of the effectiveness of the plant sanitation process.

Membrane filtration techniques are commonplace for measuring microorganisms including total coliform bacteria, *E. coli*, thermotolerant coliform bacteria, yeast and mold. These techniques involve placing samples in a container (called a 'monitor'), using a membrane to capture microorganisms, adding the appropriate media to promote growth, incubating the samples in an oven for the appropriate time and temperature and counting the number of colonies that grow. Colisure, Colilert (both available from IDEXX Laboratories) and E*Colite (from Charm Sciences) are examples of rapid methods which are gaining increasing popularity within the food industry to detect the presence or absence of total coliform bacteria and *E. coli*.

B. Protozoa

The category of microorganisms known as the "emerging pathogens" has received a high level of scrutiny since a massive disease outbreak in Milwaukee, Wisconsin (U.S.), in 1993. Reports estimated at the time that 400,000 people became sick with gastrointestinal illness, and 100+ died (8). The cause of the outbreak was the protozoan parasite *Cryptosporidium parvum*. Since then, disease outbreaks due to protozoans such as "Crypto" and *Giardia lamblia* have become increasingly prevalent around the globe. *Cryptosporidium parvum* is particularly resistant to chlorine disinfection compared to bacteria. Part of this resistance is due to the fact that the organism forms protective shells, or oocysts, which make destruction difficult. A "multiple barrier" approach is generally recommended to protect against protozoan organisms. Coagulation with sand filtration, filtration to 1 micron absolute or less (e.g. reverse osmosis, nanofiltration, cartridge filters, etc.), ozone and UV can be effective "barriers" against *Cryptosporidium* and *Giardia*. The National Sanitation Foundation (NSF International) has published a standard for cartridge filters, Standard 53, which assures removal of a large percentage of oocysts ("cyst reduction filters").

Most water samples contain few oocysts and concentration techniques are required to obtain even a small number, many of which may be dead. As a result, the current test methods for protozoan organisms are neither precise nor accurate and monitoring results may contain false positives and false negatives.

C. Algae

At certain times of the year surficial supplies can undergo "inversion" leading to increased numbers of algae. Blooms, or scums, become easily visible across the water surface. In simplified terms, this phenomenon occurs where water at the surface reaches a temperature at which it is most dense (near 3.98°C, or 39.2°F), while the water below it has not yet reached this state. A density and temperature gradient are thus formed, with the denser water descending and displacing the water below it (hence the term "inversion"). The agitation brings sediment and unwanted components with it from the bottom to the surface.

Algae can produce physiologic breakdown products, such as geosmin and methyl-isoborneol, that are extremely sensory-active. They can cause off-odors in water (dirty, musty, moldy) at concentrations as low as nanograms/liter. These require extensive treatment to remove and can place a strain on the food plant water treatment system. Furthermore, some species of algae produce toxins that can be dangerous to people if they are consumed. To put this toxicity in perspective, *Microcystis*, a commonly found blue-green algae, produces a toxin called microcystin LR which is 200 times more toxic than cyanide (43).

Municipal water treatment authorities generally know of the taste, odor and toxicity problems associated with algae, and usually swiftly apply an algaecide. This kills the algae, but doesn't remove the "bodies." Sometimes, when conditions are just right, these algae (essentially silicate-laden diatomic skeletons) can make it through to food plant water supplies. If not effectively filtered by the plant water treatment system, this can result in issues with food products. The skeletons can be easily confirmed via electron microscopy.

V. RADIOLOGICAL PARAMETERS

Radiological contamination of drinking water can result from naturally occurring and man-made sources.

A prime example of a common naturally occurring radioactive species that can be found in water sources is uranium. More than 99% of natural uranium consists of the isotope U-238 which has a half life of 4,510,000,000 years. One gram of pure U-238 has a specific activity of 12,400 Bq, which means there are 12,400 atomic transformations every second, each of which releases an energetic alpha particle. Each atomic transformation produces another radioactive chemical: first U-238 produces thorium 234 (which has a half life of 24.1 days) and then thorium 234 decays to uranium 234 (which has a half life of 247,000 years). The Becquerel, or Bq, is the S.I. unit of radioactivity, where 1 Bq = one disintegration per second.

Man-made sources of radiological contamination include those from medical or industrial areas, from the disposal of radioactive materials (such as the mining and processing of mineral sands or phosphate fertilizer production), or from materials such as depleted uranium that are used in the manufacture of anti-tank missiles and other

weaponry. Depleted uranium has a lower or "depleted" U-235 content (0.2%) and higher U-238 content (99.8%) than naturally occurring uranium.

The process of identifying individual radioactive species and determining their concentration requires sophisticated and expensive procedures. A practical approach to monitoring for radioactivity in water is to use a screening technique. For example, the World Health Organization recommend a screening approach whereby the total radioactivity present in the form of alpha and beta radiation is determined without regard to the identity of specific radionuclides (8). If gross alpha and gross beta activity are within their threshold values, then the water is suitable from a radiological perspective. Only if either the gross alpha activity or the gross beta activity concentration thresholds are exceeded is it recommended to identify the specific radionuclides and measure their individual activity concentrations.

VI. DISSOLVED GASES

Water contains varying amounts of dissolved gases such as carbon dioxide, oxygen, ozone, hydrogen sulfide and ammonia. Dissolved gases contribute different attributes to the water. For example, carbon dioxide decreases the pH of un-buffered water and can contribute significantly to corrosion, oxygen can increase microbiologic activity, ozone provides oxidation and disinfection properties and can result in the formation of by-products such as bromate and chlorate, hydrogen sulfide imparts rotten egg-like odors at thresholds above 0.25–0.25 micrograms/l in clean water (44) and ammonia imparts off-odors, off-tastes and forms chloramines (commonly referred to as combined chlorine). The relationship between dissolved gases and the various species in water can be complex. For example, the relationship between carbon dioxide and carbonate in water is very pH dependent, as shown in the following equation:

$$CO_2 + H_2O <—> HCO_3^- + H^+ <—> CO_3^{2-} + 2H^+$$

The equation indicates why one typically sees a lower pH in reverse osmosis permeate than in feed-water. This is because the pH is always dictated by the ratios of bicarbonate and carbon dioxide found in the water. As these ratios change through the system, with much of the bicarbonate being rejected and much of the carbon dioxide passing through the reverse osmosis membrane (as gases are not effectively blocked), the pH will drop. Of course, if acid addition is employed as a pre-treatment to reverse osmosis membranes, then this will also contribute to the pH decrease. The presence of entrapped dissolved gases needs to be minimized for conventional coagulation treatment processes. They can result in floating floc or sludge which, in addition to causing operational difficulties, can potentially make its way to treated water used in food processing. Many de-aeration treatments are available to minimize the presence of entrapped gases.

If pH, temperature, alkalinity and total mineral content are known then the concentration of CO_2, and any or all of the alkalinity forms, can be calculated from charts, diagrams and nomographs (45). Another way to measure CO_2 content is by titration with either sodium carbonate or sodium hydroxide using phenolphthalein indicator (46). Dissolved oxygen is typically measured using a membrane electrode procedure, which is based on the rate of diffusion of molecular oxygen across the membrane (47). A simple colorimetric test for ozone uses indigo blue reagent (48). The blue color of indigo reagent is bleached in proportion to the amount of ozone present in the water sample. Alternatively, DPD (N,N-diethyl-p-phenylenediamine) reagent, the same reagent commonly used for free and total chlorine testing, may be used to measure ozone (49). Zinc acetate and sodium chloride solutions are often used to preserve the sample prior to hydrogen sulfide analysis (50). Several qualitative tests for hydrogen sulfide are available, and quantitative methods include the methylene blue colorimetric (51) and iodometric test methods (52). Ammonia can be measured using an ammonia-selective electrode method (53) or by titration against sulfuric acid using boric acid and mixed indicator solutions (54).

VII. IMPURITIES ADDED TO OR FORMED BY WATER TREATMENT

While the matter in water may render it unfit for human consumption, treatment can also adversely affect water quality by introducing pollutants, or by modifying chemicals that are harmless prior to water treatment.

A. Chlorine

Chlorine is widely used in water treatment. It has effective microbial disinfection properties, acts as an oxidant (e.g. for oxidation of dissolved iron and manganese to form insoluble products that can be removed as soluble precipitates) and it removes, or assists in the removal of, some chemicals e.g. decomposition of easily oxidized pesticides such as aldicarb (8). Chlorine is easy to apply, provides a strong residual, is relatively inexpensive and is easily removed from the water (typically via granular activated carbon filtration or sodium metabisulfite dosing prior to membranes). Disadvantages of chlorine include its reaction with natural organic material to produce disinfection by-products such as the trihalomethanes (discussed below) and that it provides relatively poor effectiveness for control of the protozoan parasite *Cryptosporidium parvum* (also discussed below).

The most common form of chlorine used in the food industry is sodium hypochlorite solution. Calcium

hypochlorite, available in granular form, is also commonly used. Chlorine gas is used mostly by municipal water treatment works, but may be used by very large food plants. A major drawback with chlorine gas is the regulatory prerequisites and paperwork usually needed for its safe storage, handling and use.

When chlorine dissolves in water, the hypochlorite anion (OCl^-) and hypochlorous acid (HOCl) form. Figure 192.2 illustrates the equilibrium between the three, with relative amounts varying depending on the pH. Very little molecular chlorine exists in solution at pH levels above 4 (or in dilute solutions). Hypochlorous acid is dominant at the low to moderate pH range. The hypochlorite anion is dominant at higher pH values. Hypochlorous acid is roughly 100 times more potent a germicide than the hypochlorite anion. This illustrates very effectively why the pH range of typical conventional lime treatment coagulation systems (9.6–10.5) renders the chlorine far less effective than at lower pHs. A pH in the range of 6 to 7.5 is generally preferred for chlorine disinfection. This provides a reasonable balance between hypochlorous acid and the hypochlorous anion, while not allowing the chlorine solution to become corrosive to piping and materials (as chlorinated water is more corrosive at low pH values). Chlorine effectiveness also varies with contact time (more contact time, more kill) and temperature (higher temperature, more kill).

Colorimetric methods are widely available to determine chlorine in water at concentrations ranging from 0.02–10 mg/l (49). When evaluating chlorine residuals, the distinction must be made between a "free" residual, and a "combined" residual. The "free" available chlorine residual is defined as that portion of the total residual chlorine which will react chemically and biologically as hypochlorous acid. It is in this form that chlorine exerts the most potent bactericidal effect. The "combined" available chlorine residual is defined as that portion of the total residual chlorine which will react chemically and biologically as chloramines or organic chloramines. In these forms, chlorine is a relatively mild bactericide and oxidizing agent. Analytically, using available test kits with prepared reagents, both the "free" chlorine and the "total" chlorine are measured. The assumption is made that the difference between these two represents the "combined" chlorine residual. The reagent N,N-diethyl-p-phenylenediamine (DPD) is most commonly used for both "free" and "total" chlorine testing (49). Orthotolidine has been used in the past (55), but has been found to be carcinogenic, and may pose other health risks. Food plants should therefore avoid it if at all possible. Oxidation-reduction potential (ORP) is another way to measure if chlorine or any other oxidizing agent is present in water (56). If you concentrate, you may remember from your school science classes that many chemical reactions around us are proton donor/acceptor in nature. However, with oxidation-reduction (redox) reactions, one chemical species loses, while another gains electrons. ORP is a value (number) defining how much gaining/losing of electrons a system might do (an oxidizing environment has a positive value). It is measured indirectly via conductivity or resistivity as the ability of water to conduct electricity, typically in millivolts. Shifts in ORP indicate chlorine breakthrough, contamination or that something has "changed" in the system.

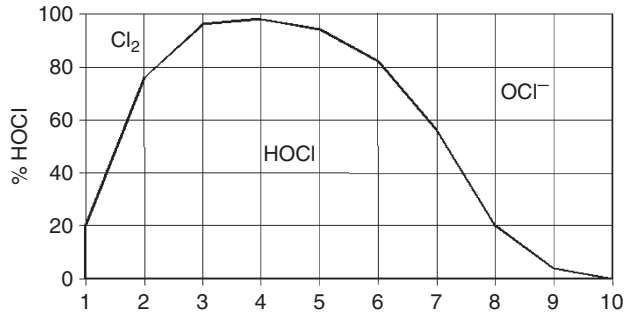

FIGURE 192.2 Relationship between chlorine, hypochlorous acid and the hypochlorite anion as a function of pH.

B. TRIHALOMETHANES

Trihalomethanes (THMs) are formed from the reaction of chlorine with natural organic matter precursors (humic and fulvic acids, for example). Surface supplies are most often implicated with respect to elevated levels of THMs. However, THMs may also be formed from ground water supplies, especially in cases where the well construction or integrity has been compromised. Four THMs are commonly found in water, namely chloroform, bromoform, bromodichloromethane and dibromochloromethane. They are closely controlled from a regulatory perspective as some THM species are known or suspect carcinogens above certain threshold levels.

THM formation is dependent on several factors including:

- Incoming raw water THM levels
- Incoming natural organic matter ("THM precursor") levels
- Water treatment conditions, such as conventional coagulation treatment systems
- Free chlorine concentration (increased chlorine levels can increase THM formation)
- pH (the higher the pH, the higher the THM formation potential)
- Chlorine contact time (the higher the contact time, the greater potential for forming THMs)
- Seasonal variations can create dramatic increases in THM precursors and subsequent THM formation

In most cases the food manufacturer's best defense against trihalomethanes such as chloroform is use and diligent maintenance of granular activated carbon (GAC) filters. Where conventional coagulation water treatment systems are in operation at a food plant, use of a "post-chlorination" technique is often employed where a high THM formation potential exists. This involves coagulating or otherwise removing the THM precursors, then chlorinating downstream. It is easier to remove the larger organic precursor molecules than the small THMs once they have been formed. For this reason, the best approach to THM control is to minimize their formation in the first place.

The same test procedures previously outlined in relation to Volatile Organic Compounds (VOC) testing may be used for THM testing (36,37). Although less precise, on-site THM test kits are offered by some companies which can be useful to track overall consistency of THM levels for some applications.

VIII. PROPERTIES OF WATER OF IMPORTANCE TO THE FOOD INDUSTRY

There are many properties of water that have particular importance for food industry applications. A number of the more common ones are described briefly below.

A. Taste, Odor and Appearance

Often, the human senses can detect contaminants in water at levels too low to be reliably measured by plant instrumentation, or in some cases the needed instrumentation may not be readily available. The concentrations at which such contaminants impact on food plant quality is dependent on individual and local plant factors, including the quality and consistency of the water available, the nature of the food products or processes with which the water is in contact and the sensitivity of the individual to the specific defect(s) that he or she is tasting.

A list of taste thresholds in water of commonly found constituents is provided in Table 192.2. Operational problems at a food plant facility can pose a major risk to the sensory profile of water and the food and beverage products it is used to prepare. One of the more common problems is the breakthrough of chlorine from a storage tank (perhaps due to the malfunction of a carbon filter). A noticeable "metallic taste" may point toward an overdose of coagulant, or an increase in metals due to an increase in the corrosivity of the water. If a carbon filter is utilized, carbon particles might sometimes penetrate the terminal polishing step of the process resulting in black specs in the treated water.

A rapid, coarse, taste, odor and appearance evaluation of water is routinely included as a step in overall food plant quality control. No chemical test is done, the tester merely relies on human senses. There should be no off-tastes, no off-odors, no turbidity (not cloudy), no suspended matter and no off-color in water used in the manufacture of food products.

TABLE 192.2
Taste Thresholds of Commonly Found Constituents in Water

Constituent	Approximate Taste Threshold
Inorganic Constituents	*mg/l*
Aluminium	0.2
Ammonia (at alkaline pH)	1.5
Calcium	100 to 300
Chloride	200 to 300
Hydrogen sulfide	0.05 to 0.1
Iron	0.3
Manganese	0.1
Sodium	200
Sulfate (highly cation specific)	250 to 1,000
Total dissolved solids	600 to 1,200
Zinc (as zinc sulfate)	4
Organic Constituents	*µg/l*
Toluene	40 to 120
Xylenes	300
Ethylbenzene	72 to 200
Chlorophenols	0.1 to 2

Source: Adapted from WHO Guidelines for Drinking Water Quality, 2nd Edition (1993).

B. Hardness

Hardness in water is caused by dissolved minerals, primarily those producing divalent, or double charged, cations including calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), strontium (Sr^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}). Calcium and magnesium ions are usually the only ones present in significant concentrations in most waters. Hardness is, therefore, generally considered by the food and water treatment industries to be a measure of the calcium and magnesium content of water. The terms "hard" water and "water hardness" stem from the fact that it is "harder" to raise a lather when more calcium and magnesium are present, i.e., more soap, detergent, or shampoo is necessary. The term "soft water" developed as "soft" is obviously an antonym of "hard." Temporary or carbonate hardness is the hardness due to the bicarbonates of calcium and magnesium in the raw water. By classical definition, this type of hardness can be removed by boiling the water. Permanent or non-carbonate hardness is the hardness due to the chlorides and sulfates of calcium and magnesium in the raw water. This type of hardness cannot be removed by boiling the water.

High level of water hardness can result in scale deposition, particularly on heating, depending on factors such as pH and alkalinity. It is critical to ensure that maximum hardness levels required by manufacturers for the equipment they supply are satisfied. Boiler make-up water, in

particular, may require softening when the hardness goes above 50 mg/l or sometimes lower. Waters with a hardness of less than 100 mg/l have a low buffering capacity and may be more corrosive to water pipes than water with higher hardness levels. The acceptability to the food industry will depend on:

- Whether water might need to be softened to prevent scale deposition. e.g., water to be used in plant operations like heat exchangers, boilers, bottle washers, cooling tunnels, etc;
- Where high hardness causes interactions with individual products (for example, tea beverages that contain oxalic acid require low hardness levels to prevent calcium oxalate precipitation from the beverage).
- Where high hardness can directly impact sensory in water, and potentially food products.
- Where soft water has a low buffering capacity and so may be more corrosive for water pipes.
- For plants that utilize coagulation water treatment systems and have sodium alkalinity present in the food plant water supply (sodium alkalinity is indicated by the hardness concentration being less than that of alkalinity, both measured as calcium carbonate equivalents). Hardness and alkalinity testing can monitor the effectiveness of coagulation systems by gauging the need for addition of a calcium salt to the reaction tank (required where effective alkalinity reduction is desired from the treatment).

C. Alkalinity

The sum of the concentrations of bicarbonates, carbonates and hydroxides present in a water sample is commonly referred to in the food industry as its alkalinity. High levels can contribute significantly to the total dissolved solids present in the sample (discussed above). Alkalinity is a critically important measurement for the beverage industry because high levels will neutralize the acidity in many beverages, especially carbonated soft drinks. This can lead to a poor sensory experience for the consumer or microbial problems in trade.

There are two widely utilized methods for measuring the alkalinity of the water. One is a colorimetric method that uses indicators that change the color of the solution under test depending on its pH (57). The second is pH-metric method which is more precise since it removes the subjectivity of interpreting a color change by using a pH meter to determine the test result. The relationship between alkalinity components, free CO_2 and pH is outlined in Figure 192.3.

D. pH

pH is a measure of the relative acidity or alkalinity of water. The pH scale runs from 0 (acid) to 14 (alkaline), with a mid-point of 7.0 denoting neutrality. In more scientific terms, pH is defined as the logarithm of the reciprocal of the hydrogen ion activity expressed in moles per liter. Like the "logarithmic units" of the Richter scale which measures earthquakes, each number on the pH scale represents a 10-fold change in the acidity/alkalinity of the water. For example, water with a pH of 5 is 10 times more acidic than water having a pH of 6.

The pH of water is a composite reading of the balance between water, free carbon dioxide, carbonate, bicarbonate and hydroxide. A shift in any one of these species will produce a shift in the pH. Since pH can be affected by chemicals in the water, it is an important indicator of water that is changing chemically. In other words, the results of a single measurement of pH are usually less important than looking at how it varies over time.

The wide range of treatment processes that food plants employ brings with it an equally wide range in treated water pH values. Treated water in the 4 to 5 range is not uncommon where the treatment system is weak acid cation (WAC) ion exchange due to the displacement of calcium and magnesium ions in the water with hydrogen (H^+) ion. An acidic pH is also common for many systems which utilize reverse osmosis or nanofiltration. This is due to the pre-membrane acid dosing used to prevent scaling/fouling and to help maximize recovery, and also due to the changed ratios of bicarbonate and carbon dioxide found in the water, as was described above in the dissolved gases section of this chapter. Conventional coagulation water treatment systems are on the other end of the spectrum. Here, the operational pH range in the reaction tank is in the area of 9.6 to 10.5 where hydrated lime addition is used in the process for effective alkalinity reduction (via precipitation as calcium carbonate). The treated waters out of these systems are typically somewhere around the pH 8–9 range.

So, a pH 4–5 would not be a concern if the treatment were WAC ion exchange or reverse osmosis, but it might suggest a process control problem with conventional coagulation treatment. Conversely, the pH 10–11 would be a concern from a conventional coagulation operational perspective, since a pH of 9.6–10.5 is expected in the reaction tank, and decreases in pH with chlorination, dechlorination with granular activated carbon filtration and other unit operations. A high pH after this type of treatment usually indicates that the treatment is not being optimally controlled (for example, excess of hydrated lime, poor selection of or exhausted carbon bed, etc.). Likewise, a pH of 10–11 for WAC, reverse osmosis or nanofiltration treatment would be highly unusual and would warrant investigation.

E. Solubility and Corrosiveness

The solubility and corrosiveness of water are two closely related properties that are of much interest to the food

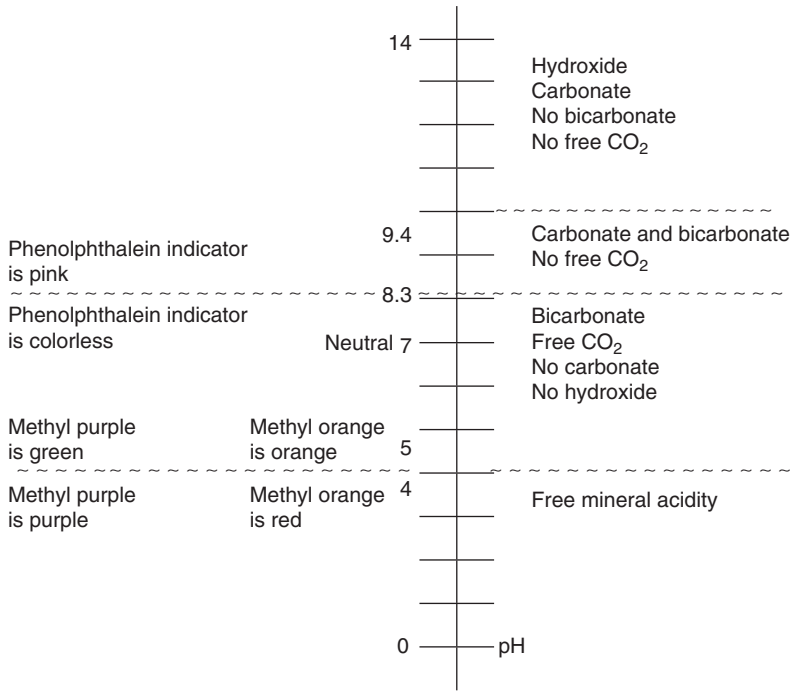

FIGURE 192.3 Relationship between alkalinity, free CO_2 and pH. (*Source*: Adapted from Pepsi-Cola training material, 2001.)

industry. The Langelier Saturation Index, or LSI, provides a measure of the solubility, or more specifically the scale forming tendency and corrosivity of water. The basic concept is that it indicates the degree of saturation of water with respect to calcium carbonate (58). LSI can also be understood as the pH change required to bring water to equilibrium. In order to calculate the LSI, it is necessary to know the alkalinity (mg/l as $CaCO_3$), the calcium hardness (mg/l Ca^{2+} as $CaCO_3$), the total dissolved solids (mg/l TDS), pH and the temperature of the water (°C). If the LSI testing result is negative, it can be interpreted that there is no potential to scale, the water will dissolve $CaCO_3$ and the water is corrosive. If the LSI test result is positive, it can be interpreted that scale can form, $CaCO_3$ precipitation may occur and the water is not corrosive.

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193 Beverage Plant Sanitation

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CONTENTS

I. INTRODUCTION

One part in 1,000,000,000,000,000 parts! Believe it or not, analytic measurements down to this detection level (parts per quadrillion, ppq) are quickly becoming routine for certain classes of organic compounds (for example, the polychlorinated dibenzo-dioxins and -furans). With the lightning-fast progress being made by chemists, physicists, and other scientists, we can only expect this trend to continue. Consequently, everyone involved with water in the food and beverage industries, either as an ingredient, product, or process chemical, will be forced to learn more about this often complex matrix—from its origin, through the paths it travels, the contaminants it meets along the way, to the point where it enters your production facility.

Water brings with it an inconceivable number of potential components—some considered contaminants, others considered therapeutic, and still others considered essential for life. The treatment technologist's challenge then becomes determining which components should be retained (and how), which should be reduced (and how)—and what the consequences of this selection might be. These consequences could potentially range from a minor, aesthetic defect in finished product, to a beverage or food

product which results in widespread public illness. The seriousness of a thorough understanding of water treatment is a unifying principle throughout this writing.

Over the past five years, the beverage industry's usable technology focus has moved from coagulation, ion exchange, filtration, carbon adsorption and ultraviolet disinfection to the use of membranes in technologies ranging from reverse osmosis, nanofiltration, ultrafiltration, and microfiltration; continuous electrodeionization, electrodialysis, selective exchangers and sophisticated controls and automation. Our quality focus has expanded to include organics, inorganics, and pathogens that are serious health concerns and present new treatment obstacles; and detection capability at a level to defy imagination which promises tighter and tighter regulatory guidelines. Our business focus is now largely driven and supported by regulatory guidelines and compliance requirements that demand in-house programs and reporting protocols. Over the next few years, millions of dollars will be spent on replacing or upgrading existing water treatment equipment or technology to keep pace with these new challenges. It will be a transition period of substantial turbulence, and we intend to offer insights into the advantages and shortcomings of all technology to aid the water technologist in making the most correct decisions for a given water supply.

II. WATER SOURCES

Perhaps the best place to begin a discussion of water sources is with an initial reference to the hydrologic cycle. As its name implies, the hydrologic cycle describes the continuous movement of water throughout its phases of state (solid, liquid, and vapor) within our hydrosphere, and is pictorially summarized in Figure 193.1. This "movement," or transformation, can be imagined in five major steps (1):

1. Water moving from the earth to the sky. In this part of the cycle, the movement of water from the earth to the sky involves a combination of three pathways: 1) evaporation, where water absorbs solar radiation which allows its transformation from water liquid to water vapor; 2) transpiration, where water is released to the atmosphere by plants as part of their normal physiologic processes; and 3) sublimation, where water (in its solid state as ice and snow) passes directly to the vapor state. The first two pathways above are often combined, and referred to simply as "evapotranspiration."
2. Water vapor forming water liquid. The active pathway in this step of the hydrologic cycle is one of condensation, due to temperature gradients within the atmosphere. Water vapor, which "moved from earth to sky," and which is currently stored in cloud formations, begins to form small droplets of water and/or small crystals of ice. As this condensing water continues to form, and eventually "saturates" the sky, the next step, below, is imminent.
3. Water falling back to earth, in the form of snow, ice, rain, sleet, and hail. The hydrologic cycle may be compared, on a much smaller and simplified scale, to a laboratory distillation. The aqueous sample in the "pot" is forced to move from liquid to vapor state, then the vapor is forced to condense back to liquid, and this purified liquid falls back to a collecting vessel. Ironically, despite the constant recycling of water through the hydrologic cycle, Table 193.1 illustrates that it may not be as pure as you think! These data, though reprinted in 1990 (2), were originally collected decades ago. A comparable analysis using today's environment and current analytic methodology would be interesting; unfortunately, it is likely that the table would need to be expanded, especially to include parameters as the result of acid precipitation, industrial discharge to the atmosphere, and emerging pathogens.
4. Water penetrating the ground. A single pathway describes this movement of water downward through the soil, but it is generally divided into two components: 1) infiltration, where water soaks into the soil and moves toward the root zones of area plant life. As part of the cycle, this water is then incorporated by the plants, and eventually re-introduced via transpirative processes; and 2) percolation, which describes a similar movement of water through the soil, but generally to greater depths—past the root zones and toward the aquifers, or water bearing geologic strata, which will be discussed in more

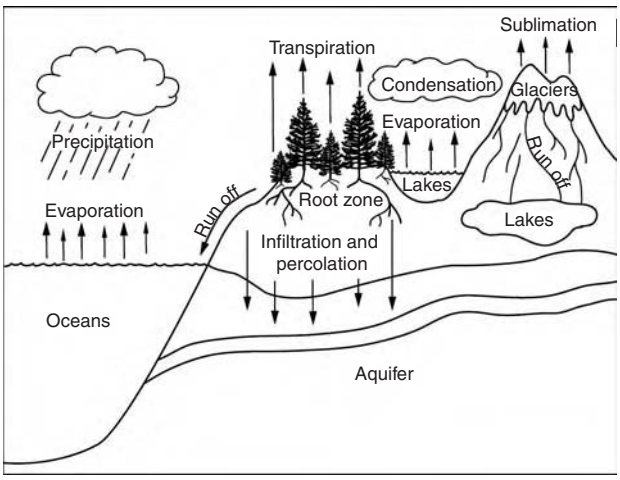

FIGURE 193.1 The hydrologic cycle.

TABLE 193.1
Chemical Analyses of Rain, Snow, and Hail

Parameter, in ppm	Rain, after 4 Hours	Rain, after 22 Hours	Snow	Hail
Total hardness, as $CaCO_3$	43	8	18	28
Calcium hardness	42	8	14	25
Magnesium hardness	1	11	4	3
Alkalinity	19	5	—	4
Sodium	5	0.11	5	—
Ammonia	1.5	2	6	1
Chloride	7	4	12	7
Sulfate	26	3	21	17
Nitrate	1	—	1	—
Iron	0.9	0.1	1.2	2.4
Silica	0.15	0.15	3	1

Source: Adapted from Morelli (1990).

detail in the next sections. It is this movement of water through the soil that represents a "double-edged sword." On one hand, infiltration provides a perfect opportunity for the water to become contaminated on its journey—from underground storage tanks, septic systems, naturally occurring contaminants, and other sources. On the other hand, it also provides one of the most effective attenuative mechanisms for many contaminants. That is, through complex ion-exchange, adsorptive, and microbiologic processes in the soil, contaminants may be reduced in concentration, or completely transformed chemically into some other compound before reaching many groundwater supplies.

5. Water returning to the oceans. This explains the pathway of surface runoff. The soil becomes "saturated" (this term is used loosely here, since saturated and unsaturated zones have very specific meanings to hydrogeologists), and flows downhill (piezometrically, and usually downhill topographically—but not always) over the surface and near the surface. The moving water can incorporate anything in its path including physical debris, chemical contaminants, and microorganisms, as it moves toward the rivers, streams, etc., and on back to the oceans, where it again joins the evapotranspirative section of the hydrologic cycle.

Since this effect is cyclical, it may be entered at any point along its route, and followed through to some endpoint. Though the hydrologic cycle is often viewed as basic and fundamental, it does underscore the conservative and recyclable nature of these processes. As a result, the quality and safety of the water used in our products and processes is, to some extent, directly linked to how that water supply traverses the hydrologic cycle, and with which contaminants and impurities it comes in contact. Our knowledge of the basic and allied sciences is ever increasing, and the importance of water quality and overall water resources management increases in direct response. Now, the toxicologic and other health-related impacts of our ingredient water are under more study than ever before. As the skills of the analytic chemist lead to ever-decreasing lower limits of detection, the chemicophysical and microbiologic integrity of our water supplies will be under more scrutiny and of greater importance than at any other time in history. This chapter addresses the selection, treatment, and safety of this dynamic ingredient—water—as used in the beverage industry.

As an introduction, Table 193.2 affords an overall view of the world's water distribution (3). These numbers will vary depending on which source, and what year of publication, are cited. The overall illustration, however, is the same. The key things to glean from the table are these: 1) the vast majority of the world's water is trapped in the oceans, which are not yet considered economically feasible as useable drinking water supplies on a large scale; 2) if we remove the oceans from consideration, and we remove the water

TABLE 193.2
Estimated World Water Balance

Parameter	Surface Area ($km^2 \times 10^{-6}$)	Volume ($km^3 \times 10^{-6}$)	Volume (%)	Equivalent Depth (m)	Residence Time
Oceans and seas	361	1370	94	2500	~4000 years
Lakes and reservoirs	1.55	0.13	<0.01	0.25	~10 years
Swamps	<0.1	<0.01	<0.01	0.007	1 to 10 years
River channels	<0.1	<0.01	<0.01	0.003	~2 weeks
Soil moisture	130	0.07	<0.01	0.13	2 weeks to 1 year
Groundwater	130	60	4	120	2 weeks to 10,000 years
Icecaps and glaciers	17.8	30	2	60	10 to 10,000 years
Atmospheric water	504	0.01	<0.01	0.025	~10 days
Biospheric water	<0.1	<0.01	<0.01	0.001	~1 week

Source: Adapted from Nace (1971).

trapped in glaciers and the polar ice caps, only 1 to 5% of the world's water (depending on the source cited) is commonly considered treatable for use; and 3) of this small percentage, the vast majority of the supply (in some cases close to 100%) is found in the form of ground water.

Unfortunately, the citation of *available water*, alone, does not automatically mean that these supplies are *accessible*—or *safe*. According to Daniel A. Okun (4), in 1980, nearly two billion people did not have access to water supply and sanitation services. In 1990, it was estimated that more than 2.7 billion people in developing countries lacked access to these basic services (1.7 billion without access to sanitation, over 1 billion without access to water). Ten years later, at the first World Water Conference held at the Hague, a full 20% of the global population was cited as being without access to safe drinking water (5). Many feel that even these numbers are grossly underestimated due to the classification of exactly what water supply and sanitation "services" mean. As a country's geographic region, economic standing, and political infrastructure vary, so do the extent of what is "acceptable" in the provision of water supplies. Since we now know that the overall supply of water is a finite entity, let us examine the different water sources from which we may select our supply.

A. Water Source Categories

Water sources may be classified into two major categories: 1) ground water, and 2) surface water. Ground water examples include consolidated and unconsolidated aquifers (discussed later), artesian supplies, springs, etc. In short, water supplies that invade the saturated zone of the subsurface environment. Surface water examples include reservoirs, oceans, lakes, rivers, etc. The two categories differ greatly in the characteristics of the water they supply, as does water from groups even within the same category. Surface waters are usually higher in suspended solids, color, and turbidity than ground waters, and lower in total dissolved solids. The temperature of ground waters (depending on depth) is remarkably consistent—sometimes within a few degrees per year; in contrast, surface supplies, subject to the sun's radiation, are remarkably variable in temperature. As far as flow characteristics, surface waters are usually turbulent, while ground waters are usually laminar. "Typical" flows for ground water supplies may be one meter *per day*, in contrast to some surface water flows of one meter *per second*! Due to this wide variability, the often asked question of, "what does a 'typical' surface water look like in terms of its chemistry?" is impossible to answer with certainty. The best we can do is provide generalities, with the condition that there are truly exceptions to every rule. Table 193.3 was compiled by the author to present a relative comparison of surface and ground water supplies. Just when you think you have a thorough understanding of a particular supply—tread carefully—you may not!

TABLE 193.3
Relative Comparison of Ground and Surface Supplies

Parameter	Ground Water	Surface Water
Total dissolved solids	Higher	Lower
Suspended solids	Lower	Higher
Turbidity and color	Lower	Higher
Alkalinity	Higher	Lower
Total organic carbon	Lower	Higher
Microbiology:		
Protection from bacteria and viruses	Highly protected	Highly susceptible
Protection from protozoa	Almost completely protected	Highly susceptible
Presence of iron and/or manganese bacteria	Common	Rare
Hydrogen sulfide gas	Common	Uncommon
Aeration/dissolved oxygen	Lower	Higher
Temperature	More consistent	More variable
Flow rate	Very slow (1 m/day)	Very fast (1 m/sec)
Flow pattern	Laminar	Turbulent
Susceptibility to pollution through surface run-off	Low	High
Time for a contaminant plume to resolve	Very long—often decades, potentially centuries!	Usually short—day/months; sometimes years

Source: Developed by the author (1997).

1. Surface Water Supplies

In general, surface supplies can be highly variable in every respect—chemically (total dissolved solids, alkalinity, etc.), microbiologically (bacteria, viruses, etc.), and physically (turbidity, color, etc.). Many surface waters are easily subject to contamination which may present in many forms, including: 1) bacteria and other organisms from animal wastes via direct introduction (animals) or indirect introduction (poorly or untreated wastewater); 2) algae blooms, which are typically acute, seasonal events; 3) "natural" chemical contamination as evidenced by high levels of natural organic matter (primarily the humic substances from decaying vegetation and animal waste); and 4) "synthetic" chemical contamination from surface runoff (outflow) of agricultural chemicals (pesticides, herbicides, insecticides, etc.).

Again, it must be underscored that the characteristics above, and those that follow, are intended to provide general trends in terms of water composition and characteristics. During the author's industrial tenure, exceptions to virtually every characteristic described have been observed.

a. Streams

In general, streams are often of reasonable chemical/physical composition. Due to their locations and physical dimensions, they offer easy access for a multitude of animal life.

With this comes the frequent introduction of microorganisms of fecal origin, in addition to appreciable amounts of organic matter. This organic material is typically considered the precursor material of trihalomethanes and a host of other chemical by-products which can be formed once this water supply is disinfected. Smaller streams are often influenced by rain events, whereby their flows are increased, with subsequent increase in suspended solids and turbidity. Larger streams are generally at higher risk of having industrial waste (often poorly or inadequately treated) discharged into them. They are often more of a concern, due in part to their larger surface area, in terms of accepting surface runoff and subsurface drainage.

b. Lakes

Natural lakes, due to their relatively stagnant flow patterns, coupled with their long water residence time, are usually of consistent composition insofar as surface supplies are concerned. One major climatic event which may have drastic changes in lake water quality, is the phenomenon of seasonal inversion. This refers to the phenomenon whereby water at the surface of the lake reaches a temperature at which it is most dense (3.98ºC, or 39.2ºF). The water below it has not yet reached this state, therefore, a density and temperature gradient are formed. This "heavy" water then begins to descend, and displaces the water below it. This displaced water then inverts (hence the term "inversion"), and moves from the bottom of the lake toward the upper portion. This agitation brings with it much of the sediment, and associated unwanted components, which make municipal and industrial monitoring even more critical during this time. As you might imagine, this phenomenon of inversion and its related thermal stratification, are actually much more complex, and their effects on aquatic life and the eutrophication process (basically, the nutrient enrichment of a body of water, usually lakes or ponds, which results in certain forms of algae growth and some higher plant life) have been studied under many other scientific disciplines. Unfortunately, as is the case with many large streams, industrial waste effluent and sewage treatment plant discharge are often re-introduced into the same lake that originally supplied the influent water. These practices are coming under more scrutiny, and there is ever increasing political pressure for regulatory reform.

c. Reservoirs

Impounding reservoirs, or "man-made" lakes, are similar in overall characteristics to those described above for natural lakes. They are often regarded as huge storage reservoirs for municipal water supplies prior to treatment. As surface supplies go, reservoirs are of fairly consistent quality, reasonable turbidity (due in large part to natural oxidation and settling mechanisms), and often afford lower bacteria counts than other surface supplies (6). However, as a consequence of their relatively low flow patterns and lack of agitation, algae blooms are often a problem.

d. Rivers

Rivers represent perhaps the most difficult of the surface supplies to address. In general, they are of highly inconsistent quality, very high turbidity and suspended solids, prone to considerable temperature fluctuations, and vary widely with respect to their flow patterns (that is, areas of excessive turbulence, and areas of minimal movement may exist within close proximity to each other). Rivers, as a result of their great length and flows, are recipients of surface runoff from many types of areas. For example, a river might flow across areas of agrarian activity, with the potential to incorporate pesticides, herbicides, nitrate, and other contaminants along its route. This same river might later flow through an industrial zone, pick up run-off from poorly contained chemical storage tanks, drains, sewers, etc., and possibly even meet the discharge of one or more municipal waste treatment plants! Imagine the contaminant "soup" that would result. For this reason, any methods of treatment for river water must be preceded by thorough characterization of the supply. The treatment itself must be capable of addressing a wide range of quickly changing water quality.

2. Ground Water Supplies

In comparison to surface supplies, ground water supplies are generally more consistent in every respect—thermally, microbially, chemically, and physically. They have historically been considered a much safer supply, or one which produces "purer" water. Two hundred years ago, this blanket statement might have been more universally true. Along with the development of the chemical and related industries came the increased potential for ground water contamination. Prior to this, the concerns over ground water were few—maybe the well was dug a little too close to the septic tank or cess pool, and incidences of diarrhea and other gastrointestinal ailments were increasing (so "let's dig another one farther away," or "let's dig this one a little deeper" were the seeming solutions). Possibly the well was under the influence of a salt water or brackish supply, and intrusion of high levels of salts were becoming evident (usually noticed by taste). In many cases, the two former solutions would have applied here as well.

Though this previous discussion has been a gross over-simplification, the facts remain that, with increasing industry, came increasing underground chemical storage—increasing contaminant spills—increasing numbers of poorly located and/or poorly constructed septic systems, along with a host of other problems for the ground water environment that needed to be addressed (and, in fact, many still do).

As with surface water supplies, there are different classifications of ground water—some more preferred than others. A brief discussion of each, in terms understood by the novice hydrogeologist (which is a discipline becoming increasingly important for anyone involved with water operations to know), follows below.

a. Aquifers and the underground environment

An aquifer (7) is defined as "a geologic formation with sufficient interconnected porosity and permeability to store and transmit significant quantities of water under natural hydraulic gradients." Critical terms in this description are "store," "transmit," and "significant quantities." All three should be satisfied to consider a supply an aquifer. The first two are straightforward—huge amounts may be stored, but unable to be transmitted—in which case, this supply, however large, should not be considered an aquifer. The third term, "significant quantities" is more nebulous and linked to the intended use of the supply—for example, a residential well, a large municipality, or multiple industrial users tapped into a community well. Clearly, "significant quantities" would be defined differently for these three applications. Before discussing aquifers in more detail, Figure 193.2 which follows should be reviewed. It describes the different layers or zones encountered as we move downward from the surface of the ground to the water bearing strata below it.

As we move from the surface, the first zone encountered is the "unsaturated zone" (also referred to as the "vadose zone"). In this area, the geologic media (dirt, clay, sand, etc.) contains a mixture of water and void spaces with air—hence, the terms "unsaturated" or "variably saturated." Continuing downward, we reach the "capillary fringe," which is generally considered the beginning of the "saturated zone," but is sometimes considered a distinct entity. This interface between unsaturated and saturated zones is not completely understood, and is the subject of much study with regard to movement of certain contaminants within it. The "saturated zone" is the area where air is at a minimum, and water is at a maximum. The geologic media here are saturated with water. Dissolved oxygen in the saturated zone is extremely rare; many deep ground water formations exist under anaerobic or hypoxic conditions. It is within the saturated zone where actual ground water supplies are found, and where most production wells are placed. Throughout the saturated zone, many strata of varying permeability will be found. Most importantly, this is where we find our aquifers.

b. Unconfined and confined aquifers

Aquifers may be grouped into two broad categories: 1) unconfined aquifers, and 2) confined aquifers. Unconfined aquifers (sometimes referred to as "water table aquifers") are those water bearing, geologic formations which are under atmospheric pressure at their upper boundary. The "water table" (sometimes referred to as the "phreatic surface") is the upper boundary of the saturated zone. Water levels in wells which tap unconfined aquifers should be the same as the level of the water table. The plane which connects the upper levels of water in all wells which penetrate unconfined aquifers is known as the "potentiometric surface." Confined aquifers (sometimes called "artesian aquifers") are those water bearing, geologic formations whose upper and lower boundaries are comprised of geologic material of low permeability, and which are under pressure greater than atmospheric. Older definitions may describe the boundary layers (or "confining layers") of a confined aquifer as being of no permeability (that is, "impermeable"). This is inaccurate, as

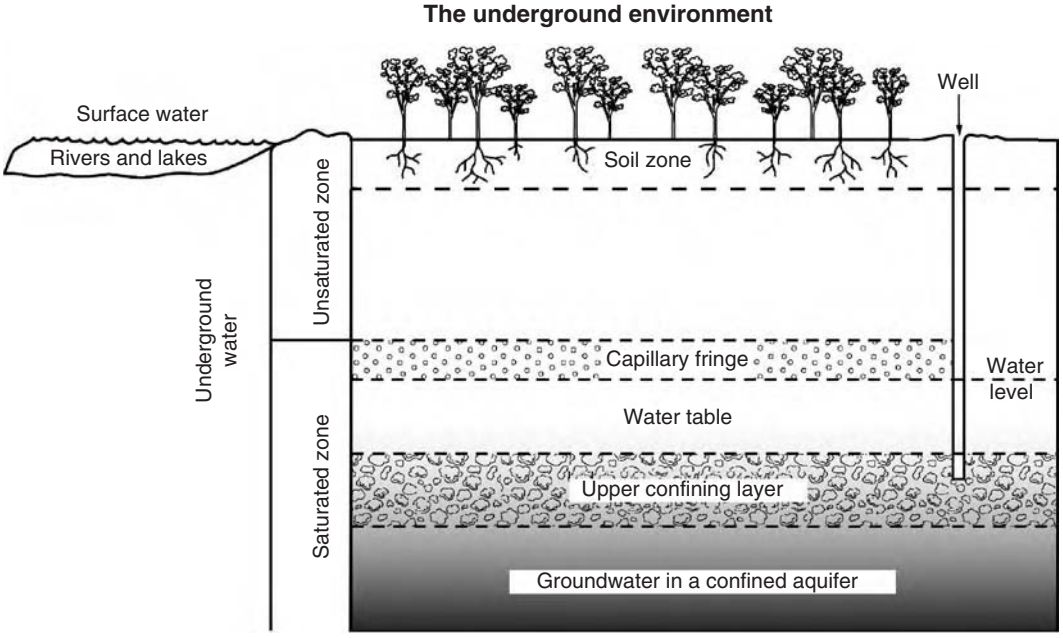

FIGURE 193.2 The underground environment.

even the least permeable geologic materials still exhibit some degree of permeability. Some hydrogeologists further classify these confining layers accordingly, as aquitards, aquicludes, and aquifuges. While all three exhibit very low permeabilities, aquitards are the most permeable of the three, followed by aquicludes, and finally aquifuges, which are as close to impermeable as we know. Confined aquifers are sometimes further described as "semiconfined" (also, "partially confined," or "leaky confined") or "highly confined" (also, "fully confined"), depending on the leakage or seepage through the confining layers.

Just as the water in unconfined aquifers will form a potentiometric surface, or water table plane, the water levels in wells tapping confined aquifers will also form a plane. It may still be referred to as a "potentiometric surface," but, intuitively, not a "water table plane." In the case of confined aquifers, due to the internal pressures, the water levels in wells which tap them may often exceed the level of the water table (which may result in a "flowing artesian well"). In the beverage industry, conversational knowledge of confined and unconfined aquifers will account for nearly all of the hydrogeologic discussions into which a beverage technologist might become engaged. For purposes of completeness, two more topics will be briefly addressed below.

In addition to aquifers being classified as confined or unconfined, the geologic material of the aquifer may be described as consolidated or unconsolidated. "Unconsolidated" deposits are formed from loose geologic material, such as sand, clay, silt, gravel, and even sea shell remains. "Consolidated" deposits are formed by mineral particles combining from heat and pressure, or via chemical mechanisms. They include sedimentary (previously unconsolidated) rocks, such as limestone, dolomite, shale, and sandstone; igneous (formed from molten) rocks, such as granite and basalt; and metamorphic rocks, such as limestone and gneiss (8). Fractured rock formations almost always refer to fractures or fissures in consolidated deposits. Ground water and contaminant flows through this type of formation are highly unpredictable, since it is difficult to determine which route the water will take through this hard rock maze. Carbonate aquifers (also "karstic formations") are formations of limestone and other water-soluble rocks whose fractures have been widened by erosion to form sinkholes, caves, or tunnels (9). As you might expect, with such little resistance, the flows through fractured and carbonate formations can be rapid enough to rival surface water sources. Flows, though rare, have been reported up to 1500 feet per day.

B. Source Selection Considerations

The categories above briefly discussed the major sources of supplies for potable water. Obviously, there are others (oceans, lagoons, glaciers, etc.), and many possible combinations of supplies. Remember, the keys to any consideration of a water source are:

1. First and foremost, its sanitary quality, wherever possible. In some areas of the world, the potability of a supply—even a municipal supply—may not be guaranteed.
2. Chemical/physical quality—is it safe? Is it too high a risk to even consider? Can it be treated economically and within regulatory guidelines? These questions must be answered on a case-by-case basis, and will depend on the degree of due diligence desired by a parent company, presiding regulations, corporate policies, and the risk assessment of the impurities themselves.
3. Consistency of composition—is it consistent? Will it vary beyond the capability of the proposed treatment? Gathering any and all available data will aid in answering this question. Make use of municipal monitoring data, rainfall data, hydrogeologic or surface water surveys, etc.
4. Volumes/supply—can it currently meet your needs? Will it in the future? In addition to the quality and safety components, supply is a key parameter to help ensure that the volume of water will be available for the long-term needs of the business. In many areas, the volume of withdrawal of water from an aquifer falls under government control or guidance, and this must be considered.
5. Its recharge—is run-off a concern? This is related to the previous discussion in this chapter of the hydrologic cycle. The volume of recharge, or replenishment, of the aquifer is important, as is the quality and origin of the water being used for that recharge.
6. Future plans for the source or surrounding areas—is a municipality planning to develop the source and treat it? Are there multiple taps? Is there planned construction or industrial entry to the area? These questions highlight the value of considering water a dynamic ingredient throughout its supply chain. Many beverage plant issues have resulted from municipal water treatment plant operators effecting a change to the municipal treatment without alerting the beverage plant personnel. For example, if polyphosphate use is instituted by a municipality for corrosion control within their distribution system, this could result in a gross upset to the floc formation in conventional lime treatment systems.

Water, unlike virtually any other raw material, often does not provide an opportunity for sourcing from an

alternate supplier. Consequently, selection of a source after thorough characterization is paramount, and subsequent treatment design is critical to helping assure that only a safe, consistent, high quality treated water is used by food and beverage plants.

C. Specifications and Guidelines

Frankly, in the Unites States, Canada, the European Union, and most other first-world countries, the expectations for water treatment are clearly delineated by the regulatory agency having jurisdiction. That is, drinking water must meet the National Primary Drinking Water Standards (10, 11) promulgated by the Unites States Environmental Protection Agency, the Directive of the European Union on the Quality of Water Intended for Human Consumption (12), or some analogous drinking water standard. Indeed, many of these standards are founded, to varying degree, on the Guidelines for Drinking Water Quality established by the World Health Organization (13, 14). Packaged water, or the water used to make carbonated beverages, usually has corresponding regulations. In the US, for example, the Food and Drug Administration established a bottled water standard of identity, which, with only a few exceptions, mirrors the EPA's Drinking Water Standards.

The challenges come when we establish our beverage businesses in second- and third-world countries where the regulatory standards are perhaps not as refined as for those countries mentioned previously. In these countries, the beverage producer is not afforded the luxury of knowing that the water entering their plant will consistently meet USEPA drinking water standards—or the EU Standards for water intended for human consumption. In these situations, it becomes even more critical that rigorous water source assessment, careful treatment selection, and conscientious long-term monitoring are performed with even greater diligence.

Table 193.4 was compiled by the author for informational purposes only to compare the major standards for drinking water around the globe. The National Primary Drinking Water Standards of the USEPA are compared to the EU Standards for Water Intended for Human Consumption, and finally with the Guidelines for Drinking Water Quality of the World Health Organization.

For a beverage producer, as is likely the case with any of our food industry allies, we cannot—and should not—rely on any external body to assure the quality and consistency we require for the production of our trademark products. Certainly, a conscientious municipal monitoring scheme established by the EPA or analogous regulatory organization often increases our confidence that a particular water supply will reach our plant with some level of safety and consistency, but this should not be expected; rather, it should be viewed as an added benefit to complement our already-dependable in-plant treatment system.

III. WATER TREATMENT

A. Goals of Treatment

In light of the mounting scientific evidence for the myriad chemical compounds related to their adverse health effects—at a time when carcinogen, mutagen, and teratogen are practically becoming part of the vernacular—the primary reason for water treatment is to safeguard public health and safety. All other reasons for treatment fall second, and include the following:

1. To assure compliance with all levels of regulatory guidelines and mandates. The product water for carbonated soft drinks has historically had to meet not only the primary drinking water standards of the U.S. Environmental Protection Agency (in the United States), but all applicable local standards as well. This same philosophy of multiple levels of compliance holds true as we look at the international arena as well. Here, compliance will be driven not only by national standards (often founded to varying degree on the World Health Organization Guidelines for Drinking Water), but also by local (state, regional, etc.) regulations and codes germane to each product category (bottled water, natural mineral waters, juice drinks, teas, etc.).
2. To achieve specific product characteristics and improve product shelf-life. Certain soft-drink products require ingredient water of varying composition. By utilizing a variety of water treatment unit operations, the requisite composition can be achieved (for example, ion-exchange demineralization for those products sensitive to the profile of dissolved ionic solids). In addition, the shelf-life of the individual product is often prolonged by removal of troublesome components that could potentially result in aesthetically displeasing precipitation (for example, preventing the precipitation of calcium oxalate salts in tea by limiting the incoming calcium load).

As mentioned above, the wide range of chemicophysical characteristics required of our ingredient water mandates the utilization of a variety of water treatment technologies. Selection of the appropriate treatment chain is based upon several factors, including, but not limited to: 1) water source—aquifer and watershed characteristics, municipal supply/consistency, location, etc.; 2) proposed technology capability relative to raw water analyses and desired product characteristics—removal of organics, inorganics, disinfection/inactivation, color and odor control, etc.; 3) recommended support technology—filtration, preozonation, iron/manganese removal, etc.; 4) costs vs. finished water profile—initial investment, operating costs,

TABLE 193.4
Comparison of Major Drinking Water Standards and Guidelines

Parameter	WHO Health	WHO Aesthetics	USEPA 1° MCL Health	USEPA 2° MCL Aesthetics	EU	EU Indicator Parameters
Inorganic Constituents	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Aluminum		0.2		0.05–0.2		0.2
Ammonia		1.5				0.5 (ammonium)
Antimony	0.005P		0.006		0.005	
Arsenic	0.01P		0.05 (interim)		0.01	
Asbestos	U		7MFL (>10 μ)			
Barium	0.7		2			
Beryllium	NAD		0.004			
Boron	0.5P				1	
Cadmium	0.003		0.005		0.005	
Calcium						
Chloride		250		250		250
Chromium	0.05P		0.1 (total)		0.05	
Copper	2P	1	1.3 (action level)	1	2	
Cyanide	0.07		0.2 (as free)		0.05	
Fluoride	1.5		4	2	1.5	
Hydrogen sulfide		0.05				
Iron		0.3		0.3		0.2
Lead	0.01		0.015 (action level)		0.01	
Magnesium						
Manganese	0.5P	0.1		0.05		0.05
Mercury (total)	0.001		0.002 (inorganic)		0.001	
Molybdenum	0.07					
Nickel	0.02P		0.1		0.02	
Nitrate (as NO_3)	50		10 (as N)		50	
Nitrite (as NO_2)	3P, acute 0.2P, chronic		1 (as N)		0.5	
Nitrate/nitrite	Sum of [conc:GV] ≤ 1		10, total (as N)			
Potassium						
Selenium	0.01		0.05		0.01	
Silver	U			0.1		
Sodium		200				200
Sulfate		250	500P	250		250
Thallium			0.002			
Tin	U					
Zinc		3		5		
Organic Constituents	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L
Chlorinated Alkanes:						
Carbon tetrachloride	2		5			
1,1-Dichloroethane	NAD					
1,2-Dichloroethane	30		5		3	
Dichloromethane	20		5			
1,2-Dichloropropane			5			
1,1,1-Trichloroethane	2000P		200			
1,1,2-Trichloroethane			5			
Chlorinated Ethenes (or Ethylenes):						
1,1-Dichloroethene	30		7			
1,2-Dichloroethene	50		70 (cis-) 100 (trans-)			
Trichloroethene	70P		5		10 total	
Tetrachloroethene	40		5			
Vinyl chloride	5		2		0.5	

(*Continued*)

TABLE 193.4 (*Continued*)

Parameter	WHO Health	WHO Aesthetics	USEPA 1° MCL Health	USEPA 2° MCL Aesthetics	EU	EU Indicator Parameters
Aromatic Hydrocarbons:						
Benzene	10		5		1	
Benzo(a)pyrene	0.7		0.2		0.01	
Ethylbenzene	300	2–200	700			
Fluoranthene	U					
Phenols						
Polycyclic aromatic hydrocarbons (PAHs), as sum of:					0.1	
Benzo(ghi)perylene						
Benzo(b)fluoranthene						
Benzo(k)fluoranthene						
Indeno(1,2,3-cd)pyrene						
Styrene	20	4–2600	100			
Toluene	700	24–170	1000			
Xylenes (o, m, and p)	500	20–1800	10,000 total			
Chlorinated Benzenes:						
Monochlorobenzene	300	10–120	100			
1,2-Dichlorobenzene	1000	1–10	600			
1,3-Dichlorobenzene	NAD					
1,4-Dichlorobenzene	300	0.3–30	75			
1,2,4-Trichlorobenzene			70			
Trichlorobenzenes (total)	20	5–50				
Miscellaneous Organics:						
Acrylamide	0.5		TT		0.1	
Dialkyltins	NAD					
Di(2-ethylhexyl)adipate	80		400			
Di(2-ethylhexyl)phthalate	8		6			
Edetic Acid (EDTA)	600					
Epichlorohydrin	0.4P		TT		0.1	
Hexachlorobutadiene	0.6					
Microcystin-LR cyanobacterial toxin	1P					
Nitrilotriacetic acid	200					
Polychlorinated biphenyls (PCBs), as decachlorobiphenyl			0.5			
Tributyltin oxide	2					
Pesticides:	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Total pesticides				0.5 (and 0.1 each)		
Alachlor	20		2		0.1	
Aldicarb	10		3		0.1	
Aldicarb sulfone			2		0.1	
Aldicarb sulfoxide			4		0.1	
Aldrin/dieldrin	0.03				0.03	
Atrazine	2		3		0.1	
Bentazone	300				0.1	
Carbofuran	7		40		0.1	
Chlordane	0.2		2		0.1	
4-Chloro-2-Methylphenoxy acetic acid (MCPA)	2				0.1	
Chlorotoluron	30				0.1	
Cyanazine	0.6				0.1	
Dalapon			200		0.1	
1,2-Dibromo-3-Chloropropane (DBCP)	1		0.2		0.1	
1,2-Dibromoethane	0.4–15P				0.1	

(*Continued*)

TABLE 193.4 (*Continued*)

Parameter	WHO Health	WHO Aesthetics	USEPA 1° MCL Health	USEPA 2° MCL Aesthetics	EU	EU Indicator Parameters
Dichlorodiphenyltrichloroethane (DDT)	2				0.1	
2,4-Dichlorophenoxyacetic acid (2,4-D)	30		70		0.1	
1,2-Dichloropropane	40P		5		0.1	
1,3-Dichloropropane	NAD				0.1	
1,3-Dichloropropene	20				0.1	
Dinoseb			7		0.1	
Diquat	10P		20		0.1	
Dioxin (2,3,7,8-TCDD)			0.00003		0.1	
Endothall			100		0.1	
Endrin			2		0.1	
Ethylene Dibromide	NAD		0.05		0.1	
Glyphosate	U		700		0.1	
Heptachlor	0.03		0.4		0.03	
Heptachlor epoxide	TOTAL OF BOTH		0.2		0.03	
Hexachlorobenzene	1		1		0.1	
Hexachlorocyclopentadiene			50		0.1	
Isoproturon	9				0.1	
Lindane	2		0.2		0.1	
Methoxychlor	20		40		0.1	
4(2-Methyl-4-Chlorophenoxy) butyric acid (MCPB)	NAD				0.1	
Metolachlor	10				0.1	
Molinate	6				0.1	
Oxamyl (Vydate)			200		0.1	
Pendimethalin	20				0.1	
Pentachlorophenol	9P		1		0.1	
Permethrin	20				0.1	
Picloram			500		0.1	
Propanil	20				0.1	
Pyridate	100				0.1	
Simazine	2		4		0.1	
Terbuthylazine	7				0.1	
Toxaphene			3		0.1	
Trifluralin	20				0.1	
Chlorphenoxy Herbicides other than 2,4-D and MCPA:						
4(2,4-Dichlorophenoxy) butyric acid (2,4-DB)	90				0.1	
Dichlorprop	100				0.1	
Fenoprop	9				0.1	
Mecoprop	10				0.1	
Silvex (2,4,5-TP)			50		0.1	
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	9				0.1	
Disnfectants and Disinfection By-Products (D-DBPs):	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Bromate	25P		10		10	
Monochloramine	3000					
Chloral hydrate (Trichloroacetal dehyde)	10P					
Chloramines (total)			4000 MRDL			

(*Continued*)

TABLE 193.4 (*Continued*)

Parameter	WHO Health	WHO Aesthetics	USEPA 1° MCL Health	USEPA 2° MCL Aesthetics	EU	EU Indicator Parameters
Chlorate	NAD					
Chlorine	5000	600–1000	4000 MRDL			
Chlorine dioxide			800 MRDL			
Chlorite	200P		1000			
Chloroacetone	NAD					
3-Chloro-4-dichloromethyl-5-hydroxy-2(5H)-furanone (MX)	NAD					
Chloropicrin	NAD					
Cyanogen chloride (as CN)	70					
Dichloramine	NAD					
Formaldehyde	900					
Trichloramine	NAD					
Other disinfectants						
Chlorophenols:						
2-Chlorophenol	NAD	0.1–10				
2,4-Dichlorophenol	NAD	0.3–40				
2,4,6-Trichlorophenol	200	2–300				
Halogenated Acetic Acids:						
Monochloroacetic acid	NAD					
Dichloroacetic acid	50P					
Trichloroacetic acid	100P					
Haloacetic Acids (HAA-5), includes mono-, di-, and trichloroacetic acid, and mono- and dibromoacetic acid)			60 total			
Halogenated Acetonitriles:						
Bromochloroacetonitrile	NAD					
Dibromoacetonitrile	100P					
Dichloroacetonitrile	90P					
Trichloroacetonitrile	1P					
Trihalomethanes:						
Bromodichloromethane	60					
Bromoform	100					
Chloroform	200					
Dibromochloromethane	100					
Total THMs	Sum of [conc:GV] ≤ 1		80		100	
Other Chemical/Physical Parameters						
Color		15TCU		15 Co-Pt		Acceptable to consumers and no abnormal change
Conductivity @20°C						2500 uS/cm
Corrosivity				Non-corrosive		
Foaming Agents				0.5 mg/l		
Odor		Acceptable		TON=3		Acceptable to consumers and no abnormal change
Oxidizability						5 mg/L O_2
pH		<8 for effective disinfection w/chlorine		6.5–8.5		≤ 6.5 and ≤ 9.5

(*Coninued*)

TABLE 193.4 (*Continued*)

Parameter	WHO Health	WHO Aesthetics	USEPA 1° MCL Health	USEPA 2° MCL Aesthetics	EU	EU Indicator Parameters
Taste		Acceptable				Acceptable to consumers and no abnormal change
Total Dissolved Solids (TDS)		1000 mg/L		500 mg/L		
Total Organic Carbon (TOC)						No abnormal change
Turbidity		5NTU	TT			Acceptable to consumers and no abnormal change
Radiologic Constituents						
Alpha activity, gross	0.1 Bq/L		15 pCi/L (includes Ra-226; excludes radon and uranium)			
Beta activity, gross	1 Bq/L		4 mRem/year			
Combined radium-226 and radium-228			5 pCi/L			
Radium-226			20P pCi/L			
Radium-228			20P pCi/L			
Radon			300P pCi/L			
Total indicative dose						0.1 mSv/year
Tritium						100 Bq/L
Uranium	0.002P mg/l		0.02P mg/l			
Microbiologic Constituents:						
All Water Intended for Drinking:						
Clostridium perfringens (including spores)						0/100 mL
Colony Count @22°C						No abnormal change
Cryptosporidium			TT (MCLG=0)			
E. coli or thermotolerant coliform bacteria	0/100 mL		<5% samples positive		0/100 mL	
Enterococci					0/100 mL	
Giardia lamblia			TT (99.9% reduction)			
Legionella			TT			
Heterotrophic plate count			TT (500 cfu/mL)			
Total coliform bacteria						0/100 mL
Total plate count @35°C						
Viruses			TT (99.99% reduction)			
Water for Sale in Bottles or Containers:						
E. coli					0/250 mL	
Enterococci					0/250 mL	
Pseudomonas aeruginosa					0/250 mL	
Colony count @22°C					100/mL	
Colony count @37°C					20/mL	

Notes and abbreviations:

- Blank cells indicate the absence of a standard for that parameter
- Chemicals that have been assigned a "provisional guideline" value by the WHO, or a "proposed MCL" by USEPA, are included in the table above, and are followed by the letter "P."
- Chemicals listed by WHO as "not of health significance at concentrations normally found in drinking water" are designated with the letter "U."

(*Continued*)

TABLE 193.4 (*Continued*)

- Chemicals evaluated by WHO and assigned the status of having "no adequate data to permit recommendation of a health-based guideline value are designated with the letters "NAD."
- 1° refers to the primary maximum contaminant levels (MCL) established by EPA, which are enforceable limits.
- 2° refers to the secondary maximum contaminant levels (SMCL) established by EPA, which are generally not enforceable on a federal level.
- MCLG = Maximum contaminant level goal
- GV = Guideline Value, a maximum level recommended by WHO for the provision of safe drinking water
- MRDL = Maximum Residual Disinfectant Level, established by the USEPA
- WHO = World Health Organization
- EPA = United States Environmental Protection Agency
- EU = Member States of the European Union
- TT = Treatment technique, an approach developed by EPA to provide direction to municipalities as to which parameters require installation of an acceptable treatment technique to demonstrate reduction of the respective contaminant(s).
- TCU = Total Color Units
- Co-Pt = Cobalt Platinum Color Units
- NTU = Nephelometric Turbidity Units
- TON = Threshold Odor Number
- uS/cm = microsiemens per centimeter, a standard expression of conductivity
- mSv/year = milliSievert per year, a standard expression of committed effective dose of radiation
- pCi/L = picoCurie per liter, the U.S. expression of the activity of ionizing radiation
- Bq/L = Becquerel per liter, the Standard International (SI) system of expressing activity for ionizing radiation:

  1 Curie = 3.7×10^{10} Becquerel

  1 pCi = 10^{-12} Ci

  therefore, pCi/L $\times$ 0.037 = Bq/L
- mrem/year = milliroentgen equivalent man per year, the U.S. expression of radiation dose; applies to total body and individual organ exposure, calculated on the basis of a two liter per day drinking water intake.
- In most cases, standard IUPAC chemical nomenclature was applied, so the unsaturated alkene family of compounds will end in the suffix "-ene." Be aware that some regulatory agencies continue to use the older common names, which bear the suffix "-ylene." So, for example, "trichloroethene" and "trichloroethylene" are the identical compound.

Table References:

(1) *Guidelines for Drinking Water Quality, Second Edition, Volume 1*, *Recommendations*, 1993, World Health Organization.

(2) *Guidelines for Drinking Water Quality, Second Edition, Addendum to Volume 1, Recommendations*, 1998, World Health Organization.

(3) National Primary Drinking Water Regulations, *Code of Federal Regulations*, Title 40, Part 141, 1 July 1999, United States Environmental Protection Agency.

(4) National Secondary Drinking Water Regulations, *Code of Federal Regulations*, Title 40, Part 143, 1 July 1999, United States Environmental Protection Agency.

(5) *Official Journal of the European Communities*, L330/32, Council Directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption.

equipment serviceability/parts accessibility, etc.; and 5) contribution to plant effluent—total dissolved solids, water volume to drain, etc.

Where ingredient water for production of soft drinks is concerned, the typical "fleet" of treatment technology available to the beverage industry includes:

1. Conventional Lime Treatment Systems (CLTS)—coagulation/flocculation, hydrated lime, superchlorination. This treatment chain represents the majority of most beverage treatment armadas worldwide. Historically, and as little as 25 years ago, this combination of treatment was regarded as the "ideal" treatment for raw water of virtually any quality. Indeed, this system, coupled with the required support technology—fine sand filtration, granular activated carbon, polishing filtration, and ultraviolet irradiation—does address a broad range of water contaminants.
2. Ion-exchange. This technology is routinely utilized for partial or complete demineralization,

softening, dealkalization, or can be customized for selective removal of a specific contaminant (for example, denitratization).

3. Membrane technology. Clearly, this has seen the most growth in recent years with the advent of more resistant membrane materials of construction and more flexible rejection characteristics. Included in this category is the prototype of the cross-flow, polymeric membrane filtration systems—reverse osmosis, along with nanofiltration and ultrafiltration (both polymeric and ceramic). Also among this group is electrodialysis technology for removal of ionic species in water, and continuous electrodeionization.

B. Primary Treatment Technology

1. Conventional Lime Treatment Systems

Conventional Lime Treatment Systems (CLTS), sometimes also referred to as traditional coagulation or the cold lime process, remains one of the most common treatment techniques employed in the carbonated beverage industry. Five years ago, one estimate placed approximately 85% of beverage water systems using conventional lime treatment (personal communication, Harry Delonge, 1996), but this number has been steadily decreasing over the years, with this treatment modality being replaced by membrane systems. Operationally, at its most fundamental level, CLTS involves mixing three chemicals—a chlorine source, a coagulant, and hydrated lime—together in a reaction tank, and allowing a contact time of at least two hours for the ensuing reactions to proceed. The hydrated lime (traditionally called "lime," even though calcium oxide is rarely used) increases the pH of the water in the reaction tank to above 9.6, thereby converting the naturally occurring bicarbonate alkalinity components to carbonate alkalinity. This is a critical reaction, since at this pH, calcium carbonate is virtually insoluble in water, and begins to precipitate from solution. At the same time, the coagulant, aided by steady, non-turbulent mixing, is overcoming the repulsive negative charges present on natural organic debris in the water supply. This charge neutralization and subsequent destabilization allows the coagulant to form a floc of ever-increasing size and density. As it forms, it incorporates the precipitating calcium carbonate, along with a host of other particulates present in the incoming water. The chlorine source in conventional systems serves a dual purpose: 1) oxidation—both of the soluble ferrous sulfate coagulant to the insoluble ferric form, and of metallic contaminants like iron, manganese, arsenic, and others; and, 2) disinfection of the water in the reaction tank. The efficiency of disinfection in these systems will be discussed in more detail at a later point in this chapter. However, at high pH values (typically the range used in CLTS), the dissociated chlorine equilibrium favors the existence of the hypochlorite anion, which is approximately 100 times less effective as a germicide than the dominant species at lower pH (hypochlorous acid). From a microbial safety perspective, if the adequate free chlorine dosage is maintained in the reaction tank over the entire course of the two-hour contact time, this will afford excellent bacterial and viral destruction. The protozoans, like *Cryptosporidium* and *Giardia*, being more resilient to chlorine, will be less affected by the chlorine at the high pH employed in conventional lime treatment systems. However, the chlorine, aided by the physical entrapment and removal of these organisms during the coagulation process, will still typically result in a substantial reduction in their numbers. Indeed, conventional coagulation is one of the effective barriers in a multiple barrier approach against protozoans.

This floc that forms over the course of the two-hour reaction time in conventional systems continues to grow in size and bulk, enmeshing suspended solids, particulates, oxidized metals, organic debris, and a host of other impurities, and eventually settles toward the bottom of the reaction tank. The settled floc comprises the "sludge" associated with these conventional lime treatment systems, which must be frequently discharged in order to keep this system in equilibrium. As the impurity-laden floc is discharged from the bottom of the reaction tank, the treated water—now free of debris, low in alkalinity, sanitized, and of a generally high quality—is withdrawn from the top of the reaction tank to undergo further treatment through support processes prior to being used for final product. Table 193.5 summarizes the advantages and disadvantages of these treatment systems. From a water safety perspective, conventional lime treatment continues to offer excellent removal of a variety of water impurities.

TABLE 193.5
Advantages and Disadvantages of Conventional Lime Treatment Systems

Advantages	Disadvantages
Removes alkalinity and hardness	Does not effectively reduce nitrate, sulfate, or chloride concentration
Removes organic debris, particulates, and natural organic matter (NOM)	Sludge formation and disposal requirements
Reduces metal concentrations (iron, manganese, arsenic, others) and some radionuclides	May promote the formation of disinfection by products (trihalomethanes) under certain conditions
Reduces some color compounds (tannins), off-tastes, and off-odors	Often difficult to operate consistently in waters with very low dissolved solids
Reduces bacteria, virus, and protozoan populations	Relatively large space requirements on plant floor ("foot print")

Source: Developed by the author (2000).

It is often adequate as a primary treatment for many beverage water purification applications, but may also be used as a superb pretreatment for membrane processes, ion-exchange, or electrodialysis.

2. Membrane Technology

Membrane technology, in the application of water treatment for the beverage industry, encompasses a broad range of polymeric and ceramic impurity removal techniques. This may range from the use of a simple ten-micron microfilter to help remove granular activated carbon fines to employment of the prototype polymeric membrane technology—reverse osmosis. Figure 193.3 depicts the Filtration Spectrum, which provides a visual representation of the relative particle size removal which may be expected of the common membrane processes, including—in order of decreasing pore size—microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. As we can see from the spectrum, particulate filters may be used for the removal of relatively large suspended matter, and are often employed at the end of a water treatment chain as a "polishing filter" to remove any small floc particles, oxidized iron, carbon, or precipitated calcium carbonate that might have carried over from the primary treatment process. Microfilters are often used for their controlled pore size distribution (when absolute rated), which makes mechanical removal of bacteria from water streams commonplace. Often, for this application, a stepped removal approach is employed which will include filters of decreasing pore size oriented in series, so as to minimize the plugging potential of the smallest pores (for example, a five or ten micron particulate filter, followed by a 0.45 micron microfilter, and finally as low as a 0.2 micronfilter to help assure adequate bacterial reduction). Ultrafiltration (either polymeric or ceramic) is an excellent tool for the removal of particulates, large organicmatter (for example, the humic and fulvic acids which comprise natural organic matter in water supplies), and many types of microorganisms, including viruses, bacteria, and protozoa. However, for removal at the level of dissolved inorganic salts, nanofiltration and reverse osmosis are our only two feasible options. Table 193.6 comparesthe typical removal percentages of reverse osmosis, nanofiltration, and ultrafiltration for a variety of water impurities (15).

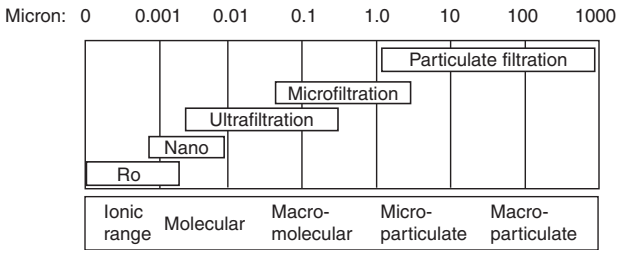

FIGURE 193.3 The Filtration Spectrum.

TABLE 193.6
Comparison of Reverse Osmosis, Nanofiltration, and Ultrafiltration Membrane Processes

Component	Reverse Osmosis	Nano Filtration	Ultrafiltration
Alkalinity	95 to 98%	50 to 70%	None
TDS	95 to 98%	50 to 70%	None
Particulates	nearly 100%	nearly 100%	nearly 100%
Organic matter	most >100 MW	most >200 MW	some >2000 MW
THM precursors	90+%	90+%	30 to 60%
Sodium	90 to 99%	35 to 75%	None
Chloride	90 to 99%	35 to 60%	None
Hardness	90 to 99%	50 to 95+%	None
Sulfate	90 to 99%	70 to 95+%	None
Nitrate	90 to 95%	20 to 35%	None
Protozoa	near 100%	near 100%	near 100%
Bacteria	near 100%	near 100%	near 100%
Viruses	near 100%	near 100%	near 100%
Operating pressure	200 to 450 psi	100 to 200 psi	80 to 150 psi

Approximate removal percentages. Actual performance is system-specific.

Source: Adapted from Brittan (1997).

The driving force behind the membranes used in water treatment applications for the beverage industry is pressure, which is applied across the membrane to force the filtered or purified water through the membrane, leaving the unwanted impurities behind. This concept becomes even more critical when describing the operation of a reverse osmosis membrane system. To understand "reverse" osmosis, we must first understand "osmosis." According to The Drinking Water Dictionary (16), osmosis is a "natural phenomenon whereby water (or some other solvent) diffuses from the lower-concentration side to the higher-concentration side of a permselective (semipermeable) membrane barrier in a process of equalizing concentrations on both sides." The corresponding osmotic pressure is the pressure exerted on the solution at equilibrium as a result of osmosis. This is illustrated in Figure 193.4.

In "reverse" osmosis, we apply a pressure to the concentrated side which is greater than the osmotic pressure, which thereby reverses the osmotic flow. The result is that the water now flows across the reverse osmosis membrane in the opposite direction to what was just described above with osmosis. The water is forced from areas of high to low solute concentration, thereby leaving a very concentrated salt stream behind on the waste side of the membrane, and a very dilute, purified water stream on the product or permeate side of the membrane. This is depicted in Figure 193.5. This is an important concept to visualize, since it is this osmotic pressure control which

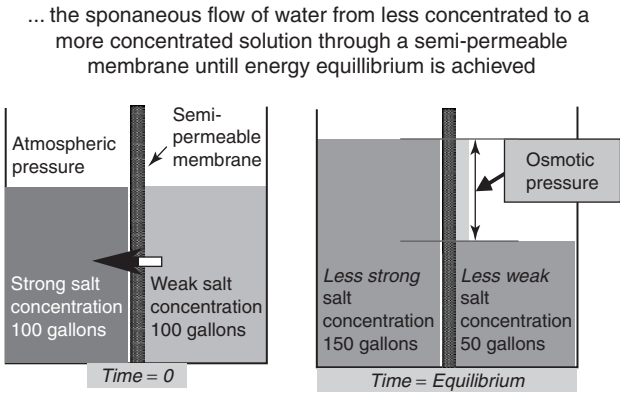

FIGURE 193.4 Osmosis.

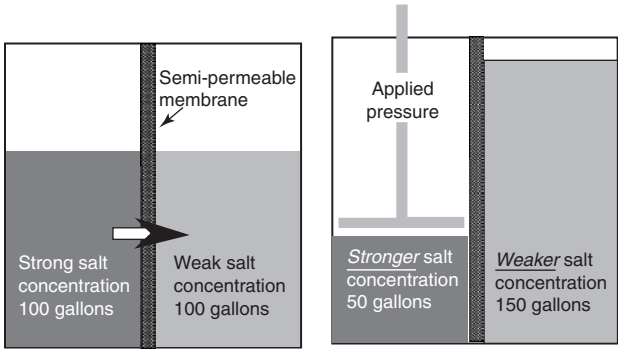

FIGURE 193.5 Reverse osmosis.

allows reverse osmosis to remove impurities from water down to the level of dissolved ionic species. In fact, reverse osmosis can afford removal of from 95 to greater than 99% of many dissolved salts, resulting in a treated water exiting the system with a total dissolved solids (TDS) concentration often below 10 mg/L, within the range of many distilled water products.

Though ultrafiltration, nanofiltration, and reverse osmosis all afford efficient removal of many microbial impurities, a critical point which must be understood is that none of these processes—not even reverse osmosis—produces a commercially sterile water. This is still a common misconception among many in the beverage and allied industries. Owing to the nature of cross-flow technology, the high pressures used, integrity of the seals, and variability in the pore structure of the membrane materials, these membrane treatment operations will remove a large percentage of the microorganisms to which they are introduced, though not all. If these processes are used as a primary treatment, this should not preclude the need for additional disinfection of the water supply.

Key considerations when selecting and designing nanofiltration and reverse osmosis treatment systems are the choice of proper pre- and post-membrane treatment operations. The primary goal of pretreatment processes is to protect the integrity of the membranes, since they often represent a substantial portion of the capital cost. These polymeric membranes, usually some form of polyamide, though traditionally also available as cellulose acetate, are susceptible to a variety of processes and impurities which may foul or degrade them, thereby rendering them inefficient, ineffective, or, in the worst case, totally destroyed. In general, the pretreatment processes will involve reducing the silt density index (plugging potential) of the feed water to the membrane. This is often accomplished by filtration through sand or mixed media, or via in-line coagulation. Pretreatment also includes some form of chlorine control—either assuring that it is removed (in the case of polyamide membranes), or assuring that it is present to prevent biologic degradation of cellulose acetate membrane materials. Finally, the pretreatment processes will normally include the in-line dosing of acid, antiscalant, or both. These steps help prevent a loss of membrane performance due to metal oxide fouling, scaling, or related processes which might occlude the membrane pores. Table 193.7 summarizes the various processes which may cause fouling (usually partially or completely reversible) or degradation (usually irreversible) of nanofiltration and reverse osmosis membranes. Potential solutions are also listed.

The post-treatment of water exiting nanofiltration and reverse osmosis membrane systems is also important, and should be carefully considered. Unlike prior to treatment, once the water has passed the membrane modules, it will be considerably more pure than when it entered. As a result, much of the microbial load is gone, as are many of the chemico-physical impurities. However, as we stressed previously, these membrane processes should not be relied upon to produce a consistently commercially sterile water. Consequently, post-treatment operations may include chemical or ultraviolet disinfection, granular activated carbon treatment, and polishing filtration. In-plant process monitoring is as critical with membrane processes as it is for any other treatment modality, and will usually include microbial monitoring to help gauge biofouling of the membranes, total dissolved solids or conductivity to assess the gross rejection of the membranes toward inorganic salts, pH, silt density index, chlorine residuals, and any other parameters assigned by the parent beverage company or suggested by the equipment supplier.

The advantages and disadvantages of reverse osmosis, as the "gold standard" of membrane removal processes, are summarized in Table 193.8

3. Ion-Exchange

The process of ion-exchange, like conventional lime treatment systems, has been known to the beverage industry for decades. However, its early applications did not typically include the water to be used for product, but rather

TABLE 193.7
Causes of Fouling and Degradation of Reverse Osmosis and Nanofiltration Membranes, Along with Possible Solution

FOULING		DEGRADATION	
Problem	**Potential Solutions**	**Problem**	**Potential Solutions**
Suspended solids (in feed water)	• Multimedia filtration (sand, greensand, carbon) • Ultrafiltration • Microfiltration • Plate and frame filtration with diatomaceous earth • Cartridge filtration • In-line coagulation • Coagulation	Oxidation	• Proper membrane selection • Disinfectant removal if required by membrane (carbon, bisulfite) • Proper dosing and feedback controls
Oxidation of metallic components (iron, manganese, etc.)	• Oxidation (aeration, ozonation, chlorination, etc.); filtration • Greensand filtration • Coagulation/lime treatment • Acid feed	Hydrolysis (membrane break down at low pH)	• Proper membrane selection • pH control (both operating range and cleaning range) • Controlled acid feed
Precipitation/scaling (sulfates, carbonates, silicates, etc.)	• Limit recovery (in design phase) • Antiscalant addition • pH control • Lime softening • Ion-exchange	Bacterial attack (especially for unprotected cellulose acetate membranes)	• Proper membrane selection • Periodic membrane cleaning and sanitizing • Proper pretreatment
Microbial growth (biofilm formation)	• Chemical disinfection • Ultraviolet disinfection • Periodic membrane cleaning and sanitizing	Solubilization (not very common in the beverage industry, but due to high concentrations of organic compounds with solvent properties)	• Removal of compounds prior to membrane contact; usually via aeration or granular activated carbon

Source: Developed by the author (1996).

involved softening of the water (removal of calcium and magnesium) used for auxiliary plant purposes in order to prevent scaling or avoid loss of efficiency in heat exchangers, boilers, and bottle washers. Today, with the advent of many categories of resin materials, ion-exchange is another of the valuable tools used by the beverage water technologist to help assure that the treated water used meets all of the applicable standards and guidelines expected.

In its most fundamental form, ion-exchange, as the name implies, involves replacing an ion which is less desirable in a particular application with one that is more desirable. In the case of the early softeners mentioned above, natural ion-exchange materials (called zeolites) were employed to exchange the hardness components (calcium and magnesium) in water with sodium. The rationale here was that calcium and magnesium salts may often precipitate as scale inside equipment; the corresponding sodium salts, however, were much more soluble, and hence did not pose a scaling concern. In this example, the supply of sodium on the zeolite to be exchanged for calcium and magnesium is not present in endless supply. Therefore, once the resin material is exhausted, it must be replenished—or "regenerated"—with a new supply of the appropriate ion; in this case, the sodium zeolite softeners are typically regenerated with brine (sodium chloride solution). Here the excess of sodium overcomes the calcium and magnesium attached to the resin, and they are washed to the drain. The resin, now fully regenerated, is again ready to be put into service. Figures 193.6 and 193.7 illustrate the exchange of sodium for calcium and magnesium, and the regeneration of the resin, respectively.

Ion-exchange applications have broadened far beyond zeolite softening, and include complete demineralization (reduction of the total dissolved solids to near zero, if desired), dealkalization (removal of alkalinity and hardness at the same time), anion or cation exchange alone, denitratization (nitrate removal), and a variety of other specialty applications, which include removal of silica, natural organic matter, iron, and other targeted impurities. The specific application depends largely on the structure of the resin material used, the characteristics of the water being treated, and the desired treated water profile.

Ion-exchange resins for most beverage water treatment operations may be divided into two major categories:

TABLE 193.8
Advantages and Disadvantages of Reverse Osmosis Systems

Advantages	Disadvantages
Removes nearly all suspended material, and greater than 99% of dissolved salts in full-flow operation	Pretreatment must be carefully considered, and typically involves operating costs for chemicals (acid, antiscalant, chlorine removal)
Significantly reduces microbial load (viruses, bacteria, and protozoans)	Does not produce a commercially sterile water
Removes nearly all natural organic matter (NOM)	Membranes still represent a substantial portion of the capital cost, and may typically last 3-5 years
May be designed as a fully automated system with little maintenance	Low solids water may be aggressive toward piping and equipment, so this must be considered for downstream operations
Relatively small space requirements on the plant floor ("footprint")	High pressure inlet pump is required

Source: Adapted from Berkefeld Water Treatment, Inc. (2000).

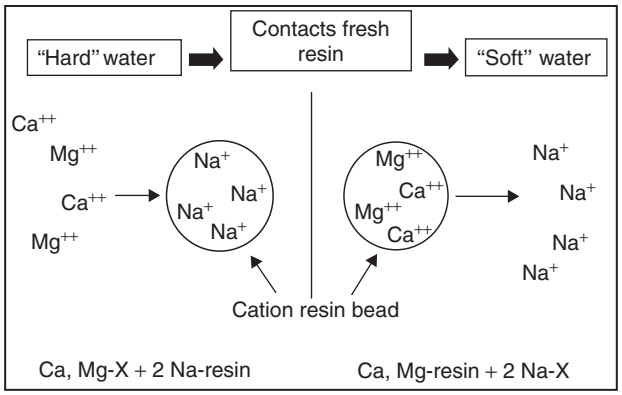

FIGURE 193.6 Ion-exchange reactions (softening).

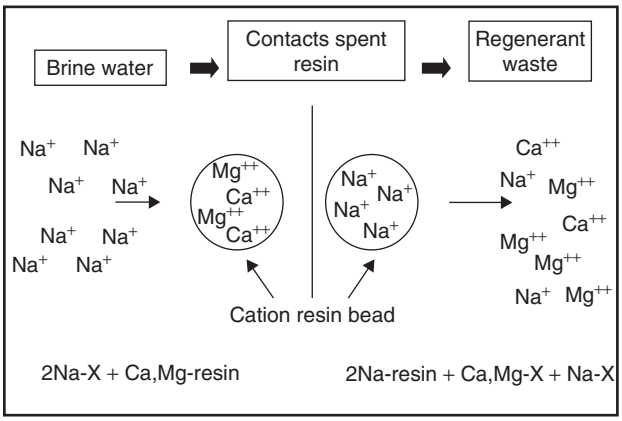

FIGURE 193.7 Ion-exchange regeneration (softening).

1) cation resins, which remove positively charged ions (cations) like calcium, magnesium, and sodium, and which require regeneration with brine or a mineral acid; and, 2) anion resins, which remove negatively charged ions (anions), like nitrate, sulfate, and chloride, and which require regeneration with brine or alkali. Each of these categories may be further divided into "weak" and "strong" subdivisions—that is, weak acid and strong acid cation exchange resins; and weak base and strong base anion exchange resins. Due to the nature of the chemistry at work, the "cation" and "acid" belong together, as do the "anion" and "base." The term "weak" refers to the functional moiety on the resin itself, and the fact that these resins will remove the "weakly" bonded ions in their respective class, whereas the "strong" resins will remove both weakly and strongly bonded ions in their groups.

An increasingly popular ion-exchange application for the beverage industry involves the use of a weak acid cation exchange resin. This resin is typically charged with acid, and, instead of sodium (as in the zeolite softener) these resins will exchange the acid proton, or hydrogen ion for weakly bonded cations in the water. The predominant cations removed are those that comprise hardness, calcium and magnesium. In addition, some sodium may be removed in the process, but only after the hardness is removed. As the hardness is removed, and exchanged for hydrogen ions, these hydrogen ions combine with the naturally occurring bicarbonate alkalinity in the water to form carbonic acid (H_2CO_3). This newly formed carbonic acid quickly dissociates into water and carbon dioxide, and the carbon dioxide is usually removed with a downstream degasifier. By forming the carbonic acid and removing the carbon dioxide, the alkalinity of the water is reduced proportionately. Hence, the weak acid cation exchange resin, in the acid form (as opposed to the sodium form, as the zeolites), removes both hardness and alkalinity in one treatment unit. The caution with this application is that, if used alone, other operations must accompany the ion-exchange—for example, disinfection, filtration, activated carbon, etc.—since softening and alkalinity removal are the major benefits of this treatment. If anion impurities are a problem in the water supply (nitrate, chloride, sulfate), then these weak acid resins will have no effect on these contaminants. Some companies, where the water source is of a demonstrated, consistent supply, use weak acid cation exchange resins preceded by in-line coagulation. This combination affords the removal of turbidity, suspended solids, and some protozoans (from the coagulation), along with the removal of alkalinity and hardness by the resin itself.

Ion exchange alone affords no disinfection or microbial protection, unlike conventional lime treatment systems and

membrane processes already discussed. Consequently, they must be augmented by the appropriate additional treatment to result in the final water quality profile desired. In some cases, the resin beds themselves may promote the growth of bacterial populations, and, once established, may be very difficult to fully overcome. Some suppliers recommend the installation of an ultraviolet disinfection loop to help protect the resin unit from microbiologic proliferation during periods when not in use. In general, cation resins are more resilient materials, and may be disinfected with a variety of sanitizers, including chlorine solutions, permanganate, peracetic acid, and formaldehyde. This is usually not true for anion resins, which may be more prone to osmotic shock from changes in ionic strength, water temperature, or pH extremes. In some cases, anion resins—usually strong base anion material—have been implicated in causing off-odors ("fishy") in the treated water exiting their beds, which may be due to the methylamine breakdown products of some anion resins. In all cases, the supplier of the resins should be consulted for the proper operational, regeneration, and disinfection procedures to be used with their particular resin.

C. Support Technology

"Support technology" is a term used to describe the ancillary unit operations which are typically not considered primary treatment. Rather, they are intended to augment the primary treatment to result in a robust, complete treatment chain designed to deliver the quality of treated water we require for food and beverage plant applications. Given this definition, a wide variety of unit operations may be grouped into this category, but this section will only address two of the more common support technologies—media filtration, and activated carbon purification.

1. Media Filtration

Media filtration, in the simplest terms, involves the passage of water through any of a variety of coarse filtration materials. Traditionally, the most common medium for beverage water treatment was simple filtering sand, supported by a bed of gravel. The major intent of these sand filters was—and still is—to provide a coarse straining of the water stream. Sand filters may be located at different points in the treatment chain, but for conventional lime treatment systems, the industry practice is to locate the sand filter downstream of the reaction tank. Logically, this was done to capture any loose floc carryover, precipitated calcium carbonate, or other particulates that might not have settled adequately in the reaction tank. In some applications, like in-line coagulation, the deep bed sand filter which is used serves not only to filter the floc which is intentionally formed in line, but to provide part of the contact time of the water with the chlorine disinfectant.

As the choice of primary treatment technologies available to the beverage technologist has increased over time, so has the choice of support operations. In addition to sand, other media are commercially available to suit a variety of applications. For example, some reverse osmosis and nanofiltration membrane systems might incorporate "greensand" as one of the pretreatment operations. Greensand is a naturally occurring mineral that consists largely of dark greenish grains of glauconite, possesses ion-exchange properties (17), and is used for the removal of soluble iron and manganese from water streams to prevent fouling of downstream membrane systems. Other media used include garnet, anthracite, and diatomaceous earth, each having its own niche application. These media may also be combined in dual media or mixed media filters. Regardless of the medium selected, the fundamental objectives of media filtration include 1) removal of particulates from the overflow of reaction tanks, 2) preventing the occlusion of carbon pores, 3) avoiding the surface occlusion or fouling of ion-exchange resins, and, 4) providing the required contact time for chlorine and coagulant. In general, the supplier of water treatment equipment will routinely suggest media with which they have had positive operating and quality histories.

Media filtration, like virtually all water treatment processes, requires diligent maintenance in order to help assure its on-going performance. Since media filters remove particulate matter from the water, these impurities must then somehow be removed from the filter itself. This is accomplished by frequent backwashing of the filter medium, often with treated water, to suspend and expand the filter bed into the freeboard of the filter vessel, thereby allowing collected impurities to be washed to the drain. Some media filters, more commonly in municipal applications, augment the water backwash by the injection of air. This air scouring also helps suspend the bed, and allows conserves the overall volume of water necessary for the backwash cycle. The frequency and conditions of backwash depend on the filter media, vessel design, supplier recommendations and corporate policies, although the range of operating conditions usually includes a frequency of anywhere from daily to monthly, and a backwash rate of from two to five times the normal flow. Intimately linked with the maintenance described above is the routine sanitation of the media filters. Several methods may be used, including heat and chemical sanitizers, but this critical operation must not be overlooked. It is possible for bacterial populations to become established in media filter beds, and subsequent mucilagenous biofilms to form in and on the filter media which may be extremely resistant to removal.

2. Carbon Purification

In the field of water treatment, carbon purification is arguably the single unit operation that provides the broadest protection against the widest range of possible contaminants. In fact, the United States Environmental Protection Agency has routinely identified activated carbon as the "best available technology" for the removal of a wide variety of volatile

and semi-volatile organic impurities (18), which are summarized in Table 193.9. In addition to these, thousands of other organic compounds show some degree of removal by activated carbon. This application of organic impurity removal is relatively new to the beverage industry, despite the fact that activated carbon has been a part of beverage water treatment systems for many decades. The primary use of activated carbon in this field has been to effect the removal of the chlorine species used to disinfect the treated water. Here, a critical distinction must be drawn between the adsorptive mechanisms of contaminant removal by carbon, and the catalytic mechanisms carbon employs to dechlorinate. Adsorption refers to the adhesion, bonding, and other chemical attractive forces which retain impurities on the surface of the carbon and within its pores. This is usually a reversible process, to varying degree. The catalytic mechanism refers to the formation of a surface oxide on the activated carbon medium as a result of the reaction of the carbon with the hypochlorous acid, according to the following reaction:

$$HOCl + C^* \rightarrow HCl + C^*O$$

Hypochlorous acid + carbon = hydrochloric acid + carbon surface oxide

TABLE 193.9
Organic Contaminants for Which Activated Carbon Has Been Identified as the Best Available Technology for Their Removal

Alachlor	Aldicarb
Aldicarb sulfone	Aldicarb sulfoxide
Atrazine	Benzene
Benzo[a]pyrene	Carbofuran
Carbon tetrachloride	Chlordane
Dalapon	2,4-D
Di (2-ethylhexyl) adipate	Di (2-ethylhexyl) phthalate
Dibromochloropropane (DBCP)	o-Dichlorobenzene
para-Dichlorobenzene	1,2-Dichloroethane
1,1-Dichloroethylene	cis-1,2-Dichloroethylene
trans-1,2-Dichloroethylene	1,2-Dichloropropane
Dinoseb	Diquat
Endothall	Endrin
Ethylbenzene	Ethylene Dibromide (EDB)
Heptachlor	Heptachlor epoxide
Hexachlorobenzene	Hexachlorocyclopentadiene
Lindane	Methoxychlor
Monochlorobenzene	Oxamyl (Vydate)
Pentachlorophenol	Picloram
Polychlorinated biphenyls (PCB)	Simazine
Styrene	2,3,7,8-TCDD (Dioxin)
Tetrachloroethylene	Toluene
Toxaphene	2,4,5-TP (Silvex)
1,2,4-Trichlorobenzene	1,1,1 –Trichloroethane
1,1,2-Trichloroethane	Trichloroethylene
Xylene	

Source: EPA (1999).

Unlike adsorption, the catalytic reaction is not reversible, and, in fact, carbon bulk is destroyed in the process.

Carbon may be obtained from a variety of different starting materials—for example, coal, wood, peach pits, coconut shells. The carbon is activated either thermally (steam) or chemically. Steam activation, the more common, involves two steps: 1) carbonization, and, 2) activation. Carbonization involves the conversion of the raw material into a disordered carbon structure with a very low volatile content. Carbonization is done at elevated temperatures in an oxygen-lean environment which keeps it from burning. In activation, some carbon atoms are vaporized, leaving behind the highly porous structure. Steam activation is carried out in temperatures of approximately 1800°F. (982°C). At these conditions, carbon reacts with steam to form carbon monoxide and hydrogen, which exit as gases. The result: a highly porous carbon material. Chemical activation is used to produce very high pore volume in wood-based carbons, particularly in the medium-size pore range. The most common process consists of mixing wood dust or some other cellulose-based material with a strong dehydrating agent and then heating to a designated temperature. The activating agent not only extracts moisture but helps prevent collapse of the pore structure during activation (19).

Activated carbon is generally available in the powdered or granular form, but granular activated carbon (GAC) is used for the vast majority of water treatment applications in the beverage industry. In addition to the volatile organic impurities mentioned above, and the removal of chlorine and chloramine, GAC also affords treated water protection against adversely sensory-active compounds, like the microbial metabolites geosmin and 2-methyl isoborneol. These compounds may be odor active in nanogram per liter concentrations, and represent a substantial proportion of off-odor complaints to municipal water treatment works (20).

Operationally, perhaps even more so than for media filtration, the activated carbon unit operation must be diligently maintained and sanitized. Many microbial complaints and sensory excursions in the beverage industry have been linked, at least in part, to inadequate carbon bed management practices. One of the reasons for this required diligence is that the core of the GAC bed has the potential to provide optimal conditions for the growth of troublesome microorganisms—specifically, the chlorine is absent, the environment can vary in its level of air or dissolved oxygen, and, in most cases, the organic microbial nutrients abound (since GAC is so proficient at removing organic compounds, including natural organic matter, from water supplies). These conditions combine to make carbon an excellent medium for the support of microbial growth. Once established, the extremely large

surface area within the carbon pores can make control of an unwanted microbial population a daunting task. As is the case with media filters, routine and diligent backwashing and sanitization of the carbon bed should be viewed as an absolute requirement for any beverage plant water treatment system. Hot water, steam, or a combination are generally employed to sanitize the carbon filters, provided their material of construction can withstand the temperature needed. When performed regularly, this helps prevent a biofilm from becoming firmly established in the bed, and helps avoid the problems often associated with poor carbon maintenance, including high bacteria counts, off-odor production within the bed, poor dechlorination or chlorine breakthrough, and loss of adsorptive capacity.

D. Disinfection

At the beginning of this chapter, the food and beverage producer's commitment to consumer and employee safety was stressed as being a paramount goal of water treatment in our industry. Microbiologic contamination, in addition to resulting in spoilage of the beverage, represents an acute potential threat to the quality of our products and the integrity of our trademarks. Therefore, overall microbial management is critical to the success of any beverage producer. In this section, we will focus on the major techniques employed for disinfection as it specifically relates to the water used for beverage products. As an introduction to this section of the chapter, a distinction between cleaning, sanitizing, and sterilizing must be drawn.

"Cleaning" may be described as the removal of soil particles from surfaces by rinsing and washing through the use of physical and chemical action. "Sanitizing," in our industry, refers to treating a cleaned surface to destroy contaminant organisms and reduce the total vegetative cell population to a safe level. Finally, "sterilizing" is the complete destruction of all organisms, including spores, through the use of chemical agents, heat, radiation, or other means. These are largely intuitive, yet critical concepts to recognize. Unlike many pharmaceutical or ultra clean room applications which may require commercially sterile water, the beverage industry does not. Our requirements, in nearly all cases, dictate a "sanitary" treated water supply, not a "sterile" one. That is, we diligently "control any contaminant organisms…to a safe level."

1. Primary Organisms of Concern

The specific organisms of concern for the water treatment system of a beverage producer must be identified by the corporate research and development functions, with probable guidance by any applicable drinking water and food regulations. The World Health Organization asserts that "infectious diseases caused by pathogenic bacteria, viruses, and protozoa or by parasites are the most common and widespread health risk associated with drinking water" (21). It would be impossible and irrational to attempt to test all potential microbial threats to a water supply. Consequently, the focus of most major regulatory bodies is on testing and monitoring recognized "indicators" of water quality. Perhaps the most notable and widely accepted group of indicator organisms is the coliform group, which refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35–37°C with the production of acid, gas, and aldehyde within 24–48 hours. They are also oxidase-negative and non-spore-forming. By definition, coliform bacteria display beta-galactosidase activity (22).

The real threat to public health and safety is from those waterborne organisms transmitted as a result of direct contact with fecal contamination. Because not all coliform organisms are of fecal origin, other indicator tests are used to help detect the possibility of unsanitary conditions in a water supply. These include fecal or thermotolerant coliform, with *Escherichia coli* being the most prominent member (23), fecal *Streptococci*, and anaerobic, spore-forming bacteria, the target of which is primarily *Clostridium perfringens*. The most common bacterial measurement in municipal water supplies and in beverage plant water treatment monitoring remain total coliform and *E. coli*. In addition, a total bacterial plate count is commonplace for routine monitoring, but more as an indicator of acceptable "good manufacturing practices" (GMPs) rather than as an indicator of the presence of fecal organisms. One notable exception to this industry practice is in the production and packaging of a natural mineral water. In most countries where regulations for natural mineral waters exist, disinfection is realistically not allowed for these products. Instead, the focus is on impeccable selection and monitoring of a source as close to pristine as possible. Part of this rigorous monitoring may include all of the organisms mentioned above, in addition to others (like *Pseudomonas aeruginosa*, for example). The rationale is to help assure optimal confidence that the natural mineral water being abstracted from the source and subjected to minimal treatment, at best, is as microbially risk-free as feasible.

Another organism that has recently become a threat to both the municipal and industrial water treatment arenas is the protozoan, *Cryptosporidium parvum*. *Cryptosporidium* is a protozoan parasite affecting the gastrointestinal tract of humans and animals. It is shed in the feces in the form of an "oocyst," which has a hard shell to protect it from the environment. This also makes it highly resistant to disinfection by chlorine and ozone; although, UV disinfection has proven to be extremely effective at its inactivation. Waterborne *Cryptosporidium* outbreaks have occurred in

both large and small communities, with the largest outbreak occurring in Milwaukee, Wisconsin in 1993, affecting an estimated 403,000 people. Such outbreaks have caused major disruption to residents, businesses, and government. Infection with the *Cryptosporidium* organism may also have contributed to the premature deaths of immunosuppressed individuals in these outbreaks. Because of this, the finding of *Cryptosporidium* oocysts in many drinking water sources (rivers, lakes, and reservoirs), and occasionally even in municipal treated water, has been a source of considerable concern to drinking water and public health officials, as well as to the public and the news media (24).

In addition to waterborne organisms of health concern, like those discussed above, water supplies may also be subject to inhabitation by "nuisance organisms." These organisms, as their name implies, are typically not associated with any direct health effects, but, rather, are known to cause aesthetic or operational concerns to the water treatment plant or distribution network. In reality, most beverage producers will not test for these nuisance organisms unless a problem is known to exist (which is usually detected initially by an off-odor in the water plant, or by metallic particles in the system). Bacteria in this broad category include the following (25):

1. Iron bacteria. These bacteria incorporate ferrous iron as part of their normal physiologic processes, and oxidize it to the insoluble ferric form. Genera include *Leptothrix, Clonothrix*, and *Gallionella*.
2. Manganese bacteria. Instead of iron, these bacteria may incorporate manganese and oxidize it. Genera include *Hyphomicrobium* and *Caulobacter*.
3. Sulfur bacteria. Many subgroups of sulfur bacteria exist, depending on the specific sulfur form utilized as a nutrient substrate. The more troublesome group to the beverage water treatment plant is the sulfate-reducing bacteria (SRB), since they produce the malodorous hydrogen sulfide. These include the genera *Desulfovibrio* and *Desulfotomaculum*. Some species of *Pseudomonas* have also been implicated in producing organo-sulfur compounds in water.

2. The "CT" Concept

A critical concept when forming the foundation for any discussion of disinfection is the "CT" concept. In this mathematic product, the "C" refers to the final residual concentration of a particular chemical disinfectant, in mg/L, and the "T" refers to the minimum contact time, in minutes, with which the material being disinfected has been in contact with the disinfectant. Therefore, the units of CT are expressed in mg-min/L. To explore this in more detail, we must first recognize that every water supply has a natural disinfectant "demand." The demand may be described as the utilization of a disinfectant by components in the water which must first be satisfied before a residual disinfectant concentration may be established. Impurities like soluble metals, particulates, natural organic matter, microorganisms, etc., all contribute to the demand of a particular water supply. Before we can confidently begin to disinfect this supply, we must first provide the water with enough disinfectant to react with these components. Once this is accomplished, we may then establish a "residual" disinfectant concentration. After this residual is established and confirmed via testing, we must then maintain this residual in contact with the water over the course of the required contact time. This concept explains why in a conventional lime treatment system, a chlorine dose as high as 12−20 mg/L is often required to result in a free chlorine residual of between 6 and 8 mg/L. The dose must be adequate to satisfy the demand, and then establish a residual. Simply put, the residual is equivalent to the arithmetic difference between the dose and the demand.

Many regulatory and industry organizations have adopted the CT concept to express relative values of a disinfectant's effectiveness against a particular organism under a defined set of conditions (temperature, pH, etc.). Often, published tables of CT data will include a subscript, for example, $CT_{99.9}$, which describes the log removal of a particular organism when tested against a particular disinfectant. The 99.9 subscript in this example indicates that for this particular CT data, a three-log, or 99.9% reduction in the target organism has been demonstrated.

Table 193.10 provides further illustration of the CT concept using real data from the United States Environmental Protection Agency. The table values include CT_{99}, or the CT ranges within which 99% of the target organisms were inactivated by the disinfectant. A bacterium, virus, and protozoan are compared insofar as their susceptibility to free chlorine, preformed chloramine, chlorine dioxide, and ozone. Many conclusions may be drawn

TABLE 193.10
Comparison of CT Values for Inactivation at 5°C (mg-min/L)

Organism	Free Chlorine pH 6–7	Preformed Chloramine pH 8–9	Chlorine Dioxide pH 6–7	Ozone pH 6–7
E. coli (bacteria)	0.034–0.05	95–180	0.4–0.75	0.02
Polio 1 (virus)	1.1–2.5	770–3740	0.2–6.7	0.1–0.2
G. lamblia (protozoan)	47–150+	—	—	0.5–0.6

Source: Langlais (1991).

from this single tabulation, which will summarize the discussion of CT:

1. Compared to the other disinfectants, preformed chloramine is virtually ineffective at inactivating polio 1 virus (note the very high CT value of 770–3740 mg-min/L).
2. In general, ozone is the most effective disinfectant against all categories of the organisms studied (note the very low CT values in the ozone column).
3. Except for preformed chloramine, the other disinfectants are markedly effective against *E. coli* and polio 1 (in most cases, CT values are well below one).

Critical concepts with regard to CT data include: 1) the disinfectant demand must first be satisfied; 2) the disinfectant residual must then be established; 3) this residual must be maintained for the minimal contact time specified in order to provide adequate protection; and 4) "dose" and "residual" must not be confused or equated.

3. Major Disinfectants

A variety of disinfectants are currently available for use in beverage water treatment applications, but this section will focus on the three most common: chlorine species, ozone, and ultraviolet irradiation. The perspective presented will be from that of direct disinfection of the water to be used for beverage production. Note, however, that the disinfection of surfaces and equipment in the water treatment room and throughout the beverage plant is often treated as a separate topic, and additional sanitizing techniques may be used for these applications, with one of the most effective being heat. However, surface and plant disinfection are beyond the scope of this chapter.

a. Chlorine species

Chlorine is commercially available to the beverage industry as compressed chlorine gas, solid calcium hypochlorite pellets, and sodium hypochlorite solution of various concentrations. The traditional and likely the most commonly used form for disinfection of treated water is the last, sodium hypochlorite solutions, although the others are also employed. Chlorine gas is usually reserved for large-volume beverage plants, and considerable drawbacks to its use are the strict transport, handling, storage, metering, permitting, and use requirements being enforced by many regulatory agencies. Calcium hypochlorite is utilized, although, in many markets, is more costly than sodium hypochlorite. Irrespective of which form is chosen, once in aqueous solution, the chlorine chemistry becomes essentially the same.

The chlorine species, when dissolved in water, will eventually dissociate into primarily two active forms: 1) hypochlorous acid (HOCl); and, 2) the hypochlorite anion (OCl^-). The ratio of these two chlorine species varies as a function of pH, with hypochlorous acid predominating at acidic pH, and the hypochlorite anion predominating at alkaline pH (26). Figure 193.8 depicts the relative equilibria, as a function of pH, for chlorine, hypochlorous acid, and the hypochlorite anion. One critical concept to link with this chlorine equilibria is that hypochlorous acid (predominant at lower pH) has been described as 80 to 100 times more potent a germicide than the hypochlorite anion (predominant at higher pH). As a result of this, the World Health Organization suggests a pH of less than 8.0 to help assure effective disinfection of water with chlorine. In conventional lime water treatment systems, where the operating pH in the reaction tank is often above 10.0, the chlorine equilibrium favors the existence of hypochlorite anion, which is why, in addition to allowing adequate floc settling time, a minimum two hours of retention must be designed in these systems.

In summary: 1) chlorine is an effective disinfectant against bacteria and viruses, although is less effective against protozoan organisms like *Giardia* and *Cryptosporidium*; 2) the effectiveness of chlorine varies markedly with pH, owing to the distribution between the more effective hypochlorous acid, and the less effective hypochlorite anion; and, 3) the preferred operating range for chlorine disinfection is roughly pH 6.0 to 7.5; below this, corrosion may occur, above this, its effectiveness declines. Though the actual disinfection criteria for your application must be decided within your own corporation, a long-standing industry practice for water disinfection using chlorine in conventional treatment systems is to maintain a free chlorine residual of 6 to 8 mg/L over the course of a two-hour contact time. For other treatments, where the pH is lower, this CT is often decreased.

b. Ozone

Ozone (O_3) is an unstable, gaseous, allotrope of oxygen (O_2). It has a distinctive pungent odor, from which its name is derived (from Greek *ozein*, "to smell"). It is formed locally in air by the ionizing effects of environmental lightning, and in the earth's stratosphere by ultra-

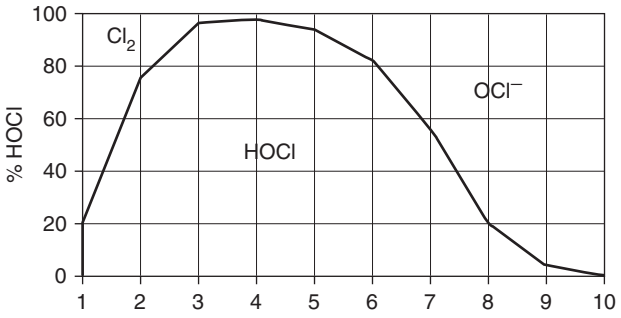

FIGURE 193.8 Chlorine species as a function of pH.

violet irradiation. It also safeguards us from the damaging effects of the sun by inhibiting the penetration of much of the sun's UV waves, and preventing them from reaching the planet's surface. It is also formed during combustion in automobile engines, and thereby contributes to the troublesome phenomenon of "photochemical smog."

Following the lead from many municipal drinking water companies that have used ozone for decades, the beverage industry more formally recognized its use in 1981 by the publication of "Ozone Treatment of Beverage Water" in the Proceedings of the International Society of Beverage Technologists (27), and then again in its 1987 Proceedings: "Applications of Ozone in Soft Drink Bottling Plants" (28). The major applications then—which continue to be currently valid—are the use of ozone as an oxidant and as a disinfectant. The major use of ozone in the beverage industry currently is the treatment of bottled water. Its use in the treatment of water for carbonated soft drinks is still uncommon.

Commercially, ozone is produced via the Corona Silent Arc Discharge process (29). The major drawback with the use of ozone, due to its very short half-life, is that it cannot be efficiently stored—it must be produced on-site at the point of use. With the Corona Discharge, a feed gas (oxygen or air) passes through an electrode pair (high and low voltage) where free electrons are of sufficient energy to split the diatomic (i.e., two atoms) oxygen molecules apart. The "single" atomic oxygen species then recombine with other diatomic oxygen to form a molecule with three atoms of oxygen—ozone (O_3). When compressed purified oxygen gas is used as the feed, in place of treated air, roughly twice the amount of ozone is produced for the same energy input. For most bottled water applications (where ozone is frequently used), even despite the increased output, the cost of the compressed oxygen usually makes its use uneconomical. There are many designs of ozone generators—tubular, plate, water-cooled, refrigerated air-cooled, etc.—but the most important design characteristic is the treatment of the feed gas. The ozone generator must include modules for: air compression and pressure regulation; cooling; particulate filtration; water vapor removal (dryers); gas impurity removal (methane, ammonia, etc.); and oil (hydrocarbon) removal. Along with the ozone generator should come some form of ozone destruct device (usually thermal or catalytic) to destroy the excess ozone "off-gas."

As a water disinfectant, the effectiveness of ozone varies widely with the specific organism of interest. For example, at 5°C, and a pH of 6–7, to obtain the same degree of inactivation (99%), the following "CT" conditions must be used (30):

1. *E. coli* bacteria: CT=0.02 mg-min/L
2. Polio 1 virus: CT=0.1–0.2 mg-min/L
3. *Giardia lamblia* cysts: CT=0.5–0.6 mg-min/L
4. *Giardia muris* cysts: CT=1.8–2.0
5. *Cryptosporidium parvum* cysts: 5–6 mg-min/L, estimated (unpublished data; personal communication)

As with any chemical disinfectant, when ozone is used for disinfecting water supplies, the ozone demand must first be met, and then a residual ozone concentration established, and maintained for the desired contact time. An industry practice, which dates back to data nearly four decades old, is to utilize an ozone CT value of 1.6 mg-min/L. This is generally done by maintaining a residual of 0.4 mg/L ozone for 4 minutes. You will notice from the data above, that a CT of 1.6 mg-min/L is adequate to provide at least a two-log reduction in bacteria, virus, and *Giardia lamblia* populations. However, as with chlorine, *Cryptosporidium parvum* remains resilient to inactivation, so higher CT values will be necessary to address this organism.

Operationally, the two major drawbacks to the more widespread application of ozone to our industry include the fact that: 1) it must be generated and used immediately on-site and cannot be stored, and, 2) due to its short half-life, it does not provide adequate residual disinfectant activity. As with other disinfectants, the dose and half life of ozone will vary as a function of pH, temperature, organic matter, and other variables, but somewhat unique to ozone is its behavior at varying levels of total dissolved solids (TDS). Table 193.11 illustrates the time, in minutes, for the disappearance of initial ozone doses of 0.64, 0.32, and 0.16 ppm in waters of varying levels of total dissolved solids (31). Note the magnitude of the inverse relationship between ozone half-life and total dissolved solids. The lower the level of total dissolved solids, the longer the

TABLE 193.11
Ozone Half-Life as a Function of Total Dissolved Solids, 70°F

		Time for Disappearance of an Initial Ozone Concentration of		
Total Dissolved Solids	**Ozone Half-Life**	**0.64 ppm O_3**	**0.32 ppm O_3**	**0.16 ppm O_3**
500 ppm	5.7 minutes	28.5 minutes	22.8 minutes	17.1 minutes
400–450 ppm	29 minutes	222 minutes	174 minutes	132 minutes
1 ppm	119 minutes	594 minutes	474 minutes	360 minutes

Source: Adapted from IBWA Technical Manual (1995).

ozone residual will last. Finally, ozone is very powerful chemical oxidant, and can be extremely aggressive toward equipment, both in air and in aqueous phase. Care must be taken to ensure that all materials used are suitable for ozone contact, and that all employee safety precautions are observed.

C. Ultraviolet irradiation

The last of the major disinfectants used for water treatment in the beverage industry is ultraviolet irradiation. UV radiation energy waves are the range of electromagnetic waves 100 to 400 nm long (between the x-ray and visible light spectrums). The division of UV radiation may be classified as Vacuum UV (100−200 nm), UV-C (200−280 nm), UV-B (280−315 nm) and UV-A (315−400 nm). In terms of germicidal effects, the optimum UV range is between 245 and 285 nm. UV disinfection utilizes a mercury source in the form of either: 1) low-pressure lamps that emit maximum energy output at a wavelength of 253.7 nm; 2) medium-pressure lamps that emit energy at wavelengths from 180 to 1370 nm; or, 3) lamps that emit at other wavelengths in a high intensity "pulsed" manner. Pulsed UV is a relatively new technology to the beverage industry, and is not widely employed at present. While both low and medium pressure designs have their own advantages and disadvantages, they have both proven to be adequate for water disinfection applications.

The degree to which the destruction or inactivation of microorganisms occurs by UV radiation is directly related to the UV dose. The UV dosage, D, is calculated as the arithmetic product of intensity, I, in milli- or micro-watt seconds per square centimeter, and time, t, in seconds. Internationally, the dose is often expressed in millijoules per square centimeter, which is exactly equivalent to milliwatt-seconds per square centimeter (1 mJ/cm^2 = 1 mW-s/cm^2 = 1000 microW-s/cm^2). Research indicates that when microorganisms are exposed to UV radiation, a constant fraction of the living population is inactivated during each progressive increment in time. This dose-response relationship for germicidal effect indicates that high intensity UV energy over a short period of time would provide the same kill as a lower intensity UV energy at a proportionally longer period of time. The UV dose required for effective inactivation is determined by site-specific data relating to the water quality and log removal required (32).

The mechanism of inactivation of microorganisms by UV is complicated, but has been reported many times in the literature. Fundamentally, the organism's genetic material (for example, bacterial deoxyribonucleic acid, or DNA) absorbs the UV radiation, which results in a chemical disruption of the DNA's chemical bases. Though many various photoproducts form as a result of this, the major rearrangement is the dimerization of the thymine base. This change renders the organism unable to replicate their DNA, and, therefore, cannot reproduce. As you might expect, ultraviolet disinfection does not provide any residual disinfectant activity.

Just as with ozone and chlorine, ultraviolet energy also has an accepted industrial "rule of thumb" which has been established for decades. A typical UV system used for the disinfection of beverage plant water treatment is sized to deliver a dose of at least 30 mJ/cm^2 at the end of its service life (typically 8,000 hours). To achieve this, since UV intensity naturally decreases over time as the lamp ages, the initial dose design is usually on the order of 60 mJ/cm^2. This design has traditionally been credited with providing at least a three-log (99.9%) inactivation of bacteria, yeast, and virus populations. Data published in late 1999 (33), and confirmed since then in several industry journals, suggest that the same 30 mJ/cm^2 dose also provides at least a three-log inactivation of *Cryptosporidium parvum*, a protozoan organism highly resistant to disinfection by chlorine and ozone. Table 193.12 summarizes the relative effectiveness of a variety of disinfectants against *Cryptosporidium parvum*. This is promising news for the water treatment industry, since it now adds UV to our armamentarium of weapons to help ensure the microbial safety of our water supplies.

4. The Multiple Barrier Approach

The "Multiple Barrier Approach," as the term implies, refers to the installation of any combination of multiple barriers in a water treatment chain to help decrease the risk of microbial contamination. These barriers may be physical (RO, microfiltration, coagulation, etc.), chemical (ozone, chlorine, UV, etc.) or a combination of the two. Multiple barrier design, though it applies correctly to the protection against any microbial threat, was the subject of increased interest after the Milwaukee *Cryptosporidium* outbreak in 1993. That outbreak, and the research done in its wake, helped demonstrate the resilience of that protozoan organism to traditional disinfection—an alternate approach was needed. That alternate approach became the multiple barrier concept. The Milwaukee outbreak also, arguably, spurred a heightened focus on the area of "emerging pathogens," with the hope of being able to

TABLE 193.12
Relative Comparison of Various Methods for 99% *Cryptosporidium* Inactivation

Disinfectant	Effectiveness	Estimated CT-99
Free chlorine	Poor	7200 mg-min/L
Chloramine	Poor	7200 mg-min/L
Mixed oxidants	Fair	1000 mg-min/L
Ozone	Good	5–15 mg-min/L
Chlorine dioxide	Good	80 mg-min/L
UV Irradiation	Excellent	2–5 mJ/cm^2

Source: Aquionìcs UV Systems, Inc. (2001).

proactively identify troublesome microorganisms, and apply appropriate treatment technology to address them.

The multiple barrier concept is becoming more recognized by both regulatory agencies and members of industry. This trend is likely to continue, since, intuitively, it should be able to address—at least to some degree—most microbial threats in the future. Considering the wide range of chemical and physical unit operations, and combinations thereof, available to the beverage water treatment technologist, we should be well-armed to design a robust treatment system to address many of the future threats which might face us in the future. The key is to work with municipalities, researchers, the private sector, and other resources to build and sustain network of expertise. Then, when a "new" pathogen arises, the industry should draw from this "skills reservoir" to take the necessary precautions to continue to protect public and consumer health, and the integrity of our brands.

In the beverage industry, many plants can confidently apply at least three microbial barriers—coagulation (conventional lime treatment systems), chlorine (primary disinfection), and UV (secondary disinfection, and extremely effective against *Cryptosporidium*). In general, the more barriers in place, the greater our confidence that we are providing treated water with adequate protection against microbial impurities. This is a simple concept, but far-reaching in implication.

IV. TESTING AND MONITORING

To keep pace with the changes in today's regulatory environment, the food and beverage industries must recognize a two-pronged approach as it relates to the testing of water in their facilities. The first prong incorporates testing required from a "regulatory" perspective. In this case, "regulatory" not only includes formal bodies like the Environmental Protection Agency or the Councils of the European Union, but also our own corporate "regulatory" departments. This type of testing is typically quite rigorous, and is concerned primarily with the protection of the consumer and the environment. The second prong incorporates the "day-to-day" testing required to keep our treatment processes functioning effectively, and to assure that our final treated water used in product consistently meets acceptable standards. This section is intended as a primer, to stimulate more in-depth investigation by the individual company, rather than a complete treatise of analytic methodology that might be employed.

What should you test? An anecdote paraphrased from a supposedly "ancient un-named philosopher" was once credited with the quotation, "To some extent, water can dissolve every naturally occurring substance on earth. Proof of this is limited only by our ability to detect species." (34) As modern analytic chemistry continues to evolve, this philosopher must be heralded as the visionary he must have been. For example, Environmental Protection Agency (EPA) Method 1613B allows the quantitation of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) down to part-per-quadrillion levels (35). As the levels of detection improve, the question will no longer be, "is this compound in our water?" Rather, it will become, "we know it's there, but what level is acceptable?" To try to simplify this complex and case-specific area, the food or beverage plant should consider dividing water related testing into five categories:

1. Testing for new plant site qualification;
2. Testing for regulatory compliance;
3. In-plant testing;
4. Testing to incorporate individual company requirements;
5. Troubleshooting

A. Testing for New Plant Site Qualification

This category of testing is usually considered the most rigorous, and typically encompasses testing for the broadest range of potential contaminants. It is often driven by the federal and local regulations that apply to the transaction of commercial real estate during a due diligence period. In the United States, this may include a simple "transaction screen," which is categorized by obtaining data primarily through visual inspection, surveys and interviews, and previous testing; or may range through a Phase III Environmental Site Assessment, which is a data-based quantitation of an identified site hazard. For more information, the American Society for Testing and Materials has published "Standard Practices for Environmental Site Assessments" (36), and should be reviewed by anyone involved in this initial phase of testing.

B. Testing for Regulatory Compliance

This category of testing is also rigorous, and has the primary function of protecting public health and safeguarding the corporate trademark. It typically represents an appreciable cost to the food or beverage manufacturer. The expense may be due to the capital investment needed for compliance (for example, a gas chromatograph with mass spectrometric detector to monitor trihalomethanes and other volatile organic compounds in potable water), or may be due to the on-going operating costs of external third party laboratories or contracted companies to manage a plant's overall compliance. The food and beverage manufacturer must be aware that regulatory compliance rarely, if ever, involves a *single* regulatory agency. Two is a common number in the United States; for example, the EPA for jurisdiction over potable water as it enters the food or beverage processing plant, and the Food and Drug Administration (FDA) for jurisdiction over a finished packaged water. Outside the United States, the maze of

presiding regulatory bodies may be even more complicated. For example, some years ago, at a natural mineral water facility in Poland, the plant was expected to comply with (1) the Council Directive of the European Union (since the country followed E.U. trends); (2) the Federal Regulations for Poland; (3) the regional Codes of the Vovoidship of Radom; and (4) the local laws as promulgated by the Warsaw authorities. The chemical, physical, microbiologic, and radiologic testing had to, therefore, follow suit.

Tracking regulatory trends is best left to the experts, and virtually all major food and beverage companies have entire departments devoted to doing just that. Historically, compliance monitoring has been criticized for the tedium and analyst sophistication necessary in the official analytic methods. Also, though the water-related agencies of the federal government have been doing a laudable job, they have been doing so with ever-dwindling resources. This has led to an official documentation system which is sometimes months or years behind the most current developments. However, the future is bright for regulatory compliance monitoring as many regulatory bodies join the Internet age (37).

C. In-plant Testing

This category should include the more routine, "plant floor friendly" testing that is required to operate a food or beverage plant on a day-to-day basis. It is generally intended for use as a surrogate system for fast, easy monitoring; the parameters measured should serve as a "red flag" assessment for when the next phase of more intensive testing is warranted.

For example, in beverage water treatment systems, especially for those utilizing polymeric membrane technology (i.e., reverse osmosis or nanofiltration), the measurement of total dissolved solids (TDS) is an excellent aggregate parameter to use as a surrogate measure of the overall rejection performance of the membrane. Simply put, TDS provides a fast, easy, in-line, reproducible method by which to monitor how effectively your membrane is rejecting dissolved salts. If the TDS changes suddenly, it raises a "red flag" that further data collection is necessary (for example, visual inspection of the membrane elements; speciation of the components of TDS—sulfate, chloride, sodium, etc.; among others). From the standpoint of normal daily operation, however, we may not need to measure all the anions and cations that comprise TDS. The decision as to which parameters should be included on an in-plant testing protocol will vary with the type of industry, the specific application of the process, an assessment of the potential risks associated with a process or product, hazard analysis and critical control point (HACCP) evaluation, the presiding regulations and guidelines that apply, and the company's own internal mandates.

D. Testing to Incorporate Individual Company Requirements

This category is often a hybrid of the other testing categories included in this section. The testing may be driven by: 1) regulatory mandates or voluntary ascription to impending regulatory trends; 2) "historical lessons" learned; 3) product- or process-specific testing (for example, individual stability or sensory standards, consumer-driven requirements, etc.); and, 4) parameters that may affect plant effluent treatment (for example, pH, TDS, biochemical oxygen demand, etc.)

Most of the points above are self-explanatory, but, in the author's experience, the "historical lessons" category is often the most worthwhile when it comes to monitoring parameters of importance. Every food and beverage company must be replete with anecdotes, for example, from a Principal Scientist with 40 years of corporate tenure, which describe problems that occurred decades ago. Though some might scoff at these "war stories," most will admit that there is empirical validity to the quote, "those who do not learn from their mistakes are destined to repeat them."

E. Troubleshooting

This category may be the most encompassing of all, and cannot be distilled to a few concise guidelines. The testing performed as a result of a troubleshooting exercise is often not planned; is often forcibly undertaken under the threat of an impending plant shut down; may potentially mean the difference between a minor, easily-remedied plant operations issue, and a serious breach which warrants a product recall; and must always be performed in a scientific, methodical fashion to maintain the integrity of the data and the value of the conclusions which will be drawn.

As we continue to develop the *ability* to detect compounds at lower levels than ever thought possible, we must also face the reality that we will undoubtedly *find* them in many of the places we look. As contaminants move more toward ubiquity, this makes the thoughtful development of appropriate testing protocols, acquisition of accurate and precise data, formulation of valid conclusions, and data-based action plans key to the future success of food, beverage, pharmaceutical, and municipal water treatment industries.

ACKNOWLEDGEMENT

The information in this chapter has been modified from "Beverage plant sanitation," by D. Bena. In *Food Plant Sanitation*. Editors: Y. H. Hui et al., 2004, Marcel Dekker, New York.

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Part W

Food Safety and Security

194 Contaminants

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CONTENTS

I. INTRODUCTION

Contrary to popular consumer perception about the risk of chemicals in foods, major hazards associated with foodborne illness are clearly of biological origin (1). The Centers for Disease Control and Prevention (CDC) has published summaries of foodborne diseases by etiology for the years 1993 through 1997 (Table 194.1) (2). CDC groups foodborne disease agents in four categories; bacterial, parasitic, viral, and chemical. Greater than 95% of all reported outbreaks foodborne illnesses are caused by microorganisms or their toxins. Fully 97% of reported cases are likewise linked to a microbial source. Only around 3% of the outbreaks and less than 1% of cases can be truly linked to chemical (heavy metals, monosodium glutamate, and other chemicals) contamination of foods. Furthermore, 97% of reported deaths are due to microbial sources. These data are from reported outbreaks. CDC estimates for the actual number of cases of foodborne disease caused by microbial agents is much higher due to under reporting (Table 194.2).

Bacterial agents are by far the leading cause of illness, with total numbers estimated as high as 76 million cases per year and deaths as high as 5,000 annually in the U.S. (3). Costs are estimated to be $9.7 billion annually in medical expenses and lost productivity in the U.S. (3). The high

TABLE 194.1
Reported Foodborne Diseases in the United States, 1993–1997

	Outbreaks		Cases		Deaths	
Etiologic Agent	**No.**	**%**	**No.**	**%**	**No.**	**%**
BACTERIAL						
Bacillus cereus	14	0.5	691	0.8	0	0.0
Brucella	1	0.0	19	0.0	0	0.0
Campylobacter	25	0.9	539	0.6	1	3.4
Clostridium botulinum	13	0.5	56	0.1	1	3.4
Clostridium perfringens	57	2.1	2,772	3.2	0	0.0
Escherichia coli	84	3.1	3,260	3.8	8	27.6
Listeria monocytogenes	3	0.1	100	0.1	2	6.9
Salmonella	357	13.0	32,610	37.9	13	44.8
Shigella	43	1.6	1,555	1.8	0	0.0
Staphylococcus aureus	42	1.5	1,413	1.6	1	3.4
Streptococcus, Group A	1	0.0	122	0.1	0	0.0
Streptococcus, other	1	0.0	6	0.0	0	0.0
Vibrio cholera	1	0.0	2	0.0	0	0.0
Vibrio parahaemolyticus	5	0.2	40	0.0	0	0.0
Yersinia enterocolitica	2	0.1	27	0.0	1	3.4
Other bacterial	6	0.2	609	0.7	1	3.4
Total bacterial	655	23.8	43,821	50.9	28	96.6
PARASITIC						
Giardia lamblia	4	0.1	45	0.1	0	0.0
Trichinella spiralis	2	0.1	19	0.0	0	0.0
Other parasitic	13	0.5	2,261	2.6	0	0.0
Total parasitic	19	0.7	2,325	2.7	0	0.0
VIRAL						
Hepatitis A	23	0.8	729	0.8	0	0.0
Norwalk/Norwalk-like	9	0.3	1,233	1.4	0	0.0
Other viral	24	0.9	2,104	2.4	0	0.0
Total viral	56	2.0	4,066	4.7	0	0.0
CHEMICAL						
Ciguatoxin	60	2.2	205	0.2	0	0.0
Heavy metals	4	0.1	17	0.0	0	0.0
Monosodium glutamate	1	0.0	2	0.0	0	0.0
Mushrooms	7	0.3	21	0.0	0	0.0
Scrombotoxin	69	2.5	297	0.3	0	0.0
Shellfish	1	0.0	3	0.0	0	0.0
Other chemical	6	0.2	31	0.0	0	0.0
Total chemical	148	5.4	576	0.7	0	0.0
Unknown Etiology	1,873	68.1	35,270	41	1	3.4
GRAND TOTAL	**2,751**	100.0	86,058	100.0	29	100

Source: Data from Reference 2.

incidence of foodborne disease is paralleled in other developed countries (4). Enteric viruses are now recognized as the leading cause of foodborne infections, although the bacteria are better known. Predominant bacterial agents are *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Clostridium perfringens*. Foodborne bacterial hazards are classified based on their ability to cause infections or intoxications. Foodborne infections are usually the predominant type of foodborne illness reported. Foodborne outbreaks most often occur with foods prepared at food service establishments and at home (Table 194.3). Improper holding temperatures and poor personal hygiene are the leading factors contributing to reported outbreaks (Table 194.4).

Bacterial hazards are further classified based upon the severity of risk (5). Severe hazards are those capable of causing widespread epidemics. Moderate hazards can be

TABLE 194.2
Reported and Estimated[a] Illnesses, Frequency of Foodborne Transmission, and Hospitalization and Case-Fatality Rates for Known Foodborne Pathogens, United States

Disease or Agent	Estimated Total Cases	Reported Cases by Surveillance Type			% Foodborne Transmission	Hospitalization Rate	Case-Fatality Rate
		Active	Passive	Outbreak			
Bacterial							
Bacillus cereus	*27,360*		*720*	*72*	*100*	0.006	0.0000
Botulism, foodborne	*58*		29		*100*	0.800	0.0769
Brucella spp.	*1,554*		111		*50*	0.550	0.0500
Campylobacter spp.	*2,453,926*	*64,577*	37,496	146	*80*	0.102	0.0010
Clostridium perfringens	*248,520*		*6,540*	654	*100*	0.003	0.0005
Escherichia coli O157:H7	*73,480*	*3,674*	2,725	500	*85*	0.295	0.0083
E. coli, non-O157 STEC	*36,740*	*1,837*			*85*	0.295	0.0083
E. coli, enterotoxigenic	*79,420*		*2,090*	209	*70*	0.005	0.0001
E. coli, other diarrheogenic	*79,420*		*2,090*		*30*	0.005	0.0001
Listeria monocytogenes	*2,518*	*1,259*	373		*99*	0.922	0.2000
Salmonella typhi[b]	*824*		412		*80*	0.750	0.0040
Salmonella, nontyphoidal	*1,412,498*	*37,171*	*37,842*	3,640	*95*	0.221	0.0078
Shigella spp.	*448,240*	*22,412*	17,324	1,476	*20*	0.139	0.0016
Staphylococcus food poisoning	*185,060*		*4,870*	487	*100*	0.180	0.0002
Streptococcus, foodborne	*50,920*		*1,340*	134	*100*	0.133	0.0000
Vibrio cholerae, toxigenic	*54*		27		*90*	0.340	0.0060
V. vulnificus	*94*		47		*50*	0.910	0.3900
Vibrio, other	*7,880*	*393*	112		*65*	0.126	0.0250
Yersinia enterocolitica	*96,368*	*2,536*			*90*	0.242	0.0005
Subtotal	*5,204,934*						
Parasitic							
Cryptosporidium parvum	300,000	*6,630*	2,788		*10*	0.150	0.005
Cyclospora cayetanensis	*16,264*	*428*	98		*90*	0.020	0.0005
Giardia lamblia	*2,000,000*	*107,000*	22,907		*10*	n/a	n/a
Toxoplasma gondii	*225,000*		15,000		*50*	n/a	n/a
Trichinella spiralis	*52*		26		*100*	0.081	0.003
Subtotal	*2,541,316*						
Viral							
Norwalk-like viruses	*23,000,000*				*40*	n/a	n/a
Rotavirus	*3,900,000*				*1*	n/a	n/a
Astrovirus	*3,900,000*				*1*	n/a	n/a
Hepatitis A	*83,391*		27,797		*5*	0.130	0.0030
Subtotal	*30,883,391*						
Grand Total	*38,629,641*						

[a] Numbers in italics are estimates; others are measured.

[b] >70% of cases acquired abroad.

Source: Data from http://www.cdc.gov/ncidod/eid/vol5no5/mead.htm and http://www.cdc.gov/epo/mmwr/preview/mmwrhtml/ss4901a1.htm.

those that have potential for extensive spread, with possible severe illness, complication, or sequelae in susceptible populations. Mild hazards can also cause outbreaks but have limited ability to spread. Those involved with food production, processing, and service should pay careful attention to controlling these biological hazards by: 1) destroying or minimizing the hazard, 2) preventing contamination of food with the hazard, or 3) inhibiting growth or preventing toxin production by the hazard. Control steps will follow in later sections of this chapter.

When investigating foodborne disease outbreaks, the most important factor is time (6). Prompt reporting of an outbreak is essential to identifying implicated foods and stopping potentially widespread epidemics. Initial work in

TABLE 194.3
Places where Foodborne Outbreaks Occurred, 1993–1997

Place	Number	Percentage
Home	582	21.3
Deli, café, restaurant	1185	43.1
School	91	3.3
Picnic	34	1.2
Church	63	2.3
Other	664	24.1
Unknown	99	3.6

Source: Data from Reference 2.

TABLE 194.4
Contributing Factors Leading to Foodborne Outbreaks, 1993–1997

	Number	Percentage
Improper holding temperature	938	37.0
Inadequate cooking	274	10.8
Contaminated equipment	400	15.8
Food from unsafe source	153	6.0
Poor personal hygiene	490	19.3
Other	282	11.1

Source: Data from Reference 2.

the investigation should be inspection of the premises where the outbreak occurred. Look for obvious sources, including sanitation and worker hygiene. Food preparation, storage, and serving should be carefully monitored. Interview those involved in the outbreak. Obtain case histories of victims and healthy individuals. Discuss health history and work habits of food handlers. Collect appropriate specimens for laboratory analysis, including stool samples, vomitus, and swabs of rectum, nose, and skin. Attempt to collect suspect foods, including leftovers or garbage if necessary. Specific tests for pathogens or toxins will depend on potential etiological agents and food type. Analysis of data should include case histories, illness specifics (incubation time, symptoms, and duration), lab results, and attack rates. All foodborne disease outbreaks should be reported to local and state health officers and to the CDC.

II. BACTERIAL INFECTIONS

Predominant bacterial infections transmitted via foods are salmonellosis, campylobacteriosis, yersiniosis, vibriosis, and shigellosis (7). Most causative agents are Gram-negative rod shaped bacteria that are inhabitants of the intestinal tract of animals. Indeed, federal and most state regulatory agencies consider foods of animal origin (meat, poultry and eggs, fish and shellfish, and milk and dairy products) potentially hazardous foods. One look at epidemiological data confirms this suspicion. That said, fresh produce (fruits and vegetables) is increasingly being implicated in outbreaks of both bacterial and viral agents.

A. SALMONELLOSIS

Salmonella resides primarily in the intestinal tract of animals (humans, birds, wild animals, farm animals, and insects) (8). Many people are permanent, often asymptomatic carriers. Salmonellosis varies with species and strain, susceptibility of host, and total number of cells ingested. Several dozen serotypes cause foodborne outbreaks. Incubation time is 24–36 hours, which may be longer or shorter. Symptoms include nausea, vomiting, abdominal pain, and diarrhea, which may be preceded by headache, fever, and chills. Weakness and prostration may occur. Duration is 1 to 4 days with a low mortality rate (0.1%). High risk very young and elderly may have a considerably higher mortality rate (3.8%) (9). The condition needed for an outbreak is the ingestion of live cells (10,000) present in the food. For high fat foods such as chocolate, 50 cells may be a sufficient infectious dose due to protective enrobement of cells by fat allowing survival in high acid gastric fluid during intestinal transit. Foods primarily involved in outbreaks include meat, poultry, fish, eggs, and milk products. *S. enteritidis* is present in raw uncooked eggs even with sound shells (10). Most often the bacterium is transferred from a raw food to a processed food via cross contamination. Control of *Salmonella* in foods can be accomplished in several ways. Avoidance of contamination by using only healthy food handlers and adequately cleaned and sanitized food contact surfaces, utensils, and equipment works best. Heat treatment of foods by cooking or pasteurization is sufficient to kill *Salmonella*. Refrigeration temperatures at or below 5°C are sufficient, as the minimum temperature for growth is 7 to 10°C. The prevalence of salmonellosis as a foodborne disease has prompted regulatory agencies to adopt a zero tolerance for the genus in ready-to-eat foods. Presence of the bacterium in these foods (luncheon meats, dairy products, pastries, produce, etc.) renders them unwholesome and unfit for consumptions. These foods must then be destroyed or reprocessed to eliminate the pathogen.

B. SHIGELLOSIS

Four species are associated with foodborne transmission of dysentery, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* (11). The disease is characterized with an incubation period of 1 to 7 days (usually less than 4 days). Symptoms include mild diarrhea to very severe with blood, mucus, and pus. Fever, chills, and vomiting also occur. Duration is long, typically 4 days to 2 weeks. *Shigella* spp. have a very low infectious dose of around 10 to 200 cells. Foods most often associated with shigellosis are any that are contaminated with human fecal material,

with salads frequently implicated. Control is best focused on worker hygiene and avoidance of human waste.

C. Vibriosis

Most vibrios are obligate halophiles that are found in coastal waters and estuaries (12). Consequently most foodborne outbreaks are associated with consumption of raw or undercooked shellfish (oysters, crabs, shrimp) and fish (sushi or sashimi) (13). *V. parahaemolyticus* causes most vibriosis outbreaks in developed countries and is primarily foodborne. *V. cholerae* is primarily waterborne, but has been associated with foods from aquatic origin (14). Because *V. cholerae* is halotolerant it can survive and grow in non-salt foods. Hence, the bacterium has been spread through foods of terrestrial origin in addition to nonsaline fresh water. *V. vulnificus* is capable of causing very serious infections leading to septicemia and a high mortality rate (30–40%) (15). This very high mortality rate is the highest of all foodborne infectious agents. Fortunately the incidence of *V. vulnificus* infections is extremely low. Consumption of raw oysters harvested from warm waters (U.S. Gulf Coast) among high risk individuals (chronic alcoholics, severely immunocompromised) are factors involved with fatalities (16). Several other *Vibrio* species may be pathogenic (16). Incubation period for vibriosis is 2 to 48 hours, usually 12 hours. Symptoms include abdominal pain, watery diarrhea, usually nausea and vomiting, mild fever, chills, headache, and prostration. Duration is usually 2 to 5 days. Cholera typically expresses profuse rice water stools as a characterizing symptom. *V. vulnificus* infections can include septicemia and extremity cellulitis. Prevention of vibriosis includes cooking shellfish and fish, harvesting shellfish from approved waters, preventing cross contamination, and chilling foods to less than 10°C (17).

D. *Escherichia coli*

There are six pathogenic types of *E. coli* associated with foodborne illness (18). The infectious dose for most strains is high (10^6 to 10^8 cells), although enterohemorrhagic strains may be much lower (2–45 cells). Enteropathogenic (EPEC) strains are serious in developing countries but rare in the U.S. These strains are a leading cause of neonatal diarrhea in hospitals. Likewise, diffusely adherent (DAEC) and enteroaggregative (EAEC) *E. coli* strains are associated with childhood diarrhea. Enteroinvasive (EIEC) strains have an incubation period of 8 to 24 hours, with 11 hours most often seen. Symptoms are similar to *Shigella* infections, with bloody diarrhea lasting for several days. Enterotoxigenic (ETEC) strains are a notable cause of traveler's diarrhea. Onset for illness by these strains is 8 to 44 hours, 26 hours normal. Symptoms are similar to cholera, with watery diarrhea, rice water stools, shock, and maybe vomiting lasting a short 24 to 30 hours. Enterohemorrhagic or verotoxigenic strains (EHEC) are the most serious *E. coli* found in foods, especially in developed countries. *E. coli* O157:H7 is the predominant serotype among these shiga-like toxin producing bacteria, although other serotypes are found. EHEC cause three syndromes (19,20). Hemorrhagic colitis (red, bloody stools) is the first symptom usually seen. Hemolytic uremic syndrome (HUS), which is the leading cause of renal failure in children, is characterized by blood clots in kidneys leading to death or coma in children and the elderly. Rarely, individuals may acquire thrombotic thrombocytopenic purpura (TTP), which is similar to HUS but causes brain damage and has a very high mortality rate. Verotoxic strains have an incubation period of 3 to 4 days. Symptoms include bloody diarrhea, severe abdominal pain, and no fever. Duration ranges from 2 to 9 days. Vehicles of transmission include untreated water, cheese, salads, raw vegetables, and water. For 0157:H7, ground beef, raw milk, and raw apple juice or cider are common vehicles. Prevention of *E. coli* outbreaks includes treatment of water supplies and proper cooking of food. Complete cooking of hamburgers is necessary for destruction of verotoxigenic strains.

E. Yersiniosis

Most environmental *Yersinia enterocolitica* strains are avirulent; however, pathogenic strains are often isolated from porcine or bovine foods (21). The disease is predominately serious to the very young or the elderly and is more common in Europe and Canada compared to the U.S. Incubation period for the disease is 24 hours to several days with symptoms including severe abdominal pain similar to acute appendicitis, fever, headache, diarrhea, malaise, nausea, vomiting, and chills. It is not uncommon for children involved in outbreaks to experience unnecessary appendectomies. Duration is usually long 1 week to perhaps several months. The majority of foods involved in yersiniosis outbreaks are pork and other meats. Milk, seafood, poultry, and water may also serve as vehicles. Control is achieved by adequate pasteurization and cooking and avoiding cross-contamination. Refrigeration is not adequate because the bacterium is psychrotrophic.

F. Campylobacteriosis

Three species are linked to foodborne diseases, *C. jejuni*, *C. coli*, and *C. laridis* (22). *C. jejuni* is most often associated with poultry, *C. coli* with swine, and *C. laridis* with shellfish. *C. jejuni* gastroenteritis is the most frequent infection among the bacterial agents of foodborne disease (Table 194.1). Campylobacters and related pathogens *Arcobacter* spp. and *Helicobacter pylori* are microaerophilic and are thus sensitive to normal atmospheric oxygen concentrations (21% O_2) and very low oxygen concentrations (less than 3%). Growth is favored by 5% O_2. Disease characteristics

are an incubation period of 1 to 10 days, 3–5 days normal. Symptoms include fever, abdominal pain, vomiting, bloody diarrhea, and headache, which last for 1 day to several weeks. Relapses are common. The infectious dose is low, 10 to 500 cells. Foods linked to outbreaks include raw milk, animal foods, raw meat, and fresh mushrooms. Control is achieved by adequate cooking, pasteurization, and cooling and by avoiding cross-contamination. Although gastroenteritis is the predominant clinical presentation of campylobacteriosis, chronic sequelae may occur. Guillian-Barré syndrome, which is a severe neurological condition, and Reiter's syndrome, which is reactive arthritis are rare but serious consequences of campylobacteriosis. *H. pylori* is associated with chronic peptic ulcers.

G. Listeriosis

Listeria monocytogenes emerged as a cause of foodborne disease in 1981 (23,25). Susceptible humans include pregnant women and their fetuses, newborn infants, the elderly, and immunocompromised individuals due to cancer, chemotherapy, and AIDS. The disease has a high 30% mortality rate. Incubation period is variable, ranging from 1 day to a few weeks (25). In healthy individuals, symptoms are mild fever, chills, headache, and diarrhea. In serious cases septicemia, meningitis, encephalitis, and abortion may occur. The duration is variable. The infectious dose is unknown, but for susceptible individuals it may be as low as 100 to 1000 cells. Foods associated with listeriosis are milk, soft cheeses, meats, and vegetables. Like *Y. enterocolitica*, the bacterium is psychrotrophic and will grow at refrigeration temperatures, though slowly. Control is best done by avoiding cross-contamination and adequately cooking food.

H. Clostridium perfringens

C. perfringens is a moderate thermophile showing optimal growth at 43 to 47°C, with a maximum of 55°C (26). Large numbers of viable cells ($>10^8$) must be consumed, which then pass through the stomach into the intestine. The abrupt change in pH from stomach to intestine causes sporulation to occur, which releases an enterotoxin. Furthermore, the bacterium can grow in the intestine leading to a toxicoinfection. The illness is characterized by an incubation period of 8 to 24 hours. Symptoms are abdominal pain, diarrhea, and gas. A cardinal symptom is explosive diarrhea. Fever, nausea, and vomiting are rare. Duration is short, 12 to 24 hours. Because of the large infectious dose foods often associated with outbreaks are cooked meats and poultry that have been poorly cooked, such as gravy (anaerobic environment at bottom of pot), stews, and sauces. Outbreaks frequently occur in food service establishments where large quantities of food are made and poorly cooled. Control is best achieved by rapidly cooling cooked food to less than 7°C, holding hot foods at greater than 60°C, and reheating leftovers to greater than 71°C.

I. Other Bacterial Foodborne Infections

Many other bacteria have been linked to foodborne diseases including *Plesiomonas shigelloides* (raw seafood), *Aeromonas hydrophila* (raw seafood), *Arizona hinshawii* (poultry), *Streptococcus pyogenes* (milk, eggs), and perhaps *Enterococcus faecalis* (27). Their contribution to foodborne illness appears to be minimal but they may contribute to opportunistic infections.

III. NON-BACTERIAL FOODBORNE INFECTIONS

Numerous infectious viruses and parasitic worms are capable of causing foodborne illness. All are easily controlled by proper heat treatment of foods. Difficulty with laboratory confirmation of viral agents as causes of foodborne illness leads to probable under reporting (28,29).

A. Infectious Hepatitis

Hepatitis A virus is a fairly common infectious agent having an incubation period of 10 to 50 days, mean of 4 weeks (30). Symptoms include loss of appetite, fever, malaise, nausea, anorexia, and abdominal distress. Approximately 50% of cases develop jaundice that may lead to serious liver damage. The duration is several weeks to months. The infectious dose is quite low, less than 100 particles. The long incubation period and duration of the disease means that affected individuals will shed virus for a prolonged period. Foods handled by an infected worker or those that come in contact with human feces are likely vehicles (raw shellfish, salads, sandwiches, and fruits). Filter feeding mollusks concentrate virus particles from polluted waters. Control is achieved by cooking food, stressing personal hygiene, and by avoiding shellfish harvested from polluted waters.

B. Enteroviruses

Norwalk-like viruses in the calicivirus family (Coxsackie, ECHO, Norwalk, Rotavirus, Astrovirus, Calicivirus, Parvovirus, and Adenovirus) are now considered the leading cause of foodborne gastroenteritis in the U.S. (1). Other viruses most certainly are involved but our ability to isolate them from infected consumers and foods is limited. Incubation period is typical for infectious organisms, 27 to 72 hours (30). Symptoms are usually mild and self limiting and include fever, headache, abdominal pain, vomiting, and diarrhea. Duration is from 1 to 6 days. The infectious dose for these agents is thought to be very low, 1 to 10 particles. Foods associated with transmission of viral agents are raw shellfish, vegetables, fruits, and

salads. Control is primarily achieved by cooking and personal hygiene.

C. Parasites

Nematodes (roundworms) linked to foodborne illness in humans include *Trichinella spiralis, Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Anisakis* spp., and *Pseudoterranova* spp. (31). *T. spiralis* can invade skeletal muscle and cause damage to vital organs leading to fatalities. Incubation period of trichinosis is 2 to 28 days, usually 9 days. Symptoms include nausea, vomiting, diarrhea, muscle pains, and fever. Several days duration is common. Foods linked to the disease are raw or undercooked pork and wild game meat (beaver, bear, and boar). Control in pork is accomplished by: 1) cooking to 60°C for 1 minute, 2) frozen storage at −15°C for 20 days, −23°C for 10 days, or −30°C for 6 days, or 3) following USDA recommendations for salting, drying, and smoking sausages or other cured pork products. *Anisakis simplex* and *Pseudoterranova decipiens* are found in fish and are potential problems for consumers of raw fish. The incubation period is several days with irritation of throat and digestive tract as primary symptoms. Control of these nematodes is by thoroughly cooking fish or by freezing fish prior to presenting for raw consumption. *A. lumbricoides* is commonly transmitted by use of improperly treated water or sewage fertilizer on crops.

Cestoda (tapeworms) are common in developing countries. Examples include *Taenia saginata* (raw beef), *Taenia solium* (raw pork), and *Diphyllobothrium latum* (raw fish) (31). Incubation period is 10 days to several weeks with usually mild symptoms including abdominal cramps, flatulence, and diarrhea. In severe cases weight loss can be extreme. Control methods are limited to cooking and freezing. Salting has been suggested as an additional control technique.

Protozoa cause a large number of foodborne and waterborne outbreaks each year. *Entamoeba histolytica, Toxoplasma gondii, Cyclospora cayetanensis*, *Crytosporidium parvum*, and *Giardia lamblia* cause dysentery-like illness that can be fatal (31). Incubation period is a few days to weeks leading to diarrhea. Duration can be several weeks, with chronic infections lasting months to years. Those foods that contacted feces or contaminated water are common vehicles. Control is best achieved by proper personal hygiene and water and sewage treatment.

D. Prions

Prions are small proteins found in animal nervous tissues (brain, spinal cord) (30). They are capable of forming holes in brains of affected animals leading to neurological deficits. In cattle, prions are associated with bovine spongiform encephalopathy (BSE) and consumers of beef from affected animals are at risk of obtaining the human form of the disease called variant Creutzfeldt-Jakob disease (vCJD). Although this link is tenuous, a few human cases in Europe are thought to be based on consumption of contaminating nervous tissue in beef. The disease is characterized by progressive brain dysfunction ultimately leading to death. Little is known about the incubation period or the infectious dose, as this is a newly emerged condition. Meat and milk from affected animals are not considered a transmission risk.

IV. FOODBORNE BACTERIAL INTOXICATIONS

Foodborne microbial intoxications are caused by a toxin in the food or production of a toxin in the intestinal tract. Normally the microorganism grows in the food prior to consumption. There are several differences between foodborne infections and intoxications. Intoxicating organisms normally grow in the food prior to consumption, which is not always true for infectious microorganisms. Microorganisms causing intoxications may be dead or nonviable in the food when consumed; only the toxin need be present. Microorganisms causing infections must be alive and viable when food is consumed. Infection-causing microorganisms invade host tissues and symptoms usually include headache and fever. Toxins usually do not cause fever and toxins act by widely different mechanisms.

A. *Staphylococcus aureus* Enterotoxin

Certain strains of *S. aureus* produce a heat stable enterotoxin that is resistant to denaturation during thermal processing (cooking, canning, pasteurization) (32). The bacterium is salt (10 to 20% NaCl) and nitrite tolerant, which enables survival in cured meat products (luncheon meats, hams, sausages, etc.). Conditions that favor optimum growth favor toxin production, i.e. high protein and starch foods. *S. aureus* competes poorly with other microorganisms, so if competitors are removed by cooking and *S. aureus* is introduced, noncompetitive proliferation is possible. The toxin affects the vagus nerve in the stomach causing uncontrolled vomiting shortly after consumption (1 to 6 hours). Other symptoms include nausea, retching, severe abdominal cramps, and diarrhea, which clear in 12 to 48 hours. Fortunately, fatalities are rare. Sources of the bacterium are usually from nasal passages, skin, and wound infections of food handlers. Hence, suspect foods are those rich in nutrients, high in salt, and those that are handled, with ham, salami, cream filled pastries, and cooked poultry common vehicles. Control is accomplished by preventing contamination, personal hygiene, and no hand-food contact. Refrigeration below 5°C prevents multiplication, and heating foods to greater than 60°C will not destroy the toxin but will kill the bacterium. Prolific

growth of the bacterium is possible in the 5 to 40°C range. Problems with the bacterium occur most frequently with foods prepared at home or at food service establishments, where gross temperature abuse has occurred.

B. *Bacillus cereus* Enterotoxin

This spore-forming bacterium produces a cell-associated endotoxin that is released when cells lyse upon entering the digestive tract (33). There are two distinct types of disease syndromes seen with this bacterium. The diarrheal syndrome occurs 8 to 16 hours after consumption. Symptoms include abdominal pain, watery diarrhea, with vomiting and nausea rarely seen. Duration is a short 12 to 24 hours. Foods linked to transmission of this syndrome are pudding, sauces, custards, soups, meat loaf, and gravy. The second, emetic syndrome, is similar to *S. aureus* intoxication. The incubation period is very short, 1 to 5 hours. Symptoms commonly are nausea and vomiting, with rare occurrence of diarrhea. Duration again is short, less than 1 day. This syndrome is commonly linked to consumption of fried rice in Oriental restaurants. Other foods include mashed potatoes and pasta. The infectious dose for both is thought to be at least 500,000. Because the bacterium forms spores, prevention of outbreaks is by proper temperature control. Hot foods should be held at greater than 65°C, leftovers should be reheated to greater than 72°C, and chilled foods should be quickly cooled to less than 10°C.

C. Botulism

This rare disease is caused by consumption of neurotoxins produced by *Clostridium botulinum* (34). This spore-forming bacterium grows anaerobically and sometimes produces gas that can swell improperly processed canned foods. The bacterium produces several types of neurotoxins that are differentiated serologically. The toxins are heat-labile exotoxins. Two main food poisoning groups (proteolytic and nonproteolytic) are found in nature. Nonproteolytic strains can be psychrotrophic and grow at refrigeration temperatures without the food showing obvious signs of spoilage (no swollen cans or off odor). Incubation period is 12 to 48 hours, but may be shorter or longer. Early symptoms, which may be absent, include nausea, vomiting, and occasionally diarrhea. Other symptoms are dizziness, fatigue, headache, constipation, blurred vision, double vision, difficulty in swallowing, breathing, and speaking, dry mouth and throat, and swollen tongue. Later, paralysis of muscles followed by the heart and respiratory system can lead to death due to respiratory failure. Duration is 3 to 6 days for fatal cases, several months for non-fatal cases. Treatment of suspect cases is by immediate administration of antisera, which can be useful if given early. Respiratory assistance is usually required.

Foods frequently linked to botulism are inadequately home canned foods, primarily low-acid vegetables, preserved meats, and fish (more common in Europe), cooked onions and leftover baked potatoes. The bacterium generally will not grow at a pH of less than 4.6 or at a water activity below 0.85. Thus, high-acid foods, like tomatoes and some fruits, generally are safer than low-acid foods, like corn, green beans, peas, muscle foods, etc. Control is by applying a minimum botulinum cook (12 D) to all thermally processed foods held in hermetically sealed containers. Each particle of food must reach 120°C (and be held at that temperature for 3 minutes to reach a 12 D process). Consumers should reject swollen or putrid cans of food. Properly cured meats hams, bacon, luncheon meats should not support growth and toxin production by the bacterium.

A related illness caused by *C. botulinum* is infant botulism. The bacterium can colonize and grow in the intestinal tract of some newborn infants who have not developed a desirable competing microflora. The toxin is then slowly released in the intestines leading to weakness, lack of sucking, and limpness. Evidence suggests that infant botulism may be associated with sudden infant death syndrome. Consumption of honey by young infants has been linked to this type of disease.

V. CHEMICAL INTOXICATIONS

Chemical hazards are minimally important as etiological agents of foodborne disease (Table 194.1). It should be noted that a number of chemicals, whether naturally occurring or intentionally added, have tolerance limits in foods. These limits are published in the Code of Federal Regulations, Title 21. Informal limits are available through FDA Compliance Policy Guidelines (Center for Food Safety and Applied Nutrition, Washington, D.C.). Prohibited substances (CFR 21, Part 189) are not allowed in human foods either because they have been shown to be a public health risk or they have not been shown to be safe using sound scientific data (36). Safe food additives are oftentimes referred to as Generally Recognized as Safe (GRAS) substances. There are no documented occurrences of foodborne disease associated with the proper use of insecticides, herbicides, fungicides, fertilizers, food additives, package material migration chemicals, and other industrial use chemicals.

Most human-made chemicals associated with foodborne disease find their way into foods by nonintentional means. Accidental or inadvertent contamination with heavy metals, detergents, or sanitizers can occur (36). Although infrequently reported to CDC, most chemical intoxications are likely to be short in duration with mild symptoms. CDC does not attempt to link exposure to these chemicals with chronic diseases. There are measurable levels of pesticides, herbicides, fungicides, fertilizers, and veterinary drugs and antibiotics in most foods.

The vast majority of instances where these residues are found, levels are well below tolerance. Heavy metal poisonings have occurred primarily due to leaching of lead, copper, tin, zinc, or cadmium from containers or utensils in contact with acidic foods. Although usually considered minor contributors to human illness, toxic chemicals in foods may be significant contributors to morbidity and mortality of consumers. A number of toxic chemicals found in foods are of microbial origin. For example, mycotoxins are secondary metabolites produced by fungi (37). The aflatoxins were the first fungal metabolite in foods regulated by the U.S. government. Grains and nut products are common carriers of these and other mold toxins. Other fungal toxins not associated with microscopic molds include toxic alkaloids associated with certain mushrooms. In this case direct consumption of wild mushrooms that are frequently confused with edible domesticated species can lead to acute toxicity (38). There are no current food processing or sanitation methods that can render these mushrooms acceptable as human food.

A number of seafood toxins are naturally associated with shellfish and some predatory reef fish (39). Again, the ultimate cause of these intoxications is traced to the presence of microorganisms. Under favorable environmental conditions, populations of planktonic algae (dinoflagellates) are high (algal bloom) in shellfish growing waters. The algae are removed from the water column during filter feeding of molluscan shellfish (oysters, clams, mussels, cockles, and scallops). The shellfish then concentrate the algae and associated toxins in their edible flesh. Four primary shellfish intoxications have been identified: amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), and paralytic shellfish poisoning (PSP). ASP has been linked to mussels, DSP with mussels, oysters, and scallops, NSP with oysters and clams, and PSP with all mentioned shellfish. Control of shellfish toxins is best accomplished by monitoring harvest waters for the toxic algae. Post-harvest control is not presently possible; however, depuration or relaying may be of some use.

Some marine fish harvested from temperate or tropical climates may contain toxic chemicals. Scombroid fish (anchovy, herring, marlin, sardine, tuna, bonito, mahi mahi, tuna, mackerel, bluefish, and amberjack) under time/temperature abuse during storage can support growth of bacteria that produce histidine decarboxylase (39). This enzyme releases free histamine from the fish tissues. High histamine levels lead to an allergic response among susceptible consumers. Prompt and continued refrigeration of these fish after harvesting will limit microbial growth and enzyme activity. Fish most often associated with histamine scombrotoxicity are mahi mahi, tuna, mackerel, bluefish and amberjack. Another form of naturally occurring chemical food poisoning found in tropical and subtropical fish is ciguatera. Like shellfish toxicity, ciguatera results when fish bioconcentrate dinoflagellate toxins through the food chain. Thus, large predatory fish at the top of the food chain can accumulate enough toxin to give a paralysis-type response among consumers. Fish associated with ciguatera poisoning are grouper, barracuda, snapper, jack, mackerel, and triggerfish. Again, monitoring of harvest waters is the essential control step to avoid human illness.

VI. PHYSICAL HAZARDS

Consumers frequently report physical defects with foods, of which presence of foreign objects predominate (8). Glass is the leading object consumers report and is evidence of manufacturing or distribution error. Most physical hazards are not particularly dangerous to the consumer, but their obvious presence in a food is disconcerting. Most injuries are cuts, choking, and broken teeth. Control of physical hazards in foods is often difficult, especially when these hazards are a normal constituent of the food, such as bones and shells. Good manufacturing practices and employee awareness are the best measures to prevent physical hazards. Metal detectors and X-ray machines may be installed where appropriate.

VII. SUMMARY

Because of the predominance of hazardous biological contaminants found in raw foods most food processing unit operations are designed to reduce or eliminate these hazards. Successful implementation of these processing steps can greatly minimize the risk of foodborne disease transmission. Unsuccessful implementation or failure to recognize the need for interventions sets the stage for production of potentially dangerous products. Because of the varied nature of foods it is imperative that prudent processors understand the inherent risks of their products and ensure the proper application of interventions to reduce these risks. This fundamentally sound recommendation will help keep processed foods competitive in the marketplace and will help maintain and enhance consumer confidence in the safety of their food supply.

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195 Personal Hygiene

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CONTENTS

I. INTRODUCTION

More than 215 million cases of infectious disease are caused by foodborne illness in industrialized countries each year, but the true incidence is difficult to determine because actual illness cases are probably underreported (1,2). The five major risk factors related to employee behaviors and preparation practices in retail and food service establishments that have been identified as contributing to foodborne illness are improper holding temperatures, inadequate cooking, contaminated equipment, food from unsafe sources, and poor personal hygiene (3). The five key public health interventions to protect consumer health are demonstration of knowledge, employee health controls, controlling hands as a vehicle of contamination, time and temperature parameters for controlling pathogens, and the consumer advisory notices (3). Three of the important interventions rely directly or indirectly on the employee and individual personal hygiene.

Personal hygiene refers to the cleanliness of a person's body, with skin, hands, hair, eyes, mouth, nose, nasopharynx, respiratory tract, and excretal organs as body parts that can potentially contaminate food (4). Poor personal hygiene contributed 38% of foodborne illness outbreaks in the U.S. from 1973 to 1987 (5) and 36% of outbreaks from 1988 to 1992 (6). From 1983 to 1992, 79% of outbreaks were bacterial in origin, with disease incidence contributed most by improper holding temperature and poor personal hygiene of food handlers (7). This trend continued in the U.S. from 1993–1997, with 75% of foodborne outbreaks from bacterial pathogens, 33% being attributed to improper holding temperature, and 15% to poor personal hygiene (8). The most common factors contributing to outbreaks in England and Wales between 1992 and 1996 were improper cooking, inadequate storage, cross-contamination, and raw ingredients in prepared foods, with 63% of outbreaks involving foods of animal origin (9). A key concept in proper personal hygiene is that appropriate individual behavior and actions can prevent transmission of pathogenic microorganisms from the body or from contaminated surfaces to food by the food handler. The recommended practices of sanitation and personal hygiene are similar in food processing plants, foodservice preparation establishments, and home kitchens, indicating a common goal in preventing foodborne illnesses. Personal hygiene forms the basis for effective food processing sanitation programs and manufacture of safe food products. Good Manufacturing Practices (GMPs), Sanitation Standard Operating Procedures (SSOPs), Sanitation Performance Standards (SPSs), Sanitation Control Procedures (SCPs), and Hazard Analysis Critical Control Point (HACCP) programs all depend upon the consistent application of desirable habits by employees. HACCP is a preventive system of food control that relies upon the other prerequisite programs for success (10).

II. SOURCES AND TYPES OF FOOD CONTAMINATION

Contamination of food can be categorized into three hazard areas of physical, chemical, and biochemical sources. A hazard is an agent that is reasonably likely to cause illness or injury in the absence of its control (11) or any biological, chemical, or physical property that may cause an

unacceptable consumer health risk (12). Physical hazards are usually considered to cause less severe or shorter durations of illness than the other types of hazards, but may be caused by human activities or behaviors. Physical hazards include foreign objects or extraneous materials not normally found in food, such as wood, plastic, glass, and metal that might be contributed by lack of personal hygiene regarding toothpicks, jewelry, bottles, eating utensils, and other objects not desired in food areas. Finding these items in foods causes discomfort or personal injury to the consumer, but usually does not result in death (13). Physical hazards that are less likely to be contributed by improper personal hygiene are materials like bone fragments in meat or poultry, insects or insect fragments, and sand or soil due to improper harvesting or processing practices.

Chemical hazards are substances that may cause toxicity or acute diseases. The actual risk of illness or injury to consumers from many of the chemicals used in food production and processing is controversial (14). Some chemicals may be used in processing, such as cleaners, detergents, sanitizers, lubricants, and water treatment chemicals (14). Other chemicals are used for specific purposes, ingredients in food products, antimicrobial solutions on carcasses, or as processing adjuncts (14). Other sources of chemical hazards might be pesticides, herbicides, antibiotics, hormones, or heavy metals from raw materials; personal care-related materials such as cosmetics, perspiration, and medicines; or naturally occurring substances such as myotoxins, allergens, or color extractives. Foodborne illness may be caused by chemical poisoning, but illnesses caused by microorganisms are more common than from chemicals (15). Generally, biological hazards are considered the most serious of the foodborne agents. These include bacteria, viruses, fungi, and parasites as well as the toxins from those biological sources (16,17). Humans have been regarded as an excellent harbor for most of the pathogenic microorganisms, with human disease conditions linked to the contamination of foodstuffs. Gram-negative pathogenic microorganisms (*Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter*, and *Vibrio* species) may be present in the intestines and feces of humans, animals, and birds (18). These microorganisms are not heat resistant and cause problems because of poor sanitation, inadequate personal hygiene, and cross-contamination (18).

The major classifications of biological foodborne illnesses are intoxication caused by ingestion of toxins produced by microorganisms, infection caused by growth of microorganisms or parasite, and toxin-mediated infection that arise from toxin production by pathogenic microorganisms introduced to the intestinal tract through consumption of contaminated foods (15,16,17). Pathogenic microorganisms that cause foodborne illnesses include *Campylobacter*, *Clostridium*, *Escherichia*, *Listeria*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Vibrio*, and *Yersinia* bacteria species; viruses such as Hepatitis, Noroviruses (Norwalk), and Rotavirus; molds and fungi that produce ergotoxins, alkaloids, and aflatoxins; and parasites such as *Anisakis*, *Cryptosporidium*, *Cyclospora*, *Giardia*, *Toxoplasma*, and *Trichinella* (6,15,16,17,19,20,21,22). Minimum growth temperature for most pathogenic bacteria is 4°C or above (16,17). Incubation times for foodborne illnesses range from a few hours with *S. aureus* and *V. parahemolyticus* to several weeks for *L. monocytogenes* and Hepatitis A (6,22). An individual may be infected with foodborne illness vectors and contaminate food-contact surfaces or other individuals, even if clinical symptoms of the illness are not evident (23). Food handlers may not always recognize when they are ill and may cause contamination by handling food.

Food workers who were ill either prior to or at the time of the outbreak, depending upon the specific microorganism involved, were implicated in 93% of foodborne illness outbreaks from 1975 to 1998 (23). Staphylococcal food poisoning, shigellosis, and typhoid fever were most highly contributed by colonized persons handling the implicated foods (5). *Campylobacter* in retail chicken and lamb shared identical subtypes as those strains found in humans, which indicates the potential role of food to human foodborne illness through hand-to-mouth transmission (24). The incidence of *Campylobacter* spp. in broiler feces and corresponding processed carcasses decreased by 1 logarithmic unit from 1995 to 2001, which may have contributed to the reduction in the frequency of human disease during the same time period (25). However, *Campylobacter* was not detectable on carcasses after overnight chilling, even though it was readily recovered from composite rectal and individual colon samples from swine, indicating that the microorganism did not progress through the slaughtering operation and other means of transmission to pork should be considered (26).

Campylobacter jejuni and *Salmonella* spp. are easily transferred from raw chicken to food contact surfaces and hands and then to other foods or food contact surfaces through cross-contamination (27). *Salmonella* and *Campylobacter* were implicated in 72% of laboratory-diagnosed cases of foodborne diseases in the U.S. in 2001 (22). *Salmonella* species isolated from domestic and imported food products were resistant to rifampin antibiotic, and 49% of isolates were resistant to one or more antimicrobial agents, giving a baseline for antimicrobial-resistant *Salmonella* in the U.S. food supply (28). The incidence of *L. monocytogenes* was increased after chilling and cutting of pork and beef, with contamination originating from the processing environments of the chilling or cutting room, and the incidence of *L. monocytogenes* was lower in bovine cutting and processing than in porcine cutting and processing (29). Viral foodborne infection can be transmitted to humans and food-contact surfaces by

contaminated human hands (30,31). There are health risks of contaminated hands contacting the mouth through smoking or eating or the further contamination of ready-to-eat foods by contaminated hands (27). Foodservice employees can contribute to foodborne illness by mishandling food or as direct sources of illness (32). The infectious doses for many pathogenic microorganisms are relatively low, especially in susceptible individuals, which indicates that all measures possible should be implemented to prevent contamination of food from any source. Governmental health agencies have implemented guidelines and regulations required of personnel and their managers regarding the cleaning and composition of facilities and equipment and the health and hygienic practices expected of employees for all sectors of the food industry (2). The recognition that food handlers served as the source of infection in foodborne outbreaks and that hand contact with food was a mode for pathogen contamination has been responsible for changes in governmental oversight of food production and handling practices (23).

III. PERSONAL HYGIENE REGULATIONS

Most governments have agencies that prescribe sanitation and sanitary practices because there is potential for food handlers to transfer pathogenic microorganisms to food. The European Union (EU) European Food Safety Authority, United States Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) have enacted directives and regulations that identify the need for sanitation and cleanliness of facilities, equipment, and personnel (3,33,34,35,36). The EU passed Council Directive 93/43/EEC in 1993 to harmonize general rules of hygiene for foodstuffs to protect human health through movement of foodstuffs throughout all stages of preparation, processing, manufacturing, packaging, storing, transportation, distribution, handling, and sale or supply to the consumer (33). These incorporated the use of hazard analysis, risk assessments, and other management techniques, including microbiological criteria and temperature control when those were adopted in accordance with scientifically accepted general principles. In addition, the guides to good hygiene practice based on the Recommended International Code of Practice, General Principles of Food Hygiene of the Codex Alimentarius were developed (34). In the U.S., the Food, Drug and Cosmetic Act was enacted in 1906 and amended in 1997 and the Federal Meat Inspection Act was enacted in 1906 and substantially amended by the Wholesome Meat Act of 1967. The Food and Drug Administration of the Department of Health and Human Services and the Food Safety and Inspection Service of the Department of Agriculture are agencies responsible for ensuring the wholesomeness of food products in the U.S. (3,35,36). The many requirements for personal hygiene that are common among different country jurisdictions are summarized in Table 195.1 to show the common concerns for personal hygiene.

The common concerns for prevention of food contamination and maintenance of personal hygienic conditions are reflected by the regulatory preambles to ensure that food is safe and unadulterated, that food-contact surfaces are cleaned and maintained in an uncontaminated condition, that facilities provide for cleaning and continuation of hygienic conditions, and that employees practice appropriate hygienic behaviors in food areas. Additionally, most agency regulations describe appropriate facilities for cleaning equipment; necessary personal hygiene, training, and knowledge of food sanitary practices by employees and managers; and requirements for personal habits of individuals in food areas and manufacturing facilities. A difficulty in quantifying the role of personal cleanliness in control of infectious diseases is that other factors, including improved public services, waste disposal, water supplies, commercial food handling, and nutrition, have also changed (37).

The implementation of HACCP plans and improvements in Good Manufacturing Practices have reduced microbial contamination on equipment surfaces and educational programs that continually reinforce food safety principles have reduced total aerobic and coliform counts (38). Complex etiological relationships in food products are due to interactions among food, environment, and the food handler (39). The role of hands in disease transmission and the accompanying importance of handwashing in preventing foodborne disease has been well established (40).

IV. HAND CLEANLINESS

The aim of hand sanitation is to prevent transmission of pathogenic microorganisms from the hands through food or from food to food transferred through the handler's hands (41). Cross-contamination of bacterial and viral pathogens was thought to be a major contributing factor for sporadic and epidemic foodborne illness (42,43). The FDA database of 17,477 observations by compliance officers indicated that lack of hand washing accounted for 30 to 45% of the noncompliance observations (44). Resident bacteria are those normally present on the skin and are usually not harmful while transient bacteria are microorganisms transferred to the skin (45). Bacteria can change their status from transient and become permanent residents on the skin (39). The pathogens most commonly transmitted by hands in healthcare settings are nosocomial bacterial pathogens and lipophilic viruses, which are different than the fecal pathogens transmitted through fecal-oral routes from contaminated hands to food items in retail and food service settings (46).

Skin and hand hygiene are primary means to reduce contact and fecal-oral transmission of infectious agents (37). Removal of the bioload from the hand is a key focus

TABLE 195.1
Sanitary and Personal Hygiene Requirements in International and United States Regulations

Sanitary Guideline or Requirement	Codex (34)	Food Code (3)	21CFR (36)	9 CFR (35)	EU 93/43 Directive (33)
Ensure food is safe and suitable for intended purpose	Section III	2-102.11	110.5; 110.80	416.1	Article 2
Protect food and ingredients from contamination	3.3; 4.4.4		110.10	416.1	Article 3
Effective cleaning and maintenance	3.4	2-103.11	110.35	416.3	Chap. I
Maintain appropriate personal hygiene and cleanliness	3.4; 4.4.4; 7.3	2-301.11	110.10	416.5a	Chap. VIII-1
Adequate facilities for cleaning equipment and utensils	4.4.3	2-103.11	110.35		Chap. II-2
Adequate hot and cold potable water	4.4.3		110.37	416.2g; 416.2h	Chap. I-4; Chap. III-2e
Adequate means of washing and drying hands	4.4.4	2-301.12	110.37		Chap. I-4
Suitable lavatories and sinks	4.4.4	2-301.15	110.37	416.2h	Chap. I-3
Adequate changing facilities for personnel	4.4.4		110.10	416.2h	Chap. I-9
Knowledge of food hygiene principles and practices by managers	5.6	2-102.11	110.10		
Appropriate handling of cleaning chemicals	6.1.1	2-301.16	110.35	416.4c	
Proper cleaning	6.2	2-103.11	110.35	416.12c	Chap. III-2c; Chap. V
Prohibition of ill individuals or disease carriers from food areas	7.1	2-201.12	110.10	416.5c	Chap. VIII-2
Reporting of medical conditions to management	7.2	2-201.11	110.10		
Suitable protective clothing, head covering, footwear	7.3	2-304.11; 2-402.11	110.10	416.5b	Chap. VIII-1
Dressings to cover cuts and wounds	7.3				
Effective hand washing	7.3	2-301.11; 2-301.12; 2-301.14	110.10		Chap. III-2a
Refrain from smoking, spitting, chewing, eating, sneezing, coughing in food areas	7.4	2-301.14; 2-401.11; 2-401.12	110.10		
Prohibit jewelry, watches, pens, other items	7.4	2-303.11	110.10		
Food hygiene training of personnel	10.1	2-103.11	110.10		Chap. X

in proper hand washing (47,48). Frequent hand washing has been cited as a critical component for safe food serving in the retail industry to prevent spread of disease (49,50,51). Numerous pathogenic microorganisms can survive up to 90 minutes when artificially inoculated on the fingertips (52). Thorough hand drying is also important to reduce the incidence of sporadic infections (53). A review of the efficacy of hand washing using literature and experimental data to determine risk assessment ended with the conclusion that proper hand washing can reduce the risk of bacterial contamination on hands, with sanitizer use, soap use, and drying method being primary factors influencing final bacterial counts on hands (54). Hand washing and drying efficacy against resident microbial flora is 35% to 60% (55). Levels of microbial contamination vary with worker and type of work in food and non-food industries (56). Heavily soiled hands of meat cutters had higher bacterial levels, even after hand washing, than workers in meat packaging areas (57).

More frequent hand washing will affect the number of bacteria on the skin (58). After washing with water for one minute with rubbing, but without soap, food handler hand samples had 6,200 to 16,000,000 microorganisms per mL, which was indicative of the problem of hand hygiene among food handlers (59). Comparisons of bacterial baseline levels on hands of workers in the food industries and non-food occupations showed a correlation between the flora on the hands and that of the food that was contacted

(39). Fecal coliforms and enterococci on hands of employees increased with increased degree of food contact (39). Bacterial transfer rates among hands, foods, and food preparation surfaces are highly variable, but it is likely that contamination of hands and various surfaces occurs following the preparation of contaminated foods (43).

Microorganisms were transferred in sufficient numbers by contact of fingers or clean surfaces with contaminated surfaces or cloths to represent a potential hazard if hands then contacted food (60). Cleaning of cloths with detergent increased contamination of cloths and surfaces while quaternary ammonium disinfectant plus EDTA significantly reduced contamination of surfaces and cloths (61). After handling meat contaminated with *Enterobacter aerogenes*, hands had lower microbial counts, but microorganisms were transferred to water faucet spigots (43). Hand washing reduced microorganisms enumerated on the hands, but not to an absolute level of zero, and so the transfer rate of microorganisms by hand to food must be considered a dynamic phenomenon (43). *Campylobacter* are unlikely to survive for long periods outside the body, but transmission from contaminated hands to mouth may occur when food handlers process raw food and from cross contamination of foods and utensils when cooked food is handled immediately after uncooked food (42). The risk of foodborne illness associated with cross-contamination depends upon the level of contamination on the surfaces and the probability of contamination transfer to the foods that will be consumed (42).

Inoculum level of microorganisms is critical in transmission of foodborne pathogens by the hands (58). In one study, all beef samples became positive when food handler fingertips were inoculated with more than 600 *Salmonella*, but lower levels of inocula on fingertips yielded variable microorganism counts on beef samples (62). The efficacy of hand disinfectants and hand washing practices can be determined using several different techniques, but the major areas of contamination are the fingertips and perimeter of the palm (63). Most bacteria identified on hands are resident rather than transient-type skin microorganisms (64). Transient microorganisms represent the major concern in cross-contamination and may not act like resident or normal skin flora in a washing process (64). Hand washing by workers with low initial contamination did not eliminate transient microorganisms that contaminated the hands (57). Cleaning compounds will remove transient bacteria and sanitizers will subsequently destroy the microorganisms, but antimicrobial agents must have a contact time of more than five seconds during hand washing to reduce microbial load on the hands (45). An antibacterial agent for hands should not be chosen on the basis of activity against staphylococci and Gram-positive bacteria, but should control transient organisms by killing a wide variety of pathogenic microorganisms, have sufficient residual concentration to exert control throughout the day, and be non-irritating to the skin (41). Detergents or agents that irritate the skin may damage the stratum corneum and impair the barrier properties by removing normal skin oils (65).

Bacteria are not readily transferred from person to person through use of bar soaps, which do not support growth of bacteria under usage conditions and are inherently antibacterial by their physical-chemical nature (66). Bacteria from previously used soap bars were not transferred to the hands of individuals by routine handwashing (49). Some antimicrobial agents (0.5% 2,4,4'-trichlor-2'-hydroxy diphenyl ether, 0.5% tribromosalicylanilide, 0.325% parachloro-meta-xylenol, PCMX) did not decrease the numbers of bacteria released from hands more than a non-germicidal soap control while iodophor with 0.75% available iodine and 4% chlorhexidine gluconate gave significant reductions during short exposure times (64). Higher concentrations of 2,4,4'-trichlor-2'-hydroxy diphenyl ether and PCMX did not improve reduction in microorganisms released from fingertips (67). Iodine was the only agent that killed bacteria, had residual effect, and was not irritating to skin compared with bisphenols and quaternary ammonium compounds (41). Other studies showed that non-antibacterial liquid soap, hand wash containing PCMX, and an alcohol gel sanitizer gave equivalent reductions in microbial populations on hands after 30 seconds of washing and 30 seconds of rinsing while the combination of antibacterial lotion soap and alcohol gel sanitizer caused greater reductions in microorganisms (68). Automated hand washing systems are used in some food establishments. Manual hand washing with soap for 5 seconds gave the same relative results as automated hand cleansing for 10 seconds with 2% chlorhexidine gluconate (69). The major advantage of hand wash machines is consistency of the hand washing procedure (70). Hand washing machines improved hand-washing compliance from 22% to 38%, but nurses preferred manual hand washing and contamination of sleeves sometimes occurred in a healthcare study (71). Washing of hands is generally preferable to dipping of hands in antimicrobial agents.

Manual hand washing with soap for 5 seconds or automated hand cleansing for 10 seconds with 2% chlorhexidine gluconate resulted in greater microbial reduction than an iodine dip for 2 seconds (69). Hand dip treatments with 25% available iodine, 50 ppm available chlorine, or 930 ppm benzalkonium chloride were generally less effective than hand wash treatments, particularly those with 0.75% available iodine or 4% chlorhexidine gluconate (72). Of hand dip agents, only quaternary ammonium (930 ppm benzalkonium chloride) resulted in significant microorganism decreases compared with sodium hypochlorite (50 ppm available chlorine) and iodophor (25 ppm available iodine) (64). USDA requires that hand sanitizing compounds for dips have equivalency of 50 ppm chlorine (73). Hand sanitizer and sanitizing solutions used as a hand dip should have approved antimicrobial ingredients (3).

Different antimicrobial agents influence different types of microorganisms. Widespread use of antimicrobial products that are superior to plain soap have caused concerns about bacterial strains resistant to the agents and the alterations of skin flora (37). Iodophors and chlorhexidine gluconate reduced *E. coli* more than *P. fluorescens* (72). *S. aureus* had better tolerance to sodium hypochlorite than *Salmonella enteritidis* (74). Hepatitis A virus was highly resistant to a variety of commercial disinfectants, with 12% sodium hypochlorite most effective at inactivating the virus on food contact surfaces (31). Antiseptics like alcohols and iodophors have instant microbial killing while chlorhexidine gluconate, cloroxylenol, and triclosan have less rapid action, but a residual effect (75). Some protective and antiseptic lotion products applied after hand washing may give a residual antibacterial effect and reduce skin irritation, particularly with glove use (75). Barrier creams are widely used for skin protection, creating a thin, water repellent layer over the skin, which may decrease the effectiveness of germicidal agents (76). The benefits of hand washing are enough to require hand washing even when gloves are used (77).

The FDA Food Code requires that there be no hand contact with ready-to-eat food, reflecting the premise that use of a physical barrier such as gloves or utensils minimizes the transfer of pathogens to food (3). Plastic glove use offers benefits and disadvantages because intact gloves prevent bacteria from the skin from entering product, but the skin inside the glove may be occluded and may promote resident microorganism growth (45). Use of gloves is well established for infection control in healthcare environments (77). A vinyl or latex barrier that is intact will provide protection from transmission of bacteria contaminating the hands (78). Vinyl food-grade gloves frequently have pinhole punctures that compromise the barrier protection (77). Gloves can be easily ripped, torn, or punctured as workers perform their normal duties in food handling (78). Studies are conflicting on the necessity of glove use when food is handled. Microbiological counts on ungloved hands washed hourly were lower at 3.5 log cfu/g than on gloved hands with no hand washing and hourly glove changes (6.1 log cfu/g) after a three-hour time (79). Transfer of microorganisms from food to hands and from hands to food was 0.01% when subjects wore gloves and 10% without gloves (80). Gloving is generally recognized as an adjunct and not a replacement for hand washing (77). The frequency of glove changes depends upon length of time gloves are worn, types of food handled, material and thickness of gloves, type of work activity, and chemicals contacting the gloves (56).

Hourly glove changes with hand washing between changes and hourly washing and sanitizing of bare hands after handling of ground beef contaminated with *E. coli* were similar in minimizing microbial levels on hands after 3 hours (81). Bare hands had the greatest level of contamination while bare hands with hourly washing, but no sanitizing, and hourly glove changes with no hand washing minimally reduced microbial loads on hands (81). Knitted gloves (polyester and cotton cloth) could reduce the transfer of bacteria from hands to meat, but increased the transfer of microorganisms among meat pieces (82). Cloth gloves should not be used in direct contact with food unless the food is subsequently cooked to destroy pathogenic microorganisms (3). Cloth gloves must be laundered before being used with a different type of raw animal food (3). Rubber gloves could almost prevent transfer of bacteria from hands to meat and would greatly reduce transfer of microorganisms among meat pieces (82). Heavy contamination of hands is rare when knitted or rubber gloves are worn by meat plant workers (83). Decontamination of glove surfaces can be accomplished by washing. Washing with water, non-medicated soap, or several antimicrobial agents eradicated *S. aureus* loads from vinyl glove surfaces, but water was ineffective with latex gloves (84). Use of 4% clorhexidine gluconate and 70% ethanol was more effective in decontaminating latex gloves than non-medicated soap or sodium hypochlorite with 6,000 and 10,000 ppm available chlorine (84). The routine washing of gloved hands could not be recommended for healthcare workers (84). It should be noted that glove use may promote complacency about hand hygiene so effective hand washing must be conducted with or without the use of gloves (45). Use of gloves does ensure that fingernails and fingertips, major sites of contamination, are not exposed.

Microbial counts of 2 to 3 log CFU frequently occur beneath nails and are often more difficult to remove than from other locations on hands (85). Inoculated microbial populations were reduced the greatest by washing with liquid soap plus a nailbrush (86). Counts recovered from hands with artificial nails were higher before and after hand washing than with natural nails (86,87). Best practices for fingernail sanitation are maintaining short fingernails and scrubbing of fingernails with soap and nailbrush when hands are washed (86). The residual effectiveness of hand washing to prevent cross-contamination of foods and hand-to-mouth contamination is also affected by other factors (53). Residual moisture after washing is an important factor in cross-contamination (79). Washing hands with soap and water or water alone combined with drying on paper towels can remove a heavy inoculum of *Campylobacter* from the fingertips (53). Paper towels were the most effective way to reduce residual moisture after washing (79). Wiping of hands with a paper towel seemed to physically remove bacteria from the hand (42). Hot air driers were viewed to be safe from a bacteriological view, but a 30-second cycle time was insufficient to dry hands thoroughly (88). Electric air drying was shown to reduce the most test microorganisms on finger pads, with paper intermediate and cloth drying the least effective regardless

of washing agents among 70% isopropanol, medicated liquid soap, unmedicated liquid soap, or tap water (89). Hot air driers may create undesirable aerosols when used in processing areas or accumulate microorganisms from toilet areas when used in rest rooms (56,90).

Transfer of Hepatitis A virus from contaminated finger pads could be interrupted by hard-water rinsing and towel drying, application of a topical agent followed by water rinsing and towel drying, and exposure to a hand gel with 62% ethanol (91). Variability in virus transfer rates following water rinsing indicated that volume of water might influence virus removal from the finger pads differently (91). The studies discussed in this section verify that effective hand hygiene is highly dependent upon the habitual behavior of food handlers and the available supporting facilities and items for hygienic practices.

V. RECOMMENDED PERSONAL HYGIENIC PRACTICES

Food workers should follow several fundamental practices to maintain themselves, facilities, equipment, and the food hygienically. Personal hygiene concerns include physical health maintenance, exclusion of individuals with illnesses or disease from contact with food, development of hygienic work habits, proper washing of hands, and maintenance of personal cleanliness (45). Proper personal hygiene at a food establishment involves hand washing following any act that even remotely might cause hand contamination, covering of cuts and abrasions, and designated areas for smoking (58). Personal cleanliness should be maintained through daily bathing, frequent hair washing, daily fingernail cleaning, use of a hat or hairnet, and clean underclothing (45). The sanitary and personal hygiene requirements of controlling regulatory agencies, such as those in Table 195.1, should be used as a guide in establishing food handler and personal hygiene rules and monitoring procedures. Employees should be given adequate training, supervision, and supporting items to promote and maintain desired personal hygiene when handling food.

Managers should check each employee before each shift begins to ensure healthfulness (4). All workers should be trained to report when they are ill or have suffered from an illness. Sick employees may have food poisoning bacteria that can be spread to food (4). Workers should advise the supervisor if they feel ill, especially if there is diarrhea, sore throat, discharge from ears, eyes, or nose; if there are cuts, boils, or skin infections; or if they have been in contact with someone who has been ill (4). Employees with open lesions that cannot be adequately covered, with signs of illness such as coughing or colds, or who have been in contact with other individuals who are ill should be placed in work that does not require contact with food or food-contact surfaces (3,92).

The clothing worn by food workers should be clean (3). Food handlers should also wear appropriate head and face coverings, gloves when applicable, and boots or other cleanable footwear when floors are contaminated (92). They should maintain cleanliness of themselves, particularly their hands and exposed arms, utensils, equipment, facilities, and other food-contact surfaces that might cause food contamination (3). Hand washing should be frequent to prevent hand-to-food or food-surface-to-food-contamination, with a minimum of 20 seconds washing with soap and warm water (3).

It has been suggested that hand washing procedures may vary with activity, double hand washing when initially entering food handling areas and single hand washing frequently during the food handling operations (73). The suggested procedures for double hand washing requires wetting of hands, soap application to form a lather that is spread uniformly on all hand surfaces, use of a nailbrush on all sections of the hand, fingers, and nails, rinsing with warm water, repeat of soap lathering and physical abrasion, second rinse, and towel drying (45). Recommended double washing procedures are shown in Figure 195.1 (73). Double hand washing is used to reduce high levels of pathogens to a safe level upon beginning a work shift, after using toilets or after breaks, after contact with vomit or any fecal material, and after touching any open sores (73). Increased friction through rubbing hands together or use of a fingernail brush with the soap will reduce a larger number of transient and resident microorganisms than quick hand washing (4). The single wash procedure omits use of nailbrush, second soap lathering, and second rinsing and is recommended for use during food handling operations to reduce lesser amounts of microbial or other contamination of the hands (56). Food employees should use vigorous friction on the surfaces of lathered fingers, finger tips, areas between fingers, hands, and arms by rubbing for at least 10 to 15 seconds followed by thorough rinsing under clean and running warm water and thorough drying of cleaned hands and arms (3). Contact with antimicrobial agents less than 5 seconds will have little effect on reducing the microbial load (4).

Hands should be washed before starting work, after touching bare human body parts other than clean hands and arms, after using the toilet, after coughing, sneezing, and use of a handkerchief or disposable tissue, after using tobacco products, following eating or drinking, after handling soiled equipment or utensils, during food preparation as often as needed to remove soil and contamination, to prevent cross-contamination when changing tasks, when switching work between raw and ready-to-eat foods, before donning gloves for working with food, and after engaging in activities that contaminate the hands (3). Hands should be washed often throughout the working day and most particularly when specific activities are changed to another type of task. It is highly advisable that

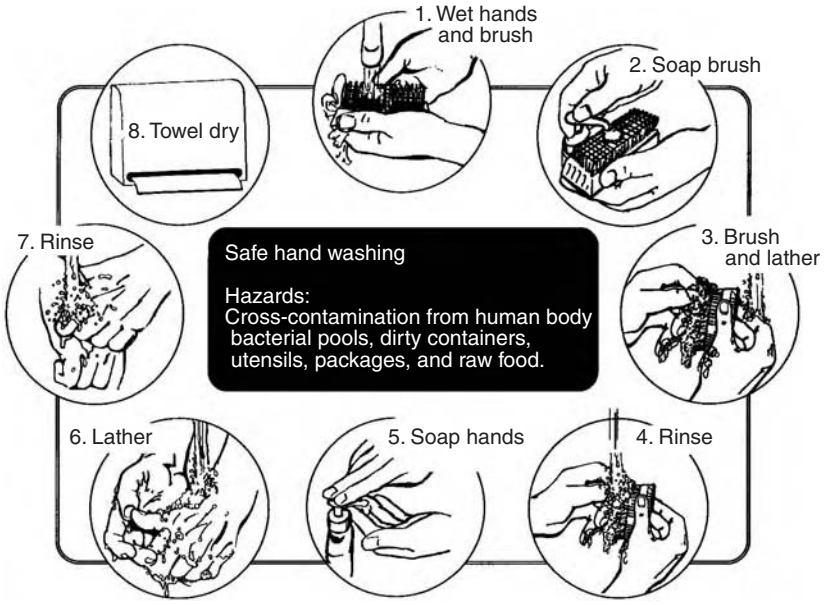

FIGURE 195.1 Double hand washing procedure advocated for elimination of pathogenic microorganisms from hands. Used with permission by OP Snyder, Jr., Ph.D., reference 73.

hand washing be conducted after each absence from the post of duty or work station, after handling cleaning chemicals or equipment, and after putting on a waterproof dressing over wounds or sores (92). Particular attention should be given to cleaning under the fingernails during hand washing (3).

Nails should be clean and short with no biting with the teeth, no nail varnish or polish, and no false nails. Artificial nails and nail polish should not be used by food workers (92). Nails and cuticles should be smooth and neatly trimmed (3,93). Workers should not wear any jewelry, such as earrings, hairgrips, rings, bracelets or wristwatches, or other items that might carry bacteria on them or be lost and cause physical contamination of food products (3). Jewelry should be removed because it interferes with hand washing and may become a physical contaminant if lost in food (89,93).

Hair should be kept clean, tidy, and covered when working with food. Hair restraints are absolutely necessary for all individuals in food processing and preparation areas (3,4). It is preferable to use disposable bouffants, hoods, or snoods to keep hair and hair contaminants from contaminating food, even with helmets, hard hats, or caps. Loosely woven hairnets should be avoided because there is inadequate control of hair (90). Individuals with facial hair should wear disposable paper beard nets. Adequate and clearly marked containers for disposal of head coverings should be immediately outside work areas and near locker and rest room areas (90).

If gloves are to be used, a means to keep them hygienic while workers are washing their hands immediately before beginning work should be available. Hands must be washed properly before gloving (3). Clean gloves should be issued at each break or when employees perform tasks other than food handling. Workers should be required to change gloves whenever they become excessively soiled or contaminated or when changing from one task to another activity (3). It is highly advisable that utensils, tools, and equipment be used to contact food rather than hands or gloves. Gloves should be chosen carefully. Green gloves frighten animals in meat processing facilities (94). Latex gloves should be avoided because latex in glove materials might be transferred to food and cause allergic reactions in the approximately 6% of U.S. consumers who are allergic to latex (94). If latex gloves are used, gloves should not be powdered since the powder, often corn starch, will bind the latex protein and increase glove contact with the skin (94). Alternatives to natural latex rubber gloves are polychloroprene (neoprene), styrene butadiene, styrene ethyl butadiene, and synthetic latex, which lack the protein that initiates allergic reactions (94).

Smoking and gum chewing should be avoided during the day (93). Workers should never eat, drink, chew gum, or use tobacco products in any room or adjacent areas where food is handled or stored. Workers must always wash their hands after eating, drinking, chewing gum, using tobacco products, or handling money before resuming activities in food areas (4). Workers should not cough, sneeze, or touch the mouth and nose during working with food and should wash their hands using the double wash technique if the mouth or nose is contacted. Sanitation training and education should be ongoing and continuous to maintain personal and environmental hygienic conditions (78).

VI. TRAINING, FACILITIES, AND SUPPORT FOR PERSONAL HYGIENE

Appropriate equipment and supplies are needed for personal hygiene (4). Survey instruments have indicated that knowledge of food safety by food service managers did not correlate with hand washing habits, with only 75% always washing hands after using the toilet (40). As many as 60% of foodservice personnel neglect to wash their hands after use of toilets (40,55). Hand washing behavior was ranked by an expert panel as the highest behavior for prevention of shigellosis while other factors such as keeping food at safe temperatures and avoidance of cross-contamination ranked more highly for prevention of other forms of foodborne illnesses (95). Hand washing should be made a part of the operation, and not treated as a separate issue apart from the food preparation (48). This may be difficult because of the location of hand wash and sanitary facilities. Barriers to frequent hand washing have been identified as understanding the necessity for frequent hand washing and the amount of time needed for hand washing (51). The solution lies in improved hand washing compliance and management of hand washing through effective and consistent training (47).

Teaching workers to wash their hands properly involves learning by doing through repetition and observing actual hand washing results (48). An effective teaching tool is to use colored spice in cooking oil or a safe fluorescent compound spread on the hands before washing and to observe the hands carefully, particularly around the fingernails and between the fingers, after washing (using UV light for fluorescent compounds) (48,55). Employees should scrub hands whenever contaminated, as may occur after touching food, contact surfaces, or the mouth, and always before performing the next or a different job function (93). Washing for 30 seconds, using a nail brush and lather to scrub fingernails, knuckle creases, backs of finger joint, and deep creases in palms is required (93).

Hand washing sinks should be located near all work and food preparation areas to encourage washing of hands frequently during work and to allow convenient use by employees (3,93). Separate sinks are required for hand washing and for food preparation or utensils (3). Hand washing sinks should have foot or knee-operated faucets to prevent contamination of faucet handles with contaminated hands (4). Hand washing lavatories should be equipped to provide water at least 38°C with a self-closing, slow-closing, or metering faucet to provide a flow of water for at least 15 seconds without reactivation (3). It is desirable that water be 43–50°C (4). Automatic hand washing facilities may be substituted for hand washing lavatories in food establishments that have at least one hand-washing lavatory (3). Each hand washing lavatory or group of adjacent lavatories should have a supply of hand cleaning liquid, powder, or bar soap and individual disposable towels, a continuous towel system that supplies the user with a clean towel, or a heated-air hand drying device (3). A sign or poster that notifies employees to wash their hands should be provided and clearly visible at all hand washing lavatories (3).

Welfare facilities must be clean, free of debris, well lighted, and conveniently located away from production areas. There should be a locker or changing area where employees change from street clothing to work garments (3). Work clothes should not be worn to work, but stored in a locker at work or carried to work in a garment bag (93). Lockers should be neat and well ventilated, with sufficient size to store clothing and other personal items (3,90). Laundry services are preferable to in-plant laundry for supply of clean clothing (90). It is highly recommended that uniforms be provided to workers to ensure that clothing is clean each day (90,94). This may be especially important when protective clothing is necessary to keep employees dry, warm, and comfortable in cold work environments (94). The choice of durability or disposability, relative weight of garments, and barrier capacity of garments depends upon the activities of the food handler (94). An effective means of reducing human contamination to food and contact surfaces in ready-to-eat facilities is clean, sanitized clean room work apparel to provide a protective barrier between employee and product (96). Clean room apparel is designed to capture and trap particles to prevent them from dispersing into the processing environment (96). Clean room clothing fabrics vary in weight, pore size, moisture vapor transmission rate, and air permeability (96).

Selection of uniforms and uniform provider services should involve consideration of garment segregation to prevent cross-contamination, a wash process formulated to decontaminate and remove particles, an efficient one-pass drying process, complete inspection process, garment packaging to maintain cleanliness, and distribution of required garment quantities (96). Cleaning of food handler clothing is a multi-step process, removing organic material first and then using sequential washing and rinsing cycles (94). Reusable uniforms should be durable and cleanable, achieving a six-log reduction of pathogens (94). Clothing of 100% polyester garments lasts longer than with cotton (94). Effective bacterial destruction relies on water temperature higher than 70°C and pH of 11 or 12 (94). Clothing should have no pockets or pockets only on the lower part of the body to prevent physical contamination by items falling from pockets (90). Pockets should have flaps that close securely, and all clothing should be routinely examined for loose buttons, snaps, closures, and frayed edges (90). Work clothing should be light in color to reveal stains and contamination (90,93). Food handlers should be encouraged to change their clothing when it becomes soiled or contaminated. Employers must anticipate the demand for

different sizes of clean garments, the purchase of replacement clothing, repair of damaged clothing, and adequate cleaning and sanitation of soiled clothing.

Rubber boots with steel toes are recommended in food processing areas to promote proper foot hygiene and prevent cross-contamination in food processing areas (92). Boot wash stations and footbaths with antimicrobial agents should be located at strategic locations, especially at entrances and exits to areas of food production. If leather boots are used in areas of the plant where food is not handled, dirt and debris should be rinsed from boots with plain water or a mild soap solution using a soft-bristle brush, damp cloth, or sponge rather than detergent or oil soap (94).

Rest room facilities should be clean at all times and adjacent to, but separated from, locker rooms and work areas. There must be at least one toilet provided and a hand washing facility in or immediately adjacent to toilet rooms (3). Toilet tissue supplies must be available at each toilet (3). Rest rooms should have self-closing doors (4). Shower areas must be kept absolutely sanitary with daily or more often cleaning and adequate ventilation (90). An area separate from lockers, changing rooms, and rest rooms should be provided for employees to store food (90). Designated areas for eating, drinking, use of tobacco products and other non-work activities should be provided away from work areas (3).

VII. SUMMARY

A major risk factor in contributing to foodborne illness is personal hygiene because food handler training and practices determine the effectiveness of eliminating and preventing contamination of food. Personal hygiene is keeping all parts of the body in a healthful and clean condition. Governmental agencies have established directives, regulations, and rules necessary for personal hygiene practices and support. Hand washing and attention to hand hygiene on a frequent basis and when hands are contaminated with pathogenic microorganisms will prevent transfer of contaminants from humans and surfaces to food. Effective hand washing depends upon rubbing of hands in warm water, preferably with an antimicrobial agent, for at least 20 seconds followed by sufficient rinsing and drying. Hands that are heavily soiled or contaminated with pathogenic bacteria should be double washed using a hand brush for nails, fingers, and creases in the hand. Sufficient numbers of hand washing facilities with knee or foot operation, soap, and towels should be located where workers enter and leave work areas and close to work activities so hands can be washed before and after handling food and when contaminated during work activities. Other hygienic practices include food handling only by healthy individuals; daily personal bathing; clean clothing; hair restraint; gloves and boots when applicable; and proper welfare facilities for toilets, lockers, and breaks for eating, drinking, and tobacco use. Proper training, constant reinforcement, and careful supervision of personal hygienic habits and supporting sanitary facilities and equipment are essential for prevention of foodborne illnesses.

ACKNOWLEDGMENTS

Appreciation is expressed to D.L. Marshall, A.P. Brock, D.M. Price, and N.J. Honeycutt for their assistance with the manuscript. This was approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript 04-18-0034.

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CONTENTS

I. INTRODUCTION

Technological advances have resulted in processing and packaging methods that produce higher quality, less expensive food (1). However, this increased productivity has also led to problems due to food contamination and waste disposal. As the food industry has expanded, sanitary practices have become more complex and regulations have become more strictly enforced (1,2). In addition, inspection has become more stringent as modern testing methods become more precise (3).

In processing food, one of the objectives should be to make it cleaner and more wholesome (2). Proper sanitation results in a better, more competitive product, a more efficiently operating plant, greater employee productivity, and fewer accidents. In addition, having a clean plant engenders employees to practice higher standards of cleanliness and hygiene (4).

An effective sanitation program can control problems due to food spoilage, can improve product quality, prolong shelflife, and ensure product safety. Such a program includes regular cleaning and sanitizing of the whole

processing plant including environmental heating and cooling equipment, ventilation ducts, and the refrigeration and storage equipment (1,2). Major effort should be expended on cleaning and sanitizing processing equipment and the plant floor. In addition, all equipment in a plant should be cleaned and sanitized before each use.

Maintaining environmental equipment is important in order to prevent the spread of dirt and microorganisms through the ventilation system and into the plant environment. As a bonus, well maintained heating and cooling equipment will run more efficiently and will thus reduce overhead costs (1,3).

II. GMPS AND REGULATIONS

Good Manufacturing Practice (GMPs) regulations were established by the U.S. Food and Drug Administration in 1969. The "umbrella GMPs" broadly cover maintenance of the facilities, cleaning and sanitizing equipment and utensils, pest control, proper use and storage of chemical compounds in the plant, and basic guidelines for restroom facilities. There are also more specific GMPs for safe food manufacturing. These are directed towards individual food industries (5,6). GMPs do not specify how often cleaning and sanitizing is to occur. This is a decision of the sanitation managers, and is based on the equipment, its frequency of use, its potential for food contact, and the type of cleaning it requires (2). International regulations by the Codex Alimentarius (FAO/WHO) are similar to GMPs. Their Recommended Codes of Practice (RCPs) make general recommendations about hygiene, and producing safe food (7).

Federal authorities have regulatory responsibility over food industries that ship their products across state lines. These companies are regulated by the federal government, with assistance from state regulatory bodies. Some states have their own local ordinances, which apply to both the food that is produced for local consumption as well as for interstate consumption (2). The majority of local regulations are in the form of GMPs. In addition, the U.S. Food, Drug and Cosmetic Act, which was established in 1938, also regulates the national food industry. This Act provides general regulations for food quality standards and identity as well as general guidelines on tolerances and specific prohibitions on what can and cannot be used as food additives (3).

III. CLEANING

Cleaning is a process where dirt, food debris, and other soils are removed from surfaces. This is a prerequisite to sanitation as the ability of sanitation routines is affected by how clean a surface is prior to application. The removal of biofilm materials from all equipment and food contact surfaces will enable best control of both spoilage and pathogenic microbes that may cross contaminate foods. Although some microbes are removed during cleaning, this is not the primary purpose of this step.

A. Types of Soil

In order to select the correct cleaning compound one has to know what type of soil is present. In a food plant the most common types of organic soils are food deposits, such as salts, sugars, lipids, and proteins. Salts are soluble in water and acid but may react with other compounds when exposed to heat and become difficult to remove (8). Sugar is soluble in water but may caramelize in the presence of heat and become more adhered to the surface. Lipids (fats and oils) are insoluble in water but soluble in alkali. The sources of lipids may be the food being processed or petroleum deposits from lubricants used to grease plant equipment (1). Both lipid sources can be removed using solvent cleaners. Protein is insoluble in water, slightly soluble in acid, and soluble in alkali. Proteins are very difficult to remove because heat denatures the protein molecules allowing them to adhere tightly to surfaces (3).

One other type of organic soil is biofilm. Biofilms are matrices of microbial cells adhering to a surface. These usually occur in areas where liquids are constantly in contact with surfaces, but they may also occur on moist surfaces, such as the exterior of meats (9,10,11,12). Biofilms inoculate any fluids passing over the colonized surfaces and are more resistant to sanitizers than planktonic (non-attached) cells because the sanitizing agent is unable to penetrate the organic matrix (9,13,11,12). Eliminating biofilms requires mechanical cleaning of the surfaces. Chlorinated alkaline detergents help detach biofilms, but scrubbing surfaces and using high-pressure jets is more effective. Acid detergents are ineffective (9).

Inorganic soils include hard water deposits or scale, rust, and alkaline film deposits from previously used cleaners. When water with bicarbonates (calcium and magnesium bicarbonate are the common sources of water hardness) is heated, the bicarbonates transform into carbonates that have low solubility and as a result precipitate out of solution and form a scale on exposed surfaces. This scale can reduce heat transfer and if allowed to build-up will also reduce pipe diameters (1,2,4). Water pretreatment systems, such as using ion exchange resins that capture calcium and magnesium ions, can prevent problems due to hardness. If no pretreatment is used, then the cleaning routine must also include an acid cleanser and an abrasive compound to remove scale build-up (1).

Rust commonly occurs near boilers or in other areas where condensation is prone to settle. Adequate ventilation and proper maintenance of plant humidity can go a long way in preventing rust. Surfactants and abrasives can help to remove rust, but will not prevent it from returning.

Any equipment that has food contact surfaces should be replaced when rust forms. Modern equipment is primarily made of rustproof aluminum and stainless steel.

B. Cleaning Basics

A properly kept plant and plant grounds will not only improve the quality of the product but will also improve the morale of the employees and serve as a reflection to the public of the quality of the food produced by the plant (4). Both the exterior and the interior of the plant need to be constantly cleaned and maintained. GMPs maintain that any grass or hedges on the exterior of the building should be properly trimmed to ensure that they do not serve as breeding ground for rodents or insects. In addition, all openings into the building should have screens to prevent animals from entering (1,5,7).

The interior of the building should be constructed in a manner that facilitates cleaning. Floors should be smooth so that any soil or food particles may be easily dislodged. In addition, drains should be present approximately every 3 meters so that water may be easily removed (1,4). Processing plants need to be well ventilated as the area may become very humid due to steam produced while processing and because large quantities of water are regularly used while cleaning. Poorly ventilated areas may facilitate microorganism growth resulting in an unsanitary and odorous plant (1,5,7).

Processing equipment used in a food plant should be designed in a manner that facilitates cleaning and sanitizing (4). Difficult to clean equipment should be disassembled to facilitate removal of debris. Improperly cleaned equipment will harbor microorganisms and serve as a reservoir of contamination of the plant. Small parts, chains, cutters, grinders, and conveyor belts are examples of equipment that tend to be difficult to clean, and as a result are conducive to biofilm formation. Despite claims by detergent manufacturers, difficult to clean equipment must be taken apart and the individual pieces must be scrubbed. Gels and foams are not abrasive, and as a result will not remove adhered material from equipment. Detergents with silicates are somewhat abrasive, but without scrubbing, they are not abrasive enough to remove food particles and biofilms (1,9,11).

When cleaning, gross dirt must be removed first. This can be done by using air jets or vacuums, by sweeping, or spraying with low-pressure water hoses. Compressed air or vacuums are good options for removing dry material. Use of compressed air is usually done at the beginning of the cleaning session as the material may be blown onto surrounding equipment. Vacuums are especially useful in storage areas, where excess humidity is undesirable. At this point, any water used should be cold as hot water is more expensive. In addition, hot water may gel or bake on food particles, making them even more difficult to remove (1,4).

While high-pressure jets can efficiently remove dirt, if used incorrectly they can simply spread loose dirt around. The resultant spray may also disseminate microbes throughout the plant in aerosols. These aerosols can also condensate on ceilings, walls, and overhanging structures, which can serve as harborages of microorganisms. For loose dirt, low-pressure sprayers and brooms are recommended. High-pressure sprayers are better recommended for difficult to reach areas and dirt that is stuck on surfaces. Keep in mind that high-pressure sprayers are more likely to have strong recoil and the water is likely to splash on the operator (1,2,3).

Cleaning should be carried out in planned stages. When planning, the operators should take into account the order in which the equipment and plant areas are being cleaned in order to prevent spreading dirt or dirty water onto surfaces that have been previously cleaned. Cleaning should be carried out in a top down manner, from the highest level of the equipment moving down towards the floor. The floors should be the last surface cleaned (1).

Cleaning equipment, including, brushes, brooms, buckets and hoses must also be cleaned after each use to prevent them from becoming sources of plant contamination. They must be washed and allowed to dry before storage. Hoses should be drained before storage. Processing plants normally have suspended hoses for use on the processing floor, and use portable hoses for cleaning the exterior of the plant or the non-processing areas of the plant. These should not be introduced into the plant, as they may have picked up dirt and microorganisms. All the cleaning and sanitizing equipment must be maintained in proper working order, and must be replaced when they start showing wear, as these old brushes, frayed brooms, and broken hoses, for example, will be less effective and will make the operators less efficient (1).

C. Cleaning Compounds

1. Detergents

An ideal detergent is water-soluble and will not leave deposits on cleaned surfaces. A good detergent is non-corrosive and should assist in conditioning (softening) the water. It should also be able to penetrate the surface of soils in order to remove them more effectively. The ideal detergent should also be able to emulsify fats and break the bonds between soil and the surface. In addition, it must be easily rinsed off after use (1). As yet, no single detergent has all the listed properties, so mixtures of detergents and other compounds are used. Most of the detergents used in food plant applications are synthetic detergents, which usually will not react with minerals in hard water.

2. Alkaline Cleaners

Alkaline cleaners have a pH above 7 and are used primarily for their abilities to dissolve protein-based soils and emulsify fats (1,3). Unfortunately, alkaline cleaners also tend to

be corrosive to metals and skin, and will also corrode glass if heated. In addition, they may also leave a film on surfaces. There are three different types of alkaline cleaners, strong, heavy duty, and mild (3).

Strong alkaline cleaners have strong dissolving properties, but are corrosive and can burn skin. In addition, the fumes they release may cause respiratory problems. These cleaners may precipitate calcium and magnesium from hard water, thereby depositing scale on the equipment surfaces. Strong alkaline cleaners are used to remove heavy, mixed soils such as those deposited on walls and equipment of smoke houses. These soils may consist of fats, sugars, and proteins and as a result require a cleaner that has strong penetrating and emulsifying properties. Examples include sodium hydroxide, sodium orthosilicate, and sodium sesquisilicate (1,2,3).

Heavy-duty alkaline cleaners are less corrosive than strong alkaline cleaners; however, prolonged exposure to skin may cause irritation. These are usually used in conjunction with high pressure cleaning. Examples include sodium carbonate and sodium metasilicate (1,2,3).

Mild alkaline cleaners are usually used for manual cleaning of equipment. These cleaners are general purpose and can be used on any surface. Examples include sodium bicarbonate and tetrasodium pyrophosphate (1,2,3).

3. Acid Cleaners

Acid cleaners are not as commonly used as alkaline cleaners. They are mainly used to remove mineral buildup (scale) and other encrusted deposits on surfaces. These cleaners are naturally corrosive, but low-corrosion formulations have been created for plant use (1). The acid cleaners react chemically with mineral deposits, making them soluble and easily removable. These chemicals are extremely corrosive and are not used as all-purpose cleaners. They are not very effective against lipid and protein soils. Their cleaning activity is mainly limited to removal of mineral and alkaline deposits (1,2,3). The most commonly used acid cleaners are hydrochloric (muriatic) acid, nitric acid, phosphoric acid, sulfuric acid, and sulfamic acid. Sulfamic acid is distributed in crystalline form and is therefore easier to transport and easier to mix with powdered detergents.

Strong acid cleaners are corrosive to most metals and even concrete. When heated they release toxic fumes. In addition, if the acid solution is at too high of a temperature, the solution will redeposit the minerals onto the equipment surfaces. Therefore, use of these cleansers has to be done in a strictly controlled manner. Examples of strong acids include hydrochloric acid, sulfuric acid, and sulfamic acid. Phosphoric acid, which is also classified as a strong acid cleaner, is the least corrosive of the strong acid cleaners (1,2,3).

Mild acid cleaners are only mildly corrosive. Examples include acetic acid, gluconic acid, and hydroxyacetic acid. These can be used in manual applications but tend to be of higher cost than the strong acid cleaners. As a result they are less popular than the strong acids.

4. Chelating Agents

Chelating agents are also called sequestrants. These additives prevent minerals from being deposited on equipment surfaces and forming scale. Chelators act by binding mineral ions to prevent precipitation and adherence to equipment surfaces. Commonly used chelators include polyphosphates or amine derivatives. Examples include ethylenediaminetetraacetic acid (EDTA), sodium acid pyrophosphate, and sodium tripolyphosphate. EDTA is more effective than the polyphosphates (1,2,3).

5. Surfactants

Surfactants are used to improve the wetting and penetrating abilities of a detergent by reducing the surface tension between water molecules and soils. Surfactants are also able to emulsify lipids with water, which results in easier removal of fats from surfaces.

Surfactants usually dissociate in solution to give positively charged ions and negatively charged ions. There are three types of surfactants, negatively charged anionic, positively charged cationic, and amphoteric in which the most active ion is determined by the pH of the solution. If the solution is alkaline, an amphoteric surfactant will behave anionically. If the solution is acidic, the surfactant will behave cationically.

Anionic surfactants are usually high foaming cleaners and are affected by water hardness. Common anionic surfactants include sodium and potassium salts of fatty acids, and sodium, potassium and ammonium sulfates, sulfonates, and sulfosuccinates. Cationic surfactants are not affected by water hardness and usually have bactericidal properties. These two advantages make cationic surfactants the most commonly used surfactants.

Surfactants that do not dissociate in solution are called non-ionic. They are not affected by water hardness. These are low foaming and can be combined with other surfactants (1,2,3).

6. Abrasive Agents

Abrasive agents are normally inert materials that are added to detergents to physically scour equipment surfaces. Abrasives can scratch smooth surfaces of stainless steel equipment leaving pockets where biofilms or miniscule food particles can accumulate. In addition, they can corrode surfaces over time. Abrasive use should be carefully monitored. Examples of abrasives include borax, volcanic ash, pumice, and feldspar. They are available in powder or paste forms and are usually used to remove baked on or encrusted material. They may also be used to

remove mineral scale in areas where the use of acid cleaners is undesirable (1,2,3).

IV. SANITIZING

Once surfaces have been cleaned they are ready for a sanitization step. The purpose of sanitization is to kill any residual microbes that may be present on surfaces. Because the efficacy of many sanitizers is affected by soils, properly cleaned surfaces will be more readily sanitized than dirty surfaces.

A. Heat

Sanitizing using heat is the oldest method used. Unfortunately heat is economically inefficient because of substantial energy requirements for production of hot water or steam. Sanitation by heat should not be carried out on surfaces that are not heat tolerant. Heat also should not be used on sectors of equipment that are lubricated as steam can melt grease. In addition, high temperatures can cause scale deposition. The most common heat sanitizing methods use steam and hot water (14).

1. Steam

Steam is not recommended for sanitizing because operators frequently mistake steam for water vapor, resulting in high probability for worker injury. In addition, steam can condense on surfaces and provide a water source for microbe proliferation (14). Water droplets from condensed steam can help spread microorganisms on food contact surfaces. This may only be a problem in plants that are inadequately ventilated. Guthrie (2) recommends steam, citing that the high temperature allows the surfaces to dry quickly.

2. Hot Water

Hot water (76°–85°C) is frequently used as a sanitizing rinse. This is a non-selective sanitizing method. The temperature of the water needs to be maintained; as a result one cannot simply pour hot water over equipment. Hot water sanitization requires the use of thermostat-controlled tanks, which will circulate the water and maintain the desired temperature (2,5,14).

3. Time-Temperature Relationships

Since heat historically has been the most widely used method of killing microorganisms, several studies have been carried out to determine the most advantageous heat treatment. It has been established that the temperature of the heat source determines the time of exposure required to sanitize a surface (14). For example, equivalent treatments can be achieved using hot water at 85°C for 15 minutes or 80°C for 20 minutes. According to Guthrie (1988), steam is more efficient at killing microorganisms than hot water because of its higher temperature. This reduces the time required to sterilize equipment making the sanitation process quicker and also reduces deterioration of equipment, as it is exposed to high temperatures for less time than it would be if hot water was used (2,5,14).

B. Chemicals

Chemical sanitizers are usually more desirable than thermal sanitizers because they reduce the likelihood of burns and require less labor and less energy input. Chemical sanitizers have various modes of activity and each is appropriate for different situations. Chemical sanitizers do not penetrate below the surfaces of the soil and as a result will only sanitize the exterior. The effectiveness of chemical sanitizers depends primarily on the initial microbial load, the exposure time, and the sanitizer concentration (3,14).

1. Chlorine

Hypochlorite solutions are the most common and most effective chemical disinfectants. Other common sources of chlorine are potassium chloride, trichlorisocycamuric acid, and dichloroisocycamuric acid. The latter two are available in granulated form in concentrations of up to 60% available chlorine. Sodium and potassium chloride are usually only available in liquid forms. Commercial solutions are available with any of these formulations in concentrations of up to 12% available chlorine. In contrast, solutions for household use are only available with up to 6.0% sodium hypochlorite. Solutions with 200 ppm or less of free chlorine do not have to be rinsed. Chlorine compounds are desirable because they can kill a wide variety of microorganisms and because they can be used in concentrations that do not need to be rinsed, which cuts down on cleaning time.

One disadvantage of chlorine is that it corrodes metal. In addition, chlorine reacts with all proteinaceous material resulting in inactivation by organic material present on surfaces. Because of this, all surfaces should be cleaned before chlorine sanitizing is attempted. Chlorine also tends to lose strength in storage and should not be purchased in amounts larger than will be used within a few months. Storage conditions will determine how quickly the chlorine will become inactivated. Temperatures above 60°C and excess light will hasten deterioration (2,3,4,14).

2. Iodine Compounds

Iodine is effective against a wide variety of microorganisms. However, iodine is of limited use in the food industry because it is extremely corrosive, toxic, and stains equipment and food. Instead, iodophors, which are iodine in

complex with a nonionic surface-active agent, are more useful in the food industry. Iodophors are less irritating than chlorine compounds and are mostly employed as skin sanitizers or in solutions used for manual washing of equipment. Iodophors are also able to remove scale build-up on equipment.

Iodophors are available in solution with surfactants and acids such as phosphoric acid. This gives them detergent properties, which allows them to be used as a sanitizing cleaning agents. Iodophors are usually used in concentrations of up to 50–70 mg/L free iodine. They have higher bactericidal activity in acid conditions and are more stable than chlorine in the presence of organic matter. However, they are more expensive than chlorine solutions and when heated above 43°C will release free iodine resulting in the negative characteristics of iodine (2,3,4,14).

3. Quaternary Ammonium Compounds

These compounds have a different mode of action than other sanitizing compounds in that they form a bacteriostatic film on surfaces. As a result, their use is usually limited to physical plant surfaces such as floors and walls, but not processing equipment. In addition, they penetrate surfaces and are very effective for porous surfaces. Quaternary ammonium compounds (QACs) are only mildly corrosive, non-irritating, and of low toxicity. They are less susceptible to inactivation by organic matter than chlorine and iodine based sanitizers. They are, however, easily inactivated by the presence of detergents (2,3,4,14).

4. Peroxyacetic Acid

This type of sanitizer is popular because it can combine an acid cleaning rinse with sanitization in one step. It is a rapid acting, broad-spectrum sanitizer. Despite being acid based, it is less corrosive than iodine. The normal, application rate is 125–250 ppm. The disadvantages of peroxyacetic acid are that its effectiveness is reduced in the presence of organic material and it is expensive. However, it is useful for automated cleaning equipment, because it is low foaming and heat stable, pH tolerant, and effective against biofilms (2,3,4,14).

V. DESIGNING CLEANING AND SANITIZING ROUTINES

A. Sanitation Standard Operating Procedures

Sanitation should not be delegated to the employee who has nothing else to do, as it requires dedication and knowledge of what needs to be done and why it needs to be done (4). The sanitary engineer or the sanitation department is responsible for the development and maintenance of a sanitation program. In addition, they must secure the support and compliance of the management and employees (3,4).

Sanitation Standard Operating Procedures (SSOPs) are closely linked to GMPs as they both are guidelines on how to maintain sanitary plant conditions. SSOPs describe the specific steps required to clean different equipment or areas of the plant. They cover the preoperational and operational procedures that must be followed to prevent contamination of the food products. In addition, SSOPs should specify: 1) the frequency and method for cleaning, 2) the frequency and method for sanitization, 3) the cleaners/disinfectants to be used, 4) the specific equipment that will make the process more efficient and effective, and 5) the employee responsible for cleaning and sanitation (3,4,7).

B. Designing Schedules

The most efficient sanitation program is accomplished by establishing a routine that is preventative (2). Having a designed schedule facilitates cleaning and avoids problems or downtime. The workers are aware of what their assignments are and what equipment they will need, which makes cleaning more efficient. Schedules should show who has been designated for each specific job. In addition, these schedules should also have a space for the workers signature. This can serve as proof that the particular assignment was carried out. Schedules should be prepared for every cleaning and sanitizing task in the processing plant (2,3).

C. Stop Production and Continuous Production Processing Plants

Stop production plants are those that process for a specific number of hours daily. In these plants all the equipment is cleaned and sanitized after the last shift. Continuous production plants process 24 hours a day, 7 days a week. There is no time when the plant is shut down for cleaning and maintenance. In these situations, a carefully constructed cleaning and sanitation schedule must be prepared and rigorously adhered to on a daily basis. In continuous plants, the preferred cleaning methods are those that will reduce downtime. Thus, use of sanitizing detergents, acid sanitizers, and sanitizers that do not need to be rinsed are widely used (2).

D. Cleaning out of Place and Cleaning in Place

Cleaning out of place (COP) is the traditional cleaning method. Equipment is dismantled and the parts are washed. First, gross pieces of dirt are removed using brushes. Then the pieces are manually cleaned using water, detergent, and brushes to scrub the parts. Scrubbing may

also be carried out in tanks or vats — this saves water, but the operators also have to be aware to regularly change the water. Then the parts are rinsed with hot water (76°C–85°C), which would rinse, sanitize, and help the parts dry quickly. Parts can also be rinsed with cold water first then immersed in sanitizers. Concentrations of sanitizers to be used should be carefully monitored using test kits or a Bactometer as sanitizer effectiveness is dependent on concentration (15). In addition some sanitizers degrade with time, with exposure to light or exposure to organic material (15). Some sanitizers may need to be rinsed off. This should be done with hot water if possible to facilitate drying. If not, then special areas of the plant will have to be designated for drying. Drying ovens, which circulate warm dry air, are useful for quickly drying equipment or utensils. Drying cloths are not recommended as they can easily contaminate the equipment if dirty (3,14).

Small parts can also be washed using commercial dishwashers, which usually have a sanitizing cycle. If the dishwasher does not sanitize, then the parts must be manually sanitized as described above. Circulation cleaners may also be used to wash parts. These are tanks that circulate detergent solutions. The cleaning solutions in circulation tanks are usually held at higher temperatures than for manual cleaning. In addition more caustic detergents may be used, as no employees will actually be handling the solution. This has to be followed by manual rinsing and sanitizing (2,3).

Cleaning in place (CIP) is used more frequently now. In this system, a tank containing detergent solution is connected to the processing equipment, and the solution is piped into the equipment lines and pumped through. The proper concentration of chemicals, the required temperature, and the pressure must be maintained in order to effectively clean using CIP (14). CIP can reduce downtime and requires less man-hours than COP as the equipment does not have to be taken apart. The drawback, however, is that it is initially expensive to refit existing equipment to CIP (1,2,3,14).

VI. SUMMARY

Sanitation programs, no matter how well designed, are only as good as the operators who implement them. As a result, regular testing of cleaned and sanitized equipment is recommended. Common tests include the use of sterile swabs, replicate organism detection and counting plates (Rodac), and ATP bioluminescence tests. Testing with swabs simply requires dipping a sterile swab in a sterile buffer solution and swabbing the test area. The swab is then returned to the buffer solution and swirled to deposit bacteria into the solution, and the buffer solution is plated and incubated. Rodac plates are surface contact plates that are simply pressed against the area being tested, then incubated. Plate counts are used as a measure of sanitation, for both the swab tests and the rodac tests. ATP bioluminescence test measures adenosine triphosphate (ATP), which is the energy store in living cells. When ATP comes into contact with luciferin, luciferase catalyzes a chemical reaction that produces light. ATP bioluminescence tests will measure the amount of light produced, which is directly related to the amount of ATP present, which in turn is related to the amount of cellular debris on surfaces. All these testing methods can be used to evaluate the sanitation operators, but can also be used when a plant adopts new sanitation procedures or equipment. Sanitation programs are never complete. Managers must constantly look for ways to improve their sanitation program and make it more efficient.

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CONTENTS

I. GENERAL CONSIDERATIONS

The information in this chapter have been derived from the following sources:

1. Food and Drug Administration (FDA) documents: Code of Federal Regulations, Current Good Manufacturing Practices (CGMPs), the Food Code, Hazard Analysis and Critical Control Point (HACCP) programs;
2. Department of Agriculture (USDA) documents: Code of Federal Regulations, HACCP programs, inspection manuals, directives, etc.;
3. Recommendations developed and distributed by major trade associations representing food, warehousing, and transport and related industries.

Food processing equipment used in all food plants (meat and non-meat) is "predetermined" under usual circumstances:

1. FDA and USDA mandatory specifications. Most of those used in processing low acid (acidified, etc.) foods in hermetically sealed containers (thermal processing) and meat and poultry products are directly regulated by regulations promulgated by the FDA and USDA, in relation to safety and sanitation.
2. New establishments. Equipment intended for use in newly constructed establishments should take into consideration all aspects of good manufacturing practices before their construction or purchases.
3. Most specialty equipment (for bakery, dairy, pasta, oil, etc.) is under voluntary/mandatory requirements established and distributed by trade associations such as the American Institute of Baking, Dairy and Food Industries Supply Association, American Oil Chemical Society, and the National Sanitation Foundation. Again, such equipment is in compliance with FDA/USDA GMP provided that all instructions relating to maintenance and repair are adhered to.
4. With built-in designs to comply with FDA/USDA GMP, this equipment saves food processors time and money in looking for the "right" equipment.
5. Custom made equipment. Plant personnel may build their own equipment or have an outside contractor fabricate equipment for them. Even though it is custom made and not intended for resale, such equipment should be built to comply with good manufacturing practices. The same standards are applicable to custom made equipment as are applicable to commercially available equipment.
6. Many food processing plants require some equipment that is custom made for particular operational requirements. It may not be possible to comply with certain good manufacturing practices in the same way as other conventional equipment. If so, it is always advisable to inform the appropriate state and federal regulators of the circumstances.
7. All other food processing equipment that is commercially available.

The bottom line is that all food processing equipment, no matter how they are "predetermined," must comply with FDA, USDA and State GMP.

Some equipment manufacturers or brokers are sometimes not interested in complying with FDA/USDA GMP. In such events, equipment is considered the same as custom made and food establishment operators should be aware of this responsibility before they purchase any equipment.

All food processors using a variety of equipment, especially custom made ones, should focus on correcting problems during the initial development of equipment instead of resolving problems which may result when improperly designed or constructed equipment is put into widespread use. This preventive mode of action benefits equipment manufacturers, food processors, state and federal regulators, and American consumers.

In general, the following basic and standard equipment is usually considered to comply with good manufacturing practice or their compliance is of minor significance:

1. Simple hand tools.
2. Equipment used to prepare packaging materials.
3. Equipment used on fully packaged product.
4. Equipment used on operations involving inedible products that will not be mixed with edible ones.
5. Central cleaning system.
6. Utensil and equipment cleaning machinery.
7. Pails, buckets, etc.
8. Pallets for packaged product.
9. Picking fingers.
10. Tanks for fully finished oils.
11. Simple can openers.
12. Chutes, flumes, hangback racks, supporting stands, and brackets.
13. Vegetable cleaning equipment (not applicable to spin type washers/dryers.)
14. Insect control units.
15. Shipping containers.
16. Pressure storage vessels for refrigerants (not applicable to CO_2 snow making equipment).
17. Water softeners, water heaters, water meters, and chemical dispensers.
18. Can and jar washers/cleaners.
19. Mixing equipment.
20. Hot air shrink tunnels.

21. Air and water filters.
22. Devices for measuring physical characteristics (temperature, pressure, etc.).
23. Rubber floor mats.

The product contact area of this equipment must be made up of approved materials.

II. MATERIALS FOR CONSTRUCTION AND REPAIR

A. Characteristics

Materials that are used in the construction of utensils and food-contact surfaces of equipment may not allow the migration of deleterious substances or impart colors, odors, or tastes to food and under normal use conditions should be:

1. Safe;
2. Durable, corrosion-resistant, and nonabsorbent;
3. Sufficient in weight and thickness to withstand repeated warewashing;
4. Finished to have a smooth, easily cleanable surface; and
5. Resistant to pitting, chipping, crazing, scratching, scoring, distortion, and decomposition.

Multi-use equipment is subject to deterioration because of its nature, i.e., intended use over an extended period of time. Certain materials allow harmful chemicals to be transferred to the food being prepared which could lead to foodborne illness. In addition, some materials can affect the taste of the food being prepared. Surfaces that are unable to be routinely cleaned and sanitized because of the materials used could harbor foodborne pathogens. Deterioration of the surfaces of equipment such as pitting may inhibit adequate cleaning of the surfaces of equipment, so that food prepared on or in the equipment becomes contaminated. Inability to effectively wash, rinse and sanitize the surfaces of food equipment may lead to the buildup of pathogenic organisms transmissible through food. Studies regarding the rigor required to remove biofilms from smooth surfaces highlight the need for materials of optimal quality in multi-use equipment.

It must be emphasized that each food processing operation is unique which applies to the equipment used. The acceptability and non-acceptability of any food processing equipment or its component materials occasionally depends on the operation itself. This must be taken into consideration when evaluating an item of equipment in relation to the GMP.

B. Cast Iron, Use Limitation

1. In general, cast iron may not be used for utensils or food-contact surfaces of equipment.
2. It may be used as a surface for cooking.
3. It may be used in utensils for serving food if the utensils are used only as part of an uninterrupted process from cooking through service.

Cast iron is an alloy of iron and heavy metals which may leach into food if left in contact with acidic foods for extended periods of time. Heavy metal poisoning has resulted from such situations. The temporary or incidental contact that results from using cast iron as a cooking surface and for dispensing utensils used as part of an uninterrupted, short-term process is acceptable because of the brief contact time involved.

C. Some Acceptable Materials

Equipment should be constructed of materials that will not deteriorate from normal use under the anticipated environment. For example, equipment must be constructed of materials that will withstand one category of environment, e.g., generally humid operating environment and high pressure, hot water cleaning with strong chemical cleaning agents. Of course, there are other categories of food processing environments. In addition, all equipment surfaces should be smooth; corrosion and abrasion resistant; shatterproof; nontoxic; non-absorbent; and not capable of migrating into food product (staining). The following lists some acceptable food processing equipment and/or their component materials.

1. The Series 300 (18-8) Stainless Steel

The Series 300 (18-8) stainless steel is acceptable for general use. Other series have been used for construction of food equipment, but their use is limited because they tend to rust or discolor in certain applications. The abbreviation “S/S” is used throughout this publication to denote stainless steel construction.

2. Aluminum

Aluminum may pit and corrode when exposed to certain chemicals. When friction occurs between aluminum and fats a black oxide is produced which discolors the product. Anodizing the aluminum does not eliminate this problem. Therefore, the use of aluminum is limited to applications where the metal does not contact the product or in which the product is suspended in water.

3. Surface Coatings and Platings

Surface coatings and platings may be used if the base material is non-toxic and rendered non-corrosive and the plating material is USDA/FDA acceptable. Chrome, nickel, tin, and zinc (galvanization) platings will generally be acceptable for most appropriate applications. USDA/FDA clearance of other plating materials and processes can he obtained by

receiving a favorable opinion for the intended use from the FDA, Office of Premarket Approval. Surface coatings and platings must remain intact. If a surface coating or plating begins to peel or crack, the FDA/USDA inspection will request correction from the management and may even disallow the use of the equipment.

4. Hardwood

Hardwood may be used for dry curing. In addition, solid (unlaminated) pieces of hardwood are acceptable as removable cutting boards provided the wood is maintained in a smooth, sound condition and is free from cracks. Hardwood cutting boards must be of the shortest dimension which is practical preferably not exceeding 3 or 4 feet (.91 or 1.22m.).

D. Some Unacceptable Materials

Cadmium. antimony. and lead are toxic materials that cannot be used as materials of construction either as a plating or the plated base material. Lead, however, may be used in acceptable alloys in an amount not exceeding 5%.

Enamelware and porcelain are not acceptable for handling and processing food product unless a food plant management provides reasons why they are needed.

Copper, bronze, and brass are not acceptable for direct product contact. These materials may be used in air and water lines or for gears and bushings outside the product zone. Brass is acceptable for potable water systems and direct contact with brine, but not for brine, or any solution, that is recirculated.

Leather and fabric are not acceptable materials unless a food plant management provides reasons why they are needed.

E. Nonfood-Contact Surfaces

Nonfood-contact surfaces of equipment that are exposed to splash, spillage, or other food soiling or that require frequent cleaning should be constructed of a corrosion-resistant, nonabsorbent, and smooth material.

Nonfood-contact surfaces of equipment routinely exposed to splash or food debris are required to be constructed of nonabsorbent materials to facilitate cleaning. Equipment that is easily cleaned minimizes the presence of pathogenic organisms, moisture, and debris and deters the attraction of rodents and insects.

III. DESIGN AND CONSTRUCTION

A. Durability and Strength

1. Equipment and Utensils

Equipment and utensils should be designed and constructed to be durable and to retain their characteristic qualities under normal use conditions.

Equipment should be designed so that all product contact surfaces can be readily and thoroughly cleaned with high temperature, high pressure water and caustic soap solution. Components such as electric motors, electric components, etc., which cannot be cleaned in this manner should be completely enclosed and sealed. Other considerations are:

a. All product contact surfaces should be visible (or easily made visible) for inspection.
b. All product contact surfaces should be smooth and maintained free of pits, crevices, and scale.
c. The product zone should be free of recesses; open seams; gaps; protruding ledges; inside threads; inside shoulders; bolts; rivets; and dead ends.
d. Bearings (including greaseless bearings) should not be located in or above the product zone. In addition, bearings should be constructed so that lubricants will not leak or drip or be forced into the product zone.
e. Internal corners or angles in the product zone should have a smooth and continuous radius of 1/4 inch (6.35 mm.) or greater. (Lesser radii may be used for proper functioning of parts or to facilitate drainage provided these areas can be readily cleaned).
f. Equipment should be self-draining or designed to be evacuated of water.
g. Framework of equipment (if not completely enclosed and sealed) should be designed to use as few horizontal frame members as possible. Furthermore, these components should be rounded or of tubular construction. Angle is not acceptable except as motor supports.
h. Equipment should be designed, constructed, and installed in a manner to protect personnel from safety hazards such as sharp edges, moving parts, electric shocks, excessive noise, and any other hazards. Safety guards should be removable for cleaning and inspection purposes.
i. All welds, in both product and non-product contact areas, should be smooth, continuous, even, and relatively flush with the adjacent surfaces.
j. Equipment should not be painted on areas which are in or above the product zone.
k. External surfaces should not have open seams, gaps, crevices, and inaccessible recesses.
l. Where parts must be retained by nuts or bolts, fixed studs with wing nuts should be used instead of screws to a tapped hole.
m. Gasketing, packing materials, 0-rings, etc., must be nontoxic, nonporous, nonabsorbent, and unaffected by food products and cleaning compounds.

Equipment and utensils must be designed and constructed to be durable and capable of retaining their original characteristics so that such items can continue to fulfill their intended purpose for the duration of their life expectancy and to maintain their easy cleanability. If they can not maintain their original characteristics, they may become difficult to clean, allowing for the harborage of pathogenic microorganisms, insects, and rodents. Equipment and utensils must be designed and constructed so that parts do not break and end up in food as foreign objects or present injury hazards to consumers. A common example of presenting an injury hazard is the tendency for tines of poorly designed single service forks to break during use.

2. Food Temperature Measuring Devices

Food temperature measuring devices may not have sensors or stems constructed of glass, except that thermometers with glass sensors or stems that are encased in a shatterproof coating such as candy thermometers may be used.

Food temperature measuring devices that have glass sensors or stems present a likelihood that glass will end up in food as a foreign object and create an injury hazard to the consumer. In addition, the contents of the temperature measuring device, e.g. mercury, may contaminate food or utensils.

B. Cleanability

1. Food-Contact Surfaces

Multi-use food-contact surfaces should be:

a. Smooth;
b. Free of breaks, open seams, cracks, chips, pits, and similar imperfections;
c. Free of sharp internal angles, corners, and crevices;
d. Finished to have smooth welds and joints; and
e. Accessible for cleaning and inspection by one of the following methods:
    i. Without being disassembled,
    ii. By disassembling without the use of tools, or
    iii. By easy disassembling with the use of only simple tools such as mallets, screwdrivers, or wrenches that are kept near the equipment and are accessible for use.

The purpose of the requirements for multi-use food-contact surfaces is to assure that such surfaces are capable of being easily cleaned and accessible for cleaning. Food-contact surfaces that do not meet these requirements provide a potential harbor for foodborne pathogenic organisms. Surfaces which have imperfections such as cracks, chips, or pits allow microorganisms to attach and form biofilms. Once established, these biofilms can release pathogens to food. Biofilms are highly resistant to cleaning and sanitizing efforts. The requirement for easy disassembly recognizes the reluctance of food employees to disassemble and clean equipment if the task is difficult or requires the use of special, complicated tools.

2. Clean-In-Place (CIP) Systems

Clean-In-Place or CIP is defined as follows:

a. "CIP" means cleaned in place by the circulation or flowing by mechanical means through a piping system of a detergent solution, water rinse, and sanitizing solution onto or over equipment surfaces that require cleaning, such as the method used, in part, to clean and sanitize a frozen dessert machine.
b. "CIP" does not include the cleaning of equipment such as band saws, slicers or mixers that are subjected to in-place manual cleaning without the use of a CIP system.

Sanitation procedures for CIP systems must be as effective as those for cleaning and sanitizing disassembled equipment. Only equipment that meets the following criteria may be cleaned in place. Any equipment or portions of equipment not meeting these requirements should be disassembled for daily cleaning and inspection.

a. CIP equipment should meet the characteristics as specified.
b. Cleaning solutions, sanitizing solutions, and rinse water should circulate throughout a fixed system and contact all interior surfaces of the system.
c. All internal surfaces should be either designed for self draining (of cleaning and sanitizing solutions) or physically disassembled for draining after rinsing. CIP equipment that is not designed to be disassembled for cleaning should be designed with inspection access points to assure that all interior food-contact surfaces throughout the fixed system are being effectively cleaned.
d. Pipe interiors should be highly polished (120–180 grit abrasive) stainless steel or some other acceptable, smooth surfaced material which is easy to inspect.
e. Easily removable elbows with quick disconnect mechanisms should be located at each change of direction.
f. All sections of the system should be capable of being completely disassembled for periodic inspection of all internal surfaces.

g. All sections should be available for inspection without posing any safety hazard to the inspector.

Certain types of equipment are designed to be cleaned in place (CIP) where it is difficult or impractical to disassemble the equipment for cleaning. Because of the closed nature of the system, CIP cleaning must be monitored via access points to assure that cleaning has been effective throughout the system.

The CIP design must assure that all food-contact surfaces of the equipment are contacted by the circulating cleaning and sanitizing solutions. Dead spots in the system, i.e., areas which are not contacted by the cleaning and sanitizing solutions, could result in the buildup of food debris and growth of pathogenic microorganisms. There is equal concern that cleaning and sanitizing solutions might be retained in the system, which may result in the inadvertent adulteration of food. Therefore, the CIP system must be self-draining.

3. "V" Threads Use Limitation

"V" type threads may not be used on food-contact surfaces. This section does not apply to hot oil cooking or filtering equipment.

V-type threads present a surface which is difficult to clean routinely; therefore, they are not allowed on food-contact surfaces. The exception provided for hot oil cooking fryers and filtering systems is based on the high temperatures that are used in this equipment. The high temperature in effect sterilizes the equipment, including debris in the "V" threads.

4. Hot Oil Filtering Equipment

Hot oil filtering equipment should meet the characteristics of cleanability and should be readily accessible for filter replacement and cleaning of the filter.

To facilitate and assure effective cleaning of this equipment, cleanability requirements must be followed. The filter is designed to keep the oil free of undesired materials and therefore must be readily accessible for replacement. Filtering the oil reduces the likelihood that off-odors, tastes, and possibly toxic compounds may be imparted to food as a result of debris buildup. To assure that filtering occurs, it is necessary for the filter to be accessible for replacement.

5. Can Openers

Cutting or piercing parts of can openers should be readily removable for cleaning and for replacement.

Once can openers become pitted or the surface in any way becomes uncleanable, they must be replaced because they can no longer be adequately cleaned and sanitized. Can openers must be designed to facilitate replacement.

6. Nonfood-Contact Surfaces

Nonfood-contact surfaces should be free of unnecessary ledges, projections, and crevices, and designed and constructed to allow easy cleaning and to facilitate maintenance.

Hard-to-clean areas could result in the attraction and harborage of insects and rodents and allow the growth of foodborne pathogenic microorganisms. Well-designed equipment enhances the ability to keep nonfood-contact surfaces clean.

7. Kick Plates, Removable

Kick plates should be designed so that the areas behind them are accessible for inspection and cleaning by being:

a. Removable by one of the methods specified under cleanability or capable of being rotated open; and
b. Removable or capable of being rotated open without unlocking equipment doors.

The use of kick plates is required to allow access for proper cleaning. If kick plate design and installation does not meet the above requirements, debris could accumulate and create a situation that may attract insects and rodents.

C. Accuracy

1. Food Temperature Measuring Devices

a. Food temperature measuring devices that are scaled only in Celsius or dually scaled in Celsius and Fahrenheit should be accurate to ±1°C (1.8°F).
b. Food temperature measuring devices that are scaled only in Fahrenheit should be accurate to ±2°F.

The Metric Conversion Act of 1975 (amended 1988) requires that all federal government regulations use the Celsius scale for temperature measurement. The Fahrenheit scale is included here for all other sectors of the country using Fahrenheit equivalents. The Fahrenheit equivalent will also help those jurisdictions that require Celsius readings to make the transition from Fahrenheit. Since 1°C is equivalent to approximately 2°F (1.8°F), an accuracy of ± 1°C is required.

The small margin of error specified for thermometer accuracy is due to the lack of a large safety margin in the temperature requirements themselves. The requirement for Fahrenheit thermometers to be accurate to 2°F is due to the lack of 1° increment scaling in Fahrenheit thermometers currently being used such as the metal stem thermometer.

2. Ambient Temperature Measuring Devices

a. Ambient temperature measuring devices that are scaled in Celsius or dually scaled in Celsius and Fahrenheit should be designed to be easily readable and accurate to ±1.5°C (2.7°F) at the use range.
b. Ambient temperature measuring devices that are scaled only in Fahrenheit should be accurate to ±3°F at the use range.

A temperature measuring device used to measure the air temperature in a refrigeration unit is not required to be as accurate as a food thermometer because the unit's temperature fluctuates with repeated opening and closing of the door and because accuracy in measuring internal food temperatures is of more significance.

The Celsius scale is the federally recognized scale based on The Metric Conversion Act of 1975 (amended 1988) which requires the use of metric values. The ±1.5°C requirement is more stringent than the 3°F previously required since ±1.5°C is equivalent to ±2.7°F. The more rigid accuracy results from the practical application of metric equivalents to the temperature gradations of Celsius thermometers.

If Fahrenheit thermometers are used, the 3°F requirement applies because of the calibrated intervals of Fahrenheit thermometers.

IV. FUNCTIONALITY, DESIGN AND CONSTRUCTION

A. Ventilation Hood Systems, Drip Prevention

Exhaust ventilation hood systems in food preparation and warewashing areas including components such as hoods, fans, guards, and ducting should be designed to prevent grease or condensation from draining or dripping onto food, equipment, utensils, linens, and single-service and single-use articles.

The dripping of grease or condensation onto food constitutes adulteration and may involve contamination of the food with pathogenic organisms. Equipment, utensils, linens, and single service and single use articles that are subjected to such drippage are no longer clean.

B. Equipment Openings, Closures and Deflectors

1. A cover or lid for equipment should overlap the opening and be sloped to drain.
2. An opening located within the top of a unit of equipment that is designed for use with a cover or lid should be flanged upward at least 5 millimeters (two-tenths of an inch).
3. Fixed piping, temperature measuring devices, rotary shafts, and other parts extending into equipment should be provided with a watertight joint at the point where the item enters the equipment. This assumes that a watertight joint is not provided:
4. If a watertight joint is not provided:
   a. The piping, temperature measuring devices, rotary shafts, and other parts extending through the openings should be equipped with an apron designed to deflect condensation, drips, and dust from food openings; and
   b. The opening should be flanged (see Item 2 above).

Equipment openings and covers must be designed to protect stored or prepared food from contaminants and foreign matter that may fall into the food. The requirement for an opening to be flanged upward and for the cover to overlap the opening and be sloped to drain prevents contaminants, especially liquids, from entering the food-contact area.

Some equipment may have parts that extend into the food-contact areas. If these parts are not provided with a watertight joint at the point of entry into the food-contact area, liquids may contaminate the food by adhering to shafts or other parts and running or dripping into the food.

An apron on parts extending into the food-contact area is an acceptable alternative to the watertight seal. If the apron is not properly designed and installed, condensation, drips, and dust may gain access to the food.

1. Bearings and Gear Boxes, Leakproof

Equipment containing bearings and gears that require lubricants should be designed and constructed so that the lubricant cannot leak, drip, or be forced into food or onto food-contact surfaces.

It is not unusual for food equipment to contain bearings and gears. Lubricants necessary for the operation of these types of equipment could contaminate food or food-contact surfaces if the equipment is not properly designed and constructed.

2. Condenser Unit, Separation

If a condenser unit is an integral component of equipment, the condenser unit should be separated from the food and food storage space by a dustproof barrier.

A dust-proof barrier between a condenser and food storage areas of equipment protects food and food-contact areas from contamination by dust that is accumulated and blown about as a result of the condenser's operation.

3. Temperature Measuring Devices

1. In a temperature regulated storage unit (cool for refrigerator or warm/hot for a storage room/equipment), the sensor of a temperature measuring device should be located to measure the air temperature in the warmest part of a mechanically refrigerated unit and in the coolest part of the storage unit.
2. Cold or hot holding equipment used for edible products should be equipped with at least one integral or permanently affixed temperature measuring device that is located to allow easy viewing of the device's temperature display. There are exceptions.
3. Item 2 does not apply to equipment for which the placement of a temperature measuring device is not a practical means for measuring the ambient air surrounding the edible product because of the design, type, and use of the equipment, such as calrod units, heat lamps, cold plates, bainmaries, steam tables, insulated food transport containers, and salad bars.
4. Temperature measuring devices should be designed to be easily readable.
5. Food temperature measuring devices should have a numerical scale, printed record, or digital readout in increments no greater than 1°C or 2°F.

The placement of the temperature measuring device is important. If the device is placed in the coldest location in the storage unit, it may not be representative of the temperature of the unit. Food could be stored in areas of the unit that exceed requirements. Therefore, the temperature measuring device must be placed in a location that is representative of the actual storage temperature of the unit to assure that all potentially hazardous foods are stored at least at the minimum temperature required for the specific foods.

A permanent temperature measuring device is required in any unit storing potentially hazardous food because of the potential growth of pathogenic microorganisms should the temperature of the unit exceed requirements. In order to facilitate routine monitoring of the unit, the device must be clearly visible.

The exception to requiring a temperature measuring device for the types of equipment listed is primarily due to equipment design and function. It would be difficult and impractical to permanently mount a temperature measuring device on the equipment listed. The futility of attempting to measure the temperature of unconfined air such as with heat lamps and, in some cases, the brief period of time the equipment is used for a given food negate the usefulness of ambient temperature monitoring at that point. In such cases, it would be more practical and accurate to measure the internal temperature of the food.

The importance of maintaining potentially hazardous foods at the specified temperatures requires that temperature measuring devices be easily readable. The inability to accurately read a thermometer could result in food being held at unsafe temperatures.

Temperature measuring devices must be appropriately scaled per stated requirements to assure accurate readings.

The required incremental gradations are more precise for food measuring devices than for those used to measure ambient temperature because of the significance at a given point in time, i.e., the potential for pathogenic growth, versus the unit's temperature. The food temperature will not necessarily match the ambient temperature of the storage unit; it will depend on many variables including the temperature of the food when it is placed in the unit, the temperature at which the unit is maintained, and the length of time the food is stored in the unit.

4. Case Lot Handling Equipment, Moveability

Equipment, such as dollies, pallets, racks, and skids used to store and transport large quantities of packaged foods received from a supplier in a cased or overwrapped lot, should be designed to be moved by hand or by conveniently available equipment such as hand trucks and forklifts.

Proper design of case lot handling equipment facilitates moving case lots for cleaning and for surveillance of insect or rodent activity.

V. EQUIPMENT AND WATER USAGE

A. Water Wasting Equipment

Water wasting equipment should be installed so that waste water is delivered into the drainage system through an interrupted connection without flowing over the floor, or is discharged into a properly drained curbed area. Waste water from cooking tanks, soaking tanks, chilling tanks, and other large vessels may be discharged for short distances across the floor to a drain after operations have ceased and all product has been removed from the area.

B. Protection of Water Supply

An air gap should be provided between the highest possible level of liquids in equipment and a directly connected water supply line(s). The air gap must be at least twice the diameter of the supply side orifice. If submerged lines are unavoidable due to design considerations, then the equipment must include a functional vacuum breaker which will, without fail, break the connection in the event of water pressure loss.

C. Re-circulation of Water

Equipment which re-circulates water as part of its intended function should be equipped with sanitary re-circulating

components if the water directly or indirectly contacts food product or the product contact surfaces. For examples, recirculating pumps should be accepted for direct product contact and piping must be easily demountable with quick disconnect mechanisms at each change of direction.

In addition, establishment operators using equipment or systems which re-use water may be required to have written approval of a water re-use procedure. However, the requirement is mandatory for meat and poultry processors by the USDA. Although the FDA does not require a written approval at this stage, its GMP regulations make it clear that there must be built-in safeguards in the reuse of water in a food plant.

D. Valves

Valves on drainage outlets should be easily demountable to the extent necessary for thorough cleaning. Overflow pipes should be constructed so that all internal and external surfaces can be thoroughly cleaned.

VI. PIPING SYSTEMS

Piping systems used to convey edible product (including pickle solutions) should be readily disassembled for cleaning and inspection. Pumps, valves, and other such components should comply with the sanitary requirements of good manufacturing practices promulgated by USDA/FDA. Piping systems must be designed so that product flow will be smooth and continuous, i.e., no traps or dead ends. Pipes must be either 300 series stainless steel or a USDA/FDA acceptable plastic.

Clear demountable rigid plastic piping may be used for two-way flow provided it is chemically and functionally acceptable. Opaque plastic piping may be used for one way purposes only.

The above requirements apply to systems for conveying raw fat and to re-circulate cooking and frying oils. Black iron pipes with threaded or welded joints are acceptable for conveying completely finished, rendered fats. Continuous rendering is not considered complete until after the final centrifuge.

Pipeline conveying systems for aseptic processing and packaging should comply with the requirements promulgated by the FDA and USDA in the U.S. Code of Federal Regulations.

VII. MAGNETIC TRAPS AND METAL DETECTORS

The extensive exposure of some products to metal equipment such as grinders, choppers, mixers, shovels, etc., causes the possibility of metal contamination. Magnetic traps have been found effective in removing iron particles from chopped or semi-liquid products. However, these magnetic traps are not useful for removing nonmagnetic metals such as stainless steel or aluminum. Therefore, the use of electronic metal detectors is highly recommended for sausage emulsions, can filling lines (especially baby foods), etc. Metal detectors are usually installed so an alarm (either a bell or light or both) is activated when a metal fragment is in the detection zone. The production line should stop automatically when the detector is activated. Alternatively, some systems are arranged so that the nant(s) is automatically removed from the production line.

The FDA and USDA do not currently regulate the use of metal detectors for normal production. The agencies do encourage food plant operators to voluntarily use metal detectors whenever possible. The agencies review and evaluate metal detectors using the same sanitary standards applied to other types of equipment.

The sensitivity and reliability of metal detectors varies depending on aperture size, type of food product, frequency and method of calibration, and numerous other variables. Since many of the involved factors are not related to the design of the unit itself, the agencies do not currently classify metal detectors. However, the following classification standard is offered on a voluntary basis.

Classification*	Spherical Diameter	Type of Metal
A	1/32 inch (.794 mm)	316 stainless
B	1/16 inch (1.588 mm)	316 stainless
C	1 / 8 inch (8.175 mm)	316 stainless
D**		

*To test a metal detector, a metal sphere of the size and type indicated (generally imbedded in an acceptable, non-metallic material) is passed through the center of the aperture. The detector must detect in at least 9 of the 10 pass through to qualify for the applicable classification.

**The "D" classification identifies those detectors which are either not sensitive to the 1/8 inch (3.175 mm) level or are installed in a manner that prevents testing in the described fashion.

VIII. CONVEYOR BELTS

Conveyor belts used in direct contact with food product must be moisture resistant and nonabsorbent. Convey belts should have the edges sealed with the same material as is used for the food contact surface. In addition, belting material must be chemically acceptable and approved by the FDA/USDA. Conveyors with trough like sides and bed should have a quick belt tension release device to allow cleaning under the belt.

IX. JET-VACUUM EQUIPMENT

Equipment used for cleaning jars or cans should have safety devices to indicate malfunction of either jet or

vacuum elements. If necessary, vents to the outside should be provided to control exhaust currents and to prevent dust and/or paper particles from being blown back into cleaned containers.

X. HOSES

Hoses used for product contact should comply with recommendations of trade associations or be accepted by both FDA/USDA. The hose material must be installed in a manner that allows for inspection of the interior surface. Sanitary connectors can be installed at appropriate intervals to allow breakdown for visual inspection or use of inspection devices, such as, boroscopes.

Hoses without sanitary connectors are acceptable for steam and water lines where breakdown for cleaning and inspection is not necessary. However, hoses used for re-circulating water into and out of product contact areas must satisfy the requirement for product contact hoses.

XI. PICKLE LINE

Pickle lines should be either stainless steel or some other USDA acceptable material. If re-circulated, pickle line should be filtered and re-circulated through a system that can be disassembled to the extent necessary for thorough cleaning and inspection.

XII. SMOKEHOUSES, OVENS

Smokehouses or ovens must be designed for easy cleaning and inspection of all inner and outer surfaces. Ducts should be designed to be easily disassembled to the extent necessary for thorough cleaning and inspection. Spray heads for dispensing liquid smoke must be mounted below the level of the rails and trolleys. If liquid smoke is to be recirculated, the pump and pipelines must be of sanitary type construction. Liquid smoke cannot be re-circulated if product is on rack trucks.

XIII. SCREENS AND FILTERS

Screens and straining devices should be readily removable for cleaning and inspection and should be designed to prevent incorrect installation. Permanent screens should be constructed of non-corrosive metals. Synthetic filter materials should have clearance from trade associations. The same applies to filters intended for direct product contact. Filter paper should be single service. Filter cloths should be washable. Asbestos is not acceptable for use as filtering material or for any other purpose.

XIV. VENT STACKS FROM HOODS

Vent stacks from covered cooking vats or hoods over cook tanks and CO_2 equipment should be arranged or constructed so as to prevent drainage of condensate back into the product zone.

XV. ULTRAVIOLET (UV LAMPS)

Ultraviolet lamps which generate ozone are restricted for use as described under Ozone Producing Equipment. UV lamps which do not produce ozone may be used in any area provided shields are used to prevent exposure of workers to direct or reflected UV rays. Otherwise, rooms where unshielded UV lights are used should be equipped with switches at all entry points so the units may be turned off before workers enter. These switches should be identified with suitable placards such as, “Ultraviolet Lights.” Employees should not enter areas where unshielded UV lights are burning because of possible damage to skin and eyes.

XVI. HEAT EXCHANGERS

Heat exchangers may be used to heat or cool product. Heat exchangers may also be used to heat or cool gases or liquids which directly contact product. However, extreme caution should be exercised to prevent contamination. Inspectors and plant personnel should be alert to the following conditions and requirements.

1. Only heat exchanger media authorized by trade associations, FDA, USDA and other standardization bodies in the U.S. can be used for applications involving food product. Common materials such as brine or ammonia need not be submitted for review. Under no circumstances can toxic materials be used.
2. Heat exchangers should be routinely pressure tested to ensure that pinholes, hairline cracks, loose fittings, or other similar defects are not present. Presence of off-color, off-odor, and/or off-flavor may indicate leakage. Frequent depletion of heat exchange media may also indicate leakage.
3. Pressure on the product side should be higher than the media side.

XVII. IN-PLANT TRUCKS

Trucks used to transport product within the plant should be constructed of stainless steel. However, galvanized metal is acceptable provided it is maintained in a good state of repair and is re-galvanizcd when necessary. Trucks should be free of cracks and rough seams. Metal wheels should be avoided as they cause deterioration of the floor surfaces. All trucks should have some means of affixing a tag. This can be accomplished by drilling two

holes approximately 1 inch (25.4 mm) apart in the lip of the truck to accommodate string or wire.

XVIII. AIR COMPRESSORS

Compressed air may be used to directly contact product and/or product contact surfaces provided the air is filtered before entering a compressor and it is clean and free of moisture, oil, or other foreign material when contacting product or product contact surfaces. Lubricants and coolants directly contacting air should be authorized by trade associations, FDA, USDA and other standardization bodies in the U.S.

Compressed air storage tanks should have a drain. Water and oil traps must be located between storage tanks and the point of use. Spent air must be exhausted in a manner to prevent product contamination.

Air directly contacting product or product contact surfaces should be filtered as near the air outlet as feasible. Filters should be readily removable for cleaning or replacement and should be capable of filtering out 50 micron particles (measured in the longest dimension). Air intake on votators should also be filtered.

XIX. PRODUCT RECONDITIONING EQUIPMENT

Product which is accidentally soiled may be cleaned on a separate, conveniently located wash table or sink. This wash station should be properly equipped with sprays and a removable, perforated plate to hold product off the bottom. The station should be identified as a "product wash station" and cannot be used for hand or implement washing.

XX. ELECTRIC CORDS

Accepting the use of electric cords should be based on both sanitary and safety considerations. Drop cords suspended from the ceiling may be retractable and used to connect portable equipment on an as needed basis if the cords are properly wired to the power source. Electric cords should not be strung across the floor even on a temporary basis.

XXI. ELECTRIC INSECT TRAPS

Electric insect traps may be used in edible product handling and storage areas provided the following conditions are met.

1. The equipment should be made of acceptable non-corrosive materials.
2. The traps must not be placed above uncovered product or above uncovered product traffic ways.
3. The electrified components are either apparent or properly identified; insulated from non-electrified components; and covered with a protective grille to prevent electric shock hazard.
4. The equipment should have a removable shelf or drawer which collects all trapped insects.
5. The equipment is designed and constructed so that all dead insects are trapped in the removable shelf or drawer. (Insects must not collect on the protective grille.)

Removable drawers or shelves should be emptied as often as necessary. If the drawer or shelf becomes full of dead insects, then the fourth requirement above cannot be met so the equipment should be rejected for use. Dead insects must be removed from the unit before they create an odor problem. They cannot be left in the unit as "bait."

XXII. INEDIBLE PRODUCT EQUIPMENT

Containers for handling and transporting inedible products should be watertight; maintained in a good state of repair (no rust or corrosion); and clearly marked with an appropriate identification. All inedible product containers in the plant should be uniformly identified. Inedible product containers should be cleaned before being moved into an edible products department.

Metal barrels, tanks, or trucks may be used for holding inedible poultry products in specially designated inedible product rooms. Alternatively, the containers may be stored outside the building provided the storage area is paved, drained, and conveniently located. These storage areas should also be equipped with nearby hose connections for cleanup.

XXIII. BLOW-OFF EQUIPMENT USING COMPRESSED AIR

Filters used on the compressed-air line should be readily removable for cleaning or replacement and should be capable of filtering out 50 micron particles.

The air-pressure must be measured and recorded with appropriate devices and must be set to deliver 75 to 125 psi (5.27 to 8.79 Kg/sq. cm).

The blown air must be confined so that it is captured by a water curtain or by an exhaust system that has a suction of at least 1500 cfm (425 Hectoliters/min) at the point of exhaust.

XXIV. OZONE PRODUCING EQUIPMENT

Equipment which produces ozone may be used only in coolers designated for certain types of products, e.g., aging meat. The ozone concentration in the air must be measured and recorded with appropriate devices and cannot exceed 0.1 ppm. Ozone generating equipment should be turned off and the ozone permitted to dissipate before any in-house or external inspection is performed.

XXV. OZONE WATER TREATMENT AND RECYCLING EQUIPMENT

Equipment used to ozonize and recycle water should be constructed from non-corrosive type material with safe and easy access for cleanup and sanitary inspection of all component parts. Pumps and piping should be of acceptable sanitary type, demountable, with quick disconnect mechanisms at each change of direction. Tanks, funnels, ozone generators, filter housings and filter media should have FDA/USDA acceptance and approval and they should be easily demountable for cleanup and sanitary inspection. A written approval of a water re-use procedure may be needed by state and federal regulators. There should also be written procedures for measuring and recording the total bacteria count and the total organic carbon level (TOC) in the ozone treated water returning to the source of use. [Established guidelines: a total aerobic plate count of less than 1000/mL, coliform less than 10/mL, and E. coli less than 2/mL.] All systems should have monitoring devices online:

1. to measure and record the ozone level and concentration in the immediate area (ozone concentration cannot exceed 0.1 ppm);
2. to measure and record ozone level and range of turbidity in the water being returned to the source of use; all ozone must be dissipated at this time and turbidity must be within the range of 0.5–5.0 NTU (nephelometric turbidity units); and
3. to automatically interrupt the water flow if the quality of the ozone treated water does not comply with established NTU guidelines.

XXVI. SANITIZING SOLUTIONS, TESTING DEVICES

A test kit or other device that accurately measures the concentration in mg/L of sanitizing solutions should be provided. Testing devices to measure the concentration of sanitizing solutions are required for two reasons:

1. The use of chemical sanitizers requires minimum concentrations of the sanitizer during the final rinse step to assure sanitization; and
2. Too much sanitizer in the final rinse water could be toxic.

XXVII. LOCATION AND INSTALLATION

1. Fixed Equipment, Spacing or Sealing

A unit of equipment that is fixed because it is not easily movable should be installed so that it is:

a. Spaced to allow access for cleaning along the sides, behind, and above the unit;
b. Spaced from adjoining equipment, walls, and ceilings a distance of not more than 1 millimeter or 1/32 inch; or
c. Sealed to adjoining equipment or walls, if the unit is exposed to spillage or seepage.

Table-mounted equipment that is not easily movable should be installed to allow cleaning of the equipment and areas underneath and around the equipment by being: (a) sealed to the table; or (b) elevated on legs as specified.

When the weight of the equipment exceeds 14 kg (30 pounds), it is no longer considered by the definition to be easily movable. Consequently, the following guide is noted:

a. Allows accessibility for cleaning on all sides, above, and underneath the units or minimizes the need for cleaning due to closely abutted surfaces;
b. Assures that equipment that is subject to moisture is sealed;
c. Prevents the harborage of insects and rodents; and
d. Provides accessibility for the monitoring of pests.

2. Fixed Equipment, Elevation or Sealing

Floor-mounted equipment that is not easily movable should be sealed to the floor or elevated on legs that provide at least a 15 centimeter (6 inch) clearance between the floor and the equipment. However, if no part of the floor under the floor-mounted equipment is more than 15 centimeters (6 inches) from the point of cleaning access, the clearance space may be only 10 centimeters (4 inches).

The above suggestions do not apply to display shelving units, display refrigeration units, and display freezer units located in the consumer shopping areas of a retail food store, if the floor under the units is maintained clean.

Table-mounted equipment that is not easily movable should be elevated on legs that provide at least a 10 centimeter (4 inch) clearance between the table and the equipment. However, the clearance space between the table and table-mounted equipment may be:

a. 7.5 centimeters (3 inches) if the horizontal distance of the table top under the equipment is no more than 50 centimeters (20 inches) from the point of access for cleaning; or
b. 2.5 centimeters (2 inches) if the horizontal distance of the table top under the equipment is no more than 7.5 centimeters (3 inches) from the point of access for cleaning.

3. Summary

Stationary equipment or equipment not easily moveable (i.e., no casters) should be installed far enough from walls

and support columns to allow thorough cleaning and inspection. In addition, there must be ample clearance between the floor and the ceiling. If these clearances are not possible, then equipment should be sealed watertight to the surfaces. All wall mounted cabinets, electrical connections, and electronic components should be at least 1 inch from the wall or sealed watertight to the wall.

The inability to adequately or effectively clean areas under equipment could create a situation that may attract insects and rodents and accumulate pathogenic microorganisms that are transmissible through food.

The effectiveness of cleaning is directly affected by the ability to access all areas to clean fixed equipment. It may be necessary to elevate the equipment. When elevating equipment is not feasible or prohibitively expensive, sealing to prevent contamination is required.

The economic impact of the requirement to elevate display units in retail food stores, coupled with the fact that the design, weight, and size of such units are not conducive to casters or legs, led to the exception for certain units located in consumer shopping areas, provided the floor under the units is kept clean. This exception for retail food store display equipment including shelving, refrigeration, and freezer units in the consumer shopping areas requires a rigorous cleaning schedule.

XXVIII. MAINTENANCE AND OPERATION

A. Equipment

1. Good Repair and Proper Adjustment

a. Equipment should be maintained in a state of repair and condition that meets the requirements specified in this chapter.
b. Equipment components such as doors, seals, hinges, fasteners, and kick plates should be kept intact, tight, and adjusted in accordance with manufacturers' specifications.
c. Cutting or piercing parts of can openers should be kept sharp to minimize the creation of metal fragments that can contaminate food when the container is opened.

Proper maintenance of equipment to manufacturer specifications helps assure that it will continue to operate as designed. Failure to properly maintain equipment could lead to violations of the associated requirements that place the health of the consumer at risk. For example, refrigeration units in disrepair may no longer be capable of properly cooling or holding potentially hazardous foods at safe temperatures.

The cutting or piercing parts of can openers may accumulate metal fragments that could lead to food containing foreign objects and, possibly, result in consumer injury.

Adequate cleaning and sanitization of dishes and utensils using a warewashing machine is directly dependent on the exposure time during the wash, rinse, and sanitizing cycles. Failure to meet manufacturer and stated requirements for cycle times could result in failure to clean and sanitize. For example, high temperature machines depend on the buildup of heat on the surface of dishes to accomplish sanitization. If the exposure time during any of the cycles is not met, the surface of the items may not reach the time-temperature parameter required for sanitization. Exposure time is also important in warewashing machines that use a chemical sanitizer since the sanitizer must contact the items long enough for sanitization to occur. In addition, a chemical sanitizer will not sanitize a dirty dish; therefore, the cycle times during the wash and rinse phases are critical to sanitization.

B. Cutting Surfaces

Surfaces such as cutting blocks and boards that are subject to scratching and scoring should be resurfaced if they can no longer be effectively cleaned and sanitized, or discarded if they are not capable of being resurfaced.

Cutting surfaces such as cutting boards and blocks that become scratched and scored may be difficult to clean and sanitize. As a result, pathogenic microorganisms transmissible through food may build up or accumulate. These microorganisms may be transferred to foods that are prepared on such surfaces.

XXIX. CLEANING OF EQUIPMENT AND UTENSILS

A. Objectives

1. Equipment, Food-Contact Surfaces, Nonfood-Contact Surfaces, and Utensils

a. Equipment food-contact surfaces and utensils should be clean to sight and touch. The food-contact surfaces of cooking equipment and pans should be kept free of encrusted grease deposits and other soil accumulations.
b. Nonfood-contact surfaces of equipment should be kept free of an accumulation of dust, dirt, food residue, and other debris.

The objective of cleaning focuses on the need to remove organic matter from food-contact surfaces so that sanitization can occur and to remove soil from nonfood contact surfaces so that pathogenic microorganisms will not be allowed to accumulate and insects and rodents will not be attracted.

B. Frequency

1. Nonfood-Contact Surfaces

Nonfood-contact surfaces of equipment should be cleaned at a frequency necessary to preclude accumulation of soil residues.

The presence of food debris or dirt on nonfood contact surfaces may provide a suitable environment for the growth of microorganisms which employees may inadvertently transfer to food. If these areas are not kept clean, they may also provide harborage for roaches, flies, mice, and other pests.

C. Methods

1. Dry Cleaning

a. If used, dry cleaning methods such as brushing, scraping, and vacuuming should contact only surfaces that are soiled with dry food residues that are not potentially hazardous.
b. Cleaning equipment used in dry cleaning food-contact surfaces may not be used for any other purpose.

Dry cleaning methods are indicated in only a few operations, which are limited to dry foods that are not potentially hazardous. Under some circumstances, attempts at wet cleaning may create microbiological concerns.

2. Precleaning

a. Food debris on equipment and utensils should be scrapped over a waste disposal unit, scupper, or garbage receptacle or should be removed in a warewashing machine with a prewash cycle.
b. If necessary for effective cleaning, utensils and equipment should be preflushed, scrubbed with abrasives, or presoaked.

Precleaning of utensils, dishes, and food equipment allows for the removal of grease and food debris to facilitate the cleaning action of the detergent. Depending upon the condition of the surface to be cleaned, detergent alone may not be sufficient to loosen soil for cleaning. Heavily soiled surfaces may need to be presoaked or scrubbed with an abrasive.

3. Wet Cleaning

a. Equipment food-contact surfaces and utensils should be effectively washed to remove or completely loosen soils by using the manual or mechanical means necessary such as the application of detergents containing wetting agents and emulsifiers; acid, alkaline, or abrasive cleaners; hot water; brushes; scouring pads; or high-pressure sprays.
b. The washing procedures selected should be based on the type and purpose of the equipment or utensil, and on the type of soil to be removed.

Because of the variety of cleaning agents available and the many different types of soil to be removed it is not possible to recommend one cleaning agent to fit all situations. Each of the different types of cleaners works best under different conditions (i.e., some work best on grease, some work best in warm water, others work best in hot water). The specific chemical selected should be compatible with any other chemicals to be used in the operation such as a sanitizer or drying agent.

XXX. SANITIZATION OF EQUIPMENT AND UTENSILS

A. Objective

1. Food-Contact Surfaces and Utensils

Equipment food-contact surfaces and utensils should be sanitized.

Effective sanitization procedures destroy organisms of public health importance that may be present on wiping cloths, food equipment, or utensils after cleaning, or which have been introduced into the rinse solution. It is important that surfaces be clean before being sanitized to allow the sanitizer to achieve its maximum benefit.

B. Frequency

1. Before Use and After Cleaning

Utensils and food-contact surfaces of equipment should be sanitized before use and after cleaning.

Sanitization is accomplished after the warewashing steps of cleaning and rinsing so that utensils and food-contact surfaces are sanitized before coming in contact with food and before use.

C. Methods

1. Hot Water and Chemical

After being cleaned, equipment food-contact surfaces and utensils should be sanitized in:

a. Hot water manual operations by immersion for at least 30 seconds as specified in standard manuals;

b. Hot water mechanical operations by being cycled through equipment that is set up as specified elsewhere and achieving a utensil surface temperature of 71°C (160°F) as measured by an irreversible registering temperature indicator; or

Chemical manual or mechanical operations, including the application of sanitizing chemicals by immersion, manual swabbing, brushing, or pressure spraying methods, using a solution as specified in a standard manual, FDA/USDA regulations, and trade recommendations by providing:

1. An exposure time of at least 10 seconds for a chlorine solution,
2. An exposure time of at least 30 seconds for other chemical sanitizer solutions, or
3. An exposure time used in relationship with a combination of temperature, concentration, and pH that, when evaluated for efficacy, yields sanitization as defined elsewhere.

Efficacious sanitization is dependent upon warewashing being conducted within certain parameters. Time is a parameter applicable to both chemical and hot water sanitization. The time that hot water or chemicals contact utensils or food-contact surfaces must be sufficient to destroy pathogens that may remain on surfaces after cleaning. Other parameters, such as temperature or chemical concentration, are used in combination with time to deliver effective sanitization.

XXXI. PROTECTION OF CLEAN ITEMS

A. Drying

1. Equipment and Utensils: Air-Drying Required

a. After cleaning and sanitizing, equipment and utensils may not be cloth-dried.
b. Equipment and utensils may be air-dried or used after adequate draining as specified in FDA regulations (21 CFR 178.1010 Sanitizing solutions), before contact with food.
c. Utensils that have been air-dried may be polished with cloths that are maintained clean and dry.
d. Items must be allowed to drain and to air-dry before being stacked or stored. Stacking wet items such as pans prevents them from drying and may allow an environment where microorganisms can begin to grow. Cloth drying of equipment and utensils is prohibited to prevent the possible transfer of microorganisms to equipment or utensils.

B. Lubricating and Reassembling

Lubricants should be applied to food-contact surfaces that require lubrication in a manner so that food-contact surfaces are not contaminated.

Equipment should be reassembled so that food contact surfaces are not contaminated.

C. Storing

Cleaned equipment and utensils should be stored:

1. In a clean, dry location;
2. Where they are not exposed to splash, dust, or other contamination; and
3. At least 15 cm (6 inches) above the floor.

Further, equipment and utensils should be stored:

1. In a self-draining position; and
2. Covered or inverted.

Clean equipment and multi-use utensils which have been cleaned and sanitized can become contaminated before their intended use in a variety of ways such as through water leakage, pest infestation, or other insanitary conditions.

Cleaned and sanitized equipment and utensils may not be stored:

1. In locker rooms;
2. In toilet rooms;
3. In garbage rooms;
4. In mechanical rooms;
5. Under sewer lines that are not shielded to intercept potential drips;
6. Under leaking water lines including leaking automatic fire sprinkler heads or under lines on which water has condensed;
7. Under open stairwells; or
8. Under other sources of contamination.

The improper storage of clean and sanitized equipment, and utensils may allow contamination before their intended use. Contamination can be caused by moisture from absorption, flooding, drippage, or splash. It can also be caused by food debris, toxic materials, litter, dust, and other materials. The contamination is often related to unhygienic employee practices, unacceptable high-risk storage locations, or improper construction of storage facilities.

XXXII. CONCLUSION

For any food processor, the sanitation of equipment is a permanent problem. Contamination of food or food ingredients by unclean equipment has been the cause of

foodborne diseases in human population throughout the history of modern civilization.

It is important that a food processor comply with good manufacturing practices to reduce the potential of equipment being the source of human pathogens or poisonous chemicals.

ACKNOWLEDGMENT

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198 Frozen Food Plant Sanitation

Nanna Cross
Chicago, IL

CONTENTS

I. INTRODUCTION

The Departments of Agriculture of most states in this country have issued some basic regulations governing frozen food processing plants. Such regulations have, among others, a major objective of assuring that the frozen food products are safe for public consumption.

This chapter has provided a modified version of those regulations issued by the Pennsylvania Department of Agriculture. The modification transforms a legal document into an easy-to-read scientific discussion. Consult the Pennsylvania Code for a copy of the original legal document [Title 7 Agriculture, Part III Bureau of food safety and laboratory services, Chapter 37, Frozen foods, Subchapters B-G, Sections 37.11–37.216.].

A. Definitions

Accessible. Easily exposed for cleaning and inspection with the use of simple tools, such as those normally used by maintenance personnel.

Air temperature. The equilibrated temperature of the air environment in question.

Break-up room. Any area, or space within a warehouse, used primarily for the purpose of organizing cased frozen food into lots for individual consignment on route delivery.

Display case. Any case, cabinet or other facility used for displaying frozen food for sale.

Food product zone. Those surfaces with which food is normally in contact and those surfaces with which food may come in contact during processing, conveying, holding, refrigeration and packing, and which may drain onto product contact surfaces or into the product.

Freezing cycle. Lowering the internal product temperature of a food product to a temperature of 0°F or lower.

Frozen food. Any article used for food or drink by man or other animal which is all of the following: processed; packaged and preserved by freezing in accordance with good commercial practices; intended for sale in the frozen state.

Internal product temperature. The equilibrated product temperature of frozen food.

Operator. (1) Any person, firm or corporation operating or maintaining a frozen food plant or warehouse for the purpose of commercially preparing or storing frozen food. (2) Any person, firm, or corporation, operating or offering to operate, a vehicle for the purpose of transporting frozen food.

Readily (or easily) accessible. Easily exposed without the use of tools, for cleaning and inspection.

Readily removable. When a component part shall be capable of being separated from the principal part without the use of tools.

Ready to eat frozen food. A frozen food product which has been factory processed to the point at which it is ready for use as a food and may or may not require further heating before use.

Removable. When a component part shall be capable of being separated from the principal part with the use of simple tools, such as those normally used by maintenance personnel.

Retail outlet. Any building, room, or parts thereof, where the sale of frozen food is conducted to the ultimate consuming purchaser.

Route delivery. The transportation of frozen food, with frequent stops for partial unloading.

Sale. Any and every transaction including the dispensing, giving, delivering, serving, exposing, storing or any other possessing of frozen food wherein frozen food is subject to transfer to another person.

Storage room or facility. Any area or space within a warehouse used for the purpose of storing frozen food.

Transportation. The physical movement, or the acceptance for physical movement, of frozen food by a carrier.

Vehicle. Any van, truck, trailer, automobile, wagon, ship, barge, freight car, airplane or other means for transporting frozen food.

Warehouse. Any structure, room or part thereof, used for the purpose of storing commercially processed or manufactured frozen food.

B. Temperatures

1. Air Temperature

All frozen food should be held at an air temperature of 0°F or lower, except for defrost cycles, loading and unloading or for other temporary conditions beyond the immediate control of the person under whose care or supervision the frozen food is held. Only those frozen foods destined for further processing or repackaging in smaller units should be defrosted for such purposes. All such defrosting should be in accordance with good sanitary precautions.

2. Internal Product Temperature

The internal product temperature of frozen food should be maintained at 0°F or lower except when the product is subjected to the conditions provided and relating to air temperature. When the frozen food is subjected to such conditions the internal product temperature should not exceed 10°F except during further processing. In all cases the product should be returned to 0°F as quickly as possible.

When an accurate determination of internal product temperature of any case of frozen food fails without having sacrificed the packaged frozen food, representative packages or units should be opened to allow inserting of the sensing element to the approximate center of the packages in question.

Internal product temperature of cases of consumer packages of frozen food should be determined in the following manner:

1. Open the top of the case and remove two corner packages.
2. Punch a hole in the case from the inside. The stem of the thermometer should not be used for punching. The hole should be positioned so that when the thermometer stem is inserted from the outside it fits snugly between packages.
3. The temperature may be read after 5 minutes.
4. Thermometers or other temperature measuring devices should have an accuracy of plus or minus 2°F.

II. GENERAL REQUIREMENTS

A. Separation from Living Quarters and Objectionable Conditions

Frozen food preparation plants should be completely separated from areas used as living quarters by solid, impervious floors, walls and ceilings, with no connecting openings.

Food processing plants should be located in areas reasonably free from objectionable odors, smoke, fly ash and dust or other contamination. Objectionable conditions are often prevalent in the environs of the following facilities, though not limited to such facilities:

1. Oil refineries.
2. City dumps.
3. Chemical plants.
4. Sewage treatment plants.
5. Dye-works.
6. Paper pulp mills.

B. Accessways; Parking Areas; Expansion

Adequate, dust-proof accessways for all vehicular traffic, connecting loading and unloading areas of the plant to the public streets, should be available. Employee parking areas and access roads close by the food processing plant should be hard surfaced with a binder of tar, cement or asphalt.

When planning a plant, due consideration should be given to providing for an arrangement of buildings and necessary space to permit future expansion. Coolers, freezers, and the various processing departments should be so located that they may be enlarged without adversely affecting other departments.

C. Potable Water; Nonpotable Water

The plant should have an ample supply of potable water available from an approved public or private source as specified

Whenever a nonpotable water supply is necessary it should not be used in a manner which will bring it into contact with the product or product zone of equipment. Nonpotable water systems should be kept entirely separate from the potable water supply. The nonpotable water lines should be positively identified by paint of a distinctive color.

D. Equipment Installation; Hot and Cold Water; Clean-Up; Sewage Systems

Equipment should be so installed and used that back siphonage of foreign liquids into the potable water lines is impossible.

Hot and cold water in ample supply should be provided for all plant cleanup needs. Hoses used for cleanup should be stored on racks or reels when not in use.

Disposal of liquid wastes should be through the public sewage system, if available and permitted by local ordinances, or by a properly designed and installed private facility. Private liquid waste treatment facilities should be approved by the health authority having jurisdiction.

III. PLANT LAYOUT

A. Product Preparation and Processing; Preparatory Operations Areas

Product preparation and processing (including freezing) departments should be of sufficient size to permit the installation of all necessary equipment with ample space for plant operations, and with unobstructed truckways for conveyances of raw materials and processed products. The plant should be so arranged that there is a proper production flow of materials, without undue congestion or backtracking, from the time raw materials are received until the frozen, packaged article is shipped from the plant.

Raw material storage rooms and areas where preparatory operations, such as washing and peeling of fruits and vegetables and the evisceration of poultry, are carried on should be separate from areas where frozen food is formulated, processed and packaged. Doors connecting various rooms or openings to the outside should be tightly fitted and kept in a closed position by self-closing devices.

B. Refrigeration Facilities; Quick Freezing Facilities; Waste Storage Rooms

Facilities for holding products under refrigeration until processed should be provided.

Whenever facilities for quick freezing the processed product are used, they should be so located as to be convenient to the food processing and packaging departments. Ample freezer storage should be provided, located conveniently to the quick freezing facilities. Freezer storage should not, however, be required if the frozen products are immediately removed from the establishment.

Separate rooms for storing inedible materials such as fruit and vegetable peels, and feathers and bones pending removal from the plant, should be provided in a location convenient to the various preparation and processing areas.

Waste storage rooms should be of sufficient size to permit the proper storage of filled and empty metal or other relatively nonabsorbent refuse containers and their lids. Waste storage rooms should be equipped with efficient power exhaust ventilation systems, hot and cold water outlets, and adequate floor drainage. The discharge from the exhaust system should be located well away from fresh air inlets into the plant.

C. Storage of Packaging and Labeling Materials; Facilities for Inedible Products; Cleaning Room; Dockage Areas

Packaging and labeling materials should be stored in an area separate from but convenient to the packaging department, except that small quantities of such supplies which are necessary for maintaining continuity of operations may be stored in the processing and packaging departments.

Facilities for inedible products and catch basins, should be located so as to avoid objectionable conditions affecting the preparation and handling of edible products.

A separate room or area and the proper facilities for cleaning equipment such as trays, hand trucks and implements, should be provided in a location convenient to the processing department. A power exhaust system should be provided to dispel steam and vapors from the room.

Dockage areas should be of adequate size, constructed of impervious materials and so drained as to minimize the entrance into the plant of dust, dirt and other contaminants from the receiving and shipping operations. If live animals are received a separate dock should be provided for this purpose.

D. Dressing Rooms; Toilet Rooms; Eating

Well located, properly ventilated dressing rooms and toilet rooms of ample size should be provided for employeees. Dressing rooms should be separated from adjoining toilet rooms by tight, full height walls or partitions. Toilet rooms should not be entered directly from a work room but through an intervening dressing room or a properly ventilated toilet room vestibule.

Standard building codes should govern such matters as the following:

1. Ventilation and lighting of toilet and dressing rooms.
2. Ratio of toilet, handwashing facilities, and urinals to the number of employees using such facilities.
3. Type of fixtures used.
4. Manner of installation of plumbing in such rooms.

Employees should not eat in food processing or packaging areas.

IV. PLANT CONSTRUCTION

A. Floors; Walls; Ceilings; Window Ledges; Rodents; Vermin

Floors should be constructed of durable material which is easily cleaned and skid resistant. Where floors are wet cleaned, they should be sloped to drain.

Regarding the walls of the plant:

1. Interior walls should be constructed of smooth, cleanable, surfaces applied to a suitable base.
2. Dressed lumber should be used for exposed interior woodwork. Exposed wood surfaces should be finished with nontoxic oil or plastic paint or treated with hot linseed oil or clear wood sealer.

3. Coves with radii sufficient to promote sanitation should be installed at the juncture of floors and walls in all processing rooms.

Ceilings should be of adequate height and of smooth, cleanable material.

Window ledges should be sloped at least 45° to the interior to promote sanitation.

Frozen food plants and warehouses should be so constructed as to be rodent resistant. Exterior window and door openings should be equipped with effective insect and rodent screens. Where doors in outside walls of food handling areas are used for loading or unloading, fly chaser; fans and ducts or other effective means should be provided at such doors to prevent the entrance of insects.

B. Stairs; Refrigerator Doors; Variations from Requirements

Stairs in product handling departments should be constructed with solid treads and closed risers and should have side curbs of similar material, which should be 6 inches high as measured at the front edge of the tread.

Regarding the refrigerator doors:

1. Refrigerator doors and jambs should be covered with rust-resisting metal securely affixed to the doors and jambs.
2 Joints necessary for installation should be welded, soldered or otherwise effectively sealed.
3 The juncture of the metal covering on jambs and walls should be sealed with a flexible sealing compound.
4. Doorways through which the product is transferred, either on overhead rails or on hand trucks, should be sufficiently wide to permit free passage of the largest trucks or the widest suspended products without contact with the jambs.

The requirements for building materials listed in this chapter represent minimum requirements. Variations should be acceptable provided the substitutions are equal to or exceed minimum requirements.

V. PLUMBING AND FLOOR DRAINAGE

A. Wet Processing Areas; Hand Washing Facilities; Sterilizers

Floors should be sloped and drains functionally located to provide adequate drainage. In wet processing areas the type and size of floor drains and sanitary sewage lines used and the method of installing such facilities and other plumbing equipment should conform to Commonwealth or local regulations.

Hand washing facilities should be located conveniently to all locations where products are prepared and processed. Lavatories should be supplied with the following:

1. Hot and cold or warm running water.
2. Powdered or liquid soap in a suitable dispenser.
3. An ample supply of single service towels or electric air dryers.
4. A suitable receptacle for used towels.

Where sterilizers are required they should be large enough to allow complete immersion of tools and other implements. Sterilizing facilities should have the following:

1. A water line.
2. A means of heating the water.
3. An overflow outlet.
4. A means of emptying the receptacle.

VI. LIGHTING; VENTILATION

A. Work Rooms and Dressing Rooms; Fresh Air Intakes; General Light Intensities

Work rooms and dressing rooms:

1. Work rooms and employee dressing rooms should have means for furnishing adequate natural light, which may be accomplished by having windows or skylights of an area approximately 25% that of the floor area.
2. Ventilation or efficient air conditioning or a mechanical ventilation system should be provided.
3. Adequate artificial light should be provided.

Fresh air intakes:

1. Fresh air intakes for mechanical ventilation systems should be equipped with effective replaceable filters to prevent the entrance of airborne contaminants.
2. Fresh air intakes should be located well away from power exhaust system discharges and other sources of airborne contaminants.

General light intensities:

1. The general light intensities in product preparation, processing and packaging areas should be not less than 20 foot-candles as measured 30 inches above the floor.

2. Where detailed visual tasks are required to assure a safe, wholesome product, the intensity of light on the surfaces of the product or product container should be not less than 50 foot-candles.
3. At least ten foot-candles of light should be provided in all dressing rooms and at least 5 foot-candles in all other areas of the plant.

VII. FROZEN FOOD PROCESSING EQUIPMENT: CLASSIFICATION

These specifications should apply to the design, materials, construction and installation of equipment used in the processing, holding, and packaging of ready-to-eat frozen food and the processing and holding of gravies, coating batters, and other food ingredients containing eggs, milk, broth and other food components capable of supporting rapid bacterial growth.

Design, materials, construction and installation of frozen food equipment should be easily accessible for cleaning and sanitizing.

In order to encourage the cleaning of equipment, the time factor and the ease of disassembly are important considerations. The unit of equipment should contain the fewest number of parts to permit easy reassembly by unskilled labor following cleaning.

A. Group A

Equipment in Group A should be used for the processing, conveying, holding, refrigeration and packaging of gravies, coating batters or other food ingredients containing eggs, milk or broth, alone or in combination with other food ingredients, which are capable of supporting rapid bacterial growth. This group includes, but is not limited to, the following:

1. Pumps.
2. Valves.
3. Pipelines and fittings.
4. Heat exchangers.
5. Homogenizers.
6. Containers.
7. Hoppers.
8. Fillers.

B. Group B

Equipment in Group B should be used in the processing, holding and conveying of foods or food ingredients which are intended to be incorporated in ready-to-eat frozen food. This group includes, but is not limited to, the following:

1. Reservoirs.
2. Holding tanks.
3. Kettles.
4. Mixers for liquids.
5. Mixers and blenders for powders.
6. Dough mixers.
7. Flour handling equipment.
8. Fryers.
9. Cutters.
10. Dicers.
11. Slicers.
12. Cutting boards.
13. Pumps.
14. Valves.
15. Tanks.
16. Lines and fittings for liquid sugar.
17. Lines and fittings for oil and shortening.

C. Group C

Equipment in Group C should be used in the manufacture of ready-to-eat frozen food, but applicable standards are not available.

VIII. FROZEN FOOD PROCESSING EQUIPMENT: MATERIALS, DESIGN AND CONSTRUCTION: GROUPS A AND B

Specifications and published standards for food equipment have been developed by official agencies and voluntary organizations other than those specifically mentioned in this chapter. These standards may be worthy of consideration in the evaluation of certain equipment items. The development organization and the area in which standards are published are the following:

1. National Sanitation Foundation. Food preparation and service equipment.
2. United States Department of Agriculture, Meat Inspection Division, Meat processing equipment.
3. United States Department of Agriculture, Poultry Inspection Division, Poultry processing equipment.
4. United States Department of Commerce, National Marine Fisheries Service, Fishery products handling and processing equipment.

A. Group A

Effort should be made to have equipment in Group A conform to 3A Sanitary Standards. Standards are as follows:

1. Pumps. 3A Sanitary Standards for Pumps for Milk and Milk Products, including both centrifugal and rotary pumps
2. Valves. 3A Sanitary Standards for Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers.

3. Milk and milk products equipment. 3A Sanitary Standards for Fittings and Connections Used on Milk and Milk Products Equipment.
4. Heat exchangers. 3A Sanitary Standards of Plate Type Heat Exchangers for Milk and Milk Products.
5. Pasteurizers. 3A Accepted Practices for the Sanitary Construction, Installation, Testing, and Operation of High-Temperature, Short-Time Pasteurizers.

B. Group B

Effort should be made to have equipment in this group conform to B.I.S.S.C. standards, which are promulgated by the Baking Industry Sanitation Standards Committee. Standards (always check for the latest version) are as follows:

1. Mixers or blenders for powders. B.I.S.S.C. Standards pending.
2. Horizontal and vertical dough mixers. B.I.S.S.C. Sanitary Standard No. 6, for Horizontal Mixers and Vertical Mixers.
3. Flour handling equipment. B.I.S.S.C. Sanitation Standard for Flour Handling Equipment.
4. Liquid sugar handling equipment. B.I.S.S.C..
5. Liquid oil and shortening handling equipment. B.I.S.S.C.
6. Fryers. B.I.S.S.C. Sanitation Standard No. 16, for Doughnut Equipment.
7. Depositors, fillers. B.I.S.S.C., Sanitation Standard No. 5, for Cake Depositors, Fillers and Icing Machines.
8. Conveyors. B.I.S.S.C., Sanitation Standard No. 7, for Conveyors.
9. Homogenizers, emulsifiers. B.I.S.S.C., Sanitation Standard No. 18, for Emulsifiers and Homogenizers.

IX. FROZEN FOOD PROCESSING EQUIPMENT: MATERIALS, DESIGN AND CONSTRUCTION: GROUP C

A. Materials

1. Food surfaces:
   a. Surfaces within the food product zone should be smooth, free from pits, crevices and loose scale, and should be relatively nonabsorbent. Furthermore, surfaces should be nontoxic and unaffected by food products and cleaning compounds.
   b. Sponge rubber, stone slab, linoleum, flannel and unglazed ceramic material are basically objectionable and should not be used.
   c. Wood and cloth, if used, should be indicated under specific application.
2. The finish of corrosion-resistant surfaces such as stainless steel or nickel alloy should be of 125 grit, and properly applied.
3. Finishes of cast iron, cast and forged steel, and cast nickel alloy should not exceed a surface roughness of American Standard #125 or its equivalent.
4. The use of galvanized surfaces should be minimal and where used should be of the smoothness of high quality commercial hot dip.
5. Copper and its alloys should not be used in equipment where edible oils, liquid shortening, chocolate liquor and other fatty food products come in contact with the metal.
6. Cadmium should not be used in any manner or form on the food equipment.
7. Lead should not be used within or adjacent to the food product zone, with the exception of its inclusion in dairy solder, in an amount not to exceed current specification.
8. Plastics should be in conformity with federal regulations.
9. Gasketing and packing materials should be relatively non-porous, relatively non-absorbent, and installed in a manner that results in a true fit to prevent protruding into the product zone of the creation of recesses or ledges between the gasketed joints.
10. Coatings used in the food product zone as a lining to prevent corrosion of the base material of food equipment should be in conformity with federal regulations.

B. Design and Construction in the Food Product Zone

1. All parts of the product zone should be readily accessible or should be readily removable for cleaning and inspection.
2. All parts of the food product zone should be free of recesses, dead ends, open seams and gaps, crevices, protruding ledges, inside threads, inside shoulders and bolts or rivets which form pockets and patterns.
3. Permanent joints of metal parts should be butt welded. Dissimilar metals should not be used in equipment construction if their contact with liquid products might create deleterious chemical or electrolytic action.
4. Welding within the food product zone should be continuous, smooth, even and flush with the adjacent surfaces.

5. Interior corners should be provided with a minimum radius of 1/4 inch except where a greater radius is required to facilitate drainage or cleaning.
6. Equipment should be constructed and installed to provide sufficient pitch so as to be completely self-draining.
7. Equipment which introduces air into the food product or uses air to convey the food product should be fitted with filters capable of withholding particles 50 microns or larger in size. Such filters should be readily removable for cartridge replacement or cleaning.
8. Bearings should be located outside the food product zone or outboard, and should be of the sealed or self-lubricated type. Those intended for use with a dry granular or a dry pulverized product directly adjacent to the food product zone should be of the sealed type without grease fittings. The bearings should be installed flush to eliminate any recessed areas around the shaft within the food product zone.
9. Shaft seal assemblies and packing glands should be outboard and should be readily removable. The shaft seal or packing should be retractable within a space between the assembly and bearing to facilitate easy removal of the sealing assembly and materials for cleaning and inspection.
10. Permanent screening and straining devices and surfaces
    a. All permanent screening and straining devices should be readily removable for cleaning and inspection. They should be designed to prevent replacement in an improper position.
    b. Permanent screening and straining surfaces intended for use with a liquid or a semi-liquid product should be fabricated from perforated metal.
    c. Permanent screening and straining surfaces for use with a dry granular or a dry pulverized product should be designed with sufficient strength for its intended use, and be sized to efficiently remove foreign material.
11. Filtering process
    a. Filtering surfaces should be readily removable for cleaning and inspection.
    b. Filter papers should be of the single-service type.
    c. Filter cloths and spun glass filters should be launderable.
12. Hinges and latches should be of the simple take-apart type.
13. Motors should be of the totally enclosed finless type and should be mounted on the equipment whenever possible.
14. Covers should be provided on reservoirs, hoppers or other vessels and should be readily removable and fitted with drip protective devices or facilities to prevent foreign substances from falling into the product.

C. Design and Construction in the Nonfood Products Zone

1. Safety and gear guards should be removable for cleaning and inspection.
2. External surfaces should be free of open seams, gaps, crevices, unused holes and inaccessible recesses.
3. Horizontal ledges and frame members should be kept to a minimum. External angles should be rounded and internal angles should be avoided.
4. Where lubrication of equipment is required provision should be made to prevent leaking or dripping into the food product zone.

D. Installation of Equipment

1. Equipment should be installed on a foundation of durable, easily cleaned material.
2. Equipment should be placed at adequate distance from walls, ceilings and floors for cleaning and maintenance, or sealed watertight thereto. The preferred minimum space between walls or ceilings should be 30 inches.
3. Whenever equipment passes through walls or floors it should be sealed to that partition, or sufficient clearance should be allowed to permit inspection, cleaning and maintenance.
4. Wherever there is spill or drip, drains and catch pans should be provided and should be of such dimensions to collect all spill and drip. They should be easily accessible or easily removable for cleaning.
5. Where pipes pass through ceilings of processing areas pipe sleeves should be inserted in the floor above so that their upper periphery is at least 2 inches above the floor.

E. Connections

All electrical connections, such as switch boxes, control boxes, conduits and box cables, should be installed a minimum of 3/4 inch away from the equipment or walls or be completely sealed to the equipment or walls.

X. OPERATING PRACTICES FOR THE COMMERCIAL MANUFACTURE OF FOOD

A. Handling and Storage of Materials

Requirements for food should be as follows:

1. All food ingredients received at the plant should be wholesome.
2. Storage conditions should protect against contamination from rodents, insects and other sources.
3. Storage temperature should be in accordance with the following practices:
   a. Ingredients requiring refrigeration should be stored at an air temperature of 40°F or lower. Only areas where the temperature does not exceed 40°F should be considered refrigerated.
   b. Frozen ingredients not in process should be stored at an air temperature of 0°F or lower.

Storage of packaging materials should be separate and set apart from food preparation and processing operations under conditions which should protect against contamination from rodents, insects and other sources.

General housekeeping should be conducted so that the plant and premises present a neat and orderly appearance at all times.

B. Personnel Hygiene

The services of an employeee with any open sore on an exposed portion of the body or one afflicted with an infectious or contagious disease should not be used except that services of employees with finger cuts or with bandages, finger cuts and similar type coverings may be utilized on the condition that the employeee wears rubber gloves. Any employeee with an upper respiratory infection should be assigned duties outside of the areas of food preparation, processing and packaging. Visitors to food preparation, processing and packaging areas should comply with employee requirements.

Practices for employees handling unpackaged food should be as follows:

1. Employees should wear head covering and should keep clothing in a clean condition consistent with the duty being performed.
2. Before beginning work, after each absence from post of duty and after contact with non-sanitized surfaces, each employee should:
   a. Wash his hands with liquid or powdered soap and warm water dispensed from a foot or elbow operated device (Existing faucet facilities need to be changed to a foot or elbow operated device only when a new hand washing facility is installed.).
   b. Rinse his hands in a chlorinated spray or other approved sanitizing agent, unless a bacteriostatic soap is used in washing.
   c. Dry his hands with single service towels or with electric hot air dryers.
3. Hand contact with food products should be minimized.
4. Use of a common dip bowl or tank is prohibited.
5. Whenever rubber gloves are used they should be cleaned and sanitized in accordance with hand washing specifications.
6. Use of tobacco in any form, chewing gum or eating in rooms where food products are stored, handled or prepared should not be permitted.

C. Plant and Equipment Sanitation

1. Plant and equipment should be clean when put into service.
2. All floors, tables, splash boards, work surfaces, equipment and utensils should be maintained in a clean and sanitary condition at all times. Critical areas and all food contact surfaces should be cleaned and sanitized whenever necessary or at scheduled intervals.
3. Equipment such as pipes, pumps, fillers and valves should be dismantled for cleaning and sanitizing, unless in-place cleaning and sanitizing methods are effective. Suggested criteria for acceptance of clean-in-place (CIP) systems areas are as follows:
   a. The arrangement should allow cleaning and bactericidal solutions to be circulated through the system.
   b. Solutions should touch all surfaces.
   c. The system should be self-draining or otherwise completely evacuable.
   d. The cleaning procedure should result in thorough cleaning of the equipment.
4. A thorough rinse with potable water should follow any sanitizing operation that has been completed with a chemical sanitizing agent.

D. Preparation and Processing

1. Fans, blowers or air cooling systems should not move unfiltered air from raw material or preparation rooms into processing rooms.
2. Only adequately cleaned, prepared raw materials should be introduced into areas where frozen precooked foods are cooked and subsequently handled in processing operations.

3. Preparatory operations feeding to the packing line should be so timed to permit efficient handling of consecutive packages in production, and under conditions designed to prevent contamination, loss of quality or spoilage.
4. When batter, egg wash or milk wash is an ingredient, it should be maintained at a product temperature not to exceed 45°F, except when the process temperatures required for manufacturing the product are higher. Cracked or flaked ice used to refrigerate batters should meet bacterial standards for potable water. Batter remaining in machines and equipment at cleanup time should be discarded.
5. Breading materials that have come in contact with batter and have been removed by screening should be discarded.
6. Food ingredients or mixtures that are capable of supporting rapid bacterial growth should be maintained either at a product temperature above 160°F or below 45°F, except when processing temperatures falling in this range are an integral part of the product manufactured, such as, yeast.
7. Cooked food such as meat, poultry, sauces and gravies should be all of the following:
   a. Refrigerated or incorporated into the finished product within 1 hour following preparation.
   b. Refrigerated within 30 minutes following preparation at an air temperature of 50°F or less if the product is to be held from 1 to 8 hours after preparation.
   c. Refrigerated within 30 minutes following preparation such that the internal temperature of the food product will be 40°F or lower, within 2 hours of refrigeration if the food product has been comminuted, sliced or is a liquid, and if the food is to be held more than eight hours. Large solid food components such as those that must be cooled before slicing should be refrigerated at an air temperature of 40°F or lower.
8. Trays, pans or other containers of ingredients destined for incorporation into the finished product should be protected with a clean cover unless these ingredients are used within 30 minutes of preparation. The cover should not be made from porous material.
9. Permanently legible code marks should be placed on each immediate container or package at time of packing. The code marks, as devised by management, should include date of packing and establishment where packed.
10. Packaged products should be placed in the freezer according to good commercial practice. Placement of packages in cases before freezing is prohibited unless the wholesome quality of the product is fully protected by prior processing.
11. Waste disposal:
    a. Refuse from the food operations should be promptly placed in containers that are prominently marked REFUSE and equipped with lids.
    b. The handling of refuse should be done in such a manner as not to cause a nuisance.
    c. All refuse should be removed from the premises on a daily basis and in such a manner as not to contaminate food products being manufactured within the plant.
    d. Refuse containers should be thoroughly cleaned immediately after each emptying.

E. In-Plant Freezing

1. During the freezing cycle products should be cooled to 50°F or lower within 2 hours.
2. Products should then be reduced to 0°F by approved commercial practice.
3. When necessary products should be protected so that dehydration and discoloration will not occur during the freezing cycle.
4. The freezer should be precooled to an air temperature of 0°F before loading. During loading, however, the freezer may rise to temperatures above 0°F for short periods of time.
5. If cold air is used as the freezing medium the product should be arranged by staggering the individual items or by employing dunnage, spacers or other suitable methods to permit satisfactory circulation of cold air around the products. The cold air should be circulated by a positive method; natural air circulation should not be satisfactory.
6. The freezer and associated equipment used for handling the product should be maintained in a clean and sanitary condition at all times.
7. A suitable indicating or recording instrument should be used to measure the temperature of the cooling medium, that is, air, liquid, refrigerated plates or pipe coils.
8. Packaged items should be frozen in a manner that will result in a minimum amount of bulging or distortion.
9. After the freezing cycle the frozen product should be transferred to a storage facility as quickly as possible.

XI. TRANSPORTATION EQUIPMENT

Vehicles used for transportation should be equipped with insulation and mechanical refrigeration systems, or other

refrigeration methods or facilities capable of maintaining an air and product temperature of 0°F, or lower, while loaded with frozen food.

Vehicles used for transportation should be equipped with a thermometer or other appropriate means of temperature measurement, indicating air temperature inside the vehicle. The dial or reading element of the thermometer should be mounted on the outside of the vehicle.

Vehicles used for route delivery should comply with all equipment provisions specified in this chapter for vehicles used for transportation, and should in addition be equipped with curtains or flaps in the doorway area, or with port doors, or with portable insulated chests to maintain required temperature during distribution.

XII. HANDLING PRACTICES FOR OVER-THE-ROAD TRANSPORTATION

Vehicles should be precooled to an air temperature of 20°F or lower before loading.

Frozen food shipments should not be accepted for transportation when the internal product temperature exceeds 0°F, except that shipments in transit at a higher temperature should not be considered in violation of this section if the bill of lading, signed by the shipper, specifies that the product is consigned to a warehouse or other facility for further freezing, or if the product is to be sold as fresh and is to be defrosted when offered for use or for sale.

Frozen food should be loaded in a transportation vehicle so as to provide free circulation of refrigerated air at the front, rear, top, bottom and both sides of the load, except for vehicles of envelope construction in which refrigerated air circulates within the walls of the vehicles.

The mechanical refrigerating unit of vehicles should be turned on and doors of vehicles should be kept closed or curtained during any time interval when loading or unloading operations cease.

The average product temperature of any shipment of frozen food should be determined during loading and unloading by adequate temperature readings.

XIII. HANDLING PRACTICES FOR ROUTE DELIVERY

Lots for individual consignment which are to be sold in a frozen state should be refrigerated by means of mechanical refrigeration, dry ice, or by any other means capable of maintaining an air and product temperature of 0°F or lower.

Insulated containers should be precooled to a temperature of 20°F or lower before being loaded with frozen food.

Doors of vehicles should be kept closed during any time interval that loading or unloading operations cease.

XIV. SANITARY REQUIREMENTS DURING TRANSPORTATION

Interior surfaces of vehicles and devices used for transporting frozen food should be clean and free of objectionable odors before being loaded with frozen food.

Frozen food should be securely packaged or wrapped in a sanitary manner before it is accepted for transportation.

XV. WAREHOUSING EQUIPMENT

Regarding refrigeration capacity and minimum temperature:

1. Warehouses should be equipped with suitable mechanical refrigeration capacity to maintain, under extreme outside temperature and peak load conditions, an air temperature of 0°F or lower.
2. Storage rooms and all their parts should be maintained at an air temperature of 0°F or lower.

Regarding the use of thermometers:

1. Each storage room should be equipped with a thermometer or some other temperature measuring device which is easily visible.
2. The sensing element of thermometers and other temperature measuring and recording devices should be located not more than 6 feet nor less than 5 feet from the floor and not in a direct blast of refrigerated air or near entrance doors.
3. When indicating thermometers alone are used they should be read and recorded at least once every 24 hours during each calendar day.
4. Recording thermometers equipped with charts should have a range of at least 15° above and 10° below, 0°F in graduations of 1°.
5. The use of electric or handwound clocks as well as 24-hour or 7-day charts for recording thermometers should be optional at the discretion of the operator.
6. Each chart or record of observed temperatures should be dated to show the time interval covered and should be kept on file for at least 1 calendar year.

Break-up rooms should be maintained at temperatures not in excess of 20°F.

XVI. WAREHOUSING HANDLING PRACTICES

The operator of a warehouse should not accept custody of a lot or shipment of frozen food if internal product temperature exceeds 0°F (except as relating to air temperature; and

internal product temperature). When frozen food is accepted pursuant to such exception the operator should make a written record of the incident.

Notwithstanding the above, custody of lots with an internal product temperature not in excess of 10°F may be accepted by the operator on request of the owner of the lot in question if the foods are detained from sale at retail and the temperature of such product is promptly returned to and maintained at 0°F or lower.

Before lots of frozen food are placed in storage they should be given lot numbers for effective identification.

Regarding the storage of frozen food:

1. Frozen food in storage should be placed on dunnage, pallets, racks or skids and should be stored no closer than 18 inches to the ceiling and otherwise stored so as to permit free circulation of refrigerated air.
2. Frozen food should be stored under good sanitary conditions that preclude injury and contamination from or to other food held within the warehouse.

During the defrosting of overhead coils in storage rooms stacks of frozen food should be effectively protected from contamination by condensation, drip or leakage.

Break-up rooms should not be used for storage unless the temperature is kept below 0°F.

At time of removal from warehouse custody the internal product temperature of frozen food should not exceed 0°F unless authorized by the owner to begin a defrost cycle.

XVII. WAREHOUSING SANITARY PRACTICES

Floors, walls and ceilings of a warehouse should be maintained in a good sanitary condition. Premises of a warehouse should be maintained in a good sanitary condition.

Warehouses should have water flush toilets so located as to be convenient to all employees. Toilet rooms should be well lighted and ventilated and should be maintained in a sanitary condition. The doors of all toilet rooms should be full-length and self-closing.

Adequate hand washing facilities, including hot and cold or warm running water, powdered or liquid soap in a suitable dispenser, and single service towels or hot air dryers, should be provided adjacent to all toilet rooms. Wash rooms should be well lighted and ventilated, and should be maintained in a sanitary condition. The use of a common towel is prohibited.

Warehouses should have a dressing room or rooms for the changing and hanging of wearing apparel. If individual lockers are provided, they should be well vented and maintained in a clean, sanitary condition, and should be free from disagreeable odors. The dressing room or rooms should be adequately lighted and ventilated and should be maintained in a clean, sanitary condition.

XVIII. RETAIL EQUIPMENT

Storage facilities should be equipped with suitable mechanical refrigeration capacity to maintain, under extreme outside temperature and peak load conditions, an air temperature of 0°F or lower.

When storage facilities of cabinet type are used they should be all of the following:

1. Defrosted as frequently as necessary to maintain refrigeration efficiently as specified.
2. Equipped with a thermometer indicating a representative air temperature.

When storage facilities of walk-in freezer type are used the following requirements should apply:

1. Frozen food in storage should be on dunnage, pallets, racks or skids, and should be stored so as to permit free circulation of refrigerated air.
2. The facility should be equipped with a thermometer, the sensing element of which should be located within the upper third of the distance between floor and ceiling. The sensing elements should not be placed in a direct blast of air from cooling units, cooling coils and heat exchange devices, or near the entrance door.
3. The facility should be equipped with an automatic mechanism for defrosting refrigerated coils when forced air blower refrigeration is used.

Frozen food display cases should be designed, constructed and equipped with mechanical refrigeration facilities capable of maintaining an air temperature of 0°F or lower.

Frost on refrigerator coils and in air passages of display cases should be removed as frequently as necessary to maintain refrigeration efficiency of 0°F or below.

Each display case should be equipped with a thermometer, the sensing element of which should be located in an appropriate place within the path of refrigerated air being returned to the coils.

The product load line should be designated by a distinctive line at inside terminal ends of each display case, and such lines should be at the highest point of discharge and return of refrigerated air.

Separators in display cases should be located a minimum of 1/2 inch from terminal ends to provide for free circulation of refrigerated air between the terminal ends and the displayed product.

Display cases in retail outlets should be so placed as to be relatively free of all of the following:

1. Air current resulting from door drafts, electric fans, and other factors that adversely deflect the current of refrigerated air within the display case.
2. Heat elements such as lights, heating units, and related devices that tend to raise the temperature of refrigerated air within the display case.

XIX. RETAIL HANDLING PRACTICES

Frozen food should not be accepted for delivery by a retail outlet when the internal product temperature exceeds 0°F, except as relating to air temperature; and internal product temperature. When frozen food is accepted under this exception the retail outlet should duly record that fact, preferably on the bill of lading or the delivery ticket.

The receiving of frozen foods should follow the following recommendations:

1. All frozen food received at a retail outlet should be placed in storage facilities without undue delay.
2. Retail outlets should employ the first-in, first-out basis of inventory.

Retail outlets should be equipped with storage facilities of sufficient cubic displacement to accommodate the storage of frozen food.

Regarding storage and display:

1. Frozen food should not be placed above the product food lines within any display case.
2. Frozen food in retail outlets should be stored and displayed under good sanitary conditions.

Obligations for compliance with this retail requirement should cease at the time of retail sale or when the ultimate purchaser takes custody of the product.

A product should be understood to be in the custody of the purchaser when some one else takes a delivery from a retail outlet at the request of the purchaser.

A. Preordered Frozen Foods

Frozen food that is preordered by the ultimate consumer may be sold at internal temperatures exceeding 0°F but not exceeding 45°F provided all of the following are met:

1. A pickup time and date is announced to potential customers.
2. Potential customers are advised on the sales invoice that food is subject to possible quality and perishable degradation when internal product temperature exceeds 0°F and the products are not to be resold.
3. Frozen foods are limited to foods that do not support the rapid and progressive growth of pathogenic micro-organisms.
4. Foods are not to be resold to other parties.
5. Frozen foods arrive at the pickup location with an internal temperature of 0°F or below except as relating to minimum temperature requirements.

XX. FROZEN PRE-COOKED FOODS: ESTABLISHMENT INSPECTION

The United States Food and Drug Administration has issued the following guides regarding the inspection of establishing manufacturing frozen pre-cooked foods.

1. Check for presence of rodents, birds, insects, or for other possible sources of contamination.
2. Check what tests are conducted on incoming raw materials (e.g., filth, mold, decomposition, bacterial load, etc.) if they are they received under a *Salmonella* free guarantee and if tested for *Salmonella* and other pathogens.
3. Determine the adequacy of cleaning and sanitizing steps for equipment.
4. Watch for time-temperature abuses in processing, particularly where product may be hung up in equipment or in cooling and storage processes.
5. Be sure that cooking, cooling and storage conditions are adequate and do not merely produce incubation temperatures for bacteria.
6. Consider employee hygiene and sanitary practices, mainly handwashing and sanitizing, but including health, wounds, sores or disease conditions.
7. Check food and color additives used to ascertain if permitted and used at proper levels.
8. Check labeling and net weights for compliance.

During a comprehensive inspection of frozen precooked foods establishment, cover.

A. Raw Materials

1. Examine raw materials in storage for evidence of contamination with filth (insects, rodents, birds, etc.), mold, and possible routes of other microbiological contamination.
2. Determine if raw materials are stored and handled properly (i.e., frozen products kept frozen, etc.).

3. Examine raw material area for possible misuse of pesticides, rodenticides and other dangerous chemicals.
4. Determine if critical raw materials; e.g.: NFDM, frozen eggs, dried eggs, etc., are received under a *Salmonella* free guarantee and are tested for *Salmonella* and other pathogens.
5. Check food and color additives in storage and determine if they are allowed for use.

B. Processing

1. List product flow in detail, including a flow plan.
2. Ascertain if manufacturing equipment is suitable for its intended use and is in good state of repair.
3. Check if equipment is cleaned and sanitized properly before use, as necessary during the day (i.e.: at breaks, etc.), and after use.
4. Obtain manufacturing process in detail including the conditions under which products are held prior to process, handled during process, and handled after process.
5. If applicable to the process, determine the time\temperature parameters of the manufacturing operation. Include:
   a. Temperature of raw materials prior to process.
   b. Temperatures during the process.
   c. Time\temperature during and holding periods between steps.
   d. Time\temperature of final heat treatment.
   e. Temperature of product at final packaging.
6. Check for any undue delays between final heat treatment and packaging.
7. Determine freezing process to include:
   a. Freezing equipment and process.
   b. Holding time and temperature prior to freezing.
   c. Time taken to reach hard frozen condition.
8. Check finished product handling and storage.
9. Evaluate the use of food and color additives to ascertain if permitted and used at the proper level.
10. Check use of pesticides and rodenticides to preclude their becoming incidental food additives.

C. Sanitation

1. Evaluate firms operation for compliance with GMPRs (Sanitation).
2. Check employee practices which could lead to the comtamination of the products with filth, bacteria and/or mold.
3. Determine if employees use hand dip and sanitizing solutions when necessary.

D. Economics

1. Check firm's net weights to ascertain proper container fill.
2. Review labeling for compliance with FPLA, etc.
3. Obtain significance of firm's coding system.

ACKNOWLEDGMENT

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199 Sanitation and Safety for a Fats and Oils Processing Plant

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CONTENTS

I. INTRODUCTION

Fats and oils have been recovered for thousands of years from oil bearing seeds, nuts, beans, fruits, and animal tissues. These raw materials have and continue to be important ingredients for foods, cosmetics, lubricants, and chemicals. The extracted fats and oils vary from pleasant smelling products that contain few impurities to very offensive smelling, highly impure materials. Fortunately, researchers have developed technologies for processing the fats and oils products to make them more suitable for foods and other applications. Developments in lipid processing technology have produced ingredients that have been instrumental in the development of many of the current food products available that provide the functional and nutritional requirements of discerning and better informed consumers. Processes have been developed to make them flavorless and odorless, lighter in color, modify the melting behavior, rearrange the molecular structure, remove potential disease causing impurities, capture possible harmful materials, and other changes to make them more desirable for the intended application.

Process control is practiced during fats and oils processing to assure that safe, nutritious, pleasant, cost effective products that perform as intended are produced. These products must meet all of the applicable legal requirements for a food product and the customer's standards of quality, performance, economics, and aesthetics. Each fat and oil's process has individual critical control requirements that contribute to the quality assurance of the finished product. In practice, fats and oils critical control points fall into the areas of safety, quality, compliance, and economics. Hazard Analysis Critical Control Points (HACCP), a part of the process control system, is a method that assesses and monitors food safety.

The basic concept of HACCP can be stated by the maxim "An ounce of prevention is worth a pound of cure." The HACCP system is based on the principle that food safety issues can be eliminated or minimized by prevention during production rather than by detection in the finished product. Development of a HACCP plan focuses on the identification of critical control points, and procedures or activities that will adequately control them to ensure safe production of a food product. Food safety hazards include all microbiological, chemical, and foreign materials that, if consumed could cause injury or harm [8].

II. EDIBLE FATS AND OILS COMPONENTS

The primary constituents of the extracted fats and oils are triglycerides but they also contain varying amounts of nonglyceride materials. Some of the nonglyceride components are undesirable, and can be considered a food safety hazard, while others are very beneficial. Therefore, the objective in all of fats and oils processing is to remove the objectionable materials with the least possible damage to the desirable constituents. In most cases, free fatty acids, phospholipids, moisture, color pigments, oxidation products, waxes trace metals, proteins, pesticides, meal, dirt, and other gross impurities are the materials that need to be removed. Most vegetable oils contain tocopherols, which are natural antioxidants that protect the oils from oxidation and should be retained. For some products, neither the color pigments nor waxes are detrimental and need not be removed. The major product quality concerns are with free fatty acids, phospholipids, oxidation products, proteins, and trace metals; all materials that affect the odor, flavor, and flavor stability of edible fats and oils products. In the U.S., fats and oils color is usually a major concern from a cosmetic sense to include pigments adsorption as a major impurities concern especially for products marketed directly to consumers. The major food safety concerns are with residual pesticides, mold, bacteria, and impurities developed during processing or with mishandling.

A. Pesticides

Pesticides have been used for increased agriculture production throughout the world. Studies have shown that the majority of the pesticides applied eventually reach the soil surface where they gradually spread, translocate to other environments, or eventually degrade. Translocation to oil-bearing plant seeds has also been demonstrated by studies. Processing studies have shown that neither solvent extraction nor bleaching affected the pesticide levels in vegetable oils. However, it was found that pesticides were removed by volatilization during hydrogenation and/or deodorization [1,2,7]. U.S. government agencies have recognized that the insecticides are distilled from edible oils during the deodorization process and have forbidden the use of deodorizer distillates in animal feeds.

B. Mold and Bacteria

Moisture content is one of the most important factors in determining the storability of oil seeds, fruits, nuts, and animal fats. Both molds and bacteria have critical moisture levels for proliferation and enzymes function in an aqueous substrate. Micro-toxins, or mold-derived toxins, can infest oil seeds, nuts, and fruits that are routinely used as an oil source. Only a small portion of the toxin is retained in the separated oil received during normal processing. Filtration of the extracted oil lowers the aflatoxin level, and conventional refining and bleaching effectively removes any residual aflatoxin [5].

C. In-process Tank Impurities

Storage tanks throughout the process are normally dedicated to a particular source oil or modified product. Even so these tanks are emptied and cleaned on a regular frequency to remove the sludge which collects on the tank bottoms. This sludge is composed of polymerized oils, burnt particles, acid salts, high melting oil fractions and other impurities which develop and settle out of the oils during storage. Cleaning equipment using high-pressure jets rotating horizontally and vertically are effective in the large tanks ranging from 40 thousand to million pound capacities. High temperatures, in the range of 185°F or 85°C, and an appropriate detergent suitable for use in food plants are used for washing before rinsing with clear water and finally the tanks are sweetened with freshly processed oil.

D. Separation of Impurities

From the time that crude vegetable oils are expressed from the bean, nut, fruit, or seed, and meat fat rendered from the fatty tissues, until the finished oil product is packaged, edible fats and oils pass through clarification or filtration steps in almost every stage of the processing sequence.

Even finished oils are subjected to a final filtration before packaging or loading into tank cars and trucks. The three generally used methods of separating impurities from oils are distillation, centrifugation and filtration. Pesticides and other impurities are removed from fats and oils with the deodorization which is a steam distillation process. Centrifuges are utilized to separate fines from solvent extracted and screw pressed vegetable oils and to separate the saponified materials after caustic refining and the gums participated with degumming processes. Filtration is employed as well in these processes and in all of the other major fats and oils processes to remove impurities.

Filtration is the process of passing a fluid through a permeable filter media in order to separate particles from the fluid. The particles may be either in suspension or solution. A filter medium is a porous material and one which allows a fluid to pass through yet retain the particles to be removed. Examples of filter media are filter paper, filter cloth, and filter screens. Filters of various types are used in through fats and oil processing beginning with clarification of the oils after extraction or rendering. Oils are filtered after bleaching to remove the bleaching earth which has adsorbed the color pigments and secondary oxidation products; after hydrogenation to remove the nickel catalyst which promoted the reaction, and after deodorization to remove minute quantities of burnt and polymerization particles which can develop during the high temperature process. Polish filters are utilized through the fats and oils process after movement of the product from one location to another to ensure that any impurities developed during that particular process are removed immediately.

III. PROCESSING FLOW SEQUENCE

A HACCP process hazard analysis begins with a detailed examination and evaluation of the processing and handling facilities for the purpose of identifying the location and severity of food safety hazards. Hazard analysis begins with a current flowchart of the production process which provides a means to evaluate potential hazards. A complete HACCP analysis encompasses all processes from the field to the final consumer. This evaluation reviews each process or operation for situations that could result in adulteration with injurious materials, which fall into one or more of three categories, microbiological, foreign material, and chemical. After determining the location of potential hazards, the next step is to determine where critical control points exist. Critical control points are the positions in the process flow where inadequate control would result in a contamination for which there are no downstream provisions to correct. After the critical control points are identified, it is essential to establish safe operating standards, monitoring procedures, frequencies, and responsibilities.

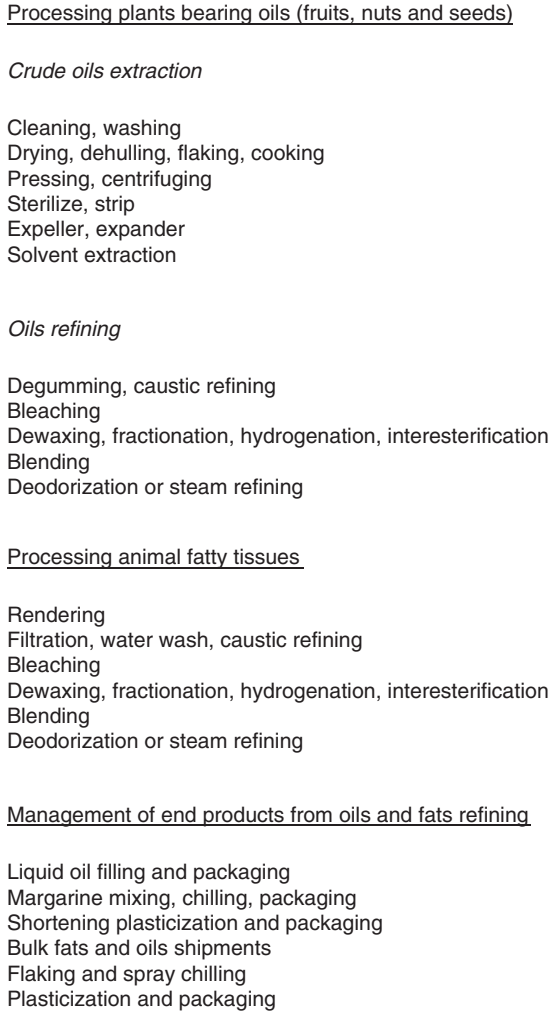

FIGURE 199.1 General steps in processing oils and fats in plant and animal products.

Edible fats and oils processing involves a series of processes in which both physical and chemical changes are made to the raw material. Figure 199.1 illustrates most of the potential processing flow sequencing to produce the various fats and oils products. The sanitation concerns for fats and oil's processing plants are similar to those of any other food processor; for example: rodent, insect and bird control, broken windows, leaking walls or roofs, unsecured doors, screened doors, dirty floor drains, cracks in floors, etc. But, the risks are somewhat lessened since all of the processing is performed within closed systems. Many of these systems are protected from the atmosphere with vacuum, pressure, or nitrogen. Practically, the only time that fats and oils products are exposed to the atmosphere is during filling into a package or a tanker for shipment. Therefore, the major product hazards concerns with fats and oils processing are the removal of impurities which

have gained access to the product prior to processing, added as a processing aid, or developed during processing.

Processing of fats and oils is initiated by an extraction or rendering process to remove the fat or oil from the seed, bean, nut, fruit, or fatty tissues. Vegetable oil's processing after extraction almost always includes neutralization or refining, bleaching, and deodorization with the major differences being the choice of equipment and techniques utilized. Rendered animal fats are normally clarified to remove impurities, bleached and deodorized, again with differences in equipment and techniques providing the major differences. Clairification, neutralization, bleaching and deodorization are all purification processes which affect the flavor, flavor stability and appearance of the fat or oil product while removing harmful impurities.

Vegetable oils and animal fats are natural products with variable characteristics contributed by nature. The fats and oils products selected for a particular application are made on the basis of functionality. Each source oil or fat has characteristic functionality traits which can be modified with the hydrogenation, interesterification, or fractionation processes. The natural or modified fats and oils products can also be blended to change the melting profiles, solidification points, crystal tendencies, and other physical characteristics of the fats and oils ingredients. A review of the major fats and oils processes follows with an emphasis on the critical control points for food safety.

A. Extraction

Cleaning is the first step in the processing of vegetable oils. Typically, oilseeds contain stems, pods, leaves, broken grain, dirt, sand, small stones, and other extraneous seeds. These foreign materials reduce the oil content, adversely effect oil quality and increase the wear and damage potential to the extraction equipment. Shaker screens are used to separate the particles on the basis of size, whereas aspiration separates on the basis of density and buoyancy in a stream of air. Tramp iron, extraneous metal acquired during harvesting, storage or transportation is removed to prevent damage to the equipment by the placement of magnets in chutes just ahead of vulnerable processing equipment.

Extraction of oil from materials of plant origin is usually done by pressing with the use of a continuous screw press or by extraction with volatile solvents. Prior to 1940, mechanical pressing was the primary method used. Mechanical pressing had limits because the oil recovery is poorer than with solvent extraction and the high temperatures generated damaged both the oil and the meal. Solvent allows a more complete oil extraction at lower temperatures. Solvent extraction plants can be either batch or continuous. The continuous extraction plants can be percolation, immersion or direct extraction plants. Generally, the oilseeds may be divided by oil content; above and below 20% oil content. In most cases, oilseeds with a low oil content are subjected to both continuous and batch solvent extraction. High oil content seeds are normally extracted in two stages; first pressing and then solvent extraction; however, many single step continuous direct solvent extraction systems are in current use.

To be used legally in the United States, oilseed extraction solvents and food processing substances must have been subjected to an approval by the U.S. Food and Drug Administration (FDA) or the U.S. Department of Agriculture (USDA), be generally recognized as safe (GRAS) for this use, or be used in accordance with food additives regulation promulgated by the U.S. FDA. Commercial hexane has been in major use since the 1940s as an oilseed extraction solvent on the determination that it is GRAS, and it may also be subject to a prior sanction. Like many other food-processing substances, there is no U.S. FDA regulation specifically listing n-hexane as GRAS or having prior sanction. However, it has been cleared in as a solvent in a number of other food products, one of them a cocoa butter substitute with a 5 ppm maximum limit. Because edible fats and oils are subjected to deodorization and other purification processes as a part of the manufacturing process before being used as a food product, they should not contain any of the extraction solvent, if proper practices are followed [9].

B. Rendering

The fatty tissue from meat animals which is not a part of the carcass or that trimmed from the carcass in preparation for sale is the raw material for which lard and tallow is obtained. Separation of fat from the fatty tissues of animals is called rendering. The rendering process consists of two basic steps. First, the meat by-product is heated to evaporate the moisture, to melt the fat present and to condition the animal fibrous tissue. Two alternative cooking temperatures are used: fat temperatures below 120°F and fat temperatures above 180°F. A more complete separation of the fat and protein is accomplished with the higher temperature processing but a better quality protein is obtained with the lower temperature processing. Normally, the value of the protein dictates that the lower temperature poorer separation technique be used which probably leaves trace quantities of protein in the rendered lard or tallow. After cooking, the fat is separated from the solid proteinaceous material. In batch rendering the cooked material is allowed to separate and the fat to drain followed by filtration to complete the separation. Continuous rendering, introduced to replace the batch systems, normally consists of a continuous cooker which requires less cooking time and is more energy efficient with better quality control [6].

C. Refining Systems

Processors have the option of approaching edible oil purification in two ways: either chemical or physical refining. The two systems utilize very similar processes with the major difference being the method used for free fatty acid removal. Chemical refining, the conventional method used

for removal of the nonglyceride impurities from edible fats and oils, consists of optional degumming, caustic neutralization, bleaching and deodorization. The alkali refining process produces good quality oil and is flexible with the ability to treat different oils and different qualities of individual oils. However, caustic refining has three major drawbacks: (1) the soap produced promotes a tendency for emulsion formation which will occlude neutral oil to increase oil losses, (2) oil losses are particularly high when processing oils with free fatty acids over 3.0%, and (3) disposal of the soapstock produced has become more difficult.

The second process, which has become known as physical refining, consists of removing the fatty acids from the oil by steam distillation under vacuum after the phosphatides have been removed by a degumming process followed by a pretreatment process before bleaching. The major advantages for physical refining are the elimination of soapstock, lower capital costs and fewer processes to operate and maintain. The objective of the initial processing step in either refining method is the removal of phosphatides, color bodies, and trace metals. Removal of these nontriglyceride impurities is crucial to ensure good product quality. Herein lies the major drawback for the physical refining system; i.e., complete phosphatide removal with degumming and bleaching is very difficult. Some of the other problems with physical refining systems can be: (1) additional bleaching earth usually required, (2) pesticides are co-distilled with the fatty acids during steam refining, (3) phosphoric acid treatment may darken the gums produced and incomplete removal can produce off flavors in the oil after deodorization, (4) steam distillation or deodorizer units must be designed to handle higher concentrations of free fatty acids, (5) cottonseed oil cannot be physically refined because the gossypol pigment must be removed with alkali refining, and (6) it may be necessary to steam refine before hydrogenation or other processing to adjust melting characteristics and deodorize again following these processes. Physical refining is favored for processing high free acidity oils with low phosphatide contents; it has been demonstrated to produce good quality product from coconut, palm kernel, palm, lard, tallow, and some of the seed oils [10].

D. Degumming

Degumming is the treatment of crude vegetable oils with water, salt solutions. or dilute acids such as phosphoric, citric, or maleic to remove phosphatides, waxes, and other impurities. Degumming converts the phosphatides to hydrated gums, which are insoluble in oil for separation as a sludge by settling, filtering, or centrifugal action. Phosphatide removal is the first process for the physical refining system and can also be for chemical refining. However, with chemical refining the processor has the option of removing the phosphatides for their by-product value as lecithin or treating them as impurities to be removed along with free fatty acids during caustic neutralization.

E. Caustic Neutralization

The conventional caustic neutralization process is the most widely used and most well known purification system. The addition of an alkali solution to a crude oil brings about a number of chemical and physical reactions: (1) the alkali combines with the free fatty acid present to form soaps, (2) the phosphatides absorb alkali and are coagulated through hydration, (3) pigments are degraded, absorbed by the gums, or made water soluble by the alkali, and (4) the insoluble matter is entrained with the other coagulable material. Efficient separation of the soapstock from the neutralized oil is a significant factor in caustic neutralization which is usually accomplished with centrifugal separators. The conventional caustic soda neutralization systems have the flexibility to efficiently refine all of the crude oils presently utilized for food products [4].

Caustic neutralization is ordinarily accomplished by treating the fat or oil with diluted sodium hydroxide. This treatment forms soapstock with the free fatty acids, phosphatides, trace metals, pigments, and other nonglyceride impurities that can be separated by settling or centrifugal force from the neutralized oil. The neutral oil is usually water washed and again separated by settling or centrifuged to remove trace impurities and residual soaps from the neutralization and separation processes. After water washing, the oil is either dried with a vacuum dryer or immediately bleached to remove the trace quantities of water remaining.

F. Bleaching

Edible fats and oils bleaching is popularly and correctly regarded as the partial or complete removal of color; however, bleaching is also an integral process in both the chemical and physical refining systems. Bleaching is relied upon to clean up the traces of soap and phosphatides remaining after caustic neutralization and water washing for the chemical refining system. For physical refining, the technical feasibility depends upon bleaching as a pretreatment to remove phosphatides, trace metals, waxes, and the color pigments. Another, very important function of bleaching, in both refining systems, is the removal of peroxides and secondary oxidation products.

The usual method of bleaching is by adsorption of the pigments and other nonglyceride impurities on bleaching earth. In a typical process, the bleaching materials are added to the oil in an agitated vessel either at atmospheric pressure or under a vacuum. The oil is heated to bleaching temperature and held to allow contact time with the bleaching earth. After the adsorbent has captured the color pigments, soap, phosphatides, trace metals, and polar

materials, it becomes an impurity which must be removed from the oil with a filtration system. Control point impurities analysis are used to monitor the removal of the potential food safety hazard.

G. Animal Fat Purification Systems

Traditionally, the method used to purify meat fats has been a form of physical refining. The two main impurities in meat fats are proteins carried over from the rendering process and free fatty acids. The pretreatment phase for meat fats is the removal of the proteinaceous materials. Typically this is easily accomplished by adding small amounts of diatomaceous earth and/or bleaching earth followed by filtration. An alternative clairification or pretreatment method is to water wash the fat to remove the proteins. This method also requires bleaching or at least drying to remove the moisture remaining in the oil after water washing. A third method for meat fat clarification is caustic refining. Chemical refining is usually reserved for poor quality animal fats or for specialty products used undeodorized to preserve the characteristic meat fat flavor. The caustic refining system consists of caustic neutralization, water washing and vacuum drying.

H. Hydrogenation

The hydrogenation process is an important tool for the edible fats and oils processor. With hydrogenation, liquid oils can be converted into plastic or hard fats more suitable for a particular food product. There are two reasons to hydrogenate a fat or oil: (1) to change the physical form for product functionality improvement, and (2) to improve oxidative stability. Hydrogenation involves the chemical addition of hydrogen to the double bonds in the unsaturated fatty acids. The reaction is carried out by mixing heated oil and hydrogen gas in the presence of a catalyst. After the hydrogenation endpoint has been achieved, the hardened oil is cooled and filtered to remove the nickel catalyst.

Most hydrogenations are preformed in batch reactors due to the variation in raw materials and the desired end products. Normally, batch hydrogenation are performed in an agitated tank reactor with heating and cooling capabilities designed to withstand pressures of 7 to 10 bar. First, the catalyst is suspended in the oil. Then, hydrogen gas, dispersed as bubbles, must be dissolved in the oil to reach the surface of the catalyst. The three reaction variables, pressure, temperature, and rate of agitation are controlled to reduce batch to batch variation for preparation of the desired hydrogenated product or basestock. The typical analytical evaluations for endpoint control which measure consistency are refractive index, iodine value, and various melting points. A food safety control point would be the incomplete removal of the nickel catalyst after the reaction is completed; however, this is not a critical control point because the post bleaching process immediately following hydrogenation is designed to remove the remaining trace catalyst impurities.

I. Post-Bleaching

A separate bleaching operation, immediately following the hydrogenation process, has three purposes: (1) insurance that all traces of the prooxidant hydrogenation catalyst that have escaped the filtration system after hydrogenation have been removed, (2) to remove undesirable colors generally of a greenish hue accentuated during hydrogenation by heat bleaching of the red and yellow pigments, and (3) removal of peroxide and secondary oxidation products. Post-bleach systems are usually batch systems for the same reasons as for hydrogenation systems; production of a wide variety of hydrogenated basestocks.

J. Fractionation

Edible fats and oils are fractionated to provide new materials more useful than the natural product. Fractionation may be practiced to remove an undesirable component, which is the case with dewaxing and winterization, or to provide two or more functional products from the same original fat or oil as is the case with cocoa butter equivalents or substitutes and high stability oils.

The three fractionation process types practiced commercially to produce the value added products are: (1) dry fractionation. (2) solvent fractionation, and (3) aqueous detergent fractionation. Dry fractionation, which includes winterization, dewaxing, hydraulic pressing, and crystal fractionation processes, is probably the most widely practiced. Solvent or aqueous detergent fractionation process provide better separation of specific fractions for the more sophisticated fats and oils products. All of these fractionation process practice the three successive stages of fractionation: (1) cooling the oil to supersaturation to form the nuclei for crystallization, (2) progressive growth of the crystalline and liquid phases, and (3) separation of the crystalline and liquid fractions. A food safety control point identified for the solvent fractionation system would naturally be removal of the solvent used. Complete solvent removal is assured with steam distillation in the deodorization process which is downstream.

K. Interesterification

The interesterification process can alter the original order of distribution of the fatty acids in the triglyceride-producing products with melting and crystallization characteristics different from the original oil or fat. Unlike hydrogenation, interesterification neither affects the degree of saturation nor causes isomerization of the fatty acid double bond. It does not change the fatty acid composition of the starting material but rearranges the fatty acids on the glycerol molecule. The process of interesterification can be considered as the removal of fatty acids

from the glyceride molecules, shuffling them, and then replacement on the glyceride molecules at random. This change in the distribution of the fatty acids affects the structural properties and melting behavior of the fats and oils. Commercially, the interesterification process has been utilized for the production of confectionery fats, margarine oils, cooking oils, frying fats, shortenings, and other special application fats and oils products.

Two types of chemical interesterification process are practiced: random or directed. Random rearrangement of fats and oils can be accomplished using either a batch or continuous process. Both random interesterification processes perform the three important rearrangement steps: (1) pretreatment of the oil, (2) reaction with the catalyst, and (3) deactivation of the catalyst. In the directed rearrangement process, one or more of the triglyceride products of the interesterification reaction is selectively removed from the ongoing reaction. Continuous processes are normally used for directed rearrangements for better control. Trisaturated glycerides are crystallized and separated from the reaction which upsets the reaction equilibrium so that more trisaturated glycerides are produced.

L. Blending

Different stocks are blended to produce the specified composition, consistency, and stability requirements for the various fats and oils products, such as shortenings, frying fats, margarine oils, specialty products, and even some salad or cooking oils. The basestocks may be composed of hydrogenated fats and oils, interesterified products, refined and bleached vegetable oils, purified animal fats, and/or fractions from winterization, dewaxing, or another form of fractionation. The products are blended to meet both the composition and analytical consistency controls identified by the product developers and quality assurance. The consistency controls frequently include analytical testing for solids fat index, iodine value, various melting points, fatty acid composition, and other evaluations designed to insure compliance with customer requirements. The blending process requires scale tanks and meters to proportion the basestocks accurately for each different product. The blend tanks should be equipped with agitators and heating to assure a uniform blend for consistency control [4].

M. Deodorization

With conventional edible oil processing, deodorization is the last in a series of process steps used to improve the taste, odor, stability, and the food safety of the fats and oils by the removal of undesirable substances. In this process, the fats and oils products are steam-distilled under vacuum. The object is to remove the volatile impurities from the oil. The foremost concern from a quality aspect is the volatile impurities are the objectionable flavors and odors; however, deodorization is also very important from a food safety aspect. Steam distillation removes any trace pesticide and "heavy" metals contents obtained during the growing process. Deodorization is primarily a high-temperature, high-vacuum, steam distillation process to remove volatile, odoriferous materials present in edible fats and oils. It is the last major processing step through which the flavor and odor and many of the stability qualities of a fat or oil product can be changed. From this point forward, efforts must directed toward retaining the quality that has been built into the fat and oil product with all of the preceding processes [4].

The odoriferous substances in fats and oils are generally considered to be free fatty acids, peroxides, aldehydes, ketones, alcohols, and other organic compounds. Experience has shown that the removal of flavor, odor, and other undesirables correlates well with the reduction of free fatty acids. Therefore, all commercial deodorization consists of steam stripping the oil for free fatty removal. Currently, batch, semicontinuous, and continuous systems of various designs are utilized by edible fats and oils processors to produce deodorized oil. All of the systems utilize steam stripping with four interrelated operating variables: (1) vacuum, (2) temperature, (3) stripping steam rate, and (4) holding time.

N. Liquid Oil Filling and Packaging

Most salad and cooking oils are packaged shortly after deodorization in containers for home, restaurant, or large food processor use. The processing necessary for most oils is oxidative stability preservation measures such as nitrogen protection, temperature control, light avoidance, and the addition of any additives required by the individual products. The oil is filtered for a final time in-line to the bottle filler. The effectiveness of this final filtration is monitored with laboratory filterable impurities testing of packaged product samples obtained utilizing a statistical sampling plan. Food safety concerns for retail liquid oils were lessen with the packaging change from glass to plastic containers. Glass breakage and contamination of other containers were major concerns when glass bottles were used. Exposure of the oil to the atmosphere is limited to a micro-second for most filling lines with a tamper-evident seal applied to the container before the cap is applied.

O. Shortening Plasticization and Packaging

Plasticized shortening products can be defined as fats with a consistency that can be readily spread, mixed, or worked. Considerably more is involved in the plasticization of shortening and margarine than merely lowering the temperature to cause solidification. Slow cooling of these products produces a grainy, pasty, non-uniform mushy product that lacks the appearance, texture, and functional

characteristics associated with plasticized products. Development of these characteristics are a function of controlled crystallization or plasticization. The final consistency of a shortening is the culmination of all the factors influencing crystallization and plasticization: chilling, working, tempering, pressure, and gas incorporation.

The plasticization process involves the rapid chilling and homogenization of shortening mixture. Most shortenings are quick-chilled in closed thin-film scraped-wall heat exchangers with extrusion valves to deliver a smooth homogeneous product to the package at 17 to 27 atm pressure. Nitrogen is injected at 13 ± 1% into most shortenings to increase the product's workability and provide a white, creamy appearance. After packaging, many processors temper shortenings at temperatures slightly above the packaging temperature to allow the crystal structure of the hard fraction to reach equilibrium and form a stable matrix. After tempering, shortenings are usually stored and shipped at controlled temperatures of 70 to 80°F (21.1 to 26.7°C) to avoid crystal change and loss of the plastic properties [3].

Shortenings are only exposed to the atmosphere during the actual filling of the product into the container which is less than 15 seconds for the slowest filling lines. Protection from contamination from the atmosphere is provided at the filling lines with covers and other protective devices. As in most of the other fats and oils processes the oil is filtered for a final time while being pumped to the plasticization equipment. Laboratory filterable impurities testing of the packaged product will determine if this filtration effectively removed any particles of polymerized or charred oil that may have developed during storage or movement to the shortening filling department. A positive finding at this point is only resolved by reworking the packaged product involved. This necessitates a remelting of the product reprocessing that may include bleaching and deodorization. Shortenings free of impurities and that meet all of the other quality requirements are released for shipment after the product has crystallized to the predetermined consistency.

Plasticization provides a safety risk that requires careful control; metal contamination from the plasticization equipment. The scraped wall heat exchangers have very tight tolerances between the floating metal scraper blades and the chilling surface. A malfunction of this equipment can distribute shreds of metal into the product. Protective devices such a magnetic traps downstream of the plasticization equipment, screens to filter the product, and metal detection after packaging are used on almost all shortening and margarine packaging lines. The magnetic traps and the screens need to be inspected on a routine basis, probably daily, to ensure that no problem developed since the last inspection. Metal particle findings necessitate a hold on the release of the product filled on that particular line during the period covered by the last inspection. Packaged product metal detectors usually isolate the package with a positive detection. Inspection of these packages must include the package itself because the detection levels must be set to very sensitive levels. At these levels the equipment will alarm with the tramp metal contained in many packages. The package metal detectors should be tested hourly with a test wand or package with metal contents at the lowest detection level.

P. Margarine Mixing, Chilling, and Packaging

Margarine was developed and continues to be a butter substitute. It is a flavored food product containing 80% fat, made by blending selected fats and oils with other ingredients, such as milk, salt, color and fortified with vitamin A, to produce a table, cooking, or baking fat product that serves the purpose of dairy butter but is different in composition and can be varied for different applications. Now, spreads have been developed as margarine substitutes. The major difference between spreads and margarine is that spreads are not required to contain a minimum of 80% fat.

Processing for margarines and spreads begins with the preparation of an emulsion of the ingredients. Emulsions are prepared by adding the oil soluble ingredients to a heated margarine oil formulations in an agitated emulsion tank. Concurrently, a pasteurized aqueous phase is prepared by mixing all of the water soluble ingredients together in another vat. The water phase is then added to oil phase to make the emulsion. The emulsion is rapidly chilled with scraped wall heat exchangers similar to those used for shortening products. The plasticized products are then formed into prints, or filled into the various containers for consumer, restaurant, or food processor use. Most margarine and spread products are stored at refrigerator temperatures immediately after packaging, except for some specialized baking products [4].

The high moisture content with or without milk makes margarine and spreads a good growth media for microorganisms. Bacteria need food, moisture, and heat to grow. They are not mobile and have to be transported from place to place, most often by hands, shoes, and clothes. Therefore, the first preventive measures to practice are the good manufacturing practices or GMPs. The generic first area for any HACCP program for all areas of the plant, but with special attention is the margarine areas, is employee hygiene to prevent the spreading of human bacteria like *E. coli.* The equipment must be kept clean and sanitized to prevent the growth of microorganisms. Normally, margarine processing equipment are cleaned and sanitized with CIP (Clean In Place) systems. In many plants, a highly acidic solution may be held in the equipment and lines during down periods to discourage bacterial growth. Additionally, any suspect ingredient should be pasteurized before use. Many margarine producers will pasteurize the water phase of the margarine or spread emulsions

using continuous in-line HTST (high-temperature-short-time) pasteurization techniques. Formulation-wise, it is desirable to add preservatives to the product, where permitted. After packaging margarine and spread products are stored, shipped, and displayed in the grocery stores under refrigeration.

The product safety preventive measures are followed up with testing for harmful microorganism contamination. The types of bacteria that most margarine manufacturers check for are [4]:

- Coliform — Some members of the coliform group are found in the intestines of all warm blooded animals. They are not generally considered pathogenic or disease producing but rather as "fellow travelers" with intestinal pathogens. Coliforms do not survive pasteurization. When found in pasteurized product, their presence is suggestive of unsanitary conditions or practices during production, packaging, or storage. Coliform testing measures the quality of the sanitation procedures.
- Standard Plate Count — Total plate count is valuable as a sanitation indicator and for quality information. The bacteria that grow at the total plate count incubation temperature are known as mesophiles and include a wide variety of microorganisms. The media used is nutrient-rich and nonselective. Both pathogenic and nonpathogenic organisms may be present.
- Yeast and Mold — Yeast and mold have very similar growth parameters. Both are able to survive extremes in conditions, such as pH, water activity, and high concentrations of sugar, that most bacteria cannot tolerate. Since yeast and mold can survive such conditions, they are important spoilage organisms in margarine and spread products. The presence of yeast and mold in these products indicates poor sanitation practices.
- Thermophile — The term thermophile is used to describe a group of microorganisms that grow in the 131° to 194°F or 55° to 90°C range. These organisms are very heat sensitive and can cause spoilage of product. They will grow into spores if held at elevated temperatures or if the product is improperly cooled.
- Pathogenic Microorganisms — A pathogen is an organism that causes disease. The two types that are of interest for margarine and spread products are infections and intoxications. *Salmonella, E. coliform,* and *Listeria* are infectious organisms that can grow in margarine-type products. These live organisms are poisonous to humans and cause food poisoning when they are ingested. The other organism that pose a problem is *Staphylococcus.* Some strains of *Staph.* produce a toxin that is poisonous to humans when ingested. Pasteurization will kill the organism but once the toxin has been formed, it remains active. This is the reason hand contamination of product after pasteurization can have very critical effects.

Q. Flaking and Spray Chilling

Fat flakes describe the higher melting fat and oil products solidified in a thin flake form for ease of handling, quick remelting, or for a specific function in a food product. Chill rolls and processed oil formulations have been adapted to produce several different flaked products that can provide distinctive performance characteristics in specialty formulated foods. The flaked products, produced almost exclusively the restaurant and food processor consumers, are hardfats or stearines, shortening chips, icing stabilizers, confectioners fats, hard emulsifiers, and other customer specific products.

The flake products are solidified on a chill roll which has been described as an endless moving chilling surface held at a temperature below the crystallization point of the applied fat or oil product to form a congealed film on the outer surface. Specifically, chill rolls are usually 4 foot diameter hollow metal cylinders, in various lengths, with a machined and ground smooth surface, internally refrigerated, that revolve slowly on longitudinal and horizontal axes, with several options for feeding the melted oil onto the surface. After application, a thin film of liquid fat is carried over the roll, and as the revolution of the roll continues, the fat is partially solidified. The solidified fat is scraped from the roll by a doctor blade positioned ahead of the feeding mechanism with all of the chill roll designs. Flakes are packaged in kraft bags, corrugated cartons with vinyl liners or other suitable containers for storage and shipment [3].

Spray chilled or powdered fats are specialized products developed for ease of incorporation, handling, melting efficiency, uniform delivery with addition systems, encapsulation, and other special purpose uses. The spray chilling process consists of atomizing a molten fat in a crystallization zone or tower, maintained under temperature conditions where a very fine mist of the melted fat is contacted with cooled air or gas to cause crystallization without marked supercooling [4].

Flaked products present the most opportunity for product contamination during processing of all the fats and oils products. During chilling the product is exposed to the atmosphere longer than any of the other packaging operations. Most chill rolls are protected from air borne contamination with shields but the chill time and slow packaging rate require constant attention by the packaging

employees. Metal contamination is also a product risk with some of the chill roll arrangements using application rolls or metal doctor blades. Metal detection after packaging and constant visual attention by the packaging employee have effectively identified any problems. Nevertheless, the preventive measures are proper equipment maintenance, proper equipment set-up, and diligent inspection of the equipment.

R. Bulk Fats and Oils Shipments

Food processors that use fats and oils in large quantities many times have the facilities to handle this ingredient liquid in bulk. All of the products packaged for shipment and use can be provided to the customers in tank cars or tank trucks, except margarine and spread mixes which contain milk and salt. The customers for these bulk products must have fats and oils bulk handling systems to receive, store, and handle the liquid products. Impurities and contamination are the two major food safety concerns which are controlled with a through inspection of the cleanliness of the tankers and in-line filtration of the oil during loading. Oil purity is monitored with laboratory filterable impurities evaluations of representative samples obtained from the tanker after loading. An impurity deviation at this point necessitates unloading of the tanker and reprocessing to remove the impurity identified. In addition, it is standard practice for the bulk oil customer to again filter the oil as it is being unloaded into his facility.

ACKNOWLEDGMENT

The information in this chapter has been modified from "Sanitation and Safety for a Fats and Oils Processing Plant," by Richard D. O'Brien, In Eds., Y. H. Hui et al., *Food Plant Sanitation*, Marcel Dekker, New York, 2003.

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200 US FDA Guidances for Food Security

Nanna Cross
Chicago, IL

CONTENTS

I. INTRODUCTION

To protect our food supply from terrorist attacks, the U.S. Food and Drug Administration (FDA) has issued a number of guidance documents for the food industry. These documents represent FDA's current thinking on the kinds of measures that food establishments may take to minimize the risk that food under their control will be subject to tampering or other malicious, criminal, or terrorist actions.

This chapter discusses some of these documents. Please note the following premises:

1. The information in each document has been modified to make easy readings. Therefore, the original documents must be consulted for details.
2. Some major abbreviations are:
   FR = U.S. Federal Register.
   CFR = U.S. Code of Federal Regulations.
3. For all documents discussed in this chapter, please note that the Emergency Point of Contact is:
   U.S. Food and Drug Administration
   5600 Fishers Lane, Rockville, MD 20857
4. If a food import establishment operator suspects that any of his/her products that are regulated by the FDA have been subject to tampering, "counterfeiting," or other malicious, criminal, or terrorist action, FDA recommends that he/she notify the FDA 24-hour emergency number at 301-443-1240 or call their local FDA District Office. FDA District Office telephone numbers are listed at http://www.fda.gov/ora/inspect_ref/iom/iomoradir.html. FDA recommends that the operator also notify local law enforcement and public health agencies.
5. Additional Resources:

A process called Operational Risk Management (ORM) may help prioritize the preventive measures that are most likely to have the greatest impact on reducing the risk of tampering or other malicious, criminal, or terrorist actions against food. Information on ORM is available in the Federal Aviation Administration (FAA) System Safety Handbook, U.S. Department of Transportation, FAA, December 30, 2000, Chapter 15, Operational Risk Management. The handbook is available at: http://www.asy.faa.gov/Risk/SSHandbook/Chap15_1200.pdf.

The U.S. Department of Transportation, Research and Special Programs Administration has published an

advisory notice of voluntary measures to enhance the security of hazardous materials shipments. It is available at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2002_register&docid=02-3636-filed.pdf. The notice provides guidance to shippers and carriers on personnel, facility and en route security issues.

The U.S. Postal Service has prepared guidance for identifying and handling suspicious mail. It is available at: http://www.usps.com/news/2001/press/mailsecurity/postcard.htm.

The Federal Anti-Tampering Act (18 USC 1365) makes it a federal crime to tamper with or taint a consumer product; to attempt, threaten, or conspire to tamper with or taint a consumer product; or make a false statement about having tampered with or tainted a consumer product. Conviction can lead to penalties of up to $100,000 in fines and up to life imprisonment. The Act is available at: http://www.fda.gov/opacom/laws/fedatact.htm.

The National Infrastructure Protection Center (NIPC) serves as the federal government's focal point for threat assessment, warning, investigation, and response for threats or attacks against U.S. critical infrastructure. The NIPC has identified the food system as one of the eight critical infrastructures, and has established a public-private partnership with the food industry, called the Food Industry Information Sharing and Analysis Center (Food Industry ISAC). The NIPC provides the Food Industry ISAC with access, information, and analysis, enabling the food industry to report, identify, and reduce its vulnerabilities to malicious attacks, and to recover from such attacks as quickly as possible. In particular, the NIPC identifies credible threats and crafts specific warning messages to the food industry. Further information is available at http://www.nipc.gov/ and http://www.foodisac.org/.

Finally, FDA encourages trade associations to evaluate the preventive measures contained in this guidance document and adapt them to their specific products and operations and to supplement this guidance with additional preventive measures when appropriate. FDA welcomes dialogue on the content of sector specific guidance with appropriate trade associations.

Finally, some trade associations have developed food security guidance that is appropriately focused for that specific industry. For example, the International Dairy Food Association has developed a food security guidance document as an aid to the dairy industry.

FDA encourages other trade associations to evaluate the preventive measures contained in this FDA guidance document and adapt them to their specific products and operations and to supplement this guidance with additional preventive measures when appropriate. FDA welcomes dialogue on the content of sector specific guidance with appropriate trade associations.

- Preparing for the possibility of tampering or other malicious, criminal, or terrorist events
- Assigning responsibility for security to knowledgeable individual(s).

When studying the information, there are many areas of overlap among the different guidance documents. There are guidelines that are the same, whether they are for food producer, food manufacturer, food importer or food transport companies, e.g., a good security record of each employee, suspicious packages in a work area, abnormal looking foods, leaking containers, and so on. Such information is repeated in each of the guidance document for several reasons:

1. To maintain the integrity of each document so that the users will understand the intention and details of each guidance in its entity. It must be emphasized that they are not legal documents.
2. Each guidance document is designed for a specific segment of the food industries. If all documents are combined to avoid over-lapping, then each specific segment of the food industries will be frustrated in the study and dissemination of the information to its intended members. This frustration may potentially lead to ignoring such documents because they are difficult to read.
3. Although the repeated information may take up space and printed pages and be responsible for a minor deviation from full professional English, its benefits to the readers outweigh economical and language considerations.

II. FOOD PRODUCERS, PROCESSORS, AND TRANSPORTERS: FOOD PREVENTIVE MEASURES GUIDANCE

This document was issued by the FDA on March 21, 2003 and covers the following:

A. Goals and Objectives

1. Purpose and Scope

This guidance is designed as an aid to operators of food establishments (firms that produce, process, store, repack, relabel, distribute, or transport food or food ingredients). This is a very diverse set of establishments, which includes both very large and very small entities.

This guidance identifies the kinds of preventive measures operators of food establishments may take to minimize the risk that food under their control will be subject to tampering or other malicious, criminal, or terrorist actions. It is relevant to all sectors of the food system, including farms, aquaculture facilities, fishing vessels, producers, transportation operations, processing facilities, packing facilities, and warehouses. It is not intended as guidance for retail food stores or food service establishments.

Operators of food establishments are encouraged to review their current procedures and controls in light of the potential for tampering or other malicious, criminal, or terrorist actions and make appropriate improvements. FDA recommends that the review include consideration of the role that unit and distribution packaging might have in a food security program. This guidance is designed to focus operator's attention sequentially on each segment of the farm-to-table system that is within their control, to minimize the risk of tampering or other malicious, criminal, or terrorist action at each segment. To be successful, implementing enhanced preventive measures requires the commitment of management and staff. Accordingly, FDA recommends that both management and staff participate in the development and review of such measures.

2. Limitations

Not all of the guidance contained in this document may be appropriate or practical for every food establishment, particularly smaller facilities and distributors. FDA recommends that operators review the guidance in each section that relates to a component of their operation, and assess which preventive measures are suitable. Example approaches are provided for many of the preventive measures listed in this document. These examples should not be regarded as minimum standards, nor should the examples provided be considered an inclusive list of all potential approaches to achieving the goal of the preventive measure. FDA recommends that operators consider the goal of the preventive measure, assess whether the goal is relevant to their operation, and, if it is, design an approach that is both efficient and effective to accomplish the goal under their conditions of operation.

3. Structure

This guidance is divided into five sections that relate to individual components of a food establishment operation: management; human element — staff; human element — public; facility; and operations.

B. Food Establishment Operations

1. Management

FDA recommends that food establishment operators consider:

a. Preparation for the possibility of tampering or other malicious, criminal, or terrorist actions

1. assigning responsibility for security to knowledgeable individual(s)
2. conducting an initial assessment of food security procedures and operations, which we recommend be kept confidential
3. having a security management strategy to prepare for and respond to tampering and other malicious, criminal, or terrorist actions, both threats and actual events, including identifying, segregating and securing affected product
4. planning for emergency evacuation, including preventing security breaches during evacuation
5. maintaining any floor or flow plan in a secure, off-site location
6. becoming familiar with the emergency response system in the community
7. making management aware of 24-hour contact information for local, state, and federal police/fire/rescue/health/homeland security agencies
8. making staff aware of who in management they should alert about potential security problems (24-hour contacts)
9. promoting food security awareness to encourage all staff to be alert to any signs of tampering or other malicious, criminal, or terrorist actions or areas that may be vulnerable to such actions, and reporting any findings to identified management (for example, providing training, instituting a system of rewards, building security into job performance standards)
10. having an internal communication system to inform and update staff about relevant security issues
11. having a strategy for communicating with the public (for example, identifying a media spokesperson, preparing generic press statements and background information, and coordinating press statements with appropriate authorities)

b. Supervision

1. providing an appropriate level of supervision to all staff, including cleaning and maintenance staff, contract workers, data entry and computer support staff, and especially, new staff
2. conducting routine security checks of the premises, including automated manufacturing lines, utilities and critical computer data systems (at a frequency appropriate to the operation) for signs of tampering or malicious, criminal, or terrorist actions or areas that may be vulnerable to such actions

c. Recall strategy

1. identifying the person responsible, and a backup person
2. providing for proper handling and disposition of recalled product
3. identifying customer contacts, addresses and phone numbers
4. investigation of suspicious activity

5. investigating threats or information about signs of tampering or other malicious, criminal, or terrorist actions
6. alerting appropriate law enforcement and public health authorities about any threats of or suspected tampering or other malicious, criminal, or terrorist actions

d. Evaluation program

1. evaluating the lessons learned from past tampering or other malicious, criminal, or terrorist actions and threats
2. reviewing and verifying, at least annually, the effectiveness of the security management program (for example, using knowledgeable in-house or third party staff to conduct tampering or other malicious, criminal, or terrorist action exercises and mock recalls and to challenge computer security systems), revising the program accordingly, and keeping this information confidential
3. performing random food security inspections of all appropriate areas of the facility (including receiving and warehousing, where applicable) using knowledgeable in-house or third party staff, and keeping this information confidential
4. verifying that security contractors are doing an appropriate job, when applicable

2. Human Element — Staff

Under Federal law, food establishment operators are required to verify the employment eligibility of all new hires, in accordance with the requirements of the Immigration and Nationality Act, by completing the INS Employment Eligibility Verification Form (INS Form I-9). Completion of Form I-9 for new hires is required by 8 USC 1324a and nondiscrimination provisions governing the verification process are set forth at 8 USC 1324b.

FDA recommends that food establishment operators consider:

a. Screening (pre-hiring, at hiring, post-hiring)

This involves examining the background of all staff (including seasonal, temporary, contract, and volunteer staff, whether hired directly or through a recruitment firm) as appropriate to their position, considering candidates' access to sensitive areas of the facility and the degree to which they will be supervised and other relevant factors (for example, obtaining and verifying work references, addresses, and phone numbers, participating in one of the pilot programs managed by the Immigration and Naturalization Service and the Social Security Administration [These programs provide electronic confirmation of employment eligibility for newly hired employees. For more information call the INS SAVE Program toll free at 1-888-464-4218, fax a request for information to (202) 514-9981, or write to US/INS, SAVE Program, 425 I Street, NW, ULLICO-4th Floor, Washington, DC 20536. These pilot programs may not be available in all states], having a criminal background check performed by local law enforcement or by a contract service provider [Remember to first consult any state or local laws that may apply to the performance of such checks]).

Note: screening procedures should be applied equally to all staff, regardless of race, national origin, religion, and citizenship or immigration status.

b. Daily work assignments

1. knowing who is and who should be on premises, and where they should be located, for each shift
2. keeping information updated

c. Identification

1. establishing a system of positive identification and recognition that is appropriate to the nature of the workforce (for example, issuing uniforms, name tags, or photo identification badges with individual control numbers, color coded by area of authorized access), when appropriate
2. collecting the uniforms, name tag, or identification badge when a staff member is no longer associated with the establishment

d. Restricted access

1. identifying staff that require unlimited access to all areas of the facility
2. reassessing levels of access for all staff periodically
3. limiting access so staff enter only those areas necessary for their job functions and only during appropriate work hours (for example, using key cards or keyed or cipher locks for entry to sensitive areas, color coded uniforms [remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes])
4. changing combinations, rekeying locks and/or collecting the retired key card when a staff member who is in possession of these is no longer associated with the establishment, and additionally as needed to maintain security

e. Personal items

1. restricting the type of personal items allowed in establishment
2. allowing in the establishment only those personal use medicines that are necessary for the health of staff and ensuring that these personal use medicines are properly labeled and stored away from food handling or storage areas
3. preventing staff from bringing personal items (for example, lunch containers, purses) into food handling or storage areas

4. providing for regular inspection of contents of staff lockers (for example, providing metal mesh lockers, company issued locks), bags, packages, and vehicles when on company property (Remember to first consult any federal, state, or local laws that may relate to such inspections)

f. Training in food security procedures

1. incorporating food security awareness, including information on how to prevent, detect, and respond to tampering or other malicious, criminal, or terrorist actions or threats, into training programs for staff, including seasonal, temporary, contract, and volunteer staff
2. providing periodic reminders of the importance of security procedures (for example, scheduling meetings, providing brochures or payroll stuffers)
3. encouraging staff support (for example, involving staff in food security planning and the food security awareness program, demonstrating the importance of security procedures to the staff)

g. Unusual behavior

This involves watching for unusual or suspicious behavior by staff (for example, staff who, without an identifiable purpose, stay unusually late after the end of their shift, arrive unusually early, access files/information/areas of the facility outside of the areas of their responsibility; remove documents from the facility; ask questions on sensitive subjects; bring cameras to work).

h. Staff health

This involves being alert for atypical staff health conditions that staff may voluntarily report and absences that could be an early indicator of tampering or other malicious, criminal, or terrorist actions (for example, an unusual number of staff who work in the same part of the facility reporting similar symptoms within a short time frame), and reporting such conditions to local health authorities.

3. Human Element — Public

FDA recommends that food establishment operators consider visitors (for example, contractors, supplier representatives, delivery drivers, customers, couriers, pest control representatives, third-party auditors, regulators, reporters, tours):

1. inspecting incoming and outgoing vehicles, packages and briefcases for suspicious, inappropriate or unusual items or activity, to the extent practical
2. restricting entry to the establishment (for example, checking visitors in and out at security or reception, requiring proof of identity, issuing visitors badges that are collected upon departure, accompanying visitors)
3. ensuring that there is a valid reason for the visit before providing access to the facility - beware of unsolicited visitors
4. verifying the identity of unknown visitors
5. restricting access to food handling and storage areas (for example, accompanying visitors, unless they are otherwise specifically authorized)
6. restricting access to locker room

4. Facility

FDA recommends that food establishment operators consider:

a. Physical security

1. protecting perimeter access with fencing or other deterrent, when appropriate
2. securing doors (including freight loading doors, when not in use and not being monitored, and emergency exits), windows, roof openings/hatches, vent openings, ventilation systems, utility rooms, ice manufacturing and storage rooms, loft areas, trailer bodies, tanker trucks, railcars, and bulk storage tanks for liquids, solids, and compressed gases, to the extent possible (for example, using locks, "jimmy plates," seals, alarms, intrusion detection sensors, guards, monitored video surveillance [remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes])
3. using metal or metal-clad exterior doors to the extent possible when the facility is not in operation, except where visibility from public thoroughfares is an intended deterrent (remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes)
4. minimizing the number of entrances to restricted areas (remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes)
5. securing bulk unloading equipment (for example, augers, pipes, conveyor belts, and hoses) when not in use and inspecting the equipment before use
6. accounting for all keys to establishment (for example, assigning responsibility for issuing, tracking, and retrieving keys)
7. monitoring the security of the premises using appropriate methods (for example, using security patrols [uniformed and/or plain-clothed], video surveillance)

8. minimizing, to the extent practical, places that can be used to temporarily hide intentional contaminants (for example, minimizing nooks and crannies, false ceilings)
9. providing adequate interior and exterior lighting, including emergency lighting, where appropriate, to facilitate detection of suspicious or unusual activities
10. implementing a system of controlling vehicles authorized to park on the premises (for example, using placards, decals, key cards, keyed or cipher locks, issuing passes for specific areas and times to visitors' vehicles)
11. keeping parking areas separated from entrances to food storage and processing areas and utilities, where practical

b. Laboratory safety

1. restricting access to the laboratory (for example, using key cards or keyed or cipher locks [remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes])
2. restricting laboratory materials to the laboratory, except as needed for sampling or other appropriate activities
3. restricting access (for example, using locks, seals, alarms, key cards, keyed or cipher locks) to sensitive materials (for example, reagents and bacterial, drug, and toxin positive controls)
4. assigning responsibility for integrity of positive controls to a qualified individual
5. knowing what reagents and positive controls should be on the premises and keeping track of them
6. investigating missing reagents or positive controls or other irregularities outside a normal range of variability immediately, and alerting appropriate law enforcement and public health authorities about unresolved problems, when appropriate
7. disposing of unneeded reagents and positive controls in a manner that minimizes the risk that they can be used as a contaminant

c. Storage and use of poisonous and toxic chemicals (for example, cleaning and sanitizing agents, pesticides)

1. limiting poisonous and toxic chemicals in the establishment to those that are required for the operation and maintenance of the facility and those that are being held for sale
2. storing poisonous and toxic chemicals as far away from food handling and storage areas as practical
3. limiting access to and securing storage areas for poisonous and toxic chemicals that are not being held for sale (for example, using keyed or cipher locks, key cards, seals, alarms, intrusion detection sensors, guards, monitored video surveillance [remember to consult any relevant federal, state or local fire codes that may apply before making any changes])
4. ensuring that poisonous and toxic chemicals are properly labeled
5. using pesticides in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (for example, maintaining rodent bait that is in use in covered, tamper-resistant bait stations)
6. knowing what poisonous and toxic chemicals should be on the premises and keeping track of them
7. investigating missing stock or other irregularities outside a normal range of variation and alerting appropriate law enforcement and public health authorities about unresolved problems, when appropriate

5. Operations

FDA recommends that food establishment operators consider:

a. Incoming materials and contract operations

1. using only known, appropriately licensed or permitted (where applicable) contract manufacturing and packaging operators and sources for all incoming materials, including ingredients, compressed gas, packaging, labels, and materials for research and development
2. taking reasonable steps to ensure that suppliers, contract operators and transporters practice appropriate food security measures (for example, auditing, where practical, for compliance with food security measures that are contained in purchase and shipping contracts or letters of credit, or using a vendor approval program)
3. authenticating labeling and packaging configuration and product coding/expiration dating systems (where applicable) for incoming materials in advance of receipt of shipment, especially for new products
4. requesting locked and/or sealed vehicles/containers/railcars, and, if sealed, obtaining the seal number from the supplier and verifying upon receipt, making arrangements to maintain the chain of custody when a seal is broken for inspection by a governmental agency or as a result of multiple deliveries
5. requesting that the transporter have the capability to verify the location of the load at any time, when practical

6. establishing delivery schedules, not accepting unexplained, unscheduled deliveries or drivers, and investigating delayed or missed shipments
7. supervising off-loading of incoming materials, including off hour deliveries
8. reconciling the product and amount received with the product and amount ordered and the product and amount listed on the invoice and shipping documents, taking into account any sampling performed prior to receipt
9. investigating shipping documents with suspicious alterations
10. inspecting incoming materials, including ingredients, compressed gas, packaging, labels, product returns, and materials for research and development, for signs of tampering, contamination or damage (for example, abnormal powders, liquids, stains, or odors, evidence of resealing, compromised tamper-evident packaging) or "counterfeiting" (for example, inappropriate or mismatched product identity, labeling, product lot coding or specifications, absence of tamper-evident packaging when the label contains a tamper-evident notice), when appropriate
11. evaluating the utility of testing incoming ingredients, compressed gas, packaging, labels, product returns, and materials for research and development for detecting tampering or other malicious, criminal, or terrorist action
12. rejecting suspect food
13. alerting appropriate law enforcement and public health authorities about evidence of tampering, "counterfeiting" or other malicious, criminal, or terrorist action

b. Storage

1. having a system for receiving, storing, and handling distressed, damaged, returned, and rework products that minimizes their potential for being compromised or to compromise the security of other products (for example, destroying products that are unfit for human or animal consumption, products with illegible codes, products of questionable origin, and products returned by consumers to retail stores)
2. keeping track of incoming materials and materials in use, including ingredients, compressed gas, packaging, labels, salvage products, rework products, and product returns
3. investigating missing or extra stock or other irregularities outside a normal range of variability and reporting unresolved problems to appropriate law enforcement and public health authorities, when appropriate
4. storing product labels in a secure location and destroying outdated or discarded product labels
5. minimizing reuse of containers, shipping packages, cartons, etc., where practical

c. Security of water and utilities

1. limiting, to the extent practical, access to controls for airflow, water, electricity, and refrigeration
2. securing non-municipal water wells, hydrants, storage, and handling facilities
3. ensuring that water systems and trucks are equipped with backflow prevention
4. chlorinating water systems and monitoring chlorination equipment, where practical, and especially for non-municipal water systems
5. testing non-municipal sources for potability regularly, as well as randomly, and being alert to changes in the profile of the results
6. staying attentive to the potential for media alerts about public water provider problems, when applicable
7. identifying alternate sources of potable water for use during emergency situations where normal water systems have been compromised (for example, trucking from an approved source, treating on-site or maintaining on-site storage)

d. Finished products

1. ensuring that public storage warehousing and shipping operations (vehicles and vessels) practice appropriate security measures (for example, auditing, where practical, for compliance with food security measures that are contained in contracts or letters of guarantee)
2. performing random inspection of storage facilities, vehicles, and vessels
3. evaluating the utility of finished product testing for detecting tampering or other malicious, criminal, or terrorist actions
4. requesting locked and/or sealed vehicles/containers/railcars and providing the seal number to the consignee
5. requesting that the transporter have the capability to verify the location of the load at any time
6. establishing scheduled pickups, and not accepting unexplained, unscheduled pickups
7. keeping track of finished products
8. investigating missing or extra stock or other irregularities outside a normal range of variation and alerting appropriate law enforcement and public health authorities about unresolved problems, when appropriate
9. advising sales staff to be on the lookout for counterfeit products and to alert management if any problems are detected

e. Mail/packages

This involves implementing procedures to ensure the security of incoming mail and packages (for example,

locating the mailroom away from food processing and storage areas, securing mailroom, visual or x-ray mail/package screening, following U.S. Postal Service guidance).

f. Access to computer systems

1. restricting access to computer process control systems and critical data systems to those with appropriate clearance (for example, using passwords, firewalls)
2. eliminating computer access when a staff member is no longer associated with the establishment
3. establishing a system of traceability of computer transactions
4. reviewing the adequacy of virus protection systems and procedures for backing up critical computer based data systems
5. validating the computer security system

If a food establishment operator suspects that any of his/her products that are regulated by the FDA have been subject to tampering, "counterfeiting," or other malicious, criminal, or terrorist action, FDA recommends that he/she notify the FDA 24-hour emergency number at 301-443-1240 or call their local FDA District Office. FDA District Office telephone numbers are listed at: http://www.fda.gov/ora/inspect_ref/iom/iomoradir.html. FDA recommends that the operator also notify appropriate law enforcement and public health authorities.

III. BULK OVER-THE-ROAD FOOD TANKER TRANSPORT SAFETY AND SECURITY GUIDELINES

A. Introduction

On October 2003, the FDA posted this industry information as a service to industry, consumers, the media, and other interested parties. This document was sponsored by the following trade associations:

American Bakers Association
American Frozen Food Institute
Concord Grape Association
Corn Refiners Association
Grocery Manufacturers of America
Institute of Shortening and Edible Oils
International Dairy Foods Association
National Food Processors Association
National Institute of Oilseed Products
National Juice Products Association
National Milk Producers Association
National Tank Truck Carriers
Northwest Food Processors Association
Processed Apples Institute
Snack Food Association
The Vinegar Institute

Information and recommendations contained in this document are intended as voluntary guidelines for the safe and secure transport and handling of over-the-road food tankers. As such, the sponsoring organizations do not guarantee or warrant, expressly or by implication, that compliance with the guidelines will prevent damage, spoilage, accidents, or injuries to persons or property. Any inference of such a guarantee or warranty is expressly and specifically disclaimed.

It is the sole responsibility of any company or person using the guidelines and related information provided in this publication, and not the responsibility of the sponsoring organizations, to ensure that such company or person is proficient in the operations and procedures discussed in this publication. The use of statements, recommendations, or suggestions contained herein creates no responsibility on the part of the sponsoring organizations for damage, spoilage, loss, accident or injury resulting from such use, or irrespective of such use. Moreover, adoption of any of the guidelines or recommendations included in this publication does not assure compliance with legal or regulatory requirements. Those involved with the production, handling and transportation of foods are advised to become familiar with all relevant and applicable local, state and federal regulations and to ensure that they comply with such requirements as appropriate.

A company policy should be established to designate authorized personnel for acceptance of incoming transportation equipment. The policy document should be maintained in company files in accordance with company policy. In addition to these guidelines, a company may provide employees with additional information and forms for use in acceptance of transportation equipment. Consult your legal counsel for guidance on related legal requirements concerning the transportation of foods.

B. Documentation

1. Recommendations

1. The contract and/or verification between a shipper and carrier should include a clause identifying the last three prior cargoes, a copy of the last wash ticket and documentation if the tanker has been converted from non-food to food grade.
2. The carrier should supply documentation to the shipper confirming that all equipment being used is for food grade purposes. This information should be kept on file at the shipper's office.
3. The commodities the carrier may haul in tankers contracted for shipper service are those agreed upon between the parties.

4. A new piece of equipment should have a sanitary cleaning prior to placement in service.
5. Except for dedicated equipment, in-service equipment should have a listing of the last three loads and a copy of the last wash out certificate.
6. If special handling requirements are necessary, they should be specified in the Bill of Lading. Special requirements may include, but are not limited to temperature specifications or restrictions, pumps and nitrogen overlays.

C. Receipt and Inspection of Empty Tanker

1. Receipt

1. Plant personnel should take all reasonable measures and precautions to assure that plant operators conform to the requirements in 21 CFR 110 (Current Good Manufacturing Practices (CGMPs) and 9 CFR Part 417 (Hazard Analysis Critical Control Point (HACCP) Systems). The loading and unloading areas should be designed and maintained in accordance with Good Manufacturing Practices or appropriate regulations in order to reduce the potential for contamination of the inbound product.
2. The tanker should be identified for use if there are specific regulation requirements — juice, seafood, meat and poultry, etc., and/or if there are specific customer requirements.
3. Confirm that access points were sealed at the wash station with numbered, tamper-evident seals to guard against subsequent contamination of the cleaned trailer before delivery. This should include at least all major points of entry and discharge. Sealing points may include the dome cover, tank outlet, vent cap, pump inlet, pump outlet, and hose tube covers.
4. In the event that transfer hoses and/or the shipper or consignee supplies piping, seals need not be applied to hose tubes mounted on the cargo tank. If seals are present and are broken or tampered with by enforcement personnel, documentation must be produced by enforcement personnel to verify such action. Tank wash facilities, shippers and consignees should be authorized to remove and replace seals.
5. Confirm that hoses and pump outlets were capped and sealed at the wash station after cleaning. Trailers hauling food grade commodities should be washed on a regular schedule regardless of whether or not they have hauled food, the interval for which will be determined under the terms of the contract.
6. All documentation, including wash certificates or tags and bills of lading, should be reviewed and seal identification checked and verified.
7. Seal numbers should be recorded on the wash ticket, or a suitable document designed for that purpose, and verified by the receiver when inspecting the vehicle. Any discrepancy should be reported to management immediately. If the driver indicates that it has been necessary to transfer the lading from one tank to another after washing, contact appropriate plant management.
8. Obtain appropriate documentation from the trucker or the truck company concerning the previous cargo(es). The prior load should be accurately documented and documentation available to assure that the tank has been in acceptable transportation service. Additionally, the trucking company should be able to present independent documentation (e.g., shipper bills of lading) of the last three prior load commodities (by fax or e-mail), upon request.
9. The cleaning certificate issued by the wash station should contain the name of the product last hauled determined via the last shipping documents and should be reviewed and copied for filing. If no cleaning certificate is presented, management should be consulted.
10. If washing is required, a copy of the wash ticket, noting the prior commodity, should be presented to the outbound truck operator. The carrier should also provide wash schedules/wash histories for a specific cargo tank upon request.
11. The prior load records should be verified as designated in the documentation section of this guideline. If the prior cargo was not an acceptable material, do not accept the load and contact appropriate plant management immediately.

2. Visual Inspection

1. The interior of the tank should be inspected visually. The interior of the tank should be clean and free of cracks and corrosion, which can harbor contaminants. If condensate is present or the interior is otherwise unacceptable, check with your supervisor prior to loading. Internal damage or corrosion, foreign objects, incompatible product residue, mold, and moisture are potential causes for rejection.
2. The presence of off-odors or of any residual material when opening the dome cover should be reported to appropriate plant management immediately.
3. Inspect the inside of the tank for evidence of residue of prior cargoes or flaking, which

indicates inadequate cleaning/rinsing of the tank or unacceptable prior cargoes. Be especially alert to those areas hard to inspect visually, such as the top inner portion of the tank.

4. It is recommended that the shippers/receivers use their own pumps and hoses. If the tanker or tractor pump and hoses are used, they should meet all applicable tanker guidelines. If the truck's pumping system is to be used for loading, all hoses and pumps should be visually inspected. Special attention should be paid to pumps located on the tractor, as the tractor unit may not be dedicated to food service use and may not have been cleaned when the trailer was cleaned. If pumps and/or hoses carried on the tractor are to be used, they should be indicated as having been cleaned on the wash station certificate. Tractor mounted blowers, used for the transfer of dry commodities should not be cleaned, but move only air.
5. Inspect all seals, gaskets, pumps, valves, hoses, and hose tubing for cleanliness, integrity, and proper capping. Cracked, corroded, or improperly protected equipment can trap [residual material] and serve as a source of contamination or create an environment conducive to bacterial growth with the potential for contaminating product coming in contact with the surface.
6. The company should have a visual inspection form for plant employees to check off during the tanker inspection. The form should have an accept/reject notation and a space for the employee and/or appropriate signature(s). (Refer to Appendix "C" for an example of an empty tanker inspection form.)
7. A clear company policy should be established to designate authorized personnel for acceptance of incoming transportation equipment. The policy document should be maintained in company files in accordance with company policy. In addition to these guidelines, a company may provide employees with additional information and forms for use in acceptance of transportation equipment. Consult your legal counsel for guidance on related legal requirements concerning the transportation of foods.

D. Tank Truck Loading

1. Recommendations

1. If the tanker is deemed acceptable for loading, sufficient care should be exercised during loading to ensure that the integrity of the product and the tanker vessel are maintained.
2. Product loading and unloading facilities should be designed and maintained in accordance with Good Manufacturing Practices 21 CFR, part 110.
3. After loading, the tanker should be closed and tamper-evident security seals affixed to any access ports, which were unsealed during inspection to preclude tampering with or adulteration of the product during shipment.
4. Seal numbers should be recorded on the bill of lading or other appropriate document.
5. The cargo should be identified on the bill of lading by the common or usual name of the food or food ingredient or as identified by DOT regulations. If the product is classified as a "hazardous material" by DOT regulation, the shipper must supply appropriate product hazard information (e.g., Material Safety Data Sheet (MSDS), vehicle placards and shipping documents.) Product hazard information will also assist carriers and wash rack personnel to determine proper cleaning and passivation procedures, thus preserving the integrity of the cargo tank for food grade service.
6. A copy of the incoming wash certificate with information about the previous cargo should be provided to the outbound truck operator if the tanker has been cleaned prior to loading.
7. Food ingredients such as food-grade chemicals should be identified by the proper shipping name (FDA or DOT regulations).
8. Shippers should ensure that they have provided the trucker with any emergency action information required. Identification will assist the receiver and those responsible for wash station operations in determining the clean-up procedure and assure that tankers dedicated to food shipments remain available.
9. When all information is recorded, sign the bill of lading indicating the shipment may proceed.
10. A clear company policy should be established to designate authorized personnel for certifying proper loading of outbound transportation equipment.

E. Loaded Tanker

1. Receipt

1. When receiving a loaded tanker, obtain a copy of the bill of lading and confirm the cargo and security seal numbers. (If seal numbers do not match or the seals appear to have been tampered with, notify appropriate management immediately. Do not open the tanker or begin unloading.)

2. Review the information provided in the wash ticket copy, and evaluate the prior load information to insure that proper food, food-grade commodities, or acceptable non-food products have been previously carried in the tank. If the prior cargo was not acceptable material, contact appropriate plant management.
3. If all documents and seals are in order, begin your inspection of the tanker and all attendant equipment, including pumps and hoses, for cleanliness and state of repair. (See Appendix "B" for an example of an appropriate checklist.)

F. Minimum Cleaning Requirements for Non-Dairy Food, Food Grade, Liquid Cargo Tanks

1. "Food to Food"

Acceptable media (depending on the prior load, and which may be applied alone or in combination) include:

1. Steam;
2. Hot or cold water;
3. Detergent, where appropriate, according to customer's specifications and the product hauled, observing manufacturer's and chemical suppliers recommendations on pH;
4. Caustic, according to customer's specifications observing manufacturer's recommendation on pH; and
5. Air drying — using an appropriate filter.

Overall criteria to be applied as appropriate to the media used:

1. When used alone or as part of another media, the term "hot water" should mean that water (and cleaning agents applied to product contact surfaces) should be appropriate to clean and sanitize internal surfaces;
2. The term "water" means potable water;
3. Only cleaning compounds as covered by appropriate food additive regulations as established by FDA (or USDA-approved cleaning substances) should be used in any cleaning media (or combinations thereof); and
4. At a minimum, the following accessories and components should be removed (and disassembled) from the unit for cleaning — gaskets, external valves, vents and caps as applicable.

Seals should be applied to all cargo tank access points after cleaning and prior to shipment of the tank to the facility for product loading.

2. "Food to Food" — Dry Bulk

Product wash cycles should follow defined industry standards, or as specified by carriers and shippers. In terms of solutions, the same basic processes used in the cleaning of liquid trailers would be, as appropriate, applicable to the cleaning of dry bulk units. In addition:

1. All lines should be disassembled and cleaned separately; note that standard washes, used routinely between compatible commodities, do not require disassembly of the product line, and conversion washes, used between incompatible commodities, do require disassembly of the product line.
2. Remove, inspect, clean and/or replace aeration pads and dust collectors. Gaskets should be removed and cleaned on all washes. If the prior load involved approved non-food products, all gaskets should be removed and replaced.
3. The internal loading tube and product piping should receive separate cleaning;
4. All fumigants, chemicals and/or detergents must be covered by and used in accordance with appropriate regulations;
5. The drying phase should utilize filtered air; and
6. Seals and tags as appropriate should be applied at the cleaning facility.

3. Additional Criteria Applicable to Both "Food to Food" and "Approved Non-Food to Food" Interior Cleaning

1. Nothing herein should be construed to prohibit internal visual inspections of the trailer, including tank entry to perform such inspection prior to the final hot water rinse.
2. If the use of seals is required, such should be applied by the tank wash facility to all external openings (manways, caps, vents, valves, inlet and outlet side of the pump, etc.) prior to the vessel's return to service.
3. In addition to the above, and whether or not seals are required, the tank wash facility should (prior to the vessel's return to service) supply appropriate documentation noting at a minimum:
   - The name, address, phone and fax numbers of the cleaning facility;
   - The certification number of the facility if Federal, state or local authorities require such information:
   - The date of interior cleaning;
   - The prior load:

- The temperature of water applied for cleaning purposes;
- The cleaning agents applied; and
- If seals are utilized, the seal numbers should be recorded.

4. In some cases, it may be necessary for a given trailer to be subjected to two (or more) cleaning processes, which may involve the use of materials not specified in this standard. Nothing herein should be construed to prohibit this practice, except that the methods, specified herein, should always be the final step in a multi-stage cleaning process.
5. All tank wash facilities performing specified tank wash services for the food industry should receive "third party" inspection and certification. If appropriate, the tank wash facility should be inspected and certified by Federal, state and/or local authorities and/or representatives of shippers, carriers and consignees. Such inspection and/or certification should verify that the facility:
   - Has the equipment and personnel to meet the standards (including document preparation and retention);
   - Has piping (integral to any wash rack mechanism) after a pre-determined filtered control point used to conduct steam, hot and cold water, and cleaning solutions involved in the cleaning of food-grade equipment that is constructed of stainless steel alloys;
   - Has safeguards to preclude the commingling of steam, water, and cleaning solutions used in the interior cleaning of food-grade cargo tanks with steam, water, and cleaning solutions used in the cleaning of non-food grade cargo tanks;
   - Possesses sufficient insurance, or otherwise evidence of financial responsibility, at levels equivalent to those for motor carriers for public liability, property damage, and environmental restoration;
   - If hot washing tankers, has a boiler capable of providing hot water on a continuous basis for a minimum needed to ensure the tanker is clean;
   - Avoids direct contact of steam with food contact surfaces. If steam injection is used to heat water, only food grade boiler treatment additives are used;
   - Utilizes appropriate sanitary wash equipment designed to ensure that all interior surfaces are cleaned and sanitized; and
   - Where appropriate, has a permanently mounted thermometer capable of monitoring and recording water temperature at the discharge valve.

G. Conversion of Trailers from "Non-Approved, Non-Food Service" to "Approved Non-Food to Food" and "Food to Food" Service

(Note: There should be clear, legally binding agreement between carriers, shippers and consignees concerning the use of converted tankers. All conversion steps should be documented.)

1. Minimum Tank Conversion Steps

1. The tank should be appropriately cleaned of prior product;
2. An internal inspection should include determination evaluation and/or removal of:
   - Finish (parent metal and welds);
   - Evidence of pitting;
   - Corrosion;
   - Weld and parent material discoloration; and
   - Odors.
3. Remove, clean, pacify and/or replace:
   - Internal and external vents, valves and caps, discs, and piping;
   - Manifolds;
   - All gaskets (including those on accessories); and
   - Tank interior metal surfaces.
4. A final wash should be performed before applying a seal.
5. Apply a "Food Grade" decal, cleaning tag, and seals (if required).

H. Tank Requirements

1. Non-Dairy Liquid Food Grade Products

1. The shell, heads, and appliances with product contact surfaces should be a minimum alloy #304 SS, low carbon;
2. Weld finish should be W-3;
3. All parent metal finish on product contact surfaces should be 2B;
4. The entire tank surface should be clean-bore (no baffles);
5. If compartments are permitted they should be equipped with double bulkheads with evacuated airspace between bulkheads;
6. With the exception of center-discharge (belly drop) tanks, all tanks should have a positive drain (minimum 4 inch slope from front to back of tankers).

2. Accessories and Fittings on Non-Dairy Liquid Tanks

1. All internal accessories should be capable of being disassembled to clean product-contact surfaces.
2. Internal valves should be acceptable (note: internal valves are mandatory if food-grade product is flammable or corrosive liquid).
3. Clean-out openings should be appropriate.
4. Gaskets should be removable and non-porous.
5. Manways, fittings, and connections should be a minimum alloy #304 SS, low carbon.
6. Vehicle-mounted product transfer equipment if used, should meet the requirements established for the tanker. Product transfer equipment (vehicle-mounted versus facility-supplied) is a matter of choice involving the shipper, carrier and consignee. If "vehicle-mounted" product transfer equipment is used, such equipment should meet the requirements established for the cargo tank.
7. If the use of seals is required by the shipper (at loading) such should be provided and affixed by shipper representatives and should be "cable type" or equivalent "tamper evident." The shipper should provide authorized seals to carriers loading from remote rail facilities where the carrier or a third party acts as the "shipper representative."

3. Dry-Bulk Food Grade Cargo Tanks

1. The use of aluminum alloys in the construction of shell, heads, and accessory equipment is appropriate.
2. Clean-out openings are appropriate.
3. All gaskets should be removable and non-porous.
4. Product transfer equipment - vehicle mounted equipment to accomplish pressure unloading is appropriate.

I. Security

1. Trucking Company

In the interest of safety and security, shippers should maintain and regularly update records of:

1. Carrier contacts (to include names, phone and fax numbers and (if applicable) e-mail contacts;
2. The carrier's "U.S. DOT Safety Rating" available via the Internet;
3. The carrier's compliance with U.S. DOT insurance regulations (available via the internet); and
4. Verification that the carrier has resources (in-house or contractual) to respond to a product spill.

2. Driver

Each cargo tank driver, entering a shipper, wash station, carrier terminal or consignee facility should produce:

1. His/her Commercial Drivers License (with photo); and
2. In light of security concerns, many trucking companies have established methods and procedures whereby parties to the transportation transaction can verify employment status of an individual driver. Shippers and consignees should consult with individual trucking companies for more details.

3. Cleaning Facilities

Shippers, carriers and consignees should maintain and update:

1. Facility contacts (to include names, phone and fax numbers, and if applicable e-mail contacts; and
2. All appropriate certificates (if required by Federal, state and local authorities and/or by carriers, shippers and consignees).

4. Receiving Facility

Follow appropriate instructions discussed in this section and include:

1. That access points are sealed and match appropriate paperwork;
2. That paperwork is verified including previous washing record, investigate and verify suspicious alterations;
3. Verification of the driver; if the driver has changed, do not unload (or load) until his/her credentials are confirmed;
4. If delivery schedule has been changed the receiver should be notified in advance, if not, confirm the reasons for the change before unloading or loading;
5. Where scales are used, reconciliation before unloading, where appropriate, of differences between the amount of product shipped and that received; and
6. Limited access of drivers to the facility.

5. General

In addition to the above, shippers and consignees should assure themselves that trucking companies and/or cleaning facilities have the resources for:

1. Collecting, maintaining and reproducing relevant documents including but not limited to;

shipping papers, records of prior hauls (on a vehicle-by-vehicle basis), cleaning certificate, inspection reports and exception reports; and
2. A written procedure regarding the use of cargo tank access seals (including "what to do" in the event that a seal is broken or shows evidence of tampering.)

Food facilities should consider:

1. Using only known, pre-approved and appropriately licensed or permitted (where appropriate) carriers and wash stations;
2. Establishing agreed upon security measures with shippers;
3. Taking reasonable steps, such as auditing, to ensure that carriers are in compliance with the company's food security measures;
4. Establishing and adhering to regular delivery schedules where feasible;
5. Exercising strict control including scheduling, egress to the facility, unloading and supervision of unloading of "hazardous" materials; and
6. Establishing a formal review process for evaluating shippers, and where appropriate, wash stations.

IV. REFERENCES AND FORMS

A. Resources

1. National Juice Processors Association (NJPA) "Model Tank Wash Guidelines for the Fruit Juice Industry:" http://www.njpa.com/
2. Northwest Food Processors Association (NWFPA) "Over-the-Road Bulk Transport Guidelines for Non-Dairy, Food Grade, Liquid Products:" http://www.belong2it.com/nwfpa/.
3. U.S. Department of Health and Human Services, Food and Drug Administration "Guidance for Industry - Food Processors and Transporters: Food Security Preventive Measure Guidance:" http://www.cfsan.fda.gov/
4. U.S. Department of Transportation, Federal Motor Carrier Safety Administration, "Hazardous Materials Company Anti-terrorism Tips" and "Hazardous Materials Driver Anti-terrorism Tips:" http://www.dot.gov/
5. National Food Processors Association, Food Marketing Institute, "Food Security Manual for Processors, Distributors and Retailers:" http://www.nfpa-food.org/
6. International Society of Beverage Technologists (ISBT) "Quality Guidelines and Analytical Procedure Bibliography for 'Bottlers' High Fructose Corn Syrup 42 and 55 (1999):" http://www.bevtech.org/
7. American Frozen Food Institute (AFFI) "Food Security Risk Management Guide" (2003): http://www.affi.com/.
8. Frozen Food Roundtable, "Frozen Food: Handling and Merchandising" (1999): http://www. affi.com/.
9. U.S. Department of Agriculture, Food Safety and Inspection Service, "FSIS Safety and Security Guidelines for the Transportation and Distribution of Meat Poultry, and Egg Products" http://www.fsis.usda.gov/.

B. Inspection Report Form [Incoming Tanker (Loaded)]

1. Vehicle Identification

Tractor Identification_____ Tanker Identification_____ Date Inspected_____
Name of Carrier_____ Name of Inspector_____ Cargo_____
Shipper_____ Drivers_____
Bill of Lading_____ Identification:_____ Cargo Verified_____

1. Is the outside of the carrier clean? Yes____ No____If no, describe_____
2. Is there written documentation or prior loads? Yes____ No___ If no, notify appropriate management immediately.
3. Source of prior load written documentation: driver_____ truck company_____ broker_____ shipper_____ other_____
4. Are all major points of entry and discharge sealed? Yes____ No____
5. Are seals numbered and recorded on the wash ticket/bill of lading? Yes___ No___
6. Do seal numbers correspond to the numbers on the wash ticket/bill of lading? Yes___ No___
7. Are seals intact with no evidence of tampering? Yes____ No____

If no to #'s 3, 4, 5, 6, or 7, Notify Appropriate Management Immediately.

8. As you open the tanker lid, do you smell off-odors? Yes___ No___. If yes, identify if possible: Describe_____
9. Appearance of the product: Does the product appear normal (color, consistency)? _____ Do you observe evidence of foreign material (identify if possible)?
   - Surface_____
   - Particles_____
10. Samples taken for testing: Yes___ No___
11. Is the following auxiliary equipment clean and in good repair?
    - Hoses: Yes___ No___ Gaskets and seals: Yes___ No___
    - Pump(s): Yes___ No___ Fittings: Yes___ No___
12. Add any other comments or remarks that you may wish regarding what you observed during the inspection:__ _____

Recommendation: Accept ___ Reject___ tanker. Inspector:_____

C. Inspection Report Form [Incoming Tanker (Empty)]

1. Vehicle Identification

Tractor Identification_____ Tanker Identification_____ Date Inspected_____
Name of Carrier_____ Name of Inspector_____
Cargo_____ Shipper_____ Drivers_____

1. Is the outside of the carrier clean? If no, describe:_____
2. Is there written documentation on prior loads? Yes___ no___ If no, notify appropriate management immediately.

Prior Loads:
a.
b.
c.

3. Source of your load written documentation:

trucker____ truck company____ broker___ other ___

4. Is there a valid wash ticket provided with the tanker? Yes___ no___ If no to #4, notify your supervisor immediately.
5. Wash Station_____ Date of Wash_____
6. Are all major points of entry and discharge sealed? Yes___ No___
7. Are seals numbered and recorded on the wash ticket? Yes___ No___
8. Do seal numbers correspond to the numbers on the wash ticket? Yes____ No____
9. Are seals intact with no evidence of tampering? Yes___ No___

If No to #'s 6, 7, 8, or 9, Notify Your Supervisor Immediately.

10. As you open the tanker lid:

    Does it smell clean Yes___ No___ Do you smell off-odors Yes___ No___

11. Condition of Inside of Tanker: Describe as appropriate_____

* Remember, this surface will come in contact with your product, and any residue could contaminate the shipment.

- Clean and in good shape?_____
- Dirty(describe)?_____
- Damaged (describe)?_____

12. Is the following auxiliary equipment clean and in good repair?

Hoses: Yes___ No___ Gaskets and seals: Yes___ No___
Pump(s): Yes___ No___ Fittings: Yes___ No___
Vents: Yes____ No____

Add any other comments or remarks that you may wish regarding what you observed during the inspection:_____
Recommendation: Accept____ Reject____ tanker. Inspector:_____

D. Food Grade Tanker Wash Facility Audit Form

1. General Information

Company:_____
Street Address:_____
City, State, Zip:_____
Phone(s):_____ Fax:_____
Mailing Address:_____
City, State, Zip:_____
Directions from nearest Interstate: _____
Hours of Operation: Monday–Friday _____ Saturday _____ Sunday_____
Contact Person:_____
Product Limitations:_____
Does this facility offer cleaning services outside of normal business hours?
[ ] Yes [ ] No If Yes, please note contact person

- Name:_____
- Phone:_____Fax:_____

Service Capabilities
(i). Check all offered at this facility:

[ ] Caustic	[ ] Exterior wash
[ ] Detergent	[ ] Drying
[ ] Steam	[ ] IBC cleaning
[ ] Hot/Cold water	[ ] ISO container cleaning
[ ] Kosher wash	[ ] Dry bulk container cleaning

(if Kosher box checked, attach copy of Kosher certificate)
If other, please note:_____
[ ] Yes [ ] No Does this facility have insurance?

- If yes, who is insurance carrier?_____
- What are the aggregate amounts?_____

[ ] Yes [ ] No Does this facility routinely check the Bills of Lading from the previous load?

(ii). Chemicals
[ ] Yes [ ] No Does this facility have a chemical use training program for its employees?
[ ] Yes [ ] No Are Material Safety Data Sheets (MSDS) for products handled and used at the facility readily accessible?
[ ] Yes [ ] No Are chemicals used to clean food contact surfaces FDA or USDA approved?
[ ] Yes [ ] No Are the boiler treatment chemicals of food grade?
[ ] Yes [ ] No Are chemicals stored in a locked cage or other restricted area?

(iii). Facilities and Equipment
[ ] Yes [ ] No Does this facility have a covered roof to prevent contamination by dirt and other residues?
[ ] Yes [ ] No Is this facility's housekeeping adequate to prevent contamination by dirt, filth, trash, and other residues?
[ ] Yes [ ] No Does this facility have a boiler capable of providing 180° F water for a minimum of 15 minutes?
[ ] Yes [ ] No Does this facility utilize a sanitary power wash spinner operated to manufacturer's recommended operating conditions?
[ ] Yes [ ] No Does this facility have a permanently mounted thermometer capable of monitoring and recording water temperature at the discharge valve?
[ ] Yes [ ] No Is the piping involved in the cleaning of food grade equipment constructed of stainless steel alloys?
[ ] Yes [ ] No Are safeguards in place to prevent co-mingling of steam, water, and cleaning solutions between food grade and non-food grade systems?

(iv). Water/Wastewater
Please describe water source and any pretreatment systems:_____
Is water source hard? If so, is it softened? Is it filtered? How?_____
[ ] Yes [ ] No Does the facility have a written waste-management program?
What is the disposal method for waste from food grade tank washes?_____
Name and address of the waste water receiving service:_____
Receiving disposal facility's EPA identification number:_____

(v). Audit Recommendations
[ ] Qualified [ ] Not Qualified
If not qualified, list recommendations for qualification:_____
Signed_____Title _____ Date_____
Company_____

V. DAIRY FARMS, BULK MILK TRANSPORTERS, BULK MILK TRANSFER STATIONS AND FLUID MILK PROCESSORS FOOD SECURITY PREVENTIVE MEASURES GUIDANCE

This guidance (July 2003) presents FDA's current thinking on the kinds of measures that operators of dairy farms, bulk milk transportation operations, bulk milk transfer stations, and fluid milk processing facilities may take to minimize the risk that fluid milk under their control will be subject to tampering or other malicious, criminal, or terrorist actions. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the telephone number listed on the title page of this guidance.

A. Purpose, Scope and Limitations

This guidance is designed as an aid to operators of dairy farms, bulk milk transportation operations, bulk milk transfer stations and fluid milk processing facilities. It identifies the kinds of preventive measures operators of these establishments may take to minimize the risk that fluid milk under their control will be subject to tampering or other malicious, criminal, or terrorist actions. Operators of these establishments are encouraged to review their current procedures and controls in light of the potential for tampering or other malicious, criminal, or terrorist actions and make appropriate improvements.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

Not all of the guidance contained in this document may be appropriate or practical for every dairy farm, bulk milk transportation operation, bulk milk transfer station, or fluid milk processing facility. FDA recommends that operators of these establishments review the guidance in each section that relates to a component of their operation, and assess which preventive measures are suitable. FDA further recommends that operators consider the goal of the preventive measure, assess whether the goal is relevant to their operation, and, if it is, design an approach that is both efficient and effective to accomplish the goal under their conditions of operation.

B. Management

FDA recommends that operators of dairy farms, bulk milk transportation operations, bulk milk transfer stations and fluid milk processing facilities consider:

1. Conducting an initial assessment of the adequacy of food security procedures and operations, which we recommend be kept confidential.
2. Developing a security management strategy to prepare for and respond to tampering and other malicious, criminal or terrorist actions, both threats and actual events, including identifying, segregating and securing affected product.
3. Developing a product recall strategy
4. Providing training in food security awareness to encourage all staff to be alert to any signs of tampering or other malicious, criminal or terrorist actions or areas that may be vulnerable to such actions, and report any findings to management. The training may also encourage staff to be alert to the presence of unidentified or unknown individuals or individuals that are in areas to which they are not designated access, and to directly question such individuals or report them to management
5. Providing appropriate supervision to all staff with access to raw and pasteurized milk storage, vitamin supplement receiving and storage,

and milk processing and packaging areas of the facility, including cleaning, maintenance and quality control staff, seasonal, temporary, contract, and volunteer staff, and especially, new staff. The supervision may include watching for unusual or suspicious behavior by staff (e.g., staff who, without an identifiable purpose, stay unusually late after the end of their shift, arrive unusually early, access files/information/areas of the facility outside of the areas of their responsibility; remove documents from the facility; ask questions on sensitive subjects; bring cameras to work)
6. Conducting routine security checks of the raw and pasteurized milk storage, vitamin supplement receiving and storage, and milk processing and packaging areas of the facility, for signs of tampering or malicious, criminal or terrorist actions or areas that may be vulnerable to such actions.
7. Alerting appropriate law enforcement and public health authorities about any threats of or suspected tampering or other malicious, criminal or terrorist actions. FDA may be contacted through its 24-hour emergency number, 301-443-1240, or through a local FDA District Office. FDA District Office telephone numbers are listed at: http://www.fda.gov/ora/inspect_ref/iom/iomoradir.html.
8. Reviewing, at least annually, the effectiveness of the food security plan, using knowledgeable in-house or third party staff, and revising the program accordingly, which we recommend be kept confidential.

C. Human Element

FDA recommends that operators of dairy farms, bulk milk transportation operations, bulk milk transfer stations and fluid milk processing facilities consider:

1. Obtaining and verifying work references, addresses and phone numbers of all staff with access to raw and pasteurized milk storage, vitamin supplement receiving and storage, and milk processing and packaging areas of the facility, including cleaning, maintenance and quality control staff, seasonal, temporary, contract, and volunteer staff.
2. Having a criminal background check performed by local law enforcement or by a contract service provider for the above listed staff, except if such staff are under direct supervision when they access the above listed areas.
3. Limiting access to raw and pasteurized milk storage, vitamin supplement receiving and storage, and milk processing and packaging areas of the facility to those staff that need to enter because of their job functions and only during appropriate work hours.
4. Preventing staff from bringing personal items (e.g., lunch containers, purses) into raw and pasteurized milk storage, vitamin supplement receiving and storage, and milk processing and packaging areas of the facility.
5. Being alert for atypical staff health conditions that staff may voluntarily report and absences that could be an early indicator of tampering or other malicious, criminal or terrorist actions (e.g., an unusual number of staff who work in the same part of the facility reporting similar symptoms within a short time frame), and reporting such conditions to local health authorities
6. Accompanying all visitors.

D. Facility

FDA recommends that operators of dairy farms, bulk milk transportation operations, bulk milk transfer stations and fluid milk processing facilities consider:

1. Securing doors (including freight loading doors, when not in use and not being monitored, and emergency exits), windows, roof openings/hatches, vent openings, ventilation systems, utility rooms, loft areas, trailer bodies, tanker trucks, and bulk storage tanks, to the extent possible.
2. Inspecting bulk unloading equipment and pumps in the receiving area before use.
3. Monitoring the security of the premises.

FDA further recommends that operators of dairy farms consider:

Locking or sealing, with serially numbered seals, all entrances to the milk house or all entry ports on the bulk milk tank from the time the bulk milk tank is washed until the time it is emptied, except when it is under direct, visual supervision (Remember to first make arrangements with the State regulatory agency that will ensure that the regulatory agency, rating agency and FDA continue to have ready access to the milk house and milking operation for routine inspections, Grade "A" IMS ratings and FDA check ratings, when applicable).

E. Operations

Vitamin supplements and laboratory supplies

FDA recommends that operators of fluid milk processing facilities consider:

1. Using only known, appropriately licensed or permitted (where applicable) sources for vitamin supplements.

2. Establishing delivery schedules for vitamin supplements, not accepting unexplained, unscheduled deliveries or drivers, and investigating delayed or missed shipments.
3. Supervising off-loading of incoming vitamin supplements, laboratory reagents and positive controls, including off-hour deliveries.
4. Reconciling the product and amount received with the product and amount ordered and the product and amount listed on the invoice and shipping documents.
5. Investigating shipping documents with suspicious alterations.
6. Inspecting incoming vitamin supplements for signs of tampering, contamination or damage (e.g., abnormal powders, liquids, stains, or odors, evidence of resealing) or "counterfeiting" (e.g., inappropriate or mismatched product identity, labeling, product lot coding or specifications).
7. Storing vitamin supplements, laboratory reagents, and positive controls in a secure location.
8. Keep track of vitamin supplements, laboratory reagents and positive controls and investigating any missing or extra stock outside a predetermined normal range of variability.

F. Labeling

FDA recommends that operators of fluid milk processing facilities consider:

1. Storing product labels in a secure location and destroying outdated or discarded labels

G. Raw Milk

FDA recommends that operators of bulk milk transfer stations and fluid milk processing facilities consider:

1. Accepting only those incoming tanker loads of raw milk for which all openings were either locked or sealed, with a serially numbered seal, from the time the tanker was last washed until the load is delivered. Exception may be provided for incoming loads for which a thorough investigation demonstrates that there is a verified, reasonable explanation for a deviation. Seals or locks need not be in place during those times that the tanker was under the direct, visual supervision of the driver.
2. Using only known, reputable transportation companies
3. Establishing delivery schedules for raw milk, not accepting unexplained, unscheduled deliveries or drivers, and investigating delayed or missed shipments. We recommend that driver identification include the name of the transportation company.
4. Supervising off-loading of incoming milk.
5. Reconciling the amount received with the amount listed on the shipping documents.
6. Verifying that operators of bulk milk transfer stations that supply raw milk adhere to the preventive measures listed in this guidance.

FDA recommends that operators of bulk milk transportation operations consider:

Locking or sealing, with a serially numbered seals, every tanker from the time it is last washed until the time the load of milk is delivered to the bulk milk transfer station or fluid milk processing facility. Seals or locks need not be in place during those times that the tanker is under the direct, visual supervision of the driver.

VI. IMPORTERS AND FILERS: FOOD SECURITY PREVENTIVE MEASURES GUIDANCE

This guidance (March 21, 2003) represents the Agency's current thinking on the kinds of measures that food importers and filers may take to minimize the risk that food under their control will be subject to tampering or other malicious, criminal, or terrorist actions. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public.

A. Purpose and Scope

This guidance is designed as an aid to operators of food importing establishments, storage warehouses, and filers. It identifies the kinds of preventive measures that they may take to minimize the risk that food under their control will be subject to tampering or other malicious, criminal, or terrorist actions. Operators of food importing establishments are encouraged to review their current procedures and controls in light of the potential for tampering or other malicious, criminal, or terrorist actions and make appropriate improvements.

This guidance is designed to focus operator's attention sequentially on each segment of the food delivery system that is within their control, to minimize the risk of tampering or other malicious, criminal, or terrorist action at each segment. To be successful, implementing enhanced preventive measures requires the commitment of management and staff. Accordingly, FDA recommends that both management and staff participate in the development and review of such measures.

B. Limitations

Not all of the guidance contained in this document may be appropriate or practical for every food importing establishment, particularly small facilities. FDA recommends that operators review the guidance in each section that

relates to a component of their operation, and assess which preventive measures are suitable. Example approaches are provided for many of the preventive measures listed in this document. These examples should not be regarded as minimum standards. Nor should the examples provided be considered an inclusive list of all potential approaches to achieving the goal of the preventive measure. FDA recommends that operators consider the goal of the preventive measure, assess whether the goal is relevant to their operation, and, if it is, design an approach that is both efficient and effective to accomplish the goal under their conditions of operation.

C. Structure

This guidance is divided into five sections that relate to individual components of food importing operations and practices: Management; Human Element — Staff; Human Element — Public; Facility; and Operations.

D. Food Importing Operations

1. Management

FDA recommends that operators of food importing establishments consider:

1. Preparing for the possibility of tampering or other malicious, criminal, or terrorist actions.
2. Assigning responsibility for security to knowledgeable individual(s)
3. Conducting an initial assessment of food security procedures and operations, which we recommend be kept confidential
4. Having a crisis management strategy to prepare for and respond to tampering and other malicious, criminal, or terrorist actions, both threats and actual events, including identifying, segregating and securing affected product
5. Planning for emergency evacuation, including preventing security breaches during evacuation
6. Becoming familiar with the emergency response system in the community
7. Making management aware of 24-hour contact information for local, state, and federal police/-fire/rescue/health/homeland security agencies
8. Making staff aware of who in management they should alert about potential security problems (24-hour contacts)
9. Maintaining any floor and food flow plan in a secure, off-site location
10. Promoting food security awareness to encourage all staff to be alert to any signs of tampering or malicious, criminal, or terrorist actions or areas that may be vulnerable to such actions, and to report any findings to identified management (for example, providing training, instituting a system of rewards, building security into job performance standards)
11. Having an internal communication system to inform and update staff about relevant security issues
12. Having a strategy for communicating with the public (for example, identifying a media spokesperson, preparing generic press statements and background information, and coordinating press statements with appropriate authorities)

2. Supervision

1. Providing an appropriate level of supervision to all staff, including cleaning and maintenance staff, contract workers, data entry and computer support staff, and especially, new staff (for example, supervisor on duty, daily visits by supervisor, two staff on duty at all times, monitored video cameras, one way and two way windows)
2. Conducting routine security checks of the premises and critical computer data systems (at a frequency appropriate to the operation) for signs of tampering or malicious, criminal, or terrorist actions, or areas that may be vulnerable to such actions

3. Recall Strategy

1. Identifying the person responsible, and a backup person
2. Providing for proper handling and disposition of recalled product
3. Identifying customer contacts, addresses, and phone numbers

4. Investigation of Suspicious Activity

1. Investigating threats or information about signs of tampering or other malicious, criminal, or terrorist actions
2. Alerting appropriate law enforcement and public health authorities about any threats of or suspected tampering or other malicious, criminal, or terrorist actions

5. Evaluation Program

1. Evaluating the lessons learned from past tampering or other malicious, criminal, or terrorist actions and threats
2. Reviewing and verifying, at least annually, the effectiveness of the security management program (for example, using knowledgeable

in-house or third party staff to conduct tampering or other malicious, criminal, or terrorist action exercises and mock recalls and to challenge computer security systems), revising the program accordingly, and keeping this information confidential
3. Performing random food security inspections of all appropriate areas of the facility (including receiving and storage, where applicable) using knowledgeable in-house or third party staff, and keeping this information confidential
4. Verifying that security contractors are doing an appropriate job, when applicable

E. Human Element — Staff

Under Federal law, operators of food importing establishments are required to verify the employment eligibility of all new hires in accordance with the requirements of the Immigration and Nationality Act, by completing the INS Employment Eligibility Verification Form (INS Form I-9). Completion of Form I-9 for new hires is required by 8 USC 1324a and nondiscrimination provisions governing the verification process are set forth at 8 USC 1324b.

FDA recommends that operators of food importing establishments consider:

1. Screening (pre-hiring, at hiring, post-hiring)
2. Examining the background of all staff (including seasonal, temporary, contract, and volunteer staff, whether hired directly or through a recruitment firm) as appropriate to their position, considering candidates' access to sensitive areas of the facility and the degree to which they will be supervised and other relevant factors (for example, obtaining and verifying work references, addresses, and phone numbers, participating in one of the pilot programs managed by the Immigration and Naturalization Service and the Social Security Administration [These programs provide electronic confirmation of employment eligibility for newly hired employees. For more information call the INS SAVE Program toll free at 1-888-464-4218, fax a request for information to (202) 514-9981, or write to US/INS, SAVE Program, 425 I Street, NW, ULLICO-4th Floor, Washington, DC 20536. These pilot programs may not be available in all states], having a criminal background check performed by local law enforcement or by a contract service provider [Remember to first consult any state or local laws that may apply to the performance of such checks])

Note: screening procedures should be applied equally to all employees, regardless of race, national origin, religion, and citizenship or immigration status.

1. Daily Work Assignments

1. Knowing who is and who should be on premises, and where they should be located, for each shift
2. Keeping assignment information updated

2. Identification

1. Establishing a system of positive identification and recognition that is appropriate to the nature of the workforce (for example, issuing uniforms, name tags, or photo identification badges, with individual control numbers, color coded by area of authorized access), when appropriate
2. Collecting the uniforms, name tag, or identification badge when a staff member is no longer associated with the establishment

3. Restricted Access

1. Identifying staff that require unlimited access to all areas of the facility
2. Reassessing levels of access for all staff periodically
3. Limiting access so staff enter only those areas or have access to only those segments of the operation necessary for their job functions and only during appropriate work hours, including access to data operating systems for purchasing, storing and distributing imported foods (for example, using key card or keyed or cipher locks for entry to sensitive areas, color coded uniforms [remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes])
4. Changing combinations, rekeying locks and/or collecting the retired key card when a staff member who is in possession of these is no longer associated with the establishment, and additionally as needed to maintain security

4. Personal Items

1. Restricting the type of personal items allowed in non-public areas of the establishment
2. Allowing in the establishment only those personal use medicines that are necessary for the health of staff and ensuring that these personal use medicines are properly labeled and stored away from food handling or storage areas

3. Preventing staff from bringing personal items (for example, lunch containers, purses) into food preparation or storage areas
4. Providing for regular inspection of contents of staff lockers (for example, providing metal mesh lockers, company issued locks), bags, packages, and vehicles when on company property (Remember to first consult and federal, state, or local laws that may related to such inspections)

5. Training in Food Security Procedures

1. Incorporating food security awareness, including information on how to prevent, detect, and respond to tampering or other malicious, criminal, or terrorist actions or threats, into training programs for staff, including seasonal, temporary, contract, and volunteer staff providing periodic reminders of the importance of security procedures (for example, scheduled meetings, providing brochures, payroll stuffers)
2. Providing periodic reminders of the importance of security procedures (for example, scheduled meetings, providing brochures, payroll stuffers)
3. Encouraging staff support (for example, involving staff in food security planning and the food security awareness program, demonstrating the importance of security procedures to the staff)

6. Unusual Behavior

Watching for unusual or suspicious behavior by staff (for example, staff who, without an identifiable purpose, stay unusually late after the end of their shift, arrive unusually early, access files/information/areas of the facility outside of the areas of their responsibility; remove documents from the facility; ask questions on sensitive subjects; bring cameras to work).

7. Staff Health

Being alert for atypical staff health conditions that staff may voluntarily report and absences that could be an early indicator of tampering or other malicious, criminal, or terrorist actions (for example, an unusual number of staff who work in the same part of the facility reporting similar symptoms within a short time frame), and reporting such conditions to local health authorities.

F. Human Element — Public

FDA recommends that operators of food importing establishments consider:

1. Visitors (for example, contractors, supplier representatives, delivery drivers, customers, couriers, pest control representatives, third-party auditors, regulators, reporters, tours)
2. Inspecting incoming and outgoing vehicles, packages and briefcases for suspicious, inappropriate or unusual items or activity, to the extent practical
3. Restricting entry to the establishment (for example, checking visitors in and out at security or reception, requiring proof of identity, issuing visitors badges that are collected upon departure, accompanying visitors)
4. Ensuring that there is a valid reason for the visit before providing access to the facility - beware of unsolicited visitors
5. Verifying the identity of unknown visitors
6. Restricting access to food handling and storage areas (for example, accompanying visitors, unless they are otherwise specifically authorized)
7. Restricting access to locker rooms

G. Facility

FDA recommends that operators of food importing establishments consider:

1. Physical Security

1. Protecting perimeter access with fencing or other deterrent, when appropriate
2. Securing doors (including freight loading doors when not in use and not being monitored, and emergency exits), windows, roof openings/hatches, vent openings and trailer bodies, to the extent possible (for example, using locks, "jimmy plates," seals, alarms, intrusion detection sensors, guards, monitored video surveillance [remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes])
3. Using metal or metal-clad exterior doors to the extent possible when the facility is not in operation, except where visibility from public thoroughfares is an intended deterrent (remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes)
4. Securing bulk unloading equipment (for example, augers, pipes, conveyor belts, and hoses) when not in use and inspecting the equipment before use
5. Minimizing the number of entrances to restricted areas (remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes)

6. Accounting for all keys to establishment (for example, assigning responsibility for issuing, tracking and retrieving keys)
7. Monitoring the security of the premises using appropriate methods (for example, using security patrols [uniformed and/or plain-clothed] and video surveillance)
8. Minimizing to the extent practical, places that can be used to temporarily hide intentional contaminants (for example, minimizing nooks and crannies, false ceilings)
9. Providing adequate interior and exterior lighting, including emergency lighting, where appropriate, to facilitate detection of suspicious or unusual activity
10. Implementing a system of controlling vehicles authorized to park on the premises (for example, using placards, decals, key cards, keyed or cipher locks, issuing passes for specific areas and times to visitors' vehicles)
11. Keeping parking areas separated from entrances to food storage and processing areas and utilities, where practical

2. Storage and Use of Poisonous and Toxic Chemicals (for Example, Cleaning and Sanitizing Agents, Pesticides)

1. Limiting poisonous and toxic chemicals in the establishment to those that are required for the operation and maintenance of the facility and those that are being held for sale
2. Storing poisonous and toxic chemicals as far away from food handling and storage areas as practical
3. Limiting access to and securing storage areas for poisonous and toxic chemicals that are not being held for sale (for example, using keyed or cipher locks, keycards, seals, alarms, intrusion detection sensors, guards, monitored video surveillance [remember to consult any relevant state or local fire codes before making any changes])
4. Ensuring that poisonous and toxic chemicals are properly labeled
5. Using pesticides in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (for example, maintaining rodent bait that is in use in covered, tamper-resistant bait stations)
6. Knowing what poisonous and toxic chemicals should be on the premises and keeping track of them
7. Investigating missing stock or other irregularities outside a normal range of variation and alerting appropriate law enforcement and public health authorities about unresolved problems, when appropriate

H. Operations

FDA recommends that operators of food importing establishments consider:

1. Incoming Products

1. Using only known and appropriately licensed or permitted (where applicable) sources for all products
2. Taking reasonable steps to encourage suppliers, distributors and transporters to practice appropriate food security measures (for example, auditing, where practical, for compliance with food security measures that are contained in purchase and shipping contracts or letters of credit or using a vendor approval program.)
3. Authenticating labeling, packaging configuration, tamper-evident packaging and product coding/expiration dating systems (where applicable) in advance of receipt of shipment, especially for new products
4. Requesting locked and/or sealed vehicles/containers/railcars, and, if sealed, obtaining the seal number from the supplier, and verifying upon receipt, making arrangements to maintain the chain of custody when a seal is broken for inspection by a governmental agency or as a result of multiple deliveries
5. Requesting that transporters have the capability to verify the location of the load at any time, when practical
6. Establishing delivery schedules, not accepting unexplained, unscheduled deliveries or drivers, and investigating delayed or missed shipments
7. Supervising off-loading of incoming materials, including off hour deliveries
8. Reconciling the product and amount received with the product and amount ordered and the product and amount listed on the invoice and shipping documents, taking into account any sampling performed prior to receipt
9. Investigating shipping documents with suspicious alterations
10. Inspecting incoming products and product returns for signs of tampering, contamination or damage (for example, abnormal powders, liquids, stains, or odors, evidence of resealing, compromised tamper-evident packaging) or "counterfeiting" (inappropriate or mismatched product identity, labeling, product lot coding or specifications, absence of tamper-evident

packaging when the label contains a tamper-evident notice), when appropriate
11. Inspecting incoming products for authenticity, packaging/product integrity, and evidence of unauthorized relabeling/repackaging (for example, shipping cases and described contents not consistent with actual contents) and verifying batch/lot/container codes
12. Verifying conformance with FDA requirements for product safety, quality, effectiveness, and labeling (may require contact with and verification from the foreign manufacturer/processor)
13. Evaluating the utility of testing incoming products and product returns for detecting tampering or other malicious, criminal, or terrorist action
14. Developing and implementing procedures for inspecting shipping containers, vehicles
15. Investigating damage and loss and alerting appropriate authority of discrepancies
16. Rejecting suspect food
17. Alerting appropriate law enforcement and food public health authorities about evidence of tampering, "counterfeiting" or other malicious, criminal, or terrorist action

2. Storage

1. Having a system for receiving, storing and handling distressed, damaged, returned, and reworked products that minimizes their potential for being compromised or to compromise the security of other products (for example, destroying products that are unfit for human or animal consumption, products with illegible codes, products or questionable origin, and products returned by consumers to retail stores)
2. Keeping track of incoming products, salvage products, and returned products
3. Minimizing reuse of containers, shipping packages, cartons, etc., where practical
4. Investigating missing or extra stock or other irregularities outside a normal range of variability and reporting unresolved problems to appropriate law enforcement and public health agencies, when appropriate

3. Outgoing Products

1. Ensuring that public storage warehousing and shipping (vehicles and vessels) practice appropriate security measures (for example, auditing for compliance with food security measures that are contained in contracts or letters of guarantee
2. Performing random inspection of storage facilities, vehicles, and vessels
3. Requesting locked and/or sealed vehicles/containers/railcars and providing the seal number to the consignee (remember to consult any relevant federal, state or local fire or occupational safety codes before making any changes)
4. Establishing scheduled pickups and not accepting unexplained, unscheduled pickups
5. Restricting access to distribution process to employees with appropriate clearance
6. Requesting that the transporter have the capability to verify the location of the load at any time
7. Advising sales staff to be on the lookout for counterfeit products during visits to customers and notify management if any problems are detected
8. Investigating missing or extra stock or other irregularities outside a normal range of variation and alerting appropriate law enforcement and public health authorities about unresolved problems, when appropriate

4. Security of Water and Utilities

1. Limiting, to the extent practical, access to controls for airflow, water, electricity, and refrigeration securing non-municipal water wells, hydrants, storage, and handling facilities
2. Ensuring that water systems and trucks are equipped with backflow prevention
3. Chlorinating water systems and monitoring chlorination equipment, where practical, and especially for non-municipal water systems
4. Testing non-municipal sources for potability regularly, as well as randomly, and being alert to changes in the profile of the results
5. Staying attentive to the potential for media alerts about public water provider problems, when applicable
6. Identifying alternate sources of potable water for use during emergency situations where normal water systems have been compromised (for example, bottled water, trucking from an approved source, treating on-site or maintaining on-site storage)

5. Security of Ventilation System (where Applicable)

1. Securing access to air intake points for the facility, to the extent possible (for example, using fences, sensors, guards, video surveillance)
2. Examining air intake points for physical integrity routinely

6. Mail/Packages

Implementing procedures to ensure the security of incoming mail and packages (for example, following U.S. Postal

Service guidance, locating the mailroom away from food handling and storage areas, securing mailroom visual or x-ray mail/package screening).

7. Access to Computer Systems

1. Restricting access to critical computer data systems to those with appropriate clearance (for example, using passwords, firewalls)
2. Eliminating computer access when a staff member is no longer associated with the establishment
3. Establishing a system of traceability of computer transactions
4. Reviewing the adequacy of virus protection systems and procedures for backing up critical computer based data systems
5. Validating and periodically challenging the computer security system and procedures

VII. FIELD INSTRUCTIONS FOOD FEDERAL FOOD INSPECTORS REGARDING "FOOD PRODUCERS, PROCESSORS AND TRANSPORTERS: FOOD SECURITY PREVENTIVE MEASURES GUIDANCE" AND "IMPORTERS AND FILERS: FOOD SECURITY PREVENTIVE MEASURES GUIDANCE"

The above named guidance documents were published in the Federal Register on 3/19/03, and are designed primarily as aids for operators of food establishments (i.e., firms that produce, process, store, repack, relabel, distribute, or transport food or food ingredients) and food importers, warehouses, and filers. This guidance is voluntary and is not tied to existing regulations. The guidance documents identify preventive measures that these kinds of establishments can take to minimize the risk that food under their control will be subject to tampering or other malicious, criminal or terrorist actions. The documents set out goals in this regard and provide example strategies for achieving those goals.

You should not perform a comprehensive food security audit of the establishment, nor should you conduct an extensive interview in an attempt to determine the level of adoption of preventive measures listed in the guidance. The goal is to facilitate an exchange of information to heighten awareness of food security. Management of the establishment should be encouraged to voluntarily implement those preventive measures that are appropriate for their operations. Not all of the guidance contained in this document is appropriate or practical for every food establishment, food importer, warehouse, or filer, particularly smaller entities.

Food regulatory officials should cover the preventive measures during regular inspections or audits of food establishments, food importers, warehouses, and evaluation of filers. Based on conditions observed during the normal course of the inspection/audit, the appropriate material in the guidance documents should be discussed with management of the establishment and a copy of the guidance should be provided during the close-out meeting. Additionally, any opportunities for improvement or enhancement of the establishment's preventive measures that were identified during the inspection/audit should be discussed with management, but should not be listed as violations (e.g., on FDA's form FDA-483 Inspectional Observations) unless they likewise constitute deviations from Current Good Manufacturing Practice.

The fact that the discussion took place and that a copy of the guidance document was provided should be recorded in the inspection report (e.g. FDA's Establishment Inspection Report [EIR]) or evaluation report, but the details of the inspectional findings in this regard should not be recorded. For example, under a section heading titled, "Food Security," the regulator should only state, "A copy of the Food Security Guidance Document was provided to and food security issues were discussed with (name of food establishment official)." Additionally, you should minimize the quantity and detail of notes taken relative to the establishment's food security program, taking only those needed to serve as a "memory jog" during the discussion with management.

If management of the establishment has suggestions for improvement of the referenced guidance he/she should be encouraged to provide comments to the Agency via Docket # 01D-0583 (see Federal Register Notice of Availability dated January 9, 2002).

VIII. RETAIL FOOD STORES AND FOOD SERVICE ESTABLISHMENTS: FOOD SECURITY PREVENTIVE MEASURES GUIDANCE

On November 2003, the FDA issed the above guidance document. A brief discussion follows.

A. Purpose and Scope

This guidance is designed as an aid to operators of retail food stores and food service establishments (for example, bakeries, bars, bed-and-breakfast operations, cafeterias, camps, child and adult day care providers, church kitchens, commissaries, community fund raisers, convenience stores, fairs, food banks, grocery stores, interstate conveyances, meal services for home-bound persons, mobile food carts, restaurants, and vending machine operators). This is a very diverse set of establishments, which includes both very large and very small entities.

This guidance identifies the kinds of preventive measures they may take to minimize the risk that food under their control will be subject to tampering or other

malicious, criminal, or terrorist actions. Operators of food retail food stores and food service establishments are encouraged to review their current procedures and controls in light of the potential for tampering or other malicious, criminal, or terrorist actions and make appropriate improvements.

This guidance is designed to focus operators' attention sequentially on each segment of the food delivery system that is within their control, to minimize the risk of tampering or other malicious, criminal, or terrorist action at each segment. To be successful, implementing enhanced preventive measures requires the commitment of management and staff. Accordingly, FDA recommends that both management and staff participate in the development and review of such measures.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

B. Limitations

Not all of the guidance contained in this document may be appropriate or practical for every retail food store or food service establishment, particularly smaller facilities. FDA recommends that operators review the guidance in each section that relates to a component of their operation, and assess which preventive measures are suitable. Example approaches are provided for many of the preventive measures listed in this document. These examples should not be regarded as minimum standards. Nor should the examples provided be considered an inclusive list of all potential approaches to achieving the goal of the preventive measure. FDA recommends that operators consider the goal of the preventive measure, assess whether the goal is relevant to their operation, and, if it is, design an approach that is both efficient and effective to accomplish the goal under their conditions of operation.

1. Structure

This guidance is divided into five sections that relate to individual components of a retail food store or food service establishment operation: management, human element-staff, human element-public, facility, and operations.

Related Guidance:

C. Management

FDA recommends that retail food store and food service establishment operators consider:

Preparing for the possibility of tampering or other malicious, criminal, or terrorist events

1. assigning responsibility for security to knowledgeable individual(s)
2. conducting an initial assessment of food security procedures and operations, which we recommend be kept confidential
3. having a crisis management strategy to prepare for and respond to tampering and other malicious, criminal, or terrorist actions, both threats and actual events, including identifying, segregating, and securing affected products
4. planning for emergency evacuation, including preventing security breaches during evacuation
5. becoming familiar with the emergency response system in the community
6. making management aware of 24-hour contact information for local, state, and federal police/fire/rescue/health/homeland security agencies
7. making staff aware of who in management they should alert about potential security problems (24-hour contacts)
8. promoting food security awareness to encourage all staff to be alert to any signs of tampering or malicious, criminal, or terrorist actions or areas that may be vulnerable to such actions, and to report any findings to identified management (for example, providing training, instituting a system of rewards, building security into job performance standards)
9. having an internal communication system to inform and update staff about relevant security issues
10. having a strategy for communicating with the public (for example, identifying a media spokesperson, preparing generic press statements and background information, and coordinating press statements with appropriate authorities)

1. Supervision

1. providing an appropriate level of supervision to all staff, including cleaning and maintenance staff, contract workers, data entry and computer support staff, and especially, new staff (for example, supervisor on duty, periodic unannounced visits by supervisor, daily visits by supervisor, two staff on duty at same time, monitored video cameras, off-line review of video tapes, one-way and two-way windows, customer feedback to supervisor of unusual or suspicious behavior by staff)

2. conducting routine security checks of the premises, including utilities and critical computer data systems (at a frequency appropriate to the operation) for signs of tampering or malicious, criminal, or terrorist actions, or areas that may be vulnerable to such actions

2. Investigation of Suspicious Activity

1. investigating threats or information about signs of tampering or other malicious, criminal, or terrorist actions
2. alerting appropriate law enforcement and public health authorities about any threats of or suspected tampering or other malicious, criminal, or terrorist actions

3. Evaluation Program

1. evaluating the lessons learned from past tampering or other malicious, criminal, or terrorist actions and threats
2. reviewing and verifying, at least annually, the effectiveness of the security management program (for example, using knowledgeable in-house or third-party staff to conduct tampering or other malicious, criminal, or terrorist action exercises and to challenge computer security systems), revising accordingly (using third-party or in-house security expert, where possible), revising the program accordingly, and keeping this information confidential
3. performing random food security inspections of all appropriate areas of the facility (including receiving and storage areas, where applicable) using knowledgeable in-house or third-party staff, and keeping this information confidential
4. verifying that security contractors are doing an appropriate job, when applicable

D. Human Element-staff

Under Federal law, retail food store and food service establishment operators are required to verify the employment eligibility of all new hires, in accordance with the requirements of the Immigration and Nationality Act, by completing the INS Employment Eligibility Verification Form (INS Form I-9). Completion of Form I-9 for new hires is required by 8 USC 1324a and nondiscrimination provisions governing the verification process are set forth at 1324b.

FDA recommends that retail food store and food service establishment operators consider:

1. Screening (Pre-Hiring, at Hiring, Post-Hiring)

This involves examining the background of all staff (including seasonal, temporary, contract, and volunteer staff, whether hired directly or through a recruitment firm) as appropriate to their position, considering candidates' access to sensitive areas of the facility and the degree to which they will be supervised and other relevant factors (for example, obtaining and verifying work references, addresses, and phone numbers, participating in one of the pilot programs managed by the Immigration and Naturalization Service and the Social Security Administration [These programs provide electronic confirmation of employment eligibility for newly hired employees. For more information call the INS SAVE Program toll free at 1-888-464-4218, fax a request for information to (202) 514-9981, or write to US/INS, SAVE Program, 425 I Street, NW, ULLICO-4th Floor, Washington, DC 20536. These pilot programs may not be available in all states], having a criminal background check performed by local law enforcement or by a contract service provider [Remember to first consult any state or local laws that may apply to the performance of such checks]).

Note: screening procedures should be applied equally to all staff, regardless of race, national origin, religion, and citizenship or immigration status.

2. Daily Work Assignments

1. knowing who is and who should be on premises, and where they should be located, for each shift
2. keeping information updated

3. Identification

1. establishing a system of positive identification and recognition (for example, issuing uniforms, name tags, or photo identification badges with individual control numbers, color coded by area of authorized access), when appropriate
2. collecting the uniforms, name tag, or identification badge when a staff member is no longer associated with the establishment

4. Restricted Access

1. identifying staff that require unlimited access to all areas of the facility
2. reassessing levels of access for all staff periodically
3. limiting staff access to non-public areas so staff enter only those areas necessary for their job functions and only during appropriate work

hours (for example, using key cards or keyed or cipher locks for entry to sensitive areas, color coded uniforms [remember to consult any relevant federal, state, or local fire or occupational safety codes before making any changes])
4. changing combinations, rekeying locks, and/or collecting the retired key card when a staff member who is in possession of these is no longer associated with the establishment, and additionally as needed to maintain security

5. Personal Items

1. restricting the type of personal items allowed in non-public areas of the establishment
2. allowing in the non-public areas of the establishment only those personal use medicines that are necessary for the health of staff (other than those being stored or displayed for retail sale) and ensuring that these personal use medicines are properly labeled and stored away from stored food and food preparation areas
3. preventing staff from bringing personal items (for example, lunch containers, purses) into nonpublic food preparation or storage areas
4. providing for regular inspection of contents of staff lockers (for example, providing metal mesh lockers, company issued locks), bags, packages, and vehicles when on company property (Remember to first consult any federal, state, or local laws that may relate to such inspections)

6. Training in Food Security Procedures

1. incorporating food security awareness, including information on how to prevent, detect, and respond to tampering or other malicious, criminal, or terrorist actions or threats, into training programs for staff, including seasonal, temporary, contract, and volunteer staff
2. providing periodic reminders of the importance of security procedures (for example, scheduling meetings, providing brochures, payroll stuffers)
3. encouraging staff support (for example, involving staff in food security planning and the food security awareness program, demonstrating the importance of security procedures to the staff)
4. encouraging staff support (for example, involving staff in food security planning and the food security awareness program, demonstrating the importance of security procedures to the staff)

7. Unusual Behavior

This involves watching for unusual or suspicious behavior by staff (for example, staff who, without an identifiable purpose, stay unusually late after the end of their shift, arrive unusually early, access files/information/areas of the facility outside of the areas of their responsibility; remove documents from the facility; ask questions on sensitive subjects; bring cameras to work).

8. Staff Health

This involves being alert for atypical staff health conditions that staff may voluntarily report and absences that could be an early indicator of tampering or other malicious, criminal, or terrorist actions (for example, an unusual number of staff who work in the same part of the facility reporting similar symptoms within a short time frame), and reporting such conditions to local health authorities.

E. Human Element — Public

FDA recommends that retail food store and food service establishment operators consider:

1. Customers

1. preventing access to food preparation and storage and dishwashing areas in the non-public areas of the establishment, including loading docks
2. monitoring public areas, including entrances to public restrooms (for example, using security guards, monitored video cameras, one-way and two-way windows, placement of employee workstations for optimum visibility) for unusual or suspicious activity (for example, a customer returning a product to the shelf that he/she brought into the store, spending an unusual amount of time in one area of the store)
3. monitoring the serving or display of foods in self-service areas (for example, salad bars, condiments, open bulk containers, produce display areas, doughnut/bagel cases)

2. Other Visitors (for Example, Contractors, Sales Representatives, Delivery Drivers, Couriers, Pest Control Representatives, Third-Party Auditors, Regulators, Reporters, Tours)

1. restricting entry to the non-public areas of the establishment (for example, checking visitors in and out before entering the non-public areas, requiring proof of identity, issuing visitors

badges that are collected upon departure, accompanying visitors)
2. ensuring that there is a valid reason for all visits to the non-public areas of the establishment before providing access to the facility - beware of unsolicited visitors
3. verifying the identity of unknown visitors to the non-public areas of the establishment
4. inspecting incoming and outgoing packages and briefcases in the non-public areas of the establishment for suspicious, inappropriate or unusual items, to the extent practical

F. Facility

FDA recommends that retail food store and food service establishment operators consider:

1. Physical Security

1. protecting non-public perimeter access with fencing or other deterrent, when appropriate
2. securing doors (including freight loading doors, when not in use and not being monitored, and emergency exits), windows, roof openings/hatches, vent openings, ventilation systems, utility rooms, ice manufacturing and storage rooms, loft areas and trailer bodies, and bulk storage tanks for liquids, solids and compressed gases to the extent possible (for example, using locks, "jimmy plates," seals, alarms, intrusion detection sensors, guards, monitored video surveillance [remember to consult any relevant federal, state, or local fire or occupational safety codes before making any changes])
3. using metal or metal-clad exterior doors to the extent possible when the facility is not in operation, except where visibility from public thoroughfares is an intended deterrent (remember to consult any relevant federal, state, or local fire or occupational safety codes before making any changes)
4. minimizing the number of entrances to non-public areas (remember to consult any relevant federal, state, or local fire or occupational safety codes before making any changes)
5. accounting for all keys to establishment (for example, assigning responsibility for issuing, tracking, and retrieving keys)
6. monitoring the security of the premises using appropriate methods (for example, using security patrols [uniformed and/or plain-clothed], monitored video surveillance)
7. minimizing, to the extent practical, places in public areas that an intruder could remain unseen after work hours
8. minimizing, to the extent practical, places in non-public areas that can be used to temporarily hide intentional contaminants (for example, minimizing nooks and crannies, false ceilings)
9. providing adequate interior and exterior lighting, including emergency lighting, where appropriate, to facilitate detection of suspicious or unusual activity
10. implementing a system of controlling vehicles authorized to park in the non-public parking areas (for example, using placards, decals, key cards, keyed or cipher locks, issuing passes for specific areas and times to visitors' vehicles)
11. keeping customer, employee, and visitor parking areas separated from entrances to non-public areas, where practical

2. Storage and Use of Poisonous and Toxic Chemicals (for Example, Cleaning and Sanitizing Agents, Pesticides) in Non-Public Areas

1. limiting poisonous and toxic chemicals in the establishment to those that are required for the operation and maintenance of the facility and those that are being stored or displayed for retail sale
2. storing poisonous and toxic chemicals as far away from food handling and food storage areas as practical
3. limiting access to and securing storage areas for poisonous or toxic chemicals that are not being held for retail sale (for example, using keyed or cipher locks, key cards, seals, alarms, intrusion detection sensors, guards, monitored video surveillance [remember to consult any relevant federal, state, or local fire codes before making any changes])
4. ensuring that poisonous and toxic chemicals are properly labeled
5. using pesticides in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (for example, maintaining rodent bait that is in use in covered, tamper-resistant bait stations)
6. knowing what poisonous and toxic chemicals should be on the premises and keeping track of them
7. investigating missing stock or other irregularities outside a normal range of variation and alerting local enforcement and public health agencies about unresolved problems, when appropriate

G. Operations

FDA recommends that retail food store and food service establishment operators consider:

1. Incoming Products

1. using only known and appropriately licensed or permitted (where applicable) sources for all incoming products
2. informing suppliers, distributors, and transporters about FDA's food security guidance, "Food Producers, Processors, and Transporters: Food Security Preventive Measures Guidance" and "Importers and Filers: Food Security Preventive Measures Guidance," available at: http://www.cfsan.fda.gov/~dms/guidance.html.
3. taking steps to ensure that delivery vehicles are appropriately secured
4. requesting that transporters have the capability to verify the location of the load at any time, when practical
5. establishing delivery schedules, not accepting unexplained, unscheduled deliveries or drivers, and investigating delayed or missed shipments
6. supervising off-loading of incoming materials, including off hour deliveries
7. reconciling the product and amount received with the product and amount ordered and the product and amount listed on the invoice and shipping documents, taking into account any sampling performed prior to receipt
8. investigating shipping documents with suspicious alterations
9. inspecting incoming products and product returns for signs of tampering, contamination, or damage (for example, abnormal powders, liquids, stains, or odors, evidence of resealing, compromised tamper-evident packaging) or "counterfeiting" (for example, inappropriate or mismatched product identity, labeling, product lot coding or specifications, absence of tamper-evident packaging when the label contains a tamper-evident notice), when appropriate
10. rejecting suspect food
11. alerting appropriate law enforcement and public health authorities about evidence of tampering, "counterfeiting," or other malicious, criminal, or terrorist action

2. Storage

1. having a system for receiving, storing, and handling distressed, damaged, and returned products, and products left at checkout counters, that minimizes their potential for being compromised (for example, obtaining the reason for return and requiring proof of identity of the individual returning the product, examining returned or abandoned items for signs of tampering, not reselling returned or abandoned products)
2. keeping track of incoming products, materials in use, salvage products, and returned products
3. investigating missing or extra stock or other irregularities outside a normal range of variability and reporting unresolved problems to appropriate law enforcement and public health authorities, when appropriate
4. minimizing reuse of containers, shipping packages, cartons, etc., where practical

3. Food Service and Retail Display

1. displaying poisonous and toxic chemicals for retail sale in a location where they can be easily monitored (for example, visible by staff at their work stations, windows, video monitoring)
2. periodically checking products displayed for retail sale for evidence of tampering or other malicious, criminal, or terrorist action (for example, checking for off-condition appearance [for example, stained, leaking, damaged packages, missing or mismatched labels], proper stock rotation, evidence of resealing, condition of tamper-evident packaging, where applicable, presence of empty food packaging or other debris on the shelving), to the extent practical
3. monitoring self-service areas (for example, salad bars, condiments, open bulk containers, produce display areas, doughnut/bagel cases) for evidence of tampering or other malicious, criminal, or terrorist action

4. Security of Water and Utilities

1. limiting, to the extent practical, access to controls for airflow, water, electricity, and refrigeration
2. securing non-municipal water wells, hydrants, storage, and handling facilities
3. ensuring that water systems and trucks are equipped with backflow prevention
4. chlorinating non-municipal water systems and monitoring chlorination equipment and chlorine levels
5. testing non-municipal sources for potability regularly, as well as randomly, and being alert to changes in the profile of the results
6. staying attentive to the potential for media alerts about public water provider problems, when applicable

7. identifying alternate sources of potable water for use during emergency situations where normal water systems have been compromised (for example, bottled water, trucking from an approved source, treating onsite or maintaining onsite storage)

5. Mail/Packages

This involves implementing procedures to ensure the security of incoming mail and packages

6. Access to Computer Systems

1. restricting access to critical computer data systems to those with appropriate clearance (for example, using passwords, firewalls)
2. eliminating computer access when a staff member is no longer associated with the establishment
3. establishing a system of traceability of computer transactions
4. reviewing the adequacy of virus protection systems and procedures for backing up critical computer-based data systems
5. validating the computer security system

If a retail food store or food service establishment operator suspects that any of his/her products that are regulated by the FDA have been subject to tampering, "counterfeiting," or other malicious, criminal, or terrorist action, FDA recommends that he/she notify the FDA 24-hour emergency number at 301-443-1240 or call their local FDA District Office. FDA recommends that the operator also notify local law enforcement and public health authorities.

FDA District Office telephone numbers are listed at: http://www.fda.gov/ora/inspect_ref/iom/iomoradir.html.

ACKNOWLEDGMENT

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201 USDA Safety and Security Guidelines for the Transportation and Distribution of Meat, Poultry, and Egg Products

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CONTENTS

I. INTRODUCTION

In May 2002, FSIS issued the FSIS Security Guidelines for Food Processors to assist Federal- and State-inspected meat, poultry, and egg product plants in identifying ways to strengthen their food security protection. The FSIS made the following statement:

"At the time the FSIS noted its commitment to providing continued guidance to businesses engaged in the production and distribution of USDA-regulated foods. We have worked with the Food and Drug Administration (FDA) and other agencies to now provide guidance for those handling food products during transportation and storage.

The FSIS Food Safety and Security Guidelines for the Transportation and Distribution of Meat, Poultry, and Egg Products are designed to assist small facilities and shippers handling these products. The guidelines provide a list of safety and security measures that may be taken to prevent contamination of meat, poultry, and egg products during loading and unloading, transportation, and in-transit storage. In these voluntary guidelines, we strongly encourage shippers and receivers, as well as transporters of these products, to develop controls for ensuring the condition of the products through all phases of distribution. Such controls are necessary to protect the products from intentional, as well as unintentional, contamination. We recognize that not all of these measures will be appropriate or practical for every facility."

Meat, poultry, and egg products are transported by air, sea, and land. Hazards may be present at any point during transportation and distribution, but are most likely at changes between transportation modes and during loading and unloading. Meat, poultry, and egg products frequently are transported multiple times on their way to the consumer and may be exposed to hazards at each step. For example, a product might be transported from a slaughter establishment to a raw-product processing establishment, next to a further processing plant, and then onto distribution sites and retail markets.

The first section of these guidelines provides food safety measures to help prevent the physical, chemical, radiological, or microbiological contamination of meat, poultry, and egg products during transportation and storage. The second section of the guidelines deals specifically with security measures intended to prevent the same forms of contamination due to criminal or terrorist acts. Both sections apply to all points of shipment from the processor to their delivery at the retail store, restaurant, or other facility serving consumers of the products. These guidelines can be applied whether the potential contamination occurs due to an intentional or unintentional act.

Protecting our Nation's food distribution network is essential to the Nation's homeland security. These guidelines are intended to assist the food industry, as well as Federal, State, and local authorities, in that effort.

II. FOOD SAFETY DURING TRANSPORTATION AND DISTRIBUTION OF MEAT, POULTRY, AND EGG PRODUCTS

Meat, poultry, and egg products are susceptible to contamination from a wide variety of physical, microbial, chemical, and radiological agents. These products are particularly vulnerable to microbiological hazards because their moisture, pH levels, and high protein content provide ideal environments for the growth of bacteria. Because of these characteristics, the products must be carefully monitored to prevent their exposure.

Food safety protection can be improved by the control of hazards through the use of preventive methods including good sanitation, manufacturing practices, and the Hazard Analysis and Critical Control Point (HACCP) system throughout the food production and distribution chain. Meat, poultry, and egg products must be refrigerated or frozen after processing and before shipment to inhibit spoilage and growth of pathogens. During transportation and storage, the challenge is to maintain proper refrigeration

temperatures and to keep the "cold-chain" from breaking during steps such as palletization, staging, loading and unloading of containers, and in storage.

A. General Guidance

In the United States, most food is transported by truck. However, meat, poultry, and egg products may be transferred to and from other modes of transportation during shipment and held at intermediate warehouses as well as at transfer or handling facilities, such as airports, break-bulk terminals, and rail sidings. Because transportation and storage are vital links in the farm-to-table food chain, effective control measures are essential at each point in the food distribution chain to prevent unintentional contamination.

The following general guidelines address food safety measures that should be taken by shippers from the point of food production through delivery. The guidelines do not cover breeding, feedlot, or any other pre-slaughter live-animal operations or pre-shipment operations at egg-laying farms.

B. Transportation Safety Plan

1. Identify Vulnerable Points and Develop a Comprehensive Transportation Sanitation and Safety Plan

1. Processors and distributors shipping products should assess and implement measures that will ensure the sanitation and safety of products from initial shipment through delivery to other destinations. A flow diagram from the point-of-origin to final destination, including all shipping modes/routes, can be a helpful assessment tool.
2. Identify all points of vulnerability where there is potential for adulteration or contamination to occur:

2. Identify Potential Hazards

1. If control points are identified, then determine the method, frequency, and limit that must be met.
2. Identify if control is possible at the point(s) of hazard and what is the most effective point to exert control.
3. This will determine where and how often monitoring and verification of the limits set should occur and what, if any, corrective and preventive actions should be taken.
4. Define what controls should be put in place to prevent product adulteration or contamination during the transportation and storage process.
5. As an additional check on product condition during and after transportation and storage, processors may want to include special arrangements with receivers to sample and conduct microbiological or other tests on products. The results could be compared with pre-shipment results to determine whether adjustments are needed in transport methods or procedures.
6. Verify that contracted transporters (e.g. air, ground, maritime, rail) and storage/warehouse facilities have a food safety program in effect. Consider including specific security measures in contracts and verify that measures are being met.
7. Include procedures for the immediate recall of adulterated products from trade and consumer channels (this applies to processors, transporters, and wholesale and retail distributors).
8. Have a system in place to track your products, including salvage, reworked, and returned products.

C. Train Personnel

1. Train managers and supervisors involved in the transportation, handling, and storage of food products in food hygiene and sanitation. They should be able to judge potential risks, take appropriate preventive and corrective actions, and ensure effective monitoring and supervision to prevent intentional and unintentional contamination from occurring.
2. Train personnel involved in all phases of transport, handling and storage in personal hygiene, vehicle inspection procedures, and transportation procedures that will ensure the safety of meat, poultry, and egg products.

D. Storage Food Safety System

1. Design and maintain a storage and warehousing food safety system.
2. The facility should permit easy access to all areas for cleaning.
3. Adequately insulate the facility and have an adequate temperature control capacity.
4. Prevent access by unauthorized persons through the use of locks and fences, etc.
5. Have an effective, systematic program for preventing environmental contamination and infestation by insects, vermin, etc.

E. Vehicles used to Transport Meat, Poultry, and Egg Products

1. Design and Construct Vehicles to Protect Product

1. Vehicles should be designed and built to make locking and sealing easy, protect the cargo against extremes of heat and cold, and prevent infestation by pests.

2. Vehicle design should permit effective inspection, cleaning, disinfection, and temperature control.
3. Interior surfaces should be made of materials that are suitable for direct food contact. For example, the surfaces may be made with stainless steel or be coated with food-grade epoxy resins.

2. Sanitize and Properly Maintain Vehicles

1. Meat, poultry, and egg product transportation vehicles, accessories, and connections should be kept clean and free from dirt, debris, and any other substance or odor that may contaminate the product. They should be disinfected as needed. Cleaning and sanitation procedures should be specified in writing.
2. Different cleaning procedures may be necessary for the different types of meat, poultry, or egg products that are to be transported. The type of product transported and the cleaning procedure used should be recorded. Generally, wash water should be at least 180°F (82°C) and an approved sanitizer may be used to reduce the number of microorganisms and dissolve any fat particles adhering to interior surfaces.
3. Cargo pallets, load securing devices, and loading equipment should be kept clean and free of potential food contaminants and be regularly washed and sanitized.
4. Equipment used in transferring meat, poultry, and egg products, such as hand trucks, conveyors, and forklifts, should be well maintained and kept in a sanitary condition.
5. Secure transport vehicles to prevent tampering when not in use.

3. Use Dedicated Transport Vehicles

1. Transport vehicles, containers, and conveyances should be designated and marked "for food use only," and be used only for transporting foods. If feasible, they should be restricted to a single commodity. This reduces the risk of cross contamination from previous cargoes.

F. Pre-Loading

1. General Guidelines

1. Loading and unloading areas should be configured, cleaned, disinfected (where appropriate), and properly maintained to prevent product contamination.
2. Loading or unloading facilities should be designed to permit easy access to all areas for cleaning.
3. Facilities should be adequately insulated and have an adequate temperature control capacity.
4. Facilities should have an effective, systematic program for preventing environmental contamination and infestation by insects, vermin, etc.

2. Examine Vehicles before Loading

1. Trailer or truck body should be sufficiently insulated and be in good repair with no holes in the body that might allow heat, dust, or other adulterants to enter the cargo area.
2. Check for residues of previous cargoes.
3. Check for residues from cleaning and sanitizing compounds.
4. The cooling unit must be in good repair and operating. Both truck drivers and plant personnel should check the functioning of the trailer refrigeration unit.
5. Trailers and trucks should be pre-cooled for at least 1 hour before loading to remove residual heat from the insulation and inner lining of the trailer as well as from the air of the trailer. For pre-cooling, the doors should be closed and the temperature setting of the unit should be no higher than 26°F. (Note, however, that poultry products labeled "fresh" must be shipped at temperatures higher than 26°F, usually between 26°F and 32°F.)
6. Inspect trailers prior to loading to determine that the air chutes, if used, are properly in place and that the ribbed floors are unclogged so that adequate air circulation can occur.
7. Examine trailer doors and seals to ensure that they can be secured and that there will be no air leaks.
8. When shipping a mixed load of products, such as both frozen and refrigerated products, it may be necessary to use a trailer with compartments that accommodate different temperature or other handling requirements.

3. Stage Loads to Facilitate Proper Stowage and Minimize Exposure during Loading and Unloading

1. Proper staging of loads is especially important when there are loads of products with different temperature requirements, or different delivery destinations.
2. Dock foremen should document that all freight is 40°F or lower before loading. Freight should not be allowed to remain on the loading dock in warm weather in order to prevent the product temperature from rising above 40°F.

3. *Note*: Federal regulations require processed poultry to be packaged and shipped at a temperature no higher than 40°F.

G. Loading

1. General Guidelines

1. Protect products from exposure to environmental contaminants such as microbes, dust, moisture, or other physical contamination.
2. Maintain the "cold chain" to ensure meat, poultry, and egg products are kept at appropriate temperatures continuously throughout all phases of transport.
3. Meat, poultry, and egg products must be kept refrigerated and protected from temperature changes. All persons involved in the transportation, storage, and handling of these products are responsible for keeping them at appropriate temperatures and preventing any break in the cold chain.
4. Maintain the appropriate temperature of the pre-cooled product by minimizing the time of loading or unloading, conducting the loading and unloading in an appropriately chilled environment, and reducing the amount of surface contact of the product with floors and walls of the storage areas or loading equipment.
5. Appropriately packaged meat, poultry, or pasteurized egg products can be stacked, provided that air circulation is sufficient to maintain the temperature of the products during shipment.
6. Product should be at the desired transit temperature before loading. The boxes and pallets should be secured within the vehicle and pallets should be center-loaded off the walls of the vehicle.
7. Seal vehicles shipping egg products from one official plant to another for pasteurization, repasteurization, or heat treatment. (A certificate stating that the products are not pasteurized or that they have tested positive for *Salmonella* should accompany applicable shipments.)

2. Use Appropriate Loading Procedures and Equipment

1. Use spacers on sidewalls and at the ends of trailers as well as pallets on the floor so that proper air circulation can be main tained.
2. Keep loading time as short as possible to prevent temperature changes (increases or decreases) that could threaten the safety or quality of food products.
3. Close doors immediately after the truck/trailer has pulled away from dock.

3. Use Special Care with Mixed or Partial Loads

1. Partial and mixed loads increase the frequency and duration of open doors, leading to a greater possibility of temperature fluctuations and exposure to tampering.
2. Other factors affecting temperature include the time of loading and unloading, the number of stops, the total length of the haul from origin to destination, and the outside temperature.
3. During periods of warm weather, loading or unloading should be done in the evening or early morning to minimize the likelihood of products warming.

H. In-Transit

1. General Guidelines

1. Establish procedures to periodically check integrity of the load during transit.
2. Check for leakage of heating or cooling fluid onto food products.
3. Monitor the temperature and function of the refrigeration unit at least every 4 hours. If there is a unit malfunction, the problem should be corrected by an authorized refrigeration mechanic before the temperature of the load rises.
4. Check for breakdown of temperature control.
5. Use time-temperature recording, indicator, or integrator devices, if they are available, to monitor the condition of cargo. Check the devices every 4 hours.

2. Establish Procedures to Ensure Product Safety during Interim Storage

1. Maintain logbook documenting product condition upon arrival and during storage.
2. Ensure proper temperatures are maintained during storage of meat, poultry, and egg products.

I. Unloading

1. Carefully Examine Incoming Products

1. Product should be inspected and sorted before being accepted at any point during transportation.
2. Develop and implement methods to check and document condition of product and packaging upon receipt at destination. Examine checks of time-temperature recording, indicating or integrator devices or, by prior arrangement with the

shipper, test to determine if bacterial growth has occurred after the product was packaged and shipped.
3. Include procedures for the safe handling and disposal of contaminated products. Identify where and how to separate contaminated products.
4. Establish policy and procedures for rejection of packages and products that are not acceptable, can't be verified against the delivery roster, or contain unacceptable changes to shipping documents. Have a monitoring plan and record-keeping system in place to document steps taken.
5. Do not accept products known to be, or suspected of being, adulterated.
6. Move product from the loading dock into cold storage immediately to minimize product exposure to heat and contaminants.

III. FOOD SECURITY DURING TRANSPORTATION AND DISTRIBUTION OF MEAT, POULTRY, AND EGG PRODUCTS

The tragic events of September 11, 2001, forever changed our world. They proved to us that the unthinkable could become reality, and that threats to our Nation's food supply are very plausible from those who want to harm us through any possible means. Since the terrorist attacks on America, security — including food security — has been the highest priority at both the Federal and State levels.

Ensuring safe food within the processing plant, during transportation, in storage, and at retail is a vital function to protect public health. We must now look at all possible threats, examine the risks, and take action to prevent any intentional attack on the food supply.

A. General Guidance

Meat, poultry, and egg products are susceptible to intentional contamination from a wide variety of physical, chemical, biological, and radiological agents. Everyone in the food distribution system is responsible for ensuring that these products are safe, wholesome, and unadulterated. Therefore, as part of this system, those responsible for transportation and delivery should implement every possible security measure to ensure the integrity of the products throughout the supply chain.

There are many potential benefits of having an effective security plan in place such as:

1. protects public health and assets;
2. increases public and customer confidence, including trading partners;
3. provides value-added component to product;
4. deters theft and tampering;
5. creates production and distribution efficiencies;
6. maintains greater control over product through supply chain; and
7. possibly reduces insurance premiums and freight rates.

The guidelines below provide a list of security measures to be considered by processing plants, shipping companies, and warehouse facilities to minimize the risk of tampering or other criminal action for each segment of the food-delivery system.

B. Security Plan

1. Assess Vulnerabilities

Identify a food protection management team and assign a leader to verify required actions are implemented and effective.

Develop a comprehensive transportation security plan and assess vulnerabilities using a recognized threat/risk/vulnerability model such as Operation Risk Management (ORM) and Systematic Assessment of Facility Risk (SAFR). A flow diagram from your point-of-origin to final destination, including all shipping modes/routes, can be a helpful tool in your assessment.

In your security plan, identify all points of vulnerability where there is the potential for intentional adulteration or contamination to occur during the transportation and distribution process:

1. identify potential biological, chemical, and physical hazards.
2. identify if control is possible at the point(s) of hazard and what is the most effective point to exert control.
3. if control points are identified, then determine the method, frequency, and limit that must be met.
4. this will determine where and how often monitoring and verification of the established limits should occur and what, if any, corrective and preventive actions should be taken.

2. Develop and Implement Procedures

1. Implement identified security measures at each point to ensure the protection of products from the time of shipment through delivery to each destination.
2. The plan should include a system to identify and track your product at any time during transportation and distribution such as the use of tamper-resistant seals corresponding to specific shipments and their documentation.

3. Verify that contracted transporters (e.g. air, ground, maritime, rail) and storage/warehouse facilities have a security program in effect. Consider including specific security measures in contracts and verify that measures are being met.
4. Include procedures for the immediate recall of adulterated products from trade and consumer channels.
5. Have a system in place to track salvaged, reworked, and returned products.
6. Include procedures for handling threats to and actual cases of product tampering.
7. Establish an evacuation plan for the facility.
8. Include procedures for the safe handling and disposal of contaminated products. Identify where and how to separate suspected products.
9. Develop and implement methods to check and document condition of product and packaging upon receipt at destination.
10. Establish policy and procedures for rejection of packages and products that are not acceptable, can't be verified against the delivery roster, or contain unacceptable changes to shipping documents. Have a monitoring strategy and record keeping system in place to document steps taken.
11. Establish policy and procedures for allowing rail crew, truckers, etc., to enter the facility and monitor their activities while on the property.
12. Food security plans should be kept in a secure location and shared on a "need-to-know" basis.

3. Emergency Operations

1. Regularly update a list of local, State, and Federal emergency contacts, local Homeland Security contacts, and local public health official contacts.
2. Develop procedures for notification of appropriate authorities if an event occurs.
3. Identify all entry and exit points available to emergency personnel in the plan.
4. Develop a strategy for communicating with the media, including identifying a spokesperson, drafting press statement templates, or referring media to trade association or corporate headquarters.

4. Train and Test

1. Train each team member in all provisions of the plan.
2. Conduct drills regularly to test and verify the effectiveness of the plan. Continually review policies and procedures in the plan. The food-protection management team leader should coordinate these activities.

5. Screen and Educate Employees

1. Screen all potential employees, to the extent possible, by conducting background and criminal checks appropriate to their positions, and verifying references (including contract, temporary, custodial, seasonal, and security personnel). When this is not practical, such personnel should be under constant supervision and their access to sensitive areas of the facility restricted.
2. Consider participating in the Immigration and Naturalization Service (INS) pilot program for screening (888-464-4218).
3. All employees should be trained on how to prevent, detect, and respond to threats or terrorist actions so they can recognize threats to security and respond if necessary.
4. Promote ongoing security consciousness and the importance of security procedures.
5. Personnel involved in the transport, handling, and storage of meat, poultry, and egg products should be trained in procedures that will ensure the security of these products (e.g., train dock and security personnel on documentation requirements for incoming and outgoing shipments).
6. Train appropriate personnel in security procedures for incoming mail, supplies, and equipment deliveries. Mail handlers should be trained to recognize and handle suspicious mail using U.S Postal Service guidelines.
7. Ensure employees know emergency procedures and contact information.
8. Encourage employees to report any suspicious activities such as signs of possible product tampering or break in the food security system. Have a tracking system in place for these reports and follow-up activities.

C. Secure the Facility

1. Access

1. Maintain a positive ID system for employees. Require identification and escort visitors at all times in your facility.
2. Collect company-issued IDs, keys and change lock combinations when a staff member is no longer employed by the company.
3. Ensure clear identification of personnel to their specific functions (e.g. colored hats or aprons, ID cards).
4. Restrict types of personal items allowed in the establishment, especially firearms or other weapons.

5. Secure and restrict access to facilities, transportation trucks, trailers, or containers, locker rooms, and all storage areas with alarms, cameras, locks and fences or other appropriate measures, to prevent access by unauthorized persons.
6. All visitors should be escorted while on the premises. Establish procedures for handling unauthorized persons in a restricted access area.
7. Control access to food products by unauthorized persons by limiting access to food delivery, storage, food ingredient, and chemical storage areas.
8. Restrict access to computer data systems. Protect them using firewalls, virus detection systems and secure passwords, changing them routinely.
9. Restrict access to outside water tanks, water supplies, ice machines, and conveying water pipes.
10. Restrict access to central controls for heating, ventilation, and air conditioning (HVAC), electricity, gas, and steam systems to prevent contamination from entering the air distribution systems.

2. Shipping/Receiving

1. Consider developing a checklist for shipping and receiving procedures (this can also help identify anomalies).
2. Loading docks should be secured to prevent unauthorized deliveries.
3. All deliveries should be scheduled and truck drivers should show proper identification upon arrival.
4. Shipping documents should contain product information, name of carrier(s), driver information, and seal numbers.
5. Establishments should require that incoming shipments be sealed with tamper-proof, numbered seals, and that the seal numbers are shown on the shipping documents for verification prior to entry to the facility.
6. Shipping documents with suspicious alterations should be thoroughly investigated. Product should be held and segregated during investigation process.
7. Properly secure transportation trucks, trailers, or containers:
8. Doors should not be left open when picking up a load from a warehouse.
9. Ensure shipping trucks, trailers, and containers are secured after loading is complete.
10. Lock transportation trucks, trailers, and containers when not in use, during meal breaks and at night.
11. Apply seals to all containers being shipped and maintain a seal log. Have a system in place to verify seal numbers and the integrity of the seals throughout the distribution process.
12. Ensure security procedures are in effect for interim storage at in-transit warehouses.

3. Facility

1. Designate limited and specific entry and exit points for people and trucks.
2. Secure all access and exit doors, vent openings, windows, outside refrigeration and storage units, trailer bodies and bulk storage tanks.
3. Ensure adequate interior and exterior lighting at the facility.
4. Parking areas for visitors should be situated away from the main facility, if practical. Vehicles of employees and visitors should be clearly marked (e.g., placards, decals). This is intended to identify vehicles authorized to be on the premises and deter bombing attempts.
5. Hazardous chemical storage areas or rooms should be secured and located away from food preparation and storage areas. In addition, they should be constructed and safely vented in accordance with national or local building codes.
6. Incoming mail should be handled in an area of the facility separate from food handling, storage, or preparation areas.
7. Install backflow devices on all water supply equipment.

D. Monitor Operations

1. Employees

1. Maintain a daily shift roster to easily identify persons who are/should be on the premises and indicate that they are in their appropriate location.
2. Provide appropriate level of supervision to all staff, including food handlers, cleaning and maintenance staff, and computer support staff.
3. Monitor employees for unusual behavior (e.g., staying unusually late, arriving unusually early, taking pictures of the establishment, or removing company documents from the facility).

2. Shipping/Receiving

1. Purchase all food ingredients, food products, and packaging materials only from known, reputable suppliers. Require Letters of Guaranty, if possible.
2. Require locked or sealed trucks, trailers, or containers for deliveries. Maintain an inbound load verification logbook. Verify inbound

trucks for seal numbers and integrity and load manifest. Document seal numbers and the truck or trailer number.
3. Hold unscheduled deliveries outside the premises pending verification of shipper and cargo. Do not accept deliveries from, or release product to, unknown shippers using only cell phone numbers or known shippers with unknown phone/fax numbers or e-mail addresses.
4. Supervise off-loading of incoming products, ingredients, packaging, labels, and product returns. Only a supervisor or other agent of the owner should break seals and sign off in the trucker's logbook.
5. Have system in place to ensure integrity of product when seal will need to be broken prior to delivery due to multiple deliveries or for inspection by government officials.
6. Verification of the last company seal put on a truck should be available throughout the delivery chain.
7. Examine incoming products and their containers for evidence of tampering or adulteration:
   a. Determine a random or other sound plan for checking incoming product;
   b. The warehouse supervisor should note on the bill of lading any problems with the condition of the product, packaging, labels, and seals;
   c. Do not accept products known or suspected of being adulterated; and
   d. Check food for unusual odor or appearance.
8. Processors may want to arrange with receivers to sample and conduct microbiological or other tests on products.
   a. This would require an in-house testing plan prior to shipment.
   b. The results should be compared with preshipment results to determine whether adjustments are needed in transport methods or procedures.
   c. Establish chain-of-command procedures providing for the proper handling of samples.
   d. Samples should be clearly marked and kept in a secure area.
   e. Ensure all trucks leaving the facility are sealed.
   f. Maintain a logbook of seal assignments.

3. Storage/Water

1. Maintain an accurate inventory of food and chemical products and check daily to allow detection of unexplained additions to, or withdrawals from, existing stocks. Include information about the sources and date of shipment. All discrepancies should be investigated immediately.
2. Perform random inspection of storage facilities (including temporary storage trailers or containers), trucks, trailers, containers, and vessels regularly. Keep a log of results. Designate an individual to conduct the inspection and have a record-keeping system in place.
3. Regularly test water and ice supply to ensure it is safe to use.
4. Inspect water storage and conveying lines inside and outside of the facility regularly for tampering or irregularities.

4. Respond

1. Be aware of and report any suspicious activity to appropriate authorities (e.g., unscheduled maintenance, deliveries, or visitors should be considered suspicious).
2. Processors, transportation managers, and wholesale and retail distributors should ensure traceability and recall of products.
3. Ensure procedures are in place to accomplish a complete, rapid recall, and removal from the market of any shipment of meat, poultry, and egg products in the event products are found to present a hazard to public health.
4. Keep detailed production records, including packaging lot or code numbers and where finished product was stored or served.
5. Trace Forward – Shippers (including operators of federally inspected meat, poultry, and egg processing establishments) and carriers should have systems in place for quickly and effectively locating products that have been distributed to wholesalers and retailers.
6. Trace Backward – Retailers, wholesalers, carriers and others who have received products from federally inspected meat, poultry, or egg processing establishments should be able to identify the source of the products quickly and efficiently.
7. Investigate threats or reports of suspicious activity swiftly and aggressively.
8. In the event of a food security emergency, first contact your local law-enforcement authority.

E. Additional Guidance for Specific Modes of Transportation

Of the approximately 200.5 billion metric tons of food shipped internationally each year, 60 percent goes by sea, 35 percent by land, and 5 percent by air. Domestically, most food products move via ground transportation (truck and rail). Thus, it is critical that everyone involved in the food

delivery system understands his or her role and responsibility to ensure the security of meat, poultry, and egg products to the end point or consignee. Recognizing the inter-modal nature of this system, a multi-layered approach to protecting food is essential.

F. General Guidance for All Modes

Make certain that contracted shippers and consignees have security measures in place to ensure product integrity and traceability and verify that they are meeting contractual security obligations. Security measures should include:

1. Physical boundaries of the facility/terminal are secure;
2. Background checks are conducted for all potential employees by shipping, trucking, and drayage companies;
3. A positive identification system is in place for all employees. (Recommend requiring participation in the Transportation Worker Identification Card (TWIC) program which is coordinated by the Transportation Security Administration);
4. A security training and awareness program for all employees on how to prevent, detect, and report suspicious activity is conducted;
5. A system is in place to track movement of products and truck, trailer, and containers/vessels (e.g., Global Positioning System);
6. Maintains record-keeping system to document chain-of-custody, which will aid in tracing product;
7. Uses a system (e.g., x-ray scanners) to detect tampering and radiological, biological, and chemical agents in shipping containers;
8. Policies and procedures are in place for the handling of suspicious product; and
9. Ensures all containers are properly secured at all times when held in storage yards.

1. Aviation

Although fewer meat, poultry, and egg products are transported by air than by other modes, it is still critical to ensure the security of these products when this mode of transport is utilized.

1. Check all trucks entering a terminal facility.
2. Trucks carrying meat, poultry, and egg products should have seal logbooks and the seals should be examined and numbers verified.
3. Inspect containers arriving at a terminal for loading before admitting them to the terminal.
4. Immediately report suspicious or inconsistent servicing of a container to terminal security.
5. Design internal and external packaging so customers will be able to determine if the product was tampered with and can immediately notify you. Provide instructions and contact information with shipment.

2. Truck

Approximately 21 million trucks transport products across the United States every day. Keeping containers secure is a huge undertaking as there can be many opportunities for tampering.

1. Develop and implement procedures for drivers to ensure security of the truck, trailer, or container when stopping for meals, gas, and repairs.
2. Transportation trucks, trailers, and containers should be designed and built to make locking and sealing easy and should permit effective inspection.
3. Examine trailer doors and seals to ensure that the trailer can be secured.
4. Keep empty trailers locked at all times.
5. Check product load periodically during transit to ensure its integrity has not been compromised (e.g., use weigh station stops as an opportunity to check condition of products).
6. Processors, distributors, and transporters should have action plans for emergencies, such as breakdowns or reporting criminal activity. The plans should include notification of the relevant Federal, State, and local authorities.
7. Drivers should be trained to take appropriate precautions while en route (e.g., do not pick up hitchhikers, do not discuss the nature of cargo at stops, be aware of surroundings, lock truck, trailer, or container when unattended and avoid low-lit areas).
8. Prevent unauthorized access to delivery truck, trailer, or containers. Require drivers to secure truck, trailer, or containers while en route, including while on break, at restaurants, at overnight stays, etc.
9. Drivers should report unusual circumstances, such as being followed, to appropriate authorities.
10. Develop procedures to be followed when reefer boxes or trailers are found unlocked.
11. Deter diversion or hijacking of cargo by keeping track of trucks. Ensure time logs for trips are maintained and provide trucks with communication and tracking equipment.
12. Hold drivers accountable for ensuring security measures are taken to prevent contamination of

meat, poultry, and egg products while under their control.

3. Maritime

Ports are vulnerable due to their size, accessibility by water and land, location in metropolitan areas, and the quantity of products moving through them. Approximately 80% of U.S. imports arrive via American seaports, yet U.S. Customs physically inspects only a fraction of all containers; the remainder are electronically screened. Therefore, enhanced security measures are necessary for products shipped by sea.

1. Check all trucks entering a terminal facility. Trucks carrying meat, poultry, and egg products should be sealed, drivers should have seal logbooks, and the seals should be verified.
2. Seals should be removed in the presence of terminal personnel so they can verify the seal number and its integrity.
3. Immediately report suspicious or inconsistent servicing of a container to terminal security.
4. Supervise opening of ship hatches.
5. When unloading product from sea-going vessels, inspect seals for evidence of tampering. Have documentation system in place.
6. Document cutting of seals (e.g., when seal is cut for inspection by government official).
7. Shipping line agents should provide importers and customs brokers with a record of vessel discharge and checks at discharge and in transit.
8. Establish policy and procedures to download reefer electronic information during inspection (this will also allow identification of anomalies);
9. Have reporting system in place when the discharging of any product looks suspicious or the product shows evidence of tampering.
10. The terminal facility should be locked during meal breaks and at night.
11. Facility doors should be closed immediately after the truck/trailer has pulled away from dock.

*Importers and Exporters may want to consider participation in government initiatives pertaining to maritime shipment of products such as:

1. Customs-Trade Partners Against Terrorism (C-TPAT)
2. Operation Safe Commerce
3. Container Security Initiative
4. Sea Carrier Initiative Agreement

4. Rail

Rail transportation is an integral part of the domestic food distribution system, therefore, it is important to recognize that unsecured containers can be easy targets for tampering and address this vulnerability.

1. Use boxcars dedicated for food products.
2. Employ measures to secure loaded and empty containers from tampering when being stored at the train yard for any length of time.
3. Locks/seals on boxcars should be inspected at pull and place.
4. Review shipping documents upon arrival at the trainyard and before the train engineer leaves.
5. Inspect integrity of seals upon arrival and before departure of the load.

IV. RESOURCES

If you have questions or need clarification about the guidelines, contact the FSIS Technical Service Center at: 1-800-233-3935.

Obtain additional copies of the guidelines at: http://www.fsis.usda.gov or call 202-720-9113.

Further information on the safe and secure transportation of food is available from:

1. USDA Agricultural Marketing Service: http://www.ams.usda.gov/tmd/tsb/
2. Transportation Security Administration: http://www.tsa.dot.gov/
3. Food and Drug Administration: http://vm.cfsan.fda.gov/~dms/secguid6.html
4. Federal Aviation Administration: http://www.faa.gov/
5. U.S. Postal Service: http://www.usps.com/cpim/ftp/pubs/pub166/welcome.htm
6. U.S. Customs: http://www. customs. ustreas. gov/
7. American Association of Railroads: http://www.aar.org/
8. American Trucking Association: http://www.trucking.org/
9. National Cargo Security Council: http://www.cargosecurity.com/ncsc/
10. The World Health Organization: http://www.who.int/fsf

*Consider researching government Internet sites to obtain funding resources (e.g., grants and loans) to enhance your security program.

ACKNOWLEDGMENT

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